Molar Heat Capacities of Aminouracils by Differential Scanning Calorimetry

Wojciech Zielenkiewicz* and Piotr Szterner

Institute of Physical Chemistry of the Polish Academy of Sciences, Kasprzaka 44/52, 01-224 Warsaw, Poland

The molar heat capacities $C_{p,\mathrm{m}}$ of four crystalline compounds (5-aminouracil, 6-aminouracil, 6-amino-1-methyluracil, and 6-amino-1,3-dimethyluracil) were determined using a differential scanning calorimeter (SETARAM TG-DSC 111). The measurements were made at constant pressure between (298.15 and 343.15) K. Then the molar heat capacities were correlated with temperature. The contributions of C-CH_3, N-CH_3, and C-NH_2 groups to the standard molar heat capacity values were evaluated. The experimentally determined $C^{\circ}_{p,\mathrm{m}}$ values were compared with those calculated by the Chickos method.

Introduction

Thermodynamic properties of uracil and its differently substituted derivatives were the subject of our previous investigations[1−8] aimed at the experimental determinations of vapor pressures, molar enthalpies of sublimation, and molar enthalpies of solution in aqueous and methanol solutions. The obtained data were used to evaluate solute−solvent interactions.

The properties of differently substituted derivatives of uracil are important in view of their medical application. Some of the 5-substituted uracils exhibit a significant pharmacological activity and have been used as antitumor, antibacterial, and antiviral drugs.[9] 5-Substituted uracil derivatives have been reported as a new class of nonsteroidal anti-inflammatory agents possessing anti-oxidative activity.[10] Amino derivatives of uracil also exhibit significant biological activity. 6-Aminouracil is a competitive inhibitor of thymidine phosphorylase activity (TP).[11] 5-Substituted 6-aminouracils have been synthesized and tested for inhibition of human placenta thymidine phosphorylase.[12] TP inhibitors include 6-aminouracil derivatives such as 6-aminothymine, 6-amino-5-bromouracil, 6-amino-5-chlorouracil,[13] and 6-(2-aminoethyl)amino-5-chlorouracil.[14]

This paper is a continuation of our previous investigations of thermodynamic properties of amino derivatives of uracil.[3] The molar heat capacities at constant pressure ($C_{p,\mathrm{m}}$) of four aminouracils were determined in the temperature range from (298.15 to 343.15) K. Then the correlations of $C_{p,\mathrm{m}}$ data with temperature were calculated. The contributions of -CH_3 and -NH_2 groups attached to the C- and N- atoms of uracil skeleton [NH_2(C), CH_3(N), and CH_3(C)] were evaluated for the standard molar capacity ($C^{\circ}_{p,\mathrm{m}}$) values. Finally, the experimentally determined $C^{\circ}_{p,\mathrm{m}}$ values were compared with those calculated by the Chickos method.[15−17]

Materials and Methods

The compounds used in the experiments were as follows: 5-aminouracil (lot 16H3450, Chemical Abstract Service Registry Number (CASRN): 932-52-5), 6-aminouracil (lot 97F0287, CASRN: 873-83-6), 6-amino-1,3-dimethyluracil (lot 123H3511, CASRN: 6642-31-5), each of purity better than 99 %. They were purchased from Sigma Chemical Co Ltd. whereas 6-amino-1-methyluracil (no.12411, CASRN: 2434-53-9) of 97 % purity was supplied by Lancaster Chemicals Co. Ltd. Their molar masses were 0.127103, 0.127103, 0.141130, and 0.155157 kg·mol^{-1}, respectively. Structural formulas of the studied compounds are presented in Figure 1.

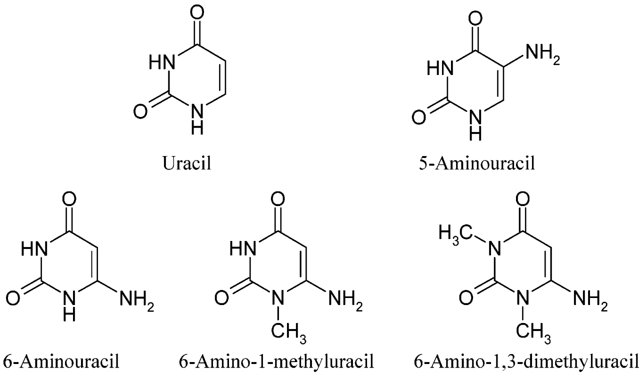
Uracil
5-Aminouracil
6-Aminouracil
6-Amino-1-methyluracil
6-Amino-1,3-dimethyluracil

Figure 1. Structural formulas of uracil and its amino derivatives.

The $C_{p,\mathrm{m}}$ determinations were made with use of Calvet TG-DSC 111 differential scanning microcalorimeter (Setaram, France).[6] The procedure for determining $C_{p,\mathrm{m}}$ was identical to the one applied in our previous study.[6] The measurements were performed differentially, relatively to the empty crucible in the range between (298.15 and 343.15) K at a scan rate of 0.5 K·min^{-1}. All the samples were prepared in the same way. The investigated compounds were placed in a stainless steal crucible of 150 μL volume. The stainless steal cover was crimped with an aluminum seal with the use of a crimping press. The sample mass was determined with the accuracy of $1 \cdot 10^{-5}$ g using the balance Mettler AT 261 Delta Range. The uncertainty of temperature was 0.1 K. The DSC device was calibrated using as a standard substance benzoic acid of purity 99.8 %, received from the Polish Committee for Standardization. The experimentally determined standard molar heat capacity $C^{\circ}_{p,\mathrm{m}}$ value of benzoic acid equal to 146.9 ± 1.32 J·K^{-1}·mol^{-1} was in agreement with those recommended by the IUPAC[18] (146.8 J·K^{-1}·mol^{-1}). The relations between the experimentally determined $C_{p,\mathrm{m}}$(exp) values and temperature T in the range from (298.15 to 343.15)K were calculated.

The contributions of NH_2(C), CH_3(N), and CH_3(C) groups to the $C^{\circ}_{p,\mathrm{m}}$(add) values at 298.15 K were evaluated according to the general additivity scheme.[6,19] The following equation was applied:

$$C^{\circ}_{p,\mathrm{m}}(\mathrm{add}) = C + \sum_i n_i C^{\circ}_{p,\mathrm{m,i}} \qquad (1)$$

---

* Corresponding author. E-mail: zivf@ichf.edu.pl.

10.1021/je6005168 CCC: $37.00

Table 1. Experimental (C_p(ex)) and Approximated (C_p(appr)) Value of Molar Heat Capacity and the Correlation Functions of Uracil and Its Derivatives $C_{p,\mathrm{m}} = f(T)$

no.	T / K	$C_{p,\mathrm{m}}$(exp) / $\mathrm{J{\cdot}K^{-1}{\cdot}mol^{-1}}$	$C_{p,\mathrm{m}}$(calc) / $\mathrm{J{\cdot}K^{-1}{\cdot}mol^{-1}}$	no.	T / K	$C_{p,\mathrm{m}}$(exp) / $\mathrm{J{\cdot}K^{-1}{\cdot}mol^{-1}}$	$C_{p,\mathrm{m}}$(calc) / $\mathrm{J{\cdot}K^{-1}{\cdot}mol^{-1}}$
1	5-Aminouracil			2	6-Aminouracil		
	$C_{p,\mathrm{m}}/\mathrm{J{\cdot}K^{-1}{\cdot}mol^{-1}} = 144.90(\pm 0.07) + 0.143(T/\mathrm{K} - 298.15)$				$C_{p,\mathrm{m}}/\mathrm{J{\cdot}K^{-1}{\cdot}mol^{-1}} = 146.94(\pm 0.02) + 0.134(T/\mathrm{K} - 298.15)$		
	RMSD^a/$\mathrm{J{\cdot}K^{-1}{\cdot}mol^{-1}} = 0.12$				RMSD^a/$\mathrm{J{\cdot}K^{-1}{\cdot}mol^{-1}} = 0.04$		
	298.15	145.0	145.0		298.15	147.0	147.0
	303.15	145.5	145.6		303.15	147.6	147.6
	308.15	146.2	146.3		308.15	148.2	148.3
	313.15	147.0	147.0		313.15	149.0	149.0
	318.15	148.0	148.0		318.15	149.6	149.6
	323.15	148.4	148.5		323.15	150.3	150.3
	328.15	149.1	149.2		328.15	151.0	151.0
	333.15	150.0	150.0		333.15	151.6	151.6
	338.15	150.6	150.6		338.15	152.3	152.3
	343.15	151.3	151.3		343.15	153.0	153.0
3	6-Amino-1-methyluracil			4	6-Amino-1,3-dimethyluracil		
	$C_{p,\mathrm{m}}/\mathrm{J{\cdot}K^{-1}{\cdot}mol^{-1}} = 166.30(\pm 0.02) + 0.149(T/\mathrm{K} - 298.15)$				$C_{p,\mathrm{m}}/\mathrm{J{\cdot}K^{-1}{\cdot}mol^{-1}} = 188.90(\pm 0.06) + 0.426(T/\mathrm{K} - 298.15)$		
	RMSD^a/$\mathrm{J{\cdot}K^{-1}{\cdot}mol^{-1}} = 0.13$				RMSD^a/$\mathrm{J{\cdot}K^{-1}{\cdot}mol^{-1}} = 0.10$		
	298.15	166.2	166.3		298.15	189.0	188.9
	303.15	167.0	167.0		303.15	191.0	191.0
	308.15	168.0	168.0		308.15	193.1	193.1
	313.15	168.5	168.5		313.15	195.2	195.3
	318.15	169.2	169.3		318.15	197.3	197.4
	323.15	170.0	170.0		323.15	199.5	199.5
	328.15	171.0	171.0		328.15	201.6	201.7
	333.15	171.4	171.5		333.15	204.0	203.8
	338.15	172.2	172.3		338.15	206.0	205.9
	343.15	173.0	173.0		343.15	208.0	208.1

[a] RMSD means root-mean-square deviation calculated from linear regression. The standard deviation of a given parameters are shown in parentheses.

where C is a constant. It was assumed that this value corresponded to the standard molar heat capacity of uracil $C^{\mathrm{o}}_{p,\mathrm{m}}$(ura). $C^{\mathrm{o}}_{p,\mathrm{m,i}}$ are the additive values of molar heat capacity for group i. n_i is a number of the group type added to the skeleton of uracil.

The value $C^{\mathrm{o}}_{p,\mathrm{m}}$ was determined in the previous study (131.8 $\mathrm{J{\cdot}K^{-1}{\cdot}mol^{-1}}$).[6] In calculation by Chickos method,[15−17] the $C^{\mathrm{o}}_{p,\mathrm{m}}$(ura) value was accepted to be equal to 127.8 $\mathrm{J{\cdot}K^{-1}{\cdot}mol^{-1}}$ assuming[6] that it corresponded to the sum of the two cyclic secondary groups -CONH- (2 × 46.4 $\mathrm{J{\cdot}K^{-1}{\cdot}mol^{-1}}$) and the two tertiary aromatics sp^2 C groups = CH- (2 × 17.5 $\mathrm{J{\cdot}K^{-1}{\cdot}mol^{-1}}$).[6]

The standard molar value of heat capacity $C^{\mathrm{o}}_{p,\mathrm{m}}$(Chickos) was calculated using the Chickos procedure at 298.15 K on the basis of heat capacities of solid-phase functional groups comprising a given compound. The $C_{p,\mathrm{m}}$(exp), $C^{\mathrm{o}}_{p,\mathrm{m}}$(ura), and $C^{\mathrm{o}}_{p,\mathrm{m,i}}$ values were estimated by using the least-squares linear regression method (Excel 2003, Microsoft).

Results and Discussion

The experimentally determined $C_{p,\mathrm{m}}$(exp) data, the relations expressing the functions $C_{p,\mathrm{m}} = f(T)$, and calculated values of $C_{p,\mathrm{m}}$(calc) of aminouracils are presented in Table 1.

The values of $C_{p,\mathrm{m}}$(exp) and $C_{p,\mathrm{m}}$(add) and standard molar heat capacities calculated using the Chickos method[15−19] $C^{\mathrm{o}}_{p,\mathrm{m}}$(Chickos) are given in Table 2. In this table there were also presented the differences between the experimental and evaluated values, namely: $\Delta_1 = C^{\mathrm{o}}_{p,\mathrm{m}}(\mathrm{exp}) - C^{\mathrm{o}}_{p,\mathrm{m}}(\mathrm{add})$; $\Delta_2 = C^{\mathrm{o}}_{p,\mathrm{m}}(\mathrm{exp}) - C^{\mathrm{o}}_{p,\mathrm{m}}(\mathrm{Chickos})$.

As can be seen from the data collected in Table 2: (a) the experimentally determined $C_{p,\mathrm{m}}$(exp) are similar to $C_{p,\mathrm{m}}$(add). The differences in these values are in the range of (−0.8 to 1.4) $\mathrm{J{\cdot}K^{-1}{\cdot}mol^{-1}}$. (b) The values $C^{\mathrm{o}}_{p,\mathrm{m}}$(Chickos) differ from the $C^{\mathrm{o}}_{p,\mathrm{m}}$(exp) data in the range (−2.8 to 6.6) $\mathrm{J{\cdot}K^{-1}{\cdot}mol^{-1}}$. These significant differences are the result of the assumption various values of standard molar heat capacity of uracil obtained experimentally and calculated on the basis of the Chickos method. This difference is equal to 4 $\mathrm{J{\cdot}K^{-1}{\cdot}mol^{-1}}$. (c) $C^{\mathrm{o}}_{p,\mathrm{m}}$ values depend on the position of substitution groups (-CH_3 or -NH_2) in the diketopirimidine rings. At the same time the differences resulting from the substitution of -NH_2 group in position 5 and 6 as well the -CH_3 group in position 1 and 3 are similar to those observed in case of halo derivatives of uracil (Table 3). The difference between the substitution in positions 5 and 6 can be explained by a strong electron coupling between a substituent and π-electron system of the pyrimidine ring that is very visible[20−22] in changes in both the UV spectra and the values of pK of dissociation of protons H(1) and H(3). It should also be pointed out that in case of the investigated derivatives

Table 2. Molar Heat Capacities Data of Uracil[6] and Its Derivatives at 298.15 K

no.	compound	$C^{\mathrm{o}}_{p,\mathrm{m}}$(exp) / $\mathrm{J{\cdot}K^{-1}{\cdot}mol^{-1}}$	$C^{\mathrm{o}}_{p,\mathrm{m}}$(add) / $\mathrm{J{\cdot}K^{-1}{\cdot}mol^{-1}}$	$C^{\mathrm{o}}_{p,\mathrm{m}}$(Chickos) / $\mathrm{J{\cdot}K^{-1}{\cdot}mol^{-1}}$	$C^{\mathrm{o}}_{p,\mathrm{m}}(\mathrm{exp}) - C^{\mathrm{o}}_{p,\mathrm{m}}(\mathrm{add})$ / $\mathrm{J{\cdot}K^{-1}{\cdot}mol^{-1}}$	$C^{\mathrm{o}}_{p,\mathrm{m}}(\mathrm{exp}) - C^{\mathrm{o}}_{p,\mathrm{m}}(\mathrm{Chickos})$ / $\mathrm{J{\cdot}K^{-1}{\cdot}mol^{-1}}$
1	uracil[6]	131.8	131.8	127.8	0.0	4.0
2	5-aminouracil	145.0	145.6	140.4	−0.6	4.6
3	6-aminouracil	147.0	145.6	140.4	1.4	6.6
4	6-amino-1-methyluracil	166.2	167.0	166.7	−0.8	−0.5
5	6-amino-1,3-dimethyluracil	189.0	188.4	191.8	0.6	−2.8

Table 3. Comparison $C^{\mathrm{o}}_{p,\mathrm{m}}$ Data for Differently Substituted Derivatives of Uracil

compound	$C^{\mathrm{o}}_{p,\mathrm{m}}$ / $\mathrm{J{\cdot}K^{-1}{\cdot}mol^{-1}}$	compound	$C^{\mathrm{o}}_{p,\mathrm{m}}$ / $\mathrm{J{\cdot}K^{-1}{\cdot}mol^{-1}}$	$\Delta C^{\mathrm{o}}_{p,\mathrm{m}}$ / $\mathrm{J{\cdot}K^{-1}{\cdot}mol^{-1}}$
5-aminouracil	145.0	6-aminouracil	147	−2
5-chlorouracil[6]	136.7	6-chlorouracil[6]	139.4	−2.7
6-amino-1-methyluracil	166.2	uracil[6]	131.8	34.4
6-amino-1,3-dimethyluracil	189.0	6-amino-1-methyluracil	166.2	22.8

Table 4. Contributions $C^{\mathrm{o}}_{p,\mathrm{m,i}}$ for CH_3(N), CH_3(C), and NH_2(C) to $C^{\mathrm{o}}_{p,\mathrm{m}}$(add)

compound	$C^{\mathrm{o}}_{p,\mathrm{m}}$(ura) / $\mathrm{J{\cdot}K^{-1}{\cdot}mol^{-1}}$	$C_{p\mathrm{NH_2(C)}}$ / $\mathrm{J{\cdot}K^{-1}{\cdot}mol^{-1}}$	$C_{p\mathrm{CH_3(N)}}$ / $\mathrm{J{\cdot}K^{-1}{\cdot}mol^{-1}}$	$C_{p\mathrm{CH_3(C)}}$ / $\mathrm{J{\cdot}K^{-1}{\cdot}mol^{-1}}$	r^2
amino derivatives of uracil	131.8 ± 1.2	13.8 ± 1.5	21.4 ± 0.75		0.998
methylated derivatives of uracil	131.6 ± 0.4		25.6 ± 0.3	30.4 ± 0.3	0.999
methylated derivatives and amino derivatives of uracil	132.8 ± 1.0	10.6 ± 1.3	24.4 ± 0.6	30.3 ± 0.8	0.996

of uracil (5-aminouracil, 6-aminouracil, 5-chlorouracil,[6] and 6-chlorouracil[6]) a vital difference is observed between the $C^{\circ}_{p,\mathrm{m}}$(exp) values and the values of the enthalpies of solvation.[5]

The general additivity scheme method was applied for the evaluation of the contribution methyl derivatives of uracil presented previously.[6] The contributions of the CH_3(N), CH_3(C), and NH_2(C) groups to the values of heat capacities calculated on the basis of: (a) the investigated aminouracils, (b) the aminouracils and methyluracils, and (c) the methyluracils only are presented in Table 4. Calculations were made by taking the $C^{\circ}_{p,\mathrm{m}}$ data from Table 2 and the data reported earlier[6] for uracil and its methylated derivatives, viz., 1-methyluracil (156.9 $\mathrm{J{\cdot}K^{-1}{\cdot}mol^{-1}}$), 3-methyluracil (157.0 $\mathrm{J{\cdot}K^{-1}{\cdot}mol^{-1}}$), 5-methyluracils (163.0 $\mathrm{J{\cdot}K^{-1}{\cdot}mol^{-1}}$), 6-methyluracil (162.5 $\mathrm{J{\cdot}K^{-1}{\cdot}mol^{-1}}$), 1,3-dimethyluracil (182.5 $\mathrm{J{\cdot}K^{-1}{\cdot}mol^{-1}}$), 1,5-dimethyluracil (187.6 $\mathrm{J{\cdot}K^{-1}{\cdot}mol^{-1}}$), 1,6-dimethyluracil (188.1 $\mathrm{J{\cdot}K^{-1}{\cdot}mol^{-1}}$), 3,6-dimethyluracil (187.4 $\mathrm{J{\cdot}K^{-1}{\cdot}mol^{-1}}$), 5,6-dimethyluracil (191.1 $\mathrm{J{\cdot}K^{-1}{\cdot}mol^{-1}}$), 1,3,6-trimethyluracil (212.6 $\mathrm{J{\cdot}K^{-1}{\cdot}mol^{-1}}$), and 1,3,5,6-tetramethyluracil (244.5 $\mathrm{J{\cdot}K^{-1}{\cdot}mol^{-1}}$). The presented data indicate that the results presented in the Table 4 differ from each other and depend on the kind and number of compounds used in the calculation.

Acknowledgment

The authors thank R. Swierzewski and M. Kozbial for their helpful assistance and fruitful discussions.

Literature Cited

(1) Zielenkiewicz, W. Thermodynamic investigations on derivatives of pyrimidine nucleic acid bases. *Thermochim. Acta.* **2003**, *405*, 155−169.

(2) Szterner, P.; Kaminski, M.; Zielenkiewicz, A. Vapour pressures, molar enthalpies of sublimation and molar enthalpies of solution in water of five halogenated derivatives of uracil. *J. Chem. Thermodyn.* **2002**, *34*, 1005−1012.

(3) Zielenkiewicz, W.; Szterner, P.; Kaminski, M. Vapor pressures, molar enthalpies of sublimation, and molar enthalpies of solution in water of selected amino derivatives of uracil and 5-nitrouracil. *J. Chem. Eng. Data* **2003**, *48*, 1132−1136.

(4) Zielenkiewicz, W.; Szterner, P. Vapor pressures, molar enthalpies of sublimation, and molar enthalpies of solution in water of 5-(trifluoromethyl) uracil. *J. Chem. Eng. Data* **2004**, *49*, 1197−1200.

(5) Zielenkiewicz, W.; Szterner, P. Thermodynamic investigation of uracil and its halo derivatives. Enthalpies of solution and solvation in methanol. *J. Chem. Eng. Data* **2005**, *50*, 1139−1143.

(6) Zielenkiewicz, W.; Kozbial, M.; Swierzewski, R.; Szterner, P. Heat capacities of uracil, thymine and its alkylated, cyclooligomenthylenated and halogenated derivatives by differential calorimetry. *J. Chem. Eng. Data* **2007**, *52*, 93−97.

(7) Zielenkiewicz, A.; Roux-Desgranges, G.; Roux, A. H.; Grolier, J. P. E.; Wierzchowski, K. L.; Zielenkiewicz, W. Apparent molar heat capacities and volumes, van der Waals volumes and accesible surface areas of alkylated derivatives of cytosine and uracil in aqueous solutions at 25 °C. *J. Solution Chem.* **1993**, *22*, 907−918.

(8) Zielenkiewicz, W.; Zielenkiewicz, A.; Wierzchowski, K. L. Thermochemistry of aqueous solutions of alkylated nucleic acid bases. VIII. Enthalpies of hydration of 6-alkyluracils. *J. Solution Chem.* **1993**, *22*, 975−981.

(9) Bednarek, E.; Dobrowolski, J.; Dobrosz-Temperek, K.; Sitkowski, J.; Kozerski, L. Theoretical and experimental ^{1}H, ^{13}C, ^{15}N, and ^{17}O NMR spectra of 5-nitro, 5-amino, and 5-carboxy uracils. *J. Mol. Struct.* **1999**, *25*, 333−337.

(10) Isobe, Y.; Tobe, M.; Inoue, Y.; Isobe, M.; Tsuchiya, M.; Hayashi, H. Structure and activity relationships of novel uracil derivatives as topical anti-inflammatory agents. *Bioorg. Med. Chem.* **2003**, *11*, 4933−4940.

(11) Miszczak-Zaborska, E.; Wozniak, K. The activity of thymidine phosphorylase obtained from human uterine leiomyomas and studied in the presence of pyrimidine derivatives. *Z. Naturforsch. C* **1997**, *52*, 670−675.

(12) Hirota, K.; Sawada, M.; Sajiki, H.; Sako, M. Synthesis of 6-aminouracils and pyrrolo[2,3-*d*]pyrimidine-2,4-diones and their inhibitory effect on thymidine phosphorylase. *Nucleic Acids Symp. Ser.* **1997**, *No. 37*, 59−60.

(13) Yano, S.; Kazuno, H.; Suzuki, N.; Emura, T.; Wierzba, K.; Yamashita, J. Synthesis and evaluation of 6-methylene-bridged uracil derivatives. Part 1: Discovery of novel orally active inhibitors of human thymidine phosphorylase. *Bioorg. Med. Chem.* **2004**, *12*, 3431−3441.

(14) Klein, R.; Lenzi, M.; Lim, T.; Hotchkiss, K.; Wilson, P.; Schwartz. E. Novel 6-substituted uracil analogs as inhibitors of the angiogenic actions of thymidine phosphorylase. *Biochem. Pharmacol.* **2001**, *62*, 1257−1263.

(15) Chickos, J. S.; Acree, W. E., Jr. Enthalpies of sublimation of organic and organometallic compounds 1910−2001. *J. Phys. Chem. Ref. Data* **2002**, *31*, 537−698.

(16) Chickos, J. S.; Hosseini, S.; Hesse, D. G.; Liebman, J. F. A. Group additivity approach for the estimation of heat capacities of organic liquids and solids. *J. Struct. Chem.* **1993**, *4*, 271−277.

(17) Chickos, J. S.; Hosseini, S.; Hesse, D. G.; Liebman, J. F. Heat capacity corrections to a standard state. A. Comparison of available methods for organic liquids and solids. *J. Struct. Chem.* **1993**, *4*, 261−269.

(18) Cox J. D. Section V: Calorimetry recast. In *IUPAC Recommended Calibration and Test Materials for the Realization of Physicochemical Properties*; Division of Chemical Standards, National Physical Laboratory: Teddington Middlesex, UK, November 1972.

(19) Cabani, S.; Gianni, P.; Mollica, V.; Lepori, L. Group contributions to the thermodynamic properties of non-ionic organic solutes in dilute aqueous solutions. *J. Solution Chem.* **1981**, *10*, 563−595.

(20) Berens, K.; Shugar, D. Ultraviolet absorption spectra and structure of halogenated uracils and their glycosides. *Acta Biochem. Pol.* **1963**, *10*, 25−47.

(21) Shugar, D.; Fox, J. Spectrophotometric studies of nucleic acid derivatives and related compounds as a function of pH. *Biochim. Biophys. Acta* **1952**, *369*, 199−218.

(22) Wempen, I.; Fox, J. Spectrophotometric studies of nucleic acid derivatives and related compounds. VI. On the structure of certain 5- and 6-halogenouracils and -cytosines. *J. Am. Chem. Soc.* **1964**, *86*, 2474−2477.

Received for review November 14, 2006. Accepted January 9, 2007.

JE6005168