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A synthetic and in silico study on the highly regioselective Diels–Alder reaction of the polyenic antifungal antibiotics natamycin and flavofungin

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Dedicated to Professor Károly Lempert on the occasion of his 85th birthday

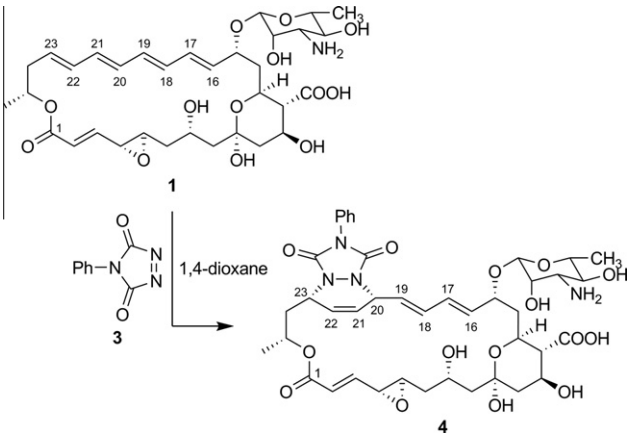
ABSTRACT

The tetraenic macrolide antibiotic natamycin and the pentaenic macrolide flavofungin gave monoadducts on Diels–Alder reaction with 4-phenyl-1,2,4-triazoline-3,5-dione. The regioselectivity of the reaction as well as the conformation of the products was studied using theoretical calculations.

Polyene macrolide antibiotics such as amphotericin B, nystatin, and natamycin are important therapeutics for the treatment of fungal infections.[1] Their structure consists of four to eight conjugated carbon–carbon double bonds with *E*-configuration. This structural unit results in the rod-shaped form of these molecules.[2] This conformation probably plays an important role in binding to fungal sterol molecules resulting in the antifungal action of these antibiotics. In this work two representatives of the polyene macrolide antibiotic family, natamycin (**1**) and flavofungin (**2**), have been chosen to study a Diels–Alder (DA) reaction on their polyenic moiety, as well as the influence of the newly formed pyridazine ring annelation on the conformation of the macrocycle and on the antifungal activity.

Only a few examples have been published on Diels–Alder reactions where the reacting diene is part of a polyene. In these cases the polyene can be regarded as a substituted diene for which the regio- and/or stereoselectivity is determined by its substituents (e.g., the remainder of the polyene). A detailed experimental and theoretical analysis of DA reactions of this type with polyenes possessing a carboxylate and hydroxyl group at the end was reported by Turner et al.[3] They found that the dienophile always reacts with the outermost diene unit of the polyene, and the theoretical calculations carried out for a model compound supported their findings. They also found that a simple model based on the frontier molecular orbital coefficients for the prediction of regioselectivity of DA reactions was not always applicable for such systems. No examples have been found, however, in the literature where geometrical constraints exist for the polyene, for example, when a linear polyene is assembled in a cyclic compound as in the case of polyene macrolide antibiotics.

Natamycin[4] (**1**) has a macrolactone structure consisting of 26 atoms. The conjugated tetraenic part of the molecule was the target for a DA reaction with the highly reactive diazadienophile **3**. The DA reaction of **1** with **3** at room temperature led to a single product which was isolated by adsorption chromatography. On the basis of NMR, MS, and UV–vis spectroscopic data (the configuration of C-20 and C-23 was assigned on the basis of TS calculations) the structure **4** was assigned to this adduct (Scheme 1) as



Scheme 1. Diels–Alder reaction of natamycin (**1**) and **3**.

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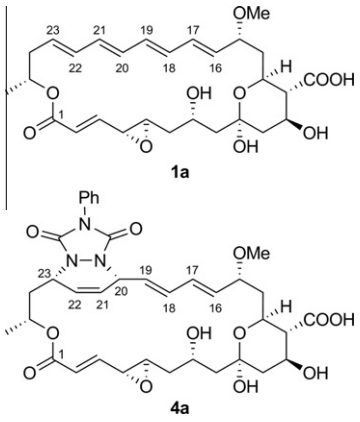


Scheme 2. Natamycin theoretical model (**1a**) and its theoretical adduct model (**4a**).

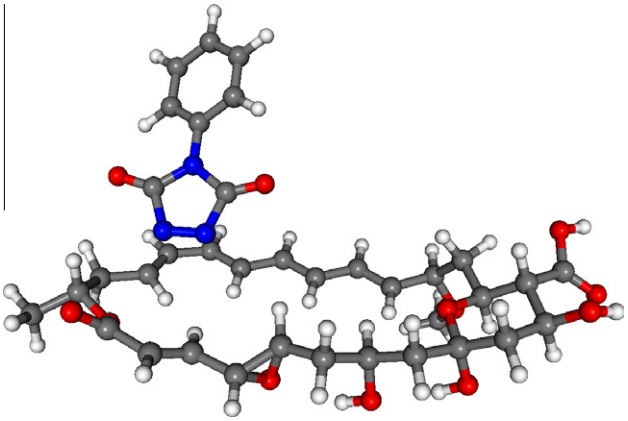


Figure 1. Most stable ONIOM(B3LYP/6-31G(D):AM1) transition state for the Diels–Alder reaction between **1a** and **3**.

detailed in the Supplementary data together with the observed antifungal activity (Fig. S1, Table S1)[†]. In an antifungal test against *Candida* strains, product **4** exhibited no activity.

Natamycin theoretical model (**1a**) (Scheme 2): Starting from a plausible all-transoid structure,[5] constant temperature (500 K) molecular dynamics (200 ns total time, 1 fs time step, GAFF force field[6]) simulations were carried out in order to explore the structures available with non-negligible populations for DA reactions. A total of 200,000 structures were saved, and each was minimized and classified into clusters regarding only the polyene chain conformation. The lowest energy representative from each cluster was then submitted to semiempirical (AM1, PM3, PM6 and RM1) and density functional (B3LYP/6-31G) calculations. Transition states for cisoid structures which contain only a single cisoid conformation (for both possible orientation of the dienophile) were calculated at AM1, PM3, PM6, RM1, and ONIOM(B3LYP/6-31G(d):AM1) levels of theory.

Classifying the optimized structures from the conformational search resulted in eight clusters within a narrow energy range which means that any cisoid conformation suitable for a DA reaction can be formed with sufficient probability. These clusters can feature from zero to three cisoids and two to five transoids (i.e., the C_{i+k}–C_{i+1+k}–C_{i+2+k}–C_{i+3+k} torsion angles close to zero or 180 degrees, respectively, where $i = 15$ and $k = 1$ and/or 3 and/or 5, see Fig. S2). The description of these clusters as well as the energy values obtained for their lowest energy representatives at various levels of theory can be found in the Supplementary data (Table S2). The transition state (TS) calculations between **3** and **1a** with single cisoid geometries (at $k = 1$ or 3 or 5) at the AM1, RM1, and PM6 as well as ONIOM(B3LYP/6-31G(d):AM1) levels of theory show the TS of cisoid diene structures correspond to $k = 5$, being the most stable in all types of computations. It is in perfect agreement with the preparative findings where only this product was formed. The graphical representations of the TSs calculated at ONIOM(B3LYP/6-31G(d):AM1 level are shown in Figures S3–S8 while the relative TS energies for all the methods and for all the single-cisoid diene subsystems are given in Table S3. The most stable transition state obtained from ONIOM(B3LYP/6-31G(d):AM1) calculations is shown in Figure 1.

The structure of natamycin theoretical model DA adduct (**4a**) (Scheme 2). The starting product geometry was obtained by minimizing the adduct derived directly from the most stable transition state (Fig. 1). Using this 'transition state like product geometry', 5 ns constant temperature molecular dynamics simulation was performed at 900 K saving 20 geometries being equidistant from the trajectory and each of these was submitted to 10 ns constant temperature ($T = 400$ K) simulations. The most stable geometries from the corresponding trajectories were minimized at the HF/6-31G and B3LYP/6-31G levels of theory.

Depiction of the energy and conformational changes during one representative molecular dynamics simulation leading to the most stable conformer is given in Figure 2 and Figure S9. Although the macrocyclic ring in the most stable TS (Figure 1) obtained from the single cisoid conformer and in the next local minimum (Fig. S10) shows appreciable similarity in shape to those observed at the global minimum of natamycin, it can be seen from these figures that product **4** does not prefer the corresponding elongated rod-shaped geometry. Instead, it adopts a more compact conformation (Fig. S12), which was reached through an intermediate product (Fig. S11). Of the 20 short molecular dynamics simulations carried out for the DA product **4a**, 19 led to the same conformation (Fig. S12) which was significantly different from the starting 'TS-like product' (Fig. S10) and was lower in energy (Table S4) than that was obtained for the geometry from the 20th simulation (Fig. S13).

Flavofungin[7] (**2**) (Scheme 3) was discovered 50 years ago at Debrecen University. Its structure consists of a 32-membered macrocycle with a conjugated pentaenic lactone moiety.[8] In the reaction with an equimolar quantity of **3** the major product isolated, on the basis of spectroscopic data, proved to have the structure **5** Scheme 3. The numbering, details on the structural elucidation as well as the observed antifungal activity of compound **5** are given in the Supplementary data (Fig. S1, Table S1). The antifungal activity of **5** was evaluated against *Candida* yeast strains and this compound proved to be inactive.

It should be mentioned that although in both cycloadditions two new chiral centers were formed, by careful analysis of the NMR spectra of **4** and **5**, only a single diastereomer could be observed.

Theoretical calculations on flavofungin (**2**): The computational protocols outlined in the case of the natamycin theoretical model were repeated with the exceptions that in this case there were more clusters found due to the longer polyene chain and the transition states were determined using AM1, PM3, PM6, RM1 as well as ONIOM (B3LYP/6-31G:AM1:AMBER, B3LYP/6-31G:HF/6-31G:AMBER, B3LYP/6-31G(d):HF/6-31G:AMBER, HF/6-31G(d):HF/6-31G:AMBER, MP2/6-31G:HF/6-31G:AMBER, and MP2/6-31G(d):HF/6-31G:AMBER) methods.

This molecule has many rotatable dihedral angles and therefore is too flexible to find the global and all the low energy local minima with full certainty. Considering, however, only the polyene part of the flavofungin (Fig. S2), it can be classified into 16 clusters

[†] Figures and tables starting with 'S' can be found in the Supplementary data.

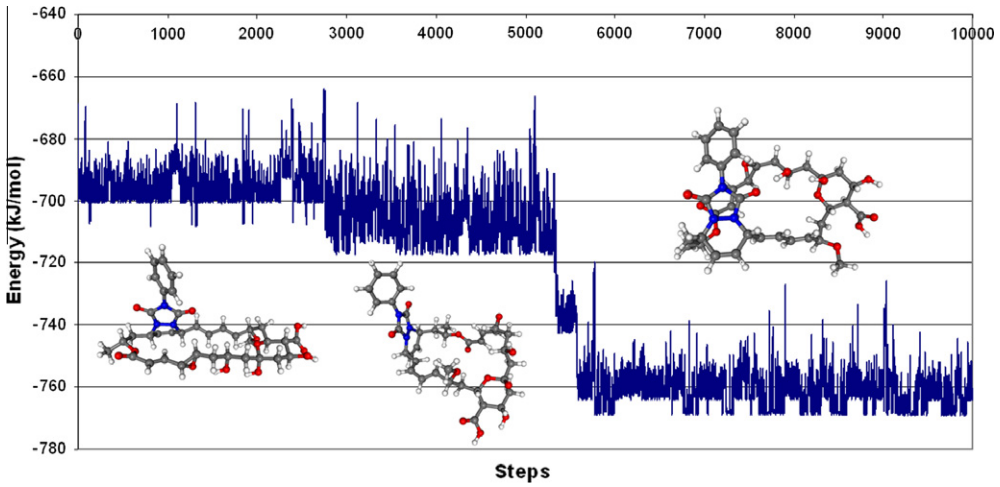
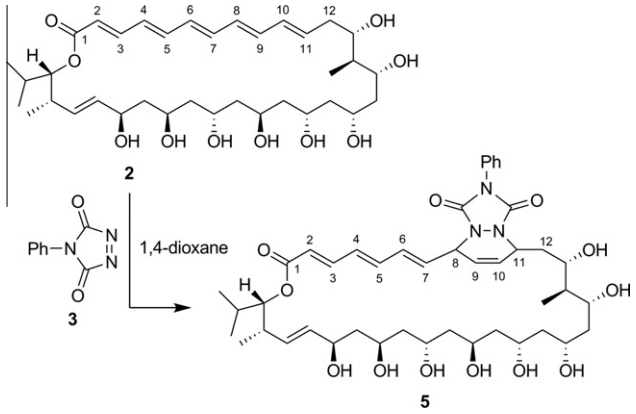


Figure 2. Total energy of the Diels–Alder adduct **4a** during 10 ns molecular dynamics simulation for one of the representative trajectories leading to the most frequently found conformation.



Scheme 3. Diels–Alder reaction of flavofungin (**2**) and **3**.

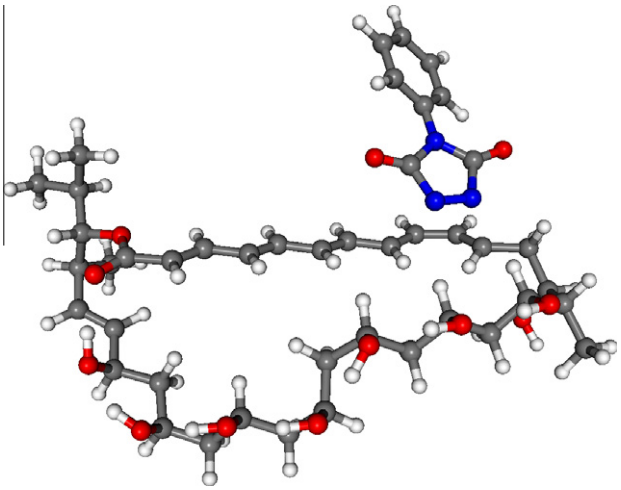


Figure 3. ONIOM(B3LYP/6-31G(d):HF/6-31G:AMBER) transition state for the Diels–Alder reaction between flavofungin (**2**) and **3** at $k = 7$.

according to its pentaenic structure. These clusters can be characterized from zero to four cisoids and three to seven transoids. Based on the energy values (Table S5) obtained for each of the 16 geometries, the probability of the presence of any cisoid conformation suitable for a DA reaction is non-negligible.

Regarding the DA transition state energies and the corresponding single cisoid diene subsystem geometries in the polyene chain, the PM3, as well as the B3LYP/6-31G:HF/6-31G:AMBER, MP2/6-31G:HF/6-31G:AMBER, HF/6-31G(d):HF/6-31G:AMBER, B3LYP/6-31G(d):HF/6-31G:AMBER, and MP2/6-31G(d):HF/6-31G:AMBER ONIOM methods predict the most stable TS with a participating cisoid diene conformation at C8═C9–C10═C11. In contrast, the AM1 and PM6 methods prefer DA reaction at C6═C7–C8═C9, while the RM1 and B3LYP/6-31G:AM1:AMBER ONIOM methods indicate reaction at the C4═C5–C6═C7 atoms. Only the PM3 and ONIOM calculations with ab initio HF/6-31G 'intermediate level' of theory resulted in a TS geometry in which the participation of the cisoid diene situated furthest from the lactone group is preferred in agreement with the experimental finding. Energy values and geometries for the possible transition states for the single cisoid polyene can be found in the Supplementary data (Table S6, Figs. S14–S21) while the most stable ONIOM(B3LYP/6-31G(d):HF/6-31G:AMBER) TS is shown in Figure 3.

Polyenoic acids as flavofungin theoretical models: the DA reaction of retinoic acid was studied experimentally and theoretically by Yamada et al.[9] Since flavofungin can be regarded as 'substituted' retinoic acid, in this work, we attempted to analyze the directing effect of the carboxyl function by means of more sophisticated calculations. Conjugated tri-, tetra-, and pentaenes with a carboxyl group at the end of the polyene chain (with the restriction that only one cisoid diene subsystem exists at the same time in the chain) were optimized at AM1, HF/6-31G(d), B3LYP/6-31G(d), and MP2/6-31G(d) levels of theory. The MO coefficients were calculated at HF/STO-6G and B3LYP/6-31G(d) levels. The AM1, HF/6-31G(d), B3LYP/6-31G(d), and MP2/6-31G(d) transition states for DA reactions between these carboxylic acids (as dienes) and the dienophile (**3**) were also calculated.

The calculations outlined for model polyenoic acids were repeated for trienoic acid systems that also possess a $-CH_2-CH_2-$OH group at the opposite end of polyene chain to the carboxyl group. The TSs were determined for the ω-Me polyenoic acids at ONIOM(MP2/6-31G(d):HF/6-31G) level of theory, as well. The ONIOM(MP2/6-31G(d):HF/6-31G) method, applying HF level only the phenyl group, was validated against the pure MP2/6-31G(d) method at ω-Me-substituted and ω-unsubstituted trienoic acids (Table S10) and was proven to give qualitatively the same results.

Transition state calculations of the DA reactions between the model polyenoic acids (as dienes) and **3** showed that the TSs involving the Cω-3–Cω-2–Cω-1–Cω (numbering started at the carboxyl carbon) atoms were the most stable for all polyenoic acids

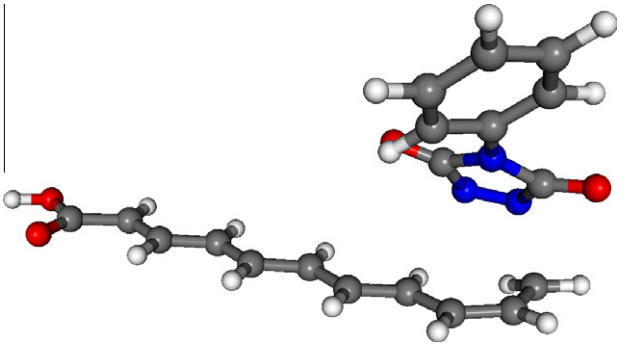


Figure 4. B3LYP/6-31G(D) transition state for the Diels–Alder reaction between a pentaene carboxylic acid (flavofungin model) and **3**.

and for all but the MP2/6-31G(d) levels of theory applied. At the MP2/6-31G(d) level the TSs involving the Cω-5–Cω-4–Cω-3–Cω-2 atoms were systematically the most stable one. Even though the B3LYP method was shown to be remarkably successful for pericyclic reactions,[3,10] additional series of calculations were carried out in order to resolve the discrepancy in the predicted (by means MP2 versus other dependable methods) DA regioselectivity. Model reactions between the Me-substituted (at the ω position relative to the carboxyl group) polyenoic acids and **3** at ONIOM(MP2/6-31G(d):HF/6-31G) level prefer again a DA reaction involving the atoms of the farthest cisoid conformation from the carboxyl group. Since the ω-Me-substituted model is expected to mirror the properties of flavofungin in a greater extent, the results of calculations are in a nice agreement with our experiments where the product corresponding to this adduct (a 'substituted pentaenoic acid') was obtained exclusively. It is also worth mentioning that this transition state corresponds to a rather asynchronous reaction step since the bond between Cω and the N of the dienophile precedes the formation of the Cω-3–N bond, like those observed by Turner[3] et al. from their theoretical calculations on symmetric polyenes (Fig. 4). Attaching the $-CH_2-CH_2-OH$ group to the other end of the polyene (triene) chain resulted qualitatively in the same findings.

Energies for each of these transition states calculated at various levels of theory can be found in Table S10 while the graphical representations of the corresponding structures are given in Figures S22–S39. It should be noted that using frontier MO coefficients to predict the regioselectivity of the DA reaction seems to work for trienoic and tetraenoic acids while it frequently fails (depending on the applied method) for the pentaenoic acid. The predicting power of MO coefficients on the directing effect can be even more doubtful in the trienoic acid case when the $-CH_2-CH_2-OH$ substituent is at the ω position. Demonstrative MO coefficients and eigenvalues are listed in Tables S8 and S9.

In conclusion, the DA reactions at compounds **1** and **2** have been carried out and the obtained products were characterized experimentally. The theoretical calculations demonstrated that despite the cyclic structures of **1** and **2,** the flexibility of these molecules allows the presence of the cisoid conformation at any position of the polyene unit of the macrocycles.

In the case of the natamycin theoretical model, all the applied quantum chemical methods supported the same regioselectivity preference in perfect agreement with the experimental findings despite the missing bulky sugar group. The applied semiempirical and ab initio quantum chemical calculations on polyenoic acids (as models of the flavofungin polyene chain) also predict a product in agreement with the experiments. From these facts we favor a mainly electronic nature of the observed DA regioselectivity.

In contrast, carrying out theoretical calculations on flavofungin itself shows a somewhat different picture since in this case the predicted regioselectivity showed method dependency. However, applying the ab initio HF/6-31G method as an 'intermediate' level of theory for the polyenoic acid part in ONIOM calculations was sufficient to predict the experimentally observed regioselectivity.

In the case of natamycin the theoretical calculations demonstrated that the rod-shaped conformation of the antibiotic was deformed as a consequence of the DA reaction, leading to a more compact conformation of **4**. The elongated shape is probably necessary for a sterol complex formation, which might explain the loss of the antifungal activity of **4** compared to the parent compound **1**.

Software packages used in this work: The AMBER software package[6] was used for empirical force-field calculations while quantum chemistry calculations were carried out using either MOPAC[11] or GAUSSIAN'03[12] suites. The conformers were classified into clusters using in-house software.[13] The MOLEKEL[14] software package was used for visualization of the results.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.07.049.

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