Syntheses and Absolute Structures of the Disaccharide and Aglycone of Acaricidal Gualamycin†

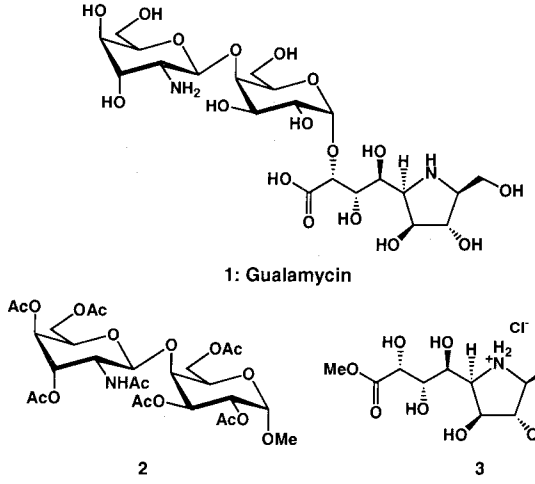
Sir:

Gualamycin is a water-soluble acaricide isolated from the culture broth of *Streptomyces* sp. NK11687[1]. The structure was deduced to be **1** mainly from the spectral and/or X-ray crystallographic analyses of its disaccharide **2** and aglycone portions **3**[2]. The absolute structure of the disaccharide, however, remained undetermined.

Herein, we confirm the absolute structure **1** by the enantiospecific syntheses of the disaccharide **2** and aglycone derivatives **3**.

When considered the molecular rotation ($[\alpha]_D$ +276°)[1] of the acetylated disaccharide **2** in comparison with those of acetylated methyl 2-amino-2-deoxy-β-D- and L-gulopyranosides[3] and methyl α-D- and L-galactopyranosides[4] according to Hudson's rule[5], the disaccharide was expected to exclusively consist of two D-sugars without any L-sugars.

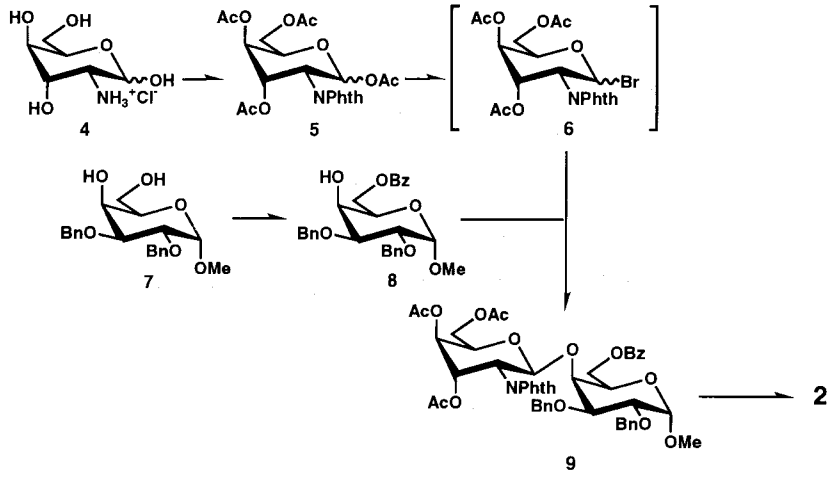
Fig. 1. Structures of gualamycin and its disaccharide and aglycone derivatives.



The starting 2-amino-2-deoxy-D-gulopyranose hydrochloride (**4**) was prepared by the GROSS's procedure[6], and led to the corresponding *O*-acetylated phthalimide **5** in 2 steps (65%): 1) phthalic anhydride/MeONa; 2) Ac_2O/DMAP/Py. This was brominated to the labile glycosyl donor **6** by 35% HBr-AcOH in CH_2Cl_2. The glycosyl acceptor **8** (mp 90°C) was prepared in 82% yield by selective benzoylation (BzCl/Py, 20°C, 15 hours) of methyl 2,3-di-*O*-benzyl-α-D-galactopyranoside[7] (**7**). The aforesaid glycosyl donor **6** reacted with **8** in the presence of AgOTf, *s*-collidine and MS 4A to give the corresponding glycoside **9** in 65% yield as shown in Table 1. The glycoside **9** was converted into the acetylated disaccharide **2** in a three-step sequence: 1) removal of the phthalimide ($MeNH_2$/EtOH); 2) hydrogenolysis (H_2/10% Pd-C/EtOH/AcOH); 3) acetylation (Ac_2O/Py). This was identical with a naturally derived disaccharide **2** in all respects[2], indicating expectedly that the sugar moiety of gualamycin (**1**) is a 4-*O*-(2-amino-2-deoxy-β-D-gulopyranosyl)-α-D-galactopyranoside.

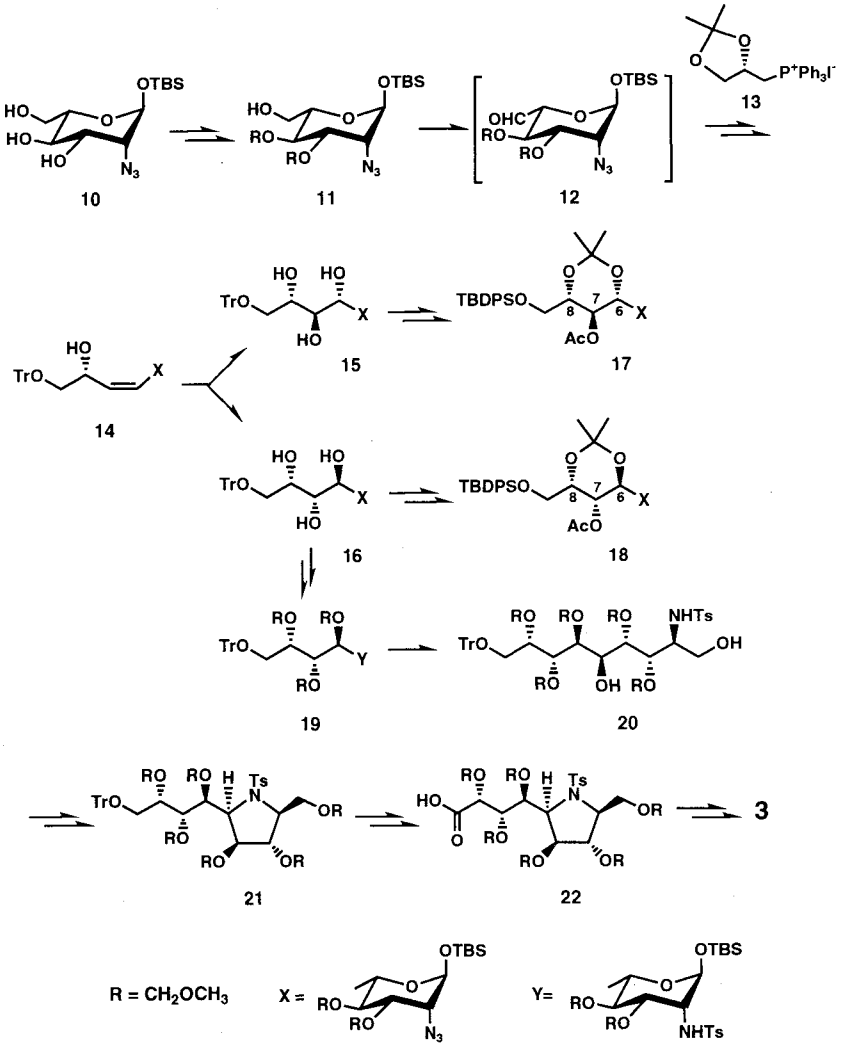
On the second stage, the pyrrolidine-containing aglycone unit **3** was synthesized from the azido sugar **10**, which was prepared by de-*O*-acetylation (MeONa/MeOH) of *t*-butyldimethylsilyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-L-mannopyranoside[8]. The azide **10** was selectively silylated (TBDMSCl/Py, 20°C, 8 hours) and methoxymethylated (MOMCl/DIPEA/CH_2Cl_2) to the fully protected product in 90% yield, which was selectively desilylated (80% aq AcOH, 40°C, 2 hours) to give the alcohol **11** in 90% yield. This was oxidized [$(COCl)_2$/DMSO/Et_3N/CH_2Cl_2, −78°C, 1 hour] to the labile aldehyde **12**, which was treated with the Wittig reagent, (4*S*)-(2,2-dimethyl-1,3-dioxolan-4-yl)methyl-triphenylphosphonium iodide (**13**)[9] (*n*-BuLi/HMPA/THF, −60°C→−10°C, 2 hours), successively followed by removal of the isopropylidene group with 80% AcOH

Scheme 1. Synthesis of the disaccharide **2**.



† Dedicated to Professor SATOSHI ŌMURA on his 60th birthday.

Scheme 2. Synthesis of the aglycone 3.



(40°C, 1.5 hours) and tritylation (TrCl/DMAP/Et_3N/DMF) of the resulting primary alcohol to yield the *cis* olefin **14** in 67% yield. The *cis* dihydroxylation of **14** by OsO_4 (aq CH_3CN, 20°C, 15 hours) gave two triols **15** and **16** (mp 169.5°C) in 46% and 34% yields, respectively. Their configurations were determined by the ^1H NMR studies (Table 1) of their corresponding isopropylidene derivatives **17** and **18**, which were derived in **4** steps: 1) 80% AcOH, 40°C, 1 hour; 2) TBDPSCl/Et_3N/DMF. 15 hours; 3) 2,2-dimethoxypropane/CSA; 4) Ac_2O/DMAP/Py. The coupling constants ($J_{6,7}$ and $J_{7,8}=10$ Hz for **17**, and $J_{6,7}=6.3$ Hz and $J_{7,8}=3.5$ Hz for **18**) showed that **16** was the desired triol for the natural aglycone **3**. Although the dihydroxylation of **14** was assayed by using a variety of conditions[10], the triol **16** could not be obtained as a major product. The triol **16** was methoxymethylated (MOMCl/DIPEA/CH_2ClCH_2Cl, 60°C, 15 hours), followed by successive treatment with Ph_3P in PhMe (100°C, 3 hours) and with 70% aq THF in refluxing to give the amino compound[11], which was tosylated (TsCl/Py) to the product **19** (mp 79°C) in 81% yield. Desilylation (*n*-Bu_4NF/AcOH/THF) and hydride reduction ($NaBH_4$/EtOH) of **19** gave the alcohol **20** in 80% yield. This was selectively methoxymethylated (MOMCl/ DIPEA/CH_2Cl_2, 20°C, 2 days) and then submitted to the S_N2-type cyclization using Ph_3P and DEAD in THF at 20°C for 1 hour to give the pyrrolidine derivative **21** in 79% yield. After detritylation (H_2/10% Pd-C/EtOH), the forming alcohol was oxidized stepwise (1) $(COCl)_2$/DMSO/Et_3N/CH_2Cl_2. −78°C, 1 hour; 2) $NaClO_2$/ H_2NSO_3H/aq *t*-BuOH, 20°C, 10 minutes) to give the carboxylic acid **22** in 82% yield. De-*N*-tosylation (Li/liq. NH_3, −30°C, 10 minutes) followed by esterification (5% HCl-MeOH, 50°C, 2 days) gave the hydrochloride of the pyrrolidine **3** (mp 182°C (dec.)) in 74% yield, which was identical with the naturally derived sample[2] in all respects (Table 1).

The completion of these syntheses supported that gualamycin (**1**) is (2*R*,3*S*,4*S*)-2-*O*-[4-*O*-(2-amino-2-deoxy-β-D-gulopyranosyl)-α-D-galactopyranosyl]-2,3,4-trihydroxy-4-[(2*S*,3*S*,4*S*,5*S*)-3,4-dihydroxy-5-hydroxymethylpyrrolidin-2-yl]butanoic acid[2].

Table 1. Physico-chemical properties of **2** and **3**, and their synthetic intermediates.

No.	FAB-MS (*m/z*)	$[\alpha]_D$	^{1}H NMR (ppm)
2	650 $(M+H)^+$	+40.0° (*c* 0.45, MeOH)	400 MHz ($CDCl_3$): δ 2.04, 2.06, 2.08, 2.08, 2.10, 2.12, 2.18 (21H, 7s), 3.42 (3H, s), 4.1 (2H, m) 4.2 (6H, m), 4.70 (1H, d, J=8.8 Hz), 4.85 (1H, dd, J=11, 2.9 Hz), 4.98 (1H, d, J=3.3 Hz), 5.35 (1H, dd, J=11, 2.9 Hz), 5.39 (1H, dd, J=11, 2.2 Hz), 5.50 (1H, d, J=3.3 Hz)
3	282 $(M+H)^+$	+51.0° (*c* 0.5, MeOH)	400 MHz (D_2O): δ 3.70 (1H, m), 3.82 (3H, s), 3.9 (2H, m), 3.98 (1H, dd, J=11.5, 5.7 Hz), 4.1 (2H, m), 4.21 (1H, dd, J=9.8, 8.2 Hz), 4.43 (1H, d, J=2.9 Hz), 4.65 (1H, d, J=1.5 Hz)
5	478 $(M+H)^+$	α, β anomers	[α-anomer] 500 MHz (C_6D_6): δ 1.64, 1.89, 1.91 (9H, 3s), 4.28 (1H, dd, J=12, 7 Hz), 4.38 (1H, dd, J=12, 6.5 Hz), 4.95 (1H, ddd, J=7, 6.5, 2 Hz), 5.30 (1H, dd, J=4, 2 Hz), 5.42 (1H, t, J=3.5 Hz), 5.77 (1H, ddd, J=1, 3.5, 4 Hz), 6.68 (1H, dd, J=3.5, 1 Hz), 6.8~7.3 (4H, m) [β-anomer] 500 MHz (C_6D_6): δ 1.42, 1.57, 1.66 (9H, 3s), 4.24 (1H, dd, J=11, 7 Hz), 4.37 (1H, dd, J=11, 6 Hz), 4.54 (1H, ddd, J=7, 6, 2 Hz), 5.13 (1H, dd, J=3.5, 2 Hz), 5.17 (1H, dd, J=9, 2.5 Hz), 5.82 (1H, dd, J=3.5, 2.5 Hz), 6.8~7.3 (4H, m), 7.57 (1H, d, J=9 Hz)
8	479 $(M+H)^+$	+22.5° (*c* 0.5, $CHCl_3$)	400 MHz ($CDCl_3$): δ 2.51 (1H, bs), 3.37 (3H, s), 3.86 (1H, dd, J=8.9, 3.2 Hz), 3.91 (1H, dd, J=8.9, 3.0 Hz), 4.05 (2H, m), 4.49 (1H, dd, J=12, 7.9 Hz), 4.56 (1H, dd, J=12, 5.0 Hz), 4.68 (1H, d, J=3.0 Hz), 4.69 (2H, dd, J=17, 12 Hz), 4.83 (2H, dd, J=12, 3.0 Hz), 7.2~8.1 (15H, m)
9	896 $(M+H)^+$	+8.6° (*c* 0.28, $CHCl_3$)	270 MHz ($CDCl_3$): δ 2.02, 2.12, 2.21 (9H, 3s), 3.22 (3H, s), 3.51 (1H, dd, J=10, 3.4 Hz), 3.71 (1H, dd, J=10, 2.7 Hz), 3.90 (1H, dd, J=9.2, 4.6 Hz), 4.04 (1H, d, J=2.7 Hz), 3.94~4.34 (5H, m), 4.40 (1H, dd, J=12, 9.2 Hz), 4.41 (1H, d, J=3.4 Hz), 4.43 (1H, d, J=14 Hz), 4.55 (1H, dd, J=12, 4.6 Hz), 4.58 (1H, d, J=14 Hz), 4.63 (1H, dd, J=9.2, 3.5 Hz), 4.91 (1H, dd, J=3.5, 1.1 Hz), 5.34 (1H, t, J=3.5 Hz), 6.04 (1H, d, J=9.2, 7.1~8.1 (19H, m)
11	408 $(M+H)^+$	+16.3° (*c* 1.1, $CHCl_3$)	90 MHz ($CDCl_3$): δ 0.01 (2H, 2s), 0.76 (9H, s), 2.27 (1H, bs), 3.20 (1H, m), 3.27 (3H, s), 3.33 (3H, s), 3.5~3.9 (5H, m), 4.57 (1H, d, J=6 Hz), 4.67 (2H, s), 4.73 (1H, d, J=6 Hz), 4.84 (1H, s)
14	728 $(M+Na)^+$	+10.7° (*c* 2.2, $CHCl_3$)	400 MHz ($CDCl_3$): δ 0.11, 0.13 (6H, 2s), 0.90 (9H, s), 2.67 (1H, bs), 3.12 (4H, m), 3.25 (1H, dd, J=9.6, 4.3 Hz), 3.44 (3H, s), 3.55 (2H, m), 3.91 (1H, d, J=3.8 Hz), 3.96 (1H, t, J=9.6 Hz), 4.36 (1H, d, J=6.7 Hz), 4.54 (2H, m), 4.75 (2H, s), 4.87 (s, 1H), 5.57 (1H, dd, J=12, 9.6 Hz), 5.67 (1H, dd, J=12, 8.6 Hz), 7.2~7.5 (15H, m)
15	762 $(M+Na)^+$	+14.9° (*c* 0.6, $CHCl_3$	270 MHz ($CDCl_3$): δ 0.13, 0.15 (6H, 2s), 0.90 (9H, s), 3.00 (1H, d, J=4.5 Hz), 3.05 (1H, d, J=4.5 Hz), 3.33 (3H, s), 3.40 (2H, m), 3.45 (3H, s), 3.51 (1H, m), 3.54 (1H, dd, J=10, 2.5 Hz), 3.61 (1H, dd, J=8.8, 3.2 Hz), 3.93 (5H, m), 4.72 (1H, d, J=5.7 Hz), 4.77 (2H, s), 4.81 (1H, d, J=5.7 Hz), 4.88 (1H, d, J=0.9 Hz), 7.2~7.5 (15H, m)
16	762 $(M+Na)^+$	+3.9° (*c* 0.9, $CHCl_3$)	270 MHz ($CDCl_3$): δ 0.10 (6H, s), 0.88 (9H, s), 2.70 (1H, d, J=4.1 Hz), 2.79 (1H, d, J=3.9 Hz), 2.82 (1H, d, J=6.0 Hz), 3.26 (1H, dd, J=6.6, 3.7 Hz), 3.38, 3.46 (6H, 2s), 3.50 (1H, dd, J=6.6, 2.9 Hz), 3.55 (1H, dd, J=6.6, 1.2 Hz), 3.68 (1H, dd, J=5.8, 2.9 Hz), 3.74 (1H, ddd, J=5.8, 4.2, 1.2 Hz), 3.82 (1H, dd, J=13.9, 5.8 Hz), 3.86~3.95 (2H, m), 4.13 (1H, m), 4.65 (1H, d, J=4.4 Hz), 4.78 (2H, d, J=1.5 Hz), 4.83 (1H, d, J=4.4 Hz), 4.92 (1H, d, J=1.2 Hz), 7.2~7.5 (15H, m)
17	840 $(M+Na)^+$		500 MHz ($CDCl_3$): δ 0.12, 0.17 (6H, 2s), 0.90, 1.04 (18H, 2s), 1.41, 1.47 (6H, 2s), 1.96 (3H, s), 3.18 (1H, d, J=9.2) 3.38, 3.44 (6H, 2s), 3.49 (1H, dd, J=9.2, 4.6 Hz), 3.66 (1H, t, J=9.2 Hz), 3.68 (2H, m), 3.79 (1H, ddd, J=10, 6.9, 3.5 Hz), 3.90 (1H, dd, J=4.6, 1.2 Hz), 4.15 (1H, d, J=10 Hz), 4.76 (4H, m), 4.82 (1H, d, J=1.2 Hz), 4.98 (1H, t, J=10 Hz), 7.4 (6H, m), 7.8 (4H, m)
18	818 $(M+H)^+$ 840 $(M+Na)^+$		400 MHz ($CDCl_3$): δ 0.20, 0.23 (6H, 2s), 0.93, 1.01 (18H, 2s), 1.33, 1.36 (6H, 2s), 1.97 (3H, s), 3.37, 3.47 (6H, 2s), 3.42 (1H, dd, J=9.2, 2.1 Hz), 3.60 (1H, dd, J=9.2, 4.0 Hz), 3.69 (1H, dd, J=10, 6.3 Hz), 3.76 (1H, dd, J=10, 8.1 Hz), 3.80 (1H, t, J=9.2 Hz), 3.82 (1H, dd, J=6.3, 2.1 Hz), 3.92 (1H, dd, J=4.0, 1.2 Hz), 4.10 (1H, ddd, J=8.1, 6.3, 3.5 Hz), 4.73 (2H, s), 4.79 (2H, m), 4.96 (1H, d, J=1.2 Hz), 5.52 (1H, dd, J=6.3, 3.5 Hz), 7.3~7.7 (10H, m)
19	1000 $(M+H)^+$	−1.9° (*c* 0.8, $CHCl_3$)	270 MHz ($CDCl_3$): δ 0.03, 0.06 (6H, 2s), 0.80 (9H, s), 2.38 (H, s), 3.20 (1H, m), 3.24 (6H, 2s), 3.31, 3.33, 3.50 (9H, 3s), 3.54 (1H, dd, J=11, 5.4 Hz), 3.63 (2H, m), 3.89 (3H, m), 4.02 (2H, s), 4.34 (1H, d, J=6.8 Hz), 4.42 (1H, d, J=6.8 Hz), 4.53 (1H, d, J=6.8 Hz), 4.69 (6H, m), 4.91 (2H, t, J=6.8 Hz), 6.11 (1H, d, J=9.5 Hz), 7.2~7.5 (17H, m), 7.80 (2H, d, J=9.0 Hz)
20	888 $(M+H)^+$ 910 $(M+Na)^+$	−37.7° (*c* 1.3, $CHCl_3$)	400 MHz ($CDCl_3$): δ 2.40 (3H, s), 3.00 (1H, m), 3.24 (1H, dd, J=10, 5.7 Hz), 3.31 (9H, 3s), 3.35 (3H, s), 3.40 (1H, m), 3.47 (3H, s), 3.58 (1H, d, J=5.7 Hz), 3.70 (3H, m), 3.79 (1H, dd, J=9.1, 5.7 Hz), 3.90 (1H, dd, J=7.9, 1.8 Hz), 4.00 (1H, m), 4.05 (1H, dd, J=9.1, 1.8 Hz), 4.09 (1H, d, J=5.7 Hz), 4.15 (1H, dd, J=5.7, 4.6 Hz), 4.56~4.86 (10H, m), 5.56 (1H, d, J=9.1 Hz), 7.2~7.5 (17H, m), 7.78 (2H, d, J=8.2 Hz)
21	936 $(M+Na)^+$	−4.8° (*c* 0.7, $CHCl_3$)	270 MHz ($CDCl_3$: δ 2.38 (3H, s), 3.00 (1H, m), 3.09, 3.15, 3.30, 3.32, 3.39, 3.45 (18H, 6s), 3.56 (1H, dd, J=10, 5.7 Hz), 3.75 (1H, m), 3.86 (1H, dd, J=9.5, 4.8 Hz), 4.0~4.3 (7H, m), 4.3~5.0 (12H, m), 7.2~7.4 (17H, m), 7.73 (2H, d, J=9.1 Hz)
22	686 $(M+H)^+$ 684 $(M-H)^-$	+5.1° (*c* 1.2, $CHCl_3$)	270 MHz ($CDCl_3$): δ 2.43 (3H, s), 3.22 (1H, m), 3.14, 3.31, 3.39, 3.40, 3.43, 3.55 (18H, 6s), 3.70 (1H, m), 3.81 (1H, dd, J=10, 5.5 Hz), 3.94 (1H, t, J=9.2 Hz), 4.05 (1H, dd, J=6.4, 3.7 Hz), 4.12 (2H, m), 4.26 (1H, d, J=6.4 Hz), 4.39 (1H, dd, J=9.2, 1.4 Hz), 4.5~4.8 (8H, m), 4.87 (1H, d, J=6.4 Hz), 4.96 (2H, s), 5.04 (1H, d, J=6.4 Hz), 7.27 (2H, d, J=9.2 Hz), 7.75 (2H, d, J=9.2 Hz)

Acknowledgment

We are grateful to Shikoku Chemical Co. and Yamanouchi Pharmaceutical Co., Ltd. for the generous support of our program.

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(Received February 24, 1995)

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