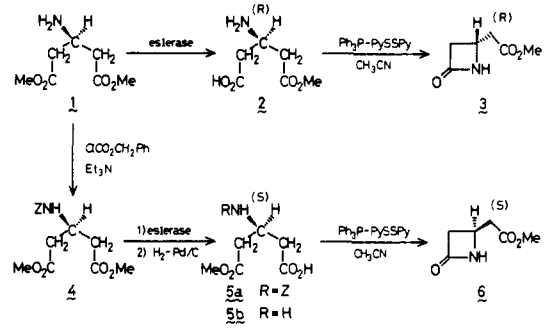
Scheme I



2-azetidinones by chemicoenzymatic approach starting from citric acid derivative as shown in Scheme I.

4-[(Methoxycarbonyl)methyl]-2-azetidinone was considered to be a versatile intermediate of the carbapenem nuclei on the basis of previous studies,[2-4] and a combination of enzymatic and chemical procedures was taken as our synthetic strategy. Cohen and Khedouri showed that α-chymotrypsin hydrolyzes the pro-*S* ester group of diethyl β-acetamidoglutarate.[6] However, the acetamido compound was considered not a good candidate for our purpose, since it may cause difficulty in further hydrolysis of the acetyl amino group followed by formation of the β-lactam ring. Dimethyl β-aminoglutarate (**1**) has been chosen as our starting material. It was prepared in excellent yield by reductive amination of dimethyl β-oxoglutarate[7] according to Borch's method[8] ($CH_3CO_2NH_4/NaBH_3CN$). The rate of hydrolysis of **1** with α-chymotrypsin was found to be extremely slow, and a large amount of the enzyme seemed necessary for synthetic purposes. Therefore, pig liver esterase was employed as shown in the case of β-hydroxy-β-methyl dimethyl glutarate by Sih and his coworkers.[9] Pig liver esterase[10] hydrolyzed **1** very efficiently and (3*R*)-half-ester **2** was formed in low optical yield. In a typical experiment, **1** (346 mg) in 0.1 M phosphate buffer (pH 8.0) (7 mL) was incubated with the esterase (400 units) at 25 °C for 1.5 h. Following usual workup, half-ester **2**, $[\alpha]^{22}_D$ +2.36° (*c* 4.23, H_2O), was obtained in 94% yield. The β-amino acid ester **2** was converted to 2-azetidinone **3**, $[\alpha]^{25}_D$ -26.03° (*c* 1.26, $CHCl_3$), with a Ph_3P-$(PyS)_2$-CH_3CN system[11] in 82% yield, and its absolute configuration and optical purity were determined by comparison with an authentic sample,[12] (*S*)-4-[(methoxycarbonyl)methyl]-2-azetidinone (**6**), prepared from L-aspartic acid.[4a] The results showed that pig liver esterase cleaved the pro-*S* methyl ester group of **1** more selectively giving **2**. However, it was also shown that the substrate **1** was partly hydrolyzed at the reaction condition even in the absence of the enzyme (about 30%), explaining the low optical yield (about 40% ee). It was assumed that the free amino group participates in the chemical hydrolysis through hydrogen bonding with a carbonyl group of the ester. Therefore, the amino group was protected by the benzyloxycarbonyl (Z) group with ZCl, affording **4** in 90% yield. Surprisingly, incubation of **4** with pig liver esterase at the same reaction condition afforded (3*S*)-half-ester **5a**, mp 97-97.5 °C, $[\alpha]^{25}_D$ +0.69° (*c* 7.45, $CHCl_3$), in 93% yield. Hydrogenolysis (H_2, Pd-C in MeOH) of **5a** afforded quantitatively **5b**, mp 175-177 °C, $[\alpha]^{25}_D$ -5.52° (*c* 3.26, H_2O). It was converted to monocyclic β-lactam **6** with Ph_3P-$(PyS)_2$-CH_3CN[11] in 84% yield, affording (*S*)-4-[(methoxycarbonyl)-methyl]-2-azetidinone in high optical purity, $[\alpha]^{25}_D$ +65.34°[12] (*c* 1.11, $CHCl_3$). In the absence of the enzyme, essentially no hydrolysis took place for **4**. This confirms that the esterase stereospecifically cleaved the pro-*R* methyl ester group of **4** giving **5a** and **5b**. It is worthy of note that the protection with ZCl of the amino group at the prochiral center in **1** reversed the chirality of the product with the same enzyme by hydrolyzing selectively one of the enantiotopic (methoxycarbonyl)methyl groups. *tert*-Butyloxycarbonyl- and benzylamino derivatives of **1** both afforded the corresponding half-esters with *S* configuration upon enzymatic hydrolysis, but the acetyl derivative afforded the corresponding half-ester with *R* configuration. These findings are synthetically useful and important, since formation of β-amino acid with desired configuration can be selectively chosen at the stage of enzymatic hydrolysis.

The key features of the present methodology include the following: (1) dimethyl β-aminoglutarate (**1**) and dimethyl β-[(benzyloxycarbonyl)amino]glutarate (**4**) were efficiently hydrolyzed with pig liver esterase to (*R*)- and (*S*)-half-esters, **2** (40% ee) and **5a** (>96% ee), respectively; (2) the half-esters were converted to optically active 2-azetidinone **3** and **6** with Ph_3P-$(PyS)_2$-CH_3CN system in high yields; (3) (*S*)-azetidinone **6** having the desired absolute configuration is now easily available in quantity as a versatile synthon for carbapenem nuclei; (4) **2** and **5a** or **5b** are useful for the synthesis of chiral β-amino acids of biological interest.

Further investigation of the enzymatic process and the synthetic work to carbapenem β-lactam antibiotics will be reported in due course.

Acknowledgment. We express our gratitude to Professor H. Umezawa of Institute of Microbial Chemistry for his interest and support of this work and also Dr. H. Nakai for the preparation of the authentic sample **6**.

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(10) This was purchased from Sigma Co.

(11) After a systematic investigation of the condensing reagents for β-lactam formation from β-amino acids, this excellent system has been found and is the subject of the following publication.

(12) (*S*)-4-(Iodomethyl)-2-azetidinone[4a] was converted into **6**, $[\alpha]^{25}_D$ +63.95° (*c* 1.34, $CHCl_3$), in the following procedures: (a) silylation with $ClSi(Me)_2Bu$-*t* (94%), (b) tris(ethylthio)methane/*n*-BuLi (82%), (c) esterification with MeOH-$HgCl_2$ (43%), (d) desilylation with aqueous MeOH-HCl (46%).

(13) All materials described here gave MS, IR, and NMR (^{13}C and ^1H) spectra consistent with their structures.

Ph_3P-$(PyS)_2$-CH_3CN as an Excellent Condensing System for β-Lactam Formation from β-Amino Acids

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In the preceding publication[1] we reported that the enzymatic hydrolysis of dimethyl β-aminoglutarate afforded (*R*)-3-amino-4-(methoxycarbonyl)butyric acid selectively and that the protection of the amino group at the prochiral center with benzyloxycarbonyl chloride reversed the chirality of the product, affording the corresponding (*S*)-β-amino acid ester (**1a**) in excellent yield and with remarkable optical purity. This communication describes a new and efficient methodology for the formation of β-lactam compounds from β-amino acids by using triphenylphosphine and 2,2′-dipyridyl disulfide in acetonitrile.

A great deal of synthetic work has been already carried out in the formation of β-lactam compounds from β-amino acids.[2,3]

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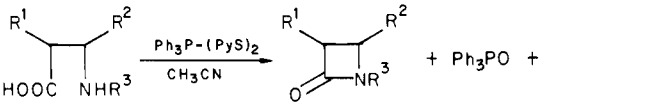
However, known methods have some limited use because of dependency upon the structural features of a substrate, requirement of basic or acidic conditions, and a large excess of reagents or limitation of functional groups located at other parts of the molecule. For instance, thermal dehydration is limited to 2,2-disubstituted β-amino acids because of the ease with which β-amino acids undergo β elimination.[2] The cyclization through the use of reagents such as AcCl, PCl_3, and $SOCl_2$ has been accomplished in a limited number of cases.[2,4] The base-catalyzed cyclization of a β-amino acid ester using a Grignard reagent as the base is often used if the substituents are inert to the reagent[5] and was improved recently by silylation of the amino group.[6] The cyclization of a β-amino acid ester was achieved with organo aluminum compounds in a special case.[7] The DCC method is most commonly applied to β-lactam formation from β-amino acids, but the yields markedly depend upon the structural features and solvents employed.[8]

Application of these known reagents to **1a** did not give any good results, yielding **2a** only in poor yields, so a systematic investigation of the condensing system was carried out, and Ph_3P–$(PyS)_2$–CH_3CN system has been found most satisfactory for the formation of (*S*)-4-[(methoxycarbonyl)methyl]-2-azetidinone (**2a**) from (*S*)-β-aminoglutaric acid monomethyl ester (**1a**) and extended to other β-amino acids as shown in Table I.



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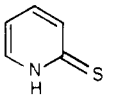

a, $R^1 = R^3 = H$; $R^2 = CH_2COOMe$, *S*
b, $R^1 = R^3 = H$; $R^2 = CH_2COOMe$, *R*
c, $R^1 = R^2 = R^3 = H$
d, $R^1 = R^3 = H$; $R^2 = Ph$, *RS*
e, $R^1 = H$; $R^2 = CH_3$, *RS*; $R^3 = CH_2Ph$
f, $R^1 = R^2 = H$; $R^3 = CH_2Ph$
g, $R^1 = NH_2$, *RS*; $R^2 = R^3 = H$
h, $R^1 = R^2 = H$; $R^3 = CH_2CH_2OH$

Mukaiyama's reagent, Ph_3P–$(PyS)_2$, is well-known as being useful for peptide synthesis through the oxidation–reduction condensation first introduced in 1970.[9] However, no successful report has appeared for the application of the reagent to form a β-lactam compound from a β-amino acid by intramolecular condensation. Actually, we found that treatment of 3-amino-3-phenylpropionic acid (**1d**) with Ph_3P–$(PyS)_2$ in usual solvents such as CH_2Cl_2 and DMF afforded polymers as main products and formation of β-lactam **2d** was confirmed only in trace amount and 9% yield, respectively (see Table I). The choice of acetonitrile as a solvent remarkably increased the yields of β-lactam compounds. In a typical experiment, (*S*)-3-amino-4-(methoxycarbonyl)butyric acid was suspended in CH_3CN (0.05 M solution), Ph_3P (1.2 equiv) and $(PyS)_2$ (1.2 equiv) were added at room temperature, and the reaction mixture was heated to 55–60 °C for 12 h with stirring, showing homogeneity with yellow color at the end of the reaction. After removal of the solvent in vacuo, the product was separated from triphenylphosphine oxide and thione by chromatography on silica gel by using 1:1 ether–methylene chloride for elution, and the fractions containing the desired β-lactam compound (TLC R_f values in a mixed solvent system, 1:1 CH_2Cl_2–Et_2O, 0.20) were further purified by chromatography on silica gel, affording (*S*)-4-[(methoxycarbonyl)-methyl]-2-azetidinone in 84% yield, showing mp 67.5–68 °C (recrystallized from ether) and $[\alpha]^{25}_D$ +65.34° (*c* 1.11, $CHCl_3$). Then, the present method was successfully applied to (*R*)-β-amino acid ester (**1b**),[10] β-alanine (**1c**),[11] *dl*-3-amino-3-phenylpropionic acid (**1d**),[12] *dl*-3-benzylaminobutyric acid (**1e**),[13] 3-benzylaminopropionic acid (**1f**)[14] and *dl*-2,3-diaminopropionic acid (**1g**),[11] affording the corresponding β-lactams in good to excellent yields (Table I). The efficiency of the condensing system, Ph_3P–$(PyS)_2$–CH_3CN, is indeed remarkable, since the DCC method afforded β-lactams in 10–30% yield in the most cases under similar conditions (CH_3CN, 0.01 M).[15]

Table I. Synthesis of β-Lactams from β-Amino Acids with Ph_3P–$(PyS)_2$–CH_3CN

β-amino acid	solvent	concn, M	temp, °C	time, h	β-lactam[a]	yield, %
1a	CH_3CN	0.05	55	12	2a	84
1b	CH_3CN	0.01	reflux	12	2b	82
1c	CH_3CN	0.1	55	24	2c	39
1c	CH_3CN	0.01	reflux	4.5	2c	80
1d	CH_3CN	0.1	55	4.5	2d	34
1d	CH_3CN	0.1	reflux	4.5	2d	60
1d	CH_3CN	0.01	reflux	4.5	2d	97
1d	DMF	0.1	55	4.5	2d	9
1d	CH_2Cl_2	0.1	reflux	4.5	2d	trace
1d	CH_3NO_2	0.01	reflux	4.5	2d	26
1e	CH_3CN	0.01	reflux	4.5	2e	96
1f	CH_3CN	0.1	reflux	4.5	2f	44
1f	CH_3CN	0.01	reflux	4.5	2f	91
1g	CH_3CN	0.02	reflux	5.5	2g	56
1h	CH_3CN	0.02	reflux	4.5	2h	68

[a] All β-lactams described here gave MS and ^1H NMR spectra consistent with their structures and showed IR absorptions at 1760 (1a and 1b), 1720 (1c),[17] 1745 (1d),[18] 1740 (1e),[18a] 1750 (1f), 1735 (1g), and 1720 cm^{-1} (1h).

The noteworthy features of the present methodology include the following: (1) Ph_3P–$(PyS)_2$–CH_3CN system is generally applicable to a variety of β-amino acids under neutral condition; (2) high dilution (0.01–0.05 M) and higher temperatures (reflux) are preferable; (3) other functional groups such as amino, hydroxyl, and ester groups do not hinder the cyclization; (4) direct synthesis of **2a** and **2g** from **1a** and **1g** provide potential intermediates for carbapenem and nocardicin,[16] respectively; (5) even moist acetonitrile can be conveniently used for water-soluble β-amino acids.

The synthetic extension of this methodology to current β-lactam antibiotics of interest and the role of acetonitrile in the cyclization mechanism are now under investigation, and the results will be reported in due course.

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24-Methyl-25-azacycloartanol, an Analogue of a Carbonium Ion High-Energy Intermediate, Is a Potent Inhibitor of (*S*)-Adenosyl-L-methionine:Sterol C-24-Methyltransferase in Higher Plant Cells

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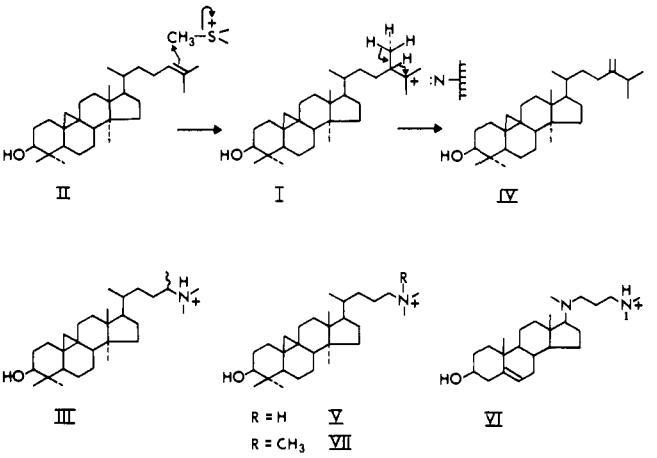
Theoretical considerations have established that catalysis of a reaction by an enzyme implies that the activated form of the substrate, occurring during the reaction pathway, is bound more energetically by the active site than the substrate in its ground state.[1,2] The result is that molecules which bear a structural and electronic resemblance to metastable intermediates should be very strong inhibitors of catalyzed reactions.[2,3]

Studies on (*S*)-adenosyl-L-methionine (SAM):sterol C-24-methyltransferases[4-7] have shown that the C-methylation reaction can be viewed as a nucleophilic attack by the Δ^{24} double bond of various sterols on the methyl group of the sulfonium group of SAM. This reaction leads to the formation of a high-energy intermediate (HEI, I) possessing a methyl at C-24 and a carbonium ion at C-25. After a hydride transfer from C-24 to C-25, an elimination of a proton at C-28 occurs giving a 24-methylene sterol (Scheme I). For an explanation of the stereochemical features of this reaction, it has been postulated that the C-25 carbonium ion could be stabilized by an electron-bearing residue of the active site of the enzyme, leading to a transient interaction of I with the enzyme.[6,8] With these considerations in mind, we thought that it was possible to mimic the carbonium ion of I by replacing the C-25 by a nitrogen atom in the structure of the intermediate. The resulting 25-aza derivative being essentially protonated under physiological conditions presents certain electronic similarities with the HEI (I), i.e., tetrahedral ammonium ion compared to the trigonal carbonium ion, and could therefore behave as a potent inhibitor of the methylation reaction. Since in photosynthetic eukaryotes cycloartenol (II) has been shown to be the best substrate in vivo and in vitro of the C-methylation reaction, (24-*RS*)-24-methyl-25-azacycloartanol (III) has been synthesized and assayed as an inhibitor of (*S*)-adenosyl-L-methionine:cycloartenol C-24-methyltransferase (CMT).

(24-*RS*)-24-Methyl-25-azacycloartanol was prepared as follows: To the known[9a] 3β-acetoxy-9β,19-cyclo-25,26,27-trinorlanostan-24-one (114 mg) taken in a 2:1 mixture of dry MeOH–THF (10 mL) was added 10 mL of methanol solutions of dimethylamine hydrochloride (stock solution was prepared by dissolving 2.17 g of $(CH_3)_2NH_2^+Cl^-$ in 50 mL of MeOH) and 60 mg of $NaCNBH_3$ under N_2 atmosphere. After stirring at room temperature for 72 h, the reaction mixture was diluted with water and the organic part was extracted in ether and dried (Na_2SO_4) and then treated with 40 mg of LAH for 4 h at room temperature. After conventional workup, 103 mg of 24-methyl-25-azacycloartanol (epimeric at C-24), mp 110–114 °C, 120–123 °C, was obtained. The assigned structure was in full agreement with its spectral data (NMR, IR, Mass).[9b]

Scheme I



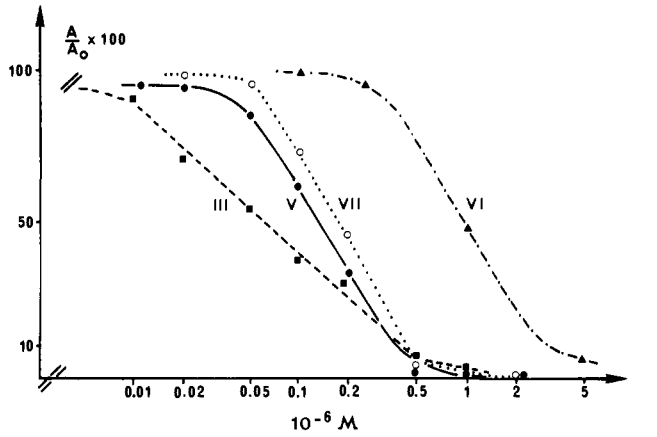


Figure 1. Inhibition of the (*S*)-adenosyl-L-methionine:cycloartenol C-24-methyltransferase by high-energy analogues: III (-■-■-), V (-●-●-), VI (··▲--▲--), and VII (··O··O··O··). A_0, A, activities measured, respectively, in the absence or presence of inhibitor, the concentration of substrate II being 100 μM.

Microsomes (0.5 mL) from maize seedlings were incubated in the presence of [*Methyl*-^{14}C]SAM (100 μM, 0.1 μCi), cycloartenol (100 μM), and various concentrations of III for 1 h at 30 °C and pH 7.4. A control which lacked III was incubated in parallel. The reactions were stopped by 6% methanolic KOH (1 mL), and the neutral lipids were extracted and analyzed as described.[10,11] The radioactivity incorporated in the 4,4-dimethyl sterol fraction was shown to be associated with 24-methylenecycloartanol (IV).[10,11] Figure 1 shows the inhibitory effect of III on the enzymatic activity. From the observed curve it was possible to calculate a I_{50} (inhibitor concentration required to reduce the reaction velocity by half) value of 0.05 μM. Under the assay conditions used in this study where the concentration of the substrate was close to its K_M value (100 μM), I_{50} values are of the order of the inhibition constants[12] and the value found for III indicates that CMT has a much higher affinity for the HEI analogue than for its substrate II. For an assessment of the inhibition specificity, the inhibitory power of III has been compared with that of 25-azacycloartanol (V) and 20,25-diazacholesterol (VI).[10,11] Results showed that III was more efficient than V to inhibit the CMT, while VI was the least potent compound. Thus III appeared to be the best among the inhibitors tested, and this

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