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

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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 10 other known angucyclin(on)es. The structures of the new compounds were established from their NMR and mass spectrometry data. The activity of these angucyclin(on)es 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 ($IC_{50} = 1.8\ \mu M$). Compounds with shorter saccharidal moieties were less potent against MCF-7. The fact that the most active landomycins have either 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 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 two, five, or six 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 the most 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] and 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 *S. 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**), landomycins A (**16**), B (**14**), D (**12**), F (**13**), M (**8**), and O (**4**), and tetrangomycin (**19**). On the basis of 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 mass of 678 Da (HRESIMS), leading to the molecular formula $C_{37}H_{42}O_{12}$. 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 and 1.21 revealed three anomeric protons and three doublet methyl signals, consistent with two β-D- and one α-L-glycosidic 6-deoxysugar moiety. The ^{13}C NMR/HSQC spectra of **5** established tetrangulol (**1**) as the 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

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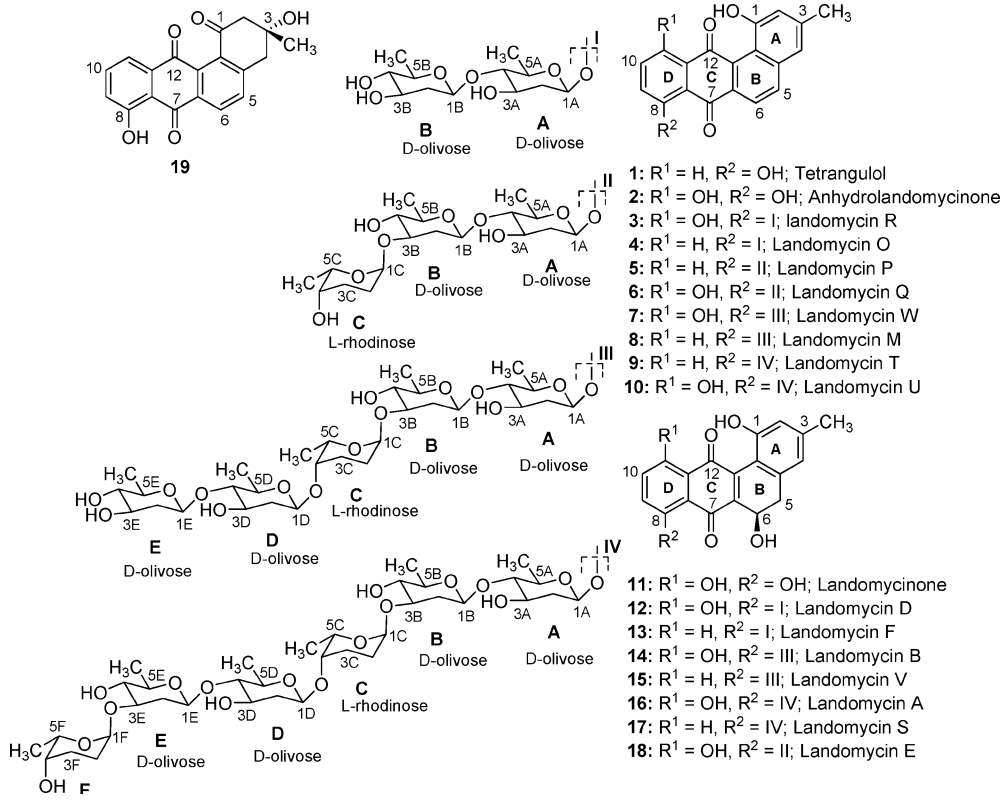
Chart 1



anomeric carbon signals (δ 101.1, 98.9, and 97.8) were observed along with 10 methine, four methylene, and four methyl signals.

The HMBC and ^{1}H−^{1}H COSY correlations of **5** (Figure 1) revealed two partial structures, the tetrangulol-aglycone (**1**) and a trisaccharide system, also supported by the ESIMS^2 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-rhodinosyltetrangulol, 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 ^{1}H NMR spectrum revealed an additional phenolic OH singlet (at δ 12.23, Table 1) and two aromatic AB systems instead of the AB/ABC systems found in **5**. Accordingly, a molecular mass of 16 amu higher than that of **5** corresponding to a molecular formula of $C_{37}H_{42}O_{13}$ 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 ESIMS^2 spectrum, the NMR data also revealed the same trisaccharide chain as in compound **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 ^{1}H 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 $C_{31}H_{32}O_{11}$ of **3** was established by ESIHRMS. 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

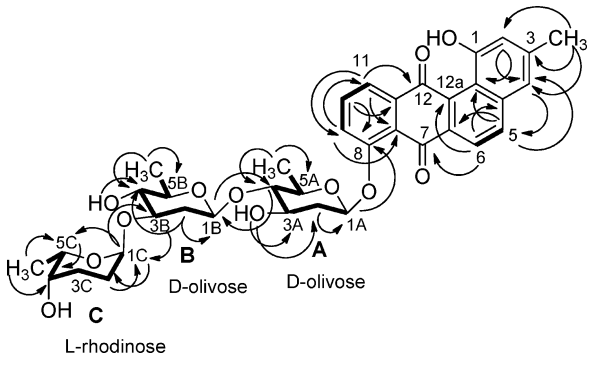


Figure 1. Selected HMBC long-range couplings (→) and ^{1}H−^{1}H COSY correlations (bold lines) of landomycin P (**5**).

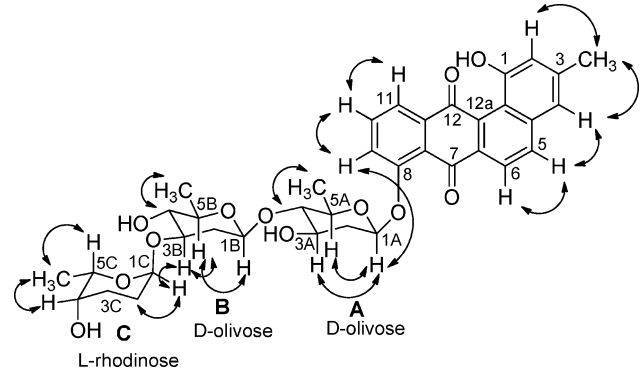


Figure 2. Selected NOESY correlations (↔) determining the sugar ring conformations in landomycin P (**5**).

Table 1. ^{13}C and ^{1}H NMR Assignments of the New Landomycins P (**5**), Q (**6**), and R (**3**) [δ in ppm relative to TMS (multiplicity, *J*/Hz)]

	landomycin P (**5**)[a]		landomycin Q (**6**)[a]	landomycin R (**3**)[a]
position	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 (12.7, 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)
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] $CDCl_3$. [c] 125 MHz. [d] DMSO-d_6. [e] Not observed.

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 mass of compound **17** was 16 amu lower than that of landomycin A (**16**), with a molecular formula of $C_{55}H_{74}O_{21}$ proven by ESIHRMS. The full assignment of compound **17** was deduced from the $^{1}H-^{1}H$ COSY, HSQC, and HMBC experiments (Figure 3 and Table 2). On the basis of 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-deoxylandomycinone 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 HRESIMS-derived molecular formula, $C_{55}H_{72}O_{20}$, was 18 amu less than landomycin S (**17**). Most of the ^{1}H 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 $^{1}H-^{1}H$ 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 landomycin T.

Landomycin U (10). The (+)-HRESIMS of compound **10** showed a mass of *m*/*z* 1091.4447 $[M + Na]^+$, consistent with the molecular formula $C_{55}H_{72}O_{21}$ of a hexasaccharidal compound. The

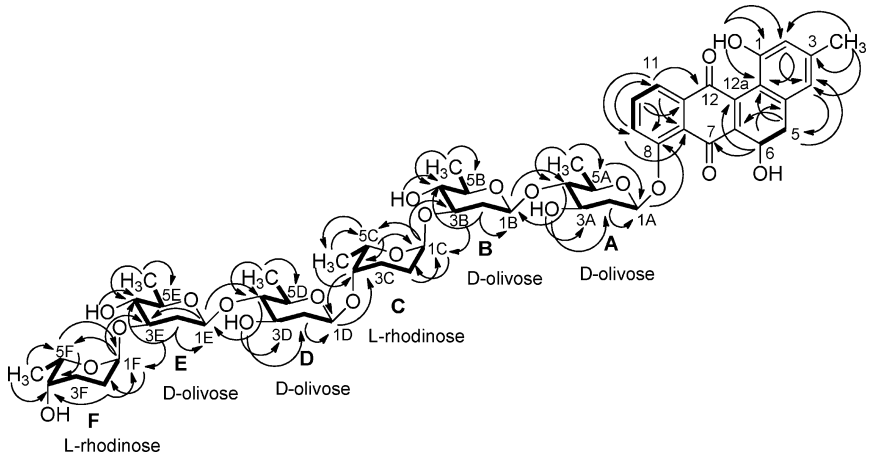


Figure 3. Selected HMBC connectivities (→) and $^1H-^1H$ COSY correlations (bold lines) of landomycin S (**17**).

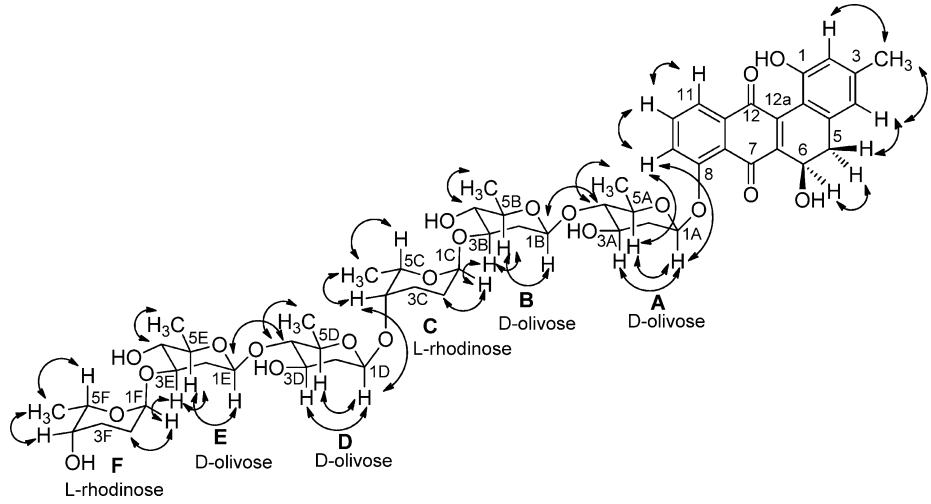


Figure 4. Selected NOESY correlations (↔) of landomycin S (**17**).

latter conclusion also was confirmed by the ^1H NMR data that revealed six signals in the anomeric region between δ 5.07 and 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 Figure S3). The (−)HRESIMS (*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 molecular mass of 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, along with 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-deoxylandomycin 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-CH_2 found in **14**). Thus, compound **7** was deduced as 5,6-anhydrolandomycin 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 (IC_{50} = 1.8 μM); compounds **10**, **11**, and **16** were moderately active (IC_{50} = 2.1, 2.1, and 2.5 μM, respectively), compounds **7**, **8**, **12**, **14**, **15**, and **17** (IC_{50} = 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** showed no cytotoxic activity. In MDA-231 cells compounds **11** and **16** (IC_{50} = 1.4 μM) were most active, compounds **1**−**3**, **8**, **9**, **12**−**15**, **17**, and **19** (IC_{50} = 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** (IC_{50} = 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

Table 2. ^{13}C and ^{1}H NMR Assignments of Landomycins S (**17**), T (**9**), and U (**10**) in $CDCl_3$ [δ in ppm relative to TMS (multiplicity, *J*/Hz)]

	landomycin S (**17**)[a]		landomycin T (**9**)[b]		landomycin U (**10**)[a]
position	δ_C[b]	δ_H (500 MHz)	δ_C[b]	δ_H (500 MHz)	δ_H (500 MHz)
1	155.9, C		155.1, C		
1-OH		9.59, br s		11.11, s	10.63, s
2	120.1, CH	6.74, br s	119.9, CH	7.06, d (1.5)	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
4	123.7, CH	6.70, br s	121.4, CH	7.20, d (1.5)	7.27, br s
4a	137.0, C		138.5, C		
5	36.5, CH_2	2.86, dd (16.0, 4.7, H_α) 3.03, dd (16.0, 4.7, H_β)	137.8, CH	8.06, d (8.8)	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)
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)
10	134.8, CH	7.63, t (8.0)	134.9, CH	7.65, t (8.2)	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
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)
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)
3A	69.5, CH	3.68, m	69.4, CH	3.74, m	3.73, m
3A-OH		4.65, br s		4.72, br s	4.69, br s
4A	88.0, CH	3.09, dd (8.8, 8.8)	88.0, CH	3.12, dd (8.7, 8.1)	3.10, dd (8.4, 8.4)
5A	71.1, CH	3.43, m	71.0, CH	3.45, m	3.49, m
6A	18.0, CH_3	1.28, d (6.1)	18.0, CH_3	1.29, d (6.2)	1.28, d (6.1)
sugar B, β-D-olivose					
1B	101.1, CH	4.50, dd (9.8, 1.8)	101.1, CH	4.51, dd (9.8, 1.6)	4.51, dd (9.6, 1.2)
2B	37.3, CH_2	1.64, m (complex, Ha), 2.21, ddd (13.1, 3.8, 1.5, He)	37.3, CH_2	1.63, m (complex, Ha) 2.22, ddd (10.5, 5.0, 1.5, He)	1.70−1.48, m (complex, Ha) 2.21, ddd (10.5, 5.0, 1.5, He)
3B	80.7, CH	3.48, ddd (12.2, 8.3, 5.2)	80.7, CH	3.47, ddd (12.2, 8.3, 5.2)	3.46, ddd (12.2, 8.3, 5.2)
4B	75.4, CH	3.07, dd (8.3, 8.1)	75.3, CH	3.07, dd (8.1, 8.2)	3.08, dd (8.4, 8.4)
4B-OH		4.28, br s		4.36, br s	4.32, br s
5B	72.5, CH	3.40−3.31, m	72.4, CH	3.40−3.31, m	3.37, m
6B	18.0, CH_3	1.35, d (6.1)	18.0, CH_3	1.35, d (6.1)	1.36, d (6.1)
sugar C, α-L-rhodinose					
1C	98.0, CH	4.94, br s	98.0, CH	4.94, br s	4.94, br s
2C	25.7, CH_2	1.73, m (complex, Ha) 2.03, m (complex, He)	25.6, CH_2	1.75, m (complex, Ha) 1.96, m (complex, He)	1.75, m (complex, Ha) 1.96, m (complex, He)
3C	25.3, CH_2	1.52, m (complex, Ha) 2.11, m (complex, He)	25.2, CH_2	1.52, m (complex, Ha) 2.11, m (complex, He)	1.70−1.48, m (complex,Ha) 2.12, m (complex, He)
4C	75.9, CH	3.51, br s	75.9, CH	3.51, br s	3.52, br s
4C-OH		n.o.[c]		n.o.[c]	n.o.[c]
5C	67.9, CH	4.06, dq (6.6, 1.1)	67.8, CH	4.06, dq (6.4, 0.8)	4.06, q (6.6)
6C	17.2, CH_3	1.18, d (6.6)	17.2, CH_3	1.18, d (6.4)	1.19, d (6.4)
sugar D, β-D-olivose					
1D	101.6, CH	4.46, dd (9.8, 1.0)	101.6, CH	4.46, br d (9.7)	4.47, dd (9.4, 1.2)
2D	38.5, CH_2	1.64, m (complex, Ha)2.28, m (complex, He)	38.4, CH_2	1.64, m (complex, Ha)2.28, ddd (11.8, 5.4, 1.5, He)	1.70−1.48, m (complex, Ha)2.29, ddd (11.8, 5.4, 1.5, He)
3D	69.7, CH	3.56, m	69.7, CH	3.46, m	3.57, m

Table 2. Continued

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_H (500 MHz)
3D-OH		4.57, br s		4.61, br s	4.59, br s
4D	88.7, CH	2.95, dd (8.8, 8.8)	88.6, CH	2.95, dd (8.6, 8.6)	2.96, dd (8.7, 8.7)
5D	70.6, CH	3.27, m	70.4, CH	3.26, m	3.27, m
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-olivose					
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.[c]		n.o.[c]	n.o.[c]
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] Not observed.

seems important: Anhydrolandomycinone (**2**) displayed relatively the 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 nonaromatic 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 the 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 a Varian Vnmr 500 (^{1}H, 500 MHz; ^{13}C, 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 (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). Samples were introduced by a syringe pump. HPLC purifications were carried out using a Symmetry Prep C_{18} 10 μm column (10 × 150 mm) on a binary LC system. HPLC-MS analyses were carried out using a Symmetry Anal C_{18} 5 μm column (4.6 × 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/UV_{254} (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 was determined using the trypan blue exclusion assay as described earlier,[36,37] where 50 × 10^3 cells in 0.5 mL of 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 test compounds, and the plates were incubated for 24 h at 37 °C. At the end of the treatment period both adherent and floating cells were collected and resuspended in PBS, and trypan blue staining was performed using 0.4% stain for 3 min. Stained (dead) and unstained (live) cells were counted using a hemocytometer, cell viability in response to specific compounds was studied, a dose−response curve was plotted, and the IC_{50} 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.

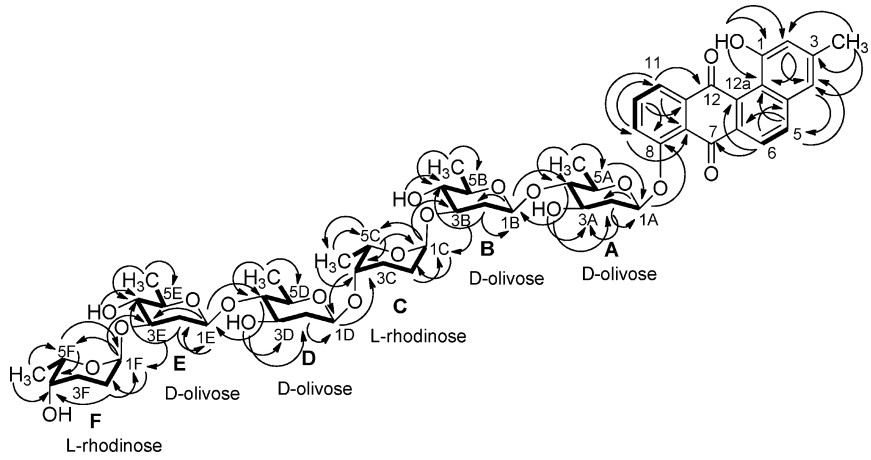


Figure 5. Selected HMBC connectivities (→) and $^1H-^1H$ COSY correlations (bold lines) of landomycin T (**9**).

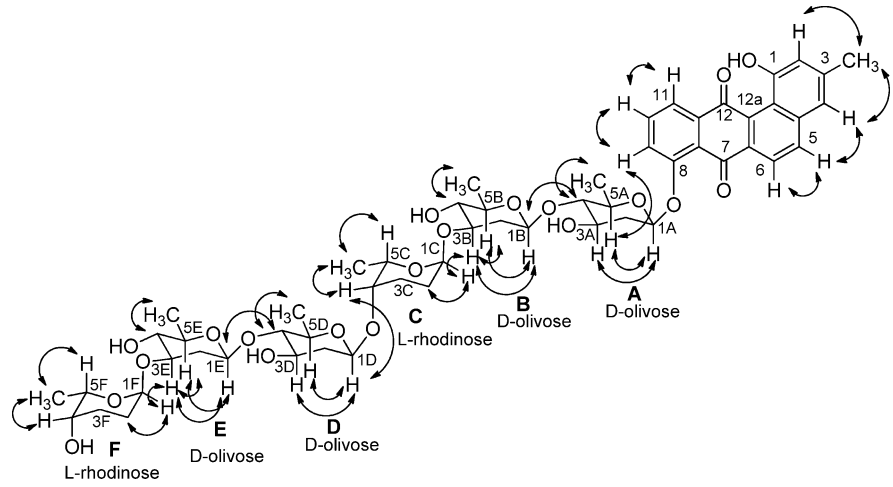


Figure 6. Selected NOESY correlations (↔) of landomycin T (**9**).

SG-Medium. Glucose (20 g), yeast extract (5 g), Soytone (10 g), $CoCl_2 \cdot 6H_2O$ (1 mg), and calcium carbonate (2 g) were dissolved in 1 L 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 L 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 *S. cyanogenus* S-136, containing 100 mL of SG-medium, was inoculated and cultivated at 28 °C (250 rpm) for 40 h. The obtained 100 mL preculture was used to inoculate 60 250 mL Erlenmeyer flasks (each with 1.5 mL of preculture), each containing 100 mL of SG medium, which was harvested after 48 h incubation at 28 °C at 250 rpm. The reddish-brown culture broth was centrifuged. The mycelium was extracted with MeOH (6 × 200 mL), while the water phase was extracted with EtOAc (3 × 2 L). 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 × 50 cm) using a stepwise $MeOH/CH_2Cl_2$ 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% $MeOH/CH_2Cl_2$) 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, $MeOH/CH_2Cl_2$ gradient 0% MeOH to 100%, each step 5% increased, 200 mL solvent) followed by Sephadex LH-20 (2 × 50 cm, 50% $MeOH/CH_2Cl_2$) yielded tetrangomycin (**19**, 25.0 mg) and landomycinone (**11**, 45.0 mg). Size exclusion chromatography (3 × 70 cm, 40% $MeOH/CH_2Cl_2$), PTLC (5% $MeOH/CH_2Cl_2$), and HPLC (CH_3CN/H_2O) 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 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/CH_2Cl_2), blue coloration with 2 N NaOH; UV/vis (MeOH) λ_{max} (log ε) 251 (5.07), 310 (4.62), 395 (4.22), 439 sh (3.53) nm; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1; (−)-ESIMS *m/z* 677 $[M - H]^-$; (−)-ESIMS/MS *m/z* (%) 679 $[M + 2H - H]^-$ (12), 545 ($[M - (L\text{-rhodinose} - H_2O) - H]^-$, 10), 433 ($[M - (L\text{-rhodinose} + D\text{-olivose}) - H]^-$, 5), 303 ($[M - (L\text{-rhodinose} + D\text{-olivose} + D\text{-olivose}) - H]^-$, 100), 275 (12); (−)-HRESIMS *m/z* 677.2593 $[M - H]^-$ (calcd for $C_{37}H_{41}O_{12}$, 677.2598).

Landomycin Q (6): dark red solid; R_f 0.37 (silica gel, 5% MeOH/CH_2Cl_2), blue coloration with 2 N NaOH; UV/vis (MeOH) λ_{max} (log ε) 251 (5.22), 311 (4.51), 399 (4.30), 457 sh (3.56) nm; ^1H NMR ($CDCl_3$, 500 MHz), see Table 1; (−)-ESIMS *m/z* 693 $[M - H]^-$; (−)-ESIMS/MS *m/z* (%) 693 $[M - H]^-$ (5), 533 (1), 318 ($[M - (L\text{-rhodinose} + D\text{-olivose} + D\text{-olivose}) - H]^-$, 100), 291 (8); (−)-HRESIMS *m/z* 693.2594 $[M - H]^-$ (calcd for $C_{37}H_{41}O_{13}$, 693.2552).

Landomycin R (3): dark red solid; R_f 0.23 (silica gel, 5% MeOH/CH_2Cl_2), blue coloration with 2 N NaOH; UV/vis (MeOH) λ_{max} (log ε) 251 (4.76), 311 (4.06), 399 (3.84), 457 sh (3.11) nm; ^1H NMR

Table 3. ^{13}C and ^{1}H NMR Assignments of Landomycins V (**15**) and W (**7**) [δ in ppm relative to TMS (multiplicity, *J*/Hz)]

	landomycin V (**15**)[a]		landomycin V (**15**)[a]	landomycin W (**7**)[a]
position	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_α) 2.87, d (15.8, H_β)	2.87, dd (16.0, 4.4, H_α) 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)
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] $CDCl_3$. [e] Not observed.

(DMSO-d_6, 500 MHz), see Table 1; (−)-ESIMS *m*/*z* 579 $[M - H]^-$; (−)-HRESIMS *m*/*z* 579.1861 $[M - H]^-$ (calcd for $C_{31}H_{31}O_{11}$, 579.1871).

Landomycin S (17): orange solid; R_f 0.14 (silica gel, 5% MeOH/CH_2Cl_2), 0.76 (15% MeOH/CH_2Cl_2), blue coloration with 2 N NaOH; UV/vis (MeOH) λ_{max} (log ε) 255 (5.25), 288 sh (4.73), 398 (4.49), 445 sh (4.04) nm; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2; (−)-ESIMS *m*/*z* 1069 $[M - H]^-$; (−)-ESIMS/MS *m*/*z* (%) 1070 ($[M - H + H]^-$, 5), 1052 ($[M - H_2O]^-$, 30), 909 ([M − (L-rhodinose − $2H_2O$)$^-$, 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 $C_{55}H_{73}O_{21}$, 1069.4649).

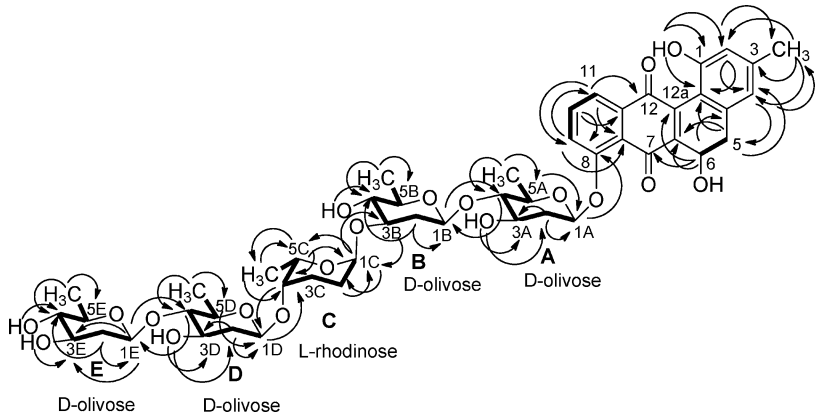


Figure 7. ^{1}H−^{1}H COSY (bold lines) and selected HMBC (→) couplings in landomycin V (**15**).

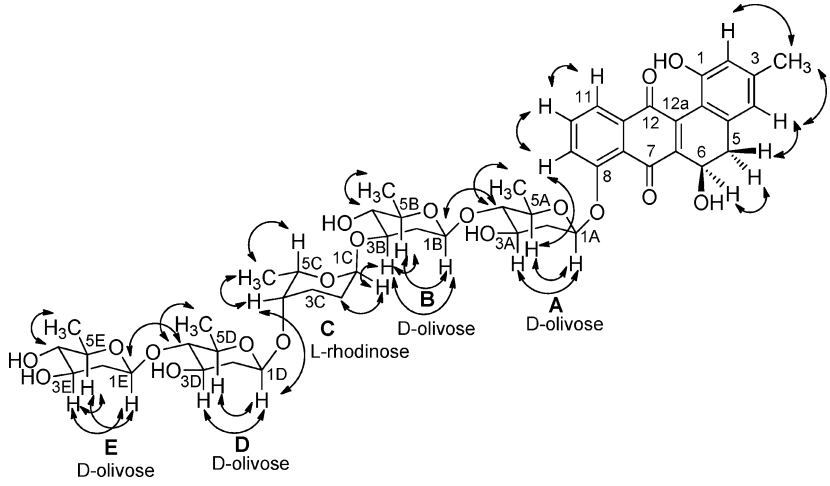


Figure 8. Selected NOESY correlations (↔) of landomycin V (**15**).

Table 4. Comparison of Cytotoxic Potencies of Angucyclin(on)es **1**−**18** (mean IC_{50} from three measurements, μM)

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

[a] MCF-7 cell assay. [b] MDA-231 cell assay. [c] From ref 18. NA = not active up to 20 μM. NT = not tested.

Landomycin T (9): orange solid; R_f 0.24 (silica gel, 5% MeOH/CH_2Cl_2), blue coloration with 2 N NaOH; UV/vis (MeOH) λ_{max} (log ε) 251 (5.00), 311 (4.44), 399 (4.10), 439 sh (3.39) nm; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2; (−)-ESIMS *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 2 N NaOH; UV/vis (MeOH) λ_{max} (log ε) 251 (5.24), 314 (4.78), 399 (4.36), 461 sh (4.07) nm; ^{1}H NMR ($CDCl_3$, 500 MHz), see Table 2; (−)-ESIMS *m/z* 1067 [M − H]$^-$; (+)-ESIMS *m/z* 1091 [M + Na]$^+$; (−)-ESIMS/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 2 N NaOH; UV/vis (MeOH) λ_{max} (log ε) 255 (5.12), 295 sh (4.49), 399 (4.30), 446 sh (3.80) nm; ^{1}H NMR ($CDCl_3$, 500 MHz), ^{1}H NMR (DMSO-d_6, 500 MHz), and ^{13}C NMR (DMSO-d_6, 125 MHz), see Table 3; (−)-ESIMS *m/z* 955 [M − H]$^-$; (−)-ESIMS/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 2 N NaOH; UV/vis (MeOH) λ_{max} (log ε) 251 (5.16), 311 (4.45), 399 (4.23), 452 sh (3.47) nm; ^{1}H NMR ($CDCl_3$, 500 MHz), see Table 3; (+)-ESIMS *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; ^{1}H 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, H_2-4), 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, CH_3-3); ^{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 (CH_3-3).

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Supporting Information Available: HPLC analysis chromatogram of the crude extract obtained from *Streptomyces cyanogenus* S136 strain; workup procedure scheme; ^{1}H and ^{13}C NMR spectra of the angucyclin(on)es (**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.

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