SYNTHETIC APPROACH TOWARD THE DEVELOPMENT OF NEW β-LACTAMASE INHIBITORS

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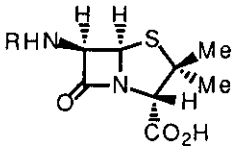
Abstract- New racemic thienamonobactams (**21**) and (**28**), designed on the basis of hybridization between thienamycin (**5**) and aztreonam (**6**), were successfully synthesized by exploiting the [2+2] cycloaddition reaction of diketene with imine (**7**). Compound (**28**) exhibited significant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



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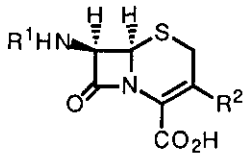

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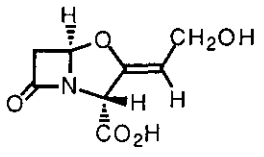

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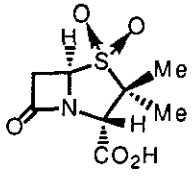

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We attempted to develop different new β-lactamase inhibitors from the earlier ones (**3**) and (**4**) in the following manner.

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]

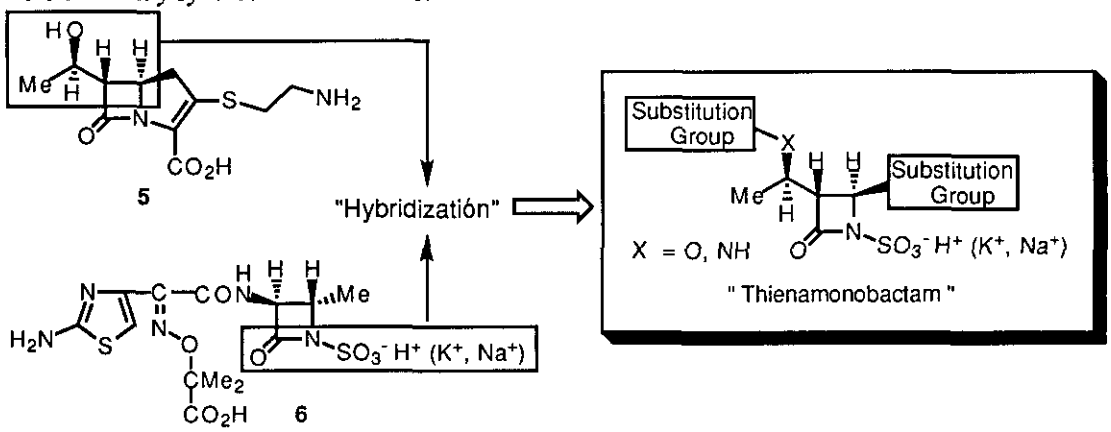
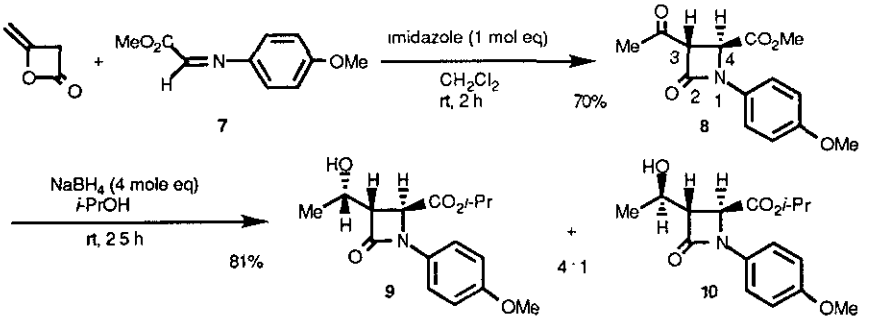


Figure 1. Molecular design for new β-lactamase inhibitors

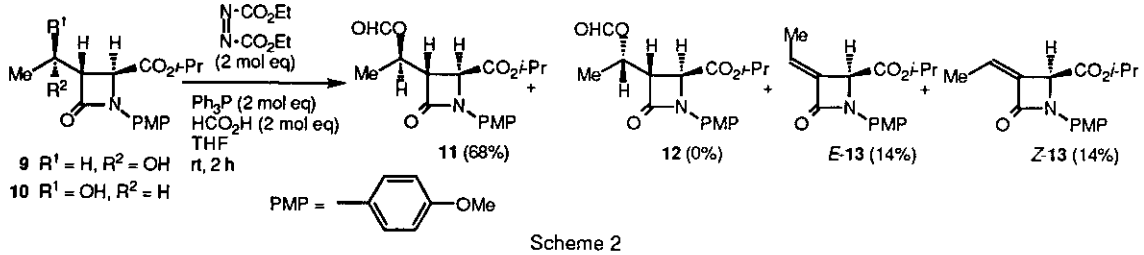
Stereoselective construction of the starting azetidinone compound (**8**) (mp 103-105 °C, CH_2Cl_2-*n*-hexane)[7] was carried out by utilizing the Sunagawa [2+2] cycloaddition reaction[8] of diketene with imine (**7**) in the presence of imidazole. The stereochemistry of **8** was assigned by its ^{1}H 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-CO_2Me was converted to C4-CO_2*i*-Pr in the products. The mixture of **9** and **10** was submitted to the Mitsunobu reaction[10] with formic acid in THF to obtain the desired formic ester (**11**) as the major product. The reaction furnished the S_N 2 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_N 2 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

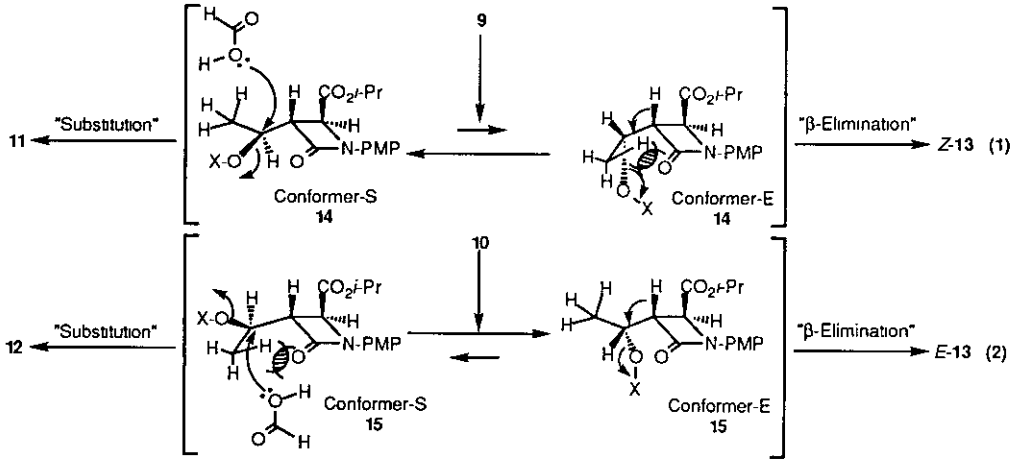
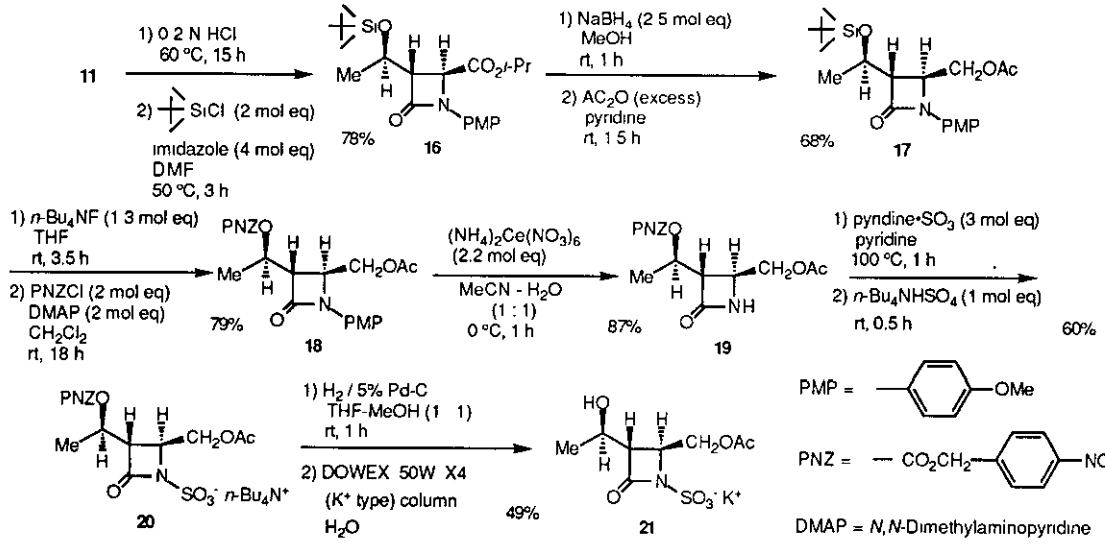


Figure 2. Possible reaction mechanisms toward substitution or β-elimination.

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**

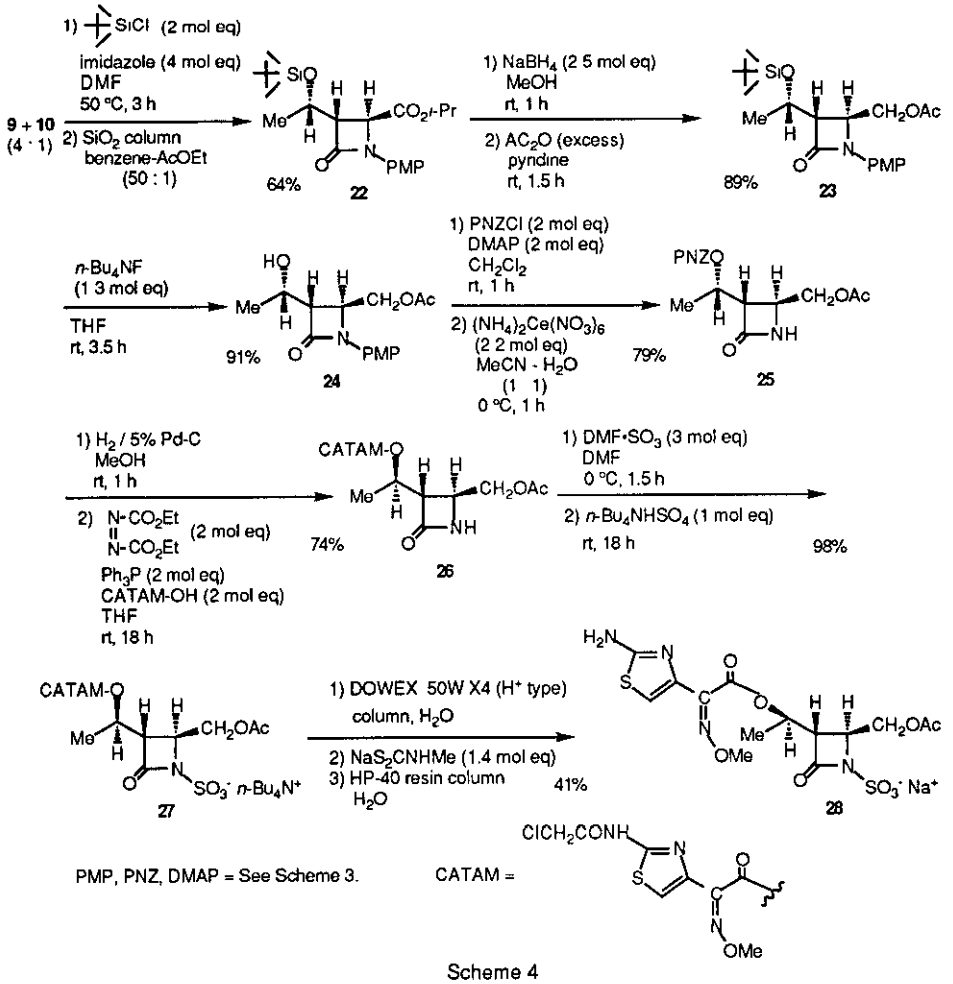


Scheme 3

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

of **17** with *n*-Bu_4NF 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) nitrate[12] 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 $NaBH_4$ in MeOH, the resultant alcohol was treated with acetic anhydride in pyridine to give acetate (**23**) in 89% yield. Desilylation of **23** with *n*-Bu_4NF 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-Bu_4NHSO_4 gave **27** in 98% yield. Exchange of the counter cation species (n-$Bu_4N^+ \rightarrow H^+$) 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 ℃ (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 $C_{14}H_{15}NO_5$: 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 ^{1}H 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 μg/mL).

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