Pergamon

Tetrahedron: *Asymmetry* 11 (2000) 1719–1727

TETRAHEDRON: *ASYMMETRY*

# A new, scalable preparation of a glucopyranosylidene-spiro-thiohydantoin: one of the best inhibitors of glycogen phosphorylases

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Received 4 January 2000; accepted 20 March 2000

**Abstract**

Benzobromo-glucose was converted into per-*O*-benzoylated β-D-glucopyranosyl cyanide by mercury(II) cyanide in nitromethane. Partial hydrolysis of the nitrile with hydrogen bromide in acetic acid gave per-*O*-benzoylated *C*-(β-D-glucopyranosyl)formamide. Photobromination using bromine in carbon tetrachloride, chloroform, or dichloromethane gave the corresponding per-*O*-benzoylated 1-bromo-1-deoxy-β-D-glucopyranosyl cyanide and *C*-(1-bromo-1-deoxy-β-D-glucopyranosyl)formamide. Reaction of the latter with ammonium thiocyanate in nitromethane gave the per-*O*-benzoylated C-6*S* configured glucopyranosylidene-spiro-thiohydantoin together with a small amount of the per-*O*-benzoylated *C*-(1-hydroxy-β-D-glucopyranosyl)formamide. Debenzoylation of the spiro-thiohydantoin with sodium methoxide in methanol gave gram amounts of the title inhibitor. The described sequence should be suitable for scaling up and the target compound can be prepared in ~30% overall yield starting from D-glucose. 

## 1. Introduction

Diabetes is one of the most dangerous diseases killing people throughout the world.[1] Its complications, such as blindness, higher risks for heart or cerebrovascular as well as renal impairments, just to mention a few, are also severe drawbacks. While continuous insulin treatment can maintain normal blood sugar level in type I of this illness, in non-insulin dependent diabetes mellitus (NIDDM or type II diabetes) the blood glucose concentration is irrespective of the presence of this hormone.[1] Type II diabetes can be controlled mainly by dietary regulation or administration of several hypoglycemic agents; however, these methods are not very efficient. Therefore, considerable attention has been paid to understand the mode of action as well as to elicit inhibition of glycogen phosphorylase (GP) enzymes[2–9] responsible for the regulation of blood sugar level. An indole-carboxamide derivative was shown to be a very efficient I-site inhibitor of human liver GP.[10] Due to the efforts of Fleet's group one of the most potent glucose

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0957-4166/00/$ - see front matter 
PII: S0957-4166(00)00107-5

analogue inhibitors of muscle GP*b* known to date is a glucopyranosylidene-spiro-hydantoin[4,5,11] **9** ($K_i = 3.1$ μM[11] or 4.2 μM[12] for muscle GP*b*). Several synthetic routes[4,5,11] have been suggested for the preparation of **9**. Unfortunately, all of them consist of rather many steps (8–10 from an available starting material or even more from the appropriate free sugar) or are stereochemically inefficient, producing the C-6 epimer of **9** as the major product which is a much weaker inhibitor ($K_i = 320$ μM[4] or 105 μM[12]). These problems clearly hinder more sophisticated biochemical or biological investigations with inhibitor **9**.

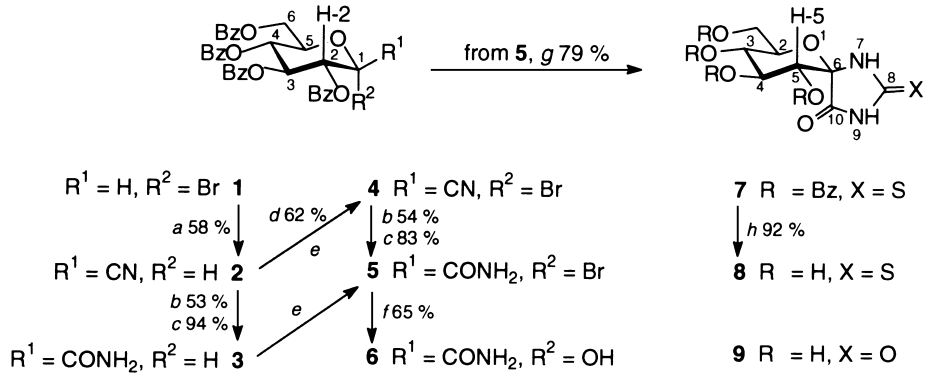
Recently we have shown[12] that a slight modification of **9**, namely changing the C-8 carbonyl to a thiocarbonyl group as in **8**, brings about practically no change in the inhibition of muscle GP*b* ($K_i = 5.1$ μM[12]). Similar inhibitor constants for **8** and **9** have been obtained for muscle GP*a* and liver GP*a* and *b* enzymes[12] as well, rendering these two compounds equipotent inhibitors. In contrast to **9**, compound **8** can advantageously be synthesized in a simple, six-step, highly stereoselective procedure starting from D-glucose. This method could open the way for preparing larger amounts of **8** required for more elaborate biological investigations, in order to understand further the mechanism of action of GPs and validate the concept of their inhibition as a potential therapy of NIDDM. However, to have a really practical synthesis several modifications have still been needed to improve the ~2% overall yield obtained for **8** from D-glucose by the published protocol. Thus, the seemingly unavoidable formation of hydroxy-amide **6** (Ac instead of Bz) accompanied in the ring closing step has been significantly reduced, as will be described in a forthcoming paper.[13] Next, the preparation of the key starting material β-D-glucopyranosyl cyanide (like **2**) has had to be amended, because the low yield (11%) of its acetyl-protected derivative has been the main reason for the very low overall yield of the sequence. The use of benzoyl protecting groups, as disclosed in this paper, has also reduced the number of chromatographic separations and facilitated the preparation of the important enzyme inhibitor **8** in gram quantities.

## 2. Results and discussion

The preparation of acetylated D-glycopyranosyl cyanides was studied with extreme care by Myers and Lee.[14,15] The best procedure proposed by them for 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl cyanide, i.e. fusion of acetobromo-glucose with mercury(II) cyanide[15] gave in our hands generally 10–12% of the target compound isolated by several consecutive crystallizations and/or chromatographic purification. There is no information in the literature to explain why the cyanation of acetobromo-glucose is so unselective under various conditions. The most important by-products are 1,2-*O*-(1-cyano-ethylidene) derivatives formed by an attack of the cyanide on the probable 1,2-acetoxonium ion intermediate and penta-*O*-acetyl-D-glucopyranoses.[14,15] Another possibility for the preparation of the acetylated β-D-glucopyranosyl cyanide could have been the procedure of Köll and Förtsch starting from the corresponding acetylated *C*-glycosyl nitromethane.[16] However, chromatographic separations would have been required even in this case. Therefore, we decided to use benzoyl protecting groups with the expectation of also promoting a higher tendency for crystallization of the benzoylated sugar derivatives. Another expected advantage was that in certain cases a lower tendency for formation of 1,2-orthoester derivatives from the intermediate 1,2-acyloxonium ion has been reported for 2-*O*-benzoylated sugars relative to their 2-*O*-acetylated counterparts.[17]

Benzobromo-glucose **1** obtained by a known procedure from penta-*O*-benzoyl-D-glucopyranoses with HBr in acetic acid[18] was treated with mercury(II) cyanide in dry nitromethane at

room temperature (Scheme 1). Crystallization of the worked-up mixture gave regularly 54–58% yields of 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl cyanide **2**. Chromatographic purification of the mother liquor gave a further 16–19% crop of **2**, and the presence of 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl cyanide[19] as well as 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranose[20] as minor by-products could also be detected by NMR spectroscopy.




Scheme 1. (*a*) $Hg(CN)_2$, $CH_3NO_2$, rt; (*b*) $TiCl_4$, AcOH, $H_2O$, rt; (*c*) HBr, AcOH, rt; (*d*) NBS, $CCl_4$, $Bz_2O_2$, reflux; (*e*) $Br_2$, solvent, hν, reflux; see Table 1; (*f*) $Ag_2O$, $H_2O$, DMSO, rt; (*g*) $NH_4SCN$, $CH_3NO_2$, $S_8$, $N_2$ atm., 80°C; (*h*) NaOMe, MeOH, reflux

For the partial hydrolysis of the nitrile moiety in **2** HBr in acetic acid[21] proved superior to the $TiCl_4$ mediated procedure[22] used in the acetylated series, giving **3** in 94% yield as an almost analytically pure crystalline product.

Radical mediated bromination[23] of **2** could be performed by *N*-bromosuccinimide in refluxing carbon tetrachloride in the presence of catalytic benzoyl-peroxide.[24] However, the insolubility of the 1-bromoglucosyl-cyanide **4** in this solvent made the work-up of the reaction mixture difficult (see Experimental) and this decreased the yield of **4** to 62%. Photobromination of **2** with bromine in refluxing carbon tetrachloride[24] gave **4** as an essentially pure product in an almost quantitative yield. The above conditions could not be used for the bromination of **3** because of its very poor solubility in carbon tetrachloride even at reflux. Replacement of this solvent partially or fully with chloroform in the photobromination reaction gave excellent yields of **5** (Table 1). The product formed quantitatively in the chloroform reaction was again sufficiently pure for the further transformations. Dichloromethane was also tried as a solvent for the photobrominations and gave similar yields for both **4** and **5**. These experiments show that the use of solvents other than carbon tetrachloride is possible in photobrominations of carbohydrate derivatives. Note that very

Table 1
Isolated yield (%) of the brominated products under conditions (*e*) shown in Scheme 1

| Solvent | 4 | 5 |
|---|---|---|
| $CCl_4$ | 80 (95*) | – |
| $CCl_4$–$CHCl_3$ 1 : 2 | – | 87 |
| $CHCl_3$ | 67 (quant.*) | 89 (quant.*) |
| $CH_2Cl_2$ | 70 (quant.*) | 60 (97*) |

*Yield of the crude product if sufficiently pure for further transformations

recently 1,1,1-trichloroethane was suggested as a replacement for carbon tetrachloride in similar radical-mediated halogenations of *C*-glycosyl formates.[25]

Reaction of **5** with ammonium thiocyanate in nitromethane in the presence of elemental sulfur to suppress radical-mediated pathways[13] under nitrogen atmosphere gave spiro-thiohydantoin **7** (79%) and hydroxy-amide **6** (6%). Compound **6** was also prepared independently from **5** by silver oxide-promoted hydrolysis in dimethylsulfoxide. Debenzoylation of **7** was accomplished by the Zemplén method in methanol at reflux to give inhibitor **8** in 92% yield.

Structure elucidation of the new compounds was straightforward using NMR methods and requires no comment for compounds **2** and **3**. In the case of compounds **4–6** having a quaternary anomeric center the conformation of the sugar ring was established as $^4C_1$ from the vicinal proton–proton couplings. Knowledge of this allowed the configuration of the quaternary carbons to be deduced from the three bond heteronuclear couplings between H-2 and the *C*-substituent of the anomeric center, as described earlier.[26] Thus, the observed couplings (**4** $^3J_{\text{H-2,CN}} = 2.1$ Hz; **5** $^3J_{\text{H-2,CONH2}} < 1$ Hz; **6** $^3J_{\text{H-2,CONH2}} \cong 1$ Hz) prove the depicted structures for **4–6**. In the same manner the configuration of the spiro carbon (C-6) in **7** was assigned as *S* based on the $^3J_{\text{H-5,C-10}} = 6.6$ Hz value. Compound **8** proved identical with a sample prepared by the previously published route.[12]

As benzobromo-glucose **1** can be made from D-glucose via the pentabenzoate in 86% yield, for the two steps the overall yield for **8** is ~30%. Most reactions were clean and the crude products could be used without purification. Even if further treatment is necessary it needs only to be crystallization. The sole chromatography required has been the separation of **7** from the minor by-products. This highly practicable synthetic sequence allowed **8** to be prepared in gram quantities. Thus, in vitro and in vivo studies of hepatic glycogen metabolism became possible, whose results have been published elsewhere.[27]

## 3. Experimental

Melting points were measured in open capillary tubes or on a Kofler hot-stage and are uncorrected. Optical rotations were determined with a Perkin–Elmer 241 polarimeter at room temperature. NMR spectra were recorded with Bruker WP 200 SY (200/50 MHz for $^1H/^{13}C$) or Bruker AM 360 (360/90 MHz for $^1H/^{13}C$) spectrometers. Chemical shifts are referenced to $Me_4Si$ ($^1H$), or to the residual solvent signals ($^{13}C$). TLC was performed on DC-Alurolle Kieselgel 60 $F_{254}$ (Merck) (eluent EtOAc:hexane 1:2, unless stated otherwise), and the spots were visualized under UV light and by gentle heating. For column chromatography Kieselgel 60 (Merck, particle size 0.063–0.200 mm) was used. Organic solutions were dried over anhydrous $MgSO_4$ and concentrated in vacuo at 40–50°C (bath temperature). Nitromethane and carbon tetrachloride were distilled from $P_4O_{10}$ and stored over molecular sieves (3 Å). Other solvents were of commercial analytical grade quality and have been used without further purification.

### 3.1. 2,3,4,6-Tetra-O-benzoyl-β-D-glucopyranosyl cyanide† 2

Benzobromo-glucose[18] **1** (9.89 g, 15 mmol) was dissolved in dry nitromethane (40 ml) and mercury(II) cyanide (3.79 g, 15 mmol, dried at 100°C in vacuo for 20 h) was added. The mixture

† This compound was prepared for the first time 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 1992–93 with the participation of K. Hiruma.

was stirred at rt for 2 days. The solids were then filtered off, washed with nitromethane, and the solvent was removed from the combined filtrate and washings. The residue was dissolved in chloroform, the solution filtered if necessary and washed with 1 M aq. KBr solution (2×). After drying the solvent was removed and the remaining syrup was crystallized from diethyl ether to give 5.09 g (56%) of **2**. Chromatographic purification (eluent EtOAc:hexane 1:5) of a 0.41 g crop of the material (4.06 g) obtained after evaporating the solvent from the mother liquor gave 0.18 g (corresponding to another 19%) of **2**. Mp 114–116°C; $[\alpha]_D$ +52 ($c=1.06$, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 8.1–7.7, 7.6–7.2 (m, 20H, 4×$C_6H_5$), 5.9–5.8, 5.73–5.67 (strongly coupled signals, 3H, H-2,3,4), 4.67 (d, 1H, H-1, $J_{1,2}=9$ Hz), 4.65 (dd, 1H, H-6, $J_{6,6'}=12.4$ Hz), 4.47 (dd, 1H, H-6′, $J_{5,6'}=5.4$ Hz), 4.20 (ddd, 1H, H-5, $J_{4,5}=10.1$ Hz); $^{13}$C NMR ($CDCl_3$) $\delta$ 166.25, 165.98, 165.22, 164.81 (C=O), 114.52 (CN), 77.45, 73.25, 69.83, 68.72, 67.11 (C-1–C-5), 62.76 (C-6). Anal. calcd for $C_{35}H_{27}NO_9$ (605.60): C 69.41, H 4.49, N 2.31. Found: C 69.05, H 4.47, N 2.11. Two other minor components of the mother liquor were identified on the basis of their $^1$H NMR spectra as 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl cyanide[19] and 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranose.[20]

### 3.2. *C-(2,3,4,6-Tetra*-O-*benzoyl*-β-D-*glucopyranosyl)formamide* **3**

(a) *With HBr/AcOH*: Glucosyl cyanide **2** (13.1 g, 20 mmol) was suspended in a solution of HBr in acetic acid (20 ml, 20% m/m) and the mixture was stirred at rt for 3 h. The resulting solution was poured into ice-water (200 ml), which was then extracted with chloroform (2×200 ml). The unified $CHCl_3$ phases were washed with satd aq. $NaHCO_3$ (2×100 ml), then with water (100 ml), dried, and the solvent removed. The crystalline residue (**3** 11.7 g, 94%) was sufficiently pure for the further step. An analytical sample was obtained by dissolving the crude material in chloroform and precipitating it with diethyl ether. Mp 226–228°C; $[\alpha]_D$ +26 ($c=0.98$, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 8.15–7.8, 7.6–7.2 (m, 20H, 4×$C_6H_5$), 6.52 (br s, 1H, CON$H_2$), 5.96 (pseudo t, 1H, H-3, $J_{2,3}\sim J_{3,4}\sim 9.2$ Hz), 5.8–5.6 (m, 3H, H-2,4, CON$H_2$), 4.71 (dd, 1H, H-6, $J_{5,6}=2.2$ Hz), 4.51 (dd, 1H, H-6′, $J_{6,6'}=12.8$ Hz), 4.26 (d, 1H, H-1, $J_{1,2}=10.1$ Hz), 4.20 (ddd, 1H, H-5, $J_{5,6'}=4.8$ Hz); $^{13}$C NMR ($CDCl_3$) $\delta$ 169.40 *C*ONH$_2$), 166.04, 165.80, 165.57, 165.50 (C=O), 76.54, 76.43, 73.80, 70.32, 69.31 (C-1–C-5), 63.02 (C-6). Anal. calcd for $C_{35}H_{29}NO_{10}$ (623.62): C 67.41, H 4.69, N 2.25. Found: C 67.14, H 4.69, N 2.49.

(b) *With $TiCl_4$*: Glucosyl cyanide **2** (9.94 g, 16 mmol) was suspended in acetic acid (16 ml), the suspension was cooled to 0°C in an ice-bath, and $TiCl_4$ (3.58 g, 32 mmol) followed by water (0.0295 μl, 16 mmol) was added. The mixture turned yellow and was stirred at rt until TLC had indicated disappearance of the starting material (3–4 days). It was then diluted with ice-water (100 ml) and extracted with chloroform (3×80 ml). The combined chloroform phases were washed with ice-cold satd aq. $NaHCO_3$ until neutral, then with water, dried and the solvent evaporated. The residue was crystallized from diethyl ether to give 6.2 g (53%) of **3**.

### 3.3. *2,3,4,6-Tetra*-O-*benzoyl-1-bromo-1-deoxy*-β-D-*glucopyranosyl cyanide* **4**

(a) *With bromine*: Glucosyl cyanide **2** (400 mg, 0.66 mmol) was dissolved in carbon tetrachloride (10 ml) and bromine (0.14 ml, 2.64 mmol) and some $BaCO_3$ were added. The mixture was placed in an Erlenmeyer flask above a heat lamp (375 W, white, distance from the lamp ~1–2 cm, height of the solution 1–1.5 cm) and refluxed until TLC showed complete transformation (~2.5 h). It was then filtered, and the filtrate washed with 5% aq. $NaHSO_3$ and satd aq. $NaHCO_3$ solutions.

Drying and evaporation of the solvent gave 431 mg (95%) of **4** as white crystals from which an analytical sample was obtained by recrystallization from ethanol. Mp 152–154°C; $[\alpha]_D$ +119 ($c = 0.95$, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 8.13–7.23 (m, 20H, 4×$C_6H_5$), 6.11 (t, 1H, H-3, $J_{3,4} = 9.6$ Hz), 5.84 (t, 1H, H-4, $J_{4,5} = 9.6$ Hz), 5.82 (d, 1H, H-2, $J_{2,3} = 9.6$ Hz), 4.72 (ddd, 1H, H-5, $J_{5,6} = 2.2$ Hz), 4.69 (dd, 1H, H-6, $J_{6,6'} = 12.7$ Hz), 4.56 (dd, 1H, H-6′, $J_{5,6'} = 4.7$ Hz); $^{13}$C NMR ($CDCl_3$) $\delta$ 167.20, 165.80, 165.33, 165.10 (C=O), 113.90 (CN, $J_{\text{H-2,CN}} = 2.1$ Hz), 93.43 (C-1), 75.16, 72.22, 70.59, 67.99 (C-2–C-5), 61.82 (C-6). Anal. calcd for $C_{35}H_{26}BrNO_9$ (684.49): C 61.41, H 3.82, N 2.04. Found: C 61.42, H 3.62, N 1.97.

The reactions in $CHCl_3$ and $CH_2Cl_2$ were performed similarly except that instead of $BaCO_3$ $K_2CO_3$ was added as the acid scavenger. In the $CHCl_3$ reaction additional portions of $Br_2$ had to be added to the mixture (see also the procedure for the preparation of **5**). Work-up was effected by adding 5% aq. $NaHSO_3$ solution to the cooled reaction mixture. After shaking and separating the phases the organic solution was washed with water, dried, and the solvent removed to give the crystalline crude product which was recrystallized from ethanol. Yields are indicated in Table 1.

(b) *With N-bromosuccinimide*:‡ Glucosyl cyanide **2** (6.75 g, 11.15 mmol) was dissolved in carbon tetrachloride (140 ml); bromotrichloromethane (60 ml), *N*-bromosuccinimide (4.96 g, 27.86 mmol) and some benzoyl-peroxide were added. The mixture was heated under reflux for 2 h, during which time the product precipitated as a white solid. After cooling to rt dichloromethane was added to the mixture until dissolution of all of the solids, and the solution was washed with 5% aq. $Na_2SO_3$ (2×), satd aq. $NaHCO_3$, and water. Drying and evaporation of the solvents left a solid which was suspended in a small amount of ethanol, boiled under reflux for several minutes and the warm suspension was filtered to give a crystalline substance. Recrystallization from ethyl acetate gave **4** (4.78 g, 62%).

### *3.4. C-(2,3,4,6-Tetra-*O*-benzoyl-1-bromo-1-deoxy-*β*-D-glucopyranosyl)formamide* **5**

(a) *By photobromination of* ***3***: *C*-Glucosyl formamide **3** (200 mg, 0.32 mmol) was dissolved in chloroform (6 ml), bromine (0.07 ml, 1.28 mmol) and some $BaCO_3$ were added, and the mixture was irradiated and refluxed by a heat lamp as described in procedure (a) for **4**. After 1 h the mixture decolorized and 0.1 ml $Br_2$ was added again. This was repeated after another 0.5 h. After TLC had shown complete transformation (~2 h from the start) the mixture was filtered, washed with 5% aq. $NaHSO_3$ and satd aq. $NaHCO_3$ solutions, dried, and the solvent removed. The residual syrup (264 mg) crystallized on addition of diethyl ether to give 201 mg (89%) of **5**. Mp 170–173°C; $[\alpha]_D$ +101 ($c = 1.02$, $CH_3OH$); $^1$H NMR ($CDCl_3$) $\delta$ 8.15–7.74, 7.68–7.21 (m, 20 H, 4×$C_6H_5$), 6.63 (br s, 1H, CON$H_2$), 6.14 (t, 1H, H-3, $J_{3,4} = 9.5$ Hz), 5.81 (t, 1H, H-4, $J_{4,5} = 9.5$ Hz), 5.76 (d, 1H, H-2, $J_{2,3} = 9.5$ Hz), 5.44 (br s, 1H, CON$H_2$), 4.85 (dd, 1H, H-6, $J_{5,6} = 2.2$ Hz), 4.72 (ddd, 1H, H-5, $J_{5,6'} = 4.2$ Hz), 4.52 (dd, 1H, H-6′, $J_{6,6'} = 12.4$ Hz); $^{13}$C NMR ($CDCl_3$) $\delta$ 167.31 *C*$ONH_2$, $J_{\text{H-2,CONH2}} < 1$ Hz), 166.30, 165.41, 164.89, 164.70 (C=O), 93.19 (C-1), 74.74, 71.99, 70.21, 67.62 (C-2–C-5), 61.80 (C-6). Anal. calcd for $C_{35}H_{28}BrNO_{10}$ (702.52): C 59.84, H 4.02, N 1.99. Found: C 60.15, H 4.08, N 2.23.

The photobrominations in other solvents (Table 1) were performed similarly. In the $CH_2Cl_2$ reaction no further addition of $Br_2$ was necessary.

‡ This protocol for the preparation of **4** was worked out 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 1992–93 with the participation of K. Hiruma.

(b) *By partial hydrolysis of* ***4*** *with* $TiCl_4$: Prepared from bromo-cyanide **4** (7.7 g, 11.31 mmol) as described for the preparation of amide **3** to give 4.28 g (54%) of **5**.

(c) *By partial hydrolysis of* ***4*** *with HBr/AcOH*: Prepared from bromo-cyanide **4** (0.5 g, 0.73 mmol) as described for the preparation of amide **3** to give 425 mg (83%) of **5** after crystallization from diethyl ether.

### 3.5. *C-(2,3,4,6-Tetra-*O*-benzoyl-1-hydroxy-*β*-D-glucopyranosyl)formamide* ***6***

Bromo-amide **5** (200 mg, 0.285 mmol) was dissolved in DMSO (12 ml); silver oxide (66 mg, 0.285 mmol) and water (5.12 μl, 0.285 mmol) were added. The mixture was stirred at rt in the dark for 3 h. It was than filtered and the filtrate diluted with water. The precipitate was filtered off, dissolved in dichloromethane, and the solution was washed with 10% aq. $NH_4SCN$ solution. After drying and solvent removal the residue was recrystallized from ethanol to give 119 mg (65%) of **6**. Mp 255–258°C; $[\alpha]_D$ +48 ($c=0.89$, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 8.15–7.15 (m, 20H, $4\times C_6H_5$), 6.51 (br s, 1H, CON$H_2$), 6.22 (pseudo t, 1H, H-3, $J_{3,4}=9.6$ Hz), 5.81 (pseudo t, 1H, H-4, $J_{4,5}=9.8$ Hz), 5.67 (d, 1H, H-2, $J_{2,3}=9.9$ Hz), 5.63 (br s, 1H, CON$H_2$), 5.23 (br s, 1H, OH), 4.70 (ddd, 1H, H-5, $J_{5,6}=2.8$ Hz), 4.65 (dd, 1H, H-6, $J_{6,6'}=12.5$ Hz), 4.48 (dd, 1H, H-6′, $J_{5,6'}=4.8$ Hz); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 168.88 *C*ONH$_2$, ($J_{\text{H-2,CONH2}} \leq 1$ Hz), 165.49, 165.16, 164.81, 164.65 (C=O), 94.47 (C-1), 72.15, 71.64, 69.13, 68.70 (C-2–C-5), 62.96 (C-6). Anal. calcd for $C_{35}H_{29}NO_{11}$ (639.62): C 65.72, H 4.57, N 2.19. Found: C 65.53, H 4.71, N 2.31.

### 3.6. *(2*R*,3*R*,4*S*,5*R*,6*S*)3,4,5-Tribenzoyloxy-2-benzoyloxymethyl-7,9-diaza-1-oxa-spiro[4,5]decane-10-one-8-thione 7*

*C*-(1-Bromoglucosyl)formamide **5** (4 g, 5.69 mmol) was dissolved in dry nitromethane (23 ml). Molecular sieves (3 Å), ammonium thiocyanate (1.732 g, 22.79 mmol) and elemental sulfur (18 mg, 0.56 mmol) were added, and the mixture was stirred in an 80°C bath under $N_2$ atmosphere for 7 h. The syrupy residue obtained after filtration and solvent removal was dissolved in dichloromethane, the solution filtered, washed with satd aq. $NH_4Cl$ solution, dried, and concentrated. The remaining syrup was separated by silica gel column chromatography with EtOAc:hexane 2:5 eluent. The first fraction which crystallized from methanol gave 3.09 g (79%) of thiohydantoin **7**. Mp 199–202°C; $[\alpha]_D$ +26 ($c=1.33$, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 9.08 (br s, 1H, NH), 8.09–7.13 (m, 21H, $4\times C_6H_5$, NH), 6.67 (t, 1H, H-4, $J_{3,4}=10$ Hz), 5.95 (d, 1H, H-5, $J_{4,5}=9.7$ Hz), 5.91 (pseudo t, 1H, H-3, $J_{2,3}=10$ Hz), 5.21 (ddd, 1H, H-2, $J_{2,CH2a}=2.5$ Hz), 4.75 (dd, 1H, $CH_2$a, $J_{CH2}=12.6$ Hz), 4.44 (dd, 1 H, $CH_2$b, $J_{2,CH2b}=4.0$ Hz); $^{13}$C NMR ($CDCl_3$) $\delta$ 181.93 (C-8), 169.90 (C-10, $J_{\text{H-5,C-10}}=6.6$ Hz), 165.73, 165.60, 165.51 (C=O), 87.75 (C-6), 71.69, 70.90, 70.41, 68.91 (C-2–C-5), 62.84 (*C*H$_2$). Anal. calcd for $C_{36}H_{28}N_2O_{10}S$ (680.68): C 63.52, H 4.15, N 4.12. Found: C 63.50, H 4.16, N 4.24.

The second fraction (227 mg) contained three substances (monitored in diethyl ether:hexane 1:1) which were not characterized. The third fraction gave 231 mg (6%) of hydroxy-amide **6**.

### 3.7. *(2*R*,3*S*,4*S*,5*R*,6*S*)3,4,5-Trihydroxy-2-hydroxymethyl-7,9-diaza-1-oxa-spiro[4,5]decane-10-one-8-thione* ***8***

Thiohydantoin **7** (3.09 g, 4.53 mmol) was dissolved in absolute methanol (20 ml) and 1 M methanolic sodium methoxide solution (1.5 ml) was added. The reaction mixture was refluxed for

2 h, cooled to rt and then neutralized with a cation exchange resin Amberlyst 15 ($H^+$ form). Filtration and solvent removal left a syrup which solidified to an amorphous product on addition of diethyl ether. This material was filtered off and washed several times with hexane to remove traces of methyl-benzoate to give 1.1 g (92%) of **8** which proved identical to the reported compound. $[\alpha]_D$ +18 ($c = 1.03$, $CH_3OH$) (lit.[12] $[\alpha]_D$ +19 ($c = 2.34$, $CH_3OH$)); $^1H$ NMR ($D_2O$) $\delta$ 4.23 (m unres., 1H, H-2), 4.13 (t, 1H, H-4, $J_{3,4} \sim J_{4,5} \sim 9.5$ Hz), 3.87–3.62 (m, 3H, H-5, $CH_2$), 3.49 (t, 1H, H-3, $J_{2,3} \sim 9.5$ Hz); $^{13}C$ NMR ($D_2O$) $\delta$ 187.16 (C-8), 175.97 (C-10), 91.81 (C-6), 77.63, 74.83, 74.68, 71.37 (C-2–C-5), 63.14 ($CH_2$).

## Acknowledgements

This work was supported by the Hungarian Scientific Research Fund (Grant No. OTKA T19339).

## References

1. Hengesh, E. J. In *Principles of Medicinal Chemistry*; Foye, W. O.; Lemke, T. L.; Williams, D. A., Eds.; Williams and Wilkins: Baltimore, 1995; pp. 581–600.
2. Johnson, L. N. *FASEB J*. **1992**, *6*, 2274–2282.
3. Oikonomakos, N. G.; Kontou, M.; Zographos, S. E.; Tsitoura, H. S.; Johnson, L. N.; Watson, K. A.; Mitchell, E. P.; Fleet, G. W. J.; Son, J. C.; Bichard, C. J. F.; Leonidas, D. D.; Acharya, K. R. *Eur. J. Drug Metabol. Pharmakokin*. **1994**, 185–192.
4. Krülle, T. M.; Fuente, C.; Watson, K. A.; Gregoriou, M.; Johnson, L. N.; Tsitsanou, K. E.; Zographos, S. E.; Oikonomakos, N. G.; Fleet, G. W. J. *Synlett* **1997**, 211–213.
5. Fuente, C.; Krülle, T. M.; Watson, K. A.; Gregoriou, M.; Johnson, L. N.; Tsitsanou, K. E.; Zographos, S. E.; Oikonomakos, N. G.; Fleet, G. W. J. *Synlett* **1997**, 485–487.
6. Krülle, T. M.; Fuente, C.; Pickering, L.; Aplin, R. T.; Tsitsanou, K. E.; Zographos, S. E.; Oikonomakos, N. G.; Nash, R. J.; Griffiths, R. C.; Fleet, G. W. J. *Tetrahedron: Asymmetry* **1997**, *8*, 3807–3820.
7. Agasimundin, Y. S.; Mumper, M. W.; Hosmane, R. S. *Bioorg. Med. Chem*. **1998**, *6*, 911–923.
8. Heightman, T. D.; Vasella, A.; Tsitsanou, K. E.; Zographos, S. E.; Skamnaki, V. T.; Oikonomakos, N. G. *Helv. Chim. Acta* **1998**, *81*, 853–864.
9. Gregoriou, M.; Noble, M. E. M.; Watson, K. A.; Garman, E. F.; Krülle, T. M.; Fuente, C.; Fleet, G. W. J.; Oikonomakos, N. G.; Johnson, L. N. *Protein Sci*. **1998**, *7*, 915–927.
10. Martin, W. H.; Hoover, D. J.; Armento, S. J.; Stock, I. A.; McPherson, R. K.; Danley, D. E.; Stevenson, R. W.; Barrett, E. J.; Treadway, J. L. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 1776–1781.
11. Bichard, C. J. F.; Mitchell, E. P.; Wormald, M. R.; Watson, K. A.; Johnson, L. N.; Zographos, S. E.; Koutra, D. D.; Oikonomakos, N. G.; Fleet, G. W. J. *Tetrahedron Lett*. **1995**, *36*, 2145–2148.
12. Ősz, E.; Somsák, L.; Szilágyi, L.; Kovács, L.; Docsa, T.; Tóth, B.; Gergely, P. *Bioorg. Med. Chem. Lett*. **1999**, *9*, 1385–1390.
13. Somsák, L.; Kovács, L., in preparation.
14. Myers, R. W.; Lee, Y. C. *Carbohydr. Res*. **1984**, *132*, 61–85.
15. Myers, R. W.; Lee, Y. C. *Carbohydr. Res*. **1986**, *154*, 145–163.
16. Köll, P.; Förtsch, A. *Carbohydr. Res*. **1987**, *171*, 301–315.
17. Veeneman, G. H. In *Carbohydrate Chemistry*; Boons, G. J., Ed.; Blackie Academic and Professional: London, 1998; pp. 98–174.
18. Lockhoff, O. In *Methoden der organischen Chemie (Houben-Weyl)*; Hagemann, H.; Klamann, D., Eds.; Thieme: Stuttgart, 1992; Vol. E14a/3, p. 708.
19. Gyóllai, V.; Somsák, L., unpublished results.
20. Salinas, A. E.; Sproviero, J. F.; Deulofeu, V. *Carbohydr. Res*. **1987**, *170*, 71–99.

21. Myers, R. W.; Lee, Y. C. *Carbohydr. Res.* **1986**, *152*, 143–158.
22. BeMiller, J. N.; Yadav, M. P.; Kalabokis, V. N.; Myers, R. W. *Carbohydr. Res.* **1990**, *200*, 111–126.
23. Somsák, L.; Ferrier, R. J. *Adv. Carbohydr. Chem. Biochem.* **1991**, *49*, 37–92.
24. Somsák, L.; Batta, G.; Farkas, I. *Carbohydr. Res.* **1983**, *124*, 43–51.
25. Smith, M. D.; Long, D. D.; Martín, A.; Campbell, N.; Blériot, Y.; Fleet, G. W. J. *Synlett* **1999**, 1151–1153.
26. Somsák, L.; Sós, E.; Györgydeák, Z.; Praly, J.-P.; Descotes, G. *Tetrahedron* **1996**, *52*, 9121–9136.
27. Somsák, L.; Nagy, V.; Docsa, T.; Tóth, B.; Gergely, P. *Tetrahedron: Asymmetry* **2000**, *11*, 405–408.