Molecular Recognition of *N*-Acetylneuraminic Acid by Acyclic Pyridinium- and Quinolinium-Based Receptors in Aqueous Media: Recognition through Combination of Cationic and Neutral Recognition Sites

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Supporting Information

ABSTRACT: Compounds **5b**–**8b** and **10b**, and **11b**, containing a triethylbenzene scaffold substituted with both neutral and cationic recognition sites, were shown to be effective receptors for *N*-acetylneuraminic acid (NeuAc), the most common occurring sialic acid, in highly competitive solvents. These compounds were established to be more powerful receptors for NeuAc than the symmetrical pyridinium- and quinolinium-based compounds **9b** and **12b** in aqueous media. As in natural protein–sialic acid complexes,



the combination of neutral/charge-reinforced hydrogen bonds, ion pairs, $CH-\pi$, and van der Waals interactions seems to be responsible for the effective binding of this naturally widespread anionic carbohydrate in aqueous media.

INTRODUCTION

N-Acetylneuraminic acid (NeuAc; the most common occurring sialic acid) and NeuAc-containing ligands play a key role in a wide range of biological processes.^{1,2} As the terminal sugar of cell surface glycoproteins, NeuAc is known to participate in carbohydrate-protein interactions that mediate recognition phenomena. NeuAc is frequently used as a recognition unit by proteins of numerous viruses, such as influenza, Sendai, Newcastle disease, and polyoma viruses. This naturally widespread carbohydrate is furthermore known to be overexpressed on the cell surface of tumor cells. The biological recognition processes involving sialoglycoconjugates use both neutral and charge-reinforced hydrogen bonds, as well as ion pairing for sugar binding;² some examples of these interactions are shown in Figure 1. The natural binding motifs have inspired the design of artificial receptors for the recognition of NeuAc,³⁻⁵ which may serve as a basis for the development of new therapeutics or agents for the detection of bound and free NeuAc (for a recent discussion on the importance of the detection of free sialic acid in biological samples, see ref 6).

Our previous studies showed that acyclic molecules of types I and II (see Figure 2) are able to complex neutral and/or ionic carbohydrates in organic and aqueous media.^{3a,b,7,8} The acyclic scaffold facilitates many synthetic modifications of the receptor structure, providing a base for systematic studies. Such studies showed the suitability of the aminopyridine, aminopyrimidine, aminonaphthyridine, indole, imidazole, benzimidazole, pyrrole, guanidinium, carboxylate, crown ether, hydroxy, amide, and oxime based groups as recognition groups for carbohydrates. Mimicking the binding motifs observed in the crystal structures of protein–carbohydrate complexes, by using natural recog-



Figure 1. Examples of neutral (a) and charge-reinforced (b, c) hydrogen bonds as well as ion pairing (d) in the crystal structures formed between NeuAc-containing ligands (red units) and different proteins (black units): (a) NeuAc(α 2-3)Gal β 4Glc and polyoma virus; (b, c) methyl α -sialoside and rhesus rotavirus hemagglutinin; (d) NeuAc and influenza neuraminidase complex.²

nition groups or their analogues, was shown to be a powerful strategy for the design of effective and selective carbohydrate receptors. Depending on the nature of the recognition groups and the mode of their connection with the aromatic platform, a wide range of receptors with different binding properties could be obtained. Among these acyclic compounds, the compounds 1-3 (see Figure 3), consisting of neutral and cationic recognition units appended to a triethylbenzene core, were

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Figure 2. Examples of acyclic carbohydrate receptors (see refs 3a,b and 7).



Figure 3. Structures of the previously described receptors 1-3 and of N-acetylneuraminic acid (4a; 4b and 4c represent the salts used for the binding studies).



Figure 4. Structures of the prepared pyridinium and quinolinium based compounds 5–12 (Br⁻ and PF₆⁻ salts).

shown to be effective receptors for *N*-acetylneuraminic acid^{3a,b} (for a recognition of NeuSAc with receptors containing a diphenylmethane or biphenyl core, see ref 3c). The studies indicated that a combination of cationic and neutral recognition groups provides powerful receptors for the recognition of anionic sugars in aqueous media.

The aim of the present study was to evaluate the potential of compounds containing pyridinium or quinolinium groups as cationic binding sites in the complexation of carbohydrates. As the first representatives of this group we have prepared compounds 5–8, 10, and 11 (see Figure 4), incorporating one or two cationic units and aminopyridine groups as neutral recognition sites. In our previous studies the aminopyridine group was established as a valuable building block for carbohydrate receptors⁹ and was used as a heterocyclic analogue of the primary amide group of the Asn/Gln side chain. Compounds 5–8, 10, and 11, incorporating both cationic and neutral recognition sites, were expected to participate in the formation of both neutral and chargereinforced hydrogen bonds as well as ion pairs with the anionic

Scheme 1^a



^{*a*}Reaction conditions: (a) 5 equiv of **19**, CH₂Cl₂, reflux, 48 h (46%); (b) 9 equiv of NaPF₆, MeOH, room temperature (40%); (c) 6 equiv of **17**, CH₂Cl₂, reflux, 8 h (89%); (d) 9 equiv of NaPF₆, MeOH, room temperature (50%); (e) 2 equiv of 2-amino-4,6-dimethylpyridine (**14**), CH₃CN/THF, K₂CO₃, room temperature, 3 days (20% **15**, 30% **16**); (f) 2.5 equiv of **19**, CH₂Cl₂, reflux, 24 h (33%); (g) 6 equiv of NaPF₆, MeOH, room temperature (75%); (h) 2 equiv of **17** or **18**, CH₂Cl₂, reflux, 8 h (63% **7a**; 25% **8a**); (i) 6 equiv of NaPF₆, MeOH, room temperature (53% **7b**, 65% **8b**); (j) 1 equiv of **19**, CH₂Cl₂, reflux, 16 h (45%); (k) 3 equiv of NaPF₆, MeOH, room temperature (80%); (l) 1 equiv of **17** or **18**, CH₂Cl₂, reflux, 8 h in the case of **17** and 12 h in the case of **18** (41% **5a**, 47% **6a**); (m) 3 equiv of NaPF₆, MeOH, room temperature (81% **5b**, 78% **6b**).

sugar. The participation of the central phenyl ring of 5-8, 10, and 11 in interactions with sugar CHs^{10,11} was expected to provide additional stabilization of the receptor-sugar complexes. The binding properties of these compounds (used as PF_6^- salts **5b-8b**, **10b**, and **11b**) were compared with those of the symmetrical pyridinium and quinolinium based compounds 9b and 12b. It should be noted that symmetrical triethylbenzene based compounds containing pyridinium or quinolinium groups as cationic recognition sites have been used by Steed¹² as receptors for anions, such as halides, nitrate, and acetate. Developments in the molecular recognition of anions by acyclic and macrocyclic pyridinium based receptors have recently been reviewed by Kilah and Beer.¹³ In the area of sugar recognition by receptors employing noncovalent interactions, the potential of pyridinium and guinolinium based receptors has not been evaluated.

RESULTS AND DISCUSSION

The syntheses of compounds 5-12 (Br⁻ and PF₆⁻ salts) are summarized in Scheme 1 and described in the Experimental Section. The binding properties of **5b**-**8b**, **10b**, and **11b** toward *N*-acetylneuraminic acid (used as the tetramethylammonium salt **4b**) were analyzed in mixtures of D₂O and DMSO- d_6 (1/9, 1/5, and 1/2.5 v/v) by ¹H NMR spectroscopic titrations. In the case of the symmetrical molecules **9b** and **12b**, additional tests were carried out in CD₃CN (Neu5Ac was used as the tetrabutylammonium salt **4c**).¹⁴

The interactions between the binding partners were analyzed by titrations in which the concentration of the receptor was held constant and that of the anionic sugar was varied, as well as by inverse titrations in which the concentration of the sugar was held constant (for examples, see the Supporting Information). The stoichiometry of the receptor–sugar complexes was determined by mole ratio plots¹⁵ and by the curve-fitting analysis of the titration data.

The complexation between the receptors 5b-8b, 10b, and 11b and NeuAc (used as mixture of anomers)¹⁶ was evidenced by several changes in the NMR spectra; examples are given in Figure 6. The ¹H NMR titration data were analyzed using the EQNMR program;¹⁷ the binding constants are summarized in Table 1.

In a 1/9 (v/v) $D_2O/DMSO-d_6$ mixture the interactions between the binding partners are too strong to be accurately analyzed by the NMR method; the analysis of the titration data indicated the formation of very strong 2/1 receptor-sugar complexes ($K_{21} > 100000 \text{ M}^{-1}$; for an energy-minimized structure of a 2/1 receptor-sugar complex, see Figure 5).

Table 1. Examples of Association Constants^{a,b} for the Tested Receptors and Neu5Ac (Used as Salt 4b or 4c)

receptor	solvent	${K_{11} \choose M^{-1}}$	$K_{21}^{e} (M^{-1})$	$ \beta_{21} = K_{11} K_{21} \\ (M^{-2}) $
5b	1/9 (v/v) D ₂ O/ DMSO- <i>d</i> ₆ ^c	f	>100000 ^f	
	1/5 D ₂ O/DMSO-d ₆ ^c	3750	43050	1.61×10^{8}
	1/2.5 D ₂ O/DMSO-d ₆ ^c	6330	180	1.13×10^{6}
7b	1/9 D ₂ O/DMSO- <i>d</i> ₆ ^c	f	>100000 ^f	
	1/5 D ₂ O/DMSO-d ₆ ^c	2540	61200	1.55×10^{8}
	1/2.5 D ₂ O/DMSO-d ₆ ^c	7680	2600	1.99×10^{7}
9b	1/5 D ₂ O/DMSO-d ₆ ^c	h	h	
	CD_3CN^d	g	g	
10b	1/5 D ₂ O/DMSO-d ₆ ^c	4240	43500	1.84×10^{8}
11b	1/5 D ₂ O/DMSO-d ₆ ^c	f	>100000 ^f	
	1/2.5 D ₂ O/DMSO-d ₆ ^c	4220	460	1.94×10^{6}
12b	1/5 D ₂ O/DMSO-d ₆ ^c	h	h	
	CD_3CN^d	g	g	
	$5/95 (v/v) D_2O/$ CD_3CN^d	h	h	

^aAverage K_a values from multiple ¹H NMR titrations. ^bErrors in K_a are less than 15%. ^cNeu5Ac used as salt **4b**. ^dNeu5Ac used as salt **4c**. ^e K_{21} corresponds to 2/1 receptor-sugar association constant. ^fBinding constants too large to be accurately determined by NMR titrations. ^gPrecipitate. ^hComplexation-induced shifts too small to be used for the determination of the binding constants.



Figure 5. Energy-minimized structure of the 2/1 complex formed between **5b** and NeuSAc (MacroModel V.9.8, OPLS_2001 force field, MCMM, 50000 steps). Color code: receptor N, blue; receptor C, gray; the sugar molecule is highlighted in yellow.

In a more competitive solvent, such as a $1/5 D_2O/DMSO-d_6$ mixture, the binding of **4b** by **5b**-7**b**, **10b**, and **11b** was also shown to be very effective. The curve fitting of the titration data indicated the existence of 1/1 and 2/1 receptor-sugar complexes in the used medium. The binding constants for the 2/1 receptor-sugar complexes were determined to be significantly higher than those for the 1/1 binding. During the titration of 7b, incorporating two pyridinium units, with **4b** the saturation occurred after the addition of about 0.50 equiv of **4b** (see Figures 6 and 7), indicating strong binding and the formation of 2/1 receptor-sugar complexes under the titration conditions. In the case of **5b** and **6b** the saturation was reached after the addition of **4b**.

In the same medium $(1/5 D_2O/DMSO-d_6 \text{ mixture})$, the formation of strong 2/1 receptor-sugar complexes was also indicated by the titrations of quinolinium-based receptors 10b and 11b with 4b. Binding of 4b by 11b, consisting of two

quinolinium groups, proved to be too strong to be followed quantitatively $(K_{21} > 100000 \text{ M}^{-1})$.¹⁸ In the case of **10b**, incorporating one quinolinium group, the interactions between the binding partners are also strong $(K_{11} = 4240 \text{ and } K_{21} = 43500 \text{ M}^{-1})$ but less effective as for **11b**·4b. The addition of 0.65 equiv of **4b** leads to practically complete complexation of the receptor **10b**, whereas in the case of **11b** the saturation occurs before the addition of 0.5 equiv of **4b**.

As the solvent polarity increases, the magnitude of the complexation-induced chemical shifts decreases and the addition of more **4b** is necessary to reach the saturation (for example, see parts a and b of Figure 6). When going from 1/5 to 1/2.5 (v/v) mixture of D₂O and DMSO- d_{6} , the 2/1 receptor-sugar complexes become much less stable, but the 1/1 binding is markedly improved; examples of association constants for **5b·4b**, **7b·4b**, and **11b·4b** are given in Table 1. Thus, the compounds containing both neutral and cationic recognition sites are able to complex **4b** even in the presence of about 30% D₂O.

In the case of the symmetrical compounds 9b and 12b, possessing only cationic recognition sites, the interactions with 4b in aqueous media were shown to be significantly weaker than those observed for the other receptors. For example, the complexation induced chemical shifts observed during the titrations of 9b/12b with 4b in a 1/5 mixture of D_2O and DMSO- d_6 are too small to be used for the determination of the binding constants. In addition, no significant shifts of *N*-acetylneuraminic acid signals (used as tetramethylammonium salt 4b) were observed during the titrations of 4b with the pyridinium-based compound 9b (inverse titrations). These results are in contrast with those of the inverse titrations of 4b with receptor 7b, containing both aminopyridine- and pyridinium-based recognition sites.

As the solvent polarity decreases, the interactions of the symmetrical pyridinium- and quinolinium-based compounds with N-acetylneuraminic acid (used as salts 4b and 4c) become expectedly more strong. Experiments in CD₃CN indicated effective binding of 4c by 9b and 12b, but precipitation of a solid, which was identified as a receptor-sugar complex, prevented the analysis of the binding constants in this solvent by ¹H NMR spectroscopic titrations. In a mixture of CD₃CN and D_2O (95/5 v/v) the ¹H NMR titrations could be carried out; however, the complexation-induced shifts were too small to be used for the determination of the binding constants. The results obtained with **9b** and **12b** in $D_2O/DMSO-d_6$ and $D_2O/$ CD₃CN mixtures indicate that aqueous media are too competitive for 9b and 12b to bind Neu5Ac effectively. Similar observations (strong binding in CD₃CN and almost no binding in water) have recently been described by Steed et al. for the complexation of halides by a triethylbenzene-based anion receptor incorporating three quinolinium groups as recognition sites.^{12e}

CONCLUSION

Nature's use of both neutral and cationic recognition groups, such as primary amide (Asn, Gln), hydroxyl (Ser), or quanidinium groups (Arg), to bind anionic carbohydrates has inspired the design of the receptors **5b**–**8b**, **10b**, and **11b**, which contain both cationic (pyridinium or quinolinium groups) and neutral (aminopyridine) binding groups. These compounds were expected to bind *N*-acetylneuraminic acid, the most naturally abundant sialic acid, by a combination of neutral/charge-reinforced hydrogen bonds, ion pairs, $CH-\pi$,



Figure 6. (a) Partial ¹H NMR spectra (400 MHz) of receptor 7b ([7b] = 0.59 mM) after addition of 0.00–1.52 equiv of 4b in D₂O/DMSO-d₆ 1/5 (v/v). (b) Partial ¹H NMR spectra of receptor 7b ([7b] = 0.82 mM) after addition of 0.00–1.71 equiv of 4b in D₂O/DMSO-d₆ 1/2.5 (v/v). Shown are chemical shifts of the pyridine CH₃ signals of 7b.



Figure 7. Mole ratio plot: titration of receptor 7b with 4b in $D_2O/DMSO-d_6 1/5$ (v/v) (analysis of the complexation-induced shift of the CH₂ signal of 7b).

and van der Waals interactions. ¹H NMR spectroscopic titrations indicated that compounds **5b**–**8b**, **10b**, and **11b** are able to bind NeuAc (used as the salt **4b** or **4c**) even in the presence of 30% D_2O . Thus, compounds **5b**–**8b**, **10b**, and **11b** combine ease of synthesis and the ability to complex NeuAc in highly competitive aqueous media. In contrast, D_2O -containing media were shown to be too competitive for compounds **9b** and **12b**, containing only cationic recognition sites, to bind NeuSAc effectively. As expected, experiments in less polar

media, such as acetonitrile, indicated the effective binding of NeuSAc by 9b/12b.

In summary, pyridinium and quinolinium groups were shown to be useful building blocks for the construction of receptor molecules for anionic carbohydrates. As in natural complexes, the combination of cationic and neutral recognition groups in 5b-8b, 10b, and 11b seems to be responsible for effective binding of NeuAc in highly competitive media; the binding properties of compounds 5-12 have now been analyzed in more detail (detailed analysis of the different interactions contributing to complex stability). Interesting complexation properties are expected for compounds of types 20-25, in which pyridinium/quinolinium or imidazolium units are combined with such neutral recognition groups as imidazole, indole, pyrrole, and 8-hydroxyquinoline (see Figure 8); the syntheses of these compounds and the analysis of their binding properties are in progress.

EXPERIMENTAL SECTION

Analytical TLC was carried out on silica gel 60 F_{254} plates; column chromatography was carried out on silica gel. Melting points are uncorrected. Binding studies are described in the Supporting Information.

1-[(3-Methylpyridinium-1-yl)methyl]-3,5-bis[((4,6-dimethylpyridin-2-yl)amino)methyl]-2,4,6-triethylbenzene Bromide (5a). 1-(Bromomethyl)-3,5-bis[((4,6-dimethylpyridin-2-yl)amino)-methyl]-2,4,6-triethylbenzene (16; 0.35 g, 0.67 mmol) and 3-methylpyridine (0.065 mL, 0.67 mmol) were dissolved in dry



Figure 8. Further examples of acyclic receptors containing neutral and cationic recognition sites.

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dichloromethane (35 mL), and the resulting mixture was refluxed for 8 h. The solvent was removed under reduced pressure, and the crude product was purified via column chromatography (chloroform/ methanol 7/1 (v/v) $\rightarrow 3/1$ (v/v)). Yield: 41%. Mp: 157–158 °C. ¹H NMR (400 MHz, CDCl₃): δ 1.13 (t, J = 7.6 Hz, 6H), 1.28 (t, J = 7.6 Hz, 3H), 2.25 (s, 6H), 2.42 (s, 6H), 2.68 (s, 3H), 2.75 (q, J = 7.6 Hz, 4H), 2.80 (q, J = 7.6 Hz, 2H), 4.48 (s, 4H), 6.17 (s, 2H), 6.34 (s, 2H), 6.40 (s, 2H), 8.04 (m, 1H), 8.16 (d, J = 8.0 Hz, 1H), 8.90 (s, 1H), 9.69 (s, 1H) ppm. ¹³C NMR (100 MHz, CDCl₃): δ 16.1, 16.3, 18.6, 21.1, 22.3, 23.0, 23.5, 40.5, 58.1, 105.2, 113.6, 124.1, 128.1, 133.2, 139.5, 140.8, 143.5, 145.09, 145.3, 147.4, 150.9, 152.8, 156.1 ppm. HR-MS (ESI): calcd for C₃₅H₄₆BrN₅ 268.69103 [M⁺ + H⁺], 536.37477 [M⁺]; found 268.69110, 536.37476. $R_{\rm f}$ = 0.10 (chloroform/methanol 7/1 v/v).

1-[(3-Methylpyridinium-1-yl)methyl]-3,5-bis[((4,6-dimethylpyridin-2-yl)amino)methyl]-2,4,6-triethylbenzene Hexafluorophosphate (5b). To a solution of 5a (145 mg, 0.24 mmol) in methanol (10 mL) was added a solution of NaPF₆ (119 mg, 0.71 mmol) in methanol (2 mL), and the resulting mixture was stirred at room temperature for 48 h. Afterward, the solvent was evaporated and the crude product was purified via column chromatography (chloroform/methanol 7/1 (v/v)). Yield: 81%. Mp: 115-116 °C. ¹H NMR (600 MHz, DMSO- d_6): δ 0.80 (t, J = 7.3 Hz, 6H), 0.94 (t, J = 7.2 Hz, 3H), 2.15 (s, 6H), 2.26 (s, 6 H), 2.55 (s, 3H), 2.64 (q, J = 6,2 Hz, 4H), 2.69 (q, J = 6.2 Hz, 2H), 3.40 (br. s, 2 H), 4.41 (s, 4H), 5.94 (s, 2H), 6.20 (s, 2H), 6.30 (s, 2H), 8.04 (t, J = 7.0 Hz, 1H), 8.50 (d, J = 7.7 Hz, 1H), 8.54 (m, 1H), 8.90 (s, 1H) ppm. 13C NMR (150 MHz, DMSO d_6): δ 16.0, 16.1, 17.9, 20.5, 22.8, 22.9, 39.2, 57.4, 105.1, 112.5, 125.3, 127.5, 134.2, 138.8, 140.6, 143.5, 144.6, 146.1, 146.2, 147.2, 155.9, 157.9 ppm. HR-MS (ESI): calcd for $C_{35}H_{46}F_6N_5P$ 536.37477 [M⁺], $682.34677 [M^+ + PF_6^- + H^+]$; found 536.37550, 682.34899. $R_f = 0.24$ (chloroform/methanol 7/1 (v/v)).

1-[(2-Amino-5-methylpyridinium-1-yl)methyl]-3,5-bis[((4,6dimethylpyridin-2-yl)amino)methyl]-2,4,6-triethylbenzene Bromide (6a). 1-(Bromomethyl)-3,5-bis[((4,6-dimethylpyridin-2-yl)amino)methyl]-2,4,6-triethylbenzene (16; 1.020 g, 1.95 mmol) and 2amino-5-methylpyridine (18; 0.211 mg, 1.95 mmol) were dissolved in dry dichloromethane (50 mL), and the resulting mixture was refluxed for 8 h. The solvent was removed under reduced pressure, and the crude product was purified via column chromatography (chloroform/ methanol 7/1 (v/v)). Yield: 47%. Mp: 163-164 °C. ¹H NMR (400 MHz, CDCl₃): δ 1.26 (t, J = 7.5 Hz, 3 H), 1.27 (t, J = 7.4 Hz, 6 H), 2.27 (s, 6H), 2.14 (s, 3H), 2.36 (s, 6H), 2.79 (q, J = 7.4 Hz, 6H), 4.38 (s, 4H), 4.80 (br. s, 2H), 5.44 (s, 2H), 6.18 (s, 2H), 6.37 (s, 2H), 6.77 (s, 1H), 7.46 (m, 1H), 7.85 (d, J = 9.0 Hz, 1H), 9.12 (s, 2H) ppm. ¹³C NMR (100 MHz, CDCl₃): δ 16.1, 16.6, 17.9, 21.2, 23.1, 23.4, 23.6, 40.5, 52.3, 103.7, 114.1, 116.1, 123.0, 125.1, 131.3, 133.6, 142.9, 145.2, 146.6, 150.2, 153.6, 155.3, 157.3 ppm. HR-MS (ESI): calcd for $C_{35}H_{47}BrN_6$ 276.19675 $[M^+ + H^+]$, 551.38567 $[M^+]$; found 276.19649, 551.38571. $R_f = 0.35$ (chloroform/methanol 7/1 (v/v)).

1-[(2-Amino-5-methylpyridinium-1-yl)methyl]-3,5-bis[((4,6dimethylpyridin-2-yl)amino)methyl]-2,4,6-triethylbenzene Hexafluorophosphate (6b). To a solution of 6a (110 mg, 0.174 mmol) in methanol (7 mL) was added a solution of NaPF₆ (88 mg, 0.522 mmol) in methanol (2 mL). The resulting mixture was stirred at room temperature for 48 h, the solvent was removed through rotary evaporation, and the crude product was purified via column chromatography (chloroform/methanol 3/1 (v/v)). Yield: 78%. Mp: 145 °C. ¹H NMR (600 MHz, DMSO- d_6): δ 1.07 (t, J = 7.3 Hz, 3H), 1.23 (t, J = 7.3 Hz, 6H), 2.11 (s, 3H), 2.14 (s, 6H), 2.29 (s, 6H), 2.58 (m, 6H), 2.79 (q, J = 7.3 Hz, 6H), 4.45 (s, 4H), 5.12 (s, 2H), 6.25 (s, 2H), 6.30 (s, 2H), 6.93 (s, 1H), 7.15 (d, J = 7.8 Hz, 1H), 7.80 (m, 1H), 8.62 (s, 2H) ppm. ¹³C NMR (150 MHz, CDCl₃): δ 15.9, 16.2, 16.9, 20.2, 22.8, 39.2, 49.2, 105.1, 112.4, 114.7, 122.4, 124.7, 132.3, 134.4, 143.9, 144.2, 145.8, 147.2, 153.0, 155.2, 157.9 ppm. HR-MS (ESI): calcd for $C_{35}H_{47}F_6N_6P$ 551.38567 [M⁺], 697.35040 [M⁺ + PF₆ + H^+]; found 551.38570, 697.35077. $R_f = 0.10$ (chloroform/methanol 7/1 (v/v)).

1,3-Bis[(3-methylpyridinium-1-yl)methyl]-5-[((4,6-dimethylpyridin-2-yl)amino)methyl]-2,4,6-triethylbenzene Bromide (7a). 1,3-Bis-(bromomethyl)-5-[((4,6-dimethylpyridin-2-yl)amino)- methyl]-2,4,6-triethylbenzene (15; 0.244 g, 0.52 mmol) and 3methylpyridine (17; 0.10 mL, 1.025 mmol) were dissolved in dry dichloromethane (15 mL), and the resulting mixture was refluxed for 8 h. The solvent was evaporated, and the crude product was purified via column chromatography (chloroform/methanol 7/1 (v/v) \rightarrow 3/1 (v/ v)). Yield: 63%. Mp: 183–184 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ 0.83 (t, *J* = 7.3 Hz, 3H), 1.06 (t, *J* = 7,3 Hz, 6H), 2.36 (s, 3H), 2.17 (s, 3H), 2.47 (q, *J* = 7,3 Hz, 2H), 2.57 (s, 3H), 2.66 (q, *J* = 7.3 Hz, 4H), 4.50 (s, 2H), 5.98 (s, 4H), 6.40 (s, 1H), 6.60 (s, 1H), 8.02 (m, 2H), 8.46 (m, 2H), 8.86 (s, 2H), 8.99 (s, 2 H) ppm. ¹³C NMR (100 MHz, CDCl₃): δ 15.5, 15.9, 18.0, 20.8, 23.1, 40.1, 57.1, 104.0, 111.6, 125.0, 127.5, 137.5, 139.9, 142.0, 144.9, 145.1, 147.1 ppm. HR-MS (ESI): calcd for C₃₄H₄₄Br₂N₄ 254.17775 [M²⁺], 587.27439 [M²⁺ + Br⁻]; found 254.17818, 587.27506. *R*_f = 0.10 (chloroform/methanol 7/1 (v/ v)).

1,3-Bis[(3-methylpyridinium-1-yl)methyl]-5-[((4,6-dimethylpyridin-2-yl)amino)methyl]-2,4,6-triethylbenzene Hexafluorophosphate (7b). A solution of NaPF₆ (360 mg, 2.14 mmol) in methanol (5 mL) was added to a solution of 7a (220 mg, 0.357 mmol) in dry methanol (10 mL), and the resulting mixture was stirred at room temperature for 48 h. The precipitated product was filtered, washed several times with methanol, and dried under reduced pressure; 7b was obtained as a white powder. Yield: 53%. Mp: 181-182 °C. ¹H NMR (600 MHz, DMSO- d_6): δ 0.70 (t, J = 7.3 Hz, 3H), 0.94 (t, J = 7.3 Hz, 6H), 2.15 (s, 3H), 2.26 (s, 3H), 2.55 (q, J = 7,3 Hz, 2H), 2.64 (s, 6 H), 2.70 (q, J = 7.3 Hz, 4H), 4.41 (s, 2H), 5.94 (s, 4 H), 6.20 (s, 1H), 6.30 (s, 1H), 8.04 (t, J = 5.6 Hz, 2H), 8.50 (m, 2H), 8.54 (s, 2H), 8.90 (s, 2H) ppm. ¹³C NMR (150 MHz, DMSO- d_{κ}): δ 15.5, 17.9, 20.6, 23.1, 23.3, 39.3, 57.2, 104.6, 112.7, 126.6, 127.6, 135.7, 138.9, 139.1, 140.6, 143.6, 146.5, 148.0, 155.3, 157.7 ppm. HR-MS (ESI): calcd for $C_{34}H_{44}F_{12}N_4P_2$: 254.17775 [M²⁺], 327.16376 [M²⁺ + $PF_{6}^{-} + H^{+}], 653.32023 [M^{2+} + PF_{6}^{-}], 799.29224 [M^{2+} + 2PF_{6}^{-} + H^{+}]$; found 254.17808, 327.16393, 653.32089, 799.29304. $R_{\rm f} = 0.15$ (chloroform/methanol 7/1 (v/v)).

1,3-Bis[(2-amino-5-methylpyridinium-1-yl)methyl]-5-[((4,6dimethylpyridin-2-yl)amino)methyl]-2,4,6-triethylbenzene Bromide (8a). 1,3-Bis-(bromomethyl)-5-[((4,6-dimethylpyridin-2yl)amino)methyl]-2,4,6-triethylbenzene (15; 0.600 g, 1.24 mmol) and 2-amino-5-methylpyridine (18; 0.310 mg, 2.45 mmol) were dissolved in dry dichloromethane (20 mL), and the resulting mixture was refluxed for 12 h. The solvent was evaporated and the crude product was purified via column chromatography (chloroform/ methanol 7/1 (v/v) \rightarrow 3/1 (v/v)). Yield: 25%. Mp: 218–219 °C. ¹H NMR (400 MHz, DMSO- d_6): δ 1.06 (t, J = 3.2 Hz, 3H), 1.21 (t, J = 7.3 Hz, 6H), 2.22 (s, 6H), 2.12 (m, 2H), 2.24 (s, 3H), 2.45 (s, 3H), 2.67 (m, 4H), 4.54 (s, 2H), 5.12 (s, 4H), 6.54 (s, 1H), 6.90 (s, 1H), 7.39 (d, J = 9.0 Hz, 2H), 7.75 (dd, J = 9.0/1.6 Hz, 2H), 8.70 (s, 4H) ppm. ¹³C NMR (100 MHz, DMSO-*d*₆): δ 14.9, 15.9, 16.9, 19.5, 21.1, 22.9, 23.2, 40.4, 49.7, 113.4, 114.5, 123.1, 125.7, 132.3, 144.2, 145.8, 148.1, 153.1 ppm. HR-MS (ESI): calcd for C34H46Br2N6 269.18865 $[M^{2+}]$, found 269.18940. $R_{f} = 0.10$ (chloroform/methanol 7/1 (v/v)).

1,3-Bis[(2-amino-5-methylpyridinium-1-yl)methyl]-5-[((4,6dimethylpyridin-2-yl)amino)methyl]-2,4,6-triethylbenzene Hexafluorophosphate (8b). To a solution of 8a (140 mg, 0.201 mmol) in methanol (8 mL) was added a solution of $NaPF_6$ (119 mg, 0.706 mmol) in methanol (4 mL). The resulting mixture was stirred at room temperature for 48 h, the solvent was removed through rotary evaporation, and the crude product was purified via column chromatography (chloroform-methanol 3/1 (v/v)). Yield: 65%. Mp: 152–153 °C. ¹H NMR (600 MHz, MeOD- d_4): δ 1.11 (t, J = 7.1 Hz, 3 H), 1.27 (t, J = 7.6 Hz, 6H), 2.18 (s, 6H), 2.37 (m, 2H), 2.40 (s, 3H), 2.53 (s, 3H), 2.72 (m, 4H), 4.81 (s, 2H), 5.23 (s, 4H), 6.69 (s, 1H), 6.89 (s, 1H), 7.13 (s, 2H), 7.15 (d, J = 6.1 Hz, 2H), 7.76 (dd, J = 9.1/1.8 Hz, 2H) ppm. ¹³C NMR (150 MHz, MeOD-*d*₄): δ 15.3, 15.9, 17.4, 18.9, 21.9, 24.4, 24.7, 26.5, 41.8, 50.6, 115.9, 116.1, 125.7, 127.9, 133.6, 133.6, 145.8, 147.8, 148.7, 150.4, 153.9, 155.0 ppm. HR-MS (ESI): calcd for $C_{34}H_{46}F_{12}N_6P_2$ 269.18865 [M²⁺], 683.34203 [M²⁺ + PF_6^{-}] 829.31458 $[M^{2+} + 2PF_6^{-} + H^+]$, found 269.18970, 683.34410, 829.31521. $R_f = 0.10$ (chloroform/methanol 7/1 (v/v)).

1,3,5-Tris[(3-methylpyridinium-1-yl)methyl]-2,4,6-triethylbenzene Bromide (9a). 3-Methylpyridine (17; 0.132 mL, 1.362 mmol) and 1,3,5-tris(bromomethyl)-2,4,6-triethylbenzene (13; 0.100 g, 0.227 mmol) were dissolved in dry dichloromethane (50 mL), and the mixture was refluxed for 24 h. Afterward, the resulting precipitate was filtered, washed several times with dichloromethane, and dried under reduced pressure; 9a was obtained as a white powder. Yield: 89%. Mp: 208–209 °C. ¹H NMR (400 MHz, D₂O): δ 0.93 (t, *J* = 7.6 Hz, 9H), 2.60 (s, 9H), 2.72 (q, *J* = 7.4 Hz, 6H), 6.08 (s, 6H), 8.00 (t, *J* = 6.2 Hz, 3H), 8.46 (d, *J* = 8.3 Hz, 3H), 8.54 (d, *J* = 6.1 Hz, 3H), 8.72 (s, 3H) ppm. ¹³C NMR (100 MHz, D₂O): δ 16.9, 20.7, 26.8, 60.6, 130.8, 130.9, 143.2, 143.5, 145.9, 149.9, 153.4 ppm. HR-MS (ESI): calcd for C₁₃H₄₂Br₃N₃ 160.11208 [M³⁺], found 160.10580.

1,3,5-Tris[(3-methylpyridinium-1-yl)methyl]-2,4,6-triethylbenzene Hexafluorophosphate (9b). A solution of NaPF₆ (420 mg, 2.502 mmol) in methanol (5 mL) was added to a solution of 9a (200 mg, 0.278 mmol) in dry methanol (30 mL), and the resulting mixture was stirred at room temperature for 48 h. The precipitated product was filtered, washed several times with methanol, and dried under reduced pressure; 9b was obtained as a white powder. Yield: 50%. Mp: 309–310 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ 0.70 (t, *J* = 7.4 Hz, 9H), 2.54 (s, 9H), 2.66 (q, *J* = 7.0 Hz, 6H), 5.97 (s, 6H), 8.04 (dd, *J* = 7.8/6.2 Hz, 3 H), 8.50 (d, *J* = 8.0 Hz, 3H), 8.59 (d, *J* = 6.1 Hz, 3H), 8.72 (s, 3H) ppm. ¹³C NMR (100 MHz, DMSO): δ 14.7, 17.9, 23.5, 57.1, 127.7, 128.1, 139.1, 140.9, 143.7, 146.5, 149.8 ppm. HR-MS (ESI): calcd for C₃₃H₄₂F₁₈N₃P₃ 160.11208 [M³⁺], 312.65048 [M³⁺ + PF₆⁻], 770.26569 [M³⁺ + 2PF₆⁻]; found 160.11216, 312.65057, 770.26564.

1-[(3-Methylguinolinium-1-yl)methyl]-3,5-bis[((4,6-dimethylpyridin-2-yl)amino)methyl]-2,4,6-triethylbenzene Bromide (10a). 1-(Bromomethyl)-3,5-bis[((4,6-dimethylpyridin-2-yl)amino)methyl]-2,4,6-triethylbenzene (16; 0.400 g, 0.75 mmol) and 3methylquinoline (19; 0.105 mL, 0.77 mmol) were dissolved in dry dichloromethane (30 mL) and heated at reflux for 16 h. The solvent was removed through rotary evaporation, and the crude product was purified via column chromatography (chloroform-methanol 5/1 (v/ v)). Yield: 45%. Mp: 134–135 °C. ¹H NMR (300 MHz, CDCl₃): δ 1.23 (t, J = 7.7 Hz, 6H), 1.31 (t, J = 7.5 Hz, 3H), 2.19 (s, 6H), 2.44 (s, 6H), 2.53 (m, 4H), 2.86 (q, J = 7.5 Hz, 2H), 2.87 (s, 3H), 4.59 (d; J = 3.5 Hz, 4 H), 6.17 (s, 2H), 6.27 (s, 2H), 6.47 (s, 2H), 8.00 (m, 1H), 8.27 (m, 1H), 8.29 (m, 1H), 8.74 (s, 1H), 8.92 (s, 1H), 9.04 (d, J = 8.9 Hz, 1H) ppm. ¹³C NMR (75 MHz, CDCl₃): δ 16.5, 16.7, 20.1, 21.1, 23.2, 24.0, 29.7, 40.9, 55.4, 106.2, 113.5, 118.5, 122.7, 129.7, 130.1, 130.6, 134.2, 134.7, 135.6, 137.1, 141.3, 145.3, 146.4, 146.9, 148.9, 157.1, 157.2 ppm. HR-MS (ESI): calcd for C₃₉H₄₈BrN₅ 293.69885 $[M^{+} + H^{+}]$, 586.39042 $[M^{+}]$; found 293.69889, 586.39017. $R_{\rm f} = 0.22$ (chloroform/methanol 5/1 (v/v)).

1-[(3-Methylquinolinium-1-yl)methyl]-3,5-bis[(4,6-dimethylpyridin-2-yl)aminomethyl]-2,4,6-triethylbenzene Hexafluorophosphate (10b). A solution of NaPF₆ (183 mg, 0.476 mmol) in methanol (5 mL) was added to a solution of 10a (0.110 mg, 0.165 mmol) in methanol (30 mL), and the resulting mixture was stirred at room temperature for 48 h. The solvent was then evaporated, and the crude product was purified via column chromatography (chloroform/ methanol 3/1 (v/v)). Yield: 80%. Mp: 124-125 °C. ¹H NMR (300 MHz, CDCl₃): δ 1.17 (t, J = 7.5 Hz, 6H), 1.30 (t, J = 7.4 Hz, 3H), 2.19 (s, 6H), 2.36 (s, 6H), 2.53 (m, 4H), 2.61 (s, 3H), 2.85 (q, J = 7.5 Hz, 2H), 4.48 (d; J = 3.8 Hz, 4H), 4.98 (s, 2 H), 6.04 (s, 2H), 6.32 (s, 2H), 6.36 (s, 2H), 7.97 (m, 1H), 8.25 (m, 3H), 8.73 (m, 2H) ppm. ¹³C NMR (75 MHz, CDCl₃): δ 15.8, 16.3, 18.7, 20.9, 23.1, 23.3, 40.1, 54.1, 105.3, 113.9, 117.6, 123.1, 129.9, 130.3, 130.8, 133.7, 134.7, 136.1, 137.3, 145.3, 145.4, 147.1, 148.5, 149.8, 154.9, 157.4 ppm. HR-MS (ESI): calcd for $C_{39}H_{48}F_6N_5P{:}\ 293.69885\ [M^+$ + $H^+],\ 586.39042$ $[M^+]$, 732.36243 $[M^++PF_6^-+H^+]$, found 293.69896, 586.39037, 732.36236. $R_f = 0.41$ (chloroform/methanol 3/1 (v/v)).

1,3-Bis[(**3-methylquinolinium-1-yl)methyl]-5-**[((**4,6-dimethylpyridin-2-yl)amino)methyl]-2,4,6-triethylbenzene Bromide** (**11a**). 1,3-Bis(bromomethyl)-5-[((**4,6-dimethylpyridin-2-yl)amino**)methyl]-2,4,6-triethylbenzene (**15**; 0.360 g, 0.75 mmol) and 3methylquinoline (**19**; 0.25 mL, 1.88 mmol) were dissolved in dry dichloromethane (40 mL) and heated at reflux for 24 h. The solvent was removed through rotary evaporation, and the crude product was purified via column chromatography (chloroform/methanol 3/1 (v/v)). Yield: 33%. Mp: 170–171 °C. ¹H NMR (400 MHz, CDCl₃): δ 1.23 (m, 9 H), 2.12 (s, 3H), 2.29 (m, 4H), 2.56 (s, 3H), 2.86 (m, 2H), 2.99 (s, 6H), 4.75 (s, 2H), 6.19 (s, 1H), 6.30 (s, 4H), 6.33 (s, 1H), 7.96 (m, 2H), 8.23 (dd, *J* = 8.3/1.0 Hz, 2H), 8.31 (ddd, *J* = 8.7/7.1/1.3 Hz, 2H), 8.75 (s, 2H), 9.47 (s, 2H), 9.53 (d, *J* = 8.7 Hz, 2H) ppm. ¹³C NMR (100 MHz, CDCl₃): δ 15.2, 15.7, 16.1, 20.1, 21.1, 24.3, 25.1, 55.7, 65.8, 113.2, 119.2, 124.9, 130.5, 134.8, 135.7, 137.2, 146.5, 146.9, 147.4, 150.0 ppm. HR-MS (ESI): calcd for C₄₂H₄₈Br₂N₄ 304.19340 [M²⁺], 687.35069 [M²⁺ + Br⁻], found 304.19352, 687.30560. R_f = 0.52 (chloroform/methanol 3/1 (v/v)).

1,3-Bis[(3-methylquinolinium-1-yl)methyl]-5-[((4,6-dimethylpyridin-2-yl)amino)methyl]-2,4,6-triethylbenzene Hexafluorophosphate (11b). To a solution of 11a (0.085g, 0.11 mmol) in methanol (15 mL) was added a solution of $NaPF_6$ (111 mg, 0.658 mmol) in methanol (5 mL), and the resulting mixture was stirred at room temperature for 48 h. The precipitated product was filtered, washed several times with methanol, and dried under reduced pressure. The solvent of the remaining solution was removed through rotary evaporation, and the crude product was purified via column chromatography (chloroform/methanol 3/1 (v/v)). Yield: 75%. Mp: 204-205 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ 1.07 (m, 3H), 1.30 (t, J = 7.3 Hz, 6H), 2.26 (s, 6H), 2.43 (m, 4H), 2.69 (s, 6H), 3.35 (m, 2H), 4.60 (s, 2H), 6.19 (s, 4H), 6.59 (s, 1H), 6.85 (s, 1H), 8.12 (m, 2H), 8.35 (td, J = 8.5/4.2 Hz, 3 H), 8.44 (d, J = 8.2 Hz, 2H), 8.72 (s, 2H), 9.06 (d, 2 H, J = 9.1 Hz, 2H), 9.15 (s, 2H) ppm. ¹³C NMR (100 MHz, DMSO-*d*₆): δ 15.3, 15.6, 18.6, 23.3, 23.4, 27.8, 40.1, 53.7, 118.5, 126.0, 129.5, 129.9, 130.2, 132.8, 134.8, 137.3, 142.6, 145.9, 146.8, 149.1 ppm. HR-MS (ESI): calcd for C₄₂H₄₈F₁₂N₄P₂ 304.19340 [M²⁺], 753.35153 $[M^{2+} + PF_6^{-}]$; found 304.19345, 753.35126. $R_f = 0.60$ (chloroform/methanol 3/1 (v/v)).

1,3,5-Tris[(**3-methylquinolinium-1-yl)methyl]-2,4,6-triethylbenzene Bromide (12a).** 3-Methylchinoline (19; 0.304 mL, 2.270 mmol) and 1,3,5-tris(bromomethyl)-2,4,6-triethylbenzene (13; 0.200 g, 0.454 mmol) were dissolved in dry dichloromethane (20 mL), and the resulting mixture was refluxed for 48 h. Afterward the mixture was concentrated to a volume of 5 mL and diethyl ether (5 mL) was added. The resulting yellow precipitate was filtered and washed several times with diethyl ether; **12a** was obtained as a yellow powder. Yield: 46%. Mp: 143–144 °C. ¹H NMR (400 MHz, CDCl₃/MeOD-*d*₄ 10/1 (v/v)): δ 1.17 (m, 9H), 2.57 (m, 6H), 2.93 (s, 9H), 6.36 (s, 6H), 8.04 (m, 3H), 8.31 (m, 9H), 8.83 (s, 3H), 9.05 (d, *J* = 9.0 Hz, 3H) ppm. ¹³C NMR (100 MHz, CDCl₃/MeOD-*d*₄ 10/1 (v/v)): δ 15.3, 19.1, 24.8, 54.9, 118.3, 127.7, 129.7, 129.9, 130.5, 134.2, 135.6, 136.9, 146.73, 150.9 ppm.

1,3,5-Tris[(**3-methylquinolinium-1-yl)methyl]-2,4,6-triethylbenzene Hexafluorophosphate (12b).** To a solution of **12a** (135 mg, 0.155 mmol) in methanol (10 mL) was added a solution of NaPF₆ (235 mg, 1.396 mmol) in methanol (5 mL). The resulting mixture was stirred at room temperature for 48 h. The precipitated product was filtered, washed several times with methanol, and dried under reduced pressure; **12b** was obtained as a white powder. Yield: 40%. Mp: 207–208 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ 1.12, (m, 9H), 2.46 (m, 6H), 2.63 (s, 9H), 6.26 (s, 6H), 8.12 (m, 3H), 8.36 (m, 6H), 8.45 (dd, *J* = 8.3/1.1 Hz, 3H) 9.00 (m, 3H), 9.17 (s, 3H) ppm. ¹³C NMR (100 MHz, DMSO-*d*₆): δ 15.0, 18.5, 24.1, 54.5, 118.7, 127.7, 129.4, 130.0, 130.3, 132.6, 134.9, 137.1, 146.5, 146.7, 150.9 ppm.

Binding Studies. The ¹H NMR titrations were carried out in $D_2O/DMSO-d_6$ and D_2O/CD_3CN mixtures at 25 °C. The titration data were analyzed by nonlinear regression analysis, using the program EQNMR. Dilution experiments show that the receptors do not self-aggregate in the concentration range used. Stock solutions in a $D_2O/DMSO-d_6$ or D_2O/CD_3CN mixture were prepared for the receptor and the anionic sugar (used as the tetramethylammonium or tetrabutylammonium salt). These solutions and the corresponding solvent were combined in such a manner that the concentration of the receptor was kept constant and that of *N*-acetylneuraminic acid was varied (in the case of inverse titrations the concentration of the anionic sugar was kept constant and that of the receptor was varied). For each

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titration 15–20 samples were prepared (the probes were equilibrated at room temperature) and the ¹H NMR spectra were recorded. For each receptor–sugar system at least three ¹H NMR titrations were carried out; examples are given in Tables S1–S7 (see the Supporting Information).

ASSOCIATED CONTENT

S Supporting Information

Tables and figures giving a description of the binding studies and ¹H and ¹³C NMR spectra of 5b-12b. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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