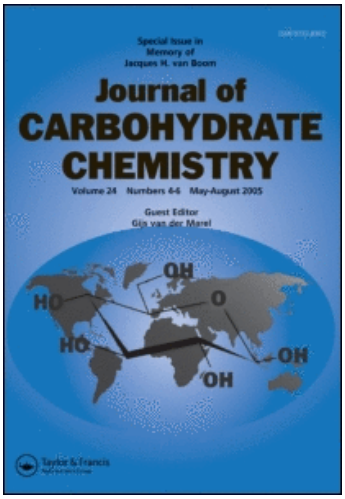

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Combined Chemical and Enzymatic Synthesis of a Disialylated Tetrasaccharide Analogous to M and N Bloodgroup Determinants of Glycophorin a

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COMBINED CHEMICAL AND ENZYMATIC SYNTHESIS OF A DISIALYLATED TETRASACCHARIDE ANALOGOUS TO M AND N BLOODGROUP DETERMINANTS OF GLYCOPHORIN A

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ABSTRACT

Reaction of 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide (1) with phenyl 2-acetamido-2-deoxy-4,6-O-(4-methoxybenzylidene)-α-D-galactopyranoside (3) mediated by mercuric salts, followed by removal of the 4-methoxybenzylidene group and O-deacylation afforded phenyl 2-acetamido-2-deoxy-3-O-β-D-galactopyranosyl-α-D-galactopyranoside (6). Compound 6 was used as a substrate for the selective introduction of two neuraminic acid residues with partially purified sialyltransferase preparations. First, disaccharide 6 was treated with CMP-[^{14}C]-NeuAc as donor substrate and CMP-NeuAc: Gal-β(1-3)-GalNac-α(2-3)sialyltransferase from human placenta to afford trisaccharide 7 (yield 85%), sialylated at C-3 of the galactose residue. Treatment of 7 with CMP-[^{3}H]-NeuAc and a microsomal fraction from regenerating rat liver, containing the CMP-NeuAc: NeuAc-α(2-3)-Gal-β(1-3)GalNAc-α(2-6) sialyltransferase activity, gave the disialylated tetrasaccharide 8 in 10% yield.

INTRODUCTION

The biological importance of sialic acids is reflected by their widespread occurrence in nature. They are found in glycolipids and glycoproteins[1].

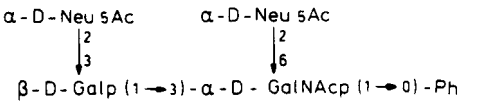

FIG. 1.

Several microorganisms also contain large polymers of sialic acids as a part of their capsular polysaccharides[2]. Not only do many kinds of sialic acids exist, but they are also involved in a great variety of linkages[1].

Organic synthesis of sialylated carbohydrates is difficult due to the instability of sialic acid and the ketosidic character of this sugar. Therefore, relatively few sialylated saccharides have been prepared[3-13]. Despite the application of several catalysts,[mercuric(II) cyanide and bromide, silver carbonate, silicate or triflate] anomeric mixtures were obtained in all cases. Consequently, the pure desired products were obtained in modest yields (20-40%).

We have shown that partially purified sialyltransferase preparations could be used in the synthesis of sialylated milk sugars[14]. The preparation of several sialylated oligosaccharides by combined chemical and enzymatic synthesis was also recently reported by Sabesan et al.,[15] and Thiem et al.[16]. We now report on the application of sialyltransferases as biocatalysts in the regio- and stereospecific synthesis of a disialylated tetrasaccharide, analogous to M and N blood-group determinants of Glycophorin A (see FIG. 1).

RESULTS AND DISCUSSION

Treatment of commercially available phenyl-2-acetamido-2-deoxy-α-D-galactopyranoside (2) with 4-methoxybenzaldehyde

dimethyl acetal and acid catalyst afforded compound 3 in excellent yield. Aglycon 3 thus obtained was glycosylated with 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide in the presence of $Hg(CN)_2$ and $HgBr_2$[17-19]. Silica gel column chromatography, followed by gel filtration on a Sephadex LH-20 column gave disaccharide 4 as a white solid in 45% yield. Hydroxyl protecting groups were then removed. The 4-methoxybenzylidene acetal was completely hydrolysed by treatment with dilute acetic acid. Zemplén deacylation of compound 5 followed by neutralization with a cation-exchange resin (H^+-form) afforded dimer 6. The analytical data (^1H NMR, see Table 1) agree well with those reported for similar "β-D-Galp-(1-3)-α-D-GalNAcp-O-R" structures[17,18]. In combination with the finding that two sialic acid residues can be incorporated in a one to one ratio into disaccharide 6 (see below), we conclude that during glycosylation no isomerization of the dioxane acetal[20] in compound 3 into a dioxolane derivative has taken place. Such isomerization would have led to extension at C-6.

Unprotected disaccharide 6 was used as a substrate for the regio- and stereoselective introduction of two neuraminic acid residues using partially purified sialyltransferase preparations. In order to introduce a sialic acid group at C-3' of compound 6, a CMP-NeuAc: Galβ(1-3)GalNAc-α2-3-sialyltransferase (α2-3STase) isolated from human placenta[21] was incubated with 6 in aqueous buffer using CMP-[^{14}C]NeuAc as donor substrate. Compound 7 was isolated by Bio-Gel P-4 gel filtration and purified by HPLC (see Experimental).

As can be seen in Table 1 the neuraminic acid group was attached at C-3' of the D-galactose residue in an α-linkage

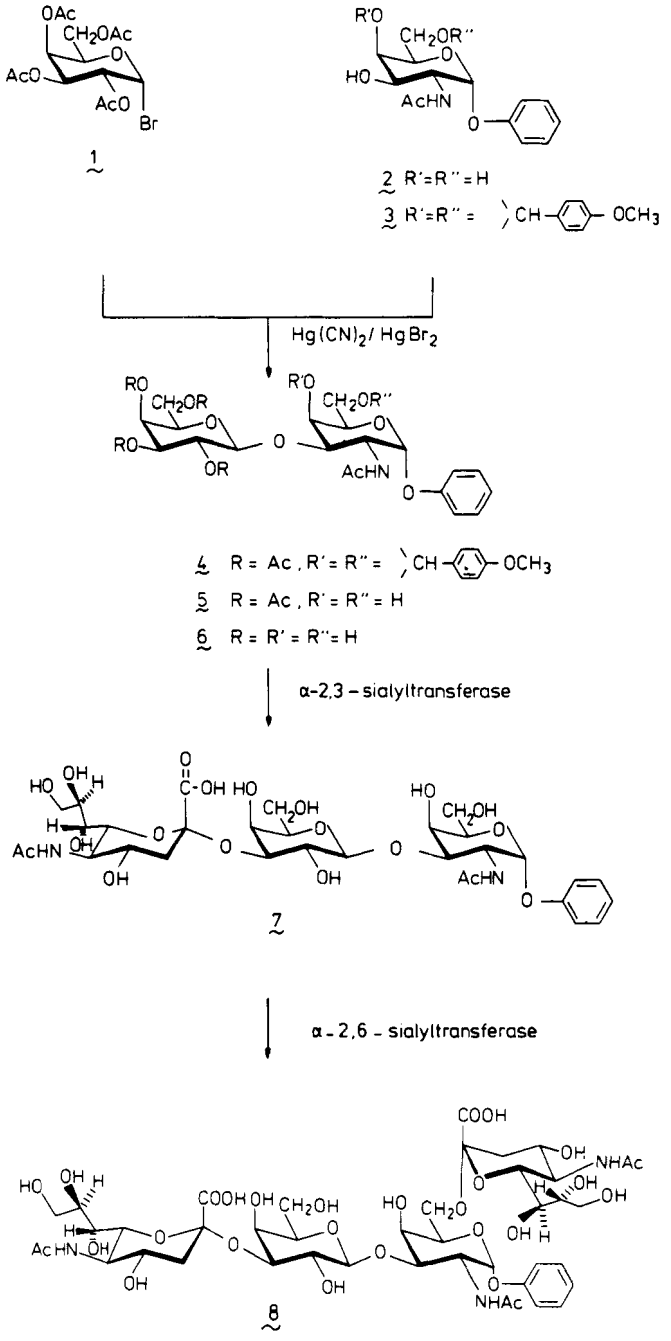

TABLE 1. ^{1}H NMR First Order Chemical Shift Data (ppm) of Compounds 6-8 in D_2O.

		Compound		
Residue		**6**	**7**	**8**
D-Galp	H-1	4.54	4.62	4.62
	H-2	3.56	-	-
	H-3	3.66	4.11	4.11
	H-4	3.93	3.96	3.96
D-GalNAcp	H-1	5.64	5.65	5.57
	H-2	4.53	4.51	4.50
	H-3	4.29	4.29	4.28
	H-4	4.32	4.32	4.36
	H-5	4.13	4.13	4.27
	NAc	2.03	2.03	2.04
D-NeuAcpα2,3	H-3ax		1.80	1.80
	H-3eq		2.77	2.77
	NAc		2.03	2.03
D-NeuAcpα2,6	H-3ax			1.54
	H-3eq			2.64
	NAc			2.04

since the set of chemical shift values of the H-3 protons of NeuAc in compound 7 (H3ax: δ 1.80; H3eq: δ 2.77 ppm) are similar to those reported for an analogous monosialylated compound[22]. Furthermore, the chemical shift increments of H-1'and H-3' of the D-galactopyranose moiety of compound 6, are characteristic for the α-D-Neup5Ac-(2-3)-β-D-Galp-(1-3) sequence[22].

Trisaccharide 7 was subsequently incubated in aqueous buffer with CMP-[^{3}H]-NeuAc and a microsomal fraction from

regenerating rat liver, which contained the CMP-NeuAc: [Neu-Acα(2-3)Galβ(1-3)]GalNAc-α1-R α2-6-sialyltransferase (α2-6STase) activity[16]. The disialylated tetrasaccharide 8 was isolated by Bio-Gel P-4 gelfiltration and purified by HPLC as described before. The identity of compound 8 was confirmed by 400-MHz ^{1}H NMR spectroscopy (see Table 1). The two sets of the equatorially and axially orientated H-3 protons of the sialyl groups confirm the α-linkage of the newly introduced neuraminic acid residue. The chemical shift values of the D-GalNAcp residue in compound 8 are changed in comparison with those of this sugar residue in compound 7. The ppm values of the H-2 and H-3 protons are decreased, while the chemical shifts of others, H-1, H-4 and H-5, are increased. The same trends were observed by Vliegenthart et al.[22] for an analogous disialylated reduced tetrasaccharide. We suppose that the differences in chemical shift values of the protons in 7 and 8 compared to the reduced compounds are due to the non-reduced nature of 7 and 8 in addition to an extra shielding effect of the α-glycosidically-linked phenyl group.

Further structural evidence is given by the finding that the sialyl groups were introduced into disaccharide 6 in a one to one ratio, as calculated from the ^{14}C to ^{3}H ratio. Moreover, alkaline hydrolysis of the phenyl ether bond[24,25] in the disialylated-tetrasaccharide 8 afforded a compound containing ^{3}H and ^{14}C radioactivity in the same ratio as in the starting material 8. Analysis of this product on a Bio-Gel P-4 column revealed that it had the same molecular size as authentic α-D-Neup5Ac-(2-3)-β-D-Galp-(1-3)-[α-D-Neup5Ac-(2-6)]-D-GalNAc-ol[25]. Special attention should be paid to the substrate specificity of sialyltransferase, especially the

α2-6STase. For instance, it was shown by Bergh and Van den Eijnden[24] that the α2-6STase isolated from porcine submaxillargy glands is virtually inactive with p-nitrophenyl-glycosides which are structurally analogous to compound 6 and 7.

In fetal calf liver[24,25] and in regenerating rat liver[14] an α2-6STase is found which can transfer a sialic acid group to α-(p-nitrophenyl)glycoside derivatives of GalNAcp provided the glycoside is substituted at C-3 by the α-D-Neup5Ac-(2-3)-β-D-Galp-group. Thus, in order to attach the sialic acid residue to N-acetyl-D-galactosamine, the β-galactopyranose moiety must be sialylated first[14,24,25], indicating that the sequence of introduction of both neuraminic acids is strictly prescribed.

Several advantages of the approach outlined here over standard organic chemistry can be mentioned: (i) the application of biocatalysts opens new ways and possibilities for the synthesis of labile compounds such as sialylated oligosaccharides, (ii) no anomeric mixtures are formed, (iii) protective groups are unnecessary and (iv) the purification of sialylated products can be achieved easily by gelfiltration and common HPLC methods. Furthermore, the conversion of disaccharide 6 into the monosialated trisaccharide 7 was shown to proceed in high yield (85%). Unfortunately, the low amount of disialylated tetrasaccharide 8 (10%) finally obtained is a serious disadvantage. For the preparation of such complex oligosaccharides further research, *e.g.* on immobilisation of sialyltransferases, is necessary.

EXPERIMENTAL

Phenyl 2-acetamido-2-deoxy-α-D-galactopyranoside was purchased from Koch-Light. Human placenta was obtained from the Department of Obstetrics and Gyneacology, Academisch Ziekenhuis van de Vrije Universiteit, Amsterdam. Regenerating rat livers were dissected from laboratory animals. Unlabelled CMP-Neup5Ac, α-D-Neup5Ac-(2-3)-β-D-Galp-(1-3)-D-GalNAc-ol and α-D-Neup5Ac-(2-3)-β-D-Galp-[α-D-Neup5Ac-(2-6)]-D-GalNAc-ol were prepared as described before[23,26]. CMP-[^{3}H]Neup6Ac (18.9 Ci/mol) and CMP-[^{14}C]Neup5Ac (1.6 Ci/mol) were purchased from New England Nuclear Corp. Boston, Mass.

^{1}H NMR spectra were measured at 400-MHz with a Bruker MSL 400 spectrometer under control of an ASPECT-3000 computer, operating in the Fourier-transform mode. Chemical shifts are given in ppm (δ) relative to sodium 4,4-dimethyl-4-silapentane-1-sulphonate as internal standard. For routine product identification, samples were analyzed on a Spectra-Physics SP 8700 liquid chromatograph, equipped with a Rheodyne 7105 injection valve and a Hewlett-Packard HP 1040 A diode-array detector operating at 195 nm. Spectra were recorded with a Hewlett-Packard HP 3390 A integrator. Chromatography was performed on a column (4 x 250 mm) Lichrosorb-NH_2 (5 um, Merck) at an ambient temperature of 20°C and a pressure of 11 mPa essentially as previously described[23]. The mobile phase consisted of a mixture of acetonitrile and deionized, distilled water containing 15 mM potassium phosphate pH 5.2. Starting at a ratio of 4:1 the acetonitrile content was decreased after an isocratic run of 30 min at a rate of 0.5% min. The flow was maintained at 2 mL

per min. Appropriate amounts of the fractions were assayed for radioactivity to establish the labelled compounds.

Phenyl 2-Acetamido-2-deoxy-4,6-O-(4-methoxybenzylidene)-α-D-galactopyranoside (3). To compound 2 (1.8 g, 6.1 mmol) in dry DMF (60 mL) 4-methoxybenzaldehyde dimethyl acetal (1.3 g, 7.1 mmol) was added together with a catalytic amount of p-toluenesulfonic acid. After stirring overnight at 20°C, the mixture was neutralized with aqueous $NaHCO_3$ (10%; 5 mL) and concentrated *in vacuo*. The residue was dissolved in ether, washed with water, dried ($MgSO_4$) and concentrated to dryness. The product crystallized from ethanol: Yield 2.0 g (80%); ^1H NMR (400 MHz) δ 2.024 (s, 3H, NHAc), 3.800 (d, 1H, H5), 3.819 (s, 3H, OCH_3), 4.039 (dd, 1H, H6b, $J_{5,6b}$ = 1.65 Hz, $J_{6a,6b}$ = 12.60 Hz), 4.092 (dd, 1H, H3, $J_{2,3}$ = 11.07 Hz, $J_{3,4}$ = 3.48 Hz), 4.2 (dd, 1H, H6a, $J_{5,6a}$ = 11.84 Hz), 4.300 (dd, 1H, H4), 4.595 (dd, 1H, H2), 5.571 (s, 1H, CH-O), 5.704 (d, 1H, H1, $J_{1,2}$ = 3.42 Hz), 6.8-7.5 (c, 1H, CH-O), 5.704 (d, 1H, H1, $J_{1,2}$ = 3.42 Hz), 6.8-7.5 (c, 9H, aromatic), 7.996 (d, 1H, NH, J 8.89 Hz).

Anal. Calcd for $C_{36}H_{43}NO_{15}$: C, 59.3; H, 5.8. Found: C, 59.2; H, 5.8.

Phenyl 2-Acetamido-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-4,6-O-(4-methoxybenzylidene)-α-D-galacto-pyranoside (4). To a mixture of anhydrous nitromethane (9.5 mL) and toluene (7.0 mL) were added compound 3 (0.10 g, 0.24 mmol), $Hg(CN)_2$ (625 mg), $Hg(Br)_2$ (125 mg) and powdered molecular sieves 4A (600 mg). In order to remove traces of water, several mL of the solvent mixture were evaporated. Then, under an atmosphere of oxygen-free nitrogen and exclusion of light, the mixture was heated to 50°C and

compound 1 (197 mg, 0.48 mmol), dissolved in a mixture of nitromethane (5 mL) and toluene (5 mL), was added dropwise in 1 h. After 2 h, an equal quantity of compound 1 was added to the reaction mixture. After 24 h, the mixture was filtrated, washed with water and concentrated *in vacuo*. The residual white solid was purified by chromatography on Sephadex LH-20 (eluent $CHCl_3/CH_3OH$, 2/1, v/v), followed by short column chromatography (silica gel 60, eluent toluene-acetone, 6/1, v/v) giving 4 yield 82 mg (45%); ^1H NMR (400 MHz) δ 1.991-2.184 (5xs, 15H, 5xCH_3CO), 3.813 (s, 3H, $CH_3OC_6H_5$), 4.358 (d, 1H, H4), 4.851 (dd, 1H, H2, $J_{2,3}$ = 8.2 Hz), 4.874 (d, 1H, H1', $J_{1',2'}$ = 7.87 Hz), 5.025 (dd, 1H, H3', $J_{3',4'2}$ = 3.44 Hz), 5.240 (dd, 1H, H2', $J_{2',3'}$ = 10.30 Hz), 5.532 (s, 1H, CH-C_6H_5), 5.791 (d, 1H, H1, $J_{1,2}$ = 3.16 Hz), 6.8-7.5 (c, 9H, aromatic).

Anal. Calcd for $C_{28}H_{37}NO_{14}$: C, 55.0; H, 5.6. Found: C, 55.1; H, 5.6.

Phenyl 2-Acetamido-2-deoxy-3-O-β-D-galactopyranosyl-α-D-galactopyranoside (6). Compound 4 (0.50 g, 0.67 mmol) was dissolved in aqueous acetic acid (80%) and kept at 50°C. After 2 h, the mixture was cooled, neutralized with aqueous $NaHCO_3$ (10%), concentrated to dryness and coevaporated with toluene (3 x 10 mL). The residual oil was dissolved in anhydrous methanol (5 mL) and sodium methoxide (0.2 mL, 1 M) was added. After 18 h, the solution was neutralized by adding Dowex 50W cation-exchange resin (100-200 mesh, H^+-form) and subsequently concentrated *in vacuo*. The product 6 crystallised from methanol/ether (1/1, v/v: yield 150 mg (49%); mp 210-214°C (dec); $[\alpha]^{20}$ + 190.7 (c 0.5, H_2O). ^1H NMR data: see Table 1.

Anal. Calcd for $C_{20}H_{29}NO_{11}$: C, 52.2; H, 6.3. Found: C, 52.1; H, 6.2.

Preparation of partially purified α2-3-sialyltransferase. CMP-NeuAc: Gal-β(1-3)GalNAc α2-3-sialyltransferase was partially purified from human placenta as described by Joziasse et al.[21]. The reaction mixture for the assay of α2-3-STase activity contained in a volume of 30 uL: 2.5 mM CMP-[^{14}C]-NeuAc (0.8 Ci/mol), 0.1 M Tris-maleate buffer pH 6.7, 0.1% Triton X-100, 1.0-2.0 mg of protein and asialo-afuco-porcine submaxillary mucin (220 nmol theoretical acceptor sites). The mixture was incubated for 30 min at 37°C and incorporation was measured by precipitating the sialylated mucin with phosphotungstic acid[27].

Preparation of partially purified α2-6-sialyltransferase. Adult male rats of about 250 g were subjected to partial hepatectomy by removing the left lateral and medial lobes of the liver according to standard procedures. Four days after operation the rats were killed and the liver-tissue (approximately 10 g) thus obtained was homogenized in 50 mL of 0.25 M sucrose containing 1 mM EDTA and 1 mM dithiothreitol by use of a Potter-Elvehjem system with a Teflon pestle, rotating at 1400 rpm for 2 min at 0°C. The homogenate was centrifuged at 3000 x g for 20 min. The resulting supernatant was subsequently centrifuged at 100.000 x g for 1 h. The pellet was resuspended in a small volume of the sucrose solution to yield a microsomal preparation at a protein concentration of 30-40 mg/mL.

The assay of α2-6-sialyltransferase activity was performed essentially as described before[14]. The reaction mixture contained in a volume of 30 uL:1 mM LS-tetrasaccha-

ride, 2.5 mM CMP-[^{14}C]NeuAc (0.8 Ci/mol), 1% Triton X-100, 0.1 M Tris-maleate (pH 6.7) and the microsomal enzyme preparation (30-40 ug of protein). The mixture was incubated for 30 min at 37°C whereafter 0.5 mL 0.05 M ammonium acetate (pH 5.2) was added in order to quench the reaction. This mixture was then analysed on a Bio-Gel P-4 column (1.6 x 200 cm) equilibrated at 37°C and eluted with 0.05 M ammonium acetate at a flow of 15 mL/h. Fractions (4 mL) were collected and the radioactivity was determined by liquid scintillation counting.

Synthesis of Neup5AC-α-(2-3)-Galp-β-(1-3)-GalNAcp-α-(1-0)-phenyl (7). An aqueous solution of 5.7 mM CMP-[^{14}C]NeuAc (0.1 Ci/mol), 0.1 M Tris-maleate buffer pH 6.7, 0.1% Triton X-100, 3.6 mM (3 mg/mL) compound 6 and 31 mU of α2-3-STase from placenta were incubated in a total volume of 1.4 mL at 37°C. After 20 h the reaction was quenched by the addition of 2 mL 0.05 M ammonium acetate (pH 5.2) and product 7 was isolated by Bio-Gel P-4 gel filtration (see before) and subsequently purified by HPLC: yield 4 mg (85%); ^{1}H NMR (400 MHz): see Table 1.

Synthesis of Neup6Ac-α-(2-3)-Galp-β-(1-3)-[Neup5Ac-α-(2-6)]-GalNAcp-α-(1-0)-phenyl (8). Several parallel runs of a mixture containing 1.5 mM CMP-[^{3}H]NeuAc (0.1 Ci/mol), 0.25 M sucrose, 0.1 M Tris-maleate buffer pH 6.7, 0.9 mM (1 mg/mL) compound 7, 1% Triton X-100 and 30-40 mg of regenerating rat liver microsomal fraction were incubated at 37°C. After 20 h, the reaction mixture was quenched by the addition of 2 mL 0.05 M ammonium acetate (pH 5.2) and centrifuged for 1 h at 100.000 x g to sediment the microsomal membrane proteins. The resulting supernatant was applied to a column (1.6 x 200 cm)

of Bio-Gel P-4 (230-400 mesh) eluted with 0.05 M ammonium acetate (see before). Compound **8** was purified by HPLC. Yield: 0.6 mg (10%). ^{1}H NMR (400 MHz): see Table 1.

REFERENCES

1. J. Montreuil, *Adv. Carbohydr. Chem. Biochem., 37,* 157 (1980).
2. H.J. Jennings, *Adv. Carbohydr. Chem. Biochem., 41,* 155 (1983).
3. D.J.M. van der Vleugel, F.R. Wassenburg, J.W. Zwikker, and J.F.G. Vliegenthart, *Carbohydr. Res., 104,* 221, (1982).
4. H. Kunz, and H. Waldmann, *J. Chem. Soc., Chem. Commun.,* 638 (1985).
5. A. Ya. Khorlin, I.M. Privalova, and I.B. Bystrova, *Carbohydr. Res., 19,* 272 (1971).
6. R. Brossmer, H. Friebolin, G. Keilich, B. Loser, and M. Supp, *Hoppe Seyler's Z. Physiol. Chem., 359,* 1064 (1978).
7. H. Paulsen, U. von Deessen, and H. Tietz, *Carbohydr. Res., 137,* 63 (1985).
8. D.J.M. van der Vleugel, J.W. Zwikker, J.F.G. Vliegenthart, S.A.A. van Boeckel, and J.H. van Boom, *Carbohydr. Res., 105,* 19 (1982).
9. H. Paulsen, and H. Tietz, *Carbohydr. Res., 125,* 47 (1984).
10. H. Paulsen, and H. Tietz, *Angew. Chem. Int. Ed. Engl., 24,* 128 (1985).
11. H. Paulsen, and H. Tietz, *Carbohydr. Res., 144,* 205 (1985).
12. H.H. Brandstetter, and E. Zbiral, *Monatsh. für Chem., 114,* 1247 (1983).
13. T. Kitajima, M. Sugimoto, T. Nukada, and T. Ogawa, *Carbohydr. Res., 127,* C1 (1984).
14. J.T. de Heij, P.L. Koppen, and D.H. van den Eijnden, *Carbohydr. Res., 149,* 85 (1986).

15. S. Sabesan, and J.C. Paulson, *J. Am. Chem. Soc., 108,* 2068 (1986).

16. J. Thiem and W. Treder, *Angew. Int. Ed. Engl., 25,* 1096 (1986).

17. R.M. Ratcliffe, D.A. Baker, and R.U. Lemieux, *Carbohydr. Res., 93,* 35 (1981).

18. H. Paulsen, and J.P. Holck, *Carbohydr. Res., 109,* 89 (1982).

19. K.L. Matta, and J.J. Barlow, *Carbohydr. Res., 48,* 65 (1976).

20. P. Hoogerhout, M. Kloosterman, and J.H. van Boom, *Recl. Trav. Chim. Pays-Bas, 104,* 235 (1985).

21. D.H. Joziasse, M.L.E. Bergh, H. ter Hart, P.L. Koppen, G.J.M. Hooghwinkel, and D.H. van den Eijnden, *J. Biol. Chem., 260,* 4941 (1985).

22. J.F.G. Vliegenthart, L. Dorland, H. van Halbeek, and J. Haverkamp, in R. Schauer (ed.), *Sialic Acids,* Cell Biology Monographs Vol. 10, Springer, Wien, 1982, p. 127.

23. M.L.E. Bergh, P.L. Koppen, and D.H. van den Eijnden, *Carbohydr. Res., 94,* 225 (1981).

24. M.L.E. Bergh, and D.H. van den Eijnden, *Eur. J. Biochem., 136,* 113 (1983).

25. M.L.E. Bergh, G.J.M. Hooghwinkel, and D.H. van den Eijnden, *J. Biol. Chem., 258,* 7430 (1983).

26. D.H. van den Eijnden, and W. van Dijk, *Hoppe-Seyler's Z. Physiol. Chem., 353,* 1817 (1972).

27. D.H. van den Eijnden, M.L.E. Bergh, B. Dieleman, and W.E.C.M. Schiphorst, *Hoppe-Seyler's Z. Physiol. Chem., 362,* 113 (1981).