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Synthesis and biological evaluation of penem inhibitors of bacterial signal peptidase

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ABSTRACT

We report the first synthesis of a 5*S* penem, known to bind bacterial type I signal peptidase, from the commercially available and inexpensive 6-aminopenicillanic acid. We report the first in vivo activity of the compound and use structure–activity relationship studies to begin to define the determinants of signal peptidase binding and also to begin to optimize the penem as an antibiotic.

The emergence of drug resistant bacteria poses a serious threat to human health, and the preservation of the 'antibiotic era' will likely require the discovery of novel antibiotics that act via novel mechanisms. One potential novel mechanism is the inhibition of bacterial type I signal peptidase (SPase). SPase is conserved across both Gram-positive and Gram-negative bacteria and is required to process cell surface bound preproteins during export from the cytoplasm.[1,2] Its location in the outer leaflet of the cytoplasmic membrane should make it accessible to small molecule drugs. In addition, its unique catalytic mechanism, which relies on an atypical Ser-Lys catalytic dyad and attack of the peptide substrate from the *si*-face, rather than the *re*-face characteristic of the common Ser-His-Asp catalytic triad serine proteases,[3] should limit off-target toxicity. Moreover, SPase is not only required for viability, but is also required to process and secrete proteins involved in a number of bacterial processes including adhesion, antibiotic resistance, virulence, lateral gene transfer, and toxin production.[4,5] SPase inhibitors should thus attenuate virulence, potentially increasing their utility as therapeutics.

Consistent with its unconventional proteolysis mechanism, standard serine protease inhibitors do not inhibit SPase.[6–9] However, researchers at SmithKline Beecham Pharmaceuticals identified a racemic penem with moderate in vitro activity from a screen against the *Escherichia coli* protein.[10–13] Interestingly, all inhibitor activity was found in the 5*S* diastereomer of the penem, which is the stereochemistry opposite to that of other known β-lactam drugs.[14]

Generally, β-lactam antibiotics are effective irreversible covalent inhibitors of serine proteases and hydrolases, such as elastase and β-lactamase.[16,17] These antibiotics act by forming long lived acyl enzyme intermediates via the electrophilicity of their cyclic amide carbonyl. A medicinal chemistry effort at SmithKline Beecham directed at the optimization of the initially identified penem culminated in the identification of the (*5S,6S*)-6-[(*R*)-hydroxyethyl]-penem-3-carboxylate (Fig. 1A, R^1,R^2 = H) which inhibits *E. coli* SPase with an IC_{50} of 180 nM.[10–13] In addition, highly strained 5*S* tricyclic penems, in which a third heterocyclic ring is fused to the C2 and C3 positions of the (*5S,6S*)-6-[(*R*)-hydroxyethyl]-penem core, inhibit *E. coli* and MRSA SPase in vitro with IC_{50} values of 0.2 μM and 5 μM, respectively.[18] Thus, potent in vitro SPase inhibition has been demonstrated by derivatization of the *5S,6S*-penem, however; antibacterial activity in vivo has not been reported for any of these compounds.

Since the efforts of SmithKline Beecham, Paetzel et al. reported the crystal structure of the *E. coli* SPase complexed with the allyl (*5S,6S*)-6-[(R)-acetoxyethyl]-penem-3-carboxylate (Fig. 1A, R^1 = acetyl, R^2 = allyl).[15] In addition to confirming the unique mechanism of SPase catalysis, the structure offered insight into the binding of both the inhibitor and the natural leader peptide substrates. In general, an *E. coli* signal peptide consists of a C-terminal cleavage recognition sequence containing small uncharged residues at the P1 and P3 sites, with the former almost always Ala and the latter Ala, Val, Leu, or Ile.[19,20] The P1 and P3 side chains are thought to bind in the corresponding substrate binding pockets S1 and S3 in SPase. The crystal structure reveals that the penem does not access the S3 pocket, but its C10 methyl group is oriented towards the S1 pocket. The structure also reveals that substituents attached to the

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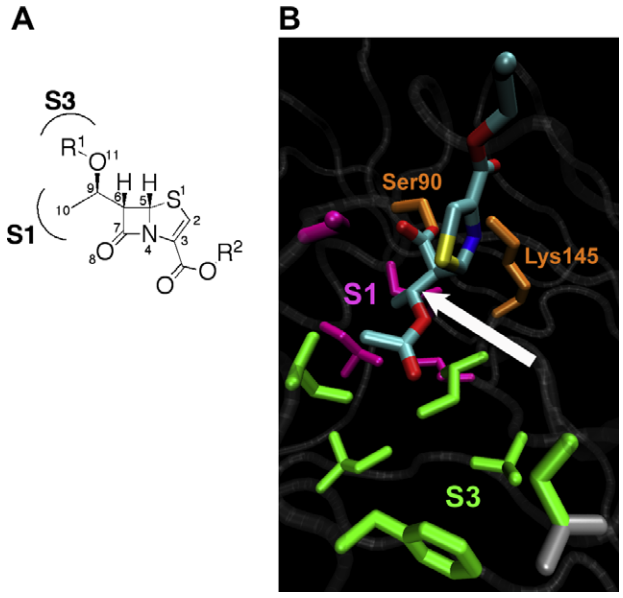
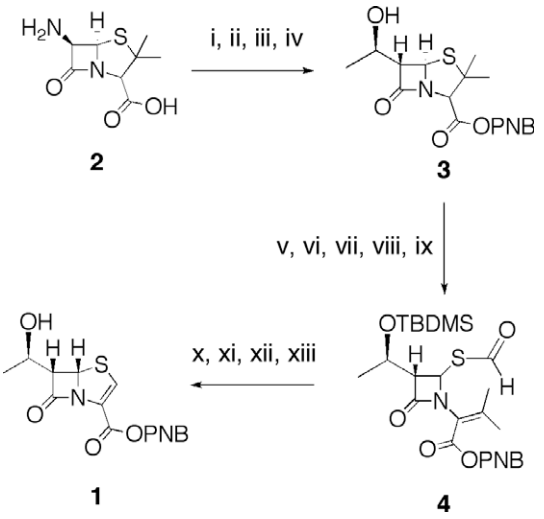


Figure 1. (A) Structure of (5*S*,6*S*)-6-[(*R*)-hydroxyethyl] (R^1, R^2 = H) and 3-allyl ester (R^1 = acetyl and R^2 = allyl) penems identified by SmithKline Beecham.[12,13] (B) Structure of the 3-allyl ester penem bound to *E. coli* SPase. The S1 and S3 binding pockets as well as the catalytically essential serine (Ser90) and lysine (Lys145) are highlighted.[15] The C6 hydroxyethyl group is indicated with an arrow.



Scheme 1. Reagents and conditions: (i) Br_2 (3.0 equiv), $NaNO_2$ (2.0 equiv), 2.5 N H_2SO_4 (2.0 equiv), CH_2Cl_2, 5 °C; (ii) K_2CO_3 (0.9 equiv), *p*-nitrobenzyl bromide (1.0 equiv), DMF, 40 °C, (70% for i–ii); (iii) MeMgBr (3.0 M in Et_2O) (1.0 equiv), MeCHO (6.5 equiv), THF, −78 °C, (35%); (iv) Bu_3P (1.6 equiv), MeOH, 0 °C, (95%); (v) *t*-$BuMe_2SiCl$ (1.5 equiv), imidazole (3.0 equiv), DMF, rt, (98%); (vi) MCPBA (0.9 equiv), CH_2Cl_2, 0 °C, (80%); (vii) mercaptobenzothiazole (0.99 equiv), toluene, reflux; (viii) Et_3N (0.1 equiv), toluene, 0 °C; (ix) HCO_2H (4.4 equiv), Ac_2O (4.4 equiv), pyridine (1.03 equiv), PPh_3 (1.01 equiv), toluene, −15 °C, (65% for vii–ix); (x) O_3, EtOAc, −78 °C, (98%); (xi) $P(OMe)_3$ (5.0 equiv), toluene, 70 °C, (40%); (xii) hυ, EtOAc, rt, (60%); (xiii) Bu_4NF (1.0 M in THF) (4.0 equiv), HOAc (12.0 equiv), rt, (90%).

penem at its C2 and C3 positions roughly correspond to substrate side chains P2 and P4, and that they offer little to no opportunity for optimization of inhibitor binding because they are oriented into solvent. This structural data is consistent with results from SmithKline Beecham[12,13] as well the natural substrate sequence diversity at P2 and P4.[19]

This structural data suggest that the optimization of binding within S1 and/or accessing the S3 pocket of the enzyme offers the best opportunity to increase the affinity of the penem for SPase and that both of these sites should be accessible from the C6 position of the inhibitor core (Fig. 1). Toward exploring this possibility, we report the first synthesis of a 5*S* penem from the commercially available and inexpensive 6-aminopenicillanic acid, which has proven to be a useful starting material for the synthesis of numerous *5R* β-lactam drugs.[21,22] With access to the compound, we explored its activity against several bacteria and demonstrated the first in vivo activity. Finally, we report an initial structure–activity relationship (SAR) study designed to probe the accessibility of the S1 and/or S3 pockets of SPases from both Gram-positive and Gram-negative bacteria from the C6 position of the 5*S* penem core.

For our initial SAR studies we examined modifications of the 5*S*,6*S*-penem core with a C3 *p*-nitrobenzyl protected carboxylic acid and a C6 hydroxyethyl moiety (**1**). Preliminary experiments showed little differences in activity with a free acid at position C3 or with *p*-nitrobenzyl-, allyl-, or methyl-esters. The C6 hydroxyethyl substituent is known to be important for activity[12,13] and we also found that it facilitated stereoinversion during synthesis. Synthesis of this penem core (Scheme 1) commenced with preparation of *p*-nitrobenzyl 6,6-dibromopenicillanate which is readily obtained from aminopenicillanic acid **2** by diazotization-bromination, followed by esterification of the crude di-bromo acid.[23] Metal–halogen exchange with methylmagnesium bromide in THF at −78 °C, gave an enolate intermediate, which upon quenching with an excess of acetaldehyde, afforded the hydroxyethyl product.[24,25] Diastereoselective reduction of the 6-bromo-6-substituted penicillanate was achieved by treatment with tributylphosphine yielding the 6-substituted penicillanate ester **3** under mild conditions.[26] After *tert*-butyldimethylsilyl protection of the hydroxyethyl group, oxidation with *m*-chloroperbenzoic acid (MCPBA) afforded the sulfoxide. Heating of the sulfoxide to reflux in toluene induced a sigmatropic rearrangement that resulted in the formation of the sulfenic acid, which was intercepted by mercaptobenzothiazole to yield the disulfide product. Base-catalyzed double-bond isomerization produced the more stable conjugated ester disulfide, and the C4 formylthio substituent was introduced via reductive formylation with pyridine, acetic formic anhydride, (in situ from formic acid and acetic anhydride), and triphenylphosphine, yielding the aldehyde **4**.[27] Ozonolysis of this material followed by trialkyl phosphite-mediated cyclization of the oxalamide by reductive carbonyl-carbonyl coupling yielded the hydroxyethyl penem ester.[28] Photochemical isomerization at C5 converted the core 5*R*,6*S*-*trans*-penem to the 5*S*,6*S*-cis stereochemistry.[29] Tetrabutylamonium fluoride (TBAF)-mediated desilylation gave the desired hydroxyethyl penem.

Before commencing our structure–activity studies we first determined the susceptibility of several important human pathogens to the parent penem **1** (Table 1). Specifically, we determined the sensitivity of *E. coli* (strain ATCC25922 treated with permeabilizing agent polymyxin b (PMBn) to eliminate penetrance issues), two *Staphylococcus aureus* strains (8325 and the methicillin resistant clinical isolate USA300, which for convenience will be referred to as MRSA), and *Staphylococcus epidermidis* (strain RP62A). In each case, the minimal inhibitory concentration (MIC) was determined using the liquid dilution method. As reported previously, the parent 5*S*-OH penem **1** showed no significant activity against permeabilized *E. coli* or *S. aureus* (MIC ⩾ 200 μg/mL). However, we did observe activity against *S. epidermidis* (MIC = 50 μg/mL), which is the first demonstration of antibacterial activity for a penem SPase inhibitor on whole cells. In contrast, the 5*R* diastereomer had no activity, suggesting that the antibacterial activity was indeed associated with SPase inhibition.

Our initial SAR studies focused on attaching simple ethers to the C6 hydroxyethyl moiety. Synthetic challenges limited our ability to introduce branched ethers, but we were able to introduce the methoxy methyl ether (**5**), ethoxy methyl ether (**6**), and methoxy

Table 1
MICs of penem inhibitors **1**–**18**[a]

Compound #	*E. coli*+PMBn (μg/mL)	*S. aureus* (μg/mL)	MRSA (μg/mL)	*S. epidermidis* (μg/mL)
1	>200	200	>200	50
5	>200	>200	>200	50
6	>200	>200	>200	50
7	>200	200	>200	100
8	>200	>200	>200	>200
9	>200	>200	>200	>200
10	>200	>200	>200	>200
11	>200	>200	>200	>200
12	>200	>200	>200	100
13	>200	>200	>200	100
14	>200	>200	>200	100
15	>200	>200	>200	50
16	>200	>200	>200	100
17	>200	>200	100	100
18	>200	>200	100	100

[a] MICs were determined using compounds that were serially diluted 2-fold in cation-adjusted Mueller–Hinton broth to a total volume of 100 μL in 96-well plates. Bacteria were grown to 10^8 cfu/mL, and 5 μL of a 10-fold dilution was used to inoculate 100 μL of penem-containing media to a final concentration of 5×10^5 cells/mL. Microplates were incubated at 37 °C for 18 h with MICs defined as the lowest concentration necessary to inhibit visible growth.

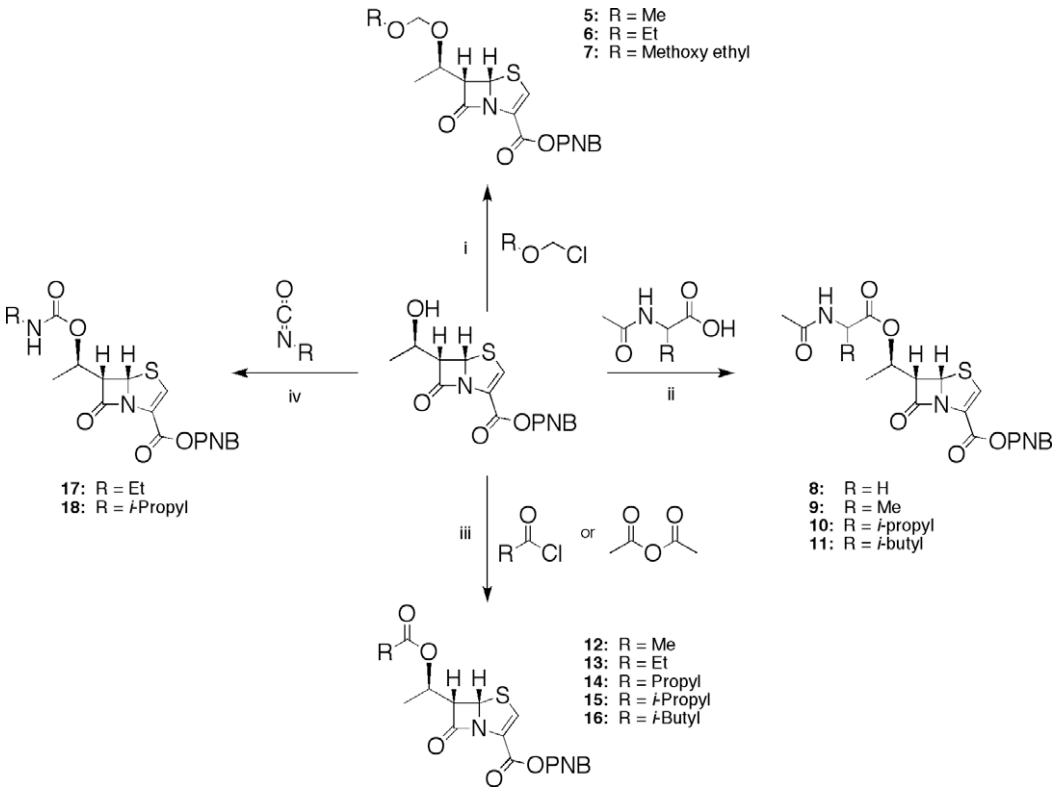
ethoxy ether (**7**) substituents via diisopropylethylamine (DIEA) treatment followed by the corresponding chloride (Scheme 2).[11] These derivatizations did not confer activity against *E. coli* or *S. aureus*, and had little to no effect on the sensitivity of *S. epidermidis* (Table 1).

We next explored the attachment of different amino acids via their C-terminus to the hydroxyethyl moiety of the penem core (**8**–**11**, Scheme 2). We hypothesized that these compounds might mimic a natural leader peptidase substrate. Acetyl protected glycine, alanine, valine, or leucine was coupled with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and catalytic dimethyl amino pyridine (DMAP). In each case, we found that the modifications did not bestow the penem with activity against *E. coli* or *S. aureus* and actually eliminated activity against *S. epidermidis* (MICs > 200 μg/mL) (Table 1). Thus, at least when linked via an ester, these amino acids do not appear to mimic the constituents of a natural leader sequence substrate.

We reasoned that poor recognition may be the result of the penem scaffold mispositioning the peptidic side chain or heteroatom functionality of **8**–**11** in the SPase binding site, relative to the natural substrates. To begin to explore this possibility, we examined a series of simple alkyl esters (**12**–**16**, Scheme 2). The esters were synthesized by triethylamine treatment of the penem followed by the appropriate acetic anhydride or acid chloride and a catalytic quantity of DMAP.[11] Again, none of the ester substituents conferred activity against *E. coli* or *S. aureus*. However, they all restored activity against *S. epidermidis*, and the isopropyl ester **15** was as active as the parent penem (Table 1). This data suggests that while simple ester derivatization reduces SPase binding, appropriate derivatization of the alkyl group can restore affinity, presumably by accessing the S1 and/or S3 pockets.

To examine the reintroduction of peptide-like functionality into the derivatives, we synthesized and examined two carbamates (**17** and **18**, Scheme 2),[11] which restore a nitrogen atom, but in a position shifted relative to the amino acid derivatives **8**–**11**. Neither the ethyl nor the isopropyl carbamate derivatization resulted in detectable activity against *E. coli* or *S. aureus* 8325, and both slightly reduced activity against *S. epidermidis*, but surprisingly both conferred the penem with modest activity against MRSA (MIC = 100 μg/mL) (Table 1).

We have demonstrated that while the penem derivatives synthesized have no activity against the Gram-negative pathogen



Scheme 2. Reagents and conditions: (i) R-Cl (4.0 equiv), DIEA (4.0 equiv), CH_2Cl_2, rt, (80%); (ii) $COCH_3NH$-RCH-COOH (4.0 equiv), DMAP (1.0 equiv), EDC (8.0 equiv), CH_2Cl_2, −15 °C, (40%); (iii) for compound **12**: Et_3N (2.0 equiv), Ac_2O (2.5 equiv), DMAP (1.0 equiv), CH_2Cl_2, 0 °C, (90%); for compounds **13**–**16**: Et_3N (5.0 equiv), R-Cl (5.0 equiv), DMAP (1.0 equiv), rt, (75%); (iv) R-isocyanate (50.0 equiv), DMAP (1.0 equiv), rt (50%).

E. coli, even with a permeabilized outer membrane, they do generally have antibiotic activity against the important human pathogen *S. epidermidis*. *S. epidermidis* is a very common nocosomial pathogen and the major cause of indwelling device infection.[30,31] Interestingly, the penem's activity is only ~50-fold less than that of antibacterials currently used to treat *S. epidermidis* infections.[32] Importantly, the carbamate derivatives also resulted in activity against MRSA. One interpretation of the structure–activity relationship data is that the ester linkage employed mispositions the amino acid side chains or H-bonding functionality relative to the SPase binding site, and that the carbamate nitrogen, which is shifted by one bond, is better positioned to favorably engage the enzyme. This suggests that amino acids attached via different linkers might be better mimics of the natural substrate and might increase penem activity.

The structure–activity relationship data also reveal interesting differences among the SPases from the different organisms. The unique activity against *S. epidermidis* might result from unique aspects of the pathogen's SPase or from a unique aspect of its biology, perhaps being more sensitive to SPase inhibition. Also, not only is there variation in the SPases from Gram-negative and Gram-positive pathogens, but the cell wall changes associated with methicilin resistance[33,34] appear to confer *S. aureus* with sensitivity, at least to the carbamate-derivatized penems. This latter effect may result from increased or altered protein secretion in MRSA relative to methicillin sensitive *S. aureus*. Thus, the characterization of additional penem derivatives should help further define both SPase biochemistry and possibly protein secretion in general.

Increasingly, there is a pressing need for antibiotics with novel mechanisms of action, and in this regard penem inhibitors of SPase appear attractive. The demonstration that a derivative of the penem possesses at least modest in vivo antibacterial activity against two important human pathogens suggests that with further optimization more potent and broad spectrum activity may be achievable.

Acknowledgments

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References and notes

1. Paetzel, M.; Dalbey, R. E.; Strynadka, N. C.. *Pharmacol. Ther.* **2000**, *87*, 27.
2. Carlos, J. L.; Paetzel, M.; Klenotic, P. A.; Strynadka, N. C. J.; Dalbey, R. E., 3rd ed.. In *The Enzymes*; Academic Press: Newyork, 2001; Vol. 22. p. 27.
3. Tschantz, W. R.; Sung, M.; Delgado-Partin, V. M.; Dalbey, R. E. *J. Biol. Chem.* **1993**, *268*, 27349.
4. Kulanthaivel, P.; Kreuzman, A. J.; Strege, M. A.; Belvo, M. D.; Smitka, T. A.; Clemens, M.; Swartling, J. R.; Minton, K. L.; Zheng, F.; Angelton, E. L.; Mullen, D.; Jungheim, L. N.; Klimkowski, V. J.; Nicas, T. I.; Thompson, R. C.; Peng, S. B. *J. Biol. Chem.* **2004**, *279*, 36250.
5. Cao, T. B.; Saier, M. H. J. *Microbiology* **2001**, *147*, 3201.
6. Black, M. T.; Munn, J. G.; Allsop, A. E. *Biochem. J.* **1992**, *282*, 539.
7. Kim, Y. T.; Muramatsu, T.; Takahashi, K. *J. Biochem.* **1995**, *117*, 535.
8. Kuo, D. W.; Chan, H. K.; Wilson, H. K.; Griffin, P. R.; Williams, H.; Knight, W. B. *Arch. Biochem. Biophys.* **1993**, *303*, 274.
9. Zwizinski, C.; Date, T.; Wickner, W. *J. Biol. Chem.* **1981**, *256*, 3593.
10. Allsop, A. E.; Brooks, G.; Bruton, G.; Coulton, S.; Edwards, P. D.; Hatton, I. K.; Kaura, A. C.; McLean, S. D.; Pearson, N. D.; Smale, T. C.; Southgate, R. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 443.
11. Allsop, A. E.; Brooks, G.; Edwards, P. D.; Kaura, A. C.; Southgate, R. *J. Antibiot. (Tokyo)* **1996**, *49*, 921.
12. Allsop, A. E. et al In *Anti-Infectives: Recent Advances in Chemistry Activity Relationships*; Bently, P. H., O'Hanlon, P. J., Eds.; Royal Society of Chemistry: Cambridge, 1997; p 61.
13. Black, M. T.; Bruton, G. *Curr. Pharm. Des.* **1998**, *4*, 133.
14. Anderrson, I.; van Scheltinga, A. C.; Valegård, K. *Cell. Mol. Life Sci.* **2001**, *58*, 1897.
15. Paetzel, M.; Dalbey, R. E.; Strynadka, N. C. J. *Nature* **1998**, *396*, 186.
16. Green, B. G.; Chabin, R.; Mills, S.; Underwood, D. J.; Shah, S. K.; Kuo, D.; Gale, P.; Maycock, A. L.; Liesch, J. *Biochemistry* **1995**, *34*, 14331.
17. Massova, I.; Mobashery, S. *Acc. Chem. Res.* **1997**, *30*, 162.
18. Hu, X. E.; Kim, N. K.; Grinius, L.; Morris, C. M.; Wallace, C. D.; Meiling, G. E.; Demuth, T. P., Jr. *Synthesis* **2003**, *11*, 1732.
19. von Heijne, G. *J. Mol. Biol.* **1985**, *184*, 99.
20. Izard, J. W.; Kendall, D. A. *Mol. Microbiol.* **1994**, *13*, 765.
21. Maruyama, H. *J. Org. Chem.* **1986**, *51*, 399.
22. Rolinson, G. N.; Geddes, A. M. *Int. J. Antimicrob. Agents* **2007**, *29*, 3.
23. Micetich, R. G.; Maiti, S. N.; Tanaka, M.; Yamazaki, T.; Ogawa, K. *J. Org. Chem.* **1986**, *51*, 853.
24. DiNinno, F.; Beattie, T. R.; Christensen, B. G. *J. Org. Chem.* **1977**, *42*, 2960.
25. DiNinno, F.; Leanza, W. J. *Tetrahedron* **1983**, *39*, 2505.
26. Ishiwata, A.; Kotra, L. P.; Miyashita, K.; Nagase, T.; Mobashery, S. *Org. Lett.* **2000**, *2*, 2889.
27. Abe, T.; Sato, C.; Ushirogochi, H.; Sato, K.; Takasaki, T.; Isoda, T.; Mihira, A.; Yamamura, I.; Hayashi, K.; Kumagai, T.; Tamai, S.; Shiro, M.; Venkatesan, A. M.; Mansour, T. S. *J. Org. Chem.* **2004**, *69*, 5850.
28. Osborne, N. F.; Atkins, R. J.; Broom, N. J. P.; Coulton, S.; Harbridge, J. B.; Harris, M. A.; Stirling-François, I.; Walker, G. C. *J. Chem. Soc., Perkin Trans. 1* **1994**, 179.
29. Iwata, H.; Tanaka, R.; Imajo, S.; Oyama, Y.; Ishiguro, M. *J. Chem. Soc., Chem. Commun.* **1991**, 285.
30. *Catheter-Related Infections*; Seifert, H., Jansen, B., Farr, B. M., Eds.; Marcel Dekker: New York, 1997.
31. *Infections Associated with Indwelling Medical Devices*; Waldvogel, F. A., Bisno, A. L., Eds.; American Society for Microbiology: Washington, DC, 2000.
32. John, M. A.; Pletch, C.; Hussain, Z. *J. Antimicrob. Chemother.* **2002**, *50*, 933.
33. McAleese, F.; Wu, S. W.; Sieradzki, K.; Dunman, P.; Murphy, E.; Projan, S.; Tomasz, A. *J. Bacteriol.* **2006**, *188*, 1120.
34. Utaida, S.; Dunman, P.; Macapagal, D.; Murphy, E.; Projan, S. J.; Singh, V. K.; Jayaswal, R. K.; Wilkinson, B. J. *Microbiology* **2003**, *149*, 2719.