(m, 4 H), 6.18 (d, 1 H, J = 3.6 Hz), 5.38 (ddd, 1 H, J = 7.2, 4.8, 3.6 Hz), 3.47 (dd, 1 H, J = 16.5, 7.2 Hz), 2.82 (dd, 1 H, J = 16.5, 4.8 Hz), 2.09 (s, 3 H), 2.05 (s, 3 H).

***cis*-Dihydro-1*H*-indene-1,2-diol diacetate (38)**:[48] IR 3050, 3030, 2960, 1740, 1370, 1250, 1065, 755, 735 cm^{-1}; NMR δ 7.45–7.10 (m, 4 H) 6.18 (d, 1 H, J = 5.4 Hz), 5.65–5.37 (m, 1 H), 3.14 (dd, 2 H, J = 6.2, 1.8 Hz), 2.08 (s, 3 H), 2.05 (s, 3 H); mp 47.5–48.5 °C (lit. mp 48.0–48.5 °C).

Inden-2-ol acetate (39): IR 3060, 3020, 2930, 1740, 1230, 1025, 750, 715 cm^{-1}; NMR δ 7.60–6.95 (m, 4 H), 6.79 (br s, 1 H), 3.41 (br s, 2 H), 2.10 (s, 3 H).

***trans*-Dihydro-4-methyl-5-phenyl-2(3*H*)-furanone (40)**:[28] IR 3070, 3035, 2965, 2930, 1785, 1495, 1455, 1280, 1215, 1140, 1000, 945, 755, 700 cm^{-1}; NMR δ 7.34 (s, 5 H), 4.91 (d, 1 H, J = 8.0 Hz), 2.90–1.95 (m, 3 H), 1.16 (d, 3 H, J = 6.2 Hz); exact mass calcd for $C_{11}H_{12}O_2$ m/z 176.0834, found 176.0833.

***cis*-Dihydro-4-methyl-5-phenyl-2(3*H*)-furanone (41)**:[28] IR 3060, 3030, 2970, 2930, 1780, 1495, 1455, 1240, 1155, 985, 750, 700 cm^{-1}; NMR δ 7.65–7.05 (m, 5 H), 5.55 (d, 1 H, J = 6 Hz), 3.00–2.05 (m, 3 H), 0.69 (d, 3 H, J = 7.0 Hz).

***trans*-Dihydro-4-phenyl-5-methyl-2(3*H*)-furanone (42)**:[28] IR 3070, 3030, 2980, 2930, 1780, 1495, 1455, 1385, 1200, 1170, 1070, 940, 755, 700 cm^{-1}; NMR δ 7.60–7.05 (m, 5 H), 4.53 (d, 1 H, J = 8.3 Hz), 3.4–3.05 (m, 1 H), 2.9–2.65 (m, 2 H), 1.42 (d, 3 H, J = 6.1 Hz).

Methyl 4-hydroxy-4-phenyl-5-methylbutanoate (threo/erythro mixture, 1.5:1; 43-Me ester):[49] IR 2970, 2930, 2850, 1775, 1435, 1370, 1235, 1020, 760, 700 cm^{-1}; NMR δ 7.30 (br s, 5 H), 5.69 (d, 0.4 H, J = 6 Hz), 5.54 (d, 0.6 H, J = 7.2 Hz), 3.66 (s, 1.8 H), 3.63 (s, 1.2 H), 2.75–1.95 (m, 3 H), 2.08 (s, 1.2 H), 2.04 (s, 1.8 H), 0.96 (d, 1.2 H, J = 6.7 Hz), 0.86 (d, 1.8 H, J = 6.6 Hz); exact mass calcd for $C_{13}H_{18}O_4$ m/z 250.1200, found 250.1195.

***threo*-1-Phenyl-1,2-propanediol diacetate (44)**:[50] IR 3070, 3040, 2990, 2949, 1737, 1455, 1370, 1240, 1040, 1020, 760, 700 cm^{-1}; NMR δ 7.33 (br s, 5 H), 5.75 (d, 1 H, J = 7.2 Hz), 5.22 (dq, 1 H, J = 7.2, 6.4 Hz), 2.07 (s, 3 H), 2.02 (s, 3 H), 1.08 (d, 3 H, J = 6.4 Hz).

***erythro*-1-Phenyl-1,2-propanediol diacetate (44)**: IR 3070, 3040, 2990, 2940, 1735, 1495, 1455, 1370, 1235, 1020, 755, 700 cm^{-1}; NMR δ 7.33 (br s, 5 H), 5.87 (d, 1 H, J = 4.5 Hz), 5.20 (dq, 1 H, J = 4.5, 6.4 Hz), 2.13 (s, 3 H), 1.99 (s, 3 H), 1.16 (d, 3 H, J = 6.4 Hz).

***2-threo*-1-Phenyl-1,2-propanediol acetate (45)**: IR 3450, 3060, 3030, 2980, 2935, 1730, 1455, 1370, 1240, 1040, 760, 700 cm^{-1}; NMR δ 7.33 (br s, 5 H), 5.04 (dq, 1 H, J = 3.5, 7 Hz), 4.60 (d, 1 H, J = 7 Hz), 2.80 (br, 1 H), 2.06 (s, 3 H), 1.10 (d, 3 H, J = 6.4 Hz).

***2-erythro*-1-Phenyl-1,2-propanediol acetate (45)**: IR 3490, 3060, 3030, 2970, 2930, 1730, 1455, 1370, 1240, 750, 700 cm^{-1}; NMR δ 7.65–7.05 (m, 5 H), 5.07 (dq, 1 H, J = 4, 6.5 Hz), 4.82 (d, 1 H, J = 4 Hz), 2.04 (s, 3 H), 1.15 (d, 3 H, J = 6.5 Hz), OH not observed.

***trans*-Dihydro-4-methyl-5-(butoxycarbonyl)-2(3*H*)-furanone (46)**: IR 2960, 2930, 2875, 1790, 1745, 1460, 1270, 1145, 1095, 1050 cm^{-1}; NMR δ 4.49 (d, 1 H, J = 4.7 Hz), 4.21 (t, 2 H, J = 6.4 Hz), 2.90–1.85 (m, 3 H), 1.80–1.05 (m, 7 H), 1.29 (d, 3 H, J = 6.6 Hz), 0.94 (t, 3 H, J = 6.3 Hz); exact mass calcd for $C_{10}H_{16}O_4$ $(m + 1)/z$ 201.1122, found 201.1120, calcd m/z 200.1044, found 200.1041.

***trans*-Dihydro-5-methyl-4-(butoxycarbonyl)-2(3*H*)-furanone (47)**: IR 2960, 2935, 2875, 1785, 1735, 1455, 1420, 1385, 1260, 1195, 1055, 940 cm^{-1}; NMR δ 4.85 (quint, 1 H, J = 6 Hz), 4.16 (t, 2 H, J = 6.4 Hz), 3.20–2.62 (m, 3 H), 1.85–1.10 (m, 7 H), 1.51 (d, 3 H, J = 6.2 Hz), 0.94 (t, 3 H, J = 6.3 Hz); exact mass calcd for $C_{10}H_{16}O_4$ $(m + 1)/z$ 201.1122, found 201.1140.

***trans*-Dihydro-5-phenyl-4-(carbomethoxy)-2(3*H*)-furanone (48)**:[51] IR 3070, 3040, 3010, 2960, 1785, 1735, 1495, 1435, 1360, 1310, 1260, 1195, 1005, 765, 695 cm^{-1}; NMR δ 7.36 (s, 5 H), 5.64 (d, 1 H, J = 6.8 Hz), 3.75 (s, 3 H), 3.33 (dd, 1 H, J = 6.8, 2.9 Hz), 3.02 (d, 1 H, J = 11 Hz), 2.76 (dd, 1 H, J = 11, 2.9 Hz), exact mass calcd for $C_{12}H_{12}O_4$ m/z 220.0732, found 220.0740.

***cis*-Dihydro-5-phenyl-4-(carbomethoxy)-2(3*H*)-furanone (49)**:[51] IR 3060, 3030, 3005, 2950, 1785, 1732, 1495, 1437, 1380, 1210, 1170, 1005, 750, 700 cm^{-1}; NMR δ 7.55–6.93 (m, 5 H), 5.75 (d, 1 H, J = 7.8 Hz), 3.28 (s, 3 H), 3.70 (br dt, 1 H, J = 6, 9 Hz), 5.10 (dd, 1 H, J = 18, 6 Hz), 2.75 (dd, 1 H, J = 18, 9 Hz).

***trans*-Dihydro-4-phenyl-5-(carbomethoxy)-2(3*H*)-furanone (50)**: IR 3060, 3035, 3005, 2960, 1785, 1735, 1495, 1435, 1370, 1005, 765, 695 cm^{-1}; NMR δ 7.65–7.10 (m, 5 H), 4.91 (d, 1 H, J = 4.6 Hz), 3.82 (s, 3 H), 4.2–3.4 (m, 1 H), 3.05 (dd, 1 H, J = 18, 9 Hz), 3.62 (dd, 1 H, J = 18, 6 Hz).

1-Hydroxy-4-methyl-3-penten-2-one acetate (51): IR 2980, 2935, 1750, 1705, 1630, 1445, 1370, 1235, 1030 cm^{-1}; NMR δ 6.05 (septet, 1 H, J = 1.3 Hz), 4.65 (s, 2 H), 2.19 (d, 3 H, J = 1.1 Hz), 2.17 (s, 3 H), 1.91 (d, 3 H, J = 1.2 Hz), exact mass calcd for $C_8H_{12}O_3$ m/z 156.0783, found 156.0795.

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6-(Methoxymethylene)penicillanic Acid: A New β-Lactamase Inactivator

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The β-lactamase inactivator 6-(methoxymethylene)penicillanic acid is the first compound in a new class of penams having a heteroatom-substituted methylene group at the 6-position conjugated to the β-lactam carbonyl. Treatment of benzyl 6-oxopenicillanate with the anion of methoxy(trimethylsilyl)methane gives a pair of β-silyl alcohols. Quantitative acetylation of either of the alcohols and subsequent fluoride-promoted elimination of acetate and trimethylsilyl fluoride yields an equilibrium mixture of the *Z* and *E* isomers of the title compound. Close examination of the elimination reaction by ^{1}H NMR permitted the assignment of the chirality in the C-6 side chain of the β-silyl alcohols that was confirmed by X-ray crystallographic analysis. After separation of the enol ether geometrical isomers, it was found that whereas the *E* isomer does not interact perceptibly with the purified RTEM-2 β-lactamase from *E. coli*, the *Z* isomer irreversibly inactivates the enzyme.

The enzyme β-lactamase catalyzes the hydrolysis of β-lactam antibiotics to the therapeutically impotent penicillanic acids, and bacterial strains that possess this enzyme are generally resistant to the killing effects of hydrolytically labile but otherwise potent β-lactam antibiotics. β-Lactamase is therefore regarded as an important target, in the effort to retain the clinical utility of susceptible but powerfully therapeutic β-lactams. The past

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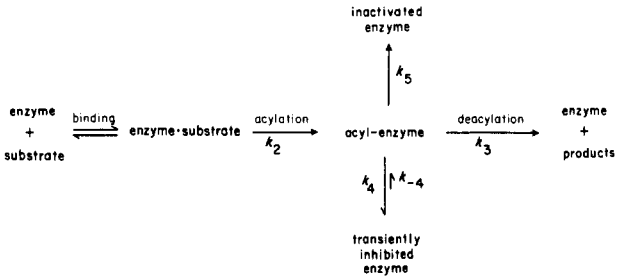
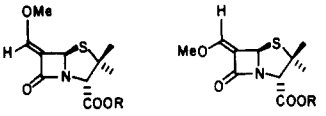
Scheme I



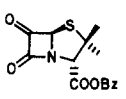
seven years have witnessed the discovery and synthesis of a number of β-lactam derivatives that inhibit or inactivate the β-lactamase.[1] From investigations of the interaction with the enzyme of a number of these compounds, including clavulanic acid, penam sulfones, and 6-halopenams, the view has emerged that catalytic activity can be disrupted if the first-formed acyl–enzyme intermediate has fates open to it other than the normal deacylation pathway as illustrated in Scheme I. In particular, a branched reaction pathway has been well documented in a number of instances, and there is much evidence supporting the notion that the formation of a β-amino acrylate system involving the active site serine residue is often responsible for enzyme inactivation.[1] With clavulanic acid and with penicillanic acid sulfone, for example, this phenomenon arises from the facile cleavage of the five-membered ring subsequent to β-lactam acylation of the enzyme.[2] In these cases, the imine formed may either tautomerize directly to a β-amino acrylate (thus forming a transiently inhibited enzyme species), or undergo transimination to a putative enzyme lysine residue followed by tautomerization to a conjugated enamine which results in irreversible inactivation of the enzyme by chemical cross-linking of the active site.[1,3a]

In a novel approach to create a more efficient inhibitor of the β-lactamase, compounds were sought for which the *first-formed* acyl–enzyme intermediate would be a relatively stable vinylogous ester. To this end, two prototypic molecules, the *Z* and *E* isomers of 6-(methoxymethylene)penicillanic acid, **1a** and **2a**, were chosen. We report here the synthesis of these compounds, and briefly relate their effect on the catalytic activity of the β-lactamase.



1a R = H **2a** R = H
1b R = Bz **2b** R = Bz

Synthesis of 6-(Methoxymethylene)penicillanic Acid. The approach is based on the compound, benzyl 6-oxopenicillanate, **3**.[4,5] The ketone carbonyl of **3**, which



3

forms a crystalline cyanohydrin,[6] reacts with carbonyl-stabilized phosphonium ylids to form olefins[7] as well as with the anion of nitromethane.[8] All of these addition reactions employ nucleophiles the conjugate acids of which have pK_a values of about 9–10.[9] Since most β-lactams are relatively stable at pH 9, the success of these transformations is not surprising. In contrast, the protonated form of the phosphonium reagent required to make an enol ether (such as a methoxymethyltriphenylphosphonium halide) has a much higher pK_a, and the Wittig reaction with such compounds is slow even at room temperature.[10] Thus, efforts to form an enol ether from the ketone of **3** by using reagents such as the ylid of methoxymethyltriphenylphosphonium bromide resulted either in unreacted **3** at low temperatures (between −78 and −20 °C) or in the complete loss of the β-lactam functionality at more elevated temperatures. Similar problems were encountered with phosphonate-stabilized anions[11] which were investigated since they are more readily formed and are more stable than the corresponding Wittig ylids. As an alternative to the normal Wittig reagent, silicon-stabilized anions have been employed by Magnus and Roy.[12] For example, (methoxy(trimethylsilyl)methyl)lithium [generated from (methoxymethyl)trimethylsilane (**4**) with *sec*-butyllithium] adds cleanly to a variety to ketones to form stable β-silyl alcohol adducts. Treatment of these adducts with potassium hydride effects an elimination reaction to form the methyl vinyl ether in high yield. The overall route of using **4** to convert a ketone into a methyl vinyl ether has shown its utility in natural product syntheses where such a functionality is desired.[13] One of the attractions of using such silicon reagents lies in being able to isolate the intermediate alcohol adduct, from which the required protecting group on the C-3 carboxylic acid group can be removed, before elimination to give the desired enol ether.

Oxidation of benzyl 6α-hydroxypenicillanate gives the 6-oxo compound **3**.[8] This material can be converted into the cyanohydrin (which is purified by recrystallization), but the latter compound is not cleanly converted back to the ketone upon treatment with silver oxide, contrary to a report in the literature.[6] However, "flash" chromatography[14] on silica yields crystalline benzyl 6-oxopenicillanate **3**, which is stable at ambient temperature in the dark for several weeks.

When the lithium salt of (methoxymethyl)trimethylsilane **4** is slowly added at −100 °C to a solution of **3** at −100 °C, a 1:1 adduct with the ketone is formed. The silicon-stabilized anion from **4** is so reactive that its chemoselectivity for the ketone in such a highly functionalized

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(2) The question of synchronous vs. subsequent ring cleavage has been investigated in detail (Page, M. I. *Acc. Chem. Res.* **1984**, *17*, 144–151) and the evidence strongly favors the latter mechanism.
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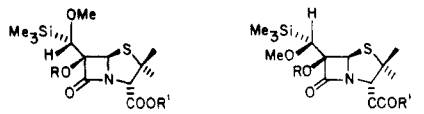
molecule is **3** can be controlled only at very low temperatures. At these temperatures, the reagent shows marked stereospecificity for the ketone of **3**, producing a single diastereoisomeric mixture of **5a** and **6a** by attack of the chiral anion upon the less hindered α-face (i.e., the *si*-face) of the ketone.[4-6,8] The diastereoisomeric pair which would result from the attack of the anion on the β-face (i.e., the *re*-face) of **3** could not be detected by ^{1}H NMR.



5a R = H, R' = Bz
5b R = Ac, R' = Bz
5c R = Ac, R' = H

6a R = H, R' = Bz
6b R = Ac, R' = Bz
6c R = Ac, R' = H

After chromatographic separation of the diastereomeric pair **5a** and **6a**, an attempt was made to effect the elimination reaction by using potassium hydride. This reaction did not yield isolable amounts of enol ether and an alternative approach, involving treatment of the silyl adducts **5a** or **6a** with fluoride ion, was taken. Although Magnus and Roy[12] have reported that the addition of cesium fluoride in dry dimethyl sulfoxide to a β-hydroxy silane usually results only in desilylation, in two examples, competitive elimination (to give the enol ethers) was also observed. In the present case, the silyl adducts from benzyl 6-oxopenicillanate do react with cesium fluoride and undergo both desilylation and elimination, though the yields of the elimination product are low (<20%). To promote the desired elimination, the β-alcohol at C-6 was made into a better leaving group by acetylation of **5a** and **6a** to **5b** and **6b**. Exposure of either **5b** or **6b** to cesium fluoride results in the *quantitative* conversion of the acetate to the enol ethers **1b** and **2b** by elimination of the elements of trimethylsilyl acetate. No desilylated alcohol could be detected. The acetylation of the alcohol is evidently sufficient to define the course of the reaction with fluoride ion.

Olefin-forming elimination reactions of β-hydroxy silanes are highly stereospecific. Under basic conditions, the geometry of the four-centered transition state results in an overall syn elimination.[15] Acid-catalyzed eliminations, on the other hand, proceed with anti geometry,[15] like other solvolytic β-elimination reactions. In the reaction of a β-acyloxy silane with fluoride ion, it appears that fluoride ion attacks at silicon, and the olefin is formed with concomitant departure of the β-acyloxy group. The fluoride-promoted elimination of an α-iodo-α-(trimethylsilyl)-β-trifluoro acetate seems to follow this pattern, and proceeds with anti stereochemistry.[16] By analogy with this study, the β-acetoxy silanes **5b** and **6b** should each give a single enol ether.

Surprisingly, ^{1}H NMR of the elimination products showed that both the *E* and *Z* enol ethers are produced in the same ratio from each of the two diastereomeric acetates. The configurations of the geometrical isomers were assigned on the basis that (i) the vinyl proton of the *Z* isomer **1b** is downfield from that of the *E* isomer **2b** (by analogy with previous assignments of similar compounds[7]), and (ii) a larger allylic coupling[17] is observed for the *Z* (1.0 Hz) than for the *E* (0.3 Hz) isomer. Each product mixture contained *Z*:*E* in a ratio of 4:1. The *Z*-isomer is the less hindered, based on inspection of molecular models. An identical result was obtained by using the free acids **5c** and **6c** (produced from the corresponding esters **5b** and **6b** by hydrogenolysis) to generate the enol ethers **1a** and **2a** in a ratio of 4:1. It is apparent that the two isomers must equilibrate under the conditions of the elimination.

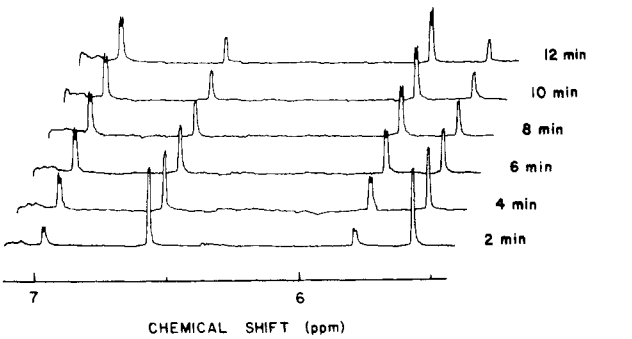


Figure 1. ^{1}H NMR spectra from the reaction of **5b** with CsF in perdeuteriodimethyl sulfoxide at 80 °C. The C-5 and vinylic protons from the products **1b** and **2b** are at δ 5.88 and 7.12 (J = 1.0 Hz) and at 5.64 and 6.71 (J = 0.3 Hz), respectively.

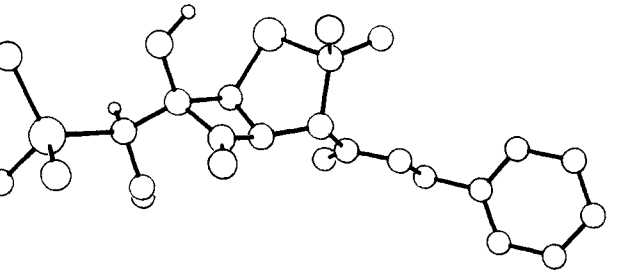


Figure 2. Crystal structure of **5a**.

When the time-course of the elimination reaction was monitored by ^{1}H NMR, it was found that one of the two acetates (**5b** or **6b**) was converted rapidly into predominantly the more hindered *E* isomer, which under the conditions of the reaction isomerized to the equilibrium mixture of the *Z* and *E* compounds (see Figure 1). The other acetate (**5b** or **6b**) suffered the elimination reaction more slowly, to yield the *Z* and *E* isomers in a 4:1 ratio during the whole conversion. These results are consistent with an elimination reaction proceeding with anti stereochemistry, and with the acetate diastereoisomer that reacts more slowly to the *Z*-*E* mixture having the *S* configuration in the C-6 side chain, that is, **5b**. The acetate **6b** has a severe steric interaction between the methoxy group and the C-5 hydrogen in the conformation required for anti elimination, and this compound would be expected to react more slowly and allow the possibility of geometrical isomer equilibration as fast as the olefin is formed.

The configurational assignments of **5** and **6** have been confirmed by X-ray crystal structure analysis of **5a**, which confirms the configuration in the C-6 side chain, since the natural configuration at C-5 is known to be *R* (Figure 2). It may also be noted that the adduct **5a** is indeed formed by attack on the 6-oxo compound **3** from the less hindered α face.

The isomerization reaction is rather curious, and a further observation is worth noting. When either of the purified *E* or *Z* isomers was heated to 80 °C in dimethyl sulfoxide, no change occurred in the geometry of the double bond. Isomerization occurred only in the presence of cesium fluoride. The equilibration can be viewed as resulting from the Michael addition of fluoride ion to the β carbon of the conjugated enol ether to form an anion stabilized by the β-lactam carbonyl. Rotation about the carbon-carbon single bond between C-6 and the side chain

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(17) In perdeuteriodimethyl sulfoxide as solvent.

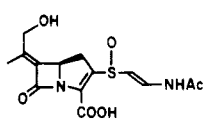
and subsequent collapse of the enolate (eliminating fluoride ion) equilibrate the olefins. There is procedent for this reaction, in the reversible addition of fluoride ion to perfluoroolefins in polar aprotic solvents to give perfluorocarbanions.[18]

Even though the elimination reaction does not produce a unique enol ether from a given β-silyl acetate, the reaction does yield the mixture of *E* and *Z* isomers quantitatively. The two isomers of the enol ethers (as the free acids **1a** and **2a**) can then be separated by reverse-phase HPLC.

A modification of this route may be applicable to the synthesis of other 6-(heteroatom-substituted-methylene)penicillanic acids. For example, it may be possible to synthesize penicillanic acid having a silyl-substituted spiroepoxide at the 6-position.[19] This compound could be converted by known methods[15] and by application of the work described in this paper into various enol ethers, enamines, and thio enol ethers. While such work has not been carried out, it is worth mentioning to indicate the potential versatility of silicon-stabilized anions with regard to functional group introduction in penams.

β-Lactamase Inhibitory Properties of 6-(Methoxymethylene)penicillanic Acids. The potassium salts of **1a** and **2a** were prepared for enzymatic studies and for biological testing. Neither substance displayed significant antimicrobial activity when tested in vitro against strains of *Escherichia coli* and *Proteus mirabilis*. Furthermore, compound **2a** showed no inhibitory properties against the purified RTEM-2 β-lactamase from *E. coli*. The enol ether **2a** is evidently not recognized by the enzyme: no hydrolysis of the β-lactam ring could be detected in the presence of the purified β-lactamase ($k_{cat} < 10^{-3}$ s^{-1}), and the enzyme is not inhibited by this penam ($K_i > 0.3$ mM). In contrast, the *Z* isomer, **1a**, was found to be a potent inhibitor and an irreversible inactivator of the β-lactamase. The kinetic characteristics of the interaction of **1a** with the enzyme are reminiscent of the behavior of clavulanic acid and of penicillanic acid sulfone[1] and indicate that the first-formed acyl–enzyme intermediate can partition three ways: to give the products of hydrolysis and the regeneration of free enzyme, to yield a transiently inhibited form of the enzyme, and to produce irreversibly inactivated enzyme. The details of these interactions have been published elsewhere.[3b]

Very recently, two β-lactamase inactivators have been reported that are related to 6-(methoxymethylene)-penicillanic acid in having substituted methylene groups at the 6-position. Asparenomycin A, **7**, is a naturally occurring carbapenem that is a broad spectrum antibiotic against many non-β-lactamase producing organisms.[20] in addition, synergistic activities were observed with a combination of **7** and ampicillin against various resistant bacteria, presumably as a result of inhibition of the β-lactamase (including the RTEM enzyme) by **7**. From other studies, it was concluded that asparenomycin A progressively inhibits β-lactamases produced by Gram-negative bacteria (such as the *E. coli* RTEM β-lactamase) by acylation of the enzyme.[21] The second β-lactamase inhibitor having a 6-methylene substituent is the semisynthetic compound, 6-(acetylmethylene)penicillanic acid, **8**.[22] This β-lactam forms a 1:1 complex with the RTEM-1 β-lactamase after brief incubation with the enzyme, and it appears that, after acylation of the enzyme, further reactions result in the formation of a stable inactive adduct.[22]



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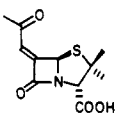

8

Experimental Section

Proton magnetic resonance spectra and carbon magnetic resonance spectra were recorded on a JEOL FX-270 spectrometer; chemical shifts are reported relative to tetramethylsilane. Infrared spectra were determined on a Perkin-Elmer 457A spectrometer. Ultraviolet measurements were made on a Perkin-Elmer 554 instrument. Mass spectra were measured on an AEI MS-9 instrument, and exact mass determinations were kindly made by P. Briggs on a Kratos MS-50 instrument. Optical rotations were measured on a Perkin-Elmer 241 polarimeter at ambient temperature at the sodium D line (589 nm). Analytical thin-layer chromatography (TLC) was carried out with E. Merck F-254 silica gel plates. Column chromatography was performed as described by Still et al.[14] with silica gel 60 (particle size 0.040–0.063 mm, E. Merck). Preparative high-pressure liquid chromatography was performed on a DuPont series 8800 chromatograph with a DuPont Zorbax ODS column (2.12 × 25 cm).

Benzyl 6-Oxopenicillanate (3). Benzyl 6α-hydroxypenicillanate[23] was oxidized by the method of Chandrasekaran et al.[8] The alcohol (9.8 g, 32 mmol) was dissolved in a mixture of dry benzene (70 mL) and dry dimethyl sulfoxide (70 mL) at 25 °C, and *N,N'*-dicyclohexylcarbodiimide (19.8 g, 96 mmol) was added with stirring. After 5 min, dichloroacetic acid (1.27 mL, 15 mmol) was added. After a further 10 min, during which dicyclohexylurea began to precipitate, the mixture was dilute with ether (350 mL), and a methanolic solution (30 mL) of oxalic acid (7.2 g, 80 mmol) was added. After the evolution of gas had ceased, the dicyclohexylurea was removed by filtration, and the solution was washed successively several times with water, aqueous $NaHCO_3$ (5% w/v), and brine. The solution was dried over anhydrous $MgSO_4$, and the solvent removed by evaporation. The residue was then chromatographed on silica gel, eluting with CH_2Cl_2:ether (10:1, v/v). The ketone **3** was obtained as a yellow crystalline solid (7.1 g, 73%): mp 53–55 °C; R_f (on silica, eluted with benzene:acetone, 4:1, v/v), 0.58; IR (Nujol) 1830 (sh), 1780, 1745 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.47 (s, 3 H), 1.53 (s, 3 H), 4.81 (s, 1 H), 5.23 (s, 2 H), 5.78 (s, 1 H), 7.38 (s, 5 H); ^{13}C NMR ($CDCl_3$) δ 25.17 (q, J = 129.9 Hz), 34.10 (q, J = 128.1 Hz), 64.32 (s), 67.85 (t, J = 150.1 Hz), 71.66 (d, J = 148.2 Hz), 76.81 (d, J = 179.4 Hz), 128.78 (d, J = 159.2 Hz), 134.31 (s), 166.65 (s), 168.08 (s), 190.45 (s).

Addition of [Methoxy(trimethylsilyl)methyl]lithium to the α-Keto Amide 3. (Methoxymethyl)trimethylsilane[24] (1.48 mL, 9.5 mmol) and dry tetrahydrofuran (40 mL) were added under argon to a dried 100-mL round-bottom flask equipped with a thermometer, a rubber septum, and a magnetic stirrer, and the solution was cooled to −78 °C. To this was added *sec*-butyllithium (8.6 mL, 9.5 mmol, 1.1 M in cyclohexane) over 15 min, and the solution was then stirred at −23 °C for 30 min to complete the formation of the anion.[12] The resulting pale yellow solution was cooled to −100 °C and added via canula under argon over 30 min to a stirred solution of the ketone **3** (3.05 g, 10 mmol, previously dried in vacuo for 24 h) dissolved in tetrahydrofuran (40 mL) at −100 °C. When the addition was complete, the solution was warmed to −78 °C and stirred at this temperature for 3 h. The

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mixture was then poured into a rapidly stirred solution (100 mL) of aqueous NH_4Cl at 0 °C. The product was extracted into ether and the combined organic extracts were washed with saturated aqueous NH_4Cl and then dried over anhydrous Na_2SO_4. The solvent was removed by evaporation to give an oil (3.0 g) which contained the β-silyl alcohols **5a** and **6a**. These diastereoisomers were separated by chromatography on silica gel, eluting with CH_2Cl_2:ether (25:1, v/v) to give **5a** (0.82 g, 20%) and **6a** (0.80 g, 20%).

6α-[(*S*)-Methoxy(trimethylsilyl)methyl]-6β-hydroxypenicillanic acid, benzyl ester (5a): R_f (on silica, eluting with CH_2Cl_2:ether, 20:1, v/v) 0.26; $[\alpha]_D$ +73° (*c* 0.095, $CHCl_3$); IR (film) 3390, 2920, 1775, 1740 cm^{-1}; ^1H NMR ($CDCl_3$) 0.11 (s, 9 H), 1.42 (s, 3 H), 1.60 (s, 3 H), 3.25 (s, 1 H), 3.37 (s, 1 H), 3.49 (s, 3 H), 4.52 (s, 1 H), 5.18 (s, 2 H), 5.29 (s, 1 H), 7.37 (s, 5 H); ^{13}C NMR ($CDCl_3$) −2.29 (q, J = 119.0 Hz), 26.09 (q, J = 129.9 Hz), 32.57 (q, J = 130.0 Hz), 61.95 (q, J = 148.2 Hz), 64.05 (s), 66.99 (t, J = 148.3 Hz), 69.18 (d, J = 148.2 Hz), 72.82 (d, J = 173.9 Hz), 78.75 (d, J = 129.9 Hz), 88.63 (s), 128.32 (d, J = 161.1 Hz), 134.49 (s), 167.25 (s), 175.21 (s); mass spectrum, m/z 423 (M^+), 408, 322, 278, 250; exact mass calcd for $C_{20}H_{29}NO_5SSi$ 423.15356, found 423.15310.

X-ray Analysis of 5a. The crystals were orthorhombic, space group $P2_12_12_1$. The cell parameters were a = 6.257 Å, b = 17.090 Å, c = 21.340 Å; d_c = 1.225 g·cm^{-1}. Intensity data were collected on a Nicolet R3 diffractometer with Mo Kα radiation (λ = 0.71073 Å) in the $\theta - 2\theta$ mode. The structure was solved by direct methods. In the refinement, 954 reflections [$F^2 > 2.5(F^2)$] were used. The positional and isotropic thermal parameters of the 28 non-hydrogen atoms were refined by using full-matrix least-squares calculations with the phenyl ring fixed as a rigid group. Inclusion of the parameters for the hydrogen atoms, fixed assuming standard geometries, permitted further refinement until R had reached 0.07 (see supplementary material).

6α-[(*R*)-Methoxy(trimethylsilyl)methyl]-6β-hydroxypenicillanic acid, benzyl ester (6a): R_f (on silica, eluting with CH_2Cl_2:ether, 20:1, v/v) 0.39; $[\alpha]_D$ +136° (*c* 0.07, $CHCl_3$); IR (film) 3440, 2950, 2850, 1780, 1750, 1695, 1500, 1455 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.10 (s, 9 H), 1.43 (s, 3 H), 1.58 (s, 3 H), 3.36 (s, 1 H), 3.42 (s, 3 H), 4.52 (s, 1 H), 5.17 (s, 1 H, J = 12.0 Hz), 5.23 (d, 1 H, J = 12.0 Hz), 5.62 (s, 1 H), 7.37 (s, 5 H); ^{13}C NMR ($CDCl_3$) δ −2.21 (q, J = 119.0 Hz), 25.82 (q, J = 129.9 Hz), 33.13 (q, J = 128.1 Hz), 61.68 (q, J = 146.4 Hz), 64.10 (s), 66.91 (t, J = 147.3 Hz), 69.18 (d, J = 144.6 Hz), 73.57 (d, J = 177.5 Hz), 75.00 (d, J = 126.3 Hz), 88.63 (s), 128.34 (d, J = 161.1 Hz), 134.63 (s), 167.03 (s), 175.88 (s); mass spectrum, m/z 423 (M^+), 408, 322, 278, 250; exact mass calcd for $C_{20}H_{29}NO_2SSi$ 423.15356, found 423.15443.

6α-[(*S*)-Methoxy(trimethylsilyl)methyl]-6β-acetoxypenicillanic Acid, Benzyl Ester (5b). The alcohol **5a** (20 mg, 0.05 mmol) was allowed to react with excess acetic anhydride (0.3 mL) in the presence of triethylamine (0.2 mL) and a catalytic amount of 4-(dimethylamino)pyridine (2 mg) at 25 °C. After 4 h, the solvents were removed under reduced pressure, and the residue was chromatographed on silica gel eluting with CH_2Cl_2 to give **5b** (19 mg, 80%): R_f (on silica, eluting with CH_2Cl_2) 0.52; IR (Nujol) 1780, 1745, 1710, 1675 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.13 (s, 9 H), 1.39 (s, 3 H), 1.54 (s, 3 H), 2.12 (s, 3 H), 3.47 (s, 3 H), 3.73 (s, 1 H), 4.58 (s, 1 H), 5.13 (d, 1 H, J = 12.2 Hz), 5.21 (d, 1 H, J = 12.2 Hz), 5.62 (s, 1 H), 7.36 (s, 5 H); ^{13}C NMR ($CDCl_3$) δ −2.40 (q, J = 119.0 Hz), 21.31 (q, J = 129.9 Hz), 25.50 (q, J = 128.7 Hz), 33.46 (q, J = 129.1 Hz), 62.24 (s), 62.59 (q, J = 140.9 Hz), 67.13 (t, J = 148.2 Hz), 69.34 (d, J = 146.4 Hz), 73.57 (d, J = 177.5 Hz), 74.30 (d, J = 133.6 Hz), 94.83 (s), 128.51 (d, J = 159.2 Hz), 134.76 (s), 167.14 (s), 168.49 (s), 169.35 (s); mass spectrum, m/z 465 (M^+), 450, 422, 405, 322, 250.

6α-[(*R*)-Methoxy(trimethylsilyl)methyl]-6β-acetoxypenicillanic Acid, Benzyl Ester (6b). The alcohol **6a** (20 mg, 0.05 mmol) was acetylated as described for **5a**. The product from the reaction was chromatographed on silica eluting with CH_2Cl_2 to give **6b** (20 mg, 85%): R_f (on silica, eluting with CH_2Cl_2) 0.54; IR (Nujol) 1778, 1750, 1715, 1668 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.12 (s, 9 H), 1.38 (s, 3 H), 1.58 (s, 3 H), 2.14 (s, 3 H), 3.45 (s, 3 H), 3.71 (s, 1 H), 4.54 (s, 1 H), 5.14 (d, 1 H, J = 12.1 Hz), 5.20 (d, 1 H, J = 12.1 Hz), 5.48 (s, 1 H), 7.56 (s, 5 H); ^{13}C NMR ($CDCl_3$) δ −2.56 (q, J = 119.0 Hz), 21.34 (q, J = 129.9 Hz), 26.30 (q, J = 129.9 Hz), 31.86 (q, J = 130.0 Hz), 62.62 (q, J = 140.9 Hz), 63.02 (s), 67.34 (t, J = 147.3 Hz), 69.80 (d, J = 146.4 Hz), 70.42 (d, J = 177.5 Hz), 75.30 (d, J = 126.3 Hz), 93.13 (s), 128.64 (d, J = 161.1 Hz), 134.68 (s), 167.33 (s), 169.33 (s), 170.35 (s); mass spectrum, m/z 465 (M^+), 450, 422, 405, 322, 250.

Reaction of the β-Silyl Acetates 5b and 6b with Cesium Fluoride. The β-silyl acetate **5b** or **6b** (10. mg, 0.22 mmol) in dry dimethyl sulfoxide (2.0 mL) containing anhydrous cesium fluoride (3.6 mg, 0.024 mmol) was heated at 80 °C for 2 h.[12] The mixture was cooled, water (5.0 mL) was added, and the product was extracted into ether. The combined extracts were washed with brine and then dried over anhydrous $MgSO_4$. After evaporation of the solvent, the isomers were separated by preparative TLC on silica with CH_2Cl_2:ether (50:1, v/v) as the eluent to give **1b** (5.5 mg, 77%) and **2b** (1.4 mg, 20%).

6-[(*Z*)-Methoxymethylene]penicillanic acid, benzyl ester (1b): R_f (on silica, eluting with CH_2Cl_2:ether, 50:1, v/v) 0.28; UV (methanol) 242 nm (ε 12 500 M^{-1} cm^{-1}), 303 (ε 5300 M^{-1} cm^{-1}); IR (film) 1770 (sh), 1750, 1710, 1680, 1594 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.38 (s, 3 H), 1.58 (s, 3 H), 3.83 (s, 3 H), 4.49 (s, 1 H), 5.19 (s, 2 H), 5.88 (d, 1 H, J = 1.0 Hz), 6.92 (d, 1 H, J = 1.0 Hz), 7.37 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 26.09 (q, J = 126.3 Hz), 33.02 (q, J = 128.1 Hz), 60.16 (q, J = 170.2 Hz), 67.18 (t, J = 148.2 Hz), 67.31 (d, J = 170.2 Hz), 69.99 (d, J = 144.6 Hz), 118.74 (s), 128.53 (d, J = 159.2 Hz), 128.59 (d, J = 159.2 Hz), 134.98 (s), 146.45 (d, J = 183.9 Hz), 168.25 (s), 170.11 (s); mass spectrum, m/z 333 (M^+), 91; exact mass calcd for $C_{17}H_{19}NO_5S$ 333.10347, found 333.10303.

6-[(*E*)-Methoxymethylene]penicillanic acid, benzyl ester (2b): R_f (on silica, eluting with CH_2Cl_2:ether, 50:1, v/v) 0.40 $[\alpha]_D$ −32° (*c* 0.031, $CHCl_3$); UV (methanol) 261 nm (ε 10 300 M^{-1} cm^{-1}), 303 nm (ε 5200 M^{-1} cm^{-1}); IR (film) 1760 (sh), 1742, 1720 (sh), 1684, 1670, 1594 cm^{-1}; ^1H NMR ($CDCl_3$) 1.39 (s, 3 H), 1.59 (s, 3 H), 4.00 (s, 3 H), 4.48 (s, 1 H), 5.19 (s, 2 H), 5.66 (s, 1 H), 6.37 (s, 1 H), 7.37 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 26.33 (q, J = 128.2 Hz), 32.70 (q, J = 127.9 Hz), 61.73 (q, J = 136.8 Hz), 65.43 (s), 66.24 (d, J = 174.5 Hz), 67.21 (t, J = 148.2 Hz), 69.96 (d, J = 146.2 Hz), 117.34 (s), 128.56 (d, J = 159.4 Hz), 128.61 (d, J = 161.2 Hz), 128.80 (d, J = 160.3 Hz), 128.91 (d, J = 159.2 Hz), 135.03 (s), 148.36 (d, J = 184.2 Hz), 166.76 (s), 168.31 (s); mass spectrum, m/z 333 (M^+), 91; exact mass calcd for $C_{17}H_{19}NO_4S$ 333.10347, found 333.10328.

6α-[(*S*)-Methoxy(trimethylsilyl)methyl]-6β-acetoxypenicillanic Acid (5c). The benzyl ester group of the acetate **5b** (95 mg, 0.2 mmol) was cleaved by catalytic hydrogenolysis in ethyl acetate (5 mL) using activated palladium on carbon (10% w/w, 0.1 g) under H_2 (50 psi) for 5 h at 25 °C. The catalyst was removed by filtration through Celite, and the solvent was evaporated to give the free acid **5c** as an oil (68 mg, 88%): IR (film) 1782, 1745 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.12 (s, 9 H), 1.50 (s, 3 H), 1.55 (s, 3 H), 2.10 (s, 3 H), 3.54 (s, 3 H), 3.71 (s, 1 H), 4.52 (s, 1 H), 5.61 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ −2.40 (q, J = 120.8 Hz), 21.34 (q, J = 129.9 Hz), 25.71 (q, J = 124.5 Hz), 32.94 (q, J = 130.0 Hz), 62.05 (s), 62.75 (q, J = 135.4 Hz), 69.31 (d, J = 146.4 Hz), 73.06 (d, J = 177.5 Hz), 74.41 (d, J = 133.6 Hz), 94.48 (s), 168.95 (s), 169.57 (s), 171.35 (s); mass spectrum (trimethylsilyl ester), m/z 447 (M^+), 432, 404, 232.

6α-[(*R*)-Methoxy(trimethylsilyl)methyl]-6β-acetoxypenicillanic Acid (6c). The benzyl ester group of **6b** (0.2 g, 0.43 mmol) was cleaved as described for **5b**. The product **6c** was isolated as a solid (0.15 g, 93%): IR (film) 1780, 1750 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.14 (s, 9 H), 1.53 (s, 3 H), 1.61 (s, 3 H), 2.14 (s, 3 H), 3.46 (s, 3 H), 3.71 (s, 1 H), 4.51 (s, 1 H), 5.47 (s, 1 H); ^{13}C NMR ($CDCl_3$) −2.48 (q, J = 119.0 Hz), 21.29 (q, J = 131.8 Hz), 26.52 (q, J = 131.8 Hz), 31.46 (q, J = 128.2 Hz), 62.62 (q, J = 141.8 Hz), 62.81 (s), 69.85 (d, J = 139.1 Hz), 70.20 (d, J = 179.4 Hz), 75.19 (d, J = 135.4 Hz), 92.89 (s), 169.35 (s), 170.76 (s), 172.32 (s); mass spectrum (trimethylsilyl ester), m/z 447 (M^+), 432, 404, 232.

Reaction of the β-Silyl Acetates 5c and 6c with Cesium Fluoride. The β-silyl acetate **5c** or **6c** (3.0 mg, 8.0 μmol) in dry dimethyl sulfoxide (2.0 mL) containing anhydrous cesium fluoride (1.3 mg, 8.6 μmol) was heated at 80 °C for 2 h. The mixture was cooled, cold water (5.0 mL) was added, and the pH of the solution was adjusted to 3 with 0.01 N HCl. The solution was extracted with ethyl acetate, and the extracts were washed with brine and dried over $MgSO_4$. After removal of the solvent, the mixture of isomers (1.9 mg, 98%) was redissolved in ethanol (5.0 mL), and

$KHCO_3$ (0.78 mg, 1 equiv) in water (1.0 mL) was added to form the carboxylate salts. This solution was concentrated under reduced pressure, and the isomers were then separated by HPLC on a reverse-phase column eluting with 20% methanol in 1% ammonium acetate buffer, pH 7.0. The isomers **2a** and **1a** were eluted quantitatively at 6.5 and 7.4 retention volumes, respectively, in the ratio of 1:4.

6-[(*Z*)-Methoxymethylene]penicillanic acid (1a): UV (ammonium salt in water) 245 nm (ϵ 12 400 M^{-1} cm^{-1}); IR (film) 1770, 1752, 1712, 1685, 1595 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.55 (s, 3 H), 1.65 (s, 3 H), 3.84 (s, 3 H), 4.44 (s, 1 H), 5.87 (d, 1 H, J = 0.7 Hz, 6.96 (d, 1 H, J = 1.7 Hz); mass spectrum (trimethylsilyl ester), m/z 315 (M^+).

6-[(*E*)-Methoxymethylene]penicillanic acid (2a): UV (ammonium salt in water) 252 nm (ϵ 10 300 M^{-1} cm^{-1}); IR (film) 1763, 1745, 1723, 1688, 1592 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.55 (s, 3 H), 1.65 (s, 3 H), 4.01 (s, 3 H), 4.43 (s, 1 H), 5.65 (s, 1 H), 6.40 (s, 1 H); mass spectrum (trimethylsilyl ester), m/z 315 (M^+).

β-Lactamase Inhibition and Inactivation by 1a. The TEM-2 β-lactamase was purified to homogeneity from *E. coli* W3110 carrying the RP4 plasmid. The inhibition of the β-lactamase by **1a** was followed by assay of the remaining enzyme activity. The enzyme (10–40 μL of a 22 μM solution) was incubated with 6-(methoxymethylene)penicillanic acid (20–50 μL of a 4.7 mM solution). Portions (5 μL) were withdrawn at appropriate intervals and mixed with a buffered solution (3.0 mL) of benzylpenicillin (3 mM), and the hydrolysis of the latter was followed at 240 nm.

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Supplementary Material Available: Lists of atomic coordinates, thermal parameters, bond distances, bond angles (4 pages). (Observed and calculated structure factors are available from the author.) Ordering information is given on any current masthead page.

Total Synthesis of (±)-Isoclovene

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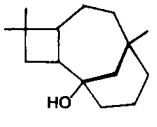
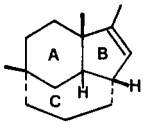
A total synthesis of racemic isoclovene (**2**), the most abundant sesquiterpene artifact derived from acid treatment of caryolan-1-ol (**1**), is presented. The characteristic tricyclo[6.2.2.$0^{5,12}$]dodecane skeleton of **2** has been set up creating the seven-membered ring C through an internal Michael addition of the suitably functionalized hydrindenone system **5**. The latter was in turn obtained starting from the readily available *cis*-4,5,6,7,8,9-hexahydro-8-methylindan-1,5-dione (**7**) in 15 steps, the most significant of which was a [3,3]-sigmatropic rearrangement which allowed the establishment of the correct stereochemistry of the quaternary center at C-5.

Acid treatment of caryolan-1-ol **1** represents a source of a wide variety of rearranged sesquiterpene artifacts, all but one featured by basic carbon frameworks common to many other natural compounds.[1] In fact only isoclovene **2** incorporates an unprecedented and unusual tricyclo[6.2.2.$0^{5,12}$]dodecane skeleton. Its structure was determined by X-ray crystallographic analysis of the crystalline derivative resulting by addition of hydrogen chloride.[2] Its possible mode of formation from **1** was investigated and a plausible mechanistic scheme was proposed.[3] The rarity of the carbon skeleton of **2** unlike the other compounds having the same origin, probably accounts for the limited literature on its chemistry.[4-7] The year 1983 marked a



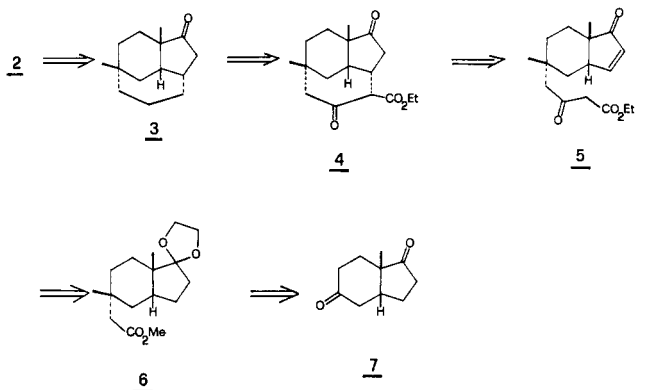
1



2

renaissance of interest in the chemical synthesis of **2** and two confirmatory syntheses of its structure, tackling the problem from a significantly different point of view, were reported by Kellner and Loewenthal[8] and by our group.[9]

Scheme I



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