Biomimetic Total Syntheses of (+)-Chloropupukeananin, (–)-Chloropupukeanolide D, and Chloropestolides

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ABSTRACT: Chloropupukeananin, chloropupukeanolides, and chloropestolides are a family of structurally complex bioactive natural products that possess highly functionalized tricyclo[4.3.1.0^{3,7}]decane or bicyclo[2.2.2]octane skeletons. Biosynthesis of the chloropupukeananin family is triggered by the intermolecular heterodimeric Diels-Alder reaction between maldoxin and iso-A82775C; however, the enzymes involved have not yet been identified. We herein report the one-pot biomimetic synthesis of chloropupukeananin and chloropupukeanolide D. Moreover, the effect of solvent on the intermolecular Diels-Alder reaction of siccayne and maldoxin suggested that the biosynthesis of the chloropupukeananin family involves a Diels-Alderase catalyzed heterodimeric Diels-Alder reaction.

INTRODUCTION

Natural products owe their structural diversity to enzymes. Enzymes, such as prenyl transferases[1] and terpene cyclases,[2] which catalyse the carbon-carbon bond formation and cyclisation reactions, are responsible for the diversity in the carbon skeleton of natural products. The Diels-Alder reaction is one of the most powerful tools to introduce molecular complexity through biosynthetic,[3] and chemical synthesis approaches.[4] Several natural products are synthesized through the Diels-Alder reaction in nature. However, most of these are only artifacts or products of spontaneous cycloaddition.[5] Since the discovery of a Diels-Alderase in the biosynthesis of solanapyrone,[6a] only a few classes of this enzyme, that can catalyze intramolecular reactions in polyketide synthesis,[6b-e] have been identified. Very recently, enzymes promoting the intermolecular Diels-Alder reactions to produce pseudo-dimeric resveratrols have been identified.[7] Artificial Diels-Alderases, such as antibody catalysts,[8] artificial enzymes,[9] and super molecules,[10] have been developed to realize intermolecular Diels-Alder reactions owing to the high synthetic convergence and broad diversity of products of this process. If an intermolecular heterodimeric Diels-Alderase is identified, its genetic modifications should pave the way for the versatile production of bioactive natural product-like compounds.[11]

Chloropupukeananin (**1**), an inhibitor of HIV-1 replication, was isolated from the plant endophytic fungus *Pestalotiopsis fici* by Che et al. as the first chlorinated pupukeanane derivative (Figure 1).[12] Simultaneously, iso-A82775C (**2**) and pestheic acid (**3**) were also isolated and proposed as the biosynthetic precursors of **1**. One of the prominent structural features of **1** is its highly functionalized tricyclo[4.3.1.0^{3,7}]decane moiety that bears the vinyl allene moiety of **2** and the methyl benzoate moiety of **3**. Thus, we proposed that (*R*)-maldoxin (**4**), which is generated by the asymmetric oxidative dearomatization of **3**, is the actual biosynthetic precursor instead of **3**.[13] Subsequently, an intermolecular reverse-electron-demand Diels-Alder reaction between **2** and **4** and an intramolecular carbonyl-ene reaction leads to the construction of the tricyclo[4.3.1.0^{3,7}]decane skeleton. Finally, migration of the *p*-orsellinate moiety results in **1**. Continuous efforts by Che et al. to unveil the biosynthetic pathway of **1** led to the identification of many natural heterodimeric congeners.[14] These included (i) chloropestolides A–F (**5–10**),[14a,b] the isomers of the Diels-Alder reactions, (ii) chloropupukeanolide D and C (**11**, **12**),[14c] a carbonyl-ene products of **7** and **8**, and (iii) other degradation products.[15] Finally, optically active (*R*)-**4**,[14d] which was the missing link in the biosynthesis of **1**, was isolated from the related fungus, *P. theae*. These results support our hypothesis on the biosynthesis of **1**. Chloropestolides H–K (**13–16**)[14e] were also isolated during the identification of the biosynthetic gene cluster of **2** using a prenyl transferase gene-disrupted strain. By inhibiting the biosynthesis of **2**, intermediate **17** was found to undergo intermolecular Diels-Alder reactions with **4** to afford **13–16**. Isolation of the chloropestolides (**5–8**, **13–16**) indicates poor facial selectivity of the Diels-Alder reaction during biosynthesis (Figure 2). Briefly, during the formation of these bicyclo[2.2.2]octane-containing cycloadducts, the dienophiles approach the *Re*- or *Si*-face of C4' of diene **4**, with *syn*- or *anti*-orientation between the R group of the dienophiles and the C2 acetal moiety of **4**. The intermolecular Diels-Alder reaction produces all the possible cycloadducts, with some *Si-syn* selectivity; these adducts are artifacts owing to the poor selectivity. Thus, the chemical synthesis of a chloropupukeananin family through the Diels-Alder reaction between (+)-**2** and (−)-**4** can elucidate the biosynthetic pathway, especially clarifying whether a non-enzymatic Diels-Alder reaction occurs or not. If an enzymatic reaction occurs, this enzyme might be the first example of an intermolecular heterodimeric Diels-Alderase that could construct a highly functionalized bicyclo[2.2.2]octane skeleton. Thus, we herein report the biomimetic total synthesis of (+)-**1** from (+)-**2** and (−)-**4** using a one-pot Diels-Alder/carbonyl-ene cascade reaction and migration of the *p*-orsellinate group.

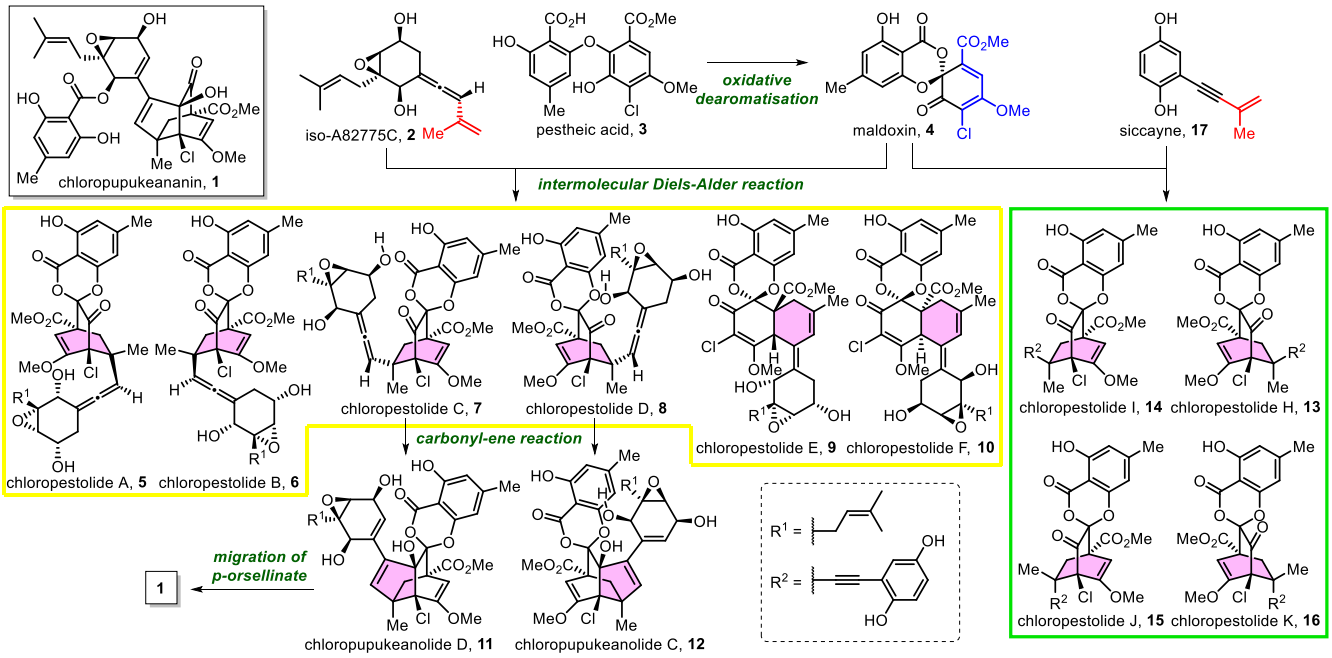


Figure 1. Proposed biosynthetic pathway of the chloropupukeananin family (the Diels-Alder adducts from **2** and **4** are framed in yellow box and those from **4** and **17** are framed in green box)

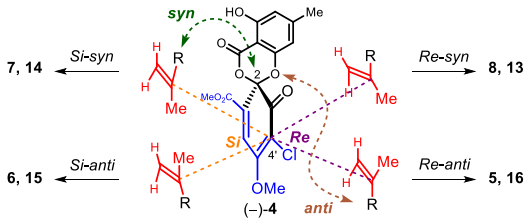
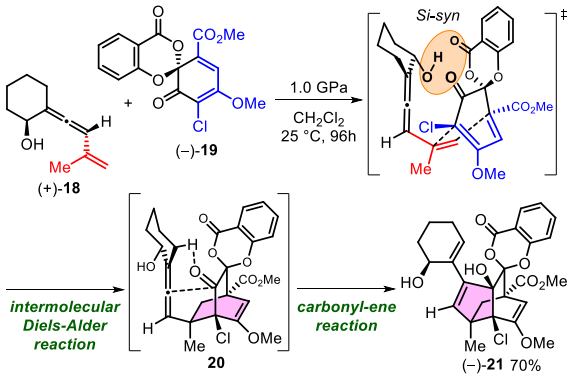


Figure 2. Four orientations of the diene and dienophile moieties in the intramolecular Diels-Alder reaction

RESULTS AND DISCUSSION

Our previous synthetic study on chloropupukeananin[16] demonstrated the selective construction of a tricyclo[4.3.1.0^{3,7}]decane skeleton under high pressure conditions (Scheme 1). The Diels-Alder/carbonyl-ene cascade reaction of model compounds (+)-**18** and (−)-**19** results in tricyclic compound (−)-**21** in 70% yield via *Si-syn* cycloadduct **20**. The *Si-syn* transition state is stabilized by hydrogen bonding networks. Therefore, the chemical Diels-Alder reactions between (+)-**2** and (−)-**4** should selectively produce *Si-syn* cycloadduct **7**, whereas that in nature exhibits poor selectivity, forming cycloadducts **5–10**. This is contradictory to the usual biosynthetic enzyme reactions. However, it is also possible that *Si-syn* **7** is biosynthetically obtained as a major product, without the involvement of enzymes. The subsequent reactions, such as the carbonyl-ene reaction or other degradation reactions, consumed most of **7** in the presence or absence of enzymes. Consequently, all the cycloaddition products, **5**-**10**, are isolated in a non-selective manner. Hence, a simple system to directly compare the cycloadducts of the chemical synthesis with the natural cycloadducts is required.

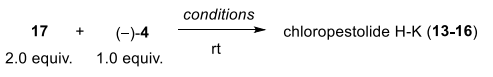
Scheme 1. Diels-Alder/carbonyl-ene cascade reaction in our privious study



Accordingly, we investigated the biosynthetic Diels-Alder reaction of **17**[17] and (−)-**4** to produce **13–16** (Table 1). The reaction was initially conducted under neat conditions (entry 1), resulting in a mixture of products **13–16** (**13**:**14**:**15**:**16** yield ratio = 9:32:51:8) produced in quantitative yield. Owing to the nature of (−)-**4**,[18] the *Si*-face was preferred. Performing the reaction in MeOH/H$_2$O (entry 2) resulted in a mixture containing predominantly *Si-anti* **15** (**13**:**14**:**15**:**16** = 4:11:74:11), which was incompatible with the *Si-syn* selectivity of isolation.[14e] Surprisingly, the product ratio obtained in the aqueous solvent differed from that of the isolated sample; generally, hydrophilic solvents can simulate the biosynthetic reaction conditions. Next, we considered the relationship between the product ratio and solvent to reproduce the isolated ratio of **13–16**. After investigating the Diels-Alder reaction in common organic solvents (entries 3–10; for the complete solvent screening data, see the Supporting Information), we established that the product ratio, especially for **14**:**15**, depended on the solvent basicity (SB)[19] and not on the dielectric constant, dipole moment, or solvent polarity parameter.[20] In low-basicity solvents (SB < 0.2), **14** and **15** were obtained in a ratio of nearly 1:1. The best result

for the *Si-syn* selectivity was obtained in CH_2Cl_2 (entry 4), giving **14** and **15** in isolated yields of 39% and 34%, respectively.[21] In contrast, in high-basicity solvents (SB > 0.2), the Diels-Alder reaction resulted in predominantly **15**. The same trends were observed under high-pressure conditions (entries 11 and 12). Comparing the ratio of **13–16** isolated from nature[14e] revealed that the preference for **14** was not reproduced in any of organic solvents. Comparatively similar results were obtained in lipophilic solvents (entries 3 and 4) rather than in hydrophilic solvents (entries 2, 7, and 9). Thus, there was no possibility that cycloadducts **13–16** are artifacts during cell cultivation and isolation. These results suggest that the biosynthetic production of the chloropupukeananin family involves the heterodimeric Diels-Alderase catalyzed the intermolecular Diels-Alder reaction of (−)-**4**.

Table 1. Intermolecular Diels-Alder reaction between (−)-4 and 17

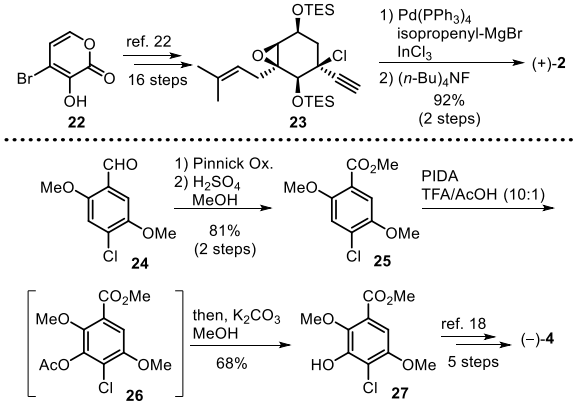


entry	conditions	SB	time (h)	ratio[a]			
				13	**14**	**15**	**16**
	isolation (ref.14e)			17	52	18	13
1	neat		24	9	32	51	8
2	$MeOH/H_2O$ (4/1)		48	4	11	74	11
3	benzene	0.124	48	9	42	44	5
4[b]	CH_2Cl_2	0.178	48	13	44 (39)	37 (34)	6
5	CH_3CN	0.286	48	10	28	53	9
6	acetone	0.475	48	6	20	64	10
7	MeOH	0.545	48	4	11	75	10
8	Et_2O	0.562	48	6	17	69	7
9	EtOH	0.658	48	3	7	80	10
10	*i*-PrOH	0.762	48	3	5	81	10
11[c]	1.0 GPa, MOH/CH_2Cl_2 (10/1)		1	3	11	75	11
12[c]	1.0 GPa, CH_2Cl_2		1	15	46	33	6

[a] Ratio was determined by ^{1}H-NMR. [b] Isolated yield in parentheses. [c]1.1 Equiv. of **17** was used.

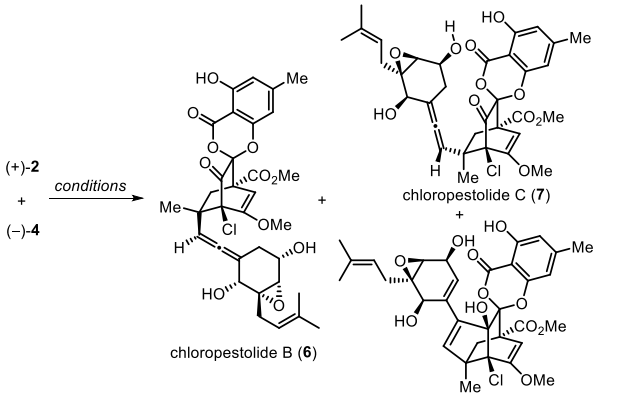
Subsequently, the next stage of the biomimetic total synthesis of chloropupukeananin, based on our proposed biosynthetic pathway, was considered. Scaling up of the previously reported syntheses of (+)-**2**[22] and (−)-**4**[18] is associated with limitations, such as low conversion in the *anti*-S_N2' reaction of **23** using a vinyl copper reagent in the total synthesis of (+)-**2** (Scheme 2). This problem was addressed by using an organoindium reagent and Pd catalyst,[23] and subsequently deprotecting the triethylsilyl (TES) groups of the resulting allene, resulted in (+)-**2** in 92% yield on a sub-gram scale.[24] In the original synthesis of (−)-**4**, phenol **27** was prepared from 5-methoxysalicylic acid in five steps, one of which was a non-selective chlorination reaction.[13] The alternative synthesis was initiated with **24**, which was prepared by the formylation of commercially available 2-chloro-1,4-dimethoxybenzene.[25] The Pinnick oxidation and esterification of **24** gave salicylate **25** in 81% yield. Under Kita's conditions,[26] site-selective oxidation using (diacetoxyiodo)benzene (PIDA) in a trifluoroacetic acid (TFA)/acetic acid mixed solvent[24] gave acetate **26**, which underwent a one-pot hydrolysis to form **27** in 68% yield. Phenol **27** was converted to (−)-**4** according to our previously reported synthetic protocol.[18]

Scheme 2. Improved synthesis of (+)-2 and (−)-4.



Next, we investigated the Diels-Alder/carbonyl-ene cascade reaction using the biosynthetic precursors (+)-**2** and (−)-**4** (Table 2). A mixture of (+)-**2** and (−)-**4** was first subjected to a high pressure in CH_2Cl_2 resulting in the formation of the desired *Si-syn* **7** and one diastereomer, *Si-anti* **6**, after 1 h (entry 1). Notably, no other cycloadducts were detected except for a small

Table 2. Intermolecular Diels-Alder/carbonyl-ene cascade reactions (+)-2 and (−)-4 under high pressure and atmospheric pressure conditions

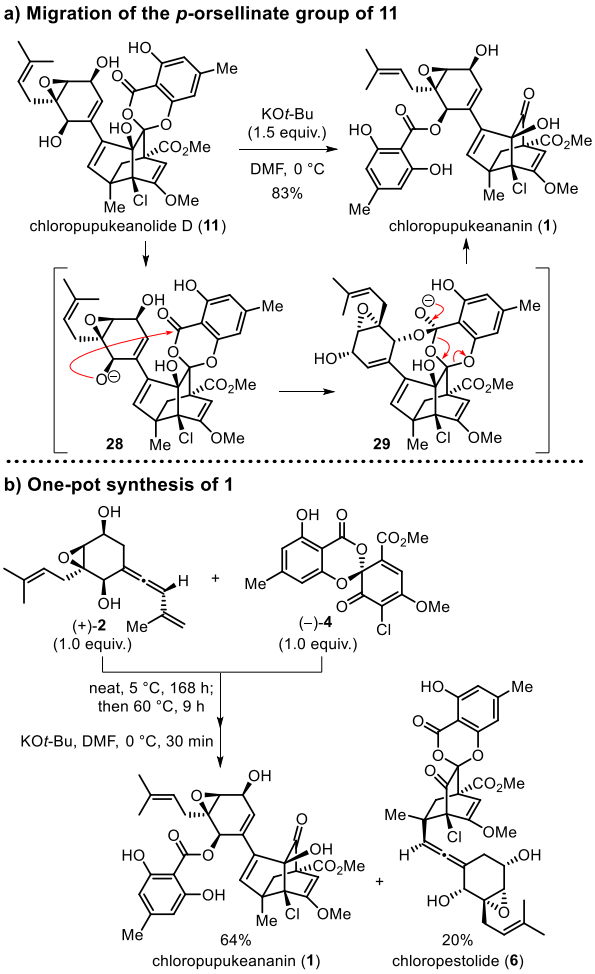


entry	ratio (**2**/**4**)	conditions	temp. (°C)	time (h)	yield (%)[a]		
					6	**7**	**11**
1	1.3/1.0	1.0 GPa, CH_2Cl_2	25	1	17	67	3
2	1.3/1.0	1.0 GPa, CH_2Cl_2	25	4	17	62	13
3	1.3/1.0	1.0 GPa, CH_2Cl_2	25	16	17	42	40
4	1.3/1.0	1.0 GPa, CH_2Cl_2	25	64	17	5	71
5[b]	1.3/1.0	(i) 1.0 GPa, CH_2Cl_2 (ii) 0.1 MPa, neat	25 60	64 9	21	-	69
6	1.0/1.5	0.1 MPa, $(CH_2Cl)_2$	70	30	21	13	19
7[b]	1.0/1.0	(i) 0.1 MPa, neat (ii) 0.1 MPa, neat	25 60	120 68	25	-	57

[a] NMR yield otherwise noted. [b] Isolated yield.

amount of **11**. The *Si-syn* selectivity would be attributed to the hydrogen bonding networks, as shown in Scheme 1. Prolonging the reaction time facilitated the intramolecular carbonyl-ene reaction; when the reaction was conducted for 64 h (entry 4), **11** was obtained in 71% yield. Since the mixture of **7** and **11** was inseparable, it was heated under atmospheric pressure for the carbonyl-ene reaction to reach completion. After the high-pressure reaction (entry 5), the reaction mixture was concentrated and heated to 60 °C to afford **11** and **6** in isolated yields of 69% and 21%, respectively. We also investigated the Diels-Alder reaction under atmospheric pressure. The thermal reaction (entry 6) produced a mixture of **6**, **7**, and **11** in low yields owing to the decomposition of the starting materials. Next, the reaction was conducted under neat conditions (entry 7), followed by heating to 60 °C to produce the desired compound **11** in 57% yield, along with **6** in 25% yield. These results indicated that a high pressure significantly accelerated the Diels-Alder reactions between (+)-**2** and (−)-**4** but did not affect the facial selectivity. This synthetic protocol demonstrates the intriguing *Si*-face selectivity of the intermolecular Diels-Alder reaction between (+)-**2** and (−)-**4**; i.e., only two cycloadducts, **6** and **7**, were obtained in the chemical synthesis, while six types of cycloadducts **5–10** were isolated as natural products.[14a,b] This suggests that the biosynthetic intermolecular Diels-Alder reactions of **5–10** and **13–16** involve enzymes that activate the substrates to form diverse Diels-Alder products.

Scheme 3. Completion of biomimetic total synthesis of chloropupukeananin



Finally, the migration of the *p*-orsellinate group of **11** was examined (Scheme 3a). We postulated that the secondary alkoxide group of **28** generated from **11** in basic conditions attacks the carbonyl carbon of *p*-orsellinate group to form intermediate **29**. Then, elimination reaction completes the migration to give **1**. Treatment of **11** with KO*t*-Bu in DMF afforded **1** in 83% yield. Because the Diels-Alder/carbonyl-ene cascade reaction did not require any reagents, we attempted the one-pot biomimetic transformation from (+)-**2** and (−)-**4** (Scheme 3b). The atmospheric pressure cascade reaction (neat, 5 °C, 7 days; then 60 °C, 9 h) and the following migration reaction provided **1** in 64% yield and **6** in 20% yield. Thus, the first asymmetric total synthesis of **1** was accomplished. The spectral data of **1**, **6**, and **11**[27] were identical to those of the corresponding natural compounds.[12,14b,c]

CONCLUSION

The biomimetic total synthesis of (+)-chloropupukeananin and (−)-chloropupukeanolide D through an intermolecular Diels-Alder reaction using (−)-maldoxin and (+)-iso-A82775C and a carbonyl-ene reaction was achieved. The biomimetic synthesis of chloropestolides H–K using (−)-maldoxin and siccayne unveiled the effect of solvent on the intermolecular Diels-Alder reaction. The origin of the intermolecular Diels-Alder reaction stereoselectivity is still elusive; however, biosynthesis of the chloropupukeananin family possibly involves an enzymatically catalyzed intermolecular heterodimeric Diels-Alder reaction. We expected that our chemical synthesis of the family of chloropupukeananin advance the identification of the first intermolecular heterodimeric Diels-Alderase, which can produce diverse highly functionalized bicyclo[2.2.2]octanes.

ASSOCIATED CONTENT

Supporting Information

This material is available free of charge via the Internet at http://pubs.acs.org.
Experimental procedures and compound characterization (PDF)

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Notes

The authors declare no competing financial interests.

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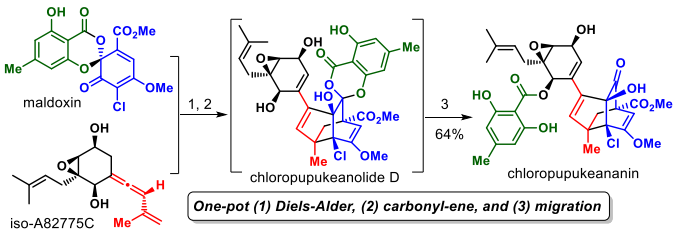
maldoxin
iso-A82775C
1, 2
chloropupukeanolide D
3
64%
chloropupukeananin
One-pot (1) Diels-Alder, (2) carbonyl-ene, and (3) migration