Chapter 8
Structural Analysis of Oligosaccharides and Glycoconjugates Using NMR

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Abstract Carbohydrate chains play critical roles in cellular recognition and subsequent signal transduction in the nervous system. Furthermore, gangliosides are targets for various amyloidogenic proteins associated with neurodegenerative disorders. To better understand the molecular mechanisms underlying these biological phenomena, atomic views are essential to delineate dynamic biomolecular interactions. Nuclear magnetic resonance (NMR) spectroscopy provides powerful tools for studying structures, dynamics, and interactions of biomolecules at the atomic level. This chapter describes the basics of solution NMR techniques and their applications to the analysis of 3D structures and interactions of glycoconjugates in the nervous system.

Keywords Nuclear magnetic resonance • Structure • Dynamics • Interaction • Oligosaccharide • Glycolipid

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Abbreviations

Aβ	Amyloid β
DIS	Deuterium-induced isotope shift
FID	Free induction decay
HSQC	Heteronuclear single-quantum coherence
MD	Molecular dynamics
NMR	Nuclear magnetic resonance
NOE	Nuclear Overhauser effect
NOESY	NOE spectroscopy
PCS	Pseudocontact shift
PRE	Paramagnetic relaxation enhancement
REMD	Replica exchange MD
RF	Radio frequency
STD	Saturation transfer difference
TRNOE	Transferred NOE
TROSY	Transverse relaxation optimized spectroscopy

8.1 Introduction

In the nervous system, cellular processes, including division, development, migration, and morphological changes, are dynamically controlled through molecular recognition events on cell surfaces. In these bio-organization processes, carbohydrate chains that modify proteins and lipids play critical roles in recognition and adhesion during cell–cell communication. To better understand the molecular mechanisms underlying these neurophysiological functions, atomic views are desirable to describe dynamic interactions of biomolecules such as glycoconjugates. In addition, recent evidence has demonstrated that gangliosides on neuronal cell membranes are targets for various amyloidogenic proteins that are associated with neurodegenerative disorders, e.g., α-synuclein in Parkinson's disease, amyloid β (Aβ) in Alzheimer's disease, and prion proteins in Creutzfeldt–Jakob disease (Taylor and Hooper 2006; Ariga et al. 2008; Fantini and Yahi 2010; Matsuzaki et al. 2010). Atomic descriptions of these pathological processes provide a basis for designing novel therapeutic molecules.

Nuclear magnetic resonance (NMR) spectroscopy is one of the most widely used techniques for atomic visualizations of biomolecules. The unique feature of this method is its ability to determine atomic coordinates of biomacromolecules in solution and those embedded in membranes and to characterize their dynamic motion at the atomic level. NMR spectroscopy also serves as a powerful tool for detailed analyses of functional intermolecular interactions and is now routinely used by a wide range of researchers, including glycobiologists. This chapter provides the basics of biomolecular NMR spectroscopy needed for applying this useful technique to address neuroglycobiological issues.

8.2 Basic NMR Phenomena

NMR is a physical phenomenon that reflects quantum mechanical magnetic properties of atomic nuclei in orientation with a strong magnetic field (Abragam 1961; Levitt 2008). Although all isotopes containing odd numbers of protons and/or neutrons have intrinsic magnetic moments and are therefore NMR active, the most commonly studied nuclei are ^{1}H and ^{13}C, which have spin quantum numbers of 1/2 and therefore exhibit high-resolution NMR spectra. These nuclei have two spin states with energy differences that depend on intrinsic magnetic moments and a given magnetic field. In comparison with ^{1}H-NMR, ^{13}C-NMR measurements suffer from low sensitivity because of the lower natural abundance of this isotope (1.1%) and its smaller magnetic moment. Hence, NMR samples are often enriched with ^{13}C using metabolic labeling and chemical synthesis (Yamaguchi and Kato 2007b; Ohki and Kainosho 2008; Kato et al. 2010; Zhang et al. 2013).

Alignment of the nuclear magnetic moment in the magnetic field is perturbed by an electromagnetic field with a resonant radio frequency (RF) pulse that corresponds with the energy difference. After applying the RF pulse, nonequilibrium magnetization, as the sum of all the individual nuclear magnetic moments in the sample, precesses around the magnetic field with the resonant frequency and produces corresponding voltage oscillations in the detection coil. The duration of the oscillating signal is limited and decays exponentially. This time-domain NMR signal, known as free induction decay (FID), is Fourier transformed to produce a frequency-domain spectrum. Figure 8.1a shows FID and Fourier-transformed ^{1}H-NMR spectrum of the pentasaccharide of ganglioside GM1 dissolved in D_2O. The real part of the complex spectrum is typically displayed as the NMR spectrum, showing the absorptive Lorentzian line shape.

8.3 Chemical Shifts as Structural Probes

Individual protons in molecules are generally surrounded by differing electronic environments, which shield each proton against the magnetic field with various modes. As a result, resonant frequencies differ among protons depending on chemical environments, even under the same magnetic field. Hence, a ^{1}H-NMR spectrum exhibits a number of peaks at different positions, and these chemical shifts are each assigned to distinct protons in the molecule. The same is true for the other NMR-active nuclei.

In practice, chemical shift (δ) is measured in parts per million (ppm) relative to a reference resonance signal from a standard compound:

$$\delta = \left(\nu - \nu_{\mathrm{ref}}\right) / \nu_{\mathrm{ref}} \times 10^{6}$$

In the equation above, ν and ν_{ref} represent the resonance frequencies of sample and reference signals, respectively.

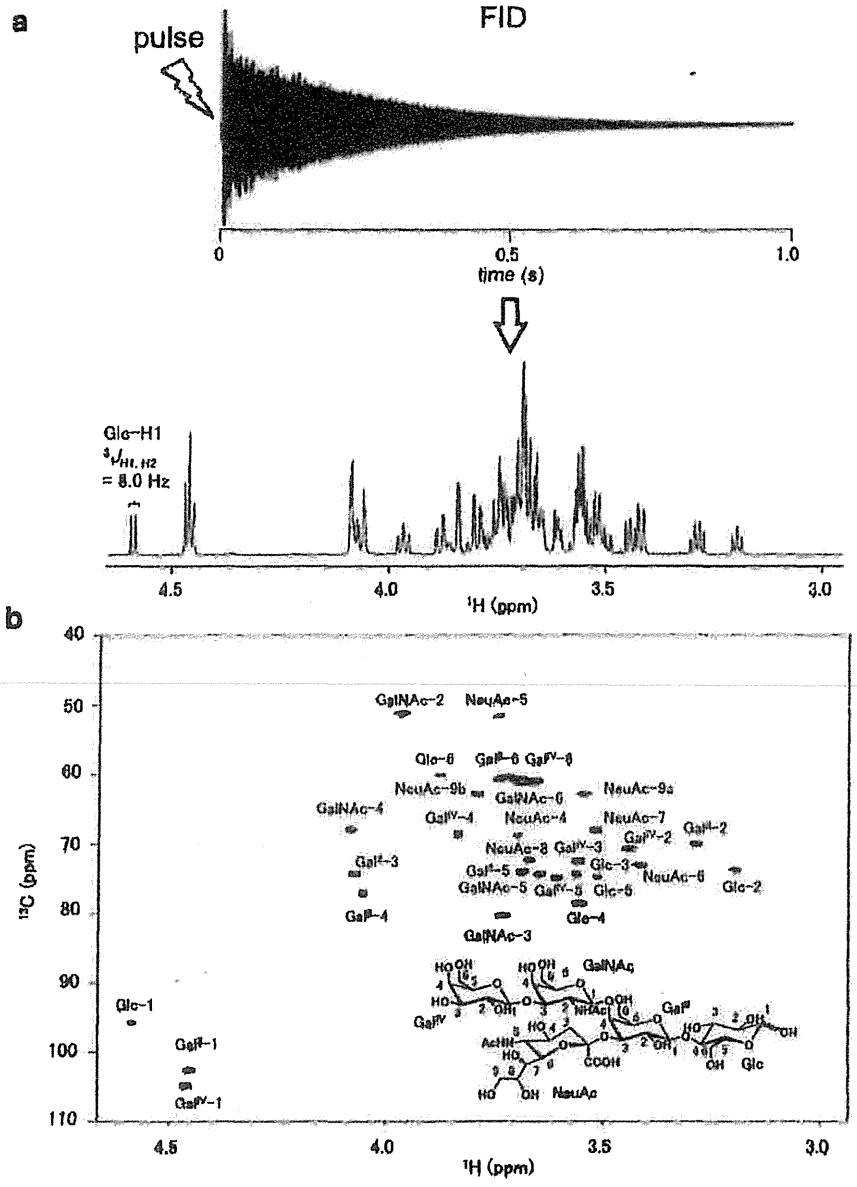

Fig. 8.1 NMR spectral examples of the GM1 pentasaccharide. (a) A free induction decay (FID) and Fourier-transformed ^{1}H-NMR spectrum and (b) ^{1}H–^{13}C HSQC spectrum of the GM1 pentasaccharide

In carbohydrate NMR spectroscopy, ^{1}H-NMR chemical shifts have traditionally been used as *structural reporters* for identifying chemical structures of oligosaccharides (Vliegenthart 1980). Chemical shifts also offer unique conformational probes for biomolecules. For example, secondary structures of proteins can be determined by inspecting chemical shifts of backbone ^{1}H and ^{13}C atoms (Wishart and Sykes 1994; Cornilescu et al. 1999).

In carbohydrate NMR spectra, signals are typically in very close proximity of one another (Fig. 8.1a). To resolve chemical shift degeneracy, applications of higher magnetic fields are certainly advantageous (Kato et al. 2008).

8.4 Through "BOND" and Through "SPACE" Interactions

Chemical shift values are influenced by surrounding chemical environments. In contrast, scalar coupling (or *J*-coupling) splits NMR peaks and reflects indirect interactions between NMR-active nuclei that are mediated by the electrons participating in chemical bonds between nuclei. Scalar coupling constants (J) are defined by magnitudes of peak splitting and are independent of molecular orientations with respect to the magnetic field but depend on molecular geometry. The vicinal scalar coupling constant ^3J, which pertains to atoms separated by three covalent bonds, is typically employed for conformational analyses of biomolecules. This constant is related to the dihedral angle θ as described by the Karplus equation as follows:

$$^3J(\theta) = \mathrm{A}\cos 2\theta + \mathrm{B}\cos\theta + \mathrm{C}$$

In this equation, A, B, and C are coupling coefficients, and θ is the dihedral angle. This relationship has been applied to determinations of sugar-ring stereochemistry and characterization of glycosidic linkage conformations of oligosaccharides (Zhao et al. 2007). In Fig. 8.1a, the anomeric proton (H1) of the β-glucose residue at the reducing terminus of the GM1 pentasaccharide exhibits peak spits that originate from vicinal scalar coupling between H1 and H2 protons ($^3J_{H1,H2} = 8.0$ Hz).

In many two- and multidimensional NMR experiments, migration of magnetization among correlated nuclei is a crucial process. Magnetization transfer that proceeds from one spin to another is mostly achieved through scalar coupling. Due to increased numbers and line widths of resonances, 2D homonuclear ^{1}H-NMR methods are ineffective for biomolecules with molecular masses >10 kDa. In addition, larger line widths result in decreased sensitivity for ^{1}H correlation experiments that rely on small (<10 Hz) homonuclear ^3J scalar coupling for coherence transfer. The efficiency of magnetization transfer in heteronuclear NMR spectroscopy is improved by employing relatively large one-bond scalar coupling interactions of ^{1}H with ^{15}N or ^{13}C. To measure ^{1}H–^{13}C heteronuclear single-quantum coherence (HSQC) spectra (Bodenhausen and Ruben 1980) (Fig. 8.1b), one-bond scalar coupling $^1J_{C\text{-}H}$ (approximately 145 Hz) is used to transfer magnetization from ^{1}H to ^{13}C and vice versa.

In addition to the through-bond scalar coupling interaction, through-space dipolar interactions are utilized to transfer magnetization between spins. Due to the nuclear Overhauser effect (NOE), perturbation of populations of stationary states within a spin system causes time-dependent changes in the intensity of dipolar-coupled resonance signals, which follows polarization transfers between spin populations via dipolar cross-relaxation. The efficiency of the NOE depends on the

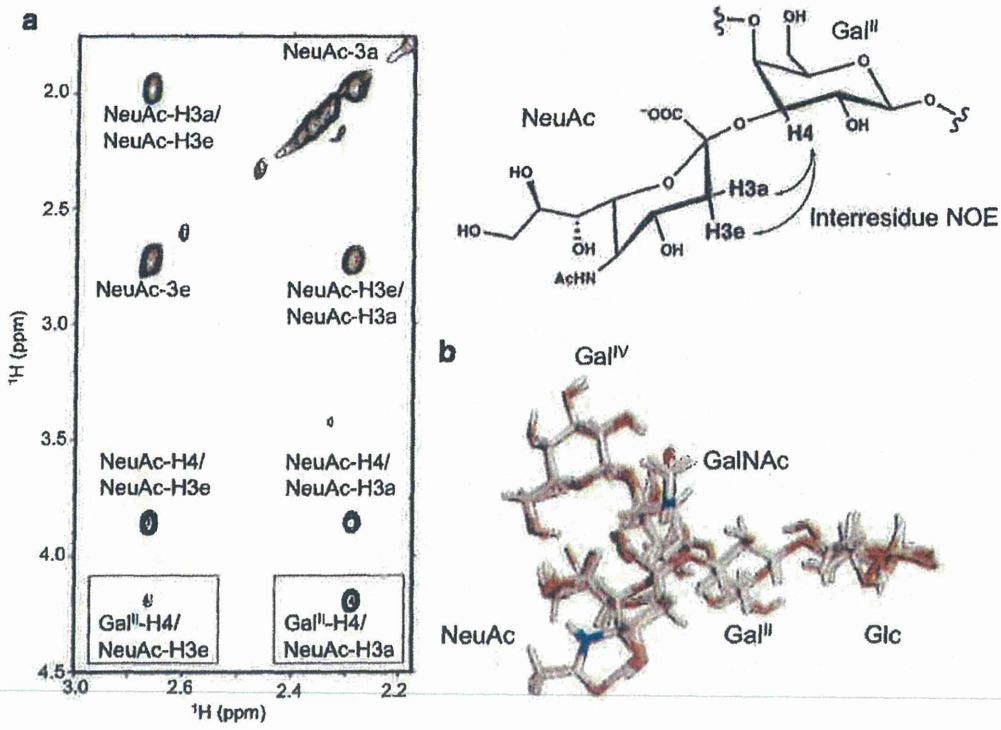

Fig. 8.2 Atomic distance information provided through NOEs. (**a**) A part of ^{1}H–^{1}H NOESY spectrum of the micellar lysoGM1 and (**b**) the lowest-penalty 3D models of the GM1 pentasaccharide calculated from interresidue NOE data. Reprinted from (Yagi-Utsumi et al. 2010) with permission from Elsevier

distance between interacting spins. Thus, through-space rather than through-bond magnetization transfer generates cross-peaks according to the NOE in NMR spectra. ^{1}H–^{1}H NOE spectroscopy (NOESY) provides a measure of interproton distances of up to 5 Å, enabling identification of atomic coordinates of biomacromolecules such as proteins (Wüthrich 1986). The intensity of the NOE (I) is related to the distance (r) between proton pairs, as in

$$I = f(\tau_c) \times r^{-6},$$

where $f(\tau_c)$ is a function of the rotational correlation time τ_c of the molecule. Figure 8.2a shows a part of the ^{1}H–^{1}H NOESY spectrum of micellar lyso-GM1. In addition to intraresidue NOEs, interresidue NOEs are observed between spatially proximal pairs of protons, as exemplified by the NeuAc H3 (axial)–Gal^{II} H4 proton pair. Interresidual NOE observations are used to identify glycosidic linkages of unknown compounds and estimate dihedral angles along glycosidic bonds (Homans et al. 1987; Cumming and Carver 1987; Voisin et al. 2005). Combined NMR analyses based on through-bond and through-space interactions enable sequence-specific resonance assignments of oligosaccharide NMR signals and subsequently provide 3D structural information (Acquotti et al. 1990; Brocca et al. 2000; Prestegard et al. 1982; Yagi-Utsumi et al. 2010; Yu et al. 1986) (Fig. 8.2b).

8.5 Relaxation and Molecular Motion

In principle, NMR experiments begin from the equilibrium state, in which all populations of energy levels of the system are described by the Boltzmann distribution. Although multiple pulses and multidimensional NMR techniques permit generation of nonequilibrium states, the equilibrium state is eventually restored. As in other spectroscopic techniques, recovery from the nonequilibrium state to equilibrium is called relaxation. Relaxation in NMR involves recovery of the nuclear spin magnetization component with an orientation that is parallel to the static magnetic field (called spin–lattice relaxation) and/or loss of phase coherence of individual nuclear spins (called spin–spin relaxation). Time constants of these two processes are termed T_1 and T_2, respectively. In solution NMR spectroscopy, relaxation is governed by the dynamic properties of molecules, including overall molecular tumbling and internal motions. For example, T_2 determines natural line widths of resonances detected during the acquisition period. In the comparison of ^{1}H-NMR spectra for micellar ganglioside GM1 and the free oligosaccharide derived from it shown in Fig. 8.3, molecules with slower tumbling rates exhibit broader signal line widths, which are inversely proportional to T_2. Thus, T_2 is a critical factor for detecting NMR peaks with higher signal-to-noise ratios. A sophisticated pulse sequence (transverse relaxation optimized spectroscopy (TROSY)) has been developed for

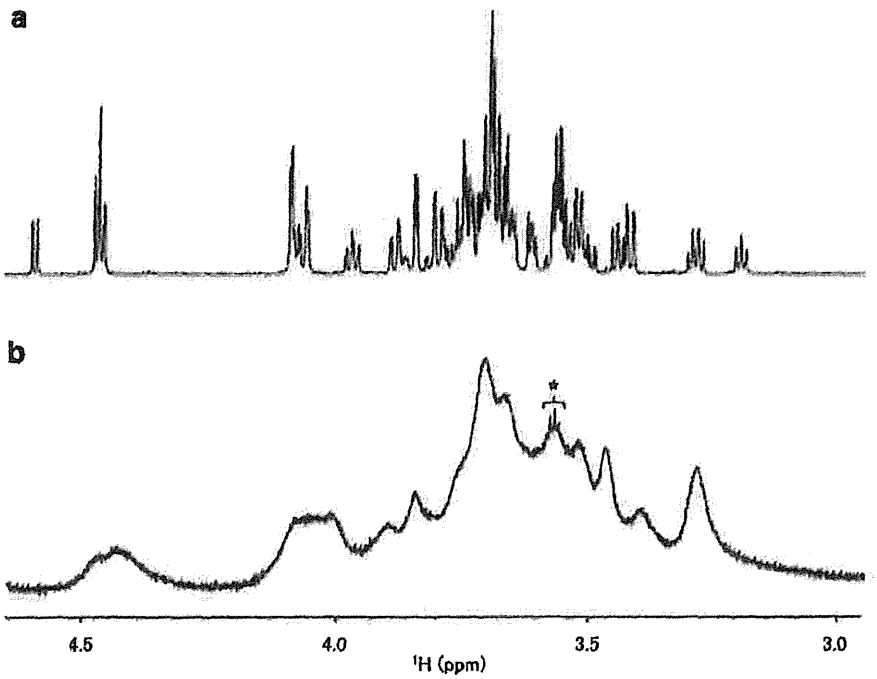

Fig. 8.3 One-dimensional ^{1}H-NMR spectra of (**a**) liberated GM1 pentasaccharide (1.0 kDa) and (**b**) GM1 micelle with an approximate molecular mass of 140 kDa. Peaks originating from a low-molecular-weight contaminant are indicated by an asterisk

NMR analyses of larger macromolecular complexes, in combination with optimal (generally higher) magnetic fields and sample deuteration for suppressing the magnetic dipole–dipole interactions caused by protons that are strong sources of relaxation (Pervushin et al. 1997).

8.6 Paramagnetic Effects as Sources of Long-Distance Information

Unpaired electrons can dramatically perturb NMR spectra, due to stronger dipole–dipole interactions with nuclei that have much larger magnetic moments than atomic nuclei. For example, through-space interactions between a paramagnetic center and neighboring protons cause increased relaxation rates with r^{-6} dependence between paramagnetic spin-proton distances (Solomon 1955). Such paramagnetic relaxation enhancement (PRE) offers long-distance information. Paramagnetic probes such as nitroxide radicals are used to characterize oligosaccharide conformations and lectin–carbohydrate interactions (Johnson et al. 1999; Jain et al. 2001; Yamaguchi et al. 2013a).

Chemical shifts can also be modulated in the presence of paramagnetic lanthanide ions (such as Er^{3+} and Tm^{3+}). This perturbation is known as pseudocontact shift (PCS), which occurs when the magnetic susceptibility of metal ion is anisotropic. PCS is exploited to determine geometrical arrangements of individual nuclei in relation to the position of the metal ion with r^{-3} dependence (McConnell and Robertson 1958; Kurland and McGarvey 1970). Therefore, the atomic long-distance information for determining biomacromolecular conformations can be obtained by observing PCS following introduction of lanthanide probes into specific target molecule sites. Several NMR studies have used PCS to restrain protein and oligosaccharide conformations (Bertini et al. 2005; Otting 2010; Zhang et al. 2013). Recently, PCS of the GM3 trisaccharide (NeuAcα2-3Galβ1-4Glc) and the GM2 tetrasaccharide (GalNAcβ1-4(NeuAcα2-3) Galβ1-4Glc) were analyzed by attaching a lanthanide-chelating tag to reducing ends (Yamamoto et al. 2012; Zhang et al. 2012). Using two-dimensional ^{1}H–^{13}C HSQC spectra, PCS values were measured as differences between ^{1}H and ^{13}C chemical shifts and those of diamagnetic compounds (Fig. 8.4). These analyses provide conformational information related to oligosaccharides. However, in general, oligosaccharide conformations dynamically fluctuate in solution. Therefore, observed PCS should be interpreted as averages of the dynamic conformational ensemble (*vide infra*).

8.7 Chemical Exchange: Dynamic Aspects in NMR

NMR spectroscopy provides unique information on the *exchange* of nuclei between different environments due to conformational transitions and/or intermolecular interactions (Lian and Roberts 1993). Suppose that a given nucleus exchanges with

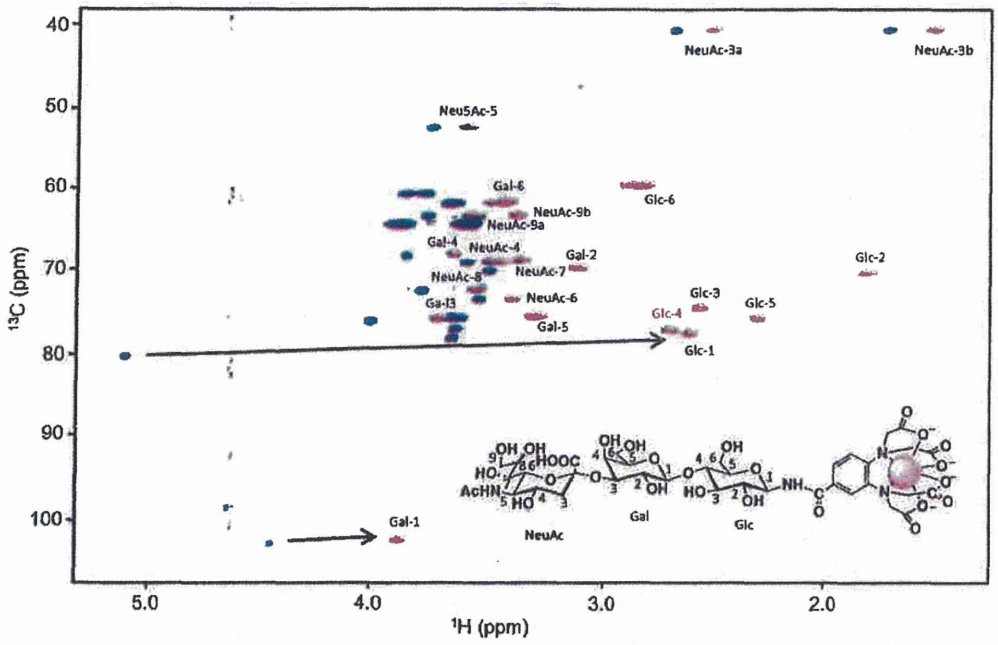

Fig. 8.4 Comparison of ^{1}H–^{13}C HSQC spectra of the GM3 trisaccharide with lanthanide-chelating tag complexed with paramagnetic Tm^{3+} (*red*) and diamagnetic La^{3+} (*blue*). Reprinted from (Yamamoto et al. 2012) with permission from The Royal Society of Chemistry

rate constant k between two sites with resonance frequencies that differ by $\Delta\omega$ (Fig. 8.5a). If k is slow in terms of the frequency of chemical shift differences ($k << \Delta\omega$), then two distinct signals corresponding to the nuclei of two sites are observed. In contrast, if the exchange rate is fast ($k >> \Delta\omega$), then a single resonance is observed, which reflects the population-weighted average chemical shift. Because conformational transitions of free oligosaccharides occur in nanosecond time range (Yamamoto et al. 2012), the observed PCS shown in Fig. 8.4 are analyzed in the fast exchange regime. If the exchange rate is of the order of the chemical shift difference between two sites, the lines become considerably broad and coalesce at $k \sim \Delta\omega$. This is known as the intermediate exchange regime or coalescence, where k and $\Delta\omega$ as well as populations of individual states can be estimated using sophisticated relaxation dispersion experiments (Loria et al. 1999; Mittermaier and Kay 2006; Sugase et al. 2007).

Biomolecules possess exchangeable protons, such as those in hydroxyl and amide groups. Proton exchange rates of these groups provide useful probes for characterizing conformational fluctuations and interactions of biomolecules such as oligosaccharides, because slower exchange rates indicate protecting factors such as hydrogen-bonding interactions at corresponding sites (Englander and Mayne 1992; Englander et al. 2007). Exchanges between protein amide protons and water occur in the slow exchange regime. The rates of these processes can be measured in several ways depending on the rate of exchange. When the exchange rate is comparable to or faster than the spin–lattice relaxation rate (typically, $k_{ex} > 0.1\ s^{-1}$), the rate constant

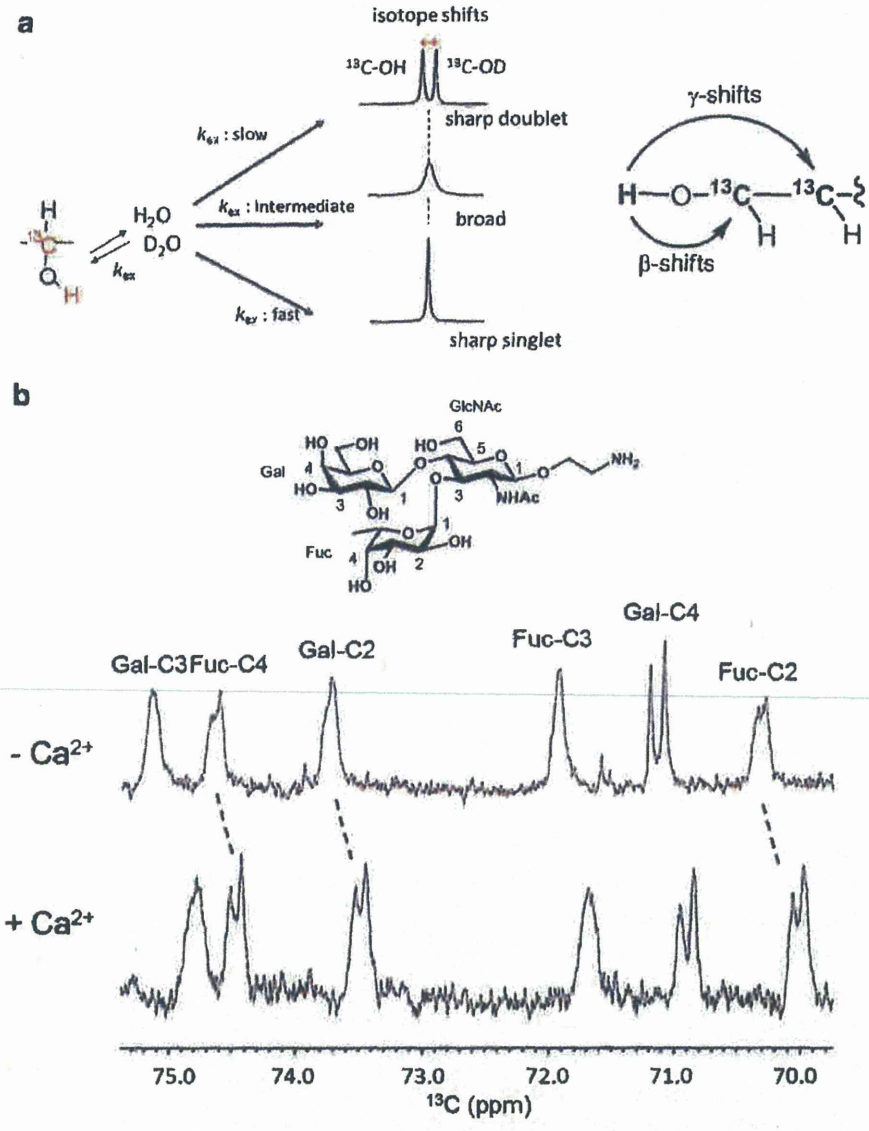

Fig. 8.5 ^{13}C-NMR isotope shifts for analyzing proton exchange rates of sugar hydroxyl groups. (**a**) Schematic of NMR experiment using isotope shifts. All hydroxyl protons on glycans rapidly exchange to deuterons in $H_2O/D_2O = 50:50$ solution. The ^{13}C-NMR signal of the geminal carbons at the H/D exchanging hydroxyl protons shows characteristic signal shape, which is dependent on the H/D exchanging rate. Especially under significantly slow exchange conditions, a set of sharp doublet is provided due to isotope shifts (β-shifts). The chemical shifts difference is ~0.15 ppm. k_{ex}; exchanging rates of protons. (**b**) Parts of ^{13}C-NMR spectra of 40 mM Lewis X trisaccharide without 1.0 M $CaCl_2$ (*upper*) and with 1.0 M $CaCl_2$ (*lower*) at 5 °C. Adapted with modifications from (Hanashima et al. 2011) with permission from The Royal Society of Chemistry

is most easily determined in saturation transfer experiments (Forsén and Hoffman 1963, 1964). In these experiments, saturation is performed by selectively irradiating the frequency of the water signal, and the exchange of amide protons with saturated water protons is quantitatively assessed according to NMR signal intensities (Spera

et al. 1991). For slower rates ($k_{ex} < 0.01\ s^{-1}$), exchanges are measured by observing progressive changes of NMR spectra, which exhibit time-dependent attenuation of peak intensities after rapidly transferring proteins from H_2O into D_2O (Jeng et al. 1990; Paterson et al. 1990).

Deuterium exchanges of rapidly exchanging protons, such as hydroxyl protons of oligosaccharides, cannot be quantitatively characterized using conventional H_2O/D_2O exchange monitoring. To overcome this difficulty in aqueous solution, an NMR strategy has been developed using deuterium-induced isotope shifts (DIS; Hanashima et al. 2011). This method provided detailed characterization of Ca^{2+}-dependent homophilic interactions of Lewis X trisaccharides (Fucα1-3(Galβ1-4) GlcNAc), which have been implicated as having a role in mediating compaction of the mouse embryo at the morula stage (Fenderson et al. 1984; Eggens et al. 1989). ^{13}C-NMR of this trisaccharide was measured in a 50:50 mixture of H_2O/D_2O (Fig. 8.5). DISs were observed to be dependent on Ca^{2+} concentrations. Sample conditions of 1.0 M Ca^{2+} provided doublets originating from Fuc-C2, Fuc-C4, and Gal-C2. In contrast, under Ca^{2+}-free conditions, no doublets originated from these carbon atoms, indicating that proton exchange became significantly slower upon Ca^{2+} coordination.

8.8 NMR Tools for Intermolecular Interaction Analysis

8.8.1 Oligosaccharide–Protein Interactions

Analysis of sugar–protein interactions is an important step for elucidating structure–function relationships of glycans and designing drugs that target carbohydrate recognition systems (Kamiya et al. 2011). NMR spectroscopy provides invaluable tools for this purpose because it allows detection of weak sugar–protein interactions ($K_d \sim$ mM), identification of glycotopes that are recognized by proteins, characterization of protein-bound oligosaccharide conformations, and determination of the modes of atomic interaction between oligosaccharides and proteins in solution (Fig. 8.6) (Yamaguchi and Kato 2007a).

Saturation transfer difference (STD) NMR is now frequently used to analyze protein–ligand interactions (Mayer and Meyer 1999, 2001). One of the great advantages of this method is that it does not require expensive time-consuming stable-isotope labeling of either proteins or ligands. STD-NMR requires the alternate collection of an on-resonance spectrum for saturation of protein protons and an off-resonance spectrum, for reference. Upon irradiation of the protein with a saturation pulse, the saturation effect immediately spreads from irradiated points over the entire protein–ligand complex (Fig. 8.7). If ligand exchange between free and bound states is fast in terms of the time scale of spin–lattice relaxation, the saturation effect is readily transferred to the free fraction of the ligand, particularly to

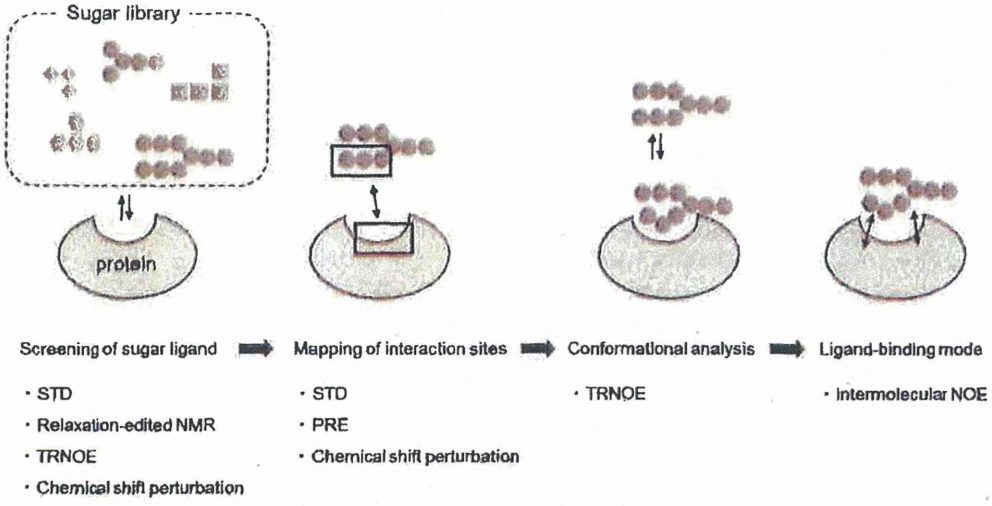

Fig. 8.6 A strategy for analyzing carbohydrate–protein interactions. Adapted with modifications from (Yamaguchi and Kato 2007a) with permission from Yodosha Co., Ltd.

ligand protons located at the interaction interface. An example of STD-NMR is shown in Fig. 8.7, in which the interaction between trisialic acid and a specific monoclonal antibody is analyzed (Hanashima et al. 2013). The nonreducing terminus residue (c) and the central residue (b) showed higher relative values of STD amplification factor compared with the reducing terminal residue (a). The protons at C4, C6, and C7 on residues b and c also had higher values than those at C3, C5, and C8, indicating that this antibody preferentially binds to the α-face of pyranose rings at residues b and c.

Atomic contacts can be identified by observing intermolecular NOE correlations between proteins and cognate ligands. Conformations of protein-bound ligands can also be determined by analyzing intramolecular NOE connectivities within ligands. The sign of the NOE signal depends on τ_c of the molecular tumbling motion and becomes opposite when a fast-tumbling low-molecular-weight ligand binds to a slow-tumbling protein. Under conditions of excess ligand, if the ligand undergoes chemical exchange between the free and bound state more rapidly than longitudinal relaxation, intramolecular NOE connectivities reflecting the protein-bound state can be observed even for peaks exhibiting chemical shifts of free ligand (Clore and Gronenborn 1982, 1983; Glaudemans et al. 1990; Ni and Scheraga 1994). This type of NOE is referred to as transferred NOE (TRNOE). Figure 8.8 displays TRNOE data that characterize conformations of a trimannosyl ligand bound to the carbohydrate recognition domain of VIP36, an animal lectin involved in vesicular transport of glycoproteins between the endoplasmic reticulum and the Golgi (Yamaguchi and Kato 2008). TRNOE can also determine atomic contacts between proteins and carbohydrate ligands (Satoh et al. 2010).

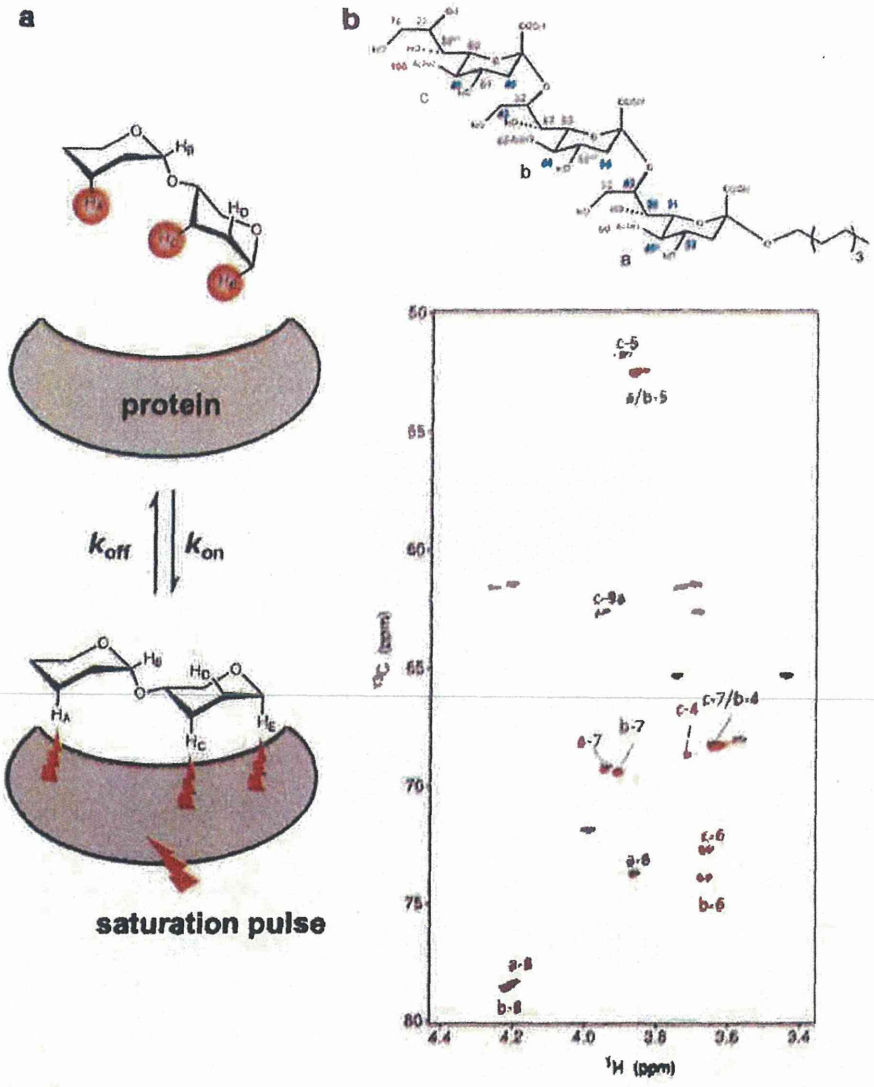

Fig. 8.7 STD experiment. (**a**) Schematic of saturation transfer used for characterizing a carbohydrate–protein interaction; in this experiment, saturation transfer from protein to ligand is observed, thereby identifying the protons involved in the interaction, i.e., H_A, H_C, and H_E; (**b**) Overlay of 2D ^{1}H–^{13}C STD-HSQC spectra of octyl-$(NeuAc)_3$ (50 equiv) with 20 μM anti-oligo/polysialic acid IgM antibody 12E3 (*red*) and 2D ^{1}H–^{13}C HSQC spectrum (*black*) in PBS with 99 % D_2O; Protein signal at 7 ppm was irradiated for saturation. Adapted with modifications from (Hanashima et al. 2013) with permission from Elsevier

8.8.2 Protein Binding to Glycolipid Clusters

In cell membranes, glycolipids such as gangliosides form clusters and play important roles in various biomolecular recognition events (Hakomori 2004). Because of their dynamic properties, crystallographic structural analyses of these glycolipid clusters

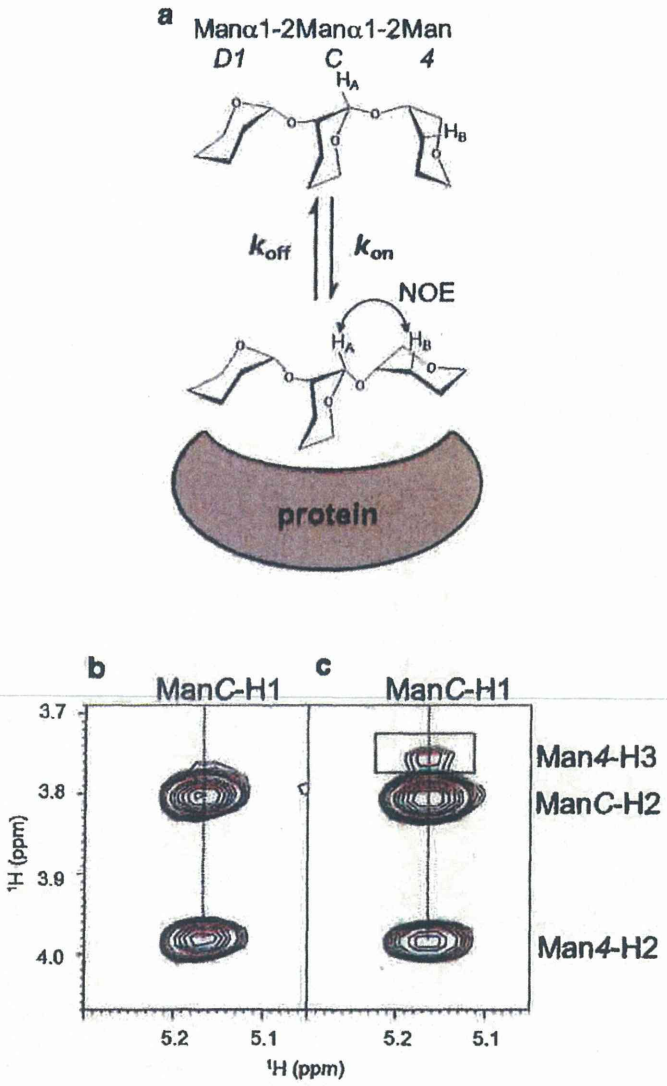

Fig. 8.8 TRNOE experiment. (**a**) Schematic of TRNOE used for characterizing carbohydrate–protein interactions; in this experiment, a spatially proximal proton pair, H_A and H_B, in the protein-bound state provides an NOE peak corresponding to chemical shifts of the unbound state under conditions of excess ligand. A part of NOESY spectrum of Manα1-3Manα1-3Man in (**b**) the absence and (**c**) the presence of the carbohydrate recognition domain of VIP36. An interglycosidic NOE between Man*C*-H1 and Man*4*-H3 (*boxed*) is observed only in the presence of lectin, indicating that these protons are spatially in close proximity of each other in the lectin-bound state of the trimannose. Reprinted from (Yamaguchi and Kato 2008) with permission from Springer

are difficult. In contrast, NMR techniques provide detailed structural analyses of such dynamic clusters of glycolipids and their specific interactions with proteins.

Aβ has been reported to interact with GM1 gangliosides in Alzheimer's disease patients, thereby undergoing conformational transitions that result in pathogenic assemblies (Matsuzaki et al. 2010). To determine the interaction modes of Aβ with ganglioside clusters, NMR experiments were conducted using deuterated ^{15}N-labeled Aβ(1–40) and aqueous gangliosidic micelles (Utsumi et al. 2009). Analyses of backbone chemical shift data of Aβ(1–40) indicated that this peptide forms discontinuous α-helices upon binding to GM1 micelles. The saturation transfer data demonstrate that Aβ(1–40) lies on the hydrophobic/hydrophilic interface of the ganglioside clusters, exhibiting an up-and-down topological mode in which the two α-helices (His14-Val24 and Ile31-Val36) and the C-terminal dipeptide are in contact with the hydrophobic interior (Fig. 8.9).

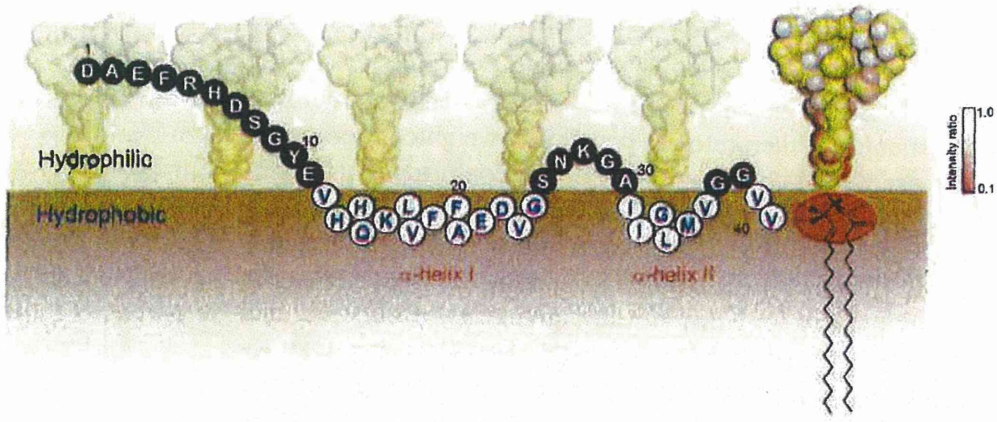

Fig. 8.9 A topological model of Aβ(1–40) bound to a ganglioside cluster, as deduced from NMR experiments; the regions of Aβ(1–40) buried inside the hydrophobic interior of lyso-GM1 micelles and those exposed to hydrophilic environments were identified using saturation transfer experiments. Moreover, the PRE effect was used to identify atomic groups of lyso-GM1 that are proximal to the spin-labeled Aβ peptide. The observed PRE effects are mapped on the 3D model of the carbohydrate moiety of lyso-GM1 with a color gradient from *red* to *white*. Adapted with modifications from (Utsumi et al. 2009; Yagi-Utsumi et al. 2010) with permission from Springer and Elsevier, respectively

The Aβ–glycolipid interaction was also characterized by PRE (Yagi-Utsumi et al. 2010). The Aβ(1–40) peptide with an extra C-terminal cysteine residue was recombinantly produced and conjugated through a disulfide bond with a spin-labeled probe. On addition of the spin-labeled Aβ peptide to the solution containing micellar lyso-GM1, the ^{1}H–^{13}C HSQC peaks originating from Glc and Gal^{II} and those originating from the head group of the lyso-GM1 lipid moiety exhibited significant attenuation of intensity due to PRE line broadening (Fig. 8.9). These results indicate that the sugar–lipid interface was primarily perturbed upon interactions of Aβ with the micelles.

Ganglioside micelle assemblies vary in size and curvature, depending on the size of the carbohydrate moiety. Such morphological variability can be a determining factor for ganglioside–protein interactions. Hence, for structural characterization of biomolecular interactions of glycolipid clusters, it is crucial to design appropriate membrane models that are suitable for sophisticated high-resolution spectral measurements. Small bicelles, in which a series of gangliosides were successfully embedded, have been developed as mimics of ganglioside-containing membranes for detailed NMR studies (Yamaguchi et al. 2013b). Using these standardized bicelles, chemical shift perturbation and relaxation data clearly indicated the ganglioside-specific involvement of N-terminal regions of α-synuclein in membrane interactions (Yamaguchi et al. 2013b).

8.9 Liaisons Between NMR and Computation

As mentioned above, NMR data for flexible oligosaccharides, including chemical shifts, *J*, NOE, and PCS, should be interpreted as population-weighted averages of dynamic conformational ensembles rather than as one or two conformational states. Therefore, quantitative interpretations of NMR data are supported by theoretical calculations such as molecular dynamics (MD) simulations (Fadda and Woods 2010; Woods and Tessier 2010). Although motional properties of systems can be obtained from suitable conditions under Newton's law, an inherent problem of this approach is its heavy dependence on simulation protocols, including initial structures, computational times, and force fields. It is therefore important to validate simulations by comparing with experimental observations. For example, the PCS-assisted NMR method has been successfully used to validate oligosaccharide conformational spaces sampled by MD simulations (Yamamoto et al. 2012; Zhang et al. 2012). Replica exchange MD simulations (REMD) can enhance sampling using a parallel tempering technique (Sugita and Okamoto 1999). This method overcomes the multiple-minima problem by exchanging noninteracting replicas of the system at several temperatures. REMD simulations were recently applied to biantennary *N*-glycan and were consistent with both experimental NMR data (Re et al. 2011; Nishima et al. 2012) and with the collisional cross sections determined using ion mobility spectrometry (Yamaguchi et al. 2012). Thus, the combination of NMR spectroscopy and theoretical approaches promises atomic descriptions of dynamic conformations and interactions of glycoconjugates of neurophysiological and neuropathological interest.

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