Quantitation of the Anomeric Effect in Adenosine and Guanosine by Comparison of the Thermodynamics of the Pseudorotational Equilibrium of the Pentofuranose Moiety in N- and C-Nucleosides

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Abstract: The effect of N-aglycones at C1' on the drive of two-state north (N (C3'-endo-C2'-exo)) = south (S (C2'-endo-C3'-exo)) pseudorotational equilibrium in β-D-ribofuranosyl-N-nucleosides consists of two counteracting contributions from (i) the anomeric effect (stereoelectronic interactions between furanose O4' and the nucleobase nitrogen at C1'), which places the aglycone in the pseudosxial orientation, and (ii) the inherent steric effect of the nucleobase, which opposes the anomeric effect by its tendency to take up pseudoequatorial orientation. The maximum pseudoequatorial orientation of the nucleobase is sterically possible only in the S-type conformations. Therefore, the extent of this pseudoequatorial orientation can be experimentally determined by the extent of the thermodynamic stabilization of the S conformer in the two-state N = S equilibrium (ref 1). This means that a direct measurement of the extent of thermodynamic stabilization of the S conformer among a set of various β-D-ribofuranosyl-C-nucleosides, where the absence of the anomeric effect has been previously established from X-ray studies (ref 6), should give us a reference point for the maximally pseudoequatorially oriented C-nucleobase. We report here the characterization of this reference β-D-ribofuranosyl-C-nucleoside. The subtraction of the ΔH° of its pseudorotational equilibrium from the ΔH° of β-D-ribofuranosyl-N-nucleosides gave the quantitative insight of the anomeric effect in adenosine (+9.1 kJ/mol) and guanosine (+10.5 kJ/mol) for the first time. We have found that the C-aglycones in formycin A (1) and B (2) constitute the optimal reference point (ref 12) for our purpose because they take up the predominantly favored pseudoequatorial orientation (closer to the limit), where the steric control is almost the exclusive determinant, owing to the negligible presence of any stereoelectronic interaction with the lone pairs of furanose-O4' (see Table 3).

Introduction

The drive of the two-state N = S pseudorotational equilibrium of the sugar moiety of β-D-ribofuranosyl-N-nucleosides in solution is energetically controlled by various stereoelectronic gauche and anomeric effects.3,11 The gauche effects of O4' → C4' → C3' → O3' and O2' → C2' → C1' → O1' fragments drive the sugar pseudorotational equilibrium toward S,1a whereas it is driven to N by the gauche effect of O4' → C1' → C2' → O2' (Scheme 1).

The X-ray crystal structures of N-nucleosides show the shortening of the O4' → C1' bond relative to C4' → O4' by about 0.03 Å, which has been considered as a manifestation of the anomeric effect. The origin of the anomeric effect has been previously attributed to the following: (i) favorable n → σ* molecular orbital overlap which favors pseudosxial orientation of the N-substituent, (ii) destabilization of the pseudoequatorial

Scheme 1. Dynamic Two-State Equilibrium of N = S Pseudorotamers of β-D-Ribofuranosyl-C-nucleosides in Solution

North sugar (C3'-endo-C2'-exo) South sugar (C2'-endo-C3'-exo)

In order to achieve minimal dipole-dipole electrostatic interaction, or (iii) a hyperconjugative effect.5 In β-D-ribofuranosyl-C-nucleosides, however, the difference between O4' → C1' and O4' → C4' bond lengths is much smaller (0.01 Å) compared to β-D-ribofuranosyl-N-nucleosides (0.03 Å), which suggests that the aglycone in the former does not induce any anomeric effect. On the basis of a regression analysis performed in a parallel study,1a on a set of ΔH° values for the N = S pseudorotational equilibrium of (S)-tetrahydrofurfuryl alcohol, 1-deoxy-D-ribofuranose, 1,2-dideoxy-D-ribofuranose, and 2',3'-dideoxy-β-D-ribofuranose, 2'-deoxy-β-D-ribofuranosyl-, and β-D-ribofuranosyluridine and -pyrimidine derivatives, we derived the ΔH° contribution for the sum of the intractable anomeric and steric effects in adenosine, guanosine, and cytidine,1a and also the gauche effect involving O2' and anomeric nitrogen (i.e., O2' = C2' = C1' = N-purine).9 A qualitative comparison of the ΔH° values,7a for the N-substituent effect, however, clearly showed that the larger ΔH° value for cytidine compared to


The gauche effects of $O^4' - C^4' - C^3' - O^3'$ and $O^4' - C^2' - C^1' - O^2'$ fragments and the effect of the 4'-CH$_2$-OH group are a constant factor in both $\beta$-d-ribofuranosyl-N- and -C-nucleosides, while the strengths of the gauche effect of the $O^2' - C^2' - C^1' - N$-substituent in N-nucleosides or the aromatic and stereoelectronic effects of the aglycone in 8-aza-9-deazad-adenosine (formycin A 1), 8-aza-9-deazainosine (formycin B 2), 9-deazaadenosine (3), tiazofurin (4), $\Psi$-isocytidine (5), $\Psi$-isocytidine hydrochloride (6), 1-methyl-$\Psi$-uridine (7), 1,3-dimethyl-$\Psi$-uridine (8), and $\beta$-d-ribofuranosyl-N-nucleosides 9-14 on the drive of the pseudorotational equilibrium of the constituent sugar depend on the chemical nature of purine or pyrimidine heterocycles at C1' compared to the reference compound 1-deoxy-d-ribofuranose 9.14

![Diagram](image)

We have earlier shown14 through a comparison of the $\Delta$H$^o$ values of the pseudorotational equilibria in 5-8 that the substituent

\[(\text{5}) (\text{a}) \text{ The differences between } C^4' - O^4' \text{ and } O^4' - C^1' \text{ bond distances in X-ray crystal structures of most } \beta\text{-d-ribofuranosyl-N-nucleosides are smaller (} \approx 0.01 \text{ A} \text{) than in N-counterparts (} \approx 0.03 \text{ A} \text{), and therefore we conclude that the anomic effect is absent in } \beta\text{-d-ribofuranosyl-C-nucleosides. For the X-ray crystal structures of pyrimidine } \beta\text{-d-ribofuranosyl-C-nucleoside 4-thio-} \Psi\text{-uridine, see: } \text{Barnes, C. L.; Hawkinson, S. W.; Wigles, P. W. Acta Crystallogr., Sect. B 1980, 36, 2399. For } \Psi\text{-isocytidine hydrochloride, see: } \text{Birnbaum, G. I.; Watanabe, K. A.; Fox, J. J. Can. Chem. 1980, 58, 1633. For } 2\text{-}[^{13}C] \text{-d-ribofuranosyl-N-nucleosides, see: } \text{Pankiewicz, K. W.; Watanabe, K. A.; Takayanagi, H.; Itoh, T.; Ogura, H. J. Comput. Chem. 1985, 22, 1703. The X-ray crystal structures of some } \beta\text{-d-ribofuranosyl-C-nucleosides have also been elucidated by the following: Koyama, G.; Nakamura, H.; Umezawa, H.; Iitaka, Y. Acta Crystallogr., Sect. B 1986, 42, 813 (for formycin B 2); Abola, J. E.; Sims, M. J.; Abraham, D. J.; Lewis, A. F.; Townsend, L. S. J. Chem. Soc., Perkin Trans. 1989, 11, 1996. (b) From a X-ray study (Goldstein, B. M.; Takusagawa, T.; Panzica, R. P.; Smith, J. C.; Pugmire, R. J.; Grant, D. M.; Townsend, L. S. J. Chem. Soc., Perkin Trans. 1992, 76, 1291 (for formycin C 2); Abola, J. E.; Sims, M. J.; Abraham, D. J.; Lewis, A. F.; Townsend, L. S. J. Chem. Soc., Perkin Trans. 1993, 11, 1996.) We have also been able to determine the pseudorotational equilibria of the $\beta$-d-ribofuranosyl-N-nucleosides by the Karplus-Altona equation,29 and the errors have been assessed in terms of root mean square and the largest deviation between the experimental and calculated coupling constants ($\Delta$Jcalc) using the

Table 1. Vicinal $^{1}J_{HH}$ Coupling Constants and Distribution of the Rotamers around the C4'-C5' Bond for 1-4 at Two Extreme Temperatures

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\( ^{a} J_{HH} \) (In Hz, error $\pm 0.1$ Hz) were extracted from 1D 500-MHz $^{1}H$-NMR spectra recorded in D$_2$O solution in 10 K steps between 278 and 358 K for 1, 2, and 4, between 278 and 363 K for 3. All spectra were simulated using the DAISY program.30 The populations of the $\gamma$-rotamers across the C4'-C5' bond were calculated following the procedure of Haasnot et al. (Recl. Trav. Chim. Pays-Bas 1979, 98, 576). Negligible changes in the chemical shift (<0.05 ppm) of all protons over the whole temperature range (278-358 K) suggest the absence of aggregation at 20 mM concentration for 1-4.\( ^{b} J_{HH} \) values have been calculated using the DAISY, Spin Simulation Program, was provided by Bruker.\( ^{c} J_{HH} \) values have been recalculated using the PSEURO$^0$ program (version 5.4 with the latest $\chi$-electronegativity set from July, 1992); see footnotes of Table 2). These new $\Delta$H$^o$ values have been the basis for an updated regression analysis using our methodology that is almost described in ref 1a. In this new regression analysis we have also taken into consideration the $\Delta$H$^o$ values for the pseudorotational equilibrium of thymidine, uridine, 3'-AMP, 2'-dAMP, 3'-ethylphosphate of 2'-dA, 3'-GMP, 2'-dGMP, 3'-ethylphosphate of 2'-dG, 3'-UMP, 2'-dUMP, 3'-TMP, and 3'-ethylphosphate of T$^{\text{II}}$ in addition to the ones described29 (a total set of 30 compounds for the new extended version of the old regression analysis).41 As a result of this extended regression analysis, the standard deviations of the values of the stereoelectronic effects varied between 0.4 and 1.1 kJ/mol and are reduced compared to old standard deviations (1.3 $\pm$ 0.5) described in ref 1a. (Unpublished work.)


(10) DAISY, Spin Simulation Program, was provided by Bruker. For the IUPAC nomenclature of these heterocycles see: Chenon, M.-T.; Panzica, R. P.; Smith, J. C.; Pugmire, R. J.; Grant, D. M.; Townsend, L. B. J. Am. Chem. Soc. 1976, 98, 4736.

(11) PROFIT II 4.1, Quantum Soft, Postfach 6613, CH-8023 Zürich, Switzerland, 1993.
Table 2. Hyperspace of Geometries of the N and S Sugar Pseudorotamers Assessed by the PSEUROT analyses of vicinal $J_{HH}$ of C-Nucleosides 1-4

<table>
<thead>
<tr>
<th>compds</th>
<th>(i) $\Psi_N = \Psi_S$ fixed</th>
<th>(ii) $P_N$ and $\Psi_N$ fixed</th>
<th>rms (Hz)</th>
<th>$\Delta J_{max}$ (Hz)</th>
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<td>1</td>
<td>$[42^\circ &lt; P_N &lt; 11^\circ]$ = $[126^\circ &lt; P_S &lt; 138^\circ]$</td>
<td>$127^\circ &lt; P_S &lt; 144^\circ$ with $32^\circ &lt; \Psi_N &lt; 46^\circ$</td>
<td>$&lt;0.2$</td>
<td>$&lt;0.5$</td>
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<td>2</td>
<td>$[42^\circ &lt; P_N &lt; 11^\circ]$ = $[125^\circ &lt; P_S &lt; 134^\circ]$</td>
<td>$126^\circ &lt; P_S &lt; 141^\circ$ with $32^\circ &lt; \Psi_N &lt; 46^\circ$</td>
<td>$&lt;0.2$</td>
<td>$&lt;0.5$</td>
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<td>3</td>
<td>$[-48^\circ &lt; P_N &lt; -17^\circ]$ = $[128^\circ &lt; P_S &lt; 138^\circ]$</td>
<td>$132^\circ &lt; P_S &lt; 146^\circ$ with $30^\circ &lt; \Psi_N &lt; 40^\circ$</td>
<td>$&lt;0.2$</td>
<td>$&lt;0.5$</td>
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<td>4</td>
<td>$[-30^\circ &lt; P_N &lt; 33^\circ]$ = $[120^\circ &lt; P_S &lt; 148^\circ]$</td>
<td>$120^\circ &lt; P_S &lt; 148^\circ$</td>
<td>$&lt;0.2$</td>
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* The PSEUROT analyses (24 calculations for 1-3, 11 for 4) of the temperature-dependent $J_{HH}$ have been performed either (i) by fixing $\Psi_N$ of both N and S pseudorotamers to an identical value in the range 35-45$^\circ$ for 1 and 2, 35-43$^\circ$ for 3, and 34-44$^\circ$ for 4 (1$^\circ$ resolution) or (ii) by constraining successively $P_N$ of the minor N pseudorotamer to $-36^\circ$, $-18^\circ$, $0^\circ$, $18^\circ$, and $36^\circ$ with $\Psi_N$ set to $37^\circ$, $41^\circ$, and $45^\circ$ for 1 and 2 and to $35^\circ$, $39^\circ$, and $43^\circ$ for 3. The following $\lambda$-electron negativity values were used in the PSEUROT input files for the substituents on $C_\text{C}$-$C$-$H$ fragments: $O^\dagger$, 1.27; $C$-aglycone, 0.45; $OH$, 1.26; $C_1'$, $C_2'$, $C_3'$, $C_4'$ 0.62; and $C_5'$, 0.68. $\Delta J_{max}$ is the largest deviation observed between experimental $J_{HH}$ and the coupling constants back-calculated using both PSEUROT fitting processes.

Figure 1. Van't Hoff plots of $\ln(X_N/X_S)$ as a function of 1000/T for 1 (A), 2 (B), 3 (C), and 4 (D). The natural logarithm of the mole fractions $X_N$ and $X_S$ from various PSEUROT analyses of temperature-dependent $J_{HH}$ was used to perform least-squares fitting processes with PROFIT to give as many straight lines as possible (see Table 2 for the conformational space covered by the PSEUROT analyses). For clarity we only show representative plots based on the following PSEUROT analyses: $\Psi_N$ of both N and S conformers were constrained to $37^\circ$ (+), $41^\circ$ (X), and $45^\circ$ (O) for 1 and 2, to $35^\circ$ (+), $39^\circ$ (X), and $43^\circ$ (O) for 3, and to $36^\circ$ (+), $37^\circ$ (+), $38^\circ$ (X), $39^\circ$ (O), $40^\circ$ (O), and $41^\circ$ (O) for 4. $P_N$ was also set to $0^\circ$ for 1 and 2 ($\Psi_N = 37^\circ$ (O)), $41^\circ$ (X), $45^\circ$ (A)) and 3 ($\Psi_N = 35^\circ$ (O), $39^\circ$ (X), and $43^\circ$ (O)). Individual enthalpy ($\Delta H^\circ$) and entropy ($\Delta S^\circ$) values were derived from the slopes and intercepts, respectively, of each van't Hoff plot according to the relation $\ln(X_N/X_S) = -(\Delta H^\circ/R)(1000/T) + \Delta S^\circ/R$ and were used subsequently to calculate average $\Delta H^\circ$ and $\Delta S^\circ$ contributions to the N $\Rightarrow$ S equilibrium of 1-4 (Table 3).

Results and Discussion

At 298 K, for the purine $\beta$-d-ribofuranosyl-C-nucleosides 1-3 and $\psi$-isocytidine (S), the $\Delta H^\circ$ contribution prevails over the countering $-T\Delta S^\circ$ term and drives the N $\Rightarrow$ S pseudorotational equilibrium to the S conformer (Table 3). On the other hand, the $\Delta H^\circ$ contribution for the $\beta$-d-ribofuranosyl-C-nucleoside 4, 7, and 8 drives the sugar conformation to the N, and it is overridden at 298 K by the $-T\Delta S^\circ$ term, which favors S-type pseudorotamers, as evident from the ca. 1:1 ratio of N and S conformers at the pseudorotational equilibrium. A particular heterocycle at $C_1'$ in purine $\beta$-d-ribofuranosyl-C-nucleoside 3 or in thiazolo 4 and

Program PSEUROT (see Table 2). Figure 1 shows the typical van't Hoff plots of the natural logarithm of the ratio of the populations of N and S conformers obtained from PSEUROT analyses, whereas Table 3 shows experimental enthalpy ($\Delta H^\circ$) and entropy ($\Delta S^\circ$) values of the two-state N $\Rightarrow$ S pseudorotational equilibria of the sugar moieties of 1-4 and 5-8 (see Experimental Section for the details of the methodology). Note that we have only considered a two-state dynamic N $\Rightarrow$ S pseudorotational equilibrium for the van't Hoff-type analysis since there is no experimental evidence (NMR) for a third state (see refs 15-17 in ref 1d and ref 17 in ref 1e) in the solution.
pyrimidine β-D-ribofuranosyl-C-nucleosides 5–8 influences the drive of the N = S pseudorotational equilibrium by combination of both steric and stereoelectronic effects,18b whereas the orientation of the purine heterocycle at C1' in 1 and 2 is almost exclusively determined by the steric effect (vide infra).

The overall C-substituent effect of a specific aglycone can be assessed by simple subtraction of the ΔH° value for 1-deoxyribonanosyl (9) from that of a β-D-ribofuranosyl-C-nucleoside 1–8. (For ΔH° (i.e. C-substituent) effect values see column 5 in Table 3. The larger positive to larger negative ΔH° and ΔΔH° values in Table 3 suggest more N to more S sugar stabilization, respectively.)

Since steric hindrance in a β-D-ribofuranosyl-C-nucleoside is reduced by placing its C1'-substituent in the pseudorotational orientation, which is achieved in S-type conformations, the more the S sugar pseudorotamer is stabilized, the larger the negative ΔΔH° value, and the larger the magnitude of the pseudorotational orientation of the C1' substituent. A perusal of the data reported in Table 3 clearly shows that the purine aglycones in 1 and 2 are in purer pseudorotational orientation than in 3. The order of the purity of sterically dictated pseudorotational orientation amongst thiazolo and pyrimidine β-D-ribofuranosyl-C-nucleosides is difficult to distinguish wholly on the basis of the steric bulk because in tiazofurin (4) a specific interaction involving thiazole-sulfur and furanose-O4' is evident from its X-ray structure.65 It is, however, more straightforward to classify the C-aglycones in pyrimidine β-D-ribofuranosyl-C-nucleosides 1–3 and pyrimidine β-D-ribofuranosyl-5-C-nucleosides 5–8 on the basis of the extent of the pseudorotational orientation based on ΔΔH° values, as shown in Table 3: purine (1 = 2 > 3) >> pyrimidine (5 > 7 > 8 > 6).

Within both groups, the steric contributions are, however, counteracted by the inherent stereoelectronic effect, which increases in the reverse order as the C-aglycone takes up the pseudooxial orientation, which in turn pushes the N = S pseudorotational equilibrium to the more N-type sugar.

The negative sign and magnitude of the ΔH° values of the N = S equilibria in formycin A (1) and B (2) suggest that the interaction between the π-electron system involving the N8–C9 double bond and O4', owing to the conjugation of the N8–C9 double bond with the conjugated fused π-deficient pyrimidine system, is minimal or is closer to the limit where the steric control is almost exclusively dominant, resulting in the maximal pseudooxial orientation of the C-aglycone.12 The almost identical ΔH° values for formycin A (1) and B (2) show that π-electron clouds involving the N8–C9 double bonds are delocalized to a similar extent in both pyrazolo[4,3-d]pyrimidine moieties. Thus, the nature of the pyrimidine part in the pyrazolo[4,3-d]pyrimidine moieties in 1 and 2 has almost no specific and significant influence on the pseudooxial preference of the aglycone. The comparison of ΔH° values in 9-deazaadenosine (3) with formycin A (1) or formycin B (2) also shows that the π-electrons of the C9–C8 double bond in the pyrrole moiety are less delocalized than those of N8–C9 in the pyrazole moiety and therefore more available for a stereoelectronic interaction with O4', which in turn counteracts the steric effect of the bulk and pushes the N = S pseudorotational equilibrium to the more N-type conformation in 3 (Table 3). These observations are consistent with the relative 13N-NMR chemical shifts,13 where the 13N deshielding increases in the order pyrrole < imidazole < pyrazole, suggesting that the pyrazole system fused with the π-electron-deficient pyrimidine is less π-electron excessive than the fused pyrrole system.

In our set of three purine β-D-ribofuranosyl-C-nucleosides 1–3, we have chosen pyrazolo[4,3-d]pyrimidines as the C-substituents in 1 or 2 as the optimal isosteric and isoelectronic C-aglycone analogs of the adenine or guanine base in β-D-ribofuranosyl-N-nucleosides, whereas the pyrrolo[3,2-d]pyrimidine moiety in 9-deazaadenosine (3) can be considered clearly as the undesirable alternative. ΔH° values17b of the pseudorotational equilibria of adenine (ΔH° = −4.6 kJ/mol) and guanosine (ΔH° = −3.2 kJ/mol) are the net result of the following driving forces: (i) the gauche effects of 2'-OH and 3'-OH with O4' and the effect of 4'-CH2OH, which are exclusively accounted for in the ΔH° value of 9 (0.4 kJ/mol), (ii) the gauche effect of the O2'–C2'–C1'–N(purine) fragment (ΔH° = −6.3 kJ/mol), which is clearly accounted for in our previous studies with 2'-deoxyribonucleosides,7c (iii) the steric effect of adenine and guanine, which has been quantified in the present work by the average C-substituent effect (average ΔH° = −7.8 kJ/mol, Table 3) in 1 and 2, and (iv) the anomic effect in adenosine and guanosine. The above considerations have enabled us to calculate for the first time the magnitude of the O4'–C1'–N9 anomeric effect

\[ \Delta H° \]
Anomeric Effect in Adenosine and Guanosine

in the biologically ubiquitous adenosine (eq 1b) and guanosine (eq 1c) on the basis of eq 1a:

\[
\text{anomeric effect} = \Delta H^o \text{ of adenosine or guanosine} - \\
(\Delta H^o \text{ from (i)} + \Delta H^o \text{ from (ii)} + \Delta H^o \text{ from (iii)})
\]

In the absence of an appropriate maximally pseudoequatorially
oriented reference pyrimidine \(\beta\)-d-ribofuranosyl-C-nucleoside or a bulky \(\text{C-alkylglycoside}^{12}\)

Clearly, the most important use of this work is the reparam-
eterization of the molecular mechanics force field parameters, which presently use only the generalized atom type for the
modeling of nucleosides and their analogs without taking the
 constituent aromatic system into consideration. We believe this
study should also be useful to understand how the local structure
variation takes place in DNA and RNA depending upon the
local nucleobase sequence. The further implication of this work
is to understand why the catalytic self-cleavage in some RNAs
(e.g. the hammerhead ribozyme) takes place in a sequence-specific
manner.

Experimental Section

Vicinal proton-proton coupling constants \(J_{HH}\) were measured (Table 1) at 500 MHz in \(\text{D}_2\text{O}\) solution (=20 mM) in 10 K steps in the ranges
278-338 K for 1-3 and 278-363 K for 4 (Table 1). The computer program
PSEUROT (version 5.4) was used to calculate the best fit of the five
conformational parameters \(P\) and \(\Phi_n\) for both N and S pseudorotamers and the
mole fraction of the N (\(\Phi_N\)) or S (\(\Phi_S\)) conformer to the three
temperature-dependent coupling constants \(J_{12T}, J_{23T}, \text{and } J_{34T}\). The PSEUROT fitting processes were performed by constraining
either \(\Phi_n\) of the N and S forms to an identical value for 1-4 or \(P_N\) and
\(\Phi_S\) of the minor conformer during the optimization (for 1-3) (see
footnotes of Table 2). The quality of the fits has been assessed through the
rms error of the fits and the number of the final optimized points (for 1-4)
according to our PSEUROT analyses is also reported in Table 2. The enthalpy
\(\Delta H^o\) and entropy \(\Delta S^o\) contributions to the free energy \(\Delta G^{298}\) at 298
K characterizing the N = S pseudorotational equilibrium of 1-4 were
determined by plotting \(\ln(\Phi_S/\Phi_N)\) versus 1000/\(T\). The equations of the
straight lines were calculated through a least-squares fitting process (see
Figure 1 for typical van't Hoff plots and Table 3 for the energetics).

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