TOTAL SYNTHESIS OF BICYCLOMYCIN

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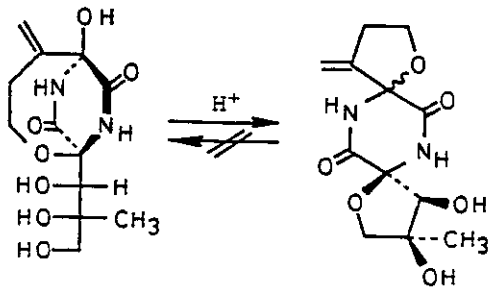
Abstract —— Total synthesis of (±)-bicyclomycin was achieved in 19 steps from diketopiperazine by applying mainly the following two newly developed reactions: (1) regiospecific cyclization of the terminal hydroxy group of the side-chain on the diketopiperazine ring into the bicyclo[4.2.2] ring system; and (2) stereospecific aldol condensation of the aldehyde and the carbanion on the bridge-head position of the bicyclo[4.2.2] ring; two chiral centers in the side chain being controlled by double stereodifferentiation with mutual kinetic resolution to afford preferentially the product having the stereochemistry same to natural bicyclomycin.

Bicyclomycin is an antibiotic found in Japan independently by two groups: Imanaka group[1] (Fujisawa Pharmaceutical Co. Ltd.) from Streptomyces sapporonensis and Ogasawara group[2] (Niigata University) from S. aizunensis. It has a unique spectrum of antibacterial activities.[3] It shows activity against Gram-negative bacteria only, including Escherichia coli, Klebsiella and Salmonella, and no cross resistance with streptomycin, kanamycin, chloramphenicol, tetracycline, aminobenzyl penicillin and nalidixic acid. No relation was noted to any groups of the known antibiotics. Its toxicity is very low (more than 2 g/kg mouse by intravenous injection). Its primary action is due to interference with the biosynthesis of lipoprotein and its assembly to peptidoglycan in the cell envelope of E. coli.[4]

Structure of bicyclomycin (1) has been elucidated by chemical[5] and X-ray[6] analyses, and absolute configuration by X-ray diffraction method.[7] It has a novel bicyclo[4.2.2] system containing oxidized diketopiperazine ring and a side chain at its bridge-head position. A variety of functional groups and asymmetric centers in such a small molecule would make its stereocontrolled synthesis very difficult. Because the diketopiperazine part consists of two α-oxidized amino acids equivalent to the hemiketal of α-keto acids, number of reactions such as ring-opening and reclosing to a new ring with a different hydroxyl group would be possible. Indeed, bicyclomycin (1)

is very susceptible toward acids and bases, and even in neutral aqueous solution it decomposes under reflux for 10 min.[8] Two reactons we have found, that lead to new ring systems are given in Figure 1. Acid treatment (0.1N perchloric acid at 100 °C for 15 min) of bicyclomycin (1) afforded a stereoisomeric mixture of the bis-spiro compounds 2, which could not be converted to the original bicyclomycin.[9] Thus, bicyclomycin is not the thermodynamically most stable form among its isomeric compounds. Although many chemical modifications[10a] and synthetic approaches[10b] toward bicyclomycin (1) had been studied recently, no total synthesis of bicyclomycin had been reported. This paper reviews our total synthesis of (±)-bicyclomycin (1).



Bicyclomycin (**1**) **2**

STEREOSPECIFIC ALKYLATION OF THE OXIDIZED DIKETOPIPERAZINES[11]

Bicyclomycin contains a hydroxy group at 3 position and an alkoxy group at 6 position. Although syntheses of such oxidized diketopiperazines have been widely studied[12] good methods applicable to our case are scarce. We have developed a stereospecific alkylation of the dimethoxydiketopiperazine 4 to produce the 3-alkyl or acyl derivative 5.

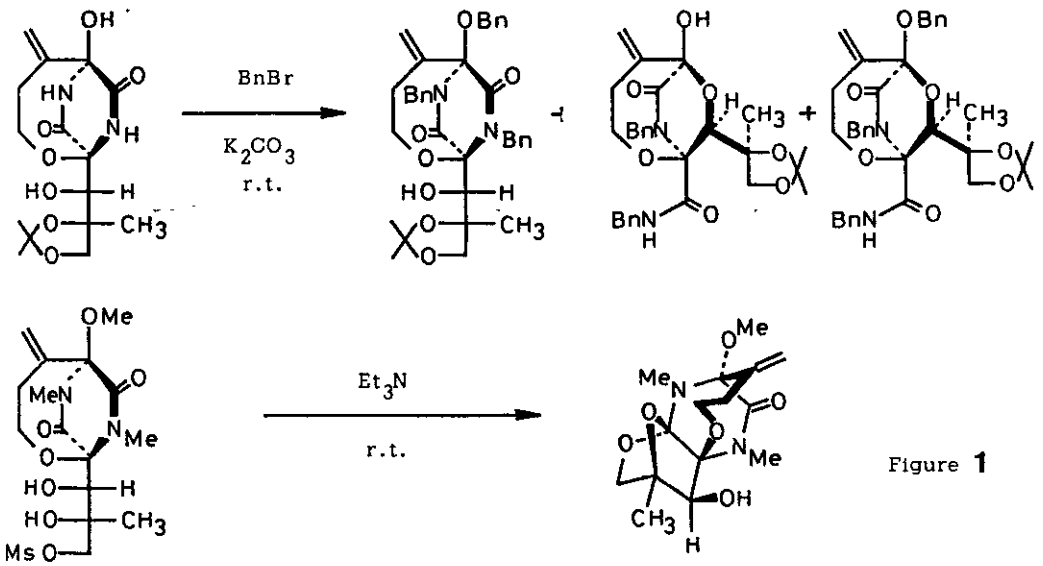
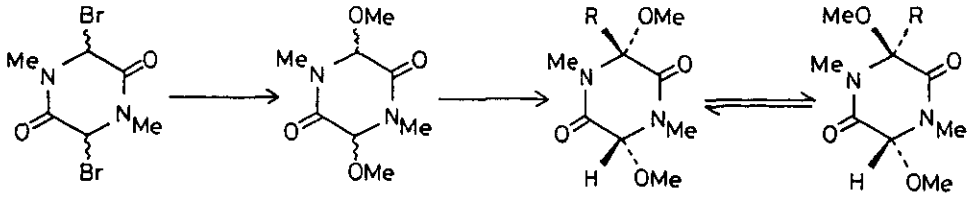


Figure **1**

Starting material 4a was obtained from the dibromide 3 prepared from N,N'-dimethyldiketopiperazine with bromine,[13] by treatment with methanol in the presence of triethylamine. Chromatography on silica gel gave 3:1 mixture of two diastereomers. The minor product 4b was crystallized out from the mixture by dissolving it in ether-hexane (1:1). The remaining major product 4a (oil; purity over 95%) was employed for the alkylation.



3 **4** **5** **6**

The minor compound 4b gave an equilibrium mixture of 4a and 4b (3:2) by treatment with camphorsulfonic acid in methanol under reflux. Stereochemistry of these compounds has not yet been clarified. Crystalline 4b was prevented from use for alkylation reaction, for its solubility in tetrahydrofuran is two low to use it, although it was considered to give the same anion to that from 4a. Alkylation of 4a was carried out in tetrahydrofuran with 1.2 eq of n-BuLi at -78 °C, followed by addition of 1.2 eq of alkyl halide. Acylation could also be carried out in a similar manner by using acyl halide instead of alkyl halide. The yields and PMR spectral data are listed in Table 1.

TABLE 1

Compd.	5a	5b	5c	5d	5e	5f	6d	6f
R	CH_3	$CH_2CO_2CH_3$	$COCH_3$	COC_6H_5	$CH_2CH{=}CH_2$	$CH_2C_6H_5$	COC_6H_5	$CH_2C_6H_5$
Yield (%)	63	66	68	72	65	63	–	–
PMR(H-6)	4.76	4.88	4.82	4.92	4.68	3.66	4.95	4.64

Stereochemistry of 5 was determined as follows: Treatment of the benzyl derivative 5f with camphorsulfonic acid in methanol under reflux gave an equilibrium mixture of 5f and its isomer 6f in a ratio of about 1:1. They were easily separated on silica gel tlc. The methine signal of the benzyl derivative 5f appeared in an abnormally high field (3.66 ppm),[14] while the corresponding signal of its isomer 6f appeared in a normal position (4.64 ppm). Only in the isomer in which the methine proton and the benzyl group in the same side, such an anisotropy effect can be expected as shown in Fig. 2. This stereospecificity[15] seems to be controlled by the steric hindrance of the methoxy group at 6 position. The methine signal of both of the benzoyl derivatives (5d and

6d) appeared in normal position because 5d exists as the conformer in which the carbonyl and the benzene ring are in the same plane. Compounds 5a-e also gave a mixture of 5a-e and corresponding 6a-e (about 1:1 ratio), respectively, by acid treatment. Thus, the kinetic alkylation and acylation products 5a-f must have the configuration in which two methoxy groups are in the same side.

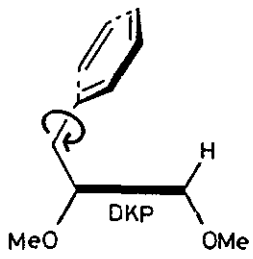
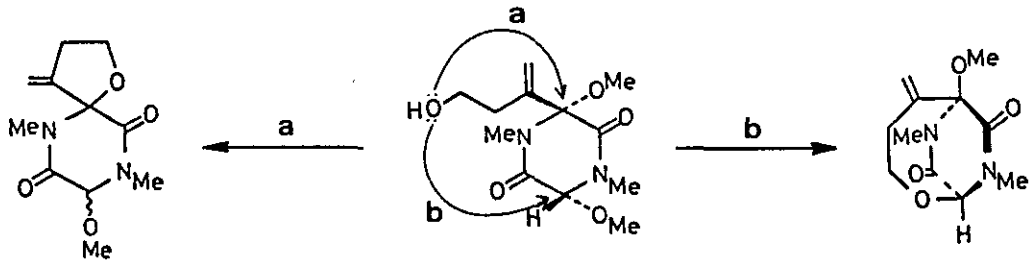


Fig. **2**

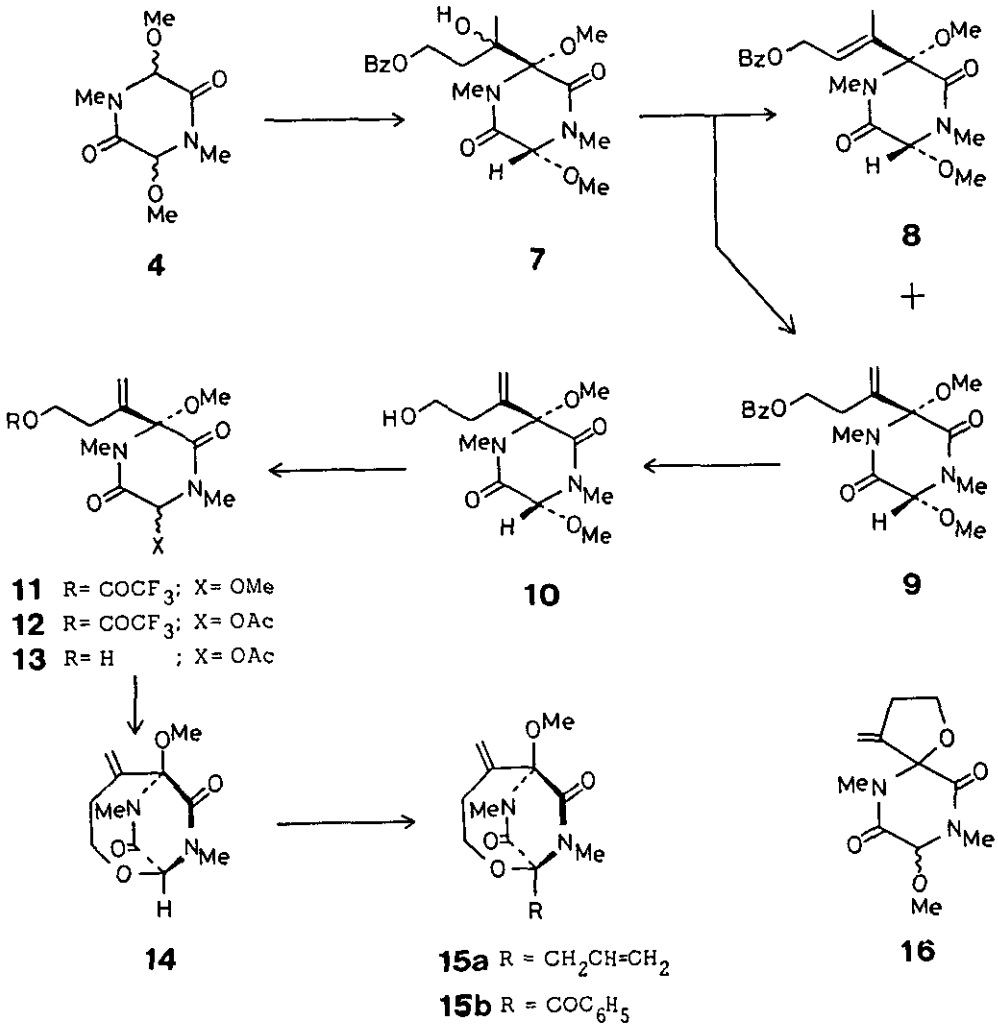
SYNTHESIS OF THE BICYCLO[4.2.2] SYSTEM FOUND IN BICYCLOMYCIN[16]

In the total synthesis of bicyclomycin (1), the key step would be the cyclization to form the bicyclo[4.2.2] ring. How can we differentiate cyclization to the bicyclo compound instead to the spiro compound such as 2, since the latter must be the thermodynamically more stable form and easy to produce ? If we could make any differentiation between the two methoxy groups, and of the cyclization proceeds kinetically, exclusive formation of the bicyclo ring might be possible.



We have succeeded in the selective activation of the methoxy group at 6 position. Thus, the stereocontrolled condensation[11] of 3,6-dimethoxydiketopiperazine derivative 4 (oily isomer) with $PhCO_2(CH_2)_2COCH_3$ in the presence of 1.2 eq of n-BuLi in THF at -78 °C gave in 68% yield the monoalkylated cis compound 7, which is a 1:1 mixture of side chain stereoisomers. Treatment of 7 with thionyl chloride and pyridine at room temp gave the endo-olefin 8 (33% yield) and the exo-olefin 9 (40% yield). Hydrolysis of the exo-olefin 9 with 1N NaOH in methanol at room temp afforded the alcohol 10 (95% yield), which was employed for the following cyclization.

The selective activation of the methoxy group at 6 position was carried out as follows: the alcohol 10 was treated with trifluoroacetic anhydride at room temp to give crude trifluoroacetate 11. It was heated in acetic anhydride and trifluoroacetic acid (1:1) at 40 °C for 2 h to give the monoacetoxy compound 12. Introduction of the acetoxy group at 6 position was confirmed by ca 1.6 ppm down-field shift of the PMR signal of the proton at 6 position of 12 in comparison with that of 11.



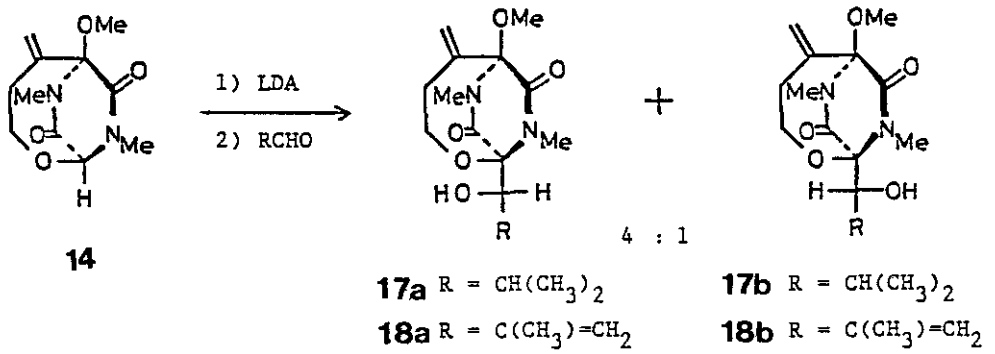
Removal of trifluoroacetyl group was carried out by treatment of 12 with 20% aqueous Na_2HPO_4–dioxane (1:1) to give a mixture of stereoisomers 13 (55% from 9; ratio of the isomers 3:2).

Cyclization of 13 in dichloroethane in the presence of pyridinium tosylate afforded solely the desired compound 14 containing the bicyclo ring system (50% yield). Like bicyclomycin, the bicyclo compound 14 was isomerized by treatment with camphorsulfonic acid in methanol under reflux to the spiro compound 16 (over 80% yield), which was a 1:1 mixture of two stereoisomers.

The reverse reaction from 16 to 14 did not proceed at all, indicating that the spiro compound 16 is energetically favored over the bicyclo compound 14 as suggested by the Hoffmann-La Roche group.[9]

ALKYLATION AND ALDOL CONDENSATION AT THE BRIDGE-HEAD POSITION OF THE BICYCLO-[4.2.2] SYSTEM[16,17]

The next step was the alkylation at the bridge-head position of the bicyclo[4.2.2] compound 14 through its bridge-head carbanion. Formation of such a bridge-head carbanion had not been reported until our paper had appeared.[16] Shortly later, Williams[18] found accidentally the formation of the bridge-head carbanions. We had expected the formation of this carbanion from our experiences on the diketopiperazine field so far studied. In the coruse of the total synthesis of gliotoxin and sporidesmin, one of us, Kishi, and Fukuyama[19] carried out the alkylation on the bridge-head position of bicyclo[3.2.2] compounds containing diketopiperazine ring, but in this case the carbanion might be stabilized with a sulfur atom. The present case gave the expected

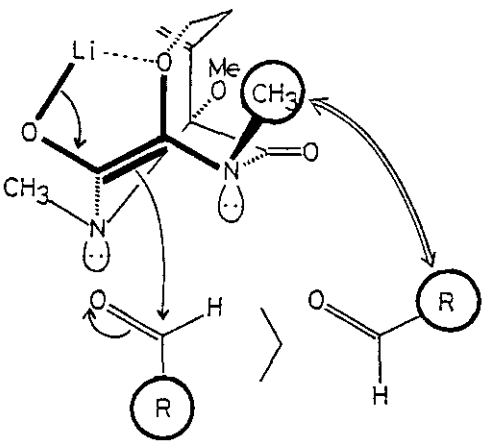


14

17a R = $CH(CH_3)_2$ **17b** R = $CH(CH_3)_2$

18a R = $C(CH_3)=CH_2$ **18b** R = $C(CH_3)=CH_2$

Figure **3**

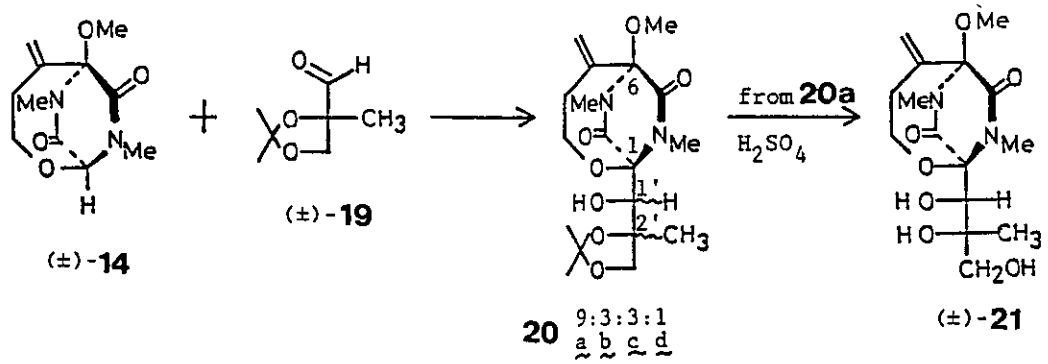


carbanion with lithium diisopropylamide (LDA). Thus, the addition of 1.2 eq of LDA at -78 °C to a THF solution of 14, followed by addition of allyl bromide, afforded the allyl derivative 15a. Similarly the benzoyl derivative 15b was obtained in good yield using benzoyl chloride instead of allyl bromide.

Stereoselectivity of the aldol condensation of bicyclo[4.2.2] compound 14 with aldehyde was studied by using isobutyraldehyde. Treatment of 14 with 1.2 eq LDA at -78 °C, followed by addition of isobutyraldehyde afforded a mixture of stereoisomers, 17a and 17b. In this reaction, stereochemistry concerning with the newly formed secondary alcohol was highly controlled (74% yield; ratio of 17a and 17b is 4:1). Inspection through the CPK model clearly indicates that the major product has the desired configuration as shown in 17a, because of the steric hindrance between the isobutyl group and the N-methyl group (Figure 3). The allyl alcohol 18a and 18b were also obtained in 60% yield using methacrolein instead of isobutyraldehyde. The ratio of the products 18a and 18b was also 4:1. These highly stereocontrolled aldol condensations prompted us to apply this reaction to the protected aldehyde 19.

SYNTHESIS OF (±)-N,N',O-TRIMETHYLBICYCLOMYCIN[17]

Aldol condensation of (±)-14 with the protected aldehyde (±)-19 smoothly proceeded to give a mixture of four kinds of stereoisomers 20a-d (46% yield), which were separable by silica gel TLC. If the stereoselectivity is assumed to be controlled only by the chirality of the diketopiperazine nucleus, the ratio of the isomers is estimated to be 4:4:1:1. But, the ratio of the



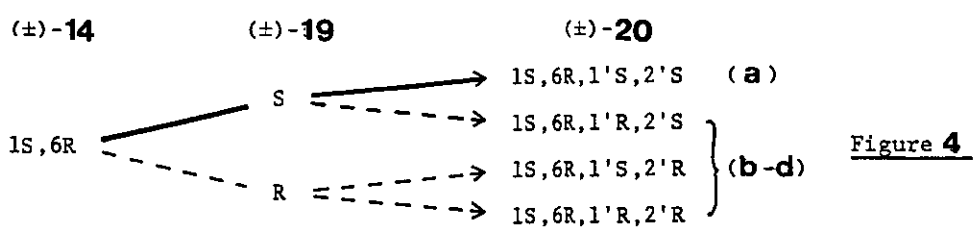


Figure 4

four products were 9:3:3:1. Surprisingly, in this reaction not only the chiral center of the newly formed secondary alcohol but also the combination of the chiral centers of 14 and 19 was controlled by each other (Figure 4). This is an example of "double stereodifferentiation with mutual kinetic resolution".[20] PMR, CMR and mass spectra as well as the Rf values on TLC of the major product 20a were completely identical with those of 20[21] derived from natural bicyclomycin (1). Hydrolysis of the acetonide group of 20a gave (±)-N,N',O-trimethylbicyclomycin 21 as reported in the literature.[21]

SYNTHESIS OF THE KEY INTERMEDIATE HAVING THE BICYCLO[4.2.2] RING SYSTEM OF THE TOTAL SYNTHESIS OF BICYCLOMYCIN[22]

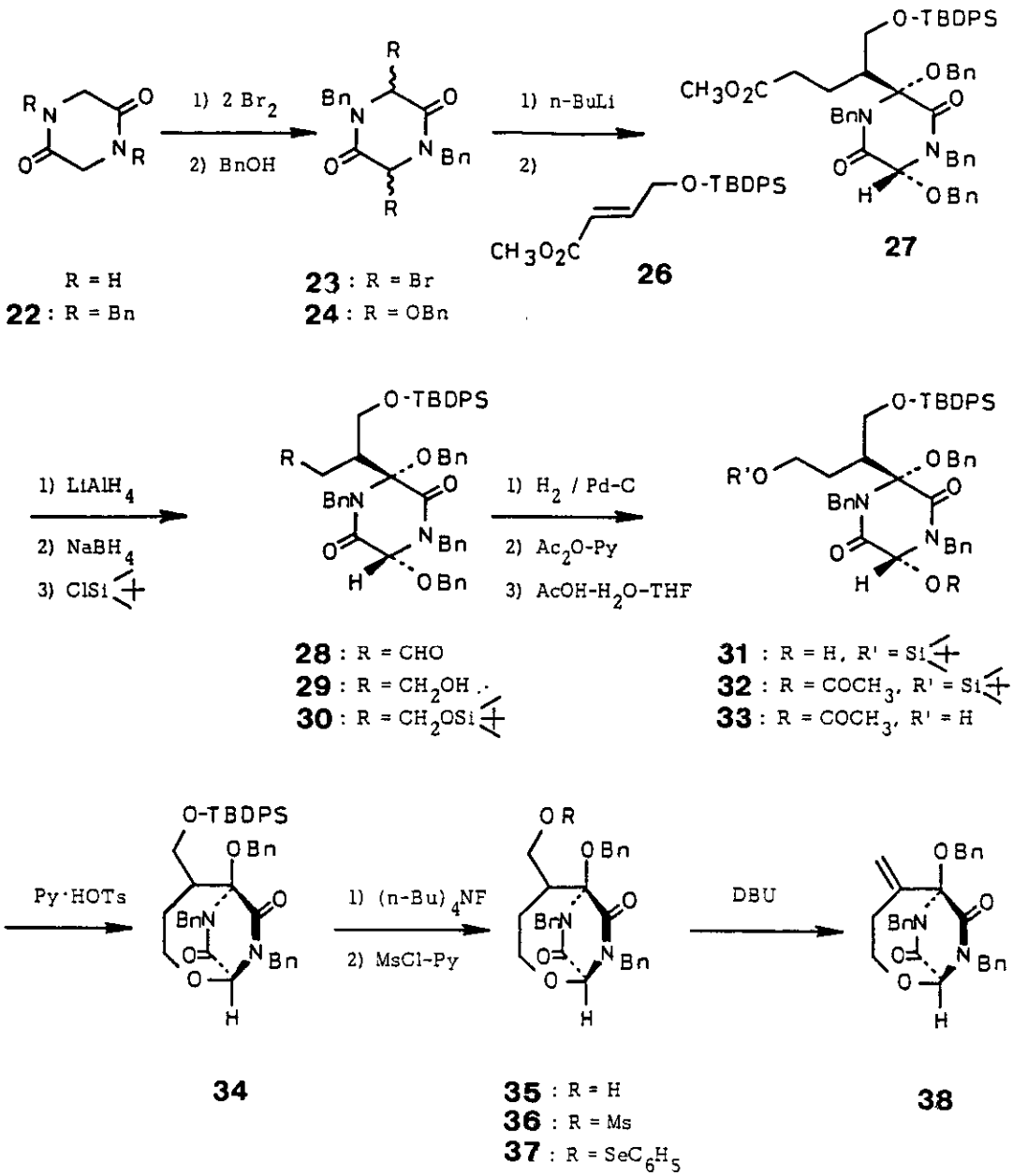
We have now challenged to the total synthesis of unprotected bicyclomycin (1). We have selected benzyl groups for N,N',O-protection of the bicyclo ring. p-Methoxybenzyl group may be a better protecting group than benzyl group for easy removal, but in this case it cannot be used, for it is susceptible toward bromination.

Diketopyperazine was protected by benzyl group to 22 and then brominated with bromine at an elevated temperature. On heating with benzyl alcohol at 80 °C the dibromide 23 afforded the dibenzyl ether 24 in 85% yield as a 3:1 mixture of cis and trans forms; they could be separated by fractional crystallization, but either isomer or the mixture could be used in the next step.

Methyl γ-hydroxycrotonate 25, the component for the side chain, was prepared from methyl crotonate by oxidation with selenium dioxide followed by reduction with $NaBH_4$. The hydroxy group was protected with diphenyl-t-butylsilyl group (TBDPS) by treatment with TBDPS chloride and imidazole in DMF at room temp to give methyl γ-TBDPS-oxycrotonate 26 (76% yield).

The monoanion prepared from the dibenzyl ether 24 and n-BuLi at -110 °C was treated with the crotonate 26 at -78 °C. This conjugate addition proceeded stereospecifically and gave only one condensed product 27. Stereochemistry of 27 on the diketopiperazine ring was as shown.[11] It was reduced with $LiAlH_4$ in THF at -78 °C afforded the crude aldehyde 28, which was further reduced with $NaBH_4$ in methanol followed by silica gel column chromatography to give alcohol 29. The free primary alcohol of 29 was protected with t-butyldimethylsilyl group (TBDMS) by treatment with TBDMS chloride and imidazole in DMF at room temp to 30 in almost quantitative yield.

In the synthesis of N,N',O-trimethylbicyclomycin the secondary methoxy group corresponding to the secondary benzyl group in 30 was displaced smoothly by acetoxy group by treatment with acetic anhydride and trifluoroacetic acid[16], but the secondary benzyl group in 30 could not be displaced by acetoxy group under the similar conditions. We had to, therefore, develop a new route. 30 was hydrogenated in ethanol in the presence of 20% Pd-charcoal and pyridine. Only



one benzyl group (secondary rather than tertiary) was selectively removed and the mono-debenzylated product 31 was crystallized from hexane (88% yield). Acetylation of the secondary alcohol of 31 with acetic anhydride and pyridine afforded the acetate 32. The TBDMS group was selectively removed by heating in acetic acid:water:tetrahydrofuran (1:1:2) at 80 °C for 2.5 h to give the mono-ol 33 (80% yield), which was heated in dichloroethane at 80 °C in the presence of pyridinium tosylate, affording the bicyclo compound 34 (84% yield).

The TBDPS group of the bicyclo compound 34 was removed with 1M n-Bu_4NF to give the free alcohol 35 (93% yield). To a solution of the alcohol 35 in dichloromethane was added pyridine and mesyl chloride to give the mesylate 36 (87% yield), which in xylene was treated with DBU at 120 °C to yield the olefin 38 (67% yield).

TOTAL SYNTHESIS OF BICYCLOMYCIN[22]

In the preceding section we synthesized the key intermediate 34 having the bicyclo[4.2.2] system. Aldol condensation of the bicyclo compound 34 and the aldehyde 19 would give four stereoisomers. Stereoselectivity of this condensation has been extensively studied in the previous section using the bicyclo compound 14 and the aldehyde 19. We have found[17] that the major isomer was formed in more than 50% of the four stereoisomers and that it had the same relative stereochemistry of that of bicyclomycin (1).

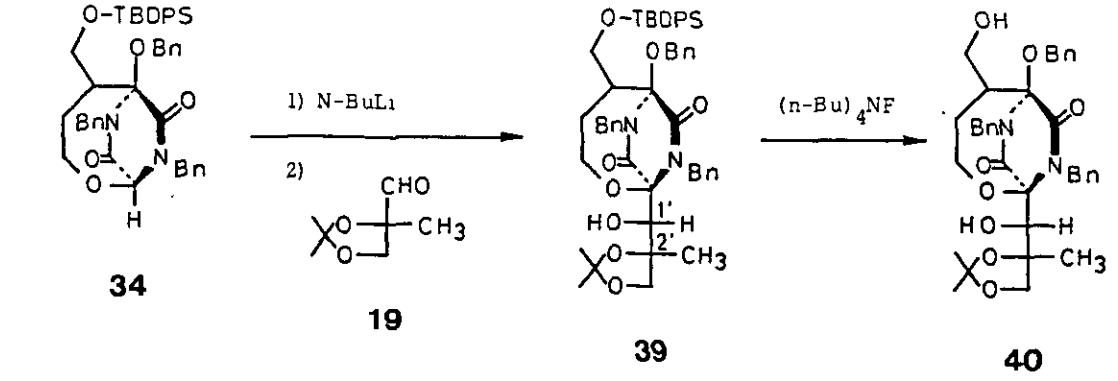
The bicyclo compound 34 in THF was converted to its monoanion with n-BuLi at -110 °C and condensed with (±)-2-methylglyceraldehyde acetonide (19) at -78 °C to give a mixture of four stereoisomers in a 3:1:1:~0 ratio, from which the major product 39 was isolated in 41% yield. The yields of the other stereoisomers were: B 13%, C 12%, and D detected but not isolated. For desilylation this major isomer 39 was treated with 1M n-Bu_4NF in THF at room temp for 1 h to give the primary alcohol 40 in almost quantitatively.

All of the benzyl protecting groups of 40 were removed by catalytic hydrogenation in ethanol under H_2 atmosphere in the presence of 20% Pd-charcoal at 80 °C for 12 h to yield the completely debenzylated product 41 (57% yield). Mesylation of 41 in pyridine with mesyl chloride at room temp afforded the monomesylate 42 (83% yield).

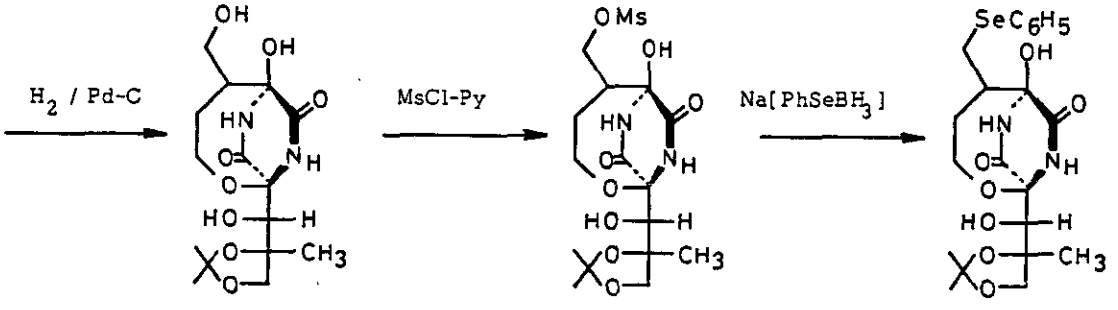
Contrary to the benzyl-protected mesylate, the unprotected mesylate 42 could not be converted to the olefin 45 by treatment with DBU in xylene at 130 °C, for the olefin 45 did not survive under the reaction condition. Unprotected bicyclomycin system is much less stable than protected one such as N,N',O-trimethyl and -tribenzyl derivatives.

The mesylate 42 was then dissolved in absolute ethanol under nitrogen atmosphere and treated at room temp with 0.5M Na[$PhSeBH_3$] solution prepared from $NaBH_4$ and PhSeSePh in ethanol. The selenide 43 was isolated in 40% yield. To a solution of the selenide 43 in dichloromethane was added m-chloroperbenzoic acid in dichloromethane and the reaction mixture was poured on a silica gel column. Elution with methanol-dichloromethane (1:9) gave the selenoxide 44, which was dissolved in dichloroethane and the solution was heated at 60 °C for 20 min to give bicyclomycin acetonide 45 (69% yield).

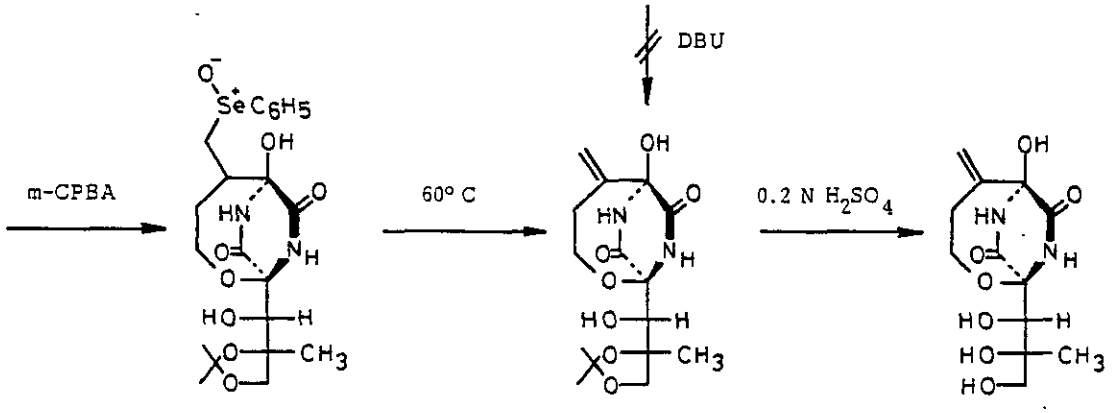
The acetonide 45 was hydrolyzed by careful treatment with two equivalents of 0.2N sulfuric acid initially at 0 °C and then at 25 °C for 8 h. Purification using a silica gel column and an ODS



34 **19** **39** **40**



41 **42** **43**



44 **45** Bicyclomycin (**1**)

RP-18 column, and crystallization from methanol-acetone afforded (±)-bicyclomycin (1) (66% yield), whose PMR and CMR spectra as well as Rf values on tlc were completely identical with those of natural bicyclomycin.[23]

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23. After thin-layer chromatography using Merck silica gel 60 PF_{254}, bicyclomycin (natural and synthetic) showed quite different PMR spectra in DMSO-d_6 from the original one. Treatment of DMSO-d_6 solution of bicyclomycin with a minute amount of $Ba(OH)_2$ or $Sr(OH)_2$ also caused same phenomenon. Facilitation of proton exchange by inorganic impurities would have caused it.