



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 NeuAc α -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 NeuAc α -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, NeuAc α -2,6-GlcNBzNO₂OMe (**7**). This cluster, TRIS(NeuAc α -2,6-GlcNBzNO₂)₃ (**52**), displayed a more than 58-fold higher affinity for CD22 than the reference structure NeuAc α -2,6-LacOMe (**10**). To our knowledge, the cluster **52** is one of the most potent antagonists for CD22 yet synthesised. © 2000 Elsevier Science Ltd. All rights reserved.

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*-acetylactosamine (Sia α -2,6-LacNAc).⁹ Unlike in the case of the selectins,¹⁴ the presence of the Sia moiety, and in particular the C₇–C₈–C₉ 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

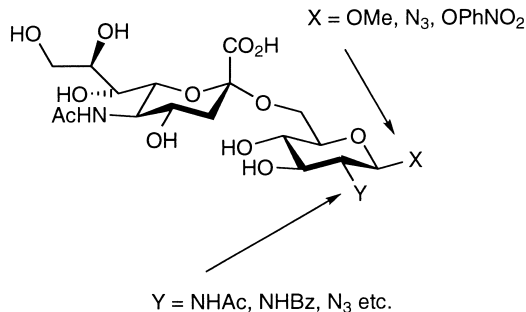
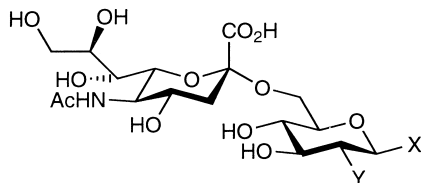
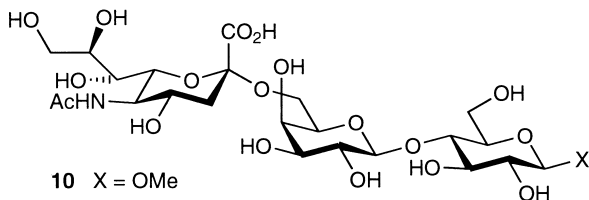


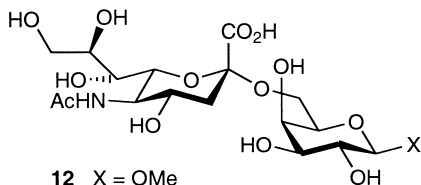
Figure 1. Possible variations in NeuAc α -2,6-Glc(Y)X type ligands for CD22.



- 1 X = OMe, Y = NHAc
- 2 X = OMe, Y = N₃
- 3 X = OMe, Y = NH₂
- 4 X = OMe, Y = NHBz
- 5 X = OMe, Y = NHOct
- 6 X = OMe, Y = NH(2-Napht)
- 7 X = OMe, Y = NH(4-NO₂)Bz
- 8 X = N₃, Y = NHAc
- 9 X = O(4-NO₂)Ph, Y = NHAc



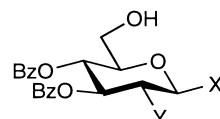
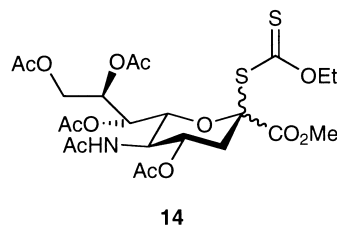
- 10 X = OMe
- 11 X = N₃



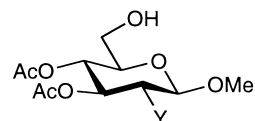
- 12 X = OMe
- 13 X = O(4-NO₂)Ph

Figure 2. Structures of monovalent ligands 1–13.

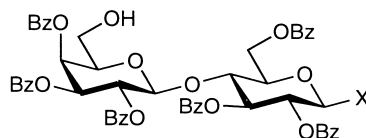
moiety by a halogenated acetyl, a formyl, or an aminoacetyl group did not affect binding by either human or murine CD22. In contrast, in the same study, binding to the siglec myelin associated glycoprotein (MAG) was shown to improve by a factor 17 by substitution of the *N*-acetyl by an *N*-fluoroacetyl 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



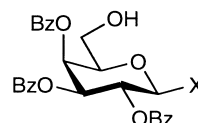
- 15 X = OMe, Y = NHAc
- 16 X = N₃, Y = NHAc
- 17 X = O(4-NO₂)Ph, Y = NHAc
- 18 X = OMe, Y = N₃



- 19 Y = NHBz
- 20 Y = NHOct
- 21 Y = NH(2-Napht)
- 22 Y = NH(4-NO₂)Bz



- 23 X = OMe
- 24 X = N₃



- 25 X = OMe
- 26 X = O(4-NO₂)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)

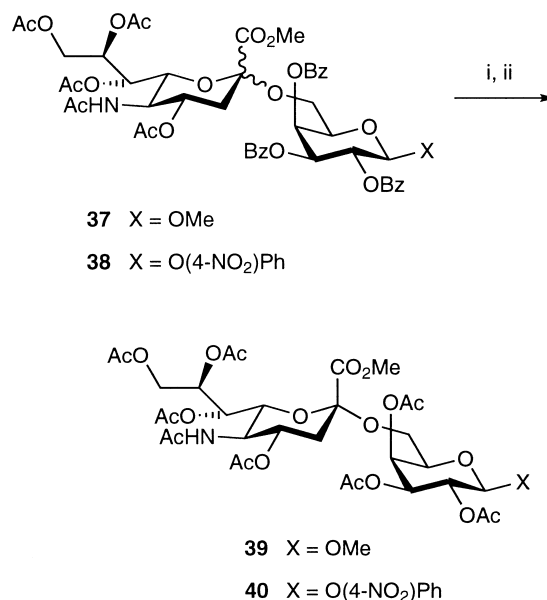
^a α/β Mixture.^bAs gauged by ¹³C NMR analysis.

disaccharide NeuAc α -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 NeuAc α -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 NeuAc α -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 NeuAc α -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, nitrophenoxo) and at the C-2 position (e.g., Y = aceta-

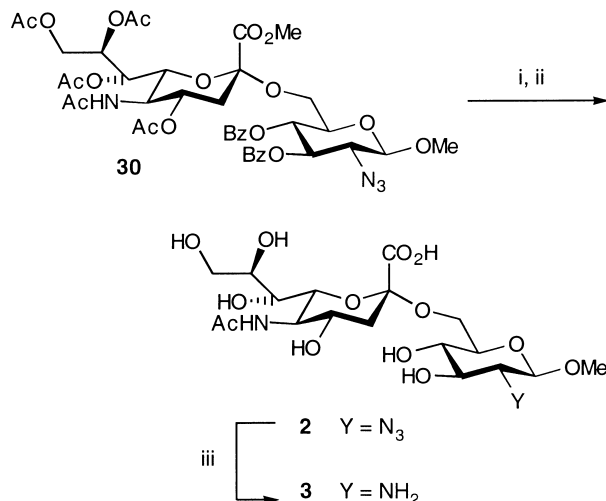
mido, benzoylamido, azido) of the glucosamine. In the second stage, a trivalent cluster, based on the monovalent motif that displayed the highest affinity for murine CD22, was prepared and tested, to establish whether the affinity of a synthetic oligosaccharide could be further enhanced by multivalent presentation.

**Scheme 1.** Protecting group exchange of NeuAc α -2,6-GalX type disaccharides. Reagents: (i) NaOMe; (ii) Ac₂O.

Results and Discussion

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



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

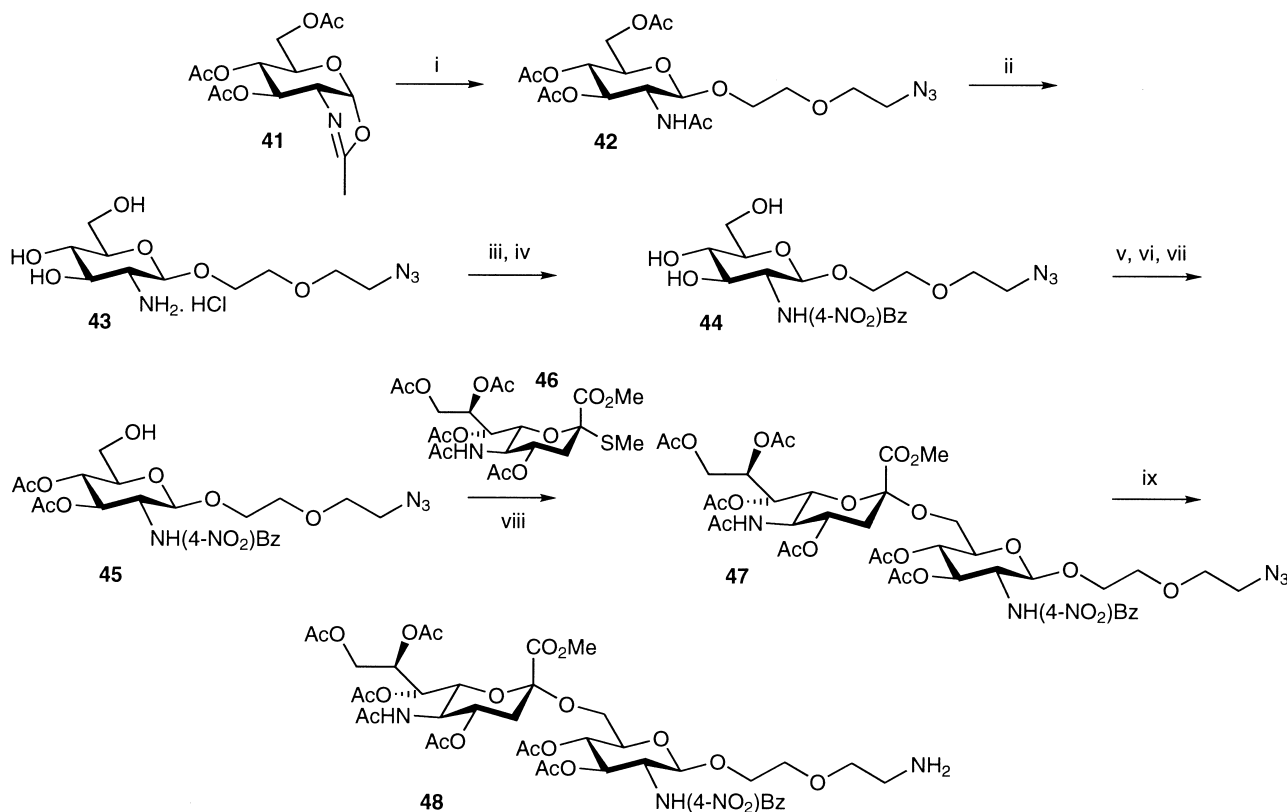
with the sialyl xanthate **14**¹⁶ (Fig. 3) in combination with *N*-iodosuccinimide/triflic acid (NIS/TfOH)^{17,18} as promoter system. The results of the glycosylation reactions are depicted in Table 1.

The α -isomers of the di- and trisaccharides **27–36** were identified by the characteristic ¹H NMR chemical shifts of the H-3eq [$\delta(\alpha\text{-isomer}) > \delta(\beta\text{-isomer})$] and H-4 ($\delta \pm 4.8$ 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

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

Compound		Relative IC ₅₀
1	NeuAc α -2,6-GlcNAcOMe	6.9
2	NeuAc α -2,6-GlcN ₃ OMe	1.2
3	NeuAc α -2,6-GlcNH ₂ OMe	2.0
4	NeuAc α -2,6-GlcNHBzOMe	2.2
5	NeuAc α -2,6-GlcNHOctOMe	1.6
6	NeuAc α -2,6-GlcNHNaphtOMe	1.8
7	NeuAc α -2,6-GlcNHBzNO ₂ OMe	9.2
8	NeuAc α -2,6-GlcNAcN ₃	2.1
9	NeuAc α -2,6-GlcNAcOPhNO ₂	7.9
10	NeuAc α -2,6-LacOMe	1.0
11	NeuAc α -2,6-LacN ₃	2.2
12	NeuAc α -2,6-GalOMe	2.4
13	NeuAc α -2,6-GalOPhNO ₂	3.6

^aThe relative IC₅₀ was calculated by dividing the average IC₅₀ found for compound **10** by the average IC₅₀ of the respective compound. A relative IC₅₀ > 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) HOCH₂CH₂OCH₂CH₂N₃, TfOH; (ii) 1 M NaOH, reflux; (iii) excess 4-NO₂BzCl; (iv) NaOMe; (v) tBDMS-Cl; (vi) Ac₂O; (vii) *p*-TsOH; (viii) NIS, TfOH; (ix) P(Ph)₃, H₂O.

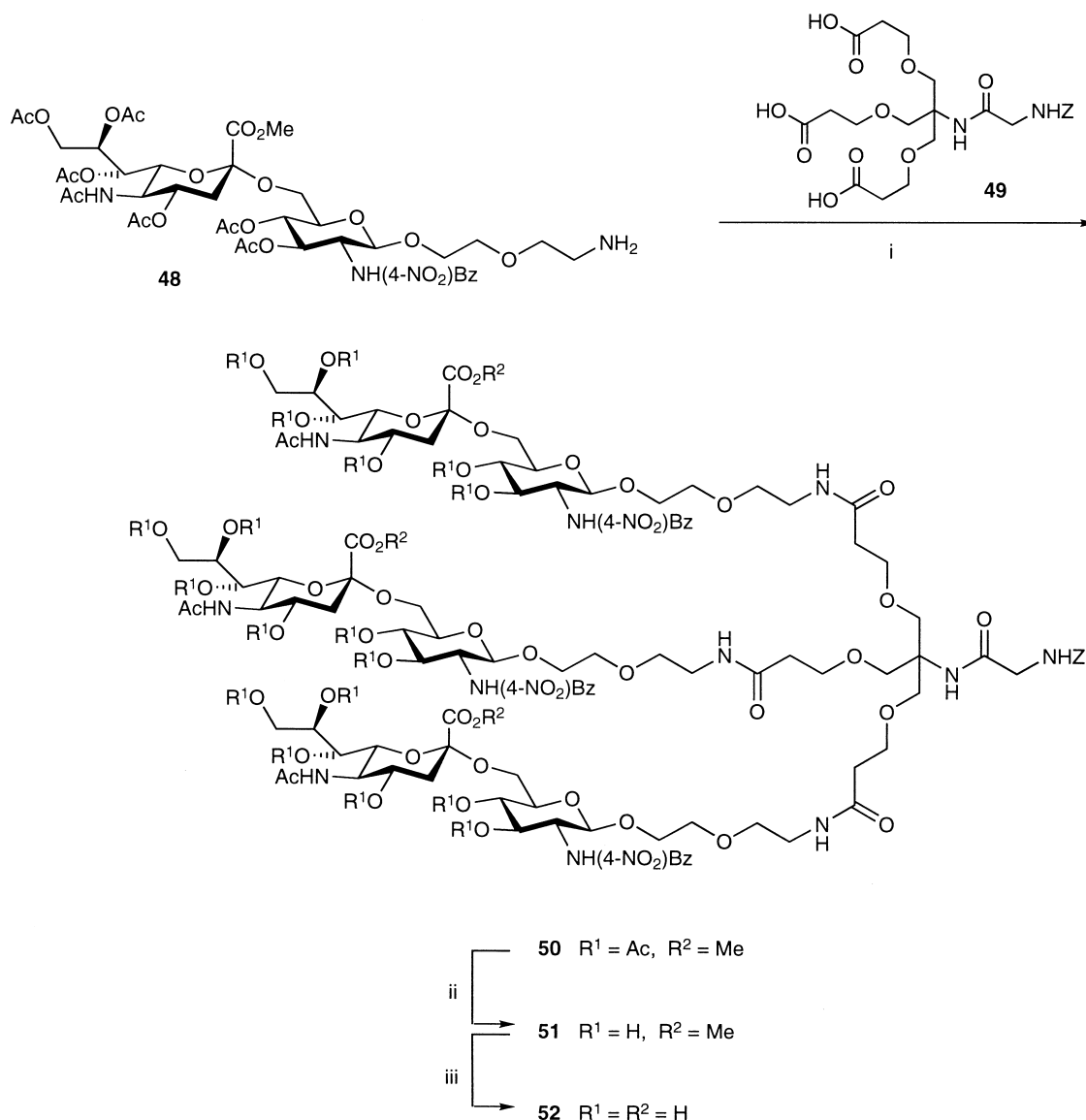
the respective α - and β -isomers could only be accomplished after replacing the benzoyl groups on the galactose ring by acetyl groups (see Scheme 1). Having the fully protected α -isomers of **27–36**, **39** and **40** in hand, two-step basic hydrolysis of the protecting groups then gave the respective 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-propanedithiol¹⁹ (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 NeuAc α -2,6-LacOMe trisaccharide **10**, which was selected as the reference compound, displayed an average IC₅₀ of 244 μ M. This compares well with the value reported by Powell et al.¹⁰ for binding of NeuAc α -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 IC₅₀ 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 nitrophenoxo 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 NeuAc α -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 found²² 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 known³⁰ 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 Staudinger³² 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 NeuAc α -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.

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, NeuAc α -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 F₂₅₄ 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). ¹H NMR spectra (200 MHz) and ¹³C{¹H} NMR spectra (50.1 MHz) were recorded with a Jeol JNM-FX200 spectrometer. ¹H NMR spectra (300 MHz) were recorded with a Bruker WM-300 spectrometer. Matrix Assisted Laser Desorption Ionisation (MALDI) Time-of-Flight (TOF) mass spectrometry³⁴ 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

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

Compound		Relative IC ₅₀
10	NeuAc α -2,6-LacOMe	1.0
7	NeuAc α -2,6-GlcNHBzNO ₂ OMe	9.2
52	TRIS(NeuAc α -2,6-GlcNHBzNO ₂) ₃	58.1

^aThe relative IC₅₀ was calculated by dividing the average IC₅₀ found for compound **10** by the average IC₅₀ of the respective compound. A relative IC₅₀ > 1 therefore represents a better binding to CD22 than the reference compound **10**.

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 μ M 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 routinely purified from the pooled media by protein-A Sepharose chromatography (yield $\pm$ 200 μ g/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 μ mol), donor **14** (130 mg; 218 μ mol)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 μ L; 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 $\text{Na}_2\text{S}_2\text{O}_3$ (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 $\rightarrow$ 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 μ mol, 37%, α/β 5:1). The α -isomer was isolated from the latter mixture by silica gel chromatography, with toluene:ethanol (1:0 $\rightarrow$ 8:2, v/v) as eluent, in a yield of 45 mg (49 μ mol). $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3): δ 171.1–128.3 ($\text{C}=\text{O}$ 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, $J_{\text{NH}-2}$ = 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, H-9'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 $\times$ 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 μ mol) and donor **14** (130 mg; 218 μ mol) as described above for the synthesis of **27**. Yield: 83 mg (89 μ mol, 41%, α/β 4:1). Isolated yield of α -isomer: 54 mg (58 μ mol). $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3): δ 171.5–164.6 ($\text{C}=\text{O}$ 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, $J_{\text{NH}-2}$ = 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, 1H, 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 $\times$ 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 μ mol) and donor **14** (130 mg; 218 μ mol) as described above for the synthesis of **27**. Yield: 61 mg (60 μ mol, 28%, α/β 6:1). Isolated yield of α -isomer: 23 mg (22 μ mol). $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3): δ 171.9–164.9 ($\text{C}=\text{O}$ 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, $J_{\text{NH}-2}$ = 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, $J_{\text{NH}-5'}$ = 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 $\times$ 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 μ mol) and donor **14** (119 mg; 200 μ mol) as described above for the synthesis of **27**. Yield: 90 mg (100 μ mol, 50%, α/β 5:1). Isolated yield of α -isomer: 51 mg (56 μ mol). $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3): δ 170.7–164.8 ($\text{C}=\text{O}$ 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, OCH₃), 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, CH₃ 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). ¹³C{¹H} NMR (CDCl₃): 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 (OCH₃), 54.5 (C-2), 52.6 (OCH₃ ester), 49.3 (C-5'), 37.6 (C-3'), 23.0 (N-acetyl), 20.7 (CH₃ O-acetyl). β-Isomer δ 98.3 (C-2'). ¹H NMR (CDCl₃): δ 7.75–7.38 (m, 5H, CH_{arom}), 6.59 (d, 1H, NH 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, OCH₃ 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, OCH₃), 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, CH₃ 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). ¹³C{¹H} NMR (CDCl₃): 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 (OCH₃), 54.2 (C-2), 52.6 (OCH₃ ester), 49.4 (C-5'), 37.6, 36.7 (C-3', CH₂ octanoyl), 31.6–22.5 (CH₂ octanoyl), 23.0 (N-acetyl), 20.6 (CH₃ O-acetyl), 13.9 (CH₃ octanoyl). β-Isomer δ 98.3 (C-2'). ¹H NMR (CDCl₃): δ 5.64 (d, 1H, NH 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, NH 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, $J_{9'a-9'b}$ = 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, OCH₃ ester), 3.46 (s, 3H, OCH₃), 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, CH₃ acetyl), 1.94 (dd, 1H, H-3'ax, $J_{3'ax-4'}$ = 12.4 Hz), 1.56 (t, 2H, CH₂ octanoyl), 1.25 (broad, 10H, CH₂ octanoyl), 0.86 (t, 3H, CH₃ 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). ¹³C{¹H} NMR (CDCl₃): δ 171.3–167.3 (C=O acetyl, naphthoyl, C-1'), 134.5–131.0 (C_q naphthoyl), 128.6–123.3 (CH_{arom} naphthoyl), 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 (OCH₃), 54.1 (C-2), 52.2 (OCH₃ ester), 48.5 (C-5'), 37.3 (C-3'), 22.0 (N-acetyl), 20.1 (CH₃ O-acetyl). β-Isomer δ 98.1 (C-2'). ¹H NMR (CDCl₃): δ 8.27–7.48 (m, 7H, CH_{arom}), 6.73 (d, 1H, NH naphthoyl, 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, NH 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, OCH₃ 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, OCH₃), 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, CH₃ 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). ¹³C{¹H} NMR (CDCl₃): 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 (OCH₃), 54.8 (C-2), 52.6 (OCH₃ ester), 49.3 (C-5'), 37.6 (C-3'), 22.9 (N-acetyl), 20.6 (CH₃ O-acetyl). β-Isomer δ 98.4 (C-2'). ¹H NMR (CDCl₃): δ 8.29 (m, 4H, CH_{arom}), 7.17 (d, 1H, NH nitrobenzoyl, J_{NH-2} = 9.2 Hz), 5.48 (d, 1H, NH 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, OCH₃ 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, OCH₃), 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, CH₃ 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 μ mol) and donor **14** (149 mg; 250 μ mol) as described above for the synthesis of **27**. Yield: 49 mg (34 μ mol, 26%, α/β 2:1). Isolated yield of α -isomer: 23 mg (15 μ mol). $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl₃): 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 (OCH₃), 52.5 (OCH₃ ester), 49.5 (C-5'), 37.9 (C-3'), 23.1 (*N*-acetyl), 20.7 (CH₃ *O*-acetyl). β -Isomer δ 99.1 (C-2'). ^1H NMR (CDCl₃): 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, $J_{\text{NH}-5''} = 9.7$ 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, OCH₃), 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, CH₃ 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 μ mol) and donor **14** (149 mg; 250 μ mol) as described above for the synthesis of **27**. Yield: 98 mg (67 μ mol, 33%, α/β 3:1). Isolated yield of α -isomer: 59 mg (40 μ mol). $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl₃): 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 (OCH₃ ester), 49.4 (C-5'), 37.8 (C-3''), 23.0 (*N*-acetyl), 20.7 (CH₃ *O*-acetyl). β -Isomer δ 99.1 (C-2'). ^1H NMR (CDCl₃): 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, OCH₃ 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, CH₃ 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 μ mol) and donor **14** (119 mg; 200 μ mol) as described above for the synthesis of **27**. Yield: 92 mg (94 μ mol, 47%, α/β 6:1). $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl₃): 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 (OCH₃), 52.5 (OCH₃ ester), 49.4 (C-5'), 37.9 (C-3'), 23.0 (*N*-acetyl), 20.6 (CH₃ *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 μ mol) 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 μ mol). ^1H NMR (CDCl₃): 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, $J_{\text{NH}-5'} = 9.6$ 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, OCH₃ ester), 3.53 (s, 3H, OCH₃), 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, CH₃ 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 μ mol) and donor **14** (119 mg; 200 μ mol) as described above for the synthesis of **27**. Yield: 114 mg (105 μ mol, 52%, α/β 4:1). $^{13}\text{C}\{^1\text{H}\}$

NMR (CDCl₃): 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 (OCH₃ ester), 49.2 (C-5'), 37.7 (C-3'), 23.0 (N-acetyl), 20.7 (CH₃ 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 μ mol) was treated with sodium methanolate and then acetylated as described above for the synthesis of **39**. Silica gel chromatography, with ethanol:toluene (1:0 $\rightarrow$ 1:9, v/v) as eluent, afforded the pure α -isomer of **40**. Isolated yield of α -isomer: 38 mg (42 μ mol). ¹H NMR (CDCl₃): 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, OCH₃ 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 $\times$ s, 24H, CH₃ 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 μ mol) 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 μ mol, 90%). Characteristic ¹H NMR (D₂O) signals: δ 4.40 (d, 1H, H-1, J_{1-2} = 8.4 Hz), 3.47 (s, 3H, OCH₃), 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 $\times$ s, 6H, CH₃ acetyl), 1.69 (dd, 1H, H-3'ax, $J_{3'ax-4'}$ = 12.0 Hz). HRMS: calculated for [C₂₀H₃₄N₂O₁₄ + 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 μ mol, 89%). ¹H NMR (D₂O): δ 4.41 (d, 1H, H-1, J_{1-2} = 8.2 Hz), 3.57 (s, 3H, OCH₃), 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, CH₃ acetyl), 1.69 (dd, 1H, H-3'ax, $J_{3'ax-4'}$ = 12.1 Hz). HRMS: calculated for [C₁₈H₃₀N₄O₁₃ + 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 μ mol, 51%). ¹H NMR (D₂O): δ 7.78–7.49 (m, 5H, CH_{arom}), 4.57 (d, 1H, J_{1-2} = 8.5 Hz), 3.50 (s, 3H, OCH₃), 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, CH₃ acetyl), 1.73 (dd, 1H, H-3'ax, $J_{3'ax-4'}$ = 12.0 Hz). HRMS: calculated for [C₂₅H₃₆N₂O₁₄ + 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 μ mol, 74%). ¹H NMR (D₂O): δ 4.39 (d, 1H, H-1, J_{1-2} = 8.4 Hz), 3.48 (s, 3H, OCH₃), 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, CH₂C=O octanoyl, J = 7.2 Hz), 2.01 (s, 3H, CH₃ acetyl), 1.70 (dd, 1H, H-3'ax, $J_{3'ax-4'}$ = 12.0 Hz), 1.58 (bt, 2H, CH₂ octanoyl), 1.27 (bs, 8H, CH₂ octanoyl), 0.84 (t, 3H, CH₃ octanoyl, J = 6.8 Hz). HRMS: calculated for [C₂₆H₄₆N₂O₁₄ + 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 μ mol, 70%). ¹H NMR (D₂O): 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, OCH₃), 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, CH₃ acetyl), 1.78 (dd, 1H, H-3'ax, $J_{3'ax-4'}$ = 12.0 Hz). HRMS: calculated for [C₂₉H₃₈N₂O₁₄ + 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 μ mol, 74%). ¹H NMR (D₂O): 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, OCH₃), 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, CH₃ 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 μ mol, 90%). ¹H NMR (D₂O): 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 $\times$ s, 6H, CH₃ acetyl), 1.69 (dd, 1H, H-3'ax, $J_{3'ax-4'}$ = 11.9 Hz). HRMS: calcd for [C₁₉H₃₁N₅O₁₃ + 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 μ mol, 79%). ¹H NMR (D₂O): δ 8.24, 7.18 (2 $\times$ 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, CH₃ acetyl), 1.65 (dd, 1H, H-3'ax, $J_{3'ax-4'}$ = 12.0 Hz). HRMS: calcd for [C₂₅H₃₅N₃O₁₆ + 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 μ mol, 99%). ¹H NMR (D₂O): 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, OCH₃), 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, CH₃ acetyl), 1.72 (dd, 1H, H-3''ax, $J_{3''ax-4''}$ = 12.1 Hz). HRMS: calcd for [C₂₄H₄₁NO₁₉ + 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 μ mol, 99%). ^1H 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, CH_3 acetyl), 1.71 (dd, 1H, H-3'ax, $J_{3'ax-4'}$ = 12.1 Hz). HRMS: calcd for $[\text{C}_{22}\text{H}_{38}\text{N}_4\text{O}_{18} + \text{Na}^+]$ 681.2079, found 681.2118.

Methyl 6-*O*-(*N*-acetyl- α -D-neuraminate-2-yl)- β -D-galactopyranoside (12**).** Yield: 13 mg (26 μ mol, 55%). ^1H NMR (D_2O): δ 4.27 (d, 1H, H-1, J_{1-2} = 7.9 Hz), 3.53 (s, 3H, OCH_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, CH_3 acetyl), 1.66 (dd, 1H, H-3'ax, $J_{3'ax-4'}$ = 12.0 Hz). HRMS: calcd for $[\text{C}_{18}\text{H}_{31}\text{NO}_{14} + \text{Na}^+]$ 508.1642, found 508.1639.

4-Nitrophenyl 6-*O*-(*N*-acetyl- α -D-neuraminate-2-yl)- β -D-galactopyranoside (13**).** Yield: 12 mg (20 μ mol, 91%). ^1H NMR (D_2O): δ 8.25 (d, 2H, CH_{arom} , J = 9.3 Hz), 7.23 (d, 2H, CH_{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, CH_3 acetyl), 1.61 (dd, 1H, H-3'ax, $J_{3'ax-4'}$ = 11.8 Hz). HRMS: calcd for $[\text{C}_{23}\text{H}_{32}\text{N}_2\text{O}_{16} + \text{Na}^+]$ 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 μ mol) was dissolved in dry methanol (140 μ L). To this solution were added triethylamine (20 μ L; 140 μ mol) and 1,3-propanedithiol (14 μ L; 140 μ mol). 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 $\times$ 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 μ mol, 18%). ^1H NMR (D_2O): δ 4.61 (d, 1H, H-1, J_{1-2} = 8.1 Hz), 3.57 (s, 3H, OCH_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, CH_3 acetyl), 1.70 (dd, 1H, H-3'ax, $J_{3'ax-4'}$ = 12.1 Hz). HRMS: calcd for $[\text{C}_{18}\text{H}_{32}\text{N}_2\text{O}_{13} + \text{H}^+]$ 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 known³⁰ 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 μ L, 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 $\rightarrow$ 96:4, v/v) as eluent. Yield: 5.02 g

(10.9 mmol, 72%). $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3): δ 170.4–168.8 ($\text{C}=\text{O}$ acetyl), 99.9 (C-1), 71.8, 70.6, 68.1 (C-3,4,5), 69.1, 69.0, 68.0 (CH_2O glycol, C-6), 53.3 (C-2), 49.7 (CH_2N_3), 21.7 (CH_3 *N*-acetyl), 19.4 (CH_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 $\times$ 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** ($\sim$ 3 mmol) was evaporated with dry toluene (2 $\times$ 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 $\times$ 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 $\times$ 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 $\times$ 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*-toluene-sulfonic 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 $\rightarrow$ 9:1, v/v). Yield: 0.51 g (0.97 mmol, 32% based on **42**). $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3): δ 170.6–166.1 ($\text{C}=\text{O}$ acetyl, benzoyl), 149.1, 139.5 (C_q benzoyl), 127.9, 123.1 (CH_{arom} benzoyl), 100.2 (C-1), 73.9, 72.5, 68.7

(C-3,4,5), 69.7, 69.3, 68.4 (OCH_2 glycol), 60.6 (C-6), 54.4 (C-2), 50.1 (CH_2N_3), 19.8 (CH_3 O-acetyl). ^1H NMR (CDCl_3): δ 8.00–7.67 (m, 4H, CH_{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, CH_2 glycol), 1.79, 1.71 (2×s, 6H, CH_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 $\text{Na}_2\text{S}_2\text{O}_3$ (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). $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3): δ 171.0–165.4 ($\text{C}=\text{O}$ 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 (OCH_2 glycol), 63.6, 62.5 (C-6, C-9'), 54.8 (C-2), 52.6 (OCH_3 ester), 50.7 (CH_2N_3), 49.2 (C-5'), 37.5 (C-3'), 22.9 (CH_3 N-acetyl), 20.6 (CH_3 O-acetyl). β -Isomer: δ 98.3 (C-2'). ^1H NMR (CDCl_3): δ 8.25–7.96 (m, 4H, CH_{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, CH_2 glycol), 3.80 (s, 3H, OCH_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, CH_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**.

[Ac₄NeuAcMeAc₂GlcN(BzNO₂)O(CH₂)₂O(CH₂)₂NHC(O)(CH₂)₂OCH₂]₃CNHC(O)CH₂NHZ (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%). $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3): δ 171.7–165.8 ($\text{C}=\text{O}$ acetyl, benzoyl, C-1'), 156.7 ($\text{C}=\text{O}$ 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 (OCH_2 TRIS, propanoyl, glycol, CH_2 Z), 63.6, 62.6 (C-6, C-9'), 60.0 (C_q TRIS), 55.1 (C-2), 52.7 (OCH_3 ester), 49.4 (C-5'), 44.7 (CH_2 Gly), 39.3, 37.6, 36.5 (C-3, $\text{CH}_2\text{C}=\text{O}$ propanoyl, CH_2NH), 23.0 (CH_3 N-acetyl), 21.0, 20.6 (CH_3 O-acetyl). ^1H NMR (CDCl_3): δ 8.30–7.19 (m, 12H, CH_{arom} benzoyl), 7.31 (bs, 5H, CH_{arom} Z), 6.87 (d, 3H, NH 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, CH_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, CH_2 Gly, OCH_2 propanoyl, CH_2 glycol, OCH_2 TRIS, $\text{CH}_2\text{C}=\text{O}$ propanoyl), 3.81 (s, 9H, OCH_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, CH_3 acetyl), 1.94 (dd, 3H, H-3'ax).

[NeuAcGlcN(BzNO₂)O(CH₂)₂O(CH₂)₂NHC(O)(CH₂)₂OCH₂]₃CNHC(O)CH₂NHZ (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%). ^1H NMR (D_2O): δ 8.26–7.91 (m, 12 H, CH_{arom} benzoyl), 7.30 (bs, 5H, CH_{arom} Z), 5.02 (s, 2H, CH_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, CH₃ acetyl), 1.71 (dd, 3H, H-3'ax, $J_{3'ax-4'}=12.0$ Hz). Mass: calcd for C₁₀₇H₁₅₂N₁₄O₆₀ 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 haemoglobin in the bound erythrocytes was determined using *o*-phenylenediamine hydrochloride as substrate in 50 mM phosphate citrate buffer (pH=5) + 0.014% H₂O₂ 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 IC₅₀ (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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