Carbohydrate Research, 245 (1993) 137–144
Elsevier Science Publishers B.V., Amsterdam

Chemical synthesis and kinetic characterization of UDP-2-deoxy-D-lyxo-hexose("UDP-2-deoxy-D-galactose"), a donor-substrate for β-(1 → 4)-D-galactosyltransferase

Geeta Srivastava, Ole Hindsgaul * and Monica M. Palcic

Department of Chemistry, University of Alberta, Edmonton, Alberta T6G 2G2 (Canada)

(Received October 16th, accepted in revised form December 30th, 1992)

ABSTRACT

Bovine β-(1 → 4)-galactosyltransferase (GalT) transfers galactose from UDP-galactose to β-D-Glc*p*NAc-terminating oligosaccharides to produce *N*-acetyllactosamine sequences. We report here the chemical synthesis, structural characterization and enzymatic evaluation of the very labile UDP-2-deoxy-D-*lyxo*-hexose ("UDP-2-deoxy-galactose," **2**) as an alternate donor for GalT. Donor **2** had kinetic parameters, including a K_m value of 51 μM, almost identical to those for the natural substrate UDP-galactose when β-D-Glc*p*NAc-O$(CH_2)_8$COOMe was used as the acceptor. The product of the enzymatic transfer was isolated and confirmed to have the expected 2′-deoxy-*N*-acetyllactosamine sequence.

INTRODUCTION

An increasing number of glycosyltransferases are being cloned with the promise that these will soon become available as tools to assist in the rapid assembly of complex oligosaccharides[1,2]. Clearly, these enzymes can be utilized to perform in vitro the same function that they perform in vivo, i.e., the cloned glycosyltransferases can be used to add the natural sugars from their natural sugar nucleotides to the natural endogenous oligosaccharide acceptors[3,4]. It is not so clear, however, the extent to which glycosyltransferases can add unnatural sugars and thus become more generally useful in the synthesis of oligosaccharide analogs.

Several laboratories, including our own, have reported that sugar nucleotide analogues containing deoxygenated and otherwise modified sugars can indeed act as donors for glycosyltransferases[5–13]. The only readily available commercial glycosyltransferase, bovine β-(1 → 4)-galactosyltransferase (GalT, EC 2.4.1.90), has also been extensively studied in this regard. The natural donor substrate for this

* Corresponding author.

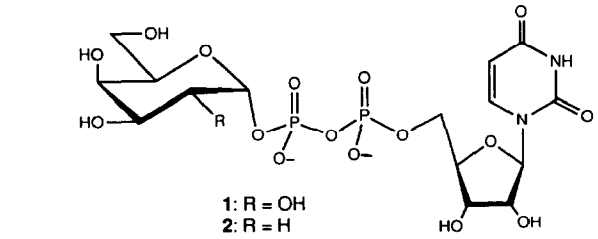
enzyme is UDP-galactose (UDP-Gal, **1**), but it has been shown to transfer also a variety of modified galactose residues from their UDP-Gal derivatives[6,12,13]. These include 3-deoxy- and 4-deoxy-D-*xylo*-hexose (3-deoxy- and 4-deoxy-D-galactose) that transfer at rates of 0.15 and 4%, respectively, of that of the native galactose. Several analogues of CMP-sialic acid have also been shown to be good substrates for a panel of sialyltransferases[8,9].

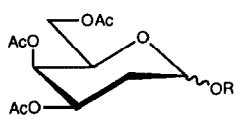
Recently, Thiem and Wiemann[14,15] reported that 2-deoxy-D-*arabino*-hexose 6-phosphate ("2-deoxy-D-glucose 6-phosphate") could be used with an in situ recycling enzyme system which sequentially converted it to 2-deoxy-glucose 1-phosphate, then to UDP-2-deoxy-D-glucose which was subsequently epimerized to UDP-2-deoxy-Gal (UDP-2-deoxy-D-*lyxo*-hexopyranose, **2**). GalT could then utilize this compound as a donor, and, in the presence of D-GlcNAc as the acceptor, 2′-deoxy-*N*-acetyllactosamine was produced in 40% yield. Wong et al.[16] subsequently showed that UDP-2-deoxy-Gal could be prepared in situ by a more direct recycling system wherein 2-deoxy-Gal was directly 1-O-phosphorylated using galactose kinase. The glycosylphosphate was then converted transiently, in a recycling system, to UDP-2-deoxy-Gal, which served as a donor for the synthesis of 2′-deoxy-*N*-acetyllactosamine (36% yield). These two papers significantly widen the use of deoxygenated sugar nucleotides as potential donors for the preparative synthesis of deoxy oligosaccharides. 2′-Deoxy-*N*-acetyllactosamine is an inhibitor for an α-(1 → 2)-fucosyltransferase[17].

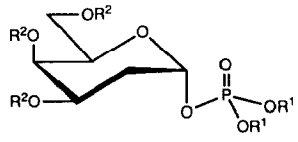
The UDP-2-deoxy-Gal that served as the donor in the studies described above was formed only transiently, in equilibrium with other structures, and could be neither isolated nor kinetically characterized as a donor substrate in the enzymatic transfer reaction. Glycosyltransferase reactions occurring with inversion of configuration at the anomeric center of the transferring sugar are likely to proceed via a cyclic oxo-carbonium ion[17–19]. UDP-2-deoxy-Gal is expected to form such a carbonium ion much more easily than UDP-Gal due to the removal of the electronegative substituent at C-2 (ref. 18). The rate of acid-catalyzed hydrolysis of 2-deoxy-D-glucose 1-phosphate, for example, is ~ 2500 times faster than that of D-glucose 1-phosphate[20]. On the other hand, if OH-2 of UDP-Gal is an important recognition element for the GalT, then the intrinsic ease of formation of the oxo-carbonium ion would be offset by a decreased efficiency in the enzyme catalysis. It was therefore possible that UDP-2-deoxy-Gal was either a very good or a very poor donor substrate, compared to UDP-Gal. In order to better understand the donor recognition properties of GalT, we therefore decided to chemically synthesize and kinetically characterize UDP-2-deoxy-galactose as a donor substrate.

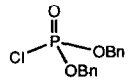
RESULTS AND DISCUSSION

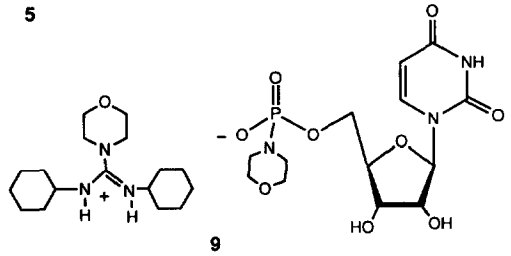
Literature reports exist on the preparation of UDP-2-deoxy-D-*arabino*-hexose ("UDP-2-deoxy-glucose") using the McDonald procedure[21,22]. This reaction involves the fusion of a peracetylated sugar with anhydrous phosphoric acid. Use of







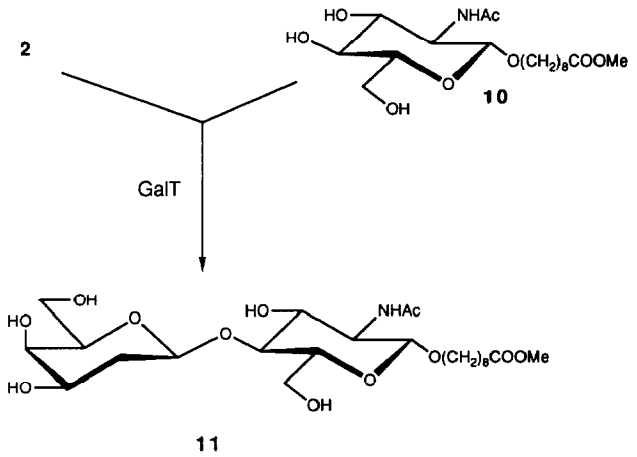




Scheme 1.

this procedure could be predicted to be problematic since the sought-after product, in this case 3,4,6-tri-*O*-acetyl-2-deoxy-D-*lyxo*-hexose 1-phosphate, is itself very acid sensitive. Indeed, it has been reported that the originally described procedure produces a complex mixture of products[20,23]. We therefore elected, as in the past[13], to employ reactions for syntheses of deoxy-glycosyl-phosphates which proceed under basic conditions.

Commercial 2-deoxy-D-*lyxo*-hexose ("2-deoxy-D-galactose") was peracetylated to produce **3** (Scheme 1) with the expectation that the 1-*O*-acetyl group could be selectively removed by the extraordinarily useful hydrazinolysis procedure of Excoffier et al.[24]. Surprisingly, this generally reliable method produced only complex mixtures in which the desired **4** was a minor component. Likewise, a mild deacetylation procedure[25] using KOH–2-propanol failed. We suspect that the lack of an electronegative substituent at C-2 in **3** renders the 1-*O*-acetyl group much less electrophilic at the carbonyl carbon and that the other *O*-acetyl groups



Scheme 2.

therefore have similar reactivity. Compound **4** was finally obtained on mild acid hydrolysis of the anomeric acetate in **3** in a yield of 50%. Ionization (BuLi, −78°C) of OH-1 in **4**, followed by phosphoryation[26] using dibenzylphosphorochloridate (**5**), yielded the phosphotriester **6**, which decomposed on attempted purification by silica gel chromatography. This product was therefore directly hydrogenated in the presence of triethylamine to give the glycosyl phosphate **7** (27% from **4**), which was *O*-deacetylated and converted to its sodium salt **8**. Condensation[27] of **8** with uridine 5′-monophosphate morpholidate (**9**), followed by purification of the product by ion-exchange chromatography, then yielded the required UDP-2-deoxy-Gal (**2**, 25%). The purity of **2** achieved was only ~80% with co-chromatographing uridine monophosphate (20%) contaminating the sample. All attempts to further purify this very sensitive material by ion-exchange chromatography resulted in extensive decomposition. It was therefore used as such in the following kinetic experiments.

The deoxy sugar-nucleotide **2** (see Scheme 2) was evaluated as a donor substrate for the commercial GalT using the known[28] acceptor, 8-methoxycarbonyloctyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**10**, K_m 134 μM). Using a published spectrophotometric assay method[29], which couples the formation of UDP to the reduction of NADH, the rate of transfer of a sugar from a UDP derivative can be estimated. In this manner the kinetic parameters for the transfer of galactose from UDP-Gal to **10** were found to be $K_m = 44 \pm 2$ μM with $V_{max} = 5.50 \pm 0.09$ μmol/min under standard assay conditions. Under identical conditions, UDP-2-deoxy-Gal (**2**) had $K_m = 51 \pm 6$ μM and $V_{max} = 4.50 \pm 0.17$ μmol/min. Although the sample of **2** used to derive these kinetic parameters was not pure, the contaminating UMP is known[30] to be a poor inhibitor for GalT and should therefore not imporantly affect these kinetic parameters. This prediction was experimentally verified by independently measuring the kinetic parameters for

UDP-Gal in the presence of 20% added UMP. As predicted, there was no effect of added UMP on V_{max} and the measured K_m was increased by 18%. For all practical purposes the kinetic parameters for UDP-Gal and UDP-2-deoxy-Gal are therefore the same.

In order to ascertain that the spectrophotometric assay was indeed measuring the rate of transfer of 2-deoxy-Gal to OH-4 of **10**, a preparative enzymatic reaction was performed. The expected 2′-*N*-acetyllactosamine derivative **11** (88%) was isolated and its structure confirmed by comparison of its ^{1}H NMR spectrum with that of a previously chemically synthesized sample[31]. The product also showed the expected molecular weight in the fast atom bombardment mass spectrum (M + Na, *m/z* 560). Deoxygenation of C-2 of the galactosyl residue in UDP-Gal is thus demonstrated to have an insignificant effect on its efficiency as a donor for GalT.

EXPERIMENTAL

General methods.—TLC was performed on Silica Gel 60-F_{254} (E. Merck) with detection by quenching of fluorescence or by charring with H_2SO_4. Unless otherwise noted, column chromatography was performed on Silica Gel 60 (E. Merck, 40–63 μm). Iatrobead refers to a beaded silica gel 6RS-8060 manufactured by Iatron Laboratories (Tokyo). C_{18} SepPak sample-preparation cartridges were from Waters Associates. UDP-galactose, UMP-morpholidate and β-(1 → 4)galactosyltransferase were from Sigma Chemical Co. Optical rotations were measured with a Perkin–Elmer 241 polarimeter at 22 ± 2°C. ^{1}H NMR spectra were recorded at 300 MHz (Bruker AM-300) or at 360 MHz (Bruker WM-360) in $CDCl_3$ or CD_3OD (internal Me_4Si, δ 0), or D_2O (internal acetone, δ 2.225). ^{13}C NMR spectra were recorded at 75 MHz on the same instruments in $CDCl_3$ or CD_3OD (internal Me_4Si, δ 0) or D_2O (external dioxane (1%), δ 67.4). Only partial NMR data are reported, as the other data were in accord with the proposed structures. FAB mass spectra were obtained on a Kratos AEIMS9 instrument with Xe as the bombarding gas and the sample suspended in Clelland's matrix (1:5 1,4-dithiothreitol–1,4-dithioerythritol). Elemental analyses were carried out on a Carlo Erba EA1108 instrument.

3,4,6-Tri-O-acetyl-2-deoxy-α,β-D-lyxo-hexopyranose (**4**).—A solution of **3** (1.1 g, 3.31 mmol) in 4:1 acetic acid–H_2O (30 mL) was kept for 15 h at 40°C and a further 6 h at 80°C, then concentrated. Column chromatography (1:1 hexane-EtOAc) of the residue gave **4** (487.5 mg, 50.7%) as an approximately 3:1 mixture of α,β anomers: R_f 0.28 (1:1 hexane–EtOAc). NMR data ($CDCl_3$): ^{1}H, δ 5.50 (d, $J_{3,4}$ 2.5 Hz, H-4α), 5.26 (d, $J_{3,4}$ 3.0 Hz, H-4α), 4.42 (dd, $J_{5,6} = J_{5,6'} = 7.0$ Hz, H-5α), 3.86 (dd, $J_{5,6} = J_{5,6'} = 7.0$ Hz, H-5β), 3.25 (br, OH), 2.15, 2.05, 2.00 (each s, αAc).

3,4,6-tri-O-acetyl-2-deoxy-α-D-lyxo-hexopyranosyl phosphate.—(a) Mixed pyridinium–triethylammonium salt (**7**). To a solution of **4** (381.9 mg, 1.32 mmol) in dry tetrahydrofuran (8 ml) was added 1.6 M butyllithium in hexane (813 μL, 1.32 mmol) at −70°C. After 5 min, a solution of dibenzyl phosphochloridate (**5**; 1.9 mL,

6.58 mmol) in tetrahydrofuran (2 mL) was added slowly at the same temperature, and the mixture was stirred for a further 10 min at −60°C. Triethylamine was added to pH 8. The crude product **6** decomposed on attempted column chromatography and was therefore hydrogenated in the presence of Et_3N (200 μL) and MeOH (5 ml), using 5% Pd–C (190 mg). Removal of the catalyst and evaporation of the solvent gave a syrup which was purified by chromatography (60:35:6 CH_2Cl_2–MeOH–H_2O containing 0.02% Et_3N) on Iatrobeads. A drop of pyridine was added to all fractions (~2 mL) containing **7**, and these fractions were then pooled and concentrated to leave a clear syrup (170 mg, 27.4%): R_f 0.42 (60:35:6 CH_2Cl_2–MeOH–H_2O). NMR data (CD_3OD): ^1H, δ 5.73 (m, 1 H, $J_{1,P}$ 7.0 Hz, H-1), 5.29–5.36 (2 H, H-2 and 3), 4.43 (dd, 1 H, $J_{5,6} = J_{5,6'} = 7.0$ Hz, H-5), 4.15 (dd, 1 H, $J_{6,6'}$ 10.5 Hz, H-6), 4.01 (dd, 1 H, H-6′), 2.15–1.95 (2H, H-2), 2.09, 1.98, 1.94 (each s, 3 H, Ac); ^{13}C, δ 172.2 (2 × $COCH_3$), 171.7 ($COCH_3$), 95.4 (d, $J_{C,P}$ 5 Hz, C-1), 68.8, 67.8, 67.5 (C-3,4,5), 62.9 (C-6), 32.0 (d, $J_{2,P}$ 7 Hz, C-2), 20.8, 20.6, 20.5 (3 × $COCH_3$).

(b) Disodium salt (**8**). Compound **7** (150 mg, 0.32 mmol) was dissolved in 0.5 M NaOH (10 mL) and stirred for 3 h at room temperature. The solution was neutralized with Dowex 50 (triethylammonium) resin, filtered, and concentrated, and the residue was passed through a column of Dowex 50 X-8 (Na^+) resin (20 mL) to give **8** as a white foam (85 mg, 92.7%): $[\alpha]_D$ +16.9° (*c* 0.86, H_2O), R_f 0.14 (7:3:1 2-propanol–H_2O–NH_4OH). NMR data (D_2O): ^1H, δ 5.57 (ddd, 1 H, $J_{1,2a} = J_{1,2e} = 2.0$, $J_{1,P}$ 7.5 Hz, H-1), 4.18–4.08 (m, 2 H, H-3 and 5), 3.85 (d, 1 H, $J_{3,4}$ 2.5 Hz, H-4), 3.78–3.66 (m, 2 H, H-6′s), 1.9 (m, 2 H, H-2a and 2e); ^{13}C, δ 94.6 (d, $J_{C,P}$ 5 Hz, C-1), 72.5, 70.2, 68.6 (C-3,4,5), 62.6 (C-6), 33.4 (d, $J_{2,P}$ 6 Hz, C-2); ^{31}P: δ 1.52. Anal. Calcd for $C_6H_{11}Na_2O_8P \cdot H_2O$: C, 23.54; H, 4.28. Found: C, 23.92; H, 4.69.

Uridine 5′-(2-deoxy-α-D-lyxo-hexopyranosyl diphosphate), disodium salt (**2**).—A solution of 4-morpholine *N,N′*-dicyclohexyl carboxamidinium uridine 5′-phosphoromorpholidate (**9**, 82.6 mg, 0.12 mmol) in anhyd pyridine (5 mL) was concentrated to dryness in vacuo. The process of dissolution and concentration was repeated thrice, dry N_2 being admitted into the flask after each concentration. A solution of disodium salt (**8**; 35 mg, 0.12 mmol) in water was passed slowly through a column of Dowex 50 X-8 (triethylammonium) resin, and the column was thoroughly washed with water. The eluate was evaporated with pyridine and concentrated in vacuo to ~2 mL, and a solution of trioctylamine (105 μL, 0.24 mmol) in pyridine was added. The solution was then concentrated to dryness, and the residue was rendered anhydrous by four times dissolving it in dry pyridine and evaporating the solvent in vacuo. A solution of the residue in pyridine was added to the dry phosphomorpholidate described above. The solution was concentrated in vacuo, and a solution of the residue in anhydrous pyridine (2 mL) was kept at room temperature for 5 days. Concentration of the product gave a syrup, which was diluted to a volume of 50 mL with water. It was then slowly passed through Dowex X-2-200 (2.5 × 15 cm, Cl^-). The column was washed with water (100 mL)

and then eluted with a linear gradient of LiCl. The mixing vessel contained 600 mL water. The reservoir contained 600 mL of 0.7 M LiCl in water. The column was operated at 23°C at a flow rate of 2 mL/min, and 15-mL fractions were collected with monitoring at 280 nm. The fractions eluting at 0.27 M LiCl were pooled and adsorbed on Darco G-60 (0.25g). The charcoal was collected by centrifugation and washed three times with water (40 mL). The product was eluted from the charcoal by three washes (25 mL) with 100:100:1 EtOH–H_2O–NH_4OH. Adsorption and elution were carried out at 23°C. The eluate was concentrated to a small volume and converted into the disodium salt by passage through the Dowex 50 X-8 (Na^+) resin and lyophilized to give the product **2** (18 mg, 25.2%), R_f 0.52 (3:1 2-propanol–H_2O). NMR data (D_2O): ^1H, δ 7.95 (d, 1 H, $J_{5,6}$ 8.0 Hz, H-6), 5.95 (2 H, H-1′ and 5), 5.94 (d, 1 H, H-5), 5.72 (m, 1 H, $J_{1'',P}$ 7.0 Hz, H-1″), 3.87 (d, 1H, $J_{3'',4''}$ 3.0 Hz, H-3″), 2.05–1.85 (m, 2 H, H-2); ^{13}C, δ 95.8 (d, $J_{1'',P}$ 5.0 Hz, C-1″), 89.2 (C-1′), 84.0 (d, $J_{4',P}$ 9 Hz, C-4′), 74.6, 72.9, 70.4, 68.4, 65.3 (C-2′,3′,3″,4″,5″), 65.7 (d, $J_{5',P}$ 5 Hz, C-5′), 62.3 (C-6″), 32.9 (C-2″); ^{31}P (D_20), δ −11.35 and −13.25 (each d, $J_{P,P}$ 21 Hz).

*Enzymatic Synthesis. 8-Methoxycarbonyloctyl 2-acetamido-2-deoxy-4-*O*-(2-deoxy-β-*D*-lyxo-hexopyranosyl)-β-*D*-glucopyranoside* (**11**).—A solution of 8-methoxycarbonyl octyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**10**, 1.40 mg, 3.6 μmol), galactosyltransferase (5 unit), and UDP-2-deoxy-galactose (4.26 mg, 7.2 μmol) in 100 mM sodium cacodylate (11 mL; pH 7.5) containing 10 mM $MnCl_2$ was kept at ambient temperature for 24 h. TLC examination of the mixture in 40:10:1 $CHCl_3$–MeOH–H_2O showed complete conversion into a new product (R_f 0.26). The mixture was diluted with water (10 mL) and passed directly onto a SepPak C_{18} cartridge that had been prewashed with 20 mL of each of MeOH, 1:1 $CHCl_3$–MeOH, 1:1 MeOH–H_2O, and finally water. The cartridge was washed with water (20 mL), and then product (**3**) was eluted with MeOH (20 mL). Evaporation left a residue that was purified by chromatography (40:10:1 CH_2Cl_2–MeOH–H_2O) on Iatrobeads to give **3** (1.7 mg, 88.3%) as a white powder. NMR data (D_2O): ^1H, δ 4.712 (dd, 1 H, $J_{1',2'a}$ 9.5, $J_{1',2'e}$ 2.0 Hz, H-1′), δ 4.510 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 3.687 (s, 3 H, OMe), 2.389 (t, 2 H, J 7.5 Hz, CH_2COO), 2.086 (ddd, 1 H, $J_{2a,2e}$ 11.5, $J_{2e,3}$ 4.5 Hz, H-2e), 2.030 (s, 3 H, NAc), 1.695 (ddd, 1 H, $J_{2a,3}$ 11.5, H-2a); ^{13}C, δ 175.2 ($COOCH_3$), 101.9 and 101.4 (C-1 and 1′), 62.2 and 61.0 (C-6 and 6′), 56.0 (C-2), 52.9 ($COOCH_3$), 34.5 (CH_2COOCH_3), 34.2 (C-2′), 23.0 ($COCH_3$); the FAB mass spectrum showed the expected intense signal at m/z 560 ($M + Na^+$).

Enzyme Kinetics.—The initial velocities of the enzymatic reactions were determined by measuring the rate of UDP formation at 37°C using a modification of the method of Fitzgerald et al.[29]. All reactions were carried out in plastic cuvettes containing 1 mM phosphenolpyruvate, 50 units of lactate dehydrogenase, 0.34 mM NADH, 56 mM KCl, 8 mM $MnCl_2$, 50 units of pyruvate kinase, 920 μM **10**, and 5 munits of galactosyltransferase in a total volume of 0.89 mL. Six different concentrations of **1** or **2** were used in the assays varying from 11 to 437 μM. The decrease in absorbance at 340 nm was monitored with a Beckman DU-8 spectrophotometer,

and reaction rates were estimated using a millimolar extinction coefficient of 6.2 mM^{-1} cm^{-1} for NADH oxidation. The initial rate data were analyzed by the statistical method of Wilkinson[32].

ACKNOWLEDGMENT

This work was supported by the Natural Sciences and Engineering Research Council of Canada through Research/Operating grants to both O.H. and M.M.P.

REFERENCES

1 J.C. Paulson and K.C. Colley, *J. Biol. Chem.*, 264 (1989) 17615–17618.
2 J.B. Lowe, *Semin. in Cell Biol.*, 2 (1991) 289–307.
3 H. Schachter, *Current Opin. Struct. Biol.*, 1 (1991) 755–765.
4 Y. Ichikawa, G.C. Look, and C.-H. Wong, *Anal. Biochem.*, 202 (1992) 215–238.
5 V.N. Shibaev, *Pure Appl. Chem.*, 50 (1978) 1421–1436.
6 L.J. Berliner and R.D. Robinson, *Biochemistry*, 21 (1982) 6340–6343.
7 W. McDowell, T.J. Grier, J.R. Rasmussen, and R.T. Schwarz, *Biochem. J.*, 248 (1987) 523–531.
8 H.J. Gross, A. Bunsch, J.C. Paulson, and R. Brossmer, *Eur. J. Biochem.*, 168 (1987) 595–602.
9 H.J. Gross, U. Rose, J.M. Krause, J.C. Paulson, K. Schmid, R.E. Feeney and R. Brossmer, *Biochemistry*, 28 (1989) 7386–7392.
10 U.B. Gokhale, O. Hindsgaul, and M.M. Palcic, *Can. J. Chem.*, 68 (1990) 259–276.
11 G. Srivastava, G. Alton, and O. Hindsgaul, *Carbohydr. Res.*, 207 (1990) 259–276.
12 M.M. Palcic and O. Hindsgaul *Glycobiology*, 1 (1991) 205–209.
13 O. Hindsgaul, K.J. Kaur, U.B. Gokhale, G. Srivastava, G. Alton, and M.M. Palcic, *ACS Symp. Ser.*, 466 (1991) 38–50.
14 J. Thiem and T. Wiemann, *Angew. Chem. Int. Ed. Engl.*, 30 (1991) 1163–1164.
15 J. Thiem and T. Wiemann, *Synthesis*, (1992) 141–145.
16 C.-H. Wong, R. Wang and Y. Ichikawa, *J. Org. Chem.*, 57 (1992) 4343–4344.
17 O. Hindsgaul, K.J. Kaur, G. Srivastava, M. Blaszczyk-Thurin, S.C. Crawley, L.D. Heerze, and M.M. Palcic, *J. Biol. Chem.*, 266 (1991) 17858–17862.
18 B. Capon, *Chem. Rev.*, 69 (1969) 407–498.
19 M.L. Sinnot, in M.I. Page (Ed.), *The Chemistry of Enzyme Action* , New Comprehensive Biochemistry Ser., Vol. 6, Elsevier, Amsterdam, 1984, pp 389–431.
20 S.G. Withers, M.D. Percival, and I.P. Street, *Carbohydr. Res.*, 187 (1989) 43–66.
21 V.N. Shibaev, Y.Y. Kusov, S. Kuchar, and N.K. Kochetkov, *Izv. Akad. Nauk SSSR, Ser. Khim.*, (1973) 922–926.
22 W. McDowell and R.T. Schwarz, *FEBS Lett.*, 243 (1989) 413–416.
23 M.D. Percival and S.G. Withers, *Can. J. Chem.*, 66 (1988) 1970–1972.
24 G. Excoffier, D. Gagnaire, and J.P. Utille, *Carbohydr. Res.*, 39 (1975) 368–373.
25 K. Watanabe, K. Itoh, Y. Araki, and Y. Ishido, *Carbohydr. Res.*, 154 (1986) 165–176.
26 M. Inage, H. Chaki, S. Kusumoto, and T. Shiba, *Chem. Lett.*, (1982) 1281–1284.
27 S. Roseman, J.J. Distler, J.G. Moffat, and H.G. Khorana, *J. Am. Chem. Soc.*, 83 (1961) 659–663.
28 M.M. Palcic, L.D. Heerze, M. Pierce, and O. Hindsgaul, *Glycoconj. J.*, 5 (1988) 49–63.
29 D.K. Fitzgerald, B. Colvin, R. Mawal and K.E. Ebner, *Anal. Biochem.*, 36 (1970) 43–61.
30 J.T. Powell and K. Brew, *Biochemistry*, 15 (1976) 3499–3505.
31 D.P. Khare, O. Hindsgaul, and R.U. Lemieux, *Carbohydr. Res.*, 136 (1985) 285–308.
32 G.N. Wilkinson, *Biochem. J.*, 80 (1961) 324–332.