Interactive design and synthesis of a novel antibacterial agent[1]

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β-Lactam compounds act on penicillin-recognizing enzymes via acylation of the hydroxyl group of an active site serine. When the resulting acyl enzyme is kinetically stable, as in the case of a penicillin-binding protein (PBP), the biosynthesis of a bacterial cell wall is inhibited, and death of the organism results. The de novo design of an antibacterial agent targeted to a PBP might be possible if the three-dimensional structural requirements of the equilibrium (i.e., *fit*) and catalytic (i.e. *reactivity*) steps of the aforementioned enzymatic process could be determined. For a model of the active site of a PBP from *Streptomyces* R61, the use of molecular mechanics calculations to treat "fit," and ab initio molecular orbital calculations to treat "reactivity," leads to the idea that the carboxyl group (G_1) and the amide N-H (G_2) of the antibiotic are hydrogen bonded to a lysine amino group and a valine carbonyl group in the enzyme–substrate complex. These two hydrogen bonds place the serine hydroxyl group on the convex face of the antibiotic, in position for attack on the β-lactam ring by a neutral reaction, catalyzed by water, that involves a direct proton transfer to the β-lactam nitrogen. Molecular orbital calculations of structure–reactivity relations associated with this mechanism suggest that C═N is bioisosteric to the β-lactam N-C(═O), comparable to a β-lactam in its reactivity with an alcohol, and that the product RO(C-N)H is formed essentially irreversibly ($-\Delta E > 10$ kcal/mol). Accordingly, structures containing a G_1 and a G_2 separated by a C═N, and positioned in different ways with respect to this functional group, have been synthesized computationally and examined for their ability to fit to the PBP model. This strategy identified a 2*H*-5,6-dihydro-1,4-thiazine substituted by hydroxyl and carboxyl groups as a target for chemical synthesis. However, exploratory experiments suggested that the C═N of this compound equilibrates with endocyclic and exocyclic enamine tautomers. This required that the C2 position be substituted, and that the hydroxyl group not be attached to the carbon atom adjacent to the C═N. These conditions are met in a 2,2-dimethyl-3-(2-hydroxypropyl)-1,4-thiazine, which also exhibits the necessary fit to the PBP model. Two epimers of this compound have been synthesized, from D- and L-serine. The compound derived from L-serine is not active. The compound derived from D-serine exhibits antibacterial activity, but is unstable, and binding studies with PBP's have not been performed. It is hoped that these studies can be carried out if modification of the lead structure leads to compounds with improved chemical stability.

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Les β-lactames agissent sur les enzymes qui reconnaissent la pénicilline par le biais d'une acylation du groupe hydroxyle d'un site actif de la sérine. Lorsque l'enzyme acylé qui en résulte est cinétiquement stable, comme dans le cas d'une pénicilline se liant à une protéine (PBP), la biosynthèse d'une paroi de la cellule bactérienne est inhibée et la mort de l'organisme s'en suit. Le «design» de novo d'un agent antibactérien orientée vers une PBP peut être possible s'il est possible de déterminer les besoins structuraux des étapes d'équilibre (à savoir l'*ajustement*) et catalytique (à savoir la *réactivité*) des processus enzymatiques mentionnés plus haut. Pour un modèle du site actif d'une PBP du *Streptomyces* R61, l'utilisation de calculs de mécanique moléculaire pour examiner l'adjustement et des calculs d'orbitales moléculaires ab initio pour traité de la «réactivité» conduisent à la suggestion que, dans le complexe enzyme–substrat, le groupe carboxyle (G_1) et le groupe amide (G_2) de l'antibiotique sont reliés à un groupe amino de la lysine et à un groupe carbonyle de la valine par des liaisons hydrogènes. Ces deux liaisons hydrogènes placent le groupe hydroxyle de la sérine sur la face convexe de l'antibiotique, dans une position permettant une attaque sur le noyau β-lactame par une réaction neutre, catalysée par l'eau, qui implique un transfert direct de proton à l'azote du β-lactame. Des calculs d'orbitales moléculaires des relations structure–réactivité associées avec ce mécanisme suggèrent que le C═N est bioisostérique avec le β-lactame, N-C(═O), comparable au β-lactame en ce qui a trait à sa réactivité avec un alcool, et que le produit RO(C-N)H se forme d'une façon qui est essentiellement irréversible ($-\Delta E > 10$ kcal/mol). Sur cette base, on a synthétisé par ordinateur des structures contenant les groupes G_1 et G_2 séparés par un C═N et placés dans des positions diverses par rapport à ce groupe fonctionnel et on a examiné leur habilité à s'insérer dans le modèle PBP. Cette stratégie a permis d'identifier une 2*H*-5,6-dihydro-1,4-thiazine substituée par des groupes hydroxyles et carboxyles comme cibles pour une synthèse chimique. Toutefois, des essais préliminaires ont suggéré que le C═N de ce composé serait en équilibre avec les énamines endocycliques et exocycliques tautomères. Cette caractéristique nécessite que la position C2 soit substituée et que le groupe hydroxyle ne soit pas attaché au carbone adjacent du C═N. On rencontre ces conditions dans une 2,2-diméthyl-3-(2-hydroxypropyl)-1,4-thiazine qui présente aussi les qualités requises pour s'insérer dans le modèle PBP. On a synthétisé deux épimères de ce composé à partir des D- et L-sérine. Le composé dérivé de la L-sérine n'est pas actif. Le composé dérivé de la D-sérine présente une activité antibactérienne, mais il est instable et on n'a pas pu effectuer d'études de liaisons avec les PBP. On espère que ces études pourront être effectuées sur des modifications de cette structure qui auraient une meilleure stabilité chimique.

[Traduit par la Rédaction]

[1]This paper is dedicated to the twin patriarchs of alkaloid synthesis and biosynthesis in Canada, David B. MacLean and Ian D. Spenser, on the occasion of their retirements from McMaster University.

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Introduction

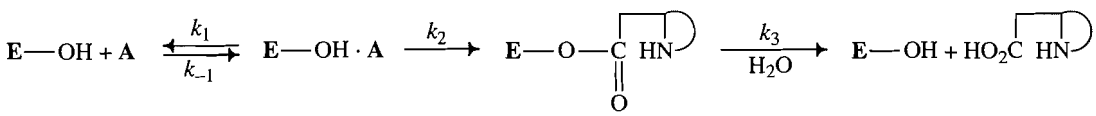
β-Lactam antibiotics interact with their target enzymes in three stages (Scheme 1) (1). In the first stage, the antibiotic (**A**) "fits" reversibly (k_1, k_{-1}) to the enzyme (**E**-OH), to form an enzyme–substrate complex (**E**-OH·**A**). Then OH, the hydroxyl group of a serine residue, "reacts" (k_2), and is acylated by the β-lactam carbonyl group. In the final stage, the acyl-enzyme (**E**-OCOR) is hydrolysed (k_3). This regenerates **E**-OH, and produces an inactive degradation product of the antibiotic.

When $k_3 \ll k_2$, Scheme 1 leads to inactivation of the enzyme, and an enzyme that exhibits these kinetics is termed a penicillin-binding protein (PBP) (2). The inactivation of a PBP comprises a killing mechanism, because these enzymes play an essential role in the biosynthesis of bacterial cell walls. When the biosynthesis is interrupted, the wall is weakened, becomes permeable to water, and the cell swells, bursts, and dies. On the other hand, if k_3 is large in Scheme 1, it is the substrate that is inactivated; an enzyme having this property is termed a β-lactamase (3), and its presence, or its ability to be induced when an organism is challenged by a β-lactam antibiotic, are primarily responsible for bacterial resistance to these antibiotics.

Theoretical considerations: "fit" and "reactivity"

We have recently discussed the abilities of penicillins (**1**) and cephalosporins (**2**) to "fit" to a computer-generated model (Fig. 1) of the peptide Ac-Val^1-Gly^2-Ser^3-Val^4-Thr^5-Lys^6-NHMe (**3**) (4). This peptide contains the residues that surround the active site serine of a PBP from *Streptomyces* R61, which has been studied in some detail (5). Since (*i*) structure–activity and conformation–activity relationships project important roles for the carboxyl group and the amide N-H of a β-lactam antibiotic in its interaction with a PBP (4); (*ii*) pH-dependence studies suggest that the carboxyl group of the antibiotic is charge-paired to the terminal amino group of Lys^6 (5*b*); (*iii*) in **3**, the carbonyl group of Val^1 and the Ser^3 and Lys^6 side chains are in proximity, we "fit" β-lactam antibiotics to **3** with one hydrogen bond from the side-chain N-H to the carbonyl group of Val^1, and a second hydrogen bond from the amino group of Lys^6 to the carboxyl group.

When this docking strategy is used to "fit" the crystal structure of penicillin G (6) to **3**, the complex which results (Fig. 2) has the Ser^3 hydroxyl group positioned on the convex face of the bicyclic molecule, poised for attack on the β-lactam group. The positions of the C-O-H and N-C=O reacting groups of Fig. 2 with respect to one another (C-O approximately *syn* to C=O, and O-H approximately *syn* to N-C on the convex face) are the same as those seen in the most recent modelling of a β-lactam antibiotic into the active site of the *crystal structure* of the *Streptomyces* R61 PBP (7). However, there is a mechanistic problem associated with these structures, because addition to the carbonyl group to form the expected tetrahedral intermediate of β-lactam alcoholysis cannot occur from a geometry in which C-O and C=O are *syn* to one another.



SCHEME 1

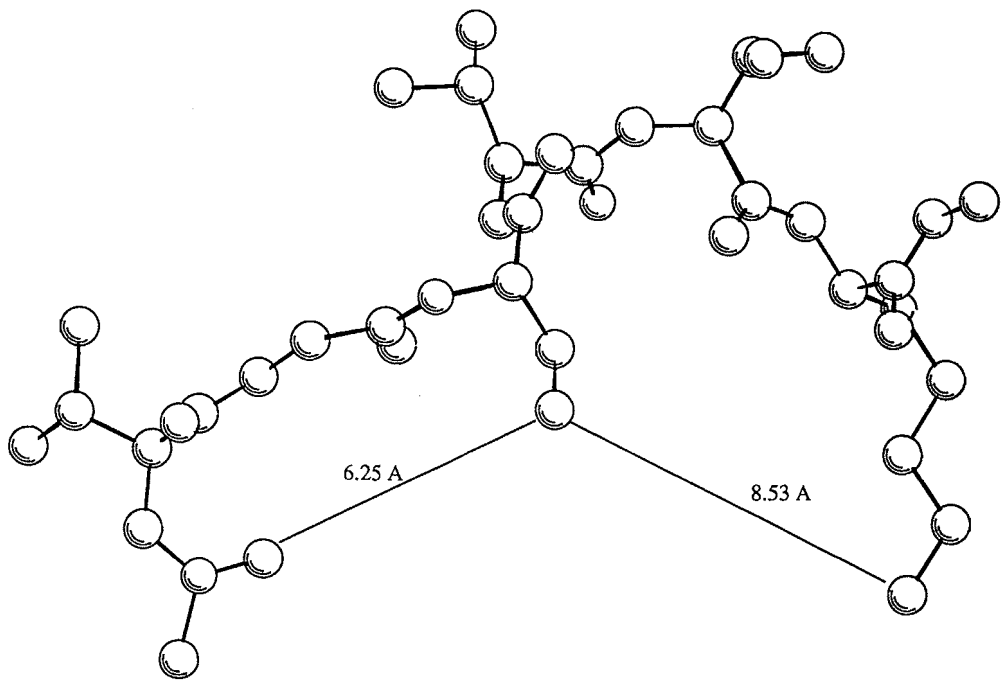


FIG. 1. A computer-generated model of the active site of a penicillin-binding protein having the sequence val-gly-ser-val-thr-lys. The model shows close contact, on the concave face, between the serine hydroxyl, the lysine amino, and a valine carbonyl.

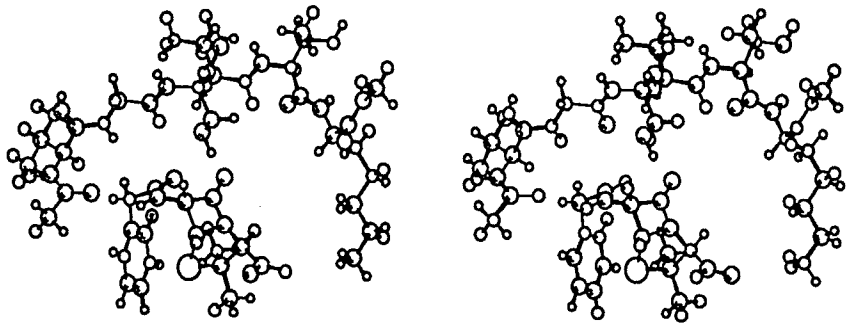


FIG. 2. Docking of the crystal structure of penicillin G to the active site model of Fig. 1. The creation of hydrogen bonds between the carboxyl group of the antibiotic and the amino group of lysine, and between the amide N-H of the antibiotic and the valine carbonyl, results in close contact between the serine hydroxyl group and the convex face of the penicillin.

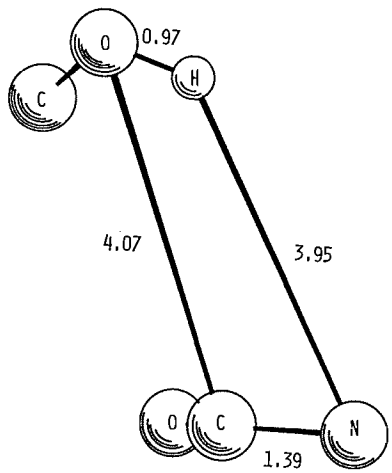


FIG. 3. A closeup view of the C-O-H/N-C=O relationship in Fig. 2.

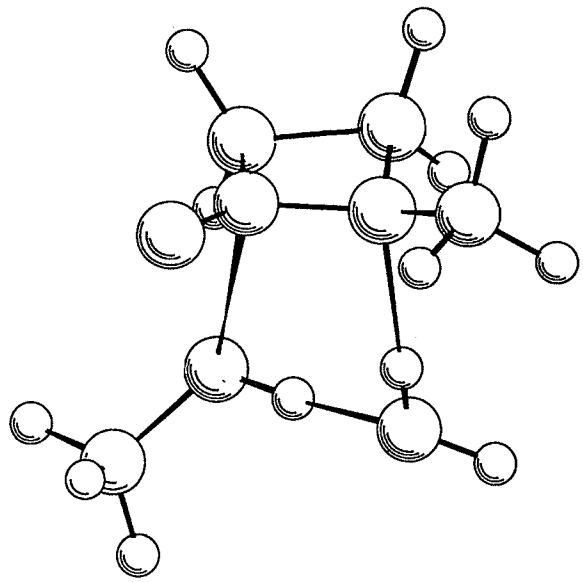


FIG. 4. Ab initio (3-21G) structure of the complex formed on the reaction coordinate when the reaction of methanol with *N*-methylazetidinone is catalyzed by one molecule of water.

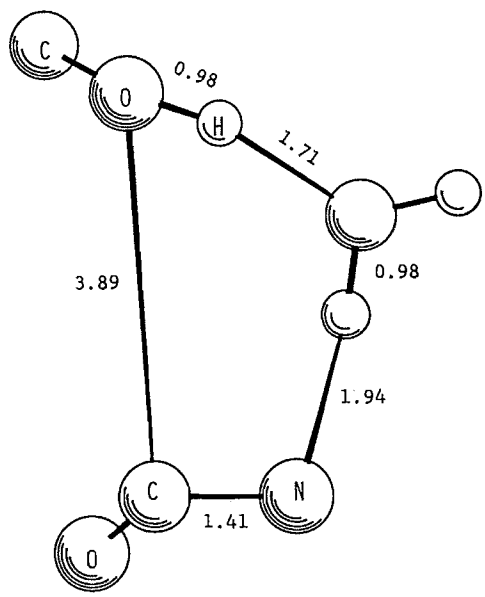


FIG. 5. A closeup view of the C-O-H/N-C=O/H-O-H bond lengths of the reactant complex of Fig. 4.

The nature of the problem is seen more clearly in Fig. 3, a closeup view of the C-O-H/N-C=O relationship in Fig. 2. This arrangement of the reacting groups suggests that the "reactivity" step of Scheme 1, which produces the acyl-enzyme, involves C=O bond formation and proton transfer to nitrogen, *concerted with the fission of the β-lactam bond.* As we report elsewhere (8), ab initio MP2/6-31G*//3-21G* calculations (9) of the neutral hydrolysis and alcoholysis of *N*-methylazetidinone (**4**) and penam (**5**) confirm that a concerted mechanism, with proton transfer to nitrogen, exists, and is 5–10 kcal/mol energetically more favorable than addition to the carbonyl group, with proton transfer to oxygen. In addition, attack on **5** from the convex face is more favourable than attack from the concave face.

What emerges from this theoretical analysis of the first two steps of Scheme 1 is the idea that a β-lactam antibiotic "fits" to a PBP in such a way as to permit acylation of a hydroxyl group via the mechanistically and energetically most favourable chemical "reaction". The realization that the action of the enzyme involves both "fit" and "reactivity" (10) is a variation of Emil Fischer's lock and key analogy (11), in which a key fits into a lock, and then turns to open the lock.

The methanolysis of **4** exhibits significant catalysis by one water molecule (8). In this case, based on 3-21G calculations, the reaction proceeds via a ternary complex (Fig. 4), whose C-OH/N-C=O/H-O-H bond lengths are highlighted in Fig. 5.

If the water molecule is deleted from Fig. 5, what remains resembles Fig. 3. This suggests that the structure of Fig. 3 is

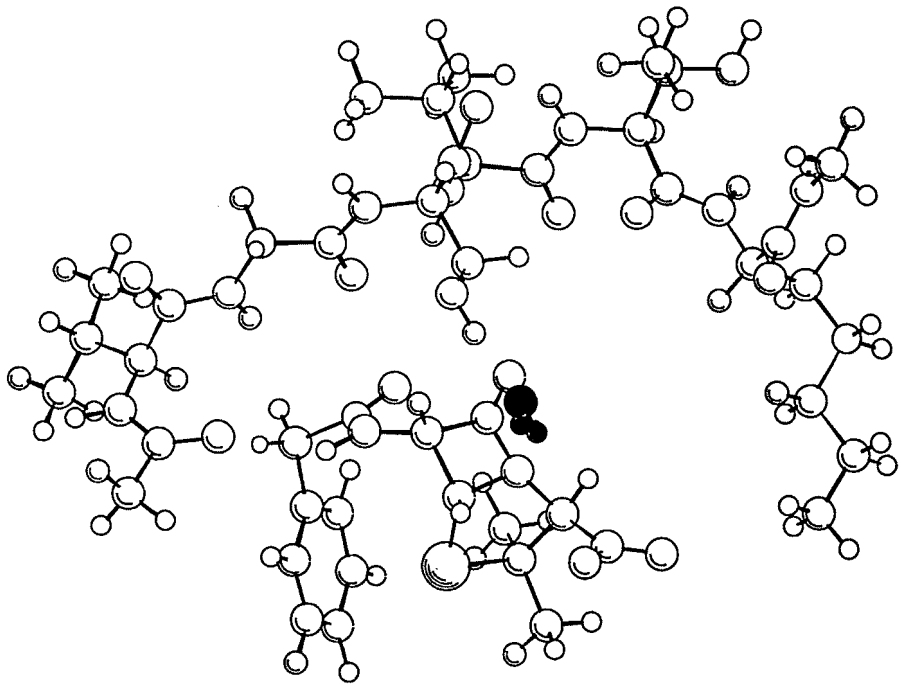


FIG. 6. Addition of one water molecule to the complex of penicillin G with the receptor model. The water molecule is in the centre of the structure.

able to accommodate a water molecule, and that at least one water molecule should also be included in the active site of Fig. 2. This would be consistent with the limited information concerning water in the active sites of β-lactamase crystal structures (12). Based on these considerations, the program developed to obtain the supermolecule of Fig. 2 (4) was modified[6] to allow a third molecule, in this case, water, to be included, with the result shown in Fig. 6 and, in close-up, in Figs. 7 and 8.

It is important to emphasize that the (nearly identical) Figs. 5 and 8 are obtained in very different ways. Figure 5, an ab initio result, is a closeup view of a stationary point on a reaction coordinate; Fig. 8, a molecular mechanics result, is a closeup view of an "enzyme"–water–substrate complex. Because of its ability to assemble the reactants in the proper manner, the "enzyme" has overcome the entropic disadvantage of a termolecular process.

Targeting a PBP

We wish to use the insights gained from the work just described to attempt the de novo design of a molecule that will "fit" to the active site of a PBP in the same manner as penicillin, "react" with the hydroxyl group of the active site serine through a geometry similar to that seen in Figs. 3 or 8, and exhibit antibacterial activity (13). As depicted in Fig. 9, our synthetic objective should possess a carboxyl group or its bioisosteric equivalent (G_1), to interact with the Lys^6 amino group of **3**, and also a hydrogen-bonding donor group X-H (G_2), to interact with the Val^1 carbonyl group of **3**. The distance between G_1 and G_2 can be determined from, e.g., the corresponding distance in the crystal structure of penicillin G.

The formation of the hydrogen bonds to G_1 and G_2 must now cause a functional group 𝔽 to be oriented into a four-centred relationship with respect to the serine hydroxyl group (cf. Fig. 3). A decision concerning the choice of 𝔽 is possible if a functional group can be found that reacts with a hydroxyl group via a four-centred geometry, possibly with catalysis by water, and with an activation energy comparable to that exhibited by a β-lactam. The search for 𝔽 was carried out computationally.

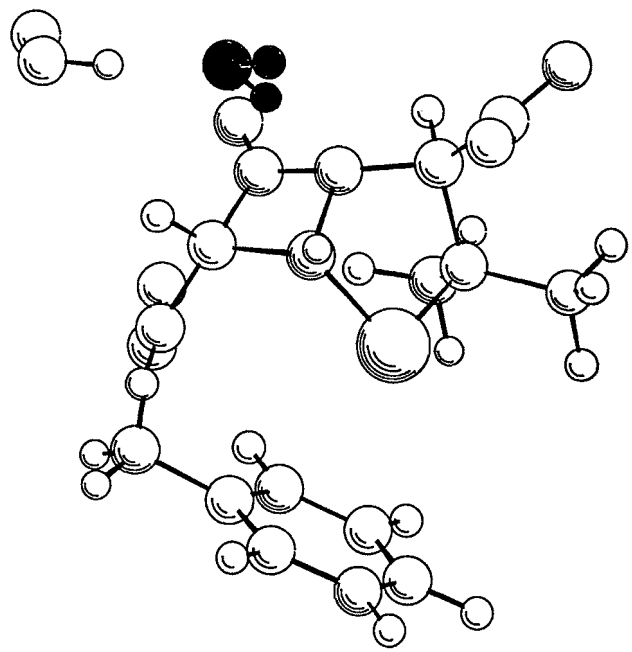


FIG. 7. Truncation of the structure of Fig. 6, showing the C-O-H of serine and the water molecule on the convex face of the penicillin.

Figure 10 is the N-protonated transition structure calculated, using MINDO/3, for the four-centred reaction of 2,2-dimethyl-3α-carboxypenam (**6**) with methanol. The activation energy of this reaction is 49.6 kcal/mol, 7.6 kcal/mol lower than that required for the alternative process of addition to the β-lactam carbonyl group. This trend is typical. As discussed elsewhere (14), MINDO/3, but not MNDO, MNDO/H, AM1,

[6]K. Yang and S. Wolfe. To be published.

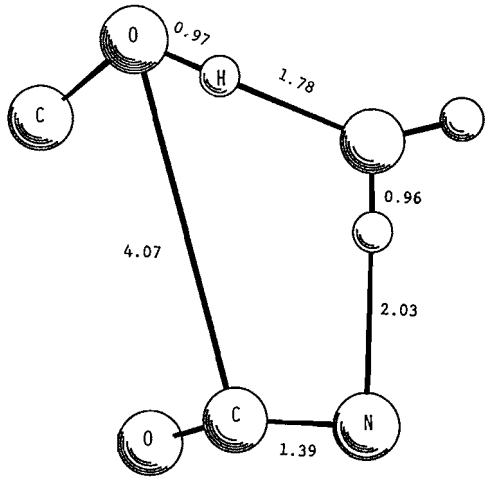


FIG. 8. A closeup view of the C-O-H/N-C=O/H-O-H bond lengths in Figs. 6 and 7.

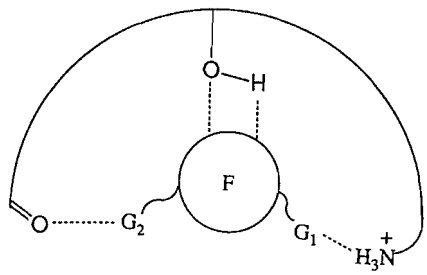


FIG. 9. Targeting the penicillin receptor.

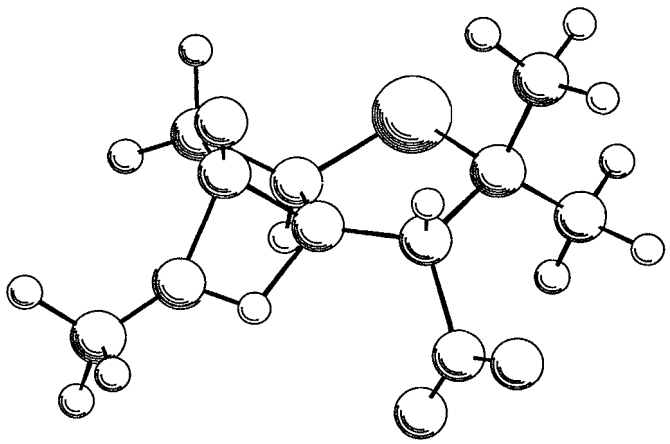


FIG. 10. MINDO/3 transition structure for the methanolysis of 2,2-dimethyl-3α-carboxypenam.

or PM3 reproduces the ab initio findings concerning the relative energies of the N- and O-protonated reaction pathways. In addition, MINDO/3 calculations of the activation energies for the methanolyses of azetidinones provide structure–reactivity trends that parallel the trends in the structure–activity relations of penicillins and cephalosporins (14). Therefore, the MINDO/3 semiempirical procedure was used for a preliminary survey of more general relationships between structure and four-centred reactivity towards methanol.

Table 1 lists the activation energies calculated for the four-centred reactions of methanol with the β-lactam (N-C=O), γ-lactam (N-C=O), β-lactone (O-C=O), γ-lactone

TABLE 1. Calculated activation energies (kcal/mol, MINDO/3), relative to 2,2-dimethyl-4-carboxypenam (6), for the neutral methanolysis of various compounds via a four-centred mechanism

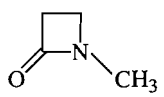
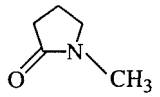
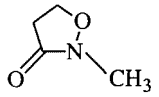
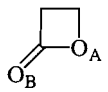
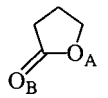
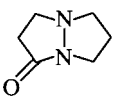
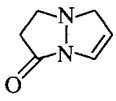
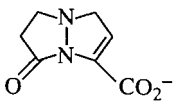
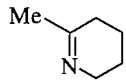
		
4.90 (N)	9.70 (N)	11.05 (N)
		
A 18.10 B 8.28	A 22.98 B 12.05	
		
10.52 (N)	10.52 (N)	9.92 (N)

TABLE 2. Calculated activation energies (kcal/mol, MINDO/3), relative to **6**, for the four-centred attack of methanol on some C=N containing compounds

				
−18.35	−15.33	−8.32	−14.09	−20.99

(O-C=O), oxazolidinone (O-N-C=O) (13*f*, 13*g*, 13*h*), and pyrazolidinone (N-N-C=O) (13*d*, 13e) functional groups, relative to the 49.6 kcal/mol calculated for **6**. The designations N, A, and B in Table 1 refer to the site to which the O-H proton of methanol is added. All of the compounds of Table 1 are calculated to be less reactive than **6**.

On the other hand, the data of Table 2 suggest that the imino group (C=N) is inherently more reactive towards methanol than the β-lactam ring of **6**, and that the reactivity of this group can be varied by variation of the ring size and the nature of the substituents.

The reactivities of several cyclic imines with methanol, with and without catalysis by water, were therefore examined more closely, using the 3-21G basis set for ab initio geometry optimizations, followed by one-point calculations of energies at the MP2/6-31G* level. In Fig. 11, A and B are the reactant complex and transition structure for the four-centred reaction with pyrroline, which produces the carbinolamine ether E; C and D are stereoscopic views of the complex and transition structure of the water-catalyzed process.

Table 3 summarizes the calculated reactivities towards methanol of *N*-methylazetidinone, 1-pyrroline, 2-oxazoline, 2-thiazoline, and also *N*-methyloxazolidinone. The first and second columns of Table 3 give the energy differences between separated reactants and transition structures for the uncatalyzed and catalyzed reactions; the third column shows the energy differences between the ternary reactant complexes and the transition structures of the catalyzed reactions.

The enhanced C=N reactivity predicted by the four-centred

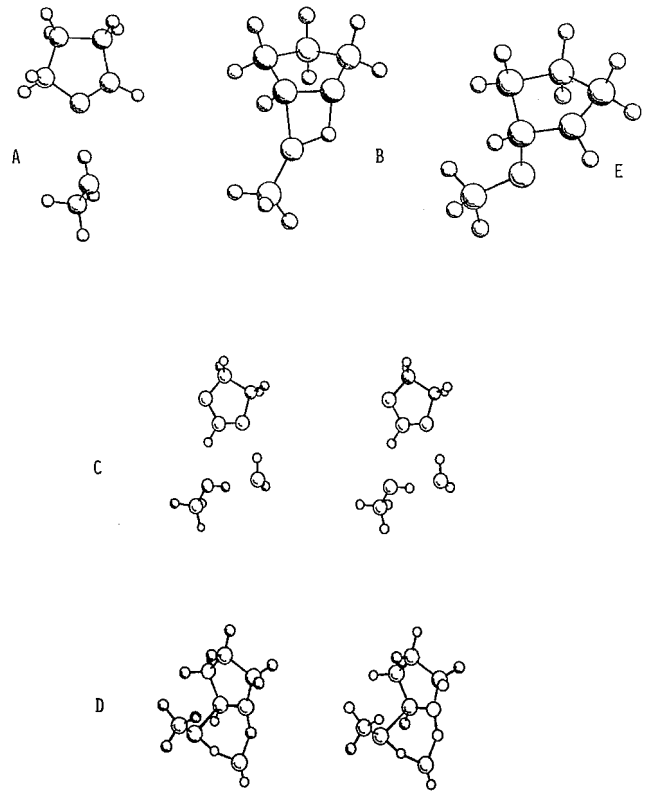


FIG. 11. Ab initio (3-21G) structures for the reaction of methanol with 1-pyrroline. A: reactant complex for the four-centred reaction. B: transition structure of the four-centred reaction. C: reactant complex for the water-catalyzed reaction. D: Transition structure of the water-catalyzed reaction. E: the carbinolamine ether product of the reaction.

TABLE 3. Ab initio activation energies[a] for the reaction of methanol with *N*-methylazetidinone (**4**),[b] 1-pyrroline, 2-oxazoline, 2-thiazoline, and *N*-methyl-1,2-oxazolidin-3-one (**A**)

Substrate	$\Delta E^{\ddagger}$ for reaction with: Methanol[c]	Methanol + water[d]	ΔE
4[e]	32.20[f]	16.62[f] (31.71)[g]	
1-Pyrroline[e]	40.42[f]	11.58[f] (33.25)[g]	−16.16[h]
2-Oxazoline[e]	39.86[f]	13.97[f] (35.45)[g]	
2-Thiazoline[i]	41.32[f]	— —	
A	29.37[f]	— —	

[a]In kcal/mol.
[b]Proton transfer to the β-lactam nitrogen.
[c]Four-centred reaction. See, e.g., Fig. 10.
[d]Reaction catalyzed by water. See, e.g., Fig. 4.
[e]MP2/6-31G*//3-21G calculations.
[f]Refers to the energy difference between separated reactants and transition structure.
[g]Data in parentheses refer to the energy difference between reactant complex and transition structure. See, e.g., C and D of Fig. 11.
[h]Energy change for MeOH + 1-pyrroline → 2-methoxy-1,3-oxazolidine.
[i]MP2/6-31G*//3-21G* calculations.

MINDO/3 calculations of Table 2 is not reproduced by the four-centred ab initio calculations of Table 3. In the β-lactam N-C=O, the lone pair of N and the π-orbital of C=O are approximately parallel, and the formation of the N—H and C—O bonds of the four-centred structure in a *syn* manner is not inhibited. In N=C, however, the lone pair of N and the

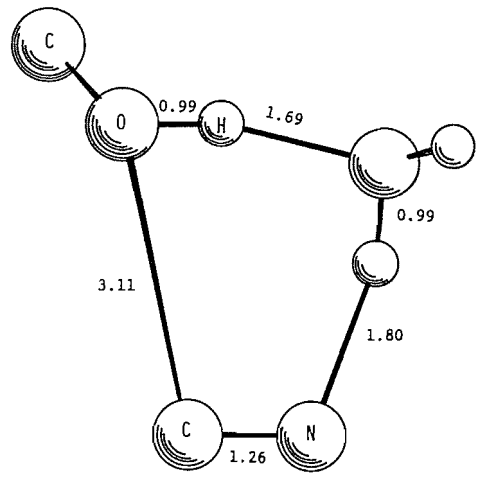


FIG. 12. A closeup view of the C-O-H/C=N/H-O-H bond lengths in the reactant complex C of Fig. 11.

π-orbital of N=C are approximately perpendicular, and *syn* formation of the N—H and C—O bonds is more difficult. This effect is taken into account in the ab initio calculations, but not by MINDO/3 (15). As can be seen in D of Fig. 11, the catalytic water molecule is important, because it allows the H-N-C-O dihedral angle to increase. The trends in the second and third columns of Table 3 are therefore more reliable than the four-centred trends, and suggest that the *bioreactivity* of C=N should be comparable to that of the β-lactam N-C=O. As seen in Fig. 12, the C=N group is also *bioisosteric* to N-C=O; this figure is a closeup view of the C-O-H/C=N/H-O-H bond lengths in C (Fig. 11), and can be compared to Fig. 5.

The final column of Table 3 contains the important result that the formation of a carbinolamine ether is an exoergic process. This means that the capture of a hydroxyl group by an imine function will be effectively irreversible, a necessary requirement for a compound targeted to a penicillin receptor. Furthermore, since the final deacylation step of Scheme 1 has no counterpart in the case of a carbinolamine ether, the incorporation of C=N into a properly designed compound could lead to the inhibition of both PBP's *and* β-lactamases.

It should be emphasized that the role of water could not be examined in the MINDO/3 calculations of Tables 1 and 2, because MINDO/3 does not treat intermolecular hydrogen bonds well, and predicts that water *inhibits* all of these processes (10). In addition, although AM1 correctly predicts catalysis, the use of AM1 for a systematic analysis of the catalyzed reactions fails for other reasons (10). Nevertheless, despite all of these limitations of the semiempirical calculations, these calculations do agree with the ab initio identification of C=N as a candidate for 𝔽.

In this connection, a referee has commented that excessive reliance on the MINDO/3 calculations of Table 1 would not have identified the X-N-C=O functionality of oxazolidinones (X = OR) and pyrazolidinones (X = NR_2) as potential candidates for 𝔽, and the existence of antibacterial agents containing these ring systems and targeted towards penicillin-binding proteins (13*d*, 13*e*, 13*f*, 13*g*, 13*h*) would not have been predicted.

The reason for the failure of MINDO/3 in the case of molecules having adjacent electron pairs (15) is that the neglect of one-centre overlap in the INDO procedure causes N—N (N—O) bonds to be too short and R-N-N (R-N-O) angles to be too large. The molecules then behave as though they have an

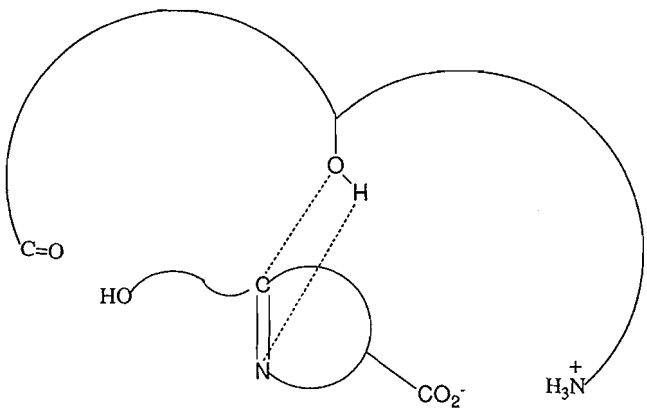


FIG. 13. Updated requirements for the targeting of the penicillin receptor.

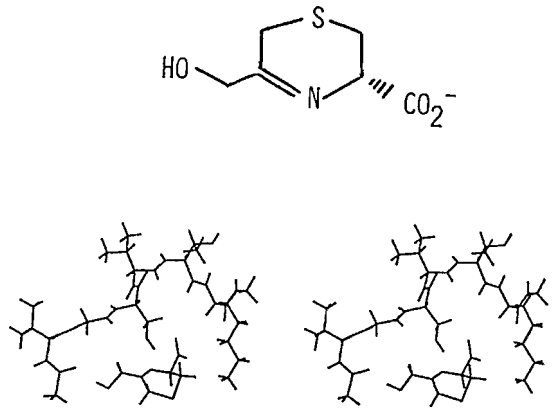


FIG. 14. Stereoscopic view of the initial synthetic target **7**, showing the *S* enantiomer docked to the penicillin receptor model.

electron-withdrawing substituent attached to a planar nitrogen, and proton transfer to this nitrogen becomes more difficult. As seen in the final entry of Table 3, the 6.15 kcal/mol reactivity preference for *N*-methylazetidinone over *N*-methyloxazolidinone that is predicted by MINDO/3 (Table 1) has been reversed at MP2/6-31G*//3-21G, to a 2.83 kcal/mol preference for the reaction of *N*-methyloxazolidinone. Clearly, ab initio calculations should be given priority in future computational studies related to 𝔽.

The identification of C=N as our current candidate for 𝔽 allowed Fig. 9 to be revised to Fig. 13, in which G_1 is CO_2^-, and G_2 is OH, and it was now necessary to create the desired spatial relationship between G_1, C=N, and G_2. This was done, crudely at first, by inspection of molecular models. For example, based on the dihedral angles in penicillin G, a compound having the part structure H-O-C1-C2=N-C3-CO_2^- would require O-C-C=N and C=N-C-C dihedral angles of −155° and +155°, respectively. Structures in which C1 and C3, or C2 and C3 were connected by different sized rings were, therefore, examined; these, in turn, suggested other possibilities.

A more careful conformational analysis of each candidate structure identified in this way was then performed, using MM2 (28). Previously unknown MM2 parameters were found, where necessary, using established procedures (14, 16). The parameters thus determined for the compounds to be discussed below are available as Supplementary Material.[7]

Each candidate was docked to **3** via the two hydrogen bonds already noted, and the relationship between the C=N of the candidate and the O-H of Ser^3 in the resulting complex was examined. This procedure eventually identified a number of targets for synthesis, and a programme directed towards the 2*H*-5,6-dihydro-1,4-thiazine **7** (cf Fig. 14), was initiated. Because it was known (17) that dihydrothiazines exhibit imine–enamine tautomerism (e.g., **8***a–c*), some preliminary experiments were necessary to assess the possible complications resulting from such tautomerism.

Preliminary Synthetic Studies

The reaction of L-cysteine with 1-benzoyloxy-3-chloropropanone (**9**), prepared in three steps from epichlorohydrin (18), produced an unstable product from which, after esterification with diazomethane, only the enamine **10** could be isolated. The same compound was obtained when **9** was reacted with the methyl ester of cysteine. Attempts to shift the double bond of **10** were not successful.

After numerous variations of the above synthesis had been examined, a compound with the gross structure **12***a* was isolated from the reaction of L-cysteine methyl ester with methyl bromoacetoacetate (**11**). However, this compound is a special case, since its ^{1}Hmr spectrum is consistent with the zwitterion structure **12***b* (no side-chain CH_2, but N^+-H at 9.00 and C=C-H at 4.63 ppm).

It was concluded that these 5,6-dihydro-1,4-thiazines prefer the 4H-tautomeric structure **8***b* and that, to avoid this structure, it would be necessary to place substituents at C2. The original objective **7** was, therefore, modified to **13***a*, which is the cyclodehydration product of **14***a*.

Scheme 2 summarizes the synthesis of the fully protected cysteine derivative **14***b*. Bromination of methyl isopropyl ketone afforded **15**, which was oxidized to **16** with lead tetraacetate, and then thiolated to **17** with triethylamine – hydrogen sulfide. Condensation of the lithium salt of **17** with the Vederas lactone **18** (19) proceeded smoothly to yield the acid **14***c*, and thence **14***b*.

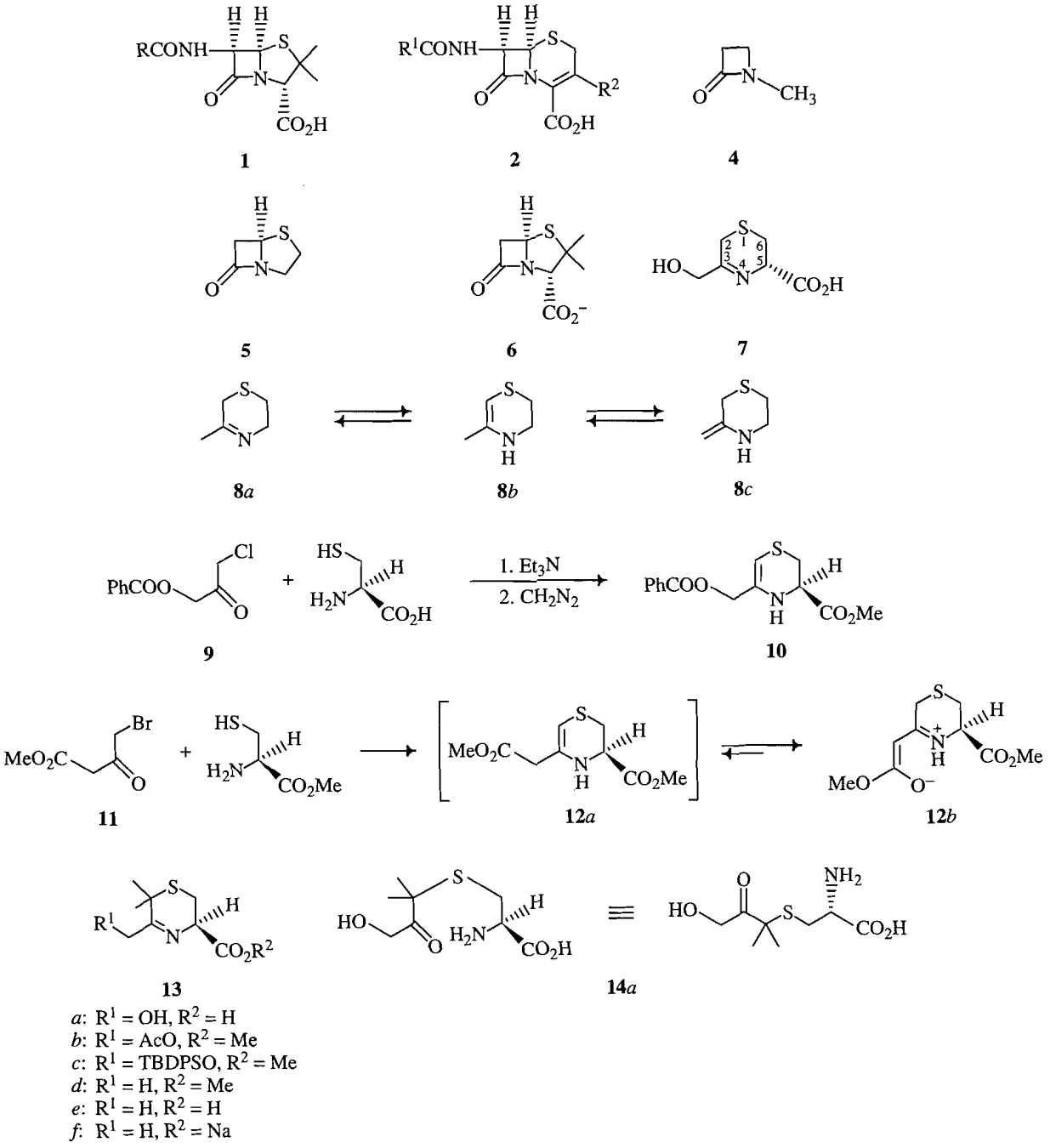
Removal of the *tert*-butoxycarbonyl protecting group of **14***b* with anhydrous formic acid (20) led directly to the protected dihydrothiazine **13***b*. That cyclization had taken place in the desired manner was immediately apparent from the observation of ^{1}Hmr coupling, through the C=N double bond, between the exocyclic methylene protons and H5.

Unfortunately, **13***b* was not stable, and decomposed completely within 1 day to acetic acid and the thiazine **19** (Scheme 3). This decomposition may proceed by 1,4-elimination of acetic acid from **13***b*, to form **20**, which tautomerizes to **19**. The alternative tautomerization, from **13***b* to **21**, could not be observed. However, the experiments summarized in Scheme 4 demonstrate that the latter process does occur. Lithium hydroxide hydrolysis of **14***b*, and remethylation, afforded **14***d*, which was silylated to **14***e*. The deprotection of **14***e* with formic acid did not proceed as with **14***b*; instead the product was the exocyclic enamine **22**, and not the dihydrothiazine **13***c*. Treatment of **14***d* with formic acid gave a complex reaction mixture from which no identifiable product could be isolated.

The imine tautomer is sometimes stable

The observations summarized so far seem to be consistent

[7]Supplementary data may be purchased from: The Depository of Unpublished Data, Documentary Delivery, CISTI, National Research Council Canada, Ottawa, Canada K1A 0S2.



1 2 4 5 6 7 8a 8b 8c 9 10 11 12a 12b 13 14a

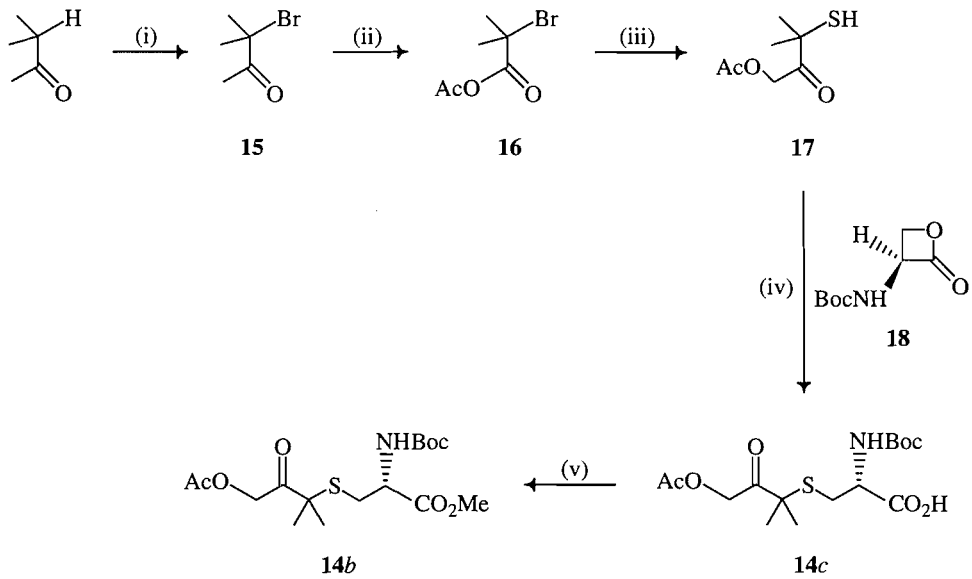
a: R^1 = OH, R^2 = H
b: R^1 = AcO, R^2 = Me
c: R^1 = TBDPSO, R^2 = Me
d: R^1 = H, R^2 = Me
e: R^1 = H, R^2 = H
f: R^1 = H, R^2 = Na

with prior work (17): in the C2-unsubstituted system the endocyclic enamine is the preferred tautomeric structure; when this structure is blocked by the introduction of methyl groups, the relative stabilities of the imine and exocyclic enamine structures depend upon the nature of R^1 of **13**. This latter point was examined further with the deoxy compound **13***d*, prepared from the bromoketone **15** by thiolation, condensation with **18** as in Scheme 2, methylation, and deprotection with formic acid.

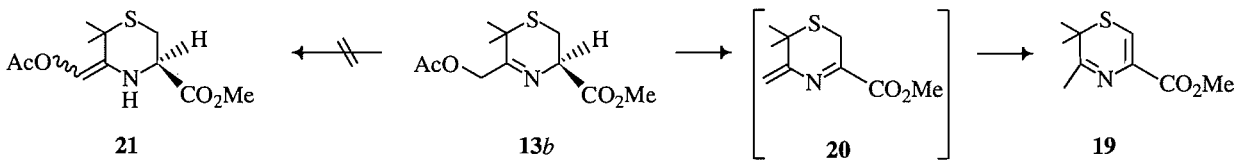
The imine structure **13***d* was readily identified by the 2.0 Hz coupling between H5 and the C3 methyl protons. Only this structure could be observed in pure chloroform solvent, and the compound appeared to be stable for at least several days. In one preparation, however, the chloroform was not pure, and **13***d* underwent complete rearrangement to the enamine tautomer **23***a* in 1 day (N-H at 5.20 Hz; C=CH_2 singlets at 4.59, 4.60 Hz).

A knowledge of the chemical shifts of **23***a* proved useful in the next set of experiments. Hydrolysis of **13***d* to the acid **13***e* was successful, using lithium hydroxide. Only the imine tautomer of the sodium salt **13***f* could be observed in D_2O solvent during a period of 3 days at room temperature. However, during this period, the C3 methyl protons underwent hydrogen–deuterium exchange. The solution of **13***f* was lyophilized at this point, and the compound was redissolved in H_2O. Because of the isotope effect, the original spectrum of **13***f* required 5 days to reappear fully. At no time during these experiments in D_2O and H_2O was any other compound (e.g., **23***b*) observable by nmr.

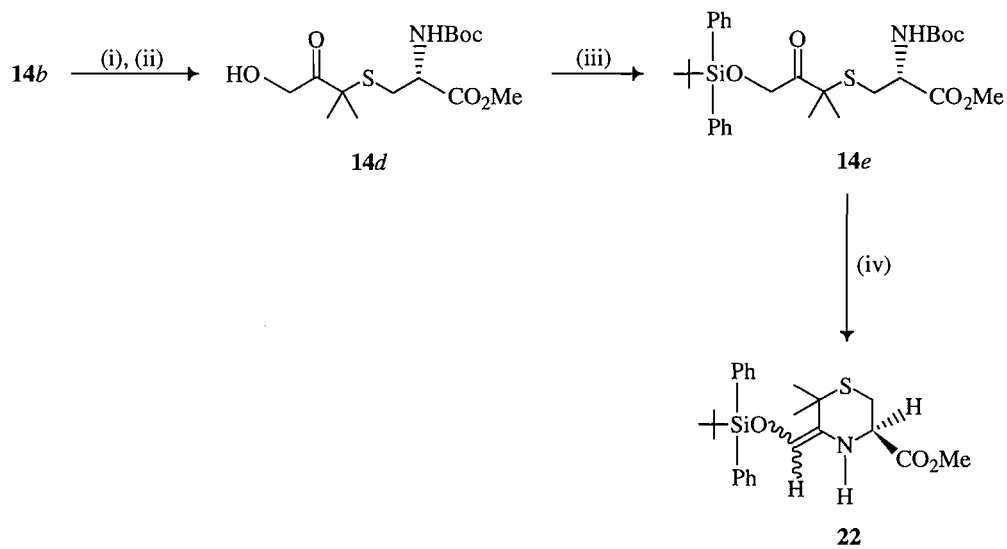
The simplest explanation of these observations is that the imine–enamine equilibrium **13***f* ⟶ **23***b* lies on the side of the imine tautomer in water, and this leads to the exchange of the methyl protons in a deuterated solvent.

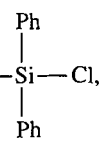


SCHEME 2: (i) Br_2, KCl, H_2O, *hν*; (ii) HOAc, LTA, 100°C; (iii) CH_2Cl_2, Et_3N, H_2S, –20°C; (iv) LDA, DMF–THF, –60°C; (v) CH_2N_2



SCHEME 3



SCHEME 4: (i) LiOH; (ii) CH_2N_2; (iii)  ; (iv) HCO_2H

Interaction of theory and synthesis: the 2-hydroxypropyl side chain

It was now clear that the desired ring system could be attained, and the imine tautomer maintained in water solvent. However, it was also clear that the molecule does not tolerate oxygen on the carbon atom adjacent to the C=N double bond. The hydroxyl group of **13***a* would therefore have to be repositioned. Replacement of the hydroxymethyl side chain of **13***a* by

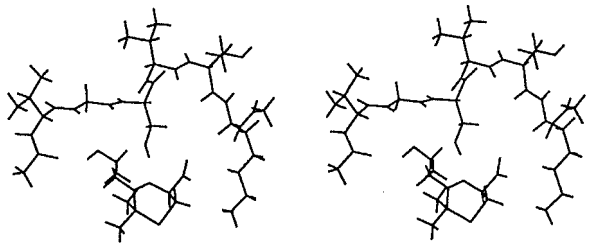


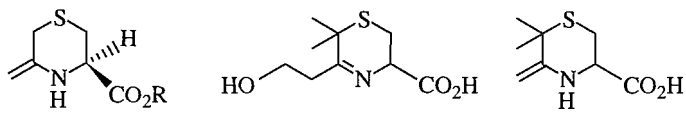
FIG. 15. Stereoscopic view showing one of the conformations of one of the stereoisomers of **26** docked to the penicillin receptor model.

a hydroxyethyl side chain leads to **24**. However, this compound is, formally, the primary product of the reaction of the enamine **25** with formaldehyde, and was feared to be unstable with respect to the reverse of the latter process. The hydroxypropyl side chain (**26**) was, therefore, examined.

Figure 15 is a stereoscopic view of the structure obtained by docking **3** to one of the conformations of the 3**S**,8**R** isomer of **26** (21). This structure is compatible with the formal design requirement depicted in Fig. 13, and the synthesis of **26**, the cyclodehydration product of **27** ($R^1 = R^2 = H$), was undertaken.

Based on the preliminary studies already described, it was expected that **27** would be accessible from the reaction of the mercaptoketone **28** with **18**, and that **28** could be synthesized from the ketol **29**. If an olefin-to-ketol oxidation **30** → **29** could be achieved, the starting materials for the synthesis of **26** would then be 5-methyl-4-hexen-2-ol (**30**, R = H) and serine. Since the racemic bromide **31** was known from the work of Julia et al. (22), the first synthesis of **26** was directed towards the 3**R** and 3**S** isomers, starting with **31** and, respectively, L- and D-serine.

The bromide **31** was converted, via the acetate (**30**, R = OAc) and alcohol (**30**, R = OH), to the *tert*-butyldimethylsilyl (*t*-BDMS) compound (**30**, R = *t*-BDMS). As shown in Scheme 5, the key olefin-to-ketol transformation was accomplished

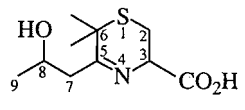


23

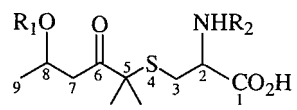
a: R = Me
b: R = Na^+

24

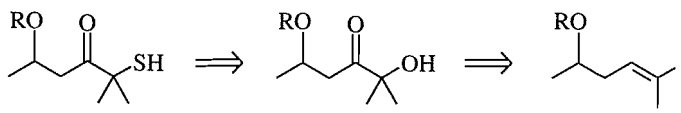
25



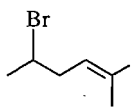
26



27



28 **29** **30**



31

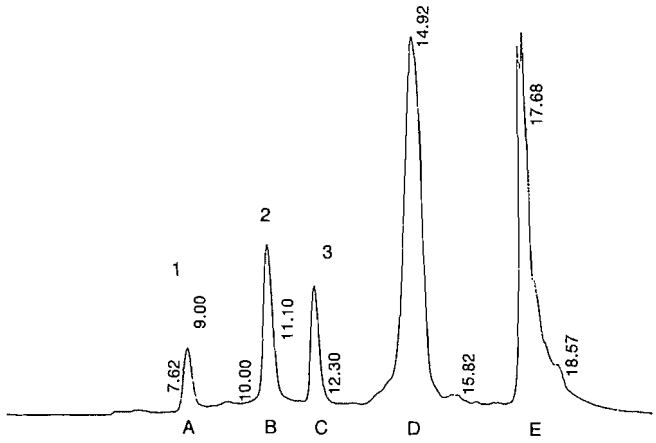
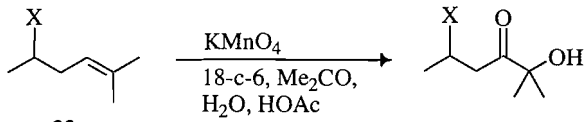


FIG. 16. HPLC examination of **26**. Peak D has the assigned structure.



32 **33**

SCHEME 5

using potassium permanganate (23). Although the reaction conditions are rather special, this is a general reaction, which usually proceeds in 70–80% yield.[8]

The silylated ketol **29** (R = *t*-BDMS) was converted to the thiol **28** (R = *t*-BDMS), following activation as the mesylate and, as the lithium salt, **28** reacted with D- and L-**18** to produce the 2**S** and 2**R** isomers of **27** (R^1 = *t*-BDMS, R^2 = *t*-Boc) in over 80% yield.

In addition to **27** (R^1 = *t*-BDMS), the compounds **27** (R^1 = Ac) and **27** (R^1 = *tert*-butyldiphenylsilyl) were also synthesized. The latter two did not undergo clean removal of the *t*-Boc protecting group. Formic acid treatment of the acetate or trifluoroacetic acid treatment of the *tert*-butyldiphenylsilyl ether resulted in concomitant elimination of the C8 substituent of **27**. On the other hand, treatment of **27** (R^1 = *t*-BDMS) with formic acid led to the removal of both protecting groups, and cyclization to **26**.

The 3**R** and 3**S** epimers of **26**, synthesized separately from L- and D-serine, are not particularly stable. Figure 16 is the analytical chromatogram obtained, using an acetonitrile–water gradient with detection at 250 nm, for a sample of the amorphous sodium salt of 3**S**-**26**, after this had been maintained at 0°C for several weeks, or at room temperature for 1 day. The peak labelled D comprises ca. 45% of the mixture, and is also the initial product of the deprotection of **27**; the nmr and high-resolution mass spectra of D confirm structure **26**. Each of the peaks seen in Fig. 16 begins to reappear after several hours when the sodium salt of **26** is dissolved in water. The bioassay of **26** was, therefore, carried out as soon as possible after the isolation of the compound, and always within 1 day.

Bioassay of 26S and 26R: 26S is an antibacterial agent

The bioassay of **26S** and **26R** was carried out at 37°C using

[8]H. Jin and S. Wolfe. To be published. See also ref. 29.

TABLE 4. Inhibition of *S. aureus* by the thiazine **27**[a]

Amount (mg)	Zone of Inhibition (mm)
0.05	6.0
0.10	8.5
1.0	19
1.0	18

[a]Potassium penicillin V (0.10 μg) gives a 13 mm zone of inhibition.

4.0 mm filter discs, loaded with known weights of material, and placed on (*i*) *Sarcina lutea* seeded plates prepared by Mr. Dennis Muncey, Department of Microbiology, Queen's University; (*ii*) *Escherichia coli* seeded plates prepared by Dr. G.J. Delisle, Hotel Dieu Hospital, Kingston; (*iii*) penicillin sensitive *Staphylococcus aureus* plates prepared by Dr. J.A. Smith, Vancouver General Hospital.

The *R* isomer, synthesized from L-serine, did not exhibit activity against any of these organisms, in amounts up to 1000μg. At this level, the *S* isomer showed activity against all of the organisms. Direct comparisons with authentic antibiotics indicated that **26S** has 1/800 the activity of penicillin G versus *S. lutea*, and 1/10 the activity of cephalexin versus *E. coli*. As seen in Table 4, inhibition of *S. aureus* could be observed using 50 μg of **26S.**

Although the activity thus observed with the *S* isomer of **26** is extremely modest in comparison to that of penicillin G, as a lead structure representing the prototype of a designed antibacterial agent, **26S** is already comparable to a Δ^2-cephalosporin (10), which requires only one structural change (Δ^2 to Δ^3 isomerization) to become a useful antibiotic. Furthermore, the much lower activity of the *R* isomer of **26** suggests that **26S** is not a general bactericidal agent, but functions via a three-dimensional fit to a specific receptor. An understanding of the nature of this fit will require bioassay data for each of the four stereoisomers of **26**, and, eventually, kinetic studies of the reactions of these compounds with penicillin-recognizing enzymes. The syntheses and bioassays of the four stereoisomers of **26** are reported in an accompanying paper (21). However, because of its chemical instability, binding studies with **26** are not currently planned, unless systematic modification of the lead structure is unable to improve the stability.

Experimental

General experimental

All syntheses with sulfur-containing compounds were performed under dry nitrogen. Anhydrous magnesium sulfate was used routinely as a drying agent. Melting points were determined on a Fisher–Johns apparatus, and are uncorrected. Solvents were distilled before use and dried, as necessary, by literature procedures. Optical rotations were obtained on Perkin Elmer 141 or Rudolph Automatic polarimeters. Infrared (ir) spectra were recorded on a Perkin–Elmer 599B spectrometer. Nuclear magnetic resonance (nmr) spectra were run at 400 MHz on Bruker WM or Bruker AM spectrometers. High-resolution mass spectra refer to direct inlet EI measurements on a Kratos MS50 Spectrometer.

Analytical thin-layer chromatography was carried out on precoated Merck Silica Gel 60 F-254 plates with aluminium backing. Spots were observed under short-wavelength ultraviolet light, or were visualized with iodine vapour, ninhydrin, or ceric sulfate. Preparative layer chromatography (plc) was performed on Silica gel 60 F-254 precoated 20 × 20 cm plates (layer thickness 2mm) or, alternatively, glass plates were coated with a slurry of Macherey Nagel silica gel G-HR and calcium sulfate. Flash chromatography was carried out with 230–400 mesh Merck silica gel. The high-performance liquid chromatography (HPLC) system consisted of an M600 gradient module, U6K universal sample injector, 486 tunable absorbance detector, 170 sample loader, M746 data module, and fraction collector plus diverter valve, all from Waters. Analytical work was carried out using a 8 × 10 10-μm 125 Å μBondapak column. Preparative work was performed using a 25 × 10 10-μm 125 Å μBondapak column, with a loading of 25 mg per run. Acetonitrile and methanol for HPLC analyses were HPLC grade. Water for HPLC was collected through a Millipore purifier and degassed prior to use.

Elemental analyses are by M.K. Yang on a Carlo Erba model 1106 elemental analyzer at Simon Fraser University.

Theoretical

Ab initio molecular orbital calculations were carried out using GAUSSIAN 86 (24), GAUSSIAN 90 (25), or GAUSSIAN 92 (26). Semiempirical molecular orbital calculations were performed using AMPAC Version 2.1, kindly provided by Professor M.J.S. Dewar, or MOPAC Version 6.0 (27). Molecular mechanics calculations employed an extensively locally modified version of MMP2(85) (28). Docking of two molecules to form an intermolecular complex was accomplished as follows: first, preliminary MM2 calculations were carried out on these molecules to obtain, for predetermined conformations of each, their input data files. Then the numbering of the atoms that had been selected to engage in intermolecular hydrogen bond formation was determined. Next, the energy of the supermolecule created by these hydrogen bonds was minimized to produce an MM2 input file for the supermolecule. The docking of three molecules was carried out in two steps: in the first, the strategy just described was employed to dock two of the three molecules. Then the third molecule was added, using the appropriate numbers to identify the atoms engaging in the hydrogen bonds. Structures are drawn using a locally modified version of CHEMGRAF. We thank Dr. David Watkin, Oxford University, for a copy of the source code of this programme.

*Synthesis of the endocyclic enamine **10***

A solution of epichlorohydrin (10 mL) in water (10 mL) containing *p*-toluenesulfonic acid (50 mg) was refluxed for 9 h, cooled, neutralized by addition of sodium bicarbonate (22 mg), and distilled under reduced pressure. After removal of the water, 2-chloropropane-1,2-diol (9.3 g) was collected at 55°/2 Torr (1 Torr = 133.3 Pa). A solution of this diol (1.1 g, 10 mmol) in methylene chloride (10 mL) was cooled in an ice-bath, and treated successively with triethylamine (2.09 mL, 1.52 g, 15 mmol) and benzoyl chloride (1.16 mL, 1.40 g, 10 mmol). After 1 h, additional methylene chloride (10 mL) was added, and the solution was washed successively with water (2 × 10 mL), 0.1 N hydrochloric acid (2 × 10 mL), saturated sodium bicarbonate (1 × 10 mL), water (1 × 10 mL), and saturated sodium chloride (1 × 10 mL), dried, and evaporated. The crude monobenzoate (1.89 g, 8.8 mmol) was used directly in the next step. It was dissolved in methylene chloride (30 mL), and pyridinium chlorochromate (PCC) (9.5 g) was added in portions. The reaction mixture was stirred at room temperature for 6 h and additional PCC (5.0 g) was then added. Stirring was continued for 30 h. The mixture was then filtered, and after washing with methylene chloride (2 × 10 mL), the combined filtrates were washed successively with 0.5 N hydrochloric acid (2 × 10 mL), saturated sodium bicarbonate (2 × 10 mL), water (1 × 10 mL), and saturated sodium chloride (1 × 10 mL), dried, and evaporated. The residue was chromatographed on 5 g of silica gel. Successive elution with 10, 15, 25, and 50% ethyl acetate – hexane yielded 483 mg of the crystalline chloroketone **9** mp 94°C (lit. (18) mp 94°C).

To a solution of L-cysteine (12.1 mg, 0.1 mmol) in methanol (0.3 mL) were added consecutively triethylamine (28 μL, 0.2 mmol), dimethylformamide (DMF) (0.1 mL), and a solution of **9** (21.3 mg, 0.1 mmol) in DMF (0.2 mL). After 10 min, acetic acid (6 μL) was added, followed by ethereal diazomethane until the yellow colour per-

sisted. After an additional 10 min, ethyl acetate (5 mL) was added, and the reaction mixture was washed successively with water (2 × 5 mL) and saturated sodium chloride (1 × 5 mL), dried, and concentrated to yield 26 mg of crude product. This was purified by plc (silica gel, methylene chloride:ethyl acetate:acetic acid, 1.2:0.8:0.1, one elution) to give 7 mg, (24%) of **10**, $[\alpha]_D^{25}$ 172 (*c* 5, MeOH); ^{1}Hmr ($CDCl_3$), δ: 8.06 (2H, d, 7 Hz), 7.59 (1H, t, 7 Hz), 7.48 (2H, t, 7 Hz), 6.06 (1H, br, N-H), 5.50 (1H, C═C-H), 4.96 (1H, d, 12 Hz, O-C-H), 4.89 (1H, d, 12 Hz, O-C-H), 4.53 1H, d, 12 Hz, N-C-H), 3.87 (3H, s, ester), 3.35 (1H, d, 12 Hz, S-C-H), 2.31 (1H, t, 12 Hz, S-C-H). Decoupling experiments established that the protons at δ 4.53 and 3.35 are not coupled.

In a second experiment, the methyl ester hydrochloride of L-serine (207 mg, 1.2 mmol), in methanol (2 mL), was cooled in an ice-bath and triethylamine (336 μL, 2.4 mmol) and a solution of **9** (256 mg, 1.2 mmol) in dimethylformamide (3.7 mL) were added. The clear solution was stirred for 35 min, and then concentrated under reduced pressure to remove most of the methanol. Methylene chloride (50 mL) was added, and the resulting solution was washed successively with saturated sodium bicarbonate (2 × 10 mL), water (2 × 10 mL), and saturated sodium chloride (1 × 10 mL), dried, and evaporated. The residue was chromatographed on silica gel (11 g); successive elution with 10, 15, 30, 40, 50, and 60% acetone–hexane afforded, in the latter eluate, 48 mg (14%) of **10**.

*Synthesis of the zwitterionic imine **12**b*

Ketene dimer (1.05 g, 12.5 mmol, purified by distillation at 48–54°C/90 Torr) was dissolved in carbon tetrachloride (8 mL), cooled in an ice-bath, and a solution of bromine (2.0 g, 12.5 mmol, freshly treated with concentrated sulfuric acid), in carbon tetrachloride (5 mL) was added dropwise. Stirring was continued for 5 min, and methanol (0.6 mL, 0.47 g, 14.8 mmol) was then added. After 10 min, the reaction mixture was washed with water until the aqueous washings were neutral (3 × 10 mL), and then successively with saturated sodium bicarbonate (1 × 10 mL), water (1 × 5 mL), and saturated sodium chloride (1 × 5 mL), dried, and concentrated to yield 1.95 g of the bromoacetoacetate **11**.

The methyl ester hydrochloride of L-serine (17.2 mg, 0.1 mmol), in methanol (0.1 mL), was cooled in an ice-bath, and **11** (19.5 mg, 0.1 mmol), in methanol (1.1 mL), was added. The solution was stirred, allowed to warm to room temperature, and, after 30 min, triethylamine (28 μL, 0.2 mmol) was added. After 10 h, the solvent was removed and the residue, in methylene chloride (15 mL), was washed successively with saturated sodium bicarbonate (1 × 5 mL), water (1 × 5 mL), and saturated sodium chloride (1 × 5 mL). The solvent was dried and evaporated, and the product purified by chromatography on a column containing 1.5 g of silica gel. Elution with 20% ethyl acetate–hexane afforded 10 mg (43%) of ***12b***; ^{1}Hmr ($CDCl_3$), δ: 9.00 (1H, br, N^+-H), 4.63 (1H, s, C═C-H), 4.27 (1H, dd, 4, 9 Hz, N-C-H), 3.82 (3H, s), 3.66 (3H, s), 3.36 (1H, d, 14 Hz, S-C-H), 3.29 (1H, dd, 4, 14 Hz, S-C-H), 3.17 (1H, d, 14 Hz, S-C-H), 3.02 (1H, dd, 9, 14 Hz, S-C-H).

*Synthesis of **14**b*

*A. Preparation of the thiol **17***

Methyl isopropyl ketone (15 mL, 140 mmol) was added to a solution of potassium chloride (1.1 g, 14.8 mmol) in water (9.6 mL). The mixture was stirred, warmed to 60°C, and illuminated with a 350-W tungsten lamp mounted beside the flask. Bromine (11.9 g, 74.4 mmol) was then added dropwise. When the colour of the first few drops had disappeared, the heating bath was replaced by a cold water bath, and the 350-W bulb was replaced by a 60-W bulb. Addition of bromine was continued at a rate sufficient to maintain the internal temperature at 40–45°C. When the addition was complete (25 min), the reaction mixture was allowed to stand for 2 h and the organic phase was then separated, washed with water – magnesium oxide, and dried over anhydrous calcium chloride. Fractional distillation afforded 7.0 g (30%) of **15**, bp 82–86°C/145 Torr; ^{1}Hmr ($CDCl_3$), δ: 2.36 (3H, s), 1.77 (6H, s).

The bromoketone **15** (4.65 g, 28 mmol) was dissolved in glacial acetic acid (40 mL), and freshly recrystallized lead tetraacetate (12.5 g, 28.2 mmol) was added. The mixture was heated at 100°C, with stirring, for 2 h and cooled to room temperature. Ethylene glycol (2 mL) was then added to destroy unreacted lead tetraacetate. The reaction mixture was diluted with ether (100 mL), washed successively with 10% sodium carbonate, water, and saturated sodium chloride, dried, and evaporated. The residue was distilled, and the fraction boiling at 57–60°C/120 Torr was further purified by chromatography (silica gel, 5% → 10% → 15% ether–hexane) to give the bromoketoacetate **16** (750 mg, 11%); ^{1}Hmr ($CDCl_3$), δ: 5.16 (2H, s), 2.13 (3H, s), 1.87 (6H, s).

Triethylamine (140 μL) was added to methylene chloride (3 mL). The solution was cooled to −20°C, and gaseous hydrogen sulfide was introduced during 10 min. Then the bromoketoacetate **16** (200 mg), in methylene chloride (1.0 mL), was added dropwise with stirring during 10 min. The yellow solution was diluted with methylene chloride (30 mL), washed successively with 2 N hydrochloric acid, water, and saturated sodium chloride, dried over anhydrous sodium sulfate, and evaporated to yield the mercaptoketoacetate **17** (145 mg, 96%); ^{1}Hmr ($CDCl_3$), δ: 5.16 (2H, s), 2.18 (3H, s), 1.57 (6H, s) 1.55 (1H, s).

*B. Preparation of the β-lactone **18***

To triphenylphosphine (258 mg, 0.98 mmol) in dry tetrahydrofuran (1.0 mL) was added dropwise with stirring at −78°C a solution of dimethylacetylenedicarboxylate (144 mg, 0.99 mmol) in tetrahydrofuran (1.0 mL). The white slurry was maintained at −78°C for 10 min, and a solution of Boc-L-serine (184 mg, 0.90 mmol) in tetrahydrofuran (1.0 mL) was added dropwise. The temperature was maintained at −78°C for 20 min and the reaction mixture was then allowed to warm to room temperature (2 h). The solvent was removed and the residue was chromatographed on silica gel. Elution with 15% → 22% → 30% → 35% ethyl acetate – hexane afforded the β-lactone L-**18**. ^{1}Hmr ($CDCl_3$), δ: 5.29 (1H, br), 4.92 (1H, br), 4.34 (2H, br), 9.07 (9H, s). Anal. calcd. for $C_8H_{13}NO_4$: C 51.33, H 6.99, N 7.48; found: C 51.59, H 7.05, N 7.62.

*C. Reaction of **17** with **18***

To a solution of **17** (79.6 mg, 0.45 mmol) in dry degassed dimethylformamide (1.5 mL) was added dropwise a solution of lithium diisopropylamide (0.8 mmol) in tetrahydrofuran (1.5 mL). The addition was carried out under nitrogen at −60°C. The reaction mixture was allowed to warm to −25°C during 50 min, cooled again to −55°C, and a solution of **18** (56.4 mg, 0.30 mmol) in dry degassed dimethylformamide (0.5 mL) was added dropwise. When the addition was complete, the mixture was warmed to −20°C, stirred for 25 min, and then diluted with ethyl acetate (30 mL) and washed with 0.5 N hydrochloric acid (2 mL). The aqueous layer was extracted with ethyl acetate (2 × 10 mL) and the combined organic extracts were washed with water (2 × 5 mL) and saturated sodium chloride (1 × 5 mL), dried, and evaporated. The oily residue was purified by preparative layer chromatography on a 10 × 20 cm plate coated with silica gel, using methylene chloride:ethyl acetate:acetic acid (1.7:0.3:0.05) as eluant to give **14***c* (77 mg, 70%); ^{1}Hmr ($CDCl_3$), δ: 5.43 (1H, br), 5.20 (1H, d, 18 Hz), 5.04 (1H, d, 18 Hz), 4.46 (1H, br), 2.97 (1H, br), 2.78, 2.74 (1H, dd, 4.5, 9.0 Hz), 2.17 (3H, s), 1.48 (3H, s), 1.47 (3H, s), 1.44 (9H, s).

The acid **14***c* (77 mg) was dissolved in methylene chloride (10 mL) and treated at 0°C with an ethereal solution of diazomethane. The solvent was removed and the residue was purified on a 5 × 10 cm silica gel plate using hexane:ethyl acetate (1.4:0.6) as eluant to give the ester **14***b* (48.2 mg); ^{1}Hmr ($CDCl_3$), δ: 5.32 (1H, br d, N-H), 5.15 (1H, d, 11 Hz, O-C-H), 5.07 (1H, d, 11 Hz, O-C-H), 4.48 (1H, br q, N-C-H), 3.76 (3H, s,ester), 2.91 (1H, dd, 4, 12 Hz, S-C-H), 2.74 (1H, dd, 5.5, 12 Hz, S-C-H), 2.17 (3H, s, Ac), 1.48 (3H, s, C-Me), 1.47 (3H, s, C-Me), 1.44 (9H, s, *t*-Boc).

*Deprotection and cyclization of **14**b. Formation of **13**b and **19***

The methyl ester acetate **14***b* (3.7 mg) was treated at room temperature with anhydrous formic acid (0.2 mL). After 10 min, the solution was frozen and the solvent removed by lyophilization to give **13***b*;

^{1}Hmr ($CDCl_3$), δ: 4.82 (1H, q, 1.8, 14.3 Hz, O-C-H coupled to O-C-H and N-C-H), 4.71 (1H, q, 2.4, 14.3 Hz, O-C-H coupled to O-C-H and N-C-H), 4.56 (1H, td, 1.8, 2.4, 7.8 Hz, N-CH), 3.79 (3H, s, ester), 2.94 (2H, d, 7.8 Hz), 2.14 (3H, s, Ac), 1.36 (6H, s, C-Me). During a period of 24 h, the spectrum of **13***b* gradually disappeared, and the spectra of **19** and acetic acid (2.09 ppm) appeared; ^{1}Hmr of **19** ($CDCl_3$), δ: 7.29 (1H, q, S-C-H coupled to C=C-Me), 3.86 (3H, s, ester), 2.29 (3H, d, N=C-Me), 1.36 (6H, s, C-Me).

*Conversion of **14**b to the exocyclic enamine **22***

The ester **14***b* (46 mg), in tetrahydrofuran (1 mL), was treated at room temperature with 0.25 M lithium hydroxide (0.4 mL). After 25 min an additional 0.4 mL of lithium hydroxide was added. The mixture was stirred for 35 min and then diluted with ethyl acetate (10 mL) and washed with 0.5 N hydrochloric acid (2 × 5 mL). The aqueous layer was extracted with ethyl acetate (2 × 5 mL) and the combined organic extracts were washed with water (1 × 5 mL), followed by saturated sodium chloride (1 × 5 mL), dried, and evaporated. The residue was dissolved in the minimum of methylene chloride, treated with ethereal diazomethane, concentrated, and the residue was purified on a 10 × 20 cm silica gel plate. Elution with hexane:ethyl acetate (1.4:0.6) gave **14***d* (14.4 mg); ^{1}Hmr ($CDCl_3$), δ: 5.22 (1H, br), 4.58 (2H, d), 4.48 (1H, br), 3.75 (3H, s), 3.06 (1H, br), 2.92 (1H, br), 2.74 (1H, dd, 5, 11 Hz), 1.46 (9H, s), 1.44 (6H, s).

To a solution of **14***d* (5 mg, 0.015 mmol) in freshly dried pyridine (0.2 mL) were added successively silver nitrate (3.4 mg, 0.02 mmol) and *tert*-butyldiphenylchlorosilane (6.3 mg, 0.023 mmol). The solution was stirred for 15 min at room temperature. The solvent was then removed and the product was purified by preparative layer chromatography to give **14***e* (5.5 mg); ^{1}Hmr ($CDCl_3$), δ: 7.69 (4H, m), 7.41 (6H, m), 5.07 (1 H, br), 4.70 (2H, s), 4.41 (1H, br), 3.72 (3H, s), 2.70 (1H, dd, 6, 15 Hz), 2.55 (1H, dd, 6, 15 Hz), 1.43 (9H, s), 1.28 (3H, s), 1.26 (3H, s), 1.10 (9H, s).

The silylated ester **14***e* (5 mg) was treated at room temperature with formic acid (0.2 mL). After 33 min the reaction mixture was frozen and the solvent was removed by lyophilization to yield the enamine **22**. ^{1}Hmr ($CDCl_3$), δ: 8.31 (1H, br, N-H), 7.69 (4H, m, Ar), 7.40 (6H, m, Ar), 5.90 (1H, s, C=C-H) 4.65 (1H, br, N-C-H), 3.79 (3H, s, ester), 3.17 (1H, dd, 10, 15 Hz, S-C-H), 3.00 (1H, dd, 3, 15 Hz, S-C-H), 1.49 (3H, s, C-Me), 1.31 (3H, s, C-Me), 1.08 (9H, s, *t*-Bu).

*Synthesis of the deoxythiazine **13**d*

To methylene chloride (3 mL), saturated with hydrogen sulfide, were added successively at −20°C triethylamine (0.33 mL, 0.24 g, 2.36 mmol) and the bromoketone **15** (330 mg, 2.0 mmol). The reaction was complete in 10 min, and the thiol (101 mg) was isolated as described above for **17**, and used directly in the next step.

To the thiol (38 mg, 0.32 mmol), in dimethylformamide (1 mL), was added dropwise at −55°C 1.0 mL of a 0.64 M solution of lithium diisopropylamide in tetrahydrofuran. The temperature was allowed to rise to −20°C during 45 min, then lowered again to −55°C, and **18** (30 mg, 0.16 mmol), in dimethylformamide (0.4 mL), was added. Stirring was continued at −20°C for 25 min, and the reaction mixture was then worked up as described above for **14***c*. The product was purified by plc (silica gel, 0.5 mm thickness, 10 × 20 cm plate, methylene chloride: ethyl acetate:acetic acid 1.7:0.3:0.05, one elution), to give 43.5 mg (89%) of the cysteine derivative Me-CO-C(Me)$_2$-S-CH$_2$-CH(NHBoc)-CO_2H.

This acid (20 mg), in methylene chloride (0.5 mL), was treated with ethereal diazomethane. The reaction was instantaneous. The solvent was removed and the residue purified by plc (silica gel, 10 × 20 cm, hexane – ethyl acetate 1.4:0.6, one elution) to give the methyl ester (18 mg); ^{1}Hmr ($CDCl_3$), δ: 5.19 (1H, d, 4 Hz, N-H), 4.48 (1H, br, N-C-H), 3.73 (3H, s, ester), 2.82 (1H, dd, 5, 16 Hz, S-C-H), 2.73 (1H, dd, 7, 16 Hz), 2.26 (3H, s, Me-CO), 1.41 (9H, s, Boc), 1.39 (6H, s, C-Me).

The methyl ester (14 mg) was dissolved in anhydrous formic acid (0.4 mL) and the solution was maintained at room temperature for 50 min. The solvent was then removed by lyophilization, and the product was purified by plc (alumina, 10 × 20 cm, methylene chloride:ethyl acetate 0.8:1.2, two elutions) to give the thiazine **13***d*; ^{1}Hmr ($CDCl_3$), δ: 4.48 (1H, ddq, 2.0, 5.0, 8.8 Hz, N-C-H coupled to S-C-H, S-C-H, and N=C-Me), 3.81 (3H, s, ester), 2.88 (1H, dd, 5.0, 14.2 Hz, S-C-H), 2.94 (1H, dd, 8.8, 14.2 Hz), 2.10 (1H, d, 2.0 Hz, N=C-Me coupled to N-C-H), 1.52 (3H, s, CMe), 1.47 (3H, s, C-Me).

The thiazine **13***d* was relatively stable. However, one $CDCl_3$ solution underwent isomerization to the enamine **23***a*; ^{1}Hmr, δ: 5.20 (1H, br s, N-H), 4.60 (1H, s, C=C-H), 4.59 (1H, s, C=C-H), 4.51 (1H, m), 3.77 (3H, s, ester), 2.94 (1H, dd, 5.0, 13 Hz), 2.77 (1H, dd, 5.8, 13 Hz), 1.47 (3H, s, C-Me), 1.46 (3H, s, CMe).

*Hydrolysis of **13**d*

The methyl ester (1.3 mg, 0.0055 mmol) was treated at 0°C with methanol (0.2 mL) and 0.5 M lithium hydroxide (80 μL). After 20 min, 1 M sulfuric acid (17.25 μL) was added, the methanol was removed under reduced pressure, and the remaining solvent was removed by lyophilization. For nmr studies the residue was dissolved in 0.6 mL of a solution prepared from 4 mg of sodium bicarbonate and 1.0 mL of D_2O; ^{1}Hmr of **13***f* (D_2O), δ: 4.12 (1H, br), 2.87 (1H, q, 4.9, 14 Hz), 2.78 (1H, q, 7.3, 14 Hz), 1.95(3H, br, exchanges), 1.37 (3H, s), 1.35 (3H, s).

*Synthesis of **26**. Biologically active series*

*A. Synthesis of the bromide **31***

A solution of ethyl 2-methylcyclopropanecarboxylate (5.0 g, 38.9 mmol) in dry ether (5 mL) was added dropwise, with stirring under nitrogen, to the Grignard reagent prepared from magnesium turnings (1.935 g, 0.080 g-atom) and methyl iodide (12.43 g, 87.6 mmol) in dry ether (42 mL). The addition required 30 min; stirring was continued for 2.75 h at room temperature and then for 2 h under reflux. The reaction mixture was cooled in an icebath and saturated ammonium chloride (10 mL) was added, with stirring. The layers were separated and the aqueous layer was extracted with ether (2 × 20 mL). The combined organic phase was dried, evaporated, and the residue distilled at 132–136°C to give the tertiary alcohol product (4.24 g, 95%).

To this alcohol (4.24 g, 37 mmol), cooled in an ice-bath, was added ice-cold 48% hydrobromic acid (15 mL). The mixture was shaken vigorously in the ice-bath for 30 min. The two layers were then separated, the aqueous layer extracted with hexane (2 × 20 mL), and the combined organic phase was washed successively with saturated bicarbonate (2 × 10 mL), water (2 × 10 mL), and saturated sodium chloride (2 × 10 mL), dried over anhydrous sodium sulfate, and evaporated. Distillation afforded 3.72 g (60%) of the bromide **31**, bp 46–54°C/10 Torr; ^{1}Hmr ($CDCl_3$) δ: 5.18 (1H, br t), 4.11 (1H, q), 2.59 (1H, m), 2.51 (1H, m), 1.72 (3H, s), 1.68 (3H, d, 8 Hz), 1.62 (3H, s).

*B. Conversion of **31** to **30** (R = Ac)*

To a solution of the bromide **31** (3.72 g, 21 mmol) in glacial acetic acid (20 mL) was added potassium acetate (3.1 g, 31.6 mmol). The mixture was heated under reflux for 12 h, cooled, and poured into water (30 mL). Extraction with ether (3 × 30 mL), followed by successive washing of the organic phase with saturated sodium carbonate, water, and saturated sodium chloride, drying, and evaporation at room temperature yielded the acetate 2.82 g (85%); ^{1}Hmr ($CDCl_3$), δ: 5.10 (1H, br t), 4.88 (1H, q, 6 Hz), 2.30 (1H, m), 2.19 (1H, m), 2.02 (3H, s), 1.71 (3H, br s), 1.62 (3H, br s), 1.20 (3H, d, 6 Hz).

C. Hydrolysis of the acetate

The acetate (320 mg, 2.05 mmol) was dissolved in methanol (2 mL) and treated dropwise with a 1.5 M solution of potassium hydroxide in methanol (1.38 mL). The reaction mixture was allowed to stand for 6 h and was then neutralized with 1.5 M methanolic hydrogen chloride, and the solvent was removed. The residue was dissolved in methylene chloride, and this solution was washed successively with water and saturated sodium chloride, dried, and evaporated to give the alcohol **30**

(R = H) (208 mg, 99%); ^{1}Hmr ($CDCl_3$) δ: 5.14 (1H, tq, 1.5, 8 Hz), 3.79 (1H, sextet, 6 Hz), 2.16 (2H, br t, 7.5 Hz), 1.72 (3H, br s), 1.63 (3H, br s), 1.18 (3H, d, 6 Hz).

D. tert-*Butyldimethylsilylation of the alcohol* ***30*** *(R = H)*

The alcohol (312 mg, 2.73 mmol) was dissolved in dimethylformamide (2 mL) and to this solution were added successively *tert*-butyldimethylchlorosilane (535 mg, 3.55 mmol), pyridine (0.29 mL, 284 mg, 3.59 mmol), and silver nitrate (603 mg, 3.55 mmol). The mixture was stirred for 2 h and then filtered. The insoluble material was triturated with ether (20 mL) and the combined organic material was washed successively with saturated sodium bicarbonate, water, and saturated sodium chloride, dried, and evaporated to give the silylated compound **30** (R = *t*-BDMS) (620 mg, 100%); ^{1}Hmr ($CDCl_3$), δ: 5.13 (1H, br t), 3.77 (1H, m), 2.17 (1H, m), 2.08 (1H, m), 1.69 (3H, br s), 1.60 (3H, br s), 1.11 (3H, d, 6.1 Hz), 0.88 (9H, s), 0.05 (3H, s), 0.03 (3H, s).

E. Permanganate oxidation of the silylated alcohol. Preparation of the ketol ***29*** *(R = t-BDMS)*

The olefin **30** (R = *t*-BDMS) (624 mg, 2.73 mmol) was dissolved in acetone (3 mL) and 18-crown-6 (100 mg, 0.27 mmol) and acetic acid (0.16 mL) were added successively followed, dropwise, by a solution of potassium permanganate (603 mg, 3.82 mmol) in water (7.5 mL). The mixture was stirred for 1 h and then diluted with methylene chloride (50 mL). The organic phase was washed successively with 20% sodium bisulfite, 0.5 N hydrochloric acid, saturated sodium bicarbonate, water, and saturated sodium chloride, dried, and evaporated. The residue was subjected to flash chromatography on silica gel (7 g). Elution with 4 → 15% ethyl acetate – hexane gave 479 mg (70%) of **29** (R = *t*-BDMS); ^{1}Hmr ($CDCl_3$), δ: 4.42 (1H, m), 3.95 (1H, s), 2.83 (1H, dd, 7.6, 16.0 Hz), 2.47 (1H, dd, 4.8, 16.0 Hz), 1.34 (3H, s), 1.33 (3H, s), 1.18 (3H, d, 6.1 Hz), 0.84 (9H, s), 0.06 (3H, s), 0.02 (3H, s). Mass spectrum (CI, isobutane, *m/e*): 261 (M + 1). Anal. calcd. for $C_{13}H_{28}O_3Si$: C 59.95, H 10.84; found: C 59.78, H 10.99.

F. Mesylation of ***29*** *(R = t-BDMS)*

To a solution of the ketol (478 mg, 1.83 mmol) in methylene chloride (6 mL) were added successively triethylamine (0.76 mL, 4.0 mmol) and methanesulfonyl chloride (0.24 mL, 3.1 mmol). The reaction mixture was stirred for 5 h at room temperature and then diluted with methylene chloride (80 mL). The solution was washed successively with water, 0.5 N hydrochloric acid, saturated sodium bicarbonate, water, and saturated sodium chloride, dried, and evaporated. Flash chromatography on silica gel (3 g) and elution with 7% → 8% → 9% → 10% ethyl acetate – hexane gave the mesylate Me-CH(O*t*BDMS)-CH_2-CO-CH(Me)$_2$-OSO_2CH_3 (432 mg, 70%); ^{1}Hmr ($CDCl_3$), δ: 4.40 (1H, m), 3.11 (3H, s), 2.94 (1H, dd, 7.5, 17.3 Hz), 2.59 (1H, dd, 4.6, 17.3 Hz), 1.68 (3H, s), 1.63 (3H, s), 1.18 (3H, d, 6.1 Hz), 0.84 (9H, s), 0.07 (3H, s), 0.02 (3H, s).

G. Synthesis of the thiol ***28*** *(R = t-BDMS)*

Methylene chloride (5 mL) was saturated with hydrogen sulfide at −20°C, and triethylamine (0.14 mL, 1 mmol) and a solution of the above mesylate (233 mg, 0.5 mmol) were added successively. The solution was stirred for 10 min at −20°C and for 45 min at −20° to 0°C, and was then diluted with methylene chloride (30 mL), washed successively with 0.5 N hydrochloric acid, water, and saturated sodium chloride, dried, and evaporated to give, after drying at 0.1 Torr, the silylated mercaptan **28** (R = *t*-BDMS) (170 mg, 85%); ^{1}Hmr ($CDCl_3$), δ: 4.37 (1H, m), 2.98 (1H, dd, 5, 11 Hz), 2.63 (1H, dd, 4, 11 Hz), 1.98 (1H, s), 1.49 (3H, s), 1.48 (3H, s), 1.17 (3H, d, 6 Hz), 0.84 (9H, s), 0.05 (3H, s), 0.01 (3H, s). Mass spectrum (CI, *m/e*): 277 (M^+). Anal. calcd. for $C_{13}H_{28}O_2SSi$: C 56.47, H 10.21; found: C 56.25, H 10.07.

H. Coupling of the thiol ***28*** *(R = t-BDMS) with* ***D-18****: 27 (R^1 = t-BDMS, R^2 = Boc)*

Under nitrogen, the mercaptan **28** (100 mg, 0.36 mmol) was dissolved in degassed dimethylformamide (1.0 mL). The solution was cooled to −55°C and treated with 0.46 mL of a solution of lithium diisopropylamide prepared from *n*-butyllithium (0.8 mL of a 1.6 M hexane solution) and diisopropylamide (0.36 mL, 0.259 g, 2.56 mmol) in degassed tetrahydrofuran (0.8 mL). The reaction mixture was stirred at −45°C for 30 min, and a solution of the β-lactone **D-18** (56.8 mg, 0.30 mmol) in degassed dimethylformamide (0.8 mL) was added. The mixture was stirred at −30°C for 20 min, then diluted with methylene chloride (10 mL), and washed with 0.5 N hydrochloric acid. The aqueous layer was extracted with methylene chloride (2 × 5 mL) and the combined organic extracts were washed with water, then saturated sodium chloride, dried, and evaporated. The residue was dried under high vacuum and purified by flash chromatography (silica gel, 4 g; 0% → 8% ethyl acetate – methylene chloride (1% acetic acid)) to give the coupled product **27** (R^1 = *t*-BDMS, R^2 = Boc) having the *S* configuration at C2 in 89% yield; ^{1}Hmr ($CDCl_3$), δ (one isomer): 5.28 (1H, br t), 4.48 (1H, br), 4.32 (1H, m), 2.83, 2.71 (2H, m), 2.71, 2.62 (2H, m), 1.44 (6H, s), 1.16 (3H, d, 6 Hz), 0.85 (9H, s), 0.05 (3H, s), 0.00 (3H, s). The nmr spectrum shows a 1:1 mixture of epimers in the 2-hydroxypropyl side chain. Anal. calcd. for $C_{21}H_{41}NO_6SSi$: C 54.40, H 8.91, N 3.02; found: C 54.16, H 8.72, N 2.96.

I. Formation and characterization of 3S- ***26***

To **27** (R^1 = *t*-BDMS, R^2 = Boc) (22.7 mg, 0.049 mmol) was added formic acid (0.3 mL). The solution was shaken for 20 min at room temperature and the solvent was then removed by lyophilization. The residue was dissolved in a mixture of ether (3 mL) and water (1 mL). The ether phase was extracted with water (1 mL), and the combined aqueous phase was neutralized with 5% sodium bicarbonate and lyophilized to give **26** (5 mg, 40%) as a mixture of epimers in the 2-hydroxypropyl side chain; ir (KBr, cm^{-1}): 3422 (OH), 1696 (C=O), 1636 (C=N); ^{1}Hmr (D_2O), δ: 4.23 (1H, m), 3.80 (1H, m), 3.30 (1H, q), 2.70–2.85 (3H, m), 1.40 (6H, s), 1.15 (3H, d). High-resolution mass spectrum (*m/e*): 231.0924 (M^+), 213.0823 (M − H_2O).

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