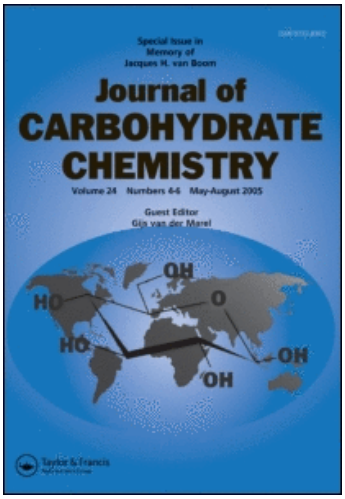

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Özlem Akpınar[a]; Michael H. Penner[b]
[a] Department of Food Engineering, Gaziosmanpaşa University, Taşlıçiftlik, Tokat [b] Department of Food Science and Technology, Oregon State University, Corvallis, OR, USA

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Preparation of Methyl 6-O-*p*-Nitrobenzoyl-β-D-Glucoside

Özlem Akpınar[1] and Michael H. Penner[2]

[1]Department of Food Engineering, Gaziosmanpaşa University, Taşlıçiftlik, Tokat
[2]Department of Food Science and Technology, Oregon State University, Corvallis, OR, USA

Methyl 6-O-*p*-nitrobenzoyl-*β*-D-glucoside was synthesized by reacting methyl 4,6-O-*p*-nitrobenzylidine-*β*-D-glucoside with N-bromosuccinimide (NBS). First, methyl *β*-D-glucoside was converted into methyl 4,6-O-*p*-nitrobenzylidine-*β*-D-glucoside with p-nitrobenzaldehyde. Later, methyl 4,6-O-*p*-nitrobenzylidine-*β*-D-glucoside was opened oxidatively with NBS to give methyl 6-O-p-nitrobenzoyl-*β*-D-glucoside.

Keywords Nonreducing end, NBS, Cyclic acetals, Oxidation, Nitrobenzoyl

INTRODUCTION

Cyclic acetals have been used in carbohydrate chemistry for protecting groups. The main advantage of cyclic acetals is that they can block a pair of diols in one step.[1] Generally an aldehyde and a sugar are mixed, either directly (benzaldehyde) or in solution by using a solvent (dioxane) in the presence of catalyst. The catalyst can be either soluble acid (sulfuric acid, *p*-toluonesulfonic acid) or an insoluble one (amberlyst resins).[2] Treatment of unprotected hexopyronoside with aldehyde in the presence of catalytic acid gives selectively protected 4,6-O-benzylide acetals since the least hindered hydroxyl group (the primary at C-6) is more reactive than other hydroxyl groups, which leads to the formation of 4,6-O-benzylide acetals.[3] Another alternative is *p*-nitrobenzylidene acetal (Fig. 1).

Benzylidene acetals can be opened selectively by acid-catalyzed reduction to yield either 4-O or 6-O benzyl ether monohydroxyl derivatives. The selectivity of the reduction is determined by the reducing agents (metal hydride), acid catalysts, solvents, and steric effects.[3] Reduction of 4,6-O-benzylidene acetals with lithium aluminium hydride-aluminium chloride gives 4-O-benzyl ethers. Lewis acids like aluminium chloride coordinate with the less hindered 6-O to give a

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Address correspondence to Özlem Akpınar, Department of Food Engineering, Gaziosmanpaşa University, Taşlıçiftlik 60100, Tokat. E-mail: oakpinar@gop.edu.tr

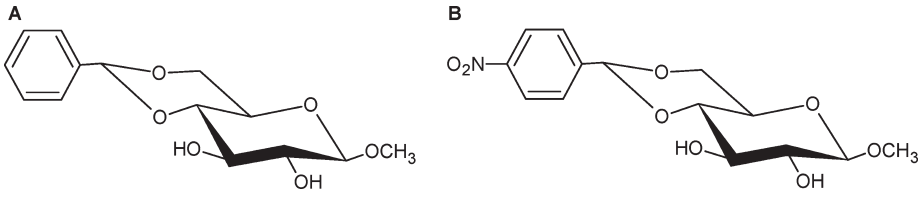

Figure 1: A: 4,6-O-Benzylide acetals. **B:** 4,6-O-*p*-Nitrobenzylidine acetals.

reactive complex that is reduced by lithium aluminium hydride to produce 4-O-benzyl derivatives. Proton-catalyzed reduction (sodium cyano borohydride-ethereal hydrogen chloride) gives 6-O-benzyl derivatives. This is because the 4-O oxygen is more basic than the 6-O oxygen and the proton is small enough to reach the 4-O oxygen; thus, protonation of 4-O oxygen produces an intermediate that reacts with sodium cyanoborohydride to give the 6-O benzyl derivative.[4]

However, *p*-nitrobenzylidene acetals are more resistant to acid-catalyzed reduction than are simple benzylidene acetals. The nitro substituents on the aromatic rings make them less reactive toward electrophilic attack. Electrons can flow from the benzene ring to the substituent, thus leaving a positive charge in the ring, destabilizing carbocation intermediates and thus making the ring less susceptible to the electrophilic attack.[5] Another way to open benzylidene acetals is by oxidative ring opening. Benzylidene acetals can be oxidized with NBS (N-bromosuccinimide) in the presence of water. The mechanism of this reaction has been studied extensively for the synthesis of 4-O-benzoyl-6-bromo-6-deoxy-α-D-glucopyranoside.[6–9] The purpose of this study was to explore possible approaches for the opening 4,6-*p*-nitrobenzylidene acetals with NBS oxidation. This reaction has not been tried for regioselective ring opening for 4,6-*p*-nitrobenzylidene acetals in carbohydrate chemistry.

MATERIALS AND METHODS

General Method

All organic solutions were dried with anhydrous Na_2SO_4. The ^1H NMR spectra recorded at 400 MHz with a Bruker AM 400 spectrometer using tetramethylsilane as an internal standard. Assignments were confirmed by 2D-COSY, HMBC, and HSQC experiments. Spots on TLC were detected by exposure to UV light and by spraying with *p*-anisaldehyde-sulfuric acid visualizing reagent.

Methyl 4,6-O-*p*-nitrobenzylidine-β-D-glucoside

Methyl-β-glucoside (2.04 g), *p*-nitrobenzaldehyde (1.46 g), and concentrated sulfuric acid (0.8 mL) in *p*-dioxane (20 mL) were stirred together at rt for 2 h.

The solution was then diluted with dichloromethane (50 mL), neutralized with solid sodium carbonate, filtered, and concentrated under reduced pressure. The concentrate was dissolved in dichloromethane, washed with water, and then dried. The solution was evaporated and crystallized from ethanol as a white crystal (0.9635 g). ^{1}H (Me_2SO-*d6*) data: δ 4.22 (s, 1H, $J_{1,2} = 7.7$ Hz H-1), δ 4.23 (dd, 1H, $J_{6a,6b} = 10.3$ Hz, $J_{6a,5} = 3.88$ Hz H-6a), δ 3.73 (dd, 1H, $J_{6a,6b} = 10.3$ Hz H-6b), δ 3.43–3.41 (m, 3H, H-3,4,5), δ 3.05 (dd, 1H, $J_{1,2} = 7.7$ Hz, $J_{2.3} = 13.1$ Hz H-2), δ 5.37 (OH), δ 5.35 (OH), δ 3.39 (s, 3H-OCH_3), δ 5.73 (s PhCH), δ 8.24 (d $J_{meta,ortho} = 8.82$ Hmeta), δ 7.71 (d $J_{meta,ortho} = 8.82$ Hortho). ^{13}C: δ 106 (C-1), δ 74.7 (C-2), δ 76.2 (C-3), δ 82.6 (C-4), δ 67.5 (C-5), δ 70.1 (C-6), δ 58.5 (OCH3), δ 101 (PhC), δ 130 (Ortho), δ 125 (Meta).

Methyl 6-O-*p*-nitrobenzoyl-β-D-glucoside

Methyl 4,6-O-*p*-nitrobenzylidine-β-D-glucoside (30 mg) and NBS (214 mg) in carbon tetrachloride containing 2 equiv of water (2 equiv) was refluxed for 2 h. The solution was filtered and concentrated under diminished pressure. The concentrate was dissolved in ethyl acetate and washed with saturated $NaHCO_3$ and cold water, dried with Na_2SO_4, and evaporated. The target compound was purified by silica gel chromatography (70–230 mesh, Sigma Chemical Co., St. Louis, MO), using ethyl acetate:ethanol:water (30:9.5:0.5). Column fractions were monitored by thin layer chromatography using silica plates ($LK6DF_{254}$, Whatman Inc., Clifton, NJ) with the same mobile phase used for column chromatography. The compound of interest was identified on TLC plate by exposing to UV light (phenyl group) and by spraying with *p*-anisaldehyde-sulfuric acid visualizing reagent (carbohydrate). Fractions containing the target compound were pooled, evaporated to dryness under reduced pressure, and then crystallized from ethanol to yield methyl 6-O-*p*-nitrobenzoyl-β-D-glucoside (6.75 mg). ^{1}H (Me_2SO-*d6*) data: δ 4.11 (d, 1H, $J_{1,2} = 7.7$ Hz H-1), δ 3.24–3.15 (m, 2H, H-2,3), δ 2.985 (1H H-4), δ 3.50 (1H, H-5), δ 4.37 (dd, 1H, $J_{6a,6b} = 11.7$, $J_{6a,5} = 6.1$ Hz H-6a), δ 4.57 (dd, 1H, $J_{6a,6b} = 11.7$, $J_{6b,5} = 2.10$ Hz H-6b), δ 3.29δ (s, 3H-OCH_3), δ 8.36 (d Hmeta), δ 8.17 (d Hortho). ^{13}C: δ 104 (C-1), δ 70.0 (C-2), δ 76 (C-3), δ 72.2 (C-4, C-5), δ 60.1 (C-6), δ 55.3 (OCH3), δ 126 (Ortho), δ 132 (Meta).

RESULTS AND DISCUSSIONS

Methyl 4,6-O-*p*-nitrobenzylidine-β-D-glucoside was prepared by selectively protecting the hydroxyl groups at the C-4 and C-6 position with *p*-nitrobenzaldehyde according to the method described by Collins and Oparaeche.[10] Methyl β-D-glucoside was directly mixed with *p*-nitrobenzaldehyde in the presence of an acid catalyst to give methyl 4,6-O-*p*-nitrobenzylidine-β-D-glucoside (Fig. 2). The ^{1}H NMR spectra of this compound showed signals at

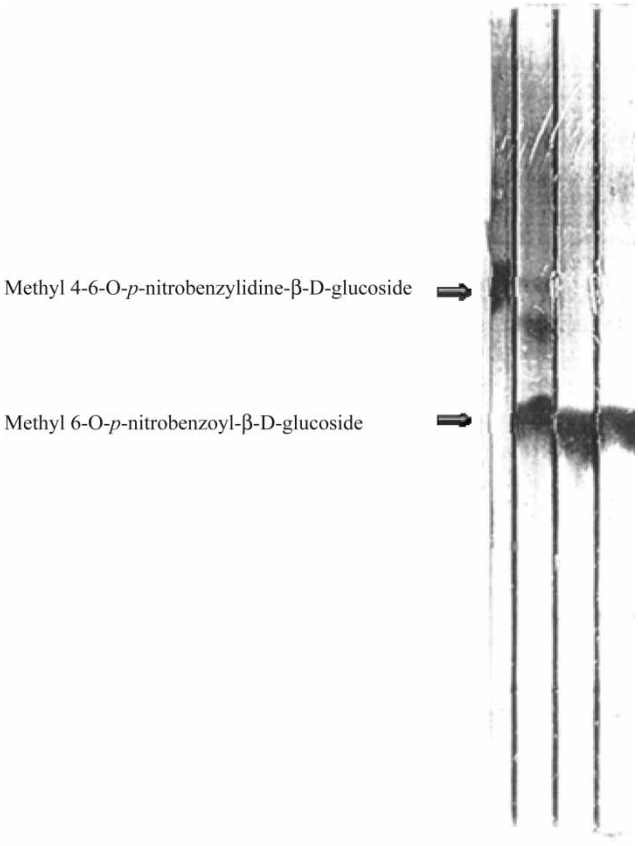

Figure 2: Oxidation of methyl 4,6-*O*-*p*-nitrobenzylidine-*β*-D-glucoside with NBS. **LN1:** Methyl 4,6-*O*-*p*-nitrobenzylidine-*β*-D-glucoside. **LN2:** Reaction mixture. **LN3:** Methyl 6-*O*-*p*-nitrobenzoyl-*β*-D-glucoside. **LN4:** Same as LN3.

δ 8.24 (d $J_{\mathrm{meta,ortho}} = 8.82$ Hz Hmeta) and δ 7.71 (d $J_{\mathrm{meta,ortho}} = 8.82$ Hz Hortho) characteristic of the aromatic ring and at δ 5.73 (s PhCH) resulting from the proton belonging to the *p*-nitrobenzylidene (Fig. 1). These assignments were confirmed with 2D-COSY, HMBC, and HSQC experiments.

The last reaction was the regioselective opening of methyl 4,6-O-*p*-nitrobenzylidine-*β*-D-glucoside with NBS (N-bromosuccinimide). When methyl 4,6-O-*p*-nitrobenzylidine-*β*-D-glucoside was oxidized with NBS (N-bromosuccinimide) in the presence of water, the resulting reaction mixture gave three different products on TLC plate (Fig. 2). The first one was unreacted starting material, the second one was an unknown side reaction product, and the third one was methyl 6-O-*p*-nitrobenzoyl-*β*-D-glucoside, confirmed by NMR. The ^{1}H NMR spectra of this compound showed that the signal at δ 5.73

(s PhCH) arising from the proton belong to the *p*-nitrobenzylidene group disappeared. The lowest field sugar proton resonance at δ 4.57 (dd, 1H, $J_{6a,6b} = 11.7$ Hz, $J_{6b,5} = 2.10$ Hz H-6b) indicated the *p*-nitrobenzoate group at position 6 of the glucose ring (Fig. 1). The other 2D-COSY, HMBC, and HSQC experiments supported this conclusion.

As shown in the Figure 3, free radical bromination of the benzylidene acetal carbon atoms gives a bromo derivative A, which generates stabilized carbocation B. The carbocation intermediate is attacked by water to form very reactive orthoacetal C, which may undergo ring opening by either of two paths depending on the conditions used. Path "1" leads to the formation of 6-O-NO_2Bz ester, whereas path "2" gives the 4-O-NO_2Bz ester.

Although the origin of the regioselectivity of the ring opening has not been fully explained, steric factors may affect this reaction. The nitro group plays an important role in this reaction, since the selectivity is somewhat different from that of other *O*-benzylidene acetals. Since the formation 4-O oxygen is more basic than 6-O, enhancing the acidity of the reaction solution is expected to increase the percentage of 4-O-Bz ester. However, the dominant product is 6-O-Bz ester. In addition, excess acid may lead to a general glycoside hydrolysis reaction.[1]

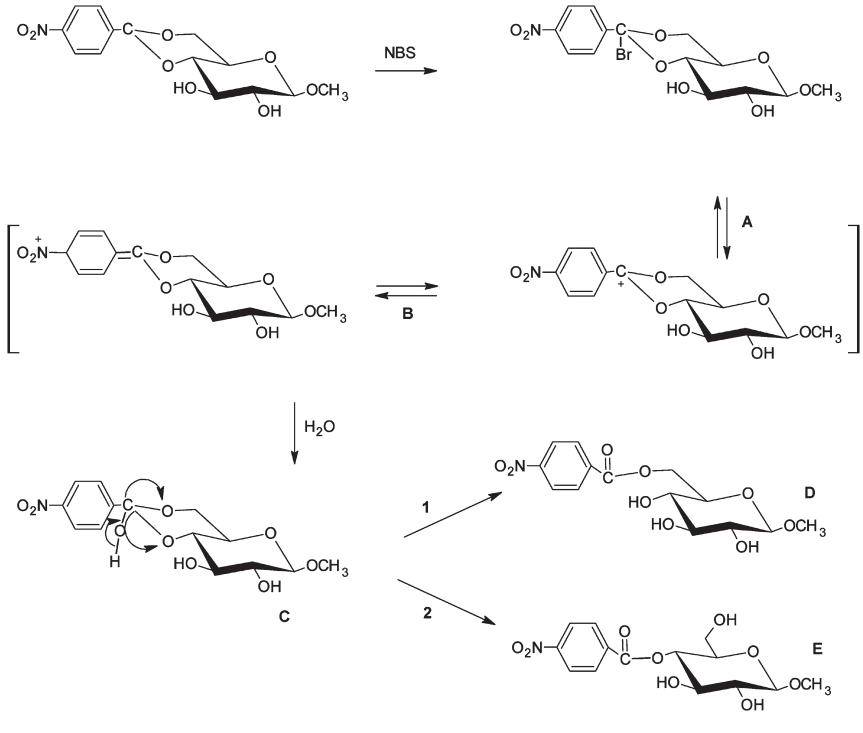

Figure 3: Opening of benzylidene acetals selectively by NBS oxidation.

The synthetic method described here can be used as a general method for the modification of the nonreducing terminus of oligosaccharides. In this method the anomeric carbon needs to be protected due to its high reactivity. This was accomplished by blocking with a methyl group due to its stability under various reaction conditions. Protection of 4′-6′-positions of methyl oligosaccharides with *p*-nitrobenzylidene acetals followed by regioselective ring opening gives nonreducing end-modified oligosaccharides. This method is simple because it does not require many protection and deprotection steps. Such modified oligosaccharides might be used for testing of the chain end-specificity of exoenzymes. The nitro group on the nonreducing end of the substrate will have another advantage. Since the nonreducing end of the substrate will be derivatized to give a *p*-nitro group, the *p*-nitro group can then be reduced with catalytic hydrogenation under hydrogen atmosphere in the presence of palladium on activated carbon, to give the nonreducing end of the substrate a *p*-aminophenyl group. This compound can be immobilized to the insoluble support by using CnBr- or NHS-activated agarose. Thus, modified oligosaccharides can be used as affinity ligands for the separation of exoenzymes.

SUPPORTING INFORMATION

1. NMR data for Methyl β-D-glucoside

^{1}H (Me_2SO-*d6*) data: δ 4.02 (d, 1H, $J_{1,2} = 7.62$ Hz H-1), δ 3.66 (dd, 1H. $J = 10.1$ Hz, $J = 4.27$ Hz), δ 3.43 (1H, $J = 10.3$ Hz), δ 3.12–2.90 (m 4H), δ 4.99 (OH), δ 4.88 (OH), δ 4.86 (OH), δ 4.47 (OH), δ 3.37 (s, 3H-OCH_3). (Figures S1–S9).

2. NMR data for Methyl 4-6-O-*p*-nitrobenzylidine-β-D-glucoside

^{1}H (Me_2SO-*d6*) data: δ 4.22 (s, 1H, $J_{1,2} = 7.7$ Hz H-1), δ 4.23 (dd, 1H, $J_{6a,6b} = 10.3$ Hz, $J_{6a,5} = 3.88$ Hz H-6a), δ 3.73 (dd, 1H, $J_{6a,6b} = 10.3$ Hz H-6b), δ 3.43–3.41 (m 3H, H-3,4,5), δ 3.05 (dd, 1H, $J_{1,2} = 7.7$ Hz, $J_{2.3} = 13.1$ Hz H-2), δ 5.37 (OH), δ 5.35 (OH), δ 3.39 (s, 3H-OCH_3), δ 5.73 (s PhCH), δ 8.24 (d $J_{meta,ortho} = 8.82$ Hmeta), δ 7.71 (d $J_{meta,ortho} = 8.82$ Hortho). ^{13}C: δ 106 (C-1), δ 74.7 (C-2), δ 76.2 (C-3), δ 82.6 (C-4), δ 67.5 (C-5), δ 70.1 (C-6), δ 58.5 (OCH3), δ 101 (PhC), δ 130 (Ortho), δ 125 (Meta).

3. NMR data for Methyl 6-O-*p*-nitrobenzoyl-β-D-glucoside

^{1}H (Me_2SO-*d6*) data: δ 4.11 (d, 1H, $J_{1,2} = 7.7$ Hz H-1), δ 3.24–3.15 (m, 2H, H-2,3), δ 2.985 (1H, H-4), δ 3.50 (1H, H-5), δ 4.37 (dd, 1H, $J_{6a,6b} = 11.7$, $J_{6a,5} = 6.1$ Hz H-6a), δ 4.57 (dd, 1H, $J_{6a,6b} = 11.7$, $J_{6b,5} = 2.10$ Hz H-6b), δ 3.29δ (s, 3H-OCH_3), δ 8.36 (d Hmeta), δ 8.17 (d Hortho). ^{13}C: δ 104 (C-1), δ 70.0 (C-2), δ 76 (C-3), δ 72.2 (C-4, C-5), δ 60.1 (C-6), δ 55.3 (OCH3), δ 126 (Ortho), δ 132 (Meta).

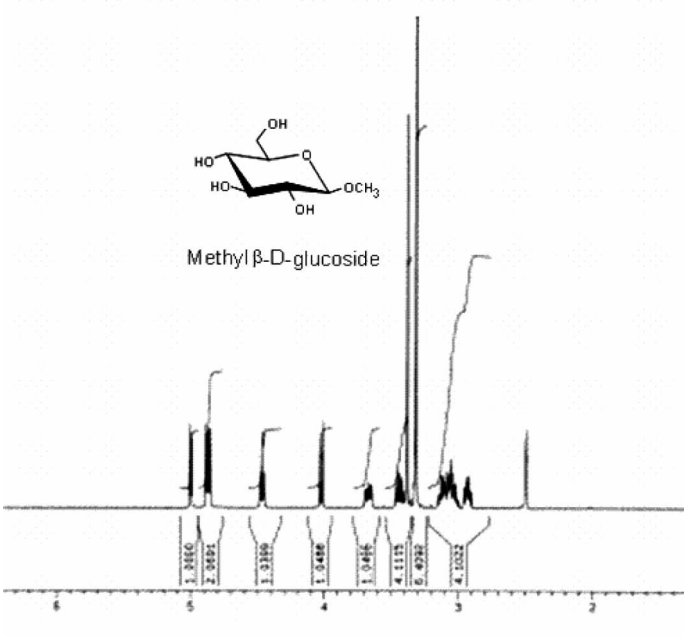

Figure S1: H NMR spectrum for methyl-*β*-D-glucoside (Me_2SO-*d6*).

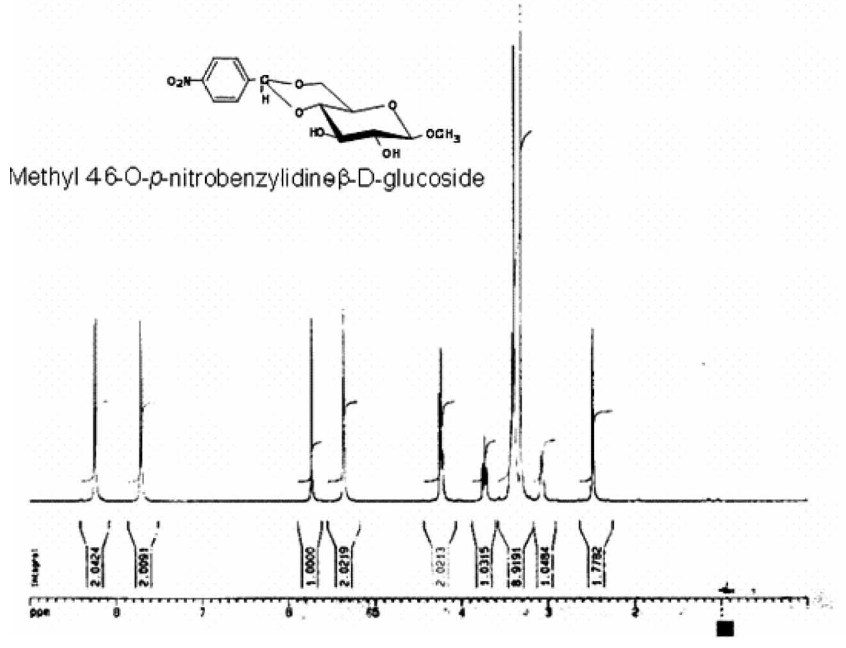

Figure S2: ^{1}H NMR spectrum data for Methyl 4-6-O-*p*-nitrobenzylidine-*β*-D-glucoside (Me_2SO-*d6*).

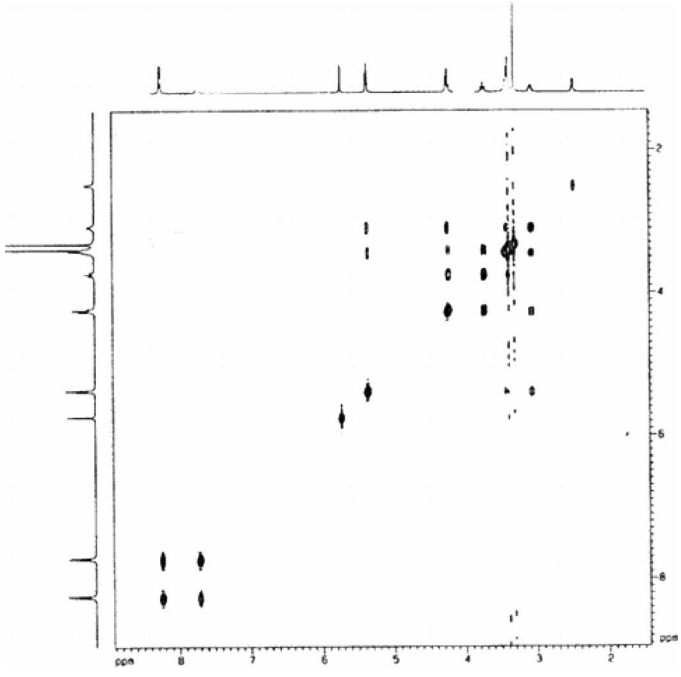

Figure S3: 2D COSY spectrum for methyl 4-6-O-*p*-nitrobenzylidine-β-D-glucoside (Me_2SO-*d6*).

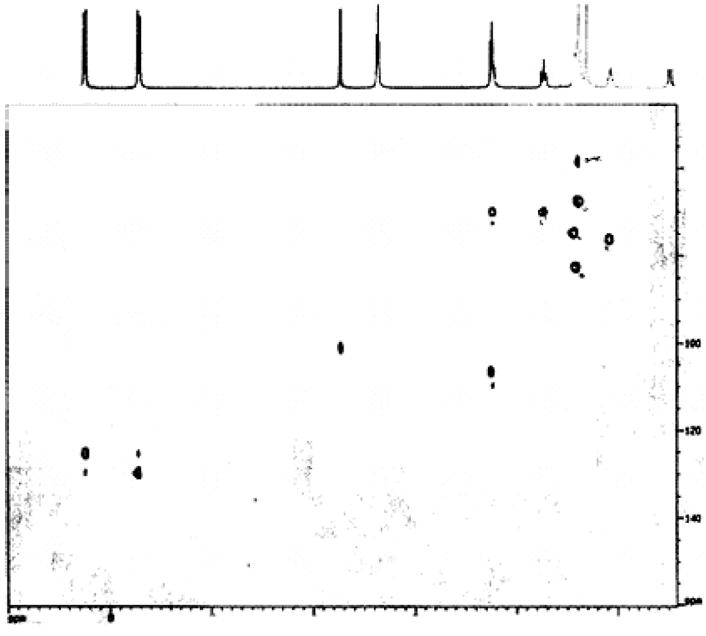

Figure S4: HSQC spectrum for methyl 4-6-O-*p*-nitrobenzylidine-β-D-glucoside (Me_2SO-*d6*).

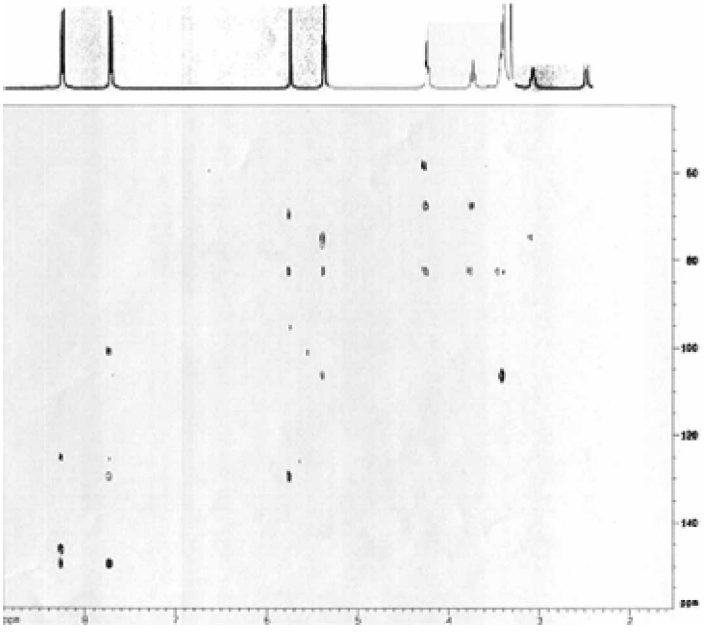

Figure S5: HMBC spectrum for methyl 4-6-O-*p*-nitrobenzylidine-*β*-D-glucoside (Me_2SO-*d6*).

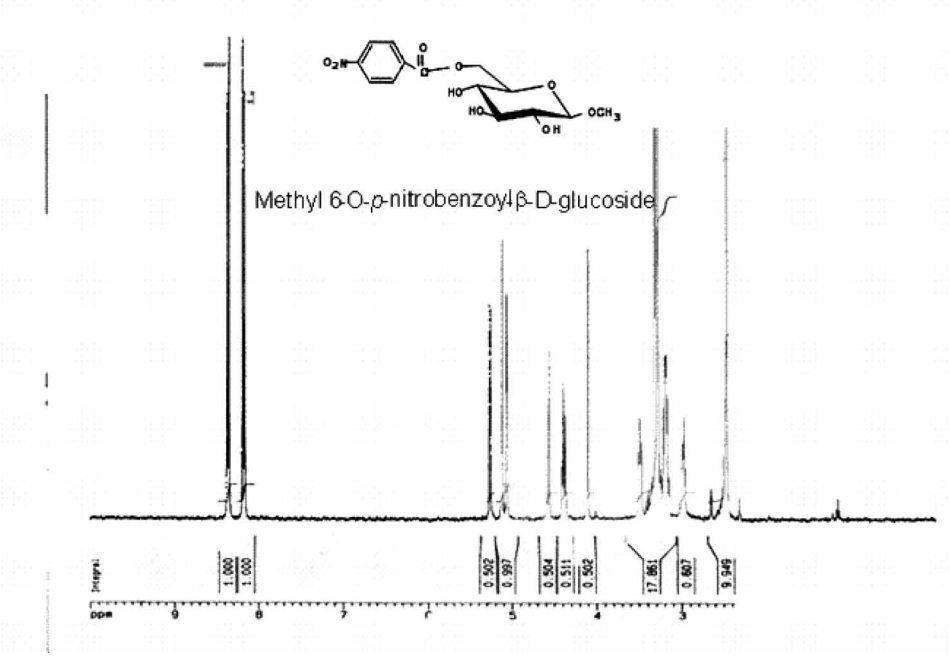

Figure S6: ^1H NMR spectrum for Methyl 6-O-*p*-nitrobenzoylidine-*β*-D-glucoside (Me_2SO-*d6*).

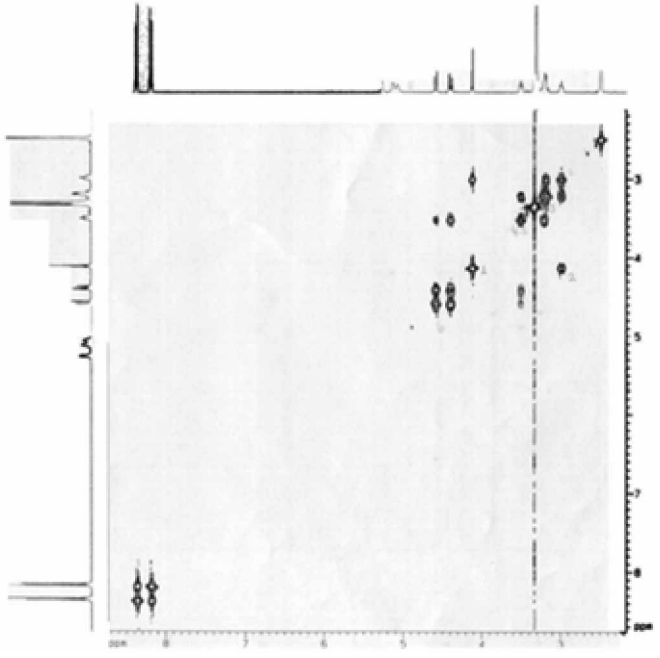

Figure S7: 2D COSY spectrum for methyl 6-O-*p*-nitrobenzoyl-β-D-glucoside (Me_2SO-*d6*).

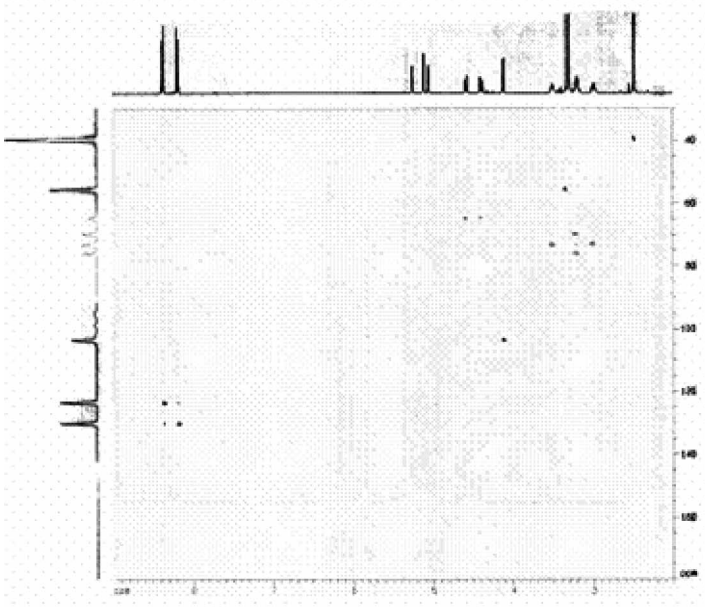

Figure S8: HSQC spectrum for methyl 6-O-*p*-nitrobenzoyl-β-D-glucoside (Me_2SO-*d6*).

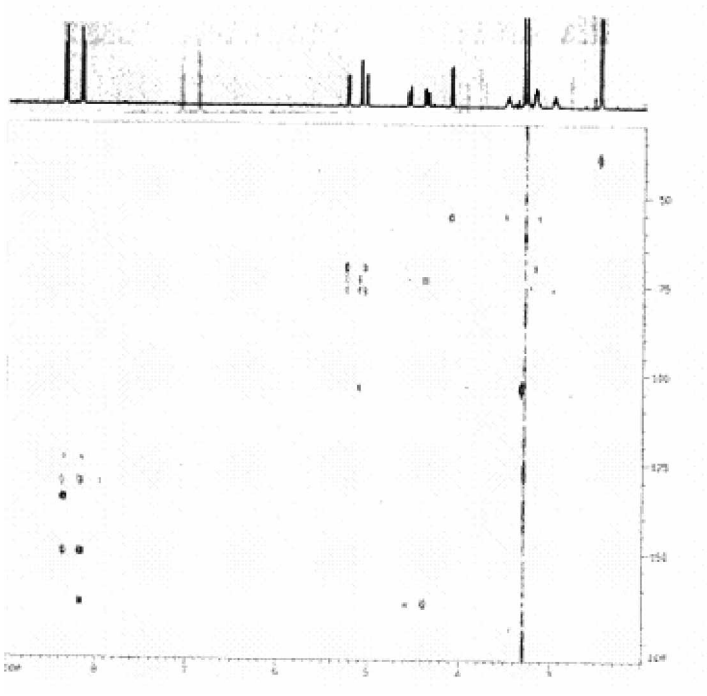

Figure S9: HMBC spectrum for methyl 6-O-*p*-nitrobenzoyl-β-D-glucoside (Me_2SO-*d6*).

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