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ARTICLE *in* THE JOURNAL OF PHYSICAL CHEMISTRY B · APRIL 2008

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Design of a New Warhead for the Natural Enediyne Dynemicin A. An Increase of Biological Activity

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Received: September 13, 2007; In Final Form: October 29, 2007

A concept for designing nontoxic enediyne-based antitumor drugs that was previously suggested (*J. Am. Chem. Soc.* **2000**, *122*, 8245) is converted into reality by merging amidines with the natural enediyne dynemicin A. The dynemicin−amidines (DADs) resulting from this combination are biologically not active because they form extremely labile singlet biradicals that can no longer abstract H from DNA. However, if protonated in the acidic environment of the tumor cell, they possess increased biological activity, as is reflected by a lowering of the activation enthalpy for the Bergman cyclization from 16.7 (dynemicin A) to 11−12 kcal/mol (DADs), kinetic stability of the singlet biradicals formed in the cyclization reaction, increased H abstraction ability of the singlet biradicals, and improved docking properties in the minor groove of the duplex 10-mer B-DNA sequence d(CTACTACTGG)·d(CCAGTAGTAG) throughout the triggering and Bergman reactions. The implications and the consequences of using DADs to exploit the differences between normal and tumor cells and to design a nontoxic antitumor drugs are discussed.

I. Introduction

Naturally occurring enediynes such as dynemicin A, calicheamicin, esparamicin, kerdarcidin, or neocarzinostatin are known for their high biological activity.[1−5] They are able to bind to the minor groove of DNA, to rearrange if properly triggered to a compound that undergoes Bergman cyclization,[6,7] and to form a highly reactive singlet biradical out of the class of the *p*-didehydrobenzenes (*p*-benzynes)[8] or the α,3-dehydrotoluenes.[9] The biradicals can in turn abstract H from a proximal DNA sugar backbone, which initiates DNA cleavage and cell death (apoptosis).[1−5] There has been a considerable amount of research to exploit the biological activity of the natural enediynes as the basis for a powerful antitumor drug.[1−3,10−12] However all attempts in this direction are hampered by the fact that enediynes are toxic;[1] that is, they cause apoptosis in both the tumor cell and the normal cell.

In a recent project, which comprised the investigation of the parent enediyne ((*Z*)-3-hexene-1,5-diyne (**1**), Scheme 1),[13,14] the spectroscopic identification of its biradical *p*-benzyne (**2**, Scheme 1),[8] and the Bergman cyclization of analogous compounds,[15,16] we investigated the possibility of converting the active part of the enediyne (the warhead) in such a way that its reactivity becomes pH dependent.[17] These studies were based on the fact that in a tumor cell the pH value is somewhat lower than that in the normal cell (6.6 vs 7.2).[18−22] Moreover, the pH can be further reduced to 5.5 by administering ionophores such as amiloride,[20] by invoking hyperglycemia (injection of glucose),[19] causing hyperthermia (local heating by microwaves),[20] or applying monocarboxylate transport inhibitors.[21] With the small pH difference of $7.2 - 5.5 = 1.7$, a suitable enediyne warhead with a pH detector can be triggered in the weakly acidic medium of the tumor cell and generate the active biradical.

Our previous investigations[15−17] focused on designing a suitable warhead that is sensitive to the pH value of the tumor cell. It was found that by including an amidine into the warhead, leading to a *C*,*N*-dialkinylamidine, **3** (Scheme 1), one interrupts π-conjugation in the enediyne unit by a formal N−C single bond, thus reducing its tendency to undergo a Bergman cyclization via **TS(3−4)** to form the singlet biradical **4-S**. In Scheme 1, it is also indicated that **4-S** may not be stable and rearrange immediately via a retro-Bergman reaction, that is, via **TS(4−5)**, to cummulene **5**.[17] The tendency of undergoing a Bergman reaction can be restored for amidine **3** by protonation, yielding **6** (Scheme 1). Amidinium cation **6** possesses an enediyne structure and forms via cyclization the biradical **7-S**, which should not easily open to the enediyne **8**. Accordingly, it should possess a sufficiently long lifetime to abstract H atoms from DNA.

The activation enthalpies of the Bergman cyclization of model enediynes **1**, **3**, and **6** have been calculated to be between 27 and 31 kcal/mol,[14,17,22] and where experimental results were available (**1**),[22] the calculated and measured values were in good agreement. Therefore the reaction is too slow to be observed at room temperature for these compounds. However, it is possible to lower the barrier to cyloaromatization by applying the strain principle:[13a,23]

Incorporation of the enediyne unit into a strained ring system raises the energy of the reactant. In the TS of the Bergman cyclization there is always a release of strain by forming the benzene unit. As a net effect a lower barrier results.

Natural enediynes such as dynemicin A or calicheamicin γ_1^I contain in their triggered form the warhead in a 10-membered ring, which is strained in various ways thus effectively lowering the barrier to Bergman cyclization.[24−27] In the cases of **3** or **6**, incorporation in a 10-membered ring does not suffice to reduce the barrier significantly.[17] However as soon as a double bond is included as in **9** or **12** (Scheme 1), the barrier to Bergman cyclization is substantially lowered.[17]

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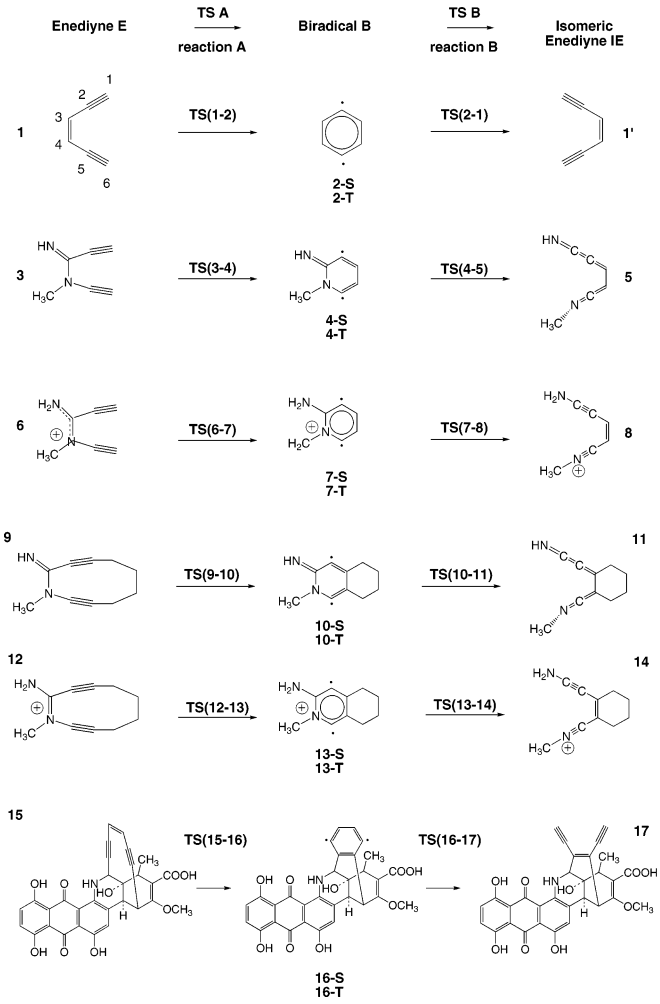
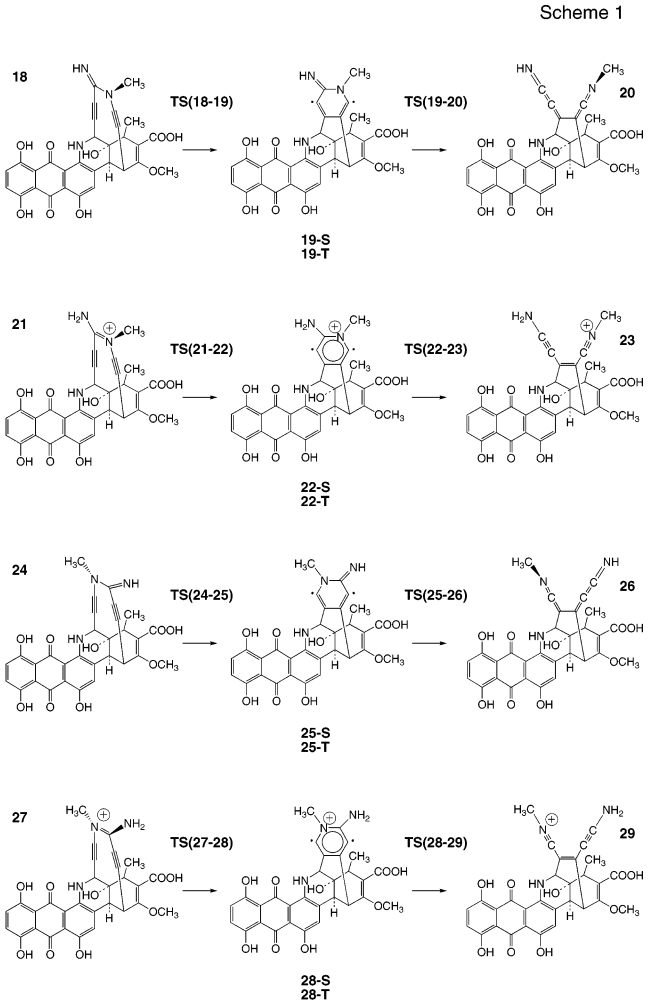
10.1021/jp0773536 CCC: $40.75
Published on Web 02/13/2008

SCHEME 1





Our previous investigations focused on the energetics of the Bergman cyclization of amidines and amidinium cations **3**, **6**, **9**, or **12**.[15−17] Despite the fact that these compounds represented promising pH-dependent warheads, it was not known how insertion of these warheads into a naturally occurring enediyne would change their properties. In this work, we will investigate the reactivity of a natural enediyne for which the enediyne unit has been exchanged by an amidine unit. Since we have recently described the biological activity of dynemicin A (**15**, Scheme 1) in detail,[25,26] this compound is a suitable candidate for making a nontoxic antitumor lead based on a native enediyne.

There are two amidines and amidinium cations that can be obtained by the merging of dynemicin A with either **3** or **6**: In **18** and in the protonated molecule **21** (Scheme 1) the imine N is at the same side of N1 (for numbering, see Scheme 2) whereas in **24** and in the protonated molecule **27** (Scheme 1) the imine N is at the opposite side side of N1.

Dynemicin-amidines (DADs) **18** and **21** investigated in this work are considered as prototypes of a new class of nontoxic antitumor drugs insofar as they fulfill a number of important prerequisites needed for the final drug. Nonetheless, unsubstituted amidines cannot represent the actual target of the design goal because they are sensitive to hydrolysis, which however can be avoided by appropriate substitution at the imino N atom.[28,29] In this connection, it has to be borne in mind that the pK_a values of amidines stretch over a large range (4.4−11) in dependence of their substitution pattern;[28,29] i.e., once they have been found as suitable headgroups they can always be fine-tuned to react at the actual pH value of a cancer cell. Before we consider these questions, we have to clarify for simple representatives such as **18** and **21** whether the basic requirements formulated by Kraka and Cremer[17] are fulfilled for a DAD. Does the incorporation of the amidine warhead in a naturally occurring enediyne such as dynemicin A change its properties with regard to (a) carrying out the Bergman cyclization, (b) forming a kinetically stable but at the same time highly reactive singlet

SCHEME 2

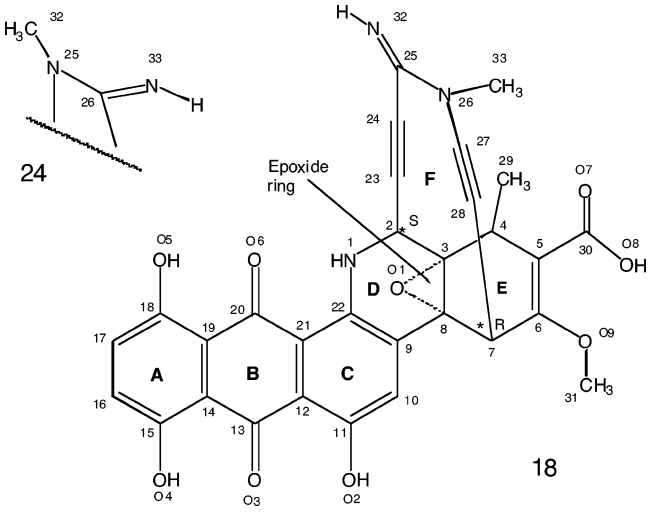


TABLE 1: Energies *E*, Enthalpies *H*, Free Enthalpies *G*, and Dipole Moments μ Calculated along the Reaction Path of the Bergman Cyclization of DADs 18 and 24[a]

molecule	R/U	ref	ΔE	$\Delta H(298)$	$\Delta G(298)$	μ
			B3LYP/3-21G			
18	R		−1987.21348	−1986.68514	−1986.78529	3.7
TS(18−19)	R	**18**	17.0	14.9	16.6	4.1
19S	B-U	**18**	9.2	7.7	9.1	4.7
19T	U	**19-S**	4.5	4.0	3.9	4.4
TS(19−20)	R	**19-S**	2.7	1.8	3.0	5.2
20	R	**19-S**	−35.9	−35.2	−38.3	4.5
			B3LYP/6-31G(d)//B3LYP/3-21G			
18	R		−1998.14881	−1997.62047[b]	−1997.72062[b]	3.0
TS(18−19)	R	**1**	16.5	14.4	16.1	
19S	BS−U	**18**	4.1	2.6	4.0	4.2
19T	U	**19-S**	2.8	2.3	2.2	3.9
TS(19−20)	R	**19-S**	3.7	2.8	4.0	4.0
20	R	**19-S**	−31.7	−31.0	−34.1	3.5
			BD(T)/cc-pVDZ;B3LYP/3-21G//B3LYP/3-21G[c]			
18	R		−1988.15523	−1987.62689[b]	1987.72704[b]	
TS(18−19)	R	**18**	14.2	12.1	13.8	
19-S	U	**18**	3.4	1.9	3.3	
19-T	U	**19-S**	4.6	4.1	4.0	
TS(19−20)	R	**19-S**	0.0	−0.9	0.3	
20	R	**19-S**	−21.9	−21.2	−23.4	
			B3LYP/3-21G			
24	R		−1987.212991	−1986465[b]	−1986.78480[b]	
TS(24−25)	R	**24**	17.8	15.7	17.4	
25-S	U	**24**	10.0	8.5	9.9	
25-T	U	**25-S**	3.8	3.3	3.2	
TS(25−26)	R	**25-S**	*d*			
26	R	**25-S**	−38.3	−37.6	−40.7	
			B3LYP/6-31G(d)//B3LYP/3-21G			
24	R		−1998.148574	−1997.620234[b]	−1997.72038[b]	
TS(24−25)	R	**24**	17.4	15.3	17.0	
25-S	U	**24**	3.9	2.8	3.8	
25-T	U	**25-S**	3.1	2.6	3.4	
TS(25−26)	R	**25-S**	*d*			
26	R	**25-S**	−31.7	−31.0	−34.1	

[a] Absolute energies and enthalpies in hartree, relative energies and enthaplies in kcal/mol, and dipole moments in debye. R, U, or BS-U indicate whether restricted, unrestricted, or broken-symmetry unrestricted DFT was used. ref denotes which molecule is used as a reference for calculated energy (enthalpy) differences ΔE (ΔH, ΔG). [b] Thermochemical corrections calculated at the B3LYP/3-21G level of theory. [c] ONIOM2 calculations using a BD(T) description of the warhead (six carbon and two hydrogen atoms) and a B3LYP/3-21G description of the remainder of the molecule. [d] Not found

biradical, (c) docking into the minor groove, and (d) the ability to abstract H from proximal DNA groups.

We will describe in the following the computational methods and strategies used. In section 3, we will first investigate the energetics of the Bergman cyclization of the new DADs **18** and **21** and their protonated counterparts. On the basis of this analysis we will investigate the docking properties of the DADs in section 4. Finally, in section 5 we will discuss the relevance of the results for the design of the first nontoxic enediyne antitumor drugs based on the DAD principle.

2. Computational Methods

As in the previous work on dynemicin A,[26] we use both density functional theory (DFT)[30,31] and wave function theory (WFT) to describe the new ligands whereas molecular mechanics is applied to describe the receptor and the docking process. The cycloaromatization of the ligands was calculated employing the hybrid functional B3LYP[32,33] in connection with Pople's 3-21G[34] and 6-31G(d) basis sets.[35] Results were tested with a two-layer ONIOM (ONIOM2) description.[36] For this purpose the reaction complex was partitioned into (i) a kernel comprising the six enediyne carbon atoms 23 to 28, N32, C33, and the H atoms sitting at the latter two atoms (Scheme 2) and (ii) a periphery comprising the rest of the DAD investigated (Scheme 1). The kernel was described with coupled cluster theory using Brueckner orbitals,[37] Dunning's cc-pVDZ basis set,[38] and all double excitations. Triple excitations were included in a perturbative manner thus leading to BD(T), which is known to yield very accurate results.[39] The rest of the molecule was described at the B3LYP/3-21G or B3LYP/6-31G(d) levels of theory. The separation between kernel and periphery was accomplished by replacing C2 and C7 by H atoms. The ONIOM2 energy was obtained with the help of eq 1

$$E(\text{ONIOM2}) = E(\text{BD(T),kernal}) + E(\text{B3LYP,Real}) - E(\text{B3LYP,kernal}) \quad (1)$$

where E(B3LYP,Real) is the energy of the total molecule calculated with the lower level method.

Frequency calculations were carried out to obtain zero point energies, entropies $S(298)$, as well as thermal corrections and to characterize the stationary points along the reaction path as minima or first-order TSs (one single imaginary frequency). The thermal corrections were used to obtain activation enthalpies $\Delta H^a(298)$, reaction enthalpies $\Delta H_R(298)$, and singlet−triplet splittings $\Delta H(S-T)$ at 298 K as well as the corresponding free energies $\Delta G^a(298)$ or $\Delta G_R(298)$. Since the calculated values at 298 K hardly change when recalculated for body temperature

TABLE 2: Energies *E*, Enthalpies *H*, Free Enthalpies *G*, and Dipole Moments μ Calculated along the Reaction Path of the Bergman Cyclization of Protonated DADs 21 and 27[a]

molecule	R/U	ref	ΔE	$\Delta H(298)$	$\Delta G(298)$	μ
			B3LYP/3-21G			
21	R		−1987.60858	−1987.06693	−1987.16619	17.0
TS(21−22)	R	**21**	19.0	16.7	17.5	14.4
22-S	BS-U	**21**	1.5	−0.2	1.4	12.7
22-T	U	**22-S**	2.4	2.2	1.3	12.3
TS(22−23)	R	**22-S**	12.7	11.6	10.0	13.4
23	R	**22-S**	−38.1	−36.6	−41.0	16.5
			B3LYP/6-31G(d)//B3LYP/3-21G			
21	R		−1998.53497	−1977.99332[b]	−1998.09258[b]	
TS(21−22)	R	**21**	15.9	13.6	14.4	
22-S	BS-U	**21**	−4.9	−6.6	−5.0	
22-T	U	**22-S**	2.8	2.6	1.7	
TS(22−23)	R	**22-S**	16.1	15.0	13.4	
23	R	**22-S**	−29.3	−27.8	−32.2	
			BD(T)/cc-pVDZ;B3LYP/3-21G//B3LYP/3-21G[c]			
21	R		−1988.53805	−1987.99640[b]	−1988.09566[b]	
TS(21−22)	R	**21**	12.3	10.0	10.8	19.0
22-S	U	**21**	−7.5	−9.2	−7.6	18.9
22-T	U	22-S	5.2	5.0	4.1	
TS(22−23)	R	22-S	15.8	14.7	13.1	
23	R	22-S	−18.1	−16.6	−21.0	
			B3LYP/3-21G			
27	R		−1987.60516	−1987.063510[b]	−1987.162770[b]	
TS(27−28)	R	**27**	18.7	16.4	17.2	
28-S	BS-U	**27**	−0.8	−2.5	−0.9	
28-T	U	**28-S**	2.7	2.5	1.6	
TS(28−29)	R	**28-S**	14.8	13.7	12.1	
29	R	**28-S**	−38.3	−36.8	−41.2	
			B3LYP/6-31G(d)//B3LYP/3-21G			
27	R		−1998.532254	−1997.99060[b]	−1998.08986[b]	17.50
TS(27−28)	R	**27**	15.8	13.5	14.4	
28-S	BS-U	**27**	−7.2	−8.9	−7.3	
28-T	U	**28-S**	3.4	3.2	2.3	
TS(28−29)	R	**28-S**	19.0	17.9	16.3	
29	R	**28-S**	−28.7	−27.2	−31.6	

[a] Absolute energies and enthalpies in hartree, relative energies and enthaplies in kcal/mol, dipole moments in debye. R, U, or BS-U indicate whether restricted, unrestricted, or broken-symmetry unrestricted DFT was used. ref denotes which molecule is used as a reference for calculated energy (enthalpy) differences ΔE (ΔH, ΔG). [b] Thermochemical corrections calculated at the B3LYP/3-21G level of theory. [c] ONIOM2 calculations using a BD(T) description of the warhead (six carbon and two hydrogen atoms) and a B3LYP/3-21G description of the remainder of the molecule.

(310 K), enthalpies and free energies at 298 K will also be used when discussing reactions at body temperature.

Restricted DFT (RDFT) calculations were carried out for the singlet states; however, in each case the internal and external stability of the RDFT solution was tested.[40] If the latter was not fulfilled, as in the case of the singlet biradical and some TSs, a broken-symmetry unrestricted DFT (BS-UDFT) calculation was performed. BS-UDFT is known to provide a useful description of the singlet biradical generated in the Bergman cyclization reaction provided the S−T splitting is not large.[14,41−43] The triplet states investigated in this work were calculated at the UDFT level of theory.

We note in this connection that B3LYP/3-21G is able to describe the Bergman cyclization rather accurately[25,26] because of the lower sensitivity (compared to WFT) with regard to basis set truncation errors[31,43] and the property of approximate exchange functionals to include nondynamic correlation effects via the self-interaction error.[44] Of course, a fortuitous cancellation of basis set and method errors also plays a role, which becomes obvious for the amidines. Therefore, an improvement of B3LYP/3-21G results via basis set enlargement and ONIOM calculations becomes necessary. For the DFT and WFT calculations, the program packages COLOGNE2007[45] and Gaussian03[46] were used.

Each of the new DAD structures and their follow-up products along the Bergman cyclization path was docked into the duplex 10-mer B-DNA sequence d(CTACTACTGG)·d(CCAGTAGTAG). The sequence was selected as it has previously been used in DNA cleavage experiments with the natural enediyne **15**.[47] The duplex was constructed in HyperChem[48] and was optimized in vacuo to 0.01 kcal/(mol Å). The Amber94[49] Parameter set was used for the minimization procedure. Docking of the new DAD structures was carried out with the program AutoDock v 3.0.5,[50] using grid dimensions of 60 × 60 × 40 npts and a grid spacing of 0.375 Å. (For details, see Supporting Information.)

Since MM force fields fail to describe the Bergman cyclization in a reasonable way,[26] the geometry of the ligand was kept fixed during the docking tests. In view of this necessity, it did not seem appropriate to adjust the receptor to the ligand by reoptimizing its geometry in the complex situation at the MM level of theory, i.e., employing a QM + MM rather than QM/MM methodology. This decision was also made on the background that the AutoDock program used does not contain fragmental volume or solvation parameters for DNA and insofar as the full free binding energy calculated by AutoDock had to be reduced to an intermolecular energy function based on the Weiner force field.[51] The freezing of ligand and receptor leads

to an underestimation of the binding energy whereas the neglect of solvation effects implies an exaggeration of the binding energy. For a valididation of our QM + MM approach, we have carried out an extensive QM/MM investigation for the parent dynemicin A.[26] In this study, we could show that the energetic effects of simplifications used in the QM + MM protocol cancel each other to a large degree,[26] thus yielding reasonable binding energies ΔE_b and approximate inhibition constants K_i calculated from ΔE_b rather than ΔG_b.

The calculation of the energy function was carried out as recently described.[25] It implied the evaluation of the electrostatic interaction energy (E_{elec}), which in turn requires that the atoms in the complex be assigned a partial charge. For the receptor component Gasteiger−Hckel charges[52] were calculated using the charge calculation function available in SYBYL,[53] whereas the ligand charges were taken from a Mulliken population analysis of the B3LYP/3-21G wave function at optimized geometries.

The interactions between ligand and receptor were analyzed by projecting electrostatic, hydrogen bonding, and the lipophilicity potential on their Connolly surfaces[54−61] (for details, see Supporting Information).

3. Energetics and of the Bergman Cyclization and Kinetic Stability of the Biradical

The anthraquinone part of **15**, which is responsible for the triggering of the compound in the cell environment, does not change significantly upon replacing the enediyne warhead by an amidine. In addition, the puckering of the two rings D and E in the DADs is similar to that observed for the corresponding dynemicin structures.[26] This indicates that the triggering mechanism does not change for the DADs investigated in this work. Therefore, we concentrate in the following on the Bergman cyclization of the new DADs. In Tables 1 and 2, the calculated energetics for the cyclization reactions as obtained with different methods are given. Table 3 gives the best enthalpy differences obtained in this work, which are the basis for the profile diagram shown in Figure 1. Reference data for Bergman systems **1**−**2**, **3**−**4**−**5**, **6**−**7**−**8**, **9**−**10**−**11**, **12**−**13**−**14**, and **15**−**16**−**17** are listed in Table 4.

Comparison of the energy data listed in Tables 1 and 2 reveals that the stability of the amidine singlet biradical **19-S** is differently described by different methods. B3LYP/3-21G underestimates the stability of nonplanar **19-S** as reflected by relatively long bonds C25−N26 (1.504) and C23−C28 (1.499 Å) and a clearly endothermic Bergman reaction energy (Table 1). Reference calculations for **3** or **9** reveal that either single point calculations at the BD(T)/cc-pVDZ level of theory or a repetition of the geometry optimization at the B3LYP/6-31G-(d) level of theory lead to a substantial improvement in the description of the singlet amidine biradicals. We carried out BD(T)/cc-pVDZ calculations for systems **18**−**19**−**20** and **21**−**22**−**23** at B3LYP/3-21G geometries within the ONIOM approach (see Tables 1 and 2). However, a stronger energy lowering was found upon B3LYP/6-31G(d,p) geometry optimizations (4 and 9 kcal/mol with regard to Bergman cyclization barrier and reaction energy), which reflects the need of an augmented basis set at the DFT level to obtain a reliable biradical geometry.

Since 6-31G(d,p) geometry optimizations are too expensive to be carried out for $4 \times 6 = 24$ structures, we estimated the effect of the larger basis set by reference calculations for the cyclic amidine **30** shown in Scheme 3. The energy lowerings obtained in this way are listed in Table 4 and are used to estimate the best DFT set of relative energies given in Table 3. In most cases (apart from the cummulenes formed by the DAD after Bergman and retro-Bergman reactions, Scheme 1) these estimates are in reasonable agreement with the BD(T)/cc-pVDZ results. In the case of our protonated system **27**−**28**−**29**, we carried out in addition B3LYP/6-31G(d) geometry optimizations, which led to small changes in the geometry and energy changes smaller than 1 kcal/mol. Therefore, we will discuss in the following exclusively the energetics of the Bergman cyclization as documented in Table 3 and Figure 1.

TABLE 3: Estimated B3LYP/6-31G(d,p) Energies *E*, Enthalpies *H*, and Free Enthalpies *G*, for the Bergman and Retro-Bergman Reaction of DADs 18 and 24 and Their Protonated Counterparts[a,b]

molecule	R/U	ref	correc	ΔE	$\Delta H(298)$	$\Delta G(298)$
18	R			0	0	0
TS(18−19)	R	**18**	−3.8	13.2	11.1	12.8
19-S	BS-U	**18**	−8.9	0.3	1.2	0.2
19-T	U	**19-S**	−0.9	3.6	3.1	3.0
TS(19−20)	R	**19-S**	2.0	4.7	3.8	5.0
20	R	**19-S**	6.4	−29.5	−28.8	−31.9
21	R			0	0	0
TS(21−22)	R	**21**	−5.2	13.8	11.5	12.3
22-S	BS-U	**21**	−8.9	−7.4	−9.1	−7.5
22-T	U	**22-S**	−0.8	1.6	1.4	0.5
TS(22−23)	R	**22-S**	3.3	16.0	14.9	13.2
23	R	**22-S**	10.3	−27.8	−26.3	−30.7
24	R			0	0	0
TS(24−25)	R	**24**	−3.8	14.0	11.9	13.6
25-S	BS-U	**24**	−8.9	1.1	−0.4	1.0
25-T	U	**25-S**	−0.9	2.9	2.4	2.3
TS(25−26)	R	**25-S**	2.0	*c*	*c*	*c*
26	R	**25-S**	6.4	−31.9	−31.2	−34.3
27	R			0	0	0
TS(27−28)	R	**27**	−5.2	13.5	11.2	12.0
28-S	BS-U	**27**	−8.9	−9.7	−11.4	−9.8
28-T	U	**28-S**	−0.8	1.9	1.7	0.8
TS(28−29)	R	**28-S**	3.3	18.1	17.0	15.4
29	R	**28-S**	10.3	−28.0	−26.5	−30.9

[a] Relative energies, enthalpies, and free energies in kcal/mol. R, U, or BS-U indicate whether restricted, unrestricted, or broken-symmetry unrestricted DFT was used. ref denotes which molecule is used as a reference for calculated energy (enthalpy) differences ΔE (ΔH, ΔG). [b] B3LYP/6-31G(d,p) estimates are obtained from the B3LYP/3-21G results (see Tables 1 and 2) using correction increments Correc. derived from B3LYP/3-21G and B3LYP/6-31(d,p) energy differences for the 10-membered amidiones **30** and **33** (see Scheme 3). [c] Not found.

The activation enthalpy for the Bergman cyclization of **18** is 12.1 kcal/mol (11.9 kcal/mol for isomer **24**) and by this 5.6 kcal/mol smaller than that for the parent dynemicin **15** (Table 3 and Figure 1). The reaction is slightly exothermic ($\Delta H_R(298) = -1.2$ kcal/mol, Table 3), whereas the reaction enthalpy is thermoneutral for the isomer (Table 3). There is a striking difference between parent enediyne **15** and amidine **18**: The retro-Bergman reaction to the open enediyne **17** requires an activation enthalpy of 21.8 kcal/mol; however, the corresponding reaction leading to the cummulene **20** has an activation enthalpy of just 3.8 kcal/mol. Hence, the observations made for the amidine **3** (Table 4) are fully confirmed for the DAD: The intermediate biradicals (either **19-S** or **4-S**) are kinetically not stable, have a very short lifetime, and, therefore, are not capable of abstracting H from DNA. Once the cummulene **20** has been formed in a highly exothermic reaction ($\Delta H_R(298) = -28.8$ kcal/mol, Table 3), the amidine **18** is taken effectively out of the system because it has no chance to regenerate biradical **19-S**.

For the isomer **24**, the energetics of the Bergman and retro-Bergman reactions are similar (see Tables 1 and 2) although

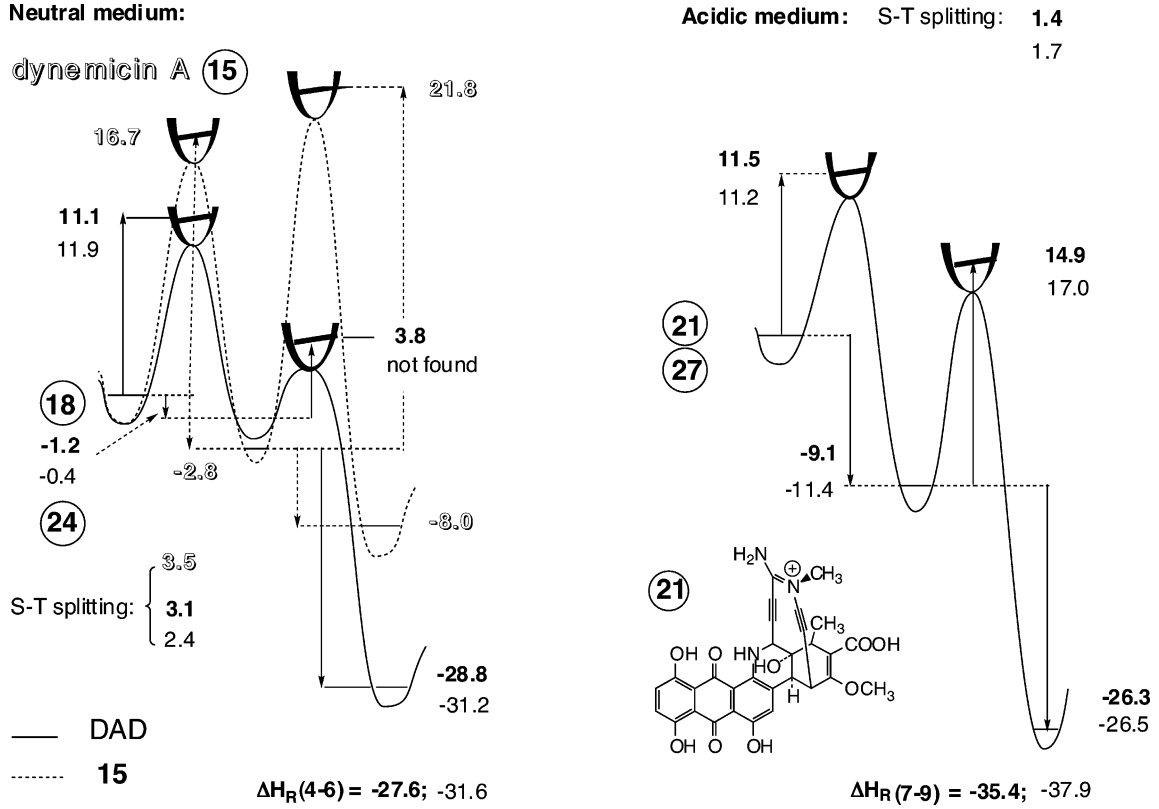


Figure 1. Qualitative reaction enthalpy profile for the Bergman cyclization of DADs **18** (left side, bold print), **24** (left side, normal print), and their protonated counterparts **21** (right side, bold print) and **27** (right side, normal print), respectively. For reasons of comparison the enthalpy profile of **15** is also given. Enthalpies in kcal/mol. Optimizations and frequency calculations obtained with the B3LYP hybrid functional and the 3-21G basis set.

the second TS, **TS(25−26)**, cannot be found at either the B3LYP/3-21G or the B3LYP/6-31G(d) level of theory. However, for the objectives of this study, a methodological error of 1 or 2 kcal/mol is unimportant as the qualitative result (the short lifetime of a biradical intermediate) is not changed in this way.

At this point it is appropriate to reconsider the design concept for a pH-dependent warhead. In the neutral medium of the normal cell, the biological of the intermediate biradical formed by Bergman reaction should be suppressed. This is accomplished by replacing the double bond in the (*Z*)-3-hexene-1,5-diyne unit by a single bond, yielding **36** (see Scheme 4). Whereas the Bergman reaction corresponds to a symmetry-allowed electrocyclic reaction, **36** can react via a symmetry-allowed[3,3] sigmatropic shift via **TS(36−38)** to the hexatetraene **38**. We calculate a barrier of 32.5 kcal/mol at B3LYP/6-31G(d,p) (starting from a C_2-symmetrical gauche form of **36**), which is 2.4 kcal/mol higher than the Bergman reaction barrier for **1** (30.1 kcal/mol[14]). By IRC calculations we confirmed that **TS(36−38)** is on the sigmatropic reaction path leading from **36** to **38**. Its geometry is characterized by two long C,C interactions of 1.78 Å (bond to be broken) and 1.93 Å (bond to be formed). The reaction is exothermic by 13.5 kcal/mol.

The possible biradical intermediate **37-S** is 28.7 kcal/mol higher in energy than **36** at BS-UB3LYP/6-31G(d,p); however the TS leading to **37-S** is 34.3 kcal/mol and by this just 2 kcal/mol higher in energy than that of the sigmatropic reaction. The intermediate biradical is short-lived as its barriers to backward or forward reaction are just 5.6 and 1.9 kcal/mol, respectively. The reaction complex of the sigmatropic reaction **36** → **38** has C_{2v}-symmetry (the σ-CC bond migrates in plane) whereas it has C_2-symmetry in the case of the formation of the twisted (nonplanar) biradical **37-S**. Since the energies of the three TSs and of intermediate **37-S** are close in energy, it is easy to foresee that incorporation of N atoms into **36** as in **3** can lead to changes in the energy ordering, which may involve the vanishing of one reaction path.

For **3**, the C−C single bond of model **36** is replaced by a N−C single bond of an amidine, which, because of the π-type lone pair of the amide N, can adopt some small double bond character by lone pair delocalization. Hence, amidine **3** takes a position between the model cases **1** and **36** and can react easier via electrocyclic Bergman reaction than sigmatropic shift as **36** does (Table 4). However, the intermediate biradical is slightly puckered as **37-S** rather than planar as **2-S**. Typical of a singlet biradical is the lengthening of the C−N and C−C bonds in β-position to the radical C atoms, which results from through-bond delocalization.[16,17,62] The latter effect cannot be as effective as in a planar ring system. Accordingly, the singlet biradical is less stabilized for the amidine systems and the singlet−triplet (S−T) splitting is smaller, relative to **2-S**. This in turn leads to a larger H-abstraction ability, which however does not matter in view of the short lifetime of biradicals such as **4-S**, **19-S**, or **25-S**.

A priori one cannot predict whether substituent or strain effects direct the amidine toward the electrocyclic or sigmatropic reaction path. In any case, one can foresee that protonation at the imine N atom leads to much stronger double bond character for the CN bond so that the amidinium ion **6** is similar to the parent enediyne **1** and undergoes the Bergman cyclization exclusively. This is confirmed by the larger relative stability of the intermediate biradical **7-S** and the much higher activation enthalpies to retro-Bergman reaction (20.3 and 14.5 kcal/mol, **TS(7−6)** and **TS(7−8)**, Table 4). In a cyclic amidinium system such as **12**, the kinetic stability of the intermediate biradical is even increased as reflected by $\Delta H^a(298)$ values of 20.2 and 17.6 kcal/mol (Table 4).

TABLE 4: Energetics for the Bergman and Retro-Bergman Reaction of Suitable Reference Systems[a]

		B3LYP/3-21G		best results	
molecule	ref	ΔE	$\Delta H(298)$	ΔE	$\Delta H(298)$
1		0	0	0[b]	0[b]
TS(1−2)	**1**	30.8	29.4	30.1	28.2
2-S	**1**	7.7	8.4	7.8	8.5
2-T	**2-S**	3.2	3.5	3.8	3.5
3		0	0	0[c]	0[c]
TS(3−4)	**3**	33.1	31.9	29.2	27.0
4-S	**3**	21.1	21.5	16.2	16.6
4-T	**4-S**	3.9	4.1	3.5	3.7
TS(4−5)	**4-S**	3.4	2.0	4.1	2.7
5	**4-S**	−38.4	−38.5	−26.4	−26.5
6		0	0	0[c]	0[c]
TS(6−7)	**6**	32.6	31.6	27.1	25.6
7-S	**6**	12.1	12.8	4.6	5.3
7-T	**7-S**	2.7	2.9	4.1	4.3
TS(7−8)	**7-S**	14.2	12.2	16.5	14.5
8	**7-S**	−45.6	−45.7	−26.5	−26.6
9		0	0	0[d]	0[d]
TS(9−10)	**9**	20.9	18.6	19.0	17.3
10-S	**9**	12.6	11.1	5.4	4.7
10-T	**10-S**	3.3	3.2	2.3	2.2
TS(10−11)	**10-S**	*e*	*e*	5.8	4.5
11	**10-S**	−36.6	−36.5	−31.9	−32.1
12		0	0	0[d]	0[d]
TS(12−13)	**12**	24.0	22.0	21.2	19.3
13-S	**12**	7.7	6.6	−0.4	−0.9
13-T	**13-S**	2.2	2.4	1.0	0.4
TS(13−14)	**13-S**	15.6	14.4	19.5	17.6
14	**13-S**	−42.8	−41.4	−33.5	−33.6

[a] Relative energies and enthalpies, and free energies in kcal/mol. ref denotes which molecule is used as a reference for calculated energy (enthalpy) differences ΔE (ΔH). [b] Experimental results from refs 14 and 23. [c] BD(T)/6-31G(d,p)//B3LYP/6-31G(d,p) calculations. [d] B3LYP/6-31G(d,p) calculations. [e] Not found.

This trend is also observed for the two protonated amidines **21** and **27**. The $\Delta H^a(298)$ values for the Bergman cyclization are just 11.5 and 11.2 kcal/mol, respectively (Table 3, Figure 1). The reactions are exothermic by 9.1 and 11.4 kcal/mol, respectively, reflecting the improved stability of the intermediate biradical, which however is not as strongly stabilized by through-bond interactions as in the case of **2**. S−T splittings of just 1.4 and 1.7 kcal/mol suggest increased H-abstraction ability, which is confirmed when using the parent amidine biradical **4-S** to abstract H from methanol (barrier of just 5 kcal/mol).[63] The $\Delta H^a(298)$ values for the retro-Bergman reactions of **22-S** and **28-S** are 20.6, 14.9, 22.6, and 17.0 kcal/mol, respectively (Table 3), which are sufficiently large to make H-abstraction the clearly faster reaction.

We conclude that the energetics calculated for the two new DADs fulfill the criteria for an antitumor drug. (1) They are nontoxic in their neutral form because a possible biradical as a cyclization product does not exist at all or is very labile with a lifetime much too short to abstract H from DNA. (2) In their protonated form they become biologically active because (a) a singlet biradical is quickly formed by Bergman cyclization and (b) the latter is kinetically stable but (c) reactive enough to abstract H from DNA. The question remains whether any variation in the structure of **15** does not change the docking properties of the naturally occurring enediyne. Therefore, we will investigate this question in the next section.

4. Docking of DADs in the Minor Groove

In Table 5, the results of the docking studies are presented. The docking arrangements of amidinium ions **21** and **27** are shown in Figure 2 where on the Connolly surface the H-bonding ability is mapped (for lipophilicity and electrostatic potential, see the Supporting Information). Since the neutral amidines **18** and **24** are not relevant for the biological activity as discussed above, their docking properties (which are actually similar to those of the amidinium cations) are not discussed.

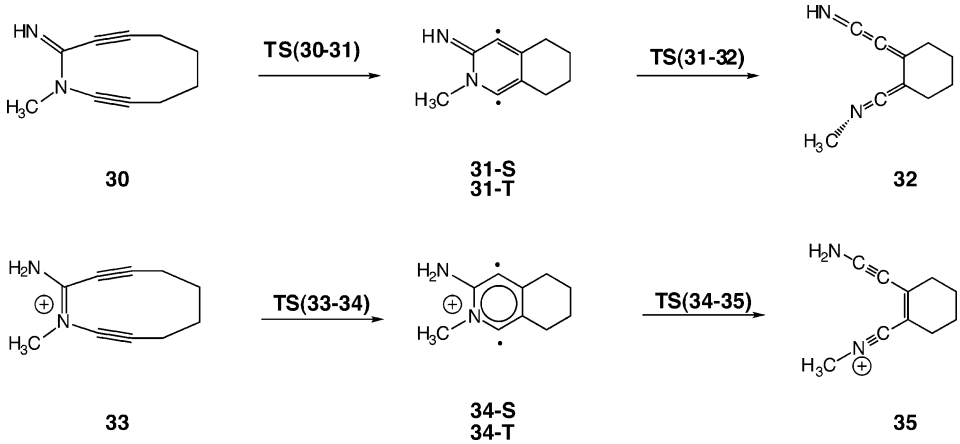
The two amidinium cations **21** and **27** in their untriggered as well as in their triggered form show similar docking preferences as the natural enediyne **15**. No matter which of the two possible amidinium ions is considered, the ligand is always oriented in a way that N1H (see Scheme 2 for numbering) points out of the minor groove (Figure 2). We have shown in previous work[25,26] that the position of N1H follows from the overall shape of the molecule and an edgewise insertion into the minor groove. Due to the in vacuo limitations enforced upon the docking simulation, it is necessary to consider whether the orientation presented would be supported in the solvated environment, in particular, potential H-bonding interactions could be overlooked in the simplified model. However, the edgewise inserted model presented is also supported by the orientation of the COOH group at C5. In aqueous solution this will be present in form of a carboxylate anion strongly solvated by water molecules. The DNA structure does not offer any H-bond donors in the vicinity of the carboxylate group that can interact with the latter; on the contrary, the ligand has to orient in such a way that it keeps the carboxylate anion away from the negatively charged phosphate groups. Hence, the carboxylate group has to point to the outside of the minor groove to keep its solvation shell and to avoid unfavorable electrostatic interactions. This is also true for some degree for the N1H group, which also does not find a H-bond partner in its vicinity (see Figure 2) and therefore should have its strongest interactions with the solvent molecules.

As soon as the triggering occurs the OH group resulting from epoxide opening can establish a H-bond with a PO_4-rest of the A6 nucleotide of the lower strand (see H-bond surface in Figure 2; H-bond donors are indicated by red, H-bond acceptors by blue). The OH group is on the same side as the carboxylate and the N1H group and by this further stabilizes the positioning of the ligand in the receptor bay. We note that upon triggering there is a slight shift of the enediyne part into the H4′ direction (Table 5). Apart from this, the overall positioning of the ligand is not changed during triggering and Bergman cyclization as is documented by the data in Table 5.

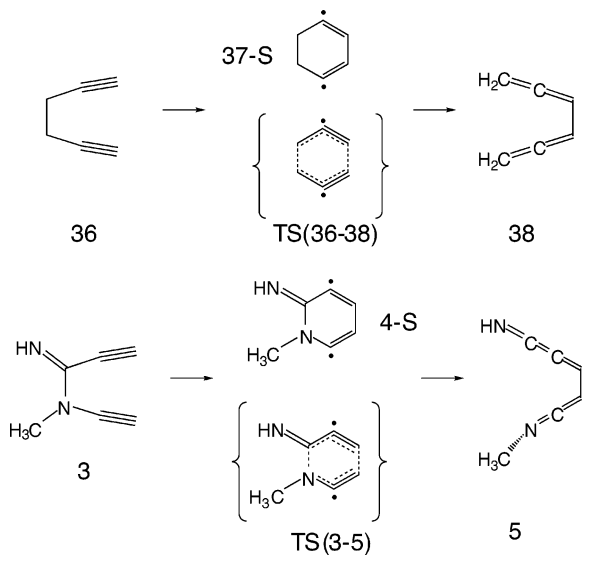
In the amidinium cation, the positive charge leads to additional electrostatic interactions with DNA and, therefore, it is understandable that the binding energies of **21** and **27** are always somewhat larger than that of the parent dynemicin **15** (see Table 5). However, it is difficult to foresee which of the two isomers (**21** and **27**, respectively) has a better binding profile. The positive charge is delocalized between the two N atoms, which withdraw electrons from H atoms and methyl group. The amidinium rest is positioned between two phosphate groups (Figure 2) and therefore it is the question whether NH_2 or NMe group carries a larger positive charge. According to Mulliken or NBO charges, the NH_2 group does (0.40 vs 0.28 electron); however the methyl group has stronger contacts with the phosphate oxygen atoms than the NH_2 group. Therefore, it is not surprising that isomer **21** with the NH_2 group on the N1H site and the NMe group to the back has slightly larger docking energies than isomer **27** (−8.1 vs −7.8 kcal/mol, Table 5).

Contrary to dynemicin **15**, amidinium cations **21** and **27** will abstract a H atom from the deoxyribose part of nucleotide A6 (rather than nucleotide G7; see Figure 2). For singlet biradical **22-S**, A6(H5′) is close to radical center C27 (2.175 Å, Table 5)

SCHEME 3



SCHEME 4



whereas for biradical **28-S** atom C27 is close to A6(H4′) (2.165 Å, Table 5). In both cases, H abstraction should be easier for the DADs than for the parent dynemicin. However, again there is no chance for a double strand scission: As found for the parent dynemicin **15**, the ligand will have to leave the minor groove when abstracting H.[26] A second ligand molecule has to come in to complete the scission through the two DNA strands.

5. Chemical Relevance of Results

This work has confirmed that a change of the warhead of the natural enediyne **15** does not reduce its biological activity as far as docking, triggering, and biradical formation are concerned.

(1) Replacement of the warhead by an amidine group does not change the geometry in rings A to E significantly (Scheme 2), and therefore the electronic and strain requirements for the triggering of the DADs are the same as for the parent dynemicin **15**.

(2) There is a considerable change in the energetics of the Bergman reaction insofar as the activation enthalpy of the triggered amidines and amidinium ions decreases from 16.7 to 11−12 kcal/mol thus enhancing the reaction rate significantly.

(3) The amidine biradicals formed are rather labile and react immediately in a retro-Bergman reaction to a cummulene. For isomer **24**, the biradical may not exist at all. After protonation, the kinetic stability of the biradicals is considerably increased raising the activation enthalpy for a retro-Bergman reaction to a minimum of 15 kcal/mol (maximum 22.6 kcal/mol, Figure 1).

TABLE 5: Comparison between the Calculated Docking Properties of the Parent Dynemicin 15 and the Two Protonated DAD Derivatives 21 and 27[a]

structure	ΔE	K_i	abs site	C27···H
untrig **15**	−7.61	1.46×10^{-6}	G7(H5′)	2.707
15	−7.32	4.34×10^{-6}	G7(H5′)	2.764
16-S	−6.54	1.60×10^{-5}	G7(H5′)	2.694
untrig **21**	−8.24	9.08×10^{-7}	A6(H5′)	2.702
21	−8.08	1.19×10^{-6}	A6(H4′)	2.503
22-S	−7.16	5.66×10^{-6}	A6(H5′)	2.175
untrig **27**	−7.77	2.03×10^{-6}	A6(H4′)	2.776
27	−7.82	1.86×10^{-6}	A6(H5′)	2.607
28-S	−7.05	6.78×10^{-6}	A6(H4′)	2.165

[a] Binding energies in kcal/mol, inhibition concentration in mol, distances in Å. The abbreviation untrig denotes the untriggered enediyne. Abs site is the abstraction site, indicating the nucleotide and the hydrogen aligned for abstraction.

(4) The reactivity of the amidinium biradicals is much higher than that of the dynemicin biradical as is reflected by a decrease in the S−T splitting from 3.5 to 1.4 and 1.7 kcal/mol (the smaller the S−T splitting the larger H abstraction ability[17]).

(5) The docking properties of the amidinium ions are improved relative to those of natural enediyne **15** as is reflected by the larger docking energy and a closer contact of the radical center C27 with the proximal H atom to be abstracted. The overall orientation of the amidinium ligand in the minor groove is similar to that of dynemicin **15**; however the headgroup is shifted by one nucleotide to the left (Figure 2) so that the A6 hydrogens are attacked rather than those at G7 (Table 5). As for dynemicin **15**, there is a tendency of the anthraquinone part to orient the N1H group to the outside of the minor groove, which is due primarily to the shape of the molecule and is supported by the orientation of the functional groups. In the triggered form, the alcohol resulting from the epoxide is oriented to the front and establishes a H bond with a phosphate group of the lower strand, which fixes the ligand in the position kept during the Bergman cyclization.

(6) There is a difference in the docking properties of the two isomers **21** and **27** of just 0.26 kcal/mol (Table 5), which is too small to derive from there a stronger docking of **21** compared to that of **27**. However, a somewhat stronger docking energy of **21** is reasonable in view of the fact that there are close contacts between the phosphate groups of nucleotides G7 and A6 of the upper strand in Figure 2 and the *N*-methyl group of **21**, which carries a substantial part of the positive charge.

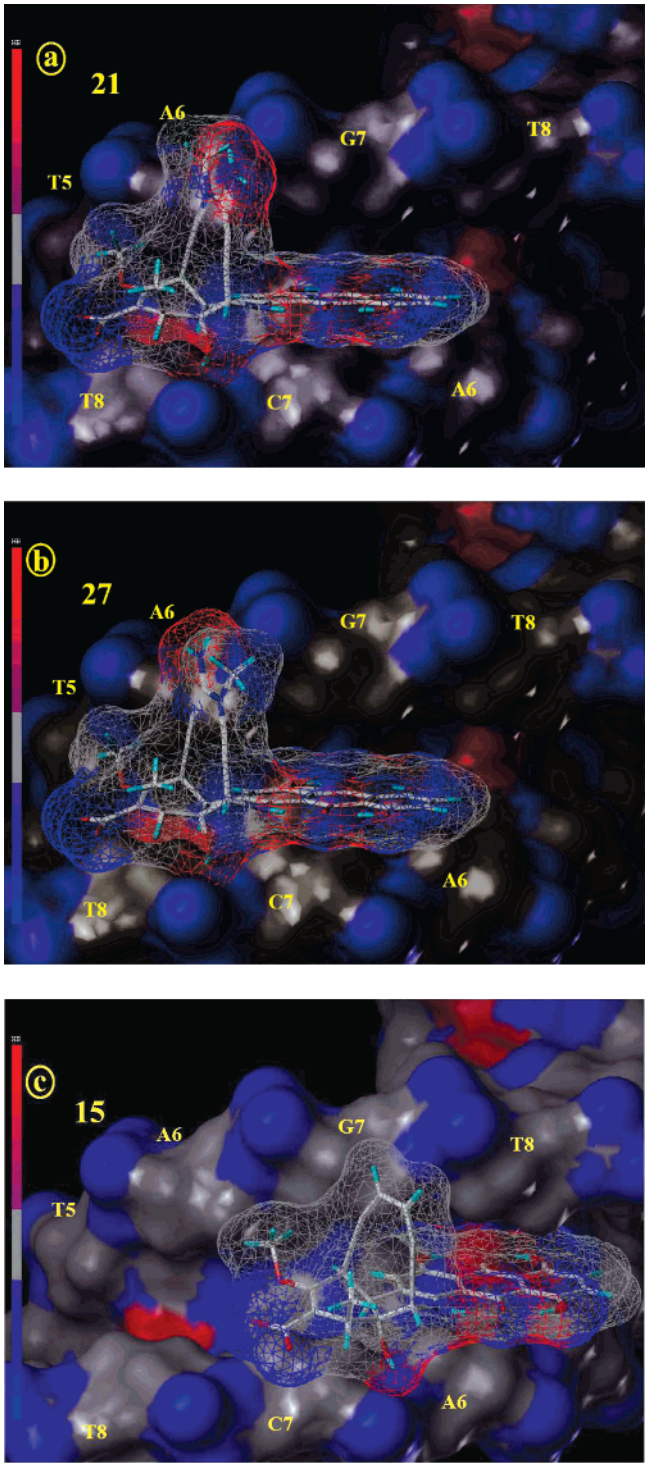


Figure 2. Positioning of (a) **21** and (b) **27** in the minor groove of DNA. (c) The parent compound, **15**, is shown for reference purposes. On the Connolly surfaces representing receptor and ligand (in the latter case a net representation to see the receptor structure in a capped stick representation) the H-bonding surface is mapped. Blue corresponds to H-acceptor, red to H-donor sites. The receptor is geometry-optimized at B3LYP/3-21G, DNA minimized with the Amber94 force field.

Contacts are stronger than those between the NH_2 group and phosphate groups. It can however be foreseen that inclusion of alkyl or aryl groups (with or without polar substituents) may lead to a different docking situation.

The amidine/amidinium warhead increases considerably the biological activity of the compound. This could not be expected on the basis of the general concept of tumor cell selectivity worked out in previous papers (replacement of the enediyne double bond by a formal single bond that is converted into a double bond by protonation); however it is accepted as a fact that improves the use of DADs as new antitumor leads.

So far, we have not considered the question whether the amidine warhead can be manipulated in such a way that it is protonated just in the acidic medium of the tumor cell and not in the normal cell. If the pK_a value of the parent amidine **3** can be estimated to be close to 10.5,[64,65] it will be clear that protonation will take place already in a neutral aqueous solution; i.e., both in the tumor and the normal cell the active amidinium cation will be generated. Another problem results from the fact that amidines are hydrolyzed in aqueous solution.[28,29] Finally one has to consider that with the generation of the biradical center close to the NH_2 group, a H shift may take place leading to a new biradical. In the case of biradical **22-S** this would concern only radical center C24, which is oriented to the outside of the minor groove and does not play any role in connection with H abstraction. If the amidinium group is switched, as in **28-S**, a H transfer will block the H abstracting potential of radical center C27 and by this the biological activity of the compound, considering that abstractable H atoms of DNA are too far away from an N radical.

All three problems are related to the type of amidine used as headgroup. In this work, we have investigated energetic and docking prerequisites to be fulfilled by the new drug candidate. Work is in progress to tune the amidine properties in such a way that the three remaining problems are solved. For example, it is well-known that trisubstituted formamidines no longer hydrolyze[29] and in addition by keeping bulky substituents R at a preferred site (outward oriented) a H-shift from the HNR group to C27 can be largely suppressed. Also it is known that the substituents of a formamidine lead to a variation in the pK_a values from 4 to 13,[64,65] which facilitates the selection of substituents that ensure that the amidine is only protonated in the tumor cell.

Acknowledgment. E.K. and D.C. thank the University of the Pacific for generous support of this work.

Supporting Information Available: Details of the docking calculations, a comparison of results obtained with the QM+MM and an advanced QM/MM description, and graphical representations of the electrostatic and lipophilicity potentials mapped on the Connolly surface of **21** and **27**, which are docked in the minor groove of DNA. This material is available free of charge via the Internet at http://pubs.acs.org.

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