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Design and Synthesis of a Multivalent Homing Device for Targeting to Murine CD22

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Abstract—CD22 is a cell-surface glycoprotein uniquely located on mature B-cells and B-cell derived tumour cells. Current evidence suggests that binding of endogenous ligands to CD22 leads to modulation of B-cell activation by antigen. Incidentally, however, B-cell activation may derail, and lead to an undesired immune response, for example in cases of allergy, rheumatoid arthritis and Crohn's disease. In this situation, synthetic high-affinity ligands for CD22 may be of therapeutic value as inhibitors of B-cell activation. Recent studies have revealed that natural ligands for CD22 contain the trisaccharide NeuAcα-2,6-Lac as the basic binding motif. In addition, it has been demonstrated that binding to CD22 is strongly enhanced by multivalent presentation of the basic binding motif (cluster effect). In this paper, the stepwise development of a novel multivalent high-affinity ligand for CD22 is described. In the first stage, a series of monovalent NeuAcα-2,6-Glc(Y)X type binding motifs was prepared, and their affinity for murine CD22 was monitored, to obtain more insight into the effect of separate structure elements on ligand recognition. In the second stage, we prepared a trivalent cluster, based on the monovalent motif that displayed the highest affinity for CD22, NeuAcα-2,6-GlcNBzNO$_2$OMe (**7**). This cluster, TRIS(NeuAcα-2,6-GlcNBzNO$_2$)$_3$ (**52**), displayed a more than 58-fold higher affinity for CD22 than the reference structure NeuAcα-2,6-LacOMe (**10**). To our knowledge, the cluster **52** is one of the most potent antagonists for CD22 yet synthesised.

Introduction

Sialoadhesins, or siglecs,[1] are a group of cell adhesion molecules within the immunoglobulin superfamily (IgSF) that bind to glycans with terminal sialic acid (Sia) residues. The siglec CD22 is a cell-surface glycoprotein uniquely located on mature B-cells and B-cell derived tumour cells.[2,3] Binding of endogenous ligands to CD22 triggers a cascade of intracellular events, leading to B-cell activation and proliferation.[4–6] Incidentally, however, B-cell activation may derail, and lead to an undesired immune response, for example in cases of allergy, rheumatoid arthritis and Crohn's disease. In this situation, synthetic high-affinity ligands for CD22 may be of therapeutic value as inhibitors of B-cell activation. The structural features that determine the affinity of (synthetic) ligands for human and/or murine CD22 have been the subject of a number of studies.[7–13] From these studies, it appeared that CD22 binds α-2,6-sialylated glycoproteins, whereas it does not recognise α-2,3-sialylated glycoproteins.[7,8] The basic monovalent binding motif in natural ligands for CD22 was identified as α-2,6-sialyl-*N*-acetyllactosamine (Siaα-2,6-LacNAc).[9] Unlike in the case of the selectins,[14] the presence of the Sia moiety, and in particular the C_7–C_8–C_9 glycerol side arm and the axial anomeric carboxyl function, is imperative for binding to CD22. The presence of a so-called cluster effect for CD22 was also suggested, as multiple sialylated structures were bound more avidly than monovalent sialosides.[9,10] While sharing the above mentioned binding characteristics, human and murine CD22 differ in their preference for the substituent at the C-5 position of Sia. Human CD22 binds equally well to

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N-acetyl (NeuAc) and *N*-glycoloyl (NeuGc) sialic acid-containing glycans, whereas murine CD22 prefers NeuGc to NeuAc conjugates.[12] In a recent report, Kelm et al.[13] investigated the binding of human and murine CD22 to erythrocytes which presented different *N*-modified sialyl glycans. Remarkably, replacement of the respective native *N*-acyl group on the erythrocyte Sia moiety by a halogenated acetyl, a formyl, or an aminoacetyl group did not affect binding by either human or murine CD22. In contrast, in the same study, binding to the siglec myelin associated glycoprotein (MAG) was shown to improve by a factor 17 by substitution of the *N*-acetyl by an *N*-fluoroacetyl group. The most extensive QSAR study for CD22 was reported by Powell et al.,[10] revealing that the minimal motif required for recognition by human CD22 is a

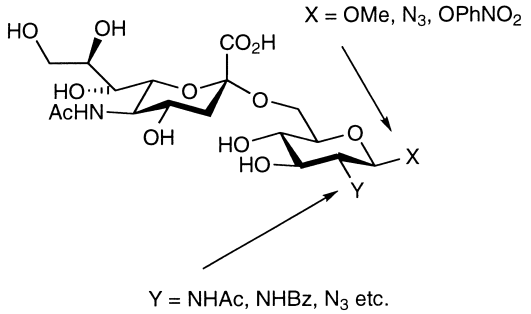
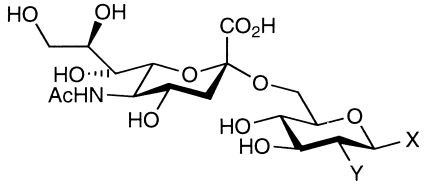


Figure 1. Possible variations in NeuAcα-2,6-Glc(Y)X type ligands for CD22.



1 X = OMe, Y = NHAc

2 X = OMe, Y = N_3

3 X = OMe, Y = NH_2

4 X = OMe, Y = NHBz

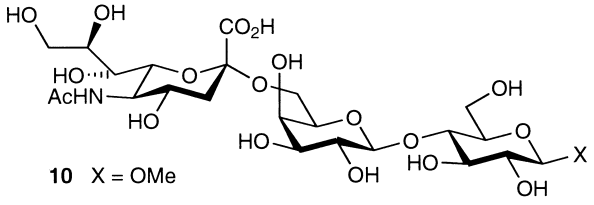
5 X = OMe, Y = NHOct

6 X = OMe, Y = NH(2-Napht)

7 X = OMe, Y = NH(4-NO_2)Bz

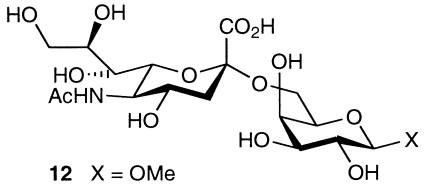
8 X = N_3, Y = NHAc

9 X = O(4-NO_2)Ph, Y = NHAc



10 X = OMe

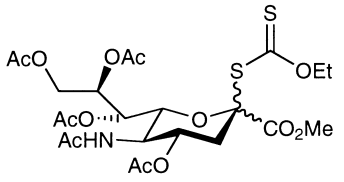
11 X = N_3



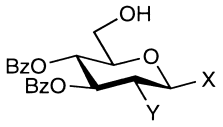
12 X = OMe

13 X = O(4-NO_2)Ph

Figure 2. Structures of monovalent ligands **1**–**13**.



14

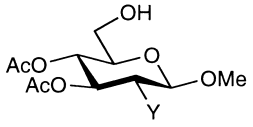


15 X = OMe, Y = NHAc

16 X = N_3, Y= NHAc

17 X = O(4-NO_2)Ph, Y = NHAc

18 X = OMe, Y = N_3

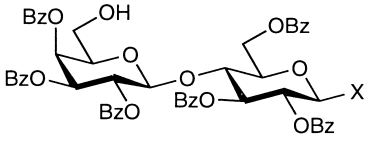


19 Y = NHBz

20 Y = NHOct

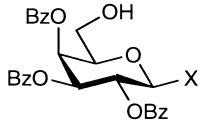
21 Y = NH(2-Napht)

22 Y = NH(4-NO_2)Bz



23 X = OMe

24 X = N_3



25 X = OMe

26 X = O(4-NO_2)Ph

Figure 3. Structures of sialoside donor **14** and acceptors **15**–**26**.

Table 1. Structures, yields and α/β ratios of protected di- and trisaccharides prepared from donor **14** and acceptors **15–26**

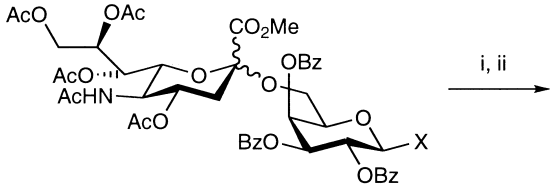
Acceptor	Product	X	Y	Yield[a] (α/β)[b]
15	**27**	OMe	NHAc	37% (5/1)
16	**28**	N_3	NHAc	41% (4/1)
17	**29**	O(4-NO_2)Ph	NHAc	28% (6/1)
18	**30**	OMe	N_3	50% (5/1)
19	**31**	—	NHBz	42% (3/1)
20	**32**	—	NHOct	30% (3/1)
21	**33**	—	NH(2-Napht)	25% (5/1)
22	**34**	—	NH(4-NO_2)Bz	44% (4/1)
23	**35**	OMe	—	26% (2/1)
24	**36**	N_3	—	33% (3/1)
25	**37**	OMe	—	47% (6/1)
26	**38**	O(4-NO_2)Ph	—	52% (4/1)

[a]α/β Mixture.
[b]As gauged by ^{13}C NMR analysis.

disaccharide NeuAcα-2,6-Hex(NAc), Hex(NAc) being Gal, GalNAc or GlcNAc. The most potent synthetic binding motifs in this study displayed a threefold higher affinity for CD22 than the reference structure NeuAcα-2,6-Lac.

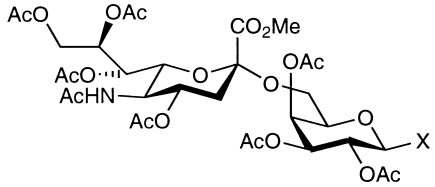
In summary, from the literature it appears that (a) the presence of a complete Sia ring structure is essential for binding to CD22, (b) the group at the C-5 position of the Sia moiety does not have a dramatic effect on binding, and (c) the affinity for CD22 can be enhanced by further optimisation of the (carbohydrate) structure attached to the reducing end of the Sia moiety.

We were interested in developing a novel synthetic high-affinity ligand for CD22. The molecular structure of this ligand should warrant maximal (selective) binding to CD22, but also be easily accessible from a synthetic point of view. These two requirements can be met by preparing a trivalent glycoconjugate, based on a TRIS dendritic core used by our group in earlier studies,[15] and a NeuAcα-2,6-Hex(NAc) derived minimal motif as proposed by Powell et al., in which (a) the terminal NeuAc moieties are intact and unmodified, and (b) glucosamine serves as the basic skeleton for the underlying carbohydrate. In search of this high-affinity ligand, a two-stage approach was pursued. In the first stage, a series of monovalent NeuAcα-2,6-Glc(Y)X type binding motifs (Fig. 1) was prepared, and their affinity for murine CD22 was monitored, to obtain insight into the effect of modifications at both the C-1 position (X = methoxy, azido, nitrophenoxy) and at the C-2 position (e.g., Y = acetamido, benzoylamido, azido) of the glucosamine. In the second stage, a trivalent cluster, based on the monovalent motif that displayed the highest affinity for murine CD22, was prepared and tested, to establish whether the affinity of a synthetic oligosaccharide could be further enhanced by multivalent presentation.



37 X = OMe

38 X = O(4-NO_2)Ph



39 X = OMe

40 X = O(4-NO_2)Ph

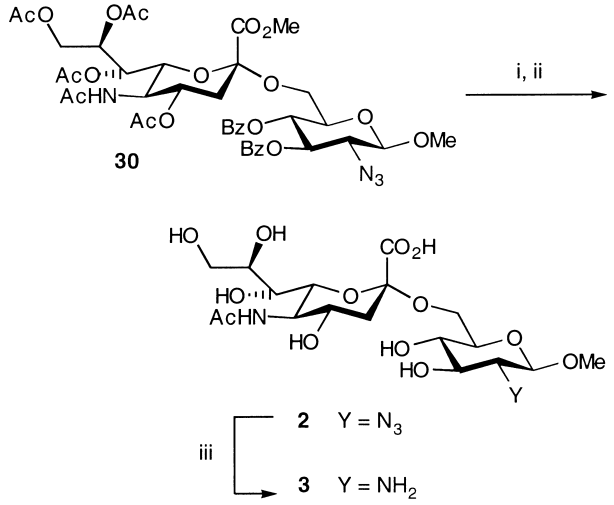
Scheme 1. Protecting group exchange of NeuAcα-2,6-GalX type disaccharides. Reagents: (i) NaOMe; (ii) Ac_2O.

Results and Discussion

For the first phase of our study, nine NeuAcα-2,6-Glc(Y)X type ligands were prepared (**1**–**9**, see Fig. 2), together with, as a frame of refererence, two NeuAcα-2,6-LacX (**10** and **11**) and two NeuAcα-2,6-GalX (**12** and **13**) type ligands.

The synthesis of the di- and trisaccharides **1**–**13** involved sialylation of the partially protected acceptors **15**–**26** with the sialyl xanthate **14**[16] (Fig. 3) in combination with *N*-iodosuccinimide/triflic acid (NIS/TfOH)[17,18] as promoter system. The results of the glycosylation reactions are depicted in Table 1.

The α-isomers of the di- and trisaccharides **27**–**36** were identified by the characteristic ^{1}H NMR chemical shifts of the H-3eq [δ(α-isomer) > δ(β-isomer)] and H-4 (δ±4.8 ppm) of the NeuAc moieties, and were separated from the β-isomers by silica gel chromatography. For the galactose derived disaccharides **37** and **38**, separation of

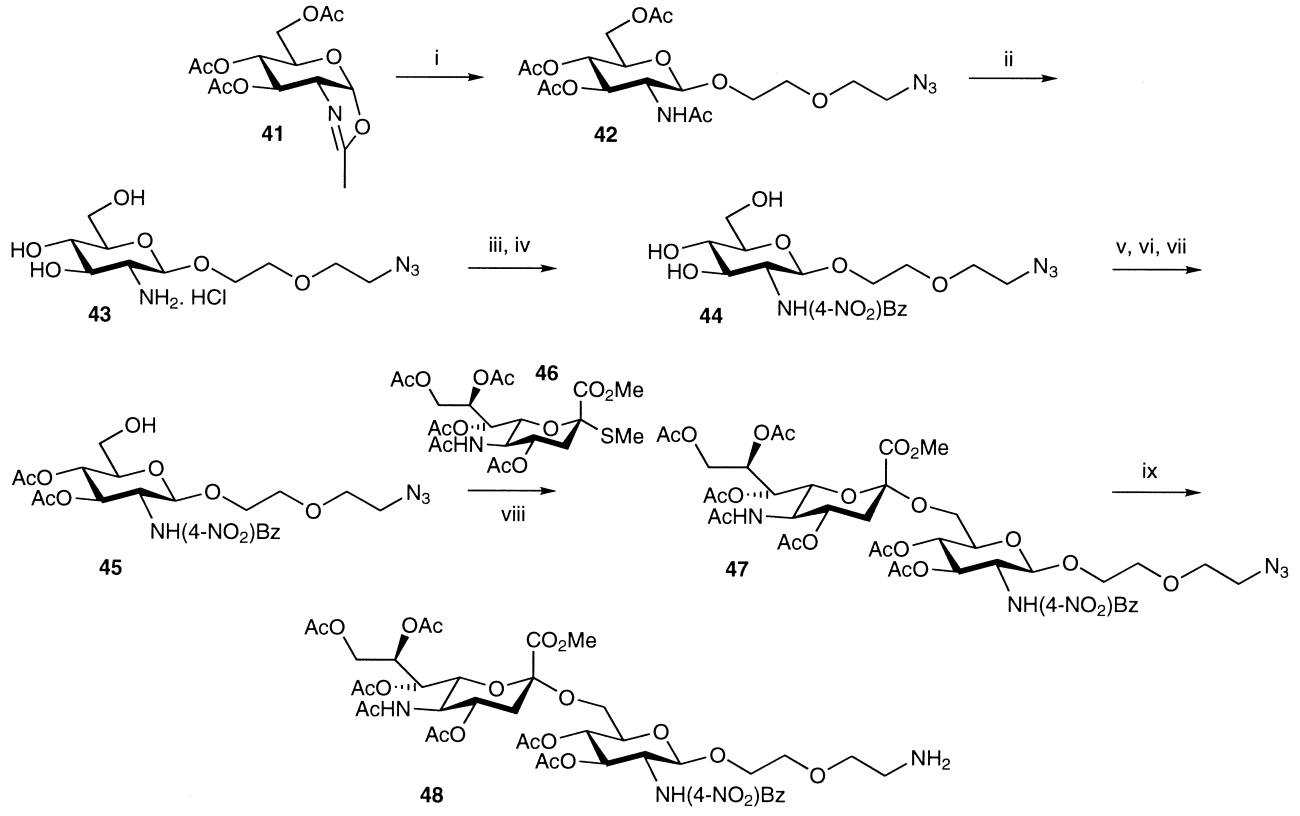


Scheme 2. Synthesis of 2-amino disaccharide **3**. Reagents: (i) NaOMe; (ii) NaOH; (iii) 1,3-propanedithiol.

Table 2. Affinity data of oligosaccharides **1**–**13**[a]

Compound		Relative IC_{50}
1	NeuAcα-2,6-GlcNAcOMe	6.9
2	NeuAcα-2,6-GlcN_3OMe	1.2
3	NeuAcα-2,6-GlcNH_2OMe	2.0
4	NeuAcα-2,6-GlcNHBzOMe	2.2
5	NeuAcα-2,6-GlcNHOctOMe	1.6
6	NeuAcα-2,6-GlcNHNaphtOMe	1.8
7	NeuAcα-2,6-GlcNHBzNO_2OMe	9.2
8	NeuAcα-2,6-GlcNAcN_3	2.1
9	NeuAcα-2,6-GlcNAcOPhNO_2	7.9
10	NeuAcα-2,6-LacOMe	1.0
11	NeuAcα-2,6-LacN_3	2.2
12	NeuAcα-2,6-GalOMe	2.4
13	NeuAcα-2,6-GalOPhNO_2	3.6

[a]The relative IC_{50} was calculated by dividing the average IC_{50} found for compound **10** by the average IC_{50} of the respective compound. A relative $IC_{50} > 1$ therefore represents a better binding to CD22 than the reference compound **10**.



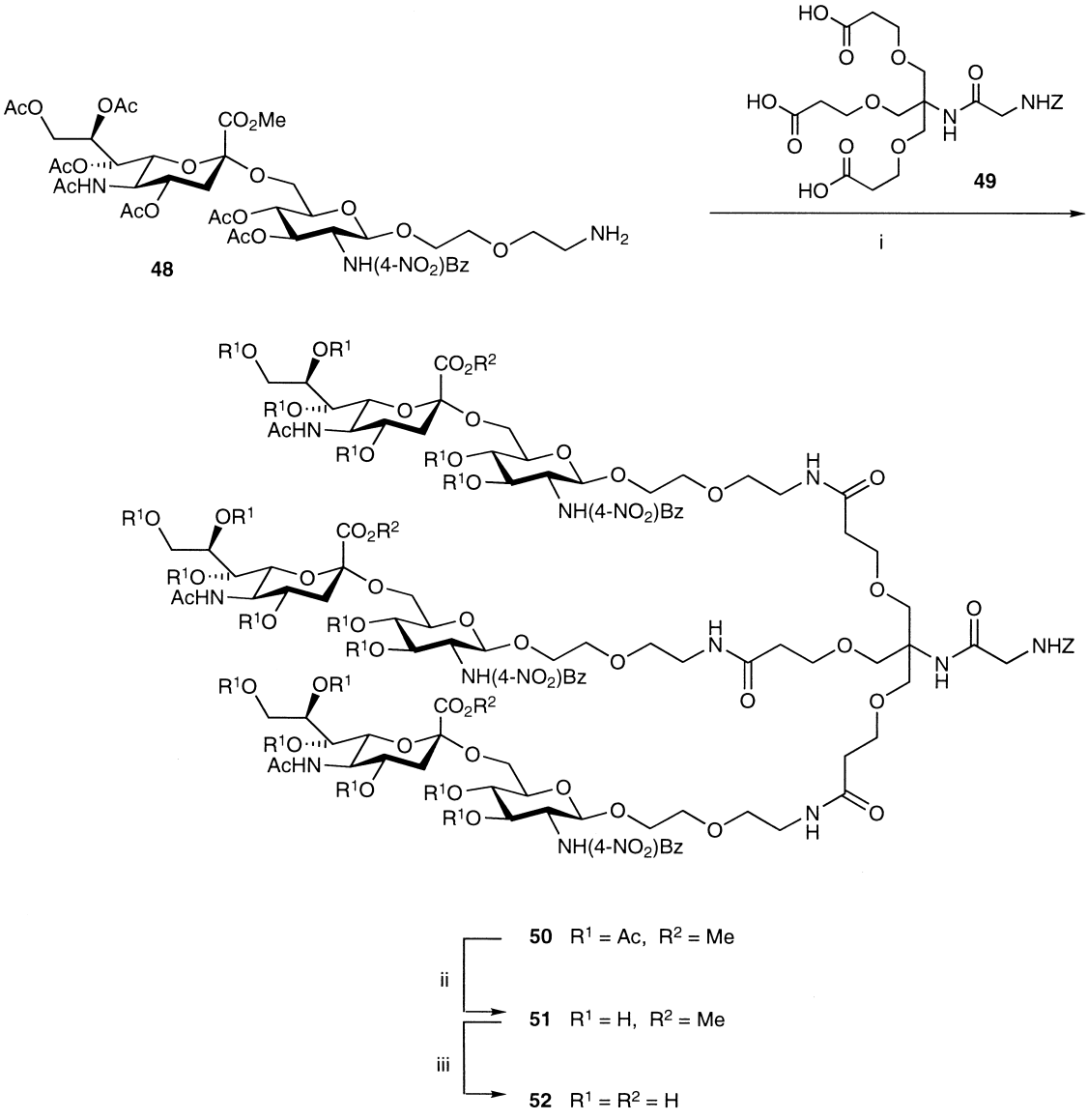
Scheme 3. Synthesis of disaccharide fit for coupling to TRIS dendritic core. Reagents: (i) $HOCH_2CH_2OCH_2CH_2N_3$, TfOH; (ii) 1 M NaOH, reflux; (iii) excess 4-NO_2BzCl; (iv) NaOMe; (v) tBDMS-Cl; (vi) Ac_2O; (vii) *p*-TsOH; (viii) NIS, TfOH; (ix) $P(Ph)_3$, H_2O.

the respective α- and β-isomers could only be accomplished after replacing the benzoyl groups on the galactose ring by acetyl groups (see Scheme 1). Having the fully protected α-isomers of **27**–**36**, **39** and **40** in hand, two-step basic hydrolysis of the protecting groups then gave the respective target ligands **1**–**2** and **4**–**13**.

Finally, the 2-amino ligand **3** was prepared from the deprotected 2-azido ligand **2** by mild reduction with 1,3-propanedithiol[19] (see Scheme 2).

The monovalent ligands **1**–**13** were tested on their affinity for CD22 in an in vitro competition assay based on the binding of porcine erythrocytes to immobilised murine CD22D1-3-Ig.[20,21] The NeuAcα-2,6-LacOMe trisaccharide **10**, which was selected as the reference compound, displayed an average IC_{50} of 244 μM. This compares well with the value reported by Powell et al.[10] for binding of NeuAcα-2,6-Lac to human CD22, taking into account that the affinity of NeuAc glycoconjugates is slightly lower for murine CD22 than for human CD22.[12] The affinity of **10** was defined as 1 and the IC_{50} values of all other ligands were expressed relative to that of **10** (see Table 2).

From Table 2 it can be seen that, in line with the results of Powell et al.,[10] the glucosamine derived dimers **1**–**9** all have a comparable or higher affinity for CD22 than the trisaccharide **10**. Remarkably, substitution of an anomeric methoxy by an azido group appears to have a negative effect (**1** versus **8**, respectively) at the disaccharide level, but a beneficial effect at the trisaccharide level (**10** versus **11**, respectively). An anomeric nitrophenoxy instead of a methoxy group has a positive effect on binding, both in the case of glucosamine- (**9** versus **1**, respectively) and galactose-derived (**13** versus **12**, respectively) disaccharides. Focusing on the C-2 position of the glucoside in the NeuAcα-2,6-Glc(Y)X template, substitution of the acetamide in **1** by an azide or an amine impairs binding to CD22 (**2** and **3** versus **1**, respectively). With



Scheme 4. Synthesis of the cluster based on **7**. Reagents: (i) HBTU, HOBT; (ii) NaOMe; (iii) NaOH.

respect to **3**, it should be noted that Kelm et al.[13] also reported that the presence of a free amine reduced the affinity for CD22, as well as for the other siglecs sialoadhesin (Sn) and MAG. By contrast, azido and amino groups at the C-2 position of the glucose ring of sialyl-LewisX were found[22] to enforce the binding to E-selectin by a factor of 4–6. Introduction of a more lipophilic moiety at the C-2 position of a disaccharide by replacing the *N*-acetyl in **1** by either an aromatic (benzoyl, naphthoyl) or aliphatic (octanoyl) group negatively affects binding to CD22 (**4**, **5** and **6** versus **1**, respectively). The latter result may look rather unexpected in view of recent reports in which lipophilic groups confer stronger binding in many different ligand–receptor systems.[23–29] On the other hand, addition of a single nitro group to the aromatic benzoyl ring leads to a dramatic increase in affinity for CD22 (**7** versus **1**, **7** versus **4**). The nitrobenzoyl disaccharide **7** displayed an almost 10-fold higher affinity than the reference **10**, establishing **7** as the most potent ligand of all the monovalent carbohydrates tested in this study. The disaccharide **7** was therefore selected as the basic motif for the multivalent ligand to be prepared in the second phase of our study.

The synthesis of the multivalent ligand based on **7** started with the introduction of a masked diethylene glycol amino spacer moiety at the anomeric position of the glucosamine ring. This spacer should allow smooth attachment of the disaccharide to the TRIS dendritic core, and warrant an optimal resemblance of the resulting cluster to the native multivalent ligand structure, in terms of spacing of the terminal sialic acid residues.

To this end, the azide **42** was prepared by the addition of 2-(2-azidoethoxy)ethanol to the known[30] oxazoline **41** of glucosamine (see Scheme 3). Conversion into the nitrobenzoyl derivative **45** proceeded via a sequence of straightforward protection and deprotection steps. Sialylation of **45** with the methyl thiosialoside **46**[31] and subsequent Staudinger[32] reduction of the azido group of the dimer **47** afforded the amine **48**, fit for coupling to the TRIS dendritic core.

Condensation of TRIS-derived tricarboxylate **49**[15] with **48** (Scheme 4), under standard peptide coupling conditions, furnished the protected cluster **50** in an excellent yield. Finally, mild two-step removal of the protective ester groups afforded the desired sialoside **52**.

It was found that the multivalent sialoside **52** displayed a 6.2-fold higher affinity for CD22 than **7** (see Table 3). Compared to our initial NeuAcα-2,6-LacOMe reference structure **10**, a 58.1-fold improvement in binding was observed. It cannot be excluded that the presence of the lipophilic benzyloxycarbonyl group in **52** contributes to the binding of the TRIS cluster. However, the results found for the lipophilic monovalent ligands **19** and **21** suggest that the effect of a single phenyl ring on binding will not be very significant. Therefore, we assume that the strong gain in affinity found for the multivalent sialoside **52** is mainly due to the cluster effect.

Table 3. Affinity data of oligosaccharides **10**, **7** and the cluster **52**[a]

Compound		Relative IC_{50}
10	NeuAcα-2,6-LacOMe	1.0
7	NeuAcα-2,6-GlcNHBzNO_2OMe	9.2
52	TRIS(NeuAcα-2,6-GlcNHBzNO_2)$_3$	58.1

[a]The relative IC_{50} was calculated by dividing the average IC_{50} found for compound **10** by the average IC_{50} of the respective compound. A relative $IC_{50} > 1$ therefore represents a better binding to CD22 than the reference compound **10**.

Conclusion

We have prepared 13 sialoside oligosaccharides that carry different structural features on the hexose moieties at the reducing terminus, and tested these oligosaccharides on their ability to bind to CD22. The main result of these tests is that the presence of 4-nitroaryl groups at either the C-1 or C-2 position of the reducing hexose enhances binding, while the presence of either lipophilic or positively charged groups at C-2 does not. The most potent disaccharide, the nitrobenzoyl derivative **7**, displays an almost 10-fold higher affinity for CD22 than the reference structure, NeuAcα-2,6-LacOMe. Based on disaccharide **7**, a trivalent cluster (**52**) was prepared. This trivalent sialoside **52** displayed a 6.3-fold higher affinity for CD22 than disaccharide **7**, thereby providing strong evidence that ligand valency is indeed an important determinant for high-affinity binding to CD22.[9,10] To our knowledge, the cluster **52** is one of the most potent ligands for CD22 yet synthesised, and may find application as (ant)agonist or homing device for CD22-expressing B-cells.

Experimental

General

All solvents were of analytical grade. Dry solvents were stored over 4 Å molecular sieves. Merck Kieselgel 60 F_{254} DC Alufolien was used for TLC analysis. Carbohydrate compounds were visualised by charring with sulfuric acid:ethanol (1:4, v/v). Compounds containing NH functions were visualised by charring with a solution of 4,4′-tetramethyldiamino-diphenylmethane (TDM)[33] after treatment of the TLC plates with chlorine, or visualised by charring with a 0.3% solution of ninhydrine in acetic acid:*n*-butanol (3:100, v/v). Column chromatography was performed with Kieselgel 60, 230–400 mesh (Merck). Gel filtration was performed with Sephadex LH-20 (Pharmacia). ^{1}H NMR spectra (200 MHz) and ^{13}C{^{1}H} NMR spectra (50.1 MHz) were recorded with a Jeol JNM-FX200 spectrometer. ^{1}H NMR spectra (300 MHz) were recorded with a Bruker WM-300 spectrometer. Matrix Assisted Laser Desorption Ionisation (MALDI) Time-of-Flight (TOF) mass spectrometry[34] was performed with a Perkin Elmer/PerSeptive Biosystems Voyager-DE-RP MALDI-TOF mass spectrometer equipped with delayed extraction.[35] Exact masses were obtained by nano–ESI–TOF–MS on a Micromass LCT with a resolution of 5000 FWHM using pentaphenylalanine as reference mass. Murine

CD22/IgG was purified from a stable transfected CHO cell line (kindly provided by Dr. P. Crocker, Dundee, Scotland). Cells were grown in GMEM (First link UK) containing 5% IgG-poor FCS (Gibco), 20 U pen/strep and 400 μM MSX (Sigma). Production of CD22 was induced by addition of 2 mM sodium butyrate (Sigma), and medium was collected for 3 weeks. Murine CD22 was routineously purified from the pooled media by protein-A Sepharose chromatography (yield±200 μg/mL). Purity was routinely checked by PAGE analysis.

Methyl 2-acetamido-3,4-di-*O*-benzoyl-2-deoxy-6-*O*-(methyl 4,7,8,9-tetra-*O*-acetyl-*N*-acetyl-α-D-neuraminate-2-yl)-β-D-glucopyranoside (27). A mixture of acceptor **15** (145 mg; 327 μmol), donor **14** (130 mg; 218 μmol)[16] and powdered molecular sieves (3 Å) in acetonitrile (3 mL) was stirred for 1 h under argon atmosphere. The temperature of the mixture was lowered to −50 °C, and a solution of NIS (74 mg; 0.33 mmol) and TfOH (3 μL; 0.03 mmol) in acetonitrile (1 mL) was added. The mixture was gradually warmed to room temperature over a period of 3 h. The reaction was quenched by the addition of triethylamine (0.1 mL). The mixture was filtered over Hyflo Super Cel (Fluka), diluted with ethyl acetate (50 mL), washed with aq $Na_2S_2O_3$ (1 M; 50 mL), aq $NaHCO_3$ (1 M; 50 mL) and water (50 mL), dried ($MgSO_4$), concentrated, and applied to a silica gel column. Elution was performed with DCM:methanol (1:0→96:4, v/v). The fractions containing the product were pooled, concentrated, and applied to a Sephadex LH-20 column. The disaccharide **27** was obtained as α/β mixture. Yield: 73 mg (80 μmol, 37%, α/β 5:1). The α-isomer was isolated from the latter mixture by silica gel chromatography, with toluene:ethanol (1:0→8:2, v/v) as eluent, in a yield of 45 mg (49 μmol). $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 171.1–128.3 (C=O acetyl, benzoyl, C-1′), 133.2–128.3 (CH_{arom} benzoyl), 128.9 (C_q benzoyl), 101.9 (C-1), 99.2 (C-2′), 73.4, 72.3, 71.8, 70.0, 68.8, 67.8, 67.1 (C-3,4,5, C-4′,6′,7′,8′), 64.6, 62.8 (C-6, C-9′), 56.5 (OCH_3), 52.6 (OCH_3 ester), 52.2 (C-2), 48.9 (C-5′), 37.3 (C-3′), 23.1 (*N*-acetyl), 20.9, 20.6 (CH_3 *O*-acetyl). β-Isomer δ 98.0 (C-2′). ^1H NMR ($CDCl_3$): δ 8.01–7.33 (m, 10H, CH_{arom}), 6.19 (d, 1H, NH acetyl, J_{NH-2} = 9.3 Hz), 5.62 (dd, 1H, H-3, J_{3-4} = 9.5 Hz), 5.55 (dd, 1H, H-4, J_{4-5} = 9.6 Hz), 5.33 (ddd, 1H, H-8′, $J_{8'-9'a}$ = 2.6 Hz, $J_{8'-9'b}$ = 6.6 Hz), 5.24 (dd, 1H, H-7′, $J_{6'-7'}$ = 1.2 Hz, $J_{7'-8'}$ = 10.0 Hz), 4.73 (ddd, 1H, H-4′, $J_{4'-5'}$ = 9.9 Hz), 4.65 (d, 1H, H-1, J_{1-2} = 8.4 Hz), 4.29 (dd, 1H, H9′a, $J_{9'a-9'b}$ = 12.3 Hz), 4.23 (ddd, 1H, H-2, J_{2-3} = 10.1 Hz), 4.05–3.95 (m, 3H, H-5′,6′,9′b), 3.86–3.71 (m, 3H, H-5,6a,6b), 3.74 (s, 3H, OCH_3 ester), 3.54 (s, 3H, OCH_3), 2.39 (dd, 1H, H-3′eq, $J_{3'eq-4'}$ = 4.6 Hz, $J_{3'eq-3'ax}$ = 12.7 Hz), 2.11, 2.03, 1.96, 1.86, 1.85 (5×s, 18H, CH_3 acetyl), 1.55 (dd, 1H, H-3′ax, $J_{3'ax-4'}$ = 12.3 Hz).

2-Acetamido-3,4-di-*O*-benzoyl-2-deoxy-6-*O*-(methyl 4,7,8,9-tetra-*O*-acetyl-*N*-acetyl-α-D-neuraminate-2-yl)-β-D-glucopyranosyl azide (28). Compound **28** was prepared from acceptor **16** (149 mg; 327 μmol) and donor **14** (130 mg; 218 μmol) as described above for the synthesis of **27**. Yield: 83 mg (89 μmol, 41%, α/β 4:1). Isolated yield of α-isomer: 54 mg (58 μmol). $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 171.5–164.6 (C=O acetyl, benzoyl, C-1′), 133.3–128.3 (CH_{arom} benzoyl), 129.3–128.7 (C_q benzoyl), 99.5 (C-2′), 88.9 (C-1), 73.6, 73.1, 72.5, 69.9. 68.6, 67.6, 67.3 (C-3,4,5, C-4′,6′,7′,8′), 65.0, 63.2 (C-6, C-9′), 53.7 (C-2), 52.7 (OCH_3 ester), 48.7 (C-5′), 37.2 (C-3′), 22.9 (*N*-acetyl), 20.9, 20.8, 20.6 (CH_3 *O*-acetyl). β-Isomer δ 98.0 (C-2′). ^1H NMR ($CDCl_3$): δ 8.05–7.33 (m, 10H, CH_{arom}), 6.52 (d, 1H, NH acetyl, J_{NH-2} = 9.8 Hz), 5.65 (dd, 1H, H-3, J_{3-4} = 9.6 Hz), 5.57 (dd, 1H, H-4, J_{4-5} = 9.5 Hz), 5.37 (ddd, 1H, H-8′, $J_{8'-9'a}$ = 2.7 Hz, $J_{8'-9'b}$ 7.1 Hz), 5.23 (dd, 1H, H-7′, $J_{6'-7'}$ = 1.2 Hz, $J_{7'-8'}$ = 9.5 Hz), 4.90 (d, 1H, H-1, J_{1-2} = 9.3 Hz), 4.72 (ddd, 1H, H-4′, $J_{4'-5'}$ = 10.0 Hz), 4.35 (dd, 1H, H-9′a, $J_{9'a-9'b}$ = 12.2 Hz), 4.30 (ddd, 1 = H, H-2, J_{2-3} = 9.9 Hz), 4.19 (m, 3H, H-5′,6′,9′b), 3.82 (dd, 1H, H-6a, J_{5-6a} = 7.4 Hz, J_{6a-6b} = 12.2 Hz), 3.78–3.70 (m, 2H, H-5,6b), 3.75 (s, 3H, OCH_3 ester), 2.33 (dd, 1H, H-3′eq, $J_{3'eq-4'}$ = 4.7 Hz, $J_{3'eq-3'ax}$ = 12.8 Hz), 2.16, 2.14, 2.04, 1.95, 1.88, 1.86 (6×s, 18H, CH_3 acetyl), 1.42 (dd, 1H, H-3′ax, $J_{3'ax-4'}$ = 12.3 Hz).

4-Nitrophenyl 2-acetamido-3,4-di-*O*-benzoyl-2-deoxy-6-*O*-(methyl 4,7,8,9-tetra-*O*-acetyl-*N*-acetyl-α-D-neuraminate-2-yl)-β-D-glucopyranoside (29). Compound **29** was prepared from acceptor **17** (180 mg; 327 μmol) and donor **14** (130 mg; 218 μmol) as described above for the synthesis of **27**. Yield: 61 mg (60 μmol, 28%, α/β 6:1). Isolated yield of α-isomer: 23 mg (22 μmol). $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 171.9–164.9 (C=O acetyl, benzoyl, C-1′), 161.6, 142.6 (C_q phenyl), 133.6–128.0 (CH_{arom} benzoyl), 125.6, 116.7 (CH_{arom} phenyl), 99.9 (C-2′), 98.5 (C-1), 72.5, 71.7, 70.3, 68.5, 67.2 (C-3,4,5, C-4′,6′,7′,8′), 65.5, 63.7 (C-6, C-9′), 54.2 (C-2), 52.8 (OCH_3 ester), 48.7 (C-5′), 37.0 (C-3′), 23.2 (*N*-acetyl), 20.6 (CH_3 *O*-acetyl). β-Isomer δ 98.1 (C-2′). ^1H NMR ($CDCl_3$): δ 8.21–7.29 (m, 14H, CH_{arom}), 6.56 (d, 1H, NH acetyl, J_{NH-2} = 9.4 Hz), 5.81 (dd, 1H, H-3, J_{3-4} = 9.7 Hz), 5.61 (dd, 1H, H-4, J_{4-5} = 9.6 Hz), 5.56 (ddd, 1H, H-8′, $J_{8'-9'a}$ = 2.8 Hz, $J_{8'-9'b}$ = 8.0 Hz), 5.50 (d, 1H, H-1, J_{1-2} = 8.2 Hz), 5.23 (dd, 1H, H-7′, $J_{7'-8'}$ = 9.8 Hz), 5.16 (d, 1H, NH acetyl, $J_{NH-5'}$ = 9.6 Hz), 4.68 (ddd, 1H, H-4′, $J_{4'-5'}$ = 9.3 Hz), 4.66 (ddd, 1H, H-2, J_{2-3} = 10.3 Hz), 4.46 (dd, 1H, H-9′a, $J_{9'a-9'b}$ = 12.1 Hz), 4.33 (ddd, 1H, H-5, J_{5-6a} = 6.7 Hz, J_{5-6b} = 5.0 Hz), 4.10 (ddd, 1H, H-5′, $J_{5'-6'}$ = 10.6 Hz), 4.03 (dd, 1H, H-6′, $J_{6'-7'}$ = 1.7 Hz), 3.90–3.82 (m, 2H, H-6a, H-9′b), 3.71 (s, 3H, OCH_3 ester), 3.68 (dd, 1H, H-6b, J_{6a-6b} = 13.0 Hz), 2.26 (dd, 1H, H-3′eq, $J_{3'eq-4'}$ = 4.7 Hz, $J_{3'eq-3'ax}$ = 12.9 Hz), 2.20, 2.19, 1.94, 1.88, 1.86 (5×s, 18H, CH_3 acetyl), 1.29 (dd, 1H, H-3′ax, $J_{3'ax-4'}$ = 12.7 Hz).

Methyl 2-azido-3,4-di-*O*-benzoyl-2-deoxy-6-*O*-(methyl 4,7,8,9-tetra-*O*-acetyl-*N*-acetyl-α-D-neuraminate-2-yl)-β-D-glucopyranoside (30). Compound **30** was prepared from acceptor **18** (128 mg; 300 μmol) and donor **14** (119 mg; 200 μmol) as described above for the synthesis of **27**. Yield: 90 mg (100 μmol, 50%, α/β 5:1). Isolated yield of α-isomer: 51 mg (56 μmol). $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 170.7–164.8 (C=O acetyl, benzoyl, C-1′), 133.1–128.2 (CH_{arom} benzoyl), 129.2, 129.0 (C_q benzoyl), 102.9 (C-1), 98.6 (C-2′), 73.0, 72.5, 72.4, 69.2, 69.0, 68.4, 67.1, 64.1 (C-2,3,4,5, C-4′,6′,7′,8′), 63.4, 62.1 (C-6, C-9′), 57.1 (OCH_3), 52.4 (OCH_3 ester), 49.1 (C-5′), 37.4 (C-3′), 23.0 (*N*-acetyl), 20.6 (CH_3 *O*-acetyl). β-Isomer δ 98.1 (C-2′). ^1H NMR ($CDCl_3$): δ 8.04–7.29 (m, 10H, CH_{arom}), 5.51

(dd, 1H, H-4, $J_{4-5}=9.6$ Hz), 5.40 (dd, 1H, H-3, $J_{3-4}=9.6$ Hz), 5.24 (dd, 1H, H-7′, $J_{7'-8'}=8.6$ Hz), 5.19 (ddd, 1H, H-8′, $J_{8'-9'a}=2.5$ Hz, $J_{8'-9'b}=5.0$ Hz), 4.79 (ddd, 1H, H-4′, $J_{4'-5'}=9.8$ Hz), 4.45 (d, 1H, H-1, $J_{1-2}=8.0$ Hz), 4.10 (dd, 1H, H-9′a, $J_{9'a-9'b}=12.5$ Hz), 3.99 (dd, 1H, H-6′, $J_{5'-6'}=6.0$ Hz, $J_{6'-7'}=1.4$ Hz), 3.97–3.94 (m, 2H, H-5′, H-6a), 3.86–3.82 (m, 1H, H-5), 3.80 (dd, 1H, H-9′b), 3.65 (dd, 1H, H-2, $J_{2-3}=10.1$ Hz), 3.61 (dd, 1H, H-6b, $J_{5-6b}=3.0$ Hz, $J_{6a-6b}=12.1$ Hz), 3.74, 3.63 (2×s, 6H, OC$\underline{H}_3$), 2.49 (dd, 1H, H-3′eq, $J_{3'eq-4'}=4.7$ Hz, $J_{3'eq-3'ax}=12.6$ Hz), 2.08, 2.02, 2.00, 1.99, 1.83 (5×s, 15H, C$\underline{H}_3$ acetyl), 1.78 (dd, 1H, H-3′ax, $J_{3'ax-4'}=12.5$ Hz).

Methyl 2-benzoylamido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-(methyl 4,7,8,9-tetra-*O*-acetyl-*N*-acetyl-α-D-neuraminate-2-yl)-β-D-glucopyranoside (31). Compound **31** was prepared from acceptor **19** (164 mg; 430 μmol) and donor **14** (170 mg; 285 μmol) as described above for the synthesis of **27**. Yield: 102 mg (119 μmol, 42%, α/β 3:1). Isolated yield of α-isomer: 70 mg (81 μmol). $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 171.0–166.9 (C=O acetyl, benzoyl, C-1′), 134.1 (C_q benzoyl), 131.5–126.9 (CH_{arom} benzoyl), 102.0 (C-1), 98.7 (C-2′), 73.0, 72.4, 72.3, 69.0, 68.3, 67.4 (C-3,4,5, C-4′,6′,7′,8′), 63.6, 62.6 (C-6, C-9′), 56.5 (OC$\underline{H}_3$), 54.5 (C-2), 52.6 (OC$\underline{H}_3$ ester), 49.3 (C-5′), 37.6 (C-3′), 23.0 (*N*-acetyl), 20.7 (C$\underline{H}_3$ *O*-acetyl). β-Isomer δ 98.3 (C-2′). ^1H NMR ($CDCl_3$): δ 7.75–7.38 (m, 5H, C$\underline{H}_{arom}$), 6.59 (d, 1H, N$\underline{H}$ benzoyl, $J_{NH-2}=9.1$ Hz), 5.40 (ddd, 1H, H-8′, $J_{8'-9'a}=2.6$ Hz, $J_{8'-9'b}=6.1$ Hz), 5.35 (dd, 1H, H-3, $J_{3-4}=9.6$ Hz), 5.28 (dd, 1H, H-7′, $J_{7'8'}=8.7$ Hz), 5.20 (dd, 1H, H-4, $J_{4-5}=9.5$ Hz), 4.88 (ddd, 1H, H-4′, $J_{4'-5'}=9.7$ Hz), 4.57 (d, 1H, H-1, $J_{1-2}=8.3$ Hz), 4.36 (dd, 1H, H-9′a, $J_{9'a-9'b}=12.4$ Hz), 4.22 (ddd, 1H, H-2, $J_{2-3}=10.2$ Hz), 4.07 (ddd, 1H, H-5′, $J_{5'-6'}=10.7$ Hz), 4.02 (dd, 1H, H-9′b), 4.00 (dd, 1H, H-6′, $J_{6'-7'}=1.9$ Hz), 3.88 (dd, 1H, H-6a, $J_{5-6a}=4.1$ Hz, $J_{6a-6b}=11.4$ Hz), 3.81 (s, 3H, OC$\underline{H}_3$ ester), 3.74–3.68 (m, 1H, H-5), 3.65 (dd, 1H, H-6b, $J_{5-6b}=3.0$ Hz), 3.47 (s, 3H, OC$\underline{H}_3$), 2.63 (dd, 1H, H-3′eq, $J_{3'eq-4'}=4.6$ Hz, $J_{3'eq-3'ax}=12.7$ Hz), 2.15, 2.14, 2.08, 2.03, 2.02, 1.97 1.86 (7×s, 21H, C$\underline{H}_3$ acetyl), 1.94 (dd, 1H, H-3′ax, $J_{3'ax-4'}=12.3$ Hz).

Methyl 2-octanoylamido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-(methyl 4,7,8,9-tetra-*O*-acetyl-*N*-acetyl-α-D-neuraminate-2-yl)-β-D-glucopyranoside (32). Compound **32** was prepared from acceptor **20** (122 mg; 302 μmol) and donor **14** (119 mg; 200 μmol) as described above for the synthesis of **27**. Yield: 53 mg (60 μmol, 30%, α/β 3:1). Isolated yield of α-isomer: 34 mg (38 μmol). $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 173.3–167.7 (C=O acetyl, octanoyl, C-1′), 101.8 (C-1), 98.7 (C-2′), 72.9, 72.5, 72.3, 69.0, 68.4, 67.4 (C-3,4,5, C-4′,6′,7′,8′), 63.4, 62.5 (C-6, C-9′), 56.5 (OC$\underline{H}_3$), 54.2 (C-2), 52.6 (OC$\underline{H}_3$ ester), 49.4 (C-5′), 37.6, 36.7 (C-3′, $\underline{C}H_2$ octanoyl), 31.6–22.5 ($\underline{C}H_2$ octanoyl), 23.0 (*N*-acetyl), 20.6 (C$\underline{H}_3$ *O*-acetyl), 13.9 (C$\underline{H}_3$ octanoyl). β-Isomer δ 98.3 (C-2′). ^1H NMR ($CDCl_3$): δ 5.64 (d, 1 H, N$\underline{H}$ octanoyl, $J_{NH-2}=9.0$ Hz), 5.36 (ddd, 1H, H-8′, $J_{8'-9'a}=2.7$ Hz, $J_{8'-9'b}=5.9$ Hz), 5.31 (d, 1H, N$\underline{H}$ acetyl, $J_{NH-5'}=8.4$ Hz), 5.29 (dd, 1H, H-7′, $J_{7'-8'}=8.6$ Hz), 5.21 (dd, 1H, H-3, $J_{3-4}=9.4$ Hz), 5.12 (dd, 1H, H-4, $J_{4-5}=9.4$ Hz), 4.85 (ddd, 1H, H-4′, $J_{4'-5'}=9.7$ Hz), 4.48 (d, 1H, H-1, $J_{1-2}=8.3$ Hz), 4.31 (dd, 1H, H-9′a, $J_{9a'-9'b}=12.4$ Hz), 4.04 (ddd, 1H, H-2, $J_{2-3}=10.4$ Hz), 4.03 (dd, 1H, H-9′b), 3.97 (dd, 1H, H-6′, $J_{5'-6'}=10.8$ Hz, $J_{6'-7'}=2.0$ Hz), 3.94–3.56 (m, 4H, H-5′, H-5,6a,6b), 3.79 (s, 3H, OC$\underline{H}_3$ ester), 3.46 (s, 3H, OC$\underline{H}_3$), 2.61 (dd, 1H, H-3′eq, $J_{3'eq-4'}=4.7$ Hz, $J_{3'eq-3'ax}=12.7$ Hz), 2.14, 2.13, 2.05, 2.03, 2.02, 2.00, 1.86 (7×s, 21H, C$\underline{H}_3$ acetyl), 1.94 (dd, 1H, H-3′ax, $J_{3'ax-4'}=12.4$ Hz), 1.56 (t, 2H, C$\underline{H}_2$ octanoyl), 1.25 (broad, 10H, C$\underline{H}_2$ octanoyl), 0.86 (t, 3H, C$\underline{H}_3$ octanoyl).

Methyl 2-naphthoylamido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-(methyl 4,7,8,9-tetra-*O*-acetyl-*N*-acetyl-α-D-neuraminate-2-yl)-β-D-glucopyranoside (33). Compound **33** was prepared from acceptor **21** (177 mg; 410 μmol) and donor **14** (160 mg; 268 μmol) as described above for the synthesis of **27**. Yield: 63 mg (69 μmol, 25%, α/β 5:1). Isolated yield of α-isomer: 39 mg (43 μmol). $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 171.3–167.3 (C=O acetyl, naphtoyl, C-1′), 134.5–131.0 (C_q naphtoyl), 128.6–123.3 (CH_{arom} naphtoyl), 101.6 (C-1), 98.4 (C-2′), 73.0, 72.0, 71.8, 69.2, 68.9, 68.2, 67.2 (C-3,4,5, C-4′,6′,7′,8′), 63.0, 62.3 (C-6, C-9′), 56.3 (OC$\underline{H}_3$), 54.1 (C-2), 52.2 (OC$\underline{H}_3$ ester), 48.5 (C-5′), 37.3 (C-3′), 22.0 (*N*-acetyl), 20.1 (C$\underline{H}_3$ *O*-acetyl). β-Isomer δ 98.1 (C-2′). ^1H NMR ($CDCl_3$): δ 8.27–7.48 (m, 7H, C$\underline{H}_{arom}$), 6.73 (d, 1H, N$\underline{H}$ naftanoyl, $J_{NH-2}=9.0$ Hz), 5.41 (ddd, 1H, H-8′, $J_{8'-9'a}=2.5$ Hz, $J_{8'-9'b}=6.3$ Hz), 5.40 (dd, 1H, H-3, $J_{3-4}=9.6$ Hz), 5.34 (d, 1H, N$\underline{H}$ acetyl, $J_{NH-5'}=9.5$ Hz), 5.30 (dd, 1H, H-7′, $J_{7'-8'}=8.7$ Hz), 5.23 (dd, 1H, H-4, $J_{4-5}=9.5$ Hz), 4.88 (ddd, 1H, H-4′, $J_{4'-5'}=9.8$ Hz), 4.60 (d, 1H, H-1, $J_{1-2}=8.3$ Hz), 4.37 (dd, 1H, H-9′a, $J_{9'a-9'b}=12.3$ Hz), 4.28 (ddd, 1H, H-2, $J_{2-3}=10.2$ Hz), 4.08 (ddd, 1H, H-5′, $J_{5'-6'}=10.7$ Hz), 4.04 (dd, 1H, H-9′b), 4.02 (dd, 1H, H-6′, $J_{6'-7'}=1.9$ Hz), 3.90 (dd, 1H, H-6a, $J_{6a-6b}=11.5$ Hz, $J_{5-6a}=4.1$ Hz), 3.81 (s, 3H, OC$\underline{H}_3$ ester), 3.76–3.70 (m, 1H, H-5), 3.65 (dd, 1H, H-6b, $J_{5-6b}=3.0$ Hz), 3.49 (s, 3H, OC$\underline{H}_3$), 2.64 (dd, 1H, H-3′eq, $J_{3'eq-4'}=4.6$ Hz, $J_{3'eq-3'ax}=12.7$ Hz), 2.16, 2.15, 2.09, 2.04, 2.02, 1.97, 1.87 (7×s, 21H, C$\underline{H}_3$ acetyl), 1.95 (dd, 1H, H-3′ax, $J_{3'ax-4'}=12.3$ Hz).

Methyl 2-(4-nitrobenzoyl)amido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-(methyl 4,7,8,9-tetra-*O*-acetyl-*N*-acetyl-α-D-neuraminate-2-yl)-β-D-glucopyranoside (34). Compound **34** was prepared from acceptor **22** (180 mg; 422 μmol) and donor **14** (160 mg; 268 μmol) as described above for the synthesis of **27**. Yield: 109 mg (121 μmol, 44%, α/β 4:1). Isolated yield of α-isomer: 67 mg (74 μmol). $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 171.1–165.3 (C=O acetyl, benzoyl, C-1′), 149.6, 139.6 (C_q benzoyl), 128.3, 123.6 (CH_{arom} benzoyl), 101.7 (C-1), 98.8 (C-2′), 73.0, 72.5, 72.2, 69.0, 68.9, 68.3, 67.4 (C-3,4,5, C-4′,6′,7′,8′), 63.7, 62.7 (C-6, C-9′), 56.5 (OC$\underline{H}_3$), 54.8 (C-2), 52.6 (OC$\underline{H}_3$ ester), 49.3 (C-5′), 37.6 (C-3′), 22.9 (*N*-acetyl), 20.6 (C$\underline{H}_3$ *O*-acetyl). β-Isomer δ 98.4 (C-2′). ^1H NMR ($CDCl_3$): δ 8.29 (m, 4H, C$\underline{H}_{arom}$), 7.17 (d, 1H, N$\underline{H}$ nitrobenzoyl, $J_{NH-2}=9.2$ Hz), 5.48 (d, 1H, N$\underline{H}$ acetyl, $J_{NH-5'}=9.5$ Hz), 5.40 (ddd, 1H, H-8′, $J_{8'-9'a}=2.4$ Hz, $J_{8'-9'b}=6.4$ Hz), 5.34 (dd, 1H, H-3, $J_{3-4}=9.6$ Hz), 5.30 (dd, 1H, H-7′, $J_{7'-8'}=8.6$ Hz, $J_{6'-7'}=1.4$ Hz), 5.18 (dd, 1H, H-4, $J_{4-5}=9.5$ Hz), 4.88 (ddd, 1H, H-4′, $J_{4'-5'}=9.8$ Hz), 4.62 (d, 1H, H-1, $J_{1-2}=8.3$ Hz), 4.40 (dd, 1H, H-9′a, $J_{9'a-9'b}=12.3$ Hz),

4.25 (ddd, 1H, H-2, J_{2-3} = 10.1 Hz), 4.07 (ddd, 1H, H-5′, $J_{5'-6'}$ = 10.6 Hz), 4.06–4.03 (m, 1H, H-6′), 4.02 (dd, 1H, H-9′b), 3.86 (dd, 1H, H-6a, J_{5-6a} = 4.5 Hz, J_{6a-6b} = 11.5 Hz), 3.81 (s, 3H, OCH$_3$ ester), 3.78–3.74 (m, 1H, H-5), 3.64 (dd, 1H, H-6b, J_{5-6b} = 3.0 Hz), 3.50 (s, 3H, OCH$_3$), 2.63 (dd, 1H, H-3′eq, $J_{3'eq-4'}$ = 4.6 Hz, $J_{3'eq-3'ax}$ = 12.8 Hz), 2.15, 2.12, 2.08, 2.05, 2.01, 1.97, 1.88 (7×s, 21H, CH$_3$ acetyl), 1.94 (dd, 1H, H-3′ax, $J_{3'ax-4'}$ = 12.3 Hz).

Methyl 2,3,6-tri-*O*-benzoyl-4-*O*-(2,3,4-tri-*O*-benzoyl-6-*O*-(methyl 4,7,8,9-tetra-*O*-acetyl-*N*-acetyl-α-D-neuraminate-2-yl)-β-D-galactopyranosyl)-β-D-glucopyranoside (35). Compound **35** was prepared from acceptor **23** (131 mg; 129 μmol) and donor **14** (149 mg; 250 μmol) as described above for the synthesis of **27**. Yield: 49 mg (34 μmol, 26%, α/β 2:1). Isolated yield of α-isomer: 23 mg (15 μmol). ^{13}C{^{1}H} NMR ($CDCl_3$): δ 170.8–164.9 (C=O acetyl, benzoyl, C-1″), 133.6–128.2 (CH$_{arom}$ benzoyl), 129.0 (C$_q$ benzoyl), 101.7, 100.6 (C-1, C-1′), 98.9 (C-2′), 75.8, 73.4, 72.9, 72.1, 71.9, 71.8, 70.2, 68.6, 67.6, 67.3 (C-2,3,4,5, C-2′,3′,4′,5′, C-4′,6′,7′,8′), 62.9, 62.7, 61.6 (C-6, C-6′, C-9′), 56.7 (OCH$_3$), 52.5 (OCH$_3$ ester), 49.5 (C-5′), 37.9 (C-3′), 23.1 (*N*-acetyl), 20.7 (CH$_3$ *O*-acetyl). β-Isomer δ 99.1 (C-2′). ^{1}H NMR ($CDCl_3$): δ 8.10–7.16 (m, 30H, CH$_{arom}$), 5.78 (dd, 1H, H-3, J_{3-4} = 9.5 Hz), 5.75 (d, 1H, H-4′), 5.61 (dd, 1H, H-2′, $J_{2'-3'}$ = 10.2 Hz), 5.49 (ddd, 1H, H-8″, $J_{8''-9''a}$ = 2.9 Hz, $J_{8''-9''b}$ = 6.7 Hz), 5.44 (dd, 1H, H-3′, $J_{3'-4'}$ = 3.1 Hz), 5.38 (dd, 1H, H-2, J_{2-3} = 9.6 Hz), 5.32 (dd, 1H, H-7″, $J_{7''-8''}$ = 8.4 Hz), 5.21 (d, 1H, NH acetyl, $J_{NH-5''}$ = 9.7 Hz), 5.00 (d, 1H, H-1′, $J_{1'-2'}$ = 7.8 Hz), 4.78 (ddd, 1H, H-4″, $J_{4''-5''}$ = 10.1 Hz), 4.66 (dd, 1H, H-6a, J_{5-6a} = 1.7 Hz, J_{6a-6b} = 12.0 Hz), 4.60 (d, 1H, H-1, J_{1-2} = 7.9 Hz), 4.55 (dd, 1H, H-6b, J_{5-6b} = 5.7 Hz), 4.43 (dd, 1H, H-9″a, $J_{9''a-9''b}$ = 12.3 Hz), 4.35 (dd, 1H, H-4, J_{4-5} = 9.6 Hz), 4.11 (dd, 1H, H-9″b), 4.10 (dd, 1H, H-6″, $J_{6''-7''}$ = 1.8 Hz), 4.03 (dd, 1H, H-5″, $J_{5''-6''}$ = 10.6 Hz), 3.91–3.86 (m, 2H, H-5, H-5′), 3.51 (dd, 1H, H-6′a, $J_{5'-6'a}$ = 5.5 Hz, $J_{6'a-6'b}$ = 9.9 Hz), 3.42, 3.41 (2×s, 6H, OCH$_3$), 2.87 (dd, 1H, H-6′b, $J_{5'-6'b}$ = 9.5 Hz), 2.40 (dd, 1H, H-3″eq, $J_{3''eq-4''}$ = 4.6 Hz, $J_{3''eq-3''ax}$ = 12.8 Hz), 2.18, 2.16, 2.09, 2.00, 1.89 (5×s, 15H, CH$_3$ acetyl), 1.81 (dd, 1H, H-3″ax, $J_{3''ax-4''}$ 12.5 Hz).

2,3,6-Tri-*O*-benzoyl-4-*O*-(2,3,4-tri-*O*-benzoyl-6-*O*-(methyl 4,7,8,9-tetra-*O*-acetyl-*N*-acetyl-α-D-neuraminate-2-yl)-β-D-galactopyranosyl)-β-D-glucopyranosyl azide (36). Compound **36** was prepared from acceptor **24** (131 mg; 129 μmol) and donor **14** (149 mg; 250 μmol) as described above for the synthesis of **27**. Yield: 98 mg (67 μmol, 33%, α/β 3:1). Isolated yield of α-isomer: 59 mg (40 μmol). ^{13}C{^{1}H} NMR ($CDCl_3$): δ 170.8–164.9 (C=O acetyl, benzoyl, C-1″), 133.3–128.3 (CH$_{arom}$ benzoyl), 128.9 (C$_q$ benzoyl), 100.3 (C-1′), 98.9 (C-2′), 87.9 (C-1), 75.6, 75.3, 72.8, 72.4, 71.9, 71.5, 70.1, 68.6, 68.5, 67.6, 67.2 (C-2,3,4,5, C-2′,3′,4′,5′, C-4′,6′,7′,8′), 62.8, 61.6 (C-6, C-6′, C-9′), 52.5 (OCH$_3$ ester), 49.4 (C-5″), 37.8 (C-3″), 23.0 (*N*-acetyl), 20.7 (CH$_3$ *O*-acetyl). β-Isomer δ 99.1 (C-2′). ^{1}H NMR ($CDCl_3$): δ 8.11–7.16 (m, 30H, CH$_{arom}$), 5.84 (dd, 1H, H-3, J_{3-4} = 9.6 Hz), 5.77 (d, 1H, H-4′), 5.62 (dd, 1H, H-2′, $J_{2'-3'}$ = 10.3 Hz), 5.50 (ddd, 1H, H-8″, $J_{8''-9''a}$ = 3.1 Hz, $J_{8''-9''b}$ 6.8 Hz), 5.46 (dd, 1H, H-3′, $J_{3'-4'}$ = 3.4 Hz), 5.37 (dd, 1H, H-2, J_{2-3} = 9.5 Hz), 5.33 (dd, 1H, H-7″, $J_{6''-7''}$ = 1.7 Hz, $J_{7''-8''}$ = 8.3 Hz), 5.04 (d, 1H, H-1′, $J_{1'-2'}$ = 7.8 Hz), 4.85 (d, 1H, H-1, J_{1-2} = 8.9 Hz), 4.79 (ddd, 1H, H-4″, $J_{4''-5''}$ = 9.6 Hz), 4.68 (dd, 1H, H-6a, J_{5-6a} = 1.9 Hz, J_{6a-6b} = 12.3 Hz), 4.59 (dd, 1H, H-6b, J_{5-6b} 5.5 Hz), 4.44 (dd, H-9″a, $J_{9''a-9''b}$ = 12.1 Hz), 4.39 (dd, H-4, J_{4-5} = 9.6 Hz), 4.12 (dd, 1H, H-9″b), 4.16 (m, 4H, H-5″5,6″, H-5′, H-5), 3.53 (dd, 1H, H-6′a, $J_{5'-6'a}$ 5.6 Hz, $J_{6'a-6'b}$ = 10.0 Hz), 3.43 (s, 3H, OCH$_3$ ester), 2.90 (dd, 1H, H-6′b, $J_{5'-6'b}$ = 9.6 Hz), 2.42 (dd, 1H, H-3″eq, $J_{3''eq-4''}$ = 4.5 Hz, $J_{3''eq-3''ax}$ = 13.0 Hz), 2.20, 2.17, 2.09, 2.00, 1.88 (5×s, 15H, CH$_3$ acetyl), 1.83 (dd, 1H, H-3″ax, $J_{3''ax-4''}$ = 12.5 Hz).

Methyl 2,3,4-tri-*O*-benzoyl-6-*O*-(methyl 4,7,8,9-tetra-*O*-acetyl-*N*-acetyl-α-D-neuraminate-2-yl)-β-D-galactopyranoside (37). Compound **37** was prepared from acceptor **25** (152 mg; 300 μmol) and donor **14** (119 mg; 200 μmol) as described above for the synthesis of **27**. Yield: 92 mg (94 μmol, 47%, α/β 6:1). ^{13}C{^{1}H} NMR ($CDCl_3$): δ 170.8–165.1 (C=O acetyl, benzoyl, C-1′), 133.8–128.1 (CH$_{arom}$ benzoyl), 129.0 (C$_q$ benzoyl), 102.3 (C-1), 99.0 (C-2′ α-isomer), 72.4, 71.9, 71.7, 69.9, 68.7, 68.4, 67.7, 67.4 (C-2,3,4,5, C-4′,6′,7′,8′), 62.7, 62.3 (C-6, C-9′), 57.1 (OCH$_3$), 52.5 (OCH$_3$ ester), 49.4 (C-5′), 37.9 (C-3′), 23.0 (*N*-acetyl), 20.6 (CH$_3$ *O*-acetyl). β-Isomer δ 98.8 (C-2′).

Methyl 2,3,4-tri-*O*-acetyl-6-*O*-(methyl 4,7,8,9-tetra-*O*-acetyl-*N*-acetyl-α-D-neuraminate-2-yl)-β-D-galactopyranoside (39). Compound **37** (78 mg; 79 μmol) was dissolved in a methanolic solution of sodium methanolate (0.05 M; 2 mL), and stirred overnight. The solution was neutralised by the addition of Dowex/H$^+$ and filtered. The filtrate was evaporated to dryness, and dissolved in a mixture of pyridine and acetic anhydride (1:1, v/v; 10 mL). This mixture was stirred overnight and then concentrated, evaporated with toluene (2×10 mL) and ethanol (10 mL), and applied to a silica gel column. Elution with ethanol:toluene (0:1→1:9, v/v) afforded the pure α-isomer of **39**. Isolated yield of α-isomer: 37 mg (47 μmol). ^{1}H NMR ($CDCl_3$): δ 5.44 (dd, 1H, H-4, J_{4-5} = 0.8 Hz, J_{3-4} = 3.3 Hz), 5.36 (ddd, 1H, H-8′, $J_{8'-9'a}$ = 2.7 Hz, $J_{8'-9'b}$ = 5.7 Hz), 5.28 (dd, 1H, H-7′, $J_{7'-8'}$ = 8.8 Hz), 5.23 (d, 1H, NH acetyl, $J_{NH-5'}$ = 9.6 Hz), 5.18 (dd, 1H, H-2, J_{2-3} = 10.5 Hz), 5.05 (dd, 1H, H-3, J_{3-4} = 3.3 Hz), 4.85 (ddd, 1H, H-4′, $J_{4'-5'}$ = 9.7 Hz), 4.46 (d, 1H, J_{1-2} = 7.9 Hz), 4.33 (dd, 1H, H-9′a, $J_{9'a-9'b}$ = 12.4 Hz), 4.09 (dd, 1H, H-6′, $J_{6'-7'}$ = 1.9 Hz), 4.06 (dd, 1H, H-9′b), 4.03 (ddd, 1H, H-5′, $J_{5'-6'}$ = 10.8 Hz), 3.95–3.91 (bt, 1H, H-5, J_{5-6a} = 6.0 Hz, J_{5-6b} = 7.6 Hz), 3.83 (dd, 1H, H-6a, J_{6a-6b} = 10.1 Hz), 3.79 (s, 3H, OCH$_3$ ester), 3.53 (s, 3H, OCH$_3$), 3.40 (dd, 1H, H-6b), 2.53 (dd, 1H, H-3′eq, $J_{3'eq-4'}$ = 4.6 Hz, $J_{3'eq-3'ax}$ = 12.9 Hz), 2.18, 2.14, 2.13, 2.06, 2.03, 2.02, 1.97, 1.88 (8×s, 24H, CH$_3$ acetyl), 1.91 (dd, 1H, H-3′ax, $J_{3'ax-4'}$ = 12.3 Hz).

4-Nitrophenyl 2,3,4-tri-*O*-benzoyl-6-*O*-(methyl 4,7,8,9-tetra-*O*-acetyl-*N*-acetyl-α-D-neuraminate-2-yl)-β-D-galactopyranoside (38). Compound **38** was prepared from acceptor **26** (184 mg; 300 μmol) and donor **14** (119 mg; 200 μmol) as described above for the synthesis of **27**. Yield: 114 mg (105 μmol, 52%, α/β 4:1). ^{13}C{^{1}H}

NMR ($CDCl_3$): δ 170.9–165.0 (C=O acetyl, benzoyl, C-1′), 161.6, 142.8 (CH_{arom} phenyl), 133.8–128.2 (CH_{arom} benzoyl), 129.2 (C_q benzoyl), 125.8, 117.0 (C_q phenyl), 99.8 (C-2′ α-isomer), 98.4 (C-1), 72.8, 72.4, 71.5, 69.5, 68.5, 67.7, 67.4 (C-2,3,4,5, C-4′,6′,7′,8′), 63.7, 62.9 (C-6, C-9′), 52.7 (OCH_3 ester), 49.2 (C-5′), 37.7 (C-3′), 23.0 (*N*-acetyl), 20.7 CH_3 *O*-acetyl). β-Isomer δ 98.8 (C-2′).

4-Nitrophenyl 2,3,4-tri-*O*-acetyl-6-*O*-(methyl 4,7,8,9-tetra-*O*-acetyl-*N*-acetyl-α-D-neuraminate-2-yl)-β-D-galactopyranoside (40). Compound **38** (93 mg, 85 μmol) was treated with sodium methanolate and then acetylated as described above for the synthesis of **39**. Silica gel chromatography, with ethanol:toluene (1:0→1:9, v/v) as eluent, afforded the pure α-isomer of **40**. Isolated yield of α-isomer: 38 mg (42 μmol). ^{1}H NMR ($CDCl_3$): δ 8.21–7.21 (m, 4H, CH_{arom}), 5.59 (d, 1H, H-4), 5.52 (dd, 1H, H-2, J_{2-3} = 10.0 Hz), 5.50 (ddd, 1H, H-8′, $J_{8'-9'a}$ = 3.0 Hz, $J_{8'-9'b}$ = 7.1 Hz), 5.44 (d, 1H, H-1, J_{1-2} = 7.9 Hz), 5.29 (dd, 1H, H-3, J_{3-4} = 3.5 Hz), 5.24 (dd, 1H, H-7′, $J_{7'-8'}$ = 9.9 Hz), 4.85 (ddd, 1H, H-4′, $J_{4'-5'}$ = 10.0 Hz), 4.37 (dd, 1H, H-9′a, $J_{9'a-9'b}$ = 12.2 Hz), 4.34 (dd, 1H, H-5, J_{5-6a} = 6.5 Hz, J_{5-6b} = 8.5 Hz), 4.16 (dd, 1H, H-6′, $J_{5'-6'}$ = 10.7 Hz, $J_{6'-7'}$ = 1.8 Hz), 4.14–4.05 (m, 1H, H-5′), 3.98 (dd, 1H, H-9′b), 3.81 (s, 3H, OCH_3 ester), 3.67 (dd, 1H, H-6a, J_{6a-6b} = 11.5 Hz), 3.55 (dd, 1H, H-6b), 2.53 (dd, 1H, H-3′eq, $J_{3'eq-4'}$ = 4.6 Hz, $J_{3'eq-3'ax}$ = 12.9 Hz), 2.24, 2.22, 2.17, 2.08, 2.03, 2.01, 1.91, 1.90 (8×s, 24H, CH_3 acetyl), 1.93 (dd, 1H, H-3′ax, $J_{3'ax-4'}$ = 12.3 Hz).

Methyl 2-acetamido-2-deoxy-6-*O*-(*N*-acetyl-α-D-neuraminate-2-yl)-β-D-glucopyranoside (1). Compound **27** (45 mg; 49 μmol) was dissolved in a methanolic solution of sodium methanolate (0.05 M; 2 mL), and stirred overnight. TLC analysis (2-propanol:water 4:1, v/v) revealed the formation of a single product. The solution was neutralised by the addition of Dowex/H^+ and filtered. The filtrate was evaporated to dryness and dissolved in a solution of NaOH (0.1 M) in 1,4-dioxane:water (3:1, v/v; 2 mL). The solution was carefully neutralised by the addition of Dowex/H^+ and filtered. The filtrate was evaporated to dryness and applied to an HW40 S gel filtration column, with water as eluent. The product fractions were pooled, concentrated and lyophilised. Yield of **1**: 23 mg (44 μmol, 90%). Characteristic ^{1}H NMR (D_2O) signals: δ 4.40 (d, 1H, H-1, J_{1-2} = 8.4 Hz), 3.47 (s, 3H, OCH_3), 2.72 (dd, 1H, H-3′eq, $J_{3'eq-4'}$ = 4.7 Hz, $J_{3'eq-3'ax}$ = 12.4 Hz), 2.01, 2.00 (2×s, 6H, CH_3 acetyl), 1.69 (dd, 1H, H-3′ax, $J_{3'ax-4'}$ = 12.0 Hz). HRMS: calculated for [$C_{20}H_{34}N_2O_{14}$ + Na^+] 549.1908, found 549.1918.

Methyl 2-azido-2-deoxy-6-*O*-(*N*-acetyl-α-D-neuraminate-2-yl)-β-D-glucopyranoside (2). Yield: 13 mg (25 μmol, 89%). ^{1}H NMR (D_2O): δ 4.41 (d, 1H, H-1, J_{1-2} = 8.2 Hz), 3.57 (s, 3H, OCH_3), 3.28 (dd, 1H, H-2, J_{2-3} = 9.4 Hz), 2.72 (dd, 1H, H-3′eq, $J_{3'eq-4'}$ = 4.5 Hz, $J_{3'eq-3'ax}$ = 12.3 Hz), 2.01 (s, 3H, CH_3 acetyl), 1.69 (dd, 1H, H-3′ax, $J_{3'ax-4'}$ = 12.1 Hz). HRMS: calculated for [$C_{18}H_{30}N_4O_{13}$ + Na^+] 533.1707, found 533.1679.

Methyl 2-benzoylamido-2-deoxy-6-*O*-(*N*-acetyl-α-D-neuraminate-2-yl)-β-D-glucopyranoside (4). Yield: 24 mg (41 μmol, 51%). ^{1}H NMR (D_2O): δ 7.78–7.49 (m, 5H, CH_{arom}), 4.57 (d, 1H, J_{1-2} = 8.5 Hz), 3.50 (s, 3H, OCH_3), 2.76 (dd, 1H, H-3′eq, $J_{3'eq-4'}$ = 4.4 Hz, $J_{3'eq-3'ax}$ = 12.3 Hz), 2.03 (s, 3H, CH_3 acetyl), 1.73 (dd, 1H, H-3′ax, $J_{3'ax-4'}$ = 12.0 Hz). HRMS: calculated for [$C_{25}H_{36}N_2O_{14}$ + Na^+] 611.2064, found 611.2079.

Methyl 2-octanoylamido-2-deoxy-6-*O*-(*N*-acetyl-α-D-neuraminate-2-yl)-β-D-glucopyranoside (5). Yield: 17 mg (28 μmol, 74%). ^{1}H NMR (D_2O): δ 4.39 (d, 1H, H-1, J_{1-2} = 8.4 Hz), 3.48 (s, 3H, OCH_3), 2.72 (dd, 1H, H-3′eq, $J_{3'eq-4'}$ = 4.6 Hz, $J_{3'eq-3'ax}$ = 12.4 Hz), 2.25 (t, 2H, CH_2C=O octanoyl, J = 7.2 Hz), 2.01 (s, 3H, CH_3 acetyl), 1.70 (dd, 1H, H-3′ax, $J_{3'ax-4'}$ = 12.0 Hz), 1.58 (bt, 2H, CH_2 octanoyl), 1.27 (bs, 8H, CH_2 octanoyl), 0.84 (t, 3H, CH_3 octanoyl, J = 6.8 Hz). HRMS: calculated for [$C_{26}H_{46}N_2O_{14}$ + Na^+] 633.2847, found 633.2863.

Methyl 2-naphthoylamido-2-deoxy-6-*O*-(*N*-acetyl-α-D-neuraminate-2-yl)-β-D-glucopyranoside (6). Yield: 19 mg (30 μmol, 70%). ^{1}H NMR (D_2O): δ 8.32–7.61 (m, 7H, CH_{arom}), 4.65 (d, 1H, H-1, J_{1-2} = 8.5 Hz), 3.56 (s, 3H, OCH_3), 2.80 (dd, 1H, H-3′eq, $J_{3'eq-4'}$ = 4.5 Hz, $J_{3'eq-3'ax}$ = 12.3 Hz), 2.07 (s, 3H, CH_3 acetyl), 1.78 (dd, 1H, H-3′ax, $J_{3'ax-4'}$ = 12.0 Hz). HRMS: calculated for [$C_{29}H_{38}N_2O_{14}$ + Na^+] 661.2221, found 661.2201.

Methyl 2-(4-nitrobenzoylamido)-2-deoxy-6-*O*-(*N*-acetyl-α-D-neuraminate-2-yl)-β-D-glucopyranoside (7). Yield: 35 mg (55 μmol, 74%). ^{1}H NMR (D_2O): δ 8.33 (d, 2H, CH_{arom}, J = 9.0 Hz), 7.95 (d, 2H, CH_{arom}), 4.58 (d, 1H, H-1, J_{1-2} = 8.5 Hz), 3.51 (s, 3H, OCH_3), 2.75 (dd, 1H, H-3′eq, $J_{3'eq-4'}$ = 4.6 Hz, $J_{3'eq-3'ax}$ = 12.3 Hz), 2.02 (s, 3H, CH_3 acetyl), 1.73 (dd, 1H, H-3′ax, $J_{3'ax-4'}$ = 12.0 Hz). Mass: calculated for C25H35N3O16 *m/e* 633.2, found 656.6 [M + Na^+].

2-Acetamido-2-deoxy-6-*O*-(*N*-acetyl-α-D-neuraminate-2-yl)-β-D-glucopyranosyl azide (8). Yield: 27 mg (50 μmol, 90%). ^{1}H NMR (D_2O): δ 4.73 (d, 1H, H-1, J_{1-2} = 9.2 Hz), 2.73 (dd, 1H, H-3′eq, $J_{3'eq-4'}$ = 4.4 Hz, $J_{3'eq-3'ax}$ = 12.3 Hz), 2.04, 2.03 (2×s, 6H, CH_3 acetyl), 1.69 (dd, 1H, H-3′ax, $J_{3'ax-4'}$ = 11.9 Hz). HRMS: calcd for [$C_{19}H_{31}N_5O_{13}$ + Na^+] 560.1816, found 560.1823.

4-Nitrophenyl 2-acetamido-2-deoxy-6-*O*-(*N*-acetyl-α-D-neuraminate-2-yl)-β-D-glucopyranoside (9). Yield: 11 mg (17 μmol, 79%). ^{1}H NMR (D_2O): δ 8.24, 7.18 (2×d, 4H, CH_{arom}, J = 9.3 Hz), 5.28 (d, 1H, H-1, J_{1-2} = 8.4 Hz), 2.73 (dd, 1H, H-3′eq, $J_{3'eq-4'}$ = 4.4 Hz, $J_{3'eq-3'ax}$ = 12.3 Hz), 2.00 (s, 6H, CH_3 acetyl), 1.65 (dd, 1H, H-3′ax, $J_{3'ax-4'}$ = 12.0 Hz). HRMS: calcd for [$C_{25}H_{35}N_3O_{16}$ + Na^+] 656.1915, found 656.1931.

Methyl 4-*O*-(6-*O*-(*N*-acetyl-α-D-neuraminate-2-yl)-β-D-galactopyranosyl)-β-D-glucopyranoside (10). Yield: 10 mg (15 μmol, 99%). ^{1}H NMR (D_2O): δ 4.40 (d, 1H, H-1′, $J_{1'-2'}$ = 7.8 Hz), 4.39 (d, 1H, H-1, J_{1-2} = 8.0 Hz), 3.55 (s, 3H, OCH_3), 3.51 (dd, 1H, H-2′, $J_{2'-3'}$ = 10.0 Hz), 3.31 (dd, 1H, H-2, J_{2-3} = 8.6 Hz), 2.69 (dd, 1H, H-3″eq, $J_{3''eq-4''}$ = 4.7 Hz, $J_{3''eq-3''ax}$ = 12.3 Hz), 2.00 (s, 3H, CH_3 acetyl), 1.72 (dd, 1H, H-3″ax, $J_{3''ax-4''}$ = 12.1 Hz). HRMS: calcd for [$C_{24}H_{41}NO_{19}$ + Na^+] 670.2170, found 670.2165.

4-*O*-(6-*O*-(*N*-Acetyl-α-D-neuraminate-2-yl)-β-D-galactopyranosyl)-β-D-glucopyranosyl azide (11). Yield: 26 mg (39 μmol, 99%). ^{1}H NMR (D_2O): δ 4.75 (d, 1H, H-1, J_{1-2} = 8.8 Hz), 4.40 (d, 1H, H-1′, $J_{1'-2'}$ = 7.8 Hz), 3.51 (dd, 1H, H-2′, $J_{2'-3'}$ = 9.9 Hz), 3.32 (dd, 1H, H-2, J_{2-3} = 8.9 Hz), 2.68 (dd, 1H, H-3′eq, $J_{3'eq-4'}$ = 4.7 Hz, $J_{3'eq-3'ax}$ = 12.3 Hz), 2.01 (s, 3H, $C\underline{H}_3$ acetyl), 1.71 (dd, 1H, H-3′ax, $J_{3'ax-4'}$ = 12.1 Hz). HRMS: calcd for [$C_{22}H_{38}N_4O_{18}$ + Na^+] 681.2079, found 681.2118.

Methyl 6-*O*-(*N*-acetyl-α-D-neuraminate-2-yl)-β-D-galactopyranoside (12). Yield: 13 mg (26 μmol, 55%). ^{1}H NMR (D_2O): δ 4.27 (d, 1H, H-1, J_{1-2} = 7.9 Hz), 3.53 (s, 3H, $OC\underline{H}_3$), 3.45 (dd, 1H, H-2, J_{2-3} = 10.0 Hz), 2.69 (dd, 1H, H-3′eq, $J_{3'eq-4'}$ = 4.6 Hz, $J_{3'eq-3'ax}$ = 12.3 Hz), 1.99 (s, 3H, $C\underline{H}_3$ acetyl), 1.66 (dd, 1H, H-3′ax, $J_{3'ax-4'}$ = 12.0 Hz). HRMS: calcd for [$C_{18}H_{31}NO_{14}$ + Na^+] 508.1642, found 508.1639.

4-Nitrophenyl 6-*O*-(*N*-acetyl-α-D-neuraminate-2-yl)-β-D-galactopyranoside (13). Yield: 12 mg (20 μmol, 91%). ^{1}H NMR (D_2O): δ 8.25 (d, 2H, $C\underline{H}_{arom}$, J = 9.3 Hz), 7.23 (d, 2H, $C\underline{H}_{arom}$), 5.16 (d, 1H, H-1, J_{1-2} = 7.3 Hz), 2.73 (dd, 1H, H-3′eq, $J_{3'eq-4'}$ = 4.2 Hz, $J_{3'eq-3'ax}$ = 12.3 Hz), 1.97 (s, 3H, $C\underline{H}_3$ acetyl), 1.61 (dd, 1H, H-3′ax, $J_{3'ax-4'}$ = 11.8 Hz). HRMS: calcd for [$C_{23}H_{32}N_2O_{16}$ + Na^+] 615.1650, found 615.1627.

Methyl 2-amino-2-deoxy-6-*O*-(*N*-acetyl-α-D-neuraminate-2-yl)-β-D-glucopyranoside (3). Compound **2** (14 mg; 28 μmol) was dissolved in dry methanol (140 μL). To this solution were added triethylamine (20 μL; 140 μmol) and 1,3-propanedithiol (14 μL; 140 μmol). The mixture was left at room temperature, until TLC analysis (2-propanol:water 4:1, v/v) indicated complete conversion of the starting material into a ninhydrine-positive product. The mixture was filtered, the filtrate was concentrated and then evaporated with 1,4-dioxane (1×10 mL). The crude product thus obtained was lyophilised from 1,4-dioxane, and then applied to an HW40 S gel filtration column, with water as eluent. The product fractions were pooled, concentrated and lyophilised. Yield: 2.4 mg (5.0 μmol, 18%). ^{1}H NMR (D_2O): δ 4.61 (d, 1H, H-1, J_{1-2} = 8.1 Hz), 3.57 (s, 3H, $OC\underline{H}_3$), 2.73 (dd, 1H, H-3′eq, $J_{3'eq-4'}$ = 4.5 Hz, $J_{3'eq-3'ax}$ = 12.3 Hz), 2.02 (s, 3H, $C\underline{H}_3$ acetyl), 1.70 (dd, 1H, H-3′ax, $J_{3'ax-4'}$ = 12.1 Hz). HRMS: calcd for [$C_{18}H_{32}N_2O_{13}$ + H^+] 485.1983, found 485.1984.

1-Azido-3-oxopentyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (42). To a solution of the known[30] oxazoline of glucosamine (**41**) (4.94 g; 15 mmol) and 2-(2-azidoethoxy)ethanol (2.95 g; 22.5 mmol) in 1,2-dichloroethane (DCE, 50 mL) was added, under an argon atmosphere, a catalytic amount of TfOH (130 μL, 1.5 mmol). The reaction was stirred overnight at 40 °C. According to TLC analysis (DCM: methanol 9:1, v/v), the starting material was completely converted into a product with lower mobility. The mixture was taken up in DCM (50 mL), washed with aq $NaHCO_3$ (1 M, 50 mL) and water (50 mL), dried over $MgSO_4$ and concentrated. The crude oil thus obtained was purified by silica gel column chromatography, with DCM:methanol (100:0→96:4, v/v) as eluent. Yield: 5.02 g (10.9 mmol, 72%). $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 170.4–168.8 ($\underline{C}$=O acetyl), 99.9 (C-1), 71.8, 70.6, 68.1 (C-3,4,5), 69.1, 69.0, 68.0 ($\underline{C}H_2O$ glycol, C-6), 53.3 (C-2), 49.7 ($\underline{C}H_2N_3$), 21.7 ($\underline{C}H_3$ *N*-acetyl), 19.4 ($\underline{C}H_3$ *O*-acetyl).

1-Azido-3-oxopentyl 2-amino-2-deoxy-β-D-glucopyranoside (43). A solution of the azido derivative **42** (1.38 g; 3.0 mmol) in aq NaOH (1 M; 150 mL) was refluxed overnight. The mixture was allowed to cool to room temperature, and was neutralised by the addition of aq HCl (1 M). The mixture was concentrated, evaporated with methanol (3×50 mL) and filtered. The filtrate, containing the crude amine **43,** was concentrated and led over a silica gel column, with ethyl acetate:methanol:water (7:2:1, v/v/v) as eluent. The product, still contaminated with salts, was used immediately for the synthesis of **45**.

1-Azido-3-oxopentyl 2-(4-nitrobenzoyl)amido-3,4-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside (45). The crude amine **43** (~3 mmol) was evaporated with dry toluene (2×20 mL), and dissolved in pyridine (30 mL). The solution was placed in an ice bath. Triethylamine (0.42 mL; 3.0 mmol) and 4-nitrobenzoyl chloride (2.22 g; 12 mmol) were added, and the mixture was stirred overnight. TLC analysis (ethanol:toluene 1:9, v/v) revealed the absence of **43**. The reaction was quenched by the addition of water (1 mL), the mixture was concentrated, taken up in DCM (50 mL), washed with aq $NaHCO_3$ (50 mL) and water (50 mL), dried ($MgSO_4$) and evaporated with toluene (2×20 mL) and ethanol (20 mL). The product mixture, obtained as a viscous oil, was dissolved in a methanolic solution of sodium methanolate (0.1 M; 50 mL), and stirred overnight. According to TLC analysis (toluene:methanol 1:1, v/v) one product was formed. The solution was neutralised by the addition of Dowex/H^+ and filtered. The filtrate, containing the crude 4-nitrobenzoylamido glucopyranoside **44**, was concentrated, evaporated with dry toluene (2×20 mL), and dissolved in pyridine (30 mL). tBDMS-Cl (0.46 g; 3.0 mmol) was added and the mixture was stirred for 3 h. TLC analysis (toluene:methanol 1:1, v/v) indicated complete conversion of the starting material. Acetic anhydride (2 mL) was added and the mixture was stirred overnight. TLC analysis (hexanes/ethyl acetate) showed the acetylation to be complete. The mixture was concentrated, taken up in DCM (100 mL), washed with aq $NaHCO_3$ (1 M, 100 mL) and water (100 mL), dried over $MgSO_4$ and evaporated with toluene (2×10 mL) and ethanol (10 mL). The fully protected product thus obtained was dissolved in acetonitrile:water (7:1, v/v; 16 mL) and the pH of the mixture was adjusted to 3 by the addition of *para*-toluenesulfonic acid monohydrate. After 2 h, TLC analysis (DCM:methanol 95:5, v/v) showed the removal of the tBDMS group to be complete. The reaction mixture was diluted with ethyl acetate (50 mL), washed with aq $NaHCO_3$ (1 M; 50 mL) and water (50 mL), dried ($MgSO_4$) and concentrated. The crude product was applied to a silica gel column, and eluted with DCM:MeOH (1:0→9:1, v/v). Yield: 0.51 g (0.97 mmol, 32% based on **42**). $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 170.6–166.1 ($\underline{C}$=O acetyl, benzoyl), 149.1, 139.5 ($\underline{C}_q$ benzoyl), 127.9, 123.1 ($\underline{C}H_{arom}$ benzoyl), 100.2 (C-1), 73.9, 72.5, 68.7

(C-3,4,5), 69.7, 69.3, 68.4 (O$\underline{C}H_2$ glycol), 60.6 (C-6), 54.4 (C-2), 50.1 ($\underline{C}H_2N_3$), 19.8 ($\underline{C}H_3$ *O*-acetyl). ^{1}H NMR ($CDCl_3$): δ 8.00–7.67 (m, 4H, C$\underline{H}_{arom}$ benzoyl), 5.13 (dd, 1H, H-3, $J_{3-4}=9.6$ Hz), 4.81 (dd, 1H, H-4, $J_{4-5}=8.9$ Hz), 4.59 (d, 1H, H-1, $J_{1-2}=8.6$ Hz), 3.93 (dd, 1H, H-2, $J_{2-3}=10.5$ Hz), 3.78 (m, 1H, H-5), 3.55–2.95 (m, 10H, H-6a,6b, C$\underline{H}_2$ glycol), 1.79, 1.71 (2×s, 6H, C$\underline{H}_3$ acetyl).

1-Azido-3-oxopentyl 2-(4-nitrobenzoyl)amido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-(methyl 4,7,8,9-tetra-*O*-acetyl-*N*-acetyl-α-D-neuraminate-2-yl)-β-D-glucopyranoside (47). A mixture of acceptor **45** (262 mg; 0.500 mmol) and donor **46** (521 mg; 1.00 mmol)[31] and powdered molecular sieves (3 Å) in acetonitrile (10 mL) was stirred for 1 h under argon atmosphere. The temperature of the mixture was lowered to −50 °C, and a solution of NIS (225 mg; 1.00 mmol) and TfOH (10 μL; 0.11 mmol) in acetonitrile (1 mL) was added. The mixture was gradually warmed to room temperature over a period of 3 h. The reaction was quenched by the addition of triethylamine (0.1 mL). The mixture was filtered over Hyflo, diluted with ethyl acetate (50 mL), washed with aq $Na_2S_2O_3$ (1 M; 50 mL), aq $NaHCO_3$ (1 M; 50 mL) and water (50 mL), dried ($MgSO_4$), concentrated, and applied to a silica gel column. Elution was performed with DCM:methanol (1:0→96:4, v/v). The fractions containing the product were pooled, concentrated, and applied to a Sephadex LH-20 column. The disaccharide **47** was obtained as α/β mixture. Yield: 214 mg (214 μmol, 45%, α/β 3:1). The α-isomer was isolated from the latter mixture by silica gel chromatography, with toluene:ethanol (1:0→9:1, v/v) as eluent. Yield: 143 mg (143 μmol). ^{13}C{^{1}H} NMR ($CDCl_3$): δ 171.0–165.4 (C=O acetyl, benzoyl, C-1′), 149.5, 139.5 (C_q benzoyl), 128.2, 123.6 (CH_{arom} benzoyl), 101.2 (C-1), 98.7 (C-2′), 72.9, 72.3, 68.9, 68.3, 67.4 (C-3,4,5, C-4′,6′,7′,8′), 70.6, 69.6, 68.5 (O$\underline{C}H_2$ glycol), 63.6, 62.5 (C-6, C-9′), 54.8 (C-2), 52.6 (O$\underline{C}H_3$ ester), 50.7 ($\underline{C}H_2N_3$), 49.2 (C-5′), 37.5 (C-3′), 22.9 ($\underline{C}H_3$ *N*-acetyl), 20.6 ($\underline{C}H_3$ *O*-acetyl). β-Isomer: δ 98.3 (C-2′). ^{1}H NMR ($CDCl_3$): δ 8.25–7.96 (m, 4H, C$\underline{H}_{arom}$ benzoyl), 5.61 (dd, 1H, H-3, $J_{3-4}=9.6$ Hz), 5.38 (ddd, 1H, H-8′, $J_{8'-9'a}=2.5$ Hz, $J_{8'-9'b}=6.0$ Hz), 5.30 (dd, 1H, H-7′, $J_{7'-8'}=8.7$ Hz, $J_{6'-7'}=11.5$ Hz), 5.17 (dd, 1H, H-4, $J_{4-5}=9.6$ Hz), 4.88 (m, 1H, H-4′), 4.84 (d, 1H, H-1, $J_{1-2}=8.5$ Hz), 4.34 (dd, 1H, H-9′a, $J_{9'a-9'b}=9.9$ Hz), 4.15 (ddd, 1H, H-2, $J_{2-3}=9.0$ Hz), 4.07–3.16 (m, 14H, H-5,6a,6b, H-5′,6′,9′b, C$\underline{H}_2$ glycol), 3.80 (s, 3H, OC$\underline{H}_3$ ester), 2.62 (dd, 1H, H-3′eq, $J_{3'eq-4'}=4.5$ Hz, $J_{3'eq-3'ax}=12.7$ Hz), 2.14, 2.13, 2.07, 2.03, 2.01, 1.96, 1.88 (7×s, 21H, C$\underline{H}_3$ acetyl), 1.93 (dd, 1H, H-3′ax).

1-Amino-3-oxopentyl 2-(4-nitrobenzoylamido)-2-deoxy-6-*O*-(*N*-acetyl-α-D-neuraminate-2-yl)-β-D-glucopyranoside (48). The azido disaccharide **47** (122 mg; 122 μmol) was dissolved in dry THF (2 mL). To the solution was added triphenylphosphine (40 mg; 0.15 mmol), and the mixture was stirred for 48 h. Water (6 μL; 350 μmol) was added, and the mixture was stirred for another 24 h. According to TLC analysis (toluene:methanol 1:1, v/v), the starting material was completely converted into a lower-running ninhydrine-positive product. Trifluoroacetic acid (TFA, 20 μL; 250 μmol) was added, followed by 1,4-dioxane (10 mL). The mixture was concentrated, and evaporated with 1,4-dioxane (2×10 mL). The crude amine **48** thus obtained was used immediately for the synthesis of the cluster **50**.

[Ac_4NeuAcMeAc_2GlcN($BzNO_2$)O$(CH_2)_2$O$(CH_2)_2$NHC(O)$(CH_2)_2$O$CH_2]_3$CNHC(O)CH_2NHZ (50). The amine **48** (90 μmol) and the tris-carboxylic acid **49** (11 mg; 20 μmol) were dissolved in DMF (5 mL), and DIPEA (41 μL; 0.24 mmol) and HOBT (10 mg; 75 μmol) were added. To this mixture was added HBTU (28 mg; 75 μmol). The reaction was stirred for 3 h, when TLC analysis (DCM:methanol 9:1, v/v) revealed the reaction to be complete. The mixture was taken up in DCM (40 mL), washed with dil. H_3PO_4 (1 M; 50 mL), aq $NaHCO_3$ (1 M; 50 mL), and water (50 mL), dried ($MgSO_4$), concentrated and evaporated with toluene (2×20 mL) and ethanol (20 mL). The crude product was purified by silica gel column chromatography, with DCM:methanol (1:0→92:8, v/v) as eluent, followed by Sephadex LH-20 gel filtration, with DCM:methanol (2:1, v/v) as eluent. Yield: 44 mg (13.0 μmol, 65%). ^{13}C{^{1}H} NMR ($CDCl_3$): δ 171.7–165.8 (C=O acetyl, benzoyl, C-1′), 156.7 (C=O Z), 149.6, 140.0 (C_q benzoyl), 136.3 (C_q Z), 128.5–123.7 (CH_{arom} benzoyl, Z), 101.0 (C-1), 98.8 (C-2′), 72.9, 72.5, 69.2, 69.0, 68.3, 67.5 (C-3,4,5, C-4′,6′,7′,8′), 70.1 69.7, 69.4, 68.6 66.9 (O$\underline{C}H_2$ TRIS, propanoyl, glycol, $\underline{C}H_2$ Z), 63.6, 62.6 (C-6, C-9′), 60.0 (C_q TRIS), 55.1 (C-2), 52.7 (O$\underline{C}H_3$ ester), 49.4 (C-5′), 44.7 ($\underline{C}H_2$ Gly), 39.3, 37.6, 36.5 (C-3, $\underline{C}H_2$C=O propanoyl, $\underline{C}H_2$NH), 23.0 ($\underline{C}H_3$ *N*-acetyl), 21.0, 20.6 ($\underline{C}H_3$ *O*-acetyl). ^{1}H NMR ($CDCl_3$): δ 8.30–7.19 (m, 12H, C$\underline{H}_{arom}$ benzoyl), 7.31 (bs, 5H, C$\underline{H}_{arom}$ Z), 6.87 (d, 3H, N$\underline{H}$ benzoyl, $J=9.3$ Hz), 5.39 (ddd, 3H, H-8′, $J_{8'-9'a}=2.7$ Hz, $J_{8-9'b}=6.2$ Hz), 5.30–5.17 (m, 11H, C$\underline{H}_2$ Z, H-3,4, H-7′), 4.87 (ddd, 3H, H-4′, $J_{4'-5'}=9.7$ Hz, $J_{3'ax-4'}=12.3$ Hz), 4.76 (d, 3H, H-1, $J_{1-2}=8.4$ Hz), 4.35 (dd, 3H, H-9′a, $J_{9'a-9'b}=12.3$ Hz), 4.27 (ddd, 3H, H-2, $J_{2-3}=9.3$ Hz, $J_{3-4}=9.4$ Hz), 4.10–3.18 (m, 62H, H-5,6a,6b, H-5′,6′,9′b, C$\underline{H}_2$ Gly, OC$\underline{H}_2$ propanoyl, C$\underline{H}_2$ glycol, OC$\underline{H}_2$ TRIS, C$\underline{H}_2$C=O propanoyl), 3.81 (s, 9H, OC$\underline{H}_3$ ester), 2.63 (dd, 3H, H-3′eq, $J_{3'eq-4'}=4.6$ Hz, $J_{3'eq-3'ax}=12.8$ Hz), 2.15, 2.09, 2.04, 2.02, 1.98, 1.88 (6×s, 63H, C$\underline{H}_3$ acetyl), 1.94 (dd, 3H, H-3′ax).

[NeuAcGlcN($BzNO_2$)O$(CH_2)_2$O$(CH_2)_2$NHC(O)$(CH_2)_2$OC$H_2]_3$CNHC(O)CH_2NHZ (52). Compound **50** (44 mg; 13 μmol) was dissolved in a methanolic solution of sodium methanolate (0.05 M; 2 mL), and stirred overnight. According to TLC analysis (2-propanol: water 4:1, v/v) one product was formed. The solution was neutralised by the addition of Dowex/H^+ and filtered. The filtrate was evaporated to dryness and dissolved in a solution of NaOH (0.1 M) in 1,4-dioxane: water (3:1, v/v; 2 mL). The solution was carefully neutralised by the addition of Dowex/H^+ and filtered. The filtrate was evaporated to dryness and applied to an HW40 S gel filtration column, with water as eluent. The product fractions were pooled, concentrated and lyophilised. Yield: 16 mg (6 μmol, 47%). ^{1}H NMR (D_2O): δ 8.26–7.91 (m, 12 H, C$\underline{H}_{arom}$ benzoyl), 7.30 (bs, 5H, C$\underline{H}_{arom}$ Z), 5.02 (s, 2H, C$\underline{H}_2$ Z), 4.68 (d, 3H, H-1,

$J_{1-2}=8.4$ Hz), 2.73 (dd, 3H, H-3′eq, $J_{3'eq-4'}=4.5$ Hz, $J_{3'eq-3'ax}=12.3$ Hz), 2.01 (s, 9H, $C\underline{H}_3$ acetyl), 1.71 (dd, 3H, H-3′ax, $J_{3'ax-4'}=12.0$ Hz). Mass: calcd for $C_{107}H_{152}N_{14}O_{60}$ *m/e* 2592.9, found 2592.7.

Erythrocyte solid-phase binding assay

The affinities of the glycosides for CD22 were determined in a solid phase binding assay, based on the binding of porcine erythrocytes to immobilised murine CD22D1-3-IgG (Costar, high binding). In short, multiwell plates were coated with Goat anti Human Fc (0.75 μg/well) by overnight incubation in 0.1 M sodium bicarbonate buffer (pH = 9.6) at 4 °C. Subsequently, the wells were blocked with 5% skimmed milk (w/w) for 1 h at 37 °C. After thorough washing, the wells were incubated with mCD22 for 3 h at 4 °C at a concentration of 0.01 μg/well in PBS + 0.25% BSA. The wells were again thoroughly washed and incubated for 1 h at 20 °C with porcine erythrocytes (1% dilution in PBS + 0.25% BSA) in the absence or presence of 1.0–1500 μM of the inhibitor. The peroxidase activity of haemoglobulin in the bound erythrocytes was determined using *o*-phenylenediamine hydrochloride as substrate in 50 mM phosphate citrate buffer (pH = 5) + 0.014% H_2O_2 after thorough washing with PBS + 0.25% BSA. CD22-specific binding was defined as the differential binding in the absence and the presence of an excess of Orosomucoid (Sigma) (3 μM). The CD22-specific erythrocyte binding amounted to a reproducible 40–60% of the total binding. From the competition curves, the IC_{50} (defined as the concentration of inhibitor giving 50% reduction of specific erythrocyte binding) and the Hill coefficient (which is indicative of the cooperativity of ligand binding) could be calculated by non-linear regression analysis (Prism, GraphPad Software Inc.). Data are means of a triplicate experiment.

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