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Structure of pyrimidinocyclophanes in solution by NMR

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Abstract—The conformations and self-associative properties of novel pyrimidinocyclophanes with a substituted nitrogen atom in a spacer have been studied using several independent NMR methods (NOE, aromatic shielding effect, 2D DOSY, GIAO DFT chemical shift calculations, DNMR) in a variety of solvents, in acidic and basic media. At room temperature the title compounds in neutral solution are in slow exchange on the NMR time-scale and exist in a folded conformation. In the acidic medium protonation occurs at the bridge nitrogen, and that leads to dramatic modifications of the structure and dynamics of the compound. The protonated form exists at the equilibrium of 'folded' and 'extended' conformers. Moreover, the protonated form is prone to association and can be effectively described as a dimer at room temperature.

1. Introduction

Nucleotide bases and their derivatives play a crucial role in different processes in biochemistry, pharmacology, and medicinal chemistry. Therefore, there have been constant efforts to investigate such systems and their properties by different experimental and theoretical methods[1]. These efforts attest to the great interest currently focused on understanding and controlling nucleotide base interactions.[1c,2] However, in spite of the remarkable progress in this area there is still a lot to be understood. There are no clear ideas about the forces that control and stabilize primary, secondary, and supramolecular structure of the synthetic and biological systems containing nucleotide bases, particularly nucleic acids and their complexes with proteins.[3] It is well known that hydrogen bonding is crucial in the determination of the conformational and supramolecular structures of the systems with nucleotide bases. However, such strong stabilization is controlled not only by hydrogen bonding, the mechanism of the interactions seems to be more complicated, and new hypotheses have been developed.[2i,4] Most of the investigations are theoretical, thus experimental studies have become a necessity.

To promote these investigations some simpler model systems are needed, which can offer an access to control and subtle structural variations the effects of which can be assessed and comprehended at the molecular level.[5] To develop such systems, modeling interactions between nucleic acids and proteins or intercalating agents, a variety of new compounds has been synthesized. Almost all of them consist of nucleic acids or their derivatives linked by a polymethylene spacer with another nucleic bases (pyrimidines or purines or their combination) or with aromatic or heteroaromatic system.[5c–i,6] The analysis of hypochromic and in very few cases of ^{1}H NMR effects can provide insights into geometry, which is controlled by weak noncovalent interactions (hydrogen bonding (HB), van der Waals (vdW)). However, the very flexible nature of the proposed acyclic models allows one to establish only the fact of folded/unfolded conformation or complexed/not complexed structure and no exact 3D solution structure can be obtained in most cases.

The use of macrocyclic molecules containing nucleic bases and their derivatives as models seems to be more promising because even with the same flexible spacers, NMR and UV effects are stronger, probably due to the higher rigidity of macrocycles versus acyclic analogues. For example, in the case of purinophanes and pyrimidinophanes high field shifts for purine and pyrimidine protons and remarkably increasing hypochromic effects were observed in comparison with their acyclic counterparts both in $CDCl_3$ and aqueous solution.[7] At the same time, although these data are an indirect indication of stacking between various nucleic bases in these macrocycles, there has been little success in the determination of the 3D geometry of macrocycles in solution by NMR.

NMR techniques have proven to be powerful tools in the conformational analysis of biologically important macrocycles such as peptides and proteins.[8] However, in the case of macrocycles containing nucleic acid bases, the problem is more complicated: due to the diversity of weak interactions of similar energy (weak HB, vdW interactions) these

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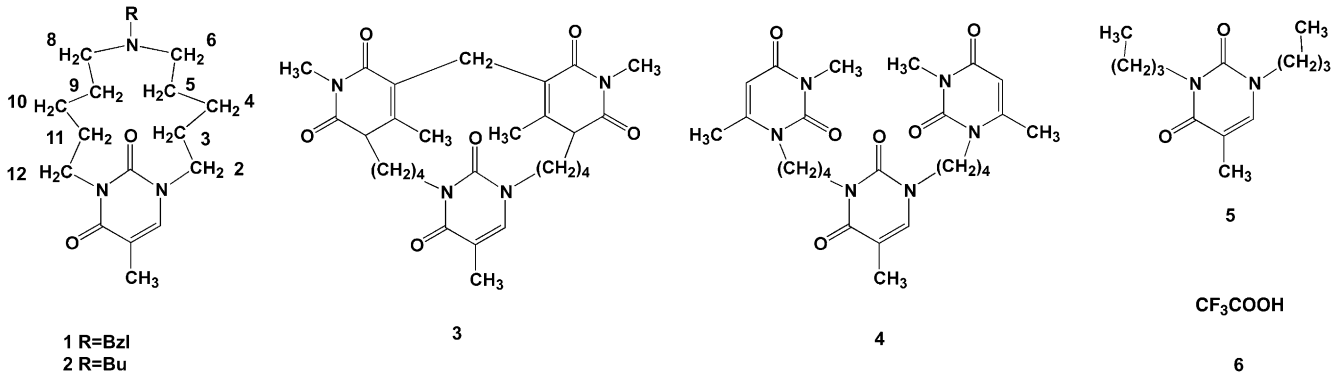


Scheme 1.

compounds in solution are in equilibrium in a variety of conformations of close energy, and no folded-structure-specific NOEs were seen.[7b,9]

Moreover, the equilibrium between different tautomeric forms and the contribution of protonated forms is very probable.[10] Thus nucleotide macrocycles have to be essentially flexible and a variety of different structural forms can be expected. Moreover, association or self-association processes may take place, thus additionally complicating the structure determination problem.[11] In addition, the dispersion of the signals in ^{1}H NMR is worse than in the spectra of peptides. Therefore, it is clear that macrocycles containing pyrimidine bases are difficult objects for NMR investigation.

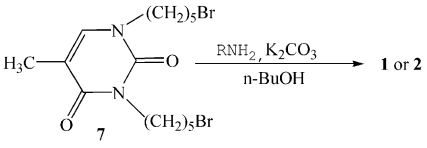
We faced such problems when we started a new project concerning macrocycles with three pyrimidine fragments.[†] Our attempts to make use of variable temperature NMR experiments were of little success: at low temperatures extensive broadening of most signals in proton NMR was observed, and it was not possible to derive conclusions about conformational and/or supramolecular structure from those experiments.

Only recently we have obtained new macrocycles (**1**, **2**) (Scheme 1), which can be considered as cyclophanes containing pyrimidine rings in place of aromatic unit, called pyrimidinocyclophanes.[12] These macrocyclic compounds allowed us insight into their 3D structures in solution and, perhaps, to propose an explanation why the line shape evolution for previous compounds was so dramatic and difficult to analyze. Here we report our recent results on the structure and association properties of new pyrimidinocyclophanes in solution.

2. Results

2.1. Synthesis

Pyrimidinocyclophanes **1** and **2** were prepared by amination of 1,3-bis(bromopentyl)thymine **7** with 2–3-fold excess of appropriate amine in *n*-BuOH in the presence of K_2CO_3 under heating (Scheme 2). The yields of the macrocycles are poor (17 and 19%), and attempts to increase them were unsuccessful. In particular, the reactions were carried out under high-dilution conditions, *n*-BuOH was substituted by other solvents, and salts of transition metals as catalysts were introduced into reaction mixtures. However, the yields of the obtained pyrimidinocyclophanes didn't exceed 20%. It seems that the reactions are subjected mainly to statistic factors.



Scheme 2.

Compounds **3**, **4**, and **5** were prepared by procedures reported earlier.[7b]

2.2. Conformational structure

The full assignment of signals in proton and carbon spectra of **1** was carried out by 2D COSY, HSQC, HMBC, and NOESY methods.[‡13] The analysis started from the thymine fragment, for which signals can be easily assigned,[14] e.g., starting from $C(6)_{thy}H$ by COSY, HSQC, and HMBC correlation techniques. The key correlations to distinguish H2 and H12 of the aliphatic spacers are between $C(6)_{thy}$/H2 and $C(4)_{thy}$/H12 in HMBC, and $C(6)_{thy}H$/H2 in NOESY. The –N–CH_2–Ph fragment was also unequivocally assigned in the same manner starting from the aromatic fragment. The H6/H8 and the C6/C8 show correlations with NCH_2–Ph carbons and protons, respectively. Thus, the fully assigned ^{1}H NMR spectrum of **1** is shown in Figure 1.The most important and interesting resonances are those of the H2 and H12 protons: it is quite unexpected that geminal CH_2 protons at C2 (and C12) are nonequivalent. One could expect rapid flipping of the long aliphatic chain around a thymine fragment and therefore the geminal protons at the benzylic positions should be chemically equivalent due to the fast exchange on the NMR time-scale and only one signal would be

[†] Results of low temperature NMR investigation of macrocycles containing one 6-methyluracil and two 2-thio-4-amino-6-methylpyrimidine units are in preparation and will be published elsewhere.

[‡] All details can be found in Supplementary data.

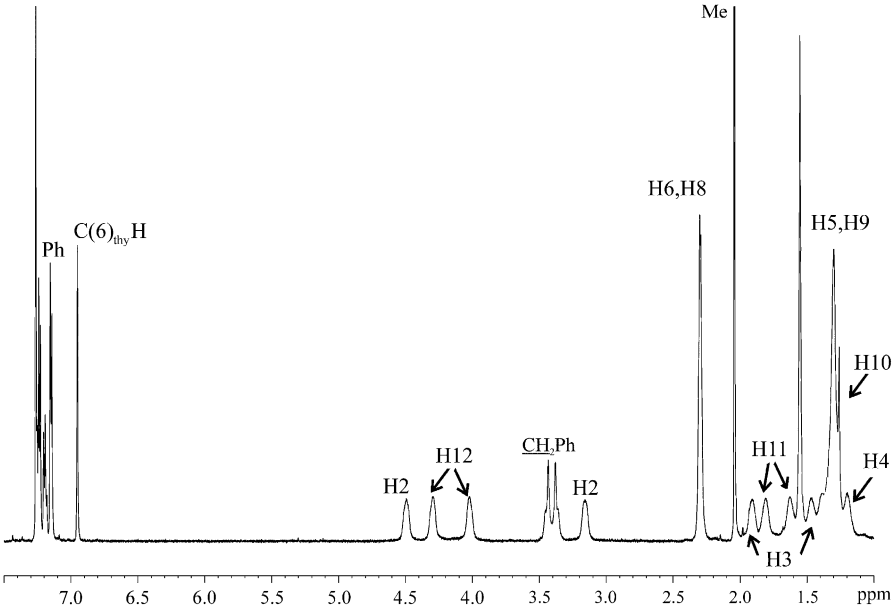


Figure 1. ^{1}H NMR spectrum of **1** in $CDCl_3$ at T=303 K.

observed for H2 and H12 protons. For example, for macrocycles possessing thymine and two 3,6-dimethyluracil moieties linked by similar aliphatic spacers (**3**) one observes ^{1}H NMR spectra for these protons that correspond to fast exchange when $n\geq4$ and these spectra are very similar to those observed for acyclic analogues (**4**).[§]

These results imply that this compound is conformationally rigid, at least in the neighborhood of the thymine fragment: the chemical shifts of the geminal H12a/H12b and the H2a/H2b protons reflect their position with respect to the plane of the thymine ring.

At the same time, there is broadening in the spectrum at T=303 K that might be due to some exchange processes. In order to explore this process, low temperature experiments were carried out. These experiments in $CDCl_3$ in the range of temperatures from 303 to 213 K did not show any remarkable modification of the NMR parameters, particularly for H2 and H12. Only some low field shifts for $C(6)_{thy}H$ and 5-Me were detected, and minimal modification of the line shape for CH_2Ph protons was observed.

Such line shape evolution can be interpreted as if the title compounds (macrocycles) exist basically in one form. Only some minor process near to the bridging nitrogen (N-inversion and/or rotation around N–CH_2) might be the reason why the N–CH_2 proton spectrum is slightly modified at lower temperature.

The conformational structure of **1** has been established by three NMR techniques. First of all, there are NOEs at T=213 K between the phenyl and 5-Me protons (Fig. 2) that are due to the close proximity of these protons. In addition, there are deshielding effects of phenyl ring on $C(6)_{thy}H$ and 5-Me protons (Fig. 2b). As one can see, there are low field shifts for these protons in comparison with the spectrum for the model compound **5** (Fig. 2b) where there is no such effect. Finally, comparison of the calculated and experimental chemical shifts for the H2 and H12 protons in this conformation is in good agreement with this conclusion.[7b] In fact, the thymine ring is magnetically anisotropic, and chemical shifts of vicinal protons depend strongly on their exact position in respect to the thymine plane (angle around N–C2 and N–C12 bonds). Therefore, we searched for the stable conformers of **1** by the MM method (program ChemOffice)[15] and found that the 'folded' structure (Fig. 3a) corresponds to energy minima, and nonempirical ^{1}H chemical shift calculation in the frame of GIAO DFT approach[16] shows quite good correspondence between the experimental and theoretical chemical shifts for this geometry (Fig. 3b). This additionally supports our conclusion about the conformational structure of **1** because even a small discrepancy in geometry would have been reflected in chemical shifts of the vicinal protons.

Thus, we have concluded that compound **1** in $CDCl_3$ predominantly exists in a folded conformation (Fig. 3a).

In addition, ^{1}H NMR experiments at different concentrations were carried out in order to see whether this molecule is prone to association. In proton spectra only minimal changes were observed when concentration changed from 1 to 50 mmol/l. Thus, we concluded that the molecule in $CDCl_3$ solution exists as a monomer in folded form.

In general, the fact that this molecule exists in a folded conformation and exchange is slow in NMR time-scale is quite an unexpected result. For example, for pyrimidinophane with one thymine and two 3,6-dimethyluracil fragments (**3**, **4**) the exchange is fast even for aliphatic spacer $(–CH_2–)_n$ with n=4, moreover, for n=5 the spectrum is very similar to that observed for acyclic analogue **4**. This suggests that there may be some extra interactions, which stabilize this folded conformation of **1**.

[§] Results of NMR investigation of pyrimidinophane **3** and acyclic analogue **4** are under preparation and will be published elsewhere.

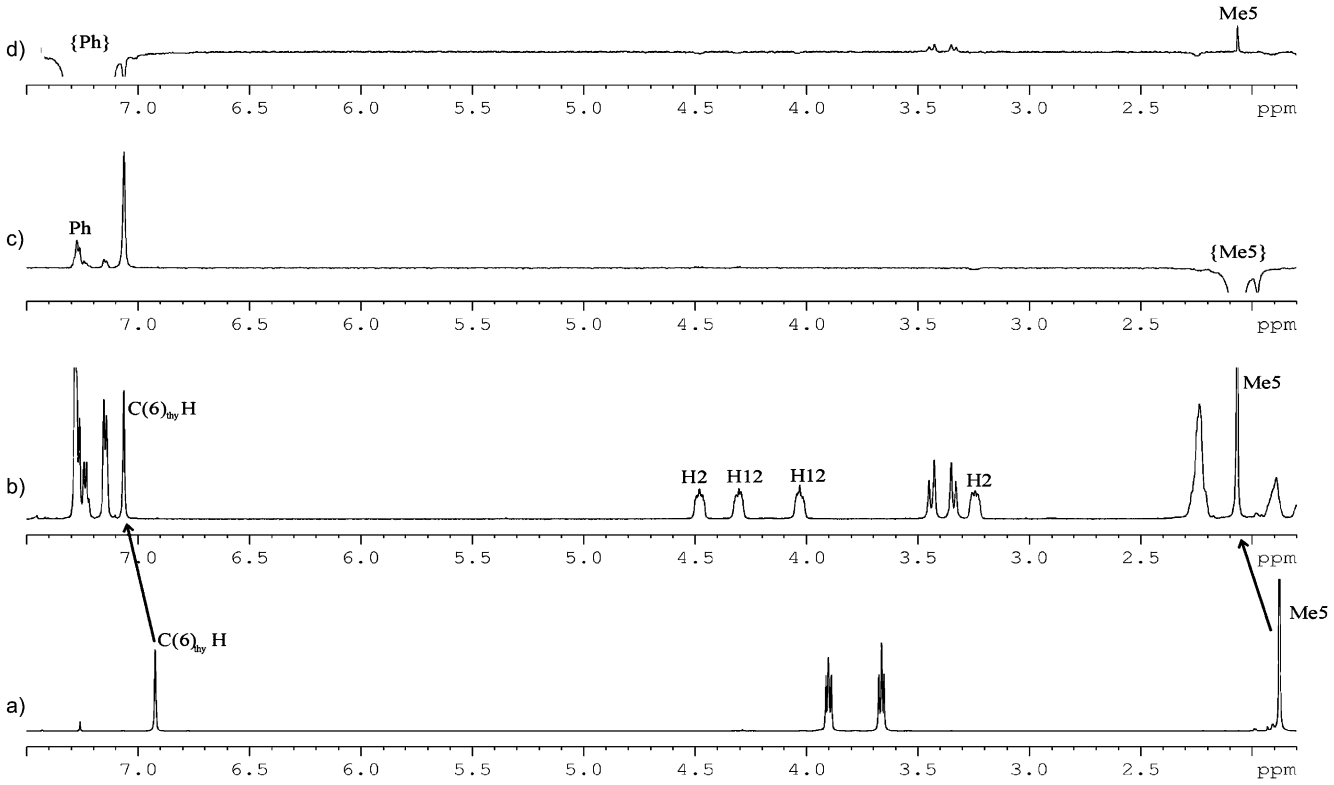


Figure 2. ^{1}H NMR (a, b) and NOE spectra (c, d) (τ_m=0.6 s) of compounds **1** and **5** in $CDCl_3$: (a) **5** at T=303 K; (b–d) **1** at T=213 K (irradiated atoms shown in braces).

In this case several noncovalent interactions may be considered as a contribution to stabilization. There are a number of publications concerning this subject but there are still many uncertainties in the energy gain due to these interactions. In fact, most of these reports are based on theoretical results and there are only little experimental data. Moreover, even amongst the theoretical estimations there is dispersion in values of energies depending on the method used to calculate particular interaction, level of theory, choice of the model system, and the model to account for the solvent effects.[3a,b,17]

In fact, from the variety of possible interactions in this system the HB is the strongest one. There are several polar atoms and groups (C═O, N, $C(5)_{thy}H$) that may be proton donors or acceptors. If this assumption is correct then polar solvents should disrupt such intramolecular interactions and destabilize the folded conformation.[1a,18] To verify this idea the ^{1}H NMR spectra in different polar solvents at room temperature were obtained, and it was found that replacement of $CDCl_3$ by acetone, acetonitrile, and methanol only has insignificant effect on the spectra, particularly on indicative H2 and H12 resonances. Therefore, we concluded that dynamics and equilibrium of **1** in these solvents are essentially the same. Thus, HB can probably be excluded from the interactions that stabilize this conformation.

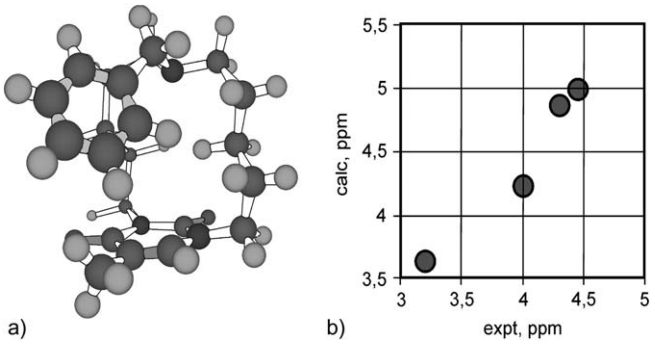
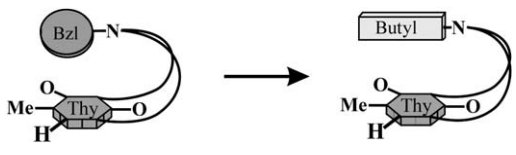


Figure 3. (a) 3D structure (left) and (b) correlation of experimental and calculated (GIAO B3LYP/6-31G(d)//RHF/6-31G) ^{1}H chemical shifts for **1** (right).

It is possible that aromatic π–π (e.g., face-to-edge) interactions may take place in this case.[3a,b] Recently, the role of different aromatic–aromatic and aromatic–aliphatic interactions has been reviewed and, according to calculations, stabilization of up to 3.2 kcal/mol could be expected.[3b] To check if these interactions work in this case the model compound with aliphatic substituent instead of phenyl ring on bridged nitrogen (**2**) was synthesized and ^{1}H NMR experiments were carried out.



The ^{1}H NMR spectrum of **2** is shown in Figure 4. For comparison the spectrum of **1** is also given. As one can see, the H2 and H12 proton signals are very similar. Taking into

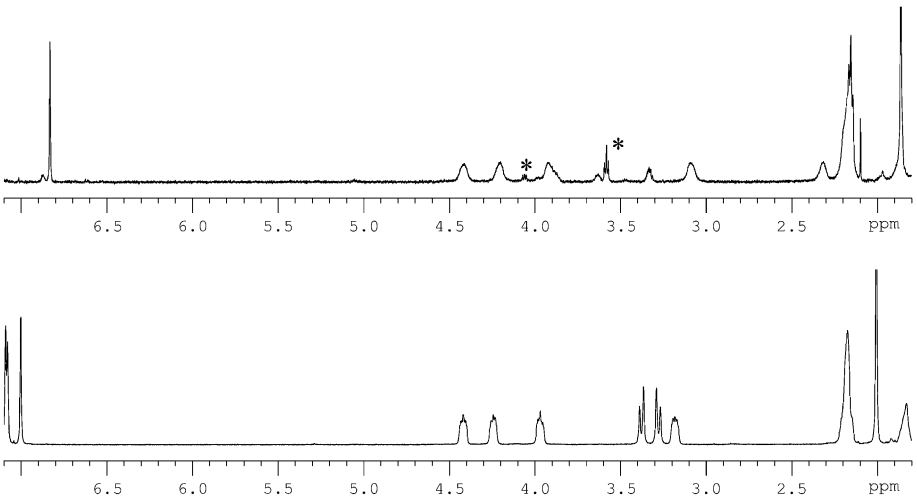


Figure 4. ^{1}H NMR spectra of **2** (top, R=butyl, C=12 mM) and **1** (bottom, R=Bzl, C=7 mM) in $CDCl_3$ at T=303 K. *Undetermined admixture.

account the strong anisotropy of the thymine ring and almost equal chemical shifts of the H2 and H12 protons in **1** and **2**, we can conclude that geometries of these molecules are effectively the same.

Low temperature ^{1}H NMR experiments were also in good agreement with this conclusion. In addition, NOE between 5-Me and the aliphatic substituent at N unambiguously proves that **2** in solution exists also in folded conformation.

Thus both compounds are in an identical folded conformation irrespective to the nature of the substituent at bridge nitrogen, and no indication of π–π 'edge-to-face' interactions was obtained for the title compounds. Most probably, the realization of the folded conformation for such systems is explained by the structure of polymethylene spacers and the number of methylene groups.

2.3. Protonation and association

Another aspect of our efforts was to explain why the ^{1}H NMR spectra of **1** still remain well resolved at lower temperature and correspond to one conformation, with no indications of association even at temperatures close to the solvent's m.p. For macrocycles containing other pyrimidine bases, however, extensive broadening of signals was seen even at moderately low temperature.

We supposed that other macrocycles are prone to association due to the existence of protonated or charged forms.[10] Therefore, we decided to try to provoke protonation of compound **1** too.

^{1}H NMR experiments in $CDCl_3$ with titration by CF_3COOH (**6**) at room temperature have demonstrated marked modification of the spectra (Fig. 5). Some changes could be expected due to protonation of the bridge nitrogen, and therefore vicinal CH_2 protons (H6, H8, CH_2Ph) should reflect this process. However, the most important is the observed line shape evolution of the H2 and H12 protons: as the concentration of acid is increased the signals of these protons start to broaden and then finally they coalesce. It is particularly apparent for the H2 protons, while in pure $CDCl_3$ the geminal CH_2 protons were not equivalent and resonate at 4.5 and 3.2 ppm. At intermediate concentrations of acid their signals broadened extensively, and then close to full protonation, one exchange averaged signal was observed for each of these protons.

Such line shape evolution can be explained either by exchange between two symmetric forms that becomes fast in NMR time-scale or/and stabilization of the additional form in which the chemical shifts of these protons differ from those in the folded conformation.

In order to determine the structure of the protonated form(s), NMR experiments with variation of the temperature were carried out (Fig. 6). As the temperature decreases, the signals of the H2 and H12 protons start to collapse and finally at T=223 K the spectrum corresponds to slow exchange of several forms. The lines, in addition, are extensively broadened, perhaps due to self-association.

Unfortunately, due to ambiguous line shape evolution and extensive broadening it is difficult to determine the number of components in equilibrium and to assign the signals in the low temperature ^{1}H spectra to corresponding groups in order to establish their conformational structure.

Therefore to the association of the title compound in solution under protonation and particularly at low temperature (corresponding extensive broadening of the lines in ^{1}H spectra) we tried to estimate effective volume of the molecule by the measurement of self-diffusion coefficient.[10a,19] Translation self-diffusion coefficients were measured by 2D DOSY method with bipolar gradients. Every value was averaged over two–three measurements. The results are shown in Figure 7. It was established that at room temperature the coefficient is approximately 1.5 times larger in pure $CDCl_3$ than in a solution of the 1:1 mixture of compounds **1** and **6**. Then according to Stock's model[19] the effective radius of the protonated form is about 1.5 of the radius of noncharged molecule. Therefore, the effective volume of the charged form is twice as large as the noncharged one. Thus if in neutral solution **1** exists as monomer, protonation leads to association of the molecule into dimer.

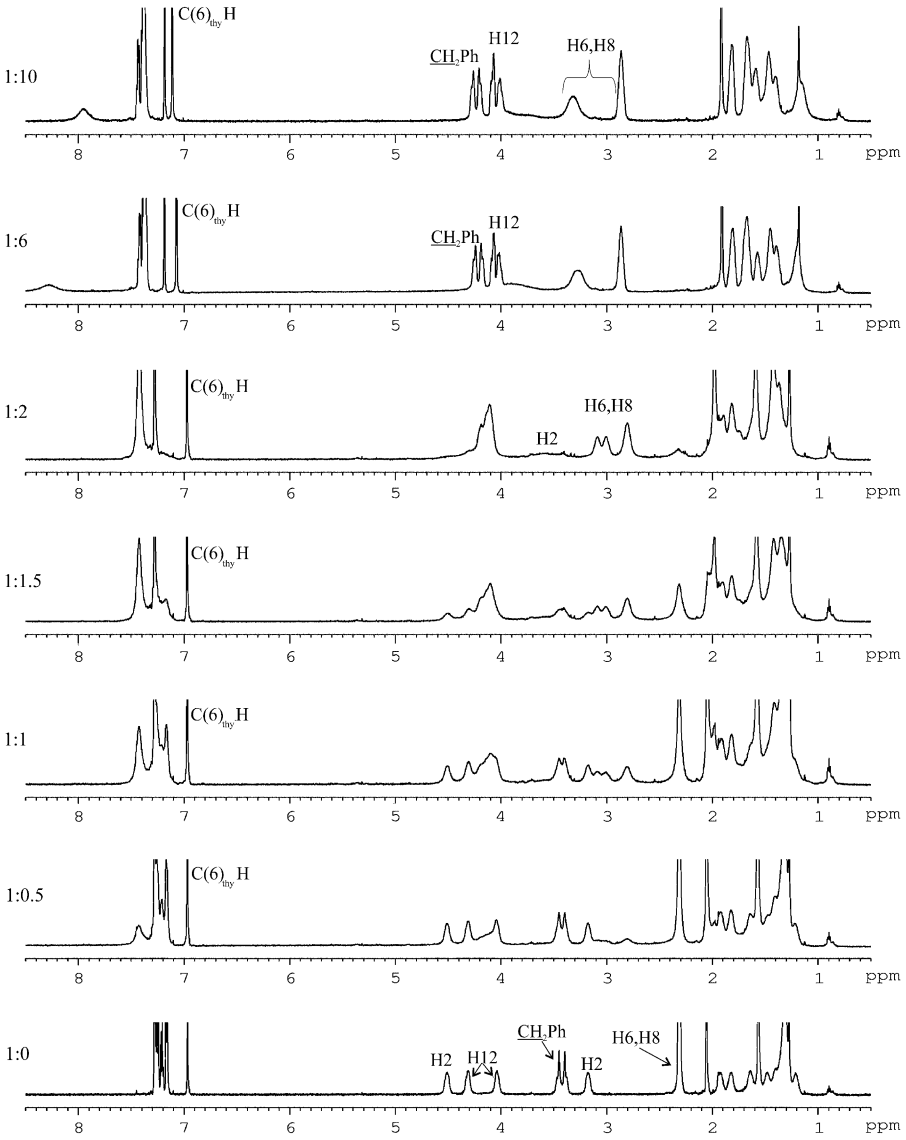


Figure 5. Dependence of ^{1}H NMR spectra of mixture **1+6** on the **1**:**6** ratio in $CDCl_3$ at T=303 K.

In addition, the self-diffusion coefficient was measured at lower temperatures (Fig. 7). The temperature dependence of the self-diffusion coefficient for neutral molecule can be well explained by its dependence directly on temperature and indirectly on viscosity. At the same time for the protonated form this dependence reflects also the fact that at low temperature higher associates become more stable and low temperature self-diffusion coefficient corresponds to four molecular associates. Thus association is thermodynamically more favorable than the monomeric form in acidic media.

In the next step we tried to get insight into the conformations of the protonated form. Room temperature ^{1}H NMR spectra of the protonated form in solution are much broadened and cannot be used for spectra-structure correlations. The ^{13}C NMR spectrum of **1** at room temperature also cannot be used to determine the conformational structure. However, a comparison of ^{13}C spectra with those for the nonprotonated form shows (Fig. 8) that they are different and this difference may be due to the contribution of some additional conformers. As can be seen from Figure 8, most spectacular differences were observed for $C(2)_{thy}$, $C(4)_{thy}$, $C(5)_{thy}$, and C6/8. None of them were seen in the acidic medium due to extensive broadening. Therefore, we concluded that this could be due to equilibrium of the forms where these carbons have large chemical shift differences.

To check this hypothesis we carried out several experiments with variation of temperature. As mentioned above, the evolution of the proton NMR spectra was dramatic but it was very difficult to follow these changes and to ascribe it either to slowing down of exchange between the conformers and/or to association effect. Therefore, we tried to run similar ^{13}C experiments at lower temperature, but due to low concentration a regular spectrum could not be accumulated, and information on ^{13}C chemical shifts of carbons directly bonded to protons was obtained only from 2D HSQC spectra (Fig. 9).

As can be seen in Figure 9, the number of carbon signals in the low temperature spectrum is twice that at room temperature (for carbons C2, C12, C6, C8, CH_2Ph). Therefore,

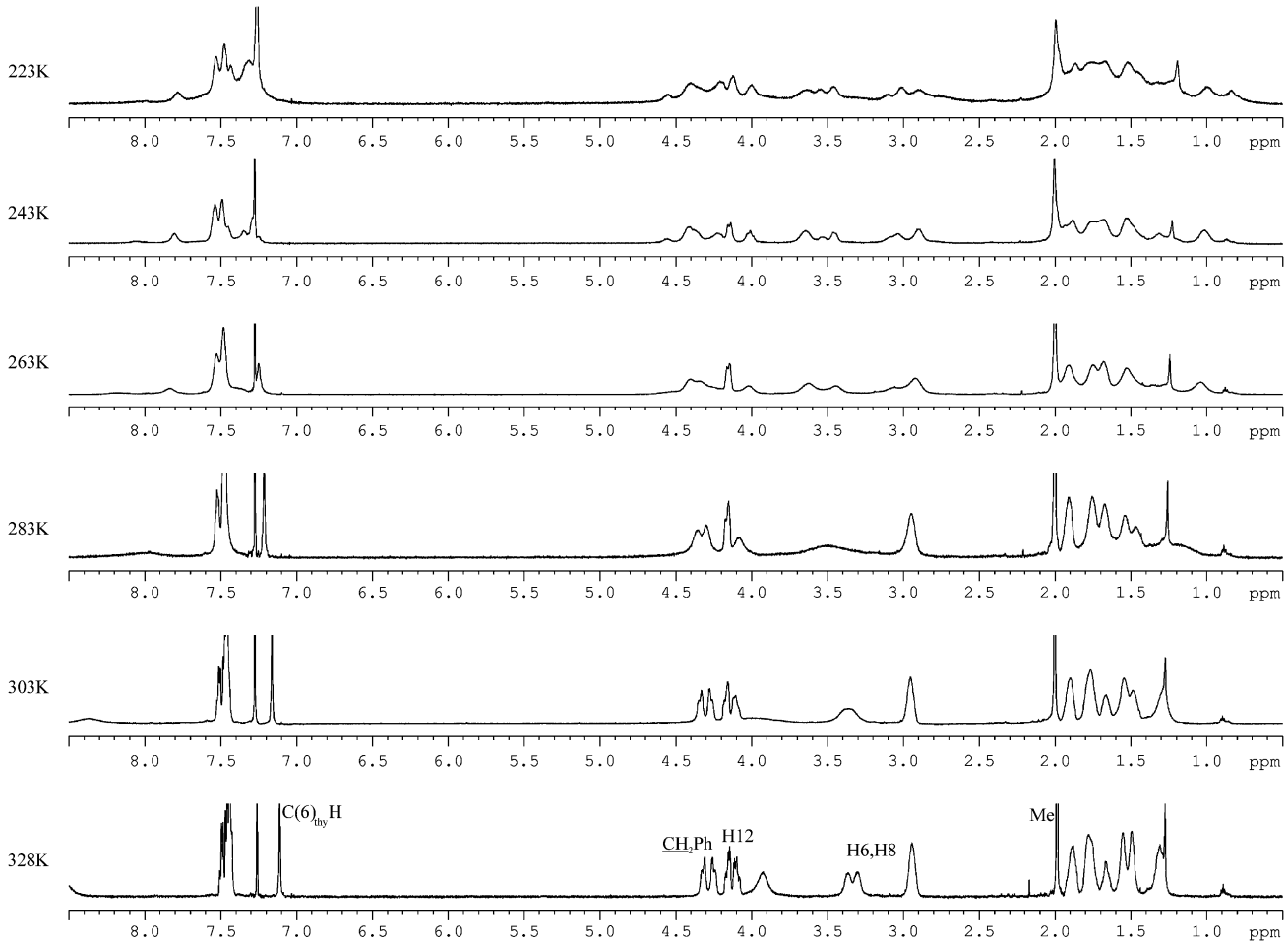


Figure 6. Temperature-dependent ^{1}H NMR spectra for a mixture of **1+6** (1:6) in $CDCl_3$.

we concluded that this spectrum corresponds to a two-component equilibrium. Moreover, comparison of room temperature chemical shifts with low temperature data and analysis of CH correlation data allowed us to assign signals as shown in Figure 9.

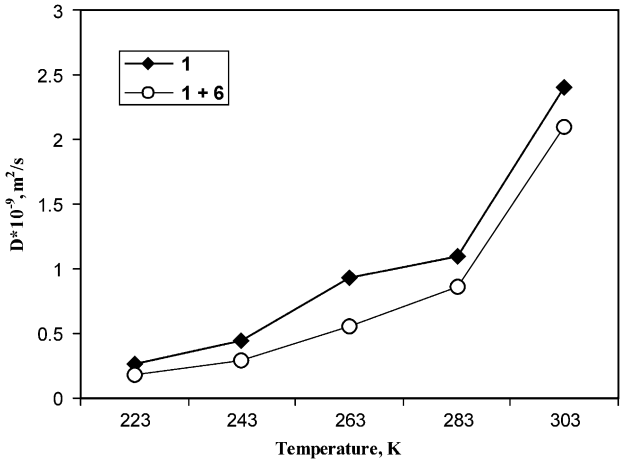


Figure 7. Temperature dependences of self-diffusion coefficients for **1** and **1+6** (1:1) in $CDCl_3$.

As can be seen, chemical shifts for the carbons in one form (except for the C6/8 and C2) are very similar to those observed in the nonprotonated form in $CDCl_3$ at room temperature, while for another conformer they are different. Particularly important is the difference for the C6/8 and C12 carbons of the spacer: $\Delta\delta$ are ca. 8 and 4 ppm, respectively.

Thus, we can conclude that the first form has a conformation similar to nonprotonated form, i.e. folded conformation. Some differences in chemical shifts of C6, C8, and C2 can be explained by the change of the local structure upon N-protonation. The analysis of the chemical shift difference for the second form allowed us to conclude that the observed low field shifts with respect to the folded form's shifts for C6, C8 carbons can be attributed to γ-effects on these carbons due to a change of orientation around C4–C5 and C10–C9 bonds that leads to extended conformation shown schematically in Figure 10. Thus, the title compound in solution upon protonation exists in equilibrium of two forms, one being folded and the second being extended.

In addition, the extended form is very prone to association and its structure can be accessed by high field shifts for H4, H10 protons at low temperature.[‡] Namely, in such dimers (trimer, tetramer, and higher associates) the protons

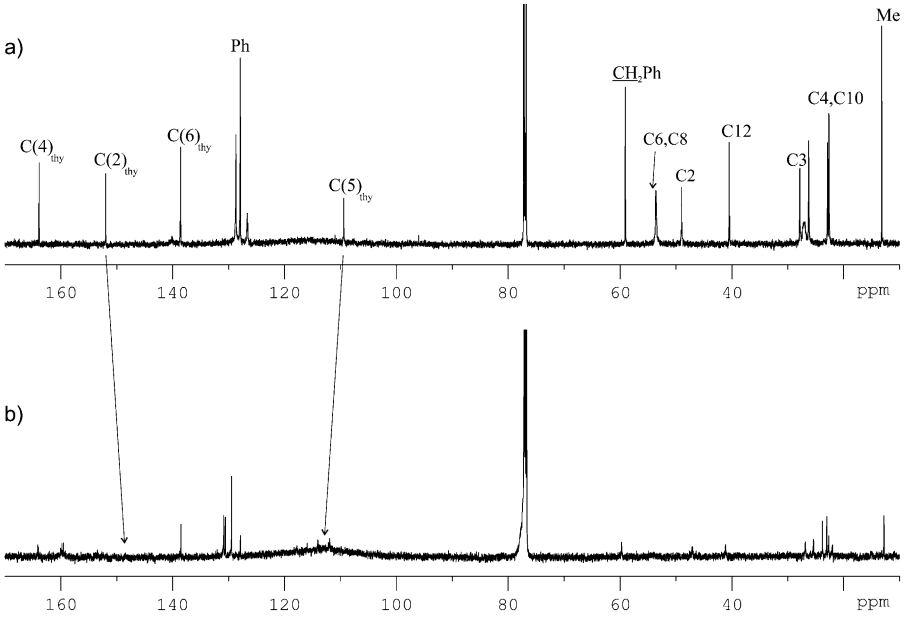


Figure 8. ^{13}C NMR spectra of **1** (a) and **1+6** (1:6) (b) at room temperature in $CDCl_3$.

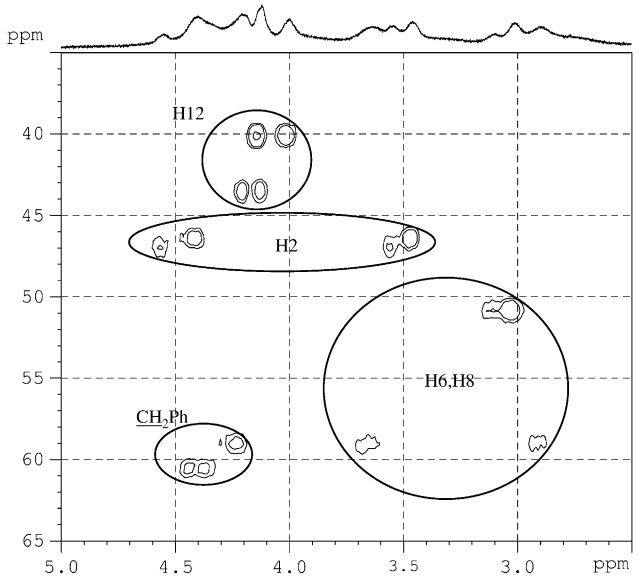


Figure 9. Fragment of 2D HSQC (^1H–^{13}C) spectrum of **1+6** (1:6) at T=223 K in $CDCl_3$.

at C4 and C10 of spacer of one molecule are located in the shielding cone of the phenyl ring of another molecule (Fig. 11). Therefore, at lower temperature the contribution of the higher associates, which have similar NMR parameters, increases and this leads to high field shift of H4, H10 protons.

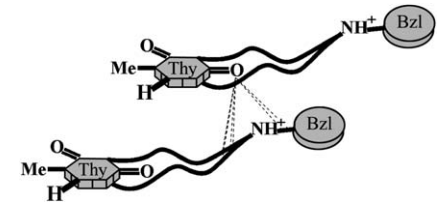


Figure 11. Schematic presentation of dimer of **1**.

3. Discussion

Besides the particular data related to this type of compounds (the protonation site and conformational structure in solution and in protonated form) there are two questions that have general interest: (1) the mechanism of stabilization of such strained forms and (2) the mechanism of stabilization of associates and their structure. (1) Folded conformations appear to be stabilized by the 'edge-to-face' π–π interactions. Different authors estimate such interactions in the range of 0.9–2.6 kcal/mol.[3b] At this point, however, its magnitude and its value in solution remain unclear. In particular there are almost no experimental data.

According to our experiments there is no indication of such interactions in the ground state in the title compounds. In the case of such interactions, the energy barrier of interconversion between symmetric conformers would increase for **1** versus **2** (when butyl is changed for Bzl) and it should affect

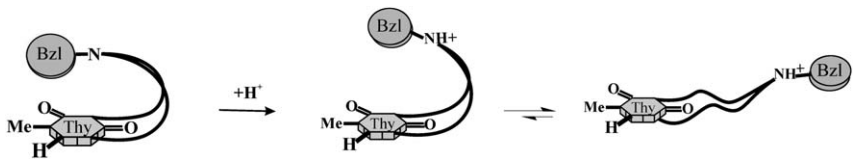


Figure 10. Schematic presentation of the equilibrium of folded and extended conformations of **1**.

NMR line shape (broadening or coalescence temperature). Some broadening at room temperature is very similar in both compounds (**1** and **2**) and this proves that exchange rate is very much the same in these compounds irrespective of the substituent at the bridge nitrogen atom.

(2) To explain the stabilization of such associates under protonation and also the mechanism an additional argument in favor of the structure depicted in Figure 11 is the chemical shift of $C(2)_{thy}$=O. It is likely that this oxygen atom participates in binding when dimer and higher associates are formed. There is no signal for this carbon in the room temperature ^{13}C spectra although other signals are present. (Unfortunately due to low *S*/*N* at low temperature we were not able to obtain the ^{13}C NMR spectra.) Based on this observation we concluded that its signal is much broadened at room temperature, which might take place if the chemical shift of this carbon in above-mentioned conformers (forms) is substantially different. This difference may originate from the bonding by the C=O group if, e.g., HB are formed in one of the forms. Indeed, GIAO DFT calculations of chemical shifts (with RHF/6-31G optimized geometry) for **1** with $C(2)_{thy}$=O involved in HB with three ethyl amine cation as example of proton donor group and for **1** which is not involved in HB predict maximal differences just for $C(2)_{thy}$ (+4.4 ppm) and $C(5)_{thy}$ (+5.4 ppm, positive signs mean low field shifts in HB complex).

Which group could donate proton to this group to form HB? There are different options: first of all, N^+H could participate in such bonding. Such bonds can be excluded as dominant, however, because the chemical shift of this proton does not depend remarkably on temperature. Second, vicinal to protonated nitrogen the N^+CH_2 protons may give HB, which are in total even stronger than classical HB, as it was recently proposed theoretically.[10b,17c] Three such groups can interact with C=O and in doing so stabilize such associates. This can explain the low field shift of these protons when the temperature decreases. In this structure high field shifts have to be observed for the protons at C4 and C10 of the bridges due to the shielding effect of the phenyl ring.

According to theory, enormous stabilization (90–95 kcal/mol in the gas phase were calculated for the [N^+–C–H$\cdots$O=C] model)[17c] can be expected due to these hydrogen bonds although solvent effects have to diminish such interactions to some extent (energy drops to 20 kcal/mol in $CHCl_3$). In order to verify this hypothesis we carried out additional ^1H NMR experiments for the protonated form in $CDCl_3$ with titration by DMSO as HB disrupting solvent. Indeed, the change of the spectra was observed and it can be ascribed to the increase of the monomeric form.

Such dependence of the conformational structure and association on acid concentration models a real situation where properties change with the solution pH.[20] In physiological systems pH in aqueous solution may vary from 1.9 up to 10.4 and this can effectively produce changes in the structure, which are responsible for definite properties.

Unfortunately, this compound dissolves poorly in water and we could not demonstrate the above statement directly in 'physiological' solvents. However, we were able to see these effects in the mixture of solvents: alcohol/water (methanol/water, 2/1). NMR experiments at room temperature with titration by HCl showed similar evolution of the line shape as observed in $CDCl_3$ under titration with CF_3COOH.[‡] The most spectacular were the changes of the H2/H12 protons signals: they start to broaden extensively upon the addition of ca. 1 equiv of HCl. Moreover, these changes are reversible: when equimolar NaOH was added the reverse evolution of the line shape was seen.

This fact demonstrates that being regulated by the solution pH, 3D and supramolecular association may be a means of recognition and modulation of that recognition.

4. Conclusion

The title macrocycles in solution exist in a folded conformation with bridge N substituents proximal to the thymine Me and H_5. The barrier of interconversion is very high.

A small amount of acid in solution may protonate the compounds and change dramatically not only the conformation but also the supramolecular structure. Interactions (HB) of protons vicinal to protonated nitrogen with carbonyl oxygen ($N^+CH\cdots O$=C) are strong when compared to other noncovalent ones, and this result strongly supports the theoretical prediction. The role of such interactions may be important if one takes into account that protonation in physiological solutions occurs very often. The supramolecular association of designed macrocycles and closely related natural systems can be regulated by the solution pH.

5. Experimental

5.1. General

NMR experiments were recorded with a Bruker AVANCE-600 spectrometer (14.1 T) equipped with a pulsed gradient unit capable of producing magnetic field pulse gradients in the *z*-direction of 56 G/cm. All spectra were acquired in a 5-mm inverse probehead in 5-mm tubes. Chemical shifts (ppm) are internally referenced to the TMS signal (0 ppm) in all cases. Complete assignments of the ^1H and ^{13}C NMR spectra of the title compounds were accomplished by 2D COSY, TOCSY, HSQC, HMBC, and NOESY experiments. In some cases 1D DPFGNOE method in rotating frame was used to measure NOEs.[21] A Hermite-shaped pulses were used for selective irradiation.

In order to minimize convection effects, 2D DOSY experiments were performed using the bipolar pulse longitudinal eddy current delay (BPPLED) pulse sequence. The duration of the magnetic field pulse gradients (δ) was optimized for each diffusion time (t) in order to obtain 1–5% residual signal with maximum gradient strength. The pulse gradients (g) were incremented from 2 to 95% of the maximum gradient strength in a linear ramp. All diffusion coefficients reported are means of at least three measurements.

For DNMR spectroscopy, a standard unit calibrated using a methanol reference controlled the probe temperature; the

samples were allowed to equilibrate for 15 min at each temperature before recording spectra.

Most of the solvents and all the reagents were commercial and used without further purification. All dry solvents were prepared according to the standard procedures and stored over molecular sieves.

Molecular mechanics (employing the MM2 force field)[22] were performed with CS Chem3D Ultra 6.0 (CambridgeSoft Corp.) on a AuthenticAMD Athlon (Im)computer. Chemical shifts were determined within the DFT framework using a hybrid exchange-correlation functional, B3LYP, at the 6-31G(d) level as implemented in Gaussian 98.[23] Full geometry optimizations were done at the ab initio RHF/6-31G level. All data were referred to TMS (^{1}H and ^{13}C) chemical shifts that were calculated at the same conditions.

The mass spectra (EI) were obtained on a Finnigan MAT-212 mass spectrometer (resolution was 1000, direct inlet of the sample into the ion source, energy of ionizing electrons was 70 eV, electron emission current was 1 mA). Melting points were measured on a Boetius hot-stage apparatus and are uncorrected. Thin-layer chromatography was performed on Silufol-254 plates; visualization was carried out with UV light. For column chromatography neutral Al_2O_3 (activity II) was used. Chemicals and reagents were purchased from Lancaster or Aldrich Chemical companies.

Thyimidinocyclophanes **1** and **2** were prepared by the reaction of 1,3-bis(5-bromopentyl)thymine (**7**) with benzylamine or butylamine.

5.1.1. 7-Benzyl-15-methyl-1,7,13-triazabicyclo[11.3.1]-heptadeca-15-en-14,17-dione (1). At 90 °C to a stirred mixture of benzylamine (2.14 g, 20 mmol) and K_2CO_3 (2.95 g, 21 mmol) in *n*-BuOH (250 mL) compound **7** (4.1 g, 9.7 mmol) in *n*-BuOH solution (100 mL) was added and stirring was continued for 7 h at 100–110 °C. The solvent was distilled off and the residue was treated by $CHCl_3$, filtered, concentrated, and transferred to a column with Al_2O_3. Elution with ether gave thyimidinocyclophane **1** in a yield of 0.6 g (17%); mp 130–131 °C; HRMS found *m/z* 369.2420, $C_{22}H_{31}N_3O_2$ required 369.2416; MS (EI) *m/z* 369 (M+, 94), 368 (M−1, 42), 340 (M−29, 33), 278 (M−91, 95), 250 (M−119, 29), 91 (100). ^{1}H NMR (600.0 MHz, $CDCl_3$, 303 K) δ_H (ppm) 1.16–1.44 (8H, m, $(CH_2)_{4,5,9,10}$), 1.48 (1H, m, H_3), 1.64 (1H, m, H_{11}), 1.8 (1H, m, H_{11}), 1.9 (1H, m, H_3), 2.04 (3H, s, $C(5)_{thy}$Me), 2.32 (4H, m, $H_{6,8}$), 3.18 (1H, m, H_2), 3.38 and 3.46 (2H, AB, J=13.0 Hz, CH_2Ph), 4.03 (1H, m, H_{12}), 4.28 (1H, m, H_{12}), 4.48 (1H, m, H_2), 6.97 (1H, s, $C(6)_{thy}$H), 7.16 (2H, d, J=6.7 Hz, Ar_{ortho}), 7.21 (1H, t, J=6.7 Hz, Ar_{para}), 7.26 (2H, t, J=7.4 Hz, Ar_{meta}); ^{13}C NMR (150.9 MHz, $CDCl_3$, 303 K) δ_C (ppm) 13.2 ($C(5)_{thy}$*Me*), 22.6 (C_9), 22.9 (C_5), 26.3 (C_{11}), 27.1 (C_{10}), 27.3 (C_4), 27.9 (C_3), 40.5 (C_{12}), 49.0 (C_2), 53.5 ($C_{6,8}$), 59.0 (CH_2Ph), 109.4 ($C(5)_{thy}$), 126.4 (Ar_{para}), 127.8 (Ar_{meta}), 128.4 (Ar_{ortho}), 138.6 (Ar_{ipso}), 152.0 ($C(2)_{thy}$), 163.9 ($C(4)_{thy}$).

5.1.2. 7-Butyl-15-methyl-1,7,13-triazabicyclo[11.3.1]heptadeca-15-en-14,17-dione (2). At 70 °C to a stirred mixture of *n*-butylamine (2.07 g, 28.4 mmol), K_2CO_3 (4.00 g, 29.0 mmol), and catalytic amount TBA·HSO_4 in *n*-BuOH (250 mL) compound **7** (3.0 g, 7.08 mmol) in *n*-BuOH solution (100 mL) was added and stirring was continued for 11.5 h at 70–75 °C. After evaporating the solvent, treating by $CHCl_3$, filtering, and concentration of $CHCl_3$ solution residue was eluated through column with Al_2O_3 by 2:1 ether/petroleum ether mixture. From the fractions of the eluent, thyimidinocyclophane **2** was obtained in a yield of 0.45 g (19%); mp 44–45 °C; HRMS found *m/z* 335.2570, $C_{19}H_{33}N_3O_2$ required 335.2573, *m/z* 292.2020, $C_{16}H_{26}N_3O_2$ required 292.2025; MS (EI) *m/z* 335 (M+, 13), 292 (M−43, 100), 278 (M−57, 8). ^{1}H NMR (600.0 MHz, $CDCl_3$) δ_H (ppm) 0.85 (3H, m, $(CH_2)_3CH_3$), 1.12 (2H, m, J=7 Hz, $(CH_2)_2CH_2CH_3$), 1.15–1.28 (9H, m, $H_{3,4,5,9,10}$), 1.34 (2H, m, J=7.4 Hz, $CH_2CH_2C_2H_5$), 1.42 (1H, m, H_{11}), 1.57 (1H, m, H_{11}), 1.75 (1H, m, H_3), 1.86 (3H, s, $C(5)_{thy}$Me), 2.11–2.25 (6H, m, $H_{6,8}$), 2.32 (2H, m, $CH_2C_3H_7$), 3.09 (1H, m, H_2), 3.92 (1H, m, H_{12}), 4.21 (1H, m, H_{12}), 4.42 (1H, m, H_2), 6.83 (1H, s, $C(6)_{thy}$H); ^{13}C NMR (150.9 MHz, $CDCl_3$) δ_C (ppm) 13.2 ($C(5)_{thy}$*Me*), 14.0 ($(CH_2)_3CH_3$), 20.4 ($(CH_2)_2CH_2CH_3$), 22.4 ($C_{5,9}$), 27.4 (C_{10}), 27.8 (C_3), 28.0 (C_4), 30.1 ($(CH_2)_2CH_2CH_3$), 40.9 (C_{12}), 49.0 (C_2), 52.8 ($C_{6,8}$), 55.0 ($CH_2C_3H_7$), 110.0 ($C(5)_{thy}$), 138.7 ($C(6)_{thy}$), 153.1 ($C(2)_{thy}$), 163.5 ($C(4)_{thy}$).

8,14,23,26,27-Pentamethyl-1,6,8,14,16,21-hexaazatetracyclo-[19,3,1,1^{6,10},1^{12,16}]heptacosa-10(27),12(26),23(24)-triene-7,9,13,15,22,25-hexaone (**3**), 1,3-bis[4-(3,6-dimethyluracil-1-yl)butyl-1-]thymine (**4**), 1,3-dibutylthymine (**5**) were prepared by known procedures.[7b]

5.1.3. 8,14,23,26,27-Pentamethyl-1,6,8,14,16,21-hexaazatetracyclo-[19.3.1.1^{6,10}.1^{12,16}]heptacosa-10(26),12(27),23(24)-triene-7,9,13,15,22,25-hexaone (3). HRMS found *m/z* 526.2530 $C_{26}H_{34}N_6O_6$ required 526.2540; MS (EI) *m/z* 527 $(M+1)^+$ (23), 526 $(M)^+$ (78), 511 $(M-15)^+$ (100), 373 (38), 333 (18), 292 (37), 235 (17), 206 (31), 193 (48), 181 (63), 166 (46), 153 (52), 141 (21), 127 (22), 122 (19). ^{1}H NMR[¶] (600.0 MHz, $CDCl_3$, 303 K) δ_H (ppm) 1.49–1.57 (4H, m, $N(1)_{thy}(CH_2)_2CH_2CH_2$; $N(3)_{thy}(CH_2)_2CH_2CH_2$), 1.62–1.69 (4H, m, $N(1)_{thy}CH_2CH_2(CH_2)_2$; $N(1)_{thy}CH_2CH_2(CH_2)_2$), 1.92 (3H, s, $C(6)_{ur1}CH_3$), 2.06 (3H, s, $C(5)_{thy}$Me), 2.11 (3H, s, $C(6)_{ur2}CH_3$) 3.39 (3H, s, $N(3)_{ur1}CH_3$), 3.40 (3H, s, $N(3)_{ur2}CH_3$), 3.75 (2H, m, $N(1)_{thy}CH_2(CH_2)$), 3.87 (2H, s, $C(5)_{ur1}CH_2C(5)ur_2$), 3.89–4.01 (6H, m, $N(3)_{thy}CH_2(CH_2)_3$; $N(1)_{thy}(CH_2)_3CH_2$; $N(3)_{thy}(CH_2)_3CH_2$), 6.91 (1H, s, $C(6)_{thy}$H); ^{13}C NMR (150.9 MHz, $CDCl_3$, 303 K) δ_C (ppm) 13.0 ($C(5)_{thy}$*Me*), 16.5 ($C(6)_{ur1}CH_3$), 16.7 ($C(6)_{ur2}CH_3$), 22.1 ($C(5)_{ur1}CH_2C(5)_{ur2}$), 23.2 ($N(1)_{thy}CH_2CH_2C_2H_4$), 23.7 ($N(3)_{thy}CH_2CH_2C_2H_4$), 27.1 ($N(1)_{thy}(CH_2)_2CH_2CH_2$), 27.8 ($N(3)_{thy}(CH_2)_2CH_2CH_2$), 28.0 ($N(3)_{ur1}CH_3$), 28.5 ($N(3)_{ur2}CH_3$), 40.7 ($N(3)_{thy}CH_2(CH_2)_3$), 44.6 ($N(1)_{thy}(CH_2)_3CH_2$), 44.9 ($N(3)_{thy}(CH_2)_3CH_2$), 48.9 ($N(1)_{thy}CH_2(CH_2)_3$), 109.6 ($C(5)_{thy}$), 110.5 ($C(5)_{ur1}$), 111.0 ($C(5)_{ur2}$), 138.4 ($C(6)_{thy}$), 148.6 ($C(6)_{ur1}$), 149.0 ($C(6)_{ur2}$), 151.2 ($C(2)_{thy}$), 151.8 ($C(2)_{ur1}$), 151.9 ($C(2)_{ur2}$), 162.8 ($C(4)_{ur1}$), 163.0 ($C(4)_{ur2}$), 163.7 ($C(4)_{thy}$).

5.1.4. 1,3-Bis[4-(3,6-dimethyluracil-1-yl)butyl-1-]thymine (4). Found (%): C, 58.41; H, 6.74; N, 16.37.

¶ thy—thymine unit, and ur2—3,6-dimethyluracil attached to N(1)thy and N(3)thy, respectively.

$C_{25}H_{34}N_6O_6$ required (%): C, 58.35; H, 6.66; N, 16.33. ^{1}H NMR[‖] (600.0 MHz, $CDCl_3$, 303 K) δ_H (ppm) 1.66–1.76 (8H, m, $N(1)_{thy}CH_2(CH_2)_2CH_2$; $N(3)_{thy}CH_2(CH_2)_2CH_2$), 2.26 (6H, s, $C(6)_{ur}CH_3$), 3.30 (6H, s, $2N(3)_{ur}CH_3$), 3.33 (3H, s, $C(5)_{thy}Me$), 3.84 (4H, t, J=7 Hz, $N(1)_{thy}(CH_2)_3CH_2$; $N(3)_{thy}(CH_2)_3CH_2$), 3.92 (4H, t, J=7 Hz, $N(1)_{thy}CH_2(CH_2)_3$; $N(3)_{thy}CH_2(CH_2)_3$), 5.59 (2H, s, 2 $C(5)_{ur}H$), 7.06 (1H, s, $C(6)_{thy}H$); ^{13}C NMR (150.9 MHz, $CDCl_3$, 303 K) δ_C (ppm) 13.0 ($C(5)_{thy}Me$), 19.7 ($C(6)_{ur}CH_3$), 25.0 ($N(1)_{thy}CH_2CH_2C_2H_4$), 25.8 ($N(3)_{thy}CH_2CH_2C_2H_4$), 26.1 ($N(1)_{thy}(CH_2)_2CH_2CH_2$), 26.3 ($N(3)_{thy}(CH_2)_2CH_2CH_2$), 27.8 ($N(3)_{ur}CH_3$), 40.7 ($N(3)_{thy}CH_2(CH_2)_3$), 44.3 ($N(1)_{thy}(CH_2)_3CH_2$), 44.9 ($N(3)_{thy}(CH_2)_3CH_2$), 48.7 ($N(1)_{thy}CH_2(CH_2)_3$), 101.6 ($C(5)_{ur}$), 110.0 ($C(5)_{thy}$), 138.5 ($C(6)_{thy}$), 151.0 ($C(6)_{ur}$), 151.3 ($C(2)_{thy}$), 152.2 ($C(2)_{ur}$), 162.3 ($C(4)_{ur}$), 163.7 ($C(4)_{thy}$).

5.1.5. 1,3-Dibutylthymine (5). Found (%): C, 65.44; H, 9.37; N, 11.85. $C_{13}H_{22}N_2O_2$ required (%): C, 65.51; H, 9.30; N, 11.75. ^{1}H NMR (600.0 MHz, $CDCl_3$, 303 K) δ_H (ppm) 0.87–0.94 (6H, m, $N(1)_{thy}(CH_2)_3CH_3$; $N(3)_{thy}(CH_2)_3CH_3$), 1.28–1.36 (4H, m, $N(1)_{thy}(CH_2)_2CH_2CH_3$; $N(3)_{thy}(CH_2)_2CH_2CH_3$), 1.56 (2H, m, J=7.8 Hz, $N(1)_{thy}CH_2CH_2C_2H_5$), 1.62 (2H, m, J=7.8 Hz, $N(3)_{thy}CH_2CH_2C_2H_5$), 1.88 (3H, s, $C(5)_{thy}Me$), 3.66 (2H, t, J=7.3 Hz, $N(1)_{thy}CH_2(CH_2)_2CH_3$), 3.9 (2H, t, J=7.3 Hz, $N(3)_{thy}CH_2(CH_2)_2CH_3$), 6.92 (1H, s, $C(6)_{thy}H$); ^{13}C NMR (150.9 MHz, $CDCl_3$, 303 K) δ_C (ppm) 13.0 ($C(5)_{thy}Me$), 13.7 ($N(1)_{thy}(CH_2)_3CH_3$), 13.8 ($N(3)_{thy}(CH_2)_3CH_3$), 19.8 ($N(1)_{thy}(CH_2)_2CH_2CH_3$), 20.3 ($N(3)_{thy}(CH_2)_2CH_2CH_3$), 29.7 ($N(3)_{thy}CH_2CH_2C_2H_5$), 31.2 ($N(1)_{thy}CH_2CH_2C_2H_5$), 41.3 ($N(3)_{thy}(CH_2)_3CH_3$), 49.2 ($N(1)_{thy}(CH_2)_3CH_3$), 109.6 ($C(5)_{thy}$), 138.3 ($C(6)_{thy}$), 151.4 ($C(2)_{thy}$), 163.8 ($C(4)_{thy}$).

5.1.6. 1,3-Bis(5-bromopentyl)thymine (7). A solution of 1,5-dibromopentane (155.9 g, 677.8 mmol) in DMF (90 mL) was added dropwise with stirring to a suspension of 14.4 g (84.7 mmol) of disodium salt of thymine in DMF (150 mL). The mixture was stirred for 5 h at 50–60 °C, after which it was evaporated in a vacuum and the residue was treated with 150 mL of $CHCl_3$. The precipitate that formed was filtered off. The solution was concentrated and submitted to chromatography over Al_2O_3. The column was successively washed with petroleum ether and a 2:1 ether/petroleum ether mixture. From ether/petroleum ether mixture fractions compound **6** was obtained as oil in a yield of 15.9 g (45%): MS (EI) m/z 426 (M+, 9), 424 (M+, 22), 422 (M+, 10), 346 (28), 345 (88), 344 (28), 343 (88), 275 (75), 195 (86), 140 (100). Anal. Calcd for $C_{15}H_{24}Br_2N_2O_2$: C, 42.47; H, 5.70; N, 6.60; Br, 37.67. Found: C, 42.48; H, 5.81; N, 6.53; Br, 37.75. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.01 (s, 1H), 3.95 (2H, t, J=7 Hz), 3.73 (2H, t, J=7 Hz), 3.42 (4H, m), 1.93 (3H, s), 1.89 (4H, m), 1.76–1.58 (4H, m), 1.50 (4H, m).

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[‖] thy—thymine unit, ur—two 3,6-dimethyluracil units.

Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.04.064

References and notes

1. (a) Saenger, W. *Principles of Nucleic Acid Structures*; Springer: New York, NY, 1984; (b) Chen, Sh.; Zhang, Q.; Wu, Xu; Schultz, P. G.; Ding, Sh. *J. Am. Chem. Soc.* **2004**, *126*, 410–411; (c) Chifotides, H. T.; Koshlap, K. M.; Perez, L. M.; Dunbar, K. R. *J. Am. Chem. Soc.* **2003**, *125*, 10703–10713.
2. (a) Corbin, P. S.; Zimmerman, S. C.; Thiessen, P. A.; Hawryluk, N. A.; Murray, T. J. *J. Am. Chem. Soc.* **2001**, *123*, 10475–10488; (b) Giedroc, D. P.; Cornish, P. V.; Hennig, M. *J. Am. Chem. Soc.* **2003**, *125*, 4676–4677; (c) Chifotides, H. T.; Koshlap, K. M.; Perez, L. M.; Dunbar, K. R. *J. Am. Chem. Soc.* **2003**, *125*, 10714–10724; (d) Sontjens, Serge H. M.; van Genderen, Marcel H. P.; Sijbesma, Rint P. *J. Org. Chem.* **2003**, *68*, 9070–9075; (e) Janke, E. M. Basilio; Limbach, H.-H.; Weisz, K. *J. Am. Chem. Soc.* **2004**, *126*, 2135–2141; (f) Lai, J. S.; Kool, E. T. *J. Am. Chem. Soc.* **2004**, *126*, 3040–3041; (g) Poner, J.; Jureka, P.; Hobza, P. *J. Am. Chem. Soc.* **2004**, *126*, 10142–10151; (h) Grunenberg, J. *J. Am. Chem. Soc.* **2004**, *126*, 16310–16311; (i) Swart, M.; Guerra, C. F.; Bickelhaupt, F. M. *J. Am. Chem. Soc.* **2004**, *126*, 16718–16719; (j) Hobza, P.; Sponer, J.; Czeck. *Chem. Rev.* **1999**, *99*, 3247–3276.
3. (a) Tsuzuki, S.; Honda, K.; Uchimaru, T.; Mikami, M.; Tanabe, K. *J. Am. Chem. Soc.* **2002**, *124*, 104–112; (b) Ribas, J.; Cubero, E.; Luque, F. J.; Orozco, M. *J. Org. Chem.* **2002**, *67*, 7057–7065; (c) Lin, C.-H.; Tour, J. *J. Org. Chem.* **2002**, *67*, 7761–7768; (d) Huang, N.; MacKerell, A. D., Jr.. *J. Phys. Chem. A* **2002**, *106*, 7820–7827; (e) Rozas, I.; Alkorta, I.; Elguero, J. *J. Am. Chem. Soc.* **2000**, *122*, 11154–11161; (f) Tatko, Ch. D.; Waters, M. L. *J. Am. Chem. Soc.* **2004**, *126*, 2028–2034; (g) Yohannan, S.; Faham, S.; Yang, D.; Grosfeld, D.; Chamberlain, A. K.; Bowie, J. U. *J. Am. Chem. Soc.* **2004**, *126*, 2284–2285; (h) Alonso, J. L.; Antolinez, S.; Blanco, S.; Lesarri, A.; Lopez, J. C.; Caminati, W. *J. Am. Chem. Soc.* **2004**, *126*, 3244–3249; (i) Arbely, E.; Arkin, I. T. *J. Am. Chem. Soc.* **2004**, *126*, 5362–5363; (j) Sinnokrot, M. O.; Sherrill, C. D. *J. Am. Chem. Soc.* **2004**, *126*, 7690–7697; (k) Gschwind, R. M.; Armbruster, M.; Zubrzycki, I. Z. *J. Am. Chem. Soc.* **2004**, *126*, 10228–10229; (l) Reiter, S. A.; Nogai, S. D.; Karaghiosoff, K.; Schmidbaur, H. *J. Am. Chem. Soc.* **2004**, *126*, 15833–15843.
4. (a) Bickelhaupt, F. M. *Chem.—Eur. J.* **1999**, *5*, 3581–3594; *Angew. Chem.* **1999**, *111*, 3120–3122; *J. Am. Chem. Soc.* **2000**, *122*, 4117–4128; (b) Rozas, I.; Alkorta, I.; Elguero, J. *J. Phys. Chem. A* **2001**, *105*, 10462–10467; (c) Chen, D.; Meena; Sharma, S. K.; McLaughlin, L. W. *J. Am. Chem. Soc.* **2004**, *126*, 70–71; (d) Wesolowski, T. A. *J. Am. Chem. Soc.* **2004**, *126*, 11444–11445; (e) Sessler, J. L.; Jayawickramarajah, J.; Sherman, C. L.; Brodbelt, J. S. *J. Am. Chem. Soc.* **2004**, *126*, 11460–11461.
5. (a) Kool, E. T. *J. Org. Chem.* **2002**, *67*, 5869–5875; (b) Blas, J. R.; Luque, F. J.; Orozco, M. *J. Am. Chem. Soc.* **2004**, *126*, 154–164; (c) Liu, H.; Gao, J.; Maynard, L.; Saito, Y. D.; Kool, E. T. *J. Am. Chem. Soc.* **2004**, *126*, 1102–1109; (d) Liu, H.; Lynch, S. R.; Kool, E. T. *J. Am. Chem. Soc.* **2004**, *126*, 6900–6905; (e) Polak, M.; Seley, K. L.; Plavec, J. *J. Am. Chem. Soc.* **2004**, *126*, 8159–8166; (f) Moody, E. M.;

Bevilacqua, P. C. *J. Am. Chem. Soc.* **2004**, *126*, 9570–9577; (g) Scott, L. G.; Geierstanger, B. H.; Williamson, J. R.; Hennig, M. *J. Am. Chem. Soc.* **2004**, *126*, 11776–11777; (h) Pattanayek, R.; Sethaphong, L.; Pan, C.; Prhavc, M.; Prakash, Th. P.; Manoharan, M.; Egli, M. *J. Am. Chem. Soc.* **2004**, *126*, 15006–15007; (i) Purwanto, M. G. M.; Weisz, K. *J. Org. Chem.* **2004**, *69*, 195–197.

6. (a) Itahara, T. *J. Chem. Soc., Perkin Trans. 2* **1996**, 2695–2700; *Bull. Chem. Soc. Jpn.* **2002**, *75*, 285–290; (b) Itahara, T. *Bull. Chem. Soc. Jpn.* **1996**, *69*, 3239–3246; (c) Paquette, L. A.; Fabris, F.; Gallou, F.; Dong, S. *J. Org. Chem.* **2003**, *68*, 8625–8634.
7. (a) Seyama, F.; Akahori, K.; Sakata, Y.; Aida, M.; Nagata, C. *J. Am. Chem. Soc.* **1988**, *110*, 2192–2201; (b) Semenov, V. E.; Akamsin, V. D.; Reznik, V. S.; Chernova, A. V.; Dorozhkina, G. M.; Efremov, Yu. Ya.; Nafikova, A. A. *Tetrahedron Lett.* **2002**, *43*, 9683; (c) Biot, Ch.; Wintjens, R.; Rooman, M. *J. Am. Chem. Soc.* **2004**, *126*, 6220–6221.
8. (a) *Peptide Pharmaceuticals: Approaches to the Design of Novel Drugs*; Ward, David J., Ed.; Open University Press: Buckingham, 1991; (b) *Computer-aided Drug Design: Methods and Applications*; Perun, Thomas J., Propst, C. L., Eds.; Marcel Dekker: New York, NY, 1989; (c) Kessler, H.; Bats, J. W.; Griesinger, Ch.; Koll, S.; Will, M.; Wagner, K. *J. Am. Chem. Soc.* **1988**, *110*, 1033–1049; (d) Sun, H.; Oldfield, E. *J. Am. Chem. Soc.* **2004**, *126*, 4726–4734; (e) Ernst, M.; Meier, M. A.; Tuherm, T.; Samoson, A.; Meier, B. H. *J. Am. Chem. Soc.* **2004**, *126*, 4764–4765; (f) Lichtenecker, R.; Ludwiczek, M. L.; Schmid, W.; Konrat, R. *J. Am. Chem. Soc.* **2004**, *126*, 5348–5349; (g) Peti, W.; Norcross, J.; Eldridge, G.; O'Neil-Johnson, M. *J. Am. Chem. Soc.* **2004**, *126*, 5873–5878; (h) Ding, K.; Gronenborn, A. M. *J. Am. Chem. Soc.* **2004**, *126*, 6232–6233; (i) Miclet, E.; Williams, D. C., Jr.; Clore, G. M.; Bryce, D. L.; Boisbouvier, J.; Bax, Ad. *J. Am. Chem. Soc.* **2004**, *126*, 10560–10570.
9. Mikhailov, A. S.; Giniyatullin, R. Kh.; Semenov, V. E.; Reznik, V. S.; Nafikova, A. A.; Latypov, Sh. K.; Efremov, Y. Y.; Sharafutdinova, D. R. *Russ. Chem. Bull.* **2003**, *52*, 1399–1402.
10. (a) Avram, L.; Cohen, Y. *J. Org. Chem.* **2002**, *67*, 2639–2644; (b) Tsou, L. K.; Tatko, Ch. D.; Waters, M. L. *J. Am. Chem. Soc.* **2002**, *124*, 14917–14921; (c) Picazo, O.; Alkorta, I.; Elguero, J. *J. Org. Chem.* **2003**, *68*, 7485–7489; (d) Holschbach, M. H.; Sanz, D.; Claramunt, R. M.; Infantes, L.; Motherwell, S.; Raithby, P. R.; Jimeno, M.-L.; Herrero, D.; Alkorta, I.; Jagerovic, N.; Elguero, J. *J. Org. Chem.* **2003**, *68*, 8831–8837; (e) Demeter, A.; Weber, C.; Brlik, J. *J. Am. Chem. Soc.* **2003**, *125*, 2535–2540; (f) Gorb, L.; Podolyan, Y.; Dziekonski, P.; Sokalski, W. A.; Leszczynski, J. *J. Am. Chem. Soc.* **2004**, *126*, 10119–10129; (g) Blancafort, L.; Bertran, J.; Sodupe, M. *J. Am. Chem. Soc.* **2004**, *126*, 12770–12771; (h) Wieczorek, R.; Dannenberg, J. J. *J. Am. Chem. Soc.* **2004**, *126*, 12278–12279.
11. (a) Zhao, D.; Moore, J. S. *J. Org. Chem.* **2002**, *67*, 3548–3554; (b) Li, X.; Sevilla, M. D.; Sanche, Leon *J. Am. Chem. Soc.* **2003**, *125*, 8916–8920; (c) Alkorta, I.; Elguero, J. *J. Org. Chem.* **2002**, *67*, 1515–1519; (d) Varnai, P.; Canalia, M.; Leroy, J.-L. *J. Am. Chem. Soc.* **2004**, *126*, 14659–14667.
12. Cram, D. J.; Steinberg, H. *J. Am. Chem. Soc.* **1951**, *73*, 5691–5704.
13. (a) Derome, A. E. *Modern NMR Techniques for Chemistry Research*; Pergamon: Oxford, 1988; (b) Breitmaier, E.; Voelter, W. *Carbon-13 NMR Spectroscopy: High Resolution Methods and Applications in Organic Chemistry and Biochemistry*; VCH: Weinheim, 1987; (c) Gunther, H. *NMR Spectroscopy: An Introduction*; Wiley: New York, NY, 1987; (d) Clore, G. M.; Kuszewski, J. *J. Am. Chem. Soc.* **2003**, *125*, 1518–1525.
14. (a) Katz, L.; Pelman, S. *J. Mol. Biol.* **1966**, *15*, 220–231; (b) Newmark, R. A.; Cantor, C. R. *J. Am. Chem. Soc.* **1968**, *90*, 5010–5017; (c) Lancelot, G.; Helen, C. *Nucleic Acids Res.* **1979**, *6*, 1063–1072.
15. © 1985–2000, CambridgeSoft.com.
16. (a) Cheeseman, J. R.; Trucks, G. W.; Keith, T. A.; Frisch, J. *J. Chem. Phys.* **1996**, *104*, 5497–5509; (b) Forsyth, D. A.; Sebag, A. B. *J. Am. Chem. Soc.* **1997**, *119*, 9483–9494; (c) Barfield, M.; Fagerness, P. *J. Am. Chem. Soc.* **1997**, *119*, 8699–8711; (d) Kolehmainen, E.; Koivisto, J.; Nikiforov, V.; Perakyla, M.; Tuppurainen, K.; Laihia, K.; Kauppinen, R.; Miltsov, S.; Karavan, V. *Magn. Reson. Chem.* **1999**, *37*, 743–747; (e) Rossi, P.; Harbison, G. S. *J. Magn. Reson.* **2001**, *151*, 1–9; (f) Gomila, R. M.; Quinonero, D.; Rotger, C.; Garau, C.; Frontera, A.; Ballester, P.; Costa, A.; Deya, P. M. *Org. Lett.* **2002**, *4*, 399–401; (g) Alkorta, I.; Elguero, J. *New J. Chem.* **1998**, 381–385; (h) Alkorta, I.; Elguero, J.; Fruchier, A.; Jagerovic, N.; Yap, G. P. A. *J. Mol. Struct.* **2004**, *689*, 251–254; (i) Alkorta, I.; Elguero, J. *Magn. Reson. Chem.* **2004**, *42*, 955–961; (j) Balandina, A. A.; Kalinin, A. A.; Mamedov, V. A.; Figadere, B.; Latypov, Sh. K. *Magn. Reson. Chem.* **2005**, *43*, 816–828.
17. (a) Vargas, R.; Garza, J.; Dixon, D. A.; Hay, B. P. *J. Am. Chem. Soc.* **2000**, *122*, 4750–4755; (b) Jeffrey, G. A.; Saenger, W. *Hydrogen Bonding in Biological Structures*; Springer: Berlin, Germany, 1991; (c) Cannizzaro, C. E.; Houk, K. N. *J. Am. Chem. Soc.* **2002**, *124*, 7163–7169; (d) Scheiner, S.; Kar, T.; Pattanayak, J. *J. Am. Chem. Soc.* **2002**, *124*, 13257–13264; (e) Latypov, Sh. K.; Fakhfakh, M. A.; Jullian, J.-Ch.; Franck, X.; Hocquemiller, R.; Figadère, B. *Bull. Chem. Soc. Jpn.* **2005**, *78*, 1296–1301.
18. (a) Chan, S. I.; Schweitzer, M. P.; Ts'O, P. O. P.; Helkamp, J. K. *J. Am. Chem. Soc.* **1964**, *86*, 4182; (b) Chan, S. I.; Schweitzer, M. P.; Ts'O, P. O. P. *J. Am. Chem. Soc.* **1965**, *87*, 5241–5247; (c) Iwahashi, H.; Kyogoku, Y. *J. Am. Chem. Soc.* **1977**, *99*, 7761–7765; (d) Ts'O, P. O. P. *Basic Principles in Nucleic Acid Chemistry*; Academic: New York, NY, 1974; (e) Turner, D. H.; Sugimoto, N.; Kierzek, R.; Dreiker, S. D. *J. Am. Chem. Soc.* **1987**, *109*, 3783–3785.
19. (a) Antalek, B. *Concepts Magn. Reson.* **2002**, *14*, 225–258; (b) Callaghan, P. T. *Principles of Nuclear Magnetic Resonance Microscopy*; Clarendon: Oxford, 1991; (c) Stilbs, P. *Prog. Nucl. Magn. Reson. Spectrosc.* **1987**, *19*, 1–45; (d) Price, W. S. *Concepts Magn. Reson.* **1997**, *9*, 299–336; (e) Cabrita, E. G.; Berger, S. *Magn. Reson. Chem.* **2001**, *39*, 142–148; (f) Cabrita, E. G.; Berger, S.; Brauer, P.; Karger, G. *J. Magn. Reson.* **2002**, *157*, 124–131.
20. (a) Kayser, V.; Turton, D. A.; Aggeli, A.; Beevers, A.; Reid, G. D.; Beddard, G. S. *J. Am. Chem. Soc.* **2004**, *126*, 336–343; (b) Miranda, C.; Escarti, F.; Lamarque, L.; Yunta, M. J. R.; Navarro, P.; Garcia-Espana, E.; Jimeno, M. L. *J. Am. Chem. Soc.* **2004**, *126*, 823–833; (c) Kwon, Ji Y.; Singh, N. J.; Kim, Ha Na; Kim, S. K.; Kim, K. S.; Yoon, J. *J. Am. Chem. Soc.* **2004**, *126*, 8892–8893; (d) Lu, J. R.; Perumal, Sh.; Hopkinson, I.; Webster, J. R. P.; Penfold, J.; Hwang, W.; Zhang, Sh. *J. Am. Chem. Soc.* **2004**, *126*, 8940–8947.

21. Stott, K.; Stonehouse, J.; Keeler, J.; Hwang, T. L.; Shaka, A. J. *J. Am. Chem. Soc.* **1995**, *117*, 4199–4200.
22. (a) Allinger, N. L. *J. Am. Chem. Soc.* **1977**, *99*, 8127–8134; (b) Allinger, N. L.; Kok, R. A.; Imam, M. R. *J. Comput. Chem.* **1988**, *9*, 591–595; (c) Lii, J.-H.; Gallion, S.; Bender, C.; Wikstrom, H.; Allinger, N. L.; Flurchick, K. M.; Teeter, M. M. *J. Comput. Chem.* **1989**, *10*, 503–513.
23. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A., Jr.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Baboul, A. G.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B. G.; Chen, W.; Wong, M. W.; Andres, J. L.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98, Revision A.6*; Gaussian: Pittsburgh, PA, 1998.