Pergamon

Tetrahedron 56 (2000) 5719–5728

TETRAHEDRON

N-Sulfonyloxy-β-lactam Inhibitors for β-Lactamases

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Received 5 November 1999; accepted 4 February 2000

Abstract—Structure-function analysis with a series of *N*-sulfonyloxy β-lactam molecules as inhibitors of β-lactamases is reported. The best of these compounds acylate the active site of the class A TEM-1 β-lactamase from *Escherichia coli* rapidly, and resist deacylation. Whereas acylation of the active site of the class C β-lactamase from *Enterobacter cloacae* was not seen, these compounds function as competitive inhibitors of this enzyme. © 2000 Elsevier Science Ltd. All rights reserved.

The emergence of pathogenic microorganisms resistant to multiple classes of antibiotics is a serious clinical challenge.[1] Among these classes of antibacterials, β-lactam antibiotics are still the most commonly used more than 50 years after their initial introduction. The success of these antibiotics is due to their bactericidal activity, good pharmacokinetics, low host toxicity and their ability to synergize with other classes of antibiotics such as aminoglycosides. In light of the fact that there are no replacements for these versatile antibiotics in the immediate future, it is essential that their clinical utility be prolonged.

The most common mechanism for resistance to β-lactam antibiotics is the ability of bacteria to produce β-lactamases.[2,3] These enzymes hydrolyze the β-lactam moiety in these drugs, a reaction that inactivates the antibiotics. There are four distinct classes of β-lactamases, of which class A enzymes are the most common.[3] A successful approach to overcoming the adverse action of these enzymes has been the use of β-lactamase inhibitors together with the typical β-lactam antibiotics, such as penicillins.[3,4] Unfortunately, this approach has been compromised as well by the discovery of the new variants of β-lactamases resistant to inhibition by known inhibitors.[5] These new variants of β-lactamases still remain highly effective in hydrolyzing β-lactam antibiotics. Therefore, development of novel β-lactam inhibitors that operate by entirely different mechanisms is highly desirable.

We have reported previously on the synthesis and mechanism of action of a novel class of monobactam mechanism-based inhibitors for the class A β-lactamases.[6–9] As exemplified by compound **1**, the inhibitor acylates the active site serine of β-lactamases, a process that is usually rapid (Scheme 1). On acylation of the active site, the tosylate is released from species **2**. From that point on, there appears to be some variations for the final structure of the inhibited enzyme. We have documented the possibility for structures **3**–**5** in inhibited β-lactamases.[6–8] An α- or β-elimination would give rise to a species such as **3**, which resists deacylation because it is an α,β-unsaturated ester.[6] The iminium species such as **4** was seen in conjunction with the movement of the ester carbonyl out of the oxyanion hole in the active site.[7] As such, the ester could not undergo the deacylation step, and the inhibited enzyme enjoyed considerable longevity. We have also seen the keto species **5** in the inhibited NMCA β-lactamase from *Enterobacter cloacae*.[8] In this case as well, a movement of the ester carbonyl out of the oxyanion hole was critical for the inhibition process. The present report expands on these findings and defines further the properties of this class of enzyme inhibitors.

Results and Discussion

Syntheses

Synthesis of the key intermediate **6** for the preparation of compounds **7a**–**f** has been described.[6] We retained the phenyl group at position 4 of the azetidinone ring in many of our compounds since a specific hydrophobic interaction in the active site of class A β-lactamases favored this group. Furthermore, the stereochemistry at position 4 appeared not to be important for inhibition,[6] so we have a mixture of isomers at this position with the new compounds. Removal

Keywords: β-lactamase; inhibition.
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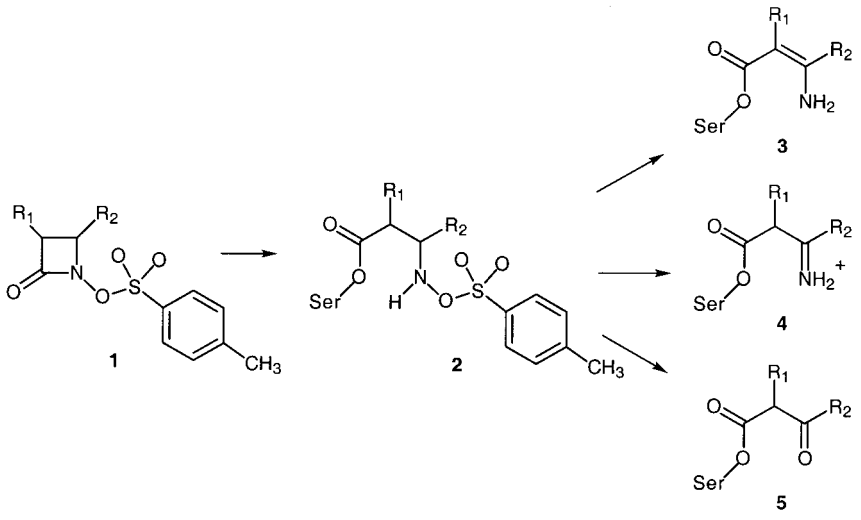
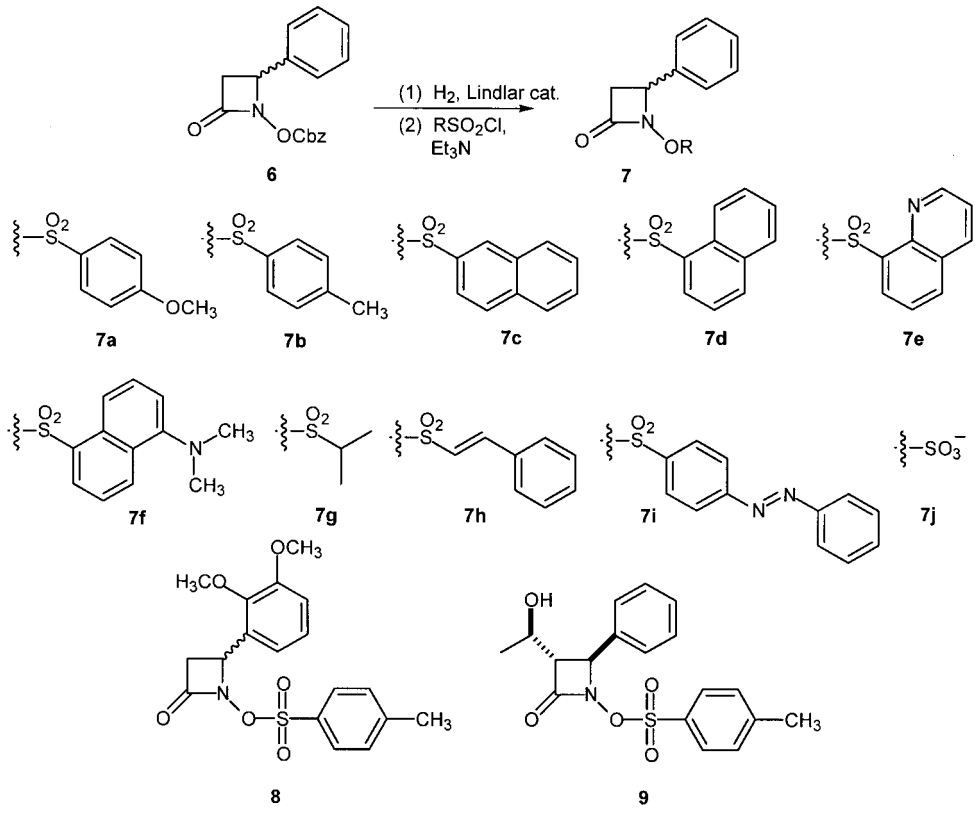
PII: S0040-4020(00)00427-0



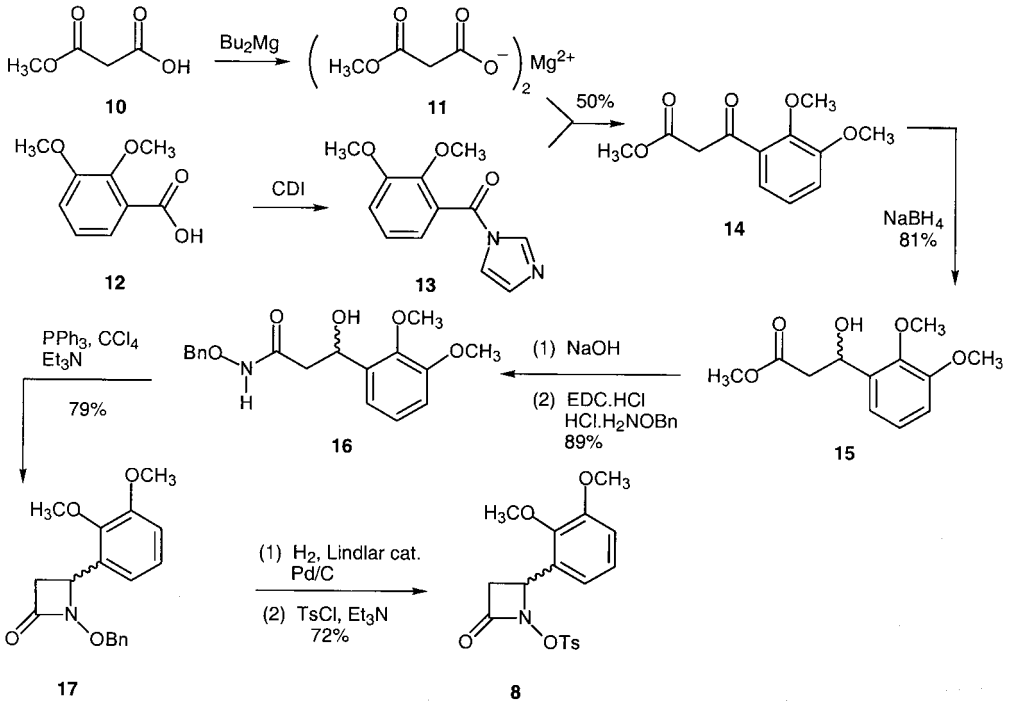
Scheme 1.

of the Cbz group of **6** by hydrogenolysis over Lindlar's catalyst ($Pd–CaCO_3–PbO$) afforded the corresponding *N*-hydroxy β-lactam, which was treated with a series of sulfonyl chlorides in the presence of triethylamine to afford the desired β-lactams **7a–i** in high yields (**6→7**) Scheme 2. The reaction of the *N*-hydroxy β-lactam, the product of hydrogenolysis with pyridine SO_3 afforded the corresponding *O*-sulfonated β-lactam **7j**, which was isolated as the tetra-*n*-butyl ammonium salt.

The structure-function analysis of the first generation of these molecules, in conjunction with molecular modeling with the X-ray structures of β-lactamases, suggested to us that certain functionalities could be introduced into the basic design to improve the interactions between the inhibitors and the target enzyme(s). β-Lactam **8**, which contains a 2,3-dimethoxyphenyl group at the C-4 position, was one such molecule. The methoxy groups of **8** were expected to have favorable electrostatic interactions with the enzyme, for example. Compound **9** is another second-generation compound. The C-3α group of **9** was introduced into the structure of the inhibitor to retard the deacylation of the



Scheme 2.



Scheme 3.

inhibited species. This designed concept finds precedent in earlier work by us.[10–14] The synthesis and properties of **9** have already been reported.[7]

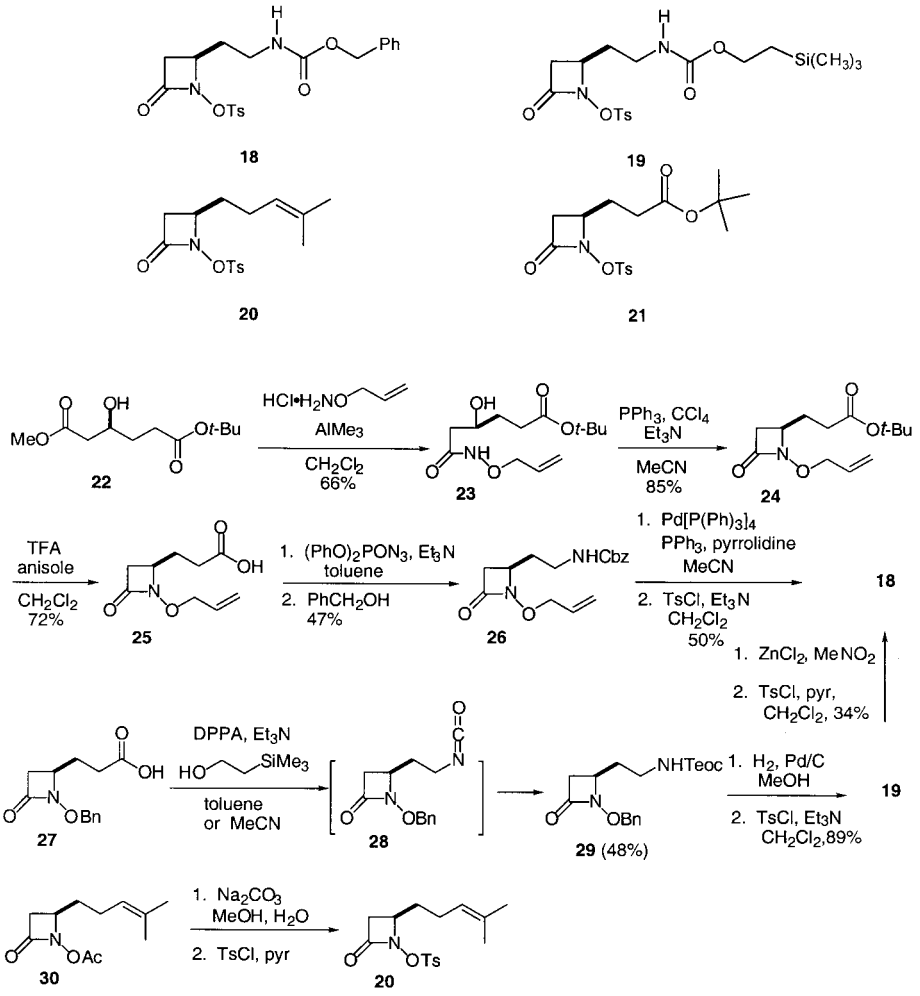
Compound **8** was synthesized according to Scheme 3. β-Ketoester **14** was synthesized from 2,3-dimethoxybenzoic acid (**12**) and monomethylmalonate (**11**)[15] using the Masamune–Brooks reaction.[16] The ketone moiety of **14** was reduced with sodium borohydride to afford **15**. Saponification of the methyl ester **15** with 1.0 M sodium hydroxide and coupling of the resulting acid with *O*-benzylhydroxylamine using EDC·HCl afforded hydroxamate **16** in 89% yield, which was cyclized to β-lactam **17** in the presence of carbon tetrachloride, triphenylphosphine, and triethylamine in 79% yield. Removal of the benzyl group of **17**, followed by tosylation of the *N*-hydroxy β-lactam afforded *N*-tosyloxy β-lactam **8** in 72% yield.

To explore the nature of the interactions of the enzyme(s) with the C-4 substituent, we synthesized four additional compounds. The syntheses of three of these compounds (**18**–**20**) are given in Scheme 4, and that of compound **16** has been reported earlier.[17]

Kinetic determinations of enzyme inhibition

The results of our inhibition studies are summarized in Table 1. The effective inhibitors modify the active site of the class A TEM-1 β-lactamase essentially instantaneously on mixing. The rate of this process could not be attenuated effectively by competition with substrates to permit evaluation of individual parameters for enzyme inhibition, such as reported by us previously for another type of inhibitor.[18] However, we have used three parameters in evaluation of these compounds with the TEM-1 β-lactamase. The second-order rate constant for inactivation (k_{inact}/K_I) is useful in comparisons of these inactivators to one another; the larger the number, the better the process. The partition ratio (k_{cat}/k_{inact}) can be evaluated directly, and is an expression of the efficiency of the inactivation process. That is, how readily the compound experiences turnover versus the rate of enzyme inactivation: the lower this number, the more favorable the inactivation process. Once the active site is modified, one can evaluate the rate constant for recovery of activity (k_{H_2O}), as a measure of the longevity of the inactive enzyme species; the smaller this rate constant, the better the inactivator.

The trends that emerged from the kinetic analyses indicated that there is a strong preference for a hydrophobic moiety at the sulfonate position for inactivation of the class A TEM-1 β-lactamase. Compound **7j** failed to inactivate the enzyme, whereas compound **7c** inactivated the enzyme most favorably, as discerned from the values for k_{inact}/K_I. It is noteworthy that the rate constants for inactivation of the enzyme for all compounds that did inactivate the enzyme are high. The TEM-1 β-lactamase also shows preference for the phenyl ring at position 4. The change of phenyl to methyl at this position reduced the efficiency of the inactivation process (lower k_{inact}/K_I, and higher partition ratio).[6] Compounds **18**–**21**, which lack the phenyl group at position 4, did not inactivate the enzyme. They either served as poor substrates or poor competitive inhibitors for the class A β-lactamase. Compounds **19**, **20** and **21** were found to be competitive inhibitors for the TEM-1 β-lactamase with dissociation constants (K_I) of 5, 1 and 200 μM, respectively. Compound **18** was neither a substrate nor an inhibitor for the TEM-1 β-lactamase, but it inhibited the Q908R enzyme. The K_I values for compounds **18** and **20** with the Q908R enzyme were 45 and 27 μM, respectively. Compounds **19** and **21** did not inhibit the class C Q908R enzyme. All inactivators showed desirable partition ratios of less than 10.



Scheme 4.

Table 1. Kinetic parameters for inhibition of β-lactamases by monocyclic *N*-oxy-substituted β-lactams

Compounds	Enzymes			
	TEM-1			Q908R
	k_{inact}/K_I (M^{-1} s^{-1})×10^{-3}	k_{cat}/k_{inact}	k_{H_2O} (s^{-1})×10^3	K_I (μM)
7a[a]	7.4±0.2	7	2.0±0.3	24±5
7b[a]	11.2±0.8	5	2.1±0.3	12±2
7c[a]	72.5±1.4	2	1.8±0.2	65±15
7d	37.7±1.1	2	3.3±0.8	220±55
7e	22.6±0.9	4	2.7±0.3	260±30
7f	13.6±0.7	4	3.6±0.5	680±110
7g	15.0±0.5	4	2.1±0.4	5±1
7h	15.8±0.5	2	2.7±0.4	37±4
7i	5.8±0.3	6	1.5±0.8	110±20
7j[b]	–	–	–	–
18	–	–	–	45±7
20	–	–	–	27±9
8	32±3	8	9±1	9±2
9[c]	3.2±0.15	7	1.6±0.4[d] 0.013±0.002	520± 40

[a] Preliminary results were reported in Ref. 6.
[b] This compound did neither inactivate nor inhibit the enzymes.
[c] Results were reported in Ref. 7.
[d] Recovery of activity was biphasic for compound **9**; the two numbers refer to the rates of recovery of activity for each phase.

The second-generation inactivator **8** was designed in order to take advantage of potential electrostatic interactions of the enzyme with the methoxy substituents in the ring. These interactions were expected to favor the enzyme modification process, and indeed they did. Compound **8** modified the enzyme with a second-order rate constant that was over three-fold more rapid than that of the parent compound without the methoxy moieties (i.e., **7b**), but regrettably, the rate of deacylation of this inhibitor from the active site was faster by the same extent. As reported earlier, compound **9** inactivated the TEM-1 β-lactamase somewhat slower than others, reflecting an attenuation of the rate of enzyme acylation due to the presence of the C-3α hydroxyethyl group.[7] This compound was designed specifically to undergo deacylation more slowly than the rest of these inactivators, thereby imparting enhanced longevity of the inhibited enzyme species. We indeed observed considerably slower deacylation in this case, which gave a longevity of the inhibited species of a number of days.[7]

It is important to note that the sulfonate moiety serves as a surrogate for the invariant carboxylate of β-lactams in recognition by the enzyme.[6] Compounds that lack it, as in *N*-alkoxy or *N*-carbonate derivatives of our β-lactams, are recognized by the enzyme with much poorer affinity or not at all (data not shown). None of these compounds appeared to acylate the active-site serine of the class C β-lactamase (Q908R). However, all of these compounds, except **19** and **21**, inhibited the class C enzyme competitively.

Conclusions

These monobactam inhibitors modify the active site serine of class A β-lactamases rapidly. On acylation of the active site, the acyl-enzyme species undergoes fragmentation, resulting in enzyme inhibition by three distinct products, depending on the nature of the funtionalities that have been incorporated into the inhibitor (species **3**, **4**, or **5**). The results reported herein for the structure-function analyses of the sulfonate moiety argue for the requirement of a hydrophobic functionality, but its size does not appear to be limiting. The absence of any hydrophobic functionality at this position (as in **7j**) impairs the ability of the molecules to inhibit β-lactamases.

Experimental

General procedures

Melting points (mp) were determined on a Thomas–Hoover capillary melting point apparatus in open capillaries and are uncorrected. Infrared (IR) spectra were obtained on a Perkin–Elmer 1420 IR spectrophotometer and were calibrated with the 1601 cm^{-1} band of polystyrene. Nuclear magnetic resonance (NMR) spectra were obtained on a General Electric GN-300, a Varian Unity Plus 300, or a Varian VXR500S spectrometer. ^1H NMR chemical shifts are reported in parts per million relative to tetramethylsilane (0.00 ppm). ^{13}C NMR spectra were referenced relative to the center peak of $CDCl_3$ (77.00 ppm). Fast-atom bombardment (FAB, xenon, 3-nitrobenzyl alcohol matrix) mass spectra were obtained on a Jeol JMS-AX505HA mass spectrometer. Thin-layer chromatography (TLC) was conducted on silica gel 60 F_{254} (0.2 mm thickness, aluminum support) and the chromatograms were visualized with ultraviolet light and by dipping in 10% phosphomolybdic acid (PMA) in ethanol, followed by heating. Flash column chromatography was performed using silica gel 60 (EM Science, 230–400 mesh ASTM). Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ. Anhydrous acetonitrile and triethylamine were freshly distilled from calcium hydride under an atmosphere of nitrogen and transferred via syringe or cannula. Bulk grade ethyl acetate (EtOAc) and Skellysolve B (referred to simply as 'hexanes') were distilled before use. All purchased reagents were of reagent grade quality and were used without further purification. Penicillin G and cephaloridine were purchased from Sigma. The wild-type class A TEM-1 β-lactamase from *Escherichia coli*[19] and the class C *E. cloacae* Q908R β-lactamase[20–22] were purified according to literature methods. All kinetic and spectral measurements were made on a Hewlett–Packard 8452 or 8453 diode-array spectrometer. The enzyme assay and methods for determination of kinetic parameters were according to published procedures.[6]

General procedure for the syntheses of compounds 7e–7i

A mixture of compound **6** (0.8 mmol) in methanol (8 mL) was treated with either 10% Pd/C (15 mg) (for compounds **7e**, **7f** and **7g**) or Lindlar catalyst (15 mg) (Pd–$CaCO_3$–PbO, Aldrich) under an atmosphere of hydrogen. The suspension was stirred for 30 min and filtered through a thin layer of celite and concentrated. The resulting crude *N*-hydroxy-β-lactam and the appropriate sulfonyl chloride (1.0 equiv., Aldrich) were dissolved in dry acetonitrile and cooled to ice–water temperature. Triethylamine (1.0 equiv.) was added to this mixture dropwise and the reaction mixture was stirred for 20 min. The reaction mixture was concentrated and the residue was subjected to flash column chromatography (25% ethyl acetate in hexanes) to obtain the corresponding product.

(±)-*N*-(8-Quinolinesulfonyloxy)-4-phenyl-2-azetidinone (7e). Three fractions were collected. Fraction 1 (21 mg) contained an unidentified compound. Fraction 2 (191 mg) contained a mixture of unidentified products and **7e**. Fraction 3 (140 mg) contained **7e** as a white solid. Fractions 2 and 3 were recrystallized from ethyl acetate in hexanes to give a total of 89 mg (35%) of **7e** as a white solid: mp>190°C; IR (KBr) 1820 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 9.07 (dd, J=1.8, 4.2 Hz, 1H), 8.51 (dd, J=1.4, 7.4 Hz, 1H), 8.04 (dd, J=1.4, 8.2 Hz, 1H), 7.60 (t, J=7.8 Hz, 1H), 7.5 (dd, J=4.3, 8.3 Hz, 1H), 7.26–7.11 (m, 3H), 7.10–6.96 (m, 2H), 4.86 (dd, J=3.4, 6.3 Hz, 1H), 3.18 (dd, J=6.4, 14.4 Hz, 1H), 2.66 (dd, J=3.4, 14.5 Hz, 1H); HRMS (FAB, MH^+) calcd for $C_{18}H_{14}N_2O_4S$ 355.0753, found 355.0750; Anal. calcd for $C_{18}H_{14}N_2O_4S$: C, 61.01; H, 3.98; N, 7.90. Found: C, 60.79; H, 4.21; N, 7.72.

(±)-*N*-(5-Dimethylamino-1-naphthalenesulfonyloxy)-4-phenyl-2-azetidinone (7f). The resulting white solid after the column chromatography was recrystallized with ethyl acetate in hexanes to yield 96 mg (33%) of **7f** as a

yellow-brown solid; mp>190°C (dec.); IR (KBr) 1810 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.56 (d, J=8.5 Hz, 1H), 8.26 (dd, J=1.2, 6.1 Hz, 1H), 7.48 (tm, J=8.7 Hz, 2H), 7.20–7.11 (m, 4H), 6.97–6.93 (m, 2H), 4.65 (dd, J=3.3, 6.2 Hz, 1H), 3.18 (dd, J=6.2, 14.5 Hz, 1H), 2.87 (s, 6H) 2.68 (dd, J=3.3, 14.5 Hz, 1H); HRMS calcd for $C_{19}H_{20}N_2O_4S$ 396.1222, found 396.1209.

(±)-*N*-(Methylethylsulfonyloxy)-4-phenyl-2-azetidinone (7g). After purification 46 mg (96%) of **7g** was obtained as a light yellow oil: R_f 0.54 (2:3, EtOAc–hexanes); IR (neat) 1795 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.51 (d, J=6.9 Hz, 6H), 2.84 (dd, J=14.3, 2.9 Hz, 1H), 3.35 (dd, J=14.3, 5.9 Hz, 1H), 3.69 (heptet, J=6.9 Hz, 1H), 5.12 (dd, J=5.7, 2.7 Hz, 1H), 7.32–7.46 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 16.33, 16.81, 42.36, 53.76, 62.65, 126.79, 129.01, 129.26, 135.17, 164.88; HRMS (FAB) calcd for $C_{12}H_{16}NO_4S$ (MH^+) 270.0800, found 270.0825.

(±)-*N*-(*trans*-2-Phenylethenesulfonyloxy)-4-phenyl-2-azetidinone (7h). Column chromatography afforded 94 mg (93%) of **7h** as a light yellow oil, which crystallized upon standing: R_f 0.31 (1:4, EtOAc–hexanes); mp 90–92 °C; IR (neat) 1800 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.83 (dd, J=14.4, 3.2 Hz, 1H), 3.332 (dd, J=14.4, 6.1 Hz, 1H), 5.08 (dd, J=6.1, 3.2 Hz, 1H), 6.73 (d, J=15.5 Hz, 1H), 7.28–7.55 (m, 10H), 7.65 (d, J=15.5 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 42.37, 62.13, 119.54, 126.87, 128.78, 128.92, 129.13, 131.49, 132.05, 135.34, 147.92, 165.46; HRMS (FAB) calcd for $C_{17}H_{16}NO_4S$ (MH^+) 330.0800, found 330.0766.

(±)-*N*-((4-Phenylazo)benzenesulfonyloxy)-4-phenyl-2-azetidinone (7i). The orange solid obtained after chromatographic purification was recrystallized from ethyl acetate in hexanes to provide 16.7 mg (13%) of **7i** as an orange solid, mp 120–121°C (dec.). IR (KBr) 1800 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.07–8.03 (m, 1H), 8.0–7.95 (m, 4H), 7.59–7.56 (m, 3H), 7.35–7.31 (m, 3H), 7.28–7.24 (m, 3H), 4.96 (dd, J=3.3, 6.2 Hz, 1H), 3.28 (dd, J=6.2, 14.6 Hz, 1H), 2.81 (dd, J=3.3, 14.6 Hz, 1H); HRMS (FAB, MH^+) calcd for $C_{21}H_{17}N_3O_4S$ 408.1018, found 408.1043. UV (CH_3CN) λ_{max} (*trans*) 321 nm (ϵ 2.4×10^4 L/mol cm); λ_{max} (*cis*) 434 nm (ϵ 1.2×10^3 L/mol·cm).

(±)-2-Oxo-4-phenyl-1-azetidinyl sulfate, tetra-*n*-butyl ammonium salt (7j). A stirred solution of 276 mg (0.928 mmol) of β-lactam **6**[6] in 10 mL of methanol was charged with 266 mg of the Lindlar catalyst (Pd–$CaCO_3$–PbO, Aldrich), and stirred for 45 min under an atmosphere of hydrogen. The catalyst was removed by filtration and the solvent was evaporated to afford a colorless oil. The oil was dissolved in 3.6 mL of pyridine and 443 mg (2.78 mmol) of pyridine SO_3 was added. The solvent was evaporated after 5.5 h of stirring at room temperature, the residue was dissolved in 75 mL of a 0.5 M KH_2PO_4 solution, and the resultant solution was washed with EtOAc to remove any organic-soluble impurities. Tetra-*n*-butylammonium hydrogen sulfate (280 mg, 0.825 mmol) was added to the aqueous solution, which was washed with CH_2Cl_2 and $CHCl_3$. The combined organic layers were dried over anhydrous $MgSO_4$, the suspension was filtered, and the filtrate was evaporated to afford 440 mg (98%) of **7j** as a colorless oil: IR (neat) 1780 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.98 (t, J=7.2 Hz, 12H), 1.40 (sextet, J=7.2 Hz, 8H), 1.53–1.70 (m, 8H), 2.58 (dd, J=13.2, 2.1 Hz, 1H), 3.14–3.22 (m, 9H), 5.39 (dd, J=5.4, 2.4 Hz, 1H), 7.23–7.45 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.62, 19.61, 23.82, 41.96, 58.49, 60.14, 126.67, 128.06, 128.50, 137.71, 162.89; HRMS (FAB) (positive ion) calcd for $C_{16}H_{36}N$ 242.2848, found 242.2841; HRMS (FAB) (negative ion) calcd for $C_9H_8NO_5S$ 242.0123, found 242.0115.

Methyl 3-oxo-3-(2,3-dimethoxyphenyl)propanoate (14). A stirred solution of 720 mg (6.10 mmol) of monomethylmalonate (**10**)[23] in 7 mL of anhydrous THF was charged with 3.0 mL (3.0 mmol) of a 1.0 M solution of dibutylmagnesium in heptane (Aldrich) at −78°C under an atmosphere of argon. A white precipitate formed after 10 min of reaction, at which time the mixture was allowed to warm to room temperature, followed by an additional 1.5 h of stirring. At this point, the solvent was evaporated to afford a white solid.

A 980 mg portion of carbonyldiimidazole (6.04 mmol) was added to a stirred solution of 1.0 g (5.5 mmol) of 2,3-dimethoxybenzoic acid **12** (Aldrich) in 7 mL of anhydrous THF at ice-water temperature under an atmosphere of argon. The reaction was allowed to warm to room temperature, and was aged for 2 h. The acyl imidazolide (**13**) solution was transferred via a cannula over to the magnesium salt and the mixture was stirred for 40 h at ambient temperature under an atmosphere of argon. Stirring became difficult due to the precipitation of salts. The reaction was diluted by addition of portions of ether and 1.0 M HCl, and the layers were separated. The aqueous layer was extracted with ether (3×), and the combined ether portions were washed with saturated $NaHCO_3$, brine, dried ($MgSO_4$), the suspension was filtered and the solvent was evaporated to afford a light yellow oil. The oil was purified on a Chromatotron device (2 mm silica gel plate), eluting with 20% EtOAc in hexanes to afford 655 mg (50%) of **14** as a colorless oil: R_f 0.60 (2:3, EtOAc–hexanes); IR (neat) 1745, 1675 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.75 (s, 3H), 3.90 (s, 3H), 3.92 (s, 3H), 4.01 (s, 2H), 6.99–7.15 (m, 2H), 7.30–7.40 (m, 1H) (a small amount of the enol tautomer was also observed); ^{13}C NMR (75 MHz, $CDCl_3$) δ 49.59, 52.12, 56.01, 61.22, 116.80, 121.49, 123.94, 131.48, 149.09, 152.83, 168.24, 193.79 (a small amount of the enol tautomer was also observed); HRMS (FAB) calcd for $C_{12}H_{15}O_6$ (MH^+) 239.0919, found 239.0954.

Methyl (±)-3-hydroxy-3-(2,3-dimethoxyphenyl)propanoate (15). Sodium borohydride (384 mg, 10.2 mmol) was added to a stirred solution of 4.84 g (20.3 mmol) of β-keto ester **14** in 140 mL of methanol at ice-water temperature under an atmosphere of nitrogen. Brine (100 mL) was added after 1 h and the reaction was stirred at room temperature for 10 min. The reaction was diluted by addition of portions of EtOAc and water, and the layers were separated. The aqueous layer was extracted with EtOAc (3×) and the combined organic layers were washed with brine, dried over $MgSO_4$, the suspension was filtered, and the solvent was evaporated to afford a colorless oil. The oil was chromatographed on silica gel eluting with 35% EtOAc in hexanes to

afford 3.94 g (81%) of **14** as a colorless oil: R_f 0.47 (2:3, EtOAc–hexanes); IR (neat) 3500, 1735 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.60–2.84 (m, 2H), 3.43 (br s, 1H), 3.70 (s, 3H), 3.85 (s, 3H), 3.86 (s, 3H), 5.38 (dd, J=9.0, 4.0 Hz, 1H), 6.80–6.90 (m, 1H), 7.01–7.10 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 42.06, 51.58, 55.54, 60.57, 65.74, 111.63, 118.11, 124.01, 135.89, 145.50, 152.18, 172.70; HRMS (FAB) calcd for $C_{12}H_{16}O_5$ ($M^{+\cdot}$) 240.0998, found 240.1007.

(±)-*O*-Benzyl-3-hydroxy-3-(2,3-dimethoxyphenyl)propanohydroxamate (16). A stirred solution of 1.56 g (6.49 mmol) of β-hydroxy ester **15** in 6 mL of THF was mixed with 7.8 mL (7.8 mmol) of a 1.0 M NaOH solution. The reaction mixture was diluted with ether after 30 min of stirring at room temperature and the layers were separated. The aqueous layer was acidified to pH 5 using 3.0 M HCl. *O*-benzylhydroxylamine hydrochloride (1.35 g, 8.46 mmol) was added and the pH was adjusted to 4.5 by the addition of saturated $NaHCO_3$ solution. EDC·HCl (1.87 g, 9.75 mmol) was added to the mixture in three portions over a period of 1 h while maintaining the pH of the reaction between 4 and 5. The reaction was stirred for an additional 30 min and was extracted with EtOAc, washed with 1.0 M HCl, saturated $NaHCO_3$, brine, dried (Na_2SO_4), filtered and the solvent evaporated to afford a light yellow oil. The oil was chromatographed on silica gel eluting with 60% EtOAc in hexanes to afford 1.28 g (60%) of **16** as a colorless oil, which solidified upon standing. An analytical sample was obtained by recrystallization from EtOAc–hexanes: mp 88–90°C; R_f 0.24 (1:1, EtOAc–hexanes); IR (KBr) 3475, 3365, 1680 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.34–2.64 (m, 2H), 3.81 (s, 3H), 3.83 (s, 3H), 4.10 (d, J=4.5 Hz, 1H), 4.81 (d, J_{AB}=11.7 Hz, 1H), 4.86 (d, J_{AB}=11.7 Hz, 1H), 5.21–5.34 (m, 1H), 6.80–6.90 (m, 1H), 6.97–7.12(m, 2H), 7.33 (br s, 5H), 8.85 (br s, 1H) (a small amount of the hydroximate was also observed); ^{13}C NMR (75 MHz, $CDCl_3$) δ 40.47, 55.64, 60.70, 66.18, 78.14, 111.70, 118.21, 124.26, 128.46, 128.64, 129.17, 135.13, 136.02, 145.37, 152.23, 169.58 (a small amount of the hydroximate was also observed); HRMS (FAB) calcd for $C_{18}H_{22}NO_5$ (MH^+) 332.1498, found 332.1483. Anal. calcd for $C_{18}H_{21}NO_5$: C, 65.24; H, 6.39; N, 4.23. Found: C, 65.33; H, 6.44; N, 4.21.

(±)-*N*-Benzyloxy-4-(2,3-dimethoxyphenyl)-2-azetidinone (17). A stirred solution of 566 mg (1.71 mmol) of hydroxamate **16** in 15 mL of anhydrous acetonitrile was charged with 500 μL (5.18 mmol) of carbon tetrachloride, 360 μL (2.58 mmol) of triethylamine, and 672 mg (2.56 mmol) of triphenylphosphine at room temperature under an atmosphere of nitrogen. The solvent was evaporated after 12 h to afford a dark brown solid. The solid was chromatographed on silica gel eluting with 30% EtOAc in hexanes to afford 425 mg (79%) of **17** as a light yellow oil: R_f 0.33 (30% EtOAc in hexanes); IR (neat) 1775 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.59 (dd, J=13.5, 2.4 Hz, 1H), 3.06 (dd, J=13.7, 5.6 Hz, 1H), 3.77 (s, 3H), 3.86 (s, 3H), 4.87 (dd, J=5.7, 2.4 Hz, 1H), 4.90 (d, J_{AB}=11.4, 1H), 4.98 (d, J_{AB}=11.4 Hz, 1H), 6.85–6.93 (m, 2H), 7.02–7.10 (m, 1H), 7.30–7.36 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 41.27, 55.27, 55.69, 60.82, 77.77, 112.33, 118.50, 124.31, 128.44, 128.70, 128.92, 130.61, 135.13, 147.20, 152.53, 164.64; HRMS (FAB) calcd for $C_{18}H_{20}NO_4$ (MH^+) 314.1392, found 314.1409.

(±)-*N*-*p*-Toluenesulfonyloxy-4-(2,3-dimethoxyphenyl)-2-azetidinone (8). Lindlar catalyst (Pd–PbO–$CaCO_3$, 60 mg) and 10% Pd/C (5 mg) were added to a stirred solution of 60 mg (0.19 mmol) of β-lactam **17** in 5 mL of absolute methanol at room temperature under an atmosphere of hydrogen, and the mixture was stirred for 1 h. The catalyst was removed by filtration and the solvent was evaporated in vacuo to afford a colorless oil. The oil was dissolved in 2 mL of anhydrous dichloromethane and 40 mg (0.21 mmol) of *p*-toluenesulfonyl chloride and 20 μL (0.25 mmol) of anhydrous pyridine were added at room temperature under an atmosphere of nitrogen. The solvent was evaporated after 45 min of stirring and the residue was dissolved in EtOAc/1.0 M HCl. The layers were separated and the organic portion was washed with saturated $NaHCO_3$, brine, dried (Na_2SO_4), filtered and the solvent was evaporated to afford a light brown oil. The oil was radially chromatographed (1 mm silica gel plate) eluting with 30% EtOAc in hexanes to afford 52 mg (72%) of **8** as a colorless oil, which solidified upon standing: mp 104–105°C (dec.) (EtOAc–hexanes); R_f 0.43 (2:3, EtOAc–hexanes); IR (KBr) 1790 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.46 (s, 3H), 2.81 (dd, J=14.1, 3.0 Hz, 1H), 3.18 (dd, J=14.1, 6.2 Hz, 1H), 3.85 (s, 3H), 3.87 (s, 3H), 5.22 (dd, J=6.0, 3.0 Hz, 1H), 6.82 (dd, J=7.8, 1.5 Hz, 1H), 6.92 (dd, J=8.4, 1.5 Hz, 1H), 7.05 (t, J=8.1 Hz, 1H), 7.34 (d, J=7.8 Hz, 2H), 7.84 (d, J=8.4 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.80, 41.54, 55.77, 58.18, 60.90, 113.05, 119.76, 124.19, 128.29, 129.06, 129.93, 130.93, 146.24, 147.63, 152.60, 165.39; HRMS (FAB) calcd for $C_{18}H_{19}NO_6S$ ($M^{+\cdot}$) 377.0933, found 377.0935.

***t*-Butyl 4(*S*)-hydroxy-6-oxo-6-(propene-3-oxyamino)hexanoate (23).** To a suspension of 850 mg (7.8 mmol) of *O*-allylhydroxylamine hydrochloride in 17 mL of anhydrous CH_2Cl_2 at 0°C under an atmosphere of nitrogen was added 3.9 mL (7.8 mmol, 2 M solution in hexanes, Aldrich) of trimethylaluminum over 10 min. The solution was warmed to room temperature for 1 h and then cooled to 0°C. Methyl ester **22** (1.0 g, 4.3 mmol) in 4 mL of anhydrous CH_2Cl_2 was added dropwise to the reaction mixture. After stirring for 18 h at room temperature, the solution was cooled to 0°C, and the reaction was quenched by the addition of 25 mL of 10% citric acid and stirred for 1 h. The aqueous layer was extracted with CH_2Cl_2, washed with 10% citric acid, saturated $NaHCO_3$, brine, dried over $MgSO_4$, and the suspension was filtered. The filtrate was evaporated to dryness and the residue was recrystallized from EtOAc–hexanes to give 780 mg (66%) of **23** as a white solid: mp 74–76°C; IR (KBr) 1730, 1660, 1170, 940 cm−1; ^1H NMR (300 MHz, $CDCl_3$) δ 1.45 (s, 9H), 1.60–1.86 (m, 2H), 2.16–2.48 (m, 2H), 2.41 (t, J=7.0 Hz, 2H), 3.86 (d, J=3.3 Hz, 1H), 3.92–4.13 (m, 1H), 4.40 (d, J=6.2 Hz, 2H), 5.26–5.47 (m, 2H), 5.84–6.18 (m, 1H), 8.84 (br s, 1H); ^{13}C NMR (75 MHz, CDCl3) (27.97, 31.72, 40.21, 67.75, 77.19, 80.65, 120.37, 132.07, 169.71, 173.44; $[\alpha]_D^{20}$=+20.8° (c=1, $CDCl_3$); HRMS (EI) calcd for $C_9H_{15}NO_5$ ($M^+-C_4H_8$) 217.0950, found 217.0940. Anal. calcd for $C_{13}H_{23}NO_5$: C, 57.13; H, 8.48; N, 5.12. Found: C, 57.13; H, 8.52; N, 5.02.

(4*R*)-1-(2-Propenoxy)-4-(2-(1,1-dimethylethoxycarbonyl)-ethyl)-2-azetidinone (24). To a stirred solution of 2.80 g (10.2 mmol) of hydroxamate **23** in 56 mL of anhydrous acetonitrile at room temperature under an atmosphere of nitrogen was added 5.0 mL (52 mmol) of carbon tetrachloride, 1.9 mL (14 mmol) of triethylamine, and 3.22 g (12.3 mmol) of triphenylphosphine. After stirring at room temperature for 36 h the solvent was evaporated to afford a brown solid. The solid was chromatographed on silica gel eluting with 30% EtOAc in hexanes to afford 2.2 g (85%) of **24** as a yellow oil: R_f 0.43 (2:3, EtOAc–hexanes); IR (neat) 1775, 1725 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.46 (s, 9H), 1.82–1.97 (m, 1H), 2.05–2.19 (m, 1H), 2.36 (dd, J=13.7, 2.5 Hz, 1H), 2.35 (t, J=7.7 Hz, 2H), 2.79 (dd, J=13.7, 5.2 Hz, 1H) 3.85–3.96 (m, 1H), 4.36–4.50 (m, 2H), 5.30–5.45 (m, 2H), 5.93–6.10 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.44, 27.55, 30.87, 37.70, 56.12, 76.43, 80.05, 120.08, 131.93, 163.40, 171.15; $[\alpha]_D^{20}$=−15.0° (c=1, $CDCl_3$); HRMS (FAB) calcd for $C_9H_{14}NO_4$ ($MH^+-C_4H_8$) 200.0923, found 200.0914.

(4*R*)-1-(2-Propenoxy)-4-(3-propanoic acid)-2-azetidinone (25). To a stirred solution of 4.30 g (16.8 mmol) of **24** in 4 mL of anhydrous CH_2Cl_2 at 0°C under an atmosphere of nitrogen was added 1.8 mL of anisole and 12 mL of TFA. After stirring for 2 h at 0°C, toluene (10 mL) was added and the solvents were evaporated. The residue was dissolved in EtOAc and extracted with a saturated $NaHCO_3$ solution. The combined aqueous layers were acidified to pH 3.0 using 3 M HCl, and the mixture was extracted with CH_2Cl_2 (3×). The combined organic extracts were dried (Na_2SO_4), filtered and the solvent was evaporated to afford 2.4 g (72%) of **25** as a yellow oil: R_f 0.24 (1:1, EtOAc–hexanes); IR (neat) 3090, 1750, 1735 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.88–2.04 (m, 1H), 2.10–2.23 (m, 1H), 2.39 (dd, J=13.8, 2.4 Hz, 1H), 2.51 (t, J=7.4 Hz, 2H), 2.83 (dd, J=13.8, 5.2 Hz, 1H), 3.88–3.98 (m, 1H), 4.35–4.50 (m, 2H), 5.27–5.44 (m, 2H), 5.92–6.17 (m, 1H), 8.57 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.60, 29.87, 37.51, 56.54, 77.22, 121.11, 132.13, 164.03, 177.45; $[\alpha]_D^{20}$=−20.0° (c=1, $CDCl_3$); MS (EI) m/z 200 (MH^+).

(4*R*)-1-(2-Propenoxy)-4-(2-benzyloxycarbonylaminoethyl)-2-azetidinone (26). To a stirred solution of 228 mg (1.14 mmol) of **25** in 4 mL of toluene under an atmosphere of nitrogen was added 270 μL (1.25 mmol) of diphenylphosphoryl azide and 180 μL (1.29 mmol) of triethylamine. After stirring at 80°C for 20 min, 240 μL (2.32 mmol) of benzyl alcohol was added. After stirring for 19 h at 80°C, the reaction was cooled and the solvent was evaporated to afford a brown oil. The oil was chromatographed on silica gel eluting with 55% EtOAc in hexanes to afford 162 mg (47%) of **26** as a yellow oil: R_f 0.30 (3:2, EtOAc–hexanes); IR (neat) 3320, 1765, 1715, 1530, 1250 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.74–1.90 (m, 1H), 1.96–2.20 (m, 1H), 2.37 (dd, J=13.7, 2.0 Hz, 1H), 2.81 (dd, J=13.7, 5.1 Hz, 1H), 3.33 (q, J=6.6 Hz, 2H), 3.81–3.93 (m, 1H), 4.35–4.50 (m, 2H), 4.99 (br s, 1H), 5.10 (s, 2H), 5.25–5.44 (m, 2H), 5.90–6.07 (m, 1H), 7.30–7.40 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 33.28, 37.66, 37.80, 55.54, 66.59, 77.00, 120.91, 127.95, 128.05, 128.39, 131.95, 136.26, 156.29, 163.92; $[\alpha]_D^{20}$=−9.2° (c=1, $CDCl_3$); HRMS (EI) calcd for $C_{16}H_{20}N_2O_4$ (M^+) 304.1423, found 304.1430.

(4*R*)-1-*p*-Toluenesulfonyloxy-4-(2-benzyloxycarbonylaminoethyl)-2-azetidinone (18). To a stirred solution of 618 mg (2.03 mmol) of β-lactam **26** in 10 mL of anhydrous acetonitrile at room temperature under an atmosphere of nitrogen was added 30 mg (0.11 mmol) of triphenylphosphine, 190 μL (2.28 mmol) of pyrrolidine, and 89 mg (0.077 mmol) of tetrakis(triphenylphosphine)palladium (0). The reaction was shielded from light. After stirring for 3 h at room temperature the solvent was evaporated to afford a yellow oil. The oil was dissolved in EtOAc and was extracted with 5% Na_2CO_3. The combined basic extracts were acidified to pH 5.0 using 3 M HCl, and then was extracted with CH_2Cl_2 (3×). The combined organic layer was washed with brine, dried (Na_2SO_4), filtered and the solvent was evaporated to afford 417 mg of a yellow oil. To a stirred solution of the oil in 10 mL of anhydrous CH_2Cl_2 at room temperature under an atmosphere of nitrogen was added 320 μL (2.30 mmol) of triethylamine, and 470 mg (2.47 mmol) of *p*-toluenesulfonyl chloride. After stirring at room temperature for 1.5 h, the solvent was evaporated to yield a yellow solid. The solid was chromatographed on silica gel eluting with 40% EtOAc in hexanes to afford 428 mg (50%) of **18** as a colorless oil: R_f 0.57 (3:2, EtOAc–hexanes); IR (neat) 1795, 1710 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.85–2.00 (m, 1H), 2.06–2.20 (m, 1H), 2.46 (s, 3H), 2.50 (dd, J=14.9, 2.9 Hz, 1H), 2.89 (dd, J=14.4, 6.0 Hz, 1H), 3.21–3.45 (m, 2H), 4.02–4.18 (m, 1H), 4.98 (br t, 1H), 5.09 (d, J_{AB}=12.4 Hz, 1H), 5.13 (d, J_{AB}=12.4 Hz, 1H), 7.36 (d, J=7.8 Hz, 2H), 7.36 (s, 5H), 7.87 (d, J=8.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.78, 32.64, 37.49, 38.12, 57.80, 66.75, 128.05, 128.15, 128.50, 129.10, 129.98, 130.26, 136.30, 146.52, 156.39, 165.08; HRMS (FAB) calcd for $C_{20}H_{22}N_2O_6S$ (MH^+) 419.1277, found 419.1281.

1-Benzyloxy-4-[2-[2-(trimethylsilyl)ethoxycarbonylamino]-ethyl]-2-azetidinone (29). To a stirred solution of 401 mg (1.61 mmol) of carboxylic acid **27**[24] in 5 mL of anhydrous MeCN was added 240 μL (1.72 mmol) of triethylamine followed by 380 μL (1.76 mmol) of diphenylphosphoryl azide (DPPA). The mixture was stirred under an atmosphere of nitrogen at 90–95°C for 1 h. 2-(Trimethylsilyl)ethanol (460 μL, 3.22 mmol) was added and the solution was stirred at 90–95°C for 19 h. The mixture was allowed to cool, and the solvent was evaporated to afford a brown oil. The oil was chromatographed eluting with 40% EtOAc in hexanes to afford 282 mg (48%) of carbamate **29** and 16 mg (4%) of isocyanate **28**, both as colorless oils. Compound **29**: IR (neat) 1770, 1710 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.03 (s, 9H), 0.94 (t, J=4.5 Hz, 2H), 1.55–1.69 (m, 1H), 1.70–1.84 (m, 1H), 2.32 (dd, J=13.7, 2.4 Hz, 1H), 2.76 (dd, J=13.7, 5.2 Hz, 1H), 3.05–3.21 (m, 2H), 3.45–3.56 (m, 1H), 4.12 (t, J=8.2 Hz), 4.94 (d, J_{AB}=11.2 Hz, 1H), 4.98 (d, J_{AB}=11.2 Hz, 1H), 7.35–7.46 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ −1.75, 17.45, 32.92, 37.33, 37.60, 55.69, 62.61, 77.88, 128.33, 128.73, 129.06, 134.84, 156.49, 163.66; MS (FAB) m/z 377 ($MH^+-C_2H_4$).

(±)-1-*p*-Toluenesulfonyloxy-4-[2-[2-(trimethylsilyl)ethoxycarbonylamino]ethyl]-2-azetidinone (19). To a stirred solution of 258 mg (0.708 mmol) of β-lactam **29** in 10 mL

of methanol under an atmosphere of nitrogen was added 71 mg of 10% Pd/C. A hydrogen atmosphere was applied to the flask. After stirring under hydrogen for 1.5 h, the catalyst was filtered and the solvent was evaporated to afford the crude *N*-hydroxy β-lactam as a colorless oil, which was used without further purification. To a stirred solution of the *N*-hydroxy β-lactam in 8 mL of anhydrous CH_2Cl_2 was added 99 μL (707 μmol) of triethylamine and 135 mg (707 μmol) of TsCl. After stirring at room temperature under an atmosphere of nitrogen for 45 min, the solvent was evaporated. The residue was chromatographed eluting with 25% EtOAc in hexanes to afford 269 mg (89%) of **19**, as a colorless oil which crystallized upon cooling: mp 76–78°C (EtOAc–hexanes); IR (KBr) 1775, 1710 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.04 (s, 9H), 0.98 (t, J=8.5 Hz, 2H), 1.82–1.98 (m, 1H), 2.05–2.20 (m, 1H), 2.47 (s, 3H), 2.48 (dd, J=11.2, 3.3 Hz, 1H), 2.92 (dd, J=14.4, 6.0 Hz, 1H), 3.19–3.45 (m, 2H), 4.10–4.25 (m, 1H), 4.14 (t, J=8.6 Hz, 2H), 4.85 (br s, 1H), 7.38 (d, J=8.3 Hz, 2H), 7.88 (d, J=8.4 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ −1.60, 17.63, 21.72, 32.72, 37.25, 38.14, 57.80, 63.00, 129.05, 129.92, 130.23, 146.44, 156.73, 165.12; HRMS (FAB) calcd for $C_{16}H_{25}N_2O_6SSi$ ($MH^+ - C_2H_4$) 401.1203, found 401.2040.

(±)-1-*p*-Toluenesulfonoxy-4-(4-methyl-3-pentenyl)-2-azetidinone (20). To a stirred solution of 85 mg (0.40 mmol) of *N*-acetoxy-β-lactam **30**[25] in 3 mL of 2:1 MeOH–H_2O was added 107 mg (1.0 mmol) of Na_2CO_3. After stirring for 4 h at 25°C, the apparent pH of the solution was adjusted to ~5 with 1 M HCl and then the solution was extracted three times with CH_2Cl_2. The extracts were combined, dried, and concentrated to afford the crude *N*-hydroxy β-lactam as a dark yellow oil. This crude product was used directly in the next reaction.

To a stirred solution of the crude *N*-hydroxy β-lactam in 2.0 mL of anhydrous CH_2Cl_2 was added 85 mg (0.45 mmol) of TsCl and 66 μL (0.82 mmol) of pyridine. After stirring for 1 h at 25°C, the mixture was concentrated on a rotary evaporator and then chromatographed eluting with 1:4 EtOAc–hexanes to afford 83 mg (64%, 2 steps) of *N*-tosyloxy β-lactam **20** as a colorless oil: R_f 0.45 (3:7, EtOAc–hexanes); IR (TL) 1800 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.50–1.63 (m, 1H), 1.60 (br s, 3H), 1.69 (br s, 3H), 1.94–2.11 (m, 3H, CH_2CH═ and one of CH_2CH$_2$CH═), 2.45 (dd, J=14.3 and 3.2 Hz, 1H), 2.47 (s, 3H), 2.84 (dd, J=14.3, 5.9 Hz, 1H), 3.94–4.03 (m, 1H), 5.06 (tm, J_t=7.2 Hz, 1H), 7.38 (app. d, J=8.0 Hz, 2, ArH), 7.88 (app. d, J=8.4 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 17.61, 21.75, 23.77, 25.60, 31.90, 38.06, 59.56, 122.39, 129.08, 129.89, 130.65, 132.94, 146.27, 165.36; HRMS (FAB) calcd for $C_{16}H_{22}NO_4S$ (MH^+) 324.1270, found 324.1274.

Acknowledgements

The Notre Dame group gratefully acknowledges support from the NIH (GM25845) and Eli Lilly & Company, as well as the use of the NMR facilities of the Lizzadro Magnetic Resonance Research Center at Notre Dame. The work at Wayne State University was supported by the NIH.

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20. The *E. cloacae* P99 and Q908R β-lactamases differ only in four amino acids, positions of which are far from the active site. Therefore, it is believed that these variations would not affect the activity nor the structure of the Q908R enzyme, compared

to P99. Moreover, the kinetic profiles of both enzymes are very similar. The Q908R enzyme has not been crystallized to date, hence we have used the structure of the P99 enzyme in our modeling.

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