JOC*Note*

Synthesis and Evaluation of 3-(Carboxymethylidene)- and 3-(Carboxymethyl)penicillinates as Inhibitors of β-Lactamase

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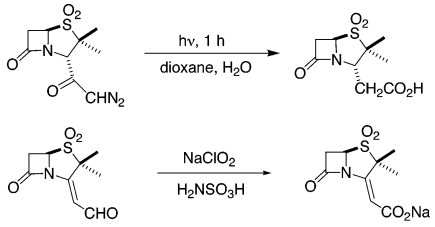

Penicillin-resistant bacteria can often be treated through the co-administration of an antibiotic and a β-lactamase inhibitor. Current inhibitors target only class A β-lactamases. We report two new series of C3-modified penicillin sulfones, having either a simple methylene group (i.e., a homologue) or exocyclic unsaturation between the thiazolidine ring and the C3 carboxylate. The homologue has 10-fold better activity against a class C β-lactamase than does sulbactam itself. By contrast, the exocyclic C3 unsaturated compounds are less active.

The most common form of bacterial resistance to the β-lactam antibiotics involves the ability to produce one or more types of β-lactamase.[1] More than 300 β-lactamases have been characterized. Classes A, C, and D are serine enzymes, while class B are zinc metalloenzymes. The class A enzymes usually prefer penicillins (Figure 1) as substrates and are known as penicillinases, while the class C β-lactamases are considered cephalosporinases. One approach to treating such resistant infections involves the co-administration of a β-lactam antibiotic and a β-lactamase inhibitor.[2] Commercial inhibitors, including clavulanic acid, sulbactam, and tazobactam (see Figure 2), are active only against class A β-lactamases.[3] In response to the rapid proliferation of resistant bacteria expressing class B, C, and D β-lactamases,[4] our group has reported nanomolar inhibitors that simultaneously inactivate classes A, C, and D serine β-lactamases (Figure 3, **1**−**3**)[5] and also inhibitors that simultaneously target both the class B metallo- and class C serine-β-lactamases (e.g., **4**).[6]

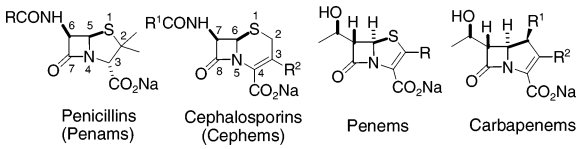

FIGURE 1. Structures of four representative classes of β-lactam antibiotics.

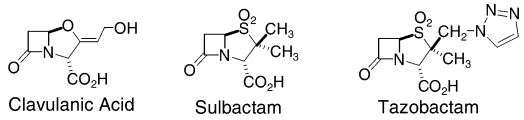

FIGURE 2. Commercial β-lactamase inhibitors.

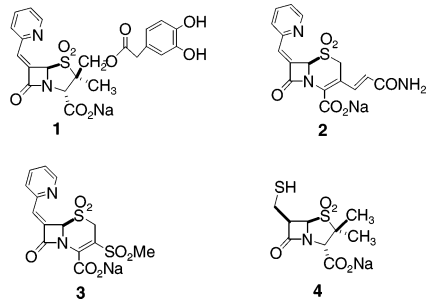

FIGURE 3. β-Lactamase inhibitors designed and synthesized in the Buynak group.

In the process of developing new inhibitors, we have already explored modification of the 2′- and the 6-position in the penam series (Figure 1 for numbering) as well as modifications at C2, C3, and C7 in the cephem series. By contrast, the C3 carboxylate of the penicillins or the C4 carboxylate of the cephalosporins are critical for recognition of these antibiotics in a positively charged pocket of the active site of the PBPs and in their descendents, the β-lactamases. Relatively few modifications have been performed at these positions.[7] The

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(2) (a) Maiti, S. N.; Phillips, O. A.; Micetich, R. G.; Livermore, D. M. *Curr. Med. Chem.* **1998**, *5*, 441−456. (b) Miller, L. A.; Ratnam, K.; Payne, D. J. *Curr. Opin. Pharm.* **2001**, *1*, 451−458. (c) Micetich, R. G.; Salama, S. M.; Maiti, S. N.; Reddy, A. V. N.; Singh, R. *Curr. Med. Chem. Anti Infect. Agents* **2002**, *1*, 193−213. (d) Sandanayaka, V. P.; Prashad, A. S. *Curr. Med. Chem.* **2002**, *9*, 1145−1165. (e) Page, M. G. P. *Drug Resist. Updates* **2000**, *3*, 109−125.

(3) Bush, K.; Mobashery, S. *Adv. Exp. Med. Biol.* **1998**, *456*, 71−98.

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(5) For a review, see: Buynak, J. D. *Curr. Med. Chem.* **2004**, *11*, 1951−1964.

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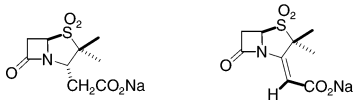


FIGURE 4. Target molecules.

geometry of the (C3 or C4) carboxylate placement with respect to the (roughly planar) β-lactam ring is different for the penicillins versus the cephalosporins. In the case of the roughly planar bicyclic cephalosporin system, the carboxylate carbon is attached to an sp^2-hybridized carbon and lies in approximately the same plane as both the 6- and the 4-membered ring of this bicyclic system. By contrast, in the more strained and bowed bicylic penam, the carboxylate carbon is attached to an sp^3 carbon and skewed to the convex (i.e., to the α) face. This geometric difference may partially account for the difference in substrate specificity between class A and class C enzymes.

A second structural feature, the endocyclic double bond of the cephalosporins, penems, and carbapenems, is an important contributor to enhanced reactivity (enhanced acylation capability). This double bond stabilizes the nitrogen leaving group (and strains the penem and carbapenem systems) leading to enhanced activity of the antibiotic to acylate the active site serine.

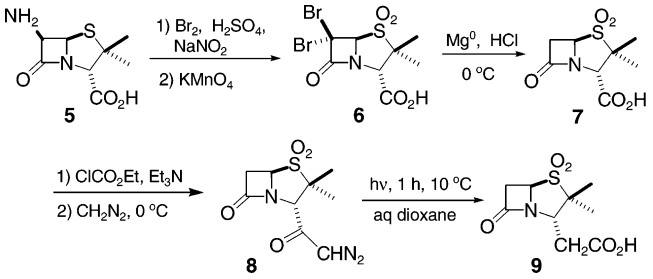
Known inhibitors of the class A, C, and D serine β-lactamases acylate the active site serine. It is logical that the commercial inhibitors are selective for class A enzymes, since they structurally resemble penicillins more closely than they resemble cephalosporins (Figure 2). As in penicillin, the carboxylates of the commercial inhibitors are bonded to an sp^3-hybridized carbon. We therefore hypothesized that a C3-homologated penam-derived β-lactamase inhibitor might have a broader specificity for both the A and C classes of β-lactamase than do current commercial inhibitors. The enhanced conformational flexibility of the carboxylate of the homologated penam derivative could enable the molecule to fulfill the geometric requirements of both A and C classes of serine β-lactamase. The longer chain might also enable the carboxylate to penetrate deeper into the positively charged pocket. Second, we hypothesized that placing a double bond in direct conjugation with the nitrogen but exocyclic to the five membered ring might also enhance acylation efficacy. We report the synthesis of these prospective inhibitors (Figure 4) and also a preliminary investigation of their capability to inactivate serine β-lactamases.

As shown in Scheme 1, 6,6-dibromopenicillanic acid sulfone (**6**) was prepared from 6-APA according to the method of Volkmann.[8] Subsequent treatment with magnesium in the presence of dilute HCl produced sulbactam **7**. Formation of the mixed anhydride and treatment with diazomethane produced diazoketone **8**. Photolysis of **8** in wet dioxane produced homologated sulbactam **9**. The X-ray structure of **9** is shown in Figure 5.

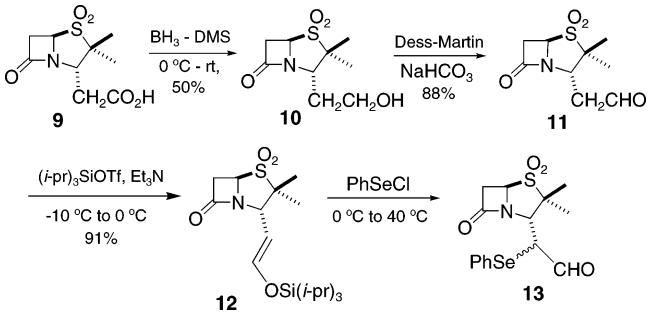
As shown in Scheme 2, the free carboxylic acid of **9** was reduced with BH_3−DMS to produce primary alcohol **10** which was subsequently oxidized to the aldehyde **11**.

(8) Volkmann, R. A.; Carroll, R. D.; Drolet, R. B.; Elliott, M. L.; Moore, B. S. *J. Org. Chem.* **1982**, *47*, 3344−3345.

SCHEME 1



SCHEME 2



Treatment with TIPS-OTf allowed isolation of a single triisopropylsilyl enol ether **12**, which reacted with PhSeCl to generate selenides **13**. Attempts to oxidize **13** to the corresponding selenoxide as a precursor to the α,β-unsaturated aldehyde were unsuccessful; thus, **13** was converted to dioxolane **14**. Treatment of **14** with *m*-CPBA produced C3-unsaturated penicillin sulfones (separable) **15** and **16** in a ratio of 15:85, respectively. The X-ray structure of **16** is shown in Figure 5. Each geometric isomer was deprotected and oxidized to the corresponding acid salt (**18** or **20**).

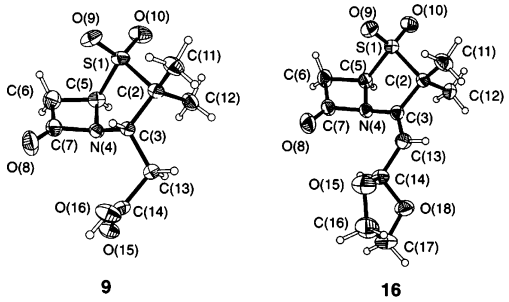


FIGURE 5. X-ray structure of **9** and **16**.

The inhibitory data presented in Table 1 indicate that the homologated sulbactam analogue, **9**, had a 10-fold improved inhibitory activity against the class C P99 enzyme, as compared with sulbactam itself. It is possible that the additional carbon now allows greater conformational flexibility, thus permitting the carboxylate of **9** to occupy a space close to that occupied in a typical cephalosporin substrate. Note, however, that the compounds incorporating the exocyclic unsaturation (**18** and **20**) did not display significant ability to inactivate either class A or class C serine β-lactamases. This could be due to the conformational rigidity imposed by the exocyclic unsaturation. These compounds are slowly hydrolyzed in buffered aqueous media and this hydrolysis is accelerated

SCHEME 3

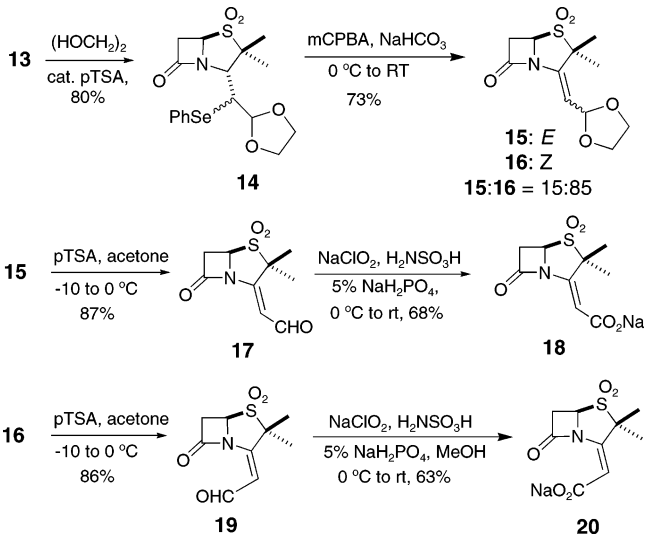


TABLE 1. Inhibition of Serine β-Lactamases (IC_{50}, μM)

compd	TEM-1 (class A) (serine)	P99 (class C) (serine)
tazobactam	0.122	53.2
sulbactam	13.0	276
9	33.5	27.7
18	>1000	>1000
20	>1000	>1000

in the presence of the TEM-1 β-lactamase, thus indicating that they are substrates. As in the penems and carbapenems, the unsaturation present on the five-membered ring (endocyclic or exocyclic) presumably further activates the β-lactam toward hydrolysis. This explains the reduced hydrolytic stability of **18** and **20**.

In summary, we succeeded in preparing and evaluating several C3-modified derivatives of sulbactam. One of these, **9**, a simple C3 homologue of sulbactam, exhibits improved ability to inhibit class C β-lactamases than does sulbactam itself. By contrast the compounds incorporating exocyclic unsaturation at C3 (**18** and **20**) were devoid of useful inhibitory activity.

Experimental Section

2-Diazoacetyl-3,3-dimethyl-4,4-dioxo-4-thia-1-azabicyclo-[3.2.0]heptan-7-one (8). To a solution of penicillanic acid *S*,*S*-dioxide **7** in anhyd CH_2Cl_2 (200 mL) was added triethylamine (31.3 mL, 225 mmol) at −20 °C. To this cold solution was added ethyl chloroformate (48.6 mL, 450 mmol) slowly over a period of 15 min, and the reaction was stirred for 30 min. Then the reaction mixture was allowed to warm to rt and stirred for an additional 30 min. After completion (TLC), the mixture was washed with cold aq $NaHCO_3$ and brine, dried over sodium sulfate, and evaporated to produce product. To a solution of above-prepared anhydride in THF was added freshly prepared diazomethane−ether solution (excess) at 0 °C. After completion (1 h), excess diazomethane was removed by bubbling Ar through the mixture. Removal of the solvent and purification by flash chromatography (100% CH_2Cl_2 to 10% EtOAc/CH_2Cl_2) produced diazoketone (39.21, 68%). Intermediate acyl carbonate: IR (neat, cm^{-1}) ν 1 800, 1789; ^1H NMR (400 MHz, $CDCl_3$) δ 4.66−4.65 (m, 1H), 4.39 (s, 1H), 4.32 (q, J = 7.1 Hz, 2H), 3.53 (dd, J_1 = 11.8 Hz, J_2 = 4.5 Hz, 1H) 1.57 (s, 3H), 1.46 (s, 3H), 1.36−1.31 (m, 3H). Diazoketone **8**: IR (neat, cm^{-1}) ν 2112, 1787, 1631. Diazoketone **8**: ^1H NMR (400 MHz, $CDCl_3$) δ 5.80 (s, 1H), 4.59 (s, 1H), 4.17 (s, 1H), 3.49 (d, J = 3.3 Hz, 2H), 1.63 (s, 3H) 1.44 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.8, 171.7, 66.3, 63.8, 60.5, 55.4, 37.8, 19.4, 18.3; HRMS (FAB) calcd for $C_9H_{11}N_3O_4S$ 258.0593 (M + H)$^+$, found *m/z* 258.0595.

(3,3-Dimethyl-4,4,7-trioxo-4-thia-1-azabicyclo[3.2.0]hept-2-yl)acetic Acid (Homologue of Sulbactam) (9). A solution of **8** (20.56, 80 mmol) in 1,4-dioxane (800 mL) and water (8 mL) was irradiated in an Ace photochemical reactor while the temperature was maintained between 5 and 10 °C using ice−water circulation. After completion (60 min), the solvent was removed. The crude product was diluted with EtOAc, washed with cold water and brine, dried Na_2SO_4, and concentrated to give crude product (10.86 g, 55%) which was used without further purification: IR (neat, cm^{-1}) ν 1773, 1657; ^1H NMR (400 MHz, $CDCl_3$) δ 4.48 (t. J = 3.4 Hz, 1H), 4.30−4.27 (m, 1H), 3.43−3.42 (m, 2H), 2.57−2.55 (m, 2H), 1.47 (s, 3H) 1.42 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 173.5, 171.3, 63.4, 61.2, 59.4, 38.6, 36.1, 19.5, 19.1; HRMS (FAB) calcd for $C_9H_{13}NO_5S$ 248.0593 (M + H)$^+$, found *m/z* 248.0596.

2-(2′-Hydroxyethyl)-3,3-dimethyl-4,4-dioxo-4-thia-1-azabicyclo[3.2.0]heptan-7-one (10). To a solution of **9** (9.88 g, 40 mmol) in anhyd THF (300 mL) was added BH_3−Me_2S (5 mL, 50 mmol). The mixture was then stirred at rt for 20 h. The solvent was then removed, and the residue was purified by flash chromatography (10% EtOAc/CH_2Cl_2) to 50% EtOAc/CH_2Cl_2) to give product (4.66 g, 50%): IR (neat, cm^{-1}) ν 1782, 3422; ^1H NMR (400 MHz, $CDCl_3$) δ 4.52−4.50 (m, 1H), 3.92−3.82 (m, 1H), 3.84−3.80 (m, 2H), 3.47−3.42 (m, 2H), 3.10−2.90 (brs), 1.73−1.69 (m, 1H), 1.49−1.44 (m, 1H) 1.42 (s, 3H) 1.41 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.3, 63.4, 60.1, 58.3, 53.5, 37.1, 32.1, 19.4, 17.3; HRMS (FAB) calcd for $C_9H_{16}NO_4S$ 234.08 (M + H)$^+$, found *m/z* 234.0793.

(3,3-Dimethyl-4,4,7-trioxo-4-thia-1-azabicyclo[3.2.0]hept-2-yl)acetaldehyde (11). To a solution of **10** (4.2 g, 18 mmol) in anhyd CH_2Cl_2 were added $NaHCO_3$ (15.12 g, 180 mmol) and Dess−Martin periodinane (15.4 mg, 36 mmol). The reaction was stirred for 3 h. Saturated sodium thiosulfate solution was added, and the reaction was stirred for 30 min. The solution was diluted with CH_2Cl_2, washed with saturated $NaHCO_3$ solution and brine, and dried over Na_2SO_4 and the solvent removed. Purification by flash chromatography (5% EtOAc/CH_2Cl_2 to 20% EtOAc/CH_2Cl_2) produced product (3.65 g, 88%): IR (neat, cm^{-1}) ν 1785, 1724; ^1H NMR (400 MHz, $CDCl_3$) δ 9.81 (s, 1H), 4.51−4.50 (m, 1H) 4.34−4.30 (m, 1H), 3.43 (d, J = 3.2 Hz, 2 H), 2.65−2.55 (m, 2H), 1.46 (s, 3H), 1.41 (s, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 198.6, 171.8, 63.1, 61.0, 57.4, 44.2, 38.3, 19.5, 18.5; HRMS (FAB) calcd for $C_9H_{14}NO_4S$ 232.0644 (M + H)$^+$, found *m/z* 232.0639.

3,3-Dimethyl-4,4-dioxo-2-[2-(triisopropylsilanyloxy)vinyl]-4-thia-1-azabicyclo[3.2.0]heptan-7-one (12). To a solution of **11** (1.8 g, 7.8 mmol) in anhyd CH_2Cl_2 (10 mL) were added Et_3N (3.8 mL, 27.3 mmol) and triisopropylsilyl triflate (6.29 mL, 23.4 mmol) at 0 °C. The solution was stirred at 0 °C for 40 min. After completion (TLC), the mixture was washed with cold aq $NaHCO_3$ and brine, dried over Na_2SO_4, concentrated, and purified by flash column chromatography (80% CH_2Cl_2/hexane, 100% CH_2Cl_2 to 2% EtOAc/CH_2Cl_2) to give pure product (2.74 g, 91%): IR (neat, cm^{-1}) ν 1787; ^1H NMR (400 MHz, $CDCl_3$) δ 9.81 (s, 1H), 4.51−4.50 (m, 1H) 4.34−4.30 (m, 1H), 3.43 (d, J = 3.2 Hz, 2H), 2.65−2.55 (m, 2H), 1.46 (s, 3H), 1.41 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.2, 146.7, 146.5, 104.1, 63.6, 62.2, 60.7, 37.3, 19.6, 17.7, 17.3, 17.2, 17.0, 16.6, 12.9, 12.3, 12.1, 12.0, 11.8; HRMS (FAB) calcd for $C_{18}H_{14}NO_4SSi$ 388.1978 (M + H)$^+$, found *m/z* 388.1978.

(3,3-Dimethyl-4,4,7-trioxo-4-thia-1-azabicyclo[3.2.0]hept-2-yl)phenylselenylacetaldehyde (13). To a solution of **12** (1.93 g, 5 mmol) in anhyd CH_2Cl_2 was added PhSeCl (0.954 g, 5 mmol) in THF (8 mL) at 0 °C. The reaction mixture was heated to 40 °C for 40 min. Volatiles were removed, and the residue was dissolved in CH_2Cl_2, dried over Na_2SO_4, concentrated, and purified by flash chromatography (100% CH_2Cl_2 to 20% EtOAc/CH_2Cl_2) to give a diastereomeric mixture (inseparable, 1.566 g, 82%): IR (neat, cm^{-1}) ν 1790, 1703; ^1H NMR (400 MHz, $CDCl_3$) δ 9.41−9.40 (s, 2H), 7.63−7.61 (m, 2H) 7.53−7.49 (m, 2H), 7.40−7.31 (m, 6H), 4.48−4.47 (m, 2H), 4.27−4.18 (m,

2H), 3.79−3.81 (m, 1H), 3.52−3.49 (m, 3H), 3.42−3.41 (m, 2H), 1.68 (s, 3H), 1.58 (s, 3H), 1.42 (s, 3H), 1.38 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.9, 188.4, 171.4, 170.9, 136.8, 135.6, 131.5, 129.6, 129.2, 125.1, 124.5, 64.3, 63.3, 61.6, 60.6, 60.2, 60.0, 54.7, 52.4, 39.3, 37.9, 20.1, 20.0, 19.4, 19.6; HRMS (FAB) calcd for $C_{15}H_{18}NO_4SSe$ 388.0122 $(M + H)^+$, found *m/z* 388.0109.

2-[[1,3]Dioxolan-2-yl(phenylselenyl)methyl]-3,3-dimethyl-4,4-dioxo-4-thia-1-azabicyclo[3.2.0]heptan-7-one (14). To a solution of **13** (2.5 g, 6.5 mmol) in anhyd C_6H_6 (30 mL) were added ethylene glycol (1 mL, 13 mmol) and cat. PTSA. The reaction mixture was refluxed for 3.5 h using a Dean−Stark apparatus. The solution was concentrated and purified by flash chromatography (100% CH_2Cl_2 to 20% EtOAc/CH_2Cl_2) to give a mixture of diastereomers (2.23 g, 80%): IR (neat, cm^{-1}) ν 1797, 1593; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64−7.60 (m, 4H), 7.31−7.26 (m, 6H), 5.31−5.30 (m, 2H), 4.45−4.44 (m, 2H), 4.20−4.00 (m, 10H), 3.28−3.40 (m, 6H), 1.67 (s, 6H), 1.28 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.9, 187.4, 141.5, 139.7, 136.8, 135.6, 129.6, 129.2, 125.1, 124.5, 65.2, 65.3, 64.7, 64.8, 63.3, 62.3, 61.6, 60.6, 59.8, 59.2, 54.7, 52.4, 39.2, 37.8, 20.3, 20.1, 19.3, 19.6; HRMS (FAB) calcd for $C_{17}H_{22}NO_5SSe$ 432.0384 $(M + H)^+$, found *m/z* 432.0379.

2*E*-[1,3]Dioxolan-2-ylmethylene-3,3-dimethyl-4,4-dioxo-4-thia-1-azabicyclo[3.2.0]heptan-7-one (15) and 2*Z*-[[1,3]-Dioxolan-2-ylmethylene]-3,3-dimethyl-4,4-dioxo-4-thia-1-azabicyclo[3.2.0]heptan-7-one (16). To a solution of **14** (2 g, 4.8 mmol) in anhyd CH_2Cl_2 (6 mL) under N_2 were added $NaHCO_3$ (1.0 g, 10 mmol) and *m*-CPBA (1.2 g, 14.8 mmol) at 0 °C. The mixture was stirred for 45 min at rt. The mixture was diluted with CH_2Cl_2, washed with aq sodium metabisulfite solution, aq $NaHCO_3$, and brine, dried over Na_2SO_4, and evaporated. The residue was purified by chromatography (100% CH_2Cl_2 to 15% EtOAc/CH_2Cl_2) to give the *E*-isomer **15** (120 mg, 10.95%) and *Z*-isomer **16** (685 mg, 62%) in a 15:85 ratio.

***E*-Isomer (15):** IR (neat, cm^{-1}) ν 1811, 1320; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.79 (d, J = 7.8 Hz, 1H, olefin CH), 5.05 (d, J = 7.8 Hz, 1H, CH), 4.71−4.69 (m, 1H, C5 CH), 4.06−3.90 (m, 4H, CH_2CH_2), 3.59−3.54 (m, 2H, CH_2), 1.59 (s, 3H, CH_3) 1.54 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.5, 141.5, 109.8, 99.9, 65.3, 65.1, 62.9, 60.9, 38.3, 26.6, 17.9; HRMS (FAB) calcd for $C_{11}H_{16}NO_5S$ 274.0749 $(M + H)^+$, found *m/z* 274.0753.

***Z*-Isomer (16):** IR (neat, cm^{-1}) ν 1799.6, 1326; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.69 (d, J = 5.8 Hz, 1H), 5.64 (d, J = 5.8 Hz, 1H), 4.62−4.60 (m, 1H), 4.03−4.99 (m, 2H), 3.91−3.89 (m, 2H), 3.62−3.52 (m, 2H), 1.65 (s, 3H), 1.61 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.05, 139.7, 114.4, 97.9, 65.03, 64.1, 60.3, 39.2, 25.3, 17.7; HRMS (FAB) calcd for $C_{11}H_{16}NO_5S$ 274.0749 $(M + H)^+$, found *m/z* 274.0743.

(3,3-Dimethyl-4,4,7-trioxo-4-thia-1-azabicyclo[3.2.0]hept-2*E*-ylidene)acetaldehyde (17). To a solution of dioxolane **15** (109 mg, 0.4 mmol) in acetone (2 mL) was added pTSA (80 mg, 0.4 mmol) at 0 °C. The reaction was stirred at rt for 30 min. The solvent was removed. The mixture was diluted with EtOAc (20 mL), washed with aq $NaHCO_3$ solution and brine, dried over Na_2SO_4, and concentrated. Purification by flash chromatography (100% CH_2Cl_2 to 10% EtOAc/CH_2Cl_2) afforded product (80 mg, 87%): IR (neat, cm^{-1}) ν 1811.53, 1663.6, and 1616.5; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.95 (d, J = 6.7 Hz, 1H), 6.23(d, J = 6.7 Hz, 1H), 4.78−4.76 (m, 1H), 3.79−3.62 (m, 2H), 1.78 (s, 3H), 1.62 (s, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 188.7, 166.7, 153.2, 112.1, 64.4, 62.1, 39.1, 26.5, 17.2; HRMS (FAB) calcd for $C_9H_{12}NO_4S$ 230.0487 $(M + H)^+$, found *m/z* 230.0490.

Sodium Salt of (3,3-Dimethyl-4,4,7-trioxo-4-thia-1-azabicyclo[3.2.0]hept-2*E*-ylidene)acetic Acid (18). To a solution of **17** (57 mg, 0.25 mmol) in MeOH (2 mL) were added 5% aq NaH_2PO_4 (0.5 mL), sodium chlorite (113 mg, 1.25 mmol), and sulfamic acid (121, 1.25 mmol) at 0 °C. The solution was stirred at rt for 45 min. MeOH was removed, and the residue was dissolved in EtOAc (5 mL), washed with water and brine, dried over Na_2SO_4, and concentrated. Purification by flash chromatography (1% MeOH/CH_2Cl_2 to 20% MeOH/CH_2Cl_2) afforded product (41 mg, 68%): IR (neat, cm^{-1}) ν 3347.05, 1806.5, 1707.5, and 1637.4; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.0 (s, 1H), 6.07 (d, J = 6.7 Hz, 1H), 4.66−4.4.65 (m, 1H), 3.76−3.71 (m, 1H), 3.63−3.58 (m, 1H) 1.78 (s, 3H), 1.62 (s, 3H); HRMS (FAB) calcd for $C_9H_{12}NO_5S$ 246.0436 $(M + H)^+$, found *m/z* 246.0443.

Forthy milligrams of this acid was dissolved in EtOAc (4 mL), extracted with aq $NaHCO_3$ (25 mg of sodium bicarbonate was dissolved in 4 mL of deionized H_2O), and purified on a column of CHP-20P-deionized water as eluent to give product (25 mg): ^{1}H NMR (400 MHz, D_2O) δ 6.04 (s, 1H), 5.00−4.98 (m, 1H), 3.63 (dd, J = 12.4 Hz, J = 4.5 Hz, 1H), 3.46 (dd, J = 15.5 Hz, J = 1.9 Hz, 1H) 1.53 (s, 3H), 1.44 (s, 3H).

(3,3-Dimethyl-4,4,7-trioxo-4-thia-1-azabicyclo[3.2.0]hept-2*Z*-ylidene)acetaldehyde (19). To a solution of **16** (229 mg, 1 mmol) in acetone (5 mL) was added PTSA (200 mg, 1 mmol) at 0 °C. The reaction mixture was stirred at rt for 30 min. The solvent was removed and the mixture diluted with EtOAc (30 mL), washed with aq $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated. Purification by flash chromatography (100% CH_2Cl_2 to 10% EtOAc/CH_2Cl_2) afforded product (196 mg, 86%): IR (neat, cm^{-1}) ν 1810.9, 1673.5, 1622.0; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.85 (d, J = 7.5 Hz, 1H), 5.60 (d, J = 7.5 Hz, 1H), 4.93−4.91 (m, 1H), 3.82−3.70 (m, 2H, C6), 1.62 (s, 3H), 1.56 (s, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 189.7, 165.7, 153.1, 112.0, 64.2, 61.9, 39.5, 26.2, 17.4; HRMS (FAB) calcd for $C_9H_{12}NO_4S$ 230.0487 $(M + H)^+$, found *m/z* 230.0490.

Sodium Salt of (3,3-Dimethyl-4,4,7-trioxo-4-thia-1-azabicyclo[3.2.0]hept-2-ylidene)acetic Acid (20). To a solution of aldehyde (171 mg, 0.75 mmol) in MeOH (6 mL) were added 5% NaH_2PO_4 solution (1.5 mL), sodium chlorite (339 mg, 3.75 mmol), and sulfamic acid (363, 3.75 mmol) at 0 °C. The mixture was stirred at rt for 45 min. MeOH was removed to give crude product. The residue was dissolved in EtOAc (15 mL), washed with water and brine, dried over Na_2SO_4, and concentrated to produce the product (80 mg, 63%): IR (neat, cm^{-1}) ν 3351.05, 1802.5, 1701.5, 1630.4; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.0 (s, 1H), 5.40 (s, 1H), 4.83−4.82 (m, 1H), 3.65−3.62 (m, 2H), 1.63 (s, 3H), 1.57 (s, 3H); HRMS (FAB) calcd for $C_9H_{12}NO_5S$ 246.0436 $(M + H)^+$, found *m/z* 246.0431.

Seventy-five milligrams of this acid was dissolved in EtOAc (4 mL) and extracted with aq $NaHCO_3$ (45 mg of $NaHCO_3$ in 4 mL of deionized H_2O). The aqueous layer was purified on CHP-2OP using water as eluent to give product (20 mg): ^{1}H NMR (400 MHz, D_2O) δ 5.45 (d, J = 6.7 Hz, 1H), 5.11−5.09 (m, 1H), 3.65 (dd, J = 12.1 Hz, J = 4.9 Hz, 1H), 3.39 (dd, J = 15.3 Hz, J = 1.7 Hz, 1H) 1.50 (s, 3H), 1.42 (s, 3H).

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Supporting Information Available: Experimental details for the preparation of compounds **6** and **7**, NMR spectra of compounds **8**−**20**, and crystal data for compounds **9** and **16**. This material is available free of charge via the Internet at http://pubs.acs.org.

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