Computer Design of Anticancer Drugs. A New Enediyne Warhead†

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Abstract: Seven criteria are developed and discussed that lead to the design of a new enediyne anticancer drug, which should have low toxicity but high biological selectivity and activity when attacking the DNA of tumor cells. These criteria concern (among others) the thermodynamic and kinetic stability of the species involved in the reaction of an enediyne, the biradical character and H-abstraction ability of the intermediate biradical generated in a Bergman reaction of the enediyne, and the basicity of the enediyne and its associated biradical. Thirteen different heteroenediynes were investigated with the help of B3LYP/6-31G(d,p) calculations to find a suitable candidate for a new enediyne anticancer drug, which fulfills the seven criteria. These calculations included the determination of reaction profiles for Bergman and retro-Bergman reactions, the calculation of singlet−triplet splittings of biradicals formed from enediynes, and the prediction of pK_a values. Results were tested by using a larger basis set (6-311+G(3df,3pd)), another functional (BLYP), and coupled cluster methods such as CCSD(T) and the Brueckner orbitals-based BD(T) method. The best candidate for a new enediyne anticancer drug is an N,C-dialkynyl aldimine incorporated into a cyclodecaene ring.

1. Introduction

Microorganisms and human beings have one thing in common: Both are attacked by toxic bacteria and viruses. However, microorganisms have been on earth at least 2 billion years longer than human beings, and therefore, they know much better how to protect themselves against bacteria and viruses. In the early 1980s, a natural product screening program was started that was aimed at the discovery of new anticancer antibiotics produced by microorganisms. This led five years later to the discovery of the enediyne antibiotics,[1] which today are divided into three families,[2] namely, (1) the calicheamicin/esperamicin family[3,4] possessing an enediyne chromophore with a methyl trisulfide group, (2) the dynemicin family,[5] where the enediyne unit is associated with the hydroxyanthraquinone chromophore, and (3) the chromoprotein family, which consists of a nonpeptidic chromophore such as neocarzinostatin,[6,7] kedarcidin,[8] or C-1027[9] complexed with an apoprotein that acts as a carrier. All members of the first two families have the enediyne unit incorporated into a 10-membered ring and do not need any additional stabilization factors while all members of the third family possess a nine-membered-ring enediyne core structure and require a specific associated protein for chromophore stabilization.

All enediyne antibiotics originally derived by fermentation of microorganisms possess the ability to destroy the DNA of toxic bacteria and viruses. The astonishing biological activity of the naturally occurring enediynes has led to increased efforts of using enediynes as anticarcinogens by exploiting their ability of destroying the DNA of tumor cells. This implies a basic understanding of the chemical mechanism leading to the enediyne activity. As is known today, an enediyne can fulfill three functions, which are associated with certain units of its molecular structure. For example, in the case of calicheamicin, a delivery system formed by a polysaccharide rest (see Figure 1) helps the enediyne to dock into the minor grove. Then, there is a trigger device consisting of a methyl trisulfide group and a conjugated cyclohexenone and, finally, an antitumor warhead

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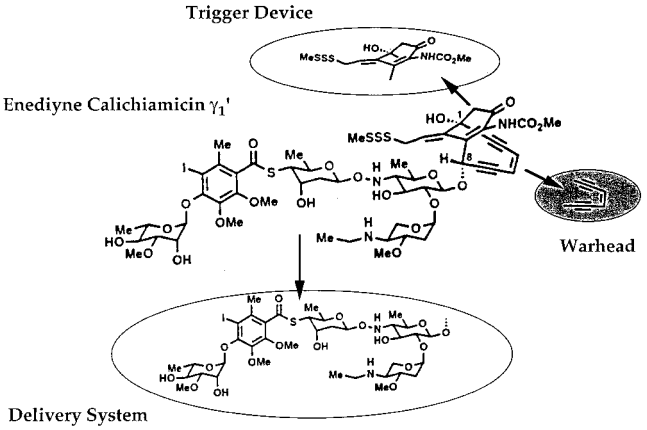

Figure 1. Delivery system, trigger device, and enediyne warhead of calichiamicin γ_1'.

identical to the enediyne part. A nucleophilic attack at the trisulfide group (Scheme 1) leads to an intramolecular Michael addition, which causes a contraction of the enediyne ring thus triggering a Bergman cyclization[10] of the enediyne part. A reactive *p*-didehydrobenzene biradical is formed, which is positioned in such a way in the minor grove that it can abstract two proximal hydrogen atoms from the deoxyribose sugars on both strands of the DNA, thus damaging DNA to form a stable arene. This leads to cleavage of the DNA at both strands and, accordingly, causes cell death. There is evidence that under physiologically relevant conditions the nucleophilic attack at the methyl trisulfide group is initiated by glutathione, which is the most prevalent thiol in mammalian cells.[11]

Hence, considerable efforts have been made to design and synthesize simpler analogues of the naturally occurring enediynes[12] and to tune their biological activity to meet the requirements of an efficient antitumor drug being highly active, selective, and nontoxic. However, this implies a long, expensive, and tedious discovery and development process. Computational chemistry can contribute to accelerate this process by predicting in which way structural changes lead to improvements of the drug efficiency.

The goal of this work is to design a new enediyne warhead that selectively attacks tumor rather than normal cells and by this loses the toxicity of natural enediynes. To accomplish this goal, we will first discuss how the requirements of a useful antitumor drug can be related to its molecular properties, in particular the structural features of the enediyne. Then, we will set the basis for an appropriate computational description of enediynes so that, by the quantum chemical screening of different enediyne compounds, the most likely candidate for a new antitumor enediyne warhead can be suggested.

2. Criteria for a Useful Enediyne Anticancer Drug

Out of the many desired drug properties that determine optimal activity, stability, selectivity, safety, and production costs, we will consider here a subset of properties that has to be fulfilled in any case. For example, it must be guaranteed that the enediyne drug is active under physiological conditions, i.e., at body temperature in the aqueous medium of a mammal cell. Also, the ability to cleave DNA must be high so that the concentration of the enediyne can be kept low, which is particular important to reduce unwanted side effects as much as possible. The stability of the drug must be large enough to avoid early reactions before penetrating the membrane of a tumor cell and docking to its DNA. Therefore, the half-life time of the enediyne in the body must be a factor of 10−1000 higher than the time needed for optimal distribution of the drug within the location of the tumor.[13] Of course, this ratio can be influenced in a positive way by administering the drug locally.

The activity, stability, and selectivity of the drug are affected by the properties of the intermediate biradical. If its lifetime is too short for attacking DNA, then the enediyne drug becomes ineffective. Alternatively, if its lifetime is too long, i.e., its reactivity too small, its effectiveness as a DNA-cleaving compound will be also too small thus requiring high doses of the drugs and, by this, raising the chances of unwanted side effects. As for the selectivity of the enediyne drug, it is most important that the drug distinguishes between normal and tumor cells by attacking only the latter. Clearly, high selectivity associated with a broad spectrum of clinical activity is the most important prerequisite for low toxicity of the drug, and therefore, we will consider this point explicitly.

(1) Activity of the Drug at Body Temperature. For a chemical reaction to proceed by 50% at body temperature (T = 310 K) within 5 min or faster, the free activation enthalpy, $\Delta G(310)^{\ddagger}$, has to be 22 kcal/mol or lower. (We prefer to use the term *free enthalpy* (used in German textbooks) rather than the terms Gibbs energy or *free energy* (used in American textbooks) for ΔG because it helps to distinguish between $\Delta G = \Delta H - T\Delta S$ as opposed to the actual *free energy* $\Delta F = \Delta E - T\Delta S$.) In the case of the Bergman reaction, which has an activation entropy $\Delta S^{\ddagger}$ of −7 eu,[14] the activation enthalpy $\Delta H(310)^{\ddagger}$ has to be 24 kcal/mol or lower. For the parent enediyne (*Z*)-hexa-1,5-diyne-3-ene (**1**), the activation enthalpy $\Delta H(310)^{\ddagger}$ is 28.7 kcal/mol according to an experimental value based on kinetic measurements made at 470 K by Roth et al.[14] and according to temperature corrections determined by Cremer and co-workers with the help of calculated vibrational frequencies for 298 K[15−17] (values of $\Delta H^{\ddagger}$ at 298 K and at body temperature differ by less than 0.1 kcal/mol, which is the reason throughout this work enthalpies are given for 298 K rather than 310 K). Such an activation enthalpy is much too high to guarantee biological activity at body temperature. On the other hand, the barrier should be high enough to avoid that the drug is too quickly consumed in the body or that the activation enthalpy of the retro-Bergman reaction becomes lower than the activation enthalpy for H-abstraction from DNA (see below).

An analysis of calculated and experimental data[18,19] suggests that the incorporation of the enediyne unit into a 9- or 10-

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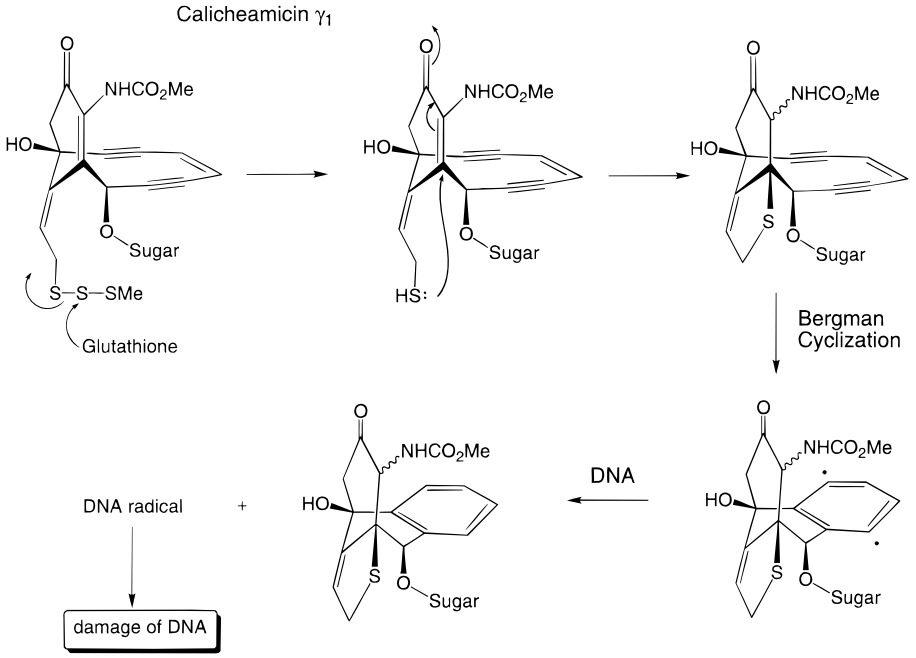
Scheme 1



membered ring as in the case of the naturally occurring enediynes lowers the activation enthalpy of the Bergmann cyclization to a value below 24 kcal/mol, which is sufficient to guarantee biological activity under physiological conditions. Nicolau, Snyder, and others[19] argued that this lowering of the activation enthalpy results from a destabilization of the enediyne caused by the strain of a 9- or 10-membered ring. High-level ab initio calculations by Kraka and Cremer[18] showed that a decrease of the 1,6-distance in **1** (as will occur if the enediyne unit is incorporated into a 9- or 10-membered ring) leads indeed to a substantial lowering of the barrier. As an alternative possibility, strain release in the transition state (TS) of the Bergman reaction due to conformational changes in a ring annelated to the enediyne unit of naturally occurring enediynes was also discussed by Magnus and co-workers.[20]

Schmittel and Kiau pointed out that electron-withdrawing substituents attached to the triple-bond termini lower the activation enthalpy of the Bergman reaction.[21] Semmelhack[22] and Nicolau[23] provided evidence that electron-withdrawing groups associated with the double bond can also facilitate the Bergman cyclization, which is in line with the fact that annelation of **1** by an heteroarene lowers the cyclization barrier: 5,6-diethynyl-2,4-dimethoxypyrimidine synthesized by Kim and Russell was found to have an activation energy of just 16.1 kcal/mol.[24] In general, benzannelation of an acyclic enediyne changes the barrier to Bergman cyclization only slightly[25] while benzannelation of a cyclic enediyne seems to slow cyclization in most cases.[23] Recently, it has been suggested that in the latter case the barrier of the retro-Bergman reaction is rather low so that the retroreaction becomes faster than H-abstraction by the intermediate biradical.[26]

(2) Ability of the Arene Biradical To Cleave DNA. *p*-Didehydroarene biradicals in their singlet (S) state are less reactive than the corresponding (phenyl-type) radicals since through-bond coupling between the single electrons leads to significant stabilization,[16,27−29] thus leading to a S ground state of the biradical. In the H-abstraction reaction of a *p*-didehydroarene S biradical, this stabilization energy has to be paid back, thus increasing the barrier relative to that of H-abstraction by a phenyl radical. Chen and co-workers[30,31] used this model to estimate the reactivity of *p*-didehydroarene S biradicals from their singlet−triplet (S−T) splittings, where the T state was considered as the appropriate reference for determining the S stabilization energy since through-bond coupling of the single electrons is not possible in the T biradical. According to Chen and co-workers, the lower reactivity of a *p*-didehydroarene S biradical as compared to the corresponding radical is the basis for its higher selectivity while the value of its S−T splitting determines its actual reactivity. In the case of *p*-didehydrobenzene **2**, the measured S−T splitting is 3.8 0.5 kcal/mol,[32] which indicates that biradicals with higher biological activity (lower selectivity) should possess a smaller S−T splitting, those with lower biological activity a larger S−T splitting than that of **2**. The biradical character of a *p*-didehydroarene S biradical (estimated for **2-S** to be close to 80%[18]) decreases with the coupling and delocalization of the single electrons by through-bond interactions and, thereby, with the stabilization of the S state and an increase of the S−T splitting. Hence, biradical

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character, stability of the S biradical, S−T splitting, the ability to abstract H atoms from DNA, and the biological activity of an *p*-didehydroarene S biradical formed from an enediyne by Bergman cyclization are all closely related and can be assessed either from measured or calculated S−T splittings or geometrical differences between S and T as will be discussed in this work.

(3) Kinetic Stability of the Arene Biradical. The activation enthalpy for abstracting H atoms from an organic substrate such as methane or methanol (used to model the sugar ring in DNA) by biradical **2-S** was calculated to be between 9 and 14 kcal/mol.[26a,30,31a] In the tumor cell, this process has to be faster than the activation enthalpy for any other reaction of the S biradical, in particular, it has to be faster than the (retro-)Bergman reaction to (the starting or) an isomeric enediyne. If the barrier of the retro-Bergman reaction is too low, than the biradical has little chance to damage and destroy the DNA by H-abstraction. Several investigations were made to compare barriers for Bergman cyclization and H-abstraction using appropriate models.[26] Also, attempts were made to enforce the H-abstraction reaction by suppressing the retro-Bergman and other side reactions. For example, Lott et al.[33] suggested increasing the H-abstraction ability of the *p*-didehydrobenzenes by invoking intersystem crossing to a T biradical with the help of a magnetic field, thus making the retro-Bergman reaction, which requires the conservation of electron spin, impossible. However, experiments were not conclusive and did not indicate that the biological activity of enediynes can be manipulated by applying a magnetic field.

(4) Low Toxicity of the Enediyne. All naturally occurring enediynes are highly toxic since they attack both normal and tumor cells.[1a,1b] Therefore, the current application of naturally occurring enediynes as chemotherapeutics is rather limited. Mostly, their chemotherapeutic index (ratio of maximum tolerable dose/minimum effective dose) is too small. Only neocarzinostatin has received approval in Japan for clinical treatment of cancer in digestive organs including stomach, pancreas, and liver, cancers of bladder and brain, and for leukemia.[34] To overcome the pharmacological drawbacks of neocarzinostatin, attempts have been made to conjugate it with appropriate polymers. Poly(styrene-*co*-maleic acid)-conjugated neocarzinostatin has shown clinical promise in comparison to alternative chemotherapies.[35] However, the basic problem of enediyne antibiotics still remains, namely, how to reduce their toxicity while retaining at the same time their biological activity.

Clearly, the structure of an enediyne has to be modified in such a way that the drug can distinguish between normal and tumor cells. One major difference between many solid tumors and their surrounding normal tissues is their metabolic and nutritional environment. Often the nutritional needs of the growing tumor cells are not met, leading to a lack of oxygen and other nutrients and, as a consequnence, to an acidic microenvironment in the tumor cell.[36,37] In general, tumor cells (pH 6.2−6.6) are more acidic than normal cells (pH 7.5).[38] This effect can be increased by invoking hyperglycemia, which also causes decreased blood flow,[39] by hyperthermia,[40] or by an addition of ionophores such as nigericin[41] or amiloride.[42] In this way, the tumor cells becomes more acidic, leading to pH values as low as 5.5.[43] Such a difference in the pH value of normal and tumor cells was already used as the basis for the design of pH-dependent organometallic anticancer drugs.[44] Similarly, one could design an enediyne anticancer drug that is only active in the weakly acidic medium of the tumor cell.

Requirements for a New Enediyne Drug. Chen and coworkers[30] suggested incorporation of N into the enediyne framework and, therefore, investigated derivatives of (*Z*)-3-aza-hex-3-ene-1,5-enediyne (**4**) and their protonated counterparts. We will follow their suggestion and show how heteroenediynes can be used to modulate the reactivity of a drug in dependence of the pH value. We will calculate the properties of the heteroenediynes to test whether they can fulfill the following requirements:

1. (a) The heteroenediyne drug (delivery system, trigger device, and warhead) must possess sufficient thermodynamic stability so that after administration optimal distribution of the drug in the tumor tissues is possible. (b) While the thermodynamic stability of the heteroenediyne in the neutral medium can (should) be large to hinder the production of the biradical, the protonated species must be destabilized thermodynamically so that (after in vivo triggering of the warhead) the formation of the biradical becomes possible.

2. The heteroenediyne should be protonated at a pH of 5.5−6.5, i.e., in the acidic medium of the tumor cell, and the basic character of the intermediate biradical must be large enough to remain in the protonated form.

3. The protonated form must also be kinetically stable against decomposition or rearrangement reactions other than the Bergman cyclization.

4. The conjugated acid of the heteroenediyne should undergo Bergman cyclization at 310 K; i.e., $\Delta H(310)^{\ddagger}$ must be smaller than 24 kcal/mol. There is no need that the heteroenediyne itself fulfills these requirements; on the contrary, it would reduce the toxicity of a potential drug if in the neutral medium of the normal cell the activation enthalpy of the heteroenediyne is larger than 24 kcal/mol and because of this does not become biologically active.

5. The activation enthalpies for the retro-Bergman cyclization to the starting enediyne or an isomeric enediyne or any other reaction the biradical may perform must be significantly larger for the protonated heteroenediyne than the activation enthalpy of the H-abstraction reaction (14 kcal/mol); i.e., kinetic stability of the biradical intermediate must be guaranteed. If the biradical is also formed in the normal cell, its kinetic stability should be low (retro-Bergman barrier significantly lower than that for H-abstraction) to reduce the toxicity of the drug.

6. The protonated heteroenediyne must lead to a biradical intermediate with relatively high biradical character and high

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H-abstraction ability, as indicated by a relatively small S−T splitting. (In any case, H-abstraction ability and selectivity of the protonated biradical must be balanced to avoid unwanted reactions more typical of radicals rather than biradicals.) The biradical character and the H-abstraction ability of the hetero-enediyne itself must be lower, its S−T splitting larger than that of the protonated biradical.

7. If (1−6) are fulfilled, a suitable warhead for a potentially useful enediyne anticancer drug will be found. However, the synthesis of such a drug must be possible in an economic way. This work focuses on the investigation of 14 different enediyne warheads and their associated Bergman systems involving the investigation of molecules **1**−**91** (shown in Schemes 2−4) to single out a possible candidate for the warhead of an efficacious new enediyne anticancer drug that fulfills requirements 1−7.

Our investigation will be totally based on quantum chemical calculations, and therefore, it cannot give any answer as to the actual synthesis of such a new enediyne warhead or as to its actual clinical usefulness. Instead we will use the results of the calculations to test requirements 1−7 for each system investigated and, by this, to shorten the long way from drug design via synthesis, safety tests, and clinical trials to the final approval of the drug for chemotherapy. This implies that the quantum chemical calculations are carried out in a reliable way, which has to be discussed in the next chapter.

3. Computational Details

The Bergman reaction of the parent enediyne **1** was investigated by various authors[18,45−49] using a variety of high-level ab initio methods. CASSCF and CASSCF-PT2 calculations turned out to overestimate the stability of **2-S**,[47b] where it seems to be a general problem to bring static and dynamic electron correlation effects into the right balance for molecules with different π systems (**1**, 10 π electrons; **2**, 6 π electrons, two single electrons in in-plane orbitals). The most reliable results on the energetics of the Bergman reaction were obtained at the CCSD(T)/VDZP level of theory by Kraka and Cremer[18] although calculations with extended basis sets showed that CCSD(T) underestimates somewhat the stability of **2**,[47b] which possesses a (low-spin) open-shell S ground state (**2-S**) and, therefore, is more difficult to calculate than its triplet (T) state (**2-T**). The biradical **2-S** was isolated for the first time at low temperature in the matrix and characterized by infrared measurements in combination with quantum chemical calculations by Sander and co-workers.[50] (See also, recent studies by Zewail et al.[51]).

In this work, we will investigate enediyne units incorporated into 10-membered rings as they occur in the natural products.[19,20,52,53] In addition, there is the need to investigate not only the enediyne warhead but also the trigger device and the docking system[1a,1b] of the naturally occurring enediynes (Figure 1). Clearly, this is no longer possible using CCSD(T) or other advanced ab initio methods because of the cost factor implied. Since density functional theory (DFT)[54] methods are considerably less costly than CCSD(T) or CAS-PT2, we recently tested the performance of DFT for the description of the Bergman reaction of the parent system and some modified enediynes. A problem encountered in the DFT description of the Bergman reaction is the treatment of the intermediate biradical **2-S**, which represents a multiconfigurational problem difficult to describe with single determinant methods such as DFT. However, a recent investigation by Gräfenstein and co-workers[15] revealed that DFT results on the Bergman cyclization can be of equal or even better accuracy than the results of high-level ab initio calculations provided one fulfills a number of criteria not (or only partially) considered in previous DFT investigations of enediyne warheads.

1. A basic prerequisite for the correct description of the Bergman reaction is to monitor the stability of a restricted DFT (RDFT) description along the reaction path. Standard density functionals lead an unstable RDFT description of the biradical intermediate, which accordingly has to be described at the unrestricted DFT (UDFT) level of theory.[15,55] This was not considered in most of the previous DFT investigations (for exceptions, see refs 48−50) and explains the disturbingly poor S−T splittings, geometries, and relative energies of the biradical intermediate obtained with some DFT methods.

2. As discussed by Gräfenstein and co-workers,[15] UDFT considerably improves the description of the biradical intermediates due to the fact that static electron correlation effects are partially covered at this level.

3. Because of its empirical calibration, the B3LYP hybrid functional[56−58] performs better than LSD or GGA functionals. The latter have a tendency of underestimating the barrier of the Bergman reaction of **1**, which has also been found in other cases.[59] The BLYP functional[57,58] leads also to a reasonable description of the Bergman reaction as observed previously by

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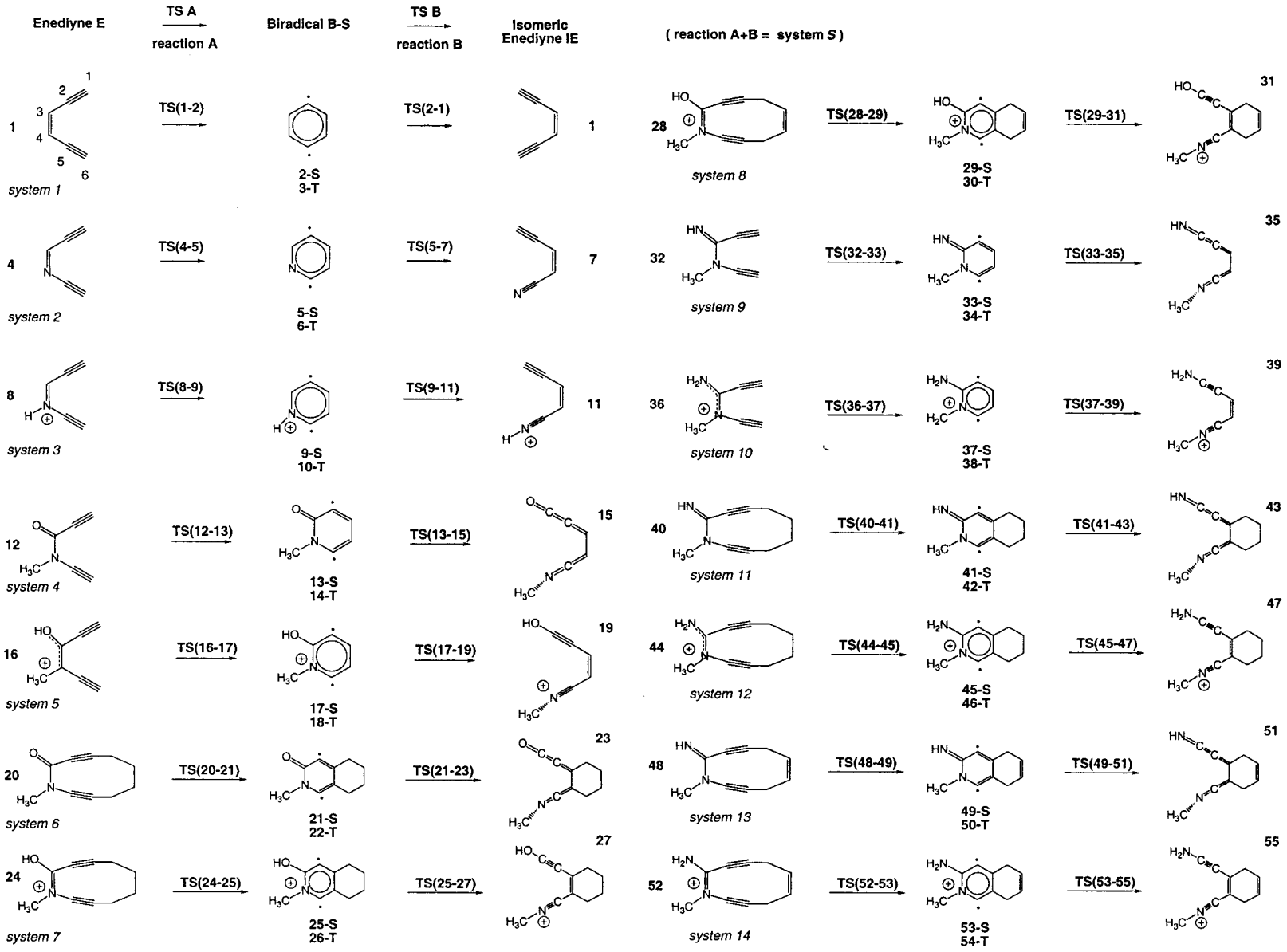
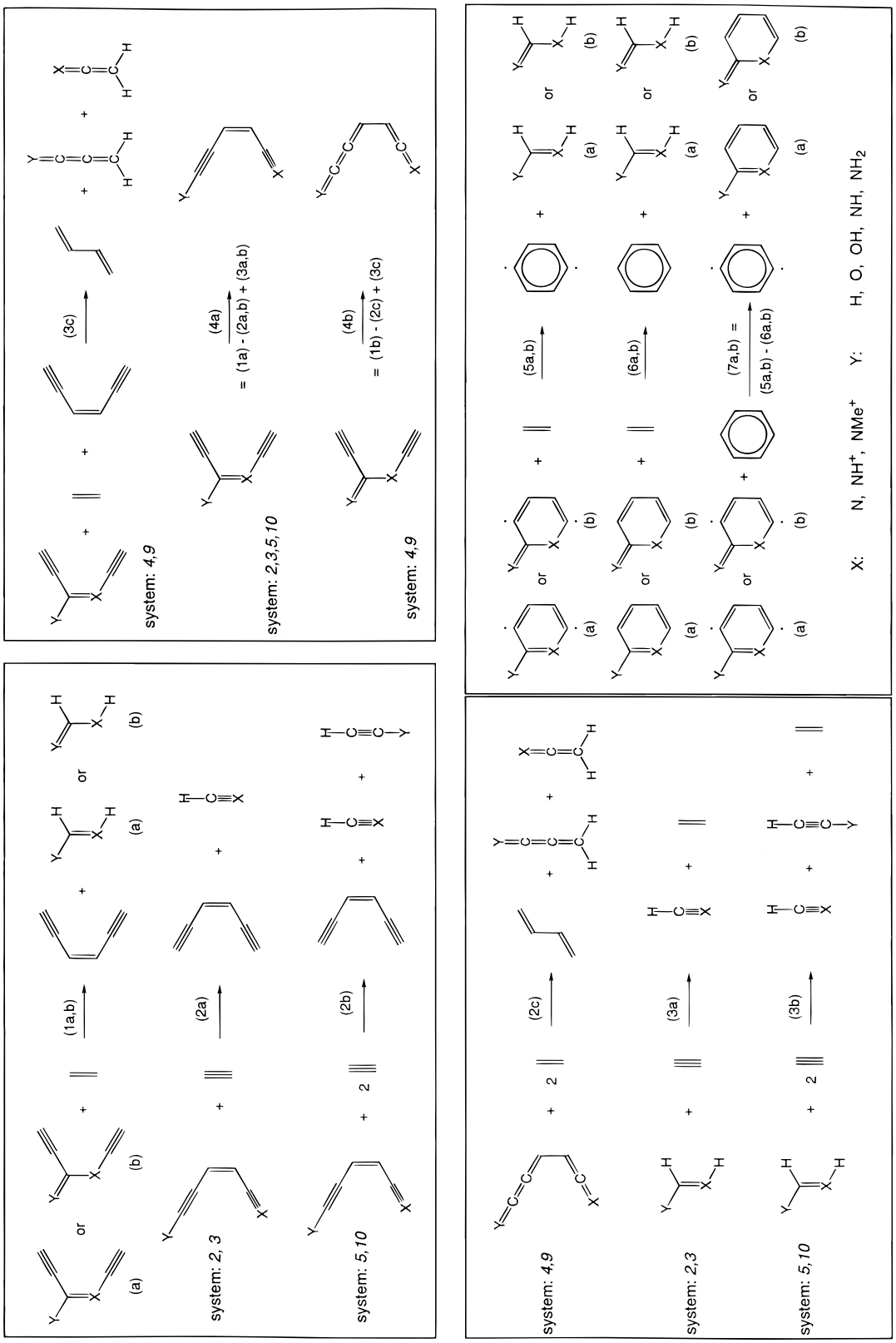
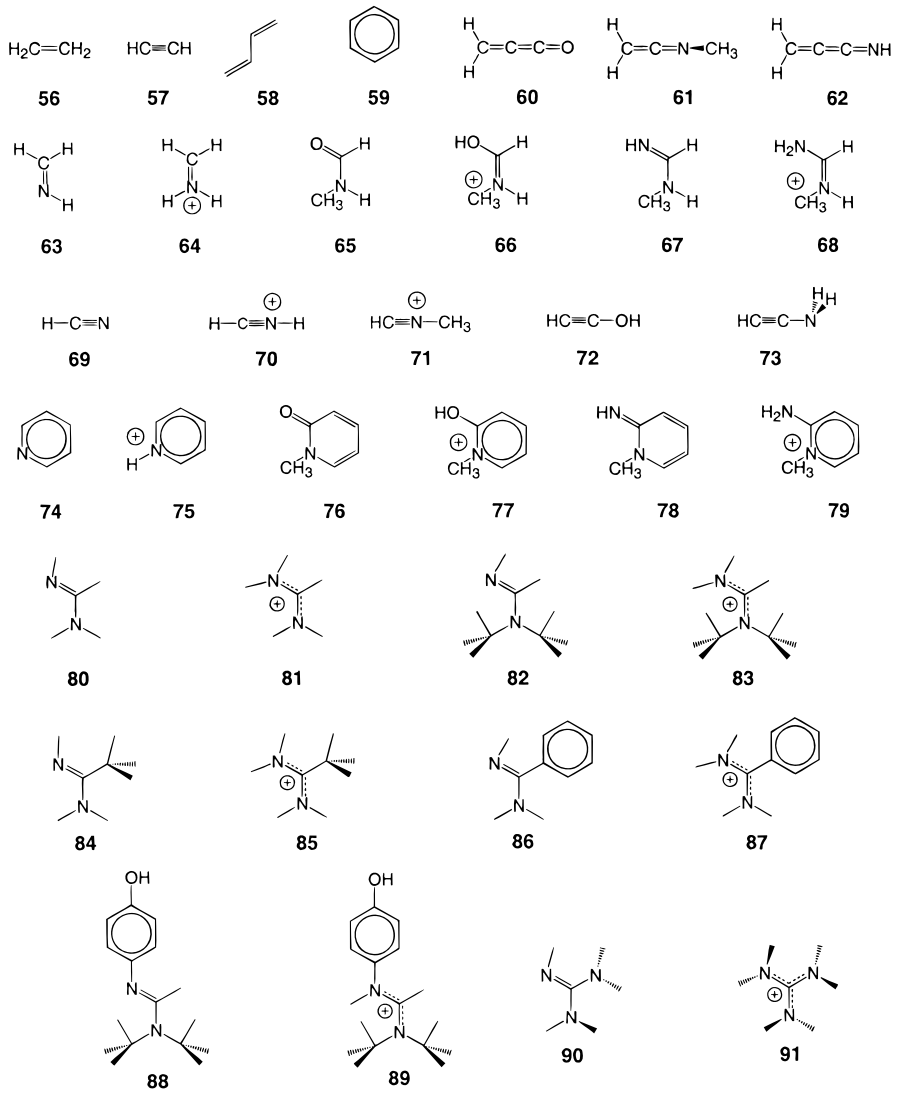
Scheme 2


Enediyne E
TS A
reaction A
Biradical B-S
TS B
reaction B
Isomeric Enediyne IE
(reaction A+B = system S)
system 1
TS(1-2)
2-S
3-T
TS(2-1)
1
28
system 8
TS(28-29)
29-S
30-T
TS(29-31)
31
system 2
TS(4-5)
5-S
6-T
TS(5-7)
7
32
system 9
TS(32-33)
33-S
34-T
TS(33-35)
35
system 3
TS(8-9)
9-S
10-T
TS(9-11)
11
36
system 10
TS(36-37)
37-S
38-T
TS(37-39)
39
system 4
TS(12-13)
13-S
14-T
TS(13-15)
15
40
system 11
TS(40-41)
41-S
42-T
TS(41-43)
43
system 5
TS(16-17)
17-S
18-T
TS(17-19)
19
44
system 12
TS(44-45)
45-S
46-T
TS(45-47)
47
system 6
TS(20-21)
21-S
22-T
TS(21-23)
23
48
system 13
TS(48-49)
49-S
50-T
TS(49-51)
51
system 7
TS(24-25)
25-S
26-T
TS(25-27)
27
52
system 14
TS(52-53)
53-S
54-T
TS(53-55)
55

Scheme 3


system: 4,9
system: 2,3,5,10
system: 4,9
(4a) = (1a) - (2a,b) + (3a,b)
(4b) = (1b) - (2c) + (3c)
(3c)
system: 2, 3
system: 5,10
(1a,b)
(2a)
(2b)
or
(a)
(b)
system: 4,9
system: 2,3
system: 5,10
(2c)
(3a)
(3b)
(5a,b)
(6a,b)
(7a,b) = (5a,b) - (6a,b)
X: N, NH+, NMe+ Y: H, O, OH, NH, NH2

Scheme 4



other authors;[48,49,52,53] however, B3LYP results are preferable for the following reasons:

(a) With increasing size of the basis set, B3LYP performs better (Table 1) while BLYP results become poorer. The astonishingly good BLYP/VDZP energies seem to result from a fortuitous cancellation of errors. Recent claims on the superiority of BLYP were based on VDZP investigations.[52] (b) B3LYP results are more consistent than BLYP results as reflected by the σ parameters of Table 1. (c) B3LYP results can substantially be improved with the help of the sum formula,[63] which is not the case for pure density functionals such as BLYP. Application of the sum formula will be justified if one adds HF exchange, however, not in the case of a pure density functional such as BLYP. (d) In general, B3LYP performs better for hydrocarbons and other molecules than BLYP, and therefore, it will lead to more reliable results if enediynes are compared with other molecules.[17]

4. The quality of the UDFT description of the biradical of the Bergman reaction should be evaluated with the help of total density $\rho(\mathbf{r})$ and on-top pair density distribution $P(\mathbf{r},\mathbf{r})$ rather than Kohn−Sham (KS) orbitals or the spin density distribution because the former do not suffer from the symmetry-breaking problem.[61−63] The correct behavior of the on-top pair density guarantees that the UDFT solution leads to a reasonable description of biradical **2-S** and, by this, also of the energetics of the Bergman reaction. This is in line with recommendations given by Perdew and co-workers.[61]

According to these observations, we carried out RB3LYP and UB3LYP geometry optimizations for 14 different enediyne systems (denoted by *1*, *2*, ..., *14*), which implied the investigation of five different stationary points along the reaction path starting at the enediyne (**E**), leading via **TS A** to the intermediate biradical **B** in its S state (**B**−**S**) (reaction A, top of Scheme 2), and proceeding via TS **B** to the isomeric enediyne **IE** (reaction B, top of Scheme 2). In addition, the T state of biradical **B** (**B**−**T**) was calculated in each case to determine the S−T splitting. Hence, in total 82 stationary points were investigated, which are shown in Scheme 2 (molecules **1**−**55** and TSs **TS**(**1**−**2**) to **TS**(**53**−**55**)). Because of the large number of calculations,

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Table 1. Energetics of the Bergman Cyclization of Enediyne **1** Calculated at Various Levels of DFT[a]

functional	basis set	$\Delta E^{\ddagger}(\mathbf{1}-\mathbf{2})$	ΔE_R	$\Delta E^{\ddagger}(\mathbf{2}-\mathbf{1})$	S−T(splitting)	μ	σ
SVWN	6-31G(d,p)	17.7	−4.6	22.3	6.1	8.3	7.1
	cc-pVTZ	19.2	0.6	18.6	6.6	7.3	3.6
BPW91	6-31G(d,p)	23.3	0.2	23.1	3.5	5.1	3.7
	cc-pVTZ	25.4	5.4	20.0	3.6	3.1	1.4
BLYP	6-31G(d,p)	25.4	6.8	18.6	4.1	3.1	1.9
	cc-pVTZ	28.6	13.6	15.1	4.5	4.8	2.9
BLYP,sum	6-31G(d,p)	26.7	6.8	18.6	6.8	4.5	2.0
	cc-pVTZ	25.8	10.8	15.1	7.3	4.5	1.9
B3LYP	6-31G(d,p)	31.2	3.3	27.9	2.5	3.7	2.3
	cc-pVTZ	34.4	10.1	24.3	2.6	2.9	1.3
	6-311+G(3df,3pd)	34.1	10.1	24.0	2.6	2.7	1.2
B3LYP,sum	6-31G(d,p)	29.9	1.1	27.9	4.7	3.6	3.0
	cc-pVTZ	32.1	7.8	24.3	4.9	1.3	0.9
	6-311+G(3df,3pd)	31.8	7.8	24.0	4.9	1.1	0.8
expertl	refs 14 and 15	30.1 ± 0.5	7.8 ± 1.0	22.3 ± 0.7	3.5 ± 0.5		

[a] Relative energies in kcal/mol. $\Delta E^{\ddagger}(\mathbf{1}-\mathbf{2})$, ΔE_R, and $\Delta E^{\ddagger}(\mathbf{2}-\mathbf{1})$ denote the barrier for the forward reaction, the reaction energy, and the barrier for the backward reaction, respectively, of the Bergman cyclization. The mean absolute deviation μ and the standard deviation σ are calculated with regard to the experimental values taken from ref 14 and corrected to energies at 0 K according to ref 15. The addition sum to the functional indicates use of the sum formula as described in ref 15. The cc-pVTZ basis was taken from Kendall, R. A.; Dunning, T. H., Jr.; Harrison, R. J. *J. Chem. Phys.* **1992**, *96*, 6796.

Pople's 6-31G(d,p)[64] basis set was employed throughout this work.

The internal and external stability of the R description of each structure was calculated following procedures described by Bauernschmitt and Ahlrichs.[65] RDFT calculations for the intermediate biradicals and some of the TSs turned out to be unstable (the external stability matrix possesses one negative eigenvalue λ indicating a breaking of the constraint $\psi_\alpha = \psi_\beta$) so that the geometry of the structure in question had to be reoptimized at the UB3LYP level of theory. All DFT geometries were confirmed as local minima or first-order transition states by computing analytically harmonic vibrational frequencies, which were also used to calculate zero-point vibrational energies (ZPE) and enthalpies $H(298)$, which include effects of ZPE and thermal corrections for the difference between 298 and 0 K.

Four different tests were made to investigate the reliability of B3LYP/6-31G(d,p) results for selected enediyne systems. First, B3LYP calculations with the 6-311+G(3df,3pd) basis[66] were performed to estimate the influence of the basis set on geometry and energy. Second, the B3LYP functional was replaced by the BLYP functional to see whether this functional leads to differing results. In the third and fourth steps, coupled cluster (CC) theory[67] was applied to scrutinize the reliability of DFT results. The CCSD(T) method, which includes all single and double excitations as well as a perturbative treatment of triple excitations,[68] was used because it is known to provide reasonable results for the parent system *1*.[18] However, CCSD(T) is also known to fail in the case of low-symmetry biradicals **B**−**S**,[69] and therefore, Brueckner (B) orbitals[70] and the more stable BD(T)[71] method were applied. In this way, a reliable description of the target systems investigated in this work was obtained.

The basicity of the heteroenediynes was probed by calculating proton affinities at 0 (PA) and at 298 K (PA(298)) using for the latter eq 1:

$$\mathrm{PA}(298,\mathrm{X}) = E(\mathrm{X}) - E(\mathrm{XH}^+) - [\Delta H_{\mathrm{therm}}(298, \mathrm{XH}^+) - \Delta H_{\mathrm{therm}}(298,\mathrm{X}) - 5/2RT] \quad (1)$$

where X is the base, XH^+ the conjugate acid, and $\Delta H_{\mathrm{therm}}(298)$ represents vibrational and thermal correction to obtain from energy values at 0 K enthalpy values at $T = 298$ K. As was documented in the literature, DFT in general, but in particular B3LYP, provides reliable PA values.[72−74] Charge distributions were investigated by employing the natural atomic orbital (NAO) population analysis.[75] All calculations were performed with the ab initio packages COLOGNE99,[76] GAUSSIAN98,[77] and ACES II[78] on a Cray C90 computer.

4. Results and Discussion

Calculated energies E or ΔE, enthalpies $H(298)$ or $\Delta H(298)$, ZPEs, entropies S, and free enthalpies $G(298)$ or $\Delta G(298)$ for the 14 enediyne systems of Scheme 2 are summarized in Table 2. Geometries for all molecules calculated are available as

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(78) Aces II: Stanton, J. F.; Gauss, J.; Watts, J. D.; Lauderdale, W. J.; Bartlett, R. J. *Int. J. Quantum Chem. Symp.* **1992**, *26*, 879.

Table 2. Energies *E*, Zero-Point Energies ZPE, Enthalpies *H*, Entropies *S*, Free Enthalpies *G*, and Dipole Moments μ of Enediyne Systems *1*−*14*[a]

molecule	R/U	ref	*E*, Δ*E*	ZPE	*H*(298), Δ*H*(298)	*S*(298)	*G*(298), Δ*G*(298)	ω_{low}	μ
					System *1*				
1	R		−230.88718	44.4	−230.80931	75.78	−230.84532	108	
TS(1−2)	R	**1**	31.2	43.9	29.9	69.4	31.8	−489	
2	U	**1**	3.2	46.6	4.3	65.9	7.2	413	
3	U	**2**	2.5	46.8	2.7				
					System *2*				
4	R		−246.91554	36.8	−246.84979	76.9	−246.88634	108	2.32
TS(4−5)	R	**4**	21.8	36.8	21.0	70.2	23.0	−446	1.71
5	U	**4**	−9.3	39.2	−8.1	68.2	−5.5	414	1.67
6	U	**5**	8.2	39.6	8.6	70.6	7.9	352	2.10
TS(5−7)	R	**5**	7.7	37.9	6.5	68.7	6.4	−490	1.70
7	R	**5**	−32.2	37.9	−32.4	76.1	−34.7	107	3.74
					System *3*				
8	R		−247.26161	45.6	−247.187185	77.1	−247.21850	110	1.23
TS(8−9)	R	**8**	26.2	45.1	24.9	70.6	26.8	−474	0.87
9	U	**8**	−5.8	47.6	−5.0	68.7	−3.5	393	1.59
10	U	**9**	2.8	47.9	3.2	71.0	3.5	352	1.68
TS(9−11)	R	**9**	20.0	44.7	17.4	70.4	16.9	−477	1.23
11	R	**9**	−22.8	44.5	−24.5	78.8	−27.5	103	3.20
					System *4*				
12	R		−361.47433	57.6	−361.37271	91.1	−361.41599	77	2.53
TS(12−13)	R	**12**	28.8	56.9	27.4	86.1	28.9	−487	3.15
13	U	**12**	7.6	59.2	8.1	82.6	10.6	107	3.26
14	U	**13**	3.3	59.6	3.7	85.0	3.0	89	3.48
TS(13−15)	U	**13**	2.9	57.8	1.6	85.8	25.7	−472	2.68
15	R	**13**	−31.3	58.6	−30.9	92.8	−34.0	52	5.26
					System *5*				
16	R		−361.82014	65.9	−361.70522	93.4	−361.74959	22	1.90
TS(16−17)	R	**16**	28.2	65.4	26.9	84.7	29.5	−479	0.83
17	U	**16**	−0.8	67.9	−0.1	81.9	3.4	129	0.58
18	U	**17**	1.5	68.1	1.8	84.1	1.1	130	0.54
TS(17−19)	R	**17**	22.7	65.2	20.4	86.3	19.1	−485	0.69
19	R	**17**	−28.2	66.3	−28.4	101.3	−35.3	3	1.32
					System *6*				
20	R		−517.54594	118.0	−517.34504	107.1	−517.39586	65	4.23
TS(20−21)	R	**20**	26.5	116.8	24.7	103.3	25.8	−474	4.03
21	U	**20**	10.8	118.2	10.2	101.8	11.8	66	4.01
22	U	**21**	2.7	118.4	2.9	104.4	2.1	51	4.16
TS(21−23)	U	**21**	3.4	116.8	2.2	104.4	1.6	−475	3.37
23	R	**21**	−30.6	117.6	−30.3	110.9	−33.0	52	5.21
					System *7*				
24	R		−517.91059	126.0	−517.74858	109.1	−517.74858	29	2.36
TS(24−25)	R	**24**	26.9	124.9	25.1	103.2	26.9	−441	2.14
25	U	**24**	4.0	126.6	3.7	100.7	6.2	80	3.16
26	U	**25**	1.2	126.7	1.4	0.7	102.9	79	3.14
TS(25−27)	R	**25**	22.8	124.3	20.8	102.8	20.1	−502	3.32
27	R	**25**	−23.9	124.9	−24.1	115.7	−28.6	25	6.58
					System *8*				
28	R		−516.66505	110.7	−516.52633	106.2	−516.52633	50	2.20
TS(28−29)	R	**28**	22.1	109.5	20.4	102.2	21.5	−469	2.12
29	U	**28**	−1.9	111.3	−2.1	101.8	−0.8	27	3.37
30	U	**1.2**	111.4	1.4		106.9	−0.1	7	3.40
TS(29−31)	R	**29**	22.3	109.0	20.3	101.9	20.3	−503	3.25
31	R	**29**	−24.2	109.6	−24.4	115.4	−28.5	31	6.28
					System *9*				
32	R		−341.59097	65.3	−341.47694	92.3	−341.52080	46	1.19
TS(32−33)	R	**32**	29.4	64.9	28.2	65.0	30.3	−464	1.18
33	U	**32**	12.1	66.8	12.5	84.4	14.9	50	1.73
34	U	**33**	2.8	67.1	3.0		1.9	23	1.95
TS(33−35)	R	**33**	7.2	65.5	5.8	83.9	6.0	−367	1.22
35	R	**33**	−34.4	65.7	−34.5	93.6	−37.2	45	2.90
					System *10*				
36	R		−341.97347	73.8	−341.84583	91.2	−341.88916	73	2.85
TS(36−37)	R	**36**	28.6	73.3	27.3	85.0	29.1	−471	1.98
37	U	**36**	2.7	75.5	3.4	83.4	5.7	131	2.17
38	U	**37**	1.8	75.7	2.0	85.8	1.3	131	2.17
TS(37−39)	R	**37**	20.6	73.4	18.6	84.3	18.4	−428	2.2
39	R	**37**	−36.5	74.0	−36.6	98.8	−41.2	20	2.58

Table 2 (Continued)

molecule	R/U	ref	E, ΔE	ZPE	$H(298)$, $\Delta H(298)$	$S(298)$	$G(298)$, $\Delta G(298)$	ω_{low}	μ
					System *11*				
40R			−497.66098	125.6	−497.44766	108.2	−497.49906	52	3.09
TS(40−41)	R	**40**	25.1	124.8	23.5	101.8	25.4	−460	2.48
41	U	**40**	13.5	125.9	12.8	101.0	14.9	60	2.16
42	U	**41**	2.7	125.9	2.7	102.8	2.2	71	2.36
TS(41−43)	R	**41**	5.6	124.6	4.3	101.4	4.2	−405	1.84
43	R	**41**	−32.0	124.7	−32.1	111.4	−35.2	42	2.9
					System *12*				
44R			−498.06129	133.9	−497.83462	107.5	−497.88569	63	4.96
TS(44−45)	R	**44**	27.8	132.8	26.0	103.0	27.4	−429	5.02
45	U	**44**	7.5	134.1	7.1	103.2	8.4	78	5.68
46	U	**45**	1.5	134.2	1.6	106.0	0.7	72	5.70
TS(45−47)	R	**45**	19.8	132.2	18.0	103.0	18.1	−445	5.41
47	R	**45**	−33.2	132.7	−33.4	118.2	−37.8	20	6.12
					System *13*				
48	R		−496.41386	110.5	−496.22495	108.7	−496.27660	39	2.90
TS(48−49)	R	**48**	19.0	109.5	17.3	102.0	19.3	−450	2.20
49	U	**48**	5.4	110.5	4.7	101.4	6.8	51	1.93
50	U	**49**	2.3	110.3	2.2	103.5	1.5	44	1.96
TS(49−51)	R	**49**	5.8	109.2	4.5	101.6	4.4	−412	1.66
51	R	**49**	−31.9	109.3	−32.1	114.4	−35.9	26	2.89
					System *14*				
52	R		−496.81279	118.7	−496.66269	109.7	−496.66269	16	5.17
TS(52−53)	R	**52**	21.2	117.4	19.3	103.4	21.1	−448	4.84
53	U	**52**	−0.4	118.8	0.89	104.5	0.6	28	5.88
54	U	**53**	1.05	118.9	0.4	108.5	0.4	15	5.95
TS(53−55)	R	**53**	19.5	117.0	17.6	102.6	18.2	−452	5.39
55	R	**53**	−33.5	117.3	−33.6	117.8	−37.6	27	6.38

[a] Absolute energies and enthalpies in hartree, relative energies, zero-point energies, and enthaplies in kcal/mol, entropies in cal/(mol·kelvin), frequencies in cm^{-1}, and dipole moments in debye. R/U indicates whether restricted (R) or unrestricted (U) DFT was used where application of the latter was a result of an external instability of RDFT. Ref denotes that molecule which is used as a reference for calculated energy (enthalpy) differences ΔE (ΔH, ΔG). The lowest calculated harmonic frequency σ_{low} is given to identify imaginary frequencies (denoted by a negative sign) and to characterize the conformational flexibility (rotation, N inversion, ring inversion) of a given molecule.

Table 3. Proton Affinities PA and PA(298) Calculated at the B3LYP/6-31G(d,p) Level of Theory[a]

molecule	PA	PA(298)	molecule	PA	PA(298)	molecule	PA	PA(298)
	System *2*			System *4*			System *9*	
4	217.2	209.9	**12**	217.0	210.1	**32**	240.0	232.9
7	204.2	199.6	**15**	222.4	214.3	**35**	251.5	242.8
5	213.7	206.7	**13**	225.4	218.2	**33**	249.4	242.1
6	219.1	212.3	**14**	227.2	218.7	**34**	250.4	243.2
74	235.6		**76**	235.3		**78**	258.0	
	System *6*			System *11*			System *13*	
20	228.8	222.1	**40**	251.2	244.3	**48**	250.3	243.4
23	228.9	222.5	**43**	258.4	251.3	**51**	257.7	250.5
21	235.6	228.6	**41**	257.1	249.9	**49**	256.2	249.0
22	237.1	230.3	**42**	258.4	251.1	**50**	257.1	249.6

[a] All values in kcal/mol. For the notation of molecules, see Schemes 2 and 4.

Supporting Information. In Table 3, calculated PA and PA(298) values are listed. In the following, electronic effects are discussed by referring to energies, but otherwise we will exclusively refer to calculated enthalpies at 298 K (reaction enthalpies, $\Delta H_R(298)$; activation enthalpies, $\Delta H^{\ddagger}(298)$) since these are of direct relevance for the experimentalist. We will not distinguish between enthalpies at room temperature (T = 298 K) and at body temperature (T = 310 K) since these differ by less than 0.1 kcal/mol (see above).

The heteroenediynes investigated can be grouped in three classes, which will be discussed separately considering points 1−4:

1. The thermodynamic stability of the enediyne **E**, intermediate biradical **B**, and isomeric enediyne **IE** will be investigated with the help of suitable reference molecules and formal reactions shown in Scheme 3. Reactions 1 and 2 compare the stability of the heteroenediynes **E** and **IE** with that of the parent enediyne **1** (in the case of a cumulene, another reference is used according to reaction 2c in Scheme 3). In reaction 3 relative stabilities of simple molecules with the C≡N and C═C linkage are compared with those containing the C≡C or C═N linkage. Reaction 4 determines the relative stability of enediyne **E** and its isomer **IE**. Reaction 5 compares electron delocalization in the intermediate biradical **B** with that in the parent *p*-benzyne **2**. Reaction 6 compares π delocalization in the saturated compounds **74**−**79** with that in benzene (**59**). Finally, reaction 7 estimates the H-abstraction ability of intermediate **B** by comparison with the parent biradical. The corresponding reaction enthalpies are listed in Table 4.

2. Differences in the geometries of **B**−**S** and **B**−**T** will be used to assess the amount of through-bond interactions between the single electrons of the biradical (Figure 2) and the possible involvement of electrons of the heteroatom(s) in these interactions. Through-bond interactions decrease the length of the four ring bonds at the C atoms with the single electrons while they increase the bond lengths of the two remaining bonds by partial

Table 4. Reaction Enthalpies $\Delta H_R(298)$ for Formal Reactions 1−7[a]

		formal reaction						
system	X, Y	1	2	3	4	5	6	7
2, aza	N, H	−6.5	−0.4	−34.3	−40.5	5.8 (−0.1)	−2.9	8.7 (2.8)
3, prot. aza	NH^+, H	−8.9	25.8	5.3	−29.4	0.4 (−0.1)	13.0	−12.6 (−13.1)
4, amide	NMe, O	−16.9	4.6	−1.4	−22.8	−20.7 (−21.7)	−19.6	−1.1 (−2.1)
5, prot. amide	NMe^+, O	−17.4	27.1	16.1	−28.4	−13.1 (−12.2)	−2.0	−11.1 (−10.2)
9, amidine	NMe, NH	−14.3	0.2	−7.4	−22.0	−22.6 (−22.9)	−21.6	−1.0 (−1.3)
10, prot. amidine	NMe^+, NH	−20.8	22.9	−33.2	−33.8	−20.0 (−19.2)	−10.2	−9.8 (−9.0)

[a] All values in kcal/mol. Enthalpy differences for triplet states are given in parentheses. For reactions 1−7 and the notation X, Y, see Scheme 3.

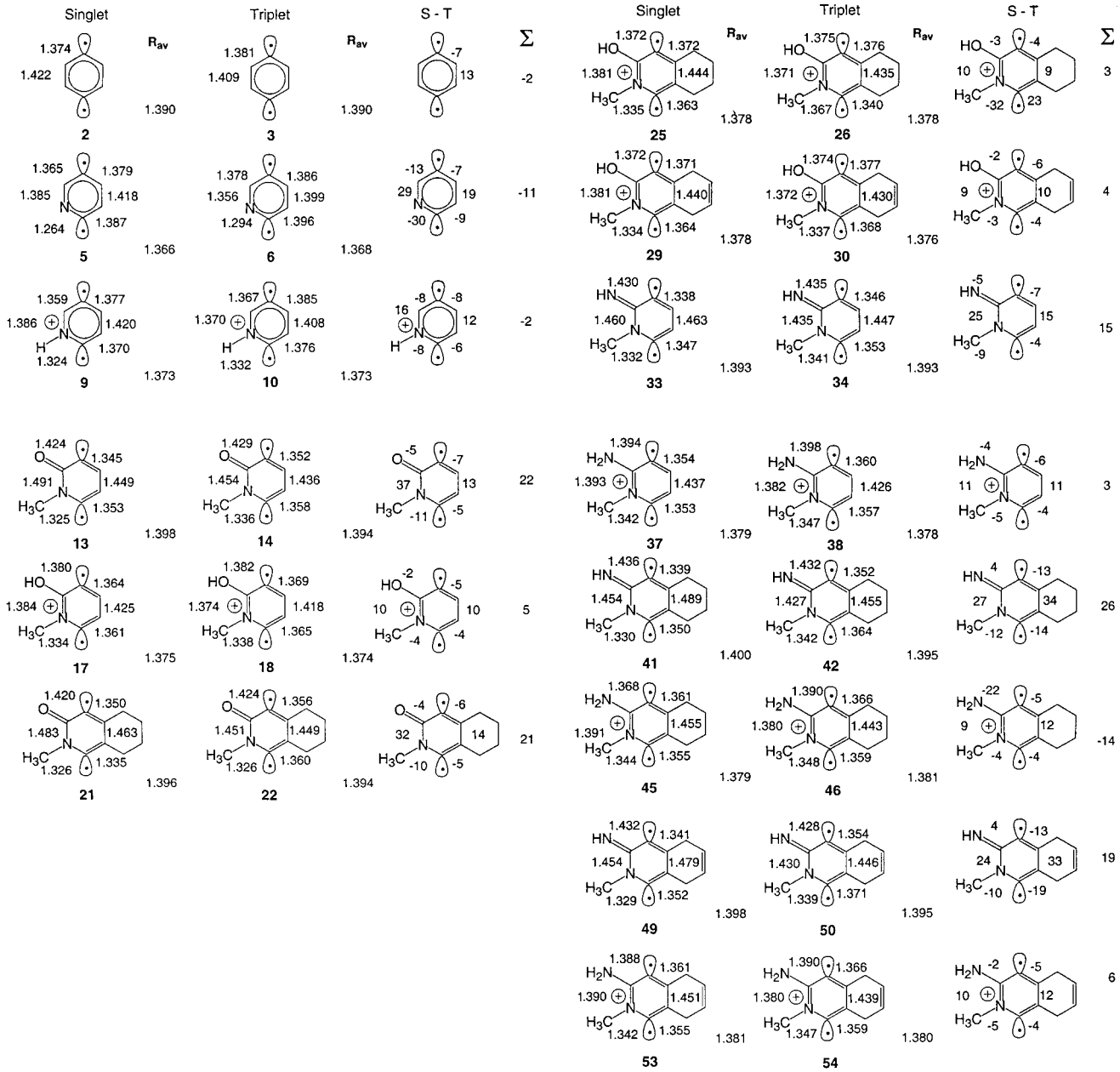


Figure 2. UB3LYP/6-31G(d,p) values of heavy atom bond lengths (in Å) of the singlet and triplet biradicals investigated in this work. R_{av} is the average ring bond length. The differences in bond lengths for singlet and triplet states are given under the heading S−T (in units of 10^{-3} Å). The sum of differences for the six ring bonds is given under the heading Σ.

occupation of a $\sigma^\star$ orbital. Since this is not possible in the **B**−**T** state, the difference between ring bond lengths in the S and the T state reflects the degree of through-bond interactions and is also useful to assess three-electron delocalization and anomeric delocalization of an electron lone pair at a N atom, which requires additional comparison with the parent **B**.[16] The sum

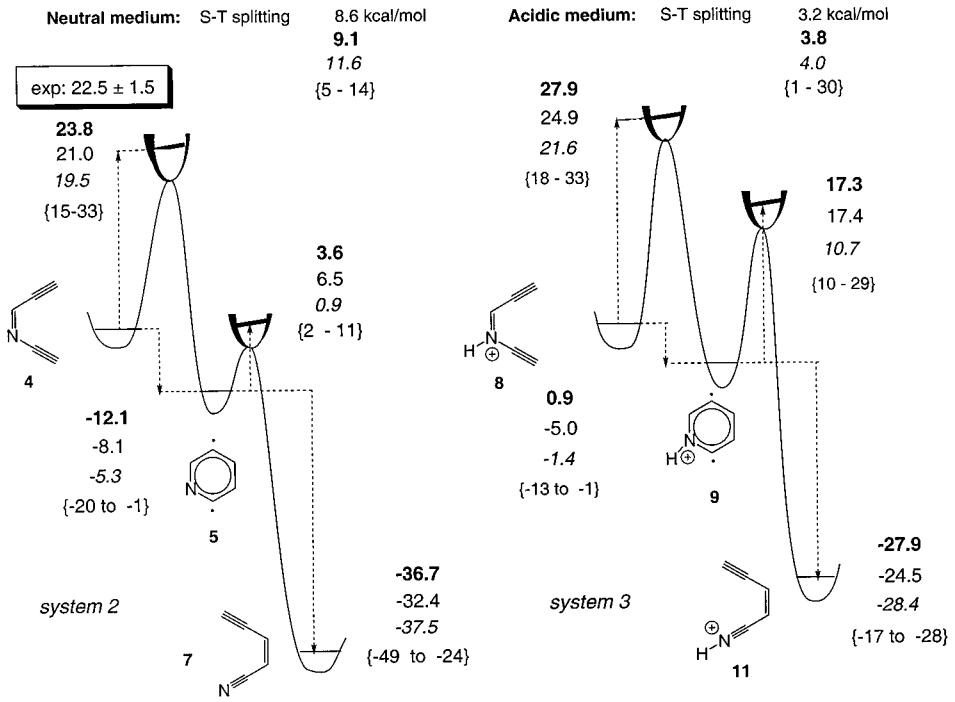


Figure 3. Reaction profiles for azaenediyne systems *2* and *3*. All enthalpies are given in kcal/mol at 298 K. B3LYP/6-31G(d,p) results are given in normal print, B3LYP/6-31+G(3df,3pd) results in bold print. Estimated values (in italics) based on BD(T) calculations and empirical corrections were taken from ref 69. Results of other ab initio and DFT calculations[30,69] are included by giving the range (given in braces) of calculated values. The experimental value for the activation enthalpy of a derivative of **4**[30] is also given.

of bond length differences (denoted by $\sum$ in Figure 2) and the average ring bond length R_{av} help to complete the description of electronic effects in the ring of **B**−**S**.

3. Calculated reaction enthalpies of reactions A and B in Scheme 2 will be discussed on the background of the thermodynamic stabilities of molecules **E**, **B**, and **IE**. The Hammond postulate will be applied to rationalize changes in reaction barriers.

4. Finally, the energetics of a given reaction system A + B (Scheme 2) will be viewed with regard to the requirements for a new enediyne warhead listed in Chapter 2.

(1) N-Containing Enediynes: (*Z*)-3-Aza-hex-3-ene-1,5-diyne (4). Aza-enediyne **4** (Scheme 2) was previously investigated by Chen and co-workers,[30] who synthesized various derivatives of **4**, measured for one of them the barrier to Bergman cyclization, and tested the reactivity of the enediyne in the presence of protic acid. These authors also carried out CASSCF(6,6)/6-31G(d) and CASMP2/6-31G(d) calculations to analyze experimental results. Later, Cramer[69] investigated systems *2* and *3* with the help of DFT-BPW91/PVDZ calculations as well as CCSD(T), BD(T), and CASPT2 single-point calculations at BPW91/PVDZ geometries. Figure 3 gives the energetics of reactions A (leading to the *p*-didehydropyridine biradical **5-S** via **TS 4**−**5**) and B (leading from **5** via **TS 5**−**7** to the isomeric nitrile **7**, (*Z*)-1-aza-hex-3-ene-1,5-diyne) as calculated in the present work. For comparison, the range of values from previous calculations[30,69] is given (in braces) as well as the best previous estimate based on BD(T) calculations combined with empirical corrections (in italics).[69]

System *2*. Chen and co-workers[30] measured the Arrhenius activation energy of 1,6-bis(4-*tert*-butylphenyl)-3-aza-4-methyl-hex-3-ene-1,5-diyne to be 23.1 ± 1.5 kcal/mol, which corresponds to a $\Delta H^{\ddagger}(298)$ value of 22.5 kcal/mol. Assuming that the methyl and aryl substituents do not influence the barrier to Bergman cyclization strongly (the aryl groups can arrange perpendicular to the plane of the enediyne), a direct comparison between the measured activation enthalpy and the corresponding calculated values is reasonable. The experimental value of 22.5 kcal/mol agrees well with the B3LYP/6-31G(d,p) and 6-311+G-(3df,3pd) results of 21.0 and 23.8 kcal/mol, respectively, obtained in this work while activation enthalpies published previously[30,69] can deviate considerably (Figure 3). In general, the B3LYP values for the Bergman reactions A and B of both azaenediyne **4** and its protonated form **8** (Scheme 2) agree reasonably (exception barriers of reaction B, Figure 3) with the BD(T) results, which in general may be considered to lead to the most reliable ab initio description. Since Cramer's BD(T) results were based on single-point calculations at BPW91/PVDZ geometries, which in view of the poor energetics obtained with BPW91 ($\Delta H^{\ddagger}$(298,**TS A**) = 14.5 kcal/mol;[69] experimental value, 22.5 kcal/mol, Figure 3) may be questionable, it is reasonable to consider the B3LYP results of this work to provide the most reliable description of the reaction system A + B.

Incorporation of an N atom into **1** at position 3 perturbs π delocalization and destabilizes the enediyne by 6.5 kcal/mol (reaction 1, Table 4). Biradical **5-S**, however, is stabilized relative to the parent biradical **2-S** by 5.8 kcal/mol (reaction 5, Table 4) because of anomeric delocalization of the electron lone pair at the N atom. In addition, three-electron interactions between the electron lone pair and the single electron at the vicinal C atom lead to stabilization as is reflected by the shortening of the NC4 bond relative to the bond length calculated for the T state **6** (Figure 2). Nitrile **7** is 40.5 kcal/mol more stable than **4** (reaction 4, Table 4), which is due to the fact that the C≡N bond is almost 35 kcal/mol more stable in relation to the C≡C triple bond than the C═N double bond in relation to the C═C double bond (reaction 4, Scheme 3). Since π delocalization is comparable in **1** and **7** (reaction 2: −0.4 kcal/mol, Table 4), the energy difference between the two azaenediynes **4** and **7** is the result of the transfer of the N atom

from the unfavorable position 3 to the more favorable position 1 (energy gain: $34 + 6 = 40$ kcal/mol, reaction 4; for numbering of atoms, see Scheme 2).

Because of the lower thermodynamic stability of **4** compared to **1** and the larger stability of **5-S** compared to **2-S**, the Bergman cyclization reaction becomes exothermic (−8.1 kcal/mol, small basis, Table 2; −12.1 kcal/mol, large basis, Figure 3), which according to the Hammond postulate explains a lowering of the activation enthalpy from 31 (parent system; exp. 30.1 kcal/mol, Table 1) to 21 kcal/mol (small basis, Table 2; 23.8 kcal/mol, large basis, Figure 3). The retro-Bergman reaction to nitrile **7** (reaction B, Scheme 2) is because of the large thermodynamic stability of the latter even more exothermic (small basis, −32.4; large basis, −36.7 kcal/mol), thus leading to a very low activation enthalpy (6.5; 3.6 kcal/mol) for reaction B.

Enediyne **4** will undergo Bergman cyclization at body temperature; however, it will lead to a **B** intermediate that is (a) kinetically not stable (isomerization reaction B is faster than H-abstraction reaction) and (b) probably not very active since the S−T splitting is relatively large (8.6; 9.1 kcal/mol) due to the stabilization of **5-S** and, accordingly, a low biradical character. This is also reflected by the fact that **5-S** is 9 kcal/mol more stable than the parent biradical **2-S**, (reaction 7, Scheme 3 and Table 4), which means that in the neutral medium of the normal cell the biological activity of **4** will be low as needed for limiting its toxicity.

System *3*. Protonation of **4** yields enediyne **8**, the π delocalization of which is even more perturbed (destabilization by 9 kcal/mol, reaction 1, Scheme 3 and Table 4). In the case of biradical **5-S**, protonation annihilates the stabilizing influence of the N electron lone pair so that through-bond coupling of the single electrons is cut back to the level found for **2-S**. This is indicated by the bond length differences shown in Figure 2 and the vanishing enthalpy value of reaction 5 (Table 4). The relative stabilities of **8** and **9-S** lead for Bergman reaction A to $\Delta H_R(298) = -5$ kcal/mol (less exothermic than in the neutral medium; large basis, 0.9 kcal/mol), which in turn implies a somewhat larger activation enthalpy (24.9 and 27.9 kcal/mol, Table 2, Figure 3) relative to enediyne system *2*.

Enediyne **11** containing a protonated N atom in the terminal position is 29.4 kcal/mol more stable than enediyne **8** with a protonated N in the central position, where the reason for the stability difference is no longer the difference in the strengths of bonds C=N and C≡N in relation to that of the corresponding CC bonds (this difference is changed from −34.4 to 5.3 kcal/mol according to Table 4, reaction 3) but mainly the more favorable charge delocalization in **11**.

The S−T splitting is reduced by protonation from 8.6 (system *2*; large basis, 9.1) to 3.2 (3.8) kcal/mol (system *3*), which indicates that in a sufficiently strong acidic medium the biradical character of the intermediate and, by this, its H-abstraction ability is increased. This is in line with the fact that **9-S** is 13 kcal/mol less stable than **2-S** (Table 4, reaction 7). The barrier for reaction B leading to the isomeric enediyne is increased (from 6.5 to 17.4 kcal/mol, Table 2; large basis, from 3.6 to 17.3 kcal/mol) in line with the reduced exothermicity of this reaction and the lower thermodynamic stability of enediyne **11**. Hence, biradical **9-S** should possess sufficient kinetic stability to abstract H from DNA. If one considers that the barrier for reaction A could be lowered by incorporating azaenediyne **4** into a ring, the system *2*/*3* with **4** and **8** as starting enediynes will fulfill requirements 3−6 for a new enediyne warhead listed in Section 2. However, enediynes **7**/**11** are not suited in this respect since the thermodynamic stability of these enediynes is too large thus leading to cyclization barriers ($\Delta H^{\ddagger}(298) = 38.9$ (neutral; large basis, 40.3); 41.9 kcal/mol (acidic; large basis, 45.2 kcal/mol), Table 2), which are too high to be reduced by incorporation into a 9- or 10-membered ring.

Our results are in line with the recent experimental work by David and Kerwin,[79] who observed that C,N-dialkynyl imines undergo a facile thermal rearrangement to β-alkynylacrylonitriles where the barrier to Bergman cyclization (reaction A) is lower than that for the parent enediyne. There were no traces of products that would arise as a result of H-abstraction by the intermediate biradical. David and Kerwin speculated that the intermediate might exist in alternative closed-shell structures which do no longer possess H-abstraction abilities. However, the absence of a H-abstraction reaction confirms the low kinetic stability of the biradical and the high thermodynamic stability of the isomeric nitrile which was produced in the experiment in quantitative yield. Chen and co-workers[30] confirmed these results and showed in addition that in acidic medium small amounts of substituted pyridine as result of an H-abstraction reaction could be observed in line with an increased kinetic stability of the intermediate 2,5-didehydropyridinium biradical of type **9-S**.

Chen and co-workers[30] observed also that an excess of acid destroys both the azaenediyne and the pyridine, which leads to a basic problem of using azaenediynes as new warheads. Apart from the fact that these compounds are difficult to synthesize in high yields,[79] they can undergo hydrolysis in the acidic medium of a tumor cell without any chance of abstracting H atoms from DNA. Hence requirement 1, namely, the stability of the enediyne warhead in the aqueous, weakly acidic medium of the tumor cell, is not fulfilled. Chen and co-workers suggested therefore as an alternative to azaenediynes N,C-dialkynyl amides such as **20** (Scheme 2) because amides are relatively stable in aqueous, weakly acidic media.[80] We will investigate in the following five different amides to check their usefulness as enediyne warheads.

(2) Enediynes with an Amide Group: Systems *4*−*8*. The amide group is a key unit in many biologically important macromolecules,[81] which already indicates that amides are stable under physiological conditions. Although N,C-dialkynyl amides such as **12** or **20** (Scheme 2) seem to be structurally different from an enediyne, we will show that they can undergo Bergman cyclization to a biradical in the same way as enediynes. This has to do with the well-known partial π character of the central CN (peptide) bond described in Scheme 5 with the help of zwitterionic resonance structures, which justify addressing amides **12** and **20** as enediynes in a formal way. However, all properties calculated in this work for **12** and **20** (geometries, charge distribution, dipole moment, vibrational frequencies, bond orders) show that these amides possess little zwitterionic character, which is also true for isomers **15** and **23** (Scheme 5).

In an amide, but in particular in an N,C-dialkynyl amide protonation occurs almost exclusively at the carbonyl oxygen[82] because in this way a delocalized enediyne structure is established while protonation at the nitrogen atom destroys any π delocalization, thus destabilizing the N,C-dialkynyl amide. Therefore, the possibility of N-protonation was not considered

(79) David, W. M.; Kerwin, S. M. *J. Am. Chem. Soc.* **1997**, *119*, 1464.

(80) *The Chemistry of Functional Groups, The Chemistry of Amides*; Zabicky, J., Ed.; Wiley: New York, 1970.

(81) See, for example: Reiman, J. E.; Byerrum, R. U. In *The Chemistry of Functional Groups, The Chemistry of Amides*; Zabicky, J., Ed.; Wiley: New York, 1970; p 601.

(82) See for example, Homer, R. B.; Johnson, C. D. In *The Chemistry of Functional Groups, The Chemistry of Amides*; Zabicky, J., Ed.; Wiley: New York, 1970; p 187.

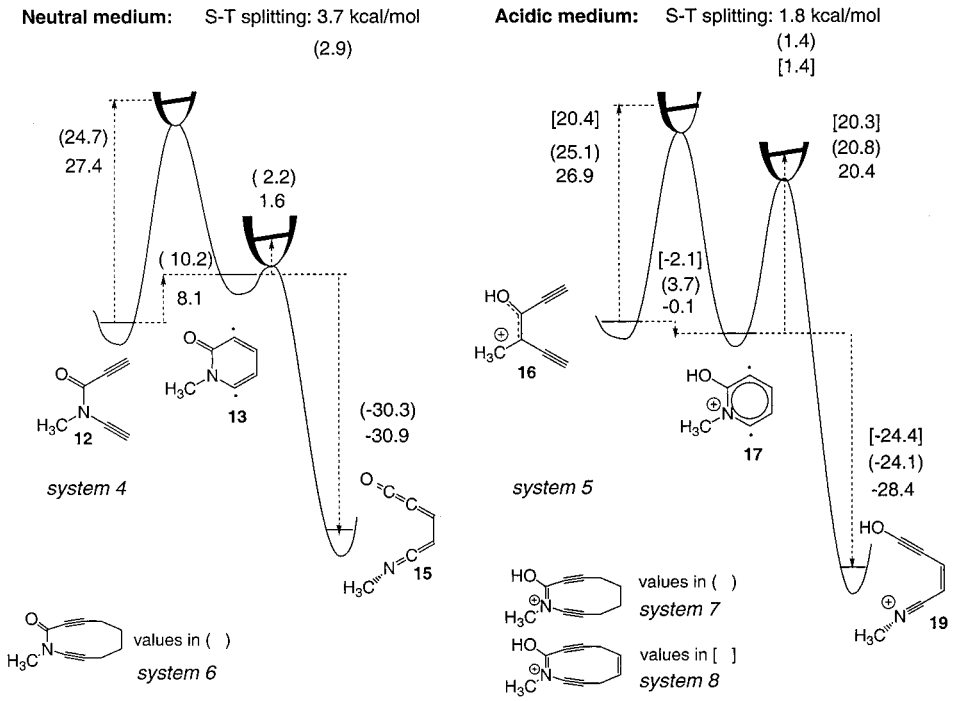
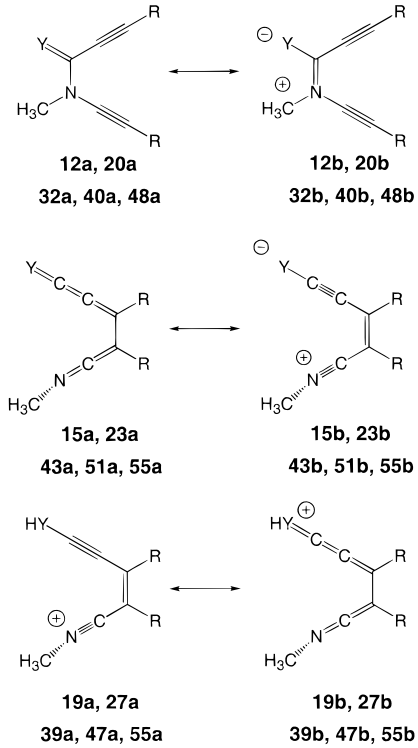


Figure 4. Reaction profiles for amide enediyne systems *4* and *5* (numbers in normal print), *6* and *7* (numbers in parentheses), and *8* (numbers in brackets) calculated at B3LYP/6-31G(d,p). All enthalpies in kcal/mol at 298 K.

Scheme 5



for amides **12** and **20**. In Figure 4, the calculated enthalpy profiles for systems *4*−*8* are shown.

The stability of amide **12** suffers from cross-conjugation, which is reflected by a destabilization enthalpy of 16.9 kcal/mol relative to 1 (Table 4, reaction 1). However, biradical **13-S** is even more destabilized relative to **2-S** ($\Delta H_R(298)(4) = -20.7$ kcal/mol, reaction 5, Table 4) because of reduction of π delocalization in the six-membered ring caused by the amide group. For example, the endocyclic CN bond length of 1.491 Å (Figure 2) and the lengthening of the average bond length R_{av} from 1.366 (**5-S**, Figure 2) to 1.398 Å (Figure 2) document this. Through-bond coupling is comparable to that in **2-S** and, by this, significantly smaller than in **5-S** (Figure 2) in line with a S−T splitting of just 3.7 kcal/mol (Table 2) and the reaction enthalpies 5−7 of Table 4. Actually, reactions 5 and 6 reveal that the amide group incorporated into benzene leads to a comparable destabilization as incorporation into biradical **2-S**.

The cumulene **15** is more stable by 22.8 kcal/mol than the isomeric amide **12** (Scheme 3, reaction 4). This is a result of the different π stabilization energies of the two isomers where the largest part of this difference is due to the destabilization of amide **12** by cross-conjugation.[17] Cummulene **15** contains an oxocumulene and an azaallene unit connected by a formal CC single bond in the same way as the two double bonds in *cis*-1,3-butadiene. It is interesting to note that neither the oxocumulene unit (OCC = 170.2, CCC = 144.9°) nor the azaallene unit (NCC = 174.6°) of **15** are linear. This is also the case for reference molecules **60** (OCC = 169.5, CCC = 150.0°), **62** (NCC = 173.1, CCC = 177.5°), and **61** (NCC = 176.0°) in line with the well-known nonlinearity of cumulenes.[83,84]

The Bergman cyclization of the amide **12** is endothermic by 8.1 kcal/mol and its activation enthalpy is 27.4 kcal/mol, thus reflecting the decreased stability of biradical **13-S**. In view of a S−T splitting of 3.7 kcal/mol, **13-S** should be as reactive as the parent compound **2-S**. Because of the stabilization of cumulene **15**, reaction B is considerably more exothermic (−30.9 kcal/mol) and possesses a much lower activation enthalpy (1.6 kcal/mol, Table 2, Figure 4) than found for the parent compound. Thus, biradical **13-S** is kinetically not stable in the neutral medium of the normal cell.

System *5*. Protonated amide **16** has a stability (17.4 kcal/mol, Table 4, reaction 1) comparable to that of amide **12** while the stability of biradical **17-S** is 7.6 kcal/mol (reaction 5, Table 4) larger than that of **13-S** because protonation improves in the former case 6π delocalization in the ring (R_{av} = 1.375 Å; Σ =

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(84) Liang, C.; Allen, L. C. *J. Am. Chem. Soc.* **1991**, *113*, 1873.

0.005, Figure 2). As a result, reaction A becomes slightly exothermic (−0.1 kcal/mol, Table 2, Figure 4) while the activation enthalpy hardly changes. Nitrilium cation **19** is 28.4 kcal/mol (reaction , Table 4) more stable than its isomer **16**. In contrast to the neutral system, an "aromatic" bond has to be broken in **17-S** to undergo reaction B, which leads to a substantial increase in the activation enthalpy (from 1.6 to 20.4 kcal/mol, Table 2, Figure 4) upon protonation, thus yielding a kinetically stable biradical **17**. Similar to that for azaenediyne **4**, protonation leads to a reduction of the S−T splitting indicating an increase of biradical character and, by this, a better ability of abstracting H atoms.

Since the activation enthalpy of reaction A (26.9 kcal/mol, system *5*, Table 2) is too high for an enediyne drug to react at body temperature, we also investigated 10-membered-ring amides **20** and **24**. As shown in Figure 4, the change in the activation enthalpy of reaction A is too small for the protonated system (Table 2: from 26.9 to 25.1 kcal/mol) and, therefore, system *8*, which possesses a double bond in the 10-membered ring, was also investigated. Because of the increased strain energy of **28**, the activation enthalpy of reaction A is 20.4 kcal/mol, which is sufficient to guarantee that it takes place at body temperature. Since the energetics of system *8* is comparable to that of both system *5* and system *7*, amide **28** and its neutral counterpart fulfill requirements 1 and 3−7 of section 2. The question is only whether an amide such as **12** or **20** can be protonated under the conditions of the tumor cell, which has to be investigated in the next section.

(3) Proton Affinities and Basicity. An efficient drug, which is biologically inert in the neutral medium of the normal cell, but biologically active in the acidic medium of the tumor cell, should be protonated by 99% or even more at pH 5.5. According to the Henderson−Hasselbach equation

$$\mathrm{pH} = \mathrm{p}K_a + \log([\mathrm{base}]/[\mathrm{acid}]) \qquad (2)$$

This implies p$K_a \geq 7.5$ in aqueous solution. The pK_a value of pyridine (**74**) is 5.25[85] and that of the corresponding biradical **5-S** should be lower in view of its stabilization as discussed above. The PA values of **4** (217 kcal/mol, Table 3) and **5-S** (214 kcal/mol) are 19 and 22 kcal/mol smaller than that of **74** (236 kcal/mol), which means that there should be a significant lowering of the pK_a value provided solvation effects do not compensate electronic effects. Considering the pK_a value of **74**, less than 50% of an azaenediyne is protonated at pH 5.5, which indicates that azaenediyne **4** or related compounds are unsuitable for a new enediyne warhead that distinguishes between normal and tumor cells.

In the case of amides **12** or **20**, the situation is even more unfavorable because amides are known to possess pK_a values between −2 to +2.[82] Hence, at pH 5.5, amides **12** or **20** will exclusively exist in their unprotonated form. Amides do not fulfill requirement 2 of section 2, and therefore, they are also not suited for a new enediyne warhead. Nevertheless, the other useful properties of amides **12** or **20** suggest that one looks after closely related compounds that hopefully possess all important amide properties but have a much higher pK_a value. These considerations lead to amidine systems *9*−*14* of Scheme 2.

Protonation of the amidino group occurs preferentially at the imino nitrogen rather than the amino nitrogen[86] since in this way a resonance-stabilized amidinium cation is formed. For example, the pK_a value of guanidine (**90**) is 13.6,[87] typical of a strong organic base.[88] This is also reflected by the calculated PA values (Table 3) although a direct correlation between PA and pK_a values was not successful. Instead, we correlated NBO charges at the imino N with experimentally known pK_a values for acetamidine (**84**),[89] benzamidine (**86**),[87] N^2-*p*-hydroxyphyenyl-N^1,N^1-dimethylformamide (**88**, Scheme 4),[90] and **90**[95] and obtained a quadratic relationship covering the range $8.2 < \mathrm{p}K_a < 13.6$ (see Table 5 and Figure 5).

Although the relationship shown in Figure 5 is just of a qualitative nature since any nonconsistency in solvation effects

Table 5. Proton Affinities PA, NBO Charges at the Imino N Atom, and pK_a Values for Selected Amidines[a]

molecule	PA	N	pK_a	ref
80	242.2	−741	(12.1)	
82	250.2	−765	(12.9)	
84	248.8	−755	12.4	89
86	252.7	−722	11.6	87
88	251.7	−549	8.3	90
90	251.6	−780	13.6	87
32	240.0	−687	(10.5)	
33-S	249.4	−744	(12.2)	
40	251.2	−704	(11.0)	
41-S	257.1	−670	(10.1)	
48	250.3	−705	(11.0)	
49-S	256.2	−691	(10.6)	

[a] PA values in kcal/mol, total charges at N in melectron. Experimental pK_a values (from refs 87, 89, and 90) refer to aqueous solution; predicted values (see Figure 5) are given in parentheses. For the notation of amidines, see Schemes 2 and 4.

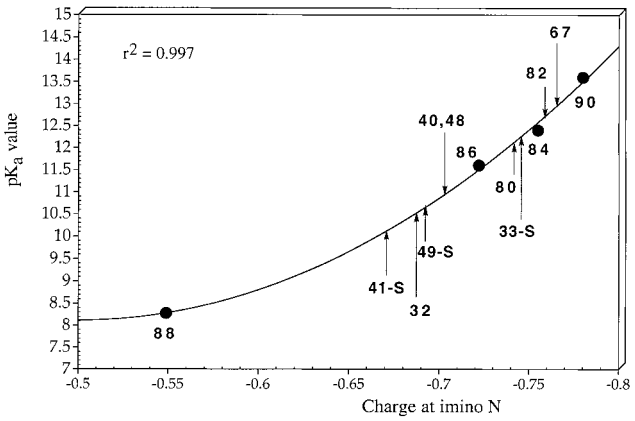

Figure 5. Experimental pK_a values (solid circles) as a function of NBO charges calculated for the imino N atom of amidines **84**, **86**, **88**, and **90** (see Scheme 4) at the B3LYP/6-31G(d,p) level of theory. The quadratic function obtained by a least-squares fit ($R^2 = 0.997$) was used to calculate pK_a values of enediynes and biradicals with an amidine or amidinium group as indicated in the diagram by arrows.

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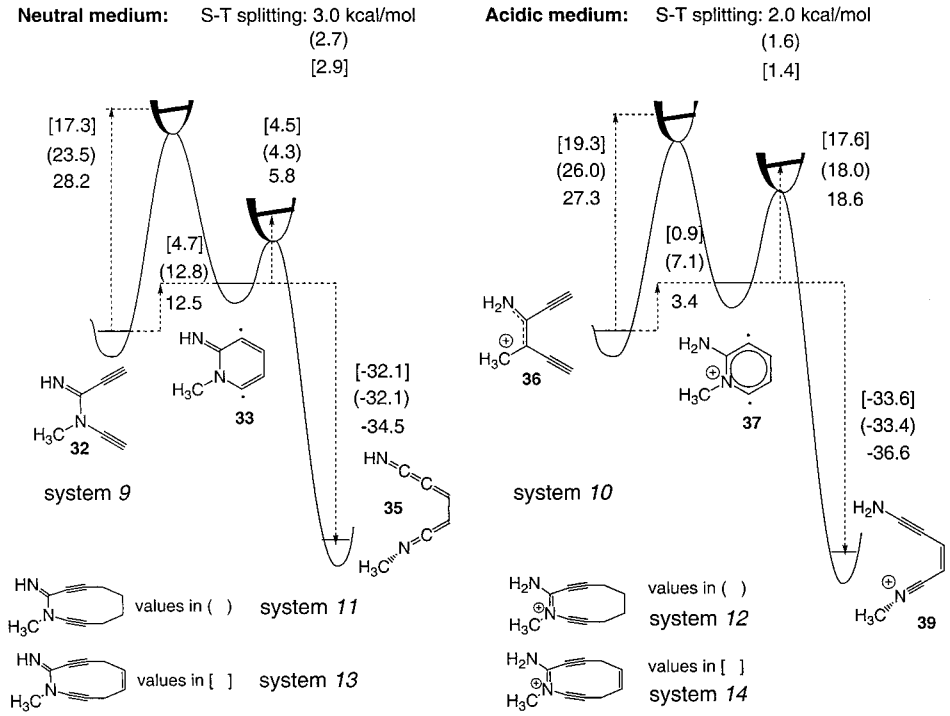


Figure 6. Reaction profiles for amidine−enediyne systems *9* and *10* (numbers in normal print), *11* and *12* (numbers in parentheses), and *13* and *14* (numbers in brackets) calculated at B3LYP/6-31G(d,p). All enthalpies in kcal/mol at 298 K.

can drastically change the dependence of the pK_a value on the charge at the imino N, it nevertheless provides some evidence that the pK_a values of the amidines investigated in this work are all larger than 10. In the acidic medium of the tumor cell, these amidines will be protonated more than 99.99%, which means that they fulfill requirement 2 of chapter 2.

Since at each stage along reaction paths A and B protonation or deprotonation can take place, the question is whether protonated amidines such as those investigated in this work can loose their proton at the stage of the biradical and, by this, become deactivated. Also, if the neutral enediyne undergoes Bergman cyclization, then it should be guaranteed that the neutral biradical should be a strong base being protonated in the slightly acidic medium of the tumor cell. Inspection of Figure 5 and Table 5 shows that biradicals **33-S**, **41-S**, and **49-S** are all strong bases with estimated pK_a values of 12.2, 10.1, and 10.6. Hence, amidines seem to be ideal candidates for a new enediyne warhead provided they fulfill the other requirements of chapter 2.

(4) Enediynes with an Amidine Group: Systems *9*−*14*. Calculations shows that N,C-dialknylamidines **32**, **40**, and **48** have properties similar to the corresponding amides although the stability of the individual enediynes or biradicals may differ by a few kilocalories per mole when comparing amidine and amide (see Table 4). Noteworthy is the decreased stability of the *N*-methylaminopyridinium biradical **37-S** (reaction 5, −20 compared to −13 kcal/mol, Table 4), which indicates stronger perturbation of 6π electron delocalization by a NH_2 than an OH π donor which is parallel to an increase in the average bond length R_{av} (**17-S**, 1.375; **37-S**, 1.379 Å, Figure 2).

In Figure 6, the calculated enthalpy profiles for systems *9*−*14* are shown. All amidines can undergo Bergman cyclization, which confirms that they have some partial enediyne character as described in Scheme 5. For example, the central CN bond of **32** has partial π character as reflected by the C−N bond length of 1.345 Å (B3LYP/6-31G(d,p)), which is shorter than that in formamidine, **80** (R(C−N) = 1.397 Å) or N^2-*p*-hydroxyphyenyl-N^1,N^1-dimethylformamide, **88** (R(C−N) = 1.366 Å). The calculated energetics of systems *9*−*14* closely resemble that of the corresponding amides: (1) In the neutral medium, the intermediate biradicals are kinetically unstable (Figure 6) while protonation increases their kinetic stability so that H-abstraction should be faster than the retro-Bergman reactions A or B. (2) The barrier of the reaction A can be decreased to 17.3 and 19.3 kcal/mol, respectively, by incorporating the amidine into a 10-membered ring with a double bond (systems *13* and *14*, Scheme 2, Figure 4). (3) Protonation leads always to a decrease of the S−T splitting corresponding to an increase in biradical character caused by a reduction of through-bond coupling as is nicely reflected by the parameter Σ of Figure 2. In the case of systems *13* and *14*, the S−T splitting changes from 2.9 to 1.4 kcal/mol (Table 2, Figure 6) because of protonation suggesting that **53-S** is one of the strongest biradicals investigated in this work.

We conclude that amidine **48** is the best candidate for a new enediyne warhead since it should have a totally different chemical behavior in the normal cell (low kinetic stability, rapid rearrangement to cumulene **51**, no H-abstraction ability, low toxicity) and in the tumor cell (relatively high kinetic stability with regard to a rearrangement to cumulene **55**, strong H-abstraction ability, strong biological activity) where the high pK_a value of **48** (Table 5 and Figure 5) guarantees that the amidine is completely converted into its protonated form in the tumor cell. In the following, we have to verify these results by appropriate calculational results at higher levels of theory.

5. Reliability of Quantum Chemical Results

Tables 6 and 7 summarize energies (enthalpies) obtained with other methods and basis sets to test (1) the basis set dependence of B3LYP/6-31G(d,p) geometries and energies, (2) the performance of B3LYP in comparison with the GGA functional BLYP, (3) the influence of electron correlation using the CCSD(T) method, and (4) the applicability of DFT in the case of biradicals by applying the BD(T) method.

Table 6. Energetics of the Amidine Systems *9* and *10* Obtained with Different Methods and Basis Sets[a]

molecule	ref molecule	Δ*E*(B3LYP/A)	Δ*E*(B3LYP/B//B3LYP/A)	Δ*E*(B3LYP/B)	diff(opt-sp)[b]	Δ*E*(CCSD(T)/A//B3LYP/A)	Δ*E*(BD(T)/A//B3LYP/A)	Δ*E*(BLYP/A//BLYP/A)
				System *9*				
32		−341.59097	−341.71097	−341.71154	−0.36	−340.68380	−340.64499	−341.46589
TS (32−33)	**32**	29.4	32.8	32.7	−0.27	29.8	29.2	23.3
33	**32**	12.1	18.4	18.5	−0.26	−14.0	16.2	10.7
34	**33 (32)**	2.8 (14.9)	2.7 (21.1)	2.7 (21.1)	−0.25	35.0 (21.0)	3.5 (19.7)	5.3 (16.0)
TS(33−35)	**33 (32)**	7.2 (19.3)	6.1 (24.5)	6.1 (24.5)	−0.31	34.7 (20.7)	34.7 (20.7)	*c*
35	**33 (32)**	−34.4 (−22.3)	−39.4 (−21.0)	−39.2 (−20.6)	0.01	−24.0 (−10.0)	−26.4 (−10.2)	−37.9 (−27.1)
				System *10*				
36		−341.97347	−342.08447	−342.08502	−0.34	−341.06702	−341.02781	−341.84792
TS (36−37)	**36**	28.6	31.8	31.9	−0.27	27.7	27.1	22.1
37	**36**	2.7	8.8	8.9	−0.26	−24.7	4.6	5.9
38	**37 (36)**	1.8 (4.5)	1.8 (10.6)	1.8 (10.7)	−0.26	34.4 (9.7)	4.1 (8.7)	2.9 (8.8)
TS(37−39)	**37 (36)**	20.6 (23.3)	19.4 (28.2)	19.4 (28.3)	−0.28	46.4 (21.6)	16.5 (21.1)	7.5 (13.4)
39	**37 (36)**	−36.5 (−33.8)	−41.4 (−32.6)	−41.5 (−32.6)	−0.38	2.7 (−22.0)	−26.5 (−21.9)	−41.9 (−36.0)

[a] Absolute energies in hartree and relative energies Δ*E* in kcal/mol. Basis A, 6-31G(d,p); basis B, 6-311+G(3df,3pd). Ref molecule denotes that molecule which is used as a reference for calculated energy differences Δ*E*. [b] Diff(opt-sp) is the difference between B3LYP/B//B3LYP/B and B3LYP/B//B3LYP/A energies. [c] No transition state was found.

Table 7. Relative Enthalpies for Azaenediyne Systems *2* and *3* and Amidine Systems *9*, *10*, *13*, and *14* Evaluated at the B3LYP/6-311+G(3df,3pd) Level of Theory[a]

molecule	ref mol	Δ*H*(298)	diff[b]	molecule	ref mol	Δ*H*(298)	diff[b]
	System *2*				System *3*		
4		0		**8**		0	
TS (4−5)	**4**	23.8	2.8	**TS (8−9)**	**8**	27.9	3.0
5	**4**	−1.9	6.2	**9**	**8**	0.9	5.9
6	**5**	9.1	0.5	**10**	**9**	3.8	0.6
TS(5−7)	**5**	3.6	−2.9	**TS(9−11)**	**9**	17.3	−0.1
7	**5**	−36.7	−4.3	**11**	**9**	−27.9	−3.0
	System *9*				System *10*		
32		0		**36**		0	
TS (32−33)	**32**	31.6	3.4	**TS (36−37)**	**36**	30.5	3.2
33	**32**	18.8	6.3	**37**	**36**	9.5	6.1
34	**33**	2.9	−0.1	**38**	**37**	2.0	0
TS(33−35)	**33**	4.7	−1.1	**TS(37−39)**	**37**	17.4	−1.2
35	**33**	−39.5	−5.0	**39**	**37**	−41.5	−4.9
	System *13*				System *14*		
48		0		**52**		0	
TS (48−49)	**48**	24.0	6.7	**TS (52−53)**	**53**	23.5	4.2
49	**48**	10.2	5.5	**53**	**52**	6.3	5.4
50	**49**	2.4	0.2	**54**	**53**	1.0	−0.4
TS(49−51)	**49**	3.2	−1.3	**TS(53−55)**	**53**	16.4	−1.2
51	**49**	−36.5	−4.4	**55**	**53**	−38.6	−5.0

[a] All values in kcal/mol. Geometries, zero-point energies, and thermal corrections were taken from B3LYP/6-31G(d,p) results. [b] Diff denotes the difference between B3LYP/6-311+G(3df,3pd) and B3LYP/6-31G(d,p) enthalpies.

The basis set dependence of calculated geometries was tested for amidine systems *9* and *10* by carrying out B3LYP/6-311+G(3df,3pd) single-point calculations at B3LYP/6-31G(d,p) geometries and then recalculating geometries with the larger basis. The differences in absolute energies thus obtained are all <0.4 kcal/mol (Table 6, column 6) so that differences in relative energies are just ≤0.1 kcal/mol (Table 6). Therefore, it is justified to determine B3LYP geometries with the 6-31G(d,p) basis set.

B3LYP/6-311+G(3df,3pd) single-point calculations using BLYP/6-31G(d,p) geometries and frequencies for aza systems *2*, *3* and amidine systems *9*, *10* and *13*, *14* reveal that the larger basis leads to a more endothermic Bergman cyclization reaction (increase by 5.5−6.3 kcal/mol, Table 7; see also Table 1). This is caused by the fact that the description of an enediyne benefits more from a larger, more flexible basis set than that of a cyclic biradical with a more rigid hexagon-related geometry. Hence, the stability of the enediyne increases relative to that of the intermediate biradical, which is also responsible for an increase of the first barrier by 3−7 kcal/mol, a more exothermic reaction B, and a somewhat lower barrier for B. S−T splittings are almost unchanged by the basis set increase, which indicates that the electronic structure of the intermediate biradical in its S and T states is consistently described by the two basis sets.

In the case of azaenediyne **4**, the B3LYP/6-311+G(3df,3pd) barrier of 23.8 kcal/mol deviates from the experimentally determined barrier[30] by just 0.7 kcal/mol and as such is the most accurate barrier calculated so far. In the case of the target systems *13* and *14*, the first barrier is raised to 24 and 23.5 kcal/mol, respectively, which suggests that Bergman reaction A of **52** is rather slow in the tumor cell and further strain raising (barrier lowering) structural changes (incorporation of electronegative substituents; see section 2 and ref 23) may be needed. In case of Bergman cyclization B, the decrease in the barrier (Table 7) improves the kinetic properties of the intermediate biradical without jeopardizing it H-abstraction ability (barriers of protonated forms are still larger than 15 kcal/mol, Table 7). Since energy changes obtained with the larger basis set are regular for all systems tested and since the conclusions drawn from B3LYP/6-31G(d,p) results do not

Table 8. Application of Requirements 1−6 to Enediyne Systems *2*−*14* for the Determination of a New Enediyne Anticancer Drug[a]

	requirement										
	(1b) thermo stab of warhead reaction 1		(2, 3) basicity pK_a value	(4) reactivity at body T $\Delta H^{\ddagger}$(310, A)		(5) kinet stab of B $\Delta H^{\ddagger}$(310, B)		(6) H-abstrn ability (S−T) splitting		(6) H-abstrn ability reaction 7		(1−6)
system	large neutral	small acidic	>9	>24 neutral	<24 acidic	<14 neutral	>14 acidic	large neutral	small acidic	>0 neutral	<0 acidic	fulfilled
(*2*,*3*)	−6.5	−8.9	≤5	21.0	24.9	6.5	17.4	8.6	3.2	8.7	−12.6	
	no	yes	no	no	no	yes	yes	yes	yes	yes	yes	no
(*4*,*5*)	−16.9	−17.4	<2	27.4	26.9	1.6	20.4	3.7	1.8	−1.1	−11.1	
	no	yes	no	yes	no	yes	yes	no	yes	(yes)	yes	no
(*6*,*7*)	<−5	<−5	10−11	28.2	27.3	5.8	18.6	3.0	2.0	<0	<0	
	no	yes	yes	yes	no	yes	yes	no	yes	(yes)	yes	no
(*9*,*10*)	−14.3	−20.8	<2	24.7	25.1	2.2	20.8	2.9	1.4	−1.0	−9.8	
	no	yes	no	yes	no	yes	yes	no	yes	(yes)	yes	no
(*11*,*12*)	<−10	<−10	10−11	23.5	26.0	4.3	18.0	2.7	1.6	<0	<0	
	no	yes	yes	no	no	yes	yes	no	yes	(yes)	yes	no
(*13*,*14*)	<−10	<−10	10−11	17.3	19.3	4.5	17.6	2.2	1.4	<0	<0	
	no	yes	yes	no	yes	yes	yes	no	yes	(yes)	yes	yes

[a] All enthalpy values in kcal/mol. Reactions 1 and 7 (see Table 4) measure energy differences with regard to the parent system 1. For reaction 1, negative energies indicate destabilization relative to **1**, for reaction 7; negative energies indicate a larger H-abstraction ability than that of **2-S**.

change when applying the more flexible 6-311+G(3df,3pd) basis set, the discussion based on B3LYP/6-31G(d,p) theory is justified.

As was discussed previously,[15] BLYP results suffer from the known deficiencies of GGA functionals, namely, to underestimate reaction barriers.[59] For systems *9* and *10*, barriers are to small by 6 and 13 kcal/mol (Table 6), respectively, where in case of the neutral system, the TS of reaction B could not be located at all. Therefore, BLYP is not an appropriate functional for the description of the enediyne systems discussed in this work, which is in line with the results summarized in Table 1 and the discussion in ref 15.

CCSD(T)/ and BD(T)/6-31G(d,p) results at B3LYP/6-31G-(d,p) geometries (Table 6) are parallel, however, with one important exception concerning the energy of biradicals **33-S** and **37-S**. CCSD(T) theory fails to provide a reasonable description of these biradicals, thus leading to an unrealistically low energy for the S states and a large S−T splitting. This result is in line with the known failure of of CCSD(T) to describe low-symmetry biradicals with small S−T splitting[69] and is caused by an external instability of the CC wave function.[91] A comparison of BD(T) and B3LYP results reveals that, apart from the reaction energy of B, changes predicted by BD(T) for the energetics of systems *9* and *10* are similar as those found for enlarging the basis set at the B3LYP level. Since these trends are obtained already with the 6-31G(d,p) basis set and since wave function methods are much more sensitive to an extension of the basis set to TZ quality than DFT methods, it is likely that BD(T)/large basis set calculations are close to B3LYP/6-311+G(3df,3pd) results. In any case, the BD(T) results obtained in this work confirm the general conclusions of section 4.

6. Chemical Relevance of Results: A New Enediyne Warhead

In Table 8, all systems investigated in this work are evaluated according to requirements 1−6 of section chapter 2 that have to be fulfilled by a useful enediyne anticancer drug.

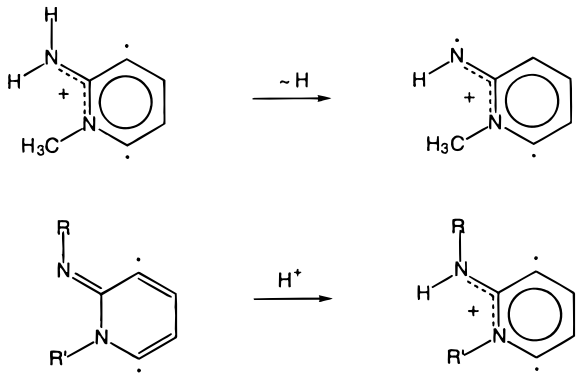
For some of these requirements, the calculations carried out in this work cannot provide any conclusive answer. For example, the question whether the thermodynamic stability of the enediyne drug (requirement 1a) is sufficient to guarantee distribution of the drug in a given body region before Bergman cyclization takes place will depend on the embedment of the warhead in the delivery system. Even an unstable enediyne can be stabilized within a drug, as was found for the naturally occurring enediynes, and therefore, requirement 1a can only be tested considering the whole drug and not just the enediyne warhead. On the other hand, quantum chemical calculations provide a basis for predicting an increased activity of the enediyne warhead (requirement 1b) due to a lower thermodynamic stability relative to the parent enediyne. As Table 8 (column 1) reveals, this is fulfilled for all heteroenediynes investigated in this work where estimates are based on energies of reaction 1 (Table 4, Scheme 3).

Of course, Table 8 also shows that an ideal change of the thermodynamic stability from a high to a low value upon protonation, which would also affect requirement 4 and strongly reduce the toxicity of the enediyne drug, cannot be expected for the systems investigated and, therefore, must be balanced by requirements 5 or 6. For the amidines, the reactivity of the intermediate biradical is always large according to calculated S−T splittings (Table 8) although it is larger in the tumor cell as shown for the S−T splittings of the amidinium ions and the energies of the H-abstraction reaction 7 of Table 4. Hence, the kinetic stability of the intermediate biradical (requirement 5) becomes actually decisive as to the toxicity of the amidine drug. There is a relatively large difference in calculated rearrangement barriers for the neutral (4 kcal/mol, Table 8) and the acidic medium (18 kcal/mol), which should guarantee that an amidine drug is biologically inactive in the normal cell, but becomes active in the tumor cell because the barrier for H-abstraction from DNA should be of the order of 14 kcal/mol. Hence, fulfillment of requirement 5 provides the only guarantee for the low toxicity of the amidine enediyne drugs suggested in this work.

As for the basic character of the heteroenediynes investigated, only amidines can guarantee stable protonated forms in the acidic medium of the tumor cell (requirements 2 and 3). Also, the corresponding biradicals are strong bases to remain in their protonated form under the conditions of the tumor cell. Connecting their basicity with the change in kinetic stability upon protonation, amidines are the ideal candidates for a new enediyne drug with low toxicity but large biological activity.

One has also to consider that there are other reaction possibilities for the intermediate biradical. Such a possibility is the H migration from an amino group of the amidinium cation to the pyridine ring, thus leading to the N analogue of the Myers−Saito biradical.[92] Calculations show that such a process

has a rather low barrier and leads to an interesting new class of active biradicals, which we are investigating presently. However, H migration can be suppressed by replacing the H atom of the imino group by a bulky substituent R, which will be anti rather than syn oriented with regard to a sterically demanding aryl (alkyl) group R′ at the pyridine N atom. Protonation would place the H atom in a position that hinders migration to the ring because the barrier to rotation at the CN(HR) bond in amidinium ions is normally larger than 14 kcal/mol and in the case of steric interactions between bulky substiuents even larger than 20 kcal/mol.[93]



It is more a technical question to select a suitable carbon frame for an amidine group so that the barrier of step A is lowered below 24 kcal/mol to guarantee biological activity at body temperature. Amidine **52**, for which the amidine part is incorporated into a cyclodecaene, fulfills requirement 4. However, calculations carried out with a larger basis set or at the BD(T) level of theory suggest that probably electronegative substituents have to be introduced into **52** to enforce an activation enthalpy for reaction A, which is sufficiently below 24 kcal/mol to guarantee activity of the drug at body temperature.

Amidine units are contained in many compounds of importance in biochemistry and medicine.[93,94] Amidines are synthesized by a few microorganism, which has attracted chemists to explore their usefulness in chemotherapy.[95] For example, N-unsubstituted amidines such as amidinomycin, which was isolated from a species related to *Streptomyces flavochromogenes*, possess antivirial properties.[96] Several synthetic amidines such as terephthalanilide[97] or methylglyoxal bis(guanylhydranzone)[98] show anticancer activities, especially against leukemia and, therefore, were suggested as potentiators of cancer chemotherapeutic agents. However, they showed severe toxicity, which must be also tested in the case of an amidine-based new enediyne drug. In any case, there should be no principal problems to synthesize a compound such as **48**.

Since the calculations discussed so far refer to the gas phase, we also considered how results might change in the aqueous medium of a tumor cell. It is well-known that a spline of water molecules is embedded in the minor grove of the DNA strands,[99] and therefore, one might assume that water molecules will solvate (or complex) the enediyne warhead if the enediyne docks into the minor grove. We have tested how the formation of the biradical is influenced in aqueous solution by performing model calculations on the parent amidine and protonated amidine systems employing the polarized continuum model of Tomasi[100a] in connection with the self-consistent isodensity approach (dielectric constant for water: 78.9).[100b] Changes in the energetics of the reaction caused by the water solvent sphere are all smaller than 1 kcal/mol while the proton affinity of **32** changes, as expected, significantly. There is a slight increase in the barrier of the Bergman reaction of **32** by 0.3 kcal/mol, which will slightly slow the reaction in water.[101]

These results differ from recent reports on a pronounced solvent dependence of the Bergman reaction. For example, the Bergman cyclization of 2,3-diethynlquinoxaline is slowed by a factor of more than 20 with increasing polarity (dielectric constant) of the solvent (THF vs acetonitrile).[102a] A similar solvent dependence was found for nine-membered cyclic enediynes.[102b] These observations could indicate specific solvation effects for the enediynes investigated**.** Apart from this, one has to consider that all investigations were carried out for free enediynes rather than enediyne−DNA complexes. Recently, Kumar and co-workers[103] carried out a molecular dynamics simulation of the structure of the calicheamicin−DNA complex in aqueous solution. They found that there is a close complementarity at the interface between the drug and the DNA minor groove spanning the entire binding site. Therefore, it seems, that the drug by entering the minor groove forces the water spline out of this region so that the actual Bergman reaction is not affected by the presence of water molecules.

Following the results by Kumar and co-workers, future work has to concentrate on the modeling of enediyne−DNA complexes derived from **32** under different environmental conditions. Most likely the solvent will not directly influence the activity of the enediyne drug, but may have an indirect effect. Unno and co-workers[104] suggested that a water-soluble enediyne may show enhanced antitumor activity and reduced toxicity due to increased bioavailability.

In conclusion, amidine **48** is a strong candidate for a new enediyne anticancer drug because it meets the seven requirements described in section 2. Investigations are in progress to determine how the delivery system and the trigger device of a natural enediyne (see Figure 1) influence the properties of amidine **48**.

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Supporting Information Available: Geometries for all the calculated molecules (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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