THE TOTAL SYNTHESIS OF NOCARDICIN A

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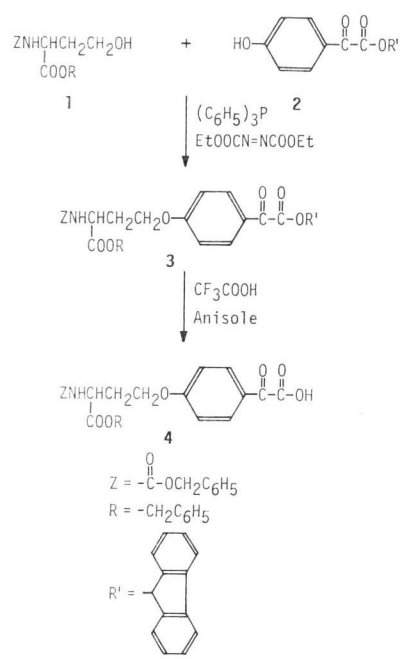
We report here a total synthesis of nocardicin A. The antibiotic was obtained as a mixture of diastereoisomers that possessed approximately one quarter of the antibacterial activity of the naturally occurring material.

Recently a new β-lactam antibiotic, nocardicin A, has been reported.[1,2] This is the first example of a monocyclic β-lactam derivative having antimicrobial activity. We report here the synthesis of this antibiotic. Two syntheses of nocardicin A have thus far been reported.[3,4] The synthesis of a hydroxymethyl analog has also recently appeared.[5]

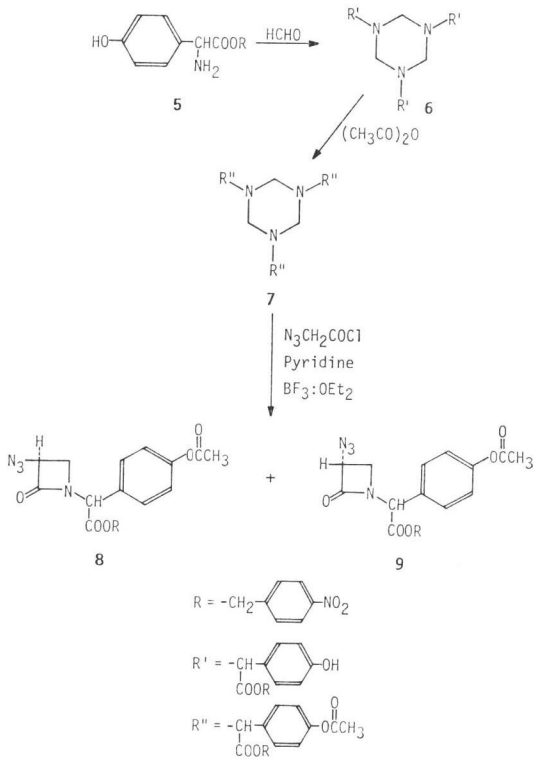
A. Synthetic Strategy

The synthesis of nocardicin A can be visualized as a three part problem involving 1) synthesis of the side chain acid containing homoserine linked at the *para* position to the oxime derivative of phenyl-

Scheme 1.



Scheme 2. Synthesis of the β-lactam moiety.



glyoxylic acid, 2) synthesis of the β-lactam moiety containing the D-*p*-hydroxyphenylglycine and 3) coupling of the two suitably protected parts of the molecule followed by removal of the protecting groups to give the desired nocardicin A.

B. Synthesis of the Side Chain Acid (Scheme 1)

Carbobenzoxy-(±)-homoserine benzyl ester **1**[6] was coupled with the fluorenyl ester of *p*-hydroxyphenylglyoxylic acid **2**[7] using diethyl azodicarboxylate and triphenylphosphine to afford compound **3** which was readily converted to the side chain acid **4** by reaction with trifluoroacetic acid and anisole.

C. Synthesis of the β-Lactam Moiety (Scheme 2)

The starting material for this synthesis was the commercially available Dane salt of D-*p*-hydroxyphenylglycine which was converted to the *p*-nitrobenzyl ester by treatment with *p*-nitrobenzyl bromide in dimethylformamide. Acid hydrolysis to the aminoester **5** followed by treatment with formaldehyde afforded the triazine **6**. This compound was acetylated to give the fully protected derivative **7** which was converted to a diastereomeric mixture of the β-lactams **8** and **9** by reacting with boron trifluoride etherate, pyridine, and azidoacetyl chloride.[8] This mixture which was composed of a 2: 1 ratio (**8** and **9**) in favor of the desired compound, as shown by proton magnetic resonance spectroscopy, was purified by high pressure liquid chromatography and converted to the corresponding amino compounds (**10**) by treating with hydrogen sulfide and triethylamine (see Scheme 3).

A similar route to some phthalimidonocardicinic acid compounds has been reported by KAMIYA *et al.*[9]

Scheme 3. Synthesis of nocardicin A.

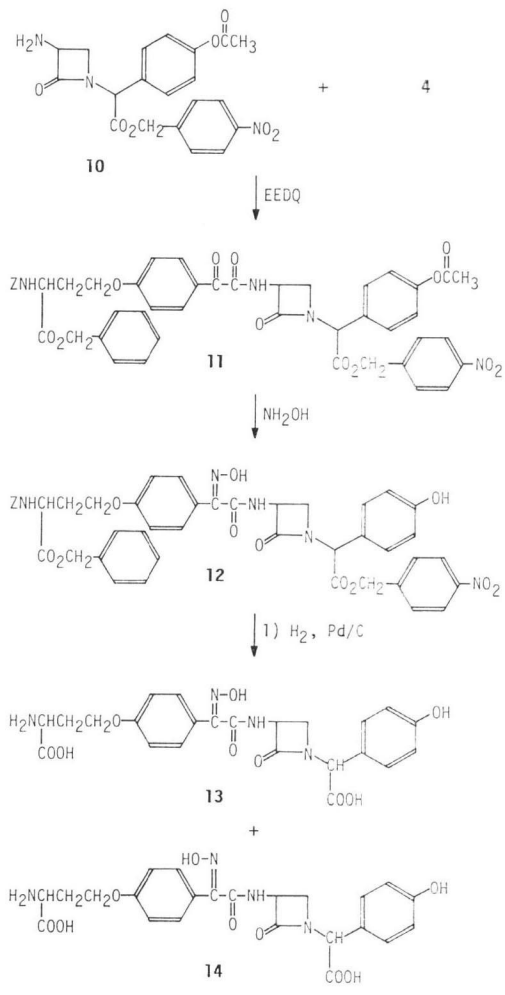


Table 1. *In vitro* activity of nocardicin A, natural and synthetic.

Culture	Strain designation	MIC (mcg/ml)	
		Nocardicin A	Synthetic sample
Pseudomonas aeruginosa	12–4–6	64	256
P. aeruginosa	UCS 76–18	16	64
Serratia marcescens	F–35	64	64
Prot. mirabilis	OSY 75–1	4	16
Prot. mirabilis	#4671	4	16
Prot. mirabilis	W–75–2	4	16
E. coli	ESS 22–31	0.5	2
Micrococcus luteus	PCI–1001	64	256

D. Synthesis of Nocardicin A (Scheme 3)

The protected side chain acid **4** was coupled with the 3-aminonocardicinic acid derivative **10** using EEDQ (ethyl 1,2-dihydro-2-ethoxy-1-quinolinecarboxylate) to give the desired product (**11**) which was purified by column chromatography. Treatment of compound **11** with hydroxylamine and pyridine at 60°C cleaved the acetate protecting group of the phenol moiety and gave the oxime **12**. Catalytic hydrogenation of compound **12** gave nocardicin A (**13**) as a mixture of diastereoisomers along with some nocardicin B (**14**) as shown by proton magnetic resonance spectroscopy.

The *in vitro* activity of the synthetic compound was approximately one quarter of that exhibited by the natural antibiotic as shown in Table 1.

Experimental

9'-Fluorenyl *p*-Hydroxyphenylglyoxylate (**2**)

A solution of diazofluorene (9.65 g) in 125 ml of ethyl acetate was added in several portions to *p*-hydroxyphenylglyoxylic acid[7] (8.3 g) in 75 ml of ethyl acetate and the solution was stored overnight at room temperature. The solution was extracted with dilute sodium bicarbonate, dried ($MgSO_4$) and concentrated to give 10.5 g of product: m.p. 172~174°C.

Anal. Calcd. for $C_{21}H_{14}O_4$: C 76.35, H 4.27.
Found: C 76.29, H 4.86.

1,2-Dibenzyl-4-(9-fluorenyl)-2-carboxyamino-4-(*p*-carboxycarbonylphenoxy)-(±)-butyrate (**3**)

A solution of compound **2** (0.991 g), carbobenzoxy-(±)-homoserine benzyl ester[6] (1.03 g), triphenylphosphine (0.787 g) and diethyl azodicarboxylate (0.522 g) in 20 ml of tetrahydrofuran was stirred at room temperature for 48 hours under nitrogen. The solution was evaporated to dryness, triturated with ether and filtered. The filtrate was evaporated to a syrup which was chromatographed on silica gel using chloroform to afford 1.26 g of product as a foam: NMR (DMSO-d_6) δ 4.18 (m, 3H, CH_2O+-CHO), 5.04 (s, 2H, ϕCH_2-), 5.16 (s, 2H, ϕCH_2-).

Anal. Calcd. for $C_{40}H_{33}NO_8$: C 73.27, H 5.07, N 2.14.
Found: C 73.04, H 5.30, N 2.21.

1,2-Dibenzyl-2-carboxyamino-4-(*p*-carboxycarbonylphenoxy)-(+)-butyrate (**4**)

A solution of 810 mg of fluorenyl ester (**3**) dissolved in anisole (1.82 ml) was cooled in an ice bath, and 8 ml of chilled trifluoroacetic acid was added with stirring. After 10 minutes in the cold, the acid was removed by high vacuum evaporation (25°C). Finally, pumping was continued for about-40 minutes while the flask was immersed in room temperature water. The residue was dissolved in 15 ml of ether and extracted with 10 ml of water containing 180 mg of sodium bicarbonate (chilled). Centrifugation was required to separate the phases. The aqueous layer was acidified to pH 2 in the cold and extracted with ether. The ether was dried and evaporated to 440 mg of a syrup; NMR (DMSO-d_6) δ 7~8 (m, 14H, ϕ), 5.02 (s, 2H, ϕCH_2-), 5.16 (s, 2H, ϕCH_2-).

Anal. Calcd. for $C_{27}H_{25}NO_8$: C 65.98, H 5.13, N 2.85.
Found: C 66.36, H 5.36, N 2.73.

N-(2-Methoxycarbonyl-1-methylvinyl)-D-4-hydroxyphenylglycine-*p*-nitrobenzyl Ester

A solution of 21.6 g (0.1 mole) of *p*-nitrobenzyl bromide in 75 ml dimethylformamide was added at room temperature in small portions over 40 minutes to a stirred solution of 28.7 g (0.1 mole) of *N*-(2-methoxycarbonyl-1-methylvinyl)-D(−)-4-hydroxyphenylglycine in 200 ml dimethylformamide. After stirring at room temperature overnight, the mixture was poured into 1.6 liter of ice water and extracted into 8×200 ml ethyl acetate. The ethyl acetate was washed with water and saturated sodium chloride. Evaporation gave a pale yellow oil which was crystallized from ethyl acetate - hexane to afford 21 g (52%) of a white, crystalline solid, m.p. 132~134°C: $[\alpha]_D^{23}$ +214±2° (*c* 0.553, CH_3OH): IR (KBr, cm^{-1}) 1742, 1655: NMR ($CDCl_3$+DMSO-d_6) δ 9.46 (d, 1H, NH), 9.12 (s, 1H, OH), 8.12 & 7.34 (dd, 4H, NO_2-*Ar*, *J*=10 Hz), 7.20 +6.84 (dd, 4H, HO*Ar*, *J*=11 Hz), 5.28 (m, 3H, OCH_2 +NCH), 4.57 (s, 1H, =CH), 3.66 (s, 3H, OCH_3), 1.83 (s, 3H, CCH_3).

Anal. Calcd. for $C_{20}H_{20}N_2O_7$: C 60.00, H 5.03, N 7.00.
Found: C 60.22, H 5.12, N 7.12.

D-4-Hydroxyphenylglycine *p*-Nitrobenzyl Ester (**5**)

N-(2-Methoxycarbonyl-1-methylvinyl)-D(−)-4-hydroxyphenylglycine *p*-nitrobenzyl ester (70 g) was dissolved in 300 ml acetone and 200 ml water. Over 40 minutes, 170 ml 1 N HCl was added dropwise, with stirring. Acetone was evaporated, and the aqueous residue was stirred with 100 ml ethyl acetate, chilled, and filtered. The filter cake was washed thoroughly with diethyl ether to give 42 g (81%) of a white solid. $[\alpha]_D^{23}$ −58°±1 (*c* 0.833, CH_3OH): IR (KBr, cm^{-1}) 1760, 1702: NMR (DMSO-d_6) δ 8.17 +7.48 (dd, 4H, NO_2 *Ar*, *J*=12 Hz), 7.22+6.73 (dd, HO*Ar*, *J*=10 Hz), 5.25 (s, 2H, OCH_2), 4.55 (s, 1H, NCH).

Anal. Calcd. for $C_{15}H_{14}N_2O_5$: C 59.60, H 4.67, N 9.27.
Found: C 59.24, H 4.74, N 9.02.

1,3,5-Tris-[D(−)-1-(4-nitrobenzyloxycarbonyl)-1-(4-hydroxyphenyl) methyl] perhydro-1,3,5-triazine (**6**)

D(−)-4-Hydroxyphenylglycine *p*-nitrobenzyl ester (**5**) (42 g, 0.14 mole) was slurried in 250 ml tetrahydrofuran and 17 ml (0.17 mole) 40% aqueous formaldehyde was added. The resulting solution was stirred overnight at room temperature, evaporated to 100 ml and 350 ml methylene chloride was added. After extraction with water and saturated sodium chloride, the solution was dried over magnesium sulfate and evaporated to give 44 g (100%) of a foam which dried as a glass. $[\alpha]_D^{23}$ −63° (*c* 0.757, CH_3OH): IR (KBr, cm^{-1}) 1750: NMR (DMSO-d_6) δ 9.49 (s, 3×1H, ArO*H*), 8.12 & 7.37 (dd, 4H, NO_2*Ar*, *J*=11 Hz), 7.12+6.65 (dd, 3×4H, HO*Ar*, *J*=11 Hz), 5.11 (s, 3×2H, OCH_2), 4.50 (s, 3×1H, NCH), 3.35 (s, 3×2H, CH_2N).

Anal. Calcd. for $C_{48}H_{42}N_6O_{15}$: C 61.14, H 4.49, N 8.91.
Found: C 60.77, H 5.02, N 7.95.

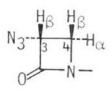
1,3,5-Tris-[D(−)-1-(4-nitrobenzyloxycarbonyl)-1-(4-acetoxyphenyl) methyl] perhydro-1,3,5-triazine (**7**)

1,3,5-Tris-[D(−)-1-(4-nitrobenzyloxycarbonyl)-1-(4-hydroxyphenyl) methyl] perhydro-1,3,5-triazine (**6**) (20.0 g) 60 ml acetic anhydride, and 150 ml pyridine were heated on a steam bath for 10 minutes. The solution was evaporated to an oil which was codistilled several times with toluene. The residue was washed with saturated sodium bicarbonate, water, and saturated sodium chloride, dried over magnesium sulfate, filtered through Magnesol, and evaporated to a pale yellow oil which was crystallized from ethyl acetate-hexane to give 10.2 g (45%) of white crystals, m.p. 103~105°C. $[\alpha]_D^{23}$ −33°±1 (*c* 0.78, CH_3OH): IR (KBr, cm^{-1}) 1750: NMR ($CDCl_3$) δ 8.12 & 7.36 (dd, 3×4H, NO_2*Ar*, *J*=11 Hz), 7.18 & 6.97 (dd, 3×4H, O*Ar*, *J*=12 Hz), 5.08 (s, 3×2H, OCH_2), 4.62 (s, 3×1H, NCH), 3.62 (s, 3×2H, CH_2N), 2.32 (s, 3×3H, CH_3C=O).

Anal. Calcd. for $C_{54}H_{48}N_6O_{18}$: C 60.67, H 4.53, N 7.86.
Found: C 60.38, H 4.35, N 7.80.

D(−)-2-(3-Azido-2-oxo-1-azetidinyl)-2-(4-acetoxyphenyl)acetic Acid 4-Nitrobenzyl Ester (**8**+**9**)

1,3,5-Tris-[D(−)-1-(4-nitrobenzyloxycarbonyl)-1-(4-acetoxyphenyl) methyl] perhydro-1,3,5-triazine [**10**] (1.95 g) was dissolved in 45 ml of dry methylene chloride and 0.55 ml of boron trifluoride etherate was added under strictly anhydrous conditions. After stirring at room temperature for 20 minutes, the mixture was cooled to −40°C and 0.55 g azidoacetyl chloride[8] in 5 ml methylene chloride was added under nitrogen over 25 minutes, followed by a solution of 0.76 ml pyridine in 5 ml methylene chloride over 25 minutes. An orange precipitate formed. After stirring for 60 minutes at −40°C and for 100 minutes at 0~5°C, the reaction mixture was washed with 1 N hydrochloric acid, aqueous sodium bicarbonate, water, and saturated sodium chloride. The organic layer was dried over magnesium sulfate and evaporated to give 2.3 g of a tan foam. Purification by Waters HPLC on silica gel (hexane-ethyl acetate, 2:1) afforded 0.7 g (29%) of a colorless oil. $[\alpha]_D^{23}$ −44° (*c* 0.28,



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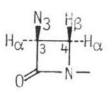

II

$CHCl_3$+CH_3OH): IR (neat, cm^{-1}) 2100, 1782, 1751: NMR ($CDCl_3$) δ 2.32 (3H, s, OC-CH_3), 3.04 ($4\beta_{II}$H, dd, J=2 and 5 Hz), 3.48 ($4\beta_I$, t, J=5 Hz), 3.62 ($4\alpha_I$, H, dd, J=2 and 5 Hz), 3.92 ($4\alpha_{II}$, H, t, J=5 Hz), 4.56 ($3\beta_I$ H, dd, J=2 and 5 Hz), 4.74 ($3\alpha_{II}$H, dd, J=2 and 5 Hz), 5.30 (2H, s, OCH_2–), 5.60 (1H, s, H–C–), 7.2 (6H, m, *Ar*), 8.18 (2H, d, J=8 Hz, *Ar*). Integration shows the mixture is composed of two parts of diastereomer II to one part of diastereomer I.

Anal. Calcd. for $C_{20}H_{17}N_5O_7$: C 54.67, H 3.90, N 15.94.
Found: C 54.68, H 4.21, N 15.99.

D-2-(3-Amino-2-oxo-1-azetidinyl)-2-(4-acetoxyphenyl)acetic Acid *p*-Nitrobenzyl Ester (**10**)

Hydrogen sulfide was bubbled into an ice cold solution of the azido β-lactams (**8** & **9**) (2 g) and triethylamine (0.64 ml, 4.0 ml) in 120 ml of methylene chloride for 15 minutes. The solution was stirred at room temperature for 1.5 hours then nitrogen was bubbled through the solution for 10 minutes. Evaporation at reduced pressure gave an oil which was redissolved in methylene chloride and again evaporated at reduced pressure. The latter procedure was repeated and the resulting oil was used directly in the next step.

3,3-Dibenzyl-*p*-nitrobenzyl 3-[2-[*p*-(3-Carboxy-3-carboxyaminopropoxy)phenyl]glyoxylamido]-α-(*p*-acetoxyphenyl)-2-oxo-1-azetidineacetic Acid (**11**)

A solution of the side chain acid (**4**) (2.0 g) the aminonocardicinic acid derivative described above and EEDQ (1.1 g) in 125 ml of methylene chloride was allowed to stand at room temperature overnight. The solution was extracted with cold 1 N hydrochloric acid, water, saturated sodium bicarbonate solution, water, and brine then dried over magnesium sulfate. The solvent was removed at reduced pressure and the resulting oil was chromatographed on 200 g of silica gel using methylene chloride 5% acetone to afford 3.2 g (90%) of a glass-like solid.

Anal. Calcd. for $C_{47}H_{42}N_4O_{14}$: C 63.65, H 4.77, N 6.32.
Found: C 63.11, H 4.51, N 6.28.

3,3-Dibenzyl-*p*-nitrobenzyl 3-[2-[*p*-(3-carboxy-3-carboxyaminopropoxy)phenyl]glyoxylamido]-α-(*p*-hydroxyphenyl)-2-oxo-1-acetidineacetic Acid, 2′ Oxime (**12**)

A solution of compound **11** and 1.0 g of hydroxylamine hydrochloride in 30 ml of ethanol and 30 ml of pyridine was heated at 60°C for 2.5 hours. The mixture was cooled and 100 ml of methylene chloride added. The resulting solution was extracted with two aliquots of 1 N hydrochloric acid, water, and brine then dried over magnesium sulfate. Removal of the solvent left a glass; yield 2.2 g (100%): The proton magnetic resonance spectrum of this compound was similar to compound **11** except the acetyl was no longer present.

Anal. Calcd. for $C_{45}H_{41}N_5O_{13}$: C 62.86, H 4.81, N 8.14.
Found: C 62.91, H 4.84, N 8.08.

Nocardicin A and B (**13** & **14**)

The protected nocardicin derivative **12** (1.1 g) was dissolved in a solution of 20 ml of methanol, 10 ml of tetrahydrofuran and 10 ml of water. Palladium on carbon (10%) (0.6 g) was added and the mixture was hydrogenated at atmospheric pressure for 2 hours. An additional 0.3 g of 10% palladium on carbon was added and hydrogenation was continued for another 0.5 hour. The catalyst was filtered and the filtrate evaporated to dryness and the resulting solid was dissolved in a solution of 10 ml of water and 5 ml of methanol by adding 1 N sodium hydroxide to pH 9. The solution was treated with decolorizing carbon, filtered and the filtrate was adjusted to pH 3.5 with 4 N HCl. The resulting solid was collected and dried to afford 0.25 g (39%). Proton magnetic resonance spectroscopy indicated that this material contained both nocardicin A and B with the former as the major product. Bioassay showed about 25% of the potency of an authentic sample of nocardicin A (see Table 1).

Anal. Calcd. for $C_{28}H_{24}N_4O_9 \cdot H_2O$: C 53.28, H 5.05, N 10.81.
Found: C 53.13, H 4.95, N 10.61.

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References

1) AOKI, H.; H. SAKAI, M. KOHSAKA, T. KONOMI, J. HOSODA, T. KUBOCHI, E. IGUCHI & H. IMANAKA: Nocardicin A, a new monocyclic β-lactam antibiotic. I. Discovery, isolation and characterization. J. Antibiotics 29: 492~500, 1976
2) HASHIMOTO, M.; T. KOMORI & T. KAMIYA: Nocardicin A, a new monocyclic β-lactam antibiotic. II. Structure determination of nocardicins A and B. J. Antibiotics 29: 890~901, 1976
3) KOPPEL, G. A.; L. MCSHANE, F. JOSE & R. D. G. COOPER: Total synthesis of nocardicin A. Synthesis of 3-ANA and nocardicin A. J. Amer. Chem. Soc. 100: 3933~3935, 1978
4) KAMIYA, T.; M. HASHIMOTO, O. NAKAGUCHI & T. OKU: Total synthesis of monocyclic β-lactam antibiotics, nocardicin A & D. Tetrahedron 35: 323~328, 1979
5) HAKIMELAKI, G. H. & G. JUST: β-Lactams. V. The synthesis of D,L-4-hydroxymethylnocardicin A, D,L-hydroxymethyl-*N*-phenylacetylnocardicinic acid and their α-epimers. Can. J. Chem. 57: 1932~1938, 1979
6) CHANG, C.D. & J. K. COWARD: Analogues of 5-adenosylhomocysteine as potential inhibitors of biological transmethylation. Synthesis of analogues with modifications at the 5′-thioether linkage. J. Med. Chem. 19: 684~691, 1976
7) BARNISH, J. T.; P. E. CROSS, J. C. DANILEWICZ, R. P. DICKINSON & D. G. STAPHER: Promotion of carbohydrate oxidation in the heart by some phenylglyoxylic acids. J. Med. Chem. 24: 399~404, 1981
8) BOSE, A. K.; B. ANJANEYULU, S. K. BHATTACHARYA & M. S. MANHAS: Studies on lactams. V. 3-Azido-2-azetidinones. Tetrahedron 23: 4769~4776, 1967
9) KAMIYA, T.; T. OKU, O. NAKAGUCHI, H. TAKENO & M. HASHIMOTO: A novel synthesis of nocardicins and their analogues. Tetrahed. Lett. 1978: 5119~5122 1978