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TOTAL SYNTHESIS OF TYLOSIN

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Summary Tylosin has been synthesized by regio- and stereoselective introduction of the amino disaccharide moiety and D-mycinose onto the previously synthesized 16-membered-ring aglycone.

Tylosin (1), a 16-membered-ring macrolide antibiotic, is extremely used as a therapeutic substance in treatment of mycoplasmosis in poultry [1] An aglycone (2) of tylosin has been stereospecifically synthesized from D-glucose in these laboratories.[2] Herein, we report the first total synthesis of tylosin (1) by regio- and stereoselective introduction[3] of the amino disaccharide, namely 4-O-(α-L-mycarosyl)-D-mycaminose, and D-mycinose onto the C-5 and C-23 hydroxyl groups of the macrolide aglycone The synthesis began with the conversion of the aglycone 2 into the ethylene acetal 5 as previously described in the total synthesis of carbomycin B [4] Acid hydrolysis (4% H_3PO_4 in 50% aq THF, 60°, 21h) of the aforesaid 2 (mp 124°, $[\alpha]_D$ +60°)[5] afforded the hemiacetal 3[5] (78%, mp 120°, $[\alpha]_D$ +25°), which was treated with ethylene glycol (TsOH, dioxane, 35°, 2 days) to give the hydroxyethyl furanoside 4[6] (62%, mp 118°, $[\alpha]_D$ +53°) and 5[4] (11%, mp 88°, $[\alpha]_D$ +23°, λ_{max} 284 nm (ε 21400)) after preparative TLC (R_f 0 37 and 0.39, PhMe-hexane-Me_2CO 1 1 1) The β-glycosidation of 5 was widely investigated using a variety of modified Koenigs-Knorr conditions, which involved recent methods [7] Thus, reaction of 5 with 1-α-bromo-2,4-diacetyl-mycaminose hydrobromide[3b] (20 equiv) in the presence of HgO and $HgBr_2$[8] (dioxane/Drierite, 20°, 17h), followed by methanolysis[3b] (MeOH, 40°, 24h), gave the desired β-glycoside 6[5] as a single isolable product (22%, mp 129°, $[\alpha]_D$ +7 5°, λ_{max} 284 nm (ε 27300), R_f 0 36 ($CHCl_3$-MeOH 8 1) identical in all respects with the authentic sample, which was derived from mycaminosyl tylonolide[9] by treatment with ethylene glycol and TsOH (MeCN, 20°, 1h) followed by tritylation (TrCl/Py, 50°, 18h) That the C-5 hydroxyl group is more reactive than the C-3 toward glycosidation is consistent with the view in the total synthesis of erythromycin by Woodward et al [6]

The second glycosidation of mycarose moiety to the C-4' hydroxyl group was stereospecifically accomplished by our method[3] after protecting the C-2' hydroxyl group Without protection, the glycosidation unexpectedly[3b] gave the C-2' glycoside as the major product Selective acetylation (Ac_2O/MeCN, 5° 0 5h) of 6 by using its own basicity gave 7[5] (60%, mp 129°, $[\alpha]_D$ +2 5°) The glycal 8[3,5] ($[\alpha]_D$ -165°) was prepared ($TsCl/Et_3N$/MeCN, 20°, 4h) from 4-O-acetylmycarose (needles, mp 103°, $[\alpha]_D$ -100°), which was in turn obtained from L-mycarose by acetylation (Ac_2O/Py) and selective hydrolysis (aq $CHCl_2COOH$/MeCN) Reaction (PhMe/MeCN, -30° to 20°, 5h) of 7 with 8 (4 equiv) in the presence of 1,3-dibromo-5,5-dimethylhydantoin (2 equiv) afforded, after silica gel column chromatography (PhH-Me_2CO 6 1 and $CHCl_3$-Me_2CO 15 1), a single condensed product 9[5] (17%, mp 132°, $[\alpha]_D$ -25°, λ_{max} 283 nm (ε 20600), and unreacted starting material 7 (recovered in

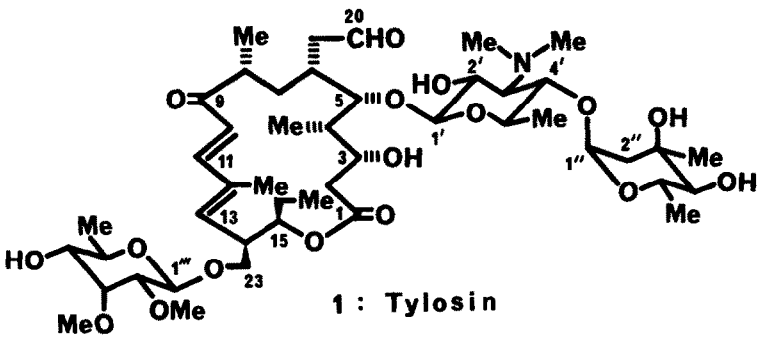
52% yield). Use of N-iodosuccinimide[7] gave no desired product possibly owing to the nonselectively occurring halogenation of the double bonds of the aglycone. In the ^{13}C-NMR spectrum of **9**, the chemical shift (δ 173 7) of the signal due to the C-1 carbon indicated the presence of an intramolecular hydrogen bonding[10] between the C-1 carbonyl and C-3 hydroxyl groups, supporting the structure of the C-4' glycoside. It has been found in these laboratories[3b] that the presence of a bromine atom at the C-2" induces the glycoside to resist even drastic acid hydrolysis, while 2-deoxyglycosides are generally labile under acidic conditions. Consequently, deprotection of **9** with 90% CF_3COOH (5°, 1h) gave needles of the hydroxy aldehyde **10**[5] (82%, mp 148°, $[\alpha]_D$ -50°) without cleavage of the α-glycosidic linkage. Debromination of **10** by using Bu_3SnH (3 equiv) with α,α'-azobisisobutyronitrile as catalyst (PhMe, Ar, 45°, 5h) afforded the 2"-deoxyglycoside **11**[5] (90%, mp 145° (needles), $[\alpha]_D$ -70°, λ_{max} 284 nm (ε 21900)), which was deacetylated (MeOH, 40°, 60h and then K_2CO_3/MeOH, 20°, 4h) to give demycinosyl tylosin **12**[5] (80%, mp 152°, $[\alpha]_D$ -59°, λ_{max} 283 nm (ε 21200)) identical in all respects with a sample of the same structure produced by a mutant strain of *Streptomyces fradiae* [11]

The third glycosidation was eventually achieved by a usual Koenigs-Knorr condition as follows. The labile 4-O-acetylmycinosyl bromide was prepared (HBr-AcOH/CH_2Cl_2, 5°, 2h) from diacetylmycinoses (α-acetate· syrup, $[\alpha]_D$ +56°, β-acetate needles, mp 106°, $[\alpha]_D$ +30°), which were obtained by acetylation (Ac_2O/Py) of D-mycinose [12] Condensation of **11** with the glycosyl bromide (10 equiv) in the presence of $Hg(CN)_2$ in $MeNO_2$ (Drierite, 40°, 6h) gave, after silica gel column chromatography (PhH-Me_2CO 4·1 and $CHCl_3$-Me_2CO 5:1), the α- and β-glycosides (**13** and **14**, R_f 0.26 and 0 34 (PhH-Me_2CO 3 1)). The β-glycoside **14**[5] was further purified on Sephadex LH-20 column chromatography (PhH-Me_2CO 4·1) to give an analytically pure sample (22%, mp 128°, $[\alpha]_D$ -51°) The other glycoside **13**, without further purification, was submitted to selective deacetylation (MeOH, 40°, 2 days) to give **15**[5] (32% from **11**, mp 120°, $[\alpha]_D$ -6.3°), which was completely deacetylated (K_2CO_3/MeOH, 20°, 2h) to yield the α-anomer (**17**)[5] of tylosin (69%, mp 123°, $[\alpha]_D$ -28°, λ_{max} 284 nm (ε 22800)). Similar deacetylation of **14** completed the synthesis, through **16**[5] (mp 185° (needles), $[\alpha]_D$ -45°), giving tylosin (**1**[5], 84%, mp 135-138° (amorphous from Me_2CO-hexane), mp 131° (monohydrate plates from Me_2CO-H_2O), $[\alpha]_D$ -55°, λ_{max} 283 nm (ε 27500)) identical in all respects (IR, UV, ^{1}H-NMR and antibacterial activity (Table 1)) with that obtained from natural sources.

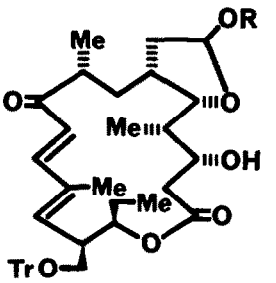
Compounds **11**, **12** and **17** also were found to show fairly strong antibacterial activities (Table 1).

Table 1 Minimal Inhibitory Concentration (mcg/ml) Of The Products

	Synthetic Tylosin(1)	Natural Tylosin	**11**	Synthetic **12**	Natural[11] **12**	**17**
Staph. aureus 209P	0 39	0 39	0 78	0 39	0.39	0.78
Sarcina lutea PCI 1001	<0.2	<0 2	<0 2	<0 2	<0.2	<0.2
B. subtilis B-558	0.39	0.39	0.78	0.78	0.78	0.78
E. coli NIHJ	50	50	3 12	3.12	3.12	50
Kl. pneumoniae PCI 602	50	50	6.25	6.25	6.25	50
Sh. dysenteriae JS 11910	6.25	6.25	3 12	3.12	3.12	25
Sal. enteritidis 1891	25	25	12 5	12 5	12.5	50



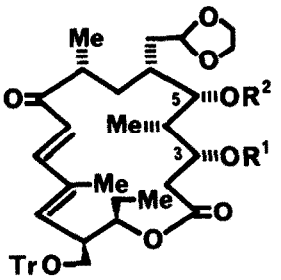
1 : Tylosin



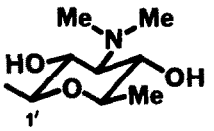
2 : R = Me

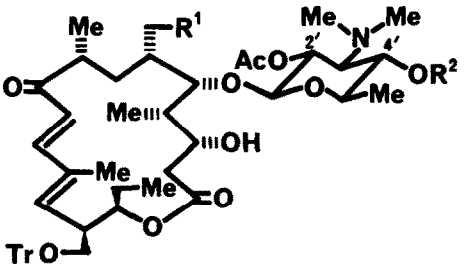
3 : R = H

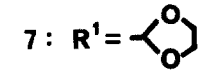
4 : R = $(CH_2)_2OH$

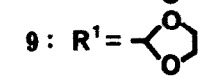


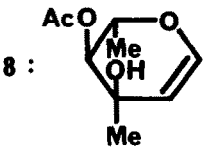
5 : $R^1 = R^2 = H$

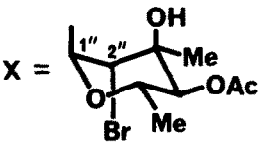
6 : $R^1 = H$, $R^2 =$ 



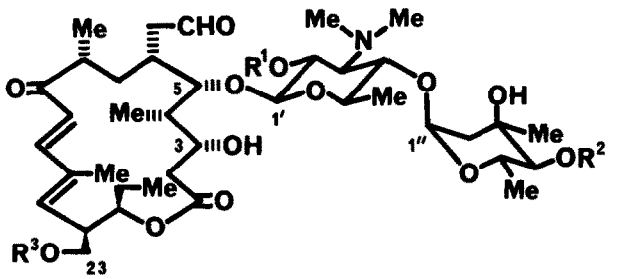
7 : $R^1 =$  , $R^2 = H$

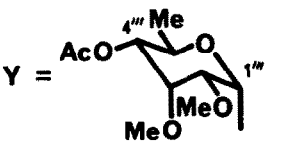
9 : $R^1 =$  , $R^2 = X$

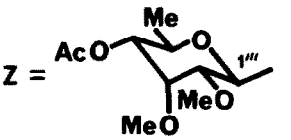




10 : $R^1 = CHO$, $R^2 = X$, Tr = H







11 : $R^1 = R^2 = Ac$, $R^3 = H$

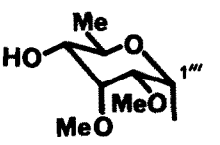
12 : $R^1 = R^2 = R^3 = H$

13 : $R^1 = R^2 = Ac$, $R^3 = Y$

14 : $R^1 = R^2 = Ac$, $R^3 = Z$

15 : $R^1 = H$, $R^2 = Ac$, $R^3 = Y$

16 : $R^1 = H$, $R^2 = Ac$, $R^3 = Z$

17 : $R^1 = R^2 = H$, $R^3 =$ 

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5) All compounds have been fully characterized by spectroscopic means and elemental analyses. Melting points were uncorrected Optical rotations were done in $CHCl_3$ at c 1 00 (20°) and UV spectra measured in MeOH R_f-values were measured on silica gel Merck TLC 60F-254 NMR (250 MHz, δ, ppm from TMS, and J in Hz) spectra were in $CDCl_3$ solution Significant ^{1}H-NMR spectral data are the following (with ^{13}C-NMR for **9** and **11**) **1** 4 22(d, J=7, H-1'), 4 57(d, J= 7 5, H-1'''), 4 99(dt, J=10 & 2 5, H-15), 5 08(d, J=3 5, H-1") **3** 4 06(dd, J=10 & 2 5, H-5), 5 46(m, H-20) **5** 4 96(t, J=5, H-20), 5 02(dt, J=9 5 & 2 5, H-15). **6** 2 43(t, J=10, H-3'), 3 55(dd, J=10 & 7 5, H-2'), 4.33(d, J=7 5, H-1') **7** 3 05(dd, J=10 5 & 8 5, H-4'), 4 41(d, J= 7 5, H-1'), 5 00(dd, J=10 5 & 7 5, H-2') **8** 4 69(d, J=6 5, H-2), 6 35(d, J=6 5, H-1) **9** 3 97(s, H-2"), 5 17(d, J=10, H-4"), 5 26(s, H-1"), ^{13}C-NMR 173 7(C-1), 104 0(C-1'), 102 3 (C-20), 100 4(C-1"), 53 1(C-2") **10** 3 96(s, H-2"), 4 28(d, J=8, H-1'), 5 17(d, J=10, H-4"), 5 24(s, H-1"), 9 67(d, J=1, CHO) **11** 4 28(d, J=8, H-1'), 4 60(d, J=10, H-4"), ^{13}C-NMR 203 1(CHO), 173 8(C-1), 101 9(C-1'), 97 1(C-1") **12.** 4 22(d, J=7 5, H-1'), 4 96(dt, J=10 & 2 5, H-15), 5 08(d, J=2 5, H-1") **14** 4 27(d, J=8, H-1'), 4 44(dd, J=10 & 2, H-4'''), 4 60(d, J=10, H-4"), 4 63(d, J=8, H-1'''), 5 01(dd, J=10 5 & 8, H-2'), 5 07(d, J=3 5, H-1") **15**· 4 24 (d, J=7 5, H-1'), 4 82(d, J=4, H-1'''), 5 07(d, J=3, H-1") **16** 3 04(dd, J=8 & 2 5, H-2'''), 3 72(d, J=8 5, H-5), 3 83(d, J=10, H-3), 4 23(d, J=7 5, H-1'), 4 44(dd, J=10 & 2, H-4'''), 4 61 (d, J=10, H-4"), 4 63(d, J=8, H-1'''), 5 07(d, J=3 5, H-1") **17** 4 23(d, J=7 5, H-1'), 4 81(d, J=3 5, H-1'''), 5.96(d, J=10, H-13), 9 70(s, CHO)

6) The product **4** can be recycled for the preparation of **5**

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