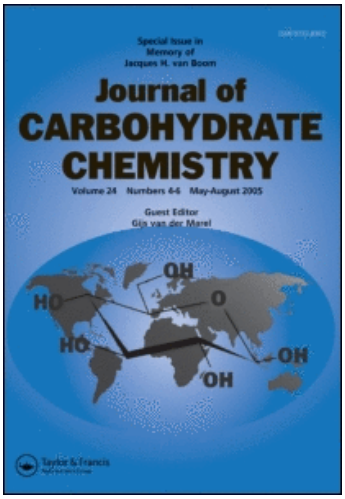

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Assignment of the NMR Parameters of the Branch-Point Trisaccharide of Ahylopectin Using 2-D NMR Spectroscopy at 500 MHz

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ASSIGNMENT OF THE NMR PARAMETERS OF THE BRANCH-POINT TRISACCHARIDE OF AMYLOPECTIN USING 2-D NMR SPECTROSCOPY AT 500 MHz

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ABSTRACT

The proton and carbon nuclear magnetic resonance spectroscopic data for methyl 4-O-α-D-glucopyranosyl-[6-O-α-D-glucopyranosyl]-β-D-glucopyranoside (1), a model for the branch-point trisaccharide of amylopectin, have been analysed using 2-D-heteronuclear correlated spectroscopy. Similar data are presented for the related disaccharide structures methyl β-D-maltopyranoside and β-D-isomaltopyranoside.

INTRODUCTION

The structural analysis of complex carbohydrates has been facilitated during the last decade by the introduction of Fourier transform NMR spectroscopy, particularly the possibility of measuring the natural abundance ^{13}C NMR spectra of small amounts of isolated or synthetic material.[1,2] The appearance of high field spectrometers combined with computer control of the spectrometer functions, which allow the use of 2-dimensional methods among them, correlated experiments, has added a new dimension to the application of NMR methods in structural analysis of complex

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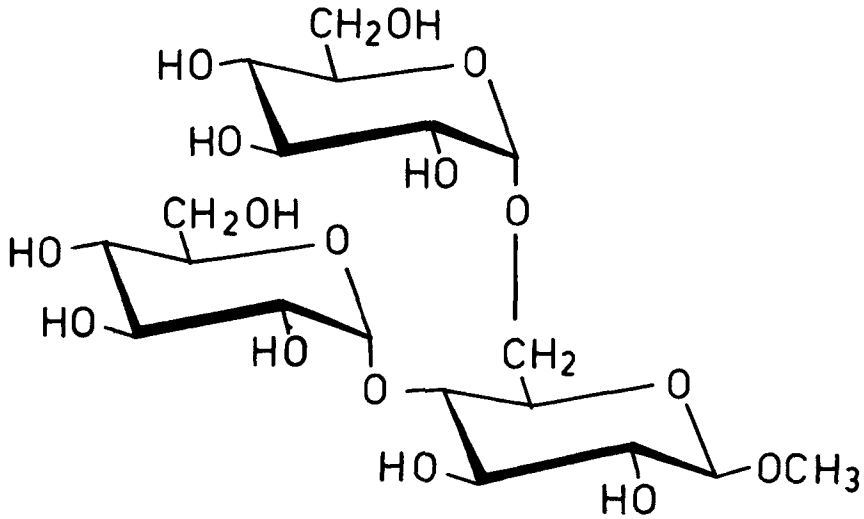
carbohydrates.[3] The present paper describes the use of heteronuclear shift correlation in the analysis of the ^{1}H NMR and ^{13}C NMR parameters of a trisaccharide, methyl 4-O-α-D-glucopyranosyl-[6-O-α-D-glucopyranosyl]-β-D-glucopyranoside (1). This trisaccharide (1) plays an important role in the three dimensional structure of starch because this molecule represents the branch-point in amylopectin.

RESULTS AND DISCUSSION

Compound 1 has been synthesized using halide catalyzed glycosidation[4] with tetra-O-benzyl-α-D-glucopyranosyl bromide as the glycosyl halide and methyl 2,3,2',3',4',6'-hexa-O-acetyl-β-D-maltopyranoside as the aglycone. After deprotection, 1 was isolated in 40% yield. The ^{1}H NMR spectrum of 1 in D_2O at 500 MHz is shown in Fig. 1A and 1B. Most of the data presented in Tables 1 and 2 can be assigned using the chemical shifts for the model



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compounds methyl β-D-maltopyranoside and methyl β-D-isomaltopyranoside and homonuclear decoupling experiments.[5,6] The assignment of the H-3 and H-5 protons with chemical shifts around 3.7-3.8 ppm is ambiguous. The chemical shift of H-3 protons can often be assigned using partially relaxed spectra[7,8] but a better method is to correlate the ^{1}H NMR spectral data with the ^{13}C NMR spectral data using 2-D-heteronuclear shift correlation.[2,3,9] The result of such an experiment performed at 500 MHz for protons and 125 MHz for carbons is shown in Fig. 2. (The carbon chemical shifts are given along the horizontal axis and the proton chemical shifts along the vertical axis.) The appearance of a signal in the 2-dimensional data matrix indicates that the carbon and the proton with these particular chemical shift values are directly bonded to each other. It is, therefore, possible to assign the proton shift data provided the chemical shifts of the carbon atoms have been determined by an independent method[2,3] or vice versa. The results can be visualised more clearly by projections of the 2-dimensional data matrix as shown in Fig. 3 for two different carbon nuclei, where the Fourier transform has been performed in the phase sensitive mode. This projection gives the proton subspectrum for a carbon nucleus; i.e., it is possible to use the carbon nuclei to filter the unwanted overlapping proton signals and analyse only the multiplicity of the proton directly bonded to the carbon atom. This makes it easy to distinguish the H-3 and H-5 protons, the former appearing as triplets with coupling constants of $\sim$ 9.6 Hz, and the latter signals as more complex coupled spin systems. From this correlated experiment it is possible to make the assignments given in Table 1. As may be seen from these results, there are still some ambiguities in the proton and carbon chemical shifts for H-3 to H-6 and C-3 to C-6, for the two almost identical α-D-glucopyranosyl units linked in either the 4- or the 6-position. For most practical purposes these ambiguities are not important for the use of the data in structural assignments but are merely a result of the very high field used which makes it possible to measure experimentally even these very small differences. It would be possible to solve this

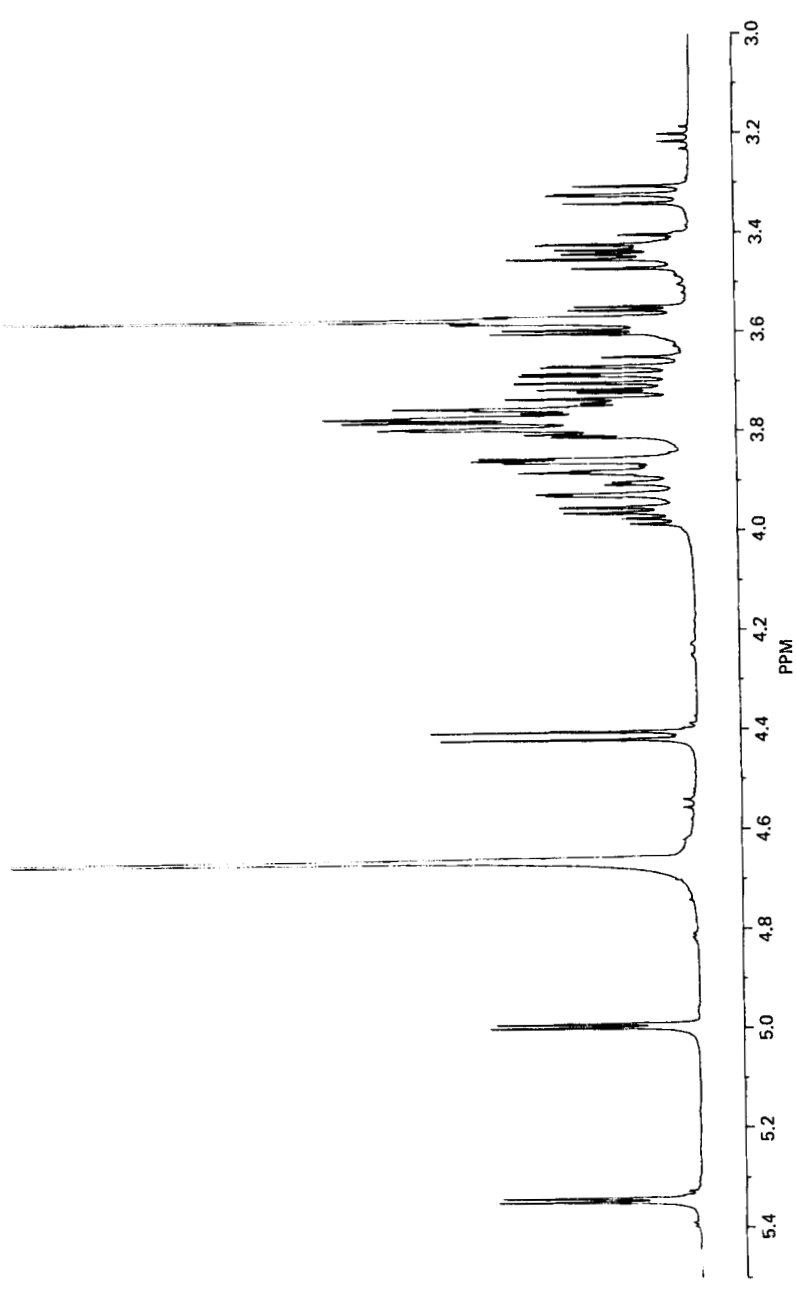

Fig. 1A. 500 MHz ^1H NMR spectrum of 1 in D_2O.

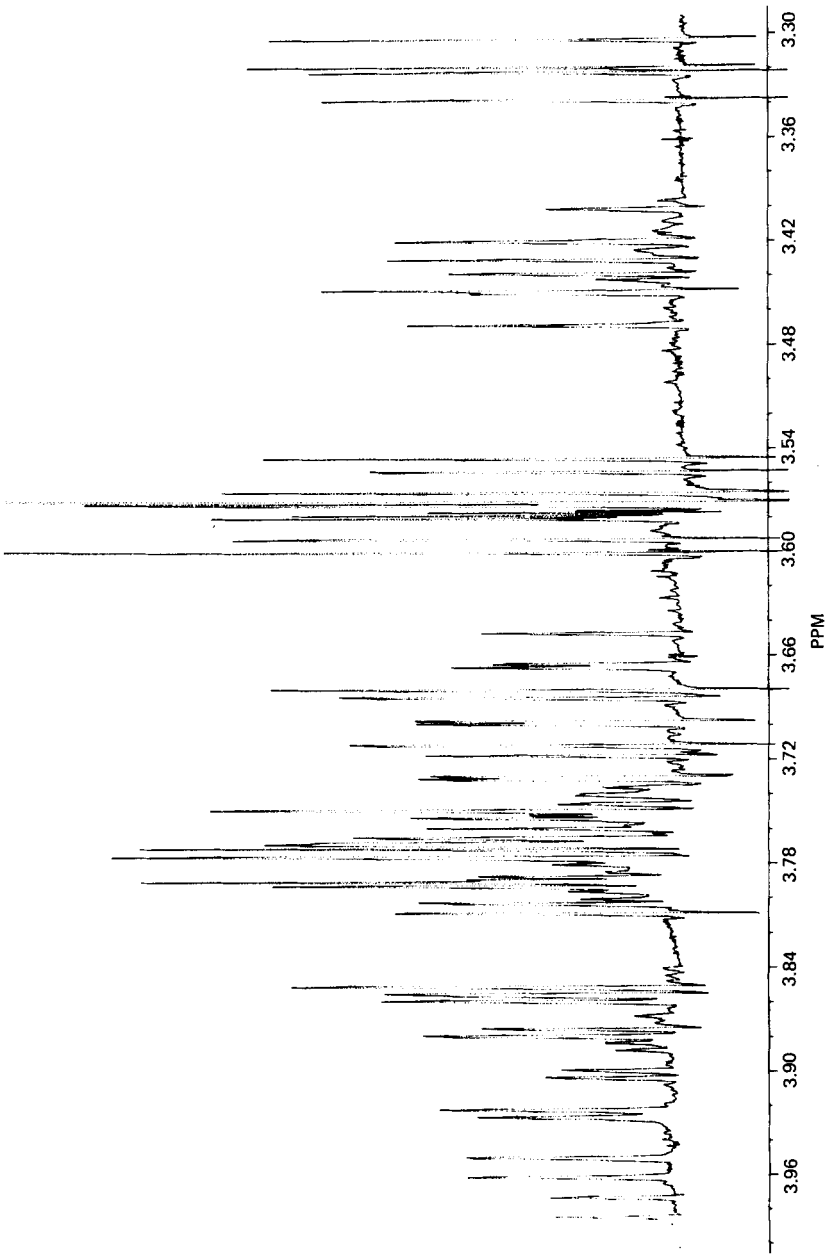

Fig. 1B 500 MHz ^{1}H NMR spectrum of 1 in D_2O.

Expansion of signals between 3.30 and 4.00 ppm after resolution enhancement.

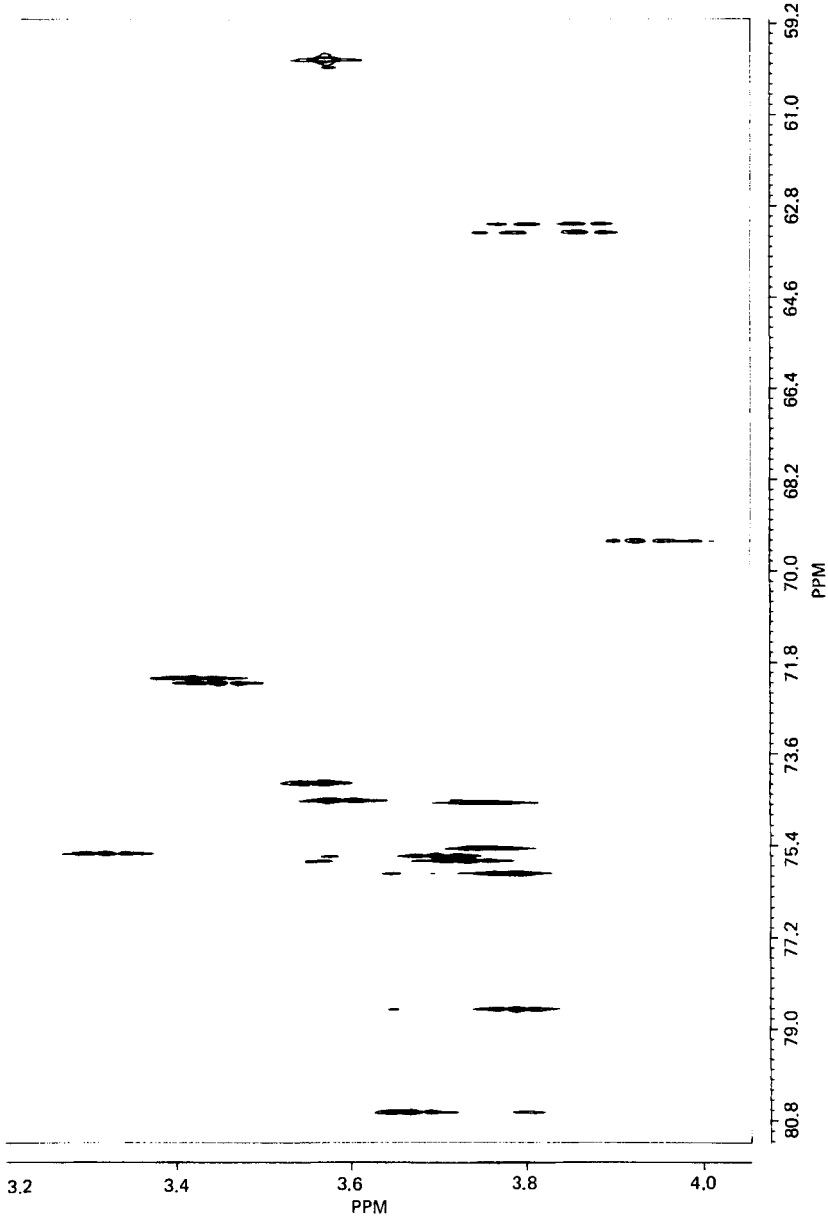

Fig. 2. Heteronuclear proton-carbon correlation of compound 1.

Anomeric signals not shown.

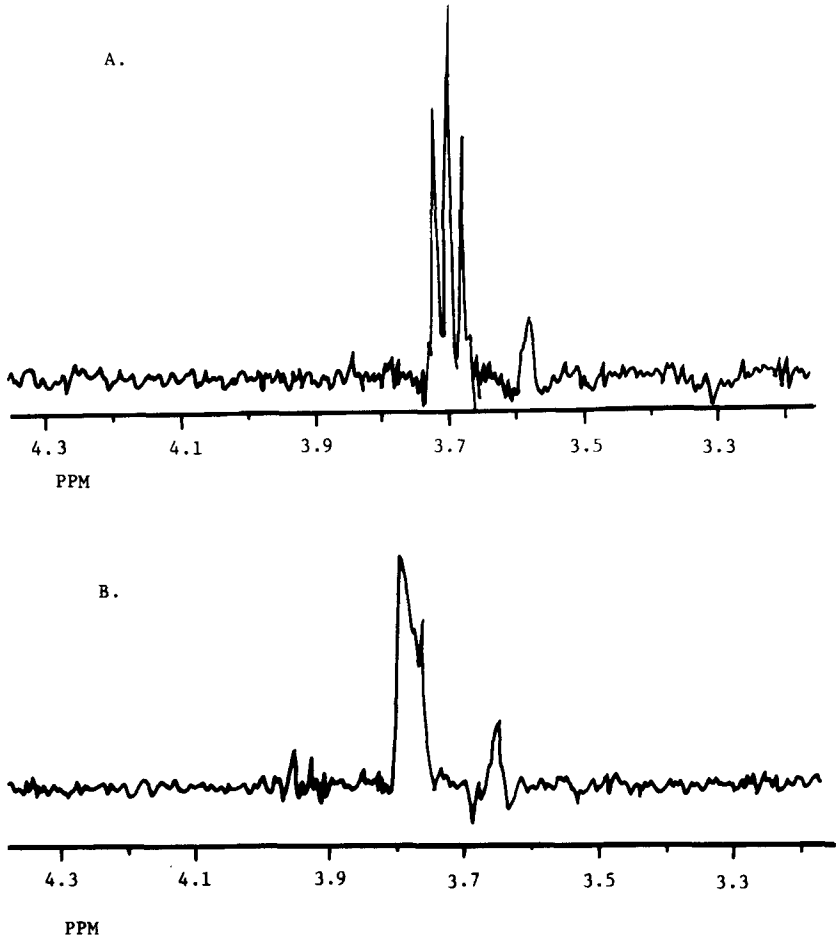

Fig. 3. Heteronuclear proton-carbon correlation for compound 1 showing proton subspectra after phase sensitive 2-D-Fourier transform for A. Carbon 3 resonating at 73.7 ppm, B. Carbon 5 resonating at 74.0 ppm.

TABLE 1.

NMR CHEMICAL SHIFT DATA FOR COMPOUND (1) AND RELATED STRUCTURES.

^{1}H NMR Data.[a]	H1	H2	H3	H4	H5	H6[b]	H6'[c]
Compound (1).							
α-D-glc(1-4)	5.34 (4.2)	3.58 (9.8)	3.74[d] (9.6)	3.44[e] (9.6)	3.77[f]	3.87	3.78[g]
α-D-glc(1-6)	5.01 (4.2)	3.56 (9.8)	3.70[d] (9.6)	3.42[e] (9.6)	3.74[f]	3.87	3.77[g]
β-D-glc OMe	4.42 (8.1)	3.32 (9.8)	3.78 (9.6)	3.67 (9.6)	3.75	3.92 (2.2)	3.97 (5.8,11.6)
Methyl β-D-maltopyranoside.							
α-D-glc(1-4)	5.40 (3.8)	3.58 (9.5)	3.68 (9.5)	3.42 (9.5)	3.72	3.85 (1.8)	3.74 (5.0,12.0)
β-D-glc OMe	4.39 (8.5)	3.29 (9.5)	3.76 (9.5)	3.60 (9.5)	3.58	3.95 (1.6)	3.74 (5.0,12.0)
Methyl β-D-isomaltopyranoside.							
α-D-glc(1-6)	4.96 (3.6)	3.55 (9.5)	3.73 (9.8)	3.44 (9.8)	3.72	3.86 (2.2)	3.78 (4.8,12.0)
β-D-glc OMe	4.42 (8.0)	3.27 (9.5)	3.49 (9.8)	3.53 (9.8)	3.65	3.77 (1.8)	3.99 (4.3,11.0)

a. Measured at 500 MHz in D_2O at 310°K as a 0.01M solution using the DOH signal as reference signal (4.60 ppm). Coupling constants are given in parentheses below the chemical shifts (in Hz).

b. Proton determined to be pro-S.

c. Proton determined to be pro-R.

d.,e.,f.,g. Assignments may have to be reversed.

TABLE 2

NMR CHEMICAL SHIFT DATA FOR COMPOUND (1) AND RELATED STRUCTURES.

^{13}C NMR Data[a]	C1	C2	C3	C4	C5	C6	OMe
Compound (1)							
α-D-glc(1-4)	100.5	72.6	73.8[b]	70.3[c]	74.0[d]	61.4[e]	
α-D-glc(1-6)	99.2	72.2	73.7[b]	70.2[c]	73.5[d]	61.3[e]	
β-D-glc OMe	103.9	73.6	76.7	78.7	72.6	67.5	58.5
Methyl β-D-maltopyranoside.							
α-D-glc(1-4)	100.4	72.5	73.7	70.2	73.5	61.6	
β-D-glc OMe	103.9	73.8	77.1	77.7	75.4	61.4	58.0
Methyl β-D-isomaltopyranoside.							
α-D-glc(1-6)	98.4	72.1	73.8	70.1	72.4	61.1	
β-D-glc OMe	103.9	73.8	76.7	70.0	74.9	66.0	58.0

a. Measured at 125 MHz in D_2O at 310°K as a 0.2M solution using the C-1 signal as reference (103.9 ppm).

b.,c.,d.,e. Assignments may have to be reversed.

problem using 1-or 2-dimensional methods where the connectivities between the ^{13}C atoms can be determined[10] but the amount of compound available at present is not sufficient to perform such experiments.

It should be noted that the assignments of the H-6 (pro-R) and H-6 (pro-S) signals differ from those suggested in reference 5, but the results presented here are based on synthetic compounds (1 and methyl β-D-isomaltopyranoside) with stereospecific deuterium labelling in the C-6 positions by published methods.[11]

In conclusion, these experiments have demonstrated that it is possible to analyse the NMR spectra of complex structures using a

combination of 1- and 2-dimensional methods, where the heteronuclear shift correlated experiment especially gives much information in the time used on the spectrometer compared with other 2-D experiments that could also have given part of the information shown in Table 1.

EXPERIMENTAL

The ^1H NMR spectra were obtained on a Bruker AM 500 MHz spectrometer from 0.01 M D_2O solutions at 310°K using the DOH signal as reference (δ4.60 ppm). The spectral width used was 5 KHz, which with a data table of 32 K gave a digital resolution of 0.3 Hz/point. 90° pulses (15 μsec) were used.

The proton-carbon shift correlated experiment was performed using the pulse sequence described by Freeman and Morris[10] with the same spectrometer mentioned above and a 0.15 M solution of 1 in D_2O at 310°K.

Methyl 2,3-di-O-Acetyl-4-O-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl)-6-O-(2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl)-β-D-glucopyranoside (2). Methyl 2,3,2',3',4',6'-hexa-O-acetyl)-β-maltoside[12] (307 mg, 0.61 mmol) and tetraethylammonium bromide (128 mg, 1.2 mmol) were dissolved in dry N,N-dimethylformamide (DMF) (0.3 mL) containing molecular sieves (4 A) and kept under dry nitrogen. 2,3,4,6-Tetra-O-benzyl-α-D-glucopyranosyl bromide[13] (735 mg, 1.2 mmol) was dissolved in dry dichloromethane (3 mL) containing molecular sieves (4 A) and added to the DMF solution and left at room temperature for 72 h under dry nitrogen. The reaction mixture was filtered and the filter was washed with dichloromethane. The combined organic phases were washed with saturated sodium hydrogen carbonate solution (3 mL), water (twice, 3 mL), dried ($MgSO_4$), and evaporated yielding 734 mg of crude product. Purification by preparative TLC, using ether : pentane (4:1) as eluant, gave as the main product (401 mg, 48%) of 2 as a syrup, $[\alpha]_D^{23} = 55.2°$ (c 3.0, $CHCl_3$). The product was further characterized

through its ^{13}C NMR data in $CDCl_3$ (125 MHz) : α-D-glc (1-6); 96.9 ppm (C-1), 81.9, 79.5 (C-2, C-3), 77.4 (C-4), 70.3 (C-5), 63.8 (C-6), α-D-glc (1-4); 94.7 (C-1), 70.2 (C-2), 69.3, (C-3), 68.0 (C-4), 68.1 (C-5), 61.8 (C-6), β-D-glc (OMe); 101.3 (C-1), 71.8 (C-2), 75.0 (C-3), 72.1 (C-4), 74.6 (C-5), 68.4 (C-6), 56.8 (OMe).

Anal. Calcd for $C_{59}H_{70}O_{22}$: C,62.65; H,6.24. Found: C, 62.00; H,6.13.

Methyl 4,6-Di-O-(α-D-glucopyranosyl)-β-D-glucopyranoside (1). The trisaccharide (2) (150 mg, 0.13 mmol) was dissolved in methanol (16 mL) and acetic acid (4 mL) was added together with Pd on carbon (5%, 50 mg). The mixture was hydrogenated at 1 atm hydrogen pressure for 24 h at room temperature. The catalyst was removed by filtration and the filtrate was evaporated to dryness. The product (101 mg) was dissolved in sodium methoxide in methanol (10 mL, 0.1%) and left at room temperature for 2 h. The reaction mixture was neutralized with solid CO_2 and evaporated to dryness. The product was purified by chromatography on a Sephadex G-15 column using methanol-water (1:1) as eluant and 1 was isolated as a syrup (48 mg, 71%), $[\alpha]_D^{23} = 85.4^\circ$ (c 1.1, water). The product was further characterized through its ^{13}C- and ^1H NMR data (see Tables 1 and 2).

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