ORGANIC LETTERS

2009
Vol. 11, No. 12
2515−2518

Regiospecific Syntheses of 6α-(1*R*-Hydroxyoctyl)penicillanic Acid and 6β-(1*R*-Hydroxyoctyl)penicillanic Acid as Mechanistic Probes of Class D β-Lactamases

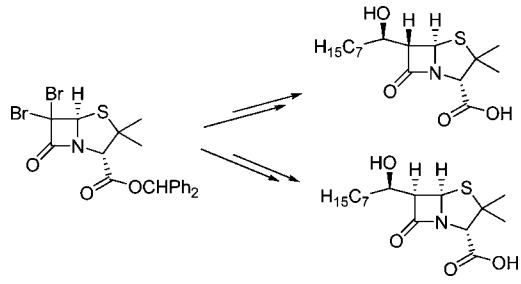
Sebastian A. Testero, Peter I. O'Daniel, Qicun Shi, Mijoon Lee, Dusan Hesek, Akihiro Ishiwata, Bruce C. Noll, and Shahriar Mobashery*

Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, Indiana 46556

mobashery@nd.edu

Received March 31, 2009

ABSTRACT



The unique hydrophobic surface patches in class D β-lactamases presented an opportunity for designing two compounds, 6α-(1*R*-hydroxyoctyl)penicillanic acid and 6β-(1*R*-hydroxyoctyl)penicillanic acid, as mechanistic probes of these enzymes. In a sequence of three synthetic steps from benzhydryl 6,6-dibromopenicillanate, the targeted compounds were prepared in a stereospecific manner.

β-Lactamases are important resistance determinants for β-lactam antibiotics (penicillins, cephalosporins, carbapenems, etc.).[1] Bacteria have evolved four classes of these enzymes, of which three (classes A, C, and D) are serine-dependent enzymes.[1,2] The antibiotic acylates an active site serine, and the acyl-enzyme species subsequently experiences hydrolysis to complete the catalytic process. Because evolution of these β-lactamases has been independent of each other, nature has devised the deacylation step on three separate occasions.[1,2] As such, the trajectory that the hydrolytic water uses for approach to the acyl-enzyme carbonyl has been either from one face of the carbonyl entity or the other.

We have described a class of 6-hydroxyalkylpenicillanate inhibitors for these enzymes.[3−5] These compounds acylate the active site serine by the virtue of the fact that they are penicillins. However, if the stereochemistry of the 6-hydroxyalkyl moiety (α or β) predisposes the functionality in the direction of the approach of the water molecule, the hydroxyl moiety would present a steric impediment to the

(1) Fisher, J. F.; Meroueh, S. O.; Mobashery, S. *Chem. Rev.* **2005**, *105*, 395–424.

(2) Massova, I; Mobashery, S. *Antimicrob. Agents Chemother.* **1998**, *42*, 1–17.

(3) Miyashita, K.; Massova, I.; Taibi, P.; Mobashery, S. *J. Am. Chem. Soc.* **1995**, *117*, 11055–11059.

(4) Maveyraud, L; Massova, I.; Birck, C.; Miyashita, K.; Samama, J. P.; Mobashery, S. *J. Am. Chem. Soc.* **1996**, *118*, 7435–7440.

(5) Golemi, D.; Maveyraud, L.; Ishiwata, A.; Tranier, S.; Miyashita, K.; Nagase, T.; Massova, I.; Mourey, L.; Samama, J. P.; Mobashery, S. *J. Antibiot.* **2000**, *53*, 1022–1027.

10.1021/ol900668k CCC: $40.75
Published on Web 05/15/2009

travel of the hydrolytic water molecule to the acyl-enzyme species.[5] The opposite stereochemistry at position 6 would render the molecule a substrate, as the approach of the hydrolytic water to the acyl-enzyme species would be unencumbered.

The work that has been performed with these penicillanate inhibitors has been limited to classes A and C of β-lactamases. However, in the past five years, the members of the class D of these enzymes have become prominent, with >140 members having been identified to date. These enzymes are distinctly different from the other two classes by having an *N*-carboxylated lysine within their active sites.[6−8] Furthermore, the environments surrounding the lysine and immediately adjacent to the active site are highly hydrophobic. This presented an opportunity for design of variants of these 6-hydroxyalkylpenicillanates that would show preference for class D enzymes. This task was undertaken by computational methods, building on the knowledge of interactions of these penicillanates with the active sites of the enzymes (Figure 1). The exercise led us to 6α- and 6β-hydroxyloctylpenicillanic acids as probes targeting class D β-lactamases. The synthetic work was also performed for 6α- and 6β-(hydroxyl-1-methylethyl)penicillanic acids with the intention of the optimization of the syntheses of these compounds, which we had reported earlier.

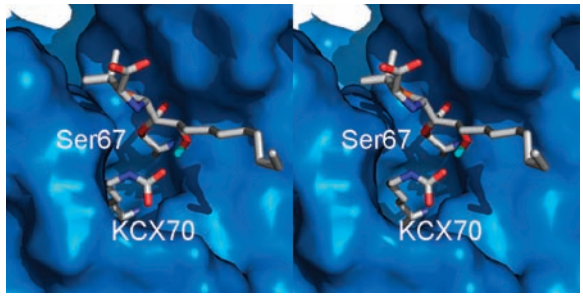
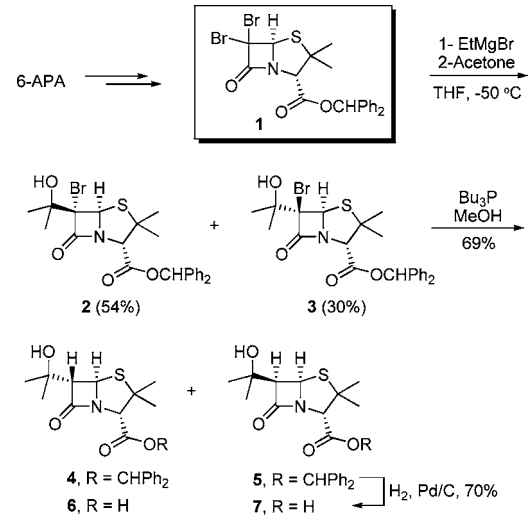


Figure 1. Stereoview representation of the acyl-enzyme species of the class D OXA-10 β-lactamase and 6-β-(1-hydroxyloctyl)-penicillanate from molecular dynamics (MD) equilibration. The penicillanate covalently bound to Ser67 (in capped sticks, carbon in gray, nitrogen in blue, sulfur in yellow and oxygen in red) is depicted within the active site, which is shown as a solvent-accessible Connolly surface (blue). *N*-Carboxylate lysine (KCX70) is at 7 o'clock and the octyl moiety is pointing to 4 o'clock. Note that the hydroxyl moiety of the C-6 functionality is hydrogen-bonded to the *N*-carboxylated lysine.

The synthesis began with the preparation of 6,6-dibromopenicillanic acid (**1**) from 6-aminopenicillanic acid (6-APA) by the procedure of Volkman.[9] Subsequent esterification of the C-3 carboxylic acid by diphenyldiazomethane gave the key intermediary compound **1** (Scheme 1), which

Scheme 1



was crystallized from ethyl acetate and the structure was confirmed by X-ray crystallography (Figure 2). Transmetalation with the use of the Grignard reagent, followed by the addition of acetone, gave a mixture of **2** and **3**, a method that has been reported for alkylation of penicillins.[3,10] As observed in the present study, a freshly prepared anhydrous THF was critical for this reaction in the presence of stoichiometric amount of the Grignard reagent. The yield of the carbon−carbon bond formation step improved over the earlier reports by nearly 3-fold by simply preparing the

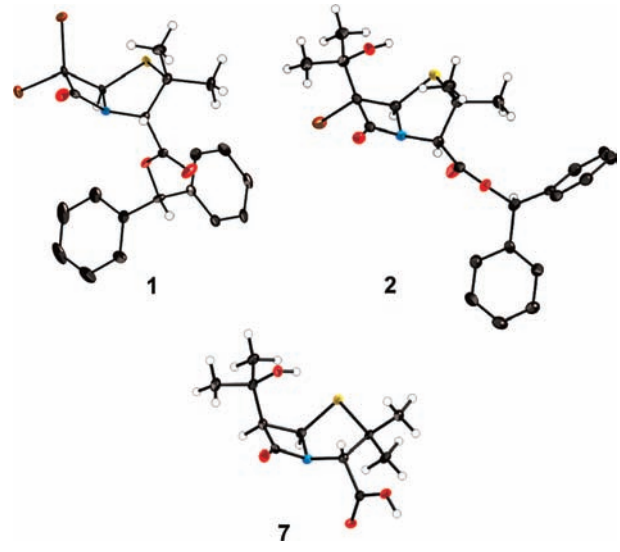


Figure 2. ORTEP diagram of X-ray structure of compounds **1**, **2**, and **7**.

(6) Maveyraud, L.; Golemi, D.; Kotra, L. P.; Tranier, S.; Vakulenko, S.; Mobashery, S.; Samama, J. P. *Structure* **2000**, *8*, 1289–1298.
(7) Golemi, D.; Maveyraud, L.; Vakulenko, S.; Samama, J. P.; Mobashery, S. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 14280–14285.
(8) Maveyraud, L.; Golemi, D.; Ishiwata, A.; Meroueh, O.; Mobashery, S.; Samama, J. P. *J. Am. Chem. Soc.* **2002**, *124*, 2461–2465.
(9) Volkmann, R. A; Carroll, R. D; Drolet, R. B.; Elliot, M. L.; Moore, B. S. *J. Org. Chem.* **1982**, *47*, 3344–3345.

anhydrous THF prior to carrying out the reaction. After purification, the yields of isomers **2** (β) and **3** (α) were 54 and 30%, respectively. The assignment of the stereochemistry was greatly helped by the determination of the crystal structure for compound **2** (Figure 2).

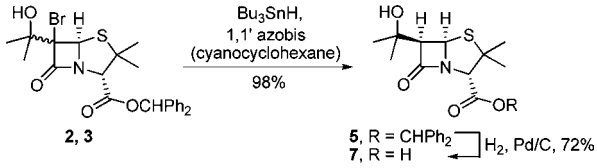
The reduction of the carbon−halide bond could be achieved by the use of tributyltin hydride,[11] zinc-mediated chemistry,[12] or hydrogenolysis over Pd−C.[13] We have previously reported the stereoselective synthesis of 6α-substituted penicillanate ester by the use of tributylphosphine, but we have noted that the stereoselectivity can be quite variable from run to run and was not reliable in its reproducibility.[14] The use of this procedure on either a mixture of **2** and **3** or on pure isomer as starting material afforded an inseparable mixture of the α and β isomers (**4** and **5**) in the ratio of 2.8/1 (determined by ^{1}H NMR of the crude) in 69%. The stereochemical assignments for C-6 of compounds **4** and **5** were based on their ^{1}H NMR coupling constants $J_{H5-H6} = 1.8$ and 4.6 Hz, respectively, which are in agreement with the Karplus equation.

Several methods for the removal of the benzhydryl group in the mixture of compounds **4** and **5** were tried (hydrogenolysis over palladium, TFA/anisole, formic acid, Lewis acids with anisole or *m*-cresol). Of these, hydrogenolysis gave good yields, but more importantly, the reaction products were clean at the end of the workup.

Fractional crystallization from chloroform-hexane afforded colorless crystals of the β-isomer **7**, whose structure was confirmed by X-ray crystallography (Figure 2). The α-isomer **6** was isolated in pure form from the solution.

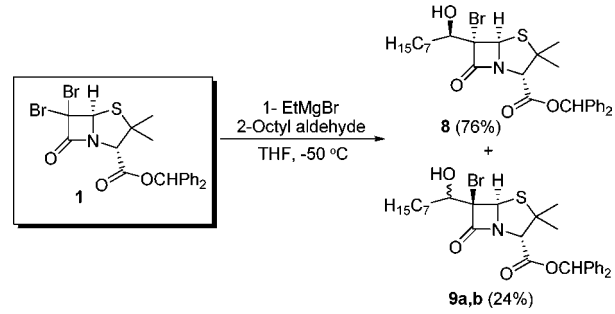
Whereas the reductive step in the previous procedure was useful in that we obtained the two isomers in pure forms (after deprotection), we observed that the use of tributyltin hydride (with 1,1′ azobis(cyanocyclohexane) as initiator) with the mixture of **2** and **3** produced the β-isomer **5** as the exclusive product in 98% yield. This allowed us to access compound **7** after hydrogenolysis by an additional route (Scheme 2).

Scheme 2



The computational design procedure with the recently emerged structural information of the class D β-lactamases revealed the opportunity for the design of a novel family of hydroxyalkylpenicillanate compounds for their inhibition. The methodology that we have described above was applied to the preparation of these new penicillanates for mechanistic studies of class D β-lactamases. The reaction of the dibromide **1** in the presence of the Grignard reagent in freshly prepared anhydrous THF and octyl aldehyde produced 6β-hydroxyoctylpenicillanate **8** in 76% yield, along with the undesired mixture of two isomers **9a,b** (24%; Scheme 3).

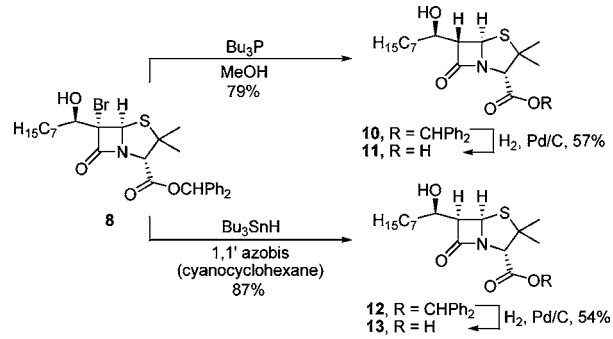
Scheme 3



The configuration at C-6 and the stereochemistry of the stereogenic carbon at the C-6 side chain in compounds **8** and **9a,b** were assigned by NMR according to DiNinno et al.[15]

Treatment of bromide **8** with tributylphosphine in methanol afforded solely the 6α-substituted penicillanate **10** in 79% yield (Scheme 4). A coupling constant of 1.7 Hz between

Scheme 4



the vicinal protons H-5 and H-6 established a *trans* relationship. This reaction would appear to proceed through a tributylphosphonium β-lactam enolate, followed by diastereoselective protonation from the β-face.[14] Finally, removal of the benzhydryl group furnished compound **11**.

(10) (a) Buynak, J. D.; Chen, H.; Vogeti, L.; Gadhachanda, V. R.; Buchanan, C. A.; Palzkill, T.; Shaw, R. W.; Spencer, J.; Walsh, T. R. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1299–1304. (b) Norris, T.; Ripin, D. H. B.; Ahlijanian, P.; Andersen, B. M.; Barrila, M. T.; Colon-Cruz, R.; Couturier, M.; Hawkins, J. M.; Loubkina, I. V.; Rutherford, J.; Stickley, K.; Wei, L.; Vollinga, R.; de Pater, R.; Maas, P.; de Lange, B.; Callant, D.; Konigs, J.; Andrien, J.; Versleijen, J.; Hulshof, J.; Daia, E.; Johnson, N.; Sung, D. W. L. *Org. Process Res. Dev.* **2005**, *9*, 432–439.

(11) For examples, see: (a) Ziegler, C. B., Jr.; Fields, T. L. *Tetrahedron* **1993**, *49*, 3919–3932. (b) Hanessian, S.; Alpegiani, M. *Tetrahedron* **1989**, *45*, 941–950. (c) Foulds, C. D.; Kosmirak, M.; Sammes, P. G. *J. Chem. Soc., Perkin Trans. 1* **1985**, 763–768. (d) Hirai, K.; Iwano, Y.; Fujimoto, K. *Tetrahedron Lett.* **1982**, *23*, 4021–4024. (e) Bitha, P.; Li, Z.; Francisco, G. D.; Rasmussen, B. A.; Lin, Y.-I. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 991–996.

On the other hand, reduction of the bromide **8** by tributyltin hydride provided the opposite configuration at C-6, to result in compound **12** as the exclusive product. The establishment of the stereochemistry at C-6 was based on the coupling constant between H-5 and H-6 ($J = 4.5$ Hz). In this case, the reaction would take place through an intermediary penicillin radical that would be quenched on the α-face of the penicillin to yield the 6-β-hydroxyoctyl product **12**.[16] Deprotection of the benzhydryl group of **12** afforded the 6β-hydroxyoctylpenicillanic acid **13** in an acceptable yield (54%).

In summary, we have described short and regiospecific methodology into preparation of penicillanate derivatives **11** and **13**, which will be valuable tools in investigations of mechanistic and structural details of class D β-lactamases.

Acknowledgment. This work was supported by the NIH.

Supporting Information Available: General experimental procedures, compound characterization data, including copies of 1D NMR spectra (^{1}H, ^{13}C NMR) and X-ray data of compounds **1**, **2**, and **7**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL900668K

(12) For examples, see: (a) Yoshida, A.; Hayashi, T.; Takeda, N.; Oida, S.; Ohki, E. *Chem. Pharm. Bull.* **1981**, *10*, 2899–2909. (b) Leanza, W. J.; DiNinno, F.; Muthard, D. A.; Wilening, R. R.; Wildonger, K. J.; Ratcliffe, R. W.; Christensen, B. G. *Tetrahedron* **1983**, *39*, 2505–2513. (c) Fujimoto, K.; Iwano, Y.; Hirai, K.; Sugawara, S. *Chem. Pharm. Bull.* **1986**, *34*, 999–1014.

(13) For examples, see: (a) Girijavallabhan, V. M.; Ganguly, A. K.; McCombie, S. W.; Pinto, P.; Rizvi, R. *Tetrahedron Lett.* **1981**, *22*, 3485–3488. (b) Rossi, R. L.; Kapilani, L. V.; Morrissey, P.; Retsema, J. A. *J. Med. Chem.* **1990**, *33*, 291–297.

(14) Ishiwata, A.; Kotra, L. P.; Miyashita, K.; Nagase, T.; Mobashery, S. *Org. Lett.* **2000**, *2*, 2889–2892.

(15) DiNinno, F; Beattie, T. R.; Christensen, B. G. *J. Org. Chem.* **1977**, *42*, 2960–2965.

(16) Norris, T.; Dowdeswell, C.; Johnson, N.; Daia, D. *Org. Process Res. Dev.* **2005**, *9*, 792–799.