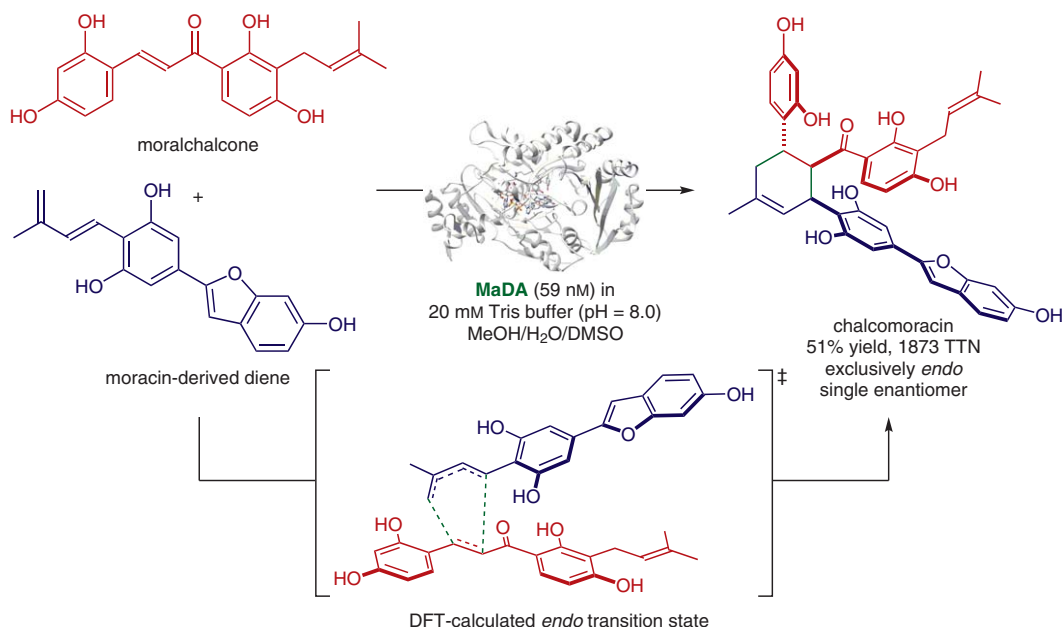


L. GAO, C. SU, X. DU, R. WANG, S. CHEN, Y. ZHOU, C. LIU, X. LIU, R. TIAN, L. ZHANG, K. XIE, S. CHEN, Q. GUO, L. GUO, Y. HANO, M. SHIMAZAKI, A. MINAMI, H. OIKAWA, N. HUANG, K. N. HOUK, L. HUANG\*, J. DAI\*, X. LEI\* (CHINESE ACADEMY OF MEDICAL SCIENCES, PEKING UNION MEDICAL COLLEGE, AND PEKING UNIVERSITY, BEIJING, P. R. OF CHINA)

FAD-Dependent Enzyme-Catalysed Intermolecular [4+2] Cycloaddition in Natural Product Biosynthesis  
*Nat. Chem.* **2020**, *12*, 620–628.

# Identification of the First Stand-Alone Intermolecular Diels–Alderase



**Significance:** The groups of Lei, Dai, and Huang report the discovery and characterization of the first naturally occurring stand-alone intermolecular Diels–Alderase. Isolated from mulberry trees by using a biosynthetic intermediate probe-based target identification strategy, the *Morus alba* Diels–Alderase (MaDA) was shown to catalyze a [4+2]-cycloaddition between a moracin-derived diene and morachalcone to complete the biosynthesis of the isoprenylated flavonoid chalconoracine. The reaction proceeds with high efficiency as well as with excellent diastereo- and enantioselectivity. Notably, the enzyme was shown to be promiscuous towards both dienes and dienophiles thus providing access to other natural products and nonnatural derivatives containing the methylcyclohexene motif. The broad applicability of MaDA was showcased in chemoenzymatic total syntheses of five other natural products found in *Morus* species.

**Comment:** The majority of enzymes capable of catalyzing Diels–Alder reactions are multifunctional or act through a stepwise rather than the characteristic concerted mechanism. Almost a decade after the discovery of the first stand-alone intramolecular [4+2]-cyclase, SpnF (H. K. Kim, M. W. Ruszczycky, S.-h. Choi, Y.-n. Liu, H.-w. Liu *Nature* **2011**, *473*, 109), the authors have identified an intermolecular [4+2] cycloaddition catalyzed by a flavin adenine dinucleotide (FAD)-dependent Diels–Alderase. Density functional theory calculations and kinetic isotope effect studies suggest that the reaction proceeds by a concerted asynchronous pathway. Control experiments indicated that the presence of the FAD cofactor is crucial for the catalytic activity of the enzyme. The authors propose that the function of the FAD cofactor might be related to sustaining substrate binding through hydrogen-bonding interactions. Identification of the exact role of the FAD cofactor is keenly anticipated.

**SYNFACTS Contributors:** Benjamin List, Mathias Turberg  
 Synfacts 2020, 16(09), 1093 Published online: 18.08.2020  
 DOI: 10.1055/s-0040-1706763; Reg-No.: B06220SF

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Category

Organo- and  
Biocatalysis

Key words

Diels–Alder reaction

Diels–Alderase

[4+2] cycloaddition

chalconoracine

flavonoids

Synfact  
of the  
Month

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