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Biotransformation of a biosynthetic intermediate mimic of nonactin by *Streptomyces griseus*

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Abstract

A mimic of a plausible biosynthetic intermediate of bishomononactic acid, one of the monomers of macrotetrolide antibiotics (polynactin), was synthesized as a thiol ester. FAB-MS analysis showed that fermentation of polynactin producing *Streptomyces griseus* with the compound, afforded the unnatural polynactins containing bishomononactic acid(s).

Keywords: antibiotics; bishomononactic acid; biotransformation; polynactin; *Streptomyces griseus*; synthesis.

Introduction

The macrotetrolide ionophore antibiotic 'polynactin' family **1**, which has been isolated from various *Streptomyces* species (Keller-Schierlein and Gerlach, 1967; Ando et al., 1971), is composed of both enantiomers of nonactic acid **2a**, homononactic acid **2b** or bishomononactic acid **2c** arranged in an alternating order (Figure 1).

The macrolides and monomers show a wide variety of biological activities (Zizka, 1998) such as antimicrobial, fungicidal, acaricidal, immunosuppressive etc. During the course of the synthetic studies of new tetramer analogs, we synthesized macrotetrolides α **1j** (Hanadate et al., 2001; Takai et al., 2011) and β **1k** (Takai et al., 2006), however, the synthesis needed over 30 chemical steps. On the other hand, a mixture of the parts **1a-1e** is used as an acaricide by effective fermentative production. Recently, their unique biosynthetic pathway was revealed (Rong et al., 2010) (Scheme 1). Assembly of the various acyl CoA fragments led to diketone **A**. Successive anti-reduction affords the enantiomeric pair diols **B** and **B'**, which are cyclized to give nonactic acid thiol esters **C** and **C'**, respectively. These monomers are alternatively assembled to form nonactin **1a**. Very recently, fermentative production of polynactin congeners by addition of various acyl CoA derivatives was reported (Rezanka et al., 2010). We assumed that a mimic of **B** or **B'** could also be incorporated into the enzyme system of this pathway, to produce new tetramer analogs effectively. Thus, a racemic analog with a larger isopropyl substituent **3**, which would lead to macrotetrolides B-G and α or new analogs, was designed for the biotransformation. This paper describes the synthesis and biotransformation of **3**.

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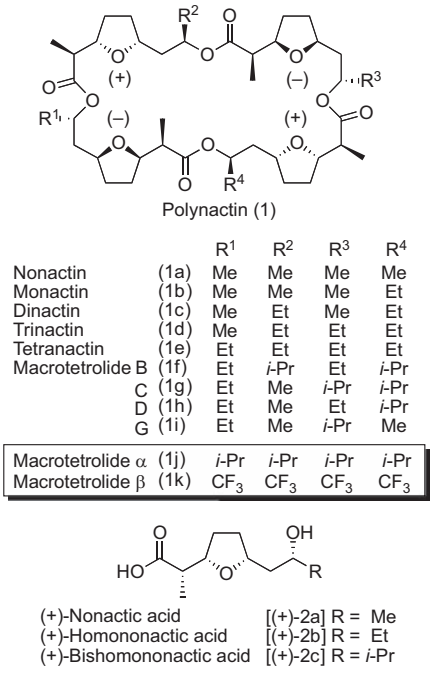
Results and discussion

Scheme 2 shows the synthetic route towards **3**. Regioselective deprotonation of methyl isopropyl ketone, followed by the addition of commercially available aldehyde **4,** afforded aldol product **5**. Stereoselective reduction of the keto group in **5** was accomplished using $Me_4NHB(OAc)_3$ (Evans et al., 1986) to give **6** (*anti*/*syn*=95:5) in 86% yield after separation of the stereoisomers. The anti-relationship of the dihydroxy functionality was determined by ^{13}C NMR analysis of the corresponding acetonide (**16**, *vide infra*). Protection of the two hydroxy groups by TBS groups **7**, deprotection of the Bn group (**8**), followed by Swern oxidation, afforded aldehyde **9**, which was subjected to the modified Horner-Wadsworth-Emmons reaction (Rathke and Nowak, 1985) to give **10** (*E*/*Z*=9:1, separated by SiO_2 chromatography). The geometry of the isomers was determined by observation of a strong NOE correlation between 3-H and 2-Me for (*Z*)-**10**. Hydrolysis of the ethoxycarbonyl group furnished carboxylic acid **11**. On the other hand, synthesis of the thiol fragment suffered from oxidative dimerization of products, such as the formation of **13** from **12**. Thus, thiol **14** was prepared *in situ* by the reduction of **13** and was subjected to condensation with **11** to afford the thiol ester **15** in 73% yield. Finally, removal of the two TBS groups gave the desired substrate **3**.

In order to determine the stereochemistry of the anti-diol moiety, **6** was converted to an acetonide **16** (Scheme 3). Then, the C_2-symmetrical nature of the dioxane ring of **16** was elucidated by ^{13}C NMR chemical shift of the geminal methyl groups (24.3 and 24.6 ppm), and that of the quaternary carbon (100.2 ppm) which is typical for this kind of structure (Kocienski, 2005). In a similar manner as described for the TBS derivatives, **16** was converted to **3**.

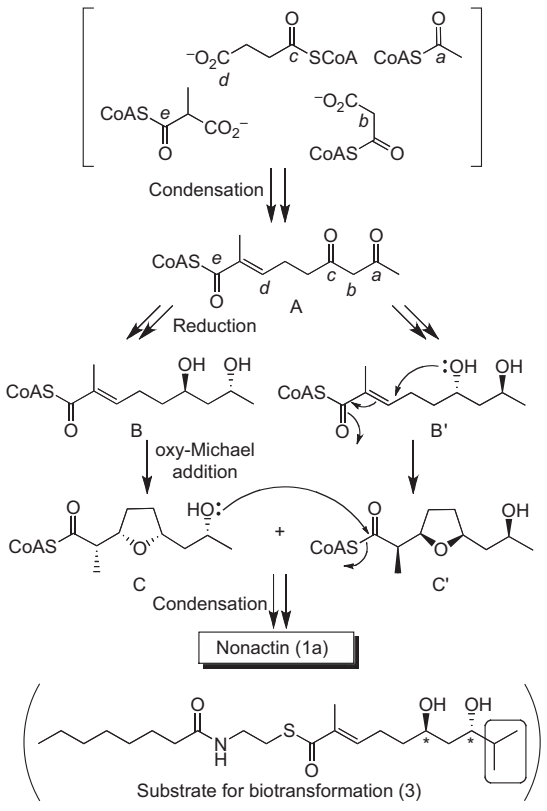
Priestley has reported the synthesis of the inhibitor **18** of polynactin biosynthesis (Earle and Priestley, 1997). To stop the polyketide biosynthesis upstream toward **A**, **B** and **B'**, we also prepared the analog **20** in a different manner (Scheme 4). Aldol reaction of acetone with **4** afforded **21** (Nogawa et al., 2006), which was converted to aldehyde **22** with anti-oxygen functions. Treatment of **22** with Ohira reagent (Ohira, 1989) gave the intermediate product **23**, the chain elongation of which gave carboxylic acid **24**. Deprotection of the thioester derivative **25** furnished Priestley's diol **20**.



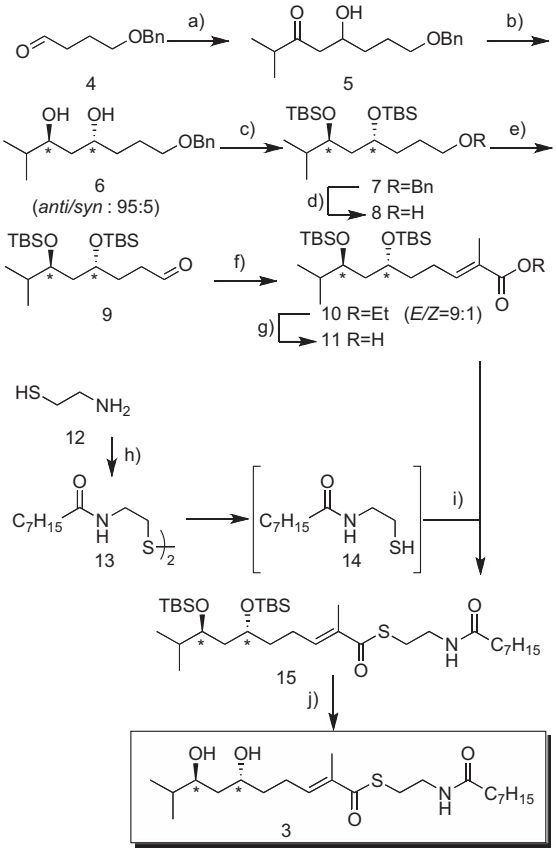
		R^1	R^2	R^3	R^4
Nonactin	(1a)	Me	Me	Me	Me
Monactin	(1b)	Me	Me	Me	Et
Dinactin	(1c)	Me	Et	Me	Et
Trinactin	(1d)	Me	Et	Et	Et
Tetranactin	(1e)	Et	Et	Et	Et
Macrotetrolide B	(1f)	Et	*i*-Pr	Et	*i*-Pr
C	(1g)	Et	Me	*i*-Pr	*i*-Pr
D	(1h)	Et	Me	Et	*i*-Pr
G	(1i)	Et	Me	*i*-Pr	Me
Macrotetrolide α	(1j)	*i*-Pr	*i*-Pr	*i*-Pr	*i*-Pr
Macrotetrolide β	(1k)	CF_3	CF_3	CF_3	CF_3

Figure 1 Polynactin antibiotics.

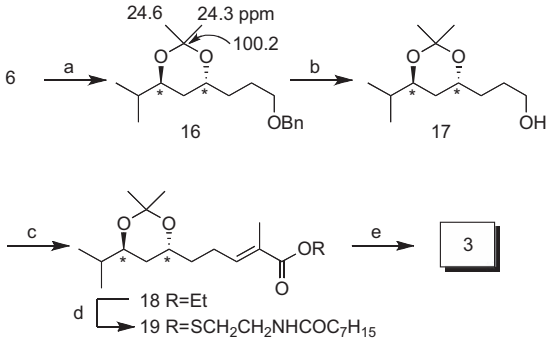
Biotransformation was performed using *Streptomyces griseus* ETH 7796. The pre-incubated strain (Bacto-tryptone, yeast extract, and maltose media, 30°C, 2 days) was incubated with and without the compounds (A: control, B: **3**, C: **3+20**, and D: **20**, each 17 mM) for 6 days by a rotary shaker, and each culture broth was filtrated through a Celite pad. The pad was washed with acetone and the washings were combined with the filtrate. Each combined organic layer was concentrated *in vacuo* and silica gel chromatography gave the crude polynactin mixture (Table 1) which was analyzed by FAB-MS (Figure 2). Addition of the inhibitor **20** (entries 3 and 4) significantly diminished the yield of polynactin, indicating that **20** actually inhibits the biosynthesis. Unexpectedly, addition of the mimic **3** also decreased the yield (entry 2) as compared with control (entry 1). The mimic **3,** with a bulky isopropyl substituent, would partly act as an inhibitor as well as the substrate. Figure 2 (A) indicates that the control fraction contained an almost equal amount of nonactin (**1a**, *m/z* 759) and monactin (**1b**, *m/z* 773). The signal (*m/z* 787) is for dinactin **1c**. On the contrary, the signals corresponding to **1a** and **1b** decreased in Figure 2 (B), and the larger peaks (*m/z* 787, 801, and 815) were predominant. These peaks could be assigned to unnatural polynactin with R^{1-4}=Me_3 and *i*-Pr (*m/z* 787 $[M+Na]^+$), R^{1-4}=Me_2, Et and *i*-Pr (*m/z* 801 $[M+Na]^+$), and R^{1-4}=Me_2 and



Scheme 1 Biosynthetic pathway of polynactin antibiotics.



Scheme 2 Synthesis of the substrate for biotransformation-1.
a) LDA, MeC(=O)*i*-Pr (88%). b) $Me_4NHB(OAc)_3$, AcOH, MeCN (86%). c) TBSCl, imidazole, DMF (88%). d) H_2, Pd/C, MeOH (89%). e) Swern oxi. f) $(EtO)_2P(=O)CHMeCO_2Et$, LiBr, Et_3N, THF (78%, 2 steps). g) 2 M aqueous KOH. h) i. 15% aqueous NaOH, Et_2O, 10°C. ii. $C_7H_{15}COCl$ (74%). i) Bu_3P, N_2, DMF, H_2O then **11**, EDCI, DMAP (73% from **10**). j) $BF_3{\cdot}Et_2O$, CH_2Cl_2, 0°C to room temperature (90%).



Scheme 3 Synthesis of the substrate for biotransformation-2.
a) 2,2-dimethoxypropane, PPTS (quant.). b) H_2, Pd/C, $NaHCO_3$, EtOH (54%). c) i. Swern oxi. ii. $Ph_3P{=}CMeCO_2Et$, toluene, reflux (98%). d) i. 1 M aqueous KOH-MeOH-THF. ii. **14**, EDCI, DMAP, CH_2Cl_2 (61%). e) PPTS, MeOH (quant.).

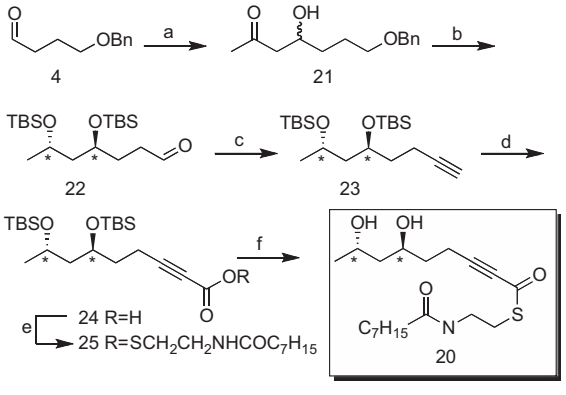
i-Pr_2 or Me, Et and *i*-Pr (*m/z* 815 $[M+Na]^+$) considering the amount of other natural polynactin congeners. A weak signal 872 $[M+Na]^+$ corresponding to macrotetrolide α **1j** was also detected. Although a total amount of polynactin production decreased, the designed mimic **3** would be incorporated by the enzyme system.

In conclusion, diol **3**, a mimic of biosynthetic intermediate of macrotetrolide antibiotics, was synthesized as a racemate for biotransformation. Although partly acting as a biosynthetic inhibitor, **3** could be incorporated into the enzyme system to produce polynactin congeners.

Experimental

General

FT-IR spectra were recorded for films on a Jasco 4100 spectrometer (ATR, Zn-Se). ^1H and ^{13}C NMR spectra were recorded with a Varian Gemini 2000 (300 MHz for ^1H and 75 MHz for ^{13}C) spectrometer in $CDCl_3$ with tetramethylsilane (δ_H 0 ppm) and $CHCl_3$ (δ_C 77.00 ppm) as internal standards. Mass spectra were recorded with a Jeol JMS–700 spectrometer. Merck silica gel 60 (70–230 mesh) was used for column chromatography. Merck silica gel 60 F_{254} (0.50 mm thickness) was used for preparative TLC. *Streptomyces griseus* ETH 7796 was purchased from Deutsche Sasmmlung von Mikroorganismen und Zellkulturen GmbH.



Scheme 4 Synthesis of the biosynthesis inhibitor.
a) i. LDA, acetone, THF, -78°C (74%). ii. b) i. $Me_4NBH(OAc)_3$, AcOH, MeCN (86%, *anti/syn*=95:5). ii. TBSCl, imidazole, DMF (80%). iii. H_2, Pd/C, MeOH, iv. Swern oxi. c) Ohira reagent, K_2CO_3, MeOH (72%, 3 steps). d) BuLi, CO_2(s), THF. e) **13**, Bu_3P, DMF, H_2O then EDCI, DMAP (54%, 2 steps). f) AcOH-THF-H_2O (85%).

Table 1 Biotransformation of the synthetic compounds by *S. griseus* ETH 7796.

Entry	Substrates	Yield (mg/100 ml broth)
1	None	67
2	**3**	5
3	**3**+**20**	<1
4	**20**	2

8-Benzyloxy-5-hydroxy-2-methyloctan-3-one (5)

To a solution of LDA [lithium diisopropylamide (ca. 88 mmol, prepared from diisopropylamine (12 ml, 88 mmol) and BuLi (1.6 M in hexane, 55 ml, 88 mmol)] in dry THF (tetrahydrofuran, 100 ml) was added dropwise 3-methylbutan-2-one (6.7 g, 78 mmol) in dry THF (20 ml) at -78°C over 1 h, and the mixture was stirred for 1.5 h. Then, to this was added dropwise a solution of **4** (14 g, 78 mmol) in dry THF (30 ml) over 30 min, and the mixture was stirred for 1 h. The reaction mixture was poured into a saturated aqueous NH_4Cl solution and extracted with ether. The combined extract was washed with water and brine, dried with $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on silica gel. Elution with hexane/EtOAc (3:1) gave **5** (18 g, 69 mmol, 88%) as a colorless oil. FT-IR: ν_{max} 3439 (s, OH), 1704 (s, C=O), 1095 (s), 736 (s), 697 cm^{-1} (s); NMR: δ_H 1.07 (3H, d, *J*=6.7 Hz, *i*-Pr), 1.08 (3H, d, *J*=7.2 Hz, *i*-Pr), 1.50–1.85 (4H, m), 2.56–2.64, 3.35 (1H, d, *J*=3.3 Hz, OH), 3.51 (2H, t, *J*=6.0 Hz, H-8), 4.00–4.10 (1H, m, 5-H), 4.51 (2H, s, C*H*$_2$Ph), 7.32–7.36 (5H, m, Ph). NMR: δ_C 138.4, 128.3, 127.6, 127.5, 72.7, 70.1, 67.3, 46.5, 41.2, 33.3, 25.6, 17.7. HR-FAB MS: *m/z* calcd. for $C_{16}H_{25}O_3$ $[M+H]^+$ 265.1804; found 265.1811.

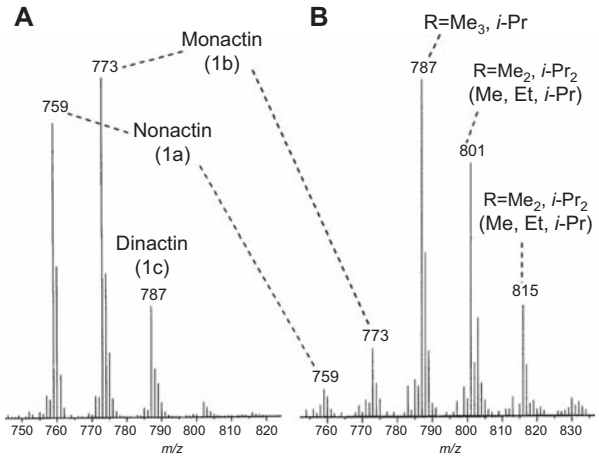


Figure 2 Parts of FAB-MS charts of the products of microbial transformation.
(A) Control; (B) +**3**.

(3SR,5RS)-8-Benzyloxy-2-methyloctane-3,5-diol (6)

To a solution of $Me_4NBH(OAc)_3$ (10 g, 38 mmol) in dry CH_3CN (11 ml) was added anhydrous AcOH (11 ml) and the mixture was stirred at 20°C for 30 min. After the mixture was cooled to -40°C, a solution of **5** (2.0 g, 7.6 mmol) in dry CH_3CN (4 ml) was added. After being stirred at -40°C for 18 h, the mixture was treated with 0.5 M aqueous solution of NaOH and allowed to warm to 20°C with stirring. The mixture was extracted with EtOAc and the combined extract was washed with a saturated aqueous NH_4Cl solution and brine, dried with $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on silica gel. Elution with hexane/EtOAc (3:1) gave **6** (1.7 g, 6.5 mmol, 86%) as a colorless oil. FT-IR: ν_{max} 3399 (s, OH), 1096 (m), 735 (m), 687 cm^{-1} (m); NMR: δ_H 0.87 (3H, d, *J*=6.9 Hz, *i*-Pr), 0.92 (3H, d, *J*=6.9 Hz, *i*-Pr), 1.46–1.66 (4H, m), 1.71 (1H, sep, *J*=6.6 Hz, 2-H), 3.18 (1H, br, OH), 3.50 (2H, t, *J*=6.0 Hz, H-8), 3.61 (1H, ddd, *J*=8.8, 5.8, 3.0 Hz, 5-H), 3.85–3.94 (1H, m, 3-H), 4.50 (2H, s, CH_2Ph), 7.24–7.37 (5H, m, Ph); NMR: δ_C 138.1, 128.4, 127.7, 127.7, 73.5, 72.9, 70.4, 69.0, 39.4, 34.47, 33.6, 26.3, 18.4, 17.9. HR-FAB MS: *m/z* calculated for $C_{16}H_{27}O_3$ $[M+H]^+$ 267.1960; found 267.1967.

(4RS,6SR)-1-Benzyloxy-4,6-bis(t-butyldimethylsilyloxy)-7-methyloctane (7)

A solution of **6** (2.0 g, 7.6 mmol), imidazole (2.4 g, 30 mmol) and TBSCl (3.0 g, 20 mmol) in DMF (50 ml) was stirred at 20°C for 16 h. The mixture was diluted with ether, washed with water, dried with $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on silica gel. Elution with hexane/EtOAc (15:1) gave **7** (3.3 g, 6.7 mmol, 88%) as a colorless oil. FT-IR: ν_{max} 1471 (w), 1361 (w), 1253 (m), 1057 (s), 833 (s), 771 cm^{-1} (s); NMR: δ_H 0.02 (3H, s, SiMe), 0.03 (6H, s, SiMe), 0.04 (3H, s, SiMe), 0.79–0.85 (6H, m, *i*-Pr), 0.86 (18H, s, *t*-Bu), 1.37–1.56 (4H, m), 1.60–1.73 (3H, m), 3.44 (2H, t, *J*=6.3 Hz, 1-H), 3.61 (1H, quint, *J*=3.6 Hz), 3.73 (1H, quint, *J*=5.7 Hz), 4.48 (2H, s, CH_2Ph), 7.22–7.28 (1H, m, Ph), 7.31 (2H, s, Ph), 7.32 (2H, s, Ph); NMR: δ_C 138.8, 128.4, 127.7, 127.5, 74.2, 72.8, 70.5, 70.2, 40.8, 34.4, 33.2, 25.8, 25.6, 25.2, 18.0, 18.0, 17.4, 16.9, -4.0, -4.3. HR-FAB MS: *m/z* calculated for $C_{28}H_{55}O_3Si_2$ $[M+H]^+$ 495.3690; found 495.3692.

(4RS,6SR)-4,6-Bis(t-butyldimethylsilyloxy)-7-methyloctan-1-ol (8)

In a similar manner as described for **17**, compound **7** (3.0 g, 6.1 mmol) was converted to **8** (2.2 g, 5.4 mmol, 89%) as a colorless oil. FT-IR: ν_{max} 3389 (m, OH), 1471 (w), 1387 (m), 1254 (m), 1053 (s), 907 (s), 833 (s), 772 (s), 733 cm^{-1} (s); NMR: δ_H 0.04 (3H, s, SiMe), 0.05 (3H, s, SiMe), 0.07 (3H, s, SiMe), 0.08 (3H, s, SiMe), 0.83 (3H, d, *J*=7.1 Hz, *i*-Pr), 0.86 (3H, d, *J*=7.1 Hz, *i*-Pr), 0.884 (6H, s, *t*-Bu), 0.889 (6H, s, *t*-Bu), 0.891 (6H, s, *t*-Bu), 1.42–1.76 (7H, m), 2.07 (1H, br, OH), 3.56–3.66 (4H, m), 3.79 (1H, quint, *J*=5.4 Hz); NMR: δ_C 74.3, 70.2, 63.1, 40.4, 34.3, 33.0, 28.0, 25.8, 25.8, 18.0, 17.9, 17.4, 16.8, -4.1, -4.3, -4.3, -4.4. HR-FAB MS: *m/z* calculated for $C_{21}H_{49}O_3Si_2$ $[M+H]^+$ 405.3220; found 405.3213.

Ethyl (2E,6RS,8SR)-6,8-bis(t-butyldimethylsilyloxy)-2,9-dimethyldec-2-enoate (10)

In a similar manner as described for **18**, compound **8** (4.4 g, 11 mmol) was converted to **10** (4.1 g, 8.6 mmol, 78%) as a pale yellow oil. FT-IR: ν_{max} 1713 (s, C=O), 1652 (w, C=C), 1471 (m), 1367 (m), 1254 (s), 1053 (s), 834 (s), 772 cm^{-1} (s); NMR: δ_H 0.04 (3H, s, SiMe), 0.05 (3H, s, SiMe), 0.06 (3H, s, SiMe), 0.07 (3H, s, SiMe), 0.83 (3H, d, *J*=6.9 Hz, *i*-Pr), 0.85–0.88 (3H, m, *i*-Pr), 0.880 (9H, s, *t*-Bu), 0.889 (9H, s, *t*-Bu), 1.29 (3H, t, *J*=7.1 Hz, CH_2CH_3), 1.45 (1H, ddd, *J*=14.7, 7.4, 5.2 Hz), 1.52–1.61 (3H, m), 1.70 (1H, dquint, *J*=3.0, 6.9 Hz), 1.84 (3H, d, *J*=1.1 Hz, 2-Me), 2.16–2.28 (2H, m, 4-H), 3.62 (1H, ddd, *J*=7.4, 4.7, 3.1 Hz), 3.76 (1H, dt, *J*=5.5, 6.6 Hz), 4.19 (2H, q, *J*=7.1 Hz, CH_2CH_3), 6.76 (1H, dt, *J*=7.4, 1.4 Hz, 3-H); NMR: δ_C 168.4, 142.2, 127.9, 74.2, 70.0, 60.3, 40.7, 36.6, 33.1, 25.8, 25.8, 24.2, 18.0, 17.9, 17.4, 16.9, 14.2, 12.2, -4.1, -4.3, -4.4. HR-FAB MS: *m/z* calculated for $C_{26}H_{55}O_4Si_2$ $[M+H]^+$ 487.3639; found 487.3645.

Bis(2-octanamidoethyl) disulfide (13)

A mixture of **12** (2.5 g, 32 mmol), 15% aqueous NaOH (75 ml) and ether (25 ml) was stirred at 10°C for 1 h, then treated with octanoyl chloride (6.8 ml, 40 mmol) and the mixture was stirred at 10°C for 2 h. The mixture was diluted with 1 M aqueous HCl and extracted with ether. The extract was washed with brine, dried with $MgSO_4$ and concentrated *in vacuo*. The residue was crystallized from hexanes to give **13** (4.8 g, 12 mmol, 74%) as a white powder. FT-IR: ν_{max} 3299 (s, NH), 3059 (w), 1635 (s, C=O), 1542 (s), 1469 (m), 1421 (m), 1204 (m), 1041 (w), 682 cm^{-1} (w); NMR: δ_H 0.86–0.90 (6H, m, 8-H), 1.2–1.4 (16H, m, 4,5,6,7-H), 1.6–1.7 (4H, m, 3-H), 2.14 (4H, t, *J*=7.7 Hz, 2-H), 2.83 (4H, t, *J*=6.3 Hz, SCH_2), 3.58 (4H, q, *J*=6.6 Hz, $NHCH_2$), 6.25 (2H, br, NH); NMR: δ_C 169.7, 38.7, 37.6, 36.2, 31.6, 29.1, 28.9, 25.7, 22.5, 13.9. HR-FAB MS: *m/z* calculated for $C_{20}H_{41}O_2N_2S_2$ $[M+H]^+$ 405.2609; found 405.2607.

S-2-Octanamidoethyl (2E,6RS,8SR)-6,8-bis (t-butyldimethylsilyloxy)-2,9-dimethyldec-2-enethioate (15)

In the same manner as described for **18**, ester **10** (19.5 mg, 0.0400 mmol) was converted to **11**. NMR: δ_H 0.04 (3H, s, SiMe), 0.05 (3H, s, SiMe), 0.06 (3H, s, SiMe), 0.07 (3H, s, SiMe), 0.83 (3H, d, *J*=6.9 Hz, *i*-Pr), 0.85–0.88 (3H, m, *i*-Pr), 0.879 (9H, s, *t*-Bu), 0.889 (9H, s, *t*-Bu), 1.40–1.78 (5H, m), 1.84 (3H, s, 2-Me), 2.17–2.32 (2H, m, 4-H), 3.58–3.65 (1H, m), 3.72–3.81 (1H, m), 6.91 (1H, t, *J*=7.4 Hz, 3-H). The carboxylic acid **11** was condensed with thiol **14** [FT-IR: ν_{max} 3302 (s, NH), 3059 (w), 1638 (s, C=O), 1550 cm^{-1} (s); NMR: δ_H 0.88 (3H, t, *J*=5.8 Hz, 8-H), 1.2–1.4 (8H, m, 4,5,6,7-H), 1.6–1.7 (2H, m, 3-H), 2.20 (2H, t, *J*=8.2 Hz, 2-H), 2.68 (2H, t, *J*=7.1 Hz, SCH_2), 3.45 (2H, q, *J*=6.1 Hz, $NHCH_2$), 5.84 (1H, br, NH); prepared from the sulfide **13**] to give **15** (19 mg, 0.029 mmol, 73% from **10**) as a pale yellow oil. FT-IR: ν_{max} 1658 (m, C=O), 1471 (w), 1254 (m), 1068 (m), 837 (m), 774 cm^{-1} (m); NMR: δ_H 0.05 (6H, br s, SiMe), 0.07 (3H, s, SiMe), 0.08 (3H, s, SiMe), 0.82–0.88 (6H, m, *i*-Pr), 0.88 (9H, s, *t*-Bu), 0.89–0.90 (9H, m, *t*-Bu), 1.24–1.64 (15H, m), 1.89 (3H, s, 2-Me), 2.15 (2H, t, *J*=7.7 Hz), 2.20–2.31 (2H, m), 3.08 (1H, t, *J*=6.0 Hz, SCH_2), 3.46 (2H, q, *J*=6.0 Hz, NCH_2), 3.58–3.68 (1H, m), 3.72–3.80 (1H, m), 5.84 (1H, br, NH), 6.77 (1H, dt, *J*=6.0, 2.4 Hz, 3-H). HR-FAB MS: *m/z* calculated for $C_{34}H_{70}O_4NSSi_2$ $[M+H]^+$ 644.4564; found 644.4557.

S-2-Octanamidoethyl (2E,6RS,8SR)-6,8-hydroxy-2,9-dimethyldec-2-enethioate (3)

From **15**: a solution of **15** (10 mg, 0.016 mmol) and $BF_3{\cdot}OEt_2$ (2 drops) in $CHCl_3$ (2 ml) was stirred at 0°C for 1 h. The mixture was diluted with a saturated aqueous $NaHCO_3$ solution and extracted

with ether. The extract was washed with brine, dried with $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on preparative TLC. Development with hexanes/EtOAc (4:1) gave **3** (5.8 mg, 0.014 mmol, 90%) as a pale yellow oil.

From **19**: a solution of **19** (1.34 g, 2.93 mmol) and PPTS (300 mg) in MeOH (50 ml) was stirred at 20°C for 4.5 h, and the reaction mixture was concentrated *in vacuo*. The residue was diluted with EtOAc, washed with saturated aqueous $NaHCO_3$ solution and brine, dried with $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on silica gel. Elution with hexanes/EtOAc (1:2) gave **3** (1.22 g, 2.93 mmol, quantitative). NMR: δ_H 0.80–1.00 (9H, m), 1.20–1.40 (8H, m), 1.52–1.80 (9H, m), 1.89 (3H, s, 2-Me), 2.15 (2H, t, *J*=7.8 Hz, CH_2C=O), 2.24–2.50 (2H, m, 4-H), 3.07 (2H, t, *J*=6.0 Hz, SCH_2), 3.42–3.50 (3H, m, NCH_2, OH), 3.66–3.74 (1H, m), 3.90–4.00 (1H, m), 5.84 (1H, br, NH), 6.77 (1H, dt, *J*=6.0, 2.4 Hz, 3-H). HR-FABMS *m/z*: calculated for $C_{22}H_{42}O_4NS$ $[M+H]^+$ 416.2835; found 416.2838.

(4RS,6SR)-1-Benzyloxy-4,6-isopropylidenedioxy-7-methyloctane (16)

A solution of **6** (30 mg, 0.11 mmol) and PPTS (pyridinium p-toluenesulfonate, 10 mg) in 2,2-dimethoxypropane (3 ml) was stirred for 2 h at 0°C. The reaction mixture was diluted with a saturated aqueous $NaHCO_3$ solution and concentrated *in vacuo*. The residue was diluted with ether, washed with water and brine, dried with $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on silica gel. Elution with hexanes/EtOAc (3:1) gave **16** (33 mg, 0.11 mmol, quantitative) as a pale yellow oil. R_f=0.70 (SiO_2, hexane/EtOAc=3:1); FT-IR: ν_{max} 3031 (w), 2984 (s), 2937 (s), 2871 (s), 1586 (w), 1496 (w), 1454 (m), 1377 (s), 1224 (s), 1171 (m), 1100 (s), 1071 (m), 998 (m), 931 (w), 908 (w), 735 (m), 697 cm^{-1} (m); NMR δ_H 0.84 (3H, d, *J*=6.6 Hz, *i*-Pr), 0.91 (3H, d, *J*=6.6 Hz, *i*-Pr), 1.31 (6H, s, acetonide), 1.46–1.81 (7H, m), 3.35–3.54 (3H, m), 3.67–3.78 (1H, m), 4.50 (2H, s, Bn), 7.26–7.34 (5H, m, Ph); NMR: δ_C 138.7, 128.4, 127.7, 127.6, 100.2 (acetonide), 72.9, 71.7, 70.2, 66.6, 36.5, 32.9, 32.4, 25.8, 24.5 (acetonide), 24.3 (acetonide), 18.7, 17.5. HR-FAB MS: *m/z* calculated for $C_{19}H_{31}O_3$ $[M+H]^+$ 307.2273; found 307.2270.

(4RS,6SR)-4,6-isopropylidenedioxy-7-methyloctan-1-ol (17)

A suspension of **16** (2.58 g, 8.42 mmol), $NaHCO_3$ (0.70 g) and 10% Pd/C (0.70 g) in EtOH (80 ml) was stirred for 3 h under hydrogen atmosphere (1 atm). The mixture was filtrated and the filtrate was concentrated *in vacuo*. The residue was chromatographed on silica gel. Elution with hexanes/EtOAc (3:1) gave **17** (0.98 g, 4.53 mmol, 54%) as a pale yellow oil. R_f=0.27 (SiO_2, hexane/EtOAc=1:1); FT-IR: ν_{max} 3419 (m, OH), 2985 (s), 2938 (s), 2874 (s), 1470 (w), 1378 (m), 1224 (s), 1169 (m), 1114 (w), 1038 (m), 996 (m), 929 (w), 907 (w), 854 (w), 669 cm^{-1} (w); NMR: δ_H 0.85 (3H, d, *J*=6.9 Hz, *i*-Pr), 0.93 (3H, d, *J*=6.6 Hz, *i*-Pr), 1.34 (3H, s, acetonide), 1.36 (3H, s, acetonide), 1.50–1.72 (7H, m), 2.44 (1H, br, OH), 3.37–3.48 (1H, m), 3.57–3.70 (2H, m), 3.72–3.82 (1H, m); NMR: δ_C 100.4, 71.8, 67.1, 62.7, 36.7, 32.6, 29.1, 24.5, 24.2, 18.6, 17.4. HR-FAB MS: *m/z* calculated for $C_{12}H_{25}O_3$ $[M+H]^+$ 217.1804; found 217.1806.

Ethyl (2E,6RS,8SR)-6,8-isopropylidenedioxy-2,9-dimethyldec-2-enoate (18)

To a solution of $(COCl)_2$ (0.520 ml, 6.07 mmol) in dry THF (50 ml) was added a solution of DMSO (0.862 ml, 12.1 mmol) in dry THF (15 ml) at -78°C, and the mixture was stirred for 15 min. To this mixture was added **17** (966 mg, 4.67 mmol) in dry THF (25 ml) and the resulting mixture was stirred for 30 min. Then Et_3N (3.30 ml, 23.40 mmol) was added and the mixture was allowed to warm to 20°C. After 30 min, the mixture was diluted with water and extracted with EtOAc. The combined extract was washed with water and brine, dried with $MgSO_4$ and concentrated *in vacuo* to give aldehyde (990 mg, *ca*. 4.63 mmol) as a pale yellow oil. The aldehyde was used in the next step without further purification.

A solution of the aldehyde (990 mg, *ca*. 4.63 mmol) and Ph_3P=$CMeCO_2Et$ (2.52 g, 6.95 mmol) in dry toluene (90 ml) was stirred at reflux for 20 h. The mixture was concentrated *in vacuo* and the residue was chromatographed on silica gel. Elution with hexanes/EtOAc (7:1) gave **18** (1.31 g, 4.39 mmol, 98%) as a pale yellow oil. R_f=0.66 (SiO_2, hexane/EtOAc=3:1); FT-IR: ν_{max} 2984 (s), 2936 (s), 1711 (s, C=O), 1651 (w), 1464 (w), 1378 (m), 1263 (m), 1225 (s), 1171 (m), 1135 (m), 1105 (m), 1020 (m), 907 (w), 744 cm^{-1} (w); NMR: δ_H 0.85 (3H, d, *J*=6.6 Hz, *i*-Pr), 0.91 (3H, d, *J*=6.6 Hz, *i*-Pr), 1.29 (3H, t, *J*=7.1 Hz, $CH_2C\mathit{H_3}$), 1.33 (3H, s, acetonide), 1.34 (3H, s, acetonide), 1.47–1.69 (5H, m), 1.84 (3H, s, 2-Me), 2.22–2.31 (2H, q, *J*=7.6 Hz, 4-H), 3.36–3.46 (1H, m), 3.66–3.78 (1H, m), 4.17 (2H, q, *J*=7.1 Hz, CH_2CH_3), 6.75 (1H, dt, *J*=7.4, 1.4 Hz, 3-H); NMR: δ_C 141.8, 100.3, 71.7, 66.1, 60.4, 36.5, 34.6, 32.8, 24.7, 24.5, 24.2, 18.7, 17.5, 14.2, 12.3. HR-FAB MS: *m/z* calculated for $C_{17}H_{31}O_4$ $[M+H]^+$ 347.1470; found 299.2225.

S-2-Octanamidoethyl (2E,6RS,8SR)-6,8-isopropylidenedioxy-2,9-dimethyldec-2-enethioate (19)

A mixture of **18** (1.42 g, 4.76 mmol), 1 M aqueous KOH (20 ml), THF (15 ml) and MeOH (10 ml) was stirred for 20 h. The mixture was concentrated and the residue was neutralized with 1 M aqueous HCl, and extracted with EtOAc. The combined extract was washed with brine, dried with $MgSO_4$ and concentrated *in vacuo* to give carboxylic acid (1.17 g, *ca*. 4.33 mmol). The carboxylic acid was used in the next step without further purification.

A mixture of the carboxylic acid (1.17 g, *ca*. 4.33 mmol), thiol **14** (1.76 g, 8.65 mmol), EDCI·HCl [1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 1.66 g, 8.66 mmol] and DMAP (4-dimethylaminopyridine, 60.0 mg, 0.50 mmol) in dry CH_2Cl_2 (10 ml) was stirred for 18 h at room temperature. The reaction mixture was diluted with a saturated aqueous NH_4Cl solution and extracted with EtOAc. The combined extract was washed with brine, dried with $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on silica gel. Elution with hexanes/EtOAc (3:1) gave **19** (1.33 g, 2.92 mmol, 61%) as a pale yellow oil. Rf=0.26 (SiO_2, hexane/EtOAc=3:1); FT-IR: ν_{max} 3289 (w, NH), 2985 (m), 2955 (s), 2929 (s), 2871 (m), 1654 (s, C=O), 1544 (m), 1462 (w), 1378 (m), 1224 (s), 1111 (w), 1066 (w), 1020 (w), 909 (w), 659 cm^{-1} (w); NMR: δ_H 0.83–0.92 (24H, m), 1.24–1.35 (15H m), 1.48–1.71 (5H, m), 1.88 (3H, s, 2-Me), 2.15 (2H, t, *J*=Hz), 2.31 (2H, q, *J*=Hz), 3.09 (2H, t, *J*=6.3 Hz), 3.36–3.50 (3H, m), 3.68–3.78 (1H, m), 5.88 (1H, br, NH), 6.75–6.82 (1H, t, 2-H, *J*=Hz, H-3).

Pre-incubation of *Streptomyces griseus* ETH 7796

Cultures of *S. griseus* ETH 7796 were maintained on Emerson agar at 4°C. Mycelium scraped from an agar slant was inoculated in a solution of glucose (0.02 g), yeast extract (0.02 g), malt extract (0.05 g) and $CaCO_3$ (0.01 g) mixed in distilled water (5 ml) in a 10 ml test tube, and incubated at 30°C for 48 h on an orbital shaker at 125 rpm (medium A). The medium A and a solution of maltose (3 g) in distilled water (10 ml) were sterilized and transferred into

a 500 ml Sakaguchi flask containing a sterilized solution of Bacto-tryptone (0.8 g), yeast extract (0.4 g), $NaNO_3$ (0.3 g), and $CaCO_3$ (0.2 g) in distilled water (90 ml). This mixture was incubated at 30°C for 48 h at 125 rpm (medium B). The production medium comprised of Bact-tryptone (0.8 g), yeast extract (0.4 g), $NaNO_3$ (0.3 g), $CaCO_3$ (0.2 g), $MnSO_4$ (0.04 g), $ZnSO_4$ (0.005 g) and distilled water (90 ml) was sterilized at 120°C for 20 min in a 500 ml Sakaguchi flask, and to this mixture was added a sterilized solution of maltose (3 g) in distilled water (10 ml), and medium B (5% v/v). The incubation was performed at 30°C for 96 h at 125 rpm (medium C).

Feeding experiments

Compounds **3**, **20**, **3+20**, and none were dissolved in distilled water-EtOH (5:1) and the solution was administrated portionwise into medium C over 3 days, to a final concentration in the broth of 17 mM. Then each flask was incubated at 30°C for 6 days at 125 rpm. Each medium was filtered through a Celite pad and the pad was washed with water. The washings were extracted with acetone for 16 h, and the extract was concentrated *in vacuo*. The residue was extracted with EtOAc and the combined organic layers were dried with $MgSO_4$, and concentrated *in vacuo*. The residue was chromatographed on preparative TLC. Development with hexanes/EtOAc (2:1) furnished polynactin congeners.

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