Chemical Delivery Systems for Some Penicillinase-Resistant Semisynthetic Penicillins†

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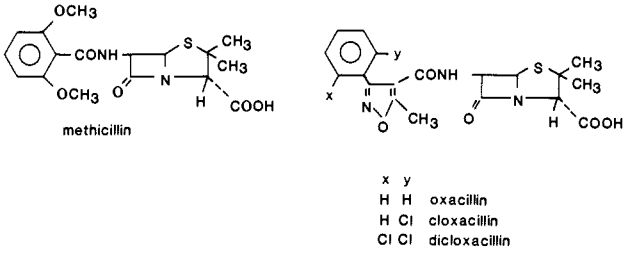
Chemical delivery systems (CDS's) based on a dihydropyridine ⇄ quaternary pyridinium ion redox system analogous to the naturally occurring NADH ⇄ NAD^+ system were synthesized for a group of staphylococcal penicillinase resistant penicillins, including methicillin, oxacillin, cloxacillin, and dicloxacillin, in order to improve their penetration of the central nervous system (CNS). The CDS's are penicillin monoesters of *gem*-diols in which the other hydroxyl group is esterified by the dihydrotrigonelline carrier. The CDS's were found to be much more lipophilic than the parent drugs by comparing their log k' values used as lipophilicity indexes. A study of the chemical oxidation of the CDS's performed by a UV spectrophotometric method showed relatively slow reaction. Stability studies were performed in buffers and different animal tissues for both the CDS's and the quaternary salt type derivatives. These studies showed that the CDS's were oxidized to the quaternary salt forms at neutral and basic pH and added water at lower pH. The quaternary salts released the parent drugs both in buffers and in vitro. A preliminary in vivo distribution study in the rat and rabbit demonstrated blood–brain barrier (BBB) penetration by the CDS, whereas no drug was detected by administering the drug itself.

The treatment of bacterial infections of the central nervous system (CNS) is difficult because of the inability of many potentially active antibiotics to pass the blood–brain barrier (BBB).[1,2] While in the case of infected meninges BBB permeability is improved somewhat, cerebritis, brain abscesses, and neurosyphilis constitute even greater therapeutic problems.[3,6]

In a previous study[7,8] successful attempts at using chemical delivery systems (CDS's) in order to improve the brain uptake of benzylpenicillin were described. The CDS's were based on a dihydropyridine ⇄ quaternary pyridinium salt redox system, analogous to the endogenous NADH ⇄ NAD^+ enzyme system.[9–12] In this approach, successfully applied to various classes of drugs, a molecular carrier is attached to agents whose ability to pass the BBB is poor, mainly because of their polar, hydrophilic character. This lipophilicity modifier increases the distribution of the drug conjugate in general and, in particular, improves the BBB transit. Under the influence of enzymes, the carrier is oxidized, producing polar species which are eliminated from the peripheral circulation, but which are "locked in" behind the BBB. The active drug is then released in the brain by hydrolysis. The principle of the CDS was presented elsewhere.[7,9–12] In the present work, the application of the same CDS approach to some important semisynthetic penicillins is described. The methicillin and isoxazolyl penicillins (Chart I) form a group of staphylococcal penicillinase resistant penicillins. Their stability toward these enzymes is explained by the increased steric hindrance around the carbon atom attached to the amide carbonyl group. This C atom is quaternary by incorporation into an aromatic (methicillin) or heterocyclic (isoxazolyl penicillins) ring.[13–17] The aromatic or heterocyclic ring is not enough to confer penicillinase resistance, other bulky groups being required (methoxy, or phenyl and methyl substituents). Methicillin is equally active against penicillin-susceptible and penicillinase-producing strains of *Staphylococcus aureus*. Although it is less active than benzylpenicillin against streptococci and pneumococci, methicillin is used in treatment of this kind of infection and also to protect susceptible penicillins

Chart I



(benzylpenicillin, ampicillin) as a β-lactamase inhibitor. The in vitro activity of the 3-phenyl-5-methyl-4-isoxazolyl penicillins against susceptible and resistant staphylococci is roughly the same as that of methicillin. They are stable to acid and can be administered orally, whereas the iv route of administration is needed for methicillin. Halogenation of the ortho positions of the phenyl group progressively

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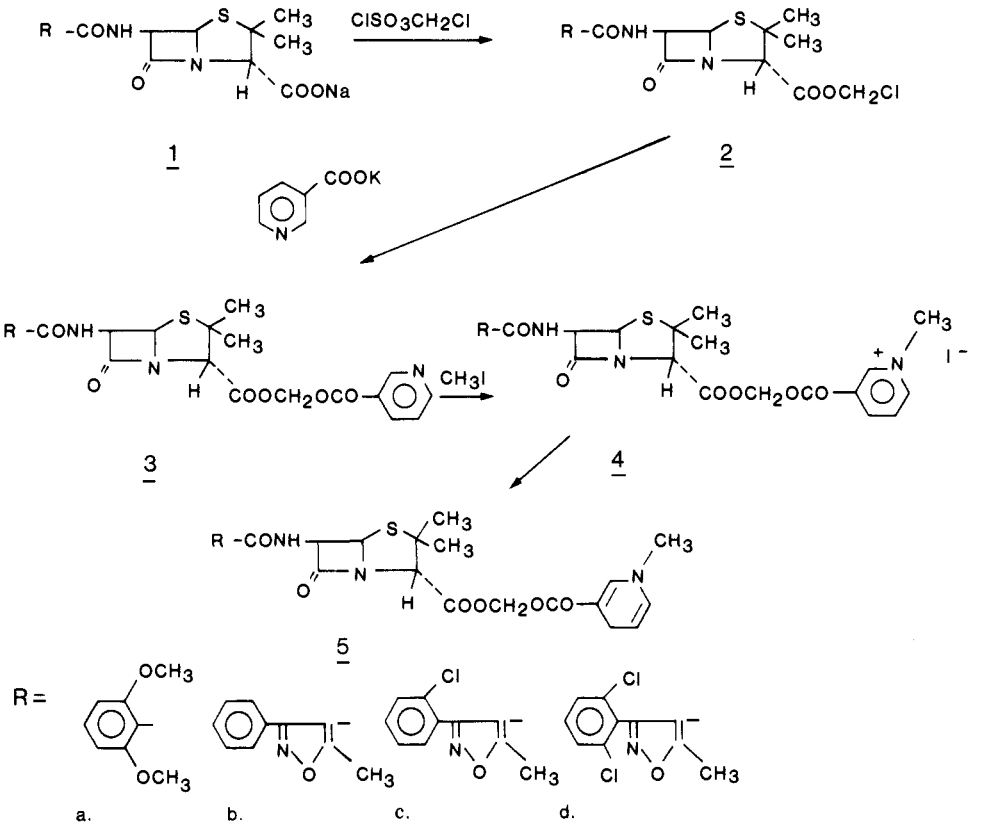
Scheme I



doubles the efficiency of oral absorbtion. However, their protein binding (over 90%) is more extensive compared to that of methicillin (approximately 50%).

The brain penetration of all of these antibiotics is poor. In the case of isoxazolyl penicillins, CSF levels in man, in the absence of meningitis, were found to be minimal or nonexistent. Methicillin penetration of the BBB is not much better, the CSF/serum ratio in normal human subjects being 0.5%.[18-21] By application of the CDS approach an improved CNS penetration and a prolonged release of these drugs are expected. Their use in the treatment of brain infections would be in this way permitted. Such compounds could also serve as site specific β-lactamase inhibitors as protectors of susceptible penicillin upon coadministration with a benzylpenicillin CDS.[17,22]

Results and Discussion

On the basis of the results of an extensive study of different types of CDS's for benzylpenicillin,[7,8] the diol diester type CDS was chosen for the semisynthetic penicillins: the penicillin CDS's are monoesters of *gem*-diols in which the second hydroxyl group is esterified by dihydrotrigonelline. The synthetic procedure is described in Scheme I.

Table I. Second-Order Oxidation Rate Constant (k_0) of the Semisynthetic Penicillin CDS's Determined by Using Ferricyanide[a]

no.	k_0, M^{-1} s^{-1}	r
5a	0.701	0.9986
5b	0.480	0.9978
5c	0.360	0.9909
5d	0.230	0.9977

[a] The k_0 of the same type of CDS of benzylpenicillin was 0.530 (r = 0.9933).

The chloromethyl esters **2a–d** were obtained by using chloromethyl chlorosulfate as chloromethylating agent and tetrabutylammonium hydrogen sulfate as a phase-tranfser catalyst, in water–methylene chloride media;[23] the penicillins were in the form of their sodium salts. Chloromethylation resulted in compounds of a remarkable purity so that no further purification of **2a–d** was necessary. The [(pyridin-3-ylcarbonyl)oxy]methyl esters **3a–d** were obtained by reacting **2a–d** with potassium nicotinate in dry DMF followed by purification by column chromatography. The quaternary salts **4a–d** were prepared by N-methylation of **3a–d** with methyl iodide in nitromethane, at room temperature (20–25 °C) in order to avoid side reactions. The CDS's **5a–d** were obtained by reducing **4a–d** with sodium dithionite in a mixture of ethyl acetate and aqueous sodium bicarbonate. The reduction led to the 1,4 isomers of the dihydrotrigonelline derivatives, structures proved by their typical UV maxima at ~360 nm and appropriate ^{1}H NMR absorbances.

All compounds were characterized by their elemental analysis UV and ^{1}H NMR spectroscopy. The IR spectra showed in all cases the characteristic maxima for the β-lactam ring at 1780 cm^{-1}. Chromatographic techniques

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Table II. Extrapolated Capacity Factors (log k) to a Mobile Phase of 100% Water of Semisynthetic Penicillins and Their CDS's

compd		log k	r^a
penicillin	1a	0.658	0.9993
	1b	1.868	0.9996
	1c	2.097	0.9900
	1d	2.435	0.9983
CDS's	5a	2.280	0.9983
	5b	2.820	0.9955
	5c	3.060	0.9965
	5d	3.410	0.9963

[a] r = correlation coefficient.

(HPLC, TLC) indicated only one component in each case; in the case of the dihydropyridine derivatives a small amount of the 1,6 isomer was generally present in the products.

All dihydropyridine derivatives were oxidized back to the corresponding quaternary pyridinium salts by methanolic silver nitrate solution or H_2O_2 in the presence of cupric ions. A ferricyanide oxidation study was performed in order to compare the oxidative stability of different CDS's. The oxidations were followed spectrophotometrically[24] and will be discussed in detail elsewhere.[25] Table I shows a summary of the results. The CDS's were relatively stable to oxidation. The rate of oxidation decreased in the studied series in the order **5a > 5b > 5c > 5d**. The values produced for the second-order rate constants (k) situated between 0.230 and 0.700 M^{-1} s^{-1} compare in an empirical sense with systems that are oxidized slowly in vivo. However, so far a real correlation between the chemical and in vivo, enzymatic oxidation was not proved. The relatively slow oxidation rate does not seem to be an obstacle for the in vivo distribution of the discussed CDS's, if compared with the rate constant for the same type of CDS of benzylpenicillin (0.530 M^{-1} s^{-1}) that gave good brain levels, after administration to rats, rabbit, or dog.

Lipophilicity is one of the main factors controlling penetration of drugs through biological membranes, including the BBB. The relative lipophilicities of the CDS's were compared with those of the parent semisynthetic penicillins. log k' values were used as lipophilicity indexes[26,27] and were calculated for various mobile-phase combinations of acetonitrile/water according to their retention characteristics on a reversed-phase HPLC. The lipophilic index was defined as

$$\log k' = \log [(t_r - t_0)/t_0]$$

where t_r is the retention time of a retained peak and t_0 is the retention time of an unretained peak (formaldehyde). log k' is considered analogous to R_m.[28] The extrapolated log k' to a mobile phase of 100% water represents the lipophilicity of the compounds.

The lipophilic indexes are presented in Table II. The CDS's are, as expected, much more lipophilic than the parent drugs: the methicillin CDS **5a** is ~35 times more lipophilic than methicillin, and the isoxazolyl penicillin CDS's are about 14–15 times more lipophilic than the respective oxacillin, cloxacillin, and dicloxacillin parent compounds. Clearly, a much improved penetration of the BBB can be expected by using the CDS's.

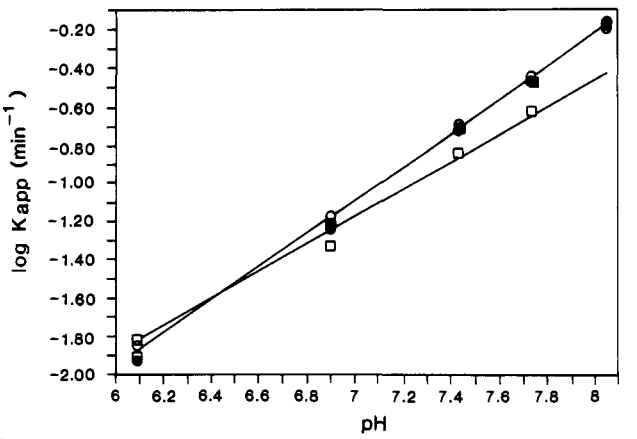


Figure 1. Buffer and pH effects on hydrolysis rate of the semisynthetic penicillin quaternary salt type derivatives at 37 °C (phosphate buffer 0.05 M; μ = 0.15): **4a** (●); **4b** (○); **4c** (■); **4d** (□).

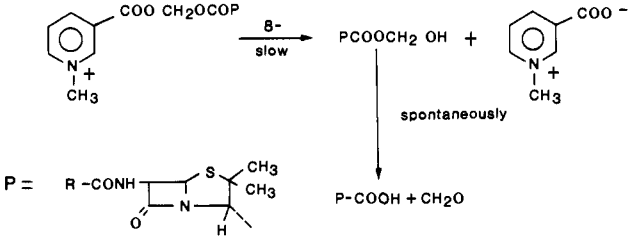
Table III. Stability (Half-Life, min) of Pyridinium Salt Type Derivatives of Semisynthetic Penicillins in Phosphate Buffer (0.05 M, μ = 0.15) at 30 °C

	half-life, min, for compd			
pH	4a	4b	4c	4d
6.07	58.6	49.0	56.0	44.4
6.88	12.0	10.3	11.3	14.7
7.43	3.6	3.4	3.5	3.9
7.73	2.0	1.9	2.1	2.9
8.05	1.0	1.1	1.0	

Table IV. Stability (Half-Life, min) of CDS's of Semisynthetic Penicillins in Phosphate Buffer (0.05 M, μ = 0.15) at 30 °C

	half-life, min, for compd			
pH	5a	5b	5c	5d
6.15	129.9	249.2	350.5	464.5
6.93	238.0	277.2	505.5	1011.0
7.45	134.4	195.6	377.1	1032.5
7.79	102.7	172.4	305.0	931.9
8.14	55.7	100.4	164.6	598.1

Scheme II



Stability in Buffers. The change in stability of the compounds due to pH shifts was investigated by determining the apparent rate constants (K_{app}) and half-lives under similar conditions (37 °C, 0.05 M phosphate buffer). Both quaternary salts and CDS's released the parent penicillins in the hydrolysis conditions. The stability of the quaternary salts **4a–d** in phosphate buffer is significantly pH dependent as shown from determinations in the pH range 6–8 (Table III, Figure 1). While at pH 6 the half-lives are in the range of 45–60 min, at physiological pH they drop more than 10-fold to a range of 3.5–4 min. At more basic pH, about 8, the half-lives were about 1 min.

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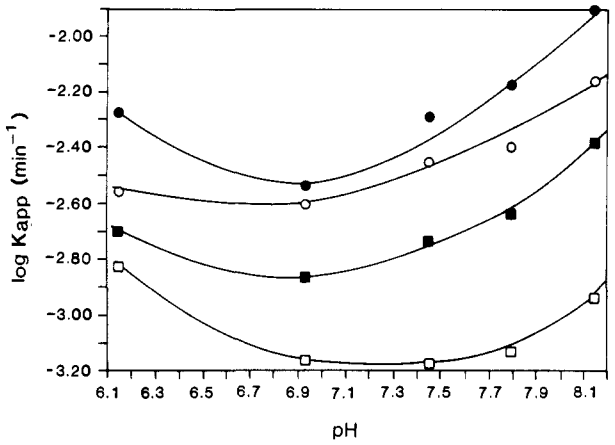

Figure 2. Buffer and pH effects on stability of semisynthetic penicillin CDS's at 37 °C (phosphate buffer 0.05 M, μ = 0.15): **5a** (●); **5b** (○); **5c** (■); **5d** (□).

Table V. Stability (Half-Life, min) of Pyridinium Salt Derivatives and CDS's of Semisynthetic Penicillins in Rat Biological Materials[a]

	half-life,[b] min		
compd	buffer, pH 7.4	rat blood	rat 10% brain homogenate
4a	3.6	1.0	1.1
5a	134.4	0.2	8.0
4b	3.4	0.7	1.2
5b	195.6	1.1	5.4
4c	3.5	0.6	1.1
5c	377.1	3.8	10.0
4d	3.9	0.7	1.0
5d	1032.5	4.1	12.6

[a] $t_{1/2}$ of **5a** in rabbit blood is 10.7, and in human blood 115.9 min; $t_{1/2}$ of **5b** in rabbit blood is 23.7, and in human blood 7.5 min. [b] Each datum point shows mean value of two determinations.

In Figure 1 a linear increase of the apparent rate constant with the increase of pH can be noticed. The mechanism of hydrolysis was discussed for similar cases; the two-step process includes a first rate-determining step, the hydrolysis of the trigonelline ester linkage to form the hydroxymethyl ester which decomposes spontaneously into the parent penicillin and formaldehyde (Scheme II).

Although all the CDS's were more stable compared to the same type derivative of benzylpenicillin ($t_{1/2}$ 2.7 min at pH 7.4), the difference is not significant. As expected (Table IV, Figure 2) the CDS's are all much more stable in buffers than are the corresponding quaternary salts. A considerable increase in stability from **5a** to **5d** was also noticed, the dicloxacillin CDS being very stable ($t_{1/2}$ = 1032.5 min) at pH 7.4. From Figure 2 it can be seen that the stability in the case of CDS is not linear; the stability increases on lowering the pH until 7; then at acidic pH water addition takes place with the formation of the 6-hydroxy-1,2,3,4-tetrahydropyridine derivatives.

Stability in Biological Materials. The stability of the CDS's and pyridinium salt derivatives of the semisynthetic penicillins was studied in rat blood and 10% brain homogenate. Hydrolysis of the compounds in the biological materials was followed by their disappearance and showed pseudo-first-order kinetics. The calculated half-lives are listed in Table V. Enzymatic hydrolysis occurred faster than chemical hydrolysis: no correlation was noticed between the two types of hydrolysis. In the case of methicillin, the half-life of the CDS was shorter than that of the quaternary salt, and in the case of the oxacillin CDS only slightly higher than that of the respective quaternary salts. The cloxacillin and dicloxacillin CDS's were much more stable than the corresponding quaternary salts. In brain homogenates the quaternary salt hydrolysis was slower than in blood; however, in the case of methicillin and oxacillin (**4a** and **4b**) the differences were not significant. The CDS's were oxidized in brain homogenate to the quaternary salt forms. Except for **5b** there was a correlation between the second-order rate of the chemical oxidation (Table I) and the half-life in brain, the stability increasing with the decrease of the oxidation rate.

Table VI. Concentration of **1b** in Blood and Brain after Iv Administration of **1b** or **5b** to Rats and Rabbits[a]

		concn, μg/mL or μg/g			
		blood		brain	
animal	time, min	**1b**	**5b**	**1b**	**5b**
rat	3	41.1	26.2	ND[b]	2.1
rabbit	5	15.3	4.6	ND	0.3
	10	9.1	2.2	ND	0.3

[a] Data show mean value of two determinations. [b] ND = undetectable. **5b** was present in both rabbit blood (3.1 μg/mL after 5 min and 1.8 μg/mL after 10 min) and brain (traces), as determined by HPLC.

The stabilities of some of the CDS's were determined in blood tissues belonging to other species, such as rabbit and human. As in the case of the benzylpenicillin CDS,[8] the differences were quite spectacular. The half-life of **5a** was 45-fold longer in rabbit and 483-fold longer in human blood than in rat; the stability of **5b** was 21-fold longer in rabbit than in rat blood.

In Vivo Distribution Study. Preliminary distribution studies on oxacillin and its CDS were performed in rat and rabbit (Table VI). In both cases when the penicillin itself was administered, no drug was identified in brain, this being in agreement with the previous available data. When the CDS **5b** was administered to rats, after 3 min the drug was found in the brain (blood:brain ratio 12:1). In rabbits, the concentration in blood and brain of oxacillin was measured 5 and 10 min after administration. While the blood concentration decreased in this time by approximately 50%, the brain concentration was constant, the blood:brain ratio being improved in this way from 14:1 to 7:1. Clearly, when the CDS was used, the drug was transported through the BBB into the brain. More extensive distribution studies performed in rabbits and dogs will be reported elsewhere. These studies will include distribution data about the other semisynthetic penicillin CDS's.

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 (J) 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. Methicillin, oxacillin, cloxacillin, and dicloxacillin were obtained from Sigma.

Chloromethyl [2*S*-(2α,5α,6β)]-3,3-Dimethyl-7-oxo-6-(2,6-dimethoxybenzamido)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (2a). To a solution of 4.02 g (0.01 mol) of methicillin sodium salt (**1a**) in 10 mL of water and 10 mL of CH_2Cl_2 were added 2.4 g of sodium bicarbonate and 0.34 g of tetrabutylammonium hydrogen sulfate, and with stirring 1.9 g (0.0115 mol) of chloromethyl chlorosulfate dissolved in 3 mL of CH_2Cl_2 was

added over 5 min, keeping the temperature below 30 °C. After an additional 30 min of stirring, the organic phase was separated, washed twice with water, and dried over $MgSO_4$. By removal of the solvent in vacuo, 4.24 g of **2a** was obtained as a yellow solid, mp 88–90 °C.

^1H NMR ($CDCl_3$) δ 1.57 (s, 3 H, CH_3), 1.66 (s, 3 H, CH_3), 3.85 (s, 6 H, CH_3), 4.45 (s, 1 H, C-2 proton), 5.65–6.10 (m, 4 H, C-5, C-6 proton, CH_2), 6.05 (d, 1 H, J = 7, NH), 6.62 (d, 2 H, J = 7, phenyl C-3, C-5 protons), 7.35 (t, 1 H, phenyl C-4 proton). Anal. ($C_{18}H_{21}ClN_2O_6S$) C, H, Cl, N, S.

Chloromethyl [2*S*-(2α,5α,6β)]-3,3-Dimethyl-6-[(5-methyl-3-phenyl-4-isoxazolyl)carboxamido]-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (2b). In the same way, from 2.12 g (0.005 mol) of oxacillin sodium salt (**1b**) with 1.2 g of $NaHCO_3$, 0.17 g of tetrabutylammonium hydrogen sulfate, and 0.95 g of chloromethyl chlorosulfate was obtained **2b** (1.87 g), mp 78–80 °C dec.

^1H NMR ($CDCl_3$) δ 1.32 (s, 3 H, CH_3), 1.43 (s, 3 H, CH_3), 4.35 (s, 1 H, C-2 proton), 5.35–5.90 (m, 4 H, CH_2, C-5, C-6 protons), 6.09 (d, 1 H, J = 9, NH), 7.50–7.60 (m, 5 H, C_6H_5). Anal. ($C_{20}H_{20}ClN_3O_5S$) C, H, Cl, N, S.

Chloromethyl [2*S*-(2α,5α,6β)]-6-[[3-(2-Chlorophenyl)-5-methyl-4-isoxazolyl]carboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (2c). According to the same procedure, from 2.38 g (0.005 mol) of cloxacillin sodium salt (**1c**) (1 mol of water) and 1.2 g of $NaHCO_4$, 0.17 g of Bu_4NHSO_4, and 0.95 g of chloromethyl chlorosulfate was obtained 2.27 g of **2c**, mp 97–100 °C dec.

^1H NMR ($CDCl_3$) δ 1.36 (s, 3 H, CH_3), 1.41 (s, 3 H, CH_3), 2.79 (s, 3 H, CH_3), 4.35 (s, 1 H, C-2 proton), 5.45 (d, 1 H, J = 4, C-5 proton), 5.80 (d, 1 H, J = 4.6, C-6 proton), 5.90 (d, 2 H, J = 6.5, CH_2), 6.05 (d, 1 H, J = 7, NH), 7.42–7.49 (m, 4 H, C_6H_4). Anal. ($C_{20}H_{19}Cl_2N_3O_5S$) C, H, Cl, N, S.

Chloromethyl [2*S*-(2α,5α,6β)]-6-[[3-(2,6-Dichlorophenyl)-5-methyl-4-isoxazolyl]carboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (2d). Similarly, from 2.55 g (0.005 mol) of dicloxacillin sodium salt (**1d**) (1 mol of water) with 1.7 g of $NaHCO_3$, 0.17 g of Bu_4NHSO_4, and 0.95 g of chloromethyl chlorosulfate was obtained 2.43 g of **2d**, mp 98–101 °C dec.

^1H NMR ($CDCl_3$) δ 1.48 (s, 3 H, CH_3), 1.49 (s, 3 H, CH_3), 2.83 (s, 3 H, CH_3), 4.35 (s, 1 H, C-2 proton), 5.46 (d, 1 H, J = 4.5, C-5 proton), 5.65–5.95 (m, 4 H, CH_2, NH, C-6 proton), 7.46–7.51 (m, 3 H, C_3H_3). Anal. ($C_{20}H_{18}Cl_3N_3O_5S$) C, H, Cl, N, S.

[(3-Pyridinylcarbonyl)oxy]methyl [2*S*-(2α,5α,6β)]-3,3-Dimethyl-7-oxo-6-(2,6-dimethoxybenzamido)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (3a). A mixture of 3.8 g (0.0089 mol) of chloromethyl ester **2a** and 1.6 g (0.01 mol) of potassium nicotinate in 70 mL of DMF was stirred for 6 days at room temperature (20–25 °C). Ethyl acetate (300 mL) was added, the resulting solid was filtered off, and the solution was extracted four times with 50 mL of concentrated aqueous NaCl and dried over $MgSO_4$. The solvent was removed in vacuo and the resulting residue purified by chromatography (silica gel), giving 3 g of **3a** as a white solid, mp 151–157 °C.

^1H NMR ($CDCl_3$) δ 1.48 (s, 3 H, CH_3), 1.62 (s, 3 H, CH_3), 3.75 (s, 6 H, CH_3), 4.45 (s, 1 H, C-2 proton), 5.65 (d, 1 H, J = 4.7, C-5 proton), 5.91 (d, 1 H, J = 4.4, C-6 proton), 6.15 (s, 2 H, CH_2), 6.45–7.55 (m, 5 H, pyridine C-5 proton, C_6H_3, NH), 8.35 (d, 1 H, J = 8, pyridine C-4 proton), 8.8 (d, 1 H, J = 4, pyridine C-6 proton), 9.25 (s, 1 H, pyridine C-2 proton). Anal. ($C_{24}H_{25}N_3O_8S$) C, H, N, S.

[(3-Pyridinylcarbonyl)oxy]methyl [2*S*-(2α,5α,6β)]-3,3-Dimethyl-6-[(5-methyl-3-phenyl-4-isoxazolyl)carboxamido]-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (3b). In the same way, from 1.81 g (0.004 mol) of **2b** and 0.75 g (0.0046 mol) of potassium nicotinate, after purification by chromatography, was obtained 0.75 g of **3b** as a white solid, mp 79–82 °C dec.

^1H NMR ($CDCl_3$) δ 1.29 (s, 3 H, CH_3), 1.35 (s, 3 H, CH_3), 2.73 (s, 3 H, CH_3), 4.45 (s, 1 H, C-2 proton), 5.55 (d, 1 H, J = 4.5, C-5 proton), 5.9 (d, 1 H, J = 5, C-6 proton), 6.15 (s, 2 H, CH_2), 6.40 (d, 1 H, J = 8.5, NH), 7.35–7.50 (m, 1 H, pyridine C-5 proton), 7.50–7.62 (m, 5 H, C_6H_5), 8.33 (d, 1 H, J = 7.5, pyridine C-4 proton), 8.85 (d, 1 H, J = 4.5, pyridine C-6 proton), 9.25 (s, 1 H, pyridine C-2 proton). Anal. ($C_{26}H_{24}N_4O_7S \cdot 0.5H_2O$) C, H, N, S.

[(3-Pyridinylcarbonyl)oxy]methyl [2*S*-(2α,5α,6β)]-6-[[3-(2-Chlorophenyl)-5-methyl-4-isoxazolyl]carboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (3c). From 2.1 g (0.0043 mol) of **2c** and 0.8 g (0.005 mol) of potassium nicotinate, in the same manner, was obtained 1.2 g of **3c**, mp 83–85 °C dec.

^1H NMR ($CDCl_3$) δ 1.36 (s, 3 H, CH_3), 1.40 (s, 3 H, CH_3), 2.79 (s, 3 H, CH_3), 4.36 (s, 1 H, C-2 proton), 5.45 (d, 1 H, J = 4.5, C-6 proton), 5.80 (d, 1 H, J = 4.5, C-6 proton), 6.10 (s, 2 H, CH_2), 6.25 (d, 1 H, J = 8, NH), 7.35–7.55 (m, 5 H, C_6H_5, pyridine C-5 protons), 8.35 (d, 1 H, J = 7, pyridine C-4 proton), 8.85 (d, 1 H, J = 5, pyridine C-6 proton), 9.3 (s, 1 H, pyridine C-2 proton). Anal. ($C_{26}H_{23}ClN_4O_7S$) C, H, Cl, N, S.

[(3-Pyridinylcarbonyl)oxy]methyl [2*S*-(2α,5α,6β)]-6-[[3-(2,6-Dichlorophenyl)-5-methyl-4-isoxazolyl]carboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (3d). From 2.27 g (0.0047 mol) of **2d** and 0.87 g (0.0054 mol) of potassium nicotinate, in the same way, was obtained 1.1 g of **2d** as a white solid, mp 87–90 °C dec.

^1H NMR ($CDCl_3$) δ 1.43 (s, 3 H, CH_3), 1.44 (s, 3 H, CH_3), 2.82 (s, 3 H, CH_3), 4.39 (s, 1 H, C-2 proton), 5.47 (d, 1 H, J = 4.5, C-5 proton), 5.75 (d, 1 H, J = 5, C-6 proton), 7.35–7.51 (m, 1 H, pyridine C-5 proton), 7.41–7.50 (m, 3 H, C_6H_3), 8.30 (d, 1 H, J = 8, pyridine C-4 proton), 8.85 (d, 1 H, J = 4.5, pyridine C-6 proton), 9.25 (s, 1 H, pyridine C-2 proton). Anal. ($C_{26}H_{22}Cl_2N_4O_7S$) C, H, Cl, N, S.

[2*S*-(2α,5α,6β)]-3-[[[[[3,3-Dimethyl-7-oxo-6-(2,6-dimethoxybenzamido)-4-thia-1-azabicyclo[3.2.0]hept-2-yl]carbonyl]oxy]methoxy]carbonyl]-1-methylpyridinium Iodide (4a). A mixture of 1.25 g (0.0024 mol) of **3a** in 35 mL of nitromethane and 1.14 g (0.5 mL, 0.008 mol) of methyl iodide was reacted in a closed system at room temperature (20–25 °C) for 7 days. The solvent was removed in vacuo, and the resulting residue was stirred with ether, filtered off, washed with ether, and dried, giving 1.6 g of **4a** as a yellow, hygroscopic material: mp 95–100 °C; UV (MeOH) 222, 268 nm.

^1H NMR (DMSO-d_6) δ 1.41 (s, 3 H), 1.62 (s, 3 H, CH_3), 3.72 (s, 6 H, CH_3), 4.42 (s, 3 H, N^+–CH_3), 4.46 (s, 1 H, C-2 proton), 5.58 (d, 1 H, J = 4.2, C-5 proton), 5.74 (d, 1 H, J = 4.5, C-6 proton), 6.15 (s, 2 H, CH_2), 6.65 (d, 2 H, J = 8, phenyl C-3, C-5 protons), 7.3 (t, 1 H, phenyl C-4 proton), 8.30 (t, 1 H, pyridinium C-4 proton), 8.97 (d, 1 H, J = 2, pyridinium C-4 proton), 9.25 (d, 1 H, J = 6, pyridinium C-6 proton), 9.7 (s, 1 H, pyridinium C-2 proton). Anal. ($C_{25}H_{28}IN_3O_8S \cdot 2.5H_2O$) C, H, I, N, S.

[2*S*-(2α,5α,6β)]-3-[[[[[3,3-Dimethyl-6-[(5-methyl-3-phenyl-4-isoxazolyl)carboxamido]-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-yl]carbonyl]oxy]methoxy]carbonyl]-1-methylpyridinium Iodide (4b). From 0.5 g (0.0009 mol) of **3b** in 25 mL of nitromethane and 0.45 g (0.2 mL, 0.003 mol) of CH_3I over 6 days was obtained 0.6 g of **4b**: mp 75–80 °C; UV (MeOH) 222, 268 nm.

^1H NMR (DMSO-d_6) δ 1.44 (s, 3 H, CH_3), 1.58 (s, 3 H, CH_3), 2.56 (s, 3 H, CH_3), 4.45 (s, 3 H, N^+–CH_3), 4.56 (s, 1 H, C-2 proton), 5.61–5.68 (m, 2 H, C-5, C-6 protons), 6.15 (s, 2 H, CH_2), 7.47–7.68 (m, 5 H, C_6H_5), 8.29 (t, 1 H, pyridinium C-5 proton), 9.01 (d, 1 H, J = 8, pyridinium C-4 proton), 9.23 (d, 1 H, J = 6, pyridinium C-6 proton), 9.35 (d, 1 H, J = 7, NH), 9.62 (s, 1 H, pyridinium C-2 proton). Anal. ($C_{27}H_{27}IN_4O_7S$) C, H, I, N, S.

[2*S*-(2α,5α,6β)]-3-[[[[[6-[[3-(2-Chlorophenyl)-5-methyl-4-isoxazolyl]carboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-yl]carbonyl]oxy]methoxy]carbonyl]-1-methylpyridinium Iodide (4c). From 0.44 g (0.0008 mol) of **3c** in 25 mL of nitromethane and 0.45 g (0.2 mL, 0.003 mol) of CH_3I was obtained 0.45 g of **4c**: mp 90–95 °C dec; UV 224, 268 nm.

^1H NMR (DMSO-d_6) δ 1.42 (s, 3 H, CH_3), 1.55 (s, 3 H, CH_3), 2.64 (s, 3 H, CH_3), 4.44 (s, 3 H, N^+–CH_3), 4.50 (s, 1 H, C-2 proton), 5.52–5.69 (m, 2 H, C-5, C-6 protons), 6.14 (s, 2 H, CH_2), 7.42–7.51 (m, 4 H, C_6H_4), 8.30 (t, 1 H, pyridinium C-5 proton), 8.76 (d, 1 H, J = 7, pyridinium C-4 proton), 8.97 (d, 1 H, J = 6.5, pyridinium C-6 proton), 9.23 (d, 1 H, J = 6, NH), 9.62 [s, 1 H, pyridinium C-2 proton). Anal. ($C_{27}H_{26}ClIN_4O_7S$) C, H, Cl, I, N, S.

[2*S*-(2α,5α,6β)]-3-[[[[[6-[[3-(2,6-Dichlorophenyl)-5-methyl-4-isoxazolyl]carboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-yl]carbonyl]oxy]methoxy]carbonyl]-1-methylpyridinium Iodide (4d). A mixture of 0.5

g (0.007 mol) of **3d** in 25 mL of nitromethane and 0.45 g (0.2 mL, 0.003 mol) of CH_3I gave 0.55 g of **4d**: mp 95–100 °C dec; UV (MeOH) 222, 268 nm.

^1H NMR (DMSO-d_6) δ 1.42 (s, 3 H, CH_3), 1.58 (s, 3 H, CH_3), 2.71 (s, 3 H, CH_3), 4.44 (s, 3 H, N^+–CH_3), 4.54 (s, 1 H, C-2 proton), 5.51–5.64 (m, 2 H, C-5, C-6 protons), 6.14 (s, 2 H, CH_2), 7.45–7.62 (m, 3 H, C_6H_3), 8.29 (t, 1 H, pyridinium C-5 proton), 8.70 (d, 1 H, J = 7, pyridinium C-4 proton), 9.00 (d, 1 H, J = 6, pyridinium C-6 proton), 9.23 (d, 1 H, J = 6, NH), 9.65 (s, 1 H, pyridinium C-2 proton). Anal. ($C_{27}H_{25}Cl_2IN_4O_7S$) C, H, Cl, I, N, S.

[[(1,4-Dihydro-1-methyl-3-pyridinyl)carbonyl]oxy]methyl [2*S*-(2α,5α,6β)]-3,3-Dimethyl-7-oxo-6-(2,6-dimethoxybenzamido)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (5a). A solution of 0.45 g (0.0007 mol) of **4a** in a mixture of 25 mL of deaerated ethyl acetate and 70 mL of water was reduced with a mixture of 0.34 g (0.004 mol) of $NaHCO_3$ and 0.48 g (0.0028 mol) of sodium dithionite at 0–5 °C over 70 min. The disappearance of the 268-nm maximum and increase of the 366-nm maximum in the UV spectrum was followed. The layers were separated, and the aqueous one was extracted with 2 × 25 mL of EtOAc; then the organics were extracted with 2 × 20 mL of cold, deaerated water. After drying on Na_2SO_4, solvent was removed in vacuo, giving 0.25 g of **5a** as a yellow solid: mp 88–90 °C dec; UV (MeOH) 220, 366 nm.

^1H NMR ($CDCl_3$) δ 1.42 (s, 3 H, CH_3), 1.62 (s, 3 H, CH_3), 2.95 (s, 3 H, N–CH_3), 3.05 (s, 2 H, pyridine C-4 protons), 3.75 (s, 6 H, CH_3), 4.45 (s, 1 H, C-2 proton), 4.75–4.80 (m, 1 H, pyridine C-5 proton), 5.45–6.10 (m, 5 H, pyridine C-6 proton, CH_2, C-5, C-6 protons), 6.40–7.45 (m, 5 H, pyridine C-2, C_6H_3, NH). Anal. ($C_{25}H_{29}N_3O_8S·0.5H_2O$) C, H, N, S.

[[(1,4-Dihydro-1-methyl-3-pyridinyl)carbonyl]oxy]methyl [2*S*-(2α,5α,6β)]-3,3-Dimethyl-6-[(5-methyl-3-phenyl-4-isoxazolyl)carboxamido]-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (5b). In the same way, from 0.17 g (0.00025 mol) of **4b**, 0.08 g (0.0001 mol) of $NaHCO_3$, and 0.51 g (0.001 mol) of $Na_2S_2O_4$, in 15 mL of water and 15 mL of EtOAc, was obtained **5b** (0.1 g): mp 93–100 °C dec; UV (MeOH) 216, 364 nm.

^1H NMR ($CDCl_3$) δ 1.35 (s, 3 H, CH_3), 1.42 (s, 3 H, CH_3), 2.75 (s, 3 H, CH_3), 2.95 (s, 3 H, CH_3), 3.05 (s, 2 H, pyridine C-4 protons), 4.45 (s, 1 H, C-2 proton), 4.89–4.95 (m, 1 H, pyridine C-5 proton), 5.45–6.15 (m, 5 H, pyridine C-6 proton, CH_2, C-5, C-6 protons), 6.35 (d, 1 H, J = 7, NH), 7.05 (s, 1 H, pyridine C-2 proton), 7.35–7.43 (m, 5 H, C_6H_5). Anal. ($C_{27}H_{28}N_4O_2S·0.5H_2O$) C, H, N, S.

[[(1,4-Dihydro-1-methyl-3-pyridinyl)carbonyl]oxy]methyl [2*S*-(2α,5α,6β)]-6-[[3-(2-chlorophenyl)-5-methyl-4-isoxazolyl]carboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo-[3.2.0]heptane-2-carboxylate (5c). From 0.18 g (0.00025 mol) of **4c**, 0.089 g of $NaHCO_3$, and 0.17 g of $Na_2S_2O_4$ was obtained **5c** (0.13 g) as a yellow solid: mp 80–85 °C dec; UV (MeOH) 218, 364 nm.

^1H NMR δ 1.36 (s, 3 H, CH_3), 1.45 (s, 3 H, CH_3), 2.75 (s, 3 H, CH_3), 2.95 (s, 3 H, CH_3), 3.05 (s, 2 H, pyridine C-4 protons), 4.45 (s, 1 H, C-2 proton), 4.75–4.90 (m, 1 H, pyridine C-5 proton), 5.45–6.10 (m, 5 H, pyridine C-6, CH_2, C-5, C-6 protons), 6.15 (d, 1 H, J = 7, NH), 7.05 (s, 1 H, pyridine C-2 proton), 7.30–7.42 (m, 4 H, C_6H_4). Anal. ($C_{27}H_{27}ClN_4O_7S$) C, H, Cl, N, S.

[[(1,4-Dihydro-1-methyl-3-pyridinyl)carbonyl]oxy]methyl [2*S*-(2α,5α,6β)]-6-[[3-(2,6-Dichlorophenyl)-5-methyl-4-isoxazolyl]carboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (5d). From 0.19 g (0.00025 mol) of **4c**, 0.08 g of $NaHCO_3$, and 0.17 g of $Na_2S_2O_4$, in the same way, was obtained **5d** (0.14 g): mp 98–102 °C dec; UV 222, 358 nm.

^1H NMR ($CDCl_3$) δ 1.41 (s, 3 H, CH_3), 1.49 (s, 3 H, CH_3), 2.75 (s, 3 H, CH_3), 2.97 (s, 3 H, CH_3), 3.05 (s, 2 H, pyridine C-4 protons), 4.45 (s, 1 H, C-2 proton), 4.70–4.85 (m, 1 H, pyridine C-5 proton), 5.45–6.05 (m, 5 H, CH_2, C-5, C-6 pyridine C-6 protons), 6.20 (d, 1 H, J = 8, NH), 7.10 (s, 1 H, pyridine C-2 proton), 7.40–7.55 (m, 3 H, C_6H_3). Anal. ($C_{27}H_{26}N_4O_7S·H_2O$) C, H, Cl, N, S.

Analytical Methods. Chemical Oxidation Studies. Ferricyanide oxidation kinetic study was performed according to a literature method:[24] 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_0) were determined from the pseudo-first-order rates at different concentrations of $Fe(CN)_6^{3-}$.

Lipophilicity. log k' was defined in the text. A high-performance liquid chromatography (HPLC) method was used to determine t_k and t_0. The mobile phases used were various concentrations of acetonitrile in water. The retention time of formaldehyde was used for the t_0 values. Solutions of penicillins and their derivatives were prepared in the concentration range between 5×10^{-3} and 1×10^{-2} M. A 5-μL sample was injected into the column. log K values were calculated by extrapolating log k' values to 0% acetonitrile.

HPLC Analysis. HPLC was used for quantitative analysis of the parent penicillin, quaternary salts, and CDS's. The HPLC unit consisted of a solvent delivery system (Kontron 410), variable-wavelength UV detector (LDC spectromonitor D), autosampler (Kontron MSI 66), and a recorder. The column was stainless steel (25 cm × 4.5 mm i.d.) packed with porous microparticular silica, permanently bound to monomolecular layer of octasilane (C_8) phase (ASI). The mobile phase consisted of various combinatiions of acetonitrile (45–60%) and aqueous phosphate buffer (10–30 mM). Flow rate of the mobile phase was 1–2 mL/min to obtain the retention times of 3.5–8.0 min. The CDS's were detected at UV 360 nm, and the quaternary compounds were detected at UV 260 nm.

Microbioassay. Penicillin concentration in buffer or biological media was determined by a paper disk simple diffusion method with *Bacillus subtillis* ATCC 6633 as a test organism. All biological samples were diluted or homogenized by at least 5 volumes of sterile water to minimize the protein binding effect, which might affect the antibacterial activity of penicillin. Standard calibration curves were prepared for each experiment. Different concentrations of compounds were added to five times diluted blood or tissue homogenates.

Stability in Buffers. Phosphate buffers of various pH (0.05 M, μ = 0.15) were used as media for kinetic studies. Stock solutions of compounds in dimethyl sulfoxide were prepared. Aliquots (50 mL) of the stock solutions were added into 10 mL of buffer at 37 °C to initiate the study. At appropriate time intervals samples were taken and analyzed by HPLC. Pseudo-first-order rate constants of the disappearance of compound in media were determined by linear regression analysis from plots of log peak height versus time.

In Vitro Stability in Biological Media. Freshly collected rat, rabbit, and human whole blood and rat and rabbit brain homogenized with pH 7.4 phosphate buffer (1/20 M) at a final concentration of 10% (w/v) were used as media. Aliquots of the DMSO stock solutions (50 μL) were added to the media, kept at 37 °C, to obtain a final concentration of 40 μM. At appropriate time intervals, samples were taken and extracted three times with 8% DMSO in acetonitrile solution and then centrifuged, and the supernatant was filtered and analyzed by HPLC. Pseudo-first-order rate constants were determined as described in the preceding paragraph.

In Vivo Distribution Study. Oxacillin **1b** (20 mg/kg) or an equimolar dose of CDS **5b** were intravenously administered in male Sprague-Dawley rats (tail vein) or male New Zealand albino rabbits (ear vein). Compounds were dissolved in a vehicle consisting of water, ethanol, and propylene glycol (1:6.3:2.7 for **1b**, 0:7:3 for **5b**) to obtain a concentration of 20 mg/mL. Rats were sacrificed by decapitation, and rabbits were sacrificed by overdose of pentobarbital at appropriate time intervals after the intravenous injection. In rats, trunk blood was collected after decapitation. In rabbits, venous blood was collected through ear vein. Brain tissues were then removed and frozen immediately. **1b** concentration in each sample was determined quantitatively by microbioassay and HPLC methods as described before. Doses were as follows: rat, 30 mg/kg/mL oxacillin or equivalent amount of CDS; rabbit, 30 mg/kg/0.4 mL of oxacillin or equivalent amount of CDS.

Conclusions

The CDS's prepared for methicillin and some isoxazolyl penicillins possess the required properties to deliver the drug through the BBB into the CNS. The lipophilicity,

oxidation, stability, and preliminary in vivo studies support this statement and justify further studies in different species. Their β-lactamase inhibitory properties can be also exploited in order to protect β-lactamase-susceptible penicillin CDS's.

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A Probe for Octopamine Receptors: Synthesis of 2-[(4-Azido-2,6-diethylphenyl)imino]imidazolidine and Its Tritiated Derivative, a Potent Reversible–Irreversible Activator of Octopamine-Sensitive Adenylate Cyclase

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In order to develop an irreversible ligand for octopamine receptors, a highly potent azido-substituted 2-(phenylimino)imidazolidine (NC-5Z, 8) and its tritiated derivative (^{3}H-NC-5Z, 11) have been designed and synthesized. Under reversible-binding conditions, NC-5Z is 50–100-fold more potent than octopamine in activating octopamine-sensitive adenylate cyclase in a variety of tissues. After photolysis, ^{3}H-NC-5Z binds irreversibly to cell membranes, and this binding is reduced by preincubation with octopamine agonists and antagonists but not by norepinephrine, dopamine, serotonin, or histamine. NC-5Z should be useful both as a potent reversible octopamine agonist and as an affinity probe for characterizing and isolating octopamine-receptor proteins.

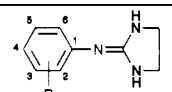
Octopamine is a physiologically significant neurotransmitter in invertebrates, having both neurohumoral and transmitter actions.[1-3] Octopamine is also found in vertebrates, although it is as yet unclear whether unique receptors exist for this amine in the higher phyla.[2] Because exogenously applied octopaminergic agonists can disrupt insect behavior and interfere with feeding, potent octopamine analogues have pesticidal and pestistatic activity.[4-7]

Recent evidence suggests that the toxic and behavioral actions of octopamine agonists in insects are, at least in part, mediated by a subclass of octopamine receptors that activate adenylate cyclase and lead to the synthesis of cyclic AMP.[7,8] The pharmacology of cyclic AMP associated octopamine receptors has been studied through physiological experiments[9] and through measurements of adenylate cyclase activation.[4,8,10,11] However, direct labeling of these receptors has been difficult because of their relatively low (20 μM) affinity for octopamine, resulting in an extremely rapid off-time for existing agonists. Because of this and because there presently exist no irreversible ligands for octopamine receptors, little is known about octopamine-receptor structure or biochemistry. It is for these reasons that we have developed a new agonist of octopamine-sensitive adenylate cyclase, 2-[(4-azido-2,6-diethylphenyl)imino]imidazolidine (NC-5Z, 8). Under conditions of dim light, this compound is 50–100 times more potent than octopamine itself and is the most potent octopamine agonist yet described. Furthermore, when photolyzed, NC-5Z and its tritiated derivative bind irreversibly to membrane octopamine receptors associated with the activation of adenylate cyclase. NC-5Z thus constitutes the first example of a covalent octopamine receptor label which should be useful in the eventual isolation of octopamine-receptor proteins.

Chemistry

The strategy for developing an irreversible octopamine agonist was influenced by recent studies of the 2-(phenylimino)imidazolidines (PIIs), a potent class of reversible octopamine receptor agonists.[7,8] In addition to being potent, a number of PII derivatives exhibit a high degree of octopamine-receptor selectivity which is clearly distinct from activity at mammalian adrenergic, dopaminergic, and serotonergic receptors.[8] Prior studies have shown that

Table I. Octopamine-Receptor Activity of Compounds[a]

compd		V_{max} (% OCT)	K_a(OCT)/K_a compd
11	2,6-$(CH_2CH_3)_2$, 3,5-$(^3H)_2$, 4-N_3	100	86
8	2,6-$(CH_2CH_3)_2$, 4-N_3	100	70
5	2,6-$(CH_2CH_3)_2$	100	12
octopamine (OCT)		100	1
7	2,6-$(CH_2CH_3)_2$, 4-NH_2	82	1.3
9	2,6-$(CH_2CH_3)_2$, 3,5-$(Br)_2$, 4-NH_2	24	15
1	2,6-$(Cl)_2$, 4-$NCH_3(CH_2CH_2Cl)$	5	6

[a] Activity (stimulation of firefly light organ adenylate cyclase) is expressed relative to the activity of octopamine (V_{max} = 100%; K_a ratio = 1) run in the same experiment. Typically, basal adenylate cyclase activity was 20–40 pmol/mg of protein per min, and octopamine stimulation at V_{max} was 50-fold.

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