ISOMERISM OF CEPHALOSPORIN ESTERS; THEORETICAL AND PRACTICAL ASPECTS

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Abstract - Attempts to prepare 1,4-dihydropyridine targetor based chemical delivery systems (CDS) for 7-(thiophene-2-acetamido) cephalosporanic acid (cephalotin) (**1**) are reported. Reaction of **1** with hydroxyethylnicotinamide resulted in a mixture of Δ^2 and Δ^3 isomers of the 3-pyridinylcarbonylamino esters of **1**; after chromatographic separation the Δ^3 isomer was *N*-methylated and the resulting quaternary pyridinium salt was reduced with sodium dithionite to give the CDS. Attempts to attach the dihydropyridine moiety to **1** as an acyloxyalkyl ester failed. Relative stabilities of the Δ^3 and Δ^2 isomers of cephalotin acid and methyl ester, as reflected by theoretically (AM1 and PM3) calculated heats of formation (ΔH_f) indicated that kinetic and mechanistic, rather than thermodynamic factors are responsible for the isomerization of the esters.

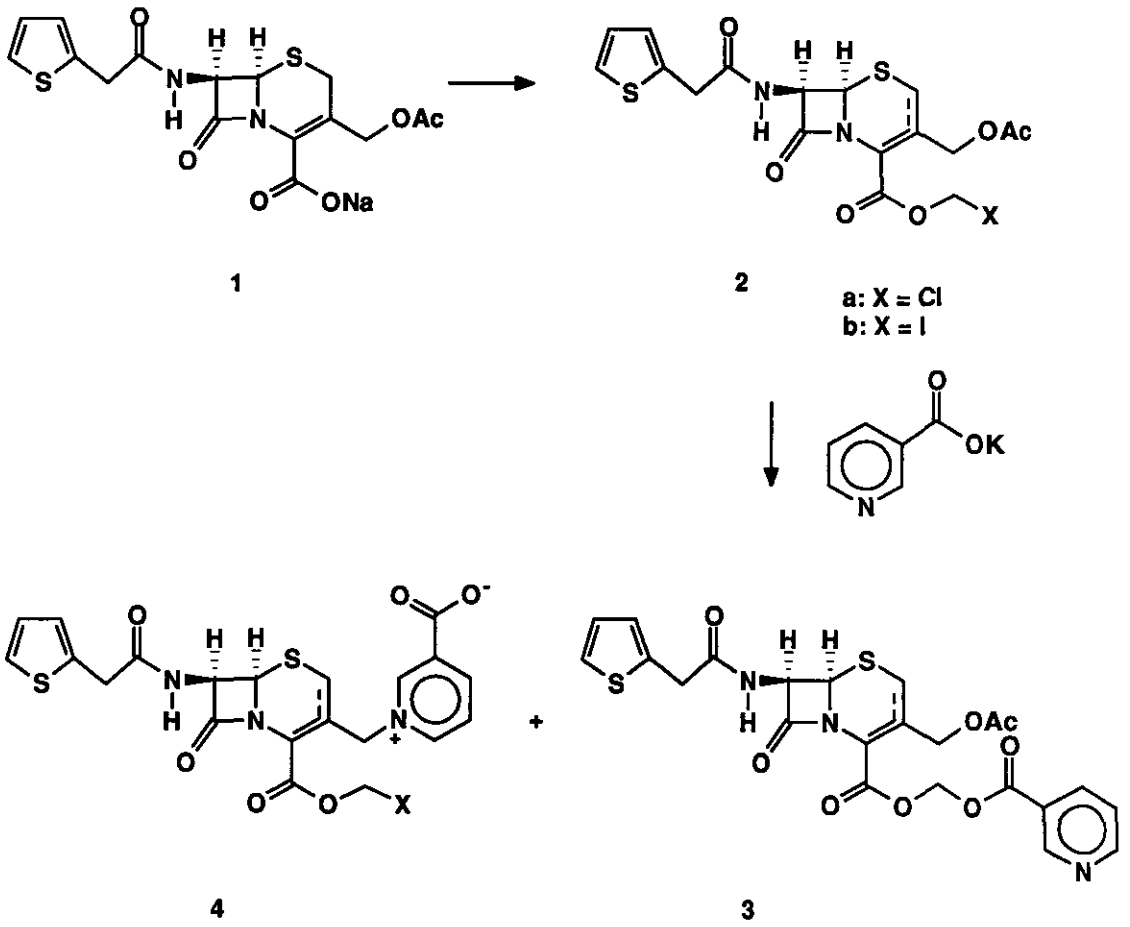
A disadvantage of most of the ß-lactam antibiotics, including penicillins and cephalosporins is their poor bioavailability. The polar character induced by the carboxylic functionality does not allow these otherwise safe and effective drugs to penetrate several biologically important barriers including the gastric mucosa and the blood brain barrier.[1,2] To overcome this problem, reversible structural modifications, usually esterification, were used which led to lipoidal prodrugs[3,4] or chemical delivery systems.[5,6] While manipulations of this type dramatically improved pharmacokinetic properties of penicillins, cephalosporins proved not to be amenable to

simple derivatization at the 4-carboxylic position. While cephalosporin acids are stable combinations, the naturally occurring Δ^3 bond of the dihydrothiazine ring of the esters or other derivatives (anhydrides, amides) of the 4-carboxylic group easily isomerize to Δ^2 position, resulting in biologically inactive compounds.[7,8] This isomerization, which in the case of cephalosporin acids is a very slow process, may occur during synthetic manipulations or subsequently *in vitro* and *in vivo*, resulting in isomeric mixtures. These changes are largely determined by structural factors.[9,10] For this and other reasons, the use of cephalosporin esters as prodrugs has not been successful. Attempts to synthesize brain specific chemical delivery systems for the widely used 7-(thiophene-2-acetamido)cephalosporanic acid (cephalotin),[11] as well as a theoretical interpretation of the $\Delta^3 \leftrightarrow \Delta^2$ isomerism are reported herein.

RESULTS AND DISCUSSION

Dihydropyridine $\leftrightarrow$ pyridinium salt targetor based chemical delivery systems were synthesized and successfully evaluated for a number of drugs including penicillins.[5,6] Two methods of reversibly attaching a pyridine moiety to the 4-carboxylic group of cephalosporin (**1**) were considered. In the carbonyloxyalkyl or diol diester type combination (**3**) (Scheme I) one hydroxyl factor of the glycol is esterified with the cephalosporin-4-carboxylic group and the other with nicotinic acid. Compounds (**6-8**) are carbamoylalkyl esters or cephalosporin esters of aminoalcohols having the amino group acylated with the pyridine-3-carboxylic acid derivative (Scheme II). Reaction of cephalosporin (**1**) with chloromethyl chlorosulfate in a mixture of aqueous sodium bicarbonate and methylene chloride in the presence of tetrabutlyammonium hydrogen sulfate used as a phase transfer catalyst, mainly the Δ^3 isomer of the chloromethyl ester (**2a**) was produced accompanied by the smaller amounts of Δ^2 isomer (ratio 7:3, as determined by integration of the ^{1}H-nmr spectra). The expectation that the fast reaction and the mild conditions used (30 min at 20-25 °C) as well as the extraction of the carboxylate anion from the basic, aqueous layer into the inert organic solution, would avoid the isomerization of the Δ^3 double bond was not confirmed. The purification of **2a** by chromatography or crystallization was not possible. It was intended to separate the isomers following the next step of the synthesis, however, the reaction of **2a** (isomer mixture) with potassium nicotinate in dimethylformamide (6 days, 20-25 °C) resulted in a mixture containing, beside the

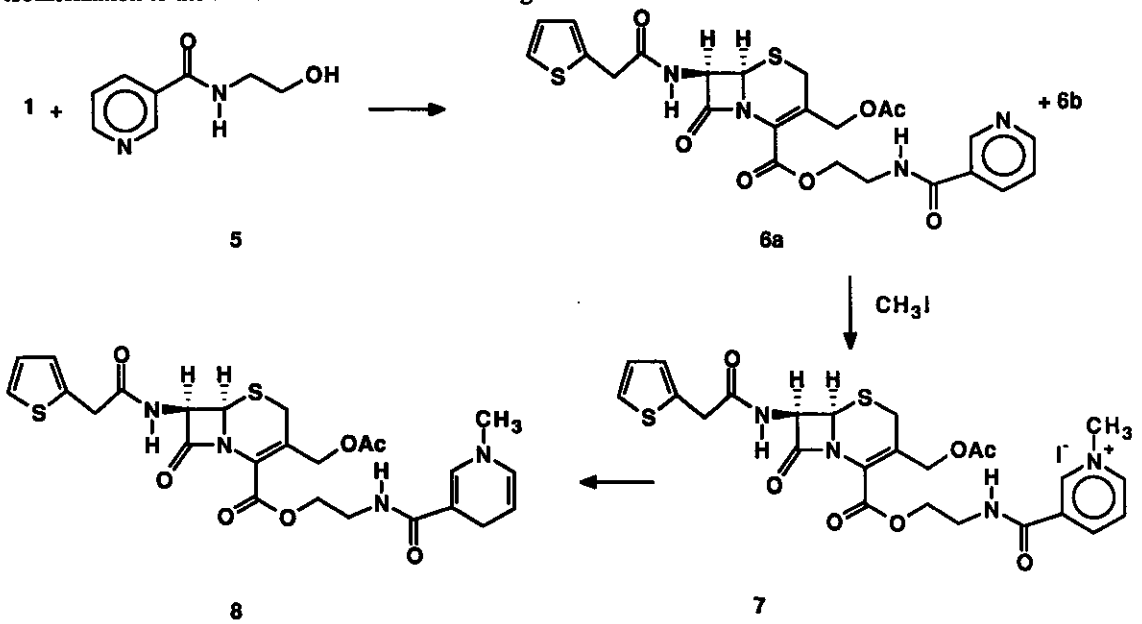
expected Δ^3 and Δ^2 isomers of **3**, the betaine type derivative (**4a**) formed by the displacement of the acetoxy group by the nucleophilic nitrogen of the nicotinic acid.[12] The same compounds were obtained by reacting the iodomethyl ester (**2b**) (prepared from **2a** by the reaction with sodium iodide in acetone) with the tetrabutylammonium salt of nicotinic acid. The synthesis was not of practical use.



Scheme I

In the synthesis illustrated in Scheme II, **1** was reacted with hydroxyethyl nicotinamide (**5**) in acetonitrile in the presence of dicyclohexylcarbodiimide (DCC) as a dehydrating agent and dimethylaminopyridine as a catalyst.

The reaction resulted in a mixture of the Δ^3 and Δ^2 isomers (**6a**) and (**6b**) (ratio 2:1) which were easily separated by column chromatography. The isomers were identified by uv and ^{1}H-nmr spectroscopy. Uv absorption in the 260 nm region is indicative of ß-lactam nitrogen Δ^3 double bond conjugation and is not observed in the Δ^2 isomer. The protons at C2 and C4 appeared at δ 6.40 and 5.00 ppm respectively for the Δ^2 isomer, replacing the methylene protons adjacent to the sulfur (doublets at 3.30 and 3.50 ppm) in the Δ^3 isomer. The single proton quartet and single proton doublet representing ß-lactam hydrogen at C7 and C6 respectively were ~ 0.4 ppm apart in Δ^2, as compared to ~ 0.8 ppm in Δ^3. The next step of the synthesis, the *N*-alkylation of the isomer (**6a**) with iodomethane resulted in the quaternary salt (**7**) which, by dithionite reduction, regioselectively gave the 1,4-dihydropyridine derivative (**8**) (the chemical delivery system of **1**). No isomerization to the Δ^2 isomer was observed during these two reactions.

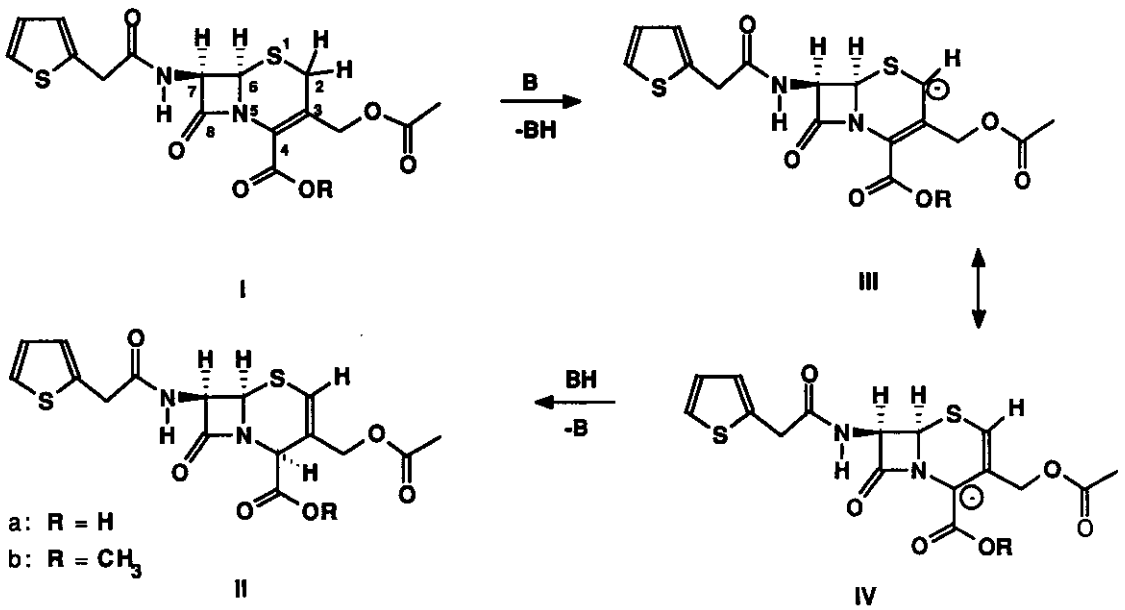


Scheme II

Preliminary *in vitro* studies performed in rat tissues indicated fast hydrolysis and release of the parent cephalosporin from all of the esters tested. The dihydropyridine combination (**8**) rapidly oxidized in brain tissues

to the quaternary salt form. Although isomerization to the inactive Δ^2 derivative might take place *in vivo*, **8** fulfills all the requirements of a successful brain-specific chemical delivery system of **1**.

A number of mechanisms have been suggested for the $\Delta^3 \rightarrow \Delta^2$ (**I**→**II**) isomerization of cephalosporins. It is likely that the double bond migration is a result of a prototropic rearrangement (Scheme III). This phenomenon is promoted by a proton extraction (by a base, **B**) at the C2 position of **I**. The resulting carbanion (**III**) can generate the carbanion (**IV**), which is stabilized by resonance. Both **III** and **IV** can then accept a proton, resulting in an equilibrium mixture of **I** and **II**. It is believed that the thermodynamically more stable isomer predominates in solution. The double bond is stabilized in a conjugative sense in both **I** and **II** by the unshared electrons of the dihydrothiazine ring N and S, respectively.



Scheme III

A study of this process is described herein using the AM1[14] and PM3[15] semiempirical molecular orbital techniques. In this examination the relative thermodynamics stabilities as reflected by the calculated heats of

formation (ΔH_f) of the pair of cephalotin isomers, both in their acid (**Ia** and **IIa**) and methyl ester (**Ib** and **IIb**) forms were estimated. The results of the computations are presented in Table 1.

Table 1. Calculated heats of formation (ΔH_f) for cephalosporin isomers

	ΔH_f (Kcal/mol)	
Compound	**AM1**	**PM3**
Δ^3 acid (**Ia**)	-143.1	-167.8
Δ^2 acid (**IIa**)	-140.7	-164.1
Δ^3 ester (**Ib**)	-136.8	-160.3
Δ^2 ester (**IIb**)	-134.0	-156.5

Calculated ΔH_f indicated that in each case the Δ^3 isomers were thermodynamically more stable than the Δ^2 isomers. The calculated energy differences, $\Delta H_f(\Delta^3) - \Delta H_f(\Delta^2)$, were smaller when the AM1 method was employed (2.4 kcal/mol for the acids and 2.8 kcal/mol for the esters) compared to PM3 (3.7 kcal/mol for the acids and 3.8 kcal/mol for the esters). The data indicate that in a chemical equilibrium, the Δ^3 isomer should predominate significantly. This data are in agreement with the experimental results and other theoretical calculations.[16] The energy differences were found to be smaller for the acids than for the esters using the AM1 method, but practically equal when the PM3 method was applied. The data would suggest that the isomerization of the esters is a result of other factors (such as kinetic or mechanistic) rather than thermodynamic. It is possible that the free carboxylic group in acids is completely ionized (having a low pk_a) which would inhibit both base and enzyme catalyst attack at C2. On the other hand, deprotonation and subsequent isomerization in the esters are permitted.

EXPERIMENTAL SECTION

Uncorrected melting points determined on an electro-thermal melting point apparatus (Fisher Scientific) are reported. Elemental microcombustion analyses were performed by Atlantic Microlabs, Inc, Atlanta, GA

Ultraviolet spectra (uv) were determined on a Hewlett-Packard 8451A diode array spectrophotometer. Proton nuclear magnetic resonance spectra (^{1}H-nmr) were recorded on a Varian XL 200 (220-MHz: FT mode) spectrometer. Samples were dissolved in an appropriate deuterated solvent, and chemical shifts were reported as parts per million (δ) relative to tetramethylsilane as an internal standard. Coupling constants are reported in hertz. Thin-layer chromatography (tlc) was performed on EM reagents DC-aluminum foil plates coated to a thickness of 0.2 mm with silica gel 60.

All chemicals were reagent grade. Cephalosporin was obtained from Sigma. Chloromethyl chlorosulfate was prepared by the method of Binderup and Hansen.[13]

7-(2-Thienylacetamido)cephalosporanic acid chloromethyl ester (2a) To a solution of 2.09 g (5 mmol) of cephalotin sodium salt, 1.2 g (14 mmol) of sodium bicarbonate and 0.17 g of (0.5 mmol) tetrabutylammonium hydrogen sulfate in water (5 ml) and methylene chloride (5 ml), 0.85 g (5.75 mmol) of chloromethyl chlorosulfate in methylene chloride (2 ml) was added in 5 min by stirring and maintaining the temperature below 30°C. The mixture was then stirred for 30 min at 20-25°C and the layers were separated. The aqueous layer was extracted with methylene chloride (5 ml) and the combined organics was washed added with water (10 ml), then dried over Na_2SO_4 and filtered with charcoal over a layer of neutral Al_2O_3. After removing the solvent *in vacuo* 1.37g (yield 62%) of white, foamy product resulted. Rf (EtOAc): 0.64; uv (MeOH) λ 208, 238, 266 nm; ^{1}H-nmr ($CDCl_3$) δ 2.06 (s, 3H, C<u>H</u>$_3$CO), 3.30, 3.50 (2d, 2H, J=18, C-2 protons), 3.85 (s, 2H, C<u>H</u>$_2$CONH), 4.85 (s, 2H, C<u>H</u>$_2$OAc), 5.00 (s, 1H, C-4 proton Δ^2), 5.20 (d, 1H, J=4.6, C-6 proton), 5.85 (s, 1H, C<u>H</u>$_2$Cl), 5.98 (q, 1H, J=5.8, 11, C-7 proton), 6.4 (br, 1H, C-2 proton Δ^2), 6.95-7.15 (m, 2H, =CH-CH=), 7.21 - 7.30 (m, 2H, S-C<u>H</u> and N<u>H</u>). Anal. Calcd for $C_{17}H_{17}N_2O_6ClS_2$: C, 45.89; H, 3.85; N, 6.30; Cl, 7.97; S, 14.41. Found: C, 45.75; H, 3.91;N, 6.27; Cl, 7.92; S, 14.32. (Δ^3: Δ^2 ratio 7:3).

7-(2-Thienylacetamido)cephalosporanic acid iodoethyl ester (2b) A solution of 0.70 g (1.5 mmol) of the chloromethyl ester (**2a**) and 1 g (6.7 mmol) of sodium iodide in dry acetone (10 ml) was stirred at 20-25°C for 20 h. The solvent was removed *in vacuo* and the residue was dissolved in ethyl acetate:water , 1:1, (30 ml). The layers were separated and the organics extracted three times with brine. After drying over $MgSO_4$ the solvent was removed *in vacuo* and 0.68 g (yield 87%) of off-white product was obtained: mp 65-70°C; uv

(MeOH) same as **2a**; ^{1}H-nmr ($CDCl_3$): similar to **2a** (CH_2I shifted to 6.05 ppm). Anal Calcd for $C_{17}H_{17}N_2O_6IS_2$: C, 38.07; H, 3.20; N, 5.22; I, 23.66; S, 11.95. Found: C, 37.96: H, 3.34; N, 5.10; I, 23.45; S, 12.26.

2-[(3-Pyridinylcarbonyl)amino]ethyl 7-(2-thienylacetamido)cephalosporanate (Δ^3; 6a and Δ^2; 6b). To a solution of 2.86 g (7.2 mmol) of cephalosporanic acid (prepared from 3.2 g of sodium salt, by treating an ethyl acetate suspension with 25% aqueous HCl (40 ml), separating the layers and removing the solvent *in vacuo*) in acetonitrile (200 ml), 1.2 g (7.2 mmol) of hydroxyethylnicotinamide, 1.57 g (7.6 mmol) of dicyclohexylcarbodiimide and 0.1 g dimethylaminopyridine was added and the mixture was stirred at 20-25°C for 20 h. The precipitated dicyclohexylurea (DCU) was filtered off and the solvent was removed *in vacuo*. By adding methylene chloride (150 ml) to the residue some more DCU precipitated. After filtering off the precipitate the solution was washed with 4% aqueous $NaHCO_3$ and water, then dried over Na_2SO_4. After removing the solvent *in vacuo*, 3.7 g off-white solid consisting of a mixture of the Δ^3 and Δ^2 isomers (**6a**) and (**6b**) resulted. The two isomers were separated by column chromatography (100g silica gel, Davisil, grade 634, 100-200 mesh, 60 Å, eluent isopropanol: chloroform, 1:8) obtaining 1.4 g (yield 35.9%) of **6a** and 0.7g (yield 17.9%) of **6b**. **6a:** mp 140-143°C; uv (MeOH), λ 210, 238, 260 nm, Rf: 0.40 (EtOAc); ^{1}H-nmr ($CDCl_3$) δ: 2.10 (s, 3H, $COCH_3$); 3.30-3.50 (2d, 2H, C-2 protons, J=18), 3.75 - 3.95 (m, 6H, CH_2CH_2 and CH_2-CONH), 4.85 (s, 2H, CH_2-OAc), 5.15 (d, 1H, J=5.6, C-6 proton), 5.80 (q, 1H, J=5.8, C-7 proton), 6.95 -7.05 (m, 2H, =CH-CH=), 7.21 - 7.37 (m, 3H, S-CH=, NH and pyridine C-5 protons), 7.58 (t, 1H, NH), 8.14 (d, 1H. J=7.9, pyridine C-4 proton), 8.67 (d, 1H, J=4.8, pyridine C-6 proton), 9.01 (s, 1H, pyridine C-2 proton); Anal. Calcd for $C_{24}H_{24}N_4O_7S_2$: C, 52.93; H, 4.4; N, 10.29; S, 11.77. Found: C, 52.94; H, 4.48; N, 10.23; S, 11.70. **6b**, mp 162-5°C; uv (MeOH) λ 212, 232 nm; Rf (EtOAc) 0.20; ^{1}H-nmr ($CDCl_3$) δ: 4.35 (s, 2H, CH_2OAc), 5.00 (s, 1H, C-4 proton), 5.28 (d, 1H, J=5.6, C-7 proton), 5.60 (q, 1H, J=5.6, C-6 proton), 6.40 (bs, 1H, C-2 proton). (The other peaks similar to **6a**.); Anal. Calc d for $C_{24}H_{24}N_4O_7S_2$: C, 52.93; H, 4.4; N, 10.29; S, 11.77. Found: C, 53.07; H, 4.52; N, 10.18; S, 11.82.

3-[[[[[[(2-Thienylacetamido)-cephalosporanoyl]carbonyl]oxy]ethyl]amino]carbonyl]-1-methylpyridinium iodide (7). A solution of 0.9 g (1.6 mmol) of **6a** in 45 ml of dry nitromethane was reacted with 1.00 g (7 mmol)

of iodomethane in a closed system at 20-25°C for 3 days (tlc showed complete reaction). The solvent was removed *in vacuo* and the solid residue was slurried with ether, filtered off and dried *in vacuo* over P_2O_5 giving 1.08 g (yield 98%) of yellow solid product: mp 110-12°C (decomp.); uv (MeOH) λ 222, 268 nm; Rf (i-propanol:$CHCl_3$, 1:8) 0.00; ^{1}H-nmr ($CDCl_3$) δ: 4.40 (s, 1H, N-CH_3), 8.87 (t, 1H, pyridinium C-5 proton), 8.90 (t, 1H, pyridinium C-4 proton), 9.14 (d, 1H, J=5.7, NH), 9.27 (t, 1H, NH), 9.39 (s, 1H, pyridinium C-2 proton). The other peaks were the same as **6a**. Anal. Calcd for $C_{25}H_{27}N_4O_7IS_2$: C, 43.74; H, 3.96: N, 8.16; I, 18.48; S, 9.34. *Found:* C, 48.86; H, 4.12; N, 7.95; I, 18.80; S, 9.26.

2-[[(1,4-Dihydro-1-methyl-3-pyridinyl)carbonyl]amino]ethyl 7-(2-thienylacetamido)-cephalosporanate (8) To a solution of 0.13 g (2 mmol) of quaternary salt **7** in water (10 ml) and ethyl acetate (10 ml), cooled at 0-5 °C and deaerated with nitrogen, a mixture of 0.11 g (13 mmol) of $NaHCO_3$ and 0.13 g (7.6 mmol) of sodium dithionite was added in 2-3 min. The mixture was stirred under the same conditions for 1 h, then the layers were separated, the aqueous layer was extracted with ethyl acetate (2 x 15 ml), and the combined organics were washed with 2 x 10 ml of cold, deaerated water. After drying over Na_2SO_4, the solvent was removed *in vacuo*, giving 0.06 g (yield 53%) yellow solid product: mp 98-100°C (decomp.): uv (MeOH) λ 212, 358 nm; Rf (i-propanol:$CHCl_3$ 1:8) 0.47. ^{1}H-nmr ($CDCl_3$) δ: 2.95 (s, 3H, N-CH_3), 3.07 (s, 2H, pyridine C-4 proton), 4.68 - 4.72 (m, 1H, dihydropyridine C-5 proton), 5.50 - 5.68 (m, 2H, dihydropyridine C-6 proton), 7.05 (s, 1H, dihydropyridine C-2 proton). (The other peaks were the same as in **6a**).

Anal. Calcd for $C_{25}H_{28}N_4O_7S_2$: C, 53.56; H, 5.03; N, 9.99; S, 11.44. Found: C, 53.72, H, 4.85; N, 10.21; S, 11.73.

Theoretical studies were performed using the AM1[14] and PM3[15] molecular orbital method included in the MOPAC (version 5.10) package. A Tektronix Computer Aided Chemistry (CAChe™) Worksystem run on an Apple Macintosh™ II computer was used for all computations. The structural input was generated using a Macintosh interface and all starting geometries were found by using molecular mechanics (MM2) to optimize the geometries. The Broyden-Flecher-Goldfarb-Shanno method was used to optimize geometries as a function of the total molecular energy. All geometric variables were optimized. The dynamic "level shift" method was used to improve the convergence of the self-consistent field (SCF). The "precise" option was implemented for

tightening the convergence criteria for all optimizations. The closed- and open-shell species were investigated using the restricted Hartree Fock (RHF) approach.

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