11-Deoxylandomycinone and landomycins X-Z, new cytotoxic angucyclin(on)es from a *Streptomyces cyanogenus* K62 mutant strain

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Abstract

Four new angucyclin(on)es, 11-deoxylandomycinone (1) and landomycins X-Z (2–4) were isolated from the crude extract of *Streptomyces cyanogenus* K62 mutant strain, along with the recently reported landomycins S, T and V (5–7) and five other known compounds. The structures of the new compounds 1–4 were elucidated by 1D and 2D NMR studies along with HRMS analyses. Unique about the structures is that the fourth sugar moiety (sugar D) in landomycins X-Z (2–4) was β-D-amicetose instead of β-D-olivose usually found in this position. The new angucyclin(on)es were biologically evaluated in comparison with previously known congeners against a small panel of MCF-7 (estrogen responsive) and MDA 231 (estrogen refractory) breast cancer cell lines. 11-deoxylandomycinone (IC₅₀ 2.1 and 1.2 μM) and landomycin Y (IC₅₀ 1.0 and 2.0 μM) showed the highest cytotoxic potencies against both cell lines.

Keywords

anticancer agents; landomycins; cytotoxicity; polyketides; angucyclines; structure-activity-relationships

INTRODUCTION

The landomycins are a subgroup of the large family of angucycline group antibiotics, which are characterized by diverse biological activities, such as antitumor, antibacterial, and enzyme inhibitory.¹⁻⁷ The chemical structures of the landomycins consist of a polyketide-derived angucyclinone decorated with a single deoxyoligosaccharide chain of various...
lengths. Landomycins A–D (C, 13) were originally found as products of *Streptomyces cyanogenus* S136. Later, several more landomycins were discovered, and analyzed for their structure-activity-relationships. It was found that the biological activities were mainly depending on the length of the saccharide chain, with those analogues possessing longer saccharide chains being more potent in general. Landomycin A, the principal product of *S. cyanogenus* S136, is the most potent antitumor agent, possessing an unusual spectrum of activity against the NCI 60 human cancer cell line panel. It contains a hexasaccharide side chain, constructed from two repeating trisaccharide patterns (D-olivose-4-1-D-olivose-3-1-L-rhodinose). Except for landomycin C (13), the sugar chains of all reported landomycins are constructed solely from L-rhodinose and D-olivose units. Landomycin C (13) was the only analogue that bears three different sugar moieties; D-olivose, L-rhodinose and D-amicetose.

During our search for further new cytotoxic landomycin analogues, a fermentation of *Streptomyces cyanogenus* K62 in SG-medium was carried out which afforded four new angucyclin(on)es: 11-deoxylandomycinone (1), and landomycins X–Z (2–4) along with the known compounds tetrangulol (11), tetrangomycin (12), landomycins M (8), F (9) and O (10). In addition, we also found again the very recently reported landomycins S, T and V (5–7).

**RESULTS AND DISCUSSION**

In our search for new landomycin analogues with altered saccharide patterns we screened the regulator-affected high producing mutants *Streptomyces cyanogenus* K62 and *Streptomyces cyanogenus* K60. The production spectrum using SG-medium was very similar for both mutant strains, based on TLC and HPLC-MS analyses (Supporting Information, Figure S1). However, the general production yields of *Streptomyces cyanogenus* K62 were significantly higher than those of *Streptomyces cyanogenus* K60. Therefore, we focused on the K62 mutant for the search of new minor congeners.

A pre-culture of *Streptomyces cyanogenus* K62 served to cultivate 40 of 0.25 L-Erlenmeyer flasks each containing 100 mL of SG-medium, on rotary shaker for 3 days. The broth was harvested, mixed with celite, filtered off and extracted with ethyl acetate, and the organic extracts from supernatant and cells were concentrated *in vacuo* to afford 6.45 g of a reddish powder crude extract (1.61 g/l). A TLC analysis of the strain extract exhibited several UV orange-red fluorescent bands at 366 nm, which turned blue on treatment with 2N NaOH, as indicative of peri-hydroxy quinones. The HPLC-MS analysis of the crude extract displayed several components with UV spectrum characteristic of 11-deoxylandomycin chromophores, which were likely new congeners (Supporting Information, Figures S4, S5). Working up and purification of 0.97 g from the strain extract using various chromatographic techniques (Figure 1) led to the isolation of four new compounds; 11-deoxylandomycinone (1) and landomycins X-Z (2–4), all three possessing 11-deoxyaglycone moiety. In addition, eight known compounds; tetrangulol (11), tetrangomycin (12), landomycins M, F and O (8–10) were isolated along with the recently reported landomycins S, T and V (5–7).
Structure elucidation

Compound 1 was obtained as an orange amorphous powder. The molecular formula of 1 was determined by HRESIMS as C_{19}H_{14}O_{5} (Tables 1, 2). The proton NMR spectrum of 1 displayed two broad singlets at δ 12.07 and 9.75, representing peri-hydroxy groups, and a 1,2,3-trisubstituted aromatic moiety revealed by an ABC system in the region of δ 7.73–7.32 (J = 7.5–8.8 Hz, Table 3). Two additional broad aromatic signals, each 1H, at δ 6.64 and 6.57 showed another highly substituted aromatic ring with two m-coupled aromatic protons. The aliphatic region revealed an oxymethine signal (δ 5.03) directly next to a methylene group (δ 2.89 and 2.76; d, J = 16.2–16.2 Hz), which was confirmed by a H,H-COSY experiment (Figure 2). Furthermore, a singlet of an aromatic-bound methyl group was observed at δ 2.26. All these structural features are typical for 11-deoxylandomycinone. The 13C NMR/HSQC spectra (Table 4) confirmed compound 1 to be 11-deoxylandomycinone, and showed the quinone carbonyls (δ 188.0 and 183.7), the small Δδ ~ 4 ppm indicating both carbonyls to be chelated with hydroxyl groups. In the sp^3 region, the three expected carbon signals representing an oxymethine carbon (δ 57.1), methylene (δ 36.5) and one methyl (δ 21.2) groups, were observed. Finally, the HMBC spectrum (Figure 2) of compound 1 showing 3J correlations between H-11 and C-12, and between H-6 and C-7, confirmed structure 1 as 11-deoxylandomycinone, with C-6 being R-configured, since it displayed the same coupling constants and NOESY correlations (Figure 2, Table 3) typically of all reported landomycins. A data base search (Chemical Abstracts) confirmed the novelty of structure 1.

Compound 2 was obtained as orange solid, with a molecular weight of 1054 Daltons corresponding to a molecular formula of C_{55}H_{74}O_{20}, as deduced by HRESIMS (Tables 1, 2). The proton NMR spectrum (Table 3) and the 13C NMR/HSQC spectra (Table 4) of 2 showed that it contains an 11-deoxylandomycinone agylcone, plus six saccharide moieties (sixanomeric 1H, δH 5.18 -4.41; δC 103.7 -97.5). Four of the anomeric protons (δ 5.18 dd, J = 9.5, 1.5 Hz; δ 4.51 dd, J = 8.6, 1.3 Hz; δ 4.48 dd, J = 9.8, 1.3 Hz; δ 4.41 dd, J = 7.9, 1.3 Hz) show large coupling constants and thus represent β-D-glycoside moieties. The remaining two anomeric protons at δ 4.94 (brs) and δ 4.92 (brs) are α-glycosidically linked L-sugars. The 2D-NMR studies revealed that all these sugars are part of one hexasaccharide chain, linked –as with all landomycins – at 8-position. Overall, structure 2 most closely resembled the recently discovered landomycin S (5), however is by 16 amu smaller, due to the lack of one oxygen atom in the hexasaccharide side, as a comparison of the MS/MS fragmentation patterns of 2 with those of landomycin S (5) revealed (Figure S3, Supporting Information). The NMR (H,H-COSY and HMBC correlations) and MS data analysis showed that the difference was in the fourth sugar moiety, with sugar D being a D-amicetose instead of the D-olivose unit usually found in this position. The data also proved the attachment of the hexasaccharide at 8-position (3J_C-H coupling between H-1A, δH 5.18 with C-8, δC 156.4), for MS-MS fragmentation see also Figure S3 (Supporting Information). All of the remaining NMR data (Tables 3,4, Figure 3) are in full agreement with structure 2. The relative configurations of the sugar residues were derived from the coupling constants and NOESY experiments (Figure 4) indicating that compound 2 has the same stereochemistry both at C-6 of the aglycone and the hexasaccharide sugar moieties as found previously for
landomycin C (13). In sum, structure 2 was determined to be 11-deoxylandomycin C, and was named landomycin X.

Closely related to landomycin X (2), compound 3 was obtained as dark red solid from the same fraction III, exhibiting a molecular formula of $\text{C}_{55}\text{H}_{72}\text{O}_{19}$ (HRESI MS), which is by 18 amu (one $\text{H_2O}$) smaller than the one of landomycin X (2), and has one more degree of unsaturation (Tables 1, 2). The $^1\text{H}$ and $^{13}\text{C}$ NMR data of 3 were similar to those of 2 (Tables 3 and 4), except that ring B of the aglycone was aromatic, as revealed by the $^1\text{H}$ NMR spectrum (two additional ortho-coupled protons at $\delta$ 8.08 (d, $J = 8.8$ Hz) and 8.23 (d, $J = 8.6$) of 3. The structure of 3 was additionally deduced by H,H-COSY, HSQC, HMBC and NOESY experiments, exhibiting the same structural and stereochemical features as in compound 2 (Figures 5, 6). Therefore, structure 3 was determined as 5,6-anhydro-landomycin X, and consequently named landomycin Y.

Structurally related to landomycin X (2) and the recently reported landomycin V (7),12 compound 4 was obtained as orange solid, with a molecular formula of $\text{C}_{49}\text{H}_{64}\text{O}_{18}$ (HREIMS), i.e. by 16 amu smaller than landomycin V (7), for physico-chemical properties see Tables 1, 2. Comparing the $^1\text{H}$ NMR data of compound 4 with those of landomycin X (2) revealed that the terminal $\alpha$-L-rhodinose moiety was missing, while the aglycone was identical to the one found in compounds 2 and 7. Compared to structure 7 an oxygen atom was missing in compound 4, again at position 3D, due a D-amicetose unit instead of a D-olivose (H,H-COSY correlations, Supporting Information, Figure S2, Table 3). Thus, compound 4 was identified as 3D-deoxy-landomycin V, and consequently named landomycin Z.

**Biological activity**

The anticancer activity of the new angucyclin(on)es 1–4 compared with landomycin A were determined using MCF-7 (estrogen responsive) and MDA 231 (estrogen refractory) breast cancer cells (Table 5). Cell viability assays showed that compounds 1 – 4 and landomycin A have comparable anticancer activities against both cells lines. Specifically, against MCF-7 cells, compound 3 was the most potent (IC$_{50}$=1.0 μM), but also compounds 1, 2 and 4 appear to have comparable activity (IC$_{50}$ = 2.1, 2.8 and 2.6 μM respectively) to landomycin A. 11-deoxylandomycinone (1) (IC$_{50}$=1.2 μM) was the most potent compound against MDA 231 cells. However compounds 2 – 4, (IC$_{50}$ = 2.0, 2.0 and 2.5 μM, respectively) also displayed significant cytotoxic activities, again comparable to landomycin A. In conclusion, unlike some of the previously discovered new 11-deoxy-landomycins, e.g. landomycins F (9), M (8), S (5), T (6), and V (7), the new angucyclin(on)es 1–4 showed potency against both MDA 231 and MCF-7 cells, previously only found for landomycin A and other landomycins bearing an 11-OH group. The exchange of the fourth sugar moiety ($\beta$-D-olivose) of landomycins S, T and V (5 – 7) with $\beta$-D-amicetose as in the new landomycins X - Z (2 – 4) slightly improve the anticancer activity (Table 5). The results suggest that a missing 4D-OH group, i.e. substitution of D-olivose by a D-amicetose unit in D-position of the saccharide chain, is advantageous, showing that subtle changes in the H-bonding properties of the saccharide chains can have a significant effect. Like discussed before, the highest activity of landomycins X–Z (2 – 4) and aglycone 1 indicate that these compounds
may have different mechanism-of-action, one for the aglycone alone, the other depending on the length of the sugar side chain, again with longer chains being advantageous. It should also be noted that the observed effects on ER-negative (MDA-231) compared to ER-positive (MCF-7) breast cancer cells could be influenced by differential gene expression patterns known from these cell lines, e.g. MDA-231 cells express higher cdc2, cyclin B1, cyclin D1, cyclin E, IGFBP-3, TGF-α, TGFβ2 compared to MCF-7 cells. Investigations of the molecular mechanism of the landomycins are currently in progress.

EXPERIMENTAL SECTION

General experimental procedures

UV spectra were recorded on a Shimadzu UV-1800 (Model TCC-240A) UV spectrometer. NMR spectra were measured on a Varian VnmrJ 500 (1H, 500 MHz; 13C, 125.7 MHz) spectrometer, the δ-values were referenced to the respective solvent signals. ESI mass spectra were recorded on a Finnigan LCQ ion trap mass spectrometer. ESIHR mass spectra were recorded 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 mass spectrometer, 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. Samples were introduced by means of a syringe pump. HPLC purifications were carried out using a Symmetry Prep C18 10μm column (10 × 150 mm) on a binary LC system. HPLC-MS analyses were carried out using a Symmetry Anal C18 5μm column (4.6 × 250 mm) on a binary LC system. Flash chromatography was carried out on silica gel MN 60 (140–270 mesh ASTM). Rf values were measured on Polygram SIL G/UV254 (Macherey-Nagel & Co.). Size exclusion chromatography was performed on Sephadex LH-20 (GE Healthcare).

Cell Viability Assay

To determine the cytotoxic activity of the new compounds 11-deoxylandomycinone (1), landomycins X-Z (2–4) and landomycin A were tested against two breast cancer cell lines, MCF-7 (estrogen responsive) and MDA 231 (estrogen refractory). Cell viability of these two cell lines in response to the various concentrations of compounds were determined using the trypan blue exclusion assay where 50 × 10^3 cells in 0.5 ml medium were plated in each well of a 24-well plate and allowed to attach overnight. The medium was replaced the following day with fresh medium containing different concentrations of the compounds to be tested 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, and resuspended in PBS for trypan blue staining 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 determined, dose response curve was plotted and finally IC50 were calculated. Each set of experiment was performed three times to confirm reproducibility of the results.
Culture material, fermentation and isolation

**SG-Medium**—Glucose (20 g, Sigma-Aldrich), yeast extract (5 g, Acros Organics), Soytone (10 g, Becton, Dickinson & Co), CoCl₂ × 6 H₂O (1 mg, Acros Organics) and calcium carbonate (2 g, Sigma-Aldrich) 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 Medium**—Glucose (4.0 g, Sigma-Aldrich), yeast extract (4.0 g, Acros Organics), malt extract (10.0 g, MP Biomedicals, LLC) and agar (15.0 g, Becton, Dickinson & Co) were dissolved in 1 liter of demineralized water.

**Fermentation, Extraction and Isolation**—Strain *Streptomyces cyanogenus* K62 was cultivated on M₂-agar plates at 28 °C for 2 days. With pieces of well-grown agar subculture of the strain, a pre-culture (0.25 L Erlenmeyer flask) of *Streptomyces cyanogenus* K62, containing 100 mL of SG-medium was prepared, inoculated and cultivated at 28 °C (250 rpm). After 2 days the grown pre-culture flask was used to inoculate 40 of 0.25 L flasks each containing 100 mL of SG-medium, which was grown at 28 °C, and harvested after 3 days. The obtained reddish brown culture broth was mixed with celite and filtered off; both biomass and filtrate were extracted with EtOAc; (5 × 500 mL, for biomass) and (4 × 2 L, for filtrate). Both extracts were combined and evaporated in vacuo at 40 °C, and afforded 6.45 g of reddish powder crude extract.

Separation of 0.97 g of crude extract on silica gel column (column 2.5 × 50 cm, 100 g), using a stepwise MeOH/CH₂Cl₂ gradient (0.2 L 0% MeOH → fraction FI, then 0.2 L 5% MeOH → fraction FII, then 0.2 L 10%, then 0.5 L 50% MeOH, combined → fraction FIII), yielded three fractions, FI (100 mg, red solid), FII (60.7 mg, orange solid), and FIII (570 mg, red solid). Fraction FI was further purified during silica gel column (0.5 L, CH₂Cl₂/20% n-hexane; 2 × 30 cm) followed by Sephadex LH-20 (2× 40 cm, 50% MeOH/CH₂Cl₂) to obtain tertangulol (11; reddish brown crystals, 38.2 mg). Purification of fraction FII was carried out by HPLC followed by Sephadex LH-20 (1 × 20 cm, MeOH) to yield tetrangomycin (12; yellow solid, 1.3 mg) and 11-deoxylandomycinone (1; orange solid, 6.1 mg.). In an analogous manner, further fractionation and purification of fraction FIII delivered landomycins F (9, 60.0 mg), O (10, 37.1 mg), V (7, 24.9 mg), S (5, 38.7 mg), M (8, 15.8 mg), T (6, 31.2 mg), along with the three new landomycins X~Z (2–4, 11.6, 9.39 and 2.1 mg, respectively) in pure form, (Figure 1, Figure S4).

**11-Deoxylandomycinone (1)**—Orange solid; Rₜ 0.87 (7% MeOH/CH₂Cl₂), blue coloration with 2N NaOH; UV (MeOH) λ_max (log ε) 263 (3.71), 288 (3.68), 319 sh (3.58), 447 (3.28) nm; ¹H NMR (DMSO-d₆, 500 MHz) δ 12.07 (1H, brs, 8-OH), 9.75 (1H, brs, 1-OH), 7.73 (1H, t, J = 8.8 Hz, H-10), 7.44 (1H, d, J = 7.5 Hz, H-11), 7.32 (1H, d, J = 8.8 Hz, H-9), 6.64 (1H, brs, H-2), 6.57 (1H, brs, H-4), 5.03 (1H, d, J = 3.9 Hz, 6-OH), 4.97 (1H, brs, H-6), 2.89 (1H, d, J = 16.2 Hz, H₂-5), 2.76 (1H, d, J = 16.4 Hz, H₂-5), 2.26 (3H, s, 3-CH₃) ppm; ¹H NMR (CDCl₃, 500 MHz) and ¹³C NMR (DMSO-d₆, 125 MHz), see Tables 3 and 4; (−)-ESI MS m/z 321 [M–H]⁻; (+)-ESI MS m/z 323 [M+H]⁺; (−)-HRESIMS m/z 321.0768 [M–H]⁻ (calcd for C₁₉H₁₃O₅, 321.0768); (−)-HRESIMS m/z 323.1001 [M+H]⁺, 305.0795
Landomycin X (2)—Orange solid; $R_f \, 0.65 \, (7\% \, \text{MeOH/CH}_2\text{Cl}_2)$, blue coloration with 2N NaOH; UV (MeOH) $\lambda_{\text{max}} \, (\log \varepsilon) \, 265 \, (4.41), \, 285 \, (4.35), \, 320 \, (3.93) \, \text{nm}; \, ^1\text{H} \, \text{NMR (CDCl}_3, \, 500 \, \text{MHz)} \, \text{and} \, ^{13}\text{C} \, \text{NMR (CDCl}_3, \, 125 \, \text{MHz)}, \text{see Tables 3 and 4; (−)-ESI MS m/z 1053 [M−H]−, (−)-ESI MS m/z 1077 [M+Na]+; (−)-ESI MS/MS m/z (%) 1053 ([M−H]−, 100), 1035 ([M−H$_2$O−H]−, 5), 893 (70), 321 ([M−(-(L-rhodinose + D-olivose + D-amicetose + L-rhodinose + D-olivose + D-olivose)-H]−, 50); (−)-HRESIMS m/z 1053.4688 [M−H]− (calcd for C$_{55}$H$_{73}$O$_{20}$, 1053.4700); (+)-HRESIMS m/z 1077.4722 [M+Na]+, 1093.4467 [M+K]+ (calcd for C$_{55}$H$_{74}$O$_{20}$Na, 1077.4665, and for C$_{55}$H$_{74}$O$_{20}$K, 1093.4405).

Landomycin Y (3)—Dark red solid; $R_f \, 0.60 \, (7\% \, \text{MeOH/CH}_2\text{Cl}_2)$, blue coloration with 2N NaOH; UV (MeOH) $\lambda_{\text{max}} \, (\log \varepsilon) \, 246 \, \text{sh} \, (4.59), \, 312 \, (4.59), \, 399 \, (4.03) \, \text{nm}; \, ^1\text{H} \, \text{NMR (CDCl}_3, \, 500 \, \text{MHz}), \text{see Tables 3 and 4; (+)-ESI MS m/z 1059 [M+Na]+; (+)-HRESIMS m/z 1059.4546 [M+Na]+ (calcd for C$_{55}$H$_{72}$O$_{19}$Na, 1059.4560).

Landomycin Z (4)—Orange solid; $R_f \, 0.35 \, (7\% \, \text{MeOH/CH}_2\text{Cl}_2)$, blue coloration with 2N NaOH; UV (MeOH) $\lambda_{\text{max}} \, (\log \varepsilon) \, 265 \, (4.14), \, 285 \, (4.05), \, 403 \, (3.79) \, \text{nm}; \, ^1\text{H} \, \text{NMR (CDCl}_3, \, 500 \, \text{MHz)}, \text{see Table 3; (+)-ESI MS m/z 963 [M+Na]+; (+)-HRESIMS m/z 963.3981 [M+Na]+ (calcd for C$_{49}$H$_{64}$O$_{18}$Na, 963.3985).

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

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**References**

1. Crow RT, et al. Landomycin A inhibits DNA synthesis and G1/S cell cycle progression. Bioorg Med Chem Lett. 1999; 9:1663–6. [PubMed: 10397496]

2. Depenbrock H, et al. Assessment of Antitumor Activity of Landomycin A (NSC 6399187-A). Ann Hematol. 1996; 73(Supl II):A80, 316.

3. Rohr J, Thiericke R. Angucycline Group Antibiotics. Nat Prod Rep. 1992; 9:103–137. [PubMed: 1620493]

4. Krohn K, Rohr J. Angucyclines: Total Syntheses, New Structures, and Biosynthetic Studies of an Emerging New Class of Antibiotics. Topics Curr Chem. 1997; 188:127–195.

5. Weber S, Zolke C, Rohr J, Beale JM. Investigations of the Biosynthesis and Structural Revision of Landomycin A. J Org Chem. 1994; 59:4211–4214.

6. Henkel T, Rohr J, Beale JM, Schwenen L. Landomycins, new angucycline antibiotics from Streptomyces sp I Structural studies on landomycins A-D. J Antibiot. 1990; 43:492–503. [PubMed: 2358402]

7. Korynevska A, et al. Mechanisms underlying the anticancer activities of the angucycline landomycin E. Biochem Pharmacol. 2007; 74:1713–1726. [PubMed: 17904109]
8. Zhu L, et al. Generation of new landomycins with altered saccharide patterns through over-expression of the glycosyltransferase gene lanGT3 in the biosynthetic gene cluster of landomycin A in Streptomyces cyanogenus S-136. Chem Bio Chem. 2007; 8:83–88.

9. Zhu L, et al. Identification of the function of gene lndM2 encoding a bifunctional oxygenase-reductase involved in the biosynthesis of the antitumor antibiotic landomycin E by Streptomyces globisporus 1912 supports the originally assigned structure for landomycinone. J Org Chem. 2005; 70:631–638. [PubMed: 15651811]

10. Luzhetskyy A, et al. Generation of novel landomycins M and O through targeted gene disruption. Chem BioChem. 2005; 6:675–678.

11. Ostash B, et al. Generation of New Landomycins by Combinatorial Biosynthetic Manipulation of the LndGT4 Gene of the Landomycin E Cluster in S. globisporus. Chem Biol. 2004; 11:547–555. [PubMed: 15123249]

12. Shaaban KA, Srinivasan S, Kumar R, Damodaran C, Rohr J, Landomycins P-W. Cytotoxic Angucyclines from Streptomyces cyanogenus S136. J Nat Prod. 2010 submitted.

13. Luzhetskyy A, Vente A, Bechthold A. Glycosyltransferases involved in the biosynthesis of biologically active natural products that contain oligosaccharides. Mol BioSyst. 2005; 1:117–126. [PubMed: 16880973]

14. Trefzer A, et al. Elucidation of the function of two glycosyltransferase genes (lanGT1 and lanGT4) involved in landomycin biosynthesis and generation of new oligosaccharide antibiotics. Chem Biol. 2001; 8:1239–1252. [PubMed: 11755402]

15. Kuntsmann MP, Mitscher LA. The structural characterization of tetrangomycin and tetragulol. J Org Chem. 1966; 31:2920–2925. [PubMed: 5919937]

16. Krohn K, Boker N, Florke U, Freund C. Synthesis of Angucyclines. 8 Biomimetic-Type Synthesis of Rabelomycin, Tetrangomycin, and Related Ring B Aromatic Angucyclinones. J Org Chem. 1997; 62:2350–2356. [PubMed: 11671566]

17. Krohn K, Khanbabae K. First total synthesis of (+/−)-rabelomycin. Angew Chem Int Ed Engl. 1994; 33:99–100.

18. Ostash I, et al. Coordination of export and glycosylation of landomycins in Streptomyces cyanogenus S136. FEMS Microbiol Lett. 2008; 285:195–202. [PubMed: 18537830]
Figure 1.

1: R^1 = OH, R^2 = OH; 11-Deoxy-landomycinone
2: R^1 = I, R^2 = OH, R^3 = H; Landomycin X
3: R^1 = I, R^2 = H, R^3 = H, Δ^5,6; Landomycin Y
4: R^1 = II, R^2 = OH, R^4 = H; Landomycin Z
5: R^1 = I, R^2 = OH, R^3 = OH; Landomycin S
6: R^1 = I, R^2 = H, R^3 = OH, Δ^5,6; Landomycin T
7: R^1 = II, R^2 = OH, R^4 = OH; Landomycin V
8: R^1 = II, R^2 = H, R^4 = OH, Δ^5,6; Landomycin M
9: R^1 = III, R^3 = OH; Landomycin F
10: R^1 = III, R^2 = H, Δ^5,6; Landomycin O
11: R^1 = OH, R^2 = H, Δ^5,6; Tetragulol
13: R^1 = I, R^2 = OH, R^3 = H; 11-OH; Landomycin C
Figure 2.
Figure 3.
Figure 4.
Figure 6.
Figure 7.
Table 1

Physico-chemical properties of 11-deoxylandomycinone (1), and landomycin X (2).

|                     | 11-Deoxylandomycinone (1)                      | Landomycin X (2)                      |
|---------------------|------------------------------------------------|-------------------------------------|
| Appearance          | Orange solid                                   | Orange solid                        |
| \( R_t \)           | 0.87 (CH\(_2\)Cl\(_2\)/7\%MeOH)              | 0.65 (CH\(_2\)Cl\(_2\)/7\%MeOH)    |
| Molecular formula   | C\(_{19}\)H\(_{14}\)O\(_5\)                   | C\(_{55}\)H\(_{74}\)O\(_{20}\)       |
| (−)-ESI MS: \( m/z \) | 321 [M−H]\(^−\)                               | 1053 [M−H]−                         |
| (+)-ESI MS: \( m/z \) | 323 [M+H]\(^+\)                               | 1077 [M+Na]\(^+\)                  |
| (−)-ESI MS/MS: \( m/z \) (%) | -                                                | 1053 ([M−H]−, 100), 1035 ([M−H\(_2\)O−H]−, 5), 893 (70), 321 ([M−(L-rhodinose + D-olivose + D-amicetose + L-rhodinose + D-olivose−H)]−, 50) |
| (+)-HRESI MS (m/z)  | Found 323.1001 [M+H]\(^+\), 305.0795 [M−H\(_2\)O+H]−, and 361.0473 [M+K]\(^+\) | 1077.4722 [M+Na]− and 1093.4467 [M+K]\(^+\) |
| Calcd.              | 323.0914 for C\(_{19}\)H\(_{14}\)O\(_5\), 305.0808 for C\(_{19}\)H\(_{14}\)O\(_4\) and 361.0473 for C\(_{19}\)H\(_{14}\)O\(_3\)K | 1077.4665 for C\(_{55}\)H\(_{74}\)O\(_{20}\)Na and 1093.4405 for C\(_{55}\)H\(_{74}\)O\(_{20}\)K |
| (−)-HRESI MS (m/z)  | Found 321.0768 [M−H]−                         | 1053.4688 [M−H]−                    |
| Calcd.              | 321.0768 for C\(_{19}\)H\(_{14}\)O\(_5\)      | 1053.4700 for C\(_{55}\)H\(_{74}\)O\(_{20}\) |
| UV/VIS (MeOH):      | \( \lambda_{max} \) (log \( \varepsilon \)) 263 (3.71), 288 (3.68), 319 sh (3.58), 447 (3.28) nm. | 265 (4.41), 285 (4.35), 320 sh (4.13), 412 (3.93) nm. |
Table 2
Physico-chemical properties of landomycins Y (3) and Z (4).

|                      | Landomycin Y (3)          | Landomycin Z (4)          |
|----------------------|---------------------------|---------------------------|
| Appearance           | Red solid                 | Orange solid              |
| $R_f$                | 0.60 (CH$_2$Cl$_2$/7%MeOH)| 0.35 (CH$_2$Cl$_2$/7%MeOH)|
| Molecular formula    | C$_{55}$H$_{72}$O$_{19}$  | C$_{49}$H$_{64}$O$_{18}$  |
| (+)-ESI MS: m/z      | 1059 [M+Na]$^+$           | 963 [M+Na]$^+$            |
| (+)-HRESI MS (m/z)   |                           |                           |
| Found                | 1059.4546 [M+Na]$^+$      | 963.3981 [M+Na]$^+$       |
| Calcd.               | 1059.45598 for C$_{55}$H$_{72}$O$_{19}$Na | 963.39846 for C$_{49}$H$_{64}$O$_{18}$Na |
| UV/VIS (MeOH):       |                           |                           |
| $\lambda_{\text{max}}$ (log $\varepsilon$) | 246 sh (4.59), 312 (4.59), 399 (4.03) nm. | 265 (4.14), 285 (4.05), 403 (3.79) nm. |
Table 3

$^1$H NMR data of 11-deoxylandomycinone (1) and landomycins X-Z (2-4) in CDCl$_3$, $\delta$ in ppm relative to TMS, multiplicities (J/Hz).

| Position | (1)$^a$ | Landomycin X (2)$^a$ | Landomycin Y (3)$^a$ | Landomycin Z (4)$^a$ |
|-----------|---------|-----------------|-----------------|-----------------|
| $\delta_H$ (500 MHz) | $\delta_H$ (500 MHz) | $\delta_H$ (500 MHz) | $\delta_H$ (500 MHz) |
| 1-OH | 9.21 brs | 9.55 brs | 11.11 s | 9.55 brs |
| 2 | 6.77 brs | 6.76 brs | 7.09 d 1.7 | 6.77 brs |
| 3-CH$_3$ | 2.31 s | 2.29 s | 2.46 s | 2.29 s |
| 4 | 6.74 brs | 6.71 (brs) | 7.22 d (1.7) | 6.71 brs |
| 5$_a$ | 2.93 dd (16.2, 4.2) | 2.87 dd (16.2, 4.3) | 8.08 d (8.8) | 2.88 dd (16.2, 4.3) |
| 5$_b$ | 3.11 dd (16.3, 4.2) | 3.05 dd (16.2, 4.3) | 3.06 dd (16.2, 4.7) | 3.06 dd (16.2, 4.7) |
| 6 | 5.20 t (4.1) | 5.10 t (4.4) | 8.23 d (8.6) | 5.10 t (4.5) |
| 8-OH | 11.97 s | - | - | - |
| 9 | 7.32 dd (8.4, 1.0) | 7.48 d (8.5) | 7.48 dd (8.4, 0.8) | 7.49 d (8.5) |
| 10 | 7.64 t (8.0) | 7.65 t (8.2) | 7.67 t (8.1) | 7.66 t (8.2) |
| 11 | 7.76 dd (7.5, 1.0) | 7.93 d (7.7) | 8.00 dd (7.7, 0.9) | 7.94 d (7.7) |

Sugar A, $\beta$-D-olivose

| 1A | 5.18 dd (9.5, 1.5) | 5.23 dd (9.6, 1.9) | 5.20 brd (9.5) |
| 2A$_a$ | 2.15 ddd (12.7, 12.0, 5.0) | 2.16 ddd (12.7, 12.0, 5.0) | 2.11 ddd (12.7, 12.0, 5.0) |
| 2A$_c$ | 2.63 ddd (12.8, 11.2, 5.1) | 2.72 ddd (12.7, 5.1, 1.7) | 2.66 ddd (12.8, 5.1, 1.7) |
| 3A | 3.70 m | 3.75 m | 3.72 m |
| 3A-OH | 4.70 brs | 4.72 brs | 4.72 brs |
| 4A | 3.05 dd (8.4, 8.4) | 3.10 dd (8.4, 8.4) | 3.09 dd (8.1, 8.1) |
| 5A | 3.48 m | 3.48 m | 3.44 m |
| 6A | 1.29 d (6.1) | 1.30 d (6.1) | 1.29 d (6.1) |

Sugar B, $\beta$-D-olivose

| 1B | 4.51 dd (8.6, 1.3) | 4.52 dd (9.9, 1.8) | 4.51 brd (9.6) |
| 2B$_a$ | 1.78–1.50 m (complex) | 1.75–1.45 m (complex) | 1.75–1.51 m (complex) |
| 2B$_c$ | 2.24 ddd (12.0, 5.0, 1.5) | 2.20 ddd (12.0, 5.0, 1.5) | 2.21 ddd (12.0, 5.0, 1.5) |
| 3B | 3.48 ddd (12.2, 8.3, 5.2) | 3.48 ddd (12.2, 8.3, 5.2) | 3.47 ddd (12.2, 8.3, 5.2) |
| 4B | 3.05 dd (8.4, 8.4) | 3.05 dd (8.4, 8.4) | 3.08 dd (8.4, 8.4) |
| 4B-OH | 4.20 brs | 4.22 brs | 4.20 brs |
| 5B | 3.22 m | 3.23 m | 3.25 m |
| 6B | 1.33 d (6.1) | 1.32 d (6.1) | 1.31 d (6.1) |

Sugar C, $\alpha$-L-rhodinose

| 1C | 4.94 brs | 4.95 brs | 4.95 brs |
| 2C$_a$ | 1.68 m (complex) | 1.58 m (complex) | 1.75–1.51 m (complex) |
| 2C$_c$ | 1.95 m (complex) | 1.78 m (complex) | 2.02–1.89 m (complex) |
| 3C$_a$ | 1.55 m (complex) | 1.55 m (complex) | 1.75–1.51 m (complex) |
| 3C$_c$ | 2.02 m (complex) | 2.05 m (complex) | 2.02–1.89 m (complex) |

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| Position | Landomycin X (1\(^a\)) | Landomycin Y (2\(^a\)) | Landomycin Z (3\(^a\)) | Landomycin Z (4\(^a\)) |
|----------|------------------------|------------------------|------------------------|------------------------|
|          | \(\delta_H\) (500 MHz) | \(\delta_H\) (500 MHz) | \(\delta_H\) (500 MHz) | \(\delta_H\) (500 MHz) |
| 4C       | 3.51 brs               | 3.51 brs               | 3.52 brs               |                        |
| 5C       | 4.07 q (6.5)           | 4.06 dq (6.6, 1.2)     | 4.07 q (6.6)           |                        |
| 6C       | 1.18 d (6.4)           | 1.18 d (6.4)           | 1.19 d (6.4)           |                        |

Sugar D, \(\beta\)-D-amicetose

1D        | 4.41 dd (7.9, 1.3)    | 4.41 dd (9.3, 1.7)     | 4.41 brd (9.0)         |                        |
2D\(_a\)  | 1.28 m (complex)      | 1.50 m (complex)       | 1.75–1.51 m (complex)  |                        |
2D\(_e\)  | 2.21 brd (12.7, 5.1)  | 2.25 brdd (12.7, 5.1)  | 2.21 brdd (12.7, 5.1)  |                        |
3D\(_a\)  | 1.88–1.96 m (complex) | 1.65 m (complex)       | 1.75–1.51 m (complex)  |                        |
3D\(_e\)  | 1.88–1.96 m (complex) | 1.92 m (complex)       | 2.02–1.89 m (complex)  |                        |
4D        | 3.10 m                 | 3.16 m                 | 3.16 m                 |                        |
5D        | 3.31 m                 | 3.31 m                 | 3.32 m                 |                        |
6D        | 1.20 d (6.1)           | 1.20 d (6.1)           | 1.20 d (6.1)           |                        |

Sugar E, \(\beta\)-D-olivose

1E        | 4.48 dd (9.8, 1.4)    | 4.48 dd (9.8, 1.8)     | 4.47 brd (9.9)         |                        |
2E\(_a\)  | 1.60 m (complex)      | 1.62 m (complex)       | 1.75–1.51 m (complex)  |                        |
2E\(_e\)  | 2.15 m (complex)      | 2.18 m (complex)       | 2.13 m (complex)       |                        |
3E        | 3.48 ddd (12.2, 8.3, 5.2) | 3.48 ddd (12.2, 8.3, 5.2) | 3.47 ddd (12.2, 8.3, 5.2) |                        |
4E        | 3.05 dd (8.4, 8.4)    | 3.06 dd (8.4, 8.4)     | 3.06 dd (8.4, 8.4)     |                        |
4E-OH     | 4.54 brs              | 4.56 brs              | 4.56 brs              |                        |
5E        | 3.38 m                 | 3.38 m                 | 3.38 m                 |                        |
6E        | 1.38 d (6.1)           | 1.39 d (6.1)           | 1.38 d (6.1)           |                        |

Sugar F, \(\alpha\)-L-rhodinose

1F        | 4.92 brs              | 4.92 brs              |                        |                        |
2F\(_a\)  | 1.55 m (complex)      | 1.52 m (complex)      |                        |                        |
2F\(_e\)  | 2.02–1.88 m (complex) | 2.02 m (complex)      |                        |                        |
3F\(_a\)  | 1.52 m (complex)      | 1.58 m (complex)      |                        |                        |
3F\(_e\)  | 2.02–1.88 m (complex) | 2.05 m (complex)      |                        |                        |
4F        | 3.61 brs              | 3.61 (br s)           |                        |                        |
5F        | 4.11 q (6.4)          | 4.11 dq (6.3, 1.0)    |                        |                        |
6F        | 1.19 d (6.5)          | 1.19 d (6.4)          |                        |                        |

\(^a\)See also Figures S7–8, S11, S13 and S15.
Table 4

\(^{13}\)C NMR data of 11-deoxylandomycine and landomycins X-Y (2, 3) compared with the reported data of landomycin C (13),\(^6\) (\(\delta_C\), mult.).

| Position | 1\(^{a,b}\) | 2\(^{a,c}\) | 3\(^{a,e}\) | 13\(^{c}\) | Position | 2\(^{a,e}\) | 3\(^{a,e}\) | 13\(^{c}\) |
|----------|------------|------------|------------|----------|----------|------------|------------|----------|
| \(\delta_C^{d}\) | \(\delta_C^{d}\) | \(\delta_C^{d}\) | \(\delta_C^{d}\) | \(\delta_C^{d}\) | \(\delta_C^{d}\) | \(\delta_C^{d}\) | \(\delta_C^{d}\) | \(\delta_C^{d}\) |
| 1        | 155.8 qC   | 155.8 qC   | 155.2 qC   | 155.1 qC | 5B       | 72.0 CH    | 72.0 CH    | 69.2\(^*\) CH |
| 2        | 115.4 CH   | 120.2 CH   | 119.9 CH   | 120.1 CH | 6B       | 18.3 CH    | 18.3 CH    | 17.8\(^*\) CH |
| 3        | 142.0 qC   | 143.9 qC   | 141.4 qC   | 143.7 qC | Sugar C, α-L-rhodinose |
| 3-CH\(_{3}\) | 21.2 CH\(_{3}\) | 21.4 CH\(_{3}\) | 21.4 CH\(_{3}\) | 21.2 CH\(_{3}\) | 1C       | 98.2 CH    | 98.1 CH    | 97.7 CH    |
| 4        | 121.1 CH   | 123.7 CH   | 121.4 CH   | 126.8 CH | 2C       | 25.7 CH    | 25.7 CH    | 25.5 CH    |
| 4a       | 138.7 qC   | 136.9 qC   | 138.5 qC   | 136.8 qC | 3C       | 25.3 CH    | 25.3 CH    | 25.1 CH    |
| 5        | 36.5 CH\(_{2}\) | 36.5 CH\(_{2}\) | 137.8 CH   | 37.1 CH\(_{2}\) | 4C       | 75.6 CH    | 75.6 CH    | 75.6\(^*\) CH |
| 6        | 57.1 CH    | 62.0 CH    | 122.9 CH   | 65.6 CH  | 5C       | 68.0 CH    | 68.0 CH    | 67.8\(^*\) CH |
| 6a       | 139.3 qC   | 145.8 qC   | 136.8 qC   | 138.8 qC | 6C       | 17.2 CH    | 17.2 CH    | 17.0\(^*\) CH |
| 7        | 188.0 qC   | 183.8 qC   | 181.9 qC   | 182.9 qC | Sugar D, β-D-amicoine |
| 7a       | 114.6 qC   | 120.6 qC   | 121.6 qC   | 114.9 qC | 1D       | 103.7 CH   | 103.7 CH   | 103.5 CH   |
| 8        | 159.8 qC   | 156.4 qC   | 156.5 qC   | 150.7 qC | 2D       | 30.2 CH    | 30.2 CH    | 30.7 CH    |
| 9        | 123.0 CH   | 125.1 CH   | 124.8 CH   | 123.7 CH | 3D       | 30.9 CH    | 30.9 CH    | 30.0 CH    |
| 10       | 136.4 CH   | 134.9 CH   | 134.9 CH   | 132.6 CH | 4D       | 80.7 CH    | 80.7 CH    | 80.7 CH    |
| 11       | 118.1 CH   | 123.1 CH   | 123.4 CH   | 159.6 CH | 5D       | 74.5 CH    | 74.5 CH    | 74.4\(^*\) CH |
| 11a      | 134.3 qC   | 134.7 qC   | 137.1 qC   | 119.1 qC | 6D       | 18.5 CH    | 18.4 CH    | 16.9\(^*\) CH |
| 12       | 183.7 qC   | 189.7 qC   | 190.7 qC   | 192.7 qC | Sugar E, β-D-olivose |
| 12a      | 144.6 qC   | 138.7 qC   | 130.8 qC   | 146.8 qC | 1E       | 101.0 CH   | 101.1 CH   | 100.9 CH   |
| 12b      | 114.1 qC   | 113.3 qC   | 119.3 qC   | 113.3 qC | 2E       | 37.4 CH    | 37.7 CH    | 37.2 CH    |
| Sugar A, β-D-olivose | | | | | 3E       | 80.7 CH    | 80.9 CH    | 75.2\(^*\) CH |
| 1A       | -         | 98.5 CH    | 98.7 CH    | 99.6 CH  | 4E       | 75.4 CH    | 75.4 CH    | 80.5 CH    |
| 2A       | -         | 37.7 CH    | 37.8 CH    | 37.6 CH\(_{2}\) | 5E       | 72.5 CH    | 72.5 CH    | 71.8\(^*\) CH |
| 3A       | -         | 69.4 CH    | 69.5 CH    | 72.3\(^*\) CH | 6E       | 18.0 CH\(_{3}\) | 18.0 CH\(_{3}\) | 18.1\(^*\) CH |
| 4A       | -         | 88.0 CH    | 88.0 CH    | 87.8 CH  | Sugar F, α-L-rhodinose |
| Position | $\delta_1$ | $\delta_2$ | $\delta_3$ | $\delta_4$ | $\delta_5$ | $\delta_6$ | $\delta_7$ | $\delta_8$ | $\delta_9$ | $\delta_10$ | $\delta_11$ | $\delta_12$ | $\delta_13$ |
|----------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| 5A       | -      | 71.1   | 71.1   | 70.8   | 97.5   | 97.5   | 97.3   |        |        |        |        |        |        |
| 6A       | -      | 18.0   | 18.1   | 17.8   | 24.7   | 24.7   | 24.6   |        |        |        |        |        |        |
| Sugar B, β-D-olivose |            | 3F     |        |        | 24.3   | 24.3   | 24.1   |        |        |        |        |        |        |
| 1B       | -      | 101.2  | 101.0  | 100.8  | 67.3   | 67.3   | 67.6   |        |        |        |        |        |        |
| 2B       | -      | 37.4   | 37.4   | 36.3   | 67.8   | 67.8   | 67.1   |        |        |        |        |        |        |
| 3B       | -      | 80.9   | 80.8   | 75.4   | 17.2   | 17.2   | 18.3   |        |        |        |        |        |        |
| 4B       | -      | 75.7   | 75.7   | 80.4   | -      | -      | -      |        |        |        |        |        |        |

$^a)$ See also Figures S9, S12 and S14,

$^b)$ DMSO-d$_6$,

$^c)$ CDCl$_3$,

$^d)$ 125 MHz,

$^e)$ 50 MHz,

$^*$ assignment is uncertain
Table 5

Anti-breast cancer potency (Trypan blue exclusion cell viability assay) of the new discovered 11-deoxylandomycinone (1) and landomycins X, Y and Z (2–4) in comparison with selected related compounds

| No. | Name                  | Structure                                  | Activities (mean IC<sub>50</sub>, μM) |
|-----|-----------------------|--------------------------------------------|---------------------------------------|
|     |                       |                                            | MCF-7 | MDA-231 |
| 1   | 11-Deoxylandomycinone | R¹ = OH, R² = OH                           | 2.1±0.3 | 1.2±0.4 |
| 2   | Landomycin X          | R¹ = hexasaccharide (I), R² = OH, R³ = H   | 2.8±0.5 | 2.0±0.3 |
| 3   | Landomycin Y          | R¹ = hexasaccharide (I), R² = H, R³ = H, Δ⁵,⁶ | 1.0±0.1 | 2.0±0.1 |
| 4   | Landomycin Z          | R¹ = pentasaccharide (II), R² = OH, R⁴ = H | 2.6±0.3 | 2.5±0.2 |
| 5   | Landomycin S          | R¹ = hexasaccharide (I), R² = OH, R³ = OH  | 6.7±1  | 1.5±0.3 |
| 6   | Landomycin T          | R¹ = hexasaccharide (I), R² = OH, R³ = OH, Δ⁵,⁶ | NP*   | 1.85±0.4 |
| 7   | Landomycin V          | R¹ = pentasaccharide (II), R² = OH, R³ = OH | 6.1±1.3 | 1.5±0.5 |
| 8   | Landomycin M          | R¹ = pentasaccharide (II), R² = H, R⁴ = OH, Δ⁵,⁶ | 7.1±1.6$ | 1.9±0.5 |
| 9   | Landomycin F          | R¹ = disaccharide (III), R² = OH            | NP*&   | 1.8±0.4 |
| 10  | Landomycin O          | R¹ = disaccharide (III), R² = H, Δ⁵,⁶       | NP*γ   | 3.55±1.1 |
| 11  | Tetrangulol           | R¹ = OH, R² = H, Δ⁵,⁶                       | NP*γ   | 1.5±0.2 |
| 12  | Tetrangomycin         | Structure 12                                | NP*γ   | 1.5±0.3 |
|     | Landomycin A          | 11-Hydroxy-landomycin S                     | 2.2±0.1 | 2.0±0.1 |

* HPLC-MS analyses showed that these compounds remained stable under assay conditions, and did not decompose into aglycone and sugar residues.

NP = Not potent, the data for compounds 5–12 were taken from reference 12

$ previously reported IC<sub>50</sub> against MCF-7 cells was 53.2 ± 0.7 μM using a sulforhodamine B assay

& previously reported IC<sub>50</sub> against MCF-7 cells was 15.9 ± 3.0 μM using a sulforhodamine B assay

γ previously reported IC<sub>50</sub> against MCF-7 cells was 46.7 ± 9.8 μM using a sulforhodamine B assay

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