



HAL
open science

Mixing water, sugar, and lipid: Conformations of isolated and micro-hydrated glycolipids in the gas phase

Ander Ander Camiruaga, Alexandra Gouasmat, Jean-Marie Beau, Yann Bourdreux, Maélie Causse, Nathan Chapelle, Gilles Doisneau, Gildas Goldsztejn, Dominique Urban, Pierre Çarçabal

► To cite this version:

Ander Ander Camiruaga, Alexandra Gouasmat, Jean-Marie Beau, Yann Bourdreux, Maélie Causse, et al.. Mixing water, sugar, and lipid: Conformations of isolated and micro-hydrated glycolipids in the gas phase. *The Journal of Chemical Physics*, 2024, 160 (21), 10.1063/5.0211435 . hal-04616644

HAL Id: hal-04616644

<https://hal.science/hal-04616644>

Submitted on 19 Jun 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Mixing water, sugar and lipid: conformations of isolated and micro- hydrated glycolipids in the gas phase.

Authors. Ander Camiruaga,^a Alexandra Gouasmat,^b Jean-Marie Beau,^b Yann Bourdreux,^b Maélie Causse,^a Nathan Chapelle,^a Gilles Doisneau,^b Gildas Goldsztejn,^a Dominique Urban,^b Pierre Çarçabal^{a,*}

Affiliations.

- a- Université Paris-Saclay, CNRS, Institut des Sciences Moléculaires d'Orsay, 91405, Orsay, France.
- b- Institut de Chimie Moléculaire et des Matériaux d'Orsay, Université Paris-Saclay, CNRS, Institut de Chimie Moléculaire et des Matériaux d'Orsay, UMR 8182, 91405, Orsay, France.

Abstract.

Both sugars and lipids are important biomolecular building blocks of exceptional conformational flexibility and adaptability to their environment. Glycolipids bring together these two molecular components in a same assembly and combine the complexity of their conformational landscapes. In the present study we have used selective double resonance vibrational spectroscopy, in combination with a computational approach, to explore the conformational preferences of two glycolipid models (3-O-acyl catechol and guaiacol α -D-glucopyranosides) either fully isolated in the gas phase or in controlled interaction with a single water molecule. We could identify the preferred conformation and structures of the isolated and micro-hydrated species and evidence the presence of a strong water pocket, which may influence the conformational flexibility of such systems, even in less controlled environments.

Introduction

The pioneering work of Simons *and co.* in the early 2000's has shown the potential of gas phase vibrational spectroscopy to determine the intrinsic conformational preferences of isolated monosaccharides, and the influence of a limited and controlled number of water molecules.¹⁻³ After the most naturally abundant species have been studied, the work has been extended to larger saccharides,⁴⁻⁷ and to modified systems such as monosaccharides based gelators.⁸ Gas phase microwave spectroscopy has also been applied to several monosaccharides, including the elusive furanose form of Ribose.⁹ More recently, Kirschbaum *et al.* have reported spectral fingerprints in the mid-IR range, which turned out to be highly diagnostic to distinguish glycan isomers for protonated glycolipids isolated in superfluid Helium nanodroplets.¹⁰

In the present work we investigate the gas phase conformational landscapes of two neutral glycolipid models, the 3-O-acyl catechol and guaiacol α -D-glucopyranosides (GLOH and GLOMe, respectively, see Scheme 1), either fully isolated, free of perturbations by any molecular environment, or in local

interaction with a single water molecule in singly hydrated complexes. We applied a well-established experimental and computational methodology where the molecules are laser evaporated and injected into a gaseous supersonic expansion for cooling and stabilization of non-covalent complexes. Their mass resolved, conformer selective, vibrational spectra are observed by double resonance IR-UV laser spectroscopy, and compared with a computational exploration of their conformational landscapes by molecular mechanics and quantum chemistry methods to relate the observed spectra to the structure and conformation of the molecules and complexes.¹¹

GLOH and GLOMe differ drastically from the monosaccharides studied so far by gas phase spectroscopy. In the common monosaccharides 3-OH of the pyranose form is often in the middle of a cooperative hydrogen bond (HB) networks¹ and adapts to the direction of the network, itself governed by the *cis* or *trans* preference of 2-OH or 4-OH (C(n-1)-Cn-On-Hn dihedral angle). Here, 3-OH substitution by a C₆ acyclic acyl chain induces two major perturbations: (i) a very strong acceptor carbonyl group, which is able to dictate the orientation of O2H and O4H groups, and (ii) the highly flexible aliphatic chain. Moreover, the aromatic chromophore was so far chosen either as a phenyl or a benzyl residue for their high efficiency to produce ions *via* the 1 color Resonant 2 Photon Ionization (1c-R2PI) process on which the IR-UV double resonance scheme relies, and for being as conformational benign as possible, avoiding any strong HB between the chromophore and the monosaccharide. Here, on the contrary, the aromatic ring is a catechol for GLOH and a guaiacol for GLOMe (Scheme 1). Besides the availability of the compounds with these aromatic substitutes have been chosen because of their ability to engage in HB with vicinal polar groups from the sugar moiety and must have a significant influence on the conformation of the molecules. The purpose of studying the effect guaiacol is to “turn off” the HB donor possibility of catechol, leaving it as an OH acceptor only.

These modifications from the natural unsubstituted monosaccharides enable testing the general rules for the role of water in the conformational preferences of monosaccharides¹² and probing untypical

conformations and the associated spectral signatures, expectedly different from those adopted by natural monosaccharides in the gas or micro-hydrated phase.



Scheme 1. The glycolipid models GLOH and GLOMe studied here. The notation and numbering of the groups of each molecules are presented.

Methods

Experimental setup

The experimental methodology employed in this work is based on mass resolved, conformer specific double resonance IR-UV spectroscopy of supersonically cooled laser desorbed non-volatile species, as already described elsewhere.¹³ Briefly, it consists of a time-of-flight mass spectrometer (TOF, Jordan) coupled to a pulsed supersonic expansion chamber. The powders of GLOH and GLOMe compounds are rubbed on the surface of a solid graphite bar to deposit a thin uniform layer of the molecules of interest. The graphite bar was placed inside the vacuum chamber next to the nozzle of a 500 μm pulsed supersonic valve (Jordan PSV), operated at 10 Hz with a backing pressure of 5 bars of Neon. The fundamental output of a Nd:YAG laser (Minilite Continuum, 500 $\mu\text{J}/\text{pulse}$) was focused on the surface to vaporize the sample. The molecules were then cooled down by the supersonic expansion by collisions with the buffer gas. To produce hydrated complexes, the carrier gas was seeded with room temperature vapour pressure of water (either H_2O or D_2O) upstream of the jet. Since the laser desorption sample is located in vacuum, it is exposed to the water isotopomers upon the collisions of the supersonic expansion, downstream the jet. This prevents H/D exchange with the desorbed molecules, thus avoiding isotopic scrambling of the analytes.¹³ The molecules and complexes under study were entrained towards the ionization region of a linear TOF mass spectrometer through a 3 mm

skimmer in the differentially pumped detection chamber, where they interacted with the spectroscopy UV and IR lasers. To perform 1c-R2PI and IR/UV double resonance experiments,¹¹ UV photons were produced by a frequency doubled tuneable UV laser (PDL3000, Lambda Physics, Coumarin 540A, 500 μ J/pulse, 265-280 nm) and IR radiation was obtained from the idler of a tuneable OPO/OPA system (LaserVision, 5 mJ/pulse, 2.5 – 4 μ m).

Computations

The overall strategy to explore computationally the conformational landscapes of these systems consisted first in generating an extensive number of conformers by running a global conformational search with a force field based Monte Carlo (MC) Molecular Mechanics (MM) algorithm. To avoid the exploration being biased by the choice of the MM force field (FF) we used several FFs: MMFFs, OPLS2005 and AMBER94 FFs.

Selected conformations resulting from these searches were then treated at the Density Functional Theory (DFT) quantum chemistry level B3LYP(D3)/cc-pVTZ to optimize their geometries, calculate their relative 0 K potential energies (ΔE) and 300 K free energies (ΔG), and compute their IR harmonic vibrational spectra. The calculated harmonic vibrational frequencies were multiplied by a scaling factor of 0.96 for all frequencies except in the case of the hydrated complexes a scaling factor of 0.98 was applied to frequencies of stretching modes of O-H groups involved as donor in an intermolecular H bond.¹⁴

The MM explorations were achieved using Macromodel from the Schrodinger suite,¹⁵ and quantum chemistry calculations were performed using the Gaussian 16 code.¹⁶

The analysis of the non-covalent interactions was performed based on the topological analysis of the electronic density function using the NCI approach, as implemented in the NCIPLOT program.^{17,18}

Results

Conformational landscapes global explorations

Given the high flexibility of the lipid chain of the molecules, the MCMM search generated tens of thousands of conformations that were neither practically tractable nor even possible to all calculate at the DFT level, a good proportion of them leading to duplicate conformations, once DFT optimized. Therefore, a different approach was designed in order to obtain a panorama of the conformational preferences of these systems. First, all the possible sugar conformations were explored by replacing the aliphatic chain of the lipid group by a methyl group in an unrestricted conformational search. This made the following quantum geometry optimizations substantially more efficient in terms of computational resources. Then, among the most stable conformations obtained by DFT studies for the methylated systems, the selected structures were reconstructed with the lipid and submitted to a second conformational search on the lipid group coordinates only, which led to about 150 conformations in total.

Not only the two steps approach (w/o then w/ lipid chain) for the CS simplified the exploration and made possible the DFT calculation by reducing considerably the computational cost, it also shed light into a conformational landscape that can be more easily apprehended. In Figure 1, choosing GLOH as an exemple, we represent its conformational panorama in a simple way, without aiming at providing accurate structural information. This allows us to extract general trends and the main governing rules for its conformation that can be summarized as follows:

- The lipid and the sugar conformations are decoupled: for each sugar conformation all lipid chain conformations can be stabilized. The relative energy of the conformation is governed by the interactions in the sugar and is “modulated” by the lipid conformation. The effect of lipid conformation on the calculated O-H stretches frequencies is non-significant. This validates *a posteriori* the choice to decompose the global search into two steps where the lipid chain is added after the monosaccharide conformations have been generated.

This is the author's peer reviewed, accepted manuscript. However, the online version of record will be different from this version once it has been copyedited and typeset.
PLEASE CITE THIS ARTICLE AS DOI: 10.1063/5.0211435

- There are 2 possible interactions in which the carbonyl group can be engaged as an H acceptor:
(i) OH2 \rightarrow O=C (Figure 1a), (ii) OH4 \rightarrow O=C (Figure 1b).
- For each of the above possibilities, the hydroxymethyl group can adopt 3 positions : (i) OH6 \rightarrow OH4 in a Tg+ conformation (Figure 1c orange), according to Talbot *et al.* notation¹⁹, OH6 \rightarrow O5 either in a (ii) G-g+ (Figure 1c green) or (iii) in a Tg+ (Figure 1c pink) conformation.
- Although the conformational possibilities of the lipid are virtually infinite (Figure 1d), clustering procedures on the first two torsional angles of the lipid reveal 3 cases: (i) the lipid adopts an extended all *trans* conformation (Figure 1e pink), or it adopts a folded conformation. In the folded conformations, a quasi-continuum of combinations of the torsional angles of the lipid makes the whole thing difficult to summarize but considering only the first two torsions of the lipid makes possible distinguishing two major families, where the lipid folds towards either (ii) OH4 (Figure 1e green) or (iii) OH2 (Figure 1e orange).

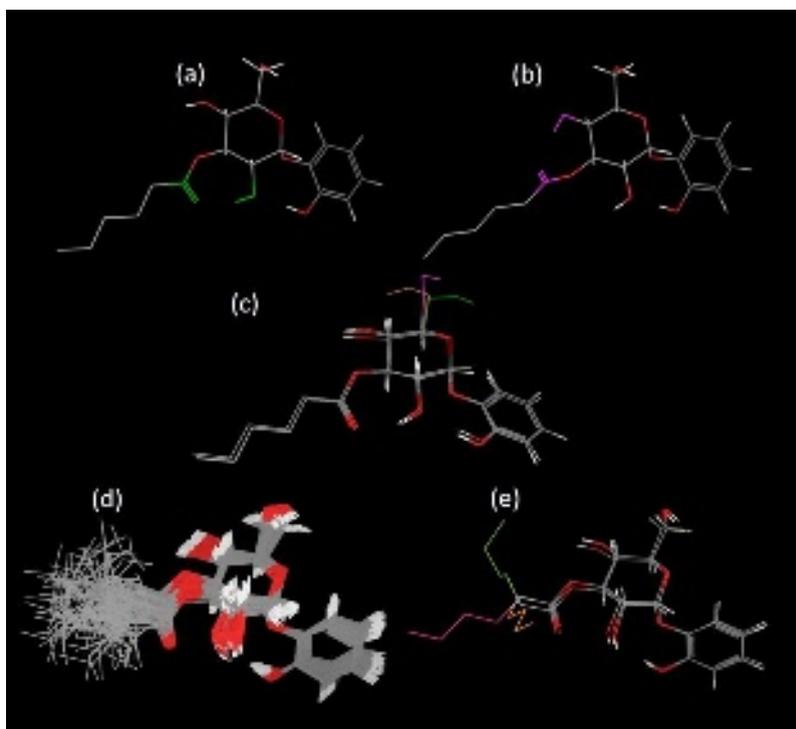


Figure 1. Conformations categories of GLOH, illustrating (a) and (b) the 2 main orientations of C=O, (a) the 3 orientations of OH6, and (d) the diversity of conformations of the lipid chain, which can be partitioned (e) into 3 major classes. For the sake of simplicity of the representation, the H atoms of the lipid chains and of the hydroxymethyl group in (c) are not shown.

The most stable conformations of GLOH are those with an $O_{cat}H \rightarrow O2H \rightarrow OC$ cooperative network. Different orientations for the hydroxymethyl group and the lipid chain appear for these conformations: the carbonyl group can point either towards $O2H$ or $O4H$, and the lipid moiety is found either folded or in a linear disposition, with all its dihedral angles in *trans* conformation. Each of these lipid conformations appear also with the different orientations of the $O6H$ moiety. For GLOMe, because of the methylation of catechol, the $O_{cat}H$ of GLOH is replaced by an OMe group. This leaves only one possibility with an H acceptor: the sugar's OH groups adapt to form $O2H \rightarrow O_{cat}$ and $O4H \rightarrow O=C$ pattern. The flexibility of the hydroxymethyl group and of the aliphatic remains the same in GLOMe as in GLOH.

The exploration of the conformational landscape of the hydrated complexes was performed using the same stepwise strategy as described for the monomers. The results of the calculations, for both molecules, show that the most stable water insertion site is between $O2H$ and carbonyl group of the sugar, forming new hydrogen bond networks. Besides, most stable structures of GLOMe+W present a tri-coordinated water molecule acting as double donor, forming a three-fold HB network between the $C=O$ group, O_{cat} and $O2H$ group. Like previously observed in the monomers, other hydroxymethyl group arrangements are possible within these conformations.

Representative samples of the explored conformational landscapes are shown in ESI, for the isolated molecules (Figure S1) and their hydrated complexes (Figure S2).

Isolated GLOH and GLOMe

The 1c-R2PI spectra of all species studied here are presented in ESI (Figure S3). They all feature rather broad, partly resolved, structures and in spite of their apparent spectral congestion we always observed a single vibrational spectrum when probing different peaks of the UV spectra by double resonance IR-UV spectroscopy. The IRID spectra shown in Figure 2 have been observed by probing the 1c-REMPI peaks at 36153 cm^{-1} for GLOH and 36509 cm^{-1} , as shown in Figure S3. The other peaks that have been probed are indicated in Figure S3.

This is the author's peer reviewed, accepted manuscript. However, the online version of record will be different from this version once it has been copyedited and typeset.
PLEASE CITE THIS ARTICLE AS DOI: 10.1063/5.0211435

The experimental InfraRed Ion Dip (IRID) spectra of GLOH and GLOMe are compared to the calculated spectra for the most stable structures for each system. In both cases, one can identify two regions: the O-H stretching bands above 3600 cm^{-1} correspond to the O-H stretches of the sugar involved in relatively weak interactions. At lower energies, more intense and broader bands appear for both monomers corresponding to stretching bands of O-H groups involved as H donor in stronger interactions. Comparison between the experimental IRID spectra of both glycolipid models suggests structural differences. The broad band in GLOH centered at 3430 cm^{-1} is much lower in frequency in comparison with the bound O-H bands of GLOMe, rising between 3500 and 3600 cm^{-1} . This GLOH band is found also at much lower frequency than usually with unsubstituted isolated monosaccharides conformers in gas phase,^{2,20,21} indicating the presence of a significantly stronger intramolecular HB.

This is the author's peer reviewed, accepted manuscript. However, the online version of record will be different from this version once it has been copyedited and typeset.
PLEASE CITE THIS ARTICLE AS DOI: 10.1063/5.0211435

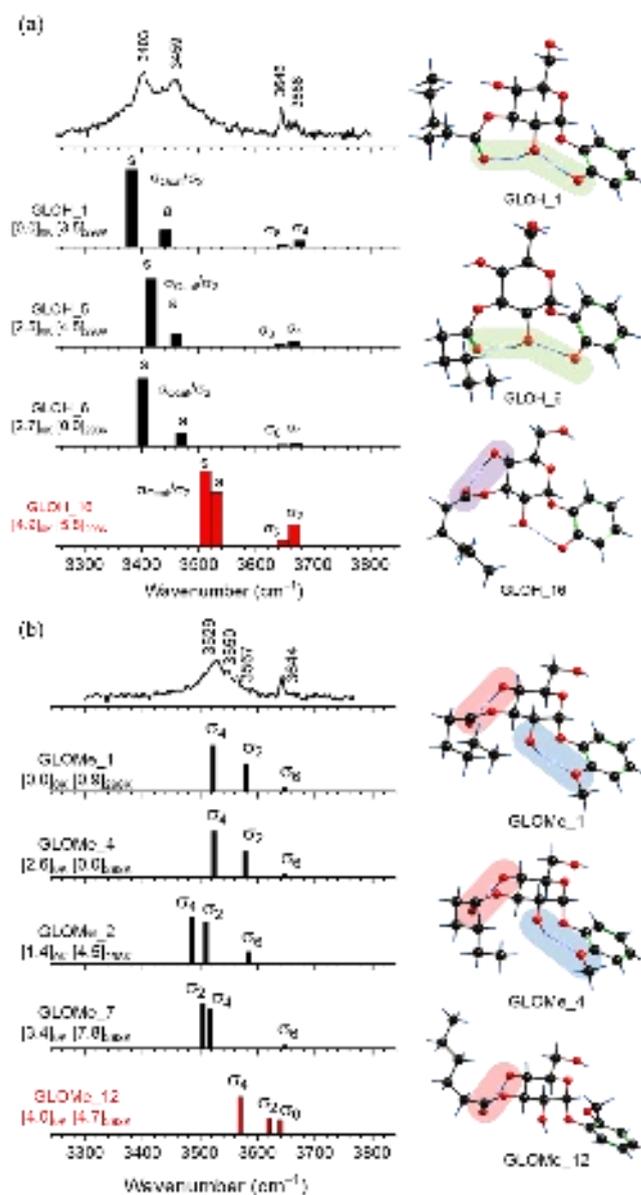


Figure 2. Comparison between experimental IRID spectra and calculated spectra of the most stable conformers of the isolated molecules: (a) GLOH and (b) GLOMe. σ_n refers to the stretching mode of the O_nH group, according to the numbering defined in Scheme 1. The symmetric and anti-symmetric stretching modes of the groups involved in the intermolecular HB are labelled as “s” and “a”, respectively. The numbers in brackets provide the relative energies of each conformer, in kJ/mol. The first one is the 0 K free energy taking into account the Zero Point Energy correction, and the second one is the 300 K free Gibbs energy (ΔG). Significant and representative conformations are shown (more conformations are shown in Figure S1).

The predicted spectrum of the most stable calculated conformation (GLOH_1) reproduces very well the experimental bands off the spectrum of GLOH. The next two conformers (GLOH_5 and GLOH_23 – see Figure S1) differ only by the orientation of the hydroxymethyl group. Since these three conformations contain the same governing interaction chain $O_{cat}H \rightarrow O_2H \rightarrow O=C$ (highlighted in green in Figure 2), their calculated spectra are similar and match satisfactorily the experimental one too.

There is a clear difference with the calculated spectra of structures characterized by the O4H→O=C interaction (in violet in Figure 2), which fail at reproducing the position of the bands in the experimental spectrum. Thus, we conclude that these conformations are not populated in our experimental conditions. The assignment of the GLOH structure further confirms the nature of the observed band around 3450 cm⁻¹. The O2H group *trans* configuration (C1-C2-O2H dihedral angle) allows O_{cat}H to interact with it, and also to form the O2H→O=C bond, resulting in a strong cooperative chain of intramolecular bonds. This leads to the symmetric and antisymmetric stretching modes of the coupled stretches of O2H/O_{cat}H to be observed at frequencies 3403 cm⁻¹ and 3459 cm⁻¹, in a range more often associated to hydrated complexes than isolated monosaccharides.^{1,2} This illustrates how strong the attraction of the carbonyl group and the cooperative effect induced by the HB network are.

Regarding GLOMe, the calculated spectra of the two most stable structures reproduce well the experimental IRID spectrum. Although GLOMe contains only three OH groups, we observed four peaks in the experimental spectrum. This suggests that more than one conformer constitutes the IRID spectrum. Given the small energy difference between GLOMe_1 and GLOMe_4, both must be populated. Hence the experimental spectrum is the superposition of the spectra of these two conformers. Both conformations have O4H→OC/O2H→O_{cat} double HB, and they differ only by the aliphatic chain conformation. Their calculated frequencies are extremely close which would explain why only one supplementary spectral feature in the 3550 cm⁻¹-3570 cm⁻¹ range is observed. Probing other peaks of the 1c-REMPI spectrum of GLOMe (see Figure S3) did not allow separating better both conformers.

As revealed by the experimental assignment, the methylation of the catechol hydroxyl group in GLOMe disrupts the strong cooperative HB network observed in GLOH. In the observed structures of GLOMe, O2H no longer interacts as H donor with the carbonyl group but with with O_{cat}, letting the carbonyl group to point towards O4H as an HB acceptor. Therefore, the cooperative effect is banished in GLOMe, leading to weaker electrostatic interactions. This is why the most red-shifted band in the IR

spectrum of GLOMe is above 3500 cm^{-1} , while the bonded stretching modes of GLOH lie below 3500 cm^{-1} .

Monohydrated GLOH and GLOMe

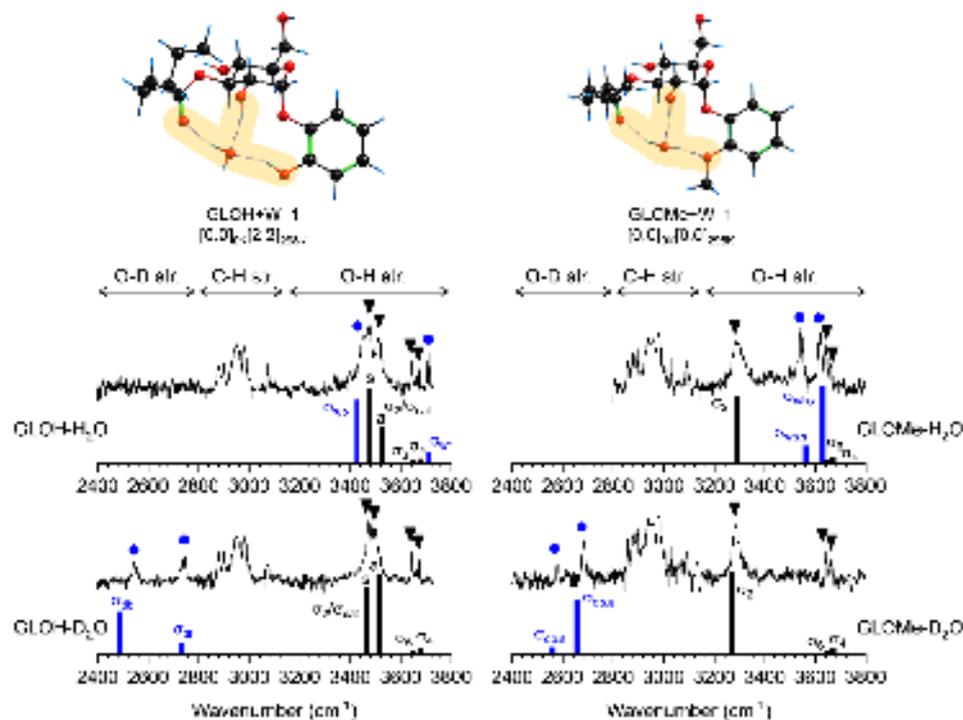


Figure 3. Gas phase IRID spectra of the glycolipid models complexed with (top) H_2O , and (bottom) with D_2O . The down arrows indicate the O-H stretching bands of (left) GLOH and (right) GLOMe, and the blue circles indicate the O-H and O-D stretching bands of H_2O and D_2O , respectively. The calculated spectrum of the most stable structure (shown above the spectra) of each system is compared to the experimental IRID. The blue sticks represent the water stretching bands (O-H and O-D) according to the normal mode analysis of the calculation.

Figure 3 compares the IRID spectra of the isolated molecules and their singly hydrated complexes with H_2O (X_W) and D_2O (X_HW). $\text{H}_2\text{O}/\text{D}_2\text{O}$ substitution reduces spectral congestion in the O-H stretching modes, and more importantly, it allows guiding conformational assignment by comparison with the quantum chemistry frequency calculation. We can easily discriminate between the O-H stretching bands of the glycolipid models that remain unchanged upon isotopic substitution and those belonging to the water molecule that disappear from the O-H stretching range above 3200 cm^{-1} to appear in the O-D stretching range below 2800 cm^{-1} . Isotopic substitution effects in hydrate clusters can also be analyzed in more detail to reveal subtle manifestations of intramolecular and intermolecular vibrational couplings but this is beyond the scope of the present study.^{13,22,23} Only conformers with

calculated water bands in agreement with those determined experimentally by isotopic substitution can be considered for conformational and structural assignment. The spectrum of GLOH_W (resp. GLOH_HW) exhibits a water band at 3713 cm^{-1} (resp. 2743 cm^{-1}), typical of a free O-H (resp. O-D) band of an inserted water molecule which is engaged in a donor-acceptor hydration site, with its donor O-H band observed at about 3450 cm^{-1} (resp. 2545 cm^{-1}).²⁰ Such typical spectral signature is not observed for GLOMe_W (resp. GLOMe_HW) where the water bands are observed at 3543 cm^{-1} and 3619 cm^{-1} (resp. 2577 cm^{-1} and 2680 cm^{-1}), indicating a bridging water molecule acting as a double donor, thus without free hydroxyl group. Still in the GLOMe IRID spectrum, the strong and broad band around 3300 cm^{-1} , assigned to one of the GLOMe O-H stretching band because it is insensitive to isotopic substitution is observed at a significantly much lower frequency than those for singly hydrated complexes of monosaccharides (3300 cm^{-1} vs. typically $3450\text{-}3500\text{ cm}^{-1}$),^{1,24} which suggests a very strong attraction exerted by the water as an acceptor to the donor O-H of GLOMe.

For both GLOH+W and GLOMe+W, there is a very good match between the calculated and observed spectra (Figure 3). The calculated positions of the water bands agree perfectly with the bands identified as originating from water stretching modes, according to the isotopic substitution experiments.

Although the conformations of these two hydrated complexes are quite different, their intermolecular HB networks show some important similarity. In both systems, the water is located in the same site creating three HB with its partner. In GLOH+W, it acts as double acceptor with OcatH and O2H, and as a single donor with C=O. Consequently, it keeps a free OH, in agreement with the observation of the typical 3713 cm^{-1} observed band. In GLOMe+W, the water bridges as a double donor between OMe and O=C and as a single acceptor with O2H. The arrangement of the carbonyl, the catechol OH in GLOH or OMe in GLOMe, and O2H, creates a hydration pocket where a single water molecule can perfectly fit to stabilize the orientation of the lipid chain by a very strong triple HB network. In GLOMe+W, the water molecule is strongly polarized by the double donor HB. This increases significantly the electronegativity of the oxygen atom, which is consistent with the low frequency of the donor O2H observed at 3300 cm^{-1} , already mentioned above.

Figure S4 and S5 compare the observed and calculated spectra of the hydrated complexes of GLOH and GLOMe, respectively. It can be seen that several conformational variations where the water sits in the hydration pocket that we have identified can reproduce the experimental spectra, while those with different hydration sites do not (in red in Figures S4 and S5).

Discussion

In comparison to the conformational preferences of the monosaccharides and their singly hydrated complexes studied so far, both the esterification in the O3 position and the catechol's hydroxyl group (or OMe group in GLOMe) affect significantly the intramolecular and intermolecular interaction regimes of the species studied here.

The structures assigned for GLOH present a strong intramolecular hydrogen bond cooperative chain that starts in the $O_{\text{cat}}\text{H}$ group, and ends in the $\text{C}=\text{O}$ group of the lipid. This interaction is by far the most stable arrangement in the isolated sugar. Moreover, we clearly evidenced the interaction site of the $\text{C}=\text{O}$ group in GLOH, with a strong cooperative effect experimentally reflected by the strong red shift of the bounded OH stretch. Methylation of catechol's hydroxyl group changed the conformational panorama. Owing to the absence of $O_{\text{cat}}\text{H}$, the HB network seen in GLOH is impossible. This results in a re-arrangement of the sugar OH groups: the carbonyl group changes its position to interact with O4H, while O2H group forms a new hydrogen bond with the lone pairs of O_{cat} . These two individual intramolecular HB of GLOMe do not benefit from the cooperative enhancement of the $O_{\text{cat}}\text{H} \rightarrow \text{O2H} \rightarrow \text{O}=\text{C}$ chain and the interactions in GLOMe are weaker, as indicated by the higher energy of the corresponding vibrational bands.

Single hydration of both glycolipid models changes significantly their conformational panorama. The observed structures resulted to be quite atypical in comparison with previous sugar-water complexes studied in the gas-phase.^{20,21,24} In non-substituted monosaccharides, hydration sites are located at the weakest points of the intramolecular hydrogen bond network of the monomer, which is often between the O4H and O6H groups of the sugar.^{12,20} Structures with such interactions are calculated several

kJ/mol higher than the most stable conformation for GLOH+W and GLOMe+W (see Figure S4 and S5), respectively, and the agreement between their predicted spectra and the experimental one is poor. In the present case, the C=O group is such a strong H acceptor that the water is strongly attracted into this binding pocket, disrupting any previous bonds inside the sugar. This behaviour is comparable to the favoured hydration site observed for GlcNac systems.²⁵ In both glycolipid models studied here, the driving parameter must be the strong electronegativity of the carbonyl group of the lipid moiety, polarized by the vicinal hydroxyl groups.

The mapping of the NCIs in the most stable conformations for GLOH+W and GLOMe+W are shown in Figure 4. In both cases, multiple interactions can be observed around the water pocket and between the lipid chain and the O2H group (green-yellow colored surface), along with stronger HBs formed between the hydroxyl groups of the sugar and water molecules (blue colored surfaces). In GLOMe, $O_{\text{cat}}\text{H} \rightarrow O_{\text{w}}$ bond is replaced by an $O_{\text{w}}\text{H} \rightarrow \text{OMe}$ interaction. As a consequence, the water molecule becomes a double donor. This polarizes the electronic density around O_{w} which becomes more electronegative, and more receptive for HBs as an acceptor. This effect was clearly observed by the experiment, as the stretching band for the O2-H group, involved in the $O2\text{H} \rightarrow O_{\text{w}}$ interaction appears 160 cm^{-1} lower in frequency in GLOMe than in GLOH. This observation was also captured by theory, in the change in the frequency predictions, and also in the values of the reduced density gradient for the $O2\text{H}\cdots O_{\text{w}}$ interaction which is much stronger for GLOMe+W_1 (in green in Figure 4) than for GLOH+W_1.

This is the author's peer reviewed, accepted manuscript. However, the online version of record will be different from this version once it has been copyedited and typeset.
PLEASE CITE THIS ARTICLE AS DOI: 10.1063/5.0211435

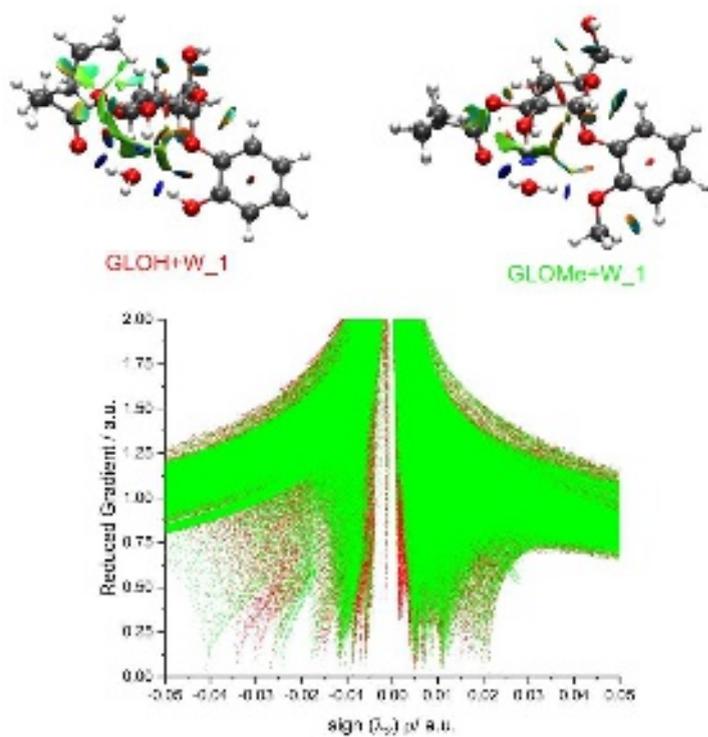


Figure 4. NCI plots of the assigned structures of GLOH+W_1 (in red) and GLOMe+W_1 (in green).

Conclusions

We present an exploration of the conformational preferences of glycolipid models both fully in the gas phase and in interaction with a single water molecule, using a combination of mass-resolved conformer selective laser spectroscopy experiments and computational conformational landscapes explorations.

The analysis of the assigned structures for the un-hydrated models revealed the big influence of the presence of a carbonyl group perturbing the typical intramolecular hydrogen bond network of monosaccharide hydroxyl groups. $O_{\text{cat}}\text{H} \rightarrow \text{O}_2\text{H} \rightarrow \text{O}=\text{C}$ hydrogen bond network is formed in GLOH, and it is disrupted in GLOMe due to the methylation of the catechol hydroxyl group.

The analysis of different lipid folding and the comparison with the predicted spectra revealed that the lipid chain and the sugar conformations do not influence each other, all chain conformations being accessible for all hydroxyl orientations. Anyhow, the O-H stretching band

vibrationally resolved spectra do not encode information on the lipid chain conformation and we have to rely on the computational global exploration, which indicates that various foldings of the lipid chain can be populated, and interconvert easily.

Upon controlled hydration with a single water molecule, both GLOH and GLOMe presented unexpected intermolecular interaction preferences, mainly located around the carbonyl group and the substituted aromatic ring. For both glycolipid models, we observed a similar strong binding site for the water molecule, which is very different from those reported for non-substituted sugar-water complexes.

All the above revealed a new glyco-conjugates conformational panorama where the insertion of voluminous groups not only alters the intramolecular arrangement of the saccharide, but also modifies the intermolecular interaction preferences. Here, we identify a clear hydration site locked by the position of the carbonyl group where the water is rigidly attached by triple HB networks. The associated spectral signatures to this hydration site show that the intermolecular forces at play are strong. Such “water pockets”, clearly identified by double resonance spectroscopy of size controlled hydrated complexes, may survive at higher temperature and in solution, with a limited number of architectural water molecules trapped in the pocket and removed from the macroscopic solvent to play a specific structural and energetic role.

Supplementary Material

Supplementary online material is available and includes:

Figure S1 and S2: selections of representative structures and conformation of the isolated and singly hydrated GLOH and GLOMe.

Figure S3: 1c-R2PI electronic spectra of GLOH, GLOH+W, GLOMe and GLOMe+W.

Figures S4 and S5: comparison between experimental and computational spectra of various structures of GLOH+W and GLOMe+W.

Acknowledgements

A.C. fellowship was supported by Investissements d'Avenir du LabEx PALM (ANR-10-LABX-0039-PALM). This work was performed using computational resources from the "Mésocentre" computing center of Université Paris-Saclay, CentraleSupélec and École Normale Supérieure Paris-Saclay supported by CNRS and Région Île-de-France (<https://mesocentre.universite-paris-saclay.fr/>).

References

- ¹ P. Çarçabal, R.A. Jockusch, I. Hunig, L.C. Snoek, R.T. Kroemer, B.G. Davis, D.P. Gamblin, I. Compagnon, J. Oomens, and J.P. Simons, "Hydrogen bonding and co-operativity in isolated and hydrated sugars: mannose, galactose, glucose and lactose.," *J. Am. Chem. Soc.* **127**(32), 11414–11425 (2005).
- ² I. Hunig, A. Painter, R.A. Jockusch, P. Çarçabal, E.M. Marzluff, L.C. Snoek, D.P. Gamblin, B.G. Davis, and J.P. Simons, "Adding water to sugar: A spectroscopic and computational study of - and - phenylxyloside in the gas phase," *Phys. Chem. Chem. Phys.* **12**, 2474–2480 (2005).
- ³ J.P. Simons, M.T. Bowers, P. Hobza, R. Weinkauff, and M.N.R. Ashfold, editors, in "*Bio-Active Mol. Gas Phase*" *Phys. Chem. Chem. Phys.* (2004), pp. 2543–2890.
- ⁴ R.A. Jockusch, R.T. Kroemer, F.O. Talbot, L.C. Snoek, P. Çarçabal, J.P. Simons, M. Havenith, J.M. Bakker, I. Compagnon, G. Meijer, and G. von Helden, "Probing the glycosidic linkage: UV and IR ion-dip spectroscopy of a Lactoside," *J. Am. Chem. Soc.* **126**, 5709–5714 (2004).
- ⁵ M. Pincu, E.J. Cocinero, N. Mayorkas, B. Brauer, B.G. Davis, R.B. Gerber, and J.P. Simons, "Isotopic Hydration of Cellobiose: Vibrational Spectroscopy and Dynamical Simulations," *J. Phys. Chem. A* **115**(34), 9498–9509 (2011).
- ⁶ P. Çarçabal, I. Hunig, D.P. Gamblin, B. Liu, R.A. Jockusch, R.T. Kroemer, L.C. Snoek, A.J. Fairbanks, B.G. Davis, and J.P. Simons, "Building up key segments of N-glycans in the gas phase: Intrinsic structural preferences of the alpha(1,3) and alpha(1,6) dimannosides," *J. Am. Chem. Soc.* **128**(6), 1976–1981 (2006).
- ⁷ C.S. Barry, E.J. Cocinero, P. Çarçabal, D.P. Gamblin, E.C. Stanca-Kaposta, S.M. Remmert, M.C. Fernández-Alonso, S. Rudić, J.P. Simons, and B.G. Davis, "'Naked' and hydrated conformers of the conserved core pentasaccharide of N-linked glycoproteins and its building blocks," *J. Am. Chem. Soc.* **135**(45), 16895–16903 (2013).
- ⁸ R. Lozada-Garcia, D. Mu, M. Plazanet, and P. Çarçabal, "Molecular gels in the gas phase? Gelator–gelator and gelator–solvent interactions probed by vibrational spectroscopy," *Phys. Chem. Chem. Phys.* **18**(32), 22100–22107 (2016).
- ⁹ P. Écija, I. Uriarte, L. Spada, B.G. Davis, W. Caminati, F.J. Basterretxea, A. Lesarri, and E.J. Cocinero, "Furanosic forms of sugars: conformational equilibrium of methyl β -d-ribofuranoside," *Chem.*

Commun. **52**(37), 6241–6244 (2016).

¹⁰ C. Kirschbaum, K. Greis, E. Mucha, L. Kain, S. Deng, A. Zappe, S. Gewinner, W. Schöllkopf, G. von Helden, G. Meijer, P.B. Savage, M. Marianski, L. Teyton, and K. Pagel, “Unravelling the structural complexity of glycolipids with cryogenic infrared spectroscopy,” *Nat. Commun.* **12**(1), 1201 (2021).

¹¹ A.M. Rijs, and J. Oomens, in *Gas-Phase IR Spectrosc. Struct. Biol. Mol.*, edited by A.M. Rijs and J. Oomens (Springer, Cham, 2014).

¹² E.J. Cocinero, and P. Çarçabal, “Carbohydrates,” *Top. Curr. Chem.* **364**, 299–333 (2015).

¹³ A. Camiruaga, G. Goldsztejn, and P. Çarçabal, “Isotopic dependence of intramolecular and intermolecular vibrational couplings in cooperative hydrogen bond networks: singly hydrated α -O-Phenyl-Mannopyranoside as a case study,” *Phys. Chem. Chem. Phys.*, (2023).

¹⁴ A. Camiruaga, A. Piquot-Lescop, G. Goldsztejn, and P. Çarçabal, “Isotopic Dependence of Intramolecular and Intermolecular Vibrational Couplings in Cooperative Hydrogen Bond Networks: Singly Hydrated α -O-Phenyl-Mannopyranoside as a Case Study,” *Soumis à PCCP* 2002, (2022).

¹⁵ F. Mohamadi, N.G.J. Richards, W.C. Guida, R. Liskamp, M. Lipton, C. Caufield, G. Chang, T. Hendrickson, and W.C. Still, “Macromodel - an Integrated Software System for Modeling Organic and Bioorganic Molecules Using Molecular Mechanics,” *J. Comput. Chem.* **11**(4), 440–467 (1990).

¹⁶ M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M. a. Robb, J.R. Cheeseman, G. Scalmani, V. Barone, G. a. Petersson, H. Nakatsuji, X. Li, M. Caricato, a. V. Marenich, J. Bloino, B.G. Janesko, R. Gomperts, B. Mennucci, H.P. Hratchian, J. V. Ortiz, a. F. Izmaylov, J.L. Sonnenberg, Williams, F. Ding, F. Lipparini, F. Egidi, J. Goings, B. Peng, A. Petrone, T. Henderson, D. Ranasinghe, V.G. Zakrzewski, J. Gao, N. Rega, G. Zheng, W. Liang, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, K. Throssell, J. a. Montgomery Jr., J.E. Peralta, F. Ogliaro, M.J. Bearpark, J.J. Heyd, E.N. Brothers, K.N. Kudin, V.N. Staroverov, T. a. Keith, R. Kobayashi, J. Normand, K. Raghavachari, a. P. Rendell, J.C. Burant, S.S. Iyengar, J. Tomasi, M. Cossi, J.M. Millam, M. Klene, C. Adamo, R. Cammi, J.W. Ochterski, R.L. Martin, K. Morokuma, O. Farkas, J.B. Foresman, and D.J. Fox, “G16_C01,” *Gaussian 16, Revision A.03*, Gaussian, Inc., Wallin (2016).

¹⁷ E.R. Johnson, S. Keinan, P. Mori-Sánchez, J. Contreras-García, A.J. Cohen, and W. Yang, “Revealing Noncovalent Interactions,” *J. Am. Chem. Soc.* **132**(18), 6498–6506 (2010).

¹⁸ J. Contreras-García, E.R. Johnson, S. Keinan, R. Chaudret, J.-P. Piquemal, D.N. Beratan, and W. Yang, “NCIPLLOT: A Program for Plotting Noncovalent Interaction Regions,” *J. Chem. Theory Comput.* **7**(3), 625–632 (2011).

¹⁹ F.O. Talbot, and J.P. Simons, “Sugars in the gas phase: the spectroscopy and structure of jet-cooled phenyl beta-D-glucopyranoside,” *Phys. Chem. Chem. Phys.* **4**(15), 3562–3565 (2002).

²⁰ P. Çarçabal, R.A. Jockusch, I. Hunig, L.C. Snoek, R.T. Kroemer, B.G. Davis, D.P. Gamblin, I. Compagnon, J. Oomens, and J.P. Simons, “Hydrogen bonding and cooperativity in isolated and hydrated sugars: mannose, galactose, glucose, and lactose,” *J. Am. Chem. Soc.* **127**(32), 11414–11425 (2005).

²¹ P. Çarçabal, T. Patsias, I. Hunig, B. Liu, C. Kaposta, L.C. Snoek, D.P. Gamblin, B.G. Davis, and J.P. Simons, “Spectral signatures and structural motifs in isolated and hydrated monosaccharides: phenyl alpha- and beta-L-fucopyranoside,” *Phys. Chem. Chem. Phys.* **8**(1), 129–136 (2006).

²² R.W. Larsen, P. Zielke, and M.A. Suhm, “Hydrogen-bonded OH stretching modes of methanol clusters: A combined IR and Raman isotopomer study,” *J. Chem. Phys.* **126**(19), 194307 (2007).

²³ T.L. Fischer, T. Wagner, H.C. Gottschalk, A. Nejad, and M.A. Suhm, “A Rather Universal Vibrational Resonance in 1:1 Hydrates of Carbonyl Compounds,” *J. Phys. Chem. Lett.* **12**(1), 138–144 (2021).

²⁴ I. Hunig, A.J. Painter, R.A. Jockusch, P. Çarçabal, E.M. Marzluff, L.C. Snoek, D.P. Gamblin, B.G. Davis, and J.P. Simons, "Adding water to sugar: A spectroscopic and computational study of alpha- and beta-phenylxyloside in the gas phase," *Phys. Chem. Chem. Phys.* **7**(12), 2474–2480 (2005).

²⁵ E.J. Cocinero, E.C. Stanca-Kaposta, M. Dethlefsen, B. Liu, D.P. Gamblin, B.G. Davis, and J.P. Simons, "Hydration of sugars in the gas phase: regioselectivity and conformational choice in N-acetyl glucosamine and glucose.," *Chemistry* **15**(48), 13427–13434 (2009).

Authors' Contribution statement

Ander Camiruaga : investigation, data analysis and interpretation, original draft preparation, editing.

Alexandra Gouasmat : Synthesis (production)

Jean-Marie Beau, Yann Bourdreux, Gilles Doisneau, Dominique Urban : Synthesis (supervision and validation), editing.

Maélie Causse, Nathan Chapelle : initial conformational searches and clustering.

Gildas Goldsztejn : editing.

Pierre Çarçabal : Supervision, funding acquisition, validation, methodology, investigation, original draft preparation, editing.