Bioorganic & Medicinal Chemistry Letters, Vol.3, No.8, pp. 1747-1750, 1993
Printed in Great Britain

0960-894X/93 $6.00 + .00

A NEW GALACTOSYL TRANSFERASE INHIBITOR

Richard R. Schmidt* and Klaus Frische

Fakultät Chemie, Universität Konstanz,

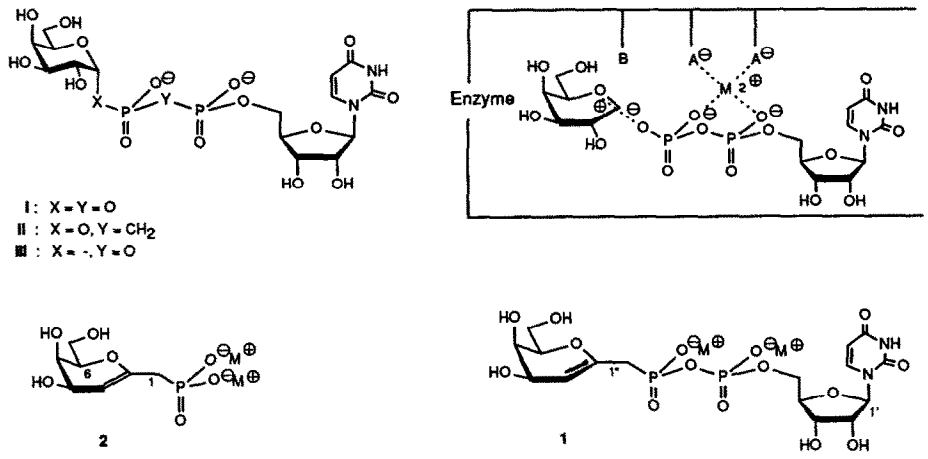
D-7750 Konstanz, Germany

(*Received in USA* 14 *May* 1993)

Abstract: Galactal-1-yl-methylphosphonates **2a,b** and uridine phosphate derivative **1** were prepared and their inhibitory activity towards β-galactosyl transferase from bovine milk investigated. **1** exhibited strong competive inhibition.

The manifold occurrence of complex oligosaccharide structures as epitopes at the surface of cells[1,2] is biosynthetically achieved with nucleoside diphosphate sugars (for instance, UDP-galactose I, Scheme 1) as glycosyl donors and glycosyl transferases as regio- and stereoselectively active catalysts[3]. Control of the biosynthesis with the help of specific enzyme inhibitors should lead to an understanding of the function of carbohydrate epitopes in cell growth and cell-cell adhesion and provide a means to influence these functions[2,4].

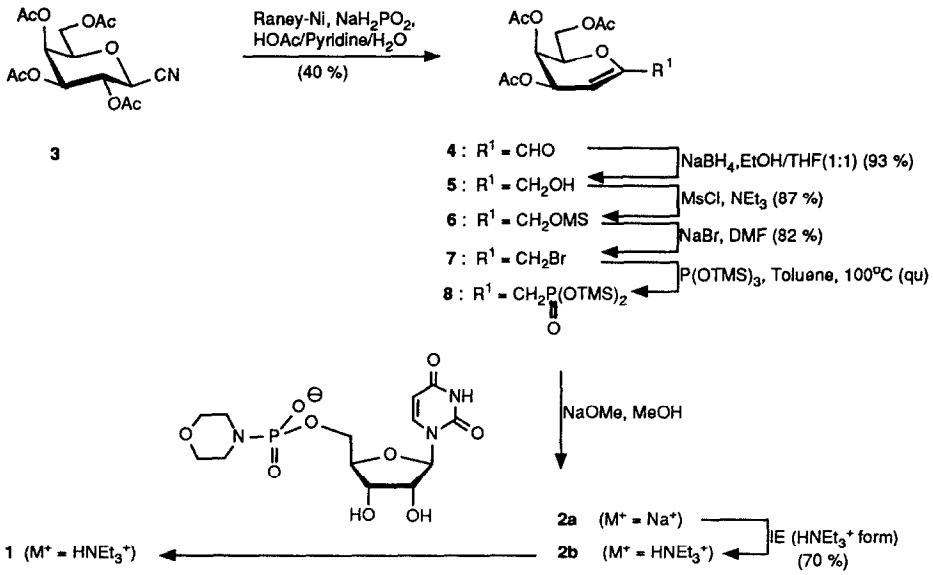
Scheme 1



The knowledge of the active site of glycosyl transferases is rather limited[5-7]. Therefore, as galactosyl transferase inhibitors structural analogs of I such as II[8] and III[9], respectively, have been synthesized which exhibited good inhibitory properties. Better inhibition is generally observed for transition state analogues[5-7]. The in vitro investigations with glycosyl phosphates as glycosyl donors support a S_N1-type transition state in the active site of the enzyme, where metal ions or protons serve as promoters for the cleavage of the nucleoside diphosphate leaving group[6]. The incipient carbenium ion may be stabilized as ion pair by the leaving group or other (possibly negatively charged) residues B within the active site (Scheme 1), thus permitting stereocontrol in the ensuing glycosylation step. From this assumption a conformational change in the sugar moiety towards a

glycal type structure in the transition state can be derived[5]. The combination of this structural moiety with a noncleavable CC-bond to the anomeric center suggested the synthesis of compound **1** as target molecule; **1** should be accessible from phosphonate **2** and activated uridine monophosphate.

Scheme 2



For the synthesis of phosphonate **2** β-galactosyl cyanide **3** was employed which can be readily obtained from acetobromogalactose[10] (Scheme 2). Reduction of the cyano to the aldehyde group and then β-elimination of an acetic acid residue to give enal **4**[11] could be carried out with Raney-nickel and sodium dihydrogenhypophosphite (NaH_2PO_2) in pyridine/acetic acid as a one-pot procedure. Reduction of the aldehyde moiety with $NaBH_4$ furnished alcohol **5** which led with methanesulfonyl chloride (MsCl) in the presence of triethylamine to mesylate **6**; subsequent Finkelstein reaction with NaBr in DMF afforded bromide **7**. Michaelis-Arbuzov reaction of **7** with tris-trimethylsilylphosphite gave bis-trimethylsilylphosphonate **8** which upon treatment with sodium methanolate in methanol furnished directly the disodium salt of the desired phosphonate **2a**; treatment of **2a** with ion exchange resin (IE amberlite IR 120, $HNEt_3^+$ form) afforded bis-triethylammonium salt **2b**. The structural assignment of **8** and all intermediates is based on the ^{1}H-NMR data (Table 1).

Compound **8** and structurally related derivatives turned out to be highly unstable. Already traces of acid seem to generate via cleavage of the allylic 4-acyloxy group a resonance-stabilized reactive oxallyl cation species enabling uncontrolled side reactions. However, reaction of **2b** with uridine-5'-morpholidophosphate[12,13] as activated UMP derivative provided the desired target molecule **1** (Scheme 2), which could be isolated and structurally assigned by NMR and MS data (Table 1).

Table 1 Physical Data of **1, 2a, 7, 8** [a]

1: δ_H 1.09 (t, J = 7.2 Hz, 18 H, NCH_2CH_3), 2.51 (d, $J_{1'',P}$ = 19.1 Hz, 2 H, H-1a", H-1b", 3.04 (q, J = 7.2 Hz, 12 H, NCH_2CH_3), 3.57 (dd, $J_{6'',7''}$ = 3.6 Hz, $J_{7a'',7b''}$ = 11.8 Hz, 1 H, H-7b"), 3.72 (m, 2 H, H-4", H-7a"), 4.23-3.89 (m, 6 H, H-2', H-3', H-4', 2 H-5', H-5"), 4.28 (m, 1 H, H-6"), 4.51 (m, 1 H, H-3"), 5.78 (d, $J_{5,6}$ = 7.8 Hz, 1 H, H-5), 5.80 (d, $J_{1',2'}$ = 6.3 Hz, 1 H, H-1'), 7.78 (d, $J_{5,6}$ = 7.8 Hz, 1 H, H-6).

δ_H - 11.1 (d, J = 22 Hz, $P(O)O_3$), 11.7 (d, J = 22 Hz, $CP(O)O_2$)

FAB-MS (70 eV, negative mode), matrix glycerol, m/z (%): 545 (85) $[M + H^+ - 2\ HNEt_3^+]^-$

2a: δ_H 2.07 (d, $J_{1,P}$ = 19.1 Hz, 2 H, H-1, H-1'), 3.48 (dd, $J_{6,7'}$ = 3.6 Hz, $J_{7,7'}$ = 11.8 H, 1 H, H-7'), 3.67 (m, 2 H, H-4, H-7), 3.84 (dd, J = 4.0 Hz, J = 8.1 Hz, 1 H, H-5), 4.21 (m, 1 H, H-6), 4.35 (m, 1 H, H-3)

δ_C 35.9 (d, $J_{C,P}$ = 123.6 Hz, C-1), 61.4 (C-7), 64.9 (C-4, C-5), 77.3 (C-6), 98.4 (d, $J_{C,P}$ = 8.1 Hz, C-3), 147.3 (d, $J_{C,P}$ = 8.8 Hz, C-2).

δ_H 16.0 Hz (s)

FAB-MS (70 eV, negative mode), matrix diethanolamine, m/z (%): 261 (43) $[M\text{-}Na^+]^-$

7: $[\alpha]_D$ - 52.8 (c = 1, $CHCl_3$); δ_H 2.02, 2.08, 2.11 (3 s, 9 H, $COCH_3$), 3.79 (d, $J_{1,1'}$ = 11.0 Hz, 1 H, H-1'), 3.85 (d, $J_{1,1'}$ = 11.0 Hz, 1 H, H-1), 4.21 (dd, $J_{6,7'}$ = 5.3 Hz, $J_{7,7'}$ = 11.1 Hz, 1 H, H-7'), 4.35 (m, 2 H, H-6, H-7), 4.94 (m, 1 H, H-3), 5.41 (m, 1 H, H-4), 5.54 (m, 1 H, H-5)

8: δ_H 0.22 (d, J = 1.2 Hz, 18 H, CH_3), 1.91, 1.98, 2.02 (3 s, 9 H, $COCH_3$), 2.51 (d, $J_{1,P}$ = 21.7 Hz, 2 H, H-1, H-1'), 4.22 (m, 3 H, H-6, H-7, H-7'), 4.62 (m, 1 H, H-3), 5.31 (m, 1 H, H-4), 5.45 (m, 1 H, H-5)

δ_C 0.4, 0.7 (CH_3), 20.3, 20.4, 20.5 (3 C, $COCH_3$), 35.2 (d, $J_{C,P}$ = 146.7 Hz, C-1), 61.4 (C-7), 63.1 (C-5), 64.3 (d, $J_{C,P}$ = 2.0 Hz, C-4), 73.1 (C-6), 97.0 (d, $J_{C,P}$ = 9.5 Hz, C-3), 147.3 (d, $J_{C,P}$ = 10.5 Hz, C-2), 169.7, 169.9, 170.1 (3 C, CO).

[a] Optical rotation at 20°C; ^1H NMR spectra, 250 MHz in $CDCl_3$ (**7, 8**), D_2O (**1, 2a**); ^{13}C NMR spectra, 62.9 MHz in $CDCl_3$ (**8**), D_2O (**2a**); ^{31}P NMR spectra, 161.7 MHz in D_2O

For the inhibition studies with **1** and **2a** β-galactosyl transferase from bovine milk[14] was employed and lactose formation from UDP-Gal and D-glucose (ratio from 1 : 30 to 1 : 2000) investigated. The rate of product formation was quantitatively persued through the release of UDP which was measured via a known pyruvate kinase and lactate dehydrogenase sequence resulting finally in NADH consumption[15]. The investigations were carried out at different concentrations (**1** : 0-200 μM; **2a** : 0-600 μM); treatment of the kinetic data according to Morrison and Ebner[16] led to the results compiled in Table 2 which indicate competitive inhibition for **1** and **2a**. Comparison of the inhibition constants K_i with literature values shows that **1** exhibits high affinity towards galactosyltransferase. Thus, for this new inhibitor type promising perspectives can be envisaged via structural modifications which favor tighter binding to the active site.

Acknowledgments

This work was supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie.

Table 2. Inhibition Constants (K_i values)[a]

Compound	K_M [M][b]	K_i [M]	References
II	$1.25 \cdot 10^{-5}$	$9.69 \cdot 10^{-5}$	8
III	$1.37 \cdot 10^{-5}$	$1.65 \cdot 10^{-4}$	9
UTP	$1.37 \cdot 10^{-5}$	$1.28 \cdot 10^{-4}$	9
2a	$2.64 \cdot 10^{-5}$	$1.43 \cdot 10^{-3}$ [c]	-
1	$2.64 \cdot 10^{-5}$	$6.20 \cdot 10^{-5}$ [c]	-

[a] For the determination, see ref. 16

[b] K_M value for UDP-Gal

[c] Referred to the K_M value of $1.3 \cdot 10^{-5}$ [M] reported for UDP-Gal in ref. 8,9 lower K_i values for **1** and **2a** can be expected

References and Notes

1. Schmidt, R.R. *Angew. Chem.* **1986**, *98*, 213-236; *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 212-235; *Pure Appl. Chem.* **1989**, *61*, 1257-1270; Paulsen, H. *Angew. Chem.* **1990**, *102*, 851-867; *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 823-839; and ref. therein.
2. Hakomori, S. *J. Biol. Chem.* **1990**, *265*, 18713-18716; and ref. therein.
3. Schwarzmann, G.; Sandhoff, K. *Biochemistry* **1990**, *29*, 10865-10871; Paulson, J.C.; Colley, K.J. *J. Biol. Chem.* **1989**, *264*, 17615-17618; and ref. therein.
4. Schmidt, R.R. Lecture, GDCh-Hauptversammlung, München, Sept. 1991.
5. Sinnott, L.M. in Page, M.I.; Williams, A. (Hrsg.): *Enzyme Mechanisms*, The Royal Society of Chemistry, London 1987, p. 259-297; *Chem. Rev.* **1990**, *90*, 1171-1202; and ref. therein.
6. Schmidt, R.R. XIVth Int. Carbohydr. Sympl., PL 8, Stockholm, Aug. **1988**; Schmidt, R.R.; Gaden, H.; Jatzke, H. *Tetrahedron Lett.* **1990**, *31*, 327-330; Schmidt, R.R. in *Carbohydrates - Synthetic Methods and Application in Medicinal Chemistry* (Eds. Ogura, H;Hasegawa, A.; Suami, T.), Kodanasha Scientific Ltd., Tokyo 1992, 68-88.
7. Palcic, M.M.; Heerze, L.D.; Srivastava, O.P.; Hindsgaul, O. *J. Biol. Chem.* **1989**, *264*, 17174-17181; Noort, D.; van der Marel, G.A.; van der Gen, A.; Mulder, G.J.; van Boom, J.H. *Recl. Trav. Chim. Pays-Bas* **1991**, *110*, 53-56; and ref. therein.
8. Vaghefi, M.M.; Bernacki, R.J.; Hennen, W.J.; Robins, R.K. *J. Med. Chem.* **1987**, *30*, 1391-1399.
9. Vaghefi, M.M.; Bernacki, R.J.; Dalley, N.K.; Wilson, B.E.; Robins, R.K. *J. Med. Chem.* **1987**, *30*, 1383-1391.
10. Nyers, R.W.; Lee, Y.C. *Carbohydr. Res.* **1984**, *132*, 61-82.
11. Dettinger, H.M.; Kurz, G.; Lehmann, J. *Carbohydr. Res.* **1979**, *74*, 301-307.
12. Moffatt, J.G.; Khorana, H.G. *J. Am. Chem. Soc.* **1961**, *83*, 649-658.
13. Heinz, E.; Schmidt, H.; Hoch, M.; Jung, K.-H.; Binder, H.; Schmidt, R.R. *Eur. J. Biochem.* **1989**, *184*, 445-453; Schmidt, R.R.; Wegmann, B.; Jung, K.-H. *Liebigs Ann. Chem.* **1991**, 121-124.
14. Ebner, K.E; Dentaon, W.L.; Brodbeck, U. *Biochem. Biophys. Res. Commun.* **1966**, *24*, 232-236.
15. Fitzgerald, D.K.; Colvin, B.; Maval, R.; Ebner, K.E. *Anal. Biochem.* **1970**, *36*, 43-61; Palcic, M.M.; Heerze, L.D.; Pierce, M.; Hindsgaul, O. *Glycoconjugate J.* **1988**, *5*, 49-63.
16. Morrison, J.F.; Ebner, K.E. *J. Biol. Chem.* **1971**, *246*, 3977-3984; 3985-3991; 3992-3998; Berliner, L.J.; Wong, S.S. *Biochemistry* **1975**, *14*, 4977-4982.