

Generation of Hybrid Elloramycin Analogs by Combinatorial Biosynthesis Using Genes from Anthracycline-Type and Macrolide Biosynthetic Pathways

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Abstract

Elloramycin and oleandomycin are two polyketide compounds produced by *Streptomyces olivaceus* Tü2353 and *Streptomyces antibioticus* ATCC11891, respectively. Elloramycin is an anthracycline-like antitumor drug and oleandomycin a macrolide antibiotic. Expression in *S. albus* of a cosmid (cos16F4) containing part of the elloramycin biosynthetic gene cluster produced the elloramycin non-glycosylated intermediate 8-demethyl-tetracenomycin C. Several plasmid constructs harboring different gene combinations of L-oleandrose (neutral 2,6-dideoxyhexose attached to the macrolide antibiotic oleandomycin) biosynthetic genes of *S. antibioticus* that direct the biosynthesis of L-olivose, L-oleandrose and L-rhamnose were coexpressed with cos16F4 in *S. albus*. Three new hybrid elloramycin analogs were produced by these recombinant strains through combinatorial biosynthesis, containing elloramycinone or 12a-demethyl-elloramycinone (= 8-demethyl-tetracenomycin C) as aglycone moiety encoded by *S. olivaceus* genes and different sugar moieties, coded by the *S. antibioticus* genes. Among them is L-olivose, which is here described for the first time as a sugar moiety of a natural product.

Search for new bioactive drugs has been usually carried out by pharmaceutical companies through wide screening programs for the isolation of microorganisms producing useful bioactive compounds. Successful screening programs have produced most of the clinically useful drugs. In addition, chemical modification of selected compounds introducing specific changes in these lead compounds has also rendered important families of bioactive compounds, as it is the case of the different generations of β -lactam

antibiotics. The development of recombinant DNA technology is beginning to offer another alternative for the generation of bioactive compounds through the genetic manipulation of biosynthetic pathways. Many bioactive compounds with pharmaceutical, veterinary or agricultural applications belong to the polyketide family. This is one of the largest families of secondary metabolites and includes well known bioactive compounds such as antibiotics (macrolides, tetracyclines), antitumor agents (anthracyclines), antiparasites (avermectins), immunosuppressant agents (rapamycin, FK506), etc. Polyketides show very diverse chemical structures. They all are synthesized in the early stages of their biosynthesis through the condensation of short-chain carboxylic acids in a series of reactions catalyzed by complex enzymes named polyketide synthases (Hopwood and Sherman, 1990; Katz and Donadio, 1993; Hutchinson and Fujii, 1995; Hopwood, 1997). An interesting feature of many polyketide drugs is the presence of sugars attached to the polyketide-derived aglycones. All these sugars belong to the family of 6-deoxyhexoses, whose members are present in a great variety of secondary metabolites (Piepersberg, 1994; Liu and Thorson 1994; Kirschning *et al.*, 1997; Trefzer *et al.*, 1999). In many cases, the presence of the sugars is important or even essential for bioactivity (Kirschning *et al.*, 1997). Therefore, engineering glycosylation in polyketide drugs could be a powerful tool for generating structural biodiversity that might conduct to the generation of novel bioactive drugs. Obviously this will require a certain degree of flexibility of glycosyltransferases.

Elloramycin is an anthracycline drug structurally related to tetracenomycin C. They differ in the absence of a C-12a-O methylation in tetracenomycin C (which is present in elloramycin) and the glycosylation of the elloramycin chromophore with a permethylated L-rhamnose moiety at the C-8 hydroxy group (Figure 1). From a cosmid library of *Streptomyces olivaceus* Tü2353 (elloramycin producer), a cosmid clone (cos16F4) was isolated that, when introduced into *S. lividans* TK21, resulted in the production of a nonglycosylated elloramycin intermediate, 8-demethyl-tetracenomycin C (Figure 1) and did also confer resistance to elloramycin and tetracenomycin C (Decker *et al.*, 1995a). It was therefore assumed that cos16F4 contained all genes necessary for the biosynthesis of the polyketide moiety of elloramycin. Interestingly, when cos16F4 was used to transform protoplasts of two glycosylated polyketide producers, novel glycosylated derivatives were produced. Thus, in *S. fradiae* Tü2717 (producer of the angucycline urdamycin), transformation of cos16F4 into a mutant lacking polyketide synthase genes produced two new glycosylated compounds: 8-demethyl-8- α -L-rhodosyl-tetracenomycin C and 8-demethyl-8- β -D-olivosyl-tetracenomycin C (Decker *et al.*, 1995b). Furthermore, transformation of *S. argillaceus* ATCC 12956, producer of

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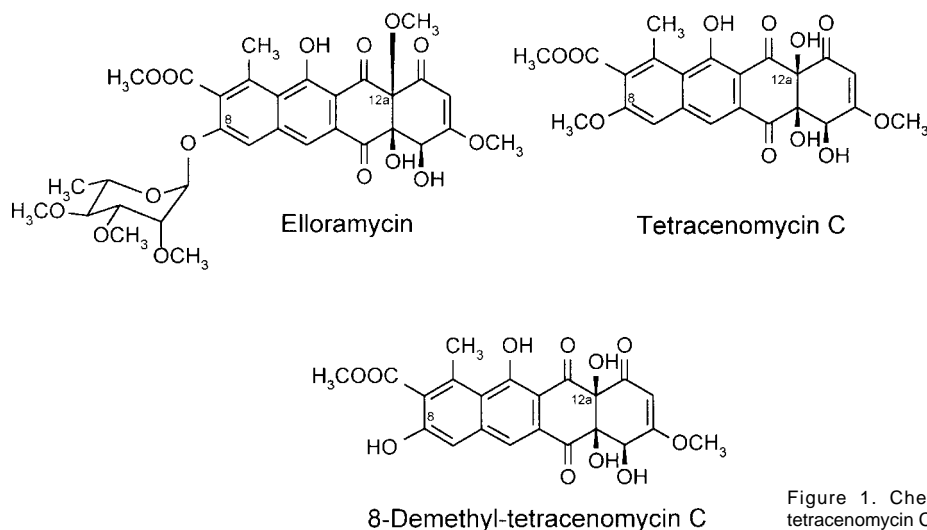


Figure 1. Chemical structures of the elloramycin, tetracenomycin C and 8-demethyl-tetracenomycin C.

the polyketide antitumor drug mithramycin, also rendered three glycosylated derivatives: two containing a monosaccharide attached to the aglycone, 8-demethyl-8- β -D-mycarosyl-tetracenomycin C and the above mentioned 8-demethyl-8- β -D-oliviosyl-tetracenomycin C. Another containing a disaccharide, 8-demethyl-8- β -D-olivo-3'-1''- β -D-oliviosyl-tetracenomycin C (Wohlert *et al.*, 1998). The formation of these glycosylated tetracenomycins suggested the existence of a "sugar flexible" glycosyltransferase. Several lines of experimental evidence demonstrated that the "sugar flexible" glycosyltransferase is present in cos16F4 and not in the transformation hosts (Wohlert *et al.*, 1998) and this has been recently confirmed through the cloning and expression of the glycosyltransferase gene

(*elmG*) from cos16F4 (G. Blanco, E. Pérez-Patallo, C. Méndez and J. A. Salas, unpublished results).

On the basis of this flexibility of the elloramycin glycosyltransferase we tested the possibility of producing novel elloramycin derivatives by combining genes from two different biosynthetic pathways, which included the goal to construct a natural product containing the in nature unprecedented deoxysugar L-olivose (while D-olivose occurs quite often). This was achieved by using on one hand genes encoding the polyketide aglycone of elloramycin harbored in cos16F4, and on the other hand, genes encoding 6-deoxysugar biosynthetic enzymes from the biosynthetic cluster of the macrolide antibiotic oleandomycin. This is a macrolide (Figure 2) consisting of

Table 1. Genes from the oleandomycin biosynthetic gene cluster from *S. antibioticus* used for construction of sugar-encoding plasmids.

Gene	Function	Involved in biosynthesis of	Homolog
<i>oleL</i>	dTDP-4-keto-6-deoxyglucose 3,5-epimerase	L-oleandrose	<i>strM</i> <i>eryBVII</i> <i>dnmU</i>
<i>oleS</i>	dTDP-D-glucose synthase	L-oleandrose and D-desosamine	<i>mtmD</i> <i>strD</i> <i>dnmL</i>
<i>oleE</i>	dTDP-glucose 4,6-dehydratase	L-oleandrose and D-desosamine	<i>mtmE</i> <i>strE</i> <i>tyIA2</i>
<i>oleU</i>	dTDP-4-ketohexulose reductase	L-oleandrose	<i>strL</i> <i>dnmV</i> <i>eryBIV</i>
<i>oleV</i>	dTDP-4-keto-6-deoxy-2,3-dehydrase	L-oleandrose	<i>dnmT</i> <i>eryBVI</i> <i>gra-orf27</i>
<i>oleW</i>	dTDP-4-keto-6-deoxy-3-keto-reductase	L-oleandrose	<i>lanT</i> <i>gra-orf26</i> <i>rdmF</i>
<i>oleY</i>	dTDP-4-keto-6-deoxy-3-O-methyltransferase	L-oleandrose	<i>snoY</i>

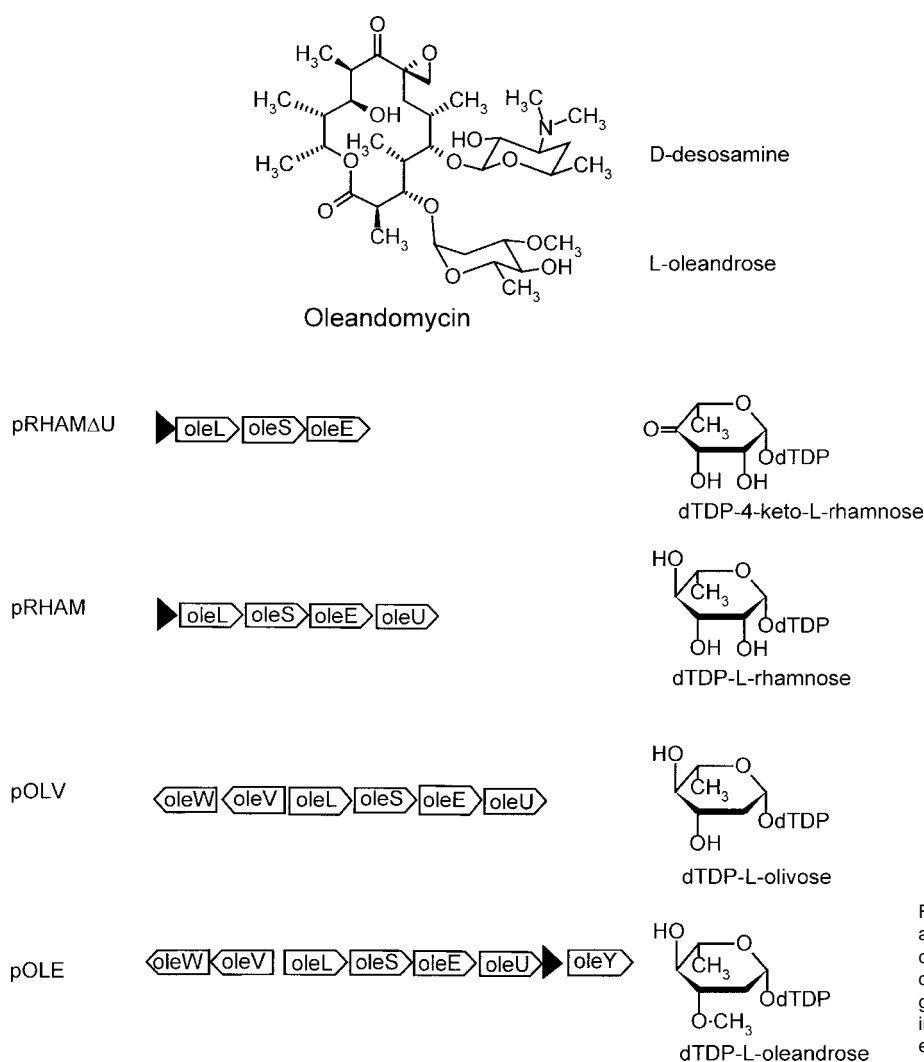


Figure 2. Chemical structure of oleandomycin and schematic representation of the constructs directing the biosynthesis of the different 6-deoxyhexoses (for details on the gene functions see Table 1). The dark triangle indicate the position and orientation of the erythromycin resistance promoter (*ermEp*).

a macrolactone ring (14-membered) to which a neutral sugar (L-oleandrose) and an aminosugar (D-desosamine) are attached to C-3 and C-5, respectively. All the genes involved in the biosynthesis of these two 6-deoxyhexoses by *S. antibioticus* (oleandomycin producer) have been identified and specific functions in 6-deoxyhexose biosynthesis assigned (Olano *et al.*, 1998; Quirós *et al.*, 1998; Aguirrezabalaga *et al.*, 2000). Using these 6-deoxyhexose biosynthetic genes from the oleandomycin cluster (Table 1), we have constructed two plasmids that direct the biosynthesis of the dTDP-activated forms of L-olivose and L-oleandrose (Aguirrezabalaga *et al.*, 2000). Plasmid pOLV (Figure 2) contains all the genes necessary for the biosynthesis of dTDP-L-olivose, an intermediate in dTDP-L-oleandrose biosynthesis: a glucose synthase (*oleS*), a 4,6-dehydratase (*oleE*), a 3,5-epimerase (*oleL*), a 2,3-dehydratase (*oleV*), a 3-reductase (*oleW*) and a 4-ketoreductase (*oleU*). Plasmid pOLE (Figure 2) is a pOLV-derived plasmid in which the *oleY* gene that encodes a sugar 3-O-methyltransferase has been incorporated; this construct directs the biosynthesis of dTDP-L-oleandrose. Experimental evidence for the plasmid-directed biosynthesis of these two deoxysugars and their transfer to a macrolide aglycone (erythronolide B) has been reported (Aguirrezabalaga *et al.*, 2000). We wanted to

determine whether the elloramycin glycosyltransferase was able to recognize and transfer these two L-deoxysugars to the elloramycin aglycone thus generating further hybrid molecules between a tetracenomycin-type compound and a macrolide. Protoplasts of *S. albus* J1074 (*ilv1*, *sal2*, R^M) (Chater and Wilde, 1980) were transformed with *cos16F4* DNA. This is a strain that can be reasonably transformed and also because, in contrast to many streptomycetes, it has a dispersed growth. Transformants were selected for resistance to apramycin (25 μ g/ml). One clone (AL16) was selected for further studies. HPLC analyses of strain AL16, performed as described (Fernández Lozano *et al.*, 2000) showed the production of 8-demethyl-tetracenomycin C at a good yield. Protoplasts of strain AL16 were independently transformed with pOLV and pOLE and transformants selected for resistance to thiostrepton (50 μ g/ml). Several transformants from both transformations were selected for further analysis. Spores of these recombinant strains simultaneously harboring *cos16F4* and either pOLV (strain AL16-OLV) or pOLE (strain AL16-OLE) were then cultivated in R5A medium (Blanco *et al.*, 2000) containing both selection antibiotics (apramycin and thiostrepton) for 4 days at 30°C. HPLC analyses of both recombinant strains revealed the presence of four new peaks when compared to the control strain AL16, all of

them showing the characteristic absorption spectra of elloramycinone (Drautz *et al.*, 1985; Fiedler *et al.*, 1986). Interestingly the four new peaks were present in both recombinant strains. The material in these peaks was purified from cultures of strain AL16-OLE (3.2 l of culture in R5A medium) by preparative HPLC. Details on extraction and purification of the compounds have been described (Fernández-Lozano *et al.*, 2000). Upon structural elucidation using standard physico-chemical methods (data given below) material in two of the peaks corresponded to two new glycosylated forms of 8-demethyl-tetracenomycin C: 8-demethyl-8- α -L-oliviosyl-tetracenomycin C and 8-demethyl-8- α -L-oleandrosyl-tetracenomycin C, respectively (Figure 3). The presence of the latter compound (*i.e.* containing L-oleandrose) in cultures of AL16-OLV was initially unexpected, since pOLV does not contain a 3-O-methyltransferase gene to render L-oleandrose. However, three methyltransferase-encoding genes (*elmMI*, *elmMII* and *elmMIII*) have been recently found in cos16F4 (E. Pérez-Patillo, G. Blanco, C. Méndez and J. A. Salas, unpublished results). They are responsible for permethylating L-rhamnose, the 6-deoxyhexose of elloramycin and, one of them (*elmMII*), could convert L-olivose into the 3-O-methylated derivative, L-oleandrose). The third peak corresponded to 2'-demethoxy-elloramycin (Figure 3). The two methyl groups at C-3' and C-4' in the sugar moiety of this compound are probably due to *elm* methyltransferases (*elmMII* and *elmMIII*). Finally, the fourth peak corresponded to elloramycin (Figure 3). All the compounds were produced in quite reasonably good yields: 41 mg/l for 8-demethyl-8- α -L-oliviosyl-tetracenomycin C, 9 mg/l for 8-demethyl-8- α -L-oleandrosyl-tetracenomycin C, 14 mg/l for 2'-demethoxy-elloramycin and 3.2 mg/l for elloramycin. Biological activity of these compounds was

tested by proliferation inhibition assays against various cancer cell lines (lung carcinoma A549, breast carcinoma MDA-MB 231, melanoma SK-Mel 30) under standard conditions (Skehan *et al.*, 1990). Elloramycin has been shown to present antitumor activity (Drautz *et al.*, 1985; Rohr and Zeeck, 1990). Of the other three compounds only 2'-demethoxy-elloramycin showed significant activity against these cell lines: IC₅₀ values ranging from 14.9 to 25.4 μ g/ml for 2'-demethoxy-elloramycin and above 40 μ g/ml for the other two compounds.

The occurrence of elloramycin, a compound with an L-rhamnose α -glycosidically attached to the aglycone, was quite unexpected. L-Rhamnose is the 6-deoxyhexose attached to elloramycin, but cos16F4 does not contain all the genes necessary for the biosynthesis of L-rhamnose since its expression into different *Streptomyces* species caused the biosynthesis of the non-glycosylated intermediate 8-demethyl-tetracenomycin C. Moreover, Southern hybridization analysis of cos16F4 with different gene probes from different 6-deoxyhexose biosynthetic pathways (including well conserved genes such as those encoding dTDP-glucose synthase and dTDP-4,6-dehydratase) did not reveal any positive signal (data not shown). Consequently, it was tentatively assumed that the L-oleandrose biosynthetic genes harbored either in pOLE or pOLV were also able to direct the L-rhamnose biosynthesis. To test this hypothesis we constructed a plasmid capable of directing the biosynthesis of dTDP-L-rhamnose. For this purpose, we subcloned a 4.1 kb *SphI*-*XmnI* DNA fragment from a cosmid clone (cosAB63), isolated from a *S. antibioticus* cosmid library (Quirós *et al.*, 1998), into the *SphI*-*SmaI* sites of pUK21 (Vieira and Messing, 1991) generating pLR2347. This fragment contains four genes of the L-oleandrose biosynthetic cluster

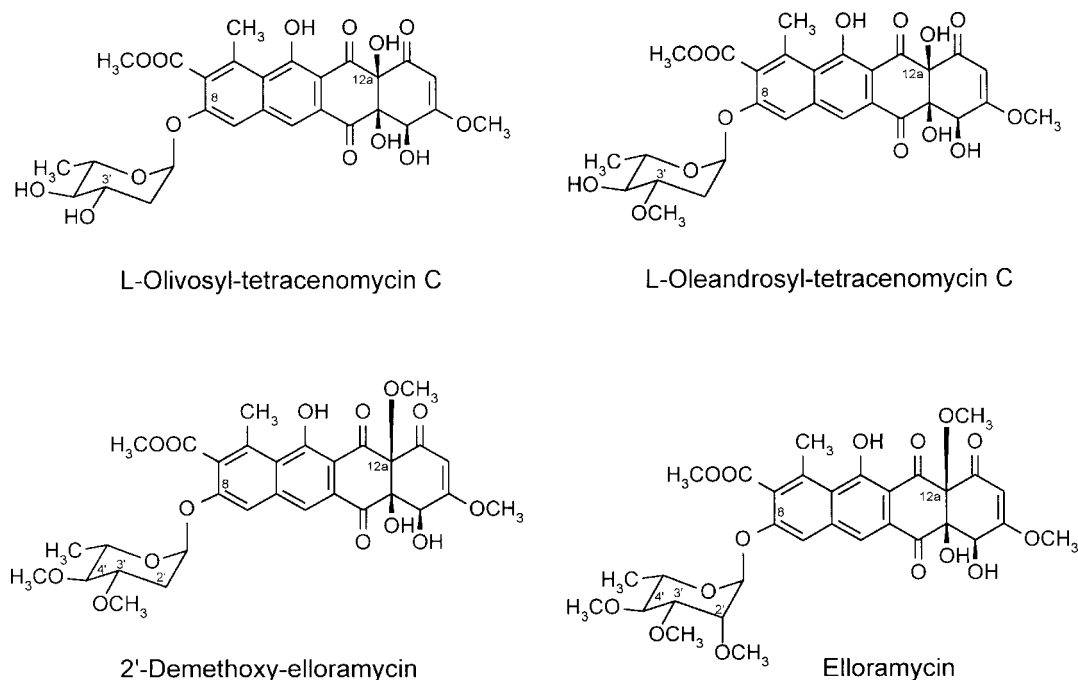


Figure 3. Chemical structures of the compounds produced by combinatorial biosynthesis between the elloramycin and oleandomycin gene clusters.

(*oleL*, *oleS*, *oleE*, and *oleU*) and the 5'-end of *oleNI* (involved in D-desosamine biosynthesis). These four genes code for the enzymatic functions necessary for the biosynthesis of dTDP-L-rhamnose. From pLR2347, the fragment was rescued using the *SpeI* and *XbaI* sites of the pUK21 polylinker and subcloned into pEM4 (Quirós *et al.*, 1998) and in the correct orientation. In this final construct, pRHAM (Figure 2), the four oleandrose genes are under the transcriptional control of the promoter of the erythromycin resistance gene from *Saccharopolyspora erythraea* (*ermEp*). As a control, we constructed a plasmid lacking the *oleU* gene that encodes a sugar 4-ketoreductase. pLR2347 was digested with *SmaI-EcoRV* and religated; in this way most of *oleU* and the 5'-end of *oleNI* were deleted and thus inactivating *oleU*. Then, the fragment was also rescued using the *SpeI* sites of pUK21 polylinker and subcloned into pEM4 generating pRHAM Δ U. This construct would direct the biosynthesis of dTDP-4-keto-L-rhamnose. Strain AL16 was then independently transformed with pRHAM and pRHAM Δ U and compounds produced by the recombinant strains analyzed. Strain AL16 transformed with pRHAM produced elloramycin but when transformed with pRHAM Δ U only 8-demethyl-tetracenomycin C was synthesized. Several conclusions can be drawn from these experiments: (i) the elloramycin glycosyltransferase (ElmG) is not able to transfer 4-keto-L-rhamnose to the aglycone, (ii) it confirms that cos16F4 does not contain a gene encoding the rhamnosyl 4-ketoreductase, and (iii) L-oleandrose biosynthetic genes can be used to synthesize L-rhamnose. This means that the 4-ketoreductase involved in L-oleandrose biosynthesis (coded by the *oleU* gene) is able to reduce deoxysugars regardless whether the 2-position is deoxygenated or not, since it operates in the L-olivose, L-oleandrose and L-rhamnose biosynthesis. This opens up the possibility of using the oleandrose 4-ketoreductase for a broad spectrum of sugar substrates.

Several examples have been reported in the meanwhile regarding the "substrate flexibility" of either the alcohol (aglycone) substrate or the sugar co-substrate of glycosyltransferases (Zhao *et al.*, 1999). For example, the picromycin glycosyltransferase of *S. venezuelae*, PikVII, has been shown to attach D-desosamine to different aglycones: both to the 12- and 14-membered macrolactones of methymycin and pikromycin, respectively (Xue *et al.*, 1998). Similarly, glycosyltransferases involved in sugar transfer during the biosynthesis of glycopeptide antibiotics have been shown to transfer sugars to different, but structurally related heptapeptides (Solenberg *et al.*, 1997). On the other hand, the oleandrosyl glycosyltransferase of *S. antibioticus*, OleG2, can transfer L-rhamnose (Doumith *et al.*, 1999) and L-olivose (Aguirrezabalaga *et al.*, 2000) to the macrolactone ring of erythromycin, erythronolide B. The results we reported here add a new example of this flexibility of antibiotic glycosyltransferases. The elloramycin glycosyltransferase, *elmG*, has been found so far to be able of transferring several L-6-deoxysugars (L-rhamnose), D-2,6-dideoxysugars (D-olivose and D-mycarose) and L-2,6-dideoxysugars (L-rhodinose and L-olivose) (Decker *et al.*, 1995b; Wohlert *et al.*, 1998; this paper) to 8-deoxy-tetracenomycin C. The new compounds shown in this paper are therefore the result of combinatorial biosynthesis of genes from two different biosynthetic pathways: elloramycin

and oleandromycin. The use of such flexible glycosyltransferases will be a powerful tool for producing novel bioactive hybrid glycosides.

Physicochemical Data of the Three New Elloramycin-Analogs

a) 8-demethyl-8- α -L-olivosyl-tetracenomycin C, C₂₈H₂₈O₁₄ (588); neg. FAB MS: 587; pos. FAB MS: 589; ¹H NMR (400 MHz, d₆-acetone): δ (ppm) 14.02 s (1H, 11-OH), 8.10 s (1H, 6-H), 7.80 s (1H, 7-H), 6.07 d (1H, *J* = 3 Hz, 1'-H), 5.81 s (1H, 12a-OH), 5.66 s (1H, 2-H), 5.18 s (1H, 4a-OH), 5.11 d (1H, *J* = 7 Hz, 4-H), 4.96 d (1H, *J* = 7 Hz, 4-OH), 4.38 s (1H, 4'-OH), 4.25 s (1H, 3'-OH), 4.00 s (3H, 9-OCH₃), 3.95 dddd (1H, *J* = 11, 6, 6, 6 Hz, 3'-H), 3.87 s (3H, 3-OCH₃), 3.67 dq (1H, *J* = 10, 6 Hz, 5'-H), 3.14 dd (1H, *J* = 9, 9 Hz, 4'-H), 2.87 s (3H, 10-CH₃), 2.32 ddd (1H, *J* = 16, 6, 2 Hz, 2'-H_e), 1.88 ddd (1H, *J* = 14, 12, 3 Hz, 2'-H_a), 1.22 d (3H, *J* = 6 Hz, 6'-CH₃); ¹³C NMR (100.6 MHz, d₆-acetone): δ (ppm) 198.0 (C-12), 194.0 (C-5), 190.8 (C-1), 175.6 (C-3), 168.0 (9-C=O), 167.8 (C-11), 155.6 (C-8), 141.2 (C-5a), 138.6 (C-10), 131.0 and 129.3 (C-10a and C-9), 122.0 (C-6a), 121.6 (C-6), 112.0 (C-7), 110.2 (C-11a), 100.2 (C-2), 97.0 (C-1'), 85.2 (C-4a), 83.8 (C-12a), 78.4 (C-5'), 70.6 (C-4), 70.6 and 69.0 (C-4' and C-3'), 57.5 (9-OCH₃), 53.0 (3-OCH₃), 38.2 (C-2'), 21.3 (10-CH₃), 18.3 (C-6'); b) 8-demethyl-8- α -L-oleandrosyl-tetracenomycin C, C₂₉H₃₀O₁₄ (602), pos. FAB MS: 625 (M+Na⁺); ¹H NMR (400 MHz, d₆-acetone): δ (ppm) 14.08 s (1H, 11-OH), 8.02 s (1H, 6-H), 7.80 s (1H, 7-H), 6.10 d (1H, *J* = 3 Hz, 1'-H), 5.81 s (1H, 12a-OH), 5.66 s (1H, 2-H), 5.18 s (1H, 4a-OH), 5.11 d (1H, *J* = 7 Hz, 4-H), 4.97 d (1H, *J* = 7 Hz, 4-OH), 4.43 d (1H, *J* = 4 Hz, 4'-OH), 4.03 s (3H, 9-OCH₃), 3.87 s (3H, 3-OCH₃), 3.69 dq (1H, *J* = 9, 6 Hz, 5'-H), 3.56 dddd (1H, *J* = 11, 6, 6, 6 Hz, 3'-H), 3.47 s (3H, 3'-OCH₃), 3.22 ddd (1H, *J* = 9, 9, 3 Hz, 4'-H), 2.86 s (3H, 10-CH₃), 2.50 ddd (1H, *J* = 13, 6, 2 Hz, 2'-H_e), 1.74 ddd (1H, *J* = 14, 12, 3 Hz, 2'-H_a), 1.22 d (3H, *J* = 6 Hz, 6'-H); ¹³C-NMR (100.58 MHz, d₆-acetone): δ (ppm): 198.0 (C-12), 194.0 (C-5), 190.8 (C-1), 174.4 (C-3), 168.2 (9-C=O), 167.8 (C-11), 155.5 (C-8), 141.3 (C-5a), 138.8 (C-10), 131.0 and 129.3 (C-10a and C-9), 122.0 (C-6a), 120.4 (C-6), 112.0 (C-7), 110.1 (C-11a), 100.1 (C-2), 96.9 (C-1'), 85.1 and 83.8 (C-4a and C-12a), 79.2 (C-5'), 76.8 (C-3'), 70.9 and 70.7 (C-4' and C-4), 57.7 and 57.6 (3'-OCH₃ and 9-OCH₃), 53.2 (3-OCH₃), 35.0 (C-2'), 21.3 (10-CH₃), 18.5 (C-6'); c) 2'-demethoxy-elloramycin, C₃₁H₃₄O₁₄ (630); pos. FAB MS: 631 (M+H⁺); ¹H NMR (400 MHz, d₆-acetone): δ (ppm) 14.02 s (1H, 11-OH), 8.02 s (1H, 6-H), 7.80 s (1H, 7-H), 6.10 s (1H, 1'-H), 5.50 s (1H, 2-H), 5.20 s (1H, 4a-OH), 5.03 d (1H, *J* = 7 Hz, 4-H), 4.96 d (1H, *J* = 7 Hz, 4-OH), 4.03 s (3H, 9-OCH₃), 3.82 s (3H, 3-OCH₃), 3.64 m (1H, 5'-H), 3.64 m (1H, 3'-H), 3.60 s and 3.58 s (3H each, 12a-OCH₃ and 4'-OCH₃), 3.48 s (3H, 3'-OCH₃), 2.88 s (3H, 10-CH₃), 2.87 dd (1H, *J* = 9, 9 Hz, 4'-H), 2.52 ddd (1H, *J* = 13, 7, 2 Hz, 2'-H_e), 1.76 ddd (1H, *J* = 14, 12, 3 Hz, 2'-H_a), 1.21 d (3H, *J* = 6 Hz, 6'-H); ¹³C-NMR (100.6 MHz, d₆-acetone): δ (ppm): 198.0 (C-12), 194.1 (C-5), 191.7 (C-1), 173.8 (C-3), 168.4 (9-C=O), 168.0 (C-11), 155.7 (C-8), 141.6 (C-5a), 139.1 (C-10), 131.2 and 129.0 (C-10a and C-9), 122.0 (C-6a), 121.1 (C-6), 112.1 (C-7), 111.0 (C-11a), 102.0 (C-2), 96.9 (C-1'), 88.0 (C-12a), 86.8 (C-3' or C-4'), 85.8 (C-4a), 79.2 (C-5'), 71.0 and 69.8 (C-3' or C-4' and C-4),

61. 2 (4'-OCH₃), 57. 7 and 57. 6 (3'-OCH₃ and 9-OCH₃), 56. 7 (12a-OCH₃), 53. 2 (3-OCH₃), 35. 4 (C-2'), 21. 5 (10-CH₃), 18. 8 (C-6'); Elloramycin was identified by comparison with literature data (Drautz *et al.*, 1985; Fiedler *et al.*, 1986).

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