Carbohydrate Research, 239 (1993) 167–176
Elsevier Science Publishers B.V., Amsterdam

Rapid production of a panel of blood group A-active oligosaccharides using chemically synthesized di- and trisaccharide primers and an easily prepared porcine (1 → 3)-α-*N*-acetyl-D-galactosaminyltransferase

Catharine A. Compston [1], Caro Condon, H. Rizk Hanna [2] and M. Abdul Mazid
Chembiomed Ltd., Edmonton Research Park, P.O. Box 8050, Edmonton, Alberta T6H 4N9 (Canada)

(Received April 7th, 1992; accepted in revised form July 28th, 1992)

ABSTRACT

A porcine (1 → 3)-α-*N*-acetyl-D-galactosaminyltransferse was obtained in a state suitable for preparative-scale (mg-scale) synthesis using simple procedures requiring only three days of effort. The enzyme thus prepared transferred GalNAc efficiently from UDP-GalNAc to six different chemically synthesized di- and tri-saccharide H-active structures to yield blood-group A-active oligosaccharides that were characterized by ^{1}H NMR spectroscopy and mass spectrometry. This work further demonstrates the efficiency and attractiveness of using glycosyltransferases in a combined chemoenzymatic approach for the rapid production of biologically active oligosaccharides.

INTRODUCTION

There is a great interest in the study of biologically active oligosaccharides, much of which stems from the appreciation of their potential in biology and medicine. This interest has created a growing need for economically produced oligosaccharides, especially for the purpose of studying their biochemical function and assessing their potential in therapeutics or as diagnostic tools. The structural characterization of the complex carbohydrate chains of mammalian glycoproteins and glycolipids as antigenic determinants of the human ABO blood group system provided the early impetus for their organic chemical synthesis[1]. These compounds, in general, are difficult to obtain from natural sources in sufficient quantities for systematic biochemical studies.

Correspondence to (present address): Dr. M.A. Mazid, Glyko, Inc., 81 Digital Drive, Novato, CA 94949, USA.
[1] Present address: Department of Food Science, University of Alberta, Edmonton, Alberta, Canada T6G 2P5.
[2] Present address: Alberta Research Council, P.O. Box 8330, Station F, Edmonton, Alberta, Canada T6H 5X2.

Powerful methods have been reported for the chemical synthesis of biologically significant oligosaccharides[2–4]. Chemical synthesis of oligosaccharides representing blood group substances, in particular, have been elegantly described by Lemieux et al.[5–7]. However, as with many oligosaccharides, traditional organic synthesis remains time-consuming, cumbersome and expensive[8]. This is partly due to the formation of isomeric mixtures in chemical glycosylation reactions. Apart from the synthesis of oligosaccharides, the glycosylation of proteins or lipoproteins without causing denaturation of the macromolecules would be even more difficult, probably impossible, by such chemical means. As a logical alternative, the enzymic approach for glycosylation is particularly attractive since it allows stereospecific synthesis where anomeric mixtures are not formed, protection and deprotection steps are not necessary, and purification of the product can be achieved relatively easily.

The enzymically assisted preparation of specifically glycosylted glycoproteins or glycoplipids will require specific glycosyltransferases with relatively high purity or specific activity. Of the various enzymes involved in the glycosylation or biosynthesis of blood group substances, the (1 → 3)-α-*N*-acetyl-D-galactosaminyltransferase occupies a key position. Purification and characterization of blood group A specific (1 → 3)-α-*N*-acetyl-D-galactosaminyltransferase (also called A-transferase) have been reported from human milk[9], human plasma[10], porcine submaxillary glands[11], and more recently from human gut mucosal tissue[12], human lung tissue[13], and human plasma[14]. Partial purification of the A-transferase from hog gastric mucosa and formation of blood group A substance from natural H substance were previously reported[15]. The synthesis of a tracer-labeled product was demonstrated in situ using a hemagglutination inhibition test; however, the product was neither isolated, purified nor structurally characterized. The characterization of a blood-group A-active tetrasaccharide synthesized by a blood-group B gene-specified glycosyltransferase has been published[16]. In the present study, we demonstrate for the first time the use of a partially purified A-transferase in synthesizing a panel of human blood group A-specific oligosaccharides from chemically synthesized H-type precursors on a preparative (mg) scale. Such a combined chemoenzymatic approach is especially efficient since the precursor acceptor substrates are readily available through chemical synthesis alone.

EXPERIMENTAL

*Partial purification of (1 → 3)-α-*N*-acetyl-D-galactosaminyltransferase from porcine gastric mucosa.*—Porcine stomachs, obtained fresh from a local slaughterhouse, were collected immediately after slaughter and placed on ice. Processing was carried out at 4°C on the day of collection. The contents of each stomach were removed, and the inner surface was rinsed with cold water. Gastric mucosa was stripped from the underlying muscle layer, rinsed with cold water and drained. Approximately 200 g of mucosa was obtained per stomach. The mucosa was minced with scissors and ground in a Waring blender. The preparation was

homogenized further in 0.05 M Tris · HCl (pH 7.6) buffer containing 0.35 M sucrose, 0.025 M KCl, 0.01 M $MgCl_2$, and 0.001 M Na_2EDTA, and the suspension was adjusted to a final concentration of 0.3 to 0.5 g mucosa per mL of buffer. The material was centrifuged at 13900*g* for 20 min. A small aliquot of the resultant homogenate supernatant was retained at 4°C for the determination of blood group A activity, and the remainder (~ 450 mL per stomach) was centrifuged at 150000*g* for 45 min to pellet the microsomal fraction. An aliquot of this high speed spin supernatant was also retained at 4°C for the determination of blood group A activity. The microsomes (5–7.5 g per stomach) were stored at −80°C until extraction of the enzyme.

All manipulations during enzyme extraction were carried out at 4°C in plastic containers. The microsomal pellets were thawed and suspended to homogeneity in 20 mM sodium cacodylate, 10 mM $MnCl_2$ buffer (pH 6.8) to a final concentration of 0.15 g microsome per mL of buffer. The suspension was sonicated in 1.5-mL aliquots, for a total of 4 min in 30-s bursts. Sonicated aliquots were pooled in the same buffer containing 0.75% Triton X-100 and homogenized in a siliconized Wheaton glass homogenizer for a total of 1.25 h. The preparation was transferred to a beaker and further diluted in buffer to give a final concentration of 1% Triton X-100 and 0.75 g microsome/mL. The suspension was stirred gently for 20 h, then spun at 100000*g* for 1 h. The Triton X-100 extract supernatant was removed and used for synthesis of blood group A-specific oligosaccharides. The blood group A activity in various samples was determined by an assay for the inhibition of agglutination of A red blood cells (data not shown), as described by Kabat[17] and reported by Tuppy and Schenkel-Brunner[15].

Determination of enzyme activity and acceptor–substrate specificity.—Extracts were assayed for A-transferase activity using a chromatographic separation of the reaction product on a C_{18} Sep-Pak column (Millipore) as described by Palcic et al.[18]. An assay mixture in a total volume of 50 μL contained, typically, 2 to 30 μL of enzyme preparation, 125 nmoles of a carbohydrate H acceptor (namely, αFuc(1 → 2)βGal-O$(CH_2)_8COOCH_3$, the spacer-linked H-disacharide), 4 nmoles of UDP-*N*-acetylgalactosamine (Sigma) with UDP-*N*-acetyl-D-[1-^{14}C]galactosamine (Amersham) and having a radioactivity of about 10000 cpm, and 5 to 33 μL of 20 mM sodium cacodylate, 10 mM $MnCl_2$ (pH 6.9) buffer. A control for endogenous acceptors which contained the same quantity of extract, but no acceptor substrate, was routinely included. All samples were incubated at 37°C for 30 to 60 min. Counts for MeOH elutions obtained for the endogenous acceptor controls for each sample (typically, 1–2% of the counts from the added donor) were subtracted from counts obtained for the sample with acceptor. The enzyme activity was calculated from the amount of product formed, one unit (U) of (1 → 3)-α-*N*-acetyl-D-galactosaminyltransferse activity being defined as that amount of enzyme catalyzing the transfer of one μmole of *N*-acetylgalactosamine to αFuc(1 → 2)βGal-O$(CH_2)_8COOCH_3$ per min at 37°C.

Synthesis of blood group A-specific oligosaccharides using the crude enzyme.—A

TABLE I

Structure of various H-type precursors and blood group A-type oligosaccharide products [a]

Precursor (trivial name)	Identification number	Product (trivial name)
αFuc(1 → 2)βGal-O-$(CH_2)_8COOCH_3$ (H-Disaccharide)	1	αGalNAc(1 → 3)βGal-O-$(CH_2)_8COOCH_3$ 2 ↑ 1 αFuc (A-Trisaccharide)
αFuc(1 → 2)βGal(1 → 3)βGlcNAc-OR (H Type I)	2	αGalNAc(1 → 3)βGal(1 → 3)βGlcNAc-OR 2 ↑ 1 αFuc (A Type I)
αFuc(1 → 2)βGal(1 → 4)βGlcNAc-OR (H Type II)	3	αGalNAc(1 → 3)βGal(1 → 4)βGlcNAc-OR 2 ↑ 1 αFuc (A Type II)
αFuc(1 → 2)βGal(1 → 3)βGalNAc-OR (H type IV)	4	αGalNAc(1 → 3)βGal(1 → 3)βGalNAc-OR 2 ↑ 1 αFuc (A Type IV)
αFuc(1 → 2)βGal(1 → 3)βGal-OR (H Type V)	5	αGalNAc(1 → 3)βGal(1 → 3)βGal-OR 2 ↑ 1 αFuc (A Type V)
αFuc(1 → 2)βGal(1 → 4)βGlc-OR (H Type VI)	6	αGalNAc(1 → 3)βGal(1 → 4)βGlc-OR 2 ↑ 1 αFuc (A Type VI)

[a] R is $-(CH_2)_8COOCH_3$ in all structures, as indicated for 1.

typical reaction mixture consisted of 2.6 mU of microsomal Triton X-100 extract (200 μL), 600 μg of a carbohydrate acceptor, and a 1.3 mol equiv of UDP-*N*-acetylgalactosamine (sodium salt) as donor. Sodium cacodylate (100 mM), 5 mM $MnCl_2$ (pH 7.2) buffer was used for the synthesis of A-trisaccharide and A type II tetrasaccharide, while 20 mM sodium cacodylate (pH 6.9) buffer containing 10 mM $MnCl_2$ was used for all other acceptors. The total volume of the reaction mixture was ~ 600 μL. It was incubated at 37°C with mild agitation for 7 h. This length of time was found to be sufficient for the completion of the reaction, as observed spectrophotometrically from a coupled reaction system with pyruvate kinase and lactate dehydrogenase (M. Palcic, unpublished observation). All acceptors were chemically synthesized, 10-carbon spacer-linked carbohydrate products representing various H-type substances (see Table I), obtained from Chembiomed Ltd.

Isolation, purification, and characterization of oligosaccharide products.—The oligosaccharide product of the enzymatic synthesis was isolated and purified as

described by Palcic et al.[18]. The reaction mixture was passed through a new C_{18} Sep-Pak cartridge which had been equilibrated and washed with MeOH and water. The product was eluted with 15 mL of abs MeOH, and the sample was reduced to dryness on a rotary evaporator. This was resuspended in 0.5 mL of MeOH and applied to a 12 cm × 8 cm regular analytical thin-layer chromatography plate (Merck Kieselgel 60 F_{254}, 0.25 mm) using a Hamilton syringe. The plate was dried under vacuum and eluted with 1:2 MeOH–CH_2Cl_2 for the A trisaccharide and A type II tetrasaccharide, or with 1:1 EtOAc–MeOH for all other products. The plate was observed under ultraviolet light for a fluorescing band of Triton X-100 which was visible just below the solvent front line. For the A trisaccharide and A type II tetrasaccharide products, all of the surface of the plate below this area was removed to the application line. The area at the application line was removed for all other products. The collected material was stirred with 10 mL of 1:1 abs MeOH–water for 30 min at room temperature. The resultant suspension was passed through a Buchner funnel containing a Whatman No. 1 filter paper and evaporated to dryness.

The dried sample was dissolved in 5 mL water and passed through a washed C_{18} Sep-Pak cartridge, and the flask was rinsed with another 10 mL of water. The cartridge was washed with 20 mL of water, and the product was eluted with 15 mL of MeOH, then passed through a 0.22-μm Millipore filter and evaporated to dryness. Finally, the sample was evaporated in D_2O, redissolved in D_2O and analysed by ^1H NMR spectroscopy. All samples were re-exchanged with pure water before lyophilization and analysis by mass spectroscopy.

^1H NMR spectra were recorded on a Bruker AM 300 instrument operating at 297K. The sharp methyl peak of the methoxy group in the 10-carbon spacer-arm of product oligosaccharides was chosen as the reference standard and assigned a chemical shift of 3.687 ppm[19]. Fast-atom bombardment mass spectrometry was performed on an AEI MS-9 spectrometer using Xe as the bombarding gas and mixtures of dithiothreitol–dithioerythritol as the matrix and positive-ion detection.

RESULTS AND DISCUSSION

The preparation of (1 → 3)-α-*N*-acetyl-D-galactosaminyltransferase used in this study had a modest specific activity in the range of 6–7 mU/mg of protein. This was easily obtained by a simple detergent solubilization of porcine gastric mucosal microsomal pellets with Triton X-100. The enzyme was found to have a rather broad acceptor–substrate specificity, as determined from initial studies with ^{14}C-labeled UDP-GalNAc and various precursor acceptor–substrates (data not shown).

The chemical structure of the various H-type precursors and the related blood group A-type oligosaccharide products are provided in Table I. The required H-type precursors, containing a glycosidically linked bridging arm, used for the enzymatic synthesis were obtained from chemical synthesis alone as described, for example, by Lemieux et al.[5–7] and Hindsgaul et al.[20]. In this work the precursor

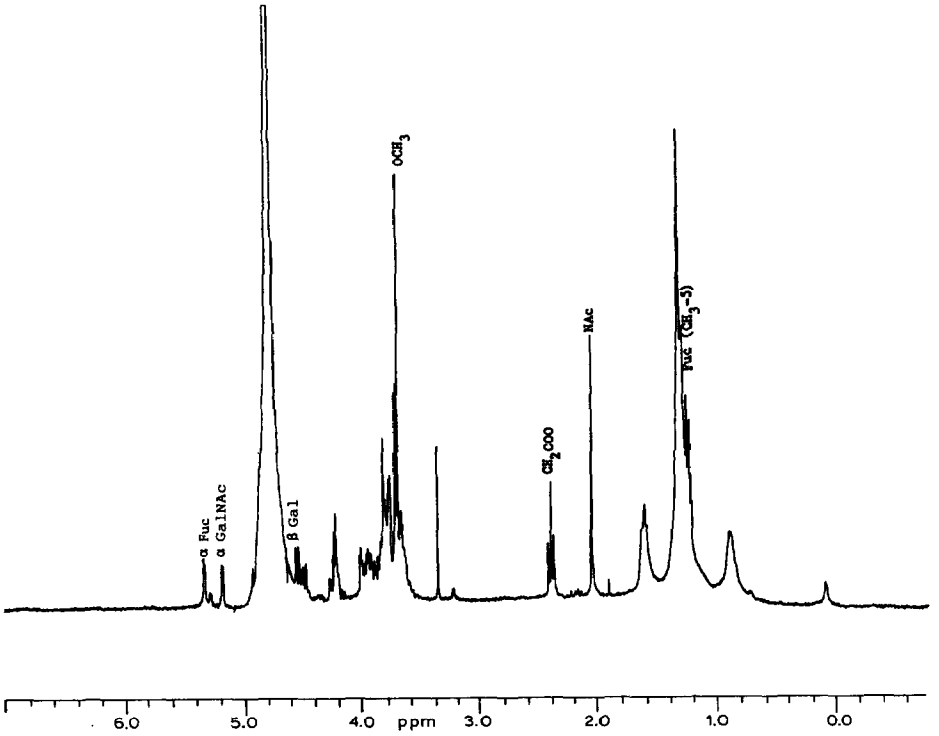

Fig. 1. ^{1}H NMR spectrum of A-trisaccharide (product 1, Table I) obtained by the enzymic synthesis using porcine (1 → 3)-α-*N*-acetylgalactosaminyltransferase.

acceptors used, and therefore the products formed enzymatically, were all attached to a 10-carbon hydrophobic spacer arm. This greatly simplified the product isolation from a rather crude incubation mixture, and the purity for the product obtained exceeded 90% in all cases as judged on the basis of NMR data. Furthermore, the conversion of the donor (UDP-GalNAc) as well as the acceptor substrate to product also appeared to be complete (> 90%) in all the cases, as determined earlier from a coupled reaction (M. Palcic, unpublished observation). It should be noted that traces of Triton X-100, which was used in the enzyme preparation, could be observed in some of the NMR spectra since the detergent was not separated by hdyrophobic chromatography on a Sep-Pak column. The method would still be useful, however, for other oligosaccharide substrates though additional chromatographic steps would have to be included in the product isolation protocols.

Fig. 1 shows the ^{1}H NMR spectrum of A-trisaccharide product obtained by the preparative-scale enzymatic synthesis from the H-disaccharide precursor and using the porcine A-transferase. A similar spectrum of the typical A-type VI tetrasaccharide product superimposed on the corresponding H-type VI trisaccharide precursor is given in Fig. 2. All other spectra are essentially similar to those shown except for minor differences in the position of anomeric protons and for two *N*-acetyl

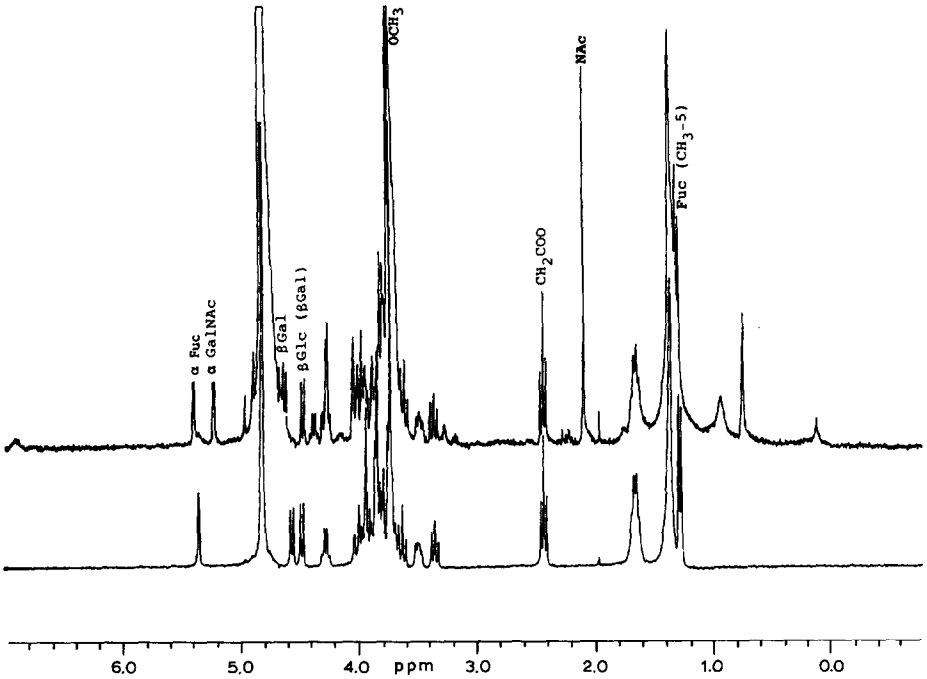

Fig. 2. ^{1}H NMR spectrum of A-type VI tetrasaccharide (product 6) superimposed on the H-type VI trisaccharide (precursor 6), obtained by the enzymic synthesis using porcine A-transferase.

signals of the products 2, 3, and 4 (see structures in Table 1). Table II summarizes some of the characteristic ^{1}H NMR as well as mass spectral data obtained for all the products.

The ^{1}H NMR spectrum shown in Fig. 1 indicates the anomeric protons as well as some other characteristic signals for the A-trisaccharide (product 1, Table I) obtained enzymatically. These assignments are consistent and completely in agreement with those reported earlier by Lemieux et al.[7,21] for the same oligosaccharide prepared by chemical synthesis alone. The additional H-1 (GalNAc) signal for the A-type VI oligosaccharide (product 6), also present in the other products, is shown clearly in Fig. 2. In the anomeric region, this doublet at 5.18 ppm (J 3.7 Hz) can be assigned to the α-anomeric proton of *N*-acetyl-D-galactosamine, while the doublet at 5.35 ppm (J 4.0 Hz) belongs to that of L-fucose. These assignments are in agreement with those given to the α-anomeric protons of these two sugars in related compounds reported by Lemieux et al.[21] and Kannagi et al.[22], and for the similar but reduced tetrasaccharide products synthesized with the A and B gene-specified transferases as described and characterized by Yates et al.[16]. The third doublet in the anomeric region at 4.65 ppm (J 7.7 Hz) was assigned by Yates et al.[16] to the β-anomeric proton of D-galactose. This is consistent with our results of 4.58 ppm (J 7.4 Hz); however, this signal assignment may be reversed with that of the fourth anomeric doublet at 4.30–4.49 ppm (J 7.4–8.5 Hz). This additional signal arises from the H-1 (β-R) where R is GlcNAc, GlcNAc, GalNAc, Gal, or

TABLE II

^{1}H NMR [a] and mass spectral data obtained for the blood group A-specific oligosaccharide products

Characteristic data	Chemical shifts (δ, ppm) and coupling constants (J, Hz) for oligosaccharide products [b]					
	A-Trisaccharide (1)	A-Type I (2)	A-Type II (3)	A-Type IV (4)	A-Type V (5)	A-Type VI (6)
H-1(αFuc)	5.32 (3.7)	5.25 (4.0)	5.35 (3.5)	5.28 (4.4)	5.32 (3.7)	5.35 (4.0)
H-1(αGalNAc)	5.17 (3.7)	5.18 (3.7)	5.18 (3.5)	5.17 (3.3)	5.18 (3.7)	5.18 (3.7)
H-1(βGal) [c]	4.53 (7.7)	4.70 (7.7)	4.60 (7.5)	4.66 (7.4)	[d]	4.58 (7.4)
H-1(βR) [d,e]		4.40 (8.1)	4.49 (8.0)	4.30 (7.4)	4.39 (8.5)	4.42 (7.7)
CH_2COO	2.39 (7.4)	2.39 (7.4)	2.39 (7.4)	2.39 (7.4)	2.39 (7.4)	2.39 (7.4)
NAc	2.04	2.05, 2.04	2.04, 2.04	2.04, 2.03	2.05	2.04
CH_3(Fuc)	1.23 (6.6)	1.22 (6.6)	1.25 (6.5)	1.22 (6.6)	1.22 (6.6)	1.25 (6.6)
MS (%) [f]	722(M + Na, 87)	925(M + Na, 12)	925(M + Na, 4)	925(M + Na, 13)	851(M + H, 34)	861(M + H, 29)

[a] Data from all spectra, essentially similar to those shown in Figs. 1 and 2 except for the minor differences in the position of anomeric protons and for the two *N*-acetyl signals of products 2, 3, and 4. [b] Structure of oligosaccharides as shown in Table I. [c] Signal assignments may be reversed for 2 to 6. [d] Obscured by residual DOH signal. [e] R is GlcNAc, GlcNAc, GalNAc, Gal, or Glc for oligosaccharide products 2, 3, 4, 5, and 6 , respectively (see Table I). [f] The mass of the molecular ions (M) are listed in combination with Na or H, the numbers in parentheses reflecting the relative abundance of the ionic species as a percent of the highest peak in the mass spectrum.

Glc for oligosaccharide products 2, 3, 4, 5, and 6, respectively (see Table I).

Outside the anomeric region, the distinctive signals of CH_2COO from the 8-(methoxycarbonyl) octyl spacer-arm (2.39 ppm), the signals at 2.03–2.05 ppm given by the CH_3 protons of the acetyl group of *N*-acetyl-D-galactosamine and *N*-acetyl-D-glucosamine, and the doublet given by the CH_3-5 of L-fucose (1.22–1.25 ppm, 6.5–6.6 Hz) were readily distinguishable. It is notable that two distinct *N*-acetyl signals could be clearly observed in the ^1H NMR spectra of products 2, 3, and 4. Also, the spectrum of each product obtained enzymatically compared very well with that of the authentic product synthesized in the laboratory by purely chemical means. The ^1H NMR spectral data (Table II) therefore confirm that the blood group A-specific oligosaccharide products obtained from the chemoenzymatic approach in this study are identical to those obtained by chemical synthesis alone. This is further corroborated by the mass spectral data which are included in Table II. The mass-spectrometric analysis of a naturally occurring A-active tetrasaccharide, α-D-GalNAc-(1 → 3)-[α-L-Fuc-(1 → 2)]-β-D-Gal-(1 → 4)-D-Glc, isolated from urine has been reported earlier[23]. Our results for the A type VI tetrasaccharide (product 6, Table I) is in accord with the assignment of this structure and also with the ^1H NMR spectrum of the tetrasaccharide reported by Yates et al.[16].

CONCLUSIONS

This work clearly demonstrates the usefulness of a combined chemoenzymatic approach for the rapid preparation of small quantities of biologically active oligosaccharides. The partially purified, albeit crude, A-transferase used in this study can be easily prepared in about three days from a readily accessible source in a form useful for synthesis with only modest effort and no specialized equipment or resins. Furthermore, the enzyme has sufficiently broad specificity to accept all of the H structures which were tested. The H-type III structure, however, αFuc(1 → 2)βGal(1 → 3)αGalNAc-OR, was not available and could, therefore, not be used for the synthesis of the corresponding A-type III tetrasaccharide product.

It may be noted that the blood group A transferase from a human stomach endothelial cell line has recently been cloned[24], but no report of its overexpression or use in synthesis has yet appeared. The use of a cloned enzyme, especially in an immobilized form, would be attractive or desirable for the eventual large-scale commercial production of oligosaccharides such as the blood group A-active substances. In the meantime, however, the enzyme from porcine stomach can be easily prepared, and it is calculated that one porcine stomach contains sufficient activity to produce, for example, about one mg of A-trisaccharide (product 1) per min at 37°C from the H-disaccharide precursor.

A recent review[25] on oligosaccharide synthesis by enzymatic transglycosylation gives a promising outlook "in our ability to develop enzymatic systems for the custom synthesis of a wide variety of useful and biologically active oligosaccharides". This study shares the same outlook and reconfirms the approach reported

previously for the synthesis of tumor-associated sialyl Lewis-a determinant[19] and other complex oligosaccharides[26,27] or carbohydrates in general[28].

ACKNOWLEDGEMENTS

The enzymatic syntheses reported herein were made possible by the availability of the required H-type precursors. We thank members of the Chemical R&D of Chembiomed Ltd. for the chemical synthesis of these precursors. Skillful technical assistance of Ken Wlasichuk and Dr. John Coffin are also gratefully acknowledged. We are indebted to Professors Monica M. Palcic and Ole Hindsgaul of the Departments of Food Science and Chemistry, respectively, of the University of Alberta, for preliminary synthetic studies, for the mass spectra, and especially for their encouragement throughout the course of the work. We are grateful to Dr. Frank M. Unger of Chembiomed Ltd. for supporting the work and particularly to Professor Hindsgaul for his valuable insight and most helpful suggestions in the preparation of the manuscript.

REFERENCES

1 W.M. Watkins, *Carbohydr. Res.*, 149 (1986) 1–12.
2 H. Paulsen, *Angew. Chem. Int. Ed. Engl.*, 21 (1982) 155–173.
3 T. Ogawa, H. Yakamoto, T. Nukeda, T. Kitijima, and M. Sugimoto, *Pure Appl. Chem.*, 56 (1984) 779–795.
4 R.R. Schmidt, *Angew. Chem. Intl. Ed. Engl.*, 25 (1986) 212–235.
5 R.U. Lemieux, D.R. Bundle, and D.A. Baker, U.S. Pat., 4 137 401 (1979).
6 R.U. Lemieux, D.R. Bundle, and D.A. Baker, U.S. Pat., 4 238 473 (1980).
7 R.U. Lemieux and R.M. Ratcliffe, U.S. Pat., 4 362 720 (1982).
8 O. Hindsgaul, K.J. Kaur, U.B. Gokhale, G. Srivastava, G. Alton, and M.M. Palcic, *ACS Symp. Ser.*, 466 (1991) 38–50.
9 A. Kobata and V. Ginsburg, *J. Biol. Chem.*, 245 (1970) 1484–1490.
10 J.S. Whitehead, A. Bella, and Y.S. Kim, *J. Biol. Chem.*, 249 (1974) 3442–3447.
11 M. Schwyzer and R.L. Hill, *J. Biol. Chem.*, 252 (1977) 2338–2345.
12 N. Navaratnam, J.B.C. Findlay, J.N. Keen, and W.M. Watkins, *Biochem. J.*, 271 (1990) 93–98.
13 H. Clausen, T. White, K. Takio, K. Titani, M. Stroud, E. Holmes, J. Karkov, L. Thim, and S. Hakomori, *J. Biol. Chem.*, 265 (1990) 1139–1145.
14 A. Takeya, O. Hosomi, and M. Ishiura, *J. Biochem.*, 107 (1990) 360–368.
15 H. Tuppy and H. Schenkel-Brunner, *Eur. J. Biochem.*, 10 (1969) 152–157.
16 A.D. Yates, J. Feeney, A.S.R. Donald, and W.M. Watkins, *Carbohydr. Res.*, 130 (1984) 251–261.
17 E.A. Kabat, *Blood Group Substances,* Academic Press, New York, 1956, p 135.
18 M.M. Palcic, L.D. Heerze, M. Pierce, and O. Hindsgaul, *Glycoconj. J.*, 5 (1988) 49–63.
19 M.M. Palcic, A.P. Venot, R.M. Ratcliffe, and O. Hindsgaul, *Carbohydr. Res.*, 190 (1989) 1–11.
20 O. Hindsgaul, T. Norberg, J. Le Pendu, and R.U. Lemieux, *Carbohydr. Res.*, 109 (1982) 109–142.
21 R.U. Lemieux, K. Bock, L.T.J. Delbaere, S. Koto, and V.S. Rao, *Can. J. Chem.*, 58 (1980) 631–653.
22 R. Kannagi, D. Roelcke, K.A. Peterson, Y. Okada, S.B. Levery, and S. Hakomori, *Carbohydr. Res.*, 120 (1983) 143–157.
23 C. Derappe, A. Lundblad, L. Messeter, and S. Svensson, *FEBS Lett.*, 119 (1980) 177–179.
24 F. Yamamoto, J. Marken, T. Tsuji, T. White, H. Clausen, and S. Hakomori, *J. Biol. Chem.*, 265 (1990) 1146–1151.
25 G.L. Cote and B.Y. Tao, *Glycoconj. J.*, 7 (1990) 145–162.
26 K.G.I. Nilsson, *Carbohydr. Res.*, 188 (1989) 9–17.
27 H.T. De Heij, M. Kloosterman, P.L. Koppen, J.H. van Boom, and D.H. van den Eijnden, *J. Carbohydr. Chem.*, 7 (1988) 209–222.
28 E.J. Toone, E.S. Simon, M.D. Bednarski, and G.M. Whitesides, *Terahedron*, 45 (1989) 5365–5422.