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Metal-binding Sites of *N*-Acetylneuraminic Acid

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Abstract. Sialic acid represents a group of thirty derivates of neuraminic acid with various substituents at the amino residue and the alcoholic hydroxy groups. We analysed the behaviour of the tetracoordinated metal ions palladium(II) and silicon(IV) against the most important derivative *N*-acetylneuraminic acid (NANA). The molecular structures were assigned by a combined ^{1}H, ^{13}C and ^{29}Si NMR-spectroscopic approach. Despite the presence of many different functional groups, the coordination chemistry of NANA with Pd^{II} follows established rules. Coordination via the *N*-acetyl-group – sterically impossible with Pd^{II} – was realised with Si^{IV}.

Introduction

The thirty derivatives of neuraminic acid occur at the non-reducing ends of glycoproteins, glycolipides and polysaccharides.[1,2] These glycoconjugates can be found in microbes, protozoa, in cells and tissues of higher animals. They are usually located on the outer cell membrane[3,4] and are involved in various biological processes.[5] *N*-Acetylneuraminic acid (NANA), the *N*-acetyl derivate of sialic acid, is the most abundant sialic acid in humans and has been isolated from body fluids. Women with endometrial cancer and patients with prostatic or bladder cancer have significantly higher NANA levels than healthy people.[6] The level of NANA concentration can also be increased by other diseases, hence it is not suitable for an initial diagnosis of cancer. Its quantitative measurement, however, may help in monitoring the progress of surgical therapy and diagnosis of metastasis.[7] NANA has gained special interest among organic and medical chemists who are engaged in synthesising a library of derivatives.

An investigation of NANA complexes with alkali and alkaline earth metals suggested that the glycerol side chain is involved in the binding;[8] ^{13}C and ^{1}H NMR studies confirmed the coordination of NANA towards calcium(II).[9,10] Furthermore, the coordination ability of NANA towards biologically important metal ions such as copper(II), cobalt(II) and towards toxic metal ions such as lead(II) and cadmium(II) was investigated in aqueous solution by means of potentiometry, UV and NMR spectroscopy. Both binary and ternary systems were studied by *Saladini* et al., where the authors suggest that NANA coordinates all metal ions through the carboxylic group, pyranosidic ring oxygen and glycerol chain alcoholic hydroxyl groups. In the pH range 2–7, species of the type $[M(NANAH_{-1})_2]$ were postulated and, above pH 7, the species $[M(NANAH_{-1})_2(OH)]^-$.[5] By potentiometric titrations the binding sites cannot be identified for sure, because the deprotonated or protonated sites cannot be localised.

In this work, new results using further metal ions coordinating NANA, Pd^{II} and Si^{IV}, are presented. By using ^{13}C NMR spectroscopy we were now able to identify the actual binding sites at NANA. To probe the metal-binding sites, $Pd^{II}N_2$-type fragments (N_2 = bidentate nitrogen ligand) which we recently introduced as tools for the analysis of carbohydrates and carbohydrate derivatives in aqueous solution were employed in this work.[11,12] For the investigation of the NANA binding sites, Pd-tmen, an aqueous solution of $[Pd(tmen)(OH)_2]$ (tmen = *N*,*N*,*N*',*N*'-tetramethylethane-1,2-diamine), and Pd-chxn, an aqueous solution of $[Pd(chxn)(OH)_2]$ (chxn = (1*R*,2*R*)-cyclohexane-1,2-diamine) were used. And to identify the silicon-binding sites, the diol-protecting reagent bis(*tert*-butyl) bis(trifluoromethanesulfonato)silicon (DTBS triflate) was used. DTBS compounds are used especially for the protection of the 3',5'-hydroxy function of ribofuranosyl moieties in RNA synthesis.[13–15] DTBS derivates of the aldopentoses were analysed in a recently published work by our group.[16]

Results and Discussion

N-Acetylneuraminic acid is a reducing sugar which exists in aqueous solution in various cyclic and acyclic forms (Figure 1). With the hydroxy function on the *N*-acetylated carbon atom C5 missing, no furanoses are observed. In the major isomer, the β-pyranose, the *N*-acetyl group, the adjacent hydroxy group, the glycerol chain on C6, and the carboxy function are all oriented equatorially in the observed $^{2}C_5$ conformation.[17,18]

In the equimolar reaction of NANA with Pd-tmen (0.45 M in D_2O) or Pd-chxn (0.30 M in D_2O), the monometallated complexes $[Pd(tmen)(NeuNAc1,2H_{-2}\text{-}\kappa^2O^{1,2})]$ (**1a**) and $[Pd(chxn)(NeuNAc1,2H_{-2}\text{-}\kappa^2O^{1,2})]$ (**1b**) were obtained

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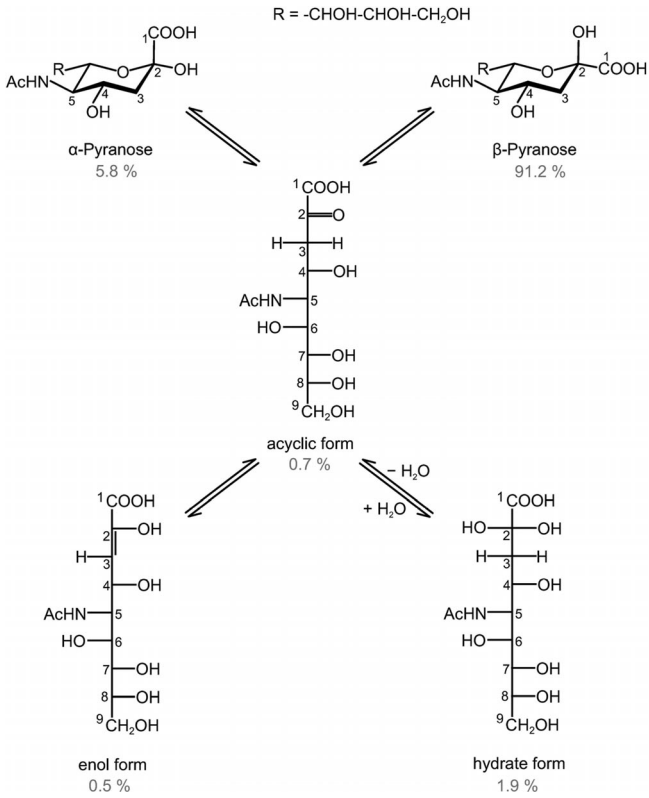
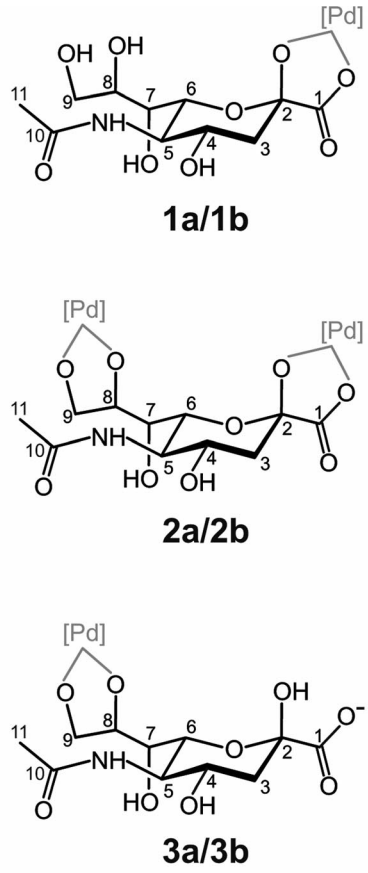


Figure 1. *N*-Acetylneuraminic acid isomers in aqueous solution. The percentages of cyclic and acyclic forms were determined at pH 2.[18]



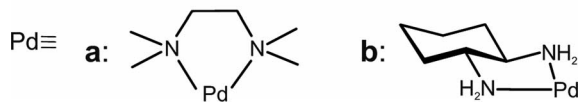


Figure 2. Species detected in Pd^{II}-containing solutions of *N*-acetylneuraminic acid (see text).

(Figure 2). On monometallation, the Pd^{II} fragment was chelated by the carboxyl residue and by the adjacent hydroxy group after its deprotonation in a five-membered chelate ring. The structures of both species were determined by standard NMR techniques, including 2D spectra (Table 1, Figure 3).

By increasing the Pd^{II} concentration to a Pd:NANA molar ratio of 2:1, the dimetallated complexes [{Pd(tmen)}$_2$(NeuNAc1,2,8,9H$_{-4}$-1κ^2*O*^{1,2}:2κ^2*O*^{8,9})] (**2a**) and [{Pd(chxn)}$_2$(NeuNAc1,2,8,9H$_{-4}$-1κ^2*O*^{1,2}:2κ^2*O*^{8,9})] (**2b**) were formed. ^{13}C NMR spectroscopy revealed that a second Pd^{II} fragment coordinated at the terminal diol function of the glycerol chain. This result matches the rules: from experiments with sugar alcohols it is known that the stability of a Pd^{II} chelate depends on the configuration of the diol moiety and decreases in the order *threo* > terminal > *erythro*.[19] With the C7-C8 diol being configured *erythro*, the observed terminal coordination confirms the rule (Table 1).

At higher Pd^{II}-tmen:NANA molar ratios, the concentration of **2a** increased. At a 5:1 ratio, only the dimetallated species was observed. Even with this high concentration of palladium(II), no coordination of a third palladium moiety via the acetyl amino function was observed. Generally, a palladium(II) atom is able to bind a deprotonated amido ligand at a relatively low pH level. As recently published, the binding properties of the palladium reagents with deprotonated acetyl amino functions depend on the steric requirements introduced by the acetyl residue which is forced into coplanar arrangement with the chelate ring. Depending on the position of the *N*-acetyl group relative to the glycerol chain, the formation of a chelate ring via the *N*-acetyl group is not possible due to vicinal acetyl-hydroxyl repulsion.[20]

By adding one equivalent of sodium hydroxide to the reaction mixture of NANA with two equivalents of Pd-tmen and Pd-chxn, the carboxylate residue was set free and the monometallated species [Pd(tmen)(NeuNAc8,9H$_{-2}$-κ^2*O*^{8,9})]$^-$ (**3a**) and [Pd(chxn)(NeuNAc8,9H$_{-2}$-κ^2*O*^{8,9})]$^-$ (**3b**) were formed. The structures of these species were derived from ^{13}C NMR spectra (Table 1, Figure 3). The shifts observed for the structure determinations lay in the typical range between 8 ppm and 14 ppm for carbon atoms bound to the coordinated carboxy and alkoxy oxygen atoms.

For the investigation of silicon binding sites on NANA, we used bis(*tert*-butyl) bis(trifluoromethanesulfonato)silicon (DTBS triflate). We performed several investigations, including aldopentoses as an example for reducing carbohydrates,[16] and tartaric acid as a model compound for the competition between carboxy and hydroxy groups. NANA combines these different functional groups and offers, in addition, the *N*-acetyl

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Table 1. ^{13}C NMR chemical shifts (δ /ppm) and shift differences ($\Delta\delta$) of [Pd(tmen)(NeuNAc1,2H$_{-2}$-κ^2*O*^{1,2})] (**1a**), [Pd(chxn)(NeuNAc1,2H$_{-2}$-κ^2*O*^{1,2})] (**1b**), [{Pd(tmen)}$_2$(NeuNAc1,2,8,9H$_{-4}$-1κ^2*O*^{1,2}:2κ^2*O*^{8,9})] (**2a**), [{Pd(chxn)}$_2$(NeuNAc1,2,8,9H$_{-4}$-1κ^2*O*^{1,2}:2κ^2*O*^{8,9})] (**2b**), [Pd(tmen)(NeuNAc8,9H$_{-2}$-κ^2*O*^{8,9})]$^-$ (**3a**) and [Pd(chxn)(NeuNAc8,9H$_{-2}$-κ^2*O*^{8,9})]$^-$ (**3b**) in D_2O, referenced to an internal methanol signal (49.5 ppm).

		C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11
NeuNAc	δ	172.7	93.2	36.7	64.6	49.9	68.0	66.1	68.2	61.0	171.4	19.9
1a	δ	185.3	102.1	42.0	67.3	52.9	69.5	69.0	70.9	63.4	174.8	22.3
	$\Delta\delta$	**12.6**	**8.9**	5.3	2.7	3.0	1.5	2.9	2.7	2.4	3.4	2.4
2a	δ	185.8	101.1	43.0	66.9	53.6	70.3	70.1	79.0	70.7	174.8	22.4
	$\Delta\delta$	**13.1**	**7.9**	6.3	2.3	3.7	2.3	4.0	**10.8**	**9.7**	3.4	2.5
3a	δ	177.9	97.2	39.7	67.2	53.8	70.2	71.5	79.4	71.4	175.3	22.9
	$\Delta\delta$	5.2	4.0	3.0	2.6	3.9	2.2	5.4	**11.2**	**10.4**	3.9	3.0
1b	δ	185.7	102.5	42.5	67.9	53.2	69.6	69.5	71.3	63.9	175.3	22.7
	$\Delta\delta$	**13.0**	**9.3**	5.8	3.3	3.3	1.6	3.4	3.1	2.9	3.9	2.8
2b	δ	186.6	102.1	42.2	67.7	53.1	70.7	70.1	80.2	70.8	175.2	22.9
	$\Delta\delta$	**13.9**	**8.9**	5.5	3.1	3.2	2.7	4.0	**12.0**	**9.8**	3.8	3.0
3b	δ	177.8	97.2	39.3	67.8	53.1	70.5	70.6	79.8	74.4	175.2	22.9
	$\Delta\delta$	5.1	4.0	2.6	3.2	3.2	2.5	4.5	**11.6**	**13.4**	3.8	3.0

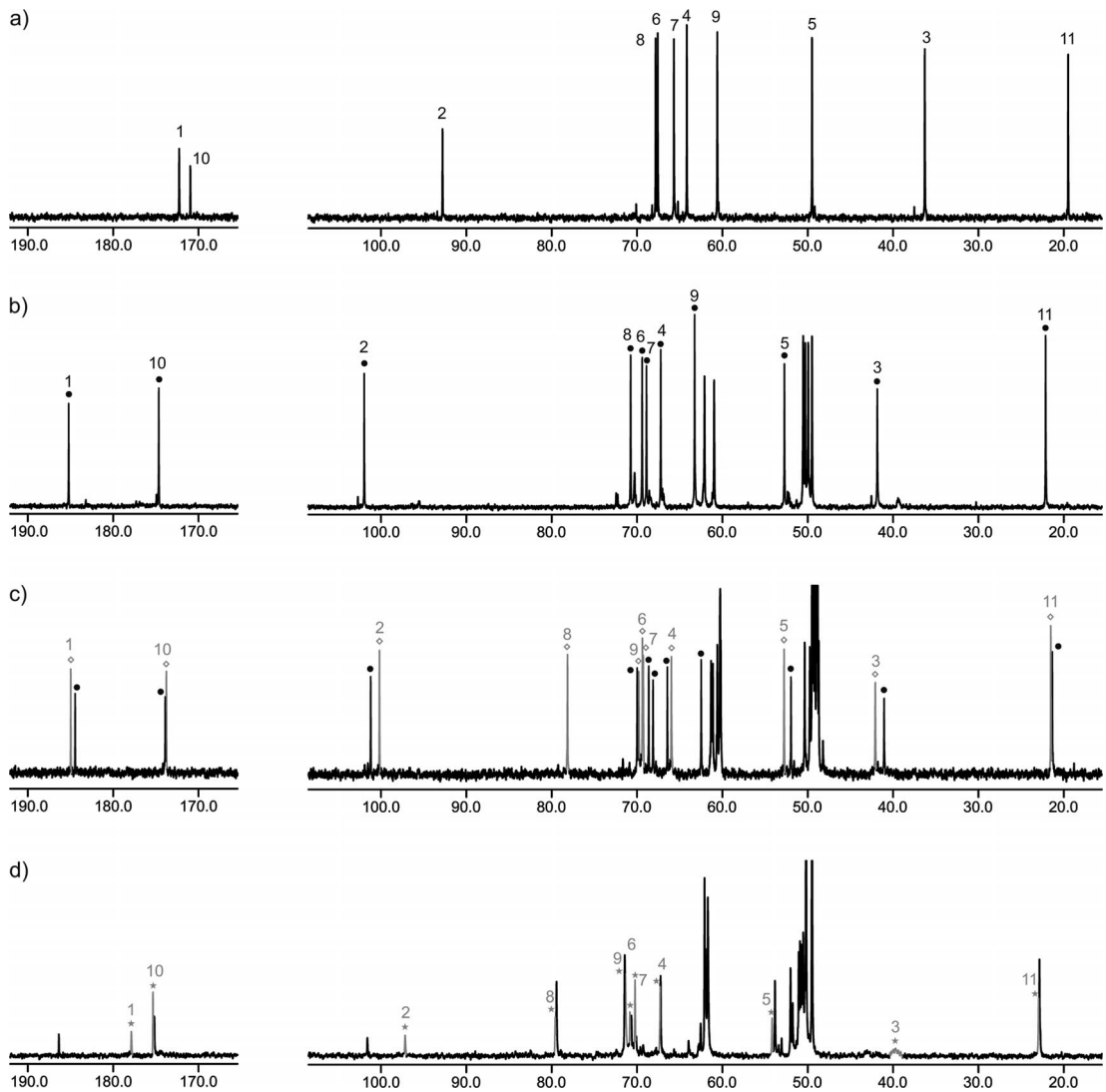

Figure 3. ^{13}C NMR spectra of *N*-acetylneuraminic acid. a) free NANA (β-pyranose), b) NANA in Pd-tmen at a molar ratio of 1:1, c) NANA in Pd-tmen at a molar ratio of 1:2 and d) NANA in Pd-tmen with NaOH at a molar ratio of 1:2:1. All measurements were performed at room temperature in D_2O. The numbering follows IUPAC rules, see Figure 1. [Pd(tmen)(NeuNAc1,2H$_{-2}$-κ^2*O*^{1,2})] (**1a**) marked with a ring, [{Pd(tmen)}$_2$(NeuNAc1,2,8,9H$_{-4}$-1κ^2*O*^{1,2}:2κ^2*O*^{8,9})] (**2a**) marked with a rhombus and [Pd(tmen)(NeuNAc8,9H$_{-2}$-κ^2*O*^{8,9})]$^-$ (**3a**) marked with a star.

group. In contrast to the Pd experiments, it was not possible to work in aqueous solution with oxophilic Si^{IV} compounds. The solvent of choice was dried *N*,*N*-dimethylformamide.

In contrast to our results with Pd^{II}, in the equimolar reaction of NANA with DTBS triflat the first chelate ring with the DTBS unit was formed at the glycerol chain. In $Si(tBu)_2$(NeuNAc7,9H$_{-2}$-$\kappa^2O^{7,9}$) (**4**) NANA is coordinated via O7 and O9 in a six-membered chelate ring (Figure 4). Si^{IV} as a small, highly charged Lewis acid obviously prefers binding the more basic oxygen atoms. Compound **4** was identified using 1D and 2D NMR spectroscopy, the ^{13}C NMR shifts are in the typical range for silicon-coordinated carbohydrates: +3.1 and +5.0 ppm for the coordinated carbon atoms C7 and C9 and –6.7 ppm for C8. Negative CIS (*coordination-induced shift*) values were characteristic for the inner carbon atom in six-membered chelate rings (Table 2). ^{29}Si NMR spectroscopy was used as a suitable method to assign the type of chelate formed. Silicon atoms, as part of five-membered chelate rings on the one hand, and those of six-membered rings or higher on the other, occur in clearly-resolved shift regions for DTBS carbohydrates.

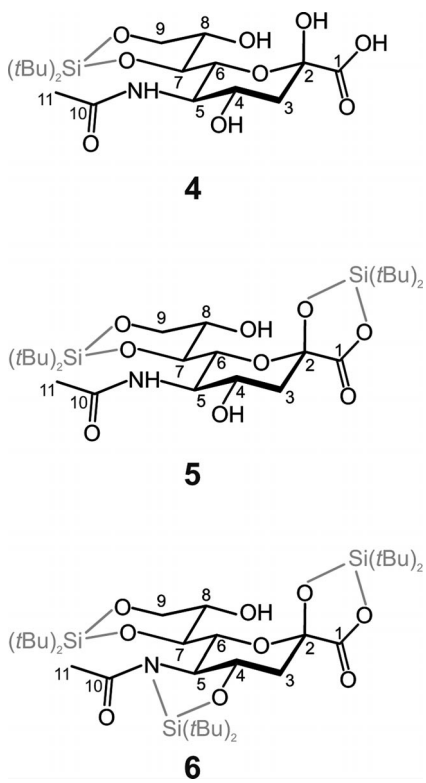

Figure 4. Species detected in DTBS-containing solutions of *N*-acetylneuraminic acid. The compounds shown were observed increasing the molar ratio from 1:1 (**4**) to 1:3 (**6**).

The analysis of the mono-metallated product thus was supported by a signal in the ^{29}Si NMR spectrum at –8.5 ppm – typical for six-membered chelate rings. By increasing the silicon concentration the coordination of the acidic carboxy oxygen was also realised. In the reaction mixture of NANA with the double molar amount of DTBS triflate, the di-metallated product $Si_2(tBu)_4$(NeuNAc1,2,7,9H$_{-4}$-1$\kappa^2O^{1,2}$:2$\kappa^2O^{7,9}$) (**5**) was identified. The CIS values for the carbon atoms C7, C8 and C9 corresponded those of the Si_1 product. The additional coordination via the acidic carboxy group with C1 and the adjacent hydroxy group on C2 caused shifts of –3.4 ppm for C1 and +3.6 ppm for C2 in the ^{13}C NMR spectrum (Figure 5). The surprising negative CIS value for the coordinated carboxy group was validated by analysing simple carbonic acids such as tartaric acid. For several carboxy groups coordinated by DTBS, negative shifts were observed and partially documented by single X-Ray analysis.[21]

In contrast to palladium, a tri-metallated product was formed using three equivalents of DTBS triflate, namely $Si_3(tBu)_6$-(NeuNAc1,2,4,5,7,9H$_{-6}$-1$\kappa^2O^{1,2}$:2κO^4,2κN^5:3$\kappa^2O^{7,9}$) (**6**). Compared to the analysed Si_1 and Si_2 species which displayed a degree of impurity (educt and species other stoichiometries), **6** is uniquely distinct and extraordinarily pure. To form a third chelate ring at the NANA backbone, the *N*-acetyl nitrogen atom had to be involved (excluding an eight-membered chelate ring formed by C4 and C8). Competing Si_3 species that were considered without the *N*-acetyl function had to be derived from the open-chain enol or ketohydrate form. The latter needs one equivalent of water for its formation and was thus excluded. Analysing the ^{13}C NMR spectrum, the enol form could be excluded as well, because, in the range for double-bonded carbon atoms, no signal was detected. In addition to the CIS values for C1, C2, C7, C8 and C9, the signals of the carbon atoms C4 and C5 were shifted as expected for **6**: C4, bonded to the coordinating oxygen, exhibits a signal shift of +5.0 ppm, C5, bonded to the coordinating nitrogen, a shift of –3.3 ppm. The ^{29}Si NMR spectrum shows, besides the two signals for the former discussed chelate rings, an additional signal at –5.1 ppm. A signal of this magnitude correlates to a six-membered chelate ring if a 1,3-diol is bonded. With a coordinating nitrogen atom, the rules of carbohydrate NMR spectroscopy seem

Table 2. ^{13}C NMR chemical shifts (δ /ppm) and shift differences ($\Delta\delta$) of $Si(tBu)_2$(NeuNAc7,9H$_{-2}$-$\kappa^2O^{7,9}$) (**4**), $Si_2(tBu)_4$(NeuNAc1,2,7,9H$_{-4}$-1$\kappa^2O^{1,2}$:2$\kappa^2O^{7,9}$) (**5**) and $Si_3(tBu)_6$(NeuNAc1,2,4,5,7,9H$_{-6}$-1$\kappa^2O^{1,2}$:2κO^4,2κN^5:3$\kappa^2O^{7,9}$) (**6**) in DMF.

		C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11
NeuNAc	δ	172.0	96.0	40.9	67.0	54.4	71.8	70.6	71.0	65.2	173.1	22.7
4	δ	170.8	94.8	39.2	67.0	53.2	69.1	75.6	64.3	68.3	173.5	22.2
	$\Delta\delta$	–1.2	–1.2	–1.7	0.0	–1.2	–2.7	**5.0**	–6.7	**3.1**	0.4	–0.5
5	δ	168.6	99.6	40.6	66.5	52.3	71.7	76.0	64.7	68.8	172.1	22.7
	$\Delta\delta$	**–3.4**	**3.6**	–0.3	–0.5	–2.1	–0.1	**5.4**	–6.3	**3.6**	–1.0	0.0
6	δ	167.8	99.0	41.1	72.0	51.1	71.6	75.7	64.6	68.6	170.5	22.7
	$\Delta\delta$	**–4.2**	**3.0**	0.2	**5.0**	**–3.3**	–0.2	**5.1**	–6.4	**3.4**	–2.6	0.0

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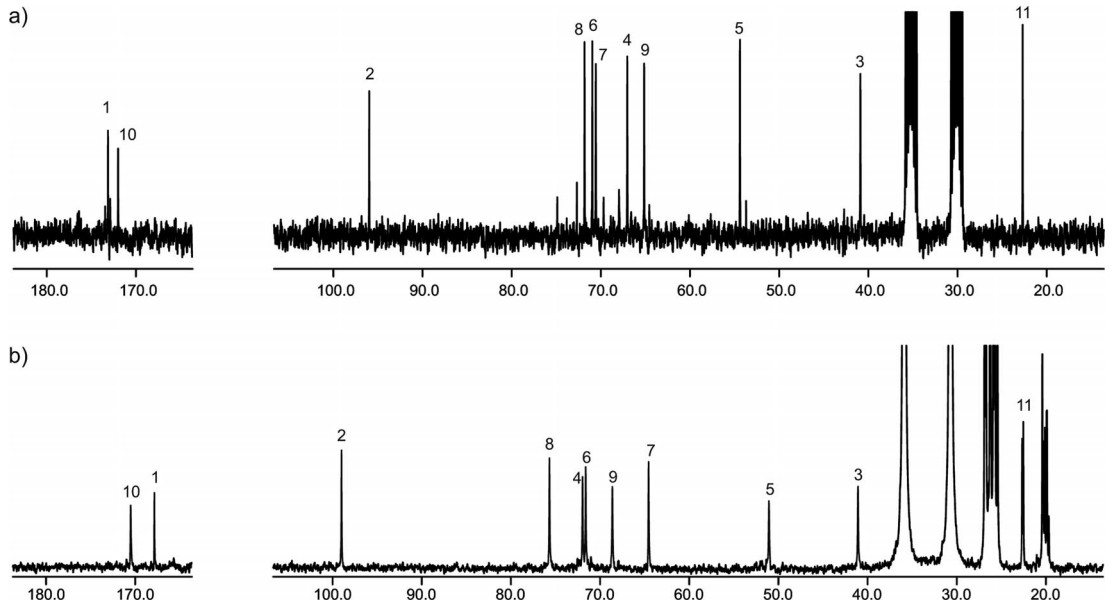

Figure 5. ^{13}C NMR spectra of *N*-acetylneuraminic acid. a) free NANA (β-pyranose), b) NANA with tBu$_2$Si(OTf)$_2$ at a molar ratio of 1:3. All measurements were performed at room temperature in DMF. The numbering follows IUPAC rules, see Figure 1.

to be not simply transferable. To confirm the result we thus analysed model compounds which have carboxy, hydroxy, amino groups or *N*-acetyl amino groups, and found equivalent values.[22]

Conclusions

N-Acetylneuraminic acid is a very important biomolecule and the knowledge of its metal-binding properties should be a matter of course. As a reducing carbohydrate, NANA exists in various cyclic and acyclic forms. The β-pyranose is the most stable one in the free carbohydrate. We analysed NANA coordination towards Pd^{II} and Si^{IV}. In recent investigations the rules of the carbohydrate chemistry of these atoms were unravelled. On this solid foundation, attempts at analysing a polyfunctional biomolecule such as NANA seems promising despite the fact that some important functional groups are present in the same molecule: various hydroxy functions, a carboxy and a *N*-acetyl group. With Pd^{II}, two different binding sites were observed: the α-hydroxy carbonic acid unit and the glycerol side chain. Complying with the rules, [Pd(tmen)(NeuNAc1,2H$_{-2}$-κ$^2O^{1,2}$)] (**1a**) formed with an equimolar amount of Pd^{II} reagent, [{Pd(tmen)}$_2$(NeuNAc1,2,8,9H$_{-4}$-1κ$^2O^{1,2}$:2κ$^2O^{8,9}$)] (**2a**) with an excess of the Pd^{II} reagent and [Pd(tmen)(NeuNAc8,9H$_{-2}$-κ$^2O^{8,9}$)]$^-$ (**3a**) after addition of NaOH. With Pd-chxn, the same coordination pattern was obtained. All compounds were analysed using the various NMR-spectroscopic techniques. Even with a high concentration of Pd^{II}, no coordination via the *N*-acetyl group was detected, but this bonding pattern was achieved with Si^{IV}. The results using DTBS triflate also agreed with the rules of carbohydrate chelation. The first DTBS unit bonded in a six-membered chelate ring on the glycerol side chain, Si(tBu)$_2$(NeuNAc7,9H$_{-2}$-κ$^2O^{7,9}$) (**4**), the second one on the α-hydroxy carbonic acid unit in a five-membered chelate ring, Si$_2$(tBu)$_4$(NeuNAc1,2,7,9H$_{-4}$-1κ$^2O^{1,2}$:2κ$^2O^{7,9}$) (**5**). In addition, the small, highly-charged Lewis acid Si^{IV} binds via the deprotonated *N*-acetyl function if three equivalents of DTBS triflate are used. Si$_3$(tBu)$_6$(NeuNAc1,2,4,5,7,9H$_{-6}$-1κ$^2O^{1,2}$:2κO^4,2κN^5:3κ$^2O^{7,9}$) (**6**) is formed: a tri-metallated compound, which was not observed even with five equivalents of Pd^{II}. As an overall result, even the binding-site pattern of as complicated a carbohydrate derivative as NANA may be unravelled through a combination of NMR results and the application of rules derived from simpler model compounds.

A final comment regarding isolated compounds: Many attempts to isolate the discussed compounds as a solid have failed despite the utilisation of several techniques successfully used with palladium and silicon carbohydrate species in the past. Based on the enormous complexity of NANA, it is necessary to develop new methods for species with hydroxy, carboxy and amido groups.

Experimental Section

Methods and Materials

All chemicals were purchased and used without further purification: *N*-acteylneuraminic acid (ABCR), *N*,*N*,*N'*,*N'*-tetramethylethane-1,2-diamine (Sigma-Aldrich), (1*R*,2*R*)-(–)-1,2-diaminocyclohexane (ABCR), deuterium oxide (Eurisotop), palladium(II) chloride (Alfa Aesar), silver(I) oxide (Merck), bis(*tert*-butyl) bis(trifluoromethanesulfonato) silicon (Aldrich: bis(*tert*-butyl)silyl bis(trifluoromethanesulfonate)), *N*,*N*-dimethylformamide D7 (Eurisotop). Reactions containing silicon compounds were carried out using standard Schlenk technique in a nitrogen atmosphere.

NMR Spectroscopy

NMR spectra were recorded at room temperature with Jeol ECX400/ECP 400 (^1H: 400 MHz, $^{13}C\{^1H\}$: 100 MHz) spectrometers. In aque-

ous solution the signals of methanol, which was added to the sample, were used as an internal secondary reference for the chemical shift. The signals of the deuterated solvent were used as an internal secondary reference for ^{13}C NMR spectra using DMF D7. ^{29}Si shift values were referenced externally to TMS. NMR signals were assigned by ^{1}H–^{1}H COSY45, ^{1}H–^{13}C HMQC and ^{1}H–^{13}C HMBC experiments. Shift differences are given as $\delta(C_{complex}) - \delta(C_{free\ sugar})$. To assign the signal sets to individual species, first of all coupling constants *J* were analysed applying the Karplus relationship to identify the correct anomer. Afterwards, CIS values were used to assign the correct chelation site. The values for the free *N*-acetylneuraminic acid were taken from our own measurements in D_2O in neutral aqueous solution and DMF D7.

Syntheses

Preparation of [Pd(tmen)(OD)$_2$]: To the brown suspension of $PdCl_2$ (2.50 g, 14.1 mmol) in water (85 mL), concentrated hydrochloric acid (2.50 mL) was added and the mixture was stirred for 30 min, yielding a brown solution. Under stirring, on the dropwise addition of *N*,*N*,*N*′,*N*′-tetramethylethane-1,2-diamine (3.25 g, 27.97 mmol) in water (90 mL) a yellow precipitate of [Pd(tmen)Cl_2] was formed. After stirring for 30 min, the yellow complex was filtered through a G4 filter, washed with cold water and dried in vacuo. The yield was 3.84 g (93%). Anal. calcd. for $C_6H_{16}Cl_2N_2Pd$: C, 24.55; H, 5.49; N, 9.54; Cl, 24.16. Found: C, 24.37; H, 5.36; N, 9.50; Cl, 24.12.

A suspension of [Pd(tmen)Cl_2] (1.32 g, 4.50 mmol) and silver(I) oxide (1.15 g, 4.95 mmol) in D_2O (15 mL) was stirred under nitrogen and the exclusion of light at 40 °C. After 30 min AgCl was removed by filtration through a G4 filter under nitrogen atmosphere, leaving a clear yellow solution of [Pd(tmen)$(OD)_2$] ("Pd-tmen"). The alkaline *ca*. 0.45 M solution was stored at –60 °C. **$^{13}C\{^{1}H\}$ NMR** (D_2O): δ = 50.2 (4C, CH_3), 62.1 (2C, CH_2) ppm.

Preparation of [Pd(chxn)(OD)$_2$]: To a suspension of $PdCl_2$ (5.00 g, 28.2 mmol) in water (50 mL) at 45 °C potassium chloride (4.20 g, 56.3 mmol) was added and the mixture was stirred for 10 min until a brown solution of $K_2[PdCl_4]$ was formed. (1*R*,2*R*)-cyclohexane-1,2-diamine (3.22 g, 28.2 mmol) dissolved in water (95 mL) and HCl (5 mL, 37%) was slowly added. After the reaction mixture was stirred for 1 h at 45 °C, the pH was raised by a solution of NaOH (2 M) to 7.0 and the complex precipitated from the solution. The reaction mixture was stirred for 3 h at 45 °C (during the first hour, the pH value was repeatedly adjusted). After the suspension was cooled to room temperature, the yellow complex was filtered through a G4 filter, washed with cold water (5 × 20 mL) and dried in vacuo. The yield was 7.7 g (93%). Anal. calcd. for $C_6H_{14}Cl_2N_2Pd$: C, 24.72; H, 4.84; N, 9.61; Cl, 24.32. Found: C, 24.70; H, 4.73; N, 9.58; Cl, 24.35.

A suspension of [Pd(chxn)Cl_2] (3.28 g, 11.25 mmol) and silver(I) oxide (2.80 g, 12.08 mmol) in D_2O (25 mL) was stirred under nitrogen and the exclusion of light at 40 °C. After 2 h AgCl was removed by filtration through a G4 filter under nitrogen atmosphere, leaving a clear yellow solution of [Pd(chxn)$(OD)_2$] ("Pd-chxn"). The alkaline *ca*. 0.3 M solution was stored at –60 °C. **$^{13}C\{^{1}H\}$ NMR** (D_2O): δ = 24.3 (2C, γ-CH_2), 33.7 (2C, β-CH_2), 60.9 (2C, α-CH_2) ppm.

***N*-Acetylneuraminic Acid Solutions in Pd-tmen:** *N*-Acetylneuraminic acid was dissolved in Pd-tmen (*ca.* 0.45 M solution in D_2O) at 4 °C. The yellow solution was stirred for about 2 h at 4 °C and subsequently either placed immediately under NMR investigation or stored at –60 °C. The used rates are listed in Table 3.

Table 3. Applied ratios for the reaction of *N*-acetylneuraminic acid with Pd-tmen (0.45 M, 2.0 mL, 0.90 mmol).

	NeuNAc	sodium hydroxide
1:1:1	280 mg (0.90 mmol)	–
1:2:1	140 mg (0.45 mmol)	–
1:2:1	140 mg (0.45 mmol)	18 mg (0.45 mmol)
1:3:1	93 mg (0.30 mmol)	–
1:5:1	56 mg (0.18 mmol)	–

^{1}H NMR Data of *N*-Acetylneuraminic Acid Complexes: [Pd(tmen)(NeuNAc1,2H$_{-2}$-κ^2*O*^{1,2})] (1a): ^{1}H NMR: δ = 3.87–4.00 (m, 4H, H9a, H4, H6, H8), 3.76 (t, 1H, H5, $^3J_{4,5}$ = 10.1 Hz, $^3J_{5,6}$ = 10.1 Hz), 3.65 (dd, 1H, H9b, $^3J_{8,9b}$ = 6.3 Hz, $^2J_{9a,9b}$ = –11.7 Hz), 3.48 (d, 1H, H7, $^3J_{6,7}$ = 8.8 Hz), 2.36 (dd, 1H, H3a, $J_{2,3a}$ = 4.5 Hz, $^2J_{3a,3b}$ = –12.4 Hz), 1.98 (s, 3H, H11), 1.66 (t, 1H, H3b, $^3J_{2,3b}$ = 12.1 Hz, $^2J_{3a,3b}$ = –12.4 Hz) ppm.

[{Pd(tmen)}$_2$(NeuNAc1,2,8,9H$_{-4}$-1κ^2*O*^{1,2}:2κ^2*O*^{8,9})] (2a): ^{1}H NMR: δ = 4.15–4.20 (m, 1H, H4 and H7), 3.96–4.02 (m, 1H, H6), 3.74–3.80 (m, 1H,H5), 3.42–3.48 (m, 1H, H9a), 3.10 (d, 1H, H9a, $^2J_{9a,9b}$ = –9.9 Hz), 3.03–3.05 (m, 1H, H8), 1.98 (s, 3H, H11), 1.68 (t, 1H, H3b, $^3J_{2,3b}$ = 12.1 Hz, $^2J_{3a,3b}$ = –12.4 Hz) ppm.

[Pd(tmen)(NeuNAc8,9H$_{-2}$-κ^2*O*^{8,9})]$^-$ (3a): ^{1}H NMR: δ = 4.10–4.14 (m, 2H, H4, H6), 4.04–4.08 (m, 1H, H7), 3.83–3.91 (m, 1H, H5), 3.36–3.46 (m, 1H, H9a), 2.94–3.08 (m, 2H, H9b, H8), 1.98 (s, 3H, H11), 1.62 (t, 1H, H3b, $^3J_{2,3b}$ = 12.1 Hz, $^2J_{3a,3b}$ = –12.4 Hz) ppm.

***N*-Acetylneuraminic Acid Solutions in Pd-chxn:** *N*-Acetylneuraminic acid was dissolved in Pd-chxn (*ca.* 0.30 M solution in D_2O) at 4 °C. The yellow solution was stirred for about 2 h at 4 °C and subsequently either placed immediately under NMR investigation or stored at –60 °C. The used rates are listed in Table 3, the respective $^{13}C\{^{1}H\}$ NMR *N*-acetylneuraminic acid shifts are listed in Table 4.

Table 4. Applied ratios for the reaction of *N*-acetylneuraminic acid with Pd-tmen (0.30 M, 2.0 mL, 0.60 mmol).

	NeuNAc	sodium hydroxide
1:1:1	186 mg (0.60 mmol)	–
1:2	93 mg (0.30 mmol)	–
1:2:1:1	93 mg (0.30 mmol)	12 mg (0.30 mmol)

^{1}H NMR Data of N-Acetylneuraminic Acid Complexes: [Pd(chxn)(NeuNAc1,2H$_{-2}$-κ^2*O*^{1,2})] (1b): ^{1}H NMR: δ = 3.92 (ddd, 1H, H8, $^3J_{7,8}$ = 9.1 Hz, $^3J_{8,9a}$ = 2.8 Hz, $^3J_{8,9b}$ = 6.6 Hz), 3.84 (d, 1H, H9a, $^3J_{8,9a}$ = 2.8 Hz), 3.77–3.83 (m, 1H, H4), 3.77 (s, 1H, H6), 3.66–3.72 (m, 1H, H5), 3.56 (d, 1H, H9b, $^3J_{8,9b}$ = 6.6 Hz), 3.39 (d, 1H, H7, $^3J_{7,8}$ = 9.1 Hz), 2.21–2.26 (m, 1H, H3a), 1.85–1.90 (m, 3H, H11), 1.63 (t, 1H, H3b, $^3J_{2,3b}$ = 12.1 Hz, $^2J_{3a,3b}$ = –12.1 Hz) ppm.

Preparation of Si(*t*Bu)$_2$(NeuNAc7,9H$_{-2}$-κ^2*O*^{7,9}) (4) in Solution: To a cooled (0 °C) suspension of *N*-acetylneuraminic acid (320 mg, 1.0 mmol) in *N*,*N*-dimethylformamide D7 (4 mL) Si(*t*Bu)$_2$(OTf)$_2$ (0.33 mL, 1.0 mmol) was added dropwise.

After stirring for 3 h, ^{1}H, ^{13}C, ^{29}Si and 2D NMR spectra were recorded.

$^{29}Si\{^{1}H\}$-NMR: δ = –8.5 ppm.

Preparation of Si$_2$(*t*Bu)$_4$(NeuNAc1,2,7,9H$_{-4}$-1κ^2*O*^{1,2}:2κ^2*O*^{7,9}) (5) in Solution: To a cooled (0 °C) suspension of *N*-acetylneuraminic

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acid (320 mg, 1.0 mmol) in *N*,*N*-dimethylformamide D7 (4 mL) $Si(tBu)_2(OTf)_2$ (0.65 mL, 2.0 mmol) was added dropwise.

After stirring for 3 h ^1H, ^{13}C, ^{29}Si and 2D NMR spectra were recorded.

^{1}H NMR: δ = 4.21 (t, 1H, *H5*, $^3J_{4,5} = {}^3J_{5,6}$ = 9.8 Hz), 4.09–4.14 (m, 1H, *H6*), 3.96–4.03 (m, 1H, *H4*), 3.90–3.86 (m, 1H, *H9a*), 3.80–3.87 (m, 1H, *H7*), 3.68–3.65 (m, 2H, *H8* and *H9b*), 2.14–1.86 (m, 2H, *H3a* and *H3b*) ppm.

^{29}Si{^{1}H}-NMR: δ = 14.1, –8.6 ppm.

Preparation of $Si_3(tBu)_6$(NeuNAc1,2,4,5,7,9H$_{-6}$-1κ^2*O*^{1,2}:2-κ*O*^{4},2κ*N*^{5}:3κ^2*O*^{7,9}) (6) in Solution: To a cooled (0 °C) suspension of *N*-acetylneuraminic acid (160 mg, 0.50 mmol) in *N*,*N*-dimethylformamide D7 (4 mL) $Si(tBu)_2(OTf)_2$ (0.52 mL, 1.6 mmol) was added dropwise.

After stirring for 3 h ^1H, ^{13}C, ^{29}Si and 2D NMR spectra were recorded.

^{1}H NMR: δ = 4.65 (dd, 1H, *H4*, $^3J_{3,4}$ = 6.9 Hz, $^3J_{4,5}$ = 9.3 Hz), 4.36 (dd, 1H, *H5*, $^3J_{4,5} = {}^3J_{5,6}$ = 9.9 Hz), 4.22 (d, 1H, *H6*, $^3J_{6,7}$ = 10.4 Hz), 3.93 (dd, 1H, *H9a*, $^3J_{8,9a}$ = 3.5 Hz, $^2J_{9a,9b}$ = –9.2 Hz), 3.86 (d, 1H, *H7*, $^3J_{7,8}$ = 8.8 Hz), 3.70–3.59 (m, 2H, *H8* and *H9b*), 2.23–2.18 (m, 1H, *H3*) ppm.

^{29}Si{^{1}H}-NMR: δ = 18.4, –5.1, –8.4 ppm.

For all compounds, the ^{13}C NMR signals of the *t*Bu function were found in the ranges 25.5–28.7 ppm (CH_3) and 19.3–22.7 ppm (C_{quart}); the shift range of ^1H NMR signals was 1.32–0.87 ppm.

Supporting Information (see footnote on the first page of this article): All coloured figures

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