Carbohydrate Research, 151 (1986) 147–156
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

THE USE OF AN IMMOBILISED CYCLIC MULTI-ENZYME SYSTEM TO SYNTHESISE BRANCHED PENTA- AND HEXA-SACCHARIDES ASSOCIATED WITH BLOOD-GROUP I EPITOPES*

CLAUDINE AUGÉ§, CÉCILE MATHIEU, AND CLAUDE MÉRIENNE

Laboratoire de Chimie Organique Multifonctionelle, Unité Associée au C.N.R.S. No. 462, Institut de Chimie Moléculaire, Bt. 420, Université de Paris-Sud, F91405 Orsay (France)

(Received August 29th, 1985; accepted for publication, October 7th, 1985)

ABSTRACT

The branched hexasaccharide β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→3)-[β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→6)]-β-D-Gal*p*-(1→4)-β-D-GlcOMe (**1**) was obtained on a 0.1-mmol scale by enzymic bigalactosylation of the tetrasaccharide β-D-Glc*p*NAc-(1→3)-[β-D-Glc*p*NAc-(1→6)]-β-D-Gal*p*-(1→4)-β-D-GlcOMe by the use of an immobilised multi-enzyme system which regenerated UDP-α-D-galactose *in situ*. The formation of **1** proceeded in two steps. An intermediate compound was identified on the basis of ^{1}H- and ^{13}C-n.m.r. data as the pentasaccharide β-D-Glc*p*NAc-(1→3)-[β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→6)]-β-D-Gal*p*-(1→4)-β-D-GlcOMe.

INTRODUCTION

The branched hexasaccharide β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→3)-[β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→6)]-β-D-Gal*p*-(1→4)-β-D-GlcOMe (**1**), which is the methyl glycoside of lacto-*N*-*neo*hexaose previously isolated by Kobata and Ginsburg[1] from human milk, has strong blood-group I activity. It was obtained on a μmol scale by Piller *et al.*[2] from β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-β-D-GlcOMe by the action of two transferases probably involved in the biosynthesis of I-active structures. Its chemical synthesis was achieved by Maranduba and Veyrières[3].

We now report a new application of our enzymic galactosylation procedure, already described[4] for simpler substrates, to the synthesis of **1** from the branched synthetic tetrasaccharide[3] β-D-Glc*p*NAc-(1→3)-[β-D-Glc*p*NAc-(1→6)]-β-D-Gal*p*-(1→4)-β-D-GlcOMe (**2**). Nunez and Barker[5] were the first to report the synthesis of *N*-acetyl-lactosamine using a partially purified UDP-D-galactose:2-acetamido-2-deoxy-D-glucoside 4-β-D-galactosyltransferase (EC 2.4.1.22) from bovine milk,

*Dedicated to Roger W. Jeanloz.

§To whom correspondence should be addressed.

0008-6215/86/$ 03.50

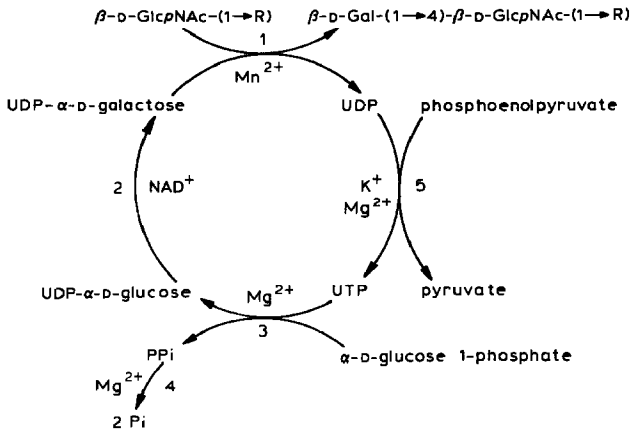


Fig. 1. The cyclic multi-enzyme system for enzymic galactosylation: 1, UDP-D-galactose:2-acetamido-2-deoxy-D-glucoside 4-β-D-galactosyltransferase (EC 2.4.1.22); 2, UDP-D-galactose 4-epimerase (EC 5.1.3.2); 3, UDP-D-glucose pyrophosphorylase (EC 2.7.7.9); 4, inorganic pyrophosphatase (EC 3.6.1.1); 5, pyruvate kinase (EC 2.7.1.40).

which catalyses the transfer of D-galactose from UDP-α-D-galactose to a non-reducing terminal β-D-Glc*p*NAc group according to the equation:

$$\text{UDP-D-Gal} + \beta\text{-D-Glc}p\text{NAc-(1}\rightarrow\text{R)} \xrightarrow{Mn^{2+}} \beta\text{-D-Gal-(1}\rightarrow\text{4)-}\beta\text{-D-Glc}p\text{NAc-(1}\rightarrow\text{R)} + \text{UDP.}$$

As this transfer occurs with a large variety of substrates[6], its adaptation to a preparative scale would provide a valuable tool for the synthesis of elaborate oligosaccharide sequences. To this end, we reported[4] an immobilised cyclic multi-enzyme system which, by regenerating UDP-α-D-galactose *in situ* from UDP, constituted an efficient route for enzymic galactosylation. In addition to the 4-β-D-galactosyltransferase, this system requires four extra enzymes, namely, pyruvate kinase (EC 2.7.1.40), UDP-D-glucose pyrophosphorylase (EC 2.7.7.9), inorganic pyrophosphatase (EC 3.6.1.1), and UDP-D-galactose 4-epimerase (EC 5.1.3.2.) (see Fig. 1). All of the enzymes were immobilised by covalent binding to an insoluble support made of 4% of agarose activated with cyanogen bromide. A similar system of six enzymes immobilised on a polyacrylamide gel allows[7] an efficient synthesis of *N*-acetyl-lactosamine.

EXPERIMENTAL

Materials. — Dithiothreitol, UDP-α-D-glucose, α-D-glucose 1-phosphate, NAD^+, pyruvate kinase (type I), UDP-D-glucose pyrophosphorylase, inorganic pyrophosphatase, and UDP-D-galactose 4-epimerase were purchased from Sigma

and used without further purification. The 4-β-D-galactosyltransferase was prepared from cow colostrum and partially purified by affinity chromatography on UDP-hexanolamine Sepharose[8] (170 units were obtained from 2 L). Phosphoenolpyruvate was synthesised according to the procedure of Hirschbein *et al.*[9]. Ultrogel A4 (4% agarose) was obtained from IBF (92390 Villeneuve-la-Garenne). Tetrasaccharide **2** was a generous gift of Dr. A. Veyrières. Silica gel (Merck, 70–230 mesh) was used for column chromatography. All the buffers were prepared with twice-distilled water.

Methods. — ^{13}C-N.m.r. spectra (62.9 MHz, internal 1,4-dioxane, δ 67.40) and ^{1}H-n.m.r. spectra (250 MHz) for solutions in $CDCl_3$ (internal Me_4Si) or D_2O (external 0.2% of Me_4Si in $CDCl_3$) were recorded with a Bruker AM-250 spectrometer. The 2D-n.m.r. measurement was performed on the same machine, using the standard Bruker software provided for carrying out a COSY experiment. It needed a 2048 × 2048 data matrix, which was symmetrised about the diagonal.

Enzymic assays. — Pyruvate kinase, UDP-D-glucose pyrophosphorylase, and UDP-D-galactose 4-epimerase were assayed spectrophotometrically, and inorganic pyrophosphatase colorimetrically, using standard procedures[10]. The 4-β-D-galactosyltransferase activity was measured by radiochemical assay[11]. For immobilised enzymes, assays were adapted as follows. An aliquot (10–50 μL) of the diluted suspension of the agarose-bound enzyme in buffer was added to the assay mixture and continuously stirred during the incubation time.

Immobilisation of the enzymes. — (*a*) *Gel activation*[12]. Ultrogel A4 (1 mL) was washed with twice-distilled water and 2M phosphate buffer (pH 11), collected, and suspended in 5M phosphate buffer (1 mL, pH 12). The suspension was diluted with twice-distilled water (1 mL), and an aqueous solution of CNBr (60 mg/mL) was added within 2 min. The temperature was kept at 5–10°. The mixture was stirred for 10 min and then filtered, and the insoluble material was washed with cold water and then with the buffer to be used in (*b*).

(*b*) *Coupling with enzyme.* The enzyme (0.2–10 mg according to the enzyme, see Table I) was gently stirred overnight at 4° under N_2 with freshly activated Ultrogel (1 mL) in 5 vol. of the immobilisation buffer. The gel was washed with M NaCl, twice-distilled water, and storage buffer, and stored at 4° under N_2 in the presence of substrates and mM dithiothreitol.

Galactosylation. — (*a*) *First galactosylation.* Tetrasaccharide **2** (114 mg, 0.15 mmol), α-D-glucose 1-phosphate (116 mg, 0.32 mmol), phosphoenolpyruvate (66 mg, 0.32 mmol), and UDP-α-D-glucose (5 mg, 0.008 mmol) were added to a solution of NAD^+ (31 mg, 0.04 mmol), $MnCl_2$ (18 mg, 0.090 mmol), $MgCl_2$ (36 mg, 0.180 mmol), KCl (235 mg, 3.15 mmol), dithiothreitol (69 mg, 0.45 mmol), and NaN_3 (4.5 mg) in 0.1M Tris buffer (pH 8.0, 5 mL). A suspension of immobilised β-D-galactosyltransferase (1.3 units), UDP-D-galactose 4-epimerase (1.6 units), UDP-D-glucose pyrophosphorylase (2.5 units), pyruvate kinase (15.5 units), and inorganic pyrophosphatase (19 units) in the same buffer (40 mL) was then added. The reaction was allowed to proceed at 30° under N_2 with gentle shaking. After

2 days, t.l.c. (4:5:3 1-butanol–acetone–water) of a deionised aliquot showed a major spot (R_F 0.44; *cf.* 0.54 for **2**). No more conversion was observed. After 2 days, UDP-α-D-glucose (0.004 mmol) was again added and, after 1 day, traces of a new compound migrating at the same rate as a reference sample of hexasaccharide[3] (R_F 0.34) were revealed by t.l.c. The reaction was stopped after 7 days, the intensity of the hexasaccharide spot showing no further increase. The gel was removed, the filtrate was deionised using Dowex 1-X2 ($HCOO^-$) and Dowex 50-X8 (H^+) resins, and concentrated to dryness. Column chromatography (8:10:3 1-butanol–acetone–water) of the residue gave **2** (0.013 mmol, 9%; fraction I), a mixture of **2** and **3** (26 mg; fraction II), pentasaccharide (51 mg, 0.054 mmol, 36%; fraction III), **3** isolated as an amorphous solid (containing traces of silica gel), and a mixture of **1** and **3** (42 mg; fractions IV). N.m.r. data (D_2O): **3**, ^1H, δ 2.05 (s, 3 H, NAc), 2.07 (s, 3 H, NAc), 3.59 (s, 3 H, OMe), 4.15 (d, 1 H, $J_{3,4}$ 2.7 Hz, H-4B), 4.42 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1A), 4.44 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1B), 4.48 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1F), 4.65 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1E), and 4.69 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1C); ^{13}C, δ 22.41, 22.66 ($NHCOCH_3$), 55.28, 55.94 (C-2C,E), 57.48 (OMe), 60.29, 60.76, 61.29 (C-6A,C,E,F), 68.68 (C-4B), 68.82 (C-4F), 68.91 (C-6B), 69.94 (C-2B), 70.13 (C-4C), 71.23 (C-2F), 72.76 (C-3F,E), 73.10 (C-2A), 73.76 (C-5B), 73.81 (C-3C), 74.70 (C-5A), 74.95 (C-3A), 75.01 (C-5E), 75.62 (C-5F), 75.94 (C-5C), 78.65, 79.19 (C-4E,A), 81.94 (C-3B), 101.23 (C-1E), 103.13 (C-1C,F), 103.29 (C-1A,B), 175.21 (C=O).

Pentasaccharide **3** (11 mg) was acetylated with 1:2 acetic anhydride–pyridine (1.5 mL) for 3 days at room temperature. Concentration of the mixture and distillation of toluene from the residue, followed by column chromatography (9:1 dichloromethane–methanol), afforded the acetylated pentasaccharide (13.5 mg, 75%) as an amorphous solid. ^1H-N.m.r. data ($CDCl_3$): δ 1.92, 1,97, 1.98, and 2.01 (4 s, each 3 H, 4 Ac), 2.02 (s, 6 H, 2 Ac), 2.05 (s, 3 H, Ac), 2.06 (s, 6 H, 2 Ac), 2.08 (s, 3 H, Ac), 2.09 (s, 6 H, 2 Ac), 2.12, 2.13, 2.14, and 2.16 (4 s, each 3 H, 4 Ac), 3.48 (s, 3 H, OMe), 5.62 (d, 1 H, $J_{2,NH}$ 7.9 Hz, NH-C), 6.42 (d, 1 H, $J_{2,NH}$ 8.6 Hz, NH-E) (for the other protons, see Table IV).

(*b*) *Second galactosylation.* Fractions II–IV were combined (119 mg) and submitted to a new galactosylation cycle using α-D-glucose 1-phosphate (40 mg, 0.11 mmol), phosphoenolpyruvate (23 mg, 0.11 mmol), UDP-α-D-glucose (1.6 mg, 0.002 mmol), and cofactors at the same concentration as previously described, together with immobilised β-D-galactosyltransferase (1 unit), UDP-D-galactose 4-epimerase (1 unit), UDP-D-glucose pyrophosphorylase (1 unit), pyruvate kinase (11.5 units), and inorganic pyrophosphatase (9.5 units), in (total volume, 30 mL) 0.1M Tris buffer (pH 8.0). The reaction was stopped after 7 days, the gel was removed, and the filtrate was worked-up as described above. No tetrasaccharide **2** was present. The mixture of **1** and **3** was fractionated by column chromatography (8:10:3 1-butanol–acetone–water) to give unreacted pentasaccharide **3** (20 mg, 0.022 mmol), a mixture of **3** and **1** (28 mg), and hexasaccharide **1** (71 mg, 0.065 mmol) as an amorphous solid (containing traces of silica gel). ^1H-N.m.r. data

(D_2O): δ 2.00 (s, 3 H, NAc), 2.02 (s, 3 H, NAc), 3.57 (s, 3 H, OMe), 4.15 (d, 1 H, $J_{3,4}$ 2.7 Hz, H-4B), 4.41 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1A), 4.43 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1B), 4.47 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1F), 4.48 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1D), 4.64 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1E), 4.70 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1C).

RESULTS AND DISCUSSION

The five enzymes required by the galactosylation cycle were separately immobilised on Ultrogel, in the presence of their substrates to preserve the active site, with an average yield of 40%, and could be stored in appropriate buffers. The conditions of immobilisation and storage were optimised for each of the enzymes and are summarised in Table I.

Galactosylation of the branched tetrasaccharide **2** was carried out at 30° and pH 8.0 in the presence of the five immobilised enzymes, their required cofactors (Mn^{2+} for β-D-galactosyltransferase, Mg^{2+} for pyruvate kinase, inorganic pyrophosphatase, and UDP-D-glucose pyrophosphorylase, K^+ for pyruvate kinase, NAD^+ for UDP-D-galactose epimerase), α-D-glucose 1-phosphate, and phospoenolpyruvate in stoichiometric amounts, and UDP-α-D-glucose in catalytic quantity. Dithiothreitol and sodium azide were added to prevent the oxidation of enzymes and bacterial contamination, respectively. The pH was controlled at 8.0, which was a compromise between the optimum pH of the β-D-galactosyltransferase (7.4) and that of UDP-D-galactose epimerase (8.8). In order to minimise the non-enzymic degradation[13] of UDP-α-D-galactose to UMP and D-galactose 1,2-(cyclic phosphate) catalysed by Mn^{2+}, the concentration of Mn^{2+} was kept low (2mM) in contrast to the high concentration (50mM) of Mn^{2+} required for maximum β-D-galactosyltransferase activity[14].

The aim was to prepare hexasaccharide **1** directly from tetrasaccharide **2** and

TABLE I

OPTIMISED CONDITIONS FOR THE PREPARATION AND STORAGE OF IMMOBILISED ENZYMES

Enzyme[a]	*Immobilisation*[b] *buffer*	*Additives for immobilisation and storage (mM)*	*Quantity of enzyme (mg/mL of gel)*	*Yield*[c] *(%)*	*Storage*[b] *buffer*
1	A	UDPG[d] (1), GlcNAc (5), $MnCl_2$ (25)	10	26–36	D
2	B	UDPG (0.5), NAD^+ (0.005)	0.4	37–46	E
3	C	UDPG (1.6), PPi (1.6)	0.3	31–41	F
4	C	PPi (1), Pi (1)	0.2	41–51	F
5	C	ADP (0.4), PEP[d] (1), $MgCl_2$ (10)	0.6	71–88	F

[a]Enzymes are numbered as in Fig. 1. [b]A, 0.1M borate (pH 8.0) containing 0.5M NaCl; B, 0.1M $NaHCO_3$ (pH 8.7) containing 0.5M NaCl; C, 0.1M $NaHCO_3$ (pH 8.0) containing 0.5M NaCl; D, 25mM cacodylate (pH 7.4); E, 0.01M Tris (pH 8.0); F, 0.01M Tris (pH 7.5). [c]Expressed as 100 (number of immobilised units)/total number of utilised units of enzyme. [d]Abbreviations used are UDPG for UDP-α-D-Glucose, and PEP for phosphoenolpyruvate.

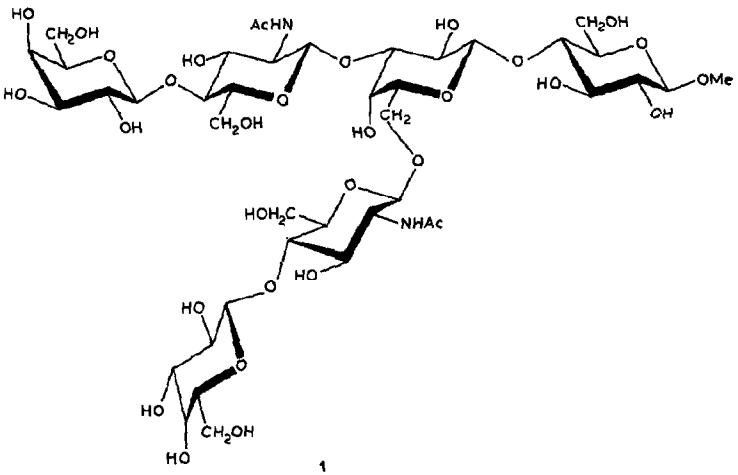
two equiv. of α-D-glucose 1-phosphate and phosphoenolpyruvate. However, the reaction was markedly retarded after the formation of a new compound (**3**), but the recovered enzymes were still active. Babad and Hassid[14] reported a 60% inhibition of β-D-galactosyltransferase activity in 5mM phosphate; our reaction could have been stopped by the accumulation of phosphate. The pure hexasaccharide **1** (44%) was obtained by recycling the first galactosylation product; in addition to **1**, column chromatography gave pentasaccharide **3** (15%) and a mixture of **1** and **3**. The ^{1}H-n.m.r. spectrum of **1** was identical with that of the chemically synthesised compound[3].

The pentasaccharide **3** was isolated in 36% yield from the first galactosylation and was characterised by n.m.r. spectroscopy. The ^{1}H- and ^{13}C-n.m.r. data of **3** were compared with those[3] for the hexa- (**1**) and tetra-saccharide (**2**). Also, the structure of acetylated **3** was investigated by two-dimensional n.m.r. spectroscopy.

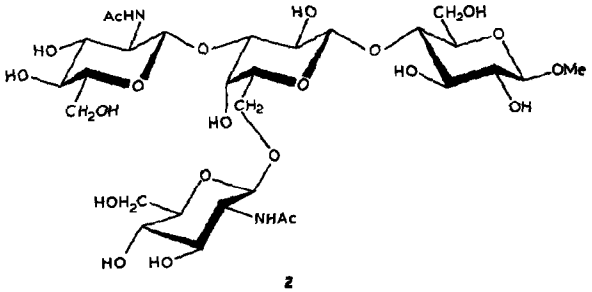
All of the signals for the anomeric protons of **1** and **2** have been unambiguously assigned[3]. The spectrum of **3** exhibited only one signal for the anomeric proton of a β-D-Gal residue (1→4)-linked to β-D-Glc*p*NAc, which clearly shows that **3** is a monogalactosylation product. In order to ascertain which GlcNAc residue had been galactosylated, an expanded scale was used to determine the precise chemical shifts of the signals of the anomeric protons of **1–3**, taking that of H-1 of unit A as the origin. Table II records the $\Delta\delta$ values observed. Unit C gave an identical $\Delta\delta$ value in **2** and **3**, as well as unit E in **1** and **3**. A comparison of the data for **1** and **2** shows that (1→4)-β-D-galactosylation caused downfield shifts of the signals for H-1 of unit C (20 mp.p.m.) and H-1 of unit E (23 mp.p.m.). Such downfield shifts have been reported[15] and are experienced by all the protons of the glycosylated unit. Vliegenthart *et al.*[16] found that a β-D-Gal residue (1→4)-linked to β-D-GlcNAc in diantennary oligosaccharides caused a 24-mp.p.m. downfield shift of the signal for H-1 of this Glc*p*NAc residue. Therefore, the β-D-Glc*p*NAc residue (1→6)-linked to Gal (unit E) was assumed to be substituted by galactose in **3**.

The same conclusion was obtained from the ^{13}C-n.m.r. data. All of the ^{13}C signals of **3** were assigned by analogy with those[3] of **1** and **2**. Since the signals for the anomeric carbon atoms of units C and E in **1** and **2** are well differentiated ($\Delta\delta$ 1.66 and 1.68 p.p.m., respectively), the chemical shifts of C-1 in **1–3** could be compared (see Table III). The chemical shifts of the signals of the anomeric carbons of unit E are similar (±0.03 p.p.m.) in **1** and **3**, but the corresponding signal in **2** is shifted downfield (0.20 p.p.m.). Those of unit C are similar in **2** and **3** (±0.20 p.p.m.), but shifted upfield (0.27 p.p.m.) in **1**. According to Bradbury and Jenkins[17], this is compatible with a shielding effect due to substitution of the GlcNAc residue at position 4.

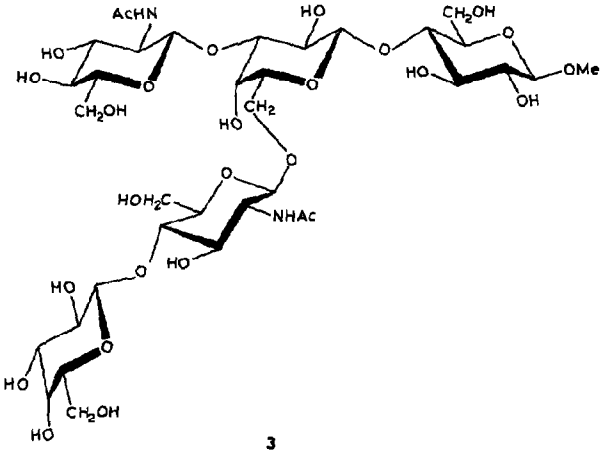
The 2D spectrum (Fig. 2) of acetylated **3** confirmed its structure. Table IV records the chemical shifts of the signals of the protons of units A–C, E, and F. The signals at δ 5.62 and 6.42 are assigned to NH of units C and E, respectively. Therefore, the signals for H-1 of units C and E are at δ 4.96 and 4.67, respectively. This



1



2



3

TABLE II

CHEMICAL SHIFTS FOR THE SIGNALS OF THE ANOMERIC PROTONS OF UNITS B–F[a] RELATIVE TO THE ANOMERIC PROTON OF UNIT A ($\Delta\delta$ IN MP.P.M.)

Compound	*B*	*C*	*D*	*E*	*F*
Hexasaccharide **1**	14	290	71	226	60
Tetrasaccharide **2**	15	270	—	203	—
Pentasaccharide **3**	16	269	—	226	60

[a]A–F refer to the monosaccharide units as indicated.

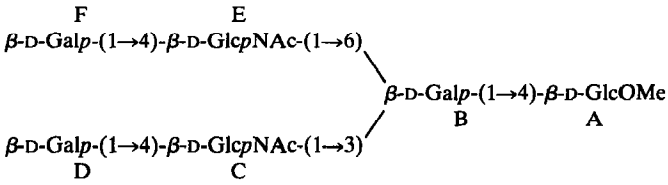


TABLE III

CHEMICAL SHIFTS FOR THE SIGNALS OF THE ANOMERIC CARBONS OF UNITS[a] E AND C (δ IN P.P.M.)

Compound	*E*	*C*
Hexasaccharide **1**	101.20	102.86
Tetrasaccharide **2**	101.43	103.11
Pentasaccharide **3**	101.23	103.13

[a]As in Table II.

TABLE IV

CHEMICAL SHIFTS FOR THE SIGNALS OF THE PROTONS OF UNITS[a] A–C, E, AND F IN THE ACETYLATED PENTASACCHARIDE **3** OBTAINED BY 2D-^{1}H-N.M.R. SPECTROSCOPY (δ IN P.P.M.)

Proton	*A*	*B*	*C*	*E*	*F*
H-1	4.40	4.35	4.96	4.67	4.51
H-2	4.87	5.05	3.39	3.71	5.14
H-3	5.11	3.80	5.43	5.32	5.01
H-4	3.78	5.41	5.08	3.75	5.32
H-5	3.60	3.81	3.67	3.67	3.89
H-6	4.17	3.54	4.14	4.14	4.05
H-6′	4.48	3.80	4.55	4.37	4.10
H-N			5.62	6.42	

[a]As in Table II.

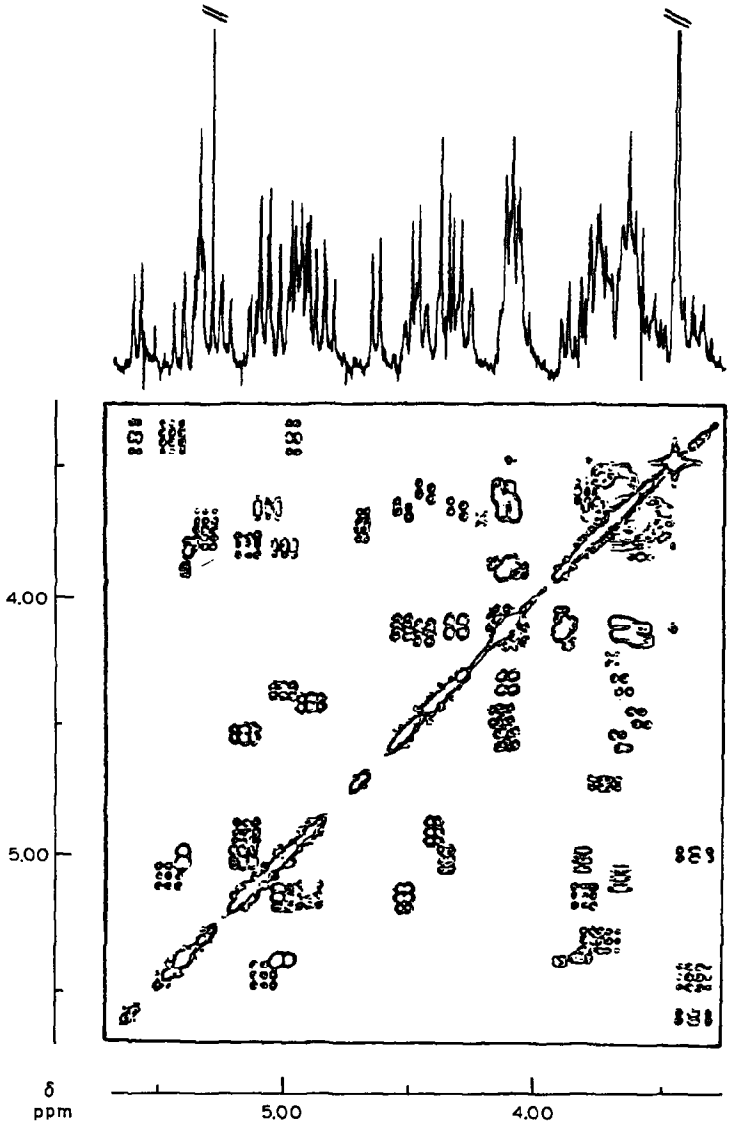


Fig. 2. The sugar ring-proton region of the 250-MHz homoscalar-correlated 2D spectrum (COSY-45) of the acetylated pentasaccharide **3**.

accords with the observation[18] that the signal of H-1 is at higher field ($\Delta\delta$ ~0.3 p.p.m.) in the (1→6)-β-linked unit (E) than in the (1→3)-β-linked unit (C). The galactosylation site at O-4 in unit E is indicated unequivocally by the upfield shift (δ 3.75) of the signal for H-4 in unit E as compared with that (δ 5.08) of H-4 in unit C which is deshielded by its acetyl group. The same shielding effect is observed for H-4 in unit A and for H-3 and H-6 in unit B, corresponding to the glycosylation

sites. All of the values recorded in Table IV agree well with those published[18] for acetylated, reduced lacto-*N*-hexaose from human milk.

It is concluded from our results that galactosylation of **2** occurred first on the β-D-GlcNAc (1→6)-linked to Gal (unit E). Thus, the β-D-galactosyltransferase displays (1→6)-branch specificity in the tetrasaccharide **2**. The same specificity has already been reported[19] for the trisaccharide β-D-Glc*p*NAc-(1→3)-[β-D-Glc*p*NAc-(1→6)]-D-Gal.

ACKNOWLEDGMENT

We thank Professor S. David for helpful discussions.

REFERENCES

1 A. KOBATA AND V. GINSBURG, *Arch. Biochem. Biophys.*, 150 (1972) 273–281.
2 F. PILLER, J. P. CARTRON, A. MARANDUBA, A. VEYRIÈRES, Y. LEROY, AND B. FOURNET, *J. Biol. Chem.*, 259 (1984) 13385–13390.
3 A. MARANDUBA AND A. VEYRIÈRES, *Carbohydr. Res.*, 151 (1986) 105–119.
4 C. AUGÉ, S. DAVID, C. MATHIEU, AND C. GAUTHERON, *Tetrahedron Lett.*, (1984) 1467–1470.
5 H. A. NUNEZ AND R. BARKER, *Biochemistry*, 19 (1980) 489–495.
6 T. A. BEYER, E. J. SADLER, J. I. REARICK, J. C. PAULSON, AND R. L. HILL, *Adv. Enzymol.*, 52 (1981) 23–175.
7 C. H. WONG, S. L. HAYNIE, AND G. M. WHITESIDES, *J. Org. Chem.*, 47 (1982) 5416–5418.
8 R. BARKER, K. W. OLSEN, J. H. SHAPER, AND R. L. HILL, *J. Biol. Chem.*, 247 (1972) 7135–7147.
9 B. L. HIRSCHBEIN, F. P. MAZENOD, AND G. M. WHITESIDES, *J. Org. Chem.*, 47 (1982) 3765.
10 T. BUCHER AND G. PFLEIDERER, *Methods Enzymol.*, 1 (1955) 435–436; R. G. HANSEN, G. J. ALBRECHT, S. T. BASS, AND S. L. SEIFERT, *ibid.*, 8 (1966) 248–249; L. A. HEPPEL, *ibid.*, 2 (1955) 570–571.
11 K. E. EBNER, R. MAWAL, D. K. FITZGERALD, AND B. COLVIN, *Methods Enzymol.*, 28 (1972) 500–502.
12 J. PORATH AND R. AXEN, *Methods Enzymol.*, 44 (1976) 28–29.
13 H. A. NUNEZ AND R. BARKER, *Biochemistry*, 15 (1976) 3843–3847.
14 H. BABAD AND W. Z. HASSID, *J. Biol. Chem.*, 241 (1966) 2672–2678.
15 U. DABROWSKI, P. HANFLAND, H. EGGE, S. KUHN, AND J. DABROWSKI, *J. Biol. Chem.*, 259 (1984) 7648–7654.
16 J. F. G. VLIEGENTHART, L. DORLAND, AND A. V. HALBECK, *Adv. Carbohydr. Chem. Biochem.*, 41 (1983) 207–374.
17 J. H. BRADBURY AND G. A. JENKINS, *Carbohydr. Res.*, 126 (1984) 125–156.
18 J. DABROWSKI, H. EGGE, AND U. DABROWSKI, *Carbohydr. Res.*, 114 (1983) 1–9.
19 W. M. BLANKEN, G. J. M. HOOGHWINKEL, AND D. H. VAN DEN EIJNDEN, *Eur. J. Biochem.*, 127 (1982) 547–552.