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On the significance of the trigger reaction in the action of the calicheamicin γ_1^I anti-cancer drug

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Abstract. The significance of the so-called trigger reaction in the reaction mechanism of the calicheamicin γ_1^I anti-cancer drug has been studied with ab initio quantum chemical methods. The structures of four fragments of calicheamicin γ_1^I, consisting of either 39 or 41 atoms, have been fully optimized using the Becke-Perdew86 density functional method and the 6-31G* basis sets. The four structures constitute members of an isodesmic reaction for which the reaction energy is a direct measure of the change in activation energy of the Bergman reaction, caused by the structural rearrangements of the preceding trigger reaction. This difference in activation energy has been calculated with density functional theory, using the exchange-correlation functional mentioned above, and with second-order Møller-Plesset perturbation theory (MP2), employing an ANO-type basis set. In both cases a value of 12 kcal/mol is obtained. The study firmly supports the hypothesis that the significance of the trigger reaction is to saturate a double bond in the vicinity of the enediyne group, which counteracts the formation of the biradical state of the drug. The MP2 computations became feasible by a novel implementation of an integral-direct, distributed-data, parallel MP2 algorithm.

Key words: Bergman reaction – Calicheamicin – Integral-direct MP2 – Parallel MP2 – Trigger reaction

1 Introduction

The structure and functionality of the "enediyne" class of anti-cancer drugs have been carefully investigated both experimentally and theoretically during the last 10 years. A typical example of these drugs is calicheamicin γ_1^I (Fig. 1). Common to this class of molecules is an enediyne subunit, which eventually undergoes a Bergman-like autoaromatization reaction (see Fig. 2) after a preceding activation step, the trigger reaction. In the autoaromatization process the drug is converted from an inert state to a fugitive and a highly reactive biradical state, which causes a cascade of reactions that finally leads to cleavage of the DNA strand in the vicinity of the former enediyne moiety.

The individual steps in the mechanism that lead to the destruction of DNA and hence to the death of the cell has been carefully mapped out. In essence, the reaction sequence of the calicheamicin γ_1^I molecule can be viewed as a three-act play (see Fig. 3). First, calicheamicin docks to the minor groove of the DNA double helix. Molecular arms of the drug ensure that it binds specifically to DNA at the recognition sequence d(TCCT) • d(AGGA). The second part of the play is the trigger reaction that activates the enediyne moiety. A nucleophilic attack on the trisulfide sequence of calicheamicin γ_1^I is followed by a subsequent Michael addition, where two sp^2 carbons (C_{11} and C_{12}) of the bridging structure in the vicinity of the enediyne complex are transformed to sp^3 carbons. The third and final act commences with the molecule undergoing a Bergman-like reaction, converting the relatively stable enediyne moiety into a biradical *para*-benzyne-like structure. The climax of the play is the abstraction of two hydrogen atoms from the adjacent sugar-phosphate backbones of DNA by the highly reactive *para*-benzyne subunit, which itself is transformed to a stable benzene analogue. After abstraction of the two hydrogen atoms, the DNA strands are bisected.

The individual stages of the play have been investigated both experimentally and theoretically. For example, the dynamics of the complexation of DNA and the drug has been studied by both 1D and 2D H-NMR spectroscopy [1, 2] and by molecular dynamics simulations [3]. These studies have provided information on life-times of the complexes, the binding mode of the drug, identify the hydrogen atoms of the DNA which are abstracted, etc.

A combination of theoretical and experimental studies have also shed light on the events of the final act. In particular, the energetics of the Bergman-like reaction has been studied, since it determines the conversion rate

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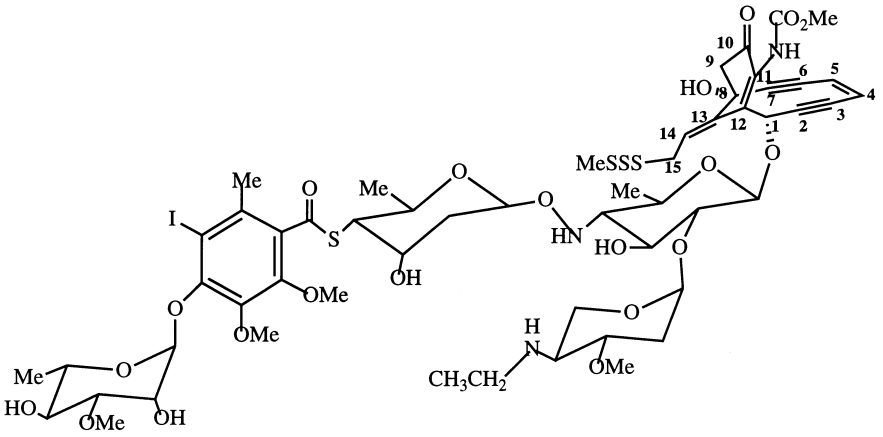

Fig. 1. A schematic representation of the structure of the calicheamicin γ_1^I anti-cancer drug

of the drug from its inactive closed-shell form to its active biradical form, which in turn defines the kinetics of the reaction sequence once the drug is activated. A number of theoretical [4–10] and experimental studies [11, 12] have concentrated on the energetics of the parent reaction. The Bergman reaction [13–16] exhibits an activation energy of about 28 kcal/mol. The biradical character of the molecule increases drastically after passing the transition state, but a closed-shell description is appropriate until the transition state is reached [7].

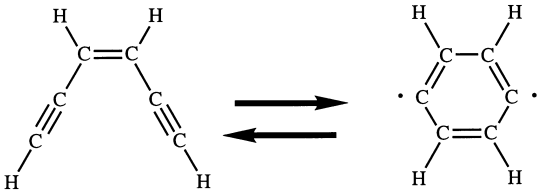

Fig. 2. The Bergman reaction, i.e. the autoaromatization of (Z)-hex-1,5-diyne-3-ene to *para*-benzyne

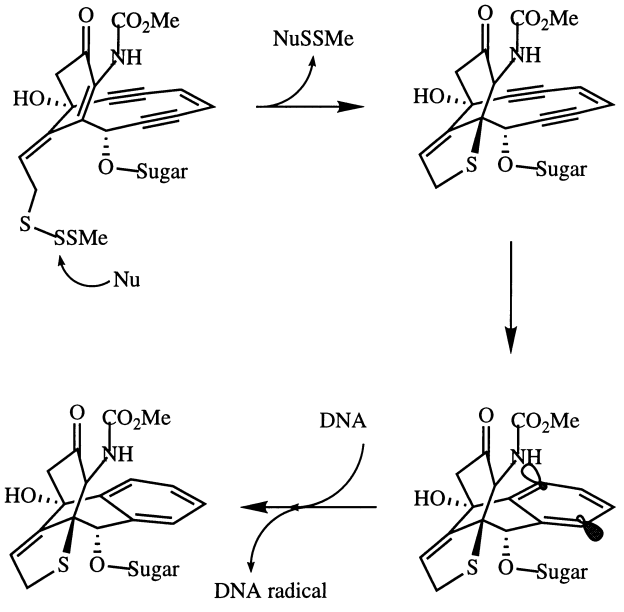

Fig. 3. The mechanism of the DNA cleaving action of calicheamicin γ_1^I

Chang and Yu [17] have studied the activation energy of Bergman-like reactions using closed-shell second-order Møller-Plesset perturbation theory (MP2). They demonstrated that introduction of substituents at the terminal acetylenic carbon positions increases the activation energy due to entropic effects. On the other hand, a bridging group, e.g. as in calicheamicin γ_1^I, lowers the activation barrier to about 16 kcal/mol, probably due to the fact that the transannular distance between the acetylenes in the enediyne subunit (C_2 and C_7) decreases. This reduction of activation energy is consistent with the CASPT2 (second-order multiconfigurational perturbation theory) energy profile computed along the reaction path converting (Z)-hex-1,5-diyne-3-ene to *para*-benzene (cf. Fig. 4). A reduction of the C_2—C_7 distance from 4.4 to 3.2 Å reduces the activation energy by approximately 6 kcal/mol [9].

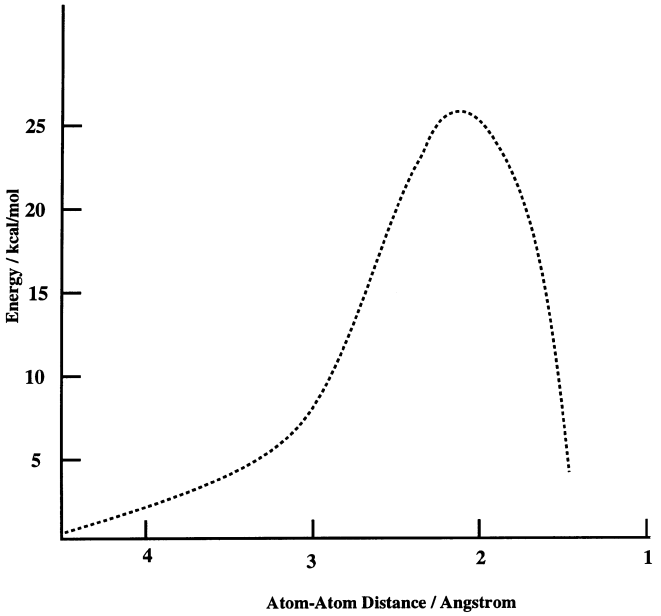

Fig. 4. The CASPT2 relative energy profile along the reaction path of the Bergman reaction. The approximate energy curve is based on the results of Lindh et al. The *ordinate* is the distance between the terminal acetylenic carbons of (Z)-hex-3-ene-1,5-diyne (corresponding to C_2—C_7 in calicheamicin)

The influence of the trigger reaction on the subsequent autoaromatization process, however, is not yet fully understood. Molecular mechanics simulations have shown that the C_2—C_7 distance is reduced by the trigger reaction [18]. Since a reduction of this distance reduces the activation energy of the Bergman reaction, it has been proposed that the major role of the trigger reaction is to reduce this distance [19] or, in other words, to increase the ring contraction [20].

However, other investigations [21–24] have shown that slight modifications of the bridging atoms in the vicinity of the enediyne moiety may significantly alter the activation barrier. For example, a PRDDO investigation of the oxy-analogue of calicheamicin showed that the trigger reaction lowers the activation energy by 21 kcal/mol, although the C_2—C_7 distance decreases by only 0.2 Å [21]. In another study, De Voss et al. [25] measured the rate of cyclization of the bridgehead-cyclized internal Michael adduct to the biradical form of calicheamicin γ_1^I. Based on these results, the half-life of the former compound was assessed to ~20 s at 23°C. Calicheamicin in the absence of thiol is stable for several months at 5°C [1]. This indicates that the difference in activation energy between the triggered and untriggered species is more than 6 kcal/mol [26]. However, referring to Fig. 4, a 0.3 Å change of the C_2—C_7 distance around 3.2 Å could only account for half of this energy. Therefore, it has been proposed that the significance of the trigger reaction is primarily to remove anti-Bredt constraints [27, 28] in the bridgehead (C_{12}), i.e. to increase the flexibility in the ring systems.

In this paper we focus on the effects of the trigger reaction for the conversion of the enediyne to benzene. We study a rather large fragment of the calicheamicin γ_1^I molecule, which is also present in other drugs, e.g. esperamicin. The study provides further evidence for the hypothesis that the significance of the trigger reaction is primarily to modify the bridgehead (C_{12}) adjacent to the enediyne moiety.

2 Computational details

In the present work the influence of strain on the transition state of the untriggered and triggered form of calicheamicin γ_1^I is investigated. At a first glance this would require calculations on the reactants (in untriggered and triggered form) and the corresponding transition states of the related Bergman reaction. However, the effect of strain on the transition state can be obtained from calculations on the corresponding products as well, because the transition state is *product-like* [4–10]. Yet, the biradical character of the products is difficult to model with the methods employed in this study. Fortunately, the enthalpy of C—H bond formation is relatively independent of the structure of the biradical product. Therefore, these effects of strain can be studied on the product molecules *after* saturation of the biradical *p*-benzyne analogue with hydrogen, or, in other words, after abstraction of the two hydrogen atoms from the DNA strands [20]. The errors introduced are significantly smaller than the effects we want to demonstrate.

To investigate the effects of the trigger reaction on calicheamicin, four different structures are considered. The first structure, **1**, consists of the whole calicheamicin γ_1^I molecule except the sugar tail responsible for the site-specific recognition of DNA. Moreover, the trisulfide moiety is replaced by a thiol group (i.e. the drug after the nucleophilic attack on the trisulfide). The Michael addition (i.e. the trigger reaction) changes fragment **1** into fragment **2**. The combination of the Bergman reaction and saturation with two hydrogen atoms changes fragment **1** into fragment **3**. Finally, the collective effect of the Michael addition, the Bergman reaction, and saturation with two hydrogen atoms changes fragment **1** into fragment **4** (see Fig. 5). The influence of the structural changes on the Bergman reaction rate, induced by the trigger reaction, can be assessed by use of an isodesmic reaction, constructed from the four fragments

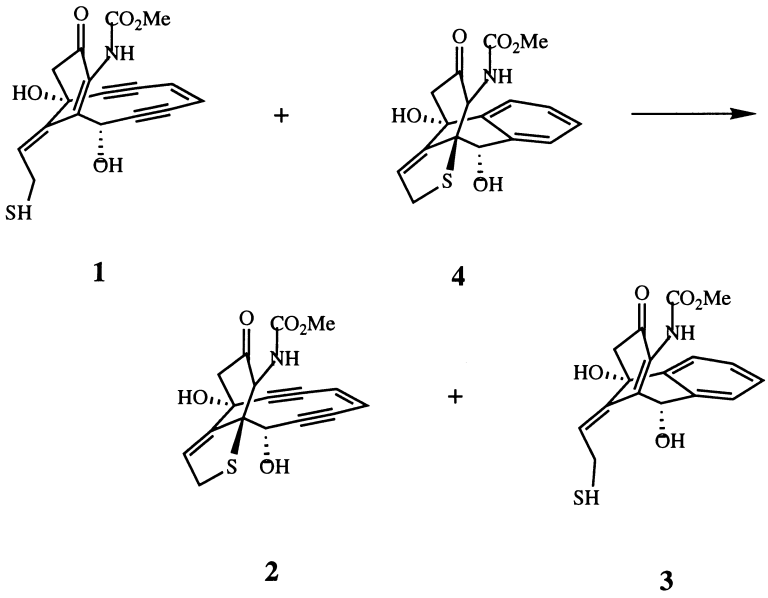

Fig. 5. The structures of fragments of the calicheamicin γ_1^I molecule, 1–4, constituting an isodesmic reaction that allows for the calculation of the change in the activation energy of the Bergman reaction due to the trigger reaction

mentioned above (cf. Fig. 5). The utilization of such an isodesmic reaction is advantageous due to favorable cancellation of systematic errors of the theoretical method and due to shortcomings in the basis set, including superposition errors.

The structures (without symmetry) are first preoptimized with the semiempirical AM1 method and the Hessian is calculated in order to speed up the forthcoming optimizations and to ensure that equilibrium states are found. The structures are then optimized with density functional theory (DFT), using the Becke-Perdew86 functional [29, 30] in connection with the 6-13 G* basis set. This resulted in 756 (764) primitive and 394 (398) contracted basis functions, and 111 (117) degrees of freedom for fragments **1** and **2** (**3** and **4**). The geometry optimizations converged in 18–46 iterations, and the total CPU time consumption was 27 days. The calculations were run with the GAUSSIAN 94 program package [31] on an IBM RS6000 model 590 machine.

At the optimized geometries, single-point energy evaluations are performed with MP2. In these calculations the ANO-S basis sets of Pierloot et al. [32] are utilized in real spherical harmonic representation with the contractions H(2*s1p*), C,N,O(3*s*2*pld*), and S (4*s*3*p*2*d*). This resulted in 1292 (1324) primitive and 420 (430) contracted basis functions for fragment **1** and **2** (**3** and **4**). 124 (126) electrons are correlated; the 56 core electrons are kept frozen in the MP2 calculations. The resulting number of unique MP2 amplitudes compiled to 209 316 030 (228 071 403) for each group of fragments.

These large-scale MP2 calculations are facilitated by the use of a newly developed integral-direct, distributed-data, parallel MP2 program [33]. The average wall-clock time for the integral-direct parallel SCF calculations (about 20 iterations) for each computation is about 12 h on 80–90 IBM SP2 nodes. The MP2 computations took on average only about 80 min wall-clock time on the same number of nodes. The total CPU time for each SCF and MP2 computation is about 1130 h or 47 days. However, the MP2 computation on a single node, with the same amount of memory as each node in the SP2 machine, would require 200–300 days. The reason for this tremendous increase in CPU time is that for single-node execution the two-electron integrals in the atomic orbital basis have to be recomputed several times since the huge MP2 amplitude list cannot be kept in the memory of a single node. For parallel execution on 80–90 nodes, the whole amplitude list is kept in global memory (i.e. the aggregate memory of the parallel computer), and only a single integral pass is required. The MP2 calculations are run with an experimental version of MOLCAS 4 [34] on an IBM SP2 machine at the Center for Parallel Computers (PDC), Stockholm, Sweden.

3 Energetics

The energies of fragments **1**–**4** at the AM1, SCF, DFT and MP2 levels of theory are presented in Table 1. The reaction enthalpy of the isodesmic reaction in Fig. 5 is computed to 16.2, 17.6, 12.9 and 12.9 kcal/mol for the AM1, SCF, DFT and MP2 methods, respectively. Thermodynamic corrections, calculated from the unscaled AM1 frequencies, change the reaction energies by −0.6 kcal/mol. It is notable that the DFT and MP2 results exactly agree. This illustrates the usefulness of the isodesmic reaction, which cancels out the systematic errors. The non-isodesmic reaction involving ring closure and subsequent saturation of the *p*-benzyne entity with two hydrogens (involving a hydrogen molecule, computed at the same levels of theory), on the other hand, has a reaction energy of −86, −115, −111, and −113 kcal/mol at the AM1, SCF, DFT, and MP2 levels of theory, respectively, for the untriggered species, while the corresponding values for the triggered species are −102, −133, −124, and −126 kcal/mol. The MP2 values of these non-isodesmic reactions are consistently 2 kcal/mol lower than the DFT results, but this systematic difference cancels out in the isodesmic reaction. Together the results show that the trigger reaction facilitates the subsequent Bergman reaction by about 12 kcal/mol for calicheamicin γ_1^I. This large differential activation energy is clearly in line with the half-life reported for calicheamicin in a neutral and reducing environment [1].

The energies of these reactions allow for some further analysis. The hydrogen affinities for the phenyl radical and *para*-benzyne have been determined experimentally to be 112 and 108 kcal/mol, respectively [35]. Furthermore, the bond energy of hydrogen is 104 kcal/mol. Using these values together with the computed MP2 reaction energies of the non-isodesmic reaction mentioned above, the reaction energies for biradical formation of the untriggered and triggered species are estimated to 3 and −10 kcal/mol, respectively. These values can be compared with the calculated energy of the

Table 1. The AM1, SCF, DFT, and MP2 energies of fragments 1–4 and the hydrogen molecule. Energies are given in hartrees

Structures	1	2	3	4	H_2
Methods:					
AM1	−0.043039	−0.049642	−0.187629	−0.220026	−0.008261
SCF[a]	−1478.807683	−1478.821727	−1480.124024	−1480.166159	−1.132436
DFT[b]	−1485.733581	−1485.742601	−1487.084314	−1487.113971	−1.173892
MP2[a]	−1482.305768	−1482.330887	−1483.643223	−1483.688883	−1.157707
TD corr.[c]	0.245692	0.251797	0.271490	0.278618	

[a] ANO-S basis set
[b] 6-31G* basis set
[c] Thermodynamic corrections to Gibbs free energy at 298 K (including zero-point vibrational energy) calculated from the unscaled AM1 harmonic vibrational frequencies

same reaction for (Z)-hex-3-ene-1,5-diyne, 4 kcal/mol [10]. Although these estimates are rather uncertain, it can be concluded that the biradical state of triggered calicheamicin γ_1^I is thermochemically stable or at least thermochemically neutral compared to the reactant state.

It can also be noted that the trigger reaction, i.e. the intramolecular Michael addition of the thiol to the enone system, is exothermic by 4.1, 8.8, 5.7, and 15.8 kcal/mol at the AM1, SCF, DFT and MP2 levels of theory, respectively. Thus, this step is not rate-limiting in the whole reaction sequence, leading to DNA cleavage.

4 Structural observations

The following analysis is based on the DFT optimized structures; the cartesian coordinates of the structures are provided in the Appendix.

Figure 6 shows the effect of the trigger reaction (the intramolecular Michael addition) on the dienyne structures. It is a superposition of the ring atoms of the optimized structures **1** and **2**. The root-mean squared (RMS) deviation of the two structures (all atoms in Fig. 6) is 0.149 Å. As has been noted before, the two acetylenic atoms C_2 and C_7 move towards each other. The distance in between decreases from 3.38 to 3.15 Å. Yet, this change is too small to account for the 12 kcal/mol decrease in the activation energy of the Bergman reaction caused by the trigger reaction. According to Fig. 4, such a ring contraction cannot account for more than 3 kcal/mol of this decrease. The largest displacement during the trigger reaction occurs for the atoms around the double bond which is saturated by the trigger reaction, i.e. C_{10}—C_{12}. Thus, the main effect of the trigger reaction seems to be a change in the structure of this region rather than a decrease in the C_2—C_7 distance.

Figures 7 and 8 show the effect of the Bergman reaction on the untriggered and triggered species, **1** and **3**, and **2** and **4**, respectively. Again, the ring atoms C_1—C_{13} have been superimposed, and the resulting movements of the atoms are compiled in Table 2. Naturally, the largest displacement is found for the carbons that form the new σ-bond of the benzene moiety, C_2 and C_7, and for the adjacent atoms. More interestingly, the atoms in the triggered molecule move less in the Bergman reaction than those in the untriggered molecule. This shows that the trigger reaction not only improves the positions of the acetylenic atoms (C_2 and C_7), but, in fact, improves the positions of almost all the ring atoms. Moreover, the largest decrease in the displacements in Table 2 is found for the centers around the double bond that is saturated by the trigger reaction, C_{10}—C_{12}. For example, the displacement of C_{10} is more than eight times larger for the untriggered molecule than for the triggered molecule.

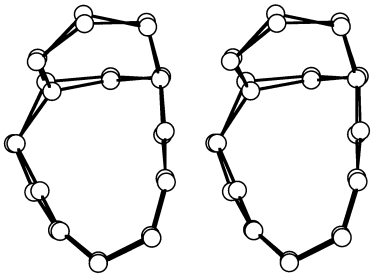

Fig. 6. A comparison of the cyclic substructures of fragments 1 and 2. The relative positions of the atoms have been derived by minimizing the RMS value of the distances between the individual carbons. This stereo plot identifies significant structural changes due to the trigger reaction

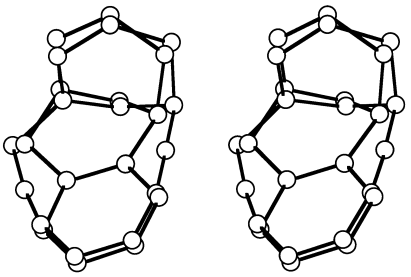

Fig. 7. A comparison of the cyclic substructures of fragments 1 and 3. The relative positions of the atoms have been derived by minimizing the RMS value of the distances between the individual carbons. This stereo plot identifies significant structural changes caused by the Bergman reaction on the untriggered species

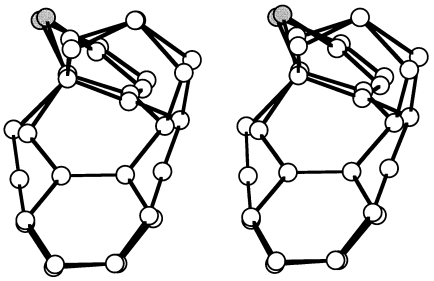

Fig. 8. A comparison of the cyclic substructures of fragments 2 and 4. The relative positions of the atoms have been derived by minimizing the RMS value of the distances between atoms C_1—C13 (the same as in Fig. 7). This stereo plot identifies significant structural changes caused by the Bergman reaction on the triggered species

Table 2. The distance between different atoms in the superimposed structures in Figs. 6–8. All distances are given in Å

Structures:	1 and 2	1 and 3	2 and 4
Atom			
1	0.072	0.434	0.346
2	0.137	1.006	0.890
3	0.045	0.124	0.075
4	0.046	0.166	0.052
5	0.073	0.141	0.074
6	0.076	0.147	0.116
7	0.016	0.955	0.840
8	0.090	0.427	0.322
9	0.124	0.368	0.378
10	0.262	0.338	0.042
11	0.189	0.511	0.265
12	0.318	0.276	0.148
13	0.089	0.143	0.169
Total RMS	0.149	0.479	0.394

Again, this indicates that the major function of the trigger reaction is to saturate the C_{11}—C_{12} double bond.

A clear illustration of the effect of the trigger reaction can be found by studying the dihedral angles around the C_{11}—C_{12} double bond. The torsional angle N—C_{11}—C_{12}—C_1 is −14, −79, −42 and −103° for fragments **1**–**4**, respectively. A relaxed double bond should have a dihedral angle around 0 or 180°. Thus, the C_{11}—C_{12} double bond of fragment **3** is significantly strained (the dihedral angle is −42°), much more than in species, **1** (−14°). Moreover, the torsional angle increases strongly during the autoaromatization, for the untriggered molecule (28°) as well as for the triggered species (61°).

The Bergman reaction creates two new bridgeheads, C_2 and C_7. Accordingly, the reaction will to a large extent be controlled by the flexibility of the ring system. The sp^2 carbons C_{12} and C_{13} in the bridge C_1—C_{12}—C_{13}—C_8, and the sp^2 carbons C_{12}, C_{11}, and C_{10} in the bridge C_1—C_{12}—C_{11}—C_{10}—C_9—C_8 will limit this ability, due to their preference for a planar arrangement. Therefore, the β-addition to the C_{11}—C_{12} double bond will significantly increase the ability of the ring system to form the bridges.

Finally, the assumption that the enthalpy of the C—H bond formation is independent of the structure of the biradical product is checked by comparing the structure of the benzene moiety before and after the trigger reaction (fragments **3** and **4**). The RMS deviation of the six atoms in the benzene ring is only 0.012 Å. This indicates that the benzene moiety is insensitive to the structure of the rest of the molecule, in accord with the assumption.

5 Concluding remarks

The significance of the trigger reaction of calicheamicin γ_1^I has been studied at the DFT and MP2 levels of theory. An isodesmic reaction is employed to compute the differential energy of the transition state for the untriggered and triggered molecule. Both methods show that the trigger reaction lowers the activation energy of the Bergman reaction by about 12 kcal/mol. The study shows that a reduction of the C_2—C_7 distance can account for, at most, a fourth of this decrease in the activation energy. Instead, the main significance of the trigger reaction for calicheamicin (and espereamicin) seems to be to reduce the strain in the bridgehead atoms and the bridges of the polycyclic product by saturating the C_{11}—C_{12} double bond.

The efficiency of an anti-cancer drug is connected to the toxicity. The drug is usually administered intravenously and the mean arrival time (MAT, the mean time from the administration of calicheamicin until it binds to the DNA) [36, 37] is governed by membrane penetration and intracellular diffusion time. The toxicity of calicheamicin is controlled by the ratio of the half-life of the drug to the MAT. To limit the damage caused by the drug, i.e. to ensure that as much as possible of the drug reaches the DNA, this ratio should be in the range of 10–1,000. The half-life of the triggered drug has been measured to be 4.5 s at 37 °C [25]. If we assume that MAT is in the 1–15 min range and limit the toxicity by a ratio of the half-life of the untriggered species to the MAT of 100, we arrive at a desired differential activation energy, for the untriggered versus the triggered species, of 4.4–6.1 kcal/mol [26]. The 0.3 Å change of the C_2—C_7 bond distance during the trigger reaction would only be able to account for half of this change in activation energy. In this respect, the presence of the C_{11}—C_{12} double bond inactivates calicheamicin to an extent which is favorable for efficient anti-cancer treatment.

A question that remains regarding the mechanism of calicheamicin is how the drug protects the trisulfide from a nucleophilic attack *before* the drug binds to the minor groove of the DNA double helix. Does the drug actively protect the trisulfide in the cytosole?

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Appendix

Below, the cartesian coordinates (in Ångströms) of the DFT optimized structures are given:

A.1 Fragment 1

S	−2.264091	−4.150097	−.323414
H	−1.684350	5.242360	−.349102
H	−.067971	4.941167	−2.209312
H	1.503255	−.218732	−1.790545
H	−.006741	−.400430	−3.518241
H	−1.442208	1.027007	3.224056
H	−1.544912	−.693987	2.752908
H	−2.466230	−2.618377	−2.129721
H	−3.939796	.773381	.773491
H	2.543934	.505946	1.741154
H	5.527120	−.731503	−1.085371
H	5.301526	−2.120338	.029982
H	6.371104	−.743178	.516009
H	−3.282505	−1.384460	.000046
H	−.806078	−2.550129	−1.505684
H	−1.424804	−3.948241	.729460
C	−1.290952	4.246605	−.584168
C	−.396378	4.078632	−1.618154
C	−1.684481	3.118938	.179516
C	.093734	2.777167	−1.890835
C	−1.862777	2.008730	.676769
C	.451900	.109781	−1.694085
C	.318786	1.567735	−1.871935
C	−2.026501	.617605	1.133311
C	−1.466030	−.357812	.050213
C	−2.253863	−1.370851	−.382249
C	−1.215339	.309603	2.417882
C	.287055	.283237	2.197066
C	.824535	.021926	.823569
C	−.023219	−.154736	−.250368
C	−1.862008	−2.559263	−1.206986
C	3.152270	−.560037	.102242
C	5.459346	−1.030683	−.026356
O	−.375140	−.603166	−2.632751
O	−3.394685	.361483	1.479177
O	1.073170	.541432	3.120527
O	2.939349	−1.291234	−.863361

O	4.389584	−.323283	.637177
N	2.221345	.171393	.819535

A.2 Fragment 2

S	.608585	−2.325253	−1.146333
H	−2.954194	4.417630	.908659
H	−1.349062	4.937039	−.920209
H	1.160843	.350647	−1.937001
H	− .378495	.339044	−3.626317
H	−1.631012	−.198404	3.096865
H	−1.741346	−1.868717	2.494597
H	−1.112035	−4.031756	−1.444209
H	−4.179211	−.568126	.581189
H	5.108947	2.026188	.625996
H	5.311408	1.061392	−.873433
H	6.221092	.597810	.620610
H	−3.043445	−2.557386	−.615866
H	−1.218297	−2.788355	−2.735807
H	.487791	.865042	.822460
H	2.400154	−1.150553	1.420704
C	−2.386333	3.616600	.423338
C	−1.496528	3.902185	−.591527
C	−2.529074	2.268003	.833541
C	−.773326	2.837256	−1.180217
C	−2.401585	1.045987	.903883
C	.072615	.335868	−1.726270
C	−.370824	−1.696228	−1.408434
C	−2.273132	−.414622	.993428
C	−1.564609	−1.062573	−.212939
C	−2.035196	−2.175418	−.805762
C	−1.420625	−.846348	2.225507
C	.089536	−.891227	2.000808
C	.665901	−.228956	.730304
C	−.110011	−.705337	−.543426
C	−1.060727	−2.948602	−1.653954
C	2.990333	.465061	.284681
C	5.281553	1.012510	.227999
O	−.669007	−.179046	−2.847269
O	−3.573292	−1.000805	1.221751
O	.830631	−1.428212	2.818391
O	2.718253	1.476927	−.360605
O	4.253516	.103544	.673297
N	2.090127	−.492343	.696115

A.3 Fragment 3

S	−1.580806	4.391357	−.613565
H	4.058132	−3.380710	−1.855175
H	−.292748	3.348009	−2.313974
H	4.188134	−.377653	1.264752
H	5.275283	−2.263003	.036099
H	−2.113859	−1.585201	2.007600
H	−.387759	−1.749194	−1.230907
H	−.868972	−.272904	−2.800464
H	1.805398	−.121202	2.912489
H	1.139578	1.521287	2.792276
H	−1.906909	3.769068	.552549
H	3.283152	1.852399	.240822
H	−4.487823	−3.113016	−1.245464
H	−5.381456	−1.639410	−.738873
H	−5.615548	−3.186903	.169375
H	1.267556	3.267944	−.246264
H	−1.338567	2.123057	−1.547664
H	1.745105	−2.638762	−2.445857
C	4.264013	−1.942215	−.237972
C	2.285269	−2.155004	−1.623988
C	2.348966	−.462201	.136400
C	.177604	−.799527	−1.279507
C	1.784039	.783365	.879045
C	.632745	1.281161	.026803
C	.501053	2.518937	−.484628
C	1.167190	.534781	2.292258
C	−.246058	−.067777	2.303044
C	−.880437	−.339160	.979299
C	−.256805	.115014	−.158242
C	−.645741	2.955808	−1.343319
C	−4.897884	−2.563527	−.382138
C	3.583117	−2.568757	−1.293343
C	1.651287	−1.113679	−.921875
C	3.650688	−.893504	.461334
C	−2.833293	−1.512814	.057522
O	.092545	−.250530	−2.597524
O	2.822710	1.742840	1.102530
O	−.738909	−.538467	3.337469
O	−2.771137	−1.105178	−1.102104
O	−3.855399	−2.258818	.571081
N	−1.884115	−1.308530	1.044272

A.4 Fragment 4

S	2.376970	−.269930	1.271464
H	−1.793303	2.532438	−.718128
H	1.939775	−.327405	3.697787
H	−.238459	−.008456	−1.335769
H	3.292350	−2.771008	−3.577519
H	4.071579	−1.598912	−4.708287
H	.293572	1.844833	3.549839
H	−5.492658	−.288210	−.176821
H	.159584	−2.073708	−.065224
H	−.629685	−2.752609	2.016363
H	−.745206	3.406041	.431274
H	2.813169	1.157932	3.207441
H	−2.294375	1.633501	2.825684
H	2.381534	1.062851	−1.783670
H	2.298383	−1.939820	−4.827290
H	−3.980865	1.511049	.689186
H	−4.548743	−2.550708	−.716906
H	−2.117988	−2.994584	−.360825
C	.608176	.489585	−.819439
C	.635391	−.072815	.668031
C	.296366	1.994650	−.796535
C	−2.211314	.274083	.666416
C	−3.900776	−1.761352	−.320066
C	−3.583149	.516262	.459183
C	−1.681137	−1.013322	.395993
C	1.995448	.467737	2.930598
C	1.870914	−.833985	−2.442018
C	3.157405	−1.833095	−4.143950
C	−2.536439	−2.009940	−.114880
C	−4.427447	−.493925	−.022883
C	−.220395	−1.367217	.700936
C	−1.318414	1.438008	1.151049
C	.000996	.896617	1.645234
C	.672797	1.171769	2.772487
C	−.948795	2.415817	−.011102
O	−.111789	−1.920919	2.021876
O	−2.008033	2.247906	2.114347
O	1.079092	−1.777739	−2.465445
O	2.969393	−.715951	−3.251222
O	1.009281	2.805215	−1.379025
N	1.801215	.242042	−1.589874

A.5 The hydrogen molecule

H	.000000	.000000	.375289
H	.000000	.000000	−.375289

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