RNase T1 (100 units). The total cell lysate was applied to nitrocellulose paper with use of a 96-well, vacuum operated dot-blot apparatus. The blots were hybridized to a ^{35}S-labeled d-ATP nick translated probe made by inserting full-length mouse mtDNA into pSP64 vector at the SacI site. The vector itself was used to generate a standard curve for each experiment. Dot blots were visualized by autoradiography and cut out, and radioactivity was determined by scintillation counting.

Flow Cytometric Analysis. Flow cytometric analysis of nuclear DNA content was performed with the RATCOM flow cytometer (RATCOM Inc., Miami, FL) interfaced with a microcomputer (IBM-XT). Cultured L1210 cells in log growth were incubated at 37 °C with 10 μM DESPM-3 and samples removed at 0, 48, 96, and 144 h were analyzed for nuclear DNA content distributions after being stained with diamidinophenylindole in a nuclear isolation media (NIM-DAPI).[20]

Cloning Assay. L1210 cells maintained between 10^5 and 10^6 cells/mL were incubated with 10 μM DESPM-3 for 96 h. At 24-h intervals, treated cells were washed (2 × 10 mL) and diluted in fresh complete media and plated in triplicate 96-well microtiter plates at 0.4 cell/well with each well containing 100 μL of sample. The plates were incubated at 37 °C in a humidified incubator in an atmosphere of 5% CO_2 and 95% air. The plates were examined with an inverse-phase microscope at 100× magnification. The final number of colonies per plate was quantitated 7 days after plating. Groups of 50 or more cells/well were identified as having been cloned from a single viable cell. Also, at each 24-h interval, treated cell samples were washed and the cells resuspended in fresh media at 10^5 cells/mL in duplicate 10-mL flasks. The regrowth of the treated cells was followed for up to 144 h.

Cell Size. Cell size was determined directly by the method of Schwartz et al.[21] In brief, uniform polymeric microspheres ranging from 4.72 to 10.2 μm in diameter (Polysciences, Warrington, PA) were diluted in Hematall (Fisher Scientific Co.). Electronic size was measured on the FACS Analyzer (Becton Dickinson, Sunnyvale, CA) with the amplifier in the log mode. The peak channel number for each size microbead was plotted against the corresponding calibrated diameter and calculated volume to obtain a calibration curve. L1210 cells were treated with 10 μM DESPM-3 for 0–144 h and samples of 10^6 cells were removed at 24-h intervals and pelleted. The cells were resuspended in 0.5 mL of Hematall and analyzed. The peak channel number of the treated cells was plotted on the calibration curve to obtain the approximate cell size directly.

Registry No. 1, 70655-37-7; 1·2HCl, 113812-27-4; **2**, 113812-18-3; **2**·4HCl, 113812-17-2; **3**, 61345-84-4; **3**·4HCl, 113812-15-0; **4**, 113812-20-7; **4**·4HCl, 113812-21-8; **5**, 113812-11-6; **5**·HCl, 113812-12-7; **6**, 113812-23-0; **6**·4HCl, 113812-24-1; **7**, 40563-84-6; **8**, 113830-94-7; **8**·4HCl, 113812-26-3; **9**, 113812-30-9; **10**, 63958-61-2; **11**, 113812-13-8; **12**, 113812-14-9; **13**, 113812-16-1; **14**, 113812-19-4; **15**, 113812-22-9; **16**, 113812-25-2; **17**, 113812-28-5; **18**, 113812-29-6; SPM·4HCl, 306-67-2; $C_2H_5NHCCH_2)_3NHC_2H_5$, 10061-68-4; $ClC(=O)(CH_2)_2COCl$, 543-20-4; $CH_2{=}CHCN$, 107-13-1; $C_2H_5NH(CH_2)_4NHC_2H_5$, 19435-68-8; $CH_3CONH(CH_2)_{12}NHCOCH_3$, 31991-77-2; $CH_3C_6H_4$-*p*-$SO_2NHC_2H_5$, 80-39-7; $Cl(CH_2)_3Cl$, 142-28-9; 1,4-bis(3-aminopropyl)piperazine, 7209-38-3.

(20) Thornwaite, J. T.; Sugerbaker, E. V.; Temple, W. J. *Cytometry* **1980**, *1*, 229–237.

(21) Schwartz, A.; Sugg, H.; Ritter, T. W.; Fernandez-Repollet, E. *Cytometry* **1983**, *3*, 456–458.

An Examination of *O*-2-Isocephems as Orally Absorbable Antibiotics

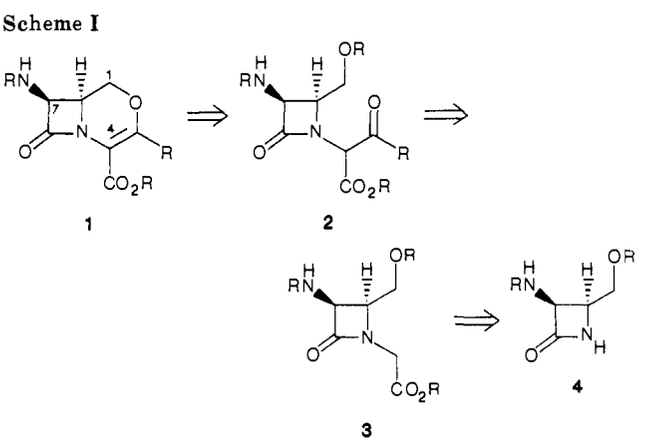
Harold Mastalerz,*† Marcel Menard,† Vivianne Vinet,† James Desiderio,‡ Joan Fung-Tomc,‡ Robert Kessler,*‡ and Yuan Tsai‡

Antiinfective Research, Bristol-Myers Pharmaceutical Group, Candiac, Quebec, Canada, J5R 1J1, and the Department of Microbiology, Bristol-Myers Pharmaceutical Group, Wallingford, Connecticut 06492-7660. *Received August 26, 1987*

The synthesis and structure–activity relationships of a series of orally absorbed *O*-2-isocephems are described. These compounds possessed a D-[(*p*-hydroxyphenyl)glycyl]amino substituent at the 7-position while the substituent at the 3-position was varied. Relative to the analogous cephems, the *O*-2-isocephems exhibited comparable to better activity against Gram-positive organisms. Against Gram-negative organisms, their activity was variable but did indicate a lower β-lactamase stability. Following oral administration, the *O*-2-isocephems generally exhibited longer half-lives but lower C_{max}'s and urinary recoveries.

The perception[1] that the use of orally administered antibiotics can be more cost-effective has spurred the search for new, long-acting, orally active β-lactam antibiotics. *O*-2-Isocephems, e.g. **1**, are nuclear analogues of the cephalosporins and have been extensively examined in these laboratories[2a] as parenterally administered antibacterials. We have conducted a reexamination of this class of compounds to see if they would show promise as orally administered antiinfectives. This consisted of examining the activity of a group of *O*-2-isocephems bearing a D-[(*p*-hydroxyphenyl)glycyl]amino substituent at the 7-position as a function of the nature of the substituent at the 3-position. The results of this effort together with the details of a new, enantioselective *O*-2-isocephem synthesis are described in this paper.

Scheme I



Chemistry

Since existing syntheses[2] were too long and did not provide much room to vary the 3-substituent, we devised a new synthesis (Scheme I), which would be more suited to our needs. We believed that the *O*-2-isocephem **1** could

† Antiinfective Research.
‡ Department of Microbiology.

(1) Scrip, 1985 Chemotherapy Report; PJB Publications: Surrey, U. K., 1985; p 22.

0022-2623/88/1831-1190$01.50/0 © 1988 American Chemical Society

Table I. In Vitro Antibacterial Activity

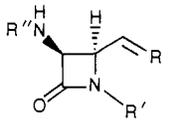
compd	minimal inhibitory concentrations (MIC),[a] μg/mL							
	S. pyogenes	*S. pneumoniae*	*S. faecalis*	*S. aureus*[b]	*E. coli*[b]	*K. pneumoniae*	*P. mirabilis*	*H. influenzae*
45	0.016	0.03	0.5	0.13 (32)	2 (32)	8	0.5	1
46	0.016	0.03	1	0.25 (32)	2 (63)	16	1	2
47	0.016	0.03	1	0.25 (125)	2 (63)	8	0.5	2
48	0.008	0.016	0.06	0.5 (125)	2 (32)	32	16	2
49	0.008	0.016	0.13	0.5 (32)	4 (32)	16	16	2
50	0.008	0.03	1	0.13 (125)	2 (63)	16	1	2
51	0.25	1	32	1 (16)	4 (8)	8	4	2
52	0.13	0.13	16	1 (32)	8 (16)	8	4	8
53	0.004	0.008	0.03	0.06 (63)	2 (32)	16	4	
54	0.016	0.03	1	0.25 (125)	4 (125)	16	2	8
55	0.03	0.03	2	0.25	4 (32)	16	4	
56	0.016	0.03	2	0.13 (32)	1 (4)	2	0.25	2
57	0.03	0.06	4	0.25 (3)	8 (32)	8	1	4
58	0.016	0.03	18	0.13 (32)	4 (8)	4	1	1
cefalexin	0.5	0.5	32	0.5	8 (16)	16	4	4
cefaclor	0.25	0.5	16	0.25	1 (32)	1	0.25	1

[a] MIC determined by 2-fold microdilution technique. [b] β-Lactamase producing strain in parentheses.

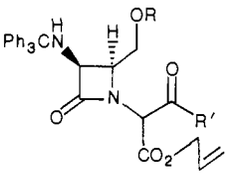
be derived from the β-keto ester **2** and that the latter could be obtained by condensation[3] of the ester enolate of **3** with the activated form of a carboxylic acid. If successful, this approach would allow us to introduce the 3-substituent as an acid derivative at a late stage of the synthesis and would thereby facilitate the preparation of this series of analogues. It would, however, require access to a source of the chiral azetidinone **4**. For the sake of expediency, we turned to an existing synthesis[4] of this material.

The 4-(hydroxymethyl)azetidinone **6** was prepared according to the literature procedure.[4a] Conversion (Chart I) of this material to the allyl acetate **10** was accomplished by the following sequence of reactions: oxidative removal of the dimethoxybenzyl group of **6**, protection of the alcohol function of **7** as a silyl ether, replacement of the benzyloxycarbonyl protecting group on **8** with a trityl group, and attachment of the allyl acetate group onto the nitrogen atom of the azetidinone **9**. With the acetate **10** in hand, we were ready to examine the proposed condensation reaction. We were pleased to find that the lithium enolate of **10** would indeed react with a variety of acid chlorides to give the β-keto esters **11–17** in good yield (48–95%). Removal of the silyl protecting group furnished the 3-hydroxymethyl compounds **18–24**, and these, when subjected to the conditions of the Mitsunobu reaction,[5] readily underwent cyclization to give the *O*-2-isocephems **25–31** (Chart II). The trityl group was then replaced by an (*R*)-*N*-(allyloxycarbonyl)(*p*-hydroxyphenyl)glycyl side chain to give the 7-acylamido derivatives **32–38**. At this stage, some additional functional group manipulation of

Chart I



5: R = O; R′ = 2,4-$(MeO)_2C_6H_3CH_2$; R″ = CO_2CH_2Ph
6: R = H, OH; R′ = 2,4-$(MeO)_2C_6H_3CH_2$; R″ = CO_2CH_2Ph
7: R = H, OH; R′ = H; R″ = CO_2CH_2Ph
8: R = H, OTBDMS; R′ = H; R″ = CO_2CH_2Ph
9: R = H, OTBDMS; R′ = H; R″ = CPh_3
10: R = H, OTBDMS; R′ = $CH_2CO_2CH_2CH{=}CH_2$; R″ = CPh_3



11: R = TBDMS; R′ = CH_2CH_2SePh
12: R = TBDMS; R′ = CH_2Cl
13: R = TBDMS; R′ = $CH_2CH_2CH_2SePh$
14: R = TBDMS; R′ = H
15: R = TBDMS; R′ = CH_3
16: R = TBDMS; R′ = *n*-Pr
17: R = TBDMS; R′ = c-C_3H_5
18: R = H; R′ = CH_2CH_2SePh
19: R = H; R′ = CH_2Cl
20: R = H; R′ = $CH_2CH_2CH_2SePh$
21: R = H; R′ = H
22: R = H; R′ = CH_3
23: R = H; R′ = *n*-Pr
24: R = H; R′ = c-C_3H_5

the 3-substituent was required in order to prepare certain analogues. The respective precursors of the 3-vinyl (**45**) and 3-allyl (**50**) compounds, **39** and **40**, were obtained from **32** and **34** by oxidation followed by selenoxide elimination. The 3-chloromethyl compound **33** served as the precursor for the 3-propenyl and 3-trifluoropropenyl compounds **46–49**. This transformation involved conversion of **33** to the iodide **41** and then reaction of the latter with triphenylphosphine to give the phosphonium salt **42**. Wittig reaction of the in situ generated ylide with the appropriate aldehyde furnished mixtures of the olefin isomers **43** and **44**. The last step was the palladium-catalyzed removal of the protecting groups. Purification and, where necessary, separation of olefin isomers was effected by reverse-phase column chromatography.

Biological Results and Discussion

The in vitro antibacterial activity of the *O*-2-isocephems (**45–54**) and several corresponding cephems[6] (**55–58**) is summarized in Table I.

(2) (a) Doyle, T. W.; Douglas, J. L.; Belleau, B.; Conway, T. T.; Ferrari, C. F.; Horning, D. E.; Lim, G.; Luh, B.-Y.; Martel, A.; Menard, M.; Morris, L. R. *Can. J. Chem.* **1980**, *58*, 2508 and earlier papers in the series. (b) Other racemic *O*-2-isocephem syntheses: Hakimelahi, G. H.; Just, G.; Ugolini, A. *Helv. Chim. Acta* **1982**, *65*, 1368. McCombie, S. W.; Metz, W. A.; Afonso, A. *Tetrahedron Lett.* **1986**, *27*, 305. Hrytsak, M.; Durst, T. *Heterocycles* **1987**, *26*, 2393. (c) Enantioselective syntheses: Tenneson, S. M.; Belleau, B. *Can. J. Chem.* **1980**, *58*, 1605. Natta, H.; Hatanaka, M.; Ishimaru, T. *J. Chem. Soc., Chem. Commun.* **1987**, 51. A preliminary account of this work has appeared: Mastalerz, H.; Vinet, V. *J. Chem. Soc., Chem. Commun.* **1987**, 1283.

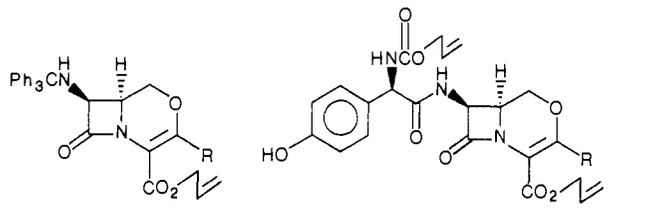
(3) A similar condensation has been used to synthesize a clavulanic acid derivative: Eglington, J. *J. Chem. Soc., Chem. Commun.* **1977**, 720.

(4) (a) Hubschwerlen, C. *Synthesis* **1986**, 962 and references therein. (b) Evans, D. A.; Sjogren, E. B. *Tetrahedron Lett.* **1985**, 3783.

(5) For the O-alkylation of β-diketones under these conditions see: Mitsunobu, O. *Synthesis* **1981**, 1.

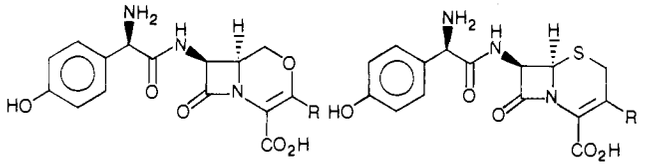
(6) (a) Leitner, F.; Pursiano, T. A.; Buck, R. E.; Tsai, Y. H.; Chisholm, D. R.; Misiek, M.; Desiderio, J. V.; Kessler, R. E. *Antimicrob. Agents Chemother.* **1987** *31*, 238. (b) Naito, T.; Hoshi, H.; Aburaki, S.; Abe, Y.; Okumura, J.; Tomatsu, K.; Kawaguchi, H. *J. Antibiot.* **1987**, *40*, 991.

Chart II



25: R = CH_2CH_2SePh
26: R = CH_2Cl
27: R = $CH_2CH_2CH_2SePh$
28: R = H
29: R = CH_3
30: R = *n*-Pr
31: R = c-C_3H_5

32: R = CH_2CH_2SePh
33: R = CH_2Cl
34: R = $CH_2CH_2CH_2SePh$
35: R = H
36: R = CH_3
37: R = *n*-Pr
38: R = c-C_3H_5
39: R = $CH{=}CH_2$
40: R = $CH_2CH{=}CH_2$
41: R = CH_2I
42: R = $CH_2PPh_3^+ I^-$
43: R = $CH{=}CHCH_3$
44: R = $CH{=}CHCF_3$



45: R = $CH{=}CH_2$
46: R = *cis*-$CH{=}CHCH_3$
47: R = *trans*-$CH{=}CHCH_3$
48: R = *cis*-$CH{=}CHCF_3$
49: R = *trans*-$CH{=}CHCF_3$
50: R = $CH_2CH{=}CH_2$
51: R = H
52: R = CH_3
53: R = *n*-Pr
54: R = c-C_3H_5

55: R = $CH{=}CH_2$
56: R = *cis*-$CH{=}CHCH_3$
57: R = *trans*-$CH{=}CHCH_3$
58: R = $CH_2CH{=}CH_2$

Substitution at the 3-position generally enhanced activity in vitro against Gram-positive bacteria (streptococci and staphylococci) as shown by comparing **45–50** and **52–54** to **51**. However, the latter was more active against the β-lactamase producing *Staphylococcus aureus* and *Escherichia coli*, suggesting lower β-lactamase stability among the substituted *O*-2-isocephems. There were relatively small or no differences in activity against the other Gram-negative bacteria, i.e., *E. coli*, *Klebsiella pneumoniae*, and *Haemophilus influenzae*. Activity against *Proteus mirabilis* was quite variable. The fluorinated compounds (**48** and **49**) in particular exhibited much lower activity against *P. mirabilis*.

Comparison of the *O*-2-isocephems **45–47** and **50** to their corresponding cephems **55–58** showed no differences in intrinsic activity against *Streptococcus pyogenes*, *Streptococcus pneumoniae*, and *S. aureus*. Compounds **56** and **58** were more active than their corresponding isocephems **46** and **50** against the β-lactamase producing *S. aureus*. The *O*-2-isocephems were 2–8-fold more active than the cephems against *Streptococcus faecalis*. The results with the Gram-negative bacteria were more variable, and no generalities were possible.

A comparison of pharmacokinetic parameters in blood and urinary recoveries following oral administration of **45–49** and **55–58** is shown in Table II. Compound **45** and its corresponding cephem **55** exhibited similar C_{max} values, but the half-life of **45** was longer. The other three *O*-2-isocephems exhibited lower C_{max} values than their corresponding cephems with longer half-lives for two of the three. Urinary recoveries of the three *O*-2-isocephems tested were significantly lower than those of the corresponding cephems.

Of these four pairs only two were compared directly in systemic infection models (Table III). Compounds **46** and **56** were similar in efficacy against *S. pneumoniae* but the cephem **56** was nearly 20-fold more effective against *E. coli*. Apparently the 3-fold higher C_{max} of **56** was more important than the somewhat longer half-life of **46** in this model.

Table II. Pharmacokinetic Parameters of Selected Cephems and *O*-2-Isocephems in Mice after an Oral Dose of 50 mg/kg

no.	C_{max}, μg/mL	$T_{1/2}$, min	AUC, μg/mL	percentage of dose recovered, 0–6 h in urine
45	26	39	30	73
46	15	52	23	25
47	11	110	30	5
50	31	32	32	46
55	22	29	21	ND[a]
56	46	29	42	85
57	35	47	45	65
58	45	34	41	79

[a] ND = not done.

Table III. Oral Therapeutic Efficacy of Selected *O*-2-Isocephems and Cephems in Systemically Infected Mice[a]

	E. coli		*S. pneumoniae*	
no.	MIC, μg/mL	PD_{50},[b] mg/kg per dose	MIC, μg/mL	PD_{50}, mg/kg per dose
45	2	2.1	0.03	0.8
46	2	19	0.03	0.30
47	2	>50	0.03	1.8
48	2	>50	0.016	2.7
49	4	>50	0.016	>12.5
50	4	9.2	0.016	1.0
51	4	6.2	1	33
52	8	11	0.13	2.1
54	4	>50	0.03	3.6
56	2	1.2	0.016	0.4
57	8	ND[c]	0.06	0.5

[a] Mice treated twice at 0 and 2 h after infection for *E. coli*, 1 and 3.5 h for *S. pneumoniae*. [b] PD_{50} = protective dose for 50% of animals tested. [c] ND = not done.

The relative importance of C_{max} was also seen with the comparison of **57** (3-fold higher C_{max}) and **47** (2–3-fold longer half-life) against *S. pneumoniae*.

Compound **45** showed the best balance in activity in vivo against *E. coli* and *S. pneumoniae* (Table III) of the *O*-2-isocephems and was nearly as active as **56**, which is currently in clinical trials.

Experimental Section

Melting points were taken on a Gallenkamp apparatus and are uncorrected. Optical rotations were obtained on a Perkin-Elmer 141 polarimeter. The UV and IR spectra were recorded on Hewlett-Packard 8451A and Perkin-Elmer 781 spectrophotometers, respectively. The ^1H NMR spectra were obtained on a Bruker AC 200 instrument with tetramethylsilane as the internal standard for organic solvents and sodium 3-(trimethylsilyl)-propionate-2,2,3,3-d_4 for deuterium oxide. TLC was performed with EM Art. 5719 Kieselgel 60 F_{254} plates. Medium-pressure column chromatography employed EM Art. 9385 Kieselgel 60 (230–400 mesh) with EtOAc/hexane mixtures being used as eluent. Where necessary, solvents were dried and reactions were conducted under an Ar atmosphere.

The final *O*-2-isocephems were purified by medium-pressure column chromatography employing the absorbant from a Waters C_{18} PrepPak-500 column as the stationary phase and CH_3CN/water mixtures as eluent. The desired fractions were combined, the organic solvent was removed under high vacuum, and then the water was removed by lyophilization. This left the isocephems as amorphous powders. Since these contained varying amounts of water of hydration, which could not be removed without some product decomposition, their final purity was judged by analytical HPLC. A Waters C_{18} Bondapak column (10 μm particle size, 3.8 mm × 30 cm) with a Waters 481 LC spectrophotometric detector was employed. Material of greater than 95% purity was used for biological evaluation.

In Vitro Antibacterial Activity. Conventional microtiter dilution procedures were used for determination of minimum

inhibitory concentrations (MICs). Organisms were grown overnight in Mueller–Hinton Broth (Difco) at 37 °C. Twofold dilutions of the stock solution of each compound (125 μg/mL) were made in Nutrient Broth (Difco) to obtain a test concentration range from 0.005 to 125 μg/mL. The wells were then inoculated with approximately 10^4 organisms. The microtiter plates were incubated at 37 °C for 18 h. The MIC was the lowest concentration of test compound that yielded no visible growth.

In Vivo Testing. Procedures for determining pharmacokinetics in mice and therapeutic efficacy in mice have been described.[6-8] Briefly, male Swiss–Webster mice (20 ± 2 g) were dosed by gavage. Determinations of blood concentrations and urine levels were done by microbiological assay. Bacterial challenges were administered intraperitoneally with sufficient numbers of bacteria to kill untreated controls within 72 h. Animals were dosed at 0 and 2 h postchallenge with *E. coli* or 1 and 3.5 h postchallenge with *S. pneumoniae*.

(3*S*,4*S*)-3-(Benzyloxycarboxamido)-1-(2,4-dimethoxybenzyl)-4-(hydroxymethyl)-2-azetidinone (6). A solution of (3*S*,4*S*)-3-(benzyloxycarboxamido)-1-(2,4-dimethoxybenzyl)-4-formyl-2-azetidine (**5**)[4a] (170 g, 0.43 mol) in CH_2Cl_2 (1.90 L) was placed in a water bath, and then $NaBH_4/Al_2O_3$[9] (850 g, 1 g of $NaBH_4$/10 g of Al_2O_3) was added slowly with vigorous stirring. After 40 min, the alumina was removed by filtration and washed with EtOAc. The solvent was then removed from the filtrate to leave the crude alcohol **6** (170 g), which was used as such without further purification. A sample was crystallized from EtOAc: mp 129–130 °C; $[\alpha]^{21}_D$ −12° (c 1.0, $CHCl_3$); IR (KBr) 3480, 3340, 1730 cm^{-1}; NMR ($CDCl_3$ + D_2O) δ 3.47–3.72 (m, 3 H), 3.78 (s, 3 H), 3.81 (s, 3 H), 4.35 (q, 2 H, δ_A 4.28, δ_B 4.42, J_{AB} = 14.4 Hz), 5.07 (m, 3 H), 6.42–7.31 (m, 8 H). Anal. ($C_{21}H_{24}N_2O_6$) C, H, N.

(3*S*,4*S*)-3-(Benzyloxycarboxamido)-4-[[(*tert*-Butyldimethylsilyl)oxy]methyl]-2-azetidinone (8). A stirred suspension of the crude alcohol **6** (170 g, ca. 0.45 mol), $K_2S_2O_8$ (161 g, 1.4 equiv), and K_2HPO_4 (96 g, 1.3 equiv) in a mixture of CH_3CN (4.4 L) and water (2.25 L) was heated at ca. 95 °C for 1 h.[10] At this point, additional quantities of $K_2S_2O_8$ (32 g, 0.28 equiv) and K_2HPO_4 (19 g, 0.26 equiv) were added, and heating was continued for an additional 3 h. After cooling, the organic solvent was removed, and the residual mixture was saturated with NaCl. This was extracted with EtOAc (4 × 900 mL), and then the combined organic phases were washed with aqueous $NaHCO_3$ (2 × 500 mL, 2.5%). After extraction of the aqueous phase with EtOAc (500 mL), the combined organic phases were dried, and the solvents were removed. Chromatography afforded the alcohol **7** (73.0 g, 69% yield), which had physical properties that were in agreement with those reported.[4b]

Triethylamine (114 mL, 2.20 equiv) was added over 0.5 h to an ice-cooled, stirred solution of the alcohol **7** (93.0 g, 0.372 mol), *tert*-butyldimethylsilyl chloride (127 g, 2.20 equiv), and 4-(dimethylamino)pyridine (8.0 g, 0.2 equiv) in DMF (900 mL). The bath was removed, and the reaction mixture was left stirring for 1 h. It was then diluted with CH_2Cl_2 (3 L) and washed with water (3 × 600 mL) followed by brine (600 mL). After drying, the solvents were removed, and the residual oil was taken up in HOAc (325 mL). KF (28.1 g, 1.30 equiv) was added, and the reaction mixture was left stirring for 1 h. The reaction was then diluted with CH_2Cl_2 (3 L), and this was washed with water (5 × 1 L). After drying and removal of the solvents, the crude material was chromatographed to give the monosilylated product **8** (113 g, 84% yield) as an oil: $[\alpha]^{21}_D$ 30° (c 1.0, $CHCl_3$); IR (neat) 3200, 1765, 1720 cm^{-1}; NMR ($CDCl_3$) δ 0.05 (s, 6 H), 0.86 (s, 9 H), 3.70–3.91 (m, 3 H), 5.10 (s, 2 H), 5.18 (q, 1 H, *J* = 5.1, 10.2 Hz), 5.92 (d, 1 H, exchangeable, *J* = 10.2 Hz), 6.05 (s, 1 H, exchangeable), 7.30 (s, 5 H). Anal. ($C_{18}H_{28}O_4N_2Si$) C, H, N.

(3*S*,4*S*)-4-[[(*tert*-Butyldimethylsilyl)oxy]methyl]-3-(tritylamino)-2-azetidinone (9). A stirred suspension of the benzyloxycarboxamido compound **8** (113 g, 0.310 mol) and Pd catalyst (29 g, 10% on charcoal) in EtOAc (1.2 L) was maintained under a H_2 atmosphere until the starting material had disappeared (ca. 3 h). The catalyst was removed by filtration, and then the solvent was removed from the filtrate. The white residue was dissolved in CH_2Cl_2 (1 L), and trityl chloride (90.0 g, 1.04 equiv) followed by NEt_3 (46.0 mL, 1.05 equiv) was added. After being stirred for 1.5 h, the reaction was diluted with CH_2Cl_2 (1.5 L) and washed with water (3 × 600 mL) and brine (100 mL). It was then dried, and the solvent was removed. Chromatography afforded the product **9** (115 g, 79%) as a pale yellow solid: mp 203–205 °C; $[\alpha]^{21}_D$ 34° (c 1.0, $CHCl_3$); IR (KBr) 3350, 3300, 1770, 1735 cm^{-1}; NMR ($CDCl_3$ + D_2O) δ −0.12 (s, 3 H), −0.06 (s, 3 H), 0.82 (s, 9 H), 2.38–3.15 (m, 3 H), 4.32 (d, 1 H, *J* = 4.6 Hz), 7.13–7.54 (m, 15 H). Anal. ($C_{29}H_{36}N_2O_2Si$) C, H, N.

Allyl 2-[(3*S*,4*S*)-4-[[(*tert*-Butyldimethylsilyl)oxy]methyl]-2-oxo-3-(tritylamino)-1-azetidinyl]acetate (10). A solution of allyl 2-bromoacetate (4.30 g, 2 equiv) in dry THF (24 mL) was added over ca. 0.5 h to a stirred suspension of the azetidinone **9** (5.66 g, 12 mmol), tetrabutylammonium bromide[11] (786 mg, 0.20 equiv), and freshly powdered KOH (876 mg, 1.30 equiv) in dry THF (24 mL). This was left stirring for 20 h after which it was diluted with EtOAc and washed with water and brine. After drying and removal of the solvents, the residual oil was chromatographed to give the acetate **10** (4.04 g, 59%) as white crystals: mp 98–101 °C; $[\alpha]^{21}_D$ 0° (c 1.0, $CHCl_3$); IR 3360, 1765, 1745 cm^{-1}; NMR ($CDCl_3$ + D_2O) δ −0.14 (s, 3 H), −0.09 (s, 3 H), 0.81 (s, 9 H), 2.47 (dd, 1 H, *J* = 3.6, 11.7 Hz), 3.10 (dd, 1 H, *J* = 2.3, 11.7 Hz), 3.32 (ddd, 1 H, *J* = 3.6, 2.3, 4.9 Hz), 3.43 (d, 1 H, *J* = 18.0 Hz), 4.36 (d, 1 H, *J* = 18.0 Hz), 4.43 (d, 1 H, *J* = 4.9 Hz), 4.52 (m, 2 H), 5.18–5.30 (m, 2 H), 5.76–5.90 (m, 1 H), 7.13–7.53 (m, 15 H). Anal. ($C_{34}H_{42}N_2O_4Si$) C, H, N.

Allyl (6*S*,7*S*)-8-Oxo-3-[2-(phenylseleno)ethyl]-7-(tritylamino)-1-aza-4-oxabicyclo[4.2.0]oct-2-ene-2-carboxylate (25). A suspension of 3-(phenylseleno)propionic acid[12] (19.0 g, 0.083 mol) in oxalyl chloride (7.60 mL, 1.05 equiv) was left stirring for 4 h. The resulting solution was then distilled to give 3-(phenylseleno)propionyl chloride (13.8 g, 67% yield) as a yellow liquid: bp 102–112 °C (0.11 mm); NMR ($CDCl_3$) δ 3.17 (m, 4 H), 7.39 (m, 5 H).

A solution of allyl 2-[(3*S*,4*S*)-4-[[(*tert*-butyldimethylsilyl)oxy]methyl]-2-oxo-3-(tritylamino)-1-azetidinyl]acetate (**10**) (3.91 g, 6.86 mmol) in dry THF (75 mL) under argon was cooled to −78 °C. To this was added a solution of lithium bis(trimethylsilyl)amide (14.1 mL, 1.0 M in THF, 2.05 equiv) dropwise. Then, after the mixture was stirred for 3 min, neat 3-(phenylseleno)-propionyl chloride (1.22 mL, 1.05 equiv) was added dropwise. The reaction mixture was left stirring for 20 min after which it was quenched by the addition of a solution of acetic acid (1.30 mL, 3.3 equiv) in THF (10 mL). After being allowed to warm to 0 °C, the reaction mixture was diluted with EtOAc (200 mL), washed with brine (2 × 30 mL), and dried, and the solvent was removed. Chromatography afforded allyl 2-[(3*S*,4*S*)-4-[[(*tert*-butyldimethylsilyl)oxy]methyl]-2-oxo-3-(tritylamino)-1-azetidinyl]-3-oxo-5-(phenylseleno)pentanoate (**11**) (2.54 g, 48% yield) as an oil: R_f 0.55 (EtOAc/hexane, 1:1).

To a solution of the azetidinone **11** (9.88 g, 12.7 mmol) in dry THF (190 mL) under argon was added a solution of tetrabutylammonium fluoride (15.2 mL, 1.0 M in THF, 1.2 equiv). After 15 min, acetic acid (0.95 mL, 1.3 equiv) was added. The reaction was then diluted with EtOAc (400 mL), washed with brine (40 mL), and dried. Removal of the solvent followed by chromatography afforded allyl 2-[(3*S*,4*S*)-4-(hydroxymethyl)-2-oxo-3-(tritylamino)-1-azetidinyl]-3-oxo-5-phenylseleno)pentanoate (**18**) (6.46 g, 76%) as a foam: R_f 0.71 (EtOAc/hexane, 1:1).

To a solution of the alcohol **18** (6.49 g, 9.73 mmol) and triphenylphosphine (2.68 g, 1.05 equiv) in dry THF (270 mL) under argon was added diisopropyl azodicarboxylate (2.07 mL, 1.05

(7) Kessler, R. E.; Bies, M.; Buck, R. E.; Chisholm, D. R.; Pursiano, T. A.; Tsai, Y. H.; Misiek, M.; Price, K. E.; Leitner, F. *Antimicrob. Agents Chemother.* **1985**, *31*, 207.
(8) Leitner, F.; Chisholm, D. R.; Tsai, Y. H.; Wright, G. E.; DeRegis, R. G.; Price, K. E. *Antimicrob. Agents Chemother.* **1975**, *7*, 306.
(9) Santaniello, E.; Ponti, F.; Manzucchi, A. *Synthesis* **1978**, 891.
(10) Conditions used are modeled after those reported by: Kishimoto, S.; Sendi, M.; Tomimoto, M.; Hashigughi, S.; Matsuo, T.; Ochiai, M. *Chem. Pharm. Bull.* **1984**, *32*, 2646.
(11) Reuschling, D.; Pietsch, H.; Linkies, A.; *Tetrahedron Lett.* **1978**, 615.
(12) Miyoshi, N.; Ishii, H.; Murai, S.; Sonoda, N.; *Chem. Lett.* **1979**, 873.

equiv). After 15 min, the solvent was removed, and the residual oil was chromatographed to afford the *O*-2-isocephem **25** (5.30 g, 73% yield) as white crystals (from EtOAc/hexane): mp 128–129 °C; IR (KBr) 1770, 1710, 1610 cm^{-1}; NMR ($CDCl_3$) δ 2.71–3.35 (m, 8 H), 4.56–4.97 (m, 3 H), 5.20–5.42 (m, 2 H), 5.83–6.00 (m, 1 H), 7.19–7.49 (m, 20 H). Anal. ($C_{37}H_{34}N_2O_4Se$) C, H, N.

(*R*)-*N*-[(Allyloxy)carbonyl]-2-(4-hydroxyphenyl)glycine. (*R*)-2-(4-hydroxyphenyl)glycine (16.7 g, 0.1 mol) was dissolved in a mixture of diethyl ether (100 mL), water (200 mL), and aqueous NaOH solution (100 mL, 1 N, 1.0 equiv). This was cooled in an ice bath, and then solutions of allyl chloroformate [12.1 g, 1.0 equiv, in dry dioxane (100 mL)] and aqueous NaOH (100 mL, 1 N, 1.0 equiv) were added contemporaneously over 1 h. The mixture was left stirring in the ice bath for 1 h after which the organic phase was separated. The aqueous phase was washed with ether (3 × 200 mL), and then its pH was adjusted to 2.5 by the addition of concentrated H_3PO_4. This mixture was then extracted with EtOAc (2 × 200 mL). The combined organic extracts were washed with water (50 mL) and dried, and then the solvents were removed. Trituration of the residual gum with a mixture of benzene (50 mL) and petroleum ether (400 mL, 30–60 °C) gave a white solid. This was taken up and digested in boiling benzene (10 mL/g). After cooling, the white solid was collected and washed successively with benzene and petroleum ether. This gave the pure *N*-allyloxycarbonyl derivative as a white powder (12.4 g, 50% yield): mp 147–149 °C; $[\alpha]^{21}_D$ −158.3° (c 1.0, MeOH); IR (KBr) 3200, 1735, 1650 cm^{-1}; NMR (DMSO-d_6) 4.47 (m, 2 H), 4.97 (d, 1 H, J = 7.9 Hz), 5.12–5.32 (m, 2 H), 5.79–5.98 (m, 1 H), 6.69 (d, 2 H, J = 8.6 Hz), 7.16 (d, 2 H, J = 8.6 Hz), 7.85 (d, 1 H, J = 7.9 Hz). Anal. ($C_{12}H_{13}NO_5$) C, H, N.

(6*S*,7*S*)-7-[[(*R*)-2-(4-Hydroxyphenyl)glycyl]amino]-8-oxo-3-vinyl-1-aza-4-oxabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid (45). A solution of the trityl derivative **25** (5.60 g, 8.64 mmol) in acetone (50 mL) was cooled in an ice bath, and *p*-toluenesulfonic acid monohydrate (1.81 g, 1.1 equiv) was added with stirring. After being allowed to stand for 19 h, the crystals were collected, washed with a little cold acetone and ether, and then dried under high vacuum. This gave the *p*-toluenesulfonic acid salt of allyl (6*S*,7*S*)-7-amino-3-[2-(phenylseleno)ethyl]-8-oxo-1-aza-4-oxabicyclo[4.2.0]oct-2-ene-2-carboxylate (3.60 g, 72% yield) as white crystals. This material (1.08 g, 1.87 mmol) was suspended in EtOAc (20 mL), and a stream of argon was allowed to bubble through the suspension. Then, sufficient saturated aqueous sodium bicarbonate solution was added with stirring to bring the pH of the aqueous phase to 8. The aqueous phase was removed and extracted with a little EtOAc. The combined organic phases were washed with brine and dried, and the solvent was removed. The residual oil was taken up in EtOAc (20 mL), and EEDQ (524 mg, 1.1 equiv) followed by (*R*)-*N*-[(allyloxy)carbonyl]-2-(4-hydroxyphenyl)glycine (516 mg, 1.1 equiv) was added. The resulting solution was left stirring at ambient temperature for 3 h after which it was washed with aqueous HCl (3 × 4 mL, 1 N), saturated aqueous $NaHCO_3$ solution (3 mL), and water (3 mL). The organic phase was dried, and the solvent was removed. Chromatography of the residual oil afforded allyl (6*S*,7*S*)-7-[[(*R*)-*N*-[(allyloxy)carbonyl]-2-(4-hydroxyphenyl)glycyl]-amino]-3-[2-(phenylseleno)ethyl]-8-oxo-1-aza-4-oxabicyclo-[4.2.0]oct-2-ene-2-carboxylate (**32**) (1.10 g, 92% yield) as a foam: NMR ($CDCl_3$) δ 3.02 (s, 4 H), 3.40 (t, 1 H, J = 10.5 Hz), 3.66 (ddd, 1 H, J = 10.5, 3.7, 3.8 Hz), 4.20 (dd, 1 H, J = 10.5, 3.7 Hz), 4.50–4.65 (m, 4 H), 5.08–5.37 (m, 6 H), 5.76–5.98 (m, 3 H), 6.15 (br s, 1 H), 6.75 (d, 2 H, J = 8.4 Hz), 6.85 (br s, 1 H), 7.10 (d, 2 H, J = 8.4 Hz), 7.18–7.48 (m, 5 H).

A solution of the selenide **32** (889 mg, 1.39 mmol) in a mixture of CH_2Cl_2 (20 mL) and water (1 mL) was cooled in an ice bath. This was stirred vigorously, and pyridine (0.224 mL, 2 equiv) followed by hydrogen peroxide (0.358 mL, 30% aqueous solution, 3 equiv) was added. The bath was removed, and the reaction mixture was left stirring for 20 min. The organic phase was separated and washed with saturated aqueous $NaHCO_3$ solution (2 × 2 mL), water (3 × 2 mL), and brine (2 mL). After drying, the solvent was removed, and the residual oil was chromatographed to afford allyl (6*S*,7*S*)-7-[[(*R*)-*N*[(allyloxy)carbonyl]-2-(4-hydroxyphenyl)glycyl]amino]-8-oxo-3-vinyl-1-aza-4-oxabicyclo[4.2.0]oct-2-ene-2-carboxylate (**39**) (539 mg, 81%) as a tan solid: NMR ($CDCl_3$ + D_2O) 3.54 (m, 1 H), 3.84 (m, 1 H), 4.30 (m, 1 H), 4.50–4.71 (m, 5 H), 5.12–5.46 (m, 6 H), 5.75–6.04 (m, 3 H), 6.60–6.73 (m, 2 H), 7.05–7.29 (m, 3 H).

A mixture of the 3-vinyl compound **39** (1.71 g, 3.54 mmol), tetrakis(triphenylphosphine)palladium(0) (204 mg, 0.05 equiv) and triphenylphosphine (200 mg, 0.20 equiv) in a flask was purged with argon. Dry CH_2Cl_2 (34 mL) followed by 2-ethylhexanoic acid (1.15 mL, 4 equiv) was added. This was left stirring for 2.25 h, during which time a precipitate formed. This was collected, washed with CH_2Cl_2, and dried. Reverse-phase chromatography followed by lyophilization of the appropriate fractions afforded the desired 3-vinyl-*O*-2-isocephem **45** (410 mg, 32%) as an off-white powder: UV (H_2O) 298 (ϵ 14 700), 230 nm (ϵ 12 900); IR (KBr) 1760, 1695, 1550 cm^{-1}; NMR (D_2O) δ 3.51 (t, 1 H, J = 10.1 Hz), 3.95 (ddd, 1 H, J = 10.1, 3.8, 4.7 Hz), 4.32 (dd, 1 H, J = 3.8, 10.1 Hz), 5.09 (s, 1 H), 5.33 (dd, 1 H, J = 1.7, 11.1 Hz), 5.62 (d, 1 H, J = 4.7 Hz), 5.70 (dd, 1 H, J = 1.7, 17.3 Hz), 6.92 (dd, 1 H, J = 11.1, 17.3 Hz), 7.00 (d, 2 H, J = 8.7 Hz), 7.37 (d, 2 H, J = 8.7 Hz).

Allyl (6*S*,7*S*)-3-(Chloromethyl)-8-oxo-7-(tritylamino)-1-aza-4-oxabicyclo[4.2.0]oct-2-ene-2-carboxylate (26). Similarly, by using chloroacetyl chloride, the azetidinone **10** was converted to allyl 2-[(3*S*,4*S*)-4-[[(*tert*-butyldimethylsilyl)oxy]methyl]-2-oxo-3-(tritylamino)-1-azetidinyl]-3-oxo-4-chlorobutanoate (**12**) (95% yield): R_f 0.58 (EtOAc/hexane, 1:4). Desilylation of **12** afforded allyl 2-[(3*S*,4*S*)-4-(hydroxymethyl)-2-oxo-3-(tritylamino)-1-azetidinyl]-3-oxo-4-chlorobutanoate (**19**) (77% yield): R_f 0.35 (EtOAc/hexane, 1:1). Cyclization of **19** gave the 3-chloromethyl derivative (**26**) (82% yield) as a white solid: mp 77–78 °C; IR (KBr) 3350, 1770, 1710, 1610 cm^{-1}; NMR ($CDCl_3$) δ 2.80 (d, 1 H, J = 5.4 Hz), 2.95–3.07 (m, 2 H), 3.56–3.68 (m, 1 H), 4.49 (q, δ_A 4.50, δ_B 4.47, J_{AB} = 11.6 Hz), 4.61–4.76 (m, 2 H), 4.81 (dd, 1 H, J = 5.4, 4.2 Hz), 5.21–5.43 (m, 2 H), 5.85–6.05 (m, 1 H), 7.20–7.39 (m, 15 H). Anal. ($C_{30}H_{27}N_2O_4Cl$) C, N; H: calcd, 5.29; found, 5.82.

(6*S*,7*S*)-7-[[(*R*)-2-(4-Hydroxyphenyl)glycyl]amino]-8-oxo-3-(*cis*-1-propenyl)-1-aza-4-oxabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid (46) and the 3-(*trans*-1-Propenyl) Isomer (47). The tritylamine **26** was converted to allyl (6*S*,7*S*)-7-[[(*R*)-*N*-[(allyloxy)carbonyl]-2-(4-hydroxyphenyl)glycyl]-amino]-3-(chloromethyl)-8-oxo-1-aza-4-oxabicyclo[4.2.0]oct-2-ene-2-carboxylate (**33**) (79% yield): R_f 0.41 (two developments, EtOAc/hexane, (1:1).

A suspension of the chloride **33** (5.27 g, 10.4 mmol) and sodium iodide (2.35 g, 1.5 equiv) in acetone (50 mL) was left stirring at room temperature for 2 h. The solvent was removed, and the residual material was suspended in EtOAc. This was washed with water and dried, and the solvent was removed. A solution of this crude iodide (**41**) in EtOAc (25 mL) was cooled in an ice bath, and triphenylphosphine (2.72 g, 1.0 equiv) was added with stirring. After 18 h, ether (25 mL) was added, and the crude phosphonium salt (**42**) (7.92 g) was collected by filtration. A solution of the crude salt (2.0 g, 2.33 mmol) in dry CH_2Cl_2 (18 mL) was cooled in an ice bath, and then acetaldehyde (526 μL, 4 equiv) followed by ethylene oxide[14] (466 μL, 4 equiv) was added. The flask was sealed with "parafilm" and then removed from the ice bath. After 24 h at room temperature in the dark, the solvent was removed, and the residual oil was chromatographed to give a mixture of the *cis*- and *trans*-propenyl isomer (**43**) (831 mg, 72%): R_f 0.24 (EtOAc/hexane, 1:1).

N-Methylaniline[13] (2.18 mL, 4 equiv) was added to a stirred solution of the olefin isomers **43** (2.50 g, 5.03 mmol), tetrakis-(triphenylphosphine)palladium(0) (190 mg, 0.03 equiv), and triphenylphosphine (190 mg, 0.12 equiv) in dry CH_2Cl_2 (50 mL) under Ar. After 50 min, the precipitate was collected, washed with CH_2Cl_2, and dried under high vacuum. Chromatography followed by lyophilization of the appropriate fractions afforded the 3-*cis*-propenyl compound **46** as a light yellow powder (455 mg, 24%): UV (H_2O) 228 (ϵ 17 700), 300 nm (ϵ 17 500); IR (KBr disk) 3250, 1755, 1600 cm^{-1}; NMR (D_2O) δ 1.78 (dd, 3 H, J = 1.7, 7.2

(13) Excess 2-ethylhexanoic acid is generally used as the acceptor for the allyl group: Jeffrey, P. D.; McCombie, S. W. *J. Org. Chem.* **1982**, *47*, 587. We have found that this deprotection is quicker when *N*-methylaniline is used as the acceptor.

(14) Buddrus, J. *Chem. Ber.* **1974**, *107*, 2050.

Hz), 3.52 (t, 1 H, J = 10 Hz), 3.91 (ddd, 1 H, J = 10, 3.9, 4.7 Hz), 4.22 (dd, 1 H, J = 3.9, 10), 4.90 (s, 1 H), 5.56 (d, 1 H, J = 4.7 Hz), 5.84 (dq, 1 H, J = 11.9, 7.2 Hz), 6.18 (dd, 1 H, J = 11.9, 1.7 Hz), 6.97 (d, 2 H, J = 8.6 Hz), 7.33 (d, 2 H, J = 8.6 Hz) followed by the *trans*-propenyl isomer **47** (245 mg, 13% yield): UV (H_2O) 230 (ϵ 9900), 300 nm (ϵ 11 300); IR (KBr) 3200, 1755, 1690, 1540 cm^{-1}; NMR (D_2O) 1.79 (dd, 3 H, J = 6.8, 1.5 Hz), 3.48 (t, 1 H, J = 10.5 Hz), 3.91 (ddd, 1 H, J = 4.7, 3.7, 10.5 Hz), 4.28 (dd, 1 H, J = 3.7, 10.5 Hz), 5.12 (s, 1 H), 5.59 (d, 1 H, J = 4.7 Hz), 6.26 (dq, 1 H, J = 15.4, 6.8 Hz), 6.63 (dd, 1 H, J = 15.4, 1.5 Hz), 6.99 (d, 2 H, J = 8.7 Hz), 7.35 (d, 2 H, J = 8.7 Hz).

(6*S*,7*S*)-7-[[(*R*)-2-(4-Hydroxyphenyl)glycyl]amino]-8-oxo-3-(1-*cis*-3,3,3-trifluoropropenyl)-1-aza-4-oxabicyclo-[4.2.0]oct-2-ene-2-carboxylic Acid (48) and the 3-(1-*trans*-3,3,3-Trifluoropropenyl) Isomer (49). To a solution of the crude phosphonium salt **42** (2.25 g, 3.08 mmol) and triethylamine (0.43 mL, 1.0 equiv) in dry CH_2Cl_2 (40 mL) under argon and at −78 °C was added a solution of trifluoroacetaldehyde[15] (4.41 mL, 2.75 g/10 mL of dry CH_2Cl_2, ca. 4 equiv), which had been cooled to −78 °C. The reaction was then placed in an ice bath and left stirring for 15 min after which it was washed with brine (5 mL) and dried, and the solvent was removed. Chromatography afforded a mixture of the olefin isomers **44** (1.16 g, 70%) as a waxy solid: R_f 0.69 (EtOAc/hexane, 3:2). Deprotection as above, followed by chromatography afforded the cis olefin isomer **48** (206 mg, 23% yield) as an off-white powder [UV (H_2O) 230 (ϵ 11 800), 308 nm (ϵ 12 800); IR (KBr) 3200, 1770, 1690, 1570 cm^{-1}; NMR (D_2O) δ 3.36 (t, 1 H, J = 10.5 Hz), 3.97 (ddd, 1 H, J = 10.5, 4.0, 4.8 Hz), 4.23 (dd, 1 H, J = 10.5, 4.0 Hz), 5.05 (s, 1 H), 5.62 (d, 1 H, J = 4.8 Hz), 5.83 (dq, 1 H, J = 9.3, 12.6 Hz), 6.90 (d, 1 H, J = 12.6 Hz), 6.98 (d, 1 H, J = 8.9 Hz), 7.36 (d, 1 H, J = 8.9 Hz)] and the trans olefin isomer **49** (165 mg, 18% yield) as a white powder [UV (H_2O) 228 (ϵ 17 000), 308 nm (ϵ 21 000); IR (KBr) 3200, 1770, 1690, 1580 cm^{-1}; NMR (D_2O + CF_3CO_2H) 3.62 (t, 1 H, J = 10.4 Hz), 4.01 (ddd, 1 H, J = 10.4, 3.9, 4.9 Hz), 4.45 (dd, 1 H, J = 10.4, 3.9 Hz), 5.13 (s, 1 H), 5.64 (d, 1 H, J = 4.9 Hz), 6.42 (dq, 1 H, J = 7.1, 15.6 Hz), 6.98 (d, 2 H, J = 8.7 Hz), 7.34 (d, 2 H, J = 8.7 Hz), 7.56 (dq, 1 H, J = 2.1, 15.6 Hz).

Allyl (6*S*,7*S*)-8-Oxo-3-[3-(phenylseleno)propyl]-7-(tritylamino)-1-aza-4-oxabicyclo[4.2.0]oct-2-ene-2-carboxylate (27). A suspension of 4-(phenylseleno)butanoic acid[16] (21.7 g, 89.3 mmol) and oxalyl chloride (10.1 mL, 1.3 equiv) was left stirring for 16 h at ambient temperature. Distillation afforded 4-(phenylseleno)butyryl chloride (18.1 g, 78%) as a pale yellow liquid: bp 132 °C (0.15 mm); NMR ($CDCl_3$) δ 2.04 (quin, 2 H, J = 7.1 Hz), 2.92 (t, 2 H, 7.1 Hz), 3.05 (t, 2 H, 7.1 Hz), 7.25–7.53 (m, 5 H).

With this acid chloride, the azetidinone **10** was converted to allyl 2-[(3*S*,4*S*)-4-[[(*tert*-butyldimethylsilyl)oxy]methyl]-2-oxo-3-(tritylamino)-1-azetidinyl]-3-oxo-6-(phenylseleno)hexanoate (**13**) (76% yield): R_f 0.44 (EtOAc/hexane, 1:4).

Desilylation of **13** afforded allyl 2-[(3*S*,4*S*)-4-(hydroxymethyl)-2-oxo-3-(tritylamino)-1-azetidinyl]-3-oxo-6-(phenylseleno)hexanoate (**20**) (73% yield): R_f 0.1 (EtOAc/hexane, 1:4).

Cyclization of **20** gave the *O*-2-isocephem (**27**) (91% yield): mp 62–63 °C; IR (KBr) 3320, 1770, 1710 cm^{-1}; NMR ($CDCl_3$) δ 1.87 (m, 2 H), 2.54 (m, 1 H), 2.84 (m, 6 H), 3.54 (m, 1 H), 4.56–4.78 (m, 3 H), 5.18–5.41 (m, 2 H), 5.84–6.03 (m, 1 H), 7.18–7.46 (m, 20 H). Anal. ($C_{38}H_{36}N_2O_4Se$) C, H, N.

Allyl (6*S*,7*S*)-7-[[(*R*)-*N*-[(Allyloxy)carbonyl]-2-(4-hydroxyphenyl)glycyl]amino]-3-[3-(phenylseleno)-propyl]-8-oxo-1-aza-4-oxabicyclo[4.2.0]oct-2-ene-2-carboxylate (34). By use of the aforementioned procedure, the tritylamine **27** was converted to the glycine derivative **34** (90% yield): mp 72–73 °C; R_f 0.33 (EtOC/hexane, 1:1); IR (KBr) 3300, 1760, 1690 cm^{-1}; NMR ($CDCl_3$) δ 1.89 (m, 2 H), 2.72 (t, 2 H, J = 7.5 Hz), 2.85 (t, 2 H, J = 7.5 Hz), 3.42 (m, 1 H), 5.10 (m, 1 H), 4.22 (dd, 1 H, J = 10.9, 3.8 Hz), 4.49–4.68 (m, 4 H), 5.06–5.38 (m, 5 H), 5.75–5.98 (m, 3 H), 6.31 (br s, 1 H), 6.64 (d, 2 H, J = 11.1 Hz), 6.97 (m, 1 H), 7.09 (d, 2 H, J = 11.1 Hz), 7.19–7.47 (m, 4 H). Anal. ($C_{31}H_{33}N_3O_8Se$) C, N; H: calcd, 5.81; found, 5.28.

(6*S*,7*S*)-7-[[(*R*)-(4-Hydroxyphenyl)glycyl]amino]-8-oxo-3-(2-propenyl)-1-aza-4-oxabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid (50). A solution of the selenide **34** (1.50 g, 2.29 mmol) in a mixture of $CHCl_3$ (30 mL) and 1,1,1-trichloroethane (120 mL) was cooled to −15 °C, and *m*-chloroperbenzoic acid (496 mg, 80%, 1 equiv) was added with stirring. After 0.5 h, pyridine (0.74 mL, 4 equiv) followed by dihydropyran (8.36 mL, 40 equiv) was added, and the reaction was placed in an oil bath (130 °C). After being heated at reflux for 1.5 h, the reaction mixture was allowed to cool to room temperature. It was diluted with CH_2Cl_2 (100 mL) and washed with an aqueous HCl solution (3 × 10 mL, 1N), a saturated aqueous $NaHCO_3$ solution (3 × 15 mL), and brine (2 × 5 mL). Drying followed by removal of the solvents left an oil, which was chromatographed to give allyl (6*S*,7*S*)-7-[[(*R*)-*N*-[(allyloxy)carbonyl]-2-(4-hydroxyphenyl)glycyl]amino]-3-(2-propenyl-8-oxo-1-aza-4-oxabicyclo[4.2.0]oct-2-ene-2-carboxylate (**40**) (998 mg, 88% yield) as a foam: R_f 0.33 (EtOAc/hexane, 1:1); NMR ($CDCl_3$) δ 3.37 (d, 2 H, J = 6.1 Hz), 3.56 (m, 1 H), 3.72 (m, 1 H), 4.23 (m, 1 H), 4.47 (m, 2 H), 4.65 (m, 2 H), 5.02–5.47 (m, 8 H), 5.65–6.00 (m, 3 H), 6.55 (d, 2 H, J = 7.2 Hz), 7.04 (d, 2 H, J = 7.2 Hz).

Palladium-catalyzed deprotection of the 3-propenyl derivative **40** gave the final product **50** (64% yield) as an off-white powder: UV (H_2O) 270 nm (ϵ 10000); IR (KBr) 1750, 1700, 1630 cm^{-1}; NMR (D_2O) δ 3.36 (m, 2 H), 3.48 (t, 1 H, J = 10.2 Hz), 3.89 (ddd, 1 H, J = 10.2, 3.9, 4.7 Hz), 4.21 (dd, 1 H, J = 10.2, 3.9 Hz), 5.09 (s, 1 H), 5.13 (m, 1 H), 5.57 (d, 1 H, J = 4.7 Hz), 5.78–5.92 (m, 1 H), 6.99 (d, 2 H, J = 8.7 Hz), 7.36 (d, 2 H, J = 8.7 Hz).

Allyl (6*S*,7*S*)-8-Oxo-7-(tritylamino)-1-aza-4-oxabicyclo-[4.2.0]oct-2-ene-2-carboxylate (28). With use of acetic formic anhydride,[17] the azetidinone **10** was converted to allyl 2-[(3*S*,4*S*)-4-[[(*tert*-butyldimethylsilyl)oxy]methyl]-2-oxo-3-(tritylamino)-1-azetidinyl]-3-oxopropanoate (**14**) (86% yield): R_f 0.63 (EtOAc/hexane, 1:4).

Desilylation of **14** gave allyl 2-[(3*S*,4*S*)-4-(hydroxymethyl)-2-oxo-3-(tritylamino)-1-azetidinyl]-3-oxopropanoate (**21**) (89% yield): R_f 0.25 (EtOAc/hexane, 19:1).

Cyclization of **21** gave the *O*-2-isocephem **28** (39% yield): R_f 0.55 (EtOAc/hexane, 1:4); mp 126–129 °C; $[\alpha]^{21}_D$ −12° (c 1.0, $CHCl_3$); IR (KBr disk) 3460, 1760, 1715, 1680, 1650 cm^{-1}; NMR ($CDCl_3$ + D_2O) δ 2.95 (m, 2 H), 3.54 (m, 1 H), 4.66 (m, 2 H), 4.81 (d, 1 H, J = 4.3 Hz), 5.17–6.00 (m, 3 H), 7.20–7.38 (m, 16 H). Anal. ($C_{29}H_{28}N_2O_5 \cdot H_2O$) C, H, N.

Allyl (6*S*,7*S*)-[[(*R*)-*N*-[(Allyloxy)carbonyl]-2-(4-hydroxyphenyl)glycyl]amino]-1-aza-4-oxabicyclo[4.2.0]oct-2-ene-2-carboxylate (35). The tritylamine **28** was converted to the glycyl derivative **35** (63% yield): R_f 0.44 (EtOAc: hexane, 3:1); mp 107–109 °C; $[\alpha]^{21}_D$ 63° (c 1.0, $CHCl_3$); IR (KBr disk) 3320, 1770, 1700, 1620 cm^{-1}; NMR ($CDCl_3$ + D_2O) δ 3.53–4.22 (m, 3 H), 4.50–4.65 (m, 4 H), 5.10–5.48 (m, 4 H), 5.76–5.96 (m, 2 H), 6.58–7.10 (m, 5 H). Anal. ($C_{22}H_{23}N_3O_8$) C, H; N: calcd, 9.19; found, 8.52.

(6*S*,7*S*)-7-[[(*R*)-2-(4-Hydroxyphenyl)glycyl]amino]-1-aza-4-oxabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid (51). Deprotection of **35** afforded the product **51** (60% yield): IR (KBr) 1765, 1690, 1620 cm^{-1}; UV (H_2O) 264 (ϵ 6900), 232 nm (ϵ 9000); NMR (D_2O) δ 3.42 (t, 1 H, J = 10.3 Hz), 3.89 (ddd, 1 H, J = 10.3, 3.9 Hz, 4.8 Hz), 4.23 (dd, 1 H, J = 10.3, 3.9 Hz), 5.11 (s, 1 H), 5.62 (d, 1 H, J = 4.8 Hz), 6.98 (d, 2 H, J = 8.7 Hz), 7.07 (s, 1 H), 7.36 (d, 2 H, J = 8.7 Hz).

Allyl (6*S*,7*S*)-3-Methyl-8-oxo-7-(tritylamino)-1-aza-4-oxabicyclo[4.2.0]oct-2-ene-2-carboxylate (29). With use of acetyl chloride, the azetidinone **10** was converted to allyl 2-[(3*S*,4*S*)-4-[[(*tert*-butyldimethylsilyl)oxy]methyl]-2-oxo-3-(tritylamino)-1-azetidinyl]-3-oxobutanoate (**15**) (87% yield): R_f 0.49 (EtOAc/hexane, 1:4).

(15) Trifluoroacetaldehyde was generated from the commercially available ethyl hemiacetal. An equal volume of concentrated H_2SO_4 was added to the hemiacetal under an Ar atmosphere. The resulting mixture was heated to 100 °C, and slightly impure aldehyde was collected in a receiver, which had been cooled to −78 °C. This was redistilled to give pure material (^1H NMR).

(16) The acid was prepared according to a literature procedure: Scarborough, R. M.; Smith, A. B. *Tetrahedron Lett.* **1977**, 4361. It was obtained as a yellow solid: bp 170–172 °C (0.25 mm); NMR ($CDCl_3$) δ 1.99 (quin, 2 H, J = 7.1 Hz), 2.50 (t, 2 H, J = 7.1 Hz), 2.94 (t, 2 H, J = 7.1 Hz), 7.21–7.51 (m, 5 H).

(17) Krimen, L. I. *Org. Synth.* **1970**, *50*, 1.

Desilylation of **15** gave allyl 2-[(3*S*,4*S*)-4-(hydroxymethyl)-2-oxo-3-(tritylamino)-1-azetidinyl]-3-oxobutanoate (**22**) (95% yield): R_f 0.23 (EtOAc/hexane, 1:4).

Cyclization of **22** gave the 3-methyl-*O*-2-isocephem **29** (95% yield): R_f 0.58 (EtOAc/hexane, 1:4); mp 88–89 °C; $[\alpha]_D$ +90° (c 0.5, $CHCl_3$); IR (KBr) 3340, 1760, 1710, 1610 cm^{-1}; NMR (C_6H_6 + D_2O) δ 2.23 (s, 3 H), 2.36 (dt, 1 H, *J* = 9.8, 4.1 Hz), 2.53 (dd, 1 H, *J* = 9.8, 4.1 Hz), 2.88 (t, 1 H, *J* = 9.8 Hz), 4.29 (d, 1 H, *J* = 4.1 Hz), 4.36–6.02 (m, 5 H), 6.89–7.29 (m, 15 H, arom). Anal. ($C_{30}H_{28}N_2O_4 \cdot H_2O$) C, H, N.

(6*S*,7*S*)-7-[[(*R*)-2-(4-Hydroxyphenyl)glycyl]amino]-3-methyl-8-oxo-1-aza-4-oxabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid (52). The 7β-tritylamine **29** was converted to the protected glycyl derivative **36** (58% yield): R_f 0.51 (EtOAc/hexane, 3:2); mp 141–143 °C; $[\alpha]^{22}_D$ +72° (c 1.0, $CHCl_3$); IR (KBr) 3320, 1720, 1680, 1620 cm^{-1}.

Deprotection gave the product **52** (44% yield) as a white powder: UV (H_2O) 266 (ε 9200), 232 nm (ε 10 500); IR (KBr) 3200, 1760, 1690, 1600 cm^{-1}; NMR (D_2O) δ 2.04 (s, 3 H), 3.44 (t, 1 H, *J* = 10.3 Hz), 3.86 (ddd, 1 H, *J* = 10.3, 4.6, 3.8 Hz), 4.19 (dd, 1 H, *J* = 10.3, 3.8 Hz), 5.11 (s, 1 H), 5.54 (d, 1 H, *J* = 4.6 Hz), 6.98 (d, 2 H, *J* = 8.6 Hz), 7.34 (d, 2 H, *J* = 8.6 Hz).

Allyl (6*S*,7*S*)-3-Propyl-8-oxo-7-(tritylamino)-1-aza-4-oxabicyclo[4.2.0]oct-2-ene-2-carboxylate (30). With use of butyryl chloride, the azetidinone **10** was converted to allyl 2-[(3*S*,4*S*)-4-[[(*tert*-butyldimethylsilyl)oxy]methyl]-2-oxo-3-(tritylamino)-1-azetidinyl]-3-oxohexanoate (**16**) (90% yield): R_f 0.53 (EtOAc/hexane, 1:4).

Desilylation of **16** gave allyl 2-[(3*S*,4*S*)-4-(hydroxymethyl)-2-oxo-3-(tritylamino)-1-azetidinyl]-3-oxohexanoate (**23**) (95% yield): R_f 0.26 (EtOAc/hexane, 1:4).

Cyclization of **23** gave the 3-propyl-*O*-2-isocephem **30** (81% yield): R_f 0.54 (EtOAc/hexane, 1:4); mp 154–156 °C; $[\alpha]_D$ +104° (c 1.0, $CHCl_3$); IR (KBr) 3430, 1770, 1705, 1600 cm^{-1}; NMR ($CDCl_3$ + D_2O) δ 0.86 (t, 3 H, *J* = 7.4 Hz), 1.50 (m, 2 H), 2.45 (m, 2 H), 2.94 (m, 2 H), 3.56 (m, 1 H), 4.57–6.01 (m, 6 H), 7.21–7.38 (m, 15 H). Anal. ($C_{32}H_{32}N_2O_4$) C, H, N.

(6*S*,7*S*)-7-[[(*R*)-2-(4-Hydroxyphenyl)glycyl]amino]-3-propyl-1-aza-4-oxabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid (53). The 7β-tritylamine **30** was converted to the glycyl derivative **37** (78% yield): $[\alpha]_D$ +61° (c 1.0, $CHCl_3$); mp 92–95 °C; IR (KBr) 3320, 1760, 1720, 1680, 1620 cm^{-1}.

Deprotection of **37** gave the product **53** (33% yield): UV (H_2O) 203 (ε 11 500), 266 nm (ε 10 300); IR (KBr) 3200, 1760, 1690, 1600 cm^{-1}; NMR (D_2O) δ 0.84 (t, 3 H, *J* = 7.4 Hz), 1.48 (m, 2 H), 2.32 (dt, 1 H, *J* = 13.9, 7.0 Hz), 2.65 (dt, 1 H, *J* = 13.9, 7.0 Hz), 3.45 (t, 1 H, *J* = 10.2 Hz), 3.87 (ddd, 1 H, *J* = 10.2, 3.8, 4.6 Hz), 4.20 (dd, 1 H, *J* = 10.2, 3.8 Hz), 5.12 (s, 1 H), 5.56 (d, 1 H, *J* = 4.6 Hz), 6.99 (d, 2 H, *J* = 8.7 Hz), 7.36 (d, 2 H, *J* = 8.7 Hz).

Allyl (6*S*,7*S*)-7-[[(*R*)-*N*-[(Allyloxy)carbonyl]-2-(4-hydroxyphenyl)glycyl]amino]-3-cyclopropyl-8-oxo-1-aza-4-oxabicyclo[4.2.0]oct-2-ene-2-carboxylate (38). With use of cyclopropanecarboxylic acid chloride, the azetidinone **10** was converted to allyl 2-[(3*S*,4*S*)-4-[[(*tert*-butyldimethylsilyl)oxy]-methyl]-2-oxo-3-(tritylamino)-1-azetidinyl]-3-oxo-3-cyclopropylpropanoate (**17**) (95% yield): R_f 0.55 (EtOAc/hexane, 1:4).

Desilylation of **17** gave allyl 2-[(2*S*,4*S*)-4-(hydroxymethyl)-2-oxo-3-(tritylamino)-1-azetidinyl]-3-oxo-3-cyclopropylpropanoate (**24**) (95% yield): R_f 0.24 (EtOAc/hexane 1:4).

Cyclization of **24** gave allyl (6*S*,7*S*)-3-cyclopropyl-8-oxo-7-(tritylamino)-1-aza-4-oxabicyclo[4.2.0]oct-2-ene-2-carboxylate (**31**) (95% yield) as a white solid: R_f 0.31 (EtOAc/hexane, 3:17); mp 141–143 °C) $[\alpha]^{21}_D$ 59° (c 1.0, $CHCl_3$); IR (KBr) 3340, 1750, 1690, 1590 cm^{-1}; NMR ($CDCl_3$) δ 0.63–0.92 (m, 4 H), 2.77–2.91 (m, 4 H), 3.53 (m, 1 H), 4.60–4.83 (m, 3 H), 5.19–5.43 (m, 2 H), 5.87–6.07 (m, 1 H), 7.20–7.37 (m, 15 H).

The tritylamine **31** was converted to the glycine derivative **38** (78% yield): $[\alpha]^{21}_D$ 9.8 (c 1.0, $CHCl_3$); mp 110–115 °C; IR (KBr) 3100, 1770, 1690, 1600 cm^{-1}; NMR ($CDCl_3$ + D_2O) δ 0.71–0.99 (m, 4 H), 2.81 (m, 1 H), 3.43 (m, 1 H), 3.71 (m, 1 H), 4.11 (m, 1 H), 4.41–4.77 (m, 4 H), 5.14–5.40 (m, 6 H), 5.75–6.07 (m, 2 H), 6.60 (m, 2 H), 7.06 (m, 2 H). Anal. ($C_{25}H_{27}N_3O_8$) C, H, N.

(6*S*,7*S*)-3-Cyclopropyl-7-[[(*R*)-2-(4-hydroxyphenyl)-glycyl]amino]-8-oxo-1-aza-4-oxabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid (54). Deprotection of **38** gave the product **54** (37% yield) as a white powder: IR (KBr) 3200, 1740, 1680, 1610 cm^{-1}; UV (H_2O) 274 (ε 10 000), 232 nm (ε 11 000); NMR (D_2O + CF_3CO_2H) δ 0.75–1.04 (m, 4 H), 2.65 (m, 1 H), 3.48 (t, 1 H, *J* = 10.3 Hz), 3.88 (ddd, 1 H, *J* = 10.3, 4.6, 3.9 Hz), 4.26 (dd, 1 H, *J* = 10.3, 3.9 Hz), 5.11 (s, 1 H), 5.56 (d, 1 H, *J* = 4.6 Hz), 6.97 (d, 2 H, *J* = 8.6 Hz), 7.32 (d, 2 H, *J* = 8.6 Hz).

Acknowledgment. We are grateful to P. Lapointe and S. Plamondon for their technical assistance.

Registry No. **5**, 86299-41-4; **6**, 86334-63-6; **7** (disilylated), 113627-36-4; **8**, 113599-60-3; **9** (detritylated), 113599-59-0; **10**, 113599-63-6; **12**, 113599-64-7; **13**, 113599-65-8; 14, 113599-66-9; **15**, 113599-67-0; **16**, 113599-68-1; **17**, 113599-69-2; **18**, 113599-70-5; **19**, 113599-71-6; **20**, 113599-72-7; **21**, 113599-73-8; **22**, 113599-74-9; **23**, 113599-75-0; **24**, 113599-76-1; **25**, 113599-77-2; **26**, 113599-78-3; **27**, 113599-79-4; **28**, 113599-80-7; **29**, 113599-81-8; **30**, 113627-35-3; **31**, 113599-82-9; **32**, 113599-83-0; **33**, 113599-84-1; **34**, 113599-85-2; **35**, 113599-86-3; **36**, 113599-87-4; **37**, 113599-88-5; **38**, 113599-89-6; **39**, 113599-90-9; **40**, 113599-91-0; **41**, 113599-92-1; **42**, 113599-93-2; *trans*-**43**, 113599-94-3; *cis*-**43**, 113666-83-4; *trans*-**44**, 113599-95-4; *cis*-**44**, 113666-84-5; **45**, 113599-96-5; **46**, 113599-97-6; **47**, 113666-85-6; **48**, 113599-98-7; **49**, 113666-86-7; **50**, 113599-99-8; **51**, 113600-00-3; **52**, 113600-01-4; **53**, 113600-02-5; **54**, 113600-03-6; allyl 2-bromoacetate, 40630-84-0; 3-(phenylseleno)propionic acid, 16599-78-3; 3-(phenylseleno)propionyl chloride, 113600-04-7; (*R*)-2-(4-hydroxyphenyl)glycine, 22818-40-2; (*R*)-[*N*-(allyloxycarbonyl)]-2-(4-hydroxyphenyl)glycine, 84792-41-6; allyl (6*S*, 7*S*)-7-amino-3-[2-(phenylseleno)ethyl]-8-oxo-1-aza-4-oxabicyclo-[4.2.3]oct-2-ene-2-carboxylate (*p*-toluenesulfonic acid salt), 113666-87-8; allyl (6S, 7*S*)-7-amino-3-[2-(phenylseleno)ethyl]-8-oxo-1-aza-4-oxabicyclo[4.2.0]oct-2-ene-2-carboxylate, 113600-05-8; chloroacetyl chloride, 79-04-9; acetaldehyde, 75-07-0; trifluoroacetaldehyde, 75-90-1; 4-(phenylseleno)butanoic acid, 23768-06-1; 4-(phenylseleno)butyryl chloride, 104680-44-6; cyclopropanecarboxylic acid chloride, 4023-34-1.