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Note

A new and improved synthesis of *p*-nitrophenyl β-D-mannopyranoside

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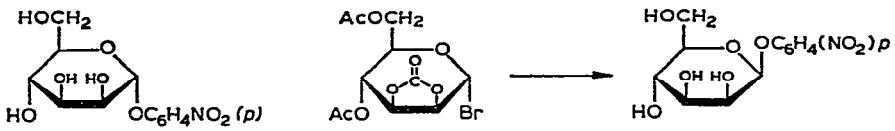
Because of the presence of β-D-mannosidic linkages in the core region of many glycoproteins[1–3], it has become important to purify β-D-mannosidases for use in identifying this residue. As in the case of the purification of other glycosidases, it is advantageous to utilize a chromogenic substrate—in this instance *p*-nitrophenyl β-D-mannopyranoside (3). Although this compound was available initially through commercial suppliers, problems encountered in its synthesis have made it very scarce and, when available, very expensive.



1 2 3

As shown by Rosenfeld and Lee[4], 3 is formed as a minor product during the preparation of *p*-nitrophenyl α-D-mannopyranoside (1) by the zinc chloride fusion procedure, but the two anomers can be separated by means of a cation-exchange column. In this paper, an alternative route to the synthesis of 3 is described, which, although requiring several intermediate steps, provides this compound almost exclusively.

The method employed was derived from a procedure described originally by Bebault and Dutton[5] for the synthesis of β-D-mannopyranosyl glycosides. However, contrary to what was expected, on treating 4,6-di-*O*-acetyl-2,3-*O*-carbonyl-α-D-mannopyranosyl bromide (2) with *p*-nitrophenol in the presence of mercuric cyanide, an α to β ratio for the *p*-nitrophenyl mannopyranoside of 4:1 was obtained. To reverse this unfavorable ratio, different solvents and metal salts were tried, and it was found that, on replacing mercuric cyanide with silver oxide, the anomer ratio could be shifted almost exclusively to β, with the initial condensation proceeding to the extent of 50%. The desired compound required only a simple extraction, followed by deacetylation and recrystallization, for its isolation. A similar procedure was described recently[6] but utilized sodium *p*-nitrophenoxide in the condensation with 2.

EXPERIMENTAL

Preparation of 4,6-di-O-acetyl-2,3-O-carbonyl-α-D-mannopyranosyl bromide (**2**). — Although the basic procedure of Bebault and Dutton[5] was used, it was found more practical to synthesize methyl 4,6-*O*-benzylidene-α-D-mannopyranoside by the method of Buchanan and Schwarz[7] and, for the synthesis of the 2,3-*O*-carbonyl derivative of this compound, consistently higher yields were obtained by the method of Doane *et al.*[8].

Condensation of **2** *with* p-*nitrophenol.* — Compound **2** (1.78 g, 5 mmol) was dissolved in redistilled acetonitrile (20 ml, stored over calcium hydride), followed by recrystallized *p*-nitrophenol (1.13 g, 7.9 mmol) and iodine (0.15 g). The reaction was initiated by adding silver oxide (1.5 g, Fisher Scientific Co., Pittsburgh, PA 15219), and the resultant suspension was stirred overnight in the dark. T.l.c. on silica gel plates with 4:1 (v/v) chloroform–acetone, followed by charring with 50% sulfuric acid to localize the compounds, revealed a major spot with an R_F 0.57 and a minor one at 0.72. Exposure of the plate to ammonia showed that both were *p*-nitrophenyl derivatives; the former was shown later to be the β-D and the latter the α-D anomer. After filtration of the reaction mixture, the solution was concentrated *in vacuo*, and the residue dissolved in chloroform (150 ml). The solution was extracted with saturated sodium hydrogencarbonate until most of the yellow *p*-nitrophenol had been removed, then extracted twice with water, and dried (sodium sulfate).

Isolation of p-*nitrophenyl* β-D-*mannopyranoside* (**3**). — The dried chloroform solution was evaporated *in vacuo*, the residue dissolved in dry methanol (30 ml) and methanolic 0.2M sodium methoxide (2.5 ml) was added. The extent of deacetylation was determined by silica gel t.l.c. with 9:4:2 (v/v) ethyl acetate–2-propanol–water and also enzymically with a β-D-mannosidase purified from *Polyporus sulfureus*[9]. The latter procedure revealed that about 50% of the starting material had been converted into **3**. T.l.c. on silica gel revealed that a small amount of the slightly faster moving **1** was also present, which by jack-bean-meal α-D-mannosidase analysis amounted to less than 5% of the total *p*-nitrophenyl mannopyranoside formed in the reaction. A product devoid of **1** and *p*-nitrophenol was obtained by concentrating the deacetylated reaction mixture *in vacuo*, dissolving the residue in water, and passing the solution through a 1 × 3 cm column of Dowex 50 (H^+); after the column was washed with several volumes of water, the combined washes were extracted with ether until residual *p*-nitrophenol was removed. On concentration of the water phase *in vacuo*, crystals began to form, whereupon the flask was placed in the cold to complete the crystallization. The crystals were collected in the cold on a sintered-glass suction funnel and washed with cold water; net yield (based on combining the first and second crop of crystals) 0.73 g.

Characterization of **3**. — The first crystallization of **3** from water yielded a product having a m.p. of 198–200°, which could not be raised above 202–203°, $[\alpha]_D^{20}$ −122° (*c* 0.706, water) on repeated crystallization from 95% ethanol. Although these values are slightly lower than those reported[4] (m.p. 206–207°, $[\alpha]_D^{20}$ −125°),

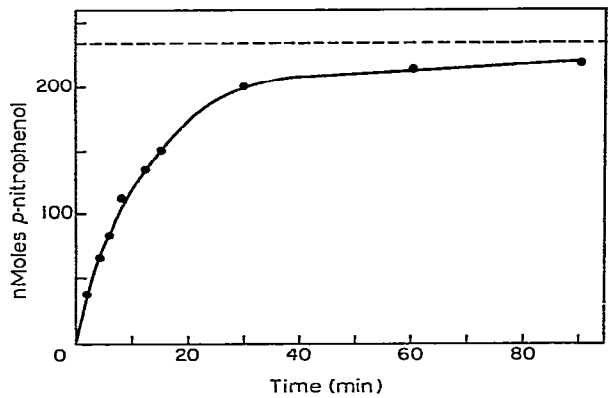


Fig. 1. Hydrolysis of **3** by β-D-mannosidase. Each reaction at 37° contained sodium citrate (0.2 ml), pH 4.0, **3** (235 nmol), and β-D-mannosidase (10 μl) purified free of α-D-mannosidase[9]. At the indicated times, 0.1M sodium carbonate (3 ml) was added and the absorbancy of the resultant solution read at 415 nm. The extinction of *p*-nitrophenol at this wavelength was determined to be $17.2.10^3.M^{-1}.cm^{-1}$. The dashed line indicates theoretical hydrolysis.

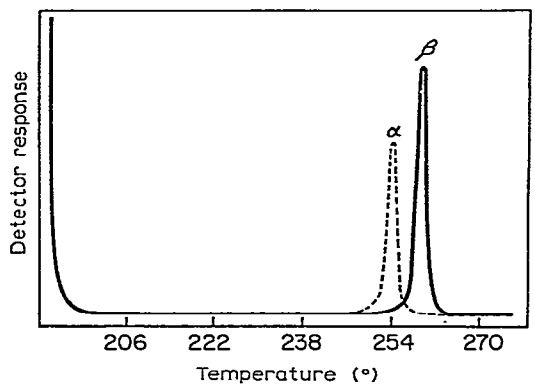


Fig. 2. Gas chromatography of per-*O*-trimethylsilyl **1** and **3**. Samples were injected onto a 1.8-m coiled column of 3% SE-30 on 80–100 mesh of Sulpelcoport (Sulpelco, Inc., Bellefonte, PA 16823) in a Hewlett–Packard 5700 gas chromatograph. The initial temperature of 190° was raised at 4°/min to 280°. The two compounds were chromatographed separately.

the product was completely hydrolyzed (within the limits of experimental error) with β-D-mannosidase (Fig. 1) and was not susceptible to hydrolysis by α-D-mannosidase.

Gas chromatography of the per-*O*-trimethylsilyl derivative of **3** on 3% SE-30 revealed only a single peak at 260°; the corresponding α-D anomer showed a peak at 254° (Fig. 2).

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