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α-D-GLUCOPYRANOSYL-PHENYLDIAZOMETHANE, A MECHANISM BASED α-GLUCOSIDASE INHIBITOR

Hansjörg Dietrich and Richard R. Schmidt*

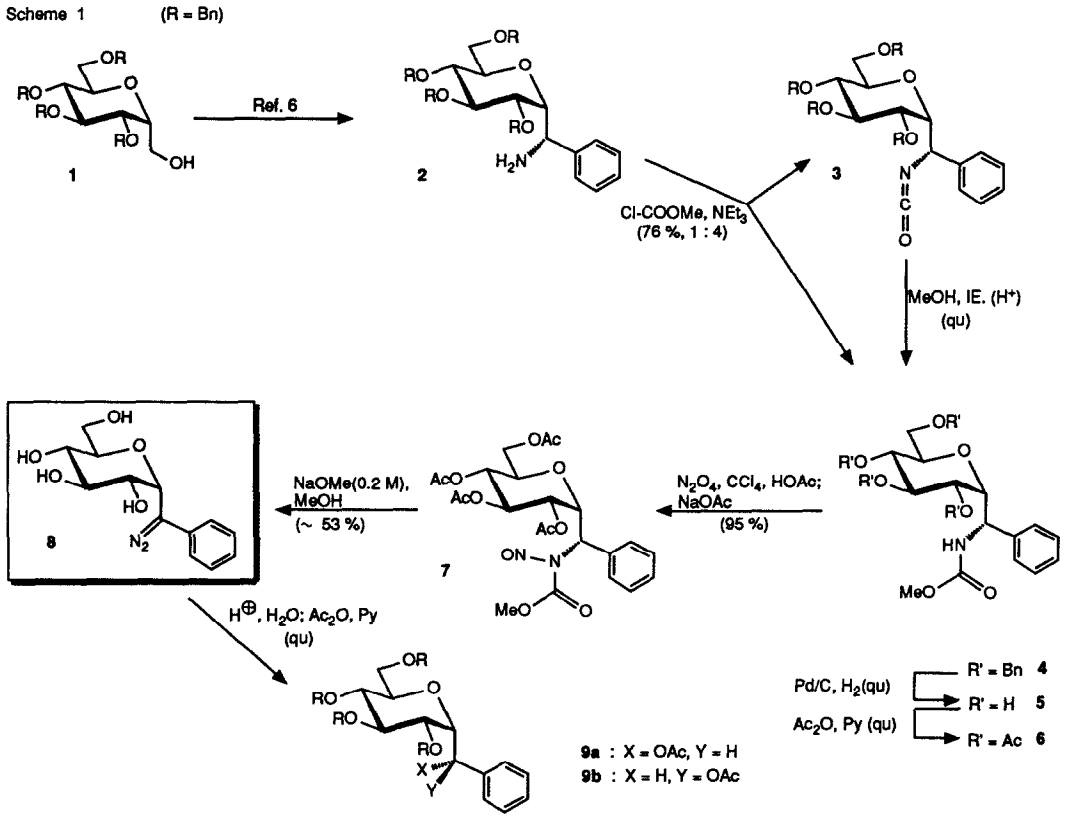
Fakultät Chemie, Universität Konstanz, Postfach 5560 M 725

D-78434 Konstanz, Germany

Abstract: The title compound **8** was readily prepared from known α-benzyl-*C*-glucoside **2**, its transformation into N-Nitroso-urethane derivative **5**, and then treatment with base. **8** showed irreversible inhibition against α-glucosidase from yeast.

The finding that amino sugar-based inhibitors have potential as anti-HIV, diabetes, and cancer therapeutic agents has led to a wide interest for such compounds[1,2]. The inhibitory action of these substances, which are structurally related to substrates, intermediates, or even transition states, is attributed particularly to the interaction of the amine nitrogen atom of the inhibitor with an acid group in the active site of the enzyme; the acidic group initiates the hydrolysis of the glycosidic bond of the substrate or (as carboxylate anion) stabilizes the nascent glycosyl cation species[2-5].

Some mechanism-based enzyme inactivators (suicide substrates) of glycosidases with varying structures have been investigated[4,6-9]. The presence of acidic groups in the active site suggests the attachment of acid sensitive moieties at the reaction center of a potential substrate; thus, an alkylating agent can be generated which by its reaction with nucleophilic groups in the active site irreversibly inhibits glycosidase activity. To this aim a galactopyranosyl-diazomethane[9] and glycopyranosylmethyl-phenyltriazenes as their precursors[8] have been investigated. However, the instability of these compounds was reason to synthesize less acid sensitive diazomethane derivatives[10]. Because phenyl glycosides are generally accepted as substrates by glycosidases, the synthesis of α-D-glucopyranosyl-phenyldiazomethane (Scheme 1, **8**) was undertaken which should exhibit lower acid sensitivity than the parent compound.



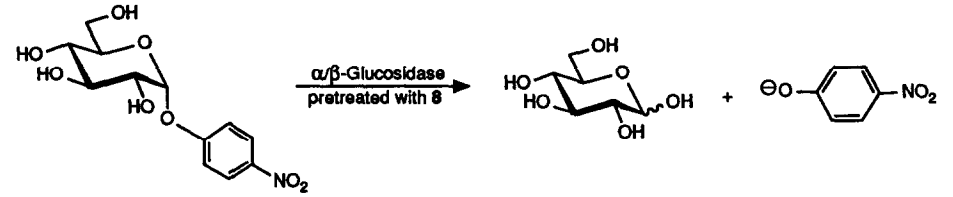
For the synthesis of **8**, readily available benzylalcohol derivative **1**[5,11] was transformed into the corresponding benzylamine derivative **2** as described previously[5]. Treatment of **2** with methyl chlorofomate and triethylamine in CH_2Cl_2 furnished the desired urethane **4** together with some isocyanate **3** (76%, **4**:**3** = 4:1), which gave in methanol in the presence of acidic ion exchange resin (Amberlite IR 120 [H^+]) quantitatively compound **4**. Hydrogenolytic O-debenzylation of **4** under standard conditions (Pd/C, H_2 in ethyl acetate/methanol, 1:1) afforded quantitatively the methoxycarbonylamino-substituted benzyl-α-C-glucoside **5** which was treated with acetic anhydride in pyridine to afford the O-acetylated derivative **6**. Reaction of **6** with gaseous N_2O_4 in a solution of CCl_4, acetic acid, and sodium acetate[12] gave the N-nitroso-urethane **7** in high yield (95 %). A 0.2 M solution of sodium methanolate in methanol afforded the desired phenylstabilized diazomethane derivative **8** in 53% yield[13] as indicated by treatment with an aqueous acidic solution and per-O-acetylation of the products with acetic anhydride in pyridine furnishing known C-glucosides **9a,b**[5] in a quantitative reaction as 1:1-diastereomeric mixture. The structural assignment of all intermediates is based on their spectroscopic and analytical data (Table 1). Under the generation conditions, **8** proved to be completely

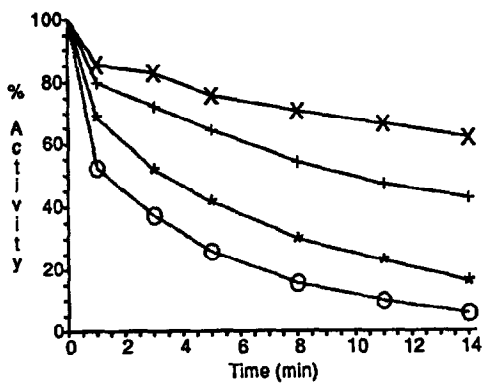
stable for several hours. Also in a buffered aqueous solution (tris-HCl, pH 8.0) no evolution of nitrogen could be observed; however, by UV-spectroscopy decomposition of **8** could be determined (~ 40-50% in 1 h).

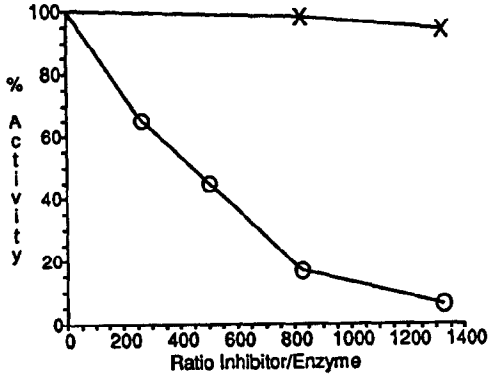
In order to determine the inhibitory activity and the type of inhibition, a freshly prepared methanolic solution of **8** (~ 10 µl; 90, 170, 280, and 450 nmole, respectively) was added to a solution of α-glucosidase from yeast[14] (23 µg protein ≙ 0.34 nmole) in 200 µl tris-HCl buffer (0.2 M, pH 8.0); the solution was incubated at 30°C. After 2,4,6,8,10,12, and 14 min (Fig 1) aliquots (20 µl) were taken from the incubation assay and added to a 1 ml cuvette containing sodium phosphate buffer (980 µl, 0.1 M, pH 6.8) and p-nitrophenyl α-D-glucopyranoside (0.4 M) at 30 °C. The time dependent loss of activity was calculated via the absorption of released p-nitrophenolate (at 400 nm); the results are exhibited in Fig. 1a.

Fig. 1 Inhibition of α-glucosidase from yeast and β-glucosidase from sweet almonds by α-Glucosyl-phenyldiazomethane (**8**).

Assay System:







(a) α-Glucosidase inhibition:
Time and concentration dependence
(× = 90, + = 170, ∗ = 280, ○ = 450 nmole **8**)

(b) α- And β-glucosidase inhibition (after 14 min):
Concentration dependence
(× = β-Glucosidase, ○ = α-Glucosidase)

In the presence of a large excess of inhibitor **8** an exponential decay of α-glucosidase activity with time was observed which is concentration dependent (Fig. 1b). The inactivation could not be reversed by dialysis of the enzyme. In order to show that compound **8** blocks the active site, inactivation of α-glucosidase was also performed in the presence of an excess of maltose (175 μmole); as expected, after dialysis higher enzyme activity compared with assays not containing this natural substrate was observed (for 450 nmole **8** after 20 min a 50% higher activity was observed). Analogous treatment of β-glucosidase from sweet almonds[14] with **8** had practically no effect on the enzyme activity (less than 5% inactivation after 14 min) (Fig. 1b).

In conclusion, **8** is a mechanism-based irreversible inhibitor specific for α-glucosidase. Promising perspectives can be envisaged via structural modifications of this readily accessible type of molecule.

Acknowledgements

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Table 1 Physical and Analytical Data of **3** - **8**, **9b**[a]

3: Colourless solid; $[\alpha]_D$ + 30° (c 1, $CHCl_3$); ^{1}H-NMR (250 MHz, $CDCl_3$): δ 3.46 (dd, $J_{4,5}$ = 5.5 Hz, $J_{5,6}$ = 4.0 Hz, 1 H, H-5), 3.53 (s, 3 H, OCH_3), 3.62-3.68 (m, 2 H, H-1a, -3), 3.73 (dd, $J_{1a,1b}$ = 10.6 Hz, $J_{1b,2}$ = 5.0 Hz, 1 H, H-1b), 3.89 (t, J = 5.5 Hz, 1 H, H-4), 3.92 (d, J = 11.4 Hz, 1 H, CH_aPh), 3.97-4.03 (m, 1 H, H-2), 4.17 (dd, $J_{5,6}$ = 4.0 Hz, $J_{6,7}$ = 9.2 Hz, 1 H, H-6), 4.25 (d, J = 11.4 Hz, 1 H, CH_bPh), 4.45-4.70 (m 6 H, 3 CH_2Ph), 4.96 (dd, $J_{6,7}$ = 9.2 Hz, $J_{7,NH}$ = 5 Hz, 1 H, H-7), 5.74 (bd, J = 5 Hz, 1 H, NH), 7.05-7.36 (m, 25 H, aryl).

Anal. Calc. for $C_{43}H_{45}N_1O_7$: C 75.08, H 6.59, N 2.04; Found: C 74.85, H 6.54, N 1.97.

4: Colourless oil; $[\alpha]_D$ + 23° (c 1, $CHCl_3$); ^{1}H-NMR (250 MHz, $CDCl_3$): δ 3.30 (dd, $J_{4,5}$ = 5 Hz, $J_{5,6}$ = 4 Hz, 1 H, H-5), 3.75-3.77 (m, 2 H, H-1a, -1b), 3.78 (dd, $J_{2,3}$ = 7.8 Hz, $J_{3,4}$ = 5 Hz, 1 H, H-3), 3.90 (t, J = 5 Hz, 1 H, H-4), 3.98 (d, J = 11.3 Hz, 1 H, CH_aPh), 4.08 (dd, $J_{5,6}$ = 4 Hz, $J_{6,7}$ = 8.6 Hz, 1 H, H-6), 4.13-4.19 (m 1 H, H-2), 4.40 (d, J = 11.3 Hz, 1 H, CH_bPh), 4.46-4.66 (m, 6 H, 3 CH_2Ph), 4.99 (d, J = 8.6 Hz, 1 H, H-7), 7.14-7.35 (m, 25 H, aryl); ^{13}C-NMR (62.9 MHz, $CDCl_3$): 59.04 (1 C, C-7), 69.10-78.40 (10C, C-1,-2,-3,-4,-5,-6, 4 CH_2Ph), 125.74 (1 C, NCO), 127.48-128.50 (25 C, o-, m-, p-Ph), 137.55-138.32 (5 C, ipso-Ph).

5: Colourless oil; $[\alpha]_D$ + 68° (c 1, MeOH); ^{1}H-NMR (250 MHz, D_2O): 3.30 (dd, $J_{4,5}$ = 6.5 Hz, $J_{5,6}$ = 3.8 Hz, 1 H, H-5), 3.36-3.77 (m, 5 H, H-1a,-1b,-2,-3,-4), 3.40 (s, 3 H, OCH_3), 4.08 (dd, $J_{5,6}$ = 3.8 Hz, $J_{6,7}$ = 10.2 Hz, 1 H, H-6), 4.77 (d, J = 10.2 Hz, 1 H, H-7), 7.15-7.24 (m, 5 H, aryl).

6: Colourless oil; $[\alpha]_D$ + 55° (c 1, $CHCl_3$); ^{1}H-NMR (250 MHz, $CDCl_3$): 1.61 (bs, 3 H, $COCH_3$), 1.99, 2.05, 2.09 (3 s, 9 H, 3 $COCH_3$), 3.58 (s, 3 H, OCH_3), 3.95 (dd, $J_{1a,1b}$ = 12.1 Hz, $J_{1a,2}$ = 2.7 Hz, 1 H, H-1a), 4.05-4.11 (m, 1 H, H-2), 4.39 (dd, $J_{5,6}$ = 3.9 Hz, $J_{6,7}$ = 10.1 Hz, 1 H, H-6), 4.53 (dd, $J_{1b,1a}$ = 12.1 Hz, $J_{1b,2}$ = 7.8 Hz, 1 H, H-1b), 4.63 (dd, $J_{4,5}$ = 6.5 Hz, $J_{5,6}$ = 3.9 Hz, 1 H, H-5), 4.82 (t, J = 6.5 Hz, 1 H, H-3), 4.94 (m, 1 H, H-7), 5.28 (t, J = 6.5 Hz, 1 H, H-4), 5.59 (d, J = 7.3 Hz, 1 H, NH), 7.20-7.34 (m, 5 H, aryl.

Anal. Calc. for $C_{23}H_{29}N_1O_{11}$: C 55.75, H 5.90, N 2.83; Found: C 55.92, H 5.92, N 3.11.

7: Greenyellowish oil; $[\alpha]_D$ + 59.5° (c 0.74, $CHCl_3$); ^{1}H-NMR (250 MHz, $CDCl_3$): ^{1}H-NMR (250 MHz, $CDCl_3$): δ 1.48, 2.01, 2.02, 2.05 (4 s, 12 H, 4 $COCH_3$), 3.47 (bs, 1 H, H-2), 4.04 (s, 3 H, OCH_3), 4.04-4.12 (m, 2 H, H-1a,-1b), 4.85-4.91 (m, 2 H, H-3,-5), 5.24 (t, J = 7.0 Hz, 1 H, H-4), 5.42 (dd, $J_{5,6}$ = 4.7 Hz, $J_{6,7}$ = 11.0 Hz, 1 H, H-6), 6.18 (bd, $J_{6,7}$ = 11.0 Hz, 1 H, H-7), 7.24-7.41 (m, 5 H, aryl); IR (CCl_4, cm^{-1}): 1443 (ν_{NO}); mass spectrum (EI), m/z 494 (M^+ - NO); UV/VIS (MeOH) max 202, 242, 388, 405, 423 nm.

8: IR (KBr, cm^{-1}): 2079 (s); UV/VIS (0.2 N NaOMe/MeOH) max 299, 475 nm.

9b: TLC (light petroleum/EtOAc 1:1) R_f = 0.52; $[\alpha]_D$ + 85° (c = 1.0, Et_2O); mp 60-61°C colourless crystaline substance; ^{1}H-NMR (250 MHz, $CDCl_3$): δ = 1.76, 2.00, 2.06, 2.07, 2.10 (5 s, 15 H, 5 $COCH_3$), 3.85 (dd, $J_{6,7a}$ = 3.9, $J_{7a,7b}$ = 11.8 Hz, 1 H, H-7a), 3.99-4.05 (m, 1 H, H-6), 4.23 (dd, $J_{6,7b}$ = 7.7, $J_{7b,7a}$ = 11.8 Hz, 1 H, H-7b), 4.31 (dd, $J_{1,2}$ = 9.1, $J_{2,3}$ = 2.2 Hz, 1 H, H-2), 4.78-4.82 (m, 1 H, H-5), 5.16-5.20 (m, 2 H, H-3,-4), 5.90 (d, $J_{1,2}$ = 9.1 Hz, 1 H, H-1), 7.27-7.40 (m, 5 H, Ph).

Anal. Calc. for **9b**: C 57.49, H 5.87; Found C 57.06, H 6.05

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[a] Optical rotation at 20°C

References and Notes

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13. During the synthesis of **8** a small amount of a byproduct with unknown structure was formed; this compound did not effect enzyme activity.
14. α-D-Glucosidase (yeast, EC 3.2.1.20) and p-nitrophenyl α-D-glucopyranoside (PNPG) were obtained from Boehringer Mannheim; also β-glucosidase from sweet almonds.

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