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Intercalators. 1. Nature of Stacking Interactions between Intercalators (Ethidium, Daunomycin, Ellipticine, and 4′,6-Diaminide-2-phenylindole) and DNA Base Pairs. *Ab Initio* Quantum Chemical, Density Functional Theory, and Empirical Potential Study

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Abstract: Properties of isolated intercalators (ethidium (E), daunomycin (D), ellipticine (EL), and 4,6′-diaminide-2-phenylindole (DAPI)) and their stacking interactions with adenine···thymine (AT) and guanine···cytosine (GC) nucleic acid base pairs were investigated by means of a nonempirical correlated *ab initio* method. All intercalators exhibit large charge delocalization, and none of them (including the DAPI dication) exhibits a site with dominant charge. All intercalators have large polarizability and are good electron acceptors, while base pairs are good electron donors. MP2/6-31G*(0.25) stabilization energies of intercalator···base pair complexes are large (E···AT, 22.4 kcal/mol; D···GC, 17.8 kcal/mol; EL···GC, 18.2 kcal/mol; DAPI···GC, 21.1 kcal/mol) and are well reproduced by modified AMBER potential (van der Waals radii of intercalator atoms are enlarged and their energy depths are increased). Standard AMBER potential underestimates binding, especially for DAPI-containing complexes. Because the DAPI dication is the best electron acceptor (among all intercalators studied), this difference is explained by the importance of the charge-transfer term, which is not included in the AMBER potential. For the neutral EL molecule, the standard AMBER force field provides correct results. The Hartree−Fock and DFT/B3LYP methods, not covering the dispersion energy, fail completely to reveal any energy minimum at the potential energy curve of the E···AT complex, and these methods thus cannot be recommended for a study of intercalation process. On the other hand, an approximate version of the DFT method, which was extended to cover London dispersion energy, yields for all complexes very good stabilization energies that are well comparable with referenced *ab initio* data. Besides the vertical dependence of the interaction, an energy twist dependence of the interaction energy was also investigated by a reference correlated *ab initio* method and empirical potentials. It is concluded that, despite the cationic (E +1, D +1, DAPI +2) or polar (EL) character of the intercalators investigated, it is the dispersion energy which predominantly contributes to the stability of intercalator···DNA base pair complexes. Any procedure which does not cover dispersion energy is thus not suitable for studying the process of intercalation.

1. Introduction

Small molecules bind to DNA through several mechanisms: (i) minor groove binding; (ii) major groove binding; (iii) intercalation; and (iv) other types of binding. The ability of planar polycyclic aromatic molecules to intercalate, i.e., to be inserted between two consecutive base pairs of DNA, is of special importance since many intercalators are active in antitumor chemotherapy.[1]

The strength of binding usually correlates with the molecule's biological activity, and several energy contributions may be responsible for the binding. All intercalators bind to DNA by noncovalent stacking with nucleic acid base pairs, often combined with H-bonding and even covalent binding involving the drug side chains. Because the majority of intercalators are highly polar or even charged systems, it is believed that electrostatic energy plays a dominant role in the intercalation process, at least in sequence preferences and drug positioning.[2−6]

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Aromatic polycyclic systems have, however, large polarizability; therefore, dispersion energy must be one of the major stabilizing components. Chemical experience tells us that, in the case of polar (or even charged) systems, the polarization (induction) energy might be important. Further, if one of the interacting systems is a good electron donor and the other one an electron acceptor, then the electron donor−electron acceptor (charge-transfer) contribution plays a role. While the electrostatic and dispersion components of stacking are well described by presently available force fields,[7,8] the polarization and charge-transfer effects are ignored by current molecular modeling tools.

Because the role of individual energy contributions for the intercalation process is not known, it would be desirable to investigate the process by means of a method which includes all interaction energy terms. This task can be achieved only by *ab initio* quantum chemical (QM) calculations with the inclusion of electron correlation effects. QM approaches such as semi-empirical quantum chemical methods, the Hartree−Fock method, and presently available DFT methods are not suitable for studies of stacking complexes, as discussed in detail elsewhere.[9] Empirical potentials include only some of the energy components mentioned, and thus it is not clear how accurately they describe the energetics of intercalation.

High-level calculations are prohibitively expensive for large complexes, and there is so far only a single paper reporting high-level QM calculations of stacking energies between nucleobases and intercalator. Bondarev and co-workers thoroughly analyzed the stacking of a single DNA base with a small monocation intercalator amiloride using the second-order Moeller−Plesset (MP2) method with a 6-311++G** basis set, and they compared their data with those obtained with the AMBER force field.[10] The authors also studied a larger cluster (amiloride···base pair) using a pair-additive empirical potential (AMBER). Their study suggests that the ligand−nucleobase binding is controlled by dispersion energy while, in optimal geometries, about a third of the stabilization is due to the Coulombic term. Rather surprisingly, they did not notice any substantial effect of induction and/or charge transfer, in contrast to our preceding study of stacking in protonated nucleobase dimers.[11] The study further indicates an excellent correlation between AMBER and MP2 data, similar to our preceding studies of base stacking.[7−9,11,24] However, the AMBER force field slightly overestimates the MP2 amiloride−base binding energies, contrasting our data for protonated stacked nucleobase dimers.[11] This is probably due to the basis set used by Bondarev *et al*. They combined standard d polarization functions with additional diffuse sp shells. Such a basis set still covers a smaller fraction of intermolecular correlation (dispersion) effects compared with basis sets having a diffuse polarization d function, which are critical for proper evaluation of the dispersion energy.[9]

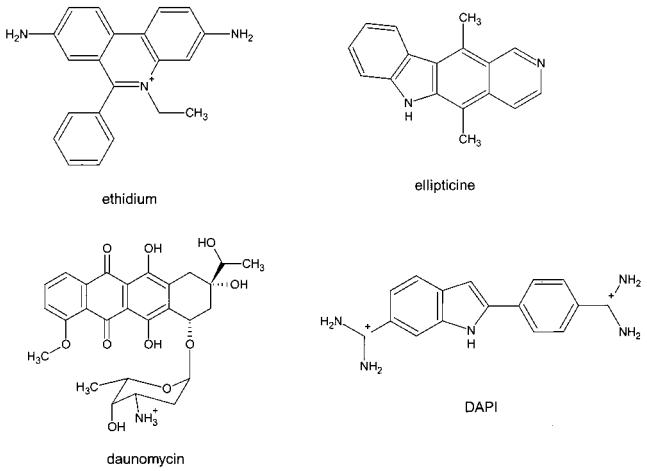
The intercalation process is, however, not governed by interaction energy or enthalpy, but the entropy term should also be considered. Thermodynamic characteristics of the interaction of intercalators with DNA can be evaluated only by using computer experiments, especially using molecular dynamics (MD) simulations. The MD simulations were already used for the description of the intercalation process.[12] Generally, MD (as well as Monte Carlo) simulations can be performed at any theoretical level, including the empirical, semiempirical, or nonempirical methods, yielding energy and forces. We have witnessed enormous progress in recent years in the so-called *ab initio* MD, but if the classical quantum chemical *ab initio* method is adapted, the calculations are limited to small systems only. MD simulations based on DFT gradients can access large systems and time scales;[13,14] however, DFT does not cover the London dispersion energy.[9]

The vast majority of MD simulations are (and will be also in the near future) based on the empirical potentials. The quality of the MD simulations depends critically on the performance of the simulation technique but also on the quality of the empirical potential used. This fact is frequently ignored, and it is often believed that sufficiently long MD simulations always yield reliable results. One of the plausible ways to evaluate the quality of an empirical potential prior to its use in MD simulations is to compare its performance by nonempirical correlated *ab initio* calculations.

The aim of this paper is to investigate properties of series of isolated intercalators and their stacking interactions with base pairs by means of a nonempirical correlated *ab initio* method capable of providing a balanced inclusion of all contributions to the interaction energies. The *ab initio* calculations will be used subsequently for verification/parametrization of cheaper methods suitable for large-scale MD simulations, namely an AMBER type of pair-additive force field, its polarization variant, and an approximate DFT method augmented by a dumped dispersion energy term.

We have considered four intercalators with different charges and electrostatic properties; their Lewis structures are presented in Chart 1. Ethidium is often used as a probe for a study of the

Chart 1



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three-dimensional structure of DNA.[15] Ethidium is also important since it provides a general model for the biological activity of various intercalation agents bound via noncovalent interactions. Daunomycin is an important drug in anticancer therapy. We have also considered two smaller molecules, namely alkaloid ellipticine and the fluorescent dye 4′,6-diaminide-2-phenylindole (DAPI). Although DAPI is a typical minor groove binder, its intercalation under certain circumstances is well established.[16−19]

2. Strategy of Calculations

The structure and properties of isolated intercalators and isolated base pairs will be determined at the *ab initio* level. Complexes between intercalators and base pairs will then be evaluated using correlated *ab initio* calculations. We believe that the smallest model for the intercalation process is represented by this cluster and not, as used in ref 10, just by a base and an intercalator. This is due to the fact that electrostatic and other one-electron properties of base pairs are significantly different from those of a single base. We will present evidence that this model yields complete information and its extension to a base pair···intercalator···base pair model (i.e., the intercalator is placed between two base pairs) does not bring any significant improvement. The correlated *ab initio* calculations will be compared with results obtained by pair-additive empirical potentials with electrostatic potential derived charges. Using the reference *ab initio* data, we will also test the recently introduced approximate DFT method (density functional tight-binding, DFTB),[20a] augmented by the empirical London dispersion energy term[20b] (acronym DFTB-D). Recent calculations performed[20b] with the DFTB-D technique for H-bonded and stacked DNA base pairs have been very promising. Because the technique is computationally very efficient, it can even be used in quantum mechanical (QM) and QM/molecular mechanical (MM) MD simulations. Finally, correlated *ab initio* characteristics will be compared with those evaluated at the Hartree−Fock (HF) and DFT/B3LYP levels. The degree of agreement between correlated *ab initio* data and other methods mentioned will give us important insights into the nature of molecular interactions in the studied complexes and will provide us with an evaluation of the accuracy limits of these methods.

3. Calculations

Geometries. The structure and geometry of ethidium, daunomycin (daunorubicin), ellipticine, and 4′,6-diaminide-2-phenylindole were optimized at the HF level using a 6-31G** basis set. The structure of Watson−Crick base pairs was determined at the HF/6-31G** level with the assumption of their planarity. Structures of ethidium···AT and daunomycin···GC complexes were taken directly from crystal data.[21] In the case of other intercalators, crystal data are not available; therefore, we used idealized geometries prepared in the following way. Intercalators (DAPI, ellipticine) and base pairs (AT and GC) were located in coplanar planes in such a way that the main system axes were parallel. Intersystem separation (vertical), twist angle, and in-plane displacements were optimized (see later). In all cases, QM-optimized geometries of base pairs and intercalators were used for QM calculation. Thus, when utilizing the crystal or idealized geometries, the interacting molecules were *overlaid* by their HF/6-31G**-optimized geometries based on the least-squares fitting method. In the case of empirical potential calculations, either the subsystem geometries were relaxed by the empirical potential or QM-optimized geometries were retained. This difference has a negligible effect on the calculated energies.

Subsystem Properties. Atomic charges of intercalators and base pairs were derived using the restrained electrostatic potential (RESP) fitting procedure[22] at the HF/6-31G* level. This charge parametrization is identical to that used in the Cornell *et al*. force field.[23] The HF/6-31G* procedure overestimates molecular dipole moments; however, this imbalance is considered to be profitable in condensed-phase simulations with Cornell *et al*. force field in a water environment, as it implicitly compensates for the missing polarization effects. For additional calculations, we derived atomic charges of bases and intercalators with the inclusion of electron correlation effects via the second-order Møller−Plesset perturbational method (MP2) with a 6-31G*(0.25) basis set. Here, d polarization functions with exponents $\alpha_d = 0.8$ used in the standard 6-31G* basis set were replaced by more diffuse ones ($\alpha_d = 0.25$). The ESP MP2/6-31G*(0.25) charges are very useful for a comparison between *ab initio* and empirical potential data, as this charge set provides an excellent approximation of electrostatic interaction energy in the MP2/6-31G*(0.25) interaction energy calculations (see below and ref 24).

Other one-electron properties (dipole moment, polarizability, energies of frontier molecular orbitals) were determined at the HF/6-31G** level. For charged species, the dipole moment is derived with respect to their center of mass, because for non-neutral molecules the calculated dipole moment depends on the origin of the coordinate system.

Reference Correlated *ab Initio* Interaction Energies. The reference interaction energies of all complexes were determined at the MP2 level (with frozen core approximation) with the 6-31G basis set augmented by diffuse polarization functions, abbreviated as 6-31G*(0.25). This basis set is well prepared to study stacked clusters. The diffuse d functions qualitatively improve the value of the intersystem correlation (dispersion) energy.[7,9,25] The dispersion energy dominates stabilization of base stacking and is obviously assumed to provide a dominating contribution to stabilization of the intercalators in DNA. Although our basis set is smaller than that used by Bondarev and co-workers,[10] it provides a better description of the dispersion energy, which is due to inclusion of diffuse polarization functions. The basis set superposition error (BSSE) was systematically removed by considering the function counterpoise method.[26] The MP2/6-31G*(0.25) method provides surprisingly good predictions of stacking energies for aromatic systems, partly due to a modest overestimation (with a given basis set) of the MP2 binding energies with respect to CCSD(T) data.[9] The reference *ab initio* method used is expected to cover properly not only the electrostatic, polarization (induction), and dispersion energy components but also the charge-transfer effects.

DFTB-D and DFT/B3LYP Calculations. Stabilization energies of selected complexes were also determined using two density functional techniques. First, DFT calculations were made using a recently introduced method based on a combination of the approximate tight-

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binding DFTB with empirical dispersion energy. DFT methods are known to be inherently very deficient for stacking interactions, as they basically ignore the dispersion attraction.[27] Thus, augmenting them by an empirical dispersion term currently appears to be a very reasonable way to improve the major deficiency of a DFT method for evaluation of molecular complexes. The DFTB-D method is described in our previous paper,[20b] where its ability to describe H-bonding and stacking of nucleic acid base pairs was also demonstrated. The key advantage of the method used is its unprecedented computational efficiency. The single-point calculations for the largest complexes (such as AT···ethidium···TA) in the present study did not exceed dozens of seconds and were done using Pentium III/800 MHz computers. The standard DFT calculations were performed with the Becke3LYP functional[28] utilizing 6-31G* and 6-31++G** basis sets.

Empirical Potential Calculations. Four empirical potential models were used. The first one is the standard Cornell *et al*. force field[23] consisting of a Lennard-Jones van der Waals (vdW) term and a Coulombic term. The missing parameters of the force field for intercalators were obtained from *ab initio* HF/6-31G* calculations: equilibrium bond lengths and angles from optimized geometry and force constants from the Hessian matrix in internal coordinates. The dihedral parameters were determined by fitting to the *ab initio* potential energy surface. The atom-centered point charges were obtained with the RESP HF/6-31G* method. Polarities of the molecules evaluated with the RESP HF/6-31G* charges are ca. 10−20% higher compared with correct values. This is believed to compensate for the missing polarization term and to improve the force field performance in simulations in an explicit inclusion of a water environment. When such charges are used in gas-phase calculations, the electrostatic component of the interaction energy is exaggerated. This potential is denoted as AMB/HF-1 in the following paragraphs.

As the second force field we utilized a polarizable potential denoted as AMB/HF-1/P. It is the AMB/HF-1 force field augmented by an explicit polarization term utilizing point polarizabilities derived by Applequist.[29]

In other calculations, we have used charges derived using MP2/6-31G*(0.25) wave functions, i.e., a level to that used in the reference *ab initio* interaction energy evaluations (denoted as AMB/MP2).[7,24] This charge distribution provides accurate gas-phase polarity of the subsystems, and the empirical electrostatic energy is then in excellent agreement with the corresponding electrostatic interaction energy component in the reference MP2/6-31G*(0.25) calculations.[7,24] This parametrization allows mining for regions of the potential energy surface notably influenced by the induction effects, anisotropic short-range repulsion, and eventually other terms not included in the force field.[30]

Finally, we tested a modified version of the AMB/HF-1 force field denoted as AMB/HF-2. Here the vdW radii of all atoms of the intercalator were enlarged by 10% with a simultaneous increase of their vdW energy well depths by a factor of 2. The purpose of this modification was to test whether one could compensate for the missing induction and charge-transfer attraction through the van der Waals term and how large parameter modifications are to be introduced in order to compensate for the missing terms.

4. Results and Discussion

Isolated Subsystems. Optimized structures and the atom numbering of ethidium, ellipticine, daunomycin, and DAPI are presented in Figure 1. Tables S1−S8 in the Supporting Information summarize those parameters, which differ from the standard Cornell *et al*. parameter set. Ellipticine is a neutral

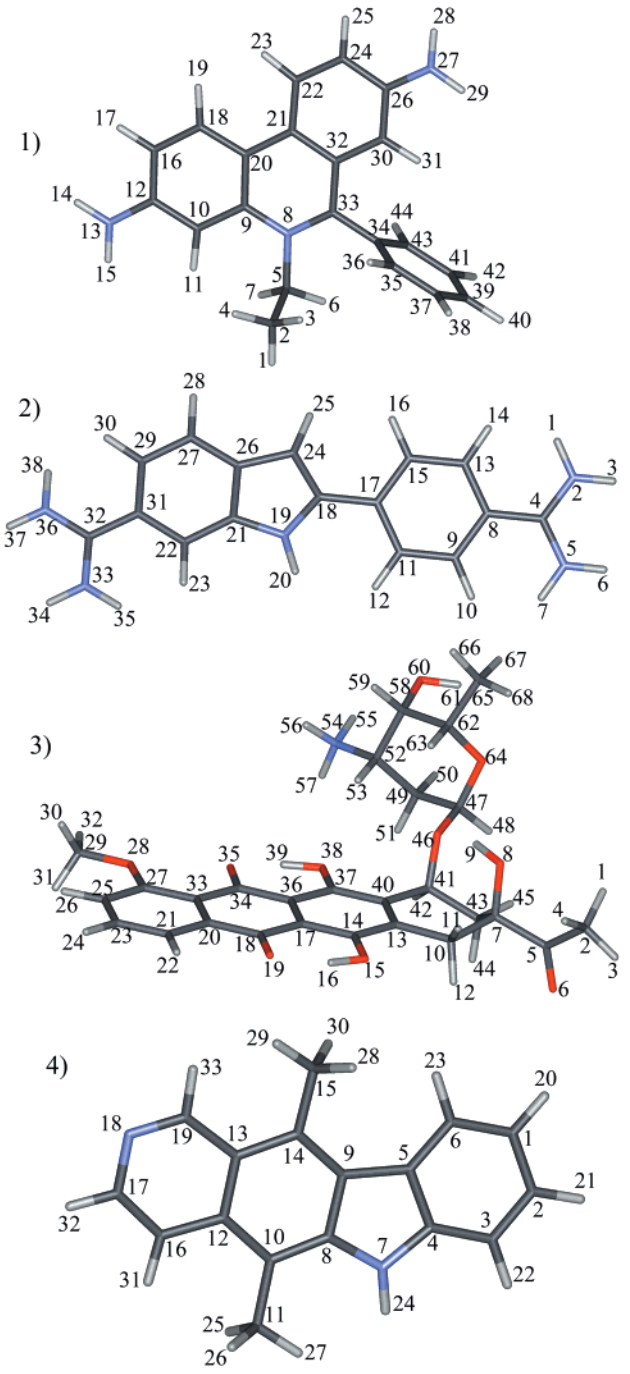

Figure 1. Optimized structures and numbering of ethidium (1), ellipticine (2), daunomycin (3), and DAPI (4).

system, ethidium and daunomycin are monocations, and DAPI is a dication. Their calculated RESP atomic charges (Table 1) nevertheless show significant delocalization of the excessive charge, and even for a DAPI dication there are no sites with a dominant positive charge. This concerns not only the presented RESP charges but also the Mulliken charges (not shown). Structures of optimized adenine···thymine (AT) and guanine···cytosine (GC) base pairs in the Watson−Crick structures are visualized in Figure 2, and Table 2 contains the RESP atomic charges of all four bases. Table 3 presents one-electron properties and energies of frontier molecular orbitals (HOMO and LUMO) of all intercalators, while frontier molecular orbitals of bases and base pairs are collected in Table 4. From Table 3

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Table 1. RESP/HF Atomic Charges of Intercalators

no.[a]	daunomycin	ethidium	DAPI	ellipticine[b]	no.[a]	daunomycin	ethidium	DAPI
1	0.08	0.06	0.40	−0.21 (−0.14)	35	−0.58	−0.12	0.40
2	−0.26	0.02	−0.66	−0.10 (−0.11)	36	−0.14	0.15	−0.66
3	0.09	0.03	0.40	−0.27 (−0.27)	37	0.12	−0.19	0.40
4	0.10	−0.05	0.53	0.18 (0.21)	38	−0.47	0.17	0.40
5	0.55	0.02	−0.66	0.09 (0.26)	39	0.43	−0.05	
6	−0.54	0.06	0.40	−0.16 (−0.27)	40	−0.03	0.15	
7	0.27	0.07	0.40	−0.43 (−0.48)	41	0.01	−0.18	
8	−0.72	0.03	−0.03	0.01 (0.14)	42	0.13	0.17	
9	0.47	−0.01	−0.17	−0.04 (−0.36)	43	−0.05	−0.15	
10	−0.08	−0.18	0.17	0.02 (0.07)	44	0.06	0.16	
11	0.09	0.18	−0.09	−0.14 (−0.46)	45	0.06		
12	0.09	0.24	0.14	0.01 (0.30)	46	−0.31		
13	−0.01	−0.87	−0.15	−0.01 (0.45)	47	0.03		
14	0.32	0.42	0.17	−0.04 (0.42)	48	0.18		
15	−0.58	0.40	−0.11	−0.16 (−0.59)	49	−0.03		
16	0.47	−0.13	0.16	−0.24 (−0.43)	50	0.07		
17	−0.27	0.17	0.08	0.17 (0.33)	51	0.05		
18	0.59	−0.21	0.06	−0.55 (−0.62)	52	0.03		
19	−0.51	0.18	−0.32	0.22 (0.44)	53	0.10		
20	−0.12	0.05	0.35	0.15 (0.13)	54	−0.31		
21	−0.10	0.10	0.09	0.14 (0.12)	55	0.31		
22	0.16	−0.24	−0.19	0.15 (0.15)	56	0.32		
23	−0.18	0.17	0.17	0.15 (0.15)	57	0.31		
24	0.19	−0.06	−0.28	0.38 (0.37)	58	0.09		
25	−0.13	0.17	0.20	0.06 (0.14)	59	0.14		
26	0.14	0.26	0.15	0.06 (0.14)	60	−0.64		
27	0.24	−0.86	−0.20	0.06 (0.11)	61	0.48		
28	−0.25	0.39	0.20	0.07 (0.15)	62	0.05		
29	−0.05	0.40	−0.16	0.07 (0.16)	63	0.10		
30	0.08	−0.28	0.16	0.07 (0.15)	64	−0.31		
31	0.09	0.19	−0.06	0.12 (0.15)	65	−0.23		
32	0.10	0.00	0.53	0.10 (0.04)	66	0.09		
33	−0.11	0.09	−0.66	0.09 (0.01)	67	0.08		
34	0.58	0.11	0.40		68	0.12		

[a] Cf. Figure 1. [b] Numbers in parentheses correspond to RESP/MP2 charges.

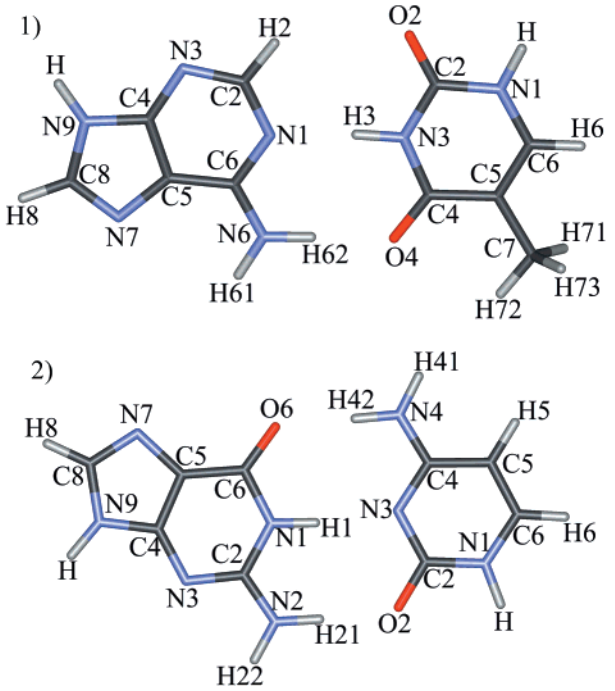

Figure 2. Optimized structures and standard numbering of the adenine···thymine (1) and guanine···cytosine (2) pairs in the Watson−Crick structures.

it follows that daunomycin has the largest polarizability among intercalators, followed by ethidium, DAPI, and ellipticine. Polarizabilities of all intercalators are large, however, which supports the fact that dispersion energy will be always important.

Table 2. Atomic Charges of Nucleic Acid Bases

adenine[a]		thymine[a]		guanine[a]		cytosine[a]	
H	0.36	H	0.32	H	0.36	H	0.31
N9	−0.45	N1	−0.32	N9	−0.39	N1	−0.38
C8	0.15	C6	−0.18	C8	0.15	C6	0.01
H8	0.17	H6	0.21	H8	0.16	H6	0.20
N7	−0.55	C5	0.04	N7	−0.56	C5	−0.46
C5	0.01	C7	−0.36	C5	0.26	H5	0.18
C6	0.68	H71	0.11	C6	0.44	C4	0.79
N6	−0.87	H72	0.11	O6	−0.53	N4	−0.96
H61	0.40	H73	0.11	N1	−0.45	H41	0.43
H62	0.40	C4	0.56	H1	0.35	H42	0.43
N1	−0.75	O4	−0.55	C2	0.60	N3	−0.73
C2	0.54	N3	−0.39	N2	−0.86	C2	0.83
H2	0.07	H3	0.31	H21	0.40	O2	−0.64
N3	−0.76	C2	0.57	H22	0.40		
C4	0.59	O2	−0.57	N3	−0.58		
				C4	0.27		

[a] Cf. Figure 2.

Investigating the energies of frontier MOs, we find that all intercalators are good electron acceptors. The lowest LUMO energy was found for dicationic DAPI, followed by ethidium and daunomycin. Ellipticine have a positive value of LUMO energy. The ability to accept electrons is closely related with molecular charge. Removing the charged alkyl group from ethidium (see Table 3) leads to a dramatic increase in LUMO energy (from −2.0 to +2.4), which means that while cationic ethidium is an electron acceptor, ethidium without a charged alkyl group becomes an electron donor. On the other hand, removing the neutral phenol group leads only to minor changes in the energies of LUMO. The electron-accepting ability of all

Table 3. One-Electron Properties and Energies of Frontier Molecular Orbitals of Intercalators

intercalator	q[a]	HOMO[b]	LUMO[c]	μ[d]	α[e]
ethidium	1	−10.2	−2.0	2.3	235.7
ethidium − $[C_2H_5]^+$ [f]	0	−6.7	2.4	3.4	211.6
ethidium − $[C_6H_5]$ [g]	1	−10.5	−2.3	2.8	177.4
ellipticine	0	−7.1	2.2	3.9	184.4
daunomycin	1	−10.5	−1.5	18.6	297.0
DAPI	2	−13.2	−4.1	5.9	200.6

[a] Total charge of intercalator [|e|]. [b] Energy of HOMO [eV]. [c] Energy of LUMO [eV]. [d] Dipole moment [D]. [e] Polarizability [B^3]. [f] Ethidium without charged ethyl group. [g] Ethidium without phenyl group.

Table 4. Energies (in eV) of Frontiers Molecular Orbitals of Bases, Base Pairs, and Nucleoside Pairs

system	HOMO	LUMO
adenine	−8.4	3.7
thymine	−9.5	3.2
guanine	−8.1	4.1
cytosine	−9.2	3.3
adenine−thymine	−8.2	3.2
guanine−cytosine	−7.5	2.9
adenosine−thymidine	−7.8	2.9
guanosine−cytidine	−7.2	2.6

intercalators becomes evident upon comparison of energies of their frontier MO with energies of frontier MO of bases. From Table 4, it clearly follows that all bases and base pairs are very poor electron acceptors (all LUMO energies are positive, in contrast to LUMO energies of intercalators, which all are negative except for ellipticine). Bases and base pairs are evidently good electron donors, and among isolated bases the best one is guanine. This is a well-known fact which is reflected by the ionization potential of bases.[31−33] The electron donor ability of all bases is further magnified by base pairing and also by the addition of sugar units. For example, the HOMO energy of guanine (−8.1 eV) increases by 0.6 eV upon pairing with cytosine and by another 0.3 eV upon addition of sugar units (cf. Table 4). From the entries of Tables 3 and 4 and the above-mentioned results, it becomes clear that a complex intercalator···base pair will be stabilized, besides electrostatic, dispersion, and induction contributions, also by a charge-transfer contribution. Among intercalators investigated, this contribution will be the most important for DAPI-containing complexes.

Dependence of Intercalator−Base Pair Stacking Interaction Energy on Their Vertical Separation. We have first investigated the dependence of stacking energy on the vertical distance between the interacting systems. Structures investigated—AT with ethidium and DAPI, and GC with daunomycin and ellipticine—are presented in Figures 3−6, and the respective interaction energies obtained by reference MP2/6-31G*(0.25) calculations and the tested methods are presented in Figures 7−10.

Because binding of ethidium to nucleic acids provides a general model for the intercalating process, interaction of ethidium with the AT base pair was investigated more thoroughly and will be discussed first. Figure 7 presents seven stacking energy curves evaluated by changing the separation of the AT base pair and ethidium. The red line 1 refers to reference correlated *ab initio* calculations. The minimum on the

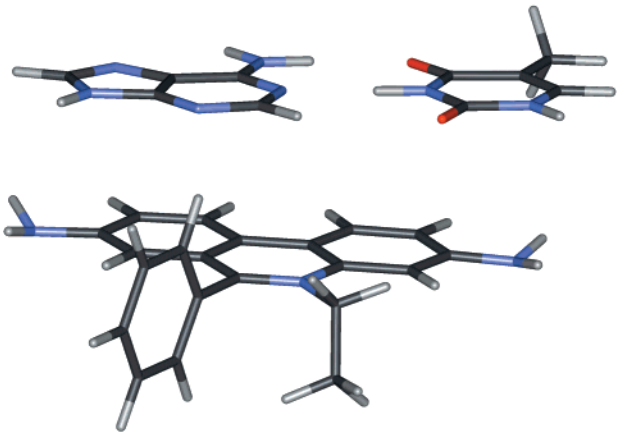

Figure 3. Structure of the adenine—thymine···ethidium.

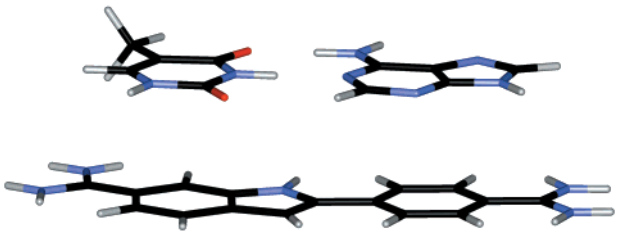

Figure 4. Structure of the adenine—thymine···DAPI.

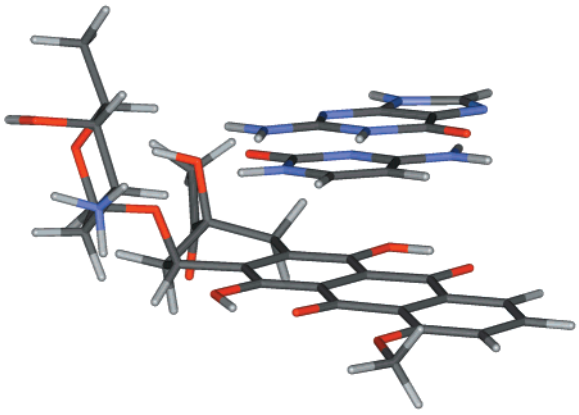

Figure 5. Structure of the guanine—cytosine···daunomycin.

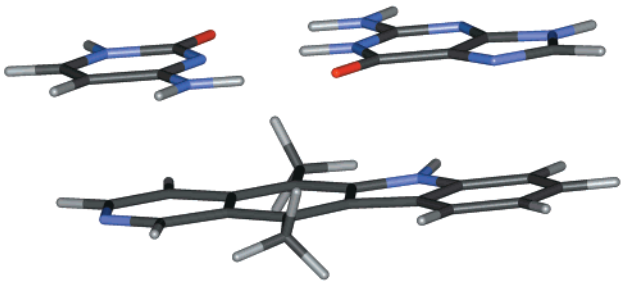

Figure 6. Structure of the guanine—cytosine···ellipticine.

respective potential energy curve was found at around 3.32 Å, which exactly corresponds to the distance from the crystal. The stabilization energy of the ethidium ···AT pair (22.48 kcal/mol; energy necessary to separate ethidium and the AT pair to infinity) is considerably larger than the stacking interaction of nucleic acid bases. Let us recall that the largest and smallest stacking energies (evaluated at the same theoretical level) were found[7] for the guanine dimer and uracil dimer (11.3 and 6.5

(31) Saito, I.; Nakamura, T.; Nakatani, K.; Yoshioka, Y.; Yamaguchi, K.; Sugiyama, H. *J. Am. Chem. Soc.* **1998**, *120*, 12686.

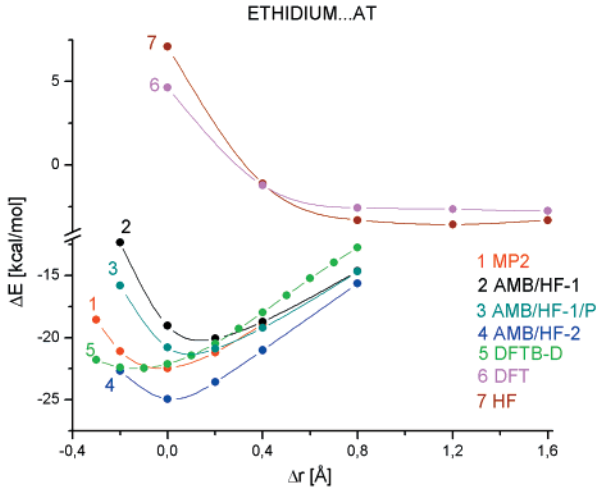

Figure 7. Stabilization energies (Δ*E*) of the adenine—thymine (AT)··· ethidium complex evaluated by correlated *ab initio* MP2 calculations and various lower-level methods as a function of vertical separation between AT and ethidium. Distances are related to the crystal distance of 3.35 Å, which is denoted as 0.0 at the *x*-axis.

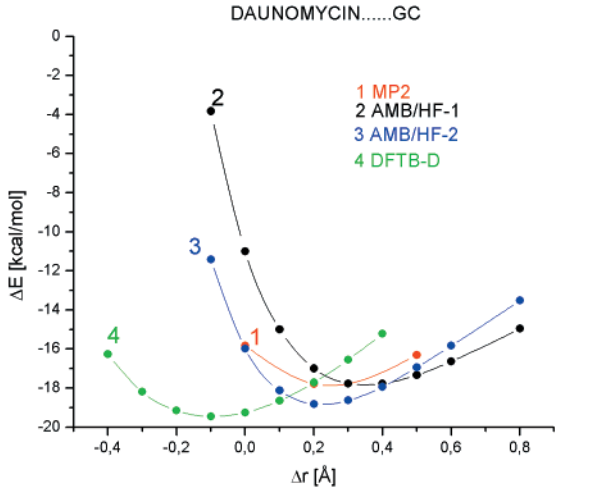

Figure 9. Stabilization energies (Δ*E*) of the guanine—cytosine (GC)··· daunomycin complex evaluated by correlated *ab initio* MP2 calculations and various lower-level methods as a function of vertical separation between GC and daunomycine. Distances are related to the crystal distance of 3.30 Å, which is denoted as 0.0 at the *x*-axis.

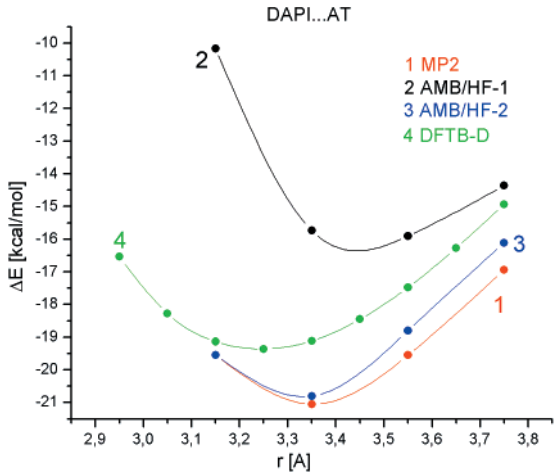

Figure 8. Stabilization energies (Δ*E*) of the adenine—thymine (AT)···DAPI complex evaluated by correlated *ab initio* MP2 calculations and various lower-level methods as a function of vertical separation between GC and planarized DAPI.

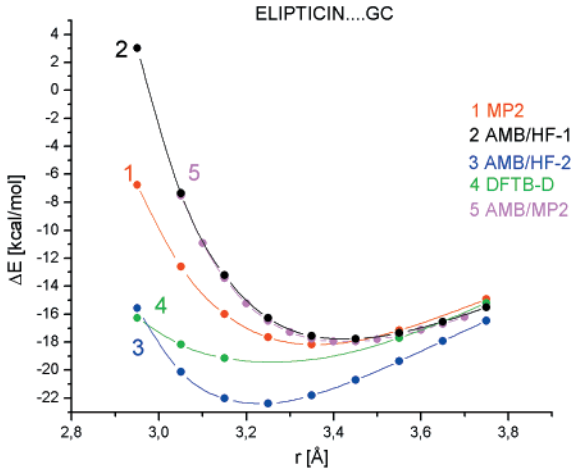

Figure 10. Stabilization energies (Δ*E*) of the guanine—cytosine (GC)··· ellipticine complex evaluated by correlated *ab initio* MP2 calculations and various lower-level methods as a function of vertical separation between GC and ellipticine.

kcal/mol for their optimized geometries), while base pair step stacking in double-helix geometries amount to 9−15 kcal/mol.[24]

Because the studied system consists of three components, we have decomposed interaction energy into three pairwise terms and the three-body term (see ref 34 for definition). The decomposition has been carried out for the optimal separation. The total trimer interaction energy is −34.98 kcal/mol (separation of all three systems to infinity), while the A···T, T···ethidium, and A···ethidium two-body terms are −12.50, −7.84, and −14.93 kcal/mol, respectively. Subtracting all pairwise terms from the total interaction energy, we obtain the three-body term of 0.29 kcal/mol. Small three-body nonadditivity in the present system supports the use of an empirical potential for the study of intercalation, because all presently used empirical potentials are pairwise additive and neglect many-body terms by definition. It must, however, be considered that many-body terms depend on the complex studied. For example, the four-body term in the GC/GC stack is as large as 2−3 kcal/mol, while for other base pair steps the many-body terms are small.[24] In contrast, very large nonadditivities in base pairs can be induced by metal cations.[34]

The HF and DFT/B3LYP curves (6 and 7) are completely away from the MP2 data and practically do not show any energy minimum. This is because these methods do not cover the London dispersion energy, which represents the dominant stabilization energy contribution. Further, the two methods do not cover completely the charge-transfer energy term, which also contributes to the stability of the complex. Increasing the AO basis set size shifts the DFT interaction energy to even more repulsive values. Evidently, HF and standard DFT/B3LYP fail to describe the intercalation process, and their use for these purposes is thus not recommended. Any study of an intercalation process performed with DFT methodology should be thus undertaken with care. The other important consequence is that, despite the highly polar or even charged character of intercalators (as well as the DNA base pair), the dominant stabilization

(32) Prat, F.; Houk, K. N.; Foote, C. S. *J. Am. Chen. Soc.* **1998**, *120*, 845.
(33) Colson, A. O.; Sevilla, M. D. *Int. J. Radiat. Biol.* **1995**, *67*, 627.
(34) Burda, J. V.; Šponer, J.; Leszczynski, J.; Hobza, P. *J. Phys. Chem. B* **1997**, *101*, 9670−9677.

stems from dispersion energy. The use of an electrostatic contribution only (as suggested in ref 12) leads thus to unreliable values. This further underlines the important role of dispersion energy in the biodisciplines, which is sometimes greatly undervalued. This finding is closely related to the similar conclusion we made about the importance of electrostatic and dispersion energy for base stacking.[7,9,24] Also in this case, the dispersion energy was dominant. Our data are also consistent with results by Bondarev and co-workers.[10] Note that the electrostatic origin of aromatic stacking in water was suggested several years ago, based on NMR analysis of stacked linked adenyl groups,[35] and this idea then became quite popular. A recent in-depth QM/MM study, however, convincingly argued against the original interpretation of the experimental data and showed that the experimental dependencies in fact strongly support the dispersion stabilization of aromatic stacking in water.[36]

The DFTB-D method, in contrast to the DFT/B3LYP method, yields an excellent stabilization energy, well approximating the MP2 values. Perhaps, the DFTB-D underestimates the intersystem repulsion at distances shorter than that of the energy minimum, leading to a too short intersystem separation—by about 0.2 Å. It must, however, be mentioned here that in the present study we did not introduce any new parameters to the original DFTB-D method, and the same parameter set was used as for stacking and H-bonding of nucleic acid bases.[20b]

Three AMBER-based potential energy curves were evaluated. All empirical potential energy curves are rather close to the MP2 data and contain a distinct energy minimum localized at about 3.2−3.4 Å. The standard AMB/HF-1 potential underestimates stabilization energy, and the shape of the potential energy curve differs. Inclusion of the polarization term (AMB/HF-1/P curve) shifts the dependence in the right direction but not sufficiently to match the reference MP2 values (see below). Evidently, the AMB/HF-2 with modified van der Waals term describes the whole potential energy curve well, giving a potential energy minimum that is deeper (with respect to MP2 *ab initio* values) by about 2.5 kcal/mol. The shape of the AMB/HF-2 potential energy curve is also remarkably good. Stabilization energies obtained by AMB/HF-2 are closer to the physical reality since the present MP2 stabilization energies are still underestimated, and their lowering by up to 20% seems to be realistic.[37] Let us recall that the main reason for modifying the vdW parameters in the AMB/HF-2 potential was the fact that all intercalators considered are good electron acceptors and the charge-transfer term (which is attractive) is missing in the empirical potential presently used. The other reason for modification of the potential was the large polarization energy, which is also not covered.

From Figures 8−10, we can see that the situation with the other three complexes is rather similar. The DFTB-D potential energy curves are in all cases rather close to the MP2 ones. In the case of DAPI···AT, the DFTB-D method yields slightly smaller stabilization (by about 1.7 kcal/mol), while in the case of daunomycin···GC and ellipticine···GC, the DFTB-D stabilization energies are slightly larger compared with the reference *ab initio* data. The agreement between the DFTB-D and MP2 values is also, in these three cases, satisfactory if we take into consideration that the original parameter set for dispersion attraction was adopted with no adjustment to the present *ab initio* data. The position of the potential energy minimum in all three complexes is shifted to smaller distances, which seems to be the general feature of the DFTB-D method. In the case of daunomycin···GC and ellipticine···GC complexes, the AMB/HF-1 potential energy curves are similar to the MP2 ones. However, also in these cases the AMB/HF-2 potential should be preferred because it yields a slightly larger stabilization energy than the MP2 method. As mentioned above, the actual stabilization energy of both complexes is expected to be larger than indicated by the MP2 method. For the ellipticine···GC complex, we have also tested the standard potential with the MP2 RESP charges (AMB/MP2 curve). Despite the fact that HF-1 and MP2 RESP charges differ (cf. Table 1), the AMB/HF-1 and AMB/MP2 potential energy curves are practically indistinguishable; in other words, the difference between the Coulombic term calculated with HF and MP2 charges is negligible, at least for this base pair−intercalator geometry.

The situation with the doubly charged DAPI is different. Here the AMB/HF-1 potential strongly underestimates (by more than 5 kcal/mol) the MP2 stabilization. On the other hand, the performance of AMB/HF-2 potential is good, and curves 1 and 3 in Figure 8 almost coincide. The large difference between AMB/HF-1 and MP2 values found for the DAPI-containing complex supports the original idea about the importance of the missing charge-transfer term, since the electron-acceptor ability of DAPI is the largest among all intercalators studied. Using the AMB/HF-2 potential, we obtained more realistic potential energy curves at the expense of modification of the van der Waals term. We did not test the polarization model, as its improvement for ethidium was not sufficient.

To demonstrate the suitability of the present model (base pair···intercalator), we extended this model for the larger one consisting of an intercalator placed between two base pairs. Specifically, the AT···ethidium···TA model was considered. The calculations were performed using the DFTB-D method which includes (in contrast to the empirical potential) the many-body terms. From Table 5, it is evident that both ethidium pair interaction energies are very similar. On the other hand, the AT···TA pair interaction is much smaller, due to rather large intersystem separation. The three-body term, determined as the difference of total interaction energy and sums of three above-

Table 5. Pair and Three-Body Interaction Energies (in kcal/mol) of the AT···Ethidium···TA System Evaluated by the DFTB-D Method

Δr^a	$\Delta E_{AT\cdots ETD}$	$\Delta E_{ETD\cdots AT}{}^b$	$\Delta E_{AT\cdots AT}{}^b$	$\Delta E_{AT\cdots ETD\cdots AT}{}^c$	$\Delta E_{total}{}^d$
−0.3	−22.105	−23.141	−0.623	0.142	−45.727
−0.2	−22.640	−23.234	−0.496	0.109	−46.261
−0.1	−22.631	−22.854	−0.392	0.085	−45.793
0	−22.196	−22.101	−0.309	0.068	−44.538
0.1	−21.409	−21.065	−0.241	0.056	−42.659
0.2	−20.338	−19.834	−0.186	0.046	−40.312
0.3	−19.063	−18.496	−0.141	0.040	−37.661
0.4	−17.670	−17.129	−0.104	0.035	−34.869
0.5	−16.245	−15.794	−0.074	0.030	−32.083
0.6	−14.858	−14.533	−0.050	0.027	−29.414
0.7	−13.559	−13.368	−0.031	0.025	−26.932
0.8	−12.371	−12.307	−0.015	0.023	−24.671

[a] Intersystem distances are related to the crystal distances of 3.35 and 3.4 Å, respectively, which are denoted as 0.0. [b] Pair interaction energy. [c] Three-body term. [d] Total interaction energy.

(35) Newcomb, L. F.; Gellman, S. H. *J. Am. Chem. Soc.* **1994**, *116*, 4993−4994.
(36) Luo, R.; Gilson, H. S. R.; Potter, M. J.; Gilson, M. K. *Biophys. J.* **2001**, *80*, 140−148.
(37) Hobza, P.; Šponer, J., manuscript in preparation.

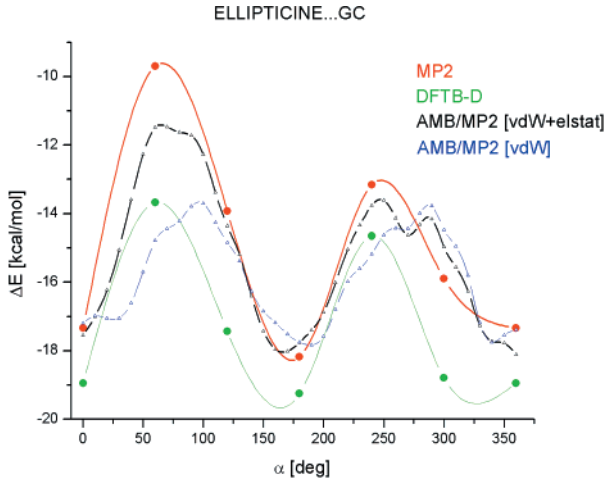

Figure 11. Twist dependence of stabilization energies (ΔE) of the guanine—cytosine (GC)···ellipticine complex evaluated by correlated *ab initio* MP2 calculations and various lower-level methods.

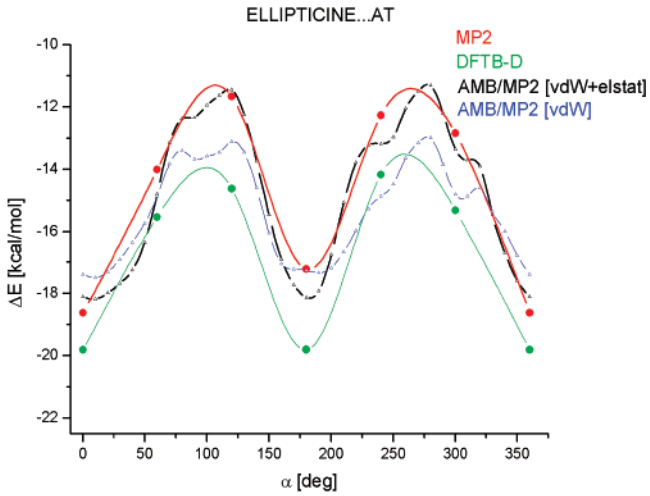

Figure 12. Twist dependence of stabilization energies (ΔE) of the adenine—thymine (AT)···ellipticine complex evaluated by correlated *ab initio* MP2 calculations and various lower-level methods.

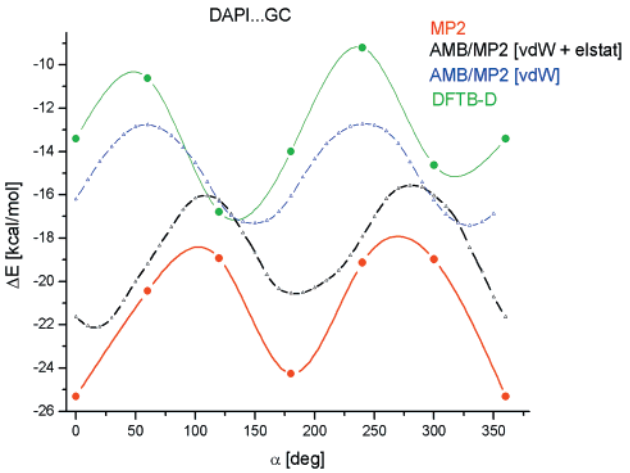

Figure 13. Twist dependence of stabilization energies (ΔE) of the guanine—cytosine (GC)···DAPI complex evaluated by correlated *ab initio* MP2 calculations and various lower-level methods.

mentioned pair interaction energies, is negligible. We believe that this table clearly demonstrates that the present model (base pair···intercalator) is representative enough to study aromatic stacking resulting from the intercalation process.

Twist Dependence of the Stacking Energy in Intercalator—Base Pair Complexes. It is known that intercalators adopt different orientations with respect to the adjacent base pairs. Some drugs intercalate in a perpendicular way with respect to the base pairs and protrude into the grooves, while other intercalators are aligned essentially along the C5—C8 base pair axis. It has been argued that the orientation of the intercalator can be attributed to the optimization of the electrostatic forces as well as to steric effects, as the intercalators often carry bulky side groups or chains. Thus, we have investigated the twist dependence of the base pair—intercalator interaction with DAPI dication and an ellipticine.[38] Both drugs as well as base pairs were assumed to have planar geometries in our calculations. We are aware of the fact that isolated DAPI as well as DAPI bound in the minor groove of a DNA duplex are nonplanar.[39] No atomic resolution structural data are available for DAPI intercalation. Nevertheless, it is fair to assume that inserting DAPI between two essentially planar base pairs should substantially planarize the drug molecule in order to optimize the molecular contacts. Note also that intercalation leads to a substantial vertical extension of the double helix which obviously is associated with a non-negligible energy penalty against any further vertical extension that would be caused by nonplanar intercalator. The intercalator—base pair complexes were initially oriented in such a way that the intercalators were aligned along the C5—C8 base pair axis and the center of mass of the intercalator was positioned exactly above the base pair center of mass. The vertical separation between the base pair and the drug was 3.38 Å, and the two systems were coplanar. Starting from initial geometries, we then twisted the interacting systems along the base pair—drug dimer axis (this axis passes through the center of mass of both subsystems) in a right-handed way. The initial geometry has been assigned by a twist value of 0°, and the twist stacking energy dependence was evaluated in the whole range of 0—360°.[7,30]

Figures 11—14 summarize the reference MP2 values with an increment of 60° and compare them with AMB/MP2 force field values and the DFTD method. The stacking energy is dependent considerably on the twist. This dependence is determined by several contributions: (i) The dispersion attraction is isotropic, attractive, and proportional to the geometrical overlap of stacked systems. Dispersion thus favors geometries with the drugs aligned along the C5—C8 base pair axes. (ii) The short-range repulsion shows the opposite dependence compared with the dispersion term but is smaller in absolute value. The sum of the short-range repulsion and dispersion attraction corresponds to the van der Waals term of the empirical force field. (iii) The electrostatic term is known to be structure-dependent and is primarily included in the HF component of stacking energy. The electron correlation still brings a small correction to the electrostatic term due to a reduction of polarity (dipole moments) of the interacting monomers. The complexes involving the DAPI dication are also affected by a non-negligible

(38) We did not study the twist dependence of stacking for daunomycin and ethidium, as their size would make the full *ab initio* treatment very costly and, more importantly, ethidium and daunomycin have bulky side groups, leading to steric clashes with base pairs while rotating the drugs.
(39) Vlieghe, D.; Šponer, J.; van Meervelt, L. *Biochemistry* **1999**, *38*, 16443—16451.

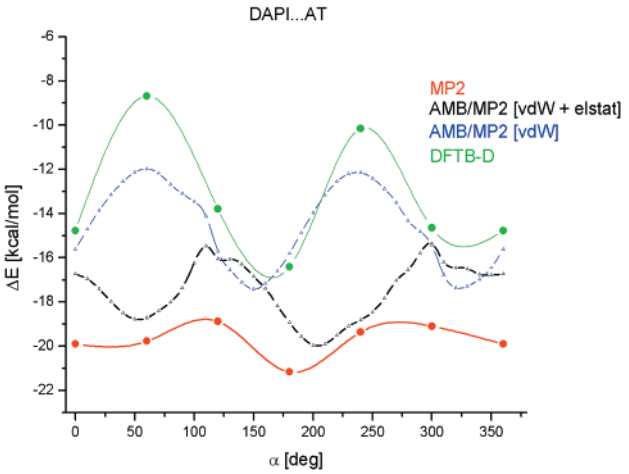

Figure 14. Twist dependence of stabilization energies (ΔE) of the adenine−thymine (AT)···DAPI complex evaluated by correlated *ab initio* MP2 calculations and various lower-level methods.

attractive induction term, which (see below) also favors the structures with a large overlap.

The twist dependence of stacking in our present systems is weaker compared with the twist dependencies calculated for stacked nucleobase dimers.[7,30] This is because, for the present extended systems, the repulsive and attractive electrostatic terms coming from different parts of the molecules compensate each other, leading to a more salient role of the overlap-dependent dispersion (van der Waals) attraction.

We will now comment on the performance of the force field. For ellipticine the *ab initio* and AMB/MP2 data show very encouraging agreement, mostly within 1 kcal/mol. This is in line with our preceding studies on stacked nucleobase dimers[7] and also the data by Bondarev *et al.*[10] For some geometries (ellipticine−AT with twist of 60°) the difference is above 1.5 kcal/mol. Such local discrepancies are likely caused by inaccuracies of the force field description of the short-range repulsion and have been discussed in detail in our preceding studies.[30,40]

For the DAPI complexes, the force field underestimates the MP2 stabilization by 0.5−4 kcal/mol, depending on the geometry. Evidently this is due to induction and charge-transfer attraction. Similar effects have been described in detail in our earlier study dealing with protonated stacked and H-bonded base pairs. Note that the magnitudes of the induction and charge-transfer effects caused by the DAPI dication (which can be roughly estimated as the difference between the force field and *ab initio* data) are not larger than those caused by protonated cytosine with a charge of +1. This again shows that extending the size of the system reduces the relative role of electrostatic and ionic effects, partly due to an efficient delocalization of the +2 charge over the DAPI molecule.

For twist values of 0° and 60°, we calculated the three-body contribution, but its values was again negligible (not shown).

Dependence of Intercalator−Base Pair Stacking Interaction Energy on Planar Displacement. To demonstrate the ability of an empirical potential to describe the complete potential energy surface of a base pair···intercalator, we performed also a systematic search of different positions of the intercalator relative to a base pair. Calculations were performed for DAPI···AT and ellipticine···GC complexes, and AMB/HF-1 empirical results were compared with DFTB-D ones. The undisplaced structure for both complexes corresponds to the energy minimum found by using the AMB/HF-1 potential (Figures 8 and 10); i.e., a base pair and intercalators were localized in coplanar planes with center of mass positioned above each other. In the second step, the intercalator was displaced along the main axis and the axis parallel to it by +2, +4, −2, and −4 Å, respectively. This means that for each intercalator we performed eight calculations. In the case of both complexes, the DFTB-D stabilization energy were systematically larger than the AMB/HF-1 ones. In the case of the first complex, the largest deviation for displacements in the *x*- and *y*-axes was 1.69 kcal/mol, or about 9% of the DFTB-D stabilization energy. In the case of the latter complex, the absolute deviations were, following expectation, slightly larger. In the case of the *x*-axis, it was 3.42 kcal/mol, which is about 18%, and for the *y*-axis it was 5.09 kcal/mol, which is about 29% of the DFTB-D stabilization energy. The values presented provide evidence that the empirical AMB/HF-1 potential describes the interaction between a base pair and an intercalator (neutral as well as charged) reliably even if the distances between the two subsystems become larger.

5. Conclusions

1. The intercalators investigated exhibit large charge delocalization, and none of them contains sites with high charge concentration. All intercalators have high polarizability and are good electron acceptors, while the AT and WC base pairs are good electron donors. This results in very favorable aromatic stacking interactions between these two systems. It is evident that only theoretical procedures properly covering dispersion, polarization, and charge-transfer effect can be used for study of intercalation processes.

2. The original AMBER force field reproduces the picture of intercalator−base pair stacking as obtained by the MP2/6-31G*(0.25) procedure in the case of the neutral intercalator ellipticine. This force field, however, neglects the induction and charge-transfer terms, and thus it underestimates the stabilization energy between non-neutral intercalators and base pairs. The discrepancies are on a scale of several kilocalories per mole, quite isotropic, and largest for the dication DAPI···GC complex. Note again that DAPI exhibits the lowest LUMO energy among all the studied intercalators. The relative magnitudes of the induction and charge-transfer effects appear to be smaller than those reported for stacking between protonated and neutral nucleobases.[11] The modified AMB/HF-2 force field with enhanced van der Waals attraction achieves a good agreement with the *ab initio* correlated MP2 calculations and can compensate for the missing induction/charge-transfer effects. On the other hand, inclusion of currently available polarization force field term did not give a sufficient correction.

3. The overall agreement between the force field and *ab initio* values suggests that the physicochemical nature of the intercalator−base pair interaction can be described as a combination of the three most common contributions to the interaction energy: the electrostatic term, the dispersion attraction, and the

(40) Šponer, J.; Leszczynski, J.; Hobza, P. *J. Phys. Chem. A* **1997**, *101*, 9489−9495.

short-range exchange repulsions.[7,9,10,24,30,40] These terms are, for cationic systems, augmented by modest induction/charge-transfer effects.[10] The electrostatic energy, which was often suggested in the past to be the dominant energy term, is less important for stabilization, and it is the dispersion energy which primarily contributes to the stabilization of the intercalator···DNA base pair stacking. The electrostatic portion of stacking is well described by the Coulombic term with simple atom-centered electrostatic-potential-fitted point charges (see also ref 10). Thus, exactly as for nucleobase stacking, no unusual aromatic-stacking-specific contributions have been evidenced.[7,24] There is no need to consider any out-of-plane π charges ("sandwich" model) to account for aromatic intercalator−base pair stacking.

4. The HF and DFT/B3LYP methods cannot be recommended for a study of intercalation since neither of them covers the London dispersion energy contributions, which are essential for intercalation process. Both methods fail completely in localizing any energy minimum at the potential energy curve of the ethidium···AT complex.

5. The DFTB-D method, which covers the dispersion energy, give stabilization energies very close to the reference MP2 values, which provides evidence that dispersion contribution is, in this case, properly taken into consideration.

6. AMB/HF-1 and AMB/HF-2 empirical potentials as well as the DFTD method will be used in subsequent papers for molecular dynamics simulations of DNA fragments with intercalators as well as for evaluation of thermodynamic characteristics for intercalation process.

7. DFTB-D calculations properly covering many-body terms demonstrated that the base pair···intercalator model is representative enough to study the intercalation process. Extension of the model by the other base pair did not bring any new information.

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