# Synthesis, Photochemistry and Enzymology of 2-*O*-(2-Nitrobenzyl)-D-glucose, a Photolabile Derivative of D-Glucose

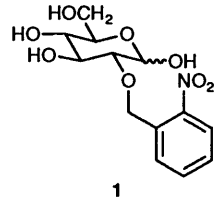
**John E. T. Corrie**
*National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK*

Alkylation of the dibutylstannylene derivative of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside **2a** with 2-nitrobenzyl bromide gave a mixture of 2-*O*- and 3-*O*-(2-nitrobenzyl) derivatives **3b** and **4b** in the ratio ~3:1. Chromatographic separation and removal of the protecting groups gave the title compound **1**. Regiospecific synthesis by 2-nitrobenzylation of the monoalcohol **8a** was examined but could not be achieved efficiently. Compound **1** was neither a substrate for nor an inhibitor of the coupled hexokinase/glucose-6-phosphate dehydrogenase enzyme system. On photolysis, it was converted into free glucose with 1:1 stoichiometry.

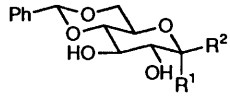
The biological properties of many small molecules such as nucleotides, neurotransmitters and other species can be blocked by alkylation at suitable sites. When the blocking group is 2-nitrobenzyl (or a derivative thereof), it can be removed by irradiation with near-UV light to regenerate the active species. With a brief but intense light pulse (typically ~100 mJ in 0.05–1000 μs), concentration changes of the active species in the region 1–5 mmol $dm^{-3}$ can be attained, with rates of release up to $10^5$ $s^{-1}$, depending on the particular species.[1] As part of a collaborative project to study the renal sodium-dependent glucose transporter protein using this technique, we needed to prepare 2-*O*-(2-nitrobenzyl)-D-glucose **1**. The choice of this particular derivative was dictated by the specificity of the transport system, for which it is known that modification of the 2-hydroxy group of glucose blocks the transport process.[2] 2-Nitrobenzyl groups have previously been used in carbohydrate chemistry for protection of the glycosidic position[3] and of the 2′-hydroxy group of ribonucleosides.[4] In seeking to extend these results to the preparation of 2-*O*-(2-nitrobenzyl)-D-glucose some difficulties were encountered, most notably in efforts to alkylate hydroxy groups on sugars with 2-nitrobenzyl bromide and sodium hydride in dimethylformamide (DMF). These problems and the successful synthesis of the required compound, together with details of its photochemical and enzymological properties, are discussed below.

## Results and Discussion

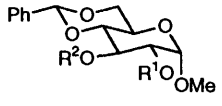
Derivatives of 2-*O*-benzyl-D-glucose have been reported by several groups, but, in all except one case, mixtures of the 2- and 3-*O*-benzyl compounds were obtained. Benzylation of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside **2a** with sodium hydride in neat benzyl chloride[5] gave predominantly the 2-ether **3a** and a similar product mix was obtained by alkylation with benzyl bromide under phase-transfer conditions.[6] By contrast, alkylation of the copper complex of **2a** with benzyl iodide in 1,2-dimethoxyethane[7] gave predominantly the 3-ether **4a**. Alkylation of the β-glucoside **2b** under phase-transfer conditions[6] gave a similar distribution of regioisomers as for the α-glucoside **2a**, while treatment of the dibutylstannylene derivative in benzene with tetrabutylammonium bromide (TBAB) and benzyl bromide gave a 1:2 ratio of the 2- and 3-*O*-benzyl ethers.[8] Alkylation of the dibutylstannylene derivative of methyl α-D-glucopyranoside with benzyl bromide in 1,4-dioxane or acetonitrile gave mixtures of the 2- and 3-*O*-monobenzyl ethers **5a** and **5b**, together with dialkylated products.[9] Although the regioselectivity for the 2-isomer **5a** was better in acetonitrile (9:1 *vs.* 3:1 in 1,4-dioxane), the overall yield of the 2-isomer was similar in both solvents because of poorer conversion efficiency in acetonitrile. Benzylation of 1-(3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl)piperidine with benzyl bromide and silver(I) oxide and transformation of the product into 2-*O*-benzyl-D-glucose has been reported,[10] but was not considered further since we were concerned about possible migration of acyl groups. The difficulties subsequently encountered in forming 2-nitrobenzyl ethers would in any case probably render this route inappropriate.



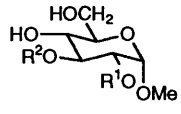
**1**



**2a** $R^1$ = OMe, $R^2$ = H
**2b** $R^1$ = H, $R^2$ = OMe



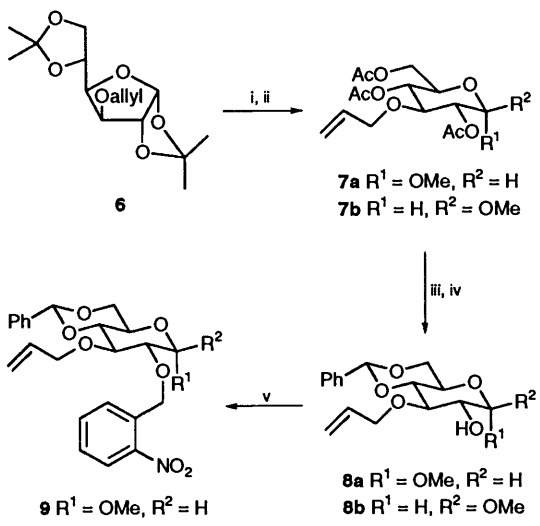
**3a** $R^1$ = Bn, $R^2$ = H
**3b** $R^1$ = 2-NBn, $R^2$ = H
**3c** $R^1$ = 2-NBn, $R^2$ = Ac
**4a** $R^1$ = H, $R^2$ = Bn
**4b** $R^1$ = H, $R^2$ = 2-NBn
**4c** $R^1$ = Ac, $R^2$ = 2-NBn
2-NBn = 2-nitrobenzyl



**5a** $R^1$ = Bn, $R^2$ = H
**5b** $R^1$ = H, $R^2$ = Bn

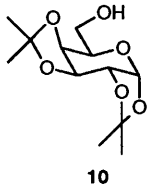
Because of the uncertainties of regiochemistry and yields in these various alkylation reactions, we chose first to examine a synthetic route which could give specifically the desired 2-*O*-(2-nitrobenzyl) ether **1**. To this end, 3-*O*-allyl-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose[11] **6** was converted by methanolysis and acetylation into a mixture of methyl-2,4,6-tri-*O*-acetyl-3-*O*-allyl-α- and -β-D-glucoside **7a** and **7b** (see Scheme 1), which were separable by chromatography. Sodium methoxide treatment of the individual triacetates, followed by acetalisation with α,α-dimethoxytoluene and camphorsulfonic acid[12] readily gave the α- and β-anomer **8a** and **8b**, which had physical constants in agreement with published data.[8,13]

Attempts to prepare the 2-nitrobenzyl ether of the free 2-hydroxy group of compound **8a** by using sodium hydride and 2-nitrobenzyl bromide in DMF were unproductive, and were invariably attended by the immediate formation of deeply coloured reaction mixtures. Similar colour development was reported in the successful, albeit low yielding 2-nitrobenzylation




**Scheme 1** *Reagents:* i, HCl–MeOH; ii, $Ac_2O$–pyridine; iii, NaOH–MeOH; iv, $PhCH(OMe)_2$–camphorsulfonic acid; v, $2\text{-}NO_2C_6H_4\text{-}CHN_2\text{–}BF_3\cdot Et_2O$

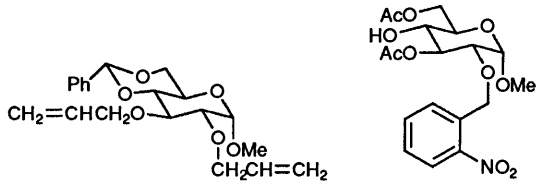
of nucleosides using the same reagents,[4b] and seems likely to be associated with formation of an anion (or perhaps radical anion) from 2-nitrobenzyl bromide. The failure to effect 2-nitrobenzylation at the 2-hydroxy group of compound **8a** compared with moderately successful derivatisation of the 2′-hydroxy group in nucleosides may be a consequence of the more crowded environment in the former compound. In model experiments with 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose **10**, which has a free primary hydroxy group at C-6, very low yields of the 2-nitrobenzyl ether were obtained by treatment with 2-nitrobenzyl bromide and sodium hydride in DMF. When silver(I) oxide was used instead of sodium hydride, formation of the ether from the galactose derivative **10** was marginally enhanced, but despite trial reactions in a variety of solvents, in no case were the results sufficiently encouraging to warrant extension to the alcohol **8a**. Fukase *et al.*[14] have reported similar conclusions from efforts to prepare 4-nitrobenzyl ethers of simple primary alcohols, namely instability of 4-nitrobenzyl bromide in the presence of sodium hydride and DMF, but excellent yields of the expected ethers in reactions catalysed by silver(I) oxide.




**10**

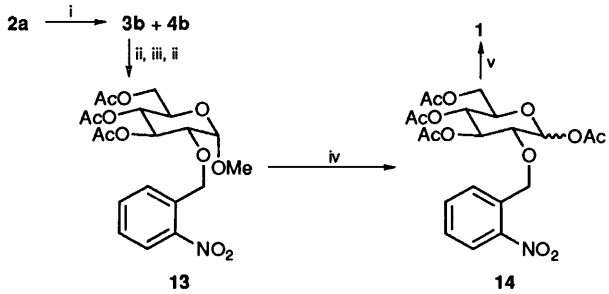
Treatment of the alcohol **8a** with 2-nitrophenyldiazomethane and boron trifluoride–diethyl ether in dichloromethane[4c,15] gave the nitrobenzyl ether **9** (among other products) in low yield, which was not improved by variations in reaction conditions. In the light of these difficulties, this synthetic scheme was abandoned and dibutyltin oxide-catalysed alkylation of the commercially available glucoside **2a** was examined instead (Scheme 2, see below). When the glucoside **2a**, dibutyltin oxide, TBAB and 2-nitrobenzyl bromide were heated in acetonitrile under prolonged reflux, a 2:1 mixture of the 2- and 3-*O*-(2-nitrobenzyl) compounds **3b** and **4b** was formed. The two isomers were readily separated by chromatography, but the required compound **3b** was contaminated with 2-nitrobenzyl alcohol and crystallised only with some loss. However, acetylation of crude compound **3b** gave the highly crystalline acetate **3c** which was easily purified. Isomer **4b** was also converted into its acetate **4c** and the $^1H$ NMR spectrum of the two acetates established the regiochemistry, since the proton geminal to the acetoxy group in compounds **3c** and **4c** shows characteristic diaxial or axial/equatorial couplings respectively to the vicinal protons.

In an effort further to confirm the assigned regiochemistry the isomer **3b** was treated under nitrogen with allyl bromide and sodium hydride in DMF in the expectation of forming the allyl ether **9**, already synthesized as described above. However, this reaction mixture immediately became deeply coloured and when worked up yielded methyl 2,3-di-*O*-allyl-4,6-*O*-benzylidene-α-D-glucoside **11** as the sole carbohydrate product. An identical result was observed for the isomer **4b**. Obviously the 2-nitrobenzyl group was cleaved under the conditions and the newly unmasked hydroxy group, as well as the one initially free, were both allylated. We have not sought further to examine this unexpected finding, although various mechanisms can be envisaged subsequent to a base-promoted removal of one of the rather acidic benzylic hydrogen atoms. Similarly as for 4-nitrobenzyl bromide, Fukase *et al.*[14] noted that 4-nitrobenzyl ethers are unstable in alkaline DMF while Gravel *et al.*[16] found that acetals derived from (2-nitrophenyl)ethylene glycol are also unstable under these conditions. Qualitatively similar chemistry presumably operates in both the *ortho* and *para* nitro compounds.




**11** **12**

With the 2-*O*-(2-nitrobenzyl) compound **3c** in hand, completion of the synthesis required only removal of the benzylidene protecting group and cleavage of the glycosidic bond. Acetic anhydride in trifluoroacetic acid has been used to effect clean acetolysis of the glycosidic bond in methyl 3-*O*-acetyl-4-azido-2-*O*-benzyl-4,6-dideoxy-α-D-glucopyranoside,[17] which on carbons 1–3 has almost identical functionality to that of glucoside **3c**. However, under the same conditions, glucoside **3c** underwent only cleavage of the benzylidene group to yield a mixture of di- and tri-acetate **12** and **13**. The latter compound was more easily obtained by treatment of acetate **3c** with methanol–aq. hydrochloric acid, followed by acetylation. Treatment of the triacetate **13** with acetic anhydride–boron trifluoride–diethyl ether[18] then effected smooth acetolysis of the glycosidic bond to yield an anomeric mixture of tetraacetates **14** and hydrolysis with NaOH in aq. methanol, followed by neutralisation with Dowex 50 ($H^+$ form), gave the required 2-*O*-(2-nitrobenzyl)-D-glucose **1** as a 1:1 anomeric mixture. The




**13** **14**

**Scheme 2** *Reagents:* i, $Bu_2SnO$–$Bu_4NBr$–$2\text{-}NO_2C_6H_4CH_2Br$; ii, $Ac_2O$–pyridine; iii, HCl–aq. MeOH; iv, $Ac_2O\text{–}BF_3\cdot Et_2O$; v, NaOH–aq. MeOH

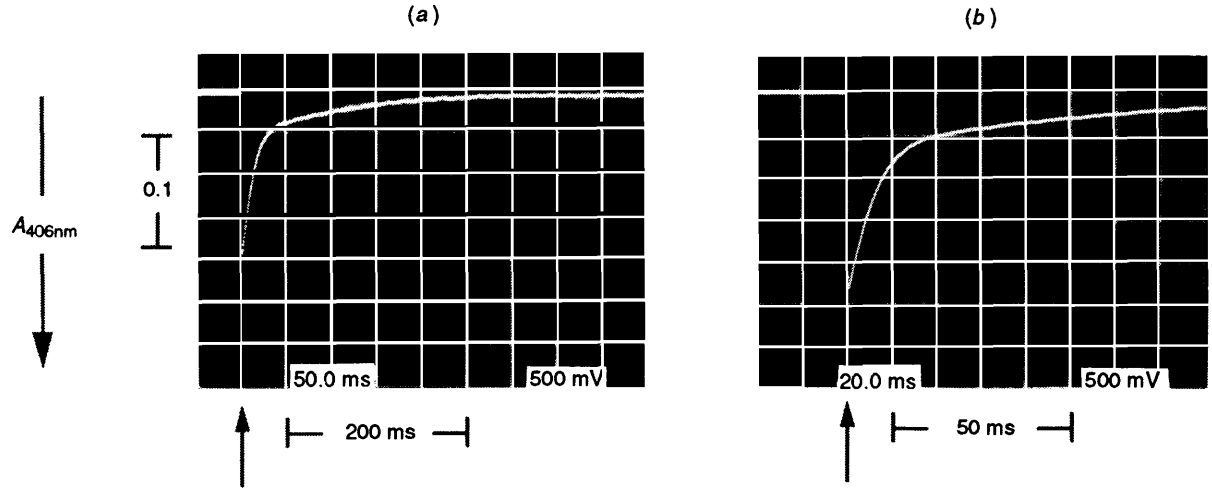
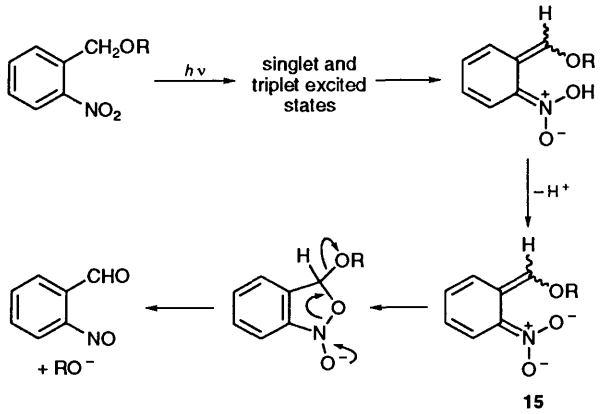



**Fig. 1** Spectrophotometric records at 406 nm of the flash photolysis of 2-*O*-(2-nitrobenzyl)-D-glucose **1** at 22 °C. The composition of the solution is given in the Experimental section. Arrows indicate the time of the 320 nm laser flash. Panel (a) shows the entire record and panel (b) is on a more rapid time-base to show the faster phase of the transient.

two anomers gave rise to separate sets of signals in the $^1$H NMR spectrum, not only for the anomeric proton but also notably for the benzylic protons, which appeared as an AB quartet centred at $\delta$ 5.19 and a singlet at $\delta$ 5.06 in the two anomers. From a ROESY experiment, the AB quartet and singlet signals were assigned to the β- and α-anomer respectively. Further details of the NMR spectrum are given in the Experimental section. The spectrum of the protons on the sugar ring was very similar to that described for glucose itself,[19] except that the signals from 1-H and 3-H were moved downfield by ~0.1 ppm.

Characterisation of photolabile precursors of biologically active molecules prior to their application in biological studies[1] requires several items of data including the rate of photolytic cleavage following flash irradiation, the product quantum yield, the extent of any contamination by the non-derivatised compound (*i.e.*, free glucose in the present case) and the presence of any residual biological activity in the derivatised compound itself.

The rate of photolytic cleavage of 2-nitrobenzyl compounds may be inferred from the decay rate of the *aci*-nitro intermediate species **15** in Scheme 3, which shows an outline of the currently accepted mechanism.[1] The *aci*-nitro intermediate **15** has a characteristic UV absorption in the region of 400 nm,[20] which can be monitored by time-resolved absorption spectrophotometry. The single-exponential decay of the *aci*-nitro intermediate has been definitively shown to be rate-limiting in the photolysis of the $P^3$-1-(2-nitrophenyl)ethyl ester of adenosine triphosphate[21] and is generally assumed to represent the rate of product formation in the flash photolysis of 2-nitrobenzyl




**Scheme 3**

compounds. For the glucose derivative **1**, flash photolysis in aqueous solution at pH and 22 °C had a double-exponential decay of the *aci*-nitro intermedite as shown in Fig. 1, with first-order rate constants of 110 and 12 $s^{-1}$. The amplitudes of the fast and slow phases were in the ratio 2.5:1. The origin of this biphasic decay is unclear, although it is noteworthy that similar results were observed for the simpler derivative methyl 2-nitrobenzyl ether[1b] and may thus be characteristic for 2-nitrobenzyl ethers. Further work is required to explain these observations.

The product quantum yield, $Q_P$, was determined from the mean ratio of the extents of conversion in equimolar mixtures of compound **1** and 1-(2-nitrophenyl)ethyl phosphate[22] following irradiation with UV light at 300–400 nm for varying lengths of time (see Experimental section for conditions). The glucose derivative **1** photolysed 1.17-fold more efficiently than did 1-(2-nitrophenyl)ethyl phosphate and comparison with the known[23] $Q_P$-value of 0.54 for the latter compound gives $Q_P$ 0.63 for compound **1**, similar to the value of 0.7 previously found for methyl 2-nitrobenzyl ether.[1b] In separate experiments (data not shown), solutions of compound **1** alone were photolysed and assayed both for disappearance of the starting material (by reversed-phase HPLC) and for formation of free glucose (by enzymic determination, see below). Stoicheiometric conversion was observed (range 0.96–1.04), which confirmed that measurement based on the consumption of the starting compound **1** was a valid estimation of $Q_P$.

The HPLC analysis of these photolysis mixtures was complicated by the elution of compound **1** as two partially resolved peaks of comparable height, presumably corresponding to the two anomers. Remarkably, upon photolysis the later eluting peak was depleted to a far greater extent than the earlier eluting one, which implies that the two anomers have differing values of $Q_P$, with that for the later eluting anomer being significantly higher than the mean value of 0.63. After re-equilibration of the anomers (which required at least 50 h at pH 7 and 4 °C), the original 1:1 ratio of peak heights was restored. We have not so far attempted to determine which anomer undergoes more efficient photolysis, although there are interesting possibilities for the study of photolytic mechanisms.

Lastly, a coupled enzyme assay with hexokinase and glucose-6-phosphate dehydrogenase has been used to examine, at least in one system, the effectiveness of the 2-*O*-(2-nitrobenzyl) group in blocking the biological properties of the glucose molecule. It was shown first that compound **1** was neither a substrate for

nor an inhibitor of the coupled enzymes, since the rate and extent of 1,4-dihydronicotinamide adenine dinucleotide (NADH) formation from $NAD^+$ and free glucose were unaffected by inclusion in the incubates of a 16-fold excess of compound **1** over free glucose. With this information in hand, it was possible to use the same assay (in the absence of added glucose) to determine the level of contamination of compound **1** with free glucose, which was found to be 0.8%.

Hexokinase is known to be less demanding in substrate specificity than glucose-6-phosphate dehydrogenase[24] and it was conceivable that compound **1** might be phosphorylated in the coupled enzyme system although not subsequently oxidised. However, incubation of compound **1** with adenosine triphosphate (ATP) and hexokinase alone gave no evidence of phosphorylation, as assessed by reversed-phase HPLC analysis (data not shown). Therefore, at least in this case the presence of the 2-nitrobenzyl ether effects complete blockage of biological activity. Details of biological applications of this new derivative will be reported elsewhere.

## Experimental

Analyses were carried out by Butterworth Laboratories, Teddington, Middlesex or the Chemical Analysis Centre, University of Kent, Canterbury. NMR spectra were determined, unless otherwise stated, in $CDCl_3$ on JEOL FX90Q or Bruker AM500 spectrometers with tetramethylsilane as internal standard. *J* Values are given in Hz. Optical rotations were determined on a Bendix 143C polarimeter. $[\alpha]_D$-Values are given in units of $10^{-1}$ deg cm$^2$ g$^{-1}$. Merck 9385 silica gel was used for flash chromatography. Light petroleum was the fraction boiling between 40–60 °C. Organic extracts were dried over anhydrous $Na_2SO_4$. Ammonium sulfate suspensions of yeast hexokinase and *leuconostoc. mesenteroides* glucose-6-phosphate dehydrogenase were from Sigma, Poole, Dorset (Product Nos. H5625 and G5760, respectively).

*Methyl* 2,4,6-*Tri*-O-*acetyl*-3-O-*allyl*-α- *and* -β-D-*glucoside* **7a** *and* **7b**.—A solution of 3-*O*-allyl-1,2:5,6-di-*O*-isopropylidene-D-glucofuranose[11] **6** (9.09 g, 30.3 mmol) in 1 mol dm$^{-3}$ methanolic HCl (90 cm$^3$) was heated under reflux for 2 h, then cooled, and basified with a saturated solution of ammonia in dry MeOH. The solvent was evaporated off and the residue was slurried with $CHCl_3$. The mixture was filtered, the filtrate was evaporated under reduced pressure, and the residue was acetylated by overnight treatment with acetic anhydride (30 cm$^3$) and pyridine (80 cm$^3$). These reagents were evaporated off under reduced pressure and a solution of the residue in $CHCl_3$ was washed successively with dil. hydrochloric acid, saturated aq. $NaHCO_3$, and brine, dried, and evaporated under reduced pressure. The residue was purified by flash chromatography [EtOAc–light petroleum (3:7)] to give a less polar fraction (6.42 g, 75%) and a more polar fraction (2.93 g, 26%). The less polar material was the syrupy α-anomer **7a**, $[\alpha]_D$ +93.6 (*c* 1.01, $CHCl_3$); $\delta_H$ (90 MHz) 4.78 (1 H, d, $J_{1,2}$ 3.5, 1-H), 3.39 (3 H, s, OMe), 2.12 (3 H, s, COMe) and 2.08 (6 H, s, 2 × COMe). The more polar material was the syrupy β-anomer **7b**, $[\alpha]_D$ −22 (*c* 1.08, $CHCl_3$); $\delta_H$ (90 MHz) 4.92 (1 H, d, $J_{1,2}$ 9.5, 1-H), 3.48 (3 H, s, OMe), 2.09 (3 H, s, COMe) and 2.08 (6 H, s, 2 × COMe).

*Methyl* 3-O-*Allyl*-4,6-O-*benzylidene*-α-D-*glucopyranoside* **8a**.—A solution of the triacetate **7a** (6.61 g, 18.4 mmol) in 0.3 mol dm$^{-3}$ NaOMe in dry methanol (180 cm$^3$) was heated under reflux for 0.5 h, then treated with Dowex 50 ($H^+$ form; 36 g) and water (36 cm$^3$). The resin was filtered off, and washed with methanol, and the combined filtrates were evaporated, then re-evaporated once with methanol to remove traces of water, and the residue was dissolved in $CHCl_3$ (95 cm$^3$) with α,α-dimethoxytoluene (4.04 g, 26.6 mmol) and camphorsulfonic acid (0.92 g, 4.2 mmol). The solution was heated under reflux for 20 min, then was treated with powdered $K_2CO_3$ (9.2 g) and heated under reflux for a further 30 min. The hot solution was filtered and the filter cake was washed with $CHCl_3$. The combined filtrates were evaporated and the solid residue was washed with light petroleum, then was crystallised from MeOH to yield the α-glucoside **8a** as needles (1.95 g), m.p. 156–157 °C; $[\alpha]_D$ +106.5 (*c* 1.06, $CHCl_3$) (lit.,[13] 154–155 °C; $[\alpha]_D$ +104). Flash chromatography of the mother liquor [EtOAc–light petroleum (2:3)] gave additional compound **8a** (0.65 g; total yield 44%); $\delta_H$(90 MHz) 7.27–7.59 (5 H, m, ArH), 5.76–6.17 (1 H, m, $CH_2$=C*H*), 5.52 (1 H, s, PhC*H*), 5.08–5.42 (2 H, m, C$H_2$=CH), 4.82 (1 H, d, $J_{1,2}$ 3, 1-H), 3.57–4.58 (8 H, m, sugar H and =CHC$H_2$O) and 3.43 (3 H, s, OMe).

*Methyl* 3-O-*Allyl*-4,6-O-*benzylidene*-β-D-*glucopyranoside* **8b**.—This compound was prepared from the triacetate **7b** (2.89 g) exactly as for the α-anomer **8a** and gave needles (0.97 g, 35%), m.p. 164–165 °C; $[\alpha]_D$ −46.5 (*c* 0.83, $CHCl_3$) (lit.,[8] 165–166 °C; $[\alpha]_D$ −43.1); $\delta_H$(90 MHz) 7.30–7.58 (5 H, m, ArH), 5.77–6.20 (1 H, m, $CH_2$=C*H*), 5.55 (1 H, s, ArC*H*), 5.08—5.44 (2 H, m, C$H_2$=CH), 4.38 (1 H, d, $J_{1,2}$ 10.4, 1-H) and 3.56 (3 H, s, OMe) superimposed on δ 3.28–4.59 (8 H, m, sugar H and =CHC$H_2$O).

*Methyl* 3-O-*Allyl*-4,6-O-*benzylidene*-2-O-(2-*nitrobenzyl*)-α-D-*glucopyranoside* **9**.—A solution of the alcohol **8a** (215 mg, 0.67 mmol) in dry $CH_2Cl_2$ (2 cm$^3$) was cooled to −5 °C and treated with a solution of boron trifluoride–diethyl ether (8.5 mg) in $CH_2Cl_2$ (0.17 cm$^3$), followed over a period of 15 min by a solution of 2-nitrophenyldiazomethane [prepared by stirring 2-nitrobenzaldehyde hydrazone (220 mg, 2 mmol) and manganese dioxide (0.73 g) in $CHCl_3$ (20 cm$^3$) for 15 min at room temp., followed by filtration, evaporation of the solvent under reduced pressure, and dissolution of the residue in dry $CH_2Cl_2$ (6.7 cm$^3$)]. The solution was stirred for a further 30 min at −10 °C to 5 °C, then was diluted with $CHCl_3$, washed with saturated aq. $NaHCO_3$, dried, and evaporated under reduced pressure. Flash chromatography [EtOAc–light petroleum (20:80)] gave the *nitrobenzyl ether* **9** (50 mg), which crystallised from MeOH as pale needles, m.p. 115–116 °C (Found: C, 62.7; H, 5.9; N, 3.2. $C_{24}H_{27}NO_8$ requires C, 63.0; H, 5.95; N, 3.1%); $\delta_H$ (90 MHz) 7.29–8.15 (9 H, m, ArH), 5.72–6.16 (1 H, m, $CH_2$=C*H*), 5.54 (1 H, s, PhC*H*), 5.02–5.40 (2 H, m, C$H_2$=CH), 5.13 (2 H, s, ArC$H_2$), 4.87 (1 H, d, $J_{1,2}$ 3.6, 1-H), 3.47–4.55 (8 H, m, sugar H and =CHC$H_2$O) and 3.44 (3 H, s, OMe).

2-*Nitrobenzylation of Methyl* 4,6-O-*Benzylidene*-α-D-*glucopyranoside*.—A mixture of the glucopyranoside **2a** (2.82 g, 10 mmol), dibutyltin oxide (6.84 g, 24 mmol) and TBAB (3.22 g, 10 mmol) in acetonitrile (200 cm$^3$) was stirred and heated under reflux for 7 h *via* a Soxhlet thimble which contained 4 Å molecular sieves (10 g). 2-Nitrobenzyl bromide (3.24 g, 15 mmol) was added and the solution was heated under reflux for a further 24 h, then was cooled to room temp., treated with triethylamine (15 cm$^3$), and stirred for a further 2 h to destroy excess of nitrobenzyl bromide. The mixture was filtered and partitioned between diethyl ether and water, and the ethereal phase (~200 cm$^3$) was stirred with an equal volume of aq. $NaHCO_3$ for 2 h. The ethereal phase was separated, washed successively with water and brine, dried and evaporated. The residue was fractionated by flash chromatography in EtOAc–light petroleum (45:55) to give a less polar fraction (1.62 g) and a more polar fraction (0.54 g).

The less polar fraction contained a mixture of the 2-*O*-(2-nitrobenzyl) ether **3b** and 2-nitrobenzyl alcohol and was acetylated with acetic anhydride–pyridine. After aqueous work-

up, the product was triturated with methanol to give *methyl* 3-O-*acetyl*-4,6-O-*benzylidene*-2-O-(2-*nitrobenzyl*)-α-D-*glucopyranoside* **3c** (1.10 g, 24% overall), which was crystallised from MeOH as fine needles, m.p. 140–141 °C (Found: 60.2; H, 5.5; N, 3.0. $C_{23}H_{25}NO_9$ requires C, 60.1; H, 5.5; N, 3.0%); $[\alpha]_D$ +67.7 (*c* 0.54, $CHCl_3$); $\delta_H$(90 MHz) 7.20–8.11 (9 H, m, ArH), 5.63 (1 H, t, *J* 9.8, 3-H), 5.47 (1 H, s, PhC*H*), 5.03 (2 H, ABq, *J* 14.7, Ar$CH_2$), 4.97 (1 H, d, $J_{1,2}$ 4, 1-H), 3.58–4.34 (5 H, m, sugar H), 3.42 (3 H, s, OMe) and 2.01 (3 H, s, COMe).

The more polar fraction from the flash chromatography was crystallised from benzene–diisopropyl ether to give *methyl* 4,6-O-*benzylidene*-3-O-(2-*nitrobenzyl*)-α-D-*glucopyranoside* **4b** as fine needles, m.p. 170–172 °C (Found: C, 60.6; H, 5.5; N, 3.3. $C_{21}H_{23}NO_8$ requires C, 60.4; H, 5.55; N, 3.35%); $[\alpha]_D$ +74.7 (*c* 1.07, $CHCl_3$); $\delta_H$(90 MHz) 7.21–8.01 (9 H, m, ArH), 5.51 (1 H, s, PhC*H*), 5.26 (2 H, ABq, *J* 14.9, Ar$CH_2$), 4.77 (1 H, d, *J* 3.6, 1-H), 3.54–4.34 (6 H, m, sugar H) and 3.41 (3 H, s, OMe).

Acetylation of the alcohol **4b** gave the *acetate* **4c**, which was crystallised from MeOH as pale needles, m.p. 117–118 °C (Found: C, 60.4; H, 5.5; N, 3.0. $C_{23}H_{25}NO_9$ requires C, 60.1; H, 5.5; N, 3.0%); $[\alpha]_D$ +48.9 (*c* 0.52, $CHCl_3$); $\delta_H$(90 MHz) 7.23–8.03 (9 H, m, ArH), 5.55 (1 H, s, PhC*H*), 5.22 (2 H, ABq, *J* 15.2, Ar$CH_2$), 4.96 (1 H, dd, *J* 3.5, 8.8, 2-H), 4.88 (1 H, d, 1-H), 3.57–4.35 (5 H, m, sugar H), 3.37 (3 H, s, OMe) and 2.09 (3 H, s, COMe).

*Allylation of Alcohols* **3b** *and* **4b**.—A solution of crude compound **3b** [contaminated with 2-nitrobenzyl alcohol as described above; total mass (177 mg)] in dry DMF (8 $cm^3$) was stirred under nitrogen with sodium hydride (60% suspension in oil; 0.30 g) for 20 min, then was treated with allyl bromide (0.5 $cm^3$). The mixture was stirred at room temp. overnight, then was treated with triethylamine (1 $cm^3$) and stirred for a further 2 h to destroy excess of allyl bromide. After quenching with excess of MeOH, the mixture was diluted with diethyl ether and the ethereal solution was washed successively with water, dil. hydrochloric acid, and brine, dried and evaporated, to leave a brown gum (200 mg), which by TLC [silica gel; EtOAc–light petroleum (25:75)] showed two UV-active components, only one of which charred on heating after spraying with conc. sulfuric acid. This material was slightly less polar on TLC than the 3-*O*-allyl ether **9**, and after isolation by flash chromatography was shown to be the 2,3-di-*O*-allyl compound **11** (50 mg), m.p. 64–65 °C (from light petroleum), (lit.,[25] 62–63 °C); $\delta_H$(90 MHz) 7.28–7.54 (5 H, m, ArH), 5.71–6.15 (2 H, m, 2 × $CH_2$=C*H*), 5.51 (1 H, s, PhC*H*), 5.02–5.39 (4 H, m, 2 × C$H_2$=CH), 4.75 (1 H, d, *J* 3.6, 1-H), 3.36–4.48 (10 H, m, sugar H and 2 × =CHC$H_2$O) and 3.40 (3 H, s, OMe).

The same sole carbohydrate product was isolated when pure compound **4b** was subjected to the identical procedure.

*Methyl* 3,4,6-*Tri*-O-*acetyl*-2-O-(2-*nitrobenzyl*)-α-D-*glucopyranoside* **13**.—A solution of the monoacetate **3c** (2.10 g, 4.57 mmol) in a mixture of MeOH (380 $cm^3$) and 1 mol $dm^{-3}$ aq. HCl (42 $cm^3$) was kept at room temp. for 3 h, then was concentrated under reduced pressure to ~100 $cm^3$, diluted with water, and extracted with ethyl acetate. The extract was washed successively with aq. $NaHCO_3$ and brine, dried, and concentrated, and the crude compound **12** was acetylated with acetic anhydride–pyridine to give an oil which, after flash chromatography [EtOAc–light petroleum (35:65)] gave the *triacetate* **13** as pale yellow crystals (1.65 g, 79%). Recrystallisation from MeOH gave hexagonal prisms, m.p. 96–97 °C (Found: C, 52.4; H, 5.45; N, 3.0. $C_{20}H_{25}NO_{11}$ requires C, 52.7; H, 5.5; N, 3.1%); $[\alpha]_D$ +117.8 (*c* 1.01, $CHCl_3$); $\lambda_{max}$(EtOH)/nm 252 (ε 5300 $dm^3$ $mol^{-1}$ $cm^{-1}$); $\delta_H$(90 MHz) 7.34–8.14 (4 H, m, ArH), 5.52 (1 H, t, *J* 9.7, 3-H), 5.03 (1 H, t, *J* 9.4, 4-H) superimposed on 5.03 (2 H, ABq, *J* 15.6, Ar$CH_2$), 4.98 (1 H, d, *J* 3.6, 1-H), 4.30 (1 H, dd, *J* 12.3, 4.4, 6-H), 4.08 (1 H, dd, *J* 12.3, 2.2, 6′-H), 3.87–4.10 (1 H, m, 5-H), 3.71 (1 H, dd, 2-H), 3.46 (3 H, s, OMe) and 2.09, 2.03 and 2.00 (9 H, 3 × s, 3 × COMe).

2-O-(2-*Nitrobenzyl*)-D-*glucose* **1**.—A solution of the triacetate **13** (1.33 g, 2.95 mmol) in acetic anhydride (15.3 $cm^3$) was cooled in an ice-bath and treated with boron trifluoride–diethyl ether (0.61 $cm^3$). The mixture was stirred for 4 h at 0 °C, quenched by addition of ice-cold, aq. $NaHCO_3$, and extracted with ethyl acetate. The extract was washed successively with aq. $NaHCO_3$ and brine, dried, and evaporated, then kept under vacuum for 16 h to remove residual acetic anhydride to leave the tetraacetate **14** as a gum (1.43 g, 100%), which ran as a single spot on TLC [EtOAc–light petroleum (35:65)] but was shown by NMR spectroscopy to be a ~1:1 mixture of the α- and β-anomer [$\delta_H$ 6.48 (d, *J* 3.5, α-anomer 1-H) and 5.42 (d, *J* 9.6, β-anomer 1-H)].

Without further purification, a portion (307 mg, 0.64 mmol) of this material was dissolved in methanol (3.8 $cm^3$) and the solution was treated with 2 mol $dm^{-3}$ aq. NaOH (1.38 $cm^3$). After 1 h at room temp., MeOH-washed Dowex 50 ($H^+$-form; 2.5 g) was added, stirred into the mixture until the solution became neutral, and removed by filtration. The filtrate was concentrated, redissolved in water (approx. 25 $cm^3$), and lyophilised to leave the 2-*nitrobenzyl compound* **1** as a pale yellow foam (158 mg, 76%) (Found: C, 47.9; H, 5.45; N, 3.9. $C_{13}H_{17}NO_8 \cdot \frac{1}{2}H_2O$ requires C, 48.1; H, 5.6; N, 4.3%); $[\alpha]_D$ +35.2 (*c*. 1.03, water); $\lambda_{max}(H_2O)$/nm 265 (ε 5300 $dm^3$ $mol^{-1}$ $cm^{-1}$); $\delta_H$(500 MHz; $D_2O$; acetone standard) 8.06–8.08 (1 H, m, Ar 3-H), 7.78 (1 H, d, *J* 7.7, Ar 6-H), 7.72–7.76 (1 H, m, Ar 5-H), 7.54–7.58 (1 H, m, Ar 4-H), 5.37 (0.5 H, d, $J_{1,2}$ 3.6, α 1-H), 5.19 (1 H, ABq, *J* 13.4, β ArOC$H_2$), 5.06 (1 H, s, α ArOC$H_2$), 4.73 (0.5 H, d, *J* 7.9, β 1-H), 3.89 (0.5 H, dd, $J_{6,6'}$ 12.3, $J_{5,6}$ 2.1, β 6-H), 3.82–3.85 (1 H, m, α 5- and 6-H), 3.81 (0.5 H, t, $J_{2,3}$ 9.8, α 3-H), 3.75 (0.5 H, dd, $J_{6,6'}$ 12.5, $J_{5,6'}$ 5.7, α 6′-H), 3.71 (0.5 H, dd, $J_{5,6'}$ 5.5, β 6′-H), 3.57 (0.5 H, t, *J* 9.0, β 3-H), 3.46 (0.5 H, dd, α 2-H), 3.38–3.45 (1.5 H, m, α 4-H and β 4- and 5-H) and 3.23 (0.5 H, dd, β 2-H). The α and β annotations denote signals arising from the α- and β-anomers respectively. Signal assignments were derived from a combination of ROESY and DQF-COSY spectra.

*Photolysis Kinetics of* 2-O-(2-*Nitrobenzyl*)-D-*glucose* **1**.—The kinetics were analysed in an absorption spectrophotometer in which light from a xenon arc lamp was passed horizontally through a monochromator set to 406 nm and the sample in a 4 mm path-length cuvette, then through a 406 nm interference filter and a second monochromator. The photolysis was initiated by a 1 μs 320 nm pulse from a frequency-doubled tunable dye laser that illuminated the optical cell orthogonally to the detection light beam. Other details of the spectrophotometer were as previously described.[21] The solution for photolysis contained the nitrobenzyl ether **1** (1 mmol $dm^{-3}$) in pH 7 sodium phosphate buffer (150 mmol $dm^{-3}$).

*Extent of Photolysis of Compound* **1**.—Aliquots (0.5 $cm^3$) of a solution of compound **1** and 1-(2-nitrophenyl)ethylphosphate (each 50.5 μmol $dm^{-3}$) with dithiothreitol (2 mmol $dm^{-3}$) in pH 7.0 sodium phosphate (10 mmol $dm^{-3}$) were exposed for varying times (8–24 s) to light from a mercury arc lamp which was passed through a 300–400 nm bandpass filter before illuminating the cell. The irradiated samples were kept at 4 °C for 70 h to allow the anomers of the residual nitrobenzyl compound **1** to re-equilibrate, and analysed by reversed-phase HPLC [Merck Lichrosphere RP8 column (Cat. No. 50832); mobile phase 10 mmol $dm^{-3}$ sodium phosphate, pH 7.0, plus

15% MeOH (v/v); flow rate 1.5 $cm^3$ $min^{-1}$; UV detection at 254 nm]. 1-(2-Nitrophenyl)ethylphosphate eluted at 1.9 min, and the glucose derivative **1** eluted as a double peak at 4.9 and 5.7 min. The extents of conversion for the phosphate ester and the glucose derivative were 17.9–42.7 and 21.0–48.1%, respectively (means of 3 determinations at each time point), with the glucose derivative **1** converted 1.17-fold more efficiently than the phosphate ester.

*Enzymic Glucose Assay.*—Aliquots (1 $cm^3$) of solutions containing ATP, $MgSO_4$, $NAD^+$ and dithiothreitol (each 1 mmol $dm^{-3}$) and glucose (0–100 μmol $dm^{-3}$) in 10 mmol $dm^{-3}$ sodium 3-(*N*-morpholino)propanesulfonate (MOPS), pH 7.6, were equilibrated at 25 °C in a Beckman DU 70 spectrophotometer operating at 340 nm. The absorbance was monitored for 30 s, when an aliquot (10 $mm^3$) of a mixture of hexokinase and glucose-6-phosphate dehydrogenase [prepared by addition of ammonium sulfate suspensions of hexokinase (2.5 $mm^3$) and the dehydrogenase (6.8 $mm^3$) to the MOPS buffer (191 $mm^3$)] was added and mixed rapidly. Absorbance was monitored until it reached a steady value (*ca.* 4 min). Glucose oxidation was quantitative at each concentration as determined from the absorbance changes [based on NADH, lit.,[26] $\lambda_{max}(H_2O)$/nm 340 (ε 6220 $dm^3$ $mol^{-1}$ $cm^{-1}$)].

*Assessment of Substrate/Inhibitor Activity and Purity of* 2-O-(2-*Nitrobenzyl*)-D-*glucose.*—Aliquots (1 $cm^3$) of solutions containing ATP, $MgSO_4$, $NAD^+$ and dithiothreitol (each 1 mmol $dm^{-3}$) in MOPS buffer as described above, together with glucose (44 μmol $dm^{-3}$) and 2-*O*-(2-nitrobenzyl)glucose (0–710 μmol $dm^{-3}$) were incubated at 25 °C and treated with an aliquot (10 $mm^3$) of the hexokinase–glucose-6-phosphate dehydrogenase mixture as described above. The extent of NADH formation, as measured by the change in absorbance at 340 nm (0.271 ± 0.005 S.D.) was 43.6 ± 0.8 μmol $dm^{-3}$, indicating that only the free glucose was oxidised. The rate of the absorbance change was identical in all incubates.

In a separate experiment, the ATP–$MgSO_4$–$NAD^+$–dithiothreitol solution also containing 2-*O*-(2-nitrobenzyl)-D-glucose (100 μmol $dm^{-3}$) was incubated with the mixed enzymes. The absorbance change at 340 nm was 0.005, corresponding to formation of 0.80 μmol $dm^{-3}$ NADH. The maximum contamination with free glucose was therefore 0.80%.

## Acknowledgements

I am grateful to Miss S. Lathwell for measurements of optical rotations, to Drs. J. Feeney and T. A. Frenkiel for high-field 1D- and 2D-NMR determinations, and to Dr. D. R. Trentham for the time-resolved flash photolysis data. I thank Dr. R. Gigg for helpful discussions.

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*Paper* 3/02781C
*Received* 17*th May* 1993
*Accepted* 28*th May* 1993