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Laboratory Experiment

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NMR Determination of Hydrogen Bond Thermodynamics in a Simple Diamide: A Physical Chemistry Experiment

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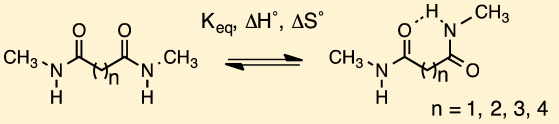
Supporting Information

ABSTRACT: Variable temperature NMR spectroscopy is used to determine the ΔH° and ΔS° of hydrogen bond formation in a simple diamide. In this two- or three-day experiment, students synthesize *N,N′*-dimethylmalonamide, dimethylsuccinamide, dimethylglutaramide, or dimethyladipamide from methylamine and the corresponding diester (typically in 50% recrystallized yield) and record NMR spectra at temperatures between 200 and 313 K. Solutions of *N*-methylacetamide in concentrations between 1.00 mM and 13.1 M (neat) are prepared and their NMR spectra recorded to determine the chemical shift of the amide proton in the hydrogen-bonded (δ_B) and nonbonded (δ_N) limits. By using these data, the equilibrium constants, ΔH°, and ΔS° for the conversion of an open-chain diamide to a cyclic structure with an intramolecular hydrogen bond are determined.



KEYWORDS: *Upper-Division Undergraduate, Laboratory Instruction, Physical Chemistry, Biophysical Chemistry, Amides, Noncovalent Interactions, Equilibrium, Hydrogen Bonding, NMR Spectroscopy, Thermodynamics*

INTRODUCTION

Hydrogen bonds are ubiquitous in biology and influence the structure of DNA, proteins, and the nature of protein−DNA interactions. In proteins alone, intramolecular hydrogen bonding contributes to the structure of the α-helix, the stability of parallel and antiparallel β-sheets, as well as type I and II hairpin turns, all of which have consequences for protein structure and function.[1,2] In polyamides such as nylon-6,6, the amide groups are aligned with significant interchain hydrogen bonding, which contributes to the stiffness, chemical resistance, and thermal stability of the polymer.[3]

In this experiment, we describe the synthesis of a series of *N,N′*-dimethyldiamide compounds of the type MeNHCO-$(CH_2)_n$CONHMe (n = 1, 2, 3, 4) following the literature synthesis of Freund.[4] Building on the model studies of Gellman and Adams,[5] the enthalpy and entropy of the hydrogen bonded form, relative to the solvated/nonbonded form, are evaluated by variable-temperature NMR (VT-NMR) spectroscopy with synthesis and NMR experiments to be completed in two or three lab periods. We have carried out this experiment in groups of four students (typically 16−24 students) who synthesize and record VT-NMR spectra for one of four *N,N′*-dimethyldiamide compounds.

This work complements other experiments published in the *Journal of Chemical Education* including computational studies of usnic acid,[6] the NMR characterization of the tautomerization of acetylacetone,[7] thermodynamics of DNA helix formation,[8] bonding in DNA salicylic acid/toluene,[9] and kinetics of amide bond rotation in *N,N*-dimethylacetamide.[10] However, to our knowledge, this is the only *JCE* experiment of its kind that models intramolecular hydrogen bonding in diamide compounds and allows for the determination of enthalpic and entropic contributions in this fashion. Although the diamides described here are known and similar in structure to diamide compounds reported in the literature, the enthalpy and entropy of hydrogen bond formation have not been reported in the literature (the *N,N,N′*-trimethyl derivatives were studied by Gellman and Adams[5]).

In addition to the minimal equipment required to perform rudimentary organic synthesis, this lab requires the availability of VT-NMR spectroscopy (^{1}H NMR spectra were initially recorded between 220 and 280 K, but we have also obtained satisfactory results between 263 and 313 K).

EXPERIMENTAL SECTION

General

All reagents were purchased from Aldrich and used without further purification. Temperature calibrations for VT-NMR experiments were performed using methanol (4% CH_3OH in CD_3OD) using the relation: T (K) = 418.39 − (49.74δ) − (19.74δ^2), where δ is the chemical shift difference (ppm) between the peaks arising from the methanol CH_3 and OH groups (note: this is different from δ measured in the solution studies of *N,N′*-dimethyldiamides).[11] VT-NMR spectra were recorded in CD_2Cl_2 or $CDCl_3$. All NMR experiments were performed on a Brüker ARX 300 MHz or Varian Avance 500 MHz NMR spectrometer, and chemical shifts are referenced to solvent residual proton shifts ($CHDCl_2$, 5.32 ppm; $CHCl_3$, 7.24 ppm). Owing to solvent exchange with amide protons, we found our results to be more reliable using oven-dried NMR tubes and new (or scrupulously dried) NMR solvents.

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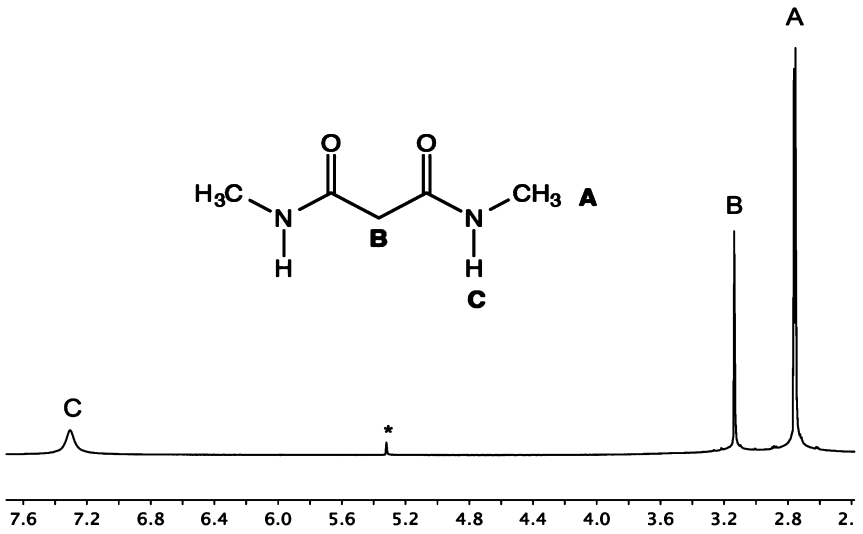


Figure 1. ^{1}H NMR spectrum of *N,N′*-dimethylmalonamide (ca. 10 mM in CD_2Cl_2) recorded at 300 K. In $CDCl_3$, the amide proton may obscure the residual solvent peak depending on the temperature.

Day 1

Synthesis of *N,N′*-Dimethylmalonamide.[4] This synthesis, including recrystallization, can be performed in one 3–4 h laboratory period. During the 1 h heating period, students can prepare the *N*-methylacetamide solutions for analysis by NMR (see the N-Methylacetamide Concentration Experiments section) so that the second day will be used only in the variable temperature measurements of *N,N′*-dimethyldiamides.

To a 50 mL round-bottom flask equipped with a reflux condenser and magnetic stir bar were added dimethylmalonate (5.79 mL, 50.0 mmol) and 41% by weight aqueous methylamine (8.66 mL, 100 mmol). The reaction mixture was heated at reflux temperature for 1 h, cooled to room temperature, and concentrated in vacuo to yield a slightly yellow oily solid. The crude product was recrystallized from hot ethyl acetate, isolated by vacuum filtration, and washed with cold diethyl ether to produce 3.06 g (47% yield) of white crystals. ^{1}H NMR (CD_2Cl_2) δ 2.75 (d, J = 9 Hz, 6H, N-CH_3), 3.14 (s, 2H, C-CH_2-C), 7.31 (br s, 2H, N-*H*).

Other *N,N′*-dimethyldiamide compounds (*N,N′*-dimethylsuccinamide, *N,N′*-dimethylglutaramide, and *N,N′*-dimethyladipamide) were synthesized from the corresponding diester. The methods for the synthesis are identical, but we noticed that the longer chain diamides required more ethyl acetate for the recrystallization than did *N,N′*-dimethylmalonamide.

Day 2

***N*-Methylacetamide Concentration Experiments.** A 10 M sample of *N*-methylacetamide in $CDCl_3$ was prepared and diluted to generate samples between 10 M and 1.0 mM. Room temperature proton NMR spectra were recorded with sufficient numbers of scans (typically 1–2 scans are necessary at the high concentration range, while 64 or more are necessary at the lower concentration range) to resolve the amide proton. For instructors who wish to use CD_2Cl_2, the published data of Gellmann and Adams are available,[5] and this step may be omitted. A plot of $\delta_{\underline{NH}}$ versus log[NMA] gives a sigmoid curve, but only the points where $\delta_{\underline{NH}}$ converge at low and high concentration are used in the determination of K_{eq}.

Day 3

VT-NMR of *N,N′*-Dimethylmalonamide. A saturated sample of *N,N′*-dimethylmalonamide (ca. 10 μM) was prepared in dry $CDCl_3$. Spectra were collected between 263 and 313 K in 5 K temperature increments starting at room temperature, and the chemical shift of the amide proton ($\delta_{\underline{NH}}$) was recorded.

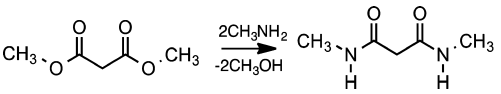
■ HAZARDS

Methylamine is corrosive and harmful if inhaled, swallowed, or absorbed through the skin. It causes eye and skin burns and may cause liver and lung damage. Chloroform is a potential carcinogen and a skin, eye, and lung irritant; it may affect the central nervous system, cardiovascular system, liver and kidneys, and may be a reproductive toxin. Dichloromethane is a probable carcinogen and a skin, eye, and lung irritant. A potent anesthetic, chronic exposure may cause bronchitis, liver, kidney, and pancreatic damage. Ethyl acetate is a skin and eye irritant and is hazardous when swallowed or inhaled.

■ RESULTS AND DISCUSSION

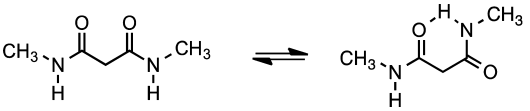
Synthesis and NMR Spectra of *N,N′*-Dimethylmalonamide

The diamide compound *N,N′*-dimethylmalonamide **1** is synthesized from dimethylmalonate and methylamine as shown in eq 1:



(1)

The ^{1}H NMR spectrum of **1** shows a single, broad resonance at 7.2 ppm in CD_2Cl_2 corresponding to the amide hydrogens, which exchange rapidly on the NMR time scale. In solution, *N,N′*-dimethylmalonamide exists in equilibrium between an open form (eq 2 at left) and a hydrogen-bonded form (eq 2 at right). In the open form,



(2)

there is a total of three rotational isomers in which each amide proton is *syn-* or *anti-* to the carbonyl oxygen, while in the

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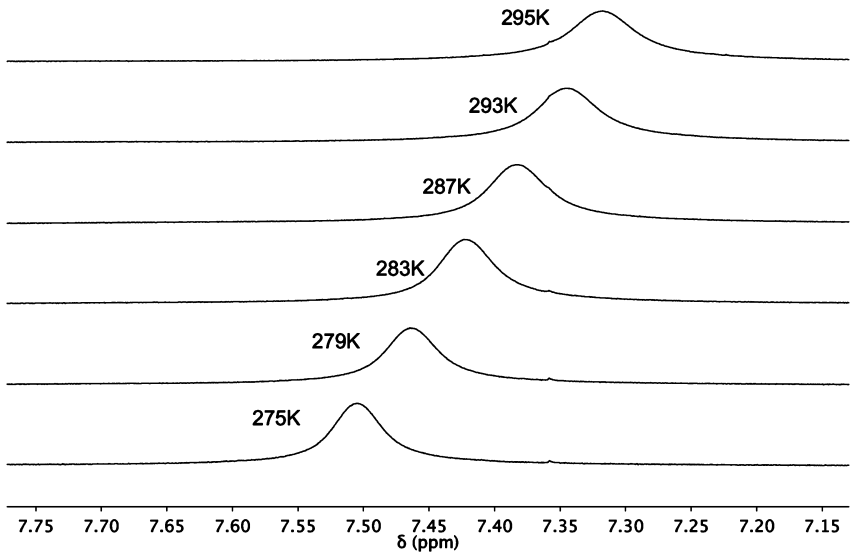

Figure 2. ^{1}H NMR spectra of about 10 μM *N*,*N*-dimethyl malonamide in CD_2Cl_2 showing the amide N-*H* region at temperatures between 2 and 22 °C.

closed form, there are two rotational isomers for the free −NH(Me) group. However, in *N*-methylamide derivatives, the *anti* form is preferred,[12] and accordingly, the NMR spectrum (Figure 1) shows a doublet for the *N*-methyl group, a narrow singlet for the methylene, and a broad singlet indicating the presence of a single rotational isomer. Moreover, the chemical shift of the amide proton depends on the environment: in the hydrogen-bonded form, the electron density at the amide hydrogen is reduced, and the chemical shift of that hydrogen moves to lower field. As the temperature is lowered, the proportion of the compound in the hydrogen-bonded form increases, and the amide proton shifts to lower field as shown in Figure 2. As demonstrated by Gellman and Adams,[5] the chemical shift of the amide proton can be used to calculate the equilibrium constant for this process.

Determination of the Equilibrium Constant from Chemical Shift Measurements

In the fast exchange limit of a chemical process in which two protons (N for the open-chain nonbonded form or B for the closed-ring hydrogen bonded form) occur in two exchangeable configurations (as open-chain vs closed-ring),

$$\mathrm{N} \rightleftarrows \mathrm{B} \tag{3}$$

the chemical shift, δ, is the weighted average of the chemical shifts of the protons in separate environments,[13] or

$$\delta = X_N\delta_N + X_B\delta_B \tag{4}$$

where X_N is the mole fraction of protons N in solution (X_N= [N]/([N]+[B]), and X_B is the mole fraction of protons B (X_B = [B]/([N]+[B]). By using these substitutions and expanding, we obtain

$$\delta = [N]\delta_N/([N] + [B]) + [B]\delta_B/([N] + [B]) \tag{5}$$

By multiplying both sides by ([N]+[B]), we obtain

$$([N] + [B])\delta = [N]\delta_N + [B]\delta_B \tag{6}$$

By solving for [B]/[N], an expression for the equilibrium constant is obtained that depends only on the observed (δ) and limiting (δ_N, δ_B) amide proton chemical shifts:

$$\frac{[B]}{[N]} = \frac{(\delta - \delta_N)}{(\delta_B - \delta)} = K_{eq} \tag{7}$$

To calculate the equilibrium constant from measurements of the amide NH chemical shift, the high and low chemical shift limits that characterize the hydrogen-bonded and nonbonded states are determined using the model compound *N*-methylacetamide (NMA, Figure 3).[14] At low (ca. 1 mM) concentration, the amide proton concentration in NMA is too low to form an intermolecular hydrogen bond, and the peak for this proton is observed at δ = 5.41 ppm. As [NMA] is increased, the chemical shift increases until it reaches 8.0 ppm at or above 10 M when all of the amide N−H bonds are fully hydrogen bonded. The chemical shifts of the fully hydrogen-bonded (δ_B) and non-hydrogen bonded (δ_N) states are similar to those reported by Gellman and Adams.[5] Although the chemical shift of the amide proton in NMA is temperature-dependent ($\Delta\delta/\Delta T$ = −0.002 ppm/K),[15] this dependence is smaller than the shift due to the formation of an intramolecular

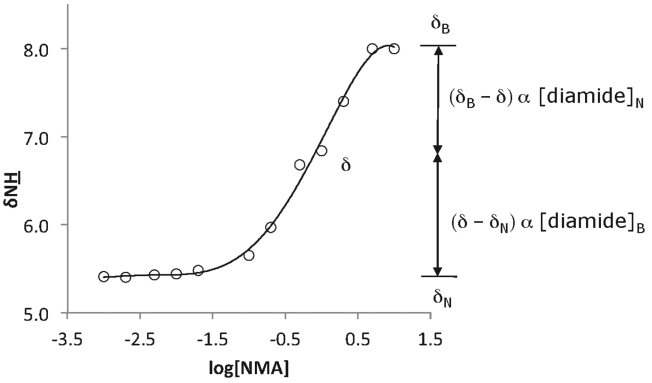

Figure 3. At left: plot of log[δ_{N-H}] shift as a function of log[*N*-methylacetamide] in $CDCl_3$ from ^{1}H NMR spectra recorded at 298 K. At low [NMA], the limiting N−H chemical shift, δ_N, is 5.41 ppm, which corresponds to the non-hydrogen-bonded form; at high [NMA], the limiting chemical shift, δ_B, is 8.00, which corresponds to the fully hydrogen-bonded form. At right: correlation of observed chemical shift with hydrogen bonding of of *N*,*N*′-dimethyldiamide.

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hydrogen bond; the choice of 5.41 as recorded at 298 K represents a compromise in the determination of thermodynamic parameters.[15]

Returning to the solution behavior of the *N*,*N*′-dimethyldiamide compounds, the amide proton chemical shift is recorded and the equilibrium constant calculated using eq 7. A qualitative explanation of the equilibrium constant as it relates to the observed chemical shift of *N*,*N*′-dimethyldiamides is also shown in Figure 3. Here, the difference between the observed shift and the nonbonded limit ($\delta - \delta_N$) is proportional to the concentration of hydrogen-bonded form, while the difference between the hydrogen-bonded limit and the observed shift ($\delta_B - \delta$) is proportional to the concentration of nonbonded form. Therefore, by measuring the chemical shift of a sample of unknown composition, it is possible to determine *K*, the equilibrium constant.

The chemical shift of the amide proton in *N*,*N*′-dimethyldiamides changes with temperature, which reflects the temperature dependence of the equilibrium constant in this system. The van't Hoff equation (eq 8) allows for the determination of the enthalpy ($\Delta H°$) and entropy ($\Delta S°$) differences between the hydrogen-bonded and nonbonded forms:

$$\ln K = \frac{\Delta S°}{R} - \frac{\Delta H°}{RT} \quad (8)$$

Van't Hoff plots are shown in Figure 4 with $\Delta H°$ (from the slope) and $\Delta S°$ (from the intercept) values recorded in Table

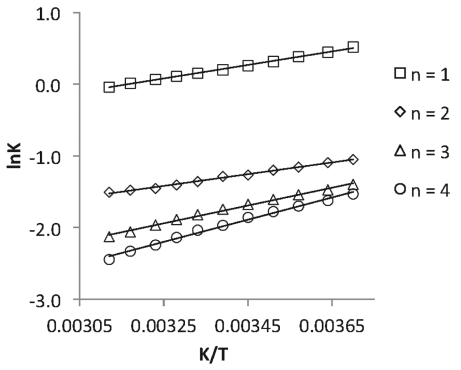

Figure 4. Plots of ln *K* versus $1/T$ for *N*,*N*′-dimethylmalonamide (n = 1), *N*,*N*′-dimethylsuccinamide (n = 2), *N*,*N*′-dimethylglutaramide (n = 3), and *N*,*N*′-dimethyladipamide (n = 4) in $CDCl_3$.

1. For *N*,*N*′-dimethylmalonamide alone, the hydrogen-bonded form is favored ($K > 1$) at most temperatures and is increasingly stabilized at lower temperature. The open-chain diamide is favored ($K < 1$) for all other diamides, but they are also increasingly stabilized at low temperature.

The negative $\Delta H°$ is similar to values (7−10 kJ/mol) obtained by Gellman and co-workers[15] and is consistent with a net stabilization of the diamide in the hydrogen-bonded form over the solvated form as observed with measurements on *N*,*N*,*N*′-trimethyl-α,ω-diamide, yet with some significant differences. Unlike the *N*,*N*,*N*′-trimethylmalonamide studied by Gellman and co-workers,[15] the range of δ_{obs} in *N*,*N*′-dimethylmalonamide indicates that this compound is not fully hydrogen-bonded at all temperatures. The low, but negative, $\Delta H°$ is consistent with the poorer hydrogen bond acceptor ability of the *N*-methylamide unit versus the *N*,*N*-dimethylamide unit as well as the 120° O−H−N bond angle required by

Table 1. Enthalpy and Entropy Changes for Hydrogen Bond Formation Among *N*,*N*′-Dimethyldiamides $MeNHCO(CH_2)_nCONHMe$ (n = 1, 2, 3, 4) in $CDCl_3$

n	$\Delta H°$ (kJ mol^{-1})[a]	$\Delta S°$ (J mol^{-1} K^{-1})[a]
1	−8.17 ± 1.10	−26 ± 5
2	−8.35 ± 1.42	−37 ± 3
3	−10.2 ± 0.9	−47.9 ± 1.7
4	−12.7 ± 0.6	−58.1 ± 1.7

[a] $\Delta H°$ and $\Delta S°$ are derived from van't Hoff plots (ln *K* vs $1/T$, shown in Figure 4 of equilibrium constant measurements obtained at temperatures between 270 and 320 K. Values at 95% confidence (N = 9) for $\Delta H°$ and $\Delta S°$ are also included.

the six-membered ring (a linear O−H−N unit is preferred[16]). While the hydrogen-bonded state is enthalpically favored, it is entropically disfavored. The negative $\Delta S°$ is consistent with the decrease in rotational and vibrational degrees of freedom in the hydrogen-bonded form. As expected, the equilibrium constant decreases with increasing temperature owing to the opposing influences of enthalpy ($\Delta H° < 0$) and entropy ($\Delta S° < 0$) in this system.

Effect of Chain Length on Enthalpy and Entropy Changes from Hydrogen Bond Formation

A comparison of the enthalpies and entropies of hydrogen bond formation across a range of *N*,*N*′-dimethyldiamides is instructive (Table 1). In general, the enthalpy change becomes more negative with increasing ring size in accord with the general preference of hydrogen bond angles (here N−H−O) toward linearity. At the same time, the entropy change becomes increasingly negative, which indicates a more significant loss of rotational and vibrational degrees of freedom for the long-chain diamides on formation of the hydrogen-bonded structure. Although the difference in enthalpy changes in n = 1 and n = 2 is not statistically significant for this data set, the only slight decrease in enthalpy for n = 2 versus n = 1 is consistent with a steric clash in the ring methylene hydrogens.[15] In the n = 1 compound, there is a single methylene group between the carbonyls, and the resulting six-membered ring hydrogen bonded structure is essentially planar. The n = 2 compound forms a seven-membered ring with adjacent methylenes in an eclipsed configuration. The influence of the increasingly linear N−H−O unit is attenuated by steric repulsion of these methylene groups. As the ring size is increased (for n = 3, 4), the methylene groups become staggered, and the effect of this steric clash is reduced.

Student Response and Suggestions for Adopters

The response from this experiment has been positive. Students and faculty appreciate the overlap of this experiment with physical chemistry, protein structure, and the use of spectroscopy to probe the populations of molecules in a two-state system. Although we highlighted only the use of NMR spectroscopy, this experiment illustrates the use of dynamic spectroscopy (the exchange is rapid on the NMR time scale) as a practical tool for evaluating a chemical equilibrium. Instructors could use this experiment as the basis for a discussion of the time scales of processes in solution as compared with the time scale of analytical techniques used to study them (see Supporting Information). In our experience, students are able to critically assess both the dynamic and thermodynamic features of particular spectroscopic techniques (we have run this experiment in parallel with an IR experiment

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that captures both the open and hydrogen-bonded form). In addition, the data that students accumulate individually are ultimately aggregated, and the resulting research "team" relies on the validity of each participant's data to draw reliable conclusions when they write-up their lab reports. In the context of an aggregated data set, this experiment provides a setting for a meaningful discussion of error analysis—both random and systematic—in research. In our experience, the van't Hoff plots are linear regardless of the amount of water in the NMR sample, and in the hands of less-experienced researchers, the contribution of water in the NMR solvent leads to an apparent increase (i.e., less negative) in $\Delta H°$. Consequently, the "best" result is not necessarily the average of many teams' efforts to study the same diamide but emerges from the sample that is most carefully prepared.

■ ASSOCIATED CONTENT

Supporting Information

A student handout with an introduction to the experiment, detailed instructions and questions to consider for a laboratory report as well as detailed notes for instructors with room temperature NMR spectra for *N*,*N*′-dimethyldiamides studied in this experiment, and suggestions for independent inquiry. This material is available via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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