The Theoretical Total Synthesis of an Aromatic Esters of the Crinane Amaryllidaceae Alkaloid Ambelline

Danyang Liu¹, Zhijian Zhou², Hao Liu^{3,*}

¹ School of Chemistry and Chemical Engineering, Baoji University of Arts and Sciences, Baoji, 721013, China

² College of Engineering, The Pennsylvania State University, State College, 16803, United States

³ Changjun High School, Changsha, 410002, China

ABSTRACT. A series of crinane-type alkaloid ambelline derivatives were assessed for their potency to inhibit both acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE), which has been shown to be effective medicine for the treatment of Alzheimer's disease. However, no enzyme modification has been reported total synthesis. In this work, two possible theoretical synthesis paths of Crinane-type alkaloid ambelline were discussed in this article. The major difficulty of the proposed synthesis was the synthesis of the quaternary carbon. One of the approaches emphasized on the reactions between cyclic and heterocyclic compounds and substrates on the intermediates to generate the quaternary carbon shown on the desired product. The other approach utilized series of amine reactions and Michael addition to create the precursor for the reactant in the Diels-Alder reaction and, therefore, the quaternary carbon, and finally, the desired natural product was obtained after a weak acid workup. The synthesis of ambelline has the potential to provide new pathways for treatment of Alzheimer's disease.

1 Introduction

Aromatic compounds are based on benzene, substitution reactions of which can make an abundance of complicated derivative compounds. For instance, the aromatic ester can be made by the aromatic compound, which is equipped with carboxyl or hydroxy[1]. Simply put, the aromatic ester is a kind of ester including the benzene. Our target molecule belongs to one kind of 20 derivatives (1–20) of the crinane-type alkaloid ambelline, which has the structural of feature an aromatic ester. Elderly people may suffer from a neurological disorder called Alzheimer's disease, which leads to reduced memory, loss of body control and, finally, death. Associated with this disease is reduced levels of acetylcholine. In later stages of the disease the amount of acetylcholinesterase, which breaks down acetylcholine, is reduced, but the levels of butyrylcholinesterase increase, suggesting that by inhibiting butyrylcholinesterase we may increase the levels of acetylcholine and so treat the disease. Therefore, in order to increase its level we are able to realize it by inhibiting the butyrylcholinesterase, it leads to the necessary synthesis of inhibitors. Cholinesterase inhibitors- especially butyrylcholinesterase inhibitors which have shown great potential in treating this disease and also prompted the the synthesis of new kinds of inhibitors[2]. Extracts from plants of the family Amaryllidaceae are already of interest in medicine, being

used to treat malaria and cancer. Esters of a crinane alkaloid called 'ambellin' could inhibit butyrylcholinesterase by binding to the active site. It would no longer be available to bind to acetylcholine; reduced breakdown of acetylcholine would lead to higher levels in the brain and possibly better brain functioning[3]. If a series of such esters could be made and tested for their value as inhibitors they would increase our understanding of the process and might provide potential drugs.

2 One strategy of retrosynthesis

Structurally, ambellin 13 is characterized by the fusion of benzene structure of the bridge, right region with a quaternary carbon center and the charge density relative concentrated around, look from the stereochemistry, N atoms is shared by the three member ring, and there is even a quaternary carbon. From a synthesis perspective, its complicated structure makes it challenging to synthesize, thus seldom people proposed the proper scheme about how to synthesize it. However we have designed two totally different strategies to get this main product. Plus owing to the scarcity of this natural product from biogenic sources, its challenging structural complexity and the promising biological function displayed by it, we were motivated to pursue a total synthesis of this important molecule.

*Corresponding author. E-mail address: liuhao171018@gmail.com

Scheme 1. Retrosynthetic Strategy

Scheme 2. Proposed Biosynthesis of 1,5 dialdehyde 4

After evaluating several potential strategies, we developed a retrosynthetic analysis of ambellin. We speculated that product in the benzene of carbon on the side of methoxyl group even one amino or hydroxyl containing carbon is indispensable, and connected with the carbon ring directly connected on the hydroxyl amino (figure 8), or between them can be through the Leuckart-Wallach reaction[4,5]directly to remove carbon to produce the imide ring formation, amine as a nucleophilic reagent and chloride on the carbon-based cyclization, generated quaternary carbon stereo center, thus the key to solve the target product stereoisomerism.

Fully synthesis starting from 1, first of all we note 1 on the primary amine is easy to react with carbonyl, so before the start of the reaction, we first through the primary amine

and acid anhydride nucleophilic addition-elimination reaction will get 2 amino protected, let 2 and 1,3 diacetone aldol condensation reaction[6]between the molecules, 2 has α -H aldehyde under alkali catalytic carbon anion, then carbon anion as nucleophilic reagent to nucleophilic addition reaction of aldehyde, generate β -hydroxy aldehyde, and reactant 1,3 diacetone structure has good symmetry, through its product of 3 in the spatial structure of a single, good selectivity, Add sodium to the reaction, react with alcohol to form sodium alcohol, react with iodomethane to convert sodium alcohol to methoxide, and get 4.

4 Under LDA and THF conditions, through intramolecular aldol condensation [6] reaction the newly formed β -hydroxy group is heated to remove a

molecule of water, that is, to form 5. For 5, under the action of alkali, the hydrogen on the newly formed carbon

ring is removed, therefore the electron cloud on the ring is redistributed, and the diene carbon ring 6 is obtained.

Scheme 3. Possible intermediates leading to the formation of 6

Scheme 4. Completion of the synthesis of 10

Scheme 5. Completion of the synthesis of 13

A product made from the previous 6 start synthesis, dienes carbon ring 6 start analysis, dienes carbon ring 6 when given its oxygen anion electronic, due to the electron density, originally the alkenyl carbon can form a negative ion as a nucleophilic reagent to attack ester carbonyl, newly generated a oxygen anion figure 7, get 8

to 7 amino to protect, then let the amino and carbonyl under the condition of formic acid, Leuckart-Wallach[4,5]. Next, the ester group on 9 is reacted with dichlorosulfoxide chloride to form acyl chloride. Then the N with lone pair electron is used as nucleophile to attack the group and form a clever cyclogenesis of 10.

10 was further acidified to get 11. In the presence of reducing agent, using McMurry coupling[7] and TiCl_3 , the reduced Ti was used to remove the two hydroxyl groups to form a double bond, namely, product 12. Then borohydrate reaction[8] took place on 12 to obtain an anti-Markov addition product 13. Butyrylcholinesterase was treated with butyrylcholinesterase to treat its efficacy as possible novel inhibitor.

In conclusion, this is one of the first use of a series of needed to construct the aldol condensation reaction substrate, then two aldol condensation reaction (aldol condensation of the one is intermolecular, one is intramolecular aldol condensation), then the amide as

nucleophilic reagent attack carbonyl twice, first to form a six-member N heterocyclic, second form 2N heterocyclic, a 7 member ring, 5 member ring, skillfully constructed quaternary carbon center, a big problem to conquer our synthesis. Finally, the reaction achieved the expected results. Owing to the lack of experiment support, there are follow-up problems related to the yield. We expect to conduct further verification of these problems through experiments.

3 Another strategy of retrosynthesis

Scheme 6. Retrosynthetic Strategy

There are several difficulties in synthesizing this natural product. One is to synthesize a more complex bridged-ring system. The other is to ensure the correct stereochemistry of the end product by selecting an appropriate reaction, especially the quaternary carbon in the product and the stereochemistry of the hydroxy group around it.

After analyzing several cutting strategies, we proposed a relatively feasible retrosynthetic analysis. We firstly cut off a carbon-nitrogen bond by a substitution reaction to obtain 2, and then cut off 2 by a reliable D-A reaction to obtain 3. In this way, it can ensure to a certain extent that the stereochemistry of the quaternary carbon and the methoxyl-carbon in the end product is correct. It should be noted that the D-A reaction is an Inverse Electron Demand Diels-Alder Reaction (IEDDA). Finally, we cut off 3 by a Michael addition reaction to obtain a substrate 4, and then simplified it into a substrate 5 by the substitution reaction.

The core idea of the entire retrosynthetic analysis is to construct a complex ring system of the natural product by the IEDDA, and then control the stereochemistry of the end product.

At the beginning of the total synthesis, the molecule 5, which was relatively simple and easily available, was used as the substrate. Firstly, we converted the helium atoms into the amino group by using the Gabriel synthesis

method[9] to effectively avoid the reduction of reaction product yield caused by the polysubstitution of the amino group, thereby obtaining a higher yield of primary amine 6. Subsequently, in order to convert 7 into 8 rather than imine by the reaction, we converted the primary amine 6 into the secondary amine by introducing the substituent group PMB of the amino group. Moreover, due to the greater steric hindrance of the PMB, the yield of the secondary amine should be much higher than that of the tertiary amine. Then, the substrate 7 was obtained by adding dibromomethane under the alkaline condition. 8 was further be obtained from 7 by the reaction between the secondary amine and the aldehyde by which the enamine can be obtained under the acidic condition. Subsequently, 8 was converted into 9 by the Michael addition reaction. It should be noted that the Michael addition reaction[10] in this step should have better regioselectivity, because the α carbon on the left side of the carbonyl group was also in the benzylic position; therefore, the acidity of hydrogen is stronger than that on the right side. After the product 9 was obtained, the enol was obtained by adding the NaOEt as the base to remove highly acidic benzylic hydrogen ions, and then the product 10 was obtained by the reaction between the enol and the MeBr. In this step, two types of enols were formed.

Scheme 7. Completion of the synthesis of 1

However, because the steric hindrance ratio of the enol that was obtained by the reaction with MeBr was smaller than that of the other enol, 10 should be the primary product with a higher yield. Then the IEDDA[11] took place by heating 10, thereby constructing a complex ring system of the natural product. The stereochemistry of the product obtained by the IEDDA will be more complex. Experiments are needed to know the specific results. But we estimate that 11 should be the primary product. After the product 11 was obtained, we introduced the formyl group on the benzene ring by the Vilsmeier reaction[12] under the milder reaction condition. It should be noted that the selectivity of the two sites on the benzene ring was relatively poor, and the possibility of introducing the formyl group at the two sites was not much different. Therefore, the product obtained should be a mixture, and 12 should be one of the more abundant products. After the product 12 was obtained, we added the sodium borohydride to reduce the carbonyl group. Due to the steric hindrance, the position of the hydroxyl group should point to the inside, that is, 2 was a relatively primary product. And then the DDQ was used in removing the protecting group PMB to obtain the secondary amine, which is prepared for the ring-forming reaction at the next step. Finally, under the weakly acidic and catalytic condition, the last six-membered ring was constructed by the nucleophilic substitution reaction between the secondary amine and the alcohol. In summary, the synthetic route is

to synthesize the substrate required by the D-A reaction by using the Michael addition, and then construct the more complex ring system of the natural product 1 by an IEDDA, and control the stereochemistry of the quaternary carbon. Regarding the yield problem in the route, and some stereochemistry-related problems, due to the lack of experiments, we can only make a rough judgment theoretically. We hope that specific data can be obtained through experiments in the future.

4 Conclusion

In summary, the most challenging part of the synthesis is the construction of the quaternary carbon center. On the first retrosynthesis, by using intermolecular and intramolecular aldol-condensation reactions around the heterolytic ring, we are able to construct a quaternary carbon center. On the second retrosynthesis, the synthetic route is to synthesize the substrate required by the D-A reaction by using the Michael addition, and then construct the more complex ring system of the natural product 1 by an IEDDA and control the stereochemistry of the quaternary carbon. Future experiments are highly recommended to examine the theoretical synthesis with empirical evidence, thereby fine-tuning the relevant stereochemistry.

ABBREVIATION

PMB, p-Methoxybenzyl. DDQ, 2,3-dichloro-5,6-dicyanobenzoquinone. DCM, Dichloromethane; LDA, Lithium diisopropylamide; THF, Tetrahydrofuran;

ACKNOWLEDGEMENT

The authors wish to thank Professor **Brian M. Stolz** from Division of Chemistry and Chemical Engineering, California Institute of Technology, and Teaching Assistant **Zitong Zheng** from Wuhan University for their continuous academic support and advice on the theoretical synthesis. We gratefully acknowledge **Dr. Patrick Whitty** who provided advice regarding the biological aspects of this work.

REFERENCES

1. Wenlai Fan and Michael C. Qian. Characterization of Aroma Compounds of Chinese “Wulianhye” and “Jiannanchun” Liquors by Aroma Extract Dilution Analysis. *Journal of Agricultural and Food Chemistry*. 2006, 54, 2695-2704.
2. Zoran Radic, Natilie A. Pickering, Daniel C. Vellom, Shelley Camp, and Palmer Taylor. Three distinct domains in the cholinesterase molecule confer selectivity for acetyl- and butyrylcholinesterase inhibitors. *Biochemistry*. 1993, 32, 45, 12074-12084.
3. Jana Maríková, Aneta Ritomska, Jan Korabecny, Rozalie Perinova, Abdullah Al Mamun, Tomas Kucera, Eliska Kohelova, Daniela Hulcova, Tereza Kobrlova, Jiri Kunes, Lucie Novakova, and Lucie Cahlikova*. Aromatic Esters of the Crinane Amaryllidaceae Alkaloid Ambelline as Selective Inhibitors of Butyrylcholinesterase. *J. Nat. Prod.*, 2020, 83, 1359-1367.
4. Michael O. Frederick, Mark A. Pietz, Douglas P. Kjell, Rachel N. Richey, Gregg A. Tharp, Taichiro Touge, Naota Yokoyama, Michio Kida, and Toshiyasu Matsuo. Development of a Leuckart-Wallach Reaction in Flow for the synthesis of Abemaciclib. *Organic Process Research & Development* **2017**, 21(9), 1447-1451.
5. A. Lukasiewicz. A study of the mechanism of certain chemical reactions—I: The mechanism of the leuckart-wallach reaction and of the reduction of schiff bases by formic acid. *Tetrahedron*. 19, 1963, 1789-1799.
6. William A. Kleschick, Charles T. Buse, and Clayton H. Heathcock. Stereoselection in the aldol condensation. *J. Am. Chem. Soc.* 1977, 99, 1, 247-248.
7. Rusli Daik, W. James Feast, Andrei S. Batsanov and Judith A. K. Howard. Stereochemical outcome of McMurry coupling. *New Journal of Chemistry*. 1998, 22, 1047-1049.
8. B. H. Liu, Z. P. Li A review: Hydrogen generation from borohydride hydrolysis reaction. *Journal of Power Sources*. 2009, 187, 527-534.
9. M. Niyaz Khan. Suggested Improvement in the Ing-Manske Procedure and Gabriel Synthesis of Primary Amines: Kinetic Study on Alkaline Hydrolysis of *N*-Phthaloylglycine and Acid Hydrolysis of *N*-(*o*-Carboxybenzoyl)glycine in Aqueous Organic Solvents. *The Journal of Organic Chemistry*. 1996, 61, 23, 8063-8068.
10. Yingze Cao, Xiaoyong Zhang, Lei Tao, Kan Li, Zhongxin Xue, Lin Feng, and Yen Wei. Mussel-Inspired Chemistry and Michael Addition Reaction for Efficient Oil/Water Separation. *ACS Appl. Mater. Interfaces* 2013, 5, 10, 4438-4442.
11. (a) Name Reactions (A Collection of Detailed Reaction Mechanisms), Jie Jack Li, Diels-Alder reaction., page 211-212. (b) Gao, S.; Wang, Q.; Chen, C. *J. Am. Chem. Soc.*, 2009, 131, 1410-1412. (c) Diels, O.; Alder, K. *Ann.*, 1928, 460, 98-122.
12. (a) Vilsmeier, A.; Haack, A. Ber., 1927, 60, 119-122. German chemists Anton Vilsmeier and Albrecht Haack discovered this reaction in 1927. (b) Lancelot, J.-C.; Laduree, D.; Robba, M. *Chem. Pharm. Bull.*, 1985, 33, 3122-3128. (c) Name Reactions (A Collection of Detailed Reaction Mechanisms), Jie Jack Li, Vilsmeier-Haack reaction, page 615-616.