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Gram-scale synthesis of a glucopyranosylidene-spiro-thiohydantoin and its effect on hepatic glycogen metabolism studied in vitro and in vivo†

László Somsák,[a,*] Veronika Nagy,[a] Tibor Docsa,[b] Béla Tóth [b] and Pál Gergely [b]

[a]*Department of Organic Chemistry, Lajos Kossuth University, PO Box 20, H-4010 Debrecen, Hungary*

[b]*Department of Medical Chemistry, University Medical School of Debrecen, H-4026 Bem tér 18/B, Debrecen, Hungary*

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Abstract

A high yielding, simple synthesis is described starting from D-glucose to produce gram quantities of a glucopyranosylidene-spiro-thiohydantoin. This compound efficiently inhibited the activity of rat liver glycogen phosphorylase *a*; moreover, it also activated phosphorylase phosphatase which, in turn, decreased the amount of glycogen phosphorylase *a*. Both effects result in the inhibition of glycogen mobilization and the formation of glucose from glycogen.

Disorders of carbohydrate uptake may cause severe health problems such as diabetes[1] or obesity[2] which threaten a continuously increasing portion of the population. Among these diseases non-insulin dependent *diabetes mellitus* (NIDDM or Type II diabetes) is represented to a very large extent which can only be controlled by dietary regulation and with the use of some hypoglycaemic agents.[1] Glycogen phosphorylase (GP) present in the liver is the major regulatory enzyme of blood sugar level. Inhibition of this enzyme can provide a means for controlling blood glucose concentration.[3]

It has recently been demonstrated that glucopyranosylidene-spiro-hydantoin **8** is the most potent glucose analogue inhibitor known to date of muscle and liver GPs.[4,5] However, **8** is not simply accessible because its known syntheses are rather lengthy on the one hand, and stereochemically unfavourable on the other, since the much less efficient C-6 epimer of **8** is the main product.[4–7] We have also reported that the more easily available spiro-thiohydantoin **7** was an equally efficient inhibitor[5] of the above enzymes.

Although the reported synthetic sequence starting from D-glucose and producing **7** in six simple steps[5] is attractive, it has several drawbacks. First, the key intermediate, acetylated β-D-glucopyranosyl cyanide (**2**, Ac instead of Bz), can only be prepared in a very low yield (11%) requiring chromatographic purification and, at least in our hands, several tedious and time-consuming crystallizations.[8] Second, two

* Corresponding author. Tel: +36-52-512-900/2453; fax: +36-52-453-836; e-mail: somsak@tigris.klte.hu

† Dedicated to Professor K. Peseke on the occasion of his 60th birthday.

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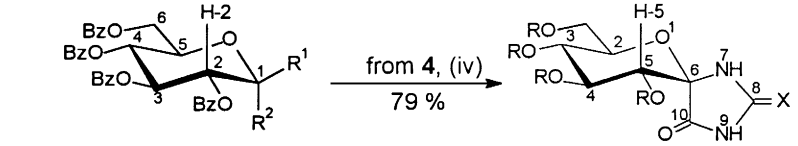
more chromatographies are needed to obtain **7** from D-glucose in an ~2% overall yield for the whole procedure. Therefore, this synthesis seemed not practical enough for the preparation of large amounts of this inhibitor required for more elaborate biological investigations.

Here we disclose a new procedure which allows **7** to be obtained in gram quantities, and demonstrate that **7** is efficient in controlling hepatic glycogen metabolism.

Benzobromoglucose[9] **1** was transformed into the benzoylated β-D-glucopyranosyl cyanide‡ **2** by mercury(II) cyanide in nitromethane. Compound **2** (mp: 114–116°C; $[\alpha]_D$ +52 (c=1.06, $CHCl_3$); NMR ($CDCl_3$) $\delta_{H\text{-}1}$ 4.67, $J_{1,2}$=9 Hz; δ_{CN} 114.52) was isolated by direct crystallization from diethyl ether in 58% yield. Partial hydrolysis of the nitrile was carried out with hydrogen bromide in acetic acid to give the *C*-glucosyl formamide **3** (mp: 226–228°C; $[\alpha]_D$ +26 (c=0.98, $CHCl_3$); NMR ($CDCl_3$) $\delta_{H\text{-}1}$ 4.27, $J_{1,2}$=10.1 Hz; δ_{CONH_2} 6.52, ~5.7; δ_{CONH_2} 169.40) in 94% yield as a crystalline raw-product of sufficient purity for the next step. Photobromination[10] of **3** with bromine in refluxing chloroform gave **4** (85% after crystallization from diethyl ether; mp: 170–175°C; $[\alpha]_D$+101 (c=1.02, MeOH); NMR ($CDCl_3$) δ_{CONH_2} 6.63, 5.44; δ_{C1} 93.19, δ_{CONH_2} 167.31, $^3J_{H\text{-}2,CONH_2} \cong 1$ Hz). Ring closure was performed with ammonium thiocyanate in nitromethane at 80°C in the presence of a small amount of elemental sulfur under nitrogen atmosphere[11] to yield after chromatographic separation spiro-thiohydantoin **6** (79%; mp: 199–202°C; $[\alpha]_D$ +26 (c=1.33, $CHCl_3$); NMR ($CDCl_3$) δ_{NH} 9.08, ~7.3; δ_{C6} 87.75, δ_{C8} 181.87, δ_{C10} 169.97, $^3J_{H\text{-}5,C\text{-}10}$=6.6 Hz) and hydroxy-amide **5** (6%; mp: 255–258°C; $[\alpha]_D$ +48 (c=0.89, $CHCl_3$); NMR ($CDCl_3$) δ_{CONH_2} 6.51, 5.23, δ_{OH} 5.65; (DMSO-d_6) δ_{C1} 94.47, δ_{CONH_2} 168.88, $^3J_{H\text{-}2,CONH_2} \cong 1$ Hz). Debenzoylation of **6** was achieved under Zemplén conditions in refluxing methanol to give **7** (92%, identical with the compound prepared previously by using acetyl protecting groups[5]).

The structure of the new compounds§ was proven by NMR data listed in the text indicating the presence of the new functional groups introduced at the anomeric centre. The configuration of the anomeric carbon was deduced from the corresponding vicinal proton–proton couplings (in **2** and **3**) or from the three-bond proton–carbon couplings (in **4**–**6**) as shown in Scheme 1.



(i) 58 % **1** R^1 = H, R^2 = Br → **2** R^1 = CN, R^2 = H

(ii) 94 % **2** → **3** R^1 = $CONH_2$, R^2 = H

(iii) 89 % **3** → **4** R^1 = $CONH_2$, R^2 = Br

5 R^1 = $CONH_2$, R^2 = OH

(v) 92 % **6** X = S, R = Bz → **7** X = S, R = H

8 X = O, R = H

Scheme 1. Reagents and conditions. (i) $Hg(CN)_2$, CH_3NO_2, rt; (ii) HBr, AcOH, rt; (iii) Br_2, $CHCl_3$, hν, reflux; (iv) NH_4SCN, CH_3NO_2, S_8, N_2 atm.; 80°C; (v) NaOMe, MeOH, reflux

‡ This compound was prepared for the first time with the participation of K. Hiruma during a stay of L.S. in F. W. Lichtenthaler's laboratory at the Technical University of Darmstadt as an Alexander von Humboldt Research Fellow in 1992–1993.

§ Each new compound gave a satisfactory elemental analysis.

The described synthetic route abandoned the difficulties in obtaining the β-D-glucopyranosyl cyanide. Since most of the reactions in this sequence were very clean the raw-products were sufficiently pure for further transformations, and no chromatographic purifications were needed, except for the isolation of **6**. These improvements allowed the overall yield for the synthesis of **7** to be raised to ~30% starting from D-glucose. Thus, we were able to get ~1 g of inhibitor **7**, and this facilitated the biological investigations described below.

Inhibitor constants (K_i) of spiro-hydantoin derivatives for purified muscle and liver GPs have recently been reported.[5] The K_i values were in the micromolar range. It could be concluded that GP*a* activities were less sensitive to the inhibitory action of hydantoin derivatives tested than GP*b*. Furthermore, thiohydantoin **7** and hydantoin **8** showed rather similar inhibitions.

Fig. 1A illustrates the effect of D-glucose and spiro-thiohydantoin **7** on the dephosphorylation of GP*a* in rat liver extracts catalyzed by phosphorylase phosphatase.[12] Both ligands can enhance the dephosphorylation (inactivation) of hepatic Gp*a*; however, **7**, applied in a much lower concentration, seems to be more effective. Fig. 1B demonstrates the effect of intravenous administration of either D-glucose or **7** on the activity of GP*a* in rat liver. It can be seen that **7** significantly decreases the active form of GP*a* in liver.

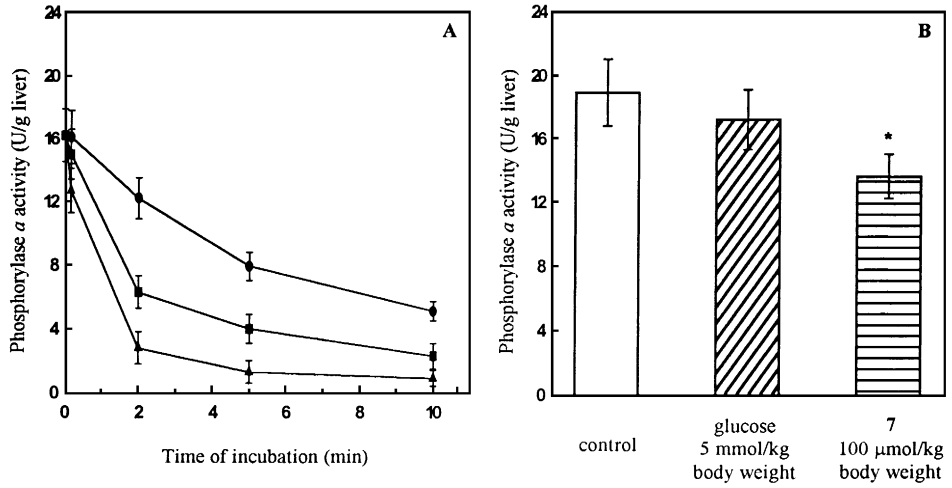


Fig. 1. Effect of D-glucose and **7** on the activity of hepatic glycogen phosphorylase *a* in vitro (A) and in vivo (B). A. Dephosphorylation of GP*a* in gel-filtered extracts from rat liver. Post-mitochondrial supernatants were prepared and filtered through Sephadex G-25 as described.[16] The filtrates were supplemented with 5 mM $(NH_4)_2SO_4$ and 1 mM magnesium acetate (●) in the presence of 5 mM D-glucose (■) or 100 μM **7** (▲). The filtrates were incubated at 30°C and samples were withdrawn at the indicated times for the assay of GP*a*.[16] The results are mean values±S.D. of four independent experiments. B. Change in the activity of GP*a* in rat liver. D-Glucose or **7** in a dose of 5 mmol or 100 μmol per body weight kg, respectively, was injected into the portal vein of Wistar rats. Liver samples were taken before (control) and 5 min after the administration of D-glucose or **7**. The tissues were homogenized for the assay of GP*a* as described.[16] Results are means±S.D. for four independent experiments. Asterisk indicates statistically significant difference compared to the control ($p<0.01$).

It is known that glucose and glucose analogues cause a sequential inactivation of liver GP*a* and activation (dephosphorylation) of glycogen synthase (GS).[13] We also tested the coordinated regulation of GP and GS in rat liver extract and observed that **7** promoted the activation of GS by significantly decreasing the latency in the dephosphorylation of GS (not documented).

The preliminary results presented here show that **7** promotes the dephosphorylation of hepatic GP*a* in cell extracts and in intact liver. The effects of **7** on GP and phosphorylase phosphatase confirm the suggested sequential mechanism.[14,15] The mechanism by which **7** inactivates GP*a* and activates GS may

be complex and requires further experiments. However, the observed effects of **7** clearly indicate that this compound and its analogues can be of potential use in controlling hyperglycaemia.

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