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Note

# Synthesis of some derivatives of *C*-(1-deoxy-1-*N*-substituted-D-glucopyranosyl)formic acid (D-*gluco*-hept-2-ulopyranosonic acid) as potential inhibitors of glycogen phosphorylase

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**Abstract**—Per-*O*-benzoylated derivatives (amide, methyl ester and glycinamide) of *C*-(1-azido-1-deoxy-α-D-glucopyranosyl)formic acid obtained by azide substitution in the corresponding *C*-(1-bromo-1-deoxy-β-D-glucopyranosyl)formic acid derivatives were debenzoylated by the Zemplén-protocol. Per-*O*-benzoylated *C*-(1-azido-1-deoxy-α-D-glucopyranosyl)formamide was dehydrated by oxalyl chloride–DMF to give the corresponding nitrile, while from its reduction mixture obtained by Raney-nickel or sodium hydrogentelluride *C*-(1-amino-1-deoxy-β-D-glucopyranosyl)formamide could be isolated. Acetylation of this amino-amide by $Ac_2O$/Py and subsequent debenzoylation gave *C*-(1-acetamido-1-deoxy-β-D-glucopyranosyl)formamide. Applying the same conditions to the crude reduction mixture allowed the α-anomer to be isolated as a minor component. An alternative pathway to produce the above β-anomer appeared in the reaction of *C*-(1-bromo-1-deoxy-β-D-glucopyranosyl)formamide with $CH_3CN$ in the presence of $Ag_2CO_3$ to yield 1-acetamido-2,3,4,6,-tetra-*O*-benzoyl-1-deoxy-β-D-glucopyranosyl cyanide, which was hydrated, in the presence of $TiCl_4$, to the formamide. Some of the new compounds were shown to be weak inhibitors of muscle glycogen phosphorylase *b*.


*Keywords:* Glycosyl azides; Glycosyl amides; Hept-2-ulopyranosonic acid derivatives; Glycogen phosphorylase inhibitors

Decreasing blood glucose levels by inhibition of glycogen phosphorylase[1–3] (GP) is one of several intensively investigated concepts,[4–8] which can be promising for finding new treatments of type 2 diabetes mellitus.[9] Pioneering studies by Fleet, Johnson and Oikonomakos on glucose analogue inhibitors of GP[1–3] culminated in discovering a glucopyranosylidene-spiro-hydantoin **1**,[10] which, together with its thio analogue **2**,[11,12] is among the most potent inhibitors[†] of rabbit muscle GP. The best glucose analogue known to date is the *N*-(2-naphthoyl)-*N*′-(β-D-glucopyranosyl) urea ($K_i = 0.4$ μM).[2] Double functionalization of the anomeric carbon atom in D-glucose other than spirocyclization[‡] has not been thoroughly studied in the above context. To the best of our knowledge, the only compounds of this type tested with rabbit muscle GP are represented by structures **3**–**5** suggesting that a –CONH– moiety at the α-position of the D-glucopyranose ring might be advantageous for the inhibition. Here, we report on the synthesis of some new anomerically bifunctionalized glucose analogues (derivatives of D-*gluco*-hept-2-ulopyranosonic acid), from which **10** and **18** have recently been investigated kinetically and by protein crystallography as inhibitors of GP.[15]

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† For other inhibitors of GP the reader is kindly referred to recent review articles[1–3] and the literature quoted there.

‡ Spirocyclization has thoroughly been reviewed with reference to chemistry[13,14] as well as enzyme inhibition and other biological effects.[1–3]

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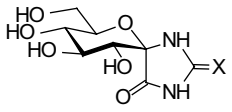
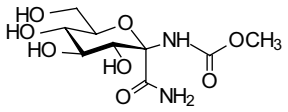
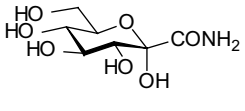
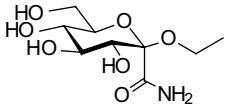




**1** X = O ($K_i$ = 3-4 μM[10,11]) **3** ($K_i$ = 16 μM[16])
**2** X = S ($K_i$ = 5 μM[11,12])





**4** ($K_i$ = 3100 μM[12]) **5** (~25 % inhibition at 625 μM[2])

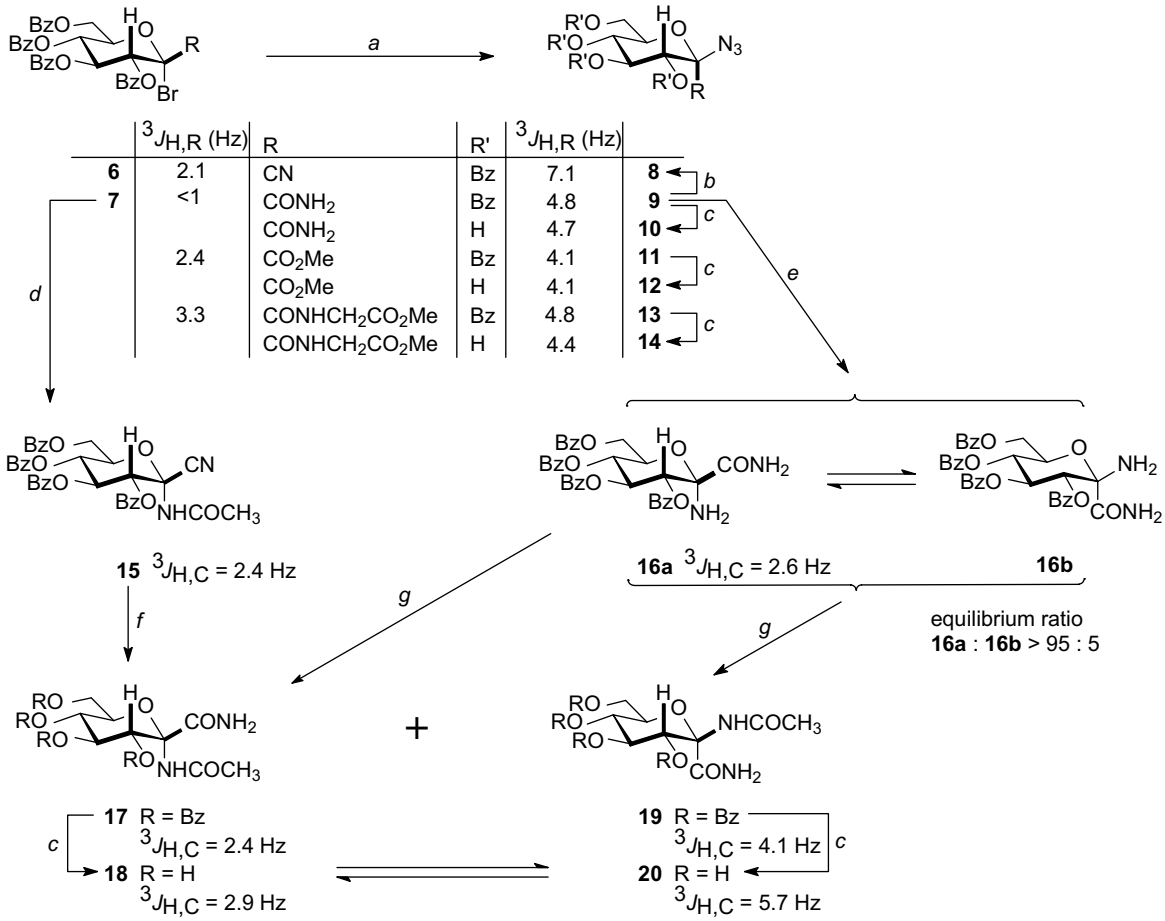
As starting materials per-*O*-benzoylated *C*-(1-bromo-1-deoxy-β-D-glucopyranosyl)formic acid derivatives **6**[17] and **7**[17] were used, both known from the literature. Azide substitution in **6** by $NaN_3$ in $Me_2SO$ as described earlier[18] gave only complex mixtures from which the isolation of **8** in reasonable yield failed. On the contrary, similar transformation of **7** gave **9** in a clean reaction in 88% yield. Nitrile **8** could be obtained by dehydration of **9** by oxalyl chloride/DMF[19] (74%) or by $POCl_3$ in pyridine (50%). Azides **11** and **13** were obtained by similar substitutions in the corresponding bromo derivatives.[20] Reduction of **9** by sodium hydrogentelluride[21] or by the more conventional Raney-Ni in hydrogen atmosphere produced a mixture of anomeric glycosylamines **16ab** (not investigated as to the ratio of anomers at this stage) from which **16a** could be isolated by crystallization in 73% yield. An equilibration experiment monitored by $^1H$ NMR in $CDCl_3$ at room temp showed that **16a** was the thermodynamically more stable anomer since **16a** and **16b** were present in the mixture in >95:5 ratio after 16 days. Acetylation of **16a** with acetic anhydride in pyridine gave 57% of **17**. Similar acetylation of a freshly prepared mixture of **16ab** gave **17** and **19** in 75% and 15% isolated yields, respectively. A shorter route to **17** was found with the transformation of **7** by $Ag_2CO_3$ in acetonitrile in a Ritter-type reaction[22] producing **15** in 78% yield, which was then hydrated by $TiCl_4$–$H_2O$/AcOH to give **17** (88%).

The protecting groups were removed according to the Zemplén-protocol and **10** (88%), **12** (26%), **14** (54%), **18** (85 %) and **20** (39%) were obtained. Several trials for the deprotection of **8** and **15** ($NH_3$–EtOH, NaOMe–MeOH at or below rt) failed by furnishing complex product mixtures.

Structure elucidation of the new compounds was based on extensive solution NMR studies. The $^4C_1$ conformations of the glucopyranose rings were unequivocally assigned with the use of vicinal proton–proton coupling constants. The configuration of the anomeric carbon was established on the basis of three-bond heteronuclear couplings[23,24] between H-2 and the exocyclic carbonyl or nitrile carbon attached to C-1 (parent glucose numbering). These couplings were measured using a sensitivity enhanced gradient long-range $^{13}C$–$^1H$ correlation experiment (G-HSQMBC),[25] and values less than or around 3 Hz indicated *gauche* while larger than 4 Hz suggested trans arrangement of the relevant atoms in the $^4C_1$ conformation.[18,26]

Anomeric configurational assignment for **18** and **20** was performed with special care since protein crystallography, as detailed in the next paragraph, revealed an unexpected anomerization. In line with the general trend, the $^3J_{H\text{-}2,CO}$ values of 2.9 and 5.7 Hz suggested the structures for **18** and **20** as shown in Scheme 1. The anomeric configuration of **18** was corroborated by a NOESY experiment run in water (10% $D_2O$) as well, showing NOE contacts of medium intensity between NH and H-3/H-5, but only very weak NOE between NH and H-2. Besides, a comparison of the chemical shifts (Table 1) for ring proton resonances of **3** and **18** exhibited characteristic differences while that of **3** and **20** showed excellent coincidences providing additional evidence of the anomeric configurations.

Protein crystallographic studies of a complex obtained by soaking rabbit muscle GP*b* crystals in a 100 mM solution of **18** in mother liquor (10 mM Bes, 0.1 mM EDTA, pH 6.7), for 2 h prior to data collection showed the presence of a glucose derivative at the catalytic site of the enzyme.[15] The crystallographic analysis gave a difference electron density map in which the glucose analogue was well defined and in which the –NHC-$OCH_3$ substituent at the C-1 position appeared to be equatorial and the $–CONH_2$ axial with respect to the glucopyranose ring, that is the bound compound corresponded to **20**. There were no trace of any positive or negative electron density corresponding to the axial $–NHCOCH_3$ or equatorial $–CONH_2$ positions, respectively. The possibility that there was a mixture of bound anomeric molecules **18** and **20** at the catalytic site of the enzyme in the crystal (with a higher proportion of anomer **20**) is therefore excluded. In order to find out whether anomerization could occur during the soaking process, a sample of **18** dissolved in the above mother liquor was investigated by NMR spectroscopy. In accordance with the NMR criteria discussed above, the observed slight increase (+0.7 Hz) of $^3J_{H\text{-}2,CO}$ coupling constant may suggest an equilibration between **18** and **20** in the presence of the buffer. More detailed investigations are needed to reveal the precise mechanism of this anomerization, although, as pointed out by an unknown referee, opening of the sugar ring and formation of a conjugated system from the aglycon seems probable. The exclusive appearance of **20** in the crystal might be due to a more favourable binding of this compound to the enzyme as compared to **18**. Anomerizations of somewhat similar character were investigated with several




| | $^3J_{H,R}$ (Hz) | R | R' | $^3J_{H,R}$ (Hz) | |
|---|---|---|---|---|---|
| 6 | 2.1 | CN | Bz | 7.1 | 8 |
| 7 | <1 | $CONH_2$ | Bz | 4.8 | 9 |
| | | $CONH_2$ | H | 4.7 | 10 |
| | 2.4 | $CO_2Me$ | Bz | 4.1 | 11 |
| | | $CO_2Me$ | H | 4.1 | 12 |
| | 3.3 | $CONHCH_2CO_2Me$ | Bz | 4.8 | 13 |
| | | $CONHCH_2CO_2Me$ | H | 4.4 | 14 |

**Scheme 1.** Reagents and conditions: (a) $NaN_3$, $Me_2SO$, rt; (b) $(COCl)_2$, DMF, $CH_3CN$, 0 °C; (c) NaOMe, MeOH, rt; (d) $Ag_2CO_3$, $CH_3CN$, rt; (e) NaTeH, EtOH, rt, or Raney-Ni, $H_2$, EtOAc, 70 °C; (f) $TiCl_4$, $H_2O$, AcOH, 0 °C to rt; (g) $Ac_2O$, pyridine, 70 °C.

**Table 1.** Chemical shifts ($\delta$, $D_2O$) for the ring protons of **3**, **18** and **20**

| | H-2 | H-3 | H-4 | H-5 | H-6 | H-6′ |
|---|---|---|---|---|---|---|
| **3**[16] | 3.69 | 3.59 | 3.47 | 4.10 | 3.67 | 3.82 |
| **18**[a] | 3.52 | 3.65 | 3.48 | 3.45 | 3.78–3.76 | |
| **20**[a] | 3.68 | 3.63 | 3.48 | 4.12 | 3.69 | 3.84 |

[a] Referenced to $CH_3OH$.

glycofuranosylidene-spiro-hydantoin[27–35] and -diketopiperazine[36,37] derivatives. In those cases rather strongly basic[30,34,35,37] or acidic[27–29,31–33,36] conditions were used to bring about anomerization. Most of these investigations showed the epimer with a β-CO moiety as the dominant component of the equilibrium mixture except for a hydantoin[28] and a diketopiperazine.[37] Obviously, this phenomenon needs and deserves further studies.

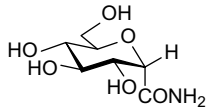
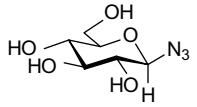
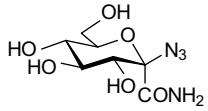
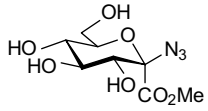
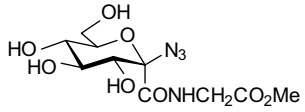
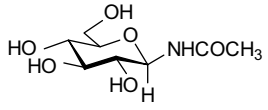
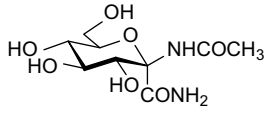
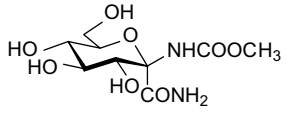
Present preliminary results and published data on the efficiency of the new compounds as inhibitors of rabbit muscle GP are collected in Table 2. They show that these derivatives are weak inhibitors of glycogen phosphorylase *b*. The observations suggest that advantageousness of the presence of a carboxamido group in the anomeric α-position of D-glucose can be judged only in conjuction with the other anomeric substituent (compare entries 1–3 and 6–8).

## 1. Experimental

### 1.1. General methods

Melting points were measured in open capillary tubes or on a Kofler hot-stage and are uncorrected. Optical rotations were determined on a Perkin–Elmer 241 polarimeter at room temperature. IR spectra were taken with a Perkin–Elmer 16 PC FT-IR spectrometer. NMR spectra were recorded with Bruker 360 (360/90 MHz for $^1H/^{13}C$) and Varian UNITY INOVA 400 WB (400/100 MHz for $^1H/^{13}C$) or Avance DRX 500 (500/125 MHz for $^1H/^{13}C$) spectrometers. Chemical shifts are referenced to $Me_4Si$ ($^1H$) or to the solvent signal ($^{13}C$). TLC was performed on DC Alurolle, Kieselgel 60 $F_{254}$ (E. Merck), the plates were visualized by gentle heating. Raney-nickel was purchased from E. Merck. For column chromatography Kieselgel 60 (E. Merck,

**Table 2.** Inhibitor constants ($K_i$) measured with rabbit muscle GP*b*

| Entry | Compound | $K_i$ [μM] |
|---|---|---|
| 1 |  | 370[38] |
| 2 |  | No inhibition[15] |
| 3 |  **10** | 1800[15] |
| 4 |  **12** | 5% Inhibition at 10 mM |
| 5 |  **14** | No inhibition at 10 mM |
| 6 |  | 32[39] |
| 7 |  **20** | 310[15] |
| 8 |  **3** | 16[16] |

particle size 0.063–0.200 mm) was used. Distilled solvents ($CH_3CN$, $CHCl_3$, $Me_2SO$, ethyl acetate) were dried by storage over 4 Å molecular sieves. Organic solutions were dried over anhydrous $MgSO_4$, and concentrated in vacuo at 40–50 °C (water bath).

### 1.2. General procedure for debenzoylation

A benzoylated compound (1 mmol) was dissolved in dry MeOH (5 mL), and a few drops of a 1 M methanolic NaOMe soln were added. The mixture was kept at rt and monitored by TLC (1:1 EtOAc–hexane, and 7:3 $CHCl_3$–MeOH). When the starting material and the partially debenzoylated products disappeared, the soln was neutralized by Amberlyst 15 resin ($H^+$ form). After solvent removal the residue was purified by column chromatography.

### 1.3. 1-Azido-2,3,4,6-tetra-*O*-benzoyl-1-deoxy-α-D-glucopyranosyl cyanide (3,4,5,7-tetra-*O*-benzoyl-β-D-*gluco*-hept-2-ulopyranosylononitrile azide) (8)

*N*-(Chloromethylene)-*N*-methylmethanaminium chloride was generated in situ from DMF (0.08 mL, 1.2 mmol) and oxalyl chloride (0.1 mL, 1.15 mmol) in dry $CH_3CN$ (2.5 mL) at 0 °C under Ar atmosphere. A soln of **9** (0.66 g, 1 mmol) in $CH_3CN$ (1 mL) was added, the mixture was stirred for 20 min and then dry pyridine (0.18 mL, 2.2 mmol) was added. After a further 15 min stirring the mixture was diluted with $CH_2Cl_2$ (30 mL), washed by satd aq NaCl soln containing 1% HCl (2 × 10 mL) and dried. Solvent removal left a syrup (0.64 g chromatographically pure crude product), which was crystallized from EtOAc–hexane to give 0.37 g (58%) of **8**. Column chromatography of the mother liquor (1:2 EtOAc–hexane) gave a further crop (0.1 g, 16%) of **8**: mp 175–176 °C; $[\alpha]_D$ +88 (*c* 1, $CHCl_3$); IR (KBr): $\nu_{max}$ 2126 $cm^{-1}$ ($N_3$); $^1H$ NMR ($CDCl_3$): $\delta$ (ppm) 8.10–7.20 (20H, m, Ph), 6.06 (1H, t, $J = 9.5$ Hz, H-3), 5.80 (1H, t, $J = 9.5$ Hz, H-4), 5.62 (1H, d, $J = 9.5$ Hz, H-2), 4.73 (1H, dd, $J = 2.1$, 12.1 Hz, H-6), 4.63 (1H, ddd, $J = 9.5$, 4.7, 2.1 Hz, H-5), 4.57 (1H, dd, $J = 4.7$, 12.1 Hz, H-6′); $^{13}C$ NMR ($CDCl_3$): $\delta$ (ppm) 165.7, 165.1, 164.8, 164.2 (CO), 134.0–127.6 (aromatics), 112.1 (*C*N, $^3J_{CN,H\text{-}2} = 7.1$ Hz), 87.8 (C-1), 74.6, 71.7, 71.2, 67.8 (C-2 to C-5), 61.7 (C-6); Anal. Calcd for $C_{35}H_{26}N_4O_9$ (646.62): C, 65.01; H, 4.05; N, 8.66. Found: C, 65.34; H, 4.26; N, 8.12.

### 1.4. *C*-(1-Azido-2,3,4,6-tetra-*O*-benzoyl-1-deoxy-α-D-glucopyranosyl)formamide (3,4,5,7-tetra-*O*-benzoyl-β-D-*gluco*-hept-2-ulopyranosylonamide azide) (9)

*C*-(2,3,4,6-Tetra-*O*-benzoyl-1-bromo-1-deoxy-β-D-glucopyranosyl)formamide (3,4,5,7-tetra-*O*-benzoyl-α-D-*gluco*-hept-2-ulopyranosylonamide bromide)[17] (**7**, 4 g, 5.7 mmol) was dissolved in dry $Me_2SO$ (6 mL), and a soln of $NaN_3$ (0.74 g, 11.4 mmol) in dry $Me_2SO$ (34 mL) was added. After stirring at rt for 5 h the mixture was diluted with ice water (100 mL). It was then extracted by $Et_2O$ (5 × 50 mL), the ethereal phase washed by water (50 mL), dried and the solvent removed. The crystalline residue (3.3 g, 88%) was pure and uniform on TLC (1:1 EtOAc–hexane). An analytical sample was obtained by recrystallization from EtOAc–hexane (77% recovery): mp 95–97 °C; $[\alpha]_D$ −32 (*c* 1, $CHCl_3$); IR (KBr): $\nu_{max}$ 2130 $cm^{-1}$ ($N_3$); $^1H$ NMR ($CDCl_3$): $\delta$ (ppm) 8.20–7.20 (20H, m, Ph), 6.64 (1H, s, CON$H_2$), 6.63 (1H, t, $J = 9.5$ Hz, H-3), 6.14 (1H, s, CON$H_2$), 5.86 (1H, t, $J = 9.5$ Hz, H-4), 5.78 (1H, d, $J = 9.5$ Hz,

H-2), 5.16 (1H, ddd, $J = 9.5$, 3.7, 2.6 Hz, H-5), 4.72 (1H, dd, $J = 2.6$, 12.1 Hz, H-6), 4.46 (1H, dd, $J = 3.7$, 12.1 Hz, H-6′); $^{13}$C NMR ($CDCl_3$): $\delta$ (ppm) 167.4 ($C$ONH$_2$, $^3J_{CO,H\text{-}2} = 4.8$ Hz), 166.0, 165.3, 165.1, 165.0 (CO), 133.7–128.2 (aromatics), 88.9 (C-1), 73.5, 71.7, 71.1, 68.5 (C-2 to C-5), 62.2 (C-6); Anal. Calcd for $C_{35}H_{28}N_4O_{10}$ (664.63): C, 63.25; H, 4.25; N, 8.43. Found: C, 63.35; H, 4.29; N, 8.02.

### 1.5. *C*-(1-Azido-1-deoxy-α-D-glucopyranosyl)formamide (β-D-*gluco*-hept-2-ulopyranosylonamide azide) (10)

According to Section 1.2 from **9** (0.7 g, 1.05 mmol). Eluent for the column chromatography: 9:1 $CHCl_3$–MeOH. Yield: 0.23 g (88%) colourless oil ($R_f = 0.54$, 2:1 $CHCl_3$–MeOH); $[\alpha]_D$ +65 ($c$ 1, MeOH); IR (film): $\nu_{max}$ 2124 cm$^{-1}$ ($N_3$); $^1$H NMR ($D_2O$): $\delta$ (ppm) 3.93 (1H, dd, $J = 1.6$, 12.2 Hz, H-6), 3.85 (1H, t, $J = 9.5$ Hz, H-3), 3.80 (1H, dd, $J = 4.4$, 12.2 Hz, H-6′), 3.75 (1H, ddd, $J = 9.5$, 4.4, 1.6 Hz, H-5), 3.65 (1H, d, $J = 10.0$ Hz, H-2), 3.58 (1H, t, $J = 9.5$ Hz, H-4); $^{13}$C NMR ($D_2O$): $\delta$ (ppm) 172.0 ($C$ONH$_2$, $^3J_{CO,H\text{-}2} = 4.7$ Hz), 92.0 (C-1), 79.1, 76.2, 75.7, 70.9 (C-2 to C-5), 62.5 (C-6); Anal. Calcd for $C_7H_{12}N_4O_6$ (248.20): C, 33.88; H, 4.87; N, 22.57. Found: C, 33.55; H, 4.28; N, 22.08.

### 1.6. Methyl *C*-(1-azido-1-deoxy-α-D-glucopyranosyl)-formate (methyl β-D-*gluco*-hept-2-ulopyranosylonate azide) (12)

According to Section 1.2 from methyl *C*-(2,3,4,6-tetra-*O*-benzoyl-1-azido-1-deoxy-α-D-glucopyranosyl)formate (methyl 3,4,5,7-tetra-*O*-benzoyl-β-D-*gluco*-hept-2-ulopyranosylonate azide)[20] (**11**, 0.40 g, 0.59 mmol). Eluent for the column chromatography: 4:1 $CHCl_3$–MeOH. Yield: 0.04 g (26%) colourless oil ($R_f = 0.34$, 7:3 $CHCl_3$–MeOH); $[\alpha]_D$ +67 ($c$ 1.1, MeOH); $^1$H NMR ($D_2O$): $\delta$ (ppm) 3.96 (1H, t, $J = 9.6$, 9.6 Hz, H-3), 3.89 (3H, s, $OCH_3$), 3.87 (1H, dd, $J = 11.8$, 1.5 Hz, H-6), 3.82 (1H, ddd, $J = 11.8$, 5.1, 1.5 Hz, H-5), 3.75 (1H, dd, $J = 11.8$, 5.1 Hz, H-6′), 3.52 (1H, d, $J = 9.6$ Hz, H-2), 3.50 (1H, t, $J = 9.6$, 9.6 Hz, H-4); $^{13}$C NMR ($D_2O$): $\delta$ (ppm) 167.1 ($C$OOCH$_3$, $^3J_{COOCH_3,H\text{-}2} = 4.1$ Hz), 90.8 (C-1), 76.4, 73.5, 72.3, 67.9 (C-2 to C-5), 60.0 (C-6), 52.7 (COO$C$H$_3$); Anal. Calcd for $C_8H_{13}N_3O_7$ (263.21): C, 36.51; H, 4.98; N, 15.96. Found: C, 36.65; H, 4.86, N, 16.16.

### 1.7. *N*-[(1-Azido-1-deoxy-α-D-glucopyranosyl)-carbonyl]glycine methylester (*N*-(β-D-*gluco*-hept-2-ulopyranosylonoyl azide)glycine methylester) (14)

According to Section 1.2 from *N*-[(2,3,4,6-tetra-*O*-acetyl-1-azido-1-deoxy-α-D-glucopyranosyl)carbonyl]glycine methylester (*N*-(3,4,5,7-tetra-*O*-acetyl-β-D-*gluco*-hept-2-ulopyranosylonoyl azide)glycine methylester)[20] (**13**, 0.17 g, 0.23 mmol). Eluent for the column chromatography: 4:1 $CHCl_3$–MeOH. Yield: 0.06 g (54%) colourless oil ($R_f = 0.42$, 4:1 $CHCl_3$–MeOH); $[\alpha]_D$ +68 ($c$ 1.34, MeOH); $^1$H NMR ($D_2O$): $\delta$ (ppm) 4.12–4.10 (2H, m, $CH_2$), 3.90 (1H, dd, $J = 13.2$, 1.0 Hz, H-6), 3.86 (1H, t, $J = 9.6$, 9.6 Hz, H-3), 3.78 (1H, dd, $J = 13.2$, 4.4 Hz, H-6′), 3.77 (3H, s, $OCH_3$), 3.74 (1H, ddd, $J = 13.2$, 4.4, 1.0 Hz, H-5), 3.65 (1H, d, $J = 9.5$ Hz, H-2), 3.55 (1H, t, $J = 9.5$, 9.5 Hz, H-4); $^{13}$C NMR ($D_2O$): $\delta$ (ppm) 171.5 ($C$OOCH$_3$), 168.4 ($C$ONH, $^3J_{CONH,H\text{-}2} = 4.4$ Hz), 89.9 (C-1), 76.9, 74.3, 73.6, 68.8 (C-2 to C-5), 60.4 (C-6), 52.9 (COO$C$H$_3$), 41.2 ($CH_2$); Anal. Calcd for $C_{10}H_{16}N_4O_8$ (320.26): C, 37.50; H, 5.04; N, 17.49. Found: C, 36.95; H, 4.96; N, 17.36.

### 1.8. 1-Acetamido-2,3,4,6-tetra-*O*-benzoyl-1-deoxy-β-D-glucopyranosyl cyanide (*N*-(3,4,5,7-tetra-*O*-benzoyl-α-D-*gluco*-hept-2-ulopyranosylononitrile)acetamide) (15)

*C*-(2,3,4,6-Tetra-*O*-benzoyl-1-bromo-1-deoxy-β-D- glucopyranosyl)formamide (3,4,5,7-tetra-*O*-benzoyl-α-D-*gluco*-hept-2-ulopyranosylonamide bromide)[17] (**7**, 0.50 g, 0.8 mmol) was dissolved in abs. $CH_3CN$ (10 mL) and dried $Ag_2CO_3$ (0.24 g, 1.1 equiv) was added. The mixture was stirred in the dark at rt until TLC (1:2 EtOAc–hexane) showed complete transformation (3 days). It was then filtered on a Celite pad and the solvent evaporated. The residue was purified by column chromatography (1:2 EtOAc–hexane) to give 0.38 g (78%) of **15** as a white crystalline product: mp 231–232 °C; $[\alpha]_D$ +67 ($c$ 1, $Me_2SO$); $^1$H NMR ($Me_2SO$-$d_6$): $\delta$ (ppm) 8.14–7.38 (21H, m, Ph, NH), 6.34 (1H, t, $J = 10.0$, 9.5 Hz, H-3), 6.24 (1H, d, $J = 10.0$ Hz, H-2), 5.80 (1H, t, $J = 9.5$, 9.5 Hz, H-4), 4.58–4.40 (3H, m, H-5, H-6, H-6′) 2.20 (3H, s, $CH_3$); $^{13}$C NMR ($Me_2SO$-$d_6$): $\delta$ (ppm) 170.3 (NH$C$OCH$_3$), 165.3, 164.8, 164.4, 163.9 (CO), 134.2–127.5 (aromatics), 115.4 (CN, $^3J_{CN,H\text{-}2} = 2.4$ Hz), 77.0 (C-1), 71.9, 70.8, 68.7, 68.1 (C-2 to C-5), 62.0 (C-6), 22.7 ($C$H$_3$); Anal. Calcd for $C_{37}H_{30}N_2O_{10}$ (662.66): C, 67.07, H, 4.56; N, 4.23. Found: C, 66.84; H, 4.36; N, 4.00.

### 1.9. *C*-(1-Amino-2,3,4,6-tetra-*O*-benzoyl-1-deoxy-β-D-glucopyranosyl)formamide (3,4,5,7-tetra-*O*-benzoyl-α-D-*gluco*-hept-2-ulopyranosylonamide amine) (16)

(A) Tellurium (0.1 g, 0.78 mmol) was suspended in EtOH (1.5 mL) under Ar, and $NaBH_4$ (0.07 g, 1.85 mmol) was added, and the mixture was refluxed for 1 h. After cooling to rt, a soln of **9** (0.2 g, 0.3 mmol) in EtOH (2 mL) was added dropwise and the mixture stirred for 20 min (longer reaction times may result in saponification of the benzoyl groups). Filtration on a Celite pad followed by solvent removal gave 0.14 g (73%) of **16a** as white crystalline product, which was

used for the next step without purification. An analytical sample was obtained by recrystallization from EtOH: mp 204–207 °C; $[\alpha]_D$ +60 (*c* 1, $CHCl_3$); IR (KBr): $\nu_{max}$ 3354, 3480 $cm^{-1}$ ($NH_2$); $^1H$ NMR ($CDCl_3$): $\delta$ (ppm) 8.2–7.2 (20H, m, Ph), 6.65 (1H, s, CON*H*$_2$), 6.19 (1H, t, $J = 9.8$ Hz, H-3), 5.76 (1H, t, $J = 9.8$ Hz, H-4), 5.68 (1H, d, $J = 9.8$ Hz, H-2), 5.55 (1H, s, CON*H*$_2$), 4.87 (1H, ddd, $J = 9.8$, 4.3, 2.4 Hz, H-5), 4.72 (1H, dd, $J = 2.4$, 12.1 Hz, H-6), 4.52 (1H, dd, $J = 4.3$, 12.1 Hz, H-6′), 2.5 (2H, s, $NH_2$); $^{13}C$ NMR ($CDCl_3$): $\delta$ (ppm) 172.8 (*C*ONH$_2$, $^3J_{CO,H\text{-}2} = 2.6$ Hz), 166.4, 165.7, 165.2, 165.0 (CO), 133.4–128.2 (aromatics), 85.6 (C-1), 71.9, 71.2, 69.4, 69.3 (C-2 to C-5), 62.6 (C-6); Anal. Calcd for $C_{35}H_{36}N_2O_{10}$ (638.64): C, 65.83; H, 4.73; N, 4.39. Found: C, 65.45; H, 4.59; N, 4.06.

(B) Compound **9** (0.5 g, 0.75 mmol) was dissolved in dry EtOAc (50 mL) and ~1.0 g Raney-Ni (washed with MeOH (2 × 10 mL), dry MeOH (2 × 10 mL) and dry EtOAc (2 × 10 mL)) were added to the mixture, which was heated at 70 °C under $H_2$ atmosphere until the transformation was complete (~1 h, TLC, 1:1 EtOAc–hexane). The mixture was filtered on a Celite pad and the solvent was removed under diminished pressure. The crude product (0.46 g, 96%) **16ab** crystallized on standing at rt.

### 1.10. *C*-(1-Acetamido-2,3,4,6-tetra-*O*-benzoyl-1-deoxy-β-D-glucopyranosyl)formamide (*N*-(3,4,5,7-tetra-*O*-benzoyl-α-D-*gluco*-hept-2-ulopyranosylonamide)acetamide) (17)

(A) Compound **16a** (0.6 g, 0.94 mmol) was dissolved in dry pyridine (9 mL) and $Ac_2O$ (0.2 mL, 2 equiv) was added, and this mixture was stirred at 70 °C for 2 days. After concentration under diminished pressure, the residue was purified by column chromatography (1:1 EtOAc–hexane) to give 0.36 g (57%) of **17** as white crystals.

(B) Compound **15** (0.1 g, 0.15 mmol) was suspended in glacial HOAc (0.5 mL), $TiCl_4$ (0.049 mL, 0.15 mmol) and water (0.011 mL, 0.6 mmol) were added at 0 °C, and the mixture was stirred for 0.5 h at 0 °C and than at rt. When TLC (1:1 EtOAc–hexane) showed disappearance of the starting material, the mixture was diluted with water (5 mL) and the product precipitated. It was filtered, dissolved in $CHCl_3$ (5 mL) and this soln was washed with satd aq $NaHCO_3$ (2×), water, dried and evaporated to give 0.09 g (88%) of **17** as a white crystalline product: mp 238–240 °C; $[\alpha]_D$ +54 (*c* 1, $CHCl_3$); $^1H$ NMR ($CDCl_3$): $\delta$ (ppm) 8.2–7.1 (21H, m, Ph, NH), 6.88 (1H, s, CON*H*$_2$), 6.14 (1H, t, $J = 9.8$ Hz, H-3), 5.86 (1H, d, $J = 9.8$ Hz, H-2), 5.84 (1H, s, CON*H*$_2$), 5.81 (1H, t, $J = 9.8$ Hz, H-4), 4.68 (1H, dd, $J = 12.5$, 2.0 Hz, H-6), 4.52 (1H, dd, $J = 12.5$, 3.9 Hz, H-6′), 4.50 (1H, ddd, $J = 12.5$, 3.9, 2.0 Hz, H-5), 2.06 (3H, s, $CH_3$); $^{13}C$ NMR ($CDCl_3$): $\delta$ (ppm) 170.8 (*C*ONH$_2$, $^3J_{CO,H\text{-}2} =$ 2.4 Hz), 168.7 (NH*C*OCH$_3$), 166.2, 164.9, 164.7 (CO), 133.6–128.3 (aromatics), 83.5 (C-1), 71.3, 70.5, 70.1, 68.8 (C-2 to C-5), 62.3 (C-6), 23.5 (*C*H$_3$); Anal. Calcd for $C_{37}H_{32}N_2O_{11}$ (680.67): C, 65.29; H, 4.74; N, 4.12. Found: C, 65.84, H, 4.56; N, 3.96.

### 1.11. *C*-(1-Acetamido-1-deoxy-β-D-glucopyranosyl)-formamide (*N*-(α-D-*gluco*-hept-2-ulopyranosylon-amide)acetamide) (18)

According to Section 1.2 from **17** (0.19 g, 0.28 mmol). Eluent for the column chromatography: 1:1 $CHCl_3$–MeOH. Yield 0.062 g (85%) colourless syrup ($R_f = 0.13$, 1:1 $CHCl_3$–MeOH); $[\alpha]_D$ +91 (*c* 0.4, MeOH); $^1H$ NMR ($D_2O$): $\delta$ (ppm) 3.78–3.76 (2H, m, H-6, H-6′), 3.65 (1H, dd, $J = 9.5$, 9.8 Hz, H-3), 3.52 (1H, d, $J = 9.5$ Hz, H-2), 3.48 (1H, t, $J = 9.8$ Hz, H-4), 3.45 (1H, m, H-5), 2.08 (3H, s, $CH_3$); $^{13}C$ NMR ($D_2O$): $\delta$ (ppm) 173.3 (*C*ONH$_2$, $^3J_{CO,H\text{-}2} = 2.9$ Hz), 175.4 (NH*C*OCH$_3$), 85.5 (C-1), 74.5 (C-5), 73.9 (C-3), 73.4 (C-2), 68.9 (C-4), 60.8 (C-6); Anal. Calcd for $C_9H_{16}N_2O_7$ (264.24): C, 40.91; H, 6.10; N, 10.60. Found: C, 40.74; H, 6.37; N, 10.80.

### 1.12. *C*-(1-Acetamido-2,3,4,6-tetra-*O*-benzoyl-1-deoxy-α-D-glucopyranosyl)formamide (*N*-(3,4,5,7-tetra-*O*-benzoyl-β-D-*gluco*-hept-2-ulopyranosylonamide)acetamide) (19)

A freshly prepared mixture of **16ab** (0.5 g, 0.78 mmol) was suspended in dry pyridine (10 mL), AcCl (0.1 mL, 2 equiv) was added and the mixture stirred at rt until completion of the transformation (~2–3 h, TLC, 4:1 EtOAc–hexane). The solvent was removed under diminished pressure, the residue diluted with EtOAc and washed with 10% HCl (15 mL), satd aq $NaHCO_3$ soln (2 × 15 mL) and water (15 mL). The organic phase was dried, the solvent removed under diminished pressure and the residue purified by column chromatography (eluent, 4:1 EtOAc–hexane) to give **17** (0.4 g, 75%) and **19** (0.08 g, 15%). Colourless oil ($R_f = 0.49$, 9:1 EtOAc–hexane); $[\alpha]_D$ +17 (*c* 0.196, $CHCl_3$); $^1H$ NMR ($CDCl_3$): $\delta$ (ppm) 8.08–7.22 (22H, m, Ph, $NH_2$), 6.24 (1H, t, $J = 7.9$, 6.6 Hz, H-3), 6.00 (1H, s, NH), 5.78 (1H, t, $J = 9.2$, 7.9 Hz, H-4), 5.68 (1H, d, $J = 7.9$ Hz, H-2), 4.83 (1H, ddd, $J = 9.2$, 5.2, 2.6 Hz, H-5), 4.70 (1H, dd, $J = 11.8$, 2.6 Hz, H-6), 4.46 (1H, dd, $J = 11.9$, 5.2 Hz, H-6′), 1.96 (3H, s, $CH_3$); $^{13}C$ NMR ($CDCl_3$): $\delta$ (ppm) 170.4 (*C*ONH$_2$, $^3J_{H\text{-}2,CONH_2} = 4.1$ Hz), 168.8 (NH*C*O), 166.4, 166.0, 165.1, 164.9 (*C*O), 133.9–128.1 (aromatics), 85.5 (C-1), 72.9, 72.1, 70.9, 68.9 (C-2 to C-5), 63.2 (C-6), 23.9 (*C*H$_3$); Anal. Calcd for $C_{37}H_{31}N_2O_{11}$ (680.37): C, 65.29; H, 4.74; N, 4.12. Found: C, 65.78; H, 4.26; N, 4.00.

### 1.13. *C*-(1-Acetamido-1-deoxy-α-D-glucopyranosyl)-formamide (*N*-(β-D-*gluco*-hept-2-ulopyranosylonamide)-acetamide) (20)

Prepared from **19** (0.1 g, 0.15 mmol) according to Section 1.2. Yield: 0.015 g (39%) colourless oil ($R_f = 0.29$, 1:1 $CHCl_3$–MeOH); $[\alpha]_D$ +91 (*c* 0.396, $H_2O$); $^1H$ NMR ($D_2O$): $\delta$ (ppm) 4.12 (1H, ddd, $J = 10.1$, 4.9, 2.2 Hz, H-5), 3.84 (1H, dd, $J = 12.6$, 2.2 Hz, H-6), 3.69 (1H, dd, $J = 12.6$, 4.9 Hz, H-6′), 3.68 (1H, d, $J = 9.5$ Hz, H-2), 3.63 (1H, dd, $J = 9.5$, 8.5 Hz, H-3), 3.48 (1H, dd, $J = 10.1$, 8.5 Hz, H-4), 2.04 (3H, s, $CH_3$); $^{13}C$ NMR ($D_2O$): $\delta$ (ppm) 172.2 ($CONH_2$, $^3J_{H\text{-}2,CONH_2} = 5.7$ Hz), 174.7 (NH*C*O), 85.2 (C-1), 77.1 (C-5), 74.1 (C-2), 73.8 (C-3), 69.7 (C-4), 61.5 (C-6), 23.0 ($CH_3$). Anal. Calcd for $C_{37}H_{31}N_2O_{11}$ (680.37): C, 65.29; H, 4.74; N, 4.12. Found: C, 65.60; H, 4.20; N, 4.58.

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