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FIRST TOTAL SYNTHESES OF (+)-POLYOXIN B AND (+)-POLYOXIN D

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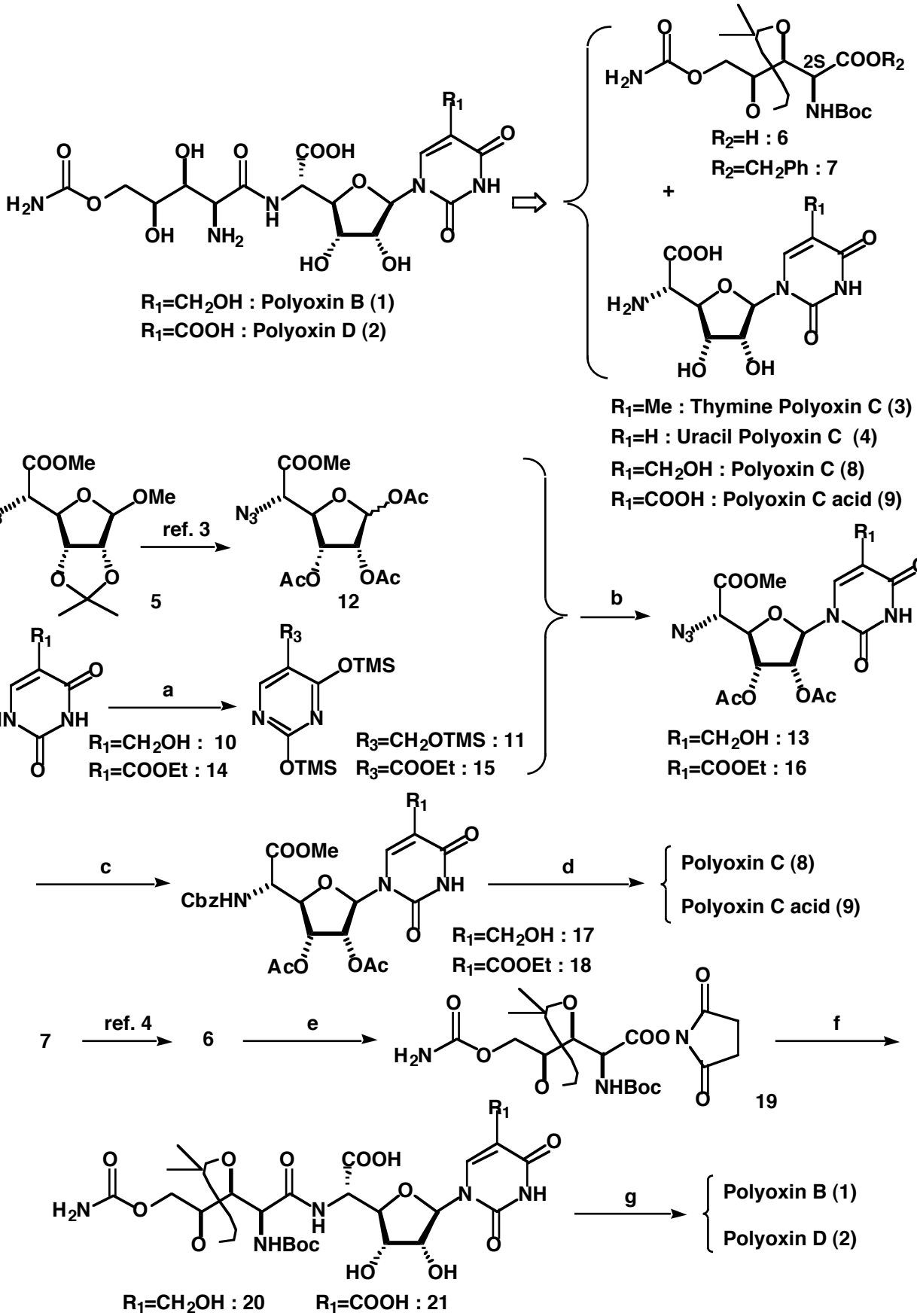
Abstract - First total syntheses of the peptidyl nucleoside antibiotics, polyoxins B (**1**) and D (**2**), are achieved based on the coupling reactions of the *N*-protected *L*-carbamoyl-polyoxamic acid derivative (**6**) with polyoxin C (**8**), **6** with polyoxin C acid (**9**), respectively.

Polyoxins B (**1**) and D (**2**) are a class of peptidyl nucleoside antibiotics isolated from the culture broths of *Streptomyces cacao*i var. *asoensis*.[1] All members of the polyoxin family possess 1-(5'-amino-5'-deoxy-β-*D*-allofuranuronosyl)pyrimidines such as thymine polyoxin C (**3**) or uracil polyoxin C (**4**) as a basic component. The biological activity of the polyoxins is very characteristic because of their specific action against phytopathogenic fungi and the human fungal pathogen (e.g. *Candida albicans*), and lack of activity against other microorganisms, plants, fish, and mammals.[1b] The site of action of the polyoxins was reported to be responsible for cell wall chitin biosynthesis.[2] In the preceding paper, we reported a short path synthesis of methyl (methyl-2,3-*O*-isopropylidene-α-*L*-talofuranoside)uronate (**5**) from methyl 2,3-*O*-isopropylidene-dialdo-*D*-ribofuranoside and its application to the total syntheses of thymine polyoxin C (**3**) and uracil polyoxin C (**4**).[3] We also reported a convenient synthesis of the *N*-protected *L*-carbamoyl-polyoxamic acid derivative (**6**) *via* the benzyl ester (**7**) which was obtained by a diastereoselective addition of vinylmagnesium bromide to 4-*O*-*tert*-butyldiphenylsilyl-2,3-isopropylidene-*L*-threose as a key step.[4] We now describe the first syntheses of polyoxin B (**1**) and polyoxin D (**2**) based on the condensation of **6** with polyoxin C (**8**), and that of **6** with polyoxin C acid (**9**), respectively.

At first, the syntheses of polyoxin C (**8**) and polyoxin C acid (**9**) are described. By applying the Vorbrüggen procedure,[5] the commercially available 5-hydroxymethyluracil (**10**) was treated with 1,1,1,3,3,3-hexamethyldisilazane and trimethylsilyl chloride to give the 5-trimethylsiloxymethyl-2,4-bis(trimethylsiloxy)pyrimidine (**11**) which reacted with the triacetate (**12**)[4] derived from **5** in the presence of trimethylsilyl trifluoromethanesufonate (TMSOTf) to afford exclusively the β-nucleoside (**13**) in 62% overall yield. Likewise, ethyl uracil 5-carboxylate (**14**)[6] was converted to the 2,4-



a; $(Me_3Si)_2NH$ /Me_3SiCl b; TMSOTf / MeCN c; 1) H_2 / 10% Pd-C 2) CbzCl
d; 1) LiOH / H_2O /THF 2) H_2 / 10% Pd-C e; DCC / *N*-hydroxysuccinimide
f; **8** / *N*,*N*-diisopropylethylamine or **9** / *N*,*N*-diisopropylamine g; CF_3COOH / H_2O / MeOH

bis(trimethylsiloxy) pyrimidine (**15**) which was condensed with the triacetate (**12**) to yield exclusively the β-nucleoside (**16**) in 98% overall yield. Hydrogenation of the azide (**13**) in the presence of 10% Pd-C gave the α-amino acid ester which was treated with benzyl chloroformate (CbzCl) in the presence of 7% $NaHCO_3$ to provide the Cbz derivative (**17**) in 61% overall yield. Alkaline hydrolysis of **17** followed by hydrogenation gave polyoxin C (**8**) ($[\alpha]_D$ +12.3° (c=0.6, H_2O), mp 261-265 °C) in 40% overall yield. The physical data of the synthesized **8** were consistent with the reported **8**[1a] ($[\alpha]_D$ +11.2° (c=0.5, H_2O), mp 260-267 °C). Conversion of **16** into polyoxin C acid (**9**; $[\alpha]_D$ +15.6° (c=0.72, 1M HCl), mp 242-250 °C) *via* the Cbz derivative (**18**) was achieved in 44% overall yield by the same way as for the preparation of **8** from **13**. The physical data of the synthetic **9** were identical with those ($[\alpha]_D$ +14.6° (c=1.15, 1M HCl), mp 240-260 °C) of the reported **9**.[1a] Catalytic deprotection of benzyl group in **7** gave the desired *N*-protected (2*S*)-carbamoyl-polyoxamic acid derivative (**6**).[4] Successful coupling of polyoxin C (**8**) with the *N*-protected (2*S*)-**6** was carried out by the *N*,*N*-dicyclohexylcarbodiimide-*N*-hydroxysuccinimide (DCC-HOSu) active ester method[7] in DMSO and *N*,*N*-diisopropylethylamine as the base. Thus, the treatment of polyoxamic acid derivative (**6**) with DCC-HOSu gave the active ester (**19**) which was condensed with **8** to afford the dipeptide (**20**) (81% from **7**). Removal of the *N*-Boc and *O*-isopropylidene protecting groups upon acid hydrolysis provided polyoxin B (**1**) ($[\alpha]_D$ +36.0° (c=0.52, H_2O), mp 150-153 °C) in 73% yield. The physical properties of the synthesized **1** were in good agreement with the literature of natural polyoxin B (**1**) ($[\alpha]_D$ +34.0° (c=1, H_2O)).[1b]

Likewise, condensation of the active ester (**19**) with polyoxin C acid (**9**) afforded the dipeptide (**21**) (50% from **7**) which was converted to polyoxin D (**2**) (($[\alpha]_D$ +30.6° (c=0.16, H_2O), mp 173-175 °C) in 90% yield. The physical properties ($[\alpha]_D$, ^{1}H-NMR and ^{13}C-NMR) of the synthesized **2** were identical with those ($[\alpha]_D$ +30° (c=1, H_2O),[1b] ^{1}H-NMR and ^{13}C-NMR) of natural polyoxin D (**2**) given by Dr. H. Osada. The syntheses described herein demonstrate an applicable synthesis of other components of polyoxin families.[1a]

EXPERIMENTAL

All melting points were measured on a Yanaco MP-S3 micro melting point apparatus and are uncorrected. NMR spectra were measured on a JEOL EX-400 spectrometer and spectra were taken as 5-10% (W/V) solutions in $CDCl_3$ with Me_4Si as an internal reference. IR spectra were measured on a JASCO FT/IR-300 spectrophotometer. FAB-MS were obtained with a JEOL JMS-DX 303 instrument (matrix: glycerol and *m*-nitrobenzyl alcohol (NBA)). Optical rotations were measured on a JASCO DIP-370 digital polarimeter. A11 the reactions were carried out in an atmosphere of argon. A11 evaporations were performed under reduced pressure.

5-Hydroxymethyl-1-(methyl 2',3'-di-*O*-acetyl-5'-azido-5'-deoxy-β-D-allofuranosyl uronate)uracil (13) A mixture of 5-hydroxymethyluracil (**10**) (355 mg, 2.5 mmol), 1,1,1,3,3,3-hexamethyldisilazane (9 mL, 42.8 mmol) and trimethylsilyl chloride (22 mg, 0.2 mmol) was refluxed for 12 h, cooled and evaporated. The residue **11** was dissolved in MeCN (3 mL) and the resulting clear solution was added

to a solution of **12** (344 mg, 0.93 mmol) in MeCN (2 mL). Trimethylsilyl trifluoromethanesulfonate (222 mg, 1.0 mmol) was added to the above reaction mixture and the reaction mixture was stirred at reflux for 1 h. The reaction mixture was diluted with $CHCl_3$ (30 mL) and the organic layer was washed with 7% $NaHCO_3$ (20 mL), dried ($MgSO_4$) and evaporated. The residue was chromatographed on silica gel (20 g) with $CHCl_3$/MeOH (40:1) to give **13** (262 mg, 62%) as a colorless oil. **13**: $[\alpha]_D^{28}$ –54.2° (c=1.3, $CHCl_3$); IR(KBr): 3020, 2117, 1751, 1689, 1468 cm^{-1}; ^1H NMR: δ 2.08 (s, 3H), 2.12 (s, 3H), 3.07 (br s, 1H), 3.86 (s, 3H), 4.44 (s, 2H), 4.47 (t, 1H, J=3 Hz), 4.55 (d, 1H, J=3 Hz), 5.35 (t, 1H, J=6.5 Hz), 5.40 (dd, 1H, J=3, 6.5 Hz), 6.20 (d, 1H, J=6.5 Hz), 7.66 (s, 1H), 9.57 (br s, 1H). MS(FAB): m/z=442 (M^++1). HRMS(FAB, matrix: glycerol): calcd for $C_{16}H_{20}N_5O_{10}$ (M^++1) 442.1210; found 442.1193

5-Ethoxycarbonyl-1-(methyl 2',3'-di-*O*-acetyl-5'-azido-5'-deoxy-β-D-allofuranosyluronate) uracil (16) A mixture of ethyl uracil-5-carboxylate (**14**) (500 mg, 3.21mmol), 1,1,1,3,3,3-hexamethyl-disilazane (10 mL, 47.5 mmol) and trimethylsilyl chloride (30 mg, 0.28 mmol) was refluxed for 4 h, cooled and evaporated. The residue (**15)** was dissolved in MeCN (3 mL) and the resulting clear solution was added to a solution of **12** (515 mg , 1.43 mmol) in MeCN (3 mL). Trimethylsilyl trifluoromethanesulfonate (250 mg, 1.13 mmol) was added to the above reaction mixture and the reaction mixture was stirred at reflux for 1 h. The reaction mixture was diluted with $CHCl_3$ (20 mL) and the organic layer was washed with 3% $NaHCO_3$ (16 mL), dried ($MgSO_4$) and evaporated. The residue was chromatographed on silica gel (20 g) with $CHCl_3$/MeOH (50:1) to give **16** (679 mg, 98%) as a colorless oil. **16**: $[\alpha]_D^{26}$ –78.6° (c=0.91, $CHCl_3$); IR(KBr): 2121, 1749, 1238, 1089 cm^{-1}; ^1H NMR: δ =1.36 (t, J=6.8 Hz, 3H), 2.09 (s, 3H), 2.13 (s, 3H), 3.89 (s, 3H), 4.36 (q, J=6.8 Hz, 2H), 4.52 (t, 1H, J=2.9 Hz), 4.61 (d, 1H, J=2.9 Hz), 5.36 (t, 1H, J=6.3 Hz), 5.40 (dd, 1H, J=2.9, 6.3 Hz), 6.21 (d, 1H, J=6.8 Hz), 8.72 (s, 1H), 8.90 (br s, 1H). *Anal.* Calcd for $C_{18}H_{21}N_5O_{11}$: C, 44.72; H, 4.38; N, 14.49. Found: C, 44.48; H, 4.11; N, 14.20. MS(FAB): m/z=522 (M^++K).

5-Hydroxymethyl-1-(methyl 2',3'-di-*O*-acetyl-5'-carbenzoloxyamino-5'-deoxy-β-D-allofuranosyl-uronate)uracil (17) A mixture of **13** (170 mg, 0.39 mmol) and 10% Pd-C (20 mg) in MeOH (6 mL) was subjected to catalytic hydrogenation at ambient temperature and the reaction mixture was filtered with the aid of Celite. The filtrate was evaporated to give the residue. A mixture of the residue, benzyl chloroformate (30% in toluene, 0.3 mL, 0.53 mmol), and 7% $NaHCO_3$ (15 mL) in dioxane (4 mL) was stirred at rt for 1 h, and diluted with brine (30 mL). The reaction mixture was extracted with EtOAc (2 x 20 mL), dried ($MgSO_4$) and evaporated. The residue was chromatographed on silica gel (10 g) with $CHCl_3$/MeOH (50:1) to afford **17** (129 mg, 61%) as a colorless oil. **17**: $[\alpha]_D^{25}$ +17.5° (c=0.4, $CHCl_3$); IR(KBr): 3429, 1711, 1239, 1056 cm^{-1}; ^1H NMR: δ 2.08 (s, 6H), 3.80 (s, 3H), 4.35 (br s, 2H), 4.41 (dd, 1H, J=3.8, 5.8 Hz), 4.86 (dd, 1H, J=3.8, 8.7 Hz), 5.11 (d, J=12.3 Hz, 1H), 5.16 (d, J=12.3 Hz, 1H), 5.32 (t, 1H, J=5.8 Hz), 5.52 (t, 1H, J=5.8 Hz), 5.96 (d, 1H, J=5.8 Hz), 6.08 (br s, 1H), 7.34 (m, 6H), 9.27 (br. s, 1H). *Anal.* Calcd for $C_{24}H_{27}N_3O_{12}\cdot H_2O$: C, 50.36; H, 4.58; N, 7.16. Found: C, 50.78; H, 5.14; N, 7.41. MS(FAB): m/z=550 (M^++1).

1-(5'-Amino-5'-deoxy-β-D-allofuranosyluronic acid)-5-hydroxymethyluracil (Polyoxin C) (8) A mixture of **17** (850 mg. 1.55 mmol) and $LiOH \cdot H_2O$ (358 mg, 8.52 mmol) in a mixed solvent (THF/H_2O (4:1), 25 mL) was stirred at O°C for 4.5 h. The reaction mixture was diluted with saturated brine (20 mL), H_2O (20 mL) and extracted with CH_2Cl_2 (50 mL). The water layer was adjusted with 10% aqueous HCl (8 mL) to pH 2-3 and extracted with EtOAc (5 x 30 mL). The organic layer was dried ($MgSO_4$) and evaporated. A mixture of the residue (390 mg) and 10% Pd-C (80 mg) in MeOH (10 mL) was subjected to catalytic hydrogenation at ambient temperature and the reaction mixture was filtered with the aid of Celite (50 mg) and activated carbon (30 mg). The residue was washed with hot water (120 ml x 3) and the combined filtrate and washing was evaporated to give **8** (220 mg, 40% overall yield). A part of **8** was crystallized from H_2O to give colorless needles. **8**: mp 261-265°C; $[\alpha]_D^{27}$ +12.3° (c=0.6, H_2O); IR(KBr): 3368, 3077, 1689, 1628,1477, 1057 cm^{-1}; ^1H NMR (*ca.* 3% DCl in D_2O, TPS-d_4 (sodium 2,2,3,3-tetradeuterio-4,4-dimethyl-4-silapentane sulfonate) as an internal reference): δ 4.39 (dd, 1H, J=2.4, 6.6 Hz), 4.45 (dd, 1H, J=3.9, 6.6 Hz), 4.59 (d, 1H, J=2.4 Hz), 4.70 (t, 1H, J=6.6 Hz), 5.79 (d, 1H, J=3.9 Hz), 7.54 (s, 1H). ^{13}C NMR (D_2O): δ 58.1 (d), 59.3 (t), 72.3 (d), 75.5 (d), 85.0 (d), 92.8 (d), 116.9 (s), 143.1 (d), 154.6 (s), 167.7 (s), 172.9 (s). *Anal.* Calcd for $C_{11}H_{15}N_3O_8 \cdot 1/2\ H_2O$: C, 40.46; H, 4.94; N, 12.88. Found: C, 40.19; H, 4.47; N, 12.80. HRMS(FAB, matrix: glycerol): calcd for $C_{11}H_{16}N_3O_8$ (M^++1) 318.0937; found 318.0943

5-Ethoxycarbonyl-1-(methyl 2',3'-di-*O*-acetyl-5'-carbobenzyloxyamino-5'-deoxy-β-D-allofuranosyluronate)uracil (18) A mixture of **16** (3 g, 6.21 mmol) and 10% Pd-C (900 mg) in AcOEt (120 mL) was subjected to catalytic hydrogenation at ambient temperature and the reaction mixture was filtered with the aid of Celite. The filtrate was evaporated to give the residue. A mixture of the residue, benzyl chloroformate (30% in toluene, 4.5 ml, 7.91 mmol), and 7% $NaHCO_3$ (25 mL) in dioxane (80 mL) was stirred at rt for 30 min. The reaction mixture was diluted with 7% $NaHCO_3$ (100 mL), and extracted with EtOAc (3 x 100 mL). The organic layer was washed with saturated brine (100 mL), dried ($MgSO_4$) and evaporated. The residue was chromatographed on silica gel (60 g) with $CHCl_3$/MeOH (100:1) to afford **18** (3.2 g, 87%) as a colorless oil. **18**: $[\alpha]_D^{26}$ +18.45° (c=0.91, $CHCl_3$); IR(KBr): 3413, 1726, 1239 cm^{-1}; ^1H NMR: δ 1.33-1.37 (m, 3H), 2.09 (s, 6H), 3.84 (s, 3H), 4.36-4.30 (m, 2H), 4.43 (t, 1H, J=5.1, Hz), 4.87 (br d, 1H, J=5.5 Hz), 5.14 (s, 2H), 5.35 (t, 1H, J=6.4 Hz), 5.61 (t, 1H, J=6.2 Hz), 5.84 (br s, 1H), 5.87 (d, 1H, J=4.6 Hz), 7.35 (br s, 5H), 8.21 (s, 1H), 9.23 (br. s, 1H). *Anal.* Calcd for $C_{26}H_{29}N_3O_{13} \cdot H_2O$: C, 51.22; H, 5.13; N, 6.90. Found: C, 51.27; H, 4.71; N, 7.34. MS(FAB): m/z=630 (M^++K).

5-Carboxy-1-(5'-amino-5'-deoxy-β-D-allofuranosyluronic acid)uracil (Polyoxin C acid) (9) A mixture of **18** (250 mg. 0.42 mmol) and $LiOH \cdot H_2O$ (178 mg, 4.24 mmol) in a mixed solvent (THF/H_2O (5:1), 12 mL) was stirred at O°C for 10 h. The reaction mixture was diluted with H_2O (20 mL) and extracted with CH_2Cl_2 (30 mL). The water layer was adjusted with 10% aqueous HCl (8 mL) to pH 2-3 and extracted with EtOAc (5 x 30 mL). The organic layer was dried ($MgSO_4$) and evaporated. A

mixture of the residue and 10% Pd-C (32 mg) in MeOH (5 mL) was subjected to catalytic hydrogenation at ambient temperature and the reaction mixture was filtered with the aid of Celite (50 mg) and activated carbon (30 mg). The residue was washed with hot water and the combined filtrate and washing was evaporated to give **9** (71 mg, 50% overall yield). A part of **9** was crystallized from H_2O to give colorless needles. **9**; mp 242-250°C; $[\alpha]_D^{24}$ +15.6° (c=0.72, 1M HCl); IR(KBr): 3459, 1712, 1646, 1052 cm^{-1}; ^1H NMR (D_2O): δ 4.29 (dd, 1H, J=2.4, 7.3 Hz), 4.34 (dd, 1H, J=3, 5.9 Hz), 4.45 (d, 1H, J=2.4 Hz), 4.57 (t, 1H, J=6.6 Hz), 5.68 (d, 1H, J=3 Hz), 8.37 (s, 1H). ^{13}C NMR (D_2O):=53.0 (d), 68.5 (d), 72.3 (d), 80.3 (d), 92.5 (d), 102.8 (s), 149.7 (s), 150.0 (d), 163.4 (s), 165.1 (s), 167.9 (s). *Anal.* Calcd for $C_{11}H_{139}N_3O_9 \cdot 1/3H_2O$: C, 39.17; H, 4.08; N, 12.46. Found: C, 39.19; H, 3.71; N, 12.49. HRMS(FAB, matrix: glycerol): calcd for $C_{11}H_{14}N_3O_9$ (M^++1) 332.0730; found 332.0715.

Polyoxin B (1) i) A mixture of **7** (228 mg, 0.52 mmol) and 10% Pd-C (15 mg) in MeOH (10 mL) was subjected to catalytic hydrogenation at ambient temperature for 1 h and the reaction mixture was filtered with the aid of Celite. The filtrate was evaporated to give a crude carboxylic acid (**6**). To a solution of a crude carboxylic acid (**6**) in AcOEt (9 mL) was added *N*-hydroxysuccinimide (63 mg, 0.55 mmol) and *N*,*N*-dicyclohexylcarbodiimide (113 mg, 0.55 mmol), and the reaction mixture was stirred for 1 h at rt. The reaction mixture was evaporated to give a crude residue **19** which was dissolved in DMSO (2 mL). A solution of polyoxin C (**8**) (157 mg, 0.5 mmol) and *i*-Pr_2NEt (67.2 mg, 0.52 mmol) in DMSO (2 mL) was added to the above DMSO solution and whole mixture was stirred for 24 h at rt. The reaction mixture was directly chromatographed on silica gel (15 g) with $CHCl_3$/MeOH (3:1 – 1:1) to give polyoxin B derivative (**20**) (274 mg, 81%) as colorless amorphous product. **20**: mp 169~170 °C; $[\alpha]_D^{25}$ +7.27° (c=0.39, MeOH); IR(KBr): 3428, 1708, 1390 cm^{-1}; ^1H NMR (CD_3OD): δ 1.36 (s, 3H), 1.37 (s, 3H), 1.46 (s, 9H), 3.94-3.98 (m, 1H), 4.14-4.18 (m, 3H), 4.24 (dd, J=4.3, 12.2 Hz, 1H), 4.27 (t, J=3.3 Hz, 1H), 4.37 (s, 2H), 4.30-4.43 (m, 1H), 4.45-4.48 (m, 1H), 4.55 (d, J=3.1 Hz, 1H), 5.93 (d, J=6.4 Hz, 1H), 7.81 (s, 1H). MS(FAB): m/z=686 (M^++K). HRMS(FAB, matrix: NBA): calcd for $C_{25}H_{37}N_5O_{15}$ Na (M^+) 670.2184; found 670.2224. ii) To a solution of polyoxin B derivative (**20**) (68 mg, 0.11 mmol) in MeOH (1 mL) and H_2O (1 mL) at 0 °C was added CF_3CO_2H (1 mL, 13 mmol) and whole mixture was stirred for 3 h at rt. The reaction mixture was evaporated to give a residue, which was chromatographed on silica gel (4 g) with $CHCl_3$/MeOH (2:1 – 1:1) to give an amorphous product. It was dissolved in H_2O (2 mL) and filtered with the aid of Celite (100 mg) and activated carbon (50 mg). The filtrate was evaporated to afford **1** (39mg, 73%) as an amorphous product. **1**; mp 150-153 °C; $[\alpha]_D^{28}$+36.0° (c=0.52, H_2O); IR(KBr): 3429, 1694, 1404, 1273 cm^{-1}; ^1H NMR (D_2O): δ 3.83-3.93 (m, 2H), 3.97-4.01 (m, 3H), 4.17 (t, J=4.9 Hz, 1H), 4.18 (t, J=5.7 Hz, 1H), 4.24 (s, 2H), 4.33 (t, J=5.4 Hz, 1H), 4.47 (d, J=4 Hz, 1H), 5.75 (d, J=5.5 Hz, 1H), 7.60 (s, 1H). ^{13}C NMR (D_2O): δ 55.8 (d), 56.0 (t), 56.2 (d), 65.1 (t), 68.1 (d), 69.3 (d), 69.5 (d), 72.5 (d), 83.9 (d), 88.4 (d), 113.5 (s), 139.3 (d), 151.3 (s), 158.6 (s), 164.4 (s), 170.5 (s), 173.3 (s). MS(FAB): m/z=546 (M^++K).

Polyoxin D (2) i) A mixture of **7** (240 mg, 0.55 mmol) and 10% Pd-C (26 mg) in MeOH (10 mL) was subjected to catalytic hydrogenation at ambient temperature for 2 h and the reaction mixture was filtered

with the aid of Celite. The filtrate was evaporated to give a crude carboxylic acid (**6**). To a solution of a crude carboxylic acid (**6**) in AcOEt (10 mL) was added *N*-hydroxysuccinimide (63 mg, 0.55 mmol) and *N*,*N*-dicyclohexylcarbodiimide (113 mg, 0.55 mmol), and the reaction mixture was stirred for 3 h at rt. The reaction mixture was evaporated to give a crude residue **19** which was dissolved in DMSO (8 mL). A solution of polyoxin C acid (**9**) (181 mg, 0.55 mmol) and *i*-Pr_2NEt (149 mg, 1.16 mmol) in DMSO (2 mL) was added to the above DMSO solution and whole mixture was stirred for 12 h at rt. The reaction mixture was directly chromatographed on silica gel (10 g) with $CHCl_3$/MeOH (3:1 – 1:1) to give polyoxin D derivative (**21**) (182 mg, 50%) as colorless amorphous product. **21**: mp 185-188 °C; $[\alpha]_D^{27}$ –31.63° (c=0.43, $CHCl_3$); IR(KBr): 3407, 1693, 1391, 1161 cm^{-1}; ^1H NMR (D_2O): δ 1.20 (s, 3H), 1.21 (s, 3H), 1.30 (s, 9H), 3.91-4.00 (m, 1H), 4.03 (dd, J=4.6, 12.2 Hz), 4.11-4.22 (m, 3H), 4.28-4.39 (m, 3H), 4.45-4.50 (m, 1H), 5.74 (d, J=4.6 Hz, 1H), 8.13 (s, 1H). ^{13}C NMR (D_2O): δ 25.3 (q), 25.5 (q), 29.8 (q), 53.9 (d), 55.5 (d), 62.9 (t), 69.0 (d), 72.9 (d), 75.1 (d), 75.9 (d), 81.2 (s), 84.4 (d), 88.3 (d), 110.3 (s), 110.3 (s), 145.9 (d), 150.6 (s), 156.8 (s), 158.3 (s), 158.3 (s), 170.2 (s), 172.9 (s), 176.3 (s). MS(ESI-positive): m/z=662 (M^++1). ii) To a solution of polyoxin D derivative (**21**) (150 mg, 0.23 mmol) in MeOH (2 mL) and H_2O (2 mL) at 0 °C was added CF_3COOH (2 mL, 26 mmol) and whole mixture was stirred for 3 h at rt. The reaction mixture was evaporated to give a residue, which was chromatographed on chromatorex (ODS, 4 g) with H_2O to give **2** (107 mg, 90%) as amorphous product. **2**: mp 173-175 °C; $[\alpha]_D^{26}$+30.63° (c=0.16, H_2O); IR(KBr): 3377, 1681, 1202, 1138 cm^{-1}; ^1H NMR (D_2O): δ 3.98-4.03 (m, 3H), 4.11 (d, J=4.4 Hz, 1H), 4.25-4.33 (m, 4H), 4.85 (d, J=3.5 Hz, 1H), 5.72 (d, J=3.5 Hz, 1H), 8.38 (s, 1H). ^{13}C NMR (D_2O): δ 53.8 (d), 55.9 (d), 64.8 (t), 68.0 (d), 68.9 (d), 69.5 (d), 72.9 (d), 82.2 (d), 90.7 (d), 103.5 (s), 148.9 (d), 149.8 (s), 158.6 (s), 162.6 (s), 167.8 (s), 171.2 (s), 176.0 (s). MS(ESI positive): m/z=522 (M^++1).

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