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TOTAL SYNTHESIS OF MONOCYCLIC β-LACTAM ANTIBIOTICS, NOCARDICIN A AND D

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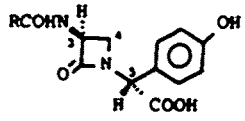
Abstract—The total synthesis of monocyclic β-lactam antibiotics, nocardicins A (1a) and D (1d), is described. 3-Aminonocardicinic acid (3-ANA, 2) was synthesized from *p*-hydroxyphenylglycine via an acid chloride-imine cycloaddition reaction. The side chain amino acid 13 was prepared via a key step of condensation of *p*-hydroxyacetophenone and α-phthalimidobutyrolactone. Acylation of 3-ANA with 13 gave nocardicin D, from which nocardicin A was obtained by oximation.

In the last decade β-lactam antibiotics have received considerable attention because of their applications.[1] The present investigations are concerned with the synthesis of various β-lactam compounds by chemical modification of the natural products or by a totally synthetic approach.[2] A variety of monocyclic β-lactams have also been synthesized from a view point of structure-activity.[3] Recently, we have found that nocardicin A (1a), a monocyclic β-lactam antibiotic isolated from a *Nocardia* species as a major product, shows relatively high activity especially against gram negative bacteria including *Pseudomonas* and *Proteus*.[4] Less abundant in the same cultures is the nocardicin family of compounds, *B*(1b), C(1c), D(1d), E(1e), F(1f) and G(1g), all rather simply varied acyl derivatives of 3-aminonocardicinic acid (3-ANA, 2) which constitutes the basic nucleus of this class of antibiotics.[5]

In the preceding paper we described the structure determination of these antibiotics on the basis of spectral and chemical evidence.[1a] We now report the total synthesis of nocardicin A. This was carried out for the final confirmation of its structure and the establishment of a synthetic sequence for analogous compounds. A minor antibiotic, nocardicin D, is an intermediate in this synthetic sequence and thus this work also constitutes the total synthesis of nocardicin D.

In approaching the synthetic problems presented by nocardicin A (1a), we first aimed at the synthesis of 3-ANA (2) in a rather straightforward manner. Up to

*This seems to be due to differences in shielding effects of the phenyl and ester groups to the 4β- and 4α-protons.[1a]



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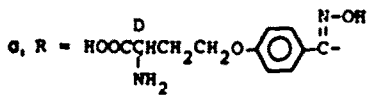
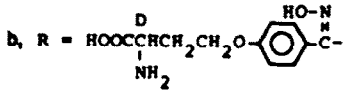
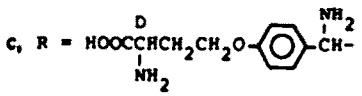


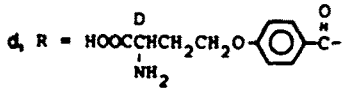
a, R = HOOCCHCH$_2$CH$_2$O– (D; NH$_2$) – C(=N–OH)–



b, R = HOOCCHCH$_2$CH$_2$O– (D; NH$_2$) – C(=N–OH)–



c, R = HOOCCHCH$_2$CH$_2$O– (D; NH$_2$) – CH(NH$_2$)–

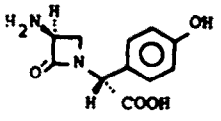


d, R = HOOCCHCH$_2$CH$_2$O– (D; NH$_2$) – C(=O)–

e, R = HO– C(=N–OH)–

f, R = HO– C(=N–OH)–

g, R = HO– CH(NH$_2$)– (D)



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Scheme 1.

date numerous methods have been devised for the preparation of the natural β-lactams and related compounds. One such route, the cycloaddition reaction of acid chlorides and imines, has proven a versatile method for the synthesis of monocyclic and bicyclic β-lactams.[6]

One important feature of the 3-ANA molecule simplifies introduction of the proper configuration at C-3 with respect to C-5. With such goal in mind we examined the acid chloride-imine method starting with *p*-hydroxyphenylglycine (3). For synthesis of 3-ANA, 3 was first converted to methylthioimine 6 as follows. Treatment of 3 with benzyl bromide in the presence of the copper ion[7] gave the *p*-benzyloxy derivative 4, which was successively converted to methyl ester 5 by treatment with MeOH–$SOCl_2$. This ester 5 was then transformed to methylthioimine 6 by thioformylation[8] and subsequent alkylation.

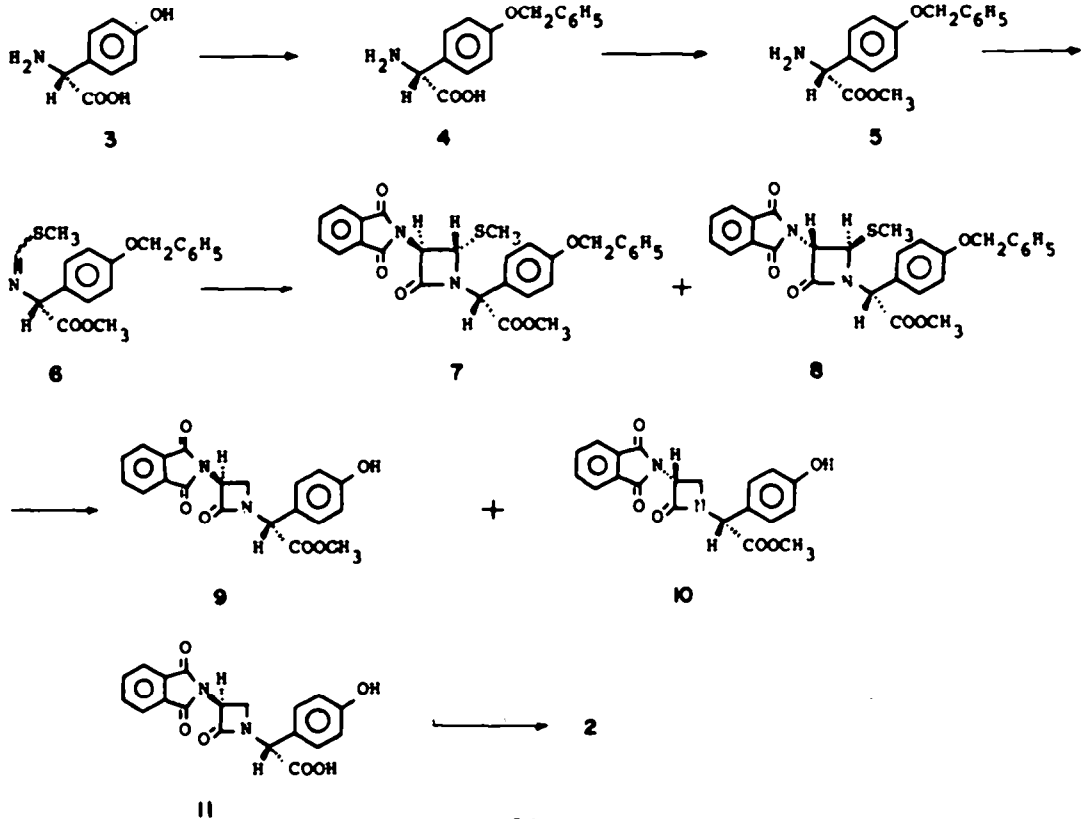
The β-lactam synthesis with this methylthioimine 6 was carried out according to a known procedure.[9] Thus, 6 was treated with phthalimidoacetyl chloride to give a 3:2 diastereomeric mixture of β-lactams 7 and 8 in 64% yield. The stereochemistry of 7 and 8 was initially assigned by inspection of their NMR spectra. The 4-H signal of the major product 7 appears as a doublet (J = 2.5 Hz, trans coupling to 3-H) at δ 4.71, whereas the minor component 8 has this corresponding doublet at δ 5.30 (J = 2.5 Hz). In view of the fact that in nocardicin series the 4β-protons always resonate at higher field than the 4α-protons,[a] the major and minor compounds were assigned as structure 7 and 8, respectively. The signal for the Me proton of the methylthio group of 7 (δ 2.14) is contrariwise at lower field than that of 8 (δ 1.87).

Since the isolation of the desired product 7 at this stage was found to be rather difficult, the mixture was directly subjected to the following reactions. Thus, the mixture was desulfrizated by treatement with Raney nickel and subsequently debenzylated by reduction with Pd-C to give a mixture of diastereomers 9 and 10 in a ratio reflecting that of the starting mixture in 37% overall yield. From this mixture the desired product 9 was separated by recrystallization.

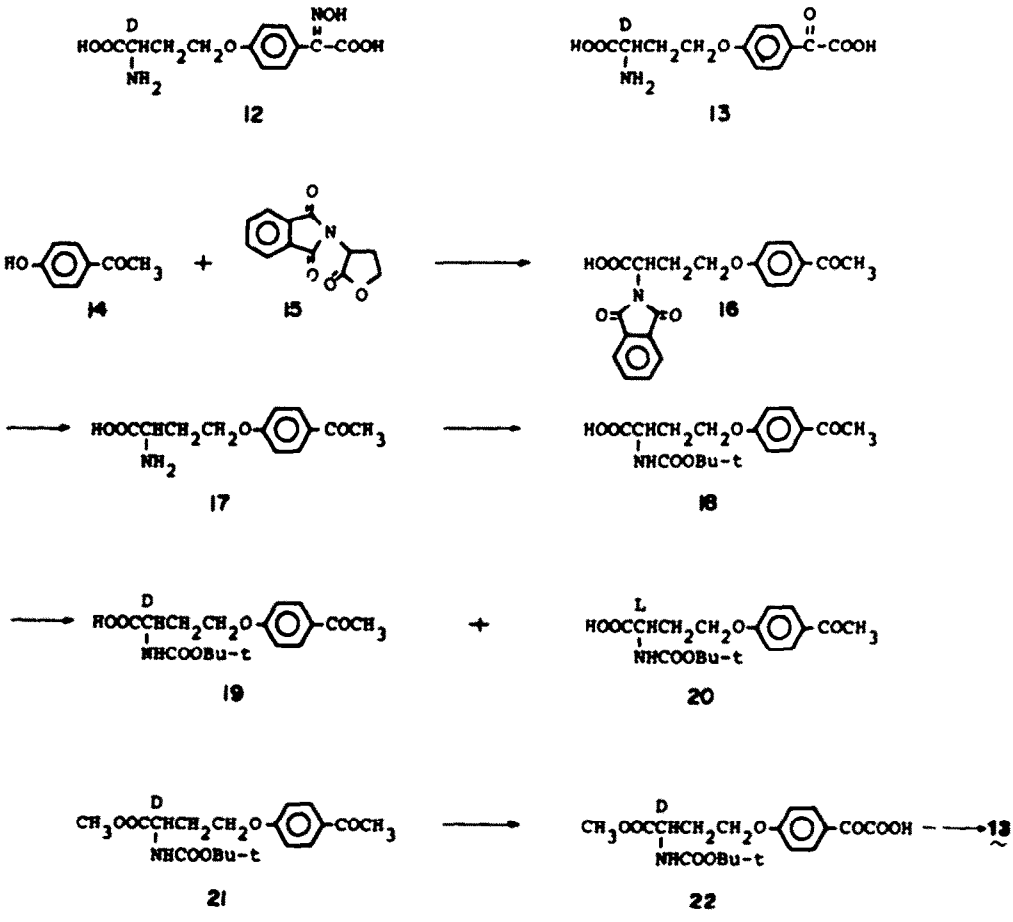
Attempted hydrolysis of 9 by treatment with one equivalent of sodium hydroxide caused epimerization of the methoxycarbonyl group of 9, resulting in a diastereomeric mixture of the corresponding acids. Having failed with the alkaline hydrolysis, we opted for the more weakly basic condition of treating 9 with lithium iodide in pyridine.[10] The reaction proceeded smoothly at reflux temperature and the desired acid 11 was thereby obtained in 80% yield. The NMR spectrum of 11 shows the 4β-proton at δ 3.47 as a double doublet (J = 2 and 5 Hz) and the 4α-proton at δ 3.86 as a triplet (J = 5 Hz) in agreement with the data of nocardicins,[1a] and this compound 11 was identified with the product obtained by phthaloylation of 3-ANA which could be derived from nocardicins by a modified Edman method.[b]

Some dephthaloylation reagents (hydrazine, hydroxylamine, ethylamine,[11] dimethylaminopropylamine and sodium sulfide-methylhydrazine[12]) were tried in an attempt to hydrolyze the phthaloyl protecting group of 11. The general result of these efforts was either a complex mixture of products or decomposition of the β-lactam ring. The most modest yield (60%) of the desired 3-ANA (2) was obtained by treatment with

[b]To be published.



Scheme 2.



Scheme 3.

dimethylaminopropylamine.[c] This synthetic sample was identical with 3-ANA derived from the natural nocardicins in m.p., optical rotation, IR and NMR data.

Having succeeded in the synthesis of the optically active 3-ANA, we next turned our attention to the preparation of the side-chain amino acid 12 in its optically active form. Since nocardicin A (1a) is the oxime derivative of nocardicin D (1d), we considered that the former can be derived from the latter by oximation and, accordingly, focused our attention to the dibasic keto amino acid 13 as our initial synthetic objective.

One important synthetic operation we had in order to construct the framework of this acid 13 was the condensation of *p*-hydroxyglyoxylic acid and D-homoserine. The most attractive route seemed to be the condensation reaction of a properly *para* substituted phenol with a protected α-aminobutyrolactone. Thus, we examined the reaction of the sodium salt of *p*-hydroxyacetophenone (14) with α-phthalimidobutyrolactone (15) and, after several attempts, secured 85% yield of the condensation product 16.

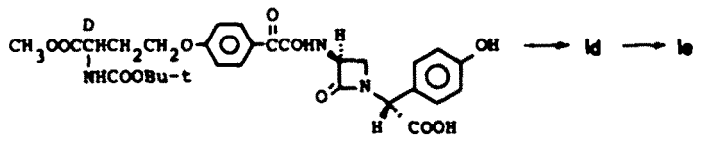
With the assurance afforded by this favorable finding, the synthetic problem was inevitably reduced to the resolution of the racemic product and the oxidation of the Me group of its acetyl function to the carboxyl group. After conversion of the phthaloyl protective group to the more easily removable t-butoxycarbonyl group by hydrolysis, followed by treatment with 2-t-butoxycarbonyloxyimino-2-phenylacetonitrile (BOC-ON)[13] in 67% overall yield, the resulting acid 18 was resolved into *d*-acid 19 ($[\alpha]_D$ + 8.1°) and *l*-acid 20 ($[\alpha]_D$ − 8.1°) by using cinchonidine (43% and 51% yield, respectively).

The identification of the stereostructures, *d*-acid 19 was converted to *d*-keto acid 13 by a four-step process: methylation of 17 gave methyl ester 21 in 92% yield; oxidation of 21 yielded the protected keto acid 22 in quantitative yield; hydrolysis of 22 with 0.15 N NaOH and subsequent 1 N HCl hydrolysis gave keto acid 13. This product was shown to be identical with the natural sample, derived from nocardicin A on hydrolysis with 6 N hydrochloric acid,[1a] by m.p., optical rotation and IR spectral comparison, being established to be D.

After completing the synthesis of the optically active side-chain acid 13, we turned to the problem of condensing this acid to 3-ANA (2), and decided to attempt the condensation at the stage of the protected keto acid 22. Thus, 22 was led to the mixed anhydride *in situ* with ethyl chloroformate and reacted with the silylated 3-ANA obtained by treatment with bis(trimethylsilyl)acetamide, resulting in 61% yield of the condensation product 23 as a crude oil. Without purification, 23 was then subjected to hydrolysis with a limited amount of 0.1 N NaOH, followed by treatment with trifluoroacetic acid to give nocardicin D (1d) in 42% yield based on 3-ANA. This synthetic product was proved to be identical with the naturally occurring nocardicin D by m.p., optical rotation, IR and NMR spectral comparison.

The final step in the total synthesis of nocardicin A (1a) was the oximation of the CO group in nocardicin D. In view of the fact that nocardicins are relatively stable under alkaline conditions in comparison with other

[c] This is a modification of the method described in Ref. 11.



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Scheme 4.

bicyclic β-lactams, the direct oximation of nocardicin D with hydroxylamine was examined, eventually resulting in 60% yield of nocardicin A. This synthetic sample was shown to be identical with the natural nocardicin A in m.p., optical rotation, IR and NMR data.

The use of this synthesis for the preparation of analogous compounds will be reported in the near future.

EXPERIMENTAL

M.ps. determined on a Thomas–Hoover capillary m.p. apparatus, are uncorrected. IR spectra were taken on a Hitachi 215 spectrophotometer. ^{1}H NMR spectra were recorded at 100 MHz on a Joel PS-100 spectrometer. Mass spectra were obtained with a Hitachi RMU-6M mass spectrometer. Optical rotations were measured on a JASCO DIP-SL automatic polarimeter. For column chromatography silica gel 60 (Merck) was used. Tlc was carried out on silica gel 60 F-254 precoated plates (Merck): visualization was with UV light or iodine vapor.

D-p-Benzyloxyphenylglycine (4)

To a solution of D-*p*-hydroxyphenylglycine (50.0 g, 0.3 mole) in 2 N NaOH (150 ml) was added a soln of $CuSO_4 \cdot 5H_2O$ (37.5 g, 0.15 mole) in H_2O (600 ml) with vigorous stirring. The mixture was warmed to 60–70° and stirred for 45 min, a green colored mass separating out. The suspension was then cooled to room temp., and MeOH (1.1 l) and 2 N NaOH (150 ml) were added. To the resulting dark green soln was added benzyl bromide (37.5 ml, 0.3 mole) in one portion with stirring and the stirring was continued for 4 hr at room temp., during which time a green colored crystalline solid separated out. This solid was collected and suspended in 1 N HCl (500 ml). After 1 hr of vigorous stirring, the resulting light brown colored solid was filtered off. The solid was then dissolved in 0.5 N NaOH (1.5 l), washed with ether and adjusted to pH 2 with 10% HCl aq to separate a colorless crystalline solid, which was filtered off and washed with H_2O to give 40.0 g (52.6%) of 4: m.p. 225–8°. An analytical sample was prepared by recrystallization from AcOH: m.p. 225–8° (dec); $[\alpha]_D$ −55°(c = 0.3, 0.1 N NaOH). (Found: C, 69.95; H, 5.78; N, 5.25. $C_{15}H_{15}O_3N$ requires: C, 70.02; H, 5.88; N, 5.44%).

Methyl D-p-benzyloxyphenylglycinate (5). To a cooled soln of $SOCl_2$ (6.96 g, 59 mmole) in MeOH (60 ml) was added 4 (10.81 g, 42 mmole) with stirring. After 20 hr of stirring at room temp., the resulting soln was filtered and concentrated under vacuum to give a crystalline solid, which was then dissolved in H_2O (300 ml), neutralized with 10% Na_2CO_3 aq and extracted with AcOEt. The extract was dried ($MgSO_4$) and evaporated to give an oil, which was crystallized on standing to yield 8.1 g (71%) of 5: m.p. 44–6°; $[\alpha]_D$ −108° (c = 1.2, MeOH). (Found: C, 71.02; H, 6.39; N, 5.19. $C_{16}H_{17}O_3N$ requires: C, 70.83; H, 6.32; N, 5.16%).

Methyl D - α - (methylthioimino) - p - benzyloxyphenylacetate (6). To a soln of 5 (5.9 g, 21.8 mmole) in $CHCl_3$ (120 ml) was added ethyl thioformate (2.94 g, 32.1 mmole), and the mixture was stirred for 15 hr at room temp. The mixture was poured into H_2O and the organic layer was successively washed with 5% HCl aq and H_2O. After drying ($MgSO_4$) and removal of the solvent, there was obtained a crude oil, which was chromatographed over 100 g of silica gel with $CHCl_3$ to give 3.82 g (55.4%) of the thioformyl derivative of 5: m.p. 98°; $[\alpha]_D$ −283° (c = 1.1, MeOH); NMR ($CDCl_3$) δ 3.66 (s, 3H, $COOCH_3$), 5.00 (s, 2H, CH_2Ar), 6.05 (d, 1H, J = 7 Hz, CHCOOMe), 8.50 (broad t, 1H, J = 6–7 Hz, NH), 9.50 (d, 1H, J = 6 Hz, CHS). (Found: C, 64.62; H, 5.39; N, 4.34. $C_{17}H_{17}O_3NS$ requires: C, 64.75; H, 5.43; N, 4.44%).

This product (3.72 g, 11.8 mmole) was dissolved in acetone (80 ml) and K_2CO_3 (0.82 g, 5.9 mmole) was added. The mixture was stirred and MeI (6.76 g, 47.2 mmole) was added. After stirring for 20 hr, the mixture was filtered and concentrated to give an oil, which was dissolved in CH_2Cl_2, washed with H_2O, dried ($MgSO_4$) and evaporated to yield 3.66 g (94.0%) of 6 (oil): NMR ($CDCl_3$) δ 2.40 (s, 3H, SCH_3), 3.65 (s, 3H, $COOCH_3$), 5.01 (s, 1H, CHCOOMe or CHSMe), 5.06 (s, 1H, CHSMe or CHCOOMe); *m/e* 329 (M^+).

Reaction of 6 with phthalimidoacetyl chloride. To a stirred soln of 6 (3.59 g, 10.9 mmole) in CH_2Cl_2 (60 ml) was added a soln of phthlimidoacetyl chloride (2.43 g, 10.9 mmole) in CH_2Cl_2 (10 ml) over 10 min at 5°. After 15 min of stirring, a soln of Et_3N (1.10 g, 10.9 mmole) in CH_2Cl_2 (10 ml) was added over 40 min at the same temp. After the stirring was continued for an additional 40 min, the mixture was washed successively with H_2O, 5% HCl aq, H_2O, 5% $NaHCO_3$ aq and H_2O, dried ($MgSo_4$) and evaporated to give a crude oil, which was chromatographed over 130 g of silica gel ($CHCl_3$) to yield a pure oil (3.57 g, 64%). A NMR ($CDCl_3$) spectrum indicated the presence of 7 and 8 in a ratio of 3:2: for 7, δ 2.14 (s, 3H, SCH_3), 3.79 (s, 3H, $COOCH_3$), 4.71 (d, 1H, J = 2.5 Hz, 4-H), 5.04 (s, 3H, CHCOOMe, and CH_2Ar), 5.33 (d, 1H, J = 2.5 Hz, 3-H); for 8, δ 1.87 (s, 3H, SCH_3), 3.81 (s, 3H, $COOCH_3$), 5.05 (s, 2H, CH_2Ar), 5.30 (s, 2H, 3-H and 4-H). This oil was used as such in subsequent reactions.

Methyl 3-phthalimidonocardicinate (9). To a soln of the 3:2 mixture of 7 and 8 (3.00 g) in dioxane (100 ml) was added Raney Ni (20 ml) and the mixture was stirred for 3 hr at 45–50°. After removal of the catalyst by filtration, the filtrate was concentrated under vacuum, and the residue was dissolved in AcOEt, washed with H_2O, dried ($MgSO_4$) and evaporated to give a crude oil (1.80 g).

This oil was dissolved in a mixture of MeOH (20 ml) and AcOH (20 ml) and 10% Pd–C (1.0 g) was added. The mixture was shaken with H_2 under its atmospheric pressure until the absorption of H_2 was ceased. After removal of the catalyst by filtration, the filtrate was concentrated under vacuum. The residue was then dissolved in AcOEt, washed with H_2O, dried ($MgSO_4$), evaporated and chromatographed over 40 g of silica gel ($CHCl_3$ and MeOH (99:1)) to give 0.82 g (37%) of 9 and 10 (3:2). Recrystallization from EtOH gave 0.22 g of 9: m.p. 203–4°; $[\alpha]_D$ −236° (c = 0.025, MeOH); NMR ($CDCl_3$) δ 3.47 (dd, 1H, J = 2.5, 6 Hz, 4β-H), 3.80 (s, 3H, $COOCH_3$), 3.95 (t, 1H, J = 6 Hz, 4α-H), 4.89 (dd, 1H, J = 2.5, 6 Hz, 3α-H), 5.71 (s, 1H, 5-H). (Found: C, 63.00; H, 4.21; N, 7.31. $C_{20}H_{16}O_6N_2$ requires: C, 63.15; H, 4.24; N, 7.37%). For 10, NMR ($CDCl_3$) δ 3.63 (t, 1H, J = 6 Hz, 4β-H), 3.82 (s, 3H, $COOCH_3$), 4.10 (dd, 1H, J = 2.5, 6 Hz, 4α-H), 5.37 (dd, 1H, J = 2.5, 6 Hz, 3β-H), 5.64 (s, 1H, 5-H).

3-Phthalimidonocardicinic acid (11). (a) To a soln of 9 (380 mg, 1 mmole) in pyridine (3 ml) was added LiI (400 mg, 3 mmole) and the mixture was refluxed for 2.5 hr. The mixture was poured into ice-water, adjusted to pH 2 with 10% HCl aq and extracted with AcOEt. The extract was washed with H_2O, dried ($MgSO_4$), and evaporated to give a crystalline solid, which was filtered and washed with AcOEt, yielding 0.31 g (80%) of 11: m.p. 202–3° (dec); $[\alpha]_D$ −301° (c = 0.6, MeOH); NMR (DMSO-d_6) δ 3.47 (dd, 1H, J = 2, 5 Hz, 4β-H), 3.86 (t, 1H, J = 5 Hz, 4α-H), 5.39 (dd, 1H, J = 2, 5 Hz, 3α-H), 5.39 (s, 1H, 5-H), 7.07 (ABq, 4H, J = 8 Hz, aromatic Hs), 7.87 (broad s, 4H, aromatic Hs). (Found: C, 62.40; H, 3.88; N, 7.43. $C_{19}H_{14}O_6N_2$ requires: C, 62.29; H, 3.85; N, 7.65%).

(b) 3-ANA (236 mg, 1 mmole), derived from nocardicin A, was dissolved in H_2O (10 ml) containing $NaHCO_3$ (250 mg, 2.98 mmole) and acetone (10 ml) was added. To this soln was

added a solution of N-ethoxycarbonylphthalimide (265 mg, 1.2 mmole) in acetone (5 ml) at 5°, and the mixture was stirred for 1 hr at the same temp. and then for 1.5 hr at room temp. Acetone was evaporated under vacuum and the resulting aqueous soln was washed with AcOEt. The aqueous layer was then acidified to pH 2 with 10% HCl aq and extracted with AcOEt. The extract was washed with H_2O, dried ($MgSO_4$) and evaporated to give a crystalline solid, which was washed with AcOEt, giving 520 mg of 11: m.p. 202–3° (dec); $[\alpha]_D$ −296° (*c* = 0.6, MeOH). Identified with the synthetic sample by comparison of the IR and NMR spectra.

3-Aminonocardicinic acid (2). To a suspension of 11 (366 mg, 1 mmole) in MeOH (6 ml) was added Et_3N (202 mg, 2 mmole) and N, N-dimethylaminopropylamine (240 mg, 2.2 mmole) at 5° and the resulting soln was stirred for 24 hr at room temp. The mixture was evaporated under vacuum and the residue was dissolved in 50% aqueous MeOH (20 ml). To this solution was added Amberlite IRC-50 resin (H^+ form) at 5° in order to adjust the pH to 6.0. After removal of the resin, the solvent was evaporated to give a crystalline solid, which was filtered off and washed with EtOH, yielding 140 mg (60%) of 2: m.p. 194–9° (dec). An analytical sample was prepared by washing with MeOH: m.p. 207–9° (dec); $[\alpha]_D$ −241° (*c* = 0.9, 0.1 N $NaHCO_3$). (Found: C, 55.69; H, 4.98; N, 11.59. $C_{11}H_{12}O_4N_2$ requires: C, 55.93; H, 5.12; N, 11.86%). The IR and NMR spectra were identical with those of 3-ANA (m.p. 198–200° (dec), $[\alpha]_D$ −252° (*c* = 1.1, 0.1 N $NaHCO_3$)) derived from the natural nocardicins.

dl - 4 - (4 - *Acetylphenoxy*) - 2 - *phthalimidobutyric acid* (16). *p*-Hydroxyacetophenone (21.2 g, 0.156 mole) was dissolved in diglyme (600 ml) and treated with 50% NaH (oil dispersion, 6.25 g, 0.13 mole) for 30 min at room temp. Then α-phthalimidobutyrolactone (30.0 g, 0.13 mole) was added in one portion and the mixture was refluxed for 6 hr. After cooling, the solvent was evaporated under vacuum and the residue was dissolved in H_2O, washed with AcOEt, acidified with 10% HCl aq and extracte with AcOEt. The organic layer was then extracted with sat $NaHCO_3$ aq and the aqueous layer was acidified with 10% HCl aq and extracted with AcOEt. The extract was dried ($MgSO_4$) and evaporated to give 40.5 g (85.0%) of 16 as a crystalline solid; m.p. 164–6° (dec); IR (Nujol) 1780 and 1745 (phthalimido CO), 1720 (COOH), 1650 (acetyl CO) cm^{-1}; NMR (DMSO-d_6) δ 2.50 (s, 3H, $COCH_3$), 2.67 (m, 2H, 3-CH_2), 4.17 (t, 2H, *J* = 6 Hz, 4-CH_2), 5.07 (t, 1H, *J* = 7 Hz, 2-CH), 7.33 (ABq, 4H, *J* = 9 Hz, aromatic Hs), 7.73 (s, 4H, aromatic Hs). (Found: C, 65.18; H, 4.74; N, 3.84. $C_{20}H_{17}O_6N$ requires: C, 65.39; H, 4.66; N, 3.81%).

dl - 4 - (4 - *Acetylphenoxy*) - 2 - *aminobutyric acid* (17). To a suspension of 16 (40.0 g) in AcOH (130 ml) was added 6 N HCl (600 ml), and the mixture was refluxed for 2.5 hr. After cooling, the ppt was removed by filtration and the filtrate was evaporated to dryness. The residue was dissolved in H_2O, treated with active charcoal and filtered. The filtrate was neutralized to pH 4 with 30% NH_4OH aq and the resulting crystalline solid was filtered and washed with H_2O to give 20.9 g (81%) of 17: m.p. 193–4° (dec); IR (Nujol) 1660 (acetyl CO), 1600 (COO^-) cm^{-1}; NMR (D_2O-NaOD) δ 2.14 (m, 2H, 3-CH_2), 3.55 (dd, 1H, *J* = 6, 8Hz, 2-CH), 4.17 (t, 2H, *J* = 6 Hz, 4-CH_2), 7.33 (ABq, 4H, *J* = 9 Hz, aromatic Hs). (Found: C, 60.59; H, 6.43; N, 5.79. $C_{12}H_{15}O_4N$ requires: C, 60.75; H, 6.37; N, 5.90%).

dl - 4 - (4 - *Acetylphenoxy*) - 2 - (*t* - *butoxycarbonylamino*) *butyric acid* (18). To a mixture of 17 (20.2 g, 85 mmole) and Et_3N (12.9 g, 128 mmole) in 50% aqueous dioxane (200 ml) was added 2-t-butoxycarbonyloxyimino-2-phenylacetonitrile (BOC-ON, 23.0 g, 93.5 mmole), and the mixture was stirred for 2 hr at room temp. The mixture, after removal of dioxane under vacuum, was washed with AcOEt, acidified (pH 2) with 10% HCl aq and extracted with AcOEt. The extract was washed with H_2O, dried ($MgSO_4$) and evaporated to give 23.8 g (83.0%) of 18 as a crystalline solid. An analytical sample was prepared by recrystallization from ether-isopropyl ether: m.p. 100–1° (dec); IR (Nujol) 1730 (COOH), 1690 (NHCOOBu-t), 1640 (acetyl CO) cm^{-1}; NMR ($CDCl_3$) δ 1.43 (s, 9H, $COOC(CH_3)_3$), 2.37 (m, 2H, 3-CH_2), 2.52 (s, 3H, $COCH_3$), 4.15 (t, 2H, *J* = 6 Hz, 4-CH_2), 4.52 (m, 1H, 1-CH), 7.40 (ABq, 4H, *J* = 9 Hz, aromatic Hs). (Found: C, 60.49; H, 6.89; N, 4.18. $C_{17}H_{23}O_6N$ requires: C, 60.52; H, 6.87; N, 4.15%).

Resolution of 18. *dl*-Acid 18 (25.66 g, 0.076 mole) and cinchonidine (22.40 g, 0.076 mole) were dissolved in hot acetonitrile (600 ml) and the soln was allowed to stand overnight. The resulting crystals were filtered and further recrystallized four times from acetonitrile to give 13.6 g (56.7%) of L-acid 20 cinchonidine salt: m.p. 144–7° (dec); $[\alpha]_D$ −64.1° (*c* = 2.2, MeOH). The mother liquor was concentrated to about 300 ml and allowed to stand overnight. The crystals were filtered off and further recrystallized three times from acetonitrile to give 11.2 g (46.7%) of D-acid 19 cinchonidine salt: m.p. 130–3° (dec); $[\alpha]_D$ −77.5° (*c* = 2.0, MeOH).

D-Acid 19 cinchonidine salt (10.0 g) was dissolved in $CHCl_3$ and washed twice with 5% HCl aq. The organic layer was dried ($MgSO_4$) and evaporated to an oil, which was crystallized from ether-isopropyl ether to give 4.60 g (43% from *dl*-acid 18) of D-acid 19: m.p. 90–2°; $[\alpha]_D$ + 18.1° (*c* = 2.0, MeOH). (Found: C, 60.30; H, 6.97; N, 4.14. $C_{17}H_{23}O_6N$ requires: C, 60.52; H, 6.87; N, 4.15%).

L-Acid 20 cinchonidine salt (11.0 g) was treated as for D-acid 19 to give 5.70 g (51% from *dl*-acid 18) of L-acid 20: m.p. 90–2°; $[\alpha]_D$ −8.1° (*c* = 2.0, MeOH). (Found: C, 60.59; H, 7.03; N, 4.19. $C_{17}H_{23}O_6N$ requires: C, 60.52; H, 6.87; N, 4.15%).

Methyl D - 4 - (4 - acetylphenoxy) - 2 - (t - butoxycarbonylamino) butyrate (21). A soln of 19 (2.10 g) in AcOEt (45 ml) was treated with ethereal diazomethane at 5° until 19 was disappeared on tlc. After removal of the solvent, the residue was crystallized from isopropyl ether to give 2.02 g of 21: m.p. 81–2°; $[\alpha]_D$ + 18.2 (*c* = 1.9, MeOH); IR (Nujol) 3390 (NH), 1740 (COOMe), 1710 (NHCOOBu-t), 1680 (acetyl CO) cm^{-1}; NMR ($CDCl_3$) δ 1.43 (s, 9H, $COOC(CH_3)_3$), 2.30 (m, 2H, 3-CH_2), 2.53 (s, 3H, $COCH_3$), 3.73 (s, 3H, $COOCH_3$), 4.12 (t, 2H, *J* = 6 Hz, 4-H), 4.50 (m, 1H, 2-H), 5.32 (d, 1H, *J* = 8 Hz, NH), 7.40 (ABq, 4H, *J* = 9 Hz, aromatic Hs). (Found: C, 61.33; H, 7.22; N, 3.97. $C_{18}H_{25}O_6N$ requires: C, 61.52; H, 7.17; N, 3.99%).

p-(D-3-t-Butoxycarbonylamino-3-methoxycarbonylpropoxy) phenylglyoxylic acid (22). To a soln of 21 (0.70 g) in pyridine (10 ml) was added SeO_2 (0.50 g) and the mixture was stirred for 5 hr at 80°. After removal of pyridine, the residue was dissolved in 5% $NaHCO_3$ aq and filtered. The filtrate was washed with AcOEt, acidified (pH 2) with 5% HCl aq and extracted with AcOEt. The extract was dried ($MgSO_4$) and evaporated to give 22 as an oil: NMR ($CDCl_3$) δ 1.46 (s, 9H, $COOC(CH_3)_3$), 2.36 (m, 2H, 2-CH_2), 3.80 (s, 3H, $COOCH_3$), 4.18 (t, 2H, *J* = 6 Hz, 1-CH_2), 4.52 (m, 2H, 3-H), 5.46 (d, 1H, *J* = 8 Hz, NH), 7.58 (ABq, 4H, *J* = 9 Hz, aromatic Hs); *m/e* 336 (M^+ − 45).

p-(D-3-Amino-3-carboxypropoxy) phenylglyoxylic acid (13). To a stirred soln of 22 (0.40 g) in MeOH (5 ml) was added 0.15 N NaOH (5 ml) and the stirring was continued for 30 min at room temp. The mixture was acidified (pH 2) with 5% HCl aq and extracted with AcOEt. The extract was dried ($MgSO_4$) and evaporated to give an oil (0.40 g), which was dissolved in MeOH (5 ml) and 1 N HCl (5 ml) was added. The mixture was stirred for 2 hr at room temp. and further refluxed for 20 min. After cooling, the mixture was adjusted to pH 2.5 with 1 N NaOH (5 ml) to separate a crystalline solid, which was filtered off and washed with H_2O to give 0.18 g of 13: m.p. 185–7° (dec); $[\alpha]_D$ −15.3° (*c* = 2.7, 1 N NaOH); IR (Nujol) 1740 (COOH), 1653 (CO), 1600 (COO^-) cm^{-1}. (Found: C, 53.83; H, 4.86; N, 5.20. $C_{12}H_{13}O_6N$ requires: C, 53.93; H, 4.90; N, 5.24%). The m.p., IR and optical rotation were identical with those of the sample derived from the natural nocardicin A.[4a]

Acylation of 3-*ANA* (2) *with* 22. To a suspension of 22 (1.15 g, 3 mmole) in CH_2Cl_2 (10 ml) was added Et_3N (0.13 g, 3 mmole) and N, N-dimethylbenzylamine (two drops). The resulting soln was cooled to −60°, and a soln of ethyl chloroformate (0.33 g, 3 mmole) in CH_2Cl_2 (5 ml) was added dropwise. After stirring for 1 hr at −40°, the mixture was again cooled to −70° and a soln of 3-ANA silyl ester, prepared from 3-ANA (0.71 g, 3 mmole) and bis(trimethylsilyl)acetamide (1.83 g) in CH_2Cl_2 (15 ml) containing DMF (0.5 ml) by stirring for 2 hr at room temp., was added all at once. The mixture was stirred for 30 min at the same temp. and then for 2 hr at −30°. The mixture was poured into ice-water and the aqueous layer was brought to pH 8 with 5% $NaHCO_3$ aq. After removal of the organic layer, the aqueous layer was acidified to pH 2 with 5% HCl aq and extracted with AcOEt. The

extract was washed with H_2O, dried ($MgSO_4$) and evaporated to give an oil, which was powdered with isopropyl ether, yielding 1.10 g of 23 (crude powder). This was directly subjected to the next reaction.

Nocardicin D (1d). To a soln of 23 (1.00 g) in MeOH (10 ml) was added dropwise 1 N NaOH (7 ml) at 0°, during which time the pH was kept at 9.5–10. The time required was 5 hr until 23 was disappeared on tlc. The mixture was neutralized to pH 8 with 1 N HCl and washed with AcOEt. The aqueous layer was then acidified to pH 2 with 1 N HCl and extracted with AcOEt. The extract was washed with H_2O, dried ($MgSO_4$) and evaporated to give 1.00 g of a crude oil. This oil was dissolved in benzene (15 ml) and anisole (1 ml) was added. To this mixture was added trifluoroacetic acid (5 ml) at 5° and the mixture was stirred for 2 hr at the same temp. Ether was added and the resulting ppt was collected to give a crude powder, which was dissolved in H_2O (20 ml) and adjusted to pH 3 with 5% $NaHCO_3$ aq to separate a crystalline solid. This solid was filtered off and washed with H_2O to give 0.62 g of 1d. An analytical sample was prepared as follows. To a suspension of the crude solid in H_2O was added 1 N NaOH and the pH was brought to 8. The resulting soln was treated with active charcoal and filtered. The filtrate was acidified to pH 3 with 1 N HCl to separate a crystalline solid, which was filtered off and washed with H_2O: m.p. 232–6° (dec); $[\alpha]_D$ −192° (c = 1.1, 1% $NaHCO_3$). (Found: C, 55.60; H, 4.70; N, 8.46. $C_{23}H_{23}O_9N_3 \cdot 1/2H_2O$ requires: C, 55.87; H, 4.90; N, 8.50%). The m.p., optical rotation, IR and NMR spectra were identical with those of the natural nocardicin D.[4a]

Nocardicin A (1a). To a suspension of nocardicin D (0.50 g) in H_2O (10 ml) was added hydroxylamine hydrochloride (0.15 g) and adjusted to pH 7.0 by adding $NaHCO_3$. The resulting soln was stirred for 1 hr at 50°. The mixture was adjusted to pH 3.5 with 5% HCl aq and treated with active charcoal. After filtration, the filtrate was concentrated to about 5 ml. On standing a crystalline mass was precipitated out, which was filtered and washed with H_2O to give 0.31 g of 1a: m.p. 215–7° (dec); $[\alpha]_D$ −150° (c = 1.0, 1% $NaHCO_3$). (Found: C, 53.03; H, 4.86; N, 10.51. $C_{23}H_{24}O_9N_4 \cdot H_2O$ requires: C, 53.28; H, 5.05; N, 10.81%). The m.p., optical rotation, IR and NMR spectra were identical with those of the natural nocardicin A ($[\alpha]_D$ −146° (c = 1.0, 1% $NaHCO_3$)).[4a]

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