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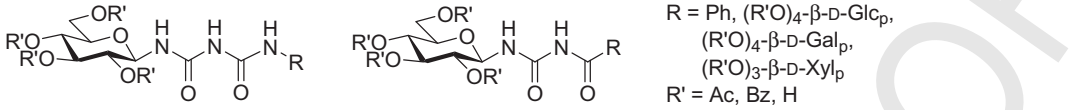
Graphical abstract

Synthesis of new glycosyl biuret and urea derivatives as potential glycoenzyme inhibitors **pp xxx–xxx**

Nóra Felföldi, Marietta Tóth, Evangelia D. Chrysina, Maria-Despoina Charavgi, Kyra-Melinda Alexacou, László Somsák *



The deprotected biuret derivatives showed moderate inhibitory effect against rabbit muscle glycogen phosphorylase *b* and human salivary α-amylase.

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Synthesis of new glycosyl biuret and urea derivatives as potential glycoenzyme inhibitors

Nóra Felföldi [a], Marietta Tóth [a], Evangelia D. Chrysina [b], Maria-Despoina Charavgi [b], Kyra-Melinda Alexacou [b], László Somsák [a,*]

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ABSTRACT

O-Peracetylated 1-(β-D-glucopyranosyl)-5-phenylbiuret was prepared in the reaction of O-peracetylated β-D-glucopyranosylisocyanate and phenylurea. The reaction of O-peracetylated *N*-β-D-glucopyranosylurea with phenylisocyanate furnished the corresponding 1-(β-D-glucopyranosyl)-3,5-diphenyl- as well as 3-(β-D-glucopyranosyl)-1,5-diphenyl biurets besides 1-(β-D-glucopyranosyl)-3-phenylurea. O-Peracetylated 1-(β-D-glucopyranosyl)-5-(β-D-glycopyranosyl)biurets were obtained in one-pot reactions of O-peracetylated β-D-glucopyranosylamine with OCNCOCl followed by a second glycopyranosylamine of β-D-*gluco*, β-D-*galacto* and β-D-*xylo* configurations. *O*-Acyl protected 1-(β-D-glucopyranosyl)-3-(β-D-glycopyranosylcarbonyl)ureas were obtained from the reaction of β-D-glucopyranosylisocyanate with *C*-(glycopyranosyl)formamides of β-D-*gluco* and β-D-*galacto* configurations. The *O*-acyl protecting groups were removed under acid- or base-catalyzed transesterification conditions, except for the *N*-acylurea derivatives where the cleavage of the *N*-acyl groups was faster than deprotection. Some of the new compounds exhibited moderate inhibition against rabbit muscle glycogen phosphorylase *b* and human salivary α-amylase.

1. Introduction

Carbohydrate processing enzymes (glycoenzymes) catalyze the assembly and degradation of vital oligo- and polysaccharides. Discovery of inhibitors of glycoenzymes and revealing their structure–activity relationship (SAR) is a principal trend in the development of carbohydrate-based drugs.[1] During our previous research several inhibitors of glycosidase[2–5] and glycogen phosphorylase[6,7] (GP) enzymes were synthesized and characterized. The first nanomolar glucose-based inhibitor of rabbit muscle GP*b* (RMGP*b*) was identified among *N*-acyl-*N*′-β-D-glucopyranosyl ureas **B** (for selected examples see Table 1, entries 5–7). The strong binding of the 2-naphthyl derivative (entry 7) was attributed to its extensive interactions upon binding with the residues lining the so-called β-pocket of the catalytic channel of the enzyme.[8] GP's β-pocket is located next to the catalytic site of the enzyme in the direction of the β-anomeric substituent of bound D-glucose derivatives surrounded by both polar and apolar amino acid side chains.[9] In the native RMGP*b*, this site is occupied by water molecules the positions of which give insights for the design of new glucose analogues with substituents that would optimize the network of interactions with the residues in close vicinity. In order to track down the nature of interactions in the β-pocket, and the role of the linker between the sugar and the aromatic part of the molecule some *N*-aryl-*N*′-β-D-glucopyranosyl ureas **A** (for selected examples see Table 1, entries 1–3) have been investigated so far. Derivatives **A** exhibited weaker binding to RMGP*b* in comparison to **B**. To study the effect of a longer linker of similar composition, synthesis of biuret derivatives **C** was envisaged. As the series of compounds **B** investigated so far contained mainly apolar residues[7] (R = e.g., methyl, cyclohexyl, (substituted)phenyl and naphthyl), an effort to exploit polar interactions in the β-pocket by substituting sugar rings for R in both **B** and **C** was also planned.

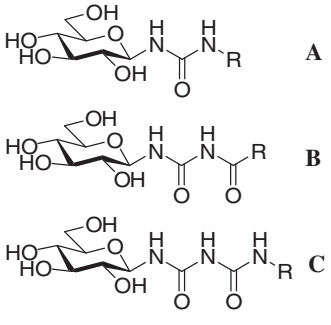

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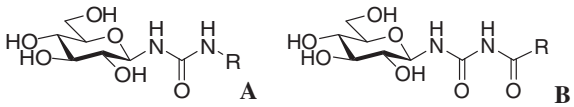
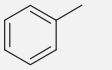
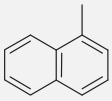
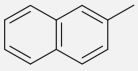
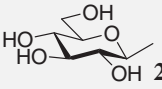
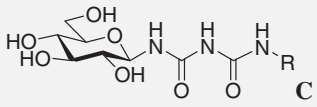
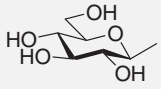
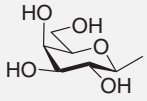
Table 1
Inhibition of rabbit muscle glycogen phosphorylase *b* (RMGP*b*) by selected glucose derivatives and the new compounds



Entry	Inhibition (μM)	R	Entry	Inhibition (K_i (μM))
1	K_i 18[7]		5	4.6[8]
2	IC_{50} 350[7]		6	15.2[6,7]
3	K_i 5.2[7]		7	0.35[6,7]
4	IC_{50} 1209 ± 8 K_i[a] 725 ± 5	 **22**		



		Inhibition	
		IC_{50} (μM)	K_i[a] (μM)
8	 **5**	41.6 ± 4	20.8 ± 2
9	 **13**	37% at 1 mM	
10	 **15**	32% at 1 mM	
11	 **17**	987 ± 127	592 ± 76

[a] K_i values were calculated for comparison purposes by the Cheng–Prusoff equation:[25] $K_i = IC_{50}/(1 + [S]/K_m)$.

2. Results and discussion

The synthesis of the protected 1-β-D-glucopyranosyl-5-phenyl biuret **4** was achieved by a reaction of phenylurea with β-D-glucopyranosylisocyanate **3**[10] generated in situ from glucosylamine **2**[11] obtained by catalytic reduction of glucosylazide **1**[12] (Scheme 1). Compound **4** was isolated by crystallization from MeOH, and deprotection was effected by acid-catalyzed transesterification to give **5**.

We have also attempted to produce **4** in a somewhat shorter way from β-D-glucosyl urea **6**[13] and PhNCO. In refluxing EtOAc no reaction occurred between **6** and 1.5 equiv of PhNCO. Using the same ratio of the reagents in boiling toluene allowed isolation of 1-β-D-glucosyl-3-phenyl urea **7**[10] in 41% yield. Performing the reaction in neat, boiling PhNCO gave compound **7** as well as biuret derivatives **8** and **9** in 4%, 24% and 31% isolated yields, respectively. This unexpected result could be explained by the presence of water in the reaction mixture. When **6** was reacted with 1 equiv of PhNCO in refluxing toluene in the presence of 1 equiv of H_2O, the formation of **7** could be observed. Urea **7** was also formed when **6** and 1 equiv of $PhNH_2$ were reacted in boiling toluene. Finally, heating of **7** in neat PhNCO gave biuret **9**. All attempts to avoid the formation of these products by carefully drying the solvents and reactants as well as the use of molecular sieves in the reaction mixtures failed. Structural elucidation of the new biurets **8** and **9** was straightforward by MS and NMR measurements as follows from the selected characteristic data shown in Scheme 1.

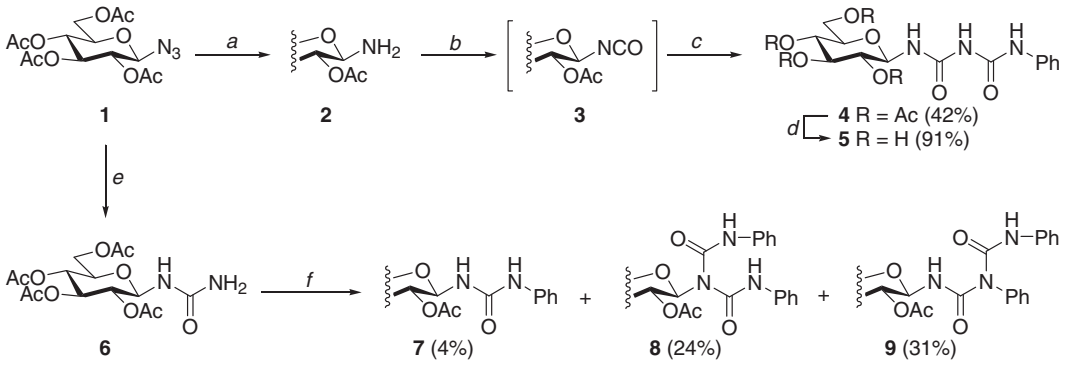
Coupling of a glycosylurea and a glycosylisocyanate could give 1,5-bis-glycosyl biuret derivatives. However, the use of commercial OCNCOCl as a bielectrophilic reagent[14,15], and glycosylamines as nucleophiles offered a simpler and shorter synthetic pathway towards such target compounds. Thus, reaction of glucopyranosylamine **2**[11] with 0.5 equiv of OCNCOCl gave cleanly the expected 1,5-bis-glucosyl biuret **12** (Scheme 2). Asymmetric derivatives could also be obtained in two-step, one-pot reactions from **2** by the addition of 1 equiv of OCNCOCl followed by a second glycosylamine **10**[16] or **11**[17,18] to give **14** or **16**, respectively. Deacetylation was performed by the Zemplén protocol to result in high yields of biurets **13**, **15** and **17**.

To obtain *N*-acyl-*N'*-β-D-glucopyranosyl ureas with a sugar part in the acyl group the reaction of isocyanate **3**[10] with O-peracetylated anhydro-aldonamide **18**[19] was investigated first (Scheme 3). When **3** and **18** were reacted in refluxing EtOAc in equimolar amounts the conversion of **18** was 25%, and the expected acylurea **20** could be isolated in 32% yield. Raising the temperature to the boiling point of toluene gave a 56% conversion of **18** and 50% isolated yield for **20**. A satisfactory result was achieved by applying **3** in a twofold excess for a full conversion of **18**, and the yield of **20** increased to 89%. From a reaction of **3** and O-perbenzoylated anhydro-aldonamide **19**[20] (molar ratio 2:1) in boiling toluene **23** was obtained in a 98% yield. In each of the above reactions bis-glucopyranosyl urea **21** was also isolated in various amounts which could be due to the presence of traces of water in the mixtures.[21] Attempted deprotection of acyl ureas **20** and **23** was successful under neither basic nor acidic transesterification conditions because cleavage of the *N*-acyl moiety was faster than removal of the *O*-acyl-protecting groups. Bis-glucopyranosyl urea **21** was deprotected under Zemplén conditions to give **22**[22] in satisfactory yield.

The deprotected compounds were tested for their potency to inhibit rabbit muscle glycogen phosphorylase *b* activity according to the protocol described earlier,[23,24] and the results are summarized in Table 1. Compounds with two sugar moieties attached to the terminal nitrogens of either urea **22** (entry 4) or biurets **13**, **15** and **17** (entries 9–11) showed very low inhibition of the enzyme activity. Comparison with the inhibition shown by the phenylbiuret derivative **5** (entry 8) this may reveal that the highly polar sugar residues opposite to the β-D-glucopyranosyl part of the compounds are unfavourable for the binding. Among biurets the β-D-xylopyranosyl derivative **17** proved the most efficient, and this may be in accord with the less polar character of this residue with three OH groups compared to four ones in the β-D-gluco- and galactopyranosyl parts of **13** and **15**, respectively. A comparison of the phenyl substituted derivatives (entries 1, 5 and 8) allows to conclude that the acyl urea linker is superior to the urea and the biuret type ones.

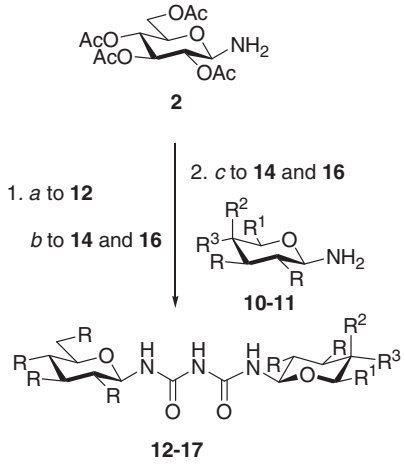
Compounds **13** and **22** were also tested against human salivary α-amylase according to the method reported earlier,[3] and exhibited inhibition in the low millimolar range (IC_{50} 10.7 and 8.3 mM, respectively).

In conclusion, synthesis of 1-(β-D-glucopyranosyl) biurets with an aromatic and several β-D-glycopyranosyl residues in the 5-position allowed to extend structure–activity relationships of glucose analogue inhibitors of glycogen phosphorylase. Introduction of the highly polar sugar moieties resulted in weak binding. The length of the linker composed of NHCO elements between the β-D-glucopyranosyl and the aromatic parts of the inhibitors proved to be optimal in the acyl urea series.



$[M+Na]^+$	489.14	608.56	608.56
NH (δ, ppm)	7.69 (s); 6.24 (d, ^3J = 9.2 Hz)	10.73 (s); 8.75 (s)	10.24 (s); 6.44 (d, ^3J = 8.8 Hz)
H-1 (δ, ppm)	5.28 (pseudo t, ^3J = 9.2 Hz)	6.32 (d, ^3J = 10.0 Hz)	5.11 (pseudo t, ^3J = 9.6 Hz)
C=O (δ, ppm)	154.6	151.8; 151.1	155.8; 152.1

Scheme 1. Reagents and conditions: (a) H_2, Raney-Ni, EtOAc, rt; (b) $(Cl_3CO)_2CO$, $NaHCO_3$, CH_2Cl_2, H_2O, rt; (c) $PhNHCONH_2$, toluene, reflux; (d) AcCl, $CHCl_3$–MeOH, rt; (e) PPh_3, EtOAc, NH_3, CO_2, rt; and (f) neat PhNCO, reflux.



	R	R^1	R^2	R^3	Yield
12	OAc	CH_2OAc	H	OAc	91%
↓d					
13	OH	CH_2OH	H	OH	96%
10, 14	OAc	CH_2OAc	OAc	H	83%
↓d					
15	OH	CH_2OH	OH	H	98%
11, 16	OAc	H	H	OAc	86%
↓d					
17	OH	H	H	OH	96%

Scheme 2. Reagents and conditions: (a) OCNCOCl, Et_3N, dry THF, N_2 atm, rt; (b) OCNCOCl, dry THF, N_2 atm, −26 °C; (c) **10** or **11**, Et_3N, dry THF, N_2 atm, 0–25 °C; and (d) cat. NaOMe, abs MeOH, rt.

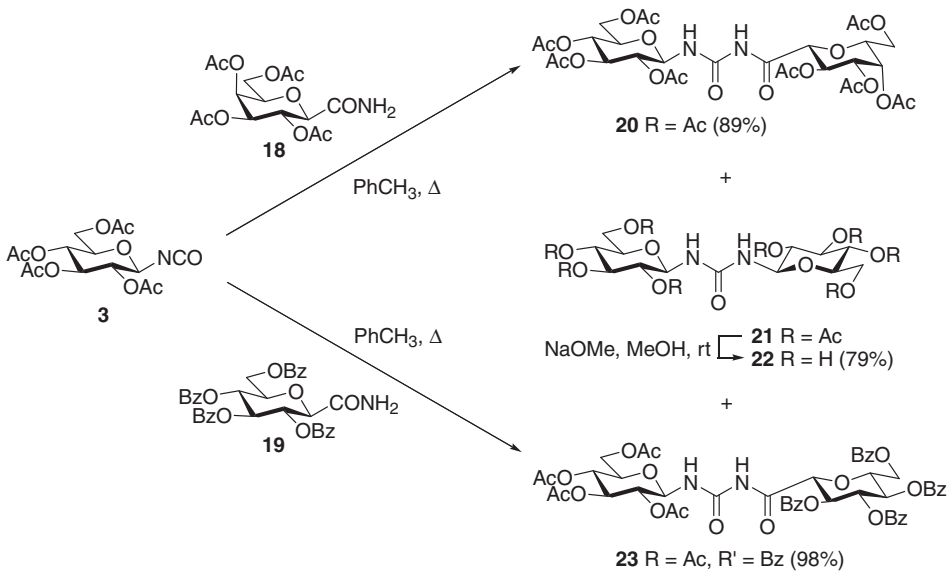
3. Experimental

3.1. General methods

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 rt. NMR spectra were recorded with Bruker 360 (360/90 MHz for $^1H/^{13}C$) or Bruker 400 (400/100 MHz for $^1H/^{13}C$) or Avance DRX 500 (500/125 MHz for $^1H/^{13}C$) spectrometers. Chemical shifts are referenced to internal TMS (^1H), or to the residual solvent signals (^{13}C). ^1H NMR assignments were established on the basis of gradient enhanced DQF-COSY spectra.[26] Proton chemical shifts and scalar coupling constants were extracted from the resolution enhanced 1D proton spectra. COSY spectra were recorded with 512 × 2 k data points, spectral widths 4000 Hz, number of transients 4 and recycle delay of 1.8 s. Microanalyses were performed on a Carlo-Erba analyser Type 1106. ESIMS were recorded with a Bruker micrOTOF-Q instrument. TLC was performed on DC-Alurolle Kieselgel 60 F_{254} (Merck), and the plates were visualized under UV light and by gentle heating. For column chromatography Kieselgel 60 (Merck, particle size 0.063–0.200 mm) was used. Flasks were flame-dried before performing the reactions. Organic solutions were dried over anhydrous $MgSO_4$, and concentrated under diminished pressure at 40–50 °C (water bath).

3.2. 1-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-5-phenyl biuret (4)

2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosylisocyanate (**3**) prepared in situ by Ichikawa's method[10] from glucosylamine **2**[11] (0.6 g, 1.73 mmol) was dissolved in toluene (12 mL) and then treated with phenyl urea (0.47 g, 3.46 mmol). The mixture was refluxed and monitored by TLC (2:1 EtOAc–hexane). When the reaction was complete, the solvent was evaporated, and the residue was crystallized from MeOH to give 0.37 g (42%) of **4**. Mp: 207–209 °C; $[\alpha]_D$ −23 (*c* 1.68, acetone); ^1H NMR ($CDCl_3$, 360 MHz) δ (ppm) 9.42 (s, 1H, N*H*), 7.44–7.12 (m, 6H, Ar, N*H*), 5.33 (t, 1H, $^3J_{3,4}$ = 10.0 Hz, H-3), 5.23 (t, 1H, H-1), 5.10 (t, 1H, $^3J_{4,5}$ = 9.5 Hz, H-4), 4.98 (t, 1H, $^3J_{2,3}$ = 9.2 Hz, H-2), 4.33 (dd, 1H, $^2J_{6,6'}$ = 12.6 Hz, H-6), 4.13 (dd, 1H, $^3J_{5,6'}$ = 2.1 Hz, H-6′), 3.87 (ddd, 1H, $^3J_{5,6}$ = 5.0 Hz, H-5), 2.44 (s, 1H, N*H*), 2.10, 2.08, 2.05 (s, 12H, 4 × $OCOCH_3$). ^{13}C NMR ($CDCl_3$, 90 MHz) δ (ppm) 170.7, 170.4, 170.0, 169.5 (CO), 155.0, 152.3 (NHCONH), 136.8, 129.4, 129.1, 124.4, 120.5 (Ar), 78.9 (C-1), 73.3, 72.9, 70.0, 68.0 (C-2–C-5), 61.7 (C-6), 20.7, 20.6, 20.56, 20.5 (CH_3). ESIMS: $[M+Na]^+$ calcd 532.46,



Scheme 3.

found: 532.16. Anal. Calcd for $C_{22}H_{27}N_3O_{11}$ (509.47): C, 51.87; H, 5.22; N, 7.51. Found: C, 51.97; H, 5.24; N, 7.50.

3.3. 1-(β-D-Glucopyranosyl)-5-phenyl biuret (5)

Biuret **4** (250 mg, 0.49 mmol) was dissolved in a mixture of MeOH and $CHCl_3$ (1:1). A catalytic amount of AcCl was added and the mixture was stirred at rt. The reaction was monitored by TLC (1:1 $CHCl_3$–MeOH). When the reaction was complete, it was neutralized with solid $NaHCO_3$, and then filtered and the solvent was evaporated. The residue was purified by column chromatography (9:1 $CHCl_3$–MeOH) to give 162 mg (97%) of **5** as a white powder. Mp: 191–193 °C; $[\alpha]_D$ +4 (*c* 0.68, MeOH);. ^{1}H NMR (DMSO-d_6 + D_2O, 360 MHz) δ (ppm) 7.41 (d, 2H, Ar), 7.29 (t, 2H, Ar), 7.04 (t, 1H, Ar), 4.68 (d, 1H, $^3J_{1,2}$ = 8.9 Hz, H-1), 3.63 (dd, 1H, $^3J_{5,6'}$ = 1.6 Hz, H-6′), 3.42 (dd, 1H, $^2J_{6,6'}$ = 12.1 Hz, H-6), 3.19–3.14 (m, 1H, $^3J_{5,6}$ = 5.8 Hz, H-5), 3.22, 4.26, 4.25 (t, 1H, $^3J_{2,3}$ = $^3J_{3,4}$ = $^3J_{4,5}$ = 8.9 Hz, H-2,3,4). ^{13}C NMR (DMSO-d_6, 90 MHz) δ (ppm) 154.6, 152.2 (NHCONH), 138.2, 128.9, 123.1, 119.0 (Ar), 80.3 (C-1), 78.5, 77.3, 72.9, 69.8 (C-2–C-5), 60.8 (C-6). Anal. Calcd for $C_{14}H_{19}N_3O_7$ (341.32): C, 49.27; H, 5.61; N, 12.31. Found: C, 49.35; H, 5.66; N, 12.30.

3.4. Reaction of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl urea[13] (6) with phenylisocyanate

Urea **6** (100 mg, 0.26 mmol) was refluxed in neat phenylisocyanate(1 mL). The reaction was monitored by TLC (2:1 EtOAc–hexane). When the reaction was complete, the excess amount of phenylisocyanate was removed by diluting the mixture with hexane. The formed precipitate was filtered, dissolved in CH_2Cl_2 and washed with satd aq $NaHCO_3$. The organic layer was separated, dried and the solvent was evaporated. The residue was separated by column chromatography (50:1 CH_2Cl_2–acetone) to give, in the order of elution, compounds **8**, **9** and **7**[10] (4%).

3.4.1. 3-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-1,5-diphenyl biuret (8)

Yield: 36 mg (24%), colourless syrup. R_f = 0.89 (25:1 $CHCl_3$–acetone) $[\alpha]_D$ +0.2 (*c* 1.71, DMSO). ^{1}H NMR ($CDCl_3$, 360 MHz) δ (ppm) 10.73 (s, 1H, N(CONHC_6H_5)$_2$), 8.75 (s, 1H, N(CONHC_6H_5)$_2$), 7.51–7.09 (m, 10H, Ar), 6.32 (d, 1H, $^3J_{1,2}$ = 10.0 Hz, H-1), 5.66 (t, 1H, $^3J_{3,4}$ = 9.5 Hz, H-3), 5.39 (t, 1H, $^3J_{4,5}$ = 9.5 Hz, H-4), 5.16 (t, 1H, $^3J_{2,3}$ = 10.0 Hz, H-2), 4.52 (dd, 1H, $^2J_{6,6'}$ = 12.6 Hz, H-6), 4.18 (dd, 1H, $^3J_{5,6'}$ = 2.1 Hz, H-6′), 4.05 (ddd, 1H, $^3J_{5,6}$ = 3.7 Hz, H-5), 2.13, 2.06, 2.04, 2.00 (s, 12H, 4×OCOCH_3). ^{13}C NMR ($CDCl_3$ + DMSO-d_6, 90 MHz) δ (ppm) 168.6, 167.9, 167.8, 167.7 (CO), 151.8, 151.1 (NCON), 138.3–116.0 (Ar), 79.0 (C-1), 73.1, 71.4, 66.3, 66.1 (C-2–C-5), 60.1 (C-6), 19.2, 19.1, 19.0, 18.9 (CH_3). ESIMS: $[M+Na]^+$ calcd for $C_{28}H_{31}N_3O_{11}$ (585.57): 608.56, found: 608.19.

3.4.2. 1-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-3,5-diphenyl biuret (9)

Yield: 46 mg (31%), white powder. R_f = 0.80 (25:1 $CHCl_3$–acetone). Mp: 226–229 °C; $[\alpha]_D$ −4 (*c* 0.64, DMSO). ^{1}H NMR ($CDCl_3$, 360 MHz) δ (ppm) 10.24 (s, 1H, NHCON(C_6H_5)CON*H*C_6H_5), 7.56–7.06 (m, 10H, Ar), 6.44 (t, 1H, $^3J_{H\text{-}1,NH}$ = 8.8 Hz, N*H*CON(C_6H_5)CON-HC_6H_5), 5.28 (t, 1H, $^3J_{3,4}$ = 9.6 Hz, H-3), 5.11 (t, 1H, $^3J_{1,2}$ = 9.6 Hz, H-1), 5.02 (t, 1H, $^3J_{4,5}$ = 9.6 Hz, H-4), 4.78 (t, 1H, $^3J_{2,3}$ = 9.6 Hz, H-2), 4.31 (dd, 1H, $^2J_{6,6'}$ = 12.3 Hz, H-6), 4.10 (dd, 1H, $^3J_{5,6'}$ = 1.8 Hz, H-6′), 3.81 (ddd, 1H, $^3J_{5,6}$ = 4.4 Hz, H-5), 2.09, 2.024, 2.019, 1.98 (s, 12H, 4 × OCOCH_3). ^{13}C NMR ($CDCl_3$, 90 MHz) δ (ppm) 170.6, 170.1, 169.8, 169.4 (CO), 155.8, 152.1 (NCON), 137.4, 135.6, 130.3, 129.9, 129.4, 128.9, 124.1, 120.1 (Ar), 79.8 (C-1), 73.5, 72.5, 69.8, 67.9 (C-2–C-5), 61.5 (C-6), 20.7, 20.5 (CH_3). ESIMS: $[M+Na]^+$ calcd for $C_{28}H_{31}N_3O_{11}$ (585.57): 608.56, found: 608.18.

3.5. 1,5-Bis-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)biuret (12)

Glucosylamine **2**[11] (400 mg, 1.15 mmol) was dissolved in dry THF (5 mL), then Et_3N (80 μL, 0.58 mmol) and OCNCOCl (46 μL, 0.58 mmol) were added. The mixture was stirred at rt under nitrogen atmosphere. After the reaction was complete (TLC, 10:1 EtOAc–hexane) the mixture was diluted with water (5 mL), and washed with EtOAc (3 × 5 mL). The organic phase was dried and the solvent was evaporated under reduced pressure to yield 402 mg (91%) colourless syrup. R_f = 0.83 (10:1 EtOAc–hexane); $[\alpha]_D$ −19 (*c* 0.97, $CHCl_3$); ^{1}H NMR (DMSO-d_6): δ (ppm) 9.11 (s, 1H, NH), 8.02 (d, 2H, *J* = 9.5 Hz, 2 × NH), 5.42, 5.34, 4.92, 4.86 (4 pseudo t, 8H, *J* = 9.5, 9.6 Hz in each, 2 × H-1, 2 × H-2, 2 × H-3, 2 × H-4), 4.16–3.94 (m, 6H, 2 × H-5, 2 × H-6, 2 × H-6′), 2.00, 1.99, 1.98, 1.95 (4s, 24H, 8 × CH_3); ^{13}C NMR ($CDCl_3$): δ (ppm) 170.6, 170.3, 169.9, 169.4 ($COCH_3$), 154.3 (2 × NHCO), 78.7 (C-1), 73.1, 72.8, 70.0, 67.9 (C-2 to C-5), 61.5 (C-6), 20.6, 20.5 (CH_3). Anal.

Calcd for $C_{30}H_{41}N_3O_{20}$ (763.65): C, 47.18; H, 5.41; N, 5.50. Found: C, 47.23; H, 5.50; N, 5.58.

3.6. General procedure I for the synthesis of 1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-5-(per-*O*-acetyl-β-D-glycopyranosyl)biurets 14 and 16

Glucosylamine **2**[11] (100 mg, 0.29 mmol) was dissolved in dry THF (2 mL), and some freshly heated molecular sieves were added. The mixture was cooled to −20 °C, OCNCOCl (23 μL, 0.29 mmol) was added, and stirred at −26 °C under nitrogen atmosphere for a day. Then a solution of 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosylamine[16] (**10**, 100 mg, 0.29 mmol) or 2,3,5-tri-*O*-acetyl-β-D-xylopyranosylamine[17,18] (**11**, 80 mg, 0.29 mmol) in dry THF (2 mL) and Et_3N (40 μL, 0.29 mmol) were added, and the mixture was allowed to warm up to rt. When the reaction was complete (TLC, 10:1 EtOAc–hexane) the insoluble materials were filtered off with suction, and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (7:1 EtOAc–hexane).

3.6.1. 1-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-5-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)biuret (14)

Prepared according to General procedure I (Section 3.6) from glucosylamine **2** (100 mg, 0.29 mmol) and galactosylamine **10** (100 mg, 0.29 mmol). Yield: 183 mg (83%) colourless syrup. R_f = 0.58 (10:1 EtOAc–hexane); $[\alpha]_D$ −15 (*c* 0.98, $CHCl_3$); ^{1}H NMR (CD_3CN): δ (ppm) 7.83 (br s, 1H, NH), 7.60–7.41 (m, 2H, 2 × NH), 5.37 (pseudo d, 1H, *J* = 3.0 Hz, H-4-Gal), 5.34 (pseudo t, *J* = 9.6 Hz H-3-Glc), 5.23 (pseudo t, 1H, *J* = 9.3, 9.4 Hz, H-1-Glc), 5.20–5.15 (m, 2H, H-1-Gal, H-3-Gal), 5.09 (pseudo t, 1H, *J* = 9.3 Hz, H-2-Gal), 5.07–4.98 (m, 2H, H-2-Glc, H-4-Glc), 4.18 (dd, 1H, *J* = 4.5, 12.4 Hz, H-6a-Glc), 4.13–4.09 (m, 1H, H-5-Gal), 4.09–4.00 (m, 3H, H-6a-Gal, H-6b-Gal, H-6b-Glc), 3.94 (m, 1H, H-5-Glc), 2.21, 2.11, 2.01, 1.99, 1.98, 1.96 (6br s, 24H, 8 × CH_3); ^{13}C NMR ($CDCl_3$): δ (ppm) 170.6, 170.3, 169.9, 169.4 ($COCH_3$), 154.3 (2 × NHCO), 79.1, 78.9 (C-1-Glc, C-1-Gal), 73.2, 72.8, 71.9, 71.0, 70.1, 68.0, 67.8, 67.2 (C-2-Glc to C-5-Glc, C-2-Gal to C-5-Gal), 61.6, 61.0 (C-6-Glc, C-6-Gal), 20.6, 20.5 (CH_3). Anal. Calcd for $C_{30}H_{41}N_3O_{20}$ (763.65): C, 47.18; H, 5.41; N, 5.50. Found: C, 47.29; H, 5.54; N, 5.61.

3.6.2. 1-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-5-(2,3,5-tri-*O*-acetyl-β-D-xylopyranosyl)biuret (16)

Prepared according to General procedure I (Section 3.6) from glucosylamine **2** (100 mg, 0.29 mmol) and xylosylamine **11** (80 mg, 0.29 mmol). Yield: 171 mg (86%) colourless syrup. R_f = 0.58 (10:1 EtOAc–hexane); $[\alpha]_D$ −28 (*c* 0.58, $CHCl_3$); ^{1}H NMR (CD_3CN): δ (ppm) 7.75 (br s, 1H, NH), 7.49 (br s, 2H, 2 × NH), 5.35 (pseudo t, 1H, *J* = 9.4 Hz, H-3-Glc), 5.29 (pseudo t, 1H, *J* = 9.1 Hz, H-3-Xyl), 5.23 (pseudo t, 1H, *J* = 9.4 Hz, H-1-Glc), 5.13 (pseudo t, 1H, *J* = 9.1 Hz, H-1-Xyl), 5.06–4.88 (m, 4 H, H-4-Glc, H-2-Glc, H-2-Xyl, H-4-Xyl), 4.17 (dd, *J* = 4.8, 12.4 Hz, H-6a-Glc), 4.04 (dd, 1H, *J* = 2.0, 12.4 Hz, H-6b-Glc), 3.98 (dd, *J* = 5.4, 11.5 Hz, H-5a-Xyl), 3.93–3.88 (m, 1 H, H-5-Glc), 3.51–3.44 (ddd, 1H, *J* = 1.3 Hz, 11.5 Hz, H-5b-Xyl), 2.18, 2.01, 2.00, 1.99, 1.98, 1.96 (5br s, 21 H, 7 × CH_3); ^{13}C NMR ($CDCl_3$): δ (ppm) 170.6, 170.3, 169.9, 169.8, 169.4 ($COCH_3$), 154.4 (2 × NHCO), 79.0, 78.7 (C-1-Glc, C-1-Xyl), 73.1, 72.8, 71.8, 70.0, 69.9, 68.6, 68.0 (C-2-Glc to C-5-Glc, C-2-Xyl to C-4-Xyl), 63.7, 61.5 (C-6-Glc, C-5-Xyl), 20.5, 20.4 (CH_3). Anal. Calcd for $C_{27}H_{37}N_3O_{18}$ (691.59): C, 46.89; H, 5.39; N, 6.08. Found: C, 46.99; H, 5.31; N, 6.15.

3.7. General procedure II for the removal of *O*-acyl protecting groups

An O-peracetylated compound (100 mg) was dissolved in dry MeOH (1 mL), and a solution of NaOMe (1 M in MeOH) was added to the solution in a catalytic amount. The reaction mixture was kept at rt. When the reaction was complete (TLC, 7:3 $CHCl_3$–MeOH) the solution was neutralized with a cation exchange resin Amberlyst 15 (H^+ form). Filtration and removal of the solvent resulted in the corresponding deacetylated sugar derivatives.

3.7.1. 1,5-Bis-(β-D-glucopyranosyl)biuret (13)

Prepared according to General procedure II (Section 3.7) from biuret **12** (100 mg, 0.13 mmol). Yield: 54 mg (96%) colourless syrup. R_f = 0.45 (1:3 $CHCl_3$–methanol); $[\alpha]_D$ −4 (*c* 0.51, MeOH); ^{1}H NMR (D_2O): δ (ppm) 4.91 (d, 2H, *J* = 9.3 Hz, 2 × H-1), 3.86 (dd, 2H, *J* = 1.6, 12.3 Hz, 2 × H-6a), 3.70 (dd, 2H, *J* = 5.3, 12.3 Hz, 2 × H-6b), 3.52, 3.43, 3.40 (3 pseudo t, 6H, *J* = 9.0, 9.3 Hz in each 2 × H-2, 2 × H-3, 2 × H-4), 3.51–3.47 (m, 2H, 2 × H-5); ^{13}C NMR (Me_2SO-d_6): δ (ppm) 154.2 (NHCO), 80.3, 78.4, 77.3, 72.8, 69.7 (C-1-C-5), 60.8 (C-6). Anal. Calcd for $C_{14}H_{25}N_3O_{12}$ (427.36): C, 39.35; H, 5.90; N, 9.83. Found: C, 39.46; H, 5.99; N, 9.90.

3.7.2. 1-(β-D-Galactopyranosyl)-5-(β-D-glucopyranosyl)biuret (15)

Prepared according to General procedure II (Section 3.7) from biuret **14** (100 mg, 0.13 mmol). Yield: 53 mg (98%) colourless syrup. R_f = 0.35 (1:3 $CHCl_3$–MeOH); $[\alpha]_D$ −7 (*c* 0.54, H_2O); ^{1}H NMR (DMSO-d_6): δ (ppm) 4.65–4.56 (m, 2H), 3.69–3.57 (m, 2H), 3.47–3.00 (m, 9H), 2.95 (t, 1H); ^{13}C NMR (D_2O): δ (ppm) 159.6 (2 × NHCO), 81.5 (C-1-Glc, C-1-Gal), 77.9, 77.2, 72.6, 70.0 (C-2-Glc to C-5-Glc, C-2-Gal to C-5-Gal), 61.3 (C-6-Glc, C-6-Gal). Anal. Calcd for $C_{14}H_{25}N_3O_{12}$ (427.36): C, 39.35; H, 5.90; N, 9.83. Found: C, 39.44; H, 5.98; N, 9.89.

3.7.3. 1-(β-D-Glucopyranosyl)-5-(β-D-xylopyranosyl)biuret (17)

Prepared according to General procedure II (Section 3.7) from biuret **16** (100 mg, 0.14 mmol). Yield: 55 mg (96%) colourless syrup. R_f = 0.63 (1:3 $CHCl_3$–MeOH); $[\alpha]_D$ −12 (*c* 0.52, H_2O); ^{1}H NMR (D_2O): δ (ppm) 4.86 (d, 1 H, *J* = 9.3 Hz), 3.81 (dd, 1H, *J* = 2.1, 12.6 Hz), 3.65 (dd, 1H, *J* = 5.1 Hz, 12.6 Hz), 3.51–3.25 (m, 10 H); ^{13}C NMR (D_2O): δ (ppm) 156.3 (2 × NHCO), 81.6, 80.9 (C-1-Glc, C-1-Xyl), 78.1, 77.1, 72.6, 72.4, 69.9, 69.7 (C-2-Glc to C-5-Glc, C-2-Xyl to C-4-Xyl), 67.3, 61.2 (C-6-Glc, C-5-Xyl). Anal. Calcd for $C_{13}H_{23}N_3O_{11}$ (397.34): C, 39.30; H, 5.83; N, 10.58. Found: C, 39.39; H, 5.90; N, 10.65.

3.8. 1-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosylcarbonyl)-3-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)urea (20)

C-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)formamide[19] (**18**, 100 mg, 0.27 mmol) was dissolved in dry toluene (3 mL). Then some molecular sieves and crystalline isocyanate **3**[10] (202 mg, 0.54 mmol) were added. The reaction was stirred at reflux temperature. After one day the reaction mixture was worked up: the molecular sieves were filtered off with suction and the solution was concentrated under reduced pressure. The residue was purified by column chromatography (100:1 $CHCl_3$–MeOH). Two products were isolated: **20** (177 mg, 89%) and **21**[21] (52 mg). Characterization of **20**: colourless syrup; R_f = 0.55 (5:1 EtOAc–hexane); $[\alpha]_D$ +18 (*c* 0.84, $CHCl_3$); ^{1}H NMR (CD_3CN): δ (ppm) 8.66 (br s, 1H, NH), 8.64 (d, 1H, *J* = 9.3 Hz, NH), 5.41 (pseudo d, *J* = 2.2 Hz, H-4-Gal), 5.35 (pseudo t, *J* = 9.6 Hz, H-3-Glc), 5.28 (pseudo t *J* = 9.3 Hz, H-1-Glc), 5.22–5.15 (m, 2H, H-2-Gal, H-3-Gal), 5.04, 5.03 (2 pseudo t, *J* = 9.3, 9.6 Hz in both, H-4-Glc, H-2-Glc), 4.24–4.02 (m, 6 H, H-6a-Glc, H-6b-Glc, H-1-Gal, H-5-Gal, H-6a-Gal, H-6b-Gal), 3.91 (ddd, 1H, *J* = 2.3, 4.8, 9.9 Hz, H-5-Glc), 2.12, 2.03, 2.02, 2.01, 1.99, 1.98, 1.96, 1.93 (8br s, 24H,8 × CH_3). ^{13}C NMR ($CDCl_3$): δ (ppm) 170.6, 170.3, 170.1, 169.9, 169.7, 169.4 ($COCH_3$), 168.0, 152.1 (2 × CONH), 78.8, 76.7 (C-1-Glc, C-1-Gal), 74.9, 73.5, 73.0, 70.6, 69.7, 68.2, 67.0, 65.9 (C-2-Glc to C-5-Glac, C-2-Gal to C-5-Gal), 61.7, 61.4 (C-6-Glc,

C-6-Gal), 20.7, 20.6, 20.5 (CH_3). Anal. Calcd for $C_{30}H_{40}N_2O_{20}$ (748.64): C, 48.13; H, 5.39; N, 3.74. Found: C, 48.20; H, 5.43; N, 3.79.

3.9. 1,3-Bis-(β-D-glucopyranosyl)urea (22)

Prepared according to General procedure II (Section 3.7) from urea **21** (190 mg, 0.26 mmol). Yield 80 mg (79%) amorphous solid. Lit.[27] Mp: 207 °C (dec.); R_f = 0.45 (1:3 $CHCl_3$-methanol); $[\alpha]_D$ +23 (*c* 0.59, DMSO), lit.[27] $[\alpha]_D$ −32.8 (*c* 2, water); ^{1}H NMR (D_2O): δ (ppm) 4.86 (d, 1H, *J* = 9.3 Hz, H-1), 3.86 (dd, *J* = 1.5, 12.3 Hz, H-6a), 3.69 (dd, 1H, *J* = 5.2, 12.3 Hz, H-6b), 3.53, 3.38, 3.37 (3 pseudo t, 3H, *J* = 9.2, 9.7 Hz in each, H-2, H-3 ,H-4), 3.51–3.48 (m, 1H, H-5). ^{13}C NMR (D_2O): δ (ppm) 159.6 (CO), 81.5 (C-1), 77.9, 77.2, 72.6, 70.0 (C-2 to C-5), 61.3 (C-6).

3.10. 1-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-3-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosylcarbonyl)-urea (23)

C-(2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl)formamide[20] (**19**, 54 mg, 0.086 mmol) was dissolved in dry toluene (1 mL), and some molecular sieves were added followed by crystalline isocyanate **3**[10] (64 mg, 0.172 mmol) mmol). The reaction mixture was heated to reflux temperature. When the reaction was complete (TLC, 5:1 EtOAc–hexane) the molecular sieves were filtered off with suction and the residue was concentrated under reduced pressure. The crude product was purified by column chromatography (1:1 EtOAc–hexane). Two products were isolated: **23** (84 mg, 98%) and **21**[21] (23 mg). Characterization of **23**: colourless syrup; R_f = 0.53 (1:1 EtOAc–hexane); $[\alpha]_D$ −6 (*c* 0.61, $CHCl_3$); ^{1}H NMR (CD_3CN): δ (ppm) 9.01 (br s, 1H, NH), 8.56 (d, 1H, *J* = 9.2 Hz, NH), 8.05, 7.94, 7.90, 7.78 (4d, 8H, Ar), 7.63–7.32 (m, 12H, Ar), 6.02 (pseudo t, 1H, *J* = 9.4 Hz, H-3-GlcBz), 5.77 (pseudo t, 1H, *J* = 10.1 Hz, H-4-GlcBz), 5.75 (pseudo t, 1H, *J* = 9.8 Hz, H-2-GlcBz), 5.38 (pseudo t, 1H, *J* = 9.6 Hz, H-3-GlcAc), 5.34 (pseudo t, 1H, *J* = 9.4 Hz, H-1-GlcAc), 5.00, 4.99 (2 pseudo t, 2H, *J* = 9.4, 9.8 Hz in both, H-4-GlcAc, H-2-GlcAc), 4.65 (dd, 1H, *J* = 2.3, 12.3 Hz, H-6a-GlcBz), 4.57 (dd, 1H, *J* = 4.6, 12.3 Hz, H-6b-GlcBz), 4.54 (pseudo t, 1H, *J* = 9.9 Hz, H-1-GlcBz), 4.43–4.39 (m, 1H, H-5-GlcBz), 4.14 (dd, 1H, *J* = 4.8, 12.3 Hz, H-6a-GlcAc), 4.01 (dd, 1H, *J* = 1.7, 12.3 Hz, H-6b-GlcAc), 3.94 (ddd, 1H, *J* = 1.9, 4.4, 9.9 Hz), 2.19, 1.96, 1.94 (3br s, 12H, 4 × CH_3). ^{13}C NMR ($CDCl_3$): δ (ppm) 170.5, 170.0, 169.5, 169.3, 166.0, 165.5, 165.2, 165.1 ($COCH_3$), 168.2, 152.6 (2 × CONH), 133.6, 133.3, 133.2, 129.8, 129.7, 129.6, 128.2, 128.3 (CH–Ar), 129.1, 128.7 (C–Ar), 78.4, 76.6 (2 × C-1), 76.1, 73.1, 72.8, 69.8, 69.6, 68.8, 68.1(2 × C-2 to C-5), 62.8, 61.6 (2 × C-6), 20.5, 20.4 (CH_3). Anal. Calcd for $C_{50}H_{48}N_2O_{20}$ (996.92): C, 60.24; H, 4.85; N, 2.81. Found: C, 60.19; H, 4.90; N, 2.89.

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