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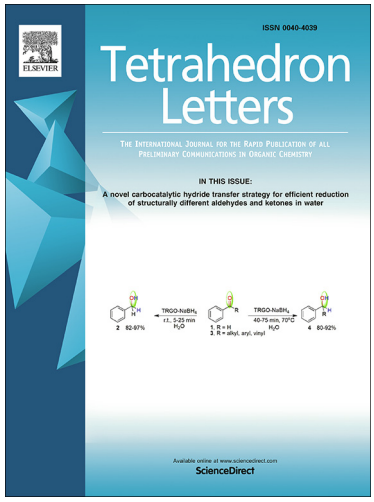

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Scalable Synthesis of Cladosporin

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ABSTRACT

Cladosporin, a secondary metabolite isolated from fungal sources like *Cladosporium cladosporioides* and *Aspergillus flavus* was found to exhibit selective nano-molar activity against malarial parasite *Plasmodium falciparum* by inhibiting parasitic protein biosynthesis. In addition, this natural product has a broad range of bioactivities including, antiparasitic, antifungal, antibacterial as well as plant growth inhibition. However, it has limited availability from the natural sources for further development. Herein, we report a modified and improved synthetic route which led us to produce this potent natural product in a gram scale. Conversion of the undesired diastereomer to desired one via Mitsunobu inversion of secondary alcohol and carbon monoxide insertion reaction towards the construction of isocoumarin unit are the key features of the present synthesis.

Cladosporin, also known as asperentin is a secondary metabolite found in diverse fungi including *Cladosporium cladosporioides*[1] and *Aspergillus flavus*[2] with the first isolation documented way back in 1971 by Scott and Walbeek.[1] Structurally it consists of a THP ring (2,6-disubstituted tetrahydropyran) connected to an isocoumarin moiety[1,3,4] (Figure 1) whose complete stereochemical elucidation was reported in 1981 by Springer et. al.[2] Cladosporin was found to illustrate a broad spectrum of bio-activities (Figure 1) such as antifungal,[1] insecticidal, plant growth inhibition[2,5] and antibacterial,[6] as well as anti-inflammatory activity.[7] While screening natural product library to identify inhibitors of *Plasmodium falciparum* (*Pf*), which happens to be the causative pathogen for malaria; Winzeler et. al. identified cladosporin to display potent antiparasitic activity (~40 nM) against both blood and liver stage proliferation of the pathogen by ceasing protein biosynthesis in the parasitic cell through inhibition of cytosolic lysyl-tRNA synthetase (*Pf*KRS).[8,9] Besides, cladosporin was found to be >100 fold selective towards parasitic KRS as compared to human enzyme (*Hs*KRS).[9] Apart from *Pf*KRS, cladosporin is known to inhibit KRSs from other species, including helminth parasites such as *Loa loa* (*Ll*) and *Schistosomamansoni* (*Sm*).[10] By considering promising potential of cladosporin, we have initiated a program towards it. Recently, we have accomplished the synthesis of all the possible eight stereoisomers (cladologs) of cladosporin and in collaboration with Sharma et. al. we successfully deciphered the stereochemical bases of cladologs' interaction with *Pf*KRS through cladolog-*Pf*KRS co-crystallization.[11] These interesting findings and impressive biological profile of cladosporin undoubtedly make it as a promising candidate towards the development of novel antimalarials. A proper bio-assessment towards drug development demands adequate quantity of the lead compound (cladosporin), which has limited access from natural sources. Hence development of an efficient and scalable synthetic strategy is worth exploring. As of today, one asymmetric total synthesis[4] followed by a formal synthesis[3] of the natural product have been documented by She et. al. and Mohapatra et. al. using independent and elegant ways, respectively. Following that, we have documented a synthesis of cladosporin, where we have adopted a strategy to access different isomers deliberately.[11] Although these synthetic routes to access cladosporin were documented, there is a need for new route which can provide sufficient material. Here we report a modified approach to access cladosporin in "gram-scale".

Our synthesis commenced with the known intermediate **1** (prepared through a reported protocol) which was subjected to epoxidation using mCPBA reagent to its corresponding epoxide (**fragment B**) as an inseparable diastereomeric mixture. The epoxide thus obtained, on Grignard reaction with commercially available 1-bromo-3,5-dimethoxybenzene (fragment A) afforded 1:1 diastereomeric mixture of alcohols (**2** and **3**) with excellent overall yield of 86%. It is worth mentioning that maintaining a low concentration of Grignard reagent (< 0.5 M) is crucial for the reaction. Higher concentrations of Grignard reagent results in an unrequired dimerization product.[13] Here, in this case, we were able to separate both the diastereomers (**2** and **3**) cleanly using simple silica gel column chromatography. In this context, we made interesting observations while analyzing ^{1}H NMR data of these isomers where identical chemical shifts of all the concerned

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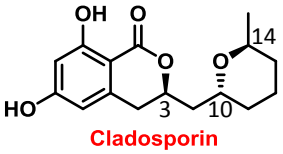


Cladosporin

EC_{50}: 40 nM (*Plasmodium falciparum*)
IC_{50}: 120 nM (*Pf*KRS)
>100 fold selective towards *Pf*KRS over *Hs*KRS

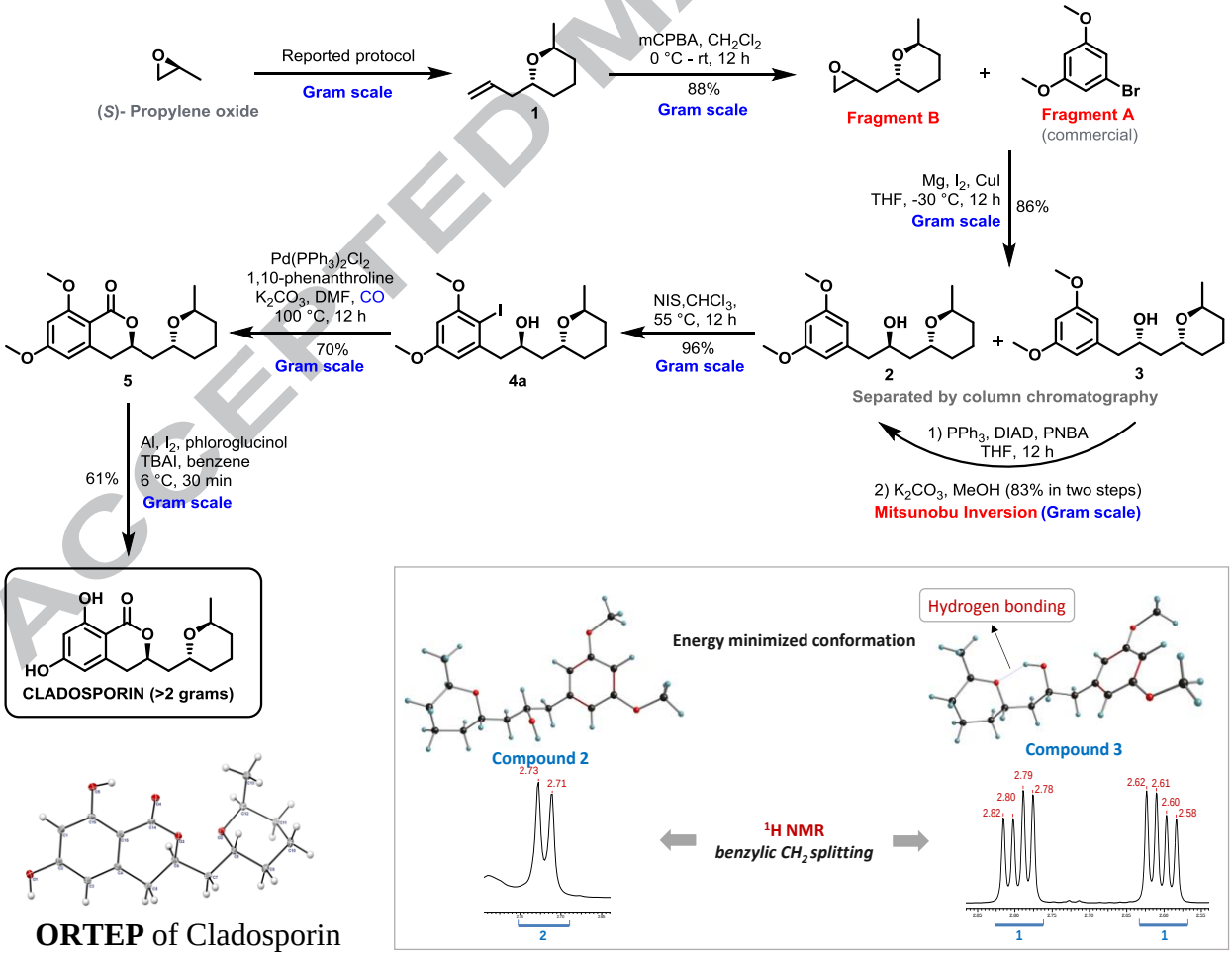
Parasites	IC_{50} (μM)
Plasmodium yoeli	0.04
Trypanosoma brucei	2.05
Leishmania donovani	2.56
Toxoplasma gondii	2.63

Fungus

Penicillium sp.	% inhibition	Aspergillus sp.	% inhibition
viridicatum	100 (20 μg/mL)	*niger*	95 (20 μg/mL)
expansum	85 (20 μg/mL)	*ochraceus*	97 (20 μg/mL)
obscurans	22.1 (30 μg/mL)	*versicolor*	<50 (20 μg/mL)
viticola	79.9 (30 μg/mL)	*clavatus*	<50 (20 μg/mL)
		nidulans	<50 (20 μg/mL)

Colletotrichum sp. (Fungus)	% inhibition
acutatum	92.7 (30 μg/mL)
fragariae	90.1 (30 μg/mL)
gloeoosporioides	95.4 (30 μg/mL)

Figure 1: Spectrum of Cladosporin bio-activities.



Scheme 1: Gram scale synthesis of cladosporin

proton signals were observed for both the compounds except the benzylic protons, which showed significant chemical shift difference for both. This difference in the ^{1}HNMR pattern could probably be explained using energy minimized conformer of both the compounds using Gaussian. Unlike compound **2**, compound **3** was found to exhibit a hydrogen bonding between the secondary alcohol and the tetrahydropyran oxygen which might be the probable cause for a constrained conformation of compound **3** and this in turn resulted in a separate splitting pattern for both the benzylic protons (Scheme 1). To make use of undesired diastereomer, compound **3** was subjected to Mitsunobu reaction protocol followed by ester hydrolysis which led to the complete inversion of the secondary alcohol center in **3** to afford the required diastereomeric alcohol **2** in good yields. Next we focused to construct the six-membered lactone present in the natural product which was envisioned using palladium catalyzed carbon monoxide insertion reaction. Accordingly, the alcohol **2** was converted to its corresponding iodo-compound **4a** in excellent yields by using *N*-iodosuccinimide (NIS) and catalytic amount of *p*TSA in chloroform solvent. The iodo-compound **4a** was treated with $Pd(PPh_3)_2Cl_2$, potassium carbonate and 1,10-phenanthroline in presence in DMF at 100 °C under blanket of carbon monoxide to obtain desired compound **5** in 70% yield.[15] Demethylation of compound **5** was achieved through aluminium triiodide mediated exhaustive demethylation[16] to furnish the natural product cladosporin. The spectral data of the synthesized compound was in complete agreement with the reported data.[3,4] Besides, structure and relative stereochemistry of the synthesized compound was further confirmed by single crystal X-ray diffraction analysis for an unambiguous assignment of its stereocenters. Herein, we would also like to document alternate ways/attempts to form the six-membered lactone ring of cladosporin, in particular using bromo

compound **4b**. Alcohol **2** upon treatment with *N*-bromo succunimide (NBS) in CH_2Cl_2 afforded bromo compound **4b** in excellent yield. The bromo compound thus obtained treated with *n*BuLi, LiHMDS to generate corresponding dianion which was subsequently quenched with electrophiles like triphosgene, carboxydiimidazole (CDI),methylchloroformate and ethyl chloroformate which did not give any fruitful results(Scheme 2). We also tried to insert copper in the carbon halogen bond followed by protodecupration to furnish the required lactone.[14]Although we were successful in obtaining the required product **5** in a moderate yield of 60%, we were unable to reproduce comparable yields at higher scale. As an alternative, we also prepared the required lactone **5** by following She's protocol.[4]

In short, we have accomplished the scalable total synthesis of cladosporin natural product. Gram-scale operations, Mitsunobu inversion to convert undesired alcohol to required one and palladium-catalyzed carbon monoxide insertion reaction to construct six-membered lactone ring are the highlights of present work. Now we have more than two grams of material in hand which is sufficient for further profiling such as in-depth assessment of the pharmacokinetics and pharmacodynamics.

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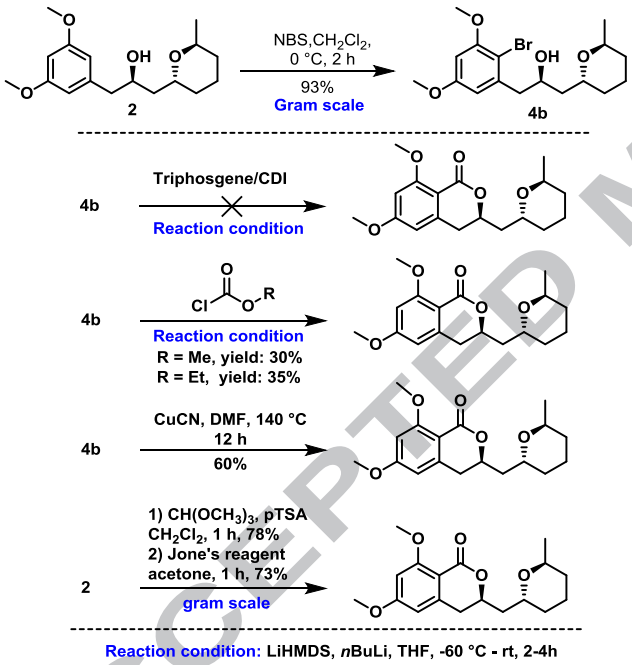
We would like to acknowledge University Grants Commission (UGC) for providing research fellowship to PD. We would also like to express our gratitude to Dr. Amit Sharma (ICGEB, New Delhi) for goading us into this project. Besides we thank Dr. Rambabu Dandela for X-ray crystallographic analysis of



Scheme 2: Alternatives towards lactonization

cladosporin and Tamal Das for conducting the energy minimization of two diastereomers. This work is a part of the project proposal submitted to SERB, New Delhi, India, under the special call "Theme-Based Call for Proposals Initiated by the Program Advisory Committee of Organic Chemistry" (Reference EMR/2016/004301/OC). This proposal is currently under evaluation.

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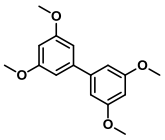
[13] Formation of dimeric compound (tentative structure shown below) at higher concentrations of Grignard reagent was observed.



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Supplementary Material

Experimental details, copies of NMR spectra are provided. Supplementary data of this article can be found online at….

Graphical Abstract

Scalable Synthesis of Cladosporin

Pronay Das[a,b], Yah Mankad[a] and D. Srinivasa Reddy*[a,b]

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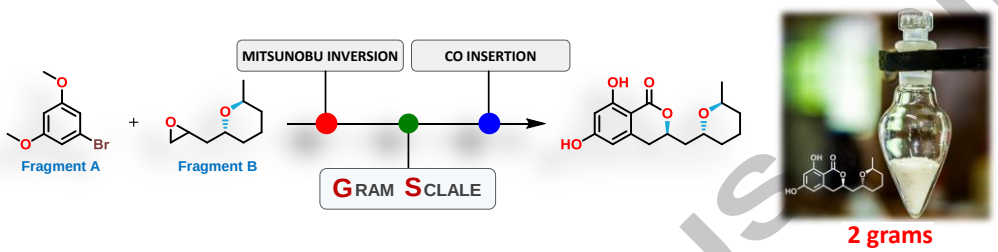

Highlights:

- Developed a scalable synthetic route to cladosporion, a potent antimalarial natural product.
- Mitsunobu inversion and carbon monoxide insertion are the key steps of the present synthesis.
- The target natural product cladosporin has been synthesized in gram scale for the first time.