Brain-Specific Chemical Delivery Systems for β-Lactam Antibiotics. Synthesis and Properties of Some Dihydropyridine and Dihydroiosquinoline Derivatives of Benzylpenicillin†

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Six chemical delivery systems (CDS) were synthesized for benzylpenicillin in order to improve its transport across the blood–brain barrier. The CDS's were based on a dihydropyridine ⇄ quaternary pyridinium ion redox system, analogous to the naturally occurring NADH ⇄ NAD^+ system. Two different types of CDS's were prepared: benzylpenicillin esters of diols in which the other hydroxyl group is esterified by dihydrotrigonelline and benzylpenicillin esters of amino alcohols in which the amine group is acylated by dihydrotrigonelline, or by 1,2-dihydro-2-methyl-4-isoquinolinecarboxylic acid. Lipophilicities of the CDS's were proved to be much higher than those of benzylpenicillin by using R_m values as lipophilicity indexes. Upon oxidation, all of the CDS's gave the quaternary ion forms. Kinetic studies in buffer (pH profiles) indicated that the quaternary salts released benzylpenicillin in pH range of 5–9 via hydrolysis. The CDS's in acidic media yielded as the major reaction product 6-hydroxy-1,4,5,6-tetrahydropyridines as a result of water addition, while in basic conditions benzylpenicillin was released. The water addition reaction was dependent on the CDS's structure, being more prevalent in the case of the "amide–esters". The dihydroisoquinoline CDS was rather stable in the pH range 5–8.

The penetration of β-lactam antibiotics into the brain has been the subject of numerous studies and reviews.[1-4] The treatment of different forms of bacterial meningitis[5-7] as well as other applications such as prophylaxis of basilar fractures and routine craniotomy, management of open cranial–cerebral wounds, or treatment of parenchymal infections, in particular abscesses, cases in which the meninges are not necessarily involved, are several examples in which the presence of the antibiotic at the infected site is essential. In spite of the significant progress made in its treatment, bacterial meningitis still represents a challenge for medicine; it requires immediate treatment with antimicrobial agents especially due to the fact that central nervous system (CNS) infections, unlike those at other sites, are seldom self-limiting. A reasonable explanation for this is the unique composition of the cerebrospinal fluid (CSF) which contains less than 0.1% of the number of immunocompetent leucocytes found in peripheral blood and almost no circulating immunoglobins.

The main problem with using β-lactam antibiotics, including penicillin and cephalosporins, for treating brain infections is their poor access to the CNS. A drug may enter the brain by crossing either the blood–brain barrier (BBB) or the blood–CSF barrier; in spite of some obvious differences between them, from a pharmacological point of view it is allowable to use the term BBB for both of them.[8] The penetration of antibiotics across the BBB is dependent upon their lipid solubility at psysiological pH, their protein binding, the pH gradient over the barrier, the size and steric complexity of the molecule, and the degree of inflammatory reactions in the meninges. Active transport systems have also been postulated for some antibiotics.[3] The β-lactam antibiotics, due to their polar, hydrophilic nature, enter the brain and CSF very slowly through the different barriers. On the other hand, they return from the extracellular fluid compartment of the brain to the CSF compartment and are actively transported by the blood–CSF barrier (choroid plexus) back into the blood. This is the reason why these types of antibiotics are not as effective in the treatment of infections in brain as they are elsewhere. Since in the case of the infected meninges BBB permeability is improved somewhat, cerebritis, neurosyphilis, and brain abscesses, for example, constitute even greater therapeutic problems than bacterial meningitis.[1,2,9,10]

It is obvious that any improvement in the CNS penetration of β-lactam antibiotics is of interest. Research in the area was directed so far toward discovering new compounds with a better CNS uptake rather than modifying the known ones in order to improve their qualities required in this direction. The prodrug approach had as its main purpose overcoming pharmacokinetic defects such as the poor oral absorption or to obtain a prolonged, depot effect.

In recent years, a new concept of brain-specific drug delivery systems has been developed, based on a redox system analogous to the endogenous NADH ⇄ NAD^+ coenzyme system.[11-13] The dihydropyridine ⇄ quaternary salt redox system based chemical delivery system (CDS) has been proved to accomplish brain-specific delivery when applied to a number of agents.[14-23] Its application to

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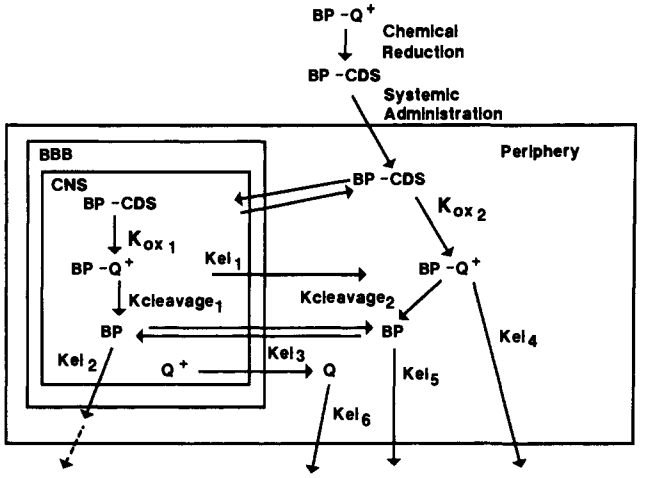
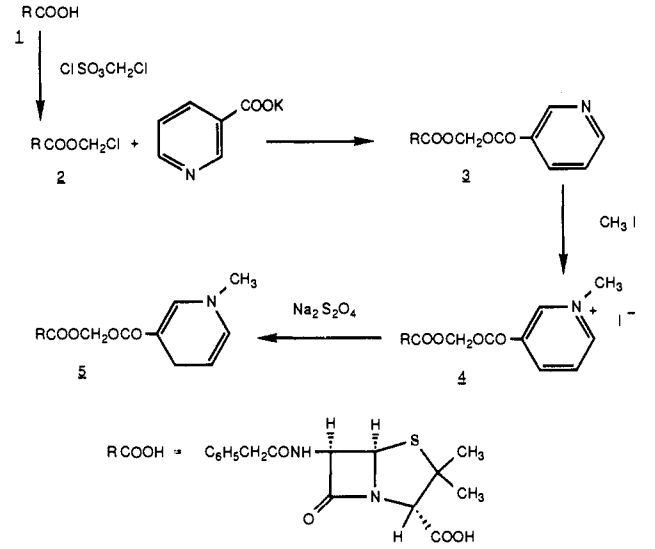
Scheme I



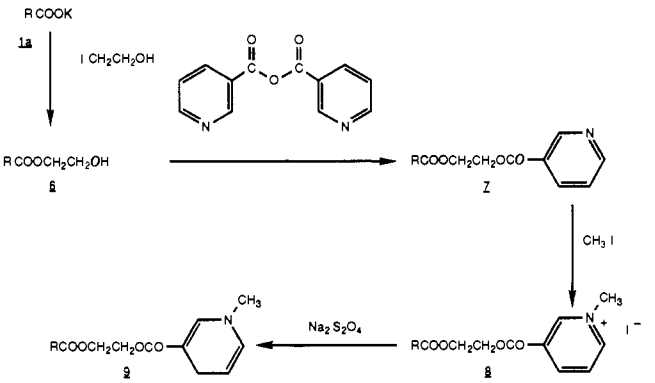
β-lactam antibiotics may open new perspectives for the brain delivery of several important penicillins or cephalosporins. It is well-known that, in spite of the discovery of a large variety of new antibiotics, the "classical" benzylpenicillin and ampicillin are still drugs of choice; although their brain penetration is rather poor, their use is justified by their low toxicity and by the low minimum inhibitory concentration (MIC). The purpose of this work was to apply the dihydropyridine-based CDS to benzylpenicillin.

Benzylpenicillin (penicillin G) is effective against pneumococci, β-hemolytic streptococci group B, and penicillinase-negative staphilococci.[7] The toxicity of benzylpenicillin is low, neurotoxic reactions being reported only when induced experimentally[24] by direct application of the drug on or into brain tissues, or in patients given excessive doses in relation to their renal function.[25,26] Benzylpenicillin has a relatively acidic 3-carboxylic group, its pK_a of 2.6 resulting in a high degree of ionization at physiological pH. Like most other β-lactam antibiotics it is also nonlipophilic ($\log_{10} P = 1.76$). The combination of low lipid solubility and high ionization at plasma and CSF pH constitute the reasons for the poor ability of benzylpenicillin to penetrate intact meninges.[1,27,28] In man penetration of benzylpenicillin into CSF is very limited, in the absence of meningeal inflammation, varying from unmeasurable concentrations to a CSF/plasma ratio of 1–2%.[29,31,32] Higher levels are obtained in the case of inflamed meninges.[30,33] In children the situation is similar.[34] By analysis of human brain tissues[2] after iv administration of a 2 g dose (average blood concentration 7.5 μg/mL) the average brain concentration was only 0.32 μg/g. The elimination by an active transport mechanism via the choroid plexus of benzylpenicillin was also studied;[29,35] in dogs a half-life of 25 min was found. Increased concentrations of the drug resulted in a prolongation of the half-life. Inflammation of the meninges induced by bacterial infection resulted in a diminished transport capacity of the choroid plexus.[36]

Scheme II



Scheme III



As mentioned, the application of the prodrug approach to benzylpenicillin[37,38] had other purposes than its brain delivery improvement. It consisted of the synthesis of and experimentation with various types of esters, such as simple alkyl and aryl[39-45] aminoalkyl[45-47] double esters like

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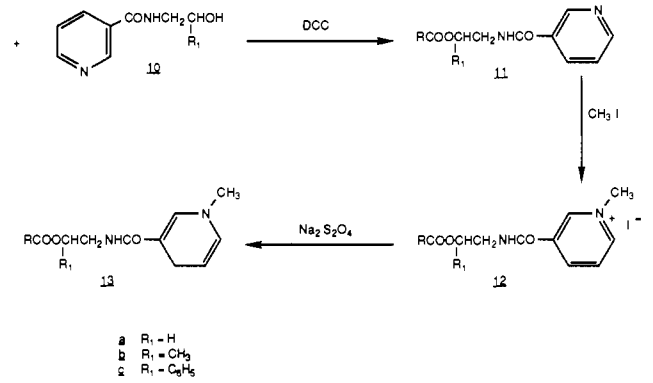
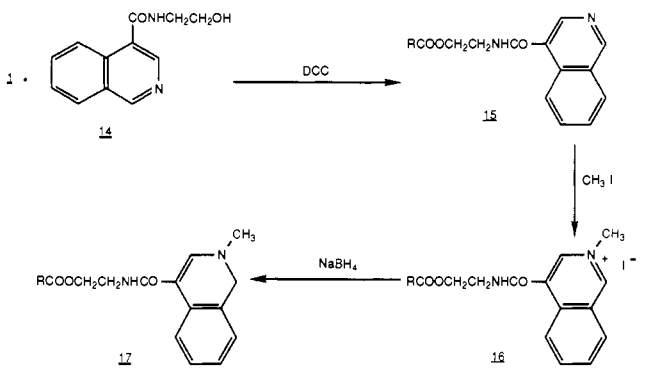
Scheme IV



Scheme V



(acyloxy)alkyl or [[(alkyloxy)carbonyl]oxy]alkyl,[48,49] and the results were a better absorption and a prolonged activity of the drug. The aim of the present investigation was to apply the dihydropyridine ⇄ pyridinium salt type CDS to the benzylpenicillin with the purpose of improving both its CNS penetration and half-life in brain.

The principle of the CDS is illustrated in Scheme I. The pyridine moiety is attached to the carboxylic group of the benzylpenicillin (BP) (mainly responsible for its polar, nonlipophilic character) through a certain linkage, and then by N-alkylation the quaternary salt form BP–Q^+ is obtained. By reducing the pyridinium salt, the corresponding dihydropyridines are obtained, which are the BP–CDS's. After systemic administration the lipophilic CDS should distribute into CNS as well as into other peripheral compartments. The BP–CDS is expected to be oxidized to BP–Q^+ in the CNS as well as in the periphery. In the CNS, BP–Q^+ is "locked in" ($k_{el1} < k_{cleavage} \ll k_{ox1}$) while it is lost rapidly from the periphery ($k_{el4} \gg k_{cleavage2}$). By enzymatic hydrolysis, BP–Q^+ slowly releases BP at the needed site. A portion of the quaternary salt is eliminated by active transport, but it is expected that BP–Q^+ will serve as a continuous source of BP. The carrier itself, Q^+, being a small molecule, can be easily eliminated from the brain and periphery ($k_{el1} < k_{el3}$) together with other small molecules resulting during hydrolysis, such as CH_2O.

A much improved CNS penetration of the drug (the BP–CDS being much more lipophilic than BP) and a prolonged release of the benzylpenicillin, due to the "lock-in" effect, are expected.

Results and Discussion

Several CDS's for benzylpenicillin were synthesized according to Schemes II–V: **5** and **9** are pyridine (carbonyloxy)alkyl or diol diester type, in which one hydroxyl group is esterified by the benzylpenicillin-3-carboxylic group and the other one by a dihydropyridine-3-carboxylic acid derivative; **13** and **17** are pyridine (or isoquinoline) carbamoylalkyl esters or penicillin esters of amino alcohols having the amino group acylated by a dihydropyridine-3-carboxylic or dihydroisoquinoline-4-carboxylic acid derivative.

The chloromethyl ester of benzylpenicillin (**2**) was prepared by the method of Binderup and Hansen[50] using chloromethyl chlorosulfate as chloromethylating agent and a phase-transfer catalyst, tetrabutylammonium hydrogen sulfate, in water–methylene chloride media. The pyridine-3-carboxylic acid methyl ester **3** was obtained by reacting **2** with a pyridine-3-carboxylic acid salt (potassium, TEA) in DMF. The N-alkylation in order to obtain the quaternary salt **4** was performed with methyl iodide in nitromethane, and the reduction to the 1,4-dihydropyridine derivative (**5**) was performed by using sodium dithionite as reducing agent in a mixture of ethyl acetate and aqueous sodium bicarbonate.

The hydroxyethyl ester of benzylpenicillin (**6**) was prepared by reacting the potassium salt of benzylpenicillin (**1a**) with iodoethanol in DMF, or the tetrabutylammonium salt in methylene chloride. By esterifying **6** with nicotinic anhydride in pyridine the 3-carboxylic acid ethyl ester **7** was obtained. Its quaternization to **8**, followed by reduction to the CDS **9**, was accomplished in the conditions described above.

In order to prepare the N-substituted nicotinamide derivatives **11**, **1** was reacted with the (hydroxyalkyl)-nicotinamides **10** in acetonitrile, with dicyclohexylcarbodiimide as dehydrating agent and (dimethylamino)pyridine as a catalyst. The nicotinamides **10** were prepared from the respective amino alcohols and ethyl nicotinate by known procedures. The N-alkylations to the quaternary salts **12** and the reduction to the CDS's **13** were performed as in the other cases.

By reaction of **1** with (hydroxyethyl)-4-isoquinolinecarboxamide **14** (obtained from the ethyl ester of the isoquinoline-4-carboxylic acid and ethanolamime) **15** was obtained. The N-alkylation was performed with methyl iodide, and the reduction of the resulting, quaternary salt **16** was achieved with sodium borohydride in absolute ethanol. The 1,2-dihydro derivative was obtained.

In all cases before the N-alkylation step the intermediates were purified by chromatography. In order to avoid side reactions the N-methylations were performed at room temperature (20–25 °C) with a slight excess of alkylating agent. The reductions with sodium dithionite were accomplished by cooling at 0–5 °C in oxygen-free conditions at pH ≃ approximately 7. In the case of **13** the pH of the reaction medium was higher (~8) in order to avoid water addition. The quaternary salts are fairly stable when

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Table I. Second-Order Oxidation Rate Constants (K_o) of the Benzylpenicillin CDS's Determined by Using Ferricyanide

compd	K_o, M^{-1} s^{-1}	r^a
5	0.530	0.9933
9	1.552	0.9987
13a	26.08	0.9989
13b	11.94	0.9999
13c	15.85	0.9989

[a] r = correlation coefficient.

isolated, but the dihydropyridine derivatives are easily oxidized or hydrolyzed and in acid conditions react readily with water.

All compounds were characterized by their elemental analysis and UV and ^1H NMR spectroscopy. The IR spectra showed in all cases the characteristic maxima for the β-lactam ring at ~1780 cm^{-1}. Chromatographic techniques (HPLC, TLC) indicated only one component in each case (if not, chromatographic purification was applied). The dihydropyridine derivatives were proved to be the 1,4 isomers by their typical UV maxima at 360 nm and appropriate ^1H NMR absorbances.[51] The dihydroisoquinoline was proved to be the 1,2-isomer by the same spectroscopic methods, though in this case the UV spectra are very close to those of the corresponding quaternary salts.

All dihydropyridine derivatives and dihydroisoquinoline were oxidized back to the corresponding pyridinium and isoquinolinium salts, respectively, by methanolic silver nitrate solution or H_2O_2 in the presence of catalytic cupric ions.

A ferricyanide oxidation study was performed in order to compare the oxidative stabilities of different CDS's. The oxidations were followed spectrophotometrically by a standard method[59] and will be discussed in detail elsewhere. Table I shows a summary of the results.

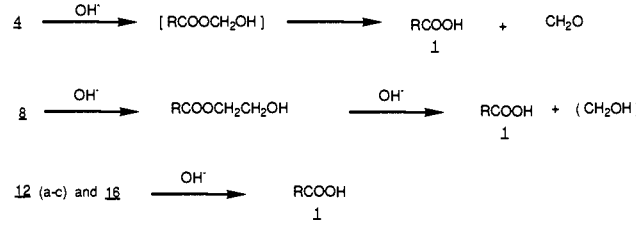
Whereas the "diester" type CDS's **5** and **9** proved to be rather stable toward oxidation, having rate constants K 0.530 and 1.552, respectively, the "amide–esters" **13a–c** were oxidized faster, K = ~12–26. The values of the second-order rate constants situate **5** and **9** among systems that are oxidized slowly in vivo, whereas **13a–c** rank among the more rapidly oxidized species. However, this is based on rather empirical observations, an accurate correlation between the chemical and in vivo, enzymatic oxidation not being proved yet.

Lipophilicity is one of the most important factors in controlling the interaction of drugs with biological systems. Lipophilic character is essential for a CDS to penetrate different barriers of the brain. It was proved that the chromatographic R_m value is related to the partition coefficient between the mobile and stationary phase of a chromatographic system and is correlated with the penetration of substances in biological cells.[53-56] The R_m values were therefore determined to be used as lipophilicity indexes in this study: the relative lipophilicities of the various CDS's (**5**, **9**, **13**, **17**) as well as of the synthetic precursors (**3**, **7**, **11**, **15**) of the quaternary salts were compared to benzylpenicillin. The R_m values [$R_m = \log (1/R_f - 1)$] were measured by means of a reversed-phase TLC method with various concentrations of acetone and water in the mobile phase; the R_m values calculated by extrapolation of the linear part of R_m value curves to a mobile phase of 0% acetone were then used as lipophilic indexes.[57]

Table II. R_m Values (Extrapolated) of Penicillin and Penicillin Dihydropyridine or Dihydroisoquinoline Derivatives

compd	R_m	compd	R_m
1	−0.72	13a	1.98
3	2.24	13b	2.08
5	2.89	13c	2.97
7	2.17	15	2.09
9	2.83	17	2.55
11a	1.54		

Scheme VI



As expected (Table II), the dihydropyridine and dihydroisoquinoline derivatives were much more lipophilic than the benzylpenicillin. The dihydro derivatives were also more lipophilic than their synthetic precursors. The diol diester type CDS's **5** and **9** were more lipophilic than the other type containing both ester and amide bonding (**13**, **16**). By increasing the length of the alkyl bridge between the penicillin and the carrier by one methylene (**9** compared to **5**) or by introducing a branched methyl substituent (**13b** as opposed to **13a**), the lipophilicity was not affected significantly. However, by introducing a phenyl substituent in the same position (**11c**), the lipophilicity was increased. The dihydroisoquinoline CDS **17** was more lipophilic than the respective dihydropyridine **13a**. The lipophilicity of the first hydrolysis product and metabolite of **9**, the hydroxyethyl ester **6**, was considerably lower compared to that of the CDS **9** (index 1.34). That should result in a low rate of its elimination from the brain through the BBB and the possibility of its further hydrolysis to the parent drug while still in brain.

From the R_m values of different CDS's it appears that all of them should be able to penetrate the BBB, the order (most lipophilic → least lipophilic) being **5** > **9** > **16** > **13c** > **13b** > **13a**.

Stability in Buffers. According to the CDS concept described, the oxidized forms, namely, the quaternary pyridinium salts, are expected to release the drug. However, the stabilities of both the quaternary salt forms (**4**, **8**, and **12**) and the CDS's (**5**, **9**, and **13**) were studied toward hydrolysis. In order to predict the transformation pathways in the body and as a stability index for the syntheses, the changes in stability of the compounds due to pH shifts were investigated. The apparent rate constants (K_{app}) were determined under similiar conditions (37 °C, 0.05 M phosphate buffer) for comparing the reactivity of the compounds. The disappearance of the CDS's or the quaternary salt forms was observed by the first-order kinetics in the investigated pH range.

It has been reported in similar cases[52] that the hydrolysis of *gem*-diol diesters, like **4**, should be a two-step process (Scheme VI): the first step, the rate-determining one,

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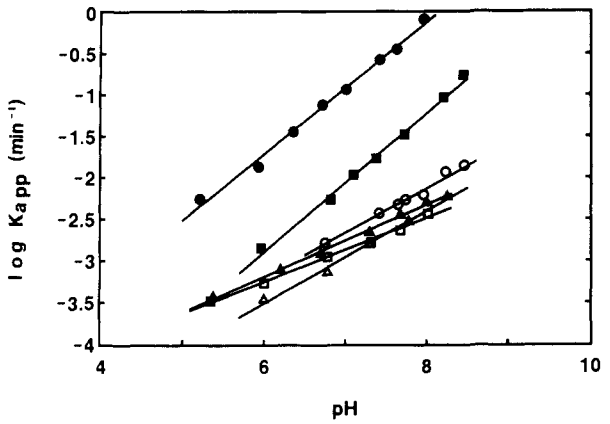

Figure 1. Buffer and pH effects on hydrolysis rate of the quaternary salts at 37 °C (phosphate buffer 0.05 M, μ = 0.15): 4 (●); 8 (■); **12a** (○); **12b** (□); **12c** (▲); 16 (△).

Table III. Stability (Half-Life, min) of CDS's and Related Compounds in pH 7.4 Phosphate Buffer (0.05 M, μ = 0.15 at 37 °C)

	compd	half-life, min
quaternary salts	4	2.7
	8	36.9
	12a	195.6
	12b	363.9
	12c	268.1
	16	377.5
dihydropyridine and dihydroisoquinoline	5	93.0
	9	115.5
	13a	12.8
	13b	25.8
	13c	35.2
	17	990.6
water addition compounds	5–OH	135.7
	9–OH	442.8
	13a–OH	148.3
hydroxyethyl penicillin	6	436.5

should be the hydrolysis of the 1-methylpyridinium-3-carboxylate (trigonelline) ester linkage with formation of the unstable hydroxymethyl ester of benzylpenicillin, which subsequently decomposes spontaneously into penicillin and formaldehyde. The mechanism is probably similar to that suggested for the (acyloxy)methyl esters.[52]

In the case of 8, a glycol diester, the first step is probably the same as above, the hydrolysis of the trigonelline ester linkage. The resulting hydroxyethyl ester of benzylpenicillin (6) is more stable than the hydroxymethyl one, and therefore, it does not decompose spontaneously; it is however hydrolyzed in a second step to benzylpenicillin, in a slower, rate-determining, step. The hydrolysis of **12** (**a**–**c**) and **16** are considered one-step reactions; the ester linkage is hydrolyzed much faster than the amide linkage, resulting in the release of the penicillin.

Figure 1 shows the pH–apparent rate profiles of the quaternary salts. It can be seen that all the ester linkages exhibited base-catalyzed hydrolysis. Compound **4** was the most reactive quaternary salt; it was hydrolyzed to yield quantitatively benzylpenicillin in buffer solutions. The rate of hydrolysis was reduced by increasing the length of the alkyl chain between the two ester linkages (8). At pH 7.4 the rate constant (k, min^{-1}) of **4** was 13.5-fold that of **8** (see Table III). The first-step hydrolysis product of **8**, the hydroxyethyl ester **6**, was quite stable in aqueous buffers (k = 0.0016 min^{-1}) at pH 7.4 but finally hydrolyzed to penicillin. The "amide–esters" **12** were more stable than the diol diesters **4** and **8**. Their hydrolysis rates were about 1/100 compared to **4** and 1/7.5 compared to **8**. By introducing branched alkyl or aryl substituents in the alkyl

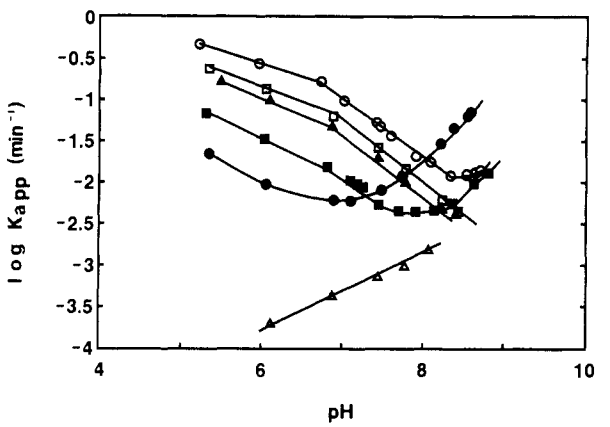

Figure 2. Buffer and pH effects on stability of CDS's at 37 °C (phosphate buffer 0.05 M, μ = 0.15): **5** (●); **9** (■); **13a** (○); **13b** (□); **13c** (▲); **17** (△).

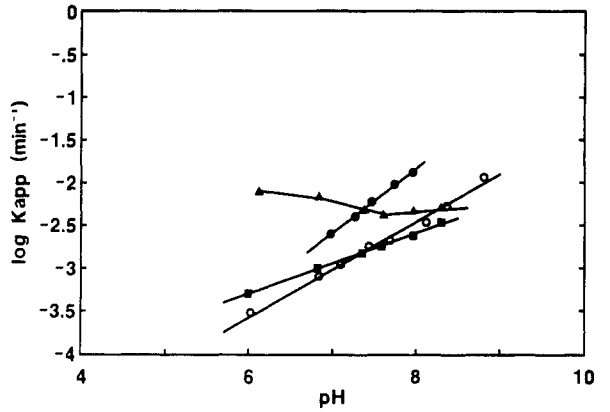

Figure 3. Buffer and pH effects on stability of water addition products of some CDS's and of metabolite **6** at 37 °C (phosphate buffer 0.05 M; μ = 0.15): **5**–OH (●); **9**–OH (■); **13a**–OH (▲); **6** (○).

bridge, near the reaction site (**12b** and **12c**), as expected, the hydrolysis rate was reduced because of steric hindrance. The isoquinolinium quaternary salt **16** was more stable than the pyridinium salts **12a**–**c**.

The stabilities of the CDS's in buffers at various pH's are shown in Figure 2; **5**, **9**, and **13a** disappeared via acid and base catalysis reactions in the pH range studied. However, **13b** and **13c** showed only acid catalysis products and **17** only base catalysis product. In the case of **5a**, **13a**, and **17** the major product of the base-catalyzed hydrolysis was benzylpenicillin. In the case of **9**, as in the case of its quaternary salt form **8**, the first hydrolysis product was the corresponding hydroxyethyl ester, which was then hydrolyzed to benzylpenicillin. In the case of **13b** and **13c**, as a result of steric hindrance and of the increased stability of the dihydropyridine compared to the quaternary salts, no hydrolysis products were identified. The hydration of the 5,6 double bond, characteristic for acid catalysis, took place with **13b** and **13c** and partially with **13a** even at higher pH's than 7.4. The water addition products, the 6-hydroxy-1,4,5,6-tetrahydropyridine derivatives, were identified by their characteristic UV absorption wavelengths at ~290 nm. Although the hydration has an acid-catalyzed mechanism consisting in the first-step protonation of C-5, followed by the nucleophilic attack (by OH^-) at C-6, it is not unusual for this reaction to take place at higher pH's, depending on the substituents present in the pyridine 1- or 3-positions.[58] As expected, the hydra-

(58) Norris, D. J.; Stewart, R. *Can. J. Chem.* **1977**, *55*, 1687–1695.

tion products were obtained in acid conditions in the case of **5** and **9**, but not in the case of the dihydroisoquinoline derivative **17**. This should be an advantage of using dihydroisoquinoline type CDS's.

Figure 3 shows the stabilities of some of the hydration products and of **6**, the first hydrolysis product of **8** (or **9**), at different pH's. It is obvious that they all are much more stable than the respective quaternary salts and dihydro derivatives in the case of "diester" type **5** and **9**. In the case of **13a** the hydration product was less stable than the quaternary salt but more stable than the dihydro derivative (in this case at pH 7.4 not the hydrolysis but the water addition occurred). Compound **6** was stable in acid and neutral conditions, being slowly hydrolyzed at alkaline pH. The irreversible formation of water addition compounds, very predominant in the "amide–esters" **13**, besides lowering the lipophilicity has the disadvantage of preventing oxidation to the quaternary ion form and the "lock-in", prolonged effect.

Conclusions

The study shows that the lipophilicities of the CDS's are much superior to those of the parent drug. This property could permit a better penetration of the drug through the biological membranes. The stabilities of the diol "diester" type CDS's at physiological pH's are superior to those of the "amide–ester" types; this instability is a result of hydration and not of hydrolysis. The isoquinoline type CDS is more stable at physiological pH than the pyridine ones. The CDS's are oxidized to the quaternary ion forms under the influence of oxidizing agents. Both CDS's and quaternary forms released benzylpenicillin by hydrolysis.

On the basis of their physical properties **5**, **9**, and **17** (and to some extent **13a**) seem to be suitable for further studies including in vivo distribution studies.

Experimental Section

Uncorrected melting points determined on an electrothermal 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 (NMR) were recorded on a Varian XL 200 (200-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. Benzylpenicillin was obtained from Fluka A.G.

Chloromethyl [2*S*-(2α,5α,6β)]-3,3-dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (2) was prepared by the method of Binderup and Hansen.[50]

[(3-Pyridinylcarbonyl)oxy]methyl [2*S*-(2α,5α,6β)]-3,3-Dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (3). A suspension of 3.83 g (0.01 mol) of chloromethyl ester **2** and 1.93 g (0.012 mol) of potassium pyridine-3-carboxylate in 100 mL of DMF was stirred at 20–25 °C for 6 days. Ethyl acetate (300 mL) was added, and the solid was filtered off. The solution was extracted with concentrated aqueous NaCl four times and then dried over $MgSO_4$. The solvent was removed in vacuo, and the resultant foamy solid (4.5 g) was purified by chromatography (135 g of silica gel, Davisil, grade 634, 100–200-mesh, 60 Å, eluent EtOAc), giving 2.5 g of **3**: mp 127–130 °C; UV (MeOH) 214, 266 nm; ^1H NMR ($CDCl_3$) δ 1.44 (s, 6 H, CH_3), 3.63 (s, 2 H, CH_2), 4.42 (s, 1 H, C-2 proton), 5.51 (d, 1 H, J = 4.3, C-5 proton), 5.66 (q, 1 H, J = 4.4, C-6 protons), 6.06 (q, 2 H, J = 5.6, CH_2), 6.21 (d, 1 H, J = 8.8, NH), 7.23–7.65 (m, 6 H, C_6H_5, pyridine C-5 protons), 8.32 (d, 1 H, J = 7.9, pyridine C-4 proton), 8.83 (d, 1 H, J = 3.2, pyridine C-6 proton), 9.24 (s, 1 H, pyridine C-2 proton). Anal. ($C_{23}H_{23}N_3O_6S$) C, H, N, S.

[2*S*-(2α,5α,6β)]-3-[[[[[3,3-Dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]hept-2-yl]carbonyl]oxy]methoxy]carbonyl]-1-methylpyridinium Iodide (4). A solution of 2.5 g (0.053 mol) of **3** in 100 mL of dry nitromethane was reacted with 2.25 g (1 mL, 0.016 mol) of methyl iodide, in a closed system at 20–25 °C for 6 days (TLC showed complete reaction). The solvent was removed in vacuo, and the solid residue was slurried with ether, filtered, and dried in vacuo over P_2O_5, giving 2.91 g of a yellow solid: mp 90–95 °C dec; UV (MeOH) 200, 268 nm; ^1H NMR (DMSO-d_6) δ 1.43 (s, 3 H, CH_3), 1.63 (s, 3 H, CH_3), 3.54 (s, 2 H, CH_2), 4.45 (s, 3 H, N^+–CH_3), 4.50 (s, 1 H, C-2 proton), 5.51–5.53 (m, 2 H, C-5, C-6 protons), 6.15 (s, 2 H, CH_2), 7.27 (s, 5 H, C_6H_5), 8.30 (t, 1 H, pyridinium C-5 proton), 8.99 (d, 1 H, J = 8.3, pyridinium C-4 proton), 9.24 (d, 1 H, J = 6.1, pyridinium C-6 proton), 9.61 (s, 1 H, pyridinium C-2 proton). Anal. ($C_{24}H_{26}IN_3O_6S\cdot H_2O$) C, H, I, N, S.

[[(1,4-Dihydro-1-methyl-3-pyridinyl)carbonyl]oxy]methyl [2*S*-(2α,5α,6β)]-3,3-Dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (5). A solution of 3.25 g (0.0053 mol) of the quaternary salt 4 dissolved in a mixture of 350 mL of water and 150 mL of EtOAc was cooled at 0–5 °C and deaerated with nitrogen. A mixture of 2.67 g (0.032 mol) of $NaHCO_3$ and 3.69 g (0.021 mol) of $Na_2S_2O_4$ 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 2 × 50 mL of EtOAc, and the combined organics were washed with 2 × 30 mL of cold deaerated water. After drying over Na_2SO_4, solvent was removed in vacuo, giving 1.7 g of a yellow solid: mp 98–100 °C; UV (MeOH) 220, 362 nm; ^1H NMR ($CDCl_3$) δ 1.44 (s, 6 H, CH_3), 2.95 (s, 3 H, N-CH_3), 3.06 (s, 2 H, pyridine C-2 proton), 3.63 (s, 2 H, CH_2), 4.38 (s, 1 H, C-2 proton), 4.80–4.84 (m, 1 H, pyridine C-5 proton), 5.50 (d, 1 H, J = 4.3, C-5 proton), 5.61–5.67 (m, 2 H, C-6, pyridine C-6 protons), 5.83 (q, 2 H, J = 5.6, CH_2), 6.25 (d, 1 H, J = 8.8, NH), 7.05 (s, 1 H, pyridine C-2 proton), 7.31 (m, 5 H, C_6H_5). Anal. ($C_{24}H_{27}N_3O_6S\cdot 1.5H_2O$) C, H, N, S.

2-Hydroxyethyl [2*S*-(2α,5α,6β)]-3,3-Dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (6). Potassium benzylpenicillin (3.72 g, 0.01 mol) suspended in 10 mL of dry DMF was reacted with 2.65 g (1.2 mL, 0.015 mol) of 2-iodoethanol by stirring under dry conditions for 24 h at 20–25 °C. Ethyl acetate (50 mL) was added, and the solid was filtered off. The solution was extracted with 3 × 25 mL of cool aqueous saturated NaCl, dried over $MgSO_4$, and filtered through Celite. After removal of the solvent in vacuo 2.65 g of a colorless oil was obtained and was used immediately in the next step of the synthesis: ^1H NMR ($CDCl_3$) δ 1.45 (s, 6 H, CH_3), 3.15 (t, 2 H, CH_2), 3.61 (s, 2 H, CH_2), 3.71 (t, 2 H, CH_2), 4.45 (s, 1 H, C-2 proton), 5.45–5.65 (m, 3 H, C-5, C-6, OH protons), 7.30 (s, 5 H, C_6H_5), 7.4 (d, 1 H, J = 8, NH).

2-[(3-Pyridinylcarbonyl)oxy]ethyl [2*S*-(2α,5α,6β)]-3,3-Dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (7). To 3 g (0.0079 mol) of hydroxyethyl benzylpenicillinate (6) dissolved in 40 mL of dry pyridine with cooling at 0–5 °C and stirring was added 2.7 g (0.019 mol) of freshly prepared nicotinic anhydride. The mixture was stirred for 4 h at 0–5 °C and then poured into 2 L of ice water. The solid was filtered off and dissolved in 50 mL of methylene chloride. The solution was washed with 3 × 25 mL of water and dried over $MgSO_4$. After removal of the solvent in vacuo 3 g of a foamy solid resulted. Purification was achieved by eluting through a silica gel column, using as eluent EtOAc, to give 2 g of pure **7**: mp 53–56 °C dec; ^1H NMR ($CDCl_3$) δ 1.44 (s, 6 H, CH_3), 3.60 (s, 2 H, CH_2), 4.44 (s, 1 H, C-2 proton), 4.55 [s, 4 H, $(CH_2)_2$], 5.50 (d, 1 H, J = 4.3, C-5 proton), 5.65 (dd, 1 H, J = 4.4, C-6 proton), 6.60 (d, 1 H, J = 8.7, NH), 7.31 (s, 5 H, C_6H_5), 7.35–7.65 (m, 1 H, pyridine C-5 proton), 8.32 (d, 1 H, J = 7.9, pyridine C-4 proton), 8.84 (d, 1 H, J = 3.2, pyridine C-4 proton), 9.25 (s, 1 H, pyridine C-2 proton). Anal. ($C_{24}H_{25}N_3O_6S\cdot 0.75H_2O$) C, H, N, S.

[2*S*-(2α,5α,6β)]-3-[[2-[[[3,3-Dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]hept-2-yl]carbonyl]oxy]ethoxy]carbonyl]-1-methylpyridinium Iodide (8). Compound **7** (4 g, 0.0083 mol) in 120 mL of nitromethane was methylated with 4.1 g (1.8 mL, 0.029 mol) of methyl iodide,

giving 5 g of a yellow solid: mp 95–100 °C dec; UV (MeOH) 224, 266 nm; ^{1}H NMR (DMSO-d_6) δ 1.45 (s, 3 H, CH_3), 1.60 (s, 3 H, CH_3), 3.52 (s, 2 H, CH_2), 4.45 [s, 5 H, $(CH_2)_2$, C-2 proton], 4.55 (s, 3 H, N^+–CH_3), 5.45–5.62 (m, 2 H, C-5, C-6 protons), 7.31 (s, 5 H, C_6H_5), 8.30 (t, 1 H, pyridinium C-5 proton), 8.85 (d, 1 H, J = 8.2, pyridinium C-4 proton), 9.25 (d, 1 H, J = 6, pyridinium C-6 proton), 9.55 (s, 1 H, pyridinium C-2 proton). Anal. ($C_{25}H_{28}IN_3O_6S·H_2O$) C, H, I, N, S.

2-[[(1,4-Dihydro-1-methyl-3-pyridinyl)carbonyl]oxy]ethyl [2*S*-(2α,5α,6β)]-3,3-Dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (9). By reduction of 1.89 g (0.003 mol) of **8**, in 240 mL of water and 150 mL of EtOAc with 1.51 g (0.018 mol) of $NaHCO_3$ and 2.1 g (0.012 mol) of $Na_2S_2O_4$ over 40 min as above, 1.25 g of **9** was obtained as a yellow solid: mp 90–95 °C dec; UV (MeOH) 224, 360 nm; ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 6 H, CH_3), 2.95 (s, 3 H, N-CH_3), 3.08 (s, 2 H, pyridine C-4 proton), 3.65 (s, 2 H, CH_2), 4.42 [s, 4 H, $(CH_2)_2$], 4.45 (s, 1 H, C-2 proton), 4.75–4.85 (m, 1 H, pyridine C-5 proton), 5.45–5.8 (m, 3 H, pyridine C-6, C-5, C-6 protons), 6.45 (d, 1 H, J = 8.5, NH), 7.05 (s, 1 H, pyridine C-2 protons), 7.31 (s, 5 H, C_6H_5). Anal. ($C_{25}H_{29}N_3O_6S·H_2O$) C, H, N, S.

2-[(3-Pyridinylcarbonyl)amino]ethyl [2*S*-(2α,5α,6β)]-3,3-Dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (11a). Benzylpenicillin (3.1 g, 0.0093 mol) prepared from its potassium salt, dissolved in 150 mL of dry acetonitrile, was reacted with 1.55 g (0.0097 mol) of (hydroxyethyl)nicotinamide in the presence of 2 g (0.0097 mol) of dicyclohexylcarbodiimide and 0.1 g of (dimethylamino)pyridine, by stirring for 4 days at 20–25 °C under dry conditions. The resulting solid (dicyclohexylurea) was filtered off, and the solvent was removed in vacuo. The residue was dissolved in methylene chloride (50 mL) and the resulting solution extracted with cold 2% aqueous $NaHCO_3$ and then with water. After drying over $MgSO_4$ and removing the solvent in vacuo, 4.7 g of an oily residue resulted. Purification was achieved by passing it through a column packed with 140 g of silica gel, using as eluent EtOAc, giving 3.1 g of a white solid: mp 112–115 °C dec; UV (MeOH) 222, 260 nm; ^{1}H NMR ($CDCl_3$) δ 1.40 (s, 6 H, CH_3), 3.61 (s, 2 H, CH_2), 3.73 (d, 2 H, J = 5.1, CH_2), 4.27–4.45 (m, 3 H, CH_2, C-2 protons), 5.49 (d, 1 H, J = 4.2, C-5 proton), 5.57 (q, 1 H, J = 8.7, C-6 proton), 6.58 (d, 1 H, J = 8.8, NH), 7.21–7.37 (m, 6 H, C_6H_5, pyridine C-5 protons), 7.58 (t, 1 H, NH), 8.14 (d, 1 H, J = 7.9, pyridine C-4 proton), 8.67 (d, 1 H, J = 4.8, pyridine C-6 proton), 9.01 (s, 1 H, pyridine C-2 proton). Anal. ($C_{24}H_{26}N_4O_5S·0.5H_2O$) C, H, N, S.

1-Methyl-2-[(3-pyridinylcarbonyl)amino]ethyl [2*S*-(2α,5α,6β)]-3,3-Dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (11b). In the same way as described above 3.3 g (0.01 mol) of benzylpenicillin, 1.98 g (0.011 mol) of *N*-(2-hydroxy-1-propyl)-3-nicotinamide, 2.27 g (0.011 mol) of dicyclohexylcarbodiimide, and 0.1 g of (dimethylamino)pyridine in 150 mL of acetonitrile reacted for 60 h at 20–25 °C to give 2.85 g of **11b**: mp 97–100 °C dec; UV (MeOH) 216; ^{1}H NMR ($CDCl_3$) δ 1.31–1.39 (m, 9 H, CH_3), 3.61 (s, 2 H, CH_2), 3.45–3.80 (m, 1 H, CH_2), 4.33 (d, 1 H, J = 6.6, C-2 proton), 5.10–5.25 (m, 1 H, CH), 5.55–5.62 (m, 2 H, C-5, C-6 protons), 6.54 (d, 1 H, J = 8.9, NH), 7.21–7.43 (m, 7 H, C_6H_5, NH, pyridine C-5 protons), 8.13 (d, 1 H, J = 7.5, pyridine C-4 proton), 8.67 (d, 1 H, J = 4.9, pyridine C-6 proton), 8.99 (s, 1 H, pyridine C-2 proton). Anal. ($C_{25}H_{28}N_4O_5S$) C, H, N, S.

1-Phenyl-2-[(3-pyridinylcarbonyl)amino]ethyl [2*S*-(2α,5α,6β)]-3,3-Dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (11c). From 1.65 g (0.0049 mol) of benzylpenicillin, 1.32 g (0.0054 mol) of *N*-(2-hydroxy-2-phenylethyl)-3-nicotinamide, 1.11 g (0.0054 mol) of dicyclohexycarbodiimide, and 0.05 g of (dimethylamino)pyridine in 75 mL of acetonitrile, reacted for 4 days at 20–25 °C, was obtained 2 g of **11c** as a white solid: mp 87–90 °C dec; UV (MeOH) 220 nm; ^{1}H NMR ($CDCl_3$) δ 1.27 (s, 3 H, CH_3), 1.34 (s, 3 H, CH_3), 3.60 (s, 2 H, CH_2), 3.70–3.95 (m, 2 H, CH_2), 4.38 (d, 1 H, J = 4.6, C-2 proton), 5.54–5.62 (m, 2 H, C-5, C-6 protons), 6.00–6.12 (m, 1 H, CH), 6.64 (d, 1 H, J = 8, NH), 7.21–7.36 (m, 11 H, C_6H_5, NH), 7.49–7.55 (m, 1 H, pyridine C-5 proton), 8.07 (d, 1 H, J = 7.5, pyridine C-4 proton), 8.64 (d, 1 H, J = 4, pyridine C-6 proton), 8.94 (s, 1 H, pyridine C-2 proton). Anal. ($C_{30}H_{30}N_4O_5S·0.5H_2O$) C, H, N, S.

[2*S*-(2α,5α,6β)]-3-[[[2-[[[3,3-Dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]hept-2-yl]carbonyl]oxy]ethyl]amino]carbonyl]-1-methylpyridinium Iodide (12a). A solution of **2** g (0.0041 mol) of **11a** in 100 mL of nitromethane was alkylated in the usual manner with 1.82 g (0.8 mL, 0.013 mol) of methyl iodide for 4 days to give 2.5 g of a yellow solid: mp 115–118 °C dec; UV (MeOH) 222, 270 nm; ^{1}H NMR (DMSO-d_6) δ 1.39 (s, 3 H, CH_3), 1.60 (s, 3 H, CH_3), 3.55 (s, 2 H, CH_2), 3.66 (d, 2 H, J = 4.3, CH_2), 4.30–4.43 (m, 6 H, CH_2, N^+–CH_3, C-2 protons), 5.46–5.51 (m, 2 H, C-5, C-6 protons), 7.27 (s, 5 H, C_6H_5), 8.78 (t, 1 H, pyridinium C-5 proton), 8.90 (t, 1 H, pyridinium C-4 proton), 9.14 (d, 1 H, J = 5.7, NH), 9.27 (t, 1 H, NH), 9.39 (s, 1 H, pyridinium C-2 proton). Anal. ($C_{25}H_{29}IN_4O_5S·0.5H_2O$) C, H, N, S.

[(2*S*-(2α,5α,6β)]-3-[[[2-[[[3,3-Dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]hept-2-yl]carbonyl]oxy]propyl]amino]carbonyl]-1-methylpyridinium Iodide (12b). Compound **11b** (0.5 g, 0.001 mol) in 25 mL of nitromethane was alkylated with 0.45 g (0.2 mL, 0.003 mol) of methyl iodide, for 6 days at 20–25 °C, giving 0.6 g of a yellow solid: mp 90–95 °C dec; UV (MeOH) 222, 270 nm; ^{1}H NMR (DMSO-d_6) δ 1.39–1.65 (m, 9 H, CH_3), 3.45–3.62 (m, 4 H, CH_2), 4.45 (s, 3 H, N^+–CH_3), 4.50 (s, 1 H, C-2 proton), 5.05–5.20 (m, 1 H, CH), 5.45–5.51 (m, 2 H, C-5, C-6 protons), 7.28 (s, 5 H, C_6H_5), 8.30 (t, 1 H, pyridinium C-5 proton), 8.90 (t, 1 H, pyridinium C-4 proton), 9.18 (d, 1 H, J = 5.8, pyridinium C-6 proton), 9.30 (t, 1 H, NH), 9.45 (s, 1 H, pyridinium C-2 proton). Anal. ($C_{26}H_{31}IN_4S·0.5H_2O$) C, N, I, N, S.

[2*S*-(2α,5α,6β)]-3-[[[2-[[[3,3-Dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]hept-2-yl]carbonyl]oxy]-2-phenylethyl]amino]carbonyl]-1-methylpyridinium Iodide (12c). From 0.56 g (0.001 mol) of **11c** in 25 mL of nitromethane reacted with 0.45 g (0.2 mL, 0.003 mol) of methyl iodide for 7 days at 20–25 °C, 0.6 g of yellow powder was obtained: mp 140–145 °C; UV (MeOH) 224, 270 nm; ^{1}H NMR (DMSO-d_6) δ 1.25 (s, 3 H, CH_3), 1.59 (s, 3 H, CH_3), 3.55 (s, 2 H, CH_2), 3.85 (m, 2 H, CH_2), 4.41 (s, 3 H, N^+–CH_3), 4.43 (s, 1 H, CH_2), 5.40–5.58 (m, 2 H, C-5, C-6 protons), 5.95–6.06 (m, 1 H, CH), 7.27 (s, 5 H, C_6H_5), 7.36–7.48 (m, 5 H, C_6H_5), 8.29 (t, 1 H, pyridinium C-5 proton), 8.71–8.95 (m, 2 H, NH, pyridinium C-4 protons), 9.14 (d, 1 H, J = 5.3, pyridinium C-6 proton), 9.37 (s, 1 H, pyridinium C-2 proton). Anal. ($C_{31}H_{33}IN_4O_5S·0.5H_2O$) C, H, I, N, S.

2-[[(1,4-Dihydro-1-methyl-3-pyridinyl)carbonyl]amino]ethyl [2*S*-(2α,5α,6β)]-3,3-Dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (13a). Compound **12a** (2.4 g, 0.0038 mol) in 300 mL of water and 200 mL of EtOAc was reduced with 1.92 g (0.023 mol) of $NaHCO_3$ and 2.58 g (0.015 mol) of $Na_2S_2O_4$ over 60 min. (The pH was maintained basic by addition of $NaHCO_3$.) Compound **13a** (1.5 g) was obtained as a light yellow solid: mp 85–87 °C dec; UV (MeOH) 218, 356 nm; ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 6 H, CH_3), 2.91 (s, 3 H, N-CH_3), 3.07 (s, 2 H, pyridine C-4 protons), 3.57 (s, 2 H, CH_2), 3.61 (s, 2 H, CH_2), 4.25 (d, 2 H, J = 3.5, CH_2), 4.38 (s, 1 H, C-2 proton), 4.68–4.72 (m, 1 H, pyridine C-5 proton), 5.50 (d, 1 H, J = 4.1 pyridine C-4 proton), 5.63–5.70 (m, 3 H, NH, C-6, pyridine C-6 protons), 6.55 (d, 1 H, J = 8.9, NH), 6.97 (s, 1 H, pyridine C-2 proton), 7.31 (d, 5 H, J = 6.1, C_6H_5). Anal. ($C_{25}H_{30}N_4O_5S·0.5H_2O$) C, H, N, S.

2-[[(1,4-Dihydro-1-methyl-3-pyridinyl)carbonyl]amino]-1-methylethyl [2*S*-(2α,5α,6β)]-3,3-Dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (13b). Compound **12b** (0.25 g, 0.0004 mol) in 15 mL of EtOAc and 20 mL of water was reduced with 0.23 g (0.0027 mol) of $NaHCO_3$ and 0.28 g (0.0016 mol) of $Na_2S_2O_4$ over 60 min to give 0.1 g of a light yellow solid: mp 70–73 °C dec; UV (MeOH) 224, 356 nm; ^{1}H NMR ($CDCl_3$) δ 1.38–149 (m, 9 H, CH_3), 2.90 (s, 3 H, N-CH_3), 3.05 (s, 2 H, pyridine C-4 protons), 3.65 (m, 4 H, CH_2), 4.35 (s, 1 H, C-2 proton), 4.75–4.95 (m, 2 H, CH, pyridine C-5 proton), 5.48 (d, 1 H, J = 4, C-5 proton), 5.50–5.68 (m, 3 H, NH, C-6, pyridine C-6 proton), 6.71 (d, 1 H, J = 8.8, NH), 7.01 (s, 1 H, pyridine C-2 proton), 7.30 (d, 5 H, J = 5.9, C_6H_5). Anal. ($C_{26}N_{32}N_4O_5·0.5H_2O$) C, H, N, S.

2-[[(1,4-Dihydro-1-methyl-3-pyridinyl)carbonyl]amino]-1-phenylethyl [2*S*-(2α,5α,6β)]-3,3-Dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (13c). As described above, **12c** (0.41 g, 0.0006 mol)

in 15 mL of EtOAc and 30 mL of water was reduced with 0.35 g (0.0041 mol) of $NaHCO_3$ and 0.42 g (0.0024 mol) of $Na_2S_2O_4$ in 60 min to give 0.2 g of yellow solid: mp 104–106 °C dec; UV (MeOH) 212, 356 nm; ^1H NMR ($CDCl_3$) δ 1.35 (s, 6 H, CH_3), 2.89 (s, 3 H, N-CH_3), 3.05 (s, 2 H, pyridine C-4 protons), 3.56 (s, 2 H, CH_2), 3.85–4.00 (m, 2 H, CH_2), 4.36 (s, 1 H, C-2 proton), 4.65–4.79 (m, 1 H, pyridine C-5 proton), 5.48–5.71 (m, 4 H, C-5, C-6, NH pyridine C-6 proton), 6.70 (d, 1 H, J = 8.6, NH), 7.05 (s, 1 H, pyridine C-2 proton), 7.20–7.35 (m, 10 H, C_6H_5). Anal. ($C_{31}H_{34}N_4O_5S\cdot 2H_2O$) C, H, N, S.

2-[(4-Isoquinolinylcarbonyl)amino]ethyl [2*S*-(2α,5α,6β)]-3,3-Dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (15). Benzylpenicillin (1.78 g, 0.0053 mol) dissolved in 60 mL of dry acetonitrile was reacted with 1.15 g (0.0053 mol) of *N*-(hydroxyethyl)-4-isoquinolinecarboxamide in the presence of 1.09 g (0.0053 mol) of dicyclohexylcarbodiimide and 0.4 g of (dimethylamino)pyridine by stirring for 3 days at 20–25 °C in dry conditions. The resulting solid was filtered off, and the solvent was removed in vacuo. The residue was purified by column chromatography on silica gel using EtOAc as eluent to give 1.74 g of white solid: mp 85–90 °C dec; UV (MeOH) 214, 280, 324 nm; ^1H NMR ($CDCl_3$) δ 1.36 (s, 3 H, CH_3), 1.42 (s, 3 H, CH_3), 3.58 (s, 2 H, CH_2), 3.81 (d, 2 H, J = 5, CH_2), 4.35–4.57 (m, 3 H, CH_2, C-2 proton), 5.48–5.53 (m, 2 H, C-5, C-6 protons), 6.39 (d, 1 H, J = 8.7, NH), 7.17–7.37 (m, 6 H, C_6H_5, NH proton), 7.64 (t, 1 H, isoquinoline C-7 proton), 7.76 (t, 1 H, isoquinoline C-6 proton), 7.96 (d, 1 H, J = 6.6, isoquinoline C-8 proton), 8.33 (d, 1 H, J = 6.5, isoquinoline C-5 proton), 8.59 (s, H, isoquinoline C-1 proton), 9.18 (s, 1 H, isoquinoline C-3 proton). Anal. ($C_{28}H_{28}N_4O_5S\cdot 0.5H_2O$) C, H, N, S.

[2*S*-(2α,5α,6β)]-4-[[[2-[[[3,3-Dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]hept-2-yl]carbonyl]oxy]ethyl]amino]carbonyl]-2-methylisoquinolinium Iodide (16). One gram (0.0019 mol) of **15** in 30 mL of nitromethane was alkylated with 0.9 g (0.4 mL, 0.006 mol) of methyl iodide for 6 days, giving 1.15 g of a yellow solid: mp 105–110 °C dec; UV (MeOH) 226, 276, 338 nm; ^1H NMR (DMSO-d_6) δ 1.62 (s, 3 H, CH_3), 3.41 (s, 2 H, CH_2), 3.72 (d, 2 H, J = 5, CH_2), 4.37–4.48 (m, 6 H, N^+–CH_3, CH_2, C-2 protons), 5.53 (m, 2 H, C-58 C-6 protons), 7.26 (s, 5 H, C_6H_5), 8.13 (t, 1 H, isoquinolinium C-7 proton), 8.30 (t, 1 H, isoquinolinium C-6 proton), 8.43 (d, 1 H, J = 8.5, isoquinolinium C-8 proton), 8.56 (d, 1 H, J = 8, isoquinolinium C-5 proton), 8.90 (s, 1 H, isoquinolinium C-1 proton), 9.26 (t, 1 H, NH), 10.12 (s, 1 H, isoquinolinium C-3 proton). Anal. ($C_{29}H_{31}IN_4O_5S\cdot 0.5H_2O$) C, H, N, S.

2-[[(1,2-Dihydro-2-methyl-4-isoquinolinyl)carbonyl]amino]ethyl [2*S*-(2α,5α,6β)]-3,3-Dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (17). A solution of 0.17 g (0.00025 mol) of **16** in 12 mL of absolute ethanol was reduced at 15–20 °C by stirring for 10 min with 0.01 g (0.00025 mol) of sodium borohydride. The colorless solution was concentrated in vacuo, removing the solvent, and the residue was dissolved in a mixture of water/EtOAc, 1:1 (25 mL of each). The layers were separated, the aqueous layer was extracted with EtOAc, and then the combined organics were washed with concentrated aqueous NaCl solution. After drying (Na_2SO_4), the solvent was removed in vacuo and 0.15 g of white product was obtained: mp 80–85 °C dec; UV (MeOH) 214, 276, 340 nm; ^1H NMR ($CDCl_3$) δ 1.35 (s, 3 H, CH_3), 1.43 (s, 3 H, CH_3), 2.85 (s, 3 H, N-CH_3), 3.65 (s, 2 H, CH_2), 3.80 (d, 2 H, J = 5, CH_2), 4.15–4.40 (m, 4 H, CH_2, isoquinoline C-1, C-3 protons), 5.45–5.59 (m, 2 H, C-5, C-6 protons), 6.39 (d, 1 H, J = 7, NH), 7.21 (s, 1 H, isoquinoline C-3 proton), 7.31 (m, 6 H, C_6H_5, NH), 7.60–8.61 (m, 4 H, isoquinoline C-5, C-6, C-7, C-8 protons). Anal. ($C_{29}H_{32}N_4O_5S\cdot H_2O$) C, H, N, S.

Chemical Oxidation Studies. Ferricyanide oxidation kinetic study was performed according to a literature method:[59] oxidations were followed by UV spectroscopy (350 nm) in solutions containing both $Fe(CN)_6^{3-}$ and $Fe(CN)_6^{4-}$ in 20% acetonitrile/water under an oxygen-free atmosphere at 37 °C; pseudo-first-order conditions were used; the concentration of $Fe(CN)_6^{3-}$ was much higher than the concentrations of CDS's; pH was held constant. The second-order rate constants of oxidation (K_o) were determined from the pseudo-first-order rates at different concentrations of $Fe(CN)_6^{3-}$.

Lipophilicity Measurements; Determination of R_m Values. Chromatography was carried out on TLC plates [Baker, Si-C18F 19C, 20 × 20 glass plates precoated with octadecylsilane (C_{18}) reversed phase bonded to silica gel, approximately 20-μm particle size, 200-μm hard surface layer with 254-nm fluorescent indicator and 19 channels each of 8-mm width]. The compounds were dissolved in distilled water or acetone, and 1 μL of a 3 mg/mL solution was applied onto each channel along a line 2 cm above the bottom of the plate in random locations. The mobile phase of 200 mL of water or various concentrations of acetone in water was allowed to run 14 cm from the origin. The developed plates were dried, and the compounds were detected by an ultraviolet illumination. The corresponding R_m values were calculated from the R_f values by means of the equation: $R_m = \log(1/R_f - 1)$. The theoretical values at 0% of acetone in mobile phase were calculated by the least-squares method from the R_m values in the linearity range of the curves, which were plotted as R_m values versus acetone concentrations.

Stability in Buffers. Kinetic Studies. Phosphate buffers of various pH (0.05 M, μ = 0.15) were used as media for kinetic studies. Test compounds in dimethyl sulfoxide stock solutions (8 mM) were prepared fresh. An aliquot (50 μL) of the stock solution was added into 10 mL of 37 °C buffer to initiate the kinetic study. At appropriate time intervals, samples were taken and applied directly to the HPLC system. The pH of the solutions was determined before and after the studies.

Analytical Method. A high-performance liquid chromatography (HPLC) method was developed for quantitative analysis of the various compounds. The HPLC systems consisted of a solvent delivery system (Kontron 410), a variable-wavelength UV detector (LDC spectromonitor D), an autosample (Kontron MSI 66), and a recorder. For benzylpenicillin determination, a Nova-Pak reversed-phase C_{18} column (15 cm × 3.9 mm i.d.) was used. The mobile phase was 12% acetonitrile in 5 mM phosphate buffer (pH 7.0) containing 10 mM tetramethylammonium perchlorate. With a flow rate of 2 mL/min, benzylpenicillin showed a retention time of 4.6 min. For separation of CDS's and related compounds, an ASI reversed-phase C_8 column (30 cm × 3.9 mm i.d.) was used. The mobile phase consisted of various solutions (5–10 mM NaH_2PO_4) at a flow rate of 2 mL/min. The retention times obtained were 3–10 min. Benzylpenicillin was detected at 254 nm; the CDS's were detected at 360 nm and other derivatives at 230 nm.

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