2-Chloromethyl-4-nitrophenyl α-D-Glucopyranoside: an Enzyme-activated Irreversible Inhibitor of Yeast α-Glucosidase

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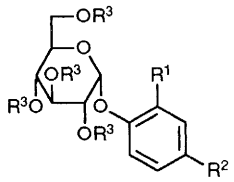
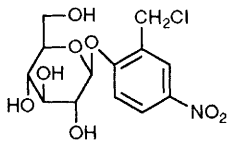
2-Chloromethyl-4-nitrophenyl α-D-glucopyranoside, prepared by a novel, four-step route from 2-methylphenyl α-D-glucopyranoside tetraacetate, is highly effective as an enzyme-activated irreversible inhibitor of yeast α-glucosidase and is much superior in this respect to 2-chloromethylphenyl α-D-glucopyranoside.

Glycosidase inhibitors are of considerable current interest in view of potential applications in the treatment of certain diseases[1] and, in particular, because of anti-HIV activity shown by the natural competitive inhibitors nojirimycin, castanospermine, and some of their derivatives.[2] Relatively little attention has been given, however, to *enzyme-activated irreversible inhibitors* of glycosidases despite their potential for highly specific action. Although conduritol epoxides,[3] aziridine derivatives[4] and glycosyl methyltriazines[5] belong to this class, they are not, in the usual sense, substrates for the glycosidases. To our knowledge, the only examples of glycoside-based, enzyme-activated irreversible inhibitors are, for β-glucosidases, 2,4-dinitrophenyl 2-deoxy-2-fluoro-β-D-glucopyranoside[6] and *o*- and *p*-(difluoromethyl)aryl β-D-glucopyranosides,[7] and, for α-glucosidases, 1′,1′-difluoroalkyl α-D-glucopyranosides.[8] We now report on a new inhibitor of this type for an α-glucosidase, 2-chloromethyl-4-nitrophenyl α-D-glucopyranoside **1**. The possible mode of action involves



1; $R^1 = CH_2Cl$, $R^2 = NO_2$, $R^3 = H$
2; $R^1 = Me$, $R^2 = H$, $R^3 = Ac$
3; $R^1 = Me$, $R^2 = NO_2$, $R^3 = Ac$
4; $R^1 = CH_2Br$, $R^2 = NO_2$, $R^3 = Ac$
5; $R^1 = CH_2Cl$, $R^2 = NO_2$, $R^3 = Ac$
7; $R^1 = CH_2Cl$, $R^2 = R^3 = H$
8; $R^1 = R^3 = H$, $R^2 = NO_2$



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initial enzymic liberation of the aglycone, 2-chloromethyl-4-nitrophenol, which, by analogy with 2-bromomethyl-4-nitrophenol (Koshland's reagent),[9] would be expected to rapidly lose hydrogen chloride with formation of a quinone methide, which would then undergo attack by a nucleophilic centre in the enzyme active site leading to alkylation and deactivation. Support for such a mechanism comes from the work of Halazy and coworkers[7] and recent studies[10] on enzymic hydrolysis of the natural β-glucoside salicortin.

Glycoside **1** was prepared from 2-methylphenyl α-D-glucopyranoside tetraacetate[11] **2** which was nitrated [concentrated nitric acid in $(CF_3CO)_2O$] to give 2-methyl-4-nitrophenyl α-D-glucopyranoside tetraacetate† **3** (45%) after chromatographic separation from the 2-methyl-6-nitrophenyl isomer (12%). Radical bromination of **3** [1,3-dibromo-5,5-dimethylhydantoin)–azoisobutyronitrile (AIBN)–CCl_4] gave 2-bromomethyl-4-nitrophenyl α-D-glucopyranoside tetraacetate **4** (80%) which, on halogen exchange (Bu^n_4NCl–MeCN), gave 2-chloromethyl-4-nitrophenyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside **5** (89%) which was deacylated‡ (MeO^-–MeOH) to give **1** (48%). A similar series of reactions in the β-series afforded β-glucoside **6**. Similar reactions on **2** but with omission of the nitration step gave 2-(chloromethyl)phenyl α-D-glucopyranoside **7**.

Comparison of the action of yeast α-glucosidase (Sigma, type VI, EC 3.2.1.20) on 4-nitrophenyl α-D-glucopyranoside **8** and **1** suggests that this enzyme is rapidly deactivated on liberation of the aglycone from **1** since, under the conditions [piperazine-*N*,*N*′-bis(ethanesulfonic acid) (PIPES) buffer, pH 6.8, 30 °C, 40 min] which led to 50% hydrolysis of **8**, less than 4% of **1** was hydrolysed.§ Incubation of β-glycoside **6** with the enzyme had no effect on α-glucosidase activity and indicated that enzymic hydrolysis of **1** was a prerequisite for deactivtion.

Incubation experiments with yeast α-glucosidase and **1** showed a time-dependent loss of enzyme activity [Fig. 1(*a*)], the deactivation rate depending on the inhibitor concentration. Pseudo-first-order kinetics were observed and a plot of reciprocals of the apparent rate constants (k_{app}) against reciprocals of the inhibitor concentrations ([I]), according to the method of Kitz and Wilson,[12] [Fig. 1(*b*), curve (*i*)] gave a dissociation constant for the initial reversible complex (K_i) of $(2.5 \pm 0.1) \times 10^{-3}$ mmol dm^{-3} and a rate constant for conversion of the reversible complex to the irreversibly inhibited enzyme of $(3.05 \pm 0.03) \times 10^{-3}$ s^{-1}. The initial ($t = 0$) enzymic activity decreased slightly but progressively with increasing inhibitor concentration, suggesting that **1** might be acting as a competitive inhibitor before cleavage by the enzyme. A related observation has been made with some irreversible inhibitors of sweet almond β-glucosidase.[13]

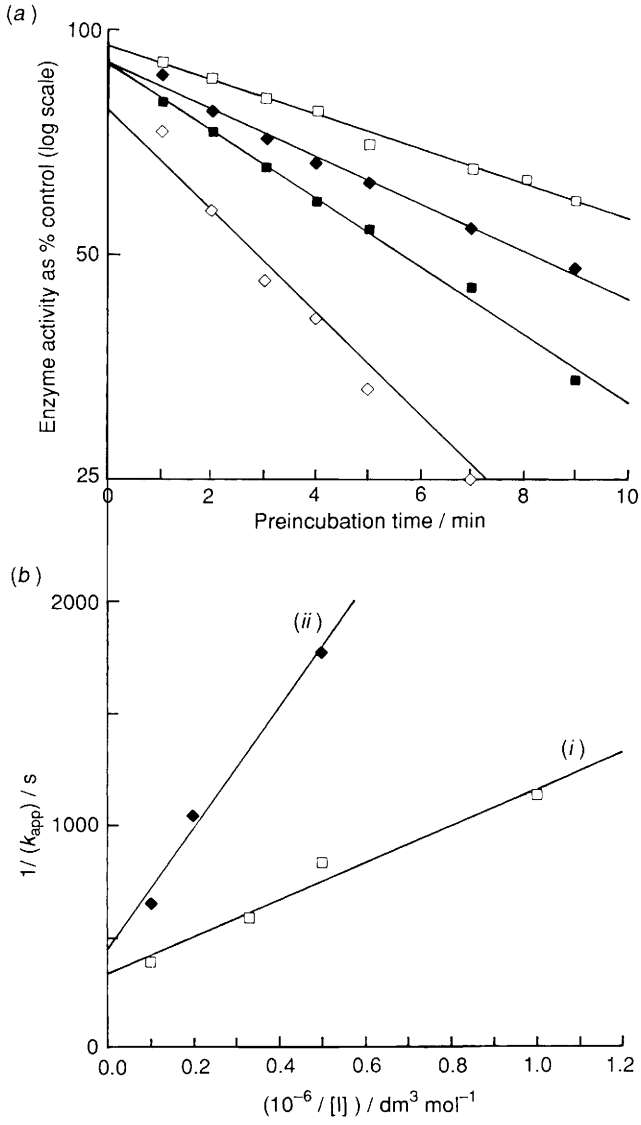

Fig. 1 (*a*) Progressive inhibition of yeast α-glucosidase with time on preincubation (30 °C) with **1**, measured at four different inhibitor concentrations and plotted as a semi-logarithmic curve. Assays performed with substrate **8** (0.4 mmol dm^{-3}) in PIPES buffer, pH 6.8, at 30 °C. □: 1 μmol dm^{-3}; ◆: 2 μmol dm^{-3}; ■: 3 μmol dm^{-3}; ◇: 10 μmol dm^{-3}. (*b*) Curve (*i*): dependence of first-order rate constants obtained from Fig. 1(*a*) on inhibitor concentration [I]; curve (*ii*): dependence of first-order rate constants on [I] in the presence of the reversible inhibitor 5-thio-D-glucose (0.19 mmol dm^{-3}).

Protection of the α-glucosidase was achieved when incubation with **1** was conducted in the presence of the competitive inhibitor of the enzyme, 5-thio-D-glucose[14] [Fig. 1(*b*), curve (*ii*)], providing further evidence for involvement of the active site in the inhibitory process. Covalent linkage between this active site and the inhibiting species from **1** seems to occur since extended dialysis of the enzyme deactivated by incubation (10 min) with **1** gave only 10% of the activity of the control. In contrast, enzymic activity was fully restored in a similar incubation experiment with 5-thio-D-glucose. Comparative incubation experiments indicated that glycoside **1** was very much more effective than **7** in inhibiting the enzyme. Thus, enzyme activity was reduced by 80% after incubation with **1** at 0.02 mmol dm^{-3} concentration for 5 min whereas a similar reduction in enzyme activity required incubation with **7** at 2 mmol dm^{-3} concentration for 30 min.

† Satisfactory analytical and spectral data were obtained for all new compounds.

‡ Deacylation of **4** under similar conditions led to concomitant displacement of bromide by methoxide ion.

§ The half-life for chemical hydrolysis of **1** in the buffer medium is 6 h.

In an anti-HIV screen, the tetraacetate of **7** showed weak activity in reducing virus (HIV-1 IIIB) progeny in infected cell (C 8166) cultures by 50% at 40 μmol dm^{-3} but compounds **1, 5** and **7** were inactive. Compounds **1** and **5** showed high cell toxicity, cell growth being reduced by 50% at 5 μmol dm^{-3}. Interestingly, separate experiments using a T cell clone (CEM 4) suggest that **7**, but not its tetraacetate, does inhibit, to a limited extent, glucosidase trimming of N-linked oligosaccharides. The anti-HIV activity of the tetraacetate of **7** seems to arise from a cause other than an effect on enzyme trimming.

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References

1 E. Truscheit, W. Frommer, B. Junge, L. Muller, D. D. Schmidt and W. Wingender, *Angew. Chem., Int. Ed. Engl.*, 1981, **20**, 744; P. S. Liu, *J. Org. Chem.*, 1987, **52**, 4717; M. J. Humphries, K. Matsumoto, S. L. White, R. J. Molyneux and K. Olden, *Proc. Natl. Acad. Sci. USA*, 1986, **83**, 1752; L. E. Fellows and R. J. Nash, *Sci. Progress*, 1990, **74**, 245.

2 A. S. Tyms, D. L. Taylor, P. S. Sunkara and M. S. Kang, in *Design of Anti-AIDS Drugs*, ed. E. De Clercq, Elsevier, Amsterdam, 1990, pp. 257–318.

3 G. Legler, *Methods Enzymol.*, 1977, **46**, 368.

4 M. K. Tong and B. Ganem, *J. Am. Chem. Soc.*, 1988, **110**, 312; G. Caron and S. G. Withers, *Biochem. Biophys. Res. Commun.*, 1989, **163**, 495.

5 P. J. Marshall, M. L. Sinnott, P. J. Smith and D. Widdows, *J. Chem. Soc., Perkin Trans. 1*, 1981, 366.

6 S. G. Withers, I. P. Street, P. Bird and D. H. Dolphin, *J. Am. Chem. Soc.*, 1987, **109**, 7530.

7 S. Halazy, V. Berges, E. Ehrhard and C. Danzin, *Bioorg. Chem.*, 1990, **18**, 330.

8 S. Halazy, C. Danzin, E. Ehrhard and G. Gerhart, *J. Am. Chem. Soc.*, 1989, **111**, 3484.

9 D. E. Koshland, Jr., V. D. Karkhanis and H. G. Latham, *J. Am. Chem. Soc.*, 1964, **86**, 1448; H. R. Horton and D. E. Koshland, Jr., *J. Am. Chem. Soc.*, 1965, **87**, 1126.

10 T. P. Clausen, J. W. Keller and P. B. Reichardt, *Tetrahedron Lett.*, 1990, **31**, 4537.

11 B. Helferich, U. Lampert and G. Sparmberg, *Berichte.*, 1934, **67**, 1808.

12 R. Kitz and I. B. Wilson, *J. Biol. Chem.*, 1962, **237**, 3245.

13 M. L. Shulman, S. D. Shiyan and A. Y. Khorlin, *Biochem. Biophys. Acta*, 1976, **445**, 169.

14 T. Kajimoto, K. K. C. Liu, R. L. Pederson, Z. Zhong, Y. Ichikawa, J. A. Porco, Jr., and C. H. Wong, *J. Am. Chem. Soc.*, 1991, **113**, 6187.