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Towards Selective Recognition of Sialic Acid Through Simultaneous Binding to Its *cis*-Diol and Carboxylate Functions

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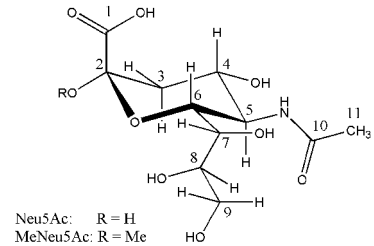
A series of receptors containing phenylboronic acid and urea or thiourea units have been designed for simultaneous recognition of the *cis*-diol and carboxylate functions of sialic acids, which are known to be overexpressed on the surfaces of tumor cells. The interaction of the receptors with 5-acetylneuraminic acid (Neu5Ac) and 2-α-*O*-methyl Neu5Ac (MeNeu5Ac) in DMSO solution has been investigated by means of spectrophotometric titrations and ^{1}H, ^{13}C, and ^{11}B NMR spectroscopy. Additionally, we have also investigated the binding of these receptors with competing monosaccharides such as D-(+)-glucose, D-fructose, methyl α-D-galactoside, and methyl α-D-mannoside. Our results show that 2-{[3-(4-nitrophenyl)thioureido]methyl}phenylboronic acid (**3a**) recognizes both Neu5Ac and MeNeu5Ac with good selectivity with regard to the remaining monosaccharides investigated. DFT calculations performed at the B3LYP/6-31G(d) level show that this selectivity is due to a cooperative two-site binding of Neu5Ac through 1) ester formation by interaction at the phenylboronic acid function of the receptor and 2) hydrogen-bond interaction between the thiourea moiety and the carboxylate group of Neu5Ac. Compound **3a** can therefore be considered a promising synthon for the design of contrast agents for magnetic resonance imaging of tumors. In contrast, the analogue of **3a** containing a urea moiety – compound **3b** – displays strong binding to all monosaccharides investigated, due to two-site binding through interaction on the phenylboronic acid function of the receptor and a hydrogen-bond interaction between the urea moiety and the sugar hydroxy groups.

Introduction

Most of the currently available contrast agents for magnetic resonance imaging (MRI) are small Gd^{III} complexes with polyaminocarboxylate ligands such as DTPA and DOTA (DTPA = diethylenetriamine-*N*,*N*,*N*′,*N*′′,*N*′′-pentaacetate; DOTA = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetate).[1] However, these MRI contrast agents distribute over the extracellular space in a non-specific way. There is therefore currently great interest in the development of more specific contrast agents that would be able either to accumulate in the target tissue[2] or to report on their biological environments through molecular recognition mechanisms.[3] A specific MRI contrast agent could take advantage of these molecular recognition processes to respond to functional groups occurring at higher concentrations in the diseased tissue. Sialic acid, for instance, is considered to be a tumor marker because it is known to be overexpressed on the surfaces of tumor cells.[4]

The most common member of the sialic acid family is the nine-carbon amino sugar 5-acetylneuraminic acid (Neu5Ac, Scheme 1).[5] Neu5Ac and its derivatives generally occupy the terminal positions of carbohydrate chains of glycoproteins and glycolipids in biological membranes, and they appear to play important roles in cellular recognition processes.[6] Neu5Ac exists in solution in equilibrium between its α-pyranose (5–8%) and β-pyranose forms (92–95%), but the furanose type of Neu5Ac is absent because of the acetamide moiety present at the C5 position.[7] It must be pointed out, however, that all known glycosides of Neu5Ac are α-linked, often to galactose or *N*-galactosamine residues through α(2→3) or α(2→6) linkages.[8]



Scheme 1. α-5-Acetylneuraminic acid (Neu5Ac) and 2-α-*O*-methyl-5-acetylneuraminic acid (MeNeu5Ac).

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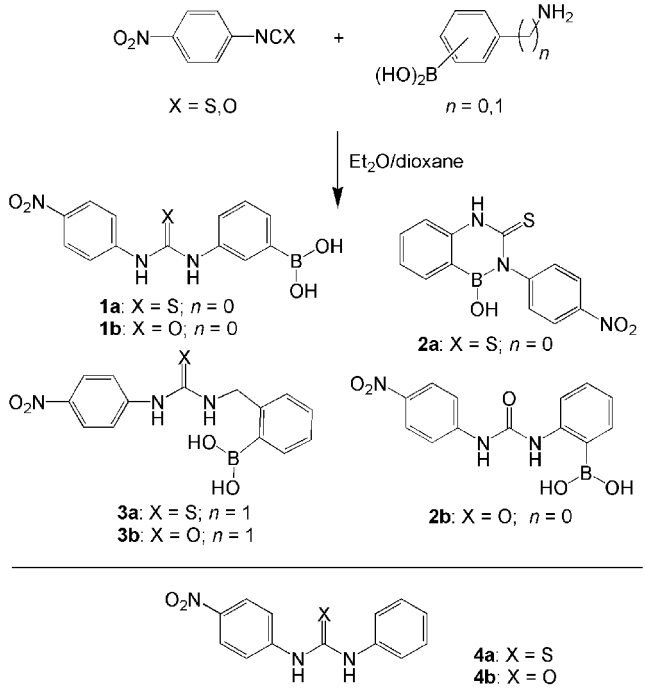
Boronic acids are a promising class of recognition moieties for the synthesis of artificial receptors and sensors for saccharides.[9] The use of boronic acids for this purpose is based on their ability to form reversible covalent complexes with 1,2- and 1,3-diol units in saccharides. Detailed investigations of the interactions of Neu5Ac with phenylboronic acid[10] and phenylboronic acid derivatives[11] have been reported in the literature. Moreover, a few conjugates of phenylboronic acid and lanthanide(III) complexes of DTPA,[12] DTPA-bisamides,[13] and DOTA-tetraamides[14] have also been reported. It has been suggested that a more selective artificial receptor for sialic acid residues in glycoproteins should contain both a phenylboronic acid function and a group capable of recognizing the negatively charged COO^- groups of sialic acids (Scheme 1).[13] In particular, a specific MRI contrast agent for sialic acid recognition should bind selectively with sialic acids in preference both to other sugar residues present in glycan chains and to saccharides such as glucose and fructose, which occur in relatively high concentrations in the blood.[15] Positively charged benzimidazolium and guanidinium-based receptors have recently been used for molecular recognition of Neu5Ac.[16] It has also been shown that bis-boronates can be designed to bind both the glycerol tail and the hydroxy acid at the anomeric center.[17]

Urea- and thiourea-based receptors are known to be suitable for anion recognition and, in particular, can establish complementary hydrogen-bond interactions with Y-shaped anions such as carboxylates.[18] In view of this, we envisaged that synthetic receptors containing urea or thiourea moieties might be also useful for recognition of the COO^- group of sialic acid. A suitable receptor for sialic acid recognition might therefore be based on urea or thiourea units containing phenylboronic acid functions for the recognition of the 1,2- and 1,3-diol groups of sialic acids. However, the design of new targeting contrast agents selective for sialic acids requires insight into the optimal arrangement of the different recognition moieties in the receptor.

Here we report the receptors **1a**, **1b**, **2b** **3a**, and **3b** (Scheme 2), which contain thiourea (**1a** and **3a**) or urea (**1b** and **3b**) groups and phenylboronic acid functions. Furthermore, we also report the benzodiazaborine receptor **2a**. We show that **2a** maintains the cyclic benzodiazaborine form in solution and is therefore not able to interact with 1,2- and 1,3-diol groups of sugars. Compound **2b**, however, is capable of recognition of sialic acids, due to its open structure. With the goal of evaluation of a possible synergetic effect of the (thio)urea and pheylboronic acid functions on the binding of sialic acids we also report the receptors **4a** and **4b**. The interactions of the eight receptors with Neu5Ac and with other competing monosaccharides such as D-(+)-glucose or D-fructose were also investigated in DMSO solution by means of spectrophotometric titrations and ^1H, ^{13}C, and ^{11}B NMR spectroscopy. Additionally, we have also investigated the interactions of these receptors with methyl α-D-galactoside and methyl α-D-mannoside, which are models of units commonly present in glycan chains. As a model for neuraminic residues present in the terminal positions of carbohydrate chains we selected 2-α-*O*-methyl Neu5Ac (MeNeu5Ac). The X-ray crystal structures of four of the eight receptors are reported. Finally, the binding trends of the different receptors were interpreted with the aid of DFT calculations at the B3LYP/6-31G(d) level.



Scheme 2. Receptors reported in this work.

Results and Discussion

Synthesis and Characterization of the Receptors

Compounds **1a**, **1b**, **2b**, **3a**, and **3b** were easily obtained each in one step by the standard method involving the reactions between amines and phenyl iso(thio)cyanates. However, the reaction between (2-aminophenyl)boronic acid and 4-nitrophenyl isothiocyanate under analogous conditions resulted in the formation of the benzodiazaborine **2a** (Scheme 2).[19] All six receptors were fully characterized by conventional techniques. The ^1H and ^{13}C NMR spectroscopic data for these receptors are summarized in Tables S1 and S2 in the Supporting Information. The ^1H NMR spectra of compounds **1a**, **1b**, **2b**, **3a**, and **3b** (500 MHz, [D_6]-DMSO) each show, for instance, two signals due to (thio)-urea protons and one signal due to the –OH protons of the phenylboronic acid function. The ^1H NMR spectrum of **2a** confirms the formation of the benzodiazaborine unit, because it shows only one resonance due to NH protons, together with one signal of the same intensity due to the –OH protons. The structure proposed for compound **2a** on the basis of the spectroscopic data was confirmed by single-crystal X-ray diffraction analysis (Figure 1). The B(1)–N(2)

distance [1.465(2) Å] is considerably shorter than that expected for a B–N single bond [1.57–1.63 Å], which suggests a partial double bond character of this bond in the benzodiazaborine **2a**. The benzodiazaborine unit is essentially coplanar (mean deviation from planarity 0.02343 Å) and forms an angle of 74.6° with the plane described by the nitrophenyl unit.

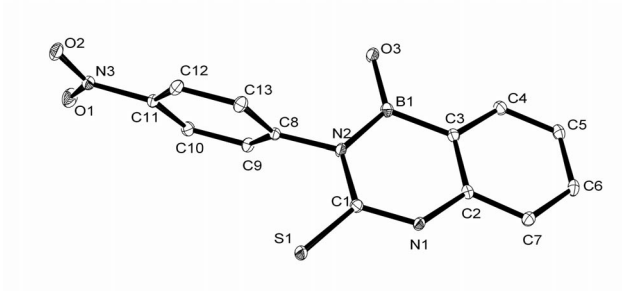


Figure 1. ORTEP diagram (30% probability level) of the crystal structure of **2a**.

Receptors **4a** and **4b** were prepared by a slight modification of the reported procedure.[20]

Single crystals of **1b**, **4a**, and **4b** were grown in DMSO/H_2O (1:1, v:v) mixtures. Compounds **1b** and **4a** crystallized as DMSO solvates, whereas **4b** crystallized in the unsolvated form. The X-ray crystal structure of a DMF solvate of **4b** has been reported previously.[20]

Compound **4b** crystallizes in the monoclinic $P2_1$ space group and the asymmetric unit contains one molecule. Self assembly in **4b** occurs through the characteristic urea α-network of N–H···O hydrogen bonds (Figure 2).[21] The urea groups are aligned along the *a*-axis, and the phenyl and nitrophenyl groups are twisted out of the urea plane by 43.1 and 43.7°, respectively, to accommodate the hydrogen bonded α-network. Weak C–H···O interactions appear to provide additional support to the crystal packing.

Compound **1b**·DMSO crystallizes in the monoclinic $P2_1/c$ space group and the asymmetric unit contains two urea and two DMSO molecules. The urea molecule adopts a flat planar conformation stabilized by intramolecular C–H···O hydrogen bonds involving the O atom of the urea group, further stabilized by resonance in the extended aromatic molecule (Figure 2).[22] The presence of the phenylboronic acid function disrupts the urea α-network through intermolecular N–H···OBC_6H_4 hydrogen bonds. The DMSO molecules stabilize the crystal packing through the formation of DMSO···$HOBC_6H_4$ hydrogen bonds.

Compound **4a**·DMSO crystallizes in the monoclinic $P2_1/n$ space group and the asymmetric unit contains one thiourea and one DMSO molecule. The phenyl and nitrophenyl groups are twisted out of the thiourea plane by 39.8 and 26.3°, respectively, as previously observed for different diaryl thioureas.[23] The sulfoxide oxygen of DMSO acts as a bifurcated acceptor to the thiourea NH donors (Figure 2).

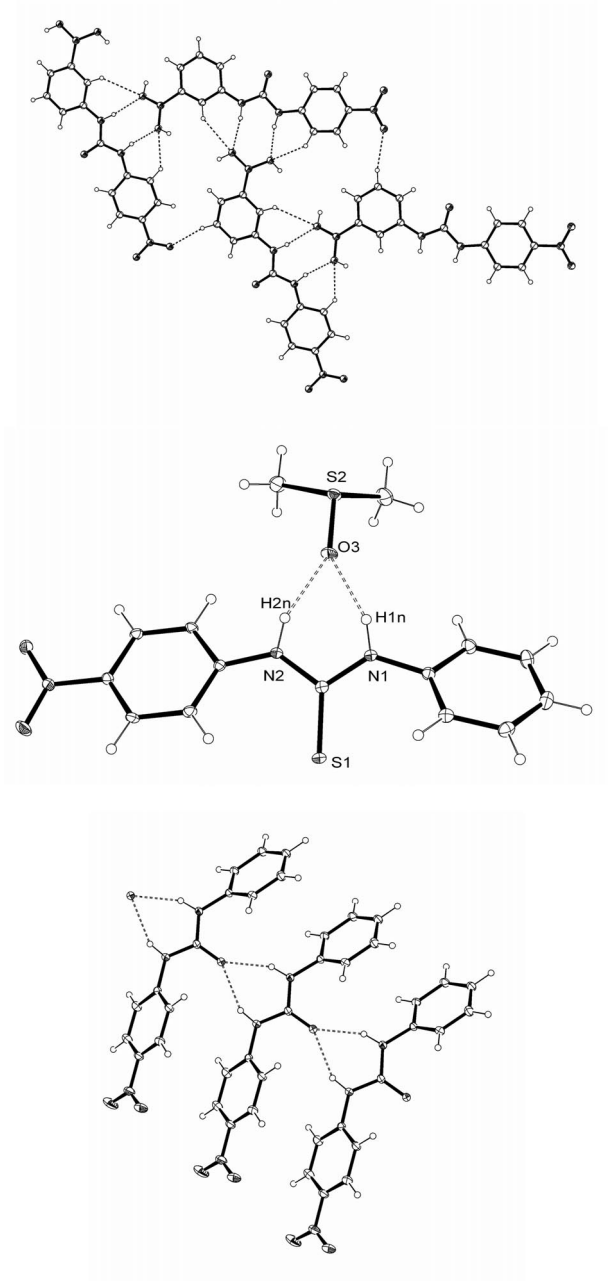


Figure 2. ORTEP diagrams (30% probability) of the crystal structures of **1b** (top), **4a** (middle), and **4b** (bottom), highlighting the H-bond interactions involving the (thio)urea units.

Study of the Interactions of 1a/b–4a/b with CH_3COO^-

Receptors **1a/b**–**3a/b** were designed for simultaneous recognition of the *cis*-diol and carboxylate functions of Neu5Ac. We therefore initially investigated the interactions of these receptors with acetate as a model for the carboxylate function of Neu5Ac (Scheme 1). The binding of receptors **1a/b**–**3a/b** with CH_3COO^- was followed by means of spectrophotometric titrations in DMSO solution: a solution of the receptor (10^{-4} M) was titrated with a standard solution of the anion (as the tetrabutylammoniun salt) up to a five- to tenfold excess. The absorption spectra of the thioureas **1a** and **3a** each show a maximum at ca. 360 nm,

whereas those of the ureas **1b**, **2b**, and **3b** each show a maximum at ca. 350 nm. These absorption bands are typical of the nitrophenyl chromophore.[24] Nonlinear least-squares fits of the UV/Vis titration profiles allowed us to determine the binding constants listed in Table 1.

Table 1. Binding constants ($\log K$ values) determined for the interaction of receptors **1a/b**–**4a/b** with acetate in DMSO solution at 25 °C.[a]

1a[b]	$\log K_1$	5.28(6)	**3a**	$\log K_1$	5.07(1)
	$\log K_2$	4.45(5)			
1b	$\log K_1$	3.61(1)	**3b**	$\log K_1$	3.59(1)
2a	$\log K_1$	5.39(3)	**4a**[b]	$\log K_1$	5.49(2)
				$\log K_2$	5.05(5)
2b	$\log K_1$	2.87(2)	**4b**	$\log K_1$	3.35(5)

[a] The errors given correspond to one statistical deviation; the experimental errors are estimated to be ± 0.2 $\log K$ units. [b] The titration data were fitted according to the equilibria described by Equations (1) (K_1) and (2) (K_2).

The experimental data can be interpreted in terms of the equilibria shown in Equations (1) and (2).[25]

$$LH + CH_3COO^- \leftrightarrows [CH_3COO\cdots HL]^- \quad (1)$$

$$[CH_3COO\cdots HL]^- + CH_3COO^- \leftrightarrows L^- + [(CH_3COO)_2H]^- \quad (2)$$

All the investigated receptors undergo the first equilibrium [Equation (1)], characterized by the association constant K_1, giving a more or less stable hydrogen-bond complex. The second process [Equation (2)], characterized by an association constant K_2, involves the deprotonation of the receptor by a second CH_3COO^- anion, and so it is expected to occur in those cases in which the receptor is particularly acidic. This is indeed the case for thiourea **1a**, for which the titration data are best fitted on the assumption that the two consecutive processes described by Equations (1) and (2) are taking place. Upon addition of CH_3COO^- to a solution of **1a** in DMSO, the intensity of the band at 362 nm progressively decreases, while three new maxima at 290, 398, and 470 nm develop (Figure 3). The new band at 470 nm is characteristic for deprotonation of the receptor.[18c,26] A comparison of the equilibrium constants determined for **1a** and **4a** (Table 1) indicates that the introduction of the boronic acid function of **1a** does not have an important effect on the K_1 and K_2 values.

Unlike **1a** and **4a**, thiourea **3a** does not undergo deprotonation upon addition of CH_3COO^-, probably because **3a** is a weaker acid than the former receptors. Indeed, upon addition of CH_3COO^- to a solution of **3a** in DMSO the intensity of the band at 360 nm progressively decreases, while the formation of a new band at 389 nm is observed (Figure 3). However, no band characteristic of the deprotonation of the receptor at ca. 470 nm is observed. The bathochromic shift of the absorption maximum at 360 nm indicates anion recognition through hydrogen bonding.[27] Indeed, the electronic excitation in (4-nitrophenyl)thioureido receptors is accompanied by charge transfer from the donor nitrogen of the thiourea to the acceptor substituent of the chromophore ($-NO_2$).[28] The coordination of acetate to the

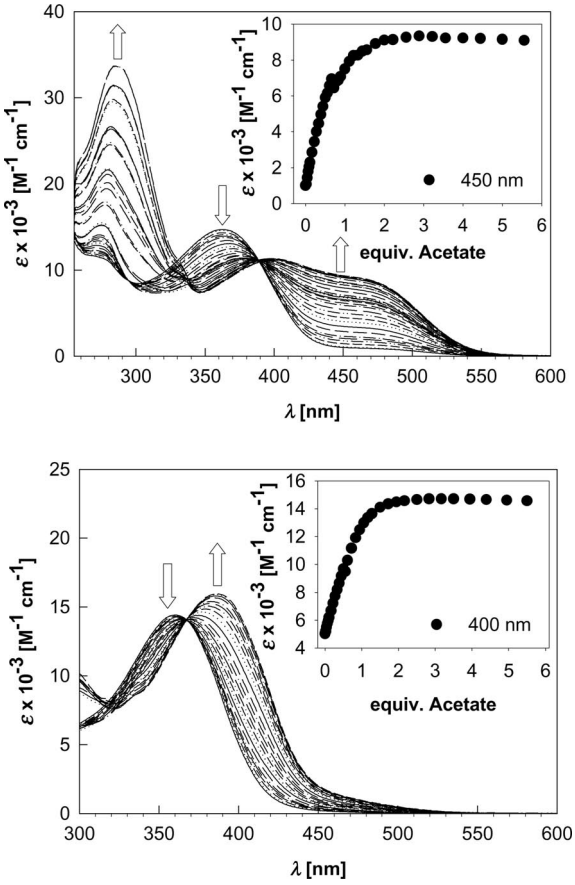


Figure 3. Spectra taken during the course of a titration of a DMSO solution of **1a** (top) or **3a** (bottom) with a standard solution of $[Bu_4N]CH_3COO$. Insets: changes in the molar absorbance at selected wavelengths upon addition of CH_3COO^-.

thiourea moiety increases the electron-donor properties of the donor, due to the negative charge of the anion. Anion binding provides stabilization of the excited state, resulting in a bathochromic shift. The $\log K_1$ value obtained for the interaction between **3a** and CH_3COO^- is only slightly lower than those obtained for **1a** and **4a**, indicating similar capabilities of the three receptors to enter into H-bond interactions with acetate.

The data reported in Table 1 indicate that the thiourea-containing receptors enter into stronger H-bond interactions with CH_3COO^- than their urea-containing counterparts. This would be expected, however, because thiourea is a much stronger acid than urea (pK_a = 21.1 and 26.9, respectively in DMSO).[29] This is also the reason why thioureas **1a** and **4a** undergo deprotonation of the N–H groups in the presence of CH_3COO^-, whereas for the urea analogues **1b**, **2b**, **3b**, and **4b** this situation is not observed. The spectral variations observed in the ureas **1b**, **2b**, **3b**, and **4b** are also less pronounced than those observed for the thiourea analogues. Figure 4 shows the representative example of **1b**. It can be seen that the band of the free receptor at 353 nm decreases upon anion addition, while a new maximum is formed at ca. 370 nm. As mentioned above, the bathochromic shift of the absorption maximum indicates

anion recognition. The binding constants reported in Table 1 show that receptor **2b** enters into weaker H-bond interactions with CH_3COO^- than **1b**, **3b**, and **4b**. This is attributed to the steric hindrance in the vicinity of the urea moiety caused by the presence of the $-B(OH)_2$ group in the *ortho* position.

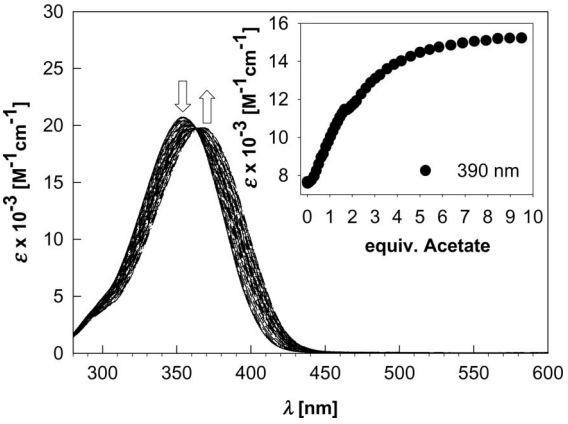


Figure 4. Spectra taken during the course of a titration of a DMSO solution of **1b** with a standard solution of $[Bu_4N]CH_3COO$. Inset: changes in the molar absorbance at 390 nm upon addition of CH_3COO^-.

^1H NMR spectroscopy confirms the binding of acetate to the receptors **1b**, **2b**, **3b**, and **4b**. Indeed, addition of CH_3COO^- to solutions of the receptors in DMSO causes important downfield shifts of the two N–H proton signals, which reflects the establishment of H-bond interactions with the anion (Figure 5).[18a,30] We also note that in the cases of **1b**, **2b**, and **3b** the –OH protons of the phenylboronic acid groups undergo significant downfield shifts upon addition of acetate. In contrast, the positions of the signals due to aromatic protons are less affected by the interaction with acetate. Even so, the protons of the nitrophenyl unit closer to the urea moiety experience a relatively significant downfield shift (ca. 0.18 ppm), whereas those positioned further away from the urea unit are shifted upfield by ca. 0.07 ppm (Figure 5). These spectral variations also indicate H-bond interaction with the anion.[18a] The $\Delta\delta$ values experienced by the –NH and –OH protons show saturation profiles that confirm the 1:1 stoichiometries of the supramolecular adducts (Figure 5).

The benzodiazaborine compound **2a** also shows interesting properties in relation to the recognition of CH_3COO^-. The absorption spectrum of **2a** has two maxima, at 290 and 350 nm (Figure 6). Upon addition of acetate the intensities of these maxima decrease, while two new bands at 280 and 390 nm are formed. The spectrum of the free receptor is very different to that recorded for, for instance, receptor **1a**, which indicates that the benzodiazaborine unit remains intact in DMSO solution (see Figure 3 and Figure 6). This is also confirmed by the ^1H NMR spectra (see Figure S1 in the Supporting Information). The ^1H NMR spectrum of the free receptor shows two signals of equal intensity at 12.14 and 9.67 ppm, which are attributed to –NH and –OH protons, respectively. Anion addition results in a dramatic

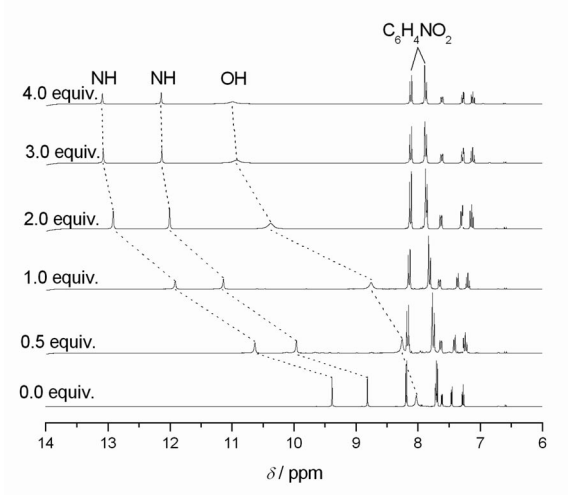


Figure 5. ^1H NMR spectra taken over the course of a titration of a DMSO solution of **1b** (10 mM) with $[Bu_4N]CH_3COO$.

upfield shift of the resonance due to the –NH proton. Upon addition of 3 equiv. of the anion this signal is observed at δ = 9.69 ppm, which clearly confirms acetate recognition by the receptor. It has long been established that triarylboranes interact with anions such as fluoride or cyanide to form the corresponding borate complexes.[31] The results reported here for **2a** demonstrate that benzodiazaborines, which also contain a trigonal planar boron center, can be also used for anion recognition purposes.

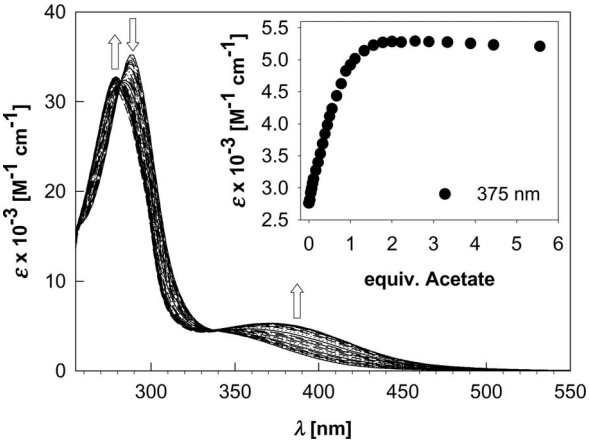


Figure 6. Spectra taken during the course of a titration of a DMSO solution of **2a** with a standard solution of $[Bu_4N]CH_3COO$. Inset: changes in the molar absorbance at 375 nm upon addition of CH_3COO^-.

Study of the Interactions between 1a/b-4a/b and Carbohydrates

The interactions between the receptors **1a/b**–**3a/b** and Neu5Ac and MeNeu5Ac were followed by means of spectrophotometric titrations on DMSO solutions of the receptors (10^{-4} M). These experiments were performed in the presence of equimolar amounts of triethylamine to ensure the deprotonation of both Neu5Ac and MeNeu5Ac. To

confirm that the functioning of the phenylboronic moieties of the receptors were not affected by triethylamine, ^{11}B NMR shift measurements were performed on phenylboronic acid (PBA) upon addition of the base in DMSO (Figure S2 in the Supporting Information). The chemical shift of 9 ppm indicated that the boron was remaining in the trigonal planar configuration.[10] In addition to Neu5Ac and MeNeu5Ac, we also investigated the affinities of these receptors for competing monosaccharides such as D-(+)-glucose, D-fructose, methyl α-D-galactoside, and methyl α-D-mannoside. Methyl α-D-galactoside and methyl α-D-mannoside are models of units commonly present in glycan chains, whereas glucose and fructose occur at relatively high concentrations in the blood. Aiming to understand whether or not binding to the (thio)urea moiety plays an important role in the recognition of Neu5Ac, we also investigated the interactions of these sugars with receptors **4a** and **4b**, which each contain a (thio)urea unit, but lack a phenylboronic acid moiety (Scheme 2). Furthermore, spectrophotometric titrations to investigate the interactions of these sugars with PBA in DMSO solution were also carried out.

The spectrophotometric titration of **1b** with Neu5Ac shows two inflection points at Neu5Ac/**1b** molar ratios close to 1 and 2, indicating the stepwise interaction of two Neu5Ac molecules with the receptor (Figure 7). ^1H NMR spectroscopy confirms the stepwise binding of two Neu5Ac units to this receptor. Indeed, **1b** shows two signals at δ = 9.39 and 8.82 ppm due to the –NH groups of the urea moiety. Addition of Neu5Ac to a solution of **1b** in DMSO (30 mM) results in the formation of two new signals for the –NH protons at ca. 9.28 and 8.60 ppm. Moreover, the signals due to the protons of the phenylboronic acid function at δ = 7.28, 7.46, and 7.62 ppm undergo sgnificant shifts upon addition of Neu5Ac. In contrast, the signals of the proton nuclei of the nitrophenyl unit at δ = 8.19 and 7.69 ppm are almost unaffected (Figure 7). This indicates that the interaction between **1b** and Neu5Ac occurs through the phenylboronic acid function of the receptor. ^{11}B NMR spectra additionally demonstrate the change of boron hybridization from trigonal to tetrahedral (chemical shift around –15 ppm), which is a result of covalent binding to Neu5Ac (Figure S3 in the Supporting Information). The binding would be expected to involve both the glycerol tail and the α-hydroxycarboxylic acid group of sialic acid. These two binding profiles were shown to be feasible by measurement of ^{11}B NMR spectra of **1b** with equimolar amounts of fructose and lactic acid in DMSO. In both cases the tetrahedral configuration of boron observed by NMR indicated binding through boronate ester formation (Figure S4 in the Supporting Information). After addition of two equivalents of Neu5Ac the signals corresponding to the free receptor in the proton spectrum have nearly disappeared. Addition of a larger excess of Neu5Ac causes significant downfield shifts of the two N–H proton signals, which reflects the establishment of a H–bond interaction between the sugar and the thiourea moiety of the receptor.[18a] A similar situation is observed in the ^1H NMR spectrum of **1a** upon addition of Neu5Ac (Figure S5 in the Supporting Information).

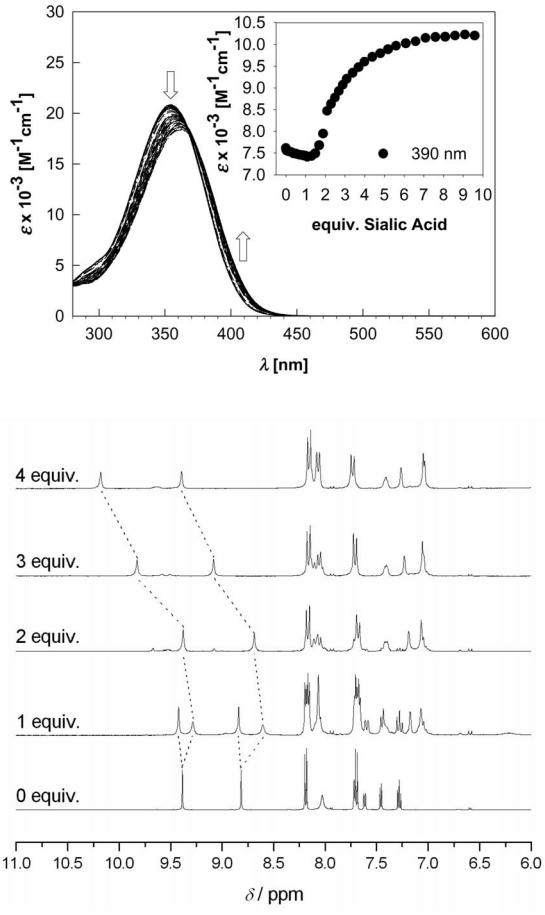


Figure 7. Top: UV/Vis spectra taken during the course of a titration of a DMSO solution of **1b** (10^{-4} M) with a standard solution of Neu5Ac and Et_3N. Inset: changes in the molar absorbance at 390 nm upon addition of Neu5Ac. Bottom: ^1H NMR spectra taken over the course of a titration of a DMSO solution of **1b** (30 mM) with Neu5Ac and equimolar Et_3N.

^{13}C NMR spectra taken after addition of different amounts of Neu5Ac provide a better insight into the binding mode. Notably, no peaks corresponding to the free Neu5Ac were found even in the presence of 2 equiv. This indicates that after the binding at the phenylboronic acid group of the receptor has occurred, an additional interaction at the thio(urea) moiety takes place. It also explains the strong chemical shift of the thiourea carbon (from 179 to 169 ppm), not observed when the equimolar amounts of Neu5Ac and the receptor are used. These data support a 1:2 (receptor:Neu5Ac) binding ratio at high concentrations of sugar. We thus conclude that receptors **1a** and **1b** are not able to recognize Neu5Ac through its simultaneous binding to the phenylboronic acid and (thio)urea functions of the receptors. Instead, Neu5Ac binds preferentially to the phenylboronic acid unit, with use of an excess of Neu5Ac also resulting in binding to the (thio)urea moiety. This picture is also in line with the UV/Vis spectral changes observed upon addition of Neu5Ac (Figure 7). Indeed, at the first stages of the titration addition of Neu5Ac causes only a slight decrease in the absorption band of **1b** at 353 nm, whereas

addition of an excess of Neu5Ac induces the characteristic bathochromic shift that signals carboxylate recognition.

As expected, the benzodiazaborine compound **2a** binds Neu5Ac, but no interaction between this receptor and the other monosaccharides studied in this work is observed. The spectral changes observed in the UV/Vis spectrum of **2a** upon addition of Neu5Ac are very similar to those observed upon interaction with acetate (see Figure 6 and Figure S6 in the Supporting Information). These results indicate that Neu5Ac binds to **2a** through the carboxylate function. ^{11}B NMR spectra additionally demonstrate the change in the boron hybridization from trigonal to tetrahedral (chemical shift around –15 ppm) resulting from covalent binding to Neu5Ac (Figure S6 in the Supporting Information). However, the binding constant obtained for the interaction with Neu5Ac ($\log K_1$ = 4.71, Table 2) is slightly lower than that obtained for the interaction with CH_3COO^- ($\log K_1$ = 5.39). This can be attributed to the larger steric demand of Neu5Ac.

Table 2. Binding constants ($\log K$ values) determined for receptors **2a/b**–**4a/b** in DMSO solution at 25 °C.[a]

	Neu5Ac	MeNeu5Ac	Fructose	Glucose	MeGal	MeMann
2a	4.71(1)	[c]	[b]	[b]	[b]	[b]
2b	3.41(1)	3.19(4)	2.19(1)	2.32(1)	2.06(1)	2.77(2)
3a	4.78(1)	4.38(4)	3.49(4)	3.24(4)	2.75(2)	2.95(4)
3b	4.65(1)	3.14(6)	5.51(5)	4.69(2)	5.02(1)	4.58(3)
4a	2.91(2)	[c]	[b]	[b]	[b]	[b]
4b	2.63(3)	[c]	[b]	[b]	[b]	[b]
PBA	3.10(1)	[c]	3.28(7)	3.21(4)	2.29(8)	2.28(8)

[a] The errors given correspond to one statistical deviation; the experimental errors are estimated to be ±0.2 $\log K$ units. [b] No apparent binding, as reflected in the lack of changes observed in the UV/Vis spectral titrations upon addition of saccharide. [c] Not determined.

The UV/Vis spectrum of **2b** shows a maximum at 290 nm, the intensity of which quickly diminishes upon addition of Neu5Ac; addition of Neu5Ac also results in the formation of a new band at ca. 350 nm (Figure S7 in the Supporting Information). Spectrophotometric titrations of **2b** with D-(+)-glucose, D-fructose, methyl α-D-galactoside, or methyl α-D-mannoside result in similar spectral variations. The binding constants obtained from the non-least-squares fit of the UV/Vis titration data are shown in Table 2. The $\log K_1$ values obtained for D-(+)-glucose, D-fructose, and methyl α-D-galactoside are slightly lower than those obtained for the interactions of these sugars with PBA. However, the $\log K_1$ value obtained for the interaction between **2b** and Neu5Ac is slightly higher than that obtained for phenylboronic acid (ca. 0.3 $\log K$ units), and also slightly higher than the binding constant of **2b** with acetate (ca. 0.5 $\log K$ units). Furthermore, **2b** binds more strongly to Neu5Ac than **4b**. This can be attributed to simultaneous recognition of the carboxylate and *cis*-diol functions of Neu5Ac by this receptor, which results in a certain degree of selectivity for Neu5Ac with respect to the remaining saccharides studied in this work.

The UV/Vis spectra of receptors **3a** and **3b** show maxima at 360 and 350 nm, respectively (Figure 8; see also Figure S8 in the Supporting Information). Addition of Neu5Ac results in a red shifting of the absorption maxima, while their intensities remain almost unaffected. These spectral variations are indicative of the recognition of the carboxylate function of Neu5Ac by the (thio)urea moiety (see above). At the same time, ^{11}B NMR shift measurements reported binding at the phenylboronic group of the receptor, through the presence of boron in its tetrahedral configuration, upon addition of different amounts of Neu5Ac (Figure S8 in the Supporting Information). ^{13}C NMR spectra taken at different Neu5Ac/**3a** ratios indicate simultaneous binding of the receptor to the glycerol tail and hydrogen-bond formation with the thiourea unit. At Neu5Ac/**3a** molar ratios of 1 and 2, no peaks corresponding to the free receptor were detected. After addition of an excess of the receptor the peaks of free **3a** appeared, but all resonances corresponding to the bound species remained unchanged in the spectrum. This indicates that the simultaneous binding through the phenylboronic group and the thio(urea) moiety takes place regardless of the amount of Neu5Ac used for the interaction. The overall complexity of the ^{13}C NMR spectra of the receptors upon binding with Neu5Ac can be explained in terms of the variety of the bound species due to the diastereomeric character of the boronate esters that are formed. Analysis of the spectrophotometric titrations provides especially high binding constants for the interactions of **3a** and **3b** with Neu5Ac: $\log K_1$ = 4.78(1) and 4.65(1), respectively. These binding constants are substantially higher than those obtained for the interaction of **4a** or **4b** with Neu5Ac. Moreover, they are also ca. 1.6 $\log K$ units higher than those obtained for the interaction of Neu5Ac with phenylboronic acid. It can thus be concluded that receptors **3a** and **3b** bind to Neu5Ac both through their (thio)urea and through their phenylboronic acid functions, such cooperative binding being responsible for the especially high binding constants observed. Of these two receptors, **3a** displays an important selectivity for Neu5Ac with

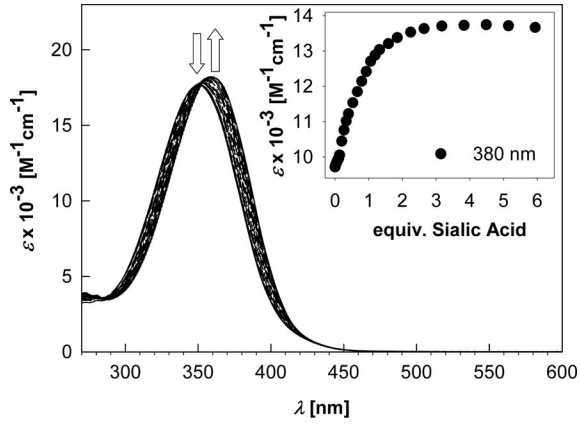


Figure 8. UV/Vis spectra taken during the course of a titration of a DMSO solution of **3b** with a standard solution of Neu5Ac and Et_3N. Inset: changes in the molar absorbance at 380 nm upon addition of Neu5Ac.

respect to potentially competing monosaccharides such as D-(+)-glucose, D-fructose, methyl α-D-galactoside, and methyl α-D-mannoside. This, however, is not the case with receptor **3b**, because the binding constants determined for the interaction with these monosaccharides are higher than that with Neu5Ac. This receptor has a particularly high affinity for D-fructose [$\log K$ = 5.51(5), Table 2]. The binding constants reported in Table 2 show that the substitution of the thiourea moiety of **3a** by a urea group does not substantially affect the affinity of the receptor for Neu5Ac, but increases the binding constants towards the remaining saccharides by 1.45–2.27 $\log K$ units.

Neu5Ac exists in solution in equilibrium between its α-pyranose (5–8%) and its β-pyranose forms (92–95%).[7] Thus, in solution the major form of free Neu5Ac is expected to be the β-pyranose form, whereas all known glycosides of Neu5Ac are α-linked.[8] We therefore investigated the interactions of the receptors **2b**, **3a**, and **3b** with 2-α-*O*-methyl Neu5Ac (MeNeu5Ac) as a model for neuraminic residues present in the terminal positions of carbohydrate chains. The changes observed in the absorption spectra of the receptors are similar to those observed upon addition of Neu5Ac (Figure S9 in the Supporting Information). The binding constants obtained are slightly lower than those obtained for Neu5Ac, which suggests that the β-pyranose form provides stronger binding to these receptors than the α-pyranose one. Even so, **3a** shows an important selectivity for MeNeu5Ac over other possible competing saccharides such as glucose, as well as over methyl α-D-galactoside and methyl α-D-mannoside, which are models of units commonly present in glycan chains. The receptor **2b** also shows a certain selectivity for MeNeu5Ac over these monosaccharides, whereas for **3b** this is not the case (Table 2).

To explain the selectivity of **3a** for Neu5Ac, we characterized the **3a**·Neu5Ac$^-$ system with the aid of DFT calculations [B3LYP/6-31G(d)]. This theoretical approach has previously been used to investigate hydrogen-bond interactions established between (thio)urea receptors and different substrates,[32] including carboxylates.[33] Phenylboronic acid is known to bind covalently and reversibly to 1,2- and 1,3-diols to give five- and six-membered cyclic esters.[10] It has previously been shown that the formation of a five-membered ester at C7 and C8 is limited by the unfavorable *erythro* configuration of the glycerol tail. In our calculations we therefore only considered the five-membered cyclic ester formed at C8 and C9 and the six-membered cyclic ester formed at C7 and C9 (Scheme 1). ^{11}B NMR studies demonstrate that the boron atom of the receptor is in tetrahedral hybridization upon binding to *cis*-diol functions (see above). For the reasons given above, our calculations were performed both for the α-pyranose and for the β-pyranose forms of Neu5Ac.

For the α-pyranose form of Neu5Ac our results show that the adduct formed at C8 and C9 is less stable than that formed at C7 and C9 by 15.9 kJ mol^{-1}. The optimized geometry of the adduct (Figure 9) indeed shows a cooperative two-site binding of Neu5Ac through 1) ester formation by interaction with the phenylboronic acid function of the receptor, and 2) a hydrogen-bond interaction between the thiourea moiety and the carboxylate group of Neu5Ac. Indeed, one of the oxygen atoms of the carboxylate group of Neu5Ac is involved in hydrogen-bond interaction with a –NH group of the thiourea unit [O···N 2.79 Å, O···HN 1.77 Å, O···H–N 166.0°]. Furthermore, a weak C–H···O interaction appears to provide additional support for the interaction of the carboxylate group and the receptor [O···C 3.33 Å, O···HN 2.25 Å, O···H–N 169.0°]. For the β-pyranose form, however, our calculations predict that the ester formed at C8 and C9 should be more stable than that formed at C7 and C9 by 30.8 kJ mol^{-1}. One of the oxygen atoms of the carboxylate group of Neu5Ac is involved in hydrogen-bond interaction with the two NH groups of the thiourea unit [Figure 9; O···N(1) 2.73 Å, O···HN(1) 1.71 Å, O···H–N(1) 164.6°; O···N(2) 2.95 Å, O···HN(2) 2.02 Å, O···H–N(2) 148.8°].

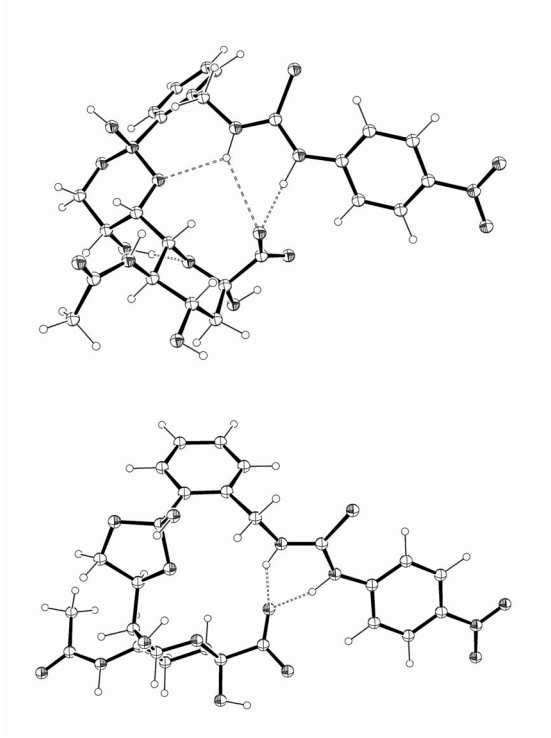


Figure 9. Structures of (top) the **3a**·α-Neu5Ac$^-$ and (bottom) the **3a**·β-Neu5Ac$^-$ systems obtained from DFT calculations at the B3LYP/6-31G(d) level.

According to our calculations the **3a**·β-Neu5Ac$^-$ system is more stable than the **3a**·α-Neu5Ac$^-$ one by 27.3 kJ mol^{-1}. This result is consistent with the lower association constants determined for the interaction of **3a** with MeNeu5Ac, which exists in its α-pyranose form, than with Neu5Ac, the major form of which adopts the β-pyranose form in solution.

DFT calculations at the B3LYP/6–31G(d) level were also performed on the **3b**·methyl α-D-galactoside system in an attempt to explain the reasons for the high affinities of this receptor for D-(+)-glucose, D-fructose, methyl α-D-

galactoside, and methyl α-D-mannoside. Methyl α-D-galactoside can potentially form five-membered esters at C2 and C3 or at C3 and C4 (Figure S10 in the Supporting Information). Additionally, a six-membered ester at position C4 and C6 is also possible. According to our calculations the formation of the six-membered ester at positions C4 and C6 is more favorable than the formation of five-membered esters at C2–C3 and C3–C4. This is nicely in agreement with experimental data reported in the literature.[34] The minimum-energy conformation obtained for the **3b**·methyl α-D-galactoside system (see Figure S10 in the Supporting Information) shows the hydroxy group at the C3-position involved in a hydrogen-bond interaction with the oxygen atom of the urea group (O···O 2.98 Å, O···HO 2.12 Å, O···H–N 146.2°). The high binding constants obtained for the interactions between **3b** and D-(+)-glucose, D-fructose, methyl α-D-galactoside, and methyl α-D-mannoside can thus be attributed to two-site binding through interaction at the phenylboronic acid function of the receptor and a hydrogen-bond interaction between the urea moiety and the hydroxy groups of the saccharides. The sulfur atom of thiourea is a poorer hydrogen-bond acceptor than the oxygen atom of urea,[35] so this cooperative effect is not observed for **3a**.

Conclusions

We have designed a series of receptors containing (thio)-urea and phenylboronic acid functions for the recognition of sialic acids. Our results show that receptors **1a** and **1b** are able to recognize Neu5Ac preferentially through their phenylboronic acid units. Additional binding to the (thio)-urea moiety can be observed when an excess of Neu5Ac is applied. No simultaneous binding of Neu5Ac to the functional groups was observed. The receptors **2b**, **3a**, and **3b**, however, bind to Neu5Ac through cooperative two-site binding involving 1) ester formation by interaction at the phenylboronic acid function of the receptor, and 2) a hydrogen-bond interaction between their (thio)urea moieties and the carboxylate group of Neu5Ac. Of these receptors, **3a** displays the highest binding affinity for Neu5Ac, combined with the best selectivity with respect to other competing saccharides. Furthermore, this receptor also shows good selectivity for MeNeu5Ac over methyl α-D-galactoside and methyl α-D-mannoside, which are models of units commonly present in glycan chains, as well as over glucose, which occurs in relatively high concentrations in blood. The results presented here may be useful for the design of specific MRI contrast agents for the recognition of sialic acid residues on the surfaces of tumor cells.

Experimental Section

General: 1-(4-Nitrophenyl)-3-phenylthiourea (**4a**)[36] and 1-(4-nitrophenyl)-3-phenylurea (**4b**)[20] were prepared by a slight modification of the literature methods with use of diethyl ether as solvent. All other chemicals were purchased from commercial sources and used without further purification. Solvents were of reagent grade and purified by the usual methods.

Elemental analyses were carried out with a ThermoQuest Flash 1112 elemental analyzer. ESI-TOF mass spectra were recorded with a LC-Q-q-TOF Applied Biosystems QSTAR Elite spectrometer in the negative mode. IR spectra were recorded with a Bruker Vector 22 spectrophotometer with a Golden Gate Attenuated Total Reflectance (ATR) accessory (Specac). ^{1}H and ^{13}C NMR spectra were run with Bruker Avance 300, Bruker Avance 500, or Varian Inova 300 spectrometers. Chemical shifts are reported in δ values. Spectral assignments were based in part on two-dimensional COSY, HMQC, and HMBC experiments. ^{11}B NMR was measured with a Bruker Avance 400 spectrometer with use of a solid-state probe and 4 mm zirconia rotors, spinning at the magic angle with a rate of 20 Hz. Peak positions were measured with respect to the resonance of a solution of boric acid (0.1 M, δ = 0 ppm). Electronic spectra in the UV/Vis range were recorded at 25 °C with a Perkin–Elmer Lambda 900 UV/Vis spectrophotometer in 1.0 cm quartz cells. Acetate- and sugar-binding studies were performed with DMSO solutions of the receptors (10^{-4} M, 10 mL). Typically, aliquots of a fresh standard solution of the envisaged substrate (0.1–0.01 M) in the same solvent were added and the UV/Vis spectra of the samples were recorded. For titrations with Neu5Ac and MeNeu5Ac one equivalent of triethylamine was used to ensure deprotonation of the carboxylate group of the sialic acids. All spectrophotometric titration curves were fitted with the aid of the HYPERQUAD program.[37] Binding constants were obtained from a simultaneous fit of the UV/Vis absorption spectral changes at six to eight selected wavelengths. A minimum of 25 absorbance data points at each of these wavelengths was used.

Computational Methods: All calculations were performed with the aid of hybrid DFT, the B3LYP exchange-correlation functional,[38,39] and the Gaussian 03 package (revision C.01).[40] Full geometry optimizations of the **2b**·Neu5Ac$^-$, **3a**·Neu5Ac$^-$, and **3b**·methyl α-D-galactoside systems were performed in vacuo with use of the standard 6–31G(d) basis set. The stationary points found on the potential energy surfaces as a result of the geometry optimizations were tested to ensure they represented energy minima rather than saddle points by frequency analysis. The relative free energies of the different conformations calculated for each system include non-potential-energy contributions (that is, zero point energy and thermal terms) obtained by frequency analysis.

3-[3-(4-Nitrophenyl)thioureido]phenylboronic Acid (1a): 3-Aminophenylboronic acid (0.130 g, 0.842 mmol) and 4-nitrophenyl isothiocyanate (0.152 g, 0.842 mmol) were dissolved in a diethyl ether/dioxane mixture (5:1, v:v, 5 mL). The resulting solution was stirred at room temperature for 14 h. The precipitate formed was collected by filtration and washed with diethyl ether to give **1a** (0.270 g, 89% yield) as a white solid; m.p. 124 °C. ^{1}H NMR (500 MHz, [D_6]-DMSO, 25 °C, Me_4Si): δ = 10.30 (s, 1 H, NH), 10.20 (s, 1 H, NH), 8.20 (d, $^{3}J_{H,H}$ = 9.2 Hz, 2 H, $C_6H_4NO_2$), 8.07 (s, 2 H, OH), 7.84 (d, $^{3}J_{H,H}$ = 9.2 Hz, 2 H, $C_6H_4NO_2$), 7.76 [s, 1 H, $C_6H_4B(OH)_2$], 7.61 [d, $^{3}J_{H,H}$ = 7.3 Hz, 1 H, $C_6H_4B(OH)_2$], 7.55 [d, $^{3}J_{H,H}$ = 9.0 Hz, 1 H, $C_6H_4B(OH)_2$], 7.34 [m, 1 H, $C_6H_4B(OH)_2$] ppm. ^{13}C NMR (125.8 MHz, [D_6]DMSO, 25 °C, Me_4Si): δ = 179.4, 146.3, 142.2, 138.1, 135.0, 130.9, 129.7, 127.6, 126.0, 124.2, 121.5 ppm. IR (ATR): $\tilde{\nu}$ = 3471 (OH), 3374 (NH), 1490 (NO_2), 1327 (NO_2) cm^{-1}. MS (ESI^-): *m/z* = 316 [M – H]$^-$. $C_{13}H_{12}BN_3O_4S{\cdot}0.5\,C_4H_8O_2$ (361.18): calcd. C 49.88, H 4.47, N 11.63; found C 49.81, H 4.42, N 11.60.

3-[3-(4-Nitrophenyl)ureido]phenylboronic Acid (1b): 3-Aminophenylboronic acid (0.132 g, 0.851 mmol) and 4-nitrophenyl isocyanate

(0.140 g, 0.851 mmol) were dissolved in diethyl ether and the mixture was stirred at room temperature for 14 h. The precipitate formed was collected by filtration and washed with diethyl ether to give **1b** (0.202 g, 79% yield) as a yellow solid; m.p. 267 °C. ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C, Me$_4$Si): δ = 9.39 (s, 1 H, NH), 8.82 (s, 1 H, NH), 8.19 (d, $^3J_{H,H}$ = 9.3 Hz, 2 H, $C_6H_4NO_2$), 8.03 (s, 2 H, OH), 7.72 [s, 1 H, $C_6H_4B(OH)_2$], 7.70 (d, $^3J_{H,H}$ = 9.3 Hz, 2 H, $C_6H_4NO_2$), 7.62 [d, $^3J_{H,H}$ = 8.1 Hz, 1 H, $C_6H_4B(OH)_2$], 7.46 [d, $^3J_{H,H}$ = 7.4 Hz, 1 H, $C_6H_4B(OH)_2$], 7.28 [m, 1 H, $C_6H_4B(OH)_2$] ppm. ^{13}C NMR (125.8 MHz, [D$_6$]DMSO, 25 °C, Me$_4$Si): δ = 151.9, 146.4, 140.8, 138.0, 134.9, 128.4, 127.8, 125.1, 124.5, 120.5, 117.3 ppm. IR (ATR): $\tilde{\nu}$ = 3403 (OH), 3327 (NH), 3308 (NH), 1673 (C=O), 1487 (NO_2), 1323 (NO_2) cm^{-1}. MS (ESI$^-$): *m*/*z* = 300 [M – H]$^-$. $C_{13}H_{12}BN_3O_5$ (301.06): calcd. C 51.86, H 4.02, N 13.96; found C 51.92, H 4.12, N 13.90.

Single crystals of **1b·DMSO** suitable for X-ray diffraction analysis were grown from an oversaturated solution of the isolated solid in DMSO/H$_2$O (1:1, v:v), which was heated until complete dissolution of the solid and then allowed to cool down to room temperature.

1-Hydroxy-2-(4-nitrophenyl)-1,2-dihydrobenzo[*c*][1,5,2]diazaborinine-3(4*H*)-thione (2a): The receptor was prepared as described for **1a** from 2-aminophenylboronic acid (0.132 g, 0.962 mmol) and 4-nitrophenyl isothiocyanate (0.173 g, 0.962 mmol) to give **2a** (0.281 g, 85% yield) as white solid; m.p. 224 °C. ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C, Me$_4$Si): δ = 12.14 (s, 1 H, NH), 9.67 (s, 1 H, OH), 8.27 (d, $^3J_{H,H}$ = 8.9 Hz, 2 H, $C_6H_4NO_2$), 8.06 [d, $^3J_{H,H}$ = 6.6 Hz, 1 H, $C_6H_4B(OH)$], 7.60 [m, 1 H, $C_6H_4B(OH)$], 7.44 [m, 3 H, $C_6H_4NO_2$ + $C_6H_4B(OH)$], 7.21 [m, 1 H, $C_6H_4B(OH)$] ppm. ^{13}C NMR (125.8 MHz, [D$_6$]DMSO, 25 °C, Me$_4$Si): δ = 179.1, 148.8, 146.0, 144.8, 133.2, 132.5, 130.6, 123.8, 122.8, 115.4 ppm. IR (ATR): $\tilde{\nu}$ = 3498 (OH), 3398 (NH), 1482 (NO_2), 1343 (NO_2) cm^{-1}. MS (ESI$^-$): *m*/*z* = 298 [M – H]$^-$. $C_{13}H_{10}BN_3O_3S{\cdot}0.5\,C_4H_8O_2$ (343.17): calcd. C 52.50, H 4.41, N 12.24; found C 52.43, H 3.85, N 12.42.

Single crystals of **2a** suitable for X-ray diffraction analysis were grown from an oversaturated solution of the isolated solid in DMSO/H$_2$O (1:1, v:v), which was heated until complete dissolution of the solid and then allowed to cool down to room temperature.

2-[3-(4-Nitrophenyl)ureido]phenylboronic Acid (2b): The receptor was prepared as described for **1a** from 2-aminophenylboronic acid (0.131 g, 0.956 mmol) and 4-nitrophenyl isocyanate (0.157 g, 0.957 mmol). The precipitate was washed with dioxane and dried under vacuum to give **2b** (0.140 g, 45% yield) as a pale yellow solid; m.p. 206 °C. ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C, Me$_4$Si): δ = 10.54 (s, 1 H, NH), 9.34 (s, 1 H, NH), 8.26 (d, $^3J_{H,H}$ = 9.1 Hz, 2 H, $C_6H_4NO_2$), 8.01 [d, $^3J_{H,H}$ = 7.4 Hz, 1 H, $C_6H_4B(OH)_2$], 7.50 [m, 3 H, $C_6H_4NO_2$, $C_6H_4B(OH)_2$], 7.09 [d, $^3J_{H,H}$ = 8.1 Hz, 1 H, $C_6H_4B(OH)_2$], 7.07 [m, 1 H, $C_6H_4B(OH)_2$], 6.52 (s, 2 H, OH) ppm. ^{13}C NMR (125.8 MHz, [D$_6$]DMSO, 25 °C, Me$_4$Si): δ = 153.4, 145.7, 145.6, 145.3, 132.7, 132.6, 130.2, 123.5, 120.9, 114.3 ppm. IR (ATR): $\tilde{\nu}$ = 3611 (OH), 3536 (OH), 3208 (NH), 1672 (C=O), 1496 (NO_2), 1338 (NO_2) cm^{-1}. MS (ESI$^-$): *m*/*z* = 282 [M – H]$^-$. $C_{13}H_{10}BN_3O_4{\cdot}0.5\,C_4H_8O_2$ (327.10): calcd. C 55.08, H 4.31, N 12.85; found C 54.80, H 3.95, N 12.51.

2-{[3-(4-Nitrophenyl)thioureido]methyl}phenylboronic Acid (3a): A mixture of 2-(aminomethyl)phenylboronic acid (0.101 g, 0.668 mmol) and 4-nitrophenyl isothiocyanate (0.120 g, 0.668 mmol) in dioxane (10 mL) was heated at reflux under Ar over a period of 24 h. The precipitate formed was filtered off and washed with dioxane. The filtrate was concentrated to dryness to give an orange oil that was purified by column chromatography on SiO$_2$ with a CH_2Cl_2/MeOH (5%) mixture as eluent to give **3a** (0.067 g, 27% yield) as a pale yellow solid; m.p. 124 °C. ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C, Me$_4$Si): δ = 10.30 (s, 1 H, NH), 8.38 (s, 1 H, NH), 8.23 (s, 2 H, OH), 8.17 (d, $^3J_{H,H}$ = 9.2 Hz, 2 H, $C_6H_4NO_2$), 7.84 (d, $^3J_{H,H}$ = 9.2 Hz, 2 H, $C_6H_4NO_2$), 7.59 [d, $^3J_{H,H}$ = 7.2 Hz, 1 H, $C_6H_4B(OH)_2$], 7.35 [m, 2 H, $C_6H_4B(OH)_2$], 7.24 [m, 1 H, $C_6H_4B(OH)_2$], 4.88 (d, $^3J_{H,H}$ = 5.2 Hz, 2 H, -CH_2-) ppm. ^{13}C NMR (125.8 MHz, [D$_6$]DMSO, 25 °C, Me$_4$Si): δ = 180.0, 146.5, 142.2, 141.9, 134.3, 129.6, 128.2, 126.5, 124.6, 120.8, 47.8 ppm. IR (ATR): $\tilde{\nu}$ = 3519 (OH), 3395 (NH), 1469 (NO_2), 1320 (NO_2) cm^{-1}. MS (ESI$^-$): *m*/*z* = 313 [M – H]$^-$. $C_{14}H_{14}BN_3O_4S{\cdot}0.5\,C_4H_8O_2$ (375.21): calcd. C 51.22, H 4.84, N 11.20; found C 51.60, H 4.86, N 11.49.

2-{[3-(4-Nitrophenyl)ureido]methyl}phenylboronic Acid (3b): The receptor was prepared as described for **3a** from 2-(aminomethyl)-phenylboronic acid (0.102 g, 0.678 mmol) and 4-nitrophenyl isocyanate (0.111 g, 0.678 mmol) to give **3b** (0.058 g, 24% yield) as a pale yellow solid; m.p. 217 °C. ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C, Me$_4$Si): δ = 9.51 (s, 1 H, NH), 8.32 (s, 2 H, OH), 8.12 (d, $^3J_{H,H}$ = 9.3 Hz, 2 H, $C_6H_4NO_2$), 7.59 (d, $^3J_{H,H}$ = 9.3 Hz, 2 H, $C_6H_4NO_2$), 7.55 [d, $^3J_{H,H}$ = 7.5 Hz, 1 H, $C_6H_4B(OH)_2$], 7.32 [m, 2 H, $C_6H_4B(OH)_2$], 7.21 [m, 1 H, $C_6H_4B(OH)_2$], 6.85 (s, 1 H, NH), 4.42 (d, $^3J_{H,H}$ = 5.8 Hz, 2 H, -CH_2-) ppm. ^{13}C NMR (125.8 MHz, [D$_6$]DMSO, 25 °C, Me$_4$Si): δ = 154.7, 147.2, 143.7, 140.6, 134.1, 129.5, 128.0, 126.2, 125.3, 117.0, 43.4 ppm. IR (ATR): $\tilde{\nu}$ = 3521 (OH), 3317 (NH), 1672 (C=O), 1495 (NO_2), 1322 (NO_2) cm^{-1}. MS (ESI$^-$): *m*/*z* = 296 [M – H]$^-$. $C_{14}H_{14}BN_3O_5{\cdot}0.5\,C_4H_8O_2$ (359.14): calcd. C 53.51, H 5.05, N 11.70; found C 53.72, H 4.90, N 12.09.

X-ray Crystal Structures: Three-dimensional X-ray data were collected with a Bruker SMART 1000 CCD for **2a** and with a Bruker X8 APEXII CCD for **1b**·DMSO, **4a**·DMSO, and **4b**. Data were corrected for Lorentz and polarization effects and for absorption by semiempirical methods[41] based on symmetry-equivalent reflections. Complex scattering factors were taken from the program SHELX97[42] running under the WinGX program system[43] implemented on a computer with an Intel Pentium® processor. All

Table 3. Crystal data and refinement details for **1b**, **2a**, **4a**, and **4b**.

	1b·DMSO	**2a**	**4a**·DMSO	**4b**
Emp. formula	$C_{15}H_{18}BN_3O_6S$	$C_{13}H_{10}BN_3O_3S$	$C_{15}H_{17}N_3O_3S_2$	$C_{13}H_{11}N_3O_3$
Formula weight	379.19	299.11	351.44	257.25
Crystal system	monoclinic	triclinic	monoclinic	monoclinic
Space group	$P2_1/c$	$P\bar{1}$	$P2_1/n$	$P2_1$
Temp. [K]	100.0(2)	100.0(2)	100.0(2)	100.0(2)
a [Å]	13.6530(6)	5.6677(5)	5.4120(6)	4.5871(3)
b [Å]	12.8836(6)	8.5250(7)	9.3484(9)	8.3370(5)
c [Å]	19.965(1)	13.681(1)	32.776(3)	15.3036(9)
α [°]	90	87.589(2)	90	90
β [°]	105.750(1)	79.481(2)	91.154(3)	97.009(4)
γ [°]	90	85.447(2)	90	90
V [Å^3]	3380.0(3)	647.63(9)	1657.9(3)	580.88(6)
F(000)	1584	308	736	268
Z	8	2	4	2
λ [Å]	0.71073	0.71073	0.71073	0.71073
$\rho_{calcd.}$ [g cm^{-3}]	1.490	1.534	1.408	1.471
μ [mm]$^{-1}$	0.231	0.263	0.339	0.108
θ range [°]	1.55–28.29	1.51–28.29	1.24–28.28	3.63–28.45
R_{int}	0.0441	0.0275	0.0561	0.0565
Measured refln.	8380	3211	4079	1561
Observed refln.	5638	2662	2947	1145
GOF on F^2	1.047	1.035	1.120	1.031
R_1 [$I > 2\sigma(I)$][a]	0.0407	0.0338	0.0438	0.048
wR_2 (all data)[b]	0.1388	0.0873	0.1419	0.1233

[a] $R_1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$. [b] $wR_2 = \{\Sigma[w(|F_o|^2 - |F_c|^2)^2]/\Sigma[w(F_o^4)]\}^{1/2}$.

the structures were solved by direct methods (SHELXS97 for **1b**·DMSO, **2a**, and **4a**·DMSO; SUPERFLIP[44] for **4b**) and refined[41] by full-matrix, least-squares on F^2. Hydrogen atoms were included in calculated positions and refined in riding mode. Refinement converged with anisotropic displacement parameters for all non-hydrogen atoms. Crystal data and details on data collection and refinement are summarized in Table 3.

CCDC-740810 (for **1b**·DMSO), -740808 (for **2a**), -740809 (for **4a**·DMSO) and -740810 (for **4b**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Supporting Information (see also the footnote on the first page of this article): Tables S1 and S2, giving NMR spectroscopic data for receptors **1a/b**–**3a/b**, Figures S1–S9 showing ^{1}H, ^{11}B, and UV/Vis titrations, Figures S10 and S11, showing the optimized geometries of the **3b**·methyl α-D-galactoside and **2b**·Neu5Ac$^-$ systems and optimized Cartesian coordinates obtained from DFT/6-31G(d) calculations.

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