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Molecular Recognition of *N*-Acetylneuraminic Acid with Acyclic Benzimidazolium- and Aminopyridine/guanidinium-Based Receptors

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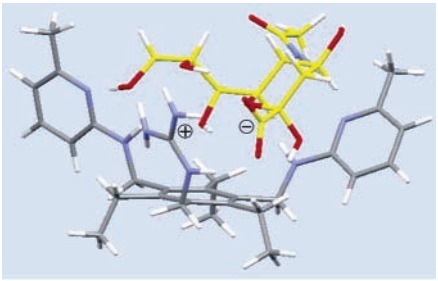

Acyclic receptors incorporating neutral and cationic recognition sites show effective binding of *N*-acetylneuraminic acid (Neu5Ac), the most naturally abundant sialic acid, in highly competitive solvents such as dimethyl sulfoxide (DMSO) and water/DMSO. Receptors **6b** and **7b** are able to form neutral/charge-reinforced hydrogen bonds and ion pairs with Neu5Ac, similar to sialic acid-binding proteins. Syntheses and binding properties of the artificial receptors are discussed.

Introduction

The molecular recognition of carbohydrates by artificial receptors remains an important challenge in artificial receptor chemistry (for reviews on carbohydrate recognition with artificial receptors, see ref 1). As part of our program aimed at the development of receptor molecules for neutral and ionic sugars, we have already reported a series of receptors for neutral carbohydrates,[2] which are particularly challenging substrates to recognize. Our interest in this area concentrates on receptors that possess a relatively simple, acyclic structure and that are expected to form complexes with carbohydrates through neutral and charge-reinforced hydrogen bonds in combination with interactions between the faces of the sugar and the aromatic rings of the receptor (similar to interactions found in the crystal structures of sugar-binding proteins[3]). The acyclic scaffold provides simplicity in the synthetic plan for many modifications of the receptor structure, supplying a base for systematic studies toward recognition motifs for carbohydrates.

In this study, we focused on the interactions of acyclic benzimidazolium- and aminopyridine/guanidinium-based receptors (**7b** and **6b**, respectively) with *N*-acetylneuraminic acid (for α- and β-anomers, see formulas **1**α and **1**β), which is the most common occurring sialic acid, in competitive media like dimethyl sulfoxide (DMSO) or water/DMSO. *N*-Acetylneuraminic acid (Neu5Ac) and Neu5Ac-containing ligands play a key

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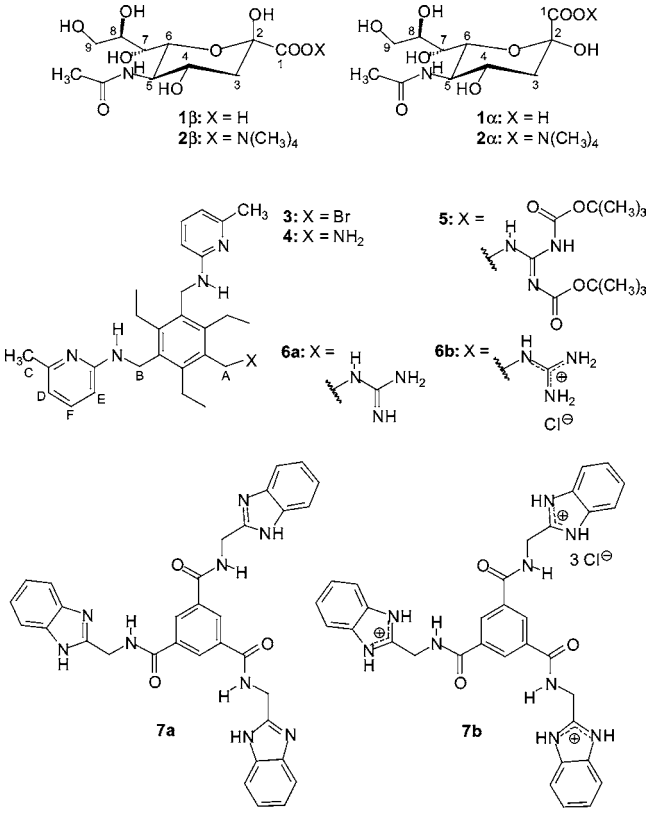

role in a variety of biological processes,[3a,4] including different cellular recognition processes. *N*-Acetylneuraminic acid is known to be overexpressed on the cell surface of tumor cells; furthermore, sialic acids are frequently used as a recognition unit in influenza viruses,[3a] including H5N1 influenza A viruses.[4b] The biological recognition processes involving sialoglycoconjugates use both neutral and charge-reinforced hydrogen bonds, as well as ion pairing for sugar binding; some examples of these interactions are shown in Figure 1.

The analyses of the crystal structures of the complexes formed between the Neu5Ac-containing ligands and sialic acid-binding lectins showed that, with the exception of polyoma virus (see Figure 1c), the carboxylate moiety of Neu5Ac interacts with the main-chain amide groups, polar side chains (especially serine, see Figure 1a), and ordered water molecules rather than fully charged side chains.[3a,b,5a−c] In contrast, formation of ion pairs with the Neu5Ac carboxylate (Figure 1e) appears to be a common feature of neuraminidases.[3b,5d−f] In the complexes of sialic acid-binding lectins, the glycerol side chain of Neu5Ac is often involved in charge-reinforced hydrogen bonds with positively charged amino acids, such as an arginine side chain, as shown in Figure 1b. The acetamido moiety of Neu5Ac is also frequently a significant recognition determinant (for example, see Figure 1d).

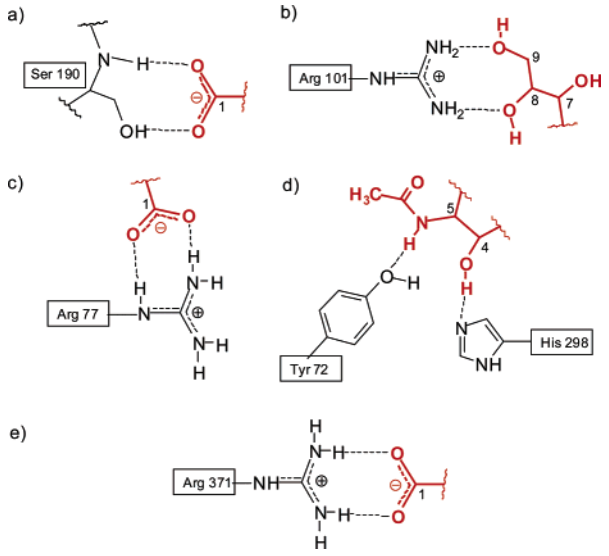

FIGURE 1. Examples of neutral/charge-reinforced hydrogen bonds and ion pairing in the crystal structures formed between Neu5Ac-containing ligands and different proteins: (a, b) methyl α-sialoside/rhesus rotavirus hemagglutinin;[3a,5c] (c, d) NeuAc(α2−3)Gal*β*4Glc/polyoma virus;[3c,5b] (e) NeuAc/influenza neuraminidase complex.[5f,g]

In the case of receptors **6b** and **7b**, both the neutral and ionic hydrogen bonds, as well as ion pairing, were expected to stabilize the complexes with Neu5Ac, similar to sugar-binding proteins. Furthermore, the participation of the central phenyl ring of **6b** and **7b** in CH···π interactions with sugar CHs was expected to provide additional stabilization of the receptor−sugar complexes. The formation of the above-mentioned interactions was also indicated by molecular modeling studies (Figures 2−5); examples of binding motifs found by molecular modeling are shown in Figures 3 and 5.

In the design of artificial receptors for anions, the guanidinium and amidinium groups have proved to be very popular motifs; both groups are strongly basic and remain protonated over a wide pH range (pK_a values typically range between 11 and 13). Several excellent reviews exist in the literature covering the use of artificial guanidinium- and amidinium-containing receptor systems in supramolecular chemistry.[6] In the area of sugar recognition, these units have mostly been incorporated into different boronic acid-based receptors.[1d,7]

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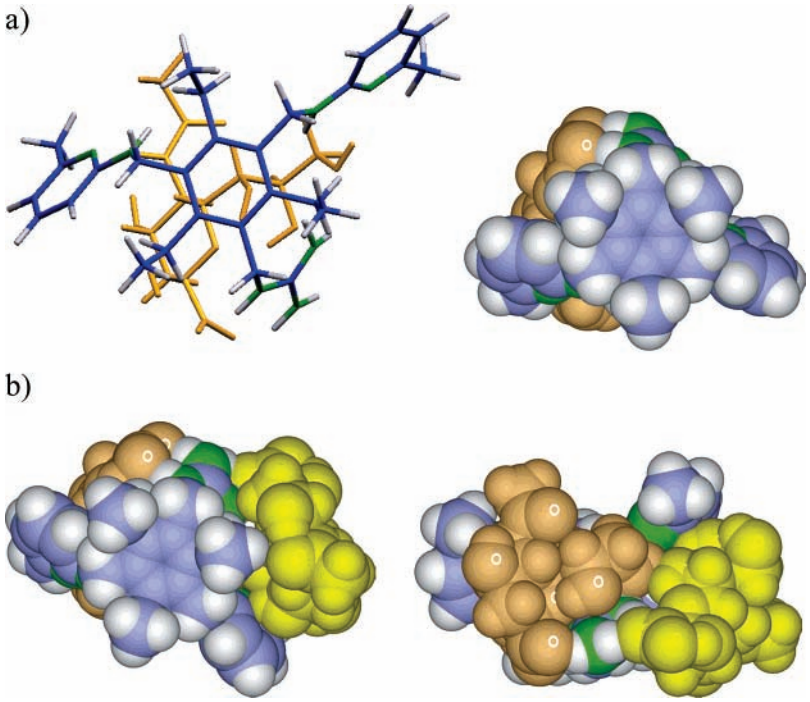

FIGURE 2. Energy-minimized structures of the 1:1 and 1:2 complexes formed between receptor **6b** and Neu5Ac **1**β (MacroModel v. 6.5, Amber* force field, Monte Carlo conformational searches, 50 000 steps). (a) Two different representations of the 1:1 receptor/sugar complex. (b) Space-filling representations of the 1:2 receptor/sugar complex. Color code: receptor C, blue; receptor N, green; the sugar molecules are highlighted in yellow or orange.

Some representatives of boronic acid-based receptors, using covalent interactions for sugar binding, were developed for the recognition of *N*-acetylneuraminic acid.[8] Kataoka and co-workers[8a] have studied the anomalous binding profile of 3-(propionamido)phenylboronic acid with Neu5Ac in aqueous solution with varying pH. The molecular recognition of sialic acid end groups by phenylboronates has been reported by Peters and co-workers.[8b] Shinkai and co-workers[8c] have described a receptor system that features two-point interactions of boronic acid−diol complexation and Zn(II)−carboxylate coordination. The interactions between Neu5Ac and xanthene dyes containing well-positioned boronic acid have been studied by Strongin and co-workers.[8d] Zhang and Anslyn[8e] have explored the binding properties of a cadmium-centered tris-boronic acid receptor toward various anionic sugars, including *N*-acetylneuraminic acid.

Results and Discussion

Synthesis of the Receptors. The synthesis of **6b** started from 1,3,5-tris(bromomethyl)-2,4,6-triethylbenzene,[9] which was converted into compound **3** via a reaction with 2 equiv of 2-amino-6-methylpyridine. The reaction of **3** with aqueous ammonia gave the amino derivative **4**, which was transformed into compound **5** by reaction with di-Boc-protected *S*-methylisothiourea in the presence of triethylamine (see Scheme 1). The crystal structure of **5** is shown in Figure 6; it should be noted that the three arms of **5** point to the same face of the central phenyl ring, while the ethyl groups point in the opposite direction. The protective groups of **5** were removed with trifluoroacetic acid, and the obtained salt was transformed into **6a**, which was further converted into the hydrochloride **6b**. The trihydrochloride **7b** was prepared from **7a**, which was synthesized by reaction of benzene-1,3,5-tricarbonyl chloride with 2-aminomethylbenzimidazole in the presence of triethylamine.

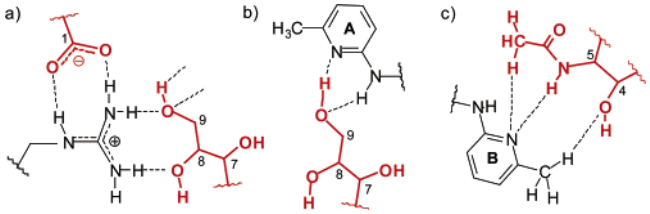

FIGURE 3. Examples of neutral/charge-reinforced hydrogen bonds and ion pairing found by molecular modeling studies of the 1:1 complex between receptor **6b** and Neu5Ac **1**β (MacroModel v. 6.5, Amber* force field, Monte Carlo conformational searches, 50 000 steps).

Binding Studies. The interactions of the receptors **6b** and **7b**, operating through noncovalent interactions, with Neu5Ac were investigated by ^{1}H NMR spectroscopy and microcalorimetry (isothermal titration calorimetry, ITC).

^{1}H NMR Titrations. The ^{1}H NMR binding titration data were analyzed by use of the Hostest 5.6[10] and the HypNMR 2006

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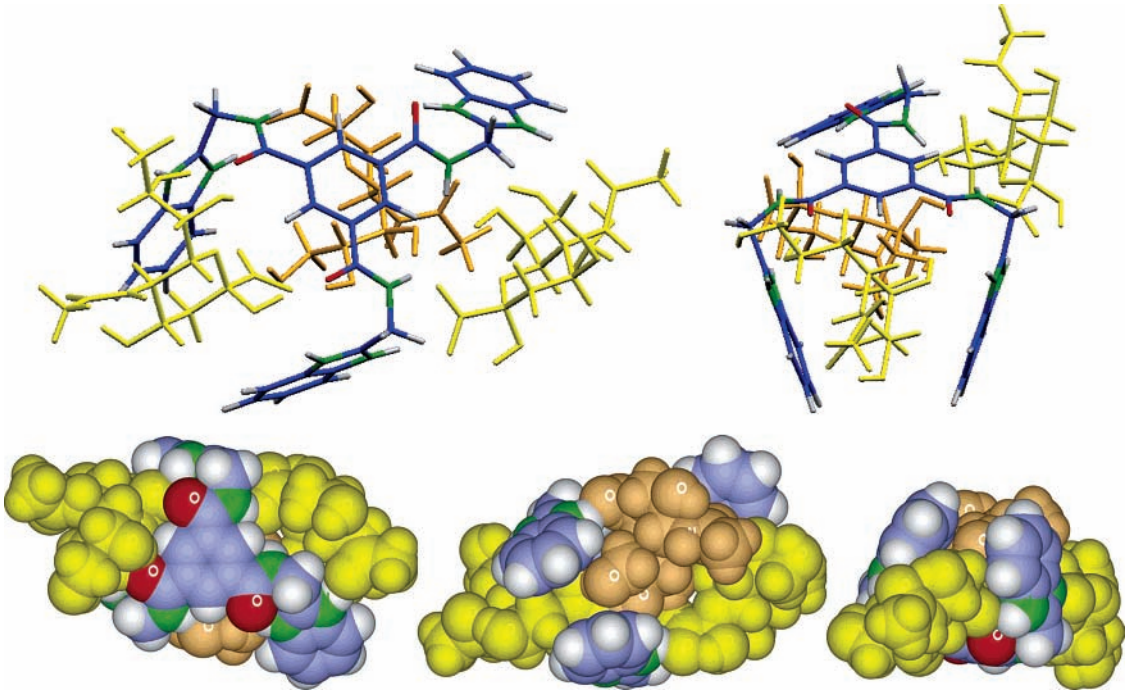


FIGURE 4. Energy-minimized structure of the 1:3 complex (different representations) formed between receptor **7b** and Neu5Ac **1**β (MacroModel v. 6.5, Amber* force field, Monte Carlo conformational searches, 50 000 steps). Color code: receptor C, blue; O, red; N, green; the sugar molecules are highlighted in yellow and orange.

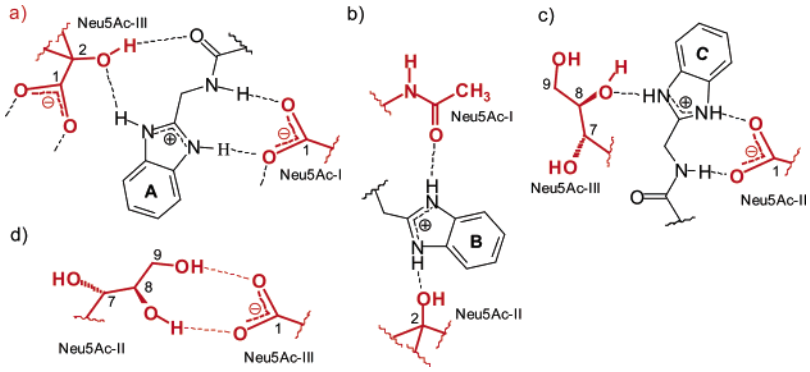


FIGURE 5. Examples of neutral/charge-reinforced hydrogen bonds and ion pairing found by molecular modeling studies of the 1:3 complex between receptor **7b** and Neu5Ac **1**β (MacroModel v. 6.5, Amber* force field, Monte Carlo conformational searches, 50 000 steps).

FIGURE 6. Crystal structure of **5** (hydrogen-bonded ethanol molecule is shown).

programs.[11] The stoichiometry of the receptor−sugar complexes was determined by mole ratio plots and by curve-fitting analysis of the titration data. The titration experiments were carried out by adding increasing amounts of the tetramethylammonium salt of Neu5Ac (**2**)[12] to a solution of the host **6b** or **7b**. In addition, inverse titrations were performed in which the concentration of **2** was held constant and that of receptor was varied. Neu5Ac in solution is in an equilibrium between α- and β-pyranose forms (structures **2**α and **2**β in the case of the tetramethylammonium salt of Neu5Ac; for studies on mutarotation of Neu5Ac, see ref 13). The ^{1}H NMR spectra[14] showed that the complexes formed with **6b** and **7b** include Neu5Ac predominantly in the β-form.

(11) Gans, P. University of Leeds, Leeds, U.K., 2006.

(12) Tetralkylammonium ions are commonly used as countercations in the binding studies of anions. For a recent discussion of the solvent and countercation effects, see Sessler, J. L.; Gross, D. E.; Cho, W.-S.; Lynch, V. M.; Schmidtchen, F. P.; Bates, G. W.; Light, M. E.; Gale, P. A. *J. Am. Chem. Soc.* **2006**, *128*, 12281−12288.

(13) The anomeric configuration of Neu5Ac as a β-anomer is more stable in solution, as demonstrated by the rapid mutarotation undergoing from the α- to the β-anomer. For studies in water, see Friebolin, H.; Kunzelman, P.; Supp, M.; Brossmer, R.; Keilich, G.; Ziegler, D. *Tetrahedron Lett.* **1981**, *22*, 1383−1386.

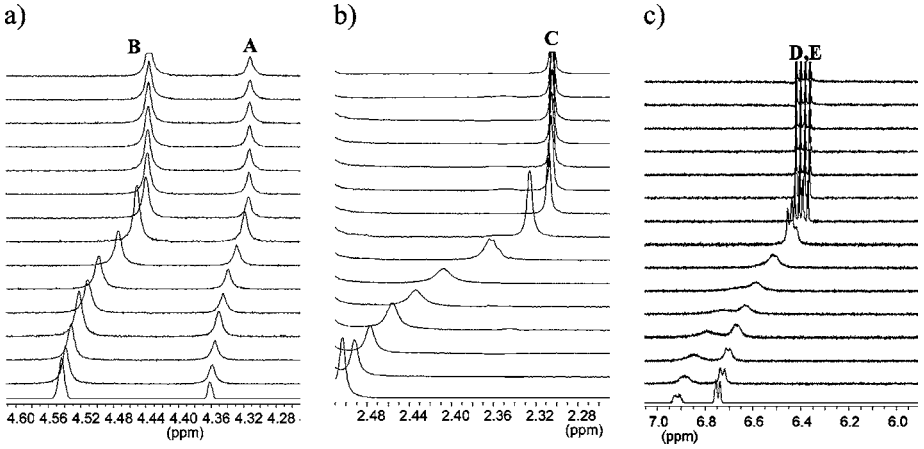


FIGURE 7. Partial ^{1}H NMR spectra (400 MHz; D_2O/DMSO-d_6, 1:9 v/v) of receptor **6b** after addition of (from bottom to top) 0.00, 0.20, 0.40, 061, 0.82, 1.02, 1.33, 1.64, 1.95, 2.26, 2.56, 2.87, 3.28, 3.70, and 4.10 equiv of **2** ([**6b**] = 0.90 mM). Shown are chemical shifts of the (a) $CH_2^{A,B}$, (b) CH_3^C, and (c) pyridine $CH^{D,E}$ resonances (for labeling, see formula **6b**).

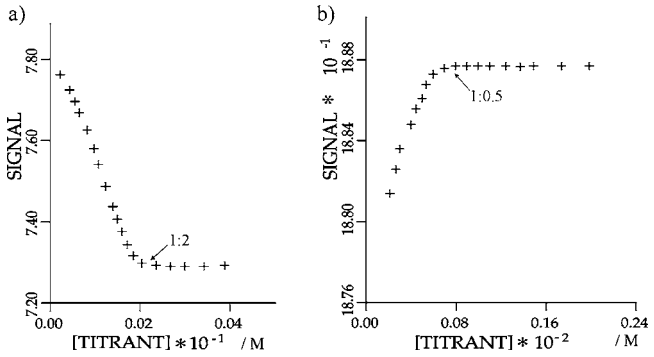
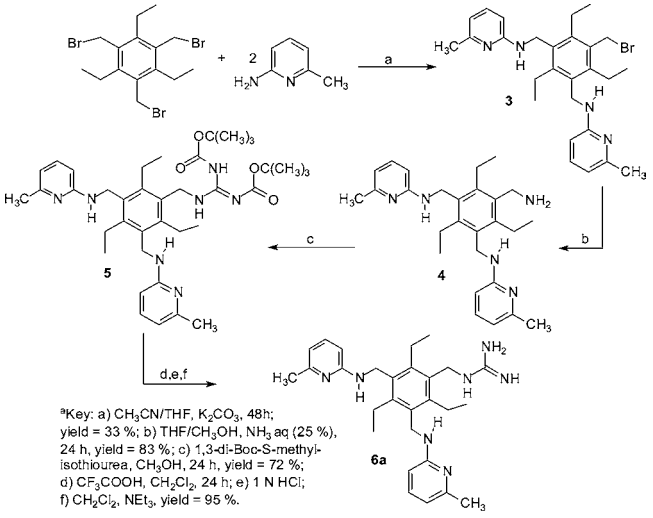


FIGURE 8. (a) Chemical shift changes observed for pyridine CH^F of **6b** during the titration of **6b** with **2** in D_2O/DMSO-d_6 (1:9 v/v). The [receptor]:[sugar] ratio is marked. (b) Chemical shift changes observed for CH_3 protons of **2** during the titration of **2** with **6b** (inverse titration). The [sugar]:[receptor] ratio is marked.

SCHEME 1[a]



Complexation between receptor **6b** and the tetramethylammonium salt of Neu5Ac (**2**) in DMSO-d_6 and D_2O/DMSO-d_6 (1:9 v/v) was evidenced by several changes in the NMR spectra (see, for example, Figure 7).[15a,b] The upfield shifts of the CH_2^B, CH_3^C (for labeling, see structure of **6b**) and pyridine CH protons of **6b** were monitored as a function of sugar concentration (typical titration curves are shown in Figures 8a and S1-1, Supporting Information). The signals for the pyridine CH protons moved in the range of 0.50−0.67 ppm, whereas those for the CH_2 and CH_3 protons shifted in the range of 0.12−0.20 ppm. The pyridine CHs broaden during the titration and became distinct near saturation, which occurred after the addition of 2 equiv of **2**. The best fit of the titration data was obtained with the mixed 1:1 and 1:2 receptor-sugar binding model; this model was further supported by the mole ratio plots (see Figure S2a, Supporting Information). Binding of **2** in DMSO proved to be too strong to be followed quantitatively ($K_a > 10^6$ M^{-1}).[16] In D_2O/DMSO-d_6 mixtures (1:9 v/v) the association constants were still at the upper limit of values, for which the ^{1}H NMR technique is meaningful (the binding constants were found to be $K_{a1} = 5.3 \times 10^5$ and $K_{a2} = 3.7 \times 10^4$ M^{-1}).[15]

The ^{1}H NMR spectra obtained during the titrations at constant sugar concentration (inverse titrations) in DMSO-d_6 showed large shifting of the sugar hydroxyl resonances; however, the strong broadening of these resonances prevented their use in the estimation of the binding constants. Consequently, chemical shift changes of the amide NH (see Figure S1-2a, Supporting Information) and CH_3 protons of **2** were monitored (downfield shifts by 0.10 and 0.03 ppm, respectively; such shifts are consistent with the binding motifs shown in Figure 3c). After the addition of 0.5 equiv of **6b**, almost no change was observed in the NMR spectra (see Figure 8b). The best fit of the titration data was obtained with the pure 1:2 receptor−sugar binding model; however, the binding constant was too large to be accurately determined by ^{1}H NMR titrations. In the first part of the inverse titration the sugar is the excess component and the receptor is the minor component, thus, the 1:1 complex is

(14) For ^{1}H and ^{13}C NMR studies on Neu5Ac in D_2O and DMSO-d_6, see ref 8a and Gervay, J.; Batta, G. *Tetrahedron Lett.* **1994**, *35*, 3009−3012.

(15) (a) Dilution experiments show that receptor does not self-aggregate in the used concentration range. (b) For each system at least four titrations were carried out (for each titration 10−21 samples were prepared). (c) The reproducibility of the K_a values was ±10−25%. (d) K_{a1} corresponds to the 1:1 association constant, K_{a2} corresponds to the 1:2 receptor/sugar association constant, and K_{a3} corresponds to the 1:3 receptor/sugar association constant.

(16) For a review discussing the limitations of the NMR method, see Fielding, L. *Tetrahedron* **2000**, *56*, 6151−6170.

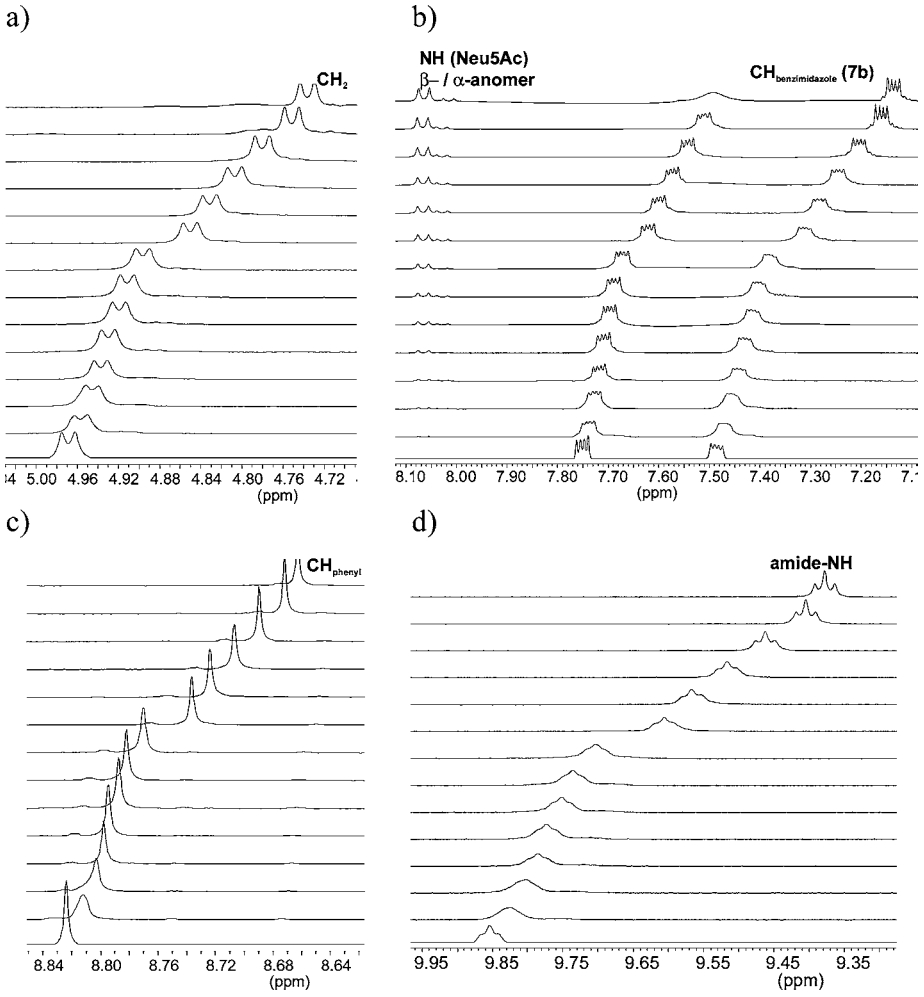


FIGURE 9. Partial ^{1}H NMR spectra (400 MHz, DMSO-d_6) of receptor **7b** after addition of (from bottom to top) 0.00, 0.15, 0.31, 0.47, 0.63, 0.79, 0.95, 1.11, 1.59, 1.83, 2.07, 2.39, 2.79, and 3.10 equiv of **2** ([**7b**] = 0.95 mM). Shown are chemical shifts of the (a) CH_2, (b) benzimidazole CH, (c) phenyl CH, and (d) amide NH resonances.

formed followed by the immediate formation of a 1:2 receptor−sugar complex.

Complexation between receptor **7b** and sugar **2** in DMSO-d_6 or water containing DMSO-d_6 ([receptor]:[water] = 1:1300; H_2O/DMSO 1:40 v/v) was evidenced by the upfield shift of the receptor amide protons (0.50 ppm) as well as changes of the chemical shifts of the CH_2 and phenyl and benzimidazole CH resonances (see Figure 9). The CH_2 and phenyl CH signals moved upfield by 0.25 and 0.17 ppm, respectively, whereas those for benzimidazole CH protons shifted in the range of 0.25−0.35 ppm. After the addition of 3 equiv of **2**, almost no change was observed in the NMR spectra (see Figures 10a and S1-3, Supporting Information). The chemical shift changes indicated that three binding processes occurred during the titration. The best fit of the titration data was obtained with a mixed 1:1, 1:2, and 1:3 receptor−sugar binding model (incorporated in the HypNMR 2006 program), indicating a very strong association constant for 1:1 binding and weaker association constants for 1:2 and 1:3 receptor−sugar complexes. However, the binding constant for the first Neu5Ac molecule (K_{a1}) derived from the model incorporating a final receptor−sugar stoichiometry of 1:3 was too large ($K_{a1} > 10^6$ M^{-1}) to be accurately determined by ^{1}H NMR titrations.

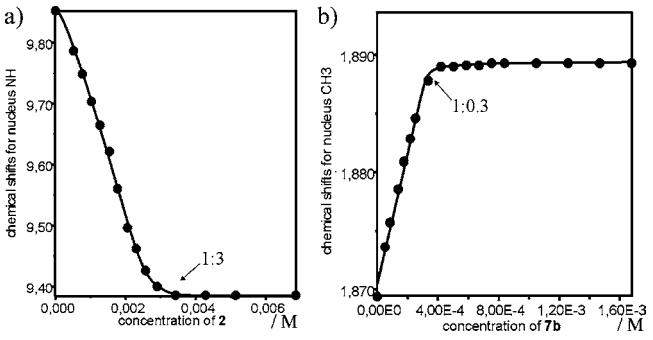


FIGURE 10. (a) Plot of the chemical shifts of the amide NH resonances of **7b** as a function of added **2** in DMSO-d_6. The [receptor]:[sugar] ratio is marked. (b) Plot of the chemical shifts of the CH_3 resonances of **2** as a function of added **7b** (inverse titration). The [sugar]:[receptor]ratio is marked.

In the case of the inverse titrations at constant sugar concentration, the addition of only 0.3 equiv of **7b** led to practically complete complexation of **2**, indicating the formation of 1:3 receptor−sugar complexes (the amide NH and CH_3 signals of **2** were monitored; see Figures 10b and S1-2b, Supporting Information). The 1:3 receptor−sugar binding stoi-

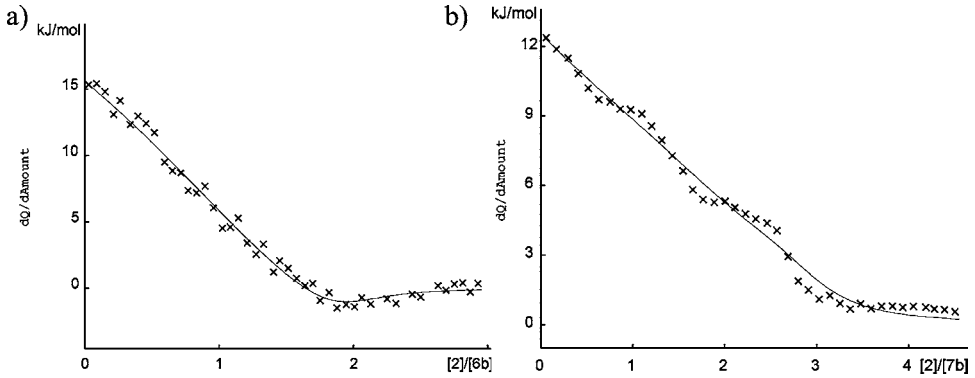


FIGURE 11. ITC titration of receptors **6b** and **7b** with Neu5Ac **2**. (a) Isotherm for titration of 1.00 mM **6b** with 10 μL aliquots of 6.18 mM **2** in H_2O/DMSO (1:9 v/v) at 25 °C. (b) Isotherm for titration of 0.70 mM **7b** with 10 μL aliquots of 7.98 mM **2** in H_2O/DMSO (1:9 v/v) at 25 °C. The molar ratio of the sugar to the receptor is given (for thermodynamic parameters, see Tables 1 and 2).

TABLE 1. Results of ITC Titrations of 6b and 7b with 2 in H_2O/DMSO (1:9 v/v) at 25 °C[a]

	β_1	β_2	β_3	ΔH_1	ΔH_2	ΔH_3	$T\Delta S_1$	$T\Delta S_2$	$T\Delta S_3$
6b·2	1.5×10^5	4.6×10^9		−15.7	−11.8		13.8	43.4	
7b·2	2.0×10^5	11.9×10^9	13.4×10^{13}	−12.6	−18.7	−22.6	17.7	39.0	58.5

[a] In the ligand binding program of Digitam, the equilibrium constants (β_i) and the reaction enthalpies for the overall reaction are determined. $\beta_1 = K_{a1}$ (M^{-1}); $\beta_2 = K_{a1}K_{a2}$ (M^{-2}); $\beta_3 = K_{a1}K_{a2}K_{a3}$ (M^{-3}). ΔH_i and $T\Delta S_i$ are given in kilojoules per mole. Errors in ΔH are less than 5%.

TABLE 2. Thermodynamic Parameters Evaluated by ITC Studies for 6b·2 and 7b·2 in H_2O/DMSO (1:9 v/v) at 25 °C[a]

	K_{a1}[b] (ΔG)	K_{a2}[b] (ΔG)	K_{a3}[b] (ΔG)	ΔH_{1s}	ΔH_{2s}	ΔH_{3s}	$T\Delta S_{1s}$	$T\Delta S_{2s}$	$T\Delta S_{3s}$
6b·2	1.5×10^5 (−29.5)	3.2×10^4 (−25.7)		−15.7	3.9		13.8	29.6	
7b·2	2.0×10^5 (−30.2)	6.1×10^4 (−27.3)	1.2×10^4 (−23.1)	−12.6	−6.1	−3.9	17.7	21.2	19.2

[a] ΔH_{is}, reaction enthalpies for the stepwise reaction. K_a is given in liters per mole; ΔG, ΔH, and $T\Delta S$ are given in kilojoules per mole. Errors in K_a range from 9% to 15%; Errors in ΔH are less than 5%. [b] See ref 15d.

chiometry was also indicated by mole ratio plots (see Figure S2b, Supporting Information). A satisfactory fit of the titration data to the 1:3 receptor−sugar binding model yielded K_a value $>10^6$ M^{-1}.

Typical binding motifs found by molecular modeling studies in the 1:3 complex between receptor **7b** and Neu5Ac **1**β are shown in Figure 5, showing interactions between receptor and sugar molecules (Figure 5a−c), as well as between the bound sugars (for example, see Figure 5d).

Microcalorimetric Titrations. The microcalorimetric titrations were carried out in a mixture of H_2O/DMSO (1:9 v/v) at 298 K by use of a Thermometric titration calorimetric system (Thermometric, Sweden). The reproducibility of the calorimeter was checked with the complexation of Ba^{2+} by 18-crown-6. A solution of Neu5Ac **2** (6−8 mM) was titrated into a 0.7−1 mM solution of receptor **6b** or **7b** (see, for example, the caption for Figure 11). The data obtained were analyzed by use of the Digitam 4.1 software provided by Thermometric (heat of dilution was corrected).

In the case of **6b**, the best fit of the titration data was obtained with the mixed 1:1 and 1:2 receptor−sugar binding model. The binding constants for **6b·2** were found to be 1.5×10^5 M^{-1} (K_{a1}) and 3.2×10^4 (K_{a2})[15d] M^{-1} and are of the same magnitude as those determined by NMR spectroscopy in D_2O/DMSO-d_6 (1:9 v/v).

In the case of **7b**, the microcalorimetric data indicated that three binding processes occurred during the titration (similar to NMR titrations in water containing DMSO-d_6). The binding constants derived from the model incorporating a final receptor−sugar stoichiometry of 1:3 were found to be $K_{a1} = 2.0 \times 10^5$ M^{-1}, $K_{a2} = 6.1 \times 10^4$ M^{-1}, and $K_{a3} = 1.2 \times 10^4$ M^{-1}.

The microcalorimetric data showed that the binding processes are exothermic and entropically favored (see Tables 1 and 2).

Conclusions

Receptors **6b** and **7b**, including neutral and cationic binding sites, proved to be very effective receptors for *N*-acetylneuraminic acid, the most naturally abundant sialic acid. As in natural complexes, the charge-reinforced hydrogen bonds and ion pairs seem to be the major driving force for receptor−Neu5Ac association in highly competitive media.

According to molecular modeling studies, the neutral and ionic recognition sites of **6b** and **7b** should be able to participate in the following interactions: (i) ion pairing and ionic hydrogen-bonding between the guanidinium/benzimidazolium residue of the receptor and the carboxylate group of Neu5Ac (for example, see Figures 3a, 5a and 5c); (ii) charge-reinforced hydrogen bonds[17] between the guanidinium/benzimidazolium residue of the receptor and the acetamido/hydroxy groups of Neu5Ac (see Figures 3a and 5a); (iii) neutral hydrogen bonds (including also CH···O/N hydrogen bonds) between the neutral recognition sites of the receptor and the acetamido/hydroxy groups of Neu5Ac

(17) The studies in the area of drug design found that a neutral−neutral hydrogen bond is worth up to about 1.5 kcal mol^{-1}, which is equivalent to a maximum 15-fold increase in binding, whereas a hydrogen bond between a charged and a neutral component can contribute up to 3000-fold to the binding of a substrate (up to 4.7 kcal mol^{-1}). See (a) Davis, A. M.; Teague, S. J. *Angew. Chem., Int. Ed.* **1999**, *38*, 736−749. (b) Fersht, A. R.; Shi, J.-P.; Knill-Jones, J.; Lowe, D. M.; Wilkinson, A. J.; Blow, D. M.; Brick, P.; Carter, P.; Waye, M. M. Y.; Winter, G. *Nature* **1985**, *314*, 845−851. (c) Williams, D. H.; Searle, M. S.; Mackay, J. P.; Gerhard, U.; Maplestone, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 1172−1178.

(see Figure 3b,c; the neutral binding sites of **7b** consist of amide groups as used by nature, whereas those of **6b** comprise aminopyridine moieties as heterocyclic analogues of the asparagine/glutamine primary amide side chains); and (iv) CH···π interactions between the CHs of Neu5Ac and the aromatic groups of the receptor (similar to sugar-binding proteins, which commonly place aromatic surfaces against patches of sugar CH groups).

^{1}H NMR titrations at constant concentration of **6b** suggested the formation of complexes with 1:1 and 1:2 receptor/sugar binding stoichiometry. Complexation between **6b** and **2** in DMSO-d_6 proved to be too strong to be accurately determined by ^{1}H NMR titrations ($K_{a1} > 10^6$ M^{-1}). In a mixture of D_2O/DMSO-d_6 (1:9 v/v), the binding constants K_{a1} and K_{a2}[15d] were found to be in the range of 10^5 M^{-1} and 10^4 M^{-1}, respectively. The microcalorimetric titrations carried out in a mixture of H_2O/DMSO (1:9 v/v) verified the mixed 1:1 and 1:2 receptor/sugar binding model. The values of the binding constants determined by microcalorimetry ($K_{a1} = 1.5 \times 10^5$ M^{-1} and $K_{a2} = 3.2 \times 10^4$ M^{-1}; see Table 2) and the NMR spectroscopy are of the same magnitude.

In the case of receptor **7b**, the ^{1}H NMR titrations in DMSO-d_6 indicated that very strong 1:1 complexes are formed ($K_{a1} > 10^6$ M^{-1}), followed by the formation of weaker 1:2 and 1:3 receptor−sugar complexes at higher Neu5Ac concentrations. The mixed 1:1, 1:2, and 1:3 receptor−sugar binding model was further supported by microcalorimetric titrations in H_2O/DMSO (1:9 v/v). The binding constants were found to be $K_{a1} = 2.0 \times 10^5$ M^{-1}, $K_{a2} = 6.1 \times 10^4$ M^{-1}, and $K_{a3} = 1.2 \times 10^4$ M^{-1} (see Table 2). The microcalorimetric titrations revealed that the enthalpy of binding is negative (apart from ΔH_{2s} for **6b·2**; see Table 2) and the binding processes are entropically favored (see Tables 1 and 2).[18] The ^{1}H NMR spectra showed that the complexes include Neu5Ac predominantly in the β-form. The receptors **6b** and **7b** combine ease of synthesis and the ability to form strong complexes with Neu5Ac in highly competitive solvents.

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Supporting Information Available: Syntheses of compounds **3**−**7**; ^{1}H and ^{13}C NMR spectra of compounds **3**−**5**, **6b,** and **7b**; examples of inverse titrations (^{1}H NMR titration of **2** with receptors **6b** and **7b**); ^{1}H NMR titrations of **6b** or **7b** with **2** (typical titration curves); representative mole ratio plots; and X-ray data for compound **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) For a discussion of the energetics of protein−carbohydrate interactions, see Toone, E. J. *Curr. Opin. Struct. Biol.* **1994**, *4*, 719−728.