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Theoretical study of the self-association of amphotericin B

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Abstract

The aim of this present work is the study of self-association of amphotericin B (AmB) at a molecular level, because of its importance in the toxicity of this antibiotic. Molecular mechanics calculations have been performed considering different conformations of the polar head of AmB, the two most stable ones we have determined (B and C) and the one issued from the X-ray data. Our calculations have shown that both *head-to-head* and *head-to-tail* stable dimers were found within an energy range between −30 and −40 kcal/mol, the very stable *head-to-head* dimer with the polar head within C conformation having an energy of −46.8 kcal/mol. We have shown that both electrostatic and Van der Waals terms contribute to the total interaction energy but their relative weight depends on the conformation of the polar head and on the *head-to-head* and *head-to-tail* structures involved in the dimer. Thus the electrostatic contribution does not particularly stabilize the *head-to-tail* dimer. Furthermore an explicit calculation of the dipole moment in the ground state of AmB has disproved the current assertion upon the greatest stabilization of *head-to-tail* dimers by electrostatic dipole–dipole interaction. Among all the dimers we have calculated, we have found a group denoted G1 with a geometrical structure consistent with absorption data, namely a blue-shift of the dimer main absorption band with regard to the monomer one. In this group G1 we have found two isoenergetic (−38.8 kcal/mol) very stable *head-to-head* and *head-to-tail* dimers. We have found that, as a rule, the self-association of AmB in dimers is more favourable than the complexation with the cholesterol and, in a less extent, with the ergosterol. It seems that these features could be also observed for some trimers, that we have roughly calculated.

Keywords: Amphotericin; Self-association; Molecular mechanics calculation

1. Introduction

Amphotericin B (AmB) is a polyene antibiotic used in deep-seated mycotic infections (for review see [1,2]). This amphiphilic molecule has a very particular structure (Fig. 1) characterized by:

(1) a rigid heptaene unit and a polyol chain fused together into a quasi planar macrolactone ring,

(2) a polar head containing a carboxylate group and a mycosamine ring which is pushed out of the mean molecular plane of the macrolactone ring.

The molecule is poorly soluble in water where it self-associates and aggregates above a certain threshold of concentration which depends on temperature, ionic strength and way of preparing solutions. The formation of these self-associated species is very important because it has been shown that they are at the origin of the inducement of permeability through membranes which contain cholesterol, the monomers being only active in fungal cells which contain ergosterol [3,4]. The origin of the toxicity of amphotericin B, which limits its usefulness, could therefore be assigned to their presence.

The absorption and circular dichroism (CD) spectra of the $II^* \rightarrow II$ transition of AmB [5–9] exhibit important changes as a function of the solvent medium and concentration. In DMSO or methanol or water at low concentration (below 10^{-7} M) a vibrational structure is observed with a weak CD. In water, above 10^{-7} M, a totally new spectrum is observed. In absorption an intense band appears, blue-shifted as compared to that observed at low concentration and in CD a strong dichroic doublet is observed. An interpretation of these spectroscopic changes is based on an aggregation phenomenon of AmB in water. Spectral effects accompanying the spontaneous organization of AmB have been analysed:

(1) Ernst et al. [10] have proposed a two stage organization of AmB molecules:

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- a close packing of few molecules which is the origin of the absorption maxima hypsochromic shifts and
- the interaction between the preceding small units inside the aggregates which is spectroscopically expressed by the intense CD couplet.

(2) According to Hemenger et al. [11] the big aggregates have a helix structure with a repeat unit containing two molecules lying in the same plane with the polyenic part parallel and facing each other.

(3) Barcwicz et al. [12] have represented the spontaneous organization of AmB in water as a tube-like hydrophobic pore formed by five amphotericin molecules; these pores may form big aggregates by *head-to-head* and side-by-side agglomeration.

An understanding of aggregate formation is of a great interest since in vitro and in vivo studies show that the Amb activity against fungal cells as compared to the toxicity on mammalian cells depends on its aggregation state.

We actually think that the knowledge of the intrinsical stability of the different AmB-AmB dimers in mutual interaction together with the solvent (water or ethanol) effects upon these molecular organization is of a prime necessity because, in one hand, they exist in solution at low concentrations [10] and, on the other hand, they are the basis of bigger aggregates.

Thus as a first strategy we performed some theoretical mechanical calculations of some possible dimers which can be built with two AmB molecules: *head-to-tail* and *head-to-head* stacked dimers. In order to have some idea on the role of the conformation of the polar head upon the dimer stability we have undergone three series of calculations with C, B conformations previously calculated and within the A conformation determined in the crystal study of *N*-iodoacetyl AmB [13] (for details see Fig. 1).

In fact such a strategy is the same as the one we assumed in a previous study of AmB-sterol (cholesterol and ergosterol) complexes [14].

2. Method

As a preliminary step, it seemed interesting to calculate the intrinsical stability of both *head-to-head* and *head-to-tail* stacked dimers.

Our calculations were performed in an isolated state with AmB polar head within a fixed conformations (open A crystal structure or B and C conformations calculated in a previous work) [14,15]. We used a method, noted INTER [16], well adapted to the study of the relative translation and rotation motions of a molecule with regard to the other one.

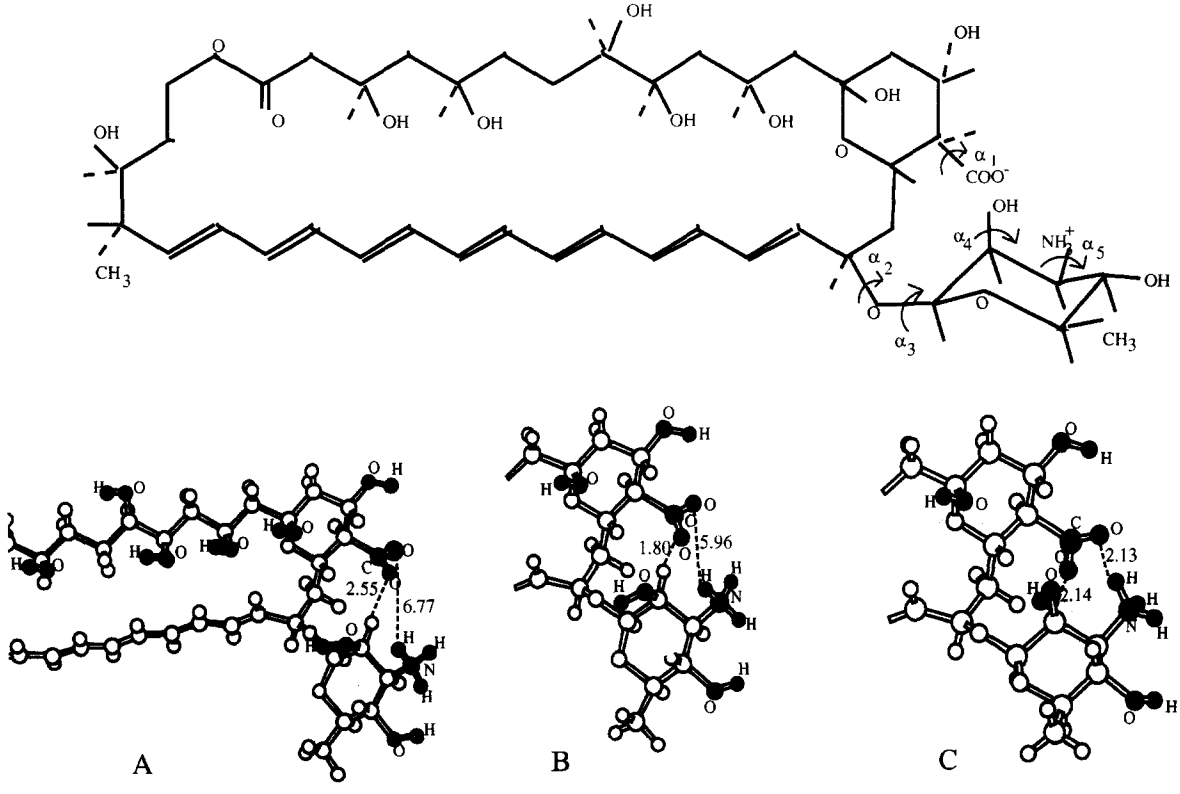


Fig. 1. Structure of amphotericin B molecule. Degrees of freedom are indicated for the polar head of AmB. Structures of the three conformations (A, B, C) of the polar head. (Distances in Å are given for similar atoms within the three conformations.).

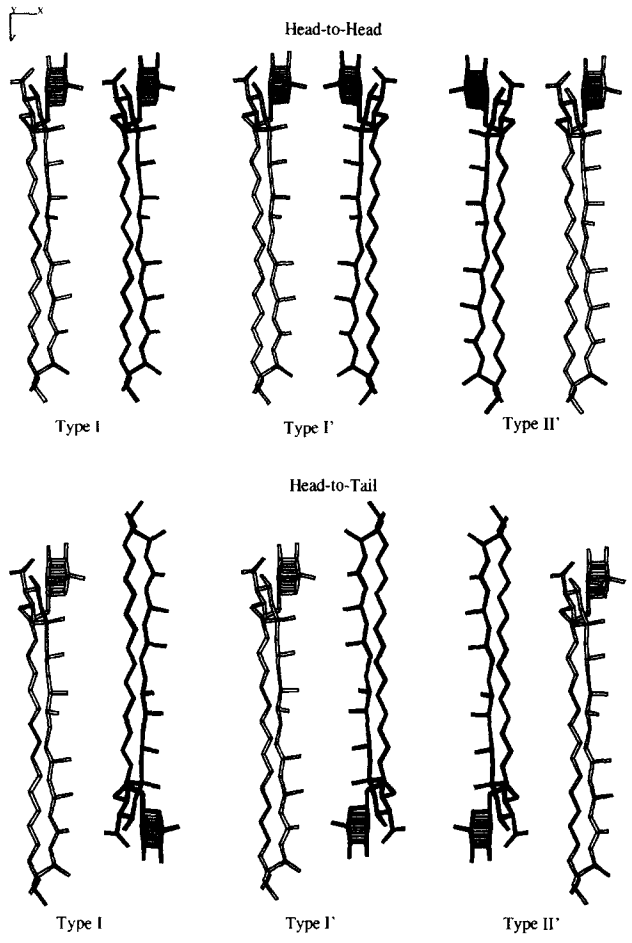


Fig. 2. Projections of the structures I, I' and II' of the *head-to-head* and *head-to-tail* AmB-AmB dimers on the XOZ plane (II is initially identical to I) for $\Delta X = 7$ Å, $\Delta Y = \Delta Z = 0$, $\Theta_Y = \Theta_Z = 0$, $\Theta_X = 0°$ for *head-to-head* dimer and $\Theta_X = 180°$ for *head-to-tail* dimer. The mycosamine rings are represented by hachured rings and the second AmB molecule is the dark molecule.

2.1. Geometrical definitions

The cartesian coordinates of AmB ($i = 1,2$) are calculated using the three main inertia axes as general coordinates axes OX_i, OY_i and OZ_i, the origin point O_i is localized on the mass gravity center. The O_iZ_i and O_iY_i axes are situated in the mean plane containing the AmB macrolide ring skeleton with the O_iZ_i axes being directed along the long axis of the molecule i. The O_iX_i axis is perpendicular to the O_iZ_i, O_iY_i plane.

In order to generate a stacked dimer, the $O_iZ_iY_i$ ($i = 1,2$) planes and the O_iX_i axe of the two molecules are first superimposed. Following that, the second molecule is translated along the OX^+ and the OX^- axis, two types of dimer denoted I or II can been obtained. Furthermore for each type of dimer (I or II) a 180° rotation of the second molecule around the OZ axis lead to dimers denoted I' and II' (Fig. 2).

Then the second molecule is allowed to move around and along the three coordinates axes. So the relative positions of the two molecules are noted by the values of the translations ΔX, ΔY, ΔZ of the second molecule along the three axis OX, OY and OZ, and the values of the rotation angles Θ_X, Θ_Y and Θ_Z around these axis. But a preliminary study has shown that:

- the most important translation motions for the stacked dimers are the ones along OX and OZ.
  - ΔZ defines the relative position of the polar heads of the two AmB molecules.
  - ΔX defines the intermolecular distance between the two AmB molecules situated in parallel planes.
- and the two most important rotation motions are the ones around OX and OZ.
  - The first one determines the relative position of the two molecules: $\Theta_X = 0°$, leads to the *head-to-head* dimer and $\Theta_X = 180°$ leads to the *head-to-tail* one.
  - The second rotation motion defined by Θ_Z leads to a loss of parallelism between the two main planes containing the AmB molecules, the two long axes remaining confused. Preliminary calculations have shown that rotation along the OY axis destabilizes the dimers. So in this present work, we have considered the interaction energy between two AmB molecules as function of four generalized coordinates (ΔX, ΔZ, Θ_X and Θ_Z).

2.2. Calculation methods

The method being previously described in details, we will only give its main characteristic features:

The interaction energy is the sum of several components, the expression of which has been fitted in such a way to satisfactorily reproduce the results of ab-initio (SCF supermolecule or perturbation treatments) calculations on small complexes:

$$E_{inter} = E_{el} + E_{pol} + E_{rep} + E_{disp} \quad (1)$$

where E_{el} and E_{pol} are respectively the electrostatic and polarization contributions calculated with an ab-initio multipolar multicentric distribution [17], E_{rep} and E_{disp} are the repulsion and dispersion terms (for more details see [16]).

3. Results

3.1. Conformational study

The conformations of AmB that we considered in that work are those we have obtained precedently. In the same way as in our previous works [14,15], only the dihedral angles are varying, all bond lengths and valence angles were fixed to the values obtained from X-ray study of *N*-iodoacetyl AmB [13] replacing the iodoacetyl group by a hydrogen atom. Because of the presence of a conjugated double bond system, we consider that the heptaenic macrolide ring remains rigid and therefore independent from the surrounding medium. Rinnert et al. [18] for AmB

and Perun et al. [19] for erythromycin, another macrolide antibiotic, have confirmed that the conformation of the macrolactone ring in solution does not change when it is compared to crystal state. Furthermore, our hypothesis has been confirmed by molecular dynamics calculations [14] which have shown that during the simulations the molecule may be considered as an association of four almost rigid moieties which move relatively to each other. Hence we have kept this part of the molecule within the conformation obtained in the crystal.

3.1.1. AmB in an isolated state

We have only been interested by the flexible polar head within a zwitterionic form. We obtained an unique folded geometry (denoted C) stabilized by an array of two intramolecular II-bonds, one of them connecting the lactone ring to the sugar moiety.

The A conformation derived from X-ray data by replacing the iodoacetyl group by an hydrogen atom is 34 kcal/mol above C conformation.

3.1.2. AmB in an hydrated state

The minimum, corresponding to the folded C conformation, remains stable but another stable conformation (denoted B) exists, characterized by a geometrical structure intermediate between the folded C and the open A ones. For these most stable conformations, C and B, nine 'hydration water molecules' form particular binding arrangements with one water molecule bridging together the NH_3^+ and CO_2^- groups. These two hydrated configurations are isoenergetic.

3.2. Complexation study

3.2.1. C conformers stacked dimers

Two series of calculations have been performed $E_{inter} = f(\Delta Z, \Delta X, \Theta_Z)$ and $E_{inter} = f(\Delta Z, \Delta X, \Theta_Z, \Theta_X)$. In order to have a simple representation of the interaction energy variations with the three (or four) generalized coordinates, we decided to represent in the same figure $E_{inter} = f(\Delta Z)_{\Delta_{X\min}, \Theta_{Z\min}}$ and $E_{inter} = f(\Delta Z)_{\Delta_{X\min}, \Theta_{Z\min}, \Theta_{X\min}}$ where the subscript 'min' below each coordinate means that the E_{inter} energy value obtained for each value of ΔZ corresponds to the minimal energy with regard to ΔX and Θ_Z (or ΔX, Θ_Z and Θ_X) values.

We will first discuss the results obtained with the AmB polar head within the C conformation. Fig. 3 relative to the *head-to-head* dimers depicts the energy profile of E_{inter} as a function of ΔZ. A similar illustration of results concerning the *head-to-tail* dimers is given in Fig. 4. The intermolecular interaction energy E_{inter} and geometrical parameters defining the most stable configurations of *head-to-head* and *head-to-tail* AmB-AmB dimers are listed in Table 1. Fig. 5 (a)–(f) illustrate the relative positions of the two AmB molecules in some most stable (*head-to-head* and *head-to-tail*) stacked dimers, respectively.

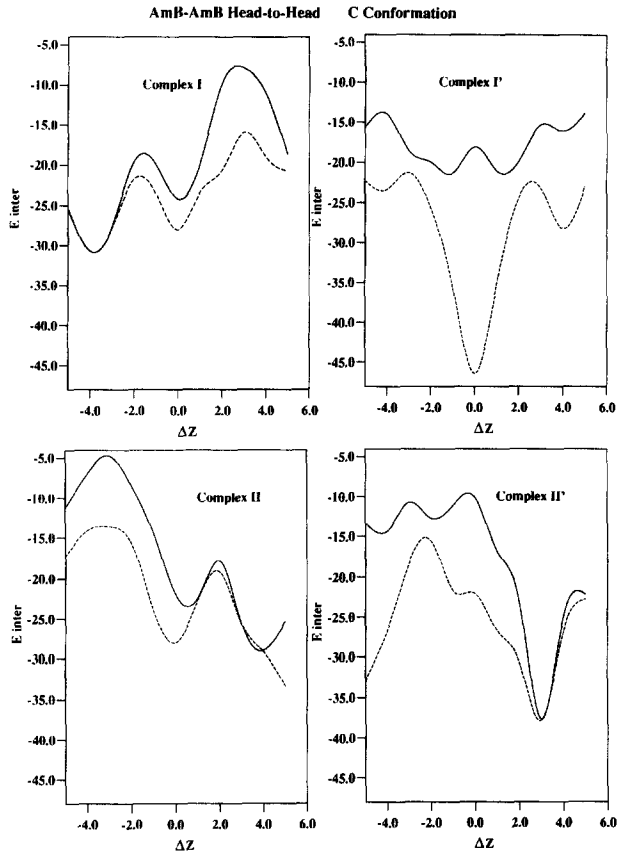


Fig. 3. Energy profiles $E_{inter} = f(\Delta Z)$ for the four *head-to-head* AmB-AmB dimers with the polar head in the C conformation. (Full line: optimized ΔX and Θ_Z, $\Theta_X = 0$; dashed line: optimized ΔX, Θ_X and Θ_Z).

We will first notice that in both *head-to-head* and *head-to-tail* dimers of type I and II the relative orientation of the two AmB molecules should be quite similar. In fact, the hydroxyl groups and the hydrogen atoms of the polyol chain are out of the macrolide mean planes, furthermore there are three methyl groups at the end of the macrolide ring. Thus, some more or less important differences in atom–atom interactions could occur following that we calculate dimers of type I or II, in particular when rotation around OX or OZ axes are taken into account. As concerns *head-to-head* dimers, Fig. 3 clearly shows that these two types of dimer are quite similar by symmetry: a translation of the second molecule along the OZ^+ axis in dimer I and a translation of the second molecule along the OZ^- axis in the dimer II are equivalent. The situation is rather different when we consider the *head-to-tail* dimers. As shown by Fig. 4, the energy profiles of these two types of dimer are quite different. The optimal complex I is more stable by 8.5 kcal/mol than the optimal complex II (see Table 1) because in *head-to-tail* dimers the out-of-plane methyl groups interact differently with the out-of-plane mycosamine group.

The role played by both translation and rotational motion is to prevent some steric hindrance between different

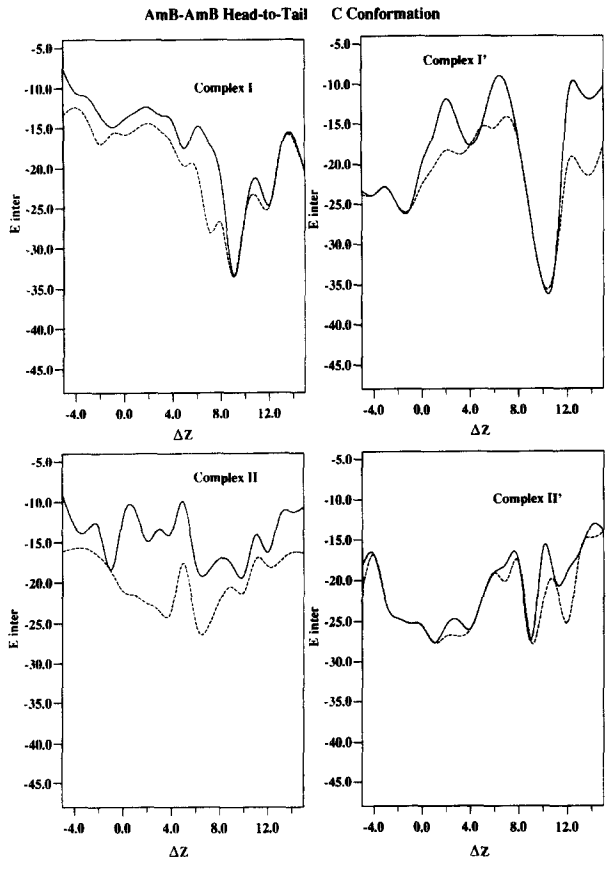


Fig. 4. Energy profiles $E_{inter} = f(\Delta Z)$ for the four *head-to-tail* AmB-AmB dimers with the polar head in the C conformation. (Full line: optimized ΔX and Θ_Z, $\Theta_X = 0$; dashed line: optimized ΔX, Θ_X and Θ_Z).

parts of the two molecules in interaction. Thus their importance should strongly depends on the type of dimer:

(a) The strongest steric hindrance occurs in dimers I′:

- In the *head-to-head* dimer, the mycosamine parts of the two molecules protrude towards each other. If we do not allow any rotation around the OX axis, the second AmB molecule may be translated along the OZ axis in a range of values between −3 Å and +2 Å with a quite constant energy: 19.5 ± 1.5 kcal/mol. The Θ_Z angle value does not vary by more than 10°, but the distance between the two molecules is quite important, 6.5 Å. When the rotation around the OX axis is allowed, the energy profile drastically changes: a very shallow energy minimum occurs for $\Delta Z = 0$ Å and $\Theta_X = -20°$; furthermore the intermolecular distance between the two molecules is now 4.5 Å. The rotation of −20° around the OX axis lead to a 25.5 kcal/mol energy stabilization.
- In the *head-to-tail* dimer, the mycosamine part of each AmB molecule interact with the end of the macrolide ring of the second molecule. The two energy profiles, obtained with or without rotation around OX, are quite identical: a very shallow minimum, $E = -34.9$ kcal/mol is obtained for $\Delta Z = 10$ Å. The steric hindrance has been removed in one hand by a translation along the OZ axis (the mycosamine part of each molecule is quite apart of the end of the macrolide ring of the second molecule) and in an other hand by a quite important rotation of 50° around the OZ axis. Thus the two molecules are not in parallel planes. We may noticed a second energy minimum ($\Delta Z = -1$ Å) which is located at ≈ 10 kcal/mol above the most stable one.

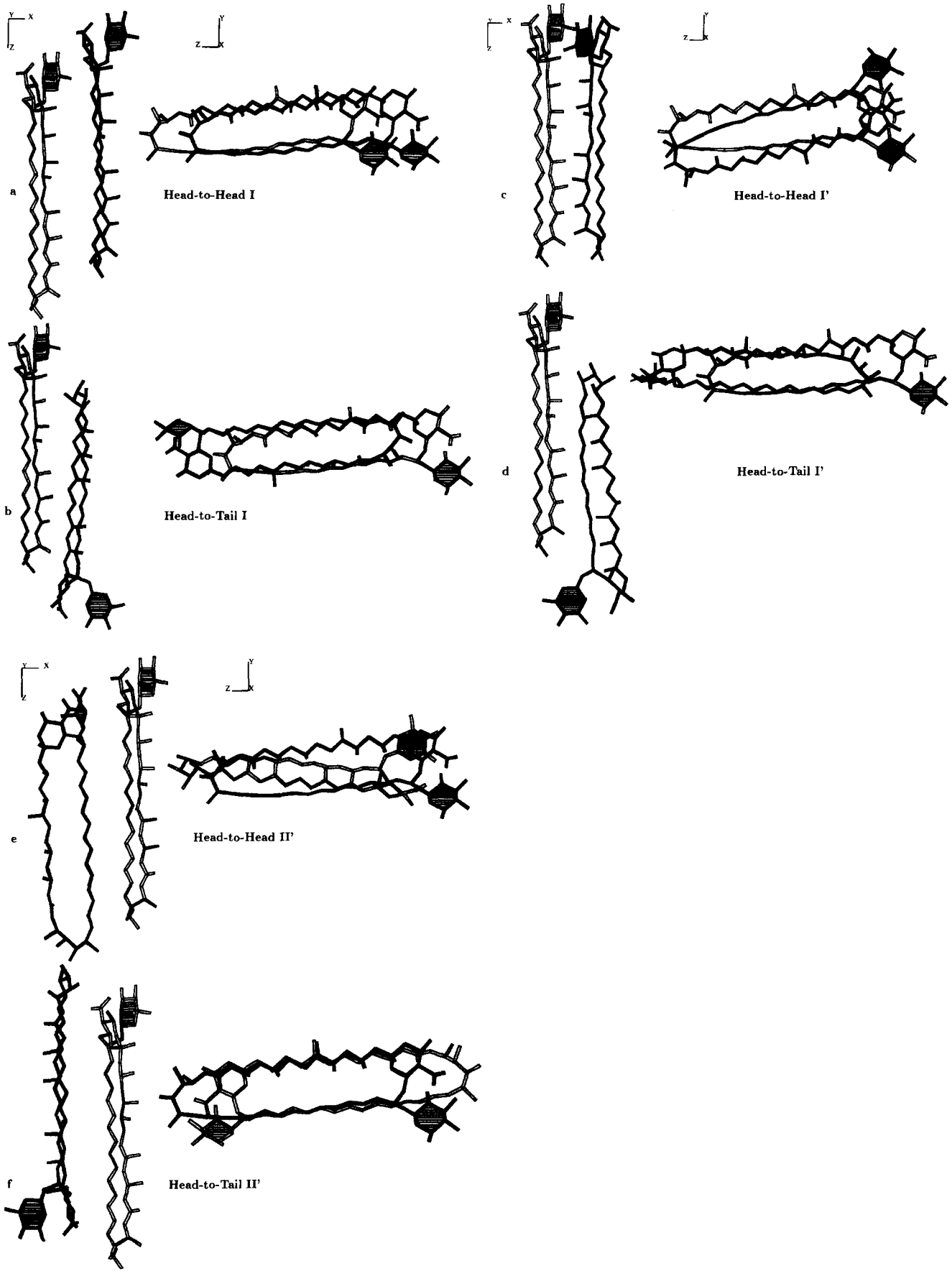
(b) Complexes of type I (or II) represent an intermediate situation, since the mycosamine part of only one molecule participates to the interaction:

- In *head-to-head* dimers the mycosamine part may be in close contact with the lactone ring of the second molecule. As shown by Fig. 3, for values of ΔZ ranging between −5 Å and −3 Å the two energy profiles precedently defined are superimposed. Then for values of $\Delta Z > -3$ Å, the shape of these two profiles are quite identical but rotation around the OX axis stabilizes the dimer. Thus, as shown by the values listed in Table 1, two quasi-isoenergetic minima have been obtained: in the first one ($E = -30.8$ kcal/mol) the steric hindrance has been removed by both a translation along the OZ axis ($\Delta Z = -4$ Å) and a small rotation around the OZ axis ($\Theta_Z = 10°$). In the second one

Table 1
Intermolecular energies calculated within the INTER method together with geometrical intermolecular parameters of the most stable AmB stacked dimers with the polar head in the C conformation

Dimer	*Head-to-head*				*Head-to-tail*			
	ΔZ	Θ_X	Θ_Z	E_{inter}	ΔZ	Θ_X	Θ_Z	E_{inter}
I	−4.0	0	10	−30.8 [a]	9.0	180	−30	−34.1 [b]
	0.0	−10	0	−28.2	7.0	160	−30	−28.4
I′	0.0	−20	180	−46.9 [c]	10.0	180	130	−34.9 [d]
					−1.0	180	180	−25.8
II	−5.0	10	−30	−33.4	7.0	170	−20	−25.6
	0.0	10	0	−28.2	4.0	170	−10	−23.6
II′	3.0	0	130	−38.3 [e]	1.0	170	160	−27.7 [f]
	−5.0	40	210	−32.8	9.0	180.0	160	−27.8

ΔZ, Θ_X and Θ_Z are defined in Section 2. For these dimers $\Delta X = 6$ Å. All distances are in Å, angles are in degrees and energies are in kcal/mol. Configurations noted by a–f are represented in Fig. 5.


a
Head-to-Head I
b
Head-to-Tail I
c
Head-to-Head I'
d
Head-to-Tail I'
e
Head-to-Head II'
f
Head-to-Tail II'

($E = -28.2$ kcal/mol) we just notice a rotation of $-10°$ around the OX axis.

- In *head-to-tail* dimer, the mycosamine part of a molecule may interact with the end of the macrolide ring. The two energy profiles are quite identical in dimer I, this is not the case for dimer II for which a small rotation around the OX axis leads to an important energy stabilization. In both *head-to-tail* dimers I and II we may notice a loss of parallelism between the main planes containing the two molecules ($\Theta_Z = -30°$ and $-20°$, respectively).

(c) In dimers of type II′, the mycosamine parts of the two molecules do not participate to the interaction:

- In *head-to-head* dimer, the two lactone rings may be in close contact. When the rotation along the OX axis is not taken into account, we only obtain one energy minimum. Allowing some motion around the OX axis does not affect this energy minimum, but a second minimum (which is less stable than the first one) appears for a value of ΔZ of -5 Å with $\Theta_X = 40°$. Once again, these minima are characterized by a loss of parallelism between the two main planes containing the two AmB molecules ($\Theta_Z = 130°$ and $210°$, respectively).
- In *head-to-tail* dimer, the lactone ring of each molecule interacts with the macrolactone ring of the second one. The energy profiles obtained when considering or not the rotation around the OX axis are quite similar. The rotation around the OZ axis is the most important one.

As a whole the interaction energies of the stacked dimers of AmB have been calculated within a range of values between -28 and -46 kcal/mol for *head-to-head* dimers and -25 and -35 kcal/mol for *head-to-tail* dimers.

3.2.2. *Influence of the polar head conformation upon stacked dimers*

Our results clearly show an influence of the conformation of the polar head upon the stability of the *head-to-head* and *head-to-tail* stacked dimers, and lead to very similar conclusions for the B and A conformations.

- In *head-to-head* dimers (excepted for dimers I′ in which the C conformation of the polar head remains the preferred one) the opening of the polar head in order to get the B or A conformation has a stabilizing effect upon the dimer.
- The four *head-to-tail* dimers we have studied are more stable when the polar head adopts the intermediate conformation B.

In order to understand the effect of the conformation on the stabilization of the dimers we have plotted E_{inter} as a function of the translation motion ΔZ in the same as we have done hereabove for conformer C. Figs. 6 and 7 have to be related, respectively, to *head-to-head* and *head-to-tail* dimers of B conformer.

For sake of information we have reported in Appendix A the tables giving the intermolecular interaction energies and the geometrical parameters defining the most stable *head-to-head* and *head-to-tail* stacked dimers obtained with the polar head of AmB within B and A conformations (Tables 3–6) and figures of variation of E_{inter} for A conformer (Figs. 10 and 11).

(a) We will first consider the stacked dimer I′.

– In *head-to-head* dimers, when the rotation around the OX axis is not allowed there is a free translation motion along the OZ axis between -3 Å and $+2.5$ Å in both B and A conformers. This result is similar to the one obtained with C conformer. But reversely to the result obtained with C conformer, allowing a rotation around the OX axis does not drastically change the energy profile calculated for A and B conformers. Furthermore because of the steric hindrance between the mycosamine parts of the two molecules, the intermolecular distance cannot be shortened to 4.5 Å as in C conformer, thus this latter one remains the most stable stacked dimer I′.

– In *head-to-tail* dimers the result concerning the poor stabilizing effect of the rotation around the OX axis is maintained by conformers B and A. The energy profiles plotted for the three conformers are quite similar. We just notice that the dimer corresponding to the most stable minimum in conformer C is destabilized when the AmB polar head opens from C to B and A conformations. Reverse result has been obtained with the second energy minimum of conformer C.

(b) In *head-to-head* dimers I and II, contrary to what was obtained with conformer C, the rotation around the OX axis has an important energy stabilizing effect on the energy minima regions of the B and A conformers. The values of the Θ_X angle, leading to the energy stabilization is quite small: $-10°$ (C and B conformers) and $-20°$ (A conformer).

– Opening the polar head of AmB does not change the location of the energy minimum of the *head-to-tail* stacked dimer I, we just notice an energy stabilizing effect due to a rotation of 10° around the OX axis in conformers B and A ($\Delta E = 6$ and 5 kcal/mol, respectively). Similarly to what was obtained with conformer C, it appears a quite important torsion angle around the OZ axis ($-40°$ and $-50°$) for conformers B and A, respectively.

(c) As clearly illustrated by Figs. 6 and 7 the energy profiles obtained for the *head-to-head* stacked dimers II′

Fig. 5. Projections of the structures of the most stable AmB-AmB dimers on the XOZ and YOZ planes. (a) *Head-to-head* and (b) *head-to-tail* dimers I. (c) *Head-to-head* and (d) *head-to-tail* dimers I′. (e) *Head-to-head* and (f) *head-to-tail* dimers II′. All these structures are characterized by a–f in Table 1. The mycosamine rings are represented by hachured rings and the second molecule AmB is the dark molecule.

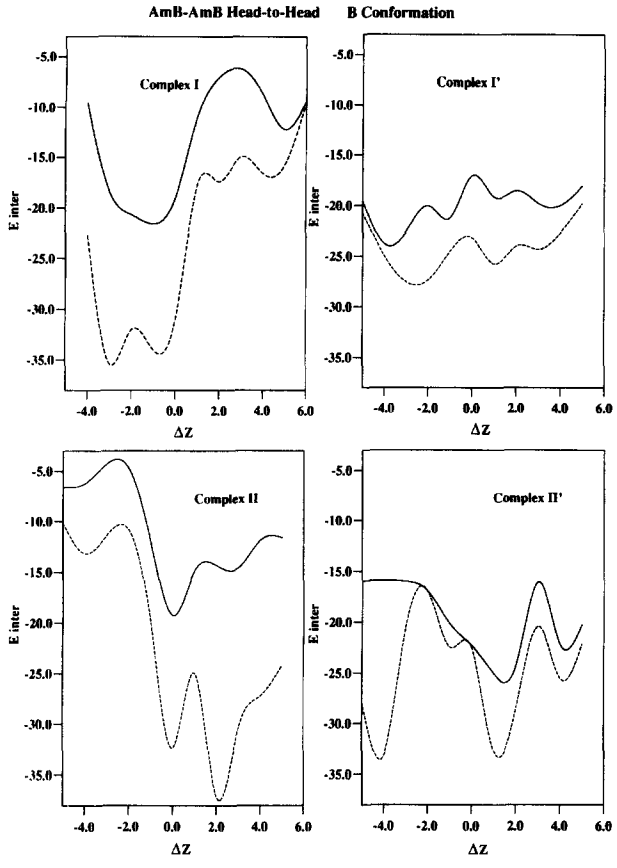


Fig. 6. Energy profiles $E_{inter} = f(\Delta Z)$ for the four *head-to-head* AmB-AmB dimers with the polar head in the B conformation. (Full line: optimized ΔX and Θ_Z, $\Theta_X = 0$; dashed line: optimized ΔX, Θ_X and Θ_Z).

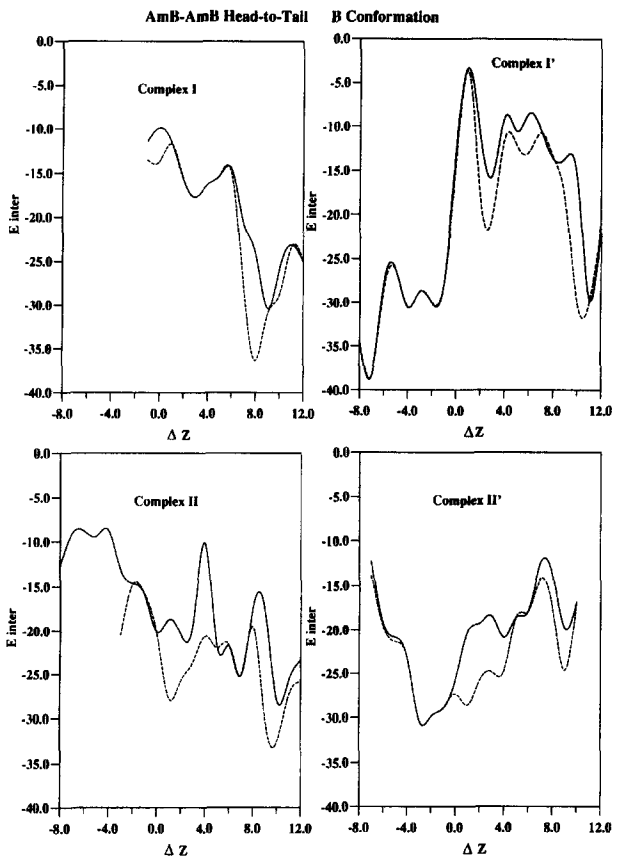


Fig. 7. Energy profiles $E_{inter} = f(\Delta Z)$ for the four *head-to-tail* AmB-AmB dimers with the polar head in the B conformation. (Full line: optimized ΔX and $\Theta_{Z,\ X} = 0$; dashed line: optimized ΔX, Θ_X and Θ_Z).

within conformation B and A are not drastically different from the one characterizing conformer C. Nevertheless some differences have to be noted:

- Besides the two most stable energy minima calculated for conformers C and B, a third energy minimum is obtained for the open conformer A.
- In conformer B, a torsion of 20° around the OX axis stabilizes by 8 kcal/mol the energy minimum located at $\Delta Z = 1$ Å.

– Same comments as hereabove may be drawn up with the *head-to-tail* dimers: once again, one has to notice that the rotation around the OX axis has a strong stabilizing effect upon some energy minima appearing in conformer B.

3.2.3. *Decomposition of the total intermolecular energy into its different contributions*

As shown by Eq. (1), the total intermolecular interaction energy is calculated as a sum of electrostatic contributions ($E_{pol} + E_{el}$) and Van der Waals contributions ($E_{disp} + E_{rep}$). It is interesting to study the relative weight of these two contributions in order to know if one contribution dominates the other one or not.

We have represented the electrostatic (E_{el} and E_{pol}) and Van der Waals ($E_{disp} + E_{rep}$) contributions together with the total intermolecular interaction energy of the most stable dimers we have obtained keeping the polar head within the C, B and A conformation (*head-to-head*) and (*head-to-tail*) (Fig. 8).

It immediately appears that:

(a) The electrostatic contribution calculated in *head-to-tail* dimers never overrides the one calculated for *head-to-head* dimers.

(b) The weight of the electrostatic contribution with regards to the total intermolecular interaction energy depends on the type of the dimer and of the conformation of the polar head.

- In *head-to-head* dimer within the C conformation, the electrostatic energy is calculated within a range of values between −7.1 kcal/mol (dimer II) and −16.9 kcal/mol (dimer II').
- When the polar head opens to B and A conformations, the absolute value of the electrostatic contribution calculated for the four types of dimer increases: in conformer A, this contribution has been calculated as -26.0 ± 1.5 kcal/mol.

An analysis of our results shows that, in fact:

- dimer I, there is a short contact between one oxygen of the carboxyl group of one molecule and one hydrogen of the ammonium group of the other one and the intermolecular distances between these two atoms varies as 3.05 Å (C), 2.69 Å (B), 2.47 Å (A). Same result has been obtained with dimer II with the following distances: 2.35 Å (C), 2.26 Å (B), 2.36 Å (A).
- In dimer I′, one intermolecular H-bond relies the OH of the mycosamine group of one molecule and the OH of the lactone ring of the other one. Once again the H-bond length depends on the conformation of the polar head: 2.44 Å (C), 2.10 Å (B) and 2.16 Å (A).

(c) When the polar head is within C conformation, the Van der Waals contribution overrides the electrostatic ones for all the *head-to-head* dimers. Then when the polar head is opened towards A conformation the amount of electro-

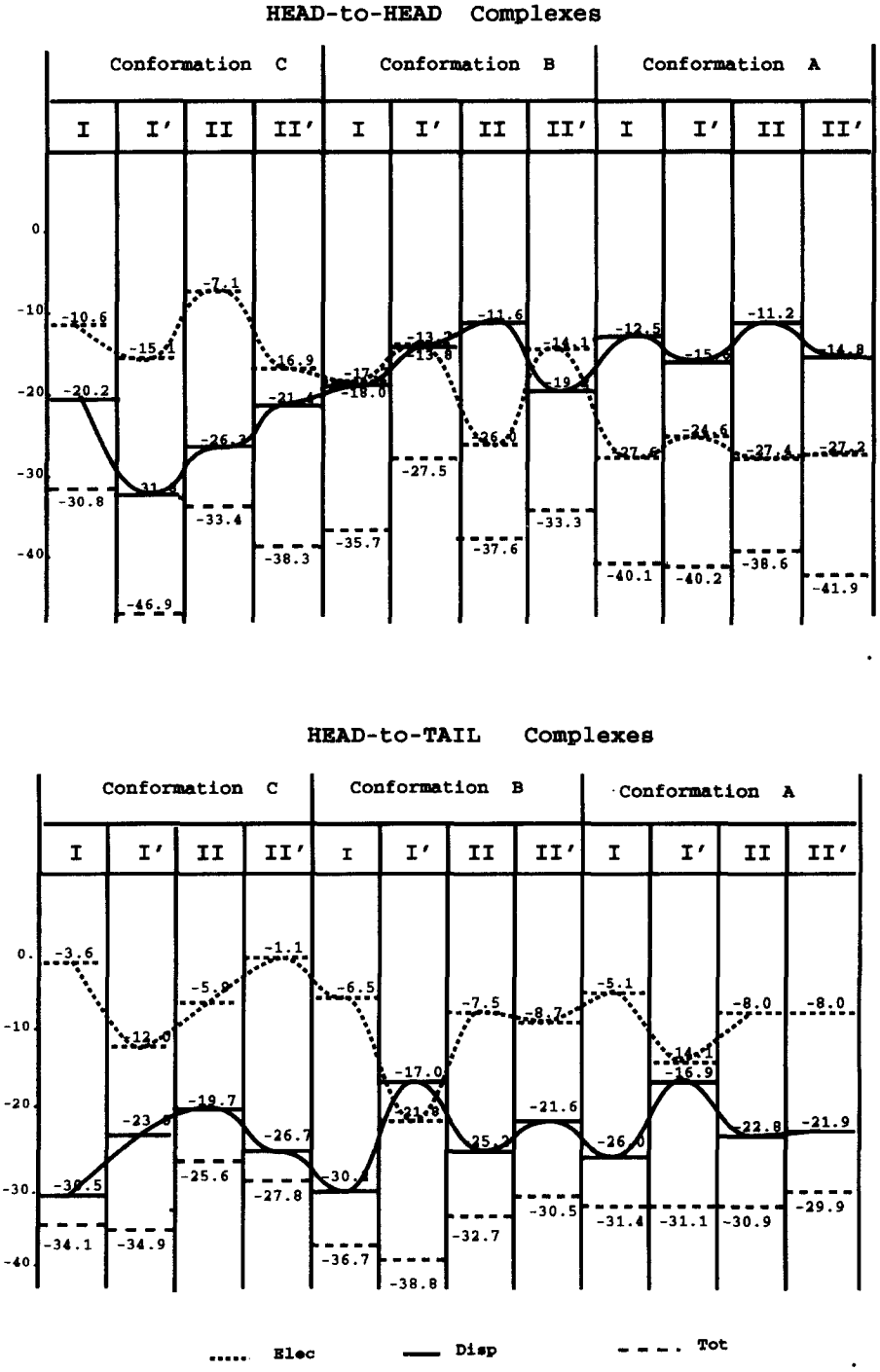


Fig. 8. Energy diagram of *head-to-head* and *head-to-tail* dimers for the three conformations of the polar head of AmB. Total intermolecular energy (- - -), electrostatic contribution (· · ·), Van der Waals contribution (————).

static contribution increases and overcomes the Van der Waals ones.

(d) Except in the case of dimer I′ (with polar head within B conformation), the Van der Waals contributions have always the dominant weight in *head-to-tail* dimer.

As a whole, it appears that:

(i) The *head-to-head* dimer I′(C) is the most stable dimer: −46.6 kcal/mol (Fig. 5c).

(ii) The intermolecular interaction energy of the less stable dimers (*head-to-head* and *head-to-tail* II(C), II′(C)) has been calculated as $-26.6 \pm 1.$ kcal/mol.

(iii) The intermolecular interaction energy of the other *head-to-head* and *head-to-tail* dimers has been calculated in a range of values between −30.0 kcal/mol and −40.0 kcal/mol.

(iv) The energy of both *head-to-head* and *head-to-tail* dimers obtained with the polar head within C or B conformation strongly depends on the type of the dimer I, I′, II, or II′, whereas all types of dimer obtained with A conformers are quite isoenergetic: $-40.0 \pm 2.$ kcal/mol for *head-to-head* dimers and -30.7 ± 0.7 kcal/mol for *head-to-tail* dimers.

4. Discussion

4.1. AmB-AmB dimers

(1) We want to discuss the current assertion that the greatest stability of the *head-to-tail* dimer (with regards to the *head-to-head* one) proceeds from the antiparallel orientation of the two molecular dipoles of the two monomers forming the dimer. Such an hypothesis proceeds from the representation of the molecular charge distribution by *one center* multipole expansion (dipole for instance).

At this level of our work, it has appeared interesting to calculate the dipole moment of the AmB monomer μ_M. We have listed in Table 2 the values of the dipole moment calculated for each conformer (C, B, A) of AmB. It appears that:

- the value of μ_M strongly depends on the conformation of the AmB polar head. The values calculated for the open A conformer (20.75 D) is twice the one obtained for the close C conformer.
- μ_M is out of the OYZ plane: the angle between μ_M and the OYZ plane has been calculated as $37 \pm 5°$ following the conformation of the polar head.
- μ_M is not aligned along the OZ axis, but nearly along the OY axis (the angle between the OY axis and μ_M has been calculated as $11 \pm 3°$).

In fact the magnitude and the direction of the dipole moment mainly depend on the orientation of the polyol OH groups and on the relative position of the two charged CO_2^- and NH_3^+ groups. In our calculations, following preliminary results we have obtained using Molecular Dynamics simulations, the polyol groups are oriented in such a way that they form an intramolecular H-bond network which is nearly parallel to the long axis of the molecule. Thus following our results it clearly appears that the magnitude and the direction of the dipole moment mainly proceeds from the two charged groups, namely CO_2^- and NH_3^+ ones.

It immediately appears, as a consequence of the results hereabove cited, that in the *head-to-tail* dimer the dipole of the two AmB molecules cannot be antiparallel. For sake of information, we have built a full overlapped dimer ($\Delta Z = 0.0$, $\Delta X = 7.0$, $\Theta_z = 180°$ and $\Theta_x = 0°$) keeping the polar head within the C conformation (Fig. 9). In such *head-to-tail* dimer, the dipole moments of the two AmB molecules involved in the dimer are nearly orthogonal (the angle θ between μ_M [1] and μ_M [2] $\cong 80°$) (Fig. 9), whereas in the corresponding *head-to-head* dimer ($\Theta_z = 0°$) the dipoles are evidently parallel.

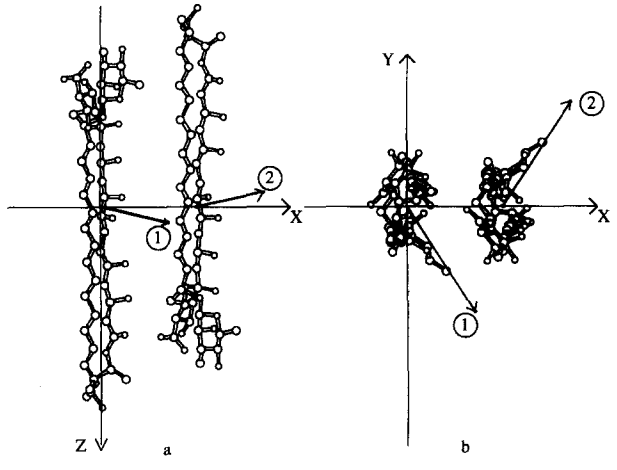


Fig. 9. Projections of the dipolar moments of a *head-to-tail* AmB-AmB dimer: (a) on XOZ plane, (b) on XOY plane.

Table 2
Dipole moment of AmB monomer and its components

Conformation	μ_M	μ_M(Z)	μ_M(Y)	μ_M(X)	ϕ_{YOZ}	θ_{OZ}	θ_{OY}
C	9.15	1.10	−7.63	−4.93	32.62	81.77	8.23
B	15.50	2.66	−10.87	10.71	43.74	76.27	13.73
A	20.75	4.04	−16.03	12.54	37.18	75.85	14.15

ϕ_{YOZ} is the angle between μ_M and the YOZ plane; θ_{OZ} and θ_{OY} are the angles between μ_M and the OZ and OY axes, respectively. Values are in Debyes.

Using the dipole-dipole approximation for the calculation of the electrostatic contribution we have obtained $E_{el} = -1.37$ kcal/mol (*head-to-tail* dimer) and $+3.51$ kcal/mol (*head-to-head* dimer) against, respectively, $+5.33$ and -1.34 kcal/mol with the multicentric multipolar approximation.

The rough results obtained for these hypothetical dimers are available for all the dimer structures that we have studied, since optimized torsions about the OZ and OX axes are rather small.

In fact, as emphasized by P. Claverie [20,21], when the molecule has not a spherical shape (this is the case with AmB), all atoms have not the same distance from the molecular center, so the potential in regions which are far from the center will be poorly represented by this one-center dipole approximation.

Furthermore, the AmB molecule presents a polar head with two charged groups situated in well defined sites of the molecule, such groups may have a very great importance as concerns the intermolecular electrostatic energy. Thus as emphasized by several authors [22–25], the electrostatic contribution will be represented with a rather good accuracy, only if the charge distribution is represented by several electrostatic centers: atoms, atoms + middle of bonds. We get what we call a multi-centric multipolar approximation [16] which is used in our methodology INTER [15].

Moreover, the electrostatic contribution is only a part of the total intermolecular interaction energy, one has also to take into account the Van der Waals energy which is the sum of both positive repulsion energy and negative dispersion energy. In fact the optimal energy of a dimer is obtained as a balance between electrostatic and Van der Waals effects.

(2) Our results clearly show that the total interaction energy of all *head-to-head* and *head-to-tail* dimers are practically within the same range of calculated values but the balance between electrostatic and Van der Waals contributions strongly depends on the relative position of the polar head of the two molecules and of the conformation of the polar head.

(3) We may try to discuss the geometrical structure that we have obtained in relation to experimental data, namely the blue-shift observed in the AmB absorption and CD spectra when the concentration increases or when water is added to the ethanol solution. Both absorption hypsochromic shift and circular dichroism may be explained in the framework of excitonic theory which connects the possible splitting of the absorption band of the monomer into two bands denoted 'dimer band' with the splitting of the excited state of a dimer into two states denoted A^+ and A^- corresponding to two excited wave functions Ψ_{A^+} and Ψ_{A^-}. The energy splitting V_{12} between the two states can be calculated, as a rough approximation, as the electrostatic interaction between the transition dipoles of the two molecules in interaction and depends on the relative orientation between the two molecules and the intermolecular distance. The absorption frequencies of the two transition $\Psi_0 \rightarrow \Psi_{A^+}$ and $\Psi_0 \rightarrow \Psi_{A^+}$ are:

$$\nu_+ = \nu_0 + V_{12}; \quad \nu_- = \nu_0 - V_{12}$$

ν_0 being the absorption frequency of the monomer. The intensity of the two dimers bands is related to D_0 the intensity of the monomer band and to the relative orientation of the two molecules involved in the dimer:

$$D_{0A^\pm} = D_0 \pm D_0 \cos\theta$$

In *head-to-head* dimers $\theta = 0°$, therefore $D_{0A^+} = 2D_0$ and $D_{0A^-} = 0$. All the absorption intensity is associated with the transition $\Psi_0 \rightarrow \Psi_{A^+}$, V_{12} is positive and the ν_+ band occurs at frequency higher than the monomer (blue-shift).

Reversely, in *head-to-tail* dimers $\theta = 180°$, therefore $D_{0A^-} = 2D_0$ and $D_{0A^+} = 0$, all intensity is associated with the transition $\Psi_0 \rightarrow \Psi_{A^-}$. In this case V_{12} is negative, thus the ν_- band occurs also at frequency higher than the monomer (blue-shift), for more details see [26].

So the spectrum of parallel (*head-to-head*) stacked dimer is indistinguishable from that of antiparallel (*head-to-tail*) stacked one.

· In this section, we have used the spectroscopic data of Ernst et al. [10], namely;

– The absorption band of the monomer located at the maximum of the envelope of the vibronic structure, $\lambda_1 = 377$ nm.

– The blue-shifted band situated at $\lambda_2 = 330$ nm.

The energy splitting V_{12} can be evaluated as 4000 cm^{-1} from the experimental blue-shift of the absorption monomer band, and the square of the transition dipole of each monomer as $\mu_1^2 = \mu_2^2 = 2 \cdot 10^{-34}$ cgs by the integration of the absorption band of the monomer.

– Using the point dipole approximation, i.e.

$$V_{12} = \mathbf{R}_{12}^{-3}\left\{\left(\vec{\mu}_1^* \cdot \vec{\mu}_2^*\right) - 3\left(\vec{R}_{12} \cdot \vec{\mu}_1^*\right)\left(\vec{R}_{12} \cdot \vec{\mu}_2^*\right)\mathbf{R}_{12}^{-2}\right\} \quad (2)$$

$\vec{\mu}_1^*$ and $\vec{\mu}_2^*$ are the transition dipoles, $\vec{R}_{12}$ is the distance between the planes containing the AmB molecules and the transition dipoles are perpendicular to R_{12}.

– Assuming the value of the angle θ between the two dipoles as 0° (or 180°), Ernst et al. [8] have evaluated the intermolecular distance $R_{12} \simeq 6$ Å.

In our calculations R_{12} and θ are, respectively, denoted ΔX and Θ_x.

· From our results listed in Tables 3–6 in Appendix A, it appears that the possible dimers we have obtained may be classified into three groups G1, G2, G3 according to the value of Θ_x.

– *Group G1*

In such dimers, the value of the angle Θ_x is 0° (in *head-to-head* dimers) and 180° (in *head-to-tail* dimers)

and ΔX has been calculated to be 6.0 ± 1 Å. This geometrical structure is consistent with the observed blue-shifted band with the evaluation of R_{12} by Ernst et al. [8]. An inspection of Tables 3–6 of Appendix A shows that three *head-to-head* dimers and eleven *head-to-tail* dimers belong to this group. Dimers of group G1 would not lead to CD spectrum since $\Theta_x = 0°$.

– *Group G2*

The angle Θ_x is very small, $\simeq 10°$, 20° (or $180 \pm 10°$ or 20°). Once again, a very intense blue-shifted band should be obtained and the intensity of the red-shifted one will be near zero. Seventeen *head-to-head* dimers and ten *head-to-tail* dimers belong to this group. The intermolecular distance calculated using these values of Θ_x is very near to the one evaluated by Ernst et al. [8], between 6.10 Å and 6.40 Å.

– *Group G3*

The value of the angle Θ_x is greater than $\pm 20°$ (or $180° \pm 20°$). Two *head-to-head* dimers namely II(A) ($\theta = 30°$, $E_{inter} = -38.6$ kcal/mol) and II'(C) ($\theta = 40°$, $E_{inter} = -32.8$ kcal/mol) and one *head-to-tail* dimer namely II(B) ($\theta = 220°$, $E_{inter} = -27.9$ kcal/mol) belong to this group. Such dimer structures will lead to both hypsochromic and bathochromic shifts of the absorption band of the monomer. Following the data of Ernst et al. [10]: the absorption band of the monomer is at $\lambda = 377$ nm, the dimer blue-shifted band will be found at $\lambda_1 = 330$ nm (same as for dimers of group A and B) and the dimer red-shifted band will be found at $\lambda_1 = 450$ nm. The intensity of this last absorption band can be calculated as 1/8 or 1/4 of the intensity of the monomer band respectively following a value of 30° and 40° for the angle θ.

Dimers of group G2 and G3 should theoretically lead to CD spectrum due to the coupled oscillator or exciton term. We should obtain two CD bands of opposite rotational strength separated by twice V_{12}, i.e. (following the data of Ernst et al. [10]) by 2×4000 cm^{-1}. The crossover point of this dimer CD should correspond to the energy at which the monomer absorption is maximum i.e. at $\lambda = 377$ nm. Such a CD spectrum is not consistent with the experimental one which is characterized by:

– A crossover point corresponding to the dimer absorption maximum.

– An energy separation $V_{12} = 600$ cm^{-1} between the two CD bands of opposite rotational strength (see [10] and [11]).

At the light of this discussion, if some dimers exist in water solution they would belong to group G1 ($\Theta_x = 0°$). It is interesting to notice that among the dimers belonging to this group we may find two isoenergetic very stable dimers ($E_{inter} = -38.8$ kcal/mol) namely the *head-to-head* II' (A) and the *head-to-tail* I' (B) ones. On the other hand the two CD bands observed in water should not be attributed to this dimer but rather to an aggregate.

· At this level of our work we cannot predict the structure of the aggregate accounting for the observed CD spectrum but we may discuss about some aggregates possibilities:

– The eventual presence in solution of some stacked dimers belonging to group G1, disproves the assumption of a single helix as a model of aggregate. This is in agreement with the results of Hemenger et al. [11] and Ernst [10]. At this step of calculation we cannot discuss about the assumption of a double helicoidal aggregate since such a model involves an in-plane dimer as basic unit cell of the aggregation.

– The important value of the torsional angle Θ_z (which leads to a loss of parallelism between the mean planes containing the two monomers involved in the dimer) is quite important in both *head-to-head* and *head-to-tail* AmB-AmB dimers, even in dimers belonging to Group G1. This result can be in favor of a cylindrical arrangement in order to form an aggregate like a channel. This is the model assumed by Barwicz et al. [12] but as admitted by these authors an asymmetry, like a slight tilt with regards to the channel axis, may be introduced to be consistent with the CD spectrum associated to aggregates in water.

Nevertheless, our calculations show that in an apolar medium like a membrane, possibility of cylindrical arrangement of AmB is an intrinsical property of the dimers.

Before ending this section we want to emphasize that we are now calculating different stacked dimers in water in order to verify our assumption concerning the presence of dimers of group G1 in water. Then our aim would be to study the different aggregates, together with the solvent effect, which are responsible to CD spectrum.

4.2. *Comparison between AmB-AmB and AmB-sterol dimers*

At this stage of our discussion, it has appeared interesting to compare these results with those obtained for the AmB-sterol (cholesterol or ergosterol) dimers which have been calculated in our previous work. We consider only the most stable dimers (AmB-AmB and AmB-sterol) for the conformer B of AmB because preliminary calculations of AmB-sterol complexes have shown that the conformation of the polar head had only a slight influence on the complexation.

Before beginning this discussion, we want to recall that even when the sterol side chain is in an all-*trans* conformation, the sterol molecules are shorter and tighter than the AmB molecule, thus they will only partially fit into the whole AmB. As a consequence, we had to discriminate the structures where the sterol is located near the AmB polar head (structure a), or repelled at the end of the macrolide ring (structure b).

Analysis of the results leads to the main differences between the complexes:

· *From an energetics point of view*, it is shown that AmB-cholesterol dimers are in an energy range between

−19 and −25 kcal/mol, these energy values are above the ones calculated for both *head-to-head* and *head-to-tail* dimers. On the other hand, the energy of three AmB-ergosterol dimers are in a range of energy values between −25 and −32 kcal/mol and quite match the values calculated for some *head-to-head* dimers (I′,II′) and for some *head-to-tail* dimers (II,II′) but remain above the energy values of the four most stable AmB-AmB dimers which have been calculated between 35. and 40. kcal/mol.

· *From a structural point of view*, several differences have been noted between AmB-AmB dimers and AmB-sterol complexes:

– The translation along the OZ axis is of a less extent in AmB-AmB dimers than in AmB-sterol complexes. This motion is very important in the AmB-sterol complexes since it determines the relative position of the AmB polar head and the sterol molecule in connection with the stability of complexes: the strongest interaction occurs when the sterol is in position a in the dimers I or I′ and in position b in II or II′.

– The translation along the OY axis has a destabilizing effect upon AmB-AmB dimers and is allowed in AmB-sterol ones.

– The motion around the OX axis has a destabilizing effect upon AmB-sterol dimers, but may be quite important in optimized AmB-AmB dimers.

The optimal values of the intermolecular distance between the two AmB molecules or AmB and sterol molecules are quite similar: 5.5 ± 0.5 Å. In both AmB-AmB dimers and AmB-sterol dimers the rotation around the OZ axis is quite important (≃ 20–40°). Such a torsion lead to a non parallelism between the main planes of the two molecules.

All these features are summarized in an energy diagram (Fig. 12) reported in Appendix A.

4.3. $(AmB)_3$ trimers

Following the ideas developed in our previous study of complexation of AmB with sterol, consisting to go beyond the dimer, we generated a trimer, in such a way that molecule 1 interacts with molecule 2 situated along the OX^+ axis and with a molecule 3 situated along the OX^- axis. The geometrical parameters defining the relative position between molecules 1 and 2, and molecules 1 and 3, are those which have been determined for the most stable dimer denoted I or I′ (interactions between molecules 1 and 2) and II or II′ (interactions between molecules 1 and 3). Thus we obtain trimers denoted I-II, I-II′, I′-II, I′-II′. The calculations have been performed for both *head-to-head* and *head-to-tail* trimers and we have also considered the three possible conformations (A, B, C) of the polar head. Since the intermolecular distance between molecules 2 and 3 are quite long (in a range of distances of 10 Å and 13 Å) we have neglected the interaction between these molecules and the interaction energy in a given trimer has been calculated as the sum of the interaction of the dimer I (or I′) and that of the dimer II (or II′). For instance, the interaction energy of trimer I-II (for AmB with polar head within conformation C) is calculated as the sum of −30.8 − 33.4 = −64.2 kcal/mol. We have verified that our approximation consisting to neglect interactions 1–3 is valid: the complete calculation of the trimer I-II has led to a value of −65.6 consistent with the one we have given hereabove.

It immediately appears that the energy of *head-to-head* trimers are calculated in a range between −85 and −60 kcal/mol separated in two groups: six trimers have their interaction energies between −85 and −80 kcal/mol and the six other ones have their energies between −70 and −60 kcal/mol. The situation is different for the twelve *head-to-tail* trimers which have all their interaction energies in a range of −70 to −60 kcal/mol.

In short (see Fig. 13 in Appendix A):

· the I′-II′ *head-to-head* trimer (C) is the most stable one (−85.2 kcal/mol); the four possible trimers (A) and the trimer I′-II (C) are nearly isoenergetic (−80 ± 2 kcal/mol) then all possible trimers (B) and both trimers I-II (C) and ′ (C) are higher in energy.

· the four possible *head-to-tail* trimers (B) are the most stable *head-to-tail* trimers with −69 ± 1.5 kcal/mol.

4.4. Comparison between $(AmB)_3$ and (AmB-sterol-AmB) trimers

Once again, these results have to be compared with the ones we had obtained with the AmB-sterol-AmB complexes. Because of the weak extent of the translation along the OZ axis, we can generate a $(AmB)_3$ trimer without any ambiguity as concerns the position of the molecule 1 with regard to molecule 2 (along the OX_+ axis) or molecule 3 (along the OX_- axis). This is not the case for AmB-sterol-AmB trimer, since the position of sterol relatively to AmB is different following that the AmB-sterol interaction is of type I (and I′) or II (and II′). So it is not possible to generate an AmB-sterol-AmB trimer with simultaneous optimal interaction of type I (or II′) and II (or II′). We discriminate two possible trimers denoted a or b where the sterol may be positioned in such a way to minimize the energy with the first AmB molecule (energy minimum of dimer I): trimer $(I\text{-}II)_a$ (respectively (I′-II′)-*a*) or with the second AmB molecule (energy minimum of dimer II (or II′)): trimer $(I\text{-}II)_b$ (respectively (I′-II′)-*b*). Following our results, for a given trimer I-II (or I′-II′), the two types a or b are isoenergetic, but this rough building of these trimers could be improved by an optimization of the position of the sterol between the two AmB molecules. Nevertheless it is less probable that such an optimization lower the energy

down to −60 kcal/mol. Finally it has appeared that the two most stable. AmB-ergosterol trimer I-II are much higher than the less stable AmB trimer (≃ −40 kcal/mol compared to −57 kcal/mol).

Acknowledgements

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Appendix A

A.1. Tables of the intermolecular energies and relative positions of the two AmB molecules in the most stable dimers within B and A conformations of the polar head (see Section 3.2.2)

In Tables 3–6, all distances are in Å, angles are in degrees and energies are in kcal/mol.

A.2. Variation of E_{inter} with ΔZ for AmB dimer within the A conformation (see Section 3.2.2)

Energy profiles $E_{inter} = f(\Delta Z)$ for the four *head-to-head* AmB-AmB dimers with the polar head in the A conforma-

Table 3
Most stable AmB dimers I

Conformation	*Head-to-head*				*Head-to-tail*			
	ΔZ	Θ_X	Θ_Z	E_{inter}	ΔZ	Θ_X	Θ_Z	E_{inter}
B	−3.0	−10	20	−35.7	8.0	170	−40	−36.7
	−1.0	−10	20	−34.0				
A	−1.0	−20	20	−40.1	8.0	170	−50	−31.4
					10.0	170	−40	−28.9

Table 4
Most stable AmB dimers I′

Conformation	*Head-to-head*				*Head-to-tail*			
	ΔZ	Θ_X	Θ_Z	E_{inter}	ΔZ	Θ_X	Θ_Z	E_{inter}
B	−3.0	−10	160	−27.5	−7.0	180	170	−38.8
	1.0	−10	160	−25.9	11.0	180	130	−30.3
					−4.0	180	170	−30.9
A	−3.0	−10	200	−39.4	−2.0	180	180	−31.1
	−1.0	−10	200	−40.2	11.0	180	130	−27.3

Table 5
Most stable AmB dimers II

Conformation	*Head-to-head*				*Head-to-tail*			
	ΔZ	Θ_X	Θ_Z	E_{inter}	ΔZ	Θ_X	Θ_Z	E_{inter}
B	0.0	10	20	−32.8	10.0	170	−50	−32.7
	2.0	20	0	−37.6	1.0	220	−10	−27.9
A	1.0	30	20	−38.6	9.0	170	−40	−30.9
	4.0	10	30	−34.3				

Table 6
Most stable AmB dimers II′

Conformation	*Head-to-head*				*Head-to-tail*			
	ΔZ	Θ_X	Θ_Z	E_{inter}	ΔZ	Θ_X	Θ_Z	E_{inter}
B	−4.0	20	200	−33.3	−3.0	180	160	−30.6
	1.0	10	160	−32.8	9.0	170	150	−24.9
A	1.0	10	160	−39.9	−1.0	180	160	−29.9
	−5.0	20	210	−41.9	1.0	170	170	−29.6
	4.0	0	150	−38.8				

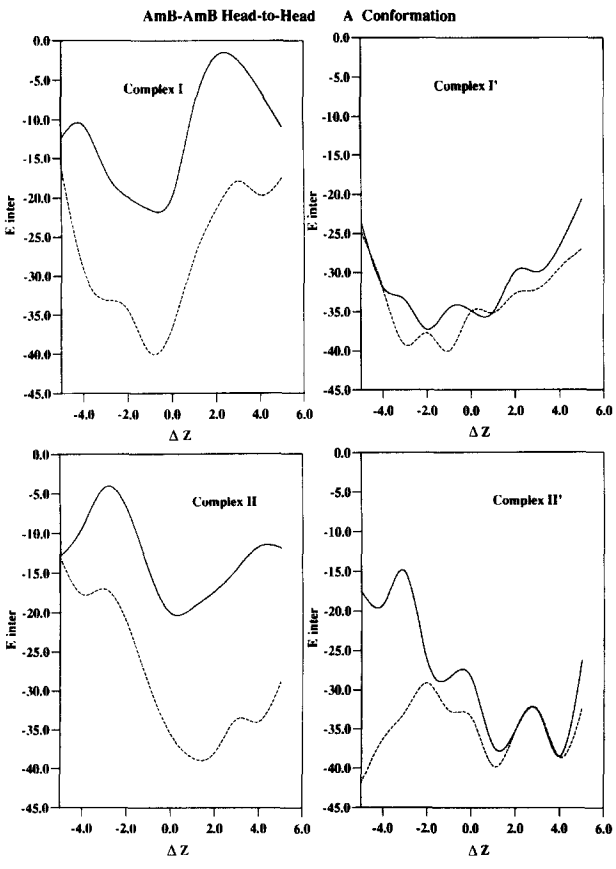

Fig. 10. Energy profiles $E_{inter} = f(\Delta Z)$ for the four *head-to-head* AmB-AmB dimers with the polar head in the A conformation. (Full line: optimized ΔX and Θ_Z, $\Theta_X = 0$; dashed line; optimized ΔX, Θ_X and Θ_Z).

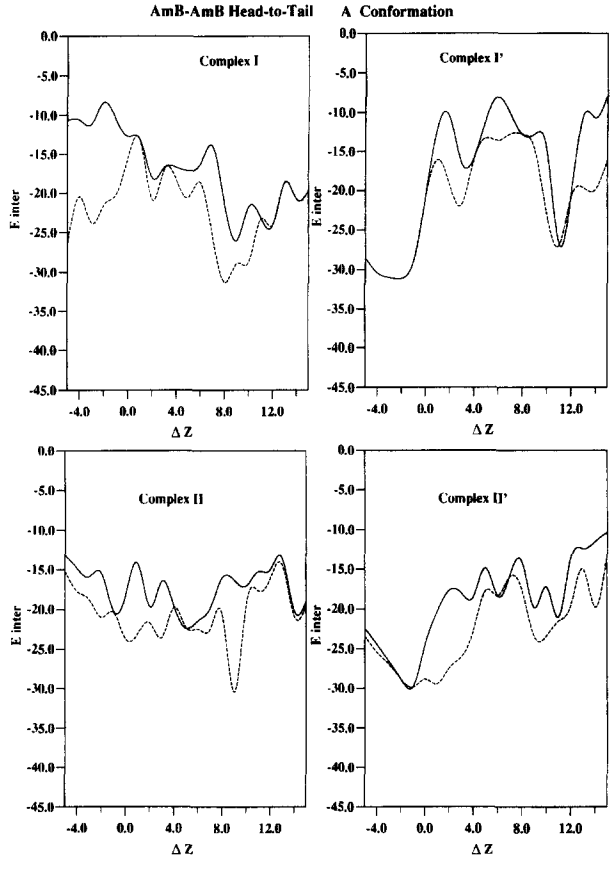

Fig. 11. Energy profiles $E_{inter} = f(\Delta Z)$ for the four *head-to-tail* AmB-AmB dimers with the polar head in the A conformation. (Full line: optimized ΔX and Θ_Z, $\Theta_X = 0$; dashed line: optimized ΔX, Θ_X and Θ_Z).

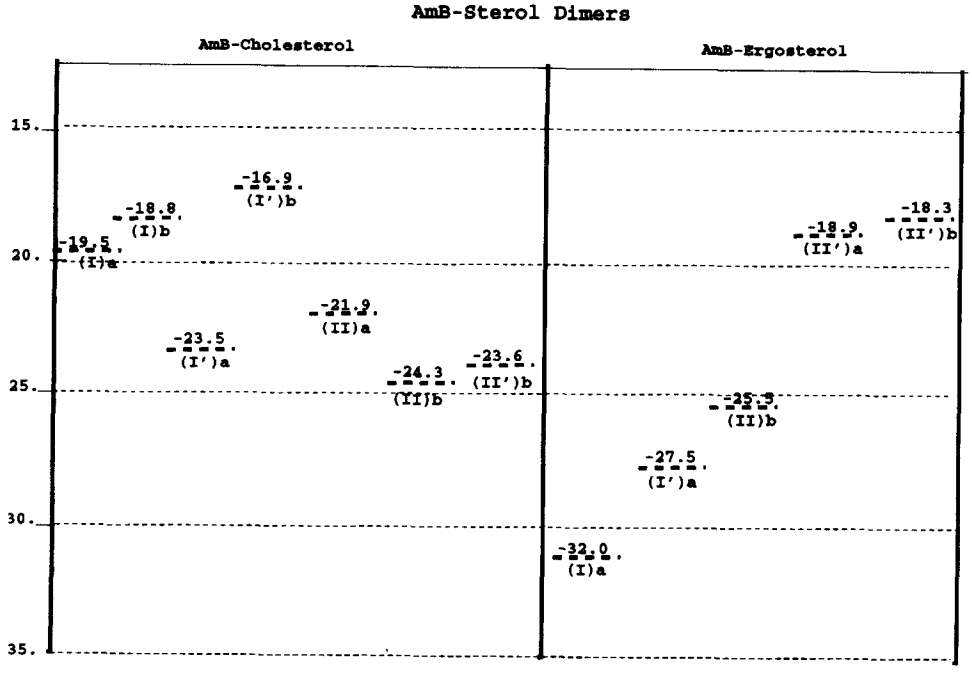

Fig. 12. Energy diagram of the most stable AmB-sterol dimers. Total intermolecular energy (- - -). (a) Structures with the sterol near the AmB polar head. (b) Structures withe the sterol at the end of the macrolide ring.

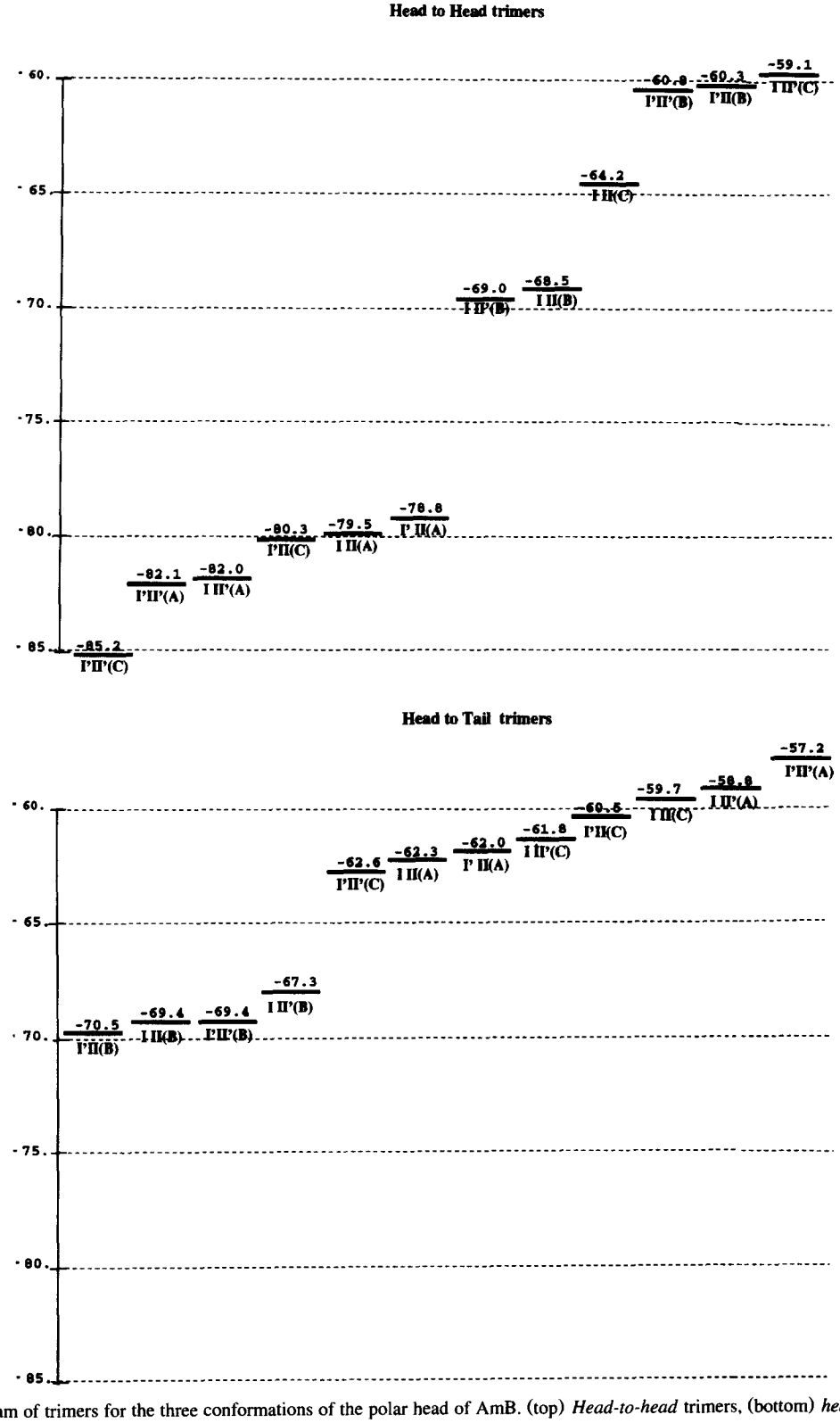

Fig. 13. Energy diagram of trimers for the three conformations of the polar head of AmB. (top) *Head-to-head* trimers, (bottom) *head-to-tail* trimers. Total intermolecular energy (---).

tion are shown in Fig. 10. In Fig. 11 the energy profiles for the four *head-to-tail* AmB-AmB dimers with the polar head in the A conformation are presented.

A.3. Energy diagrams for AmB-sterols complexes (see Section 4.2) and AmB trimers (see Section 4.3)

Fig. 12 shows the energy diagram of the most stable AmB-sterol dimers. In Fig. 13 the energy diagram of trimers for the three conformations of the polar head of AmB.

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