COMMUNICATION

Stereoselective enzymatic galactosylation of C-glucosides

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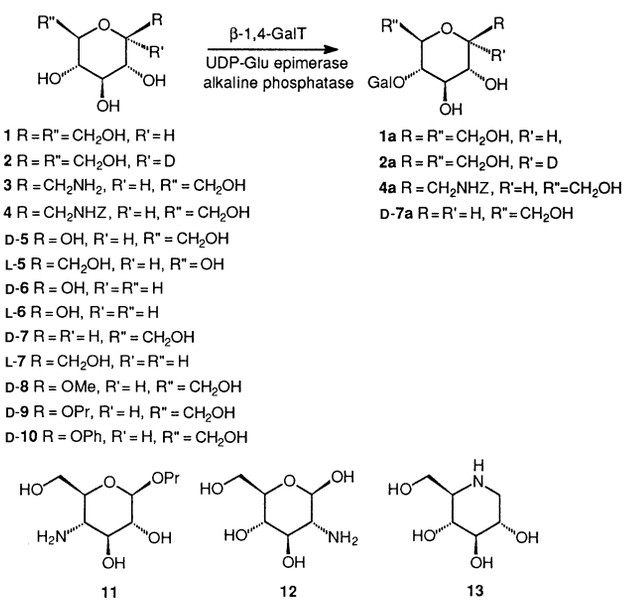
The enzyme β-1,4-galactosyl transferase from bovine colostrum (GalT) is able stereoselectively to galactosylate C-glucosides (*i.e.* 1 and 4), precursors of stable glycoconjugate analogues, and a systematic investigation of the structural modifications at C-1 and/or C-5 of the glycosides that can be accepted by this enzyme has been undertaken, adding information to the currently accepted model of substrate binding into the GalT active site.

C-Glycosides have recently become one of the major topics in carbohydrate chemistry since they are challenging synthetic targets and can be used as stable chemical analogues of naturally occurring bioactive compounds for metabolic studies. There is an increasing demand for these compounds,[1] especially in the fast growing area of glycobiology, in which they could offer easy access to stable analogues of glycoconjugates, provided that the carbohydrate part can be easily manipulated to give more complex sugar structures.

In the context of our general interest in biocatalysis,[2] we have recently become involved in the isolation and synthetic application of the β-1,4-galactosyl transferase from bovine colostrum (GalT).[3] This enzyme catalyses the transfer of D-galactose from its uridine 5′-diphosphate (UDP) derivative to the hydroxy group at C-4 of D-glucose and *N*-acetyl-D-glucosamine. Recently it has been shown that this enzyme is also able to transfer D-galactose to various substrate acceptors with some degree of structural variability.[4] However, despite the large number of reports, to our knowledge there are no examples of galactosylation of C-glycosides. We initially studied the behaviour of GalT with the simple hydroxymethyl derivative **1**, prepared by catalytic hydrogenolysis of the known *C*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)methanol[5] with Pd/C in MeOH, as this transformation could offer access to glycosylated C-glycosides and also offer additional valuable information on the substrate specificity and stereoselectivity of this bovine transferase. Even though **1** is a *meso*-compound, it was reasonable to expect, as a consequence of the stereochemical requirements of GalT described so far,[4,6] that this enzyme should be able to differentiate between the two enantiotopic hydroxy groups at the C-3 and C-5 positions of **1** (formerly C-2 and C-4 of the parent glucose), to give **1a** as the only product.

As shown in Table 1, the *meso*-C-glucoside **1** was a good substrate for GalT, its relative rate of transformation being similar to that of propyl β-D-glucopyranoside D-**9** and higher than that of methyl D-**8** or phenyl β-D-glucopyranoside D-**10**.

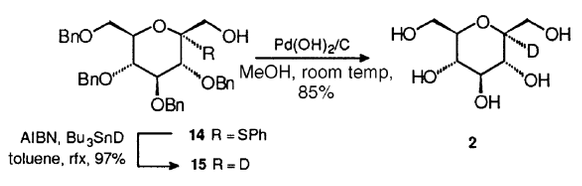
Compound **1** (40 mg, 0.205 mmol) and UDP-glucose (158 mg, 0.26 mmol) were reacted for 4 days at 30 °C in the presence of UDP-glucose epimerase (10 units), GalT (1.75 units),† alkaline phosphatase (35 units) and 5 mg of α-lactalbumine in 4 ml of 50 mM TRIS buffer (pH 7.4) containing $MnCl_2$ (1 mM),



Scheme 1

dithiothreitol (DTT) (1 mM) and NaN_3 (0.01%). The usual work-up[3] isolated 26 mg (37% isolated yield) of a single product derived from galactose and **1**. The presence of galactose is clearly shown by a broad doublet at δ 5.73 in the ^{1}H NMR spectrum of the fully benzoylated **1a**, attributable to H-4′. Although in some cases the *in situ* generation of UDP-galactose from UDP-glucose with UDP-glucose epimerase resulted in a transfer of glucose, we never observed the formation of glucose-containing products. However, the symmetry of the molecule of the acceptor C-glucoside made it difficult to determine the position of attachment of galactose to **1**.

To solve this problem, we synthesized compound **2** (from **14** *via* **15**, see Scheme 2),[7] the C-2 deuteriated analogue of **1**, to



Scheme 2

introduce an asymmetric element without modifying the reactivity of the acceptor (see Table 1). Enzymatic galactosylation gave a deuteriated pseudodisaccharide (26% isolated yield) which was fully benzoylated (BzCl, py, 60 °C, overnight) and analysed by a ^{1}H–^{1}H COSY experiment. Complete proton assignment allowed us to assign the signal at relatively high field (δ 4.22) to H-5 and to identify the product as the expected

† GalT was purified from bovine colostrum as described in ref. 3 to give an enzymatic sample with a specific activity of 67 U mg^{-1} (see ref. 3 and references cited therein).

Table 1 Relative rate of galactosylation of C-glucosides and D- and L-glucose derivatives by action of GalT

Entry	Compound	Relative rate (%)[a]	Product
1	**1**	10	β-1,5
2	D-**8**	2.5	β-1,4
3	D-**9**	10	β-1,4
4	D-**10**	3.5	β-1,4
5	**2**	10	β-1,5
6	**3**	0	—
7	**4**	10	β-1,5
8	D-**5**	100	β-1,4
9	L-**5**	0	—
10	D-**7**	0.8	β-1,4
11	L-**7**	0	—
12	D-**6**	2.5	β-1,4 and β-1,1[b]
13	L-**6**	0	

[a] Relative to D-glucose (entry 8) and determined according to literature methods.[6b,13] [b] The initial rate refers to the whole reactivity of the substrate.[11]

compound **2a**. Comparison with the NMR spectra of the previously synthesized non-deuteriated analogue, after benzoylation, allowed us to assign to it the structure **1a**. In fact, the ^{13}C NMR and ^{1}H NMR spectra of **2a** and **1a** as well as the ^{1}H NMR of their fully benzoylated derivatives were very similar. As expected, the only differences were a consequence of the presence of deuterium at C-2 of **2a**. Specifically, the ^{13}C NMR spectrum of **2a** lacks the signal at δ 79.5 (C-2) that is present in the spectrum of **1a**, while the ^{1}H NMR spectrum of benzoylated **2a** lacks a signal at δ 4.03 (ddd, H-2) and the coupling constants of the correlated protons.

To extend the scope of this preliminary work to the synthesis of precursors of stable glycopeptides we considered the amino C-glucoside **3** in which the amino group could allow the linkage with an aspartic acid to give analogues of *N*-linked glycosyl amino acids. Not only was this compound not a substrate, it was an inhibitor of the catalytic activity of the enzyme.‡ In a previous investigation we had found that propyl 4-amino-4-deoxy-β-D-glucopyranoside (**11**) was also an inhibitor of GalT ($K_i \cong$ 90 mM). In addition, it is known from the literature[6] that two other amino derivatives, **12** and **13**, are both poor substrates for the enzyme. Therefore, we might speculate that a free amino group on the sugar acceptor does not interact productively with the active site of GalT.[8] On the other hand, the *N*-protected benzyloxycarbonyl derivative **4** was a good substrate ($K_M \cong$ 20 mM) for the enzyme and the corresponding pseudodisaccharide was isolated (68% isolated yield) and characterized.[9]

Because of our interest in C-analogues of glycopeptides, we also tried the enzymatic galactosylation of the *C*-(β-D-xylopyranosyl)methanol, which corresponds to 1,5-anhydro-L-glucitol (L-**7**), since β-xylosyl glycosides of serine are present in glycoproteins. We were encouraged by the fact that Wong *et al.* succeeded in their galactosylation using GalT.[10] Surprisingly, under our conditions, derivative L-**7** was not a substrate for this enzyme. This result prompted us to determine the structural modifications at C-1 and/or at C-5 that can be accepted by the enzyme. In some cases, structural changes at these positions (as in β-D-glucopyranosides, D-xylose and our *meso*-C-glucoside) do not prevent enzymatic reaction, while in other cases (as in L-**7** or other compounds reported in the literature[11,12]) no conversion was observed.

The results, summarized in Table 1, show that modifications at either C-1 (entries 1–5, 7 and 10) or C-5 (entry 12) are accepted by bovine GalT. However, contemporaneous modifications at C-1 and C-5 lead to no reaction or, most probably, make the reaction much too slow to be detected under our conditions and, consequently, are useless for synthesis.

For this reason, despite the fact that they are so similar to our *meso*-C-glucoside **1**, in some cases differing only in the substituent R″, none of the L-sugars considered was a substrate for GalT. On the other hand, in addition to the natural substrate D-glucose and the previously described D-xylose,[11] 1,5-D-anhydroglucitol D-**7** was also accepted by this enzyme and the corresponding pseudodisaccharide D-**7a** was isolated (15% isolated yield) and characterized.

In conclusion, in addition to their interest for synthesis, our results add information to the currently accepted model of substrate binding into the active site of GalT proposed by Wiemann *et al.*[11] and Yu *et al.*,[12] especially with regard to the possible modifications at C-1 and at C-5. As it has recently been shown that this GalT can be used for galactosylation of *N*- and *O*-linked glycopeptides,[10,14] work is in progress to apply the results obtained so far with **1** and **4** to the preparation of stable analogues of glycopeptides.

‡ When 20 mM **3** was added to 20 mM glucose (approximately twice its K_M value), a 50% drop in the reaction rate was observed. A detailed kinetic analysis was beyond the purpose of this work and will be reported in due course.

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9 Selected NMR data: benzoylated **1a**: δ_H($CDCl_3$, *J*/Hz) 5.58 (t, *J* 9.7, 1 H, H-3), 4.88 (d, *J* 7.8, 1 H, H-1′), 4.22 (dd app. as t, *J* 9.5, 1 H, H-5), 4.03 (m, 1 H, H-2); benzoylated **2a**: δ_H($CDCl_3$) 5.57 (dd app. as t, *J* 9.7, 1 H, H-3), 4.88 (d, *J* 8.0, 1 H, H-1′), 4.22 (dd app. as t, *J* 9.4, 1 H, H-5); benzoylated **4a**: δ_H($CDCl_3$) 4.88 (d, *J* 8.0, 1 H, H-1′), 4.20 (dd app. as t, *J* 9.4, 1 H, H-5); **1a**: δ_C(D_2O) 103.2, 79.5, 79.0, 78.6, 76.2, 75.7, 72.9, 71.3, 69.9, 68.9, 61.4 (2C), 60.8; **2a**: δ_C(D_2O) 103.3, 79.0, 78.5, 76.3, 75.7, 72.9, 71.3, 69.8, 68.9, 61.4 (2C), 60.8; **4a**: δ_C(D_2O) 159.4, 137.4, 129.7 (2C), 129.3, 128.6 (2C), 103.8, 79.5, 79.1, 78.7, 76.6, 76.2, 73.5, 71.9, 71.6, 69.5, 68.1, 67.9, 61.9, 61.3, 42.4; **5a**: δ_C(D_2O) 103.7, 79.9, 79.5, 76.9, 76.2, 73.4, 71.8, 69.9, 69.5, 69.3, 61.8, 61.1.
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