Synthesis and Preliminary Biological Evaluation of 3′-Substituted Cephem Sulfones as Potential β-Lactamase Inhibitors

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3′-Substituted cephem sulfones, as well as the unsubstituted congener, were synthesized and biologically evaluated as β-lactamase inhibitors. Sodium 3′-substituted cephalosporanate sulfones **4** and **5** were prepared starting from 7-aminodeacetoxycephalosporanic acid (7-ADCA, **6**) by a bromodeamination reaction, followed by reduction of the 7-halo derivative. Selective, radical bromination at C-3′ on the Δ^3-isomer, followed by nucleophilic substitution and then retroisomerization at Δ^2, afforded the required derivatives. 3′-Unsubstituted and 3′-acetoxycephem sulfones **2** and **3** were prepared using routine chemistry. The 3′-substituted cephem sulfone derivatives, evaluated as β-lactamase inhibitors by IC_{50} determinations, behave as weak inhibitors of the class D "oxacillinase" OXA1 from *Escherichia coli* and the class C β-lactamase of *Enterobacter cloacae* P99. Conversely, the 3′-unsubstituted cephem sulfone **2** was shown to be comparable to sulbactam and even more active than clavulanic acid against the latter enzyme. The activity of **2** against *E. cloacae* P99 was also greatly enhanced by prolonging the pre-incubation time. Considerations as to the mechanism of inhibition are also put forward.

Introduction

Since the introduction of penicillin into therapy, at the end of the first half of the last century, bacteria have developed an incredible and growing resistance to β-lactam antibiotics, essentially due to the hydrolytic ability of extremely active β-lactamases.[1] Some of these enzymes work very efficiently. The β-lactamase molecule from P99 *Enterobacter cloacae*, a class C serine hydrolase,[2] is among the most effective enzymes: it can inactivate cephaloridine[3] at a rate from one to three orders of magnitude faster than other chromosomal β-lactamases.[1a] In order to oppose the destructive action of β-lactamases, one strategy consists of modifying the structure of the β-lactam antibiotic, in order to render it insensitive to the β-lactamase attack. A second approach uses a reagent, typically a β-lactam derivative, which incapacitates the β-lactamase, in synergy with the β-lactam antibiotic. Clavulanic acid (see Figure 1) is the archetype of β-lactamase inhibitors:[4] in synergistic mixture with amoxicillin, under the name "augmentin", it arrived to the practice some years ago.

Synthesis of new classes of β-lactamase inhibitors, and particularly studies on their mechanism of action, are the basis for the design of more active molecules. Knowles demonstrated the mechanism of inactivation of RTEM β-lactamase by penicillanic acid sulfone (sulbactam; Figure 1), and extended these results to clavulanic acid.[5] Buynak et al. synthesized 7-vinylidenecephem sulfones as β-lactamase inhibitors; in particular sodium 7-(2′α-*tert*-butylvinylidene)-

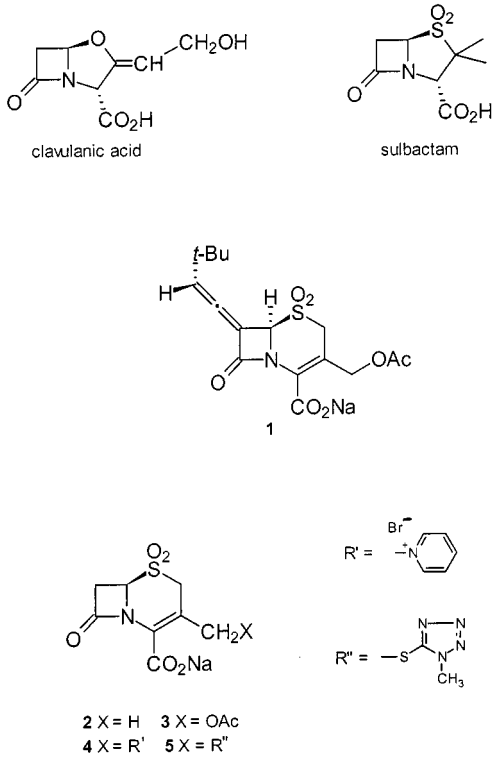

Figure 1. Structures of known, potent β-lactam inhibitors, and of the 3′-substituted cephem sulfones **2**–**5** presented in this paper

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cephalosporanate sulfone (**1**, Figure 1) was found to be a potent inhibitor of *E. cloacae* P99.[6] In their outstanding study, they proposed acetate elimination from the 3′-position of their derivatives as the necessary step towards irreversible inactivation of the enzyme. This last observation parallels the work of Doherty on the inhibition of human leukocyte elastase by cephalosporin sulfone esters,[7] as well as Pratt's study on the importance of the 3′-leaving group in the inhibition of PC1 β-lactamase of *Staphylococcus aureus* by cephalosporin sulfide derivatives.[8]

Several years ago we published a paper where, inter alia, hydrolysis under biomimetic conditions (methanolic triethylamine) of deacetoxycephalosporin sulfone derivatives were studied.[9] Following our interest in this field, we would now like to report on the synthesis of sodium 3′-substituted cephalosporanate sulfones, by analogy with sulbactam devoid of groups at the 7-position. A preliminary biological evaluation is also reported, where it is demonstrated that the leaving ability of the group at the 3′-position does not contribute to the biological potency of these compounds. Conversely, the absence of substituents at that position gives rise to the only compound with activity comparable with that of sulbactam against the β-lactamase derived from *E. cloacae* strain P99, thus allowing considerations about the structural prerequisites of cephem sulfones as possible β-lactamase inhibitors.

Results and Discussion

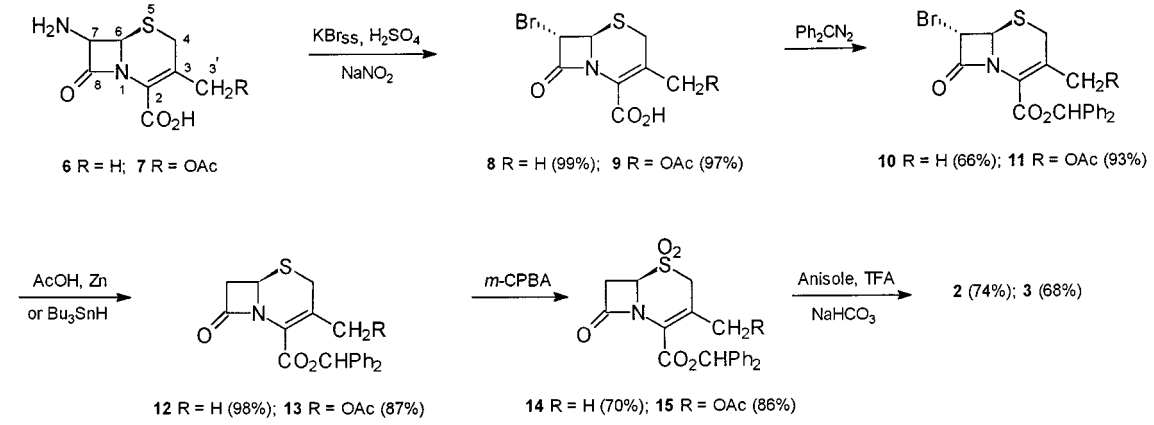
Synthesis

The key step in the preparation of cephem sulfones **2** and **3**, described in Scheme 1, consists in the reductive deamination at C-7 of 7-aminodeacetoxycephalosporanic acid (7-ADCA: **6**) and 7-aminocephalosporanic acid (7-ACA: **7**), respectively.

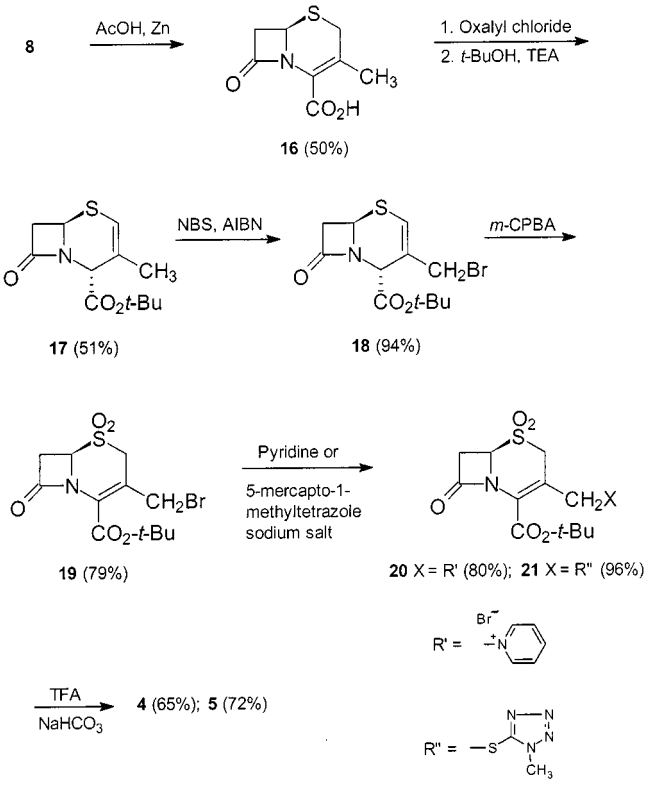
This kind of procedure proceeds by a halodeamination reaction followed by reduction of the 7-halo derivative. In order to obtain the 7-chlorocephalosporanic acids, we initially followed a well-known literature procedure,[10] by treating the substrate with $NaNO_2$/HCl in methanol/water solution. In our case, conversion yields where not satisfactory (less than 60%), the production of the chloro derivative being lowered even more by the presence in the product mixture of the 7-α-methoxy derivative (25%). Also, when the reaction was performed on the same substrate used in the reference (i.e.: 6-aminopenicillanic acid) the 6-α-methoxy derivative, not reported in the literature,[10] formed in 22% yields. Very good results were obtained when the diazotization reaction was performed in aqueous H_2SO_4, in a saturated solution of potassium bromide: isomerically pure **8** and **9** were thus produced in quantitative yields.[11] The *trans* configuration of the β-lactam protons was assigned on the basis of their coupling constant (1.5−2.0 Hz) in the ^{1}H NMR spectra.[12] By analogy with this procedure, the chlorinated analogs of **8** and **9** were also obtained, as a 3:1 mixture of the *trans* and *cis* isomers in an overall yield of 66%. The diazotization reaction in this case was performed in aqueous HCl, in the presence of saturated ammonium chloride.

The benzhydryl esters **10** and **11** were then prepared, and almost quantitatively reduced by Zn/AcOH to produce the cephem derivatives **12** and **13**. Lower yields (ca. 80%) have been obtained by using Bu_3SnH.[13] Excess *m*-CPBA was employed to oxidize the sulfur moiety to sulfone and deprotection of compounds **14** and **15** finally produced the sodium salts **2** and **3** to be used in the biological assays. As shown in Scheme 2, cephem sulfones functionalized at C-3′ were prepared by way of the 3′-bromocefem precursor **19**.

Since in this case the direct bromination at C-3′ of cephalosporanic sulfone esters[14] fails, giving rise to the 2α-bromo derivatives, we decided to adapt to our substrate, using a different approach involving bromination of a Δ^3-cephem precursor.[15] Thus, compound **8** was reduced by Zn/AcOH to produce cephalosporanic acid **16**, which was then converted into *tert*-butyl Δ^3-cephalosporanate **17** by treatment with oxalyl chloride followed by dehydrochlorination of the resulting acyl chloride with triethylamine, and final trapping of the intermediate ketene with *tert*-butyl alcohol.[15a,16] Bromination at C-3′ was performed, through a radical mechanism, using NBS to produce the bromide **18**, which due to its instability, was rapidly oxidized to the bro-



Scheme 1. Synthetic route to cephalosporin sulfones **2** and **3**



Scheme 2. Synthetic route to 3′-substituted cephalosporin sulfones **4** and **5**

mocephem sulfone **19**. During this reaction, shift of the double bond to the Δ^2-position also occurred. This material was then treated with selected nucleophiles to give, in quantitative yields, the corresponding derivatives **20** and **21**. These were finally deprotected to produce the sodium 3′-functionalized cephalosporanate sulfones **4** and **5**. It is worth noting that the variation of the 3′-substituents of cephems has proved to be particularly useful in improving antibiotic potency;[17] we have chosen pyridine and 5-mercapto-1-methyltetrazole, good leaving groups, as examples of easily accessible *N*- and *S*-substituents, respectively, also considering that the corresponding cephem antibiotics show enhanced activity against *S. aureus* as well as Gram-negative microorganisms.[18]

Biological Evaluation

Cephem sulfones **2**, **3**, **4**, and **5** were evaluated as inhibitors of the class C β-lactamase of *Enterobacter cloacae* strain P99 and the class D plasmid-mediated "oxacillinase" OXA1[19] from *Escherichia coli* by IC_{50} determination. The measured values, which are reported in Table 1, represent the concentration of inhibitor necessary to reduce the initial rate of hydrolysis of nitrocefin by 50%.

β-Lactamase preparations and inhibitors were incubated, before antibiotic administration, for 5 min at 37° C. Data were compared to the known, potent inhibitors sulbactam and clavulanic acid. Within the series of synthesized compounds, the 3′-unsubstituted cephem sulfone **2** appears to be the only efficient derivative, being fourfold better than clavulanic acid against *E. cloacae* P99 and sevenfold less active than the most potent inhibitor sulbactam. The activity of **2** was also studied in greater detail in a separate experiment, by determining the IC_{50} values against *E. cloacae* P99 as a function of the pre-incubation time (the plot is given in Figure 2).

Table 1. β-Lactamase inhibitory activity

Compound	IC_{50} (μg/mL)[a]	
	E. Cloacae P99	*E. Coli* OXA1
Sulbactam	7.6 ± 1	2.6 ± 0.7
Clavulanic acid	210 ± 36	0.43 ± 0.04
2	55 ± 2	92 ± 2
3	> 250	240 ± 5
4	> 250	> 250
5	> 250	184 ± 20

[a] Determined after 5 min pre-incubation of enzyme and inhibitor.

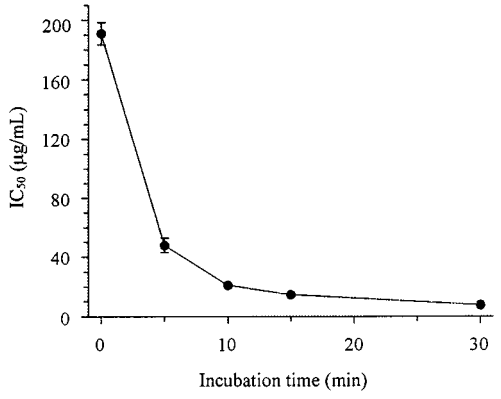


Figure 2. Time-dependent inhibition of *E. cloacae* P99 β-lactamase by the cephalosporin sulfone **2**; abscissa values indicate the pre-incubation time of the enzyme (37 °C) with compound **2** before addition of nitrocefin substrate

Discussion

While cephalosporins have been widely studied with respect to the importance of the 3′-leaving groups for antibacterial activity,[8,18] nothing is reported about this aspect in the sulfone series, as far as the β-lactamase inhibitory property is concerned. By contrast, it has been demonstrated that in the mechanism of inhibition of elastase by cephalosporin sulfone esters, a histidine residue attacks the 3′-position to form the irreversibly bound enzyme.[7] Moreover, data exist showing that in the hydrolysis of cephem sulfides by *E. cloacae* P99, a 3-*exo*-methylenecephem moiety is produced in the transiently inhibited enzyme.[8,20] Much is also known about the inhibition mechanism against β-lactamases of the 6-unsubstituted penam sulfone, sulbactam,[5] as well as of 7-alkylidenecephem sulfones.[6]

The IC_{50} data presented in Table 1 indicate that the 3′-unsubstituted cephalosporanic acid sulfone **2**, by analogy with sulbactam devoid of substituents at C-7, possess interesting inhibitory activity against serine β-lactamases, in particular against the very active *E. cloacae* P99.[21] While prac-

tically all the compounds synthesized behave, to the same extent, as weak inhibitors of the class D β-lactamase OXA1,[19] the 3′-substituted cephem sulfones **3**, **4**, and **5** do not show any measurable activity against the type C cephalosporinase P99. This result, combined with the relatively good activity shown by **2** (superior to clavulanic acid itself), rules out any possible contribution of the leaving group at C-3′, as well as of the 3-*exo*-methylene functionality,[22] to the rapid formation of a stabilized intermediate. On the positive side, the low steric demand of C-3′ in **2** is a clear indication of a significantly crowded region in *E. cloacae* P99 hydrolase close to the cephalosporin 3-position.

Compound **2**, as revealed by the plot in Figure 2, exhibits a "progressive" inhibition. This suggests a suicide mechanism or irreversible inhibition, which results in a reduction of the amount of potentially free enzyme by prolonging the pre-incubation time. Once a possible stabilization promoted by the 3′-leaving group is excluded, the explanation to this behavior still remains to be answered. It is possible that SO_2 elimination,[9] which eventually generates a partially nucleophilic carbon atom at the former cephalosporin 4-position, could favor the formation of a stabilized enzyme.

Conclusion

In summary, we have developed a high-yielding synthetic strategy for the preparation of 3′-functionalized cephem sulfones free from groups at C-7. In particular, the very high yields of the reaction for the production of isomerically pure 7α-bromocephalosporanic acid could be exploited for other synthetic purposes.

Cephalosporanic acid sulfone **2** exhibits a fairly good, progressive inhibitory activity against the serine β-lactamase of *E. cloacae* P99. On the grounds of the observed lack of activity of the 3′-functionalized congeners against the same microorganism, it is conceivable that the enzyme active structure does not accommodate any leaving group at the cephalosporin 3′-position.

Experimental Section

Biological Assays. − β-Lactamase Preparation: Strains *E. cloacae* P99 and *E. coli* J53 R455 (OXA-1) were obtained from Glaxo Wellcome cultures collection. Enzymes were prepared from 1 L of shaken cultures incubated for 16−20 h at 35 °C. The cells were harvested at 10.000 *g* for 15 min, suspended in 20 mM phosphate buffer pH = 7.0, harvested again and finally re-suspended in 5 mL of the same buffer. The cells were then disrupted by passing them twice through an Aminco French Pressure Cell. The disrupted cell suspension was ultra-centrifuged at 100.000 *g* for 2 h by using a fixed-angle TY-65 Beckman rotor. The resulting supernatant was stored at −80 °C and used as a crude enzyme preparation. − **IC_{50} Determination:** IC_{50} was determined as the concentration of inhibitor needed to reduce the initial rate of hydrolysis of nitrocefin by 50%.[23] Reactions were carried out in microtiter plates. A 10 μL water solution of inhibitor at varying concentrations was pre-incubated with 140 μL of the crude enzyme preparation (vide supra) for 5 min at 37 °C, the reaction was then started by addition of a 200 μM nitrocefin phosphate buffer solution (50 mM at pH = 7.0, 100 μL). Hydrolysis was monitored at 480 nm over a period of 10 min at 12 s intervals by using a SpectraMax 250 Microplate Reader (Molecular Dynamics). Initial rates of hydrolysis were calculated from the slope of the absorbance change vs. time over the linear range, and IC_{50} values were determined by fitting data with the Grafit program. For the determination of the time-dependent inhibition of cephalosporin sulfone **2**, experiments were carried out as described above, with the exception of the pre-incubation time that was varied from 0 to 30 min, both in the reaction mixtures containing varying inhibitor concentrations and in the reference samples. The enzyme concentrations used in these assays were adjusted to produce 75% hydrolysis of nitrocefin in 10 min under standard conditions.

7α-Bromo-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid (8): 7-ADCA (6, 10 g, 46.7 mmol) was dissolved in aqueous H_2SO_4 (400 mL, 0.5 N), saturated with potassium bromide, and the solution cooled to 0−4 °C with an ice bath. $NaNO_2$ (5 g, 72.5 mmol) was slowly added under vigorous stirring, while maintaining the reaction temperature below 4−5 °C. After the addition, the reaction mixture was allowed to warm to room temperature under constant stirring, then stirred for one additional hour and finally extracted with ethyl acetate (4 × 100 mL). The organic layer was washed with water, then brine, and dried. The solvent was removed under vacuum to give pure **8** (TLC) as a white solid (12.8 g, 99%). − IR ($CHCl_3$): $\tilde{\nu}$ = 1790 (C=O β-lactam), 1715 cm^{-1} (C=O carboxyl). − ^1H NMR (200 MHz, $CDCl_3$): δ = 2.16 (s, 3 H), 3.33 (AB system, *J* = 18.0 Hz, 2 H), 5.06 (d, *J* = 1.5 Hz, 1 H), 5.37 (d, *J* = 1.5 Hz, 1 H), 8.00 (br. s, 1 H).

3-(Acetoxymethyl)-7α-bromo-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid (9): 7-ACA (**7**, 10 g, 36.7 mmol) was dissolved in aqueous H_2SO_4 (400 mL, 0.5 N), saturated with potassium bromide, and treated with $NaNO_2$ (3.8 g, 55 mmol), as previously described for the preparation of **8**, to give pure **9** (TLC) as a white solid (12 g, 97%). − IR ($CHCl_3$): $\tilde{\nu}$ = 1800 (C=O β-lactam), 1798 cm^{-1} (C=O carboxyl). − ^1H NMR (200 MHz, $CDCl_3$): δ = 2.13 (s, 3 H), 3.65 (AB system, *J* = 18.0 Hz, 2 H), 4.76 (d, *J* = 1.5 Hz, 1 H), 4.86 (d, *J* = 1.5 Hz, 1 H), 5.00 (AB system, *J* = 14.0 Hz, 2 H), 9.30 (br. s, 1 H).

Benzhydryl 7α-Bromo-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (10): To a stirred solution of **8** (1.3 g, 4.8 mmol) in ethyl acetate (30 mL) Ph_2CN_2 (1.4 g, 7.2 mmol) was added dropwise at room temperature. After TLC showed that the starting material was consumed (ca. 8 h), the reaction solvents were evaporated to dryness in vacuo. The residue was crystallized from MeOH to give pure **10** (3.2 g, 66%). − IR ($CHCl_3$): $\tilde{\nu}$ = 1795 (C=O β-lactam) 1730 cm^{-1} (C=O ester). − ^1H NMR (200 MHz, $CDCl_3$): δ = 2.06 (s, 3 H), 3.18 (AB system, *J* = 17.5 Hz, 2 H), 4.64 (d, *J* = 1.6 Hz, 1 H), 4.75 (d, *J* = 1.6 Hz, 1 H), 6.90 (s, 1 H), 7.35 (m, 10 H).

Benzhydryl 3-(Acetoxymethyl)-7α-bromo-8-oxo-5-thia-1-azabicyclo-[4.2.0]oct-2-ene-2-carboxylate (11): Compound **9** (1.1 g, 3.3 mmol) was dissolved in ethyl acetate (30 mL) and treated with Ph_2CN_2 (0.95 g, 4.9 mmol), as previously described for the preparation of **10**. The residue was dissolved in acetone, activated decolorizing carbon was added and the hot solution was slowly stirred for 30 min and then filtered trough silica gel. The solvent was removed under vacuum to give pure **11** (1.54 g, 93%). − IR ($CHCl_3$): $\tilde{\nu}$ = 1800 (C=O β-lactam) 1740 (C=O esters) cm^{-1}. − ^1H NMR (200 MHz, $CDCl_3$): δ = 1.96 (s, 3 H), 3.43 (AB system, *J* = 17.0 Hz, 2 H), 4.65 (d, *J* = 1.5 Hz, 1 H), 4.80 (d, *J* = 1.5 Hz, 1 H), 4.85 (AB system, *J* = 13.5 Hz, 2 H), 6.95 (s, 1 H), 7.35 (m, 10 H).

Benzhydryl 3-Methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (12). − Method A: To a stirred solution of **10** (5.6 g, 12.6 mmol) in acetic acid (100 mL) Zn powder (2.5 g, 37.8 mmol) was added in three portion over a period of 1.5 h at room temperature. After TLC showed that the starting material was consumed (c.a. 3 h), the mixture was slowly poured onto a stirred mixture of crushed ice in water (12 mL), AcOEt (75 mL), and solid $NaHCO_3$ (200 g). Effervescence was controlled by dropwise additions of diethyl ether. The resulting mixture was stirred for an additional 15 min and filtered, eventually, to eliminate excess $NaHCO_3$. The organic layer was separated and the aqueous phase thoroughly extracted with ethyl acetate (3 × 100 mL). The combined organic layers were washed with water and brine, then dried and concentrated to dryness in vacuo. The crude solid was crystallized from MeOH to give pure **12** (4.5 g, 98%). − **Method B:** To a solution of **10** (200 mg, 0.45 mmol) in anhydrous toluene (5 mL), Bu_3SnH (0.16 mL, 0.6 mmol), and AIBN (85 mg, 0.52 mmol) were sequentially added. The reaction was carried out in a closed vessel at 80 °C for 4 h. The reaction mixture was then poured onto ice-cold aqueous HCl (20 mL, 0.2 N) and the layers were separated. The aqueous layer was extracted with ethyl acetate (2 × 10 mL). The combined organic layers were washed with water, aqueous HCl (0.2 N), and brine, dried, and concentrated to dryness in vacuo. Preparative-layer chromatography (eluent: toluene/ethyl acetate, 97:3) yielded **12** (140 mg, 85%). − IR ($CHCl_3$): $\tilde{\nu}$ = 1780 (C=O β-lactam) 1730 cm^{-1} (C=O ester). − ^1H NMR (200 MHz, $CDCl_3$): δ = 2.00 (s, 3 H), 2.76−2.84 (dd, *J* = 2.1, *J* = 15.5 Hz, 1 H), 3.25 (AB system, *J* = 18.2 Hz, 2 H), 3.45−3.53 (dd, *J* = 5.0, *J* = 15.5 Hz, 1 H), 4.55−4.57 (dd, *J* = 5.0, *J* = 2.1, 1 H), 6.95 (s, 1 H), 6.86 (s, 1 H), 7.35 (m, 10 H).

Benzhydryl 3-(Acetoxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (13): A solution of **11** (10.7 g, 21.3 mmol) in acetic acid (200 mL) was treated with Zn powder (4.2 g, 63.9 mmol), as previously described for the preparation of **12**, to give **13** (7.8 g, 87%). − IR ($CHCl_3$): $\tilde{\nu}$ = 1790 (C=O β-lactam) 1740 cm^{-1} (C=O esters). − ^1H NMR (200 MHz, $CDCl_3$): δ = 2.00 (s, 3 H), 3.13−3.21 (dd, *J* = 2.0, *J* = 16.0 Hz, 1 H), 3.61−3.69 (dd, *J* = 5.0, *J* = 16.0 Hz, 1 H), 3.47 (AB system, *J* = 17.3 Hz, 2 H), 4.65−4.68 (dd, *J* = 2.0, *J* = 5.0, 1 H), 4.85 (AB system, *J* = 13.0 Hz, 2 H), 7.00 (s, 1 H), 7.35 (m, 10 H).

Benzhydryl 3-Methyl-5,5,8-trioxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (14): To a solution of **12** (3 g, 8.2 mmol) in CH_2Cl_2 (80 mL) *m*-CPBA (90%, 3.6 g 18.9 mmol) was added in one portion. The reaction mixture was stirred at room temperature for 3 h and then sequentially treated with 5% $Na_2S_2O_5$, saturated $NaHCO_3$ (3 × 70 mL), and brine, then dried and concentrated to dryness in vacuo. The residue was crystallized from MeOH to give pure **14** (2.5 g, 70.%). − IR ($CHCl_3$): $\tilde{\nu}$ = 1805 (C=O β-lactam) 1730 (C=O ester) 1340 and 1120 cm^{-1} (SO_2). − ^1H NMR (200 MHz, $CDCl_3$): δ = 2.05 (s, 3 H), 3.13−3.21 (dd, *J* = 2.0, *J* = 15.8 Hz, 1 H), 3.66−3.74 (dd, *J* = 4.6, *J* = 15.8 Hz, 1 H), 3.45 (AB system, *J* = 17.7 Hz, 2 H), 4.65−4.67 (dd, *J* = 2.0, *J* = 4.6, 1 H), 6.90 (s, 1 H), 7.33 (m, 10 H).

Benzhydryl 3-(Acetoxymethyl)-5,5,8-trioxo-5-thia-1-azabicyclo-[4.2.0]oct-2-ene-2-carboxylate (15): Compound **13** (3.7 g, 8.7 mmol) was dissolved in CH_2Cl_2 (100 mL) and treated with *m*-CPBA (90%, 5.03 g, 26.2 mmol), as previously described for the preparation of **14**, to give **15** (3.4 g, 86%). − IR ($CHCl_3$): $\tilde{\nu}$ = 1790 (C=O β-lactam), 1740 (C=O esters), 1385 and 1115 cm^{-1} (SO_2). − ^1H NMR (200 MHz, $CDCl_3$): δ = 2.00 (s, 3 H), 3.26−3.34 (dd, *J* = 1.9, *J* = 15.6 Hz, 1 H), 3.51−3.59 (dd, *J* = 5.2, *J* = 15.6 Hz, 1 H), 4.3 (AB system, *J* = 18.3 Hz, 2 H), 4.7 (AB system, *J* = 13.8 Hz, 2 H), 5.35−5.38 (dd, *J* = 1.8, *J* = 5.2, 1 H), 6.95 (s, 1 H), 7.35 (m, 10 H).

Sodium 3-Methyl-5,5,8-trioxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (2): To a solution of **14** (300 mg, 0.76 mmol) in anisole (2.5 mL), kept at 0° C, trifluoroacetic acid (7 mL) was added in one portion. The mixture was stirred for 10 min and concentrated in vacuo. The residue was taken up twice with CH_2Cl_2 and concentrated in vacuo to remove residual traces of TFA. The crude product was dissolved in ethyl acetate (70 mL) and then poured into a vigorously stirred solution of $NaHCO_3$ (635 mg, in 70 mL of water). The separated aqueous layer was then lyophilized to yield **2** (142 mg, 74%). − IR (KBr): $\tilde{\nu}$ = 1720 (C=O β-lactam) 1600 (C=O carboxylate), 1330 and 1130 cm^{-1} (SO_2). − ^1H NMR (200 MHz, [D_6]DMSO): δ = 2.05 (s, 3 H), 3.30−3.37 (dd, *J* = 1.7, *J* = 15.0 Hz, 1 H), 3.58−3.65 (dd, *J* = 4.5, *J* = 15.0 Hz, 1 H), 4.06 (AB system, *J* = 18.0 Hz, 2 H), 5.13 (m, 1 H). − $[\alpha]_D^{20}$ = +34 (*c* = 1, H_2O). − ESI MS: *m*/*z* = 230.2 $[M - Na]^-$ (calcd. 230.0). − Acid: $[\alpha]_D^{20}$ = +97 (*c* = 1, MeOH); m.p. 158−159 °C (ref.[21] $[\alpha]_D$ = +78; m.p. 141−144 °C).

Sodium 3-(Acetoxymethyl)-5,5,8-trioxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxylate (3): Compound **15** (300 mg, 0.66 mmol) was dissolved in anisole (2.5 mL) and treated with TFA (7 mL), as previously described for the preparation of **2**, to give **3** (91 mg, 68%). − IR ($CHCl_3$): $\tilde{\nu}$ = 1730 (C=O β-lactam), 1600 (C=O carboxylate), 1330 and 1135 cm^{-1} (SO_2). − ^1H NMR) (200 MHz, [D_6]DMSO): δ = 2.15 (s, 3 H), 3.38−3.46 (dd, *J* = 2.1, *J* = 16.0 Hz, 1 H), 3.61−3.69 (dd, *J* = 5.2, *J* = 16.0 Hz, 1 H), 4.16 (AB system, *J* = 17.6 Hz, 2 H), 4.92 (AB system, *J* = 13.0 Hz, 2 H), 5.16 (m, 1 H). − $[\alpha]_D^{20}$ = +4.8 (*c* = 1, H_2O). − ESI MS: *m*/*z* = 288.3 $[M - Na]^-$ (calcd. 288.0). − Acid: $[\alpha]_D^{20}$ = +8.3 (*c* = 1, MeOH); m.p. 87−89 °C.

3-Methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid (16): To a stirred solution of **8** (10 g, 35.9 mmol) in acetic acid (30 mL) a suspension of Zn powder (11.3 g, 172.3 mmol) in acetic acid (20 mL) was added. The reaction mixture was heated at 70 °C for 10 min and then concentrated to dryness. The residue was dissolved in aqueous HCl (400 mL, 1 N) and stirred for an additional 15 min, then filtered. The filtrate was extracted with $CHCl_3$ (10 × 100 mL) and the combined organic layers were washed with brine and dried. The solvent was removed under vacuum to give crude **16** (3.6 g, 50%), which was used in the next step without further purification. − IR (KBr): $\tilde{\nu}$ = 1790 (C=O β-lactam) 1730 cm^{-1} (C=O carboxyl). − ^1H NMR (200 MHz, [D_6]DMSO): δ = 1.95 (s, 3 H), 2.82−2.90 (dd, *J* = 2.0, *J* = 16.0 Hz, 1 H), 3.61 (AB system, *J* = 17.8 Hz, 2 H), 3.56−3.64 (dd, *J* = 5.0, *J* = 16.0 Hz, 1 H), 4.70−4.72 (dd, *J* = 5.0, *J* = 2.0, 1 H).

***tert*-Butyl 3-Methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylate (17):** The reaction was carried out under nitrogen. To a stirred solution of **16** (2 g, 10.0 mmol) in anhydrous benzene (300 mL), containing four drops of DMF, oxalyl chloride (1.75 mL, 20.1 mmol) was added. The reaction mixture was stirred at room temperature for 10 min and then heated at 60 °C for 1 h; the solvent was then removed in vacuo and the residue dissolved in anhydrous CH_2Cl_2 (100 mL). The resulting mixture was cooled to 0 °C and a mixture of triethylamine (3.5 mL, 25.0 mmol), anhydrous CH_2Cl_2 (60 mL), and *tert*-butyl alcohol (11 mL) was rapidly added. After 10 min, the clear solution was diluted with $CHCl_3$ (50 mL) and washed with aqueous HCl (200 mL, 1 N) and brine, then dried and concentrated to dryness in vacuo. The residue was separated by column chromatography (eluent: *n*-hexane/ethyl acetate, 1:1) to give **17** (1.3 g, 51%). − IR ($CHCl_3$): $\tilde{\nu}$ = 1790 (C=O β-lactam), 1730

cm^{-1} (C=O ester). − ^1H NMR (200 MHz, $CDCl_3$): δ = 1.45 (s, 9 H), 1.90 (s, 3 H), 2.95−3.03 (dd, *J* = 1.5, *J* = 14.7 Hz, 1 H), 3.48−3.56 (dd, *J* = 4.0, *J* = 14.7 Hz, 1 H), 4.63 (s, 1 H), 5.02 (m, 1 H). 5.97 (s, 1 H).

***tert*-Butyl 3-(Bromomethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylate (18):** To a solution of **19** (1.3 g, 5.1 mmol) in CCl_4 (80 mL) NBS (1.4 g, 7.6 mmol) and AIBN. (130 mg, 0.79 mmol) were added. The reaction mixture was heated to reflux under nitrogen and stirred for 4 h. The mixture was cooled to 0 °C, filtered and the filtrate concentrated in vacuo. The crude product **18** (1.6 g, 94%) was immediately used, without further purification, for the next oxidation step. − IR ($CHCl_3$): $\tilde{\nu}$ = 1795 (C=O β-lactam), 1740 cm^{-1} (C=O ester). − ^1H NMR (200 MHz, $CDCl_3$): δ = 1.54 (s, 9 H), 3.02−3.09 (dd, *J* = 1.5, *J* = 14.2 Hz, 1 H), 3.54−3.61 (dd, *J* = 4.2, *J* = 14.2 Hz, 1 H), 4.22 (AB system, *J* = 10.8 Hz, 2 H), 5.05−5.07 (dd, *J* = 4.2, *J* = 1.5, 1 H), 5.14 (br. s, 1 H), 6.55 (br. s, 1 H).

***tert*-Butyl 3-(Bromomethyl)-5,5,8-trioxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxylate (19):** Compound **18** (1.5 g, 4.49 mmmol) was dissolved in CH_2Cl_2 (55 mL) and treated with *m*-CPBA (90%, 2.6 g, 13.5 mmol), as previously described for the preparation of **14**. The residue was purified by column chromatography (eluent: *n*-hexane/ethyl acetate, 1:1) to give **19** (1.3 g, 79%). − IR ($CHCl_3$): $\tilde{\nu}$ = 1800 (C=O β-lactam), 1740 (C=O ester), 1320 and 1130 cm^{-1} (SO_2). − ^1H NMR (200 MHz, $CDCl_3$): δ = 1.57 (s, 9 H), 3.41−3.51 (dd, *J* = 5.0, *J* = 16.0 Hz, 1 H), 3.59−3.68 (dd, *J* = 2.4, *J* = 16.0 Hz, 1 H), 3.96 (AB system, *J* = 18.0 Hz, 2 H), 4.32 (AB system, *J* = 11.0 Hz, 2 H), 4.8 (m, 1 H).

1-{[2-(*tert*-Butoxycarbonyl)-5,5,8-trioxo-5-thia-1-azabicyclo[4.2.0]-oct-2-en-3-yl]methyl}pyridinium Bromide (20): A solution of **17** (900 mg, 2.46 mmol) in dry pyridine (7 mL) was left at room temperature for 1.5 h. Diethyl ether (7 mL) was then added and the solid **20** collected by filtration and washed with diethyl ether (720 mg, 80%). − IR (KBr): $\tilde{\nu}$ = 1800 (C=O β-lactam), 1740 (C=O ester), 1330 and 1120 cm^{-1} (SO_2). − ^1H NMR (200 MHz, [D_6]DMSO): δ = 1.56 (s, 9 H), 3.44−3.52 (dd, *J* = 2.4, *J* = 16.0 Hz, 1 H), 3.81−3.89 (dd, *J* = 2.4, *J* = 16.0 Hz, 1 H), 4.32 (AB system, *J* = 17.6 Hz, 2 H), 5.48 (m, 1 H), 5.58 (AB system, *J* = 14.7 Hz, 2 H), 8.26−9.14 (m, 5 H).

***tert*-Butyl 5,5,8-Trioxo-3-(5-tetrazolylmethyl)-5-thia-1-azabicyclo-[4.2.0]oct-2-ene-2-carboxylate (21):** Sodium 1-methyltetrazole-5-thiolate (151 mg, 1.1 mmol) was added to a solution of **19** (400 mg, 1.1 mmol), in CH_3CN (40 mL). The mixture was stirred at 50 °C for 30 min and then worked up by diluting with ethyl acetate (60 mL) and washing with H_2O and brine; after drying, the solvent was evaporated in vacuo to give pure **21** (425 mg, 96%). − IR ($CHCl_3$): $\tilde{\nu}$ = 1790 (C=O β-lactam), 1730 (C=O ester), 1320 and 1130 cm^{-1} (SO_2). − ^1H NMR ($CDCl_3$): δ = 1.56 (s, 9 H), 3.41−3.49 (dd, *J* = 4.9, *J* = 16.0 Hz, 1 H), 3.56−3.64 (dd, *J* = 2.3, *J* = 16.0 Hz, 1 H), 3.93 (s, 3 H), 4.13 (AB system, *J* = 18.7 Hz, 2 H), 4.29 (AB system, *J* = 13.9 Hz, 2 H), 4.76. (m, 1 H).

Sodium Bromide Compound with 5,5,8-Trioxo-3-(pyridinium-1-ylmethyl)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (1:1) (4): Compound **20** (300 mg, 0.82 mmol) was dissolved in anisole (2.5 mL) and treated with TFA (7 mL), as previously described for the preparation of **2**, to give **4** (177 mg, 65%). − IR (KBr): $\tilde{\nu}$ = 1795 (C=O β-lactam), 1600 (C=O carboxylate), 1335 and 1120 cm^{-1} (SO_2). − ^1H NMR (200 MHz, [D_6]DMSO): δ = 3.41−3.49 (dd, *J* = 2.2, *J* = 16.0 Hz, 1 H), 3.78−3.86 (dd, *J* = 2.2, *J* = 16.0 Hz, 1 H), 4.43 (AB system, *J* = 17.8 Hz, 2 H), 5.51 (m, 1 H), 5.52 (AB system, *J* = 14.5 Hz, 2 H), 8.31−9.18 (m, 5 H). − $[\alpha]_D^{20}$ = −71 (*c* = 1, H_2O). − ESI MS: *m/z* = 309.3 $[M - NaBr + H]^+$ (calcd. 309.0). − M.p. > 120 °C (dec.).

Sodium 5,5,8-Trioxo-3-(5-tetrazolylmethyl)-5-thia-1-azabicyclo-[4.2.0]oct-2-ene-2-carboxylate (5): A solution of **21** (300 mg, 0.75 mmol) in anisole (2.5 mL) was treated with TFA (7 mL), as previously described for the preparation of **2**, to give **5** (198 mg, 72%). − IR (KBr): $\tilde{\nu}$ = 1795 (C=O β-lactam), 1600 (C=O carboxylate), 1330 and 1135 cm^{-1} (SO_2). − ^1H NMR (200 MHz, [D_6]DMSO): δ = 3.36−3.44 (dd, *J* = 2.3, *J* = 16.0 Hz, 1 H), 3.66−3.74 (dd, *J* = 2.3, *J* = 16.0 Hz, 1 H), 4.00 (s, 1 H), 4.37 (AB system, *J* = 14.0 Hz, 2 H), 4.40 (AB system, *J* = 18.0 Hz, 2 H), 5.19 (m, 1 H). − $[\alpha]_D^{20}$ = −88 (*c* = 1, H_2O). − ESI MS: *m/z* = 344.1 $[M - Na]^-$ (calcd. 344.0). − M.p. > 80 °C (dec.).

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