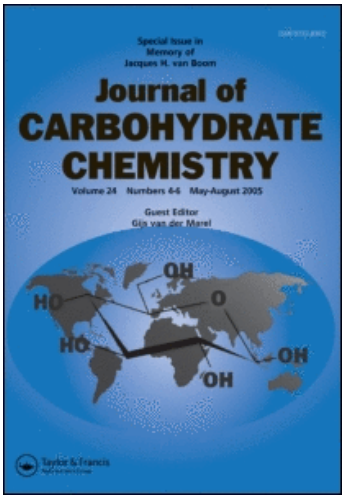

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SYNTHESIS OF SIALYL LEWIS X ANALOGUES

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ABSTRACT

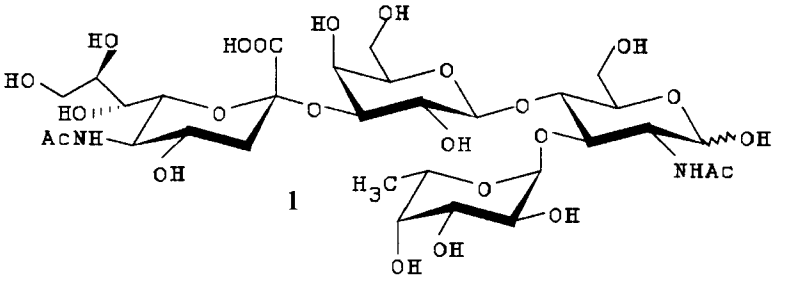
Sialyl Lewis X (SLe^x) analogs **2a** and **2b** were synthesised, where the *N*-acetyl-D-glucose and the D-galactose units of SLe^x **1** were replaced with an alkyl and a heteroalkyl spacer. Sulphate ester **6i** was also synthesised from alcohol **6b** and chlorosulphonic acid. A novel promoter, silver mercaptoethanesulphonate, was used to synthesise α-sialosides **2c**, **7b** and **7c**.

INTRODUCTION

Carbohydrate-mediated cell adhesion is an important event in inflammation initiated by tissue injury or infection and is involved in metastasis.[1,2,3] One of these adhesion processes is the interaction between E-selectin[1] (expressed on the surface of endothelial cells during inflammation) and a glycotope structure displayed on the surface of neutrophils. The ligand recognised by E-selectin was identified as the tetrasaccharide sialyl Lewis X (SLe^x, **1**).[2,4] Further studies indicated that the sulphosaccharide Le^x 3-*O*-sulphate was also a ligand of E-selectin.[5]

A mimetic of SLe^x that inhibits the interaction between E-selectin and SLe^x could be clinically useful as an anti-inflammatory or an anti-metastatic agent.

It has been demonstrated that the carboxylic acid function of the sialic acid moiety and fucose residue of SLe^x are the most significant units for binding to E-selectin.[4] It was decided that SLe^x analogues, mimicking the natural structure, would be synthesised where the *N*-acetyl-D-glucosamine and galactose units of SLe^x are replaced by an alkyl $-(CH_2)_6-$ and a heteroalkyl $-(CH_2)_2O(CH_2)_3-$ spacer. The spacer moieties in compounds **2a** and **2b**



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keep the the two essential sugars approximately the same distance apart as in SLe^x.

To synthesise the analogues **2a** and **2b** our strategy was either to build up the fucose-spacer conjugate **6b** and couple it to a sialic acid residue, or to construct the sialyl-spacer unit **7d** and couple it to the fucose unit.

RESULTS AND DISCUSSION

6-Benzyloxyhexan-1-ol, synthesised by benzylation of 1,6-hexanediol, was a suitable starting compound for the synthesis of SLe^x analogue **2a**. For the construction of compound **2b** heteroalkyl spacer ethers **5a** and **5b** were synthesised from the partially benzylated diols **3a** and **3b** via tosyl derivatives **4a** and **4b**.

Synthesis of α-fucosides: A stereospecific α-fucosylation method was sought for the synthesis of **6b**. High yields of α-fucosides had been reported using dimethyl(methylthio)sulphonium triflate (DMTST),[6,7] dimethyl(methylthio)sulphonium tetrafluoroborate (DMTSB),[8] iodonium dicollidine perchlorate (IDCP),[9] and iodonium dicollidine trifluoromethanesulfonate (IDCT)[10] as promoters, from thiofucoside donors. Halide ion catalyzed glycosylation was also applied,[11] with DMTST and tetraethylammonium bromide (TEAB) used to form bromofucosides *in situ*. 1,2-*cis* specific glycosidation had been achieved through the use of nitrile solvents.[12,13]

Using these different glycosylation methods, the α:β ratio of fucosides **6b**:**6c** was examined. In each reaction methyl 2,3,4-tri-*O*-benzyl-1-thio-ß-fucopyranoside **6a**[7,14] was used as glycosyl donor, and the acceptor was either 1,6-hexanediol or compound **5a**. The α:β ratio of products was determined by TLC (results summarised in Table 1). The best result was obtained when a mixture of bromofucosides **6d** and **6e** (prepared from **6a** by bromine treatment[15]) reacted with 1,6-hexanediol in a halide ion catalysed glycosylation in acetonitrile,[12,13] giving **6b** and **6c** as a 9:1 mixture of anomers.

Synthesis of α-sialosides: The glycosylation of simple alcohols with 2-halogeno-Neu5Ac derivatives (halogen=F, Cl, Br) under Koenigs-Knorr reaction conditions has been widely investigated. Primary alcohols reacted with 2-chloro-sialic acid derivatives in the present of insoluble silver salts (e.g., silver carbonate, salycilate, polymaleate) affording α-sialosides.[16,17,18] With less reactive alcohols, having secondary or sterically hindered hydroxyl groups, the main product was the 2,3-dehydro-Neu5Ac derivative formed by intramolecular elimination. The mechanism of this type of surface active promoters is poorly understood. Silver salts of hydroxy carboxylic acids (especially those that can form a 6-member ring structure, like silver salicylate) were more successful promoters than salts such as silver carbonate.[19] It was proposed that a concerted reaction can take place on the surface of these silver salts, leading to stereoselective glycosylation with inversion of configuration.[20]

Van der Vleugel et al.[16] employed silver salicylate in the coupling reaction of a chloro-sialic acid derivative and alcohols. In these reactions α-glycosides were isolated stereoselectively in 65% yield (the β-anomers present only in 3% yield) without elimination. Sialosides of salicylic acid can also form in the reaction as reported by Wulff and Wichelhaus.[21]

We applied silver salicylate (Ag-sal) promoted glycosylation to prepare compounds **7b** and **7c**. Compound **7a**[22,23] was reacted with 1,6-hexanediol or **5b** yielding α-sialosides **7b** and **7c** but glycosylated salicylic acid derivatives were also formed as major products with the 2,3-dehydro-derivative of sialic acid as a minor by-product.

An attempt was made to overcome this problem by the use of a novel promoter, silver 2-mercaptoethanesulphonate (Ag-MES), easily prepared from silver nitrate and sodium 2-mercaptoethanesulphonate. This promoter should be capable of forming a 6-member ring structure in the same way as silver salicylate. The possibility of

Table 1. Summary of the glycosylation reactions (D=donor, A=acceptor, hd=1,6-hexanediol)

Ref	D	A	D:A ratio	Solvent	Temp °C	Promotor system (mol eq)	α:ß **6b**:**6c**
6,7	**6a**	hd	1.2:1	CH_2Cl_2/benzene	25	DMTST (3)	1:1
6	**6a**	hd	1.2:1	CH_2Cl_2	25	DMTST/TEAB (3:5)	3:7
6	**6a**	hd	1:2	CH_2Cl_2	25	DMTST/TEAB (1:5)	4:6
6	**6a**	hd	1:2	$(CH_2)_2Cl_2$/DMF	25	DMTST/TEAB (0.9:5)	8:2
6	**6a**	**7b**	1.2:1	benzene	25	DMTST (1.4)	2:1**
8	**6a**	hd	1:1	1,2-dimethoxyethane	25	DMTSB (1.5)	3:5
9,10	**6a**	hd	1:1.7	CH_2Cl_2/ether	25	IDCT (2)	4:6
13	**6a**	**5a**	1:2.5	CH_3CN	-15	DMTST/*s*-collidine (1.2:1)	1:1*
13	**6a**	hd	1:2.5	CH_3CN	-15	DMTST (1.2)	1:1
12	**6d,6e**	hd	1:2.5	CH_3CN	-15	AgOTf (1.5)	1:1
11	**6d,6e**	hd	1:5	CH_2Cl_2	25	TEAB (5)	7:3
11	**6j,6k**	hd	1:5	CH_3CN	25	TEAB (5)	9:1

*__6l__:__6m__, **__2c__:__8c__

Table 2. Summary of the sialylation reactions (D=donor, A=acceptor, hd=1,6-hexanediol)

D	A	D:A ratio	Product	Solvent	Promoter	α/ß ratio	Yield %
7a	hd	1:10	**7b**	benzene	Ag-MES	1:0	70
7a	hd	1:10	**7b**	benzene	Ag-sal	1:0	60
7e	hd	1:3	**7b**	MeCN	DMTST	1:1	80
7a	**6b**	1:1	**2c**	benzene	Ag-sal	1:0	30
7a	**6b**	1:1	**2c**	benzene	Ag-MES	1:0	50
7a	**5b**	2:9	**7c**	benzene	Ag-sal	1:0	55
7a	**5b**	2:9	**7c**	benzene	Ag-MES	1:0	75

glycosylation of the promoter was reduced by the replacement of the carboxylic acid function with a sulphonic acid group. Glycosylation *via* the mercapto group is unlikely to occur with silver salt promotion.

^{1}H NMR examination of reaction mixtures in comparative glycosylations with silver 2-mercaptoethanesulphonate and silver salicylate showed that silver 2-mercaptoethanesulphonate promotion gave the α-sialosides **7b** and **7c** in higher yield than silver salicylate, with the 2,3-dehydro-Neu5Ac derivative as a minor product. No glycosides of the promoter or β-sialylation were observed. A comparative nitrilium-nitrile glycosylation of 1,6-hexanediol was also carried out using the methylthiosialoside donor **7e**, with DMTST promotion at -15 °C. While this method gave a high yield of sialoside, both α and ß anomers were formed. Results of the sialylation reactions are summarised in Table 2.

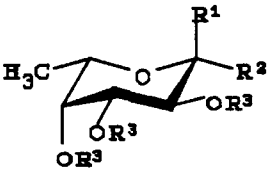
The deprotection of the side chain of **7c** was carried out by Pd-charcoal assisted hydrogenation, resulting in alcohol **7d**.

Synthesis of SLe^x analogues: For the synthesis of compound **2a**, the 9:1 mixture of **6b** and **6c** was separated by flash chromatography and α-fucoside **6b** was reacted with glycosyl chloride **7a** in the presence of silver 2-mercaptoethanesulphonate, yielding α-sialoside **2c**. The deprotection of compound **2c** was carried out by hydrogenation (Pd/C),

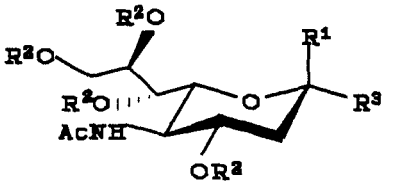
Zemplen deprotection (NaOMe) and aqueous/base hydrolysis to give the SLe^x analogue **2a**.

When sialoside **7b** was reacted with fucosyl bromides **6d** and **6e** in a bromide ion promoted glycosylation, an inseparable mixture of compounds **2c** and **8c** was formed. A similar reaction of sialoside **7d** with **6d** and **6e** resulted in an inseparable anomeric mixture of **2d** and **8d**. Catalytic hydrogenation of the mixture of **2d** and **8d** yielded compounds **2e** and **8e** which were again inseparable. Zemplen deprotection followed by base hydrolysis of **2e** and **8e** resulted in compounds **2b** and **8b**, which were separated by flash chromatography.

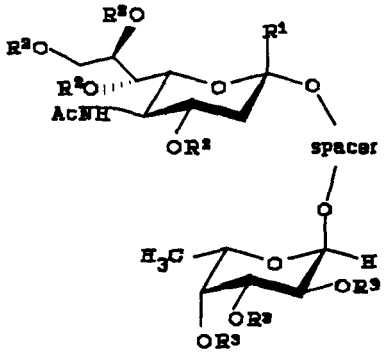
Synthesis of sulphate ester 6i: Alcohol **6b** was reacted with chlorosulfonic acid, yielding compound **6h**, which was deprotected by catalytic hydrogenation resulting in analogue **6i.**



6	R^1	R^2	R^3
a	H	SCH_3	Bzl
b	$O(CH_2)_6OH$	H	Bzl
c	H	$O(CH_2)_6OH$	Bzl
d	Br	H	Bzl
e	H	Br	Bzl
f	$O(CH_2)_2O(CH_2)_3OBzl$	H	Bzl
g	H	$O(CH_2)_2O(CH_2)_3OBzl$	Bzl
h	$O(CH_2)_6OSO_3H$	H	Bzl
i	$O(CH_2)_6OSO_3H$	H	H



7	R^1	R^2	R^3
a	Cl	Ac	$COOCH_3$
b	$COOCH_3$	Ac	$O(CH_2)_6OH$
c	$COOCH_3$	Ac	$O(CH_2)_3O(CH_2)_2OBzl$
d	$COOCH_3$	Ac	$O(CH_2)_3O(CH_2)_2OH$
e	$COOCH_3$	Ac	SCH_3



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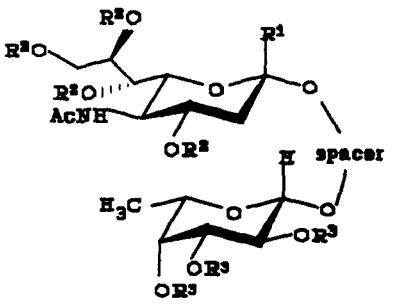

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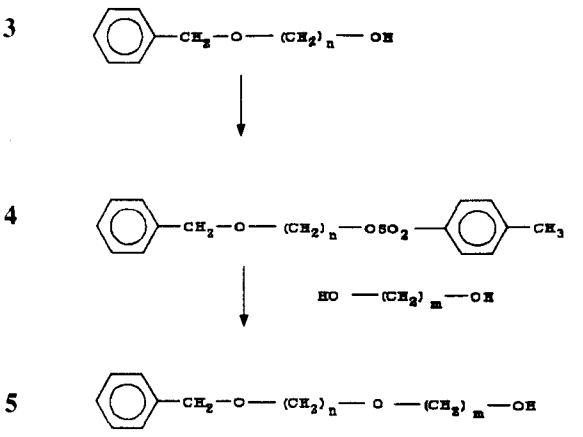
2,8	R^1	R^2	R^3	spacer
a	COOH	H	H	$(CH_2)_6$
b	COOH	H	H	$(CH_2)_3O(CH_2)_2$
c	$COOCH_3$	Ac	Bzl	$(CH_2)_6$
d	$COOCH_3$	Ac	Bzl	$(CH_2)_3O(CH_2)_2$
e	$COOCH_3$	Ac	H	$(CH_2)_3O(CH_2)_2$

3, 4	n	
a	2	
b	3	

5	n	m
a	3	2
b	2	3



EXPERIMENTAL

Purification was achieved by flash chromatography on Sorbsil C60-H40/60, using mobile phases as stated. Reaction progress was monitored by thin layer chromatography on Kieselgel 60 F_{254} using mobile phases as stated. Solvents were evaporated under reduced pressure with a rotary evaporator. ^{1}H NMR spectra were obtained with a Bruker AM 500 instrument operating at a field of 500 MHz. Chemical shifts are reported in ppm downfield from internal TMS. Mass spectra were run with a VG Analytical ZAB-SE instrument using fast atom bombardment (FAB) techniques -20 kV Cs^+ ion bombardment, with 2 µL of appropriate matrix, either 3-nitrobenzyl alcohol or thioglycerol with NaI (MeOH) solution added when necessary to produce natriated species when no protonated molecular ions were observed. The anomeric configuration of sialosides was determined by the use of empirical rules for acetyl protected sialoside when both anomers were present.[24] When only one anomer was isolated the configuration was determined by comparing H-3eq, H-4 and $J_{7,8}$ with typical values for α and ß sialosides.[25,26]

Silver 2-mercaptoethanesulphonate Sodium 2-mercaptoethanesulphonate was dissolved (1.0 g, 6.09 mmol) in THF - H_2O 5:2 v/v (7 mL) and silver nitrate (0.83 g, 4.87 mmol) in THF -H_2O 1:1 v/v (6 mL) was added dropwise at 40 °C, then the mixture was stirred for 30 minutes. The reaction mixture was cooled to room temperature and filtered.

The solid was washed with THF (30 mL) and ether 50 (mL), then dried over P_2O_5 giving the product as a yellow solid (1.1 g, 91 %).

2-Benzyloxyethyl *p*-toluenesulphonate (4a). 2-Benzyloxyethan-1-ol (**3a**) (9.57 g, 62.9 mmol) and 4-dimethylaminopyridine (96 mg, 0.78 mmol) were dissolved in pyridine (125 mL), cooled to 0 °C, and *p*-toluenesulfonyl chloride (16.73 g, 88.1 mmol) was added. The mixture was stirred for 3 hours and concentrated. The residue was taken up in ether (250 mL) and washed with water (50 mL), then with 1N HCl (50 mL) and with saturated $NaHCO_3$ solution (50 mL). The organic phase was dried over $MgSO_4$ and concentrated. The residue was purified by chromatography using hexane/EtOAc 3:1 v/v, to give **4a** (11.29 g, 81 %): R_f 0.55 (hexane/EtOAc 3:1 v/v); ^1H NMR ($CDCl_3$) δ 7.73-7.72 (dd, 2H, Ar-H), 7.22-7.18 (m, 7H, Ar-H), 4.41 (s, 2H, CH_2Ar), 4.13 (dd, 2H, CH_2O), 3.59 (t, 2H, CH_2O), 2.36 (s, 3H, CH_3); FAB MS $C_{16}H_{18}O_4S$ (306.36) *m/z* (%) 329 $[M+Na]^+$ (51), 307 $[M+H]^+$ (7), 199 (8), 155 (5), 107 (8), 91 (100).

3-Benzyloxypropyl *p*-toluenesulphonate (4b). 3-Benzyloxypropan-1-ol (**3b**) (8.4 g, 50.6 mmol) was dissolved in pyridine (110 mL), and 4-dimethylaminopyridine (84 mg, 0.68 mmol) was added. The mixture was cooled to 0 °C, then *p*-toluenesulfonyl chloride (12.52 g, 65.7 mmol) was added, and the mixture was stirred for 3 hours. The mixture was concentrated, and the residue was taken up in ether (350 mL). The undissolved solid was filtered off, and the filtrate washed with water (50 mL), 1N HCl (50 mL), saturated $NaHCO_3$ (50 mL) and with water (50 mL) again. The organic phase was dried over $MgSO_4$ and concentrated. The residue was purified by chromatography using hexane/EtOAc 3:1 v/v, to give **4b** (12.9 g, 80 %): R_f 0.40 (hexane/EtOAc 3:1 v/v); ^1H NMR ($CDCl_3$) δ 7.78 (d, 2H, 2 ArH), 7.39-7.22 (m, 7H, 7 ArH), 4.42 (s, 2H, CH_2Ar), 4.19 (t, 2H, CH_2O), 3.50 (t, 2H, CH_2O), 2.42 (s, 3H, CH_3), 1.94 (m, 2H, CH_2); FAB MS $C_{17}H_{20}O_4S$ (320.38) *m/z* (%) 343 $[M+Na]^+$ (38), 321 $[M+H]^+$ (9), 213 (8), 155 (8), 107 (9), 91 (100).

2-(3-Benzyloxypropoxy)ethanol (5a). A mixture of **4b** (670 mg, 2.09 mmol), ethylene glycol (590 mg, 9.44 mmol), potassium hydroxide (262 mg, 4.7 mmol), and xylene (0.5 mL) was stirred at 130-140 °C for 3 hours. The mixture was allowed to cool, and benzene (6 mL) and water (3 mL) were added. The organic phase was separated, dried over $MgSO_4$, filtered and concentrated. The residue was purified by chromatography using hexane/EtOAc 1:1 v/v as the mobile phase, to give **5a** (316 mg,

72.0 %): R_f 0.4 (hexane/EtOAc 1:1 v/v); ^{1}H NMR ($CDCl_3$) δ 7.30-7.18 (m, 5H, 5 ArH), 4.45 (s, 2H, CH_2Ar), 3.63 (t, 2H, CH_2O), 3.55-3.50 (m, 4H, 2 CH_2O), 3.45 (t, 2H, CH_2O), 2.40 (bs, 1H, OH), 1.83 (m, 2H, CH_2); FAB MS $C_{12}H_{18}O_3$ (210.26) *m/z* (%) 211 $[M+H]^+$ (53), 149 (15), 107 (12), 103 (16), 91 (100).

3-(2-Benzyloxyethoxy)propanol (5b). A mixture of **4a** (3.0 g, 9.8 mmol), 1,3-propanediol (3.3 g, 44.2 mmol), potassium hydroxide (1.2 g, 22.0 mmol) and xylene (2.2 mL) were heated at 130-140 °C for 5 hours. Benzene (26 mL) and water (12 mL) were added. The water phase was extracted with benzene (2x10 mL) and the benzene phase was washed with water (2x3 mL). The collected benzene phases were dried over $MgSO_4$ and concentrated. The residue was purified by chromatography using EtOAc/hexane 2:1 v/v, to give **5b** (1.42 g, 68.9 %): R_f 0.3 (EtOAc/hexane 2:1 v/v); ^{1}H NMR ($CDCl_3$) δ 7.29-7.16 (m, 5H, 5 ArH), 4.44 (s, 2H, CH_2Ar), 3.76, 3.65, 3.52, 3.45 (4t, 8H, 4 CH_2O), 2.68 (bs, 1H, OH), 1.85 (m, 2H, CH_2); FAB MS $C_{12}H_{18}O_3$ (210.26) *m/z* (%) 233 $[M+Na]^+$ (74), 211 $[M+H]^+$ (15), 91 (100).

6-Hydroxyhexyl 2,3,4-tri-*O*-benzyl-α and -ß-L-fucopyranoside (6b) and **(6c).** Compound **6a** (0.7 g, 1.50 mmol) was dissolved in dichloromethane (10 mL) and bromine (260 mg, 1.60 mmol) added, and the mixture stirred for 5 minutes. Cyclohexene was added until the bromine colour disappeared. The solvent was evaporated and the residue was taken up in MeCN (20 mL). Molecular sieves 4A (5.0 g), TEAB (1.6 g, 7.5 mmol), and 1,6-hexanediol (0.9 g, 7.5 mmol) were added and the mixture stirred for 3 days. The reaction mixture was diluted with MeCN (20 mL) and filtered. The filtrate was concentrated and the residue was taken up in CH_2Cl_2 (20 mL) and washed with $NaCO_3$ solution (2 mL), and water (2 mL). The organic phase was dried over $MgSO_4$, and concentrated. The crude product was purified by chromatography using hexane/ether/methanol 7:10:1 v/v/v as the mobile phase, to give **6b** (α-fucoside) (690 mg, 86.2 %): R_f 0.30 (hexane/ether/methanol 7:10:1 v/v/v); ^{1}H NMR ($CDCl_3$) δ 7.41-7.26 (m, 15H, 15 ArH), 4.96, 4.87, 4.81, 4.74, 4.68, 4.65 (6d, 6H, 3 CH_2Ar), 4.78 (d, 1H, H-1, $J_{1,2}$=3.66 Hz), 4.02 (dd, 1H, H-2), 3.93 (dd, 1H, H-3), 3.70 (m, 1H, H-5), 3.65 (d, 1H, H-4), 3.61 (t, 2H, CH_2O), 3.59 (m, 1H, CH_2Oa), 3.43 (m, 1H, CH_2Ob), 1.63 (m, 2H, CH_2), 1.62 (bs, 1H, OH), 1.55 (m, 2H, CH_2), 1.40-1.35 (m, 4H, 2 CH_2), 1.10 (d, 3H, H-6); FAB MS $C_{33}H_{42}O_6$ (534.67) *m/z* (%) 557 $[M+Na]^+$ (62), 181 (14), and **6c** (β-fucoside) (48 mg, 6.0 %): R_f 0.27 (hexane/ether/methanol 7:10:1 v/v/v); ^{1}H NMR ($CDCl_3$) δ 7.37-7.26 (m,

15H, 15 ArH), 4.98-4.68 (6d, 6H, 3 CH_2Ar), 4.30 (d, 1H, H-1, $J_{1,2}$=7.6 Hz), 3.79 (t, 1H, H-2), 3.54 (d, 1H, H-4), 1.66-1.30 (m, 8H, 4 CH_2), 1.16 (d, 3H, H-6); FAB MS $C_{33}H_{42}O_6$ (534.67) *m/z* (%) 557 $[M+Na]^+$ (60), 181 (15).

6-Hydroxyhexyl 2,3,4-tri-*O*-benzyl-α-L-fucopyranoside 6-hydrogensulfate (6h). Pyridine (12 mL) was cooled to 0 °C and chlorosulfonic acid (770 mg, 6.64 mmol) and compound **6b** (355 mg, 0.66 mmol) were added. The reaction mixture was stirred at 0 °C for 3 hours then dry methanol (1 mL) was added. The reaction mixture was concentrated. The crude product was purified by chromatography twice using EtOAc/methanol 10:3 v/v, then CH_2Cl_2/methanol 10:2 v/v later as the mobile phases, to give **6h** (325 mg, 79.6 %): ^1H NMR ($CDCl_3$) δ 7.39-7.24 (m, 15H, 15 ArH), 4.37-4.61 (6d, 6H, 3 CH_2Ar), 4.77 (d, 1H, H-1, $J_{1,2}$=3.0 Hz), 4.10 (t, 2H, CH_2O), 4.02 (dd, 1H, H-2), 3.96 (dd, 1H, H-3), 3.90 (m, 1H, H-5), 3.69 (d, 1H, H-4), 3.58 (m, 1H, CH_2Oa), 3.40 (m, 1H, CH_2Ob), 1.77-1.22 (m, 8H, 4 CH_2), 1.11 (d, 3H, H-6); FAB MS $C_{33}H_{42}O_9S$ (614.76) *m/z* (%) 659 $[M+Na]^+$ (45), 371 (43), 307 (35), 165 (87), 91 (100).

6-Hydroxyhexyl α-L-fucopyranoside 6-hydrogensulfate (6i). Compound **6h** (90 mg, 0.14 mmol) was dissolved in acetic acid (4 mL), and Pd/C (10%) (50 mg) was added. The reaction mixture was stirred under hydrogen for a day. The catalyst was filtered off and the filtrate was concentrated. The crude product was purified by chromatography using EtOAc/methanol/water 10:5:2 v/v/v as the mobile phase, to give **6i** (23 mg, 74.9 %): R_f 0.51 (EtOAc/methanol/water 10:5:2 v/v/v); ^1H NMR ($CDCl_3$) δ 4.72 (d, 1H, H-1, $J_{1,2}$= 3.88 Hz), 3.92 (t, 2H, CH_2O), 3.70 (dd, 1H, H-2), 3.65 (d, 1H, H-4), 3.61 (dd, 1H, H-3), 3.54 (m, 1H, CH_2Oa), 3.38 (m, 1H, CH_2Ob), 1.60-1.25 (m, 8H, 4 CH_2), 1.07 (d, 3H, H-6); FAB MS $C_{12}H_{24}O_9S$ (212.26) *m/z* (%) 257 $[M+2Na]^+$ (32).

Methyl (6-hydroxyhexyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranoside)onate (7b). A mixture of **7a** (130 mg, 0.25 mmol), 1,6-hexanediol (300 mg, 2.5 mmol) and silver 2-mercaptoethanesulphonate (92 mg, 0.37 mmol) in benzene (1 mL) was stirred for 4 days. Benzene (10 mL) was added, the mixture was filtered, and the residue rinsed with benzene (5 mL). The combined filtrate was washed with water (3 mL), saturated $NaHCO_3$ solution (3 mL), and with water (3 mL) again. The combined aqueous phases were extracted with benzene (20 mL). The combined organic phases were dried over $MgSO_4$ and concentrated. The residue was purified by chromatography using EtOAc/methanol 10:0.7 v/v, to give **7b** (103 mg, 69.8

%): R_f 0.30 (EtOAc/methanol 10:0.5 v/v); ^{1}H NMR ($CDCl_3$) δ 5.39 (m, 1H, H-8), 5.32 (dd, 1H, H-7, $J_{7,8}$=8.46 Hz), 5.17 (d, 1H, NH), 4.84 (m, 1H, H-4), 4.34 (dd, 1H, H-9'), 4.14-4.05 (m, 3H, H-5, H-6, H-9), 3.79 (s, 3H, CH_3), 3.75 (m, 1H, CH_2Oa), 3.64 (t, 2H, CH_2O), 3.26-3.21 (m, 1H, CH_2Ob), 2.58 (dd, 1H, H-3_{eq}), 1.94 (t, 1H, H-3_{ax}), 2.19, 2.04, 2.01, 1.89 (4s, 15H, 4 AcO, NAc), 1.66-1.55 (m, 4H, 2 CH_2), 1.42-1.35 (m, 4H, 2 CH_2); FAB MS $C_{26}H_{41}NO_{14}$ (591.59) *m/z* (%) 614 $[M+Na]^+$ (100), 414 (27).

Methyl [3-(2-benzyloxyethoxy)propyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranoside]onate (7c). A mixture of **7a** (370 mg, 0.72 mmol), **5b** (676 mg, 3.22 mmol) and silver 2-mercaptoethanesulphonate (392 mg, 1.58 mmol) in benzene (6 mL) was stirred for 3 days. The mixture was filtered, and the filtrate was concentrated. The residue was taken up in chloroform (20 mL) and washed with saturated $NaHCO_3$ solution (4 mL) and water (4 mL), dried over $MgSO_4$, and concentrated. The residue was purified by chromatography using hexane/ether/methanol 7:7:2.5 v/v/v, to give **7c** (370 mg, 74.6 %): R_f 0.55 (hexane/ether/methanol 7:7:2.5 v/v/v); ^{1}H NMR ($CDCl_3$) δ 7.35-7.26 (m, 5H, 5 ArH), 5.38 (m, 1H, H-8), 5.31 (dd, 1H, H-7), 5.14 (d, 1H, NH), 4.83 (m, 1H, H-4), 4.56 (s, 2H, CH_2Ar), 4.30 (dd, 1H, H-9'), 4.11-4.03 (m, 3H, H-5, H-6, H-9), 3.79 (m, 1H, CH_2Oa), 3.76 (s, 3H, CH_3O), 3.52 (m, 2H, CH_2O), 3.34 (m, 1H, CH_2Ob), 2.57 (dd, 1H, H-3_{eq}, $J_{4,3}$=4.5 Hz), 2.13, 2.12, 2.02, 2.01, 1.87 (5s, 15H,4 OAc, NAc), 1.93 (t, 1H, H-3_{ax}, $J_{4,3}$=12.5 Hz), 1.84 (m, 2H, CH_2). FAB MS $C_{32}H_{45}NO_{15}$ (683.74) *m/z* (%) 706$[M+Na]^+$ (95), 624 (5), 413 (30), 91 (27).

Methyl [(3-hydroxyethoxy)propyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranoside]onate (7d). Compound **7c** (175 mg, 0.25 mmol) was dissolved in methanol (5 mL) and Pd/C (10%) catalyst (100 mg) was added. The mixture was stirred under hydrogen for 3 days, then filtered. The filtrate was concentrated and the residue was purified by chromatography using hexane/ether/methanol 4:7:3 v/v/v as the mobile phase, to give **7d** (143 mg, 94.0 %): R_f 0.47 (hexane/ether/methanol 4:7:3 v/v/v); ^{1}H NMR ($CDCl_3$) δ 5.42 (m, 1H, H-8), 5.31 (dd, 1H, H-7), 5.12 (d, 1H, NH), 4.83 (m, 1H, H-4), 4.30 (dd, 1H, H-9'), 4.12-4.05 (m, 3H, H-5, H-6, H-9), 3.87 (m, 1H, CH_2Oa), 3.80 (s, 3H, OCH_3), 3.72 (m, 2H, CH_2O), 3.57-3.35 (m, 4H, 2 CH_2O), 3.35 (m, 1H, CH_2Ob) 2.57 (dd, 1H, H-3_{eq}), 2.15, 2.14, 2.04, 2.02, 1.88 (5s, 4 OAc, NAc), 1.94 (t, 1H, H-3_{ax}), 1.82 (m, 2H, CH_2): FAB MS $C_{25}H_{39}NO_{15}$ (593.58) *m/z* (%) 616 $[M+Na]^+$ (32).

Methyl [6-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyloxy)-1-hexyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranoside]onate (2c). A mixture of **7a** (116 mg, 0.227 mmol), **6b** (120 mg, 0.224 mmol) and silver 2-mercaptoethanesulphonate (99 mg, 0.40 mmol) in benzene (1.2 mL) was stirred at room temperature for 24 hours. Benzene (15 mL) was added and the mixture was filtered. The filtrate was washed with saturated $NaHCO_3$ solution (2.5 mL) and water (2.5 mL). The organic phase was dried over $MgSO_4$, filtered and concentrated. The residue was purified by chromatography using hexane/ether/methanol 7:7:2.5 v/v/v as the mobile phase, to give **2c** (112 mg, 49.8 %): R_f 0.40 (hexane/ether/methanol 7:7:2.5 v/v/v): ^1H NMR ($CDCl_3$) δ 7.41-7.26 (m, 15H, 15 Ar-H), 5.41 (m, 1H, H-8_{sia}), 5.33 (dd, 1H, H-7_{sia}), 5.12 (d, 1H, NH), 4.90-4.64 (m, 6H, 3 CH_2Ar), 4.85 (m, 1H, H-4_{sia}), 4.81 (d, 1H, H-1_{fuc}, $J_{1,2}$=3.6 Hz), 4.32 (dd, 1H, H-$9'_{sia}$), 4.14-4.07 (m, 3H, H-5_{sia}, H-6_{sia}, H-9_{sia}), 4.03 (dd, 1H, H-2_{fuc}), 3.94 (dd, 1H, H-3_{fuc}), 3.87 (m, 1H, CH_2Od), 3.79 (s, 3H, OCH_3), 3.70 (m, 1H, H-5_{fuc}), 3.67 (d, 1H, H-4_{fuc}), 3.61 (m, 1H, CH_2Ob), 3.43 (m, 1H, CH_2Oc), 3.23 (m, 1H, CH_2Oa), 2.58 (dd, 1H, H-$3_{eq,sia}$), 2.15, 2.14, 2.03, 2.01, 1.88 (5s, 15H, 4 OAc, NAc), 1.95 (t, 1H, H-$3_{ax,sia}$), 1.62-1.27 (m, 8H, 4 CH_2), 1.11 (d, 3H, H-6_{fuc}): FAB MS $C_{57}H_{69}NO_{18}$ (1008.84) *m/z* (%) 1031 $[M+Na]^+$ (5), 1008 (10), 948 (20), 620 (10), 591 (10), 532 (10), 474 (15), 414 (68), 91 (100).

Methyl [6-(2,3,4-tri-*O*-benzyl-α and -β-L-fucopyranosyloxy)hexyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranoside]onate (2c) and (8c). Compound **6a** (39 mg, 0.081 mmol) in CH_2Cl_2 (6 mL) was stirred at room temperature and bromine (15.6 mg, 0.096 mmol) in CH_2Cl_2 (1 mL) was added. The mixture was stirred for 5 minutes. Cyclohexene was added until the bromine colour disappeared. The solvent was evaporated and the residue was taken up in MeCN (6 mL). Molecular sieves 4A (300 mg), TEAB (96 mg, 0.45 mmol), and **7b** (48 mg, 0.081 mmol) were added and the mixture stirred for 3 days. The reaction mixture was diluted with acetonitrile (10 mL) and filtered. The filtrate was concentrated and the residue was taken up in CH_2Cl_2 (15 mL) and washed with saturated $NaHCO_3$ solution (3 mL) and water (3 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by chromatography using hexane/ether/methanol 7:7:2.5 v/v/v as the mobile phase, to give a mixture of **2c** and **8c** (30 mg, 41.9 %): R_f 0.35 (hexane/ether/methanol 7:7:2.5 v/v/v/): ^1H NMR ($CDCl_3$) δ 7.38-7.25 (m, 15H, 15 Ar-H),

5.38 (m, 1H, H-8_{sia}), 5.31 (dd, 1H, H-7_{sia}), 5.13 (d, 1H, NH), 4.98-4.65 (m, 6H, 3 CH_2Ar), 4.75 (m, 1H, H-4_{sia}), 4.29 (dd, 1H, H-9_{sia}), 3.77 (s, 3H, OCH_3), 3.54 (dd, 1H, H-4_{fuc}), 3.51 (dd, 1H, H-3_{fuc}), 3.44 (m, 1H, H-5_{fuc}), 2.57 (m, 1H, H-$3_{eq,sia}$), 2.13, 2.12, 2.02, 2.01, 1.87 (5s, 15H, 4 OAc, NAc), 1.93 (t, 1H, H-$3_{ax,sia}$), 1.64-1.33 (m, 8H, 4 CH_2), 1.18, 1.12 (2d, 3H, H-6_{fuc}); FAB MS *m/z* (%) $C_{53}H_{69}NO_{18}$ (1008.84) 1031 $[M+Na]^+$ (7), 1008 (12), 948 (23), 620 (12), 591 (13), 532 (14), 474 (18), 414 (70), 91 (100).

6-(α-L-Fucopyranosyloxy)hexyl 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranoside (2a). Compound **2c** (30 mg, 0.03 mmol) was dissolved in methanol (5 mL) and was hydrogenated over Pd/C (10 %) (30 mg) catalyst for 3 days. The mixture was filtered and sodium methoxide (20 mg, 0.26 mmol) was added. The reaction mixture was stirred at room temperature for 24 hours. Water (0.5 mL) was added, and the mixture was stirred at 40 °C for 12 hours. The mixture was neutralised with Amberlite IR-120 (H^+) ion exchange resin and filtered. The filtrate was concentrated, and then lyophilised to give **2a** (17 mg, 96 %): R_f 0.45 (chloroform/methanol/water 5:6:2 v/v/v); ^1H NMR (D_2O) δ 4.72 (d, 1H, H-1_{fuc}, $J_{1,2}$=3.86 Hz), 4.61 (s, 7H, OH), 3.91 (m, 1H, CH_2Oa), 3.73-3.30 (m, 14H, 11 OH, 3 $CH_2O_{b,c,d}$), 2.60 (dd, 1H, H-$3_{eq,sia}$, $J_{4,3}$=4.75 Hz), 1.89 (s, 3H, NAc), 1.82 (s, 1H, H-$3_{ax,sia}$), 1.46 (m, 4H, 2 CH_2), 1.22 (m, 4H, 2 CH_2), 1.07 (d, 3H, H-6_{fuc}); FAB MS $C_{23}H_{41}NO_{14}$ (555.58) *m/z* (%) 600 $[M+Na]^+$ (20).

Methyl {3-[2-(2,3,4-tri-*O*-benzyl-α and -β-L-fucopyranosyloxy)ethoxy]propyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranoside}onate (2d) and **(8d).** Thioglycoside **6a** (123 mg, 0.26 mmol) was dissolved in dry CH_2Cl_2 (5 mL) and bromine (45.7 mg, 0.28 mmol) in CH_2Cl_2 (2 mL) was added. The mixture was stirred at room temperature for 5 minutes and cyclohexene (0.5 mL) was added then the colourless solution was concentrated. The residue was taken up in dry MeCN (5 mL), and tetraethylammonium bromide (273 mg, 1.3 mmol), compound **7d** (143 mg, 0.14 mmol) and molecular sieves 4Å (1.0 g) were added. The reaction mixture was stirred at room temperature for 2 days. The mixture was diluted with CH_2Cl_2 (40 mL) and the solids present were filtered off. The filtrate was extracted with saturated $NaHCO_3$ solution (3 mL) and water (3 mL). The organic phase was dried over $MgSO_4$ and concentrated. The residue was purified by chromatography using hexane/ether/methanol 4:7:2 v/v/v as the mobile phase, to give the mixture of **2d** and **8d** (170 mg, 69.9 %): R_f 0.45 (hexane/ether/methanol 4:7:2 v/v/v); ^1H NMR ($CDCl_3$) δ 7.38-

7.23 (m, 15H, 15 Ar-H), 5.32 (m, 1H, H-8_{sia}), 5.31 (dd, 1H, H-7_{sia}), 5.15 (d, 1H, NH), 4.85 (d, 1H, H-$1_{fuc\alpha}$), 4.83 (m, 1H, H-4_{sia}), 4.37, 4.84, 4.79, 4.73, 4.68, 4.64 (6d, 6H, 3 CH_2Ar), 4.36 (d, 1H, H-$1_{fuc\beta}$), 4.30 (dd, 1H, H-$9'_{sia}$), 4.14-4.06 (m, 3H, H-5_{sia}, H-6_{sia}, H-9_{sia}), 4.08 (dd, 1H, H-2_{fuc}), 3.35 (dd, 1H, H-5_{fuc}), 3.78 (m, 1H, CH_2Oa), 3.76 (s, 3H, OCH_3), 3.72 (m, 1H, CH_2Ob), 3.65 (d, 1H, H-4_{fuc}), 3.64-3.44 (m, 5H, CH_2Oc, 2 CH_2O), 3.33 (m, 1H, CH_2Od), 2.56 (dd, 1H, H-$3_{eq,sia}$), 2.13, 2.11, 2.02, 2.01 (4s, 12H, 4 OAc), 1.92 (t, 1H, H-$3_{ax,sia}$), 1.79 (m, 2H, CH_2), 1.16, 1.10 (2d, 3H, H-$6_{fuc\alpha,\beta}$), α/β ratio = 70/30; FAB MS $C_{52}H_{65}NO_{19}$ (1010.10) *m/z* (%) 1032 $[M+Na]^+$ (100), 91 (65).

Methyl {[3-(2-α and -β-L-fucopyranosyloxy)ethoxy]propyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranoside}onate (2e) and (8e). Mixture of **2d** and **8d** (165 mg, 0.16 mmol) was dissolved in methanol (5 mL) and Pd/C (10%) (50 mg) was added. The mixture was stirred under hydrogen for 3 days. The catalyst was filtered off, and the filtrate was concentrated. The residue was purified by flash chromatography, using ether/methanol 10:3 v/v as the mobile phase, to give the mixture of **2e** and **8e** (110 mg, 91.6 %): R_f 0.38 (ether/methanol 10:3 v/v); ^1H NMR ($CDCl_3$) δ 5.37 (m, 1H, H-8_{sia}), 5.31 (m, 2H, H-7_{sia}, NH), 4.89, 4.25 (2d, 1H, H-$1_{fuc\alpha}$, H-$1_{fuc\beta}$), 4.84 (m, 1H, H-4_{sia}), 4.34 (dd, 1H, H-$9'_{sia}$), 4.13-4.02 (m, 3H, H-5_{sia}, H-9_{sia}), 3.79 (s, 3H, OCH_3), 3.37 (m, 1H, CH_2Oa), 2.57 (dd, 1H, H-$3_{eq,sia}$), 2.13, 2.03, 2.01, 1.87 (4s, 15H, 4 OAc, NAc), 1.92 (t, 1H, H-$3_{ax,sia}$), 1.82 (m, 2H, CH_2), 1.32, 1.28 (2d, 3H, H-$6_{fuc\beta,\alpha}$); FAB MS $C_{31}H_{49}NO_{19}$ (739.73) *m/z* (%) 762 $[M+Na]^+$ (100).

3-[2-(α-L-Fucopyranosyloxy)ethoxy]propyl 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranoside (2b). A mixture of **2e** and **8e** (80 mg, 0.01 mmol) and sodium methoxide (30 mg, 0.05 mmol) was dissolved in dry methanol (6.5 mL) and stirred at room temperature for 24 hours. Water (1 mL) was added and the reaction mixture stirred at 40 °C for 10 hours. The reaction mixture was diluted with water (10 mL) and the methanol was evaporated. The water solution was neutralized with Amberlite IR-120 (H^+), and filtered. The filtrate was lyophilised. The residue was purified by chromatography using chloroform/methanol/water 10:10:3 v/v/v as the mobile phase, to give the product **2b** (30 mg, 50 %): R_f 0.35 (chloroform/methanol/water 10:10:3 v/v/v); ^1H NMR (D_2O) δ 4.73 (d, 1H, H-1_{fuc}, $J_{1,2}$=4.0 Hz), 3.95 (m, 1H, CH_2Oa), 3.75-3.48 (m, sugar protons, 4 CH_2O), 2.58 (dd, 1H, H-$3_{eq,sia}$), 1.88 (s, 3H, NAc), 1.72 (m, 2H, CH_2), 1.56 (t, 1H, H-$3_{ax,sia}$), 1.07 (d, 3H, H-6_{fuc}); FAB MS $C_{22}H_{39}NO_{15}$ (557.55) *m/z* (%) 602 $[M+2Na]_+$ (38), 329 (37), 286 (56).

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