Structure-Based Design of β-Lactamase Inhibitors. 2. Synthesis and Evaluation of Bridged Sulfactams and Oxamazins

Christian Hubschwerlen,* Peter Angehrn, Klaus Gubernator,[†] Malcolm G. P. Page, and Jean-Luc Specklin

Preclinical Research, F. Hoffmann-La Roche Ltd., CH-4070 Basle, Switzerland

A series of bridged monocyclic β-lactams activated by various groups on the β-lactam nitrogen (X = OCH_2CO_2H, OSO_3H) has been synthesized and evaluated. Among them, the bridged sulfactams (X = OSO_3H) were found to be effective β-lactamase inhibitors. They inhibit both class A and class C β-lactamases.

Introduction

The development of β-lactamase-mediated resistance to β-lactam antibiotics is becoming a major threat to these antibiotics. Bridged monobactams **1** have been found to be potent inhibitors of class C β-lactamases and to display useful synergy with cephalosporins.[1,2] The nature of the substitution on the pyrrolidine ring was found to play an important role both in the efficacy of the compounds as β-lactamase inhibitors and in their ability to protect the cephalosporin in the presence of intact bacteria.[1] It was also of interest to study other activating groups attached to the β-lactam nitrogen, which are known to confer antibacterial activity in other classes of β-lactam antibiotics, and show their effect on the efficacy of the bridged compounds. In this paper we describe the synthesis and the evaluation of bridged sulfactams **2** and oxamazins **3**.
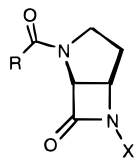
1 X = SO_3H
2 X = OSO_3H
3 X = OCH_2CO_2H

Chemistry

The key intermediate **16** was synthesized as described in Scheme 1. The alcohol **6** was obtained from the acetylenic derivative **5**[3] by condensation of its anion with formaldehyde. Selective reduction into the allylic alcohol **7** was achieved with LAH in the presence of sodium methoxide. Sharpless epoxidation[4] followed by oxidation with ruthenium oxide led to the formation of the acid **9**. Opening of the epoxide with ammonia and in situ protection with di-*tert*-butyl dicarbonate gave the amino acid **10** which was reacted with *O*-benzylhydroxylamine to give the amide **11**. Miller[5] ring closure gave the β-lactam **12** which was transformed in a two-step process into the mesylate **14**. The bicyclic lactam **15**, obtained after treatment with NaH, was deprotected by hydrogenolysis over Pd/C to give the key intermediate **16**.

Compound **16** was reacted with pyridine−SO_3 complex to give the protected sulfactam **2a**. The BOC group was removed by treatment with TFA, and the intermediate betaine was reacted with the activated side chain **18** to give compound **2b**. The intermediate **16** was treated with benzyl bromoacetate affording the bridged oxamazin **17** which was hydrogenolyzed to give **3a**.

Results and Discussion

The bridged oxamazin **3a** was not a β-lactamase inhibitor (Table 1) possibly due to insufficient activation of the β-lactam nitrogen. In contrast, the bridged sulfactams **2a**,**b** were potent inhibitors and were more active than the corresponding bridged monobactams **1a**,**b**. This was particularly true against class A β-lactamases, where the latter were inactive. The IC_{50} is a composite value of intrinsic affinity (K_S), rate of acylation (k_{on}), and rate of deacylation (k_{off}). An analysis of the kinetic parameters revealed that the low IC_{50} values of the bridged sulfactams stem primarily from a high affinity and a high rate of acylation (Table 2). However, the deacylation rate was also high, and therefore the steady-state occupancy of the enzyme, which is set by the ratio of k_{on}/k_{off}, was not as high as with the bridged monobactams. This incomplete inactivation was reflected in the in vitro data of the combination with ceftriaxone, where only a weak synergy was observed (Table 1).

The bridged sulfactams evidently have a higher chemical reactivity than the bridged monobactams (high acylation rates achieved), but the active site of the class C β-lactamase is less tightly blocked by the sulfonyloxy group than by a sulfonyl group.

Conclusions

The fact that the bridged oxamazins **3** are devoid of any β-lactamase inhibitory activity seems to indicate that the structural requirements for β-lactamase inhibition are very different from those for antibacterial activity. Despite their lower IC_{50} values, the bridged sulfactams **2** are less attractive β-lactamase inhibitors than the bridged monobactams **1** because of their high chemical reactivity and their less stable acyl−enzyme complexes.

Experimental Section

General Methods. All reagents were obtained from commercial sources and used without further purification. Sol-

---

* To whom correspondence should be addressed.
† Present address: CombiChem Inc., 9050 Camino Santa Fe, San Diego, CA 92121.

Table 1. Enzyme Inhibition and in Vitro Synergy with Ceftriaxone

| | IC$_{50}$[a] (nM) | | | MIC[b] (μg/mL) ceftriaxone:inhibitor = 1:4 | | |
| | *C. freundii* 1982 (class C) | *P. aeruginosa* 18 SH (class C) | *E. coli* TEM-3 (class A) | *C. freundii* 1982 | *P. aeruginosa* 18 SH | *E. coli* TEM-3 |
compd						
ref[c]				128	128	16
1a[d]	225	1500	>1000000	8	8	16
2a	20	28	165	4	32	4
1b[d]	53	217	9000000	0.25	4	16
2b	12	26	580	1	128	8
17	26000	185000	1000000	>16	>16	>16
taz[e]	900	800	15	8	>32	0.25

[a] Inhibition of the isolated enzymes. [b] Against the strains that produce the β-lactamases. [c] MICs of ceftriaxone in the absence of any inhibitor. [d] Values from ref 1. [e] Values obtained with tazobactam, reference inhibitor.

Scheme 1[a]
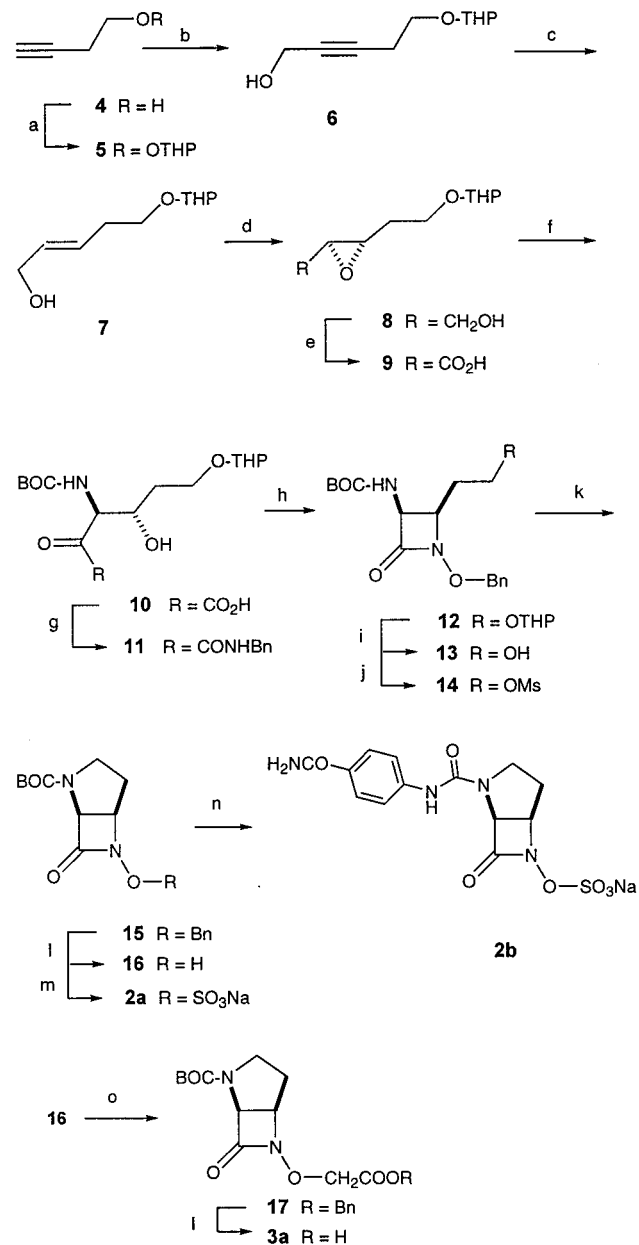
[a] Reagents: (a) 2,3-dihydropyran; (b) CH$_2$O (gaz); (c) LAH, MeONa; (d) Sharpless epoxidation, (L)-diethyl tartrate; (e) RuO$_2$/NaIO$_4$; (f) NH$_4$OH then (BOC)$_2$O; (g) BnONH$_2$, EDC; (h) DEAD/P(Ph)$_3$; (i) pTSA; (j) MsCl/TEA; (k) LiN[Si(CH$_3$)$_3$]$_2$; (l) H$_2$, Pd/C; (m) pyridine·SO$_3$; (n) TFA.

Table 2. Detailed Kinetic Parameters of the Inhibitors **1a,b**

compd	K_S (μM)	k_{on} (s^{-1})	k_{off} (10^{-6} s^{-1})
1a	127	12.5	<0.8
2a	600	20	120000

extraction were washed with water and brine and dried over MgSO$_4$. The solvent was evaporated under reduced pressure with a water bath temperature below 35 °C. Flash chromatography was performed using Merck silica gel 60 (particle size 40−63 μm), the fractions containing the substance of interest were pooled, and the solvent was evaporated. TLC was performed on Merck TLC plates (silica gel 60 F$_{254}$), and spots were visualized with aqueous KMnO$_4$. Proton NMR spectra were recorded on a Bruker FT AC-250 spectrometer. Chemical shifts (δ) are reported in ppm relative to Me$_4$Si as internal standard; values are given in Hz. As many derivatives were mixtures of rotamers and/or diastereoisomers (THP ethers), signals are not assigned. IR spectra were recorded on a Nicolet FT IR 170 SX spectrophotometer as KBr pellets (unless indicated otherwise). Ion spray mass spectra were recorded on a Finnigan MAT SSQ 7000 instrument and EI mass spectra on a Perkin-Elmer Siex API III instrument. Elemental analyses are indicated by the symbols of the elements; analytical results were within 0.4% of the theoretical values. Optical rotation measurements were performed on a Perkin-Elmer 241 polarimeter at 20 °C. Melting points were determined with a Buchi 510 melting point apparatus and are uncorrected.

2-(3-Butynyloxy)-tetrahydro-2*H*-pyran (5). A solution of 3-butyn-1-ol (105.1 g, 1.5 mol) in CH$_2$Cl$_2$ (0.5 L) was reacted at 0 °C with *p*-toluenesulfonic acid (0.2 g) and 3,4-dihydro-2*H*-pyran (163 mL, 1.8 mol, 1.2 equiv). The reaction mixture was stirred for 48 h at room temperature and then poured into 2% aqueous NaHCO$_3$ (0.1 L). The organic layer was worked up, and the residue was distilled under vacuum affording 223.7 g (96%) of **5**, bp$_{4 \text{ mm}}$ = 52−53 °C (lit.[5] bp$_{12 \text{ mm}}$ = 90 °C).

(*R,S*)-5-(Tetrahydropyran-2-yloxy)pent-2-yn-1-ol (6). A solution of **5** (15.4 g, 0.10 mol) in absolute THF (0.15 L) was reacted at −78 °C with dipyridyl (2 mg) and, dropwise, with a 1.6 N *n*-BuLi solution in hexane (72 mL, 115 mmol). The deep red solution was reacted for 1 h at −30 °C with gaseous formaldehyde.[7] The formaldehyde gas was transferred into the reaction flask via a 1-mm diameter Teflon tube. The mixture was allowed to reach 0 °C before quenching with saturated NH$_4$Cl solution (25 mL) and dilution with Et$_2$O. The organic phase was separated, worked up and chromatographed (hexane/AcOEt, 75:25) affording 15.6 g (85%) of **5**. IR (film): 3418, 2943, 2872, 2300, 2240 cm^{-1}. MS (EI): 153 (M − CH$_2$-OH). NMR (CDCl$_3$): 1.54−1.76 (6H, m), 2.20 (1H, b), 2.52 (2H, m), 3.49−3.90 (2 × 2H, 2m), 4.24 (2H, m), 4.63 and 4.81 (1H, 2t, *J* = 3.5).

(*E*)-(*R,S*)-5-(Tetrahydropyran-2-yloxy)pent-2-en-1-ol (7). A suspension of LAH (9.6 g, 253 mmol) in absolute THF (0.8 L) was reacted with NaOMe (27.4 g, 0.508 mol). After 10 min, the mixture was treated dropwise, over 45 min, with **6** (15.6 g, 84.67 mmol) in THF (70 mL) and refluxed for 1 h. The reaction mixture was sequentially treated at 5 °C with water (9 mL), 3 N NaOH (9 mL), and water (26 mL). The slurry was stirred for 1.5 h at room temperature and filtered. The

vents were dried by filtration through Al$_2$O$_3$ (neutral, Brockmann number 1) when necessary and stored over a bed of molecular sieves (3 Å). All the organic solutions obtained after

filtrate was evaporated and distilled affording 13.3 g of **7** (84%), $bp_{0.11 mm} = 83-85$ °C. NMR (CDCl$_3$): 1.42−1.90 (6H, m), 2.34 (2H, m), 2.54 (1H, t, $J = 5.5$), 3.43 (2H, m), 3.70−3.95 (2H, m), 4.06 (2H, b), 4.59 (1H, t, $J = 3$), 5.70 (2H, m).

1:1 Mixture of (2*R*,3*S*)-3-{2-[(*R*)- and (*S*)-Tetrahydropyran-2-yloxy]ethyl}oxiran-2-ylmethanol (8). Molecular sieve (3 Å) was activated at 120 °C under high vacuum for 12 h and finely pulverized before use. A suspension of molecular sieve (20 g) and L-(+)-diisopropyl tartrate (3.4 mL, 16.25 mmol) in absolute CH$_2$Cl$_2$ (0.25 L) was reacted at −30 °C with tetraisopropyl orthotitanate (3.8 mL, 13 mmol). After 15 min the suspension was treated with *tert*-butyl hydroperoxide in isooctane (3 M; 108 mL, 325 mmol) for 30 min. A solution of **7** (25 g, 130 mmol) in absolute CH$_2$Cl$_2$ (50 mL) was added at −20 °C and further stirred for 4 h. The reaction mixture was quenched at this temperature with a solution of NaOH (1 g) and NaCl (1 g) in water (10 mL). After 30 min at 10 °C, the reaction mixture was diluted with CH$_2$Cl$_2$. The organic solution was worked up and chromatographed (AcOEt/hexane, 1:1) giving 18.5 g (70%) of **8**. $[\alpha]_D = -22.9°$ (*c* 1, CHCl$_3$). NMR (CDCl$_3$): 1.52−1.90 (6H, m), 1.85 (2H, m), 2.60 (1H, 2t, $J = 6$), 3.00 (1H, m), 3.10 (1H, m), 3.49−3.92 (2 × 3H, 2m), 4.60 (1H, t, $J = 3$).

1:1 Mixture of (2*R*,3*S*)-3-[2-[(*R*)- and (*S*)-Tetrahydropyran-2-yloxy]ethyl]oxirane-2-carboxylic Acid (9). A solution of **8** in MeCN (0.2 L) and CCl$_4$ (0.2 L) was diluted with water (0.4 L) and reacted with RuO$_2$ (0.2 g) and NaIO$_4$ (80.2 g, 0.375 mol) for 4 h. The temperature of the reaction mixture was kept below 30 °C by occasional cooling, and the pH was adjusted to pH 4.5 by addition of 2 N Na$_2$CO$_3$. The reaction mixture was diluted with CH$_2$Cl$_2$. The aqueous layer was saturated with NaCl and extracted with CH$_2$Cl$_2$ (3×). The combined organic layers were worked up leaving an oil which was dissolved in ether and filtered over Dicalite. The filtrate was evaporated giving a foam (15.11 g, 76%). MS: 215 (M − H). NMR (CDCl$_3$): 1.52−2.00 (6H, m), 1.88 (2H, m), 3.33 (2H, m), 3.40−3.53 and 3.80−3.90 (2 × 2H, 2m), 4.60 (1H, m), 7.30 (1H, b).

1:1 Mixture of (1*S*,2*S*)-1-Carboxy-2-hydroxy-4-[(*R*)- and (*S*)-tetrahydropyran-2-yloxy]butylcarbamic Acid *tert*-Butyl Ester (10). A solution of **9** (2.58 g, 11.9 mmol) in MeOH (10 mL) was treated with KHCO$_3$ (1.31 g, 13.1 mmol) in water (20 mL). The solvents were evaporated. The residue was taken up in concentrated NH$_4$OH (40 mL), placed in a sealed tube, and warmed to 50 °C for 20 h. Excess ammonia was evaporated, and the residue was dried under high vacuum. It was dissolved in dioxane/water (60 mL; 2:1) and treated with (BOC)$_2$O. After stirring for 3 h, the organic solvent was evaporated and the aqueous layer was extracted twice with AcOEt. The combined organic extracts were discarded. The pH of the aqueous layer was adjusted to pH 3.5 with 1 N HCl and extracted with AcOEt (3×). The combined organic phases were worked up affording 2.62 g (66%) of **10**. NMR (CDCl$_3$): 1.45 (9H, s), 1.50−1.98 (8H, m), 3.50−4.05 (4H, m), 4.13 (1H, m), 4.36 (1H, m), 4.55 (1H, m), 5.65 (1H, d, $J = 9$).

1:1 Mixture of (1*S*,2*S*)-1-(Benzyloxycarbamoyl)-2-hydroxy-4-[(*R*)- and (*S*)-tetrahydropyran-2-yloxy]butylcarbamic Acid *tert*-Butyl Ester (11). The pH of a solution of **10** (2.62 g, 7.85 mmol) and BnONH$_2$·HCl (1.63 g, 10.2 mmol) in THF/water (0.1 L; 1:1) was adjusted to pH 4.5 with 1 N NaOH. The solution was reacted with a solution of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (2.85 g, 15.71 mmol) in water (10 mL) at pH 4.5 (kept by the addition of 1 N HCl). After the mixture stirred for 4 h, the pH was adjusted to 6.7, and the reaction mixture was diluted with AcOEt, the organic phase separated, and the aqueous layer extracted with AcOEt. The combined organic phases were worked up and chromatographed (hexane/ethyl acetate, 3:7) affording 1.74 g (51%) of **11**, mp 87−88 °C (hexane). $[\alpha]_D$ −9.4°(*c* 1, CHCl$_3$). MS (ISP): 461.4 (M + Na), 439.4 (M + H). NMR (CDCl$_3$): 1.43 (9H, s), 1.50−1.98 (9H, m), 3.45−4.10 (6H, m), 4.55 (1H, m), 4.91 (2H, 2m), 5.5−5.7 (1H, m), 7.35 (5H, s), 9.50 (1H, 2s). Anal. (C$_{22}$H$_{34}$N$_2$O$_7$) C, H, N.

1:1 Mixture of (2*R*,3*S*)-1-(Benzyloxy)-4-oxo-2-[[(*R*)- and (*S*)-tetrahydropyran-2-yloxy]ethyl]azetidin-3-ylcarbamic Acid *tert*-Butyl Ester (12). A solution of **11** (1.74 g, 3.97 mmol) and triphenylphosphine (1.15 g, 4.37 mmol) in absolute THF (25 mL) was reacted with diisopropyl azodicarboxylate (0.88 g, 4.37 mmol) for 2 h at room temperature. The solvent was evaporated; the residue was chromatographed (hexane/Et$_2$O/CH$_2$Cl$_2$, 5:4.5:0.5) affording an oil which was crystallized from Et$_2$O/hexane (820 mg, 50%), mp 104−08 °C. $[\alpha]_D$ −195° (*c* 1, CHCl$_3$). IR: 3343, 1784, 1691, 1531 cm^{-1}. MS (FAB): 421.3 (M + H)$^+$. NMR (CDCl$_3$): 1.42 (9H, s), 1.50−1.98 (8H, m), 3.30−4.00 (5H, m), 4.30−4.80 (2H, m), 4.90−5.1 (2H, m), 5.5−5.75 (1H, 2d, $J = 10$), 7.40 (5H, s). Anal. (C$_{22}$H$_{32}$N$_2$O$_6$) C, H, N.

(2*R*,3*S*)-1-(Benzyloxy)-2-(2-hydroxyethyl)-4-oxoazetidin-3-ylcarbamic Acid *tert*-Butyl Ester (13). A solution of **12** (5.1 g, 12.13 mmol) and *p*-toluenesulfonic acid (0.5 g) in EtOH (0.1 L) was warmed to 80 °C for 2 h. The solvent was evaporated, and the residue, dissolved in CH$_2$Cl$_2$, was worked up and crystallized from Et$_2$O/hexane (3.54 g, 87%), mp 102−103 °C. $[\alpha]_D$ +123.3 ° (*c* 1, CHCl$_3$). IR: 3429, 3355, 1765, 1680, 1536 cm^{-1}. MS (EI): 337.8 (M + H)$^+$, 281.8 (M − (CH$_2$=C(Me)$_2$)). NMR (CDCl$_3$): 1.42 (9H, s), 1.71 (2H, dd, $J = 7$ and 12), 3.67 (2H, t, $J = 7$), 3.75 (1H, dt, $J = 5$), 4.75 (1H, dd, $J = 6$ and 8), 4.99 (2H, ab, $J = 10$), 5.75 (1H, bd, $J = 8$), 7.40 (5H, s). Anal. (C$_{17}$H$_{24}$N$_2$O$_5$) C, H, N.

(2*R*,3*S*)-1-(Benzyloxy)-2-[2-(methylsulfonyloxy)ethyl]-4-oxoazetidin-3-ylcarbamic Acid *tert*-Butyl Ester (14). A solution of **13** (3.44 g, 10.2 mmol) and triethylamine (1.13 g, 11.2 mmol) in CH$_2$Cl$_2$ (50 mL) was treated dropwise with MeSO$_2$Cl (1.28 g, 11.2 mmol) at 0 °C. The reaction mixture was stirred for 1 h at room temperature, then diluted with CH$_2$Cl$_2$, and washed with ice water and a cooled 10% NaCl solution. The organic layer was dried over MgSO$_4$, filtered, and evaporated, leaving an oil which was crystallized from AcOEt/hexane. The yield was 4.2 g (99%), mp 148 °C. $[\alpha]_D$ +83.2° (*c* 1, CHCl$_3$). IR: 3324, 1777, 1683, 1533 cm^{-1}. MS (ISP): 437.3 (M + Na), 415.3 (M + H). NMR (CDCl$_3$): 1.43 (9H, s), 1.87 (2H, m), 2.98 (3H, s), 3.75 (1H, dt, $J = 3$ and 5), 4.19 (2H, m), 4.70 (1H, t, $J = 5$), 4.95 and 5.02 (2H, ab), 5.20 (1H, d, $J = 5$), 7.41 (5H, s). Anal. (C$_{22}$H$_{34}$N$_2$O$_7$) C, H, N.

(1*R*,5*R*)-6-(Benzyloxy)-7-oxo-2,6-diazabicyclo[3.2.0]heptane-2-carboxylic Acid *tert*-Butyl Ester (15). A solution of **14** (4.14 g, 10 mmol) in absolute THF (40 mL) was treated dropwise at −10 °C with 1 M lithium bis(trimethylsilyl)amide in THF (11.5 mL, 11.5 mmol). After stirring at room temperature overnight, the reaction mixture was quenched with saturated NH$_4$Cl solution (25 mL) and extracted with AcOEt. The organic layer was worked up, chromatographed (hexane/AcOEt, 6:4) and crystallized from Et$_2$O/hexane (819 mg, 25%), mp 88−90 °C. $[\alpha]_D$ −195° (*c* 0.9, CHCl$_3$) (lit.[8] $[\alpha]_D$ −214.9° (*c* 1, CHCl$_3$)). IR: 1767, 1687, 1421 cm^{-1}. MS (EI): 290 (M − CO). NMR (CDCl$_3$): 1.46 (9H, s), 1.52 (1H, m), 1.75 (1H, dd, $J = 6$ and 14), 3.10 (1H, dt, $J = 6$ and 10), 3.80 (1H, bt, $J = 10$), 4.09 (1H, b), 4.75 and 5.00 (1H, b), 4.96 (2H, s), 7.40 (5H, s). Anal. (C$_{17}$H$_{22}$N$_2$O$_4$) C, H, N.

(1*R*,5*R*)-6-Hydroxy-7-oxo-2,6-diazabicyclo[3.2.0]heptane-2-carboxylic Acid *tert*-Butyl Ester (16). A solution of **15** (769 mg, 2.41 mmol) in EtOH (15 mL) was hydrogenated over 10% Pd/C. The catalyst was filtered off, and the filtrate was evaporated under reduced pressure and crystallized giving 0.48 g (87%) of **15**, mp 150−52 °C (AcOEt/*n*-hexane). IR: 3437, 1754, 1721, 1697, 1404 cm^{-1}. MS (EI): 211 (M − OH). NMR (DMSO-d_6): 1.40 (9H, s), 1.68 (1H, m), 2.0 (1H, dd, $J = 6$ and 12), 3.09 (1H, m), 3.81 (1H, dd, $J = 9$ and 12), 4.39 (1H, t, $J = 4.5$), 4.8 (1H, broad), 10.32 (1H, s). Anal. (C$_{10}$H$_{16}$N$_2$O$_4$) C, H, N.

Sodium [(1*S*,5*R*)-2-(*tert*-Butoxycarbonyl)-7-oxo-2,6-diazabicyclo[3.2.0]heptan-6-yl]sulfate (2a). A solution of **16** (114 mg, 0.5 mmol) in pyridine (2 mL) was reacted with C$_5$H$_5$N·SO$_3$ complex (95 mg, 0.6 mmol) and molecular sieve (4 Å). The reaction mixture was filtered, and the filtrate was evaporated. The residue was dissolved in EtOH (3 mL) and precipitated by the addition of Et$_2$O. The first oily crystals

were removed by filtration, and the filtrate was further treated with Et_2O. The crystals were filtered, affording the pyridinium salt of **2a**, 160 mg (82%). NMR (DMSO-d_6): 1.40 (9H, s), 1.65 (1H, m), 2.15 (1H, dd, $J = 6$ and 12), 3.15 (1H, m), 3.80 (1H, t, $J = 9$ and 12), 4.60 (1H, t, $J = 4.5$), 4.80 (1H, broad), 8.03 (2H, m), 8.54 (1H, m), 9.90 (2H, m).

The pyridinium salt (160 mg, 0.413 mmol) was treated with $NaHCO_3$ (34.7 mg, 0.413 mmol) in water (10 mL). The solution was concentrated under reduced pressure to about 3 mL and purified by gel filtration (MCI gel; water then 10% MeOH in water) affording 52 mg of **2a** (38%) after lyophilization. IR: 1775, 1701 cm^{-1}. MS (ISN): 307.2 (M − Na). NMR (DMSO-d_6): 1.40 (9H, s), 1.68 (1H, m), 2.15 (1H, dd, $J = 6$ and 12), 3.15 (1H, m), 3.78 (1H, dd, $J = 9$ and 12), 4.62 (1H, t, $J = 4.5$), 4.85 (1H, broad). Anal. ($C_{10}H_{15}N_2O_7SNa$) C, H, N.

Sulfuric Acid (*1S,5R*)-2-(4-Carbamoylphenylcarbamoyl)-7-oxo-2,6-diazabicyclo[3.2.0]heptan-6-yl Ester Sodium Salt (2b). TFA (0.9 mL) was added to **2a** (0.46 g, 1018 mmol) at 0 °C. After 1 h at room temperature, Et_2O was added and the crystals were filtered and washed with Et_2O affording 308 mg (81%) of intermediate sulfuric acid mono [(*1S,5R*)-7-oxo-2,6-diazabicyclo[3.2.0]heptan-6-yl] ester. NMR (DMSO-d_6): 1.75 (1H, m), 2.58 (1H, dd, $J = 6$ and 15), 3.20 (1H, m), 3.59 (1H, dd, $J = 6$ and 12), 4.75 (1H, t, $J = 3.5$), 4.91 (1H, d, $J = 3.5$).

A solution of this betaine (0.13 g, 0.46 mmol) in water (4 mL) was reacted at 0 °C with $NaHCO_3$ (82 mg, 0.98 mmol) and a solution of 4-$H_2NCOC_6H_4NHCO_2NSu^2$ (**18**; 142 mg, 0.51 mmol) in MeCN (5 mL). The reaction mixture was filtered, the solvent evaporated, and the residue, dissolved in water, purified by gel filtration, affording 47 mg (26%) of **2b**. IR: 1772, 1660 cm^{-1}. MS (ISN): 369.2 (M − Na). NMR (DMSO-d_6): 1.75 (1H, m), 2.35 (1H, dd, $J = 6$ and 12), 3.25 (1H, m), 4.0 (1H, dd, $J = 9$ and 12), 4.70 (1H, t, $J = 4.5$), 7.17 (1H, broad), 7.55 (2H, d, $J = 8$), 7.8 (3H, m), 8.80 (1H, s).

(*1S,5R*)-[2-(*tert*-Butoxycarbonyl)-7-oxo-2,6-diazabicyclo-[3.2.0]heptan-6-yloxy]acetic Acid (17). A solution of **16** (74.4 mg, 0.326 mmol) in THF (2 mL) was reacted at 0 °C with DBU (53 μL, 0.36 mmol) and benzyl bromoacetate (57 μL, 0.35 mmol) for 1 h. The reaction mixture was diluted with AcOEt, worked up, and chromatographed (AcOEt/hexane, 4:6), affording 127 mg of **17** (100%). IR (CH_2Cl_2): 1783 cm^{-1}.

(*1S,5R*)-6-[(Benzyloxycarbonyl)methoxy]-7-oxo-2,6-diazabicyclo[3.2.0]heptane-2-carboxylic Acid *tert*-Butyl Ester (3a). A solution of **17** (127 mg, 0.325 mmol) in EtOH (3 mL) was hydrogenated over Pd/C. The catalyst was filtered off, the filtrate evaporated, and the residue crystallized from Et_2O/hexane giving 57 mg of **3a** (62%). IR (CH_2Cl_2): 1783 cm^{-1}. Anal. ($C_{12}H_{18}N_2O_6$) C, H, N.

Enzyme Inhibition. β-Lactamases were purified to homogeneity, and their hydrolytic activity was measured using standard methods.[9]

In Vitro Antibacterial Activity. The minimal inhibitory concentrations (MICs) of the test compounds were determined according to a standard method.[10] The compounds were incorporated, following a serial 2-fold dilution pattern, into Mueller-Hilton agar (Difco Laboratories, Detroit, MI). An inoculum of approximately 10^4 test organisms was applied to the agar surface using a mulipoint inoculator. The MIC of a compound or a combination was defined as the lowest concentration that prevented visible growth of bacteria after incubation at 35 °C for 18 h.

References

(1) Heinze-Krauss, I.; Angehrn, P.; Charnas, R. L.; Gubernator, K.; Gutknecht, E.; Hubschwerlen, C.; Kania, M.; Oefner, C.; Page, M. G. P.; Sogabe, S.; Specklin, J.-L.; Winkler, F. K. Structure-based design of β-Lactamase Inhibitors. 1. Synthesis and Evaluation of Bridged Monobactams. *J. Med. Chem.* **1998**, *41*, 3961−3971.

(2) Livermore, D. M.; Chen, H. Y. Potentiation of β-Lactams against *Pseudomonas aeruginosa* strains by Ro 48-1256, a bridged monobactam inhibitor of AmpC β-lactamases. *J. Antimicrob. Chemother.* **1997**, *40*, 335−343.

(3) Girard, S.; Deslongchamp, P. Formation of 14-membered Carbocycles by Intramolecular Michael Addition on Ynones and Enones. *Can. J. Chem.* **1992**, *70*, 1265−1273.

(4) Gao, Y.; Hanson, R. M.; Klunder, J. M.; Ko, S. Y.; Masamune, H.; Sharpless, K. B. Catalytic Asymmetric Epoxidation and Kinetic Resolution: Modified Procedures Including in situ Derivatization. *J. Am. Chem. Soc.* **1987**, *109*, 5765−5780.

(5) Miller, M.; Mattingly, P. G.; Morrison, M. A.; Kerwin, J. R., Jr.; Synthesis of β-Lactams from Substituted Hydroxamic Acids. *J. Am. Chem. Soc.* **1980**, *102*, 7026−7032.

(6) Breuer, H.; Straub, H.; Treuner, U. D.; Drossard, J.-M.; Höhn, H.; Lindner, K. R. [(2-Oxo-1-azetidinyl)oxy]acetic Acids: A New Class of Synthetic Monobactams. *J. Antibiot.* **1985**, *38*, 813−818.

(7) Smith, A. B., III; Branca, S. J.; Guarciaro, M. A.; Wovkulich, P. M.; Korn, A. 2-Hydroxymethyl-2-cyclopentenone. In *Organic Synthesis*; Stevens, R. V., Ed.; J. Wiley and Sons: New York, 1983, Vol. 61, pp 65−70.

(8) Belleteni, J. R.; Miller, M. J. A Short Synthesis of an Important Precursor to a New Class of Bicyclic β-Lactamase Inhibitors. *Tetrahedron Lett.* **1997**, *38*, 167−168.

(9) Page, M. G. P. The Kinetics of Non-Stoichiometric Bursts of β-Lactam Hydrolysis Catalysed by Class C β-Lactamases. *Biochem. J.* **1993**, *295*, 295−304.

(10) Heinze-Krauss, I.; Angehrn, P.; Guerry, P.; Hebeisen, P.; Hubschwerlen, C.; Kompis, I.; Page, M. G. P.; Richter, H. G. F.; Runtz, V.; Stalder, H.; Weiss, U.; Wei, C.-C. Synthesis and Structure−Activity Relationship of (Lactamylvinyl)cephalosporins Exhibiting Activity against Staphylococci, Pneumococci, and Enterococci. *J. Med. Chem.* **39**, 1864−1871.

JM9800245