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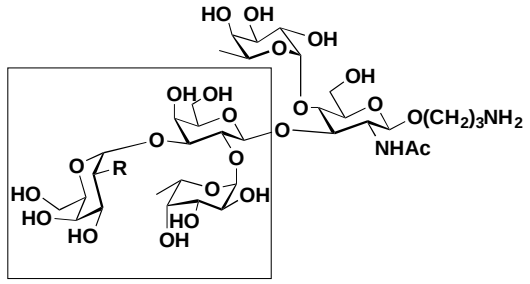
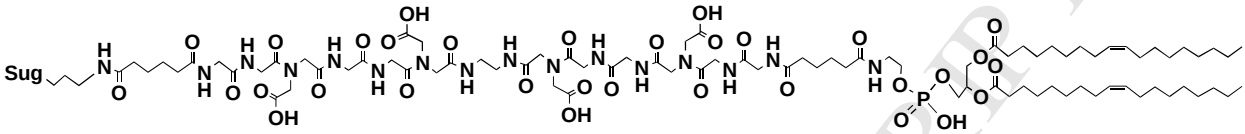
Graphical abstract



ALe^b: R=NHAc
BLe^b: R=OH



Sug=Le^a, Le^b, Le^y, ALe^b, BLe^b, ALe^y, BLe^y

Function-Spacer-Lipid Constructs of Lewis and Chimeric Lewis/ABH Glycans. Synthesis and Use in Serological Studies

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Abstract

Seven lipophilic constructs containing Lewis (Le^a, Le^b, Le^y) or chimeric Lewis/ABH (ALe^b, BLe^b, ALe^y, BLe^y) glycans were obtained starting from corresponding oligosaccharides in form of 3-aminopropyl glycosides. ALe^b and BLe^b pentasaccharides were synthesized via [3+1] blockwise approach. The constructs (neoglycolipids, or FSLs) were inserted in erythrocyte membrane, and obtained "kodecytes" were used to map the immunochemical specificity of historical and contemporary monoclonal and polyclonal blood group system Lewis reagents.

Keywords

Neoglycolipid; Lewis antigens; ABO antigens; Lewis antigens; Block synthesis; FSL constructs; kodecytes; ALe^b; BLe^b

1. Introduction

Lewis antigens are a family of structurally related fucosylated oligosaccharides,[1] represented by trisaccharide Galβ1→3(Fucα1→4)GlcNAcβ (Le^a) and tetrasaccharide Fucα1→2Galβ1→3(Fucα1→4)GlcNAcβ (Le^b). Lewis glycans are found on cell surface in composition of both glycoproteins and glycolipids. Glycans Galβ1→4(Fucα1→3)GlcNAcβ (Le^x) and Fucα1→2Galβ1→4(Fucα1→3)GlcNAcβ (Le^y) in spite of "Lewis" names and tightly

related (isomeric) chemical structure in reality are not conventional antigens of the Lewis blood group system, which leads to confusion, especially in chemical literature. As R.U. Lemieux demonstrated in 1970s,[2] stereochemically Le^a and Le^x are very similar because in both molecules the Fuc and Gal residues form the same spatial pair (the same is true for Fuc/Fuc-Gal pair in case of Le^b *vs*. Le^y), and that due to similar conformations a number of serological anti-Lewis reagents display cross-reactions with Le^x or Le^y.[3] Of note, naturally occurring human antibodies (*allo*-antibodies) against Le^a never cross-react with constitutively expressed Le^x antigen on human blood and epithelium cells, otherwise we could have auto-immune reactions.

The story is complicated by existence of "chimeric" structures, ALe^b, ALe^y, BLe^b, and BLe^y possessing structural features of both Lewis antigens (the presence of α-Fuc residue at C-3 or C-4 of GlcNAc) and A or B motifs (the presence of α-GalNAc or α-Gal units at C-3 of β-galactose). Also, Le^x and Le^a exist in form of sialylated and or sulfated glycans, known to be ligands for selectins.[4] The structural/conformational similarity in combination with occurrence of resembling glycans makes immunochemical (with antibodies) identification and quantitation of particular Lewis antigens a non-trivial task, requiring very precise tools for selection and characterization of monoclonal reagents and polyclonal anti-Lewis sera. Here, we describe synthesis of most complicated representatives of Lewis glycan superfamily, chimeric Le/ABH glycans, and lipid-conjugated forms of the chimeric and their more simple "partial" glycans.

Recently a new class of glycolipid-like constructs has been developed.[5,6] These compounds designed as conjugates with both variable glycans (having biological functions), spacer-arms and lipids, are known as FSLs (Function-Spacer-Lipid, Figure 1).

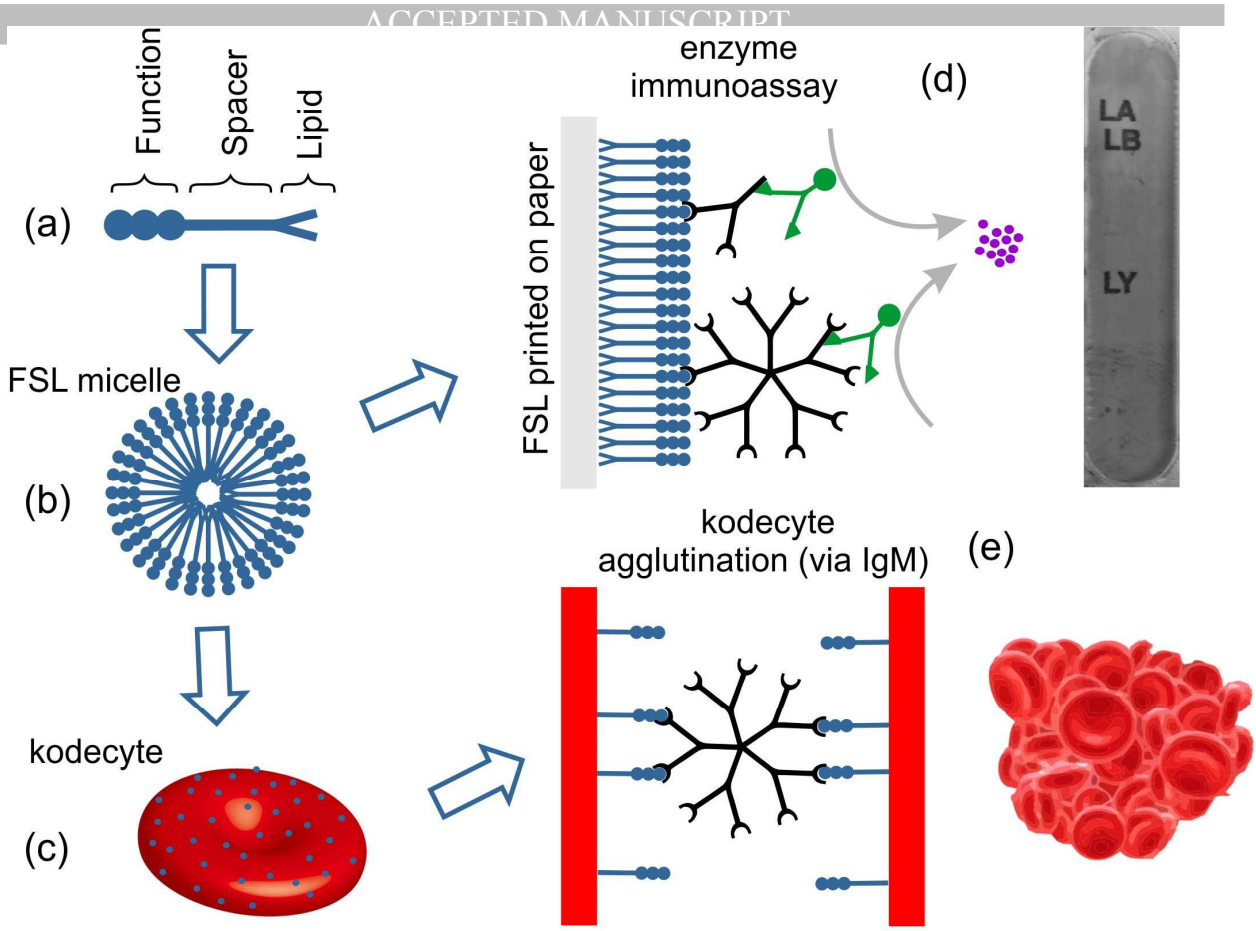


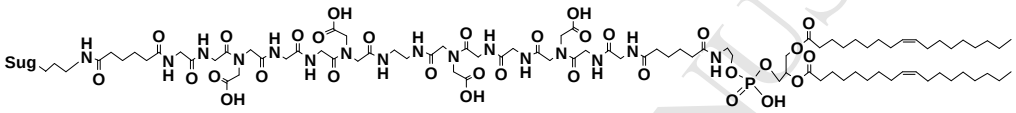
Figure 1. Schematic presentation of (a) FSL molecule design, (b) micelle formed by FSL in aqueous solutions, (c) erythrocyte modified by FSL (kodecyte), (d) monolayer of FSL on the solid support, its interaction with IgG and IgM antibodies followed by color reaction mediated by secondary antibody (in green), actual result image (in grey) is shown as well, (e) agglutination of erythrocytes due to bridging of cells with IgM antibody against glyco-part of FSL.

Unlike natural glycolipids, FSLs are easily dispersible in water, saline, and biological media. They can incorporate into cell membrane without affecting of functionality or vitality.[5,6] Attachment of FSLs to live cells can be achieved in precise and controlled ways, and modified cells, “kodecytes”, possess known glycan density.[5,6] Due to the properties described function-spacer-lipid constructs are powerful tools for a wide range research into biological functions of glycans on cell surfaces. The synthesized FSLs described in this paper were used for glycomapping of monoclonal and polyclonal serological Lewis reagents and clinical samples.[7]

These FSL constructs were used to create both Lewis kodecytes expressing single Le^a, Le^b, ALe^b, BLe^b, Le^x, Le^y, ALe^y, or BLe^y glycans, and the same FSLs were inkjet printed in a paper-based microplate and analyzed by enzyme immunoassay – for comparison with the cell assay.

2. Results and discussion

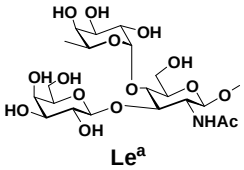
Function-spacer-lipid constructs **1**–**7** were obtained from 3-aminopropyl glycosides of oligosaccharides **8**–**14** (Scheme 1). Synthesis of derivatives **8** (Le^a)[8], **9** (Le^b)[8], **10** (Le^y)[9,10], **13** (ALe^y)[11], and **14** (BLe^y)[11] were described earlier. Aminopropyl glycosides **11** (ALe^b) and **12** (BLe^b) were obtained via [3+1] block scheme. Their synthesis is described below.



1-7

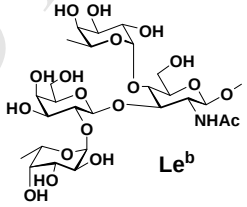
8-14

1, 8: Sug =



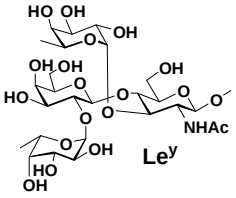
Le^a

2, 9: Sug =



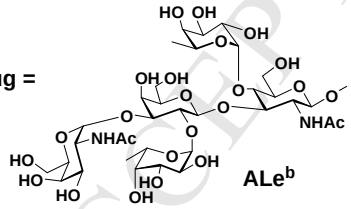
Le^b

3, 10: Sug =



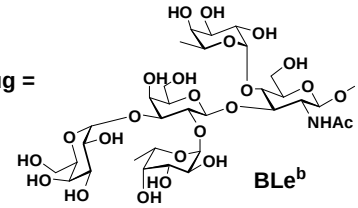
Le^y

4, 11: Sug =



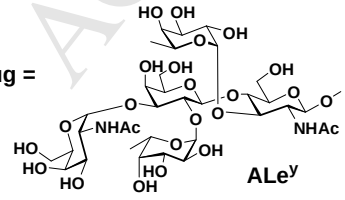
ALe^b

5,12: Sug =



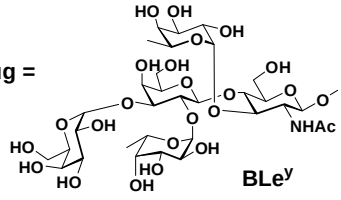
BLe^b

6, 13: Sug =



ALe^y

7, 14: Sug =



BLe^y

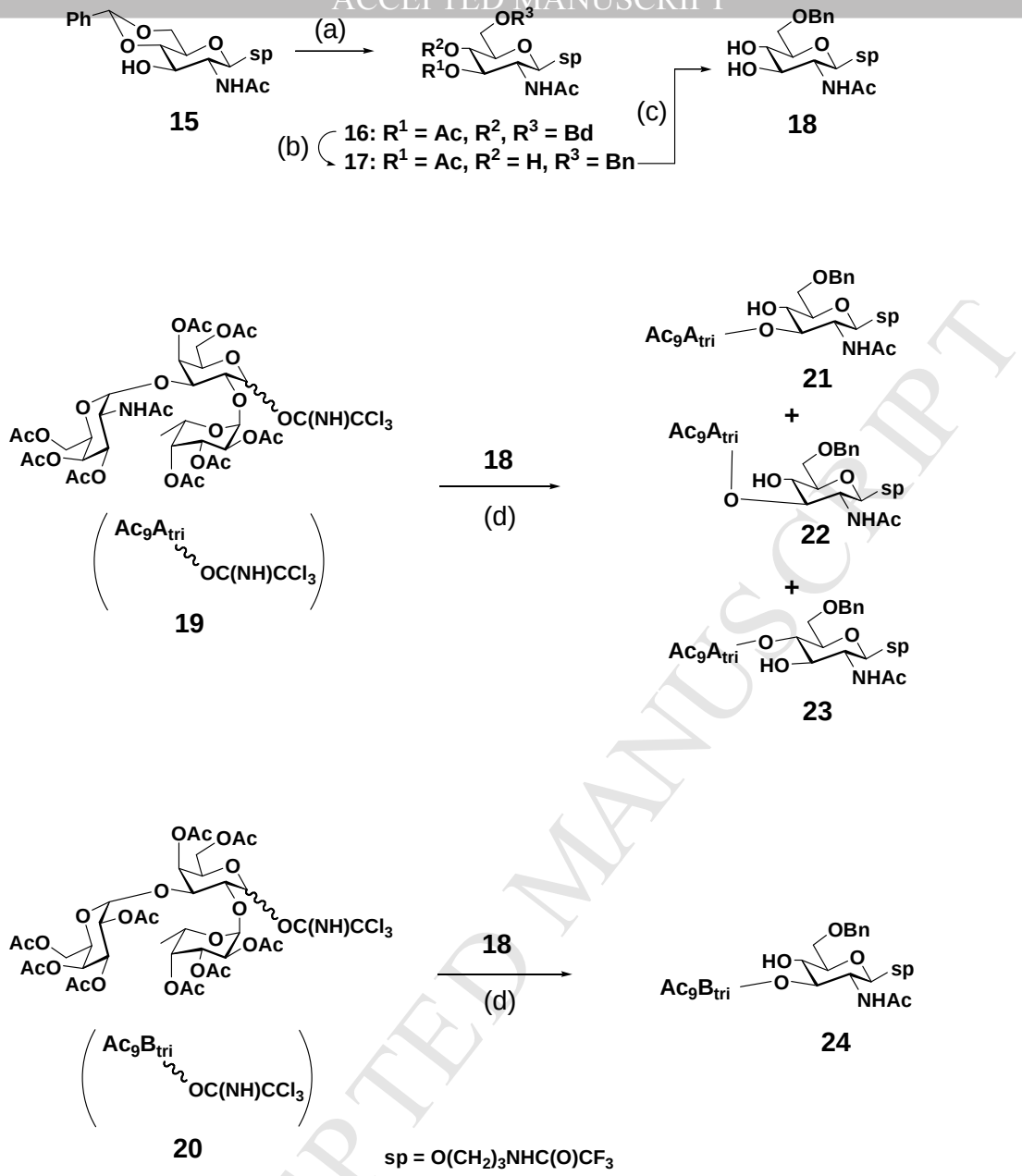
Scheme 1.

2.1 Synthesis of pentasaccharides ALe^b and BLe^b

Recently we proposed new approach to the synthesis of blood group A and B tetrasaccharides of various types, namely block scheme [3+1].[12–14] The main advantage of this strategy is employment of common A or B trisaccharide glycosyl donors – peracetylated trichloracetimidetes of A or B trisaccharides **19**[12] and **20**[13] (Scheme 2). For the synthesis of each type of target tetrasaccharides suitably protected monohydroxyl monosaccharide glycosyl acceptor was used.

The use of glucosamine diol **18** as glycosyl acceptor in block scheme [3+1] could potentially provide both tetrasaccharides of type 1 and type 2 simultaneously. Moreover, free hydroxyl group at C-3 or C-4 of GlcNAc of obtained tetrasaccharides could give an opportunity to synthesize fucosylated pentasaccharides ALe^b (BLe^b) or ALe^y (BLe^y), respectively.

Glucosamine diol **18** was easily obtained from known benzylidene derivative **15**[8] in three stages: acetylation of 3-OH, regioselective reductive ring-opening of benzylidene cycle,[15] and deacetylation (Scheme 2).



Scheme 2. Reagents and conditions: (a) Ac_2O/Py, 97%, (b) $NaBH_3CN$, MsOH, THF, 80%, (c) MeONa/MeOH, 96%, (d) TMSOTf, CH_3CN–CH_2Cl_2, 1:1, 33% (**21**), 10% (**22**), 5% (**23**), 43% (**24**).

The glycosylation reactions of the acceptor **18** with the peracetylated trichloracetimidates of A trisaccharide **19** and B trisaccharide **20** were carried out in CH_3CN–CH_2Cl_2 (1:1) mixture to enhance β-stereospecificity via nitrile effect.[16] TMSOTf was used as catalyst. Coupling of the diol **18** and the A trisaccharide imidate **19** provided the mixture of products (Scheme 2), from which three tetrasaccharides: β-1→3-glycoside **21** (33%), α-1→3-glycoside **22** (10%), and β-

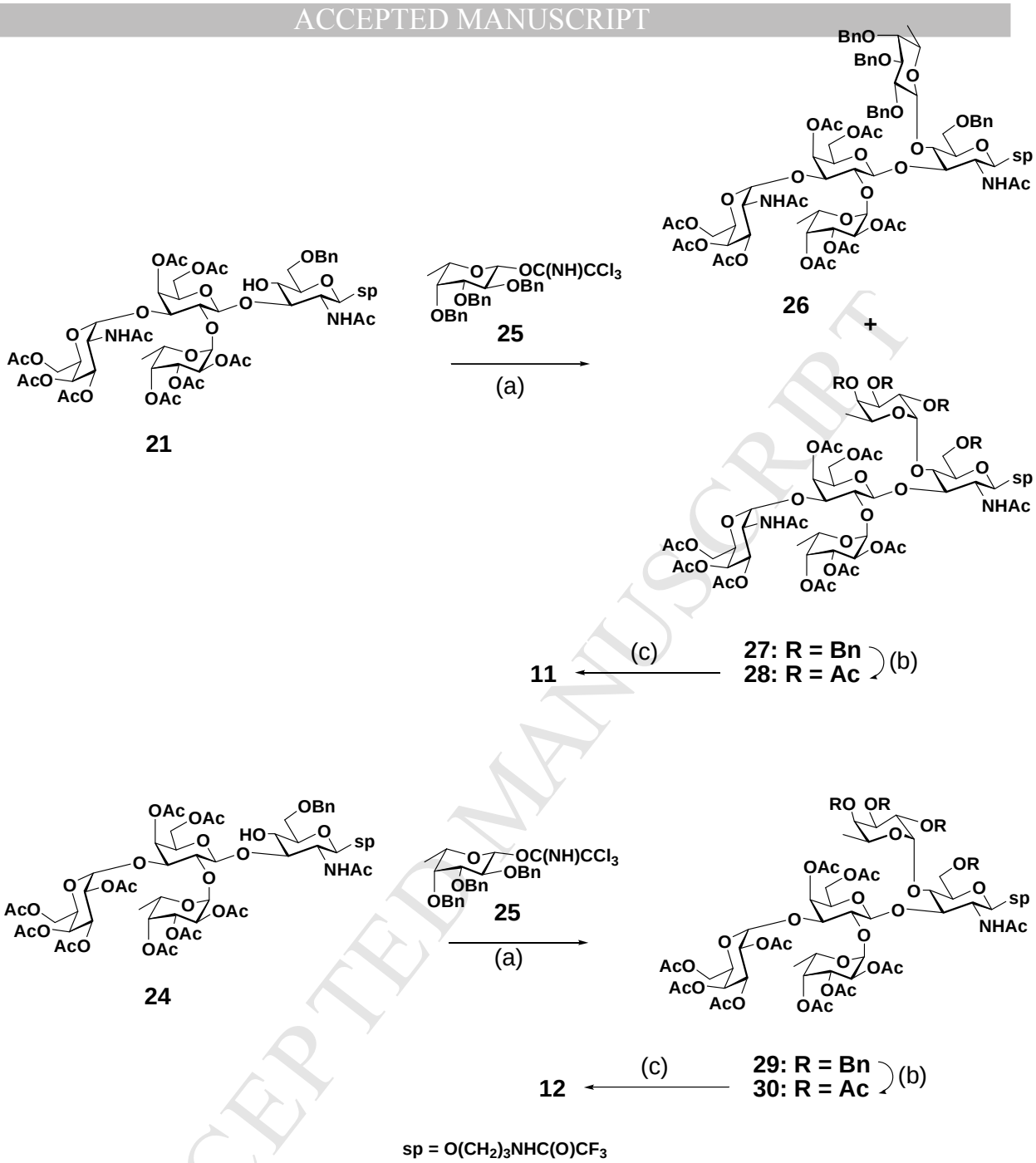
1→4-glycoside **23** (5%), were isolated. Condensation of the B trisaccharide trichloracetimidate **20** with the glycosyl acceptor **18** also resulted in complex multicomponent mixture. Only one product, β-1→3-glycoside **24** was isolated as individual compound in 43% yield (Scheme 2). Acetylated analogues **21Ac**–**24Ac** were obtained in analytical amounts. All the signals in ^{1}H and ^{13}C NMR spectra of the tetrasaccharides **21**–**24** and **21Ac**–**24Ac** were identified with the use 2D-^{1}H,^{1}H-COSY and 2D-^{1}H,^{13}C-HSQC experiments. Stereochemistry of newly formed glycosidic linkages in **21**–**24** was confirmed using characteristic values of chemical shift and coupling constant for H-1^{II} signal in ^{1}H NMR spectra (Table 1). To identify the position of the formed glycosidic linkages ^{1}H NMR spectra of the tetrasaccharides **21**–**24** were compared with the spectra of acetylated analogues **21Ac–24Ac**. Acetylation of free OH-group at C-3 or C-4 in GlcNAc unit led to substantial (1–1.5 ppm) shift of signal of H-3 or H-4, respectively, in lower field (Table 1). Thus, the position of the glycosidic linkages and the hydroxyls in **21**–**24** was determined.

Table 1. Determination of configuration and position of newly formed glycosidic bonds in tetrasaccharides **21–24**.

Compound	Configuration and position of formed glycosidic linkage	H-1^{II}, δ(ppm), $J_{1,2}$ (Hz)	H-3^{I}, δ(ppm)	H-4^{I}, δ(ppm)
21	β-1→3	4.50, $J_{1,2}$ 7.6	4.56–4.62	3.43
21Ac			4.67–4.75	4.81
22	α-1→3	5.35, $J_{1,2}$ 3.6	4.03	3.72–3.78
22Ac			4.39	5.03
23	β-1→4	4.08, $J_{1,2}$ 7.7	3.85–3.89	3.78
23Ac			4.98	3.90–4.09
24	β-1→3	4.55–4.65	3.98–4.04	3.59–3.64
24Ac		4.42, $J_{1,2}$ 7.6	4.23–4.35	4.92

The yields of the tetrasaccharides A (type 1) **21** and B (type 1) **24** were lower comparing with the glycosylation of monohydroxyl derivative **15**.[12,13] The A (type 2) tetrasaccharide **23** was isolated in minor quantities (5%), the B (type 2) tetrasaccharide was not isolated at all. Nevertheless, the main products of glycosylation – the tetrasaccharides **21** and **24** with the free OH-group at C-4 of the glucosamine unit – were convenient intermediates for the synthesis of the fucosylated pentasaccharides ALe^b **11** and BLe^b **12**. ALe^y and BLe^y pentasaccharides were synthesized according to an alternative strategy.[11]

Fucosylation of the acceptors **21** and **24** was carried out with the use of the known fucosyl imidate **25**[17] in Et_2O–CH_2Cl_2 (3:1) mixture in the presence of TMSOTf. The glycosylation of the A tetrasaccharide derivative **21** with the imidate **25** gave the mixture of two products: required pentasaccharide **27** (60%) and its β-anomer **26** (13%) (Scheme 3). In ^{1}H NMR spectra the H-1 signal of 1→4-linked fucose appeared at δ 5.09 with $J_{1,2}$ 3.7 Hz (consistent with α-glycosidic linkage) for **27** and at δ 4.52 with $J_{1,2}$ 9.0 Hz (consistent with β-glycosidic linkage) for **26**.



Scheme 3. Reagents and conditions: (a) TMSOTf, Et_2O–CH_2Cl_2, 3:1, 13% (**26**), 60% (**27**), 75% (**29**), (b) (i) MeONa/MeOH, (ii) H_2, Pd/C, MeOH, (iii) Ac_2O/Py, 85% (**28**), 88% (**30**), (c) (i) MeONa/MeOH, (ii) NaOH/H_2O, 94% (**11**), 94% (**12**).

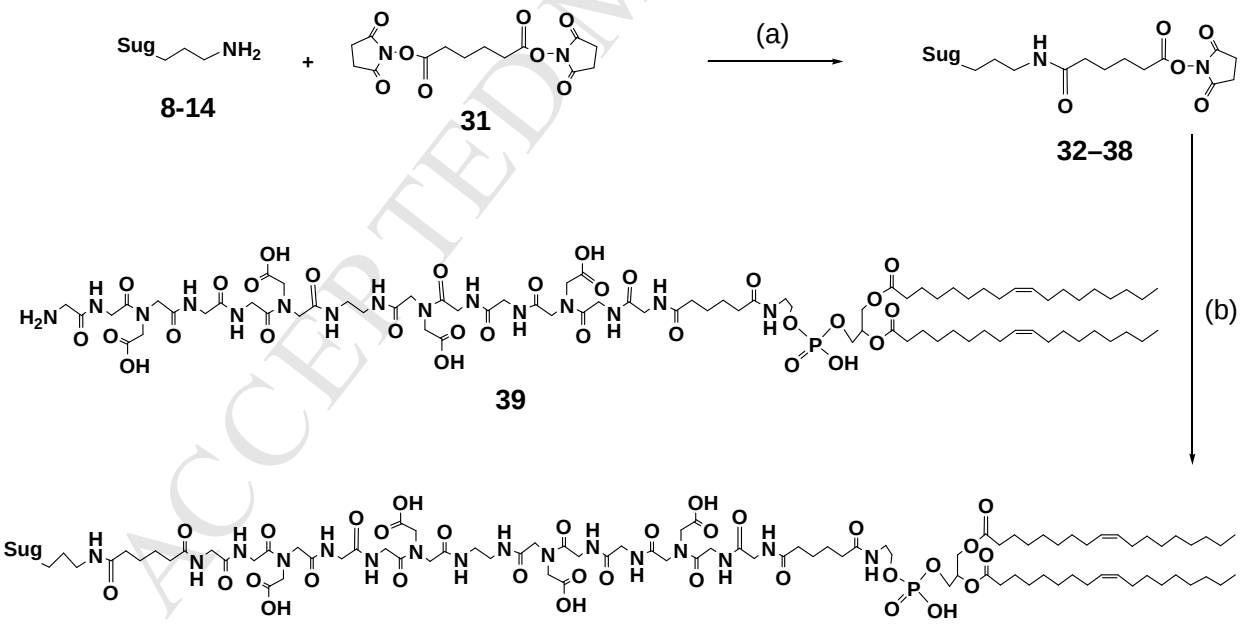
Condensation of the derivative **25** with the B tetrasaccharide **24** provided only α-fucosylated pentasaccharide **29** in 75% yield (Scheme 3). In ^{1}H NMR spectrum of **29** H-1 of 1→4-linked fucose appeared as the doubled at δ 5.14 with $J_{1,2}$ 3.7 Hz, confirming formation of α-glycosidic linkage.

Removal of protecting groups by conventional methods (Scheme 3) provided aminopropyl glycosides ALe^b **11** and BLe^b **12**.

2.2 Synthesis of function spacer lipids (FSLs)

Carboxymethylglycine peptide (CMG) spacer was chosen for FSLs **1**–**7**. It was earlier shown, that CMG possessed optimal length (~7 nm), enough for spacing glycan away from membrane, does not bind any serum proteins and inducing maximal specific serological reactivity.[5,6]

The synthesis of FSLs **1**–**7** was conducted in two steps: the aminopropyl glycosides **8**–**14** were reacted with Ad(ONSu)$_2$ **31** to give activated ethers **32**–**38** (75–97%), which were then condensed with the CMG-derivative **39** (Scheme 4). The compounds **1**–**7** were isolated from reaction mixtures by gel filtration on Sephadex LH-20 in 71–90% yields. Structures of the derivatives **1**–**7** were confirmed by ^{1}H NMR spectroscopy and mass-spectrometry.



1, 8, 32: Sug = Le^a; **2, 9, 33**: Sug = Le^b; **3, 10, 34**: Sug = Le^y; **4, 11, 35**: Sug = ALe^b; **5, 12, 36**: Sug = BLe^b; **6, 13, 37**: Sug = ALe^y; **7, 14, 38**: Sug = BLe^y

Scheme 4. Reagents and conditions: (a) DMSO, 97% (**32**), 81% (**33**), 77% (**34**), 75% (**35**), 79% (**36**), 79% (**37**), 77% (**38**), (b) 50 mM $NaHCO_3$ aq–*i*-PrOH, 2:1, DMSO, 78% (**1**), 74% (**2**), 78% (**3**), 82% (**4**), 71% (**5**), 85% (**6**), 90% (**7**).

2.3 Kodecyte and printed FSL-EIA biological assays

The extended biological analysis of monoclonal and polyclonal Lewis reagents, together with clinical samples with Lewis antibodies using the FSL constructs described in this paper are reported in detail elsewhere[7]. Using red cells modified with FSL constructs (kodecytes) and the same FSL constructs printed in solid phase on paper, a large series of monoclonal anti-Lewis serological reagents were mapped. A compiled summary of the different observable reaction patterns using natural cells, kodecytes and solid phase FSL constructs is shown in Figure 2. The monoclonal reagents tested originally only mapped against natural red cells as either anti-Le^a or anti-Le^b. However, when mapped against kodecytes prepared to carry a specific Lewis antigen, these reagents could be seen to have significant serological cross-reactivity. The kodecytes were prepared by mixing Lewis negative red cells with a Lewis FSL construct, which spontaneously and stably inserts into the cell membrane, and creates a red cell with controlled levels of a specific Lewis antigen.

When results from the kodecytes are compared with the same FSLs printed in solid phase and visualized by EIA (Figure 2) they strongly correlated with each other, and revealed a continuum of cross-reactivity from Le^x through to H. The FSL printed assay uses the same constructs as used to prepare the kodecytes except they are applied to paper using an inkjet printer (and printed in the name of the specificity they represent).

Together or alone the FSLs in the kodecytes and in the solid phase EIA assays were both able to reveal fine specificity of the monoclonal reagents tested. Most monoclonal anti-Le^a reagents could be shown to either crossreact with Le^x or Le^b, while most anti-Le^b reagents were shown to crossreact with Le^a and Le^y. Significant differences were also observed in the specificity of historical polyclonal reagents with modern monoclonal reagents[7]. Obtained results

clearly demonstrate that kodecytes, i.e. red cells inserted with neoglycolipids (FSLs), are an indispensable analytical tool capable of discriminating the fine specificity of serological reagents.

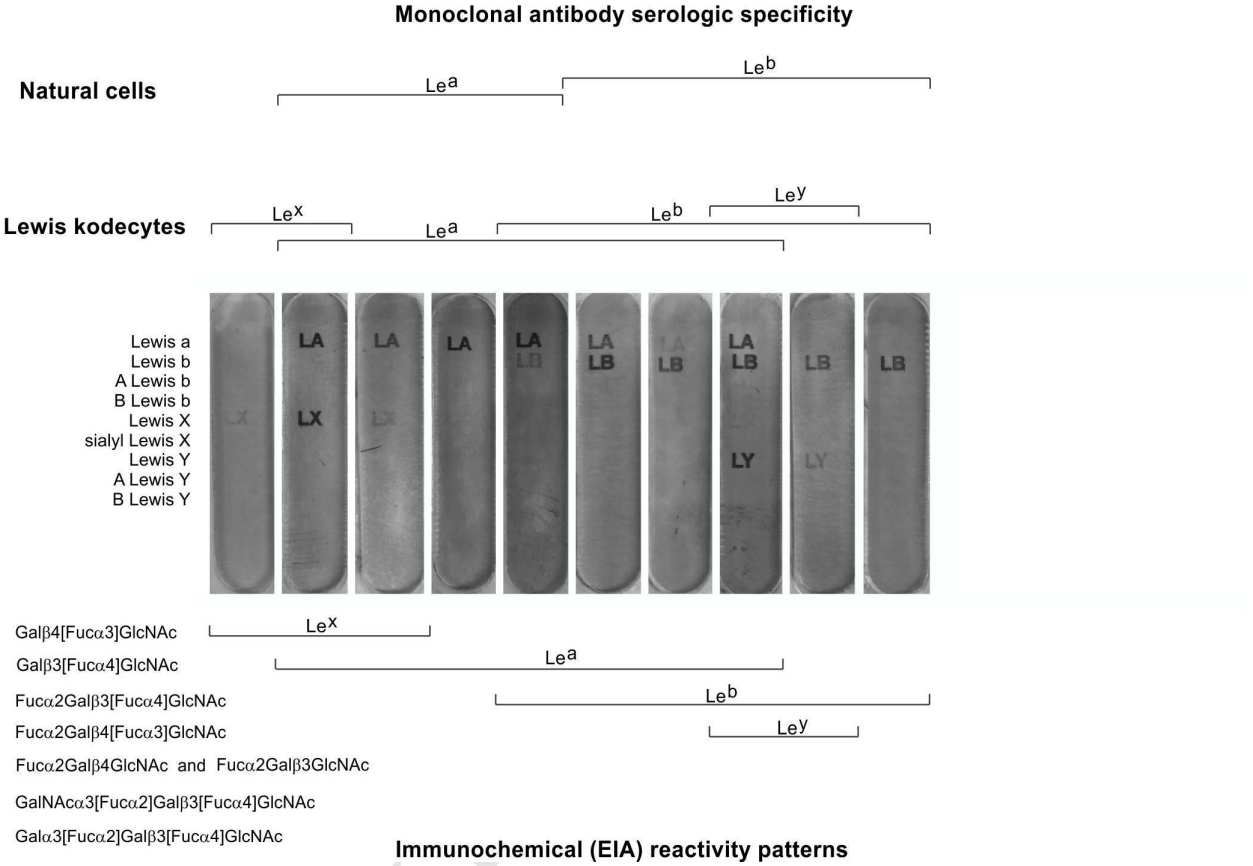


Figure 2. Mapping of monoclonal Lewis reagents with red cells modified with FSL constructs (Lewis kodecytes) and the same FSL constructs printed in solid phase on paper revealed almost identical results. Monoclonal anti-Le^a reagents that showed only Le^a specificity against natural red cells were subdivided into three related activities, Le^{a+x}, Le^a, and Le^{a+b}. The anti-Le^b reagents were subdivided into four related specificities, Le^{a+b}, Le^{a+b+y}, Le^{b+y} and Le^b. These fine specificities of the reagents including their cross-reactivity with related structures were not evident when tested against natural red cells. These results highlight the risk of defining reagents purely on the basis of reactivity against natural cells and then using these serologically defined Lewis reagents in assays other red cell serology.

3. Conclusion

A blockwise approach employing A and B blood group trisaccharides as glycosyl donors was developed for the synthesis of ALe^b and BLe^b pentasaccharides. This methodology was shown to be reasonable for obtaining of large library of structurally similar blood group antigens (ALe^b, BLe^b, and also A and B (types 1, 2, 3, and 4) tetrasaccharides, synthesis of which was described earlier).

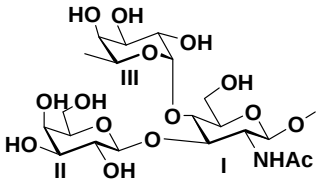
Conversion of C3 spacer-armed glycans into neoglycolipids (so called Functional Spacer Lipids, FSLs) according to a standardized protocol proceeds with yields ~80%. Seven lipophilic constructs (Le^a, Le^b, Le^y, ALe^b, BLe^b, ALe^y, BLe^y) were obtained.

FSLs where L is phosphatidyl ethanolamine residue spontaneously insert into erythrocytes in a controllable fashion giving rise to monospecific-antigen modified erythrocytes (kodecytes). Naturally impossible kodecytes bearing only a single antigen of interest can be rapidly created and used in routine serological methods to map the fine immunochemical specificity of monoclonal and polyclonal blood group reagents.

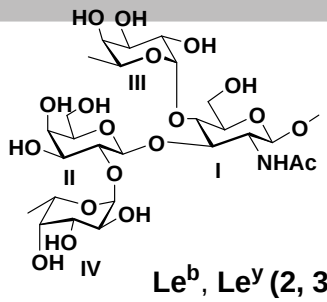
4. Experimental

4.1 General procedures

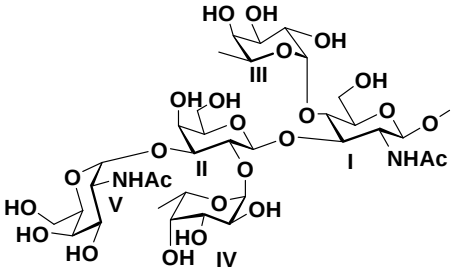
Reactions were performed with the use of commercial reagents (Acros, Aldrich, and Fluka); anhydrous solvents were purified according to the standard procedures. Column chromatography was performed on Silica gel 60 0.040–0.063 mm (Merck), gel filtration was carried out on Sephadex LH-20 (Pharmacia) columns. Solvents were removed in vacuum at 30–40 ^oC. Thin layer chromatography (TLC) was performed on Silica gel 60 F_{254} aluminum-backed plates (Merck). Spots of compounds were visualized by dipping a TLC plate into aqueous solution of H_3PO_4 (8%) and subsequent heating (>150 ^oC). Deacetylation was carried out in absolute MeOH by the addition of catalytic amount of 2M MeONa in MeOH (according to Zemplen). Na^+ ions were then removed by Dowex 50X4-400 (Acros) (H^+) cation exchanger and solution was evaporated. Hydrogenolysis was carried out on 10% Pd/C (Merck) in the atmosphere of H_2.



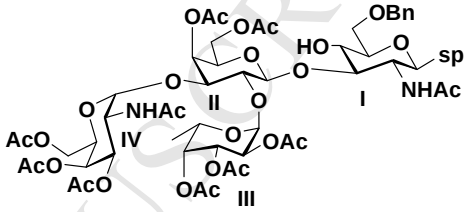
Le^a (1)



Le^b, Le^y (2, 3)



ALe^b, BLe^b, ALe^y, BLe^y
(4, 5, 6, 7, 11, 12, 26, 27, 28, 29, 30)



Tetrasaccharides 21-24, 21Ac-24Ac

Scheme 5. Symbols of monosaccharide units in NMR spectra.

The values of optical rotations were measured on a Perkin Elmer 341 polarimeter at 21±2 ^{o}C. ^{1}H NMR spectra were recorded on a Bruker BioSpin GmbH (700 MHz) spectrometer at 30 ^{o}C; chemical shifts (δ, ppm) were referred to the peak of internal D_2O (δ4.750), $CDCl_3$ (δ 7.270), or CD_3OD (δ3.500); coupling constants (J) were measured in Hz. Signals of ^{1}H NMR spectra were assigned to the corresponding protons using 2D spectroscopy (COSY). ^{13}C NMR spectra were recorded at 150 MHz. Symbols of monosaccharide residues in NMR spectra are shown on Scheme 5. ESIMS spectra were recorded on an Exactive Orbitrap (Thermo Fisher Scientific, Germany) spectrometer; MALDI MS spectra were recorded on Brucker Daltonics Ultraflex MALDI TOF/TOF Mass Spectrometer (Germany), HRESIMS spectra were recorded on an Agilent 6224 TOF LC/MS instrument (USA).

4.2 Synthesis of pentasaccharides ALe^b and BLe^b

4.2.1 3-Trifluoroacetamidopropyl 2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (18)

4.2.1.1 3-Trifluoroacetamidopropyl 2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (17)

Compound **15** (1.47 g, 3.18 mmol) was acetylated (Ac_2O/Py, 1:1, 50 ml, 20 h), the reaction mixture was poured into ice and extracted with $CHCl_3$ (3×250 ml). Organic fraction was washed with H_2O (3×300 ml), water fraction was extracted with $CHCl_3$ (2×50 ml). Combined $CHCl_3$ fractions were dried by filtration through cotton wool and concentrated. Column chromatography on silica gel ($CHCl_3$–*i*-PrOH, 10:1) afforded 1.56 g (97%) of compound **16,** white foam; R_f 0.25 ($CHCl_3$–*i*-PrOH, 10:1); $[\alpha]_D$ –56° (*C* 1, $CHCl_3$–MeOH, 1:1); ^{1}H NMR (DMSO-d_6): δ 1.69–1.74 (m, 2H, CC*H*$_2$C), 1.78 and 1.96 (2s, 6H, OC(O)C*H*$_3$, NHC(O)C*H*$_3$), 3.16–3.24 (m, 2H, NC*H*$_2$), 3.46–3.53 (m, 2H, OC*H*$_2$), 3.72–3.80 (m, 4H, H-2, H-4, H-5, H-6a), 4.24 (dd, 1H, $J_{5,6b}$ 5.0, $J_{6a,6b}$ 10.2, H-6b), 4.65 (d, 1H, $J_{1,2}$ 8.4, H-1), 5.12 (dd, 1H, $J_{2,3}$ 9.9, $J_{3,4}$ 9.9, H-3), 5.63 (s, 1H, PhC*H*), 7.35–7.39 (m, 5H, Ar*H*), 7.91 (d, 1H, $J_{2,NH}$ 9.3, N*H*Ac), 9.32–9.36 (m, 1H, N*H*C(O)CF_3); MALDI MS: *m/z* calculated for $[C_{22}H_{27}N_2O_8F_3]H^+$ 505.18, found 505.14.

Compound **16** (1.63 g, 3.23 mmol) and THF (70 ml) were stirred with molecular sieves 4 Å (13 g) for 30 min, $NaBH_3CN$ (1.83 g, 29.1 mmol) was added, and the mixture was stirred for 30 min. MsOH (14 ml of 2M solution in THF, 28 mmol) was then added to pH 3, and the mixture was stirred for 3 h. The mixture was neutralized with Et_3N (50 µl), filtered, and concentrated. The residue obtained was dissolved in $CHCl_3$ (200 ml), washed with saturated $NaHCO_3$ aq (100 ml) and NaCl aq (0.9%, 6×100 ml). Aqueous fraction was extracted with $CHCl_3$ (2×100 ml). Combined $CHCl_3$ fractions were dried by filtration through cotton wool and concentrated. Column chromatography on silica gel ($CHCl_3$–*i*-PrOH, 10:1) and crystallization from EtOAc–*n*-C_6H_{14} provided 1.31 g (80%) of compound **17**, white crystals (T_m 131 °C); R_f 0.23 ($CHCl_3$–*i*-PrOH, 6:1); $[\alpha]_D$ –68° (*C* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.81–1.88 (m, 2H,

CC*H*$_2$C), 1.95, 2.11 (2s, 6H, C(O)C*H*$_3$), 3.09–3.21 (m, 1H, O*H*), 3.23–3.32 (m, 1H, NHC*H*H), 3.53–3.64 (m, 3H, H-5, NHCH*H*, OC*H*H), 3.71 (dd, 1H, $J_{3,4}$ 9.2, $J_{4,5}$ 9.4, H-4), 3.75–3.79 (m, 2H, H-6a, H-6b), 3.90 (m, 1H, OCH*H*), 3.95 (dd, 1H, $J_{1,2}$ 8,4, $J_{2,3}$ 10.6, H-2), 4.49 (d, 1H, $J_{1,2}$ 8.4, H-1), 4.57 (dd, 2H, *J* 11.9, C*H*$_2$Ph), 4.91 (dd, 1H, $J_{2,3}$ 10.6, $J_{3,4}$ 9.2, H-3), 6.04 (d, 1H, *J* 9.0, N*H*Ac), 7.28–7.40 (m, 5H, Ar*H*), 7.50–7.57 (m, 1H, N*H*C(O)CF$_3$); ^{13}C NMR (DMSO-d_6): δ 20.7 (OC(O)*C*H$_3$), 22.6 (NC(O)*C*H$_3$), 28.3 (C*C*H$_2$C), 36.5 (N*C*H$_2$), 53.1 (C-2), 66.1 (O*C*H$_2$), 68.0 (C-5), 69.3 (C-6), 72.3 (Ph*C*H$_2$), 75.3 (C-3), 76.0 (C-4), 100.4 (C-1), 115.1 (C(O)*C*F$_3$), 127.3 (*C*-Ar), 128.1 (*C*-Ar), 138.6 (*C*-Ar), 156.2 (*C*(O)CF$_3$), 168.9 (*C*(O)CH$_3$), 169.8 (*C*(O)CH$_3$); MALDI MS: *m/z* calculated for [$C_{22}H_{29}N_2O_8F_3$]H$^+$ 507.20, found 507.22.

4.2.1.2 3-Trifluoroacetamidopropyl 2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (18)

The derivative **17** (600 mg, 1.19 mmol) was deacetylated under Zemplen conditions. In 3 h the mixture was neutralized with AcOH (10 μl) and concentrated. Crystallization from acetone–*n*-C_6H_{14} provided 530 mg (96%) of diol **18**, white crystals (T_m 135 °C); [α]$_D$ –30° (*c* 1, $CHCl_3$–MeOH, 1:1); ^{1}H NMR ($CDCl_3$–CD_3OD): δ 1.93–2.05 (m, 2H, CC*H*$_2$C), 2.23 (s, 3H, NHC(O)C*H*$_3$), 3.40–3.46 (m, 1H, NHC*H*H), 3.55 (d, 1H, $J_{3,4}$ 9.5, H-4), 3.58–3.72 (m, 4H, H-3, H-5, NHCH*H*, OC*H*H), 3.81 (dd, 1H, $J_{2,3}$ 10.1, H-2), 3.85 (dd, 1H, $J_{5,6a}$ 6.0, $J_{6a,6b}$ 10.9, H-6a), 4.03 (dd, 1H, $J_{5,6b}$ 7.8, H-6b), 4.05–4.09 (m, 1H, OCH*H*), 4.55 (d, 1H, $J_{1,2}$ 8.3, H-1), 4.76 (d, 2H, C*H*$_2$Ph), 7.43–7.61 (m, 5H, Ar*H*); ^{13}C NMR ($CDCl_3$): δ 22.2 (NC(O)*C*H$_3$), 28.5 (C*C*H$_2$C), 36.7 (N*C*H$_2$C), 56.2 (C-2), 66.6 (O*C*H$_2$), 69.7 (C-6), 71.1 (C-4), 73.4 (Ph*C*H$_2$), 75.0 (C-5), 75.4 (C-3), 101.2 (C-1), 114.4 (NHC(O)*C*F$_3$), 127.58, 127.63, 128.2, 137.9 (*C*-Ar), 157.9 (NH*C*(O)CF$_3$) 172.9 (N*C*(O)CH$_3$); MALDI MS: *m/z* calculated for [$C_{20}H_{27}N_2O_7F_3$]K$^+$ 503.14, found 503.50.

4.2.2 3-Aminopropyl 2-acetamido-2-deoxy-α-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→4)]-2-acetamido-2-deoxy-β-D-glucopyranoside (11)

4.2.2.1 3-Trifluoroacetamidopropyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl-(1→3)-[2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl-(1→2)]-4,6-di-*O*-acetyl-β-D-galactopyranosyl-(1→3)-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (21)

A mixture of trichloracetimidate **19** (279 mg, 0.276 mmol), diol **18** (256 mg, 0.552 mmol), anhydrous CH_3CN (7 ml), and anhydrous CH_2Cl_2 (7 ml) was stirred with molecular sieves 3Å (1 g) for 30 min. A solution of TMSOTf (5 μl, 0.03 mmol) in CH_2Cl_2 (1 ml) was then added. The mixture was stirred for 48 h, neutralized with Et_3N (10 μl), filtered, and concentrated. Gel filtration and subsequent column chromatography on silica gel (*n*-C_6H_{14}–$CHCl_3$–*i*-PrOH, 4:3:1→3:3:1) gave products **21** (119 mg, 33%), **22** (36 mg, 10%), and **23** (18 mg, 5%).

Compound **21**: white foam; R_f 0.18 (*n*-C_6H_{14}–$CHCl_3$–*i*-PrOH, 3:3:1); $[\alpha]_D$ +23° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): see Table 2; ^{13}C NMR (DEPT, $CDCl_3$): see Table 3; MALDI MS: *m/z* calculated for $[C_{56}H_{76}N_3O_{29}F_3]Na^+$ 1334.44, found 1334.46.

Compound **22**: white foam; R_f 0.27 (*n*-C_6H_{14}–$CHCl_3$–*i*-PrOH, 3:3:1); ^{1}H NMR ($CDCl_3$): see Table 2; ^{13}C NMR (DEPT, $CDCl_3$): see Table 3; MALDI MS: *m/z* calculated for $[C_{56}H_{76}N_3O_{29}F_3]Na^+$ 1334.44, found 1334.40.

Compound **23**: white foam; R_f 0.13 (*n*-C_6H_{14}–$CHCl_3$–*i*-PrOH, 3:3:1); ^{1}H NMR ($CDCl_3$): see Table 2; ^{13}C NMR (DEPT, $CDCl_3$): see Table 3; MALDI MS: *m/z* calculated for $[C_{56}H_{76}N_3O_{29}F_3]Na^+$ 1334.44, found 1334.43.

Tetrasaccharides **21** (15 mg, 0.011 mmol), **22** (10 mg, 0.008 mmol), and **23** (10 mg, 0.008 mmol) were acetylated (Ac_2O/Py, 1:1, 1 ml, 40 h). The reaction mixtures were coevaporated with $PhCH_3$. Column chromatography on silica gel (*n*-C_6H_{14}–$CHCl_3$–*i*-PrOH, 4:3:1) provided products **21Ac** (14 mg, 94%), **22Ac** (9 mg, 83%), and **23Ac** (9 mg, 83%), respectively.

Compound **21Ac**: white foam; R_f 0.13 (*n*-C_6H_{14}–$CHCl_3$–*i*-PrOH, 4:3:1); $[\alpha]_D$ +17° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): see Table 2,; ^{13}C NMR (DEPT, $CDCl_3$): see Table 3; MALDI MS: *m/z* calculated for $[C_{58}H_{78}N_3O_{30}F_3]Na^+$ 1376.45, found 1376.47.

Compound **22Ac**: white foam; R_f 0.39 (*n*-C_6H_{14}–$CHCl_3$–*i*-PrOH, 3:3:1); $[\alpha]_D$ +32° (*c* 0.82, $CHCl_3$); ^{1}H NMR ($CDCl_3$): see Table 2,; ^{13}C NMR (DEPT, $CDCl_3$): see Table 3; MALDI MS: *m/z* calculated for $[C_{58}H_{78}N_3O_{30}F_3]Na^+$ 1376.45, found 1376.41.

Compound **23Ac**: white foam; R_f 0.25 (*n*-C_6H_{14}–$CHCl_3$–*i*-PrOH, 3:3:1); $[\alpha]_D$ –20° (*c* 0.60, $CHCl_3$); ^{1}H NMR ($CDCl_3$): see Table 2,; ^{13}C NMR (DEPT, $CDCl_3$): see Table 3; MALDI MS: *m/z* calculated for $[C_{58}H_{78}N_3O_{30}F_3]Na^+$ 1376.45, found 1376.50.

4.2.2.2 3-Trifluoroacetamidopropyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl-(1→3)-[2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl-(1→2)]-4,6-di-*O*-acetyl-β-D-galactopyranosyl-(1→3)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→4)]-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (27)

A mixture of tetrasaccharide **21** (95 mg, 0.071 mmol), trichloracetimidate **25** (82 mg, 0.14 mmol), anhydrous Et_2O (3 ml), and anhydrous CH_2Cl_2 (1 ml) was stirred with molecular sieves 3Å (300 mg) in the atmosphere of Ar for 30 min. A solution of TMSOTf (6.5 μl, 0.036 mmol) in CH_2Cl_2 (100 μl) was added. The mixture was stirred for 4 h, neutralized with Et_3N (10 μl), filtered, and concentrated. Gel filtration and subsequent column chromatography on silica gel (*n*-C_6H_{14}–$CHCl_3$–*i*-PrOH, 3:3:1) gave products **26** (16 mg, 13%), **27** (74 mg, 60%), and starting tetrasaccharide **21** (14 mg, 15%).

Compound **26**: white foam; R_f 0.36 (*n*-C_6H_{14}–$CHCl_3$–*i*-PrOH, 3:3:1); $[\alpha]_{Hg}$ –10° (*c* 1.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$): see Table 4; ^{13}C NMR ($CDCl_3$, DEPT): see Table 5; MALDI MS: *m/z* calculated for $[C_{83}H_{104}N_3O_{33}F_3]K^+$ 1767.62, found 1767.93.

Compound **27**: white foam; R_f 0.21 (*n*-C_6H_{14}–$CHCl_3$–*i*-PrOH, 3:3:1); $[\alpha]_{Hg}$ –17° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): see Table 4; ^{13}C NMR ($CDCl_3$, DEPT): see Table 5; MALDI MS: *m/z* calculated for $[C_{83}H_{104}N_3O_{33}F_3]H^+$ 1728.66, found 1767.85.

4.2.2.3 3-Trifluoroacetamidopropyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl-(1→3)-[2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl-(1→2)]-4,6-di-*O*-acetyl-β-D-

galactopyranosyl-(1→3)-[2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl-(1→4)]-2-acetamido-6-*O*-acetyl-2-deoxy-β-D-glucopyranoside (28)

Pentasaccharide **27** (72 mg, 0.042 mmol) was deacetylated under Zemplen conditions and subjected to (45 mg Pd/C, 4 ml MeOH, 2 h). The mixture was filtered and concentrated. The residue obtained was acetylated (Ac_2O/Py, 1:1, 3 ml, 20 h) and coevaporated with $PhCH_3$. Column chromatography on silica gel (*n*-C_6H_{14}–$CHCl_3$–*i*-PrOH, 3:2:1) afforded pentasaccharide **28** (55 mg, 85%), white foam; R_f 0.18 (*n*-C_6H_{14}–$CHCl_3$–*i*-PrOH, 2:3:1); $[\alpha]_{Hg}$ –42° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): see Table 4; ^{13}C NMR ($CDCl_3$, DEPT): see Table 5; MALDI MS: *m/z* calculated for $[C_{63}H_{89}N_3O_{37}F_3]K^+$ 1575.48, found 1575.69.

4.2.2.4 3-Aminopropyl 2-acetamido-2-deoxy-α-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→4)]-2-acetamido-2-deoxy-β-D-glucopyranoside (11)

Pentasaccharide **28** (55 mg, 0.036 mmol) was deacetylated under Zemplen conditions. MeOH was then partially evaporated, H_2O (1 ml) added, and the mixture was kept for 4 h at rt. Ion exchange chromatography on Dowex 50XW-400 (H^+) (elution with 5% NH_3 aq) provided 32 mg (94%) of product **11**, white foam; R_f 0.13 (EtOH–BuOH–Py–H_2O–AcOH, 100:10:10:10:3); $[\alpha]_{Hg}$ –18° (*c* 1.2, H_2O–CH_3CN, 1:1); ^{1}H NMR (D_2O, characteristic signals): δ 1.27–1.33 (m, 6H, H-6^{III}, H-6^{IV}), 1.89–2.00 (m, 2H, CC*H*$_2$C), 2.04, 2.09 (2s, 6H, 2 NHC(O)C*H*$_3$), 3.05–3.10 (m, 2H, NC*H*$_2$), 4.39 (q, 1H, $J_{5,6}$ 6.8, H-5^{IV}), 4.42 (d, 1H, $J_{1,2}$ 8.5, H-1^{I}), 4.72 (d, 1H, $J_{1,2}$ 7.6, H-1^{II}), 4.86 (q, 1H, $J_{5,6}$ 6.7, H-5^{III}), 5.04 (d, 1H, *J1,2* 3.9, H-1^{III}), 5.22–5.24 (m, 2H, H-1^{IV}, H-1^{V}); ^{13}C NMR (D_2O, DEPT): δ 15.4, 15.5 (C-6^{III}, C-6^{IV}), 26.8 (C*C*H$_2$C), 37.5 (N*C*H$_2$), 49.9 (C-2^{V}), 55.6 (C-2^{I}), 59.5 , 61.5 , 61.6 (C-6^{I}, C-6^{II}, C-6^{V}), 62.3, 66.5, 67.2, 67.6, 67.8, 67.9, 68.0, 68.6, 69.2, 69.8, 71.0, 72.0, 72.1, 72.4, 74.2, 74.5, 74.5, 75.5, 76.0 (O*C*H$_2$, C-3^{I}, C-4^{I}, C-5^{I}, C-2^{II}, C-3^{II}, C-4^{II}, C-5^{II}, C-2^{III}, C-3^{III}, C-4^{III}, C-5^{III}, C-2^{IV}, C-3^{IV}, C-4^{IV}, C-5^{IV}, C-3^{V}, C-4^{V}, C-5^{V}), 91.0 (C-1^{V}), 98.0 (C-1^{III}), 99.3 (C-1^{IV}), 100.4 (C-1^{I}), 101.9 (C-1^{II}); MALDI MS: *m/z* calculated for $[C_{37}H_{65}N_3O_{24}]H^+$ 959.39, found 959.29.

4.2.3. 3-Aminopropyl α-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→4)]-2-acetamido-2-deoxy-β-D-glucopyranoside (12)

4.2.3.1 3-Trifluoroacetamidopropyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl-(1→3)-[2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl-(1→2)]-4,6-di-*O*-acetyl-β-D-galactopyranosyl-(1→3)-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (24)

A mixture of trichloracetimidate **20** (200 mg, 0.198 mmol), diol **18** (184 mg, 0.397 mmol), anhydrous CH_3CN (5 ml), and hydrous CH_2Cl_2 (5 ml) was stirred with molecular sieves 3Å (500 mg) under the atmosphere of N_2 for 30 min. A solution of TMSOTf (4 μl, 0.02 mmol) in CH_2Cl_2 (100 μl) was added. The mixture was stirred for 20 h, neutralized with Et_3N (10 μl), filtered, and concentrated. Gel filtration and subsequent column chromatography on silica gel (*n*-C_6H_{14}–$CHCl_3$–*i*-PrOH, 6:3:1) and repeated column chromatography on silica gel ($PhCH_3$–EtOAc, 1:5) gave product **24** (122 mg, 43%), white foam; R_f 0.20 ($PhCH_3$–EtOAc, 1:5); $[\alpha]_{Hg}$ +8° (*c* 0.70, $CHCl_3$); ^{1}H NMR ($CDCl_3$): see Table 2; ^{13}C NMR ($CDCl_3$, DEPT): see Table 3; MALDI MS: *m/z* calculated for $[C_{56}H_{76}N_2O_{30}F_3]K^+$ 1352.40, found 1352.43.

Tetrasaccharide **24** (10 mg, 0.0076 mmol) was acetylated (Ac_2O/Py, 1:1, 1 ml, 20 h), the mixture was coevaporated with $PhCH_3$. Column chromatography on silica gel (*n*-C_6H_{14}–$CHCl_3$–*i*-PrOH, 5:3:1) provided compound **24Ac** (9 mg, 87%), white foam; R_f 0.17 (*n*-C_6H_{14}–$CHCl_3$–*i*-PrOH, 5:3:1); $[\alpha]_{Hg}$ +18° (*c* 0.79, $CHCl_3$); ^{1}H NMR ($CDCl_3$): see Table 2; ^{13}C NMR ($CDCl_3$, DEPT): see Table 3; MALDI MS: *m/z* calculated for $[C_{58}H_{78}N_2O_{31}F_3]Na^+$ 1378.44, found 1378.43.

4.2.3.2 3-Trifluoroacetamidopropyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl-(1→3)-[2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl-(1→2)]-4,6-di-*O*-acetyl-β-D-galactopyranosyl-(1→3)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→4)]-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (29)

A mixture of tetrasaccharide **24** (75 mg, 0.057 mmol), trichloracetimidate **25** (65 mg, 0.11 mmol), anhydrous Et_2O (3 ml), and anhydrous CH_2Cl_2 (1 ml) was stirred with molecular sieves 3Å (300 mg) under the atmosphere of Ar for 30 min. A solution of TMSOTf (5 μl, 0.03 mmol) in CH_2Cl_2 (100 μl) was added. The mixture was stirred for 3 h, neutralized with Et_3N (5 μl), filtered, and concentrated. Gel filtration and subsequent column chromatography on silica gel (*n*-C_6H_{14}–$CHCl_3$–*i*-PrOH, 7:3:1) provided product **29** (75 mg, 76%), white foam; R_f 0.33 (*n*-C_6H_{14}–$CHCl_3$–*i*-PrOH, 5:3:1); $[\alpha]_{Hg}$ –10° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): see Table 4; ^{13}C NMR ($CDCl_3$, DEPT): see Table 5; MALDI MS: *m/z* calculated for $[C_{63}H_{88}N_2O_{38}F_3]K^+$ 1576.46, found 1576.45.

4.2.3.3 3-Trifluoroacetamidopropyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl-(1→3)-[2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl-(1→2)]-4,6-di-*O*-acetyl-β-D-galactopyranosyl-(1→3)-[2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl-(1→4)]-2-acetamido-6-*O*-acetyl-2-deoxy-β-D-glucopyranoside (30)

Pentasaccharide **29** (75 mg, 0.043 mmol) was deacetylated under Zemplen conditions, subjected to hydrogenolysis (70 mg Pd/C, 5 ml MeOH, 3 h), filtered, and concentrated. The residue obtained was acetylated (Ac_2O/Py, 1:1, 3 ml, 24 h) and coevaporated with $PhCH_3$. Column chromatography on silica gel (*n*-C_6H_{14}–acetone, 1:1) provided pentasaccharide **30** (59 mg, 88%), white foam; R_f 0.23 (*n*-C_6H_{14}–acetone, 1:1); $[\alpha]_{Hg}$ –29° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): see Table 4; ^{13}C NMR ($CDCl_3$, DEPT): see Table 5; MALDI MS: *m/z* calculated for $[C_{63}H_{88}N_2O_{38}F_3]K^+$ 1576.46, found 1576.45.

4.2.3.4 3-Aminopropyl α-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→4)]-2-acetamido-2-deoxy-β-D-glucopyranoside (12)

Pentasaccharide **30** (59 mg, 0.038 mmol) was deacetylated under Zemplen conditions. MeOH was then partly evaporated, H_2O (1 ml) was added, and the mixture was kept for 4 h at rt.

Ion exchange chromatography on Dowex 50XW-400 (H^+) (elution with 5% NH_3 aq) provided 32 mg (94%) of product **12**, white foam; R_f 0.16 (EtOH–BuOH–Py–H_2O–AcOH, 100:10:10:10:3); $[\alpha]_{Hg}$ –155° (*c* 0.27, H_2O–CH_3CN, 1:1); ^{1}H NMR (D_2O, characteristic signals): δ 1.28–1.31 (m, 6H, H-6^{III}, H-6^{IV}), 1.89–2.02 (m, 2H, CCH_2C), 2.11 (s, 3H, NHC(O)CH_3), 3.06–3.10 (m, 2H, NCH_2), 4.40 (q, 1H, $J_{5,6}$ 6.9, H-5^{IV}), 4.42 (d, 1H, $J_{1,2}$ 8.5, H-1^{I}), 4.73–4.74 (m, 1H, H-1^{II}), 4.84 (q, 1H, $J_{5,6}$ 6.6, H-5^{III}), 5.05 (d, 1H, $J_{1,2}$ 3.9, H-1^{III}), 5.19 (d, 1H, $J_{1,2}$ 4.2, H-1^{V}), 5.25 (d, 1H, $J_{1,2}$ 3.8, H-1^{IV}); ^{13}C NMR (D_2O, DEPT): δ 15.4, 15.5 (C-6^{III}, C-6^{IV}), 26.8 (CCH_2C), 37.5 (NCH_2), 55.7 (C-2^{I}), 59.6, 61.3, 61.6 (C-6^{I}, C-6^{II}, C-6^{V}), 63.5, 66.5, 67.2, 67.8, 68.1, 68.6, 69.1, 69.3, 69.4, 69.7, 71.3, 72.1, 72.1, 72.2, 74.0, 74.4, 74.8, 75.5, 76.5, 77.0 (C-3^{I}, C-4^{I}, C-5^{I}, C-2^{II}, C-3^{II}, C-4^{II}, C-5^{II}, C-2^{III}, C-3^{III}, C-4^{III}, C-5^{III}, C-2^{IV}, C-3^{IV}, C-4^{IV}, C-5^{IV}, C-2^{V}, C-3^{V}, C-4^{V}, C-5^{V}), 93.5 (C-1^{V}), 97.9 (C-1^{III}), 99.4 (C-1^{IV}), 100.7 (C-1^{I}), 102.0 (C-1^{II}); MALDI MS: *m/z* calculated for $[C_{35}H_{63}N_2O_{24}]H^+$ 896.38, found 896.10.

4.3 Synthesis of FSL (Sug-Ad-CMG-Ad-DOPE) 1–7

4.3.1 Synthesis of N-succinimide derivatives (general procedure)

A solution of aminopropyl glycoside (0.010 mmol, 5.9 mg for **8** (Le^a), 7.3 mg for **9** (Le^b) and **10** (Le^y), 9.4 mg for **11** (ALe^b) and **13** (ALe^y), 9.0 mg for **12** (BLe^b) and **14** (BLe^y)) in 0.5 ml of DMSO was added by 5 equal portions (100 μl in every 10 min) to a solution of Ad(ONSu)$_2$ (34 mg, 0.10 mmol) in 0.5 ml of DMSO. After the last portion was added, the mixture was kept for 20 min at rt and subjected to gel filtration (CH_3CN–H_2O, 0.3% AcOH) to give N-succinimide derivatives **32**–**38**.

Le^{a}AdONSu (**32**): 7.9 mg (97%), R_f 0.42 ($CHCl_3$–EtOH–H_2O, 4:9:2, 0.1% AcOH), Le^{b}AdONSu (**33**): 7.6 mg (81%), R_f 0.38 ($CHCl_3$–EtOH–H_2O, 4:9:2, 0.1% AcOH), Le^{y}AdONSu (**34**): 7.4 mg (77%), R_f 0.45 ($CHCl_3$–EtOH–H_2O, 4:9:2, 0.1% AcOH), ALe^{b}AdONSu (**35**): 8.7 mg (75%), R_f 0.37 ($CHCl_3$–EtOH–H_2O, 4:9:2, 0.1% AcOH), BLe^{b}AdONSu (**36**): 8.9 mg (79%), R_f 0.40 ($CHCl_3$–EtOH–H_2O, 4:9:2, 0.1% AcOH), ALe^{y}AdONSu (**37**): 9.2 mg (79%), R_f 0.41

($CHCl_3$–EtOH–H_2O, 4:9:2, 0.1% AcOH), BLe^{y}AdONSu (**38**): 8.6 mg (77%), R_f 0.37 ($CHCl_3$–EtOH–H_2O, 4:9:2, 0.1% AcOH).

4.3.2 Synthesis of FSLs (general procedure)

A solution of N-succinimide derivative (0.0043 mmol, 3.5 mg for **32**, 4.1 mg for **33** and **34**, 5.0 mg for **35** and **37**, 4.8 mg for **36** and **38**) in 300 μl of DMF was added by 3 equal portions (100 μl every 1.5 h) to the solution of CMG-Ad-DOPE **39** (5.3 mg, 0.0029 mmol) in the mixture of $NaHCO_3$ aq (50 mM, 0.5 ml) and *i*-PrOH (0.25 ml) (pH 8.5). The mixture was left overnight, then neutralized with AcOH (2 μl), and subjected to gel filtration (*i*-PrOH–H_2O, 1:2, 0.25% AcOH, 0.5% Py) to give neoglycolipids **1**–**7**.

Le^a-Ad-CMG-Ad-DOPE (**1**): 6.0 mg (78%), white foam; R_f 0.39 (*i*-PrOH–MeOH–CH_3CN–H_2O, 4:3:6:4); ^{1}H NMR (D_2O–CD_3OD, 1:1, characteristic signals): δ 5.51–5.57 (m, 4H, 2 -C*H*=C*H*-), 5.43–5.47 (m, 1H, OCH_2CHOCH_2O-), 5.20 (d, 1H, $J_{1,2}$ 3.9, H-1^{III}), 5.04 (q, 1H, $J_{5,6}$ 6.6, H-5^{III}), 4.68 (d, 1H, $J_{1,2}$ 8.4, H-1^{II}), 4.66 (d, 1H, $J_{1,2}$ 7.7, H-1^{I}), 4.63 (dd, 1H, *J* 2.4, *J* 12.3, -C(O)OC*H*HCHOCH_2O-), 2.41–2.57 (m, 12H, 6 -CH_2CO), 2.17–2.22 (m, 11H, 2 -CH_2-CH=CH-CH_2- and NHC(O)CH_3), 1.90–1.98 (m, 2H, $OCH_2CH_2CH_2NH$), 1.74–1.84 (m, 12H, 2 $COCH_2CH_2CH_2CH_2CO$ and 2 $COCH_2CH_2$-), 1.41–1.55 (m, 40H, 20 CH_2), 1.37 (d, 3H, $J_{5,6}$ 6.6, H-6^{III}), 1.06 (t, *J* 7.1, 6H; 2 CH_3); MALDI MS: *m/z* 2549 $(M+Na+H)^+$, 2565 $(M+K+H)^+$; 2571 $(M+2Na+H)^+$, 2587 $(M+Na+K+H)^+$, 2593 $(M+3Na+H)^+$, 2609 $(M+2Na+K+H)^+$, 2615 $(M+4Na+H)^+$, 2631 $(M+3Na+K+H)^+$, 2647 $(M+2Na+2K+H)^+$, 2653 $(M+4Na+K+H)^+$, 2669 $(M+3Na+2K+H)^+$.

Le^b-Ad-CMG-Ad-DOPE (**2**): 5.7 mg (74%), white foam; R_f 0.41 (*i*-PrOH–MeOH–CH_3CN–H_2O, 4:3:6:4); ^{1}H NMR (D_2O–CD_3OD, 1:1, characteristic signals): δ 5.51–5.57 (m, 4H, 2 -C*H*=C*H*-), 5.43–5.47 (m, 1H, -OCH_2CHOCH_2O-,), 5.33 (br.s, 1H, H-1^{IV}), 5.19 (d, 1H, $J_{1,2}$ 3.8, H-1^{III}), 5.03 (q, 1H, $J_{5,6}$ 6.8, H-5^{III}), 4.83 (d, 1H, $J_{1,2}$ 7.3, H-1^{II}), 4.63 (dd, 1H, *J* 2.4, *J* 12.3, -C(O)OC*H*HCHOCH_2O-), 4.45 (q, 1H, $J_{5,6}$ 6.7, H-5^{IV}), 4.53 (d, 1H, $J_{1,2}$ 8.5, H-1^{I}), 2.49–2.56 (m, 12H, 6 CH_2CO), 2.24 (s, 3H, NHC(O)CH_3), 2.17–2.22 (m, 8H, 2 -CH_2CH=CHCH_2-), 1.88–1.96

(m, 2H, $OCH_2CH_2CH_2NH$), 1.74–1.84 (m, 12H, 2 ($COCH_2CH_2CH_2CH_2CO$) and 2 ($COCH_2CH_2$-), 1.41–1.56 (m, 46H, H-6^{III}, H-6^{IV}, 20 CH_2), 1.06 (t, *J* 7.0, 6H; 2 CH_3); MALDI MS: *m/z* 2698 $(M+Na+H)^+$, 2714 $(M+K+H)^+$; 2720 $(M+2Na)^+$.

Le^y-Ad-CMG-Ad-DOPE (**3**): 6.0 mg (78%), white foam; R_f 0.45 (*i*-PrOH–MeOH–CH_3CN–H_2O, 4:3:6:4); ^1H NMR (D_2O–CD_3OD, 1:1, characteristic signals): δ 5.51–5.56 (m, 4H, 2 -C*H*=C*H*-), 5.43–5.46 (m, 1H, OCH_2CHOCH_2O-), 5.43 (d, 1H, $J_{1,2}$ 3.3, H-1^{IV}), 5.26 (d, 1H, $J_{1,2}$ 3.9, H-1^{III}), 5.05 (q, 1H, $J_{5,6}$ 6.5, H-5^{III}), 4.66–4.70 (m, 2H, H-1^{I}, H-1^{II}), 4.63 (dd, 1H, *J* 2.3, *J* 12.0, -C(O)OC*H*HCHOCH_2O-), 2.40–2.56 (m, 12H, 6 -CH_2CO), 2.17–2.22 (m, 11H, 2 -CH_2-CH=CH-CH_2-, NHC(O)CH_3), 1.90–1.97 (m, 2H, $OCH_2CH_2CH_2NH$), 1.74–1.84 (m, 12H, 2 $COCH_2CH_2CH_2CH_2CO$ and 2 $COCH_2CH_2$-), 1.40–1.55 (m, 46H, H-6^{III}, H-6^{IV}, 20 CH_2), 1.06 (t, *J* 7.1, 6H; 2 CH_3); MALDI MS: *m/z* 2697 $(M+Na)^+$, 2719 $(M+2Na)^+$; 2742 $(M+3Na)^+$.

ALe^b-Ad-CMG-Ad-DOPE (**4**): 6.8 mg (82%), white foam; R_f 0.42 (*i*-PrOH–MeOH–CH_3CN–H_2O, 4:3:6:4); ^1H NMR (D_2O–CD_3OD, 1:1, characteristic signals): δ 5.51–5.57 (m, 4H, 2 -C*H*=C*H*-), 5.44–5.47 (m, 1H, -OCH_2CHOCH_2O), 5.41, 5.39 (d, 1H, $J_{1,2}$ 3.8 and br.s, 1H, H-1^{IV}, H-1^{V}), 5.20 (d, 1H, $J_{1,2}$ 3.8, H-1^{III}), 5.01 (q, 1H, $J_{5,6}$ 6.9, H-5^{III}), 4.89 (d, 1H, $J_{1,2}$ 7.4, H-1^{II}), 4.63 (dd, 1H, *J* 2.2, *J* 12.1, -C(O)OC*H*HCHOCH_2O-), 4.61 (q, 1H, $J_{5,6}$ 6.6, H-5^{IV}), 4.53 (d, 1H, $J_{1,2}$ 8.3, H-1^{I}), 2.41–2.56 (m, 12H, 6 -CH_2CO), 2.24 (s, 3H, NHC(O)CH_3), 2.22 (m, 11H, NHC(O)CH_3, 2 CH_2CH=CHCH_2-), 1.86–1.96 (m, 2H, O-$CH_2CH_2CH_2$-NH), 1.72–1.84 (m, 12H, 2 -$COCH_2CH_2CH_2CH_2CO$ and 2 -$COCH_2CH_2$-), 1.40–1.56 (m, 46H, H-6^{III}, H-6^{IV}, 20 CH_2), 1.06 (t, *J* 7.1, 6H; 2 CH_3); MALDI MS: *m/z* 2923 $(M+2Na)^+$, 2945 $(M+3Na)^+$, 2962 $(M+2Na+K)^+$.

BLe^b-Ad-CMG-Ad-DOPE (**5**): 5.8 mg (71%), white foam; R_f 0.52 (*i*-PrOH–MeOH–CH_3CN–H_2O, 4:3:6:4); ^1H NMR (D_2O–CD_3OD, 1:1, characteristic signals): δ 5.52–5.58 (m, 4H, 2 -C*H*=C*H*-), 5.43–5.47 (m, 1H, -OCH_2CHOCH_2O-), 5.39 (d, 1H, $J_{1,2}$ 3.8, H-1^{IV}), 5.34 (br. s, 1H, H-1^{V}), 5.20 (d, 1H, $J_{1,2}$ 3.5, H-1^{III}), 5.00 (q, 1H, $J_{5,6}$ 6.8, H-5^{III}), 4.90 (d, 1H, $J_{1,2}$ 7.2, H-1^{II}), 4.63 (dd, 1H, *J* 2.1, *J* 12.0, -C(O)OC*H*HCHOCH_2O-), 4.61 (q, 1H, $J_{5,6}$ 6.7, H-5^{IV}), 4.53 (d, 1H, $J_{1,2}$ 8.7, H-1^{I}), 2.40–2.60 (m, 12H, 6 CH_2-CO), 2.25 (s, 3H, NHC(O)CH_3), 2.17–2.22 (m, 8H, 2 -

CH_2CH=CHCH_2-), 1.88–1.96 (m, 2H, OCH$_2$CH_2CH$_2$NH), 1.71–1.84 (m, 12H, 2 (COCH$_2$CH_2CH_2CH$_2$CO) and 2 (COCH$_2$CH_2-), 1.41–1.55 (m, 46H, H-6^{III}, H-6^{IV}, 20 CH_2), 1.04–1.08 (m, 6H; 2 CH_3); MALDI MS: *m/z* 2860 (M+Na+H)$^+$, 2882 (M+2Na+H)$^+$, 2904 (M+3Na+H)$^+$.

ALe^y-Ad-CMG-Ad-DOPE (**6**): 7.1 mg (85%), white foam; R_f 0.60 (*i*-PrOH–MeOH–CH$_3$CN–H$_2$O, 4:3:6:4); ^{1}H NMR (D$_2$O–CD$_3$OD, 1:1, characteristic signals): δ 5.51–5.57 (m, 4H, 2 -C*H*=C*H*-), 5.50 (d, 1H, J1,2 4.1, H-1^{IV}), 5.43–5.47 (m, 1H, -OCH$_2$C*H*OCH$_2$O-) 5.38 (d, 1H, $J_{1,2}$ 3.7, H-1^V), 5.28 (d, 1H, $J_{1,2}$ 4.0, H-1^{III}), 5.05 (q, 1H, $J_{5,6}$ 6.8, H-5^{III}), 4.75 (d, 1H, $J_{1,2}$ 7.3, H-1^{II}), 4.68 (d, 1H, $J_{1,2}$ 7.8, H-1^I), 4.64 (dd, 1H, *J* 2.4, *J* 12.2, -C(O)OC*H*HCHOCH$_2$O-), 4.52 (q, 1H, $J_{5,6}$ 6.8, H-5^{IV}), 2.40–2.57 (m, 12H, 6 CH_2CO), 2.16–2.23 (m, 14H, 2 -CH_2CH=CHCH_2-, 2 NHC(O)CH_3), 1.90–1.96 (m, 2H, OCH$_2$CH_2CH$_2$NH), 1.74–1.84 (m, 12H, 2 (COCH$_2$CH_2CH_2CH$_2$CO) and 2 (COCH$_2$CH_2-), 1.41–1.55 (m, 46H, H-6^{III}, H-6^{IV}, 20 CH_2), 1.06 (t, *J* 7.1, 6H; 2 CH_3); MALDI MS: *m/z* 2901 (M+Na+H)$^+$, 2923 (M+2Na)$^+$; 2939 (M+Na+K)$^+$, 2945 (M+3Na)$^+$; 2962 (M+2Na+K)$^+$, 2967 (M+4Na)$^+$.

BLe^y-Ad-CMG-Ad-DOPE (**7**): 7.4 mg (90%), white foam; R_f 0.45 (*i*-PrOH–MeOH–CH$_3$CN–H$_2$O, 4:3:6:4); ^{1}H NMR (D$_2$O–CD$_3$OD, 1:1, characteristic signals): δ 5.51–5.57 (m, 4H, 2 -C*H*=C*H*-), 5.47 (d, 1H, $J_{1,2}$ 4.1, H-1^{IV}), 5.43–5.46 (m, 1H, -OCH$_2$C*H*OCH$_2$O-), 5.40 (d, 1H, $J_{1,2}$ 3.1, H-1^V), 5.27 (d, 1H, $J_{1,2}$ 3.7, H-1^{III}), 5.06 (q, 1H, $J_{5,6}$ 6.5, H-5^{III}), 4.76 (d, 1H, $J_{1,2}$ 7.6, H-1^{II}), 4.66–4.70 (m, 1H, H-1^I), 4.63 (dd, 1H, *J* 2.3, *J* 12.3, -C(O)OC*H*HCHOCH$_2$O-), 4.51 (q, 1H, $J_{5,6}$ 6.5, H-5^{IV}), 2.40–2.56 (m, 12H, 6 CH_2-CO), 2.16–2.22 (m, 11H, 2 -CH_2CH=CHCH_2- and NHC(O)CH_3), 1.90–1.96 (m, 2H, OCH$_2$CH_2CH$_2$NH), 1.74–1.84 (m, 12H, 2 -COCH$_2$CH_2CH_2CH$_2$CO- and 2 -COCH$_2$CH_2-), 1.40–1.55 (m, 46H, H-6^{III}, H-6^{IV}, 20 CH_2), 1.06 (t, *J* 7.0, 6H; 2 CH_3); MALDI MS: *m/z* 2859 (M+Na)$^+$, 2881 (M+2Na)$^+$; 2903 (M+3Na)$^+$.

Table 2. ^{1}H NMR spectra (δ(ppm) and J (Hz)) of tetrasaccharides **21**–**24** and **21Ac**–**24Ac**.

Proton	**21**	**21Ac**	**22**	**22Ac**	**23**	**23Ac**	**24**	**24Ac**
H-1^{I}	4.82, d $J_{1,2}$ 8.1	4.96–5.03	4.77, d $J_{1,2}$ 8.2	5.23, d $J_{1,2}$ 7.7	4.81, d $J_{1,2}$ 8.2	4.47, d $J_{1,2}$ 8.3	4.55–4.65	4.99, d $J_{1,2}$ 7.8
H-2^{I}	2.97–3.08	2.92–2.99	3.46, ddd, $J_{1,2}$ 8.2, $J_{2,3}$ 9.6, $J_{2,NH}$ 7.9	3.09, ddd, $J_{1,2}$ 7.7, $J_{2,3}$ 8.9, $J_{2,NH}$ 6.7	3.37–3.43	3.90–4.09		3.28–3.41
H-3^{I}	4.56–4.62	4.67–4.75	4.03, dd $J_{2,3}$ 9.6, $J_{3,4}$ 9.1	4.39, dd $J_{2,3}$ 8.9, $J_{3,4}$ 8.9	3.85–3.89	4.98 $J_{2,3}$ 10.4, $J_{3,4}$ 9.6	3.98–4.04	4.23–4.35
H-4^{I}	3.43, dd $J_{3,4}$ 9.0, $J_{4,5}$ 9.4	4.81, dd $J_{3,4}$ 9.5, $J_{4,5}$ 9.5	3.72–3.78	5.03, dd $J_{3,4}$ 8.9, $J_{4,5}$ 9.4	3.78, dd $J_{3,4}$ 9.2, $J_{4,5}$ 9.2	3.90–4.09	3.59–3.64	4.92, dd $J_{3,4}$ 9.1, $J_{4,5}$ 9.1
H-5^{I}	3.54, ddd, $J_{4,5}$ 9.4, $J_{5,6a}$ 6.0, $J_{5,6b}$ 1.8	3.69–3.74	3.52–3.57	3.81–3.85	3.44–3.48	3.37–3.45	3.49–3.52	3.63–3.69
H-6a^{I}	3.66, dd $J_{5,6a}$ 6.0, $J_{6a,6b}$ 10.8	3.53, dd $J_{5,6a}$ 5.8, $J_{6a,6b}$ 10.7	3.72–3.78	3.55, dd $J_{5,6a}$ 5.2, $J_{6a,6b}$ 10.8	3.85–3.89	3.84, dd $J_{5,6a}$ 1.0, $J_{6a,6b}$ 10.8	3.71, dd $J_{5,6a}$ 5.8, $J_{5,6b}$ 10.7	3.55, dd $J_{5,6a}$ 5.9, $J_{6a,6b}$ 10.6
H-6b^{I}	3.84, dd $J_{5,6b}$ 1.8, $J_{6a,6b}$ 10.8	3.56, dd $J_{5,6b}$ 2.6, $J_{6a,6b}$ 10.7	3.81, dd $J_{5,6b}$ 3.5, $J_{6a,6b}$ 10.4	3.59, dd $J_{5,6b}$ 2.8, $J_{6a,6b}$ 10.8	3.93, dd $J_{5,6b}$ 2.8, $J_{6a,6b}$ 10.9	3.90–4.09	3.80–3.84	3.59, dd $J_{5,6b}$ 3.4, $J_{6a,6b}$ 10.6
H-1^{II}	4.50, d $J_{1,2}$ 7.6	4.38, d $J_{1,2}$ 7.5	5.35, d $J_{1,2}$ 3.6	4.96, d $J_{1,2}$ 3.0	4.08, d $J_{1,2}$ 7.7,	3.90–4.09	4.55–4.65	4.42, d $J_{1,2}$ 7.6
H-2^{II}	3.94, dd $J_{1,2}$ 7.6, $J_{2,3}$ 9.5	3.81, dd $J_{1,2}$ 7.5, $J_{2,3}$ 9.4	3.99, dd $J_{1,2}$ 3.6, $J_{2,3}$ 10.2	3.90, dd $J_{1,2}$ 3.0, $J_{2,3}$ 9.5	3.72, dd $J_{1,2}$ 7.7, $J_{2,3}$ 9.1	3.56–3.62	3.73–3.78	3.63–3.69
H-3^{II}	3.81–3.84	3.74–3.77	4.20, dd $J_{2,3}$ 10.2, $J_{3,4}$ 3.4	4.08, dd $J_{2,3}$ 9.5, $J_{3,4}$ 3.7	3.52–3.59	3.37–3.45	3.87, dd $J_{2,3}$ 9.8, $J_{3,4}$ 2.6	3.81, dd $J_{2,3}$ 10.4, $J_{3,4}$ 2.7
H-4^{II}	5.41, br. d $J_{3,4}$ 2.6	5.32–5.37	5.44, br.s	5.47, br. d $J_{3,4}$ 1.6	5.24–5.29	5.19, br. d $J_{3,4}$ 3.3	5.38–5.42	5.37–5.41
H-5^{II}	3.87–3.91	3.74–3.77	4.28, dd $J_{5,6a}$ 6.8, $J_{5,6b}$ 6.8	4.34–4.37	3.52–3.59	3.37–3.45	3.80–3.84	3.71, dd $J_{5,6a}$ 6.9, J5,6b 6.9
H-6a^{II}	4.05–4.15	4.05–4.09	4.07–4.12	4.13–4.16	4.05–4.08	3.90–4.09	3.98–4.04	4.01–4.06
H-6b^{II}	4.05–4.15	4.05–4.09	4.07–4.12	4.18, dd $J_{5,6b}$ 5.0, $J_{6a,6b}$ 11.5	4.05–4.08	3.90–4.09	4.17, dd $J_{5,6b}$ 4.2, $J_{6a,6b}$ 11.5	4.01–4.06
H-1^{III}	5.67, d $J_{1,2}$ 3.3	5.62, d $J_{1,2}$ 1.6	5.37, d $J_{1,2}$ 3.3	5.31, d $J_{1,2}$ 3.8	5.50, d $J_{1,2}$ 3.6, H-1^{III}	5.45, d $J_{1,2}$ 3.6	5.55, br. s	5.55–5.58
H-2^{III}	5.25, dd $J_{1,2}$ 3.3, $J_{2,3}$ 11.0	5.32–5.37	5.31, dd $J_{1,2}$ 3.3, $J_{2,3}$ 10.8	5.38–5.41	5.37, dd $J_{1,2}$ 3.6, $J_{2,3}$ 10.9	5.37–5.41	5.12–5.22	5.27, dd $J_{1,2}$ 3.8, $J_{2,3}$ 11.0
H-3^{III}	5.33, dd $J_{2,3}$ 11.0, $J_{3,4}$ 3.0	5.32–5.37	5.35–5.37	5.27, dd $J_{2,3}$ 11.1, $J_{3,4}$ 3.2	5.24–5.29	5.23, dd $J_{2,3}$ 10.9, $J_{3,4}$ 3.3	5.12–5.22	5.08–5.14
H-4^{III}	5.26, br. d $J_{3,4}$ 3.0	5.25, br. s	5.27, br. d $J_{3,4}$ 3.4,	5.34, br. d $J_{3,4}$ 3.2	5.24–5.29	5.25, br. d $J_{3,4}$ 3.3,	5.24–5.29	5.24, br. d $J_{3,4}$ 2.3
H-5^{III}	4.56–4.62	4.67–4.75	4.38, q $J_{5,6}$ 6.5	4.13–4.16	4.42–4.48	4.48, q $J_{5,6}$ 6.5	4.55–4.65	4.63, q $J_{5,6}$ 6.4
H-6^{III} (3H)	1.19, d $J_{5,6}$ 6.5	1.20, d $J_{5,6}$ 6.5	1.14, d $J_{5,6}$ 6.5	1.15, d $J_{5,6}$ 6.5	1.11, d $J_{5,6}$ 6.5	1.14, d $J_{5,6}$ 6.5	1.25, d $J_{5,6}$ 6.4	1.24, d $J_{6,5}$ 6.4

H-1^{IV}	5.21, d $J_{1,2}$ 3.6	5.22, d $J_{1,2}$ 3.5	5.23, d $J_{1,2}$ 3.4	5.20, d $J_{1,2}$ 3.4	5.20, d $J_{1,2}$ 3.5	5.15, d $J_{1,2}$ 3.4	5.35, d $J_{1,2}$ 3.8	5.34, d $J_{1,2}$ 3.7
H-2^{IV}	4.51–4.54	4.44–4.48	4.50–4.55	4.46–4.50	4.42–4.48	4.43–4.47	5.24–5.29	5.29–5.33
H-3^{IV}	5.05, dd $J_{2,3}$ 11.3, $J_{3,4}$ 3.1	4.96–5.03	5.04, dd $J_{2,3}$ 11.2, $J_{3,4}$ 3.1	5.00, dd $J_{2,3}$ 11.4, $J_{3,4}$ 3.1	4.95, dd $J_{2,3}$ 11.4, $J_{3,4}$ 3.1	4.90, dd $J_{2,3}$ 11.7, $J_{3,4}$ 2.9	5.38–5.42	5.37–5.41
H-4^{IV}	5.42, br. s	5.40 , br. s	5.46 , br. s	5.38, br. d $J_{3,4}$ 3.1	5.42, br. s	5.37–5.41	5.57, br. s	5.55–5.58
H-5^{IV}	4.05–4.15	4.11–4.15	4.22–4.26	4.13–4.16	4.16–4.20	4.10–4.14	4.36–4.44	4.42–4.46
H-6a^{IV}	4.05–4.15	3.97–4.03	4.07–4.12	3.99–4.05	3.97–4.04	3.90–4.09	3.98–4.04	3.99, dd $J_{5,6a}$ 7.7, $J_{6a,6b}$ 11.1
H-6b^{IV}	4.05–4.15	4.05–4.09	4.13, dd $J_{5,6b}$ 5.3, $J_{6a,6b}$ 12.1	4.11, dd $J_{5,6b}$ 6.0, $J_{6a,6b}$ 11.7	4.10, dd $J_{5,6b}$ 4.2, $J_{6a,6b}$ 11.7	3.90–4.09	4.24–4.30	4.23–4.35
N*H*Ac^{I}	6.99, d $J_{2,NH}$ 5.0	6.86, d $J_{2,NH}$ 6.3	6.14, d $J_{2,NH}$ 8.0	6.44, d $J_{2,NH}$ 6.7	5.90, d $J_{2,NH}$ 7.1	5.71, d $J_{2,NH}$ 8.7	7.00–7.04	6.81–6.92
N*H*Ac^{IV}	6.02–6.14	6.31–6.45	5.98, d $J_{2,NH}$ 7.9	6.03, d $J_{2,NH}$ 8.8	6.37–6.44	6.52–6.62		
-$CH_2CH_2CH_2$-	1.84–1.94	1.83–1.94	1.75–1.82 1.85–1.91	1.78–1.90	1.80–1.86 1.87–1.93	1.79–1.86 1.87–1.94	1.80–1.85 1.86–1.91	1.79–1.91
-CH_2CH_2N-	3.47–3.51	3.44–3.54	3.34–3.40 3.52–3.57	3.36–3.43 3.44–3.50	3.37–3.43 3.52–3.59	3.25–3.32 3.63–3.70	3.33–3.40 3.53–3.58	3.41–3.50
-OCH_2CH_2-	3.72–3.77 3.87–3.91	3.69–3.74 3.88–3.93	3.58–3.63 3.89–3.94	3.64–3.70 3.91–3.95	3.59–3.64 3.97–4.04	3.56–3.62 3.90–4.09	3.65–3.69 3.92–3.96	3.63–3.69 3.91–3.97
-C(O)CH_3	1.97–2.18 (9s, 30H)	1.96–2.17 (11s, 33H)	1.93–2.16 (10s, 30H)	1.97–2.17 (9s, 33H)	2.00–2.16 (9s, 30H)	1.97–2.16 (8s, 33H)	1.92–2.17 (8s, 30H)	1.92–2.17 (10s, 33H)
-N*H*C(O)CF_3	7.42–7.49	7.24–7.28	7.31–7.34	7.27–7.38	7.35–7.42	7.30–7.35	7.69–7.87	7.45–7.56
PhCH_2-	4.56–4.62	4.49, d, *J* 12.1 4.55,d, *J* 12.1	4.57, d, *J* 11.8 4.62, d, *J* 11.8,	4.46, d, *J* 12.1 4.58, d, *J* 12.1	4.42–4.48 4.80, d, *J* 12.4	4.43, d, *J* 12.1 4.83, d, *J* 12.1	4.55–4.65	4.51, d, *J* 12.0 4.54, d, *J* 12.0
Ar*H* (5H)	7.27–7.36	7.28–7.36	7.28–7.30, 7.31–7.34	7.27–7.38	7.35–7.42	7.35–7.42	7.26–7.36	7.25–7.37
O*H*	3.81–3.84							

Table 3. ^{13}C NMR spectra (DEPT, δ(ppm)) of tetrasaccharides 21–24 and 21Ac–24Ac.

Carbon	**21**	**21Ac**	**22**	**22Ac**	**23**	**23Ac**	**24**	**24Ac**
C-1^{I}	99.8	99.3	100.7	99.2	100.6	101.8	101.1	100.0
C-2^{I}	57.6	59.1	56.3	58.6	57.9	54.0		57.3
C-3^{I}	81.2	75.7	81.8	77.0	71.6	72.6	71.0	71.7

C-4^{I}	69.7	69.8	72.3	69.9	77.8	72.8	70.0	70.6
C-5^{I}	75.2	73.3	74.2	72.7	74.2	74.8	75.1	70.4
C-6^{I}	69.3	69.1	69.8	68.7	67.5	67.3	69.6	69.5
C-1^{II}	101.8	101.6	98.7	96.8	100.6	99.9	102.5	102.9
C-2^{II}	72.3	72.7	73.5	75.7	74.4	74.3	70.9	73.4
C-3^{II}	77.2	70.5	73.1	74.1	79.3	70.2	72.4	73.1
C-4^{II}	65.6	66.9	67.2	68.3	67.3	68.0	64.0	64.1
C-5^{II}	70.9	70.5	66.9	68.2	70.7	70.2	71.1	70.4
C-6^{II}	61.3	60.7	61.5	62.3	61.1	60.9	62.3	60.8
C-1^{III}	95.4	95.8	96.5	98.0	96.5	96.4	96.6	97.2
C-2^{III}	67.6	67.3	67.3	66.6	66.9	66.5	67.8	67.7
C-3^{III}	67.7	68.0	67.7	67.8	67.8	68.0	67.2	67.5
C-4^{III}	70.8	71.5	71.1	71.0	71.2	71.4	70.9	70.9
C-5^{III}	65.4	65.6	66.2	65.7	65.1	65.0	66.3	66.2
C-6^{III}	15.5	15.6	15.9	16.0	15.8	15.6	16.2	16.1
C-1^{IV}	95.7	98.2	96.8	97.7	99.2	100.3	91.8	92.5
C-2^{IV}	47.6	48.1	48.2	48.4	48.3	48.3	67.2	67.1
C-3^{IV}	67.3	67.4	67.8	67.6	67.3	67.1	67.1	66.9
C-4^{IV}	67.1	67.4	67.5	67.3	67.4	67.5	67.7	67.8
C-5^{IV}	67.9	68.5	68.2	68.2	68.7	68.8	66.7	66.3
C-6^{IV}	62.2	62.8	62.3	62.2	62.9	63.2	61.2	61.1
-$CH_2C\mathit{H}_2CH_2$-	28.0	28.3	28.6	28.4	28.5	28.6	28.4	28.2
-$CH_2C\mathit{H}_2$N-	37.3	37.5	37.4	37.6	37.6	37.5	37.5	37.3
-O$C\mathit{H}_2CH_2$-	67.5	67.9	67.1	68.1	67.4	67.0		67.3
-OC(O)$C\mathit{H}_3$	20.0–20.6 (8C)	20.4–21.0 (9C)	20.5–20.8 (8C)	20.5–20.9 (9C)	20.4–21.0 (8C)	20.5–21.0 (9C)	20.1–21.1 (9C)	20.1–21.1 (10C)
-NC(O)$\mathit{C}H_3$	22.7, 22.9	23.1, 23.4	23.1, 23.5	23.2, 23.6	23.1, 23.6	23.1, 23.4	23.0	23.2
Ph$\mathit{C}H_2$-	73.3	73.6	73.6	73.5	73.5	73.8	73.6	73.5

C-Ar (5C)	127.4, 128. 5, 129.4	127.9, 127.9, 128.5	127.8, 128.4	127.9, 128.0, 128.5	128.1, 128.7, 128.8	128.3, 128.8, 128.9	127.4–128.6	127.7–128.5

Table 4. ^{1}H NMR spectra (δ(ppm) and J (Hz)) of pentasaccharides **26**–**30**.

Proton	**26**	**27**	**28**	**29**	**30**
H-1^{I}	4.60, d $J_{1,2}$ 5.9	4.78–4.81	4.88–4.99	4.90–4.94	5.24–5.26
H-2^{I}	3.94–3.97	3.40–3.48	3.21–3.27	3.84–3.90	3.30–3.40
H-3^{I}	4.13–4.17	4.36–4.42	4.52–4.57	4.04–4.11	4.27–4.35
H-4^{I}	4.13–4.17	3.94, dd $J_{3,4}$ 8.2, $J_{4,5}$ 8.2	3.68–3.72	4.04–4.11	3.84, dd $J_{3,4}$ 8.5, $J_{4,5}$ 8.5
H-5^{I}	3.97-4.01	3.68–3.73	3.62–3.67	3.84–3.90	3.66–3.69
H-6a^{I}	3.65, dd $J_{5,6a}$ 7.5, $J_{6a,6b}$ 10.0	3.62, dd $J_{5,6a}$ 2.3, $J_{6a,6b}$ 10.5	3.97, dd $J_{5,6a}$ 3.5, $J_{6a,6b}$ 12.2	3.68–3.72	3.94, dd $J_{5,6a}$ 4.1, $J_{6a,6b}$ 12.2
H-6b^{I}	3.72–3.79	3.88–3.92	4.52–4.57	3.84–3.90	4.57–4.61
H-1^{II}	4.70, d $J_{1,2}$ 8.8	4.68–4.74	4.64, d $J_{1,2}$ 7.8	4.63, d $J_{1,2}$ 7.6	4.57–4.61
H-2^{II}	3.72–3.79	3.73–3.77	3.72–3.75	3.56–3.60	3.57–3.61
H-3^{II}	3.81, dd $J_{2,3}$ 9.4, $J_{3,4}$ 3.0	3.77–3.84	3.81, dd $J_{2,3}$ 9.3, $J_{3,4}$ 3.1	3.84–3.90	3.87–3.92
H-4^{II}	5.33, br. d $J_{3,4}$ 3.0	5.34–5.38	5.41–5.47	5.40, br.s	5.44–5.48
H-5^{II}	3.66–3.69	3.77–3.84	3.75–3.78	3.76, dd $J_{5,6a}$ 5.7, $J_{5,6b}$ 8.0	3.75, dd $J_{5,6a}$ 8.0, $J_{5,6b}$ 6.0
H-6a^{II}	3.91, dd $J_{5,6a}$ 5.5, $J_{6a,6b}$ 11.1	3.97, dd $J_{5,6a}$ 5.6, $J_{6a,6b}$ 10.9	4.20–4.26	3.92, dd $J_{5,6a}$ 5.7, $J_{6a,6b}$ 10.8	4.18, dd $J_{5,6a}$ 8.0, $J_{6a,6b}$ 11.4
H-6b^{II}	4.03,dd $J_{5,6b}$ 8.7, $J_{6a,6b}$ 11.1	4.08–4.12	4.38, dd $J_{5,6b}$ 5.6, $J_{6a,6b}$ 11.2	4.04–4.11	4.27–4.35
H-1^{III}	4.52, d $J_{1,2}$ 9.0	5.09, d $J_{1,2}$ 3.7	5.02, d $J_{1,2}$ 3.4	5.14, d $J_{1,2}$ 3.7	5.06, d $J_{1,2}$ 3.5
H-2^{III}	3.72–3.79	4.15, dd J1,2 3.8, $J_{2,3}$ 10.1	5.25–5.31	4.13, dd $J_{1,2}$ 3.8, $J_{2,3}$ 10.0	5.23, dd $J_{1,2}$ 3.5, $J_{2,3}$ 11.0
H-3^{III}	3.49, dd $J_{2,3}$ 9.8, $J_{3,4}$ 2.1	3.88–3.92	5.21, dd $J_{2,3}$ 11.1, $J_{3,4}$ 3.1	3.84–3.90	5.19, dd $J_{2,3}$ 11.0, $J_{3,4}$ 3.2
H-4^{III}	3.60, br. s	3.68–3.73	5.34, br. d $J_{3,4}$ 3.1	3.68–3.72	5.29–5.32

H-5^{III}	3.38–3.44	4.52, q $J_{5,6}$ 6.4	4.88–4.99	4.33–4.39	4.92, q $J_{5,6}$ 6.5
H-6^{III} (3H)	1.17, d $J_{5,6}$ 6.3	1.21, d $J_{5,6}$ 6.4	1.16–1.20	1.16–1.18	1.11, d $J_{5,6}$ 6.5
H-1^{IV}	5.58, br. s	5.59, d $J_{1,2}$ 3.0	5.58, d $J_{1,2}$ 3.2	5.63, d $J_{1,2}$ 3.7	5.58–5.61
H-2^{IV}	5.25–5.29	5.34–5.38	5.41–5.47	5.16–5.20	5.29–5.32
H-3^{IV}	5.25–5.29	5.24–5.29	5.25–5.31	5.04–5.08	5.01–5.05
H-4^{IV}	5.32, br. s	5.24–5.29	5.25–5.31	5.23, d $J_{3,4}$ 2.7	5.24–5.26
H-5^{IV}	4.64–4.67	4.68–4.74	4.73, q $J_{5,6}$ 6.2	4.48–4.56	4.66, q $J_{5,6}$ 6.5
H-6^{IV} (3H)	1.11, d, $J_{5,6}$ 6.3	1.13, d $J_{5,6}$ 6.4	1.16–1.20	1.16–1.18	1.23, d $J_{5,6}$ 6.5
H-1^{V}	5.21, d $J_{1,2}$ 3.0	5.22, d $J_{1,2}$ 3.2	5.25–5.31	5.32–5.37	5.37, br. s
H-2^{V}	4.46–4.50	4.44–4.49	4.43–4.49	5.32–5.37	5.38–5.42
H-3^{V}	5.01, dd $J_{2,3}$ 11.3, $J_{3,4}$ 2.1	4.95–4.99	4.88–4.99	5.42–5.47	5.44–5.48
H-4^{V}	5.45, br. s	5.44, br. s	5.41–5.47	5.61, br. s	5.58–5.61
H-5^{V}	4.25, dd $J_{5,6a}$ 5.8, $J_{5,6b}$ 5.8	4.20–4.24	4.20–4.26	4.48–4.56	4.49–4.54
H-6a^{V}	4.06–4.11	4.00–4.05	4.00–4.03	3.99, dd $J_{5,6a}$ 8.3, $J_{6a,6b}$ 10.7	3.87–3.92
H-6b^{V}	4.06–4.11	4.08–4.12	4.04–4.08	4.25–4.30	4.27–4.35
N*H*Ac^{I}	6.25, d $J_{2,NH}$ 8.2	6.56–6.63	6.57, d $J_{2,NH}$ 6.7	6.71, d $J_{2,NH}$ 7.0	6.57, d $J_{2,NH}$ 6.8
N*H*Ac^{V}	6.18–6.24	6.20–6.45	6.24–6.52		
-$CH_2CH_2CH_2$-	1.80–1.91	1.81–1.88	1.83–1.93	1.84–1.89	1.84–1.89
-CH_2CH_2N-	3.38–3.44 3.51–3.55	3.32–3.38 3.40–3.48	3.42–3.52	3.31–3.39 3.49–3.55	3.30–3.40 3.48–3.55
-OCH_2CH_2-	3.51–3.55 3.86–3.89	3.56–3.60 3.77–3.84	3.62–3.67 3.84–3.88	3.49–3.55 3.92–3.95	3.61–3.64 3.87–3.92
-C(O)CH_3	1.77–2.14 (9s, 30H)	1.89–2.14 (8s, 30H)	1.97–2.21 (12s, 42H)	1.77–2.16 (10s, 30H)	1.91–2.17 (13s, 42H)
-N*H*C(O)CF_3	7.67–7.71	7.38–7.41	7.27–7.29	7.62–7.68	7.45–7.48
PhCH_2-	4.51–4.52 4.56, d, *J* 12.0	4.36–4.42 (2H) 4.66, d, *J* 11.4		4.43–4.48 (2H) 4.66, d, *J* 11.2	

	4.69, d, *J* 12.5 4.75–4.79 (3H) 4.88, d, *J* 11.2 4.98, d, *J* 11.6	4.68–4.74 4.78–4.81 4.83, d, *J* 11.7 4.87, d, *J* 11.3 5.00, d, *J* 11.7	4.71, d, *J* 11.2 4.78–4.82 (3H) 5.00, d, *J* 11.2
Ar*H* (20H)	7.22–7.40	7.24–7.37 7.41–7.44	7.25–7.42

Table 5. ^{13}C NMR spectra (DEPT, δ(ppm)) of pentasaccharides **26**–**30**.

Carbon	**26**	**27**	**28**	**29**	**30**
C-1^{I}	100.5	99.0	99.3	99.6	99.4
C-2^{I}	54.5	56.8	58.2	54.2	57.7
C-3^{I}	72.7	76.7	75.5	77.7	76.7
C-4^{I}	77.3	72.5	73.7	70.8	72.4
C-5^{I}	76.3	74.1	73.2	72.5	72.9
C-6^{I}	70.8	68.2	61.6	68.9	61.9
C-1^{II}	101.9	101.4	101.9	103.9	103.8
C-2^{II}	74.2	74.1	75.1	76.9	77.7
C-3^{II}	78.6	70.1	74.9	75.9	72.5
C-4^{II}	66.6	67.1	66.8	64.0	64.1
C-5^{II}	70.2	69.1	70.8	70.2	70.8
C-6^{II}	60.7	60.1	60.7	60.3	60.4
C-1^{III}	100.9	97.0	96.4	95.2	95.6
C-2^{III}	79.3	75.8	67.5	75.9	67.8
C-3^{III}	82.8	80.5	67.9	80.1	67.9
C-4^{III}	76.3	76.6	71.4	76.9	71.2
C-5^{III}	70.9	66.3	64.6	66.4	64.4
C-6^{III}	16.8	15.6	16.0	15.9	16.0

C-1^{IV}	95.9	96.2	96.7	97.2	98.0
C-2^{IV}	67.5	67.1	67.5	68.3	67.7
C-3^{IV}	67.9	68.0	67.9	67.4	67.2
C-4^{IV}	71.4	71.4	71.4	70.8	70.6
C-5^{IV}	65.4	65.5	65.6	65.8	66.5
C-6^{IV}	16.1	17.0	15.6	16.8	16.0
C-1^{V}	98.0	92.5	94.5	92.5	92.3
C-2^{V}	48.1	48.2	48.3	67.1	67.0
C-3^{V}	67.5	67.3	67.2	66.9	66.7
C-4^{V}	67.4	67.3	67.2	67.8	67.6
C-5^{V}	68.4	68.5	68.4	66.1	65.8
C-6^{V}	62.5	62.6	62.5	60.9	60.8
-$CH_2CH_2CH_2$-	28.3	28.2	28.4	28.3	28.3
-CH_2CH_2N-	37.2	37.2	37.2	37.2	37.2
-OCH_2CH_2-	66.9	66.6	67.3	66.6	67.4
-OC(O)CH_3	20.1–20.9 (8C)	20.4–20.9 (8C)	20.3–21.1 (12C)	20.1–21.0 (9C)	20.2–20.8 (13C)
-NC(O)CH_3	23.0 (2C)	23.0, 23.3	23.0, 23.5	23.0	23.3
PhCH_2-	72.8, 73.1, 74.9, 75.4	72.5, 73.1, 74.3, 74.6		72.7, 73.1, 74.2, 74.5	
C-Ar	127.3–128.6 (24C)	127.1–128.6 (24C)		127.1–128.6 (24C)	

4.4 Kodecyte and printed FSL-EIA biological assays

Kodecytes for serological analyses were prepared by incubating a 500 µL volume of 50 µMol FSL with 500 µL of packed group O Le(a-b-) human erythrocytes for 2 hours at 37° C.[7] Serology involved incubating 50 µL of a 3% kodecyte suspension with 50 µL of Lewis reagent or serum and observing for IgM induced agglutination after incubation at 5 minutes at RT, followed by centrifugation.

Inkjet printed paper-based microplates were prepared with the same FSL constructs to make kodecytes, as described. [7]

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- 3-Aminopropyl glycosides of ALe^b and BLe^b pentasaccharides were synthesized
- FSLs containing Lewis or ABH/Lewis oligosaccharides were obtained
- FSLs were used for glycomapping of serological Lewis reagents