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Note

5′-Uridyl derivatives of *N*-glycosyl allophanic acid and biuret

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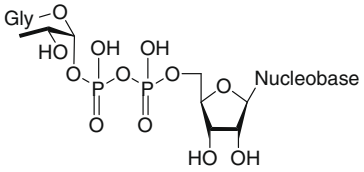
ABSTRACT

(2′,3′-*O*-Isopropylidene-5′-uridyl) 4-(2,3,4,6-tetra-*O*-acetyl-β-D-glycopyranosyl)allophanates were obtained in the reactions of 2′,3′-*O*-isopropylidene-uridine and O-peracetylated β-D-gluco-, galacto-and xylo pyranosylamines, and OCNCOCl. 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl isocyanate and *N*-(2′,3′-*O*-isopropylidene-5′-uridyl)urea gave 1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-5-(2′,3′-*O*-isopropylidene-5′-uridyl)biuret. Deprotection of the β-D-*gluco* configured allophanate and biuret was carried out by standard methods.

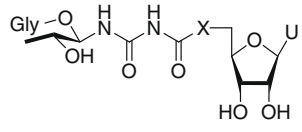
Nucleoside diphosphate (NDP) sugars (Chart 1) are natural substrates for Leloir type glycosyltransferases.[1] A large variety of molecular structures have been designed, synthesized and tested as inhibitors of these enzymes,[2–4] fuelled by the need of suitable means to elucidate the mechanism of action of such catalysts.[5] Efficient inhibitors also have the potential to be further developed to drug candidates.[6] Because of some similarities between allophanic acid and biuret derivatives and the pyrophosphate moiety (cf. target compounds in Chart 1), we have carried out some reactions to study the possibility of attaching β-D-glycosyl residues and uridine via these linkers. The experiences of these model studies can then be applied to prepare similar α-D-glycosyl derivatives to mimic NDP sugars.

A simple way to assemble allophanates is the reaction of the commercially available bielectrophilic reagent chlorocarbonyl isocyanate[7] (OCNCOCl) with the corresponding nucleophiles. Thus, the reaction of isopropylidene uridine **1**[8] and β-D-glucopyranosylamine **6**[9] with OCNCOCl was investigated first (Scheme 1 and Table 1). The expectedly less nucleophilic alcohol **1** was reacted with OCNCOCl in the first step in order to possibly avoid the formation of symmetric products[10] like **12**. However, under slow addition of **1** to OCNCOCl in THF (Table 1, entries 1 and 2) followed by **6**, this was not achieved, and in addition to the desired allophanate **9**, iminodicarboxylate **12** and carbamate **14**[11] were also obtained. Appearance of **14** could be due to the presence of traces of water in the reaction mixture. Addition of OCNCOCl in one portion to **1** and keeping the mixture at a low temperature (Table 1, entry 3) allowed the avoidance of the formation of by-products **12** and **14** in significant amounts, and **9** was isolated in 81% yield. Under the same conditions **1** was also reacted with glycosylamines **7**[12] and **8**[13,14] to give 70% and 68% yields of the corresponding allophanates **10** and **11**, respectively.

For the preparation of biurets an analogous route was envisaged by using 5′-amino-5′-deoxy-uridine derivative **5** and glycosylamines **6**–**8** with OCNCOCl. To this end, **1** was transformed into azide **4**,[15,16] via a 7:3 mixture of tosylate **2**[16] and chloride **3**[17] obtained on tosylation of **1**, according to published protocols. For the reduction of **4** to **5**, contradictory reports can be found in the literature: hydrogenation[15] with Pd–C in CH_3OH was reported to be succes-

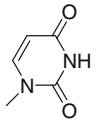


NDP sugars



X = O, NH

U =



Target compounds

Chart 1.

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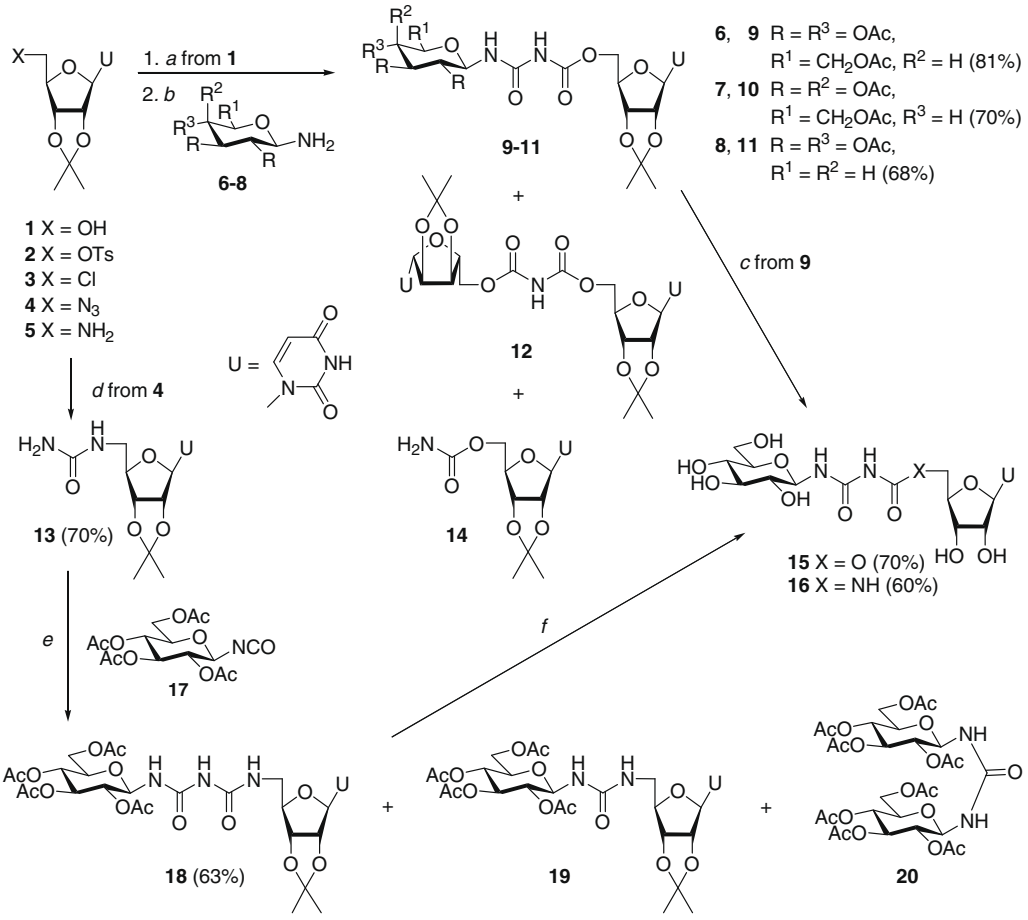

Scheme 1. Reagents and conditions: (a) THF soln of 1 equiv of OCNCOCl added in one portion to **1** in THF, −26 °C, 1 d; (b) THF soln of 1 equiv of **6**–**8** and 1 equiv of Et_3N added dropwise to the above-mentioned soln, 0 °C to rt; (c) AcCl, CH_3OH, rt; (d) PPh_3, NH_3, CO_2, EtOAc, rt; (e) **17**, toluene, reflux, 1 d; (f) (1) cat. $NaOCH_3$, CH_3OH, rt, (2) CF_3COOH (90%), rt.

Table 1
Reactions of 2′,3′-isopropylidene uridine (**1**) and 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylamine (**6**) with OCNCOCl

Entry	Reaction conditions[a]	Isolated yield (%)		
		9	**12**	**14**
1	1. THF soln of **1** added dropwise to OCNCOCl in THF, −20 to 0 °C, 2 h 2. THF soln of **6** and Et_3N added dropwise to the above-mentioned soln, 0 °C to rt	45	10	23
2	1. THF soln of **1** added dropwise to OCNCOCl in THF, −26 °C, 1 d 2. THF soln of **6** and Et_3N added dropwise to the above-mentioned soln, 0 °C	10	24	14
3	1. THF soln of OCNCOCl added in one portion to **1** in THF, −26 °C, 1 d 2. THF soln of **6** and Et_3N added dropwise to the above-mentioned soln, 0 °C to rt	81	[b]	[b]

[a] Two-step, one-pot procedures with added molecular sieves under N_2 atm: step 1 is the reaction of **1** and OCNCOCl, step 2 is the reaction of the intermediate formed in step 1 with **6**.
[b] Observed on TLC, but not isolated.

ful;[16,18,19] however, in another report **4** was found to be 'unreactive to all reduction conditions tested, including Staudinger conditions.[20] In our experiments no discrete product could be isolated under catalytic hydrogenation over Pd–C, and in Ph_3P or $(CH_3)_3P$-mediated reductions. Therefore, we have investigated another possibility to produce the desired biuret by reaction of 5′-uridyl urea **13** and crystalline glucosyl isocyanate[21] **17**. Urea **13** was prepared from **4** by applying Staudinger-type conditions[22] (PPh_3, NH_3, CO_2). In this way, **13** could be obtained in 70% yield, contrary to experiments where commercial NH_4OCONH_2 was used in the presence of either Ph_3P or $(CH_3)_3P$ which gave only ~50% of **13**. Equimolar amounts of compounds **13** and **17** showed no reaction in EtOAc at rt over one day. Boiling the mixture for two days resulted in 34% conversion of **13**, and the expected biuret **18** was isolated in 81% yield together with urea **19** (11%). The conversion of **13** was complete in one day in the presence of 2.2 equiv of **17** in boiling toluene, and **18** (63%) and some **19** (4%) were isolated. The formation of **19** can be due to the presence of traces of water in the reaction mixtures. In each of the above-mentioned reactions, bis-glucopyranosyl urea **20** was also isolated in various amounts that could be attributed to some traces of water present in the mixtures.[23]

Deprotected derivatives were obtained by treating allophanate **9** with AcCl in CH_3OH to give **15** (70%), and biuret **18** with a catalytic amount of $NaOCH_3$ in CH_3OH in the first step and CF_3COOH (90%) in the second step to furnish **16** (60%).

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With the availability of 5′-uridyl-carbamate **14**[11,24] and the propensity of carbamates to react with isocyanates[25], an alternative pathway opens for allophanates of types **9**–**11**. Since α-D-glycosyl-isocyanates[23] can be synthesized in a stereoselective manner, the reactions of urea **13** and carbamate **14** can expectedly be extended to those isocyanate derivatives as well to obtain mimics of UDP sugars.

1. Experimental

1.1. General methods

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 Avance DRX 500 (500/125 MHz for $^1H/^{13}C$) spectrometers. Chemical shifts are referenced to internal $(CH_3)_4Si$ (^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. 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). OCNCOCl was purchased from Sigma–Aldrich.

1.2. General procedure for the synthesis of (2′,3′-*O*-isopropylidene-5′-uridyl) 4-(2,3,4,6-tetra-*O*-β-D-glycopyranosyl)allophanates (9–11)

2′,3′-*O*-Isopropylidene-uridine[8] (**1**, 100 mg, 0.35 mmol) was dissolved in dry THF (2 mL) under N_2 atm and cooled to −26 °C. Some freshly heated molecular sieves were added and after ~5 min stirring OCNCOCl (28 μL, 0.35 mmol) was added in one portion. The reaction mixture was kept at −26 °C for a day. Then a solution of a O-peracetylated β-D-glycopyranosylamine (**6** or **7** or **8**, 0.35 mmol) and Et_3N (49 μL, 0.35 mmol) in dry THF (2 mL) was added dropwise at 0 °C. The reaction mixture was allowed to warm to rt. When the reaction was complete (TLC 10:1 EtOAc/hexane), the mixture was concentrated under reduced pressure and the residue was purified by column chromatography (5:1 EtOAc/hexane).

1.3. (2′,3′-*O*-Isopropylidene-5′-uridyl) 4-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)allophanate (9)

Prepared by the general procedure (Section 1.2) from 2′,3′-*O*-isopropylidene-uridine **1** (100 mg, 0.35 mmol) to give 200 mg (81%) of **9** as a colourless syrup. R_f = 0.32 (15:1 EtOAc/hexane); $[\alpha]_D$ −41 (*c* 1.0, CH_2Cl_2); ^1H NMR (DMSO-d_6): δ (ppm) 11.42 (br s, 1H, NH), 10.46 (br s, 1H, NH), 8.20 (d, 1H, $J_{1\text{-Glc, NH}}$ = 9.5 Hz, NH), 7.65 (d, 1H, *J* = 8.3 Hz, CH-uracyl), 5.80 (d, 1H, $J_{1,2\text{-U}}$ = 2.3 Hz, H-1-U), 5.59 (d, 1H, *J* = 8.3 Hz, CH-uracyl), 5.42 (pseudo t, 1H, $J_{3,4\text{-Glc}}$ = 9.6 Hz, H-3-Glc), 5.38 (pseudo t, 1H, $J_{1,2\text{-Glc}}$ = 9.7 Hz, H-1-Glc), 5.07 (dd, 1H, $J_{2,3\text{-U}}$ = 6.3 Hz, H-2-U), 4.93 (pseudo t, 2H, $J_{2,3\text{-Glc}}$ = 9.5 Hz, H-2-Glc, H-4-Glc), 4.79 (dd, 1H, $J_{3,4\text{-U}}$ = 2.2 Hz, H-3-U), 4.35–4.23 (m, 3H, H-4-U, H-5a-U, H-5b-U), 4.16–3.92 (m, 3H, H-5-Glc, H-6a-Glc, H-6b-Glc), 2.00, 1.99, 1.98, 1.95 (4br s, 12H, 4 × $COCH_3$), 1.48, 1.29 (2br s, 6H, 2 × CH_3-isopropylidene); ^{13}C NMR ($CDCl_3$): δ (ppm) 170.7, 170.2, 169.8, 169.3 (*C*OCH3), 163.5, 151.1 (2 CO-uracyl), 153.1, 153.0 (2 NHCO), 143.8, 102.4 (2 CH-uracyl), 113.9 (C-isopropylidene), 97.2, 87.0, 84.7, 82.1, 78.6, 73.1, 70.0, 68.0 (C-1-U to C-4-U, C-1-Glc to C-5-Glc), 66.4, 61.7 (C-5-U, C-6-Glc), 27.0, 25.4 (CH_3-isopropylidene), 20.6, 20.4 ($COCH_3$); Anal. Calcd for $C_{28}H_{36}N_4O_{17}$ (700.62): C, 48.00; H, 5.18; N, 8.00. Found: C, 48.09; H, 5.27; N, 7.93.

1.4. (2′,3′-*O*-Isopropylidene-5′-uridyl) 4-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)allophanate (10)

Prepared by the general procedure (Section 1.2) from 2′,3′-*O*-isopropylidene-uridine **1** (100 mg, 0.35 mmol) to give 171 mg (70%) of **10** as a colourless syrup. R_f = 0.36 (10:1 EtOAc/hexane); $[\alpha]_D$ −7 (*c* 0.9, $CHCl_3$); ^1H NMR (DMSO-d_6): δ (ppm) 11.41 (br s, 1H, NH), 10.45 (br s, 1H, NH), 8.21 (d, 1H, $J_{1\text{-Gal, NH}}$ = 9.6 Hz, NH), 7.65 (d, 1H, *J* = 8.1 Hz, CH-uracyl), 5.80 (d, 1H, $J_{1,2\text{-U}}$ = 2.3 Hz, H-1-U), 5.59 (d, 1H, *J* = 8.1 Hz, CH-uracyl), 5.34 (dd, 1H, $J_{3,4\text{-Gal}}$ = 3.5 Hz, H-3-Gal), 5.33 (pseudo t, 1H, $J_{1,2}$ = 10.0 Hz, H-1-Gal), 5.28 (pseudo d, 1H, H-4-Gal), 5.07 (dd, 1H, $J_{2,3\text{-U}}$ = 6.3 Hz, H-2-U), 4.98 (pseudo t, 1H, $J_{2,3\text{-Gal}}$ = 9.8 Hz, H-2-Gal), 4.80 (dd, 1H, $J_{3,4\text{-U}}$ = 2.8 Hz, H-3-U), 4.34–4.25 (m, 4H, H-4-U, H-5a-U, H-5b-U, H-5-Gal), 4.02 (dd, 1H, $J_{5,6a\text{-Gal}}$ = 6.2, $J_{6a,6b\text{-Gal}}$ = 11.3 Hz, H-6a-Gal), 3.98 (dd, 1H, $J_{5,6b\text{-Gal}}$ = 6.8 Hz, H-6b-Gal), 2.13, 2.01, 1.99, 1.93 (4br s, 12H, 4 × $COCH_3$), 1.49, 1.30 (2br s, 6H, 2 × CH_3-isopropylidene); ^{13}C NMR ($CDCl_3$): δ (ppm) 170.5, 170.3, 170.1, 169.8 (*C*OCH_3), 163.7, 150.6 (2 CO-uracyl), 153.8, 153.4 (2 NHCO), 142.4, 102.3 (2 CH-uracyl), 114.1 (C-isopropylidene), 95.8, 85.0, 84.7, 81.6, 79.0, 71.9, 70.9, 67.7, 67.1 (C-1-U to C-4-U, C-1-Glc to C-5 Glc), 65.7, 61.1 (C-5-U, C-6-Glc), 27.0, 25.4 (CH_3-isopropylidene), 20.6, 20.5, 20.4 (*C*O*C*H_3); Anal. Calcd for $C_{28}H_{36}N_4O_{17}$ (700.62): C, 48.00; H, 5.18; N, 8.00. Found: C, 48.05; H, 5.24; N, 7.96.

1.5. (2′,3′-*O*-Isopropylidene-5′-uridyl) 4-(2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl)allophanate (11)

Prepared by the general procedure (Section 1.2) from 2′,3′-*O*-isopropylidene-uridine **1** (100 mg, 0.35 mmol) to give 150 mg (68%) of **11** as a colourless syrup. R_f = 0.25 (5:1 EtOAc/hexane); $[\alpha]_D$ −17 (*c* 0.9, $CHCl_3$); ^1H NMR (DMSO-d_6): δ (ppm) 8.22 (d, 1H, $J_{1\text{-Xyl, NH}}$ = 9.4 Hz, NH), 7.64 (d, 1H, *J* = 8.1 Hz, CH-uracyl), 5.79 (d, 1H, $J_{1,2\text{-U}}$ = 2.5 Hz, H-1-U), 5.58 (d, 1H, *J* = 8.0 Hz, CH-uracyl), 5.31 (pseudo t, 1H, $J_{3,4\text{-Xyl}}$ = 9.1 Hz, H-3-Xyl), 5.21 (pseudo t, 1H, $J_{1,2\text{-Xyl}}$ = 9.2 Hz, H-1-Xyl), 5.06 (dd, 1H, $J_{2,3\text{-U}}$ = 6.3 Hz, H-2-U), 4.91 (pseudo t, 1H, $J_{2,3\text{-Xyl}}$ = 9.0 Hz, H-2-Xyl), 4.85 (ddd, 1H, $J_{4,5b\text{-Xyl}}$ = 9.7 Hz, H-4-Xyl), 4.79 (dd, 1H, $J_{3,4\text{-U}}$ = 3.2 Hz, H-3-U), 4.32 (dd, 1H, $J_{4,5a\text{-U}}$ = 2.7, H-5a-U), 4.28 (ddd, 1H, $J_{4,5b\text{-U}}$ = 5.3 Hz, H-4-U), 4.25 (dd, $J_{5a,5b\text{-U}}$ = 10.9 Hz, H-5-Ub), 3.89 (dd, 1H, $J_{4,5a\text{-Xyl}}$ = 5.3 Hz, H-5a-Xyl), 3.56 (pseudo t, $J_{5a,5b\text{-Xyl}}$ = 11.3 Hz, H-5b-Xyl), 1.99, 1.98, 1.97 (3br s, 9H, 4 × $COCH_3$), 1.48, 1.29 (2br s, 6H, 2 × CH_3-isopropylidene); ^{13}C NMR ($CDCl_3$): δ (ppm) 170.3, 169.8, 169.7 (*C*OCH_3), 163.9, 150.5 (2 CO-uracyl), 153.8, 153.3 (2 NHCO), 142.5, 102.1 (2 CH-uracyl), 114.0 (C-isopropylidene), 95.9, 85.3, 84.7, 81.5, 78.9, 71.8, 70.0, 68.7 (C-1-U to C-4-U, C-1-Gal to C-5-Gal), 65.8, 63.8 (C-5-U, C-6-Gal), 27.0, 25.4 (CH_3-isopropylidene), 20.6, 20.5 (*C*O*C*H_3); Anal. Calcd for $C_{25}H_{32}N_4O_{15}$ (628.54): C, 47.77; H, 5.13; N, 8.91. Found: C, 47.81; H, 5.19; N, 8.99.

1.6. *O,O*′-Bis-(2′,3′-*O*-isopropylidene-5′-uridyl) iminodicarboxylate (12)

Prepared by the general procedure (Section 1.2). Colourless syrup. R_f = 0.12 (15:1 EtOAc/hexane); $[\alpha]_D$ −36 (*c* 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ (ppm) 9.93 (br s, 2H, 2 × NH-uracyl), 8.59 (br s, 1H,OOCN*H*COO), 7.44 (d, 2H, *J* = 8.0 Hz, 2 × CH-uracyl), 5.80 (d, 2H, *J* = 8.0 Hz, 2 × CH-uracyl), 5.70 (d, 2H, $J_{1,2}$ = 1.9 Hz, 2 × H-1), 5.13 (dd, 2H, $J_{2,3}$ = 6.5 Hz, 2 × H-2), 4.92 (dd, 2H, $J_{3,4}$ = 3.4 Hz, 2 × H-3), 4.52–4.36 (m, 6H, 2 × H-4, 2 × H-5, 2 × H-5′), 1.59, 1.38 (2br s, 12H,4 × CH_3); ^{13}C NMR ($CDCl_3$): δ (ppm) 163.9, 150.3 (2 CO-uracyl), 151.0 (OO*C*NH*C*OO), 142.8, 102.5 (2 CH-uracyl), 114.5 (C-isopropylidene), 94.8 (C-1), 84.7, 84.4, 80.6 (C-2, C-3, C-4),

65.3 (C-5), 27.1, 25.3 (CH_3); Anal. Calcd for $C_{26}H_{31}N_5O_{14}$ (637.55): C, 48.98; H, 4.90; N, 10.98. Found: C, 49.05; H, 4.97; N, 11.02.

1.7. *N*-(2′,3′-*O*-Isopropylidene-5′-uridyl)urea (13)

Gaseous NH_3 (dried with KOH) and CO_2 (dried with $CaCl_2$) were bubbled through dry EtOAc (1.5 mL) for 15 min; ammonium carbamate was formed as a white solid. Then a solution of 5′-azido-5′deoxy-2′,3′-*O*-isopropylidene-uridine[16] (**4**, 100 mg, 0.32 mmol) in dry EtOAc (1 mL) followed by a solution of Ph_3P (95 mg, 0.36 mmol) in dry EtOAc (2 mL) was added. The reaction mixture was stirred at rt. When the reaction was complete (TLC, 7:3 $CHCl_3/CH_3OH$), the solvent was removed by evaporation under reduced pressure. The residue was purified by column chromatography (4:1 $CHCl_3/CH_3OH$) to give **11** as a colourless oil (74 mg, 70%). R_f = 0.38 (7:3 $CHCl_3/CH_3OH$); $[\alpha]_D$ +6 (*c* 1.1, CH_3OH); ^1H NMR (DMSO-d_6): δ (ppm) 7.71 (d, 1H, *J* = 8.0 Hz, CH-uracyl), 6.18 (t, 1H, $J_{NH,5a}$ = 5.8 Hz, $J_{NH,5b}$ = 5.8 Hz, NH), 5.76 (d, 1H, $J_{1,2}$ = 2.8 Hz, H-1), 5.63 (d, 1H, *J* = 8.0 Hz, CH-uracyl), 5.52 (br s, 2H, NH_2), 5.00 (dd, 1H, $J_{2,3}$ = 6.6 Hz, H-2), 4.67 (dd, 1H, $J_{3,4}$ = 4.1 Hz, H-3), 3.97–3.91 (m, 1H, $J_{4,5a}$ = 6.0 Hz, $J_{4,5b}$ = 6.9 Hz, H-4), 3.34–3.28 (m, 1H, H-5a), 3.17–3.10 (m, 1H, $J_{5a,5b}$ = 13.3 Hz, H-5b), 1.47, 1.27 (2br s, 6H, CH_3); ^{13}C NMR (DMSO-d_6): δ (ppm) 163.2, 150.2 (2 CO-uracyl), 158.5 (NHCONH), 142.8, 101.8 (2 CH-uracyl), 113.3 (C-isopropylidene), 91.7 (C-1), 85.4, 83.4, 81.3 (C-2, C-3, C-4), 41.2 (C-5), 26.9, 25.1 (CH_3); Anal. Calcd for $C_{13}H_{18}N_4O_6$ (326.31): C, 47.85; H, 5.56; N, 17.17. Found: C, 47.92; H, 5.63; N, 17.21.

1.8. *O*-(2′,3′-*O*-Isopropylidene-5′-uridyl)carbamate (14)

Prepared by the general procedure (Section 1.2). Colourless syrup. R_f = 0.23 (15:1 EtOAc/hexane); $[\alpha]_D$ +36 (*c* 0.8, $CHCl_3$); lit.[11] mp 184–186 °C, lit.[24] 178–179 °C. ^1H NMR ($CDCl_3$): δ (ppm) 9.78 (s, 1H, NH), 7.30 (d, 1H, *J* = 8.0 Hz, CH-uracyl), 5.72 (d, 1H, *J* = 8.0 Hz, CH-uracyl), 5.60, (d, 1H, $J_{1,2}$ = 2.1 Hz, H-1), 5.25 (br s, 2H, NH_2), 5.08 (dd, 1H, $J_{2,3}$ = 6.6 Hz, H-2), 4.84 (dd, 1H, $J_{3,4}$ = 3.1 Hz, H-3), 4.42–4.28 (m, 3H, H-4, H-5a, H-5b), 1.57, 1.36 (2br s, 6H, CH_3); The ^1H NMR spectrum recorded in DMSO-d_6 fully corresponded to the reported data.[11] ^{13}C NMR ($CDCl_3$): δ (ppm) 163.4, 150.0 (2 CO-uracyl), 156.5 (NHCOO), 142.4, 102.4 (2 CH-uracyl), 114.4 (C-isopropylidene), 95.7 (C-1), 85.8, 84.6, 81.2 (C-2, C-3, C-4), 64.8 (C-5), 27.1, 25.2 (CH_3).

1.9. 5′-Uridyl 4-(β-D-glucopyranosyl)allophanate (15)

Allophanate **9** (100 mg, 0.14 mmol) was dissolved in dry CH_3OH (2 mL) and one drop of AcCl was added. The reaction mixture was stirred at rt. The precipitate formed was filtered off to yield **15** as an amorphous solid (50 mg, 71%). R_f = 0.65 (1:3 $CHCl_3/CH_3OH$); $[\alpha]_D$ −4 (*c* 0.4, H_2O); ^1H NMR (DMSO-d_6 + D_2O); δ (ppm) 7.65 (d, 1H, *J* = 8.0 Hz, CH-uracyl), 5.81 (d, 1H, *J* = 5.8 Hz), 5.68 (d, 1H, *J* = 8.0 Hz, CH-uracyl), 4.72 (d, 1H, *J* = 9.0 Hz), 4.31–4.28 (m, 2H), 4.16 (pseudo t, 1H, *J* = 5.6 Hz, 5.9 Hz), 4.05 (dd, 1H, *J* = 3.4 Hz, 7.2 Hz), 3.97 (pseudo t, 1H, *J* = 4.5 Hz, 4.7 Hz), 3.43 (dd, 1H, *J* = 5.3 Hz, 11.9 Hz), 3.22–2.99 (m, 5H); ^{13}C NMR (D_2O): δ (ppm) 162.4, 147.8 (2 CO-uracyl), 151.5, 151.4 (2 NHCO), 138.0, 98.4 (2 CH-uracyl), 85.5, 80.3, 76.4, 73.6, 72.5, 69.8, 68.0, 65.5, 65.4 (C-1-U to C-4-U, C-1-Glc to C-5-Glc), 56.9, 56.7 (C-5-U, C-6-Glc); Anal. Calcd for $C_{17}H_{24}N_4O_{13}$ (492.40): C, 41.47; H, 4.91; N, 11.38. Found: C, 41.52; H, 5.00; N, 11.42.

1.10. 1-(β-D-Glucopyranosyl)-5-(5′-uridyl)biuret (16)

Biuret **18** (100 mg, 0.14 mmol) was dissolved in dry CH_3OH (1 mL), and a solution of $NaOCH_3$ (1 M in CH_3OH) was added to the solution in a catalytic amount. The reaction mixture was kept at rt. When the reaction was complete (TLC, 7:3 $CHCl_3/CH_3OH$), the solution was neutralized with a cation exchange resin Amberlyst 15 (H^+ form). Filtration and removal of the solvent resulted in 76 mg crude product, which was dissolved in 0.5 mL TFA/water (9:1) and stirred at rt. When the reaction was complete (TLC, 1:2 $CHCl_3/CH_3OH$), the solution was diluted with water and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography (1:2 $CHCl_3/CH_3OH$) to yield **16** as a colourless oil (42 mg, 60%). R_f = 0.37 (1:2 $CHCl_3/CH_3OH$); $[\alpha]_D$ +24 (*c* 0.3, DMSO); ^1H NMR (D_2O); δ (ppm) 7.66 (d, 1H, *J* = 7.9 Hz), 5.87 (d, 1H, *J* = 7.9 Hz), 5.81 (d, 1H, *J* = 3.5 Hz), 4.91 (d, 1H, *J* = 9.5 Hz), 4.37 (pseudo t, 1H, *J* = 3.8 Hz, 4.2 Hz), 4.18–4.08 (m, 2H), 3.88 (d, 1H, *J* = 10.7 Hz), 3.72 (dd, 1H, *J* = 5.6 Hz, 11.8 Hz), 3.68–3.39 (m, 6H); ^{13}C NMR (D_2O): δ (ppm) 166.8, 156.5, 152.1 (2 CO-uracyl, 2 NHCO), 142.8, 102.9 (2 CH-uracyl), 91.1, 82.6, 80.9, 78.0, 77.1, 73.8, 72.5, 70.8, 69.9 (C-1-U to C-4-U, C-1-Glc to C-5-Glc), 61.2, 40.8 (C-5-U, C-6-Glc); Anal. Calcd for $C_{17}H_{25}N_5O_{12}$ (491.41): C, 41.55; H, 5.13; N, 14.25. Found: C, 41.58; H, 5.17; N, 14.32.

1.11. 1-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-5-(2′,3′-*O*-isopropylidene-5′-uridyl)biuret (18)

Urea **13** (100 mg, 0.306 mmol) was dissolved in dry toluene (6 mL), then some freshly heated molecular sieves and crystalline isocyanate **17**[21] (252 mg, 0.675 mmol) were added. The reaction mixture was stirred at reflux temperature until the reaction was complete (~1 day, TLC, 10:1 EtOAc/hexane). Then the molecular sieves were filtered off with suction and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography (1:1 to 5:1 EtOAc/hexane). Three products were isolated in the order of elution: **20** (122 mg), **19** (9 mg, 4%) and **18** (134 mg, 63%).

Characterization of **18**: Colourless oil; R_f = 0.13 (5:1 EtOAc/hexane); $[\alpha]_D$ −38 (*c* 1.0, $CHCl_3$); ^1H NMR (DMSO-d_6): δ (ppm) 11.44 (br s, 1H, NH), 8.93 (br s, 1H, NH), 8.02 (d, 1H, $J_{1\text{-}Glc,\ NH}$ = 10.2 Hz, NH), 7.70 (d, 1H, *J* = 8.4 Hz, CH-uracyl), 7.55 (br s, 1H, NH), 5.78 (d, 1H, $J_{1,2\text{-}U}$ = 1.9 Hz, H-1-U), 5.64 (d, 1H, *J* = 8.4 Hz, CH-uracyl), 5.41 (pseudo t, 1H, $J_{3,4\text{-}Glc}$ = 9.6 Hz, H-3-Glc), 5.33 (pseudo t, 1H, $J_{1,2\text{-}Glc}$ = 9.2 Hz, H-1-Glc), 5.02 (dd, 1H, $J_{2,3\text{-}U}$ = 6.5 Hz, H-2-U), 4.92 (pseudo t, 1H, $J_{4,5\text{-}Glc}$ = 9.5 Hz, H-4-Glc), 4.85 (pseudo t, 1H, $J_{2,3\text{-}Glc}$ = 9.5 Hz, H-2-Glc), 4.70 (dd, 1H, $J_{3,4\text{-}U}$ = 4.5 Hz, H-3-U), 4.16–3.95 (m, 4H, H-4-U, H-5a-U, H-5b-U, H-5-Glc), 3.46–3.30 (m, 2H, H-6a-Glc, H-6b-Glc), 2.00, 1.99, 1.95 (3br s, 12H, 4 × $COCH_3$), 1.47, 1.28 (2br s, 6H, 2 × CH_3-isopropylidene); ^{13}C NMR ($CDCl_3$): δ (ppm) 170.7, 170.1, 169.5 ($COCH_3$), 163.7, 154.9, 154.8 (2 CO-uracyl, 2 NHCO), 143.8, 102.7 (2 CH-uracyl), 114.4 (C-isopropylidene), 86.3, 84.0, 81.5, 78.8, 73.2, 73.0, 70.1, 68.0 (C-1-U to C-4-U, C-1-Glc to C-5-Glc), 61.6, 41.1 (C-5-U, C-6-Glc), 27.1, 25.3 (CH_3-isopropylidene), 20.7, 20.6, 20.5 ($COCH_3$); Anal. Calcd for $C_{28}H_{37}N_5O_{16}$ (699.63): C, 48.07; H, 5.33; N, 10.01. Found: C, 48.12; H, 5.25; N, 9.93.

1.12. 1-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-3-(2′,3′-*O*-isopropylidene-5′-uridyl)urea (19)

Isolated from the reaction described in Section 1.11. Colourless oil; R_f = 0.50 (5:1 EtOAc/hexane); $[\alpha]_D$ +28 (*c* 0.4, $CHCl_3$); ^1H NMR (DMSO-d_6): δ (ppm) 11.41 (br s, 1H, NH), 7.74 (d, 1H, *J* = 8.3 Hz, CH-uracyl), 7.01–6.95 (m, 2H, 2 × NH), 5.78 (d, 1H, $J_{1,2\text{-}U}$ = 1.8 Hz, H-1-U), 5.63 (d, 1H, *J* = 8.0 Hz, CH-uracyl), 5.33 (pseudo t, 1H, $J_{3,4\text{-}Glc}$ = 9.4 Hz, H-3-Glc), 5.21 (pseudo t, 1H, $J_{1,2\text{-}Glc}$ = 9.5 Hz, H-1-Glc), 5.07 (dd, 1H, $J_{2,3\text{-}U}$ = 6.5 Hz, H-2-U), 4.89 (pseudo t, 1H, $J_{3,4\text{-}Glc}$ = 9.5 Hz, H-4-Glc), 4.80 (pseudo t, 1H, $J_{2,3\text{-}Glc}$ = 9.5 Hz, H-4-Glc), 4.73 (dd, 1H, $J_{3,4\text{-}U}$ = 4.2 Hz, H-3-U), 4.12 (dd, 1H, $J_{5',6a}$ = 4.9 Hz, $J_{6a,6b}$ = 12.3 Hz, H-6a-Glc), 4.05–3.94 (m, 3H, H-5-Glc, H-4-U, H-6b-Glc), 3.25–3.14 (m, 2H, H-5a-U, H-5b-U), 1.99, 1.98, 1.96, 1.93 (4br s, 12H, 4 × $COCH_3$), 1.49, 1.29 (2br s, 6H, 2 × CH_3-isopropylidene); ^{13}C NMR ($CDCl_3$): δ

(ppm) 171.5, 170.6, 170.0, 169.4 ($COCH_3$), 162.7, 150.4 (2 CO-uracyl), 151.8 (NHCONH), 143.8, 103.0 (2 CH-uracyl), 114.4 (C-isopropylidene), 97.3, 86.5, 84.5, 81.4, 80.3, 73.7, 72.7, 70.4, 68.0 (C-1-U to C-4-U, C-1-Glc to C-5-Glc), 61.5, 47.3 (C-5-U, C-6-Glc), 27.2, 25.3 (CH_3-isopropylidene), 20.8, 20.7 ($COCH_3$); Anal. Calcd for $C_{27}H_{36}N_4O_{15}$ (656.59): C, 49.39; H, 5.53; N, 8.53. Found: C, 49.44; H, 5.59; N, 8.60.

1.13. *N,N'*-Bis-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)urea (20)

Isolated from the reaction described in Section 1.11. White crystals; mp 152–153 °C; lit.[23] mp 153 °C. ^{1}H NMR ($CDCl_3$): δ (ppm) 6.21 (d, 2H, $J_{1,\ NH}$ = 9.2 Hz, NH), 5.30, 5.12, 5.05, 4.90 (4 pseudo t, 8H, J = 9.2 Hz, 10.1 Hz in each, H-1, H-2, H-3, H-4), 4.32 (dd, 2H, $J_{5,6a}$ = 4.5 Hz, $J_{6a,6b}$ = 12.3 Hz, H-6a), 4.10 (dd, 2H, $J_{5,6b}$ = 1.7 Hz, H-6b), 3.85 (ddd, 2H, $J_{4,5}$ = 10.1 Hz, H-5), 2.08, 2.07, 2.04, 2.02 (4br s, 24H, 8 × $COCH_3$); ^{13}C NMR ($CDCl_3$): δ (ppm) 171.0, 170.6, 169.9, 169.6 ($COCH_3$), 155.7 (CO-urea), 79.8, 73.1, 72.8, 70.4, 68.2 (C-1 to C-5), 61.7 (C-6), 20.6, 20.5, 20.4 ($COCH_3$). The NMR data correspond to the published ones.[23]

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