ELSEVIER

Thermochimica Acta 375 (2001) 93–107

thermochimica acta

www.elsevier.com/locate/tca

A consistent set of formation properties of nucleic acid compounds
Purines and pyrimidines in the solid state and in aqueous solution

C.B. Ould-Moulaye, C.G. Dussap, J.B. Gros*

Laboratoire de Génie Chimique et Biochimique, Université Blaise Pascal, CUST, 24 Avenue des Landais, BP 206, 63174 Aubière Cedex, France

Received 21 November 2000; received in revised form 19 March 2001; accepted 19 March 2001

Abstract

Enthalpy, Gibbs energy of formation, absolute entropy and heat capacity of purines and pyrimidines were calculated in the solid state and in dilute aqueous solution from a single reference state. Properties in the solid state were calculated from combustion data. Properties in dilute aqueous solution were calculated from solubility data and p*K* values after reconciliation. From this complete set of consistent data, the transformed properties were calculated. Missing data were made up using chemical group contribution techniques giving the formation properties of some chemical bonds specific to purines and pyrimidines. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Bioenergetics; Thermodynamics; Formation properties; Dissociation constants; Purine and pyrimidine bases

1. Introduction

Nucleotide phosphates (NMP, NDP and NTP) serve a central function in energy coupling within cells. Because of their unique roles in various metabolic processes, such as the citric acid cycle, glyconeogenesis, amino-acids synthesis pathways, etc., the energetics of hydrolytic cleavage of ATP to ADP and ADP to AMP has been extensively studied [1–12]. Additional information is available concerning ITP/IDP [13,14]. However, most of these studies are limited to the calculation of hydrolysis reaction properties choosing zero values for the formation properties for all nucleotides [3]. This ignores the energetics of purine and pyrimidine salvage pathways [15], and more generally purine and pyrimidine metabolism, which is especially important in the overall regulation of chemical energy utilisation in living cells and the biosynthetic pathways of nucleic acids.

In this work, we estimate the values of formation properties of bases in the purine (adenine, guanine, hypoxanthine and xanthine) and pyrimidine (cytosine, thymine and uracil) groups both in the solid state and at infinite dilution in aqueous solution. This was achieved by reconciling different sources of data, such as combustion energies, solid solubilities, enthalpies of solution, dissociation constants and enzymatic

---

* Corresponding author. Tel.: +33-4-73-40-74-30;
fax: +33-4-73-40-78-29.
E-mail address: gros@gecbio.univ-bpclermont.fr (J.B. Gros).

0040-6031/01/$ – see front matter © 2001 Elsevier Science B.V. All rights reserved.
PII: S0040-6031(01)00522-6

Nomenclature

$C_{p,\mathrm{aq}\ i}$	heat capacity of compound i in aqueous solution (J mol^{-1} K^{-1})
$C_{p,\mathrm{S}\ i}$	heat capacity of compound i in the solid state (J mol^{-1} K^{-1})
$\Delta_{\mathrm{r}}C_p$	heat capacity change for proton dissociation (J mol^{-1} K^{-1})
$\Delta_{\mathrm{r}}C_{p\mathrm{d}}$	heat capacity change for solubilisation of solid in aqueous solution (J mol^{-1} K^{-1})
G	extensive Gibbs energy of the solution (kJ)
$\Delta_{\mathrm{f}}G_i^0(T;\mathrm{aq})$	molar Gibbs energy of formation or standard chemical potential of formation of species i in the ideal 1 molal solution of i in water (kJ mol^{-1})
$\Delta_{\mathrm{f}}G_i^0(T;\mathrm{L})$	Gibbs energy of formation of compound i in the pure liquid reference state (kJ mol^{-1})
$\Delta_{\mathrm{f}}G_i^0(T;\mathrm{S})$	Gibbs energy of formation of compound i in the pure solid reference state (kJ mol^{-1})
$\Delta_{\mathrm{f}}G_i'(T,m_i;\mathrm{aq})$	transformed molar Gibbs energy of formation of species i at given T, P, pH, and I_{m} (kJ mol^{-1})
$\Delta_{\mathrm{r}}G_{\mathrm{comb}}$	Gibbs energy of combustion of compound i (kJ mol^{-1})
$\Delta_{\mathrm{f}}G_{\mathrm{p}}^0(T;\mathrm{aq})$	Gibbs energy of proton dissociation of an acid (kJ mol^{-1})
H	extensive enthalpy of the solution (kJ)
$\Delta_{\mathrm{f}}H_i^0(T;\mathrm{aq})$	molar enthalpy of formation of species i (kJ mol^{-1})
$\Delta_{\mathrm{f}}H_i^0(T;\mathrm{L})$	enthalpy of formation of compound i in the pure liquid reference state (kJ mol^{-1})
$\Delta_{\mathrm{f}}H_i^0(T;\mathrm{S})$	enthalpy of formation of compound i in the pure solid reference state (kJ mol^{-1})
$\Delta_{\mathrm{f}}H_i'(T;m_i;\mathrm{aq})$	transformed molar enthalpy of formation of species i at given T, P, pH, and I_{m} (kJ mol^{-1})
$\Delta_{\mathrm{r}}H_{\mathrm{comb}}$	enthalpy of combustion of compound i (kJ mol^{-1})
$\Delta H_{\mathrm{d}i}^0(T;\mathrm{aq})$	enthalpy of dilution of compound i (kJ mol^{-1})
$\Delta_{\mathrm{r}}H_{\mathrm{p}}^0(T;\mathrm{aq})$	enthalpy of proton dissociation of an acid (kJ mol^{-1})
I_{m}	molal ionic strength calculated with ionic molality (mol kg^{-1})
K	equilibrium constant of proton dissociation
K_{c}	equilibrium constant of complex formation
m_i	molality of compound i (mol kg^{-1})
M_1	water molar mass (0.01802 kg mol^{-1})
n	total number of moles in the system (mol)
P	pressure (atm)
R	gas constant (J K^{-1} mol^{-1})
$S_i^0(T;\mathrm{S})$	absolute entropy of compound i in solid state at T; $P = 1$ atm (J mol^{-1} K^{-1})
$\Delta_{\mathrm{f}}S_i^0(T;\mathrm{aq})$	standard molar entropy of formation of species i (J mol^{-1} K^{-1})
$\Delta_{\mathrm{f}}S_i^0(T;\mathrm{S})$	entropy of formation of i in the pure solid reference state (J mol^{-1} K^{-1})
$\Delta_{\mathrm{r}}S_{\mathrm{p}}^0(T;\mathrm{aq})$	entropy of proton dissociation of an acid (J mol^{-1} K^{-1})
T	temperature (K)
T_{r}	reference temperature (298.15 K)
z_i	charge of compound i

Greek letters

λ_{ij}	elemental composition of compound i
γ_i	activity coefficient of compound i in molality basis
γ_i^{∞}	activity coefficient of compound i at infinite dilution in the molar fraction scale and a standard state pure liquid at T
$\mu_i(T;m_i;\mathrm{aq})$	chemical potential of solute i in aqueous solution at T (kJ mol^{-1})

Subscripts

c	complex formation
comb	reaction of combustion
d	dilution
p	reaction of proton dissociation of an acid
sat	saturated solution

Superscripts

0	standard conditions
∞	property at infinite dilution

equilibria constants, and by deriving thermodynamic relations whenever needed. From a compilation of existing data obtained by various experimental methods, we propose a consistent data set for Gibbs energy of formation, enthalpy of formation, absolute entropy and heat capacity of purines and pyrimidines in the solid state and at infinite dilution in aqueous solutions, including in the latter case the formation properties of charged species formed by successive acid dissociations.

These data were used to calculate the transformed properties of pseudo-isomers [9,12] which take into account the partial molar properties of all compounds (neutral and charged species) resulting from dissociation and/or complex formation at given pH, metal ion concentration and ionic strength [3,16,17]. The values obtained are to be used to evaluate partial molar properties of these compounds in complex solutions approaching biological media compositions for the purpose of performing a thermodynamic analysis of biosynthetic and degradation pathways of purines and pyrimidines.

2. Chemical structure of purines and pyrimidines

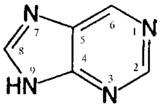
Nitrogenous bases are important in biochemistry because they are part of the building blocks of both DNA and RNA, and the different carbohydrate skeletons of chemical energy carriers such as ATP, GTP, ITP, etc. They form two groups: purines and pyrimidines. Their formulas and formula weights are given in Tables 1–3.

3. Principles of calculation of formation properties

Estimation of formation properties in aqueous solutions was done as follows:

1. selection of a data set for formation properties in the solid state, mainly from reliable experimental energies of combustion,
2. selection of a data set for solid solubilities and enthalpies of solution at infinite dilution in water,
3. selection of a data set for pK and heat of proton dissociation,
4. estimation of formation properties of transformed species defined as the composition weighted mean properties of each neutral and ionic species present in the solution at the pH and the temperature of the solid solubility measurement,
5. estimation of the formation properties of each individual species,
6. extrapolation to other conditions of pH, temperature, and solute concentration and comparison of the results with other sources of data.

Table 1
Formula and formula weight of purine and pyrimidine

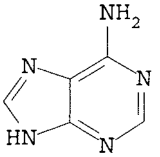
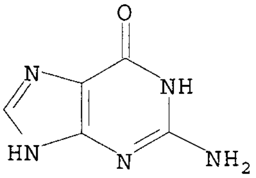
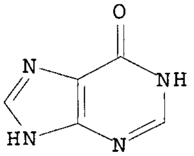
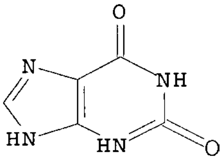
Compound	Formula	Formula weight (g mol^{-1})
Pyrimidine ($C_4H_4N_2$)		80.09
Purine ($C_5H_4N_4$)		120.11

The first three operations which concern the selection of experimental data, can also involve additional methods such as group contribution methods for estimating energies of formation when the data are lacking and for checking the consistency of the experimental data. The other operations require fitting the formation properties in aqueous solution to various equilibrium data. This requires a pertinent analysis of simultaneous chemical (acid dissociation) and physical (solid solubility) equilibria including activity corrections for neutral and charged species.

3.1. Standard states and general expressions of chemical potential

The standard state chosen for calculating the molar properties of dissolved species in solution is the classical standard state in the molality scale defined

Table 2
Formula and formula weight of several purine bases

Base	Formula	Formula weight (g mol^{-1})
Adenine (C_5 H_5 N_5)		135.13
Guanine ($C_5H_5N_5O$)		151.13
Hypoxanthine ($C_5H_4N_4O$)		136.11
Xanthine ($C_5H_4N_4O_2$)		152.11

as an ideal aqueous solution of molality 1 mol kg^{-1} at 25°C. The chemical potential and the molar enthalpy of solute i in any aqueous solution are given by

$$\mu_i(T;m_i;\mathrm{aq}) = \mu_i^0(T;\mathrm{aq}) + RT\ln\left(\frac{\gamma_i m_i}{m^0}\right) \tag{1}$$

$$H_i(T;m_i;\mathrm{aq}) = H_i^0(T;\mathrm{aq}) - RT^2\left(\frac{\partial \ln \gamma_i}{\partial T}\right)_{P,m_i} \tag{2}$$

Here m_i is the molality of the solute, $m^0 = 1$ mol kg^{-1} and γ_i the activity coefficient in the molality scale. $\mu_i^0(T;\mathrm{aq})$ and $H_i^0(T;\mathrm{aq})$ denote for the standard chemical potential and enthalpy of species i, that is the properties in a 1 molal ideal solution. For actual calculations, these properties are replaced by the corresponding formation properties, and so the former equations become [6]

$$\Delta_f G_i(T;m_i;\mathrm{aq}) = \Delta_f G_i^0(T;\mathrm{aq}) + RT\ln\left(\frac{\gamma_i m_i}{m^0}\right) \tag{3}$$

$$\Delta_f H_i(T;m_i;\mathrm{aq}) = \Delta_f H_i^0(T;\mathrm{aq}) - RT^2\left(\frac{\partial \ln \gamma_i}{\partial T}\right)_{P,m_i} \tag{4}$$

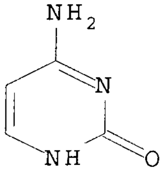
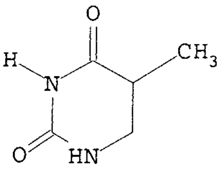
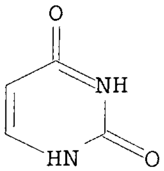
$\Delta_f G_i(T;m_i;\mathrm{aq})$ and $\Delta_f H_i(T;m_i;\mathrm{aq})$ represent, respectively, the Gibbs energy and the enthalpy of formation in the aqueous solution of molality m_i. $\Delta_f G_i^0(T;\mathrm{aq})$ and $\Delta_f H_i^0(T;\mathrm{aq})$ are the corresponding formation properties in the ideal 1 molal solution. From the above relations, the entropy of formation is given by

$$\Delta_f S_i^0(T;\mathrm{aq}) = \frac{\Delta_f H_i^0(T;\mathrm{aq}) - \Delta_f G_i^0(T;\mathrm{aq})}{T} \tag{5}$$

The enthalpy of formation at temperature T is calculated from the enthalpy of formation at $T_r = 298.15$ K and heat capacity of the dissolved species i

$$\Delta_f H_i^0(T;\mathrm{aq}) = \Delta_f H_i^0(T_r;\mathrm{aq}) + C_{p,\mathrm{aq}\,i}(T - T_r) \tag{6}$$

Table 3
Formula and formula weight of several pyrimidine bases

Base	Formula	Formula weight (g mol^{-1})
Cytosine ($C_4H_5N_3O$)		111.10
Thymine ($C_5H_6N_2O_2$)		126.11
Uracil ($C_4H_4N_2O_2$)		112.09

In the same way

$$\Delta_f S_i^0(T;\mathrm{aq}) = \Delta_f S_i^0(T_r;\mathrm{aq}) + C_{p,\mathrm{aq}\,i}\ln\left(\frac{T}{T_r}\right) \tag{7}$$

so that

$$\Delta_f G_i^0(T;\mathrm{aq}) = \Delta_f G_i^0(T_r;\mathrm{aq}) + (C_{p,\mathrm{aq}\,i} - \Delta_f S_i^0(T_r;\mathrm{aq})) \times (T - T_r) - C_{p,\mathrm{aq}\,i}T\ln\left(\frac{T}{T_r}\right) \tag{8}$$

Also, the formation properties $\Delta_f G_i^0(T_r;\mathrm{aq})$ and $\Delta_f H_i^0(T_r;\mathrm{aq})$ which refer to a 1 molal ideal solution are related to the formation properties of pure liquid i at T_r

$$\Delta_f G_i^0(T_r;\mathrm{aq}) = \Delta_f G_i^0(T_r;\mathrm{L}) + RT_r\ln(\gamma_i^\infty M_1 m^0) \tag{9}$$

$$\Delta_f H_i^0(T_r;\mathrm{aq}) = \Delta_f H_i^0(T_r;\mathrm{L}) - RT_r^2\left(\frac{\partial \ln\gamma_i^\infty}{\partial T}\right)_{P,x_1\cong 1} \tag{10}$$

where M_1 is the water molar mass ($M_1 = 0.01802\ \mathrm{kg\,mol^{-1}}$), $m^0 = 1\ \mathrm{mol\,kg^{-1}}$ and γ_i^∞ the activity coefficient at infinite dilution calculated in the molar fraction scale with the pure liquid reference state.

3.2. Chemical equilibria: actual and transformed partial molar properties

Most of the compounds involved in biochemical reactions, in particular, purine and pyrimidine bases, dissociate and/or form complexes with cations. This requires simultaneous analysis of acid dissociation and other physical equilibria (solid/liquid, vapour/liquid). This has to be taken into account when calculating the formation properties of the pure substances and of the different species in solution.

In the simple case of a single acid dissociation ($C_{\mathrm{neutral}} \Leftrightarrow C^- + H^+$), the chemical equilibrium is characterised by the equilibrium constant K

$$K = \frac{\gamma_{C^-} m_{C^-} \gamma_{H^+} m_{H^+}}{\gamma_{C\,\mathrm{neutral}}\, m_{C\,\mathrm{neutral}}} \tag{11}$$

The material balance in the solution is written as a function of the total molality $m_{C\ \mathrm{total}}$ of all compounds containing the C skeleton, which is generally a fixed quantity

$$m_{\mathrm{C\,total}} = m_{\mathrm{C\,neutral}} + m_{\mathrm{C^-}} \tag{12}$$

where $m_{\mathrm{C\,total}}$ represents the molality of the pseudo-isomer [9,12].

Using the usual definitions of pK (p$K = -\log_{10} K$) and pH ($\mathrm{pH} = -\log_{10}\gamma_{\mathrm{H^+}} m_{\mathrm{H^+}}$ [37]), relations (11) and (12) afford the fractions of neutral and charged species

$$z_{\mathrm{C\,neutral}} = \frac{m_{\mathrm{C\,neutral}}}{m_{\mathrm{C\,total}}} = \left[1 + \frac{\gamma_{\mathrm{C\,neutral}}}{\gamma_{\mathrm{C^-}}} \times 10^{\mathrm{pH}-\mathrm{p}K}\right]^{-1} \tag{13}$$

$$z_{\mathrm{C^-}} = \frac{m_{\mathrm{C^-}}}{m_{\mathrm{C\,total}}} = \left[1 + \frac{\gamma_{\mathrm{C^-}}}{\gamma_{\mathrm{C\,neutral}}} \times 10^{\mathrm{p}K-\mathrm{pH}}\right]^{-1} \tag{14}$$

The measurement of the heat of protonation $\Delta_r H_p^0(T;\mathrm{aq})$ and relations (13) and (14) give the expressions of the partial molar properties.

$$\Delta_f G_{\mathrm{C^-}}(T; m_{\mathrm{C^-}};\mathrm{aq}) = \Delta_f G^0_{\mathrm{C^-}}(T;\mathrm{aq}) + RT\ln(\gamma_{\mathrm{C^-}} z_{\mathrm{C^-}} m_{\mathrm{C\,total}}) \tag{15}$$

$$\Delta_f G_{\mathrm{C\,neutral}}(T; m_{\mathrm{C\,neutral}};\mathrm{aq}) = \Delta_f G^0_{\mathrm{C\,neutral}}(T;\mathrm{aq}) + RT\ln(\gamma_{\mathrm{C\,neutral}} z_{\mathrm{C\,neutral}} m_{\mathrm{C\,total}}) \tag{16}$$

Considering that pK values characterise the dissociation equilibria at infinite dilution in aqueous solution, the formation properties in the ideal 1 molal solution are related to pK and $\Delta_r H_p^0(T;\mathrm{aq})$ by

$$\Delta_f G^0_{\mathrm{C\,neutral}}(T;\mathrm{aq}) = \Delta_f G^0_{\mathrm{C^-}}(T;\mathrm{aq}) + \Delta_f G^0_{\mathrm{H^+}}(T;\mathrm{aq}) - RT\,\mathrm{p}K\ln 10 \tag{17}$$

$$\Delta_f H^0_{\mathrm{C\,neutral}}(T;\mathrm{aq}) = \Delta_f H^0_{\mathrm{C^-}}(T;\mathrm{aq}) + \Delta_f H^0_{\mathrm{H^+}}(T;\mathrm{aq}) - \Delta_r H_p^0(T;\mathrm{aq}) \tag{18}$$

If the charged species C^- forms a complex with a metal ion M^+ by the reaction $CM \Leftrightarrow C^- + M^+$, the chemical equilibrium is characterised by the constant K_c

$$K_c = \frac{\gamma_{\mathrm{C^-}} m_{\mathrm{C^-}} \gamma_{\mathrm{M^+}} m_{\mathrm{M^+}}}{\gamma_{\mathrm{CM}} m_{\mathrm{CM}}} \tag{19}$$

By analogy with Eq. (12), we can write

$$m_{\mathrm{C\,total}} = m_{\mathrm{C\,neutral}} + m_{\mathrm{C^-}} + m_{\mathrm{CM}} \tag{20}$$

which gives

$$z_{\mathrm{C^-}} = \frac{m_{\mathrm{C^-}}}{m_{\mathrm{C\,total}}} = \left[1 + \frac{\gamma_{\mathrm{C^-}}}{\gamma_{\mathrm{C\,neutral}}} \times 10^{\mathrm{p}K-\mathrm{pH}} + \frac{\gamma_{\mathrm{C^-}}}{\gamma_{\mathrm{CM}}} \times 10^{\mathrm{p}K_c-\mathrm{pM}}\right]^{-1} \tag{21}$$

where $\mathrm{pM} = -\log_{10}\gamma_{\mathrm{M^+}} m_{\mathrm{M^+}}$ and $\mathrm{p}K_c = -\log_{10} K_c$.

$$z_{\mathrm{C\,neutral}} = \frac{m_{\mathrm{C\,neutral}}}{m_{\mathrm{C\,total}}} = \left[1 + \frac{\gamma_{\mathrm{C\,neutral}}}{\gamma_{\mathrm{CM}}} \times 10^{\mathrm{p}K_c-\mathrm{p}K+\mathrm{pH}-\mathrm{pM}} + \frac{\gamma_{\mathrm{C\,neutral}}}{\gamma_{\mathrm{C^-}}} \times 10^{\mathrm{pH}-\mathrm{p}K}\right]^{-1} \tag{22}$$

$$z_{\mathrm{CM}} = \frac{m_{\mathrm{CM}}}{m_{\mathrm{C\,total}}} = \left[1 + \frac{\gamma_{\mathrm{CM}}}{\gamma_{\mathrm{C\,neutral}}} \times 10^{\mathrm{p}K-\mathrm{p}K_c-\mathrm{pH}+\mathrm{pM}} + \frac{\gamma_{\mathrm{CM}}}{\gamma_{\mathrm{C^-}}} \times 10^{\mathrm{pM}-\mathrm{p}K}\right]^{-1} \tag{23}$$

It is also necessary to define the formation properties characterising the overall properties of all dissolved species containing the chemical skeleton C. These properties are referred as transformed formation properties of the pseudo-isomer in the solution [9,12] and they actually depend on solution composition. When pH and pM are specified, the formation properties of the pseudo-isomer are strictly defined by [9,10,16]

$$\Delta_f G'_{\mathrm{C}}(T; m_{\mathrm{C\,total}};\mathrm{aq}) = \left(\frac{\partial G}{\partial n_{\mathrm{C\,total}}}\right)_{T,P,\mathrm{pH},\mathrm{pM},n_{\mathrm{H_2O}}} \tag{24}$$

$$\Delta_f H'_{\mathrm{C}}(T; m_{\mathrm{C\,total}};\mathrm{aq}) = \left(\frac{\partial H}{\partial n_{\mathrm{C\,total}}}\right)_{T,P,\mathrm{pH},\mathrm{pM},n_{\mathrm{H_2O}}} \tag{25}$$

where G and H denote for the extensive Gibbs energy and extensive enthalpy of the solution. In buffered solutions, it has been shown [16] that close approximations of $\Delta_f G'_{\mathrm{C}}(T; m_{\mathrm{C\,total}};\mathrm{aq})$ and $\Delta_f H'_{\mathrm{C}}(T; m_{\mathrm{C\,total}};\mathrm{aq})$ are provided by the following expressions:

$$\begin{aligned}\Delta_f G'_{\mathrm{C}}(T; m_{\mathrm{C\,total}};\mathrm{aq}) &= z_{\mathrm{C\,neutral}}\Delta_f G_{\mathrm{C\,neutral}}(T; m_{\mathrm{C\,neutral}};\mathrm{aq}) \\ &\quad + z_{\mathrm{C^-}}(\Delta_f G_{\mathrm{C^-}}(T; m_{\mathrm{C^-}};\mathrm{aq}) + \Delta_f G_{\mathrm{H^+}}(T; m_{\mathrm{H^+}};\mathrm{aq})) \\ &\quad + z_{\mathrm{CM}}(\Delta_f G_{\mathrm{CM}}(T; m_{\mathrm{CM}};\mathrm{aq}) \\ &\quad - \Delta_f G_{\mathrm{M^+}}(T; m_{\mathrm{M^+}};\mathrm{aq})) + \Delta_f G_{\mathrm{H^+}}(T; m_{\mathrm{H^+}};\mathrm{aq})\end{aligned} \tag{26}$$

which gives (Eqs. (11), (15)–(17) and $z_{\mathrm{C\,neutral}} + z_{\mathrm{C^-}} + z_{\mathrm{CM}} = 1$)

$$\Delta_f G'_C(T; m_{\mathrm{C\,total}}; \mathrm{aq}) = \Delta_f G_{\mathrm{C\,neutral}}(T; m_{\mathrm{C\,neutral}}; \mathrm{aq}) \tag{27}$$

$$\begin{aligned}\Delta_f H'_C(T; m_{\mathrm{C\,total}}; \mathrm{aq}) &= z_{\mathrm{C\,neutral}} \Delta_f H_{\mathrm{C\,neutral}}(T; m_{\mathrm{C\,neutral}}; \mathrm{aq}) \\ &\quad + z_{\mathrm{C^-}}(\Delta_f H_{\mathrm{C^-}}(T; m_{\mathrm{C^-}}; \mathrm{aq}) + \Delta_f H_{\mathrm{H^+}}(T; m_{\mathrm{H^+}}; \mathrm{aq})) \\ &\quad + z_{\mathrm{CM}}(\Delta_f H_{\mathrm{CM}}(T; m_{\mathrm{CM}}; \mathrm{aq}) \\ &\quad - \Delta_f H_{\mathrm{M^+}}(T; m_{\mathrm{M^+}}; \mathrm{aq}))\end{aligned} \tag{28}$$

3.3. Estimation of activity coefficients

To a first approximation, the activity coefficient in Eq. (1), γ_i, can be taken as equal to 1 for all neutral compounds (assumption of Henry's law) provided the total concentration of all dissolved species in the solution does not exceed $1\ \mathrm{mol\,l^{-1}}$. For higher concentrations, solution models are required to predict the activity coefficients and the group-contribution models [18] are the most useful. For charged species, activities deviate from ideality even at low concentrations and/or at low ionic strengths. A simplified model is recommended for correlating activity coefficients at ionic strengths below $0.3\ \mathrm{mol\,kg^{-1}}$ [8,16]

$$\ln \gamma_i = -A \frac{I_m^{1/2}}{1 + 1.6 I_m^{1/2}} z_i^2 \tag{29}$$

where $A = N_a e^3 \sqrt{2 \times 10^3} / 8\pi(\varepsilon RT)^{3/2}$ is the Debye–Hückel parameter, $N_a = 6.02214 \times 10^{23}\ \mathrm{mol^{-1}}$, the Avagadro's number, $e = 4.802654 \times 10^{-10}$ esu, the electronic charge, and $R = 8.3144\ \mathrm{J\,mol\,K^{-1}}$, the gas law constant.

The molal ionic strength I_m is given by

$$I_m = \frac{1}{2} \sum_i m_i z_i^2 \tag{30}$$

where z_i is the ionic charge, and the summation covers all the charged species in the solution.

3.4. Reference state of the elements

For proper comparison of the formation properties of the different purine and pyrimidine bases, the reference state for chemical substances is reported in Table 4 [22].

As this state must also concern charged species, it also includes the formation properties of H^+ ($\Delta_f G^0_{\mathrm{H^+}}(T; \mathrm{aq}) = 0, \Delta_f H^0_{\mathrm{H^+}}(T; \mathrm{aq}) = 0$). These values are also used to express, from Eqs. (17) and (18), the variations of Gibbs energy and variations of enthalpy relevant to the proton dissociation

$$\begin{aligned}RT\,\mathrm{p}K \ln 10 &= \Delta_r G^0_p(T; \mathrm{aq}) \\ &= \Delta_f G^0_{\mathrm{C^-}}(T; \mathrm{aq}) - \Delta_f G^0_{\mathrm{C\,neutral}}(T; \mathrm{aq})\end{aligned} \tag{31}$$

$$\Delta_r H^0_p(T; \mathrm{aq}) = \Delta_f H^0_{\mathrm{C^-}}(T; \mathrm{aq}) - \Delta_f H^0_{\mathrm{C\,neutral}}(T; \mathrm{aq}) \tag{32}$$

Table 4

Gibbs energies and enthalpies of formation, absolute entropies and heat capacities of reference gases and elements in the state $T_r = 298.15$ K, $P = 1$ atm, $m^0 = 1\ \mathrm{mol\,kg^{-1}}$ given by Cox et al. [22]

Compound	State	$D_f H_i^0$ ($\mathrm{kJ\,mol^{-1}}$)	S_i^0 ($\mathrm{J\,K^{-1}\,mol^{-1}}$)	$C_{p,i}$ ($\mathrm{J\,K^{-1}\,mol^{-1}}$)
H_2	Gas	0	130.57	28.82
O_2	Gas	0	205.04	29.36
N_2	Gas	0	191.50	29.12
C	Crystal, graphite	0	5.74	8.53
Mg	Crystal	0	32.67	24.89
Na	Crystal	0	51.3	28.24
K	Crystal	0	64.68	29.58
Ca	Crystal	0	41.59	25.31
P	Crystal, white	0	41.09	23.84
H^+	Aqueous ($m^0 = 1\ \mathrm{mol\,kg^{-1}}$)	0	0	0
e^-	Aqueous ($m^0 = 1\ \mathrm{mol\,kg^{-1}}$)	0	65.28	14.41

This enables to compute the absolute entropy of any compounds from the entropy of formation in the reference standard state

$$\Delta_\mathrm{f} S_i^0(T;\mathrm{aq}) = S_i^0(T;\mathrm{aq}) + \sum_j \lambda_{ij} S_j^0(T;\mathrm{aq}) - z_i S_{e^-}^0(T;\mathrm{aq}) \qquad (33)$$

where λ_{ij} are the elemental compositions of compound i. The summation stands for all the elements of the compound including its charge z_i.

3.5. Characterisation of solid–liquid equilibria

The link between the formation properties of a compound in the solid state $\Delta_\mathrm{f} G_i(T;\mathrm{S})$ and the formation properties in the standard state requires

1. measuring the solubility and enthalpy of dilution of the compound and
2. solving the solid–liquid equilibrium relations.

For a non-dissociating solute, the saturation equilibrium at 25°C is characterised by

$$\Delta_\mathrm{f} G_i(T;\mathrm{S}) = \Delta_\mathrm{f} G_i^0(T;\mathrm{aq}) + RT_\mathrm{r} \ln \gamma_{i\,\mathrm{sat}} m_{i\,\mathrm{sat}} \qquad (34)$$

The enthalpy of dilution at 25°C at infinite dilution is given by

$$\Delta H_{\mathrm{d}i}^0(T;\mathrm{aq}) = \Delta_\mathrm{f} H_i^0(T;\mathrm{aq}) - \Delta_\mathrm{f} H_i(T;\mathrm{S}) \qquad (35)$$

where $m_{i\ \mathrm{sat}}$ and $\Delta H_{\mathrm{d}i}^0(T;\mathrm{aq})$ are measured quantities, Eqs. (2), (34) and (35) link $\Delta_\mathrm{f} G_i(T;\mathrm{S})$ to $\Delta_\mathrm{f} G_i^0(T;\mathrm{aq})$ and $\Delta_\mathrm{f} H_i(T;\mathrm{S})$ to $\Delta_\mathrm{f} H_i^0(T;\mathrm{aq})$ and $\Delta_\mathrm{f} H_i(T;m_{i\,\mathrm{sat}};\mathrm{aq})$.

When the solute is dissociating, relations (34) and (35) remain valid but it must be emphasised that the variables $\Delta_\mathrm{f} G_i^0(T;\mathrm{aq}), \Delta_\mathrm{f} H_i^0(T;\mathrm{aq})$, $m_{i\ \mathrm{sat}}$ and $\Delta H_{\mathrm{d}i}^0(T;\mathrm{aq})$ are solely representative of the non-dissociated neutral fraction of the solute that is actually in equilibrium with the solid compound. Given that the measured quantities are the total quantities $m_{i\ \mathrm{total\ sat}}$ and $\Delta H_{\mathrm{d}i\,\mathrm{total}}^0(T;\mathrm{aq})$, this requires both chemical and physical equilibria to be taken into account. This is achieved using Eqs. (13)–(18), (20)–(23), (34) and (35), where $\Delta_\mathrm{f} H_{\mathrm{H}^+}^0(T;\mathrm{aq}) = 0$, $\Delta_\mathrm{f} G_{\mathrm{H}^+}^0(T;\mathrm{aq}) = 0$ (Table 4), pK, pK_c, pH, pM, $\Delta_\mathrm{r} H_\mathrm{p}^0(T;\mathrm{aq})$, $\Delta_\mathrm{f} G_i(T;\mathrm{S}), \Delta_\mathrm{f} H_i(T;\mathrm{S})$ are assumed to be known, $m_{\mathrm{C\ total\ sat}}$ and $\Delta H_{\mathrm{d}i}^0(T;\mathrm{aq})$ are assumed to be measured and m_{C^-}, $m_{\mathrm{C\,neutral}}$, m_{CM}, $\Delta_\mathrm{f} G_{\mathrm{C}^-}(T;m_{\mathrm{C}^-};\mathrm{aq})$, $\Delta_\mathrm{f} G_{\mathrm{C\,neutral}}(T;m_{\mathrm{C\,neutral}};\mathrm{aq})$, $\Delta_\mathrm{f} G_{\mathrm{CM}}(T;m_{\mathrm{CM}};\mathrm{aq})$, $\Delta_\mathrm{f} G_{\mathrm{C}^-}^0(T;\mathrm{aq})$, $\Delta_\mathrm{f} G_{\mathrm{C\,neutral}}^0(T;\mathrm{aq})$, $\Delta_\mathrm{f} G_{\mathrm{CM}}^0(T;\mathrm{aq})$, $\Delta_\mathrm{f} H_{\mathrm{C}^-}^0(T;\mathrm{aq})$, $\Delta_\mathrm{f} H_{\mathrm{C\,neutral}}^0(T;\mathrm{aq})$, $\Delta_\mathrm{f} H_{\mathrm{CM}}^0(T;\mathrm{aq})$ are the 12 unknowns calculated by solving the above system of 12 equations.

4. Sources of data

From the above, the calculation of enthalpy and Gibbs energy of formation of a compound in the solid state and in the standard state in aqueous solution requires experimentally determining the combustion data for solids, and the solubility data, if necessary together with dissociation properties. To calculate the formation properties of purines and pyrimidines, several types of data were used and compared.

1. Values of formation properties in the solid state which are available [19–22] for adenine, guanine and xanthine.
2. Values of combustion energies of the purines and pyrimidines in the solid state, to check the validity of the previous data. Given that the combustion reaction is written

$$\mathrm{C}_\alpha H_\beta \mathrm{O}_\gamma \mathrm{N}_\delta + (\alpha + \tfrac{1}{4}\beta - \tfrac{1}{2}\gamma)\mathrm{O}_{2(\mathrm{g})} \rightarrow \alpha \mathrm{CO}_{2(\mathrm{g})} + \tfrac{1}{2}\delta \mathrm{N}_{2(\mathrm{g})} + \tfrac{1}{2}\beta \mathrm{H_2O}_{(\mathrm{l})} \qquad (36)$$

and using the formation properties of $\mathrm{O}_{2(\mathrm{g})}$, $\mathrm{N}_{2(\mathrm{g})}$, $\mathrm{H_2O}_{(\mathrm{l})}$ and $\mathrm{CO}_{2(\mathrm{g})}$ (Table 4), the formation properties of $\mathrm{C}_\alpha H_\beta \mathrm{O}_\gamma \mathrm{N}_\delta$ are calculated

$$\Delta_\mathrm{f} H(T;\mathrm{S}) = -\Delta_\mathrm{r} H_\mathrm{comb} - 393.51\alpha - 285.83(\tfrac{1}{2}\beta) \qquad (37)$$

$$\Delta_\mathrm{f} G(T;\mathrm{S}) = -\Delta_\mathrm{f} G_\mathrm{comb} - 394.24\alpha - 237.18(\tfrac{1}{2}\beta) \qquad (38)$$

were $\Delta_\mathrm{f} H_\mathrm{comb}$ and $\Delta_\mathrm{f} G_\mathrm{comb}$ are the properties of combustion of the compound to $\mathrm{CO_2}$ (1 atm, 25°C, $\mathrm{H_2O}$ liquid, $\mathrm{N_2}$ (1 atm, 25°C) from oxygen (1 atm, 25°C).

In the same way, Roels [24] proposed a method to predict the enthalpy and Gibbs energy of combustion of a compound according to its degree of reduction ($d_\mathrm{r} = 4\alpha + \beta - 2\gamma$). The relations suggested are, for the compound $\mathrm{C}_\alpha H_\beta \mathrm{O}_\gamma \mathrm{N}_\delta$

$$d_\mathrm{r} = 4\alpha + \beta - 2\gamma \qquad (39)$$

$$\Delta_r H_{\text{comb}} = -115 d_r \,(\text{kJ mol}^{-1}) \tag{40}$$

$$\Delta_r G_{\text{comb}} = -94.4 d_r - 86.6\alpha \,(\text{kJ mol}^{-1}) \tag{41}$$

$$\Delta_r S_{\text{comb}} = -69.09 d_r + 290.5\alpha \,(\text{J mol}^{-1}\,\text{K}^{-1}) \tag{42}$$

The signs in Eqs. (40)–(42) have been changed from those as reported [24] to be consistent with the definition of combustion properties.

Using the formation properties of $O_{2(g)}$, $N_{2(g)}$, $H_2O_{(l)}$ and $CO_{2(g)}$, we obtain

$$\Delta_f H(T;\text{S}) = 115 d_r - 142.92\beta - 393.51\alpha \,(\text{kJ mol}^{-1}) \tag{43}$$

$$\Delta_f G(T;\text{S}) = 94.4 d_r - 118.59\beta - 307.64\alpha \,(\text{kJ mol}^{-1}) \tag{44}$$

$$\Delta_f S(T;\text{S}) = 69.09 d_r - 81.80\beta - 288.01\alpha \,(\text{J mol}^{-1}\,\text{K}^{-1}) \tag{45}$$

3. pK values of purines and pyrimidines taken from [25–28]. Comparison of the different data sources shows high consistency of the data provided all data are extrapolated to zero ionic strength using the activity coefficient correction (Eqs. (28) and (30)).
4. Solid solubilities and enthalpies of solution reported in series of papers [29–32,36]. The recent data are in satisfactory agreement with earlier experimental measurements.
5. A group contribution method proposed by Mavrovouniotis [34] to predict the Gibbs energy of formation of carbohydrate in aqueous solution, at pH 7 and 25°C. This method is known to be applicable to purines and pyrimidines. However, it must be kept in mind that the predictions given by Mavrovouniotis [33,34] do not account for the different degrees of dissociation of the compounds at pH 7. Therefore, the values of Gibbs energies of formation obtained by this predictive group contribution method are probably valid only for the neutral (or overall molar) properties. Consequently, these predictions will be used only for comparison.

5. Results

5.1. Formation properties in the solid state

Table 5 gives the properties of purines and pyrimidines in the solid state. The enthalpy of formation was available for each compound. Four data sources were compared [19–21,24]. The data are in close agreement for adenine [19,20], guanine [20,21,24], hypoxanthine [20,21] and xanthine [20,21]. Wider discrepancies are

Table 5
Available values of formation properties of purine and pyrimidine bases in the solid state

Compound	$\Delta_f H_i^0(T;\text{S})$ (kJ mol^{-1})	$\Delta_f G_i^0(T;\text{S})$ (J mol^{-1} K^{-1})	$S_i^0(T;\text{S})$	$C_{p,\text{S}\ i}$
Adenine (cr) ($C_5H_5N_5$)	97.1 [19] 96.0 [20]	300.4 [19]	151.0 [19]	143 [19]
Guanine (cr) ($C_5H_5N_5O$)	−182.9 [21] −183.9 [20]	47.8 [35]		
Hypoxanthine (cr) ($C_5H_4N_4O$)	−109.8 [21] −110.8 [20]		145.6 [20]	134 [20]
Xanthine (cr) ($C_5H_4N_4O_2$)	−378.6 [21] −379.6 [20]		161.1 [20]	151 [20]
Cytosine (cr) ($C_4H_5N_3O$)	−221.3 [20]			133 [20]
Thymine (cr) ($C_5H_6N_2O_2$)	−468.2 [21] 462.8 [20]			151 [20]
Uracil (cr) ($C_4H_4N_2O_2$)	−429.4 [20]			120 [20]

Table 6
Selected values for formation properties of purine and pyrimidine bases in the solid state[a]

Compound	$\Delta_f H_i^0(T;S)$ (kJ mol^{-1})	$\Delta_f G_i^0(T;S)$ (J mol^{-1} K^{-1})	$S_i^0(T;S)$	$C_{p,S\ i}$
Adenine (cr)	96.0 [20]	299.3	152.0	143 [19]
Guanine (cr)	−183.9 [20]	**47.5**	**160.2**	**151**
Hypoxanthine (cr)	−110.8 [20]	77.1	145.1[b] [20]	134 [20]
Xanthine (cr)	−379.6 [20]	−165.7	160.5[b] [20]	151 [20]
Cytosine (cr)	−221.3 [20]	−42.9	**140.8**	133 [20]
Thymine (cr)	−462.8 [20]	−286.4	**160.1**	151 [20]
Uracil (cr)	−429.4 [20]	−264.6	**128.0**	120 [20]

[a] Values in bold figures estimated in this work.

[b] Values of the absolute entropy readjusted to the reference state defined in Table 4.

observed for the pyrimidine group between references [20,21,24]. Data from [24] were not retained, the state (solid or solution) and the reference state not being clearly identified.

We selected the values of Lide [20] which are recommended by the National Bureau of Standards, and are available for all compounds (Table 6).

Regarding the Gibbs energy of formation, only three out of seven absolute entropies of compounds were available (adenine, hypoxanthine and xanthine). In addition, six heat capacities out of seven had been measured [19,20], the heat capacity of guanine being still unreported.

To complete the table of Gibbs energy of formation in the solid state, we proceeded as follows.

1. The absolute entropy was calculated starting from the available data for Gibbs energy and enthalpy (Eq. (33)).
2. Table 5 was first consistently completed for all the properties $\Delta_f H_i(T;S)$, $\Delta_f G_i(T;S)$, $\Delta_f S_i(T;S)$, $S_i^0(T;S)$ and $C_{p,S\ i}$, only three independent data points being necessary to calculate the others,
3. From a complete data set for absolute entropies and heat capacities for amino acids and other nitrogenous compounds in the solid state [20,23], the simple empirical correlation was established

$$\frac{S^0(T;S)}{C_{p,S}} = 1.062 \pm 0.013 \qquad (46)$$

The relatively small confidence interval (absolute error in the determination 2.5%) clearly indicates that this type of correlation is suitable for determining the absolute entropy starting from the C_p value, and conversely. Relation (46) was used to calculate the absolute entropies of the three bases of the pyrimidine group (cytosine, thymine, uracil) from the values of the heat capacities. The values obtained are reported in Table 6. The other properties, standard entropy and Gibbs energy, were calculated as previously.
4. For guanine, the value of absolute entropy was estimated assuming that the ratio of absolute entropy over formula weight of purine was constant and equal to 1.06, this value being calculated from data on hypoxanthine and xanthine, which has a chemical formula similar to guanine. We estimate that this assumption provides an estimation of the absolute entropy of ±2 J mol^{-1} K^{-1}, which corresponds to an estimation of the standard Gibbs energy of ±1 kJ mol^{-1}. The value obtained, $\Delta_f G^0_{\text{guanine}}(T;S) = 47.5$ kJ mol^{-1}, is comparable to the published value of $\Delta_f G^0_{\text{guanine}}(T;S) = 47.8$ kJ mol^{-1} [24,35], for which the state and the reference are not given. Table 6 is a complete compilation of formation properties of purines and pyrimidines in the solid state.

5.2. Formation properties in aqueous solution at infinite dilution

The proton dissociation properties pK, $\Delta_r H_p^0(T;\text{aq})$ from several references are reported in Table 7.

These data were also used to calculate $\Delta_r G_p^0(T;\text{aq})$ (Eq. (31)) and $\Delta_r S_p^0(T;\text{aq})$. The unmeasured values of $\Delta_r C_p$ were calculated assuming that the ratio

Table 7
pK, $\Delta_r H_p^0(T;\mathrm{aq})$, $\Delta_r S_p^0(T;\mathrm{aq})$ and $\Delta_r C_p$ values for proton ionisation from several bases at 25°C and for $I_m = 0$ in sites indicated in Table 1[a]

Compound	Ionisation site	Reaction[b]	pK	$\Delta_r H_p^0(T;\mathrm{aq})$ (kJ mol^{-1})	$\Delta_r S_p^0(T;\mathrm{aq})$ (J mol^{-1} K^{-1})	$\Delta_r C_p$ (J mol^{-1} K^{-1})
Adenine	N_1H^+	$H_2A^+ \Leftrightarrow HA + H^+$	4.20 [25]	20.1 [26]	−12.9	−23.3[c]
Adenine	N_9H	$HA \Leftrightarrow A^- + H^+$	9.87 [25]	40.4 [26]	−53.6	−96.4[c]
Guanine	N_7H^+	$H_2G^+ \Leftrightarrow HG + H^+$	3.3 [27]	17.2 [27]	−5.4	−8[c]
Guanine	N_1H	$HG \Leftrightarrow G^- + H^+$	9.63 [27]	41.5 [27]	−45.3	−81.5[c]
Hypoxanthine	N_7H^+	$H_3Hy^+ \Leftrightarrow H_2Hy + H^+$	1.79 [26]	12.3 [26]	7.1	12.8[c]
Hypoxanthine	$N_1H–C_6O$	$H_2Hy \Leftrightarrow HHy^- + H^+$	8.81 [26]	33.0 [26]	−58.1	−104.5[c]
Hypoxanthine	N_9H	$HHy^- \Leftrightarrow Hy^{2-} + H^+$	12.07 [26]	39.1 [26]	−99.9	−179.8[c]
Xanthine	$N_1H–C_6O$	$H_2X \Leftrightarrow HX^- + H^+$	7.53 [26]	26.5 [26]	−55.3	−99.6[c]
Xanthine	N_9H	$HX^- \Leftrightarrow X^{2-} + H^+$	11.84 [26]	40.2 [26]	−91.8	−165.3[c]
Cytosine	N_1H^+	$H_2C^+ \Leftrightarrow HC + H^+$	4.58 [28]	21.5 [28]	−15.6	−37.7 [28]
Cytosine	N_5H	$HC \Leftrightarrow C^- + H^+$	12.15 [28]	48.1 [28]	−71.2	−138.1 [28]
Thymine	$N_1H–C_6O$	$H_2T \Leftrightarrow HT^- + H^+$	9.90 [28]	34.1 [28]	−75.2	−100.4 [28]
Uracil	$N_1H–C_6O$	$H_2U \Leftrightarrow HU^- + H^+$	9.46 [28]	32.8 [28]	−71.0	−113.0 [28]

[a] The neutral forms correspond to the formula reported in Tables 2 and 3. $\Delta_r S_p^0(T;\mathrm{aq})$ values are calculated from $\Delta_r G_p^0(T;\mathrm{aq})$ and $\Delta_r H_p^0(T;\mathrm{aq})$ values.

[b] In the reactions, A, G, Hy, X, C, T and U refer to adenine, guanine, hypoxanthine, xanthine, cytosine, thymine and uracil, respectively.

[c] This work (Eq. (44)).

$\Delta_r C_p/\Delta_r S_p^0(T;\mathrm{aq})$ could be considered constant [16]

$$\frac{\Delta_r C_p}{\Delta_r S_p^0(T;\mathrm{aq})} = 1.8 \pm 0.3 \tag{47}$$

This assumption was made after examination of $\Delta_r C_p$ and $\Delta_r S_p^0(T;\mathrm{aq})$ values for a larger data set than pyrimidines which included amino-acids, carboxylic acids and bases. The accuracy of this prediction remains low (30%) but (i) the uncertainties of $\Delta_r C_p$ measurements are generally high, and (ii) the calculated values have to be used for temperature corrections of dissociation properties over a relatively narrow interval, from 0 to 50°C.

Two sources of data solubilities [29–32,36] were used to determine the Gibbs energies of formation of adenine, guanine, hypoxanthine, cytosine, thymine and xanthine in dilute aqueous solution. The two data sources are perfectly consistent. The data of Kilday [29–32], corresponding to more recent measurements, were selected whenever there was ambiguity. The two sources of data also provide the enthalpy of solution. Using the characterisation of solid–solute equilibrium (Eq. (34)), the standard Gibbs energy in solution was calculated from the formation properties in the solid state. Formation properties in solution of adenine, guanine, cytosine, thymine and uracil were thus calculated. The Gibbs energy of formation of hypoxanthine was also calculated using the solubility data.

Gibbs energy of formation of xanthine was estimated noting that the difference in chemical functions between guanine and xanthine is the same as between cytosine and uracil, namely, the replacement of a N=C–NH$_2$ group by a HN–C=O group (Tables 2 and 3). For pyrimidines, this corresponds to (Table 5)

$$\Delta_f G^0_{\mathrm{cytosine}}(T;\mathrm{S}) - \Delta_f G^0_{\mathrm{uracil}}(T;\mathrm{S}) = 221.7\,\mathrm{kJ\,mol^{-1}}$$

and

$$\Delta_f G^0_{\mathrm{cytosine}}(T;\mathrm{S}) - \Delta_f G^0_{\mathrm{uracil}}(T;\mathrm{S}) = 219.1\,\mathrm{kJ\,mol^{-1}}$$

For purines in the solid state, we obtain (Table 5)

$$\Delta_f G^0_{\mathrm{cytosine}}(T;\mathrm{S}) - \Delta_f G^0_{\mathrm{xanthine}}(T;\mathrm{S}) = 213.2\,\mathrm{kJ\,mol^{-1}}$$

Assuming that the passage from the solid to the aqueous state is accompanied by the same variation in the Gibbs energy change, 219.1 − 221.7 = −2.6 kJ mol^{-1}, we determined

$$\Delta_f G^0_{\mathrm{guanine}}(T;\mathrm{aq}) - \Delta_f G^0_{\mathrm{xanthine}}(T;\mathrm{aq}) = 213.2 - 2.6 = 210.6\,\mathrm{kJ\,mol^{-1}}$$

Table 8
Available data for purine and pyrimidine bases at $T = 298.15$ K[a]

Compound	$\Delta H_d^0(T;\text{aq})$ (kJ mol^{-1})	$m_{i\ \text{sat}}$ (mol kg^{-1})	$\Delta_r C_{pd}$ (J mol^{-1} K^{-1})	$\Delta_f H_i^0(T;\text{aq})$ (kJ mol^{-1})	$\Delta_f G_i^0(T;\text{aq})$ (kJ mol^{-1})	$C_{p,\text{aq}\ i}$ (J mol^{-1} K^{-1})	$S_i^0(T;\text{aq})$ (J mol^{-1} K^{-1})
Adenine ($C_5H_5N_5$)	33.5 [29,36]	7.7×10^{-3} [36], 8×10^{-3} [29]	78.7 [29]	129.5	311.3	221.8	224.1
Guanine ($C_5H_5N_5O$)	27.2 [36]	4.5×10^{-4} [36]		−156.7	66.6		187.4
Hypoxanthine ($C_5H_4N_4O$)		5.1×10^{-3} [36]			90.2		
Xanthine ($C_5H_4N_4O_2$)	–	–	–	–	–	–	–
Cytosine ($C_4H_5N_3O$)	27.2 [31,36]	6.6×10^{-2} [36], 7.6×10^{-2} [31]	76 [31]	−194.1	−36.5	208.6	210.5
Thymine ($C_5H_6N_2O_2$)	23.4 [36], 24.3 [30]	2.8×10^{-2} [36], 2.8×10^{-2} [30]	106 [30]	−438.5	−277.5	256.8	211.6
Uracil ($C_4H_4N_2O_2$)	29.4 [36], 29.3 [32]	2.4×10^{-2} [36], 2.7×10^{-2} [32]	57 [32]	−400.1	−255.6	177.5	196.0

[a] The standard state is the hypothetical ideal solution of unit molality and at $P = 1$ atm.

giving

$$\Delta_f G^0_{\mathrm{xanthine}}(T;\mathrm{aq}) = 66.6 - 210.6 = 144.0\,\mathrm{kJ\,mol^{-1}}$$

This made it possible to estimate the solubility of xanthine, i.e. 1.6×10^{-4} mol kg^{-1}. Estimations of the Gibbs energies of the neutral forms of purines and pyrimidines in the aqueous state were completed and are reported in Table 8.

The same method was used to calculate the enthalpy of formation of xanthine. Since guanine and cytosine have the same enthalpy of solubilisation (27.2 kJ mol^{-1}), it was assumed that the enthalpy of solubilisation of xanthine was equal to that of uracil (29.3 kJ mol^{-1}), giving

$$\Delta_f H^0_{\mathrm{xanthine}}(T;\mathrm{aq}) = -350.3\,\mathrm{kJ\,mol^{-1}}$$

The enthalpy of formation of hypoxanthine was calculated considering that from the chemical group contribution analysis, hypoxanthine was formed by the sum (xanthine + adenine − guanine). In other words, the C=O bond in the purine heterocycle is given by the differences (xanthine − hypoxanthine) or (guanine − adenine) (Table 2). Applying this method to the absolute entropy in the solid state (Table 6) gives

$$S^0_{\mathrm{xanthine-hypoxanthine}}(T;\mathrm{S}) = 15.4\,\mathrm{J\,mol^{-1}\,K^{-1}}$$

$$S^0_{\mathrm{guanine-adenine}}(T;\mathrm{S}) = 8.2\,\mathrm{J\,mol^{-1}\,K^{-1}}$$

Assuming that the differences in the evaluation of the C=O contribution are the same in the solid state and at infinite dilution (7.2 J mol^{-1} K^{-1}), this affords the estimation of $\Delta_f S^0_{\mathrm{hypoxanthine}}(T;\mathrm{aq})$

$$\begin{aligned}\Delta_f S^0_{\mathrm{hypoxanthine}}(T;\mathrm{aq}) &= \Delta_f S^0_{\mathrm{xanthine}}(T;\mathrm{aq}) - \Delta_f S^0_{\mathrm{guanine-adenine}}(T;\mathrm{aq}) - 7.2\\ &= -691.9 - (-749.0 + 609.8) - 7.2\\ &= -559.9\,\mathrm{J\,mol^{-1}\,K^{-1}}\end{aligned}$$

This made it possible to calculate the enthalpy of formation of hypoxanthine at 25°C in aqueous solution. The heat capacities when not available were estimated as the absolute entropies, which can be regarded as a good approximation when the heat

Table 9
Selected values of standard molar formation properties of purine and pyrimidine bases at $T = 298.15$ K[a]

Compound	$\Delta_f H_i^0(T;\mathrm{aq})$ (kJ mol^{-1})	$\Delta_f G_i^0(T;\mathrm{aq})$ (J mol^{-1} K^{-1})	$\Delta_f S_i^0(T;\mathrm{aq})$	C_p
Adenine$^+$	109.4	287.3	−596.8	245
Adenine	129.5	311.3	−609.8	222
Adenine$^-$	169.9	367.6	−663.6	125
Guanine$^+$	−173.9	47.8	−743.5	195
Guanine	−156.7	66.6	−749.0	187
Guanine$^-$	−115.1	121.6	−793.7	105
Hypoxanthine$^+$	−89.0	80.0	−566.9	202
Hypoxanthine	−76.7	90.2	−559.8	215
Hypoxanthine$^-$	−43.7	140.5	−617.9	110
Hypoxanthine^{2-}	−4.6	209.4	−717.7	70
Xanthine	−350.3	−144.0	−691.9	186
Xanthine$^-$	−323.8	−101.0	−747.3	86
Xanthine^{2-}	−283.6	−33.4	−839.1	−79
Cytosine$^+$	−215.6	−62.6	−513.0	246
Cytosine	−194.1	−36.5	−528.6	209
Cytosine$^-$	−146.0	32.9	−600.0	70
Thymine	−438.5	−277.5	−540.0	257
Thymine$^-$	−404.4	−221.0	−615.2	156
Uracil	−400.1	−255.6	−484.7	177
Uracil$^-$	−367.3	−201.6	−555.6	64

[a] The standard state is the hypothetical ideal solution of unit molality and at $P = 1$ atm.

capacities were measured. All the results concerning the formation properties of neutral form of purines and pyrimidines at infinite dilution are reported in Table 8 using the reference state previously defined. The formation properties of all species including neutral and charged species are reported in Table 9.

6. Discussion

When a biochemical reaction system contains many species, the calculation of the equilibrium composition in terms of species involved is very tedious. It is preferable to make calculations with a smaller number of reactants, i.e. with overall formation properties taking into account the influence of pH, T and ionic strength.

From the data for dissociation and data for formation in the standard state (Table 8), the transformed formation properties $\Delta_f G'(T; m_{\mathrm{C\,total}} = 1; \mathrm{aq})$ and $\Delta_f H'(T; m_{\mathrm{C\,total}} = 1; \mathrm{aq})$ of purines and pyrimidines at pH 7 and $I_m = 0.2\ \mathrm{mol\,kg^{-1}}$ were calculated [16] (Table 10). We note the followings.

1. The values of $\Delta_f G'$ obtained by the groups contribution method of Mavrovouniotis [33] is in reasonable agreement with the values calculated in this work (standard deviation of $10\ \mathrm{kJ\,mol^{-1}}$), except for guanine, for which earlier value is clearly over-estimated.
2. The predictions obtained by Eqs. (43) and (44), starting from the degrees of reduction, are inaccurate (standard deviation of $21\ \mathrm{kJ\,mol^{-1}}$ for $\Delta_f G'(T; m_{\mathrm{C\,total}} = 1; \mathrm{aq})$ and $37\ \mathrm{kJ\,mol^{-1}}$ for $\Delta_f H'(T; m_{\mathrm{C\,total}} = 1; \mathrm{aq})$) which is understandable given the empirical character of these correlations, indicating that these relations can be used to calculate a global energy balance, but not solubility or concentration of species at chemical equilibrium.
3. The values obtained at 25 and 37°C are appreciably different ($+6\ \mathrm{kJ\,mol^{-1}}$ for $\Delta_f G'(T; m_{\mathrm{C\,total}}; \mathrm{aq})$ and $+3\ \mathrm{kJ\,mol^{-1}}$ for $\Delta_f H'(T; m_{\mathrm{C\,total}}; \mathrm{aq})$).
4. The sum $\Delta_f G'_{\mathrm{adenine}}(T; m_{\mathrm{adenine}}; \mathrm{aq}) + \Delta_f G'_{\mathrm{thymine}}(T; m_{\mathrm{thymine}}; \mathrm{aq}) = 33.8\ \mathrm{kJ\,mol^{-1}}$ at 25°C ($47.4\ \mathrm{kJ\,mol^{-1}}$ at 37°C) is approximately equal to the sum $\Delta_f G'_{\mathrm{guanine}}(T; m_{\mathrm{guanine}}; \mathrm{aq}) + \Delta_f G'_{\mathrm{cytosine}}(T; m_{\mathrm{cytosine}}; \mathrm{aq}) = 30.1\ \mathrm{kJ\,mol^{-1}}$ at 25°C ($45.3\ \mathrm{kJ\,mol^{-1}}$ at 37°C). This means that the pairing of the two pairs of bases (adenine–thymine) and (cytosine–guanine) on a molecule of DNA corresponds to the same Gibbs energy of formation. We go no further, but it is interesting that the various units of the double helix of DNA have the same Gibbs energy of formation.

7. Conclusion

Starting from several sources of data (combustion properties, solid solubility, enthalpy of solution, etc.) the formation properties of purines and pyrimidines were calculated in the solid state and in dilute aqueous

Table 10
Transformed properties $\Delta_f H'_i(T; m_i; \mathrm{aq}), \Delta_f G'_i(T; m_i; \mathrm{aq})$ in $\mathrm{kJ\,mol^{-1}}$ of several purine and pyrimidine bases at 298.15 K, pH 7, $I_m = 0.2\ \mathrm{mol\,kg^{-1}}$ and 37°C

Compound	Conditions				$(\Delta_f G')^a$ [33]	$(\Delta_f G')^{a,b}$ Eq. (41)	$(\Delta_f H')^{a,b}$ Eq. (40)
	pH = 7, I_m = 0.2 M and T = 25°C		pH = 7, I_m = 0.2 M and T = 37°C				
	$\Delta_f G'$	$\Delta_f H'$	$\Delta_f G'$	$\Delta_f H'$			
Adenine	311.3	129.5	318.5	132.3	321.0	229	193
Guanine	66.6	−156.6	75.5	−154.2	120.0	40	−37
Hypoxanthine	90.1	−76.0	96.8	−73.0	81.0	65	−90
Xanthine	−144.8	−342.5	−137.0	−337.7	−120.0	−124	−239
Cytosine	−36.5	−194.2	−30.2	−191.7	−42.0	−29	−103
Thymine	−277.5	−438.4	−271.1	−435.3	−254.0	−173	−295
Uracil	−255.6	−399.9	−249.8	−397.7	−282.0	−194	−305

[a] For comparison, these values were calculated from Eqs. (40) and (41).

[b] These data are given at 25°C but the values of pH and ionic strength are not specified.

solution. Using these properties, and using dissociation constants, the transformed properties of these bases were calculated at different values of temperature, pH, and ionic strength. The results are in close agreement with available data. This offers a reliable and thermodynamically consistent set of enthalpies, Gibbs energies, entropies and heat capacities for these bases.

References

[1] R.W. Guynn, R.L. Veech, J. Biol. Chem. 248 (1973) 6966.
[2] R.W. Guynn, L.T. Webster, R.L. Veech, J. Biol. Chem. 249 (1974) 3248.
[3] R.A. Alberty, R.N. Goldberg, Biochemistry 31 (1992) 10610.
[4] W.E. Teague, G.P. Dobson Jr., G.P. Dobson, J. Biol. Chem. 267 (1992) 14084.
[5] W.D. Dewolf, F.A. Emig, V.L. Shramm, Biochemistry 25 (1986) 4132.
[6] R.A. Alberty, Biochim. Biophys. Acta 1207 (1994) 1.
[7] Y.B. Tewari, R.N. Goldberg, J.V. Advani, Biophys. Chem. 40 (1991) 263.
[8] R.A. Alberty, Proc. Natl. Acad. Sci. U.S.A. 88 (1991) 3268.
[9] R.A. Alberty, Biophys. Chem. 43 (1992) 239.
[10] R.A. Alberty, Biophys. Chem. 42 (1992) 117.
[11] R.A. Alberty, Arch. Biochem. Biophys. 307 (1993) 8.
[12] R.A. Alberty, Biophy. Chem. 70 (1998) 109.
[13] J.W. Larson, Y.B. Tewari, R.N. Goldberg, J. Chem. Thermodyn. 25 (1993) 73.
[14] R. Wollfenden, J. Biol. Chem. 242 (1967) 4711.
[15] Y.A. Kim, M.T. King, W.E. Teague, G.A. Rufo, R.L. Veech, J.V. Passonneau, Am. J. Physiol. 62 (1992) E344.
[16] C.B. Ould-Moulaye, Ph.D. Thesis, Université Blaise Pascal, Clermont-Ferrand, 1998.
[17] C.B. Ould-Moulaye, C.G. Dussap, J.B. Gros, Biotechnol. Tech. 13 (1999) 187.
[18] C. Achard, C.G. Dussap, J.B. Gros, AIChE J. 40 (1994) 1210.
[19] R.C. Wilhoit, in: H.D. Brown (Ed.), Biochemical Microcalorimetry, Academic Press, New York, 1969, p. 306.
[20] D.R. Lide, Handbook of Chemistry and Physics, 76th Edition, CRC Press, New York, 1996.
[21] E.S. Domalski, J. Phys. Chem. Ref. Data 1 (1972) 221.
[22] J.D. Cox, D.D. Wagman, V.A. Medvedev, CODATA Key Values for Thermodynamics, Hemisphere, New York, 1989.
[23] E.S. Domalski, E.D. Hearing, J. Phys. Chem. Ref. Data 22 (1993) 805.
[24] J.A. Roels, Energetics and Kinetics in Biotechnology, Elsevier, Amsterdam, 1983.
[25] J.J. Christensen, R.M. Izatt, J. Phys. Chem. 66 (1962) 1030.
[26] J.J. Christensen, J.H. Rytting, R.M. Izatt, Biochemistry 9 (1970) 4907.
[27] R.M.C. Dawson, D.C. Elliott, W.H. Elliott, K.M. Jones, Data for Biochemical Research, 2nd Edition, Clarendon Press, Oxford, 1969.
[28] J.J. Christensen, J.H. Rytting, R.M. Izatt, J. Chem. Soc. B (1970) 1643.
[29] M.V. Kilday, J. Res. Nat. Bur. Stand. 83 (1978) 347.
[30] M.V. Kilday, J. Res. Nat. Bur. Stand. 83 (1978) 529.
[31] M.V. Kilday, J. Res. Nat. Bur. Stand. 83 (1978) 539.
[32] M.V. Kilday, J. Res. Nat. Bur. Stand. 83 (1978) 547.
[33] M.L. Mavrovouniotis, Biotechnol. Bioeng. 36 (1990) 1070.
[34] M.L. Mavrovouniotis, J. Biol. Chem. 266 (1991) 14440.
[35] S.I. Sandler, Fluid Phase Equilib. 70 (1991) 141.
[36] S. Cabani, P. Gianni, in: H.J. Hinz (Ed.), Thermodynamic Data for Biochemistry and Biotechnology, Springer, Berlin, 1986, Chapter 10, p. 271.
[37] H. Rilbe, pH and Buffer Theory — A New Approach, Wiley, New York, 1996.