A Structure-Function Study of Ligand Recognition by CD22β*

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B-cell-specific CD22 is a member of a group of cell adhesion molecules within the immunoglobulin superfamily that display binding to glycans with terminal sialic acid residues. Binding of endogenous ligands to CD22 triggers B-cell activation and proliferation. It is therefore conceivable that high affinity ligands for CD22 may be of value as inhibitors of B-cell activation in allergy and chronic inflammation. In this study, we aimed to delineate the structural requirements for ligand binding to CD22. A library of 20 mono-, di-, and trisaccharide analogs of the basic binding motif Neu5Ac(α2,6)Lac was synthesized and screened for affinity for CD22β. In general, CD22 ligand recognition appeared to be rather tolerant with respect to structural modifications of the anomeric sugar on a mono-, di-, and trisaccharide level, although affinity was increased by the presence of a nitro aromatic group at C-2. The most potent multivalent ligand, Neu5Ac-4-nitrobenzoyl-Glc, was selected to generate multivalent ligands based on either a glutamate or Tris cluster core. All multivalent ligands displayed at least a 10-fold increased affinity for CD22 compared with the corresponding monovalentglycoside. Interestingly, a maximal gain in affinity was already obtained for bivalent ligands, regardless of the terminal glycoside. A trivalent Tris-based cluster of Neu5Ac-4-nitrobenzoyl-Glc displayed a 300-fold higher affinity compared with the basic binding motif, which makes it, to our knowledge, the most potent antagonist for CD22 yet synthesized. As our in vitro fluorescence-activated cell sorting studies demonstrated efficient cellular uptake of a CD22 substrate, the most potent ligand in this study may hold promise as a homing device for immunomodulatory compounds and cytostatics.

The sialoadhesin CD22 is a member of a group of cell adhesion molecules within the immunoglobulin superfamily that display binding to glycans with terminal sialic acid residues (1–3). It structurally resembles the carcinoembryonic antigen subfamily, with members like the adhesion molecules myelins-associated glycoprotein and CD33 (4–7).

Two isoforms of CD22 have been identified: 110- and 130-kDa proteins termed CD22α and CD22β, respectively. CD22β contains seven extracellular immunoglobulin-like domains, a short transmembrane sequence, and a 78-amino acid cytoplasmic tail. CD22α lacks two of the seven immunoglobulin-like domains, but is otherwise identical to CD22β (8, 9).

CD22 is a cell-surface glycoprotein that is uniquely located on B-cells and B-cell-derived tumor cells (10, 11). Upon activation of B-cells, the expression level of cell-surface CD22 initially increases, but is subsequently down-regulated upon differentiation into antibody-producing cells. The essential role of CD22β in B-cell activation offers an excellent possibility for the development of agents that interfere with B-cell-mediated immune responses. CD22β antagonists may prove valuable in preventing unwanted immune responses like allergy and chronic inflammatory processes, whereas CD22β agonists may be used to trigger the B-cell immune system in vaccination therapy (3, 7, 12, 13). Alternatively, ligands for CD22β may function as homing devices for the specific delivery of radionuclides and cytostatics to B-cell-derived tumors, e.g. in the case of acute leukemia.

These interesting perspectives have prompted us and others to map the binding characteristics of CD22β (3, 8, 14–19). From these studies, it has appeared that CD22β binds α2,6-sialylated glycoproteins, whereas it does not recognize α2,3-sialylated ligands (14, 8, 4). The basic monovalent binding motif in endogenous ligands for CD22β has been identified as Neu5Ac(α2,6)-N-acetyllactosamine (15). Further studies by Powell et al. (16) suggested that this basic monovalent binding motif for human CD22β could be further stripped to the disaccharide Neu5Ac(α2,6)Hex (N-acetylhexosaminyl glycoside) without loss of affinity, in which Hex may be N-acetyl-β-D-glucosamine (GalNAc), β-D-galactose (Gal), or N-acetyl-β-D-glucosamine (GlcNAc). In fact, the most potent disaccharide displayed a 3-fold higher affinity for CD22β compared with the reference trisaccharide structure Neu5Ac(α2,6)Lac (9).

Although the binding characteristics of murine and human CD22β are essentially similar, murine CD22β tends to have a slight preference for 5-glycylneuraminic acid, whereas human CD22β prefers Neu5Ac. A second remarkable difference between human and murine CD22β is that only halogenated Neu5Ac derivatives are recognized by human CD22β (3).

Summarizing the above data, it appears that (a) the presence of an intact sialic acid moiety is essential for binding to CD22β; (b) modification of the sialic acid group at C-5 does not dramatically affect ligand binding; and (c) the affinity for CD22β may be enhanced by further optimization of the sialic acid-terminated glycoside.

The aim of this study was to identify structural features of a

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† The abbreviations used are: Neu5Ac, 5-acetylneuraminic acid; Lac, lactose; BSA, bovine serum albumin; FITC, fluorescein 5-isothiocyanate; Ab, antibody; PBS, phosphate-buffered saline; FACS, fluorescence-activated cell sorting; OMe, methoxy.
CD22β ligand that determine the interaction with its receptor. A library of mono-, di-, and trisaccharide analogs of NeuAcα(2,6)Lac was synthesized and evaluated to elucidate the basic structural elements for high affinity recognition. In addition, we have addressed the effect of saccharide valency on the affinity for CD22β; based on the above criteria, we have devised a potent inhibitor of CD22β binding that may be of use in CD22β-directed immune therapy. We postulate that the most potent synthetic ligands can also serve as homing devices in B-cell tumor-specific targeting of cytostatics.

**EXPERIMENTAL PROCEDURES**

**Materials**—Bovine serum albumin (BSA; fraction V, delipidated), Dulbecco's modified Eagle's medium, RPMI 1640 medium, fetal calf serum, and penicillin/streptomycin were obtained from BioWhittaker Europe (Verviers, Belgium). Glasgow minimum essential medium (First Link UK); 5% IgG-poor fetal calf serum (Life Technologies, Inc., Breda, The Netherlands); sodium butyrate, Nt-methionine Nt-sulfoxide, fluorescein 5-isothiocyanate (FITC), goat anti-human IgG Fe fragment (referred to below as anti-Fc), and human orosomucoid (Sigma, Zwijndrecht, The Netherlands); horseradish peroxidase (Baker-deve, Deventer, The Netherlands); dimethyl sulfoxide (Baker-analyzed Deventer, NL); and multiwell plates (Costar 3590 flat-bottomed high bind- ing, Corning Inc., Corning NY) were obtained from the indicated manu- facturers. Anti-CD22 antibody (Ab1) was kindly provided by Dr. P. A. Van der Merwe (21). Anti-CD22 antibody (Ab2) was purified from ascites kindly provided by the Central Laboratory for Blood Transfu- sion (Amsterdam).

A library of mono-, di-, and trisaccharides was synthesized, as will be described elsewhere.2 Synthesis of multivalent saccharides will be pub- lished elsewhere.

**Production of Chimeric Murine CD22D3-IgG**—Murine CD22D3-IgG was purified from a stable transfected Chinese hamster ovary cell line kindly provided by Dr. P. R. Crocker (5). Cells were grown in Glasgow minimum essential medium containing 5% IgG-poor fetal calf serum, 20 units of penicillin/streptomycin, and 400 μg/ml methionine Nt-sulfox- ide. After the cell culture was 80% confluent, production of CD22 was induced by addition of 2 mM sodium butyrate, and the culture medium was collected for 3 weeks. Murine CD22 was purified from the pooled media by protein A-Sepharose chromatography at a yield of ≥200 μg/ml as previously described by Nath et al. (5). Purity was established by polyacrylamide gel electrophoresis analysis.

**FITC Labeling of Ab2**—Ab2 was dissolved in 0.5 mM sodium carbonate buffer (pH 9.5). FITC (1 mg) dissolved in 0.5 ml of Me2SO was added to Ab2 (80 μg of FITC/mg of Ab2), and the mixture was incubated for 1 h at room temperature. FITC-labeled Ab2 was purified by protein A- Sepharose chromatography and stored in 10 mM Tris and 150 mM NaCl (pH 8.2).

**Erythrocyte Isolation**—Porcine erythrocytes were isolated as follows. 70 ml of citrate buffer (210 mM citric acid, 0.9 M sodium citrate, 210 mM NaH2PO4, and 1.3 M glucose) was added to 500 ml of porcine blood. The blood was then centrifuged at 3000 rpm for 10 min at 4 °C; the plasma was removed; and erythrocytes were washed three times with PBS. 6 volumes of erythrocytes were stored in 1 volume of buffer containing 750 mM NaCl, 145 mM mannitol, 230 mM glucose, and 6 mM adenine for 4 weeks at 4 °C.

**Erythrocyte Solid-phase Binding Assay**—The solid-phase binding as-
say is based on the binding of porcine erythrocytes to murine CD22D3-IgG, which is immobilized on anti-Fc-coated multiwell plates. Erythrocyte binding to CD22 was monitored by measuring the peroxidase binding that may be of use in CD22β-directed immune therapy. We postulate that the most potent synthetic ligands can also serve as homing devices in B-cell tumor-specific targeting of cytostatics.

**RESULTS**

**Synthesis**—The aim of this study was to investigate the contribution of structural features of the ligand for CD22β (henceforth CD22) ligand binding to its receptor and to develop a synthetic high affinity ligand for CD22 for use as a homing device in B-cell tumor-specific targeting of cytostatics. For this purpose, the molecular structure of the ligand should warrant optimal and selective binding to CD22, but should also be easily accessible from a synthetic point of view.

To this end, we systematically stripped the presumed basic motif Neu5Acα(2,6)Lac (Fig. 1, compounds 1 and 2) by synthesizing a series of tri-, di-, and monosaccharide analogs. At the trisaccharide level, we tested the effect of a methoxy (compound 1) or an azido (compound 2) group at the anomeric position. Compound 1 was structurally identical to the basic binding motif Neu5Acα(2,6)Lac(NAc) and served as a reference for subsequent binding studies.

In addition, we devised a series of disaccharides, in which Neu5Ac was coupled to a modified glucosyl or galactosyl group (Fig. 1, compounds 3-11 and compounds 12 and 13, respective- ly), that enabled us to assess the effect of groups at C-1, C-2, and C-4 of the anomic sugar. (For more details about the synthesis, see Footnote 2.)

Finally, we synthesized a number of Neu5Ac-derived mono-
saccharides with structural elements at C-2 and C-5. The acetyl group at C-5 was replaced by an octanoyl (compound 14), benzoyl (compound 15), naphthoyl (compound 16), thiophenoyl (compound 17), or nitrobenzoyl (compound 18) group (see Fig. 1). In addition, the effect of substitution of the C-2 methoxy group by a C-2 thiomethoxy group on the binding affinity for CD22 was investigated (Fig. 1, compound 20).

**Analysis of the Monomeric Ligands in the Erythrocyte Solid-
phase Binding Assay**—The monovalent ligands 1–20 were tested for their affinity for CD22 in an in vitro competition assay, which is based on the binding of porcine erythrocytes to immobilized CD22 (5). First, the binding assay was optimized in terms of the coating level of anti-Fc, CD22 concentration, and erythrocyte species and concentration. Fig. 2 shows the optimization of the CD22 concentration. The erythrocyte binding signal was clearly dependent on the CD22 concentration and increased steadily with increasing CD22 concentrations, leveling off at >1.1 μg/ml CD22. For subsequent competition studies, a concentration of 0.1 μg/ml CD22 appeared to be optimal. The optimization studies also showed that human and murine erythrocytes bound less avidly to CD22 compared with...
porcine erythrocytes (data not shown).

To calibrate the assay, competition studies were performed using two established substrates for CD22, i.e. human orosomucoid and bovine thyroglobulin, and a specific anti-CD22 monoclonal antibody (Ab1) (15, 21). Both CD22 substrates were able to inhibit erythrocyte binding to CD22 in a monophasic fashion and to a similar extent as Ab1 (Fig. 3). From the competition curves, the IC50 could be calculated by nonlinear regression analysis. For proper evaluation, specific binding to CD22 was defined as the differential binding in the absence and presence of an excess of orosomucoid (3 mM).

Next, competition experiments were performed using the synthetic CD22 ligands from the ligand library. All competition curves were monophasic and showed >90% inhibition of erythrocyte binding to CD22 in a monophasic fashion and to a similar extent as Ab1 (Fig. 3). From the competition curves, the IC50 could be calculated by nonlinear regression analysis. For proper evaluation, specific binding to CD22 was defined as the differential binding in the absence and presence of an excess of orosomucoid (3 mM).

Ligand Recognition by CD22

**Fig. 1.** Chemical structures of the monomeric glycosides used in this study. All structures are based on the presumed basic binding motif NeuAcα2,6Lac. Structures are classified as mono-, di-, and trisaccharide variations. Listed are the compounds with their respective number; the modifications are displayed under X and Y. SMe, thiomethoxy; N3, azide; Bz, benzoyl; Naph, naphthoyl; NO2Bz, 4-nitrobenzoyl; OPPhNO2, 4-nitrophenoxy; Oct, octanoyl; ThioPh, thiophenoyl. Asterisks indicate that OH at C-4 has an equatorial orientation (instead of axial).

**Fig. 2.** Effect of CD22 coating concentration on erythrocyte binding. Porcine erythrocytes were incubated in wells that have been coated with 0–3 µg/ml CD22. Binding and inhibition were determined as described under “Erythrocyte Solid-phase Binding Assay” under “Experimental Procedures.” From the curve, the apparent IC50 for erythrocyte binding to CD22 can be calculated as 0.12 µg/ml. Data points are means from two independent experiments of nine data points in triplicate.

**Fig. 3.** Competition of erythrocyte binding to murine CD22 by human orosomucoid (■), bovine thyroglobulin (▲), and Ab1 (○). Porcine erythrocytes were incubated for 1 h at room temperature with CD22 (0.1 µg/ml) in the absence or presence of the inhibitor. Erythrocyte binding was determined as described under “Experimental Procedures.” The IC50 values for orosomucoid, thyroglobulin, and Ab1 were 77, 181, and 0.28 nM, respectively. Data points are means ± S.D. from three independent experiments of 10 data points in triplicate.

**Fig. 4.** Typical inhibition curve of the reference compound 1. Porcine erythrocytes were incubated for 1 h at room temperature with CD22 (0.1 µg/ml) in the absence or presence of the inhibitor. Erythrocyte binding was determined as described under “Experimental Procedures.” The IC50 calculated for the reference compound 1 was 244 µM. Data points are means ± S.D. from four independent experiments of 10 data points in triplicate.

ligands were expressed relative to that of compound 1 (Table I).

From Table I, it can be seen that, in line with the results of Powell et al. (16), the β-D-glucosamine-derived disaccharides 3–11 all displayed similar to higher affinity for CD22 compared with trisaccharide 1. Substitution of the anemic methoxy by an azido group reduced the affinity effect at the disaccharide
To address whether the observed gain in affinity was caused by the glutamate backbone itself, we also synthesized multivalent clusters based on a Tris dendritic core (Fig. 5). The Tris core has already been successfully applied in the synthesis of trivalent galactoside ligands for the asialoglycoprotein receptor (27) and offers the advantage that it is symmetrical and more suitable for large-scale synthesis. From Fig. 6, it can be concluded that the glutamate- and Tris-based clusters of compound 9 displayed a similarly enhanced affinity for CD22, although the Tris-based cluster appeared to be slightly more potent than the glutamate-based cluster.

To assess the significance of the above finding, we also studied whether the extent of the clustering effect depended on the terminal sugar moiety. To this end, a Tris-based cluster of the Neu5Ac monosaccharide 19 was synthesized and tested. Analogs of this trivalent Neu5Ac cluster 23 displayed a 10-fold higher affinity for CD22 compared with the monovalent parent compound 9, which may be the limiting factor in preventing optimal recognition by CD22.

Finally, we investigated whether the affinity of a multivalent CD22 ligand can be further improved by optimization of the spacing of the terminal saccharides within a cluster glycoside. This would allow us to verify that the affinity of multimeric compounds could be further improved beyond the bivalent level by increasing the distance between vicinal glycoside groups, which may be the limiting factor in preventing optimal recog-
dition of the separate glycoside groups. Therefore, a series of tetravalent glutamate-based saccharides (compounds 21a, 21b, and 21c, respectively) with that of the monovalent reference compound 9. Clearly, the multivalent compounds all displayed an ~10-fold higher affinity than the monovalent glycosides. B, effect of the valency of Tris-based mono- and disaccharides (compounds 19 and 9, respectively) on CD22. The multivalent compounds showed a 10–30-fold increase in affinity. C, effect of saccharide spacing (C4, C6, and C10) on the affinity of the ligands for CD22. No significant effect was observed with compounds 24–26. Data points are means ± S.D. from three independent experiments of at least nine data points in triplicate. Compound numbers are given on the x axis; the y axis shows the increase in affinity relative to that of the reference compound. The chemical structures of the multivalent compounds 21–26 are given in Fig. 5, whereas those of the monomeric compounds 9 and 19 are given in Fig. 1.

**FIG. 6.** Effect of multivalent presentation on the affinity of a sialylated saccharide for CD22. A, comparison of the affinity of di-, tri-, and tetravalent glutamate-based saccharides (compounds 21a, 21b, and 21c, respectively) with that of the monovalent reference compound 9. Clearly, the multivalent compounds all displayed an ~10-fold higher affinity than the monovalent glycosides. B, effect of the valency of Tris-based mono- and disaccharides (compounds 19 and 9, respectively) on CD22. The multivalent compounds showed a 10–30-fold increase in affinity. C, effect of saccharide spacing (C4, C6, and C10) on the affinity of the ligands for CD22. No significant effect was observed with compounds 24–26. Data points are means ± S.D. from three independent experiments of at least nine data points in triplicate. Compound numbers are given on the x axis; the y axis shows the increase in affinity relative to that of the reference compound. The chemical structures of the multivalent compounds 21–26 are given in Fig. 5, whereas those of the monomeric compounds 9 and 19 are given in Fig. 1.

**FIG. 7.** Uptake of FITC-labeled anti-CD22 antibody by Ramos (CD22+) cells. Ramos cells (10^5 cells/ml) were incubated with FITC-labeled Ab2 (10 nM) for up to 60 min at 37 °C. At the indicated time points, cells were washed, and the extracellular bound antibody was then removed by incubating the cells for 5 min at 4 °C with 0.1 M glycine (pH 3) and analyzed for fluorescence and forward scatter using a FACScan. The graph indicates the uptake of FITC-labeled Ab2 during the incubation time. From the curve, the half-life time (6.2 min) can be calculated using nonlinear regression analysis.

**DISCUSSION**

In this study, we developed new high affinity ligands for CD22 that can be utilized as homing devices in B-cell-directed delivery of cytostatics. A library of 20 Neu5Ac-terminated mono-, di-, and trisaccharides that carry different structural features was synthesized, and the compounds were tested for their ability to inhibit erythrocyte binding to CD22 in an in vitro competition assay. To calibrate the competition assay, the IC_{50} values of orosomucoid and thyroglobulin (natural ligands for CD22) were determined. The IC_{50} values were in close agreement with values reported in the literature (16). Combined with the finding that Ab1 and the CD22 substrates (orosomucoid and thyroglobulin) inhibited erythrocyte binding to a similar extent, the results establish that erythrocyte binding is completely mediated by CD22. The reproducibility of this assay is sufficiently high as judged from the small variation in IC_{50} values for orosomucoid in five independent experiments (individual values of 77, 82, 81, 86, and 93 nM).

The library of monovalent mono-, di-, and trisaccharide structures was tested with this assay. The reference compound Neu5Ac-LacOMe (compound 1), which is also the OMe derivative of the minimal basic motif (15), displayed an average IC_{50} value of 244 μM. Taking into account that the affinity of
Neu5Ac derivatives for murine CD22 is slightly lower than that for human CD22 (18), this compares well with the value reported by Powell and Varki (15) for human CD22 (30–100 μM).

Comparison of the relative IC_{50} values of the 1-O-methyl-derivatized (compounds 3 (di), 1 (tri), and 12 (di)) saccharides showed that the orientation of the C-4 hydroxyl group of the glycoside proximal to Neu5Ac (i.e., GlcNAc) does not affect CD22 recognition. The observation that the GlcNAc-derivatized disaccharide (equatorial C-4, compound 3) tends to be more potent than the trisaccharide (equatorial C-4, compound 1) and the Gal-derivatized disaccharide (axial C-4, compound 12) can also be attributed to an effect of the 2-N-acetyl group (compound 3) versus the 2-OH group (compound 12).

Azido and amino groups at C-2 of the glucosyl group of sialyl-Le^a were found to enhance the binding to E-selectin by a factor of 4–6 (29). In the case of CD22 ligands, azido and amino groups at C-2 of the anomerically free sugar did not increase the affinity. Substitution of the acetyl group at C-2 of the disaccharide with a more lipophilic moiety (benzoyl, naphthoyl, or octanoyl) impaired binding to CD22 (compounds 4–6 versus 1, respectively). The latter results may seem somewhat unexpected in view of reports in which insertion of lipophilic groups confers more avid binding in various ligand-receptor systems (4, 30–34). Generally, the in vitro competition data of dis- and trisaccharides demonstrate that (a) the affinity is enhanced by introducing 4-nitroaryl groups at C-1 or C-2 of the reducing hexose; (b) the affinity is not influenced by insertion of either lipophilic or cationic groups at C-2 of the reducing end glycoside; and (c) ligand recognition is rather tolerant toward modification of the 1- or 2-position, although an electron-rich 4-nitrobenzoyl group at C-2 significantly improves the affinity. The most potent saccharide, the nitrobenzoyl derivative 9, displayed an almost 10-fold higher affinity for CD22 compared with the reference compound Neu5Ac(a2,6)LacO-Me. Apparently, optimization of the penultimate carbohydrate moiety is an effective entry to improving the affinity for CD22.

To further strip the minimal binding motif for high affinity binding to CD22, we also synthesized and tested a series of monosaccharide analogs. Insertion of side groups at C-2 and C-5 only marginally affected the affinity for CD22 (2, 15, 16). The binding affinity of both mono- and disaccharides tends to be increased by the presence of a nitro aromatic group. In general, the affinity of the monosaccharides is essentially similar to that of the di- and trisaccharides. The above data may therefore suggest that the minimal motif required for binding to CD22 may be the Neu5Ac monosaccharide rather than the disaccharide Neu5Ac(a2,6)Hex, as has previously been proposed (16). The penultimate sugar may then confer receptor specificity rather than a gain in binding energy.

It has been established for various lectins, including the asialoglycoprotein receptor, the mannose receptor, E-selectin, and myelin-associated glycoprotein, that ligand recognition greatly benefits from multimeric presentation (4, 22–26). The fact that the endogenous substrates for CD22 (orosomucoid and thyroglobulin) have multiple terminal sialoside groups suggests that the affinity of a ligand for CD22 may be further improved by enhancing its valency as well. To map the effect of ligand valency, we prepared bi-, tri-, and tetravalent glutamate-derived clusters (compounds 21a, 21b, and 21c) based on the most potent disaccharide, compound 9. The di-, tri-, and tetravalent clusters all displayed a 10-fold higher affinity for CD22 compared with the monomeric compound 9. Apparently, optimal recognition by CD22 is already attained for bivalent glycosides. This contrasts with the results of Powell and Varki (15), who observed, in a column binding assay, that the apparent affinity for CD22 is consistently increased upon increasing the number of sialic acid residues to up to 4. However, their study was quantitative, and no absolute affinities were given for the tri- and tetravalent clusters. Accordingly, their study does not allow firm conclusions on the actual gain in affinity after di-, tri-, and tetravalent presentation. In addition, due to the high flexibility of the cluster backbones used in our study, we anticipate that the ligand configuration giving optimal receptor binding is more readily accomplished than in the case of the rigid glycoside backbone of Powell and Varki (15).

Likewise, symmetric clusters of trisaccharide 9 and monosaccharide 19 based on a Tris dendritic core (compounds 22 and 23) showed a 10–30-fold higher affinity for CD22 compared with the corresponding monovalent motif. This conclusively establishes the presence of a “cluster” effect for CD22 (15, 16) and excludes that the observed gain in affinity could be attributed to the backbone itself. The trivalent monosaccharide (compound 23), being 10-fold more potent than the monosaccharide sialoside 19, may represent a good compromise between high affinity for CD22 on the one hand and synthetic accessibility on the other. It remains to be determined how the affinity for CD22 compares with that for other siglecs such as sialoadhesin and P-selectin (20).

In the final stage of ligand design, we investigated whether the affinity of multimeric compounds for CD22 can be further...
improved beyond the bivalent level by proper spacing of the terminal glycosides. Three tetravalent clusters of compound 19 were synthesized with C₆, C₈, and C₁₀ spacers interconnecting the disubstituted glutamyls. Insertion of a flexible elongated spacer did not further enhance the affinity. Apparently, the observation that optimal binding to CD22 was already attained for bivalent ligands is not caused by suboptimal spacing of the terminal sugar moieties within a tri- or tetravalent cluster. The higher flexibility and symmetry of the Tris cluster backbone may explain the 3-fold higher affinity of the Tris-based cluster 22 compared with the glutamate cluster 21c.

In conclusion, multivalent ligands display a 10-fold higher affinity for CD22 compared with the corresponding monovalent motifs regardless of the terminal sugar moiety, the spacing of the terminal glycosides, and the nature of the backbone. Optimal recognition was attained for bivalent structures. To our knowledge, compound 22 is the most potent antagonist for CD22 yet synthesized. Further evaluation of the specificity of the terminal glycosides, and the nature of the backbone. Optimal recognition was attained for bivalent structures. To our knowledge, compound 22 is the most potent antagonist for CD22 yet synthesized. Further evaluation of the specificity of the terminal glycosides, and the nature of the backbone. Optimal recognition was attained for bivalent structures. To our knowledge, compound 22 is the most potent antagonist for CD22 yet synthesized. Further evaluation of the specificity of the terminal glycosides, and the nature of the backbone. Optimal recognition was attained for bivalent structures. To our knowledge, compound 22 is the most potent antagonist for CD22 yet synthesized. Further evaluation of the specificity of the terminal glycosides, and the nature of the backbone. Optimal recognition was attained for bivalent structures. To our knowledge, compound 22 is the most potent antagonist for CD22 yet synthesized. Further evaluation of the specificity of the terminal glycosides, and the nature of the backbone. Optimal recognition was attained for bivalent structures.

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