^{1}H NMR Elucidation of Observed Stable Sugar-NaCl-water Complexes in Aqueous Solution

Gan Zhu[♀ a b], Hui Li[♀a], Yiqun Li[b]* and Liuqun Gu[a]*

[a] Department of Biomedical Engineering, Jinan University; 601, West Huangpu Avenue, Guangzhou, China; E-mail: guliuqun@yahoo.com

[b] Department of Chemistry, Jinan University; 601, West Huangpu Avenue, Guangzhou, China; E-mail: tlyq@jnu.edu.cn

[♀]These authors contributed equally.

KEYWORDS. ^{1}H NMR elucidation; mono/disaccharides; stable sugar-NaCl-water complex; NaCl effect; aqueous solution.

Abstract:
The solvation of sugars in aqueous media matters in the understanding of biological systems and carbohydrate transformations. Generally, 2 – 4 water units were proposed to interact with each hydroxyl group in monosaccharides via different types of hydrogen bondings at room temperature in previous studies. Presence of NaCl is known to perturb hydrogen bondings of sugar hydrates. However, direct evidence to elucidate mechanism at atom level is very rare even though the "NaCl Effect" was well known in biomass chemical transformations. Here we report ^{1}H NMR elucidation evidences of mono/disaccharides hydrates in different concentrations of NaCl aqueous solutions. We here conclude two new findings: 1) under ideal usage of NaCl, different mono/disaccharides hydrates are likely to be converted into a stable sugar-NaCl-water form via a stepwise mechanism; 2) reactivity order (acidity difference) of different hydroxyls in mono/disaccharides has intangible influence on hydrate form change induced by NaCl. An ideal NaCl usage based on maximum of ^{1}H NMR shift was proposed.

1.0 Introduction

Monosaccharides as basic units of glycogen, starch and cellulose, play very important roles in biological systems and water is the only media[1]; hence solvation of monosaccharides by water was studied for decades for better understanding of molecule mechanism. However, due to lack of direct experimental evidence, exact interaction between monosaccharides like D-glucose and water in solution is not yet fully understood, particularly regarding on how each hydroxyl group is locally hydrated. In 1976, J. M. Harvey *et.al* reported that each hydroxyl group of D-glucose forms at least two hydrogen bonds with two water molecules, and four solvated forms with different hydrogen bindings were proposed (**Figure 1**)[2,3], via direct measuring hydroxyl proton at low temperature. T. Suzuki's simulation work also resulted into a similar conclusion in 2008.[4]

Figure 1. D-Glucose and its four different solvated forms in water.

D-glucose (α,β) Vicinal form **A1** Vicinal form **A2**
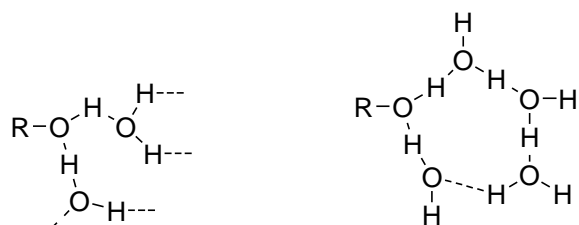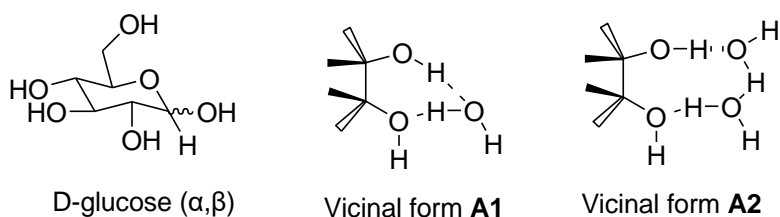
Solvated form **B1** Solvated form **B2**

The presence of sodium chloride was proved to perturb the hydrogen bonding network of
water[5], resulting into sodium-saccharide interaction in water[6]. Early studies via paper
electrophoresis and optical rotation by S. J. Angyal[7] showed that as a univalent cation sodium
cation is highly hydrated in solution and a single hydroxyl group on monosaccharides cannot
compete with water molecular in coordination; only two or three hydroxyl groups in a suitable
arranged combination may result in sodium-monosaccharide complex in a very weak manner.
A Raman-spectra study by F. Franks[8] *et.al* also indicated that sodium cation-glucose
perturbation rather than sodium-glucose complex formation caused observed spectrum
changes; and cations effect study also confirmed that the interaction induced by sodium
cation is much weaker than other multivalent cations like Ca^{2+}. Recently, NaCl usage in
aqueous solution as an additive[9-12] or a promoter[13-15] for biomass transformations into valuable
chemicals or biofuels is increasingly popular because they are abundant in nature and are
very cheap; and "NaCl Effect" (or "salt effect") became well known in biomass conversion and
carbohydrate chemistry in the recent decade. Further system study of the role of metal halide
in enhancing the dehydration of xylose to furfural by K. R. Enslow *et.al*[16] and insights from
quantum mechanics/molecule dynamics on sodium ion interaction by H. B. Mayes *et.al*[17] both
confirmed the previous hypothesis, which states that the cation has a stronger effect than the
anion on glucose with the anions acting to stabilize critical intermediates. However, very
recent higher yield productions of chemicals from monosaccharides in aqueous solution
promoted by NaCl[18-20] challenged the minor role hypothesis of chloride anion in a NaCl
promoted system. The nature of complex-formation between sodium and monosaccharides
is not yet well understood, particularly at atom level. No direct experimental evidence
mapping bonding interaction between monosaccharides like glucose and NaCl in water has
been reported to our best of knowledge, probably because of the instability nature of
complex in solution and NMR spectrum was different depending on many factors[5,6,16]. There
are two important concerns yet to be answered: 1) would the presence of NaCl change
reactivity order (acidity difference) of all hydroxyls in mono/disaccharides via perturbing
hydrogen bonding networks in aqueous solution? 2) could maximum promotion (or activation)
usage of NaCl be predictable? To answer these two concerns, here we reported ^{1}H NMR

71 evidences of a relatively stable sugar-NaCl-water complexes observed on different
72 mono/disaccharides in concentrated NaCl solution, which is not yet reported before.
73 Combining with observed ^{1}H NMR shifts correlated with a concentration of NaCl solution, a
74 more detailed mechanism on role of chloride anion is proposed here for a better elucidation
75 of "NaCl Effect".[21]
76 It was known that solid state NMR and IR spectra both showed no obvious change, especially
77 in spectral regions characteristic of the ordered and disordered regions of cellulose in the
78 presence of NaCl[22,23], probably because of cellulose's poor solubility in water. Inspired by a
79 sharp difference of ^{1}H NMR shifts of glucosamine in saturated NaCl solution, we envisaged
80 an NMR angle by measuring ^{1}H NMR shifts of monosaccharides and disaccharides might
81 deliver more experimental evidences to understand the mechanism of "NaCl Effect". Hence,
82 water soluble monosaccharides including D-glucose, D-glucosamine hydrogen chloride, N-
83 acetyl-D-glucosamine and D-fructose were initially chosen in order to gain some insights on
84 the "NaCl effect" on the intramolecular/intermolecular hydrogen bonding in water via regular
85 ^{1}H NMR measurement. Variant concentration and saturated NaCl solutions were prepared for
86 comparison study.
87
88 **2.0 Experimental Section**
89
90 *2.1 Preparation for different concentrations of NaCl solutions M (wt%)*
91 1. 0.08 M (0.5 %) NaCl solution: 29.2 mg NaCl was dissolved in 6 mL deionized water.
92 2. 0.33 M (1.9%) NaCl solution: 116.8 mg NaCl was dissolved in 6 mL deionized water.
93 3. 0.42 M (2.4%) NaCl solution: 146.1 mg NaCl was dissolved in 6 mL deionized water.
94 4. 0.85 M (4.8%) NaCl solution: 1 g NaCl was dissolved in 20 mL deionized water.
95 5. 1.71 M (9.1%) NaCl solution: 2 g NaCl was dissolved in 20 mL deionized water.
96 6. 2.56 M (13.0%) NaCl solution: 3 g NaCl was dissolved in 20 mL deionized water.
97 7. 3.42 M (16.7%) NaCl solution: 4 g NaCl was dissolved in 20 mL deionized water.
98
99 *2.2 General procedure for ^{1}H NMR investigation on monosaccharides*
100 Monosaccharide (0.5 mmol) was added into a different concentration of NaCl solution (6 mL)
101 and the mixture was stirred for 6 h at room temperature. After then, 1 mL of the reaction
102 mixture was taken out and was mixed with some ethanol (for fast evaporation); and the
103 solvent mixture was evaporated under reduced pressure at 37℃. Removal of residual solvent
104 in *vacuum* gave a crude product (dissolved in 0.4 mL D_2O) for ^{1}H NMR to determine chemical
105 shift.
106
107 *2.3 General procedure for ^{1}H NMR investigation volume impact (1.71 M NaCl solution) of*
108 *"NaCl effect" on N-acetyl-D-glucosamine*
109 N-Acetyl-D-glucosamine (0.5 mmol) was added into 1.71 M (9.1 wt%) NaCl solution (1.5 mL,
110 or 3 mL or 6 mL or 12 mL) and the mixture was stirred for 6 h at room temperature. After that,
111 1 mL of the reaction mixture was taken out and was mixed with some ethanol (for fast
112 evaporation); and the solvent mixture was evaporated under reduced pressure at 37℃.
113 Removal of residual solvent in *vacuum* gave a crude product (dissolved in 0.4 mL D_2O) for [1]H
114 NMR to determine chemical shift.

Note: sample concentration means the concentration of NaCl in NMR tube. In procedures 2.2 -2.3, 1 mL of solution was taken out and the water was evaporated along with ethanol added to give a crude solid; the solid was redissolved into 0.4 mL D$_2$O and it led to a concentration increase in NMR tube (2.5 times of prepared solution).

2.5 Procedure for ^{1}H NMR comparison study (glucose)

2.5.1 Preparation of 1.71 M (9.1 wt%) NaCl solution
2 g NaCl was dissolved in 20 mL deionized water.

2.5.2 Procedure
D-glucose (0.5 mmol) was added into a NaCl solution (1.71 M, 9.1 wt%, 6 mL) and the procedure is similar to procedure 2.2. Removal of residual solvent in *vacuum* gave a crude product (dissolved in 0.4 mL D$_2$O) for ^{1}H NMR to determine chemical shift. Meanwhile another 1 mL of the reaction mixture was taken out and was mixed with some ethanol (for fast evaporation); and the solvent mixture was evaporated under reduced pressure at 37℃. Removal of residual solvent in *vacuum* gave a crude product (dissolved in 1 mL D$_2$O), part of them for ^{1}H NMR to determine chemical shift for comparison.

2.5.3 Procedure for ^{1}H NMR study of NaCl solution directed preparation in D$_2$O.
Preparation of 1.71 M (9.1 wt%) NaCl solution: 0. 2 g NaCl was dissolved in 2 mL D$_2$O.
D-glucose (0.1 mmol) was added into above NaCl solution (1.2 mL) and the mixture was stirred for 6 h at room temperature. After then, 0.5 mL of the reaction mixture was taken out (2 times of concentration in preparation) for ^{1}H NMR to determine chemical shift.

*2.5.4 Procedure for preparation a control (D-glucose in D$_2$O).*
D-glucose (0.05 mmol) was added into D$_2$O (0.6 mL); and the reaction mixture was taken out for ^{1}H NMR to determine chemical shift.

3.0 Results and Discussion

3.1 ^{1}H NMR Study of monosaccharides in NaCl solution (0.20 M (1.25 wt%) to saturated solution (5.40 M))

Initially, 0.5 mmol of N-acetyl-D-glucosamine was dissolved into 6 mL of NaCl solution in a different concentration and the mixture continued to stir for 6 hours at room temperature before one portion was taken out for ^{1}H NMR measurement. Five easily identified peaks, including hydrogens on 1-position carbon of both α-anomer and β-anomer[24], were marked (**Figure 2**) in order to track their changes in different concentrations of NaCl solutions.

Figure 2. ^{1}H NMR Spectra of N-Acetyl-D-glucosamine and Five Peaks Marked for Tracking.

N-acetyl-glucosamine

5.18 --- (1)   4.67 --- (2)   3.84 --- (3)   3.65 --- (4)   3.46 --- (5)
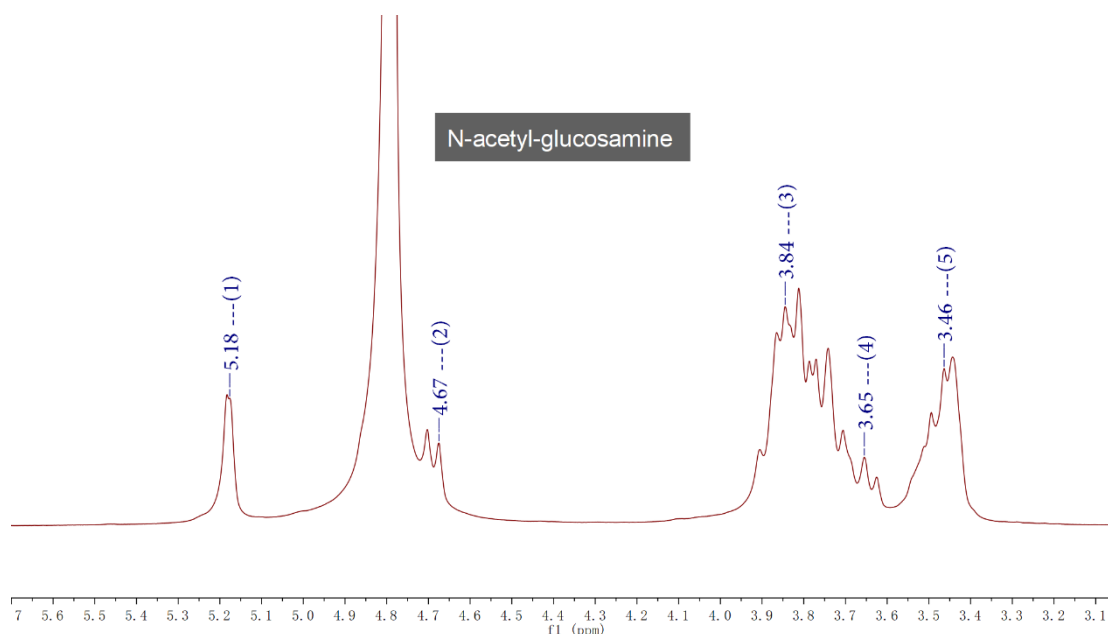
For N-acetyl-D-glucosamine, protons on 1-position carbon of α-anomer and β-anomer displayed at δ 5.18 and 4.67 respectively on ^{1}H NMR spectrum in D_2O, the former was at left side of D_2O peak while the latter was at right side. In 0.20 M (1.25 wt%, in NMR tube) NaCl solution, influence of NaCl to ^{1}H NMR shifts of all marked five peaks ($\triangle\delta <= -0.02$ ppm) were almost intangible; meanwhile the shifts became obvious ($\triangle\delta = 0.03 - 0.13$ ppm) in 0.83 M (4.75 wt%, in NMR tube) NaCl solution (**Figures 3** and supporting information). An interesting observance was that the shift ($\triangle\delta = 0.13$ ppm) of the second marked peak (H_1 of β-anomer) was the bigger than that ($\triangle\delta = 0.05$ ppm) of the first one (H_1 of α-anomer) in 0.83 M (4.75 wt%) NaCl solution, indicating the presence of NaCl had a stronger influence on β-anomer. A significant deshielding effect was observed for all protons of N-acetyl-D-glucosamine ($\triangle\delta = 0.26$ ppm) in the presence of 2.13 M (12 wt%) NaCl solution (in NMR tube), which was induced by hydrated NaCl (**Figures 3**). A remarkable downfield shifting ($\triangle\delta = 0.18$ ppm) on ^{1}H NMR shift for all five peaks was also observed by increasing of 2.13 M (12 wt%) NaCl solution to 4.28 M (22.8 wt%) NaCl solution (in NMR tube). Such an obvious shift is comparable to what K. R. Enslow *et.al*[16] had observed with D-xylose in NaCl solution. Change ($\triangle\delta <= 0.02$ ppm) became intangible when further increase of concentration to saturated NaCl solution (in NMR tube). Based on these data (**Figures 2**), it could be concluded that maximized perturbation of intramolecular/intermolecular hydrogen bonding within N-acetyl-D-glucosamine molecule was reached in 4.28 M (22.8 wt%) NaCl solution and in which relatively stable sugar-NaCl-water complex might form. Meanwhile, such a stable complex was never observed before to our best knowledge.

Figure 3. ^{1}H NMR Changes of N-Acetyl-D-glucosamine and D-Glucosamine Hydrogen Chloride Correlating with Concentration of NaCl solution and Molar Ratio by Tracking Marked Five/Six Peaks

5

Δδ ppm   N-Acetyl-glucosamine

Δδ ppm   Glucosamine hydrochloride

183

184
185
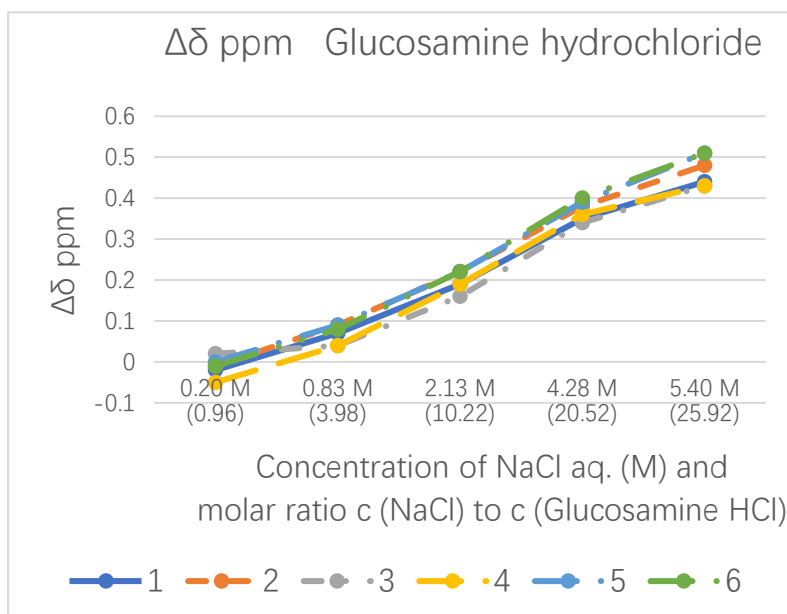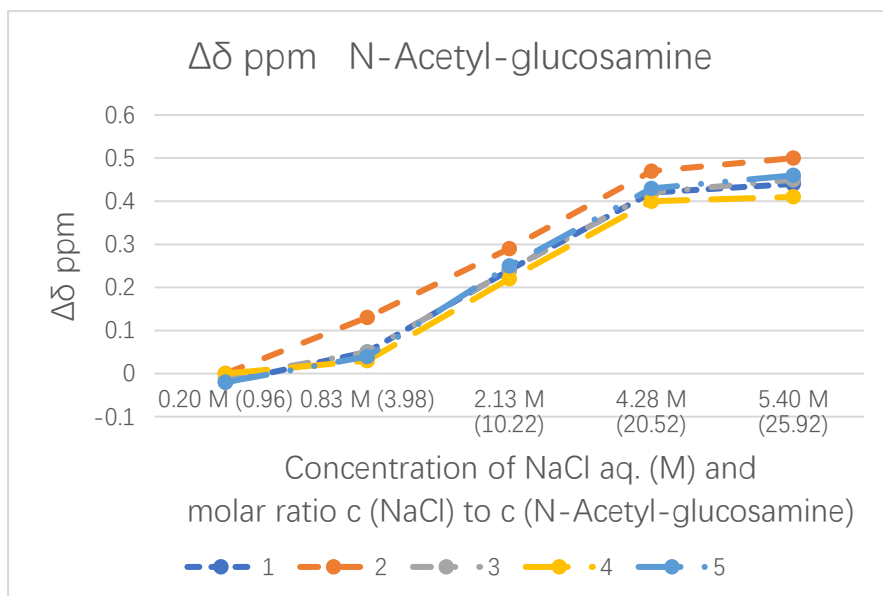
186 In order to gain more clues, similar ^{1}H NMR tracking investigation in different concentrations
187 of NaCl solution were also performed with other three abundant monosaccharides (D-
188 glucose, D-fructose and D-glucosamine hydrogen chloride) in nature (**Figure 3 and 4**). Above
189 three monosaccharides were known to interact with sodium cation in aqueous solution
190 differently.[7] Similarly, in 0.20 M (1.25 wt%) NaCl solution, influence of NaCl to ^{1}H NMR shifts of
191 all marked six peaks ($\triangle\delta <= -0.05$ ppm) of D-glucosamine hydrogen chloride was almost
192 intangible; meanwhile the shifts became obvious ($\triangle\delta = 0.04 - 0.09$ ppm) in 0.83 M (4.75 wt%)
193 NaCl solution (**Figures 3** and supporting information). The shift change ($\triangle\delta = 0.09$ ppm) of
194 the second marked peak (H_1 of β-anomer) and that ($\triangle\delta = 0.07$ ppm) of the first one (H_1 of
195 α-anomer) was narrow in 0.83 M (4.75 wt%) NaCl solution, indicating the presence of acetyl
196 group had an obvious influence and the anomeric effect became not obvious. Interestingly,
197 the possible stable complex could only be obtained when NaCl concentration is increased to
198 saturated solution (in NMR tube). The significant delay to a stable sugar-NaCl-water complex

199 was likely due to free amine moiety of D-glucosamine hydrogen chloride (stronger
200 inter/intramolecular bonding).

201

202 **Figure 4. ¹H NMR Changes of D-Glucose and D-Fructose Correlating with Concentration**
203 **of NaCl solution and Molar Ratio by Tracking Marked Six Peaks**.

204

205

206
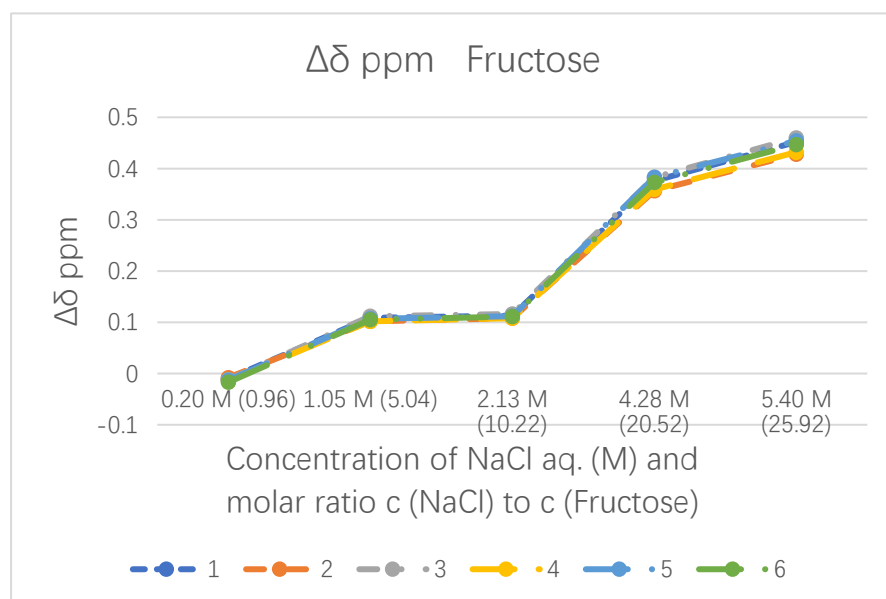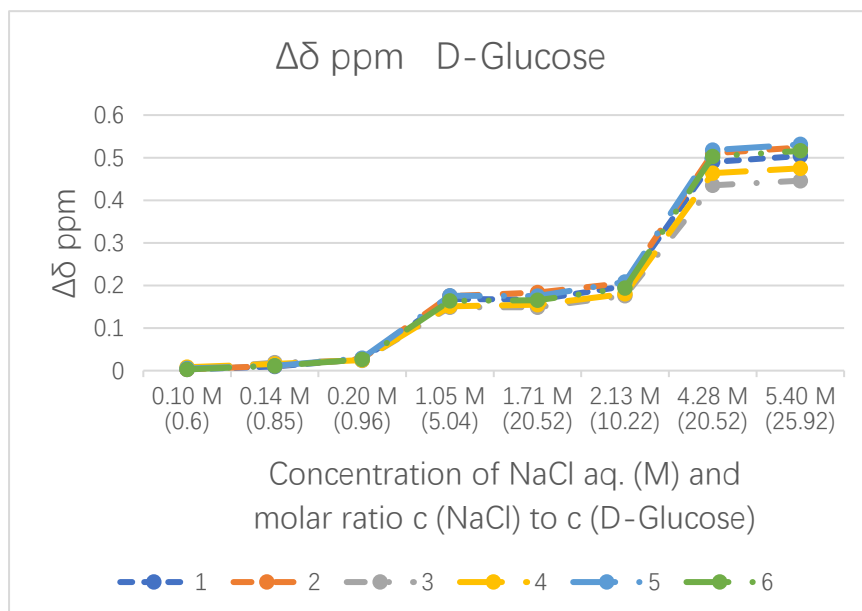
207 In 0.20 M (1.25 wt%) NaCl solution, a downfield effect ($\triangle\delta$ = 0.02 - 0.03 ppm) to all marked
208 six peaks of D-glucose was observed (**Figures 4** and supporting information), unlike the
209 existence of both upfield effect and downfield effect to the marked five/six peaks of N-acetyl-
210 D-glucosamine/D-glucosamine hydrogen chloride. Significant downfield changes ($\triangle\delta$ =
211 0.15 - 0.18 ppm) were observed upon the increase of concentration of NaCl solution from
212 0.20 M (1.25 wt%) to 1.05 M (6 wt%) (in NMR tube). No change at all or little change ($\triangle\delta$ = 0
213 - 0.03 ppm) for all marked peaks was found upon further increase of the concentration of
214 NaCl solution from 1.05 M (6 wt%) to 1.71 M (9.1 wt%) or from 1.71 M (9.1 wt%) to 2.13 M (12

wt%) or from 4.28 M (22.8 wt%) to saturated solution); meanwhile significant changes ($\triangle\delta$ = 0.26 - 0.32 ppm) were observed upon the increase of concentration of NaCl solution from 2.13 M (12 wt%) to 4.28 M (22.8 wt%). A possible stable sugar-water-NaCl complex was formed in 4.28 M (22.8 wt%) NaCl solution. Trends of ^{1}H NMR shifts of D-glucose in marked six peaks were pretty like a staircase, not a line, indicating a stepwise formation of hydrogen (H_2O) - chloride bonding.

For D-fructose, an upfield effect ($\triangle\delta$ <= -0.02 ppm) to all marked six peaks was observed in 0.20 M (1.25 wt%) NaCl solution, probably because of its rigid furanose structure. Similar to the observance for D-glucose, obvious ^{1}H NMR shifts ($\triangle\delta$ = 0.10 -0.11 ppm) could still be observed for all six marked peaks when further increase of concentration to 1.05 M (6.0 wt%) from 0.20 M (1.25 wt%); meanwhile little change ($\triangle\delta$ <= 0.01 ppm) was shown upon further increase of concentration from 1.05 M (6.0 wt%) to 2.13 M (12 wt%). Surprisingly, anomeric effect was intangible for D-fructose. Continued increase to saturated NaCl solution led to small changes ($\triangle\delta$ <= 0.08 ppm) for all six marked peaks on ^{1}H NMR spectrum. Such observed staircase-like trend indicated that correlation of ^{1}H NMR shift of other monosaccharides (without amine moiety) to concentration of NaCl solution might also be similar, which is an important insight for understanding of "NaCl effect" at atom level.

Theoretically the change of *J* coupling constants upon NaCl addition could be possible, however, it was concluded that the *J* coupling constants rarely changed upon NaCl addition in our system based on tracking ^{1}H NMR data on another random selected five peaks between 3.0 to 4.0 ppm (data and zoom-up spectrum with peaks picked see supporting information). Hence, in our system the method to track marked peaks in our manuscript is trustable. It is likely because of the excellent stability of stereochemistry structure and the lack of foldable structure in monosaccharides; and intramolecular interaction is also negligible under our conditions (relatively low concentration).

It is worth being noted that shifting trends of all marked peaks were similar in all four monosaccharides which indicated that acidity of proton has no observable effect on perturbation of hydrogen bonding, and generation of new hydrogen bonding networks was non-selective at relatively high concentration of NaCl solution. However, anomeric difference did exist for some monosaccharides in relatively low concentration of NaCl solution, in which such selective bonding might be present. Another important insight is that chloride anion likely plays major role on induction of ^{1}H NMR shift because of two reasons: 1) sodium cation is known to have less specific locations of binding due to its univalent and readily hydrated property in water.[25] Typically, two or three hydroxyl groups of monosaccharides are necessary to bind with each sodium cation and it is not so surprising that only one or two hydrated sodium cations can interact with each monosaccharide weakly. 2) Sodium cation is known to be buried inside water shells in its hydrated form meanwhile chloride anion is on the top of water cluster in its hydrated form;[26] in addition, strong perturbation of anions by exerting on adjacent H atoms was reported[27] through experimental Raman spectral measurements with classical MC simulations.

3.2 ^{1}H NMR Study of disaccharides/oligosaccharide in NaCl solution (2.13 M (12 wt%) to saturated solution) at room temperature and 60℃.

259   Disaccharides and oligosaccharides have stronger hydrogen bonds with water because of
260   more chelating bonds involved, and self-associate to form cluster is also involved in aqueous
261   solution. Next, two disaccharides (sucrose and trehalose) and one tetrasaccharide (stachyose)
262   were selected as targets for ^{1}H NMR comparison study in higher concentrations of NaCl
263   solutions at room temperature and 60℃ (supporting information) in order to provide more
264   viable references for NaCl promoted perturbation of hydrogen bonding of polysaccharides
265   such as cellulose. As a disaccharide formed by a 1,1-glycosidic bond between two α-glucose
266   units, trehalose has a stronger hydrogen bonding network than D-glucose. Disaccharide
267   containing fructose moiety and fructose need a higher concentration of NaCl solution to
268   generate the possible sugar-NaCl-water complexes since the ring of fructose hydrate is more
269   rigid than that of glucose hydrate, leading to less mobility of H-bonded water.

270   At room temperature in 2.13 M (12 wt%) NaCl solution (in NMR tube), ^{1}H NMR shifts ($\triangle\delta$ =
271   0.27 -0.30 ppm) for all four marked peaks of trehalose were much bigger than those observed
272   ($\triangle\delta$ = 0.15 -0.16 ppm) with sucrose (supporting information). It was easy to be understand
273   because one molecule sucrose composed of two monosaccharides (glucose and fructose)
274   and the moiety (fructose) in slow rate determined the rate of sucrose in perturbation of
275   hydrogen bonding. Such difference between shifts of all four peaks of trehalose ($\triangle\delta$ = 0.34
276   -0.40 ppm) and those of sucrose ($\triangle\delta$ = 0.39 -0.43 ppm) became very little in case that
277   concentration of NaCl solution was over 4.28 M (22.8 wt%) (in NMR tube).

278   Replacement of sucrose to tetrasaccharide (stachyose) led to little change on the correlation
279   between ^{1}H NMR shift and concentrations of NaCl solution (supporting information). Such
280   similar change indicates that NaCl promoted stable sugar (moiety)-NaCl-water complexes
281   are likely present as well for oligosaccharides in NaCl solution.

282   An increase of mixing temperature to 60℃ had positive effect ($\triangle\delta$ = 0.11 ppm) on
283   perturbation of hydrogen bonding of sucrose (**Table 1** and supporting information) in
284   relatively low concentration of NaCl solution (2.13 M (12 wt%) in NMR tube); smaller shifts
285   ($\triangle\delta$ = 0.02 ppm) for all four marked peaks of stachyose were also observed upon the increase
286   to 60℃ (see supporting information). However, influence became intangible in relatively high
287   concentration of NaCl solution (4.28 M, 22.8 wt%) for both disaccharides and stachyose (**Table**
288   **1** and supporting information). Such observance indicated the formed sugar-NaCl-water
289   complexes are relatively stable and could be prepared in relatively low concentration of NaCl
290   solution under elevated temperature or in higher concentration at room temperature. For
291   trehalose, a remarkable upfield effect ($\triangle\delta$ = -0.13 -0.14 ppm) was observed in 2.13 M (12
292   wt%) NaCl solution, probably because self-associate of trehalose was strongly accelerated at
293   60℃.

294

295   **Table 1.** ^{1}H NMR shifts (Four marked peaks of sucrose) under different concentrations resulted
296   from increase of temperature (60 $^{\circ}$C).

Concentration (NMR sample)	1 ($\triangle\delta$)	2 ($\triangle\delta$)	3 ($\triangle\delta$)	4 ($\triangle\delta$)
0	-0.011	-0.010	-0.010	-0.011
2.13 M (12.0%)	0.109	0.107	0.106	0.111
4.28 M (22.8%)	-0.032	-0.030	-0.029	-0.032

	Saturated (5.40 M)	0	0	0.001	0.001

The significant shifts for all marked peaks of sucrose in the presence of 2.13 M (12 wt%) NaCl solution at room temperature and 60℃ indicated the importance of temperature; particularly the temperature might play a more important role for polysaccharides that have poor solubility in water due to large molecular weight. NaCl concentration was well known to have strong effect on hydrogen bonding in water due to solvation[29-31]. The higher concentration of NaCl solution, the better effect on perturbation of hydrogen bonding network based on ^{1}H NMR shifts.

3.3 NMR instrument impact on ^{1}H NMR shifts of D-glucose in D_2O.

Table 2. ^{1}H NMR shifts (six marked peaks of D-glucose) on different NMR instruments at different date.

Entry (E)	Date (brand)	1	2	3	4	5	6
1	Dec. 2021 (JEOL)	5.198	4.607	3.883	3.713	3.460	3.215
2	Sept. 2021 (JEOL)	5.230	4.638	3.909	3.740	3.491	3.246
3	△δ (E2 – E1)	0.032	0.031	0.026	0.027	0.031	0.031
4	2018 (Bruker)	5.143	4.551	3.841	3.665	3.408	3.161
5	△δ (E4 – E1)	-0.045	-0.056	-0.042	-0.048	-0.052	-0.051

As we discussed in the introduction part, many factors might have influence on absolute value of shifts of ^{1}H NMR, here we investigated the impact of NMR instruments on six marked peaks of D-glucose by measuring the sample on different NMR instruments at different date. Both NMR instruments were in default working conditions at room temperature for users from chemistry laboratories. The error (△δ= 0.03 ppm) between two ^{1}H NMR spectrum (in D_2O) recorded on JEOL NMR instrument at Shanghai in Sept.2021 and Dec. 2021 was in acceptable error range (Entries 1 – 3, **Table 2**). The difference (△δ= 0.04 – 0.06 ppm) for ^{1}H NMR spectrum of D-glucose in D_2O (Entries 1, 4 and 5, **Table 2**) was slightly bigger when samples were recorded on different NMR instruments (JEOL at Shanghai, Dec. 2021 and Bruker at Guangzhou, 2018). Based on these comparison data, a general guideline is that a control sample is strongly suggested to be done at the same time for comparison in case samples in very low concentration of NaCl solution (< 0.20 M (1.25 wt%)) or a change to a new NMR instrument.

3.4 Volume effect

In procedures 2.1 -2.4 in experimental section, 1 mL of solution was taken out and the water was evaporated along with ethanol added to give a crude solid; the solid was dissolved into 0.4 mL D_2O and it led to a concentration increase in NMR tube (2.5 times of prepared solution). Sample concentration in **Table 3** means the concentration of NaCl in NMR tube. A recent comparison study (Entries 1 – 3, **Table 3**) on another NMR instrument (JEOL) indicated the

difference ($\triangle\delta$= 0.06 – 0.08 ppm) of six marked peaks of D-glucose was very small between the two samples mixed in 4.28 M (22.8 wt%) solution (in NMR tube), considering the obvious shifts ($\triangle\delta$= 0.44 – 0.52 ppm) in comparison with D-glucose in the absence of NaCl (supporting information). Not surprisingly, an increase on concentration in NMR tube (2.5 times) led to a sharp change on shifts of all six peaks (Entries 2 and 4, **Table 3**). A control study in NaCl/D_2O solvent (1.71 M, 9.1 wt%) was also carried out and part of solvent mixture was directly taken out for ^{1}H NMR measurement (Entries 5, **Table 3**); the shifts of all six marked peaks had little difference ($\triangle\delta$ <= 0.02 ppm) with those via evaporation/dissolving process (Entries 4, **Table 3**).

Table 3. ^{1}H NMR shifts (six marked peaks of D-glucose) on different NMR instruments at different date.

Entry (E)	Concentration (sample)	1	2	3	4	5	6
1	1.71 M (4.28 M, 0.4 mL D_2O) (2018, on Bruker)	5.633	5.061	4.276	4.129	3.926	3.664
2	1.71 M (4.28 M, 0.4 mL D_2O) (Dec. 2021, on JEOL)	5.568	4.992	4.217	4.051	3.850	3.588
3	$\triangle\delta$ (E2-E1)	-0.07	-0.07	-0.06	-0.08	-0.08	-0.07
4	1.71 M (1.71 M, 1.0 mL D_2O) (Dec. 2021, on JEOL)	5.365	4.790	4.032	3.868	3.635	3.380
5	1.71 M (D_2O) (Dec. 2021, on JEOL)	5.383	4.799	4.053	3.885	3.654	3.397

Effects of volume of 1.71 M (9.1 wt%) NaCl solution (4.28 M (22.8 wt%) in NMR tube) was also investigated with N-acetyl-D-glucosamine (0.5 mmol) (**Table 4**). Downfield shift was observed along with increase of volume till 6 mL, indicated that after the bonding network became steady no change was observed on ^{1}H NMR spectrum. The total usage of NaCl was 10 mmol under the turning point condition (6 mL) and molar ratio of NaCl to total hydroxyls (2.5 mmol) of monosaccharide was 4: 1.

Table 4. ^{1}H NMR Spectra of N-acetyl-D-glucosamine after mixing with different volume of 4.28 M (22.8 wt%) NaCl solution (in NMR tube) at room temperature.

| Volume (Molar ratio of c_{NaCl}/c_{NAG})	1 ($\triangle\delta$)	2 ($\triangle\delta$)	3 ($\triangle\delta$)	4 ($\triangle\delta$)	5 ($\triangle\delta$)
1.5 mL (5.13)	0	0	0	0	0
3 mL (10.26)	0.050	0.054	0.048	0.048	0.058
6 mL (20.52)	0.236	0.229	0.206	0.227	0.231
12 mL (41.04)	-0.017	-0.003	-0.003	0	-0.004

359 3.5 ^{1}H NMR chemical shifts referencing.

360

361 Peak of HDO was a reference (4.79 ppm) in processing all spectrum. The HDO peak position

362 is known to be sensitive to salt concentration[28a], such shift was mainly due to the same

363 mechanism of "salting-out effect" which was quite well understand; salts is dissolved in water

364 and water clusters collapse around the ions, leading to a volume contraction,

365 electrostriction.[28b,c] Such salt induced cavitation change of water structure (global changes of

366 the water H-bonding network and hydration upon salt addition) likely has similar effect on

367 monosaccharides soluble in NaCl solution, resulting to some changes on ^{1}H NMR shift of

368 monosaccharides. Hence, by using HDO as a reference, the chloride-water-sugar bonding

369 effect resulted ^{1}H NMR shift could be decoupled from those that resulted from salt induced

370 cavitation change of water structure, and this is crucial to this work to half-quantify the

371 bonding ratio of the stable sugar-NaCl-water complex.

372 Key evidence to prove existence of both global salt solution effect and chloride bonding effect

373 on ^{1}H NMR shifts is the data from Table 4. Because all experiments were carried out under

374 exactly the same concentration in reaction vials (1.71 M) and their samples were measured

375 under exactly the same concentration in NMR tubes (4.28 M), ^{1}H NMR shift change that

376 resulted from salt induced cavitation change of water structure was supposed to be the same

377 for all solute in the salt solution; however, significant ^{1}H NMR shift changes were still observed

378 when the same amount of sugar was dissolved in different volumes of salt solution leading

379 to the change on the ratio of sugar to salt in NMR tubes. The change became intangible when

380 the mole ratio of NaCl to hydroxyls (of saccharides) increased to 4:1, indicating all possible

381 stable (OH)H-Cl bondings at adjacent position were all formed (it is easy to be understand

382 that some Cl- would still remain in the solution as free hydrate anion in bulky water). Based

383 on above reasons and data, HOD is the very suitable reference in our current investigation.

384

385 3.6 Structure of stable sugar-NaCl-water complexes.

386

387 **Figure 5. Stable Complex of D-Glucose-NaCl-water in NaCl Solution.**

388

389
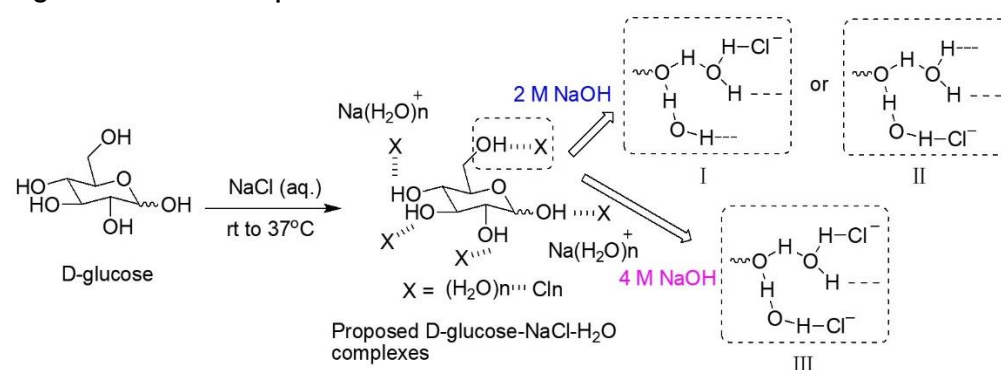
390 The staircase trend in correlation of ^{1}H NMR shifts (D-glucose/fructose) showed the formation

391 of hydrogen (H_2O)-chloride bond was likely stepwise (**Figure 4**). Both sodium cations and

392 chloride anions play an important role in perturbing hydrogen bonds with bulk water in

393 proposed hydrated forms and intramolecular hydrogen bonding of mono/disaccharides;

394 however, chloride anions may contribute much more to stabilize the sugar-NaCl-water

12

395 complexes and to the observed ^{1}H NMR shifts due to the very weak coordination between
396 sugar and sodium cations.

397 Both J. M. Harvey'[2,3] and T. Suzuki's[4] findings indicated that each hydroxyl group of D-glucose
398 forms two hydrogen bonds with two water molecules (**Figure 1**), and another discovery by J.
399 D. Smith *et.al* found that[27] changes of hydroxyl (from monosaccharides) vibrational spectrum
400 is induced by the action of halide anion's electric fields on adjacent water molecule near to
401 hydroxyl. Based on these findings, chloride anions likely bind with two water molecules
402 adjacent to each hydroxyl (or acetyl amide) respectively forming sugar-NaCl-water
403 complexes; only one or two hydrated sodium cations will bind with sugars and most are very
404 mobile. Since theoretically only three protons on the two adjacent water molecules of each
405 hydroxyl are available to bind with chloride anions, two possible sugar-NaCl-water complexes
406 (Ⅰ and Ⅱ) with single hydrogen (H_2O)-chloride bond, one possible sugar-NaCl-water
407 complexes (Ⅲ) with two hydrogen (H_2O)-chloride bonds as shown in **Figure 5**, and a
408 theoretically possible sugar-NaCl-water complexes (IV) with three hydrogen (H_2O)-chloride
409 bonds would be very bulky. Relatively stable sugar-NaCl-water complex I or II or both for
410 monosaccharides likely form under 1 ~ 2 M NaCl solution with a molar ratio of NaCl to
411 monosaccharide over 5 (the first staircase in **Figure 4** and data with 1.5/3 mL in **Table 4**),
412 meanwhile a much higher concentration of NaCl solution with a molar ratio of NaCl to
413 monosaccharide over 20 is quite necessary to enable formation of the second hydrogen
414 (H_2O)-chloride bond generating stable complexes Ⅲ (the second staircase in **Figure 4** and
415 data with 6/12 mL in **Table 4**); the complexes (IV) with three chloride anions per hydroxyl
416 might be unstable due to negative charge repulsion of two chloride anions binding with the
417 same adjacent water molecule. It is consistence with our observance that molar ratio of NaCl
418 to total hydroxyls (2.5 mmol) of monosaccharide was 4 : 1 when a stable sugar-water complex
419 Ⅱ formed since the presence of half of chloride anion in hydrated in bulk water might be
420 quite necessary in stabilization of the formed complex (data with 6/12 mL in **Table 4**). The
421 structure of complexes in NaCl solution is likely the same when they reach a stable status
422 regardless of initial parameters of concentration, volume and temperature, this is of much
423 importance for understanding of the well-known "NaCl effect".

424

425 3.7 ^{1}H NMR Study of D-glucose in biological relevant NaCl solution (< 0.20 M (1.25 wt%)).

426

427 ^{1}H NMR shifts of D-glucose in 0.20 M (1.25 wt%) showed clear downfield change on the
428 spectrum for all six marked peaks ($\triangle\delta$ = 0.03 ppm); meanwhile an upfield change ($\triangle\delta$ = -
429 0.01 - -0.02 ppm) for all six peaks of fructose was observed under the same condition (**Figure**
430 **4** and supporting information). Such observance might be of much value to understanding
431 of sugars in the human body via NMR analysis techniques[32], as well as for understanding of
432 biological mechanism of Na-dependent cotransport of sugars like glucose and fructose. It is
433 known that Na dependent glucose transport has an approximate half maximal Na
434 concentration of around 50-70 mM for the Na dependent glucose cotransporter, whereas
435 fructose is not transported in a Na dependent manner.[33,34] A further exploration on ^{1}H NMR
436 shifts of D-glucose in biological relevant NaCl solution (100 mM and 140 mM) at room
437 temperature was also performed; similar downfield changes were still observable (**Table 5**).
438 This small but clear downfield change indicated glucose-NaCl-water complex (more like

complexes I or II with only some (not all) hydroxyls bonding with chloride anions) might generate in biological system, meanwhile similar fructose-NaCl-water could not form in low concentration of NaCl solution (< 0.20 M (1.25 wt%)); such difference may explain some of the stereospecificity of this process. Further work with more sensitive ^{13}C NMR to verify such difference would be performed in the future.

Table 5. ^{1}H NMR shifts of D-glucose in biological relevant NaCl solution at room temperature.

Concentration (In NMR tube)	1 ($\triangle\delta$)	2 ($\triangle\delta$)	3 ($\triangle\delta$)	4 ($\triangle\delta$)	5 ($\triangle\delta$)	6 ($\triangle\delta$)
50 mM (100 mM, 0.58%)	0.003	0.003	0.003	0.008	0.005	0.003
70 mM (140 mM, 0.82%)	0.010	0.011	0.019	0.016	0.011	0.011

3.8 A proposed ideal NaCl usage.

Concerning frequent optimization on NaCl usage in literatures, we tentatively propose a calculation model recommending the best usage of NaCl for maximum perturbation of hydrogen bonding network of mono/disaccharides. It is also very relevant for reference in depolymerization of polysaccharides including starch, chitin and cellulose. Recommended absolute amount of NaCl in solution is 3.5 – 4.0 equivalent to total hydroxyl of saccharides (including protons on amine moiety if glucosamine-based saccharides were used) based on observed maximum ^{1}H NMR shift forming stable sugar-NaCl-water complexes. This recommended ideal usage is consistent with optimized condition in recent reported literature[20]. For temperature, room temperature is usually sufficient for monosaccharides and oligosaccharides, which are soluble in water; however, typical increase of temperature is pretty necessary for cellulose depolymerization according to a recent report[22], stating that "NaCl effect" is only obvious when the temperature is above 210℃.

4.0 Conclusion

In summary, ^{1}H NMR evidences of stable sugar-NaCl-water complexes with monosaccharides and oligosaccharides at room temperature were obtained, and the staircase trend in correlation of ^{1}H NMR shifts (D-glucose/fructose) showed that the formation of hydrogen (H_2O)-chloride bond was likely stepwise; this observance is of much importance to the further understanding of prevailing "NaCl effect" at atom level. The shifting trends of all marked peaks correlation to concentration of NaCl solutions were very similar in all four monosaccharides; that indicated that the reactivity order (acidity difference) has no observable effect on ^{1}H NMR shifts and generation of new hydrogen bonding network (H-Cl bond) is non-selective. However, anomeric effect did exist for some monosaccharides at relatively low concentration of NaCl solution. Although both sodium cations and chloride anions promote perturbation of hydrogen bonding network (global salt solution effect), chloride anions may play a key role in the stabilization of sugar-NaCl-water complexes and the induction of ^{1}H NMR shifts via bonding as proposed in **Figure 5**. Based on the maximum of the induced ^{1}H NMR shifts reaching stable sugar-NaCl-water complexes, a general recommended NaCl usage of 3.5 –

478   4.0 equivalent mole of hydroxyls (including amines or amides) on saccharides was proposed.

479   We envisage more insights may be achieved when a full characterization of the stable sugar-

480   NaCl-water complexes in aqueous media is available in the future.

481

482   **Supporting Information**.

483   Completed general information, all reaction procedures, ^{1}H NMR shifts summarized in tables, figures

484   for shift trends of disaccharides/stachyose, reproducing note and all ^{1}H NMR spectrum with marked

485   peaks as well as J coupling constants data (and zoom-up spectrum with peaks picked) were all included

486   in supporting information.

487   **Author Contributions**

492   ACKNOWLEDGMENT

499

500   References:

501   [1] Kosaka, A.; Aida, M.; Katsumoto, Y.; Reconsidering the activation entropy for anomerization

502   of glucose and mannose in water studied by NMR spectroscopy. *J. Mol. Struc.* **2015**, *1093*,

503   195-200.

504   [2] Harvey, J.M.; Symons, M.C.R.; Naftalin, R.J.; Proton magnetic resonance study of the hydration

505   of glucose. *Nature*, **1976**, *261*, 435-436.

506   [3] Harvey, J.M.; Symons, M.C.R.; The Hydration of monosaccharides an NMR study. *J. Solution*

507   *Chem.*, **1978**, *7*, 571-586.

508   [4] Suzuki, T.; The hydration of glucose: the local configurations in sugar–water hydrogen

509   bonds. *Phys. Chem. Chem. Phys.*, **2008**, *10*, 96–105.

510   [5] Cappa, C. D.; Smith, J. D.; Wilson, K. R.; Messer, B. M.; Gilles, M. K.; Cohen, R. C. and Saykally,

511   R. J.; Effects of alkali metal halide salts on the hydrogen bond network of liquid water. *J. Phys.*

512   *Chem. B* **2005**, *109*, 7046-7052.

513   [6] Angyal, S. J.; Complexes of metal cations with carbohydrates in solution. I. Determination

514   of the extent of complexing by N.M.R. Spectroscopy. *Aust. J. Chem.*; **1972**, *25*, 1957-66.

515 [7] Book chapter. Angyal, S. J.; Complexes of metal cations with carbohydrates in solution.
516 Advances in carbohydrate chemistry and biochemistry. **1989**, vol.*47*, 1-43.

517 [8] Franks, F.; Hall, J. R.; Irish, D. E.; and Norris, K.; The effect of cations on the anomeric
518 equilibrium of D-glucose in aqueous solution – A Raman-spectral study. *Carbohydr. Res.*,
519 **1986**, *157*, 53-64.

520 [9] Chen, X.; Chew, S. L.; Kerton, F. M.; Yan, N., Direct conversion of chitin into a N-containing
521 furan derivative. *Green Chem.* **2014,** *16* (4), 2204-2212.

522 [10] Omari, K. W.; Dodot, L.; Kerton, F. M., A simple one-pot dehydration process to convert N-
523 acetyl-D-glucosamine into a nitrogen-containing compound, 3-acetamido-5-acetylfuran.
524 *ChemSusChem* **2012,** *5* (9), 1767-72.

525 [11] Hansen, T. S.; Mielby, J.; Riisager, A., Synergy of boric acid and added salts in the catalytic
526 dehydration of hexoses to 5-hydroxymethylfurfural in water. *Green Chem.* **2011,** *13* (1), 109-114.

527 [12] Wang, C.; Zhang, Q.; Chen, Y.; Zhang, X.; Xu, F., Highly Efficient Conversion of Xylose Residues
528 to Levulinic Acid over FeCl3 Catalyst in Green Salt Solutions. *ACS Sustainable Chemistry &*
529 *Engineering* **2018,** *6* (3), 3154-3161.

530 [13] Li, M.; Li, W.; Lu, Y.; Jameel, H.; Chang, H.-M.; Ma, L., High conversion of glucose to 5-
531 hydroxymethylfurfural using hydrochloric acid as a catalyst and sodium chloride as a promoter in
532 a water/γ-valerolactone system. *RSC Advances* **2017,** *7* (24), 14330-14336.

533 [14] Jiang, Z.; Budarin, V. L.; Fan, J.; Remón, J.; Li, T.; Hu, C.; Clark, J. H., Sodium Chloride-Assisted
534 Depolymerization of Xylo-oligomers to Xylose. *ACS Sustainable Chemistry & Engineering* **2018,** *6*
535 (3), 4098-4104.

536 [15] Amoah, J.; Hasunuma, T.; Ogino, C.; Kondo, A., 5-Hydroxymethylfurfural production from salt-
537 induced photoautotrophically cultivated Chlorella sorokiniana. *Biochemical Engineering Journal*
538 **2019,** *142*, 117-123.

539 [16] Enslow, K. R. and Bell, A. T.; The role of metal halides in enhancing the dehydration of xylose
540 to furfural. *ChemCatChem*, **2015,** *7*, 479-489.

541 [17] Mayes, H. B.; Tian, J.; Nolte, M. W.; Shanks, B. H.; Beckham, G. T.; Gnanakaran, S. and
542 Broadbelt L. J.; Sodium ion interactions with aqueous glucose: insights from quantum
543 mechanics, molecular dynamics, and experiment. *J. Phys. Chem. B* **2014,** *118*, 1990−2000.

544 [18] Mellmer, M. A.; Sanpitakseree, C.; Demir, B. Ma, Kaiwen; Elliott, W. A.; Bai, P.; Johnson, R.
545 L.; Walker, T. W.; Shanks, B. H.; Rioux, R. M.; Neurock M. & Dumesic, J. A.; Effects of chloride
546 ions in acid-catalyzed biomass dehydration reactions in polar aprotic solvents. *Nat. Commun.*
547 **2019,** *10*. 1132.

548 [19] Pyo, S.-H.; Glaser, S. J.; Rehnberg, N. and Hatti-Kaul, R.; Clean production of levulinic acid
549 from fructose and glucose in salt water by heterogeneous catalytic dehydration. *ACS Omega*
550 **2020,** *5*, 14275−14282

551 [20] Jing, Y.; Zhang, Y.; Lv, Q.; Guo, Y.; Liu, X. and Wang, Y.; Boosting the utilization efficiency
552 of glucose via a favored C–C coupling reaction. *Green Chem.*, **2019,** *21*, 6236–6240.

553 [21] Part of this work was presented in ACS Spring 2021 National Meeting (Organic Chemistry
554 Division) (live). A recorded presentation video see: https://doi.org/10.1021/scimeetings.1c00812.

555 [22] Jiang, Z.; Fan, J.; Budarin, V. L.; Macquarrie, D. J.; Gao, Y.; Li, T.; Hu, C.; Clark, J. H., Mechanistic
556 understanding of salt-assisted autocatalytic hydrolysis of cellulose. *Sustainable Energy & Fuels*
557 **2018,** *2* (5), 936-940.

558 [23] Fan, J.; Bruyn, M. D.; Zhu, Z.; Budarin, V.; Gronnow, M.; Gomez, L. D.; Macquarrie, D.; Clark, J.

559   H. Microwave-enhanced formation of glucose from cellulosic waste. *Chem. Eng. Process.*, **2013**,

560   *71*, 37-42.

561   [24] Usually a mixture of α-anomer and β-anomer both exist due to dissociated equivalent.

562   [25] Carrell, C. J.; Carrell, H. L.; Erlebacher, J. and Glusker J. P.; Structural aspects of metal ion-

563   carboxylate interactions. *J. Am. Chem. Soc.* **1988**, *110*, 8651-8656.

564   [26] Chandrasekhar J. and Jorgensen W. L.; The nature of dilute solutions of sodium ion in water,

565   methanol, and tetrahydrofuran. *J. Chem, Phys.* **1982**, *77*, 5080-5089.

566   [27] Smith, J. D.; Saykally, R. J. and Geissler P. L.; The effects of dissolved halide anions on hydrogen

567   bonding in liquid water. *J. Am. Chem. Soc.* **2007**, *129*, 13847-13856.

568   [28] a) Wishart, D. S.; Bigam, C. G.; Yao, J.; Abildgaard, F.; Dyson, H. J.; Oldfield, E.; Markley, J. L.

569   and Sykes, B. D.; ^{1}H, ^{13}C and ^{15}N chemical shift referencing in biomolecular NMR *J. Biomolecular*

570   *NMR* **1995**, *6*, 135-140; b) Long, F. A. and McDevit, W. F.; Activity coefficients of nonelectrolyte

571   solutes in aquesous salt solutions, *Chem. Rev.*, **1952**, *51*, 119-169; c) Breslow, R.; Hydrophobic

572   effects on simple organic reactions in water, *Acc. Chem. Res.*, **1992**, *24*, 159 – 164.

573   [29] Dedonder-Lardeux, C.; Gregoire, G.; Jouvet, C.; Martrenchard, S.; and Solgadi, D. Charge

574   separation in molecular clusters: dissolution of a salt in a salt-(solvent)$_n$ cluster. *Chem. Rev.*, **2000**,

575   *100*, 4023 – 4038.

576   [30] Beladjine, S.; Amrani, M.; Zanoun, A.; Belaidi, A,; Vergoten, G. Structure and hydrogen bonding

577   in aqueous sodium chloride solutions using theoretical water model AB_4: effects of concentration.

578   *Comput. Theor. Chem.* **2011**, *977*, 97 – 102.

579   [31] Shalit, A.; Ahamed, S.; Savolainen, J. and Hamm, P. Terahertz echos reveal the inhomogeneity

580   of aqueous salt solutions. *Nat. Chem.*, **2017**, *9*, 273-278.

581   [32] Duus, J.; Gotfredsen, C. H.; and Bock, K. Carbohydrate Structural Determination by NMR

582   Spectroscopy: Modern Methods and Limitations. *Chem. Rev.*, **2000**, *100*, 4589 – 4614.

583   [33] Holman, G. D. and Naftalin R. J.; Transport of 3-O-Methyl D-Glucose and β-methyl D-

584   glucoside by Rabbit Ileum. *Biochim. et Biophys. Acta*, **1976**, *433*, 597-614.

585   [34] Loo, D. D. F.; Zeuthen, T.; Chandy, G. and Wright, E. M. Cotransport of water by the

586   Na^+/glucose cotransporter. *Proc. Natl. Acad. Sci.*, **1996**, *93*, 13367–13370.

587