PROPOSAL OF A FEASIBLE SYNTHETIC ROUTE TO ORMOSIANINE A VIA RETROSYNTHETIC ANALYSIS

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Abstract
Ormosianine A, a novel quinolizidine alkaloid isolated from Ormosia yunnanensis, has demonstrated potential acetylcholinesterase (AChE) inhibitory activity, with a Half-Maximal Inhibitory Concentration (IC_{50}) value of 1.55 μM, stronger than most Alzheimer's Disease (AD) drugs currently being used. Despite its therapeutic potential in the treatment of AD, the compound's natural extraction process is extremely inefficient, yielding only 6.0 milligrams of the compound from 8.0 kilograms of the plant. To address this limitation, this study promises a retrosynthetic route to Ormosianine A using commercially available starting materials. The retrosynthesis started with an oxidation level analysis, which is then followed by a number of disconnections and synthetic techniques, most notably the Stork-Danheiser method. Strategic choices like keeping an olefin and controlling proton acidity with protective groups increase the plausibility of the retrosynthesis route. While challenges such as the incorporation of nitrogen exist, processes like reductive amination offer viable solutions. Overall, this retrosynthetic plan lays out a practical foundation for the lab-scale synthesis of Ormosianine A.

Keywords
Ormosianine A; Retrosynthetic Analysis; Acetylcholinesterase Inhibitors; Alzheimer's Disease; Synthetic Organic Chemistry

Introduction

Alzheimer's Disease (AD) is a neurodegenerative disorder characterized by the deterioration of memory and cognitive function (Murray et al, 2013, p. 388). According to the cholinergic hypothesis, AD patients suffer from a decrease in the levels of acetylcholine (ACh), a neurotransmitter responsible for transmitting electrical signals from one nerve cell to another, due to its hydrolysis by the enzyme acetylcholinesterase (AChE). Therefore, AChE inhibitors are key to alleviating the effects of AD[1].

Numerous naturally occurring compounds from plants are potential sources of AChE inhibitors. For example, Rivastigmine, an AD medicine approved in 2000 by the FDA, originates from the initial compound physostigmine, which is a naturally occurring AChE inhibitor alkaloid. Similarly, Galanthamine and Huperzine A, extracted from *Galanthus spp.* and *Huperzia spp.* respectively, are widely used as AD medicine, yielding improved cognitive performance for their patients[2].

In 2023, the plant *Ormosia yunnanensis*, which had previously not been studied, was selected to extract compounds with AChE inhibitory activities. The compound Ormosianine A (Figure 1), among 15 other novel quinolizidine alkaloids, was isolated from the stems and leaves of the plant in powdered form and was determined by high-resolution electrospray ionisation mass spectrometry (HRESIMS) to have a molecular formula of $C_{20}H_{32}N_2O_2$. Ormosianine A was the strongest AChE inhibitor among active compounds extracted from *Ormosia yunnanensis*, with an IC_{50} value of 1.55 μM. These values are lower than Rivastigmine, Galantamine, and Huperzine A, with IC_{50} values of approximately 501 ± 3.08, 4 ± 0.13, and 13.62 μM, respectively[3,4]. Moreover, Ormosianine A formed hydrogen bonds with serine at position 238 and histidine at position 480, both key residues of the catalytic triad in the crystal structure of the AChE enzyme, further showing its potential to inhibit AChE5. Taken together, these pieces of evidence all indicate that Ormosianine A is a strong candidate for alleviating AD symptoms.

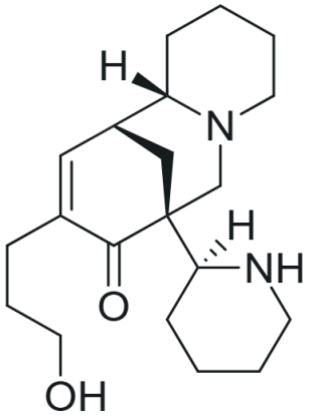


Figure 1. The structure of Ormosianine A, a novel quinolizidine alkaloid extracted from the plant Ormosia yunannensis.

However, in the status quo, extracting Ormosianine A from nature and using it as a pharmaceutical is challenging. First, the plant Ormosia yunnanensis is native only to the subtropical biome of South and Central China, particularly Yunnan province, making the extraction of the compound in other parts of the world difficult. Second, the current extraction process has extremely low yields and is complicated, with 8.0 kilograms of the plant only yielding 6.0 milligrams of the compound, displaying a yield of 7.5 x 10-5 %. The residue after powdering and extracting 8.0 kilograms of Ormosia yunnanensis with MeOH was 850 grams in mass. The residue was acidified, filtered, basified, and extracted with EtOAc to yield 40 g of total alkaloids. This was fractionated by silica gel chromatography into four parts (Fr. A–D). Compound 1 (6.0 mg) was isolated from Fr. B4 (2.0 g), a subfraction of Fr. B, via semipreparative HPLC using a MeOH / H_2O gradient[5].

Despite the difficulties in extracting Ormosianine A from nature and its promising potential against AD, no prior study has suggested a synthetic route for the production of the compound. Therefore, to efficiently produce large amounts of Ormosianine A for AD treatment, this paper proposes a synthetic route from commercially available starting materials to Ormosianine A.

Background

This section defines two key chemical processes involved in the proposed retrosynthesis: oxidation level analysis and the Stork-Danheiser approach.

Oxidation level analysis helps synthetic chemists break complex target molecules into simpler precursors by assigning oxidation states to carbon atoms based on the number of electronegative atoms attached to them. Carbons bonded to no electronegative atom are assigned as alkane level, those bonded to one electronegative atom as alcohol level, those bonded to two electronegative atoms as aldehyde/ketone level, and those bonded to three electronegative atoms as acid level. Because molecules at the same oxidation level can often be interconverted with minimal transformation, chemists treat carbons as if they already possess the functional groups corresponding to their assigned oxidation levels. For example, if a carbon is assigned the alcohol level, it is assumed the carbon has one alcohol group attached to it, even if the actual functional group is different. After performing oxidation level analysis, chemists work toward synthesizing the simplified "oxidation-mapped" intermediate. Once this intermediate is obtained, it is then converted into the original target molecule since moving between molecules at the same oxidation level is typically straightforward.

The Stork-Danheiser approach is a synthetic strategy developed by Professor Gilbert Stork and his student Rick L. Danheiser for the stepwise installation of R groups onto a cyclohexane framework[6]. The sequence typically begins with 1,3-cyclohexanedione, a commercially available compound. Then, a series of chemical reactions ensues until the desired R groups are attached to the six-membered ring. Multiple variations of the Stork-Danheiser approach exist depending on how many R groups are to be incorporated and at which ring positions. Outlined below in Figure 2 is the specific variant employed in this study. The product of this variant produces a structure resembling the oxidation-mapped intermediate.

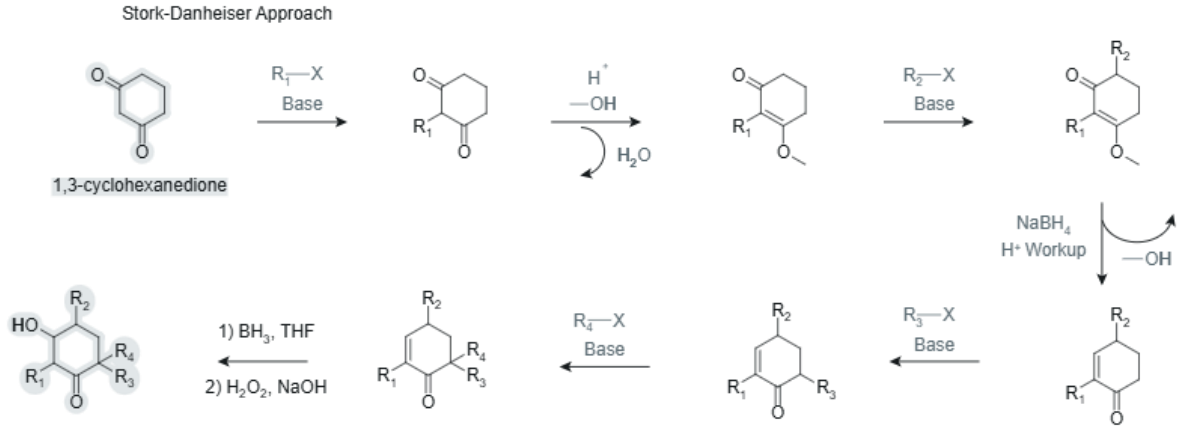
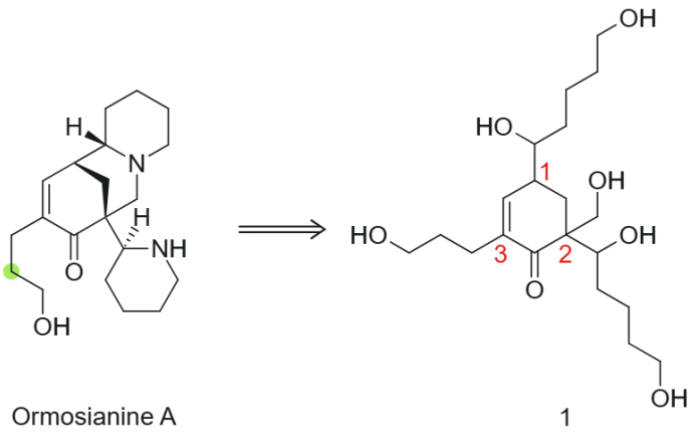


Figure 2. An illustration of the variant of the Stork-Danheiser approach, a synthetic strategy used to add side chains to a cyclohexane framework, employed in this study. The mechanism originates from Stork and Danheiser's seminal work, in which 1,3-cyclohexanedione is transformed into a more highly substituted cyclohexane[6].

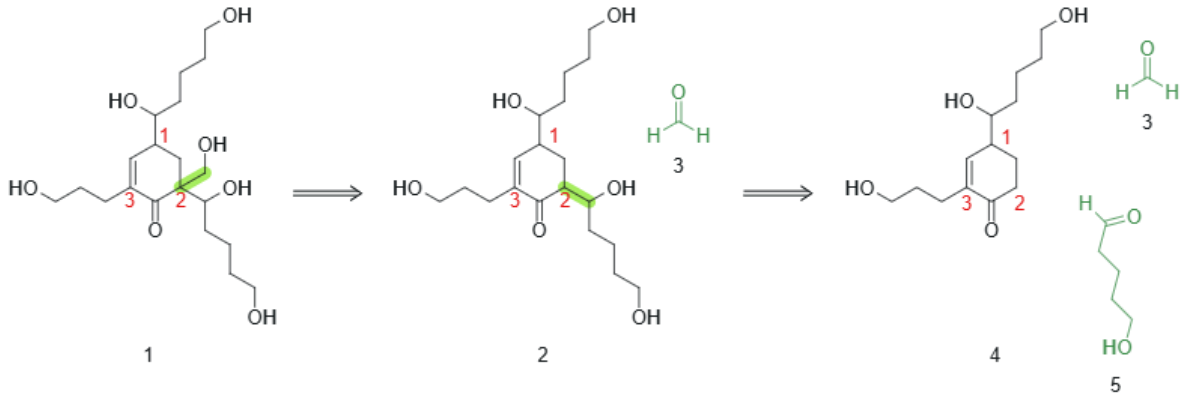
Results

The compound Ormosianine A was simplified into compound 1 via oxidation level analysis (Scheme 1). The result of the oxidation level analysis yielded a compound with a six-membered ring with R groups attached to three of its carbons. Even though the olefin on carbon 3 could have been eliminated via oxidation level analysis, it was intentionally retained to enhance the feasibility of the synthetic route, as demonstrated below.

Compound 1 was retrosynthesized into compound 2 by detaching the methanol group attached to carbon 2 (Scheme 2). Then, compound 2 can be further simplified into compounds 4 and 5 by detaching the butanol group attached to the same carbon. From a synthetic perspective, compounds 2 and 3 can be combined under basic conditions to form compound 1 if the alcohol groups are protected by being converted into ethers. Then, the most acidic protons would be attached to carbon 2, so the R groups will be attached to the following carbon. Likewise, compounds 4 and 5 can be coupled under basic conditions to generate compound 2.



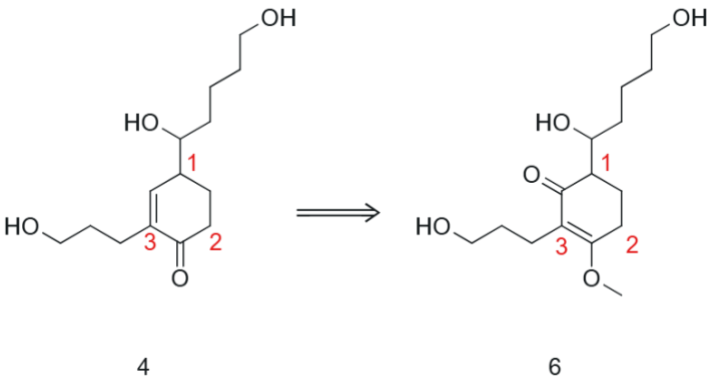
Scheme 1. A depiction of the oxidation level analysis of the compound Ormosianine A employed to simplify the molecule.



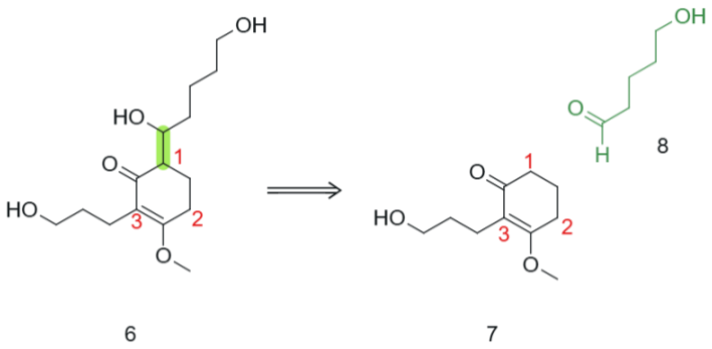
Scheme 2. Detachment of R groups attached to carbon 2. All green compounds from the current and subsequent schemes are commercially available and are therefore not further simplified.

Compound 4 was retrosynthesized into compound 6 following the implementation of the Stork-Danheiser approach (Scheme 3). Here, from a synthetic perspective, compound 6 can be converted into 4 by adding $NaBH_4$ and doing a H^+ workup, with methanol as a byproduct (Mechanism 1). Compound 4 is retrosynthesized into 6 in order to place the most acidic proton on carbon 1 so that Scheme 4 can take place.

Compound 6 was retrosynthesized into compounds 7 and 8 by detaching the butanol group from carbon 1 (Scheme 4). From a synthetic perspective, compounds 7 and 8 can be converted into compound 6 under basic conditions if the alcohol group in 7 is converted into an ether. Then, the most acidic proton would be located on carbon 1, so the R group would attach to that carbon.



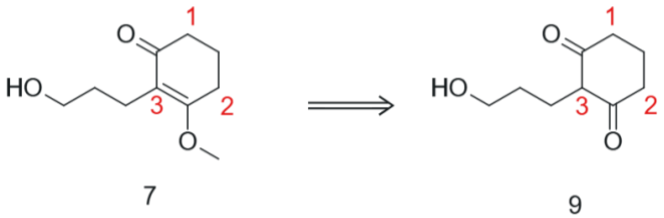
Scheme 3. Implementation of the Stork-Danheiser Approach (1). Adding $NaBH_4$ and protonating compound 6 produces compound 4 and methanol.



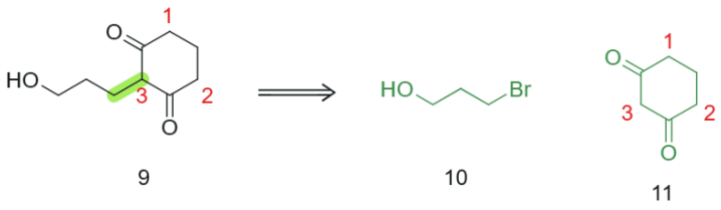
Scheme 4. Detachment of an R group attached to Carbon 1. Assuming the alcohol group in compound 7 is protected, adding compound 8 to compound 7 under acidic conditions would produce compound 6.

Compound 7 was retrosynthesized into compound 9 following the implementation of the Stork-Danheiser approach (Scheme 5). Here, from a synthetic perspective, compound 9 can be converted into 7 by adding methanol and an acid, with water as a byproduct (Mechanism 2). Compound 7 is retrosynthesized into 9 in order to remove the olefin and place the most acidic proton on carbon 3 so that Scheme 6 can take place.

Compound 9 was retrosynthesized into compounds 10 and 11 by detaching the propanol group from carbon 3 (Scheme 6). From a synthetic perspective, adding compounds 10 and 11 under basic conditions would produce compound 9 because the most acidic proton in compound 11 is located on carbon 3. Unlike in previous schemes, where R groups were detached from carbons, we do not need to convert anything to an ether since compound 11 does not contain an alcohol group.



Scheme 5. Implementation of Stork-Danheiser Approach (2). Adding methanol and an acid to compound 9 produces compound 7.



Scheme 6. Detachment of an R group attached to Carbon 3. Adding compounds 10 and 11 under acidic conditions would produce compound 9.

The final products are compounds 3, 5, 8, 10, and 11 (Figure 3). Compounds 5 and 8 are the same molecule.

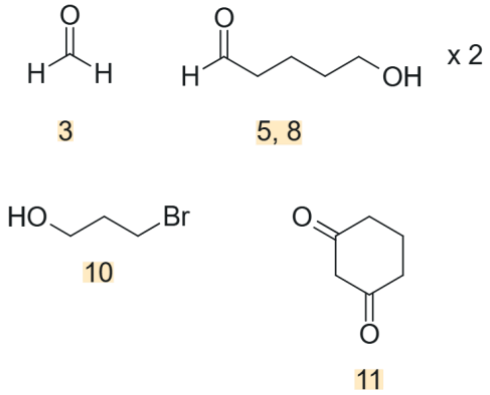
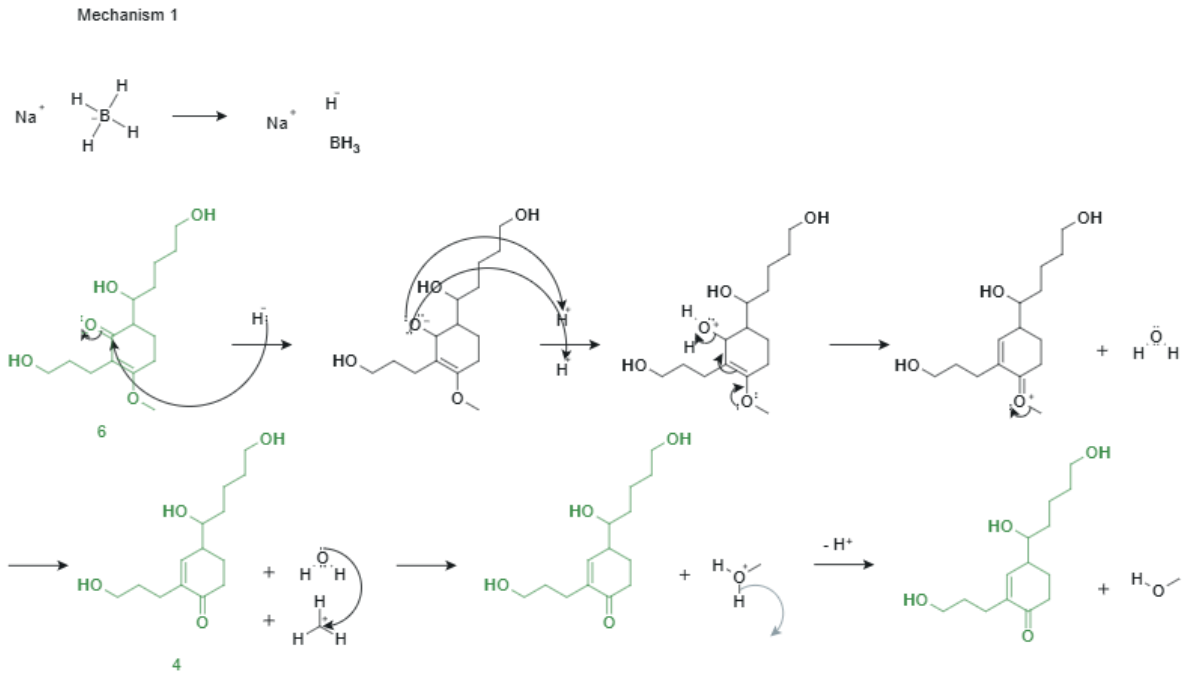
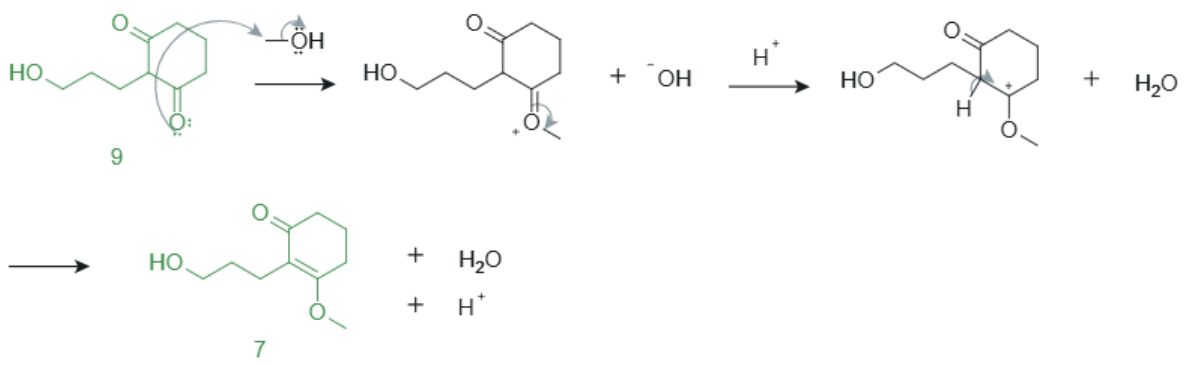


Figure 3. The final products of the retrosynthesis. Using these molecules, along with other readily available compounds such as acids, the target molecule can be synthesized.



Mechanism 1. Depiction of a significant step in the Stork-Danheiser approach, in which compound 6 is converted into compound 4. As depicted above, $NaBH_4$ is added, and a H^+ workup is done. Methanol is a byproduct.



Mechanism 2. Depiction of another vital step in the Stork-Danheiser approach, in which compound 9 is converted into compound 7. Methanol and an acid are added for synthesis, with water as the byproduct.

Discussion

The proposed retrosynthesis of Ormosianine A is a theoretically feasible retrosynthesis route. By systematically applying oxidation level analysis and breaking carbon-carbon bonds, the complex structure of Ormosianine A was broken down into simpler, commercially available building blocks. Each retrosynthetic step was guided by principles of acidity, protecting group strategy, and reactivity under basic conditions, showing that the forward synthesis in a lab is chemically plausible.

The retention of the olefin in Scheme 1, even though the oxidation level analysis suggested its removal, turns out to be a strategic decision to facilitate later coupling reactions. For example, from a synthetic perspective, when adding compounds 2 and 3 to form compound 1, assuming the alcohol groups are protected as ethers, the methanol group adds to carbon 2 due to the presence of the most acidic proton at that position. However, if the olefin were absent, and an alcohol group were added, carbon 3 would have had the most acidic protons because it is the α-position for both the alcohol and ketone groups (Figure 4). Therefore, the methanol group would add to carbon 3, an undesired consequence.

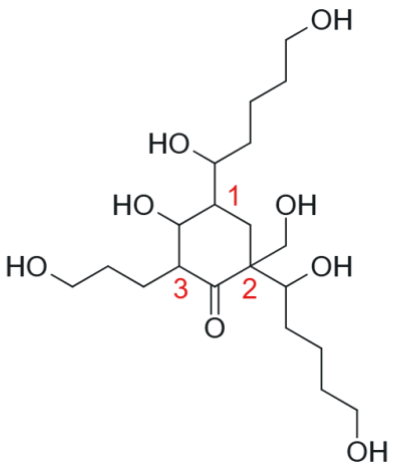


Figure 4. An alternate oxidation level analysis of Ormosianine A. The result of this oxidation level analysis produces undesired consequences, such as the addition of R-groups to undesired carbons.

Moreover, the fact that all final precursors, 3, 5, 8, 10, and 11, are either commercially available or easily synthesizable further supports the practicality of this retrosynthetic route. Furthermore, the repeated application of the Stork–Danheiser strategy reinforces the plausibility of the retrosynthesis, as its experimental conditions are well established and supported by numerous precedents validating the mechanism, thereby enhancing its commercial scalability.

As mentioned above, it would be essential in the synthesis process to protect the alcohol group as an ether. If the alcohol groups are not protected, the precedents with alcohol groups would have protons more acidic than those attached to carbons, so R groups would attach to the oxygen rather than to our desired carbons. For instance, when combining compounds 7 and 8 to synthesize compound 6, if the sole alcohol group in compound 7 is not protected, the butanol group would attach to the oxygen because its hydrogen has the lowest pKa value.

One potential challenge in the forward synthesis process would be converting compound 1 into Ormosianine A. This is especially concerning because Ormosianine A has nitrogens, even though compound 1 does not have them. Implementing the Mannich reaction would help in this case. The nitrogens can be installed at carbons 1 and 2 as imine intermediates via a Mannich reaction, in which iminium ions formed from an amine and formaldehyde are attacked by the enolizable α-carbons

adjacent to the ketone. These steps form new C–N bonds at C1 and C2 and generate a bis-aminated intermediate, which then undergoes subsequent cyclization and functional group adjustments to produce the final compound.

Overall, the proposed pathway represents a significant step toward the efficient and scalable production of Ormosianine A, which is an efficient AChE inhibitor and thus a highly promising AD medicine. Given the extremely low availability and yield from Ormosia yunnanensis, a synthetic alternative is essential for any future pharmacological development of the compound. This retrosynthesis lays the framework for experimental synthesis from simple compounds to our final product.

Conclusion

In conclusion, the proposed pathway represents a significant step toward the efficient and scalable production of Ormosianine A, which is an efficient AChE inhibitor and thus a highly promising AD medicine. Given the extremely low availability and yield from Ormosia yunnanensis, a synthetic alternative is essential for any future pharmacological development of the compound. This retrosynthesis lays the framework for experimental synthesis from simple compounds to our final product.

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