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Synthetic studies of thiazoline and thiazolidine-containing natural products. Part 3: Total synthesis and absolute configuration of the siderophore yersiniabactin

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Abstract—Total synthesis of yersiniabactin, a siderophore from cultures of the bacterium *Yersinia enterocolitica*, was accomplished. Chirality at the readily racemizable C-9 carbon was preserved during cyclization of β-hydroxythioamide by means of Burgess reagent leading to thiazoline. Based on its synthesis, the absolute configuration of natural yersiniabactin has been determined as 9*R*, 10*RS*, 12*R*, 13*S* and 19*S*.

In our previous papers,[1] we reported the total synthesis of micacocidin (**1**),[2] which comprises two thiazoline and one thiazolidine moieties. In our continuing studies, we have accomplished total synthesis of yersiniabactin[3a] (yersiniophore[3b]), a siderophore produced by the gram-negative coccoid bacterium *Yersinia enterocolitica*.

Yersiniabactin was initially isolated as a mixture of two diastereomers (isomers I and II) in regard to its C-10 configuration, and the proposed plane structure closely resembled micacocidin (**1**) as well as in pyochelin I (**2**), another siderophore (Fig. 1). Through gallium and aluminum complex formation, yersiniabactin can be readily unified into a single stereoisomer.[3] As reported previously, micacocidin (**1**) also readily formed complexes with some metal ions such as zinc, iron and copper. From the result of molecular modeling of **1**, we assumed that a molecular structure with natural stereochemistry was most favorable for forming these metal complexes. In addition, we found that the C-10 isomer of **1** could be readily isomerized to the natural C-10*R* form through metal-chelation.[1] This ready complex-formation of yersiniabactin and micacocidin (**1**) suggested that both compounds might have common stereochemical characteristics.

We inferred the absolute configuration of yersiniabactin to be identical with that of micacocidin (**1**) as shown by structure **3**, except the C-10 configuration. Consequently, a retrosynthetic analysis for yersiniabactin (**3**) was designed in which the product was constructed from two segments A and B, and for the synthesis of segment B, an intermediary compound prepared in the synthesis of **1** could be utilized. On the other hand, stereocontrol at C-9 was most critical in our micacocidin synthesis.[1] However, lack of the 5-pentyl moiety in yersiniabactin suggested that stereocontrol at C-9 in the thiazoline moiety might be achieved more easily. So, the method for synthesis of segment A was designed starting from D-serine via thioamide **4** (Scheme 1).

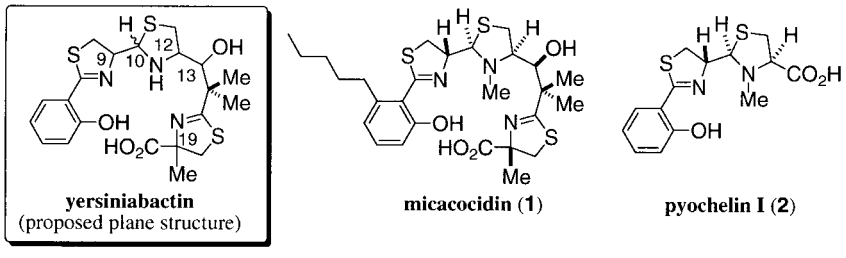

Figure 1.

Keywords: siderophores; thiazolines; thiazolidines; configuration.
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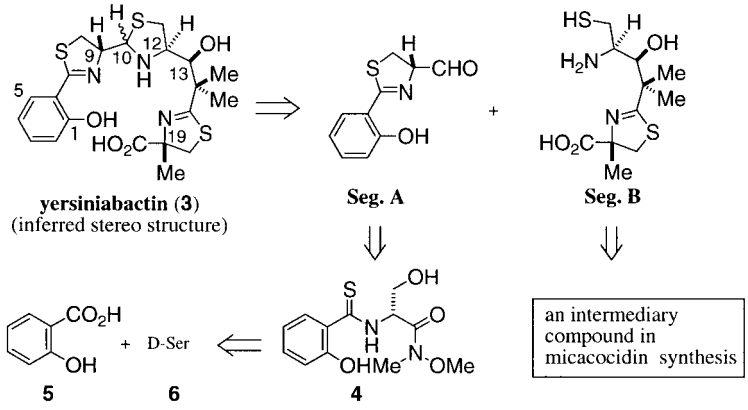
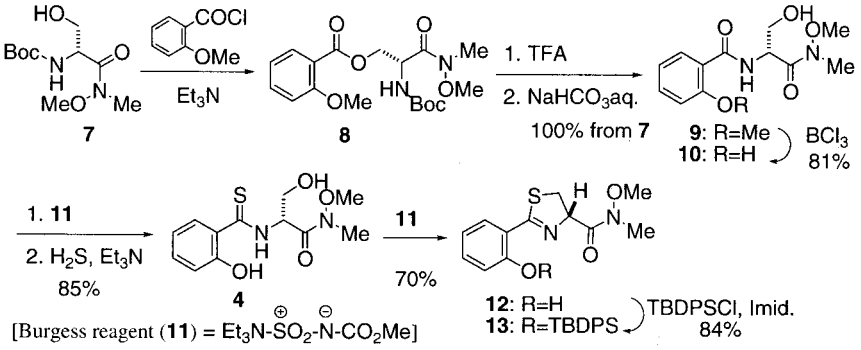
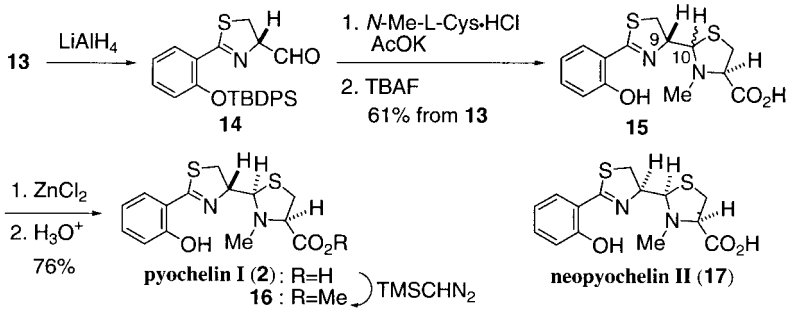


Scheme 1. Retrosynthetic analysis.



Scheme 2.



Scheme 3.

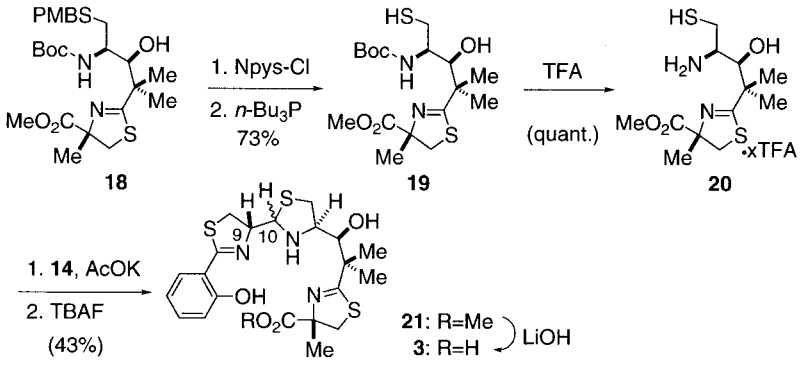
1. Synthesis of segment A

Ester **8**, which was prepared by condensation of Weinreb amide **7**[4] and 2-methoxybenzoyl chloride, was treated with TFA and then subjected to alkaline-mediated acyl-migration to afford amide **9** in quantitative yield. After demethylation by treatment with boron trichloride giving **10**, the amide was converted to thioamide **4** via an oxazoline intermediate by treatment first with Burgess reagent (**11**) and then with H_2S and Et_3N. Treatment of **4** again with **11** gave thiazoline **12** in high enantiomerical purity (73–89% ee).[5] Finally, the phenol residue of **12** was protected with a *t*-butyldimethylsilyl (TBDPS) group to give the protected segment A **13**, which was provided to the following reactions without separation of the enantiomer (Scheme 2).

2. Stereocontrolled synthesis of pyochelin I

Since protected segment A (**13**) was available, we next performed stereoselective synthesis of another siderophore, pyochelin I (**2**),[6] which is also known to exist in nature as a mixture of two diastereomers in regard to the C-10 configuration, in order to clarify the stereochemical preference at C-9 and C-10 chiral centers, which were crucial in our synthetic route for yersiniabactin (**3**). In accordance with our method for micacocidin synthesis and with that for pyochelin synthesis reported by Cox et al.,[6b,†] the condensation of labile aldehyde **14**, which was prepared by reduction of Weinreb amide **13**, with *N*-methyl-L-cysteine

† In this synthesis, the C-9 chiral center of pyochelin was not defined stereoselectively.



Scheme 4.

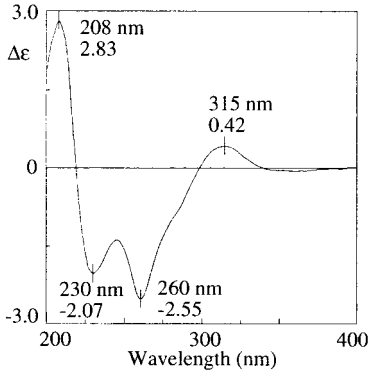


Figure 2. CD spectrum of synthesized **3**. c=9.24×10^{-5} M in H_2O.

hydrochloride[7] and subsequent deprotection of the TBDPS group gave pyochelins (a mixture of four diastereomers). Treatment of the mixture with zinc chloride was shown to unify the C-10 chiral center into *R* configuration to yield pyochelin I (**2**) and neopyochelin II (**17**) (as a ca. 5:1 mixture) (Scheme 3).

3. Total synthesis of yersiniabactin

Favorable condensation reaction of two segments for synthesis of yersiniabactin was achieved through a procedure as carried out in our total synthesis of micacocidin (**1**).[1] Thus, the protecting groups of thiol and amino groups in alcohol **18**,[1b] which was an intermediary compound in our previous synthesis of micacocidin, were removed via three successive reactions to provide **20**. Condensation reaction of **20** with **14** through the same procedure afforded thiazolidine **21** as a mixture with the C-9 isomers. Finally, alkaline hydrolysis of the terminal ester moiety and the purification by HPLC furnished **3** (Scheme 4).

4. Absolute stereochemistry of yersiniabactin

The ^{1}H NMR spectrum of synthesized **3** in DMF-d_7 was shown to be identical to that of natural yersiniabactin.[3,8,‡] So, we concluded that yersiniabactin possesses the same relative stereochemistry as micacocidin (**1**), except for the C-10 configuration which is an *R*,*S* mixture in nature, as shown by **3**.

Furthermore, the CD spectrum of synthesized **3** shown in Fig. 2 was identical to that of natural yersiniabactin.[3,§] Thus, the absolute configuration of yersiniabactin was determined as 9*R*, 10*RS*, 12*R*, 13*S* and 19*S* as shown **3**.

5. Experimental

5.1. General procedure

Melting points were determined on a Yanagimoto micro melting point apparatus. IR spectra were recorded on a JASCO FT/IR-300E spectrometer. The samples were prepared as KBr pellets. ^{1}H NMR spectra were recorded on a JEOL GSX-270 or JMN A-400 spectrometer. Tetramethylsilane was used as an internal standard for the spectra taken in $CDCl_3$, and DMF-d_7. All solvents were dried over 3A or 4A molecular sieves before use. Chromatography was carried out on Merck silica gel 60. Preparative thin-layer chromatography (p.TLC) was carried out on 2.00 mm Merck silica gel 60F_{254} plates.

5.1.1. (2*R*)-2-*tert*-Butoxycarbonylamino-*N*-methoxy-3-(2-methoxybenzoyloxy)-*N*-methylpropionamide (8). To an ice-cold solution of **7** (1.24 g, 5.00 mmol), Et_3N (1.53 ml, 2.20 equiv.) and DMAP (31.0 mg, 0.05 equiv.) in THF (15.0 ml) was added *o*-methoxybenzoyl chloride (0.82 ml, 1.10 equiv.), and the mixture was stirred at room temperature overnight. The reaction mixture was diluted with AcOEt and washed with sat. aq. NH_4Cl, sat. aq. $NaHCO_3$ and brine, dried over Na_2SO_4 and then concentrated in vacuo. Chromatography (SiO_2 100 g, AcOEt/hexane=2:3) of the residue afforded **8** (1.92 g, 100%) as a colorless caramel. $[\alpha]^{24}_D$=−12.8 (c 1.00, $CHCl_3$); IR ν_{max} 3342, 2975, 1717, 1669, 1491, 1250 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.45 (9H, s), 3.23 (3H, s), 3.80 (3H, s), 3.93 (3H, s), 4.46 (1H, dd, J=11.0, 3.7 Hz), 4.63 (1H, dd, J=11.0, 4.3 Hz), 5.01 (1H, m), 5.65 (1H, br-d, J=7.9 Hz), 6.97 (2H, m), 7.47 (1H, br-t, J=7.9 Hz), 7.80 (1H, dd,

‡ The C-9 isomer of **3**, which was synthesized separately from an *S*-isomer of Weinreb amide **7** through the same procedure, did not show a spectrum identical to that of natural yersiniabactin.

§ We considered the subtle difference observed around 250 nm in the CD spectra of natural yersiniabactin and synthesized **3** to be due to the nonidentical composition of the two diastereomers with regard to the C-10 configuration.

J=7.9, 1.8 Hz); LSIMS m/z 765 $[2M+H]^+$, 383 $[M+H]^+$, 327, 135; HR-LSIMS m/z 383.1821 $[M+H]^+$ (calcd 383.1818 for $C_{18}H_{27}N_2O_7$).

5.1.2. (2*R*)-3-Hydroxy-*N*-methoxy-2-(2-methoxybenzoylamino)-*N*-methylpropionamide (9). To an ice-cold solution of **8** (1.90 g, 4.97 mmol) in CH_2Cl_2 (20.0 ml) was added TFA (5.00 ml), and the mixture was stirred at room temperature for 3.5 h and then concentrated in vacuo. The residue was taken up into AcOEt and the extract was treated with sat. aq. $NaHCO_3$. The aqueous layer was saturated with NaCl and extracted with AcOEt. The combined organic layer was dried over Na_2SO_4 and then concentrated in vacuo. Chromatography (SiO_2 20 g, AcOEt only) of the residue afforded pure **9** (1.40 g, 100%) as a colorless caramel. $[\alpha]^{26}_D$=−40.8 (c 1.00, $CHCl_3$); IR ν_{max} 3372, 2945, 1640, 1600, 1523, 1483, 1241 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 3.19 (1H, br-s) 3.28 (3H, s), 3.83 (3H, s), 3.85–4.02 (2H, m), 4.03 (3H, s), 5.30 (1H, br-s), 7.00 (1H, d, J=7.9 Hz), 7.06 (1H, t, J=7.9 Hz), 7.47 (1H, td, J=7.9, 1.8 Hz), 8.19 (1H, dd, J=7.9, 1.8 Hz), 9.12 (1H, br-d); FABMS m/z 565 $[2M+H^+]$, 283 $[M+H]^+$, 222, 194, 135; HR-FABMS m/z 283.1292 $[M+H]^+$ (calcd 283.1294 for $C_{13}H_{19}N_2O_5$).

5.1.3. (2*R*)-3-Hydroxy-2-(2-hydroxybenzoylamino)-*N*-methoxy-*N*-methylpropionamide (10). To a solution of **9** (1.20 g, 4.25 mmol) in CH_2Cl_2 (13.0 ml) was added BCl_3 (1.00 M in hexane, 5.10 ml, 1.20 equiv.) at −78°C. After stirring at room temperature overnight, further BCl_3 (1.00 M in hexane, 4.25 ml, 1.00 equiv.) was added and the mixture was stirred at the same temperature for 1 h. After addition of ice, the mixture was poured into brine and extracted with AcOEt. The combined AcOEt layer was dried over Na_2SO_4 and then concentrated in vacuo. Chromatography ($SiO_2$50 g, AcOEt/hexane=5:1) of the residue afforded **10** (926 mg, 81%) as a colorless caramel. $[\alpha]^{26}_D$=−36.0 (c 1.00, $CHCl_3$); IR ν_{max} 3349, 2942, 1637, 1601, 1536, 1492 cm^{-1}, ^1H NMR (270 MHz, $CDCl_3$) δ 2.66 (1H, br-s), 3.29 (3H, s), 3.84 (3H, s), 3.98 (2H, br-s), 5.25 (1H, dt, J=7.3, 3.7 Hz), 6.87 (1H, t, J=7.9 Hz), 6.98 (1H, d, J=7.9 Hz), 7.41 (1H, br-t, J=7.9 Hz), 7.51 (2H, m), 12.02 (1H, s); FABMS m/z 537 $[2M+H]^+$, 269 $[M+H]^+$, 208, 180, 121; HR-FABMS m/z 269.1132 $[M+H]^+$ (calcd 269.1137 for $C_{12}H_{17}N_2O_5$).

5.1.4. (2*R*)-3-Hydroxy-2-(2-hydroxythiobenzoylamino)-*N*-methoxy-*N*-methylpropionamide (4). To a solution of **10** (338 mg, 1.26 mmol) in THF (6.30 ml) was added Burgess reagent **11** (360 mg 1.20 equiv.), and the mixture was stirred at room temperature for 30 min and then refluxed for 1 h. After cooling to room temperature, the mixture was concentrated in vacuo. Chromatography (SiO_2 15 g, AcOEt/hexane=2:1) of the residue afforded a colorless oil (oxazoline, 281 mg, 89%). IR ν_{max} 2974, 1668, 1639, 1492, 1261 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 3.28 (3H, s), 3.86 (3H, s), 4.51 (1H, dd, J=10.4, 8.5 Hz), 4.84 (1H, br-t, J=7.9 Hz), 5.40 (1H, br-t, J=8.5 Hz), 6.88 (1H, td, J=7.9, 1.2 Hz), 6.99 (1H, dd, J=8.5, 1.2 Hz), 7.38 (1H, td, J=8.5, 1.2 Hz), 7.68 (1H, dd, J=7.9, 1.2 Hz), 11.76 (1H, s).

A solution of the oil (281 mg, 1.12 mmol) in MeOH (6.60 ml) and Et_3N (3.30 ml) was saturated with H_2S gas and stirred at room temperature for 5 days. The reaction mixture was evaporated in vacuo. Chromatography (SiO_2 14 g, AcOEt/hexane=5:1 to AcOEt only) of the residue afforded **4** (305 mg, 85% from **10**) as a yellow caramel. $[\alpha]^{26}_D$=−4.6 (c 1.00, $CHCl_3$); IR ν_{max} 3278, 2940, 1653, 1539, 1461, 1360, cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 3.31 (3H, s), 3.88 (3H, s), 4.14 (2H, m), 5.76 (1H, br-s), 6.89 (1H, t, J=7.9 Hz), 7.01 (1H, d, J=7.3 Hz), 7.36 (1H, t, J=7.3 Hz), 7.52 (1H, d, J=7.9 Hz), 8.90 (1H, br-s); FABMS m/z 569 $[2M+H]^+$, 285 $[M+H]^+$, 224, 137; HR-FABMS m/z 285.0900 $[M+H]^+$ (calcd 285.0909 for $C_{12}H_{17}N_2O_4S$).

5.1.5. (4*S*)-2-(2-Hydroxyphenyl)-4,5-dihydrothiazole-4-carboxylic acid *N*-methoxy-*N*-methylamide (12). To a solution of **4** (260 mg, 0.91 mmol) in THF (4.60 ml) was added Burgess reagent **11** (261 mg, 1.20 equiv.), and the mixture was stirred at room temperature for 40 min and then refluxed for 30 min. After cooling to room temperature, the mixture was concentrated in vacuo. Chromatography (SiO_2 15 g, AcOEt/hexane=1:2) of the residue afforded **12** (170 mg, 70%). IR ν_{max} 2973, 2938, 1669, 1621, 1593, 1491 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 3.30 (3H, s), 3.48 (1H, dd, J=10.8, 9.2 Hz), 3.79 (1H, br-t, J=10.4 Hz), 3.84 (3H, s), 5.70 (1H, br-t, J=9.2 Hz), 6.88 (1H, t, J=8.5 Hz), 6.98 (1H, d, J=7.9 Hz), 7.36 (1H, td, J=8.5, 1.2 Hz), 7.43 (1H, dd, J=7.9, 1.2 HZ), 12.32 (1H, br-s); LSIMS m/z 267 $[M+H]^+$, 251, 178; HR-LSIMS m/z 267.0797 $[M+H]^+$ (calcd 267.0803 for $C_{12}H_{15}N_2O_3S$).

5.1.6. (4*S*)-2-[2-(*tert*-Butyldiphenylsilyloxy)phenyl]-4,5-dihydrothiazole-4-carboxylic acid *N*-methoxy-*N*-methylamide (13). To an ice-cold solution of **12** (50.0 mg, 0.19 mmol) in DMF (1.50 ml) were added TBDPSCl (0.10 ml, 2.10 equiv.) and imidazole (55.0 mg, 4.30 equiv.). After stirring at room temperature for 2 h, the mixture was diluted with AcOEt and washed with water and brine, dried over Na_2SO_4, then concentrated in vacuo. Chromatography (SiO_2 5 g, AcOEt/hexane=1:3) of the residue afforded **13** (79.8 mg, 84%) as a pale yellow caramel. Due to lability of the *O–Si* bond, obtained **13** was used immediately for the next reaction. IR ν_{max} 2932, 2858, 1668, 1590, 1486, 1113, 702 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 1.11 (9H, s), 3.32 (3H, s), 3.50 (1H, dd, J=11.0, 9.2 Hz), 3.84 (1H, m), 3.87 (3H, s), 5.57 (1H, br-s), 6.40 (1H, d, J=7.9 Hz), 6.85 (2H, m), 7.39 (6H, m), 7.75 (5H, m); FABMS m/z 505 $[M+H]^+$, 447; HR-FABMS m/z 505.1966 $[M+H]^+$ (calcd 505.1981 for $C_{28}H_{33}N_2O_3SSi$).

5.2. Determination of the optical purity

Enantiomeric excess of the intermediate was determined by HPLC with a chiral column (Chiralcel OD for amide **9**, **10** or OJ for thiazoline **12**); **9**,**10**>95% ee, **12**=73% ee. (9-epi-**12**=89% ee).

5.2.1. Pyochelin I (2). To an ice-cold solution of freshly prepared TBDPS ether **13** (64.0 mg, 0.13 mmol) in THF (1.50 ml) was added $LiAlH_4$ (5.30 mg, 1.10 equiv.), and the mixture was stirred at the same temperature for 30 min. The reaction was quenched with AcOEt, and then the mixture was poured into sat. aq. NH_4Cl and extracted with AcOEt. The AcOEt extract was washed with aq.

Seignette salt and brine, dried over Na_2SO_4, then concentrated in vacuo to give crude aldehyde **14** as a bright yellow amorphous solid.

Without purification, the **14** was dissolved in CH_2Cl_2 (3.00 ml) and MeOH (0.60 ml) and the solution was treated with AcOK (125 mg, 10.0 equiv.) and *N*-Me-L-Cys·HCl (ca. 50%, 109 mg, 2.50 equiv.). After stirring at room temperature overnight, the reaction mixture was diluted with AcOEt, washed with sat. aq. NH_4Cl and brine, dried over Na_2SO_4, then concentrated in vacuo.

To an ice-cold solution of the residue in THF (1.50 ml) was added TBAF (1.00 M in THF, 0.13 ml, 1.00 equiv.), and the mixture was stirred at room temperature for 30 min. The reaction mixture was diluted with AcOEt and washed with 5% aq. $KHSO_4$ and then extracted with sat. aq. $NaHCO_3$. The combined aqueous layer was acidified (pH=4) with conc. HCl and extracted again with AcOEt. The AcOEt layer was washed with brine, dried over Na_2SO_4, and then concentrated in vacuo to give pyochelins **15** (25.0 mg, 61% from **13**) as a mixture of four diastereomers.

To a solution of **15** (25.0 mg, 7.70×10^{-5} mol) in MeOH (2.00 ml) was added $ZnCl_2$ (26.0 mg, 2.5 equiv.), and the mixture was stirred at room temperature overnight. The reaction mixture was diluted with AcOEt, washed with 5% aq. $KHSO_4$ and brine, dried over Na_2SO_4, then concentrated in vacuo to give a mixture of pyochelin I (**2**) and neopyochelin II (**17**) (ca. 5:1 by ^{1}H NMR, 19.0 mg, 76%) as a yellow caramel. **2**: IR ν_{max} 3012, 2940, 1718, 1592, 1490, 1221 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) (major isomer) δ 2.71 (3H, s), 3.26 (1H, dd, J=11.4, 7.9 Hz), 3.27–3.47 (2H, m), 3.48 (1H, dd, J=11.4, 8.6 Hz), 3.85 (1H, t, J=6.9 Hz), 4.36 (1H, d, J=7.9 Hz), 4.87 (1H, q, J=8.1 Hz), 6.90 (1H, t, J=7.9 Hz), 7.01 (1H, d, J=8.2 Hz), 7.38 (1H, t, J=8.2 Hz), 7.41 (1H, dd, J=7.9, 1.5 Hz); FABMS *m/z* 649 $[2M+H]^+$, 325 $[M+H]^+$, 178, 146; HR-FABMS *m/z* 325.0676 $[M+H]^+$ (calcd 325.0681 for $C_{14}H_{17}N_2O_3S_2$).

5.2.2. Pyochelin I methyl ester (16). A solution of **2** (19.0 mg, 5.85×10^{-5} mol, containing **17** ca. 20%) in CH_2Cl_2 (3.00 ml) and MeOH (1.00 ml) was treated with $TMSCHN_2$ (1.00 M in THF) until generation of N_2 gas ceased. The reaction was quenched with AcOH and the mixture was concentrated in vacuo. The residue was purified by p.TLC (AcOEt/hexane=2:3) to give pure pyochelin I methyl ester **16**) (11.0 mg). IR ν_{max} 2950, 2858, 1747, 1592, 1490, 1220 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.59 (3H, s), 3.11 (1H, dd, J=10.6, 6.4 Hz), 3.18 (1H, dd, J=10.6, 9.1 Hz), 3.40 (1H, dd, J=11.4, 9.1 Hz), 3.47 (1H, dd, J=11.4, 8.6 Hz), 3.65 (1H, dd, J=9.1, 6.4 Hz), 3.76 (3H, s), 4.52 (1H, d, J=5.1 Hz), 5.07 (1H, td, J=8.6, 5.1 Hz), 6.87 (1H, td, J=8.4, 1.2 Hz), 6.98 (1H, dd, J=8.4, 1.2 Hz), 7.35 (1H, td, J=8.4, 1.5 Hz), 7.41 (1H, dd, J=7.9, 1.5 Hz).

5.2.3. Neopyochelin II (17). Through the same procedure for **2** from epi-**7**, was obtained a mixture of **2** and neopyochelin II (**17**) (ca. 1:5 by ^{1}H NMR). **17**; ^{1}H NMR (270 MHz, $CDCl_3$) (major isomer) δ 2.65 (3H, s), 3.29 (1H, dd, J=11.9, 7.1 Hz), 3.30–3.48 (2H, m), 3.54 (1H, dd, J=11.4, 8.9 Hz), 3.82 (1H, t, J=6.9 Hz), 4.37 (1H, d, J=5.4 Hz), 4.98 (1H, ddd, J=8.9, 7.1, 5.4 Hz), 6.89 (1H, t, J=7.8 Hz), 7.01 (1H, d, J=8.2 Hz), 7.37 (1H, t, J=8.2 Hz), 7.41 (1H, dd, J=7.8, 1.2 Hz).

5.2.4. (4*S*)-2-[(2*S*,3*R*)-3-*tert*-Butoxycarbonylamino-2-hydroxy-4-mercapto-1,1-dimethyl]butyl-4-methyl-4,5-dihydrothiazole-4-carboxylic acid methyl ester (19). To an ice-cold solution of **18** (113 mg, 0.22 mmol) in CH_2Cl_2 (4.30 ml) was added freshly prepared Npys-Cl (49.0 mg, 1.20 equiv.), and the mixture was stirred at the same temperature for 30 min, and then concentrated in vacuo. Purification of the residue with p.TLC (AcOEt/hexane=3:1) afforded a yellow caramel (120 mg).

To a solution of the caramel (120 mg, ca. 0.21 mmol) in acetone (4.00 ml) and water (1.00 ml) was added *n*-Bu_3P (0.06 ml, ca. 1.05 equiv.), and the mixture was stirred at room temperature for 20 min. The acetone was removed in vacuo and the residue was taken up in AcOEt. The AcOEt extract was washed with 10% aq. citric acid, water and brine, dried over Na_2SO_4, then concentrated in vacuo. Purification of the residue by p.TLC (AcOEt/hexane=3:2) afforded thiol **19** (64.0 mg, 73% from **18**). $[\alpha]^{25}_D=-75.9$ (c 1.00, $CHCl_3$); IR ν_{max} 3416, 3370, 2977, 2556, 1741, 1710, 1601, 1505 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.25 (3H, s), 1.38 (3H, s), 1.41 (9H, s), 1.51 (1H, t, J=9.2 Hz), 1.58 (3H, s), 2.71 (2H, m), 3.09 (1H, d, J=11.6 Hz), 3.61 (1H, d, J=11.6 Hz), 3.80 (3H, s), 3.81 (1H, m), 3.96 (1H, br-d, J=6.7 Hz), 5.28 (1H, br-d, J=9.2 Hz), 5.52 (1H, br-d, J=6.7 Hz); LSIMS *m/z* 813 $[2M+H]^+$, 407 $[M+H]^+$, 333, 230; HR-LSIMS *m/z* 407.1676 $[M+H]^+$ (calcd 407.1674 for $C_{17}H_{31}N_2O_5S_2$).

5.2.5. Yersiniabactin methyl ester (21). To an ice-cold solution of **19** (64.0 mg, 0.16 mmol) in CH_2Cl_2 (2.00 ml) was added TFA (0.40 ml), and the mixture was stirred at the same temperature for 15 min and at room temperature for a further 2 h. The mixture was concentrated in vacuo to give Segment B TFA salt **20** (quant.) as a pale yellow oil. Compound **20** thus obtained was used without further purification for next reaction. ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.34 (3H, s), 1.50 (3H, s), 1.63 (1H, t, J=9.2 Hz), 2.88 (1H, dd, J=14.0, 6.7 Hz), 2.98 (1H, dd, J=14.0, 7.3 Hz), 3.26 (1H, d, J=11.6 Hz), 3.56 (1H, m), 3.68 (1H, d, J=11.6 Hz), 3.79 (1H, m), 3.81 (3H, s).

To an ice-cold solution of freshly prepared TBDPS ether **13** (79.8 mg, 0.16 mmol) in THF (2.00 ml) was added $LiAlH_4$ (6.60 mg, 1.10 equiv.), and the mixture was stirred at the same temperature for 30 min. The reaction was quenched with AcOEt and then the whole mixture was poured into sat. aq. NH_4Cl and extracted with AcOEt. The AcOEt extract was washed with aq. Seignette salt and brine, dried over Na_2SO_4, then concentrated in vacuo to give crude aldehyde as a bright yellow amorphous solid.

Under a nitrogen atmosphere, to a suspension of the amorphous product and AcOK (155 mg, 10.0 equiv.) in CH_2Cl_2 (2.50 ml), was added a solution of TFA salt **20** (1.00 equiv.) in CH_2Cl_2 (1.50 ml) dropwise over 30 min. After stirring at room temperature for 18 h, the reaction mixture was diluted with AcOEt, washed with water and brine, dried over

Na_2SO_4, then concentrated in vacuo to give a bright yellow caramel.

To an ice-cold solution of the above caramel in THF (3.00 ml) was added TBAF (1.00 ml in THF, 0.19 ml, 1.20 equiv.), and the mixture was stirred at room temperature for 20 min. The reaction mixture was diluted with AcOEt, washed with sat. aq. NH_4Cl and brine, dried over Na_2SO_4, then concentrated in vacuo. Chromatography (SiO_2 6 g, AcOEt/hexane=1:3) of the residue afforded yersiniabactin methyl ester (**21**) (34.0 mg, 43% from **13**, ca. 6:1 mixture of diastereomers) as a bright yellow caramel. IR ν_{max} 3287, 2965, 2932, 1736, 1593, 1489, 1456, 1221, 755 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) (major isomer) δ 1.35 (3H, s), 1.36 (3H, s), 1.57 (3H, s), 2.97 (1H, dd, J=9.6, 5.6 Hz), 3.08 (1H, d, J=11.6 Hz), 3.11 (1H, m), 3.29 (1H, dd, J=10.8, 9.2 Hz), 3.35 (1H, m), 3.50 (1H, dd, J=10.8, 8.4 Hz), 3.67 (3H, s), 3.73 (1H, d, J=11.6 Hz), 3.87 (1H, d, J=1.2 Hz), 4.88 (1H, d, J=6.8 Hz), 4.94 (1H, td, J=8.8, 6.8 Hz), 5.30 (1H, brs), 6.86 (1H, t, J=7.6 Hz), 6.97 (1H, d, J=8.4 Hz), 7.34 (1H, t, J=8.4 Hz), 7.39 (1H, d, J=7.6 Hz), 12.38 (1H, brs); FABMS m/z 496 $[M+H]^+$, 317, 295, 518 $[M+Na]^+$; HR-FABMS m/z 518.1218 $[M+Na]^+$ (calcd 518.1218 for $C_{22}H_{29}N_3O_4S_3Na$).

5.2.6. Yersiniabactin (3). To a solution of yersiniabactin methyl ester (**21**) (a mixture of two diastereomers, 31.0 mg, 6.25×10^{-5} mol) in DMF (1.50 ml) and water (0.50 ml) was added $LiOH\cdot H_2O$ (5.50 mg, 2.10 equiv.), and the mixture was stirred at room temperature for 2.5 h. The reaction mixture was diluted with AcOEt, washed with sat. aq. NH_4Cl, water and brine, dried over Na_2SO_4, then concentrated in vacuo to give crude yersiniabactin (26.0 mg).

The synthesized crude yersiniabactin (19.0 mg) was purified by HPLC (ODS HG-5 (50×250 nm), 33% MeCN+1 mM phosphate buffer (pH=7), 10.0 ml/min, det. UV 254 nm: rt; 16.6 min) to give pure yersiniabactin (**3**) (Isomer I/II= ca. 6:1, 7.00 mg). IR ν_{max} 3098 (broad), 2971, 2931, 1592, 1483, 1455, 1389, 1299, 1221 cm^{-1}; CD (c 9.24×10^{-5} M, H_2O) shown in Fig. 2; ^1H NMR (400 MHz, DMF-d_7) (major isomer) δ 1.31 (6H, s), 1.48 (3H, s), 2.87 (1H, t, J=9.6 Hz), 2.99 (1H, dd, J=9.6, 5.6 Hz), 3.21 (1H, d, J=11.2 Hz), 3.35 (1H, m), 3.40 (1H, dd, J=10.8, 9.6 Hz), 3.67 (1H, m), 3.71 (1H, d, J=11.2 Hz), 3.97 (1H, d, J=1.2 Hz), 5.00 (1H, d, J=4.8 Hz), 5.15 (1H, td, J=9.2, 4.8 Hz), 6.97 (1H, t, J= 8.0 Hz), 6.99 (1H, dd, J=8.8, 1.2 Hz), 7.45 (2H, m); FABMS m/z 482 $[M+H]^+$, 303, 295; HR-FABMS m/z 482. 1245 $[M+H]^+$ (calcd 482.1242 for $C_{21}H_{28}N_3O_4S_3$).

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