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TOTAL SYNTHESIS OF THE DISACCHARIDE OF BLEOMYCIN, 2-O-(α-D-MANNOPYRANOSYL)-L-GULOPYRANOSE

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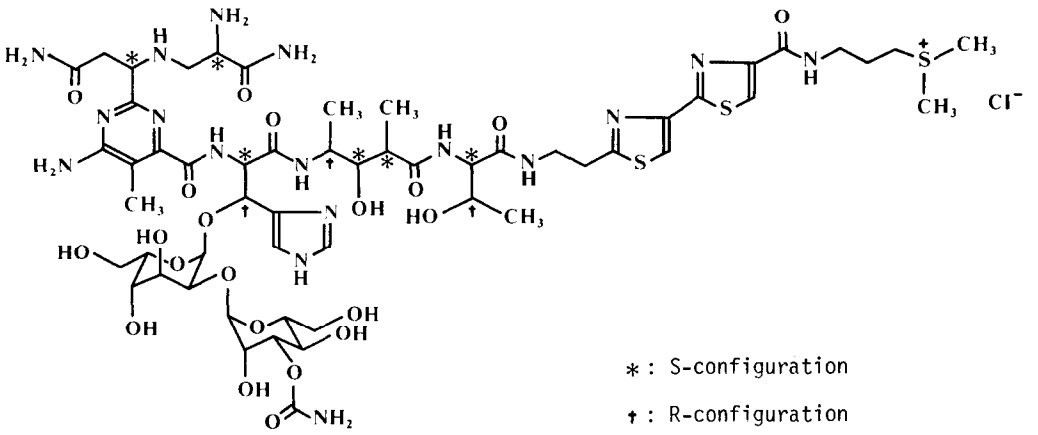
Summary: 2-O-(α-D-Mannopyranosyl)-L-gulopyranose, the sugar portion of bleomycin has been synthesized.

Bleomycin (BLM) is an antitumor antibiotic clinically used in the treatment of several types of cancer.[1] The definitive structure has been established[2,3] as shown below.



* : S-configuration

† : R-configuration

Total synthesis of BLM attracted attention and the peptide part of BLM has recently been synthesized by Takita et al.[4] This paper describes the first synthesis of the disaccharide part of BLM, namely 2-O-(α-D-mannopyranosyl)-L-gulose[5] (9).

As for a synthetic block for total synthesis of BLM, L-gulose[6] and its 3,4-di-O-benzyl derivatives[7] could be the candidates. However, these compounds seem not suitable because the

introduction of the mannose portion into their 2-hydroxyl groups is extremely difficult. We chose, instead, to prepare 2-O-(D-mannosyl)-L-gulose (9) as a suitable precursor for total synthesis of BLM.

The characteristic points in the disaccharide synthesis are the early-stage condensation of D-mannose to the 5-OH group of a D-glucofuranose derivative, and successive head-to-tail inversion of the D-glucofuranose moiety, the C-5 of the D-glucose moiety being converted to C-2 in the new-born L-gulose moiety as a result of the inversion, thus completing the synthesis of 9.

Readily preparable 3-O-acetyl-1,2-O-isopropylidene-6-O-tosyl-D-glucofuranose[8] (1) was treated with sodium azide in N,N-dimethylformamide to give the corresponding 6-azido derivative (2) (syrup, 71%), $[\alpha]_D^{25}$ - 6° (c 1, chloroform), i.r. 2100 cm^{-1} (N_3). This compound is apt to suffer 3-O- to 5-O-acetyl migration to give 5-O-acetyl isomer (2'), during the reaction. However, condensation of 2 with 2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl bromide[9] (4) in CH_2Cl_2 in the presence of $Hg(CN)_2$ gave 3-O-acetyl-6-azido-6-deoxy-1,2-O-isopropylidene-5-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-D-glucofuranose (5) (52%, after chromatography), $[\alpha]_D^{25}$ - 36° (c 1, chloroform); Found (Calcd): C, 48.80 (48.62), H, 5.66 (5.71), N, 6.48 (6.81). The possibility of condensation between 2' and 4 was negligible, because 5 retains, in its ^{1}H-NMR spectrum (in $CDCl_3$), almost identical δ and J values with those of 2 in respect to $(CH_3)_2C$, (δ 1.34, 1.58), 3-O-Ac (2.17), H-1 (5.90 d, $J_{1,2}$ 3.5 Hz), H-2 (4.56 d) and H-3 (5.32 d, $J_{3,4}$ 3.0 Hz). Deacetylation of 5 gave 6 (85%) as needles, m.p. 170 - 170.5°, $[\alpha]_D^{25}$ + 34° (c 1, water); ^{1}H-NMR (in D_2O): δ 5.09 (a slightly unresolved s, H-1'), 6.00 (d, $J_{1,2}$ 3.5 Hz, H-1). Carbon-13 NMR spectrum of 6 was shown in Table 1. The assignments were made on the basis of comparison with the shift data of 6-azido-6-deoxy-1,2-O-isopropylidene-D-glucofuranose (3) (deacetylated product of 2) [needles, m.p. 107.5 - 108.5°. $[\alpha]_D^{25}$ - 11° (c 1, water)] and of methyl α-D-mannopyranoside (Me α-D-Man). Down-field shift[10] (7.4 ppm) of C-5 from that position in 3 shows that glycosidation occurred at the carbon. The anomeric configuration (α-D) of the mannoside was verified by the $J_{C1'-H1'}$ value (that of β-D-mannoside is expected[11] to be ca. 160 Hz) and the shift-values (C-1' – C-6'); methyl β-D-mannoside gave different values.[12,13]

Acidic hydrolysis of 6 gave deacetonated product (7), which was reduced with sodium borohydride to give a non-reducing sugar, 6-azido-6-deoxy-5-O-(α-D-mannopyranosyl)-D-glucitol (8) (69% from 6 after purification), $[\alpha]_D^{24}$ + 24° (c 0.9, water); i.r. 2100 cm^{-1}; ^{1}H-NMR (in D_2O): δ 5.07 (1 H d, $J_{1',2'}$ 1.5 Hz, H-1').

Conversion of the $-CH_2N_3$ group of 8 to aldehyde group was successfully carried out by photolysis, which was first applied to carbohydrates by Horton et al.[15] A 2.5% aqueous solution of 8 was, after bubbling nitrogen, irradiated (3000Å, 5.5 h) at room temperature, and, after addition of Dowex 50W x8 resin (H^+ form), the mixture was stirred for 1 h. During the reaction, intermediary imine[15] was converted to an aldehyde and the latter condensed with the hydroxyl group at C-5 (former C-2 of glucofuranose) to form L-gulopyranose. The crude product obtained after evaporation was purified by silica gel column-chromatography (Wakogel C-200, 2:1:1 butanol-acetic acid-water) to give 9 as a hygroscopic solid (48%) $[\alpha]_D^{25}$ + 96° (c 1, water) (final value); Found (Calcd as hemihydrate): C, 41.01 (41.02), H, 6.46 (6.59).

Acetylation of 9 with acetic anhydride in pyridine gave 1,3,4,6-tetra-O-acetyl-2-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-β-L-gulopyranose (10) (82%), $[\alpha]_D^{25}$ + 36° (c 0.5, chloroform); Found (Calcd); C, 49.30 (49.56), H, 5.69 (5.64). The ^{1}H-NMR data (Table 2) indicates the conformation as shown in 10. The i.r. and the ^{1}H-NMR spectra of the compound were identical with those of the compound obtained by acetylation of natural decarbamoyl disaccharide.[5] We are now challenging the total synthesis of bleomycin by utilizing the synthesized disaccharide (9).

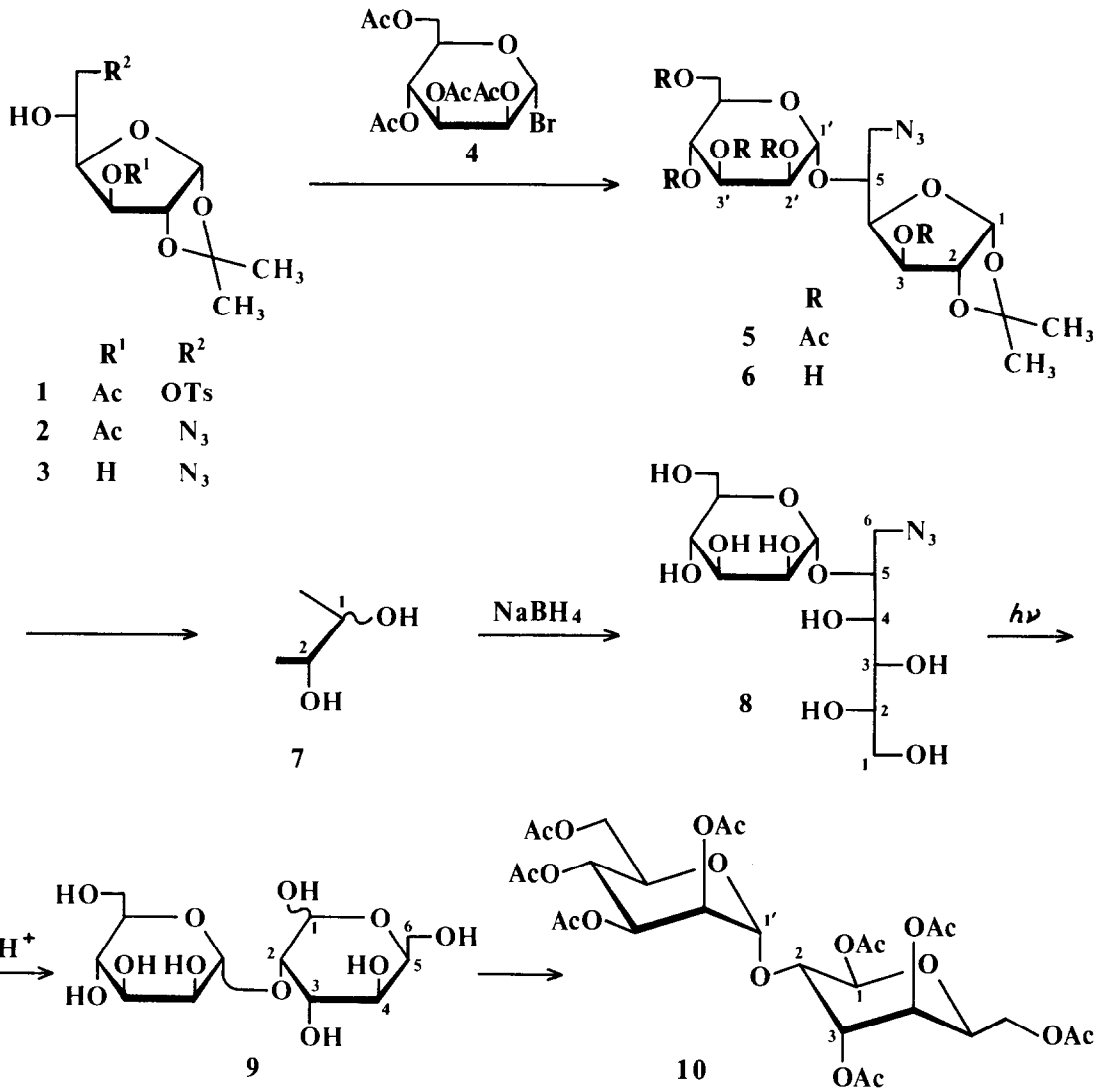


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Table 1. ^{13}C Chemical shift data[a] (62.9 MHz in D_2O) of 6

	6	3[b]
C-1	105.5	105.4
	($^1J_{C,H}$ 186.0 Hz)	($^1J_{C,H}$ 185.9 Hz)
-2	85.4	85.2
-3	74.4[c]	74.1
-4	79.5	80.9
-5	75.3	67.9
-6	53.4	55.0
CH_3	26.0, 26.5	26.0, 26.4
$\underline{C}(CH_3)_2$	113.6	113.5
		Me α-D-Man[d]
C-1'	102.1	101.6
	($^1J_{C,H}$ 171.3 Hz)	($^1J_{C,H}$ 170.7 Hz)
-2'	71.1	70.7
-3'	71.1	71.4
-4'	67.4	67.5
-5'	74.0[c]	73.3
-6'	61.8	61.7
OCH_3		55.5

a: Ppm downfield from TMS calculated as $\delta^{TMS} = \delta^{dioxane} + 67.4$. b: Shift assignments were made based on the data of 1,2-O-isopropylidene-D-glucofuranose.[14] c: The values of C-3 and C-5' may be interchangeable. d: Measured in D_2O; the shift-values were almost identical to those reported.[13,14]

Table 2. ^1H-NMR spectrum of 10 (250 MHz in $CDCl_3$)

H-1	5.90 d	$J_{1,2}$	8.5
-2	4.00 dd	$J_{2,3}$	3.5
-3	5.45 t	$J_{3,4}$	3.5
-4	5.02 dd	$J_{4,5}$	1.5
-1'	4.99 d	$J_{1',2'}$	1.8
-2'	5.10 dd	$J_{2',3'}$	3.4
-3'	5.16 dd	$J_{3',4'}$	10.1
-4'	5.28 t		

a: Assignments were made by decoupling technique as well as inspection of the signal patterns.

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