

# Enthalpy and entropy contributions to the equilibrium of the hydrogen bonding interaction between 1-octylthymine and 9-octyladenine

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## Abstract

Hydrogen bonding interaction between 1-octylthymine (**1**) and 9-octyladenine (**2**) was analyzed by dynamic proton NMR spectroscopy. A mathematical model that takes into account the independent *self*-association of **1** and **2** was developed in order to determine the equilibrium constant of the interaction between 1-octylthymine and 9-octyladenine  $K_{1-2}$ .

The measurement of  $K_{1-2}$ , in  $CDCl_3$ , in the range of temperatures of  $-30$  to  $40$  °C permitted estimation of the contributions of enthalpy  $\Delta H^\circ = -6.74$  kcal/mol, and entropy  $\Delta S^\circ = -13.95$  cal  $mol^{-1} K^{-1}$ , to the *hetero*-association process. From the enthalpic point of view the association of 1-octylthymine with 9-octyladenine is 0.56 kcal/mol stronger than the *self*-association of 1-octylthymine, and 0.09 kcal/mol stronger than the *self*-association of 9-octyladenine. X-ray diffraction of a monocrystal of mixed 1-octylthymine and 9-octyladenine indicated that the interaction between these nucleobases in the solid state resemble Haschemeyer-Sobell more than Watson-Crick hydrogen bonding.

**Keywords:** H-Bonding, nucleobases, 1-octylthymine, 9-octyladenine, thymine-adenine adduct, association, thermodynamic parameters, crystal packing

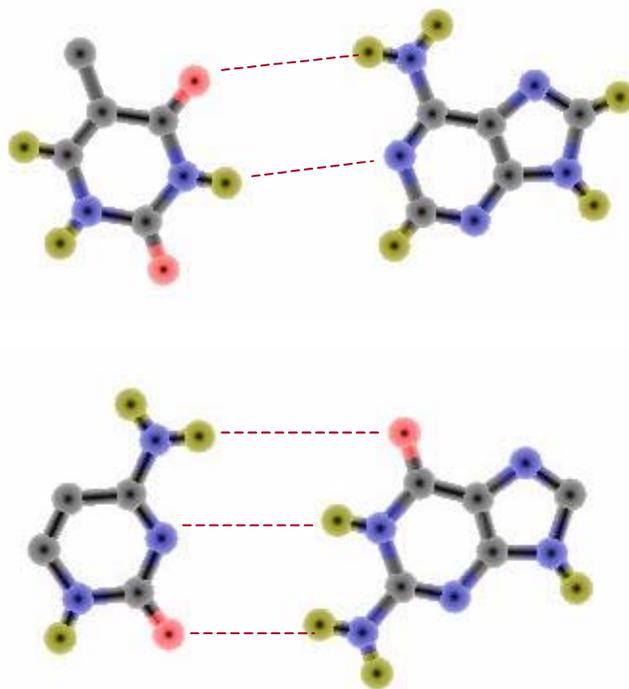
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## Introduction

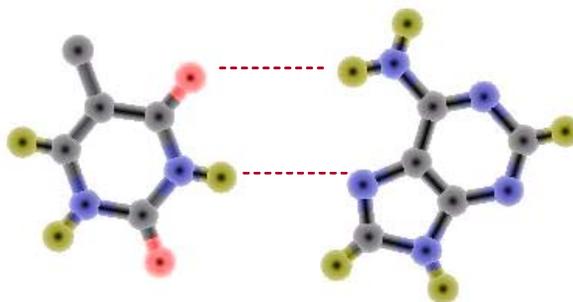
Hydrogen bonding plays a ubiquitous role in the assembly and molecular recognition properties of supramolecular structures,<sup>1-4</sup> biomolecules,<sup>5-7</sup> and nanomaterials.<sup>8-10</sup>

One of the most important biological processes, that is controlled by hydrogen bonding, is the selective recognition between the two strands of helical DNA. This weak *non*-covalent interaction is then responsible for the self-replication of DNA as well as the transcription of the information from a mother to a daughter cell.<sup>5</sup>

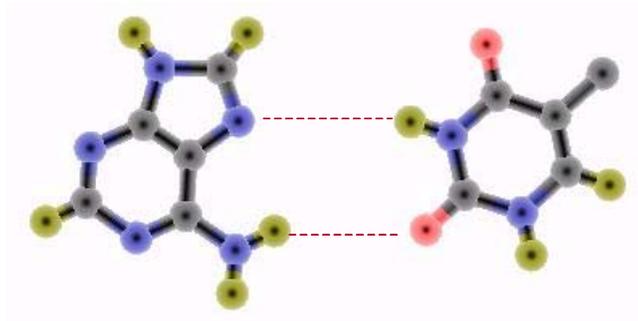
In 1954 Watson and Crick proposed that the nucleobases of DNA interact through a unique H-bonding that links a pyrimidine and purine at a time, by complementarity in shape, size, and number of bonding sites. Thus, thymine and adenine recognize each other forming two H-bonds and cytosine and guanine forming three (Scheme 1).<sup>7</sup> Watson-Crick hydrogen bonding is not the only way of interaction between the nucleobases in DNA; however, they give rise to the most common tertiary structure, the helical B-form of DNA.<sup>7,11</sup> The other important H-bonding interactions between nucleobases are the Hoogsteen, and the Haschemeyer-Sobell. Examples of these configurations are included in Scheme 2 and 3, respectively.<sup>12,13</sup>



**Scheme 1.** Watson-Crick H-bonding in nucleobases.



**Scheme 2.** Hoogsteen H-bonding in the adduct thymine-adenine.



**Scheme 3.** Haschemeyer-Sobell (or reverse Hoogsteen) H-bonding in the adduct thymine-adenine.

To this date, there have been several research groups interested in the experimental determination of the equilibrium constant of the nucleobases of DNA. Table 1 shows a compilation of methods and thermodynamic parameters, reported for the interaction of thymine and adenine. In addition, Sartorius and Schneider<sup>16</sup> have proposed an empirical rule to determine a K value for the H-bonding interaction in a substrate-receptor system. The K value calculated, by this rule, for the pair thymine-adenine is  $56.62 \text{ M}^{-1}$ .

**Table 1.** Determination of the K of equilibrium for the pair thymine/adenine

Compound	Solvent	Method	$\Delta H^\circ$ (kcal/mol)	$\Delta G^\circ$ (kcal/mol)	$K_{TA}$ (L/mol)	Ref.
9-Ethyladenine-1-cyclohexylthymine	$\text{CDCl}_3$ (25 °C)	IR	-	-2.88	130	14
9-Ethyladenine-1-cyclohexylthymine	$\text{CDCl}_3$ (- 56 °C)	$^1\text{H}$ NMR	-	-2.95	940	15
9-Hexyladenine-1-hexylthymine	$\text{CDCl}_3$ (25 °C)	NMR titration	-	-2.1	33.50	16
1-Methyladenine-1-methylthymine	Water (25 °C)	Refractive method	$-7.3 \pm 0.2$	-	-	17
1-Methyladenine-1-methylthymine	Water (25 °C)	Mass spectrometry	-13.0	-	-	18

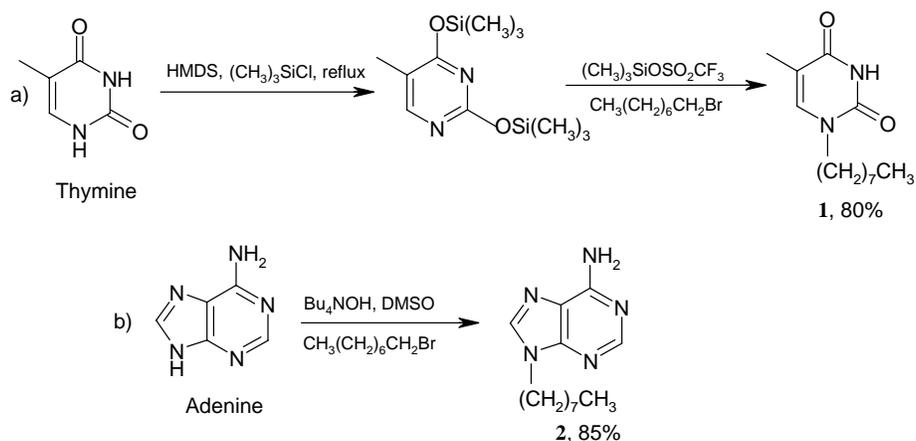
Even though  $K$  of equilibrium (eq. 1) provides information regarding the strength of the interaction between two associated species, the enthalpy  $\Delta H^\circ$  term is more relevant in order to rationalize it, because, in most of the cases, it does not depend on the solvent or the temperature which the determination is done.<sup>19</sup> In this regard, there are only few reports of the interaction between thymine-adenine. Furthermore, values of the reported enthalpy for the pair thymine-adenine, vary widely (-7.3 vs. -13 kcal/mol) as shown in Table 1, therefore we deemed it important to re-evaluate them. Thus, reported in this work is a mathematical method to analyze the proton NMR data obtained from the H-bonding interaction between 1-octylthymine (**1**) and 9-octyladenine (**2**). The method takes into account the equilibrium constant of the *self*-association of each nucleobase  $K_{1-1}$  and  $K_{2-2}$ . The NMR experiments were carried out at several temperatures, to be able to obtain the enthalpy and entropy contributions for the *self*-association of (**1**) or (**2**), as well as the cross-linked (**1-2**) interaction. X-ray structures of 9-octyladenine and the 1-octylthymine/9-octyladenine pair are also reported.

$$\Delta G^\circ = -RT \ln K = \Delta H^\circ - T\Delta S^\circ \quad (1)$$

## Results and Discussion

### Synthesis

1-Octylthymine (**1**) and 9-octyladenine (**2**) were prepared as shown in Scheme 4. Compound **1** was purified by column chromatography using hexane/ethyl acetate (80:20) as solvent (80% yield). Sample **2** was obtained as crystals through recrystallization from acetonitrile, in a 85% yield. Compound **2** was analyzed by X-ray diffraction, and the structure of **1** is already reported.<sup>20</sup> A mixed sample of 1-octylthymine/ 9-octyladenine (1:1) afforded good quality crystals for X-ray diffraction when recrystallized from DMSO.



**Scheme 4.** Synthesis of (a) *n*-octylthymine; (b) *n*-octyladenine.

### Thermodynamic parameters of the *self*-association process

The equilibrium constants for the *self*-association of 1-octylthymine  $K_{1-1}$  or 9-octyladenine  $K_{2-2}$  (eq. 2 and 3) were evaluated by means of the observed chemical shift ( $\delta_{\text{obs}}$ ) of the proton NH for 1-octylthymine and  $\text{NH}_2$  for 9-octyladenine. Equation 4 relates  $\delta_{\text{obs}}$  with the percentage of the free N and *self*-associated N-N species in equilibrium.

The proton NMR spectra of the samples were recorded in  $\text{CDCl}_3$  at several concentrations and temperatures. The proton chemical shift for the NH group of the free 1-octylthymine ( $\delta_1$ ) was taken as 7.90 ppm; this is the chemical shift of the more diluted solution ( $1 \times 10^{-3}$  M) at the highest temperature (40 °C) registered.<sup>21</sup> For 9-octyladenine, the proton chemical shift for the  $\text{NH}_2$  group ( $\delta_2$ ) was taken as 5.53 ppm (solution  $1 \times 10^{-4}$  M, at 40 °C).<sup>21</sup> We believe that these chemical shifts are indeed of the free species because the chemical shift of both NH and  $\text{NH}_2$  signals at low concentrations and low temperature (0 to -30 °C) remained practically the same ( $\pm 0.15$  ppm of variation in both cases). On the other hand, the proton chemical shift of the NH group for the *self*-association of 1-octylthymine ( $\delta_{1-1}$ ) was taken as 11.17 ppm, because this signal was registered at the highest concentration studied and the lowest temperature (0.5 M at -50 °C).<sup>21</sup> Similarly, for the *self*-association of 9-octyladenine ( $\delta_{2-2}$ ), the  $\delta_{\text{NH}_2}$  was taken as 7.45 ppm (0.5 M at -10 °C).<sup>21</sup>



Where N= 1 or 2

$$K_{\text{N-N}} = \frac{C_{\text{N-N}}}{(C_{\text{N}})^2} = \frac{C_{\text{N-N}}}{(C_{\text{No}} - C_{\text{N-N}})^2} \quad (3)$$

$$\delta_{\text{obs}} = \frac{C_{\text{N}}}{C_{\text{No}}} \delta_{\text{N}} + \frac{C_{\text{N-N}}}{C_{\text{No}}} \delta_{\text{N-N}} \quad (4)$$

The equilibrium constant for *self*-association (eq. 3) depends on the initial concentration of the solution ( $C_{\text{No}} = 0.1$  M). By rearranging equation 4, the concentration of *self*-associated species, can be calculated through equation 5.

$$C_{\text{N-N}} = \left( \frac{\delta_{\text{obs}} - \delta_{\text{N}}}{\delta_{\text{N-N}} - \delta_{\text{N}}} \right) C_{\text{No}} \quad (5)$$

The observed changes in the chemical shift ( $\delta_{\text{obs}}$ ) for the NH signal with temperature, that are involved in the interaction of (1-1) at the intermediate concentration of 0.1 M, are shown in Table 2. As for the interaction of (2-2) at the same concentration, the results are collected in Table 3. Calculations of  $C_{1-1}$  or  $C_{2-2}$  were performed using equation 5, and the values were

substituted in equation 3 to calculate the equilibrium constants at different temperatures (Table 2 and 3). By applying the Gibbs equation 1, we were able to calculate the free energy ( $\Delta G^\circ$ ) involved in the *self*-association of 1-octylthymine (Table 2) and 9-octyladenine (Table 3). A comparison of these values at 20 °C, indicate that the *self*-association of (1) in  $\text{CDCl}_3$  is stronger than the *self*-association of (2) by 0.27 kcal/mol.

**Table 2.**  $^1\text{H}$  NMR Chemical shifts (in ppm) for 1-octylthymine (1), in  $\text{CDCl}_3$ , and the calculated equilibrium constant  $K_{1-1}$  of the *self*-association process

Temperature (K)	Chemical Shift ( $\delta_{\text{NH}}$ )	$K_{1-1}$ ( $\text{M}^{-1}$ )	$\Delta G^\circ_{1-1}$ (kcal/mol)
223	10.66	328.12	- 2.57
233	10.48	179.14	- 2.40
243	10.28	100.14	- 2.22
253	10.05	57.09	- 2.03
263	9.83	35.10	- 1.86
273	9.61	22.57	- 1.69
283	9.40	15.77	- 1.55
293	9.22	11.11	- 1.40
303	9.05	8.28	- 1.27
313	8.89	6.12	- 1.13

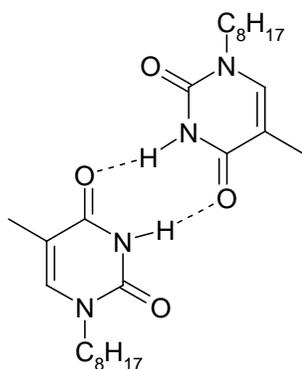
**Table 3.**  $^1\text{H}$  NMR Chemical shifts (in ppm) for 9-octyladenine (2), in  $\text{CDCl}_3$ , and the calculated equilibrium constant  $K_{2-2}$  of the *self*-association process

Temperature (K)	Chemical Shift ( $\delta_{\text{NH}_2}$ )	$K_{2-2}$ ( $\text{M}^{-1}$ )	$\Delta G^\circ_{2-2}$ (kcal/mol)
253	6.71	40.10	- 1.86
263	6.55	23.99	- 1.66
273	6.40	14.88	- 1.46
283	6.26	9.88	- 1.29
293	6.14	6.92	- 1.13
303	6.02	4.44	- 0.90
313	5.92	3.12	- 0.71

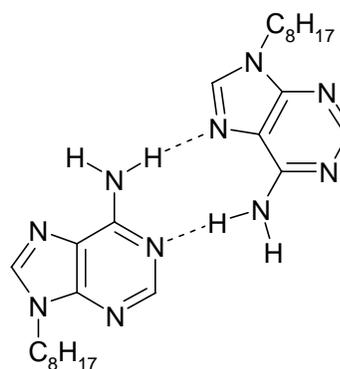
The evaluation of the enthalpy ( $\Delta H^\circ$ ), and entropy ( $\Delta S^\circ$ ) components for the *self*-association process was performed through the van't Hoff equation (6). The linear regression value for the plot  $\ln K$  vs.  $1/T$  was excellent for 1-octylthymine ( $r = 0.999$ ), as well as for 9-octyladenine ( $r = 0.999$ ). The data have a standard deviation of 0.057 for 1-octylthymine and 0.021 for 9-

octyladenine (average from 3 data set). The enthalpy was calculated from the slope of the curves and the entropy from the intercept to the ordinate. Values are shown in Scheme 5.

$$\ln \mathbf{K} = -\frac{\Delta \mathbf{H}^{\circ}}{\mathbf{RT}} + \frac{\Delta \mathbf{S}^{\circ}}{\mathbf{R}} \quad (6)$$



$$\begin{aligned} \Delta \mathbf{H}^{\circ}_{1-1} &= -6.18 \text{ kcal/mol} \\ \Delta \mathbf{S}^{\circ}_{1-1} &= -16.27 \text{ cal mol}^{-1} \text{ K}^{-1} \end{aligned}$$



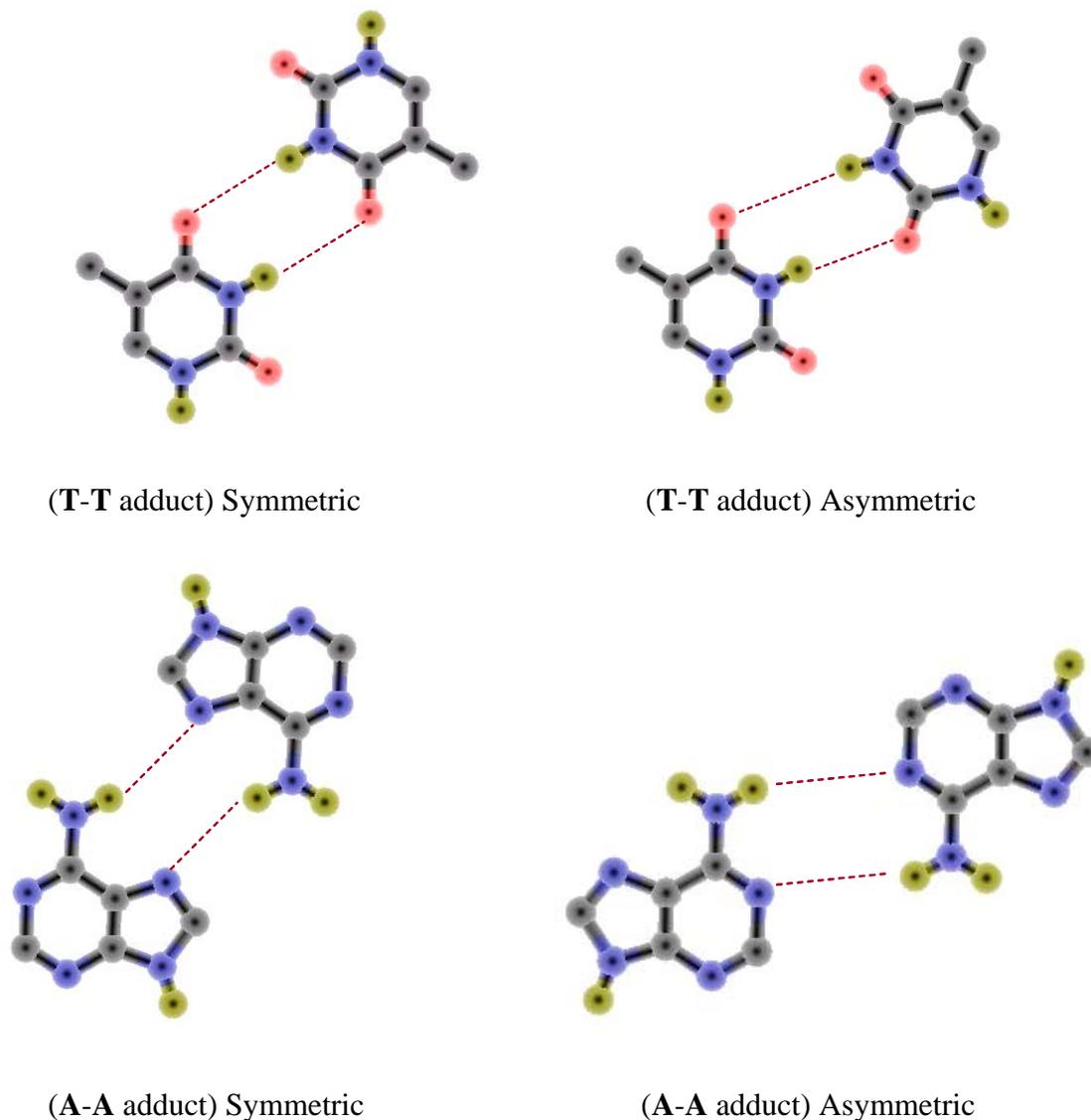
$$\begin{aligned} \Delta \mathbf{H}^{\circ}_{2-2} &= -6.65 \text{ kcal/mol} \\ \Delta \mathbf{S}^{\circ}_{2-2} &= -18.94 \text{ cal mol}^{-1} \text{ K}^{-1} \end{aligned}$$

**Scheme 5.** Enthalpy and entropy values of the *self*-association of *n*-octylthymine and *n*-octyladenine.

As it can be observed, the values of enthalpy for the *self*-association process indicate that the H-bonding linking between two 9-octyladenines are 0.47 kcal/mol more stable than the one between two 1-octylthymines; however, as expected, the formation of the dimers (**1-1**) or (**2-2**) is not favored by entropy. Actually, entropy disfavors the adenine more than the thymine *homo* adduct ( $\Delta \Delta \mathbf{S}^{\circ} = 2.67 \text{ cal mol}^{-1} \text{ K}^{-1}$ ), so that the free energy ends by favoring the *self*-association of thymine over the adenine, at least in  $\text{CDCl}_3$ , as it was observed above. On the other hand,  $T\Delta \mathbf{S}^{\circ}$  contributions at 25 °C are, for 1-octylthymine (-4.85 kcal/mol) and for 9-octyladenine (-5.64 kcal/mol); they are indeed similar to the enthalpy of their corresponding systems, as it would have been expected for *non*-covalent interactions.

The fact that the enthalpies of both *self*-association processes are close to one another (-6.18 vs. -6.65 kcal/mol) is indicating that at least the number of H-bonding interactions in each *homo* adduct is the same. The most likely ways for *self*-association between the nucleobases are the symmetric or the asymmetric H-bonding structures of Scheme 6. Nevertheless, the symmetric and asymmetric interactions are not the same, both configurations lead to two [O...H-N] for thymine and two [N-H...N] for adenine. Thus we can conclude that every [O...H-N] bonding contributes with 3.09 kcal/mol of enthalpy to the stabilization of the adduct (**1-1**) and that the interaction [N...H-N] contributes with 3.33 kcal/mol to the stabilization of (**2-2**). In short, we

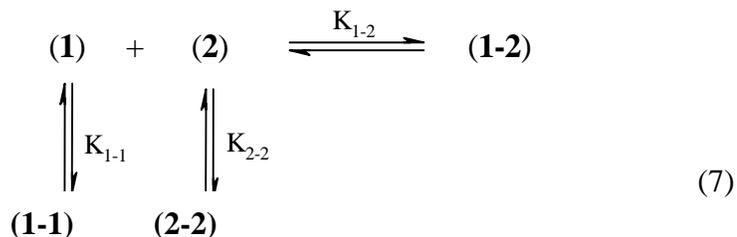
found that the H-bonding [N...H-N] is slightly stronger (0.24 kcal/mol) than the [O...H-N] interaction. An explanation for this behavior could rely on the distance of approach of the donor-acceptor, which is shorter in the *homo* adduct of 9-octyladenine [(N...H-N) bond length 2.11 Å (average), a value obtained from the X-ray structure below] than in the *homo* adduct of 1-octylthymine [(O...H-N) bond length 2.86 Å (average)].<sup>20</sup>



**Scheme 6.** H-bonding structures for the *homo* adducts of thymine and adenine.

**Thermodynamic parameters of the *hetero*-association process**

The H-bonding for the cross-linked system (1-2) was analyzed by a mathematical model that contemplates the equilibrium constants of the *self*-association process of each nucleobase, as it is shown in equation 7. Thus, the equilibrium constants of each process can be calculated by means of equations 8 and 9.



$$K_{1-2} = \frac{C_{1-2}}{C_1 C_2} \quad (8)$$

$$K_{N-N} = \frac{C_{N-N}}{(C_N)^2} \quad (9)$$

Where N= 1 or 2

The  $\delta_{\text{obs}}$  was taken as the chemical shift of the NH group for *n*-octylthymine in the pair, and can be expressed in terms of the free and associated species, as shown in equation 10. The chemical shift for the mixed pair  $\delta_{1-2}$  was taken as 14.32 ppm, this value was obtained from the NH signal of 1-octylthymine, in an independent experiment in which the proton NMR spectra of mixed 1-octylthymine/9-octyladenine (1:8) was held at high concentration (0.5 M).<sup>21</sup> If we consider the initial concentration  $C_{10} = 0.1$  M, and the previously obtained chemical shifts of the free  $\delta_1 = 7.90$  ppm and *self*-associated 1-octylthymine  $\delta_{1-1} = 11.17$  ppm respectively, equations 11 and 12 can be derived and used to characterize the system in the equilibrium.

$$\delta_{\text{obs}} = \frac{C_1}{C_{10}} \delta_1 + \frac{C_{1-1}}{C_{10}} \delta_{1-1} + \frac{C_{1-2}}{C_{10}} \delta_{1-2} \quad (10)$$

$$\delta_{\text{obs}} = 79C_1 + 111.7C_{1-1} + 143.2C_{1-2} \quad (11)$$

$$C_{1-2} = C_{10} - 2C_{1-1} - C_1 \quad (12)$$

The value of  $K_{1-2}$  was calculated from an iterative method. Proposed initial values of  $C_1$  were used to perform the calculation of  $C_{1-1}$ , according to equation 9.  $C_{1-2}$  was then calculated

according to equation 12. These values were substituted in equation 11 to obtain a calculated chemical shift  $\delta_{\text{calc}}$ . Ultimate values were those obtained when convergence was reached, that is, when  $\delta_{\text{calc}} = \delta_{\text{obs}} \pm 0.01$ . Once  $C_1$  and  $C_{1-2}$  are defined by this procedure,  $C_2$  was determined by solving the second degree equation 13, which finally allowed us to calculate the equilibrium constant  $K_{1-2}$  (equation 8).

$$C_2 = C_{20} - 2C_{2-2} - C_{1-2} = C_{20} - 2K_{2-2}C_2^2 - C_{1-2} \quad (13)$$

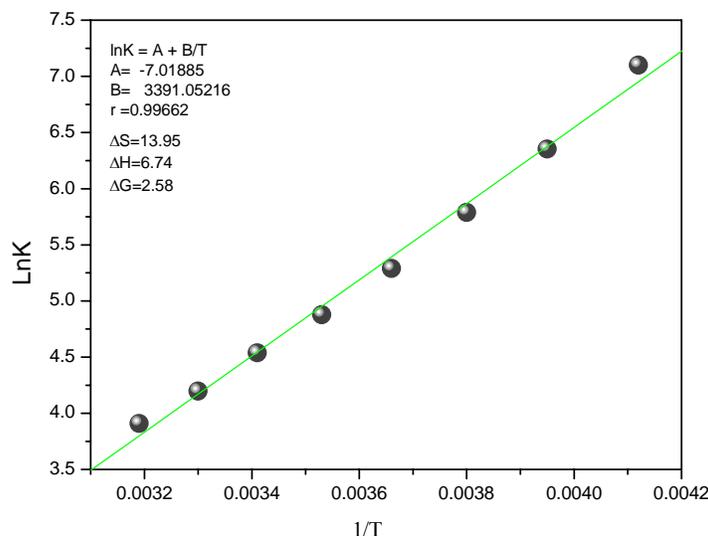
The chemical shift variation of the NH signal for the 1-octylthymine in the mixed (1)/(2) solution (0.1 M of each compound) at different temperatures is summarized in Table 4. The  $\Delta G^\circ$  at 20 °C is - 2.64 kcal/mol, around 1.2-1.5 kcal/mol stronger than the *self*-association of the nucleobases. This value matches well with those reported in Table 1 and the calculated by the empirical rule of Sartorius and Schneider ( $\Delta G_{25}^\circ = - 2.39$  kcal/mol).<sup>16</sup>

**Table 4.** <sup>1</sup>H NMR Chemical shift (in ppm) for 1-octylthymine (1)/9-octyladenine (2) in CDCl<sub>3</sub>, and the calculated equilibrium constant K of the association process

Temperature (K)	Chemical shift ( $\delta_{\text{NH}}$ )	$K_{1-2}$ (M <sup>-1</sup> )	$\Delta G_{1-2}$ (kcal/mol)
243	13.85	1211.20	- 3.43
253	13.66	574.38	- 3.19
263	13.45	326.64	- 3.02
273	13.21	198.30	- 2.87
283	12.95	131.28	- 2.74
293	12.71	93.57	- 2.64
303	12.40	66.52	- 2.53
313	12.10	49.86	- 2.43

The van't Hoff's curve for the system is shown in Figure 1 ( $r = 0.997$ ), the enthalpy  $\Delta H^\circ_{1-2}$  and entropy  $\Delta S^\circ_{1-2}$  contributions were calculated as - 6.74 kcal/mol and -13.95 cal mol<sup>-1</sup> K<sup>-1</sup>, respectively. If a comparison of enthalpy values between the *hetero* and *homo* association is made, we can conclude that even though there are also two hydrogen bonding interactions in the pair thymine-adenine (Scheme 1), the enthalpy is favoring this cross linked system over the independent *self*-association for 0.56 kcal/mol in the case of 1-octylthymine and for 0.09 kcal/mol in the case of 9-octyladenine. Regardless of structure (Schemes 1-3), the types of H-bonding in the pair thymine-adenine are [O...H-N] and [N...H-N], thus by taking the previously calculated enthalpy contribution of - 3.09 kcal/mol for [O...H-N] and - 3.33 kcal/mol for [N...H-N], the calculated  $\Delta H^\circ$  is - 6.42 kcal/mol. The small difference between the calculated and experimental enthalpy (0.32 kcal/mol) is due to subtle changes in H-bonding distances which are smaller in the 1-octylthymine/9-octyladenine pair than in the *homo* pairs [(O..H-N) = 2.09 Å

(thymine-adenine) vs. 2.86 Å (thymine-thymine);<sup>20</sup> (N...H-N) = 1.94 Å (thymine-adenine) vs. 2.11 Å (adenine-adenine)] as it is observed in the X-ray structures shown below. These finding might certainly indicate, that the intrinsic nature of the H-bonding is not always the same, even though the same kind of species are involved in the interaction. The entropic contribution is also disfavoring less the interaction between the pyrimidine-purine bases (**1-2**), than the independent (**1-1**) or (**2-2**) association.

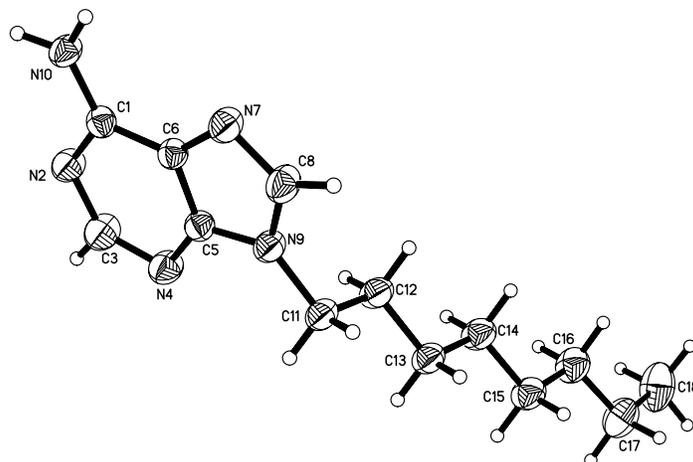


**Figure 1.** Linear regression  $\ln K$  vs.  $1/T$  for the mixed pair (**1-2**).

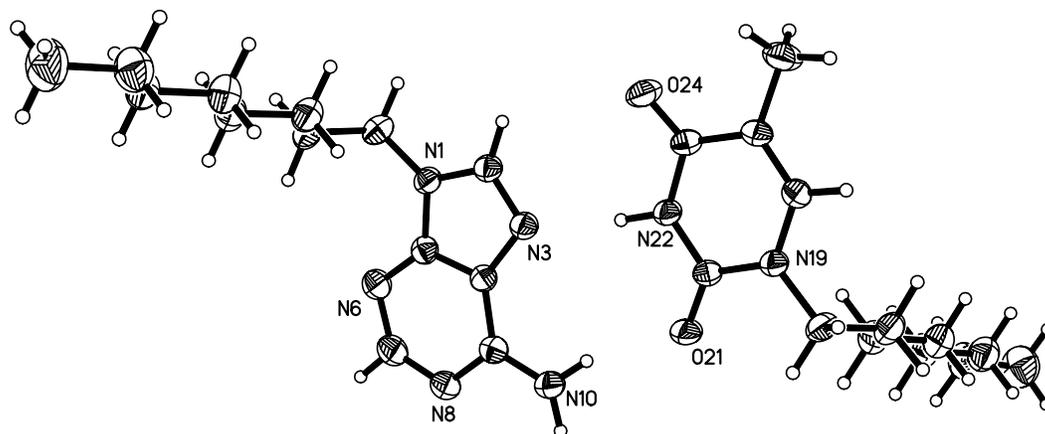
### X-Ray crystallography

In order to gain support for the number and geometrical characteristics of hydrogen bonding interactions in the studied systems, we obtained good single crystals for the *n*-octyladenine and the mixed 1-octylthymine/9-octyladenine, (the X-ray structure of *n*-octylthymine is already reported<sup>20</sup>). The ORTEP structures are shown in Figures 2 and 3, respectively. The adenine ring in Figure 2 is almost planar with a distortion of planarity of no more than 1.05 degrees, the *n*-octyl chain adopts a staggered conformation. In the 1:1 adduct of thymine-adenine (Figure 3) the thymine ring is more distorted from planarity (around 2 degrees) than the adenine ring. The *n*-octyl chains are arranged in such way that they minimize hindrance between them.

The H-bonding is visualized easily in the unit cell of the supramolecular structures shown in Figures 4 and 5. As observed, both 1:1 adducts (**2-2**) and (**1-2**) share two hydrogen bonds between the nucleobases. In the adenine *homo* adduct, the H-bonding interaction is asymmetric. On the other hand, the thymine-adenine adduct is in Haschemeyer-Sobell configuration. It is worth of note that although Watson-Crick configuration has been found in the X-ray structures of oligonucleotides of DNA, none of the adenine-thymine pairs studied in solid state, have the Watson-Crick configuration.<sup>12,13</sup>

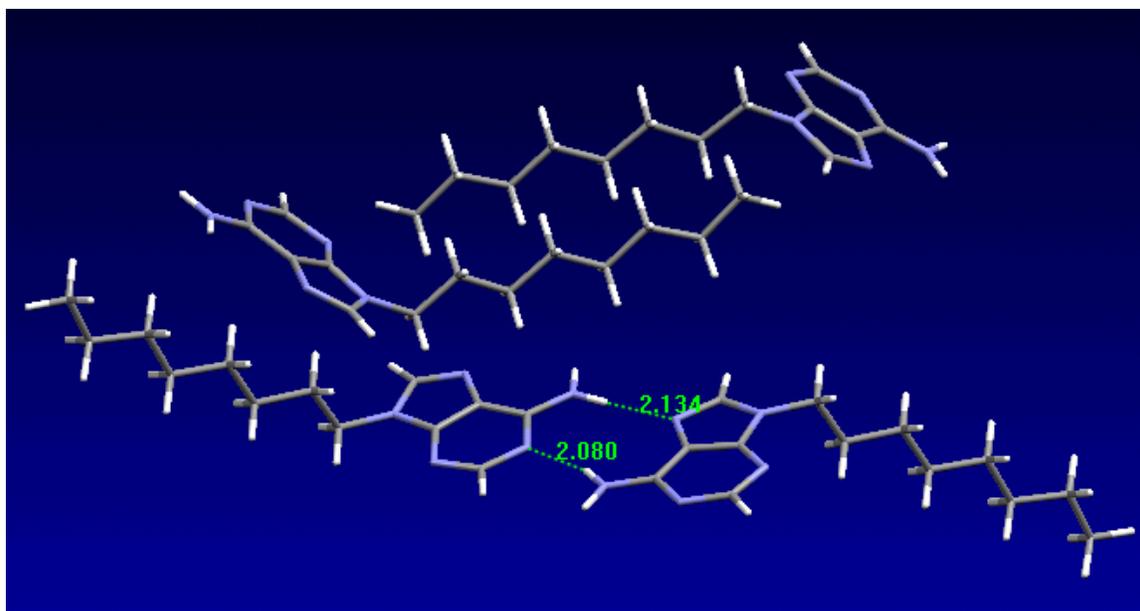


**Figure 2.** ORTEP projection of the molecular structure of 9-octyladenine (**2**). (Atoms H3, H8, H10a and H10b were located from a difference Fourier map and refined. All others H atoms were placed in calculated positions and refined using a riding model).

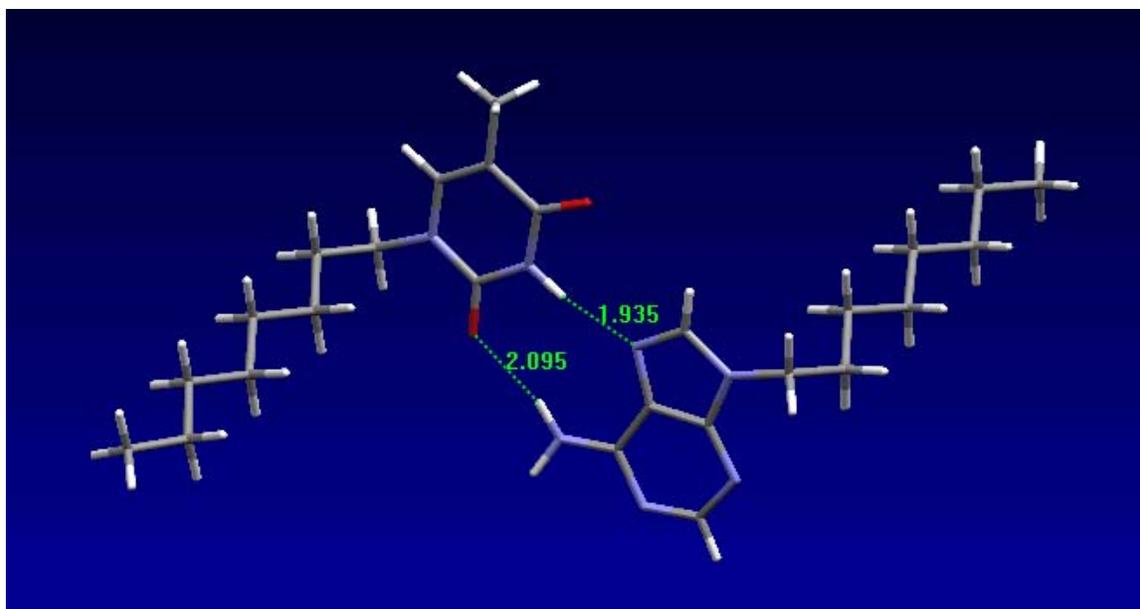


**Figure 3.** ORTEP projection of the molecular structure of mixed 1-octylthymine/9-octyladenine (1:1). (Atoms H2, H7, H10a, H10b, and H22 were located from a difference Fourier map and refined. All others H atoms were placed in calculated positions and refined using a riding model).

In the structure of the **2-2** adduct (Figure 4), the purine-purine rings are approximately coplanar with dihedral angles in the range of 2.4-3.8 Å. The bond distances between the *non*-bonded atoms [N...N] of the hydrogen bonds [N...H-N] are 2.95 Å and 3.09 Å, and the bond distances between H...N are 2.08 Å and 2.13 Å respectively. These values are within the range of expected bond lengths for the [N...H-N] bonding (3.1 Å for N...N and 2.2 Å for H...N).<sup>12,22</sup> The N...H-N interaction is almost at 180° [(N3...H-N) bond angle = 179.2°; (N10...H-N) = 163.2°). Because of all these characteristics, the H-bonding in the **2-2** adduct can be considered moderate and mainly electrostatic in nature.<sup>4</sup> In the structure of **1-2** adduct (Figure 5) the purine-pyrimidine rings are also almost coplanar (dihedral angles in the range of 3.4-6.75 Å). The geometry of the [N...H-N] interaction is as follow: bond angle = 172.3°, bond distances of *non*-bonded atoms N...N = 2.89 Å; N...H = 1.93 Å. These values are in fact smaller than those expected for the bond lengths of [N...H-N] bonding (see above). As for the [O...H-N] interaction, the geometry is: bond angle = 164.3°, bond distances of *non*-bonded atoms O...N = 2.97 Å; O...H = 2.09 Å, and the expected values are very close [(O...H-N) 2.9 Å for O...N, and 2.0 Å for H...O].<sup>12,22</sup> From these values, it is evident that the H-bonding between 1-octylthymine/9-octyladenine is also moderate;<sup>4</sup> however, the fact that the approach between the thymine and adenine is at closer distance than the *homo* pairs of adenine or thymine [(O...H-N), 2.09 Å vs. 2.86 Å<sup>19</sup> and (N...H-N) 1.94 Å vs 2.11 Å (average) for the *hetero* and *homo* adducts, correspondingly] stabilize the thymine-adenine complex over the thymine-thymine or adenine-adenine complexes.



**Figure 4.** View of the crystal packing in the unit cell of 9-octyladenine (**2**).



**Figure 5.** View of the crystal packing in the unit cell of the pair 1-octylthymine/9-octyladenine.

## Conclusions

The enthalpy and entropy contributions to the equilibrium of the *self*-association of *n*-octylthymine [ $\Delta H_{1-1}^0 = -6.18$  kcal/mol;  $\Delta S_{1-1}^0 = -16.27$  cal mol<sup>-1</sup> K<sup>-1</sup>] and *n*-octyladenine [ $\Delta H_{2-2}^0 = -6.65$  kcal/mol;  $\Delta S_{2-2}^0 = -18.94$  cal mol<sup>-1</sup> K<sup>-1</sup>] were evaluated, as well as those of the pair 1-octylthymine/9-octyladenine [ $\Delta H_{1-2}^0 = -6.74$  kcal/mol;  $\Delta S_{1-2}^0 = -13.95$  cal mol<sup>-1</sup> K<sup>-1</sup>]. From these values, it is evident that in the competition for the H-bonding association between nucleobases, the thymine-adenine pair is favored by enthalpy as compared to the *self*-association of each nucleobase. As expected, the entropy is not favoring the formation of neither the *hetero* nor the *homo* adducts. However, the fact that the loss of entropy is less in the pair thymine-adenine helps to drive the equilibrium towards the *hetero* association. In the solid state, thymine-adenine are closer in distance than the adenine-adenine or thymine-thymine dimers. That evidently is accompanied with stronger H-bonding between the *hetero* than the *self*-associated species. If we take these simple models to study the molecular recognition of the two strands of the helical DNA, we can conclude that the chemical nature of the linking sites is as important as the complement in shape or size in the H-bonding interactions of nucleobases. Finally, taking into account the energy and structural data reported in this work, one can realize how beautifully nature takes advantage of these *non-covalent* interactions to form one of the most exciting and intriguing biomolecules of all, the DNA.

## Experimental Section

**General Procedures.** Melting points were obtained in a Melt-temp II apparatus with an open capillary tube and are uncorrected. Nuclear magnetic resonance spectra were recorded in  $\text{CDCl}_3$  on a Jeol Eclipse 400 spectrometer at 399.8 MHz for  $^1\text{H}$  and at 100.5 MHz for  $^{13}\text{C}$ . Chemical shifts are referred to internal  $(\text{CH}_3)_3\text{Si}$ . Mass spectra were measured on a Hewlett Packard 5989A spectrometer using electron impact (EI) at 70 eV. Microanalysis were recorded in Thermo Finnigan Flash 1112 analyzer. X-ray diffraction data were collected in an Enraf-Nonius Kappa CCD. Data collection: COLLECT software (Nonius BV 1997).<sup>23</sup> Cell refinement and data reduction: Denzo/Scalepack software.<sup>24</sup> An integer system of programs was used for the solution, refinement and analysis of X-ray-diffraction data, WinGX v-1.64.<sup>25</sup> The structures were solved by direct methods with Shelxs-97 (Sheldrick 1997)<sup>26</sup> and refined with Shelxl-97 (Sheldrick 1997).<sup>27</sup> Molecular graphics with WinGX software.<sup>25</sup> All chemicals used here were of reagent grade and were obtained from Aldrich Chemical Co.

**NMR experiments for thermodynamic measurements.** Proton NMR spectra were taken on previously temperature calibrated instrument, with the following acquisition parameters: PW1 = 6.7 s, Acq. Time = 2.048 s, SW = 8 kHz, data points = 16384, relaxation delay = 1.6 s.

**X-ray crystallography of the adduct 1-octylthymine/9-octyladenine.** The complex was recrystallized from a solution of 1 to 1 equivalents of (1) and (2) in DMSO. *Crystal data:*  $\text{C}_{26}\text{H}_{43}\text{N}_7$ ,  $M = 485.67$ , Triclinic, P-1,  $a = 8.468$  (2),  $b = 11.312$  (3),  $c = 16.401$  (6)  $\alpha = 71.176$  (12),  $\beta = 78.503$  (12)  $\gamma = 71.175$  (16),  $V = 1399.4$  (7)  $\text{\AA}^3$ ,  $Z = 2$ , (MoKa)  $\lambda = 0.71073$  T=293 K, 5473 independent measured reflection, reflections 3264 [ $F > 4\sigma(F)$ ], final R indices [ $F > 4\sigma(F)$ ],  $R_1 = 0.0541$ ,  $wR_2 = 0.1267$ . Crystallographic data (excluding structure factors) for this structure have been deposited in the Cambridge Crystallographic Centre as supplementary publication numbers CCDC 218676.

**5-Methyl-1-octyl-1H-pyrimidine-2,4-dione [1-octylthymine] (1).** To a mixture of 0.1 g (0.79 mmol) of thymine in 0.3 mL hexamethyldisilazane (2.37 mmol), was added a catalytic amount of chlorotrimethylsilane. The mixture was heated to reflux and maintained at 80 °C, under stirring, for 12 h. The excess of hexamethyldisilazane was removed under vacuum (50 °C/ 1.5 mm Hg) and 0.14 mL of 1-bromooctane (0.79 mmol) were added along with 0.014 mL of trimethylsilyl trifluoromethanesulfonate (0.08 mmol) to the residue. When the addition was completed, the temperature of the mixture was raised to 60 °C and maintained, under stirring, for 12 h. The crude material was transferred to a chromatographic column and eluted with a mixture of hexane/ethyl acetate (80:20). The product was obtained as colourless crystals after recrystallization from acetone, mp 121-122 °C. Yield 0.151 g (80%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  (ppm): 0.86 (3H, t, chain- $\text{CH}_3$ ), 1.27 (10H, m, chain- $\text{CH}_2$ 's), 1.64 (2H, m,  $\text{CH}_2\text{CH}_2\text{N}$ ), 1.90 (3H, d, T- $\text{CH}_3$ ), 3.67 (2H, t,  $\text{CH}_2\text{N}$ ), 6.97 (1H, d, T-CH), 9.60 (1H, s, NH) ppm.  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ),  $\delta$  (ppm): 12.4 (T- $\text{CH}_3$ ), 14.1 (chain- $\text{CH}_3$ ), 22.6, 26.4, 29.1, 29.2, 30.9, 31.7, 48.6 (chain- $\text{CH}_2$ 's),

110.5 (C-5), 140.4 (C-6), 151.01 (C-2), 164.5 (C-4). MS, m/z 238 ( $M^+$ ), 223 ( $M^+-15$ ), 126 ( $M^+-112$ ), 96 ( $M^+-142$ ), 55 ( $M^+-183$ ), 41 ( $M^+-197$ ).

**9-Octyl-9H-purin-6-ylamine [9-octyladenine] (2).** To a solution of 0.1 g (0.74 mmol) of adenine in 50 mL of DMSO, was added 0.156 g (0.74 mmol) of tetrabutylammonium hydroxide and 0.13 mL (0.74 mmol) of 1-bromooctane. The mixture was stirred at 25 °C, for 3 h. To quench the reaction, 100 mL of H<sub>2</sub>O were added, the product precipitated as a white solid from the mixture and it was filtered and used without further purification. Yield 0.155 g (85%). mp 122-123 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ (ppm): 0.84 (3H, t, chain-CH<sub>3</sub>), 1.27 (10H, m, chain-CH<sub>2</sub>'s), 1.87 (2H, m, CH<sub>2</sub>CH<sub>2</sub>N), 4.17 (2H, d, CH<sub>2</sub>N), 5.88 (2H, s, NH<sub>2</sub>), 7.77 (1H, s, H-8), 8.35 (1H, s, H-2) ppm. <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ (ppm): 14.1 (CH<sub>3</sub>), 22.6, 26.7, 29.1, 30.1, 31.8, 44.0, 48.6 (chain-CH<sub>2</sub>'s), 119.4 (C-5), 140.4 (C-8), 150.2 (C-4), 153.0 (C-2), 155.7 (C-6). MS, m/z 247 ( $M^+$ ), 148 ( $M^+-99$ ), 204 ( $M^+-43$ ), 190 ( $M^+-57$ ), 176 ( $M^+-71$ ), 162 ( $M^+-85$ ), 135 ( $M^+-112$ ). Anal. Calc. for C<sub>13</sub>H<sub>21</sub>N<sub>5</sub>: C, 63.11; H 8.56; N, 28.33. Found C, 63.00; H, 8.62; N, 28.77. A crystal suitable for x-ray diffraction was obtained through recrystallization from acetonitrile. *Crystal data*: C<sub>13</sub>H<sub>21</sub>N<sub>5</sub>, M = 247.35, Orthorhombic, P2<sub>1</sub>2<sub>1</sub>2<sub>1</sub>, *a* = 8.3746 (2), *b* = 8.4248 (2), *c* = 20.089 (7) Å, α = 90.0, α = β = γ, *V* = 1417.37(7) Å<sup>3</sup>, *Z* = 4, (MoKα) λ = 0.71073 Å, T = 293 K, 3214 independent measured reflection, reflections 2129 [(F>4σ(F))], final R indices [F>4σ(F)], R<sub>1</sub> = 0.0508, wR<sub>2</sub> = 0.1098. Crystallographic data (excluding structure factors) for this structure have been deposited in the Cambridge Crystallographic Centre as supplementary publication numbers CCDC 218677.

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