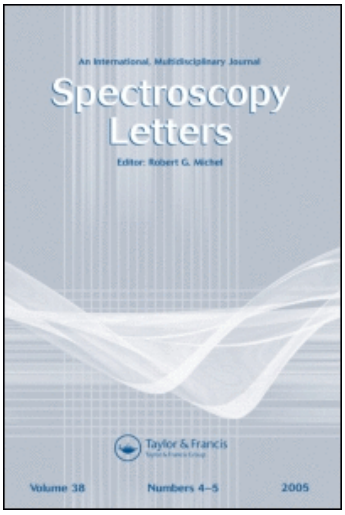


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# A Convenient Synthesis and a Nuclear Magnetic Resonance Spectroscopic Study of a β-D Linked Trisaccharide

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# A CONVENIENT SYNTHESIS AND A NUCLEAR MAGNETIC RESONANCE SPECTROSCOPIC STUDY OF A β-D LINKED TRISACCHARIDE.

**Key words: trichloroacetimidate, Trehalose heptabenzoate, coupling.**



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## ABSTRACT

The building block derivatives of the monosaccharide, O-(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl) trichloroacetimidate **(1)**, and the disaccharide α, α-trehalose, 2',3' 6'-tri-O-benzoyl-α-D-glucopyranosyl-2,3,4,6-tetra-O-benzoyl-α-D-glucopyranoside **(2)** were coupled to produce trisaccharide **3**. A detailed n.m.r. analysis [$^{1}H$, $^{13}C$, $^{13}C$-$^{1}H$ correlation, and 2D $^{1}H$-$^{1}H$ correlation experiment (COSY)] was used for the structural elucidation of the building blocks **1, 2** and the trisaccharide product **3**.

# INTRODUCTION

α, α-Trehalose is widely distributed in nature and plays an important role as an energy source as well as a carbohydrate reserve in insects[1]. Trehalose and its derivatives have been the target for systematic studies of their properties towards trehalase in order to determine the mechanism of the hydrolysis by this enzyme[2,3]. Recently, several groups of investigators have reported the synthesis of cord factor[4], 4-amino-4-deoxy-trehalose, together with its 4-epimer[5,6].

Furthermore, the conformational preferences in solution have been studied by n.m.r. spectroscopy[7-9] for both symmetrical and unsymmetrical derivatives of trehalose.



The present paper describes the preparation of the trisaccharide **3**. Full details of NMR analysis [$^{1}H$, $^{13}C$, $^{13}C$-$^{1}H$ correlation, and 2D $^{1}H$-$^{1}H$ correlation experiment (COSY)] of the unsymmetrical trehalose derivatives **2** and **3** are now reported.

# RESULTS AND DISCUSSION

The trisaccharide namely, 2',3',6',tri-O-benzoyl-4'-O-(3", 4", 6"-tri-O-acetyl-2"-deoxy-2"-phthalimido-β-D-glucopyranosyl)-α-D-glucopyranosyl-2,3,4,6-tetra-O-benzoyl-α-D-glucopyranoside (**3**), was assembled from the two building blocks **1** and **2**. The monosaccharide building block O-(3,4,6- tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)richloroacetimidate[10,11] (**1**), was useful as a precursor of β-linked, internal-2-acetamido-2-deoxy-D-glucopyranose ("N-acetyl-D-glucosamine"). 2',3',4'-Tri-O-benzoyl-α-D-

glucopyranosyl 2,3,4,6-tetra-O-benzoyl-α-D-glucopyranoside[6](2), having a hydroxy group open for chain extension at position 4, was chosen as the precursor of the trehalose moiety.

The structure of the acceptor **2** was proved through a study of the $^1$H-n.m.r., decoupling experiments, 2D $^1$H-$^1$H correlation experiment (COSY), attached proton test (APT), and $^{13}$C-$^1$H correlation experiment. The ability of high resolution n.m.r. spectroscopy to provide information about the structural details is firmly established.

The $^1$H-n.m.r. spectrum shown in Figure 1 is characterized by a multiplet at δ 8.10-7.10 corresponding to 35 aromatic protons, a triplet centered at δ 6.23 (J 10.0 Hz) which is attributed to H-3 indicating a *trans*-diaxial disposition of H-2 and H-4, a triplet at δ 5.99 due to the H-3', and a multiplet at δ 5.70-5.60 with a three protons intensity. Two overlapping doublet of doublets appear at δ 5.49-5.40. A multiplet at δ 4.38-3.72 is attributed to the sugar ring protons. A doublet at δ 3.36 corresponds to one proton and undergoes deuterium exchange, is ascribed to a hydroxyl group at position 4.

Decoupling experiments shown in Figure 1 were used to search for the signals of greatest interest, such as H-1,1', H-2,2', H-4, 4', and H-5,5' of the sugar. To interpret the results of the decoupling experiments one requires a knowledge of the exact position of at least one proton. As previously described, the signal at δ 6.23 was ascribed to the H-3, irradiation at that position simplified the multiplet at δ 5.70 - 5.60, which proves that H-4 signal lies within this range, and changed the doublet of doublets at δ 5.48 to a doublet with a small splitting (H-2). Irradiation of the H-4 resonance at δ 5.67 simplified the triplet at δ 6.23 to a doublet (H-3), and simplified the multiplet at δ 4.37-4.30 (H-5). Also, the irradiation of the H-2 pattern at δ 5.48 collapsed the triplet at δ 6.23 into a clean doublet with a large spacing (H-3), and simplified the multiplet at δ 5.70-5.60 (H-1). Irradiation of the H-5

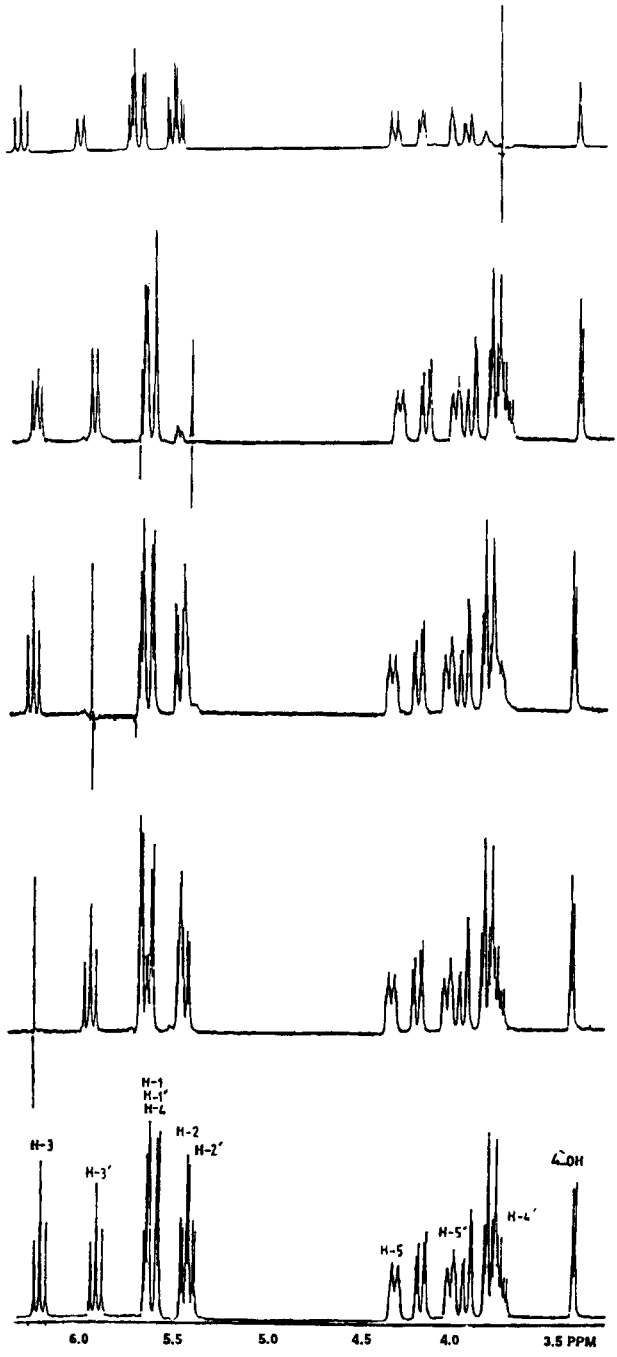



**Figure (1): $^1$H-N.m.r. spectrum and decoupling experiments for compound 2.**

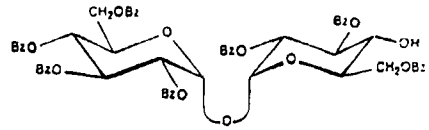
resonance at δ 4.35 simplified both the multiplet at δ 5.70-5.60 (H-4), and the multiplet at δ 4.00-3.72 (H-$6_{a,b}$).

Irradiation of the H-3' resonance at δ 5.99 changed the doublet of doublets centered at δ 5.43 to a doublet with small splitting (H-2'), and simplified the multiplet at δ 3.87-3.72, which proves that H-4' pattern lies within this range. Irradiation of H-2' signal at δ 5.43 simplified the multiplet at δ 5.70-5.60 (H-1') and collapsed the triplet at δ 5.99 into a clean doublet (H-3'). Irradiation at δ 3.78 changed the triplet at δ 5.99 into a doublet (H-3'), simplified the multiplet at δ 4.09-4.02 (H-5'), and changed the doublet at δ 3.36 to a singlet (OH-4').

A further elucidation of the structure of compound **2** is obtained through a study of the 2D $^{1}H$-$^{1}H$ correlation experiment **(COSY)**, that was used for the assignment of the sugar ring protons shown in Figure 2.

The attached proton test **(APT)** experiment was used for the assignment of the methylene and methine carbons. The inverted peaks at δ 62.94 and 62.47 correspond to the absorptions of the methylene carbons (C-6,6'). The upright peaks at δ 74.08, 71.75, 71.63, 71.17, 70.67, 69.70, 69.34, and 69.10 correspond to the rest of the sugar ring methine carbons. Unambiguous assignments of the sugar ring carbons were achieved by the use of $^{13}C$-$^{1}H$ shift correlation experiment (Figure 3) revealed the following δ assignments: 93.62, 93.42 (C-1,1'), 74.04 (C-3'), 71.75 (C-2), 71.63 (C-5'), 71.17 (C-2'), 70.67 (C-3), 69.70 (C-4'), 69.34 (C-4), and 69.10 (C-5).

The fully protected trisaccharide glucoside **(3)** was prepared by the coupling of the donor O-(3,4,6-tir-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl) trichloroacetimidate[10,11] **(1)**, with the unreactive acceptor, 2',3',6'-tri-O-benzoyl-α-D-glucopyranosyl-2,3,4,6-tetra-O-benzoyl-α-D-glucopyranoside[6] **(2)** under acidic conditions using boron trifluoride etherate in dichloromethane at-20°C; shown in the scheme.

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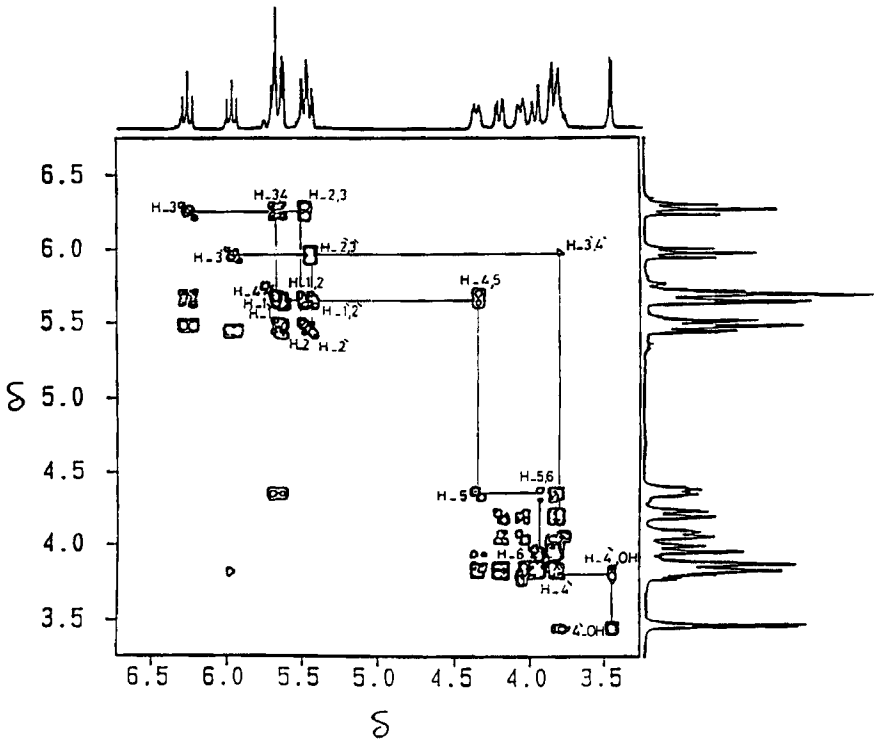



**Figure (2): COSY Experiment for compound 2.**

The initial characterization of the coupling product indicated the formation of the trisaccharide **3**. The $^1$H-n.m.r. spectrum ($CDCl_3$) showed a multiplet at $\delta$ 8.20-7.25 corresponding to the aromatic protons, and three singlets at $\delta$ 2.10, 1.90, and 1.75 due to the acetyl methyl groups. The interpretation of the complicated trisaccharide sugar ring protons signals was

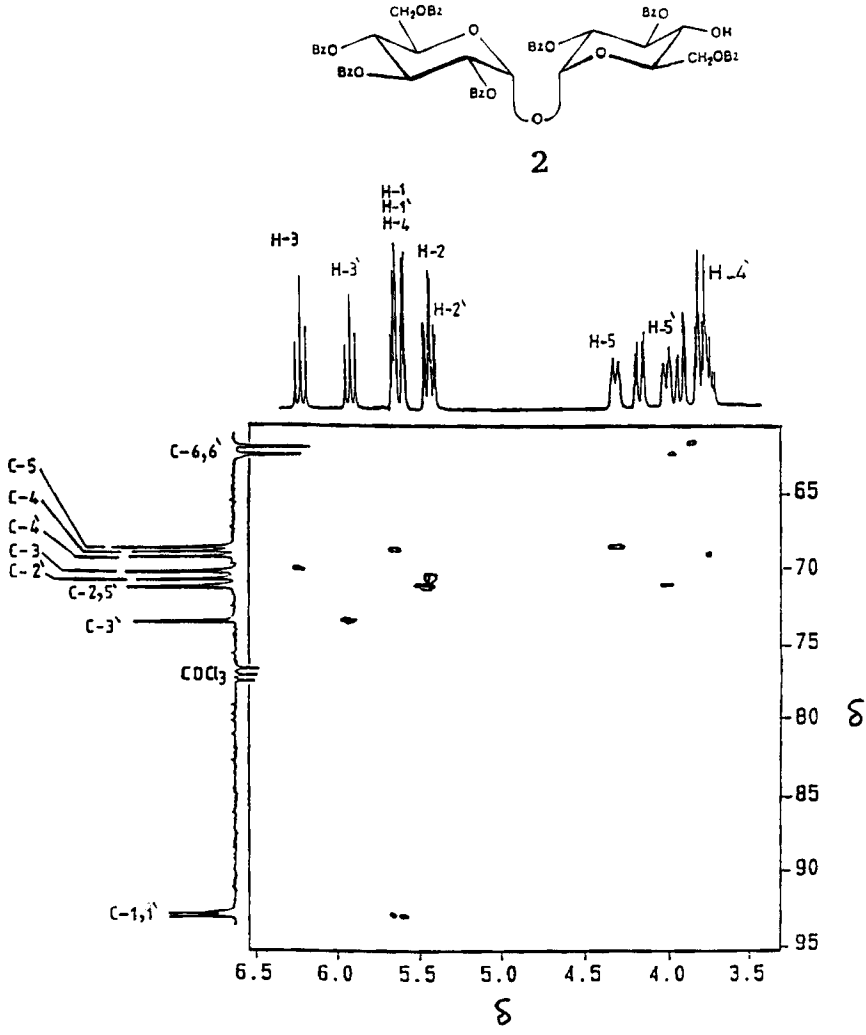
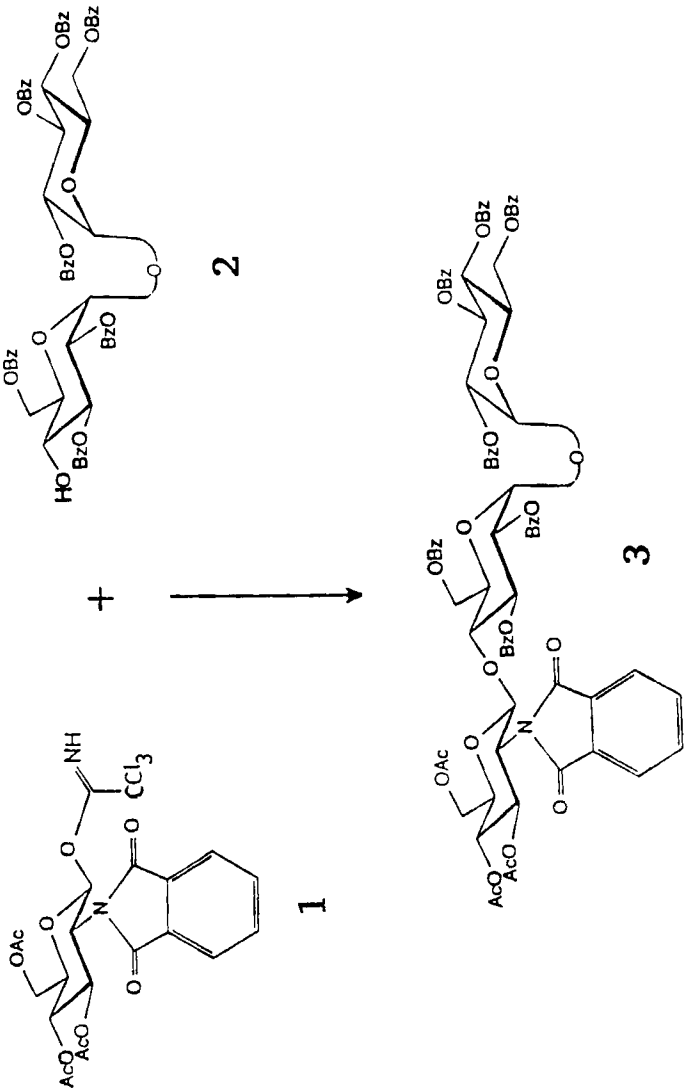


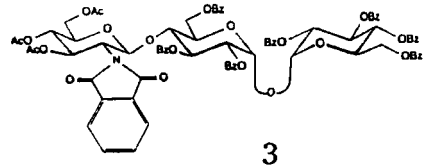

**Figure (3): C-H Correlation experiment for compound 2.**



SCHEME

quite difficult and hence, demanded a 2D $^1H$-$^1H$ correlation experiment (COSY). As previously mentioned, the interpretation of the (COSY) experiment chart requires knowing the exact position of at least one proton for each sugar ring. The COSY experiment chart (Figure 4) showed a triplet at $\delta$ 6.24 (J 9.4 Hz) attributed to H-3' and indicative of the *trans*-diaxial disposition of the H-2' and H-4'. A triplet at $\delta$ 6.19 corresponding to H-3 with large splitting. A doublet at $\delta$ 5.72 with a coupling constant of 8.4 Hz, indicating a β-configuration, corresponding to H-1". A triplet at $\delta$ 5.65 due to H-4 with J 10 Hz, a multiplet at 5.57-5.45 with four protons intensity attributable to H-1, 1',2 and 3". A doublet of doublets at $\delta$ 5.23 with small and large spacing (H-2'), a triplet at $\delta$ 5.12 (H-4") with large spacing, a multiplet at $\delta$ 4.30-4.14 (H-5, H-4', and H-2"), and a multiplet at $\delta$ 4.08-3.15 with 8 protons intensity due to the sugar ring protons.

The most adequate spectroscopic tool for the elucidation of the structure of the trisaccharide 3 was the $^{13}C$-n.m.r. spectrum (Figure 5), which simply and clearly showed three anomeric carbon signals indicating the presence of three sugar moieties. The signal at $\delta$ 99.32 is attributed to an anomeric carbon having a β-configuration (C-1"), while the two signals at $\delta$ 94.07 and 93.74 indicate an α-configuration (C-1,1'). The peak at $\delta$ 76.71 is attributed to C-4', while the signal at $\delta$ 55.60 corresponds to C-2", and the three signals at $\delta$ 21.43, 21.09, and 20.87 correspond to three methyl groups. The rest of the sugar ring carbon atoms appear at $\delta$ 72.40- 61.29. Moreover, the spectrum shows three carbonyl groups at $\delta$ 171.50, 171.00 and 170.00 corresponding to the acetyl carbonyl groups, and multiplet at $\delta$ 166.50-165.00 due to the benzoyl carbonyl carbon atoms. The aromatic multiplet appeared at $\delta$ 134.50-124.00. The APT chart revealed inverted peaks at $\delta$ 130.50, and 129.50 corresponding to the carbonyl groups of the phthalimido-moiety. The inverted peak at $\delta$ 62.29 corresponds to C-6", while the two signals at $\delta$ 61.37 and 61.29 are attributed to C-6,6'.



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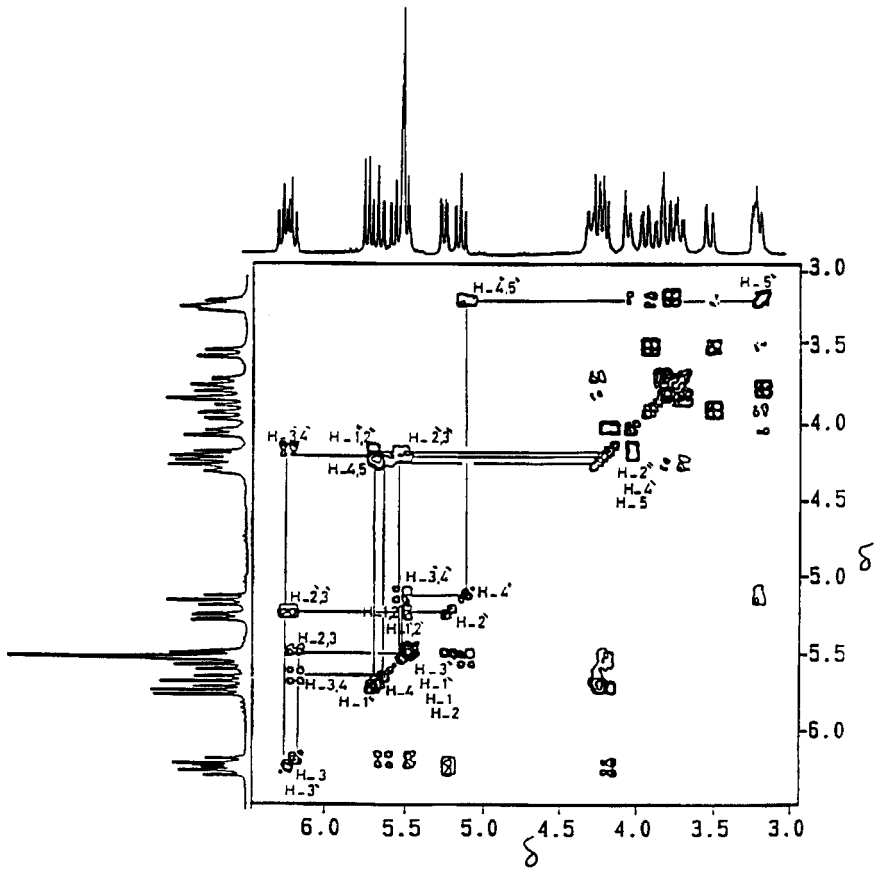


**Figure (4): COSY Experiment for compound 3.**

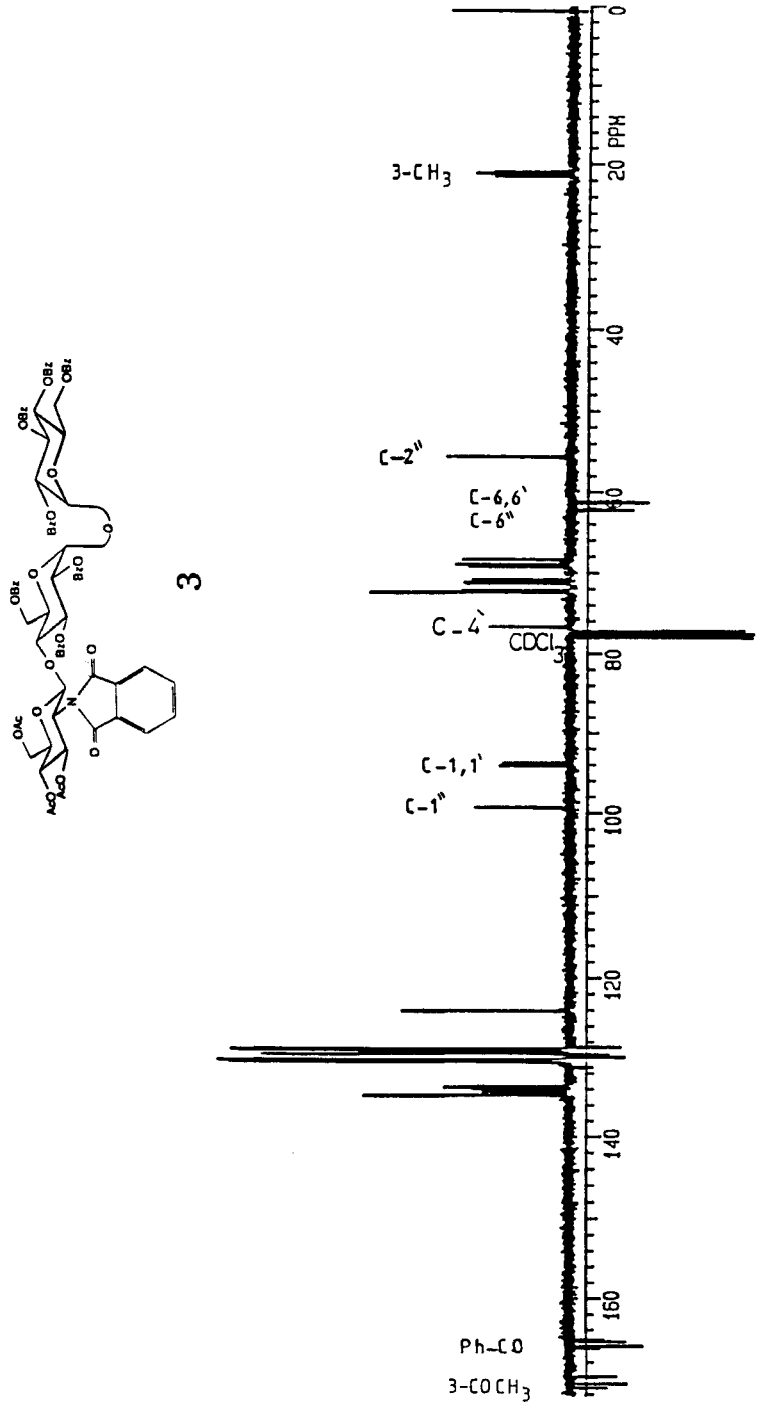


**Figure (5): $^{13}$C-N.m.r. spectrum for compound 3.**

These NMR studies have established unambiguously that the synthetic route employed here has led to the preparation of the purely β-D-linked trisaccharide 3, unmixed with the α-D-glycosidically-linked isomer.

# EXPERIMENTAL

***General Methods:***

Melting points were measured with a Büchi 510 melting point apparatus and are uncorrected. Optical rotations were determined with a Perkin-ELmer model 241 polarimeter. The NMR spectra [$^{1}H$, $^{13}C$, $^{13}C$-$^{1}H$ correlation, 2D $^{1}H$-$^{1}H$ correlation experiment (COSY)] were recorded at Glycomed Inc., U.S.A., on a Varian Gemini 300 MHz spectrometer at ambient temperature, liquid secondary-ion mass spectrometry (L.s.i.m.s) performed on a Finnigan Mat TSQ-70, triple-stage quadrupole mass spectrometer equipped with an Antek cesium ion gun.



***2',3',6'-Tri-O-benzoyl-4'-O-(3",4",6"-tri-O-acetyl-2"-deoxy-2"-phthalimido-β-D-glucopyranosyl)-α-D-glucopyranosyl-2,3,4,6-tetra-O- benzoyl-α-D-glucopyranoside (3).***

A mixture of the β-trichloroacetimidate derivative 1 (500 mg, 0.86 mmol), glycosyl acceptor 2 (840 mg, 0.78 mmol), and powdered 4 A° activated molecular sieves (1g) were dried under vacuum for 4-6 hours then dry dichloromethane (20 ml) was added. The reaction mixture was stirred at ambient temperature for 15 minutes, then treated at -20°C with 0.1 M boron trifluoride etherate (0.46 ml, 0.05 mmol). The reaction mixture was stirred for 30 minutes, when *t.l.c* [chloroform-acetone, 29: (v/v)] showed a complete disappearance of the glucosyl donor. The reaction mixture was diluted with dichloromethane, filtered over a Celite-bed, the filtrate washed with sodium hydrogen carbonate solution, and water. The organic layer was dried over

anhydrous sodium sulphate, filtered, and evaporated to dryness under reduced pressure.

The residue obtained was purified by passing through a column of silica gel using [toluene-ethyl acetate, 19:1 (v/v)] as an eluent to give 400 mg (34%) of the pure trisaccharide 3, m.p. 122°C; $[\alpha]_D$ +189°, $[\alpha]_{436}$ + 408° (c, 1.19, dichloromethane); **$^1$H-n.m.r.** (300 MHz, $CDCL_3$): $\delta$ 8.20-7.25 (m, 39 H, aromatic), 6.24 (t, 1H, J 9.4 Hz, H-3'), 6.19 (t, 1H, J 9.5 Hz, H-3), 5.72 (d, 1H, J 8.4 Hz, H-1"), 5.65 (t, 1H, J 10.0 Hz, H-4), 5.57-5.45 (m, 4H, H-1,1',2,3"), 5.23 (dd, 1H, J 4.0 Hz, J 10.1 Hz, H-2'), 5.12 (t, 1H, J 9.7 Hz, H-4"), 4.30-3.15 (m, 11 H, sugar C*H* and $CH_2$), 2.10, 1.90, 1.75 (3s, 9H, 3 $CH_3CO$); **$^{13}$C-n.m.r.** ($CDCl_3$): $\delta$ 171.50-165.00 (m, CO-groups), 130.50, 129.50 [$(CO)_2N$], 134.50-124.00 (m, aromatic), 99.32 (C-1"), 94.07, 93.74 (C-1,1'), 76.71 (C-4'), 62.29 (C-6"), 61.37, 61.29 (C-6,6'), 55.60 (c-2"), and 21.43, 21.09, 20.87 (3 $CH_3$); positive ion liquid s.i.m.s.: *m/z* 1488.9 $(M+H)^+$.

# REFERENCES


1. Lee C.K. in Lee C.K. (Ed.), *Development in Food Carbohydrate,* vol. 2, Applied Science Publishers, London, 1990, 1-89.

2. Robert J.L., in Lee C.K. and Lindley M.G. (Eds), *Development in Food carbohydrate,* Vol. 3, Applied Science Publishers, London, 1982, 81-106.

3. Defaye J., Driguez H., Henrissat B., and Bar-Guilloux E., in Marshall J.J. (Ed.), *Mechanisms of Saccharide Pohymerization and Depolymerization,* Academic Press, New York, 1980, 331-353.

4. Datta A.K., Takayama K., Nashed M.A., and Anderson L., *Carbohydr Res.,* 1992, *218,* 95-109.

5. Garcia R.C., Hough L., and Richardson A.C., *Carbohydr. Res.,* 1990, *200,* 307-317.

6. Bassily R.W., El-Sokkary R.I., silwanis B.A., Nematalla A.S., and Nashed M.A., *Carbohydr. Res.,* 1993, *239, 197-207.*

7. Ram P., Mazzola L., and Prestegard J.H., *J. Am. Chem. Soc.,* 1989, *111,* 3176-3182.

8. Tvaroska I., and vaclavik L., *Carbohydr. Res.,*1987, *160,* 137-149.

9. Bock K., Defaye J., Driguez H., and Bar-Guilloux E., *Eur. J. Biochem.,* 1983, *131,* 595-600.

10. Grundler G., and Schmidt R.R., *Carbohydr. Res.,*1985, *135,* 203-218.

11. Silwanis B.A., El-Sokkary R.I., Nashed M.A., and Paulsen H., J. Carbohydr. *Chem.,* 1991, *10(6),* 1067-1078.

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