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Transfer Hydrogenation as a Redox Process in Nucleotides

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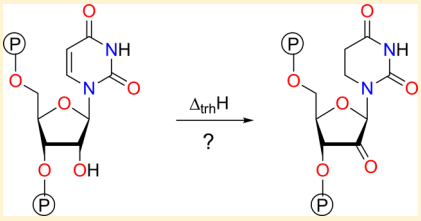
Supporting Information

ABSTRACT: Using a combined theoretical and experimental strategy, the heats of hydrogenation of the nucleotide bases uracil, thymine, cytosine, adenine, and guanine have been determined. The most easily hydrogenated base is uracil, followed by thymine and cytosine. Comparison of these hydrogenation enthalpies with those of ketones and aldehydes derived from sugar models indicates the possibility of near-thermoneutral hydrogen transfer between uracil and the sugar phosphate backbone in oligonucleotides.



The fidelity of biological information storage and information transmission is tightly linked to the chemical stability of DNA and RNA oligonucleotides. Aside from simple hydrolytic cleavage processes the stability of DNA and RNA is also threatened by a number of redox processes such as single electron oxidation or reduction steps.[1,2] We are studying here the thermodynamics of hydrogen transfer between the sugar phosphate backbone and the individual nucleotide bases as an additional redox process. That this is a potential threat to oligonucleotide stability follows from large differences in hydrogenation enthalpies of molecular systems containing C−C and C−O double bonds. The hydrogenation of formaldehyde (**2**), for example, is exothermic by 92.4 kJ mol^{-1}, while that of ethylene (**4**) is exothermic by 136.3 kJ mol^{-1} (Figure 1).[3−7] Taken together this implies that the transfer hydrogenation between ethylene and methanol as expressed in eq 3 in Figure 1 is exothermic by 43.4 kJ mol^{-1}. Similar results are obtained for a large variety of alkenes and carbonyl compounds,[8−10] and the question therefore arises how oligonucleotides avoid reduction of nucleobases (all of which contain C−C double bonds) by the sugar phosphate backbone. Aside from reductively active HO groups at free C5′ and C3′ termini of oligonucleotide strands, a major redox partner exists with the C2′ HO groups in RNA, but not in DNA. Reaction energies for the transfer hydrogenation between the sugar phosphate backbone and nucleobases as described in eq 4 in Figure 1 for the example of uridine are currently not available, due to the lack of appropriate thermochemical data. In the following we describe the results of a combined experimental and theoretical study in which the heats of formation of nucleobases and their dihydro isomers have been determined, thus allowing for the quantification of transfer hydrogenation enthalpies.

As a first step the hydrogenation enthalpies of the individual redox components were analyzed. These include the individual

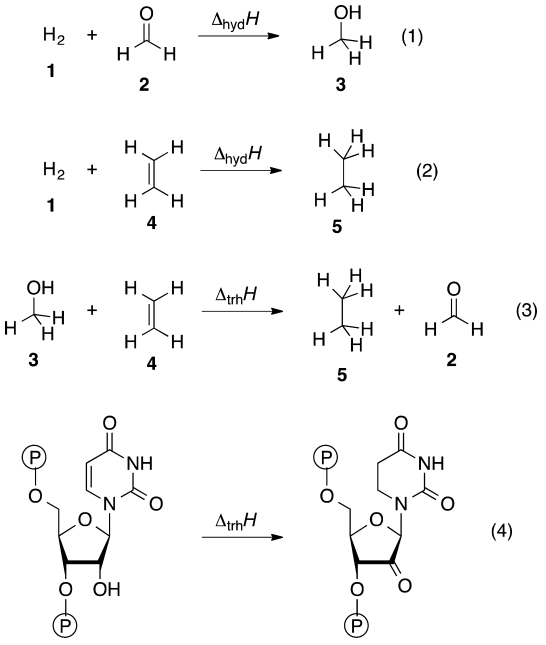


Figure 1. Hydrogenation of formaldehyde (**2**) and ethylene (**4**), and transfer hydrogenation between alcohol donors and alkene acceptors.

nucleobases present in RNA and DNA systems as well as simple sugar models. Given the general lack of experimental results, the required hydrogenation energies have been obtained through combination of energies calculated with the G3B3 compound model with experimental data for well-known reference compounds such as ethylene (**4**) (Table 1 and Figure

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Table 1. Heats of Hydrogenation $\Delta_{hyd}H$ (kJ mol^{-1}) for the Nucleobase and Sugar Models Shown in Figure 2

system	$\Delta_{hyd}H$(calcd)	$\Delta_{hyd}H$(expt)
guanine (**35**)	+7.6	n/a
adenine (**33**)	+4.9	n/a
1-methylcytosine (**31**)	−49.3	n/a
cytosine (**29**)	−54.7	n/a
1′-anhydro-3′-keto-2′- desoxyribose (**28**)	−62.7	n/a
1,3-dimethylthymine (**41**)	−65.7	−66.4 ± 3.5[c]
acetaldehyde (**26**)	−68.4	−69.1 ± 0.4[a]
		−64.5 ± 1.4[a]
		−68.6[b]
1-methylthymine (**24**)	−69.6	n/a
3′-ketoanhydroerythritol (**22**)	−70.4	n/a
thymine (**20**)	−70.5	−67.6 ± 2.3[c]
1,3-dimethyluracil (**18**)	−72.3	−67.6 ± 2.6[c]
	−70.5[g]	
1′-anhydro-2′-ketoribose (**17**)	−74.6	n/a
1-methyluracil (**15**)	−77.8	n/a
uracil (**13**)	−78.1	−81.5 ± 2.6[c]
		−75.7 ± 3.0[f]
1′-anhydro-5′-keto-2′-desoxyribose (**11**)	−81.0	n/a
1′-anhydro-5′-ketoribose (**10**)	−83.5	n/a
1′-anhydro-3′-ketoribose (**8**)	−88.1	n/a
formaldehyde (**2**)	−90.1	−92.4[b]
propene (**6**)	−125.2	−125.0 ± 0.2[d]
ethylene (**4**)	−136.3	−136.3 ± 0.2[e]
		−136.4[b]

[a]References 8−11. [b]Calculated from the individual heats of formation.[12] [c]This work using the data in Table S5 in the Supporting Information. [d]Reference 5. [e]Reference 4. [f]Reference 13. [g]G3 results from ref 14.

2; see Supporting Information for further information). Redox data for the sugar models are provided here in a comparable way (that is, as the hydrogenation enthalpies of the respective oxidized forms) in order to allow for a side-by-side comparison of all redox partners. For the sake of comparison we also include results obtained for smaller alkenes and aldehydes.

As is readily seen in Figure 2 the hydrogenation of pyrimidine bases is much more exothermic than that of purine bases. The most easily reduced base is uracil (**13**) with $\Delta_{hyd}H$(**13**) = −78.1 kJ mol^{-1}. This value is hardly affected by alkylation at the N1 position as can be seen from the value for 1-methyluracil (**15**) of $\Delta_{hyd}H$(**15**) = −77.8 kJ mol^{-1}. Introduction of a second alkyl group at N3 position as in *N*,*N*-dimethyluracil (**18**) leads to $\Delta_{hyd}H$(**18**) = −72.3 kJ mol^{-1} and thus to a more significant change.

This effect is almost identical to the introduction of a methyl group in C5 position as in thymine (**20**), which reduces the hydrogenation enthalpy by 7 kJ mol^{-1} to $\Delta_{hyd}H$(**20**) = −70.5 kJ mol^{-1}. Alkylation of the N1 and N3 positions in thymine again reduces the hydrogenation enthalpy by a small margin to $\Delta_{hyd}H$(**41**) = −65.7 kJ mol^{-1} in 1,3-dimethylthymine (**41**). A similar reduction of hydrogenation enthalpies on moving to more highly substituted systems can also be observed for the ethylene (**4**)/propene (**6**) and the formaldehyde (**2**)/acetaldehyde (**26**) pairs and can thus be considered to be a general phenomenon. The most difficult pyrimidine base to reduce is cytosine with $\Delta_{hyd}H$(**29**) = −54.7 kJ mol^{-1}. Hydrogenation of the purine bases adenine (**33**) and guanine (**35**) is significantly more difficult than any of the pyrimidine bases and overall positive hydrogenation enthalpies of $\Delta_{hyd}H$(**33**) = +4.9 kJ mol^{-1} and $\Delta_{hyd}H$(**35**) = +7.6 kJ mol^{-1} are obtained for these two systems. The most stable reaction products are in both cases those involving reduction of the C8−N7 double bond and the canonical tautomeric forms on all other centers. The large differences in hydrogenation enthalpies between pyrimidines and purines reflect the intrinsically large differences in hydrogenations of C−C and C−N double bonds. This can readily be seen already in hydrogenation enthalpies of smaller model systems such as propene ($\Delta_{hyd}H$(**6**) = −125.0 kJ mol^{-1}) and *N*-methylimine ($\Delta_{hyd}H(CH_3N{=}CH_2)$ = −62.8 kJ mol^{-1}).[15] We note in passing that the order of hydrogenation enthalpies found here in the gas phase parallels that of the experimentally measured one-electron reduction potentials in the gas phase and in polar organic solvents.[16,17] The hydrogenation enthalpies of uracil (**13**) and thymine (**20**) as well as their 1,3-dimethyl substituted derivatives were subsequently tested experimentally using a combination of methods to quantify the heat of combustion as well as the heat of sublimation for the nucleobases and their respective reduced derivatives. As depicted schematically in Figure 3 for the example of uracil (**13**), the combustion experiments yield the heat of formation of solid nucleobases as Δ_fH_{cr}(**13**) = −430.5 kJ mol^{-1} and Δ_fH_{cr}(**14**) = 494.9 kJ mol^{-1}. Combination of these data with the heats of sublimation $\Delta_{cr}^{g}H$ determined using the transpiration method yield gas phase heats of formation of Δ_fH_g(**13**) = −298.6 kJ mol^{-1} and Δ_fH_g(**14**) = −380.1 kJ mol^{-1}. Together with the gas phase heat of formation of dihydrogen of $\Delta_fH_g(H_2)$ = 0.0 kJ mol^{-1} these data yield the overall heat of hydrogenation of uracil $\Delta_{hyd}H$(**13**) = −81.5 ± 2.6 kJ mol^{-1}. In contrast to measuring the hydrogenation enthalpies directly through hydrogenation of **13**, the indirect approach used here does not require the use of any hydrogenation catalysts and, more importantly, allows an accurate characterization of the hydrogenation products. In

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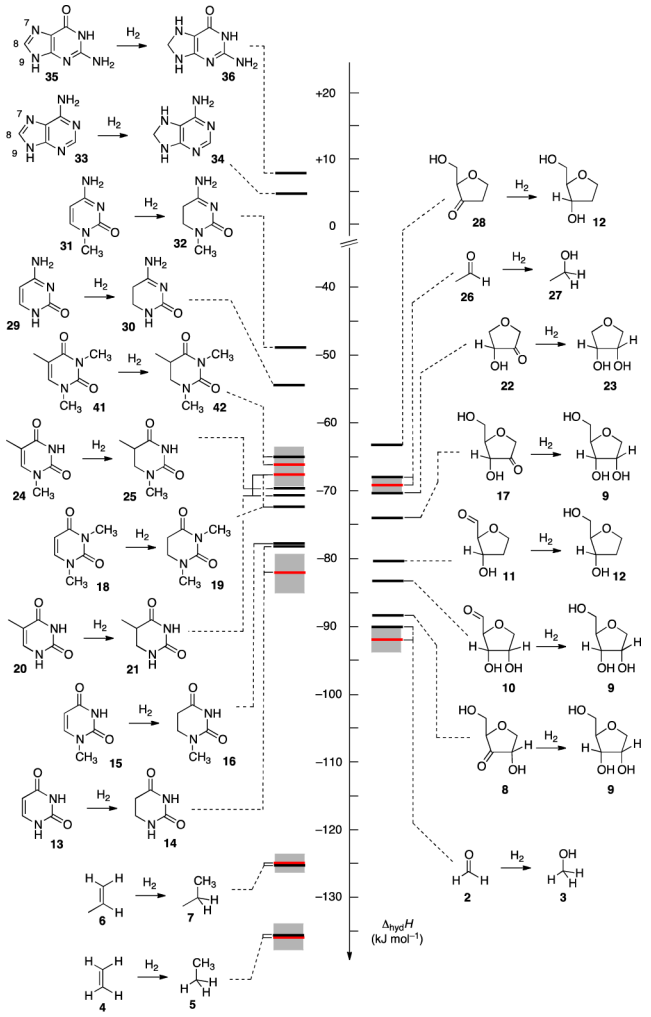

Figure 2. Heats of hydrogenation $\Delta_{hyd}H$ at 298.15 K of selected nucleobases and carbonyl compounds. Experimental hydrogenation enthalpies are shown together with their standard deviations as gray bars.

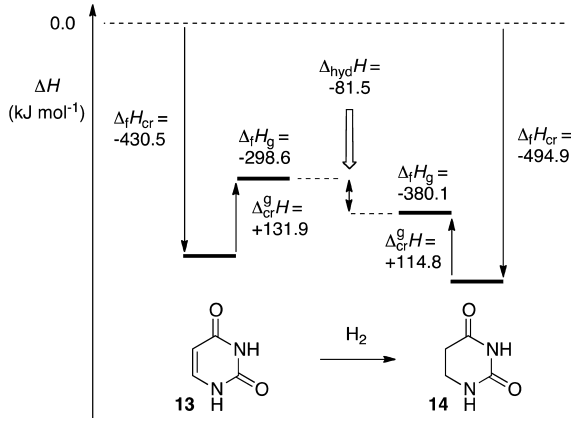

Figure 3. Experimental determination of the heat of hydrogenation of uracil (**13**).

the particular case of dihydrouracil (**14**) all analytical data are indicative of the tautomeric form shown in Figure 3. The experimentally measured heats of hydrogenation for **13**, **18**, **20**, and **41** are closely similar to the theoretically calculated values and thus confirm, that the G3B3 results compiled in Table 1 provide a quantitative guideline for the redox properties of nucleobases with respect to dihydrogenation.

The hydrogenation enthalpies for the sugar-derived reaction partners collected in Table 1 and shown graphically in Figure 2 are all located in a range from −62.7 to −88.1 kJ mol^{-1} and thus bracket the hydrogenation enthalpies for uracil, its *N*-methylated derivatives, and for thymine. Uracil (**13**) can be reduced by all sugar building blocks with a less favorable (less negative) hydrogenation enthalpy. In pictorial terms this includes all sugar building blocks in Figure 2 located above uracil (**13**). The most effective reducing agent found in the RNA sugar models is the C2′ hydroxy group in C1′-desoxyribofuranose **9**, whose transfer hydrogenation with uracil to yield dihydrouracil **14** and the C2-oxidized sugar **17** is predicted to be exothermic by −78.1 − (−74.6) = −3.5 kJ mol^{-1}. Removal of the C2′ hydroxy group as is the case in DNA sugar model **12** leaves only the C3′ and C5′ hydroxy groups as reducing agents. The former of these is reactive enough to reduce uracil and thymine in an exothermic fashion. The redox data collected in Figure 2 for individual RNA and DNA components thus suggest that transfer hydrogenation from sugar components to uracil may be thermochemically favorable.

In order to assess the thermodynamics of such a process in complete nucleosides, intramolecular transfer hydrogenation reactions have been studied theoretically for uridine (Figure 4).

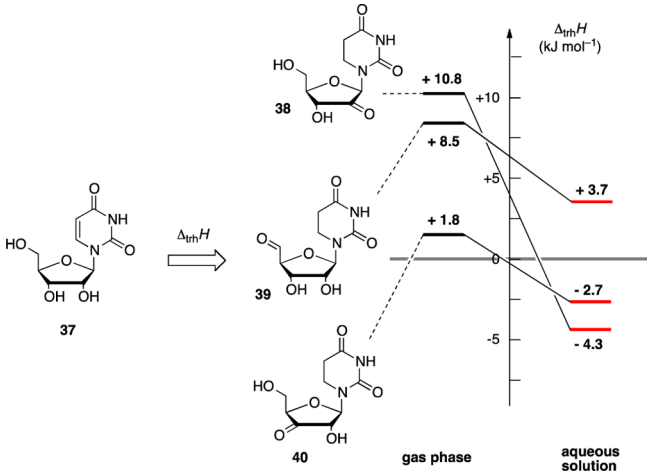

Figure 4. Heats of transfer hydrogenation in uridine (**37**) to the three possible reaction products as obtained at 298.15 K in the gas phase (left side) and in aqueous solution (right side).

Comparison of the data for individual components in Figure 2 with those for the nucleosides in Figure 4 shows that sugar-to-base transfer hydrogenation energetics become more positive on covalent coupling both redox partners. However, even for the least favorable process found here, the formation of C2′ oxidized nucleoside **38** from uridine (**37**), the endothermicity amounts to only +10.8 kJ mol^{-1}. Using either the C3′ (as in **40**) or the C5′ (as in **39**) hydroxy groups as redox partners leads to even less positive reaction energies. This difference, which is obtained from Boltzmann-averaged enthalpies for fully flexible nucleosides,[18−20] can potentially be modified through intermolecular interactions as present in base-paired systems or in polar solvents. In order to obtain an estimate of the magnitude of these effects, solvation free energies in water were calculated for all conformers identified for nucleosides **38**−**40** using the PCM/UAHF/RHF/6-31G(d) solvation model.

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Combination with the gas phase results clearly indicates that intermolecular interactions or solvation effects will stabilize reaction products **38**–**40** more than uridine itself, thus leading to a larger driving force for intramolecular transfer hydrogenation. We should add that these types of hydrogen exchange reactions are equally possible between sugar components of one nucleotide and the base of the adjacent nucleotide in 3′ or 5′ position.

The rather similar hydrogenation enthalpies found here for some of the sugar and the base components of nucleosides raise the question how the information stored in oligonucleotides can be preserved under the potential threat of internal hydrogen transfer reactions. Most RNA maturing steps such as C5′ end-capping and methylation of the C2′ hydroxy groups of nucleotides located at the C3′ terminus, or the polyadenylation of the C3′ terminus all reduce the potential of intramolecular transfer hydrogenation by simply removing the respective hydroxy groups as redox partners or by positioning redox-inactive bases at the respective termini. DNA systems, in contrast, are much less prone to this type of redox process due to removal of the C2′ hydroxy group and the replacement of uridine by thymine. This limits the risk of transfer hydrogenation to the terminal C3′ and C5′ positions carrying thymine as the most easily reduced base. Avoidance of this base at the very last position as well as any end-capping process (including the formation of cyclic DNA) will, of course, eliminate the risk of transfer hydrogenation as an unwanted redox process.

■ ASSOCIATED CONTENT

Supporting Information

Full experimental details for the synthesis of nucleobase derivatives and measurements of their heats of hydrogenation, energies and coordinates for all theoretical calculations, and NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Cooke, M. S.; Evans, M. D.; Dizdaroglu, M.; Lunec, J. Oxidative DNA damage: mechanisms, mutation, and disease. *FASEB J.* **2003**, *17*, 1195–1214.

(2) Pogozelski, W. K.; Tullius, T. D. Oxidative Strand Scission of Nucleic Acids: Routes Initiated by Hydrogen Abstraction from the Sugar Moiety. *Chem. Rev.* **1998**, *98*, 1089–1107.

(3) Calculated using the following standard heats of formation $\Delta_f H$: $\Delta_f H(H_2, \mathbf{1}) = 0.0$ kJ mol^{-1}; $\Delta_f H(CH_2O, \mathbf{2}) = -108.6 \pm 0.5$ kJ mol^{-1};[4] $\Delta_f H(CH_3OH, \mathbf{3}) = -201.5 \pm 0.1$ kJ mol-1;[4] and for reaction 2 the heats of hydrogenation reported in ref 4 and ref 5.

(4) Cox, J. D.; Pilcher, G. *Thermochemistry of Organic and Organometallic Compounds*; Academic Press: San Diego, CA, 1970.

(5) Kistiakowsky, G. B.; Nickle, A. G. Ethane–Ethylene and Propane–Propylene Equilibria. *Faraday Discuss. Chem. Soc.* **1951**, *10*, 175–187.

(6) Pedley, J. P.; Naylor, R. D.; Kirby, S. P. *Thermochemical Data of Organic Compounds*, 2nd ed.; Chapman and Hall: London, 1986; see also values compiled in ref 7.

(7) Chase, M. W., Jr. NIST-JANAF Themochemical Tables, 4th ed. *J. Phys. Chem. Ref. Data, Monogr.* **1998**, *9*, 1–1951.

(8) Dolliver, M. A.; Gresham, T. L.; Kistiakowsky, G. B.; Smith, E. A.; Vaughan, W. E. Heats of Organic Reactions. VI. Heats of Hydrogenation of Some Oxygen-Containing Compounds. *J. Am. Chem. Soc.* **1938**, *60*, 440–450.

(9) Kistiakowsky, G. B.; Romeyn, H., Jr.; Ruhoff, J. R.; Smith, H. A.; Vaughan, W. E. Heats of Organic Reactions. I. The Apparatus and the Heat of Hydrogenation of Ethylene. *J. Am. Chem. Soc.* **1935**, *57*, 65–75.

(10) Rogers, D. W. *Experimental and Computational Hydrogen Thermochemistry of Organic Compounds*; World Scientific Publishing Company: Singapore, 2006).

(11) Wiberg, K. B.; Crocker, L. S.; Morgan, K. M. Thermochemical Studies of Carbonyl Compounds. 5. Enthalpies of Reduction of Carbonyl Compounds. *J. Am. Chem. Soc.* **1991**, *113*, 3447–3450.

(12) Lide, D. R. *CRC Handbook of Chemistry and Physics*, 89th ed.; CRC Taylor & Francis: Boca Raton, FL, 2008.

(13) Galvao, T. L. P.; Rocha, I. M.; Ribeiro da Silva, M. D. M. C.; Ribeiro da Silva, M. A. V. Is Uracil Aromatic? The Enthalpies of Hydrogenation in the Gaseous and Crystalline Phases, and in Aqueous Solution, as Tools to Obtain an Answer. *J. Phys. Chem. A* **2013**, *117*, 5826–5836.

(14) Galvao, T. L. P.; Ribeiro da Silva, M. D. M. C.; Ribeiro da Silva, M. A. V. From 2,4-Dimethoxypyrimidine to 1,3-Dimethyluracil: Isomerization and Hydrogenation Enthalpies and Noncovalent Interactions. *J. Phys. Chem. A* **2014**, *118*, 4816–4823.

(15) Using $\Delta_f H(CH_3N{=}CH_2) = +44 \pm 8$ kJ mol^{-1} from Peerboom, R. A. L.; Ingemann, S.; Nibbering, N. M. M.; Liebman, J. F. Proton Affinities and Heats of Formation of the Imines $CH_2{=}NH$, $CH_2{=}$NMe and PhCH=NH. *J. Chem. Soc., Perkin Trans. 2* **1990**, 1825–1828 and $\Delta_f H(CH_3NHCH_3) = -18.8$ kJ mol^{-1} from ref 12.

(16) Seidel, C. A.; Schulz, A.; Sauer, M. H. Nucleobase-Specific Quenching of Fluorescent Dyes. 1. Nucleobase One-Electron Redox Potentials and Their Correlation with Static and Dynamic Quenching Efficiencies. *J. Phys. Chem.* **1996**, *100*, 5541–5553.

(17) Aflatooni, K.; Gallup, G. A.; Burrow, P. D. Electron Attachment Energies of the DNA Bases. *J. Phys. Chem. A* **1998**, *102*, 6205–6207.

(18) Green, E. A.; Rosenstein, R. D.; Shiono, R.; Abraham, D. J.; Trus, B. L.; Marsh, R. E. The Crystal Structure of Uridine. *Acta Crystallogr.* **1975**, *B31*, 102–107.

(19) Leulliot, N.; Ghomi, M.; Scalmani, G.; Berthier, G. Ground State Properties of the Nucleic Acid Constituents Studied by Density Functional Calculations. I. Conformational Features of Ribose, Dimethyl Phosphate, Uridine, Cytidine, 5′-Methyl Phosphate-Uridine, and 3′-Methyl Phosphate-Uridine. *J. Phys. Chem. A* **1999**, *103*, 8716–8724.

(20) Saenger, W. Structure and Function of Nucleosides and Nucleotides. *Angew. Chem., Int. Ed.* **1973**, *12*, 591–682.