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Synthesis of 4′-*O*-demycarosyl-3-*O*-(α-L-rhamnopyranosyl)tylosin

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Summary – Reaction of 2′,4″-di-*O*-acetyl-4′-*O*-demycarosyl-3′-*N*-oxide-tylosin ethylene acetal **6** with phenyl 2,3,4-tri-*O*-acetyl-1-thio-α-L-rhamnopyranoside in the presence of *N*-iodosuccinimide and trifluoromethanesulfonic acid furnished with complete regioselectivity the corresponding 3-*O*-α-L-glycoside **7** in 60% yield. Sequential deprotection afforded the title compound **10**.

macrolide antibiotic / tylosin / desmycosin / glycosylation / L-rhamnose

In view of the continuing interest in 16-membered macrolide antibiotics [1], we decided to further explore the chemistry of tylosin **1**. Two goals were considered as high priority: (a) synthesis of the title compound **10**; and (b) replacement of mycarose in tylosin **1** by a structurally similar carbohydrate, L-rhamnose, which may allow the introduction of an electronegative substituent at C-2, and thus a strong glycosidic linkage. Application of the strategy outlined below was thought to allow simultaneous accomplishment of both goals.

The title compound **10** appeared to be of considerable interest from the point of view of structure activity relationships in antibacterial studies [1]. In **10**, the right-hand moiety closely imitates the clinically important erythromycin A, whose C-3 oxygen is α-glycosylated by L-cladinose [1]. As a consequence, 4′-*O*-demycarosyl-3-*O*-(α-L-rhamnopyranosyl)tylosin **10** was expected to exhibit a broad spectrum Gram-positive activity. The synthesis of such hybrid antibacterials has only been reported once [2].

The idea of substitution of mycarose by L-rhamnose in tylosin **1** also appeared to be highly justified. The presence of a 2-deoxy-sugar, mycarose, makes tylosin quite sensitive to degradation under the acidic pH values of the stomach. 4′-*O*-Demycarosyltylosin **2** (desmycosin) is well known for its good *in vitro* antibacterial activity [1]. However, in the absence of mycarose, the dimethylamino group of this macrolide is poised to form stable complexes with cytochrome P450, resulting in a considerable increase of hepatotoxicity [3].

Simultaneous synthesis of the two compounds of interest, **10** and **11**, required the preparation of a common intermediate **6**, derived from desmycosin **2**, in which only two hydroxyl groups at C-3 and at C-4′ were free. Glycosylation of **6** was expected to occur without significant regioselectivity. Thus, separation of the glycosylation products followed by elimination of their protecting groups appeared to be a reasonable approach to the preparation of both required α-L-rhamnosyl macrolides. Literature precedents as well as our experience suggested that good glycosylation yields could be expected with iodonium-ion-promoted reactions [4] of phenyl 2,3,4-tri-*O*-acetyl-1-thio-α-L-rhamnopyranoside [5]. This strategy implied prior protection of the basic dimethylamino group of the appropriate desmycosin derivative. Furthermore, the protection of the C-20 aldehyde group, prior to glycosylation, was also planned in order to avoid problems at the final deacetylation step [6].

Mild acidic hydrolysis [1] of 2′,4″-di-*O*-acetyltylosin **3** [7] gave the corresponding 4′-*O*-demycarosyl compound **4** (78%). The aldehyde group of **4** was protected by ethylene glycol treatment (84%) furnishing **5**. In the presence of *m*-chloroperbenzoic acid, the 3′-*N*-oxide **6** (72%) was obtained. The crucial glycosylation of **6** by phenyl 2,3,4-tri-*O*-acetyl-1-thio-α-L-rhamnopyranoside [5] was performed as described [4], in the presence of *N*-iodosuccinimide and trifluoromethanesulfonic acid. However, unexpectedly, a complete regioselectivity was observed, resulting in the exclusive formation of the C-3 α-*O*-glycoside **7** (60%). The isomeric C-4′ *O*-glycoside was not detected in the crude reaction mixture. Structural proof for the unique glycosylation product **7** was afforded by spectroscopic data, and, particularly, by the strong downfield shift of C-3 and the upfield shift of C-1 in its NMR spectrum [8]. The downfield shift of C-3 is due to the β-effect of the glycosylation while the upfield C-1 shift results from the cleavage of the intramolecular hydrogen bond between the C-3 hydroxyl group and the lactone carbonyl of tylosin derivatives [8].

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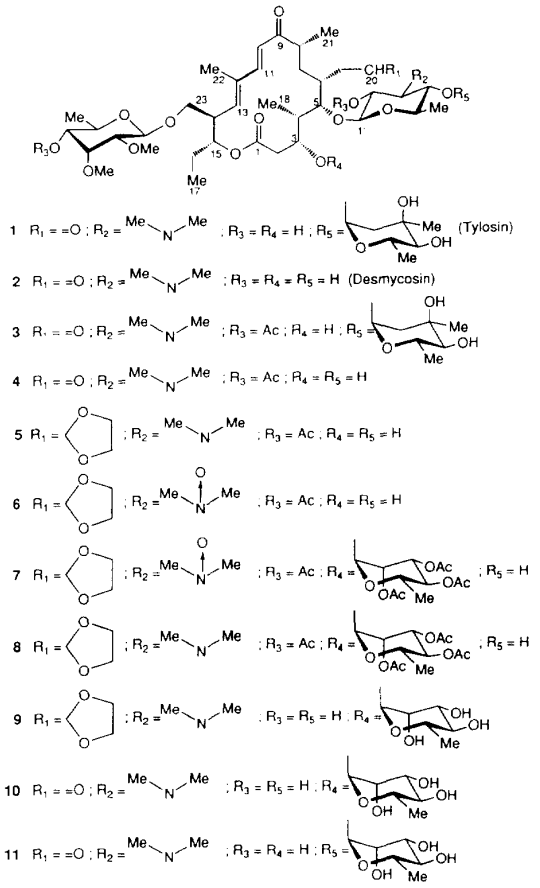
The regioselectivity of the glycosylation is surprising in view of previous results on related systems [9]. In the total synthesis of tylosin **1**, using a different glycosylation procedure, Tatsuta *et al* [9] observed preferential reaction at the C-4′ site with respect to the C-3 hydroxyl. Our result may be due to the steric hindrance induced by the 3′-*N*-oxide, but further investigations are necessary to elucidate this point. Deprotection of **7** was carried out by treatment with triphenylphosphine leading to **8** (80%). Rapid sodium methanolate treatment liberated the five hydroxyl groups affording **9** (67%) and the C-20 dioxolane was eliminated in the presence of 1 N hydrochloric acid giving the title compound **10** (80%). Disappointingly, 4′-*O*-demycarosyl-3-*O*-(α-L-rhamnopyranosyl)tylosin **10** appears to exhibit low antibacterial activity towards *Staphylococcus, Streptococcus, Moraxella* and *Haemophilus influenzae* strains.



Experimental section

General

All reactions were carried out under argon, solvents were purified and dried by standard techniques.

Thin layer chromatography (TLC) was performed using E Merck plates of silica gel 60 with fluorescent indicator. Column chromatography was carried out on the same support. Visualization was effected by spraying plates with 5% H_2SO_4 in ethanol, followed by heating at 120–140°C.

The term "usual work-up" means CH_2Cl_2 extraction, followed by washing with a dilute $NaHCO_3$ water solution, drying the organic layer over Na_2SO_4 and evaporation under reduced pressure.

^1H and ^{13}C NMR spectra were recorded on Bruker spectrometers (WP250 or WP300). Chemical shifts are expressed in ppm relative to tetramethylsilane.

Optical rotations were measured with a Perkin-Elmer 241 polarimeter.

Mass spectra (MS) and high resolution mass spectra (HRMS) were run on a MS80 and a VG-ZAB-SEQ spectrometer, respectively.

In vitro *evaluation*

These data were obtained by the standard microdilution methodology.

• *2′,4″-Di-*O*-acetyl-4′-*O*-demycarosyltylosin* **4**

To a solution of CH_3CN (55 mL) containing 0.1 N hydrochloric acid in the ratio 2.5:1 was added 2′,4″-di-*O*-acetyltylosin **3** (2.6 g, 2.69 mmol) and the mixture was stirred for 16 h at room temperature. After neutralization with amberlite IR400 (OH^-), filtration, washing with CH_3OH and evaporation, the residue was purified by flash chromatography ($CH_2Cl_2/CH_3OH/NH_4OH$ 30:1:0.05) to give **4** (1.79 g, 78%) as a foam.

Rf = 0.46 with $CH_2Cl_2/CH_3OH/NH_4OH$ 30:1:0.05.

$[\alpha]_D = -12$ ($c = +0.9$, $CHCl_3$).

^1H NMR ($CDCl_3$, 250 MHz): δ 1.78 (s, 3H, H-22), 2.06 and 2.12 (2s, 2 × 3H, 2 $OCOCH_3$), 2.38 (s, 2 × 3H, 3′-$N(CH_3)_2$), 3.48 (s, 3H, 2″-OCH_3), 3.51 (s, 3H, 3″-OCH_3), 4.00 (dd, 1H, $J_{14,23eq} = 3.9$ Hz, $J_{23ax,23eq} = 9.4$ Hz, H-23eq), 4.30 (d, 1H, $J_{1',2'} = 7.5$ Hz, H-1′), 4.22 (dd, 1H, $J_{3'',4''} = 2.4$ Hz, $J_{4'',5''} = 9.8$ Hz, H-4″), 4.61 (d, 1H, $J_{1'',2''} = 7.9$ Hz, H-1″), 4.94 (dt, 1H, $J_{14,15} = 1.2$ Hz, $J_{15,16} = 7.3$ Hz, H-15), 4.98 (dd, 1H, $J_{2',3'} = 10.5$ Hz, H-2′), 5.89 (d, 1H, $J_{13,14} = 10.5$ Hz, H-13), 6.27 (d, 1H, $J_{10,11} = 15.4$ Hz, H-10), 7.31 (d, 1H, H-11), 9.68 (s, 1H, H-20).

^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 17.6 (5′-CH_3), 20.7 and 21.3 (2 $OCOCH_3$), 41.0 (3′-$N(CH_3)_2$), 66.5 (C-3′), 68.9 (C-4′), 70.2 (C-3), 70.4 (C-2′), 72.8 (C-5′), 80.3 (C-5), 101.7 (C-1′), 168.9 and 169.8 (2 $OCOCH_3$), 173.5 (C-1), 202.8 (C-20).

MS (LSMIS): *m/z* 964 ($M^+ + H$ + thioglycerol), 856 ($M^+ + H$).

HRMS (FAB) calc for $C_{43}H_{70}NO_{16}$ ($M^+ + H$): 856.4695. Found: 856.4663.

• *2′,4″-Di-*O*-acetyl-4′-*O*-demycarosyltylosin ethylene acetal* **5**

To a solution of CH_3CN (21 mL) containing **4** (1.34 g, 1.57 mmol, 1 equiv) was added ethylene glycol (1.31 mL, 23.5 mmol, 15 equiv) and then oxalic acid (1.18 g, 9.4 mmol, 6 equiv). The mixture was stirred at room temperature for 24 h. Dilution with a cold saturated solution of $NaHCO_3$ and the usual work-up furnished a residue which was further purified by flash chromatography ($CH_2Cl_2/CH_3OH/NH_4OH$ 10:1:0.05) to give pure **5** (1.15 g, 84%) as a foam.

Rf = 0.51 with $CH_2Cl_2/CH_3OH/NH_4OH$ 10:1:0.05.

$[\alpha]_D = -15$ ($c = 1$, $CHCl_3$).

^1H NMR ($CDCl_3$, 250 MHz): δ 1.78 (s, 3H, H-22), 2.07 and 2.10 (2s, 2×3H, 2 $OCOCH_3$), 2.41 (s, 2×3H, 3′-$N(CH_3)_2$),

3.47 (s, 3H, 2″-OCH_3), 3.53 (s, 3H, 3″-OCH_3), 4.39 (d, 1H, $J_{1',2'}$ = 7.6 Hz, H-1′), 4.43 (dd, 1H, $J_{3'',4''}$ = 2.4 Hz, $J_{4'',5''}$ = 9.8 Hz, H-4″), 4.62 (d, 1H, $J_{1'',2''}$ = 7.9 Hz, H-1″), 4.90–5.01 (m, 2H, H-15 and H-20), 5.02 (dd, 1H, $J_{2',3'}$ = 10.0 Hz, H-2′), 5.88 (d, 1H, $J_{13,14}$ = 10.4 Hz, H-13), 6.28 (d, 1H, $J_{10,11}$ = 15.4 Hz, H-10), 7.33 (d, 1H, H-11).

^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 17.9 (5′-CH_3), 21.0 and 21.7 (2 $OCOCH_3$), 41.4 (3′-$N(CH_3)_2$), 64.6 and 64.7 (2 CH_2), 67.0 (C-3′), 69.6 (C-4′), 70.7 (C-3 and C-2), 73.0 (C-5′), 81.6 (C-5), 102.5 (C-1), 104.0 (C-20), 169.3 and 170.2 (2 $OCOCH_3$), 173.7 (C-1).

MS (LSMIS): m/z 900 (M^+ + H).

HRMS (FAB) calc for $C_{45}H_{74}NO_{17}$, (M^+ + H): 900.4957. Found: 900.4986.

• *2′,4″-Di-*O*-acetyl-4′-*O*-demycarosyl-3′-*N*-oxide-tylosin ethylene acetal* **6**

To a solution of **5** (0.95 g, 1.06 mmol, 1 equiv) in CH_2Cl_2 (60 mL) was added rapidly 3-chloroperoxybenzoic acid (0.4 g, 1.27 mmol, 1.2 equiv) and the mixture was stirred for 15 min at room temperature. Dilution with a cold saturated solution of $NaHCO_3$ and the usual work-up furnished a residue which was purified by flash chromatography (CH_2Cl_2/CH_3OH/NH_4OH 10:1:0.05) to give pure **6** (0.71 g, 72%) as a foam.

Rf = 0.22 with CH_2Cl_2/CH_3OH/NH_4OH 10:1:0.05.

$[\alpha]_D = -1$ (c = 1; $CHCl_3$).

^{1}H NMR ($CDCl_3$, 250 MHz): δ 1.75 (s, 3H, H-22), 2.10 and 2.14 (2s, 2 × 3H, 2 $OCOCH_3$), 3.05 (dd, 1H, $J_{1'',2''}$ = 7.9 Hz, $J_{2'',3''}$ = 2.4 Hz, H-2″), 3.22 and 3.28 (2s, 2 × 3H, 3′-$N(O)(CH_3)_2$), 3.48 (s, 3H, 2″-OCH_3), 3.53 (s, 3H, 3″-OCH_3), 4.39 (dd, 1H, $J_{3'',4''}$ = 2.1 Hz, $J_{4'',5''}$ = 9.8 Hz, H-4″), 4.41 (d, 1H, $J_{1',2'}$ = 7.2 Hz, H-1′), 4.58 (d, 1H, H-1″), 4.88–4.95 (m, 2H, H-15 and H-20), 4.97 (dd, 1H, $J_{2',3'}$ = 10.3 Hz, H-2′), 5.88 (d, 1H, $J_{13,14}$ = 9.9 Hz, H-13), 6.26 (d, 1H, $J_{10,11}$ = 15.1 Hz, H-10), 7.31 (d, 1H, H-11).

^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 17.1 (5′-CH_3), 20.7 and 21.0 (2 $OCOCH_3$), 53.2 (3′-$N(O)CH_3$), 62.4 (3′-$N(O)CH_3$), 64.2 and 64.3 (2 CH_2), 67.2 (C-3), 70.2 (C-2′), 72.1 (C-4′), 72.4 (C-5′), 79.1 (C-3′), 80.6 (C-5), 100.6 (C-1′), 103.4 (C-20), 169.1 and 169.7 (2 $OCOCH_3$), 173.3 (C-1).

MS (LSMIS): m/z 916 (M^+ + H).

HRMS (FAB) calc for $C_{45}H_{74}NO_{18}$, (M^+ + H): 916.4907. Found: 916.4907.

• *2′,4″-Di-*O*-acetyl-3-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-4′-*O*-demycarosyl-3′-*N*-oxide-tylosin ethylene acetal* **7**

To a solution of **6** (0.68 g, 0.74 mmol, 1 equiv) and phenyl 2,3,4-tri-*O*-acetyl-1-thio-α-L-rhamnopyranoside (0.34 g, 0.89 mmol, 1.2 equiv) in degassed CH_2Cl_2 (27 mL) was added activated molecular sieve 4 Å (1.5 g) and the mixture was stirred for 10 min at room temperature and, then, cooled to −30°C. Freshly crystallized *N*-iodosuccinimide (0.2 g, 0.89 mmol, 1.2 equiv) was added to the mixture, followed by an immediate dropwise addition of a 0.15 M solution of trifluoromethanesulfonic acid in CH_2Cl_2 (3.45 mL, 0.7 equiv). After about 15 min of stirring, the mixture was diluted in CH_2Cl_2, filtered and the filtrate washed with a 10% aqueous $Na_2S_2O_3$. After the usual work-up, the residue was purified by flash chromatography (CH_2Cl_2/CH_3OH/NH_4OH 15:1:0.05) to give pure **7** (0.53 g, 60%) as a foam.

Rf = 0.36 with CH_2Cl_2/CH_3OH/NH_4OH 10:1:0.05.

$[\alpha]_D = -18$ (c = 1, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.22 (d, 3H, $J_{5''',6'''}$ = 5.3 Hz, H-6‴), 1.77 (s, 3H, H-22), 1.97, 2.08, 2.12 and 2.07 (4s, 5 × 3H, 5 $OCOCH_3$), 3.26 (s, 2 × 3H, 3′-$N(CH_3)_2$), 3.44 (s, 3H, 2″-OCH_3), 3.53 (s, 3H, 3″-OCH_3), 4.41–4.46 (m, 2H, H-1′ and H-4″), 4.60 (d, 1H, $J_{1'',2''}$ = 7.9 Hz, H-1″), 4.92 (bs, 1H, H-1‴), 4.97–5.04 (m, 3H, H-15, H-20 and H-2′), 5.13 (t, 1H, $J_{3''',4'''} = J_{4''',5'''}$ = 10.0 Hz, H-4‴), 5.22 (dd, 1H, $J_{2''',3'''}$ = 3.2 Hz, H-3‴), 5.30 (bd, 1H, H-2‴), 5.86 (d, 1H, $J_{13,14}$ = 10.4 Hz, H-13), 6.20 (d, 1H, $J_{10,11}$ = 15.4 Hz, H-10), 7.25 (d, 1H, H-11).

^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 17.4 (5‴-CH_3), 17.6 (5′-CH_3), 20.6, 20.7 and 21.2 (5 $OCOCH_3$), 53.1 (3′-$N(O)CH_3$), 62.4 (3′-$N(O)CH_3$), 63.3 and 63.4 (2 CH_2), 67.4, 68.9 and 70.1 (C-2‴, C-3‴, C-4‴ and C-5‴), 70.7 (C-2′), 72.1 (C-4′), 72.3 (C-5′), 75.2 (C-3), 78.7 (C-5), 79.0 (C-3′), 97.3 (C-1‴), 100.0 (C-1′), 102.7 (C-20), 169.1, 169.6, 169.8 and 169.9 (5 $OCOCH_3$), 170.9 (C-1).

MS (FAB): m/z 1 189 (M^+ + H).

HRMS (FAB) calc for $C_{57}H_{90}NO_{25}$, (M^+ + H): 1188.5801. Found: 1188.5797.

• *2′,4″-Di-*O*-acetyl-3-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-4′-*O*-demycarosyltylosin ethylene acetal* **8**

To a solution of **7** (0.5 g, 0.42 mmol, 1 equiv) in CH_2Cl_2 (40 mL) was added triphenylphosphine (1.1 g, 4.2 mmol, 10 equiv) and the mixture was stirred for 3 days at room temperature. After the usual work-up, the residue was purified by flash chromatography (CH_2Cl_2/CH_3OH/NH_4OH 20:1:0.05) to give pure **8** (0.39 g, 80%) as a foam.

Rf = 0.47 with CH_2Cl_2/CH_3OH/NH_4OH 10:1:0.05.

$[\alpha]_D = -22$ (c = 0.7, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.20 (d, 3H, $J_{5''',6'''}$ = 6.2 Hz, H-6‴), 1.78 (s, 3H, H-22), 1.97, 2.06, 2.11 and 2.13 (4s, 5 × 3H, 5 $OCOCH_3$), 2.42 (s, 2 × 3H, 3′-$N(CH_3)_2$), 3.44 (s, 3H, 2″-OCH_3), 3.51 (s, 3H, 3″-OCH_3), 4.35 (d, 1H, $J_{1',2'}$ = 7.5 Hz, H-1′), 4.42 (dd, 1H, $J_{3'',4''}$ = 2.5 Hz, $J_{4'',5''}$ = 9.9 Hz, H-4″), 4.60 (d, 1H, $J_{1'',2''}$ = 8.0 Hz, H-1″), 4.88–5.06 (m, 5H, H-15, H-20, H-2′, H-1‴ and H-4‴), 5.16 (dd, 1H, $J_{1''',2'''}$ = 1.2 Hz, $J_{2''',3'''}$ = 3.3 Hz, H-2‴), 5.19 (dd, 1H, $J_{3''',4'''}$ = 9.9 Hz, H-3‴), 5.84 (d, 1H, $J_{13,14}$ = 10.6 Hz, H-13), 6.20 (d, 1H, $J_{10,11}$ = 15.5 Hz, H-10), 7.28 (d, 1H, H-11).

^{13}C NMR ($CDCl_3$, 75 MHz): δ 17.2 (5‴-CH_3), 17.4 (5′-CH_3), 20.5 and 21.3 (5 $OCOCH_3$), 41.0 (3′-$N(CH_3)_2$), 64.1 (2 CH_2), 67.1 (C-3′), 67.1, 69.0, 70.1 and 70.3 (C-2‴, C-3‴, C-4‴ and C-5‴), 70.1 (C-4′), 70.9 (C-2′), 72.6 (C-5′), 78.4 (C-5), 76.3 (C-3), 98.3 (C-1‴), 101.3 (C-1′), 102.7 (C-20), 168.8 and 169.6 (5 $OCOCH_3$), 171.0 (C-1).

MS (LSMIS): m/z 1 172 (M^+ + H).

HRMS (FAB) calc for $C_{57}H_{90}NO_{24}$, (M^+ + H): 1172.5853. Found: 1172.5892.

• *4′-*O*-Demycarosyl-3-*O*-(α-L-rhamnopyranosyl) tylosin ethylene acetal* **9**

To a solution of **8** (0.36 g, 0.31 mmol, 1 equiv) in anhydrous CH_3OH (2 mL) was added dropwise a 0.2 M solution of sodium methanolate in CH_3OH (7.75 mL, 1.55 mmol, 5 equiv). After stirring for an additional 15 min at room temperature, the mixture was neutralized with Amberlite IRN 77 (H^+). The resin was filtered off and washed with CH_3OH. The filtrate was concentrated under reduced pressure and the residue purified by flash chromatography (CH_2Cl_2/CH_3OH/NH_4OH 5:1:0.05) to give pure **9** (0.2 g, 67%) as a foam.

Rf = 0.05 with CH_2Cl_2/CH_3OH/NH_4OH 10:1:0.05.

$[\alpha]_D = -22$ (c = 1, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.22 (d, 3H, $J_{5''',6'''}$ = 6.3 Hz, H-6'''), 1.77 (s, 3H, H-22), 2.43 (s, 2 × 3H, 3'-N(CH_3)$_2$), 3.42 (s, 3H, 2''-OCH_3), 3.56 (s, 3H, 3''-OCH_3), 4.24 (d, 1H, $J_{1',2'}$ = 7.2 Hz, H-1'), 4.55 (d, 1H, $J_{1'',2''}$ = 7.5 Hz, H-1''), 4.88 (dt, 1H, $J_{14,15}$ = 1.3 Hz, $J_{15,16}$ = 7.3 Hz, H-15), 4.99 (bs, 1H, H-1'''), 5.12 (bd, 1H, $J_{19ax,20}$ = 6.0 Hz, H-20), 5.86 (d, 1H, $J_{13,14}$ = 10.3 Hz, H-13), 6.21 (d, 1H, $J_{10,11}$ = 15.5 Hz, H-10), 7.34 (d, 1H, H-11).

^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 17.8 (5'-CH_3 and 5'''-CH_3), 41.8 (3'-N(CH_3)$_2$), 64.4 (2 CH_2), 68.8 (C-3'''), 70.1 (C-3'), 70.6 (C-2'), 71.4 (C-5'''), 71.7 (C-4'''), 72.7 (C-4'), 73.2 (C-5'), 76.6 (C-2'''), 79.5 (C-3), 79.7 (C-5), 101.7 (C-1'''), 102.8 (C-20), 103.4 (C-1'), 171.8 (C-1).

MS (LSMIS): m/z 984 (M^+ + Na), 962 (M^+ + H).

HRMS (FAB) calc for $C_{47}H_{80}NO_{19}$, (M^+ + H): 962.5324. Found: 962.5267.

• *4'-O-Demycarosyl-3-O-(α-L-rhamnopyranosyl) tylosin* **10**

A solution of **9** (0.16 g, 0.17 mmol, 1 equiv) in a mixture of THF/HCl 0.1 N (1:1) (10 mL) was stirred at room temperature for 16 h. After neutralization with solid $NaHCO_3$, THF was distilled off under reduced pressure and the concentrate was extracted with ethyl acetate (3×). The organic layer was dried over Na_2SO_4, filtrated and concentrated under reduced pressure to furnish a residue which was further purified by flash chromatography (CH_2Cl_2/CH_3OH/NH_4OH 10:1:0.05) to give pure **10** (0.12 g, 80%) as a foam.

Rf = 0.24 with CH_2Cl_2/CH_3OH/NH_4OH 5:1:0.05.

$[\alpha]_D$ = −26 (c = 1; $CHCl_3$).

^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.79 (s, 3H, H-22), 2.43 (s, 2 × 3H, 3'-N(CH_3)$_2$), 3.02 (dd, 1H, $J_{1'',2''}$ = 7.7 Hz, $J_{2'',3''}$ = 2.5 Hz, H-2''), 3.48 (s, 3H, 2''-OCH_3), 3.60 (s, 3H, 3''-OCH_3), 3.76 (bd, 1H, H-3''), 3.81 (bs, 1H, H-2'''), 3.82–4.03 (m, 4H, H-3, H-5, H-23 and H-3'''), 4.22 (d, 1H, $J_{1',2'}$ = 7.1 Hz, H-1'), 4.56 (d, 1H, H-1''), 4.88 (dt, 1H, $J_{14,15}$ = 1.3 Hz, $J_{15,16}$ = 7.3 Hz, H-15), 5.03 (bs, 1H, H-1'''), 5.93 (d, 1H, $J_{13,14}$ = 10.6 Hz, H-13), 6.22 (d, 1H, $J_{10,11}$ = 15.3 Hz, H-10), 7.33 (d, 1H, H-11), 9.68 (s, 1H, H-20).

^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 9.6 (C-17), 10.2 (C-18), 13.0 (C-2), 17.7 (5'-CH_3 and 5'''-CH_3), 17.6 (5''-CH_3), 17.9 (C-22), 25.4 (C-16), 32.4 (C-7), 33.8 (C-6), 40.4 (C-2), 41.8 (3'-N(CH_3)$_2$), 43.1 (C-4), 44.6 (C-19), 45.0 (C-8), 45.2 (C-14), 59.7 (3''-OCH_3), 61.8 (2''-OCH_3), 69.2 (C-3'''), 69.3 (C-23), 70.3 (C-3'), 70.8 (C-2'), 71.1 (C-5''), 71.5 (C-5'''), 71.7 (C-4'''), 72.8 (C-4'), 73.3 (C-5' and C-4''), 75.3 (C-15), 76.5 (C-2'''), 79.6 (C-3), 79.9 (C-3''), 80.7 (C-5), 82.2 (C-2''), 101.2 (C-1''), 101.4 (C-1'''), 103.3 (C-1'), 118.0 (C-10), 134.5 (C-12), 143.6 (C-13), 148.6 (C-11), 171.7 (C-1), 203.2 (C-9), 203.4 (C-20).

MS (LSMIS): m/z 918 (M^+ + H), 237, 174, 158.

HRMS (FAB) calc for $C_{45}H_{76}NO_{18}$, (M^+ + H): 918.5062. Found: 918.5065.

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