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A Short Stereoselective Synthesis of Prepiscibactin Using a SmI_2-Mediated Reformatsky Reaction and Zn^{2+}-Induced Asymmetric Thiazolidine Formation

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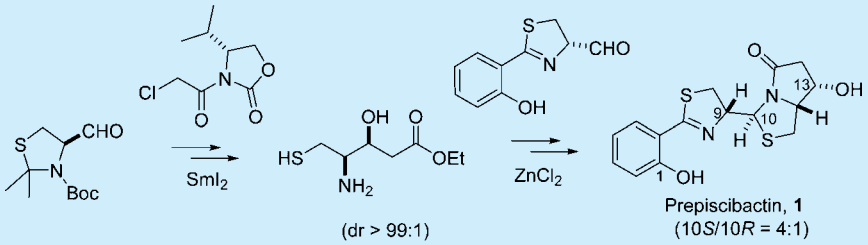
Supporting Information

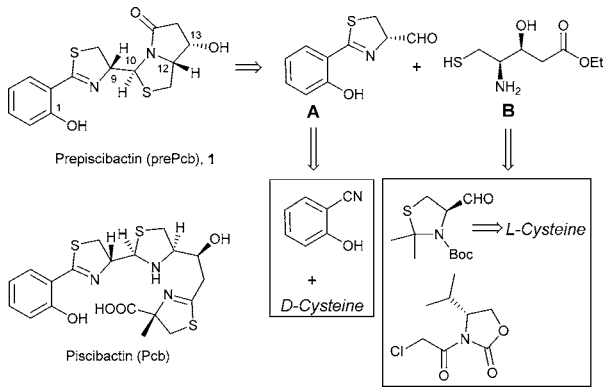


ABSTRACT: Prepiscibactin (**1**) is a possible intermediate in the biosynthesis of piscibactin, the siderophore responsible for the iron uptake of the bacterium *Photobacterium damselae* subsp. *piscicida*, the aethiological agent of fish pasteurellosis. Compound **1** was synthesized by a convergent approach starting from L-/D-cysteine and 2-hydroxybenzonitrile. The key steps were a highly diastereoselective SmI_2-mediated Reformatsky reaction and Zn^{2+}-induced asymmetric thiazolidine formation followed by lactamization. The absolute configuration 9*R*,10*S*,12*R*,13*S* was established for **1**, and this confirmed the previous relative stereochemistry proposed on the basis of NOE and computational methods.

Pasteurellosis is a fish disease that causes significant economic losses in marine aquaculture worldwide, and since 1990 it has been the major pathological problem in the culture of sea bream and sea bass in Mediterranean countries, including Spain.[1] Piscibactin (Pcb) is the siderophore responsible for the iron uptake of the bacterium *Photobacterium damselae* subsp. *piscicida*, the aethiological agent of pasteurellosis. During the isolation of Pcb from iron-deficient cultures in CM9 medium, a smaller metabolite, namely prepiscibactin (prePcb, **1**), was isolated and characterized.[2] The partial structures of prePcb and Pcb were predicted by analysis of the gene cluster involved in the siderophore biosynthesis. From the proposed biosynthetic pathway for Pcb, we envisaged that prePcb, **1**, could be an intermediate in the biosynthesis of Pcb. The relative stereochemistry of prePcb, **1**, was proposed on the basis of a combination of NOE correlations and DFT studies.[2] Since siderophores[3] are critical for the growth and virulence of the producer pathogens, the bacterial iron acquisition systems are promising targets for the design of new antibiotic strategies.[4] Consequently, the synthesis of prePcb, **1**, would be very helpful in elucidating the biosynthetic pathway of Pcb and thus for the development of new rationally designed antibacterials against pasteurellosis. Herein, we present the first total stereoselective synthesis of prePcb, **1**, in a process that features two key diastereoselective steps: a SmI_2-mediated Reformatsky reaction (dr > 99:1) of an α-chloroacetyl-2-oxazolidinone with a thiazolidinic aldehyde and Zn^{2+}-induced asymmetric thiazolidine formation followed by lactamization.

The structure of prePcb, **1**, is closely related to those of the siderophores pycohelin[5a] and yersiniabactin,[5b] the reported syntheses[6] of which were very useful in planning our synthetic strategy (Scheme 1). Our retrosynthetic analysis of prePcb, **1**, involved the preparation of key synthetic intermediates **A** and **B** from D- and L-cysteine, respectively, as chiral sources for C9*S* and C12*S*. The secondary alcohol with the *S* configuration at C13 would be generated in a stereoselective manner using a SmI_2-mediated Reformatsky reaction, while the chiral center at

Scheme 1. Retrosynthetic Analysis for Prepiscibactin (1)



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C10*S* would be constructed through the diastereoselective formation of the thiazolidine ring in a metal-chelation/lactamization sequence.

Although the classical Reformatsky reaction was introduced over 125 years ago,[7] the stereoselective version is a more recent development.[8] One of the great advantages of this approach is that the enolate is formed under neutral conditions and therefore base or acid is not required to generate the enolate or to activate the electrophile, respectively.[9] Metal-catalyzed enantio- and diastereoselective Reformatsky reactions between aldehydes or ketones and various electrophiles are now possible.[10] In particular, the asymmetric samarium iodide mediated Reformatsky[11] process developed by Fukuzawa et al.[12] seems to be very useful for the diastereoselective preparation of β-hydroxy derivatives from aldehydes and α-bromoacetyl-2-oxazolidinones. This process provides an alternative approach to asymmetric aldol reactions in the synthesis of this kind of compound. However, very few examples of this reaction have been reported in the literature.[13] One of the few examples of diastereoselective Reformatsky reactions to give β-hydroxy-γ-amino acids without the double-differentiating effect of α-substituents was reported by Burke's group. They used this coupling reaction for the synthesis of the β-hydroxy-γ-amino acids *N*-Boc-isostatine and *N*-Boc-dolaisoleucine with complete diastereoselectivity and in good yields.[14] However, additions of α-haloacetyl-2-oxazolidinones to *N*-Boc-(*S*)-prolinal gave diastereoselectivities that ranged from low (dr 5:1 for *syn*) to moderate (dr 14:1 for *anti*). Validation of this methodology for the construction of β-hydroxy-γ-amino acid derivatives requires more examples to cover a range of different substrates. Moreover, examples involving the use of other heterocycles have not been reported, e.g., thiazolidines, in which the presence of an additional sulfur atom could affect the outcome of the reaction.

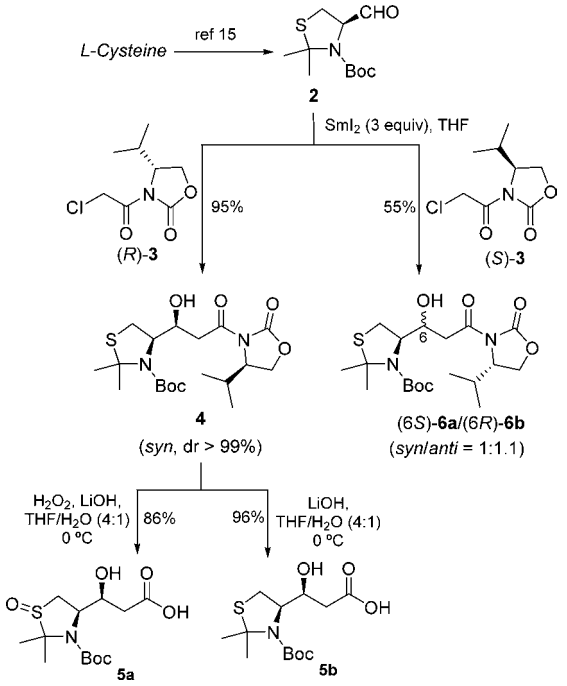
Our synthetic efforts started with the preparation of the thiazolidinic aldehyde **2** from commercially available L-cysteine according to the procedure reported by Duthaler et al. with appropriate modifications (see Supporting Information (SI)).[15] At the same time, (*R*)-α-chloroacetyl-2-oxazolidinone [(*R*)-**3**)] was prepared using the methodology developed by Roush and Brown.[16]

With these products in hand, the next step was the crucial SmI_2-induced Reformatsky reaction (Scheme 2). Reaction of a mixture of the freshly prepared thiazolidinic aldehyde **2** and α-chloroacetyl-2-oxazolidinone (*R*)-**3** with an excess (3 equiv) of SmI_2 resulted in the formation of secondary alcohol **4** as a single diastereoisomer (dr >99%, see Scheme 2), as demonstrated by HPLC and NMR (see SI). Standard oxidative removal of the chiral auxiliary of **4** with H_2O_2/LiOH gave **5a**, in which the sulfur had been oxidized to sulfoxide. In order to avoid this oxidation LiOH alone was used to obtain the acid **5b**.

The *syn* stereochemistry of **4** was determined by applying *J*-based configurational analysis to the hydrolyzed product **5b** (see SI).[17] An HSQC-HECADE experiment allowed the values of the $^2J_{CH}$ and $^3J_{CH}$ coupling constants of **5b** to be determined. The measurement gave a set of coupling constant values [$^3J_{(H3H4)}$ = 6.4 Hz, $^3J_{(C5H3)}$ = 4.5 Hz, $^3J_{(C2H4)}$ = 4.7 Hz, and $^2J_{(C3H4)}$ = −3.6 Hz, medium, large/medium, large/medium, and large, respectively] that are consistent with the presence of a mixture of two staggered conformers of the *syn* configuration (see SI).

The reaction of **2** was repeated with α-chloroacetyl-2-oxazolidinone (*S*)-**3** under similar reaction conditions, and this gave a mixture of diastereoisomers (6*S*)-**6a**/(6*R*)-**6b** (Scheme 2). HPLC separation and analysis of the NMR ^{1}H−^{1}H homonuclear and ^{13}C−^{1}H heteronuclear coupling constants indicated a 1:1.1 ratio for the *syn*-**6a**/*anti*-**6b** compounds (see SI). These results again confirm that the stereochemical outcome of the Sm^{II}-mediated Reformatsky coupling is highly dependent on the chirality of the auxiliary employed in the reaction.

Scheme 2. Samarium Iodide Mediated Reformatsky Synthesis of Alcohols 4 and 6a−b



The high diastereoselectivity of the *syn* product obtained using α-chloroacetyl-2-oxazolidinone (*R*)-**3**, in relation to the results reported by Burke and co-workers for the additions of α-haloacetyl-2-oxazolidinones to *N*-Boc-(*S*)-prolinal, is probably due to the more sterically hindered environment created by the two methyl groups and the sulfur atom in the heterocyclic ring. However, a 1:1.1 *syn*/*anti* mixture was obtained with (*S*)-α-chloroacetyl-2-oxazolidinone [(*S*)-**3**)]. In this case, the result can be explained by the similar steric environments in the chelated transition states for the samarium enolate attack at the *re* or *si* face of the aldehyde.

On following the procedure of Mislin et al.,[18] condensation of D-cysteine hydrochoride and 2-hydroxybenzonitrile gave a thiazolinic carboxylic acid, which was treated without purification with *N*,*O*-dimethylhydroxylamine in the presence of EDCI and DIPEA to yield the corresponding Weinreb amide **7a**.

Reduction of the resulting hydroxamic ester with $LiAlH_4$ afforded the thiazolinic aldehyde **8a**. In order to study the influence of the free phenolic OH group in the condensation with the thiol amine, the TBDPS-protected **7b** was also obtained from **7a** and reduced to **8b** using a procedure similar to that described above (Scheme 3).

Treatment of thiazolidine **4** with 75% TFA in CH_2Cl_2 led to the concomitant removal of the acetonide and Boc protecting moieties to give **9**. Subsequent condensation of **9** with thiazolinic aldehyde **8b**, in the presence of potassium acetate in MeOH, produced the unexpected bis-thiazoline **10**. The

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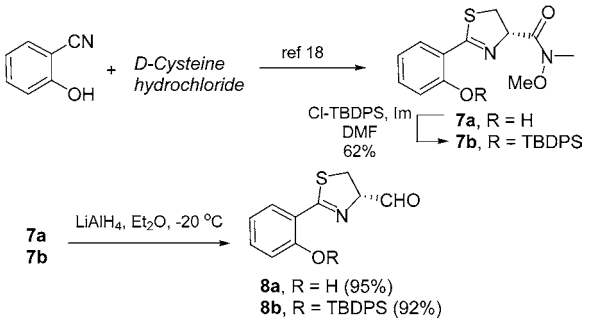
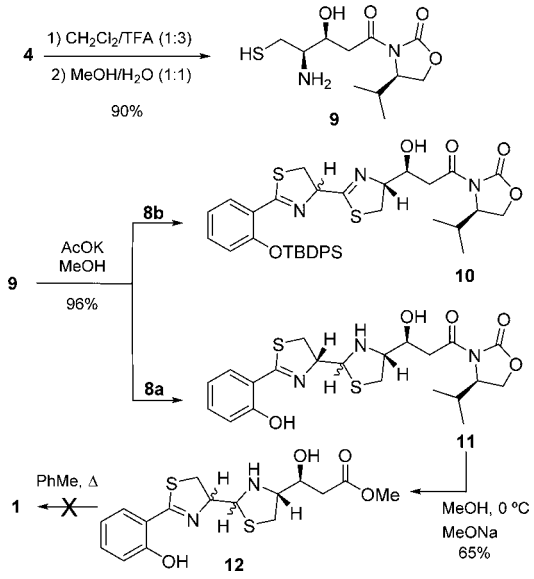
Scheme 3. Synthesis of Fragment A



cyclization reaction between the deprotected thiol amine **9** and thiazolinic aldehyde **8a** yielded a mixture of two diastereoisomers at C10 (**11**) in a 1:1.5 ratio, as deduced by ^{1}H NMR spectroscopy. Removal of the chiral auxiliary with a methanolic solution of sodium methoxide at 0 °C gave compound **12**. This proved to be very unstable, and all attempts at cyclization were unsuccessful (Scheme 4). It was therefore decided to reverse the order of the reactions.

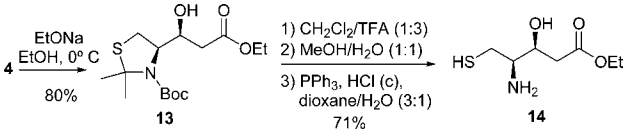
Scheme 4. First Approach for the Synthesis of 1



Removal of the chiral auxiliary from thiazolidine **4** with an ethanolic solution of sodium ethoxide at 0 °C gave the thiazolidine ethyl ester **13**. Treatment of **13** with 75% TFA in CH_2Cl_2 to remove the acetonide and Boc protecting groups afforded **14**. The dimer of **14** was formed through a disulfide bridge, and the product was therefore treated with PPh_3 and HCl(c) in dioxane/water (Scheme 5).

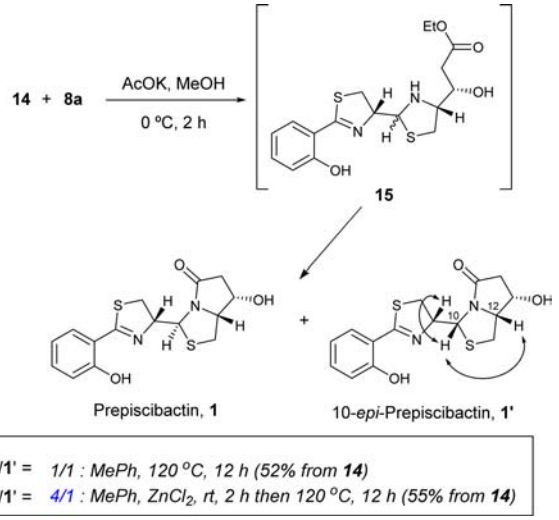
Condensation of thiazolinic aldehyde **8a** with **14** in the presence of potassium acetate in MeOH at 0 °C gave **15**, which was immediately heated under reflux in toluene for 12 h to give

Scheme 5. Synthesis of Fragment B



prepiscibactin, **1**, along with its epimer at C10, 10-*epi*-prepiscibactin, **1′**, in a 1:1 ratio (Scheme 6). The synthetic

Scheme 6. Final Steps of the Synthesis of 1 and Key NOE Correlations Found in 1′



product **1** was spectroscopically indistinguishable from the natural product, and it also had a very similar CD spectrum (see SI). 10-*epi*-Prepiscibactin, **1′**, showed an NOE between H12 and H10, clearly indicating a *cis* disposition (see Scheme 6). The absence of an NOE in the similar pair of protons in prepiscibactin, **1**, was key in the structural elucidation of the natural compound, as mentioned in the discussion of its isolation.[2]

Once the conditions to access **1** had been established, modifications to improve the diastereoselective formation of the product were examined. It is well-known that 2-substituted thiazolidine rings are rapidly equilibrated into an approximately 1:1 mixture of the C2 epimers due to ring opening−reclosure.[19] However, once the nitrogen is acylated such epimerization is not observed. Thiazolidine formation was tested using several catalysts, including scandium trifluoromethanesulfonate,[20] without success. Gratifyingly, when the intermediate **15** was treated with $ZnCl_2$[21] followed by lactamization under reflux in toluene, formation of the required isomer prepiscibactin, **1**, in relation to 10-*epi*-prepiscibactin, **1′**, was enhanced to give a 4:1 ratio. This result can be understood by considering that complexation of **15** with Zn^{2+} would shift the equilibrium to the required *trans*-thiazolidine ring, which cyclizes to give **1** as the major diastereoisomer.

In summary, we have accomplished the first total synthesis of prepiscibactin, **1**, a possible intermediate in the biosynthesis of piscibactin, the siderophore responsible for the iron uptake of the bacterium *Photobacterium damselae* subsp. *piscicida*. A highly diastereoselective SmI_2-mediated Reformatsky reaction (dr > 99:1) between an *α*-chloroacetyl-2-oxazolidinone and a thiazolidinic aldehyde was employed to obtain the chiral center at C13*S*. Furthermore, diastereoselective formation of the required isomeric thiazolidine ring after treatment of **15** with $ZnCl_2$ followed by lactamization diastereoselectively provided the chiral center at C10*S*. The synthetic product allowed us to establish the absolute configuration 9*R*,10*S*,12*R*,13*S* for **1**. The knowledge acquired in the synthesis of **1** is being exploited further in our ongoing studies into the synthesis of piscibactin.

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Siderophores are critical for the growth and virulence of the producer pathogens, and the synthesis of prePcb can therefore be useful to confirm the proposed biosynthesis pathway of Pcb and enable its total synthesis. This knowledge could be applied in the development of novel treatments against pasteurellosis based on the iron uptake mechanism of these bacteria.

■ ASSOCIATED CONTENT

Supporting Information

Synthesis and spectroscopic data for all intermediates and copies of ^{1}H and ^{13}C NMR spectra details; HRESIMS and HPLC chromatograms. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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