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Anomalous Binding Profile of Phenylboronic Acid with *N*-Acetylneuraminic Acid (Neu5Ac) in Aqueous Solution with Varying pH

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Abstract: Borates are known to interact with carbohydrate moieties expressed on the surface of biological membranes of a variety of cells, viruses, bacteria, and fungi. This study revealed the anomalous binding profile of borate in aqueous solution with *N*-acetylneuraminic acid (Neu5Ac, sialic acid) as a potential receptor site on the surfaces of biological membranes using ^{11}B, ^{1}H, ^{13}C, and ^{15}N nuclear magnetic resonance spectroscopies. 3-(Propionamido)phenylboronic acid (PAPBA) was chosen as the model borate compound. The equilibrium constant (*K*) for Neu5Ac binding to PAPBA was compared with those for glucose, mannose, and galactose, which are the major carbohydrate constituents of glycoproteins and glycolipids expressed on biological membranes. In the Neu5Ac/PAPBA system, the unusual pH dependency of the *K* values, a decrease in *K* with increasing pH, was observed, suggesting the formation of a trigonal-formed complex stabilized by the coordination of an amide group of Neu5Ac at the C-5 position to the boron atom, forming intramolecular B−N or B−O bonding. Furthermore, the anomalously high complexing ability at physiological pH 7.4 was confirmed for this system, with the *K* value 37.6 which is approximately 7 times higher than that for glucose. This exceptionally high value of *K* at physiological pH, compared to those of other sugars, strongly suggests that the boronic acid selectively recognizes the Neu5Ac residues of the glycosylated components including glycoproteins and gangliosides existing on the surface of the biological membranes.

Introduction

Borate has been demonstrated to make stable complexes with carbohydrates, vitamins, coenzymes, and ribonucleic acids (RNAs),[1−11] which contain hydroxyl groups in a favorable position for borate complexation. The borate interaction with these polyol compounds attracts growing interest as a scientific basis to construct a novel molecular recognition system as well as a platform of diverse applications related to polyol compounds, including chromatographic and membrane separation,[12−14] sensing systems,[15−17] drug delivery and discovery extended by use of boradeption,[18−20] and a particular cancer treatment known as boron neutron capture therapy (BNCT).[21−26] Furthermore, borate is known to interact with the natural biological mem-

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(1) Kitano, S.; Kataoka, K.; Koyama. Y.; Okano, T.; Sakurai, Y. *Makromol. Chem., Rapid Commun.* **1991**, *12*, 227.
(2) Miyazaki, H.; Okano, T.; Sakurai, Y.; Kataoka, K *Biochem. Biophys. Res. Commun.* **1993**, *195* (2), 829.
(3) Kataoka, K.; Miyazaki, H. *Macromolecules* **1994**, *27*, 1061.
(4) Aoki, T.; Nagano, Y.; Sanui, K.; Ogata, N.; Kikuchi, A.; Sakurai, Y.; Kataoka, K.; Okano, T. *J. Biomater. Sci., Polymer Ed.* **1997**, *9* (3), 1.
(5) Kataoka, K.; Miyazaki, H.; Bunya, M.; Okano, T.; Sakurai, Y. *J. Am. Chem. Soc.* **1998**, *120*, 12694.
(6) Schott, H.; Rudloff, E.; Schmidt, P.; Roychoudhury, R.; Kossel, H. *Biochemistry* **1973**, *12*, 932.
(7) Weith, H. L.; Weibers, J. L.; Gilham, P. T. *Biochemistry* **1970**, *9*, 4396.
(8) Yoshimura, K.; Miyazaki, Y.; Sawada, S.; Waki, H. *J. Chem. Soc., Faraday Trans.* **1996**, *92* (4), 651.
(9) James, T. D.; Sandanayake, K. R. A. S.; Shinkai, S. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1910.
(10) Wulff, G.; Vesper, W. *J. Chromatogr*. **1978**, *167*, 171.
(11) Burgemeister, T.; Grobe-Einsler, R.; Grotstollen, R.; Mannschreck, A.; Wulff, G. *Chem. Ber.* **1981**, *114*, 3403.
(12) Shinbo, T.; Nhishimura, K.; Yamaguchi, T.; Sugiura, M. *J. Chem. Soc., Chem. Commun.* **1986**, 349.
(13) Morin, G. T.; Hughes, M. P.; Paugam, M.-F.; Smith, B. D. *J. Am. Chem. Soc.* **1994**, *116*, 8895.
(14) Westmark, P. R.; Gardiner, S. J.; Smith, B. D. *J. Am. Chem. Soc.* **1996**, *118*, 11093.
(15) James, T. D.; Sandanayake, K. R. A. S.; Shinkai, S. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1910.
(16) James, T. D.; Sandanayake, K. R. A. S.; Shinkai, S. *Nature* **1995**, *374*, 345.
(17) James, T. D.; Sandanayake, K. R. A. S.; Iguchi, R.; Shinkai, S. *J. Am. Chem. Soc*. **1995**, *117*, 8982.
(18) Pan, X. Q.; Wang, H.; Shukla, S.; Sekido, M.; Adams, D. M.; Tjarks, W.; Barth, R. F.; Lee, R. J. *Bioconjugate Chem*. **2002**, *13* (3), 435.
(19) Stiriba, S.-E.; Frey, H.; Haag, R. *Angew. Chem., Int. Ed.* **2002**, *41*, 1329.
(20) Gallop, P. M.; Paz, M. A.; Henmson, E. *Science* **1982**, 217, 166.
(21) (a) Lawler, A. *Science* **1995**, *267*, 956. (b) Flam, F. *Science* **1994**, *265*, 468. (c) Anderson, C. *Science* **1993**, *262*, 329.
(22) Hawthore, M. F. *Angew. Chem., Int. Ed. Engl*. **1993**, *32*, 950.
(23) Barth, R. F.; Yang, W.; Rotaru, J. H.; Moeschberger, M. L.; Joel, D. D.; Nawrocky, M. M.; Goodman, J. H.; Soloway, A. H. *Cancer Res*. **1997**, *57*, 1129.
(24) Soloway, A. H.; Tjarks, W.; Barnum, B. A.; Rong, F.-G.; Barth, R. F.; Codogni, I. M.; Wilson, G. *Chem. Rev*. **1998**, *98*, 1515.

branes of a variety of cells, viruses, bacteria, and fungi through the membrane-constituting carbohydrate moieties.[27−34] For example, the inhibitory effect of borate on virus infection has been observed for such viruses as vaccinia,[32] chicken tumor I,[33] and *E. coli* T_7 bacteriophage.[34] Nevertheless, the recognition site for borate on cellular carbohydrates has not been well-known, although it is important to determine the site of interaction to gain chemical insight into the biological action of borate compounds.

In this study, we focused on the borate interaction with *N*-acetylneuraminic acid (Neu5Ac), which is the most common occurring sialic acid, in aqueous milieu particularly at physiological pH 7.4. It should be noted that Neu5Ac and its derivatives exist as the principal carbohydrate components and generally occupy the terminal positions of the carbohydrate chains of glycoproteins and glycolipids in biological membranes of most of the higher animals including mammalians and a few microorganisms.[35−38] They appear to play important roles as ligands in cellular recognition processes[39] and modulate the permeability of cellular membranes.[40] It is further documented that alternations in the amount of sialic acid on the cellular surface have a significant influence on many biological recognition process,[41−43] correlating with the host defensive activity against viral infection or tumor cells.[44]

As a borate compound, 3-(propionamido)phenylboronic acid (PAPBA) was used in this study because arylboronic compounds are stable in aqueous media and have been used as ligands for the affinity selection of various sugar compounds[45,46] and for the cellular recognition (boradeption)[20,47] and stimulation.[2,4,30] In particular, PAPBA is a model of the monomer unit in the water-soluble copolymer of 3-(acrylamido)phenylboronic acid which was shown previously by our group to work as a synthetic lectin to induce the proliferation of lymphocytes.[2,30]

Recently, a few studies have been done to clarify the structure of the complexes between glucose and arylboronic acid in aqueous solution using high resolution ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopies, which provides detailed structure information, particularly the binding sites on monosaccharides.[48,49] In addition, the ^{11}B NMR measurement can provide an equilibrium constant for the complexation of borate with saccharide compounds, because the concentrations of the borate/saccharide complex and other free species can all be determined without ambiguity by ^{11}B NMR. Thus, in this study, the binding profile of PAPBA with Neu5Ac was mainly investigated using ^{1}H, ^{13}C, ^{15}N and ^{11}B NMR spectroscopies, highlighting the anomalous interaction of Neu5Ac with the borate compound compared with the other common nonionic hexoses.

Experimental Section

Preparation of 3-(Propionamido)phenylboronic Acid (PAPBA). PAPBA was prepared by the condensation reaction of propionic acid (75 mmol; Wako Pure Chemical Industries Co., Ltd., Japan) with 3-aminophenylboronic acid monohydrate (75 mmol; Sigma) in water (100 mL) at pH 4.8, 0 °C for 1 h using [1-ethyl-3-(dimethylamino)-propyl]carbodiimide hydrochloride (EDC-HCl) (75 mmol; Peptide Institute, Inc., Japan) as the condensation reagent.[3] The reaction mixture was then extracted 3 times with 500 mL of diethyl ether, followed by recrystallization from water to obtain the PAPBA crystals. The structure of PAPBA was confirmed by the 600 MHz ^{1}H NMR (JEOL JNM-A600) spectrum in DMSO-d_6 [δ = 9.75(s, 1H, NH), 7.82−7.23(m, 4H, arom. H), 2.29(q, 2H, CH_2), 1.07(t, 3H, CH_3)]. All other chemicals were of reagent grade and were used as received. Water was purified using an ion-exchange column, followed by distillation.

Determination of pK_a. The acid−base titration of PAPBA was performed to determine the apparent pK_a value. A total of 4 mg of PAPBA was dissolved in 30 mL of water and titrated with 0.05 N NaOH at 37 °C after all the boronic acid groups were converted into the nonionized form by adding a small amount of 0.1 N HCl. Both the acid−base titrations and ^{11}B NMR measurements of PAPBA at various pHs were performed to determine the apparent pK_a value.

NMR Measurements. A. ^{11}B NMR. The ^{11}B NMR spectra were measured using a JEOL EX-400 Fourier transform spectrometer operating at 128.15 MHz for ^{11}B. Field stability was such that no field-frequency lock was required. To avoid the broad signal from the boron incorporated in glasses, measurements were carried out in a 10 mm tube made of poly(tetrafluoroethylene). The sample temperature was regulated at 25 ± 1 °C. The peak areas for spectra with resolved line shapes were calculated using the standard NMR integration software. For unresolved lines, the peak areas were obtained with a computer program which allows the operator to fit experimental line shapes with a series of Lorentzian functions. The ^{11}B chemical shifts were measured relative to external $BF_3{\cdot}O(C_2H_5)_2$ with positive values for signals at frequencies higher than this reference. Susceptibility corrections for externally referenced ^{11}B shifts are usually small; therefore, none were applied.

Stock sample solutions were prepared by dissolving an appropreate amount of sugar in deionized-distilled water with stirring at room temperature. A boronic acid solution was then added to the sample solution so that the total boron concentration $[B]_0$ became 0.01 M. After the solution was allowed to stand for 24 h at the desired temperature, its ^{11}B NMR spectrum was recorded. Buffers used in the ^{11}B NMR measurements conducted at varying pHs were citric acid/sodium citrate buffer solution(pH < 6.5), phosphate buffer solution (6.5 < pH <8.0),

(25) Nakanishi, A.; Guan, L.; Kane, R. R.; Kasamatsu, H.; Hawthorne, M. F. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 238.
(26) Pan, X. Q.; Wang, H.; Shukla, S.; Sekido, M.; Adams, D. M.; Tjarks, W.; Barth, R. F.; Lee, R. J. *Bioconjugate Chem.* **2002**, *13* (3), 435.
(27) Chen, X.; Scgauder, S.; Potier, N.; Dorsselaer, A. V.; Pelczer, B. L.; Bassler, B. L.; Hughson, F. M. *Nature*, **2002**, *415*, 545.
(28) O′Neill, M. A.; Eberhard, S.; Albersheim, P.; Darvill, A. G. *Science* **2001**, *294*, 846.
(29) Höfte, H. *Science* **2001**, *294*, 795.
(30) Uchimura, E.; Otsuka, H.; Okano, T.; Sakurai, Y.; Kataoka, K. *Biotechnol. Bioeng.* **2001**, *72* (3), 307.
(31) Zitte, C. A. *Adv. Enzym.* **1951**, *12*, 493.
(32) Dunham, W. B.; MacNeal, W. J. *J. Bacteriol.* **1942**, *44*, 413.
(33) Bryan, W. R.; Maver, M. E.; Moloney, J. B.; Thomas, M. A.; Sells, M. T. *J. Natl. Cancer Inst.* **1949**, *10*, 647.
(34) Kerby, G. P.; Gowdy, R. A.; Dilon, E. S.; Dillon, M. L.; Csaky, T. Z.; Sharp, D. G.; Beard, J. W. *J. Immunol.* **1949**, *63*, 93.
(35) Mammen, M.; Choi, S.-K.; Whitesides, G. M. *Angew. Chem., Int. Ed.* **1998**, *37*, 2754.
(36) Hood, D. W.; Makepeace, K.; Deadman, M. E.; Rest, R. F.; Thibault, P.; Martin, A.; Richards, J. C.; Moxon, E. R. *Mol. Microbiol.* **1999**, *33* (4), 679.
(37) Torgov, V. I.; Shashkov, A. S.; Jann, B.; Jann, K. *Carbohydr. Res.* **1995**, *272* (1), 73.
(38) Mattos-Guaraldi, A. L.; Cappelli, E. A.; Previato, J. O.; Formiga, L. C. D.; Andrade, A. F. B. *FEMS Microbiol. Lett.* **1999**, *170*, 159.
(39) Roseman, S. *Chem. Phys. Lipids* **1970**, *5*, 270. Roth, S.; McGuire, E. J.; Roseman, S. *J. Cell Biol.* **1971**, *51*, 536.
(40) Glick, J. L.; Githens, S. *Nature (London)* **1965**, *88*, 208. Rahman, H.; Rosner, H.; Breer, H. *J. Theor. Biol.* **1976**, *57*, 231.
(41) Schauer, R.; Schukla, A. K.; Schroder, C.; Muller, E. *Pure Appl. Chem.* **1984**, *56*, 907.
(42) Morell, A. G.; Gregoriadis, G.; Scheinberg, I. H.; Hickman, J.; Ashwell, G. *J. Biol. Chem.* **1971**, *246*, 1461.
(43) Chavin, S. I.; Weidner, M. *J. Biol. Chem.* **1984**, *259*, 3387.
(44) Hirsch, R. L.; Griffin, D. E.; Winkelstein, J. A. *J. Immunol.* **1981**, *127*, 1740.
(45) Wulff, G. *Pure Appl. Chem.* **1982**, *54*, 2093.
(46) Akparov, V. H.; Stepanov, V. M. *J. Chromatogr.* **1978**, *155*, 329.
(47) Gallop, P. M.; Paz, M. A.; Henson, E.; Latt, S. A. *BioTechniques* **1984**, *2*, 32.
(48) Norrild, J. C. *J. Chem. Soc., Perkin Trans. 2* **2001**, 719.
(49) Bielecki, M.; Eggert, H.; Norrild, J. C. *J. Chem. Soc., Perkin Trans. 2* **1999**, 449.

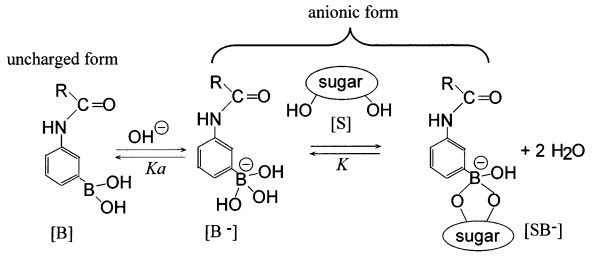

Figure 1. Equilibria of (alkylamido)phenylboronic acid in an aqueous solution in the presence of sugar.

R: $-(CH_2CH)_n-$ or CH_3CH_2-

B, B^-, S, and SB^- denote the free boronic acid, free boronate, free sugar, and sugar/boronate complex, respectively.

sodium *p*-phenosulfonate/NaOH buffer solution ($8.0 < pH < 9.0$), and $NaHCO_3$/NaOH/NaCl buffer solution ($pH > 9.0$), respectively. The ionic strength of all the buffer solutions was kept at 0.15 by adding the appropriate amount of NaCl.

B. ^{1}H, ^{13}C, ^{1}H−^{1}H COSY/TOCSY, ^{1}H−^{13}C PFG-HMQC/HMBC, ^{1}H−^{15}N PFG-HMQC, and NOESY NMR. The sugar/PAPBA samples (0.3 M) were dissolved in D_2O or DMSO-d_6. The NMR spectra were recorded on a JEOL JNM-A600 (600 MHz) spectrometer at 298 K using a glass NMR tube (5 mm o.d.). Chemical shifts are reported in parts per million (ppm). For ^{1}H NMR spectra, 3-(trimethylsilyl)-propionic-2,2,3,3-d_4 acid sodium salt (TSP) was used as the internal reference at 0 ppm for aqueous solutions and residual DMSO-d_5 and at 2.49 ppm for DMSO-d_6 solutions. For ^{13}C NMR spectra, TSP at 0 ppm and the central solvent peak of DMSO-d_6 at 39.5 ppm were used as the reference. In 2D ^{1}H−^{13}C PFG-HMQC and PFG-HMBC experiments, the matrix size was 2048 data points in the F2 (^{1}H) frequency domain and 1024 data points in the F1 (^{13}C) frequency domain. PFG-HMBC spectra were recorded using a 60 ms duration time for long-range coupling with the *z*-axis pulsed field gradient. In ^{1}H−^{15}N PFG-HMQC experiments, the matrix size was 1024 × 256 data points and $^{15}NH_4NO_3$ in DMSO-d_6 solution at 0 ppm was used as an external reference.

Boronate Affinity Chromatography. A chromatographic column was filled with swollen Matrex Gel PBA (Amicon, Massachusetts, U.S.A.) which contains up to 100 μmol of phenylboronate/mL of gel to be covalently affixed to the cross-linked agarose support.[46,50,51] Measurements were performed at room temperature at a flow rate of 0.2 mL/min using a liquid chromatograph (JASCO, Japan) equipped with UV−vis (Abs = 260 nm, UV-1575, JASCO) and RI (RI-930, JASCO) detectors. A 0.05 M phosphate buffer of pH 6.5 was used as the eluent. The sample was injected into the column circuit through a 5 μL loop.

Results and Discussion

1. Complexing Ability of PAPBA to Sugars Characterized by ^{11}B NMR. Phenylboronic acid in water exists as an equilibrium mixture of the nonionic trigonal boronic form, $PhB(OH)_2$ (:B), and the ionic tetrahedral monoboronate form, $PhB(OH)^-_3$ ($:B^-$). The acid/base equilibrium between these two species is shown in Figure 1.[3,52] It is known that the tetrahedral-formed phenylboronate ($PhB(OH)^-_3$) can form significantly stable covalent bonds with polyol compounds in aqueous solution.[3−5,53] A pK_a of 8.6 was obtained for PAPBA[3] by both acid−base titration and ^{11}B NMR. Thus, at least 6% of the total

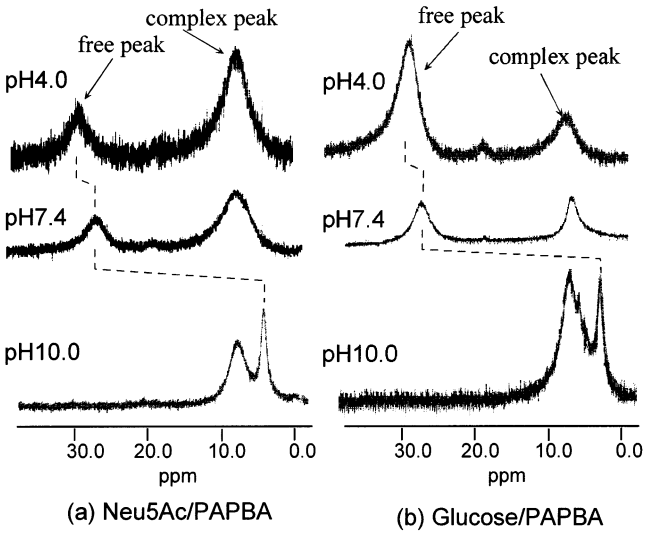

Figure 2. ^{11}B NMR spectra for PAPBA/Neu5Ac (a) and PAPBA/glucose (b) at different pHs. Solution: 0.01 M PAPBA/0.12 M sugar ($I = 0.15$).

PAPBA in the solution exists as the tetrahedral-formed boronates at pH 7.4, allowing the inducement of complexation with the sugar compounds. To confirm the interaction between phenylboronic acid and Neu5Ac, the change in the^{11}B NMR spectra of PAPBA with the addition of Neu5Ac was investigated at various pHs. The ^{11}B NMR spectra of the PAPBA solutions (0.01 M) equilibrated with Neu5Ac (0.12 M) at different pH values are compared with those of glucose (0.12 M) in Figure 2. Since the exchange between the free and the sugar-complexed form of boronic acid/boronate is slow relative to the ^{11}B NMR time scale for data acquisition, the spectrum consists of two peaks; a free boronic/boronate peak ($B + B^-$), whose chemical shift shows an upfield shift with pH increment, and a peak corresponding to boronic acid/boronate complexed with Neu5Ac or glucose at a higher field (~8 ppm). For the glucose/PAPBA system (Figure 2b), the peak intensity for the free PAPBA decreased with an increase in pH, compensating with an increased peak intensity of the glucose/PAPBA complex. Obviously, this is due to a shift in the boronic acid/boronate equilibrium shown in Figure 1 to the direction of the complexed form with glucose with the increasing mole fraction of B^- at higher pH. It should be noted this is a general trend observed for the ^{11}B NMR spectra of boronate complexing with sugar compounds including glucose, mannose, and galactose.[54] On the other hand, the opposite trend was clearly observed for the Neu5Ac/PAPBA system (Figure 2a): the relative peak intensity of the complex compared with the free form decreased with a pH increase.

To elucidate the complexing ability of PAPBA to Neu5Ac, equilibrium constants (K) were calculated from the peak area ratio of the two peaks corresponding to the free PAPBA ($B + B^-$) and the complex (SB and SB^-, abbreviating complex of sugar with the trigonal $PhB(OH)_2$ and tetrahedral $PhB(OH)_3^-$, respectively) in the ^{11}B NMR spectra, and then, the result was compared with the K values for glucose, mannose, and galactose/PAPBA systems at physiological pH 7.4. The ^{11}B NMR precisely determines an equilibrium constant for the complexation of boronate with the monosaccharide, because the

(50) Fulton, S. *Boronate Ligands in Biochemical Separations*; Amicon: Danvers, Massachusetts, 1981.
(51) Singhal, R. P.; DeSilva, S.; Shyamali, M. *Adv. Chromatogr.* **1992**, *31*, 293.
(52) Barker, S. A.; Chopra, A. K.; Hatt, B. W.; Somers, P. J. *Carbohydr. Res.* **1973**, *26*, 33.
(53) Sienkiewicz, P.; Roberts, D. *J. Inorg. Nucl. Chem.* **1980**, *42*, 1559.
(54) Ferrier, R. J. *Adv. Carbohydr. Chem. Biochem.* **1978**, *35*, 31.

Table 1. Equilibrium Constants (M^{-1}) for Neu5Ac, Glucose, Mannose, and Galactose/PAPBA Systems at Physiological pH 7.4

Neu5Ac/PAPBA	glucose/PAPBA	mannose /PAPBA	galactose/PAPBA
37.6 ± 3.1 (1/1)	5.1 ± 1.2 (1/7.4)	8.5 ± 0.9 (1/4.4)	15.0 ± 2.2 (1/2.5)

concentration of the monosaccharide/boronate complex and other free species (B and B^-) can all be determined without ambiguity. Note that the sugars are known generally to form a stable complex only with tetrahedral $PhB(OH)_3^-$ in aqueous solution, because the sugar complex with trigonal $PhB(OH)_2$ is highly susceptible to hydrolysis. The equilibrium constant for the complexation of PAPBA with a sugar molecule is defined as

$$K = [SB + SB^-]/([S][B + B^-]) \cong [\text{complexed boron}]_{area}/([S][\text{free boron}]_{area}) \quad (1)$$

The mass balance equation applicable to this boron-sugar equilibrium[55] is

$$[S] = [S_0] - [\text{complexed boron}] \quad (2)$$

The concentrations of the free $B + B^-$ and the complexes can be determined by evaluating the peak areas of the two components: $[\text{free boron}]_{area}$ and $[\text{complexed boron}]_{area}$.[56] The relative fractions of B and B^- can be estimated from the chemical shift of the free boronic/boronate peak according to Sinton.[57] In this way, one is able to calculate the concentrations of all the species which are necessary to determine the equilibrium constant from the NMR data using eq 1.

The peak area ratio of [complexed boron] to [free boron] plotted versus the free sugar concentration [S] for different pHs closely followed a linear relaionship for all the sugars examined in this study (data not shown). Eventually, the slopes of the straight lines obtained by the least-squares fit of the data gave the equilibrium constants. A minimum of three measurements for each sugars at various mixing ratios were carried out, and the calculated equilibrium constants at pH 7.4 are given in Table 1. Note that the *K* values for glucose, mannose, and galactose are in agreement with those reported previously[58] where the equilibrium constants obtained by the UV method in pH 7.4 solution are 7.08 (glucose), 12.59 (mannose), and 19.05 (galactose), respectively. This order is indicative of the inherent selectivity of all monoboronic acids.[59] Obviously, Neu5Ac has a significantly higher *K* value compared with those of the other sugars under physiological condition (pH 7.4); the *K* value for Neu5Ac is 7.4, 4.4, and 2.5 times higher, respectively, than those for glucose, mannose, and galactose. The plots for the *K* values versus pH are shown in Figure 3 for Neu5Ac and the other sugars. Worth noting is that, by increasing the pH from 4 to 10, the *K* value for Neu5Ac gradually decreased while those for the neutral hexoses such as glucose, mannose, and galactose began to increase steeply at a pH slightly higher than 7.4. The

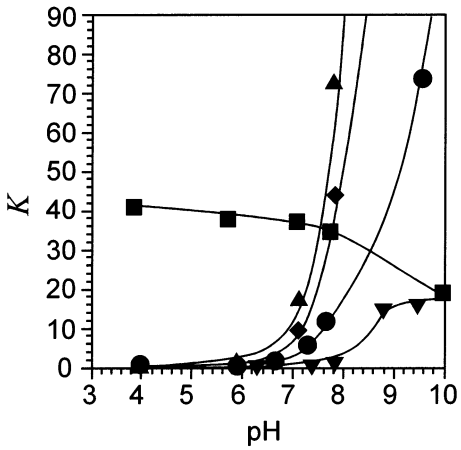

Figure 3. Plots of K (M^{-1}) vs pH for the Neu5Ac/PAPBA (■), glucose/PAPBA (●), mannose/PAPBA (◆), galactose/PAPBA (▲), and glycerin/PAPBA (▼) systems (all data within 10% of SD).

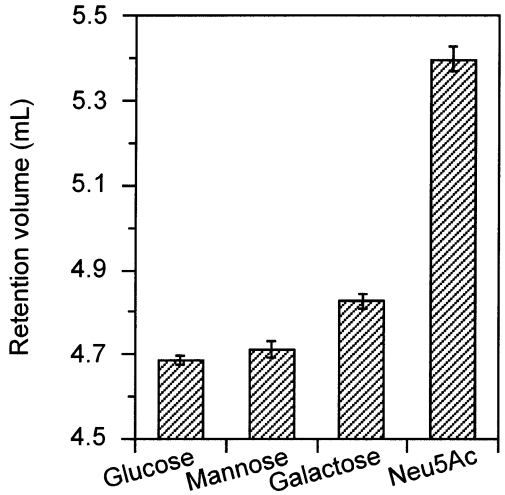

Figure 4. Differential PAPBA affinity to various sugars at pH 6.5 evaluated by retention volumes on boronate affinity chromatography.

complexing ability of sugar compounds with boronate was also estimated from the boronate affinity chromatography at pH 6.5, as seen in Figure 4. A significantly larger retention volume for Neu5Ac is quite obvious and is consistent with its higher *K* value at corresponding pH, as shown in Figure 3.

2. Structural Analysis of the PAPBA−Neu5Ac System. This unprecedentedly strong binding of PAPBA with Neu5Ac at acidic to physiological pH suggests that, unlike other sugars, the trigonal-formed complex with $PhB(OH)_2$ (SB) may be favored by Neu5Ac at a lower pH. The ^1H and ^{13}C NMR spectra are established tools for identifying the sites of binding to boronic acid in polyols.[48,49,60,61] To gain a deeper insight into the binding sites of Neu5Ac toward boronic acid, we examined the ^1H and ^{13}C NMR spectra of Neu5Ac in the presence of PAPBA (Figures 5 and 6). As the NMR measurement in pure D_2O provides spectra with only a low resolution, the complex between Neu5Ac and PAPBA was at first studied in DMSO-d_6 under neutral nonaqueous conditions and then studied in D_2O at pD = 7.4. For comparison, data for pure Neu5Ac measured under equivalent experimental conditions are summarized in Table 2. Neu5Ac in solution is in an equilibrium between

(55) S_0 denotes the total sugar.
(56) Each spectrum was resolved into two components, and the peak areas were calculated with the Lorentzian function.
(57) Sinton, S. W. *Macromolecules* **1987**, *20*, 2430.
(58) Mori, Y.; Suzuki, A.; Yoshino, K.; Kakihana, H. *Pigment Cell Res.* **1989**, *2*, 273.
(59) Sandanayake, K. R. A. S.; Shinkai, S. *J. Chem. Soc., Chem. Commun.* **1994**, 1083.
(60) Makkee, M.; Kieboom, A. P. G.; van Bekkum, H. *Recl. Trav. Chim. Pays-Bas* **1985**, *104*, 230.
(61) Norrild, J. C.; Eggert, H. *J. Am. Chem. Soc.* **1995**, *117*, 1479.

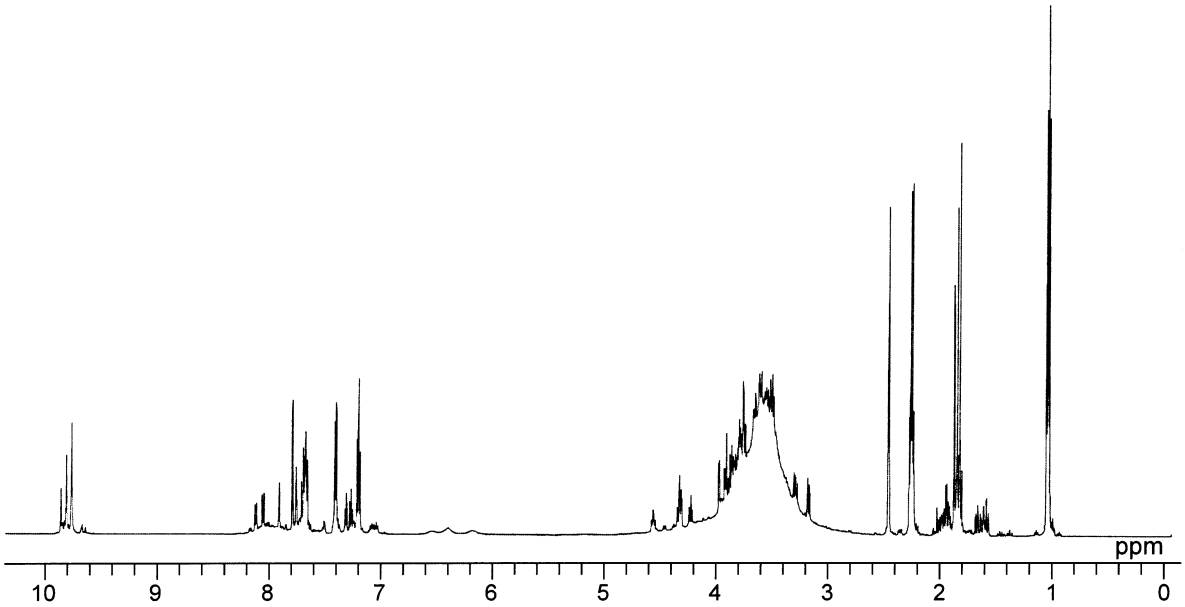

Figure 5. ^{1}H NMR spectrum of the Neu5Ac/PAPBA system (1:1 molar ratio) in DMSO-d_6.

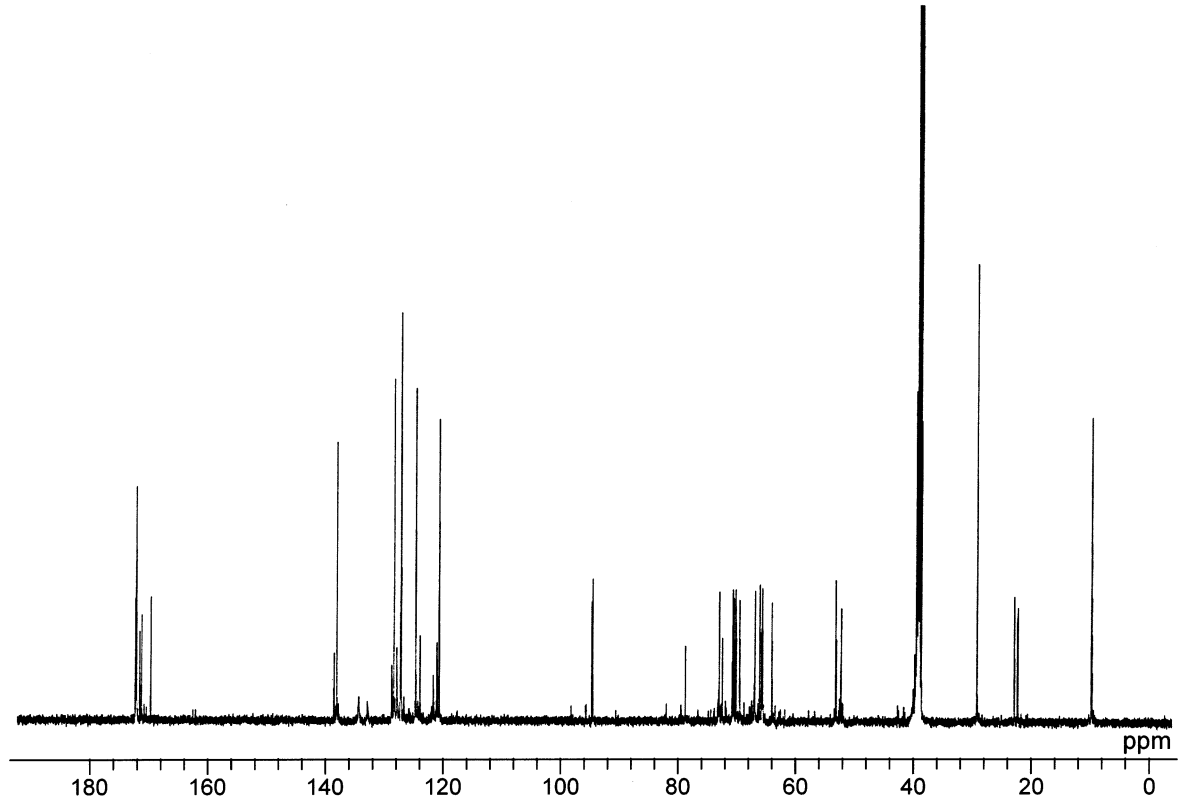

Figure 6. ^{13}C NMR spectrum of the Neu5Ac/PAPBA system (1:1 molar ratio) in DMSO-d_6.

α-pyranose (5−8%) and β-pyranose (92−95%) forms, but the furanose type of Neu5Ac is absent due to the acetamide moiety substituted at the C-5 position.[62] It is empirically known that 3-H_{eq} of the α-anomer (∼2.5 ppm) has a slightly higher chemical shift than that of the β-anomer (∼2.0 ppm).[63]

A mixture of the Neu5Ac and PAPBA in a 1:1 stoichiometric ratio in DMSO-d_6 (0.1 M for ^{1}H NMR and 0.3 M for ^{13}C NMR) was prepared, and the ^{1}H and ^{13}C NMR spectra were recorded. The assigned ^{1}H chemical shifts and J_{HH} coupling constants for the Neu5Ac in the mixture of Neu5Ac and PAPBA are summarized in Table 3. Table 4 summarizes the ^{13}C chemical shifts for Neu5Ac in the mixture of Neu5Ac and PAPBA. The chemical shift assignments for all species are in agreement with the information obtained from ^{1}H−^{1}H COSY/TOCSY and ^{1}H−^{13}C PFG-HMQC/HMBC spectra and refer to the atom numbering given in Figure 7. The ^{1}H and ^{13}C NMR spectra for

(62) Friebolin, H.; Kunzelmann, P.; Supp, M.; Brossmer, R.; Keilich, G.; Ziegler, D. *Tetrahedron Lett.* **1981**, *22*, 1383.

(63) Ogura, H.; Furuhata, K.; Itoh, M.; Shitori, Y. *Carbohydr. Res.* **1986**, *158*, 37.

Table 2. ^{1}H and ^{13}C NMR Chemical Shifts (ppm) and J_{HH} Coupling Constants in Parentheses (Hz) for the Pure Neu5Ac in DMSO-d_6[a]

^{1}H NMR chemical shifts and J_{HH} coupling constants

	β form	α form
3-H_{eq}	1.97 dd (13.2, 4.9)	2.48 dd (12.2, 4.4)
3-H_{ax}	1.68 dd (13.2, 10.7)	1.48 dd (12.2, 11.7)
4-H	3.81 ddd (10.7, 10.7, 4.9)	3.59 m
5-H	3.49 ddd (10.7, 10.7, 8.8)	3.46 m
6-H	3.72 br d (10.7)	3.16 m
7-H	3.16 br d (9.8)	3.27 m
8-H	3.48 m	3.58 m
9-H	3.27 dd (11.2, 6.8)	3.35 dd (11.7, 5.9)
9-H'	3.59 dd (11.2, 2.0)	3.58 m
NH	8.05 d (8.8)	8.01 d (8.3)
2-OH	6.17 br	
4-OH	4.79 br s	
7-OH	4.52 br s	4.69 br s
8-OH	4.33 br s	4.99 br s
9-OH	4.22 br	
NAc	1.87 s	1.86 s

^{13}C NMR chemical shifts

	β form	α form
1-C	171.35	171.76
2-C	94.69	95.69
3-C	39.85	41.82
4-C	65.74	66.68
5-C	53.05	52.37
6-C	70.36	73.50
7-C	69.16	68.39
8-C	69.83	71.53
9-C	63.62	63.08
NAc(−CO−)	171.86	172.14
NAc(−CH_3)	22.62	22.57

[a] The geminal and vicinal proton coupling constants could be obtained directly from the 1D spectrum.

Table 3. ^{1}H NMR Chemical Shifts (ppm) and J_{HH} Coupling Constants in Parentheses (Hz) for the Neu5Ac Complexed with PAPBA in DMSO-d_6[a]

	1(free)	2(major complex)	3(minor complex)
3-H_{eq}	1.98 dd (12.7, 4.9)	1.96 dd (12.7, 4.9)	2.03 dd (12.7, 4.9)
3-H_{ax}	1.69 dd (12.7, 11.2)	1.62 dd (12.7, 11.2)	1.64 dd (12.7, 11.2)
4-H	3.83	3.80	3.85
5-H	3.51	3.86	3.54
6-H	3.74 br d (10.3)	3.91 br d (10.7)	3.65 br d (9.3)
7-H	3.18 br d (9.8)	3.97 br d (3.9)	3.75 br d (3.3)
8-H	3.49	4.38 m	4.56 ddd (8.3, 8.3, 3.3)
9-H	3.28 dd (11.2, 6.8)	3.78 m	4.22 dd (9.3, 8.3)
9-H'	3.60 dd (11.2, 2.4)	4.31 dd (10.7, 2.9)	4.32 dd (9.3, 8.3)
NH	8.06 d (8.3)	7.74 d (7.8)	8.12 d (8.3)
2-OH			
4-OH			
7-OH			
8-OH			
9-OH			
NAc	1.88 s	1.86 s	1.91 s

[a] The geminal and vicinal proton coupling constants could be obtained directly from the 1D spectrum.

Table 4. ^{13}C NMR Chemical Shifts (ppm) for the Neu5Ac Complexed with PAPBA in DMSO-d_6

	1(free)	2(major complex)	3(minor complex)
1-C	171.45	171.13	171.19
2-C	94.79	94.65	94.60
3-C	39.9	39.7	40.1
4-C	65.83	66.63	65.53
5-C	53.11	52.22	53.00
6-C	70.43	70.22	72.16
7-C	69.22	72.74	69.83
8-C	69.92	65.44	78.49
9-C	63.69	65.32	66.70
NAc(−CO-)	172.00	169.56	172.21
NAc(−CH_3)	22.67	23.25	22.74

the equilibrated solution of 1:1 mixture of Neu5Ac and PAPBA apparently contained signals from the free Neu5Ac in β-pyranose form (species 1), as cited in Table 2. There exist additional peaks which may be ascribed to Neu5Ac complexed with PAPBA. As will be discussed later, two types of the complexes (species 2 and 3) were assigned, as summarized in Table 3. Contributions of free Neu5Ac (species 1), the major complex (species 2), and the minor complex (species 3) are estimated to be ∼34%, ∼40%, and ∼26%, respectively, from peak integration of both NAc methyl protons of Neu5Ac and NH protons of PAPBA. The data shown in Table 3 suggests that the boronic acid components in the system involve Neu5Ac in the β-pyranose with a negligible contribution from the α-pyranose form. The values determined for $^2J_{3eq,3ax}$ (12.7), $^3J_{3eq,4}$ (4.9), and $^3J_{3ax,4}$ (11.2) of these components are in close agreement with the calculated values of species 1, 2, and 3, assuming the β-pyranose form. The small value of $^3J_{3eq,4}$ and large value of $^3J_{3ax,4}$ in the complexes suggest the vicinal equatrial-axial and diaxial arrangements of the 3-H and 4-H hydrogen atoms, respectively, as should be the case for a pyranose form.

A unique structural feature of Neu5Ac is that it has a glycerin moiety at the C-6 position (see the structure in Figure 7). The hydroxyls on carbons 7, 8, and 9 appear to be involved in the complexation, as will be discussed later in detail. On the other hand, a separate ^{11}B NMR study for the glycerin/PAPBA system revealed that the equilibrium constant of glycerin itself with PAPBA is as low as $K = 0.72$ at pH 7.4.[64,65] This means that the exceptionally high K value (= 37.6) of Neu5Ac/PAPBA is not due to only the binding of boronic acid to the 7-, 8-, and 9-hydroxyl groups (glycerine structure) of Neu5Ac. There may be an additional and significant reason for this anomalously high K value of Neu5Ac at lower pH. Generally, the efficient complexation with polyol compounds requires the boron atom to be sp^3 hybridized (tetrahedral anion, $-B(OH)_3^-$), which is efficiently achieved only at high pH near the pK_a of the original boronate. Neutral esters of polyol with nonionic trigonal-PhB-$(OH)_2$ should be hydrolyzed easily in aqueous solution, due to the strong electrophilic property of the trigonal boron with the

(64) Hisamitsu, I. Ph.D. Thesis, Science University of Tokyo, Noda, Chiba, 1997.

(65) Simple diols, such as ethylene glycol, assume a trans conformation in solution due to repulsion of the hydroxyls and, thus, have scarce interaction with borate. The strength of borate interaction with other polyols, as measured by titration, was in the order of glycerol < erythritol < adonitol < arabitol < mannitol.

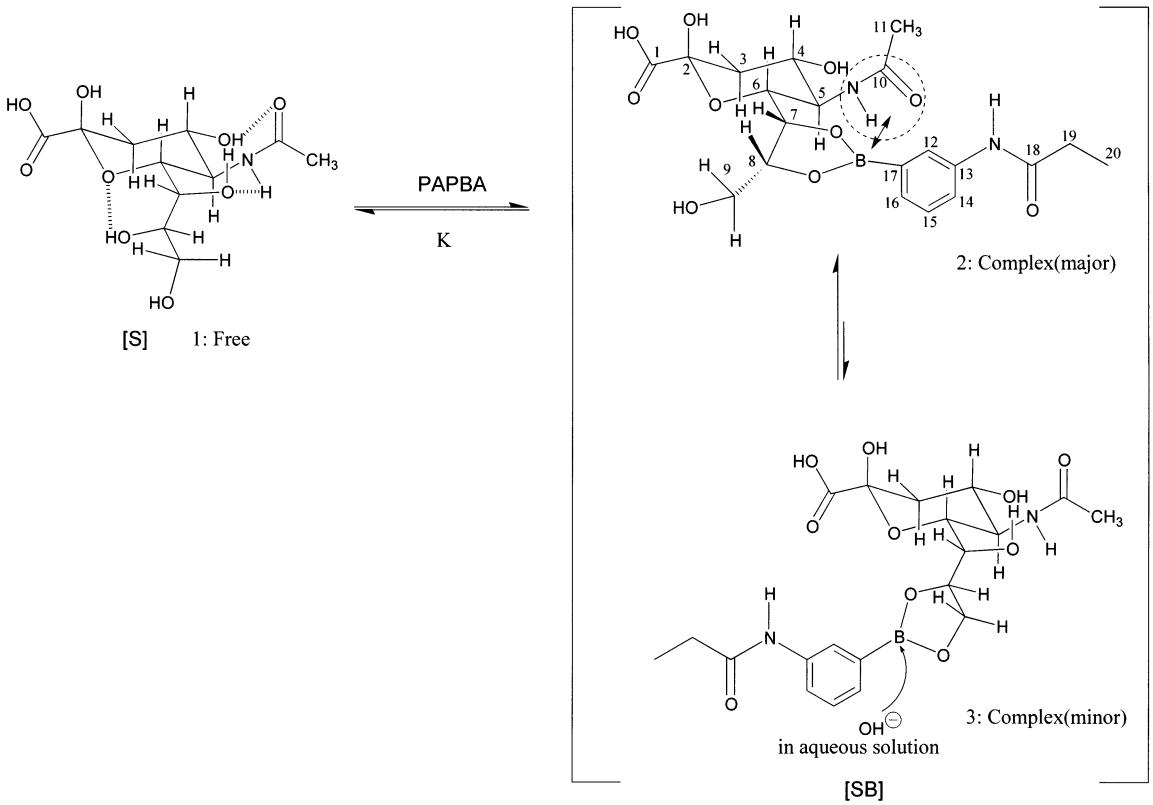

Figure 7. Conformational model for the Neu5Ac and the complex of the Neu5Ac/PAPBA system.

deficiency of a valence electron. However, an amide group attached to Neu5Ac at the C-5 position may stabilize the $B(OH)_2$ complexation with the glycerine moiety via an intramolecular B−N or B−O interaction. Gallop et al. suggested that an electron-donating group such as an amino group can make a coordination bond (B←:N) with the boron atom to stabilize the complex of phenylboronic acid with polyol.[20,48,66] Actually, polyacrylamide-based copolymers containing both phenylboronic acid and amine moieties as a side chain formed a more stable complex with the polyol compounds at pH 7.4 than when having only the phenylboronic acid group.[67] On the other hand, Keana et al. reported that *o*-acetamidophenylboronic acid stabilizes the boronate ester toward hydrolysis by an intramolecular O−B (−$NH(CH_3)CO$→B) interaction and accelerates esterification in contrast to the nonortho-substituted boronic acid.[68] As schematically shown in Figure 7, the Neu5Ac/PAPBA system nicely fits the spatial arrangement with an amide group in the vicinity of the boronic acid moiety, facilitating it to take the sp^3 hybridized complex.

Czarniecki et al.[69] performed a conformational analysis of Neu5Ac using ^{13}C NMR spin−lattice-relaxation (T_1) measurements; the structure of Neu5Ac drawn in Figure 7 as [S] represents the conformational model showing the proposed hydrogen bonds and preferred conformation of the glycerol side chain based on his analysis. The C-8 hydroxyl proton is hydrogen-bonded to the pyranose ring oxygen through a six-membered ring structure. The oxygen at C-7 can then make a hydrogen bond with the amide NH. Furthermore, additional stabilization for this conformation is attained through the hydrogen bond formation of the acetamido carbonyl oxygen with the C-4 hydroxyl proton in a seven-membered ring structure. The C-9 hydroxyl proton seems to exist as the free form without any hydrogen bonding. This conformation, a spatial arrangement formed between the acetamide group and the glycerol side chain, is favorable for inducing the stable complexation of boronic acid with Neu5Ac including the B−N/B−O bond. The most feasible structural model of the Neu5Ac/PAPBA complex, species 2, is also shown as [SB] in Figure 7, considering the original spatial arrangement of Neu5Ac formed by the glycerol moiety and the amide group. The minor complex, species 3, is likely to be a complex form without amide involvement. Actually, evidence for the B−N/B−O interaction was demonstrated by $^1H-^{15}N$ PFG-HMQC measurement (Figure 8). Table 5 summarizes obtained ^{15}N chemical shifts including pure Neu5Ac and PAPBA for comparison. The ^{15}N chemical shift of NAc amide nitrogen in the species 2 significantly shifted upfield (shielded by −4.1 ppm). As shown in Figure 9, the existence of the B−N/B−O interaction is also indicated by the ^1H NMR spectra for the mixed Neu5Ac/PAPBA solution with the significant shift of amide NH in the major complex. With an increased amount of PAPBA, the amide NH peak progressively shifted upfield (shielded by −0.31 ppm) with a decrease in the peak intensity (Figure 10), which is likely attributed to a shielding effect brought about through the formation of the B−N/B−O bond. Then, the electron density should increase in the boron atom through such a B−N/B−O bond. This was confirmed by ^{11}B NMR, in which the 27.6 ppm resonance of ^{11}B of PAPBA underwent an upfield shift from 7.7 to 8.5 for each of the complexes. These results indicate that the amide

(66) Wiskur, S. L.; Lavigne, J. J.; Ait-Haddou, H.; Lynch, V.; Chiu, Y. H.; Canary, J. W.; Anslyn, E. V. *Org. Lett.* **2001**, *3*, 1311.
(67) Hisamitsu, I.; Kataoka, K.; Okano, T.; Sakurai, Y. *Pharm. Res.* **1997**, *14*, 289.
(68) Cai, S. X.; Keana, J. F. W. *Bioconjugate Chem.* **1991**, *2*, 317.
(69) Czarniecki, M. F.; Thornton, E. R. *J. Am. Chem. Soc.* **1977**, *99*, 8273.

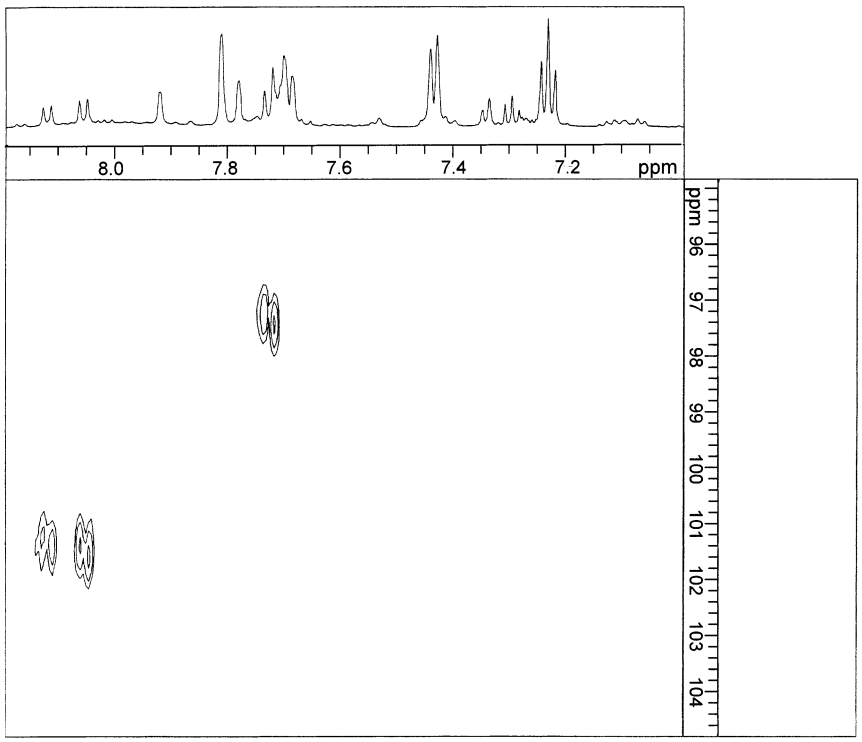

Figure 8. $^{1}H-^{15}N$ PFG-HMQC NMR spectrum of the Neu5Ac/PAPBA system (1:1 molar ratio) in DMSO-d_6.

Table 5. ^{15}N NMR Chemical Shifts (ppm) Measured by $^{1}H-^{15}N$ PFG-HMQC Measurement for the Pure Neu5Ac and Neu5Ac Complexed with PAPBA in DMSO-d_6

	pure Neu5Ac		Neu5Ac–PAPBA system		
	β form	α form	1(free)	2(major)	3(minor)
NAc(Neu5Ac)	101.5	101.5	101.5	97.4	101.3
NAc(PAPBA)	110.1	110.1	110.1	110.1	110.0

group in the structure of Neu5Ac is of a great importance for the increased stability of the Neu5Ac/PAPBA complex through the B−N/B−O interaction with the boron atom of PAPBA.

It is of further importance to discuss in detail an involvement of the hydroxyl groups on C-7, -8, and -9 in the complexation from ^{1}H and ^{13}C NMR chemical shifts: shielding effects observed on both ^{1}H and ^{13}C absorptions upon boronate formation may be used for structural determination of the complexes. Although deshielding is generally observed in the ^{13}C atoms within the cyclic boronate esters,[70,71] this rule collapses in some cases depending on the structure. In our study, we found this exceptional case at C-8 in species 2, where C-8 is shielded by 4.39 ppm (Table 2). On the other hand, C-7 in species 2 is deshielded by 3.58 ppm. These data make a conflicting structural picture around the 7,8-binding site. The deviations from the general rule of the ^{13}C (de)shielding in species 2 may be ascribed to several factors. Particularly, the restricted conformation of the C−O bond around C-7,8 due to the formation of the B−N/B−O bond may have a pronounced effect. The ^{1}H chemical shifts provide further evidence. Protons H-7 and H-8 in species 2 are most deshielded by +0.81 and +0.90 ppm, respectively, compared to free Neu5Ac, suggesting an involvement of C-7,8 in complexation. Further, there exist residual peaks assigned to species 3: here, C-8 and C-9 are largely deshielded by 8.66 and 3.08 ppm, respectively, suggesting the C-8,9 to be binding sites. This is further supported by the deshielding of protons H-8, H-9, and H-9′ by +1.08, +0.95, and +0.73 ppm, respectively.

Based on these structural analyses of complexes in DMSO, the Neu5Ac complexation with PAPBA in aqueous solution was then investigated. A mixture of the Neu5Ac and PAPBA in a 1:1 stoichiometric ratio in D_2O at pD = 7.4 (0.1 M for ^{1}H NMR and 0.3 M for ^{13}C NMR) was prepared, and the ^{1}H and ^{13}C NMR spectra were recorded. A structure corresponding to species 2 in DMSO was also suggested to form in an aqueous solution, although the peaks are not well characterized due to the spectral broadening (the assigned ^{1}H and ^{13}C chemical shifts for the Neu5Ac in the complex form are summarized as Supporting Information). Note that the main evidence of a pyranose B−N complex can be based on the pH profile of the binding constant together with the high ^{1}H coupling constants of, for example, H-3 and H-4 observed in D_2O indicative of pyranose species. Species 3 in DMSO may take a tetrahedral anionic form, $-B(OH)_3^-$ in aqueous solution (species 3′) because nonionic trigonal-$PhB(OH)_2$ should be stabilized by OH^- in aqueous solution,[72,73] as shown in the following equation.

$$\begin{array}{ccc} \text{Neu5Ac+B(OH)}_2 & \rightleftarrows & \text{Neu5Ac-B(OH)}_2\text{(major complex)}\cdots\text{stabilized by amide group} \\ \uparrow\downarrow & & \uparrow\downarrow \\ \text{Neu5Ac+B(OH)}_3^- & \rightleftarrows & \text{Neu5Ac-B(OH)}_3^-\text{ (minor complex)} \end{array}$$

It is interesting to note that the *N*-acetyl group of Neu5Ac plays a critical role in its appreciable binding to the sialic-acid-

(70) van den Berg, R.; Peters, J. A.; van Bekkum, H. *Carbohydr. Res.* **1994**, *253*, 1.
(71) Gorin, P. A. J.; Mazurek, M. *Can. J. Chem.* **1973**, *51*, 3277.
(72) Norrild, J. C.; Eggert, H. *J. Chem. Soc., Perkin Trans. 2* **1996**, 2583.
(73) Shull, B. K.; Spielvogel, D. E.; Head, G.; Gopallaswamy, R.; Sankar, S.; Devito, K. *J. Pharm. Sci.* **2000**, *89*, 215.

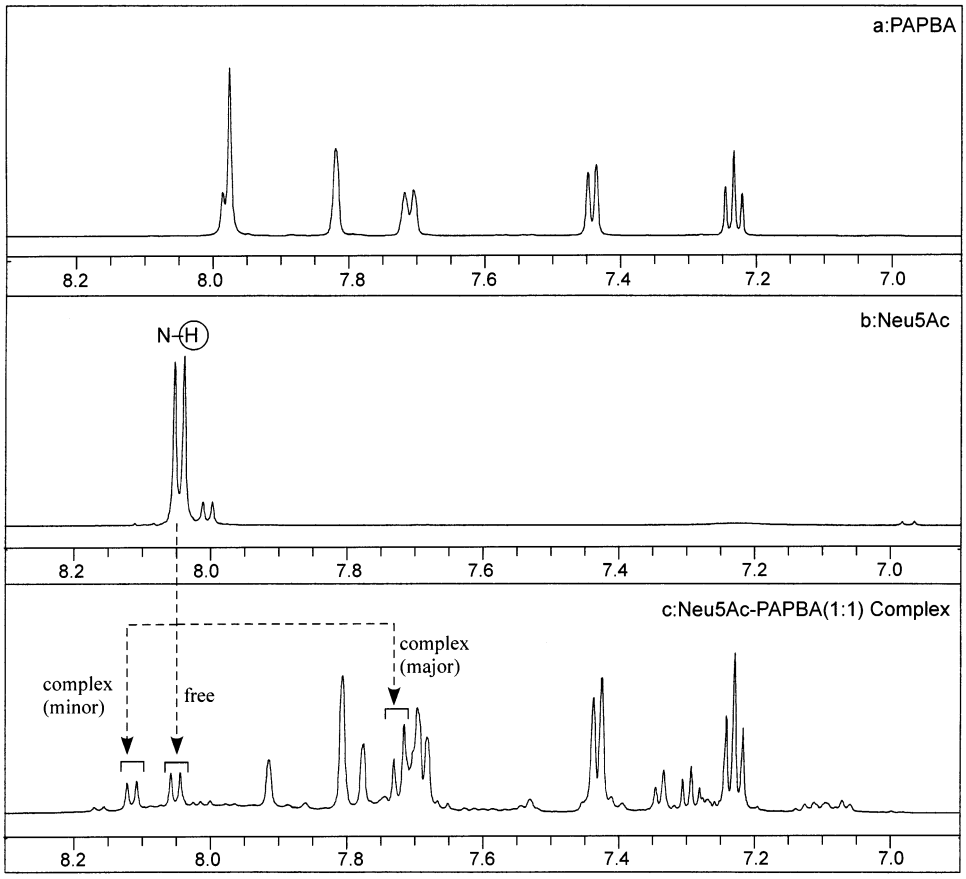

Figure 9. ^{1}H NMR spectra in DMSO-d_6: (a) PAPBA, (b) Neu5Ac, (c) Neu5Ac/PAPBA systems (1:1 molar ratio).

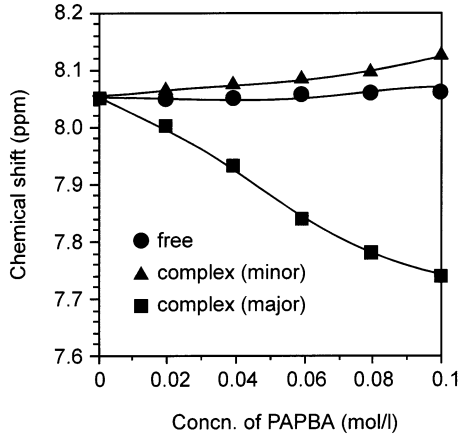

Figure 10. Change in chemical shift for amide-NH in the Neu5Ac(0.1 M)/PAPBA system with increasing concentration of PAPBA.

specific lectin, the slug *Limax flavus* agglutinin (LFA).[74,75] The glycerol moiety also appears to be critical for Neu5Ac binding to LFA, since acetylation of the C-9 hydroxyl group or periodate cleavage of carbons 8 and 9 resulted in a 20- to 50-fold decrease in the binding affinity.[74] At a high pH of 10, the assumed complexed structure of species 2 shown in Figure 7 may be destabilized due to the coordination of OH^- to boron, shifting an equilibrium toward $PhB(OH)_3^-$ and $Neu5Ac-PhB(OH)_3^-$ (species 3′). Indeed, the Neu5Ac/PAPBA system took almost an identical *K* value with the glycerin/PAPBA system at pH 10 (Figure 3), indicating that only the glycerol moiety of Neu5Ac participates in the complexation under alkali conditions.

Neu5Ac as a component of the plasma membrane glycoproteins mainly exists at the terminal of the carbohydrate moieties. The anomeric configuration of Neu5Ac as a β-anomer is more stable in solution as demonstrated by the rapid mutarotation undergoing from the α-anomer to the β-anomer. In contrast, the configuration of the Neu5Ac residue in the oligosaccharides (carbohydrates) has been shown to be the α-type.[76] Thus, *N*-acetylneuraminic acid α-methylglycoside (Neu5Acα2Me) as a model for terminal Neu5Ac residues was mixed with PAPBA in PBS at physiological pH 7.4. Of interest, the solution became immediately turbid, showing an agglutination due to the reaction between PAPBA and Neu5Acα2Me. Although the ^{11}B NMR was unable to be measured due to a solubility problem, this result clearly indicates the intensified complexation of phenylboronic acid with Neu5Acα2Me at physiological pH 7.4. On the other hand, boronate complexation with the C-1 substituted pyranose (model for terminal sugar moiety of cellular surface) such as methyl-α-D-glucopyranoside (Me-Glc) and methyl-α-D-galactopyranoside (Me-Gal) was confirmed to be very weak due to steric reasons,[31,50,61,77] which is in line with the higher boronate binding to the furanose-formed aldohexose than to pyranose. Thus, the binding of boronate to the terminal sugar residues of the glycoproteins and/or glycolipids existing on cells and tissues may be exclusive to the Neu5Ac compounds,

(74) Kibbis, R. N.; Osborne, S. E.; Glick, G. D.; Goldstein, I. J. *J. Biol. Chem.* **1993**, *268* (25), 18524.
(75) Miller, R. L.; Collawn, J. F., Jr.; Fish, W. W. *J. Biol. Chem.* **1982**, *257*, 7574.
(76) Melton, L. D.; Morris, E. R.; Rees, D. A.; Thom, D. *J. Chem. Soc., Perkin Trans. 2* **1979**, *2*, 10.
(77) Boeseken, J. *Adv. Carbohydr. Chem.* **1949**, *4*, 189.

lending boronate to be an Neu5Ac-selective ligand in cellular recognition.

Conclusion

The complexation behavior of PAPBA with Neu5Ac as well as with other monosaccharides including glucose/mannose/galactose was studied by ^{11}B, ^{13}C, ^{15}N, and ^{1}H NMR spectroscopies from the viewpoint of exploring the sugar structures and conformations suitable for boronic acid/boronate binding. The Neu5Ac/PAPBA system has an anomalously higher binding constant than those of the other neutral hexoses/PAPBA systems at lower pH. The glycerol side chain of Neu5Ac appears to be a binding site critical for boronic acid, and furthermore, the complex is stabilized through the coordination of the amide NH or CO located at the C-5 position of Neu5Ac, forming a B−N or B−O linkage. The plausible structure of the Neu5Ac/PAPBA complex thus formed fits well with the original spatial arrangement of Neu5Ac stabilized through the intramolecular interaction of the glycerol moiety located at the C-6 position with the amide group located at the C-5 position formed by the glycerol moiety and the amide group. This result suggests that the terminal Neu5Ac moiety may work as a specific recognition site during the interaction of boronic acid with the biological carbohydrates existing on the biological membranes of cells, viruses, and microorganisms.

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Supporting Information Available: ^{1}H NMR and ^{13}C NMR chemical shifts and J_{HH} coupling constants for the pure Neu5Ac and Neu5Ac complexed with PAPBA in aqueous solution. This material is available free of charge via the Internet at http://pubs.acs.org.

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