Microorganism Coculture-independent Synthesis of Berkeleypenostatin A

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Abstract: In 2021, berkeleypenostatins A-G have been biosynthesized by the coculture fermentation of microbes - Penicillium fuscum and P. camembertii/clavigerum - isolated from the Berkeley Pit Lake (Stierle, Stierle, Decato, Alverson, Apedaile, 2021). Tested by the NCI Developmental Therapeutics Program, berkeleypenostatin A effectively inhibited the migration of human pancreatic carcinoma cells (HPAF-II) (Stierle, Stierle, Decato, Alverson, Apedaile, 2021). However, despire the potent anti-tumor activity demonstrated by berkeleypenostatin A, its production from terrestrial extremophilic fungi presents challenges such as low-cell growth and high shear sensitivity (Ludlow, Clark 1991). Considering the potential of berkeleypenostatin A in pancreatic cancer treatment, this report proposes a laboratory synthesis of berkeleypenostatin A as an alternative to fungal coculture. The report analyzes berkeleypenostatin A's common atoms, deduces its retrosynthesis disconnections, and plans an effective synthesis route. To maximize the convergency of the synthesis, it begins with the reaction between a strong nucleophile and electrophile, proceeds to the McMurry coupling and the Diels-Alder reaction, and ends with the addition of glucose. Such a universal and simple synthesis introduces a series of rapid steps in producing berkeleypenostatin A, a potential anti-cancer material, which offers an innovative insight to the future treatment of pancreatic cancer.

1 INTRODUCTION

With a 5-year survival rate of 10%, pancreatic ductal adenocarcinoma (PDAC) is leading the cancer-related deaths worldwide (Osuna de la Peña, D., Trabulo, S.M.D., Collin, E. et al. 2021). Most patients have advanced or metastatic disease at diagnosis (Park, W., Chawla, A., & O'Reilly, E. M. 2021). Existing treatments, including gemcitabine and nab-paclitaxel, have limited efficacies due to various complex factors affecting PDAC, such as desmoplasia and hypervascularization (Osuna de la Peña, D., Trabulo, S.M.D., Collin, E. et al. 2021). Although chemotherapy with gemcitabine is the standard therapy for advanced or metastatic disease, its efficacy is highly limited by undesirable qualities of rapid plasma degradation, toxicity, and drug resistance (Tada 2011, Correia, Xavier, Duarte, Ferreira, Moreira, Vasconcelos, Vale 2020). Meanwhile, nab-paclitaxel demonstrates multiple adverse side-effects, including alopecia, neutropenia and nausea (Vishnu, Roy 2011). Therefore, there is an urgent demand for developing effective therapeutics for pancreatic cancer.

As a recently isolated compound, berkeleypenostatin A displays the anti-tumor qualities for a potentially effective therapeutic. The structure and configuration of berkeleypenostatins A-G have been deduced from spectral data and single-crystal X-ray crystallography (Stierle, Stierle, Decato, Alverson, Apedaile, 2021). After being produced in coculture, berkeleypenostatins were tested for anti-cancer activity. Among these molecules, berkeleypenostatin A was identified as a moderate inhibitor (50−100 μM) of MMP-3, an enzyme that promotes metastasis in pancreatic tumor cells (Suhaimi, Chan, Rosli 2020, Yang et al 2020). Berkeleypenostatin A also induces reduced cell migration of human pancreatic carcinoma cells (HPAF-II) by 30% at a concentration of 1.25 μM over a 24 h period (Stierle, Stierle, Decato, Alverson, Apedaile, 2021). The results shed light on the research for pancreatic cancer, which has been a challenging issue with patients exhibiting a low survival rate within five years after diagnosis and

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diagnosed in the advanced stages (Rawla, Sunkara, Gaduputi 2019, American Cancer Society. 2021). Although the current results indicate its potential in cancer treatment, further biological assays are essential to understanding more about the biological activity of berkeleypenostatin A. Such inquiry introduces the need to prepare berkeleypenostatin A for analysis and evaluation.

However, the existing synthesis of berkeleypenostatin A comes with various drawbacks introduced by the complexity and time-consuming nature of fungal coculture. Berkeleypenostatins are examples of secondary metabolites grown in axenic culture from microorganisms in the Berkeley Pit, acidic metal-rich waste lake (Giddings, Newman 2015). To combat the inconveniences of producing berkeleypenostatin A from microorganism coculture, this report proposes a coculture-independent method to synthesize berkeleypenostatin A with simple and fast steps. It is expected to prepare berkeleypenostatin A using the devised synthesis outlines below.

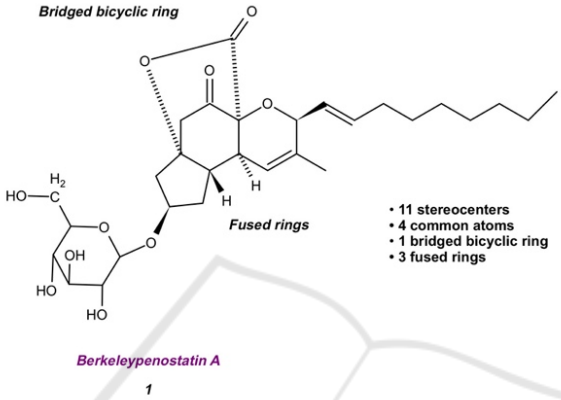

Figure 1. Structure of Berkeleypenostatin A

2 METHOD

As shown in Figure 1, the core of berkeleypenostatin A has 11 stereocenters, which results in 2^{11} potential stereoisomers. Therefore, the final products of the synthesis may include stereoisomers of this target molecule. To maximize the simplification and convergency of each retrosynthetic step, the strategy is to disconnect bonds linking two or more common atoms of the polycyclic core.

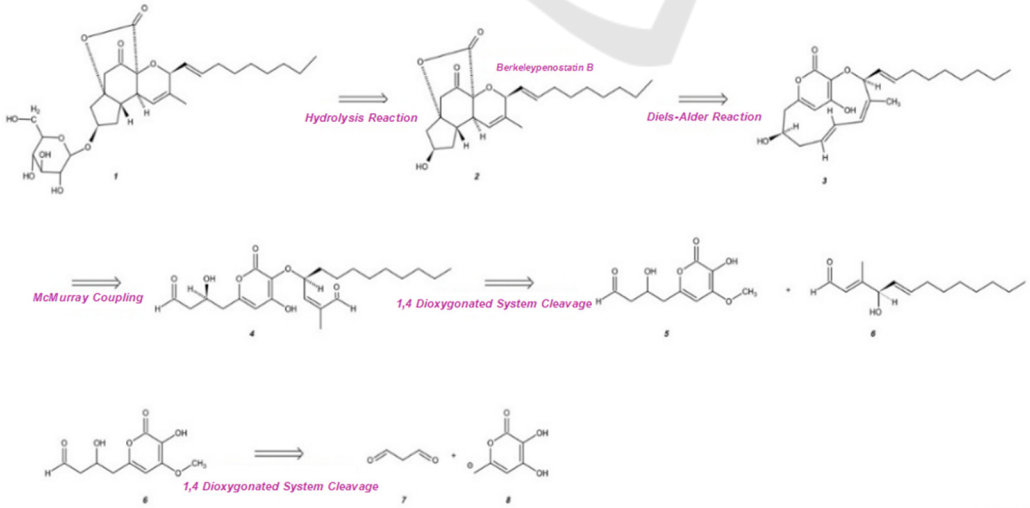

Figure 2. Retrosynthesis Strategy of Berkeleypenostatin A.

After evaluating several retrosynthesis methods, the final version, with the highest expected efficiency, is presented in Figure 2. The retrosynthesis is proposed to begin with a hydrolysis reaction under acidic conditions, separating the R group, β-D-glucose, from the rest of berkeleypenostatin A **1**. Berkeleypenostatin B **2** is expected to undergo a Diels-Alder reaction to disassemble the fused rings resulting in a bridged bicyclic ring system composed of a pyrone and a 10-carbon ring. Followed by a McMurry coupling, the alkene group of molecule **3** will split into two chains ending with an aldehyde group. When the 1,4 dioxygenated system of an ether group and an aldehyde group cleave, molecules **5** and **6** will be produced. These synthesized molecules will react as the starting materials of berkeleypenostatin A.

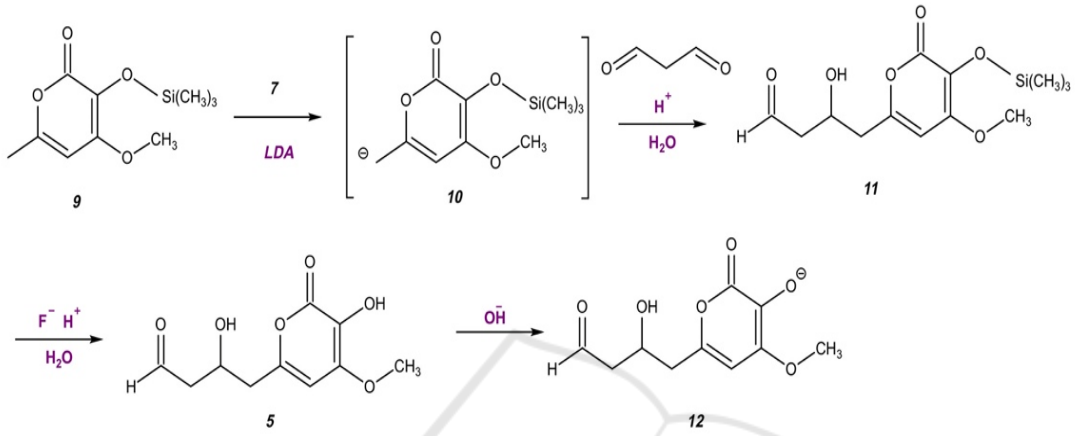

Figure 3. Preparation of Molecule 5 as an Improved Nucleophile.

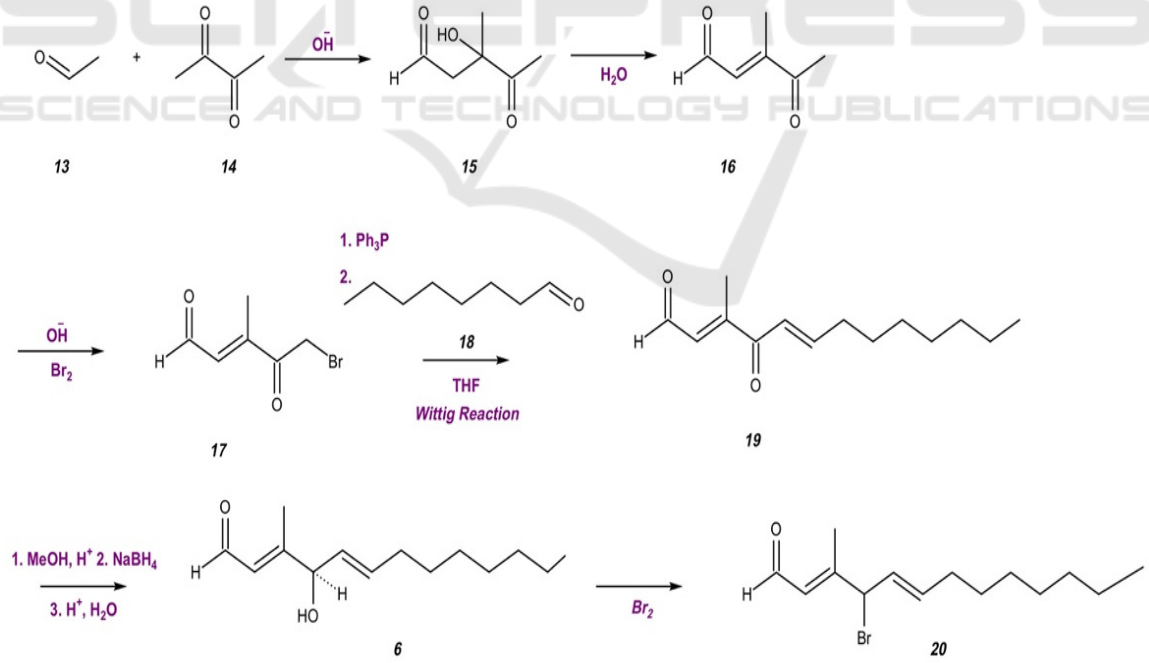

Figure 4. Preparation of Molecule 6 as an Improved Electrophile.

Before the formal synthesis, molecules **5** and **6** are prepared and tuned using the procedure presented in Figures 3 and 4. The hydroxyl groups on molecule **5** are reactive, which may later interfere with essential

steps in the synthesis. Therefore, the hydroxyl groups need to be masked with protective agents with varying strengths. With lithium diisopropylamide (LDA), molecule **9** forms a carbanion, which will react with malonaldehyde under acidic conditions with the existence of water to form molecule **11**. Under these conditions, the weak protective agent on molecule **11**, the silyl ether, will eventually be converted to a hydroxyl group, forming molecule **5**. Under alkaline conditions, the hydroxyl group on molecule **5** will be ionized to form an oxygen anion, resulting in a stronger nucleophile.

Molecule **6** cannot be found as a commercial reagent, so it is synthesized using simpler, more accessible molecules. The starting materials, acetaldehyde **13** and 2,3-butanedione **14**, will join together to form 2,5-hexanedione **15** under alkaline conditions. 2,5-Hexanedione **15** will undergo a functional group interconversion by eliminating water, which transforms the hydroxyl group to an alkene group. Next, a bromide ion will be added to 3-Hexene-2,5-dione **16** using bromine liquid under strong alkaline conditions. In the Wittig reaction, the phosphorus of triphenylphosphine (Ph_3P) attacks the carbon next to the bromide group in molecule **17**, forming a ylide that reacts with heptaldehyde **18**, generating molecule **19**. The ketone group on molecule **19** is then converted to a hydroxyl group, forming molecule **6**. To improve the electrophilic properties of molecule **6**, the hydroxyl group is converted to a bromide group. The enhanced nucleophilic and electrophilic properties of molecules **20** and **12**, respectively, prepare for their reaction.

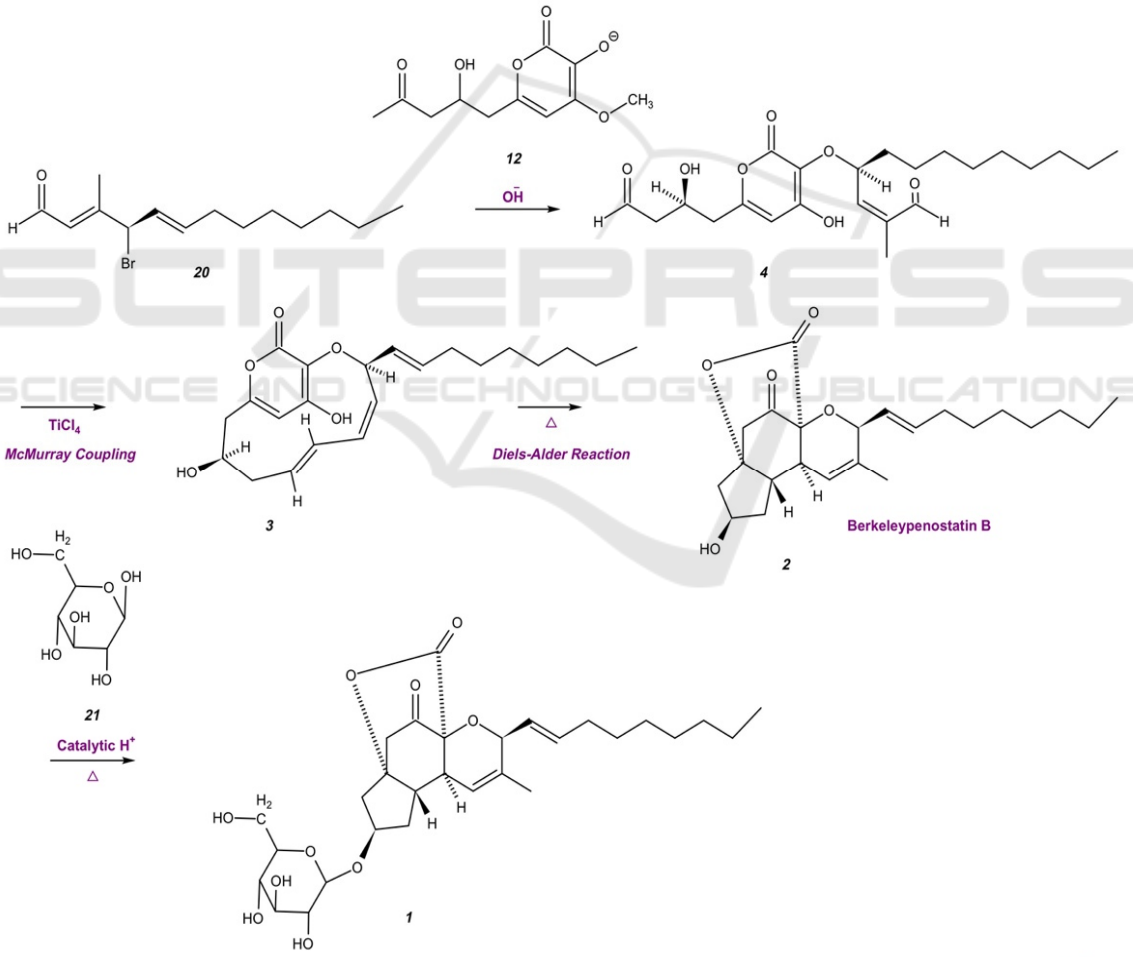

Figure 5. Synthesis Strategy of Berkeleypenostatin A.

The steps to synthesizing berkeleypenostatin A are shown in Figure 5. Molecules **12** and **20** will be mixed under alkaline conditions, forming molecule **4**. With titanium chloride acting as a reducing agent, the

two aldehyde groups at the ends of molecule **4** will connect to form an alkene group, undergoing the McMurry reaction. The bicyclic bridged system of pyrone 3 will fuse to form three fused rings under heat, carrying out the Diels-Alder reaction. Finally, by eliminating water via heating, β-D-glucose may be added to berkeleypenostatin B **2** in a condensation reaction, synthesizing berkeleypenostatin A.

3 RESULTS & DISCUSSION

By following the synthesis proposal, berkeleypenostatin A is expected to be produced with a few challenges. In the Wittig reaction, the aldehyde group on the heptaldehyde may be affected given that it is more reactive than the ketone group on molecule **17**, producing an undesired outcome as the aldehyde group converts into a hydroxyl group. If such possibility is verified by experiment that it hugely impacts the result, an alternative step should be devised.

A notable limitation of the synthesis is the production of undesired stereochemical outcomes. For instance, when the malonaldehyde is added to molecule **10**, the product consists of one stereocenter, forming two enantiomers of molecule **11** in a 1:1 ratio. Therefore, it is expected that berkeleypenostatin A will be mixed with some unexpected stereoisomers in the final products.

To boost the yield of berkeleypenostatin A, the future direction of the synthesis is to develop an enantioselective strategy of adding the malonaldehyde to molecule **10**, which may involve the use of a catalyst.

4 CONCLUSION

In conclusion, this report suggests a synthesis strategy of berkeleypenostatin A with significantly maximized convergency. The logistics of the route may be improved by experiments and testing the optimal temperatures at different stages of the synthesis, including the necessary heating during the Diels-Alder reaction and the condensation reaction. The yield of the synthesis may be enhanced by stereoselective steps. Nevertheless, the report devised a strategic laboratory method to synthesize berkeleypenostatin A by reacting a nucleophile with an electrophile in a minimal number of steps. Overall, this microbe coculture-independent synthesis route of berkeleypenostatin A has high prospects, offering a new way of producing this anti-tumor reagent without limitations of fungal coculture. Such a facile and universal synthesis of berkeleypenostatin A would make the material more readily available for biological assays in evaluating its efficacy for pancreatic cancer treatment.

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