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A TOTAL SYNTHESIS OF NOCARDICINS†

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Abstract—Four-component-condensation of D-isoserine or L-isoserine, diphenylmethyl isocyanide and *p*-(benzyloxy)benzaldehyde was used to construct a functionalized β-lactam ring system which was transformed in 4 steps into 3-aminonocardicinic acid. A protected side chain of nocardicin D was synthesized from D-asparagine in 6 steps. Coupling of these units and further conversion to nocardicins A, B and D followed published procedures. Using L-asparagine for the synthesis of the side chain led to unnatural isonocardicin A. Biological activities of the products are compared.

Nocardicin A (**19**) was the first example of a monocyclic β-lactam derivative having a potentially useful antibacterial activity. It was isolated by a Fujisawa research group as fermentation product of a *Nocardia* species together with six minor congeners named nocardicins B–G.[1-3] All nocardicins share 3-aminonocardicinic acid (3-ANA; **8**) as a common nucleus and differ only in the structure of the N-acyl-side chain.[4]

The discovery of these antibiotics was first disclosed in 1975.[5] It received wide attention because the unique yet simple structures invalidated some long accepted concepts of structure-activity relationships. In the following years a number of reports have appeared describing syntheses of racemic 3-ANA[6-8] and its natural (3*S*)-enantiomer.[9-15] Total syntheses of nocardicin A were also described by research groups of Fujisawa[10] and Lilly.[11]

In our studies of nocardicin analogs we were primarily interested in modifying the 3-ANA nucleus. However, because the comparison of the antibacterial effectiveness of the natural nocardicins clearly demonstrated that the presence of the D-homoserine fragment and of the *Z*-hydroxyimino function in the side chain is essential for good activity[4] we felt the need to also have a practical synthesis of the nocardicin A side chain. Our studies finally led to a complete synthesis of nocardicin A (**19**) and B (**20**) which we now wish to report.‡

Synthesis of 3-ANA

In a previous publication[16] we have described the synthesis of various azetidinone-1-acetic acid derivatives based on the "four-component-condensation" (4CC or Ugi reaction)[17] of β-amino-acids, aldehydes and isocyanides. We had found that the reaction did not proceed satisfactorily when N^2-protected 2,3-diaminopropionic acid was used as amino acid component together with diphenylmethyl isocyanide. However, good results were obtained with isoserine. Thus, when L-isoserine (**1**) was condensed with *p*-(benzyloxy)benzaldehyde (**2**) and diphenylmethyl isocyanide (**3**) a 1:1 mixture of epimeric (3*S*)-3-hydroxyazetidinone-1-acetamides **4** was formed in fair yield. The (3*S*)-OH group was transformed into a (3*S*)-NH_2 group by a double inversion process. First the OH group was treated with diethyl azodicarboxylate, triphenylphosphine and methyl bromide[18] to yield the (3*R*)-3-bromoazetidinones **5** with inverted configuration. In a second step bromide was displaced under inversion with the aid of sodium azide in DMSO to give (3*S*)-3-azidoazetidinone-1-acetamides which were transformed smoothly to a 1:1 mixture of the diphenylmethyl esters **6** and **7** by treatment with dinitrogen tetroxide in chloroform. At this stage the epimers could be easily separated by column chromatography on silica gel. Whereas **7** was obtained as an oil the desired epimer **6** was a crystalline solid.

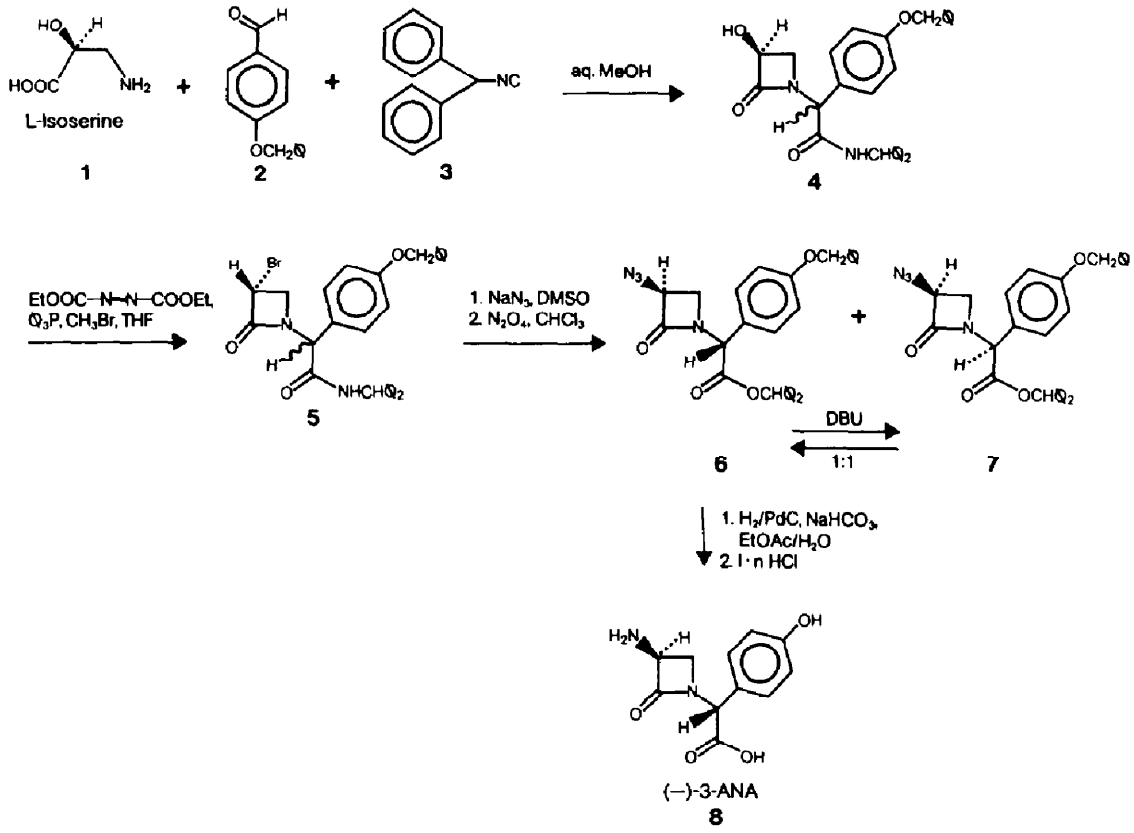
The stereochemistry of the two products was assigned from their NMR spectra based on the observation that in the nocardicin series the 4β-protons resonate at higher field than the 4α-protons whereas in the unnatural epimeric compounds the shifts are reversed and the shift differences small.[10] The unwanted isomer **7** could easily be epimerized with DBU in dichloromethane to a 1:1 mixture of **6** and **7**. Attempts to shift the equilibrium in favour of **6** by changing solvent and base were not successful. The optical rotation of **6** was not affected by the base treatment showing that no isomerization occurred at the ring position 3.

Hydrogenation of **6** into (3*S*)-3-ANA (**8**) was smoothly achieved in a mixture of ethyl acetate and water in the presence of 1 mol sodium bicarbonate and Pd/C as catalyst. **8** was isolated as a crystalline Na-salt. Its NMR data are identical with those reported for authentic 3-ANA.[19]

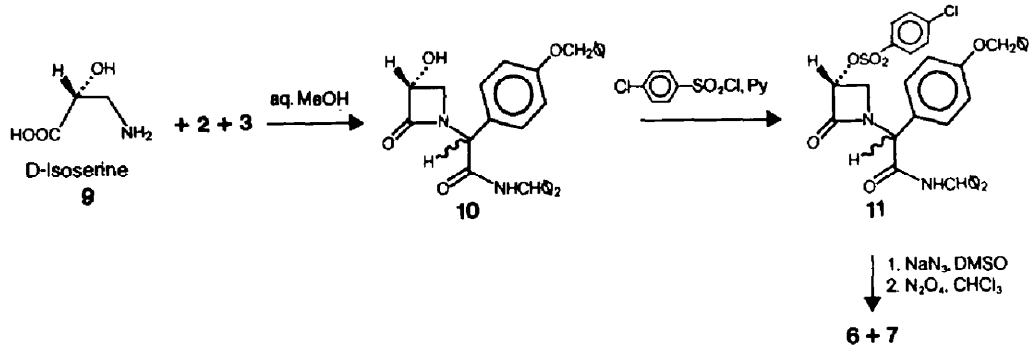
A similar sequence could also be performed starting with D-isoserine (**9**). The initial products **10** of the 4CC were the enantiomers of **4** and consequently required a conversion into azides **6** and **7** with a single inversion. This could be cleanly effected by first activating the OH group with *p*-chlorobenzenesulfonyl chloride followed by displacement of the sulfonate by azide.[20] Nitrosation as described above finally led to the same 1:1 mixture of **6** and **7**. Both reaction sequences offer equally practical 5-step routes to 3-ANA from inexpensive starting materials. The overall yield is around 30% for both syntheses provided **7** is recycled by base-catalyzed isomerization. L- and D-isoserine are both readily available

†Dedicated to Prof. Dr. A. Hürlimann on the occasion of his 60th birthday.

‡Part of this work was reported at the ESOC II, Stresa, 1–5 June 1981 (abstract No. p. 31 C).



Scheme 1.



Scheme 2.

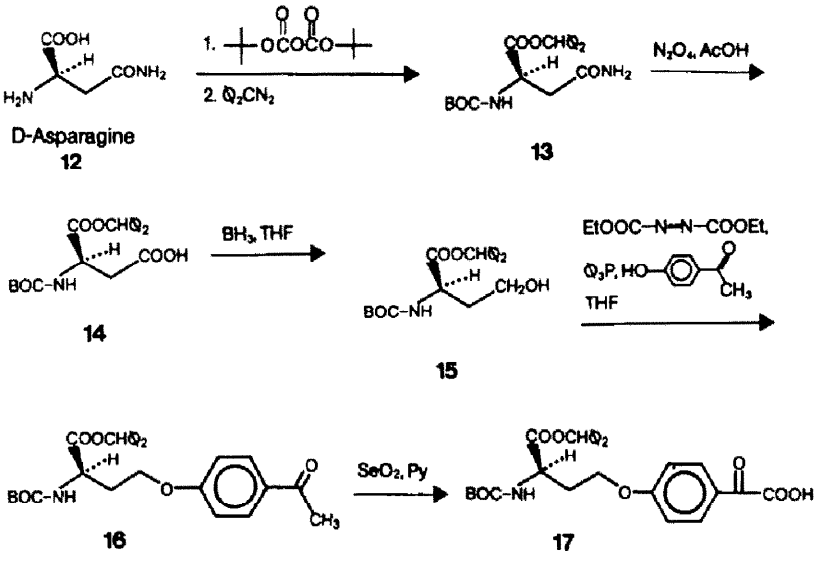
by the same procedure[21] from cheap precursors, the former from D-serine, the latter from L-serine.

Synthesis of the side chain

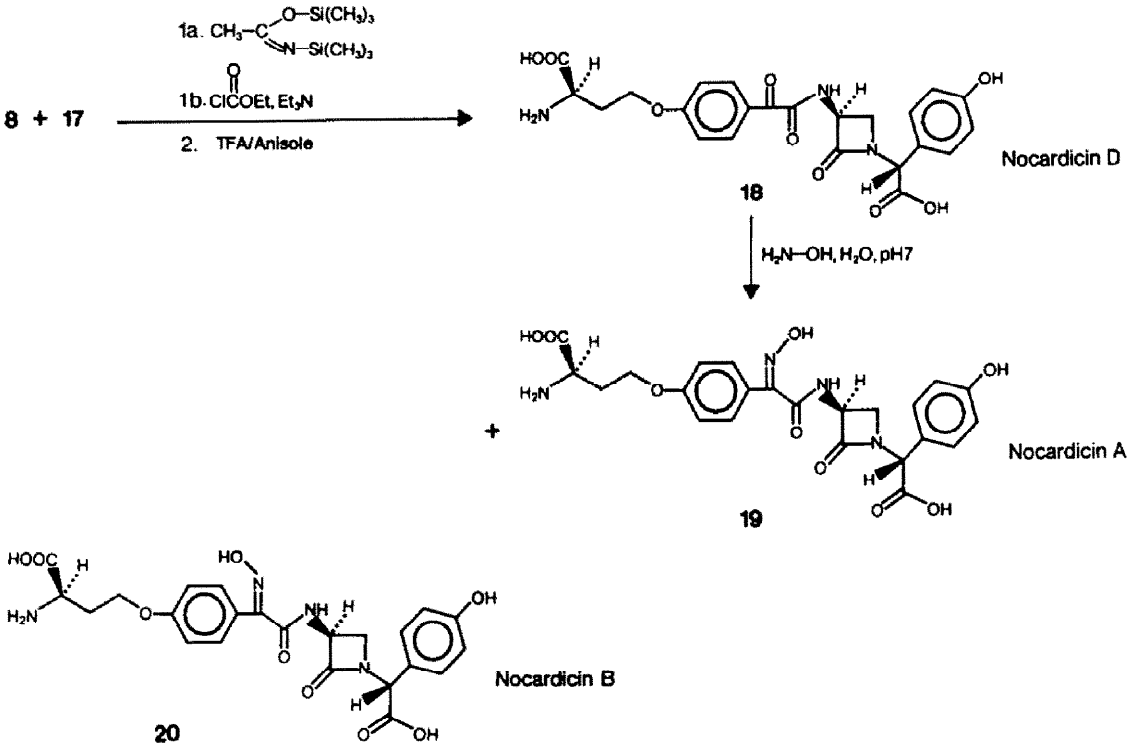
The first synthesis of the optically pure side chain of nocardicin A was accomplished by the Fujisawa group. It startet with racemic α-phthalimido-butyrolactone and involved a resolution step.[10] A second synthesis developed by a Lilly group started from D-methionine.[22] Our own synthesis was based on the conversion of D-asparagine (**12**), one of the cheapest D-aminoacids, into the protected D-homoserine derivative **15**. This was accomplished by protecting **12** as N-BOC-diphenylmethyl ester **13** which was deaminated to the aspartic acid derivative **14** by nitrosation. Diborane reduction of **14** led directly to **15** which was condensed with *p*-hydroxyacetophenone under Mitsunobu's conditions[23] to **16**. This intermediate was oxidized to the ketoacid **17** with selenium dioxide as described by the Fujisawa group. The whole procedure is shorter than any of the previously reported ones and gave a most practical access to key intermediate **17**.

Synthesis of nocardicins A and B

The protected side chain acid **17** was condensed by the mixed anhydride method with the silylated 3-ANA essentially as described by the Fujisawa group.[10] Deprotection with trifluoroacetic acid/anisole led to crude nocardicin D (**18**) which was purified by chromatography on Sephadex G 15 and crystallized. Treatment of **18** with hydroxylamine in



Scheme 3.

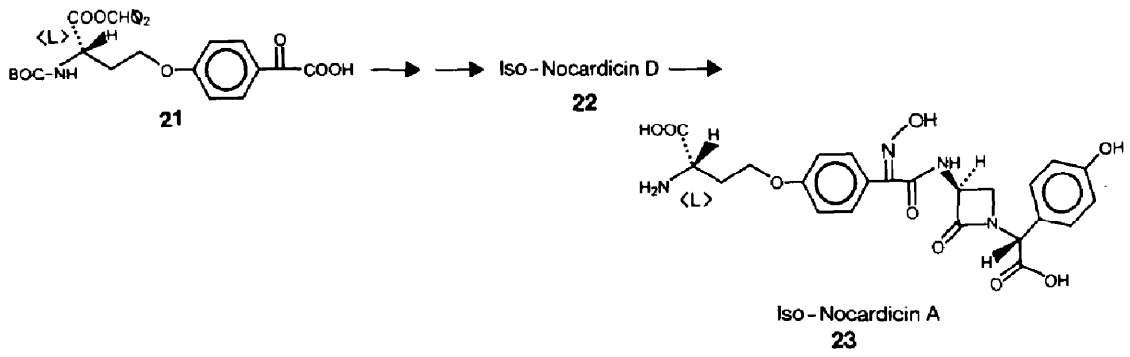


Scheme 4.

water at pH 7.0 gave nocardicin A (**19**) as the major product and a small amount of nocardicin B (**20**). Separation was effected by chromatography on a reversed phase silica. Crude products were desalted on Sephadex G 15 and crystallized from acidified aqueous solution. $[\alpha]_D$, IR and NMR of nocardicin A (**19**) and $[\alpha]_D$ and IR of nocardicin B (**20**) were identical with reported data.[4,10]

Synthesis of isonocardicin A

With the procedures described above it became possible to prepare "isonocardicin A" containing a L-homoserine instead of the D-homoserine moiety in the side chain with equal ease. **21** was prepared from L-asparagine by the same reaction sequence used for the D-enantiomer **16**. Oxidation to the keto acid and coupling with 3-ANA was again done according to



Scheme 5.

Fujisawa's procedure and led to crystalline isonocardicin D (**22**). Treatment with hydroxylamine gave isonocardicin A (**23**) which was purified by reversed phase chromatography and isolated after desalting on Sephadex G 10 by crystallization from an acidified aqueous solution.

Isonocardicin A was tested against *E. coli* 1024, *Proteus vulgaris* 1028 and *Serratia marcescens* 80315, three gram-negative bacterial strains sensitive towards nocardicin A. Whereas the minimum inhibitory concentration of nocardicin A was 6.3 μ g/ml for all three test organisms, isonocardicin A was inactive at 200 μ g/ml. These results again clearly demonstrate the crucial role played by the D-homoserine moiety in the biological activity of nocardicin A.

EXPERIMENTAL

(3S - α - [p - *Benzyloxy*)*phenyl*] - *N* - (*diphenylmethyl*) - 3 - *hydroxy* - 2 - *oxo* - 1 - *azetidineacetamide* (*mixture of epimers*) (**4**)

A mixture of **1** (10.5 g, 0.1 mol), **2** (21.2 g, 0.1 mol) and **3** (19.3 g, 0.1 mol) in MeOH (140 ml) and water (14 ml) was refluxed with stirring under argon for 3 days. After evaporation of the solvent *in vacuo* the residue was taken up in CH_2Cl_2 (400 ml) and cooled to 0°. L-isoserine **1** (2.5 g, 24 mmol) was recovered by filtration. The filtrate was concentrated and purified on a silicagel column (2 kg Kieselgel 60, 0.063–0.2 mm, Merck, EtOAc/cyclohexane 2:1). Crystallization from EtOAc/cyclohexane afforded **4** (12.7 g, 34% based on consumed L-isoserine: m.p. 99.2°; IR (KBr), 1749 cm^{-1}; NMR ($CDCl_3$) δ 2.95–3.90 (br, m, 2H), 4.02 (s, 1H), 4.05 (s, 1H), 4.50–4.80 (br, m, 2H), 5.47 (s, 1H), 6.20 (d, J 8 Hz, 1H), 6.85–7.50 (m, 20H); *m/e* 492 (M^+), 474, 407, 314, 289, 254, 267, 91; $[\alpha]_{546} - 20.6°$ (CH_2Cl_2, $c = 1.0\%$). (Found: C, 75.29; H, 5.93; N, 5.34. Calc. for $C_{31}H_{28}N_2O_4$: C, 75.59; H, 5.73; N, 5.69%).

(3*R*) - α - [p - (*Benzyloxy*)*phenyl*] - 3 - *bromo* - N - (*diphenylmethyl*) - 2 - *oxo* - 1 - *azetidineacetamide* (*mixture of epimers*) (**5**)

A mixture of **4** (12.4 g, 25.2 mmol) and triphenylphosphine (6.6 g, 25.2 mmol) was dissolved in benzene (130 ml) and THF (25 ml) at RT. During 20 min a soln of diethyl azodicarboxylate (4.4 g, 25.2 mmol) in benzene (25 ml) was added dropwise while keeping the temp below 30°. A rapid stream of MeBr was blown into the soln for 10 sec (*c.* 1.2 g, 12.6 mmol). The temp rose to 35°. As soon as the temp had dropped to RT, more triphenylphosphine (6.6 g, 25.2 mmol) was added followed by diethyl azodicarboxylate (4.4 g, 25.2 mmol) in benzene (25 ml). The same amount of MeBr was added two times more. After 2.5 hr **4** had disappeared as seen by TLC (silicagel, EtOAc/cyclohexane 2:1). The solvent was evaporated and the residue chromatographed on a silicagel column (500 g Kieselgel 60, 0.063–0.2 mm, Merck, EtOAc/cyclohexane 1:4). As soon as triphenylphosphine was eluted, the eluent was changed to EtOAc/cyclohexane 1:1. Yield: 8.0 g (57%) pure amorphous **5**: IR (KBr) 1760 cm^{-1}. (Found: C, 67.49; H, 5.20; N, 4.88. Calc. for $C_{31}H_{27}BrN_2O_3$: C, 67.03; H, 4.90; N, 5.04%).

Diphenylmethyl (α R,3S) - 3 - *azido* - α - [p - (*benzyloxy*)-*phenyl*] - 2 - *oxo* - 1 - *azetidineacetate* (**6**) *and its* (α S)-*epimer* (**7**) *from* L-*isoserine*

A mixture of **5** (3.6 g, 6.5 mmol) and sodium azide (3.6 g, 55 mmol) was stirred in DMSO (50 ml) for 5 hr at 50° and then poured into ice water (200 ml) and extracted with EtOAc. The organic layer was dried (Na_2SO_4), concentrated *in vacuo*, and chromatographed on silicagel (500 g Kieselgel 60, 0.04–0.063 mm, Merck, EtOAc/cyclohexane 2:1) to yield 2.4 g of an oil. This was dissolved in $CHCl_3$ and added dropwise at 0° to a soln prepared by adding N_2O_4 (0.86 ml, 13.9 mmol) dissolved in $CHCl_3$ (11.5 ml) to a suspension of NaOAc (1.3 g, 16.1 mmol) in $CHCl_3$. The yellow mixture was stirred for 90 min at 0°. $NaHCO_3$ (1.5 g, 17.9 mmol) in water (50 ml) was added and the $CHCl_3$ distilled off *in vacuo*. The aqueous residue was extracted with EtOAc, the extract dried (Na_2SO_4), concentrated, and chromatographed on silicagel (Lobar column size C, E. Merck, EtOAc/cyclohexane 1:4) to yield 1.02 g **6** and 0.97 g **7** (62%). **6** was crystallized from CH_2Cl_2/diisopropyl ether: m.p. 68.3°; IR (KBr), 1767, 1739 cm^{-1}; NMR ($CDCl_3$) δ 2.95 (dd, J 2.5 Hz and J 5.5 Hz, 1H), 3.80 (dd, J 5.5 Hz and J 5.5 Hz, 1H), 4.60 (dd, J 2.5 Hz and J 5.5 Hz, 1H), 5.08 (s, 2H), 5.68 (s, 1H), 6.97 (d, J 9 Hz, 2H), 6.98 (s, 1H), 7.20 (s, 5H), 7.23 (d, J 9 Hz, 2H), 7.32 (s, 5H), 7.41 (s, 5H); *m/e* 490 ($M^+ - N_2$), 307, 279, 238, 224, 167, 91; $[\alpha]_{546} - 274°$ (EtOH, $c = 1.0\%$). (Found: C, 71.47; H, 4.94; N, 10.86. Calc. for $C_{31}H_{26}N_4O_4$: C, 71.80; H, 5.05; N, 10.80%). **7** was an oil: NMR ($CDCl_3$) δ 3.4 (m, 2H), 4.45 (dd, J 2.5 Hz and 5 Hz, 1H), 5.06 (s, 2H), 5.66 (s, 1H), 6.96 (s, 1H), 6.98 (d, J 9 Hz, 2H), 7.22 (d, J 9 Hz, 2H), 7.21 (s, 5H), 7.32 (s, 5H), 7.42 (s, 5H).

When **7** was dissolved in a small amount of CH_2Cl_2 and treated with a catalytic amount of DBU at RT a 1:1 mixture of the two epimers **6** and **7** was formed within 1 min. After chromatographic separation, **6** was crystallized from CH_2Cl_2/diisopropyl ether: m.p. 68.3°, $[\alpha]_{546} - 272°$; a 2. crystallization gave: m.p. 68.7°, $[\alpha]_{546} - 274°$.

(α R,3S) - 3 - *Amino* - α - (p - *hydroxyphenyl*) - 2 - *oxo* - 1 - *azetidine* - *acetic acid* (**8**; Na *salt*)

The azidoester **6** (2.59 g, 5.0 mmol) was hydrogenated in the presence of $NaHCO_3$ (0.42 g, 5.0 mmol) and Pd-C (0.5 g, 10% Pd) in a mixture of water (50 ml) and EtOAc (50 ml). Within 1 hr 210 ml of H_2 had been consumed. The mixture was passed through a pad of Celite, the phases were

separated and the aqueous phase evaporated to give amorphous Na salt of **8** (1.4 g, 100%). For analysis the material was recrystallized from water/EtOH: IR (KBr) 1744 cm^{-1}; NMR (D_2O) δ 2.91 (dd, J 3 and 6 Hz, 1H), 3.81 (t, J 6 Hz, 1H), 4.25 (m, 1H), 5.31 (s, 1H), 6.98 (d, J 9 Hz, 2H), 7.31 (d, J 9 Hz, 2H) [almost identical data have been reported for **8**].[19] $[\alpha]_{546} - 269°$, $[\alpha]_D - 224°$ (H_2O, $c = 1.0\%$; reported value is $[\alpha]_D - 242°$ ($c = 1.1\%$, 0.1 n $NaHCO_3$).[9] (Found: C, 47.91; H, 5.38; N, 9.21; Na 7.71. Calc. for $C_{11}H_{11}N_2O_4Na + H_2O + 1/2$ EtOH: C, 48.16; H, 5.39; N, 9.36; Na, 7.68%).

Diphenylmethyl (α R,3S) - 3 - *azido* - α - [p - *benzyloxy*)-*phenyl*] - 2 - *oxo* - 1 - *azetidineacetate* (**6**) *and its* (α S)*-epimer* **7** *from* D*-isoserine*

A mixture of **9** (10.5 g, 0.1 mol), **2** (21.2 g, 0.1 mol) and **3** (19.3 g, 0.1 mol) in MeOH (150 ml) and water (15 ml) was refluxed with stirring under argon for 3 days. The solvent was evaporated *in vacuo* and the residue was stirred in CH_2Cl_2 (300 ml) for 15 min. After evaporation of the filtered soln the residue was purified by flash chromatography (340 g Kieselgel 60, 0.04–0.063 mm, Merck, hexane/EtOAc 1:1) to give 28.7 g **10** (58%) as a foam. This material was dissolved in pyridine (350 ml) and *p*-chlorobenzene-sulfochloride (24.5 g, 116 mmol) was added at 0°. The soln was kept overnight at 7°. The dark brown mixture was then poured onto crushed ice (400 g) and conc HCl (400 ml). The product was extracted with CH_2Cl_2 (900 ml) in three portions, the organic layer dried (Na_2SO_4) and the solvent removed by distillation to give **11** as a red oil (36 g; 54 mmol).

The crude **11** was dissolved in DMSO (320 ml) and sodium azide (33.4 g, 513 mmol) was added. After stirring overnight at 50°, the mixture was poured onto crushed ice (700 g) and extracted with EtOAc (800 ml) in four portions. The combined organic layer was washed with brine (200 ml), dried (Na_2SO_4) and evaporated. The residue was purified by flash chromatography (300 g Kieselgel 60, 0.04–0.063 mm, Merck, EtOAc/cyclohexane 1:1). The crude product was rechromatographed through an identical column but with EtOAc/cyclohexane 1:2 to give 21.9 g of a 1:1 mixture of epimeric azidoamides. This mixture was dissolved in $CHCl_3$ (200 ml) and added dropwise to a suspension prepared by adding N_2O_4 (8.1 ml, 130 mmol) in $CHCl_3$ (110 ml) to a suspension of NaOAc (12.4 g, 151 mmol) in $CHCl_3$ (110 ml) at 0°. The yellow suspension was stirred 1 hr at 0° and 2 hr at RT. $NaHCO_3$ (15 g, 178 mmol) in water (500 ml) was added and the $CHCl_3$ removed *in vacuo*. The product was extracted with three portions of EtOAc (800, 500 and 300 ml). The combined organic phases were washed with brine (400 ml), dried (Na_2SO_4) and evaporated.

The crude product (20.8 g) was separated into the two epimers by column chromatography (Jobin Yvon Chromatospac Prep 100, 1.5 kg Kieselgel 60, 0.04–0.063 mm, Merck, EtOAc/hexane 1:4, 50 ml/min). Yield: 8.1 g **6** and 8.1 g **7**. The overall yield from D-isoserine was 31%. **6** was crystallized from diisopropyl ether and had the same physical properties as the material obtained from L-isoserine.

BOC-D-*Asparagine diphenylmethyl ester* (**13**)

To a soln of BOC-D-asparagine (31.8 g, 137 mmol; prepared from D-asparagine in 64% yield according to the procedure of Moroder *et al.*[24]) in DMF (290 ml) diphenyldiazomethane (31.8 g, 164 mmol) was added in portions over a period of 40 min at a temp between 40 and 50°. After stirring the soln for 1 hr, the solvent was evaporated *in vacuo* (0.1 mm Hg). The residue partly crystallized on addition of EtOAc (200 ml) to give a first crop of 17 g **13**. The filtrate was washed with $NaHCO_3$ aq, dried (Na_2SO_4) and evaporated. Crystallization from toluene afforded a second crop of **13** (25 g), total yield: 42 g (78%): m.p. 171°; IR (KBr) 1745, 1706, 1695, 1680, 1613, 1540 and 1498 cm^{-1}; NMR ($CDCl_3$) δ 1.40 (s, 9H), 2.8 (m, 2H), 4.6 (m, 1H), 5.8 (m, 3H), 6.84 (s, 1H), 7.27 (s, 10H); $[\alpha]_D + 30.2°$C (DMF, $c = 1\%$). (Found: C, 66.47; H, 6.67; N, 7.01. Calc. for $C_{22}H_{26}N_2O_5$: C, 66.32; H, 6.58; N, 7.03%).

BOC-D-*Aspartic acid* 1-*diphenylmethyl ester* (**14**)

Dinitrogen tetroxide (10.6 ml, 167 mmol) dissolved in AcOH (130 ml) was added within 30 min to a mixture of **13** (33.4 g, 84 mmol) and NaOAc (13.7 g, 167 mmol) in AcOH (170 ml) at a temp between 13 and 15°. After stirring for 5 hr at RT the yellow mixture was evaporated in HV. The residue was dissolved in benzene (200 ml) and refluxed for 6 hr. The solvent was evaporated and the oily residue crystallized from EtOAc/hexane. Yield: 16.6 g **14** (50%): m.p. 155.7°; IR(KBr) 3442, 1741, 1718, 1701, 1590, 1510, 1205, 1165, 746 and 702 cm^{-1}; NMR ($CDCl_3$) δ 1.42 (s, 9H), 2.98 (t, J 5 Hz, 2H), 4.7 (m, 1H), 5.5 (m, 1H), 6.92 (s, 1H), 7.30 (s, 10H), 10.01 (s, 1H); $[\alpha]_D + 26.9°$ (EtOH, $c = 1\%$). (Found: C, 66.10; H, 6.35; N, 3.52. Calc. for $C_{22}H_{25}NO_6$: C, 66.15; H, 6.31; N, 3.51%).

BOC-D-*Homoserine diphenylmethyl ester* (**15**)

To a soln of **14** (18.8 g, 47 mmol) in THF (235 ml) a 1.0 molar borane-THF soln (47 ml, 47 mmol) was added under stirring at 0° within 20 min. After 5 hr at 0° MeOH (33 ml) was added slowly and the soln was allowed to warm to RT. The solvent was evaporated and the residue dissolved in ether (150 ml). After washing with water (100 ml) the ether was evaporated and the residue purified by chromatography (500 g Kieselgel 60, 0.04–0.063 mm, Merck, EtOAc/cyclohexane 2:1) to give 8.8 g (48%) of **15** as an oil: NMR ($CDCl_3$) δ 1.44 (s, 9H), 2.7 (broad, 1H), 3.7 (m, 2H), 4.6 (m, 1H), 5.4 (m, 1H), 6.93 (s, 1H), 7.35 (s, 10H). Almost identical data have been reported.[22]

Diphenylmethyl D - 4 - (p - *acetylphenoxy*) - 2 - (1 - *t* - *butoxyformamido*)*butyrate* (**16**)

Compound **15** (8.8 g, 22.8 mmol), *p*-hydroxyacetophenone (3.2 g, 22.8 mmol) and triphenylphosphine (5.9 g, 22.8 mmol) was dissolved in dry THF (70 ml). Diethyl azodicarboxylate (3.6 ml, 22.8 mmol) in dry THF (43 ml) was dropped into this soln below 30°. After stirring for 30 min at RT the solvent was evaporated and the residue dissolved in toluene/acetone 9:1, filtered, concentrated, and chromatographed (500 g Kieselgel 60, 0.04–0.063 mm, Merck, toluene/acetone 9:1). The product **16** was crystallized from diisopropyl ether to give 8.2 g (71%): m.p. 101°; IR (KBr) 1745, 1718, 1681, 1599, 1579, 1508, 1260, 1240 cm^{-1}; NMR ($CDCl_3$) δ 1.41 (s, 9H), 2.3 (m, 2H), 2.51 (s, 3H), 4.01 (t, J 6 Hz, 2H), 4.8 (m, 1H), 5.3 (m, 1H), 6.75 (d, J 9 Hz, 2H), 6.96 (s, 1H), 7.34 (s, 10H), 7.90 (d, J 9 Hz, 2H); $[\alpha]_D + 18°$ (EtOH, $c = 1\%$). (Found: C, 71.02; H, 6.60; N, 2.92; Calc. for $C_{30}H_{33}NO_6$: C, 71.55; H, 6.61; N, 2.78%).

[p - [(R) - 3 - (1 - *t* - *Butoxyformamido*) - 3 - [(*diphenylmethoxy*)*carbonyl*] - *propoxy*]*phenyl*]*glyoxylic acid* (**17**)

To a stirred soln of **16** (1.0 g, 2 mmol) in pyridine (4 ml) selenium dioxide (388 mg, 3.5 mmol) was added in portions over a period of 2 hr and at a temp of 90°. After 4.5 hr the mixture was cooled to RT and filtered and the solvent removed *in vacuo*. The residue was dissolved in 50 ml water and adjusted to pH 3 with dilute HCl. Extraction with EtOAc and evaporation gave 1.06 g **17** (100%) as an oil: IR (KBr) 1743, 1718, 1680, 1600, 1574, 1510 cm^{-1}; NMR ($CDCl_3$) δ 1.39 (s, 9H), 2.35 (m, 2H), 4.08 (t, J 6 Hz, 2H), 4.65 (m, 1H), 5.4 (m, 1H), 6.74 (d, J 9 Hz, 2H), 6.93 (s, 1H), 7.31 (s, 10H), 7.9 (broad, 1H), 8.18 (d, J 9 Hz, 2H). Almost identical data have been reported.[22]

Nocardicin D (**18**)

3-ANA Na-salt (**8**) (1.29 g, 5.0 mmol) was dissolved in water (5 ml), cooled to 0° and treated with 1 N HCl (5 ml, 5.0 mmol). The resulting pH was 3.5. The soln was frozen immediately after the addition of the acid and lyophilized. The colourless powder was suspended in dry CH_2Cl_2 (37.5 ml) and treated with bis(trimethylsilyl)-acetamide

(3.75 ml, 15.1 mmol) and DMF (0.25 ml). The mixture was stirred for 4.5 hr (soln I). Acid **17** (2.66 g, 5 mmol) was dissolved in dry CH_2Cl_2 (40 ml) and treated at $-60°$ with Et_3N (0.7 ml, 5 mmol), N,N-dimethylbenzylamine (7 drops) and ethyl chloroformate (0.48 ml, 5 mmol). The soln was allowed to warm up to $-15°$ kept at this temp for 10 min and again cooled to $-70°$. Solution I was then added within 15 min at $-70°$ and stirring at the same temp was continued for 2 hr. The cooling bath was removed and the soln evaporated as soon as it had reached RT. The residue was dissolved in water, HCl was added to pH 2 and the soln extracted with EtOAc. After evaporation of the solvent a foam (4.51 g) was obtained which was stirred for 2 hr at RT in a mixture of anisole (5 ml) and TFA (50 ml) and then evaporated. Anisole (5 ml) was twice added and evaporated again.

The crude product was then dissolved in water the pH adjusted to 7.2 with $NaHCO_3$. The aqueous soln was washed with EtOAc and purified in two equal portions by chromatography on Sephadex G 15 (500 g; eluent water, 60 ml/hr). Lyophilization gave an amorphous product which was dissolved in water (50 ml). On adjustment to pH 3.0 with 1 N HCl **18** precipitated as colourless crystals. Yield: 942 mg (39%): m.p. ~230° dec.; IR (KBr) 3244, 2640, 1739, 1660, 1604, 1575, 1519, 1410, 1261 and 849 cm^{-1}; NMR (D_2O + NaOD) δ 2.4 (m, 2H), 3.03 (dd, J 6 Hz and 2 Hz, 1H), 3.81 (t, J 6 Hz, 1H), 4.00 (t, J 5 Hz, 1H), 4.29 (t, J 6 Hz, 2H), 5.01 (dd, J 5 Hz and 2 Hz, 1H), 5.34 (s, 1H), 6.94 (d, J 9 Hz, 2H), 7.03 (d, J 9 Hz, 2H), 7.28 (d, J 9 Hz, 2H), 7.97 (d, J 9 Hz, 2H); $[\alpha]_D - 178°$ (1% $NaHCO_3$, $c = 1\%$). (Found: C, 55.63; H, 4.86; N, 8.41; Calc. for $C_{23}H_{23}N_3O_9 + 0.5\ H_2O$: C, 55.87; H, 4.90; N, 8.50%). These data are in agreement with values from the literature.[4]

Nocardicin A (**19**) *and nocardicin B* (**20**)

Hydroxylamine hydrochloride (0.3 g, 4.3 mmol) was added to a soln of **18** (1.0 g, 2.02 mmol) in water (20 ml) adjusted to pH 7.0 with $NaHCO_3$. The soln was stirred for 2 hr at 50° and then lyophilized. The residue was purified by chromatography on reversed phase C_{18} (Waters Prep 500, eluent 0.01 m $(NH_4)_2HPO_4$/0.01 m KH_2PO_4/MeOH 50:50:5). Fractions containing **19** were concentrated and desalted by passing through a Sephadex column (500 g Sephadex G 15, eluent water, 60 ml/hr. The concentrated fractions were adjusted to pH 3.5 and the colourless ppt was collected and dried in HV. Yield of **19**: 300 mg (28%): m.p. ~209° dec.; IR (KBr) 3276, 3230, 2674, 2606, 1731, 1657, 1611, 1514, 1399, 1260, 1244, 936 and 846 cm^{-1}; NMR (DMSO-d_6) δ 2.04 (m, 1H), 2.22 (m, 1H), 3.06 (dd, J 2 and 5 Hz, 1H), 3.48 (t, J 6 Hz, 1H), 3.82 (t, J 5 Hz, 1H), 4.14 (t, J 6 Hz, 2H), 4.96 (m, 1H), 5.31 (s, 1H), 6.77 (d, J 9 Hz, 2H), 6.96 (d, J 9 Hz, 2H), 7.15 (d, J 9 Hz, 2H), 7.42 (d, J 9 Hz, 2H), 9.18 (d, J 8 Hz, 1H), 11.40 (broad, 1H); $[\alpha]_D - 147°$ (1% $NaHCO_3$, $c = 1\%$). (Found: C, 52.55; H, 5.04; N, 10.75; H_2O, 2.88. Calc. for $C_{23}H_{24}N_4O_9 + 1.0\ H_2O$: C, 53.28; H, 5.05; N, 10.81; H_2O, 3.48%). These data are almost identical with published values.[4]

Fractions containing **20** were concentrated and desalted in the same way and the product was isolated from the aqueous soln at pH 2.3. Yield of **20**: 60 mg (6%): m.p. ~260° dec.; IR (KBr) 3234, 3070, 1740, 1710, 1661, 1630, 1612, 1418, 1254, 1230, 1021, 846 and 819 cm^{-1}; $[\alpha]_D - 163°$ (1% $NaHCO_3$, $c = 1\%$). (Found: C, 53.50; H, 4.72; N, 10.89; H_2O, 2.22. Calc. for $C_{23}H_{24}N_4O_9 + 0.7\ H_2O$: C, 53.84; H, 4.99; N, 10.92; H_2O, 2.45%).

Diphenylmethyl L - 4 - (p - *acetylphenoxy*) - 2 - (1 - *t* - *butoxyformamido*)*butyrate* **21**

Compound **21** was prepared as **16** using identical conditions: m.p. 102.5°; IR (KBr) and NMR ($CDCl_3$) identical with those of **16**; $[\alpha]_D - 19°$ (EtOH, $c = 1\%$). (Found: C, 71.28; H, 6.71; N, 2.78. Calc. for $C_{30}H_{33}NO_6$: C, 71.55; H, 6.61; N, 2.78%).

(α R,3S) - 3 - [2 - [p - [(S) - 3 - *Amino* - 3 - *carboxypropoxy*]*phenyl*]*glyoxylamido*] - α - (p - *hydroxyphenyl*) - 2 - *oxo* - 1 - *azetidineacetic acid* (**22**); *isonocardicin D*

The desired product was prepared by oxidizing **21** and condensing the protected L-sidechain acid with 3-ANA (**8**) in the same way as described for **18**. Deprotection with TFA and purification gave the product as an amorphous solid in 25% yield.

IR (KBr) 3250, 1745, 1601 cm^{-1}; NMR (D_2O + NaOD) δ 2.26 (m, 2H), 3.09 (dd, J 6 and 2 Hz, 1H), 3.81 (t, J 6 Hz, 1H), 3.8 (broad, 1H), 4.24 (t, J 6 Hz, 2H), 4.99 (dd, J 6 and 2 Hz, 1H), 5.29 (s, 1H), 6.81 (d, J 8.5 Hz, 2H), 6.98 (d, J

Isonocardicin A (**23**)

Oxidation of **22** was done under the same conditions as described for **18**. IR (KBr): 3230, 1736, 1657, 1615, 1519, 1252 cm^{-1}; NMR (D_2O) δ 2.4 (m, 2H), 3.30 (dd, J 2 and 6 Hz, 1H), 3.88 (t, J 5.5 Hz, 1H), 3.97 (dd, J 5 and 6 Hz, 1H), 4.27 (m, 2H), 5.02 (dd, J 2 and 5 Hz, 1H), 5.36 (s, 1H), 6.93 (d, J 8.5 Hz, 2H), 7.06 (d, J 8.5 Hz, 2H), 7.27 (d, J 8.5 Hz, 2H), 7.53 (d, J 8.5 Hz, 2H).

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