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Large Scale Synthesis of Linker-Modified Sialyl Lewis^X, Lewis^X and N-Acetyllactosamine

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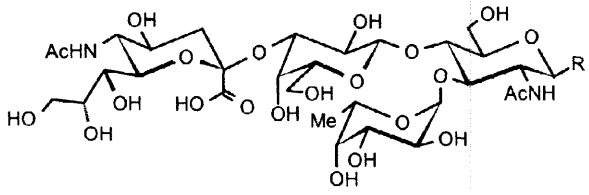
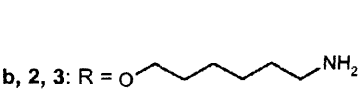
Abstract: The synthesis of sialyl-Lewis^X (**1b**), Lewis^X (**2**) and N-acetyllactosamine (**3**), each being attached to the 1β-O-(6-amino)hexyl handle, were scaled up to gram amounts to obtain sufficient material for thorough pharmaceutical evaluations and for derivatisations aiming at more potent selectin antagonists. The disaccharide **3** was synthesised from inexpensive lactose to provide a versatile building block, either to be used for alternative approaches to the Lewis type oligosaccharides, or to prepare polyvalent LacNAc templates to be further elaborated by glycosyltransferase reactions. All syntheses were directed to reasonable large scale procedures, especially by minimising the number of steps and the use of heavy metal salts in glycosylations.

Introduction

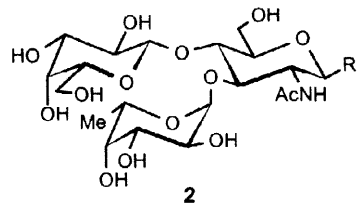
A variety of disease states are characterised by excess inflammation, including arthritis, allergies and the tissue damage (reperfusion injury) suffered following lung injury or heart attack. Much of the recent interest surrounding potential new anti-inflammatory drugs has been centred on the sialyl Lewis^X (sLe^X, Figure 1) tetrasaccharide and the proteins that bind it, known as the selectins, owing to their roles in inflammatory responses.[1] The recognition of cell-surface glycoproteins bearing the sLe^X epitopes by selectins, present either on the walls of blood vessels (E-, P-selectins) or on leukocytes (L-selectin), causes the leukocytes to



1a: R = OH



1b, 2, 3: R =



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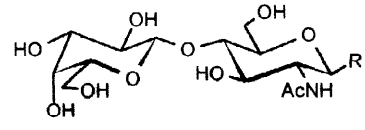

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Figure 1: sialyl Lewis^X (**1a**) and the title compounds **1b**, **2** and **3**.

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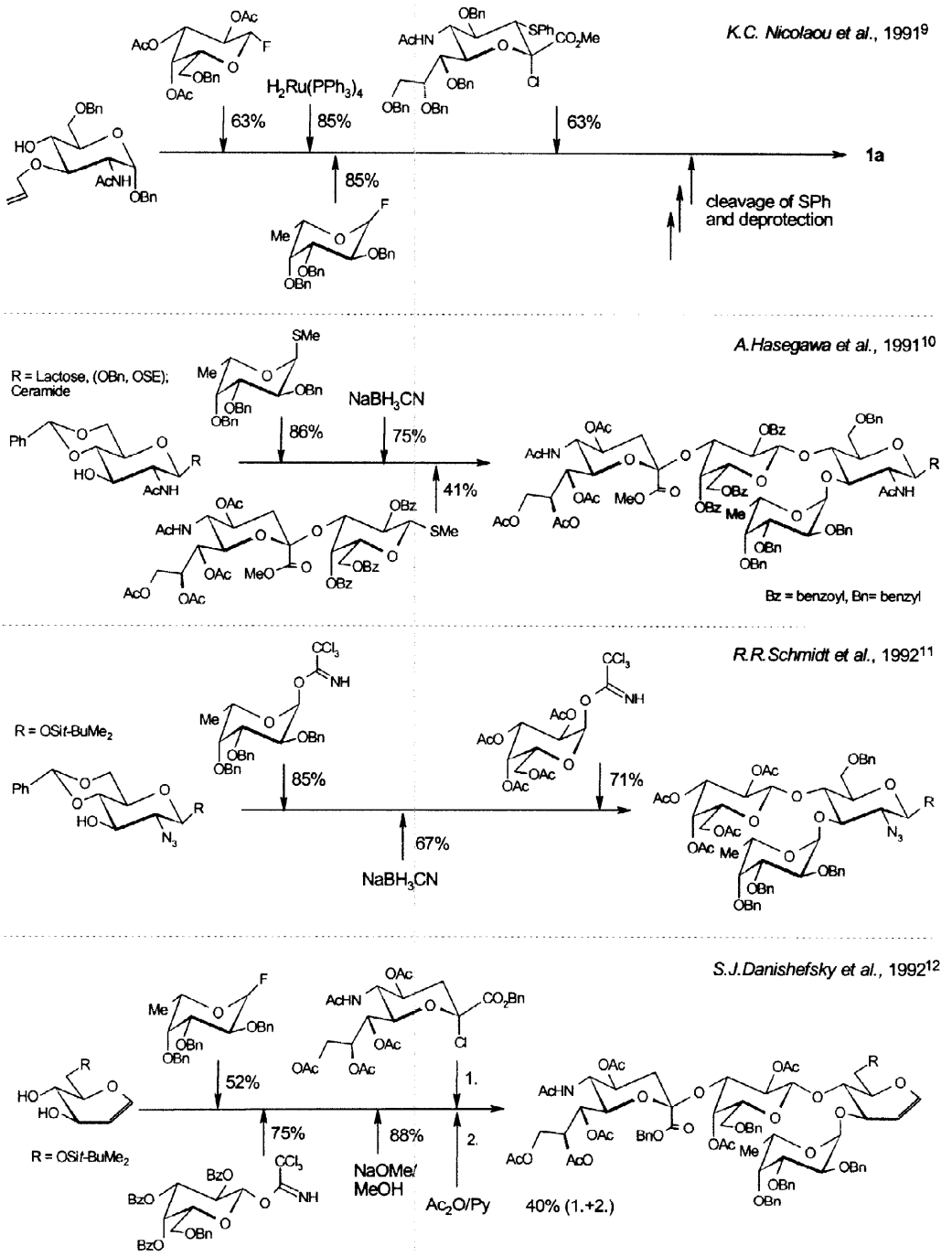
move into sites of injury or infection from the bloodstream. This migration involves three distinct steps in which the leukocytes initially roll along the vascular surface because of sLe^X binding by selectins, before adhering firmly to the vascular endothelium by protein-protein interaction, and finally escaping through the



Scheme 1: Synthesis concepts for (s)Le^X oligosaccharides reported in the literature.

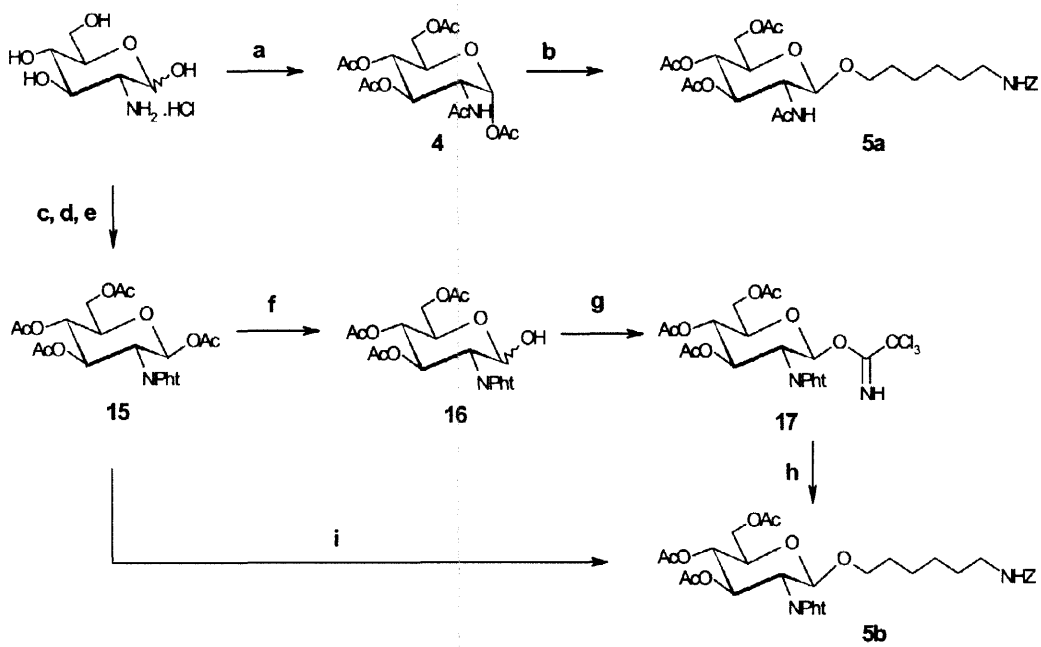
vessel wall into surrounding tissues. Consequently, the blocking of the initial adhesion mechanism with soluble sLe^X itself or with glycomimetics derived therefrom is seen as a way of counteracting excess inflammation. Furthermore, the sLe^X-type carbohydrate determinants are involved in the adhesion of cancer

cells to the vascular endothelium and thus contribute to hematogenous metastasis of cancer through selectin mediated recognition processes.[2] Although sLe^X and a sLe^X-propanolamine conjugate recently became available as tools in glycobiology research, gram amounts of material required for preclinical research studies are neither available nor affordable from commercial sources.[3] Similar arguments apply to the biomimetic syntheses of structures closely related to **1a**, in particular performed by the *Cytel Corp.* to prepare their *CY 1503* (*Cylexin*) clinical development product, since this approach using a variety of cloned enzymes is not fully accessible for large scale applications.[4] The derivative **1b** with an sLe^X epitope attached to a suitable linker group was needed in quite large amounts, exceeding one gram quantities, to prepare and evaluate reduced derivatives,[5] clustered conjugates,[6] and some more complex model compounds[7] to study in detail the inhibition of leukocyte adhesion *in vitro* and *in vivo*. The flexible 1β-O-linked hexanolamine anchor group of medium length in **1b** was chosen to keep any attachments far enough away from the tetrasaccharide moiety, to prevent the spacer chain from backfolding and to provide the reactive amino group onto which virtually any residue could be fixed to the ligand. The synthesis of the tetrasaccharide moiety NAcNeu α(2→3)Gal ß(1→4) [Fuc α(1→3)] GlcNAc, although not designated as sLe^X, for the first time was reported by *Chembiomed* chemists,[8] and the non-enzymatic and chemo-enzymatic preparations reported later mostly followed analogous strategies, presumably to provide milligram amounts of **1a**.[9-12] They were either based on consecutive glycosylations of the respective monosaccharide building blocks, or on linking up the disaccharides NAcNeu α(2→3) Gal ß(1→4) and Fuc α(1→3) GlcNAc, the first option in general being preferred over the [2+2]-strategy. Persistent problems in synthesising sLe^X mainly consist in the spacial proximity of the D-galactose and α-L-fucose in positions 4 and 3 of the N-acetylglucosamine, respectively, in the low reactivity of the 4-OH group in the galactosylations, in the pronounced acid lability of the α-L-Fuc linkage, and in the difficult anomeric control and side reactions in the sialylation. Although the procedures referred to[8-12] provided considerable improvements on laboratory synthesis scales to afford milligram quantities of the deprotected final compounds, they do not automatically translate into larger scales under a sustainable laboratory setting. For instance, most procedures for **1a** and related saccharides hitherto reported (Scheme 1), extensively make use of heavy metals in excess to promote the glycosylations. To illustrate this, *Nicolaou*[9] used 2.5 equivalents of $AgClO_4$ and of $SnCl_2$ to yield the lactosamine by galactosylation of the 3-allyl protected GlcNAc precursor, followed by deallylation and repeated use of this metal system (3 equiv. of each) in the fucosylation step. The Le^X-trisaccharide was then treated with 4 equivalents of $Hg(CN)_2/HgBr_2$ to give the sialoside which was liberated from the directing phenylthio group with 5 equivalents of triphenyltin hydride. The two additional steps required by using the temporary allyl group in the 3-position of GlcNAc might only prove advantageous if the overall yield obtained here would compensate for a yield significantly lower than about 48% for the galactosylation of a directly fucosylated intermediate. *Danishefsky*[12a] used the glycal strategy to perform the (in part) regioselective fucosylation promoted with $AgClO_4$ and $SnCl_2$ (2 equiv. of each). The glycal moiety was transformed into the GlcNAc unit *after* the pseudotetrasaccharide assembly.[12b] However, this strategy certainly might not be particularly rewarding to prepare large quantities e.g. of **1b**, given the overall low-yielding multistep sequence to introduce the 2-acetamido group (22%, including the general final deprotection steps) at the end of the synthesis. *Toepfer*[11] consistently used the elegant imidate procedure up to the Le^X- trisaccharide. For large scale applications, *Hasegawa's*[10] synthesis may suffer from difficulties in the purification of the NAcNeu α(2→3) Gal ß(1→4) thioglycoside donor, which is acetylated at the

NAcNeu moiety, as well as from the moderate yields obtained in coupling this valuable building block. The combined chemoenzymatic approach performed by *Wong*[13] involved the fucosylation of the NAcNeu α(2→3) Gal ß(1→4) GlcNAc trisaccharide by a cloned α-1,3-fucosyltransferse and GDP-fucose. Very recently, a fully thioglycoside-based synthesis of **1a**, at the expense of stereoselectivity in the galactosylation step, has been reported.[14,15] The objective of our work was to provide a concise and reliable route to sufficient amounts of pure **1b** for extensive pharmacological studies *in vivo* and as a standard material to be used in cell-based screening assays to determine the selectin inhibitory potency of sLe^X mimetics.[6] Moreover, **1b** has been used to prepare much more potent selectin antagonists.[5] It was intended to minimise the consumption of heavy metal promoters and not to spend on more synthetic steps for only little improved overall yields. The most valuable building block, the sialic acid, should be introduced at the end of the synthesis. Furthermore, the large scale synthesis of the corresponding spacer-modified Le^X (**2**) and N-acetyllactosamine (**3**) is reported. The precursor molecule **23** is readily available from inexpensive lactose and represents the potential starting point for a convergent new approach to **1b**. Compound **3** has also been used for grafting the LacNAc units onto biocompatible backbones which are suitable for the synthesis of polyvalent oligosaccharide ligands by glycosyltransferase reactions.

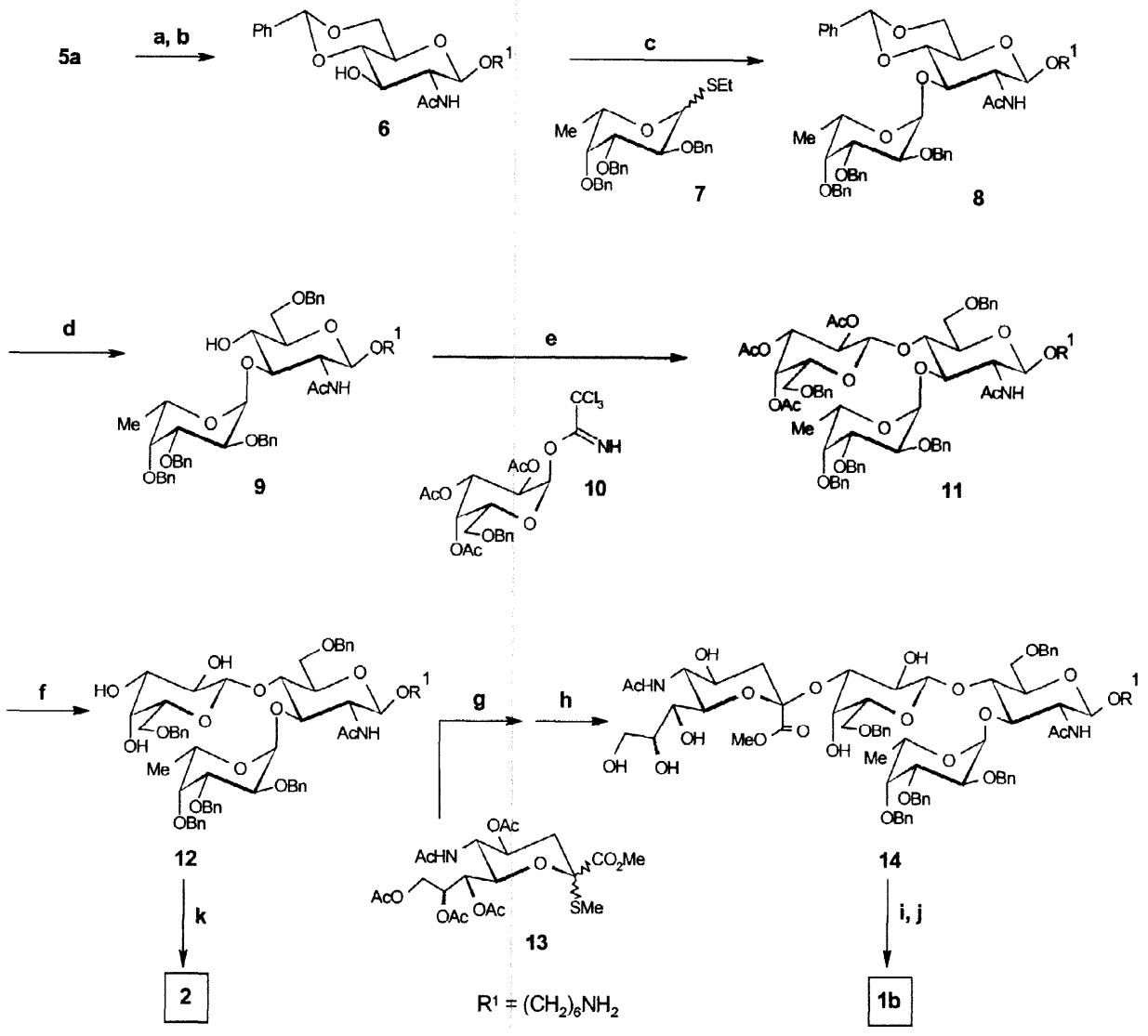
Preparative Results and Discussion

Preliminary attempts to use an allyl-protected GlcNAc building block, in analogy to the synthesis described by *Nicolaou*[9a], did not give superior overall yields. Therefore, the shorter sequence as outlined in Schemes 2 and 3 was pursued by which 100 gram batches of the disaccharide **8**, 20-30 gram batches of the trisaccharide **11** and 0.7-1.5 gram batches of **1b** could be reproduced several times. The fucosylation and sialylation were



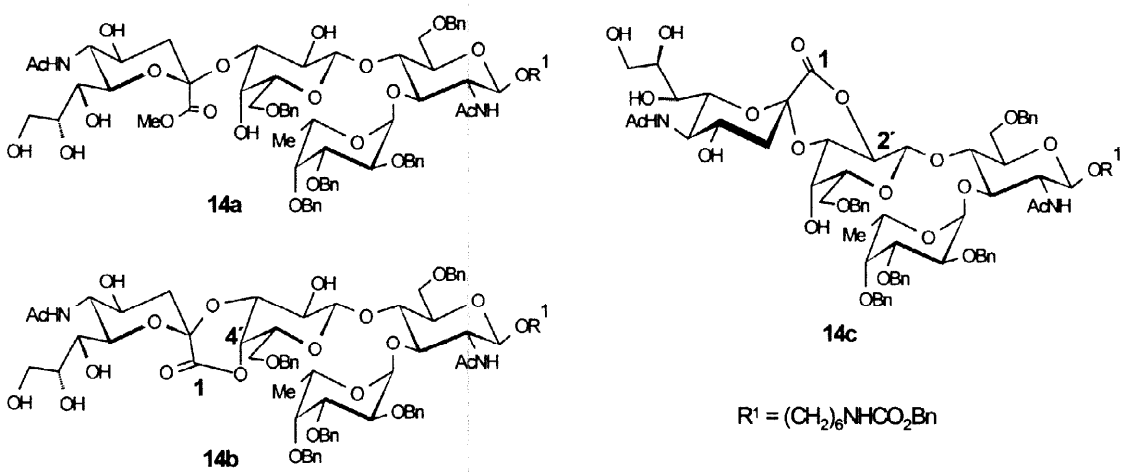
Scheme 2: Comparison of alternative pathways to the GlcNAc precursors **5a,b**: a) Ac_2O, pyridine, NEt_3, CH_2Cl_2 (78%); b) 1.5 equiv. 6-N-Z-aminohexanol, $(CH_2Cl)_2$, mol. sieves 4 Å, 60°C, 1.2 equiv. TMSOTf (51%); c) NaOMe/MeOH; d) phthalic anhydride, MeOH, NEt_3; e) Ac_2O, pyridine, 40-50%, 3 steps; f) N_2H_4-HOAc, DMF, 2 h, 20°C (65%); g) DBU/CCl_3CN (84%); h) 6-N-Z-aminohexanol, mol. sieves 4 Å, cat. TMSOTf, 20°C, CH_2Cl_2 (98%); i) 1.5 equiv. 6-N-Z-aminohexanol, $(CH_2Cl)_2$, mol. sieves 4 Å, 40°C, 1.2 equiv. TMSOTf (87%).

performed with the stable thioglycosides **7**[16] and **13**[17], respectively, and the galactosylation by using the galactose imidate **10**.[12a] All these donor sugar units were available in >100 gram quantities in analogy to the reported methods. Because of the stepwise character of this approach, much effort was directed at a quick and large scale supply of the GlcNAc precursor **5a** (Scheme 2). The recommended literature procedures for glycosylations with GlcNAc donors[18] proved to be not satisfactory in terms of shortness and overall yields. Whereas relatively reactive and easily removable acceptors like allyl and benzyl alcohol can be used in large



Scheme 3: Large scale synthesis of **1b** and **2**: a) NaOMe in MeOH, 1 h, 20°C, then H^+ (97%), b) 1.6 equiv. benzaldehyde dimethylacetal, DMF, 30°C, cat. CSA (84%); c) **7** (2.0 equiv.), CH_2Cl_2/ DMF (2.6:1), mol. sieves 4 Å, *n*-$Bu_4N^+Br^-$ (2.4 equiv.), $CuBr_2$ (2.2 equiv.), 2 h, 25°C, (77%); d) $NaBH_3CN$ (10 equiv.), HClg in Et_2O, THF, 25°C, (71%); e) **10** (1.3 equiv.), $(CH_2Cl)_2$, TMSOTf, tetramethylurea, 2 h, 55-60°C (not isol.); f) NaOMe (cat.), MeOH, 2.5 h, 25°C, (64%, 2 steps); g) **13** (3.2 equiv.), mol. sieves 4 Å, -70 to -35°C, CH_3CN/CH_2Cl_2, MSB/AgOTf, then h) cat. NaOMe in MeOH, 2 h, 25°C, SC on silica gel (55% **14**, 23% **12**); i) Pd-black, MeOH, HCO_2H; j) $NaOH/H_2O$, pH 10.5, then HCl to pH 6, then SC on Biogel P2 (80%, 2 steps); k = i (91%).

excesses in such reactions, presumably proceeding *via* the GlcNAc-oxazoline intermediate,[19] the trimethylsilyltriflate (TMSOTf) catalysed reaction of this isolated intermediate gave very poor yields (< 20%, 3 equiv. of the crystalline 6-[N-benzyloxycarbonyl]aminohexanol) in our hands. Following the procedure of *Lemieux*,[20] kg-quantities of the pure α-acetate **4** were easily prepared in good yield, whereas the phthalimido donor **15** was obtained as an anomeric mixture that became very dark during attempts to achieve the recommended anomerisation on such scale by heating ($NaOAc/Ac_2O/100°C$). The pure β-acetate of **15** could only be prepared in moderate yield by repeated crystallisations and column chromatography. Although the imidate donor **17**, prepared from **15** *via* **16**, nearly quantitatively provided the GlcNAc unit **5b**,[21] the direct TMSOTf-catalysed glycosylation[22] using **15** proved to be even better. The outcome of the last reaction is well in accord with *Paulsen's* work,[23] suggesting that the TMSOTf-catalysed reaction should only proceed here by assistance of the neighbouring group positioned *trans* to the anomeric β-acetate leaving group. Clearly, this raises questions about the mechanism of the reaction pathway to **5a**, namely by using the less reactive α-acetate donor **3**.[24] For the corresponding more reactive β-acetate, an oxazoline intermediate being activated by an electrophile has been proposed.[25] Probably the α-acetate **4** anomerises under the reaction conditions employed here, prior to formation of the presumed oxazoline intermediate. Taken together, the least efficient glycosylation to give **5a** turned out to be the preferred one, in terms of shortness, overall yield and the advantage not to have to replace the phthalimido group by the acetyl group at the end of the synthesis. The fucosylation of the 4,6-O-benzylidene protected derivative **6** to give the disaccharide **8** proceeded smoothly in the presence of copper(II) bromide and tetrabutylammonium bromide, but a metal-free promoter like dimethyl(methylthio)sulfonium triflate (DMTST)[26] could equally be used here. The scale-up of the fucosylation from mg-batches up to 100 g quantities of **8**, while switching from column chromatography to a more practicable isolation procedure on large scales, caused a decrease in the yields from 95% to 77% (Table 1). The reductive ring-opening of the benzylidene acetal in **8** was performed with



Scheme 4: Structures of the deacetylated intermediates **14**.

sodium cyanoborohydride and HCl in THF,[27a,b] the huge excess of hydride reagent needed here and the difficult reaction control not being acceptable for economical large scale preparations. However, no alternative for this procedure seems to be available. Modified reaction conditions using the borane

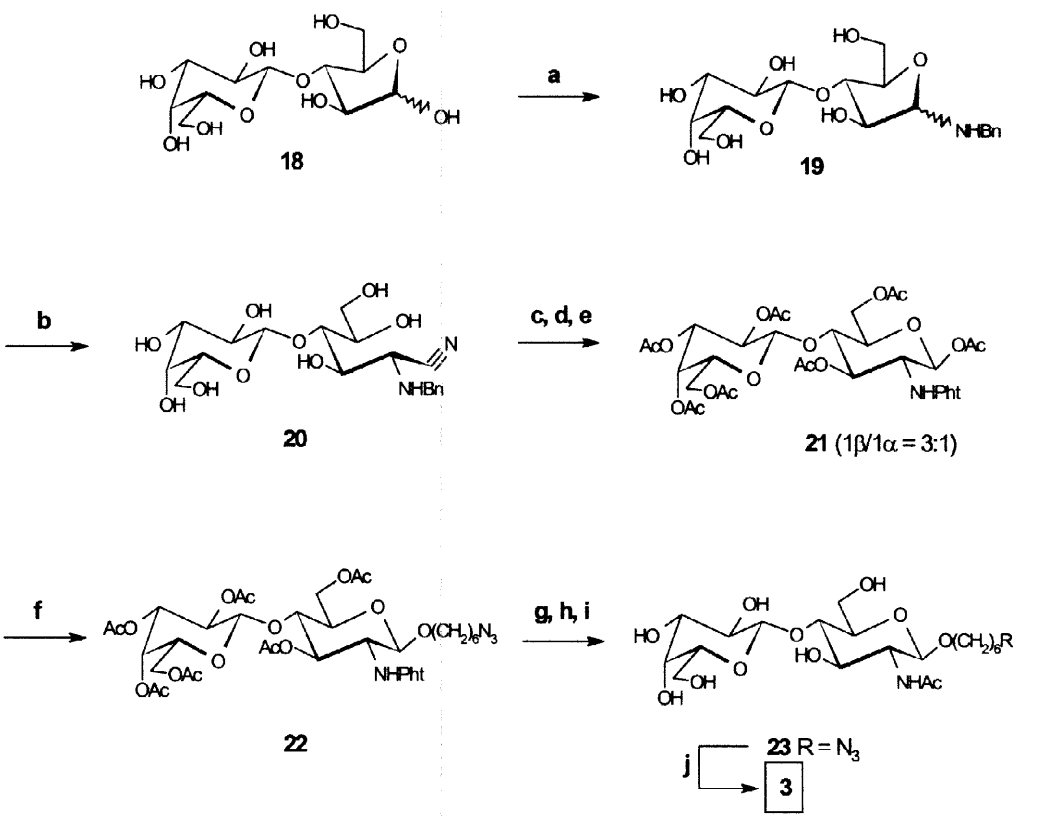
trimethylamine complex and aluminium chloride[27c] proved to be unsuccessful. Due to the poor reactivity of the hydroxyl group in the 4-position of **9**, the reactive imidate **10** was chosen to perform the difficult galactosylation reaction which was promoted by boron trifluoride etherate in dichloromethane. On much larger scales (Table 1), quite good results could be achieved using only 1.3 equivalents of the donor **9** and TMSOTf as promoter, buffered by tetramethylurea, to give **12** after subsequent O-deacetylation with sodium methoxide in methanol in 64% overall yield. Trisaccharide **12** was further deprotected by catalytic transfer hydrogenation with formic acid to give the free Le^X epitope **2** in 91% yield. Generally these conditions were found to be superior to the standard method (using a 10% Pd/C catalyst) to achieve the removal of the benzyl protecting groups, probably due to the neutralisation of the deactivating primary amino group formed in the linker unit. Acceptors like **12** which are deprotected at the 2,3 and 4 positions of the galactose unit have the suitable features for regioselective sialylations at position 3 using either N-iodosuccinimide (NIS)/ trifluoroacetic acid or DMTST in acetonitrile to favour the α/β ratio, as was previously demonstrated.[17] The best method to prepare the tetrasaccharide **14** proved to be the methylensulfenyl bromide (MSB)/silver trifluormethane-sulfonate (AgOTf) system at low temperatures (-70 to -35°C) in a dichloromethane/ acetonitrile mixture, while adding an excess of thioglycoside **13** in portions to compensate for side reactions caused by the decomposition of **13**. The product mixture obtained was directly subjected to the standard O-deacetylation procedure and purified at this stage. In this manner 23% of recovered **12** and 55% of the desired product **14** which in fact consisted of a mixture of tetrasaccharides (Scheme 4) could be obtained. It was not attempted to separate and characterise these partially lactonised intermediates more in detail, because this laborious task has already been accomplished in a similar case,[28] and comparable 1-4′- and 1-2′-lactones have already been described.[29] The tetrasaccharide mixture **14** was deprotected by transfer hydrogenation and O-deacetylation to give the pure title compound **1b** in 80% yield. The ligand **1b** has been used extensively as a reference compound in bioassays measuring the inhibition of HL60 tumor cell adhesion to recombinant E- and P-selectin-IgG fusion proteins.[5,6] The concentrations of **1b** required to block adhesion of 50% of the cells compared with a negative control (IC_{50}) typically ranged from 1.0 to 1.5 mM and from 2.0 to 3.0 mM for E and P-selectin, respectively.

Table 1: Isolated yields (%) of small and large scale batches.

Synth. step	Small scale	Large scale	Scale-up factor
6 to **8**	95	77	417
8 to **9**	90	71	100
9 to **12**	71	64	138
12 to **14**	-	55% (23% **12**)	-

In comparison to the yields which one can obtain on small scales, the losses suffered due to our scaling up (Table 1) show that even larger batches may not provide acceptable results using the synthesis methodology employed here. Particularly the hydride reduction to **9** and the sialylation step would require much improved methods in order to compete with the enzymatic synthesis process. For the sialylation, the alternative phosphite method recently reported still remains to be explored for large scale applications.[30] However, as regards the potential therapeutic applications of pharmaceuticals derived from the sLe^X epitope, the potent

multivalent versions of **1b**[6] may be more suitable and easier to prepare by combining chemical synthesis in solution and ensuing enzymatic synthesis, the latter preferably to be accomplished on a water soluble support simultaneously serving as the biocompatible drug delivery system. To achieve this goal, an expedient synthesis of the lactosamine precursor **3** which corresponds to the final epitopes **1b** and **2** has been developed (Scheme 5): The 3-O-(β-D-galactopyranosyl)-D-arabinose **18** which is commercially available or can be readily prepared from inexpensive lactose, was transformed into 100 gram amounts of the crystalline 2-benzylamino-2-deoxy derivate **19**.[31] This compound was converted into the known LacNAc derivative **21** *via* the crystalline aminonitrile **20** by following reported procedures.[22a,31] Disaccharide **21** has been used to prepare the β-methyllactoside by reaction of the corresponding β-lactosyl chloride with methanol (50 equiv.) and $HgO/HgCl_2$ (5 equiv.).[22a] Fortunately, our proven method to prepare the linker-modified glucosamines **5a,b** could be applied successfully to the donor **21** to give **22** in 77% yield, while using only a slight excess (1.2 equiv.) the 6-azidohexanol acceptor and completely omitting heavy metal promoters. Recently, a synthetic strategy to prepare sulfated Le^X by selective benzoylation of 3,4-O-isopropylidene lactosides into



Scheme 5: a) $PhCH_2NH_2$, EtOH (81%); b) NaCN, HOAc, EtOH (75%); c) H_2, Pd-$BaSO_4$, HCl/H_2O, then d) $NaHCO_3$, phthalic anhydride, then e) Ac_2O, pyridine, (55%, 3 steps); f) 6-azidohexanol (1.2 equiv.), $(CH_2Cl)_2$, TMSOTf (77%); g) cat. NaOMe in MeOH, then h) N_2H_4-H_2O, MeOH, reflux, 4 h, then i) Ac_2O, MeOH, 0°C (80%, 3 steps); j) $PdCl_2$, MeOH, HCO_2H (91%).

2,2′,6,6′-tetra-O-benzoyl lactosides as the key steps has been reported.[32] However, it is worth mentioning here that this strategy has already been explored earlier[33] and did not lead to the compounds designated as sulfated "Le^X", but in fact gave the respective glucose analogs (GlcNAc replaced by Glc). Thus, the spacer-modified precursor molecule **23** may provide a new entry to prepare **1b** by following an analogous strategy.

Moreover, the free amine **3** can be used for grafting the LacNAc units *via* the linker group onto biocompatible polymer backbones which then can be further elongated by glycosyltransferase reactions to give artificial structures similar to the natural ligands of the lectins. The preparation of these polyvalent oligosaccharide ligands will be reported elsewhere.[34]

Experimental Part

General: Thin layer chromatography (TLC) was carried out on precoated Kieselgel 60 F_{254} plates (0.25 mm thickness, E. Merck) with the specified solvent mixtures. Spots were visualised by spraying the plates with sulfuric acid/anisaldehyde reagent, followed by heating. E.Merck silica gel (60, particle size 0.040-0.063 mm) was used for flash column chromatography. Yields refer to chromatographically (TLC) and spectroscopically (NMR) homogeneous materials. Optical rotations were measured using a Perkin Elmer 241 polarimeter (1 dm). NMR spectra were recorded on a Bruker WT 300 and AM 600. NMR chemical shifts are given as δ-values with reference to tetramethylsilane (TMS) as internal standard, if not otherwise noted. The spectra recorded in D_2O as solvent were locked to deuterium. Fast Atom Bombardment (FAB) and Electrospray Ionisation (ESI) mass spectra were recorded on a TSQ 700 (Finnigan/MAT) spectrometer.

2-Acetamido-1,3,4,6-tetra-O-acetyl-2-deoxy-α-D-glucopyranose (4): The procedure to prepare **4** from D-GlcNAc hydrochloride was analogous to that reported by *Lemieux*[20] to obtain the pure α-acetate after crystallisation from ethanol (1.16 kg, 78%; DC: R_f = 0.66 CH_2Cl_2/MeOH 20:1). The assignment of H-1β is in accord with reported data[24] and was confirmed by comparison with the β-acetate, bought from SIGMA (5.70 ppm; d, $J_{1,2}$ = 9.0 Hz, H-1α). ^{1}H NMR (300 MHz, $CDCl_3$): 6.17 (d, $J_{1,2}$ = 3.5 Hz, 1 H, H-1β), 5.62 (d, J = 9.0 Hz, 1 H, NH), 5.22 (m, 2 H, H-3, H-4),), 4.48 (m, H-2), 4.25 (dd, J = 4.0, 12.6 Hz, 1 H, H-6a), 4.07 (dd, J = 2.5, 12.6 Hz, 1 H, H-6b), 4.00 (m, 1 H, H-5), 2.20 (s, 3 H, 1-OAc), 2.09, 2.06, 2.04, (3s, 9 H, OAc), 1.94 (s, 3 H, NAc).

6-(N-Benzyloxycarbonylamino)hexyl-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy)α-D-glucopyranoside (5a): The α-acetate **4** (93.25 g, 0.25 mol), 6-(N-benzyloxycarbonylamino)hexanol (62.72 g, 0.25 mol) and molecular sieves 4 Å (10 g) in dry $(CH_2Cl)_2$ (250 ml) were stirred at 60°C for 30 min. Trimethylsilyl trifluoromethane-sulfonate (TMSOTf, 55 ml, 0.304 mol) was added dropwise over 45 min. After stirring for 1 h at 65°C, more 6-(N-benzyloxycarbonylamino)hexanol (32.0 g, 0.125 mol) was added, and stirring continued for 1 h at the same temperature. The dark reaction was quenched with NEt_3 (45 ml, 0.32 mol), filtered and washed with 1N aqueous HCl (2x 200 ml), aqueous $NaHCO_3$ and brine. After drying ($MgSO_4$) and evaporation of the solvents, **5a** crystallised from diethyl ether: 75.0 g (52%); $[\alpha]_D^{20}$ = -17.8° (c = 1, CH_2Cl_2); mp.: 114°C; ^{1}H NMR (300 MHz, [d_6]DMSO): 7.93 (d, J = 9.0 Hz, 1 H, AcN<u>H</u>), 7.34 (m, 5 H, Ph), 7.21 (t, J = 6.0 Hz, 1 H, N<u>H</u>Z), 5.08 (*pseudo*-t, J = 10.0 Hz, 1 H, H-3), 5.00 (s, 2 H, OC<u>H</u>$_2$Ph), 4.82 (*pseudo*-t, J = 10.0 Hz, 1 H, H-4), 4.58 (d, $J_{1,2}$ = 9.0 Hz, 1 H, H-1α), 4.18 (dd, $J_{5,6}$ = 5.0 Hz, J_{6ab} = 12.0 Hz, 1 H, H-6a),), 4.00 (dd, $J_{5,6}$ = 2.0 Hz, J_{6ab} = 12.0 Hz, 1 H, H-6b), 3.81 (ddd, $J_{4,5}$ = 10.0 Hz, H-5), 3.69 (m, 2 H, H-2, OC<u>H</u>$_a$[CH_2]$_5$), 3.42 (m, 1 H, OC<u>H</u>$_b$[CH_2]$_5$), 2.97 (*pseudo*-q, 2 H, NC<u>H</u>$_2$[CH_2]$_5$), 2.01, 1.97, 1.91 (3s, 9 H, OAc), 1.75 (s, 3 H, NAc), 1.46, 1.38 (2m, 4 H, OCH$_2$C<u>H</u>$_2$C$_2$H$_4$C<u>H</u>$_2$CH$_2$), 1.24 (m, 4 H, OC$_2$H$_4$C$_2$<u>H</u>$_4$). ^{13}C NMR (75.41 MHz, [d_6]DMSO): 169.94 (3-OAc), 169.54 (4-OAc), 169.18 (6-OAc), 169.02 (NAc), 155.96 (N<u>C</u>=O), 137.22 (C-1$_{Ph}$), 128.22 (C-2$_{Ph}$), 127.61 (C-3$_{Ph}$), 127.59 (C-4$_{Ph}$), 100.13

(C-1), 72.54 (C-3), 70.54 (C-5), 68.72 ($O\underline{C}H_2CH_2$), 68.54 (C-4), 64.96 ($O\underline{C}H_2Ph$), 61.76 (C-6), 53.08 (C-2), 40.13 ($N\underline{C}H_2$), 29.31, 28.77, 25.83, 24.94, ($4C_{spac.}$) 22.52 (NAc), 20.41/20.32/20.25 (3 OAc).

*Synthesis of 6-(N-Benzyloxycarbonylamino)hexyl-O-(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido)-β-D-glucopyranoside (**5b**), from 1,3,4,6-tetra-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose (**15**):* Compound **15** was synthesised by the reported method[20] from $GlcNH_2HCl$ in 40-50% yield. After recrystallisations and column chromatography, the pure β-acetate **15** was obtained: $[\alpha]_D^{20} = 55.8°$ (c = 1, CH_2Cl_2); mp.: 91-94°C (90-94°C);[20] ^1H NMR (300 MHz, $CDCl_3$): 6.51 (d, $J_{1,2} = 8.0$ Hz, H-1α) ppm. A mixture of **15** (100 g, 210 mmol), 6-(N-benzyloxycarbonylamino)hexanol (62.7 g, 250 mmol) and molecular sieves 4 Ä (10 g) in dry $(CH_2Cl)_2$ (250 ml) was stirred at 20°C for 30 min. TMSOTf (28 ml, 154 mmol) was added dropwise over 45 min. After stirring for 2 h at 35-40°C, the reaction was quenched with NEt_3 (63 ml, 45 mmol) and washed with 1N aqueous HCl (2x 200 ml), aqueous $NaHCO_3$ and brine. After drying ($MgSO_4$) and concentration *i.vac.*, the residue was filtered over silica gel (500 g, ethyl acetate/*n*-hexane 2:1) to give **5b** (122 g, 87%); $R_f = 0.45$ (toluene/ethyl acetate 2:1); $[\alpha]_D^{20} = -33.4°$ (c = 1, CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$): 7.85, 7.70 (2dd, 4 H, Pht), 7.29-7.40 (m, 5 H, Ph), 5.79 (dd, $J_{2,3} = 11.0$ Hz, $J_{3,4} = 9.0$ Hz, 1 H, H-3), 5.35 (d, $J_{1,2} = 8.0$ Hz, 1 H, H-1), 5.17 (dd, $J_{3,4} = 9.0$ Hz, $J_{4,5} = 10$ Hz, 1 H, H-4), 5.09 (s, 2 H, $PhC\underline{H}_2$), 4.33 (dd, 1 H, H-6a), 4.31 (dd, 1 H, H-2), 4.17 (dd, 1 H, H-6b), 3.85 (m, 2 H, H-5, CH_aO_{spacer}), 3.45 (m, 1 H, CH_bO_{spacer}), 3.00 (m, 2 H, CH_2N), 2.11, 2.03, 1.87 (3s, 9 H, OAc), 1.10-1.50 (m, 8 H, (C_4H_8) ppm.

*Synthesis of **5b** via the imidate 17:* **15** (185.4 g, 0.389 mol) was stirred with hydrazine acetate (39.0 g, 0.433 mol) in dry DMF (600 ml) for 2 h at 20°C. The reaction was diluted with ethyl acetate (600 ml) and washed with half-saturated aqueous NaCl solution (3x 200 ml). The organic layer was dried ($MgSO_4$) and concentrated to yield the 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-D-glucopyranose (**16**) by stirring the residue in diethyl ether/diisopropyl ether as a colourless solid (108 g, 64%). $R_f = 0.50$ (ethyl acetate/toluene 1:1); ^1H NMR (300 MHz, [d_6]DMSO): 7.90 (m, 4 H, Pht), 7.52 (d, J = 6.0 Hz, OH), 5.65 (dd, J = 11.0, 9.0 Hz, 1 H), 5.46 (dd, J = 8.0, 6.0 Hz, 1 H), 4.98 (*pseudo*-t, J = 9.0 Hz, 1 H), 3.95-4.25 (m, 4 H) ppm. To a solution of compound **16** (131 g, 0.30 mol) in CH_2Cl_2 (1 l) was added at 5°C 1,8-diazabicyclo-[5.4.0]-undec-7-ene (DBU, 880 µl), and then dropwise trichloroacetonitrile (100 g, 0.346 mol). After repeated addition of DBU (880 µl), stirring was continued for 2 h at 10°C. The solution was concentrated and the residue was purified by chromatography using CH_2Cl_2 as the mobile phase to give **17** (148 g, 84%); $R_f = 0.46$ (ethyl acetate/toluene 1:1); ^1H NMR (300 MHz, $CDCl_3$): 8.66 (*br*s, 1 H, NH), 7.84, 7.74 (2 dd, 4 H, Pht), 6.62 (d, $J_{1,2} = 9.0$ Hz, 1 H, H-1α), 5.93 (dd, $J_{3,4} = 9.0$, $J_{2,3} = 10$ Hz, 1 H, H-3), 5.30 (dd, $J_{3,4} = 9.0$ Hz, $J_{4,5} = 10$ Hz, 1 H, H-4), 4.40 (dd, 1H, H-6a), 4.20 (dd, 1 H, H-6b), 4.08 (m, 1 H, H-5) ppm. A mixture of **17** (180 g, 0.310 mol), 6-(N-benzyloxycarbonylamino)hexanol (84.1 g, 0335 mol) and molecular sieves 4 Ä (75 g) was stirred for 1 h at 20°C in dry CH_2Cl_2 (1 l). TMSOTf (1 ml, 5.5 mmol) was added within 5 min at 20°C. The mixture warmed up to ca. 30°C, and the reaction was completed after 15 min, as indicated by TLC. After addition of NEt_3 (1.1 ml) and filtration, the solution was concentrated and the residue taken up in diethyl ether/diisopropyl ether (2:1) to crystallise the TLC-pure **5b** (205 g, 98%, for data see above).

*6-(N-Benzyloxycarbonylamino)hexyl-O-(2-acetamido-4,6-O-benzylidene-2-deoxy)-β-D-glucopyranoside (**6**):* A solution of **5a** (93.0 g, 160 mmol) and NaOMe (3 ml of a 0.1N solution in MeOH) in dry MeOH (500 ml)

was stirred for 1 h at 20°C. The neutralised (HOAc) reaction was filtered and concentrated to give the deacetylated intermediate (70.5 g, 97%) [^{1}H NMR (300 MHz, CD_3OD): 7.30 (m, 5 H, Ph), 5.02 (s, 2 H, $PhCH_2$), 4.38 (d, $J_{1,2}$ = 8.0 Hz, 1 H, H-1_{GlcNAc}), 3.86 (m, 2 H, H-6_{GlcNAc}), 3.64 (m, 2 H, $CH_2O_{spac.}$), 3.10 (t, J = 7.0 Hz, 2 H, CH_2N), 1.95 (s, 3 H, Ac), 1.20-1.60 (m, 8 H, C_4H_8). This product (65.4 g, 144 mmol), benzaldehyde dimethylacetal (32.4 ml, 216 mmol) and CSA (1.62 g, 7.00 mmol) were stirred for 3 h at 60°C in dry DMF (200 ml). More benzaldehyde dimethylacetal (3.24 ml, 21.6 mmol) was added, and after 1 h reaction at 60°C the cooled solution was poured into diethyl ether (3.2 l). After standing for 1 h, the precipitate was filtered off, dissolved in a minimum amount of CH_2Cl_2/MeOH, precipitated again in diethyl ether, filtered and dried *i.vac.* to give **6** (65.4 g, 84%). mp.: 205°C; $[a]_D^{20}$ = -110.2° (c = 1, CH_2Cl_2). ^{1}H NMR (300 MHz, [d_6]DMSO): 7.80 (d, J = 9.0 Hz, 1 H, AcN<u>H</u>), 7.27-7.49 (m , 10 H, 2 Ph), 7.23 (t, J = 6.0 Hz, 1 H, N<u>H</u>Z), 5.59 (s, 1 H, $CH_{benzylidene}$), 5.25 (J = 5.0 Hz, 1 H, OH), 5.00 (s, 2 H, N[C=O]O<u>C</u>H_2), 4.46 (d, $J_{1,2}$ = 8.0 Hz, 1 H, H-1α), 4.19 (dd, $J_{5,6}$ = 5.0 Hz, J_{6ab} = 11.0 Hz, 1 H, H-6a), 3.72 (m, 1 H, H-6b), 3.60 (m, 1 H, H-3),), 3.63 (m, 1 H, O<u>C</u>$H_aC_5H_{10}$), 3.50 (*pseudo*-t, J = 8.0 Hz, 1 H, H-2), 3.41 (m, 1 H, H-4), 3.38 (m, 1 H, O<u>C</u>$H_bC_5H_{10}$), 3.32 (m, 1 H, H-5), 2.98 (*pseudo*-q, 2 H, NCH_2), 1.80 (s, 3 H, NAc), 1.32-1.50 (m, 4 H, $OCH_2CH_2C_2H_4CH_2$), 1.24 (m, 4 H, C_2H_4). ^{13}C NMR (75.41 MHz, [d_6]DMSO): δ = 168.93 (NAc), 155.97 (N<u>C</u>=O), 137.66, 137.22 (C-1_{Ph}, C-$1'_{Ph}$), 128.73 (C-$4'_{Ph}$), 128.22 (C-2_{Ph}), 127.90 (C-$3'_{Ph}$), 127.61 (C-3_{Ph}), 127.58 (C-4_{Ph}), 126.24 (C-$2'_{Ph}$), 100.47 (C-1), 100.55 ($C_{benzylidene}$), 81.23 (C-4), 70.30 (C-3), 68.55 (O<u>C</u>H_2CH_2), 67.80 (C-6), 65.86 (C-5), 64.96 (O<u>C</u>H_2Ph), 56.12 (C-2), 40.15 (N<u>C</u>H_2), 29.32, 28.89, 25.87, 24.94, ($4C_{spac.}$) 22.93 (Ac).

6-(N-Benzyloxycarbonylamino)hexyl-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1→3)-2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside (7) <u>Small scale synthesis</u>: A mixture of **6** (0.167 g, 0.307 mmol), **7**[16] (0.206 g, 0.430 mmol) and molecular sieves 3 Å was stirred in CH_2Cl_2/DMF (7 ml / 7 ml) for 30 min at 20°C, then (n-Bu)$_4N^+Br^-$ (0.415 g, 1.29 mmol) and $CuBr_2$ (0.268 g, 1.20 mmol) were added. After stirring for 36 h at 25 °C in the dark, the solids were filtered off and washed with CH_2Cl_2 (2 x 20 ml). The combined filtrates were washed with diluted aqueous HCl, saturated aqueous $NaHCO_3$ and brine, dried ($MgSO_4$) and concentrated *i.vac.*.The residue was purified by flash chromatography (10%→30% ethyl acetate in toluene) to afford **8** (0.280 g, 95%). R_f = 0.56 (toluene/ethyl acetate 1:1); $[\alpha]_D^{20}$ = -88.0° (c = 1, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): 0.81 (d, $J_{6,5}$ = 6.6 Hz, 3 H, 6-H_{Fuc}), 1.3 (m, $CH_{2spac.}$), 1.47 (m, $CH_{2spac.}$), 1.65 (s, 3 H, NAc), 3.17 (m, $CH_{2spac.}$), 3.40 (dd, 1 H, 2-H_{GlcNAc}), 3.58 (dd, 1 H, 4-H_{Fuc}), 3.94 (dd, 1 H, 3-H_{Fuc}), 4.06 (dd, 1 H, 2-H_{Fuc}), 4.09 (dd, 1 H, 5-H_{Fuc}), 4.27 (dd, 1 H, 3-H_{GlcNAc}), 4.86 (d, 1 H, 1-H_{GlcNAc}), 5.09 (d, 1 H, 1-H_{Fuc}), 5.02 (s, 2 H, $PhCH_2O$), 5.48 (s, 1 H, $CH_{benzylidene}$), 5.77 (d, $J_{1,NH}$ = 7.2 Hz, 1 H, $NHAc_{GlcNAc}$), 7.1-7.4 (m, 20 H, Ph); FAB-MS m/z: 957 ($M+H^+$); 979 ($M+Na^+$).

<u>Large scale synthesis of **8**</u>: A mixture of **6** (67.0 g, 128 mmol), **7** (122 g, 256 mmol) and molec. sieves 3 Å was stirred in CH_2Cl_2/DMF (0.93 l / 0.35 l) for 1 h at 20°C, then (n-Bu)$_4N^+Br^-$ (98.0 g, 304 mmol) and $CuBr_2$ (63.0 g, 282 mmol) were added. After stirring for 2 h at 20 °C in the dark, the solids were filtered off and washed with CH_2Cl_2 (2 x 0.2 l). The combined filtrates were washed with water (3 l), diluted aqueous NH_3 (3 x 0.5 l), 0.5N HCl (50 ml), saturated $NaHCO_3$ (50 ml) and brine, dried ($MgSO_4$) and concentrated *i.vac.*.The residue was stirred in a mixture of *iso*-hexane (1.5 l)/diethyl ether (0.5 l) to give **8** (94.0 g, 77%). The dimethyl(methylthio)sulfonium triflate (DMTST) reagent[26] likewise can be used as the promoter.

6-(N-Benzyloxycarbonylamino)hexyl-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1→3)-2-acetamido-6-O-benzyl-2-deoxy-β-D-glucopyranoside (9) Small scale synthesis: A suspension of **8** (0.260 g, 0.271 mmol) and $NaCNBH_3$ (0.171 g, 2.71 mmol) in dry THF (10 ml) was treated very slowly at 25°C with a saturated solution (sat. at 25°C) of HCl in diethyl ether under careful control of the pH value (5.0-4.5) and by TLC monitoring. Then solid $NaHCO_3$ (1.0 g) and ethyl acetate (50 ml) were added. The mixture was washed with saturated aqueous $NaHCO_3$ and brine, dried ($MgSO_4$), concentrated *i.vac.* and purified by flash chromatography (10%→60% ethyl acetate in toluene) to give **9** (0.240 g, 90%); R_f = 0.39 (toluene/ ethyl acetate 1:1); $[\alpha]_D^{20}$ = -55.5 (c = 1, CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$): 1.14 (d, $J_{6,5}$ = 6.6 Hz, 3 H, 6-H_{Fuc}), 1.31 (m, $CH_{2spac.}$), 1.5 (m, $CH_{2spac.}$), 1.60 (s, 3 H, Ac), 3.16 (m, $CH_{2spac.}$), 3.31 (dd, $J_{2,1}$ = 8.3 Hz, 1 H, 2-H_{GlcNAc}), 3.68 (dd, 1 H, 4-H_{Fuc}), 3.93 (dd, 1 H, 3-H_{Fuc}), 4.07 (dd, 1 H, 2-H_{Fuc}), 4.10 (dd, 1 H, 5-H_{Fuc}), 3.85 (dd, 1 H, 3-H_{GlcNAc}), 4.83 (d, $J_{1,2}$ = 8.4 Hz, 1 H, 1-H_{GlcNAc}), 4.98 (d, $J_{1,2}$ = 4.1 Hz, 1 H, 1-H_{Fuc}), 5.02 (s, 2 H, $PhCH_2$), 5.53 (d, 1 H, $NHAc_{GlcNAc}$), 7.1-7.4 (m, 20 H, Ph) ppm; FAB-MS m/z: 959 ($M+H^+$); 981 ($M+Na^+$). Large scale synthesis of **9**: The reduction of **7** (26.0 g, 27.1 mmol) with $NaCNBH_3$ (17.1 g, 271 mmol) in dry THF (1 l) and the isolation of **9** were performed in an analogous manner as described above. In addition, samples were taken from the reaction and checked by ^1H NMR for the presence of the benzylidene resonance at ca. 5.48 ppm. To separate the product from boron derivatives, the concentrated reaction mixture was treated several times with methanol and evaporated. Yield of **9**: 18.6 g (71%).

6-(N-Benzyloxycarbonylamino)hexyl-O-(2,3,4-tri-O-acetyl-6-O-benzyl-β-D-galactopyranosyl)-(1→4)-O-[(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1→3)]-2-acetamido-6-O-benzyl-2-deoxy-β-D-glucopyranoside(11) Small scale synthesis: A solution of **10**[12a] (0.197 g, 0.364 mmol) and **9** (0.222 g, 0.226 mmol) in dry CH_2Cl_2 (2 ml) and molecular sieves 4 Ä were stirred for 30 min at 20°C. After addition of $BF_3(OEt_2)$ (0.24 ml) and stirring for 4 h at 25°C, the reaction was quenched with $NaHCO_3$ (1 g) and diluted with CH_2Cl_2 (25 ml). The organic phase was washed with saturated aqueous $NaHCO_3$ and brine, dried ($MgSO_4$) and concentrated *i.vac.*. Purification by flash chromatography (25%→50 % ethyl acetate in toluene) gave **11** (0.221 g, 73%); R_f = 0.61 (CH_2Cl_2/MeOH 20:1); ^1H NMR (300 MHz, $CDCl_3$): 1.11 (d, 3 H, 6-H_{Fuc}), 1.25 (m, $CH_{2spac.}$), 1.46 (m, $CH_{2spac.}$), 1.77 (s, 3 H, NAc); 1.87, 1.96, 2.0 (3s, 9 H, OAc), 3.14 (m, 2 H, $CH_{2spac.}$), 5.05 (s, 2 H, $PhCH_2$), 5.41 (d, $J_{1,2}$ = 4.0 Hz, 1 H, 1-H_{Fuc}), 5.96 (d, $J_{1,NH}$ = 8.0 Hz, 1 H, $NHAc_{GlcNAc}$), 7.1-7.4 (m, 25 H, Ph) ppm; FAB-MS m/z: 1340 ($M+H^+$); 1362 ($M+Na^+$).

6-(N-Benzyloxycarbonylamino)hexyl-O-(6-O-benzyl-β-D-galactopyranosyl)-(1→4)-O-[(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1→3)]-2-acetamido-6-O-benzyl-2-deoxy-β-D-glucopyranoside (12).

Small scale synthesis: **11** (0.204 g, 0.152 mmol) and NaOMe (1.6 ml of 0.1N solution in MeOH) in dry MeOH (6 ml) was stirred for 3 h at 25°C. The solution was neutralised (HOAc) and concentrated. Flash chromatography (5%→20% MeOH in CH_2Cl_2) gave the title trisaccharide **12** (0.180 g, 97%); R_f = 0.44 (CH_2Cl_2/MeOH 20:1); $[\alpha]_D^{20}$ = -49.9 (c = 1, MeOH); ^1H NMR (300 MHz, $CDCl_3$): 1.05 (d, $J_{6,5}$ = 6.6 Hz, 3 H, 6-H_{Fuc}), 1.22 (m, $CH_{2spac.}$), 1.4 (m, $CH_{2spac.}$), 1.82 (s, 3 H, NAc), 2.98 (t, 2 H, $CH_{2spac.}$), 5.05 (s, 2 H, $PhCH_2$), 5.19 (d, $J_{1,2}$ = 3.6 Hz, 1 H, 1-H_{Fuc}), 6.00 (d, 1 H, NH), 7.1-7.4 (m, 25 H, Ph) ppm; FAB-MS m/z: 1217 ($M+H^+$); 1239 ($M+Na^+$).

Large scale synthesis of **12**: The mixture of **10** (25.3 g, 46.8 mmol), **9** (30.0 g, 31.3 mmol), tetramethyl urea (1 ml) and molecular sieves 4 Å in dry $(CH_2Cl)_2$ (400 ml) was stirred for 1 h at 55-60°C. At 60°C TMSOTf

(1 ml) was added and after 1 h at 55-60°C the reaction was quenched by adding NEt_3 (0.5 ml) at 30°C and diluted with CH_2Cl_2 (250 ml). The organic phase was washed with saturated aqueous $NaHCO_3$ and brine, dried ($MgSO_4$) and concentrated *i.vac.*. The residue was filtered over silica gel (1.5 kg, 40-60 μ) using ethyl acetate in *iso*-hexane (gradient 25%→50%) as eluent to give crude **11** (31.0 g) which was stirred with NaOMe (1.6 ml of a 35% solution in MeOH) in dry MeOH (150 ml) for 2.5 h at 25°C. The product was isolated as above. Yield of **12**: 24.62 g (64%).

6-Aminohexyl-O-(ß-D-galactopyranosyl)-(1→4)-O-[(α-L-fucopyranosyl)-(1→3)]-2-acetamido-2-deoxy-ß-D-glucopyranoside (2): The procedure to remove the benzyl groups in **12** (4.87 g, 4.00 mol) by catalytic hydrogenation was analogous to that described for the preparation of **1b** to give the Le^X derivative **2** (2.23 g, 91%). $[\alpha]_D^{20}$ = -54.3° (c = 1, H_2O); ^1H NMR (600 MHz, D_2O): 1.17 (d, 3 H, J = 6.0 Hz, 6-H_{Fuc}), 1.30-1.42 (m, 4 H, $NC_2H_4C_2\underline{H}_4$), 1.57, 1.67 (2m, 4 H, $NCH_2C\underline{H}_2C_2H_4C\underline{H}_2$), 2.03 (s, 3 H, Ac), 2.99 (t, 2 H, J = 7.0 Hz, CH_2N), 3.59 (*pseudo*-t, 1 H, J = 9.0 Hz, 2-H_{Gal}), 3.60 (m, 3 H, 5-H_{Gal}, 5-H_{GlcNAc}, $C\underline{H}aO_{spacer}$), 3.66 (dd, J = 3.5 Hz, J = 9.5 Hz, 1 H, 3-H_{Gal}), 3.70 (dd, 1 H, $J_{1,2}$ = 4.5 Hz, $J_{2,3}$ = 11.5 Hz, 2-H_{Fuc}), 3.73 (m, 2 H, 6-H_{Gal}), 3.78 (d, J = 3.0 Hz, 1 H, 4-H_{Fuc}), 3.83-3.95 (m, 7 H, $C\underline{H}bO_{spacer}$, 4-$H_{Gal}$, 3-$H_{Fuc}$, 2-$H_{GlcNAc}$, 3-$H_{GlcNAc}$, 4-$H_{GlcNAc}$, 6a-$H_{GlcNAc}$), 4.00 (dd, J = 1.0 Hz, J = 12.0 Hz, 1 H, 6b-H_{GlcNAc}), 4.46 (d, J = 7.5 Hz, 1 H, 1-H_{Gal}), 4.53 (d, J = 7.5 Hz, 1 H, 1-H_{GlcNAc}), 4.83 (m, 1 H, H-5_{Fuc}), 5.12 (d, 1 H, J = 4.5 Hz, 1-H_{Fuc}). ^{13}C-NMR (124.7 MHz, D_2O): 173.96, 170.83 (2 Ac), 101.67 (1-C_{Gal}), 100.80 (1-C_{GlcNAc}), 98.43 (1-C_{Fuc}), 75.21 (5-C_{GlcNAc}), 74.78, 74.75 (3-C_{GlcNAc}, 5-C_{Gal}), 73.26 (4-C_{GlcNAc}), 72.35 (3-C_{Gal}), 71.75 (4-C_{Fuc}), 70.90, (2-C_{Gal}), 70.29 ($\underline{C}H_2O_{spacer}$), 69.09 (3-$C_{Fuc}$), 68.19 (4-$C_{Gal}$), 67.57, (2-$C_{Fuc}$), 66.54 (5-$C_{Fuc}$), 61.31 (6-$C_{Gal}$), 59.65 (6-$C_{GlcNAc}$), 55.69 (2-$C_{GlcNAc}$), 39.26 ($\underline{C}H_2N_{spacer}$), 28.21, 26.47, 25.07, 24.46 ($NCH_2\underline{C}_4H_8$), 22.10, (Ac), 15.13 (6-C_{Fuc}) ppm.

6-(N-Benzyloxycarbonylamino)hexyl-O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-O-(6-O-benzyl-ß-D-galactopyranosyl)-(1→4)-O-[(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1→3)]-2-acetamido-6-O-benzyl-2-deoxy-ß-D-glucopyranoside **14a**:
Two batches were run in parallel and then subjected to combined work-up: Trisaccharide **12** (4.20 g, 3.45 mmol), thioglycoside **13**[17] (2.66 g, 5.10 mmol) and powdered molecular sieves 4 Å (5 g) were stirred for 1 h at -70°C in a mixture of CH_2Cl_2 (24 ml) and CH_3CN (24 ml). Silver trifluoromethane sulfonate (AgOTf, 2.00 g) and methylene sulfenyl bromide (MSB, 5 ml) were added. After stirring for 1 h at -70°C, additional amounts of **13** (1.40 g, 2.68 mmol) and MSB (2 ml) were added, and after 50 min further **13** (1.0 g, 1.91 mmol), MSB (1 ml) and AgOTf (1.0 g). After 12 h at -35°C the combined two batches were worked-up as follows: The cold reaction mixture (-30°C) was filtered and the residue was washed with CH_2Cl_2 (2 x 30 ml). The combined organic phases were washed with cold saturated aqueous $NaHCO_3$ (2 x 50 ml) and with 20% aqueous $Na_2S_2O_3$ (2 x 50 ml), dried ($MgSO_4$) and concentrated *i.vac.* The residue (17.7 g) was dissolved in dry MeOH (200 ml) containing NaOMe (3 ml of a 35% solution in MeOH) and stirred for 2 h at 20°C. Filtration, concentration and column chromatography (5%→20% MeOH in CH_2Cl_2) gave the product mixture **14a-c** (5.80 g, 55%) and recovered **12** (2.0 g, 23%). TLC revealed the presence of the 3 components (Scheme 4) which were not separated since analogous structures already have been assigned previously.[28] For the following synthesis of **1b**, no further purification was required.

*6-Aminohexyl-O-(5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→3)-O-(β-D-galactopyranosyl)-(1→4)-O-[α-L-fucopyranosyl)-(1→3)]-2-acetamido-2-deoxy-β-D-glucopyranoside (**1b**):* The mixture of **14a-c** (2.62 g, 1.72 mmol), 0.70 g Pd-black and HCO_2H (7.5 ml, dropwise addition) in MeOH (120 ml) was stirred for 18 h at 20°C. The reaction was filtered and concentrated *i.vac.*. The residue was evaporated with toluene (3 x 10 ml) *i.vac.*, taken up in water (10 ml) and filtered. The filtrate was subjected to gel permeation chromatography (Biogel P2, column 5x50 cm, water). Freeze-drying gave the debenzylated lactone/methyl ester mixture (1.31 g, ca. 83%) which has been used to prepare diverse sLe^X conjugates.[6] This mixture, composed of the methyl ester and mainly the 1-4′-lactone derived from **14b**, was dissolved in water (40 ml) and adjusted to pH 10.5 (0.1 N NaOH). After stirring for 2 h at 20°C, the pH was carefully adjusted to 6.0 (0.1 N HCl). The freeze-dried product was purified in the same manner as described above to give the title compound **1b** (1.27 g, 80%). $[a]_D^{20}$ = -44.9° (c = 1, H_2O). ^1H NMR (600 MHz, D_2O): 1.17 (d, 3 H, J = 6.0 Hz, 6-H_{Fuc}), 1.30-1.42 (m, 4 H, $C_2H_{4spac.}$), 1.58, 1.67 (2m, 4 H, $NCH_2C\underline{H}_2$-$C_2H_4C\underline{H}_2$), 1.80 (*pseudo*-t, J = 11.0 Hz, 1 H, 3-$H_{Nana/ax}$), 2.03 (s, 3 H, NAc_{Nana}), 2.05 (s, 3 H, NAc_{GlcNAc}), 2.77 (dd, 1 H, J = 4.5, 11.0 Hz, 3-$H_{Nana/equ}$), 3.00 (t, 2 H, J = 7.0 Hz, $CH_2NH_3^+$), 3.53 (dd, 1 H, J = 9.0, 10.0 Hz, 2-H_{Gal}), 3.59 (m, 4 H, 5-H_{Gal}, 5-H_{GlcNAc}, 7-H_{Nana}, $C\underline{H}aO_{spac.}$), 3.63-3.74 (m, 6 H, 6-H_{Gal}, 4,6,9a-H_{Nana}, 2-H_{Fuc}), 3.78 (d, J = 4.5 Hz, 1 H, 4-H_{Fuc}), 3.83-3.98 (m, 10 H, $C\underline{H}bO_{spac.}$, 5,8,9b-H_{Nana}, 4-H_{Gal}, 3-H_{Fuc}, 2,3,4,6a-H_{GlcNAc}), 4.02 (dd, J = 2.5, 12.0 Hz, 1 H, 6b-H_{GlcNAc}), 4.09 (dd, 1 H, J = 3.5, 10.0 Hz, 3-H_{Gal}), 4.52 (d, J = 9.0 Hz, 1H, 1-H_{GlcNAc}), 4.53 (d, J = 9.0 Hz, 1 H, 1-H_{Gal}), 4.82 (m, 1 H, H-5_{Fuc}), 5.11 (d, 1 H, J = 4.0 Hz, 1-H_{Fuc}). ^{13}C NMR (124.7 MHz, D_2O): 174.94 (CO_2H), 173.97/173.67 (2 OAc), 101.53 (1-C_{Gal}), 100.87 (1-C_{GlcNAc}), 99.70 (2-C_{Nana}), 98.44 (1-C_{Fuc}), 75.60 (3-C_{Gal}), 75.18 (5-C_{GlcNAc}), 74.92 (5-C_{Gal}), 74.74 (3-C_{GlcNAc}), 73.30 (4-C_{GlcNAc}), 72.82 (6-C_{Nana}), 71.77, 71.74 (4-C_{Fuc}, 8-C_{Nana}), 70.32 ($CH_2O_{spac.}$), 69.15, 69.09 (2-C_{Gal}, 3-C_{Fuc}), 68.14, 68.04 (4,7-C_{Nana}), 67.63 (2-C_{Fuc}), 67.21, (4-C_{Gal}), 66.57 (5-C_{Fuc}), 62.53 (9-C_{Nana}), 61.35 (6-C_{Gal}), 59.59 (6-C_{GlcNAc}), 55.73 (2-C_{GlcNAc}), 51.61 (5-C_{Nana}), 39.70 ($\underline{C}H_2N_{spac.}$), 39.32 (3-$C_{Nana}$), 28.24, 28.54, 25.09, 24.48 ($C_4H_{8spac.}$), 22.14/21.93 (2 Ac), 15.15 (6-C_{Fuc}).

*1,3,6-Tri-O-acetyl-2-deoxy-2-phthalimido-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α/β-D-glucopyranose (**21**)*: The lactosamine derivative was synthesised by reported methods[22a,31] which were scaled up as follows: A mixture of 3-O-(β-D-galactopyranosyl)-D-arabinose (**18**) (87.5 g, 0.28 mol, prepared from lactose) and benzylamine (34.0 ml, 0.31 mol) in dry ethanol (350 ml) was heated under reflux until 150 ml of ethanol were distilled off (ca. 1.5 h). The dark-brown reaction was diluted with dry ethanol (300 ml) and stored for 48 h at 0°C. The 2-benzylamino-2-deoxy-3-O-(β-D-galactopyranosyl)-D-arabinose (**19**) was isolated as crystals (91.0 g, 81%), and then was stirred with NaCN (12.2 g, 0.25 mol) in dry ethanol (0.7 l) at 0°C. After dropwise addition of HOAc (16 ml) in dry ethanol (180 ml) over 25 min 0°C, stirring was continued for 5.5 h at 20°C. After standing for 24 h at -20°C, the crude 2-benzylamino-2-deoxy-3-O-(β-D-galactopyranosyl)-D-glucononitrile (**20**) crystallised (73.2 g, 75%). A solution of **20** (18.0 g, 42.0 mmol) in 0.5 N HCl (200 ml) was hydrogenated over $Pd/BaSO_4$ (5%, 12 g) at 20°C for 13 h to take up ca. 2.20 l of hydrogen. To the filtrate was added $NaHCO_3$ (8.20 g, 98.0 mmol), and then a solution of phthalic acid anhydride (13.06 g, 88.0 mmol, dropwise) in acetone (0.44 l), and again $NaHCO_3$ (7.4 g, 88.0 mmol) were added within 30 min. After stirring for 5 h, the reaction was concentrated *i.vac.* and the residue evaporated with toluene/ethanol (3 x 50/50 ml/ml). The residue was heated with acetanhydride (110 ml) and pyridine

(170 ml) for 10 min up to 100°C. After stirring for 4 h at 20°C, the reaction was poured into ice, stirred for 10 min and filtered. The filtrate was extracted with CH_2Cl_2 (3 x 300 ml), and the combined organic phases were washed with 1N HCl, saturated $NaHCO_3$ and brine, dried ($MgSO_4$), filtered and concentrated. The residue was purified by flash chromatography on silica gel (ethyl acetate/toluene 1:2) to give a crude product

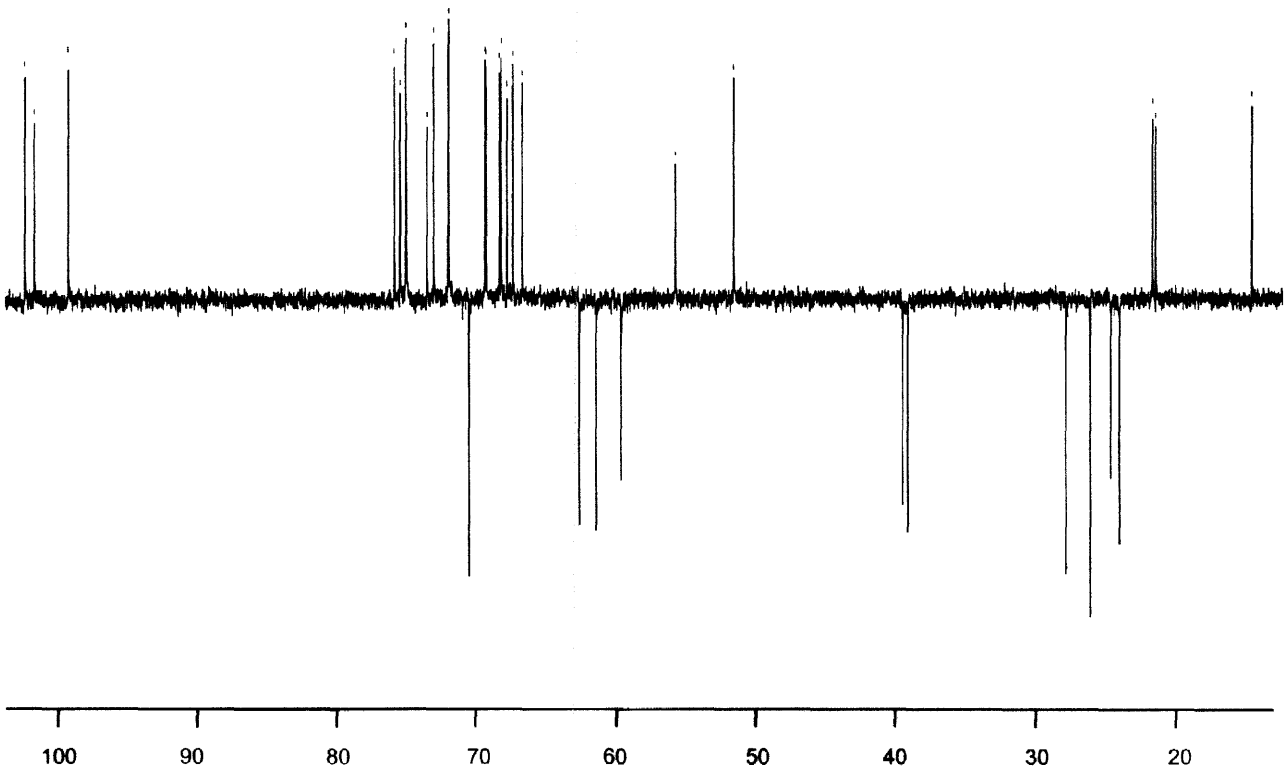


Figure 2: ^{13}C NMR (124.7 MHz, DEPT, D_2O) of the title sLe^X conjugate **1b**.

(23.4 g) which was purified by crystallisations from CH_2Cl_2/*n*-hexane and MeOH, to give **21** (17.92 g, 55%) as a 1/3 mixture of the α/β-acetates. ^1H NMR (main product β-acetate) (300 MHz, $CDCl_3$): 7.70-7.90 (m, 4 H, Pht), 6.50 (d, $J_{1,2}$ = 9.0 Hz, 1 H, H-1α$_{GlcNPht}$), 5.84 (dd, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 8.0 Hz, 1 H, H-3$_{GlcNPht}$), 5.35 (dd, $J_{3,4}$ = 3.0 Hz, $J_{4,5}$ = 1.0 Hz, 1 H, H-4$_{Gal}$), 5.12 (dd, $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ = 10.0 Hz, 1 H, H-2$_{Gal}$),), 4.95 (dd, $J_{3,4}$ = 3.0 Hz, $J_{2,3}$ = 10.0 Hz, 1 H, H-3$_{Gal}$), 4.54 (d, $J_{1,2}$ = 8.0 Hz,, 1 H, H-1$_{Gal}$), 4.36 (dd, $J_{2,3}$ = 10.0 Hz, $J_{1,2}$ = 9.0 Hz, 1 H, H-2$_{GlcNPht}$), 2.15, 2.14, 2.07, 2.05, 1.98, 1.96, 1.91 (7s, 21 H, Ac). Characteristic signals for the minor α-acetate were at 6.40 (dd, $J_{2,3}$ = 11.0 Hz, $J_{3,4}$ = 9.0 Hz, 1 H, H-3$_{GlcNPht}$) and 6.22 (d, $J_{1,2}$ = 3.0 Hz, 1 H, H-1β$_{GlcNPht}$) ppm (revised assignment[22a]).

6-Azidohexyl-O-[3,6-di-O-acetyl-2-deoxy-2-phthalimido-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)]-β-D-glucopyranoside (22): A mixture of **21** (9.27 g, 12.1 mmol), 6-azidohexanol (2.08 g, 14. 5 mmol) and molecular sieves 4 Å in dry $(CH_2Cl)_2$ (30 ml) was stirred for 30 min at 0°C, then TMS-OTf (1.54 ml, 8.50 mmol) was added. After 2.5 h at 20°C, NEt_3 (3.1 ml, 22 mmol) was added. The mixture was filtered, the filtrate washed with water, dried ($MgSO_4$) and concentrated to give a crude product (11.1 g) which was purified by flash chromatography on silica (ethyl acetate/toluene 1:4). Yield of **22**: 7.90 g (77%). ^1H NMR

(300 MHz, $CDCl_3$): 7.70-7.90 (m, 4 H, Pht), 5.74 (dd, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 8.0 Hz, 1 H, H-$3_{GlcNPht}$), 5.36 (d, $J_{1,2}$ = 8.0 Hz, 1 H, H-$1\alpha_{GlcNPht}$), 5.34 (dd, $J_{3,4}$ = 3.0 Hz, $J_{4,5}$ = 1.0 Hz, 1 H, H-4_{Gal}), 5.12 (dd, $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ = 10.0 Hz, 1 H, H-2_{Gal}),), 4.96 (dd, $J_{3,4}$ = 3.0 Hz, $J_{2,3}$ = 10.0 Hz, 1 H, H-3_{Gal}), 4.53 (d, $J_{1,2}$ = 8.0 Hz,, 1 H, H-1_{Gal}), 3.25 (t, 2 H, CH_2N_3), 2.14, 2.13, 2.07, 2.04, 1.98, 1.91 (6s, 21 H, Ac).

6-Azidohexyl-O-[2-deoxy-2-acetamido-4-O-(β-D-galactopyranosyl)]-β-D-glucopyranoside (23): **22** (7.90 g, 9.32 mmol) was stirred in dry MeOH (30 ml) with NaOMe in MeOH (0.5 ml, 30% solution) for 1.5 h at 20°C. The reaction was concentrated, dissolved in dry MeOH (100 ml) and then heated at reflux with hydrazine hydrate (5 ml, 80%) for 4 h. The mixture was concentrated, and toluene (2 x 20 ml) was distilled from the residue. The residue was taken up in MeOH (50 ml) and acetic anhydride (3.6 ml) at 0°C. The mixture was stirred for 1.7 h at 0°C, diluted with ethanol (30 ml) and stirred for 4 h at 0°C. After filtration at 0°C, the solution was concentrated nearly to dryness and the residue diluted with acetone. The precipitated azide **23** (3.79 g, 80% 3 steps) was characterised as the hydrogenated title compound **3.**

6-Aminohexyl-O-[2-deoxy-2-acetamido-4-O-(β-D-galactopyranosyl)]-β-D-glucopyranoside (3): **23** (2.42 g, 4.76 mmol) was hydrogenated on Pd-black (0.35 g) in MeOH (20 ml) with HCO_2H (2.0 ml) for 16 h at 20°C. The mixture was filtered, concentrated, and toluene (3 x 10 ml) was distilled off. The residue was dissolved in H_2O (5 ml) and applied to GPC (Biogel P2, H_2O) to give **3** (2.02 g, 91%) as a colourless solid. ^1H NMR (300 MHz, D_2O): 1.35 (m, 4 H, $CH_{2spac.}$), 1.57 (m, 4 H, $CH_{2spac.}$), 2.03 (s, 3 H, Ac), 2.82 (t, J = 7.0 Hz, 2 H, CH_2NH_2), 3.51-3.64 (m, 4 H, 2,5-H_{Gal}, 5-H_{GlcNAc}, $C\underline{H}aO_{spac.}$), 3.67 (dd, J = 3.0, 10 Hz, 1 H, 3-H_{Gal}), 3.70-3.96 (m, 8 H, 4,6-H_{Gal}, $C\underline{H}bO_{spac.}$, 2,3,4,6a-H_{GlcNAc}), 4.00 (dd, J = 2.0, 12.0 Hz, 1 H, 6b-H_{GlcNAc}), 4.48 (d, $J_{1,2}$ = 7.5 Hz, 1 H, H-1_{Gal}), 4.53 (d, J = 7.5 Hz, 1 H, H-1_{GlcNAc}). ^{13}C-NMR (75.41 MHz, D_2O): 173.97 (Ac), 102.05 (1-C_{Gal}), 100.80 (1-C_{GlcNAc}), 78.50, 75.20, 74.40, 72.35, 72.30, 70.90, (3,4,5-C_{GlcNAc}, 2,3,5-C_{Gal}), 70.05 ($\underline{C}H_2O_{spac.}$), 68.20 (4-$C_{Gal}$), 60.90 (6-$C_{Gal}$), 59.95 (6-$C_{GlcNAc}$), 54.70 (2-$C_{GlcNAc}$), 39.30 ($CH_2NH_2$), 28.20, 28.05, 24.95, 24.25 ($\underline{C}_4H_8$), 22.00, (Ac).

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21. Compound **5b** was used likewise to prepare **1b**. In addition, the whole sequence was performed using the 6-azidohexanol linker.
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