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SYNTHESIS AND REACTION OF POTENTIAL ALTERNATE SUBSTRATES AND MECHANISM-BASED INHIBITORS OF CLAVAMINATE SYNTHASE[1]

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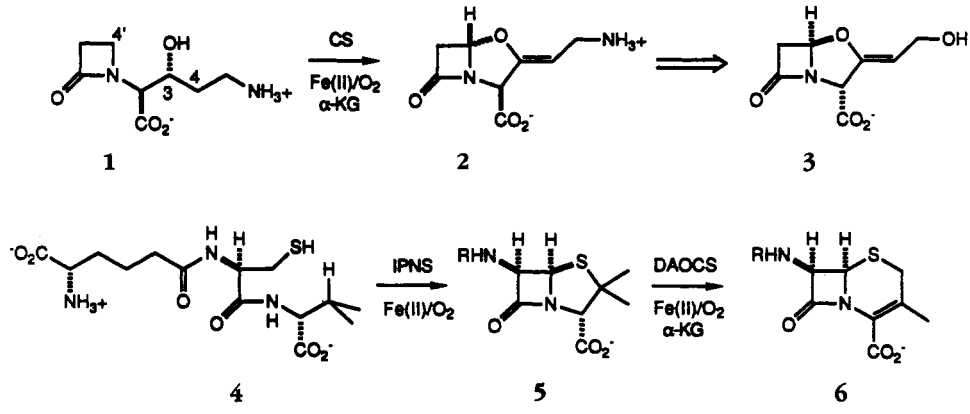
ABSTRACT.—Clavaminate synthase is an Fe^{II}/α-ketoglutarate-dependent enzyme central to the biosynthesis of the β-lactamase inhibitor clavulanic acid. In the presence of dioxygen it catalyzes the oxidative cyclization/desaturation of proclavaminic acid to clavaminic acid in a two-step process. Samples of (4′*R*)- and (4′*S*)-D,L-[4′-^{2}H]proclavaminic acid have been prepared and used to demonstrate that oxazolidine ring formation occurs with retention of configuration. The stereochemical course of oxygen insertion from substrate that takes place in this oxidative cyclization is the same as that observed from molecular oxygen in several hydroxylation reactions catalyzed by other Fe^{II}/α-ketoglutarate-dependent enzymes. The ferryl (Fe^{IV}=O) species thought to be transiently involved in each of these processes was investigated in the present work with clavaminate synthase and three structural analogues of proclavaminic acid bearing vinyl or ethynyl groups at C-4′ or a cyclopropyl at C-4. In the synthesis of the former two derivatives and proclavaminic acid stereoselectively labeled with deuterium at C-4′, introduction of the unsaturated substituents in a stereochemically defined manner at C-4′ relied upon ready access to (4*R*)-4-thiophenyl-2-azetidinone. Trimethylsilyl substitution could be easily achieved at C-3 of the optically pure starting material to give the readily separable cis and trans diastereomers. In radical chain reactions in which the thiophenyl was replaced by deuterium or in anionic reactions in which the thiophenyl was eliminated as its sulfone and replaced by addition of carbanions, the steric bulk of the trimethylsilyl group at C-3 governed the approach of incoming reagents to give the trans product. The enzymatic fate, however, of these derivatives was disappointing, yielding neither detectable reaction nor hoped-for inactivation of clavaminate synthase. Finally, as mixed competitive/noncompetitive inhibitors of catalysis, they gave unexceptional inhibition constants in the range 2–10 mM.

Oxidative cyclization plays a central role in the biosynthesis of the β-lactam antibiotics clavulanic acid [**3**], penicillin (e.g., **5**) and cephalosporin (e.g., **6**) (1–4). The enzymes clavaminate synthase (CS) (1,5), isopenicillin N synthase (IPNS) (6,7), and deacetoxycephalosporin C synthase (DAOCS, Scheme 1) (8–10) all require ferrous ion, molecular oxygen, and, apart from IPNS, α-ketoglutaric acid (α-KG) to achieve cyclization reactions in which a substrate nucleophile is oxidatively inserted into an internal C-H bond to give a cyclic/bicyclic product. The chemical potential of these strained entities is released during their ultimate expression of enzyme inhibitory activity. Of fundamental importance to understanding the catalytic cycle of these proteins is the recognition that, unlike conventional hydroxylase chemistry, molecular oxygen is reduced to H_2O and renders the overall transformation thermodynamically favorable (1).

The cornerstone of current thinking about α-KG-dependent oxygenase activity is the putative intermediacy (11) of a ferryl oxidant, $[Fe^{IV}=O]^{2+}$, analogous in its reactions to the perferryl species widely held to be involved in the hydroxylation chemistry carried out by the cyctochromes P-450 (12). These iron-oxo species are believed to carry out homolytic hydrogen abstraction at C—H bonds to give carbon-based radicals or, through one-electron oxidation, generation of carbocations and subsequent ionic reaction to product. It has been proposed that CS shares with other known α-KG-

[1]This paper is dedicated with respect and affection to the memory of Professor Edward Leete.



SCHEME 1

dependent dioxygenases a common mechanism of oxygen activation to the ferryl species, but an alternate reaction path then intervenes to give a bicyclic product in a manner having important similarities to IPNS and DAOCS (1,3).

Early in our mechanistic studies of CS we reported the stereochemical course of the clavaminate synthase reaction in which proclavaminic acid, stereospecifically deuteriated at C-4′, was synthesized and reacted with CS. These experiments showed that the CS reaction was at least 90% stereospecific in the absence of a *V/K* kinetic isotope effect, and like other α-KG dependent dioxygenases, oxygen insertion occured with overall retention of configuration (13). We report here full synthetic details for the (4′*R*)- and (4′*S*)-D,L-[4′-^{2}H]proclavaminic acids and the results of their interaction with CS. Additionally, to gather support for the involvement of a ferryl species during the CS-catalyzed reaction (oxidative cyclization rather than hydroxylation), as well as to address other mechanistic issues of the reaction, we describe an extension of this synthesis to the preparation fo the 4′-alkylated analogues of proclavaminic acid, as well as the synthesis of 4-cyclopropyl proclavaminate, and their interactions with CS.

Our goals in designing these structural analogues were twofold: the first was to obtain alternate substrates possessing functionality and defined stereochemistry to investigate specific mechanistic questions about the CS reaction, in particular the validity of the ferryl-oxo model and the nature of the chemical intermediates formed in the reaction. The second goal was to open the enzyme to structural study with analogues incorporating functional groups capable of undergoing reaction with the putative iron-oxo species to generate highly reactive intermediates which could in turn covalently modify the enzyme active site. To this end, three functional groups were chosen compatible with these objectives: vinyl, ethynyl, and cyclopropyl. These functional groups have seen extensive use in probing the chemistry of a number of iron-containing oxygenases, including the cytochromes P-450 (12) and α-KG-dependent dioxygenases. Particularly relevant to our work were recent studies with the α-KG-dependent dioxygenases thymine hydroxylase (14) and γ-butyrobetaine hydroxylase (15), as well as the β-lactam cyclases IPNS and DAOCS/DACS. These enzymes, particularly IPNS, are remarkably tolerant of substrate structural changes, and as a result, a considerable body of mechanistic information has been gleaned from the reactions of these normal substrate derivatives (4).

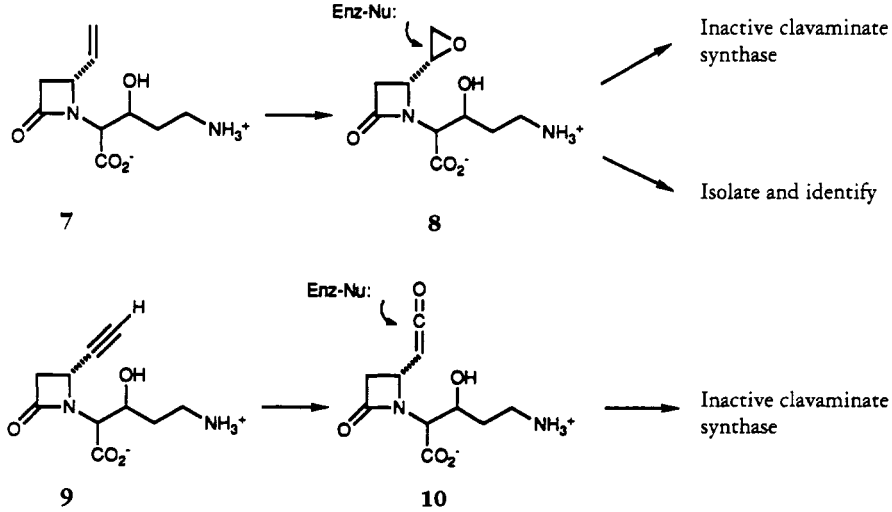
From examination of the CS reaction, it was determined that the most useful and informative introduction of potentially reactive functional groups into the proclavaminate framework would be achieved by functionalization at C-4′ and C-4. Vinyl and ethynyl

groups were chosen for C-4′ and cyclopropyl at C-4. Because the initial oxidation catalyzed by CS occurs with abstraction of the C-4′ *pro-S* hydrogen (13), introduction of unsaturated elements at C-4′ allows for two potential modes of reactivity depending on the position occupied by the substituent. If the group is located in the *pro-S* position, then the normal oxidation would be blocked, but the group may be positioned favorably to react directly with the postulated iron-oxo species. Shown in Scheme 2 are some of the possible consequences of direct reaction of the iron-oxo with either a vinyl or ethynyl substituent. By analogy to the results obtained with thymine hydroxylase, if a ferryl-oxo species is the correct description of the enzyme oxidant, then interaction with a vinyl analogue **7** could yield an epoxide as in **8**. This product might leave the active site, in which case it could be isolated and its structure confirmed, or it may cyclize to a 6- or 7-membered ring species. Alternatively, if an enzyme nucleophile is suitably situated within the active site, then reaction with the epoxide could result in covalent attachment and enzyme inactivation. Similarly, reaction of a ferryl-oxo species with the 4′ *pro-S* ethynyl analogue **9** would be expected to lead to ketene formation as in **10** followed by possible enzyme inactivation.

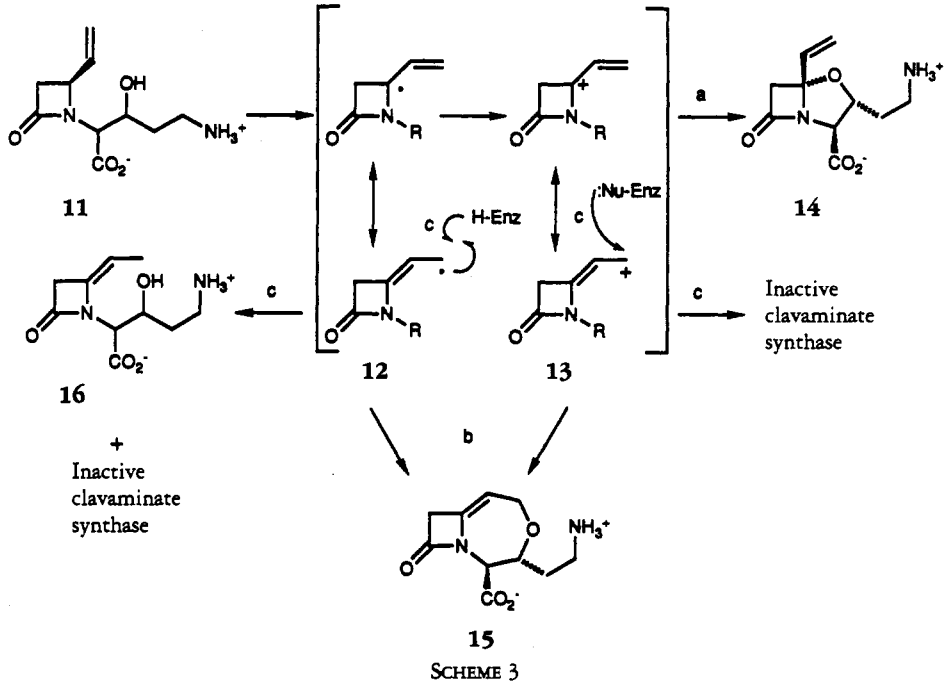
For the case in which the unsaturated substituents occupy the 4 *pro-R* position of proclavaminate, the initial abstraction of the 4 *pro-S* hydrogen is possible, giving, in the case of the 4′-vinyl substrate **11**, the allylic radical **12** or, through subsequent electron transfer, cation **13** (Scheme 3). These intermediates could potentially react in three separate ways: normal cyclization via path a would give the clavam intermediate **14**, while an alternative cyclization to the terminal position of the allylic system via path b would give the oxybicyclic azetidinone **15**. Inactivation of the enzyme could occur via path c, which could be visualized to occur by either a radical or cationic pathway.

While the fate of the clavam **14** or the 7-membered **15**, formed after a single oxidation of **11**, would probably be release into the reaction medium, the potential for a second oxidation exists for both compounds. This could follow the course of the normal oxidation of dihydroclavaminate, giving the corresponding exocyclic enol ethers. Alternatively, the position of the vinyl group in **14** might enable it to react further with the iron-oxo center, resulting in epoxidation and product release or enzyme inactivation.

An analogous set of reactions can be considered for the substrate bearing a 4′ *pro-R* ethynyl group, and these are summarized in Scheme 4. In this case, while a bicyclic



SCHEME 2

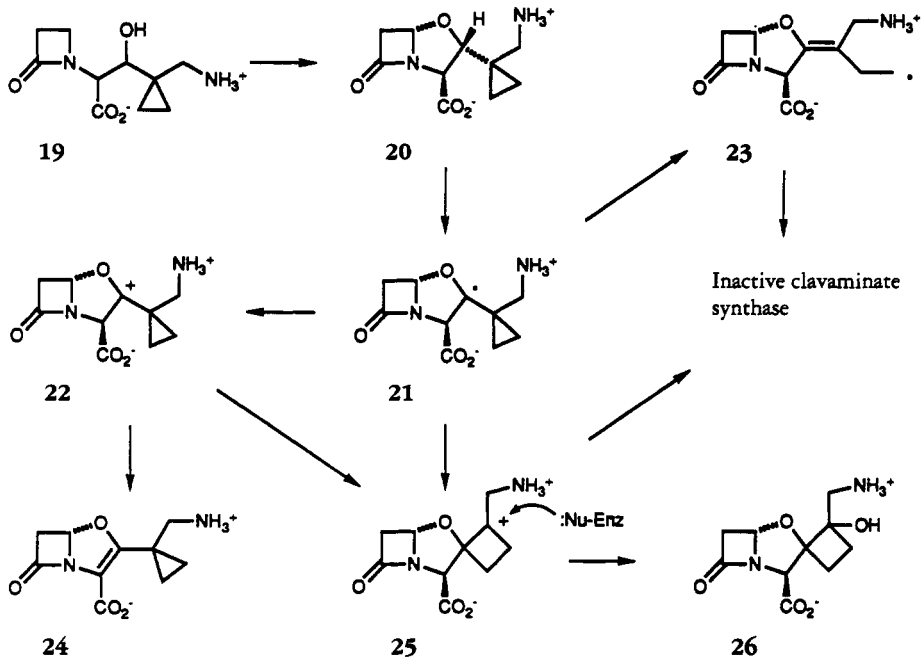


SCHEME 3

product such as **18** can be readily visualized, the intermediate allene radical or cation would be highly reactive and could lead on to enzyme inactivation.

The presence of a cyclopropyl group at C-4 of proclavaminate (**19**, Scheme 5) would not be expected to influence the initial oxidation and cyclization to the clavam intermediate **20**, provided **19** was accepted as a substrate, and instead was envisioned as a useful probe for the nature of the second oxidation at C-3. Upon hydrogen abstraction at C-2 of **20**, three possible paths would be available to the cyclopropylcarbinyl radical **21**. If the rate of cyclopropyl ring opening were competitive with either putative normal oxygen rebound or further electron transfer to the cation, then formation of the radical **23** might be accompanied by enzyme inactivation. If ring opening were not competitive, then hydroxylation or electron transfer would both ultimately yield the cation **22**, which, lacking a C-4 hydrogen, could lose the C-3 hydrogen to produce the clavem **24** with an endocyclic double bond or decompose to ring opened products. Alternatively, this stabilized cation could suffer nucleophilic attack leading to enzyme inactivation, or

SCHEME 4

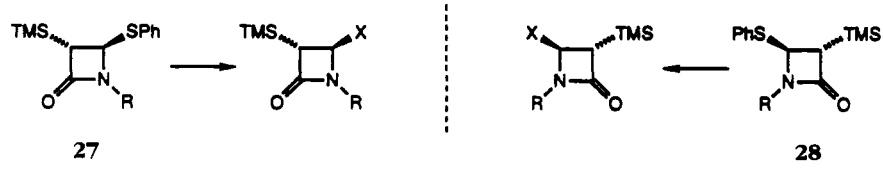


SCHEME 5

possibly undergo rearrangement through the cyclopropylcarbinyl manifold to the homoallylic cation (not shown) or the cyclobutyl cation **25** followed by addition of H_2O to **26** or inactivation.

STRATEGY FOR THE CHIRAL SYNTHESIS OF 4′-SUBSTITUTED PROCLAVAMINATE ANALOGUES.—Control of the absolute stereochemical orientation of substituents at C-4′ in proclavaminic acid was the key synthetic task to be accomplished in order to selectively probe the reactions catalyzed by CS. Linked with consideration of meeting this need was the prior stereochemical question of oxazolidine ring formation in the conversion of proclavaminic acid [**1**] to clavaminic acid [**2**]. A general solution was identified in the trimethylsilylated 4-thiophenylazetidinones **27** and **28** reported to be accessible in enantiomerically pure form from L- and D-aspartic acid, respectively (16,17). Replacement (Scheme 6) of the thiophenyl in either a radical or cationic process would proceed through a reactive intermediate that would be at once stabilized by the adjacent TMSi group whose steric bulk would be expected at the same time to govern the approach of a reagent X to occur selectively/specifically anti.

Unfortunately, the yield of **27** and **28** over several steps from the corresponding amino acid was only about 10%. This prospect was improved in an interesting way. 3-Acetoxyazetidinone could be converted to (+)-(4*R*)-thiophenylazetidinone [**29**] in about 50% enantiomeric excess (e.e.) over two steps by the procedure of Ikegami but using (−)-cinchonidine in place of (+)-cinchonine (18). Fractional crystallization gave optically pure **29** of higher melting point and optical rotation than originally reported

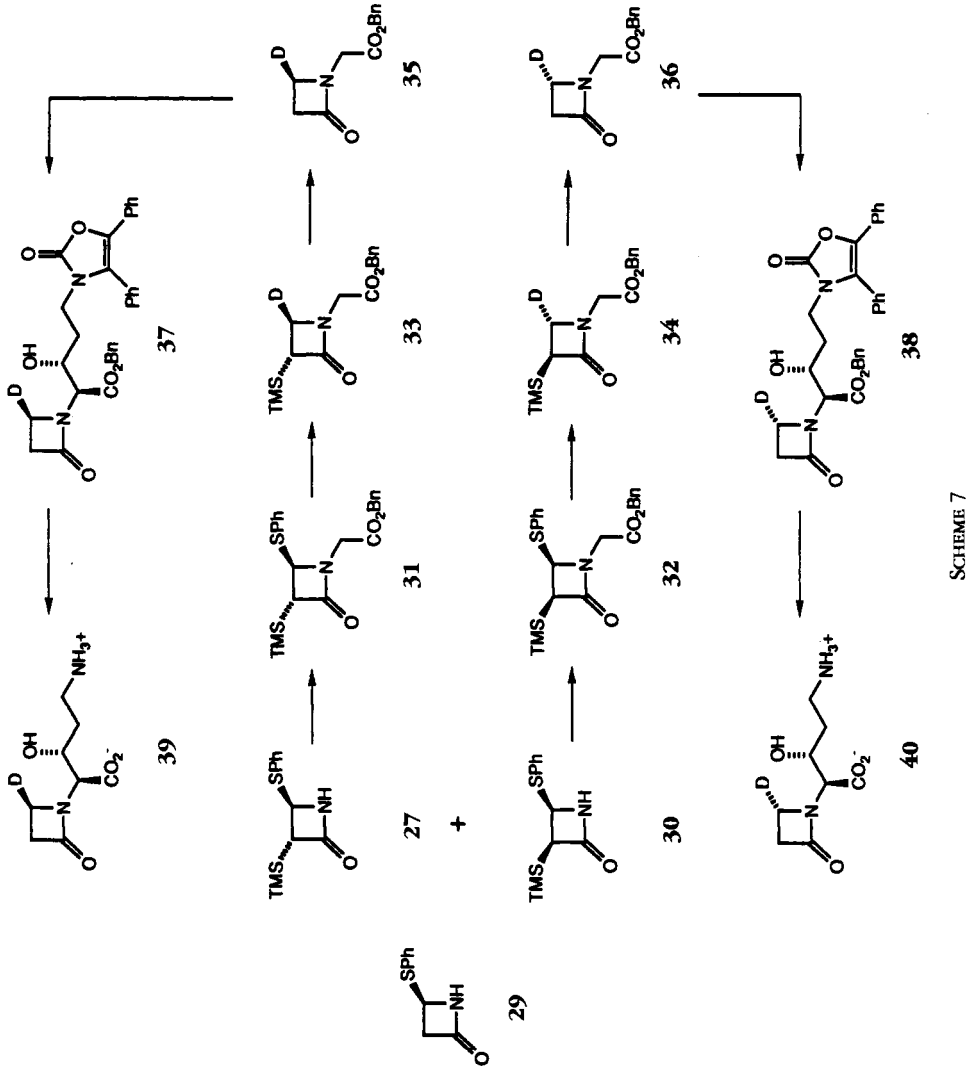


SCHEME 6

(see Experimental Section). After *N* protection with TMSiCl/Et_3N in THF, the azetidinone enolate was generated at the 3 position with LDA at −78°, followed by silylation with TMSiCl, giving the *cis*- and *trans*-azetidinones **30** and **27** in a ratio of 2:1 in 82% combined yield (Scheme 7). Although we had originally envisioned having access to the enantiomeric deuteriated azetidinones ultimately from the corresponding 4*R* and 4*S* azetidinones, the formation of the *cis*-azetidinone **30** allowed us to synthesize both compounds from a single antipode. It appears that the cis azetidinone is the kinetic product, since only the cis isomer is observed when the reaction is terminated after short reaction times, while allowing long reaction times gives exclusively the *trans*-azetidinone. These observations may be due to intramolecular ligation of lithium by the thiophenyl substituent at C-4, resulting in preferred silylation cis to the thiophenyl. Base-promoted epimerization at C-3 would then give the thermodynamically favored trans product. The pure diastereomers **30** and **27** were readily separated by Si gel chromatography and individually crystallized and identified by their vicinal C-3/4 coupling constants. These were reacted with benzyl bromoacetate in the presence of lithium bis(trimethylsilyl) amide to afford **31** and **32**. Reduction with tri-*n*-butyltin deuteride proceeded with high diastereofacial selectivity (17±1:1) of the intermediate radicals formed in the chain propagation step to give the enantiomeric products **33** and **34**. These were readily desilylated to the enantiomeric deuteriated azetidinones **35** and **36**, which were elaborated as previously described to (4′*S*)- and (4′*R*)-D,L-[4′-^{2}H]proclavaminic acids **39** and **40** (2).

Incubation of **39** and **40** with CS could be driven only to partial completion (65–70% and 50%, respectively) owing to the limited amounts of enzyme then available (13). The samples of clavaminic acid were purified by hplc, derivatized, and analyzed for their deuterium content by mass spectrometry and ^{1}H-nmr spectroscopy (19). The β-deuteriated substrate **39** substantially retained its heavy isotope in this conversion (94±1% D_1) whereas its considerably more slowly reacting α-deuteriated diastereomer **40** largely lost its label (12±1% D_1). At complete turnover of each substrate, the expected isotopic content of each product could be estimated knowing the deuterium contents of **37** and **38** (97–98% D_1) and the diastereofacial selectivity of the tri-*n*-butyltin deuteride reduction of **31** and **32** (96:6 to 95:5). Assuming a stereospecific reaction proceeding with retention of configuration during the oxygen insertion at C-4′ of proclavaminic acid, the deuterium contents at 100% conversion can be predicted to be 91–92% and 5–6% from **39** and **40**, respectively. While both of these estimates lie below the observed incorporation of deuterium at partial extents of reaction, it must be noted that primary and α-secondary *V*max/*K*m kinetic isotope effects act in the transformation (2). Taking this factor into account, oxazolidine ring formation carried out in this oxidative cyclization takes place with functionally complete stereochemical retention.

The success of this sytem for the enantioselective introduction of deuterium prompted pursuit of an analogous strategy for the asymmetric synthesis of the envisioned C-4′ alkylated compounds. However, instead of replacing the thiophenyl group in a reductive step, it would be activated for elimination by oxidation to the phenylsulfone (20). It has been previously demonstrated that a variety of nucleophilic substitution reactions can be performed at the 4 position of the simple 4-phenylsulfonylazetidinone (21); and, since this is an elimination-addition process, the steric bulk of the C-3 TMSi group could be anticipated again to govern the approach trajectory of any incoming nucleophile resulting preferentially in formation of the trans isomer. As with the radical chain reduction of **31** and **32**, the reaction proceeds analogously through enantiomeric transition states such that the phenylsulfone from the cis isomer provides the enantiomer



SCHEME 7

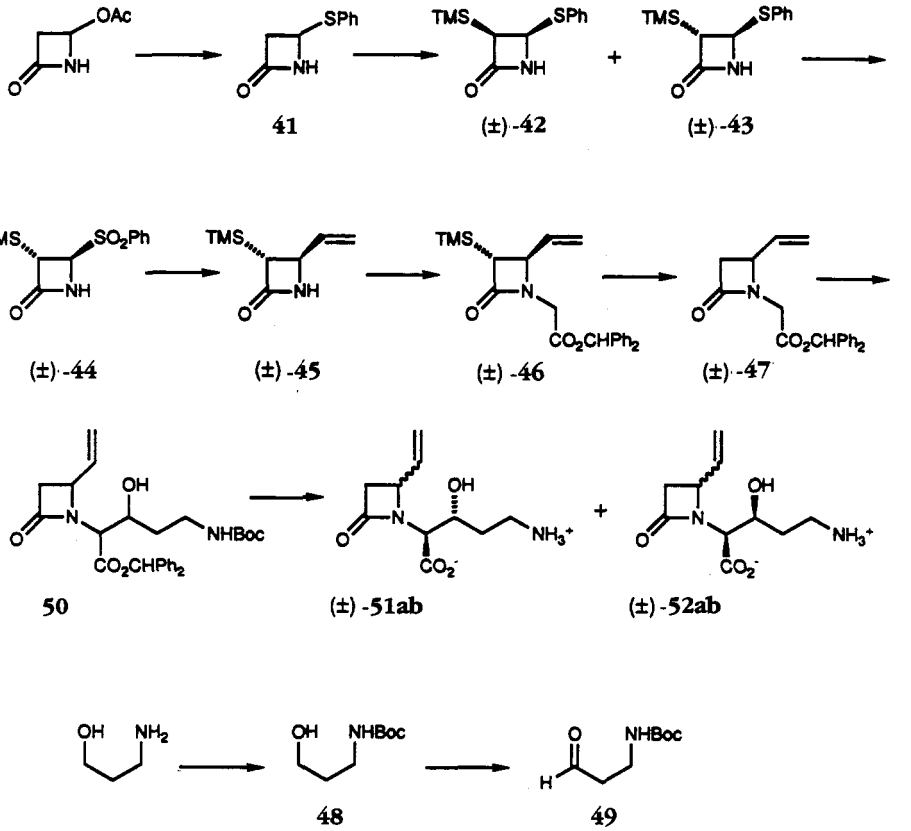
to that produced from the trans isomer. These expectations proved to be the case as trans addition was observed exclusively within the detection limits of ^{1}H-nmr spectroscopy.

SYNTHESIS OF PROCLAVAMINIC ACID ANALOGUES.—Although it is reasonable to assume that, in analogy with proclavaminate, the L-threo (2*S*,3*R*) isomers of each analogue are most likely to bind and react with CS, in principle any of the eight C-4′ substituted isomers or the four C-4 cyclopropyl isomers could undergo reaction. Recognizing this, we chose to synthesize and investigate all of the analogues initially as racemates, as this was a much less labor-intensive route to all of the compounds of interest.

The synthesis of racemic 4′-vinyl proclavaminate is shown in Scheme 8. Reaction of 4-acetoxyazetidinone with phenyl disulfide and $NaBH_4$ in EtOH gave 4-thiophenylazetidinone [**41**] in 79% yield. Although it was unnecessary to introduce the TMSi group for a racemic synthesis, all of the steps planned for the asymmetric synthesis were performed on the racemate to insure the workability of the chemistry. Thus, **41** was treated with Et_3N and TMSiCl in THF at 0°, giving the *N*-silylated azetidinone. This compound was not isolated; instead the $Et_3N{\cdot}HCl$ was removed by filtration, the filtrate cooled to −78°, and the azetidinone reacted with LDA and TMSiCl, giving the *cis*- and *trans*-3-trimethylsilyl-4-thiophenylazetidinones **42** and **43** in an overall yield of 79% (cis:trans 1.6:1). Oxidation of the *trans*-azetidinone **43** to the phenylsulfonylazetidinone **44** with *m*-CPBA was accomplished in 87% yield. Treatment of **44** with two equivalents of vinyl magnesium bromide gave exclusively the *trans*-azetidinone **45** in yields of 70–95%. *N*-Alkylation was accomplished by reacting **45** with benzhydryl bromoacetate in the presence of powdered KOH and catalytic $Bu_4N^+Br^-$ (22) in 41% yield. Desilylation of the glycylazetidinone **46** with KF in MeCN gave **47** in a yield of 84%. Aldol condensation with the *N*-BOC-protected aldehyde **49** was accomplished smoothly in the presence of LiHMDS at −78°, giving the diastereomeric protected vinyl proclavaminates **50** in a combined yield of 91%. The individual diastereomers could not be isolated from one another at this point, so were separated as racemic pairs of diastereomers. Deprotection of the diastereomeric pairs was accomplished essentially in quantitative yield in anhydrous TFA at 0° (23,24), whereupon the products **50** could be separated by reversed-phase hplc into racemic threo pairs epimeric at C-4′ (**51ab**) and the corresponding erythro pairs **52ab**.

The synthesis of 4′-ethynylproclavaminic acid (**9**/**17**) was analogous to that of the 4′-vinyl derivative; however, in this case the four product diastereomers were separable while fully protected (Scheme 9). Having established in the synthesis of the vinyl analogue above the high directing ability of the TMSi group for entering nucleophiles, the steps to introduce and remove this substituent were eliminated in the preparation of **9**/**17**. Thus trimethylsilylacetylene was added to 4-phenylsulfonyl-2-azetidinone (21) to give **53**, which was reacted as above with benzhydryl bromoacetate to give **54**. Aldol condensation with the BOC-protected aldehyde **49** gave **55** as a mixture of four racemic stereoisomers, two of which constituted the majority of the product and could be cleanly separated by Si gel chromatography (isomers 1 and 2, Scheme 9). It was subsequently discovered, however, that desilylation of each proceeded with epimerization to give a unique threo and erythro pair of enantiomers that could in turn be separated. Finally, trifluoroacetic acid deprotection and hplc purification gave each of the four possible racemic diastereomers **60**–**63** of the desired 4′-ethynyl derivative of proclavaminic acid.

4-Cyclopropylproclavaminic acid [**19**] (Scheme 5) was accessible from *N*-BOC-3-amino-2-cyclopropylpropanal [**66**] (Scheme 10). Methyl cyanoacetate was readily cyclopropanated by reaction with 1,2-dibromoethane (25), and straightforward reduction, protection, and Swern oxidation gave the desired aldehyde **66**. The acid-labile

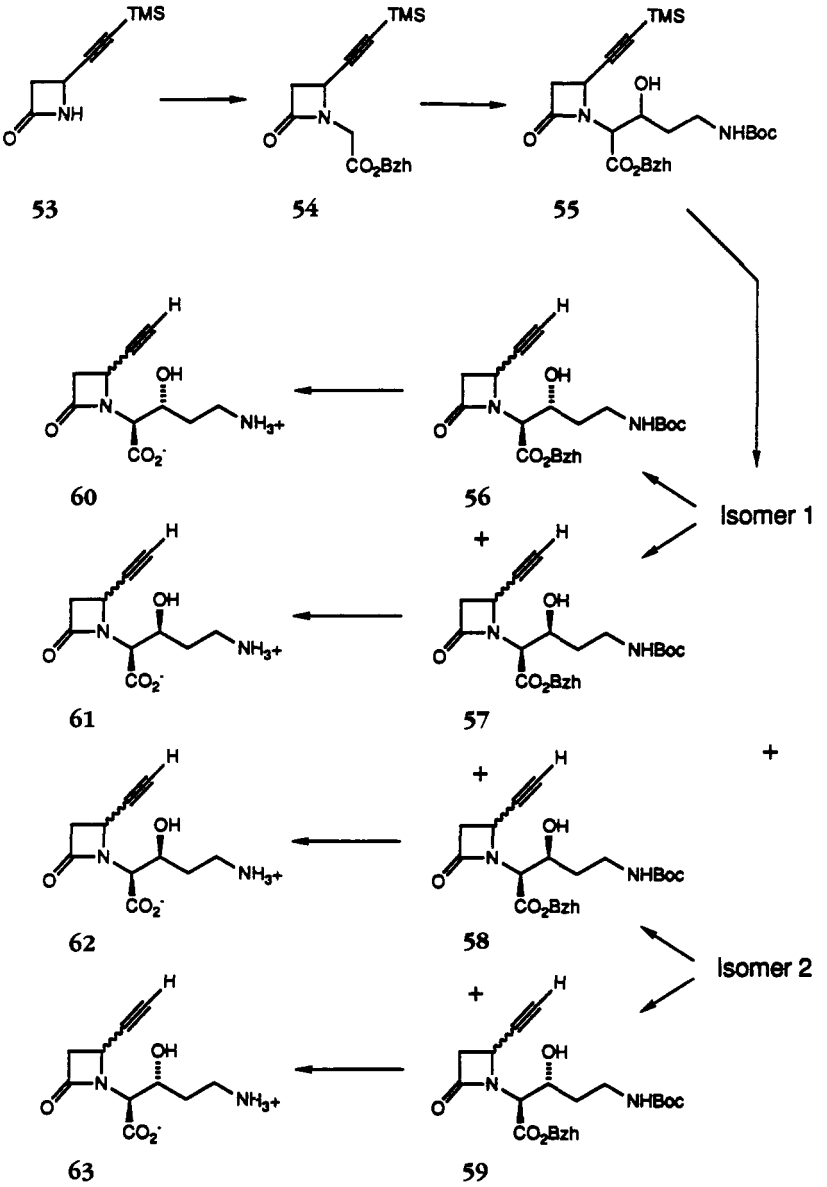


SCHEME 8

benzhydryl ester (Bzh) of (2-oxazetidinyl)acetate was prepared from the corresponding benzyl ester (2, 26, 27) by hydrogenolysis and reprotection with diphenyldiazomethane. Aldol condensation essentially as before gave the separable threo and erythro products **67** and **68**, which were readily deprotected to the corresponding **69** and **70** and purified by hplc.

PROOF OF RELATIVE CONFIGURATION.—Although the proclavaminic acid analogues had been prepared as racemates, any compound that showed significant activity with CS was to be synthesized in optically pure form of unambiguous absolute configuration. As a first step in this stereochemical assignment process, we sought to differentiate between the erythro and threo diastereomeric pairs at C-2/3. Simple comparison of the respective ^{1}H-nmr spectra with threo and erythro proclavaminate proved unsatisfactory for this purpose, so we turned to modifications of the compounds that would provide unambiguous evidence for the relative configurations, namely, the dehydrative decarboxylation of β-hydroxycarboxylic acids mediated by DMF dimethylacetal (28,29). Contrary to an earlier report (28), we observed an *anti*-elimination process such that *N*-protected *threo*-proclavaminate **71** provided the *E*-olefin **72**, while dehydrative decarboxylation of the erythro diastereomer **73** provided the *Z*-olefin **74** (Scheme 11). The products were easily distinguished by the olefinic coupling constants of 14.3 and 9.4 Hz, respectively, observed in their ^{1}H-nmr spectra.

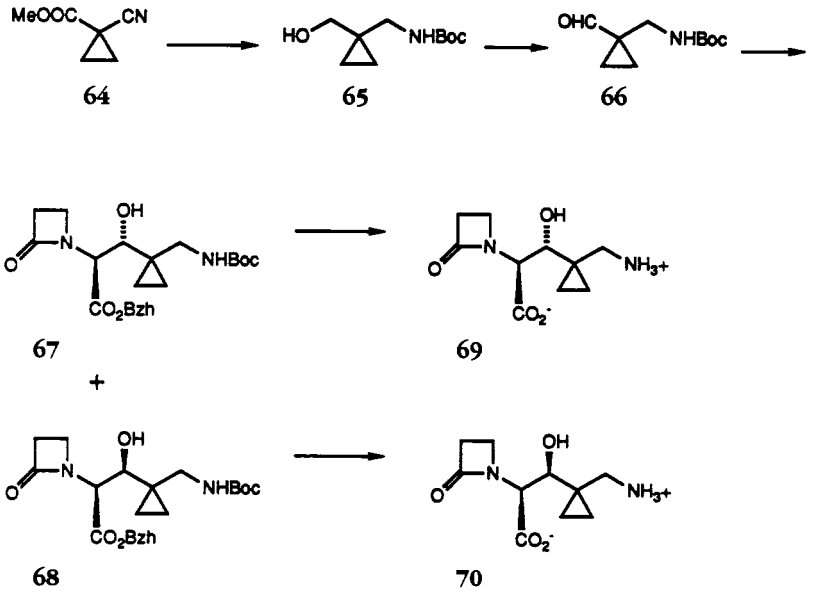
The stereochemical course of the dehydrative decarboxylation for both diastereomers of proclavaminate having been determined, the reaction was applied to the hplc-



SCHEME 9

purified proclavaminate analogues. *N*-Protection of these proclavaminate derivatives with ethyl or *t*-butyl chloroformate and reduction of the ethynyl groups to 4′-ethyl gave decarboxylative elimination products clearly having olefinic coupling constants of either 9–10 Hz or 14–15 Hz. Longer reaction times were required for the erythro diastereomers than for the threo diastereomers. The erythro and threo pairs of the 4-cyclopropyl and 4′-ethynyl derivatives of proclavaminic acid were thus readily distinguished and have been designated in the Experimental section. The 4′-vinyl stereoisomers, while identical after reduction to those obtained from the 4′-ethynyl, were not determined owing to the biological observations made below.

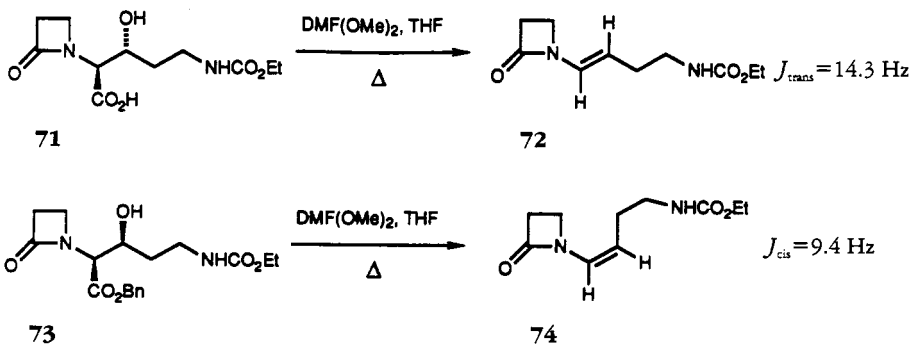
Proclavaminate and the proclavaminate analogues tended to be hygroscopic and therefore were difficult to weigh accurately. To establish concentrations for bioassays,



SCHEME 10

aqueous solutions of these compounds were prepared and standardized by ninhydrin derivatization (30,31), using β-alanine to generate a standard curve. Although this is a well-established protocol for the determination of amino acid concentrations, we discovered that a secondary absorption at lower wavelengths interfered with the normal measurement at 570 nm, giving erratic results. However, by monitoring at 600 nm, away from the λ max of the ninhydrin derivative, we were able to eliminate the interference and obtain reliable measurements.

ENZYMATIC ACTIVITY OF 4′-VINYL-, 4′-ETHYNYL-, AND 4-CYCLOPROPYL PROCLAVAMINATE WITH CLAVAMINATE SYNTHASE.—In assessing the activity of these compounds with CS, we were concerned with four potential phenomena: (1) the ability of the compounds to act as alternate substrates for CS, that is, to undergo oxidative cyclization chemistry in analogy with proclavaminate; (2) the ability of CS to chemically act on the compounds to generate non-clavam products; (3) the ability of the compounds to irreversibly inactivate CS; or, in the absence of any of the above, (4) the ability of the compounds to reversibly inhibit normal substrate processing. All of the assays contained the standard assay components, which included 50 mM MOPS (pH 7.0), 0.5 mM DTT,



SCHEME 11

0.1 mM sodium ascorbate, and 0.01 mM ferrous ammonium sulfate (1). The concentration of α-KG, CS, and D,L-proclavaminate varied depending on the type of experiment.

The assay for the formation of clavam products was based on the imidazole-mediated decomposition of the clavam nucleus to the α,β-unsaturated acyl imidazole (32,33). Nucleophilic addition of imidazole to the β-lactam carbonyl results in opening of both the β-lactam and oxazolidine rings, giving a chromophore having a λ max=312 nm with an extinction coefficient taken to be 26,900 M^{-1} based on measurements with clavulanic acid. In the assays for clavam formation, parallel incubations containing 2.0 mM α-KG and the compounds of interest or *rac*-proclavaminate at a concentration of 1.0 mM were run with CS (ca. 50 μg) for 1 h. After terminating the reactions with EDTA, 3 M imidazole at pH 6.8 was added, and the mixture was heated at 40° for 20 min. After dilution with H_2O, the solution was scanned from 272 to 352 nm for chromophore development centered at 312 nm. Testing of all four diastereomers of both 4′-vinyl and 4′-ethynyl proclavaminate, as well as the two diastereomers of 4-cyclopropyl proclavaminate, failed to show production of any detectable clavam products. The detection limit for this assay is approximately 0.1% of the proclavaminate control reaction, suggesting that at best these compounds are extremely poor substrates for the observation of oxidative cyclization chemistry.

To examine these compounds for the formation of non-clavam products, we utilized a protocol incorporating *o*-phthaldialdehyde (OPA) derivatization of the incubation mixture followed by reversed-phase hplc analysis (34). Primary amines react extremely rapidly with OPA to form an isoindole product. This derivative has a λ max ca. 340 nm with an extinction coefficient of approximately 4800 M^{-1} (35). Each of the compounds was incubated with CS and an aliquot of the reaction mixture combined with an equal volume of OPA reagent for derivatization. The derivatized mixture was then analyzed by reversed-phase hplc with a phosphate/MeOH gradient. In no case was there any evidence for the formation of products upon incubation of the analogues with CS.

A prerequisite for mechanism-based irreversible inhibition of an enzyme by a particular substrate is the demonstration that the interaction of substrate and protein results in time-dependent inactivation of the enzyme. Clavaminate synthase (ca. 2.0 mg/ml) was incubated with 1.0 mM α-KG and 0.1 mg/ml catalase in the presence and absence of the compound being tested (2.0 mM). Catalase was included as a protective agent against the inactivation that rapidly occurs in the absence of substrate due to the uncoupled activation of oxygen (1). At various time points, an aliquot of the incubation was removed and diluted into a standard assay containing 1.0 mM D,L-proclavaminate, terminated with EDTA after 2 min, and derivatized with imidazole to measure the residual enzyme activity. Disappointingly, in no case was there any indication of significant inactivation over time above the control reaction.

Having determined that none of the analogues displayed any covalent interaction with CS, we turned finally to an evaluation of their ability to reversibly inhibit the enzyme. Each analogue was initially examined for inhibition of CS by incubating it at a single concentration of 1.0 mM in a standard assay with 0.3 mM D,L-proclavaminate and measuring the rate of clavaminate production under initial velocity conditions (2-min assays) (1). All of the analogues inhibited CS to some extent, although the levels were not substantial, generally showing a rate of clavaminate production 50–85% of the rate of the control reaction. The K_i (or K_{ii} and K_{is}) (36) for each analogue was determined from reciprocal plots of the velocity data at multiple proclavaminate and analogue concentrations, with the data computer-fit to competitive, noncompetitive, and uncompetitive equations by in-house modifications of the methods of Duggleby (37). While in no instance did any of the analogues display an uncompetitive pattern, the quality of the fit

for noncompetive inhibition was generally superior for most of the analogues, with both noncompetitive and competitive giving essentially equivalent fits for a few of the analogues. The fitted parameters were uniformly unremarkable, with the inhibition parameters in the range of 2–10 mM.

CONCLUSION.—The interactions of 4′-vinylproclavaminic acid (**51ab/52ab**), 4′-ethynylproclavaminic acid (**60–63**), and 4-cyclopropylproclavaminic acid (**69/70**) with CS were singularly disappointing. Such substrate discrimination has been observed recently in even simpler proclavaminate derivatives synthesized in these laboratories (unpublished) and elsewhere (38). At this juncture clavaminate synthase appears significantly less tolerant of substrate structural variations than, for example, IPNS (4). Nonetheless, successful transformation of the γ-lactam analogue of proclavaminic acid has been observed (39, and unpublished results) and that of a limited number of other compounds that will be reported in due course.

Despite the failure of these three substrate analogues to give chemical insights into the catalytic cycle of the protein, the ready availability of (+)-(*R*)-4-thiophenylazetidinone [**29**] by extension of the earlier observations of Shibnasaki *et al.* (18) and Hiemstra and Wynberg (40) allows the optically pure and readily separable 3-trimethylsilyl derivatives **27** and **30** to be prepared in one step (Scheme 7). The versatility of these intermediates to undergo reaction in either a radical or ionic regime to give products of known absolute configuration affords a powerful approach to the synthesis of proclavaminate derivatives substituted at C-4′ as well as other C-4 substituted azetidinones. Radical reactions involving replacement of the 4-thiophenyl and ionic pathways proceeding through its elimination as phenylsulfinate each traverse enantiomeric intermediates and, hence, lead on to enantiomeric products whose absolute stereochemistry is defined by the starting trimethylsilyl configuration. Unfortunately, the introduction of substituents larger than methyl gave materials whose reactions with CS were undetectable.

Incorporation of deuterium under radical conditions gave (4′*R*)- and (4′*S*)-[4′-^{2}H]proclavaminic acids **39** and **40** (Scheme 7). While oxazolidine ring formation, the first step catalyzed by CS (2), involves the insertion of substrate oxygen during oxidative cyclization rather than the introduction of molecular oxygen as seen in conventional α-KG dependent hydroxylases (19), the stereochemical course of ring formation is clearly retention in keeping with all examined cases of oxygen insertion by the latter hydroxylases (13). Thus, while the oxidative cyclization process catalyzed by CS shares this central stereochemical feature of C—O bond formation with the α-KG dependent hydroxylases, the concomitant reduction of molecular oxygen to H_2O confers a thermodynamically favorable path to strained ring formation pivotal to the biosynthesis of clavulanic acid (1,3).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCDURES.—All air-or moisture-sensitive reactions were run under an inert atmosphere (Ar or N_2) in flame- or oven-dried (150°) glassware with magnetic stirring unless otherwise noted. Moisture-sensitive reagents were added to reaction vessels by dry syringes equipped with oven-dried needles through rubber septa. Reaction temperatures refer to bath temperatures, not internal reaction temperatures.

THF and Et_2O were freshly distilled from Na/benzophenone ketyl; CH_2Cl_2 was freshly distilled from CaH_2. All other solvents and reagents for air- or moisture-sensitive reactions were used as received or dried by standard procedures (41). ^{1}H- and ^{13}C-nmr spectra were obtained on a Varian XL/VXR-400 or a Bruker AMX 300 nmr spectrometer. Chemical shifts of hydrogen resonances are reported on the δ scale and referenced to TMS (0.00 ppm), $CDCl_3$ (7.26 ppm), or Me_2CO-d_6 (2.23 ppm). Coupling constants are reported in Hertz. ^{13}C chemical shifts are also reported on the δ scale and referenced to $CDCl_3$ (77.0 ppm), or *p*-dioxane-d_8 (66.5 ppm). Low and high resolution mass spectral data were obtained on a VG Instruments

70-S GC/MS at 70 eV and are tabulated as *m/z* under electron impact (ei) or chemical ionization (ci, NH_3 as reagent gas) conditions. Ir spectra were obtained on a Perkin-Elmer 1600 series FT-spectrophotometer ($CHCl_3$ solution or a KBr disk). Uv-visible spectra were obtained on Beckman DU 70 spectrophotometer. Optical rotations were measured with a Perkin-Elmer 141 polarimeter using a 1 dm cell at 25°, with concentrations expressed in g/100 ml. Melting points were determined in open capillary tubes with a Thomas-Hoover Uni-Melt melting point apparatus and are uncorrected. Microanalyses were performed by Atlantic Microlab, Inc. of Norcross, Ga. Flash chromatography was performed with EM Science Si gel 60 (230–400 mesh ASTM). Radial chromatography was carried out on a Chromatotron (Harrison Research), using rotors prepared with Si gel PF-254 with $CaSO_4 \cdot \frac{1}{2}H_2O$ as binder. Hplc chromatography was performed with a Waters 600 multisolvent delivery system equipped with a Rheodyne injector and a Waters 490 programmable multiwavelength detector.

All reagents used in enzyme purification were of the highest grade available. *Streptomyces clavuligerus* ATCC 27064 cells were broken by sonication using a Heat Systems-Ultrasonics Model 225R ultrasonicator. DEAE-Sepharose and Sephadex G-15 and G-75 chromatography media were obtained from Pharmacia. Bacto-agar, yeast extract, soluble starch, and tryptone were purchased from Difco Laboratories. Whole soybeans (Arrowhead Mills) were purchased from a local health food store.

(4′R)- AND *(4′S)-*D,L-[4′-^{2}H]-PROCLAVAMINIC ACIDS (**39** AND **40**).—*(4R)-4-Thiophenyl-2-azetidinone* [**29**].—4-Phenylsulfonyl-2-azetidinone (20) (4.00 g, 19.0 mmol) was reacted with thiophenol (9.74 ml, 94.8 mmol) and (−)-cinchonidine (6.70 g, 22.8 mmol) in C_6H_6 (380 ml) at 40° under Ar for 48 h. The mixture was filtered through a plug of cotton wool to remove most of the (−)-cinchonidine, and the filtrate was passed through a column of Si gel (100 g). The column was washed with C_6H_6 (200 ml) to elute the excess thiophenol, followed by EtOAc (250 ml), which on evaporation afforded 4-thiophenyl-2-azetidinone as a white solid (3.30 g, 18.4 mmol, 97%), $[\alpha]_D$ 55° ($CHCl_3$, c=1.02). The solid was recrystallized from C_6H_6/cyclohexane and filtered. From the mother liquor **29** was obtained by removal of the solvent and crystallizing the residue from CH_2Cl_2/petroleum ether as white needles (1.02 g, 5.96 mmol, 30%): mp 68–69° [lit. (18) mp 58–60°]; $[\alpha]_D$ 137.3° ($CHCl_3$, c=1.29) [lit. (18) 105.1° ($CHCl_3$, c=0.65)]; ^{1}H nmr ($CDCl_3$) δ 7.49–7.37 (m, 5H, Ar*H*), 6.27 (br s, 1H, N*H*), 5.03 (dd, J=2.4, 4.9 Hz, 1H, H-4), 3.40 (ddd, J=1.9, 5.0, 15.3 Hz, 1H, H-3α), 2.96 (dd, J=2.4, 15.3 Hz, 1H, H-3β); ^{13}C(^{1}H) nmr ($CDCl_3$) δ 166.1, 133.4, 131.3, 129.3, 128.6, 54.2, 45.3; ms *m/z* $[M]^+$ 179 (7%), 119, 110, 77, 70 (100%); accurate mass 179.0406 (calcd for C_9H_9NOS, 179.0405).

*(3R,4R) and (3S,4R)-3-Trimethylsilyl-4-thiophenyl-2-azetidinone (***27** *and* **30**).—(+)-(4*R*)-Thiophenyazetidinone (1.18 g, 6.59 mmol) in 20 ml of THF was cooled to 0° and treated with triethylamine (1.02 ml, 7.25 mmol) for 15 min. A solution of TMSiCl (921 ml, 7.25 mmol) in THF (2 ml) was added slowly (10 min), stirred for 30 min at 0°, and then drawn into a syringe. The residue of amine salt was rinsed with THF and the washings drawn into the syringe. The hazy solution in the syringe was then filtered through a dry Millex-SR 0.5 μm filter unit into a dry pear flask. The clear filtrate was added slowly over a period of 30 min to a solution of LDA at −78° [generated from *n*-butyl lithium (1.5 M) (7.25 ml, 10.88 mmol) and diisopropylamine (1.52 ml, 10.88 mmol) at −15°] and stirred for 30 min at −78°. A solution of TMSiCl (921 ml, 7.25 mmol) in THF (2 ml) was added slowly in 30 min and the solution stirred at −78° for 1 h. The reaction was quenched by the addition of saturated aqueous NH_4Cl (10 ml) and slowly warmed to 0°. The mixture was then partitioned between EtOAc and H_2O (100 ml each). The aqueous layer was re-extracted with EtOAc (2×100 ml), and the combined EtOAc layers were washed with brine (200 ml), dried over Na_2SO_4, and filtered. Evaporation gave an oil which was chromatographed over Si gel. Early hexane-EtOAc (3:1) eluates afforded the trans isomer **27** (880 mg, 3.50 mmol, 53%), recrystallized from CH_2Cl_2/petroleum ether as white needles, while the later hexane-EtOAc (1:3) eluates furnished the cis isomer **30** (435 mg, 1.73 mmol, 26.3%), also recrystallized from CH_2Cl_2/petroleum ether as needles. Trans isomer **27**: mp 59–60° [lit. (17) mp 58–60°]; $[\alpha]_D$ 95.3° ($CHCl_3$, c=1.5) [lit. (17) 857.4° ($CHCl_3$)]; ir ($CHCl_3$) 3401, 3008, 1751, 1479, 1479, 1334, 1253, 1091, 859, 847 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 7.48–7.35 (m, 5H, Ar*H*), 6.09 (br s, 1H, N*H*), 4.76 (d, J=2.4 Hz, 1H, H-4), 2.73 (dd, J=1.0, 2.4 Hz, 1H, H-3), 0.13 (s, 9H, TMSi); ^{13}C(^{1}H) nmr ($CDCl_3$) δ 168.6, 133.4, 132.1, 129.4, 128.7, 56.5, 50.9, −3.0; ms *m/z* $[M]^+$ 251 (0.5%), 236, 208, 193, 182, 167, 151, 142; accurate mass 251.0802 (calcd for $C_{12}H_{17}NOSSi$, 251.0800). *Anal.* calcd for $C_{12}H_{17}NOSSi$, C 57.33, H 6.82, N 5.57, S 12.75; found C 57.35, H 6.84, N 5.53, S 12.66. Cis isomer **30**: mp 71–73°; $[\alpha]_D$ 120.7° ($CHCl_3$, c=2.15); ir ($CHCl_3$) 3401, 3013, 1749, 1561, 1472, 1437, 1337, 1267, 1249, 1161, 1108, 1020, 950, 861, 850 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 7.40–7.29 (m, 5H, Ar*H*), 6.08 (brt s, 1H, N*H*), 5.22 (d, J=5.2 Hz, 1H, H-4), 3.23 (dd, J=1.5, 5.1 Hz, 1H, H-3), 0.31 (s, 9H, TMSi); ^{13}C(^{1}H) nmr ($CDCl_3$) δ 168.8, 134.6, 131.4, 129.4, 127.7, 58.7, 49.5, −1.2; ms *m/z* $[M-Me]^+$ 236 (0.14%), 179, 167, 151, 142, 109, 70 (100%); accurate mass 236.0565 (calcd for $C_{11}H_{14}NOSSi$ $[M-Me]^+$, 236.0565). *Anal.* calcd for $C_{12}H_{17}NOSSi$, C 57.33, H 6.82, N 5.57, S 12.75; found C 57.29, H 6.85, N 5.55, S 12.67.

Benzyl(3′R,4′R)-(4′-thiophenyl-3′-trimethylsilyl-2′-oxoazetidin-1′-yl)-acetate [**31**].—A solution of *trans-*

azetidinone [**27**] (491 mg, 2.08 mmol) in THF (5 ml) was added over 15 min to lithium bis(trimethylsilyl)amide (2.2 ml, 2.2 mmol, 1M in hexane) and THF (5 ml) at −78° and stirred for 30 min. Benzyl bromoacetate (381 ml, 2.40 mmol) in THF (2 ml) was then added slowly (10 min), and the solution was stirred at −78° for 30 min and allowed to warm to 0° over the course of 2 h. The solution was filtered through Si gel, and the silica was washed thoroughly with EtOAc. The combined filtrate and washings were evaporated, the residue was purified by flash chromatography [Si gel; hexane-EtOAc (3:1 to 1:1)], and **31** was isolated as a pale yellow oil (655 mg, 1.71 mmol, 82%): $[\alpha]_D$ −28.4° ($CHCl_3$, c=1.6); ir ($CHCl_3$) 3016, 1754, 1741, 1408, 1384, 1254, 1187, 1096, 865, 849 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.42–7.32 (m, 10H, Ar*H*), 5.09 (s, 2H, C*H*$_2$Ph), 5.01 (d, J=2.4 Hz, 1H, H-4′), 4.36 (d, J=15 Hz, 1H, H-2), 3.74 (d, J=15 Hz, 1H, H-2), 2.68 (d, J=2.4 Hz, 1H, H-3′), 0.15 (s, 9H, TMSi); $^{13}C\{^1H\}$ nmr ($CDCl_3$) δ 168.0, 167.8, 134.9, 130.8, 134.0, 129.3, 128.7, 128.6, 128.6, 128.5, 67.2, 61.3, 50.3, 41.0, −2.8; ms *m/z* $[M-Me]^+$ 384 (0.13%), 327, 290, 91 (100%); accurate mass 384.1092 (calcd for $C_{21}H_{25}NO_3SSi$ $[M-Me]^+$ 384.1090).

*Benzyl (3′*R*,4′*R*)-[4′-^{2}H]-(3′-trimethylsilyl-2′-oxoazetidin-1′-yl)-acetate* [**33**].—Ester **31** (265 mg, 0.664 mmol), AIBN (22 mg, 0.133 mmol), C_6H_6 (30 ml), and tri-*n*-butyltin deuteride (359 ml, 1.33 mmol) were heated to reflux for 6 h at 80° under Ar (13). After cooling to room temperature, the solvent was evaporated and the residue was partitioned between MeCN and hexane (50 ml each). The MeCN layer was washed with hexane (2×50 ml) and, after concentration, the residue was purified by radial chromatography [2 mm silica, hexane-EtOAc (3:1 to 1:1)], giving **33** as a clear oil (135 mg, 0.465 mmol, 70%): $[\alpha]_D$ −29.7° ($CHCl_3$, c=1.0); ir ($CHCl_3$) 3013, 2955, 2931, 1748, 1730, 1408, 1384, 1249, 1190, 1108, 873, 844 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.40–7.32 (m, 5H, Ar*H*), 5.12 (s, 2H, C*H*$_2$Ph), 4.10 (ABq, J=18.0 Hz, 1H, H-2), 3.93 (ABq, J=18.0 Hz, 1H, H-2), 3.20 (d, J=2.7 Hz, 1H, H-3′), 2.82 (d, J=2.5 Hz, 1H, H-4′), 0.13 (s, 9H, TMSi); $^{13}C\{^1H\}$ nmr ($CDCl_3$) δ 170.2, 168.4, 135.1, 128.6, 128.5, 128.5, 67.1, 43.1, 42.1, 41.1, −2.8; ms *m/z* $[M]^+$ 292 (0.33%), 277, 264, 249, 236, 220, 205, 191, 178, 157, 102, 91 (100%); accurate mass 292.1357 (calcd for $C_{15}H_{20}{}^2HNO_3Si$, 292.1353).

*Benzyl (4′*R*)-[4′-^{2}H]-(2′-oxoazetidin-1′-yl)-acetate* [**35**].—Deuteriated ester **33** (110 mg, 0.377 mmol) in MeCN (5 ml) was treated with KF (153 mg, 2.64 mmol) and the solution was stirred at room temperature for 10 h. It was then filtered through Si gel, and the silica was washed thoroughly with EtOAc. The combined filtrate and washings were evaporated and the residue purified by radial chromatography [1 mm silica, petroleum ether-EtOAc (3:1 to 1:1)] giving **35** as a clear oil (80 mg, 0.366 mmol, 97%). Ir ($CHCl_3$) 3013, 2955, 2931, 1751, 1744, 1561, 1449, 1402, 1185, 950, 691 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.38–7.34 (m, 5H, Ar*H*), 5.17 (s, 2H, C*H*$_2$Ph), 4.03 (s, 2H, H-2), 3.39 (t, J=4.1 Hz, 1H, H-4′), 3.03 (d, J=4.1 Hz, 2H, H-3′); $^{13}C\{^1H\}$ nmr ($CDCl_3$) δ 168.1, 167.9, 135.1, 128.7, 128.6, 129.4, 67.2, 43.2, 40.0, 37.7; ms *m/z* $[M]^+$ 220 (2.5%), 192, 178, 150, 129, 105, 91 (100%), 85; accurate mass 220.0961 (calcd for $C_{12}H_{12}{}^2HNO_3$, 220.0958).

Benzyl threo-*(4′*R*)-D,L-[4′-^{2}H]-5-(4,5-diphenyl-2-oxo-4-oxazolin-3-yl)-3-hydroxy-2-(2′-oxoazetidin-1′-yl)-pentanoate* [**37**].—Lithium bis(trimethylsilyl)amide (341 ml, 0.341 mmol, 1 M in hexane) and THF (2 ml) were cooled to −78°, and a solution of **35** (68.0 mg, 0.31 mmol) in THF (2 ml) was added slowly (15 min) (27). After stirring for 30 min, a solution of 4,5-diphenyl-3-(3-oxopropyl)-4-oxazolin-2-one (109 mg, 0.372 mmol) in THF (2 ml) was added slowly over 30 min (2,42). The solution was stirred at −78° for 2 h and then quenched with HOAc (20 μl) in H_2O (300 μl). The reaction mixture was warmed to 0° and partitioned between EtOAc and H_2O (50 ml each). The aqueous layer was re-extracted with EtOAc (2×50 ml). The combined EtOAc layers were washed with 5% $NaHCO_3$ and brine (100 ml each), dried with Na_2SO_4, filtered, and evaporated to leave a foamy solid. Purification by flash chromatography [Si gel, petroleum ether-EtOAc (1:1)] afforded the threo isomer **37** (35 mg) and a mixture of threo and erythro isomers (68 mg).

A solution of the erythro/threo mixture (68 mg, 0.133 mmol) in CH_2Cl_2 (4 ml) was reacted with DBN (162 μl, 0.133 mmol). The solution was stirred at room temperature for 1 h, then filtered through a small pad of Si gel, wahing with EtOAc. The filtrate was concentrated and the residue purified by flash chromatography [Si gel, petroleum ether-EtOAc (3:1 to 1:1)] to give an additional quantity of the threo isomer (60 mg) as a clear, colorless oil, **37** (95.0 mg, 0.185 mmol, 60%): ir ($CHCl_3$) 3395, 2926, 1751, 1740, 1736, 1730, 1596, 1444, 1373, 1186 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.55–7.19 (m, 15H, Ar*H*), 5.23 (ABq, J=12.2 Hz, 1H, C*H*$_2$Ph), 5.19 (ABq, J=12.2 Hz, 1H, C*H*$_2$Ph), 4.39 (d, J=7.9 Hz, 1H, O*H*), 4.22 (m, 1H, H-3), 4.20 (d, J=3.0, 1H, H-2), 3.76 (m, 1H, H-5), 3.55 (m, 1H, H-5), 3.49 (t, J=4.1 Hz, 1/2H, H-4′ of D & L), 3.41 (t, J=4.1 Hz, 1/2H, H-4′ of D & L), 2.98 (d, J=4.2 Hz, 2H, H-3′), 1.68 (m, 2H, H-4); ms *m/z* $[M]^+$ 513 (28.0%), 495, 484, 422, 404, 380, 293, 277, 265, 250, 237, 220, 206, 178, 165, 143, 132, 105, 91 (100%); accurate mass 513.2012 (calcd for $C_{30}H_{27}{}^2HN_2O_6$, 513.2010).

*(4′*R*)-D,L-[4′-^{2}H]-Proclavaminic acid* [**39**].—A small Parr hydrogenation vessel was charged with **37** (30 mg, 0.058 mmol) and a mixture of THF (2 ml) and H_2O (2 ml). 10% Pd-C (40 mg) was added and the atmosphere was exchanged for H_2 and shaken at 45 psi in a Paar apparatus for 16 h. The catalyst was filtered

through a bed of Celite, which was thoroughly washed with H_2O (25 ml). The combined filtrates were evaporated to remove the THF and then lyophilized. The residue was triturated with Et_2O to leave a pale green powder. This was dissolved in H_2O (5 ml), filtered through a 0.25 μ filter unit to remove the last traces of Pd-C, and then lyophilized to leave compound **39** as a white solid (12 mg, 0.058 mmol, 100%). This material was further purified by reversed-phase hplc (Whatman ODS-3, C-18, 9.4×250 mm, 100% H_2O, 3.0 ml/min, λ=220 nm) prior to its use in enzymic experiments to give **39** as a fluffy white solid (6 mg, 0.030 mmol, 51%): ^{1}H nmr (D_2O) δ 4.10 (m, 1H, H-3), 3.98 (d, J=5.5 Hz, 1H, H-2), 3.47 (t, J=4.2 Hz, 1/2H, H-4′), 3.40 (t, J=4.2 Hz, 1/2H, H-4′), 3.06 (m, 2H, H-5), 2.91 (d, J=4.2 Hz, 2H, H-3′), 1.74 (m, 2H, H-4); other spectral properties identical with unlabeled material (2,43,44).

Benzyl (3′S,4′R)-(4′-thiophenyl-3′-trimethylsilyl-2′-oxoazetidin-1′-yl)-acetate [**32**].—The title compound was prepared from **30** (251 mg, 1.00 mmol) as described for the preparation of **31** above. Recrystallization from Et_2O/petroleum ether provided **32** as white needles (201 mg, 0.050 mmol, 50.4%): mp 82–83°; [α]D 33.4° ($CHCl_3$, c=1.5); ir ($CHCl_3$) 2960, 1754, 1406, 1383, 1352, 1253, 1190, 1152, 1122, 936, 862, 849 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 7.36–7.24 (m, 10H, Ar*H*), 5.42 (d, J=5.2 Hz, 1H, H-4′), 5.03 (ABq, J=12.0 Hz, 1H, C*H*$_2$Ph), 5.02 (ABq, J=12.0 Hz, 1H, C*H*$_2$Ph), 4.25 (d, J=18.3 Hz, 1H, H-2), 3.76 (d, J=18.3 Hz, 1H, H-2), 3.31 (d, J=5.2 Hz, 1H, H-3′), 0.28 (s, 9H, TMSi); ^{13}C{^{1}H} nmr ($CDCl_3$) δ 168.1, 134.6, 131.3, 129.3, 128.6, 128.5, 128.0, 127.5, 67.2, 64.5, 49.8, 41.2, −1.3; ms *m/z* [M−Me]$^+$ 384 (0.24%), 290, 262, 91 (100%); accurate mass 384.1096 (calcd for $C_{21}H_{25}NO_3SSi$ [M−Me]$^+$ 384.1090; *Anal.* calcd for $C_{21}H_{25}NO_3SSi$, C 63.12, H 6.31, N 3.51, S 8.02; found C 63.19, H 6.32, N 3.51, S 7.96.

Benzyl (3′S,4′R)-[4′-^{2}H]-(3′-trimethylsilyl-2′-oxoazetidin-1′-yl)-acetate [**34**].—Ester **32** (245 mg, 0.614 mmol), AIBN (21 mg, 0.13 mmol, 0.2 equiv), C_6H_6 (30 ml), and tri-*n*-butyltin deuteride (333 ml, 1.23 mmol, 2 equiv) were heated at reflux for 12 h at 80° under argon. Additional tri-*n*-butyltin deuteride (167 ml, 0.612 mmol) and AIBN (21 mg, 0.13 mmol) were added, and the solution was refluxed another 12 h. After cooling to room temperature, the solvent was evaporated and the residue was worked up and chromatographed on Si gel as for the enantiomer to give **34** as a clear oil (91 mg, 0.313 mmol, 51%): [α]D 27.7° ($CHCl_3$, c=2.065); accurate mass 292.1357 (calcd for $C_{15}H_{20}{}^2HNO_3Si$, 292.1353); other spectral data identical with the enantiomer **33**.

Benzyl (4′S)-[4′-^{2}H]-(2′-oxoazetidin-1′-yl)-acetate [**36**].—Compound **36** was prepared from **34** (75.0 mg, 0.260 mmol) as described above for the preparation of **35** . Compound **36** was isolated as a clear oil (55 mg, 0.250 mmol, 96%). Spectral data were identical to those for the enantiomer **35**.

Benzyl threo-*(4′S)-D,L-[4′-^{2}H]-5-(4,5-diphenyl-2-oxo-4-oxazolin-3-yl)-3-hydroxy-2-(2′-oxoazetidin-1′-yl)-pentanoate* [**38**].—Compound **38** was prepared from **36** (50.0 mg, 0.227 mmol) as described above for the preparation of **37**. Compound **38** was isolated as a white foam (69.9 mg, 0.136 mmol, 60%). Spectral data were identical with those of the corresponding 4′*R* isomer **37**.

(4′S)-D,L-[4′-^{2}H]-Proclavaminic acid [**40**].—The title compound was prepared from **38** (30.0 mg, 0.058 mmol) as described above for the preparation of **39**. Compound **40** was isolated after lyophilyzation as a white solid (12 mg, 0.058 mmol, 100%) and was further purified by hplc as described above to give **40** as a fluffy white solid (7.0 mg, 0.035 mmol, 60%). Spectral data were identical with those of the corresponding 4′*R* isomer **39**.

SYNTHESIS OF D,L-4′-VINYLPROCLAVAMINIC ACID [**7**/**11**].—*(±)-4-Thiophenylazetidinone* [**41**].—Phenyl disulfide (2.6 g, 11.9 mmol) in EtOH (100 ml) was cooled in an ice bath. $NaBH_4$ (491.1 mg, 12.81 mmol) was added in one portion and the reaction mixture stirred for 30 min. A solution of 4-acetoxyazetidinone (1.5 g, 12 mmol) in EtOH (15 ml) was added dropwise over 10 min. After 30 min, the reaction was terminated by the addition of Si gel (ca. 500 mg), and the reaction mixture was filtered through additional silica, washing with EtOAc. The filtrate was concentrated in vacuo, and the crude product was purified by flash chromatography [35 g Si gel; petroleum ether-EtOAc (4:1 to 1:1)] to give, after recrystallization from EtOAc/hexane, the product as white plates (1.649 g, 9.201 mmol, 79%): mp 72–72.5° [lit. (20) mp 72°]; spectral data identical with those of **29** above.

3-Trimethylsilyl-4-thiophenylazetidinone [**42**/**43**].—4-Thiophenylazetidinone [**41**] (5.053 g, 28.19 mmol) in THF (55 ml) was cooled to 0° and freshly distilled diisopropyl amine (4.35 ml, 31.0 mmol) was added dropwise. After stirring for 15 min, freshly distilled TMSiCl (3.95 ml, 31.5 mmol) was added dropwise. The reaction was stirred for 1 h, then filtered through a dry fritted funnel washing with THF (45 ml) to remove precipitated diisopropyl ammonium hydrochloride. The clear filtrate was then cannulated dropwise to a cold (−78°) solution of LDA [prepared from diisopropyl amine (5.9 ml, 49.1 mmol) and *n*-BuLi (28.2 ml, 1.5 M in hexane)]. When the addition was complete, the solution was stirred for 20 min, and then TMSiCl (6.5 ml, 51.2 mmol) was added dropwise. After stirring for 2 h, the reaction was quenched by addition of a solution of 5 M HOAc (12 ml) in THF and warmed to room temperature. The reaction mixture was then diluted with EtOAc (450 ml) and washed with 1 N HCl (2×300 ml), 5% $NaHCO_3$ (350

ml) and brine (2×300 ml). The organic layer was dried (anhydrous Na_2SO_4), concentrated in vacuo, and the crude mixture purified by flash chromatography [200 g Si gel; EtOAc-petroleum ether (1:9 to 1:1)] to give, after recrystallization from CH_2Cl_2/pentane, the trans (2.125 g, 8.453 mmol, 30%) and cis (3.442 g, 13.69 mmol, 49%) isomers as white needles. Trans isomer **43**: mp 90–90.5°; other spectral data identical to those of **27** above. Cis isomer **42**: mp 92.5–93°; other spectral data identical to those of **30** above.

trans-*3-Trimethylsilyl-4-phenylsulfonylazetidinone* [**44**].—*trans*-Thiophenylazetidinone [**43**] (1.70 g, 6.77 mmol) was dissolved CH_2Cl_2 (30 ml) and the solution was cooled to −78°. A solution of *m*-CPBA (3.22 g, 14.9 mmol) in CH_2Cl_2 (50 ml) was added dropwise over 15–20 min. After stirring for ca. 10 min at −78°, the reaction was allowed to warm to room temperature over 2 h. After stirring an additional 1.5 h, the reaction mixture was diluted with CH_2Cl_2 (200 ml) and washed with 5% $NaHCO_3$ (2×200 ml) and brine (200 ml). The organic layer was dried (anhydrous $MgSO_4$) and concentrated to give a white solid, which upon recrystallization from CH_2Cl_2/petroleum ether gave the product as a fine white powder (1.67 g, 5.88 mmol, 87%): mp 167–168.5°; ir ($CDCl_3$) 3401, 3025, 2954, 1766, 1325, 1155, 1079, 849 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.92 (m, 2H, Ar*H*), 7.72 (m, 1H, Ar*H*), 7.61 (m, 2H, Ar*H*), 5.95 (br s, 1H, N*H*), 4.40 (d, *J*=2.2 Hz, 1H, H-4), 3.03 (dd, *J*=0.8, 2.2 Hz, 1H, H-3), 0.09 [s, 9H, Si(CH_3)$_3$]; $^{13}C\{^1H\}$ nmr ($CDCl_3$) δ 167.6, 135.1, 134.8, 129.6, 129.4, 67.1, 47.0, −3.2; cims *m/z* $[M+18]^+$ 301 (NH_3), $[MH]^+$ 284 (43%), 232, 215, 160 (100%), 142, 125, 90, 78, 70, 44; accurate mass $[MH]^+$ (NH_3) 284.0780 (calcd for $C_{12}H_{18}NO_3SSi$, 284.0777).

trans-*3-Trimethylsilyl-4-vinylazetidinone* [**45**].—*trans*-Phenylsulfonylazetidinone [**44**] (913.6 mg, 3.22 mmol) in THF (45 ml) was cooled to −78°. Vinyl magnesium bromide (7.2 ml, 7.2 mmol, 1.0 M in THF) was then added dropwise over 5 min. When the addition was complete, the reaction mixture was stirred for a few min at −78°, then allowed to warm to room temperature over 60 min. The reaction was quenched by the addition of ca. 3 g of Si gel, then concentrated in vacuo and the residue purified by flash chromatography [30 g Si gel, hexane-EtOAc (9:1 to 1:1)] to give 422.6 mg (2.49 mmol, 77%) of the vinylazetidinone **45** as a clear, colorless oil: ir ($CHCl_3$) 3410, 3013, 2957, 2910, 1743, 1424, 1345, 1318, 1252, 1170, 1118, 1076, 991, 927, 889, 860, 845 cm^{-1}; ^1H nmr ($CDCl_3$) δ 5.89 (ddd, *J*=7.2, 9.9, 17.3 Hz, 1H, H-5), 5.89 (br, 1H, N*H*), 5.25 (dt, *J*=1.0, 17.0 Hz, 1H, H-6), 5.11 (dt, *J*=1.0, 10.2 Hz, 1H, H-6), 3.86 (ddt, *J*=0.9, 2.6, 7.4 Hz, 1H, H-4), 2.54 (dd, *J*=0.9, 2.6 Hz, 1H, H-3), 0.15 [s, 9H, Si(CH_3)$_3$]; $^{13}C\{^1H\}$ nmr ($CDCl_3$) δ 170.4, 138.7, 116.2, 51.5, 50.4, 3.0; ms *m/z* $[M-NH=C=O]^+$ 126 (26%), 111 (100%), 99, 75, 73, 59, 43; accurate mass 126.0869 (calcd for $C_7H_{14}Si$ $[M-NH=C=O]^+$ 126.0865).

*Benzhydryl 2-(*trans*-4′-vinyl-3′-trimethylsilyl-2′-oxoazetidin-1′-yl)acetate* [**46**].—A dry 50-ml round-bottomed flask equipped with a stir bar was charged with KOH (165.0 mg, 2.535 mmol), tetrabutyl ammonium bromide (65.0 mg, 0.2 mmol), and THF (5 ml). A solution of the vinylazetidinone **45** (284.0 mg, 1.670 mmol) and benzhydryl bromoacetate (773.3 mg, 2.535 mmol) in THF (15 ml) was then added over 5 min, and the reaction was stirred at room temperature for 2 h. The reaction mixture was diluted with EtOAc (100 ml), and washed with 1 N HCl (100 ml), 5% $NaHCO_3$ (100 ml), and brine (2×100 ml). The organic layer was dried (Na_2SO_4), concentrated in vacuo, and the residue purified by radial chromatography [1 mm Si gel; hexane-EtOAc (9:1 to 1:1)] to give 269.8 mg (0.686 mmol, 41%) of the desired product as a clear, colorless oil: ir ($CHCl_3$) 3090, 3069, 3031, 3010, 2957, 2919, 1731, 1602, 1493, 1452, 1405, 1367, 1252, 1193, 1179, 1123, 1076, 864, 846, 700 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.32 (m, 10H, Ar*H*), 6.92 (s, 1H, C*H*Ph$_2$), 5.74 (ddd, *J*=8.6, 10.0, 17.0 Hz, 1H, H-5′), 5.25 (dt, *J*=0.7, 17.1 Hz, 1H, H-6′), 5.17 (dt, *J*=1.0, 10.1 Hz, 1H, H-6′), 4.33 (d, *J*=18.0 Hz, 1H, H-2), 4.02 (dd, *J*=2.5, 8.6 Hz, 1H, H-4′), 3.70 (d, *J*=18.0 Hz, 1H, H-2), 2.55 (d, *J*=2.4 Hz, 1H, H-3′), 0.13 [s, 9H, Si(CH_3)$_3$]; $^{13}C\{^1H\}$ nmr ($CDCl_3$) δ 169.2, 167.5, 139.5, 139.4, 136.8, 128.6, 128.1, 127.1, 119.3, 77.8, 56.2, 49.8, 41.6, −2.7; ms *m/z* 226, 182, 167, 152, 111, 102, 81, 68, 59, 41; accurate mass $[MH]^+$ (NH_3) 394.1838 (calcd for $C_{23}H_{28}NO_3Si$, 394.1844).

Benzhydryl 2-(4′-vinyl-2′-oxoazetidin-1′-yl)-acetate [**47**].—Ester **46** (496.1 mg, 1.261 mmol) in MeCN (15 ml) was treated with KF (720.0 mg, 12.39 mmol) and stirred at room temperature for 8 h. The reaction mixture was then filtered through Si gel washing with EtOAc, the filtrate concentrated in vacuo, and the crude product purified by flash chromatography [15 g Si gel; EtOAc-hexane (1:9 to 2:3)] to give 340.2 mg (1.059 mmol, 84%) of **47** as a clear, faintly yellow oil: ir ($CDCl_3$) 3090, 3066, 3028, 3010, 2960, 2922, 1757, 1745, 1496, 1455, 1424, 1405, 1368, 1264, 1198, 1179, 1124, 1080, 1062, 987, 961, 937, 700 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.32 (m, 10H, Ar*H*), 6.90 (s, 1H, C*H*Ph$_2$), 5.75 (ddd, *J*=8.5, 10.1, 17.1 Hz, 1H, H-5′), 5.28 (dt, *J*=0.6, 16.9 Hz, 1H, H-6′), 5.22 (dt, *J*=0.7, 10.6 Hz, 1H, H-6′), 4.30 (d, *J*=18.0 Hz, 1H, H-2), 4.20 (m, 1H, H-4′), 3.75 (d, *J*=18.0 Hz, 1H, H-2), 3.25 (dd, *J*=5.2, 14.8 Hz, 1H, H-3′), 2.75 (dd, *J*=2.3, 14.8 Hz, 1H, H-3′); $^{13}C\{^1H\}$ nmr ($CDCl_3$) δ 167.3, 167.0, 139.4, 135.5, 128.6, 128.2, 127.1, 127.0, 120.1, 78.1, 54.2, 44.4, 41.8; ms *m/z* $[M]^+$ 321 (2%), 167 (100%), 152, 110, 68, 56, 41; accurate mass 321.1371 (calcd for $C_{20}H_{19}NO_3$, 321.1365).

*Benzhydryl 5-(*N*-t-butyloxycarbonyl)-amino-3-hydroxy-2-(4′-vinyl-2′-oxoazetidin-1′-yl)-pentanoate* [**50**].—A dry 100-ml round-bottomed flask equipped with a stir bar was charged with the vinylazetidinone **47**

(249.0 mg, 0.7748 mmol) and dried under high vacuum. THF (20 ml) was added, the solution cooled to −78°, and lithium bis(trimethylsilyl)amide (860 µl, 1.0 M in hexane) added dropwise via syringe. After stirring for 40 min, a solution of the BOC-protected aldehyde **49** (188.8 mg, 1.090 mmol) in THF (5 ml) was added dropwise over 5 min (2). After stirring at −78° for 4.5 h, the reaction was quenched by the addition of a solution of 5 M HOAc in THF (350 µl). After warming to room temperature, the reaction mixture was diluted with EtOAc (100 ml) and washed with 1 N HCl (100 ml), 5% $NaHCO_3$ (100 ml) and brine (2×100 ml). The organic layer was dried (anhydrous Na_2SO_4), concentrated in vacuo, and the crude products purified by radial chromatography [2 mm Si gel, hexane-EtOAc (9:1 to 1:1)] to give the product (348.2 mg, 0.7040 mmol, 91%) as an inseparable mixture of diastereomers that was carried on to the next step.

5-Amino-3-hydroxy-2-(4′-vinyl-2′-oxoazetidin-1′-yl)-pentanoic acids [**51ab** *and* **52ab**].—The mixture of the two of the diastereomeric azetidinones **51** (100.8 mg, 0.2039 mmol) was treated with cold (−10°) TFA and stirred for 30 min at −5°. The TFA was then removed in vacuo followed by repeated evaporations with toluene. The residue was triturated with Et_2O and sonicated, and the Et_2O removed by pipet. The crude products were then taken up in H_2O and purified by reversed-phase hplc (Whatman Partisil ODS-3 C-18 semi-prep column, 100% H_2O, 3.0 ml/min). **51/52a**: ^{1}H nmr (D_2O/Me_2CO) δ 5.88 (ddd, J=9.0, 10.1, 17.1 Hz, 1H, H-5′), 5.43 (d, J=17.1 Hz, 1H, H-6′), 5.34 (dd, J=0.9, 10.1 Hz, 1H, H-6′), 4.29 (m, 2H, H-4′, H-3), 3.96 (d, J=5.2 Hz, 1H, H-2), 3.30 (dd, J=5.1, 15.3 Hz, 1H, H-3′$_{trans}$), 3.19 (m, 2H, H-5), 2.86 (dd, J=2.2, 15.3 Hz, 1H, H-3′$_{cis}$), 1.89 (m, 2H, H-4). **51/52a**: ^{1}H nmr (D_2O/Me_2CO) δ 5.88 (ddd, J=9.0, 10.1, 17.1 Hz, 1H, H-5′), 5.43 (d, J=17.1 Hz, 1H, H-6′), 5.34 (dd, J=1.2, 10.1 Hz, 1H, H-6′), 4.33 (m, 1H, H-4′), 4.22 (m, 1H, H-3), 3.89 (d, J=6.1 Hz, 1H, H-2), 3.25 (dd, J=5.1, 15.2 Hz, 1H, H-3′$_{trans}$), 3.18 (m, 2H, H-5), 2.82 (dd, J=2.2, 15.3 Hz, 1H, H-3′$_{cis}$), 1.98 (m, 2H, H-4); ^{13}C(^{1}H) nmr (D_2O′dioxane) δ 173.5, 170.3, 134.9, 120.7, 68.0, 62.6, 55.0, 42.0, 37.2, 30.4; cims *m/z* [MH]$^+$ (NH_3) 229 (100%), 211, 193, 169, 131, 116, 98, 70; accurate mass [MH]$^+$ (NH_3) 229.1183 (calcd for $C_{10}H_{17}N_2O_4$, 229.1188). **51/52b**: ^{1}H nmr (D_2O/Me_2CO) δ 5.96 (ddd, J=9.1, 10.1, 17.1 Hz, 1H, H-5′), 5.45 (dd, J=1.4, 17.1 Hz, 1H, H-6′), 5.29 (dd, J=1.4, 10.1 Hz, 1H, H-6′), 4.28 (ddd, J=2.3, 5.0, 9.1 Hz, 1H, H-4′), 4.14 (ddd, J=2.9, 7.3, 10.0 Hz, 1H, H-3), 3.90 (d, J=7.4 Hz, 1H, H-2), 3.24 (dd, J=5.0, 15.1 Hz, 1H, H-3′$_{trans}$), 3.18 (m, 2H, H-5), 2.82 (dd, J=2.3, 15.2 Hz, 1H, H-3′$_{cis}$), 1.92 (m, 1H, H-4), 1.79 (m, 1H, H-4); ^{13}C(^{1}H) nmr (D_2O/dioxane) δ 173.6, 171.1, 135.7, 120.3, 68.2, 64.5, 55.8, 41.7, 36.9, 30.9. **51/52b**: ^{1}H nmr (D_2O/Me_2CO) δ 5.92 (ddd, J=9.2, 10.1, 17.0 Hz, 1H, H-5″), 5.46 (ddd, J=0.6, 1.3, 17.1 Hz, 1H, H-6′), 5.32 (dd, J=1.6, 10.3 Hz, 1H, H-6′), 4.27 (ddd, J=2.4, 4.9, 9.2 Hz, H-4′), 4.16 (ddd, J=7.4, 2.6, 9.9 Hz, 1H, H-3), 3.94 (d, J=7.4 Hz, 1H, H-2), 3.24 (dd, J=5.1, 15.2 Hz, 1H, H-3′$_{trans}$), 3.14 (m, 2H, H-5), 2.81 (dd, J=2.3, 15.2 Hz, 1H, H-3′$_{cis}$), 1.99 (m, 1H, H-4), 1.85 (m, 1H, H-4); ^{13}C(^{1}H) nmr (D_2O/dioxane) δ 173.7, 170.9, 135.6, 120.8, 68.8, 62.6, 55.6, 41.8, 37.1, 30.2; cims *m/z* [MH]$^+$ (NH_3) 229 (47%), 211, 193, 183, 169, 131, 116 (100%), 98, 86, 70, 56, 44; accurate mass [MH]$^+$ (NH_3) 229.1192 (calcd for $C_{10}H_{17}N_2O_4$, 229.1188).

SYNTHESIS OF D,L-4′-ETHYNYLPROCLAVAMINIC ACID.—*4-Trimethylsilylethynyl-2-azetidinone* [**53**].—To a solution of trimethylsilylacetylene (17.95 ml, 0.1270 moles) in THF (100 ml) at −40° was added a solution of ethyl magnesium bromide (42.33 ml, 0.1270 mol) in THF (100 ml). When the addition was complete, the reaction mixture was allowed to warm to room temperature over 15 min and stirred for 1 h. The mixture was cooled again to −40° and a solution of 4-phenylsulfonyl-2-azetidinone (6.70 g, 0.0317 mol) in THF (130 ml) was added slowly. The reaction was allowed to warm to room temperature and stirred for an additional 1.5 h. The reaction mixture was then cooled to 0° and quenched with a solution of saturated aqueous NH_4Cl (10 ml). The reaction mixture was poured into H_2O (150 ml) and extracted with EtOAc (2×300 ml). The combined EtOAc extracts were washed with 5% $NaHCO_3$ (2×300 ml) and brine (300 ml), dried over $MgSO_4$, and concentrated to give an orange oil. Purification by flash chromatography [silica, hexane-EtOAc (7:1 to 3:1)] followed by recrystallization from CH_2Cl_2/petroleum ether gave the product **53** as white crystals (2.58 g, 15.4 mmol, 49%): mp 68–69°; ir ($CHCl_3$) 3414, 3013, 2962, 1771, 1411, 1335, 1250, 1100, 858, 847 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 5.97 (br s, 1H, N*H*), 4.26 (dd, J=2.7, 5.4 Hz, 1H, H-4), 3.32 (ddd, J=1.8, 5.4, 14.7 Hz, 1H, H-3α), 3.08 (ddd, J=1.7, 2.6, 14.8 Hz, 1H, H-3β), 0.18 [s, 9H, TMSi]; ^{13}C(^{1}H) nmr ($CDCl_3$) δ 166.9, 103.1, 90.3, 46.8, 37.4, −0.3; ms *m/z* [M−Me]$^+$ 152 (0.67%), 124, 111, 110, 109 (100%), 83, 53, 43; accurate mass 152.0535 (calcd for $C_7H_{10}NOSi$ [M−Me]$^+$ 152.0532). *Anal.* calcd for $C_8H_{13}NOSi$, C 57.44, H 7.83, N 8.37; found C 57.33, H 7.84, N 8.31.

Benzhydryl 2-(4′-trimethylsilylethynyl-2′-oxoazetidin-1′-yl)-acetate [**54**].—A dry 500-ml round-bottomed flask equipped with a stir bar was charged with KOH (0.94, g, 16.8 mmol), Bu_4NBr (1.23 g, 3.80 mmol), and THF (80 ml). Into this flask was cannulated a solution of the alkylated azetidinone **53** (2.55 g, 15.2 mmol) and benzhydryl bromoacetate (6.98 g, 22.9 mmol) in THF (120 ml). Four drops of H_2O were added to facilitate the reaction and stirring was continued at room temperature for 3 h. The reaction mixture was filtered through Si gel, washed with EtOAc (150 ml), and concentrated to give an orange oil. The

product was purified by flash chromatography [Si gel, hexane-EtOAc (6:1)] and recrystallized from CH_2Cl_2/petroleum ether to give **54** as white crystals (3.59 g, 9.12 mmol, 60%): mp 106°; ir ($CHCl_3$) 3030, 3014, 2960, 1763, 1748, 862, 847 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.33 (m, 10H, Ar*H*), 6.91 (s, 1H, C*H*Ph_2), 4.45 (d, J=18.0 Hz, 1H, CH_2CO_2), 4.42 (dd, J=2.5, 4.4 Hz, 1H, H-4'), 3.77 (dd, J=18.0 Hz, 1H, CH_2CO_2), 3.33 (dd, J=5.4, 14.6 Hz, 1H, H-3'α), 3.19 (dd, J=2.4, 14.6 Hz, 1H, H-3'β), 0.16 [s, 9H, TMSi]; $^{13}C\{^1H\}$ nmr ($CDCl_3$) δ 167.2, 166.3, 139.4, 128.7, 128.6, 128.3, 128.2, 127.1, 127.0, 100.6, 92.3, 78.2, 45.9, 42.2, 41.6, −0.3; ms *m/z* $[M]^+$ 391 (0.94%), 225, 183, 180, 167 (100%), 152, 138, 109, 105, 83, 77, 65; accurate mass 391.1607 (calcd for $C_{23}H_{25}NO_3Si$, 391.1604). *Anal.* calcd for $C_{23}H_{25}NO_3Si$, C 70.56, H 6.44, N 3.58; found C 70.36, H 6.45, N 3.55.

*Benzhydryl 5-(*N*-t-butyloxycarbonyl)-amino-3-hydroxy-2-(4'-trimethylsilyl-ethynyl-2'-oxoazetidin-1'-yl)-pentanoate* [**55**].—A solution of lithium bis(trimethylsilyl)amide (8.1 ml, 8.1 mmol, 1 M in hexane) and THF (30 ml) was cooled to −78°, and a solution of **55** (2.65 g, 6.80 mmol) in THF (40 ml) was added slowly over 30 min and stirred for another 30 min. A solution of BOC-protected aldehyde **49** (1.76 g, 10.2 mmol) (42) in THF (30 ml) was then added over 45 min and the reaction stirred for 2 h. The reaction mixture was quenched with HOAc (500 μl) in H_2O (5 ml), warmed to 0°, and partitioned between EtOAc and H_2O (200 ml each). The aqueous layer was extracted with EtOAc (2×200 ml), and the combined EtOAc layers were washed with 5% $NaHCO_3$ (2×250 ml) and brine (250 ml). The combined organic extracts were dried ($MgSO_4$), concentrated, and the residue purified by flash and radial chromatography [Si gel, hexane-EtOAc (8:1 to 4:1)] to give the diastereomeric products in an overall yield of 58% as colorless oils. The two major racemic diastereomers were isolated cleanly, while the two minor products were isolated as an inseparable mixture. The top band (isomer 1, 745 mg, 1.33 mmol, 19.5%), intermediate band (isomer 2, 760 mg, 1.35 mmol, 19.9%), bottom band (isomers 3 and 4, 725 mg, 1.29 mmol, 19%). Isomer 1: ir ($CHCl_3$) 3456, 3356, 3028, 3013, 2979, 1749, 1737, 1708, 1508, 1253, 1173, 847 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.33 (m, 10H, Ar*H*), 6.90 (s, 1H, C*H*Ph_2), 4.87 (br s, 1H, N*H*), 4.76 (br d, 1H, O*H*), 4.40 (m, 1H, H-3), 4.19 (dd, J=2.6, 5.4 Hz, 1H, H-4'), 4.12 (d, J=2.0 Hz, 1H, H-2), 3.32 (m, 2H, H-5), 3.30 (dd, J=5.4, 14.8 Hz, 1H, H-3'α), 3.05 (dd, J=2.7, 14.9 Hz, 1H, H-3'β), 1.75 (dm, 2H, H-4), 1.42 [s, 9H, CMe_3], 0.16 [s, 9H, TMSi]; $^{13}C\{^1H\}$ nmr ($CDCl_3$) δ 167.7, 167.5, 156.0, 139.4, 139.3, 128.6, 128.2, 128.1, 127.2, 127.0, 100.5, 92.5, 78.9, 69.1, 64.0, 43.5, 41.9, 37.7, 34.7, 28.3, −0.4; ms *m/z* $[M-C_4H_8]^+$ 508 (0.03%), 183, 167 (100%), 152, 109, 86, 57; accurate mass 508.2035 (calcd for $C_{27}H_{32}N_2O_6Si$ $[M-C_4H_8]^+$ 508.2030). Isomer 2: ir ($CHCl_3$) 3456, 3359, 3011, 2978, 1738, 1706, 1507, 1251, 1173, 846 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.35 (m, 10H, Ar*H*), 6.92 (s, 1H, C*H*Ph_2), 4.95 (br d, 2H, O*H* and N*H*), 4.35 (m, 1H, H-3), 4.12 (dd, J=2.6, 5.4 Hz, 1H, H-4'), 3.95 (d, J=2.5 Hz, 1H, H-2), 3.37 (m, 2H, H-5), 3.22 (dd, J=5.5, 14.9 Hz, 1H, H-3'α), 3.03 (dd, J=2.7, 14.9 Hz, 1H, H-3'β), 1.87 (dm, 2H, H-4), 1.44 [s, 9H, CMe_3], 0.16 [s, 9H, TMSi]; $^{13}C\{^1H\}$ nmr ($CDCl_3$) δ 167.5, 166.9, 156.2, 139.4, 139.2, 128.7, 128.6, 128.4, 128.1, 127.4, 126.8, 100.2, 92.9, 78.9, 69.8, 64.0, 44.2, 41.9, 38.1, 33.2, 28.4, −0.3; ms *m/z* $[M-C_4H_8]^+$ 508 (0.23%), 182, 167 (100%), 152, 109, 86, 57; accurate mass 508.2040 (calcd for $C_{27}H_{32}N_2O_6Si$ $[M-C_4H_8]^+$ 508.2030). Isomers 3 and 4 were not used or characterized further (see below).

*Benzhydryl 5-(*N*-t-butyloxycarbonyl)-amino-3-hydroxy-2-(4'-ethynyl-2'-oxoazetidin-1'-yl)-pentanoate (erythro and threo isomers 1)* [**56** *and* **57**].—KF (530 mg, 8.86 mmol) was added to isomer 1 of **55** (500 mg, 0.886 mmol) in MeCN (6 ml) and stirred at room temperature for 8 h. The reaction mixture was filtered through Si gel washing with EtOAc (100 ml) and concentrated. The residue was purified via radial chromatography [Si gel, hexane-EtOAc (5:1 to 2:1)] to give two erythro/threo diastereomers as white foams in an overall yield of 56%, threo isomer **56** (175 mg, 0.354 mmol, 40%), erythro isomer **57** (70 mg, 0.142 mmol, 16%). threo Isomer **56**: ir ($CHCl_3$) 3456, 3306, 3008, 2981, 2933, 1750, 1738, 1706, 1507, 1240, 1173 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.33 (m, 10H, Ar*H*), 6.91 (s, 1H, C*H*Ph_2), 4.86 (br s, 1H, N*H*), 4.66 (br s, 1H, O*H*), 4.38 (dd, J=2.8, 6.2 Hz, 1H, H-4'), 4.21 (m, 1H, H-3), 4.12 (d, J=2.5 Hz, 1H, H-2), 3.33 (m, 2H, H-5), 3.33 (dd, J=5.4, 14.9 Hz, 1H, H-3'α), 3.08 (dd, J=2.7, 14.9, 1H, H-3'β), 2.43 (d, J=2.0 Hz, 1H, H-6'), 1.73 (m, 2H, H-4), 1.42 (s, 9H, Me_3); $^{13}C\{^1H\}$ nmr ($CDCl_3$) δ 167.5, 167.4, 156.2, 139.3, 139.2, 128.6, 128.2, 128.1, 127.2, 127.0, 79.2, 79.0, 75.1, 68.9, 63.9, 43.4, 41.3, 37.6, 34.5, 28.4; ms *m/z* $[M-C_4H_8]^+$ 436 (0.05%), 167 (100%), 152, 118, 96, 86, 57; accurate mass 436.1639 (calcd for $C_{24}H_{24}N_2O_6$ $[M-C_4H_8]^+$ 436.1634). erythro Isomer **57**: ir ($CHCl_3$) 3458, 3303, 3028, 3010, 2979, 2933, 1761, 1739, 1701, 1602, 1508, 1170 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.35 (m, 10H, Ar*H*), 6.94 (s, 1H, C*H*Ph_2), 4.80 (br s, 1H, N*H*), 4.24–4.33 (br m, 3H, H-4', H-2 and H-3), 3.41 (br s, 1H, O*H*), 3.23 (dd, J=5.5, 14.8 Hz, 1H, H-3'α), 3.16 (m, 2H, H-5), 3.03 (dd, J=2.7, 15 Hz, 1H, H-3'β), 2.26 (d, J=2.0 Hz, 1H, H-6'), 1.76 (dm, 2H, H-4), 1.43 [s, 9H, $(CH_3)_3$]; $^{13}C\{^1H\}$ nmr ($CDCl_3$) δ 167.4, 166.7, 156.8, 139.5, 139.3, 128.6, 128.5, 128.14, 128.11, 127.3, 127.2, 80.2, 79.5, 78.6, 74.9, 68.4, 61.2, 43.9, 41.2, 38.1, 33.2, 28.4; ms *m/z* $[M-C_4H_8]^+$ 436 (0.03%), 167 (100%), 152, 118, 86, 57; accurate mass 436.1635 (calcd for $C_{24}H_{24}N_2O_6$ $[M-C_4H_8]^+$ 436.1634).

*Benzhydryl 5-(*N*-t-butyloxycarbonyl)-amino-3-hydroxy-2-(4'-ethynyl-2'-oxoazetidin-1'-yl)-pentanoate*

(erythro and threo isomers 2) [**58** *and* **59**].—Isomers **58** and **59** were prepared from isomer 2 of **55** (643 mg, 1.14 mmol) as described above for **56** and **57**, to give isomer **58** (125 mg, 0.254 mmol, 29%) and isomer **59** (115 mg, 0.234 mmol, 26%) as white foams in an overall yield of 55%. erythro Isomer **58**: ir ($CHCl_3$) 3455, 3306, 3029, 3013, 2981, 2934, 1739, 1707, 1507, 1173 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.32 (m, 10H, Ar*H*), 6.93 (s, 1H, C*H*Ph_2), 4.91 (br d, 2H, O*H* and N*H*), 4.34 (dd, *J*=3.0, 7.2 Hz, 1H, H-4′), 4.13 (m, 1H, H-3), 4.02 (d, *J*=2.5 Hz, 1H, H-2), 3.29 (m, 2H, H-5), 3.25 (dd, *J*=5.4, 14.9 Hz, 1H, H-3′α), 3.06 (dd, *J*=2.5, 14.7 Hz, 1H, H-3′β), 2.42 (d, *J*=2.1 Hz, 1H, H-6′), 1.88 (m, 2H, H-4), 1.44 [s, 9H, $(CH_3)_3$]; $^{13}C(^1H)$ nmr ($CDCl_3$) δ 167.2, 166.8, 156.2, 139.4, 139.2, 128.7, 128.6, 128.4, 128.1, 127.4, 126.9, 79.0, 75.4, 69.8, 63.6, 43.9, 41.2, 38.1, 33.2, 28.4; ms *m/z* $[M-C_4H_8]^+$ 436 (0.06%), 167 (100%), 152, 118, 96, 86, 57; accurate mass 436.1635 (calcd for $C_{24}H_{24}N_2O_6$ $[M-C_4H_8]^+$ 436.1634). threo Isomer **59**: ir ($CHCl_3$) 3458, 3305, 3030, 3008, 2980, 2932, 1761, 1741, 1706, 1508, 1174 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.31 (m, 10H, Ar*H*), 6.91 (s, 1H, C*H*Ph_2), 4.80 (br s, 2H, N*H* and O*H*), 4.47 (m, 1H, H-3), 4.31–4.36 (br m, 2H, H-4′ and H-2), 3.34 (dd, *J*=5.5, 14.9 Hz, 1H, H-3′α), 3.20 (m, 2H, H-5), 3.12 (dd, *J*=2.7, 14.9 Hz, 1H, H-3′β), 2.26 (d, *J*=2.2 Hz, 1H, H-6′), 1.70 (dm, 2H, H-4), 1.42 [s, 9H, $(CH_3)_3$]; $^{13}C(^1H)$ nmr ($CDCl_3$) δ 168.2, 167.7, 156.6, 139.4, 139.3, 129.6, 128.5, 128.2, 128.1, 127.3, 127.2, 80.4, 79.6, 78.7, 75.2, 68.9, 61.8, 44.9, 42.0, 37.2, 34.7, 28.4; ms *m/z* $[M-C_4H_8]^+$ 436 (0.09%) 167 (100%), 152, 118, 96, 86, 57; accurate mass 436.1635 (calcd for $C_{24}H_{24}N_2O_6$ $[M-C_4H_8]^+$ 436.1634).

5-Amino-3-hydroxy-2-(4′-ethynyl-2′-oxoazetidin-1′-yl)-pentanoic acid [**60–63**].—Each of the racemic, diastereomerically pure isomers of **9**/**17** (e.g., isomer **56**, 55 mg, 0.112 mmol) was treated with cold, freshly distilled TFA, (5 ml) and stirred at 0° for 2 h. The TFA was then removed in vacuo and followed by three evaporations with toluene. The residue was triturated with Et_2O and sonicated, and the Et_2O removed by pipet. The white precipitate was dried under vacuum and purified by hplc as described above, giving **60** (16.0 mg, 0.071 mmol, 63%) as a fluffy white solid. Deprotection of the remaining three isomers gave isomer **61** (14.0 mg, 0.062 mmol, 55%), isomer **62** (16 mg, 0.071 mmol, 63%), and isomer **63** (17 mg, 0.075 mmol, 67%). threo Isomer **60**: ir (KBr) 3357, 3281, 3199, 2969, 1719, 1683, 1627, 1405, 1397, 1341, 1316, 1206, 1142, 974, 675 cm^{-1}; ^1H nmr (D_2O) δ 4.28 (dt, *J*=2.3, 5.2 Hz, 1H, H-4′), 4.12 (m, 1H, H-3), 3.78 (d, *J*=5.7 Hz, 1H, H-2), 3.22 (dd, *J*=5.3, 15.0 Hz, 1H, H-3′$_{cis}$), 3.00 (m, 2H, H-5), 2.94 (dd, *J*=2.4, 15.0 Hz, 1H, H-3′$_{trans}$), 2.72 (d, *J*=2.1 Hz, 1H, H-6′), 1.75 (m, 1H, H-4), 1.69 (m, 1H, H-4); $^{13}C(^1H)$ nmr (D_2O) δ 172.7, 170.2, 79.4, 75.6, 68.2, 63.4, 43.2, 42.0, 37.0, 30.9; cims *m/z* $[MH]^+$ 227 (7.06%) (NH_3), 209 (100%), 191, 180, 149, 131, 114, 98, 58, 144. erythro Isomer **61**: ir (KBr) 3473, 3380, 3274, 3222, 2896, 1721, 1656, 1592, 1388, 1090, 1060, 669 cm^{-1}; ^1H nmr (D_2O) δ 4.45 (dt, *J*=2.4, 5.3 Hz, 1H, H-4′), 4.31 (m, 1H, H-3), 4.20 (d, *J*=4.3 Hz, 1H, H-2), 3.35 (dd, *J*=5.3, 15.1 Hz, 1H, H-3′$_{cis}$), 3.08 (m, 2H, H-5), 3.07 (dd, *J*=2.4, 15.1 Hz, 1H, H-3′$_{trans}$), 2.84 (d, *J*=2.0, 1H, H-6′), 1.82 (m, 2H, H-4); $^{13}C(^1H)$ nmr (D_2O) δ 173.5, 170.1, 80.4, 75.6, 68.2, 62.3, 43.6, 41.8, 37.1, 30.2; cims *m/z* $[MH]^+$ 227 (86.37%) (NH_3), 209, 191, 183, 165, 149, 131 (100%), 114, 98, 70, 44. erythro Isomer **62**: ir (KBr) 3314, 3263, 3203, 3092, 2884, 1724, 1627, 1605, 1404, 1331, 1317, 1203, 1090, 1070, 670 cm^{-1}; ^1H nmr (D_2O) δ 4.42 (dt, *J*=2.5, 5.4 Hz, 1H, H-4′), 4.20 (m, 1H, H-3), 4.00 (d, *J*=6.6 Hz, 1H, H-2), 3.22 (dd, *J*=5.4, 15.0 Hz, 1H, H-3′$_{cis}$), 3.00 (m, 2H, H-5), 2.96 (dd, *J*=2.6, 15.0 Hz, 1H, H-3′$_{trans}$), 2.75 (d, *J*=2.1 Hz, 1H, H-6′), 1.75 (m, 2H, H-4); $^{13}C(^1H)$ nmr (D_2O) δ 173.4, 169.6, 79.4, 75.8, 68.4, 63.6, 43.2, 42.0, 37.3, 30.4; cims *m/z* $[MH]^+$ 227 (7.32%) (NH_3) 209 (100%), 191, 180, 163, 149, 137, 131, 122. threo Isomer **63**: ir (KBr) 3435, 3282, 1749, 1736, 1685, 1399, 1341, 1204, 1137, 772 cm^{-1}; ^1H nmr (D_2O) δ 4.26 (dt, *J*=2.4, 5.3 Hz, 1H, H-4′), 4.15 (m, 1H, H-3), 3.78 (d, *J*=6.1 Hz, 1H, H-2), 3.18 (dd, *J*=5.3, 15.0 Hz, 1H, H-3′$_{cis}$), 3.00 (m, 2H, H-5), 2.90 (dd, *J*=2.4, 14.9 Hz, 1H, H-3′$_{trans}$), 2.74 (d, *J*=2.1 Hz, 1H, H-6′), 1.82 (m, 2H, H-4); $^{13}C(^1H)$ nmr (D_2O) δ 172.2, 170.9, 80.3, 75.7, 68.1, 62.7, 43.7, 42.3, 36.9, 30.7; cims *m/z* $[MH]^+$ 227 (18.05%) (NH_3), 209 (100%), 191, 180, 165, 149, 131, 114, 98, 58, 44.

4-CYCLOPROPYLPROCLAVAMINIC ACID [**19**].—*Methyl 2-cyano-2-cyclopropylacetate* [**64**].—Methyl cyanoacetate (4.0 g, 40.4 mmol) was reacted with 1,2-dibromoethane (10.62 g, 56.6 mmol), K_2CO_3 (12.28 g, 88.9 mmol), and DMF (44 ml) at room temperature for 20 h, then filtered through a fritted funnel and concentrated in vacuo. The residue was taken up in Et_2O and the residual salts were removed by filtration. Evaporation of the Et_2O gave the product **64** (3.28 g, 26.3 mmol, 65%) as a pale yellow oil, bp 46–47° (0.1 mm). No further purification was necessary. Ir ($CHCl_3$) 3050, 2960, 2220, 1730, 1460, 860, 740 cm^{-1}; ^1H nmr ($CDCl_3$) δ 3.81 (s, 3H, CH_3), 1.6–1.75 (2×m, 4H, CH_2-CH_2). Other spectral properties were in accord with those reported in the literature (25).

3-(N-t-butyloxycarbonyl)amino-2-cyclopropylpropanol [**65**].—Ester **64** (1.00 g, 8.00 mmol) in Et_2O (76 ml) was reacted with $LiAlH_4$ (610 mg, 16.0 mmol) for 1 h at room temperature. The reaction was quenched by the addition of H_2O (610 μl) followed by a solution of 15% NaOH (610 μl) and another addition of H_2O (1.83 ml). After stirring for 15 min, the mixture was filtered through Celite washing with EtOAc. The filtrate was evaporated in vacuo and the residue taken up in a solution of $NaHCO_3$ (2.01 g, 23.9 mmol) in H_2O (29 ml) and 1,4-dioxane (15 ml). A solution of di-*t*-butyl dicarbonate (2.74 g, 12.5 mmol) in 1,4-

dioxane (15 ml) was then added over 20 min, and the mixture was stirred at room temperature for 6 h. The reaction mixture was concentrated in vacuo to 20 ml and partitioned between EtOAc (75 ml) and H_2O (20 ml). The aqueous layer was re-extracted with EtOAc (75 ml), and the combined organic solution was washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by flash chromatography [Si gel, hexane-EtOAc (3:1)] to give **65** (640 mg, 3.36 mmol, 42%) as a clear, colorless oil: ir ($CHCl_3$) 3365, 2978, 2931, 2871, 1689, 1523, 1260, 1166, 1031 cm^{-1}; ^1H nmr ($CDCl_3$) δ 4.94 (br s, 1H, N*H*), 3.39 (s, 2H, H-3), 3.13 (s, 2H, H-1), 1.93 (br s, 1H, O*H*), 1.47 (s, 9H, $(CH_3)_3$), 0.4–0.5 (2×m, 4H, CH_2-CH_2); $^{13}C\{^1H\}$ nmr ($CDCl_3$) δ 180.0, 79.8, 67.1, 45.3, 28.3, 23.4, 9.1; ms *m/z* $[M-C_4H_8]^+$ 145 (28%), 127, 117, 99, 82, 72, 67, 57 (100%), 41; accurate mass 145.0739 (calcd for $C_6H_{11}NO_3$ $[M-C_4H_8]^+$ 145.0739).

*3-(*N*-t-butyloxycarbonyl)amino-2-cyclopropyl-propanal* [**66**].—A solution of the alcohol **65** (100 mg, 0.498 mmol) in CH_2Cl_2 (1 ml) was added over 5 min to oxalyl chloride (70.0 mg, 0.547 mmol) and DMSO (86.0 mg, 1.09 mmol) in CH_2Cl_2 (2.5 ml), and the mixutre was stirred for 15 min. Triethylamine (251 mg, 2.49 mmol) was then added over 5 min, and the reaction mixture warmed to room temperature over 45 min. The reaction mixture was washed with H_2O (15 ml), 1 N HCl (2×15 ml), 5% $NaHCO_3$ (15 ml), and brine (15 ml). The organic extracts were dried ($MgSO_4$), and concentrated in vacuo to yield the aldehyde **66** (72.0 mg, 0.362 mmol, 72%) as a pale yellow oil. This compound was carried through to the next step without further purification. Ir ($CHCl_3$) 3050, 1725, 1705, 1660, 1520 cm^{-1}; ^1H nmr ($CDCl_3$) δ 8.61 (s, 1H, H-1), 5.12 (br s, 1H, N*H*), 3.30 (d, *J*=6.2 Hz, 2H, H-3), 1.41 [s, 9H, $(CH_3)_3$], 1.15–1.19 (2×m, 4H, CH_2-CH_2); $^{13}C\{^1H\}$ nmr ($CDCl_3$) δ 201.6, 156.2, 67.8, 41.1, 33.9, 28.3, 11.9; ms *m/z* $[M-C_4H_8]^+$ 143, 99, 84; accurate mass 184.0978 $[M-Me]^+$ (calcd for $C_9H_{14}NO_3$ $[M-Me]^+$ 184.0974).

*Benzhydryl 5-(*N*-t-butyloxycarbonyl)-amino-4-cyclopropyl-3-hydroxy-2-(2′-oxoazetidin-1′-yl)*-pentanoate [**67**/**68**].—A solution of benzhydryl (2′-oxoazetidin-1′-yl)-acetate (260 mg, 0.880 mmol), prepared from the corresponding benzyl ester (26,27) by hydrogenolysis and reaction with diphenyldiazomethane, in THF (15 ml) cooled to −78° was treated with lithium bis(trimethylsilyl) amide (968 ml, 0.968 mmol, 1 M in hexane) over 5 min, and the reaction was stirred an additional 20 min. A solution of the cyclopropylaldehyde **66** (175 mg, 0.880 mmol) in THF (2.5 ml) was then added over 5 min. After stirring for 1 h, the reaction was quenched by addition of saturated NH_4Cl (6 ml) and the mixture was partitioned between EtOAc and 1 N HCl (50 ml each). The organic layer was washed with 5% $NaHCO_3$ (50 ml) and brine (50 ml), dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by radial chromatography [2 mm silica, EtOAc-$CHCl_3$ (1:9 to 2:8)] to give the faster moving *erythro*-(±)-isomer **68** (165.4 mg, 0.336 mmol) and less mobile *threo*-(±)-isomer **67** (163.7 mg, 0.333 mmol) as clear colorless oils in a combined yield of 76%. *erythro*-**68**: ir ($CHCl_3$) 3448, 3036, 3001, 2975, 1731, 1701, 1507, 1243, 1166 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.35 (m, 10H, Ar*H*), 6.92 (s, 1H, C*H*Ph_2), 5.27 (br s, 1H, N*H*), 4.66 (d, *J*=6.0 Hz, 1H, H-2), 4.22 (br s, 1H, O*H*), 3.79 (br d, *J*=6.0 Hz, 1H, H-3), 3.40–3.05 (sym m, 2H, H-5), 3.20 (app t, *J*=ca. 4 Hz, 2H, H-4′), 2.89 (app t, *J*=ca. 4 Hz, 2H, H-3′), 1.43 [s, 9H, $(CH_3)_3$], 0.62 (m, 1H, CH_2-CH_2), 0.52 (m, 1H, CH_2-CH_2), 0.35 (sym m, 2H, CH_2-CH_2); $^{13}C\{^1H\}$ nmr ($CDCl_3$) δ 168.3, 156.5, 139.5, 139.3, 128.6, 128.3, 128.1, 127.4, 127.0, 79.5, 78.7, 73.5, 59.6, 45.4, 39.6, 36.34, 28.4, 22.1, 9.1, 8.3; accurate mass $[MH]^+$ (NH_3) 495.2506 (calcd for $C_{28}H_{35}N_2O_6$ $[MH]^+$ 495.2495). *threo*-**67**: ir ($CHCl_3$) 3448, 3354, 3007, 2975, 1748, 1731, 1701, 1595, 1507, 1243, 1166 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.33 (m, 10H, Ar*H*), 6.92 (s, 1H, C*H*Ph_2), 5.04 (br s, 1H, N*H*), 4.66 (d, *J*=5.5 Hz, 1H, H-2), 4.28 (br s, 1H, O*H*), 3.97 (d, *J*=5.5 Hz, 1H, H-3), 3.33 (sym m, 2H, H-4′), 3.10 (m, 2H, H-5), 2.95 (app q, *J*=ca. 4 Hz, 2H, H-3′), 1.41 [s, 9H, Me_3], 0.69 (sym m, 1H, CH_2-CH_2), 0.48 (sym m, 1H, CH_2-CH_2), 0.39 (m, 1H, CH_2-CH_2), 0.09 (m, 1H, CH_2-CH_2); ^{13}C nmr ($CDCl_3$) δ 169.4, 168.0, 156.3, 139.4, 128.6, 128.3, 128.2, 127.4, 127.0, 79.6, 78.6, 73.3, 61.6, 45.4, 39.9, 36.0, 28.4, 22.5, 8.5, 8.1; accurate mass $[MH]^+$ 495.2501 (NH_3), (calcd for $C_{28}H_{35}N_2O_6$ $[MH]^+$ 495.2495).

5-Amino-4-cyclopropyl-3-hydroxy-2-(2′-oxoazetidin-1′-yl)-pentanoic acid (**69**/**70**).—*Erythro*-**68** (152 mg, 0.308 mmol) was deprotected in cold TFA (6 ml) and the solution was stirred at 0° for 30 min. The TFA was removed in vacuo, followed by three evaporations with toluene. The residue was triturated with Et_2O and sonicated, and the Et_2O was removed by pipet. Purification by hplc as described above provided the product **70** (30.0 mg, 0.132 mmol, 43%) as a fluffy white solid. Deprotection of the remaining isomer **67** (threo) (30.0 mg, 0.061 mmol) was performed analogously to give **69** (4.7 mg, 0.021 mmol, 34%) also as a fluffy white solid. **70** (erythro): ir (KBr) 3436, 3250, 2937, 1719, 1686, 1618, 1400, 1205, 1137, 1055; ^1H nmr (D_2O/Me_2CO) δ 4.59 (d, *J*=9.5 Hz, 1H, H-2), 3.80 (d, *J*=14.0 Hz, 1H, H-5), 3.54 (m, 1H, H-4′), 3.47 (d, *J*=9.5 Hz, 1H, H-3), 3.44 (m, 1H, H-4′), 3.02 (app t, *J*=ca. 4 Hz, 2H, H-3′), 2.52 (d, *J*=14.0 Hz, 1H, H-5), 0.85–0.75 (m, 4H, CH_2-CH_2); ^{13}C nmr (D_2O/dioxane) δ 173.9, 171.2, 75.7, 58.3, 43.9, 39.5, 35.1, 19.8, 9.2, 8.9; ms *m/z* $[MH]^+$ 229 (36%), 211, 140, 112; accurate mass $[MH]^+$ 229.1192 (NH_3) (calcd for $C_{10}H_{17}N_2O_3$ $[MH]^+$ 229.1188). **69** (threo): ^1H nmr (D_2O/Me_2CO) δ 4.38 (d, *J*=8.0 Hz, 1H, H-2), 3.52 (m, 1H, H-4′), 3.42 (d, *J*=16.0 Hz, 1H, H-5), 3.40 (d, *J*=8.0 Hz, 1H, H-3), 3.38 (m, 1H, H-4′), 2.89 (m, 2H, H-3′), 2.74 (d, *J*=16.0 Hz, 1H, H-5), 0.70–0.58 (m, 4H, CH_2-CH_2); $^{13}C\{^1H\}$ nmr (D_2O/dioxane)

δ 173.0, 172.0, 74.7, 59.7, 43.7, 39.5, 35.1, 20.3, 10.4, 9.8; ms *m/z* $[MH]^+$ 229 (22%), 211 (100%), 141; accurate mass $[MH]^+$ 229.1190 (NH_3) (calcd for $C_{10}H_{17}N_2O_3$ $[MH]^+$ 229.1188).

ASSAY SYSTEMS FOR ANALYSIS OF INTERACTION OF PROCLAVAMINIC ACID ANALOGUES WITH CLAVAMINATE SYNTHASE.—*Isolation and purification of clavaminate synthase.*—Clavaminate synthase was purified from *S. clavuligerus* (ATCC 27064) as prevously described (1). The yields and extents of purification are presented in Table 1 for the individual steps of a typical isolation.

TABLE 1. Purification of Clavaminate Synthase from 50 g of *Streptomyces clavuligerus* Cell Paste.

Fractionation Step	Total Protein (mg)	Total Activity (μmol/min)	Specific Activity μmol/min·mg	% Recovery	Purification (x-fold)
Extract	930	5.8	0.0062	(100)[a]	(1)
Streptomycin sulfate	610	5.4	0.0089	93	1.4
Sephadex G-15	400	5.6	0.014	97	2.3
DEAE-Sepharose	19	4.4	0.23	76	37
Sephadex G-75	7.0	2.8	0.40	48	65

General assay conditions.—Formation of clavulanic acid in fermentations was monitored by derivatization of the broth with imidazole and scanning from 272 to 352 nm for chromophore development at 312 nm (1,32,33). The imidazole reagent consisted of a 3 M aqueous solution of imidazole (recrystallized four times from C_6H_6 and washed with Et_2O) at pH 6.8. A 1-ml sample of broth was centrifuged (2 min at 14,000 rpm) and the supernatant filtered (0.22 μm filter) to give a clear solution. A portion of the filtrate (10 μl) was then combined with 5 μl imidazole reagent and heated at 40° for 20 min. The solution was diluted with 500 μl H_2O and scanned.

Assays for specific activity determinations (1) during the purification of CS contained 50 mM sodium MOPS buffer (pH 7.0), 0.5 mM DTT, 0.1 mM sodium ascorbate, 1 mM α-ketoglutarate, 1 mM D,L-proclavaminic acid, and 25 μM ferrous ammonium sulfate. For activity measurements of crude enzyme (prior to DEAE-Sepharose) the assays were conducted in glass test tubes in a final volume of 200 μl. Separate controls were run for each sample containing the same amount of extract but omitting the proclavaminic acid and including 0.2 mM EDTA throughout the incubation. After a 5-min incubation at room temperaature, the assays were terminated by the addition of 10 μl of 4 mM EDTA. The assay tubes were immersed in boiling H_2O for 30 sec and then cooled in an ice bath. An aliquot of 180 μl was transferred from each test tube to a plastic microfuge tube containing 90 μl of imidazole reagent. After incubating for 20 min at 40°, 270 μl of H_2O was added to each tube and the protein precipitate pelleted by centifugation (5 min at 14,000 rpm). The absorbance of the supernatant at 312 nm, corrected for the absorbance of the control, was used to compute turnover assuming an extinction coefficient of 26,900 $M^{-1}\cdot cm^{-1}$ for the α,β-unsaturated acyl imidazole derivative of clavaminic acid. Assays in the latter part of the purification procedure containing much less protein were conducted similarly, except that boiling and individual controls were not required.

Assay of proclavaminic acid analogues.—Clavaminate synthase was purified through the Sephadex G-75 step. For each enzyme activity assay below the same batch of enzyme was used to ensure mutually comparable results. All assays contained 50 mM MOPS (pH 7.0), 0.5 mM DTT, 0.1 mM sodium ascorbate, and 0.01 mM ferrous ammonium sulfate in a final volume of 200 μl. Assays were run at room temperature in plastic microfuge tubes and terminated after the specified time with EDTA.

Clavam nucleus formation.—To test the various analogues as alternate substrates undergoing the established oxidative cyclization chemistry with clavaminate synthase, an assay based on imidazole derivatization of the clavam nucleus, if generated, was used. Parallel reactions containing 2 mM α-KG and either D,L-proclavaminic acid (1 mM) or analogue (1 mM) in a final volume of 100 μl were initiated with enzyme (ca. 50 μg) along with separate controls. After 1 h of incubation at room temperature with occasional manual stirring, the assays were terminated with 5 μl 4 mM EDTA. To the assays was added 50 μl of imidazole reagent (3 M aqueous imidazole, pH 6.8) (32,33), and the solutions were heated at 40° for 20 min. After dilution with 400 μl of H_2O, the samples were scanned between 272 and 352 nm to look for chromophore development typically centered at 312 nm (1).

Formation of non-clavam products.—To test for the possibility that some of the analogues might undergo reaction with clavaminate synthase to form a non-clavam product or products, an assay system based on

amine derivatization with *o*-phthaldialdehyde (OPA) followed by hplc analysis was employed (34). Reactions were run exactly as described above, followed by reaction of an aliquot to assay (10 μl) with an equal volume of OPA reagent for 30 sec at room temperature and immediate hplc analysis. The OPA reagent consisted of 450 μl 0.6 M sodium borate buffer (pH 9.7), 50 μl 0.5 OPA in EtOH, and 2 μl β-mercaptoethanol. The isoindoles formed are detected at a λ max of 340 nm (35). Hplc analysis was performed on a Spherisorb ODS-2 C-18 column at a flow rate 0f 0.75 ml/min. The following gradient was used (A=50 mM KH_2PO_4 adjusted to pH 6.8 with KOH; B=MeOH): 50 to 80% B over 15 min, hold at 80% B for 5 min, return to 50% B over 5 min, and re-equilibrate at 50% B for a minimum of 6 min.

Inhibition of normal substrate processing.—To measure the level of inhibition exhibited by the various analogues, parallel reactions contained 1 mM α-KG, 0.3 mM D,L-proclavaminate, and either an analogue (1.0 mM) or no addition in a final volume of 390 μl. The assays were initiated by the addition of enzyme (20 μg) and terminated after 2 min with 10 μl 4 mM EDTA. Derivatization with 200 μl imidazole reagent was as described above, followed by scanning from 272 to 352 nm to measure inhibiton level. Based on the degree of inhibition observed, a series of 12 parallel reactions were run containing 1 mM α-KG, and 0.1 mM, 0.2 mM, and 0.5 mM D,L-proclavaminate along with no addition and the analogue at 3 concentrations chosen to exhibit a range of inhibition from ca. 10 to 90%. The velocity data were then computer fit to competitive, non-competitive, and un-competitive patterns, with the appropriate K_i's or K_{ii}'s and K_{is}'s reported from the best fit (39).

Irreversible inactivation.—To test the analogues for irreversible inactivation of clavaminate synthase, the enzyme was incubated at 2.0 mg/ml with the usual assay components as well as 1 mM α-KG and 0.1 mg/ml catalase (Sigma) in the presence and absence of the analogue being tested. At various time points a 10 μl aliquot was withdrawn and diluted into a 380 μl standard assay containing 0.3 mM D,L-proclavaminate. After 2 min the assay was terminated with 10 μl 4 mM EDTA, derivatized with 200 μl imidazole reagent as described above, and scanned from 272 to 352 nm to measure residual enzyme activity.

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