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Preliminary communication

Combined chemical-enzymic synthesis of an internally monofucosylated hexasaccharide corresponding to the CD-65/VIM-2 epitope: use of a terminal α2-6-linked *N*-acetylneuraminic acid as a temporary blocking group

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Sialylated and fucosylated lactosaminyl structures such as sialyl Lewis^x, sialyl dimeric Lewis^x, and its internally monofucosylated derivative have been proposed as ligands for the E- and the P-selectins[1–4]. The availability of pure synthetic materials is critical for the evaluation of the binding specificities. Glycosyltransferases have often been employed in chemo-enzymic syntheses in order to circumvent lengthy total chemical routes[5–7]. Particularly pertinent examples are the sialyl-Lewis^a and sialyl-Lewis^x oligosaccharides, which have been obtained by sequential enzymic sialylation and fucosylation[8]. Such syntheses follow the biosynthetic pathway "extension, sialylation, fucosylation"[9,10], which has also been proposed to lead to the terminal structure of the VIM-2 epitope, Neu5Ac(α2-3)Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)[Fuc(α1-3)]GlcNAc, by selective internal fucosylation[11,12]. Mutually exclusive glycosylation by the Gal(β1-4)GlcNAc α2-6-sialyltransferase and the GlcNAc α1-3/4-fucosyltransferase occurs in the synthesis of asparaginyl linked oligosaccharides in glycoproteins[13]. The conformational preference of an α-sialyl residue[14] attached to the 6-hydroxyl of the terminal galactose may prevent the enzymatic fucosylation of the neighbouring *N*-acetylglucosamine.

In this communication, we report the synthesis of the hexasaccharide determinant of the VIM-2 epitope (**5a,b**) starting from tetrasaccharide **1a**, by using

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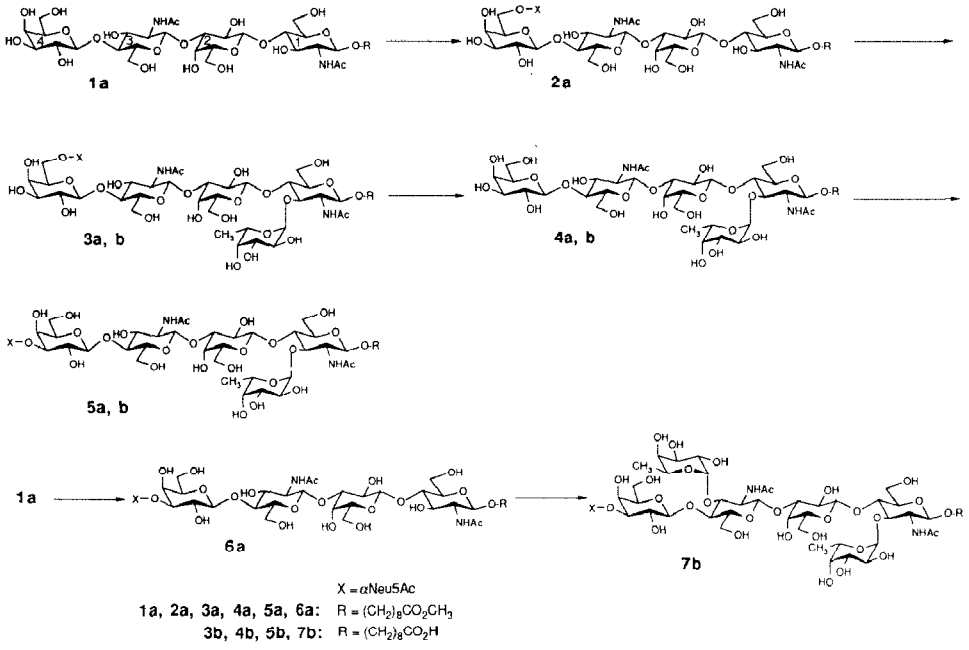

Scheme 1. Synthetic pathway to the VIM-2 epitope (**5a** and **b**) and the sialyl dimeric Lewis^x structure (**7b**).

glycosyltransferases in an appropriate sequence and an α2-6-linked Neu5Ac residue as a temporary blocking group (Scheme 1). The 8-methoxycarbonyloctyl glycoside of **1a** (prepared according to Alais et al.[15]) was used in order to take advantage of the hydrophobic properties of the aglycon for separation purposes, and to provide for the possible coupling of the products to carriers[8]. During incubations partial hydrolysis of the methyl ester group could not always be avoided.

Thus **1a** (6.5 mg) was transformed into **2a** (3.0 mg) by using the rat liver Gal(β1-4)GlcNAc α2-6-sialyltransferase. Compound **2a** was then selectively fucosylated by the GlcNAc α1-3/4-fucosyltransferase from human milk[8], giving **3a** (1.2 mg) and **3b** (0.5 mg). Quantitative desialylation of the mixture of **3a** and **3b** (1.7 mg) by immobilized sialidase from *Clostridium perfringens* (1 U) in sodium cacodylate buffer (50 mM, pH 5.2, 2 mL, 24 h, 37°) led to pentasaccharides **4a** (0.8 mg) and **4b** (0.7 mg). Compound **4b** was then transformed into **4a** by the action of diazomethane in methanol. Finally, sialylation of **4a** (1.5 mg) by the Gal(β1-3/4)GlcNAc α2-3-sialyltransferase from rat liver provided the hexasaccharides **5a** (0.7 mg) and **5b** (0.5 mg).

The heptasaccharide **7b** (1.7 mg) was obtained by sequential sialylation of **1a** (5 mg) by the Gal(β1-3/4)GlcNAc α2-3-sialyltransferase, followed by difucosylation of the intermediate **6a** (2.5 mg) by the GlcNAc α1-3/4-fucosyltransferase. Under the conditions used, only the difucosylated product was obtained.

TABLE I

Selected ^{1}H-NMR data for compounds **1a–6a** and **7b**[a]

Sugar unit	H atom (J in Hz)[b]	Chemical shifts (δ)						
		1a	**2a**	**3a**	**4a**	**5a**	**6a**	**7b**
β-GlcNAc(1)[c]	1 ($J_{1,2}$ 7.9)	4.52	4.52	4.53	4.53	4.52	4.52	4.53
β-Gal (2)	1 ($J_{1,2}$ 7.8)	4.46	4.46	4.44	4.44	4.44	4.46	4.43
	4 ($J_{3,4}$ 3.2)	4.16	4.16	4.10	4.10	4.10	4.16	4.09
β-GlcNAc(3)	1 ($J_{1,2}$ 7.9)	4.70	4.73	4.73	4.70	4.69	4.70	4.69
β-Gal(4)	1 ($J_{1,2}$ 7.8)	4.48	4.46	4.46	4.48	4.56	4.56	4.53
	3 ($J_{2,3}$ 10.0) ($J_{3,4}$ 3.2)					4.12	4.11	4.08
α-Fuc	1 ($J_{1,2}$ 3.8)			5.10	5.10	5.09		5.13, 5.09
	5 ($J_{4,5}$ 1.0)			4.82	4.81	4.81		4.82, 4.82
	6 ($J_{5,6}$ 6.5)			1.15	1.15	1.15		1.17, 1.14
α-Neu5Ac(2-3)	3*eq* ($J_{3eq,3ax}$ 12.7) ($J_{3eq,4}$ 4.5)					2.76	2.76	2.76
	3*ax* ($J_{3ax,4}$ 11.8)					1.80	1.80	1.79
α-Neu5Ac(2-6)	3*eq* ($J_{3eq,3ax}$ 12.7) ($J_{3eq,4}$ 4.5)		2.67	2.67				
	3*ax* ($J_{3ax,4}$ 11.8)		1.72	1.72				
	NCOCH_3 (s)	2.03 2.03	2.06 2.03 (two)	2.05, 2.03 2.02	2.03 2.02	2.02 (three)	2.03 (three)	2.03, 2.02 2.01

[a] 500 MHz, D_2O, ~20°, internal acetone (δ 2.225). [b] Average apparent coupling constants. The variation in these values was $< \pm 0.3$ Hz. [c] For numbering of the sugar residues see **1a**, Scheme 1.

In preparative sialylations using sialyltransferases from rat liver[16,17] the acceptors **1a** and **4a** (1.5–6.5 mg), CMP-Neu5Ac (8–17 mg), sialyltransferase (17–50 mU), calf-intestine alkaline phosphatase (5–15 U)[18], and sodium cacodylate buffer (50 mM, pH 6.5, 1.5–2.5 mL) containing Triton CF-54 (0.5%) and bovine serum albumin (BSA) (1 mg/mL) were incubated for about 48 h at 37°. For preparative fucosylations using the GlcNAc α1-3/4-fucosyltransferase purified from human milk[8], acceptors **2a** and **6a** (2.5–3.0 mg), GDP-fucose (5–8 mg), fucosyltransferase (10–19 mU), and sodium cacodylate buffer (100 mM, pH 6.5, 1.3–2.0 mL) containing $MnCl_2$ (10 mM) and ATP (1.6 mm) were incubated for 48–68 h at 37°. The products obtained in all the enzymic reactions were purified as reported earlier[8]. They were isolated as the methyl ester [^{1}H-NMR (D_2O): δ 3.687 (s, CH_3) and 2.387 (t, J 7.5 Hz, CH_2CO$_2$CH$_3$)] or acid forms [δ 2.166 (t, J 7.5 Hz, CH_2CO$_2$H)] (at the "spacer" terminus) after chromatography on Iatrobeads (Iatron Laboratories), using appropriate mixtures of chloroform, methanol, and water as eluents. Key features of the ^{1}H-NMR spectra of all the above compounds (determined on the Na^+ salts) are presented in Table I, and are in agreement with expectations[14,19]. ^{1}H-NMR data for similar sialylated natural glycolipids in $(CD_3)_2SO$–D_2O have already been reported[20,21]. Improvements of the present methodology as well as the use of other appropriate temporary blocking groups to direct the enzymic synthesis toward specific structures are being examined.

This synthesis of the hexasaccharide terminal structure of the CD-65/VIM-2 epitope is characterized by (*i*) a selective internal monofucosylation directed by a temporary α2-6-sialyl blocking group on the terminal galactose of **1a** and (*ii*) an enzymic sequence where fucosylation precedes the sialylation. In this procedure, the Gal(β1-4)GlcNAc α2-6-sialyltransferase was used to introduce a terminal sialyl group on a dimeric *N*-acetyllactosamine derivative. Interestingly, a regulatory role has been proposed for a similar sialyltransferase, which prevents the synthesis of the X and the sialyl-Lewis^x determinants by competing with a GlcNAc α1-3-fucosyltransferase for the poly-(*N*-acetyllactosaminyl) acceptors at some stages of myeloid cell maturation[22].

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