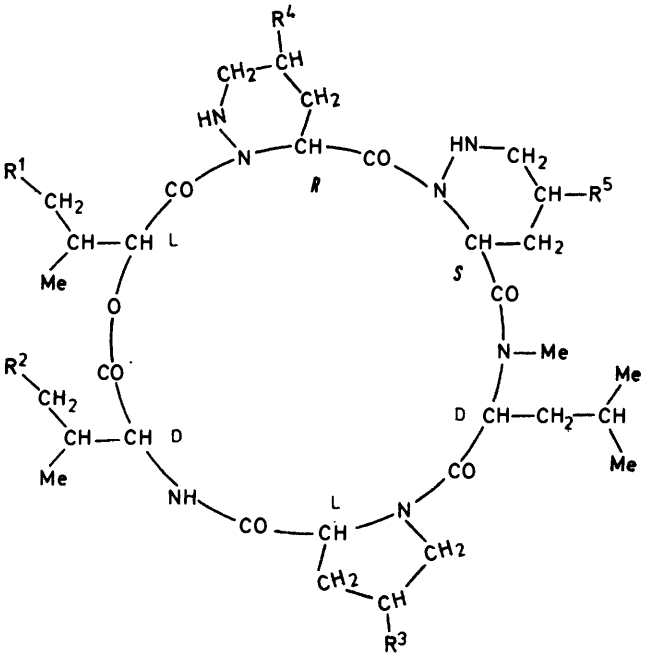
Amino-acids and Peptides. Part 21.[1] Synthesis of a Congener of the Cyclohexadepsipeptide Antibiotic, Monamycin

By **Cedric H. Hassall, William H. Johnson,** and **Colin J. Theobald,** Roche Products Limited, P.O. Box 8, Welwyn Garden City, Hertfordshire AL7 3AY

The hexadepsipeptide H-D-Val-L-Ile-(*R*)-Pip-(*S*)-Pip-NMe-D-Leu-L-Pro-OH has been prepared and cyclised to give the unnatural congener (I, X) of monamycin. It has antibacterial properties.

EARLIER papers in this series have described studies which have led to the elucidation of the molecular structures of fifteen natural congeners of the monamycin family of antibiotics (1).[2,3] These cyclohexadepsipeptides all incorporate residues of the novel amino-acids, (*R*)-piperazic [or 5-chloro-(*R*)-piperazic] acid and (3*S*,5*S*)-5-hydroxypiperazic acid. We have undertaken the synthesis of an unnatural congener, monamycin-X (1; R^1 = Me; $R^2 = R^3 = R^4 = R^5$ = H) with the



(1)

hydroxy-(*S*)-piperazic acid residue of monamycin-B_3 replaced by one of (*S*)-piperazic acid. A synthesis of (3*S*,5*S*)-5-hydroxypiperazic acid has been achieved recently,[1] so that the preparation of a natural congener such as monamycin-B_3 becomes possible, using a route similar to that described below.

The synthesis was designed to form the depsipeptide linkage at a relatively late stage and to cyclise the linear hexadepsipeptide through the only unsubstituted amide bond in the molecule (Pro ⟶ Val); model experiments with piperazic acid derivatives had shown that acylation at position 2 did not take place readily and, at least in some cases, *N*-methylamino-acids are acylated with difficulty.

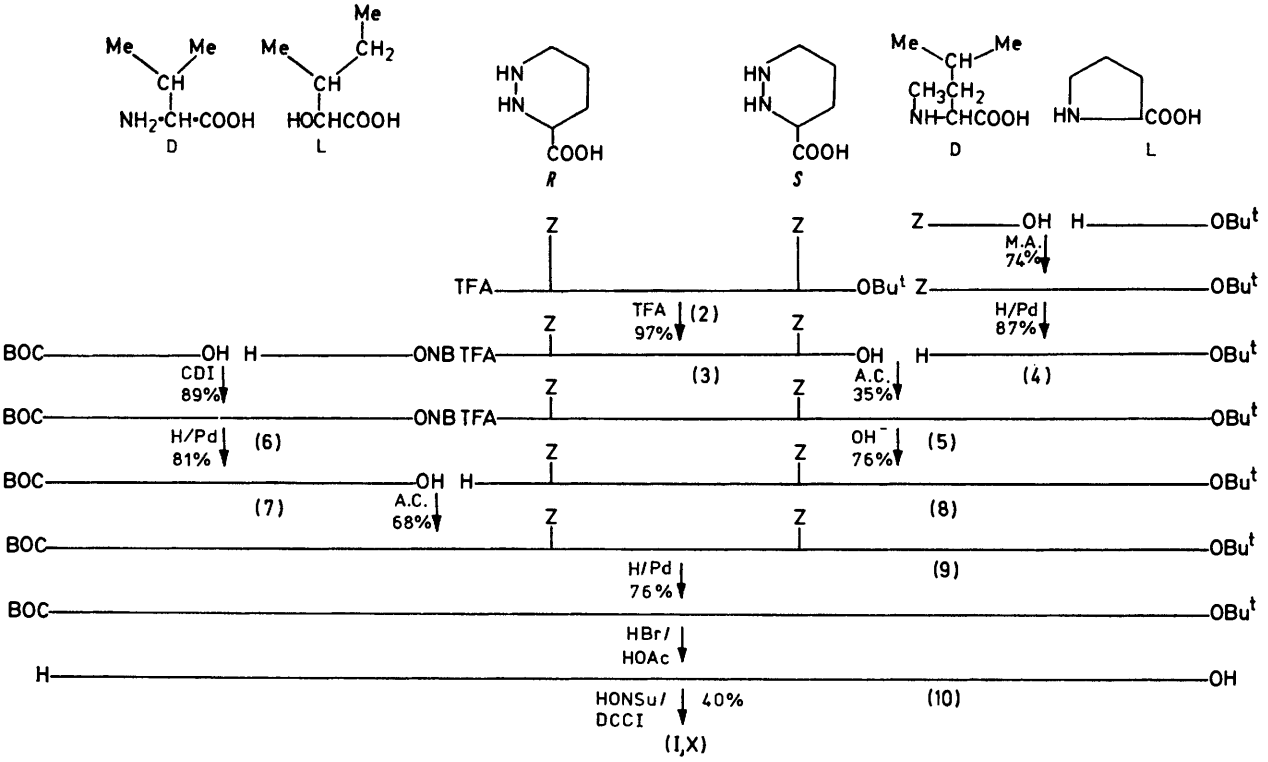
The (+)- and (−)-piperazic acids used in these studies were derived from racemic material synthesised by Diels–Alder condensation of phthalazinedione with penta-2,4-dienoic acid, followed by hydrolysis.[2] The N^1-benzyloxycarbonyl (*i.e.* Z) derivative of the product,[4] (±)-piperazic acid, was resolved with (+) and (−) ephedrines [5] to give (+)- and (−)-Z-piperazic acids, respectively. They were identified through comparison with the DNP-derivative of natural (−)-(*R*)-piperazic acid.

N^1-(Z)-N^2-Trifluoroacetyl-(*R*)-piperazyl chloride, prepared by the action of trifluoroacetic anhydride and phosphorus pentachloride in turn, on N^1-Z-(*R*)-piperazic acid was condensed with N^1-(Z)-(*S*)-piperazic acid t-butyl ester to give the fully protected dipeptide (2). The corresponding acid chloride was condensed with *N*-methyl-D-leucyl-L-proline t-butyl ester (4) to give the tetrapeptide derivative (5) which after deprotection of the *N*-terminus, condensed with the depsipeptide t-butyloxycarbonyl-D-valyl-L-isoleucic acid to give the linear hexadepsipeptide derivative (9). This was deprotected by successive hydrogenation and treatment with hydrogen bromide–acetic acid. The product, an oil, was cyclised (40% yield) by the action of a mixture of *N*-hydroxysuccinimide and dicyclohexylcarbodi-imide. This crystalline cyclohexadepsipeptide, $C_{35}H_{55}N_7O_7$, was identified as the congener (I,X) by combustion and amino-acid analysis and by ^{1}H n.m.r. and mass spectra.

This synthetic analogue differs from natural congeners, such as monamycin B_3 in having the residue of (3*S*,5*S*)-5-hydroxypiperazic acid replaced by that of (3*S*)-piperazic acid. However, it retained antibacterial activity against *Staphylococcus aureus*, similar to monamycin 1 [6] but this is only 25% of the more active congener, monamycin D.[4]

EXPERIMENTAL

M.p.s were determined with a Tottoli-type apparatus. Optical rotation measurements were with a Perkin-Elmer 141MC automatic polarimeter. ^{1}H and ^{13}C n.m.r. spectra were determined with a Varian XL100 instrument, with tetramethylsilane as internal standard. Mass spectra were obtained with an A.E.I. MS902 spectrometer. Amino-acid analyses employed a JEOL 6AH instrument. T.l.c. plates were of Kieselgel 60 F_{254} (Merck) and were developed in the systems: A, benzene–ethyl acetate (2 : 1); B, chloroform–methanol (4 : 1); C, benzene–ethyl acetate (4 : 1); D, chloroform–methanol (37 : 3); and E, n-butanol–acetic acid–water (4 : 1 : 1). Dried plates were sprayed with:



SCHEME Z = benzyloxycarbonyl; BOC = t-butyloxycarbonyl; TFA = trifluoroacetyl; ONB = 4-nitrobenzyl; M.A. = mixed anhydride method; A.C. = acid chloride method; C.D.I. = 1,1′-carbonyldi-imidazole

(a) 0.25% solution of ninhydrin in acetone (then heated at 100 °C for 5 min) and (b) 1% solution of soluble starch in 1% potassium iodide solution (after the plates had been exposed to chlorine). The plate was introduced into an atmosphere of iodine vapour to determine iodine reactivity.

N¹-*Benzyloxycarbonylpiperazic Acid* (*with S. L. Tan*).—Piperazic acid hydrochloride [2] (38.9 g, 0.233 mol) in water (50 ml) was adjusted to pH 7 with sodium hydroxide solution (4M; *ca.* 75 ml). After further sodium hydroxide had been added (4M; 58.5 ml) the solution was cooled to 0 °C; benzyl chloroformate (60.75 g, 0.35 mol) and sodium hydroxide solution (4M; 88.0 ml) were added over a period of 45 min at 0 °C. The mixture was stirred for 30 min at 0 °C and 2 h at room temperature, extracted with ether (2 × 100 ml), and acidified to pH 2 with 2M-hydrochloric acid. The oil which separated soon crystallised. Recrystallisation from water yielded N¹-*benzyloxycarbonylpiperazic acid* (42.0 g, 69%), m.p. 148—150° (Found: C, 58.9; H, 6.0; N, 10.5. $C_{13}H_{16}N_2O_4$ requires C, 59.1; H, 6.1; N, 10.6%).

Resolution of N¹-*Benzyloxycarbonylpiperazic Acid*.—*N*¹-Benzyloxycarbonylpiperazic acid (29.3 g, 0.111 mol) and (−)-ephedrine hydrate (11.3 g, 0.062 mol) were dissolved in hot ethyl acetate (800 ml) and allowed to cool overnight. The crystals that separated were filtered off, washed with ethyl acetate, and dried. The (−)-salt {16.9 g, $[\alpha]_D^{20}$ −25.8° (*c*, 0.5 in EtOH)} was recrystallised from ethyl acetate (4 × 800 ml) to yield pure (−)-salt {7.1 g, $[\alpha]_D^{20}$ −39.6° (*c*, 0.5 in EtOH)} which was dissolved in water (150 ml) and acidified with 2N-hydrochloric acid to pH 1.8. After 5 min the (−)-*N*¹-benzyloxycarbonylpiperazic acid was filtered off, washed with water, and dried (4.4 g, 15%), m.p. 168—171°, $[\alpha]_D^{20}$ −35.6° (*c*, 0.5 in MeOH).

The *N*¹-benzyloxycarbonylpiperazic acid (16.4 g, 0.062 mol) recovered from the mother liquors and (+)-ephedrine hydrate (11.3 g, 0.062 mol) were dissolved in hot ethyl acetate (400 ml) and allowed to cool overnight. The (+)-salt {19.0 g, $[\alpha]_D^{20}$ +26.4° (*c*, 0.5 in EtOH)} was filtered and recrystallised from ethyl acetate (4 × 800 ml) to yield pure (+)-salt {8.76 g, $[\alpha]_D^{20}$ +39.9° (*c*, 0.5 in EtOH)}. Acid treatment yielded (+)-*N*¹-benzyloxycarbonylpiperazic acid (5.4 g, 18%), m.p. 168—172°, $[\alpha]_D^{20}$ +35.0° (*c*, 0.5 in MeOH). Treatment of *N*¹-Z-(+)-piperazic acid with hydrogen bromide in acetic acid followed by 2,4-dinitrofluorobenzene in ethanol–sodium hydrogen carbonate solution yielded N¹-*DNP*-(R)-*piperazic acid*, m.p. 151.5—152° [from light petroleum (b.p. 30—40°)], $[\alpha]_D^{25}$ +324.6° (*c*, 1.0 in MeOH) (Found: C, 44.6; H, 4.0; N, 18.8. $C_{11}H_{12}N_4O_6$ requires C, 44.6; H, 4.1; N, 18.9%) {lit.,[2] m.p. 153—155°, $[\alpha]_D^{25}$ +307° (*c*, 0.18 in MeOH)}.

N¹-*Benzyloxycarbonyl*-N²-*trifluoroacetyl*-(R)-*piperazic Acid*.—*N*¹-(Z)-(*R*)-Piperazic acid (2.64 g, 0.01 mol) in trifluoroacetic acid (20 ml) was cooled to −10 °C and trifluoroacetic anhydride (1.67 ml, 0.012 mol) was added. After 0.5 h the solution was evaporated and the crude product dissolved in diethyl ether (80 ml), washed with water (4 × 30 ml), dried ($MgSO_4$), and evaporated to an oil. Crystallisation from light petroleum (b.p. 60—80°) yielded N¹-*Z*-N²-*TFA*-(R)-*piperazic acid* (3.38 g, 96%), m.p. 94—97°, $[\alpha]_D^{20}$ +72.0° (*c*, 1.0 in methanol) (Found: C, 50.2; H, 4.3; N, 7.7. $C_{15}H_{15}N_2O_5F_3$ requires C, 50.0; H, 4.2; N, 7.8%).

N¹-*Benzyloxycarbonyl*-(S)-*piperazic Acid t-Butyl Ester*.—*N*¹-(Z)-(*S*)-Piperazic acid (5.0 g, 19 mmol) in 1,4-dioxan (80 ml) was treated with sulphuric acid (2 ml) and isobutene (80 ml) for 4 days at room temperature. The solution was diluted with ether (250 ml), washed with 5% sodium

hydrogen carbonate solution (2 × 100 ml), dried over magnesium sulphate, and evaporated to yield N^1-Z-(*S*)-piperazic acid t-butyl ester as an oil (4.5 g, 74%), M^+ 320, R_F (system A) 0.7.

N^1-*Benzyloxycarbonyl*-N^2-*trifluoroacetyl*-(R)-*piperazyl*-N^1-*benzyloxycarbonyl*-(S)-*piperazic Acid t-Butyl Ester* (2).—N^1-(Z)-N^2-TFA-(*R*)-Piperazic acid (3.37 g, 9.38 mmol) in ether (45 ml) was treated with phosphorus pentachloride (1.95 g, 9.38 mmol) at 0 °C for 30 min and 15 min at 25 °C. The solution was evaporated to an oil which was dissolved in ether (50 ml) and added dropwise to a solution of N^1-Z-(*S*)-piperazic acid t-butyl ester (2.0 g, 6.25 mmol) and *N*-ethylmorpholine (1.98 ml, 15.6 mmol) in dry ether (45 ml) at −15 °C. The mixture was stirred at −15 °C for 1 h and then overnight at room temperature, diluted with ether (100 ml), washed with water (4 × 80 ml), dried ($MgSO_4$), and evaporated to an oil. Chromatography on silica gel (100 g) (ethyl acetate–benzene 1 : 19 as eluant), followed by treatment with light petroleum yielded N^1-*Z*-N^2-*TFA*-(R)-*piperazyl*-N^1-*Z*-(S)-*piperazic acid t-butyl ester* (2) (2.0 g, 48%), m.p. 95—97°, $[\alpha]_D^{20}$ −4.2° (*c*, 1.1 in MeOH) (Found: C, 58.1; H, 5.7; N, 8.5. $C_{32}H_{37}N_4O_8F_3$ requires C, 58.0; H, 5.6; N, 8.4%).

N^1-*Benzyloxycarbonyl*-N^2-*trifluoroacetyl*-(R)-*piperazyl*-N^1-*benzyloxycarbonyl*-(S)-*piperazic Acid* (3).—Compound (2) (1.9 g, 2.87 mmol) was stirred for 1 h in trifluoroacetic acid (10 ml) and evaporated. The residue in ether (100 ml) was washed with water (4 × 80 ml), dried, and evaporated to yield N^1-Z-N^2-TFA-(*R*)-piperazyl-N^1-Z-(*S*)-piperazic acid (3) as an oil (1.7 g, 97%), M^+ 606, R_F (system B) 0.8.

N-*Methyl*-D-*leucyl*-L-*proline t-Butyl Ester Toluene*-p-*sulphonate* (4).—*N*-Benzyloxycarbonyl-*N*-methyl-D-leucine (8.9 g, 32 mmol) in tetrahydrofuran (100 ml) at −10 °C, was treated with isobutyl chloroformate (4.34 ml, 32 mmol) and *N*-ethylmorpholine (4.05 ml, 32 mmol). The mixture was stirred for 15 min at −10 °C and a solution of proline t-butyl ester (5.46 g, 32 mmol) in *NN*-dimethylformamide (100 ml) was added. The mixture, after stirring for 1 h at 0 °C and overnight at room temperature, was evaporated to dryness. The residue was dissolved in ethyl acetate, washed with 5% citric acid, 5% sodium hydrogen carbonate, and water. dried (Na_2SO_4), and evaporated to an oil. Purification by chromatography in chloroform on silica gel (200 g), yielded *N*-Z-*N*-methyl-D-leucyl-L-proline t-butyl ester as an oil (10.5 g, 74%), M^+ 432, R_F (system C) 0.75. Hydrogenation, and the usual work-up yielded *N*-methyl-D-leucyl-L-proline t-butyl ester as an oil which was treated with toluene-*p*-sulphonic acid to give the *salt*, m.p. 170—171° (Found: C, 58.8; H, 8.1; N, 5.8. $C_{23}H_{38}N_2O_6S$ requires C, 58.7; H, 8.1; N, 5.9%).

N^1-*Benzyloxycarbonyl*-N^2-*trifluoroacetyl*-(R)-*piperazyl*-N^1-*benzyloxycarbonyl*-(S)-*piperazyl*-N-*methyl*-D-*leucyl*-L-*proline t-Butyl Ester* (5).—N^1-Z-N^2-TFA-(*R*)-Piperazyl-N^1-Z-(*S*)-piperazic acid (3) (1.7 g, 2.81 mmol) in anhydrous ether (25 ml) was cooled to 0 °C. Phosphorus pentachloride (0.584 g, 2.81 mmol) was added, the mixture was stirred at 0 °C for 30 min, the ice-bath was removed, and stirring was continued for a further 15 min. The solution was evaporated, phosphorus by-products were removed by the addition and evaporation of toluene (3 × 10 ml) and the residue dissolved in anhydrous ether (30 ml). The solution was added dropwise to a cooled solution (−15 °C) of *N*-methyl-D-leucyl-L-proline t-butyl ester toluene-*p*-sulphonate (1.32 g, 2.81 mmol) and *N*-ethylmorpholine (1.07 ml, 8.43 mmol) in dry tetrahydrofuran (25 ml). The mixture was stirred for 1 h at −15 °C, overnight at room temperature, and evaporated. The residue was dissolved in ether (80 ml), washed with 1M-hydrochloric acid (50 ml), water (50 ml), and 5% sodium hydrogen carbonate (50 ml), dried ($MgSO_4$), and evaporated to an oil. Chromatography in chloroform on silica gel (100 g) followed by Sephadex LH20 (40 × 4 cm) in methanol yielded the protected tetrapeptide (5) as a *foam* (0.86 g, 35%), $[\alpha]_D^{20}$ +15.1° (*c*, 0.2 in chloroform) (Found: C, 59.7; H, 6.3; N, 9.6. $C_{44}H_{57}N_6O_{10}F_3$ requires C, 59.6; H, 6.5; N, 9.5%).

N^1-*Benzyloxycarbonyl*-(R)-*piperazyl*-N^1-*benzyloxycarbonyl*-(S)-*piperazyl*-N-*methyl*-D-*leucyl*-L-*proline t-Butyl Ester* (9).—The protected tetrapeptide (5) (0.86 g, 0.97 mmol) in methanol (20 ml) was treated with 1M-sodium hydroxide solution (1.2 ml, 1.2 mmol) for 0.75 h at room temperature. The methanol was removed by evaporation, brine (50 ml) was added, and the resulting oil extracted into ether (3 × 40 ml). The organic layer was washed with brine (60 ml) and dried ($MgSO_4$). Evaporation yielded the partially protected tetrapeptide (8) as a *foam* (0.58 g, 76%) (M^+ 789) (Found: C, 63.6; H, 7.2; N, 10.5. $C_{42}H_{58}O_9N_6$ requires C, 63.8; H, 7.4; N, 10.6%).

t-Butyloxycarbonyl-D-*valyl*-L-*isoleucic Acid* 4-*Nitrobenzyl Ester* (6).—t-Butyloxycarbonyl-D-valine (4.56 g, 21.0 mmol) in dichloromethane (15 ml) was treated with a solution of 1,1-carbonyldi-imidazole (3.75 g, 23.1 mmol) in dichloromethane (10 ml). Isoleucic acid 4-nitrobenzyl ester (5.61 g, 21.0 mmol) was added and the mixture stirred for 2 days at room temperature and evaporated. The residue in ether (80 ml) was washed to neutrality in the usual way, dried ($MgSO_4$), and evaporated to an oil (9.7 g). Purification by chromatography in chloroform on silica gel (200 g) yielded the *ester* (6) as an oil (8.75 g, 89%), $[\alpha]_D^{21}$ −7.2° (*c*, 1.0 in benzene) (Found: C, 59.4; H, 7.3; N, 6.0. $C_{23}H_{34}N_2O_8$ requires C, 59.2; H, 7.3; N, 6.0%).

t-Butyloxycarbonyl-D-*valyl*-L-*isoleucic Acid* (7).—The 4-nitrobenzyl ester (6) (6.6 g, 14.15 mmol) in methanol (100 ml) was hydrogenated for 16 h over 10% palladium–carbon. The reaction was worked-up in the usual manner to give an oil, which crystallised on the addition of light petroleum (b.p. 40—60°). Recrystallisation from ether–light petroleum yielded the *acid* (3.8 g, 81%), m.p. 136—138°, $[\alpha]_D^{20}$ +20.9° (*c*, 1.05 in MeOH) (Found: C, 58.1; H, 8.9; N, 4.4. $C_{16}H_{29}NO_6$ requires C, 58.0; H, 8.8; N, 4.2%).

Protected Hexadepsipeptide (9).—t-Butyloxycarbonyl-D-valyl-L-isoleucic acid (0.73 g, 2.2 mmol) was converted into acid chloride by treatment with phosphorus pentachloride (0.46 g, 2.2 mmol) in ether. This was added to a solution of the partially protected tetrapeptide (8) (0.58 g, 0.73 mmol) and *N*-ethylmorpholine (0.28 ml, 2.2 mmol) in ether (45 ml) at −15 °C. The mixture was stirred for 1 h at −15 °C and overnight at room temperature, washed to neutrality with acid and base, dried ($MgSO_4$), and evaporated to an oil. Chromatography on Sephadex LH20 (methanol) and silica gel (chloroform) yielded pure protected linear hexadepsipeptide (9) (0.55 g, 68%) as a *foam*, $[\alpha]_D^{20}$ +13.8° (*c*, 0.4 in chloroform), M^+ 1 103 (Found: C, 62.6; H, 7.7; N, 8.8. $C_{58}H_{85}N_7O_{14}$ requires C, 63.1; H, 7.8; N, 8.9%).

Monamycin Congener (I, X).—The preceding protected hexapeptide (9) (0.45 g, 0.41 mmol) in methanol (45 ml) was hydrogenated for 16 h in the presence of 10% palladium–carbon (0.1 g). Purification of the product on silica gel (100 g) (2% methanol–chloroform) yielded partially protected hexadepsipeptide (0.26 g, 76%) as an *oil*, M^+ 835

(Found: C, 60.2; H, 8.7; N, 11.5. $C_{42}H_{73}N_7O_{10}$ requires C, 60.3; H, 8.7; N, 11.7%).

The above product (0.15 g, 0.18 mmol) in 4N-hydrogen bromide in acetic acid (2 ml) was stirred for 0.75 h and ether (15 ml) was added. The solid was dissolved in ethanol (3 ml) and treated with 1,2-epoxypropane (0.1 ml). Evaporation after 15 min yielded the deprotected linear sequence (10) as an oil, R_F (system B) 0.55, R_F (system E) 0.60 (ninhydrin positive).

The linear sequence in *NN*-dimethylformamide (3.5 ml) and dichloromethane (35 ml) was treated, at 0 °C, with *N*-hydroxysuccinimide (0.083 g, 0.72 mmol) and *NN'*-dicyclohexylcarbodi-imide (0.074 g, 0.36 mmol). The mixture was stirred at 0 °C for 2 h, then 72 h at room temperature, and evaporated. The residue was extracted with ether (3 × 10 ml), dried ($MgSO_4$), and evaporated to an oil. The crude product was purified on Sephadex LH20 (column: 35 × 2.4 cm) eluted with methanol. Crystallisation from methanol–water yielded *monamycin congener* (I, X) (0.046 g, 40%), m.p. 92—95°, $[\alpha]_D^{20}$ −39.8° (*c*, 0.2 in chloroform), R_F (system D) 0.60 (ninhydrin negative, iodine positive) (Found: C, 59.1; H, 8.4; N, 14.6%; *m/e* 661.418 7. $C_{33}H_{55}N_7O_7 \cdot 0.5H_2O$ requires C, 59.1; H, 8.4; N, 14.6%; *M*, 661.416 3). Amino-acid analysis (6M-HCl, 16 h at 95 °C): $Pip_{2.00}$; $NMe\cdot Leu_{0.97}$; $Pro_{0.81}$; $Val_{0.87}$.

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