Published in final edited form as:

J Nat Prod. 2011 January 28; 74(1): 2–11. doi:10.1021/np100469y.

Landomycins P-W, Cytotoxic Angucyclines from *Streptomyces cyanogenus* S-136

Khaled A. Shaaban[†], Sowmyalakshmi Srinivasan[‡], Raj Kumar[‡], Chendil Damodaran[‡], and Jürgen Rohr^{*,†}

Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, 789 South Limestone Street, Lexington, Kentucky 40536-0596, and Department of Clinical Sciences, College of Health Sciences, University of Kentucky, 900 South Limestone Street, Lexington, Kentucky 40536

Abstract

Streptomyces cyanogenus S-136 is the producer of previously reported landomycins A – D. An analysis of minor products of the strain led to isolation and structure elucidation of eight new congeners, named landomycins P – W (**5**, **6**, **3**, **17**, **9**, **10**, **15**, **7**), along with ten other known angucyclin(ones). The structures of the new compounds were established from their NMR and mass spectrometry data. The activity of these angucyclin(ones) was determined using MCF-7 (estrogen responsive) and MDA-231 (estrogen refractory) breast cancer cell lines. Cell viability assays showed that anhydrolandomycinone (**2**), landomycinone (**11**) and landomycin A (**16**) showed the best combined activities in both MCF-7 and MDA-231 assays with **2** being the most potent in the former and **11** and **16** in the latter. These data reveal that some of the aglycones are equipotent to the principle product **16**, which contains the longest saccharide chain. Specifically, anhydrolandomycinone (**2**) was the most active against MCF-7 cells (IC₅₀=1.8 μM). Compounds with shorter saccharidal moieties were less potent against MCF-7. The fact that the most active landomycins either have long penta- or hexasaccharide chains or no sugars at all suggests that the large compounds may act by a different mode of action than their small sugar-free congeners. The results presented here provide more insights into the structure activity relationship (SAR) of landomycins.

Streptomyces species play a significant role in the production of bioactive natural products, many of which are polyketides.^{1,2} The angucycline group^{3,4} is the largest group of polycyclic aromatic polyketides, with more than 120 members, and constantly growing.^{5–11} The group is rich in chemical scaffolds and various biological activities, predominantly antitumor and antibacterial,^{3,4,12} yet none of these compounds have been developed to clinically applied drugs usually due to toxicity or solubility issues.^{1,3,4} The landomycins A – D, produced by *Streptomyces cyanogenus* S-136^{13–18} consist of a polyketide-derived benz[*a*]anthracene backbone with single saccharide chains of 2, 5 or 6 sugar units. They show broad activity against many cancer cell lines, with the general tendency that compounds with longer saccharide chains show better activity.^{19–22} The main compound, landomycin A (**16**) containing a hexasaccharide side chain, has so far been shown to be most

*Corresponding author. jrohr2@email.uky.edu Fax: 859-257-7564.

[†]Department of Pharmaceutical Sciences, College of Pharmacy

[‡]Department of Clinical Sciences, College of Health Sciences

Supporting Information Available: HPLC analysis chromatogram of the crude extract obtained from *Streptomyces cyanogenus* S136 strain; workup procedure scheme; ¹H and ¹³C NMR spectra of the angucyclin(ones) (**1–17**, **19**); table of cytotoxic activities for compounds (**1–17**, **19**); and SAR table of the 18 isolated compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

potent congener, and was extensively tested by the NCI, against the NCI 60 human cancer cell line panel, and particularly against prostate cancer lines.^{23,24} Various new landomycin variants were produced through combinatorial biosynthetic approaches,^{13,16–18,20–22,25–27} differing with respect to both the oxygen and the saccharide patterns. The mode-of-action of the landomycins is still unclear.^{16,28} However, all studies indicate a new mode-of-action for which SARs (structure-activity-relationships) are needed, which could guide further development of these drugs.^{16,23,24,28} In contrast to the anthracyclines, the cytotoxic effect of the landomycins was only weakly reduced by efflux pumps, such as MRP1 or P-glycoprotein overexpression.²⁸ For studying the mechanism of action and the interrogation of biosynthetic enzymes to determine the exact sequence of events, we were looking for further landomycin variants in both oxygen and sugar pattern produced by *S. cyanogenus* S-136.

We found eight new metabolites, namely landomycins P (5), Q (6), R (3), S (17), T (9), U (10), V (15) and W (7). In addition, we isolated the known angucyclinones tetrangomycin (19) and tetrangulol (1),^{29–32} 5,6-anhydrolandomycinone (2), landomycinone (11)³³ along with the previously described landomycins A (16), B (14), D (12),¹⁵ F (13)¹³, M (8) and O (4).²⁰ Trisaccharidal landomycins have not been reported from *Streptomyces cyanogenus* S-136, although were previously found in *Streptomyces globisporus* 1912, the producer of landomycin E (18), and in mutant strains of *S. cyanogenus* upon inactivation of glycosyltransferase LandGT3.^{22,34,35} However, here we report two new analogues of this type, named landomycins P (5) and Q (6).

Results and Discussion

Cultivation, Isolation and Structure Investigations

A liquid preculture of *Streptomyces cyanogenus* S-136 using SG-medium was used to inoculate a 6 L liquid production culture, consisting of 60 250-mL Erlenmeyer flasks each containing 100 mL of the same medium. The fermentation was carried out at 28 °C for 2 days. The broth was harvested, and chromatographic purification of the crude ethyl acetate extract (6.40 g of a reddish powder) yielded the known metabolites as well as eight new congeners.

On the basis of known TLC, UV bands and HPLC-MS (Figure S2) of the crude extract, various angucycline chromophores were identified.¹⁴ Purification of half of the strain extract (3.00 g) using various chromatographic techniques (Figure S3) led to the isolation of eight new landomycins P-W (5, 6, 3, 17, 9, 10, 15, 7). In addition, we isolated the known

compounds tetrangulol (**1**), 5,6-anhydrolandomycinone (**2**), landomycinone (**11**) and landomycins A (**16**), B (**14**), D (**12**), F (**13**), M (**8**) and O (**4**), and tetrangomycin (**19**). Based on NMR data, we report here corrected NMR assignments of tetrangomycin (**19**) (Figure S6), which were previously incorrectly assigned.³²

Landomycin P (5)—Compound **5** is an orange solid with a molecular weight of 678 Da (HRESIMS), leading to molecular formula C₃₇H₄₂O₁₂. The ¹H NMR spectrum of **5** displayed the same aromatic pattern as tetrangulol (**1**), with sugar substitution at the 8-position, as in the case of the previously reported landomycins M (**8**) and O (**4**). The aliphatic region between δ 5.23 – 1.21, revealed three anomeric protons, and three doublet methyl signals, consistent with two β -D- and one α -L-glycosidic 6-deoxysugar moieties. The ¹³C NMR/HSQC spectra of **5** established tetrangulol (**1**) as aglycone moiety, with its typical carbonyls at δ 190.7 and 181.9 corresponding to the quinone system, with one carbonyl being chelated with a *peri*-hydroxy group. In the *sp*³ region, three anomeric carbon signals (δ 101.1, 98.9 and 97.8) were observed along with ten methines, four methylene and four methyl signals.

The HMBC and ¹H-¹H COSY correlations of **5** (Figure 1) revealed two partial structures, the tetrangulol-aglycone (**1**) and a trisaccharide system, also supported by the ESI-MS² spectra (peak at *m/z* 303 corresponding to [tetrangulol-H][−] as a result of the loss of three sugar moieties [(L-rhodinose + D-olivose + D-olivose)-H][−] from the parent molecule **5**, Figure S4). The attachment of the trisaccharide at the usual 8-position was confirmed by a ³J_{C-H} long range coupling between the anomeric proton of one of the β -D-olivoses (δ _H 5.23) and C-8 (δ _C 156.6) of the aglycone. All three sugars showed the same signal patterns and connectivity as previously found for the trisaccharide chain of landomycin E (**18**). The couplings and chemical shifts were in full agreement with structure **5** (Figure 1). The relative configuration of the sugar residues was further confirmed by NOESY experiments (Figure 2), determining structure **5** as 8- β -D-olivosyl-4-1- β -D-olivosyl-3-1- α -L-rhodinosyl-tetrangulol, now named landomycin P.

Landomycin Q (6)—Compound **6** was obtained as dark red solid from the same fraction as landomycin P (**5**), exhibiting similar physicochemical properties, with the sole difference that the ¹H NMR spectrum revealed an additional phenolic OH singlet (at δ 12.23, Table 1), and a two aromatic AB systems instead of the AB/ABC systems found in **5**. Accordingly, a molecular weight of 16 amu higher than that of **5** corresponding to a molecular formula of C₃₇H₄₂O₁₃ was determined by HRESIMS. The NMR data revealed the additional OH group to be in 11-position determining the aglycone of compound **6** as 5,6-anhydrolandomycinone. Along with the ESI-MS² spectrum, the NMR data also revealed the same trisaccharide chain as in compounds **5** or **18**, establishing the previously unknown structure **6**, now named landomycin Q.

Landomycin R (3)—Compound **3** was obtained as a dark red solid. The UV data and the proton NMR spectrum indicated a close structural relationship with the previously published landomycin O (**4**).²⁰ Comparing the ¹H NMR data of compound **3** with landomycin O (**4**) showed the replacement of the H-11 dd signal of **4** (δ 7.74) by an OH singlet (δ 12.17) (Table 1). The molecular formula C₃₁H₃₂O₁₁ of **3** was established by ESI HRMS. Like **4**, structure **3** has a disaccharide (β -D-olivose- β -D-olivose) attached at C-8 of 5,6-anhydrolandomycinone. The β -D-glycosidic linkage of the disaccharide system again followed from the large coupling constant of the anomeric protons and comparison with other landomycins; for NMR data see Table 1. Thus, compound **3** was elucidated as 8-(β -D-olivosyl-1,4- β -D-olivosyl)-5,6-anhydrolandomycinone and named landomycin R.

Landomycin S (17)—Compound **17** was isolated along with main product landomycin A (**16**) as red and orange solids, respectively, after alternative chromatographic purifications of fraction V. The UV data and the proton NMR spectrum of compound **17** indicated its close similarity with landomycin A **16**, except that the singlet 11-OH group (δ 12.29) in landomycin A was replaced by a methine (δ 7.90, dd) proton, which is part of an aromatic ABC system, as described above for landomycin P (**5**). The corresponding carbon signal appears at δ 122.8. The molecular weight of compound **17** was by 16 *amu* lower than that of landomycin A (**16**), with a molecular formula $C_{55}H_{74}O_{21}$ proven by ESI HRMS. The full assignment of compound **17** was deduced from the 1H - 1H COSY, HSQC and HMBC experiments (Figure 3 and Table 2). Based on NOESY experiments (Figure 4), coupling constants and comparison with landomycin A (**16**), compound **17** was established to have the same *R*-configuration of C-6 in its 11-deoxy-landomycinone moiety, and the same stereochemistry of the attached hexasaccharide chain attached to C-8. This new compound **17** was named landomycin S.

Landomycin T (9)—Compound **9** was isolated as an orange solid from fraction IV. The HRESI MS-derived molecular formula $C_{55}H_{72}O_{20}$ was 18 *amu* less than landomycin S (**17**). Most of the 1H and ^{13}C NMR data of **9** were similar to those of **17** (Table 2), except for its aromatic ring B, which showed two ortho-coupled protons at δ 8.06 (d, J = 8.8 Hz) and 8.20 (d, J = 8.6) for 5- and 6-H in **9**. The structure of **9** was confirmed by 1H - 1H COSY, HSQC, HMBC and NOESY experiments, exhibiting the same sugar chain and connection as found in **16** and **17** (Figures 5, 6). Hence the new compound **9** was named as landomycin T.

Landomycin U (10)—The (+)-HRESIMS of compound **10** showed a mass of m/z 1091.4447 $[M+Na]^+$, consistent with a molecular formula $C_{55}H_{72}O_{21}$ of a hexasaccharidal compound. The latter conclusion also was confirmed by the 1H NMR data that revealed six signals in the anomeric region between δ 5.07 and δ 4.47 (Table 2). Thus, landomycin U (**10**) was found to be closely related to landomycins A (**16**) and T (**9**). Its molecular formula indicates a loss of H_2O compared to landomycin A (**16**). The 1H NMR pattern revealed ring B to be aromatic showing the *o*-coupled protons 5-H and 6-H at δ 8.13 (d) and 8.24 (d), respectively. The new compound was named landomycin U (**10**).

Landomycin V (15)—Compound **15** was isolated as an orange solid together with the red solid landomycin B (**14**) from the same fraction FVI. The two compounds were separated by HPLC (see Fig. S3). The (–)HRESI MS (m/z 955.3932, $M-H^-$) suggested the molecular formula $C_{49}H_{64}O_{19}$ for **15** (calcd for $C_{49}H_{63}O_{19}$: 955.3968) with a Δm = 16 *amu* corresponding to one oxygen atom less than landomycin B (**14**). In the aliphatic region, the 1H NMR spectrum of **15** showed the same pattern as found for landomycin B (**14**). However, one of the chelated OH groups observed for landomycin B **14** (δ 12.32 s) was missing in the 1H NMR spectrum of **15**. Instead, an additional aromatic proton signal (δ 7.56 d, 7.5 Hz), part of an aromatic ABC system, was found, and its corresponding carbon signal at δ 119.6 (Table 3). The structure of compound **15** was confirmed using 1H - 1H COSY, HSQC, HMBC and NOESY experiments (Figure 7 and Table 3), to be a 11-deoxy-landomycin B, with the same stereochemistry and pentasaccharide chain attached at C-8 (Figure 8) as in **14**, and named landomycin V.

Landomycin W (7)—Compound **7** was a dark red solid with similar physicochemical properties and staining to those of the earlier isolated landomycins. Its molecular weight was deduced by ESIMS and HRESIMS, establishing its molecular formula to be $C_{49}H_{62}O_{19}$, 18 *amu* less than landomycin B (**14**), attributed again to the aromatization of ring B. This was confirmed by the 1H NMR spectrum (*o*-coupled protons in **7** at δ 8.24 and 8.13 instead of

the 6-oxymethine and its vicinal 5-CH₂ found in **14**). Thus, compound **7** was deduced as 5,6-anhydro-landomycin B, now named landomycin W.

Cytotoxicity assays

The cytotoxic potency of angucyclines (**1–17**, **19**) was determined using MCF-7 (estrogen responsive) and MDA-231 (estrogen refractory) breast cancer cells (Table 4, see also Supporting Information, Table S1). Cell viability assays showed that compounds **2**, **11** and **16** had similar activities on both MCF-7 and MDA-231 cells. Specifically, in MCF-7 cells compound **2** was the most active (IC₅₀ = 1.8 μM); compounds **10**, **11** and **16** were moderately active (IC₅₀ = 2.1, 2.1 and 2.5 μM, respectively), compounds **7**, **8**, **12**, **14**, **15** and **17** (IC₅₀ = 6.9, 7.1, 7.6, 4.25, 6.1 and 6.7 μM, respectively) were less active and compounds **1**, **3–6**, **9**, **13** and **19** showing no cytotoxic activity. In MDA-231 cells compounds **11** and **16** (IC₅₀ = 1.4 μM); were most active, compounds **1–3**, **8**, **9**, **12–15**, **17** and **19** (IC₅₀ = 1.5, 2, 1.7, 1.9, 1.85, 1.75, 1.8, 1.8, 1.5, 1.5 and 1.55 μM, respectively) also showed significant activity, while compounds **4–7** and **10** (IC₅₀ = 3.55, 3.55, 3.85, 2.65 and 7.3 μM, respectively) were only moderately active. The results showed that the cytotoxic activity of the molecules differed corresponding to their substitution pattern (Table 4, Supporting Information, Table 1). The compounds with aromatic B-rings (**1–10**) all showed moderate to good activities against MDA-231 cells, however, some of them were inactive against MCF-7 cells, namely compounds **1**, **3–6** and **9**. The 11-OH group seems important: Anhydrolandomycinone **2** displayed the relatively best overall cytotoxicity against both human breast cancer cell lines MCF-7 and MDA-231, and its pentasaccharidal analogue landomycin W (**7**) and its hexasaccharidal analogue landomycin U (**10**) showed similar activities against one of the tested cell lines (**7** against MDA-231, **10** against MCF-7). The observation that either a long hexa- or pentasaccharide chain or no sugar moiety at all is required for good activity, but everything in between (e.g., compounds **4–6**) seems less desirable, is intriguing, and may indicate a switch of mechanism-of-action. It is possible that the sugar-free (aglycone) compounds act through simple DNA intercalation, while the congeners with longer saccharide chains, such as **7** and **10**, have a different target. The same is observed in the set of compounds with a non-aromatic ring B (compounds **11–17**), for which landomycinone (**11**) as well as landomycin A (**16**) show the overall best activities. The fact that this set of compounds is more potent than their anhydro-analogues indicates that the saturation of the 5,6-bond and presence of the 6-OH group at C-6 is important for the cytotoxicity. In general, the landomycins appear to be more active against MDA-231 cells when compared to MCF-7 breast cancer cells. This differential sensitivity may be due to the variation in the status of hormonal receptors where MCF-7 cells are estrogen receptor (ER) positive and MDA-231 cells are ER negative.^{36,37} Additionally, MCF-7 cells lack caspase-3 (major executor of apoptosis in most cell types) expression suggesting that the mechanism of action of landomycins may be caspase-3 dependent.³⁸ Currently, we are investigating the molecular mechanism by which such angucyclines exert their cytotoxic effects on both MCF-7 and MDA-231 cells.

Experimental Section

General Experimental Procedures

UV spectra were recorded on a Shimadzu UV-1800 (Model TCC-240A) UV spectrometer. NMR spectra were measured on Varian Vnmr 500 (¹H, 500 MHz; ¹³C, 125 MHz) spectrometer. ESIMS was recorded on a Finnigan LCQ ion trap mass spectrometer. HRMS was recorded by ESIMS on an Agilent LC/MSD TOF (Resolution: 10,000; 3 ppm mass accuracy; inlet systems: Agilent Technologies 1200 Series LC pumps) Mass Spectrometer (Manufacturer: Agilent Palo Alto, CA, USA). LC/MS/MS measurements were performed on an Applied Biosystems 3200 QTRAP instrument (Applied Biosystems, Foster City, CA,

USA) using electrospray ionization in the positive and negative ionization mode (inlet systems: Agilent 1100 series HPLC; Resolution: Unit mass) mass spectrometer. Samples were introduced by a syringe pump. HPLC purifications were carried out using a Symmetry Prep C₁₈10 μ m column (10 $\times$ 150 mm) on a binary LC system. HPLC-MS analyses were carried out using a Symmetry Anal C₁₈ 5 μ m column (4.6 $\times$ 250 mm) on a binary LC system. Flash chromatography was carried out on silica gel MN 60 (140–270 mesh ASTM). *R_f* values were measured on Polygram SIL G/UV₂₅₄ (Macherey-Nagel & Co.). Size exclusion chromatography was performed using Sephadex LH-20 (GE Healthcare).

Cell Viability Assay

To determine the cytotoxicity of the angucycline group compounds (**1–17**, **19**) two breast cancer cell lines, MCF-7 (estrogen responsive) and MDA-231 (estrogen refractory) were used. Both cell lines were purchased from the American Type Culture Collection (ATCC). The cells were grown in Dulbecco's modified Eagle's medium and cell viability of these two cell lines in response to the test compounds were determined using the trypan blue exclusion assay as described earlier,^{36,37} where 50 $\times$ 10³ cells in 0.5 ml medium were plated in each well of a 24-well plate and allowed to attached overnight. The medium was replaced the following day with fresh medium containing different concentrations of the test compounds and the plates were incubated for 24 hours at 37 °C. At the end of the treatment period both adherent and floating cells were collected, resuspended in PBS, and trypan blue staining was performed using 0.4% stain for 3 minutes. Stained (dead) and unstained (live) cells were counted using a hemocytometer, cell viability in response to specific compounds were studied, dose response curve was plotted and the IC₅₀ values were determined. Each set of experiment was performed three times to confirm reproducibility of results. Landomycin A was used as a standard, ethanol as a positive control, and the medium without test compound as a negative control.

SG-Medium

Glucose (20 g), yeast extract (5 g), Soytone (10 g), CoCl₂.6H₂O (1 mg) and calcium carbonate (2 g) were dissolved in 1 liter of demineralized water. The suspension (pH 7.2) was sterilized by autoclaving for 33 min at 121 °C.

M2-Agar

Glucose (4.0 g), yeast extract (4.0 g), malt extract (10.0 g) and agar (15.0 g) were dissolved in 1 liter of demineralized water.

Fermentation, Extraction and Isolation

Streptomyces cyanogenus S-136 (originally isolated by Hoechst India, obtained from Hoechst AG, Germany, maintained as glycerol spore suspension at –80 °C) was cultivated on M2-agar plates at 28 °C for 2 days. With pieces of well-grown agar cultures of the strain, a 250 mL Erlenmeyer flask preculture of *Streptomyces cyanogenus* S-136, containing 100 mL of SG-medium, was inoculated and cultivated at 28 °C (250 rpm) for 40 hrs. The obtained 100 mL preculture was used to inoculate 60 250 mL Erlenmeyer flasks (each with 1.5 mL preculture), each containing 100 mL of SG-medium, which was harvested after 48 hrs incubation at 28 °C at 250 rpm. The reddish-brown culture broth was centrifuged. The mycelium was extracted with MeOH (6 $\times$ 200 mL), while the water phase was extracted with EtOAc (3 $\times$ 2L). Both extracts were combined and evaporated to dryness under vacuum at 40 °C, and afforded 6.40 g of a reddish powder.

Approximately 3 g of this material was chromatographed on silica gel (column 2 $\times$ 50 cm) using a stepwise MeOH/CH₂Cl₂ gradient (0–50% MeOH) to yield fractions I (0.1 g, red

solid), II (30 mg, red solid), III (0.13 g, red solid), IV (0.4 g, red solid), V (1.2 g, red solid), VI (0.95 g, red solid), and VII (0.1 g, brown solid), see also Figure S3. Purification of fractions I and II using Sephadex LH-20 (2 × 50 cm, 50 % MeOH/CH₂Cl₂) afforded tetrangulol (**1**; reddish brown crystals, 55.0 mg) and 5,6-anhydrolandomycinone (**2**; red solid, 15.0 mg), respectively. In a similar manner, purification of fraction III using silica gel column chromatography (2 × 30 cm, MeOH-CH₂Cl₂ gradient 0% MeOH to 100%, each step 5%-increased, 200 mL solvent) followed by Sephadex LH-20 (2 × 50 cm, 50 % MeOH/CH₂Cl₂) yielded tetrangomycin (**19**, 25.0 mg) and landomycinone (**11**, 45.0 mg). Size exclusion chromatography (3 × 70 cm, 40 % MeOH/CH₂Cl₂), PTLC (5% MeOH/CH₂Cl₂) and HPLC (CH₃CN/H₂O) of fraction IV yielded landomycins U (**10**, 22.0 mg), T (**9**, 25.3 mg), P (**5**, 20.0 mg) and Q (**6**, 5.1 mg). In a similar way, partial separation and purification of fractions V and VI following figure S3 gave the landomycins A (**16**, 38.9 mg), S (**17**, 27.6 mg), W (**7**, 6.3 mg), M (**8**, 4.0 mg), O (**4**, 10.0 mg), R (**3**, 3.0 mg), B (**14**, 45.3 mg), V (**15**, 35.6 mg), F (**13**, 27.3 mg) and D (**12**, 28.3 mg) in pure form. Fraction VII was excluded based on the TLC and HPLC-MS analysis, since no products were found (Figure S3).

Landomycin P (**5**)

Orange solid; *R_f* 0.39 (silica gel, 5% MeOH/CH₂Cl₂), blue coloration with 2N NaOH; UV/vis (MeOH) $\lambda_{\max}$ (log ϵ) 251 (5.07), 310 (4.62), 395 (4.22), 439 sh (3.53) nm; ¹H NMR (CDCl₃, 500 MHz) and ¹³C NMR (CDCl₃, 125 MHz), see Table 1; (–)-ESI MS *m/z* 677 [M–H][–]; (–)-ESI MS/MS *m/z* (%) 679 [M+2H–H][–] (12), 545 ([M–(L-rhodinose–H₂O)–H][–], 10), 433 ([M–(L-rhodinose + D-olivose)–H][–], 5), 303 ([M–(L-rhodinose + D-olivose + D-olivose)–H][–], 100), 275 (12); (–)-HRESIMS *m/z* 677.2593 [M–H][–] (calcd for C₃₇H₄₁O₁₂, 677.2598).

Landomycin Q (**6**)

Dark red solid; *R_f* 0.37 (silica gel, 5% MeOH/CH₂Cl₂), blue coloration with 2N NaOH; UV/vis (MeOH) $\lambda_{\max}$ (log ϵ) 251 (5.22), 311 (4.51), 399 (4.30), 457 sh (3.56) nm; ¹H NMR (CDCl₃, 500 MHz), see Table 1; (–)-ESI MS *m/z* 693 [M–H][–]; (–)-ESI MS/MS *m/z* (%) 693 [M–H][–] (5), 533 (1), 318 ([M–(L-rhodinose + D-olivose + D-olivose)–H][–], 100), 291 (8); (–)-HRESIMS *m/z* 693.2594 [M–H][–] (calcd for C₃₇H₄₁O₁₃, 693.2552).

Landomycin R (**3**)

Dark red solid; *R_f* 0.23 (silica gel, 5% MeOH/CH₂Cl₂), blue coloration with 2N NaOH; UV/vis (MeOH) $\lambda_{\max}$ (log ϵ) 251 (4.76), 311 (4.06), 399 (3.84), 457 sh (3.11) nm; ¹H NMR (DMSO-*d*₆, 500 MHz), see Table 1; (–)-ESI MS *m/z* 579 [M–H][–]; (–)-HRESIMS *m/z* 579.1861 [M–H][–] (calcd for C₃₁H₃₁O₁₁, 579.1871).

Landomycin S (**17**)

Orange solid; *R_f* 0.14 (silica gel, 5% MeOH/CH₂Cl₂), 0.76 (15% MeOH/CH₂Cl₂), blue coloration with 2N NaOH; UV/vis (MeOH) $\lambda_{\max}$ (log ϵ) 255 (5.25), 288 sh (4.73), 398 (4.49), 445 sh (4.04) nm; ¹H NMR (CDCl₃, 500 MHz) and ¹³C NMR (CDCl₃, 125 MHz), see Table 2; (–)-ESI MS *m/z* 1069 [M–H][–]; (–)-ESI MS/MS *m/z* (%) 1070 ([M–H+H][–], 5), 1052 ([M–H₂O][–], 30), 909 ([M–(L-rhodinose – 2H₂O)][–], 100), 320 ([M–(L-rhodinose + D-olivose + D-olivose + L-rhodinose + D-olivose + D-olivose)–H][–], 62); (+)-HRESIMS *m/z* 1069.4648 [M–H][–] (calcd for C₅₅H₇₃O₂₁, 1069.4649).

Landomycin T (**9**)

Orange solid; *R_f* 0.24 (silica gel, 5% MeOH/CH₂Cl₂), blue coloration with 2N NaOH; UV/vis (MeOH) $\lambda_{\max}$ (log ϵ) 251 (5.00), 311 (4.44), 399 (4.10), 439 sh (3.39) nm; ¹H NMR (CDCl₃, 500 MHz) and ¹³C NMR (CDCl₃, 125 MHz), see Table 2; (–)-ESI MS *m/z* 1051

$[M-H]^-$; (+)-ESIMS m/z 1075 $[M+Na]^+$; (+)-HRESIMS m/z 1075.4493 $[M+Na]^+$ (calcd for $C_{55}H_{72}O_{20}Na$, 1075.4508).

Landomycin U (10)

Dark red solid; R_f 0.26 (silica gel, 5% MeOH/ CH_2Cl_2), blue coloration with 2N NaOH; UV/vis (MeOH) λ_{max} (log ϵ) 251 (5.24), 314 (4.78), 399 (4.36), 461 sh (4.07) nm; 1H NMR ($CDCl_3$, 500 MHz), see Table 2; (–)-ESI MS m/z 1067 $[M-H]^-$; (+)-ESI MS m/z 1091 $[M+Na]^+$; (–)-ESI MS/MS m/z (%) 1067 ($[M-H]^-$, 15), 1049 ($[M-H_2O-H]^-$, 8), 954 ($[M-(L-rhodinose)]^-$, 10), 822 ($[M-(L-rhodinose + D-olivose)-H]^-$, 5), 675 ($[M-(L-rhodinose + D-olivose + D-olivose-H_2O)-H]^-$, 2), 319 ($[M-(L-rhodinose + D-olivose + D-olivose + L-rhodinose + D-olivose + D-olivose)-H]^-$, 100); (+)-HRESIMS m/z 1091.4447 $[M+Na]^+$ (calcd for $C_{55}H_{72}O_{21}Na$, 1091.4458).

Landomycin V (15)

Orange solid; R_f 0.06 (silica gel, 5% MeOH/ CH_2Cl_2), 0.70 (15% MeOH/ CH_2Cl_2), blue coloration with 2N NaOH; UV/vis (MeOH) λ_{max} (log ϵ) 255 (5.12), 295 sh (4.49), 399 (4.30), 446 sh (3.80) nm; 1H NMR ($CDCl_3$, 500 MHz), 1H NMR ($DMSO-d_6$, 500 MHz), and ^{13}C NMR ($DMSO-d_6$, 125 MHz), see Table 3; (–)-ESI MS m/z 955 $[M-H]^-$; (–)-ESI MS/MS m/z (%) 955 ($[M-H]^-$, 5), 937 ($[M-H_2O-H]^-$, 40), 795 ($[M-(D-olivose-H_2O-H)]^-$, 100), 320 ($[M-(D-olivose + D-olivose + L-rhodinose + D-olivose + D-olivose-H)]^-$, 62); (–)-HRESIMS m/z 955.3932 $[M-H]^-$ (calcd for $C_{49}H_{63}O_{19}$, 955.3968).

Landomycin W (7)

Dark red solid; R_f 0.15 (silica gel, 5% MeOH/ CH_2Cl_2), blue coloration with 2N NaOH; UV/vis (MeOH) λ_{max} (log ϵ) 251 (5.16), 311 (4.45), 399 (4.23), 452 sh (3.47) nm; 1H NMR ($CDCl_3$, 500 MHz), see Table 3; (+)-ESI MS m/z 977 $[M+Na]^+$; (+)-HRESIMS m/z 977.3779 $[M+Na]^+$ (calcd for $C_{49}H_{62}O_{19}Na$, 977.3777) and m/z 1932.7720 $[2M+Na+H]^+$ (calcd for $C_{98}H_{125}O_{38}Na$, 1932.7740).

Tetrangomycin (19)

Yellow solid; 1H NMR ($CDCl_3$, 500 MHz), δ 12.21 (1H, s, OH-8), 8.28 (1H, d, $J = 8.0$, H-6), 7.63 (2H, m, H-10, H-11, 7.52 (1H, d, $J = 7.9$ Hz, H-5), 7.24 (1H, dd, $J = 6.9$, 1.2 Hz, H-9), 3.14 (2H, s, H₂-4), 3.08 (1H, d, $J_{2e,2a} = 14.3$ Hz, H_a-2), 2.98 (1H, d, $J_{2e,2a} = 14.8$ Hz, H_e-2), 1.49 (3H, s, CH₃-3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 197.3 (C-1), 187.6 (C-7), 183.4 (C-12), 162.3 (C-8), 147.8 (C-4a), 137.3 (C-10), 136.3 (C-11a), 135.9 (C-12b), 135.4 (C-6a), 134.0 (C-5), 133.9 (C-12a), 129.6 (C-6), 123.9 (C-9), 119.8 (C-11), 115.6 (C-7a), 72.8 (C-3), 54.1 (C-2), 44.3 (C-4), 30.3 (CH₃-3).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Dr. Jack Goodman, University of Kentucky mass spectrometry facilities, for the ESI mass spectra. The mass spectrometry facility of the University of Wisconsin Biotechnology Centre is greatly acknowledged for the HRESI and the MS/MS mass spectra. This work was supported by a grant of the National Institutes of Health to J.R. (CA 102102).

References and Notes

1. Rohr, J.; Hertweck, C. *Comprehensive Natural Products II - Chemistry and Biology*. Mander, L.; Liu, H-w, editors. Vol. 1. Elsevier; Oxford: 2010. p. 227-303.

2. Newman DJ, Cragg GM. *J Nat Prod.* 2007; 70:461–477. [PubMed: 17309302]
3. Rohr J, Thiericke R. *Nat Prod Rep.* 1992; 9:103–137. [PubMed: 1620493]
4. Krohn K, Rohr J. *Topics Curr Chem.* 1997; 188:127–195.
5. Bringmann G, Lang G, Maksimenka K, Hamm A, Gulder TA, Dieter A, Bull AT, Stach JE, Kocher N, Muller WE, Fiedler HP. *Phytochemistry.* 2005; 66:1366–1373. [PubMed: 15907962]
6. Bruntner C, Binder T, Pathom-aree W, Goodfellow M, Bull AT, Poterat O, Puder C, Horer S, Schmid A, Bolek W, Wagner K, Mihm G, Fiedler HP. *J Antibiot.* 2005; 58:346–349. [PubMed: 16060387]
7. Sun CH, Wang Y, Wang Z, Zhou JQ, Jin WZ, You XF, Gao H, Zhao LX, Si SY, Li X. *J Antibiot.* 2007; 60:211–215. [PubMed: 17446695]
8. Fotso S, Mahmud T, Zabriskie TM, Santosa DA, Proteau PJ. *J Antibiot.* 2008; 61:449–456. [PubMed: 18776657]
9. Fotso S, Mahmud T, Zabriskie TM, Santosa DA, Sulastri, Proteau PJ. *J Nat Prod.* 2008; 71:61–65. [PubMed: 18081255]
10. Maruna M, Sturdikova M, Liptaj T, Godany A, Muckova M, Certik M, Pronayova N, Proksa B. *J Basic Microbiol.* 2010; 50:135–142. [PubMed: 20082376]
11. Poterat O, Puder C, Wagner K, Bolek W, Vettermann R, Kauschke SG. *J Nat Prod.* 2007; 70:1934–1938. [PubMed: 18044841]
12. Sasaki E, Ogasawara Y, Liu HW. *J Am Chem Soc.* 2010; 132:7405–7417. [PubMed: 20443562]
13. Ostash B, Rix U, Remsing Rix LL, Liu T, Lombó F, Luzhetskyy A, Gromyko O, Wang C, Braña AF, Méndez C, Salas JA, Fedorenko V, Rohr J. *Chem Biol.* 2004; 11:547–555. [PubMed: 15123249]
14. Henkel T, Rohr J, Beale JM, Schwenen L. *J Antibiot.* 1990; 43:492–503. [PubMed: 2358402]
15. Weber S, Zolke C, Rohr J, Beale JM. *J Org Chem.* 1994; 59:4211–4214.
16. Ostash B, Korynevskaya A, Stoika R, Fedorenko V. *Mini Rev Med Chem.* 2009; 9:1040–1051. [PubMed: 19689401]
17. Von Mulert U, Luzhetskyy A, Hofmann C, Mayer A, Bechthold A. *FEMS Microbiol Lett.* 2004; 230:91–97. [PubMed: 14734170]
18. Zhu L, Luzhetskyy A, Luzhetskaya M, Mattingly C, Adams V, Bechthold A, Rohr J. *ChemBiochem.* 2007; 8:83–88. [PubMed: 17139690]
19. Luzhetskyy A, Vente A, Bechthold A. *Mol BioSyst.* 2005; 1:117–126. [PubMed: 16880973]
20. Luzhetskyy A, Zhu L, Gibson M, Fedoryshyn M, Dürr C, Hofmann C, Hoffmeister D, Ostash B, Mattingly C, Adams V, Fedorenko V, Rohr J, Bechthold A. *ChemBioChem.* 2005; 6:675–678. [PubMed: 15812784]
21. Hoffmeister D, Weber M, Dräger G, Ichinose K, Dürr C, Bechthold A. *ChemBioChem.* 2004; 5:369–371. [PubMed: 14997529]
22. Luzhetskyy A, Liu T, Fedoryshyn M, Ostash B, Fedorenko V, Rohr J, Bechthold A. *ChemBioChem.* 2004; 5:1567–1570. [PubMed: 15515090]
23. Crow RT, Rosenbaum B, Smith R 3rd, Guo Y, Ramos KS, Sulikowski GA. *Bioorg Med Chem Lett.* 1999; 9:1663–1666. [PubMed: 10397496]
24. Depenbrock H, Bornschlegl S, Peter R, Rohr J, Schmid P, Schweighart P, Block T, Rastetter J, Hanauske AR. *Ann Hematol.* 1996; 73(Supl II):A80/316.
25. Krauth C, Fedoryshyn M, Schleberger C, Luzhetskyy A, Bechthold A. *Chem Biol.* 2009; 16:28–35. [PubMed: 19171303]
26. Luzhetskyy A, Fedoryshyn M, Dürr C, Taguchi T, Novikov V, Bechthold A. *Chem Biol.* 2005; 12:725–729. [PubMed: 16039521]
27. Luzhetskyy A, Taguchi T, Fedoryshyn M, Dürr C, Wohler SE, Novikov V, Bechthold A. *ChemBioChem.* 2005; 6:1406–1410. [PubMed: 15977274]
28. Korynevskaya A, Heffeter P, Matselyukh B, Elbling L, Micksche M, Stoika R, Berger W. *Biochem Pharmacol.* 2007; 74:1713–1726. [PubMed: 17904109]
29. Kuntzmann MP, Mitscher LA. *J Org Chem.* 1966; 31:2920–2925. [PubMed: 5919937]
30. Krohn K, Bernhard S, Florke U, Hayat N. *J Org Chem.* 2000; 65:3218–3222. [PubMed: 10814219]

31. Dann M, Lefemine DV, Barbatschi F, Shu P, Kunstmann MP, Mitscher LA, Bohonos N. *Antimicrob Agents Chemother.* 1965; 5:832–835. [PubMed: 5883506]
32. Krohn K, Khanbabaee K. *Liebigs Ann Chem.* 1994;1109–1112.
33. Roush WR, Neitz RJ. *J Org Chem.* 2004; 69:4906–4912. [PubMed: 15255715]
34. Westrich L, Domann S, Faust B, Bedford D, Hopwood DA, Bechthold A. *FEMS Microbiol Lett.* 1999; 170:381–387. [PubMed: 9933932]
35. Zhou M, O'Doherty GA. *Org Lett.* 2008; 10:2283–6. [PubMed: 18461951]
36. Srinivasan S, Koduru S, Kumar R, Venguswamy G, Kyprianou N, Damodaran C. *Int J Cancer.* 2009; 125:961–967. [PubMed: 19384950]
37. Koduru S, Kumar R, Srinivasan S, Evers MB, Damodaran C. *Mol Cancer Ther.* 2010; 9:202–210. [PubMed: 20053782]
38. Koduru S, Srinivasan S, Kumar R, Gomathinayagam R, Rohr J, Damodaran C. *BMC Cancer.* 2009; 9:41–51. [PubMed: 19183448]

Figure 1. Selected HMBC long range couplings ($\rightarrow$) and ^1H - ^1H COSY correlations (bold lines) of landomycin P (5)

Figure 2. Selected NOESY correlations ($\leftrightarrow$) determining the sugar ring conformations in landomycin P (5)

Figure 3.
Selected HMBC connectivities ($\rightarrow$) and ^1H - ^1H COSY correlations (bold lines) of
Landomycin S (**17**)

Figure 4.
Selected NOESY correlations ($\leftrightarrow$) of Landomycin S (**17**)

Figure 5.
Selected HMBC connectivities ($\rightarrow$) and ^1H - ^1H COSY correlations (bold lines) of
Landomycin T (**9**)

Figure 6.
Selected NOESY correlations ($\leftrightarrow$) of Landomycin T (9)

Figure 7.
 ^1H - ^1H COSY (bold lines) and selected HMBC ($\rightarrow$) couplings in Landomycin V (**15**)

Figure 8.
Selected NOESY correlations ($\leftrightarrow$) of Landomycin V (**15**)

Table 1

^{13}C and ^1H NMR assignments of the new landomycins P (**5**), Q (**6**) and R (**3**). δ in ppm relative to TMS (multiplicity, J/Hz).

Position	Landomycin P (5) ^{a)}		Landomycin Q (6) ^{a)}	Landomycin R (3) ^{a)}
	δ_{C} ^{b,c)}	δ_{H} (500 MHz) ^{b)}	δ_{H} (500 MHz) ^{b)}	δ_{H} (500 MHz) ^{d)}
1	155.3, C	-	-	-
1-OH	-	11.11, s	10.62, s	10.67, br s
2	120.0, CH	7.10, d (1.5)	7.16, d (1.8)	6.97, br s
3	141.4, C	-	-	-
3-CH ₃	21.4, CH ₃	2.46, s	2.48, s	2.45, s
4	121.4, CH	7.23, d (1.5)	7.27, d (1.8)	7.34, br s
4a	138.6, C	-	-	-
5	137.8, CH	8.09, d (8.6)	8.13, d (8.7)	8.09, d (8.6)
6	123.0, CH	8.24, d (8.6)	8.25, d (8.6)	8.18, d (8.8)
6a	136.8, C	-	-	-
7	181.9, C	-	-	-
7a	122.5, C	-	-	-
8	156.6, C	-	-	-
9	124.9, CH	7.49, dd (8.4, 1.0)	7.53, d (9.3)	7.52, d (9.3)
10	134.9, CH	7.67, ddd (8.0, 7.8, 1.0)	7.26, d (9.3)	7.34, d (9.8)
11	123.5, CH	8.01, dd (7.8, 1.0)	-	-
11-OH	-	-	12.23, s	12.17, br s
11a	137.2, C	-	-	-
12	190.7, C	-	-	-
12a	130.9, C	-	-	-
12b	119.4, C	-	-	-
Sugar A, β-D-olivose				
1A	98.9, CH	5.23, dd (9.5, 1.7)	5.08, dd (9.5, 2.0)	5.25, dd (9.7, 1.9)
2A	37.8, CH ₂	2.02, ddd (12.7, 12.1, 8.4, Ha), 2.73, ddd (12.0, 5.1, 1.7, He)	2.00, ddd (12.7, 12.1, 8.4, Ha), 2.80, ddd (12.7, 5.1, 2.0, He)	1.66, ddd (12.0, 11.8, 9.8, Ha), 2.78, ddd (12.7, 5.1, 1.5, He)
3A	69.5, CH	3.76, ddd (12.3, 8.4, 5.1)	3.75, ddd (12.0, 8.5, 5.0)	3.61, m
3A-OH	-	4.70, br s	4.68, br s	4.70, br s
4A	88.1, CH	3.12, dd (8.4, 8.4)	3.12, dd (8.4, 8.4)	3.08, dd (8.8, 8.8)
5A	71.1, CH	3.46, m	3.39, m	3.35, m
6A	18.1, CH ₃	1.31, d (6.1)	1.28, d (6.2)	1.22, d (6.1)
Sugar B, β-D-olivose				
1B	101.1, CH	4.55, dd (9.9, 1.6)	4.52, dd (9.8, 2.0)	4.64, dd (9.8, 1.7)
2B	37.3, CH ₂	1.66, ddd (12.0, 12.0, 8.0, Ha), 2.24, ddd (12.0, 5.0, 1.5, He)	1.66, ddd (12.0, 12.0, 8.0, Ha), 2.24, ddd (12.0, 5.0, 1.5, He)	1.35, ddd (12.1, 11.9, 8.0, Ha), 2.07, ddd (11.8, 5.0, 1.5, He)
3B	80.6, CH	3.52, ddd (12.2, 8.3, 5.2)	3.52, ddd (12.0, 8.5, 4.8)	3.48, ddd (12.2, 8.3, 5.2)
3B-OH	-	-	-	4.98, br s
4B	75.5, CH	3.12, dd (8.4, 8.4)	3.12, dd (8.4, 8.4)	3.60, ddd (12.1, 9.0, 5.2)

Position	Landomycin P (5) ^{a)}		Landomycin Q (6) ^{a)}	Landomycin R (3) ^{a)}
	$\delta_C^{b,c)}$	δ_H (500 MHz) ^{b)}	δ_H (500 MHz) ^{b)}	δ_H (500 MHz) ^{d)}
4B-OH	-	4.33, br s	4.32, br s	4.98, br s
5B	72.5, CH	3.40, m	3.39, m	3.31, m
6B	18.1, CH ₃	1.40, d (6.1)	1.39, d (6.2)	1.21, d (6.1)
Sugar C, α-L-rhodinose				
1C	97.8, CH	4.94, br s	4.94, br s	-
2C	25.7, CH ₂	1.56, m (complex, Ha), 2.02, m (complex, He)	1.56, m (complex, Ha), 2.00, m (complex, He)	-
3C	24.3, CH ₂	1.66, m (complex, Ha), 2.02, m (complex, He)	1.67, m (complex, Ha), 2.00, m (complex, He)	-
4C	67.3, CH	3.63, br s	3.63, br s	-
4C-OH	-	n.o. ^{e)}	n.o. ^{e)}	-
5C	68.0, CH	4.13, q (6.4)	4.13, q (6.7)	-
6C	17.2, CH ₃	1.21, d (6.6)	1.21, d, (6.6)	-

^{a)} See also Figures S13–S18,

^{b)} CDCl₃,

^{c)} 125 MHz,

^{d)} DMSO-*d*₆,

^{e)} not observed

Table 2

^{13}C and ^1H NMR assignments of Landomycins S (17), T (9) and U (10) in CDCl_3 , δ in ppm relative to TMS (multiplicity, J/Hz).

Position	Landomycin S (17) ^a		Landomycin T (9) ^b		Landomycin U (10) ^a	
	δ_{C}^b	δ_{H} (500 MHz)	δ_{C}^b	δ_{H} (500 MHz)	δ_{C}^b	δ_{H} (500 MHz)
1	155.9, C	-	155.1, C	-	-	-
1-OH	-	9.59, br s	-	11.11, s	10.63, s	10.63, s
2	120.1, CH	6.74, br s	119.9, CH	7.06, d (1.5)	7.16, br s	7.16, br s
3	143.8, C	-	141.4, C	-	-	-
3-CH ₃	21.4, CH ₃	2.28, s	21.4, CH ₃	2.44, s	2.48, s	2.48, s
4	123.7, CH	6.70, br s	121.4, CH	7.20, d (1.5)	7.27, br s	7.27, br s
4a	137.0, C	-	138.5, C	-	-	-
5	36.5, CH ₂	2.86, dd (16.0, 4.7, H _a) 3.03, dd (16.0, 4.7, H _b)	137.8, CH	8.06, d (8.8)	8.13, d (8.7)	8.13, d (8.7)
6	62.0, CH	5.07, t (4.1)	122.9, CH	8.20, d (8.6)	8.24, d (8.6)	8.24, d (8.6)
6a	145.9, C	-	136.7, C	-	-	-
7	183.8, C	-	181.8, C	-	-	-
7a	120.7, C	-	121.5, C	-	-	-
8	156.4, C	-	156.5, C	-	-	-
9	125.1, CH	7.47, dd (8.5, 1.0)	124.8, CH	7.47, dd (8.4, 0.7)	7.53, d (8.7)	7.53, d (8.7)
10	134.8, CH	7.63, t (8.0)	134.9, CH	7.65, t (8.2)	7.26, d (9.6)	7.26, d (9.6)
11	122.8, CH	7.90, dd (7.7, 1.0)	123.4, CH	7.98, dd (7.7, 0.8)	-	-
11-OH	-	-	-	-	12.23, s	12.23, s
11a	134.8, C	-	137.1, C	-	-	-
12	189.7, C	-	190.6, C	-	-	-
12a	138.7, C	-	130.8, C	-	-	-
12b	113.4, C	-	119.2, C	-	-	-
Sugar A, β-D-olivose						
1A	98.6, CH	5.16, dd (9.5, 1.9)	98.8, CH	5.21, dd (9.5, 1.8)	5.07, dd (9.6, 1.5)	5.07, dd (9.6, 1.5)
2A	37.7, CH ₂	1.95, m (complex, Ha) 2.61, ddd (12.7, 7.0, 5.1, He)	37.8, CH ₂	2.04, ddd (12.7, 12.0, 5.0, Ha) 2.70, ddd (12.7, 5.1, 1.5, He)	2.06-1.90, m (complex, Ha) 2.79, ddd (12.6, 5.0, 1.2, He)	2.06-1.90, m (complex, Ha) 2.79, ddd (12.6, 5.0, 1.2, He)
3A	69.5, CH	3.68, m	69.4, CH	3.74, m	3.73, m	3.73, m

Position	Landomycin S (17) ^a	Landomycin T (9) ^b	Landomycin U (10) ^d
	δ_C^b (500 MHz)	δ_C^b (500 MHz)	δ_H (500 MHz)
3A-OH	-	-	4.69, br s
4A	88.0, CH	88.0, CH	3.10, dd (8.4, 8.4)
5A	71.1, CH	71.0, CH	3.49, m
6A	18.0, CH ₃	18.0, CH ₃	1.28, d (6.1)
Sugar B, β-D-olivose			
1B	101.1, CH	101.1, CH	4.51, dd (9.6, 1.2)
2B	37.3, CH ₂	37.3, CH ₂	1.70-1.48, m (complex, Ha) 2.21, ddd (10.5, 5.0, 1.5, He)
3B	80.7, CH	80.7, CH	3.46, ddd (12.2, 8.3, 5.2)
4B	75.4, CH	75.3, CH	3.08, dd (8.4, 8.4)
4B-OH	-	-	4.32, br s
5B	72.5, CH	72.4, CH	3.37, m
6B	18.0, CH ₃	18.0, CH ₃	1.36, d (6.1)
Sugar C, α-L-rhodosose			
1C	98.0, CH	98.0, CH	4.94, br s
2C	25.7, CH ₂	25.6, CH ₂	1.75, m (complex, Ha) 1.96, m (complex, He)
3C	25.3, CH ₂	25.2, CH ₂	1.70-1.48, m (complex, Ha) 2.12, m (complex, He)
4C	75.9, CH	75.9, CH	3.52, br s
4C-OH	-	-	n.o. ^d
5C	67.9, CH	67.8, CH	4.06, q (6.6)
6C	17.2, CH ₃	17.2, CH ₃	1.19, d (6.4)
Sugar D, β-D-olivose			
1D	101.6, CH	101.6, CH	4.47, dd (9.4, 1.2)
2D	38.5, CH ₂	38.4, CH ₂	1.70-1.48, m (complex, Ha) 2.29, ddd (11.8, 5.4, 1.5, He)
3D	69.7, CH	69.7, CH	3.57, m
3D-OH	-	-	4.59, br s
4D	88.7, CH	88.6, CH	2.96, dd (8.7, 8.7)
5D	70.6, CH	70.4, CH	3.27, m

Position	Landomycin S (17) ^{a)}		Landomycin T (9) ^{b)}		Landomycin U (10) ^{d)}	
	$\delta_C^b)$	δ_H (500 MHz)	$\delta_C^b)$	δ_H (500 MHz)	$\delta_C^b)$	δ_H (500 MHz)
6D	18.0, CH ₃	1.23, d (6.1)	18.0, CH ₃	1.23, d (6.1)		1.24, d (6.1)
Sugar E, β-D-oligose						
1E	101.1, CH	4.46, dd (9.8, 1.0)	101.1, CH	4.46, brd (9.7)		4.47, dd (9.4, 1.2)
2E	37.2, CH ₂	1.72, m (complex, Ha) 2.20, ddd (12.1, 3.8, 1.5, He)	37.2, CH ₂	1.63, m (complex, Ha) 2.20, ddd (10.7, 5.6, 1.5, He)		1.70-1.48, m (complex, Ha) 2.21, ddd (10.7, 5.6, 1.5, He)
3E	80.4, CH	3.48, ddd (12.2, 8.3, 5.2)	80.5, CH	3.45, ddd (12.2, 8.3, 5.2)		3.47, ddd (12.2, 8.3, 5.2)
4E	75.5, CH	3.07, dd (8.8, 8.8)	75.4, CH	3.09, dd (8.7, 8.7)		3.09, dd (8.7, 8.7)
4E-OH	-	4.41, br s	-	4.49, br s		4.48, br s
5E	72.6, CH	3.40-3.31, m	72.5, CH	3.40-3.31, m		3.37, m
6E	18.1, CH ₃	1.37, d (6.1)	18.1, CH ₃	1.38, d (6.1)		1.38, d (6.1)
Sugar F, α-L-rhodinose						
1F	97.6, CH	4.92, br s	97.5, CH	4.92, br s		4.93, br s
2F	24.7, CH ₂	1.52, m (complex, Ha) 2.03-1.88, m (complex, He)	24.6, CH ₂	1.52, m (complex, Ha) 1.96-1.88, m (complex, He)		1.70-1.48, m (complex, Ha) 2.04-1.88, m (complex, He)
3F	24.3, CH ₂	1.52, m (complex, Ha) 2.03-1.88, m (complex, He)	24.2, CH ₂	1.54, m (complex, Ha) 2.06-1.99, m (complex, He)		1.70-1.48, m (complex, Ha) 2.04-1.88, m (complex, He)
4F	67.3, CH	3.61, br s	67.2, CH	3.61, br s		3.62, br s
4F-OH	-	n.o. ^{d)}	-	n.o. ^{d)}		n.o. ^{d)}
5F	67.9, CH	4.11, dq (6.6, 1.1)	67.9, CH	4.10, dq (6.5, 0.8)		4.12, q (6.6)
6F	17.2, CH ₃	1.19, d (6.6)	17.2, CH ₃	1.19, d (6.4)		1.20, d (6.4)

^{a)} See also Figures S40-S48,

^{b)} 125 MHz,

^{c)} 500 MHz,

^{d)} not observed

Table 3

^{13}C and ^1H NMR assignments of Landomycins V (**15**) and W (**7**), δ in ppm relative to TMS (multiplicity, J /Hz).

Position	Landomycin V (15) ^{a)}		Landomycin V (15) ^{a)}	Landomycin W (7) ^{a)}
	δ_{C} ^{b,c)}	δ_{H} (500 MHz) ^{b)}	δ_{H} (500 MHz) ^{d)}	δ_{H} (500 MHz) ^{d)}
1	155.4, C	-	-	-
1-OH	-	9.66, br s	9.56, s	10.63, s
2	115.2, CH	6.55, s	6.76, s	7.16, br s
3	141.2, C	-	-	-
3-CH ₃	21.2, CH ₃	2.24, s	2.29, s	2.48, s
4	121.0, CH	6.62, s	6.71, s	7.27, br s
4a	138.2, C	-	-	-
5	36.5, CH ₂	2.73, d (15.8, H _a) 2.87, d (15.8, H _{β})	2.87, dd (16.0, 4.4, H _a) 3.05, dd (16.0, 4.8, H _{β})	8.13, d (8.7)
6	57.0, CH	5.04, br s	5.10, t (4.5)	8.24, d (8.6)
6-OH	-	5.00, br s	-	-
6a	140.2, C	-	-	-
7	181.2, C	-	-	-
7a	120.0, C	-	-	-
8	155.6, C	-	-	-
9	121.9, CH	7.53, d (8.5)	7.48, d (7.5)	7.53, d (8.7)
10	134.6, CH	7.75, t (7.8)	7.65, t (8.1)	7.26, d (9.6)
11	119.6, CH	7.56, d (7.5)	7.93, d (7.6)	-
11-OH	-	-	-	12.23, s
11a	136.0, C	-	-	-
12	184.3, C	-	-	-
12a	141.4, C	-	-	-
12b	113.8, C	-	-	-
Sugar A, β-D-olivose				
1A	96.5, CH	5.42, d (9.6)	5.18, dd (9.6, 1.8)	5.07, dd (9.4, 1.1)
2A	38.1, CH ₂	1.84-1.68, m (complex, Ha) 2.36, ddd (12.7, 7.0, 5.1, He)	2.01-1.89, m (complex, Ha) 2.64, ddd (12.8, 5.1, 1.6, He)	2.00-1.90, m (complex, Ha) 2.79, ddd (12.8, 5.1, 1.6, He)
3A	68.3, CH	3.64, m	3.71, m	3.73, m
3A-OH	-	4.73, br s	4.71, br s	4.70, br s
4A	86.5, CH	3.11, dd (8.6, 8.6)	3.11, dd (8.8, 8.8)	3.11, dd (8.8, 8.8)
5A	70.1, CH	3.35, m	3.45, m	3.46, m
6A	17.7, CH ₃	1.16, d (6.1)	1.29, d (6.2)	1.27, d (6.2)
Sugar B, β-D-olivose				
1B	100.0, CH	4.69, d (9.4)	4.51, dd (7.8, 1.8)	4.51, dd (9.2, 1.3)
2B	35.8, CH ₂	1.39-1.26, m, (complex, Ha) 2.36, ddd (10.5, 5.0, 1.5, He)	1.70-1.56, m (complex, Ha), 2.23, m (complex, He)	1.70-1.50, m (complex, Ha), 2.23, m (complex, He),
3B	73.1, CH	3.55, ddd (12.2, 8.3, 5.2)	3.48, ddd (12.2, 8.3, 5.2)	3.46, ddd (12.2, 8.3, 5.2)

Position	Landomycin V (15) ^{a)}		Landomycin V (15) ^{a)}	Landomycin W (7) ^{a)}
	δ_C ^{b,c)}	δ_H (500 MHz) ^{b)}	δ_H (500 MHz) ^{d)}	δ_H (500 MHz) ^{d)}
4B	73.9, CH	2.94, dd (9.0, 8.8)	3.09, dd (8.3, 8.1)	3.10, dd (8.3, 8.1)
4B-OH	-	5.11, br d (5.5)	4.32, br s	4.47, br s
5B	71.9, CH	3.37, m	3.37, m	3.37, m
6B	17.8, CH ₃	1.23, d (6.1)	1.34, d (6.1)	1.34, d (6.1)
Sugar C, α-L-rhodinose				
1C	92.1, CH	4.88, br s	4.95, br s	4.94, br s
2C	24.0, CH ₂	1.82, m (complex, Ha) 1.96, m (complex, He)	1.74, m, (complex, Ha) 2.01-1.89, m (complex, He)	1.70-1.50, m (complex, Ha) 2.00-1.90, m (complex, He)
3C	24.0, CH ₂	1.32, m (complex, Ha) 1.94, m (complex, He)	1.52, m (complex, Ha) 2.12, m (complex, He)	1.70-1.50, m (complex, Ha) 2.12, m (complex, He)
4C	75.7, CH	3.43, br s	3.52, br s	3.51, br s
4C-OH	-	n.o. ^{e)}	n.o. ^{e)}	n.o. ^{e)}
5C	65.2, CH	4.11, q (6.2)	4.08, q (6.1)	4.06, q (6.4)
6C	17.0, CH ₃	1.00, d (6.2)	1.19, d (6.6)	1.18, d (6.4)
Sugar D, β-D-olivose				
1D	101.8, CH	4.52, d (9.5)	4.49, dd (7.6, 1.9)	4.49, dd (9.3, 1.4)
2D	38.8, CH ₂	1.40, m (complex, Ha) 2.07, ddd (11.8, 5.4, 1.5, He)	1.70-1.56, m (complex, Ha) 2.29, m (complex, He)	1.70-1.50, m (complex, Ha) 2.29, m (complex, He)
3D	68.7, CH	3.43, m	3.58, m	3.58, m
3D-OH	-	4.60, br s	4.56, br s	4.56, br s
4D	87.2, CH	2.94, dd (8.6, 9.0)	2.95, dd (8.8, 8.8)	2.95, dd (8.8, 8.8)
5D	69.7, CH	3.26, m	3.28, m	3.27, m
6D	17.9, CH ₃	1.23, d (6.1)	1.24, d (6.2)	1.24, d (6.1)
Sugar E, β-D-olivose				
1E	100.2, CH	4.59, br d (9.8)	4.47, dd (9.8, 1.4)	4.47, dd (9.2, 1.7)
2E	39.4, CH ₂	2.07, m (complex, Ha) 2.44, m (complex, He)	2.01-1.89, m (complex, Ha) 2.21, ddd (12.1, 3.8, 1.5, He)	2.00-1.90, m (complex, Ha) 2.22, ddd (12.1, 3.8, 1.5, He)
3E	70.2, CH	3.55, ddd (12.2, 8.3, 5.2)	3.46, ddd (12.2, 8.3, 5.2)	3.46, ddd (12.2, 8.3, 5.2)
3E-OH	-	4.96, d (4.4 Hz)	3.62, br s	3.61, br s
4E	76.4, CH	2.77, dd (8.6, 8.6)	3.07, dd (8.8, 8.8)	3.08, dd (8.8, 8.8)
4E-OH	-	5.04, br s	4.48, br s	4.47, br s
5E	71.9, CH	3.26, m	3.37, m	3.37, m
6E	17.8, CH ₃	1.17, d (6.1)	1.38, d (6.1)	1.38, d (6.1)

^{a)} See also Figures S29–32 and S36–37,

^{b)} DMSO-*d*₆,

^{c)} 125 MHz,

^{d)} CDCl₃,

^{e)} not observed

Table 4Cytotoxic potencies of angucyclin(on)es **1–18** in comparison (mean IC₅₀ from three measurements, μ M)

Sugar chain	R ¹ = H ($\Delta^5,6$)	R ¹ = OH ($\Delta^5,6$)	R ¹ = H (6 β -OH)	R ¹ = OH (6 β -OH)
None	1	2		11
	NA ^a /1.5 $\pm$ 0.2 ^b	1.8 $\pm$ 0.1 ^a /2 $\pm$ 0.2 ^b		2.1 $\pm$ 0.3 ^a /1.4 $\pm$ 0.2 ^b
I	O (4)	R (3)	F (13)	D (12)
	NA ^a /3.55 $\pm$ 1.1 ^b	NA ^a /1.7 $\pm$ 0.3 ^b	NA ^a /1.8 $\pm$ 0.4 ^b	7.6 $\pm$ 1.5 ^a /1.75 $\pm$ 0.3 ^b
II	P (5)	Q (6)		E (18)
	NA ^a /3.55 $\pm$ 0.5 ^b	NA ^a /3.85 $\pm$ 0.4 ^b		13.0 $\pm$ 2.2 ^{a,c} /NT ^b
III	M (8)	W (7)	V (15)	B (14)
	7.1 $\pm$ 4.6 ^a /1.9 $\pm$ 0.5 ^b	6.9 $\pm$ 3.2 ^a /2.65 $\pm$ 0.3 ^b	6.1 $\pm$ 1.3 ^a /1.5 $\pm$ 0.5 ^b	4.25 $\pm$ 0.8 ^a /1.8 $\pm$ 0.2 ^b
IV	T (9)	U (10)	S (17)	A (16)
	NA ^a /1.85 $\pm$ 0.4 ^b	2.1 $\pm$ 0.1 ^a /7.3 $\pm$ 2.5 ^b	6.7 $\pm$ 1.0 ^a /1.5 $\pm$ 0.3 ^b	2.2 $\pm$ 0.1 ^a /2.0 $\pm$ 0.1 ^b

^aMCF-7 and^bMDA-231 cell assays;^cfrom ref. 18;NA = not active up to 20 μ M; NT = not tested