Insight into the Diels-Alder reaction: A green chemistry revisitation of the synthesis of a cantharidine-like trypanocidal pilot-molecule

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ABSTRACT

Relying on a straightforward « Green-Chemistry » based approach, this article reports an optimized Diels-Alder (DA) experimental set-up allowing for this reaction to be cleanly operated in the presence of water used both as solvent and catalyst. This work was initiated to synthesize a cantharidine-derived trypanocidal pilot-molecule. Diels-Alder cyclo-addition between anthracene-9-methanol and maleimide in water was selected as benchmark reaction and demonstrated how water as a solvent can be used not only because of its more benign impact on the environment but also because it improves some key aspects (rate, yield, ease of isolation) of the reaction. Adjunction of lithium chloride into the reaction medium adds a significant incremental benefit to the overall yield due to its oxophilic character increasing electron demand at dienophile's double bond. Our set-up illustrates a typical condition of « on-water » reaction rather then « in-water » reaction conditions. Conclusively, in the DA reaction of our benchmark reaction, water not only behaves as a green solvent but also as a powerful catalyst.

Keywords: Diels-Alder reaction, Green chemistry, Cantharidine, antitrypanocidal agents.

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INTRODUCTION

In recent multiple years, in the context of a medicinal chemistry research program focused on antitrypanocidal agents with new modes of action, [1-3] we became interested in synthesizing a trypanocidal pilot molecule based on the cantharidine template. Cantharidine is known for its anticancer, herbicidal, pesticide, hair growth stimulation and aphrodisiac properties exploited in traditional medecine. However, cantharidine contains in its structure a cyclic anhydride moiety which reacts with nucleophiles present in the biological surrounding and this in turn induces toxicity. There are indications in the literature that cantharidine targets specifically nucleophiles such as serine, threonine, and cysteine. In particular, the latter has a prominent role in the various cathepsins and in this regard, inhibition of these protein enzymes is potentially useful for targets such as Ebola and trypanosomiasis. [4-5]
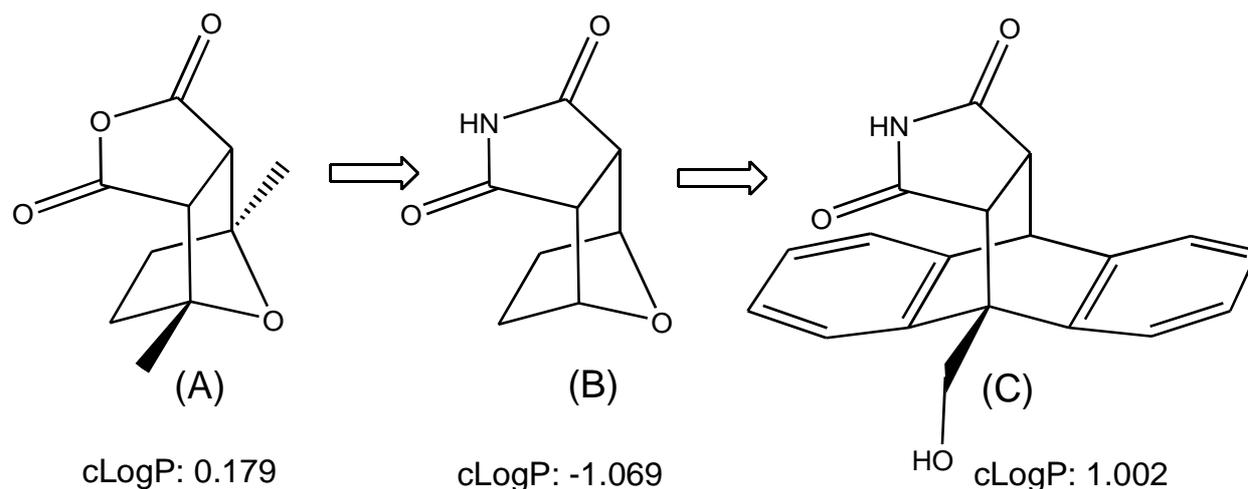
cLogP: 0.179   cLogP: -1.069   cLogP: 1.002

Figure 1. Design of cantharidine-like Structure C as trypanocidal lead

As illustrated in the Figure 1, our target molecule (Structure C) was derived from the prototypical cantharidine template (Strucure A) *via* the norcantharidine-imide analog (Structure B). Authors have mutated indeed the reactive and toxic cyclic anhydride functionality in A to a stable imide moiety (B) in physiological conditions. Structure B, derived from norcantharidine (or desmethylcantharidine) preserves some of the pharmacological properties of A [6] . This shows that the two flanking phenyl groups are not absolutely mandatory to elicit pharmacological effects. However B has got a poor degree of druggability considering its negative cLogP value. In order to correct this defect, the two methyl groups present in A were replaced by two phenylene groups. Moreover, the tetrahydrofurane oxygen was mimicked by the exo-methanol side-chain situated on the bridgehead of the central cycle. The resulting Structure C is endowed with all the attributes of a druggable chemical entity as reflected by its observance of Lipinski's « rule of five ». [8] In particular, the molecule weight is 305.33 daltons, the number of heteroatoms is 4, and the cLog Pis equal to 1.0.

A great deal of our efforts towards a more environmentally friendly approach in the context of the Green Chemistry is aimed in part at the reduction of the use of organic solvents. Usually, organic solvents are used in by far much larger amounts than the solutes they solubilize and have a propensity to leave out into the environment through evaporation and/or leakages. A lot of research is nowadays being carried out to shifting from classical organic solvents by a collection of less environmentally hazardous ones. Water is ideally suited for this purpose owing to its non-toxic character and its high specific heat. Its ubiquitous presence in our biosphere makes therefore water an inexpensive and most viable alternative option. [9]

The Diels Alder (DA) reaction remains in the realm of Organic Chemistry a major player in creating new-formed carbon-carbon bonds. This remarkable reaction is the protypical example of a [4+2] cycloaddition between a conjugated diene and a diversely substituted alkene, commonly termed the dienophile, to form a functionalized cyclohexene ring. This reaction was first described by Otto Diels and Kurt Alder in 1928, for which work they were awarded the Nobel Prize in Chemistry, some 22 years later. This chemical conversion has now got the fame of a canonical application of a concerted pericyclic reaction and it is commonly accepted that this reaction takes place *via* a single aromatic-like transition state, consequently without intervention of one or several intermediates generated during the course of this transformation. As such, the DA reaction is governed simply by orbital symmetry considerations and it has been classified as a [4πS+2πS] cycloaddition, for it proceeds through a suprafacial-suprafacial interaction of a 4π-electron system with another 2π-electron system, an interaction which is thermally allowed as a [4n+2] cycloaddition. [10]

Lewis acids such as zinc chloride, boron trifluoride, tin tetrachloride, aluminum chloride, etc. can act as catalysts of normal-demand Diels–Alder reactions by coordination to the dienophile. The resulting complexed dienophile becomes more electrophilic and consequently more reactive toward the diene, increasing the reaction rate and often improving the regio- and stereoselectivity as well. Lewis acid catalysis also enables Diels–Alder reactions to proceed at low temperatures, *i.e.* without exogenous thermal activation.[9] Much study has been performed around the questions of the mechanisms and reaction rates of this reaction. A lot of works focused on the rate acceleration of these reactions due to a potential hydrophobic effect in water [11-14]. There is indeed a significant rate enhancement when certain Diels–Alder reactions are carried out in water, as well as in some other polar solvents such as *N,N*-dimethylformamide and ethylene glycol. The reaction of cyclopentadiene and butenone for example

was found about 700 times faster in water relative to isooctane. Several explanations for this effect were proposed, such as an increase in the effective concentration due to hydrophobic collapse and/or an hydrogen-bond mediated stabilization of the transition state. [15]

Based on this afore mentioned work and some other related works, in this paper, we have thus revisited the Diels-Alder cyclo-addition of anthracene-9-methanol with maleimide in water in an effort to render this chemical transformation more efficient and greener. Indeed, the DA reaction is ideally suited for this purpose owing to its exceptionally high atomic economy, which is actually of 100 %. [16].

EXPERIMENTAL SECTION

General procedures
Melting points were determined using an Electrothermal melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 457 spectrometer using vacuum-degazed KBr pellets. Wave numbers are expressed in cm–1. ^{1}H- and ^{13}C-NMR spectra were recorded at ambient temperature using a Brucker 400 spectrometer. Compounds were dissolved in CDCl3 or DMSO-d6 to obtain a 0.1 molar solution. Chemical shifts are expressed in the δ scale with TMS (tetramethylsilane) as internal standard. Thin layer chromatography (TLC) analyses were performed on Merck TLC plates (silica gel, 60 F 254, E. Merck, Darmstadt, ref. 5735). For TLC, all the compounds reported were routinely checked in two standard solvents, that is, acetone/toluene/cyclohexane (solvent A, 5: 2: 3, v/v/v) and ethyl acetate/*n*-hexane (solvent B, 4: 6, v/v). The reverse-phase thin layer chromatography conditions were HPTLC plates RP-18 F-254 S (Merck) and methanol: water (75/25, v/v). All compounds reported were found homogenous under such TLC and HPLC conditions. All reagents were purchased from Aldrich. All solvents were of the ACS reagent grade (Aldrich). We report hereunder two procedures which are representative of the various reaction conditions shown in the Table 1.

Benchmark Diels-Alder Reaction
Synthesis of 9-(hydroxymethyl)-9, 10-dihydro-9, 10-[3,4]epipyrroloanthracene-12,14-dione.
A reaction mixture composed of anthracene-9-methanol (3 mmol, 625 mg), maleimide (10 mmol, 970 mg), lithium chloride (100 mmol, 4.24 g), and 110 mL of double-distilled water was vigorously magnetically stirred and heated under reflux in an oil bath set at 105°C until complete discharge of the slight yellow color (after approximately 30 min). It is worth noting that the reaction system never became a homogenous solution but merely consisted of a slurry slowly turning from yellow to a clear appearance. The reaction mixture was then allowed to cool down to the room temperature and was subsequently left to stand overnight in a refrigerator (~ 6°C). The resulting slightly off-white crystalline material was then collected on a Buchner funnel, washed twice with ice-cold water (twice 20 mL), and dried *in vacuo* in a dessicator over powdered tetraphosphorus decaoxide (P4O10) to give snow-white luminous crystalline flakes of the title compound in 87 % yield after a single recrystallization from ethanol:water (30:70, *v/v*) and drying *in vacuo* to constant weight. It should be noted that the rough material was slightly contaminated by a tiny impurity, *i. e.* the starting material anthracene-9-methanol, as evidenced by its intrinsic high fluorescence in TLC. Mp = 237-239°C. This material was found TLC and HPLC-pure. ^{1}H-NMR and ^{13}C-NMR data were found in full accordance with previously published data [16].
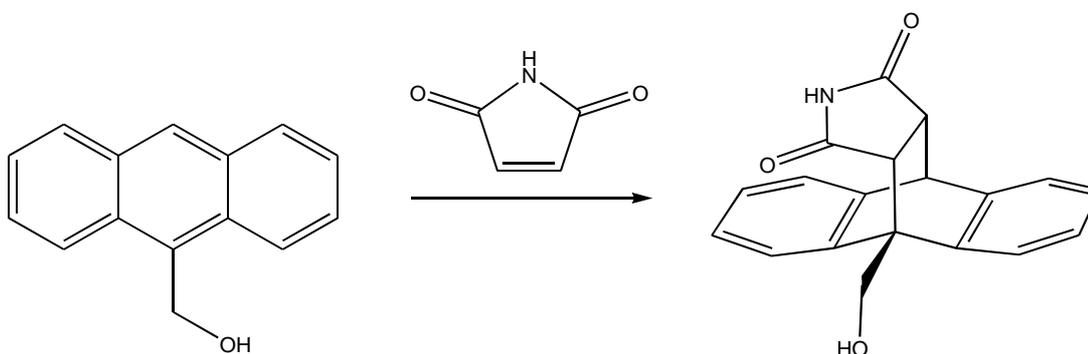

Figure 2. DA Benchmark reaction of anthracene-9-methanol with maleimide (for conditions, see Table 1)

RESULTS AND DISCUSSION

For a rather long time, water was not such a popular solvent for the Diels–Alder reaction among the synthetic organic chemist community. Before 1980, its use was reported only incidentally. However, the pioneers Diels and Alder themselves performed the reaction between furan and maleic acid in aqueous solution already in 1931, an

experiment which was later on repeated by Woodward and Baer in 1948. Yet it was not until the publications of Breslow *et al.* that it became common knowledge that water behaved as a unique solvent in the Diels–Alder reaction. In 1980, Breslow reported the acceleration of the DA reactions using water as a solvent. This came as a surprise given the relative insensitivity of Diels-Alder reactions to solvent polarity. This point was subsequently qualified by Fieser and Fieser. Therefore, the polarity of water could not be used to rationalize the reactivity. To explain the increase of the reaction rate, Breslow invoked a hydrophobic collapse induced by the aqueous surrounding on the reacting partners, *i. e.* the diene and the dienophile [11]. As a matter of fact, it is not absolutely mandatory for the two components to be completely solubilized in water. In 2007, Marcus and Jung introduced a new model which which introduced the concept of *on water* reactivity [17]. The rapid mixing required for these reactions to take place (because it maximizes the reactive surface) comes into support of the authors' hypothesis.

Moreover, there is a paramount proficiency for even further more enhancing the rates and selectivities of the DA reaction in water through the use of more specific catalysts. In organic solvents, implementation of the Lewis-acid catalysis in the DA reaction is very well established [18]. Unfortunately, in many instances, extension of the implementation of Lewis-acid catalysts in an aqueous surrounding is harmfully hampered by the strong interactions between water (a Lewis base) and these acid catalysts. In many cases indeed, there is a most severe incompatibility between the Lewis acid and water, which is leading either to a partial loss of catalytic power or – worse – to the total annihilation of the Lewis acid catalyst itself. Brönstedt acid catalyst such as the green L-proline were included for comparison in our experimental set-up (see Table 1). Briefly, conditions #1 - #3 were selected because these classical solvents show increasing polarity but relatively similar boiling point (~100°C) and they give a baseline to our benchmark. Water (#1) provides a net improvement compared to baseline conditions #1 - #4. Condition #5 is met with the best score which remains unsurpassed in conditions with other adjuvants (#6 - #9). Second best condition surprisingly enough is delivered by L-Pro (#9).

Table 1. Benchmark reaction

Experiment	Condition	t (h)	Yield (%)
#1	n-C_7H_{16}	4	42
#2	n-C_3H_7OH	4	47
#3	dioxane	4	51
#4	H_2O	1	78
#5	H_2O/LiCl	0.5	87
#6	H_2O/NaCl	1	76
#7	H_2O/G.HCl	1	73
#8	H_2O/βCD	1	71
#9	H_2O/L-Pro	0.5	78

Lithium halides are commonly classified among the mild Lewis acids and have been involved as catalysts in a lot of organic successful syntheses. Indeed, the strong « oxophilicity » of lithium cation activates oxygen-containing electrophiles and renders them more susceptible to nucleophilic attacks. In our benchmark reaction, it is clearly the carbonyls of maleimide which are targeted. Interestingly enough, according to the litterature, these oxophilic lithium salts can be used in water without significant deterioration of their catalytic power. We therefore gladly enrolled lithium chloride within our experimental set-up. [19]

To our pleasure, there was a clear promotion effect of our benchmark reaction as can be evidenced from inspection of the Table 1, upon addition of Li+ cation within the reaction medium (*cfr* also Experimental Section), albeit at a rather robust concentration (0.9 M). To our dismay, in the same context, the greener sodium chloride or the exotic guanidinium chloride was found inefficient, which reinforces the interest of the oxophilic boost effect of lithium cation. The rationale behind this phenomenon is due to a depletion of the electron density within the heterocycle leading to a higher activation of the maleimide double bond. In other words, lithium cation's oxophilic character increases the electron demand at dienophile double bond, which in turn increase its reactivity towards the diene.

A quite recent publication in 2015 reports the catalytic effect of cyclodextrines precisely in the same type of reaction as our benchmark, but involving *N*-arylmaleimide derivatives. To rationalize this catalysis, the authors invoked a clastic bidentate proton transfer between cyclodextrin's outward-oriented hydroxyls and maleimide's carbonyls, a situation very similar to the lithium-promoting effect here observed. In our hands however, the catalytic power of β-cyclodextrin was not found so obvious (Table 1, #8) and this is most likely due to the poor tethering interaction between our *N*-unsubstituted maleimide and the hydrophobic core of the cyclodextrin [20-21]. Moreover, the solubilizing effect of β-cyclodextrin for our diene may also be considered as somewhat detrimental to the reaction by increasing the steric hindrance on the central cycle due to complexation of one of the external cycles of the starting diene.

A most salient feature in our experimental set-up is the fact that maleimide (log P = -0.69) and lithium chloride are totally soluble at the reflux temperature of water. However, anthracene-9-methanol with a log P = 3.50 is only at best scarcely soluble. It is therefore conceivable that the anthracene species will organize itself into rafts at water's interface, with the methanol side-chain as anchor. As already first evidenced by Sharpless *et al.,* we are thus clearly confronted herein with a typical condition of « on-water » reaction rather than an « in-water » reaction condition [17]. We may therefore safely claim that - in the DA reaction - water not only behaves as a solvent but also as a powerful catalyst.

CONCLUSION

Possibilities still exist for further rate enhancements and even for more potent catalytic effects by using Lewis-acid catalysts in aqueous solution, we confess we have barely touched this option. Despite these obvious benefits, even nowadays nature's choice water is not generally considered as the solvent of choice for the DA reaction, and this for various reasons, among which the poor water solubility of many Diels–Alder reagents. The use of surfactants and low concentrations of cosolvents might perhaps circumvent some of these obstacles eventually transforming the DA reaction into a cleaner and greener process.

In this paper, we have highlighted that the use of water as a solvent for the Diels–Alder reaction is not merely an environmentally friendly alternative to toxic organic volatile solvents. The illustrative reaction of the Diels-Alder cyclo-addition between anthracene-9-methanol and maleimide in water - selected as benchmark reaction - illustrates how water as a green solvent can be used not only because its more benign impact on the environment but also because of it improves some other aspects (rate, yield, ease of isolation) of the reaction. Adjunction of lithium chloride in the reaction medium adds a somewhat incremental benefit. Conclusively, we may safely claim that - in the DA reaction - water not only behaves as a solvent but most of all as a powerful catalyst. Moreover, we have synthesized in a green manner a cantharidine-like pilot molecule to be tested for its ability to inhibit cathepsin-L and the growth of *Trypanosoma brucei brucei*. Preliminary results are indicative of the validity of our design.

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