Towards Cortistatin A Analogue:

Asymmetric Reduction of Diketone

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Major in Chemistry (Intensive)

Abstract

Cortistatins is a group of steroidal alkaloids, including cortistatin A, B, etc. This group of compounds contains a five-ring system. They were first isolated in 2006. The Kobayashi group from the Osaka University isolated cortistatins A, B, C and D from marine sponge Corticium simplex. Cortistatins were later discovered having anti-angiogenic and anti-HIV potentials. In 2015, Kuang Liping from research group of Professor Pauline Chiu completed the total synthesis for cortistatin A and J using 2-allyl-2-methylcyclopentane-1,3-dione as the starting material. In this study, we propose an analogue of cortistatin A. The five-membered ring E, which comes from the starting material, in cortistatin A is changed to a six-membered ring to become the proposed analogue. The starting material, hence, is proposed to be 2-methylcyclohexane-1,3-dione, a six-membered ring dione. The synthesis began with the allylation of the dione at C-2 position. After that, it is the main focus of this study, asymmetric reduction of the diketone. With reference to the previous total synthesis of cortistatin A, several asymmetric reduction methods were tested, including asymmetric transfer hydrogenation using two Ru-based catalysts and copper hydride-catalyzed asymmetric reduction. In all reductions, the diastereomeric ratio (*dr*) and enantiomeric excess (*ee*) were assessed. In asymmetric transfer hydrogenation, *(R,R)*-Ts-DENEB showed good diastereoselectivity and enantioselectivity while RuCl(p-cymene)[*(R,R)*-Ts-DPEN] were poorer in these two selectivities. Copper hydride-catalyzed reduction had low yield due to incomplete conversion, apart from low selectivities. Despite a lower reactivity of a six-membered ring, after changing the five-membered starting-material to a six-membered ring, the first few steps of the total synthesis is still feasible, with a little modification.

Background

In the structure of cortistatin A, there is a five-fused ring system. An isoquinoline group is connected to ring E (Figure 1). The isoquinoline group acts as a high affinity ligand which can binds to amino acids of enzyme and inhibits the proliferation of human umbilical vein endothelial cells (HUVECs) (Figure 2)[1]. HUVECs are responsible for the process that new blood vessels are formed from existing ones, also known as angiogenesis, which is important for cancer cells to grow. Therefore, cortistatins are expected to have anti-angiogensis effect and are potential cancer drugs.

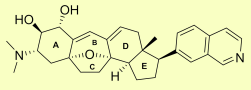


Figure 1

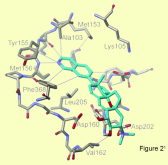
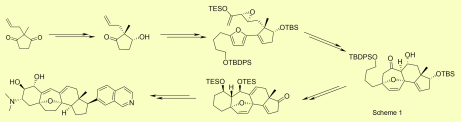


Figure 2[1]

In 2015, Kuang Liping completed a total synthesis of cortistatin A and J (Scheme 1)[2]. The starting material of the synthesis was 2-allyl-3-hydroxy-2-methylcyclopentane-1,3-dione. Diketone underwent an asymmetric reduction to give a β-hydroxyketone, which was then functionalized. [4+3] cycloaddition and aldol cyclization could complete the five-ring system. After that, an isoquinoline group was added on the alocohol.



Scheme 1

Objective

We proposed to change ring E in cortistatin A from five-membered to six-membered to produce an analogue (Figure 3). Since five-membered ring and six-membered ring have different conformations, it was expected that the position of the isoquinoline substituent would be slightly altered and the binding effect would also be affected. Before the investigation of whether the anti-angiogenic effect will be improved or not, we had to develop a synthetic route for the analogue.

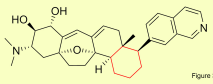
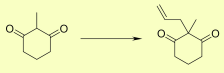


Figure 3

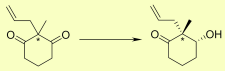
Design

Although only one carbon is added to the compound, the new synthetic route may be very different since the very beginning as ring E we concerned would come from the starting material, the diketone. Yet, we could still start by repeating the existing five-membered ring synthesis on our six-membered starting material.

Since 2-allyl-2-methylcyclohexane-1,3-dione was not readily available, preparation was required. By allylation of 2-methylcyclohexane-1,3-dione, 2-allyl-2-methylcyclohexane-1,3-dione was expected to be produced.



The second step would also be the focus of this study, the asymmetric reduction of diketone.



There would be 4 possible products in this asymmetric reduction (Figure 4).

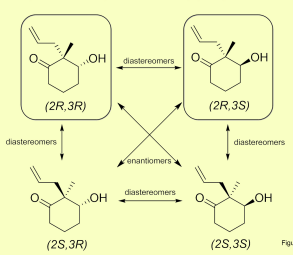


Figure 4

Stereochemistry is an important issue in drug synthesis since enantiomers may have different medical effects. In the worst case, a wrong stereochemistry may result in a lethal product.

Stereochemistry at C-2 position is a challenge in this reduction. Comparing with our target molecule, it is clear that the *(R)* configuration of the methyl group is retained until the end. Stereochemistry at C-3 is not as important as that at C-2. It can be either *(R)* or *(S)* because the hydroxyl group will later be deprotected back to a carbonyl group. *(2S,3S)* and *(2S,3R)* are undesirable and hence, the reduction should be highly enantioselective to *(2R,3R)* and *(2R,3S)*.

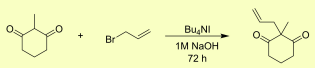
The enantioselectivity of the reaction is measured by enantiomeric excess (*ee*) which refers to the degree of excess enantiomer over the another. *ee* can be determined by high-performance liquid chromatography (HPLC) since the enantiomers can be separated in HPLC. Although both *(2R,3R)* and *(2R,3S)* are desired, it is better to have a pure diastereomer rather than a mixture of two diastereomers as the reduction product. Here, we take *(2R,3R)* as our desired product. the diastereoselectivity should be high as well. It is measured by diastereomeric ratio (*dr*) which means the ratio of the two diastereomers. *dr* can be determined by the NMR spectrum of the crude product since the diastereomers show different peaks in NMR.

In this study, we would use Ru-catalyzed asymmetric transfer hydrogenation and copper-hydride asymmetric reduction. Both methods had also been used in Kuang's cortistatin A total synthesis[2].

Result & Discussion

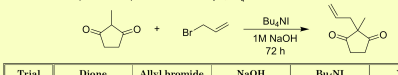
A. Allylation of 2-methylcyclohexane-1,3-dione

The allylation of 2-methylcyclohexane-1,3-dione can be achieved by α-carbonyl substitution with allyl bromide under a basic condition and the presence of phase-transfer catalyst, Bu_4NI[3].



Trial	Dione	Allyl bromide	NaOH	Bu_4NI	Yield
1	30 mmol	60 mmol	30 mL	0.3 mmol	60%
2	30 mmol	60 mmol	30 mL	0.3 mmol	50%

Table 1: Allylation of 2-methylcyclohexane-1,3-dione



Trial	Dione	Allyl bromide	NaOH	Bu_4NI	Yield
1	10 mmol	20 mmol	10 mL	0.1 mmol	50%
2	20 mmol	40 mmol	20 mL	0.2 mmol	75%
3	20 mmol	40 mmol	20 mL	0.2 mmol	62%

Table 2: Allylation of 2-methylcyclopentane-1,3-dione

In literature[3], the yield of this reaction was 80%. The yield we achieved in our laboratory was significantly lower than this (Table 1). Then, the allylation to 2-methylcyclopentane-1,3-dione was repeated for comparison. Again, in the allylation of 5-membered ring, the yield achieved in our laboratory was significantly lower than the literature value (84%) (Table 2).

B. Asymmetric Reduction of 2-allyl-2-methylcyclohexane-1,3-dione

B1. Asymmetric Transfer Hydrogenation (ATH)

In a mixture of formic acid and triethylamine under the presence of a Ru-based catalyst, the dione was successfully reduced. There were two catalysts available, RuCl(p-cymene)[*(R,R)*-Ts-DPEN] (1) and *(R,R)*-Ts-DENEB (2) (Figure 5).

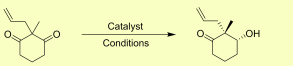


Figure 5

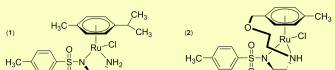


Table 3: ATH of 2-allyl-2-methylcyclohexane-1,3-dione using RuCl(p-cymene)[*(R,R)*-Ts-DPEN]

Trial	Catalyst	Conditions	Yield	*dr*
1	1 (1%)	HCOOH/Et_3N (1:1, 1.0 M), r.t., 24 h	N.C.	/
2	1 (1%)	HCOOH/Et_3N (1:1, 1.0 M), 80 °C, 4 h	Low	1:1
3	1 (1%)	HCOOH/Et_3N (1:1, 1.0 M), 40 °C, 24 h	N.C.	/
4	1 (1%)	HCOOH/Et_3N (1:1, 1.0 M), 60 °C, 48 h	36%	2:1

Trial	Catalyst	Conditions	Yield	*dr*	*ee*
5	2 (1%)	HCOOH/Et_3N (1:1, 1.0 M), r.t., 48 h	68%	7.5:1	>99%
6	2 (1%)	HCOOH/Et_3N (1:1, 1.0 M), r.t., 48 h	95%	6.5:1	>99%
7	2 (0.5%)	HCOOH/Et_3N (1:1, 1.0 M), r.t., 96 h	85%	5.3:1	87%
8	2 (1%)	HCOOH/Et_3N (1:5, 1.0 M), r.t., 24 h	82%	5.7:1	98%

Table 5: ATH of 2-allyl-2-methylcyclohexane-1,3-dione using *(R,R)*-Ts-DENEB

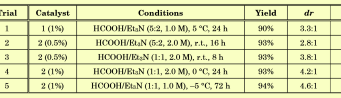


Table 4: Kuang's ATH results[2]

Trial	Catalyst	Conditions	Yield	*dr*	*ee*
1	1 (1%)	HCOOH/Et_3N (5:2, 1.0 M), 5 °C, 24 h	90%	3.3:1	79%
2	2 (0.5%)	HCOOH/Et_3N (5:2, 2.0 M), r.t., 16 h	93%	2.8:1	98%
3	2 (0.5%)	HCOOH/Et_3N (1:1, 2.0 M), r.t., 8 h	93%	3.8:1	98%
4	2 (1%)	HCOOH/Et_3N (1:1, 2.0 M), 0 °C, 24 h	93%	4.2:1	>99%
5	2 (1%)	HCOOH/Et_3N (1:1, 1.0 M), −5 °C, 72 h	94%	4.6:1	>99%

When RuCl(p-cymene)[*(R,R)*-Ts-DPEN] was used as catalyst (Table 3) and the reaction mixture was stirred at room temperature for 24 h, there was no conversion. Compared to the five-membered ring, the six-member ring showed a lower reactivity. Hence, in the second trial, we heated the reaction mixture to 80 °C and stirred for 4 h but only a small amount of reactant was consumed as seen in the TLC result. From the crude product NMR, *dr* was about 1:1. In the third trial, we only heated the reaction mixture to 40 °C but there was no conversion. In the fourth trial, the reaction temperature was 60 °C and the mixture was stirred for 2 d. The yield was 36% and *dr* was 2:1. Comparing to the Kuang's five-membered ring reduction (Table 4), the six-memebered ring was obviously much less reactive.

RuCl(p-cymene)[*(R,R)*-Ts-DPEN] showed unsatisfactory result and hence, we tried another catalyst, *(R,R)*-Ts-DENEB (Table 5). In the fifth trial, the reaction mixture was stirred at room temperature for 48 h. The progress of the reaction was examined by TLC analysis. However, a small-scale solvent extraction was required to remove highly polar HCOOH. Hence, there was a significant loss of reaction mixture, leading to a yield of 68%. The same reaction was repeated under the same condition but in a greater scale. The loss of reaction mixture relative to the total amount was much smaller. The yield was 95%. In these two trials, *dr* was very high at around 7:1.

Concern 1: High cost of the catalyst	A smaller amount of catalyst was used. However, there was a compromise between the amount of catalyst and the reaction rate. Halving the amount of catalyst led to doubling the time required for the reaction to complete.
Concern 2: Low reactivity	From Kuang's result, the more acidic the mixture, the slower the reaction is. Hence, we changed the ratio of formic acid to triethylamine to 1:5. The reaction was completed in 24 h. However, the *dr* is lower. Also, there were more side products formed.

Figure 6

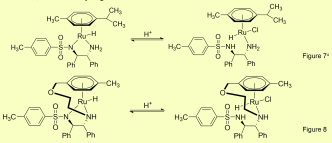


Figure 7[4]

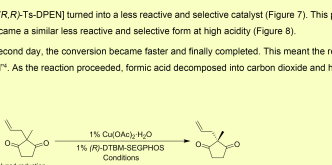


Figure 8

Why does a higher acidity lead to a lower rate?

Zhou and colleagues from Xi'an Jiaotong University conducted a study on Ru-based catalyst[4]. They proposed that at high acidity, RuCl(p-cymene)[*(R,R)*-Ts-DPEN] turned into a less reactive and selective catalyst (Figure 7). This pattern may also be applicable to other Ru-based catalyst such as *(R,R)*-Ts-DENEB. From our experimental result, we proposed that *(R,R)*-Ts-DENEB also became a similar less reactive and selective form at high acidity (Figure 8).

This could explain one phenomenon in this reduction. It was observed that in the first day of the reaction, there was only slight conversion. In the second day, the conversion became faster and finally completed. This meant the reaction rate indeed increased as the reaction proceeded. Zhou's study also mentioned this phenomenon. They stated that the reaction had an "induction period"[4]. As the reaction proceeded, formic acid decomposed into carbon dioxide and hydrogen so the acidity decreased. This explained why the reaction went faster.

B2. Copper-hydride catalyzed asymmetric reduction

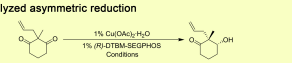


Table 6

Trial	Conditions	Yield	*dr*	*ee*
1	$(EtO)_3MeSiH$ (3 equiv.), THF, 0 °C, 24 h	N.C.	/	/
2	$(EtO)_3MeSiH$ (3 equiv.), THF, r.t., 24 h	56%	1.7:1	34%
3	$PhSiH_3$ (1 equiv.), THF, r.t., 3 h	/	/	/
4	$PhSiH_3$ (1 equiv.), THF, 0 °C, 5 h	/	/	/

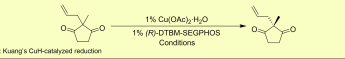


Table 7: Kuang's CuH-catalyzed reduction

Trial	Conditions	Yield	*dr*	*ee*
1	$(EtO)_2MeSiH$ (3 equiv.), Et_2O, −25 °C, 12 h	55%	2.3:1	83%
2	$PhSiH_3$ (1 equiv.), Et_2O, −25 °C, 6 h	86%	2.5:1	82%
3	$PhSiH_3$ (1 equiv.), Et_2O, −78 °C, 12 h	89%	2.9:1	89%

The result was much poorer than ATH. Using a less reactive silane $(EtO)_2MeSiH$, the reaction was slow and at a low conversion rate. When the silane was changed to more reactive $PhSiH_3$, all reactant was consumed in 3 h. However, read from NMR spectrum, it was converted to a number of side products with only a little amount of resired product. This was different to the result in five-membered ring reaction in which $PhSiH_3$ could raise the yield of the reduction.

B3. Racemic reduction

In chromatogram of HPLC, several peaks are shown corresponding to different stereoisomers. However, it is difficult to match the peaks with stereoisomers. Hence, we have to prepare a racemic mixture (a mixture of *(R)* and *(S)* enantiomers in equal amounts). With the racemic mixture chromatogram, it is much easier to analyze the peaks. Here, lithium tri-*tert*-butoxyalumnium hydride (LTBA) was used as the reducing agent[5].

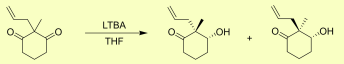


Figure 9: HPLC of racemic mixture

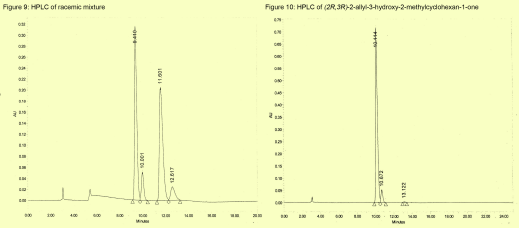
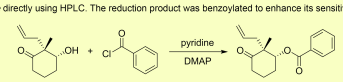


Figure 10: HPLC of *(2R,3R)*-2-allyl-3-hydroxy-2-methylcyclohexan-1-one

C. Derivatization of *(2R,3R)*-2-allyl-3-hydroxy-2-methylcyclohexane-1-ol

The reduction product was found insensitive to UV light. Since HPLC used UV light for detection, it was difficult to determine *ee* directly using HPLC. The reduction product was benzoylated to enhance its sensitivity to UV light.



Conlusion

In this study, we focused on methods of asymmetric reduction of 2-allyl-2-methylcyclohexane-1,3-dione. In ATH, *(R,R)*-Ts-DENEB gave product with high yield, *dr* and *ee* while RuCl(p-cymene)[*(R,R)*-Ts-DPEN] had poorer reuslt. In copper hydride catalyzed reduction, yield, *dr* and *ee* were much lower than ATH. These results were similar to the five-membered ring reduction in which *(R,R)*-Ts-DENEB was also the best, except in our study, the six-membered ring had a lower reactivity. ATH of six-membered ring using *(R,R)*-Ts-DENEB even gave a higher *dr* than ATH of five-membered ring. The study showed the first crucial step, asymmetric reduction, of synthesizing our proposed analogue would be feasible.

Reference

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