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A facile stereoselective synthesis of α-glycosyl ureas

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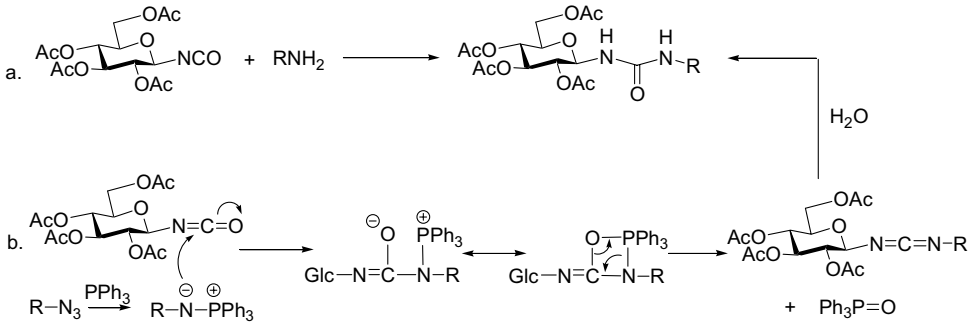
Abstract—α-Glycosyl ureas can be synthesised directly from tetra-*O*-benzyl glycosyl azides and isocyanates, using a one-pot procedure that is simple and general in scope. The benzyl protecting groups are easily removed from the urea products by catalytic hydrogenation. The synthesised α-glycosyl ureas represent a new class of neo-glycoconjugates with the potential of being resistant towards carbohydrate processing enzymes.

Keywords: Carbohydrates; Glycosyl azides; Glycosyl ureas; Neo-glycoconjugates; Synthetic methods

1. Introduction

Glycosyl ureas are found in nature in the aminoglycosidic antibiotics.[1,2] They have also been used as stable N-linked-glycopeptide mimics[3] and for the synthesis of polyvalent glycoconjugates.[4] However, only a few methods for the synthesis of glycosyl ureas have been reported,[5–8] and in particular, a practical synthesis of anomeric α-glycosyl ureas is still lacking.[3b,7d] Yet, these compounds could constitute an interesting new class of neo-glycoconjugates, with virtually unexplored physical and chemical properties and biological activity. In fact, because glycosyl derivatives can be highly sensitive to chemical or enzymatic hydrolysis, it is particularly interesting to have access to new modified structures that could be endowed with an increased stability.

Glycosyl ureas are generally prepared starting from glycosyl isocyanates (Scheme 1).[7,8] These substrates can react directly with amines[7] (Scheme 1a) or with iminophosphoranes[8] generated by reduction of azides[9] (Scheme 1b). In the latter case, carbodiimides are initially obtained and subsequently hydrolysed to the cor-



Scheme 1. Synthesis of β-glycosyl ureas from glycosyl isocyanates: (a) direct synthesis using amines,[3,7] (b) synthesis via carbodiimides, using iminophosphoranes.[8]

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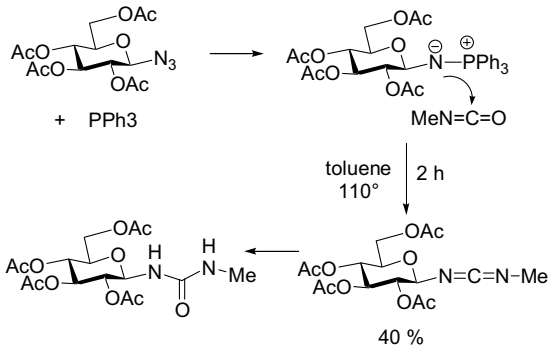


Scheme 2. Reaction of tetra-*O*-acetyl-glucopyranosyl iminophosphorane with isocyanates.[8a]

responding ureas, most conveniently in a one-pot procedure (Scheme 1b).[8a] The starting glycosyl isocyanates can be synthesised either from glycosyl halides, following the classical Fischer procedure,[6] or from the corresponding azides,[3,7] using multi-step sequences that involve glycosyl isonitriles[3,7a–c] or glycosyl carbamates[7d] as intermediates. The stereoselectivity of these transformations is complete for β azides, but 4:1 α/β ratios are typically obtained starting from the α anomers.[7]

Direct conversion of glycosyl azides to ureas could in principle be achieved by inverting the reaction partners of the carbodiimide reaction, as shown in Scheme 2. Thus, a glycosyl azide could be transformed with phosphines into a glycosyl iminophosphorane, which could react with isocyanates to give ureas through a carbodiimide intermediate (Scheme 2).

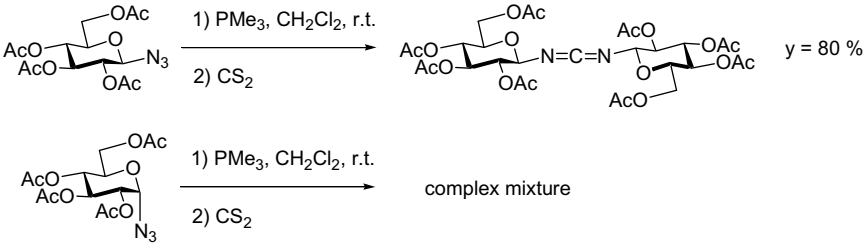
However, in practice,[8a] anomeric glycosyl iminophosphoranes of tetra-*O*-acetyl pyranoses react very sluggishly with isocyanates.[8a] For instance, at room temperature no reaction occurred between 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl phosphinimine and methyl isocyanate, while at 110 °C the reaction proceeded in two hours in low yield (Scheme 2). With more complex isocyanates, the reaction is even slower and gives rise to various side products.[8a]

In a recent example, Györgydeák and co-workers employed a similar procedure for the synthesis of symmetrical and unsymmetrical glycosyl carbodiimides.[10] The reaction of peracetylated glycosyl azides with 1 equiv of trimethylphosphine in dry dichloromethane at room temperature led to the corresponding iminophosphoranes. The in situ reaction of these compounds with carbon disulfide under mild conditions led to the symmetrical glycosyl carbodiimides. The procedure worked effectively starting from β-azides and the expected products were obtained in good yields as stable crystalline solids. However, from α-azides, complex reaction mixtures were formed and the expected products could not be isolated (Scheme 3).

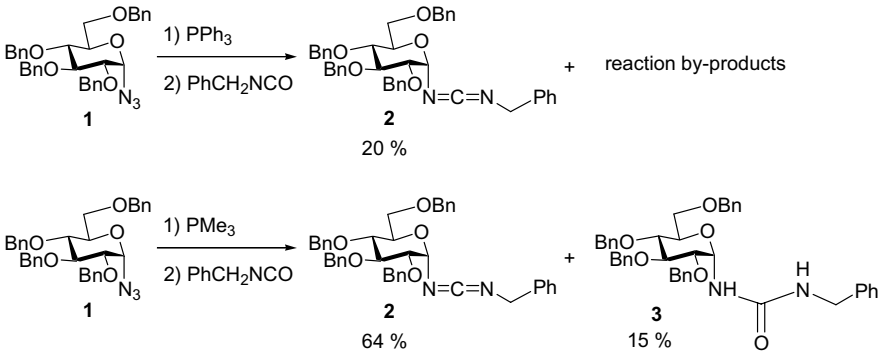
Here we report that, on the contrary, 2,3,4,6-tetra-*O*-benzyl-glycosyl azides give iminophosphoranes that do react productively with isocyanates without anomeric isomerisation. Hence, a one-pot stereoselective synthesis of α-glycosyl ureas from α-glycosyl azides can be easily achieved, provided that tetra-*O*-benzyl α-glycosyl azides are used as starting material.[11] The benzyl protecting groups are then simply removed from the urea products by catalytic hydrogenation. In general, the resulting α-glycosyl ureas were found to be configurationally stable, but one exception was found. Thus, a new class of neoglycoconjugates becomes available for further studies.

2. Results and discussion

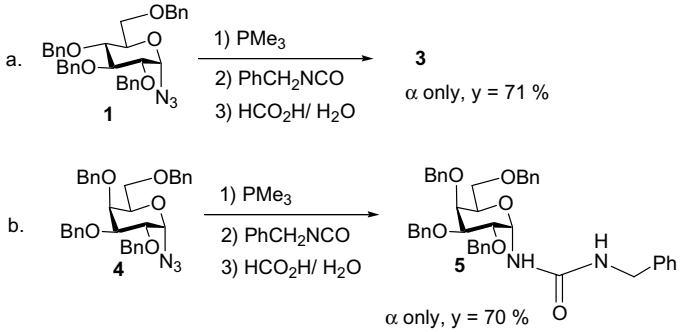
The low reactivity observed for anomeric glycosyl iminophosphoranes is likely due to the electron withdrawing effect of the pyranose moiety, which stabilises the negative charge on the ylide nitrogen (Scheme 2) and reduces its nucleophilicity.[8a] This effect is maximised for acetylated (disarmed[12]) pyranoses but it should be greatly reduced if the hydroxyl groups of sugar are protected as benzyl ethers (armed[12]). Therefore, we reasoned that the iminophosphoranes obtained by reduction of the 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl azide **1**[11a,13] (Scheme 4) ought to react with alkyl isocyanates. The reaction of **1** with triphenylphosphine in dry dichloromethane at room temperature followed by treatment with benzyl isocyanate afforded a complex mixture of reaction intermediates that contained the α anomer of carbodiimide **2** (20% yield). The reaction of **1** with trimethylphosphine was much faster. After disappearance of the starting material, benzyl isocyanate was added to the mixture and complete conversion of the intermediate into the corresponding carbodiimide **2**



Scheme 3. Synthesis of symmetrical carbodiimides from tetra-*O*-acetyl-glucopyranosyl azides.[10]



Scheme 4. Synthesis of *N*′-benzyl-*N*-2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl carbodiimide **2** and of urea **3** from azide **1**.



Scheme 5. One-pot synthesis of *N*′-benzyl-*N*-tetra-*O*-benzyl-α-D-glucopyranosyl urea **3** from α-glucopyranosyl azide **1** (a) and of *N*′-benzyl-*N*-tetra-*O*-benzyl-α-D-galactosyl urea **5** from α-galactosyl azide **4** (b).

was achieved in one hour (Scheme 4). This compound could be isolated in 64% yield by flash chromatography even if, during the purification, partial hydrolysis was observed and 15% of urea **3** was formed. The α configuration was assigned to the anomeric carbon of **2** and **3** by ^{1}H NMR, on the basis of the value of the coupling constant of the anomeric proton ($J_{1,2} = 4.1$ Hz). The reaction was found to be completely α-stereoselective. Trimethylphosphine, used as a 1 M toluene solution, has the further advantage that the corresponding trimethylphosphinoxide can be easily removed from the reaction mixture by simple aqueous work-up.

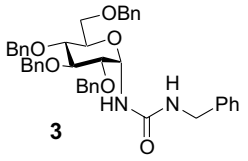
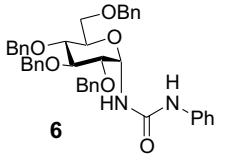
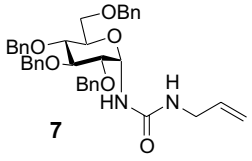
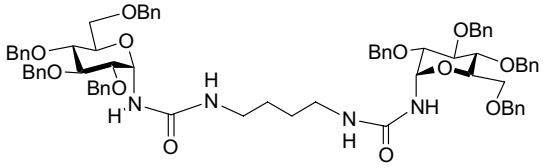
Acid-catalysed hydrolysis of **2** in aqueous THF and in the presence of different protic acids (Amberlite-IR120, acetic acid, formic acid) proved to be slow and incomplete. Better results were obtained following a slight modification of the one-pot procedure introduced by Ortiz Mellet, Garciá-Fernandéz and co-workers in 2000[8b] (Scheme 5). Thus, after the formation of carbodiimide, formic acid and water (2:1) were added to the reaction mixture, which was stirred for an additional 30 min.[†] The urea product **3** was then isolated after chromatographic purification in 71% overall yield and with complete retention of the anomeric configuration (Scheme 5a). The α-galactosyl azide **4**[11,13] reacted under the same conditions with benzyl isocyanate to afford the corresponding α-galactosyl urea **5** in 70% yield (Scheme 5b).

These optimised conditions were then used for the reactions of **1** with a series of commercially available isocyanates; the results are collected in Table 1. All the reactions examined proceeded with total retention of the α configuration at the anomeric centre, as determined by ^{1}H NMR. The reactions with phenyl isocyanate (entry 2) and allyl isocyanate (entry 3) gave the corresponding ureas **6** and **7** with 74% and 67% yield, respectively. On the contrary, using phenyl isothiocyanate (entry 4) phenyl urea **6** was isolated with only 19% yield and the formation of various side products was observed. The reaction with 1,4-diisocyanobutane (entry 5) also proved to be highly efficient and afforded divalent neo-glycoconjugate **8** in 60% global yield starting from azide **1**.

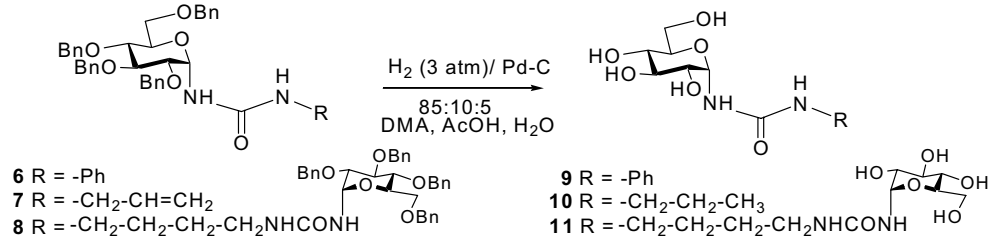
Removal of the benzyl ether protecting groups was achieved by catalytic hydrogenation (Scheme 6). After some investigation, we found that the reaction performed consistently well using a 85:10:5 *N*,*N*-dimethylacetamide/H_2O/AcOH mixture and a slight H_2 pressure (3 atm).[14] The deprotected glycosyl ureas could be isolated in good yields (85–90%) by filtration from the

[†] The use of acetic acid and water (2:1), as in the original procedure, resulted in much slower conversion of the diimide, and partially hydrolysed by-products were obtained (12%) even after 18 h of reaction.

Table 1. One-pot synthesis of glycosyl ureas from 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl azide **1**

Entry	Electrophile	Yield[a] (%)	Product	α/β Ratio[b]
1	$PhCH_2$–NCO	71	 **3**	⩾97:3
2	Ph–NCO	74	 **6**	⩾97:3
3	CH_2–CH=CH_2–NCO	67	 **7**	⩾97:3
4	Ph–NCS	19	**6**	⩾97:3
5	OCN–$(CH_2)_4$–NCO	60[c]	 **8**	⩾97:3

[a] Yield after flash chromatography. General conditions: 1.2 equiv of PMe_3 in CH_2Cl_2, then 1.2 equiv of iso(thio)cyanate for 1 h, at rt, followed by 2:1 v/v HCO_2H/H_2O (30 min).
[b] Determined by ^1H NMR on the crude reaction mixtures.
[c] 2.2 mol of sugar per mol of isocyanate 1 h at rt, followed by 2:1 v/v HCO_2H/H_2O (1.5 h).

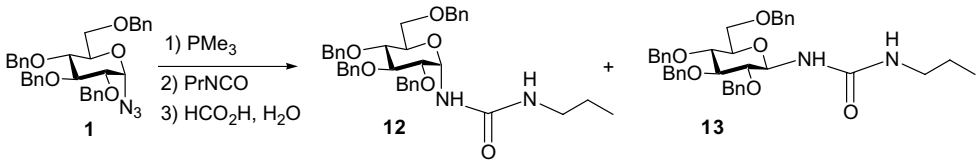


Scheme 6. Hydrogenolitic removal of the protecting groups.

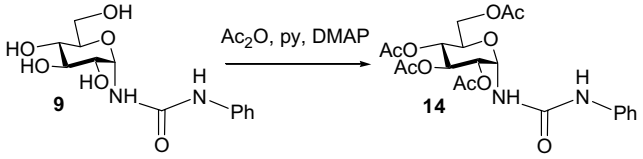
reaction mixtures, and further purified by flash chromatography, reverse phase HPLC or gel permeation chromatography.

Surprisingly, deprotection of *N*′-allyl urea **7** occurred with simultaneous anomerisation, and *N*′-propyl urea **10** was initially isolated as a 1:1 mixture of the two anomeric α- and β-*N*-propyl anomers that slowly interconverted to the pure β-anomer. Examples of anomerisation of glycosyl thioureas have been reported to occur, but only in basic media.[15] The behaviour of the propyl derivative was rather puzzling, so, at the suggestion of a referee, we independently synthesised the *N*′-propyl-*N*-2,3,4,6-tetra-*O*-benzyl-glucopyranosyl urea derivative by reaction of **1** with trimethylphosphine and *n*-propyl isocyanate (Scheme 7).

To our surprise, a 3.2:1 mixture of the α and β isomers was formed. Moreover, both α- to β- and β- to α-isomerisation were found to occur on silica gel during flash chromatography. Slow anomer interconversion (4 days) was also observed in $CDCl_3$ solution in the NMR tube. This behaviour was not observed for any of the other compounds described in this paper, which were all found to be stable on silica gel, and configurationally stable for months at 4 °C, nor was it reported for similar



Scheme 7. Reaction of **1** with *n*-propyl isocyanate.



Scheme 8. Acetylation of urea **9**.

(unprotected) β-glucopyranosyl azides recently described in the literature.[16] These results suggest that simple *N*-alkyl, *N*′-glycosyl ureas may undergo anomeric equilibration under slightly acidic conditions and that the composition of the α-β equilibrium mixture may depend on the oxygen substitution of sugar.

Finally, to confirm the product configuration, *N*′-phenyl urea **9** was acetylated (Ac_2O, pyridine, DMAP catalyst) and product **14** was fully characterised by NMR spectroscopy confirming the α configuration of the anomeric centre and the $^4C_1$ conformation of the pyranose ring (Scheme 8).

3. Conclusions

In conclusion, the reaction of α-glycosyl azides of perbenzylated monosaccharides with trimethylphosphine and isocyanates followed by one pot hydrolysis affords the corresponding α-glycosyl ureas with complete stereocontrol and good yields. Peracetylated compounds show little reactivity under these conditions,[8a] and this difference can be explained considering the arming-disarming effect of the protecting groups.[12] Trimethylphosphine was used as a 1 M solution in toluene and it was found to be more effective than triphenylphosphine in this reaction in terms of yield.

The procedure reported here works effectively for a series of isocyanates, both on the *gluco* and *galacto* series. The resulting α-glycosyl ureas appear to adopt a $^4C_1$ chair conformation, and the anomeric configuration can be established by coupling constant analysis. Removal of the benzyl protecting groups is obtained by catalytic hydrogenation, and leads to configurationally stable α-ureas, with the exception of *N*′-propyl urea **11**. Compared to the literature reports,[7d] this method allows the direct transformation of α-glycosyl azides into the corresponding ureas in a one-pot facile reaction, avoiding the four steps and one chromatographic separation involved in the best procedure previously reported. Neo-glycoconjugates are of great interest for medicinal chemistry due to their potential biological activities, their resistance towards enzymatic degradation, and their expected greater half-life in biological systems.[17] This protocol opens the way to a straightforward synthesis of an interesting class of neo-glycoconjugates, whose chemical and biological properties can now be explored in detail.

4. Experimental

4.1. General

The solvents were dried by standard procedures: CH_2Cl_2 over CaH_2, pyridine and *N*,*N*-dimethylacetamide (DMA) over activated 4 Å molecular sieves. Reactions involving trimethylphosphine were performed under argon. ^1H and ^{13}C spectra were recorded at 300 K on a Bruker AVANCE-400 or a Bruker AVANCE-600 spectrometer. Chemical shifts δ for ^1H and ^{13}C are expressed in parts per million relative to internal $(CH_3)_4Si$ as standard. Signals were abbreviated as s, singlet; br s broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Mass spectra were obtained with a MALDI-TOF (OMNIFLEX Bruker) or an ESI (LCQ Advantage Termofinnigan) apparatus, and HRMS with a FT-ICR (APEX™ II Bruker Daltonics). Optical rotations $[\alpha]_D$ were measured in a cell of 1 dm pathlength and 1 mL capacity with a Perkin–Elmer 241 polarimeter. IR spectra were recorded on a JASCO FT/IR-300E spectrometer, and frequencies are expressed in cm^{-1}. Thin layer chromatography (TLC) was carried out with precoated Merck F_{254} silica gel plates. Flash chromatography (FC) was carried out with Macherey-Nagel Silica Gel 60 (230–400 mesh). Tetra *O*-benzyl α-glycosyl azides **1** and **4** were synthesised following the reported procedures.[11,13]

4.2. General procedure for the synthesis of glycosyl ureas 3 and 5–7

Trimethylphosphine (1.2 equiv, 1 M solution in toluene) was added, at rt and under argon, to a 0.1 M solution of the α-azide (1 equiv) in dry CH_2Cl_2. The mixture was stirred for 1 h until the disappearance of the starting material from the TLC plate (4:1, toluene/EtOAc). The iso(thio)cyanate (1.2 equiv) was added and the solution was stirred for 1.5 h. A 2:1 v/v solution of formic acid and H_2O was then added (5 equiv of formic acid) and the hydrolysis of the carbodiimide was monitored by TLC (4:1, toluene/EtOAc). After 1.5 h, the solution was diluted with CH_2Cl_2 and washed with H_2O, satd aq $NaHCO_3$ and H_2O again. The organic layer was dried (Na_2SO_4) and concentrated. The crude product was purified by flash chromatography.

4.3. *N*′-Benzyl-*N*-2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl urea (3)

Flash chromatography: 4:1, toluene/EtOAc; yield: 71%; $[\alpha]_D^{25}$ +62.9 (*c* 1.0, $CHCl_3$); IR (nujol): 1652 $\nu_{C=O}$; ^1H NMR (400 MHz, $CDCl_3$): 7.30–7.10 (m, 23H, aromatics), 7.10–7.05 (m, 2H, aromatics), 5.83 (t, 1H, N*H*CH_2, $J = 7.5$ Hz), 5.15 (br s, 1H, N*H*), 5.13 (dd, 1H, H_1, $J_{1,2} = 4.6$ Hz, $J_{1\text{-NH}} = 8.0$ Hz), 4.82 (d, 1H, C*H*$_2$Ph, $J = 11$ Hz), 4.70 (AB system, 2H, C*H*$_2$Ph, $J = 6$ Hz), 4.55 (AB system, 2H, C*H*$_2$Ph, $J = 12$ Hz), 4.38 (AB system, 1H, C*H*$_2$Ph, $J = 11$ Hz), 4.36 (AB system, 1H, C*H*$_2$Ph, $J = 12$ Hz), 4.24–4.22 (m, 2H, NC*H*$_2$Ph), 4.21 (AB system, 1H, C*H*$_2$Ph, $J = 11$ Hz), 3.81 (m, 1H, H-5), 3.70–3.60 (m, 2H, H-2, H-3), 3.50–3.40 (m, 2H, H-4 H-6), 3.37 (d, 1H, H-6′, $J_{6,6'} = 8$ Hz, $J_{5,6'} = 0$ Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$, selected peaks): 158, 139.27, 138.49, 138.18, 137.77, 137.29, 128.79–127.39 (12 peaks), 82.07, 78.49, 78.34, 77.47, 76.04, 75.16, 73.56, 73.25, 69.78, 68.52, 44.13. ESIMS *m*/*z*: 673.3 ($M+H^+$), 695.3 ($M+Na^+$), 711.3 ($M+K^+$).

4.4. *N*′-Phenyl-*N*-2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl urea (6)

Flash chromatography: 9:1, toluene/EtOAc; yield: 74%. $[\alpha]_D^{25}$ +120.8 (*c* 1.0, $CHCl_3$); IR (nujol): 1665 $\nu_{C=O}$; ^1H NMR (400 MHz, $CDCl_3$): 7.55 (br s, 1H, N*H*Ph), 7.30–7.27 (m, 20H, aromatics), 7.13 (t, 2H, aniline H_m, $J = 7.3$ Hz), 7.09–7.07 (m, 2H, aromatics), 6.92 (t, 1H, aniline H_p, $J = 7.3$ Hz), 5.33 (br s, 1H, N*H*), 5.18 (br s, 1H, H-1, $J_{1,2} = 4.6$ Hz), 4.82 (d, 1H, C*H*$_2$Ph, $J = 11$ Hz), 4.74 (d, 1H, C*H*$_2$Ph, $J = 11$ Hz), 4.72 (d, 1H, C*H*$_2$Ph, $J = 11$ Hz), 4.59 (AB system, 2H, C*H*$_2$Ph), 4.48 (d, 1H, C*H*$_2$Ph, $J = 12$ Hz), 4.43 (d, 1H, C*H*$_2$Ph, $J = 11$ Hz), 4.42 (d, 1H, C*H*$_2$Ph, $J = 12$ Hz), 3.92–3.90 (m, 1H, H-5), 3.70–3.64 (m, 2H, H-2, H-3), 3.60–3.52 (m, 2H, H-6, H-6′), 3.45 (dd, 1H, H-4, $J_{4,3} = J_{4,5} = 8.3$ Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$): 156.14, 138.35, 138.29, 137.92, 137.50, 137.00, 129.19–127.80 (9 peaks), 123.75, 123.34, 120.64, 119.96, 81.84, 78.65, 78.03, 77.46, 75.87, 75.02, 73.50, 73.08, 69.86, 68.74; MALDI-TOFMS *m*/*z*: 659.2 ($M+H^+$), 681.2 ($M+Na^+$), 697.2 ($M+K^+$).

4.5. *N*′-Propenyl-*N*-2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl urea (7)

Flash chromatography: 3:1 toluene/EtOAc; yield: 67%; $[\alpha]_D^{25}$ +21.7 (*c* 1.0, $CHCl_3$); IR (nujol): 1678 $\nu_{C=O}$; ^1H NMR (400 MHz, $CDCl_3$): 7.30–7.20 (m, 18H, aromatics), 7.15–7.04 (m, 2H, aromatics), 5.75–5.65 (m, 1H, =C*H*), 5.60 (t, 1H, N*H*CH_2, $J = 5.4$), 5.16 (br s, 1H, N*H*,) 5.04 (d, 1H, H-1, $J_{1,2} = 4.0$ Hz), 5.06–5.04 (m, 1H, =CH_2), 4.98–4.94 (m, 1H, =CH_2), 4.80 (d, 1H, CH_2Ph, $J = 11$ Hz), 4.71 (d, 1H, CH_2Ph, $J = 11$ Hz), 4.69 (d, 1H, CH_2Ph, $J = 11$ Hz), 4.55 (AB system, 2H, CH_2Ph), 4.45 (d, 1H, CH_2Ph, $J = 12$ Hz), 4.38 (d, 1H, CH_2Ph, $J = 11$ Hz), 4.34 (d, 1H, CH_2Ph, $J = 12$ Hz), 3.86–3.84 (m, 1H, H-5), 3.71–3.60 (m, 4H, H-2, H-3, CH_2N) 338–3.52 (m, 2H, H-6, H-6′), 3.43 (dd, 1H, H-4, $J_{4,3} = J_{4,5} = 5.3$ Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$): 158.51, 138.36, 138.06, 137.69, 137.14, 134.99, 128.58–127.73 (six peaks), 115.44, 81.91, 80.41, 78.32, 78.19, 77.89, 77.43, 75.80, 74.94, 73.45, 72.99, 69.66, 68.63, 42.40; MALDI-TOFMS *m*/*z*: 623.4 ($M+H^+$), 645.4 ($M+Na^+$), 661.4 ($M+K^+$).

4.6. *N*′-Benzyl-*N*-2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl urea (5)

Flash chromatography: 4:1, toluene/EtOAc; yield: 70%; $[\alpha]_D^{25}$ +48.8 (*c* 1.0, $CHCl_3$); IR (nujol): 1652 $\nu_{C=O}$; ^1H NMR (400 MHz, $CDCl_3$): 7.35–7.18 (m, 25H, aromatics), 6.06 (t, 1H, N*H*CH_2, $J = 5.3$ Hz), 5.10 (br s, 1H, N*H*CO), 5.10 (d, 1H, H-1, $J_{1,2} = 4.9$ Hz), 4.84 (d, 1H, CH_2Ph, $J = 11$ Hz), 4.71–4.55 (m, 4H, CH_2Ph), 4.44 (d, 1H, CH_2Ph, $J = 11$ Hz), 4.30 (dd, 1H, CH_2NH, $J = 5.3$ Hz, 14.3 Hz), 4.21–4.18 (m, 2H, CH_2Ph), 4.10 (dd, 1H, CH_2NH $J = 5.3$, 14.3 Hz), 4.02 (dd, 1H, H-2, $J_{2,1} = 4.9$ Hz, $J_{2,3} = 9.5$ Hz), 3.95–3.90 (m, 1H, H-5), 3.74 (br s, 1H, H-4), 3.56 (dd, 1H, H-3, $J_{3,4} = 2.3$ Hz, $J_{2,3} = 9.5$ Hz), 3.34 (dd, 1H, H-6, $J_{6,5} = J_{6,6'} = 9.1$ Hz), 3.21 (dd, 1H, H-6′, $J_{6',5} = 5.1$ Hz, $J_{6,6'} = 9.1$ Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$): 159.4, 139.3, 138.4, 137.8, 137.7, 128.7–727.2 (10 peaks), 79.1, 78.6, 75.2, 74.8, 74.5, 73.6, 73.5, 69.4, 69.2, 44.0; ESIMS *m*/*z*: 673.9 ($M+H^+$), 695.9 ($M+Na^+$), 711.9 ($M+K^+$).

4.7. Synthesis of the *N*,*N*″-butane-1,4-diylbis[(*N*′-2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)urea] (8)

Trimethylphosphine (2.4 equiv, 1 M solution in toluene) was added, at rt and under argon, to a 0.1 M solution of

α-azide **1** (2.2 equiv) in dry CH_2Cl_2. The mixture was stirred for 1 h until the disappearance of the starting material from the TLC plate (4:1, toluene/EtOAc). The 1,4-diisocyanobutane (1 equiv) was added and the solution was stirred for 1.5 h. A 2:1 v/v solution of formic acid and H_2O was then added (5 equiv of formic acid) and the hydrolysis of the carbodiimide was monitored by TLC (4:1, toluene/EtOAc). After 1.5 h, the solution was diluted with CH_2Cl_2 and washed with H_2O, satd aq $NaHCO_3$ and H_2O again. The organic layer was dried (Na_2SO_4) and concentrated. The crude product was purified by flash chromatography (3:1, toluene/EtOAc); yield: 60%; $[\alpha]_D^{25}$ +41.5 (*c* 1.0, $CHCl_3$); IR (nujol): 1651 $\nu_{C=O}$; ^{1}H NMR (400 MHz, $CDCl_3$): 7.30–7.15 (m, 2 × 18H, aromatics), 7.09–7.07 (m, 2 × 2H, aromatics), 5.51 (t, 2H, 2 × N*H*CH_2, $J = 5.3$ Hz), 5.06 (br s, 2H, 2 × N*H*), 5.03 (d, 2H, 2 × H-1, $J = 5.0$ Hz), 4.80 (d, 2H, 2 × CH_2Ph, $J = 11$ Hz), 4.68 (d, 2H, 2 × CH_2Ph, $J = 11$ Hz), 4.66 (d, 2H, 2 × CH_2Ph, $J = 11$ Hz), 4.51 (AB system, 4H, 2 × CH_2Ph), 4.43 (d, 2H, 2 × CH_2Ph, $J = 12$ Hz), 4.38 (d, 2H, 2 × CH_2Ph, $J = 11$ Hz), 4.31 (d, 2H, 2 × CH_2Ph, $J = 12$ Hz), 3.81–3.78 (m, 2H, 2 × H-5), 3.66–3.63 (m, 4H, 2 × H-2, 2H-3), 3.53–3.49 (m, 4H, 2 × H-6, 2 × H-6), 3.40 (dd, 2H, 2 × H-4, $J_{4,2} = J_{4,3} = 9.2$ Hz), 3.03–3.01 (m, 2H, 2 × CH_2NH), 2.94–2.92 (m, 2H, 2 × CH_2NH), 1.33–1.29 (m, 4H, 2 × CH_2CH_2); ^{13}C NMR (100.6 MHz, $CDCl_3$): 158.66, 138.36, 138.06, 137.68, 137.15, 128.56–627.72 (six peaks), 81.90, 78.21, 77.61, 77.46, 75.77, 74.93, 73.46, 72.91, 69.56, 68.78, 39.76, 27.32; MALDI-TOFMS: *m*/*z* 1219.2 (M+H^+), 1241.2 (M+Na^+), 1257.1 (M+K^+).

4.8. General procedure for the debenzylation; synthesis of glycosyl ureas 9–11

A 0.01 M solution of the protected urea in 85:15:5 DMA/AcOH/H_2O was prepared. The catalyst (10% Pd/C, 1:1 w/w with the substrate) was added and the mixture was stirred for 12–36 h under a H_2 atmosphere (3 atm). The reaction was monitored by TLC (4:1 toluene/EtOAc and 60:35:5 $CHCl_3$/CH_3OH/H_2O). The mixture was filtered on a Celite pad, washed several times with CH_3OH and then evaporated to dryness.

4.9. *N*′-Phenyl-*N*-α-D-glucopyranosyl urea (9)

Flash chromatography 64:35:1 $CHCl_3$/MeOH/H_2O, on silica gel; yield = 85%; ^{1}H NMR (400 MHz, CD_3OD): 7.35 (d, 2H, aniline H_o, $J = 8.4$ Hz), 7.21 (7, 2H, aniline H_m, $J = 7.9$ Hz), 6.95 (t, 1H, aniline H_p, $J = 7.9$ Hz), 5.44 (d, 1H, H-1, $J_{1,2} = 5.4$ Hz), 3.77 (dd, 1H, H-6, $J_{5,6} = 1.9$ Hz, $J_{6,6'} = 11.8$ Hz), 3.68–3.60 (m, 2H, H-2, H-6′), 3.53–3.43 (m, 2H, H-3, H-5), 3.27 (dd, 1H, H-4, $J_{4,3} = J_{4,5} = 9.3$ Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$, HETCOR): 128.6, 122.5, 118.9, 78.5, 74.1, 72.6, 70.3, 70.2, 61.5; ESIMS *m*/*z*: 321.3 (M+Na^+).

4.10. *N*′-Propyl-*N*-D-glucopyranosyl urea (10)

Flash chromatography 61:35:4 $CHCl_3$/MeOH/H_2O, on silica gel; yield = 86%; Mixtures of α and β anomers were obtained, with variable composition, depending on the reaction time. The two isomers could be identified in the crude NMR spectra (CD_3OD) by the signals of their anomeric protons at 5.30 ppm (α isomer, $J_{1,2} = 4.0$ Hz) and 4.76 ppm (β isomer, $J_{1,2} = 9.4$ Hz). These two signals correlated in the HETCOR ^{1}H–^{13}C spectrum with the two anomeric carbons (82.0 ppm C-1β and 78.5 ppm C-1α). ESI mass spectrometry showed two peaks at *m*/*z* 287.3 (M+Na^+) and 303.3 (M+K^+), which are consistent with the *N*′-propyl-*N*-D-glucopyranosyl urea structure.

4.11. *N*,*N*′-Butane-1,4-diylbis[*N*′-(α-D-glucopyranosyl)urea] (11)

The compound was purified by gel permeation (Sephadex LH20, CH_3OH) chromatography. An analytical sample was purified by reverse phase HPLC (Xterra™ C18, 95:5 H_2O/CH_3CN with 0.1% TFA); yield: 70%; ^{1}H NMR (400 MHz, CD_3OD): 5.24 (d, 2H, 2 × H-1, $J_{1,2} = 5.4$ Hz), 3.75 (dd, 2H, 2 × H-6, $J_{5,6} = 2.1$ Hz, $J_{6,6'} = 11.4$ Hz), 3.56–3.49 (m, 4H, 2 × H-2 and 2 × H-6′), 3.49–3.45 (m, 2H, 2 × H-5), 3.38 (dd, 2H, 2 × H-3, $J_{2,3} = J_{3,4} = 9.6$ Hz), 3.17–3.08 (m, 6H, 2 × H-4 and 2 × CH_2NH), 1.45–1.42 (m, 4H, 2 × CH_2); ^{13}C NMR (100.6 MHz, $CDCl_3$, HETCOR): 78.7, 73.7, 72.3, 70.5, 70.0, 61.6, 38.8, 26.9; ESIMS *m*/*z*: 521.4 (M+Na^+).

4.12. Synthesis of *N*′-propyl-*N*-2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl urea (12) and *N*′-propyl-*N*-2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl urea (13)

Trimethylphosphine (2.0 equiv, 1 M solution in toluene) was added, at rt and under argon, to a 0.1 M solution of α-azide **1** (1 equiv) in dry CH_2Cl_2. The mixture was stirred for 1.5 h until the disappearance of the starting material from the TLC plate (4:1, toluene/EtOAc). The propylisocyanate (1.2 equiv) was added and the solution was stirred for 1.5 h. A 2:1 v/v solution of formic acid and H_2O was added (5 equiv of formic acid) and the hydrolysis of the carbodiimide was monitored by TLC (4:1, toluene/EtOAc). After 1 h, the solution was diluted with CH_2Cl_2 and washed with H_2O, satd aq $NaHCO_3$ and H_2O again. The organic layer was dried (Na_2SO_4) and concentrated. The crude product was purified by flash chromatography (4:1 toluene/EtOAc).

4.12.1. *N*′-Propyl-*N*-2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl urea (12).

Yield: 54%; ^{1}H NMR (600 MHz, CD_3Cl_3): 7.34–7.29 (m, 18H, aromatics), 7.17–7.14 (m,

2H, aromatics), 5.6 (br s, 1H, N*H*), 5.3 (br s, 1H, N*H*), 5.1 (d, 1H, H-1, $J_{1,2} = 4.6$ Hz), 4.93 (d, 1H, CH_2Ph, $J = 11$ Hz), 4.83 (d, 1H, CH_2Ph, $J = 11$ Hz), 4.79 (d, 1H, CH_2Ph, $J = 11$ Hz), 4.68 (AB system, 2H, CH_2Ph), 4.56 (d, 1H, CH_2Ph, $J = 12$ Hz), 4.50 (d, 1H, CH_2Ph, $J = 11$ Hz), 4.44 (d, 1H, CH_2Ph, $J = 12$ Hz), 3.94–3.92 (m, 1H, H-5), 3.76 (dd, 1H, H_3, $J_{3,2} = J_{3,4} = 9.2$ Hz), 3.71 (dd, 1H, H_2, $J_{2,1} = 4.6$ Hz, $J_{2,3} = 9.2$ Hz), 3.65–3.63 (m, 2H, H-6_6, H-6′), 3.54 (dd, 1H, H-4, $J_{4,3} = J_{4,5} = 9.2$ Hz), 3.30–3.27 (m, 2H, NHCH_2), 1.42–1.38 (m, 2H, CH_2CH$_3$), 0.85 (t, 1H, CH$_2$CH_3, $J = 7.8$ Hz); ^{13}C NMR (125 MHz, $CDCl_3$, HETCOR): 82.5, 79.5, 78.3, 77.9, 76.8, 75.8, 74.1, 73.8, 70.1, 69.0, 42.0, 24.0, 13.0.

4.12.2. *N*′-Propyl-*N*-2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl urea (13). Yield: 17%; ^{1}H NMR (600 MHz, CD_3Cl_3): 7.34–7.29 (m, 18H, aromatics), 7.17–7.14 (m, 2H, aromatics), 5.6 (br s, 1H, N*H*), 5.3 (br s, 1H, N*H*), 4.93 (d, 1H, CH_2Ph, $J = 11$ Hz), 4.86 (d, 1H, CH_2Ph, $J = 11$ Hz), 4.83 (d, 1H, CH_2Ph, $J = 11$ Hz), 4.80 (br s, 1H, H-1, $J_{1,2} = 8.0$ Hz), 4.71 (AB system, 2H, CH_2Ph), 4.58 (d, 1H, CH_2Ph, $J = 12$ Hz), 4.52 (d, 1H, CH_2Ph, $J = 11$ Hz), 4.47 (d, 1H, CH_2Ph, $J = 12$ Hz), 3.76 (dd, 1H, H-3, $J_{3,2} = J_{3,4} = 9.0$ Hz), 3.71 (dd, 1H, H-6, $J_{6,5} = 10.6$ Hz, $J_{6,6'} = 2.1$ Hz), 3.68–3.66 (m, 2H, H-4, H-6′), 3.54 (dd, 1H, H-4, $J_{4,3} = J_{4,5} = 9.2$ Hz), 3.38 (t, 1H, H-2, $J_{2,1} = J_{2,3} = 8.0$ Hz), 3.12–3.02 (m, 2H, NHCH_2), 1.48–8.42 (m, 2H, CH_2CH$_3$), 0.85 (t, 1H, CH$_2$CH_3, $J = 7.8$ Hz).

4.13. *N*′-Phenyl-*N*-2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl urea 14

A solution of urea **9** (0.03 mmol) in dry pyridine (0.3 mL) was prepared. Ac_2O (0.5 mmol) and *N*,*N*-dimethylaminopyridine (0.1 equiv) were added. The solution was stirred at rt for 18 h and the reaction was monitored by TLC (4:1, toluene/EtOAc). The solution was then concentrated, the residue was taken up with CH_2Cl_2 and washed with 5% HCl, 5% $NaHCO_3$ and H_2O. The organic layer was dried (Na_2SO_4) and concentrated. The crude product was purified by flash chromatography (6:4, toluene/EtOAc); yield = 80%; $[\alpha]_D^{25}$ +153.25 (*c* 0.2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): 7.60 (br s, 1H, NHPh), 7.41 (d, 1H, aniline H_o, $J =$ 9 Hz), 7.27 (dd, 1H, aniline H_m, $J = 9$ Hz, $J = 7.6$ Hz), 7.05 (t, 1H, aniline H_p, $J = 7.5$ Hz), 6.00 (br s, 1H, N*H*), 5.70 (dd, 1H, H-1, $J_{1,2} = 5.2$ Hz, $J_{1,NH} = 5.2$ Hz), 5.40 (dd, H-3, $J_{3,2} = J_{3,4} = 10$ Hz), 5.16 (dd, H-2, $J_{1,2} =$ 5.2 Hz, $J_{2,3} = 10.2$ Hz), 5.07 (t, 1H, H-4, $J = 9.6$ Hz), 4.22–4.19 (m, 3H, H-5, H-6, H-6′), 2.07 (s, 3H, CH_3CO), 2.03 (s, 6H, CH_3CO), 2.00 (s, 3H, CH_3CO); ^{13}C NMR (100.6 MHz, $CDCl_3$): 170.54, 170.04, 169.48, 169.13, 155.00, 137.81, 129.10, 124.02, 119.98, 77.33, 69.78, 68.84, 68.22, 67.66, 61.92, 20.56; ESIMS: *m*/*z* calcd for $[C_{21}H_{26}N_2O_{10}]Na^+$: 489.14797. Found: 489.14627; *m*/*z* calcd for $2[C_{21}H_{26}N_2O_{10}]Na^+$: 955.30671. Found: 955.31064.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2006.03.042.

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