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A Theoretical Study of the Comparative Binding Affinities of Daunomycin Derivatives to a Double-Stranded Oligomeric DNA. Proposal for New High Affinity Derivatives

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SUMMARY

Theoretical computations were performed on the comparative binding affinities of daunomycin (DM, *1*) and seven derivatives related to the double-stranded oligonucleotide d(CGATCG)$_2$. The compounds investigated were 4-demethoxy DM (*2*), and its β-anomer (*3*), 4-demethoxy-7,9-bis-epi DM (*4*) and its β anomer (*5*), a derivative with glucosamine instead of daunosamine (*6*), and two additional hypothetical DM derivatives in which the cationic NH_3^+ group of the daunosamine moiety is replaced by either a CH_2—NH_3^+ group (*7*) or a $CH_2CH_2NH_3^+$ group (*8*), so as to indicate the effect on the binding affinity of interposing one- or two-methylene groups between the sugar and the cationic charge. The conformational angles of the hexanucleotide are fixed in values found in the representative crystal structure of the d(CGTACG)$_2$-DM complex. The intermolecular drug-hexanucleotide interaction energies and the conformational energy changes of the drug upon binding are computed and optimized in the framework of the SIBFA procedure (sum of interactions between fragments computed ***ab initio***), which uses empirical formulas based on *ab initio* SCF computations. The overall binding affinity ordering of compounds *1–6* compares satisfactorily with the ordering of available experimental affinity constants. The binding affinities of compounds *7* and *8*, for which no experimental results seem to be available yet, are predicted to be significantly higher than those of the parent compound DM, with the greatest affinity found for *7*. Because of the overall correlation between binding affinity of anthracyclines to DNA and their antitumor activity, these last two compounds deserve an exploration of their chemotherapeutic efficiency.

The outstanding antitumor properties of DM have stimulated the search for synthetic derivatives, with the purpose of designing compounds with enhanced chemotherapeutic activity and/or reduced cytotoxicity (see, e.g., Refs. 1 and 2). It seems well established that the primary target of DM is DNA, to which binding occurs by means of intercalation (3–6). A remarkable illustration of the stereochemistry of this interaction was provided by the X-ray diffraction study of the d(CGTACG)$_2$-DM complex (4).

We have investigated, recently, by theoretical computations, the base pair sequence selectivity, in this respect, of DM, using as receptors a series of representative self-complementary, double-stranded hexanucleotides (7) and as starting point the indications of Ref. 4. These computations indicated that the preferred binding sequences must be expressed in term of a triplet of base pairs (and not as usually attempted in terms of the couple of such pairs), the best ones corresponding to d-ACG or d-TCG, in which the intercalation site is situated between the two GC base pairs, but is flanked on the "downstream" side by an AT base pair.

In the present study, we propose to retain one of the two preferred hexanucleotide sequences found in Ref. 7, namely, d(CGATCG)$_2$, and to investigate the effect of structural changes in DM on its binding affinity to this sequence, the intercalation site being maintained, following the procedure used in Ref. 7, for all DM derivatives between base pairs G6-C1′ and C5-G2′ (Fig. 1). It may be remarked that the model receptor contains a larger number of base pairs than the proposed recognition site of the anthracyclines. This approach is advantageous in theoretical computations because it provides a more realistic representation of the molecular electrostatic potential of DNA which, because of the importance of the electrostatic component in the overall energy of interaction (vide infra), is highly desirable (see, e.g., Refs. 8 and 9). The compounds investigated are represented in Fig. 2A. With respect to DM, these derivatives differ by the following changes: suppression of the 4-methoxy substituent (*2–5*); inversion of configuration at $C_{1'}$, i.e., replacement of the α-anomer by the β-anomer (*3*, *5*); simultaneous epimerization of the two substituents at positions 9 and 7 (*4*, *5*); replacement of daunosamine by glucosamine (*6*); and interposition of one (*7*) or two (*8*) methylene between the terminal NH_3^+ cationic group and

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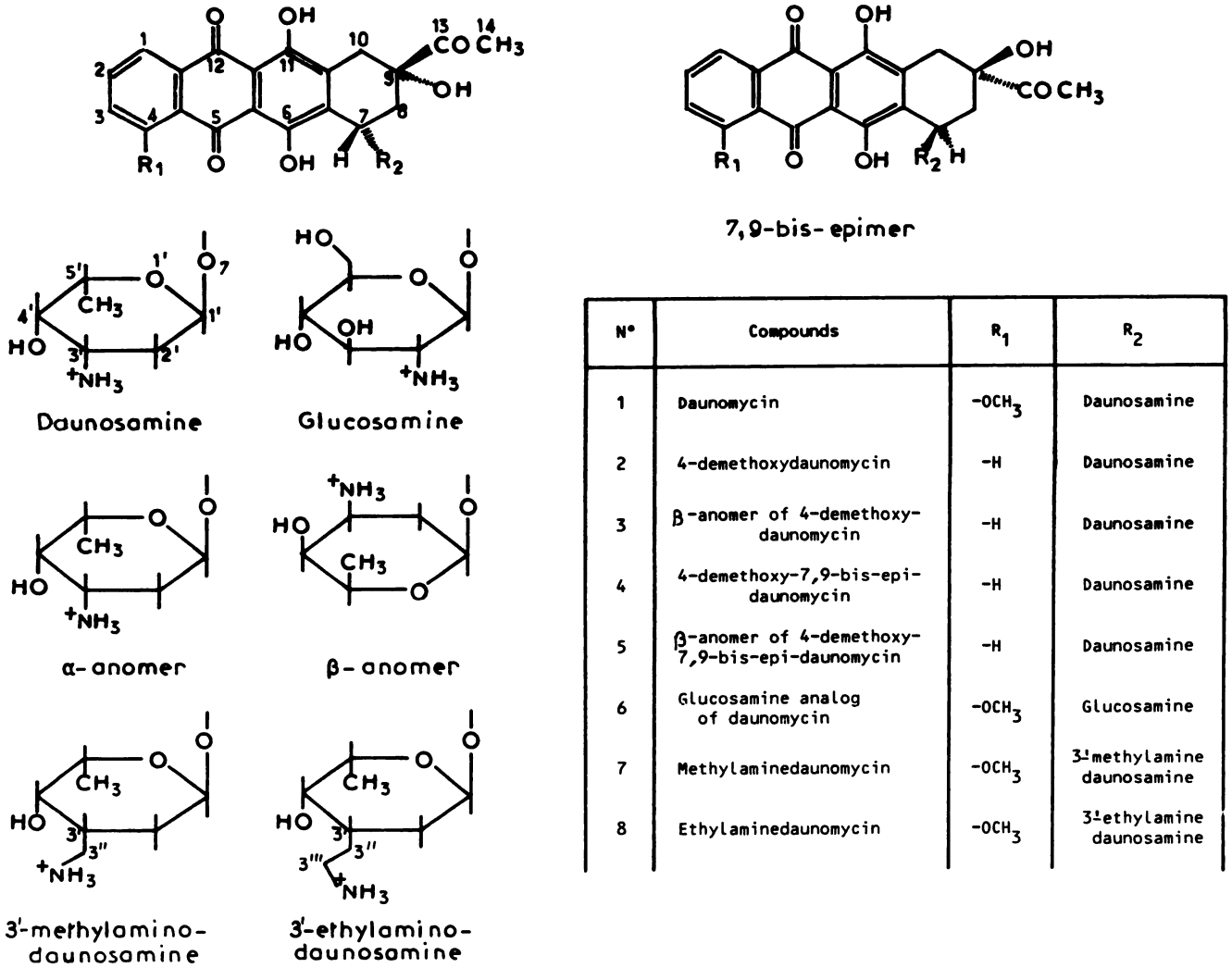
ABBREVIATIONS: DM, daunomycin; SIBFA, sum of interactions between fragments computed *ab initio*; SCF, self-consistent field.



N°	Compounds	R_1	R_2
1	Daunomycin	$-OCH_3$	Daunosamine
2	4-demethoxydaunomycin	-H	Daunosamine
3	β-anomer of 4-demethoxy-daunomycin	-H	Daunosamine
4	4-demethoxy-7,9-bis-epi-daunomycin	-H	Daunosamine
5	β-anomer of 4-demethoxy-7,9-bis-epi-daunomycin	-H	Daunosamine
6	Glucosamine analog of daunomycin	$-OCH_3$	Glucosamine
7	Methylaminedaunomycin	$-OCH_3$	3'-methylamine daunosamine
8	Ethylaminedaunomycin	$-OCH_3$	3'-ethylamine daunosamine

Fig. 1. Structural elements of the eight anthracyclines investigated.

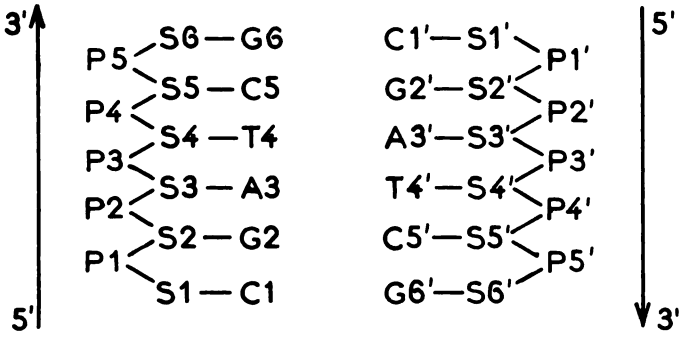


Fig. 2. The double-stranded hexamer d(CGATCG)$_2$. Base numbering.

the sugar moiety. We shall designate these last two compounds as, respectively, the methylamine and the ethylamine derivatives of DM. Throughout the series the ammonium group is held in a staggered conformation. For compounds *1–5*, the atoms H_1, H_2, and H_3 of this group are defined by the values of 180°, 60°, and −60°, respectively, of the torsion angle around bond C_3'—N in the sequence C_4'—C_3'—N—H. For compound *6*, the glucosamine analog, the corresponding torsion angle is C_3'—C_2'—N—H. For compounds *7* and *8*, the torsion angles around bonds C_3'—N and C_3'—N are defined by the sequences C_3'—C_3'—N—H and C_3'—C_3'—N—H, respectively.

The rationale behind the choice of these particular compounds, especially of molecules *2–6*, resides in their representativeness of the essential tendencies which may be produced in the antitumor activity of DM upon significant structural modifications. Thus, it appears (2, 5) that, whereas modifications at position 4 of the DM chromophore may lead to an enhanced chemotherapeutical activity, modifications at other positions tend rather to decrease this activity (10–14). The 4-demethoxy analogue of DM (compound *2*) is an outstanding example of the first case and was therefore investigated primarily. The replacement of the α-anomer by the β-anomer, the replacement of daunosamine by glucosamine, and the epimerization of the substituents at positions 7 and 9 have, on the contrary, a tendency to reduce the activity of the drugs. These structural modifications are represented by compounds *3–6*.

The reasons for the choice of compounds *7* and *8* correspond to a more ambitious endeavour. Our previous theoretical investigation on the DM-hexanucleotide complexes (7) suggested that the observed and computed location of the ammonium group was not an optimal one for its binding possibilities in the minor groove of DNA. For the d(CGATCG)$_2$ sequence used here, it was found that, whereas one DM proton (H_1) was bound to O_1' of S5 with an adequate H-bonding distance ($d_{O_1\text{—}H_1} \cong 1.8$ Å), the remaining distances with other sites of

the groove (between H_1 and O_2 of C5 and between another ammonium proton, H_3 and O_2 of T4) were elongated and not optimal (≈2.6 Å). Now, in line with a proposal put forward in Ref. 15, previous theoretical computations devoted to DNA binding of monomethylammonium (8) or of aliphatic diamines (16) showed that the optimal binding configuration of this group involves hydrogen bond formation of the three ammonium hydrogens with the "electron-rich triad" made up, in our hexamer, of O_2 of T4, O_2 of C5, and O_1· of S5. This observation incited us to search for DM derivatives in which the positioning of the cationic moiety would be modified so as to enable it to reach more effectively this most satisfactory location. It appeared to us that one way of doing so is to interpose one or two methylene groups between the sugar and the ammonium group. This is undertaken with compounds *7* and *8*.

Procedure

Intramolecular (conformational) energies. The variations of the conformational energy of the anthracyclines upon complex formation are computed in the framework of the SIBFA procedure (17). Within this methodology, the anthracycline is built of elementary constitutive fragments, separated by single bonds, and the variation of the intramolecular energy upon a conformational change is obtained as the variable part of the sum of the interactions between these fragments expressed as:

$$\Delta E_{conf} = E_{MTP} + E_{pol} + E_{rep} + E_{disp} + E_{tor} \quad (1)$$

where E_{MTP} and E_{pol} are the electrostatic and polarization contributions, computed using a multipolar expansion of the *ab initio* SCF molecular wave functions of the fragments, E_{rep} and E_{disp} are the repulsion and dispersion contributions, respectively (see Ref. 17 for details). E_{tor} is a torsional contribution, calibrated in Ref. 17 for elementary rotations around C—C and C—O bonds.

The fragments utilized to construct the anthracyclines are the daunomycinone and 4-demethoxydaunomycinone chromophores, the 9-acetoxy and 9-hydroxy substituents, and dehydroxydaunosamine and dehydroxyglucosamine, substituted both by the hydroxy groups in the appropriate positions. The fragment used to build the side chain of compounds *7* and *8* is deaminodaunosamine, substituted in its 3′-position by the additional fragments methylammonium or methane and methylammonium for *7* and *8*, respectively. Such a subdivision enables one to account for the additional conformational changes around bonds C_3—C_3· and C_3—C_3·. The ammonium groups are held in a staggered conformation. The internal geometry of the constitutive fragments (bond lengths and valence angles) is the same as in the crystal structure of the hydrochloride monohydrate salt of DM (18). A standard geometry was adopted for the glucosamine moiety (19). As indicated in the introduction, the model oligonucleotide utilized in this study is $d(CGATCG)_2$. Following our general procedure, it was constructed from its constituent fragments in the same fashion as that adopted for the computation of the molecular electrostatic potential of large macromolecules (20). These constituent fragments are the four bases, deoxyribose, and monomethylphosphate; their *ab initio* SCF wave functions were computed using our usual basis set (21). The multicenter multipole expansions of the electron densities of the constituent subunits of DNA, as those of the anthracycline fragments, were simplified according to a procedure recently developed in this laboratory (22) in which each multipole located on the center of a nonbonded pair of atoms is split between the two centers closest to it, either atom or bond barycenter.

Following the procedure used in Ref. 7, the conformation of the model hexanucleotide will be maintained in that found crystallographically in the $d(CGTACG)_2$-DM complex. The conformation of the interacting anthracyclines will, however, be reoptimized upon binding in each case, the torsional changes occurring along *all* the functional bonds. The value of ΔE_{conf} for each anthracycline will then be given as the difference between the energy of the free molecule optimized by means of energy minimization (following Ref. 23) and that of its (intramolecular) energy in its complex with the hexanucleotide.

Intermolecular interactions. The intermolecular drug-oligonucleotide interaction energies are computed by the SIBFA 2 procedure (24) as a sum of five terms:

$$\Delta E_{inter} = E_{MTP} + E_{pol} + E_{rep} + E_{disp} + E_{CT} \quad (2)$$

in which the terms E_{MTP}, E_{pol}, E_{rep}, and E_{disp} have the same meaning as in Eq. 1 and E_{CT} is the charge-transfer energy contribution. It is worth underlining that the SIBFA and SIBFA 2 procedures are calibrated so as to enable coherent simultaneous computations of intra- and intermolecular interaction energies.

The computations have been carried out in two approximations, corresponding to the absence or the presence of counterions on the oligonucleotide. In the latter approximation, one monovalent cation was bound to each phosphate group of the oligonucleotide in a configuration bridging its two anionic oxygens (for details see Ref. 21).

The search for the optimal configurations of the respective anthracycline-hexamer complexes was performed by energy minimization of the sum of ΔE_{conf} plus ΔE_{inter}. The variables involved in the minimization procedure are the dihedral angles of the investigated anthracycline together with the intermolecular variables defining its relative orientation with respect to the hexamer.

In our previous computations on DM and, also, our very recent ones on adriamycin binding to oligonucleotides (25), we have taken into account also the energy necessary to produce the intercalation site by unstacking the corresponding base pairs (term called $\Delta E_{unstack}$ in refs. 7 and 25). This was indispensable in these studies because of the variation in the base pair sequences at the intercalation site. In our present work, in which the intercalation is considered to occur, for all anthracyclines studied, at the same site in the same oligonucleotide, $\Delta E_{unstack}$ is a constant throughout all computations. Its introduction would thus have no effect on the essential feature of the results considered here, namely, the relative ordering of δE or δ values. It is therefore omitted (for those interested it may be stated that it amounts to 10.5 kcal/mol for an intercalation site between two alternating G—C base pairs (7).

Results and Discussion

The results of the computations on the anthracycline-DNA complexes are reported in Tables 1 and 2. Table 1 lists, for all investigated compounds, the intermolecular interaction energy ΔE_{inter} and its components, the conformational energy change of the anthracycline, ΔE_{conf}, the resulting energy balance $\delta E = \Delta E_{inter} + \Delta E_{conf}$, and the difference δ of these overall energy balances with respect to DM taken as energy zero, which is the most significant quantity for our considerations. For illustrative purposes, we have also reported the values of ΔE, E_{MTP}, δE, and δ obtained in the presence of counterions for the most significant cases. The values obtained in this condition are denoted by *asterisks* in Table 1. Also reported in Table 1 are the available binding constants to DNA which were measured experimentally.

The results given with superscript *b* denote (for compounds *1–5*) the concentrations (IC_{50}, in μM) of the compound necessary to inhibit the activation of 50% *Escherichia coli* DNA polymerase (taken from Ref. 11). These concentrations are inversely proportional to the binding affinities. The explicit values of the affinity constants, K_a, were not given in Ref. 11, but their ordering was indicated as: $1 \geq 2 > 3 > 4 > 5$, which is the same as that of the inverse values of the IC_{50}.

Table 2 lists the most significant interatomic distances found

TABLE 1
Interaction energies of compounds *1–8* with d(CGATCG)$_2$[a]

	1 DM	*2* 4-Demethoxy DM	*3* β-Anomer of 4-demethoxy DM	*4* 4-Demethoxy-7,9-bis-epi DM	*5* β-Anomer of 4-demethoxy-7,9-bis-epi DM	*6* Glucosamine analog of DM	*7* Methylamine DM	*8* Ethylamine DM
ΔE_{inter}	−433.9	−435.5	−401.7	−383.7	−371.4	−411.4	−450.1	−453.1
E_{MTP}	−369.2	−371.8	−344.4	−350.1	−344.0	−350.6	−381.6	−380.7
E_{pol}	−20.3	−20.6	−18.6	−22.3	−19.3	−19.2	−21.9	−22.6
E_{CT}	−14.0	−13.4	−14.6	−9.8	−5.5	−14.6	−13.4	−15.2
E_{disp}	−86.4	−84.2	−75.6	−37.2	−46.0	−82.4	−87.1	−93.1
E_{rep}	56.1	54.6	51.5	35.8	43.2	55.4	53.8	58.4
ΔE_{conf}	9.7	9.8	3.4	8.4	8.1	8.0	7.5	23.6
δE	−424.2	−425.7	−398.3	−375.3	−363.3	−403.4	−442.6	−429.5
δ	0.0	−1.5	25.9	48.9	60.9	20.8	−18.4	−5.3
ΔE^*_{inter}	−147.6	−149.9	−118.9			−124.2	−160.7	−163.5
E^*_{MTP}	−91.4	−94.1	−71.3			−73.9	−100.9	−100.4
δE^*	−137.9	−140.1	−115.5			−116.2	−153.2	−139.9
δ^*	0.0	−2.2	22.4			21.7	−15.3	−2.0
IC_{50} (μM)	18[b]	18	38	50	>100			
K_a (M^{-1})	3.3×10^{6}[c] 3.3×10^{6}[d]	4.4×10^{6}				$71. \times 10^{4}$[d]		

[a] See text for definitions. Energies are in kcal/mol.
[b] Ref. 11.
[c] Ref. 5.
[d] Ref. 10.

TABLE 2
Interatomic distances (Å) of ammonium and hydroxy hydrogens with binding sites in d(CGATCG)$_2$

	1 DM		*2* 4-Demethoxy DM		*3* β-Anomer of 4-Demethoxy DM		*4* 4-Demethoxy-7,9-bis-epi DM		*5* β-Anomer of 7,9-bis-epi DM	
9-OH	H_1—$O_{1'}$ (S5)	1.83	H_1—$O_{1'}$ (S5)	1.83	H_1—N_3 (A3′)	2.77	H_1—$O_{5'}$ (P4)	2.15	H_1—$O_{5'}$ (P4)	2.04
	H_1—O_2 (C5)	2.68	H_1—O_2 (C5)	2.68	H_1—$O_{1'}$ (S4′)	2.20	H_1—O_2 (P4)	2.55	H_1—O_2 (P4)	2.26
	H_3—O_2 (T4)	2.58	H_3—O_2 (T4)	2.58	H_3—$O_{1'}$ (S4′)	2.58	H_3—O_2 (P5)	1.75	H_2—O_2 (P4)	2.75
	H—N_3 (G2′)	2.73	H—N_3 (G2′)	2.73	H—N_3 (G2′)	2.48	H_3—O_1 (P5)	2.72	H_1—O_1 (P4)	2.77
	O—HN_2 (G2′)	2.33	O—HN_2 (G2′)	2.33	O—HN_2 (G2′)	2.12			H_2—O_1 (P4)	2.26

	6 Glucosamine analog of DM		*7* Methylamine DM		*8* Ethylamine DM	
9-OH	H_1—$O_{1'}$ (S5)	1.92	H_3—$O_{1'}$ (S5)	2.08	N_3—N_3 (A3′)	2.84
	H_1—O_2 (C5)	2.93	H_2—O_2 (C5)	2.70	H_1—O_2 (T4′)	1.98
			H_1—O_2 (T4)	2.75	H_3—$O_{1'}$ (S4′)	2.82
			H_2—O_2 (T4)	1.99		
	H—N_3 (G2′)	2.79	H—N_3 (G2′)	2.68	H—N_3 (G2′)	2.74
	O—HN_2 (G2′)	2.44	O—HN_2 (G2′)	2.31	O—HN_2 (G2′)	2.29
	3′-OH (on glucosamine)					
	H—N_3 (A3′)	2.26				

in the optimized complexes between the hydrogen atoms of the interacting anthracyclines and heteroatoms of the hexanucleotide. Fig. 3 (A–C) displays the representations of the optimized structures of the most significant hexanucleotide complexes, namely, those of *1*, *7*, and *8*. These figures were drawn with the help of the FIGATOM program (26) for drawing stereoscopic views using a graphic plotter.

The results of Table 1 indicate the following ordering of anthracycline affinities δ for the investigated hexanucleotide:

$$7 > 8 > 2 \geq 1 \text{ (DM)} > 6 > 3 > 4 > 5.$$

This ordering is the same as that of the corresponding ΔE_{inter} values, except for the relative position of compounds *7* and *8*: the more favorable value of ΔE_{inter} for the latter is compensated by a distinctly less favorable value of its ΔE_{conf}. In contrast, the ordering of ΔE_{inter} runs parallel to the ordering of the corresponding E_{MTP} values, which is, by and large, the preponderant contribution to the binding. In the presence of counterions (the *asterisk* values), the numerical values of ΔE and E_{MTP} are considerably reduced, but the ordering of anthracycline affinities remains the same with the exception of a minor inversion between compounds *8* and *2*.

The most striking observation relevant to the results of Table 1 is the division of the derivatives studied into two categories: those whose affinity to the model oligomeric DNA is predicted to be greater than that of the parent DM and those whose affinity is predicted to be smaller. The first group, which interests us most, includes the 4-demethoxy derivative and the two hypothetical analogs in which one or two methylene groups have been interposed between the NH_3^+ group and the sugar moiety. The second group illustrates the negative effect on the affinity of the other structural modifications considered.

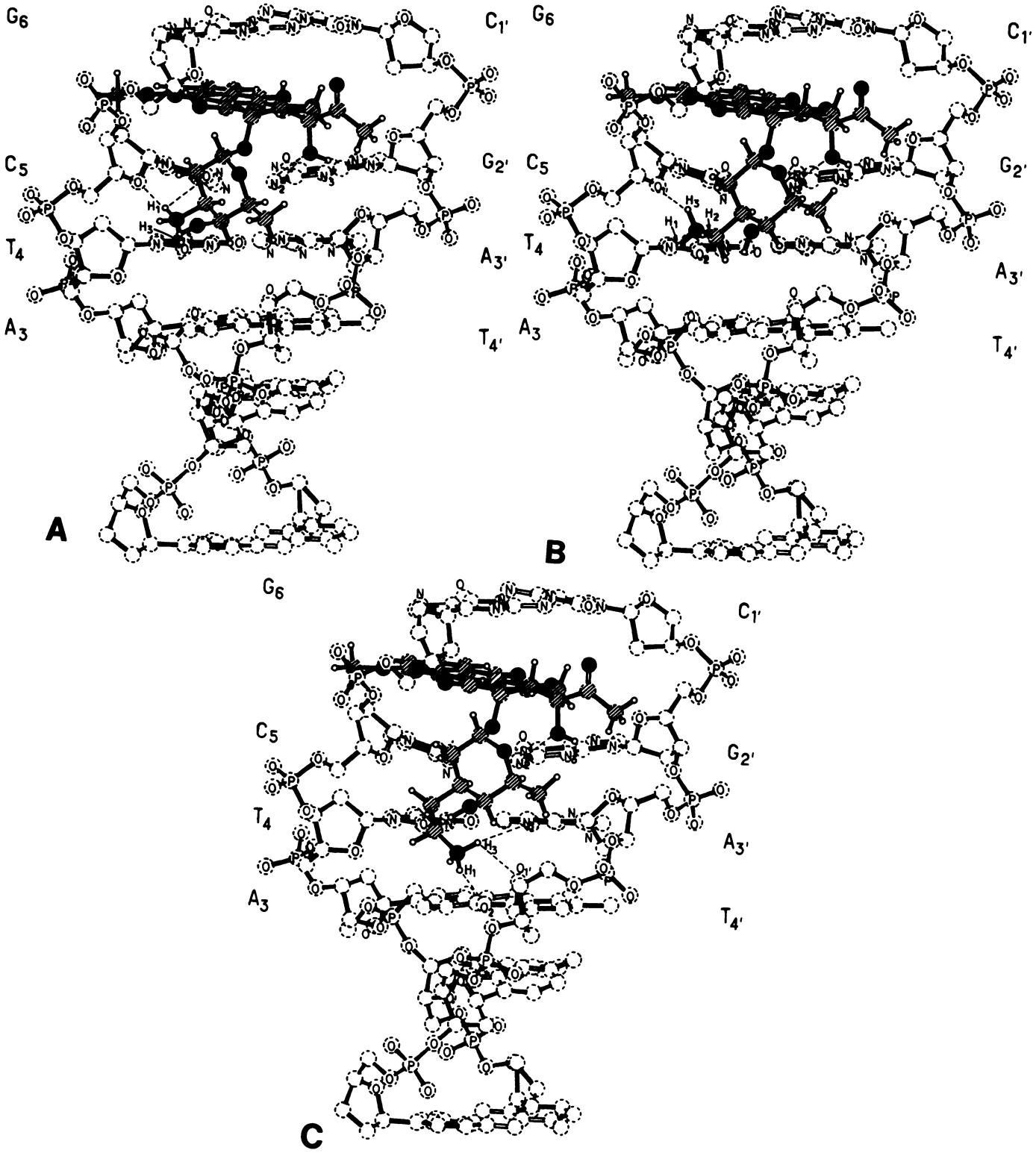


Fig. 3. Representation of the complexes of compounds *1* (A), *7* (B), and *8* (C) with d(CGATCG)$_2$.

A glance at Table 1 immediately shows that this division is determined by the different evolution of the most significant components of the interaction energy. Thus, ΔE_{inter} is more negative in compounds *2*, *8*, and *7* than it is in DM, whereas the reverse happens with compounds *5*, *4*, *3*, and *6*. It is paralleled by a similar evolution of the E_{MTP} component of the interaction energy.

To rationalize the evolution of the binding affinities of the investigated anthracyclines, we shall concentrate in the ensuing discussion on the configurational characteristics of the different complexes at the outcome of this energy minimization, as expressed by the most relevant anthracycline-DNA interatomic distances. We shall discuss the eight anthracycline complexes, following the order of their decreasing binding affinities δE.

The interaction of compound *7*, the most strongly bound methylamine derivative, with the hexamer is stabilized by two hydrogen bonds which involve atoms H_2 and H_3 of the ammonium group with O_2 of T4 and $O_{1'}$ of S5, respectively, the corresponding distances being 2.00 and 2.08 Å. Two additional, although elongated (2.7–2.8 Å), H-bonds occur between H_1 and H_2 of the ammonium and O_2 of T4 and O_2 of C5, respectively. When compared to the situation in DM (7), this result indicates that the lengthening of the cationic arm with respect to the parent compound has enabled its closer approach to the O_2 atom of T4, although at a cost of somewhat more elongated distances of the ammonium protons to $O_{1'}$ of S5. The change in stereochemistry is energetically profitable as the closer approach of the cationic head to O_2 of T4 results in an appreciable increase of the interaction energy of compound *7* as compared to compound *1*, amounting to 16.2 and 13.1 kcal/mol, in computations with the unscreened and screened phosphates, respectively. In addition, the cationic head is now more deeply imbedded in the core of the minor groove, in which the electrostatic potential reaches its highest values (27, 28). A previous study devoted to diarylamidine binding to a $(dA\text{-}dT)_{11}$ oligonucleotide indicated, in line with this result, that the deeper the cationic end in the minor groove, the more optimal the binding affinity (29).

Additional H-bonding interactions between compound *7* and the oligonucleotide involve the 9-OH oxygen and hydrogen atoms with one hydrogen of the 2-amino group and N_3 of base G2′, respectively (see Table 2). The range of distances is close to the corresponding range of distances observed in the DM complex.

A further lengthening of the cationic arm by one additional methylene to obtain compound *8* brings the NH_3^+ moiety still closer to heteroatoms O_2 of T4, O_2 of C5, and $O_{1'}$ of S5. We have derived and energy-minimized a configuration in which H_1 is bound to O_2 of T4, H_2 to O_2 of C5, and H_3 is shared by O_2 of C5 and $O_{1'}$ of its sugar S5, the respective distances being (in Å) 1.67, 2.56, 2.35, and 1.98. This configuration (not listed in Table 1) is, however, disfavored with respect to DM by an amount of 14.4 kcal/mol, due to a severe E_{rep} term (86.3 kcal/mol compared to 56.1 kcal/mol in DM) and a significantly more unfavorable ΔE_{conf} term (22.1 kcal/mol as compared to 9.7 kcal/mol in DM). In addition, the two hydrogen bonds, involving the 9-OH substituent on the one hand and the N_3 and 2-amino group of G2′ on the other, are substantially weakened, the distances $d_{H\text{—}N_3}$ and $d_{O\text{—}H(N_2)}$ being 3.22 and 2.67 Å, respectively, as contrasted to 2.73 and 2.33 Å in the DM complex. Another distinctly more stable configuration (listed in Table 1) can, however, be obtained for compound *8* by a marked but nevertheless advantageous conformational rearrangement along bonds $C_7\text{—}O_7$, $C_{3'}\text{—}C_{3'}$ and $C_3\text{—}C_{3'}$. In this configuration, the ammonium group interacts through H_1 with O_2 of base T4′ of the primed strand (distance 1.98 Å). Additional stabilization occurs by means of two elongated H-bonds involving H_3 and N_3 of A3′ as well as $O_{1'}$ of S4′. The two H-bond distances involving the 9-OH substituent and the G2′ base have values similar to those found for compound *7*. It is this configuration associated with a binding energy for the oligomeric DNA greater than that of DM which is listed in Table 1. It is interesting to note that ΔE_{inter} is slightly greater for compound *8* than for compound *7*, but that *8* is, however, altogether disfavored with respect to *7* owing to a much greater cost in conformational deformation. The methylammonium derivative of DM, compound *7*, is thus predicted to have the greatest DNA affinity among all of the compounds investigated.

The third derivative whose binding to DNA is found to be stronger than that of DM is its 4-demethoxy derivative, *2*. The interactions of compounds *1* and *2* with the hexamer are altogether similar and stabilized, at the level of the cationic head, by one H-bond between H_1 and $O_{1'}$ of S5 and two rather elongated H-bonds between H_1 and H_3 and O_2 of C5 and T4, respectively. The 9-OH substituent interacts by means of both its O and H atoms with one H of the 2-amino group and N_3 of base G2′, respectively, and such a double interaction is a prominent feature of the DM-$d(CGTACG)_2$ complex as elucidated by X-ray diffraction (4).

The more favorable value of ΔE found for compound *2* rather than for *1* is due to a slightly greater E_{MTP} contribution and a slightly smaller E_{rep} term which spring from a somewhat more favorable stacking interaction of the chromophore with the $(CG)_2$ subunit upon removal of the 4-methoxy substituent.

Our theoretical result on the increased binding affinity of compound *2* with respect to that of DM is in remarkable agreement with the recent findings of Arcamone and collaborators (5) who have established that, among the many DM analogs whose association with DNA has been investigated experimentally, the 4-demethoxy derivative is one of the few which show an *increased* affinity and thermal stabilization of DNA. These authors call this derivative a "high affinity" one.

The remaining derivatives of Table 1, compounds *3–6*, all show a significantly decreased complexation energy with the model oligonucleotide, compared to DM.

The complex of derivative *6*, which comprises a glucosamine sugar instead of daunosamine, shows, at the level of its cationic head, only one H-bond between H_1 and $O_{1'}$ of S5. An additional stabilizing interaction occurs between the hydrogen atom of 3′-OH on the sugar and N_3 of A3′ in the primed strand. The double hydrogen bonding interaction between the 9-OH substituent and G2′ is maintained but with some lengthening in the relevant H-bond distances. The strong decrease of the interaction energy of this derivative with respect to compound *1* stems predominantly from the fall of the E_{MTP} component of ΔE_{inter}. The theoretical finding that the replacement of the daunosamine sugar by a glucosamine is detrimental to the binding affinity of the anthracycline to DNA is in agreement with experimental results of Zunino *et al.* (10).

A very significant decrease of the binding affinity is observed upon the modification of the stereochemistry of the glycoside linkage by changing from the α- to the β-anomer to obtain compound *3*.

Compared to compound *1* or *2*, compound *3* has a significantly smaller ΔE_{inter}, due to a decrease of E_{MTP}, E_{pol}, and E_{disp}. Its DNA complex involves H-bonding interactions between the ammonium group and two sites on the *primed* strand, N_3 of A3′ and $O_{1'}$ of S4′. This is in distinct contrast to the configurations optimized for compounds *1*, *2*, and *7*, for which the ammonium end interacted with the unprimed strand but is reminiscent somewhat of the configuration derived for compound *8*. We observe (see Table 2) that H_1 is shared between N_3 of A3′ and $O_{1'}$ of S4′ (the H-N_3 bond being rather elongated), the latter heteroatom interacting also with H_3. The two hydrogen-bonding interactions of the 9-OH group with G2′ remain unaltered. Conversely, no hydrogen-bonding interac-

tions occur with O_2 of T4′, in contrast to the results obtained with compound *8*. The cationic head probes less deeply into the core of the minor groove with *3* than with *8* which, according to Ref. 29, is prejudicial to the binding affinity.

The marked predicted decrease in the DNA binding affinity upon inverting the configuration of the daunosamine sugar from an α-anomer to a β-anomer is again in line with experimentation (10, 11). This decrease is computed to be more severe than the one incurred by the daunosamine-to-glucosamine replacement. To our knowledge, there are no experimental results of comparative binding constants of *6* versus *3* to check this point.

We may now consider the two least stable derivatives, *4* and *5*, which involve epimerization at positions 7 and 9.

The drastic reduction in the binding energies of these compounds springs from a number of factors. In the hexanucleotide complexes of compounds *1–3* and *6–8*, the sugar fragment was located in the minor groove, in conformity to experimental (4–6, 30) and theoretical (7, 31) results of studies of DM-DNA interactions. In marked contrast, the optimal binding configurations predicted for compounds *4* and *5*, the 7,9 bis-epimers of compounds *2* and *3*, respectively, locate the ammonium moiety at the periphery of the major groove, complexes with the sugar side chain in the minor groove being markedly disfavored by intermolecular repulsions. In the optimized complex of *4*, the ammonium group bridges phosphates P4 and P5 and establishes short hydrogen bonds with O_1 (P4) and $O_{5'}$ (P4). In the optimized complex of *5*, three hydrogen bonds are established, involving O_1, O_2, and $O_{5'}$ of P4. The interaction energies for the stacking of the chromophore with the two G-C base pairs are dramatically reduced, to −3.1 and −5.0 kcal/mol for compounds *4* and *5* respectively, whereas, in the complex of the parent compound *1*, this energy amounts of −15.4 kcal/mol. These decreases are reflected predominantly in the corresponding values of E_{disp}, which are of −5.7, −14.6, and −36.0 kcal/mol in the complexes of *4*, *5*, and *1*, respectively, for the interactions of the chromophore with the two G-C base pairs of the intercalation site. The decrease in E_{disp} of *4* and *5* is paralleled also by a much less favorable E_{MTP} contribution due to the fact that, in an oligonucleotide of sufficient length, the electrostatic contribution is stronger in the grooves than at the periphery or on the phosphates (27).

Altogether, the values of ΔE_{inter} computed for compounds *4* and *5*, are thus considerably diminished when compared to the value optimized for compound *1*. Those of ΔE_{conf} are only slightly smaller. This results in values of δ of 48.9 and 60.9 kcal/mol for compounds *4* and *5*, respectively. This decrease of affinity upon epimerization is also in agreement with experimental results of Ref. 11.

Summary and conclusions. The present computations are thus able to reproduce satisfactorily the experimental ordering of binding affinities of a series of representative selected DM derivatives. They correctly account for the enhancing effect on the affinity of the suppression of the OCH_3 group at position 4 of the DM chromophore and, on the contrary, the decreasing influence on the activity of: 1) the change in the stereochemistry of the glycoside linkage from the α- to the β-anomer, 2) the replacement of daunosamine by glucosamine, and 3) the epimerization of positions 7 and 9. They account even satisfactorily for the relative ordering of these three last effects: in agreement with the computations, the experimental binding affinities to DNA are in the order (5, 6, 11):

$$2 > 1 > 3 > 4 > 5 \quad \text{and} \quad 1 > 6$$

Moreover, we have been able to conceive two compounds for which we compute a greater binding affinity to DNA than that of *1* or *2*: these are derivatives of DM in which the cationic NH_3^+ group at position 3′ of the sugar is replaced by CH_2—NH_3^+ or CH_2—$CH_2NH_3^+$, with the strongest enhancing effect predicted for the former of the two. To our knowledge, synthesis of derivatives *7* and *8* has not been reported to date. We believe that the present prediction of their enhanced DNA binding properties with respect to DM should provide a stimulating challenge to attempt their synthesis and, subsequently, to undertake experimental studies, both on their DNA binding affinities and their antitumor properties. Although the correlation between these two latter properties of anthracyclines is not always completely consistent (32), a recent work by Arcamone and collaborators (5) points to a far reaching general overall parallelism between them.

However, two precautions need to be expressed concerning our results in their present stage of elaboration.

The first precaution relates to the neglect of hydration in the energy balances, a factor probably responsible for the exaggerated values of the δE values, either positive or negative, computed for compounds *2–8*. We intend to implement solvation/desolvation effects in these balances as the next step of our treatment, particularly in connection with the prediction of the enhanced binding affinities of *7* and *8* with respect to *1*.

The second precaution pertains to the relevance of the conformation of the hexanucleotide $d(CGTACG)_2$ in its complex with DM in the crystal state, to its conformation and, thus, to that of the complexes in solution and, more generally, to the conformation of the crucial d(TpCpG) or d(ApCpG) triplets imbedded in a B-DNA structure. It may be questioned whether the crystal structure (which actually comprises *two* DM molecules intercalated between the two top and the two bottom base pairs) is a satisfactory representation of the situation in solution. Until a *detailed* structure for the DM-$d(CGTACG)_2$ or DM-$d(CGATCG)_2$ complexes in a 1:1 stoichiometry is provided (e.g., by two-dimensional NMR in solution), the adoption of the crystal structure seems the most realistic assumption. The fact that the binding affinity ordering of compounds *1–6* compares satisfactorily with the experimental ordering of their affinity constants lends support to the validity of the model adopted. The introduction of the flexibility of oligomeric DNAs is another future goal of our computations (33).

Finally, it may be contended that this study has concentrated on the sole sequence $d(CGATCG)_2$ and that, thus, its relevance with respect to experimental binding studies to DNA, in which a manifold of random sequences at a triplet level can appear as candidates for binding sites, needs to be reaffirmed. The predominance for strong binding of the triplets d-(TCG) or d-(ACG) is, however, such (7) that the significance of our present results seems to us, from that point of view, highly probable.

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