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An unexpected formation of the novel 7-oxa-2-azabicyclo[2.2.1]hept-5-ene skeleton during the reaction of furfurylamine with maleimides and their bioprospection using a zebrafish embryo model†

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An unexpected intramolecular cyclization during the reaction of furfurylamine with maleimides is reported as a novel strategy for the efficient green synthesis of the 7-oxa-2-azabicyclo[2.2.1]hept-5-ene skeleton. Under the same reaction conditions, 7-oxabicyclo [2.2.1]hept-5-enes were synthesized when furfurylamine was N-protected by the acetyl group. Both types of bicycloheptenes were screened using the zebrafish model system for genetics and developmental biology.

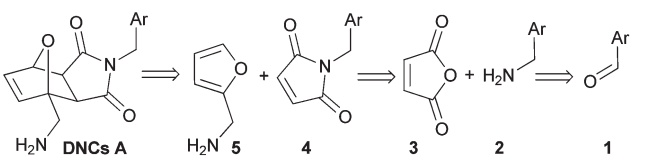
Introduction

Among the oxygen and nitrogen-containing heterocycles, 7-oxa and 7-azabicycles are the common core components of some biologically active natural alkaloids as well as potent pharmaceutical drugs like cantharidines[1] and (−)-epibatidine analogues.[2] However, the known natural and synthetic examples of oxa and/or aza bicyclic skeletons are scarce and the synthetic methods to prepare them remain the main objective of many current investigations.[3] To date the most fundamental and common strategies for the synthesis of these oxa-azabicyclo rings are based on the Diels–Alder reaction (DAR) between nitroso derivatives and cyclopentadiene to afford 2-oxa-3-azabicyclo[2.2.1]hept-5-enes[4] or *via* an intramolecular 1,3-dipolar cycloaddition using nitrones to give 7-oxa-1-azabicyclo[2.2.1]heptanes.[5] Nevertheless, both methods are in general expensive, laborious and not eco-friendly.

With our current interest in the development of new synthetic routes for the preparation of diverse heterocycles using the DARs,[6] we directed our efforts to complement the backgrounds and improve the drawbacks in the synthesis of dehydronorcantharimides (DNC), designing a logic route for the selective synthesis of functionalized DNC **A** from diverse maleimides **4** and furfurylamine **5**, according to the retrosynthetic analysis depicted in Scheme 1. Followed by our retrosynthetic analysis of functionalized DNC **A**, our study began with the three-step synthesis of the commercially unavailable *N*-arylmethylmaleimides **4a–g** derived from benzylamines **2** and maleic anhydride **3** using the respective substituted benzaldehydes **1** as the main starting materials[7] (see ESI† for details).

Having prepared the required diverse maleimides **4a–g**, we focused our study on the desired DNCs **A.** The first experiment was carried out at room temperature under an inert atmosphere, *N*-benzylmaleimide **4a** and furfurylamine **5** were chosen as model substrates, acetonitrile was employed as the solvent. After 3 hours the reaction was complete (TLC) and once the main product was purified, the structural elucidation of the isolated substance revealed surprisingly that instead of the desired molecule **A**, a new compound **6a** with the 7-oxa-2-azabicyclo[2.2.1]hept-5-ene skeleton was obtained as a single product and in moderate yield (Scheme 2).

The reaction conditions were varied (Table 1), in order to improve the yield and to establish a green method for the selective preparation of product **6a**, finding that (i) all the experiments gave in moderate to excellent yields the same product **6a** as a stable oil, (ii) this reaction can be carried out smoothly in CH_3CN or polyethylene glycol 400 (PEG-400) without any catalyst at room temperature (entries 1,2) and at 90 °C (entries 5,6), (iii) 10 mol% H_3BO_3 catalyses this process reducing the reaction times in both polar solvents, CH_3CN and PEG-400 at rt (entries 3,4), and (iv) heating the reaction at



Scheme 1 Synthetic design for the preparation of N-substituted dehydronorcantharimides.

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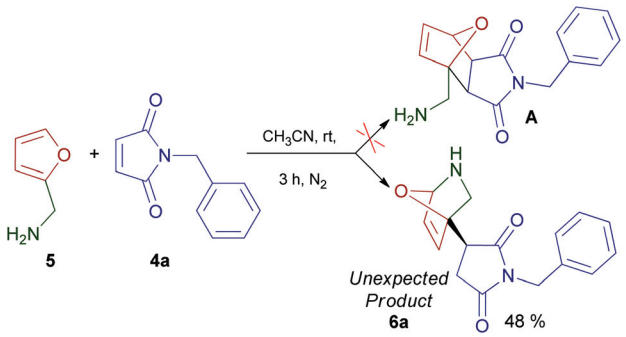


Scheme 2 Formation of the unexpected product **6a** during the model reaction between maleimide **4a** and furfurylamine **5**.

Table 1 Criteria for the selection of the best conditions for the reaction of the *N*-benzylmaleimides **4a** with furfurylamine **5**

Entry	Solvent	Catalyst (10 mol%)	Conversion[a] (%)	Time (h)	Yield of **6a**[b] (%)
1	CH_3CN	None	57[c]	3	48
2	PEG-400	None	52[c]	2.3	43
3	CH_3CN	H_3BO_3	63[c]	1	52
4	PEG-400	H_3BO_3	60[c]	1	57
5	CH_3CN	None	100[d]	4	80
6	PEG-400	None	100[d]	3	79
7	CH_3CN	H_3BO_3	100[d]	1	84
8	PEG-400	H_3BO_3	100[d]	1	82

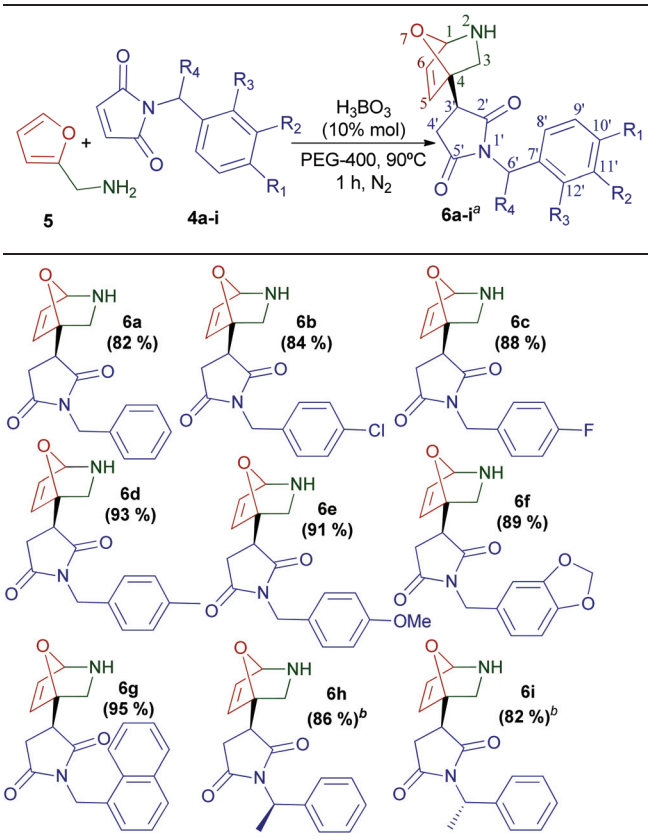
[a] Selecting maleimide **4a** as a limit reactant. [b] Isolated yield. [c] Reaction performed at room temperature. [d] Reaction performed at 90 °C.

90 °C enhances the catalytic activity of boric acid, accelerating considerably the formation of product **6a** (entries 7,8).

Next, having optimized the reaction conditions, a small library of novel and diverse 7-oxa-2-azabicyclo[2.2.1]hept-5-enes **6a–i** (Table 2) were easily prepared from the respective maleimides **4a–i**, selecting boric acid (10 mol%) and PEG-400 as a solvent (Table 1, entry 8) as standard conditions, in agreement with the current environmental concerns, designing and developing economically and environmentally benign synthesis.[8] We examined the generality of the reaction by varying the steric and electronic properties of the substituents on the maleimide ring finding that (i) the course of this reaction is not affected by the chemical nature of the *N*-benzyl maleimides and (ii) the novel series of the unexpected products **6a–i** were obtained in good yields instead of the desired DNC **A**, without the observation of any collateral product or isomers.

^{1}H NMR, ^{13}C NMR, DEPT-135 and HSQC experiments of products **6a–i** revealed the presence of the 2,5-dioxopyrrolidine and the 7-oxa-2-azabicyclo[2.2.1]hept-5-ene rings, and a new methylene group (C-4′) confirmed the saturation of the five-membered ring. The COSY experiment confirmed the formation of the 7-oxa-2-azabicyclo[2.2.1]hept-5-ene ring as a rigid system, while the HMBC experiment, through the correlations observed between H-3 to C-3′ and H-3′ to C-3, indicated the connection between C-4 (the bicyclic moiety) and C-3′ (pyrrolidine ring) (see ESI† for details).

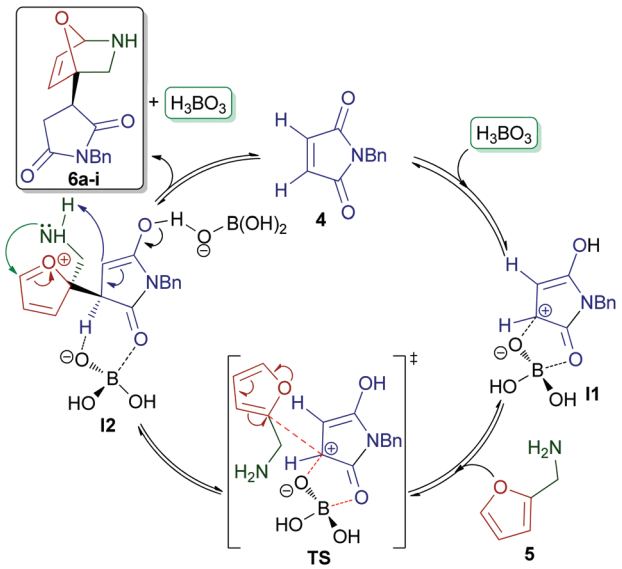
Table 2 H_3BO_3-Catalyzed reaction of furfurylamine **5** with various maleimides **4a–i** in PEG-400



[a] Isolated yields. [b] Commercial (*R*)- and (*S*)-1-phenylethanamines were used to prepare the corresponding maleimides **4h** and **4i**.

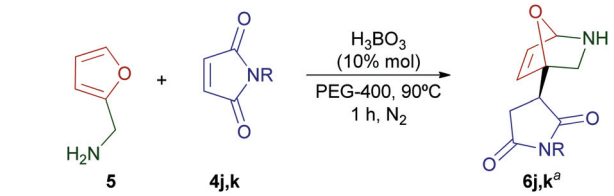
According to Corey's work, where the possible interactions of the boron atom (oxazaborolidines) with the maleimide core are mentioned,[9] we proposed a reasonable mechanistic hypothesis, in which in a first step the maleimides lose their properties of dienophiles when they interact with boric acid to rapidly form the intermediate **I1**, this species has a positive charge on one of the olefinic carbons of maleimides that is stabilized by the boronic diacid ion $H_2BO_3^-$. Due to the trigonal geometry of this anion and the symmetry of the pyrrolidine ring, the boronic diacid ion induces the stereoselective attack of nucleophilic species to the positively-charged carbon (Scheme 3).

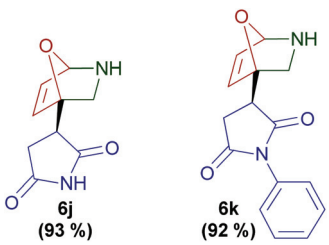
The second step involves the selective attack of furfurylamine **5** to intermediate **I1**, which adopts one of its possible resonance structures, promoted by the electron-donor nature of the furan oxygen,[10] to form the intermediate **I2** through the possible pre-transition state **TS**. Thus, we suggested a concerted rearrangement, promoted by the regeneration of H_3BO_3, in which the C=O function is restored and promoted the abstraction of one of the H bonded to furfurylamine nitrogen. This fact generates a nucleophilic centre on that atom that induces the attack on the electrophilic centre on the oxocarbenium ion and leads to the formation of the 7-oxa-2-azabicyclo-[2.2.1]hept-5-ene ring.



Scheme 3 Possible mechanistic hypothesis for the reaction of maleimides and furfurylamine under H_3BO_3 catalysis.

Table 3 Reaction between maleimides **4j** and **4k** with furfurylamine **5a** giving products **6j** and **6k**





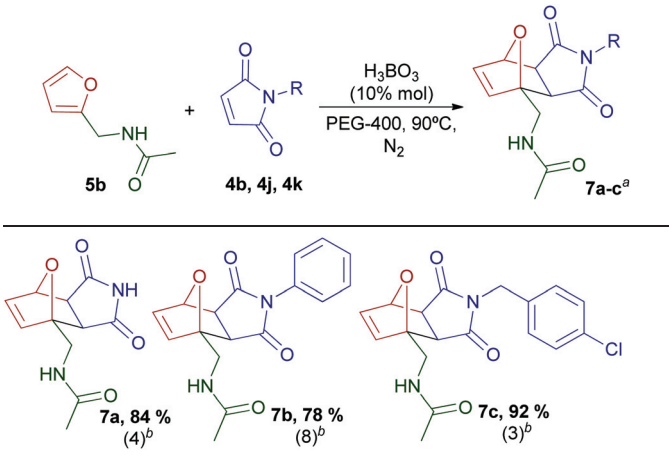
[a] Isolated yield.

Finally, according to the reaction mechanism, there is no evidence that the pre-existing chirality of maleimides **4h** and **4i** generates an asymmetric induction that affects the selective formation of the enantiomers **6h** and **6i** (see ESI† for details).

Next, we turned our attention to other functionalized maleimides in order to explore the reactivity and versatility of our reaction. Thus, making furfurylamine **5a** react with maleimides **4j** and **4k**, two new molecules **6j** and **6k** were obtained with both 7-oxa-2-azabicyclo[2.2.1]hept-5-ene and 2,5-dioxopyrrolidine rings as exclusive products (Table 3).

At the end of our investigation and with the need to accomplish our initial objective, we focused our efforts on the synthesis of the desired DNC **A**. Based on the possible reaction

Table 4 Synthesis of N-substituted DNCs **7a–c** using boric acid as a catalyst in PEG-400



[a] Isolated yield. [b] Reaction times (hours) monitored by TLC.

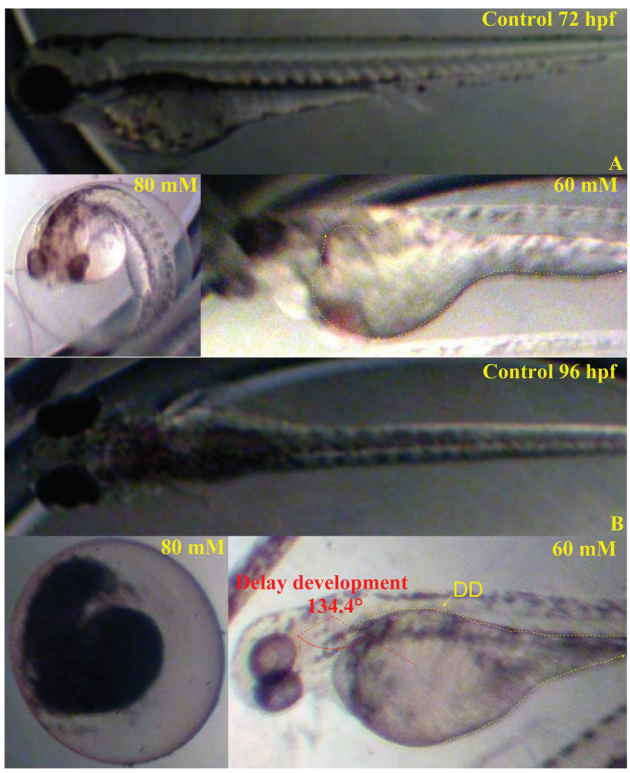


Fig. 1 **A**: Zebrafish embryos treated with compound **6g** at 72 hpf. The embryos treated at 100, 150 and 200 μM died after chemical exposure at this time. The main visual phenotype, to measure the development delay at this stage, was when the eggs hatch and the alevins could be photographed. At 80 μM after 72 hpf, the eggs have not hatched at the same rate than the control did and the digestive damage (DD) could be observed at 60 μM. **B**: Zebrafish embryos treated with compound **6g** at 96 hpf. Embryos treated at 80 μM finally died after 96 hpf. Head–trunk angle (red dotted line) 134.4° (control angle: 148°) at 60 μM indicating several development delays. The yellow dotted line indicates that the embryos, treated with 60 μM and below, did not consume their yolk at the same rate to the control fish, putting in evidence the DD induced by the 7-oxa-2-azabicyclo[2.2.1]hept-5-ene **6g**.

Table 5 Criteria for the selection of the best conditions for the reaction of the *N*-benzylmaleimides **4a** with furfurylamine **5**

Compound	IC_{50} [a] (mM)
6a	0.233 ± 0.003
6b	0.200 ± 0.004
6c	0.267 ± 0.008
6d	0.315 ± 0.014
6e	0.293 ± 0.004
6f	0.235 ± 0.011
6g	0.279 ± 0.015
6h	0.249 ± 0.012
6i	0.262 ± 0.012
6j	0.302 ± 0.008
6k	0.419 ± 0.026
7a	0.236 ± 0.006
7b	0.287 ± 0.009
7c	0.212 ± 0.008
Physostigmine	0.173 ± 0.009[b]

[a] IC_{50} values are the mean ± SEM of at least three different experiments in duplicate. [b] IC_{50} values are expressed in μM.

mechanism proposed in Scheme 3, the nucleophilicity of the furfurylamine nitrogen plays a key role in the ring closure during the formation of the bicyclic system, so the acetylation of this nitrogen probably would avoid the ring closure and allow the DAR between the reactants.

From this hypothesis we performed the reaction between *N*-acetylfurfurylamine **5b** and maleimides **4b**, **4j** and **4k** under the same conditions studied above. We were pleased that N-substituted DNCs **7a–c** were obtained in excellent yields (Table 4).

Finally and as part of our current chemical-biology program, preliminary studies were carried out to establish the possible biological activity of molecules **6a–k** and **7a–c**.

We addressed the zebrafish (*Danio rerio*, Cyprinidae) as a system for biomedical research. Through zebrafish embryo *in vivo* screening[11] we discovered that compound **6g** was a novel inhibitor of early-stage zebrafish embryo development and an extremely toxic agent at diverse concentrations. The embryos treated with 100, 150 and 200 μM died after 72 hours after chemical exposure. The head–trunk angle, used as a measure of developmental rate (normal angle from the middle of the ear and eye to the notochord: 148° at 96 hpf), indicates that the embryos treated with 60 μM, and below, are slightly delayed in development, head–trunk angle 134.4° (red dotted line). The yellow dotted line indicates that the embryos, treated with 60 μM and below, did not consume their yolk at the same rate to the control fish, putting in evidence the digestive damage (DD) (Fig. 1B).

Additionally, we followed the method described by Ellman[12] and adapted in our laboratory with some modifications.[13] Compounds **6a–k** and **7a–c** were evaluated as inhibitors of the enzyme acetylcholinesterase (AChE) from *Electrophorus electricus* (type V-S). Comparing the AChE inhibitory activity for the evaluated compounds with the obtained inhibitory activity for the reference compound (physostigmine), we found that these compounds possess moderate *in vitro* AChE inhibitory activity, highlighting compound **6b**, with an IC_{50} = 0.2 mM, as the most potent inhibitor of this enzyme (Table 5).

Conclusions

In summary, the present work is the first example of an easy, efficient and green protocol for the synthesis of both novel 7-oxa-2-azabicyclo[2.2.1]hept-5-enes and 4-aminomethyl-7-oxabicyclo [2.2.1]hept-5-enes, further investigations of the scope and the reaction mechanism of this synthetic method could extend its application to the synthesis of some natural and biologically active molecules.

Through zebrafish embryo *in vivo* screening it was discovered that **6g** is a novel inhibitor of early-stage zebrafish embryo development and an extremely toxic agent.

Possessing several degrees of structural diversity, both types of bicycloheptenes **6a–k** and **7a–c** are novel, interesting models for zebrafish embryo *in vivo* screening that encourage our synthetic and biological investigations on phenotypic characterization of a whole vertebrate organism in order to exploit this platform for medium to high throughput compound testing.

Acknowledgements

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