(Halogenomethyl)phenyl α-D-Glucopyranosides as Enzyme-activated Irreversible Inhibitors of Yeast α-Glucosidase and Potential Anti-HIV Agents

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A range of (halogenomethyl)phenyl α-D-glucopyranosides **2–7**, prepared from corresponding methylphenyl glucosides by synthetic manipulation of the aglycone moiety, have been investigated as enzyme-activated irreversible inhibitors of yeast α-glucosidase and their anti-HIV activity measured. Compounds **5–7**, which also contain a 4- or 6-nitro group in the phenyl ring of the aglycone, are much more effective inhibitors of the enzyme than are compounds **2–4** which lack this feature.

Glycosidase inhibitors are currently of interest in view of potential applications in the treatment of certain diseases.[1–4] In particular, some competitive inhibitors of α-glucosidases, such as 1-deoxynojirimycin and castanospermine, show anti-HIV activity,[5] and one such compound, *N*-butyl-1-deoxynojirimycin **1**, is undergoing clinical trials.[6] The anti-HIV activity of compound **1** is thought to result from its inhibition of α-glucosidases involved in the processing of N-linked oligosaccharides on the viral coat during assembly, thus preventing successful completion of the viral coat and hence reproduction of infectious virus.[7]

Nojirimycin and related compounds are reversible glycosidase inhibitors.[8] Irreversible inhibitors, for example bromoconduritol, are also known.[8] Relatively little attention has been given, however, to enzyme-activated irreversible inhibitors, which produce their effect as a result of being activated by the specific action of the target enzyme.[9–12] Such agents allow a given biochemical process to be precisely targetted and are thus attractive as therapeutic agents. Only a few examples of glycoside-based, enzyme-activated irreversible inhibitors of glucosidases are known, examples being, for β-glucosidases, 2,4-dinitrophenyl 2-deoxy-2-fluoro-β-D-glucopyranoside[13] and 2- and 4-(difluoromethyl)aryl β-D-glucopyranosides,[14] and, for α-glucosidases, 1,1-difluoroalkyl α-D-glucopyranosides.[15]

Recently we have synthesized a number of novel (halogenomethyl)phenyl α-D-glucopyranosides **2–7** with the aim that they might act as enzyme-activated irreversible inhibitors of α-glucosidases and, therefore, have possible application as anti-HIV agents. In a preliminary communication[16] we gave details of two of these, compounds **2** and **5**, and gave evidence that compound **5** does indeed act as an enzyme-activated irreversible inhibitor of yeast α-glucosidase. We now report the synthesis of other members of this group, further inhibition studies on yeast α-glucosidase, and results of anti-HIV tests with these compounds.

A possible mode of inhibitory action of this type of glycoside involves initial enzymic liberation of the aglycone, for example 2-chloromethyl-4-nitrophenol from glycoside **5**, within or near the active site. Compounds of this type are known to be particularly reactive and, by analogy with Koshland's reagent, 2-bromomethyl-4-nitrophenol,[17,18] should rapidly alkylate nucleophiles, most probably *via* an elmination–addition process involving a quinone methide intermediate. Support for the intermediacy of a quinone methide intermediate comes from a study of the enzymic hydrolysis of the natural β-glucoside salicortin.[19] Should this alkylation involve an important nucleophilic centre at the enzyme's active site, or lead to steric hindrance at this site, then inactivation of the enzyme might result.

Results and Discussion

Bromination of 2-methylphenyl tetra-*O*-acetyl-α-D-glucopyranoside[20] **9** with 1,3-dibromo-5,5-dimethylhydantoin (DBDMH) in benzene in the presence of azoisobutyronitrile (AIBN) gave 2-(bromomethyl)phenyl tetra-*O*-acetyl-α-D-glucopyranoside **10**. Attempts to deacetylate this compound were unsuccessful but halogen exchange using tetrabutylammonium chloride (TBAC) or fluoride (TBAF) in acetonitrile gave tetraacetates **11** and **12**, respectively, which on deacylation gave the glucosides **2** and **3**, respectively. 4-(Chloromethyl)phenyl α-D-glucopyranoside **4** was similarly prepared from 4-methylphenyl tetra-*O*-acetyl-α-D-glucopyranoside **13** *via* the 4-(bromomethyl)phenyl and 4-(chloromethyl)phenyl glycosides **14** and **15**, respectively.

In order to increase the reactivity of the phenol which is liberated on enzymic hydrolysis, it was thought desirable to introduce a nitro group into the *ortho*- or *para*-position with respect to the phenolic linkage. Nitration of compound **9** with nitric acid–trifluoroacetic anhydride (TFAA) gave a mixture of the 4- and 6-nitrophenyl glucosides **16** and **17**, which were separated by column chromatography. These were converted into the respective glucosides **5** and **7** through intermediates **18** and **19**, and **20** and **21**, respectively, by the same reaction sequence used to make the non-nitrated glucoside **2** from tetraacetate **9**. The bromination of compound **16** to give bromide **18** produced as a by-product the dibromo derivative **22** which, without purification, was converted by halide exchange and deacylation of the product into glucoside **6**.

In order to confirm enzyme specifity, the β-glucoside **27**, the anomer of compound **5**, was prepared from the glucoside tetraacetate **23**, in the usual manner, *via* intermediates **24–26**.

A comparison of the action of yeast α-glucosidase on 4-nitrophenyl α-D-glucopyranoside **8** and on compound **5** suggests that the enzyme is rapidly deactivated on liberation of the aglycone from glucoside **5** since, under conditions which led to 50% hydrolysis of compound **8**, less than 4% of compound **5** was hydrolysed. The activity of the α-glucosidase was unaffected by incubation with the β-glycoside **27**, confirming that enzymic hydrolysis of the glucoside **5** was a prerequisite for deactivation.

Time-dependent inhibition of yeast α-glucosidase by the α-glucosides **2–7** was investigated in piperazine-*N*,*N*′-bis(ethane-2-sulfonic acid) (PIPES) buffer (pH 6.8) at 30 °C, by measuring the initial rate of liberation of 4-nitrophenol from glucoside **8** (5 mmol dm^{-3}) in the presence of a certain concentration of the

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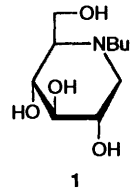

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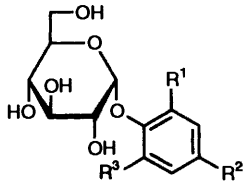


2 $R^1 = CH_2Cl$, $R^2 = R^3 = H$

3 $R^1 = CH_2F$, $R^2 = R^3 = H$

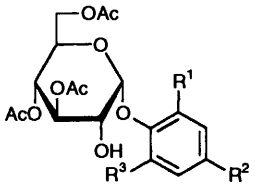
4 $R^1 = R^3 = H$, $R^2 = CH_2Cl$

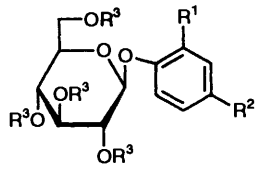
5 $R^1 = CH_2Cl$, $R^2 = NO_2$, $R^3 = H$

6 $R^1 = CHCl_2$, $R^2 = NO_2$, $R^3 = H$

7 $R^1 = CH_2Cl$, $R^2 = H$, $R^3 = NO_2$

8 $R^1 = R^3 = H$, $R^2 = NO_2$





9 $R^1 = Me$, $R^2 = R^3 = H$

10 $R^1 = CH_2Br$, $R^2 = R^3 = H$

11 $R^1 = CH_2Cl$, $R^2 = R^3 = H$

12 $R^1 = CH_2F$, $R^2 = R^3 = H$

13 $R^1 = R^3 = H$, $R^2 = Me$

14 $R^1 = R^3 = H$, $R^2 = CH_2Br$

15 $R^1 = R^3 = H$, $R^2 = CH_2Cl$

16 $R^1 = Me$, $R^2 = NO_2$, $R^3 = H$

17 $R^1 = Me$, $R^2 = H$, $R^3 = NO\text{-}2$

18 $R^1 = CH_2Br$, $R^2 = NO_2$, $R^3 = H$

19 $R^1 = CH_2Cl$, $R^2 = NO_2$, $R^3 = H$

20 $R^1 = CH_2Br$, $R^2 = H$, $R^3 = NO_2$

21 $R^1 = CH_2Cl$, $R^2 = H$, $R^3 = NO_2$

22 $R^1 = CHBr_2$, $R^2 = NO_2$, $R^3 = H$

23 $R^1 = Me$, $R^2 = H$, $R^3 = Ac$

24 $R^1 = Me$, $R^2 = NO_2$, $R^3 = Ac$

25 $R^1 = CH_2Br$, $R^2 = NO_2$, $R^3 = Ac$

26 $R^1 = CH_2Cl$, $R^2 = NO_2$, $R^3 = Ac$

27 $R^1 = CH_2Cl$, $R^2 = NO_2$, $R^3 = H$

inhibitor, after pre-incubation of the enzyme with the inhibitor for various time intervals. The resultant graphs are shown in Fig. 1, which indicate that all of the new α-glucosides do indeed act as irreversible glucosidase inhibitors, an indication which was unambiguously confirmed in the case of compound **5** (see later).

With the non-nitrated glucosides **2**–**4** at a concentration of 2 mmol dm^{-3} there was an immediate drop in enzyme activity on addition of the inhibitor ($t = 0$) to 44, 57 and 71%, respectively, suggesting that the compounds are acting as competitive inhibitors before hydrolysis occurs. This initial drop in activity was followed by a steady loss of enzyme activity over a period of 1 h to 14, 22 and 42%, respectively.

The nitrophenyl α-glucosides **5**–**7** are all much more potent inhibitors of yeast α-glucosidase than are the non-nitrated analogues. At an inhibitor concentration of 0.02 mmol dm^{-3}, a drop in enzyme activity from 75, 56 and 88%, respectively, to below 20% occurred after only 10 min (Fig. 1). Further experiments with inhibitor **5** showed that the rate of time-dependent loss of enzyme activity is related to the inhibitor concentration.[16] Pseudo-first-order kinetics were observed and a Kitz–Wilson plot[21] gave a dissociation constant for the initial

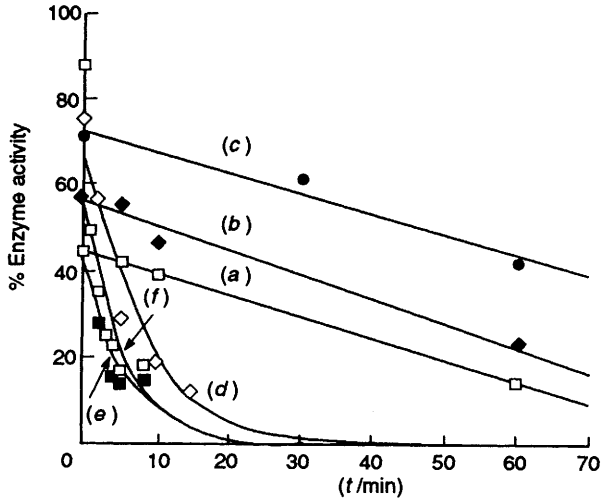


Fig. 1 Plots (a), (b), (c), (d), (e) and (f) are of %Enzyme activity against Incubation time for the inhibition of yeast α-glucosidase with compounds **2**–**7**, respectively. The concentration of inhibitors **2**–**4** during incubation with the enzyme was 2 mmol dm^{-3}; for inhibitors **5**–**7** it was 0.02 mmol dm^{-3}. Assays were performed at pH 6.8 and 30 °C.

reversible complex (K_i) of 2.5 (±0.1) × 10^{-3} mmol dm^{-3} and a rate constant for the conversion of this complex into the irreversibly inhibited enzyme of 3.05 (±0.03) × 10^{-3} s^{-1}. Protection of the α-glucosidase was achieved[16] when incubation with compound **5** was conducted in the presence of the competitive inhibitor 5-thio-D-glucose[22] providing further evidence for involvement of the active site in the inhibitory process. Furthermore, dialysis of the enzyme which had been deactivated by incubation with compound **5** for 10 min gave only 10% of the activity of the control, suggesting that covalent linkage between the active site and the inhibitory species from compound **5** had occurred. In contrast, enzymic activity was fully restored in a similar experiment where the activity had been reduced by incubation with 5-thio-D-glucose. Finally, an experiment was conducted to show that deactivation was not the result of the liberated aglycone leaving the active site and then reacting indiscriminately with the enzyme. In this experiment, deactivation of the enzyme by compound **5** was compared in the presence and in the absence of cysteine. The latter compound would reasonably be expected to react with 2-chloromethyl-4-nitrophenol, or the corresponding quinone methide, which might escape from the enzyme's active site into the bulk solution through nucleophilic attack on such species by the thiol group of the amino acid. In fact, the rate of enzyme deactivation by compound **5** was not affected by the presence of cysteine and, in a separate experiment, it was shown that cysteine did not affect the activity of the uninhibited enzyme.

Enzyme specificity was confirmed by the fact that the α-glucosides **2** and **5** did not inhibit almond β-glucosidase.

The α-glucosides **2**–**7** and their tetraacetates* were submitted for anti-HIV screening. Disappointingly, the α-glucosides **2**–**4** were inactive, possibly due to poor membrane penetration. It was hoped that the corresponding tetraacetates, compounds **11**, **12** and **15**, respectively, would cross the cell membrane more easily and that subsequent enzymic deacetylation would release the active compound inside the cell. Compounds **11** and **12**, but not **15**, did indeed show weak anti-HIV activity, reducing HIV-1 progeny in infected cell (C 8166) cultures by 50% at 40 and 400 μmol dm^{-3}, respectively. Interestingly, separate experiments using a T-cell clone (CEM 4) suggested that compound **2**, but

* The tetraacetate of glycoside **6** was not obtained pure and contained approximately 8% of the monochloro compound **19**.

not its tetraacetate **11**, does inhibit, to a small extent, glucosidase trimming of N-linked oligosaccharides. The anti-HIV activity of the tetraacetate **11** seems to arise from a cause other than an effect on oligosaccharide trimming, possibly by inhibition of oligosaccharide synthesis. This observation raises the intriguing possibility that compounds analogous to **11** could have a two-fold action, first as inhibitors of oligosaccharide synthesis and then, when enzymically deacetylated, of oligosaccharide processing.

Unfortunately, the nitrated α-glucosides **5–7** and their tetraacetates all showed high toxicity in this assay.

Experimental

^{1}H NMR spectra were recorded in $CDCl_3$ or CD_3OD (internal Me_4Si) either at 60 MHz with a JEOL PMX60si spectrometer or at 400 MHz with a JEOL GX-400 spectrometer. *J* Values are given in Hz. Accurate mass measurement was performed on a VG Analytical Mass Spectrometer (Manchester, U.K.) in the FAB mode. Optical rotations were measured with a Perkin-Elmer 141 polarimeter and $[\alpha]_D$ units are recorded in 10^{-1} deg cm^2 g^{-1}. Enzyme assays were monitored with a Pye-Unicam PU 8800 UV/Visible spectrophotometer fitted with a cell-temperature controller. TLC and column chromatography was performed on silica gel (Machery-Nagel), SIL G-25UV$_{254}$ and Silica Gel 60 (Merck, 70–230 mesh), respectively. α-Glucosidase (type VI from brewer's yeast), β-glucosidase (from almonds, chromatographically purified), 4-nitrophenyl α-D-glucopyranoside **8**, and 4-nitrophenyl β-D-glucopyranoside were purchased from the Sigma Chemical Co. Ltd. 2-Methylphenyl tetra-*O*-acetyl-α- and -β-D-glucopyranoside (**9** and **23**, respectively) were prepared by fusion of 2-methylphenol with penta-*O*-acetyl-α-D-glucopyranose, essentially as described,[20,23] but by using the improved general method of Hudson and co-workers,[24] involving zinc chloride–acetic acid–acetic anhydride as the catalyst. Light petroleum refers to the fraction with distillation range 60–80 °C.

2-(*Bromomethyl*)*phenyl Tetra*-O-*acetyl*-α-D-*glucopyranoside* **10**.—A mixture of 2-methylphenyl tetra-*O*-acetyl-α-D-glucopyranoside **9** (7.00 g, 16.0 mmol) dry benzene (20 cm^3), DBDMH (2.40 g, 8.30 mmol) and a catalytic amount of AIBN (0.05 g) was heated under reflux for 1 h, with irradiation by a 150-W tungsten lamp. A further portion (0.05 g) of AIBN was added after 30 min. The mixture was filtered while hot, the collected solid was washed with chloroform, and the combined filtrates were evaporated, leaving a syrup. Trituration with hexane, followed by crystallisation of the residue from chloroform–hexane gave, as pale yellow crystals, compound **10** (3.44 g, 42%), m.p. 86–89 °C; $[\alpha]_D$ +142 (*c* 1, $CHCL_3$) {lit.,[20] m.p. 94–95.5 °C; $[\alpha]_D$ +145.6 ($CHCl_3$)}; ν_{max}(Nujol)/cm^{-1} 1750 (CO), 1602, 1590, 1494 (Ar), 1230 and 1040 (CO–O); δ_H(60 MHz; $CDCl_3$) 2.00–2.18 (12 h, complex, 4 × MeCO), 3.98–4.31 (3 H, complex, 5-H and 6-H$_2$), 4.60 (2 H, s, CH_2Br), 4.95–5.38 (2 H, complex, 2- and 4-H), 5.60–5.84 (2 H, complex, 1- and 3-H) and 6.82–7.46 (4 H, complex, C_6H_4).

2-(*Chloromethyl*)*phenyl Tetra*-O-*acetyl*-α-D-*glucopyranoside* **11**.—A solution of compound **10** (1.90 g, 3.66 mmol) and TBAC (6.50 g, 23.9 mmol) in acetonitrile (25 cm^3) was heated under reflux for 4 h. The residue obtained upon concentration was subjected to column chromatography [light petroleum–ethyl acetate (3:2)], and recrystallisation of the major component from dichloromethane–hexane gave compound **11** (1.19 g, 69%), m.p. 100–101 °C (Found: C, 53.7; H, 5.4; Cl, 7.5. $C_{21}H_{25}ClO_{10}$ requires C, 53.3; H, 5.3; Cl, 7.5%); $[\alpha]_D$ +154 (*c* 0.9, $CHCl_3$); ν_{max}(Nujol)/cm^{-1} 1750br (CO), 1602, 1590, 1495 (Ar), 1225, 1040 (CO–O) and 672 (C–Cl); δ_H(400 MHz; $CDCl_3$) 2.05, 2.06, 2.07 and 2.08 (4 × 3 H, 4 s, 4 × MeCO), 4.09 (1 H, dd, $J_{5,6}$ 2.1, $J_{6,6'}$ 12.2, 6-H), 4.18 (1 H, m, 5-H), 4.27 (1 H, dd, $J_{5,6'}$ 4.6, 6-H'), 4.69 (1 H, d, $J_{A,B}$ 11.3, CH_AH_BCl), 4.74 (1 H, d, CH_AH_BCl), 5.10 (1 H, dd, $J_{1,2}$ 3.5, $J_{2,3}$ 10.2, 2-H), 5.19 (1 H, t, $J_{3,4} = J_{4,5}$ = 9.6, 4-H), 5.76 (1 H, d, 1-H), 5.74 (1 H, br t, 3-H), 7.08 (1 H, td, J_m 1.1, $J_o = J_{o'}$ = 7.5, 4″-H),* 7.19 (1 H, dd, J_m 0.9, J_o 8.2, 6″-H), 7.30 (1 H, m, 5″-H) and 7.40 (1 H, dd, J_m = 1.7, 3″-H).

2-(*Chloromethyl*)*phenyl* α-D-*Glucopyranoside* **2**.—To a solution of compound **11** (1.40 g, 2.96 mmol) in warm methanol (60 cm^3) was added a catalytic amount of sodium (~0.05 g) and after 10 min the solution was neutralised with Amberlite IR-120 (H^+) resin. The solution was concentrated and the product was isolated by column chromatography [ethyl acetate–methanol (9:1)], followed by recrystallisation of the major component from methanol–ethyl acetate to give *compound* **2** (0.57 g, 63%), m.p. 104–106 °C (decomp.) (Found: C, 51.5; H, 5.7. $C_{13}H_{17}ClO_6$ requires C, 51.2; H, 5.6%); $[\alpha]_D$ +192 (*c* 1.0, MeOH); ν_{max}(Nujol)/cm^{-1} 3350br (OH), 1040br (C–O) and (C–Cl); δ_H(60 MHz; CD_3OD) 3.24–4.05 (6 H, complex, 2-, 3-, 4-, 5-H and 6-H$_2$), 4.68–4.90 (6 H, complex, CH_2Cl, 4 × OH), 5.57 (1 H, d, $J_{1,2}$ 3.6, 1-H) and 6.88–7.57 (4 H, m, C_6H_4).

2-(*Fluoromethyl*)*phenyl Tetra*-O-*acetyl*-α-D-*glucopyranoside* **12**.—Compound **10** (0.50 g, 0.97 mmol) was treated with TBAF (1.50 g, 5.74 mmol) in acetonitrile (7 cm^3), as described for the preparation of compound **11**. After column chromatography [light petroleum–ethyl acetate (3:2)] to remove the ammonium salts, the bulk of the material obtained, which was compound **12** contaminated with partly deacetylated products, was used for the preparation of compound **3** without further purification.

A portion of the crude material was treated with acetic anhydride–pyridine to give material, which was purified by column chromatography [light petroleum–ethyl acetate (1:1)] to give, as a syrup, compound **12** (68%) (Found: C, 55.6; H, 5.7. $C_{21}H_{25}FO_{10}$ requires C, 55.3; H, 5.5%); δ_H(60 MHz; $CDCl_3$) 2.04 (12 H, s, 4 × MeCO), 4.06–4.51 (3 H, complex, 5-H and 6-H$_2$), 4.90–5.76 (4 H, complex, 1-, 2-, 3- and 4-H), 5.48 (2 H, d, $J_{H,F}$ 47.5, CH_2F) and 6.96–7.50 (4 H, complex, C_6H_4).

2-(*Fluoromethyl*)*phenyl* α-D-*Glucopyranoside* **3**.—Crude 2-(fluoromethyl)phenyl tetra-*O*-acetyl-α-D-glucopyranoside **12** (0.38 g) was treated in the usual manner with a catalytic amount of sodium methoxide in methanol (20 cm^3) for 10 min. Column chromatography [ethyl acetate–methanol (9:1)] of the residue obtained on evaporation gave *compound* **3** (0.17 g, 61%), m.p. 120–122 °C (Found: C, 53.9; H, 6.0. $C_{13}H_{17}FO_6$ requires C, 54.2; H, 6.0%); $[\alpha]_D$ +102 (*c* 1.0, MeOH); ν_{max}(Nujol)/cm^{-1} 3350br (OH), 1606, 1592, 1493 (Ar), 1080br (C–O), 700w (C–F); δ_H(60 MHz; CD_3OD) 3.26–3.98 (6 H, complex, 2-, 3-, 4-, 5-H and 6-H$_2$), 4.72 (4 H, s, 4 × OH), 5.55 (1 H, d, $J_{1,2}$ 3.0, 1-H), 5.56 (2 H, d, $J_{H,F}$ 48 CH_2F) and 6.76–7.46 (4 H, complex, C_6H_4).

4-*Methylphenyl Tetra*-O-*acetyl*-α-D-*glucopyranoside* **13**.—Heating of penta-*O*-acetyl-α-D-glucopyranose (100 g, 0.26 mol), 4-methylphenol (110 g, 1.02 mol) and fused zinc chloride (25 g, 0.19 mol) in an acetic acid–acetic anhydride mixture[24] (19:1 v/v; 75 cm^3) at 120 °C, and work-up by the usual procedure[24] gave the anomeric mixture of glycoside tetraacetates as a syrup, from a solution of which in ethanol crystallised the β-anomer (13.3 g, 12%), m.p. 119–120 °C; $[\alpha]_D$ −14.8 (*c*, 2 in $CHCl_3$) (lit.,[25] m.p. 120 °C). The mother liquors were concentrated, the

* Double-primed numbers refer to the aromatic moiety; $J_o = J_{ortho}$, $J_m = J_{meta}$, and $J_p = J_{para}$.

residue was deacetylated by treatment with methanol containing a catalytic amount of sodium methoxide, and the residue obtained on concentration was crystallised from methanol to give 4-methylphenyl α-D-glucopyranoside (6.04 g, 49%), m.p. 190–192 °C; $[\alpha]_D$ +177 (*c* 0.3, water) {lit., [26] m.p. 190–191 °C; $[\alpha]_D$ +178 (water); lit.,[27] m.p. 190 °C; $[\alpha]_D$ +175 (*c* 1, water)}. Reacetylation of this glycoside (4.6 g, 17 mmol) in the usual manner with acetic anhydride (11 cm^3, 0.116 mol) in pyridine (20 cm^3) and crystallisation of the product from methanol gave *compound* **13** (5.66 g, 76%); m.p. 85–87 °C (Found: C, 57.5; H. 6.0. $C_{21}H_{26}O_{10}$ requires C, 57.5; H, 6.0%); $[\alpha]_D$ +181 (*c* 2.2, CH_2Cl_2) {lit.,[26] $[\alpha]_D$ +162 ($CHCl_3$)]}; ν_{max}(Nujol)/cm^{-1} 1755 (CO), 1512 (Ar), 1226 and 1050 (CO–O); δ_H(60 MHz; $CDCl_3$) 2.04 (12 H, s, 4 × MeCO), 2.30 (3 H, s, *Me*Ar), 4.04–4.36 (3 H, complex, 5-H and 6-H_2), 4.85–5.16 (2 H, complex, 2- and 4-H), 5.52–5.88 (2 H, complex, 1- and 3-H) and 6.81–7.20 (4 H, m, AA'BB' system of 4-MeC_6H_4O).

4-(*Bromomethyl*)*phenyl Tetra*-O-*acetyl*-α-D-*glucopyranoside* **14**.—A mixture of compound **13** (2.0 g, 4.56 mmol), DBDMH (0.68 g, 2.37 mmol), and AIBN (0.05 g) in benzene (30 cm^3) was heated under reflux with irradiation, as for the preparation of compound **10**, for 90 min. Isolation of the product in the manner described for compound **10** and recrystallisation of the crude product from ethyl acetate–hexane gave *title compound* **14** (1.75 g, 74%); m.p. 96–99 °C (Found: C, 48.5; H, 4.8; Br, 15.7. $C_{21}H_{25}BrO_{10}$ requires C, 48.8; H, 4.9; Br, 15.4%); $[\alpha]_D$ +150 (*c* 1.0, CH_2Cl_2); δ_H(60 MHz; $CDCl_3$) 1.92–2.16 (12 H, m, 4 × MeCO), 3.88–4.23 (3 H, complex, 5-H and 6-H_2), 4.40 (2 H, s, CH_2Br), 4.79–5.74 (4 H, complex, 1-, 2-, 3- and 4-H), 6.92–7.40 (4 H, m, AA'BB' system of 4-$BrCH_2C_6H_4O$).

4-(*Chloromethyl*)*phenyl Tetra*-O-*acetyl*-α-D-*glucopyranoside* **15**.—Compound **14** (1.0 g, 1.93 mmol) was converted by treatment in the usual manner with TBAC (3.00 g, 11.0 mmol) in acetonitrile (14 cm^3) into the crude chloride. Column chromatography [light petroleum–ethyl acetate (3:2)] and recrystallisation of the major component from ethyl acetate–hexane gave compound **15** (0.53 g, 41%), m.p. 90–92 °C (Found: C, 53.2; H, 5.1; Cl, 7.8. $C_{21}H_{25}ClO_{10}$ requires C, 53.3; H, 5.3; Cl, 7.5%) $[\alpha]_D$ +163 (*c* 1.0, CH_2Cl_2); ν_{max}(Nujol)/cm^{-1} 1743 (CO), 1612, 1514 (Ar), 1230, 1040 (CO–O) and 662 (C–Cl); δ_H(60 MHz; $CDCl_3$) 2.04 (12 H, s, 4 × MeCO), 3.93–4.26 (3 H, complex, 5-H and 6-H_2), 4.53 (2 H, s, CH_2Cl), 4.87–5.86 (4 H, complex, 1-, 2-, 3- and 4-H), 6.93–7.45 (4 H, m, AA'BB' system of 4-$ClCH_2H_4O$).

4-(*Chloromethyl*)*phenyl* α-D-*Glucopyranoside* **4**.—Compound **15** (0.40 g, 0.85 mmol) was deacylated in the usual manner. The product was recrystallised from ethyl acetate–methanol to give *compound* **4** (0.09 g, 35%), m.p. 95 °C (gradually softens) (Found: C, 51.4; H, 6.0. $C_{13}H_{17}ClO_6$ requires C, 51.2; H, 5.6%); $[\alpha]_D$ +185 (*c* 1.0, CH_2Cl_2); ν_{max}(Nujol)/cm^{-1} 3400 (OH) and 1070 (C–O); δ_H(60 MHz; $CDCl_3$) 3.24–4.05 (6 H, complex, 2-, 3-, 4-, 5-H and 6-H_2), 4.74–5.06 (6 H, complex, CH_2Cl, 4 × OH), 5.41 (1 H, d, $J_{1,2}$ 3.4, 1-H), 6.96–7.44 (4 H, m, AA'BB' system of 4-$ClCH_2C_6H_4O$).

2-*Methyl*-4-*nitrophenyl and* 2-*Methyl*-6-*nitrophenyl Tetra*-O-*acetyl*-α-D-*glucopyranosides* **16** *and* **17**, *respectively*.—To a stirred solution of compound **9** (9.0 g, 20.5 mmol) in TFAA (12 cm^3), cooled in ice, was added a solution of nitric acid (4.6 cm^3) in TFAA (12 cm^3). After 10 min the solution was poured into ice–water (250 cm^3) and extracted with dichloromethane (2 × 50 cm^3). The combined extracts were washed successively with water, aq. sodium hydrogen carbonate and aq. sodium chloride, and then was dried and concentrated. Column chromatography [light petroleum–ethyl acetate (6:4)] of the residue separated the two isomers. 2-Methyl-4-nitrophenyl glycoside **16** (3.18 g, 32%) eluted first and was obtained as a *syrup* which then solidified, m.p. 53–55 °C (Found: C, 51.8; H, 5.0; N, 2.9. $C_{21}H_{25}NO_{12}$ requires C, 52.2; H, 5.2; N, 2.9%); $[\alpha]_D$ +181 (*c* 0.6, $CHCl_3$); ν_{max}(Nujol)/cm^{-1} 1750 (CO), 1618, 1590, 1492 (Ar), 1515, 1342 (NO_2), 1230 and 1035 (CO–O); δ_H(60 MHz; $CDCl_3$) 2.00–2.20 (12 H, m, 4 × MeCO), 2.40 (3 H, s, *Me*Ar), 3.92–4.32 (3 H, complex, 5-H and 6-H_2), 4.95–5.34 (2 H, complex, 2- and 4-H), 5.52–5.88 (2 H, complex, 1- and 3-H), 7.17 (1 H, d, J_o 9.6, 6″-H) and 7.84–8.10 (2 H, complex, 3″- and 5″-H).

The 2-methyl-6-nitro analogue **17** eluted next and was obtained as a *syrup* which, from dichloromethane–hexane, gave *crystals* (1.11 g, 10%); m.p. 177–179 °C (Found: C, 52.1; H, 5.2; N, 3.0. $C_{21}H_{25}NO_{12}$ requires C, 52.2; H, 5.2; N, 2.9%); $[\alpha]_D$ +67.9 (*c* 1.1, $CHCl_3$); ν_{max}(Nujol)/cm^{-1} 1750 (CO), 1605, 1578, 1505 (Ar), 1533, 1360 (NO_2), 1225 and 1048 (CO–O); δ_H(400 MHz; $CDCl_3$) 2.03 and 2.07 (double intensity), 2.09 (4 × 3 H, 3 s, 4 × MeCO), 2.40 (3 H, s, *Me*Ar), 4.09 (1 H, dd, $J_{5,6}$ 1.8, $J_{6,6'}$ 12.5, 6-H), 4.34 (1 H, dd, $J_{5,6'}$ 4.0, 6-H'), 4.27–4.31 (1 H, m, 5-H), 5.14 (1 H, dd, $J_{1,2}$ 3.7, $J_{2,3}$ 10.4, 2-H), 5.17 (1 H, t, $J_{3,4}$ = $J_{4,5}$ = 9.7, 4-H), 5.31 (1 H, d, 1-H), 5.66 (1 H, br t, 3-H), 7.17 (1 H, t, J_o = $J_{o'}$ = 7.6, 4″-H), 7.42 (1 H, ddq, J_o 7.6, J_m 0.6, $^4J_{H,Me}$ 0.6, 3″-H) and 7.54 (1 H, ddq, $^6J_{H,Me}$ 0.6, 5″-H).

2-*Bromomethyl*-4-*nitrophenyl and* 2-*Dibromomethyl*-4-*nitrophenyl Tetra*-O-*acetyl*-α-D-*glucopyranosides* **18** *and* **22**, *respectively*.—A solution of compound **16** (3.18 g, 6.57 mmol), DBDMH (1.95 g, 6.80 mmol) and AIBN (0.05 g) in dry benzene (40 cm^3) was heated under reflux with irradiation by light as before for 3.5 h. The crude mixture obtained on concentration of the filtered solution was crystallized from ethyl acetate–hexane to give *bromide* **18** (1.53 g, 41%), m.p. 145–146 °C (Found: C, 44.7; H, 4.1; N, 2.3; Br, 14.5. $C_{21}H_{24}BrNO_{12}$ requires C, 44.9; H, 4.3; N, 2.5; Br, 14.2%); $[\alpha]_D$ +180 (*c* 0.6, $CHCl_3$); ν_{max}(Nujol)/cm^{-1} 1740 (CO), 1612, 1589, 1482 (Ar), 1518, 1342 (NO_2), 1232, 1035 (CO–O) and 691 (C–Br); δ_H(400 MHz; $CDCl_3$) 2.05, 2.06, 2.08 and 2.10 (4 × 3 H, 4 s, 4 × MeCO), 4.05–4.12 (2 H, complex, 5- and 6-H), 4.26 (1 H, dd, $J_{5,6'}$ 4.4, $J_{6,6'}$ 12.5, 6-H'), 4.57 (1 H, d, $J_{A,B}$ 10.1, CH_AH_BBr), 4.63 (1 H, d, CH_AH_BBr), 5.14 (1 H, dd, $J_{1,2}$ 3.7, $J_{2,3}$ 10.3, 2-H), 5.22 (1 H, t, $J_{3,4}$ = $J_{4,5}$ = 9.9, 4-H), 5.79 (1 H, br t, 3-H), 5.92 (1 H, d, 1-H), 7.29 (1 H, d, J_o 9.2, 6″-H), 8.20 (1 H, dd, J_m 2.9, 5″-H) and 8.30 (1 H, d, 3″-H).

Evaporation of the mother liquid yielded, as an oil, the crude compound **22** (1.01 g), ν_{max}(Nujol)/cm^{-1} 1750 (CO), 1612, 1590, 1482 (Ar), 1520, 1345 (NO_2), 1250 and 1040 (CO–O); δ_H(60 MHz; $CDCl_3$) 2.02–2.28 (12 H complex, 4 × MeCO), 3.94–4.30 (3 H, complex, 5-H and 6-H_2), 4.98–5.44 (2 H, complex, 2- and 4-H), 5.56–5.98 (2 H, complex, 1- and 3-H), 7.14 (1 H, s, $CHBr_2$), 7.39 (1 H, d, J_o 9.6. 6″-H), 8.25 (1 H, dd, J_m 2.4, 5″-H) and 8.72 (1 H, d, 3″-H). This material was used without further purification.

2-*Chloromethyl*-4-*nitrophenyl Tetra*-O-*acetyl*-α-D-*glucopyranoside* **19**.—A solution of compound **18** (2.32 g, 4.13 mmol) in dry acetonitrile (20 cm^3) was heated under reflux with TBAC (4.90 g, 17.6 mmol) and the product was isolated in the usual manner. Purification of this material by column chromatography [light petroleum–ethyl acetate (3:2)] and recrystallisation of the major component from ethyl acetate–hexane gave *title compound* **19** (1.47 g, 69%), m.p. 158–160 °C (Found: C, 48.8; H, 4.6; N, 2.6; Cl, 6.9. $C_{21}H_{24}ClNO_{12}$ requires C, 48.7; H, 4.7; N, 2.7; Cl, 6.9%); $[\alpha]_D$ +150 (*c* 4.0, $CHCl_3$); ν_{max}(Nujol)/cm^{-1} 1755, 1742 (CO), 1612, 1590, 1485 (Ar), 1518, 1345 (NO_2), 1230, 1055 (CO–O) and 697 (C–Cl); δ_H(400 MHz; $CDCl_3$) 2.05,

2.06, 2.08 and 2.09 (4 × 3 H, 4 s, 4 × MeCO), 4.06–4.10 (2 H, complex, 5- and 6-H), 4.26 (1 H, dd, $J_{5,6'}$ 5.1, $J_{6,6'}$ 13.2, 6-H'), 4.72 (2 H, s, CH_2Cl), 5.12 (1 H, dd, $J_{1,2}$ 3.7, $J_{2,3}$ 10.3, 2-H), 5.21 (1 H, t, $J_{3,4} = J_{4,5}$ = 9.9, 4-H), 5.73 (1 H, br t, 3-H), 5.89 (1 H, d, 1-H), 7.31 (1 H, d, J_o 9.2, 6″-H), 8.22 (1 H, dd, J_m 2.9, 5″-H) and 8.33 (1 H, d, 3″-H).

2-Chloromethyl-4-nitrophenyl α-D-*Glucopyranoside* **5**.—Compound **19** (1.32 g, 2.71 mmol) was deacylated with sodium methoxide–methanol (65 cm^3) as described for the preparation of compound **2**. Recrystallisation of the crude product from methanol–ethyl acetate gave *title compound* **5** (0.49 g, 52%), m.p. 133–135 °C (Found: C, 44.2; H, 4.5; N, 3.7%; M^+, 349.0568. $C_{13}H_{16}ClNO_8$ requires C, 44.6; H, 4.6; N, 4.0%; M, 349.0565); $[\alpha]_D$ +220 (*c* 0.3, MeOH); ν_{max}(Nujol)/cm^{-1} 3350 (OH), 1612, 1596, 1482 (Ar), 1514, 1352 (NO_2) and 698 (C–Cl); δ_H(400 MHz; CD_3OD) 3.46 (1 H, t, $J_{3,4} = J_{4,5}$ = 9.2, 4-H), 3.61 (1 H, m, 5-H), 3.65–3.70 (2 H, complex, 2- and 6-H), 3.73 (1 H, dd, $J_{5,6'}$ 2.6, $J_{6,6'}$ 12.1, 6-H'), 3.97 (1 H, t, $J_{2,3} = J_{3,4}$ = 9.2, 3-H), 4.63 (2 H, s, CH_2Cl), 4.87 (4 H, s, 4 × OH), 5.77 (1 H, d, $J_{1,2}$ 3.7, 1-H), 7.46 (1 H, d, J_o 9.2, 6″-H), 8.24 (1 H, dd, J_m 2.9, 5″-H) and 8.34 (1 H, d, 3″-H).

2-Dichloromethyl-4-nitrophenyl α-D-*Glucopyranoside* **6**.—2-Dibromomethyl-4-nitrophenyltetra-*O*-acetyl-α-D-glucopyranoside **22** (0.95 g, 1.48 mmol), as a solution in dry acetonitrile (15 cm^3), was treated in the usual manner with TBAC (3.60 g, 12.9 mmol). Column chromatography [light petroleum–ethyl acetate (3:2)] and recrystallisation of the major component from ethyl acetate–hexane gave slightly impure 2-dichloromethyl-4-nitrophenyl tetra-*O*-acetyl-α-D-glucopyranoside (0.22 g, 27%), m.p. 145–146 °C; $[\alpha]_D$ +151 (*c* 0.6, $CHCl_3$); ν_{max}(Nujol)/cm^{-1} 1762, 1742 (CO), 1612, 1590, 1492 (Ar), 1523, 1347 (NO_2), 1240, 1037 (CO–O) and 702 (C–Cl); δ_H(400 MHz; $CDCl_3$) 2.06, 2.07, 2.08 and 2.10 (4 × 3 H, 4 s, 4 × MeCO), 4.06–4.12 (2 H, complex, 5- and 6-H), 4.27 (1 H, dd, $J_{5,6'}$ 4.7, $J_{6,6'}$ 12.7, 6-H'), 5.14 (1 H, dd, $J_{1,2}$ 3.7, $J_{2,3}$ 10.4, 2-H), 5.21 (1 H, t, $J_{3,4} = J_{4,5}$ = 9.9, 4-H), 5.70 (1 H, br t, 3-H), 5.87 (1 H, d, 1-H), 7.14 (1 H, s, $CHCl_2$), 7.36 (1 H, d, J_o 9.2, 6″-H), 8.27 (1 H, dd, J_m 2.8, 5″-H) and 8.75 (1 H, d, 3″-H). The product could not be separated from a small amount of the 2-chloromethyl-4-nitrophenyl glycoside and was used for the next stage without further purification.

The crude 2-dichloromethyl-4-nitrophenyl tetra-*O*-acetyl-α-D-glucopyranoside (0.17 g, 0.35 mmol) was treated in the usual manner with sodium methoxide–methanol (8 cm^3). Recrystallisation of the crude deacylated product from methanol–ethyl acetate gave *compound* **6** (0.10 g, 76%), m.p. 150–152 °C (Found: C, 40.2; H, 4.0; N, 3.4. $C_{13}H_{15}Cl_2NO_8$ requires C, 40.6; H, 3.9; N, 3.6%); $[\alpha]_D$ +271 (*c* 0.2, MeOH); ν_{max}(Nujol)/cm^{-1} 3250 (OH), 1612, 1590, 1483 (Ar), 1515, 1352 (NO_2) and 702 (C–Cl); δ_H(400 MHz; CD_3OD) 3.45 (1 H, t, $J_{3,4} = J_{4,5}$ = 9.2, 4-H), 3.56 (1 H, m, 5-H), 3.64–3.70 (2 H, complex, 2- and 6-H), 3.74 (1 H, dd, $J_{5,6'}$ 2.6, $J_{6,6'}$ 12.1, 6-H'), 3.92 (1 H, t, $J_{2,3}$ 9.2, 3-H), 4.89 (4 H, s, 4 × OH), 5.77 (1 H, d, $J_{1,2}$ 3.7, 1-H), 7.53 (1 H, d, J_o 9.2, 6″-H), 7.58 (1 H, s, $CHCl_2$), 8.31 (1 H, dd, J_m 2.6, 5″-H) and 8.68 (1 H, d, 3″-H).

2-Bromomethyl-6-nitrophenyl Tetra-O-*acetyl-α-D-glucopyranoside* **20**.—A solution of compound **17** (1.30 g, 2.69 mmol), DBDMH (0.41 g, 1.42 mmol) and AIBN (0.05 g) in dry benzene (20 cm^3) was heated under reflux with irradiation by light as before for 3 h. Work-up as described for the preparation of compound **10**, column chromatography [light petroleum–ethyl acetate (6:4)] of the crude product, and recrystallisation of the major component from ethyl acetate–hexane gave *title compound* **20** (0.61 g, 40%), m.p. 123–125 °C (Found: C, 45.1; H, 4.1; N, 2.4. $C_{21}H_{24}BrNO_{12}$ requires C, 44.9; H, 4.3; N, 2.5%); $[\alpha]_D$ +12.0 (*c* 0.2, $CHCl_3$); ν_{max}(Nujol)/cm^{-1} 1760, 1748 (CO), 1605, 1580 (Ar), 1533 (NO_2), 1230 and 1040 (CO–O); δ_H(400 MHz; $CDCl_3$) 2.04, 2.07, 2.11 and 2.13 (4 × 3 H, 4 s, 4 × MeCo), 4.11 (1 H, dd, $J_{5,6}$ 2.0, $J_{6,6'}$ 12.6, 6-H), 4.28 (1 H, m, 5-H), 4.34 (1 H, dd, $J_{5,6'}$ 4.0, 6-H'), 4.59 (1 H, d, $J_{A,B}$ 10.5, CH_AH_B), 4.85 (1 H, d, CH_AH_B), 5.21 (1 H, t, $J_{3,4} = J_{4,5}$ = 9.9, 4-H), 5.30 (1 H, dd, $J_{1,2}$ 3.8, $J_{2,3}$ 10.5, 2-H), 5.50 (1 H, d, 1-H), 5.66 (1 H, br t, 3-H), 7.30 (1 H, t, $J_o = J_{o'}$ = 7.8, 4″-H), 7.66 (1 H, dd, J_m 1.8, 3″-H) and 7.69 (1 H, dd, 5″-H).

2-Chloromethyl-6-nitrophenyl Tetra-O-*acetyl-α-D-glucopyranoside* **21**.—A solution of compound **20** (0.52 g, 0.92 mmol) in dry acetonitrile (6 cm^3) was treated in the usual manner with TBAC (1.25 g, 4.47 mmol). Purification of the crude product by column chromatography [light petroleum–ethyl acetate (3:2)] and recrystallisation from ethyl acetate–hexane gave *title compound* **21** (0.13 g, 27%), m.p. 130–131 °C (Found: C, 48.8; H, 4.5; N, 2.6. $C_{21}H_{24}ClNO_{12}$ requires C, 48.7; H, 4.7; N, 2.7%); $[\alpha]_D$ +0.3 (*c* 0.8, $CHCl_3$; ν_{max}(Nujol)/cm^{-1} 1760, 1748 (CO), 1608, 1580 (Ar), 1534 (NO_2), 1230, 1042 (CO–O) and 700 (C–Cl); δ_H(400 MHz; $CDCl_3$) 2.04, 2.07, 2.11 and 2.13 (4 × H, 4 s, 4 × MeCO), 4.11 (1 H, dd, $J_{5,6}$ 1.8, $J_{6,6'}$ 12.5, 6-H), 4.26 (1 H, m, 5-H), 4.34 (1 H, dd, $J_{5,6'}$ 3.8, 6-H'), 4.67 (1 H, d, $J_{A,B}$ 11.9, CH_AH_BCl), 4.97 (1 H, d, CH_AH_BCl), 5.20 (1 H, t, $J_{3,4} = J_{4,5}$ = 9.9, 4-H), 5.28 (1 H, dd, $J_{1,2}$ 3.4, $J_{2,3}$ 10.7, 2-H), 5.44 (1 H, d, 1-H), 5.65 (1 H, br t, 3-H), 7.33 (1 H, t, $J_o = J_{o'}$ = 7.9, 4″-H) and 7.67–7.72 (2 H, complex, 3″- and 5″-H).

2-Chloromethyl-6-nitrophenyl α-D-*Glucopyranoside* **7**.—Compound **21** (0.15 g, 0.29 mmol) as a solution in methanol (8 cm^3), was deacetylated by treatment with sodium methoxide in methanol in the usual manner. The product, initially a yellow syrup, solidified when triturated sequentially with ethyl acetate and hexane and the residue was then dried *in vacuo* to give, as a waxy, non-crystalline solid, *glycoside* **7** (0.03 g, 79%) (Found: C, 44.2; H, 4.9; N, 3.6. $C_{13}H_{16}ClNO_8$ requires C, 44.6; H, 4.6; N, 4.0%); $[\alpha]_D$ −5.3 (*c* 3.6, water); δ_H(CD_3OD) 3.25–4.04, (6 H, complex, 2-, 3-, 4-, 5-H and 6-H_2), 4.66–4.92 (6 H, complex, CH_2Cl, 4 × OH), 5.76 (1 H, d, $J_{1,2}$ 3.7, 1-H), 7.32 (1 H, t, $J_o = J_{o'}$ = 7.9, 4″-H) and 7.64–7.68 (2 H, complex, 3″- and 5″-H).

2-Methyl-4-nitrophenyl Tetra-O-*acetyl-β-D-glucopranoside* **24**.—A suspension of 2-methylphenyl tetraacetyl-β-D-glucopyranoside **23** (12.9 g, 0.029 mol) in TFAA (17 cm^3) was cooled in ice and treated with a solution of conc. nitric acid (6.4 cm^3) in TFAA (17 cm^3), as described for the preparation of the α-analogue **16**. The crude syrupy product solidified on trituration with hot light petroleum. Column chromatography of this crude material [light petroleum–ethyl acetate (6:4)] and recrystallisation of the solid so obtained from dichloromethane–hexane gave *title compound* **24** (2.02 g, 14%), m.p. 157–158 °C (Found: C, 52.4; H, 5.2; N, 2.8. $C_{21}H_{25}NO_{12}$ requires C, 52.2; H, 5.2; N, 2.9%); $[\alpha]_D$ −44.4 (*c* 1.1, $CHCl_3$); ν_{max}(Nujol)/cm^{-1} 1754 (CO), 1612, 1592, 1492 (Ar), 1525, 1350 (NO_2), 1240 and 1040 (CO–O); δ_H(60 MHz; $CDCl_3$) 2.02–2.12 (1 H, complex, 4 × MeCO), 2.23 (3 H, s, *Me*Ar), 3.74–4.31 (3 H, complex, 5-H and 6-H_2), 4.94–5.50 (4 H, complex, 1-, 2-, 3- and 4-H), 7.01 (1 H, d, J_o 9.6, 6″-H) and 7.90–8.18 (2 H, complex, 3″- and 5″-H).

2-Bromophenyl-4-nitrophenyl Tetra-O-*acetyl-β-D-glucopyranoside* **25**.—Bromination of compound **24** (2.00 g, 4.13 mmol) in refluxing benzene (25 cm^3) with DBDMH (1.23 g, 4.29 mmol) and AIBN (0.05 g) under irradiation with light as before for 3.5 h and recrystallisation of the crude product from dichloromethane–hexane gave *title compound* **25** (1.31 g, 56%), m.p. 180–183 °C (Found: C, 44.5; H, 4.1; N, 2.3.

$C_{21}H_{24}BrNO_{12}$ requires C, 44.8; H, 4.3; N, 2.5%); $[\alpha]_D$ −14.1 (*c* 0.7, $CHCl_3$); ν_{max}(Nujol)/cm^{-1} 1756 (CO), 1612, 1592, 1492 (Ar), 1525, 1350 (NO_2), 1240 and 1040 (CO–O); δ_H(60 MHz; $CDCl_3$) 2.06–2.20 (12 H, complex, 4 × MeCO), 3.82–4.34 (3 H, complex, 5-H and 6-H_2), 4.35 (1 H, d, $J_{A,B}$ 9.6, (CH_BH_BBr), 4.63 (1 H, d, CH_AH_BBr), 5.08–5.48 (4 H, complex, 1-, 2-, 3- and 4-H), 7.12 (1 H, d, J_o 8.6, 6″-H) and 8.06–8.34 (2 H, complex, 3″- and 5″-H).

2-Chloromethyl-4-nitrophenyl Tetra-O-acetyl-β-D-glucopyranoside **26**.—A solution of compound **25** (1.30 g, 2.24 mmol) in dry acetonitrile (15 cm^3) was treated with TBAC (3.00 g, 10.8 mmol) as described for the preparation of compound **11**. Column chromatography [light petroleum–ethyl acetate (3:2)] and recrystallization from dichloromethane–hexane gave *title compound* **26** (0.23 g, 64%), m.p. 174–176 °C (Found: C, 48.4; H, 4.6; N, 2.6. $C_{21}H_{24}ClNO_{12}$ requires C, 48.7; H, 4.7; N, 2.7%); $[\alpha]_D$ −20 (*c* 0.1, $CHCl_3$); ν_{max}(Nujol)/cm^{-1} 1756 (CO), 1616, 1595, 1492 (Ar), 1526, 1355 (NO_2), 1240 and 1040 (CO–O); δ_H(60 MHz; $CDCl_3$) 2.04–2.18 (12 H, complex, 4 × MeCO), 3.80–4.36 (3 H, complex, 5-H and 6-H_2), 4.46 (1 H, d, $J_{A,B}$ 12, CH_AH_BCl), 4.72 (1 H, d, CH_AH_BCl), 5.18–5.56 (4 H, complex, 1-, 2-, 3- and 4-H), 7.15, (1 H, d, J_o 9.1, 6″-H) and 8.04–8.36 (2 H, complex, 3″- and 5″-H).

2-Chloromethyl-4-nitrophenyl β-D-Glucopyranoside **27**.—Compound **26** (0.72 g, 1.39 mmol) was deacetylated by treatment with a catalytic amount of sodium methoxide in methanol (35 cm^3). Recrystallisation of the crude product from ethyl acetate gave *title compound* **27** (0.32 g, 65%), m.p. 143–144 °C (Found: C, 44.6; H, 4.5; N, 3.8. $C_{13}H_{16}ClNO_8$ requires C, 44.6; H, 4.6; N, 4.0%); $[\alpha]_D$ −94.4 (*c* 0.4, MeOH); ν_{max}(Nujol)/cm^{-1} 3350 (OH), 1616, 1593, 1492 (Ar), 1520, 1354 (NO_2) and 658 (C–Cl); δ_H(60 MHz; CD_3OD) 3.40–3.94 (6 H, complex, 2-, 3-, 4-, 5-H and 6-H_2), 4.68–4.90 (6 H, complex, CH_2Cl, 4 × OH), 5.10 (1 H, d, $J_{1,2}$ 5.8, 1-H), 7.36 (1 H, d, J_o 8.9, 6″-H) and 8.10–8.40 (2 H, complex, 3″- and 5″-H).

Enzyme Assays.—Assays with yeast α-glucosidase were performed at 30 °C and pH 6.8 [buffer: 10 mmol dm^{-3} disodium piperazine-*N*,*N*′-bis(ethane-2-sulfonate) (PIPES disodium salt)–20 mmol dm^{-3} sodium acetate–0.1 mmol dm^{-3} EDTA; pH adjusted using a Rapide Instruments AGB-M1 meter with 5 mol dm^{-3} hydrochloric acid] using 4-nitrophenyl α-D-glucopyranoside (5 mmol dm^{-3}) as substrate (K_m 0.2 mmol dm^{-3}).[28] Assays with β-glucosidase were carried out at pH 6.2 and 27 °C (buffer: as for yeast α-glucosidase assays but adjusted to pH 6.2 with 5 mol dm^{-3} hydrochloric acid) using 4-nitrophenyl β-D-glucopyranoside (5 mmol dm^{-3}) as substrate (K_m 2.5 mmol dm^{-3}).[29] Enzyme solutions were incubated at 30 °C (for yeast α-glucosidase with **2**–**7**) or at 27 °C (for β-glucosidase with **2** and **5**), with inhibitors **2**–**4** at an inhibitor concentration of 2 mmol dm^{-3} and with inhibitors **5**–**7** at an inhibitor concentration of 0.02 mmol dm^{-3}, for various periods of time (see Fig. 1). After the required incubation time, the appropriate substrate was added and the assays were performed by measuring the initial rate of release of 4-nitrophenol from the respective glycosides at 400 nm. These rate measurements were conducted so that less than 10% of the substrate was consumed. The '% Enzyme activity' of the inhibited enzyme, shown as the ordinate in Fig. 1, is calculated relative to the activity of a similarly prepared enzyme solution not containing the inhibitor which had been stored for the same length of time.

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