of dimethylformamide under N_2 were added 108 mg (1.06 mmol) of sodium methanesulfinate and the mixture was stirred at room temperature under N_2 for 16 h. The mixture was poured into ethyl acetate and the solution was washed with water and brine, dried over magnesium sulfate, and evaporated under vacuum, yielding 0.82 g of crude product. This material was purified by chromatography on silica gel with ethyl acetate, giving 0.47 g (89% yield). ^{1}H NMR ($CDCl_3$): 1.9 (1 H, m), 2.1 (1 H, m), 2.6 (2 H, m), 3.00 (3 H, s), 4.54 (2 H, s), 5.40 (3 H, m), 6.8–7.4 (5 H, m), 7.55 (2 H, d, $J_{1,2}$ = 8 Hz), 8.20 (2 H, d, $J_{1,2}$ = 8 Hz).

7β-[(Phenoxyacetyl)amino]-3-(methylsulfonyl)-1-carba-1-dethia-3-cephem-4-carboxylic Acid (7). *p*-Nitrobenzyl 7β-[(phenoxyacetyl)amino]-3-(methylsulfonyl)-1-carba-1-dethia-3-cephem-4-carboxylate (0.49 g, 0.89 mmol) was dissolved in 13 mL of DMF, 13 mL of THF, and 13 mL 1 N HCl. The solution was chilled to 0 °C and 0.40 g of powdered zinc was added in two portions. After stirring in the cold for 1 h, the mixture was poured into 175 mL of ethyl acetate and the solution was washed twice with 1 N HCl. The colorless ethyl acetate layer was dried over magnesium sulfate and evaporated to dryness under vacuum to provide the product as a yellow oil. The oil crystallized on standing to provide 265 mg (75% yield). ^{1}H NMR (DMSO-d_6): 1.8 (2 H, m), 2.4 (1 H, m), 2.6–2.7 (1 H, m), 2.72 (3 H, s), 2.90 (3 H, s), 3.10 (3 H, s), 3.91 (1 H, m), 4.59 (2 H, s), 5.53 (1 H, dd, $J_{1,2}$ = 5 and 8 Hz), 6.95 (3 H, m), 7.32 (3 H, t, $J_{1,2}$ = 7 Hz), 7.96 (1 H, s), 9.05 (1 H, d, $J_{1,2}$ = 8 Hz). IR ($CHCl_3$): 1780 cm^{-1} (β-lactam carbonyl). MS (FD): *m/e* (M + 1) 395, (M) 394. UV (EtOH): λ_{max} 269 nm (ε 12908).

Allyl 7β-[[D-α-[[(*tert*-Butyloxy)carbonyl]amino]phenylacetyl]amino]-3-[[(trifluoromethyl)sulfonyl]oxy]-1-carba-1-dethia-3-cephem-4-carboxylate (14). A solution of 1.005 g (4.0 mM) of *tert*-butyloxycarbonyl-protected D-phenylglycine, 702 mg (4.0 mM) of chlorodimethoxytriazine, and 440 μL (4.0 mM) of *N*-methylmorpholine in 28.6 mL of anhydrous methylene chloride was stirred under N_2 for 2 h at 0 °C. A solution of allyl 7β-amino-3-[[(trifluoromethyl)sulfonyl]oxy]-1-carba-1-dethia-3-cephem-4-carboxylate (~3.77 mM) in 14 mL of methylene chloride was added to the cold solution and the reaction mixture was allowed to warm to room temperature and was stirred for 2 days. The mixture was diluted with methylene chloride, extracted with 0.1 N HCl and with saturated aqueous sodium bicarbonate, dried over magnesium sulfate, and evaporated to dryness under vacuum. The crude acylation product was chromatographed over silica gel with 35% ethyl acetate/hexane, yielding 513 mg of product (23% yield). ^{1}H NMR ($CDCl_3$): 1.2–1.3 (1 H, br m), 1.43 (9 H, s), 1.7–1.9 (1 H, br m), 2.5–2.7 (2 H, m), 3.90 (1 H, m), 4.07 (1 H, s), 4.80 (2 H, m), 5.2–5.6 (3 H, m), 5.9–6.1 (1 H, m), 7.2 (5 H, br s).

Allyl 7β-[[D-α-[[(*tert*-Butyloxy)carbonyl]amino]phenylacetyl]amino]-3-(methylsulfonyl)-1-carba-1-dethia-3-cephem-4-carboxylate. The product of acylation was dissolved in 0.9 mL of anhydrous DMF under N_2. To this solution was added about 100 mg (1 mmol) of dry sodium methanesulfinate and the mixture was stirred for 15 h at room temperature. The reaction mixture was diluted with ethyl acetate and washed with water. The washes were extracted with ethyl acetate. The combined organic layers were dried over magnesium sulfate. Chromatography of the oil obtained after solvent removal was carried out on silica gel with 50% ethyl acetate/hexane (500 mL) followed by 75% ethyl acetate/hexane (300 mL) and gave 350 mg (77%) of the desired material. ^{1}H NMR ($CDCl_3$): 1.42 (9 H, s), 1.38–1.45 (1 H, m), 1.75–1.90 (1 H, m), 2.4–2.6 (2 H, m), 3.02 (3 H, s), 3.90 (1 H, m), 4.8 (2 H, m), 5.2–5.5 (4 H, m), 5.7 (1 H, m), 6.0 (1 H, m), 7.10 (1 H, br s), 7.40 (5 H, br s).

7β-[(D-α-Aminophenylacetyl)amino]-3-(methylsulfonyl)-1-carba-1-dethia-3-cephem-4-carboxylic Acid (13). The sulfone from above (350 mg, 0.66 mmol) was dissolved in 4 mL of acetonitrile and 2.6 mL of diethyl ether under N_2 and 16.2 mg of palladium(II) acetate and 138 mg (0.528 mmol) of triphenylphosphine were added. The reaction was stirred at room temperature for 20 min and cooled to 0 °C, and 0.186 mL (0.69 mmol) of tri-*n*-butyltin hydride was added. The cooling bath was removed and the mixture was stirred for 1 h and became increasingly cloudy and green. The mixture was treated with 0.057 mL (0.69 mmol) of concentrated HCl and was diluted with diethyl ether. The product formed as a gummy precipitate and after solvent removal 239 mg (73% yield) of crude product was isolated. This material was not characterized but was used directly in the next reaction. A portion of this crude material (50 mg, 0.10 mM) was dissolved in 1.0 mL of anhydrous methylene chloride and cooled to 0 °C. Trifluoroacetic acid (1 mL) was added and after 45 min acetonitrile was added, and the volatiles were removed in vacuo. This addition of acetonitrile and solvent removal was repeated three times, producing a yellow oil. The oil was dissolved in 4 mL of methanol and the crystalline trifluoroacetate salt was separated by centrifugation. The crystals were washed with diethyl ether and dried, yielding 12 mg of product. ^{1}H NMR (D_2O): 1.0–1.2 (1 H, m), 1.8 (1 H, m), 2.3–2.5 (2 H, m), 3.12 (3 H, s), 4.0 (1 H, dt, $J_{1,2}$ = 5 and 8 Hz), 5.22 (1 H, s), 5.46 (1 H, d, $J_{1,2}$ = 5 Hz), 7.55–7.6 (5 H, m). IR (KBr): 1787 cm^{-1} (β-lactam carbonyl). MS (FAB): *m/e* (M) 394. UV (EtOH): λ_{max} 270 nm (ε 15207).

3-Quaternary Ammonium 1-Carba-1-dethiacephems

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A series of structurally unique 1-carba-1-dethiacephems is described. The structural stability of the 1-carba-1-dethiacephem nucleus was essential for the preparation of this series of 3-quaternary ammonium carbacephems. The known *p*-nitrobenzyl 7β-(phenoxyacetamido)-3-[[(trifluoromethyl)sulfonyl]oxy]-1-carba-1-dethia-3-cephem-4-carboxylate served as both a quaternization substrate as well as a precursor to derivatives such as allyl 7β-[[[2-[[(allyloxy)carbonyl]amino]-4-thiazolyl](methoxyimino)acetyl]amino]-3-[[(trifluoromethyl)sulfonyl]oxy]-1-carba-1-dethia-3-cephem-4-carboxylate. Quaternization of these enol triflates was accomplished either by dissolution in acetonitrile containing the base or by dissolution in the base, with or without warming to 50 °C. Bases nucleophilic enough to displace the triflate include a variety of substituted pyridines and *N*-methylimidazole. Deprotection then produced a very active series of 1-[7β-[[(2-amino-4-thiazolyl)(methoxyimino)acetyl]amino]-2-carboxy-8-oxo-1-azabicyclo[4.2.0]oct-2-en-3-yl] quaternary ammonium hydroxide inner salts. These compounds were extremely potent antibacterials against a broad range of Gram-positive and -negative bacteria including constitutive cephalosporinase producers, such as *Enterobacter cloacae*. The compounds exhibit similar hydrolysis kinetics and pharmacokinetics to the analogous cephalosporin-3′-quaternary ammonium salts.

The 1-carba-1-dethiacephalosporins have been known for some time,[1] the first complete cephalosporin mimic having been synthesized by Guthikonda, et al. in 1974.[2] These workers showed that the methylene analogue of

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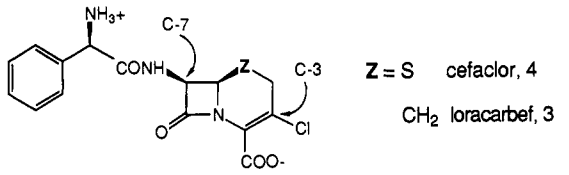


Figure 1.

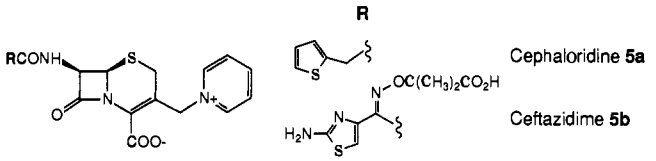
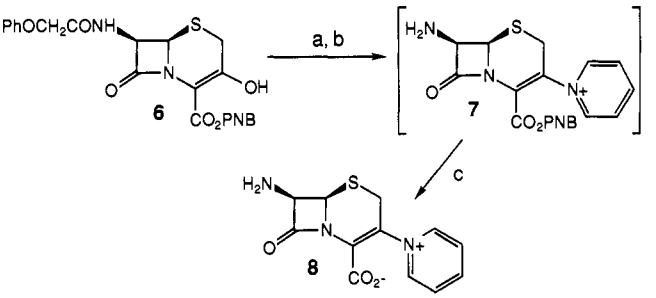


Figure 2.

cephalothin (1), 1-carbacephalothin (2), had equivalent antibacterial activity to the corresponding cephalosporin when normalized for the potency of the correct enantiomer. A number of other publications have appeared over the years concerning various aspects of 1-carba-1-dethiacephalosporin bioactivity and synthesis.[3] Our interest in this area was stimulated by the observation that the 1-carba-3-chloro-1-dethia-7-phenylglycylcephem, loracarbef (Figure 1), shows a dramatic stability increase over the corresponding cephem, cefaclor, under both neutral and basic conditions.[4] This dramatic stability increase of the carbacephem over the analogous cephem is reflected in a number of pairs of compounds synthesized and described in a forthcoming publication.[5] We decided to pursue this enhanced stability by making carbacephems whose analogues would be expected to be unstable in the cephem series.[6] Our exploration of one such structure–activity relationship, that of the 3-quaternary ammonium 1-carba-1-dethiacephems, is detailed below.

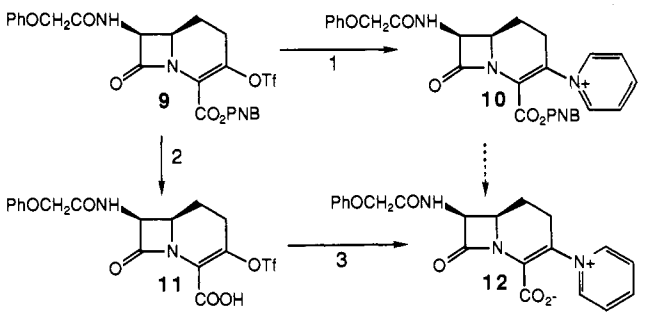
Tertiary amines quaternized by the cephalosporin nucleus at C-3′ have a long history, beginning with the fortuitous discovery of quaternization at C-3′ with pyridine by Abraham and Newton.[7] The synthesis of cephaloridine

Scheme I[a]



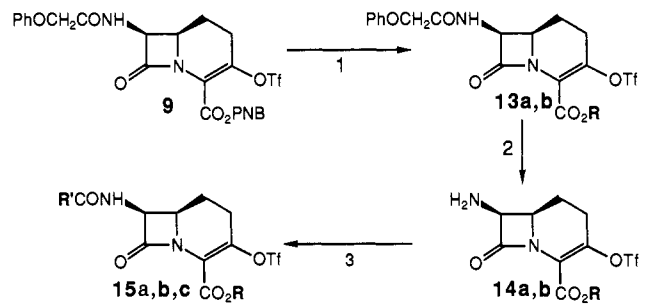
[a] (a) $(PhO)_3PCl_2$, CH_2Cl_2, py, (b) *i*-BuOH, (c) H_2O.

Scheme II[a]



[a] (1) pyridine; (2) Zn, HCl, DMF; (3) (a) $TMSCl/TMS_2NH$, (b) pyridine.

Scheme III[a]



[a] (1) (a) Zn, HCl, DMF, (b) n-Bu_4NHSO_4, $NaHCO_3$, Allyl bromide or (b) Ph_2CN_2; (2) (a) PCl_5, (b) *i*-BuOH, (c) H_2O; (3) $R'CO_2C_3N_3(OMe)_2$, NMM, CH_2Cl_2.

(**5a**) (Figure 2) followed soon after. The antimicrobial activity of cephaloridine was exciting for its time, but it also showed significant kidney toxicity in experimental models. Interest in 3′-quaternary ammonium cephalosporins waned until the discovery of the 7-[(aminothiazolyl)alkoximinoacetyl] side chain[8] led a number of groups to reinvestigate the effects of a quaternized base at C-3′. This led to the clinical evaluation of a number of third-generation compounds (e.g. ceftazidime, **5b**) all with excellent broad-spectrum activity against bacteria and lacking the renal toxicity observed with cephaloridine in animal models. The improved stability of 1-carbacephalosporins, combined with the enhanced microbiological activity of the 3′-quaternary ammonium cephalo-

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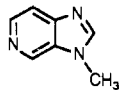
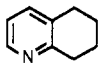
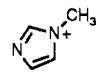
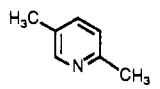
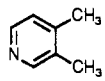
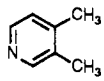
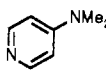
Table I. Preparation and Physical Properties of Final Products[a]

base	compd	quat condtn	% yield	deprot condtn	formula[b]	IR, cm^{-1}
	**25** (16) [11a]	CH_3CN, RT	84	Pd^0/SnH	$C_{19}H_{18}N_6O_5S$	1768.2
	**26** (17) [11a]	CH_3CN, 50 °C	71	Pd^0/SnH	$C_{21}H_{20}N_8O_5S$	1766.5
	**27** (18) [11a]	neat, 50 °C	75	Pd^0/SnH	$C_{22}H_{22}N_6O_5S$	1765.0
	**28** (19) [11a]	neat, 50 °C	65	Pd^0/SnH	$C_{23}H_{24}N_6O_5S$	1769.4
	**29** (20) [11a]	CH_3CN, RT	77	Pd^0/SnH	$C_{18}H_{19}N_7O_5S$	1765.3
	**30** (21) [11a]	neat, 50 °C	65	Pd^0/SnH	$C_{21}H_{22}N_6O_5S$	1771.4
	**31** (22) [11b]	neat, 50 °C	94	TFA/SiH	$C_{21}H_{22}N_6O_5S$	1772.5
	**32** (23) [11c]	neat, 50 °C	81	TFA/SiH	$C_{27}H_{26}N_6O_5S$	1769.3
	**33** (24) [11b]	CH_3CN, RT	81	TFA/SiH	$C_{21}H_{23}N_7O_5S$	1767.7

[a] The final products (first number) were prepared by first quaternization of the starting material (number in brackets) with the indicated base using the conditions listed (RT = room temperature), to give the intermediates (number in parentheses), and second deprotection via the indicated conditions (SnH = Bu_3SnH, SiH = Et_3SiH). [b] The formulas shown are those indicated by high-resolution mass spectroscopy and are within 1 mMU unit except for compound **32** which was +2.6 mMU.

sporins, led us to experiment with direct quaternization of aromatic heterocyclic amines with C-3 of the carbacephem nucleus.

There is precedence[9] for quaternization of a pyridine with a substituted-vinyl group in one unpublished example in the cephem series. This was the formation of a 7-amino-3-pyridiniocephem, **8**, as a byproduct in early large-scale preparations of the 3-chloro nucleus (Scheme I) where the reaction was not properly controlled. This compound is unstable and could not be isolated or acylated. Our first attempt to quaternize pyridine with a 1-carbacephem is shown in Scheme II and met with immediate success. Enol triflate **9** when stirred overnight in pyridine gave a new compound, **10**, which could be precipitated in a pure state with ether. Hydrogenolytic removal of the *p*-nitrobenzyl group of **10** was not successful, so **9** was treated with zinc and acetic acid in aqueous tetrahydrofuran to give **11**. Quaternization of **11** proceeded cleanly following in situ silylation with chlorotrimethylsilane. Reverse-phase chromatography then produced purified zwitterion **12**. This compound was stable and gave very encouraging results in initial microbiological screening.

Chemistry

(a) Preparation of Starting Materials. Our goals at this juncture were to work out a more versatile quaternization procedure as well as to understand the influence of the base on both the quaternization and the microbiological activity. We were fortunate that the work of Evans et al. allowed us to produce significant quantities of enol triflate[10] **9**. This material was homochiral and was functionalized so as to allow for ready interchange of substituents at both the 7-amino and the 4-carboxy positions (Scheme III). Toward this end, we could remove the *p*-nitrobenzyl group of **9** under reducing metal conditions as above and replace it with either an allyl or a benzhydryl group. Allyl ester **13a** was formed by alkylation of the tetra-*n*-butylammonium salt of the acid with allyl bromide in $CHCl_3$, while benzhydryl ester **13b** was prepared simply by titration of the free acid with diphenyldiazomethane. The phenoxyacetyl group could be removed by successive treatment with PCl_5, isobutanol, and water.[11] Acylation of this relatively unstable **14** with suitably protected cephalosporin side chains using 6-chloro-2,4-dimethoxy-1,3,5-triazine as activating agent[12] resulted in the fully functionalized nuclei **15a–c**. The three side chains investigated were (2-amino-4-thiazolyl)(methoxyimino)acetyl protected as either an allyl (allocATMO) or a *tert*-butyl carbamate (*t*-BocATMO) and (2-amino-4-thiazolyl)(benzyloximino)acetyl (*t*-BocATBO) protected as a *tert*-butyl carbamate.

(b) Quaternization of the Enol Triflates. A range of pyridine nuclei can be quaternized by enol triflates **15a–c** under the appropriate conditions (Scheme IV). The conditions developed for these purposes included both quaternization in the base at elevated temperatures for the least reactive heterocycles and quaternization in DMF at room temperature with the more nucleophilic bases. Isolation of the semipure product could best be accomplished by precipitation with a nonpolar cosolvent. The compounds were best purified at a later step, though ^1H NMR usually showed greater than 80% purity at this stage. The

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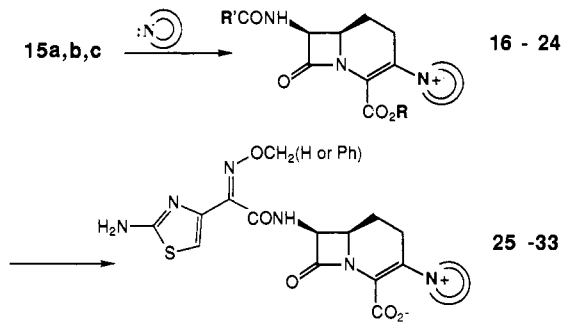
Scheme IV[a]



[a] (1) CH_3CN or neat, room temperature to 50 °C; (2) Pd^0/Bu_3SnH or TFA/Et_3SiH.

range of pyridines quaternized is demonstrated in Table I. Quaternization also proceeds with other aromatic heterocycles which possess the requisite nucleophilicity (e.g. 1-methylimidazole). A number of aromatic heterocycles could not be quaternized under a variety of conditions by the enol triflate, including thiazole and oxazole. Quinoline and isoquinoline quaternizations led to labile products, which could not be isolated. The temperature of the reaction necessary for complete quaternization correlated with the expected nucleophilicity of the base being quaternized. Thus, substituents at the 2-position of the pyridine ring required higher reaction temperatures than the corresponding unsubstituted compounds. For example, 2,5-dimethylpyridine was significantly slower to quaternize even at elevated temperatures than was 3,4-dimethylpyridine. On the other hand, the nucleophilic 1-methylimidazole was quaternized very rapidly. The desired 3-quaternary amines could then be converted to their unprotected form with either a palladium-catalyzed reduction with tributyltin hydride[13] in the case of the allyl ester/allyl carbamates or with trifluoroacetic acid for the benzhydryl ester/*tert*-butyl carbamates. Purification was usually carried out at this stage by chromatography with a water/acetonitrile gradient containing 1% acetic acid on HP-20ss polystyrene resin. The preferred protecting groups were the acid labile ones because the reaction by-products were much easier to remove. Reverse-phase HPLC was used at this stage to determine purity. The amount of Δ-2 product was always less than 10% and even though only slightly different in polarity, it could often be separated during preparative reverse-phase chromatography.

Saturated tertiary amines (e.g. *N*-methylmorpholine and *N*-methylpyrrolidine) under the usual conditions reacted extremely rapidly and produced only the Δ-2 isomer unlike the aromatic bases, which produced only small amounts of the Δ-2 isomer, generally less than 10%.

Semiempirical Calculations of Ortho-Substituted Pyridines. A second interesting class of bases quaternized in this series were the pyridines bearing substituents at the 2-position of the pyridine ring (**18/27, 19/28, 21/30**). These compounds though chromatographically pure by reverse-phase HPLC showed two distinct sets of peaks in the ^1H NMR. This indicated that these compounds might be exhibiting isomerism in which equilibration was slow on the NMR time scale. Substitution at the 2-position of a pyridinium side chain is expected to reduce its conformational freedom. Unfortunately, we were not able to conduct coalescence experiments due to decomposition of

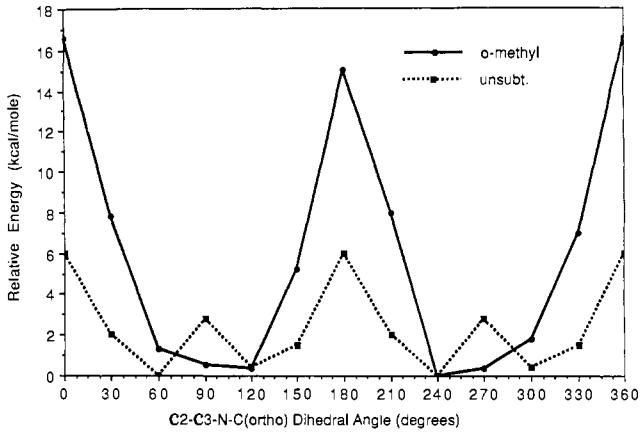


Figure 3. AM1 potential energy curves for rotation about the C3–N3′ bond in zwitterionic models of 3-pyridino-4-carboxylato-1-carba-1-dethiaceph-3-em for the unsubstituted (dotted curve) and *o*-methyl substituted (solid curve) structures. The relative energies are computed from heats of formation with respect to the most stable conformers of the unsubstituted (13.07 kcal/mol) and substituted (7.00 kcal/mol) models. All geometrical variables were free to optimize except for the C2–C3–N3′–C(ortho) torsional angle defining the conformation of the side chain and except for the pyridinium ring, which was constrained to be planar in order to facilitate and speed the calculations. Default options were used in running MOPAC 3.0 on a VAX 8800 superminicomputer.

the carbacephems at elevated temperatures. Computational chemistry experiments are an effective way to estimate the extent of the conformational freedom of molecules in general, and in this case, they were the only practical approach. In order to determine barriers to internal rotation about the C3–N3′ bond, quantum mechanical calculations were done on 3-R-4-carboxylato-1-carbaceph-3-em, where R is pyridinium or *o*-methylpyridinium. This model should be adequate for establishing the interactions between the carbacephem nucleus and the 3-position side chain. The SYBYL molecular modeling software package[14,15] was used to derive starting atomic coordinates from the crystalline state coordinates of benzyl 1α-hydroxy-7β-(phenoxyacetamido)-1-carba-1-dethiacephem-4-carboxylate, the only published carbacephem structure at the time these calculations were done.[16] The AM1[17,18] semiempirical molecular orbital method was used because, compared to Dewar's other methods (MINDO/3[19] and MNDO[20]), it gives the best representation of rotation about single bonds between sp^2 hybridized atoms. An advantage of using a quantum mechanical approach, rather than a molecular mechanics one, is that no ad hoc parameterization is necessary.[21,22] The resulting potential energy curves are shown in Figure 3, and ORTEP molecular graphics[23,24] are in Figures 4 and 5. Introduction of the

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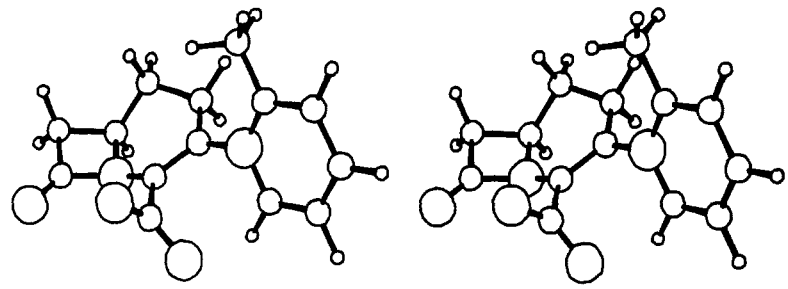

Figure 4. Stereoview of the most stable conformer of 3-*o*-methylpyridino-4-carboxylato-1-carba-1-dethiaceph-3-em determined by the AM1 molecular orbital method. Short interatomic distances occur between the 2β proton of the nucleus and a methyl proton (2.1 Å) and between the ortho hydrogen of the pyridinium ring and a carboxyl oxygen (2.2 Å). (The sum of van der Waals radii of two hydrogens is 2.4 Å and that of an oxygen and hydrogen is 2.6 Å.)

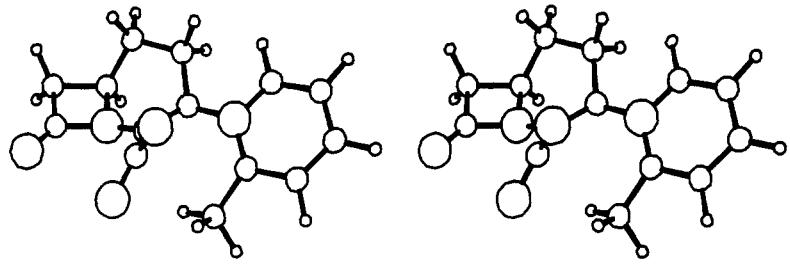

Figure 5. Stereoview of the least stable conformer of 3-*o*-methylpyridino-4-carboxylato-1-carba-1-dethiaceph-3-em determined by the AM1 molecular orbital method. Short interatomic distances occur between the ortho hydrogen of the pyridinium ring and the C2 protons (2.0 and 2.1 Å) and between a methyl hydrogen and a carboxyl oxygen (2.3 Å). This conformer tries to relieve steric hindrance by opening up the C4′–C4–C3, C4–C3–N3′, and N3′–C(ortho)–C(Me) bond angles; each distorts 3° with respect to its value in the most stable conformer (Figure 2). The bond angles at C4 sum to 359°.

methyl group in the ortho position of the pyridinium ring is seen to increase the barrier significantly from 6 to 16 kcal/mol. Hence distinguishable conformational isomers should be possible near or below room temperature with the substituted derivative.

Stability of 3-Quaternary Ammonium 7-(Acylamino)-1-carba-1-dethiaceph-3-em-4-carboxylates. The stability of β-lactams to basic hydrolysis has often been correlated with microbiological activity.[25] These correlations have been questioned in general, but nevertheless they provide important insight in specific limited examples. The carbacephem loracarbef has been shown to be significantly more stable than the parent cefaclor to base-catalyzed hydrolysis.[4] More recently, a thorough study has shown this stability increase to be a general feature of carbacephalosporins with a variety of substituents at C-3 and acyl groups at C-7.[5] Two 3-quaternary ammonium 1-carba-1-dethiacephems, **25** and **27**, were the targets of a kinetic evaluation of their base-catalyzed hydrolysis. The hydrolyses were carried out at a constant pH of 10 and the progress of the reactions were monitored by HPLC analysis. The rate constants for hydrolysis (and half-lives) are 0.746 h^{-1} (0.93 h) and 0.538 h^{-1} (1.29 h) for **25** and **27**, respectively. **34**, (Figure 6) the ATMO analogue of ceftazidime (**5b**), under identical conditions at pH = 10, has a rate constant for hydrolysis of 2.55 h^{-1} (0.272 h). If one were to extrapolate on the basis of the ~20-fold rate difference between cephalosporins and carbacephalosphorins found in the earlier study,[6] the cephem

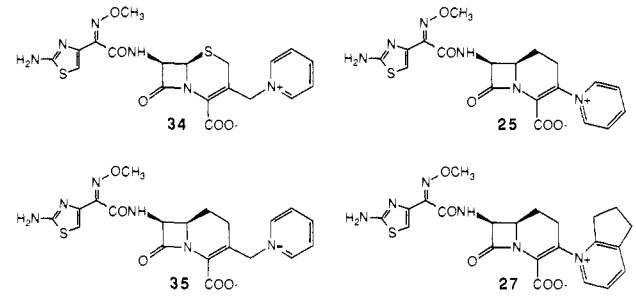

Figure 6.

counterparts of the 3-quaternary ammonium 1-carba-1-dethiacephems presented would have half-lives of less than 1 min at pH = 10. Interestingly, 4-(dimethylamino)-pyridinium **33** was quite unstable and showed no antimicrobial activity.

NMR Spectra of 3-Quaternary Ammonium 7-(Acylamino)-1-carba-1-dethiaceph-3-em-4-carboxylates. The chemical shifts are relatively straightforward for all of the quaternary ammonium carbacephems excluding the compounds with substituents at the ortho position of the quaternized pyridine ring. The protons at C-1 (cephem numbering) appear as two multiplets at δ 1.8–1.95 and 2.0–2.1. The protons on C-2 similarly show up as two multiplets at δ 2.65–2.75 and 2.7–2.8. The β-lactam hydrogens at C-6 and C-7 are at δ 3.8–4.0 (multiplet) and 5.40–5.45 (t or dd), respectively. In **18/27**, **19/28**, and **21/30**, the ^{1}H-NMR displays doubled peaks (just line broadening for **19/28**) for both protons on the base and the proton at C-7. For instance, **30** has two doublet of doublets for the C-7 proton (δ 5.60 and 5.70) as well as a doubling of the C-7 amide NH doublet (δ 9.32 and 9.38) and the pyridinium protons. The protons at C′-3 and C′-6 of the 2,5-dimethylpyridinium ring of **30** are also doubled with the C′-3 proton appearing as two overlapping doublets (7.90 and 7.93) and with the C′-6 proton as two singlets (8.72 and 8.79).

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Table II. MIC's of Final Products[a]

compd	S.a.	S.e.	S.py.	S.pn.	H.i.	E.c.	E.c.+	K.	K.+	E.cl.	E.cl.+	S.	Ps.	P.m.	P.r.
25	4.0	8.0	0.03	0.03	0.50	0.06	0.06	0.03	8.0	0.125	2.0	0.03	128	0.25	0.5
26	4.0	8.0	0.06	0.06	0.5	0.06	0.50	0.06	4.0	0.125	1.0	0.06	128	0.50	0.25
27	2.0	4.0	0.03	0.03	0.50	0.06	0.50	0.03	8.0	0.125	1.0	0.03	128	0.25	0.50
28	2.0	8.0	0.06	0.06	0.50	0.125	1.0	0.125	16.0	0.25	2.0	0.125	128	1.0	4.0
29	2.0	4.0	0.03	0.03	0.25	0.03	0.125	0.06	4.0	0.125	2.0	0.03	128	0.25	0.5
30	4.0	8.0	0.03	0.06	0.5	0.125	0.125	0.125	2.0	0.25	1.0	0.125	128	0.25	0.25
31	2.0	4.0	0.03	0.015	0.25	0.06	0.06	0.06	4.0	0.125	0.25	0.03	128	0.125	0.06
32	1.0	2.0	0.03	0.015	0.125	2.0	1.0	0.06	128	4.0	8.0	1.0	128	2.0	8.0
5b	8.0	16.0	0.06	0.06	0.06	0.06	0.125	0.06	0.50	0.25	32.0	0.125	2.0	0.50	0.25

[a] Minimal inhibitory concentrations are listed for compounds **25–32**. Organisms are abbreviated as follows: S.a. = *Staphylococcus aureus* (X1.1); S.e. = *Staphylococcus epidermidis* (222); S.py. = *Streptococcus pyogenes* (C203); S.pn. = *Streptococcus pneumoniae* (PARK); H.i. = *Haemophilus influenzae* (C.L.); E.c. = *Escherichia coli* (EC14); E.c.+ = *Escherichia coli* (TEM β-lactamase containing); K. = *Klebsiella* (X26); K.+ = *Klebsiella* (KAE β-lactamase containing); E.cl. = *Enterobacter cloacae* (EB5); E.cl.+ = *Enterobacter cloacae* (265A β-lactamase containing); S. = *Salmonella* (X514); Ps. = *Pseudomonas aeruginosa* (X528); P.m. = *Proteus morganii* (PR15); P.r. = *Proteus rettgeri* (C24).

Antibacterial Properties and Structure–Activity Relationships. The 3-quaternary ammonium carbacephems were found to have excellent antibacterial characteristics similar to those of the ATMO 3′-quaternary ammonium cephalosporins with two major exceptions (Table II). Most of the Gram-positive as well as the Gram-negative species were shown to be susceptible to concentrations of ≤4 μg/mL. One notable difference was the lack of discernible activity against the tested strains of *Pseudomonas aeruginosa* for these compounds. Inactivity against *P. aeruginosa* can be due to poor penetration of the outer membrane, rapid destruction by periplasmic β-lactamases, low affinities for the essential penicillin binding proteins, or a combination of these factors. Outer membrane permeability in *P. aeruginosa* was assessed by comparing the in vitro susceptibility of a wild type strain of *P. aeruginosa* (PS128) with that of an isogenic mutant strain (PS129), which has a highly permeable outer membrane,[26] to the action of these compounds. The susceptibility of the permeable mutant to these compounds, although weaker than that of the representative ATMO 3′-quaternary ammonium cephalosporin **34**, suggested that a major factor in their inactivity against wild type strains of this species is outer membrane permeability. A second control compound, **35**, having the same substituent at C-3 as **34**, was found to have antipseudomonal activity similar to that of the latter compound, strongly suggesting that the lack of antipseudomonal activity in the studied series is due to the absence of the methylene group in the 3-substituent rather than the difference between CH_2 and S in the nucleus.

Another difference between the two series of compounds was the potency of the carba series against the stably derepressed, β-lactamase-producing strain of *Enterobacter cloacae* (strain 265A). Such strains of this species constitutively produce high levels of a Richmond type I β-lactamase (a cephalosporinase)[27] and express a moderate to high level of resistance to most "third-generation" cephalosporins despite their observed stability to this enzyme. The current series of compounds have activity against *E. cloacae* 265A at concentrations of 4 μg/mL or lower (Table II). This degree of activity is within 10-fold of that observed against the strains which require induction for high-level production of this same enzyme (strain EB-5). By contrast, many of the ATMO 3′-quaternary ammonium cephalosporins suffer as much as 100-fold losses in potency between the inducible strains and the constitutive enzyme producers. This same characteristic was also illustrated by studying the inducible strains with and without induction. Table III shows results of a "cefoxitin disk approximation" assay[28] on three inducible strains of *Enterobacter* species. The severity of truncation of the inhibition zone on the side proximal to the cefoxitin disk is a measure of the effect of high-level β-lactamase production by the otherwise susceptible strains. In this assay, compound **31** was only slightly affected by the induced β-lactamase, a finding that was consistent with the potent activity (MIC = 0.25 μg/mL) observed against the constitutive high-level β-lactamase producing strain, 265A (Table II).

Table III. Zone Diameters (mm) and Truncation[a]

compd	*E. cloacae* strain[b] EB5	EB17	EB57
25	30/+3	30/+2	31/+3
34	30/+3	30/+2	30/+3
31	30/+1	30/+1	31/+2
35	26/+3	25/+3	26/+3

[a] A 30-μg disk of test compound was placed 15 mm from a cefoxitin disk. [b] Truncation of the zone of inhibition was graded as follows: 0 = no truncation; +1 = slight truncation; +2 = moderate truncation; +3 = severe truncation.

Table IV. Pharmacokinetic Parameters for Selected Quaternary Salts[a]

compd	*N*	half-life, min	% urinary recovery
5b	4	22.9	100.0
25	3	21.2	82.6
35	4	22.9	87.9
29	4	21.7	93.9
27	4	20.1	82.3

[a] The terminal half-lives and urinary recoveries are listed for three quaternary ammonium carbacephems, **25**, **27**, and **29**, and are compared with two controls, ceftazidime and **35**.

Pharmacokinetic Evaluation. The pharmacokinetic profiles of compounds **25**, **27**, **29**, **35**,[5] and **5b** were evaluated in rats. The results (Table IV) indicate that there are no pharmacokinetic differences between the cephem and the 1-carba-1-dethiacephem tested. All of the compounds tested had short plasma half-lives (20–26 min) and high urinary recoveries (≥83%). It was also observed that CH_2 and S, and C-3 and C-3′ quaternized derivatives were pharmacokinetically indistinguishable in the cases studied here. Thus, the novel structural aspects of the compounds described above do not provide for any pharmacokinetic advantages relative to **5b** in the compounds tested.

In conclusion, we have been able to take advantage of the substantial increase in chemical stability offered by

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the 1-carba-1-dethiacephalosporin nucleus by preparing an unprecedented series of 3-quaternary ammonium carbacephems. The most active of these 3-quaternary ammonium carbacephems is comparable to or more active than ceftazidime against most bacteria excluding *P. aeruginosa*. The compounds are quite resistant to the type I β-lactamases produced by *Enterobacter* and maintain excellent activity against Gram-positive bacteria.

Experimental Section

IR spectra were recorded on a Nicolet FT-IR Model 10-DX instrument. Mass spectra were determined with a Varian MAT 731. Mass spectra were recorded by either one of two methods, fast atom bombardment (FAB) or field desorption (FD). Proton NMR spectra were recorded on a General Electric QE-300 instrument. UV spectra were determined on a Cary Model 219 spectrometer in EtOH. Analytical HPLC was carried out on a C_{18} column eluted with aqueous 1–20% acetonitrile (constant or gradient) in aqueous ammonium acetate (1–2%) buffer and monitored at 254 nm. Thin-layer chromatography was performed on Merck F254 silica gel plates eluted with either ethyl acetate/hexane solvent mixtures or for polar compounds a mixture of ethyl acetate, acetonitrile, acetic acid, and water (25:7:9:9) sometimes diluted with varying amounts of ethyl acetate. Analytical results indicated by elemental symbols were within ±0.4% of the theoretical values. Experimental details for compounds **9**, **11**, **13a**, **13b**, **14a**, **14b**, **15a**, and **15b** are reported in a separate publication.[6]

Determination of Minimal Inhibitory Concentrations (MIC). Test compounds were diluted to an appropriate range of concentrations in 0.1 M phosphate buffer, pH 7.0, incorporated into Mueller-Hinton agar (Difco) supplemented with 1% Bacto-Supplement C (Difco) at 50 °C and allowed to solidify in petri dishes. Fresh overnight cultures of test bacteria were diluted to approximately 1×10^4 cells/μL and applied in 1-μL volumes to the surfaces of the agar plates. The inoculated plates were incubated overnight at 35 °C in ambient air. MIC endpoints were recorded as the lowest antibiotic concentrations that inhibited the development of visible growth on the plates.

Disk Diffusion Assays. Activity of the test compounds against the permeable and wild type parent strains of *P. aeruginosa* and the inducible strains of *E. cloacae* were carried out on Mueller-Hinton agar inoculated by surface swabbing with a cotton-tipped applicator moistened with a bacterial suspension containing approximately 1×10^8 cells/mL. Filter-paper disks saturated with solutions of test compounds sufficiently concentrated to give 30 μg of compound per disk were applied to the surfaces of the inoculated plates. Following overnight incubation at 35 °C in ambient air, zones of growth inhibition were measured and recorded. The cefoxitin-induction assay was performed by adding commercial cefoxitin susceptibility test disks (30 μg) at a distance of approximately 15 mm from the edge of the disks containing the test compounds.

Determination of Base Catalyzed Hydrolysis Rates. The hydrolysis rates were determined by following the loss of parent β-lactam. Constant pH was maintained by a pH stat consisting of a Metrohm 655 dosimat, 614 implusomat, and a 632 pH meter fitted with a combination electrode. Initial β-lactam concentrations were 3.9×10^{-4}–1×10^{-3} M. the pH was maintained at 10 or 11 by addition of NaOH. The ionic strength was adjusted to 0.5 with KCl. The chromatography system consisted of a Beckman 332 chromatograph, a Rheodyne 7125 injection valve fitted with a 20-μL loop, a Waters 450 or a Kratos Spectroflow 773 detector, and a Hewlett-Packard 3390A integrator. The stationary phase was a 4.4 × 250 mm Zorbax ODS (Du Pont) reverse-phase column and the detector was set at 254 nm. The flow rate was 1 mL/min. The mobile phases (v/v) were as follows: **25** and **34**, 10% CH_3CN/90% 0.025 M $NH_4H_2PO_4$; **27**, 14% CH_5CN/86% 0.025 M $NH_4H_2PO_4$.

Determination of Pharmacokinetic Profiles. Male Sprague–Dawley rats were dosed intravenously with test compounds at 20 mg/kg in saline. Dosing and blood sampling were carried out through an indwelling jugular vein cannula, thus permitting serial sampling from individual rats. Plasma levels and cumulative urinary recoveries were detemined from samples collected over a 4-h time course. Antibiotic concentrations were determined with an agar well diffusion assay employing *E. coli* (ATCC 4157) as the bacterial test strain. The detection limit of the assay was ≤0.80 μg/mL for all compounds tested. Plasma half-life was calculated as $0.693/\beta$, where β is the slope of the terminal portion of the plasma vs time curve. Urinary recovery was calculated as the percent of the administered dose recovered in the urine.

1-[2-[[(*p*-Nitrobenzyl)oxy]carbonyl]-7-(phenoxyacetamido)-8-oxo-1-azabicyclo[4.2.0]oct-2-en-3-yl]pyridinium Trifluoromethanesulfonate (10). Enol triflate **9** (480 mg, 0.80 mmol) was dissolved in 4.0 mL of anhydrous pyridine under N_2. The faintly cloudy solution obtained was stirred at ambient temperature for 20 h. At this point TLC analysis showed no starting material and a single spot upon elution with a 50:18:18:14 mixture of ethyl acetate, acetonitrile, water, and acetic acid. The reaction mixture was poured into diethyl ether giving a reddish-brown gum, which could be crystallized by heating and scratching the flask during the addition of ethyl acetate. The tan crystals were isolated by filtration, washed with diethyl ether, and dried in vacuo. This produced 508 mg (94%) of the desired product, which melted at 122–124 °C. ^{1}H NMR (DMSO-d_6): 2.0–2.15 (2 H, m), 2.8–3.0 (2 H, m), 4.06 (1 H, m), 4.64 (2 H, s), 5.23 (2 H, s), 5.79 (1 H, dd, $J_{1,2}$ = 6 and 9 Hz), 7.0 (3 H, m), 7.33 (2 H, t, $J_{1,2}$ = 7 Hz), 7.53 (2 H, d, $J_{1,2}$ = 8 Hz), 8.2–8.3 (4 H, m), 8.76 (1 H, t, $J_{1,2}$ = 8 Hz), 9.02 (1 H, d, $J_{1,2}$ = 9 Hz), 9.17 (2 H, d, $J_{1,2}$ = 6 H). IR ($CHCl_3$): 1787.2 cm^{-1} (β-lactam carbonyl). MS (FD): *m/e* (M^+) 529. UV (EtOH): λ_{max} 264 nm (ε 19631). Anal. ($C_{29}H_{25}F_3N_4O_{10}S$): C, H, N.

1-[7β-(Phenoxyacetamido)-2-carboxy-8-oxo-1-azabicyclo[4.2.0]oct-2-en-3-yl]pyridinium Hydroxide, Inner Salt (12). Free acid enol triflate **11** (130 mg, 0.28 mM) was dissolved in 10 mL of anhydrous pyridine under N_2 and treated with *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (130 mL, 0.70 mmol). The reaction was stirred for 16 h at room temperature, showing only a trace of product, and was then heated to 50 °C for 2 days. Though there was some starting material left, there was also a significant precipitate, which was isolated by centrifugation and supernatant decantation after dilution with ethyl acetate. This solid was washed twice with ethyl acetate and once with diethyl ether and dried under a stream of N_2. This produced 56 mg of a tan powder. ^{1}H NMR (D_2O): 1.8–2.0 (1 H, m), 2.0–2.1 (1 H, m), 2.8–2.9 (2 H, m), 4.15 (1 H, dt, $J_{1,2}$ = 4 and 12 Hz), 5.51 (1 H, d, $J_{1,2}$ = 4 Hz), 7.06 (2 H, d, $J_{1,2}$ = 9 Hz), 7.11 (1 H, t, $J_{1,2}$ = 7 Hz), 7.44 (2 H, t, $J_{1,2}$ = 7 Hz), 8.15 (2 H, t, $J_{1,2}$ = 7 Hz), 8.66 (1 H, t, $J_{1,2}$ = 8 Hz), 8.86 (2 H, d, $J_{1,2}$ = 7 Hz). IR ($CHCl_3$): 1758.2 cm^{-1} (β-lactam carbonyl). MS (FD): *m/e* (M + 1) 394. UV (EtOH): λ_{max} 256 (ε 11887.1), 316 nm (ε 2854).

Diphenylmethyl 7-[[[2-(Tritylamino)-4-thiazolyl](benzyloximino)acetyl]amino]-3-[[(trifluoromethyl)sulfonyl]oxy]-1-carba-1-dethia-3-cephem-4-carboxylate (15c). In an oven-dried, N_2-filled, round-bottom flask [2-(tritylamino)-4-thiazolyl](benzyloximino)acetic acid (10.81 g, 20.8 mM) was suspended in 150 mL of anhydrous CH_2Cl_2. After cooling of this suspension in an ice bath, distilled *N*-methylmorpholine (2.29 mL, 20.8 mmol) was added by syringe followed by 2-chloro-4,6-dimethoxy-1,3,5-triazine (3.67 g, 20.8 mmol) in 10 mL of anhydrous CH_2Cl_2. After 2 h, the reaction was complete and the ice bath had warmed to ambient temperature. This solution was chilled to −50 °C and crude amine **14a** (<20.8 mmol) was added dropwise via an addition funnel in 100 mL of anhydrous CH_2Cl_2. The reaction mixture was allowed to stir for an additional 16 h as the temperature gradually rose to 15 °C. The solid was filtered off and the filtrate was diluted with 300 mL of ethyl acetate. This was washed twice with 200 mL of aqueous 1 N HCl, twice with 200 mL of saturated aqueous $NaHCO_3$, and once with brine. After drying over Na_2SO_4, filtering, and solvent removal in vacuo, the product was chromatographed on silica gel eluted with 4 L of 60% ethyl acetate/40% hexane. Concentration gave 6.50 g of amorphous, white solid (31% for two steps), which was pure by ^{1}H NMR. ^{1}H NMR ($CDCl_3$): 1.04–1.2 (1 H, m), 1.6 (1 H, m), 2.0 (1 H, m), 2.2–2.4 (1 H, m), 3.83 (1 H, dt, $J_{1,2}$ = 6 and 12 Hz), 5.27 (2 H, dd, $J_{1,2}$ = 11 Hz), 5.34 (1 H, br t, $J_{1,2}$ = 6 Hz), 6.44 (1 H, d, $J_{1,2}$ = 7 Hz), 6.70 (1 H, s), 6.99 (2 H, m), 7.2–7.5 (30 H, m). IR ($CHCl_3$): 1784.1 cm^{-1} (β-lactam carbonyl). MS (FAB): *m/e* (M^+) 998. UV (EtOH): λ_{max} 263 (ε 17000), 426 (ε 213), 415 nm

(ε 415). Anal. ($C_{53}H_{42}F_3N_5O_{10}S_2$): C, H, N.

1-[7β-[[[2-[[(Allyloxy)carbonyl]amino]-4-thiazolyl](methoxyimino)acetyl]amino]-2-[(allyloxy)carbonyl]-8-oxo-1-azabicyclo[4.2.0]oct-2-en-3-yl]pyridinium Trifluoromethanesulfonate (16). 3-Triflate **15a** (1.00 g, 1.57 mmol) was dissolved in 0.80 mL of acetonitrile under a N_2 atmosphere. Anhydrous pyridine (0.14 mL, 1.73 mmol) was added via a syringe at ambient temperature. The reaction was stirred for 3 days after further additions of pyridine (0.14 mL after 1 day and 0.07 mL after 2 days). The mixture was concentrated in vacuo, redissolved in acetonitrile, and reconcentrated. The residue was chromatographed over silica gel eluting with a gradient of 75% ethyl acetate/7% acetonitrile/9% acetic acid/9% water to 50% ethyl acetate/14% acetonitrile/18% acetic acid/18% water. The desired fractions were combined and lyophilized, providing 0.95 g (84% yield) the desired, but impure, product as an orange solid. ^{1}H NMR (CD_2Cl_2): 3.96 (3 H, s), 4.08–4.24 (1 H, m), 4.54 (2 H, d, $J_{1,2}$ = 5 Hz), 4.72 (2 H, d, $J_{1,2}$ = 5 Hz), 5.04–6.20 (7 H, m), 7.20 (1 H, s), 7.92–8.20 (2 H, m), 8.32–8.60 (1 H, m), 9.12–9.36 (2 H, m).

1-[7β-[[[2-[[(Allyloxy)carbonyl]amino]-4-thiazolyl](methoxyimino)acetyl]amino]-2-[(allyloxy)carbonyl]-8-oxo-1-azabicyclo[4.2.0]oct-2-en-3-yl]-3-methyl-3*H*-imidazo[4,5-*c*]pyridin-5-ium trifluoromethanesulfonate (17) was prepared according to the above procedure except the reaction mixture was heated to 50 °C overnight. The product was precipitated with diethyl ether and the solid was washed three times with diethyl ether. After drying at 10 Torr, 78 mg of semipure product (from 100 mg of starting material) was obtained, a 71% yield. ^{1}H NMR (CD_3CN): 2.05–2.41 (2 H, m), 3.89 (3 H, s), 4.01 (3 H, s), 4.05–4.27 (1 H, m), 4.43 (2 H, d, $J_{1,2}$ = 5 Hz), 4.63 (2 H, d, $J_{1,2}$ = 5 Hz), 4.89–6.13 (8 H, m), 7.13 (1 H, s), 8.01 (1 H, d, $J_{1,2}$ = 7 Hz), 8.43 (1 H, d, $J_{1,2}$ = 6 Hz), 8.57 (1 H, s).

1-[7-[[[2-[[(Allyloxy)carbonyl]amino]-4-thiazolyl](methoxyimino)acetyl]amino]-2-[(allyloxy)carbonyl]-8-oxo-1-azabicyclo[4.2.0]oct-2-en-3-yl]-6,7-dihydro-5*H*-1-pyridinium Trifluoromethanesulfonate (18). This reaction required 5 h at 50 °C for quaternization, and **18** was obtained in 75% yield on a 100-mg scale. This salt was isolated similarly to **17** above. ^{1}H NMR (CD_3CN): 2.0–3.1 (12 H, m), 3.98 (3 H, s), 4.2–4.3 (1 H, d, $J_{1,2}$ = 5 Hz), 4.5 (2 H, m), 4.74 (2 H, m), 5.2–5.45 (3 H, m), 5.6–5.7 (2 H, m), 5.9–6.1 (1 H, m), 7.3 (1 H, s), 7.55–7.85 (2 H, m), 8.25 (1 H, d, $J_{1,2}$ = 7 Hz), 8.43 (1 H, d, $J_{1,2}$ = 6 Hz).

1-[7-[[[2-[[(Allyloxy)carbonyl]amino]-4-thiazolyl](methoxyimino)acetyl]amino]-2-[(allyloxy)carbonyl)]-8-oxo-1-azabicyclo[4.2.0]oct-2-en-3-yl]-5,6,7,8-tetrahydroquinolinium Trifluoromethanesulfonate (19). This quaternization also required 4 h at 50 °C and produced the desired product as a solid in 65%. Isolation was via precipitation with ether. ^{1}H NMR (CD_3CN): 2.00–2.32 (4 H, m), 2.68–3.00 (2 H, m), 3.04–3.50 (4 H, m), 3.92 (3 H, s), 4.04–4.28 (1 H, m), 4.62 (2 H, m), 4.66 (2 H, m), 5.04–5.32 (4 H, m), 5.38–5.68 (2 H, m), 5.72–6.04 (1 H, m), 7.28 (1 H, s), 7.40–7.82 (2 H, m), 8.29 (1 H, d, $J_{1,2}$ = 6 Hz), 8.42 (1 H, d, $J_{1,2}$ = 4 Hz).

3-[7β-[[[2-[[(Allyloxy)carbonyl]amino]-4-thiazolyl](methoxyimino)acetyl]amino]-2-[(allyloxy)carbonyl]-8-oxo-1-azabicyclo[4.2.0]oct-2-en-3-yl]-1-methyl-1*H*-imidazolium Trifluoromethanesulfonate (20). This quaternization also proceeded under conditions like those for the pyridine quaternization with a 77% yield. ^{1}H NMR (DMSO-d_6): 1.73–1.93 (1 H, m), 2.10–2.23 (1 H, m), 2.50–2.77 (1 H, m), 2.80–2.97 (1 H, m), 3.87 (3 H, s), 3.89 (3 H, s), 3.93–4.13 (1 H, m), 4.53–4.70 (4 H, m), 5.13–5.50 (4 H, m), 5.63–5.83 (2 H, m), 5.87–6.03 (1 H, m), 7.37 (1 H, s), 7.83 (1 H, s), 7.97 (1 H, s), 9.35 (1 H, s), 9.52 (2 H, d, $J_{1,2}$ = 9 Hz).

1-[7β-[[[2-[[(Allyloxy)carbonyl]amino]-4-thiazolyl](methoxyimino)acetyl]amino]-2-[(allyloxy)carbonyl]-8-oxo-1-azabicyclo[4.2.0]oct-2-en-3-yl]-2,5-dimethylpyridinium Trifluoromethanesulfonate (21). This reaction proceeded at 50 °C (neat) in 65% yield. ^{1}H NMR (CD_3CN): 1.87–2.93 (10 H, m), 4.0 (3 H, s), 4.17–4.25 (1 H, m), 4.23–4.77 (4 H, m), 5.17–5.47 (4 H, m), 5.60–5.80 (2 H, m), 5.93–6.09 (2 H, m), 7.30 (1 H, s), 7.58 (1 H, d, $J_{1,2}$ = 9 Hz), 7.95 (1 H, d, $J_{1,2}$ ≈ 7 Hz), 8.32 (1 H, d, $J_{1,2}$ = 7 Hz), 8.40 (1 H, s), 9.43 (1 H, s).

1-[7β-[[[2-[[(Allyloxy)carbonyl]amino]-4-thiazolyl](methoxyimino)acetyl]amino]-2-[(allyloxy)carbonyl]-8-oxo-1-azabicyclo[4.2.0]oct-2-en-3-yl]-3,4-dimethylpyridinium Trifluoromethanesulfonate (22). This reaction was also best carried out at 50 °C in 3,4-dimethylpyridine as solvent and produced the product in 94% yield. ^{1}H NMR (CD_3CN): 1.87–2.33 (2 H, m), 2.45 (3 H, s), 2.60 (3 H, s), 2.80–2.88 (2 H, m), 4.00 (3 H, s), 4.13–4.23 (1 H, m), 4.27–4.60 (2 H, m), 4.75 (2 H, d, $J_{1,2}$ = 6 Hz), 5.17–5.47 (4 H, m), 5.63–5.77 (2 H, m), 5.95–6.07 (1 H, m), 7.30 (1 H, s), 7.72 (1 H, d, $J_{1,2}$ = 9 Hz), 7.85 (1 H, d, $J_{1,2}$ = 9 Hz), 8.31 (1 H, d, $J_{1,2}$ = 9 Hz), 8.41 (1 H, s); 9.30–9.60 (1 H, br s).

1-[7β-[[[2-[(Triphenylmethyl)amino]-4-thiazolyl](benzyloximino)acetyl]amino]-2-[(diphenylmethoxy)carbonyl]-8-oxo-1-azabicyclo[4.2.0]oct-2-en-3-yl]-3,4-dimethylpyridinium Trifluoromethanesulfonate (23). Similar reaction gave this compound in 81% yield. ^{1}H NMR (CD_3CN): 1.62–1.83 (2 H, m), 2.25 (3 H, s), 2.40 (3 H, s), 2.53–2.64 (2 H, m), 3.96–4.07 (1 H, m), 5.14 (2 H, s), 5.45–5.58 (1 H, m), 6.60 (1 H, s), 6.73 (1 H, s), 7.10–7.60 (33 H, m), 8.28–8.38 (2 H, m).

1-[7β-[[[2-[(*tert*-Butoxycarbonyl)amino]-4-thiazolyl](methoxyimino)acetyl]amino]-2-[(diphenylmethoxy)carbonyl]-8-oxo-1-azabicyclo[4.2.0]oct-2-en-3-yl]-4-(dimethylamino)pyridinium Trifluoromethanesulfonate (24). This compound was produced in acetonitrile as solvent at room temperature in 81% yield. ^{1}H NMR (CD_3CN): 1.47 (9 H, s), 2.67 (2 H, m), 3.07 (6 H, s), 3.97 (3 H, s), 4.03–4.13 (1 H, m), 5.60–5.67 (1 H, m), 6.58 (2 H, d, $J_{1,2}$ = 9 Hz), 6.70 (1 H, s), 7.13–7.40 (10 H, m), 7.62 (1 H, d, $J_{1,2}$ = 9 Hz), 7.78 (2 H, d, $J_{1,2}$ = 9 Hz), 9.20 (1 H, s).

1-[7β-[[(2-Amino-4-thiazolyl)(methoxyimino)acetyl]amino]-2-carboxy-8-oxo-1-azabicyclo[4.2.0]oct-2-en-3-yl]pyridinium Hydroxide, Inner Salt (25). In an oven-dried, N_2-flushed, ice-cooled, round-bottom flask were combined triphenylphosphine (22 mg, 0.084 mmol), palladium(II) acetate (4.1 mg, 0.17 mmol), and 10.5 mL of anhydrous acetonitrile. Bis-allyl-protected starting material **16** (300 mg, 0.419 mmol) was added and the reaction mixture was stirred until a solution was obtained. At this time, 2.0 mL of anhydrous diethyl ether was added and the reaction was cooled in an ice bath. Tri-*n*-butylstannane (231 μL, 0.859 mmol) was added dropwise via a syringe. After 15 min, the reaction was warmed to room temperature. TLC showed no starting material after 30 min. The flask was then chilled with an ice bath and treated with 1 N aqueous HCl (880 μL, 0.88 mmol). After 10 min, the ice bath was removed and after an additional 20 min diethyl ether was added, forcing the product to precipitate out as a gum. Water was added and the aqueous solution was washed three times with 50/50 mixture of diethyl ether and hexane. The water layer was placed on a column packed with HP-20ss which had been packed in deionized water. The column was eluted with a 1600-mL linear gradient from 100% H_2O to 10% acetonitrile in H_2O. The clean fractions were lyophilized, producing 36 mg (19% yield) of very pure product. ^{1}H NMR (500 MHz, DMSO-d_6): 1.85–1.93 (1 H, m), 2.00–2.08 (1 H, m), 2.65–2.73 (1 H, m), 2.74–2.80 (1 H, m), 3.87 (3 H, s), 3.88–3.93 (1 H, m), 5.43–5.45 (1 H, m), 6.80 (1 H, s), 7.23 (2 H, s), 8.12 (2 H, t, $J_{1,2}$ = 9 Hz), 8.60 (1 H, t, $J_{1,2}$ = 9 Hz), 8.98 (2 H, d, $J_{1,2}$ = 9 Hz), 9.39 (1 H, d, $J_{1,2}$ = 10 Hz). IR: 1768.2 cm^{-1} (β-lactam carbonyl). MS (FAB): m/e (M + 1) 443.

The following compounds were prepared by employing the same procedure as above except THF was used in place of diethyl ether to precipitate the product.

1-[7β-[[(2-Amino-4-thiazolyl)(methoxyimino)acetyl]amino]-2-carboxy-8-oxo-1-azabicyclo[4.2.0]oct-2-en-3-yl]-1-methyl-1*H*-imidazolium Hydroxide, Inner Salt (29). Yield: 34%. ^{1}H NMR (DMSO-d_6): 1.70–2.00 (2 H, m), 2.49–2.63 (2 H, m), 3.77–3.87 (4 H, m), 5.35–5.43 (1 H, dd, $J_{1,2}$ = 5 and 9 Hz), 6.77 (1 H, s), 7.20 (1 H, s), 7.60 (1 H, s), 7.80 (1 H, s), 9.15 (1 H, s), 9.33 (1 H, d, $J_{1,2}$ = 9 Hz). IR: 1765.3 cm^{-1} (β-lactam carbonyl). MS (FAB): m/e (M + 1) 446.

1-[7β-[[(2-Amino-4-thiazolyl)(methoximino)acetyl]amino]-2-carboxy-8-oxo-1-azabicyclo[4.2.0]oct-2-en-3-yl]-3-methyl-3*H*-imidazolo[4,5-*c*]pyridin-5-ium Hydroxide, Inner Salt (26). Yield: 23%. ^{1}H NMR (DMSO-d_6): 1.74–2.26 (2 H, m), 2.74–2.90 (4 H, m), 3.02 (1 H, s), 5.34 (1 H, dd, $J_{1,2}$ = 5 and 9 Hz), 6.74 (1 H, s), 7.14 (1 H, s), 8.16 (1 H, d, $J_{1,2}$ = 7 Hz), 8.50

(1 H, d, $J_{1,2}$ = 7 Hz), 8.54 (1 H, s), 9.32 (1 H, d, $J_{1,2}$ = 9 Hz), 9.66 (1 H, s). IR: 1766.5 cm^{-1} (β-lactam carbonyl). MS (FAB): m/e (M + 1) 497.

1-[7β-[[(2-Amino-4-thiazolyl)(methoxyimino)acetyl]amino]-2-carboxy-8-oxo-1-azabicyclo[4.2.0]oct-2-en-3-yl]-6,7-dihydro-5*H*-1-pyrindinium Hydroxide, Inner Salt (27). Yield: 57%. ^1H NMR (DMSO-d_6): 1.80–2.27 (4 H, m), 2.43–2.73 (2 H, m), 3.07–3.40 (4 H, m), 3.83 (3 H, s), 3.87–4.00 (1 H, m), 5.40–5.50 (1 H, m), 6.77 (1 H, s), 7.20 (2 H, s), 7.81 (0.5 H, d, $J_{1,2}$ = 8 Hz), 7.86 (0.5 H, d, $J_{1,2}$ = 8 Hz), 8.34–8.40 (1 H, m), 0.5 H, d, $J_{1,2}$ = 6 Hz), 8.61 (0.5 H, d, $J_{1,2}$ = 6 Hz), 9.31 (0.5 H, d, $J_{1,2}$ = 8 Hz), 9.40 (0.5 H, d, $J_{1,2}$ = 8 Hz). IR: 1765 cm^{-1} (β-lactam carbonyl); MS (FAB): m/e (M + 1) 483.

1-[7β-[[(2-Amino-4-thiazolyl)(methoxyimino)acetyl]amino]-2-carboxy-8-oxo-1-azabicyclo[4.2.0]oct-2-en-3-yl]-5,6,7,8-tetrahydroquinolinium Hydroxide, Inner Salt (28). Yield: 56%. ^1H NMR (90 MHz, DMSO-d_6): 3.8–4.0 (1 H, m), 5.40 (1 H, dd, $J_{1,2}$ = 5 and 9 Hz), 6.70 (1 H, s), 7.12 (2 H, br s), 7.72 (1 H, br t, $J_{1,2}$ = 7 Hz), 8.20 (1 H, br d, $J_{1,2}$ = 7 Hz), 8.53 (1 H, br d, $J_{1,2}$ = 7 Hz), 9.18 (1 H, br d, $J_{1,2}$ = 9 Hz). IR: 1769.4 cm^{-1} (β-lactam carbonyl). MS (FAB): m/e (M + 1) 497.

1-[7β-[[(2-Amino-4-thiazolyl)(methoxyimino)acetyl]amino]-2-carboxy-8-oxo-1-azabicyclo[4.2.0]oct-2-en-3-yl]-2,5-dimethylpyridinium Hydroxide, Inner Salt (29). Yield: 31%. ^1H NMR (DMSO-d_6): 1.83–2.17 (2 H, m), 2.40–2.43 (3 H, s), 2.63–2.68 (3 H, s), 2.73–2.80 (2 H, m), 4.03–4.20 (2 H, m), 4.88 (3 H, s), 5.39 (0.3 H, dd, $J_{1,2}$ = 5 and 9 Hz), 5.45 (0.7 H, dd, $J_{1,2}$ = 5 and 9 Hz), 6.80 (1 H, s), 7.20 (2 H, s), 7.90 (0.7 H, d, $J_{1,2}$ = 8 Hz), 7.93 (0.3 H, d, $J_{1,2}$ = 8 Hz), 8.32 (1 H, br d, $J_{1,2}$ = 8 Hz), 8.72 (0.7 H, s), 8.79 (0.3 H, s), 9.32 (0.7 H, d, $J_{1,2}$ = 9 Hz), 9.39 (0.3 H, d, $J_{1,2}$ = 9 Hz). IR: 1771.4 cm^{-1} (β-lactam carbonyl). MS (FAB): m/e (M + 1) 471. UV: λ_{max} 234 nm (ε 17 006).

1-[7β-[[(2-Amino-4-thiazolyl)(methoxyimino)acetyl]amino]-2-carboxy-8-oxo-1-azabicyclo[4.2.0]oct-2-en-3-yl]-3,4-dimethylpyridinium Hydroxide, Inner Salt (30). Yield: 47%. ^1H NMR (DMSO-d_6): 1.73–2.07 (2 H, m), 2.35–2.77 (8 H, m), 3.77–3.90 (4 H, m), 5.42 (1 H, dd, $J_{1,2}$ = 5 and 9 Hz), 6.77 (1 H, s), 7.20 (1 H, s), 7.87 (1 H, d, $J_{1,2}$ = 8 Hz), 8.63 (1 H, d, $J_{1,2}$ = 8 Hz), 8.77 (1 H, s), 9.38 (1 H, d, $J_{1,2}$ = 9 Hz). IR: 1772.5 cm^{-1} (β-lactam carbonyl). MS (FAB): m/e (M + 1) 471.

1-[7β-[[(2-Amino-4-thiazolyl)(methoxyimino)acetyl]amino]-2-carboxy-8-oxo-1-azabicyclo[4.2.0]oct-2-en-3-yl]-4-(dimethylamino)pyridinium Hydroxide, Inner Salt (33). To 175 mg of **15b** was added at 0 °C a mixture of 1.2 mL of trifluoroacetic acid, 90 μL of triethylsilane, and 1.2 mL of methylene chloride. After stirring at 0 °C for 30 min, the external cooling bath was removed for 5 min and diethyl ether added to precipitate the product. The solid was recovered by centrifugation, washed three times with diethyl ether, and dried to provide 81 mg of crude title product. The material was dissolved in a small amount of water, filtered, and chromatographed by reverse-phase HPLC employing 8% acetonitrile and 1% acetic acid in water as the eluant. Lyophilization of the desired fractions provided 41 mg of the desired product. ^1H NMR (DMSO-d_6): 1.70–2.00 (2 H, m), 2.38–2.66 (2 H, m), 3.20 (6 H, s), 3.70–3.86 (2 H, m), 5.32 (1 H, dd, $J_{1,2}$ = 5 and 9 Hz), 6.73 (1 H, s), 6.95 (2 H, d, $J_{1,2}$ = 8 Hz), 7.68 (2 H, s), 8.10 (2 H, d, $J_{1,2}$ = 8 Hz), 9.33 (1 H, d, $J_{1,2}$ = 9 Hz). IR: 1767.7 cm^{-1} (β-lactam carbonyl). MS (FAB): m/e (M + 1) 486.

1-[7β-[[(2-Amino-4-thiazolyl)(benzyloximino)acetyl]amino]-2-carboxy-8-oxo-1-azabicyclo[4.2.0]oct-2-en-3-yl]-3,4-dimethylpyridinium Hydroxide, Inner Salt (32). Fifty milligrams of **15c** was added to 0.3 mL of trifluoroacetic acid and 9.7 μL of thiophenol under a nitrogen atmosphere and cooled by means of an external ethanol/ice bath. After stirring for 30 min at 0 °C and after 10 min with the ice bath removed, diethyl ether was added and the solid was worked up in the same manner as above. Chromatography with a continuous gradient of water to 12% acetonitrile in water and lyophilization provided 12 mg of the desired material. ^1H NMR (DMSO-d_6): 1.72–2.01 (2 H, m), 2.45 (3 H, s), 2.61 (3 H, s), 2.72–2.88 (2 H, m), 3.98–4.08 (1 H, m), 5.18 (2 H, s), 5.63 (1 H, dd, $J_{1,2}$ = 5 and 9 Hz), 6.84 (1 H, s), 7.24–7.36 (7 H, m), 8.08 (1 H, d, $J_{1,2}$ = 7 Hz), 8.94 (1 H, d, $J_{1,2}$ = 9 Hz), 9.00 (1 H, s), 9.56 (1 H, d), 7.94 (1 H, d, $J_{1,2}$ = 9 Hz). IR: 1769.3 cm^{-1} (β-lactam carbonyl), MS (FAB): m/e (M + 1) 547. UV: λ_{max} 234 (ε 21 900), 295 nm (ε 8350).

Synthesis and Biological Properties of N^{63}-Carboxamides of Teicoplanin Antibiotics. Structure–Activity Relationships

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The condensation of the carboxyl function of teicoplanin A2 (CTA) and its acidic hydrolysis pseudoaglycons (TB, TC) and aglycon (TD) with amines carrying various functional groups and chains produced amide derivatives with different isoelectric points and lipophilicities. Amide formation did not affect the ability of these compounds to bind to Ac_2-L-Lys-D-Ala-D-Ala, a model for the natural peptide binding site in bacterial cell walls. The antimicrobial activities of teicoplanin amides were found to depend mostly on their ionic and lipophilic character and on the type and number of sugars present. Positively charged amides were generally more in vitro active than the respective unmodified antibiotics against Gram-positive organisms. In particular, most basic amides of CTA were markedly more active than teicoplanin against coagulase-negative staphylococci. A few amides of TC and most of those of TD also showed a certain activity against Gram-negative bacteria. In experimental *Streptococcus pyogenes* septicemia in the mouse, some basic amides were more active than the parent teicoplanins when administered subcutaneously. Some of those of CTA were also slightly more effective than teicoplanin by oral route.

The glycopeptide antibiotic teicoplanin[1] was recently introduced in therapeutic use for the parenteral treatment of severe infections caused by aerobic and anaerobic Gram-positive bacteria, including methicillin-resistant (MR) staphylococci.[2] It is very poorly absorbed when administered orally and is inactive in vitro against Gram-

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