Carbohydrate Research, 236 (1992) 335–338
Elsevier Science Publishers B.V., Amsterdam

Note

Galactosylation of non-natural glycosides with human β-D-galactosyltransferase on a preparative scale *

Reinhold Öhrlein [a], Beat Ernst [a] and Eric G. Berger [b]

[a] *Central Research Laboratories Ciba–Geigy AG, Schwarzwaldallee 211, CH-4002 Basel (Switzerland)*
[b] *Physiologisches Institut der Universität Zürich, Winterthurerstrasse 190, CH-8057 Zürich (Switzerland)*

(Received July 15th, 1991; accepted in revised form February 3rd, 1992)

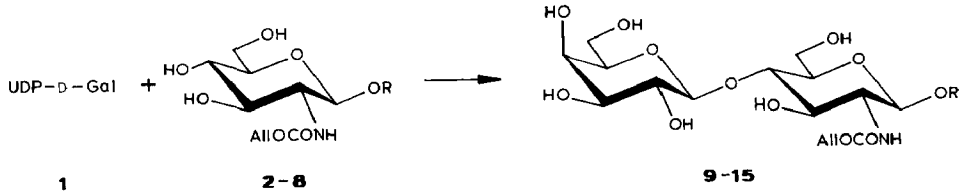
The complex oligosaccharide chains of glycoproteins or glycolipids mediate and modulate an impressive variety of biological processes by acting as information carriers or recognition molecules[1–6]. In an attempt to obtain such glycoconjugates, we were prompted to make use of combined chemical and enzymic synthesis[7], and we studied the substrate compatibility of human β-D-galactosyltransferase [EC 2.4.1.22) with regard to acceptor structure. For bovine β-D-galactosyltransferase, Geren et al.[8] found that 2-deoxy-2-fluoroacetamido- and 2-deoxy-2-(trifluoroacetamido)-D-glucose were substrates, but not the *N*-propionylacetamido and *N*-benzoylacetamido derivatives. In addition, a broader array of 2-acylamino-2-deoxy-D-glucose (including the 2-propionylamino) derivatives were found to be good substrates[9]. The use of bovine galactosyltransferase on a preparative scale was reported by Wong et al.[10], and Sabesan and Paulson[11], and successful β-D-galactosylation of a 2-acetamido-2-deoxy-D-glucosyl group linked to another sugar unit[12], asparagine[12,13], or a pentapeptide[14] has been published. Furthermore the enzymic transfer of a β-D-galactosyl group to a 2-acetamido-2-deoxy-D-glucose unit linked to lipophilic alcohols was reported by Palcic et al.[15], Lehmann and Petry[16], and Zehavi et al.[17].

We present herein the use of human β-D-galactosyltransferase, isolated according to Gerber et al.[18], with the non-natural acceptors **2–8**. These glycosides differ from the natural acceptor in both the *N*-acyl and the aglycon groups.

Acceptors **2–8** were obtained according to the procedure of Boullanger et al.[19]. On a preparative scale, the enzymic galactosylation was carried out in Tris buffer (pH 7.5) solution containing Mn^{2+}, calf intestinal alkaline phosphatase (CIAP, EC 3.1.3.1)[14,20], and UDP-D-galactose (**1**) except for **2**, which was galactosylated by use

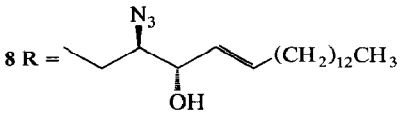
Correspondence to: Dr. R. Öhrlein, Central Research Laboratories Ciba–Geigy AG, Schwarzwaldallee 211, CH-4002 Basel, Switzerland; or to Prof. Dr. E.G. Berger, Physiologisches Institut der Universität Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland.

* Dedicated to Professor Jean Montreuil.



1 2–8 9–15

		Yield [a]	
		(%)	(mg)
2 R = H	9	96	203.0
3 R = $CH_2CH{=}CH_2$	10	93	43.4
4 R = $(CH_2)_5CH_3$	11	86	43.7
5 R = $(CH_2)_3CH{=}CH_2$	12	86	44.8 [b]
6 R = $(CH_2)_{11}CH_3$	13	42	27.5 [b]
7 R = $CH_2CH_2SiMe_3$	14	38	20.8 [b]
	15	39	34.4



[a] Starting from 504 μmol of **2** and 100.8 μmol of **3–8**. [b] Yield not optimized.

Scheme 1. Reaction of UDP-D-Gal (**1**) with 2-amino-2-deoxy-D-glucose derivatives **2–8** to give derivatives **9–15**, respectively.

of UDP-D-galactose, produced in situ by epimerization[10,14] of UDP-D-glucose by UDP-D-glucose epimerase (EC 5.1.3.2). Amounts and yields of products are given in Scheme 1.

The allyloxycarbonyl protective group was chosen because it facilitates β-D-glycosidation without formation of side-products and can be removed by a mild procedure[19]. In the enzymic galactosylation step, compound **2** proved to be an excellent substrate for human β-D-galactosyltransferase. For the galactosylations of the more lipophilic glycosides **3–8**, a high concentration of UDP-D-galactose was used to saturate the transferase for a more rapid reaction, thus giving disaccharides **10–15** in acceptable to high yields. Some selected ^{13}C NMR data for **10–15** proving the product structures are listed in Table I. The high, downfield shift of C-1 of the galactosyl groups proved the β anomery and the downfield shift (from δ ~71–72 to 80–81) of C-4 of the 2-amino-2-deoxy-β-D-glucopyranosyl residue proved the (1 → 4) linkage[21].

These results show that human β-D-galactosyltransferase can efficiently be used for the synthesis of non-natural saccharides. This agrees well with the studies of the acceptor structure of *N*-acetyl-D-glucosaminyl- (EC 2.4.1.101) and L-fucosyltransferase (EC 2.4.1.65) reported by Hindsgaul and co-workers[22,23]. The possible use of non-natural substrates in enzymic glycosidations greatly increases the scope of these reactions, as the present drawback of these glycosidations is the limited

TABLE I

Selected ^{13}C NMR data (δ) for compounds **9–15**[21]

Compound	Solvent	β-D-Gal*p* group				D-GlcN residue			
		C-1	C-4	C-5	C-6	C-1	C-2	C-4	C-6
9 (α anomer)	D_2O	106.55	72.34	79.07	64.88	98.70	58.87	82.20	63.75
9 (β anomer)	D_2O	106.55	72.34	79.07	64.88	94.73	58.87	82.38	63.75
10	CD_3OD	105.05	70.39	77.04	62.58	102.20	58.42	80.95	61.89
11	CD_3OD	105.06	70.43	76.99	62.59	103.06	58.49	81.05	61.93
12	CD_3OD	105.06	70.34	77.09	62.54	103.14	58.10	81.00	61.93
13	CD_3OD–$CDCl_3$	104.15	69.59	76.28	61.94	102.33	57.82	80.30	61.39
14	CD_3OD	105.08	68.12	77.14	62.56	102.50	58.46	81.06	61.98
15	CD_3OD	104.66	69.96	76.02	62.26	102.51	58.00	80.78	61.70

availability of transferases and activated sugars[7]. For human β-D-galactosyltransferase, however, we could show that, based on an efficient enzyme isolation procedure[18], glycoside synthesis on a preparative scale is easily achieved. It may be expected that, in the near future, virtually unlimited amounts of several glycosyltransferases will be available by their heterologuous expression[24] in bacterial or yeast cells.

EXPERIMENTAL

General procedure for the preparation of disaccharides **10–15**.—Acceptor (100.8 μmol), UDP-D-Gal (111.0 μmol), and bovine serum albumin (~ 2 mg) were stirred into 0.5 M Tris buffer solution (2 mL, pH 7.5). To this mixture were added $MnCl_2 \cdot 4H_2O$ (23.6 mg, 119.2 μmol, corresponding to a final concentration of 47.6 mM), CIAP (0.5 μL, 7 units), and electrophoretically pure β-D-galactosyltransferase[18] (0.5 mL, ~ 1.8 units). The stoppered flask was incubated at 36° until no further reaction could be detected by TLC (1–16 h, 4:2:1 ethyl acetate–ethanol–water). After lyophilization of the crude reaction mixture, the resulting white solid was chromatographed on silica gel in the same solvent system.

NOTE ADDED IN PROOF

Since submission of this manuscript in july 1991, a number of studies involving enzymic galactosylations have been published and summarized in a recent review[25]. Among them, the following address the issue of probing the acceptor specificity of galactosyltransferase by using other substrates than *N*-acetyl-D-glucosamine[26–29].

ACKNOWLEDGMENTS

The authors thank Dr. M. Watzele for his helpful assistance in the isolation of human β-D-galactosyltransferase. This work was supported, in part, by grant No. 1781 of KWF to E.G.B.

REFERENCES

1 E.G. Berger, E. Buddecke, J.P. Kamerling, A. Kobata, J.C. Paulson, and J.F.G. Vliegenthart, *Experientia*, 38 (1982) 1129–1162.
2 S. Hakomori, *Annu. Rev. Immunol.*, 2 (1984) 103–126; S. Hakomori, *Adv. Cancer Res.*, 52 (1989) 257–331.
3 R.W. Rademacher, R.B. Parekh, and R. Dwek, *Annu. Rev. Biochem.*, 57 (1988) 785–838.
4 J. Hodgson, *Biotechnology*, 8 (1990) 108–111.
5 H.F. Oettgen, *Gangliosides and Cancer*, Verlag Chemie, Weinheim, 1989.
6 J.C. Paulson, *Trends Biochem. Sci.*, 14 (1989) 272–275.
7 E.J. Toone, E.S. Simon, M.D. Bednarski, and G.M. Whitesides, *Tetrahedron*, 45 (1989) 5365–5422.
8 C.R. Geren, S.C. Magee, and K.E. Ebner, *Arch. Biochem. Biophys.*, 172 (1976) 149–155.
9 L.J. Berliner, M.E. Davies, K.E. Ebner, T.A. Beyer, and J.E. Bell, *Mol. Cell. Biochem.*, 62 (1984) 37–42.
10 C.-H. Wong, S.L. Haynie, and G.M. Whitesides, *J. Org. Chem.*, 47 (1982) 5416–5418.
11 S. Sabesan and J.C. Paulson, *J. Am. Chem. Soc.*, 108 (1986) 2068–2080.
12 J. Thiem and T. Wiemann, *Angew. Chem.*, 29 (1990) 80.
13 C. Augé, C. Gautheron, and H. Pora, *Carbohydr. Res.*, 193 (1989) 288–293.
14 C. Unverzagt, H. Kunz, and J.C. Paulson, *J. Am. Chem. Soc.*, 112 (1990) 9308–9309.
15 M.M. Palcic, L.D. Heerze, M. Pierce, and O. Hindsgaul, *Glycoconjugate J.*, 5 (1988) 49–63.
16 J. Lehmann and S. Petry, *Carbohydr. Res.*, 204 (1990) 141–144.
17 U. Zehavi, M. Herchman, R.R. Schmidt, and T. Bär, *Glycoconjugate J.*, 7 (1990) 229–234.
18 A.C. Gerber, I. Kozdrowski, S.R. Wyss, and E.G. Berger, *Eur. J. Biochem.*, 93 (1979) 453–460.
19 P. Boullanger, M. Jouineau, B.B. Bouammali, D. Lafont, and G. Descotes, *Carbohydr. Res.*, 202 (1990) 151–164.
20 A. Redlitz, Diploma Thesis, Freie Universität Berlin, 1988.
21 K. Bock and H. Thorgersen, *Annu. Rep. NMR-Spectrosc.*, 13 (1982) 1–57.
22 G. Srivastava, G. Alton, and O. Hindsgaul, *Carbohydr. Res.*, 207 (1990) 259–276.
23 U.D. Gokhale, O. Hindsgaul, and M.M. Palcic, *Can. J. Chem.*, 68 (1990) 1063–1071.
24 D. Aoki, H.E. Appert, D. Johnson, S.S. Wong, and M.N. Fukuda, *EMBO J.*, 9 (1990) 3171–3178.
25 Y. Ichikawa, G.C. Look, and C.H. Wong, *Anal. Biochem.*, 202 (1992) 215–238.
26 C.H. Wong, T. Krach, C. Gautheron-le Narvor, Y. Ichikawa, G.C. Look, F. Gaeta, D. Thompson, and K.C. Niclaou, *Tetrahedron Lett.*, 32 (1991) 4867–4870.
27 C.H. Wong, Y. Ichikawa, T. Krach, C. Gautheron-le Narvor, D.P. Dumas, and G.C. Look, *J. Am. Chem. Soc.*, 113 (1991) 8137–8145.
28 M.M. Palcic and O. Hindsgaul, *Glycobiol.*, 1 (1991) 205–209.
29 E. Yoon, and R.A. Laine, *Glycobiol.*, 2 (1992) 161–168.