

# Interaction Studies of Sialic Acids with Model Receptors Contribute to Nanomedical Therapies

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## Abstract

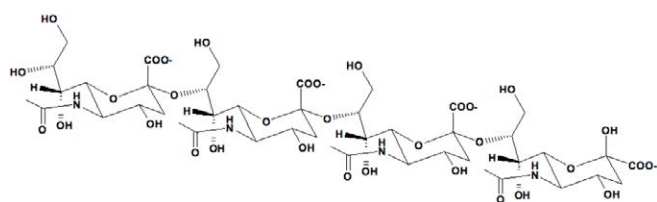
Sialic acid supports nerve cell regeneration, differentiation and neuronal plasticity. Especially, polysialic acid (polySia) chains which are built up by  $\alpha$ 2,8-linked Neu5Ac Neu5Ac residues influence by their specific interactions with polySia receptors neuronal processes related to tumor spread and differentiation processes. With a combination of biophysical and biochemical methods including molecular modeling as described here it is possible to support cell biological experiments and *in vivo* studies on a nanoscale level. The submolecular analytical approaches which are directed to crucial functional groups focus on the potential therapeutic impact of sialic acids and in particular polySia. Such results are helpful for the development of new drugs which might have a high clinical relevance in respect to the therapy of various diseases correlated to neuronal regeneration, tumor spread and infections. It is not surprising that several diseases belonging to these different clinical fields (e.g. oncology, infection diseases, neuronal disorder) can be treated as indicated because sialic acids represent essential contact structures on numerous cell surfaces in dependence to their state of differentiation.

**Keywords:** Nanomedical therapies; Polysialic acid; Cell-cell interactions

## Introduction

A deeper insight into the biological role of sialic acids particularly polysialic acid (polySia) is possible when we understand the complementarity between structure and function on a submolecular level using a strategic combination of biochemical and biophysical methods. Similarities between interactions with cell pathogens and cell surfaces, cell-cell interactions, neuro-oncological mechanisms and nerve cell regeneration processes are obvious since sialic acids and especially the  $\alpha$ 2,8-linked Neu5Ac residues which build up polySia chains (Scheme 1) are often involved in these molecular recognition processes. These characteristic recognition processes are strongly dependent on the organ, the cell type and the stage of differentiation [1-6]. For example, in the respiratory and reproductive systems polySia covalently connected with NCAM is discussed to counteract the cytotoxic characteristics of extracellular histones, which are generated during inflammation [7,8]. In contrast, in the neuronal system the interaction of polySia with histone H1 seems to be important for regeneration processes [9]. Thereby, histone H1 directly binds to polySia at an extracellular position as shown for cultured cerebellar

neurons. Immunostaining of living cerebellar neurons and Schwann cells confirmed that an extracellular pool of histone H1 colocalizes with polySia at the cell surface. Histone H1 stimulated neuritogenesis *in vitro*, process formation and proliferation of Schwann cells as well as migration of neural precursor cells via polySia-dependent mechanisms, further indicating that histone H1 is active extracellularly. These *in vitro* observations suggested an important functional role for the interaction between histone H1 and polySia not only for nervous system development but also for regeneration in the adult organism. Indeed, histone H1 improved glycan chain chafunctional recovery, axon regrowth, and precision of reinnervation of the motor branch in adult mice with femoral nerve injury [9]. Due to their important role in nervous system regeneration and neuro-oncological processes, polySia receptors (e.g. lectins) are of highest clinical importance [10-12]. The involvement of sialic acid in general and polySia in special as well as sulfated oligosaccharides (e.g. the HNK-1 epitope) in neurite outgrowth allows to develop new therapeutic strategies with strong supportive impact on nervous system regeneration in mammals [13-17]. The neural cell adhesion molecule NCAM as well as other polySia-carrying proteins, i.e. neuropilin and the synaptic cell adhesion molecule SynCAM1, interact with these receptors via the polySia



**Scheme 1:** Illustration of an  $\alpha$ 2,8-linked Neu5Ac tetramer.

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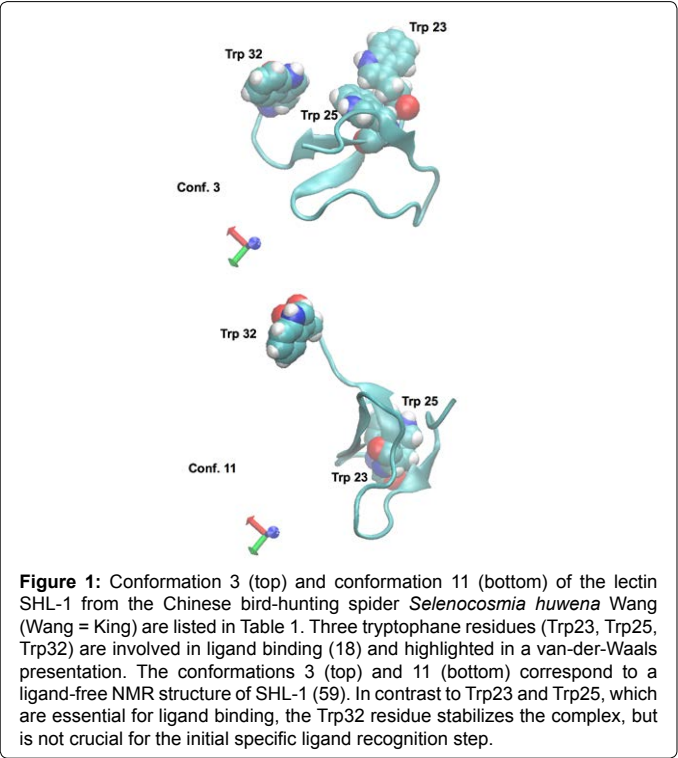
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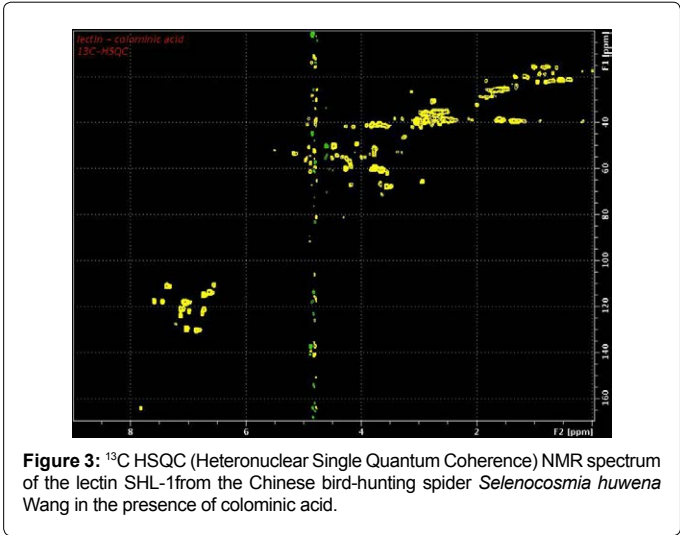
SHL-1 conf.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Trp32	No	No	Yes	Yes	Yes	No	Yes	Yes	No	Yes	No	No	No	Yes	No	Yes	Yes	No	No	No

**Table 1:** Three Trp residues are stabilizing in the SHL-1 – sialic acid complex: Trp23, Trp25 and in parts Trp32. Twenty energy minimum conformations based on the NMR conformations of the ligand-free SHL-1 structure 1QK7.pdb (59) are listed. A contribution of Trp32 in the initial part of complex formation has been detected in nearly half of the analyzed conformations. Nine-times “Yes” means participation in the sialic acid recognition process in respect to Trp32. Eleven-times “No” indicates a lack of involvement concerning Trp32 when the initial binding step with the carbohydrate-recognition region of SHL-1 is discussed.

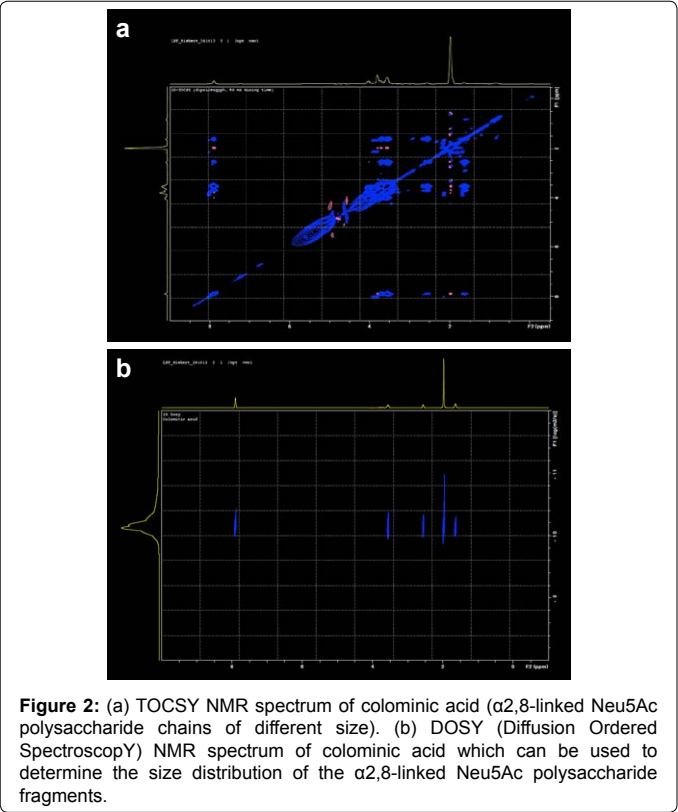


**Figure 1:** Conformation 3 (top) and conformation 11 (bottom) of the lectin SHL-1 from the Chinese bird-hunting spider *Selenocosmia huwena* Wang (Wang = King) are listed in Table 1. Three tryptophane residues (Trp23, Trp25, Trp32) are involved in ligand binding (18) and highlighted in a van-der-Waals presentation. The conformations 3 (top) and 11 (bottom) correspond to a ligand-free NMR structure of SHL-1 (59). In contrast to Trp23 and Trp25, which are essential for ligand binding, the Trp32 residue stabilizes the complex, but is not crucial for the initial specific ligand recognition step.

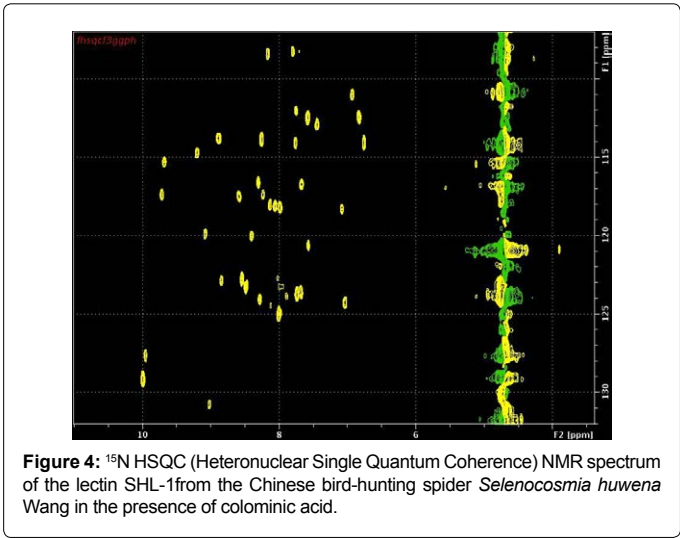
glycan chain. The building block of the polySia glycan chain is the disaccharide repeating unit  $\alpha$ 2,8-linked sialic acid which interacts with the amino acid residues highlighted in Figure 1. In order to analyze the intermolecular processes between sialic acid molecules and their receptors on a nanoscale level suited model systems are needed. Such a suited model system is provided by the lectin SHL-1 from the Chinese bird hunting spider *Selenocosmia huwena* Wang. Sialic acid receptors of different origin have structural similarities in the architecture of their carbohydrate recognition domains (CRDs), pertaining, for instance, in the three-dimensional arrangement of crucial residues [18,19]. Beside arginine residues, aromatic amino acids, such as tryptophan and tyrosine (Figure 1) are often involved in sialic acid interaction processes [18-21]. However, as indicated by the different conformational states listed in Table 1 the structural dynamics of a sialic acid receptor is of highest relevance for the carbohydrate recognition process.



**Figure 3:** <sup>13</sup>C HSQC (Heteronuclear Single Quantum Coherence) NMR spectrum of the lectin SHL-1 from the Chinese bird-hunting spider *Selenocosmia huwena* Wang in the presence of colominic acid.



**Figure 2:** (a) TOCSY NMR spectrum of colominic acid ( $\alpha$ 2,8-linked Neu5Ac polysaccharide chains of different size). (b) DOSY (Diffusion Ordered Spectroscopy) NMR spectrum of colominic acid which can be used to determine the size distribution of the  $\alpha$ 2,8-linked Neu5Ac polysaccharide fragments.

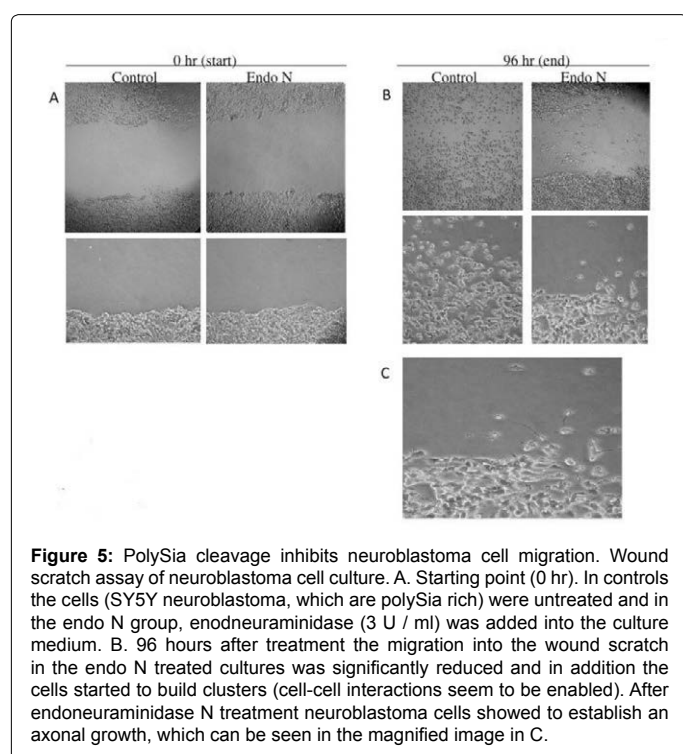


**Figure 4:** <sup>15</sup>N HSQC (Heteronuclear Single Quantum Coherence) NMR spectrum of the lectin SHL-1 from the Chinese bird-hunting spider *Selenocosmia huwena* Wang in the presence of colominic acid.

## Results and Discussion

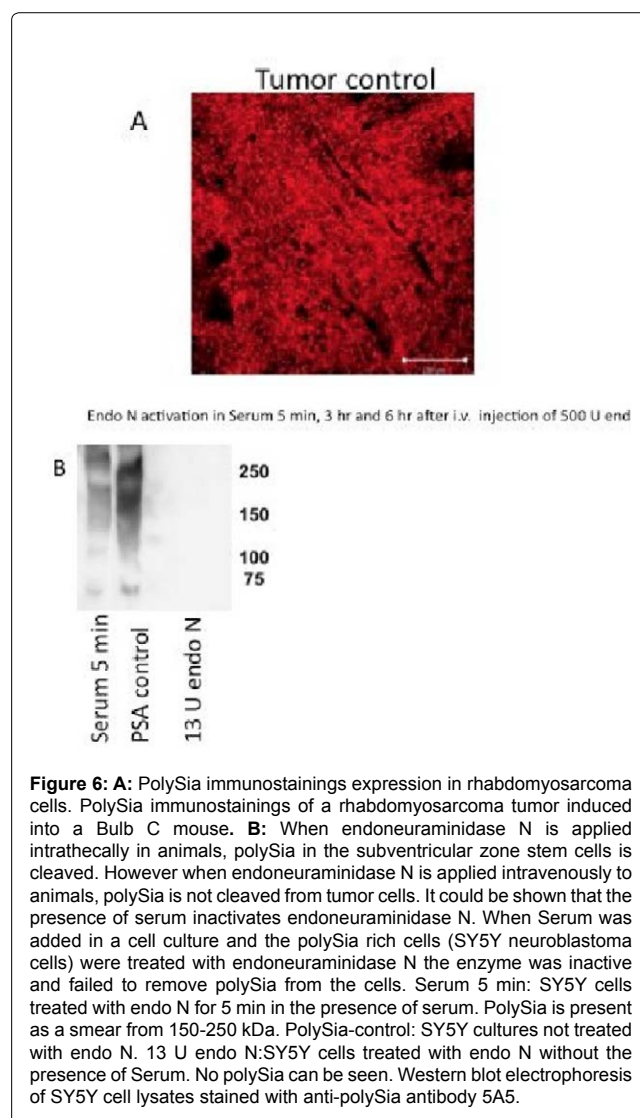
### Analytical approaches

To further validate these nanomedical role models, it is advisable, for instance, to make use of nuclear magnetic resonance (NMR) methods which monitor the interactions between polySia and its sialic acid receptors (Figures 2-4). NMR and molecular modeling studies can be further extended to define whether the ligand-binding results are in agreement with general binding principles and/or similarities in the CRD architectures of sialic acid receptors [18-21]. In this context the strategic combination of methods lead to a new nanomedical approach in neurosciences providing data that explain why bio-active molecules like cyclic and linear peptides or small organic molecules can act as glycomimetics [22-34]. Especially, polar molecules with partially equalized single and double bonds which can be analyzed with *ab initio* calculations are proper candidates for effective new drugs which can mimic sialic acid functions. O-acetylated sialic acids are suited blueprints to identify further glycomimetic molecules since O-acetylation at various positions on Neu5NAc represents an important functional group [35]. In this context we have also refined our tools to understand the molecular interactions on a nanoscale level, thereby, we considered beside polySia the binding processes of HNK-1 and related sulfated saccharides. Furthermore, bio-active peptides and small organic molecules show specific interactions with receptors such as laminin, myristoylated alanine-rich C kinase substrate (MARCKS) and various integrins. Processes of neuronal regeneration and tumor growth are related to sialic acid concentrations as we have learned from stem cell studies. Therefore, it is feasible to invent new therapeutic strategies in these important field of neurological disorders with panels of suited molecules. The physiological role of polySia shall now be discussed in order to understand how inhibition of its expression or increasing of its expression can affect cancer growth as well as central nervous system cell regeneration.



### Therapeutical and diagnostic consequences

PolySia cleavage and tumor cell differentiation are directly related to each other. A number of studies have shown that polySia is overexpressed in malignant tumors like malignant gliomas, small cell lung cancer, neuroblastomas to name a few [12,36-43]. It has been postulated that polySia enables tumor cells to keep an undifferentiated state and therefore to exist outside of the cellular “social network” and grow irrespective of regulating factors expressed by their neighbor cells. Also the characteristic polySia function, to accelerate migration of stem cells [44-46], is used by tumor cells to infiltrate normal tissue and metastasize [12]. As a logical consequence, polySia seems to act as NCAM interaction inhibitor. This means that different pathways induced by NCAM activation can be blocked by the expression of polySia on NCAM. The presence of polySia on NCAM inhibits this interaction [47] and keeps tumor cells away from differentiation back to normal cells. In relation to this it is of clinical interest that the transcription factor Pax3 involved in tumorigenesis seems to induce NCAM polysialylation on medulloblastoma cells [48]. Regarding these processes on a nanoscale level, polySia inhibits cell-cell interactions through its ability to bind significant amount of water and therefore



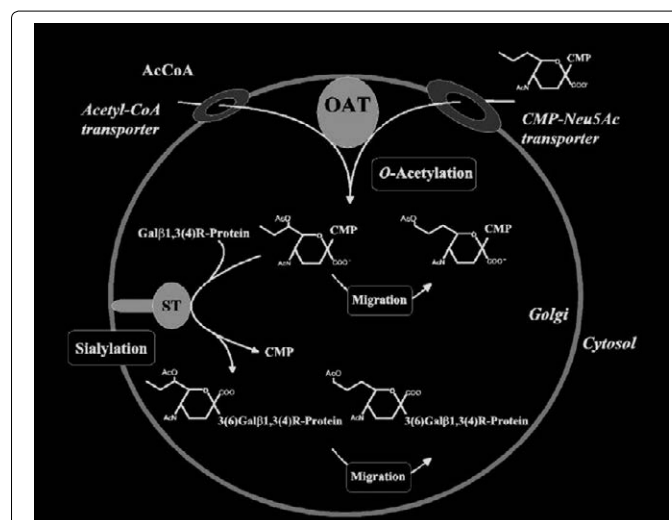


keeping receptors away from their ligand or other receptors. Some studies are postulating that polySia expression of tumor cells can be used as a prognostic factor in different tumors. Wilms tumor patients with increased polySia expression for example have a shorter survival time, therefore, polySia was claimed to be an oncodevelopmental antigen [49,50].

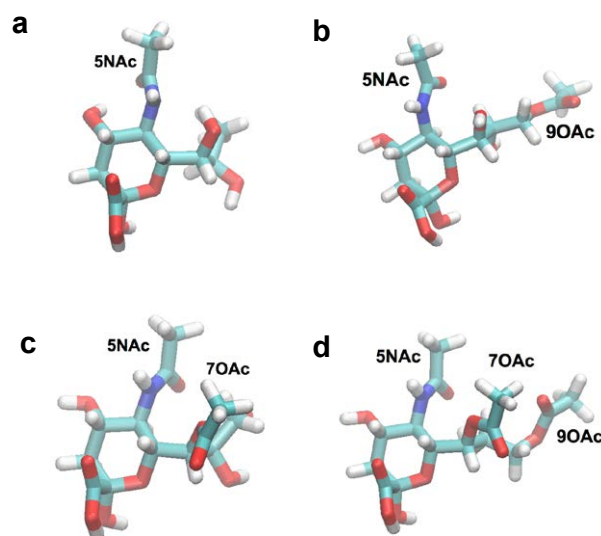
## Nanomedical improvements

Apart from using polySia as a prognostic marker in tumors there is also a therapeutic approach to tumors through cleavage of polySia from the surface of polySia expressing tumor cells.

Endoneuraminidase N is a polySia selective cleavage enzyme. A polySia cleavage in neuroblastoma cells with the non-toxic endoneuraminidase N induces differentiation of these cells with developing axons and expressing neurofilaments [38]. Additionally, the migration capacity of these cells was significantly reduced [38,51-53] (Figure 5). Unfortunately, *in vivo* experiments with intravenous application of endoneuraminidase N in animals with polySia-rich tumors failed to remove polySia from the tumor cells. Reason of this seemed to be factors in the serum, which inactivated endoneuraminidase N there (Figure 6). Therefore, different alternative delivery methods for endoneuraminidase to tumors have to be evaluated. Since the cleavage of polySia from tumor cells is a promising tool in the treatment of cancer nanomedical tools for their delivery are now under construction. These tools can also be used as vessels for polySia fragments and other molecules with an impact on neuronal differentiation. It is important to mention that polySia is not oncogenic. This molecule is expressed by tumor cells but there are no observations that it is inducing cancer. It is indeed fascinating that new drugs can be developed by a precise knowledge of the structural and functional properties of polySia, HNK-1 and the corresponding glycomimetic molecules. The high clinical relevance in respect to the therapy of diseases correlated to neuronal regeneration, tumor spread and infection opens a wide field for medical and pharmacological research projects. It should not be surprising that various diseases of different origin can be treated with drugs based on polySia because this

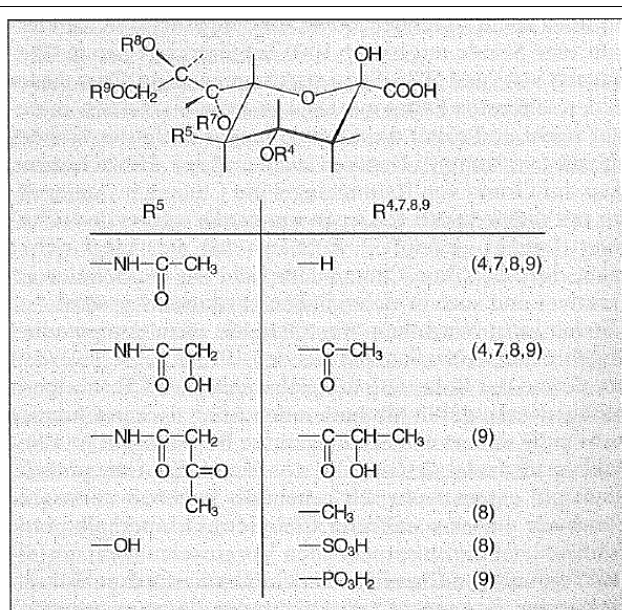


**Figure 8:** The postulated pathway for the incorporation of O-acetyl groups into sialic acids in human colon mucosa. ST, sialyltransferase; R, GlcNAc or GalNAc. The migration (or isomerisation) of acetyl groups from C-7 to C-9, as shown in the postulated pathway, can occur spontaneously under mild-alkaline conditions, or as postulated by Vandamme-Feldhaus and Schauer, 1998 (54), be catalyzed by a hypothetical 'migrase'. It is unclear whether isomerisation takes place at the nucleotide-sugar level or following sialic acid transfer to glycoprotein acceptors.



**Figure 9:** Quantum chemical calculations of (a) Neu5NAc, (b) Neu5NAc9OAc, (c) Neu5NAc7OAc, (d) Neu5NAc7,9OAc were carried out with the DFT (Density Functional Theory) method (B3LYP/6-31G\*) using the Gaussian03 program. Beside the 5-acetyl group also 9O-acetyl- and 7O-acetyl groups are important contact parts for the fine-tuning of ligand – receptor interactions when sialic acids are involved.

polysaccharide has to be considered as an essential contact structure on the cell surface related to many innovative clinical approaches in the field of nanomedicine. In particular, O-acetylation of sialic acids may play an important role in respect to a rational-based design of new drugs since the O-acetyl groups act as special recognition points (Figure 7). The postulated pathway for the incorporation of O-acetyl groups into sialic acids in human colon mucosa is described in the literature [54,55] and shown in (Figure 8). When the biological effects of sialic acid modifications caused by different functional groups have to



**Figure 7:** The carboxyl group (COOH). The N-acetyl group (left column) is always found at C-atom 5 whereas an O-acetyl group (right column) can be attached to the C-atoms 4, 7, 8 and 9 of a sialic acid residue.

be understood completely it is essential to consider all their biophysical properties. As it is the case when studying olfaction processes beside the molecular shape and its dynamics also the vibrational states of certain functional groups have to be considered [56]. Quantum chemical calculations of (a) Neu5Ac, (b) Neu5Ac9OAc, (c) Neu5Ac7OAc, (d) Neu5Ac7,9OAc were carried out with the DFT (Density Functional Theory) method (B3LYP/6-31G\*) using the Gaussian03 program in order to collect all these physical parameters. Pictures of the energy minimum conformations are displayed in (Figure 9) This knowledge opens new routes for a rational based drug design of molecules, since also the complete set of physical parameters including the vibrational states of these molecules are taken into account. In such a context therapeutical improvements could be expected in relation to sialic acid - receptor interactions especially when focusing on polySia with special patterns of O-acetylations. A combination of the biophysical methods described here with clinical studies could lead to neuro-oncological approaches related to applied patient-care [57,58].

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