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Stereoselective Synthesis of Optically Active β-Lactams, Potential Inhibitors of Pilus Assembly in Pathogenic Bacteria

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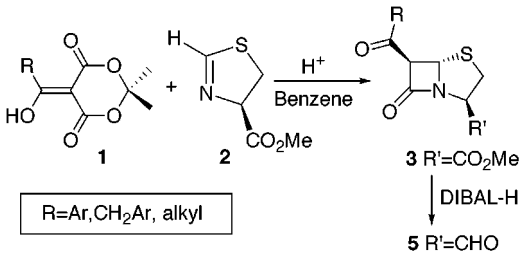
ABSTRACT



Optically active β-lactams 3 are obtained in excellent yields (up to 93%) and with complete stereoselectivity from Meldrum's acid derivatives 1 and Δ^2-thiazolines 2. A selective reduction to aldehydes 5 (R = Ar or CH_2Ar) was then accomplished by using DIBAL-H. This rigid framework, with stereochemistry different than that of penicillin, is designed to be a suitable scaffold for the development of compounds inhibiting pilus formation in uropathogenic *Escherichia coli*.

β-Lactams are known as a highly interesting class of compounds, and research directed to their antibiotic properties is continuously attracting great interest in the scientific community.[1] More recently, the uses of β-lactams as reactive intermediates and starting materials in a wide range of synthetic settings have gained attention.[2] One such example is the exploration of the β-lactam synthon method (β-LSM) by Ojima et al.[3] Our interest in this field is directed to the synthesis of a rigid framework to be used as scaffold for development of potential novel antibiotics. Since an increasing number of bacteria are developing resistance against the drugs available today, such antibiotics should preferably be directed to novel bacterial targets.[4]

Bacteria need to adhere to host tissue in order to cause disease. Many pathogenic bacteria assemble pili, i.e., extracellular protein organelles, to mediate attachment to host epithelial cells. Pilus assembly is performed by periplasmic chaperones, which bring subunits to the outer cell membrane where they are incorporated in the growing pilus.[5] A drug inhibiting pilus formation, termed a pilicide, would therefore have the potential of being an effective antibiotic.

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It has been shown, both by NMR spectroscopy[6] and X-ray crystallography,[7] that synthetic peptides from the conserved *C*-termini of the pilus proteins are bound by the PapD chaperone (found in uropathogenic *Escherichia coli*, which is the main cause of urinary tract infections). Furthermore, some of the peptides were found to inhibit complex formation between PapD and the adhesin PapG in an ELISA.[6] It was thus found that PapD binds polypeptides by anchoring of the peptide carboxyl terminus to the side chains of Arg^{8} and Lys^{112} (Figure 1), two residues that are invariant in all

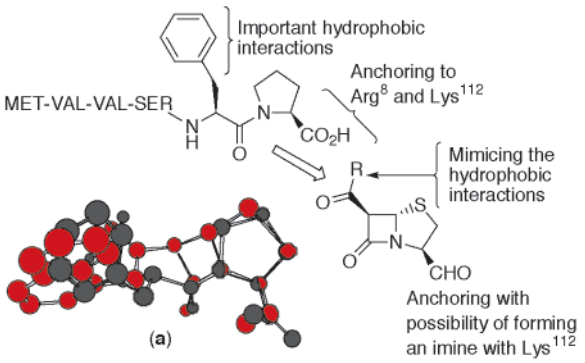

Figure 1. Careful examination of the crystal structures of peptide−PapD complexes has resulted in the design of a rigid β-lactam framework. These compounds superimpose well with the two C-terminal amino acids (**a**), which allows hydrophobic interactions via the R-group and mediates a possibility of forming an imine with a lysine residue in the chaperone.

periplasmic chaperones and required for pilus assembly. These data have recently been further confirmed when the crystal structures of the complex between the chaperones PapD and FimC together with the pilus subunits PapK and FimH were solved.[8]

Direct use of peptides as drugs has several severe drawbacks, i.e. peptides are poorly absorbed on oral administration, and they undergo rapid enzymatic degradation and are usually quickly excreted.[9] However, peptides may serve as starting points for development of drugs as demonstrated in the recent development of inhibitors of HIV protease which slow the progress of AIDS.

On the basis of the crystal structures of peptide−PapD complexes,[7] β-lactams were selected as potential chaperone inhibitors. It should be stressed that the β-lactams of interest in this report have different stereochemistry than the original penicillin's, thus having a chance to withstand enzymatic degradation by penicillin-resistant bacteria. The overall strategy has been to create small organic molecules with a rigid framework, which would locate the pharmacophores in the right position in space. In the crystal structures the backbone atoms of the two *C*-terminal amino acids of the peptides adopted a conformation which superimposed well with a bicyclic β-lactam ring (Figure 1(a)). In addition, this class of compounds allowed hydrophobic substituents (indicated by R) to interact with the chaperone while maintaining the important anchoring to Arg^{8} and Lys^{112}. Moreover, the crystal structures show that the C-terminal carboxyl group is within such a distance from Lys^{112} and that replacing it with an aldehyde would allow an imine to be formed with Lys^{112}. Although aldehydes may seem reactive, other aldehyde-containing compounds have previously been successfully employed as inhibitors and/or key substances in inhibitor development.[10]

A ketene−imine cycloaddition is one possible way of synthesizing the framework of interest.[11] Bose et al. have shown that penam derivatives were formed with the stereochemistry that we desired if Δ^2-thiazolines were condensed with ketenes, generated in situ from acid chlorides and a base.[12] Unfortunately, the reported yields were as low as 11% when Δ^2-thiazolines such as **2** were used, and the compounds were not optically active. An effort to increase the yield in the cycloaddition step via a selenium-Δ^2-thiazoline was reported later.[13] Although the yields in the cycloadditions reached 92%, four steps, with an overall yield of < 20%, were required to prepare the selenium-containing thiazoline and a reductive demethylselenation step had to be performed after the cycloaddition. Furthermore, acid chlorides are not suitable for the preparation of acyl ketenes **4**, which are the ketenes of choice for our purposes. Other reports[14] have suggested Meldrum's acid derivatives (e.g., **1**) as acyl ketene precursors. The ease with which ketenes are formed from derivatives **1** was attractive since a method tolerant of different functional groups was required in order to give optically active β-lactams. To the best of our knowledge there are no previous reports on cycloadditions between Δ^2-thiazolines and ketenes generated in situ from Meldrum's acid derivatives. We now report that these two intermediates react with each other under anhydrous and acidic conditions to give the desired optically active β-lactams with complete stereoselectivity[15] (Scheme 1).

The Δ^2-thiazoline **2** can be conveniently prepared in two steps from commercially available L-cysteine methyl ester

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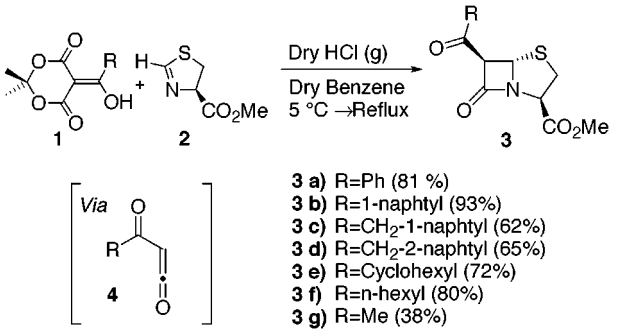
Scheme 1

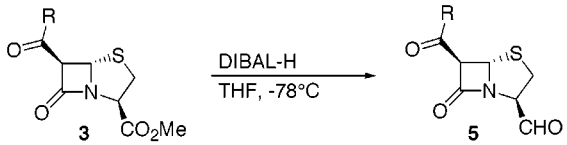


hydrochloride[16] with an overall yield of >70%. Also the derivatives **1** are easily synthesized from Meldrum's acid and acid chlorides in >80% yields.[14a] All derivatives prepared thus far are storable in the freezer for months without any detectable decomposition.

A series of penam derivatives were then synthesized with aryl-, *n*-hexyl-, and cyclohexyl substituents; excellent yields of the corresponding β-lactams **3a**, **3b**, **3e**, and **3f** were accomplished (72−93%). The yields were somewhat lower when a methylene group was introduced between the carbonyl group and the aryl moiety, and **3c** and **3d** were obtained in 62% and 65% yields, respectively. The low yield for methyl ketone **3g**, having a molecular weight of only 229 g/mol, was explained by losses during evaporation of the solvent after chromatography.

After proper assignment of the NMR spectra (HMBC), we realized that the carbonyl group in the ketone functionality of compounds **3** was more shielded than the carbonyl group in the ester functionality. Normally, one would expect the ketone moiety to be reduced easier than the ester. Because of the unusual chemical shift of the carbonyl corresponding to the ketone in our ring-fused β-lactams **3** (~164 ppm instead of ~200 ppm, as usually found for ketones), we anticipated that a selective reduction of the ester functionality, which had chemical shifts of approximately 170 ppm, could succeed. Such a direct approach would thus avoid tedious protection and deprotection steps in the synthesis of the target β-lactam aldehydes **5**. Indeed, this turned out to be correct and a selective reduction of the aromatic esters (**3a**−**3d**) to the corresponding aldehydes (**5a**−**5d**) could be achieved (Scheme 2).

Scheme 2



5 a) R=Ph (49 %)
5 b) R=1-naphtyl (44%)
5 c) R=CH_2-1-naphtyl (44%)
5 d) R=CH_2-2-naphtyl (31%)

Unfortunately, the isolated aldehydes in the aliphatic series were unstable and decomposed.[17] It is worth noting that the reduction could only be accomplished using an electrophilic reducing agent such as DIBAL-H. Use of nucleophilic reducing agents (e.g., $NaCNBH_3$, $NaBH_4$, and $LiBH_4$) resulted in unreacted starting material or, at higher temperatures, decomposition. The aryl-substituted derivatives are stable after freeze-drying and can be stored without decomposition. These compounds will be evaluated as chaperone inhibitors in the near future. The excellent and reproducible yields in the synthesis of **3** and the convenience with which Meldrum's acid derivatives **1** are prepared constitute a platform for future development of statistically diverse libraries of optically active β-lactams.

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Supporting Information Available: Experimental procedures and characterization data for all products. This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) **General procedure for the synthesis of β-lactams**: Meldrum's acid derivative **1b** (630 mg, 2.11 mmol) and thiazoline **2** (185 mg, 1.27 mmol) were dissolved in dry benzene (28 mL) and cooled to 5 °C. HCl gas was bubbled through the mixture for 10 min. The resulting turbid mixture was heated for 1.5 h at 79 °C and then cooled to room temperature. The resulting mixture was diluted with ethyl acetate and washed with ice-cooled water and brine. The water phase was extracted twice with CH_2Cl_2, and the combined organic extracts were dried with Na_2SO_4, filtered, and concentrated. Flash chromatography (heptane:ethyl acetate 1:1 → 3:7) gave **3b** (403 mg, 93%) as a white foam: $[\alpha]^{20}_D$ 2.7° (*c* 1.77, $CHCl_3$); IR λ 1739, 1664, 1508, 1388, 1211, 977, 773 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.14 (d, 1H, J = 8.23 Hz), 7.96 (d, 1H, J = 8.23 Hz), 7.89 (m, 1H), 7.66 (dd, 1H, J_1 = 7.23 Hz, J_2 = 1.19 Hz), 7.46−7.59 (m, 3H), 6.93 (s, 1H), 5.89 (s, 1H), 5.43 (d, 1H, J = 6.31 Hz), 3.84 (s, 3H), 3.60 (dd, 1H, J_1 = 11.25 Hz, J_2 = 6.40 Hz), 3.32 (d, 1H, J = 11.25); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.7, 165.7, 161.2, 133.7, 131.7, 130.6, 129.7, 128.2, 127.3, 126.4, 124.9, 124.8, 103.4, 94.4, 60.9, 53.2, 32.1; HRMS (FAB+) calcd for $C_{18}H_{16}NO_4S$ 342.0800; observed 342.0803.

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