Carbohydrate Research 344 (2009) 586–591

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

# Probing replacement of pyrophosphate via click chemistry; synthesis of UDP-sugar analogues as potential glycosyl transferase inhibitors

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ARTICLE INFO

*Article history:*
Received 24 September 2008
Received in revised form 11 December 2008
Accepted 6 January 2009
Available online 17 January 2009

*Keywords:*
Glycosyl transferases
UDP-sugars
Click chemistry
Triazoles
Huisgen cycloaddition

ABSTRACT

A series of potential UDP-sugar mimics were readily synthesised by copper(I) catalysed modified Huisgen cycloaddition of the corresponding α-propargyl glycosides with 5-azido uridine in aqueous solution. None of the compounds accessed displayed significant inhibitory activity at concentrations of up to 4.5 mM in an assay against bovine milk β-1,4-galactosyltransferase.



## 1. Introduction

The importance and implication of carbohydrates and carbohydrate–protein recognition events in a plethora of diverse biological processes are now extremely well documented.[1] Selective inhibition of glycosyl transferases represents an exciting, if as yet incompletely realised, avenue for the discovery of novel therapeutics for the treatment of certain disease states in which carbohydrates and carbohydrate–protein interactions are involved. Considerable endeavour has been expended over recent years into the search for potent and selective glycosyl transferase inhibitors.[2] However, although even nanomolar inhibitors of certain glycosyl transferases have now been identified,[3] the discovery of inhibitors that possess good activity in vivo, and which have then progressed to clinical investigation, has been less forthcoming.

Amongst the several different approaches possible for the design and synthesis of glycosyl transferase inhibitors, the development of donor substrate analogues has received considerable attention. The Leloir pathway[4] glycosyl transferases utilise sugar nucleotide diphosphates or sugar nucleotide monophosphates as the glycosyl donors. In particular, uridine diphosphate (UDP)-linked carbohydrates are employed as the donors by Gal-, Glc-, GlcNAc- and GalNAc-transferases, which are responsible for the transfer of galactose, glucose, *N*-acetylglucosamine and *N*-acetylgalactosamine units, respectively. All three portions of these UDP-sugar donors—uridine, the pyrophosphate and the sugar itself—are involved to some extent in binding to the glycosyl transferase active site.

Previous work by Wong[5] and co-workers on the use of the copper-catalysed Huisgen cycloaddition reaction[6]—the premier example of 'click chemistry'[7,8]—for the development of substrate analogue glycosyl transferase inhibitors focused on the parallel synthesis of small libraries of potential inhibitors of fucosyltransferases. Herein,[5] the fucose of the natural GDP-fucose donor was structurally varied, and several lead compounds were identified using microtiter plate screening. However, all contained an intact pyrophosphate unit, a factor that presumably precluded further medicinal and clinical development. The pyrophosphate moiety of sugar–nucleoside diphosphate donors is highly polar, and its replacement with alternative isosteric groups would be desirable in order to improve the pharmacokinetic properties of glycosyl transferase inhibitors.[9] The caveat to such an undertaking is that the pyrophosphate group is known to bind strongly to a divalent metal cation, usually $Mn^{2+}$, in the glycosyl transferase active site, and removal of the opportunity for such binding may result in complete ablation of inhibitory activity. However, the fact that natural glycosyl transferase inhibitors, such as tunicamycin, do not contain a pyrophosphate unit means that replacement is indeed possible; tunicamycin is thought to use a sugar ring to mimic the pyrophosphate group. Indeed, Wong and co-workers had also previously expended considerable effort on investigations into the use of malonic and tartaric esters, as well as sugar rings, as isosteric replacements for the pyrophosphate group, though with somewhat limited success.[10]

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doi:10.1016/j.carres.2009.01.001

With these previous studies in mind, it became clear that the union of carbohydrate and nucleoside moieties, most pertinently uridine, could be readily achieved by click chemistry. Such an approach would, for example, allow the synthesis of a series UDP mimics in which the pyrophosphate of the natural UDP-donor was replaced with a methylene triazole unit. Such mimics would still contain both carbohydrate and nucleoside fragments of the natural donor, both of which are involved in binding the glycosyl transferase active site. Subsequent screening against glycosyl transferases would reveal any effect on inhibitory activity of this replacement of pyrophosphate. Herein, we report the synthesis of a series of α-propargyl glycosides and their facile conjugation with 5-azido uridine using click chemistry in aqueous solution, to provide a novel series of analogues of natural UDP-sugar donors. Inhibitory assays of these compounds against bovine milk β-1,4-galactosyltransferase are also reported.

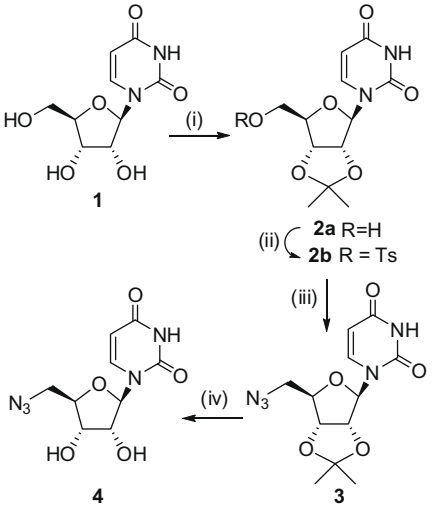
## 2. Results and discussion

5-Azido uridine **4** was accessed following standard synthetic procedures[11] as shown in Scheme 1. Thus, formation of the 2′,3′-acetonide **2a** by treatment of uridine **1** with acetone in the presence of catalytic concd sulfuric acid was followed by tosylation of the remaining primary hydroxyl to yield **2b**. Displacement by treatment with sodium azide in DMF yielded azide **3,** which underwent deprotection upon treatment with 90% aqueous trifluoroacetic acid to yield 5-azido uridine **4**.

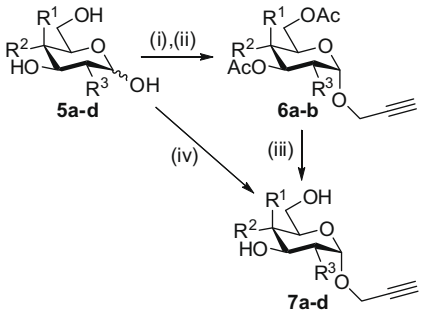
The required α-propargyl glycosides were made by Fischer glycosylation of the free sugars with propargyl alcohol. A survey of the literature indicated that sulfuric acid supported on silica gel has been recently reported[12] as an efficient catalyst for the formation of propargyl glycosides, and in many cases these were reportedly formed exclusively as the α-anomers. Sulfuric acid on silica was therefore made following the published procedure.[13] However, in our hands it was found that the silica gel so produced was not free flowing even after drying for 3 h at 100 °C, though such samples could be obtained after extended drying overnight in vacuo over $P_2O_5$. Moreover, the catalytic efficiency of the silica-sulfuric acid that was produced in this manner was considerably lower than that reported; in all cases, a considerable excess was required in order to promote dissolution of the starting materials, and more extended reaction times were required.[14] In this manner, treatment of a suspension of galactose **5a** in propargyl alcohol with silica-sulfuric acid at 65 °C for 20 h produced an inseparable ~3:1 α:β mixture of the propargyl glycosides, in rather lower yield and with considerably lower stereoselectivity than quoted in the previous report.[12] Separation of the anomers required acetylation by treatment with acetic anhydride, again catalysed by silica-sulfuric acid, to produce the acetates **6a** from which the pure α-anomer could be readily obtained by flash column chromatography. Zemplén deacetylation then yielded pure α-propargyl galactoside **7a**. A similar situation was encountered in the case of glucose; silica-sulfuric acid catalysed Fischer glycosylation again yielded a mixture of anomers, which were most readily separated by chromatography after acetylation; de-acetylation then yielded the desired pure α-glucoside **7b**. In the cases of the 2-amino sugars, the acetylation/de-acetylation sequence was not required; again silica-sulfuric acid-catalysed glycosylation of *N*-acetylglucosamine **6c** and *N*-acetylgalactosamine **6d** yielded the corresponding propargyl glycosides as anomeric mixtures (α:β ratios ~1:1), but in both cases access to the pure α-anomers was obtained by simple recrystallisation of these mixtures from methanol (Scheme 2).

With pure α-propargyl glycosides in hand, attention turned to the modified Huisgen cycloaddition reaction. Reaction of an equimolar mixture of propargyl galactoside **7a** and 5-azido uridine **4** at room temperature in a 1:1 mixture of the $^t$BuOH and water in the presence of 1 mol % $CuSO_4$ pentahydrate and 10 mol % sodium ascorbate smoothly produced the corresponding *galacto*-triazole **8a** in 86% yield. Similar reactions of the other propargyl glycosides 7b–**d** yielded the corresponding triazoles **8b–d** in good to excellent yields (Table 1). The ease with which these reactions were performed in aqueous solution with unprotected glycosides and the uridine derivative **4** highlights the utility of the Huisgen cycloaddition for the construction of water-soluble glycoconjugates of biological relevance.

Triazoles **8a–d** were then investigated as potential glycosyl transferase inhibitors. Interestingly, none of the compounds displayed any inhibitory activity at concentrations up to 4.5 mM in an assay against bovine milk β-1,4-galactosyltransferase undertaken using $^{14}$C-labelled UDP-galactose as substrate. In light of this lack of inhibitory activity, assays against other transferases were not undertaken. These results indicate that although the modified Huisgen cycloaddition allows efficient ligation of nucleoside and carbohydrate moieties in aqueous solution to provide facile access to potential UDP-sugar mimics, the triazole functionality is not a good isosteric replacement for pyrophosphate.



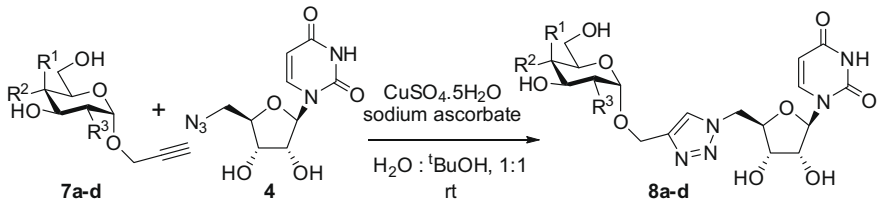

**Scheme 1.** Reagents and conditions: (i) acetone, $H_2SO_4$, rt, 74%; (ii) TsCl, pyridine, rt, 91%; (iii) $NaN_3$, DMF, 80 °C, 79%; (iv) $CF_3CO_2H/H_2O$, 9:1, rt, 97%.

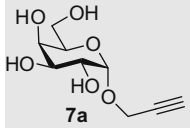
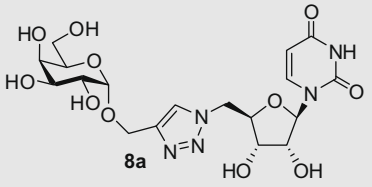
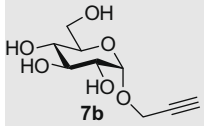
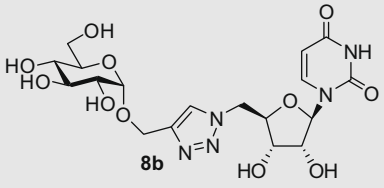
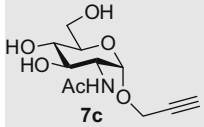
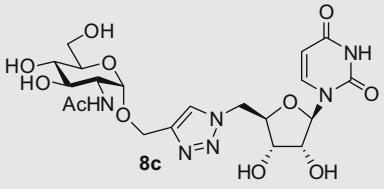
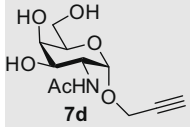
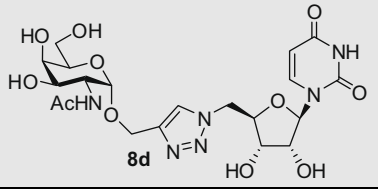



**5a, 6a, 7a** $R^1$=OH, $R^2$=H, $R^3$=OH
**5b, 6b, 7b** $R^1$=H, $R^2$=OH, $R^3$=OH
**5c, 7c** $R^1$=H, $R^2$=OH, $R^3$=NHAc
**5d, 7d** $R^1$=OH, $R^2$=H, $R^3$=NHAc

**Scheme 2.** Reagents and conditions: (i) $H_2SO_4$-silica, propargyl alcohol; (ii) $Ac_2O$, $H_2SO_4$-silica; **6a**, 50% over two steps, α:β, 3:1; **6b**, 63% over two steps, α:β, 3:1; (iii) NaOMe, MeOH, rt; **7a**, 97%; **7b**, 99%; (iv) $H_2SO_4$-silica, propargyl alcohol; **7c**, 43%, α:β, 1:1;; **7d**, 54%, α:β, 3:2.

**Table 1**
Synthesis of putative UDP-sugar donor mimics by modified Huisgen cycloaddition with 1 mol % $CuSO_4{\cdot}5H_2O$ and 10 mol % sodium ascorbate




| Propargyl glycoside | Product | Yield (%) |
|---|---|---|
|  7a |  8a | 86 |
|  7b |  8b | 66 |
|  7c |  8c | 72 |
|  7d |  8d | 97 |

## 3. Conclusions

A series of putative mimics of UDP-sugar donors were readily synthesised from the corresponding α-propargyl glycosides and 5-azido uridine in aqueous solution using Cu(I)-catalysed Huisgen cycloaddition. None of the compounds displayed significant inhibitory activity against bovine milk β-1-4-galactosyltransferase, indicating that triazole is not a good isosteric replacement for pyrophosphate. In this respect, a bis-triazole moiety may be a more appropriate replacement, particularly with respect to potential ability for coordination to $Mn^{2+}$.

## 4. Experimental

### 4.1. General

Melting points were recorded on a Kofler hot block and are uncorrected. Proton and carbon nuclear magnetic resonance ($\delta_H$, $\delta_C$) spectra were recorded on Bruker DPX 250 (250 MHz), Bruker DPX 400 (400 MHz), Bruker DQX 400 (400 MHz), Bruker AVC 500 (500 MHz) or Bruker AMX 500 (500 MHz) spectrometers. All chemical shifts are quoted in ppm using residual solvent as an internal standard. Low-resolution and high-resolution mass spectra were recorded on a Waters LCT Premier XE spectrometer and on a Bruker micrOTOF (ESI) spectrometer, respectively, using electrospray ionisation (ESI+ or ESI−) as ion source. *M/z* values are reported in Daltons, and are followed by their percentage abundance in parentheses. Optical rotations were measured on a Perkin–Elmer 241 polarimeter with a path length of 1 dm. Concentrations are given in g/100 mL. Microanalyses were performed by the Inorganic Chemistry Laboratory Elemental Analysis service, Oxford University, UK. Thin layer chromatography (TLC) was carried out on Merck Kieselgel $60F_{254}$ aluminium-backed plates. Visualisation of the plates was achieved using a UV lamp ($\lambda_{max}$ = 254 or 365 nm), and/or ammonium molybdate (5% in 2 M sulfuric acid), or sulfuric acid (5% in ethanol). Flash column chromatography was carried out using Sorbsil C60 40/60 silica. Dichloromethane was distilled from calcium hydride, or dried on an alumina column. Anhydrous DMF, pyridine and methanol were purchased from Fluka over molecular

sieves. 'Petrol' refers to the fraction of light petroleum ether boiling in the range of 40–60 °C. Bovine milk β-1,4-galactosyltransferase was purchased from Fluka at 1.05 U/mg (48279). Uridine diphospho-D-[U-$^{14}$C]galactose was purchased from Amersham at 267 mCi/mmol. $^{14}$C-Radioactivity was measured with a Perkin–Elmer Liquid Scintillation Counter.

Compounds **2a**,[11,15] **2b**,[10,11] **3**,[11,16] **4**,[11,16] **6a**,[12] **6b**,[12] **7a**,[17] and **7b**[18] were prepared as shown in Schemes 1 and 2, and exhibited spectral data in agreement with those reported previously.

### 4.2. Prop-2-ynl 2-acetamido-2-deoxy-α-D-glucopyranoside (7c)

$H_2SO_4$-silica[13] (600 mg) was added to a stirred suspension of *N*-acetyl-D-glucosamine **5c** (2.21 g, 10.0 mmol) in propargyl alcohol (8 mL, 69 mmol) at 65 °C. After 20 h, TLC ($CH_2Cl_2$/MeOH, 4:1) indicated formation of two major products ($R_f$ 0.2, 0.3), a minor product ($R_f$ 0.1) and complete consumption of starting material ($R_f$ 0.0). The reaction mixture was transferred to a short silica gel column, and the excess propargyl alcohol was first removed by elution with DCM. Further elution (DCM/MeOH, 4:1) yielded the products, which were combined, concentrated in vacuo, and the resulting residue was purified by flash chromatography (DCM/MeOH, 4:1) to afford propargyl glycoside **7c** (1.11 g, 43%) as an anomeric mixture (α:β ratio ~1:1). Recrystallisation from methanol yielded the desired pure α-glycoside **7cα** ($R_f$ 0.3, DCM/MeOH, 4:1, 0.49 g, 19% isolated yield) as a white crystalline solid; mp 172–173 °C (MeOH); $[\alpha]_D^{25}$ +155 (*c*, 1.0 in MeOH); $\nu_{max}$ (KBr disc) 3445 (s, N–H), 3285 (s, OH), 1634 (s, C=O) cm$^{-1}$; $\delta_H$ (500 MHz, $D_2O$) 2.01 (3H, s, $COCH_3$), 2.86 (1H, app. t, *J* 2.3 Hz, C≡C*H*), 3.47 (1H, app. t, *J* 9.5 Hz, H-4), 3.69–3.72 (2H, m, H-3, H-5), 3.76 (1H, dd, $J_{5,6'}$ 5.0 Hz, $J_{6,6'}$ 12.3, H-6), 3.83 (1H, dd, $J_{5,6'}$ 2.2 Hz, $J_{6,6'}$ 12.3 Hz, H-6′), 3.92 (1H, dd, $J_{1,2}$ 3.8 Hz, $J_{2,3}$ 10.7 Hz, H-2), 4.24 (1H, dd, *J* 2.3 Hz, $J_{gem}$ 16.1 Hz, C*H*HC≡C), 4.31 (1H, dd, *J* 2.3 Hz, $J_{gem}$ 16.1 Hz, CH*H*C≡CH), 5.02 (1H, d, $J_{1,2}$ 3.8 Hz, H-1); $\delta_C$ (125 MHz, $D_2O$) 21.8 (s, *C*H$_3$CO), 53.4 (d, C-2), 55.1 (t, $OCH_2$), 60.3 (t, C-6), 69.8 (d, C-4), 70.9, 72.3 (2 × d, C-3, C-5), 75.8 (d, $CH_2C$≡*C*) 79.0 (s, $CH_2C$≡C), 95.9 (d, C-1), 174.5 (s, *C*OCH$_3$); *m/z* (ESI−) 258.1 (M−H)$^-$ 100%). (Found: C, 50.88, H; 6.63; N, 5.41. $C_{11}H_{17}NO_6$ requires: C, 50.96; H, 6.61; N, 5.40%).

### 4.3. Prop-2-ynl 2-acetamido-2-deoxy-α-D-galactopyranoside (7d)

$H_2SO_4$-silica[13] (600 mg) was added to a stirred suspension of *N*-acetyl-D-galactosamine **5d** (2.21 g, 10.0 mmol) in propargyl alcohol (2.9 mL, 50.0 mmol) at 65 °C. After 3.5 h, TLC (DCM/MeOH, 4:1) indicated the formation of a major product ($R_f$ 0.4), two minor products ($R_f$ 0.3, 0.2), and the complete consumption of starting material ($R_f$ 0.0). The reaction mixture was transferred to a short silica gel column, and the excess propargyl alcohol was first removed by elution with DCM. Further elution (DCM/MeOH, 4:1) yielded the products, which were combined, concentrated in vacuo, and the resulting residue was then purified by flash chromatography (DCM/MeOH, 4:1) to afford propargyl glycoside **7d** (1.41 g, 54%), as an anomeric mixture (α:β ratio ~3:2). Recrystallisation from methanol furnished the desired pure α-glycoside **7dα** ($R_f$ 0.4, DCM/MeOH, 4:1, 0.81 g, 31% isolated yield) as a colourless crystalline solid; mp 224–226 °C (MeOH); $[\alpha]_D^{25}$ +233 (*c*, 1.0 in MeOH); $\nu_{max}$ (KBr disc) 3509 (s, NH), 3289 (s, OH), 1651 (s, C=O) cm$^{-1}$; $\delta_H$ (400 MHz, $D_2O$) 1.96 (3H, s, $COCH_3$), 2.85 (1H, t, *J* 2.2 Hz, C≡C*H*), 3.69–3.74 (2H, m, H-6, H-6′), 3.87 (1H, dd, $J_{2,3}$ 11.1 Hz, $J_{3,4}$ 3.0 Hz, H-3), 3.93–3.97 (2H, m, H-4, H-5), 4.15 (1H, dd, $J_{1,2}$ 3.8 Hz, $J_{2,3}$ 10.9 Hz, H-2), 4.24, 4.29 (2H, ABq, *J* 16.1 Hz, $OCH_2$), 5.04 (1H, d, $J_{1,2}$ 3.8 Hz, H-1); $\delta_C$ (100 MHz, $D_2O$) 21.9 (s, $COCH_3$), 48.9 (d, C-2), 55.0 (t, $OCH_2$), 61.4 (t, C-6), 67.9 (d, C-3), 68.7 (d, C-4 or C-5), 71.7 (d, C-4 or C-5), 75.8 (d, $CH_2C$≡*C*) 79.0 (s, $CH_2C$≡C), 96.3 (d, C-1), 175.0 (s, *C*OCH$_3$); HRMS (ESI+) Calcd for $C_{11}H_{17}N_1Na_1O_6$ $(M+Na)^+$ 282.0948. Found 282.0947. $C_{11}H_{17}NO_6$ requires: C, 50.96; H, 6.61; N, 5.40. Found: C, 50.76, H; 6.61; N, 5.32. Selected data for the undesired β anomer **7dβ** ($R_f$ 0.3, DCM/MeOH, 4:1); $\delta_H$ (400 MHz, $D_2O$) 1.98 (3H, s, $CH_3CO$), 2.82 (1H, app. t, *J* 2.4, C≡CH), 3.59–3.93 (5H, m, H-3, H-4, H-5, H-6, H-6′), 4.14 (1H, dd, $J_{1,2}$ 8.3, $J_{2,3}$ 10.6 Hz, H-2) 4.15–4.18 (2H, m, $CH_2C$≡C), 4.80 (1H, d, $J_{1,2}$ 8.3 Hz, H-1); *m/z* (ESI−) 258.1 (M−H)$^-$ 100%.

### 4.4. [1′-(5″-Deoxyuridin)-1′*H*-1′,2′,3′-triazol-4′-yl] methyl-α-D-galactopyranoside (8a)

Sodium ascorbate (50 μL of freshly prepared 1.0 M solution in water, 0.05 mmol) and copper(II) sulfate pentahydrate (50 μL of 0.1 M solution in water, 0.005 mmol) were added to a stirred suspension of propargyl glycoside **7a** (109 mg, 0.5 mmol) and 5′-azido-5′-deoxyuridine **4** (135 mg, 0.5 mmol) in a 1:1 mixture of water and *tert*-butyl alcohol (3 mL). After 8 h, TLC (water/*i*PrOH/ethyl acetate, 1:6:3) indicated formation of a major product ($R_f$ 0.3) and complete consumption of starting materials ($R_f$ 0.7, $R_f$ 0.8). The reaction mixture was concentrated in vacuo, and the residue was purified by flash chromatography (water/*i*PrOH/ethyl acetate, 1:6:3) to afford triazole **8a** (210 mg, 86%) as a pale yellow oil, $[\alpha]_D^{25}$ +110 (*c*, 1.0 in MeOH); $\nu_{max}$ (KBr disc) 3415 (br s, OH), 1689 (s, C=O) cm$^{-1}$; $\delta_H$ (500 MHz, $D_2O$) 3.59–3.64 (2H, m, H-6$_{Gal}$, H-6′$_{Gal}$), 3.73–3.77 (2H, m, H-2$_{Gal}$, H-4$_{Gal}$), 3.81–3.85 (1H, m, H-5$_{Gal}$), 3.89 (1H, br s, H-3$_{Gal}$), 4.10 (1H, app. t, *J* 6 Hz, H-3$_{uridine}$), 4.25 (1H, dd, $J_{1,2}$ 3.5 Hz, $J_{2,3}$ 6 Hz, H-2$_{uridine}$), 4.30–4.33 (1H, m, H-4$_{uridine}$), 4.65 (1H, d, *J* 12.6 Hz, OC*H*H), 4.71–4.76 (2H, m, OCH*H*, H-5$_{uridine}$), 4.82 (1H, dd, $J_{4,5'}$ 3.5 Hz, $J_{5,5'}$ 14.8 Hz, H-5′$_{uridine}$), 4.98 (1H, br s, H-1$_{Gal}$), 5.70 (1H, d, $J_{1,2}$ 3.5 Hz, H-1$_{uridine}$), 5.78 (1H, d, *J* 8.1 Hz, *CH*=CHN), 7.31 (1H, d, *J* 8.1 Hz, CH=C*H*N), 8.01 (1H, s, triazole); $\delta_C$ (125 MHz, $D_2O$) 50.7 (d, C-5$_{uridine}$), 60.1 (t, $OCH_2$), 61.0 (t, C-6$_{Gal}$), 68.1, 69.1, 69.4 (3 × d, C-2$_{Gal}$, C-3$_{Gal}$, C-4$_{Gal}$), 69.9 (d, C-3$_{uridine}$), 71.1 (d, C-5$_{Gal}$), 72.5 (d, C-2$_{uridine}$), 80.7 (d, C-4$_{uridine}$), 91.4 (d, C-1$_{uridine}$), 98.1 (d, C-1$_{Gal}$), 102.2 (d, *C*H=CHN), 126.3 (d, triazole C-5), 142.5 (d, CH=*C*HN), 144.0 (s, triazole C-4), 151.2, 166.1 (2 × s, C=O); HRMS (ESI+) Calcd for $C_{18}H_{25}N_5Na_1O_{11}$ $(M+Na)^+$ 510.1443. Found 510.1445. $C_{18}H_{25}N_5O_{11}$ requires: C, 44.35; H, 5.17; N, 14.37. Found: C, 44.22; H, 5.32; N, 14.32. *m/z* (ESI−) 486.1 (M−H)$^-$ 100%.

### 4.5. [1′-(5″-Deoxyuridin)-1′*H*-1′,2′,3′-triazol-4′-yl] methyl-α-D-glucopyranoside (8b)

Sodium ascorbate (100 μL of freshly prepared 1.0 M solution in water, 0.10 mmol) and copper(II) sulfate pentahydrate (100 μL of 0.1 M solution in water, 0.01 mmol) were added to a stirred suspension of propargyl glycoside **7b** (109 mg, 0.5 mmol) and 5′-azido-5′-deoxyuridine **4** (135 mg, 0.5 mmol) in a 1:1 mixture of water and *tert*-butyl alcohol (3 mL) at rt. After 24 h, TLC (water/*i*PrOH/ethyl acetate, 1:6:3) indicated formation of a major product ($R_f$ 0.3) and complete consumption of starting materials ($R_f$ 0.8, $R_f$ 0.9). The reaction mixture was concentrated in vacuo, and the residue was purified by flash chromatography (water/*i*PrOH/ethyl acetate, 1:6:3) to afford triazole **8b** (160 mg, 66%) as a colourless oil, $[\alpha]_D^{25}$ +108 (*c*, 1.0 in MeOH); $\nu_{max}$ (KBr disc) 3419 (br s, OH), 1691 (s, C=O) cm$^{-1}$; $\delta_H$ (500 MHz, $D_2O$) 3.37 (1H, app. t, *J* 9.5 Hz, H-4$_{Glc}$), 3.52 (1H, dd, $J_{1,2}$ 3.8 Hz, $J_{2,3}$ 9.8 Hz, H-2$_{Glc}$), 3.55–3.58 (1H, m, H-5$_{Glc}$), 3.62–3.70 (3H, m, H-3$_{Glc}$, H-6$_{Glc}$, H-6′$_{Glc}$), 4.13 (1H, app. t, *J* 6 Hz, H-3$_{uridine}$), 4.29 (1H, dd, $J_{1,2}$ 3.6 Hz, $J_{2,3}$ 6 Hz, H-2$_{uridine}$) 4.33–4.37 (1H, m, H-4$_{uridine}$), 4.65–4.73 (3H, m, $OCH_2$, H-5$_{uridine}$), 4.86 (1H, dd, $J_{4,5'}$ 3.3 Hz, $J_{5,5'}$ 15.0 Hz, H-5′$_{uridine}$), 4.98 (1H, d, $J_{1,2}$ 3.8 Hz, H-1$_{Glc}$), 5.72 (1H, d, $J_{1,2}$ 3.6 Hz, H-1′$_{uridine}$), 5.80 (1H, d, *J* 8.2 Hz, *CH*=CHN), 7.32 (1H, d, *J* 8.2 Hz, CH=C*H*N), 8.08

(1H, s, triazole); $\delta_C$ (125 MHz, $D_2O$) 50.7 (d, C-$5_{uridine}$), 60.2 (t, $OCH_2$), 60.3 (t, C-$6_{Glc}$), 69.4 (d, C-$4_{Glc}$), 69.9 (d, C-$3_{uridine}$), 71.2 (d, C-$2_{Glc}$), 72.0 (d, C-$5_{Glc}$), 72.5 (d, C-$2_{uridine}$), 73.0 (d, C-$3_{Glc}$), 80.7 (d, C-4 $_{uridine}$), 91.4 (d, C-$1_{uridine}$), 98.0 (d, C-$1_{Glc}$), 102.2 (d, *C*H=CHN), 126.2 (d, triazole C-5), 142.2 (d, CH=*C*HN), 144.0 (s, triazole C-4), 151.2, 166.1 (2 × s, C=O); HRMS (ESI−) Calcd for $C_{18}H_{24}N_5O_{11}$ (M−H) 486.1478. Found 486.1473. $C_{18}H_{25}N_5O_{11}$ requires: C, 44.35; H, 5.17; N, 14.37. Found: C, 44.38; H, 5.12; N, 14.28. *m/z* (ESI−) 486.1 $(M-H)^-$ 100%.

### 4.6. [1′-(5″-Deoxyuridin)-1′*H*-1′,2′,3′-triazol-4′-yl] methyl 2-acetamido-2-deoxy-α-D-glucopyranoside (8c)

Sodium ascorbate (100 μL of freshly prepared 1.0 M solution in water, 0.10 mmol) and copper(II) sulfate pentahydrate (100 μL of 0.1 M solution in water, 0.01 mmol) were added to a stirred suspension of propargyl glycoside **7c** (130 mg, 0.5 mmol) and 5′-azido-5′-deoxyuridine **4** (135 mg, 0.5 mmol) in a 1:1 mixture of water and *tert*-butyl alcohol (3 mL) at rt. After 20 h, TLC (water/*i*PrOH/ethyl acetate, 1:6:3) indicated formation of a major product ($R_f$ 0.4) and complete consumption of starting materials ($R_f$ 0.8, $R_f$ 0.9). The reaction mixture was concentrated in vacuo, and the residue was purified by flash chromatography (water/*i*PrOH/ethyl acetate, 1:6:3) to triazole **8c** (190 mg, 72%) as a pale yellow oil; $[\alpha]_D^{25}$ +126 (*c*, 1.0 in MeOH); $\nu_{max}$ (KBr disc) 3418 (br s, OH), 1691 (s, C=O) $cm^{-1}$; $\delta_H$ (500 MHz, $D_2O$) 1.92 (3H, s, $COCH_3$), 3.44 (1H, app. t, *J* 10 Hz, H-$4_{GlcNAc}$), 3.55–3.59 (1H, ddd, $J_{4-5}$ 10 Hz, $J_{5-6}$ 4.4 Hz, $J_{5-6'}$ 2.8 Hz, H-$5_{GlcNAc}$), 3.66–3.75 (3H, m, H-$3_{GlcNAc}$, H-$6_{GlcNAc}$, H-$6'_{GlcNAc}$), 3.85 (1H, dd, $J_{1,2}$ 3.8 Hz, $J_{2,3}$ 10.7 Hz, H-$2_{GlcNAc}$), 4.12 (1H, app. t, *J* 6.3 Hz, H-$3_{uridine}$), 4.30 (1H, dd, $J_{1,2}$ 3.6 Hz, $J_{2,3}$ 5.7 Hz, H-$2_{uridine}$) 4.33–4.36 (1H, m, H-$4_{uridine}$), 4.68 (1H, d, *J* 12.9 Hz, OC*H*H), 4.74–4.78 (2H, m, OCH*H*, H-$5_{uridine}$), 4.86 (1H, dd, $J_{4,5'}$ 3.3 Hz, $J_{5,5'}$ 15.0 Hz, H-$5'_{uridine}$), 4.88 (1H, d, $J_{1,2}$ 3.8 Hz, H-$1_{GlcNAc}$), 5.71 (1H, d, $J_{1,2}$ 3.6 Hz, H-$1_{uridine}$), 5.79 (1H, d, *J* 8.2 Hz, C*H*=CHN), 7.35 (1H, d, *J* 8.2 Hz, CH=C*H*N), 8.04 (1H, s, triazole); $\delta_C$ (125 MHz, $D_2O$) 21.7 (q, CO*C*$H_3$), 50.8 (d, C-$5_{uridine}$), 53.5 (d, C-2), 60.0 (t, $OCH_2$), 60.4 (t, C-$6_{GlcNAc}$), 69.8 (d, C-$3_{uridine}$), 69.9 (d, C-$4_{GlcNAc}$), 70.8 (d, C-$3_{GlcNAc}$), 72.1 (d, C-$5_{GlcNAc}$), 72.5 (d, C-$2_{uridine}$), 80.7 (d, C-$4_{uridine}$), 91.5 (d, C-$1_{uridine}$), 96.2 (d, C-$1_{GlcNAc}$), 102.2 (d, *C*H=CHN), 126.1 (d, triazole C-5), 142.5 (d, CH=*C*HN), 143.8 (s, triazole C-4), 151.2, 166.1, 174.2 (3 × s, C=O); HRMS (ESI+) Calcd for $C_{20}H_{28}N_6Na_1O_{11}$ $(M+Na)^+$ 551.1708. Found 551.1706. $C_{20}H_{28}N_6O_{11}$ requires: C, 45.45; H, 5.34; N, 15.90. Found: C, 45.19; H, 5.28; N, 15.80. *m/z* (ESI−) 527.2 $(M-H)^-$ 100%.

### 4.7. [1′-(5″-Deoxyuridin)-1′*H*-1′,2′,3′-triazol-4-yl] methyl-2-acetamido-2-deoxy-α-D-galactopyranoside (8d)

Sodium ascorbate (100 μL of freshly prepared 1.0 M solution in water, 0.10 mmol) and copper(II) sulfate pentahydrate (100 μL of 0.1 M solution in water, 0.01 mmol) were added to a stirred suspension of propargyl glycoside **7d** (130 mg, 0.5 mmol) and 5′-azido-5′-deoxyuridine **4** (135 mg, 0.5 mmol) in a 1:1 mixture of water and *tert*-butyl alcohol (3 mL) at rt. After 20 h, TLC (water/*i*PrOH/ethyl acetate, 1:6:3) indicated formation of a major product ($R_f$ 0.4) and complete consumption of starting materials ($R_f$ 0.8, $R_f$ 0.9). The reaction mixture was concentrated in vacuo, and the residue was purified by flash chromatography (water/*i*PrOH/ethyl acetate, 1:6:3) to afford triazole **8d** (256 mg, 97%) as a pale yellow oil; $[\alpha]_D^{25}$ +137 (*c*, 1.0 in MeOH); $\nu_{max}$ (KBr disc) 3405 (br s, OH), 1691 (s, C=O) $cm^{-1}$; $\delta_H$ (500 MHz, $D_2O$) 1.92 (3H, s, $CH_3CO$), 3.64–3.65 (2H, m, H-$6_{GalNAc}$, H-$6'_{GalNAc}$), 3.81 (1H, dd, $J_{2,3}$ 11.0 Hz, $J_{3,4}$ 3.2 Hz, H-$3_{GalNAc}$), 3.85–3.87 (1H, m, H-$5_{GalNAc}$), 3.90 (1H, m, H-$4_{GalNAc}$), 4.06 (1H, dd, $J_{1,2}$ 3.8 Hz, $J_{2,3}$ 11.0 Hz, H-$2_{GalNAc}$), 4.07–4.09 (1H, m, H-$3_{uridine}$), 4.26 (1H, dd, $J_{1,2}$ 3.5 Hz, $J_{2,3}$ 5.7 Hz, H-$2_{uridine}$), 4.28–4.31 (1H, m, H-$4_{uridine}$), 4.60–4.73 (3H, m, $OCH_2$, H-$5_{uridine}$), 4.81 (1H, dd, $J_{4,5}$ 3.5 Hz, $J_{5,5'}$ 15.1 Hz, H-$5'_{uridine}$), 4.88 (1H, d, $J_{1,2}$ 3.8 Hz, H-$1_{GalNAc}$), 5.67 (1H, d, $J_{1,2}$ 3.5 Hz, H-$1_{uridine}$), 5.75 (1H, d, *J* 8.1 Hz, C*H*=CHN), 7.32 (1H, d, *J* 8.1 Hz, CH=C*H*N), 7.99 (1H, s, triazole); $\delta_C$ (125 MHz, $D_2O$) 21.8 (q, CO*C*$H_3$), 49.7 (d, C-$2_{GalNAc}$), 50.8 (d, C-5″), 59.9 (t, $OCH_2$), 61.1 (t, C-$6_{GalNAc}$), 67.5 (d, C-$3_{GalNAc}$), 68.4 (d, C-$4_{GalNAc}$), 69.9 (d, C-$3_{uridine}$), 71.2 (d, C-$5_{GalNAc}$), 72.5 (d, C-$2_{uridine}$), 80.7 (d, C-$4_{uridine}$), 91.5 (d, C-$1_{uridine}$), 96.3 (d, C-$1_{GalNAc}$), 102.2 (d, *C*H=CHN), 126.1 (d, triazole C-5), 142.5 (d, CH=*C*HN), 143.8 (s, triazole C-4), 151.2, 166.0, 174.4 (3 × s, C=O); HRMS (ESI+) Calcd for $C_{20}H_{28}N_6Na_1O_{11}$ $(M+Na)^+$ 551.1708. Found 551.1705. $C_{20}H_{28}N_6O_{11}$ requires: C, 45.45; H, 5.34; N, 15.90. Found: C, 45.37; H, 5.29; N, 15.94. *m/z* (ESI−) 527.2 $(M-H)^-$ 100%.

### 4.8. Bovine milk β-1,4-galactosyltransferase assay [19]

β-1,4-Galactosyltransferase activity was assayed using $^{14}C$-radiolabelled UDP-Gal as glycosyl donor and *N*-acetylglucosamine (GlcNAc) as the acceptor, as described previously. The assay reaction mixtures contained reagents in the following final concentrations: 45 mM sodium cacodylate buffer (pH 6.8); 10 mM $MnCl_2$; 2.5 mM AMP; 10 mM GlcNAc in distilled water; 1% TX-100; 20 μU milk β-1,4-galactosyltransferase (diluted in buffer + 1 mg/mL BSA and 1% TX-100) and potential inhibitors at a range of concentrations from 0 mM (control) to 4.5 mM. Samples were incubated at 37 °C for 5 min before adding the substrate [$^{14}C$-Gal] UDP-Gal, so that the final concentration was 2 μM. Samples were then incubated for 60 min at 37 °C before the reactions were stopped by the addition of cold distilled water (1.0 mL). The reaction mixtures passed through Bio-Rad Ag-1-X8($Cl^-$ form) anion exchange resin (1 mL). The eluent was collected, and the resin was washed with distilled water (1.0 mL). Extracts were then analysed for $^{14}C$-galactose incorporation by adding 4 mL of Ultima Gold TM (Packard Bioscience Co., CT, USA) and counting for $^{14}C$ using a Wallac 1409 DSA liquid scintillation counter. Internal calibration was performed to calculate disintegrations per min (dpm). Typical incorporation of radioactivity measured using this assay was 14,000 dpm (0.24 kBq) per 60 min incubation.

## Acknowledgements

The authors gratefully acknowledge the Malaysian Government and the Universiti Sains Malaysia (Fellowship to K.K.Y.) for financial support.

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