Water Encapsulation in a Polyoxapolyaza Macrobicyclic Compound

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ABSTRACT: A new heteroditopic macrobicyclic compound (t₂pN₅O₃) containing two separate polyoxa and polyaza compartments was synthesized in good yield through a [1 + 1] “tripod−tripod coupling” strategy. The X-ray crystal structure of H₃t₂pN₅O₃³⁺ revealed the presence of one encapsulated water molecule accepting two hydrogen bonds from two protonated secondary amines and donating a hydrogen bond to one amino group. The acid−base behavior of the compound was studied by potentiometry at 298.2 K in aqueous solution and at ionic strength 0.10 M in KCl. The results revealed unusual protonation behavior, namely a surprisingly low fourth protonation constant contrary to what was expected for the compound. ¹H NMR and DOSY experiments, as well as molecular modeling studies, showed that the water encapsulation and the conformation observed in the solid state are retained in solution. The strong binding of the encapsulated water molecule, reinforced by the cooperative occurrence of a trifurcated hydrogen bond at the polyether compartment of the macrobicycle, account for the very low log K₄ value obtained.

INTRODUCTION

In the biological sciences it is widely recognized that the water−protein interactions are not only fundamental in folding, conformational stability and internal dynamics of proteins, but are also important as modulators of recognition, assembling and catalysis. In fact, the role of buried water molecules in protein stability and function is difficult to estimate experimentally, consequently it is useful to resort to model systems to investigate the modulation of physicochemical properties of host molecules by included water.

However the role played by the water in molecular recognition by synthetic receptors is often overlooked and it is rarely seen as an active player. In the very few examples where water binding was investigated, it was observed that encapsulation of water by nitrogen based receptors leads to abnormal protonation behavior.³ Lehn et al. reported that in a tetraoxatetraaza spheroidal macrotricyclic compound (L₁, Chart 1) the second protonation is as easy as the first one (log K₁H ≈ log K₂H = 10.5) whereas the third one is only possible at much lower pH values (log K₃H = 5.3). This result led to the formulation of the diprotonated species as a water cryptate, the water molecule being held in an ideal tetrahedral array of hydrogen bonds, accepting two hydrogen bonds from the ammonium sites and donating two hydrogen bonds to the amine sites.³a Reinhoudt et al. reported a study on the acidity and water binding properties of 2,6-pyridylo crown ethers of varied ring sizes (18 to 24-membered rings). In the case of the 18-membered macrocycle (L², Chart 1) the protonation constant of the pyridine nitrogen (log K₁H = 4.95) is 1.59 log units to higher than that of 2,6-bis(methoxymethyl)pyridine, its noncyclic analogue, which was attributed to a stabilization of the protonated species by the presence of a water molecule accepting a hydrogen bond from the pyridinium group and donating two hydrogen bonds to ether oxygen atoms, as evidenced by X-ray crystal structures.³b Bharadwaj et al. reported that a small polyoxapolyaza macrobicyclic compound (L₃, Chart 1) behaves in the same way as Lehn’s macrotricyclic one: the first two protonation constants are of the same

Chart 1. Compounds Used Previously in Water Binding Studies

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Scheme 1. Synthetic Procedure of t₂pN₅O₃

(Raw text content provided as an image)
tripodal compounds, representing the polyamine and polyether compartments of t2pN5O3, tbN4, and TEA, respectively (Chart 2), were also studied under the same experimental conditions for comparison purposes. The results are collected in Table 1, the corresponding species distribution diagram are represented in Figure 2, and the titration curves are presented in the Supporting Information (Figures S1−S4).

Four protonated species of t2pN5O3 were found in the working pH range, corresponding to the protonation of three secondary amines and a tertiary one.12 The tertiary amine of the tren subunit can only be protonated at very low pH values due to electrostatic repulsions.5c Interesting to note is the fact that the protonation constants of t2pN5O3 do not correspond to a combination of those of tbN4 and TEA (see Table 1). The first two protonation constants of t2pN5O3 can be unambiguously attributed to the protonation of two secondary amines (see the confirmation of the sequence of protonation in the NMR studies, below), and the values are in excellent agreement with the first two protonation constants of tbN4. The third and fourth protonation constants, however, are quite different from the expected values, taking into account the protonation constant of TEA and the third protonation constant of tbN4.

The third protonation constant of t2pN5O3 could, in principle, correspond to the protonation of either the tertiary amine of the polyether subunit or the third secondary amine of the tren subunit, as the protonation constant of the third secondary amine of tbN4 is 7.03 (in log units) and that of the tertiary amine of TEA 7.80 log units (see Table 1). The value of the fourth protonation constant is 4.41 (in log units), which is surprisingly lower than expected. This low value cannot be only explained by electrostatic repulsions within the cryptand cavity, since many polyaza cryptands of comparable size are known to be hexaprotonated at pH values of about 6,5c as is the case of t2pN8, Chart 1. The protonation constants of t2pN8, also determined at 298.2 K and ionic strength 0.10 M in KCl (see Table 1), have similar magnitude in pairs, as they correspond to protonation of amine centers at alternating positions in the macrobicyclic backbone, far from each other, and therefore the difference in values for each pair are mainly due to statistical

![Figure 1](image1.png)

**Figure 1.** Perspective views of [H3t2pN5O33+⊂H2O] showing different structural features of the inclusion compound: (a) view showing the encapsulated water molecule and the proton at the tripodal nitrogen involved in three hydrogen-bond interactions with H3t2pN5O33+; (b) space-filling model showing the water molecule encapsulated into the cryptand cage. Carbon, nitrogen, hydrogen, oxygen, and chlorine atoms are shown in gray, blue, white, red, and green, respectively. The hydrogen-bonding interactions to the tripodal proton are drawn as light blue dashed lines and the remaining ones as yellow dashed lines.

![Chart 2](image2.png)

**Chart 2.** Compounds Studied in This Work

for comparison purposes. The results are collected in Table 1, the corresponding species distribution diagram are represented in Figure 2, and the titration curves are presented in the Supporting Information (Figures S1−S4).

Four protonated species of t2pN6O5 were found in the working pH range, corresponding to the protonation of three secondary amines and a tertiary one.12 The tertiary amine of the tren subunit can only be protonated at very low pH values due to electrostatic repulsions.5c Interestingly to note is the fact that the protonation constants of t2pN5O3 do not correspond to a combination of those of tbN4 and TEA (see Table 1). The first two protonation constants of t2pN5O3 can be unambiguously attributed to the protonation of two secondary amines (see the confirmation of the sequence of protonation in the NMR studies, below), and the values are in excellent agreement with the first two protonation constants of tbN4. The third and fourth protonation constants, however, are quite different from the expected values, taking into account the protonation constant of TEA and the third protonation constant of tbN4.

The third protonation constant of t2pN5O3 could, in principle, correspond to the protonation of either the tertiary amine of the polyether subunit or the third secondary amine of the tren subunit, as the protonation constant of the tertiary amine of tbN4 is 7.03 (in log units) and that of the tertiary amine of TEA 7.80 log units (see Table 1). The value of the fourth protonation constant is 4.41 (in log units), which is surprisingly lower than expected. This low value cannot be only explained by electrostatic repulsions within the cryptand cavity, since many polyaza cryptands of comparable size are known to be hexaprotonated at pH values of about 6,5c as is the case of t2pN8, Chart 1. The protonation constants of t2pN8, also determined at 298.2 K and ionic strength 0.10 M in KCl (see Table 1), have similar magnitude in pairs, as they correspond to protonation of amine centers at alternating positions in the macrobicyclic backbone, far from each other, and therefore the difference in values for each pair are mainly due to statistical

![Figure 2](image3.png)

**Figure 2.** Species distribution diagram of the protonation of t2pN5O3 (b). C2p(NO3) = 1.0 × 10−3 M. Charges were omitted for clarity.

Table 1. Stepwise Protonation (K1H) Constants of t2pN5O3, tbN4, TEA, and t2pN6 in Aqueous Solutiona

<table>
<thead>
<tr>
<th>equilibrium reaction</th>
<th>t2pN5O3</th>
<th>tbN4</th>
<th>TEA</th>
<th>t2pN6</th>
</tr>
</thead>
<tbody>
<tr>
<td>L + H+ ⇌ HL+</td>
<td>9.25(1); [9.21(2)]</td>
<td>9.22(1)</td>
<td>7.80(1)</td>
<td>9.18(1)</td>
</tr>
<tr>
<td>HL+ + H+ ⇌ H2L2+</td>
<td>8.50(1); [8.62(2)]</td>
<td>8.43(1)</td>
<td>7.76(1)</td>
<td>7.35(1)</td>
</tr>
<tr>
<td>H2L2+ + H+ ⇌ H3L3+</td>
<td>8.18(1); [8.07(2)]</td>
<td>7.03(1)</td>
<td>7.35(1)</td>
<td>6.37(1)</td>
</tr>
<tr>
<td>H3L3+ + H+ ⇌ H4L4+</td>
<td>4.41(1); [4.74(2)]</td>
<td>1.56(2)</td>
<td>6.37(1)</td>
<td>5.73(1)</td>
</tr>
<tr>
<td>H4L4+ + H+ ⇌ H5L5+</td>
<td>6.37(1)</td>
<td>5.73(1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

T = (298.2 ± 0.1) K; I = (0.10 ± 0.01) M in KCl. Values in parentheses are standard deviations in the last significant figures. Values in brackets are log K1H values obtained after conversion of the log K1D determined in D2O from 1H NMR titration; see NMR titration below.

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Factors. Therefore, no abnormal protonation behavior was found in t2pN8.

The crystal structure of [(H5t2pN5O3)(H2O)]4+ (Figure 1) suggests that the fourth protonation occurs at a secondary amine and that its unusual low log K2H value is the result of the enclosed water molecule, which is so tightly bound that the protonation of the amine involved in the O–H···N hydrogen bond is highly disfavored. In addition, the trifurcated hydrogen bond taking place at the polyether compartment should contribute to stabilize a conformation of the macrobicycle, which reinforces the tight binding of the encapsulated water molecule. Indeed, it is very likely that the protonation of the tertiary amine of the polyether compartment is higher than that of model compound TEA, which means that in this case the diprotonated species is not found in t2pN8.

The solid lines are drawn by joining calculated values, obtained using the refined equilibrium constants and the individual chemical shifts. Both these diagrams were drawn over the corresponding speciation diagram. 

**NMR and Theoretical Studies.** In order to confirm the protonation sequence in solution, the titration of t2pN5O3 was followed by 1H NMR in D2O at 298.2 K (see Figure 3a and Figure S5 in the Supporting Information).

As pD decreases from 12.0, resonances 1, 2, 3, and 4, corresponding to protons on the polyamine compartment, shift downfield, as expected for resonances nearby secondary amines being protonated. In the 9.5–8.0 pD region, resonances 5, 6, 7, and 8, corresponding to protons in the polyether subunit, shift downfield due to the protonation of the tertiary amine. Consequently these results confirm that the third protonation occurs at the tertiary amine, as anticipated by the determined crystal structure. As the pD drops from 6.0 to 2.5, the protonation of the third secondary amine takes place, as evidenced by the downfield shift of resonances 1, 3, and 4. After conversion of the log K2H determined from this titration to log K1H13, it was found that both sets of values are in very good agreement (see Table 1).

The 1H NMR titration also provided very important information. For instance, it was found that resonance of protons 2 shift slightly upfield in the 6.0 to 2.5 pD region, instead of the expected downfield shift, which in addition should be of the same magnitude as resonance of protons 3 (Figure 3b). This suggests that a conformational rearrangement takes place in which protons 2 are positioned within the shielding region created by the p-xylyl ring, as further evidenced by the appearance of a cross peak correlating resonances of protons 2 and 4 in a NOESY spectrum recorded at pD 2.4, absent in the NOESY spectrum recorded at pD 6.8 (Figures S6 and S7 in the Supporting Information). Resonances of protons 6–8 are also shifted upfield in the 6.0–2.5 pD region (Figure 3b). As no protonation takes place near protons 6–8, the upfield shift of the corresponding resonances must also be due to the conformational rearrangement that should cause the benzene rings to change orientation in such a way that protons 6–8 become shielded. This is also in agreement with the fact that resonance 6, the closest proton to the benzene ring, is the most shifted.

The change in the value of the vicinal 1H−1H coupling constants of the protons of the ethylenic groups in the compound at the 2.5–6.0 pD region (Figure 4) gave further evidence of the conformational rearrangement. As shown in Figure 4, at pD 2.4, the resonances of protons 1 and 2 look essentially like perfect triplets, because JAB ≈ JAB = 6.8 Hz. This happens when the populations of the anti and gauche...
conformations are close to statistical values (33% anti; 67% gauche) as a consequence of unconstrained rotation of the XCH2−CH2Y bond. At pD 6.8, \( \gamma_{AB} \) and \( \gamma_{AB'} \) are no longer equal as seen by the different AA′BB′ splitting pattern (\( \gamma_{AB} = 6.7 \) Hz and \( \gamma_{AB'} = 3.2 \) Hz), which is in agreement with an increase of the population of the gauche conformations and a more restricted rotation of the XCH2−CH2Y bond, consistent with the encapsulation of a water molecule, as seen in the crystal structure. Spectra recorded in the 278.2−328.2 K temperature range and at pD = 6.8 (see Figure S8 in the Supporting Information) showed that, although the rotation of the XCH2−CH2Y bond have increased with the temperature, the free rotation is not achieved even at the highest temperature recorded.

The \( \gamma_{AB} \) and \( \gamma_{AB'} \) of the protons of the ethynyl groups of the polyether compartment are different (\( \gamma_{AB} = 6.1 \) Hz and \( \gamma_{AB'} = 3.6 \) Hz), which indicates a preference for the gauche conformers. Interestingly, the AA′BB′ splitting pattern of resonances of protons 7 and 8 is the same at both pD values which means that the conformation rearrangement of the compound does not affect the population distribution of the rotamers of the polyether subunit. This is in agreement with the trifurcated hydrogen bonding taking place at the polyether compartment which should increase the preference for the gauche rotamers at both pD values.

In order to provide further evidence of the conformational rearrangement, diffusion ordered spectroscopy (DOSY) NMR experiments were performed with samples of \( \text{t}_2\text{pN}_5\text{O}_3 \) at pD = 2.4 and 6.8. DOSY experiments allow the measurement of the diffusion coefficient of a given molecular species that relates directly to its hydrodynamic radius according to the Stokes–Einstein equation.\(^{14}\) It has been shown that this technique provides a way of determining small conformational or shape changes in molecular and supramolecular systems.\(^{15}\)

A recent study using the same NMR instrument\(^{16}\) illustrated that log \( D_t \) measurements could be measured with high precision for concentrated samples (greater than 1 mM). A change from log \( D_t = -9.394 \) at pD 2.4 to log \( D_t = -9.368 \) at pD 6.8 (+0.026 ± 0.003 log units) was determined for samples with chloride as the counterion.

The DOSY data clearly indicate that the diffusion properties of \( \text{t}_2\text{pN}_5\text{O}_3 \) are pH-dependent. The faster diffusion at higher pH indicates a smaller hydrodynamic radius, which is best explained by a more compact shape. We measured a sample of \( \text{t}_2\text{pN}_5\text{O}_3 \) with the much bulkier TsO− counterion at pD 2.4 to discriminate between shape changes related to the tightly bound water molecule observed by other methods, and the possibility of counterion association at low pD. The diffusion coefficient for the TsO− sample at pD 1.96 is log \( D_t = -9.402 \). This is slightly slower than the Cl− sample, but the difference of −0.008 ± 0.003 log units is equivalent to about 30 Da and much smaller than expected from the increased molecular mass of the TsO− complex compared to a Cl− complex (791 Da versus 637 Da). Therefore, we conclude that \( \text{t}_2\text{pN}_5\text{O}_3 \) forms a compact structure at pH 6.8 that is consistent with an organized cavity and tightly bound water molecule. This is supported by molecular dynamics simulations of the tri- and tetraprotonated species of \( \text{t}_2\text{pN}_5\text{O}_3 \), whose distributions of radius of gyration (Figure 5) correspond to an average increase of 5% with pH decrease, in agreement with the 6% increase of hydrodynamic radius indicated by the diffusion coefficients reported above; the radius of gyration is the mass-weighted root-mean-square atomic distance from the center of mass and is usually proportional to the hydrodynamic radius,\(^{17}\) so that their relative changes should be similar.

These data suggest that at low pH the XCH2−CH2Y bonds of the polyamine compartment rotate unconstrained while at higher pH such rotation is hindered because of the presence of an encapsulated water molecule, as depicted in Figure 6.

### CONCLUSION

A new heteroditopic polyoxapolyaza macrobicyclic compound was synthesized in good yield through a [1 + 1] “tripod−tripod coupling” strategy. The X-ray crystal structure of \( \text{H}_3\text{t}_2\text{pN}_5\text{O}_3^{3+} \) revealed an encapsulated water molecule strongly bound by accepting two hydrogen bonds from two protonated secondary amines and donating a hydrogen bond to an amino group. On the other hand, the study of the acid–base properties of \( \text{t}_2\text{pN}_5\text{O}_3 \) showed an unusual protonation behavior, namely a value of 4.41 log units for the forth protonation constant, which is surprisingly lower than the expected one, and that cannot be explained by electrostatic repulsions within the cryptand cavity alone. Structural information in aqueous solution provided by NMR experiments showed that rotation of the XCH2−CH2Y bonds of the polyamine compartment of \( \text{H}_3\text{t}_2\text{pN}_5\text{O}_3^{3+} \) is hindered, suggesting also the presence of one encapsulated water molecule. DOSY experiments indicated a decrease in hydrodynamic radius at higher pD, which was also supported by molecular dynamics simulations, which is consistent with a more compact molecular shape.

All these results suggest that the fourth protonation occurs at a secondary amine and that its unusual low log \( K_a^{3+} \) value is the
result of the enclosed water molecule, which is so tightly bound that the protonation of the amine involved in the O--H···N hydrogen bond is highly disfavored. In addition, the trifurcated hydrogen bond taking place at the polyether compartment should contribute to stabilize a conformation of the macrobicyclic scaffold, which reinforces the tight binding of the encapsulated water molecule. Indeed, the trifurcated hydrogen bond formation preorganizes the macrobicyclic in such a way that the binding of water is easier than that of a binding of another proton. The estimated association constant of the included H2O molecule is around 2.60 log units, which is a high value for the binding of a neutral molecule in highly polar medium.

In conclusion, a large set of experiments performed in aqueous solution, using methods so different as potentiometric measurements, NMR titration, NOESY and DOSY, and molecular dynamics, point for the presence of a water molecule encapsulated into the cavity of H3tpN5O3 forming the [H3tpN5O3]3⁻ entity in agreement with the single-crystal X-ray structure determination.

**EXPERIMENTAL SECTION**

**General Considerations.** All solvents and chemicals were commercially purchased reagent grade quality and used as supplied without further purification, except for triethanolamine hydrochloride, which was recrystallized from ethanol. The tribN₃(tpN₅s)₄ (diethoxymethylphenyl)methanol, and 1,3,5-tris(bromomethyl)-2,4,6-triethylbenzene compounds were prepared according to literature methods.¹⁸⁻²¹ NMR spectra used for characterization of products were recorded on a 400 MHz instrument. NMR spectra used in the H NMR titration were recorded on a 300 MHz instrument. The DOSY experiments were carried out in a 500 MHz instrument. TMS was used as reference for the H NMR measurements in CDCl₃ and in D₂O the experiments were carried out in a 500 MHz instrument. TMS was used based on peak integration and multiplicity for 1D H spectra and on 2D COSY, NOESY and HMBC experiments (Figures S5–S19 in the Supporting Information).

**Syntheses.** 4-(Bromomethyl)benzaldehyde. A mixture of 4-diethoxymethylphenyl)methanol (2.447 g, 11.6 mmol) and HBr 65% (2.447 g, 11.6 mmol) was added dropwise over 20 min to a magnetically stirred solution of 4-(bromomethyl)benzaldehyde (2.190 g, 11.1 mmol), triethylbenzene compounds were prepared according to literature methods.¹¹ 

Potentiometric Measurements. Reagents and Solutions. All the solutions were prepared using demineralized water. Carbonate-free solutions of the KOH titrant were prepared from a commercial ampule diluted with 1000 mL of water (freshly boiled for about 2 h and allowed to cool under nitrogen). These solutions were discarded every time carbonate concentration was about 0.5% of the total amount of base. The titration solutions were standardized (tested by measurement of the electromotive force of the cell, considering reaction constants (being values of two consecutive constants provide the stepwise (log $K_j$) determination from this titration to log $K_j$) performed using the equation log $K_j = \text{log } K_j^\circ - \text{log } K^\circ = 0.076 \text{log } K^\circ - 0.05$.

Determination of Vicinal $H-H$ Coupling Constants. Solutions of the (H$_4$t$_2$pN$_5$O$_3$)(Cl)$_4$ were prepared in D$_2$O (2.0 $\times$ 10$^{-3}$ M) and the pD was adjusted to 2.40 and 6.80 by addition of DCl or KOD with pH meter instrument fitted with a combined microelectrode. The $^1$H NMR spectra were recorded on a 400 MHz instrument. The AA'B' spin systems involving were simulated with the spectral analysis routine NUMMRIT (including iteration) within the software SpinWorks.

DOSY. Selected samples prepared in D$_2$O were analyzed by $^1$H DOSY NMR spectra, recorded on a 500 MHz instrument fitted with a triple resonance probe and Great 50/10 gradient synthesizer ($3.5$ G/cm micromolar output). This protocol followed the one published according to Groves et al.

Crystallography. The single-crystal X-ray data of [H$_3$t$_2$pN$_5$O$_3$(H$_2$O)]$^-$ were collected at 150(2) K with graphite-monochromatized Mo Kα radiation ($\lambda = 0.71073$ Å). The selected crystal was positioned $35$ mm from the CCD and the spots were measured with a counting time of $100$ s. Data reduction including a multiscan absorption correction was carried out using the SAINT-NT software package. The structure was solved by a combination of direct methods with subsequent difference Fourier syntheses and refined by full matrix least-squares on $F^2$ using the SHELEX-97 suite. Anisotropic thermal displacements were used for all non-hydrogen atoms. The hydrogen atoms of the C–H bonds were placed at geometrical positions and refined with $U_{eq} = 1.2U_{eq}$ of the atom to which they are attached. The atomic positions of the hydrogen atoms of the amine groups and of seven crystallization water molecules were discernible from difference Fourier maps, and they were included in the structure refinement with individual isotropic thermal parameters. The eighth water molecule was found to be disordered over two positions, which were inserted in the structure refinement with refined occupancies of 1 $-$ $x$ and $x$, $x$ being 0.55(1). Therefore, the hydrogen atoms of this single molecule were not considered in the last refinements of the crystal structure. Molecular diagrams were drawn with PyMOL software. The crystal data and refinement details are summarized in Table S2 in the Supporting Information.

Molecular Modeling. Molecular dynamics simulations of H$_2$t$_2$pN$_5$O$_3$ and H$_2$t$_2$pN$_5$O$_3$$^+$ in water were performed with GROMACS 4.0.4. Parameters compatible with the GROMOS96 53A6 force field were obtained using the Automated Topology Builder for both species of t$_2$pN$_5$O$_3$, and the SPC model was used for water. The nonbonded interactions were treated using a twin-range cutoff of 8/14 Å, a neighbor lists update every 10 fs, and the reaction field method to treat long-range electrostatics with a dielectric constant of 54. Berendsen coupling was used to maintain the temperature at 300 K (temperature coupling of 0.1 ps) and pressure at 1 atm (isothermal compressibility of 4.5 $\times$ 10$^{-5}$ bar$^{-1}$ and pressure coupling of 0.5 ps). The time step was 2 fs, and all bonds were constrained with the LINCS algorithm. A standard protocol was used to minimize and equilibrate the system before running the 50 ns long simulations.
ASSOCIATED CONTENT

Supporting Information

Titration curves of all studied compounds, 1H NMR spectra recorded in the course of the titration of H2t2pN5O3 with KOD in D2O; NOESY spectra of t2pN5O3 in D2O at pH = 2.4 and 6.8; variable-temperature 1H NMR spectra t2pN5O3 in D2O at pH = 6.8; NMR (1H, 13C, COSY, NOESY, and HMBC) and ESI-mass spectra of the reported compounds; table of hydrogen bonding parameters of the crystal structure; table with the crystal data and selected refinement details. This material is available free of charge via the Internet http://pubs.acs.org.

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The authors declare no competing financial interest.

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