STRUCTURE-ACTIVITY RELATIONSHIPS OF ELLORAMYCIN AND TETRACENOMYCIN C

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The structure-activity relationships of the anthracycline-related antibiotics of the tetracenomycin C/elloramycin-type were investigated by derivatization of elloramycin (**1**) and elloramycinone (**2**). During hydrolysis experiments a unique transglycosylation reaction was discovered, converting elloramycin (**1**) into isoelloramycin (**10**) by treatment with anhydrous trifluoroacetic acid. Following the proposed structure-activity relationship concept, 8-*O*-methylelloramycinone (**14**) was synthesized from elloramycinone (**2**), and was shown to be the most active derivative according to the proliferation inhibition assay against murine L1210 leukemia cells.

The elloramycins A (**1**) to F[1~3] and tetracenomycins C (**13**)[4~6] and X (**14**)[7], produced by *Streptomyces* and *Nocardia* species, form a small group of antibiotics, which is unique relative to their molecular structure as well as biological activity (mainly against Gram-positive bacteria of the genus *Streptomyces*, but also as antitumor antibiotics).

Tetracenomycin C (**13**) was the first example of this discrete group, discovered in 1979[4,5], then again in 1984[8] and, as its 12a-*O*-methyl ether (tetracenomycin X, **14**) in 1989[7].

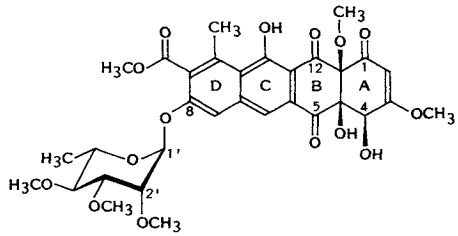
Because of the linear tetracyclic ring system and its obvious biogenetic origin from a decaketide, the tetracenomycin C-type antibiotics are related to the tetracyclines and the anthracyclines[9]. But in contrast to these antibiotics, which have several group members, tetracenomycin C has only a few relatives. Unique in the common structure of these antibiotics is the 2,3-dihydro-1,4-anthraquinone chromophore; the partially saturated ring A, which has an α,β-unsaturated keto group as structural element, resembles some anthracycline antibiotics, *e.g.*, aranciamycin (**18**)[10,11], the steffimycins (**19**)[12,13] and akrobomycin[14].

Biosynthetic investigations on tetracenomycin X (**14**)[7] focussing especially on oxidation processes proved the tetracyclic ring frame to derive from 10 acetate units, leaving only the question whether some red congeners of tetracenomycin C, namely tetracenomycins A_2 (**5**), B_3 (**6**) and D_3 (**7**, the latter two were isolated from a blocked mutant of the elloramycin-producer *Streptomyces olivaceus*) are intermediates or shunt products of the biosynthetic pathway to **1**, **13** or **14**[15~17]. The only relatives of the tetracenomycins C and X having a sugar moiety as a structural element are the elloramycins[1,3], which are phenolic bonded di- or trimethylrhamnosides. The main compound, elloramycin A (**1**) is biologically active against murine L1210 leukemia cells, but weaker than its sugarless relative **13**. This was unexpected, since most of the anthracyclines show a much higher biological activity than their aglycones. Some chemically interesting hydrolysis phenomena and the little known structure-activity relationships of this unique group of antibiotics were also reasons of our investigations.

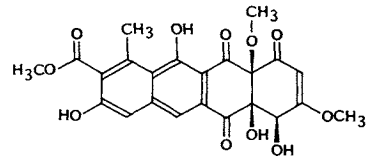
Hydrolysis and Transglycosylation

As starting material for semisynthetic studies and different biological tests, elloramycinone (**2**) had to be produced in good yields. The methanolysis of **1** with methanolic sulfuric acid gave only 37% of **2**. The reason for the low yield is an isomerization of **2** to isoelloramycinone (**9**) *via* 3-demethylelloramycinone (**8**),

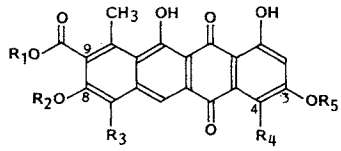
Scheme 1.

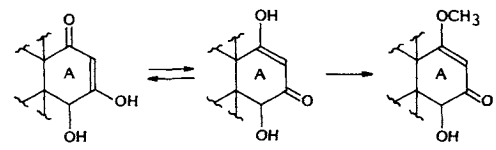


1



2





8 9

3 $R_1=CH_3$ R_2=Sugar as in **1** $R_3=H$ $R_4=OH$ $R_5=CH_3$
4 $R_1=R_2=H$ $R_3=Cl$ $R_4=OH$ $R_5=CH_3$
5 $R_1=R_2=R_5=CH_3$ $R_3=R_4=H$
6 $R_1\sim R_4=H$ $R_5=CH_3$
7 $R_1\sim R_5=H$

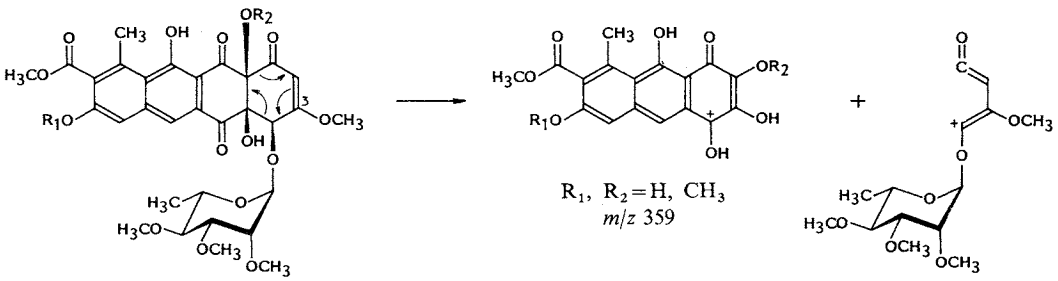
caused by the vinylogue ester function in ring A (Scheme 1), and by shunt products, of which anhydroelloramycin (**3**) could be obtained and characterized as the main compound among other unidentified red dyes. The aromatization of ring A by which the yellow 2,3-dihydro-1,4-anthraquinone chromophore of **1** is changed into a red naphthacenequinone happens partially before the scission of the glycosidic bond. The poor solubility of **3** in methanol protects it from further degradation. The use of hydrochloric acid instead of sulfuric acid increased the amount of red dyes, among which the 7-chloroanhydroelloramycinonic acid (**4**) is an unusual product, whose chlorination mechanism is not clear.

Systematic variation of the hydrolysis conditions showed trifluoroacetic acid to be the optimal reagent. In the presence of water, elloramycinone (**2**) was the only product[1], while under dry conditions, isoelloramycin (**10**) with the sugar rearranged from position 8 to position 4 was the main product. The structure of **10** was established by the NMR data, the UV spectrum under basic conditions showing a free hydroxy group at C-8 as in **2**, and the EI mass spectrum (Scheme 2)[1]. The molecular ion at *m/z* 660 and the *retro*-diene cleavage indicates that the sugar is connected to the smaller ring A fragment.

The rearrangement from **1** to **10** was of further interest considering whether the reaction is an intra- or intermolecular transglycosylation and the possibility of establishing that reaction as a general glycosylation method.

When elloramycin (**1**) was treated with dry trifluoroacetic acid in the presence of tetracenomycin C (**13**), which resembles elloramycinone (**2**), 25% 4-*O*-permethylrhamnosyl-tetracenomycin C (**11**) could be isolated from the reaction mixture besides 27% isoelloramycin (**10**). This result proves the favoured intermolecular two-step process. The liberated sugar moiety (**12**) has a higher affinity to 4-OH of elloramycinone (**2**) or tetracenomycin C (**13**) within a glycosylation reaction than to 8-OH of **2** (back reaction). We assume a mechanism with 1-*O*-trifluoroacetyl-2,3,4-tri-*O*-methyl-β-L-rhamnose (**12**) as the activated key intermediate (Scheme 3).

Scheme 2.

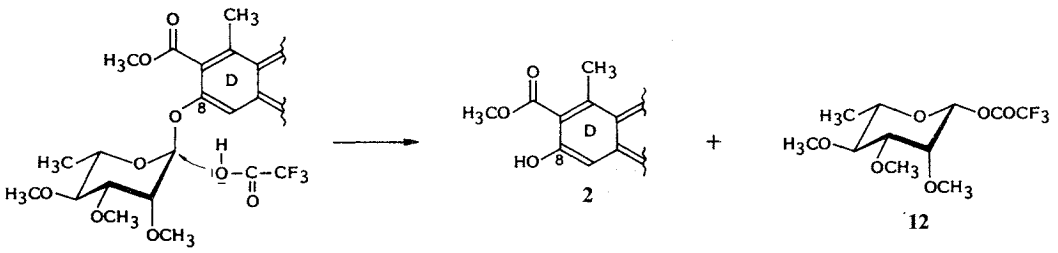


10 $R_1=H$ $R_2=CH_3$
11 $R_1=CH_3$ $R_2=H$
m/z 660

R_1, $R_2=H$, CH_3
m/z 359

m/z 301

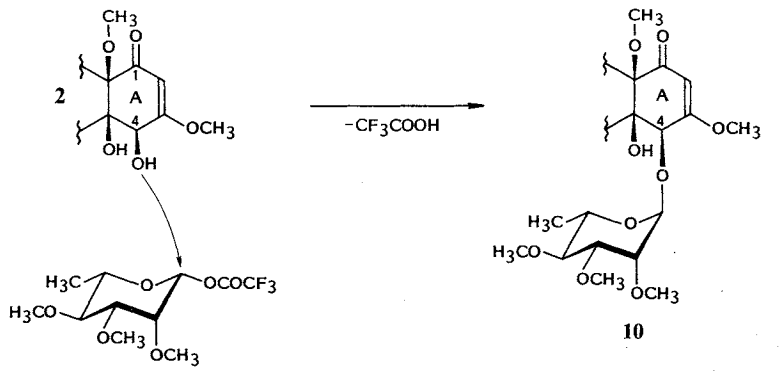
Scheme 3.



1

2

12



12

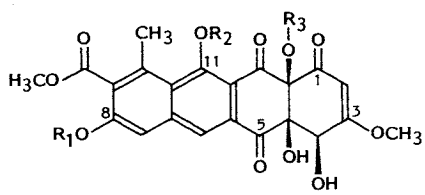
10

To prove this hypothesis, per-*O*-methyl-L-rhamnose was synthesized, demethylated in the 1-position, functionalized as 1-*O*-trifluoroacetate (**12**, α-β, 1 : 1) and added as anomeric mixture to elloramycinone (**2**) in dry trifluoroacetic acid. The obtained product mixture of isoelloramycin (**10**) and its β-isomer verifies the proposed mechanism. With **12** as a reagent, it was also possible to glycosylate tetracenomycin C (**13**) and aranciamycinone (**16**) as well, which leads to **11**, its β-isomer and to 3′,4′-di-*O*-methylaranciamycin (**17**), respectively. In the case of **16** only the α-glycosidic rhamnoside **17** was detectable.

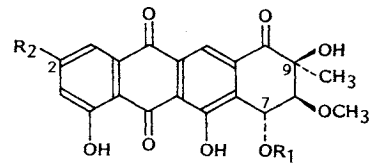
8-*O*-Methylelloramycinone

Tetracenomycin C (**13**) is assumed to intercalate with DNA, requiring a flat structural moiety to move

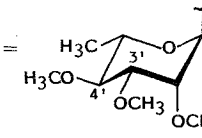
Scheme 4.

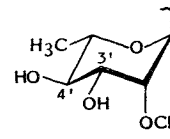


13 $R_1=CH_3$ $R_2=R_3=H$
14 $R_1=R_3=CH_3$ $R_2=H$
15 $R_1=R_2=R_3=CH_3$



16 $R_1=R_2=H$

17 $R_1=$  $R_2=H$

18 $R_1=$  $R_2=H$

19 $R_1=$Sugar as in **18** $R_2=OCH_3$

in between the base pairs. Rings B, C and D of **13** are in agreement with this condition, while ring A probably blocks the intercalation at a certain point. Elloramycin (**1**) has large substituents on both sides of its chromophore[2], which decreases its effectiveness of intercalation.

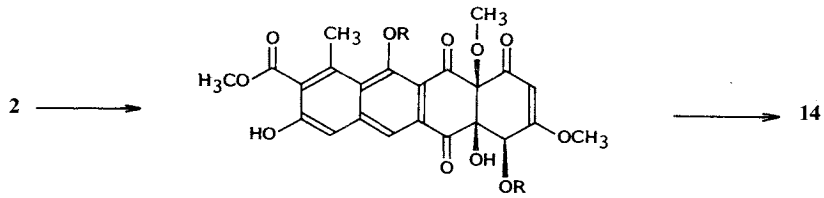
Table 1. Proliferation inhibition assay against L1210 leukemia cells[19].

Compound	IC_{50} (μg/ml)
1	3.3
2	3.1
10	10.0
13	1.2
14	0.6

Thus isoelloramycin (**10**) with the sugar moiety on the "right" side and also elloramycinone (**2**) were expected to have a better antitumor activity than **1**. But the IC_{50} value in the proliferation assay (Table 1) shows a weaker cytotoxic activity of **10** by comparison with **1**, and **2** shows only a slightly better activity than **1**, but is less active than tetracenomycin C (**13**).

Since **2** and **13** are structural isomers and differ only within the position of the methoxy group (C-8 or C-12a), it was necessary to find out which structural differences are responsible for the different biological activity. Three semisynthetic approaches to establish these structure-activity relationships were theoretically possible: (i) A selective ether cleavage of 12a-OCH_3 of elloramycinone (**2**), (ii) a selective methylation of 12a-OH of tetracenomycin C (**13**) or (iii) a selective methylation of the phenolic 8-OH position of elloramycinone (**2**). The ether cleavage of 12a-OCH_3 in **2** is chemically difficult to realize, and experiments with different reagents (BF_3, $AlCl_3$, $(CH_3)_3SiI$) showed lack of selectivity. Methylation experiments with tetracenomycin C (**13**) resulted in the desired molecule, (**14**, see below), but in very low yields, due to the various inevitable multiple methylated products. The third possibility, the methylation of 8-OH of **2**, could be established by the following reaction sequence (Scheme 5): Starting from **2**, 4-OH and 11-OH were protected as trimethylsilyl ether (**20**). After methylation of 8-OH with diazomethane and deprotection of the trimethylsilyl groups with hydrochloric acid, the goal molecule **14** could be obtained in 85% overall yield. Experiments starting from **1** using the sugar as protection group, peracetylation, hydrolysis of the sugar, methylation of 8-OH and deprotection of the acetyl groups failed because of the low yielding deprotection of the 11-*O*-acetyl group. Direct methylation of **2** always gave the 8,11-di-*O*-methylelloramycinone (**15**).

Scheme 5.



20 R = $Si(CH_3)_3$

Discussion

The experiments on elloramycin (**1**) give additional information about structure-activity relationships of the tetracenomycin C (**13**) related antibiotics, namely about importance of the functional groups at C-8 and C-12a. The fact that a phenol glycoside diminishes the biological activity was also shown within the group of anthracyclines by SETO *et al.*[18], who could glucosylate ε-rhodomycinone at the phenolic 4-position using a blocked mutant. The product, 4-*O*-(β-D-glucopyranosyl)-ε-rhodomycinone did not show any biological activity.

8-*O*-Methylelloramycinone (=tetracenomycin X, **14**) shows the best result in the proliferation-inhibition test of L1210 cell cultures (Table 1)[19], *i.e.* that 8-OH and 12a-OH cause negative interactions with the DNA, thus lowering the anti-cancer activity, and should rather be methylated. The structure and biosynthetic investigations on tetracenomycin X (**14**) were published during the preparation of this paper, but no biological activities of this newest member of the tetracenomycin/elloramycin antibiotics were described[7].

The sugar rearrangement which has been discovered during the hydrolysis experiments is until now unique. The reason for this rearrangement can be the thermodynamically stronger glycosidic bond between the secondary hydroxy group at C-4 in comparison with the phenolic hydroxy group at C-8. Two potential general synthetic approaches could follow from this discovery: The activation of a sugar as 1-*O*-trifluoroacetate or the activation of a sugar within a phenolic glycoside in the presence of trifluoroacetic acid. In the latter case the sugar can be transferred by an intermolecular transglycosylation. This method might be advantageous, especially if the accepting secondary alcohol has a α,β-double bond, usually very difficult to glycosylate. The transglycosylation experiments described in this paper prove this potential and will be the subject of further investigations.

Experimental

General

See ref 1, proton signals with an asterisk are exchangeable by D_2O or MeOH-d_4.

Methanolysis of Elloramycin

a) With H_2SO_4: 200 mg elloramycin (**1**) were dissolved in 50 ml MeOH and treated with 5 ml concd H_2SO_4. The solution was stirred for 24 hours at 25°C, and then 5 hours at 60°C. The mixture was poured on ice, the products were extracted with CH_2Cl_2. Chromatography on silica gel (plates, 20×40 cm, CH_2Cl_2-MeOH, 95:5) and Sephadex LH-20 (MeOH) gave;

15 mg (23%) methyl 2,3,4-tri-*O*-methyl-α-L-rhamnopyranoside[1],

60 mg (42%) elloramycinone (**2**)[1], yellow

35 mg (24%) isoelloramycinone (**9**), yellow

5 mg (4%) demethylelloramycinone (**8**), yellow

5 mg (3%) anhydroelloramycin (**3**), red

and several other minor unidentified red products.

b) With HCl: Using 37% HCl instead of sulfuric acid gave mostly the same products as above. Instead of **3**, 5 mg (4%) of 7-chloroanhydroelloramycinonic acid (**4**) could be isolated.

9: MP 190℃ (dec); IR (KBr) cm^{-1} 3440, 1708, 1678, 1600; UV $\lambda_{max}^{MeOH\ and\ MeOH-HCl}$ nm (ε) 290.5 (29,000), 395 (9,800), 412 (11,300), 443 (sh); $\lambda_{max}^{MeOH-NaOH}$ nm (ε) 316 (18,800), 355 (sh), 443 (10,000); ^1H NMR (80 MHz, $CDCl_3$) δ 2.62* (s, OH), 3.01 (s, 10-CH_3), 3.55 (s, 12a-OCH_3), 3.95 (s, 1-OCH_3), 4.00 (s, 9-$COOCH_3$), 4.34 (s, 4-H), 5.15 (s, 2-H), 7.20 (s, 7-H), 7.82 (s, 6-H), 14.26* (s, 11-OH); CD $\lambda_{extreme}^{MeOH}$ nm $[\theta]^{22}\times10^{-4}$) 238 (−3.0), 262 (+6.3), 286 (sh, +2.2), 348 (−1.6); EI-MS (70 eV) *m/z* (abundance, %) 472 (44%, M^+, HR calcd for $C_{23}H_{20}O_{11}$ and found 472.1006), 454 (12%, M−H_2O), 440 (23%, M−CH_3OH), 423 (29%), 411 (22%), 408 (24%), 392 (39%), 379 (26%), 359 (16%), 351 (18%), 327 (22%), 170 (16%), 155 (28%), 141 (40%), 137 (30%), 128 (100%), 113 (37%), 83 (22%), 71 (40%), 57 (46%), 44 (73%).

8: IR (KBr) cm^{-1} 3400, 1720, 1710, 1590; UV $\lambda_{max}^{MeOH\ and\ MeOH-HCl}$ nm (ε) 288 (30,600), 390 (7,200), 411 (8,100), 440 (sh); $\lambda_{max}^{MeOH-NaOH}$ nm (ε) 281 (17,900), 310 (14,900), 360 (sh), 440 (7,200); ^1H NMR (80 MHz, $CDCl_3$) δ 3.05 (s, 10-CH_3), 3.52 (s, 12a-OCH_3), 3.73* (s, OH), 3.82* (d, J=1.5 Hz, 2-H), 4.04 (s, 9-$COOCH_3$), 4.73 (d, J=1.5 Hz, 4-H), 7.25 (s, 7-H), 7.82 (s, 6-H), 14.06* (s, 11-OH); EI-MS (70 eV) *m/z* (abundance, %) 458 (3%, M^+, HR calcd for $C_{22}H_{18}O_{11}$ and found: 458.0849), 410 (23%), 378 (47%), 358 (30%), 326 (72%), 270 (18%), 72 (35%), 57 (85%), 44 (100%).

3: IR (KBr) cm^{-1} 3440, 1738, 1642 (sh), 1609, 1580; UV $\lambda_{max}^{MeOH\ and\ MeOH-NaOH}$ nm (ε) 248 (39,700), 290 (28,200), 365 (9,400), 540 (14,900), 585 (12,400); $\lambda_{max}^{MeOH-HCl}$ nm (ε) 252 (40,200), 280 (36,000), 336 (sh), 500 (17,400), 525 (16,500); ^1H NMR (100 MHz, $CDCl_3$) δ 1.30 (d, J=6 Hz, 5′-CH_3), 2.78 (s, 10-CH_3), 3.24 (dd, J=10 and 9 Hz, 4′-H), 3.60 and 3.63 (2×s and s, 2′-, 3′-, and 4′-OCH_3), 3.79 (dd, J=3 and 2 Hz, 2′-H), 4.02 (2×s, 3-OCH_3 and 9-$COOCH_3$), 5.76 (s, 1′-H), 6.90 (s, 2-H), 7.43 (s, 7-H), 7.94 (s, 6-H), 16.30* (s, OH); EI-MS (70 eV) *m/z* (abundance, %) 424 (0.5%, M−sugar), 392 (0.5%), 188 (44%, sugar), 157 (2%), 141 (4%), 129 (5%), 125 (3%), 116 (100%), 101 (6%), 88 (7%), 86 (29%), 72 (70%), 69 (15%), 59 (22%), 57 (15%), 45 (18%).

4: IR (KBr) cm^{-1} 3420～2680 (br), 1720, 1675, 1630 (sh), 1580 (sh), 1554 (sh); UV λ_{max}^{MeOH} nm (ε) 248 (28,400), 298 (24,700), 395 (9,700), 425 (sh), 550 (14,500), 600 (sh); $\lambda_{max}^{MeOH-HCl}$ nm (ε) 252 (27,700), 288 (36,000), 345 (7,600), 395 (sh), 515 (18,700), 540 (17,600); $\lambda_{max}^{MeOH-NaOH}$ nm (ε) 251 (18,700), 287 (sh), 304 (28,000), 393 (13,200), 425 (10,700), 550 sh (12,500), 600 (14,500); ^1H NMR (80 MHz, $(CH_3)_2CO$-d_6) δ 2.70 (s, 10-CH_3), 3.95 (s, 3-OCH_3), 6.45 (s, 2-H), 7.75 (s, 6-H), 11.83* (s, OH), 12.13* (s, OH), 15.33* (s, OH); EI-MS (70 eV) *m/z* (abundance, %) 444 (43%, M^+, HR calcd for $C_{21}H_{13}O_9Cl$ and found: 444.0248), 412 (100%, M−CH_3OH), 384 (11%), 378 (31%), 356 (29%), 328 (10%), 322 (13%), 192 (18%), 69 (21%).

Isoelloramycin (**10**)

Elloramycin (**1**, 100 mg) was dissolved in 10 ml anhydrous TFA and stirred in the presence of 4Å molecular sieves in a nitrogen atmosphere at 50℃ for 30 minutes. The solution was diluted by 100 ml $CHCl_3$, the acid was removed by extraction with water (3×50 ml each). The organic layer was evaporated to dryness. Chromatography on silica gel (column, 20×2.5 cm, $CHCl_3$-MeOH, 9:1) and Sephadex LH-20 (column, 100×2.5 cm, MeOH) yielded 32 mg (45%) of elloramycinone (**2**) and 52 mg (52%) of isoelloramycin (**10**). Rf 0.68 ($CHCl_3$-MeOH, 9:1), (EtOAc-pentane-AcOH, 55:40:5); $[\alpha]_D^{24}$ −114.7° (*c* 1.0, $CHCl_3$); IR (KBr) cm^{-1} 3420, 1733, 1710, 1688, 1605; UV λ_{max}^{MeOH} nm (ε) 290 (33,100), 395 (11,600), 414 (14,000), 436 (7,100); $\lambda_{max}^{MeOH-HCl}$ nm (ε) 289 (38,700), 393 (12,100), 411 (12,200); $\lambda_{max}^{MeOH-HCl}$ nm (ε) 279 (15,200), 317 (20,000), 357 (sh), 420 (12,100), 441 (13,100); ^1H NMR (80 MHz, $CDCl_3$) δ 0.85 (d, J=6 Hz, 5′-CH_3), 2.97 (dd, J=9 and 9 Hz, 4′-H), 3.05 (s, 10-CH_3), 3.21 (dd, J=9 and 3 Hz, 3′-H), 3.38, 3.49 and 3.52 (3s, 2′-, 3′- and 4′-OCH_3), *ca.* 3.4 (obscured, 5′-H), 3.62 (s, 12a-OCH_3), 3.63 (obscured, 2′-H), 3.78 (s, 3-OCH_3), 4.04 (s, 9-$COOCH_3$), 4.28* (s, 4a-OH), 4.91 (d, J=2 Hz, 4-H), 5.05 (d, J=2 Hz, 1′-H), 5.55 (d, J=2 Hz, 2-H), 7.26 (s, 7-H), 7.79 (s, 6-H), 12.20* (s, 8-OH), 14.14* (s, 11-OH); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ 17.9 (q, C-6′), 22.2 (q, 10-CH_3), 52.9 (q, 9-OCH_3), 56.5 (q, 12a-OCH_3, 56.8 (q, 3-OCH_3), 57.7 (q, 3-OCH_3), 58.5 (q, 2′-OCH_3), 60.6 (q, 4′-OCH_3), 68.8 (d, C-5), 73.4 (d, C-4), 76.9 (d, C-2′), 79.8 (d, C-3′), 81.4 (d, C-4′), 83.7 (s, C-4a), 86.5 (s, C-12a), 98.0 (d, C-1′), 102.0 (d, C-2), 108.3

(s, C-9), 113.0 (d, C-7), 120.6 (d, C-6), 121.0 (s, C-11a), 123.4 (s, C-6a), 126.6 (s, C-10a), 140.9 (s, C-10), 143.9 (s, C-5a), 158.4 (s, C-3), 167.8 (s, C-11), 169.6 (s, C-8), 171.3 (s, 9-C=O), 190.4 (s, C-1), 192.8 (s, C-5), 195.0 (s, C-12); CD $\lambda_{extreme}^{MeOH}$ nm ($[\theta]^{18} \times 10^{-4}$) 354 (−2.9), 297 (+2.1), 289 (+1.6), 266 (+12.9), 247 (−0.3), 239 (+1.8); EI-MS (70 eV) *m/z* (abundance, %) 660 (5 %, M^+, HR calcd for $C_{32}H_{36}O_{15}$ and found: 660.2054), 455 (9 %, M−sugar), 424 (2%), 359 (13%, M− 302 +H, cleavage of ring A, HR calcd for $C_{18}H_{15}O_8$ and found 359.0767), 327 (6%), 326 (6%), 302 (2%, M−359+H, cleavage of ring A, HR calcd for $C_{14}H_{22}O_7$ and found: 302.1366), 189 (100%, sugar), 157 (34%), 125 (21%), 113 (12%, 302−sugar), 101 (59%, 302−sugar), 101 (59%), 88 (40%), 75 (25%), 59 (27%).

Anal Calcd for $C_{32}H_{36}O_{15}$: C 58.18, H 5.49.
Found: C 58.61, H 5.89.

Methyl 2,3,4-Tri-*O*-methyl-α-L-rhamnoside

This sugar could be synthesized from L-(+)-rhamnose or by methanolysis of elloramycin (**1**)[1].

2,3,4-Tri-*O*-methyl-rhamnose

260 mg of the methyl 2,3,4-tri-*O*-methyl-α-L-rhamnoside were stirred in a mixture of 20 ml glacial AcOH and 3 ml of 3 M H_2SO_4 for 5 minutes at 80°C. The mixture was poured into 100 ml ice-water, the products were extracted with 50 ml of $CHCl_3$, and last traces of the acids were removed by washing the organic layer with 20 ml of a saturated aqueous solution of $NaHCO_3$. Chromatography on silica gel (column: 10×4 cm) with petroleum ether-ether (6:4) as solvent gave 196 mg (81%) 2,3,4-tri-*O*-methylrhamnose. $[\alpha]_D^{20}$ −62° (*c* 1.0, $CHCl_3$); ^{1}H NMR (80 MHz, $CDCl_3$) δ 1.27 (d, *J*=6.5 Hz, 5-CH_3), 3.08 (dd, *J*=9.5 and 9.5 Hz, 4-H), 3.35 (dd, *J*=9.5 and 3 Hz, 3-H), 3.46 and 3.54 (2×s and s, 2-, 3-, 4-OCH_3), 3.70 (s, 2-H), 5.07 (d, *J*=2 Hz, 1-H).

1-*O*-Trifluoroacetyl- 2,3,4-tri-*O*-methyl-L-rhamnose (**12**)

The sugar from b) (above) was dissolved in 20 ml of trifluoroacetic anhydride-pyridine (1:1) and stirred for 24 hours at room temperature until no starting material could be detected (TLC, phosphomolybdic acid as staining reagent), residues were removed azeotropically with toluene. Finally, the product was dried 24 hours under reduced pressure to yield 260 mg of **12** as a syrup, which was not cleaned further for the glycosylation reactions described below.

Glycosylation with **12**

Each aglycone (20 mg, elloramycinone (**2**), tetracenomycin C (**13**), aranciamycinone (**16**)) were dissolved in 5 ml anhydrous TFA (with the presence of 4Å molecular sieves) and treated with 60 mg of the 1-*O*-trifluoroacetyl- 2,3,4-tri-*O*-methyl-L-rhamnose (**12**) as a α-β (1:1) mixture. The mixture was stirred for 30 minutes at 50°C. The products were cleaned by chromatography on silica gel (column: 0.5 × 20 cm, $CHCl_3$-MeOH, 9:1) and Sephadex LH-20 (column: 0.5 × 20 cm, MeOH).

a) Isoelloramycin (**10**, Anomeric Mixture): Yield; 10 mg (36%). ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.86 (d, *J*=6 Hz, 5′-CH_3), 1.31 (d, *J*=6 Hz, β-5′-CH_3), 2.96 (dd, *J*=9 and 9 Hz, 4′-H), 3.04 (s, 10-CH_3), 3.10 (dd, *J*=9 and 9 Hz, β-4′-H), 3.20~3.40 (complex, 3′- and β-3′-H), 3.39, 3.43, 3.48, 3.51, 3.53, 3.57 (6×s, sugar−OCH_3), 3.63 (s, 12a-OCH_3), 3.64 (obscured, 2′-H), 3.81 (obscured, β-2′-H), 3.81 (s, 3-OCH_3), 4.06 (s, 9-$COOCH_3$), 4.96 (d, *J*=2 Hz, 4-H), 5.10 (d, *J*=2Hz, 1′-H), 5.26 (d, *J*=2Hz, β-1′-H), 5.61 (d, *J*=2Hz, 2-H), 7.15 (s, 7-H), 7.87 (s, 6-H). The α-anomeric diastereomer of this product mixture was identical with isoelloramycin (**10**).

b) 4-*O*-(Per-*O*-methyl-rhamnosyl)tetracenomycin C (**11**, Anomeric Mixture): Yield; 6 mg (21%). Rf 0.74 ($CHCl_3$-MeOH, 9:1); IR (KBr) cm^{-1} 3420, 1735, 1711, 1690 (sh), 1680 (sh), 1602; UV $\lambda_{max}^{MeOH\ and\ MeOH-HCl}$ nm (ε) 238 (23,700), 287 (40,100), 390 (10,400), 406 (11,200); $\lambda_{max}^{MeOH-NaOH}$ nm (ε) 252 (33,900), 279 (sh), 320 (sh), 440 (11,600); CD $\lambda_{extreme}^{MeOH}$ nm ($[\theta]^{20} \times 10^{-4}$) 400 (sh), 348 (−1.9), 284 (sh), 256 (+11.1), 235 (−0.4), 228 (−0.1); ^{1}H NMR (100 MHz, $CDCl_3$) δ 0.90 (d, *J*=6 Hz, 5′-CH_3), 1.33 (d, *J*=6 Hz, β-5′-CH_3), 2.90 (s, 10-CH_3), 3.12 (dd, *J*=9 and 9 Hz, 4′-H), 3.26 (dd, *J*=9 and 9 Hz, β-4′-H), 3.45, 3.57 (9H), 3.58, 3.61 (4×s, sugar-OCH_3), 3.64 (dd, *J*=3 and 2 Hz, 2′-H), 3.90 (s, 3-OCH_3), 4.04 (2×s, 8-OCH_3 and 9-$COOCH_3$), 5.05 (d, *J*=1.5 Hz, 4-H), 5.12 (d, *J*=2 Hz, 1′-H), 5.30 (d, *J*=2 Hz, β-1′-H), 5.76

(d, J= 1.5 Hz, 2-H), 7.18 (s, 7-H), 8.00 (s, 6-H), 13.84* (s, 11-OH); EI-MS (70 eV) m/z (abundance, %) 660 (1%, M^+, HR calcd for $C_{32}H_{36}O_{15}$ and found 660.2054), 629 (1 %, $M-OCH_3$), 590 (1%), 455 (2 %, M−sugar−O), 359 (7%, cleavage of ring A), 327 (2%), 270 (3%), 189 (60%, sugar), 157 (19%), 116 (37%), 106 (32%), 101 (100%), 99 (31%), 88 (96%), 72 (48%), 59 (63%), 44 (43%). The pure α-anomeric (as in formula **11**) could be obtained by treating elloramycin (**1**) with anhydrous TFA in the presence of tetracenomycin C (**13**) in an analogous procedure as the isoelloramycin (**10**) synthesis. Yield: 25 mg (25%). ^{1}H NMR (80 MHz, $CDCl_3$) δ 0.88 (d, J=6 Hz, 5′-CH_3), 2.88 (s, 10-CH_3), 3.10 (dd, J=9 and 9 Hz, 4′-H), 3.35 (dd, J=9 and 3 Hz, 3′-H), 3.41, 3.51 and 3.54 (3×s, sugar−OCH_3), 3.65 (dd, J=3 and 2 Hz, 2′-H), 3.85 (s, 3-OCH_3), 4.00 (2×s, 8-OCH_3 and 9-$COOCH_3$), 5.02 (d, J=1.5 Hz, 4-H), 5.08 (d, J=2 Hz, 1′-H), 5.70 (d, J=1.5 Hz, 2-H), 7.10 (s, 7-H), 7.90 (s, 6-H), 13.80* (s, 11-OH).

c) 3′,4′-Di-*O*-methylaranciamycin (**17**): Yield; 7 mg (23%). IR (KBr) cm^{-1} 3450, 2930, 2835, 1715, 1676, 1627; ^{1}H NMR (80 MHz, $CDCl_3$) δ 1.36 (d, J=6 Hz, 5′-CH_3), 1.52 (s, 9-CH_3), 3.25 (dd, J=9 and 9 Hz, 4′-H), 3.45 (s, 8-OCH_3), 3.52 and 3.55 (2×s and s, 2′-, 3′-, 4′-OCH_3), 3.74 (d, J=3 Hz, 8-H), 5.13 (d, J=3 Hz, 7-H), 5.53 (d, J=2 Hz, 1′-H), 7.26 (dd, J=8 and 2 Hz, 3-H), 7.65 (dd, J=8 and 8 Hz, 2-H), 7.81 (dd, J=8 and 2 Hz, 1-H), 8.33 (s, 11-H), 11.80* (s, 6-OH), 13.72* (s, 4-OH); EI-MS (70 eV) m/z (abundance, %) 428 (1%), 412 (1%), 367 (3 %, M−sugar), 335 (3%), 325 (7%), 323 (9%), 293 (3%), 256 (5%), 239 (4%), 205 (6 %, sugar), 189 (6%, 205−O), 173 (6%), 149 (18%), 145 (7%), 129 (10%), 117 (76%), 109 (29%), 101 (48%), 98 (25%), 97 (47%), 88 (39%), 83 (46%), 81 (40%), 73 (100%), 69 (67%), 57 (53%), 55 (43%), 43 (59%), 41 (56%). The reaction yielded no β-anomer of **17** in contrast to a) and b).

8,11-Di-*O*-methylelloramycinone (**15**)

Elloramycinone (**2**, 20 mg) was dissolved in 10 ml $CHCl_3$-MeOH-$(CH_3)_2CO$ (4:2:1) and treated with 10 ml 0.4 M etherial diazomethane solution at 0℃. After 10 minutes, no starting material was left (TLC). It was evaporated to dryness and chromatographed on Sephadex LH-20 (column 20×0.5 cm, MeOH). 11.4 mg (yield 54%) of the dimethyl ether (**15**) could be obtained as light yellow, amorphous powder by precipitating a concentrated CH_2Cl_2 solution into n-hexane; mp 152℃ (dec); IR (KBr) cm^{-1} 3460, 1735, 1706, 1697, 1609, 1555; UV $\lambda_{max}^{MeOH\ and\ MeOH\text{-}HCl}$ nm (ε) 235 (sh), 249 (sh), 284 (40,300), 342 (6,100), 365 (5,100), 385 (5,100); $\lambda_{max}^{MeOH\text{-}NaOH}$ nm (ε) 249 (33,000), 283 (sh), 342 (4,800), 385 (3,500); ^{1}H NMR (80 MHz, $CDCl_3$) δ 2.79 (s, 10-CH_3), 3.66 (s, 12a-OCH_3), 3.77 (s, 3-OCH_3), 3.91 (s, 11-OCH_3), 3.95 (s, 8-OCH_3), 3.97 (s, 9-$COOCH_3$), 4.72 (br s, 4-H), 5.49 (br s, 2-H), 7.14 (s, 7-H), 8.22 (s, 6-H); CD $\lambda_{extreme}^{MeOH}$ nm ($[\theta]^{18}\times10^{-4}$) 366 (−0.9), 316 (−0.5), 292 (+1.6), 285 (+1.3), 261 (+7.5), 237 (−1.0), 224 (+0.2); EI-MS (70 eV) m/z (abundance, %) 500 (12%, M^+, HR calcd for $C_{25}H_{24}O_{11}$ and found: 500.1319), 439 (17%), 386 (75%), 371 (84%), 355 (30%), 339 (20%), 325 (10%), 114 (100%), 85 (70%), 69 (75%), 56 (15%), 44 (39%).

4,11-Di-*O*-trimethylsilylelloramycinone (**20**)

A stirring solution of 300 mg elloramycinone (**2**) in 12 ml pyridine-hexamethyldisilazane (3:1) was treated with 5 ml trimethylchlorosilane at 40℃ for 1 hour; the stirring was continued for 12 hours at room temperature. It was evaporated to dryness, pyridine was removed azeotropically with toluene. The solid residue was chromatographed on silica gel (column: 30×5 cm, $CHCl_3$-MeOH, 95:5), to yield 248 mg (63%) **20**, 45 mg **2** could be recovered. IR (KBr) cm^{-1} 3410, 1735 (sh), 1720, 1690, 1680 (sh), 1605; UV $\lambda_{max}^{MeOH\ and\ MeOH\text{-}HCl}$ nm (ε) 242 (28,600), 290 (45,500), 393 (15,000), 412 (16,400); $\lambda_{max}^{MeOH\text{-}NaOH}$ nm (ε) 242 (34,300), 315 (27,000), 355 (sh), 420 (sh), 440 (15,700); ^{1}H NMR (200 MHz, $CDCl_3$) δ −0.12 (s, 4-TMS), 0.16 (s, 11-TMS), 1.68* (s, 4a-OH), 3.06 (s, 10-CH_3), 3.59 (s, 12a-OCH_3), 3.72 (s, 3-OCH_3), 4.06 (s, 9-OCH_3), 4.83 (d, J=1.5 Hz, 4-H), 5.45 (d, J=1.5 Hz, 2-H), 7.31 (s, 7-H), 7.82 (s, 6-H), 9.65* (s, 8-OH); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ 0.1 and 2.5 (2×q, 2×TMS), 22.3 (q, 10-CH_3), 53.0 (q, 9-OCH_3), 56.2 and 56.4 (2×q, 3-OCH_3 and 12a-OCH_3), 71.8 (d, C-4), 88.3 (s, C-12a), 88.9 (s, C-4a), 100.5 (d, C-2), 108.6 (s, C-9), 113.0 (d, C-7), 120.3 (d, C-6), 120.4 and 123.1 (2×s, C-6a and C-11a), 128.5 (s, C-10a), 141.1 (s, C-10), 143.8 (s, C-5a), 158.2 (s, C-3), 167.5 (s, C-11), 169.9 (s, C-8), 172.4 (s, 9-C=O), 192.1 (s, C-1), 192.5 (s, C-5), 196.3 (s, C-12); CD $\lambda_{extreme}^{MeOH}$ nm ($[\theta]^{22}\times10^{-4}$) 405 (−0.9), 350 (−2.6), 290 (+1.9), 280 (+0.5), 258 (+16.0), 240 (+0.7), 232 (+2.3); EI-MS (70 eV) m/z (abundance, %) 616 (1%, M^+, HR

calcd for $C_{29}H_{36}O_{11}Si_2$ and found: 616.1796), 601 (3%), 503 (2%), 383 (3%), 186 (100%), 147 (3%), 101 (2%), 89 (4%), 75 (45%), 73 (41%), 59 (4%), 57 (4%), 47 (7%), 45 (8%).

Anal Calcd for $C_{29}H_{36}O_{11}Si_2$: C 56.47, H 5.88.
Found: C 56.40, H 5.83.

8-*O*-Methylelloramycinone (**14**)

A stirred solution of 248 mg of **20** in anhydrous THF in the presence of 4Å molecular sieves was treated at 0℃ with 5 ml 4 M etherial diazomethane solution. After 10 minutes the excess diazomethane was inactivated by adding of 1 ml water. It was evaporated to dryness, and the yellow residue (240 mg) was dissolved in 10 ml of MeOH and treated with 1 ml of 0.1 M aquaeous HCl. After stirring for 20 hours at room temperature the mixture was poured into 100 ml water and extracted three times with 50 ml $CHCl_3$. Chromatography of the combined concentrated organic layer on silica gel (column: 50×2.5 cm, CH_2Cl_2-MeOH, 85:15) gave 163 mg (83%) **14**; mp 156℃ (dec); IR (KBr) cm^{-1} 3450, 1730, 1710, 1688, 1605; UV $\lambda_{max}^{MeOH\ and\ MeOH\text{-}HCl}$ nm (ε) 240 (25,000), 283 (33,900), 392 (11,300), 409 (12,400), 440 (sh); $\lambda_{max}^{MeOH\text{-}NaOH}$ nm (ε) 250 (35,500), 440 (13,000); ^1H NMR (80 MHz, $CDCl_3$) δ 2.85 (s, 10-CH_3), 3.61 (s, 12a-OCH_3), 3.77 (s, 3-OCH_3), 3.97 (2×s, 8-OCH_3 and 9-$COOCH_3$), 4.76 (d, J=1.5 Hz, 4-H), 5.50 (d, J=1.5 Hz, 2-H), 7.22 (s, 7-H), 7.88 (s, 6-H), 13.79* (s, 11-OH); EI-MS (70 eV) *m/z* (abundance, %) 486 (6%, M^+, HR calcd for $C_{24}H_{22}O_{11}$ and found: 486.1162), 485 (23%), 468 (5%), 454 (7%), 438 (6%), 386 (18%), 372 (100%, cleavage of ring A), 357 (34%), 340 (33%), 155 (7%), 141 (11%), 114 (18%), 86 (28%), 69 (13%), 56 (31%), 44 (72%).

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