

SYNTHETIC APPROACH TOWARD THE DEVELOPMENT OF NEW β -LACTAMASE INHIBITORS

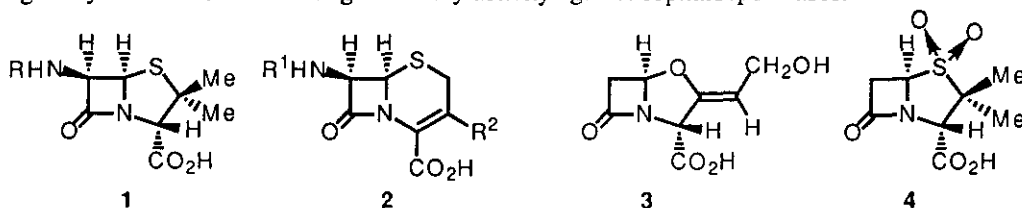
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Abstract- New racemic thienamonobactams (**21**) and (**28**), designed on the basis of hybridization between thienamycin (**5**) and aztreonam (**6**), were successfully synthesized by exploiting the [2+2] cycloaddition reaction of diketene with imine (**7**). Compound (**28**) exhibited significant inhibitory activity ($ID_{50} = 37 \mu M$) against *Citrobacter freundii* cephalosporinase.

The antibiotics bearing the β -lactam moiety such as penicillins (**1**) and cephalosporins (**2**) have been clinically using for long time because of their broad spectrum and excellent potency. However, the appearance of resistance due to the β -lactamases produced by a particular strain of pathogenic bacterias, is one of the serious problems in the chemotherapy.^{1, 2} Some β -lactamase (penicillinase) inhibitors, clavulanic acid (**3**) and sulbactam (**4**), are clinically exploited in combination with the penicillin derivatives although they do not exhibit strong inhibitory activity against cephalosporinases.^{1, 2}



We attempted to develop different new β -lactamase inhibitors from the earlier ones (**3**) and (**4**) in the following manner.

It is well known that thienamycin (**5**)^{1, 3} and aztreonam (**6**)⁴ exhibit strong antibacterial activities and excellent stability against β -lactamases. The characteristic moiety of the β -lactam antibiotics (**5**) and (**6**) seems to be hydroxyethyl or *N*-sulfonate, respectively. Based on the hybridization between both characteristic moieties involving the azetidinone skeleton, we envisaged a new type of β -lactamase inhibitor "thienamonobactam" as illustrated in Figure 1.⁵ Thus, new hybrid compounds, the thienamonobactams

[†]This paper is dedicated to the memory of the late Professor Shun-ichi Yamada, Professor Emeritus of Tokyo University.

were efficiently synthesized as follows.^{5,6}

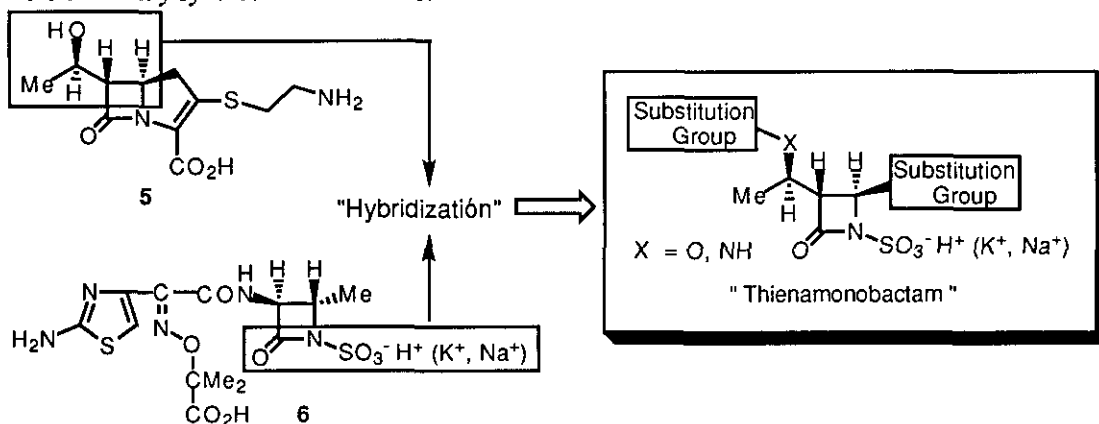
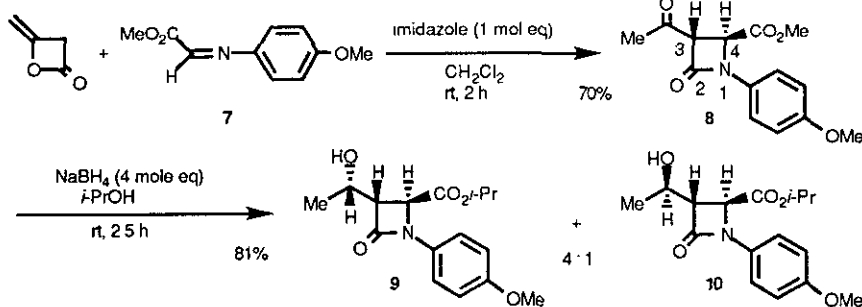


Figure 1. Molecular design for new β -lactamase inhibitors

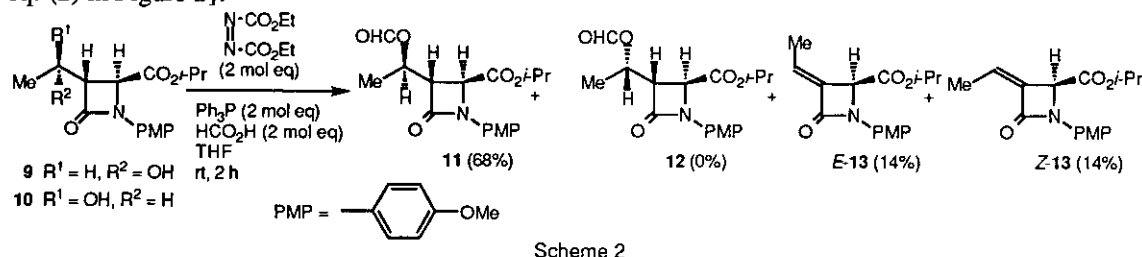
Stereoselective construction of the starting azetidinone compound (**8**) (mp 103-105 °C, CH_2Cl_2 -*n*-hexane)⁷ was carried out by utilizing the Sunagawa [2+2] cycloaddition reaction⁸ of diketene with imine (**7**) in the presence of imidazole. The stereochemistry of **8** was assigned by its ^1H NMR analysis [90 MHz, CDCl_3 ; δ 4.46 (d, 1H, $J = 3$ Hz, C3-H) and δ 5.02 (d, 1H, $J = 3$ Hz, C4-H)]. Reduction of **8** with NaBH_4 ⁹ in *i*-PrOH gave a mixture of epimeric alcohols (**9**) and (**10**) in a ratio of 4 : 1 and in 81% yield (Scheme 1).



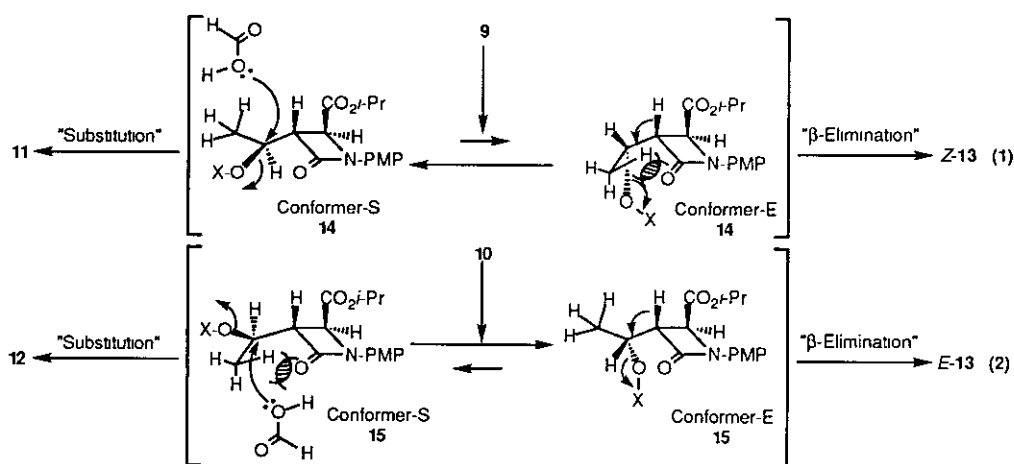
Scheme 1

Through the reduction of **8** in *i*-PrOH, C4-CO₂Me was converted to C4-CO₂*i*-Pr in the products. The mixture of **9** and **10** was submitted to the Mitsunobu reaction¹⁰ with formic acid in THF to obtain the desired formic ester (**11**) as the major product. The reaction furnished the S_N2 reaction product (**11**, 68%) together with β -elimination products, *E*-olefin (**13**, 14%)¹¹ and *Z*-olefin (**13**, 14%)¹¹ as shown in Scheme 2. These products formation can be rationalized in a stereoelectronic manner as depicted in Figure 2. There are mainly possible conformer-*E* and -*S* in the Mitsunobu reaction intermediates (**14**) and (**15**) derived from the corresponding compounds (**9**) and (**10**), respectively. Conformer-*S* and its analogs in the compounds (**14**) and (**15**) must be essential for the substitution reaction with formic acid. On the other hand, the β -elimination reaction should proceed *via* conformer-*E* with the antiperiplanar relation between C3-H and OX group. Conformer-*E* (**14**) and -*S* (**15**) bearing steric repulsion between the XO-ethyl and the lactam carbonyl, are less stable than each corresponding another conformer. In general, the β -elimination reaction with satisfactory stereoelectronic requirement seems to be much easier than the S_N2 reaction with unsatisfactory one. Thus, the compound (**11**) could be obtained from **9** *via*

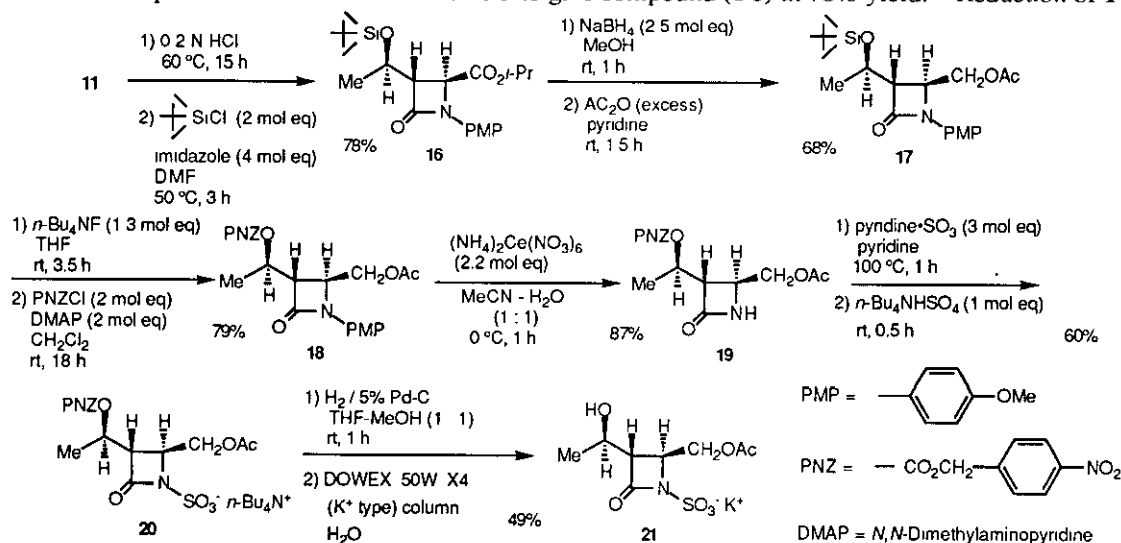
more stable conformer-S (**14**) than the conformer-E (**14**) leading to the formation of the β -elimination product (**Z-13**) [See eq. (1) in Figure 2]. **E-13** could be exclusively derived from **10** via more stable conformer-E (**15**) than the conformer-S (**15**) leading to the formation of the substitution product (**12**) [See eq. (2) in Figure 2].



Scheme 2

Figure 2. Possible reaction mechanisms toward substitution or β -elimination.

Hydrolysis of the formic ester (**11**) and then silylation of the resultant alcohol were carried out by the conventional procedure as shown in Scheme 3 to give compound (**16**) in 78% yield. Reduction of **16**

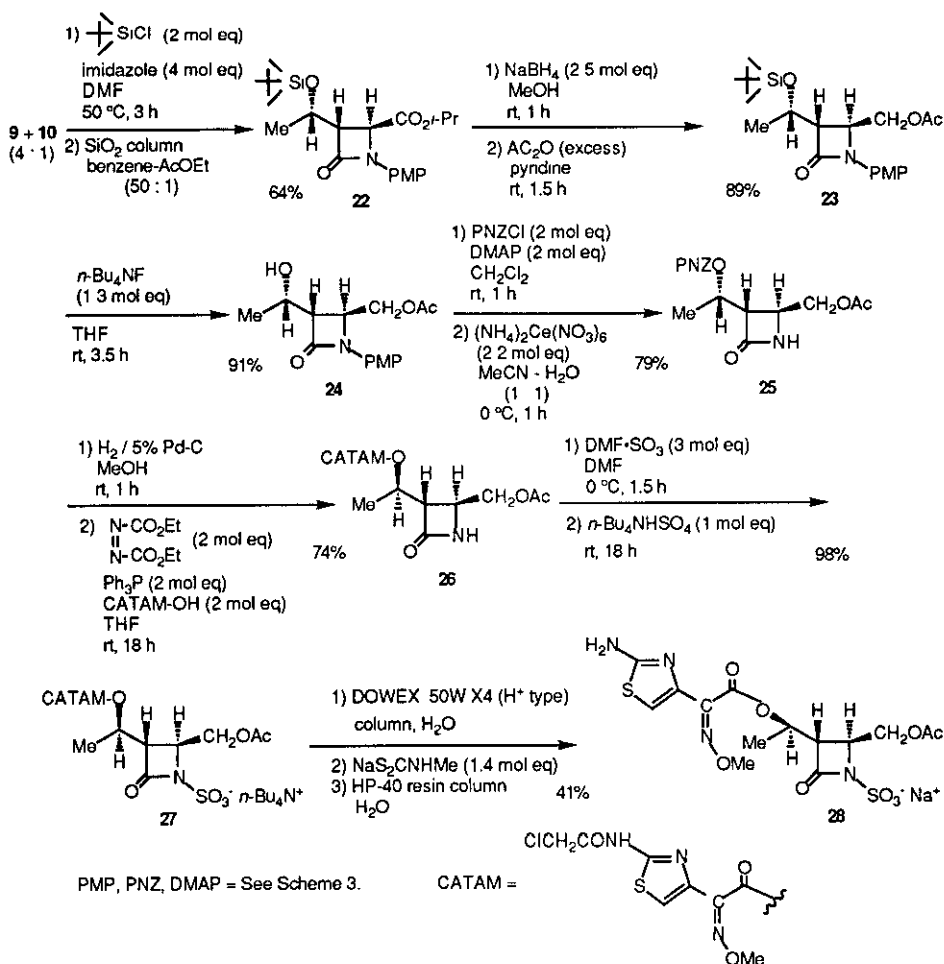


Scheme 3

with NaBH_4 followed by acetylation of the primary alcohol afforded acetate (**17**) in 68% yield. Treatment

of **17** with *n*-Bu₄NF and then with *p*-nitrobenzyloxycarbonyl (PNZ) chloride in the presence of dimethylaminopyridine (DMAP) gave compound (**18**). Oxidative removal of *p*-methoxyphenyl (PMP) group of **18** using ammonium cerium (IV) nitrate¹² furnished azetidinone (**19**) in 87% yield. Sulfonation of the lactam N atom of **19** with sulfur trioxide-pyridine complex and tetrabutylammonium hydrogen sulfate in pyridine gave compound (**20**) in 60% yield. After removal of PNZ group of **20** by hydrogenolysis, the resultant product was purified on a DOWEX 50W X4 (K⁺ type) column with water to afford the desired product (**21**)⁷ as amorphous powder in 49% yield.

Silylation of the mixture of **9** and **10** with *tert*-butyldimethylsilyl chloride followed by purification of the crude products on a silica gel column (benzene-AcOEt = 50 : 1) gave pure compound (**22**) in 64% yield. After reduction of **22** with NaBH₄ in MeOH, the resultant alcohol was treated with acetic anhydride in pyridine to give acetate (**23**) in 89% yield. Desilylation of **23** with *n*-Bu₄NF afforded alcohol (**24**, 91%). *p*-Nitrobenzyloxycarbonylation of **24** followed by oxidative removal of PMP group of the resultant product was done by same procedure as described above to give **25** in 79% yield.



Scheme 4

After hydrogenolysis of PNZ group of **25**, the resultant alcohol was submitted to the Mitsunobu reaction with CATAM-OH (Scheme 4) to furnish compound (**26**) in 74% yield. Treatment of **26** with sulfur

trioxide-DMF complex and then with $n\text{-Bu}_4\text{NHSO}_4$ gave **27** in 98% yield. Exchange of the counter cation species ($n\text{-Bu}_4\text{N}^+ \rightarrow \text{H}^+$) in the compound (**27**), dechloroacetylation of the CATAM moiety with sodium dithiocarbamate, and purification of the crude product on a HP-40 resin column were successively performed to afford the desired thienamonobactam (**28**)⁷ as colorless amorphous powder in 41% yield as shown in Scheme 4.

Inhibitory activities (ID_{50} value) of new thienamonobactams (**21**), (**28**), and sulbactam (**4**) against *Citrobacter freundii* cephalosporinase were shown to be 1080 μM , 37 μM , and 16 μM , respectively.^{13,14} Because the test sample (**28**) is racemic, the optically pure form may exhibit same inhibitory activity as that of chiral sulbactam (**4**).

In conclusion, we could disclose a new type of lead compound ("thienamonobactam") (**28**) toward the β -lactamase inhibitors based on the hybridization concept.

ACKNOWLEDGMENT

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7. Physical data of the selected compounds. Compound (**8**) : colorless needles. mp 103-105 $^{\circ}\text{C}$ (CH_2Cl_2 - n -hexane). IR ν_{max} (CHCl_3) cm^{-1} : 1750, 1715. ^1H NMR (90 MHz, CDCl_3) δ : 2.41 (s, 3H), 3.80 (s, 3H), 3.82 (s, 3H), 4.46 (d, 1H, $J = 3.0$ Hz), 5.02 (d, 1H, $J = 3.0$ Hz), 6.95 (d, 2H, $J = 9.0$ Hz), 7.33 (d, 2H, $J = 9.0$ Hz). Anal. Calcd for $\text{C}_{14}\text{H}_{15}\text{NO}_5$: C, 60.64; H, 5.45; N, 5.05. Found: C, 60.87; H, 5.46; N, 5.21. Compound (**21**) : amorphous powder. IR ν_{max} (KBr) cm^{-1} : 1750, 1230, 1050. ^1H NMR (90 MHz, D_2O) δ : 1.29 (d, 3H, $J = 6.0$ Hz), 2.12 (s, 3H), 3.15 (dd, 1H, $J = 6.0$ Hz, 3.0 Hz), 4.12-4.60 (m, 4H). Compound (**28**) : amorphous powder. IR ν_{max} (KBr) cm^{-1} : 1730, 1260, 1050. ^1H NMR (90 MHz, D_2O) δ : 1.47 (d, 3H, $J = 6.0$ Hz), 2.08 (s, 3H), 3.56-3.65 (m, 1H), 3.98 (s, 3H), 4.30-4.41 (m, 1H), 4.43-4.50 (m, 2H), 5.49-5.76 (m, 1H), 6.91 (s, 1H).
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 11. The stereochemistry (*E* or *Z*) of the compound (**13**) was assigned on the basis of the chemical shift values ("deshielding effect of the lactam carbonyl") of allylic methyl protons or vinyl proton on its ^1H NMR (90 MHz, CDCl_3) chart as follows. Compound (*E*-**13**): δ 1.87 (d, 3H, $J = 7.0$ Hz) and 6.37 (m, 1H). Compound (*Z*-**13**): δ 2.13 (d, 3H, $J = 7.0$ Hz) and 5.90 (m, 1H).
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 13. Cf. A. Yamaguchi, T. Hirata, and T. Sawai, *Antimicrob. Agents Chemother.*, 1982, **24**, 23.
 14. Compound (**28**) did not exhibit any significant antibacterial activity against *S. aureus* and *E. coli* (MIC > 50 $\mu\text{g/mL}$).

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