Synthesis and Biological Activity of 7-Alkylidenecephems

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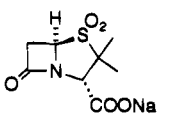
Several 7-alkylidenecephalosporins were synthesized and biologically evaluated as β-lactamase inhibitors. The three β-lactamase enzymes used in this study included two type C β-lactamases, derived from *Enterobacter cloacae* P99 and *E. cloacae* SC12368, and one type A β-lactamase, derived from *Escherichia coli* WC3310. Of the cephalosporins prepared, compound **7e**, the sodium salt of 7-[(*Z*)-(2′-pyridyl)methylene]cephalosporanic acid sulfone, was found to have excellent inhibitory properties against both type C enzymes. Also, compound **7f**, the sodium salt of 7-[(*Z*)-(*tert*-butoxycarbonyl)methylene]cephalosporanic acid sulfone showed high activity as an inhibitor of the type A enzyme. The inhibition kinetics of **7e** were further explored. The IC_{50} value of **7e** indicated that this compound was approximately 20-fold more active than tazobactam against the enzyme derived from *E. cloacae* P99 and 167-fold more active than tazobactam against the enzyme derived from *E. cloacae* SC12368. A plot of enzymatic activity vs incubation time with stoichiometric amounts of inhibitor reveals a rapid deactivation of the enzyme followed by an extremely slow reactivation. **7e** exhibited a second-order rate constant of $k_3' = 5.3 \times 10^6$ L/mol·min, and a partition ratio of approximately 20:1 inhibitor:enzyme was determined for this inhibitor. After separation of excess inhibitor with Sephadex filtration, a rate constant of enzyme reactivation was measured at $k_{reactiv} = 1.0 \times 10^{-3}$ s^{-1}. Following 24 h of incubation of enzyme with a large excess of inhibitor and sephadex filtration to remove excess inhibitor, the enzyme was able to recover only 43% of its original activity, indicating an irreversible component to the inhibition. Potential mechanisms of inhibition for both **7e** and **7f** are suggested.

Introduction

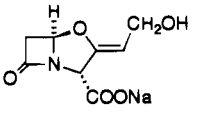
The resistance of bacteria to β-lactam antibiotics is frequently caused by the production of β-lactamase enzymes.[1] On the basis of molecular structure, the known β-lactamases have been divided into four distinct classes: A, B,[2] C,[3] and D.[4,5] Classes A, C, and D are serine hydrolases which act by hydrolyzing the β-lactam bond in antibiotics via a nontraditional activated serine complex. Class A β-lactamases, which include RTEM, are generally more specific for penicillins whereas class C β-lactamases (*Citrobacter freundii*, ECP99) are most effective against cephalosporins. Class D β-lactamases, or "oxacillinases" (OXA1, OXA2, PSE2), are relatively newly discovered proteins, and their substrate specificity has not been fully elucidated. Class B is a zinc-containing enzyme with a broad substrate selectivity.

Classes A and C represent the most studied enzymes. The class A enzymes can be chromosomally or plasmid-encoded. Particularly in the plasmid-encoded cases, the resistance can be easily transfered horizontally—not only to other members of the same strain but *even to other species*. Due to such rapid gene transfer, a patient can become infected with different organisms, each possessing the same β-lactamase. A representative of class C is found in *Enterobacter cloacae* P99, and the X-ray crystal structure at 2-Å resolution has been presented.[6] This class of enzymes often has poor affinities for inhibitors of the class A enzymes, such as clavulanic acid, and for common *in vitro* inactivators, such as 6β-iodopenicillanate.[7] Due to their rapid transference, class A enzymes have been pharmaceutically targeted as responsible for most instances of chemotherapeutic failure. However, class C cephalosporinases have also been responsible for the resistance of Gram-negative bacteria to a variety of both traditional and newly designed antibiotics.[8] The enterobacter species are now the third greatest cause of nosocomial infections in the United States.

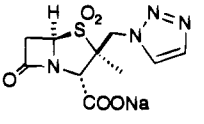
Two methods for overcoming the growing problem of bacterial resistance involve either developing antibiotics which are poor substrates of β-lactamase or designing β-lactamase inhibitors to be used in combination with known antibiotics. Reviews of β-lactamase inhibition are available.[9] Structures of a few common β-lactamase inhibitors are shown below. It is important to note that β-lactamases are a rather large and diverse family of enzymes and that inhibitors are usually active only against specific enzymes or classes. Such inhibitors frequently do not possess antibiotic activity themselves and are thus administered together with an antibiotic. Tazobactam,[10] for example, has been combined with piperacillin, and clavulanic acid is commercially distributed as Augmentin (Smith Kline Beecham), a combination of clavulanic acid and the antibiotic amoxicillin.



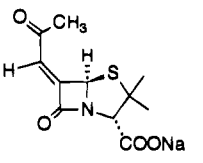
Sulbactam



Clavulanic Acid



Tazobactam



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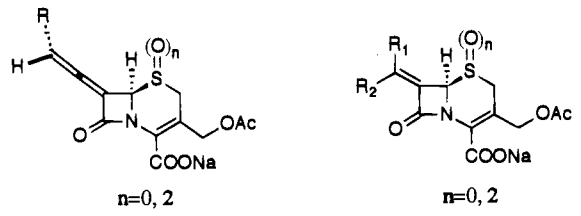


1a: R=*tert*-Bu, n = 2

7-Vinylidenecephalosporins, 1 7-Alkylidenecephalosporins, 2

Figure 1. 7-(Unsaturated)cephalosporins.

We have recently reported the synthesis of the 7-vinylidenecephalosporins (**1**, Figure 1),[11] a previously unknown class of compounds with an exocyclic allene fused to the 7-position of the cephalosporin nucleus.[12] These compounds were found to be potent inhibitors of the type C lactamase derived from *E. cloacae* P99. Through the synthesis of a deuterated analog of one such allene, we were able to elucidate the mechanism of inhibition. In the process of completing this research, we became aware of the paucity of information (both biological and chemical) available on simple 7-unsaturated cephalosporins (7-alkylidenecephems, **2**). This situation is in contrast to that of the corresponding 6-unsaturated penicillins, which were prepared and studied by Chen et al.[13] We thus decided to prepare representative 7-alkylidenecephems and study their properties as β-lactamase inhibitors.

In particular, the target molecules which interested us included the cephalosporin analogs of known biologically potent penams, such as those reported by Chen,[13] and the cephalosporin analog of 6-acetylmethylenepenicillanic acid (Ro 15-1903), another known β-lactamase inhibitor.[14] We were also interested in halogenated alkylidene side chains (**2**, R_1 or R_2 = X), both due to their probable synthetic usefulness and their potential to irreversibly alkylate a nucleophilic amino acid residue via a nucleophilic addition-elimination sequence. Lastly, we wanted to prepare a (*tert*-butylalkylidene)cephem (**2**, R_1 or R_2 = *tert*-butyl) that we could compare with our highly active (*tert*-butylvinylidene)cephem inhibitor (**1a**, R = *tert*-butyl). We felt that a comparison of the two compounds could provide more insight into the relative roles of the bulky *tert*-butyl group and the allene in the inhibitory process.

Chemistry

As in the preparation of 7-vinylidenecephalosporins, a key intermediate in the synthesis of 7-alkylidenecephalosporins is 7-oxocephalosporinate, whose preparation is described in Scheme 1. As described previously, benzhydryl 7-aminocephalosporanic acid was treated with excess triethylamine and trifluoromethanesulfonic anhydride, and the resultant trifluorosulfonyl imine hydrolyzed to produce benzhydryl 7-oxocephalosporanate **3**.[15] Due to its instability, this compound was used directly without further purification.

As shown in Scheme 1, the 7-alkylidenecephalosporanates **4** were prepared by treating 7-oxocephalosporanate **3** with the corresponding Wittig reagents. Compounds **4a-j** were prepared by standard Wittig methodology with the exceptions of **4b**, which required the addition of Zn/Cu couple[16] to produce the (dichloromethylene)cephalosporanate, and **4i**, which was prepared by the reduction of **4h** with $NaCNBH_3$. In examples **4e–j** (with the exception of **4h**), only one stereoisomer of the double bond was formed and stereochemistry was assigned by analogy with reported results on the corresponding penicillin systems.

All attempts to prepare a (*tert*-butylalkylidene)-cephem using simple Wittig methodology failed, and we decided to try another approach involving a substitution reaction of a 7-(haloalkylidene)cephalosporin. Thus compound **4a** was reduced by Zn/Cu couple to produce a single (monobromomethylene)cephem, **4k**, the stereochemistry of which was assigned by spectral comparison with reported β-bromo α,β-unsaturated amides.[17] In particular, the reduced material showed a new signal at 6.44 ppm due to the proton on the exocyclic bromomethylene group. A survey of the literature indicates that, in acyclic systems, when this proton is *cis* to the carbonyl group, a more appropriate chemical shift would be 7.5 ppm. By contrast when this proton is *trans* to the carbonyl carbon, reported chemical shifts range from 6.79 to 6.55, corresponding more closely to our observed chemical shift.

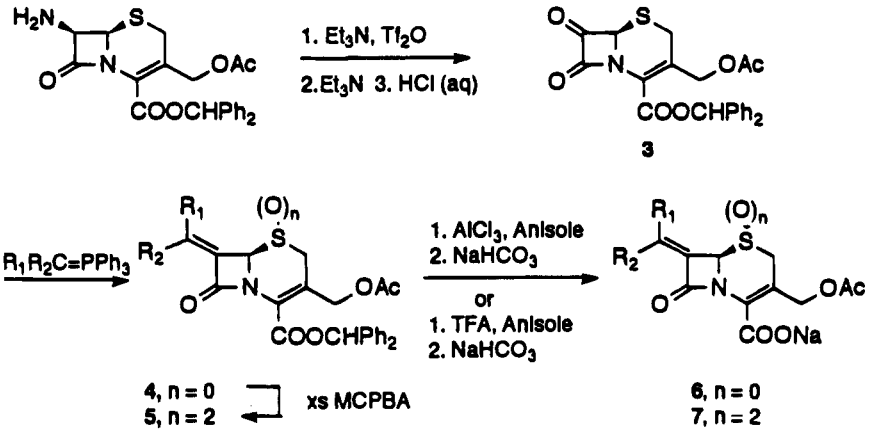
This material was further treated with the higher order cuprates derived from *t*-BuLi and CuCN to give compound **4l** as shown in Scheme 2. Once again, a single isomer was produced and the stereochemistry was assigned by analogy with structurally similar systems.[18] In particular, a new signal in the ^1H NMR appeared at 6.00 ppm, corresponding to the vinylic proton on the exocyclic methylene. In analogous acyclic systems prepared by Padwa, such a proton trans to the carbonyl carbon would have a chemical shift of approximately 5.4 ppm, while a proton cis to the carbonyl has a chemical shift of 6.05 ppm, more closely resembling our observed chemical shift. Presumedly the mechanism involves the *anti* addition of the cuprate reagent to the least-hindered side of the double bond, followed by a partial rotation and *anti* elimination of copper bromide. This sequence represents a useful new method for the preparation of α-alkylidene β-lactams.

Most of the compounds in the series **4** could be oxidized with excess *m*-CPBA to produce the corresponding sulfones **5**. In some cases, attempted oxidation failed to produce a stable product. Deprotection of compounds **4** and **5** produced the corresponding sodium salts **6** and **7** as shown in Scheme 1. A list of compounds which were successfully deprotected is shown in Table 1.

Biological Evaluation

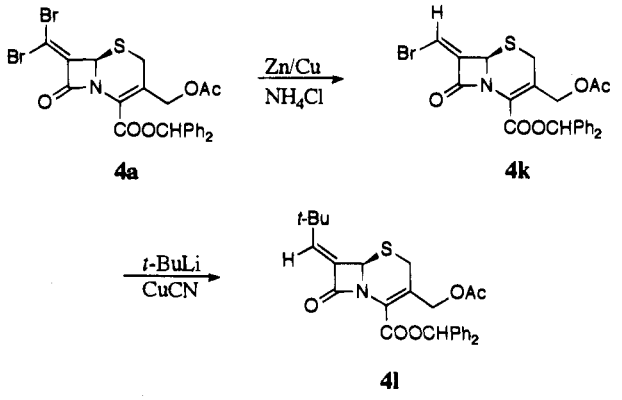
IC_{50} Determination. The aforementioned salts of 7-alkylidenecephems were evaluated as inhibitors of the class C β-lactamase of *E. cloacae* P99, the class A β-lactamase of *Escherichia coli* W3310 (TEM-2), and the class C inducible cephalosporinase of *E. cloacae* SC12368 (E-2) by relative IC_{50} analysis. The IC_{50} value, which represents the concentration of inhibitor required to effect a 50% loss of activity of free enzyme, is determined by measuring the rate of enzymatic hydrolysis of nitrocefin (the reporter substrate) after the enzyme has been preincubated with varying amounts of inhibitor for 10 min. The data is presented in Table 1. The data is standardized by measuring the IC_{50} values of tazobactam and clavulanic acid for comparison with our compounds. Two results are noteworthy. First, compound **7e**, the sodium salt of 7-[(*Z*)-(2′-pyridyl)methylene]-

Scheme 1



no.	R_1	R_2	no.	R_1	R_2
a	Br	Br	**f**	$CO_2C(CH_3)_3$	H
b	Cl	Cl	**g**	$COCH_3$	H
c	H	Ph	**h**	CHO	H
d	Ph	H	**i**	CH_2OH	H
e	2'-Py	H	**j**	$CON(CH_3)(OCH_3)$	H

Scheme 2



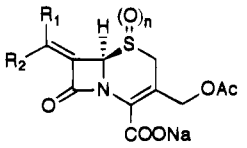
cephalosporinic acid sulfone, demonstrated extremely high activity against both class C enzymes. Secondly, compound **7f**, the sodium salt of 7-[(*Z*)-(*tert*-butoxycarbonyl)methylene]cephalosporanic acid sulfone demonstrated high activity against the type A enzyme, but not against either class C enzyme. As reported in our previous paper,[11] the allene **1a** is an excellent inhibitor of both class C enzymes, but not of this particular class A. Other compounds which show activity include **6f**, which is more active against the P99 enzyme than its corresponding sulfone, **6h**, an α,β-unsaturated aldehyde, and **7d**, a (phenylalkylidene)cephalosporin sulfone.

Inactivation Kinetics. Due the extremely high activity of compound **7e** and the availability of pure P99 enzyme, we decided to study this inhibition in further detail. In order to measure the rate of inactivation, the method of Kitz and Wilson[19] was applied. The enzyme was incubated with five different concentrations of **7e**. At each inhibitor concentration, the activity was assayed and plotted as a function of time (for a 5−10 min time period) by periodically diluting enzyme/inhibitor mixture into nitrocefin solution and continuously monitoring the rate of hydrolysis of this substrate (by measuring the change in absorbance at 481 nm) for 10 s. At each inhibitor concentration, the amount of inhibition increased with incubation time (during this 5−10 min period), indicating progressive inhibition as shown in the first plot in Figure 2. Following the conventions of Kitz and Wilson, plotting the inverse slopes of these lines ($1/k_{app}$) vs the inverse of their respective inhibitor concentrations ($1/I$) yields a double reciprocal plot as shown in the second plot in Figure 2. k_3' is a pseudo-second-order rate constant of inhibition and is inversely related to the slope of the line in this second plot. We have previously reported the rates of inhibition for **1a**, tazobactam, and clavulanic acid. Comparison of **7e** and **1a** with other known inhibitors is shown in Table 2.

Partition Ratio. While attempting to study the time dependence for inhibition by **7e**, we discovered that extremely low stoichiometric ratios of inhibitors to enzyme were sufficient to effect this inhibition. This quantity can be expressed as the partition ratio, the number of molecules of inhibitor necessary to achieve complete deactivation of one molecule of enzyme (this value is also called the turnover number). To obtain this data, a small aliquot of an incubation mixture (0.26 μM β-lactamase with stoichiometric equivalents of inhibitor) was periodically removed, diluted, and treated with substrate, and its activity was compred against a control to determine the amount of remaining active enzyme. The progress curves at various ratios of inhibitor to enzyme are shown in Figure 3. At these low concentrations of inhibitor there was a rapid deactivation of enzyme ($t_{1/2}$ = 5−15 min), and a significantly slower reactivation, usually beginning after approximately 25 min.

However, even with the addition of a stoichiometric amount of inhibitor, complete (i.e. 100%) recovery of enzymatic activity was not observed. The results of a more thorough study which allowed the enzymatic activity to recover for a full 24 h is shown below. (After this period of time, it was found that the recovery of activity had plateaued.) A plot of this remaining activity vs the molar ratio of inhibitor to enzyme produced a roughly linear plot. The partition number

Table 1. β-Lactamase Inhibitory Activity



n=0,2

compound	n	R_1	R_2	IC_{50} (nM) *E. cloacea* P99	*E. coli* WC3310	*E. cloacea* SC12368
tazobactam				943	25	4000
clavulanic acid				>20000	60	>20000
1a	2			130	>20000	260
6a	0	Br	Br	>20000	>20000	>20000
6b	0	Cl	Cl	>20000	>20000	>20000
6d	0	Ph	H	>20000	>20000	>20000
6e	0	2′-Py	H	>20000	>2000	>20000
6f	0	$CO_2C(CH_3)_3$	H	2500	>20000	>20000
6g	o	$COCH_3$	H	>20000	>20000	>20000
6h	0	CHO	H	8200	>20000	16500
6j	0	$CON(CH_3)(OCH_3)$	H	>20000	>20000	>20000
6k	0	H	Br	>20000	>20000	>20000
6l	0	*t*-Bu	H	>20000	>20000	>20000
7a	2	Br	Br	>20000	>20000	>20000
7b	2	Cl	Cl	>20000	8300	>20000
7c	2	H	Ph	>20000	>20000	>20000
7d	2	Ph	H	6250	>20000	6800
7e	2	2′-Py	H	25	800	25
7f	2	$CO_2C(CH_3)_3$	H	7800	5	5900
7i	2	CH_2OH	H	>20000	>20000	>20000
7k	2	H	Br	>20000	>20000	>20000
7l	2	*t*-Bu	H	>20000	>20000	>20000

(approximately 20 in this case) can be extrapolated from the *x*-axis in such a plot. This is shown in Figure 4. Comparison of **7e** with **1a** and commercially available inhibitors is shown in Table 2.

Gel Filtration Study. In order to ascertain the full extent of this slow reactivation of the enzyme and to determine if any of the observed inhibition was truly irreversible, inhibited enzyme was separated from excess inhibitor by gel filtration. The recovery of activity of the enzyme was then monitored over 24 h in order to determine whether or not any permanent inactivation occurred. In the gel filtration study, enzyme was incubated with a 100-fold molar excess of inhibitor **7e** for an allotted time (either 1 or 24 h). To remove excess inhibitor, the incubation mixtures were then placed on a Sephadex G-25 column at room temperature. After gel filtration, fractions containing enzyme, as determined by UV analysis, were combined, and the recovery of activity (in the absence of inhibitor) was monitored for 24 h. A control consisting of an identical solution of enzyme without inhibitor was subjected to the same process. The inhibited enzyme showed good separation from inhibitor, as demonstrated by recording an ultraviolet spectrum for each fraction.

Figure 5 shows the partial recovery of activity of enzyme for two solutions which were incubated for 1 and 24 h, respectively, then subjected to gel filtration to remove unbound inhibitor, and monitored for 24 h. After gel filtration, the inhibited enzyme showed partial recovery of enzyme activity at a very slow rate. Twenty-four hours after gel filtration, the solution which was incubated for 1 h and regained 73%, and the solution which was incubated for 24 h had regained 43% of its original activity. By plotting the log of the remaining activity vs time, the first-order rate constant of *reactivation* (bound enzyme going to free enzyme) was determined to be $k_{react} = 1.0 \times 10^{-3}$ s^{-1} at both incubation times.

Discussion

While cephalosporin sulfone esters have recently been reported as inhibitors of elastase,[20] far less is known about the antibacterial and β-lactamase inhibitory properties of these sulfone (carboxylate salts). By contrast, the corresponding penicillin sulfones have been more highly investigated. In the latter category, Knowles[21] has performed a thorough study of sulbactam, the 6-position unsubstituted penam sulfone.

Our biological data indicates that compound **7e** is a rather specific inhibitor of type C cephalosporinases. It is likely that the mechanism of inhibition of analogous to that described for the corresponding penicillin sulfone. As mentioned earlier, in their studies on the penicillin analog, Chen and co-workers observed that 6-[(2′-pyridyl)methylene]penam sulfone as well as several other 6-[(heterocyclic)methylene]penicillin sulfones were effective β-lactamase inhibitors.[13] In their case, insight was gained into the inhibitory mechanism by reacting the allyl ester of 6-[(2′-pyridyl)methylene]-penam sulfone (**8**, R = $CH_2CH{=}CH_2$) with methanolic sodium methoxide to produce the highly stabilized system **9** as shown in Scheme 3.[22] Presumably a similar rearrangement occurs in biological systems producing a stabilized acyl–enzyme. This intermediate incorporates the elements of a β-aminoacrylate (vinylogous urethane) into an aromatic system. In that case, the mechanism of inhibition was further supported by the fact that the sodium salt of 7-[(*Z*)-phenylmethylene]-penicillanic acid sulfone (**8**, R = Na) showed only weak activity in comparison to the corresponding pyridyl

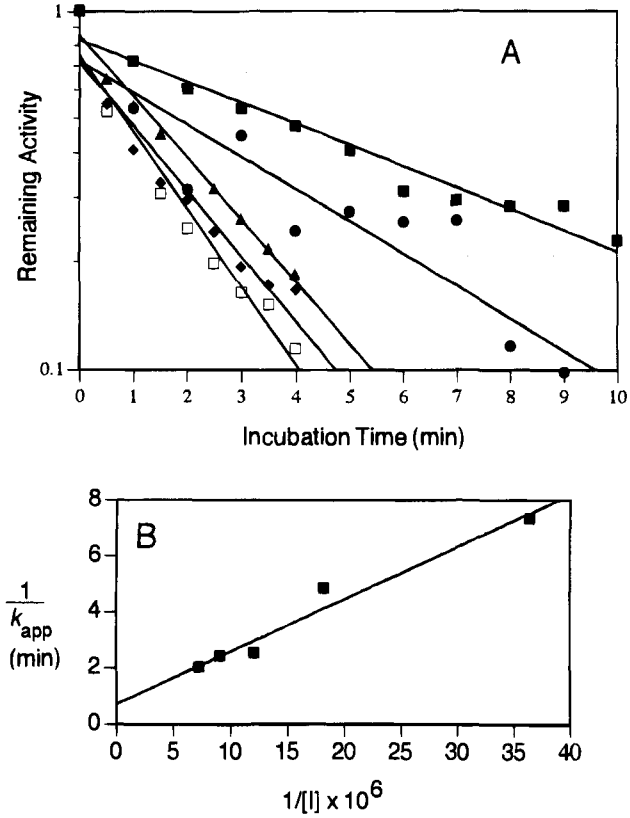


Figure 2. The inhibition of EC P99 β-lactamase by **7e**. Panel A shows the progressive inhibition in which enzyme and inhibitor were incubated at 25 °C (at various concentrations) for the times indicated before dilution into nitrocefin solution. The final assay concentration of the enzyme was 2.56 nM. Inhibitor concentrations in the assay were (□) 1.4×10^{-7} M, (◆) 1.1×10^{-7} M, (▲) 8.3×10^{-8} M, (●) 5.5×10^{-8} M, (■) 2.8×10^{-8} M. Panel B shows the plot of the reciprocals of these concentration-dependent constants vs the reciprocal of inhibitor concentrations for the same compound. k_3' was determined by linear regression to be 5.3×10^6 L/mol·min.

Table 2. Rate Constants of Inhibition and Partition Coefficients

compound	k_3' (L/mol·min)	partition coefficient
7-[(Z)-(2'-pyridyl)methylene]cephalosporanic acid sulfone (**7e**)	5.3×10^6	20
7-(2'α-*tert*-butylvinylidene)cephem sulfone (**1a**)	1.7×10^6	12
tazobactam	7.4×10^4	50[a]
clavulanic acid	799	>500 000[a]

[a] Reference 10.

analog. Furthermore, the sulfide analog was found to be only weakly active, indicating that the mechanism of enzyme inactivation required the sulfone to act as a leaving group.

Despite several attempts, we were unable to observe an analogous product upon simple reaction of **7e** with bases and nucleophiles. However, other features of our inhibitor parallel those observed by Chen. It was 250 times more active than its phenyl analog **7d** and 1300 times more active than the sulfide **6e** as shown by the IC_{50} analysis.

When the enzyme was reacted with stoichiometric ratios of **7e**, a rapid inhibition was observed followed by a slower reactivation. As shown in Figure 2 and 3, after a 24 h incubation, approximately 20 equiv of inhibitor were necessary to fully inhibit 1 equiv of

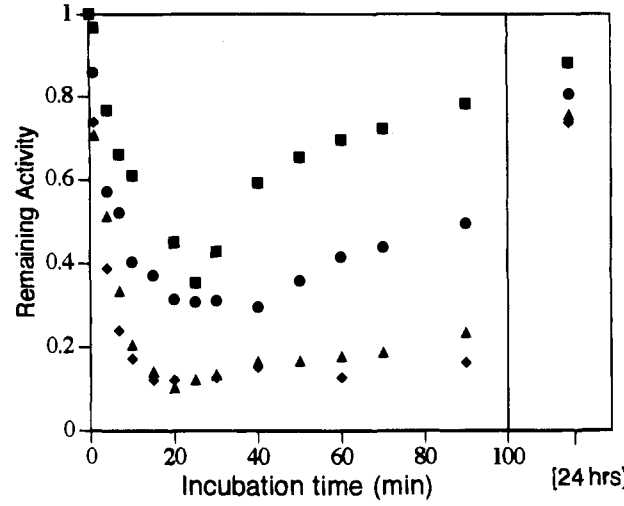


Figure 3. Inactivation of EC P99 β-lactamase by **7e**. Incubation mixture contained 0.26 μM β-lactamase solution and multiples of 0.26 mM inhibitor solution. At the prescribed times, 2 μL was diluted into 1000 μL of 200 μM nitrocefin, and the rate of hydrolysis was compared against a control mixture (no inhibitor) to determine remaining activity. Stoichiometric ratios are (■) 1:1, (●) 2:1, (▲) 3:1, (◆) 4:1.

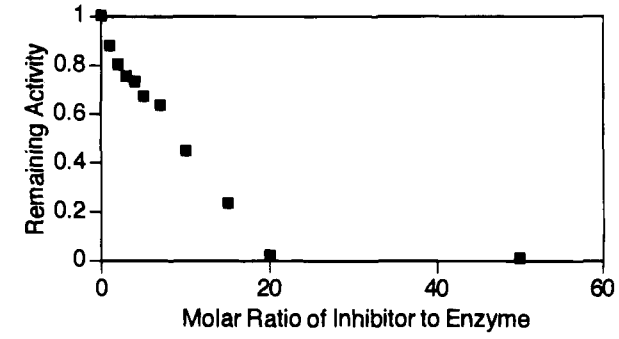


Figure 4. Plots of remaining P99 β-lactamase activity following 24 h of inubation with various stoichiometric equivalents of inhibitor **7e**.

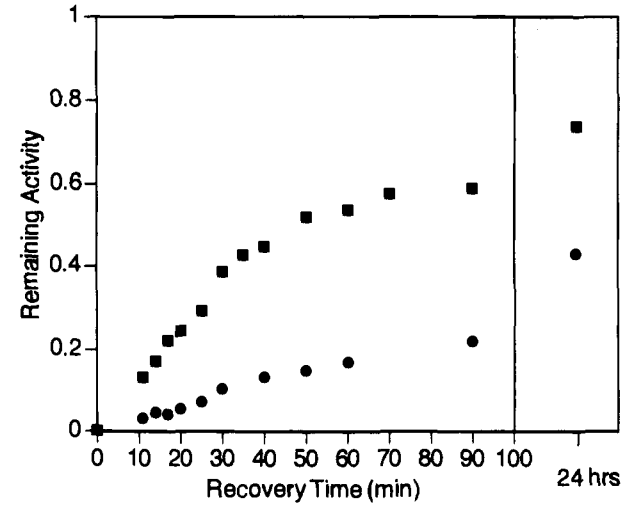
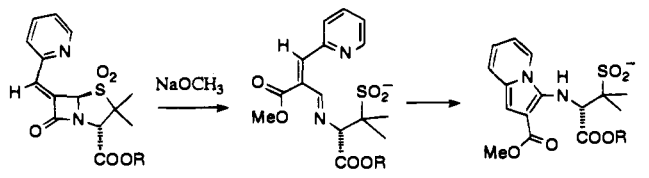


Figure 5. Plot of recovery of enzymatic activity following incubation with excess inhibitor **7e** for 1 and 24 h, respectively, followed by gel filtration to remove excess inhibitor.

enzyme. When the enzyme was incubated with excess inhibitor and subsequently subjected to gel filtration, activity was recovered in a linear first-order fashion for the first 30 min followed by a slow reactivation after this point. Full activity was never recovered, even after

Scheme 3



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24 h, and increasing incubation time (before gel filtration) increased the amount of irreversible inhibition.

A plausible mechanism which would account for the rapid enzymatic inhibition caused by **7e** is shown in Scheme 4. Following formation of the initial acyl–enzyme, **10**, the inhibitor could either be directly hydrolyzed or proceed along a pathway toward a more stabilized intermediate. The ratio between the rate of hydrolysis of **10** and the rate of formation of a more stabilized acyl–enzyme was approximately 20:1 as measured by the partition ratio. A proposed structure for the stabilized acyl–enzyme is intermediate **11**. As shown in Scheme 5, **11** would then be capable of extremely slow hydrolysis (turnover) or slow irreversible inactivation, perhaps by the addition of a nucleophilic amino acid residue to the α,β-unsaturated imine.

Our biological data also indicate that compound **7f**, a sulfone with a 7-(*Z*)-(*tert*-butoxycarbonyl)methylene side chain was a highly active and specific inhibitor for this particular class A enzyme. Three potential mechanistic possibilities for the inhibition of the class A enzyme by compound **7f** are shown in Scheme 6. The first involves an intramolecular interception of the imine by the carbonyl oxygen of the ester, subsequent loss of isobutylene, and isomerization of the double bond to form stabilized acyl–enzyme **12**. In the second potential mechanism, a simple isomerization of the double bond (presumedly following abstraction of the relatively acidic bridgehead proton) produces stabilized acyl–enzyme **13**. The last hypothetical mechanism involves an intramolecular acylation of the hydrolytically-liberated amine, producing stabilized intermediate **14**. Further mechanistic investigation of these possibilities is currently in progress.

Lastly, in contrast to the highly active 7-(*tert*-butylvinylidene)cephalosporin (**1a**), the 7-*tert*-butylmethylene compound **7a** showed little activity. This provides supporting evidence that the allene is necessary for biological activity. Surprisingly the cephalosporin analog of 6-[(*Z*)-acetylmethylene]penicillanic acid (sulfide) also did not demonstrate considerable activity.

Conclusion

We have synthesized several 7-alkylidenecephems and evaluted them as inhibitors of two type C *E. cloacae*-derived β-lactamases and one type A β-lactamase. In the process, we have developed a new method for the preparation of alkylidenecephalosporins involving the reaction of higher order cuprate with 7-(bromoalkylidene)cephalosporins.

Several of these new compounds show substantial inhibitory activity when compared with tazobactam and clavulanic acid. Among them, the sodium salt of 7-[(*Z*)-(2′-pyridyl)methylene]cephalosporonic acid sulfone, **7e** exhibited remarkably efficient inhibition of the two class C enzymes and the sodium salt of 7-[(*Z*)-(*tert*-butoxycarbonyl)methylene]cephalosporanic acid sulfone, **7f**, was an excellent inhibitor of the class A enzyme. The kinetics of the inhibition caused by **7e** were carefully examined, and potential mechanisms were proposed for the inhibition by both **7e** and **7f**. A common mechanistic motif is the formation of a β-aminoacrylate (vinylogous urethane) as a stabilized acyl–enzyme. The low activity of the 7-(*tert*-butylmethylene)cephalosporin (**7l**) is further confirmation that the allene is a key structural element of our previously reported 7-vinylidenecephalosporanate inhibitors (**1**).

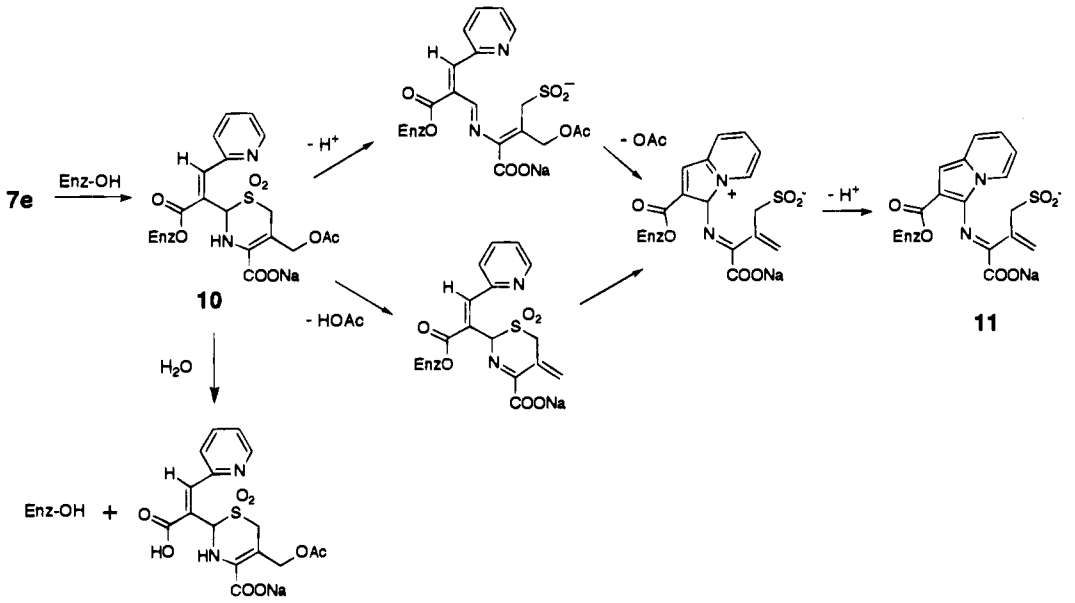
Experimental Section

All assays of β-lactamase activity were performed on a Beckman DU-650 spectrophotometer, and hydrolysis rates of the lactamase substrate, nitrocefin, were monitored at 482 nm. Nitrocefin was purchased from Becton Dickinson Microbiology Systems (Cockeysville, MD). Melting points are uncorrected and determined on a MEL TEMP capillary melting point apparatus. Infrared spectra were recorded on a Perkin-Elmer Model 710B diffraction grating spectrophotometer or a Perkin-Elmer 1600 Series Fourier transform infrared spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker WP200SY spectrometer. Proton chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane (0.0). Carbon chemical shifts are reported in parts per million (δ) by using chloroform-*d* (77.0) as the reference. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN. Mass spectral data were obtained by FAB techniques from the Midwest Center for Mass Spectrometry at the University of Nebraska–Lincoln, NE. Thin layer chromatography (TLC) was performed on Merck 0.2 mm Kieselgel 60 F_{254} silica-coated aluminum plates. The compounds were identified in one or more of the following manners: UV (254 nm), iodine chamber and/or phosphomolybdic acid spray reagent. The position of the compounds on the TLC plate is listed as an R_f value in the given solvent(s). Flash chromatography was performed by using thick-walled glass columns and Merck's 0.040–0.063 mm Kieselgel 60 silica gel. Reverse phase chromatography was performed using preparative layer plates purchased from Analtech (RPS-F, 1000 μm). All of the final sodium salts were purified by reverse phase chromatography before analysis and found to be homogeneous. The chromatography solvents were distilled from calcium hydride before use. All additional solvents were obtained from Aldrich in Sure-Seal bottles. DNAase (Deoxyribonuclease I, EC 3.1.21.1, from bovine pancreas,) was obtained from Sigma Chemical Co.

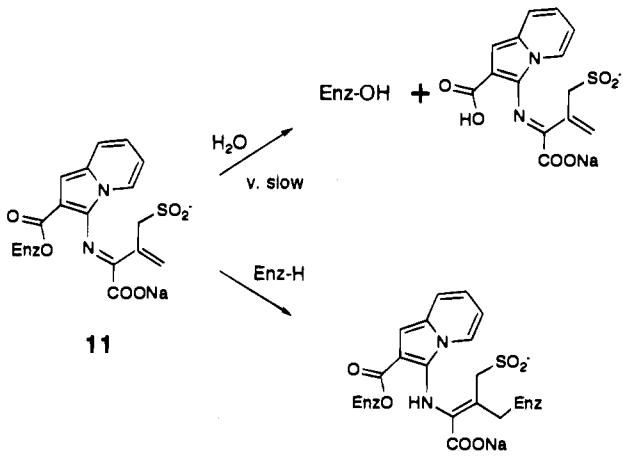
P99 β-lactamase from *E. cloacae* was purchased from the Center for Applied Microbiology and Research (Porton Down, Wilts., U.K.). Tazobactam and clavulanic acid were obtained from American Cyanamid Co. and SmithKline Beecham Pharmaceuticals, respectively. All other reagents were used as received from Aldrich unless otherwise noted. Unless otherwise specified, all yields refer to the isolation of purified material (after chromatography).

TEM-2 β-lactamase from *E. coli* W3310 was prepared as follows. Freshly cultured cells were grown in a 50 mL volume in Luria broth media for 8 h at 37 °C. Four 10 mL portions of this cell suspension were transferred to four 1 L portions of Luria broth in 3.8 L Erlynmeyer flasks and incubated at 37 °C overnight. The resulting 4 L of cell suspension was centrifuged at 9000 rpm, and the supernatant was discarded, to obtain a cell paste. From this point on, all work is carried out at <4 °C. To wash the cells, they were resuspended in 500 mL of 0.1 M phosphate buffer and again centrifuged to yield 24.19 g of cell paste which is stable at −30 °C for several months. Ten grams of cells were disrupted by a freeze–thaw technique in which the concentration of cells was 1.0 g/mL suspended in 1.0 M sodium acetate buffer (i.e., to 10 g of cells was added 10 mL of acetate buffer). Cells were frozen in dry ice/acetone and subsequently thawed at 60 °C (only until melted) three times. DNAase, 2000 Kunzt units, (Deoxyribonuclease I, EC 3.1.21.1, from bovine pancreas, 1 vial) was added to the disrupted cell suspension to reduce viscosity. This

Scheme 4



Scheme 5



suspension was centrifuged at 9000 rpm for 15 min, and the supernatant was placed directly on a Sephadex G-75 column (5 × 26 cm) and eluted with 0.4 M phosphate buffer at 1.5 mL/min, fraction size 8 mL. The UV absorbance at 254 nm of every other fraction was obtained as a rough measure of protein content, and lactamase activity was measured by monitoring the rate of hydrolysis of nitrocefin (at 481 nm). Fractions containing lactamase were combined. This technique produces a (freezer stable) solution of TEM2 enzyme suitable for IC_{50} analysis.[23]

The inducible cephalosporinase, E2 β-lactamase from *E. cloacae* SC12368, was grown in 250 mL of Luria broth overnight at 37 °C, and 50 mL was transferred to 4 × 1 L of Luria broth (i.e. 5% inoculum). After incubation for 2 h at 37 °C, the cells were treated with ampicillin to give a final concentration of 25 μg/mL. After 1 h of incubation at 37 °C, cells were harvested and purified as described above for the TEM-2 enzyme.

IC_{50} Determination. pH 7.2 phosphate buffer (50 mM) was prepared by dissolving NaH_2PO_4 (0.840 g) and Na_2HPO_4 (2.56 g) in 500 mL of deionized (Millipore) water. A solution of the β-lactamase derived from *E. cloacae* P99 (1.00 mg of enzyme was dissolved in 100 mL of 50 mM pH 7.2 phosphate buffer) was prepared. A standard solution of a lactamase substrate, nitrocefin, was prepared by dissolving 2.00 mg of nitrocefin in 50 mL of phosphate buffer and was used for evaluation of the inhibitors with the P99 enzyme. A more concentrated solution of 10 mg of nitrocefin in 100 mL of phosphate buffer was used for evaluation against the remaining two enzymes. A solution of inhibitor was prepared by dissolving a specified amount (in the range of 0.5–10 mg) of inhibitor in 10 mL of phosphate buffer. These solutions were allowed to equilibrate to 25 °C in a water bath for at least 15 min.

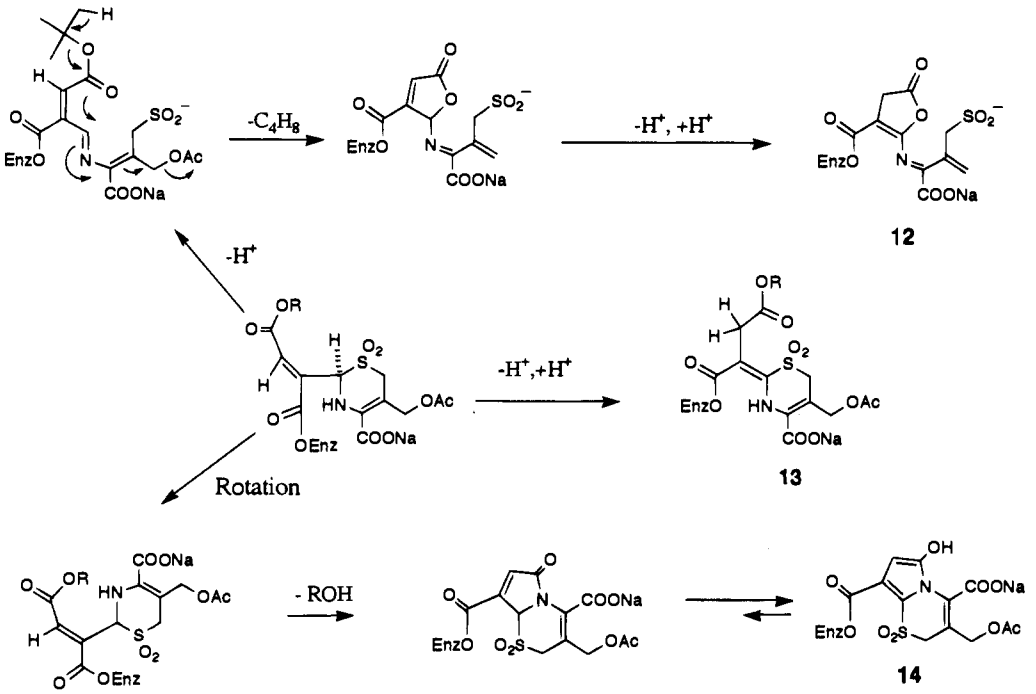
To determine the rate of enzymatic hydrolysis of nitrocefin in the absence of inhibitor, 500 μL of the standard nitrocefin solution was further diluted with 450 μL of buffer and the new solution was allowed to equilibrate to 25 °C in a water bath for 10 min. Enzyme solution (100 μL) was diluted with 400 μL of phosphate buffer, and then 50 μL of the diluted enzyme solution was added to the second nitrocefin solution (bring the total volume to 1.00 mL) and the hydrolysis rate of nitrocefin was determined spectrophotometrically by monitoring the change in the absorption at 482 nm.

For the determination of inhibitory activity, a specified volume (2.5–400 μL) of inhibitor solution was added to a solution prepared from 100 μL of enzyme standard solution and enough phosphate buffer to make the total volume 0.50 mL (total volume = volume of inhibitor solution + 100 μL of enzyme solution + volume of phosphate buffer solution), and the obtained solution was incubated at 25 °C for 10 min. The incubated solution (50 μL) was removed and added to a solution prepared from 500 μL nitrocefin standard solution and 450 μL of phosphate buffer. The hydrolysis rate of nitrocefin by the partially inhibited enzyme was determined spectrophotometrically.

Inhibition Constant Determination. For the determination of inhibitory rate, a specified volume (11, 22, 33, 44, or 55 μL) of 0.01 mg/mL inhibitor solution was added to a solution prepared from 100 μL of enzyme standard solution and enough phosphate buffer to make the total volume 0.5 mL (total volume = volume of inhibitor solution + 100 μL of enzyme solution + volume of phosphate buffer solution), and the obtained solution was incubated at 25 °C. At various time points, 50 μL of the incubated solution was removed and added to a solution prepared from 1000 μL of nitrocefin standard solution (0.19 mM), and then hydrolysis rate of nitrocefin was determined spectrophotometrically by monitoring the absorbance at 482 nm for 10 s. The extreme speed of **7e** necessitated aquisition of rates at 30 s intervals over a 5 min period. (Normally, the absorbance is monitored for 30 s to 1 min, and intervals are taken every 2–5 min over the course of 1 h.)

Dilution Reactivation Studies. For the partition coefficient studies, 100 μL of 2.56 μM was incubated with 11–550 μL of 23.6 μM **7e** and enough buffer to make a final volume of 1 mL. At various incubation times 5 mL was removed and injected into 1 mL of nitrocefin (200 μM), and the change in absorbance at 481 nm was monitored for 30 s. This rate was compared with a control which contained only enzyme and

Scheme 6



buffer in order to obtain the remaining activity. Activity was monitored repeatedly in this fashion for 24 h.

Gel Filtration Studies. For the gel filtration reactivation studies, 50 μL of 171 μM enzyme was incubated with 50 μL of 15.76 mM **7e**, and 350 μL of buffer. This corresponds to an *i/e* = 100:1. After 1 h, 50 μL was placed on an equilibrated Pharmacia Biotech PD-10 Sephadex column and eluted with 0.5 mL portions of buffer (yielding 0.5 mL fractions). At the same time, a control identical to the above except that the inhibitor was replaced with buffer, was run in a second column. The fractions containing enzyme were determined by UV analysis and pooled (fractions 5–8). This mixture was assayed by periodically injecting 5 μL portions into 1 mL of 200 μM nitrocefin and monitoring the change in absorbance at 481 nm for 30 s. This rate was compared with the control to determine remaining activity. The recovery of activity was monitored for 24 h in this fashion. After 24 h, another aliquot of the enzyme/inhibitor mixture was separated on the column along with another control on a separate column. The pooled fractions were monitored for recovery of activity for 24 h as above.

Benzhydryl 7β-aminocephalosporanate. To a suspension of 7-aminocephalosporanic acid (130.4 g, 0.48 mol) in methanol (480 mL) was added a solution of diphenyldiazomethane[24] (93.0 g, 0.48 mol) in CH_2Cl_2. The reaction mixture was then mechanically stirred at room temperature for 44 h. The remaining solid was removed by filtration. The resultant filtrate was concentrated *in vacuo* and purified by column chromatography (10% CH_3OH in CH_2Cl_2) to afford the desired ester as pale yellow solid (86.1 g, 41% yield): R_f = 0.44 in 1:9 CH_3OH:CH_2Cl_2; mp 45–46 °C; IR ($CHCl_3$) 2980, 1780, 1730 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.41 (2H, bs), 7.22 (10H, m), 6.91 (1H, s), 5.27 (1H, d, J = 2.8 Hz), 5.15 (1H, d, A of AB q, J = 14 Hz), 4.94 (1H, s), 4.84 (1H, d, B of AB q, J = 14 Hz), 3.73 (1H, d, A of AB q, J = 17 Hz), 3.33 (1H, d, B of AB q, J = 17 Hz), 1.92 (3H, s); ^{13}C NMR ($CDCl_3$) δ 169.8, 168.8, 160.6, 138.9, 138.7, 129.5, 129.3, 129.1, 128.7, 128.5, 127.97, 127.61, 127.52, 127.18, 126.52, 126.06, 125.4, 79.0, 63.3, 62.6, 58.5, 25.7, 20.1.

Benzhydryl 7-Oxocephalosporanate (3). The title compound was prepared by modifying the procedure of Hagiwara et al.[15] To a solution of benzhydryl 7β-aminocephalosporanate, (5.9 g, 13.5 mmol) in anhydrous CH_2Cl_2 (70 mL) at −78 °C was added dropwise triethylamine (5.6 mL, 40.4 mmol) with stirring. After 5 min, trifluoromethanesulfonic anhydride (6.8 mL, 40.4 mmol) was added drowise to this solution over a 5 min period. The reaction mixture was allowed to warm slowly to 0 °C over a 1 h period. It was then recooled to −78 °C, and triethylamine (5.6 mL, 40.4 mmol) was added over approximately 10 min. The reaction mixture was stirred at −78 °C for an additional 30 min and poured into 200 mL of cold 0.5 N HCl. The resultant mixture was further stirred until the ice melted. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (150 mL). The combined organic layers were washed with cold 0.5 N HCl (3 × 100 mL), dried (Na_2SO_4), and concentrated (at room temperature or below) to produce the title compound (5.8 g, 98% yield) as a brown solid which was used without further purification: IR ($CHCl_3$) 3005, 1830, 1790, 1740 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.39 (10H, m), 7.05 (1H, s), 5.32 (1H, s), 5.07 (1H, d, A of AB q, J = 14 Hz), 4.85 (1H, d, B of AB q, J = 14 Hz), 3.64 (1H, d, A of AB q, J = 18 Hz), 3.44 (1H, d, B of AB q, J = 18 Hz), 2.05 (3H, s); ^{13}C NMR ($CDCl_3$) δ 188.4 (s), 170.3 (s), 160.1 (s), 158.7 (s), 138.8 (s), 138.6 (s), 128.4, 128.2, 128.1, 127.7, 126.9, 126.2, 80.1 (d), 65.8 (d), 62.6 (t), 27.7 (t), 20.4 (q).

Benzhydryl 7-(Dibromomethylene)cephalosporanate (4a). To a solution of Ph_3P (12.0 g, 45.8 mmol) in anhydrous CH_2Cl_2 (75 mL) was added CBr_4 (7.6 g, 22.9 mmol) in one portion at 0 °C. The mixture was stirred at room temperature for 30 min. The reaction mixture was then cooled to −78 °C, and a cold (−78 °C) solution of benzhydryl 7-oxocephalosporanate **3** (500 g, 11.4 mmol) in anhydrous CH_2Cl_2 (40 mL) was added. After stirring at −78 °C for 30 min, the reaction mixture was concentrated *in vacuo* and purified by column chromatography (CH_2Cl_2) to give a pale yellow solid (4.1 g, 61% yield): R_f = 0.55 in CH_2Cl_2; mp 58–60 °C; IR ($CHCl_3$) 3030, 1780, 1745 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.37 (10H, m), 6.96 (1H, s) 5.19 (1H, s), 4.97 (1H, d, A of AB q, J = 13 Hz), 4.72 (1H, d, B of AB q, J = 13 Hz), 3.52 (1H, d, A of AB q, J = 18 Hz), 3.32 (1H, d, A of AB q, J = 18 Hz), 2.00 (3H, s); ^{13}C NMR ($CDCl_3$) δ 170.2 (s), 160.5 (s), 155.6 (s), 142.6 (s), 139.1 (s), 138.9 (s), 128.4, 128.0, 127.9, 127.0, 126.7, 125.2 (s), 92.6 (s), 79.9 (d), 63.0 (t), 60.1 (d), 27.0 (t), 20.5 (q). Anal. ($C_{24}H_{19}NO_5SBr_2$) C, H, N.

Benzhydryl 7-(Dichloromethylene)cephalosporanate (4b). CCl_4 (2 mL, 20.7 mmol) was added into a solution of PPh_3 in anhydrous CH_3CN (50 mL) and stirred at room temperature for 30 min. This solution was transferred into a solution of benzhydryl 7-oxocephalosporanate **3** (3.0 g, 8.9 mmol) in anhydrous CH_3CN (20 mL), and Zn/Cu (1.0 g, 15 mmol) was added. This reaction mixture was further stirred at room temperature for 40 min. The unreacted Zn/Cu was removed by filtration, and the filtrate was concentrated and

purified by column chromatography (CH_2Cl_2) to yield a pale yellow solid (2.70 g, 78%): R_f = 0.73 in CH_2Cl_2; mp 48–50 °C; IR ($CHCl_3$) 3050, 1780, 1740, 940 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.38 (10H, m), 6.99 (1H, s) 5.29 (1H, s), 5.02 (1H, d, A of AB q, J = 13 Hz), 4.76 (1H, d, B of AB q, J = 13 Hz), 3.57 (1H, d, A of AB q, J = 18 Hz) 3.88 (1H, d, B of AB q, J = 18 Hz), 2.04 (3H, s); ^{13}C NMR ($CDCl_3$) δ 170.0 (s), 160.2 (s), 154.5 (s), 138.8 (s), 138.7 (s), 136.2 (s), 128.1, 127.7, 127.2, 126.6, 126.2, 124.7 (s), 123.6 (s), 79.8 (d), 62.8 (t), 57.4 (d), 26.9 (t), 20.3 (q); high-resolution mass spectrum for $[C_{24}H_{19}NO_5SCl_2Na]^+$, i.e., $[M + Na]^+$, m/z calcd 526.0259, found 526.0251.

Benzhydryl 7-[(*E*)-Bebzylidene]cephalosporanate (4c) and Benzhydryl 7-[(*Z*)-benzylidene]cephalosporanate (4d). To a solution of benzyltriphenylphosphonium bromide (11.44 g, 26.4 mmol) in anhydrous THF (50 mL) was added a solution of *n*-BuLi (14.5 mL, 29.0 mmol) at −78 °C. The mixture was stirred at room temperature for 30 min. The resulting red solution was recooled to −78 °C and was added to a cold (−78 °C) solution of 7-oxocephalosporanate **3** (10.5 g, 24.0 mmol) in anhydrous THF (25 mL) and stirred at −78 °C for 5 min. The cold reaction mixture was then poured into ice-cold saturated NH_4Cl solution (25 mL), and the layers were separated. The aqueous layer was extracted with ether (2 × 50 mL). The combined organic layers were washed with water (25 mL), dried (Na_2SO_4), concentrated, and purified by column chromatography (CH_2Cl_2:hexane, 3:1) to give the *E*-isomer (0.83 g, 40%) and the *Z*-isomer (1.26 g, 60%) as white fluffy solid.

7-(*E*)-Isomer: R_f = 0.60 in CH_2Cl_2; mp 59–61 °C; IR ($CHCl_3$) 3015, 1760, 1730 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.83 (2H, m), 7.26 (13H, m), 6.93 (1H, s), 6.53 (1H, s), 4.99 (1H, s), 4.78 (1H, d, A of AB q, J = 13 Hz), 4.53 (1H, d, B of AB q, J = 13 Hz), 3.39 (1H, d, A of AB q, J = 18 Hz), 3.19 (1H, d, B of AB q, J = 18 Hz), 1.85 (3H, s); ^{13}C NMR ($CDCl_3$) δ 170.2 (s), 161.1 (s), 158.7 (s), 139.3 (s), 139.1 (s), 136.0 (s), 134.0 (d), 133.1, 130.3, 128.6, 128.3, 128.0, 127.7, 127.0, 121.7 (s), 79.6 (d), 63.1 (t), 56.1 (d), 27.9 (t), 20.5 (q). Anal. ($C_{30}H_{25}NO_5S$) C, H, N.

7-(*Z*)-Isomer. R_f = 0.50 in CH_2Cl_2; mp 45–47 °C; IR ($CHCl_3$) 3025, 1790, 1760 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.43 (15H, m), 7.21 (1H, d, J = 1.18 Hz), 7.07 (1H, s), 5.50 (1H, d, J = 1.23 Hz), 5.00 (1H, d, A of AB q, J = 13 Hz), 4.75 (1H, d, B of ABq, J = 13 Hz), 3.65 (1H, d, A of AB q, J = 18 Hz), 3.41 (1H, d, B of AB q, J = 18 Hz), 2.04 (3H, s); ^{13}C NMR ($CDCl_3$) δ 170.3 (s), 161.0 (s), 160.2 (s), 139.3 (s), 139.1 (s), 135.8 (s), 132.4 (d), 130.5, 129.7, 129.0, 128.3, 128.1, 127.9, 127.7, 127.0, 121.7 (s), 79.7 (d), 63.1 (t), 57.7 (d), 28.0 (t), 20.5 (q); high-resolution mass spectrum for $[C_{30}H_{25}NO_5SNa]^+$, i.e., $[M + Na]^+$, m/z calcd 534.1351, found 534.1352.

Benzhydryl 7-[(*Z*)-(2′-Pyridyl)methylene]cephalosporanate (4e). To a solution of 2-picolyl chloride hydrochloride (13.1 g, 80 mmol) in water (20 mL) was added K_2CO_3 (11.0 g, 80 mmol). After the carbonate was completely dissolved, the solution was extracted with ether (3 × 10 mL). The combined organic layers were washed with saturated NaCl solution (1 × 30 mL), dried (Na_2SO_4), and concentrated to give picolyl chloride (9.2 g, 90%). Picolyl chloride (8.9 g, 70 mmol), triphenylphosphine (18.3 g, 70 mmol), and 1,4-dioxane (30 mL) were mixed and refluxed for 24 h. The reaction mixture was washed with ether (2 × 30 mL), and the remaining solid was dried *in vacuo* to give a white solid (25.5 g, 94%). A mixture of 2-picolyltriphenylphosphonium chloride (5.8 g, 15 mmol) and sodium amide (0.58, 15 mmol) in THF (15 mL) was stirred at room temperature for 30 min. The resulting brown suspension was cooled to −78 °C, a solution of benzhydryl 7-oxocephalosporanate **3** (6.6 g, 15 mmol) in THF (15 mL) was added in one portion, and the mixture was stirred at −78 °C for 15 min. The reaction was quenched by the addition of saturated ammonium chloride solution (10 mL) and the reaction mixture extracted with EtOAc (2 × 20 mL). The combined organic layers were washed with water (2 × 40 mL), dried over $MgSO_4$, concentrated, and purified by column chromatography to obtain a yellow solid (2.9 g, 38%): R_f = 0.28 in 2% EtOAc in CH_2Cl_2; mp 181–183 °C; IR ($CHCl_3$) 3060, 1810, 1750 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.68 (1H, d), 7.72 (1H, t), 7.35(12H, m), 7.15 (1H, s), 7.10 (1H, s), 5.66 (1H, s), 4.96 (1H, d, A of AB q, J = 13 Hz), 4.73 (1H, d, B of AB q, J = 13 Hz), 3.63 (1H, d, A of AB q, J = 18 Hz), 3.63 (1H, D, B of AB q, J = 18 Hz), 2.01 (3H, s); ^{13}C NMR ($CDCl_3$) δ 170.3 (s), 161.0 (s), 160.2 (s), 151.6 (d), 150.1 (s), 140.6 (s), 139.3 (s), 139.1 (s), 136.6 (d), 128.3, 127.9, 127.8, 127.6, 127.2, 126.9, 125.8 (s), 123.9 (s), 123.5 (s), 79.5 (d), 63.0 (t), 58.5 (d), 28.0 (t), 20.5 (q); high-resolution mass spectrum for $[C_{29}H_{24}N_2O_5SNa]^+$, i.e., $[M + Na]^+$, m/z calcd 535.1304, found 535.1300.

Benzhydryl 7-[(*Z*)-(*tert*-Butoxycarbonyl)methylene]cephalosporanate (4f). To a solution of benzhydryl 7-oxocephalosporanate **3** (4.0 g, 9.2 mmol) in anhydrous CH_2Cl_2 (40 mL) at −78 °C was added a solution of [(*tert*-butoxycarbonyl)methylene]triphenylphosphorane (3.45 g, 9.15 mmol in 40 mL of CH_2Cl_2). The mixture was then stirred at −78 °C for 30 min. Acetic acid (1 mL) was added to quench the reaction, and the reaction mixture was concentreated and purified by column chromatography to give title compound as a pale yellow solid (yield = 55%): R_f = 0.52 in 2% EtOAc in CH_2Cl_2. mp 48–50 °C; IR ($CHCl_3$) 3050, 1780, 1730 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.36 (10H, m), 7.00 (1H, s), 6.39 (1H, s), 5.47 (1H, s), 5.00 (1H, d, A of AB q, J = 13.48 Hz), 4.77 (1H, d, B of Ab q, J = 13.48 Hz), 3.62 (1H, d, A of AB q, J = 18 Hz), 3.38 (1H, d, B of AB q, J = 18 Hz), 2.02 (3H, s), 1.54 (9H, s); ^{13}C NMR ($CDCl_3$) δ 170.2 (s), 162.4 (s), 160.5 (s), 157.8 (s), 150.1, (s), 139.0 (s), 138.8 (s), 128.3, 128.0, 127.9, 127.5, 126.9, 125.0 (s), 119.9 (d), 82.9 (s), 79.7 (d), 62.8 (t), 57.5 (d), 28.0 (q), 27.9 (t), 20.4 (q). Anal. ($C_{29}H_{29}NO_7S$) H, N; C: calcd, 65.05; found, 64.50.

Benzhydryl 7-[(*Z*)-Acetylmethylene]cephalosporanate (4g). This compound was prepared as described for compound **4f** (yield = 58%): R_f = 0.29 in 2% EtOAC in CH_2Cl_2; mp 49–50 °C; IR ($CHCl_3$) 3000, 1770, 1720 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.36 (10H, m), 7.00 (1H, s), 6.48 (1H, s), 5.50 (1H, s), 5.00 (1H, d, A of AB q, J = 13 Hz), 4.77 (1H, d, B of AB q, J = 13 Hz), 3.63 (1H, d, A of AB q, J = 19 Hz), 3.38 (1H, d, B of AB q, J = 19 Hz), 2.39 (3H, s), 2.02 (3H, s). ^{13}C NMR ($CDCl_3$) δ 195.8 (s), 170.3 (s), 160.6 (s), 158.5 (s), 149.5 (s), 139.3 (s), 139.1 (s), 128.5, 127.8, 127.1, 126.9, 126.3, 125.6 (s), 122.7 (d), 79.8 (d), 63.0 (t), 58.0 (d), 30.9 (q), 28.0 (t), 20.7 (q). Anal. ($C_{26}H_{23}NO_6S$) C, H, N.

Benzhydryl 7-[(*Z*)-Formylmethylene]cephalosporanate (4h). This compound was prepared as described for coupound **4f** (yield = 46%): R_f = 0.37 in 2% EtOAc in CH_2Cl_2; mp 113–115 °C; IR ($CHCl_3$) 3050, 1780. 1730, 1700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.80 (1H, d, J = 6.1 Hz), 7.34 (10H, m), 6.99 (1H, s), 6.60 (1H, d, J = 6.1 Hz), 5.45 (1H, s), 5.00 (1H, d, A of AB q, J = 13.51 Hz), 4.75 (1H, d, B of AB q, 13.55 Hz), 3.64 (1H, d, A of AB q, J = 18.59 Hz), 3.41 (1H, d, B of AB q, J = 18.61 Hz), 2.00 (3H, s); ^{13}C NMR ($CDCl_3$) δ 188.2 (d), 170.1 (s), 160.3 (s), 157.0 (s), 154.7 (s), 138.9 (s), 138.8 (s), 128.4, 128.1, 128.0, 127.6, 126.9, 126.7, 125.0 (s), 123.5 (d), 79.9 (d), 62.4 (t), 56.4 (d), 28.1 (t), 20.4 (q); high-resolution mass spectrum for $[C_{25}H_{21}NO_6SNa]^+$, i.e., $[M + Na]^+$, m/z calcd 486.0987, found 468.0981. Minor product *E*-isomer: ^1H NMR ($CDCl_3$) δ 10.28 (1H, d, J = 7.6 Hz), 7.34 (10H, m), 6.99 (1H, s), 6.26 (1H, d, J = 7.6 Hz), 5.28 (1H, s), 5.00 (1H, d, A of AB q, J = 13.5 Hz), 4.75 (1H, d, B of AB q, J = 13.5 Hz), 3.60 (1H, d, A of AB q, J = 18.6 Hz), 3.40 (1H, d, B of AB q, J = 18.6 Hz), 2.00 (3H, s).

Benzhydryl 7-[(*Z*)-(Hydroxymethyl)methylene]cephalosporanate (4i). To a solution of **4h** (0.75 g, 1.62 mmol) in methanol (10 mL) and acetic acid (1 mL) was added $NaCNBH_3$ (0.51 g, 8.1 mmol) in one portion, and the mixture was stirred at room temperature for 30 min. The reaction mixture was concentrated *in vacuo*, and the residue was dissolved in EtOAc (25 mL) and water (10 mL). The aqueous layer was exacted with EtOAc (1 × 30 mL), and the combined organic layer was washed with water (1 × 30 mL), dried (Na_2SO_4), concentrated, and purified by column chromatography to give a white solid (0.71 g, 94%): R_f = 0.3 in 10% EtOAc in CH_2Cl_2; mp 58–60 °C; ^1H NMR ($CDCl_3$) δ 7.39 (10H, s), 7.01 (1H, s), 6.51 (1H, s), 5.29 (1H, s), 4.94 (1H, d, A of AB q, J = 13 Hz), 4.71 (1H, d, B of AB q, J = 13 Hz), 4.60 (1H, d, A of AB q, J = 20.83 Hz), 4.42 (1H, d, B of AB q, J = 20.22 Hz), 3.56 (1H, d, A of AB q, J = 18 Hz), 3.33 (1H, d, B of AB q, J = 18 Hz), 2.01 (3H, s); ^{13}C NMR ($CDCl_3$) δ 170.5 (s), 161.2 (s), 159.9 (s), 139.0 (s), 138.8 (s), 137.4 (s), 131.8 (d), 128.3, 128.0, 127.9, 127.6, 127.4, 126.8, 122.2 (s), 79.6 (d), 63.0 (t), 60.0 (t),

56.9 (d), 28.0 (t), 20.5 (q); high-resolution mass spectrum for $[C_{25}H_{23}NO_6SNa]^+$, i.e., $[M + Na]^+$, *m/z* calcd 488.1144, found 488.1138.

Benzhydryl 7-[(*Z*)-[[*N*-Methoxy-*N*-methylamino]carbonyl]methylene]cephalosporanate (4j). To a solution of benzhydryl 7-oxocephalosporanate **3** (1.0 g, 2.3 mmol) in anhydrous CH_2Cl_2 (20 mL) at −78 °C was added *N*-methoxy-*N*-methyl-2-(triphenylphosphoranylidene)acetamide (0.73 g, 2.0 mmol). The mixture was stirred at −78 °C for 10 min, warmed to 0 °C, and further stirred for 15 min. Acetic acid (0.5 mL) was added to quench the reaction, and the reaction mixture was concentrated and purified by column chromatography (2% EtOAc in CH_2Cl_2) to give title compound as a pale yellow solid (0.53 g, 51%): IR ($CHCl_3$) 3050, 1780, 1730 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.35 (10H, m), 7.06 (1H, s), 7.00 (1H, s), 5.56 (1H, s), 4.96 (1H, d, A of AB q, *J* = 13 Hz), 4.75 (1H, d, B of AB q, *J* = 13 Hz), 3.75 (3H, s), 3.64 (1H, d, B of AB q, *J* = 19 Hz), 3.37 (1H, d, B of AB q, *J* = 19 Hz), 3.28 (3H, s), 2.01 (3H, s); ^{13}C NMR ($CDCl_3$) δ 170.4 (s), 163.1 (s), 160.8 (s), 158.5 (s), 151.2 (s), 139.2 (s), 139.0 (s), 128.5, 128.4, 128.1, 128.0, 127.8, 127.0, 124.8 (s), 115.6 (d), 79.8 (d), 63.0 (t), 62.4 (q), 58.0 (d), 32.2 (q), 28.1 (t), 20.6 (q).

Benzhydryl 7-[(*E*)-Bromomethylene]cephalosporanate (4k). To a solution of 7-[dibromomethylene]cephalosporanate **4a** (1.19 g, 2 mmol) in methanol (20 mL) and THF (10 mL) was added NH_4Cl (8.56 g, 16 mmol) in one portion at 0 °C. The mixture was stirred for 5 min. Zn/Cu (5.20 g, 8 mmol) was added in one portion, and the mixture was further stirred at room temperature for 30 min. The solvent was removed, and residue was extracted with ether (2 × 20 mL). The obtained ether was washed with water (1 × 20 mL) and brine (1 × 10 mL), dried (Na_2SO_4), concentrated, and purified by column chromatography (CH_2Cl_2) to give a white solid (0.86 g, 83% yield): R_f = 0.41 in CH_2Cl_2; mp 48−50 °C; IR ($CHCl_3$) 3025, 1780, 1730 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.32 (10H, m), 6.96 (1H, s), 6.44 (1H, s), 5.05 (1H, s) 4.92 (1H, d, A of AB q, *J* = 13 Hz), 4.67 (1H, d, B of AB q, *J* = 13 Hz), 3.46 (1H, d, A of AB q, *J* = 18 Hz), 3.26 (1H, d, B of AB q, *J* = 18 Hz), 1.96 (3H, s); ^{13}C NMR ($CDCl_3$) δ 170.15 (s), 160.60 (s), 157.04 (s), 141.77 (s), 139.05 (s), 138.86 (s), 128.32, 127.97, 127.89, 127.49, 126.92, 123.30 (s), 107.94 (d), 79.82 (d), 62.90 (t), 58.02 (d), 27.68 (t), 20.42 (q). Anal. ($C_{24}H_{20}NO_5SBr$) C, H, N.

Benzhydryl 7-[(*Z*)-*tert*-Butylmethylene]cephalosporanate (4l). To a suspension of CuCN (1.65 g, 3.2 mmol) in anhydrous THF (50 mL) at −78 °C was added *t*-BuLi (3.8 mL, 4.2 mmol). The cooling bath was removed until all the solid had gone into the solution. This cuprate solution was cooled to −78 °C again, and a solution of benzhydryl 7-[(*E*)-bromomethylene]cephalosporinate **4k** (1.65 g, 3.2 mmol in anhydrous THF, 15 mL) at −78 °C was cannulated to the cuprate solution as fast as possible. The solution was further stirred at −78 °C for 1 min before quenching with saturated NH_4Cl solution (20 mL). The reaction mixture was extracted with ether (50 mL). The combined organic layers were washed with cold saturated NH_4Cl (2 × 10 mL), dried over Na_2SO_4, concentrated, and purified by column chromatography (CH_2Cl_2) to give a white solid (1.23 g, 78% yield): R_f = 0.64 in CH_2Cl_2; mp 120−121 °C; IR ($CHCl_3$) 2950, 1765, 1730 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.35 (10H, m), 7.00 (1H, s), 6.00 (1H, s), 4.93 (1H, s), 4.86 (1H, d, A of AB q, *J* = 13 Hz), 4.63 (1H, d, B of AB q, *J* = 13 Hz), 3.48 (1H, d, A of AB q, *J* = 18 Hz), 3.28 (1H, d, B of AB q, *J* = 18 Hz), 1.96 (3H, s), 1.24 (9H, s); ^{13}C NMR ($CDCl_3$) δ 170.30 (s), 161.44 (s), 158.31 (s), 147.87 (d), 139.30 (s), 139.08 (s), 135.76 (s), 128.31, 128.02, 127.80, 127.03, 121.01 (s), 79.50 (d), 63.10 (t), 55.72 (d), 34.43 (s), 29.83 (q), 27.86 (t), 20.50 (q). Anal. ($C_{28}H_{29}NO_5S$) C, H, N.

Benzhydryl 7-(Dibromomethylene)cephalosporanate sulfone (5a). To a solution of sulfide **4a** (0.3 g, 0.5 mmol) in CH_2Cl_2 (10 mL) and pH = 6.4 buffer solution (10 mL) was added *m*-CPBA (85%, 0.35 g, 2.0 mmol) in one portion. The mixture was stirred at room temperature for 40 min, and then ether (50 mL) was added. After separating layers, the organic layers were washed with saturated $NaHCO_3$ (3 × 30 mL), dried ($NaSO_4$), concentrated, and purified by column chromatography to yield a white solid (2.5 g, 79%): R_f = 0.50 in 2% EtOAc in CH_2Cl_2; mp 62−64 °C; IR ($CHCl_3$) 3030, 1800, 1740, 1350, 1130 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.36 (10H, m), 6.95 (1H, s), 5.20 (1H, s), 5.03 (1H, d, A of AB q, *J* = 14 Hz), 4.68 (1H, d, B of AB q, *J* = 14 Hz), 4.02 (1H, d, A of AB q, *J* = 18 Hz), 3.77 (1H, d, B of AB q, *J* = 18 Hz), 2.02 (3H, s); ^{13}C NMR ($CDCl_3$) δ 170.1 (s), 159.6 (s), 154.8 (s), 138.8 (s), 138.7 (s), 135.2 (s), 128.6, 128.3, 127.5, 127.1, 126.4, 125.5 (s), 124.1 (s), 98.2 (s), 80.8 (d), 73.0 (d), 62.0 (t), 52.1 (t), 20.5 (q). Anal. ($C_{24}H_{19}NO_7SBr_2$) C, H, N, Br.

Benzhydryl 7-(Dichloromethylene)cephalosporanate Sulfone (5b). This compound was prepared from the corresponding sulfide **4b** as described for compound **5a** to give a white solid (yield = 81%): R_f = 0.38 in 2% EtOAc in CH_2Cl_2; mp 64−66 °C; IR ($CHCl_3$) 3050, 1800, 1740, 1350, 1140 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.35 (10H, m), 6.95 (1H, s), 5.28 (1H, s), 5.05 (1H, d, A of AB q, *J* = 14 Hz), 4.65 (1H, d, B of AB q, *J* = 14 Hz), 4.03 (1H, d, A of AB q, *J* = 18 Hz), 3.80 (1H, B of AB q, *J* = 18 Hz), 2.04 (3H, s); ^{13}C NMR ($CDCl_3$) δ 170.2 (s), 159.6 (s), 153.9 (s), 138.6 (s), 138.5 (s), 134.3 (s), 130.2 (s), 128.9, 128.6, 128.3, 127.6, 127.3, 127.1, 124.3 (s), 80.7 (d), 70.7 (d), 61.9 (t), 51.7 (t), 20.5 (q). Anal. $C_{24}H_{19}NO_7SCl$: C, H, N.

Benzhydryl 7-[(*E*)-Benzylidene)cephalosporanate Sulfone (5c). This compound was prepared from the sulfide **4c** (0.51 g, 1.0 mmol) as described for **5a** to give a white solid (0.350 g, yield = 65%): R_f = 0.27 in CH_2Cl_2; mp 194−196 °C; IR ($CHCl_3$) 2975, 1775, 1730, 1340, 1125 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.00 (2H, m), 7.41 (13H, m), 7.03 (1H, s), 6.94 (1H, s), 5.24 (1H, s), 5.04 (1H, d, A of AB q, *J* = 14 Hz), 4.70 (1H, d, B of AB q, *J* = 14 Hz), 4.05 (1H, d, A of AB q, *J* = 18 Hz), 3.77 (1H, d, B of AB q, *J* = 18 Hz), 2.05 (3H, s); ^{13}C NMR ($CDCl_3$) δ 170.3 (s), 160.1(s), 157.7 (s), 138.9 (s), 138.8 (s), 138.5(d), 132.5, 131.5, 131.0, 128.9, 128.6, 128.3, 127.7, 127.1, 126.7, 122.8 (s), 80.4 (d), 69.5 (d), 62.1 (t), 51.2 (t), 20.5 (q); high-resolution mass spectrum for $[C_{30}H_{25}NO_7SNa]^+$, i.e., $[M + Na]^+$, *m/z* calcd 566.1249, found 566.1248.

Benzhydryl 7-[(*Z*)-Benzylidene)cephalosporanate Sulfone (5d). This compound was prepared from the sulfide **4d** (0.68 g, 1.3 mmol) as described for **5a** to give a white solid (yield = 57%, 0.410 g): R_f = 0.40 in CH_2Cl_2; mp 61−63 °C; IR ($CHCl_3$) 3025, 2925, 1780, 1730, 1340, 1130 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.42 (15H, m), 7.12 (1H, s), 6.98 (1H, s), 5.53 (1H, s), 4.95 (1H, d, A of AB q, *J* = 13 Hz), 4.65 (1H, d, B of AB q, *J* = 13 Hz), 4.04 (1H, d, A of AB q, *J* = 18 Hz), 3.77 (1H, d, B of AB q, *J* = 18 Hz), 1.96 (3H, s); ^{13}C NMR ($CDCl_3$) δ 170.1 (s), 159.9 (s), 159.7 (s), 138.8 (s), 138.7 (s), 134.12 (s), 131.6 (d), 131.0, 129.8, 129.1, 128.4, 128.2, 128.1, 127.6, 127.0, 126.7, 126.2, 121.8 (d), 80.3 (d), 71.7 (d), 691.9 (t), 51.6 (t), 20.3 (q); high-resolution mass spectrum for $[C_{30}H_{25}NO_7SNa]^+$, i.e., $[M + Na]^+$, *m/z* calcd 566.1249, found 566.1262.

Benzhydryl 7-[(*Z*)-(2′-Pyridyl)methylene]cephalosporanate Sulfone (5e). This compound was prepared from the corresponding sulfide **4e** (0.45 g, 0.88 mmol) as described for **5a** to give a white solid (yield = 90%): R_f = 0.26 in 2% EtOAc in CH_2Cl_2; mp 120−122 °C; IR ($CHCl_3$) 2975, 2950, 1780, 1720, 1340, 1130 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.67 (1H, d), 7.71 (1H, t), 7.40 (13H, m), 7.00 (1H, s), 5.91 (1H, s), 5.14 (1H, d, A of AB q, *J* = 14 Hz), 4.80 (1H, B of AB q, *J* = 14 Hz), 4.11 (1H, d, A of AB q, *J* = 18 Hz), 3.78 (1H, d, B of AB q, *J* = 18 Hz), 2.05 (3H, s); high-resolution mass spectrum for $[C_{29}H_{24}N_2O_7SNa]^+$, i.e., $[M + Na]^+$, *m/z* calcd 567.1202, found 567.1198.

Benzhydryl 7-[(*Z*)-(*tert*-Butoxycarbonyl)methylene]-cephalosporanate sulfone (5f). This compound was prepared from the corresponding sulfide **4f** as described for **5a** to give a white solid (yield = 73%): R_f = 0.68 in 5% EtOAc in CH_2Cl_2; mp 58−60 °C. IR ($CHCl_3$) 3025, 1800, 1730, 1350, 1160 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.36 (10H, m), 6.98 (1H, s), 6.59 (1H, s), 5.58 (1H, s), 5.14 (1H, d, A of AB q, *J* = 14 Hz), 4.80 (1H, d, B of AB q, *J* = 14 Hz), 4.12 (1H, d, A of AB q, *J* = 18 Hz), 3.77 (1H, d, B of AB q, *J* = 18 Hz), 2.04 (3H, s), 1.52 (9H, s); ^{13}C NMR ($CDCl_3$) δ 170.0 (s), 161.5 (s), 159.4 (s), 157.1 (s), 142.3 (s), 138.6 (s), 138.5 (s), 128.8, 128.4, 128.3, 127.2, 127.0, 125.9 (s), 123.5 (d), 83.8 (s), 80.2 (d), 71.6 (d), 61.3 (t), 52.8 (t), 27.6 (q), 20.2 (q); high-resolution mass spectrum for $[C_{29}H_{29}NO_9SNa]^+$, i.e., $[M + Na]^+$, *m/z* calcd 590.1461, found 590.1447.

Benzhydryl 7-[(*Z*)-Acetylmethylene]cephlosporanate Sulfone (5g). This compound was prepared from the corresponding sulfide **4g** as described for **5a** to give a white solid

(yield = 79%): R_f = 0.66 in 25% EtOAc in CH_2Cl_2; mp 176–178 °C; IR ($CHCl_3$) 3050, 1800, 1730, 1350, 1140 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.38 (10H, m), 6.99 (1H, s), 6.94 (1H, s), 5.64 (1H, s), 5.13 (1H, d, A of AB q, J = 14 Hz), 4.81 (1H, d, B of AB q, J = 14 Hz), 4.12 (1H, d, A of AB q, J = 18 Hz), 3.80 (1H, d, B of AB q, J = 18 Hz), 2.46 (3H, s), 2.07 (3H, s); ^{13}C NMR ($CDCl_3$) δ 194.7 (s), 170.1 (s), 159.5 (s), 157.5 (s), 141.2 (s), 138.7 (s), 138.6 (s), 128.6, 128.3, 127.5, 127.1, 126.8 (s), 125.3 (d), 80.5 (d), 72.2 (d), 61.7 (t), 53.1 (t), 31.0 (q), 20.5 (q); high-resolution mass spectrum for $[C_{26}H_{23}NO_8SNa]^+$, i.e., $[M + Na]^+$, *m/z* calcd 532.1042, found 532.1045.

Benzhydryl 7-[(*Z*)-(Hydroxymethyl)methylene]cephalosporanate sulfone (5i). This compound was prepared form the corresponding sulfide **4i** as described in **5a** to give a white solid (yield = 68%): R_f = 0.32 in 25% EtOAc in CH_2Cl_2; mp 58–60 °C; IR ($CHCl_3$) 3050, 1780, 1730, 1330, 1130 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.36 (10H, m), 6.97 (1H, s), 6.81 (1H, s), 5.53 (1H, s), 5.05 (1H, d, A of AB q, J = 14 Hz), 4.74 (1H, d, B of AB q, J = 14 Hz), 4.61 (1H, d, A of AB q, J = 19 Hz), 3.98 (1H, d, B of AB q, J = 19 Hz), 4.06 (1H, d, A of AB q, J = 17.0 Hz), 3.76 (1H, d, B of AB q, J = 17.80 Hz), 2.04 (3H, s); ^{13}C NMR ($CDCl_3$) δ 170.4 (s), 160.0 (s), 158.6 (s), 138.9 (s), 138.8 (s), 136.8 (d), 128.6, 128.1, 127.8, 127.6, 127.5, 127.2, 126.7, 124.3 (s), 80.8 (d), 71.4 (d), 61.9 (t), 60.7 (t), 51.4 (t), 20.6 (q); high-resolution mass spectrum for $[C_{25}H_{23}NO_6SNa]^+$, i.e., $[M + Na]^+$, *m/z* calcd 488.1144, found 488.1138.

Benzhydryl 7-[(*Z*)-[(*N*-Methoxy-*N*-methylamino)carbonyl]methylene]cephlosporanate Sulfone (5j). This compound was prepared from the corresponding sulfide **4j** as described for **5a** to give a white solid (yield = 68%): R_f = 0.44 in 25% EtOAc in CH_2Cl_2; mp 81–82 °C; IR ($CHCl_3$) 3050, 1800, 1740, 1360, 1140 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.36 (10H, m), 7.28 (1H, s), 6.98 (1H, s), 5.72 (1H, s), 5.10 (1H, d, A of AB q, J = 14 Hz), 4.82 (1H, d, B of AB q, J = 14 Hz), 4.11 (1H, d, A of AB q, J = 17 Hz), 3.78 (1H, d, B of AB q, J = 17 Hz), 3.78 (3H, s), 3.31 (3H, s), 2.06 (3H, s); ^{13}C NMR ($CDCl_3$) δ 170.1 (s), 162.1 (s), 159.7 (s), 157.8 (s), 142.78 (s), 138.9 (s), 138.8 (s), 128.7, 128.4, 127.7, 127.4, 127.1, 126.9, 125.7 (s), 119.3 (d), 80.3 (d), 72.3 (d), 62.5 (q), 61.8 (t), 52.9 (t), 32.3 (q), 20.5 (q); high-resolution mass spectrum for $[C_{27}H_{26}N_2O_9SNa]^+$, i.e., $[M + Na]^+$, *m/z* calcd 577.1257, found 577.1247.

Benzhydryl 7-[(*E*)-Bromomethylene]cephalosporanate Sulfone (5k). This compound was prepared from the corresponding sulfide **4k** as described for **5a** to give a white solid (yield = 71%): R_f = 0.43 in 2% EtOAC in CH_2Cl_2; mp 80–82 °C; IR ($CHCl_3$) 3030, 1800, 1730, 1350, 1130 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.33 (10H, m), 6.94 (1H, s), 6.91 (1H, s), 5.10 (1H, s), 5.00 (1H, d, A of AB q, J = 14 Hz), 4.67 (1H, d, B of AB q, J = 14 Hz), 3.97 (1H, A of AB q, J = 18 Hz), 3.69 (1H, d, B of AB q, J = 18 Hz), 1.99 (1H, s); ^{13}C NMR ($CDCl_3$) δ 170.1 (s), 159.7 (s), 156.3 (s), 138.7 (s), 138.6 (s), 134.0 (s), 128.4, 128.1, 127.3, 126.9, 125.7, 124.9 (s), 112.5 (d), 80.57 (d), 70.9 (d), 61.8 9t), 51.2 (t), 20.4 (q). Anal. $C_{24}H_{20}NO_7SBr$ C, H, N.

Benzhydryl 7-[(*Z*)-*tert*-butylmethylene]cephalosporanate Sulfone (5l). This compound was prepared from the corresponding sulfide **4l** as described in **5a** to give a white solid (yield = 84%): R_f = 0.48 in 2% EtOAc in CH_2Cl_2; mp 147–149 °C; IR ($CHCl_3$) 3050, 3000, 1785, 1740, 1340, 1130 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.37 (10H, m), 6.97 (1H, s), 6.26 (1H, d, J = 1.0 Hz), 5.01 (1H, d, J = 1.0 Hz), 4.97 (1H, d, A of AB q, J = 13.8 Hz), 4.65 (1H, d, B of AB q, J = 13.8 Hz), 3.96 (1H, d, A of AB q, J = 18 Hz), 3.82 (1H, d, B of AB q, J = 18 Hz), 2.01 (3H, s), 1.29 (9H, s); ^{13}C NMR (acetone-d_6) δ 170.40 (s), 160.92 (s), 158.49 (s), 152.29 (d), 140.44 (s), 140.24 (s), 128.89, 128.63, 128.00, 127.58, 126.43, 124.24 (s), 80.42 (d), 69.70 (d), 62.41 (t), 51.15 (t), 35.40 (s), 29.67 (q), 20.33 (q); high-resolution mass spectrum for $[C_{28}H_{29}NO_7SNa]^+$, i.e. $[M + Na]^+$, *m/z* calcd 546.1562, found 546.1551.

Sodium Salt of 7-(Dibromomethylene)cephalosporanic Acid (6a). To a solution of benzhydryl 7-(dibromomethylene)cephalosporinate **4a** (0.3 g, 0.5 mmol) in anhydrous CH_2Cl_2 (10 mL) was added anisole (0.54 mL, 5 mmol) at −78 °C followed by the addition of $AlCl_3$ solution (1.25 mL, 1.25 mmol) in one portion. The mixture was stirred at −78 °C for 15 min and poured into rapidly stirred cold water (30 mL) containing $NaHCO_3$ (0.42 g, 5 mmol), followed by the addition of EtOAc (30 mL). It was further stirred for 5 min and filtered using Celite 545. The aqueous layer was separated and concentrated *in vacuo* to about 2 mL and purified by reverse phase chromatography followed by lyophilization to yield a pink solid (180 mg, 80%): R_f = 0.62 in 10% EtOH in water; UV λ_{max} = 252 nm (50 mM phosphate buffer, pH = 7.2), ϵ = 14 434 cm^{-1}·mol^{-1}·L; IR (KBr) 2950, 1730, 1600, 1390 cm^{-1}; ^1H NMR (DMSO-d_6) δ 5.42 (1H, s), 4.91 (1H, d, A of AB q, J = 12 Hz), 4.71 (1H, d, B of AB q, J = 12 Hz), 3.50 (1H, d, A of AB q, J = 17 Hz), 3.22 (1H, d, B of AB q, J = 17 Hz), 1.99 (3H, s); high-resolution mass spectrum for $[C_{11}H_8NO_5SBr_2Na_2]^+$, i.e., $[M + Na]^+$, *m/z* calcd 469.8285, found 469.8277.

Sodium Salt of 7-(Dichloromethylene)cephalosporanic Acid (6b). This compound was prepared from the corresponding ester **4b** (0.3 g, 0.6 mmol) as described in **6a** to give a pale yellow fluffy solid (yield = 62%): R_f = 0.66 in 10% EtOH in water; UV λ_{max} = 242 nm (50 mM phosphate buffer, pH = 7.2), ϵ = 11 789 cm^{-1}·mol^{-1}·L; IR (KBr) 2950, 1740, 1600, 1390 cm^{-1}; ^1H NMR (DMSO-d_6) δ 5.52 (1H, s), 4.93 (1H, d, A of AB q, J = 12 Hz), 4.73 (1H, d, B of AB q, J = 12 Hz), 3.53 (1H, d, A of AB q, J = 18 Hz), 3.27 (1H, d, B of AB q, J = 18 Hz), 1.99 (3H, s); high-resolution mass spectrum for $[C_{11}H_9NO_5SCl_2Na]^+$, i.e. $[M + H]^+$, *m/z* calcd 359.9473, found 359.9476.

Sodium Salt of 7-[(*Z*)-Benzylidene]cephalosporanic Acid Sulfone (6d). This compound was prepared from the corresponding ester **4d** (100 mg, 0.46 mmol) as described in **6a** to give title compound as a white fluffy solid (15 mg, 17% yield): R_f = 0.80 in 5% EtOH in water; ^1H NMR (D_2O) δ 7.34 (4H, m), 7.09 (1H, s), 5.59 (1H,s) 4.71 (1H, d, A of AB q, J = 12 Hz), 4.54 (1H, d, B of AB q, J = 12 Hz), 3.56 (1H, d, A of AB q, J = 18 Hz), 3.24 (1H, d, B of AB q, J = 18 Hz), 1.96 (3H, s).

Sodium Salt of 7-[(*Z*)-(2′-Pyridyl)methylene]cephalosporanic Acid (6e). This compound was prepared from the corresponding ester **4e** (0.4 g, 0.78 mmol) as described for **6a** to give a yellow solid (149 mg, 52%): R_f = 0.56 in 10% EtOH in water; UV λ_{max} = 296 nm (50 mM phosphate buffer, pH = 7.2), ϵ = 11 257 cm^{-1}·mol^{-1}·L; IR (KBr) 2950, 1720, 1600, 1390 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.64 (1H, d), 7.83 (1H, t), 7.63 (1H, d), 7.37 (1H, t), 7.34 (1H, s), 5.63 (1H, s), 4.92 (1H, d, A of AB q, J = 12 Hz), 4.77 (1H, d, B of AB q, J = 12 Hz), 3.56 (1H, d, A of AB q, J = 18 Hz), 3.27 (1H, d, B of AB q, J = 18 Hz), 2.00 (3H, s); high-resolution mass spectrum for $[C_{16}H_{14}N_2O_5SNa]^+$, i.e., $[M + H]^+$, *m/z* calcd 369.0518, found 369.0506.

Sodium Salt of 7-[(*E*)-(*tert*-Butoxycarbonyl)methylene]cephalosporanic Acid (6f). To a solution of benzhydryl 7-[(*Z*)-(*tert*-butoxycarbonyl)methylene]cephalosporanate (**4f**) (0.155 g, 0.2733 mmol) in anisole (0.9 mL, 8.2 mmol) at 0 °C was added trifluroacetic acid (2.5 mL, 32 mmol). The mixture was stirred for 8 min, concentrated *in vacuo* (to remove all of the TFA), dissolved in 25 mL of EtOAc, and then poured into rapidly stirred $NaHCO_3$ solution (0.230 g in 25 mL of H_2O). The aqueous layer was separated, concentrated *in vacuo* to 2 mL, and further purified by reverse phase chromatography (R_f = 0.4 in 5% EtOH in water) followed by lyopholization to yield a pale yellow fluffy solid. This compound was prepared from the corresponding ester **4f** (0.3 g, 0.56 mmol) as described to give a yellow fluffy solid (176 mg, 81%): R_f = 0.53 in 5% EtOH in water; UV λ_{max} = 225 nm (50 mM phosphate buffer, pH = 7.2), ϵ = 11 324 cm^{-1}·mol^{-1}·L; IR (KBr) 2950, 1720, 1600, 1400 cm^{-1}; ^1H NMR (DMSO-d_6) δ 6.26 (1H, s), 5.47 (1H, s), 4.92 (1H, d, A of AB q, J = 12 Hz), 4.72 (1H, d, B of AB q, J = 12 Hz), 3.56 (1H, d, A of AB q, J = 18 Hz), 3.22 (1H, d, B of AB q, J = 18 Hz), 1.99 (3H, s), 1.47 (9H, s); high-resolution mass spectrum for $[C_{16}H_{18}NO_7SNa_2]^+$, i.e., $[M + Na]^+$, *m/z* calcd 414.0600, found 414.0604.

Sodium Salt of 7-[(*Z*)-Acetylmethylene]cephalosporanic Acid (6g). This compound was prepared from the corresponding ester **4g** (0.4 g, 0.84 mmol) as described for **6f** to give a yellow fluffy solid (217 mg, 78%): R_f = 0.8 in 5% EtOH in water; UV λ_{max} = 235 nm (50 mM phosphate buffer, pH = 7.2); ϵ = 9031 cm^{-1}·mol^{-1}·L; IR (KBr) 2950, 1750, 1600, 1390 cm^{-1}; ^1H NMR (DMSO-d_6) δ 6.68 (1H, s), 5.56 (1H, s), 4..93 (1H, d, A of AB q, J = 12 Hz), 4.75 (1H, d, B of AB q, J

= 12 Hz), 3.56 (1H, d, A of AB q, J = 17 Hz), 3.25 (1H, d, B of AB q, J = 17 Hz), 2.34 (3H, s), 2.00 (3H, s); high-resolution mass spectrum for $[C_{13}H_{13}NO_6SNa]^+$, i.e., $[M + H]^+$, m/z calcd 334.0361, found 334.0360.

Sodium Salt of 7-(Formylmethylene)cephalosporanate](Mixture of *Z* and *E* Isomers) (6h). This compound was prepared from the corresponding ester **4g** (0.3 g, 0.65 mmol) as described for **6f** to give a yellow fluffy solid (160 mg, 77%). Two inseperable isomers are produced (Z:E = 2:1): R_f = 0.8 in 5% EtOH in water; ^{1}H NMR (DMSO-d_6) E-isomer δ 9.65 (1H, d, J = 4 Hz), 6.58 (1H, d, J = 4 Hz), 5.66 (1H, s), 4.93 (1H, d, A of AB q, J = 13 Hz), 4.73 (1H, d, B of AB q, J = 13 Hz), 3.58 (1H, d, A of AB q, J = 17.00 Hz), 1.98 (3H, s); Z-isomer δ 10.10 (1H, d, J = 8 Hz), 6.52 (1H, d, J = 8 Hz), 5.49 (1H, s), 4.93 (1H, d, A of AB q, J = 13.05 Hz), 4.73 (1H, d, B of AB q, J = 13.05 Hz), 3.58 (1H, d, A of AB q, J = 17.00 Hz), 1.98 (3H, s).

Sodium Salt of 7-[(*Z*)-[(*N*-methylamino)carbonyl]-methylene]cephlosporanic Acid (6j). This compound was prepared from the corresponding ester **4j** as described for **6f** to give yellow a fluffy solid (yield = 55%): R_f = 0.81 in 5% EtOH in water; UV λ_{max} = 231 nm (50 mM phosphate buffer, pH = 7.2), ϵ = 11 300 cm^{-1}·mol^{-1}·L; IR (KBr) 2950, 1720, 1600, 1390 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 6.84 (1H, s), 5.44 (1H, s), 4,89 (1H, d, A of AB q, J = 12 Hz), 4.72 (1H, d, B of AB q, J = 12 Hz), 3.72 (3H, s), 3.51 (1H, d, A of AB q, J = 18 Hz), 3.17 (3H, s), 3.15 (1H, d, B of AB q, J = 18 Hz), 1.98 (3H, s).

Sodium Salt of 7-[(*E*)-Bromomethylene]cephalosporanic Acid (6k). This compound was prepared from the corresponding ester **4k** (0.4 g, 0.78 mmol) as described for **6a** to yield a white fluffy solid (192 mg, 67%): R_f = 0.77 in 10% EtOH in water; UV λ_{max} = 243 nm (50 mM phosphate buffer, pH = 7.2), ϵ = 10 478 cm^{-1}·mol^{-1}·L; IR (KBr) 2950, 1730, 1600, 1390 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.21 (1H, s), 5.28 (1H, s), 4.93 (1H, d, A of AB q, J = 12 Hz), 4.70 (1H, d, B of AB q, 12 Hz), 3.48 (1H, d, A of AB q, J = 17 Hz), 3.21 (1H, d, B of AB q, J = 17 Hz), 1.98 (3H, s); high-resolution mass spectrum for $[C_{11}H_9NO_5SBrNa_2]^+$, i.e., $[M + Na]^+$, m/z calcd 391.9178, found 391.9180.

Sodium Salt of 7-[(*Z*)-*tert*-Butylmethylene]cephalosporanic Acid (6l). This compound was prepared from the corresponding ester **4l** (0.4 g, 0.81 mmol) as described for **6a** to obtain a white fluffy solid (105 mg, 37%): R_f = 0.55 in 10% EtOH in water; UV λ_{max} = 228 nm (50 mM phosphate buffer, pH = 7.2), ϵ = 12 760 cm^{-1}·mol^{-1}·L; IR (KBr) 2950, 1730, 1600, 1390 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 5.98 (1H, s), 5.00 (1H, s), 4.90 (1H, d, A of AB q, J = 12 Hz), 4.68 (1H, d, B of AB q, J = 20 Hz), 3.43 (1H, d, A of AB q, J = 18 Hz), 3.17 (1H, d, B of AB q, J = 18 Hz), 1.98 (3H, s), 1.17 (9H, s); high-resolution mass spectrum for $[C_{15}H_{19}NO_5SNa]^+$, i.e., $[M + H]^+$, m/z calcd 348.0877, found 348.0870.

Sodium Salt of 7-(Dibromomethylene)cephalosporanic Acid Sulfone (7a). This compound was prepared from the corresponding ester **5a** (0.3 g, 0.5 mmol) as described in **6a** to give a white fluffy solid (110 mg, 48%): R_f = 0.83 in 10% EtOH in water; UV λ_{max} = 260 nm (50 mM phosphate buffer, pH = 7.2), ϵ = 12 535 cm^{-1}·mol^{-1}·L; IR (KBr) 2950, 1740, 1600, 1390, 1330, 1130 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 5.89 (1H, s), 4.94 (1H, d, A of AB q, J = 12 Hz), 4.66 (1H, d, B of AB q, J = 12 Hz), 4.12 (1H, d, A of AB q, J = 18 Hz), 3.84 (1H, d, B of AB q, J = 18 Hz), 1.99 (3H, s); high-resolution mass spectrum for $[C_{11}H_9NO_7SBr_2Na]^+$, i.e., $[M + H]^+$, m/z calcd 479.8361, found 479.8349.

Sodium Salt of 7-(Dichloromethylene)cephalosporanic Acid Sulfone (7b). This compound was prepared from the corresponding ester **5b** (0.4 g, 0.76 mmol) as described in **4a** to give a white fluffy solid (yield = 50%): R_f = 0.84 in 10% EtOH in water; UV λ_{max} = 245 nm (50 mM phosphate buffer, pH = 7.2), ϵ = 16 679 cm^{-1}·mol^{-1}·L; IR (KBr) 2950, 1730, 1600, 1390, 1330, 1130 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 5.99 (1H, s), 4.93 (1H, d, A of AB q, J = 12 Hz), 4.64 (1H, d, B of AB q, J = 12 Hz), 4.15 (1H, d, A of AB q, J = 18 Hz), 3.90 (1H, d, B of AB q, J = 18 Hz), 1.97 (3H, s); high-resolution mass spectrum for $[C_{11}H_8NO_7SCl_2Na_2]^+$, i.e., $[M + Na]^+$, m/z calcd 413.9191, found 413.9197.

Sodium Salt of 7-[(*E*)-Benzylidene]cephalosporanic Acid Sulfone (7c). This compound was prepared from the corresponding ester **5c** (300 mg, 0.55 mmol) as described for **6a** to give title compound as a white fluffy solid (30 mg, 13% yield); R_f = 0.70 in 5% EtOH in water; UV λ_{max} = 308 (50 mM phosphate buffer, pH = 7.2), ϵ = 15 515 cm^{-1}·mol^{-1}·L; IR (KBr) 2950, 1710, 1600, 1390, 1330, 1130 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.05(2H, m), 7.47 (3H, m), 6.93 (1H, s), 5.72 (1H, s), 4.95 (1H, d, A of AB q, J = 12 Hz), 4.67 (1H, d, B of AB q, J = 12 Hz), 4.12 (1H, d, A of AB q, J = 18 Hz), 3.79 (1H, d, B of AB q, J = 18 Hz), 2.00 (3H, s); high-resolution mass spectrum for $[C_{17}H_{15}NO_7SNa]^+$, i.e., $[M + H]^+$, m/z calcd 400.0463, found 400.0451.

Sodium Salt of 7-[(*Z*)-Benzylidene]cephalosporanic Acid Sulfone (7d). This compound was prepared from the corresponding ester **5d** (250 mg, 0.46 mmol) as described in **6a** to give title compound as a white fluffy solid (77 mg, 42% yield): R_f = 0.80 in 5% EtOH in water; UV λ_{max} = 302 (50 mM phosphate buffer, pH = 7.2), ϵ = 20 543 cm^{-1}·mol^{-1}·L; IR (KBr) 2950, 1740, 1600, 1390, 1330, 1130 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.79 (2H, m), 7.44 (4H, m), 6.34 (1H, s), 4.95 (1H, d, A of AB q, J = 12 Hz), 4.70 (1H, d, B of AB q, J = 12 Hz), 4.12 (1H, d, A of AB q, J = 18 Hz), 3.88 (1H, d, B of AB q, J = 18 Hz), 2.02 (3H, s); high-resolution mass spectrum for $[C_{17}H_{15}NO_7SNa]^+$, i.e., $[M + H]^+$, m/z calcd 400.0463, found 400.0464.

Sodium Salt of 7-[(*Z*)-(2′-Pyridyl)methylene]cephalosporanic Acid Sulfone (7e). This compound was prepared from the corresponding ester **5e** as described in **6f** as a pale yellow fluffy solid (yield = 67%): R_f = 0.78 in 10% EtOH in water; UV λ_{max} = 301 nm (50 mM phosphate buffer, pH = 7.2), ϵ = 8624 cm^{-1}·mol^{-1}·L; IR (KBr) 2950, 1720, 1600, 1390, 1330, 1130 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.59 (1H, d), 7.88 (1H, t), 7.72 (1H, d), 7.42 (2H, m), 6.22 (1H, s), 4.92 (1H, d, A of AB q, J = 11 Hz), 4.72 (1H, B of AB q, J = 11 Hz), 4.19 (1H, D, A of AB q, J = 18 Hz), 3.77 (1H, d, B of AB q, J = 18 Hz), 2.00 (3H, s); high-resolution mass spectrum for $[C_{16}H_{13}N_2O_7SNa_2]^+$, i.e., $[M + Na]^+$, m/z calcd 423.0239, found 423.0227.

Sodium Salt of 7-[(*Z*)-(*tert*-Butoxycarbonyl)methylene]cephalosporanic Acid Sulfone (7f). This compound was prepared from the corresponding ester **5f** (0.3 g, 0.53 mmol) as described in **6f** to give a white fluffy solid (163 mg, 73%): R_f = 0.74 in 5% EtOH in water; UV λ_{max} = 226 nm (50 mM phosphate buffer, pH = 7.2), ϵ = 18 171 cm^{-1}·mol^{-1}·L; IR (KBr) 2950, 1720, 1600, 1390, 1330, 1130 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 6.52 (1H, s), 5.97 (1H, s), 4.97 (1H, d, A of AB q, J = 12 Hz), 4.72 (1H, d, B of AB q, J = 12 Hz), 4.16 (1H, d, A of AB q, J = 18 Hz), 3.79 (1H, d, B of AB q, J = 18 Hz), 1.99 (3H, s), 1.45 (9H, s); high-resolution mass spectrum for $[C_{16}H_{19}NO_9SNa]^+$, i.e., $[M + H]^+$, m/z calcd 424.0678, found 424.0684.

Sodium Salt of 7-[(*Z*)-(Hydroxymethyl)methylene]-cephalosporanic Acid Sulfone (7i). This compound was prepared from the corresponding ester **5i** (0.2 g, 0.4 mmol) as described in **6f** to give a white fluffy solid (130 mg, 91.5%): R_f = 0.90 in water; UV λ_{max} = 223 nm (50 mM phosphate buffer, pH = 7.2), ϵ = 9428 cm^{-1}·mol^{-1}·L; IR (KBr) 2950, 1750, 1600, 1390, 1330, 1130 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 6.54 (1H, s), 5.74 (1H, s), 4.89 (1H, d, A of AB q, J = 12 Hz), 4.66 (1H, d, B of AB q, J = 12 Hz), 4.14 (2H, s), 4.08 (1H, d, A of AB q, J = 18 Hz), 3.73 (1H, d, B of AB q, J = 18 Hz), 1.99 (3H, s); high-resolution mass spectrum for $[C_{12}H_{13}NO_8SNa]^+$, i.e., m/z calcd 354.0260, found 354.0274.

Sodium Salt of 7-[(*E*)-Bromomethylene]cephalosporanic Acid Sulfone (7k). This compound was prepared from the corresponding ester **5k** (0.3 g, 0.55 mmol) as described in **6a** in yield a white fluffy solid (128 mg, 58%): R_f = 0.88 in 10% EtOH in water; UV λ_{max} = 246 nm (50 mM phosphate buffer, pH = 7.2), ϵ = 10 856 cm^{-1}·mol^{-1}·L; IR (KBr) 2950, 1730, 1600, 1390, 1330, 1130 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.44 (1H, s), 5.79 (1H, s), 4.96 (1H, d, A of AB q, J = 12 Hz), 4.68 (1H, d, A of AB q, J = 12 Hz), 4.03 (1H, d, A of AB q, J = 18 Hz), 3.82 (1H, d, B of AB q, J = 18 Hz), 2.00 (3H, s); high-resolution mass spectrum for $[C_{11}H_{10}NO_7SBrNa]^+$, i.e., $[M + H]^+$, m/z calcd 401.9256, found 401.9245.

Sodium Salt of 7-[(*Z*)-*tert*-butylmethylene]cephalosporanic Acid Sulfone (7l). This compound was prepared from

the corresponding ester **5l** (0.34 g, 0.65 mmol) as described in **6a** to yield a white fluffy solid (2.0 g, 82%): $R_f = 0.79$ in 10% EtOH in water; UV $\lambda_{max} = 228$ nm (50 mM phosphate buffer, pH =7.2), $\epsilon = 14\ 215\ cm^{-1} \cdot mol^{-1} \cdot L$; IR (KBr) 2950, 1730, 1600, 1390, 1330, 1130 cm^{-1}; ^1H NMR (DMSO-d_6) δ 6.08 (1H, s), 5.50 (1H, s), 4.91 (1H, d, A of AB q, $J = 12$ Hz), 4.65 (1H, d, B of AB q, $J = 12$ Hz), 4.06 (1H, d, A of AB q, $J = 18$ Hz), 3.72 (1H, d, B of AB q, $J = 18$ Hz), 1.99 (3H, s); high-resolution mass spectrum for $[C_{15}H_{18}NO_7SNa]^+$, i.e., $[M + H]^+$, *m/z* calcd 380.0775, found 380.0770.

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