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NMR experiments for the detection of NOEs and scalar coupling constants between equivalent protons in trehalose-containing molecules

Ana Poveda [a], Cristina Vicent [b], Soledad Penadés [b], Jesús Jiménez-Barbero [b,*]

[a] *Servicio Interdepartamental de Investigación, Universidad Autónoma de Madrid, Cantoblanco, 28049 Madrid, Spain*

[b] *Grupo de Carbohidratos, Instituto de Química Orgánica, C.S.I.C., Juan de la Cierva 3, 28006 Madrid, Spain*

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Abstract

NOEs between chemically equivalent protons in trehalose-containing molecules have been measured by using selective ^{13}C NMR editing experiments. In addition, average interglycosidic long range couplings H1–C1–O1–C1′ have been also measured for this compounds using a selective inverse detection quantitative method. © 1997 Elsevier Science Ltd.

Keywords: Trehalose; Symmetry; ^{13}C NMR edited ROESY; Selective pulses; Long range heteronuclear coupling constants

1. Introduction

Molecular recognition processes are the key step of many biological events. In the past years, organic chemistry and, particularly carbohydrate chemistry, has provided a number and variety of hosts with different structural topologies and binding abilities [1].

Symmetric hosts have been shown to improve the design of both the macrocyclic cavity and size [2]. In addition, the study of the complex geometries is also easier [2]. In this context, we have shown that trehalose (α,α-glucopyranosyl glucopyranose)-containing macrocycles and glycophanes are adequate hosts for a variety of cations, ammonium salts and organic ligands [3–5]. Trehalose-containing molecules are also widely present in biologically relevant glycoconjugates and play significant roles in a variety of cell recognition events [6]. However, the C_2 symmetry of trehalose poses a problem for the characterization of the solution conformation of these molecules, since few distance constraints are available to be measured by regular NOE (ROE) experiments [7]. This fact, which is fairly common in carbohydrate molecules [8], is even more pronounced in trehalose-containing molecules, since the two (in principle) closest protons on both sides of the glycosidic linkage (H-1 and H-1′) are symmetry related and therefore no direct NOE can be detected under regular conditions. In principle, only one interresidue, H1/H5′ [9], NOE can be measured via ordinary 1D or 2D methods. Following

* Corresponding author. Fax: +34-1-5644853; e-mail: iqojj01@pinar1.csic.es.

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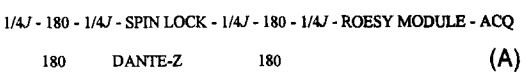
the size of this interresidue NOE, the major NMR-derived solution conformation of trehalose [9] has been shown to be in agreement with those deduced by other means, including that observed in the solid state and that calculated by molecular mechanics for trehalose and different derivatives thereof [10]. However, in O-substituted trehaloses and in several trehalose-containing macrocycles we have postulated the occurrence of major conformational changes around the glycosidic linkages [3–5] depending on the position and nature of the substituent and on the size of the polyethylene chain. The conformational changes are in agreement with the existence of a secondary local minimum deduced from semiempirical calculations by Tvaroska for the corresponding tetrahydropyranyl analogue of trehalose [11].

We here report on the application of selective, DANTE-Z based [12], ^{13}C NMR editing experiments [13] which allow the measurement of NOEs between chemically equivalent protons in trehalose-containing molecules. In particular, the edition of a given $^{1}H-^{13}C$ pair followed by a ROESY module is shown to provide an adequate means to measure NOEs between $^{1}H-^{13}C/^{1}H-^{12}C$ proton pairs. This selective experiment is a fairly simple and good alternative to the use of regular 2D-HMQC-ROESY [14] or HSQC-NOESY [15] experiments, which have been previously used for other regular organic molecules. Usually, for small and medium size organic molecules such as trehalose, only one or two NOEs between symmetric protons may be detected and therefore, the selective method proposed herein satisfactorily competes with the time consuming 2D-sequences. In fact, just one resonance needs to be selected by the DANTE-Z-based editing module. Moreover, and in order to provide additional constraints to define the conformation of trehalose containing molecules, average interglycosidic long range couplings H1–C1–O1–C1′ have been also measured using a recently published inverse detection quantitative method [16].

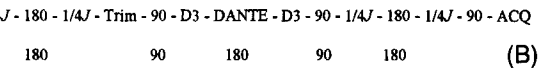
2. Experimental

NMR spectra were obtained at 300 MHz on a Bruker AMX spectrometer and at 500 MHz on a Varian Unity spectrometer at 303 K using 5–10 mmolar solutions of the different compounds in D_2O solutions. Measurements of NOEs between symmetric protons were performed using the inverse-detection pulse sequence depicted below:



The lengths of the DANTE-Z inversion pulses were between 20 and 40 ms. The DANTE-Z module [12] consisted of a train of 1–2 μs pulses separated by 200–500 μs delays. During the DANTE-Z inversion, the proton magnetization was spin-locked, following the protocol proposed by Nuzzillard and Bernassau [13]. The ROESY module consisted of a long constant phase pulse of 300, 350, and/or 400 ms. No decoupling was used during acquisition. Several independent measurements were performed and averaged. One bond coupling constants were set to 174 Hz to calculate the corresponding delays. Estimated errors in NOEs are smaller than 20% of their absolute value.

Measurements of vicinal long range heteronuclear coupling constants were performed using the inverse-detection pulse sequence depicted in B, as proposed by Lindon and coworkers [16].



The desired long range coupling constants evolve during D3 periods, and can be easily measured using a reference spectrum. In particular the intensity ratios of the spectrum in which a given proton has been selectively inverted and the reference one inverted off-resonance amounts to $I/I^{\circ} = \cos \pi J(2 * \mathrm{D3} + \mathrm{sel.P180})$, where D3 is the evolution period, and sel. P180 the length of the selective inversion pulse.

Total evolution times ($2 * \mathrm{D3} + \mathrm{sel.P180}$) were between 30 and 90 ms, while the lengths of the DANTE inversion pulses were between 12 and 24 ms. The DANTE module consisted of a train of 1–2 μs pulses separated by 50–100 ms delays. No decoupling was used during acquisition. Several independent measurements were performed at both magnetic fields and averaged. One bond coupling constants were set to 174 Hz to calculate the corresponding delays. The estimated accuracy of the couplings is 0.3 Hz.

Molecular mechanics calculations were performed by using the MM3* program as integrated in MACROMODEL 4.5 [17] on a Silicon Graphics Indy workstation. A bulk dielectric constant of 80 was used. Exhaustive minimization using conjugate gradient iterations of different starting structures generated

by the Monte Carlo search facility implemented in the MACROMODEL package was performed in order to look for the global minima structures. Glycosidic torsion angles are defined as Φ H-1–C-1–O-1–C-1′ and Φ' H-1′–C-1′–O-1–C-1.

3. Results and discussion

NOEs between symmetric protons.—For trehalose (**1**), the selection (inversion) of the C-1 resonance signal by pulse sequence A (Fig. 1) (or by the 2D HMQC-ROESY experiment) allowed estimation a H-1/H-1′ NOE of 4%. After comparing this NOE intensity with the corresponding intraresidue H-1/H-2 NOE (which corresponds to 0.249 nm, according to MM3* molecular mechanics calculations), an average H-1/H-1′ distance of 0.28 ± 0.2 nm was estimated. This distance is in fair agreement with a conformational distribution around the global minimum found in trehalose [10] by using MM3* calculations (0.284 nm, with Φ/Φ' ca. $-50/-50°$, Fig. 2). A second interresidue NOE is also observed between protons H-1/H-5′. This effect is also easily detected in regular ROE/NOE experiments and corresponds to an average distance between these two protons around 0.25 ± 0.2 nm (0.269 nm, according to MM3*).

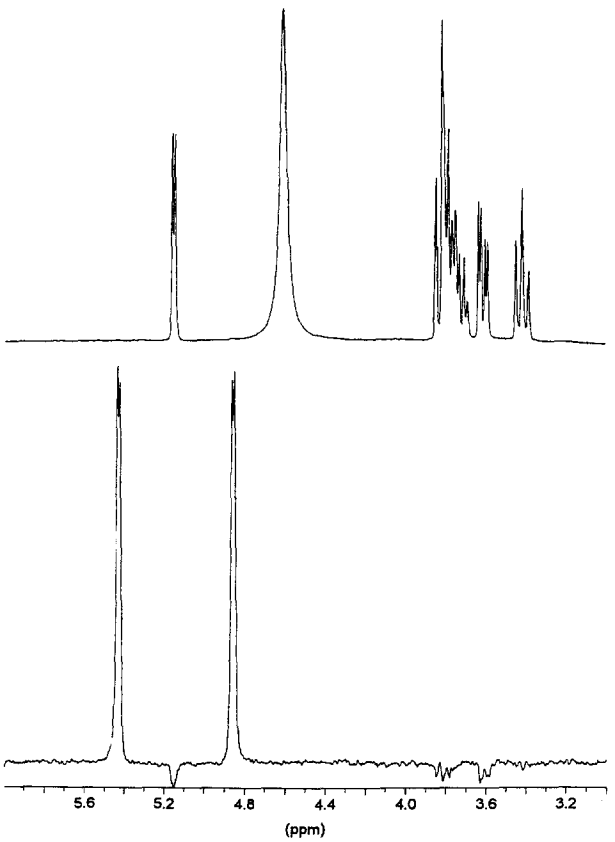


Fig. 1. 1D-selective ^{13}C edited ROESY spectrum of trehalose (**1**) after selection of proton H-1. The inverted proton appears doubled by the direct $^{1}J_{CH}$. The NOE to its symmetry-related H-1′ proton appears (with opposite sign) in the middle of the splitting signal. The intraresidue H-1/H-2 and interresidue H-1/H-5′ NOEs may also be observed.

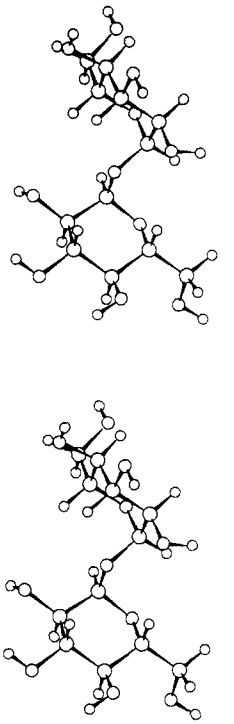
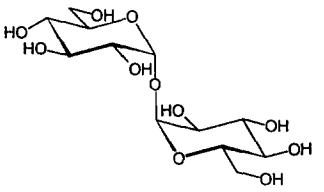


Fig. 2. MM3* calculated global minimum of trehalose (**1**). Interglycosidic torsion angles are Φ, Φ' -50, $-50°$.



1

The same experiment was repeated for the 2,2′-tetraethyleneglycol macrocycle (**2**). In this case, a strong H-1/H1′ NOE was measured (12%, Fig. 3a). Again, the comparison of this NOE value with that measured for the intraresidue H-1/H-2 proton pair allowed to estimate a much shorter distance between both anomeric protons. This distance should amount to ca. 0.22 ± 0.2 nm, that is, a much smaller distance than that estimated for trehalose itself. This change in relative NOEs indicates unambiguously a change in

the conformation around the glycosidic torsion angles Φ/Φ' of trehalose. This torsional change brings both anomeric protons in close proximity, simultaneously producing the separation of protons H-1 and H-5′. This H-1/H-5′ NOE is barely detectable in both regular or ^{13}C-edited NOE(ROE) experiments for this compound **2**, indicating a lower limit of the corresponding interproton distance of ca. 0.35 nm. These interresidue distances are indeed in close agreement with those estimated via MM3* molecular mechanics calculations for the global minimum geometry of this molecule which presents a significant variation of Φ/Φ' torsion angles to $-11/-28°$ and which present an average distance between both anomeric protons of only 0.213 nm (Fig. 4a). On the other hand, H-1 and H-5′ are 0.376 nm apart.

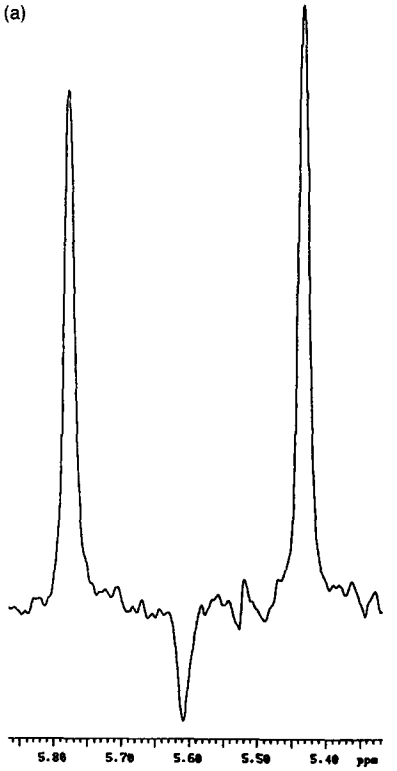


Fig. 3. (a) 1D-selective ^{13}C-edited ROESY spectrum of **2** after selection of proton H-1. The inverted proton appears doubled by the direct $^{1}J_{CH}$. The strong NOE to its symmetry-related H-1′ proton appears (with opposite sign) in the middle of the splitting signal. (b) Same experiment for compound **3**.

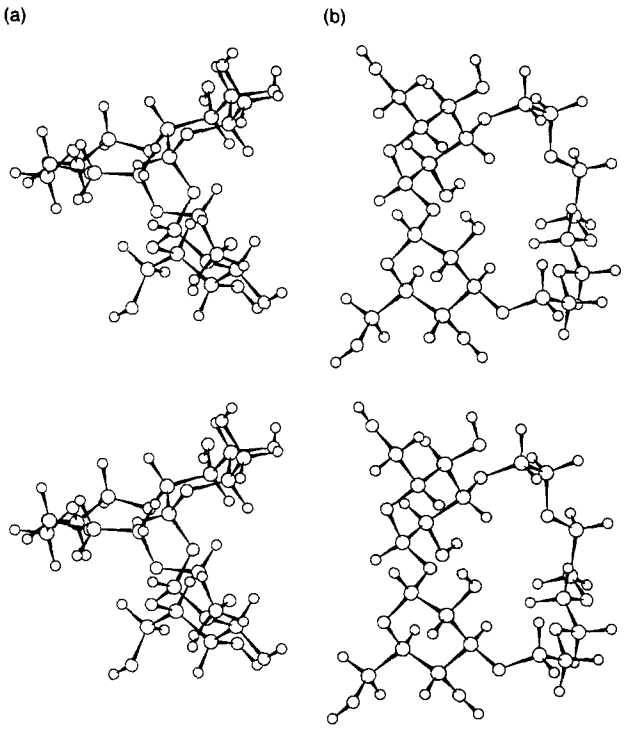
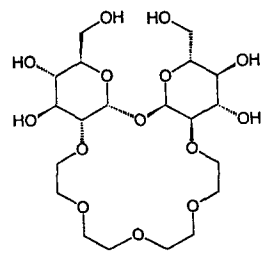
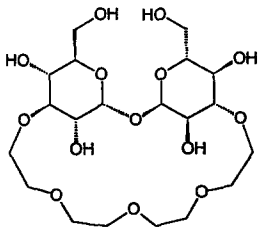


Fig. 4. (a) MM3* calculated global minimum of **2**. Interglycosidic torsion angles are Φ, Φ' ca. -30, $-10°$. (b) MM3* calculated global minimum of **3**. Interglycosidic torsion angles are Φ, Φ' ca. -50, $-50°$.



2

Macrocycle **3**, with a tetraethylene glycol moiety linking O-3 and O-3′ positions, provided a H-1/H-1′ NOE value (Fig. 3b) of 5%, which accounts for a interproton distance of 0.28 ± 0.2 nm after comparison with the intraresidue H-1/H-2 enhancement. Once again, the experimentally estimated distance is in good agreement with that derived from MM3* calculations (Fig. 4b, Φ/Φ' $-53/-55°$, H-1/H-1′ 0.293 nm).



3

Additional constraints to deduce the conformation of these molecules was obtained from the measurement of long-range heteronuclear coupling constants. These couplings present a Karplus-type relationship with the corresponding vicinal torsional angle [18,19]. The application of the pulse sequence [16] depicted in B, allows to deduce the heteronuclear long range couplings to the proton selectively inverted. The comparison of the signal intensities of two spectra in which one signal has been selectively inverted with a second one in which the selective pulse has been applied off-resonance allows to quantitatively estimate the long range coupling value, since during both D3 periods only this coupling effectively evolves. In order to test the efficiency of the sequence, several D3 periods as well as different selective pulse lengths were employed in order to compare the results.

For trehalose (**1**), the measured coupling is 3.0 ± 0.3 Hz. The derivation of the corresponding average torsion angle (H1–C1–O1–C1′) according to the most frequently used Karplus-type equation for carbohydrates [18] produced a value around $-45 \pm 5°$, also in good agreement with the average torsion angle estimated from MM3* calculations (ca. $-50/-50°$).

The measured couplings, following the same methodology, for the 2,2′- (**2**) and 3,3′- (**3**) tetraethyleneglycol macrocycles are 3.8 ± 0.3 and 2.6 ± 0.3 Hz, respectively. Thus, the expected torsion angles would also be around $-30 \pm 5°$ and $-50 \pm 5°$, respectively. Previous studies [3,4] and molecular mechanics calculations with the MM3* program (see above) have demonstrated that the conformation around the glycosidic linkage for the 3,3′-tetraethyleneglycol macrocycle (**3**) is fairly similar [3,4] to that of the regular disaccharide (Φ/Φ' ca. $-50/-50°$) and thus, the coupling constant value obtained herein (2.6 ± 0.3 Hz) is in fair agreement with this conclusion. On the other hand, for **2**, molecular mechanics calculations (see also above) and regular NOE experiments already demonstrated that the conformation around the glycosidic torsion angles is fairly different [3,4] to that of free trehalose and average values around -20 ± 10 ° (Fig. 4) would describe the observed NOEs for this linkage. According to the Karplus type equation, a coupling constant value around 5 Hz would be expected for this NOE-derived/MM3*-calculated conformation, in contrast with the experimental observation of 3.8 ± 0.3 Hz. Although the obtained value is indeed relatively close to the expected result, there is not a complete agreement between both quantities. Several reasons could account for the observed discrepancy. It could indicate a failure in the parametrization of the Karplus-type equation for trehalose derivatives. The coupling proton (H-1) is also attached to an anomeric carbon (C-1) which, in turn, additionally bears a highly electronegative substituent (O-5). The parametrization of the currently used Karplus-type equation [18] did not consider this arrangement. Since it has been reported that the presence of electronegative substituents on the carbon which bears the coupled proton indeed decreases the coupling values [19], this could probably be the origin of the discrepancy. Nevertheless, equally likely hypothesis are that the neglect of flexibility is an oversimplification or that there are variations of the torsion angles provided by the model.

In conclusion, we have shown that selective heteronuclear NMR methods may provide, fairly rapidly, distance constraints involving symmetry-related protons. These constraints are of relevant importance to deduce the three dimensional structure of these symmetric molecules. As a particular case, we have demonstrated the application of these methods for trehalose itself and for two trehalose-containing macrocycles, to unequivocally demonstrate that one of them (**2**) presents a fairly different conformation around the glycosidic linkages. In addition, it should be considered that care should be taken when using only vicinal heteronuclear coupling constants to deduce molecular conformations. In this context, more work seems to be necessary to improve the

parametrization of the current corresponding Karplus-type equation. The use of new experimental methods may help to the obtention of this conformationally relevant parameter.

References

[1] (a) D.J. Cram, *Angew. Chem. Int. Ed. Engl.*, 27 (1988) 1009–1020; (b) J.M. Lehn., *Angew. Chem. Int. Ed. Engl.*, 27 (1988) 89–112; (c) F. Diederich, *Angew. Chem. Int. Ed. Engl.*, 27 (1988) 362–386; (d) R.R. Bokownik and C.S. Wilcox, *J. Org. Chem.*, 53 (1988) 463–471.

[2] J.F. Stoddart, *Chem. Soc. Rev.*, 8 (1978) 85–110.

[3] F.H. Cano, J.L.G. de Paz, C. Foces-Foces, J. Jimenez-Barbero, M. Martin-Lomas, S. Penades, and C. Vicent, *Tetrahedron*, 49 (1993) 2109–2114.

[4] C. Vicent, C. Bosso, F.H. Cano, J.L.G. de Paz, C. Foces-Foces, J. Jimenez-Barbero, M. Martin-Lomas, and S. Penades, *J. Org. Chem.*, 56 (1991) 3614–3618.

[5] J. Jimenez-Barbero, E. Junquera, S. Sharma, C. Vicent, and S. Penades, *J. Am. Chem. Soc.*, 117 (1995) 10115–11204.

[6] C.K. Lee, in C.K. Lee (Ed.), *Developments in Food Carbohydrate*, Vol. 2, Applied Science Publishers, London, 1980, pp 1–89.

[7] D. Neuhaus and M.P. Williamson, *The Nuclear Overhauser Effect in Structural and Conformational Analysis*, VCH, New York, 1989.

[8] J.P. Carver, *Pure Appl. Chem.*, 65 (1993) 763–770.

[9] K. Bock, J. Defaye, H. Driguez, and E. Bar-Guilloux, *Eur. J. Biochem.*, 131 (1983) 595–600.

[10] (a) G.M. Brown, D.C. Rohrer, B. Berking, C.A. Beevers, R.O. Gould, and R. Simpson, *Acta Crystallogr.*, B28 (1972) 3145–3150; (b) T. Taga, M. Senma, and K. Osaki, *Acta Crystallogr.*, B28 (1972) 3258–3265; (c) G.A. Jeffrey and R. Nani, *Carbohydr. Res.*, 137 (1985) 21–30; (d) M.K. Dowd, P.J. Reilly, and A.D. French, *J. Comput. Chem.*, 13 (1992) 102–114; (e) C.A. Duda and E.S. Stevens, *J. Am. Chem. Soc.*, 112 (1990) 7406–7411; (f) P. Ram, L. Mazzola and J.H. Prestegard, *J. Am. Chem. Soc.*, 111 (1989) 3176–3182; (g) K. Bock, J. Fernandez-Bolaños Guzman, J. Duus, S. Ogawa, and S. Yokoi, *Carbohydr. Res.*, 209 (1991) 51–65.

[11] I. Tvaroska and L. Vaclavik, *Carbohydr. Res.*, 160 (1987) 137–149.

[12] D. Boudot, D. Canet, J. Brondeau, and J.C. Boubel, *J. Magn. Res.*, 83 (1989) 428–433.

[13] (a) J.M. Bernassau and J.M. Nuzillard, *J. Magn. Res. Ser. A*, 104 (1993) 212; (b) J.M. Nuzillard and J.M. Bernassau, *Tetrahedron Lett.*, 34 (1993) 469–472.

[14] J. Kawabata, E. Fukushi, and J. Mizutani, *J. Am. Chem. Soc.*, 114 (1992) 1115–1116.

[15] R. Wagner and S. Berger, *Abstracts XIII European Experimental NMR Conference, Paris*, 1996, p. 75.

[16] M. Liu, R.D. Farrant, J.M. Gilliam, J.K. Nicholson, and J.C. Lindon, *J. Magn. Reson. Ser. B*, 109 (1995) 275–283.

[17] F. Mohamadi, N.G.I. Richards, W.C. Guida, R. Liskamp, C. Canfield, G. Chang, T. Hendrickson, and W.C. Still, *J. Comput. Chem.*, 11 (1990) 440–467.

[18] I. Tvaroska, M. Hricovini, and E. Petrakova, *Carbohydr. Res.*, 189 (1989) 359–363.

[19] F.H. Cano, C. Foces-Foces, A. Alemany, J. Jimenez-Barbero, M. Martin-Lomas, and M. Bernabe, *J. Org. Chem.*, 52 (1987) 3614–3618.