Landomycin A, a Conquerable but Sacrificial Challenge in Total Synthesis
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Imagine a synthesis chemist working on a target complex molecule. He is halfway into the synthesis and his thesis and deadlines for this project are approaching soon. Attaining the product that he believes is the correct conformer, he analyzes it in spectroscopy and to his dismay it is a dissimilar structure to the actual one bacterial cultures make. His project eventually falls apart, and he decides to report his findings in a paper that reads incomplete. Years later, another group of synthesis chemists in a different country manages to use his ideas to create a total synthesis off that molecule successfully.

Synthesis chemists in labs make money when they get the synthesis right on target. If they don’t, it’s back to the drawing board. It becomes a race to figure out who is going to be the first one to publish the synthesis of that molecule in a highly coveted database, like ACS. Chemistry is evolving to the point where making natural products is possible in a lab. Once assigned a project, the challenge is to either create from scratch or recreate a synthesis while getting around the surprising chemistry encountered during these processes. However, it is really hard to conclude that everything is going to work out at the end of the day. By chance, the chemistry may be surprising.

The story of landomycin A started in the 1990s when the Rohr group reported a new drug isolated from a culture broth of *streptomycesm cyanogenus*. Genetically engineering processes made it possible to grow an entire culture of this bacterium containing this molecule, but it was possible that bacteria could naturally produce this antibiotic overtime. There were different isolated derivatives of these molecules deemed landomycins A-D then on. They were characterized as “angucycline antibiotics,” a new group of quinone natural products. The phrase “angucycline” applies to molecules that possess a benz[a]anthracene ring system. Figure 1 shows the aglycone of landomycin A bearing this fragment (i.e. the 4-ring system A-D). For this reason, landomycins are also referred to as benz[a]anthraquinone aglycones. The term “aglycone” is the part of the sugar that the functional groups are attached on. The “antibiotics” in its characterization implies that this molecule is used in anti-bacterial activity. However, this molecule was later discovered to be so potent, that it is classified as an anti-cancer agent. At a glance, the molecule looks to be difficult to make in lab. Because this molecule is an antibiotic, it can get costly, timely and even more laborious to be semi-naturally attained.

Bearing in mind that this molecule would be difficult to synthesize, in 1997 this class of carbohydrates became a target selection to create. Instead of growing bacteria for a long period of

Figure 1. Landomycin A (C56H76O22, MW=1087.18 g/mol)5, structure reported by Xiaoyu Yang, Boqiao Fu, and Biao Yu
time in order to get this natural product, it was desirable to synthesize it in the lab. Additionally, growing cultures with this molecule also includes the possibility of it being unsuccessful because this molecule is an antibiotic. Many synthetic chemists studied and attempted to formulate retrosynthetic and total synthesis for this molecule. Around 1998, synthesis chemists jumped at the opportunity of synthesizing this molecule.

Around 2000 to 2004, the Roush group successfully devised a synthesis of what they believed the landomycinone would be. They also studied and published several papers in JACS on the longest deoxygenated hexasaccharide residue, and showed how they attempted to recreate it. The Yu group also mentioned it was evident that the Roush group’s synthesis of the aglycone was the key component of their own total synthesis. It was because the Roush group had later discovered in the studies of its $^1$H NMR spectra that the landomycin A structures they synthesized was dissimilar to the naturally occurring one in the bacteria cultures. They published a paper worth mentioning their unsuccessful generation of the correct stereocenters for the aglycone, and this molecule went back to the drawing board.

Of course, it was not enough to disprove that the Roush group failed to create a landomycin. The Yu group reported that this synthesis of the landomycinone was one of the important factors when deciding how the target would form. Other groups such as Sulikowski and Takahashi that approached similarly are reported by the Yu group in their publication of their total synthesis. Although it would be worth mentioning the synthesis in detail for this report, many of the synthetic chemists were only able to create the longest landomycinone residues, or fragments of the total molecule. Within the summer of 2011, Xiaoyu Yang, Boqiao Fu, and Biao Yu were able to produce a total synthetic method of this carbohydrate. It took them a total of 15 years to develop it.

![Diagram of Roush and Neitz synthesis of landomycinone](image)

Figure 2. Roush and Neitz synthesis of landomycinone: end product aglycone is non-identical to natural product

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Landomycins are antitumor agents in which it specifically regulates the concentrations of thymidine in cell cycle activity. Thymidine, more consistently referred to as deoxythymidine in this case, is the DNA nucleoside T that base pairs with the deoxyadenosine A. As such, it synchronizes cells in the S phase or the part of the DNA replication cycle where regulation of this takes place. Landomycin is known to inhibit activity and regulate this base-pair formation in the event that the cells are cancerous. They also induce apoptosis at very low concentrations of thymidine to regulate bodily functions that would otherwise become a problem if the cell is cancerous. While other forms of landomycin also perform this, the form landomycin A was proved to be the most potent of the class because it tested to contribute stronger activity against cancerous cells, and contains the longest running hexasaccharide residue (Figure 1, the carbohydrate strands 1 to 6). These molecules also contain a special stereospecific ring on a 4-ring linked aglycone called the landomycinone. In Figure 1, this is the B-ring with the hydroxyl group point up, or the β-face. The glycan, or the pyranose rings 1-6 that make it carbohydrate, consists of di- and trideoxy D-olivose and L-rhodinose segments.

L-rhodinose

\[ \text{L-rhodinose} \]

D-olivose

\[ \text{D-olivose} \]

Figure 2. Sugars found on the hexasaccharide of landomycin A

In most carbohydrate syntheses, a chemist might say that the hardest task is to perform the coupling of the aglycone to the glycan while preserving the stereochemistry of both components. For this molecule, the phenolic linkage mentioned earlier was difficult to initiate this stitching of this complex molecule under the ideal conditions of creating carbohydrates that Sulikowski and Takahashi used. The Yu group, who developed this clever total synthesis of this natural product, had to design another way of attaching this, and decided to attach a part of the glycan to the landomycinone (Figure 3).

Figure 3. Retrosynthetic breakdown by the Yu group\(^1\)
Another obvious challenge when examining the molecule for possible disconnections is the preservation the hydroxyl group on the stereogenic ring (Figure 1, ring B) during the formation of the landomycinone in the β-position. It is incredibly unstable, and under certain conditions can undergo a dehydration aromatization because of the neighboring aromatic ring (Figure 1, ring A) during the synthesis of it. This process is very difficult to avoid, furthering the yield suffering towards the final target. Lastly, the synthesis of the glycan (i.e. the hexasaccharide fragments) was difficult to synthesize not only based on what previous literature instigated. Observing the structure of this carbohydrate, it is difficult to avoid destruction of the defined stereochemistry in acidic or basic conditions (for which the synthesis of the glycan required).

This retrosynthetic approach began with the disassembly of the glycan from the landomycinone. At a glance, the initial disconnection of a β-selected glycosylation would have been achieved with neighboring group active glycosyl donors. In other words, an equatorially disposed heteroatom within the glycosyl donor can be removed in the coupling process to the aglycone. Other ways that had been previously done had failed: using 2-deoxy-2-iodoglycosyl, etc. with Mitsunobu conditions. Unfortunately, the Yu group had to try another approach because this further caused issues when trying to link them together. From there, the Yu group tried a Hauser annulation and an intramolecular Michael addition to form the rings of the landomycinone derivative. Using research gained from the synthesis of the glycan by Sulikowski, the Yu group developed another way to attach the aglycone. More importantly, studying the synthesis of the landomycinone from Roush, the total synthesis of landomycin A was formulated.

Scheme 1. Synthesizing the Landomycinone
The chemistry that the Yu group proposed was rigorous and required a lot of thought going into the scheme of stitching the target together. As said, sometimes the chemistry does not happen the way it is designed. An example of this is through the reactive intermediate that produces about half of the stable enolate rather than the desired ketone. Observation of the end product, which is the ketone molecule, and reading the deductions made about the poor yield demonstrates that the synthesis is extremely difficult. The desired goal of avoiding loss of starting materials is inevitable. One reason would be the extended conjugation from the aromatic ring, and there is a resonance hybrid where the negative charge on oxygen and a positive charge on carbon can delocalize, further stabilize the enolate form.

Another clever trick was that they used the Roush group’s version of a Michael addition using NaOEt/EtOH and air. In this case, the Yu group modified this type of reaction to better suit the synthesis of the active form of the landomycinone. The modified conditions included the NaOEt/EtOH, but molecular sieves and a fair bit of heat were used. Unfortunately and apparently expected, the production yield had suffered drastically. When comparing the two synthetic methods of forming this landomycinone, the Roush’s group reported synthesis indicated a yield 5% more than Yu’s group when the Michael addition was taking place. One possible reason for this event to occur might be in terms of stability. However, the Yu group was able to correctly form the B-ring while keeping the protecting group on the ring β.

**Scheme 2. Adding a Glycosidic Linkage**

The sensitivity of that stereo-center is difficult to manage. There might have been a possibility that, because of the nearby conjugated system and the ketones, the ring could become aromatic which is a stable process. Therefore any sudden extremity would cause damage to the derivative when attaching the glycan to it. After testing, the Yu group tried a protocol for adding a fragment of the glycan to the landomycinone. The protocol, developed by the Gervay-Hague group, unexpectedly produced favorable results. The glycosidic linkage, now formally attached to the landomycinone, was formed in a simple bimolecular substitution using the glycosyl iodide. With this component added, it became clear what direction the Yu group wanted to proceed to attain the landomycin A.
The use of protecting groups was the key to the formation of the pentasaccharidetrifluoroacetimidate. The Yu group tested other forms of glycosidic linkages and found that using a trifluoroacetimidate saccharide coupling cleaner, higher-yielding and easier. The Yu group started with an iodide-pyranose residue (15) with L-rhodinosyl acetate (16) in the presence of TBSOTf with a polar solvent like DCM. The use of this reagent for adding to the acetyl groups, as well as providing a better substitution, this formed a thermodynamically favored α-face disaccharide. The disaccharide’s TBS group is then de-protected with TBAF in THF. For the remaining steps of this glycan synthesis the use of TBSOTf in DCM was used to create the carbohydrate, while using certain reagents to deprotect the TBS and the acetyl groups. Using $\text{N}_2\text{H}_4\cdot\text{H}_2\text{O}$ in DCM along with MeOH at room temperature, and then adding N-phenyl-trifluoroacetimidoyl chloride, the pentasaccharidetrifluoroacetimidate was created.

The penultimate step was coupling the two components of landomycin A in the presence of TBSOTf and molecular sieves of different sizes to yield 78% of the landomycin A derivative. The deprotection of the B-ring was initiated with Raney nickel and bubbled hydrogen gas in THF at room temperature. The hydroquinone was oxidized with DDQ to give the deoxyhexasaccharide and then the alkyl halides were removed from the pyranose rings. The overall yield of this step led to
44% remaining starting material. The last step of this total synthesis was to use a base to remove the acetyl groups on the sugar residue, and then reduce them to alcohols. The ending yield of the entire synthesis means that for every one run of this 63 step process, 0.34% landomycin A will be produced. Though the yield isn’t pretty good, it’s well expected that after all of these steps that are penalizing, this molecule still deserves some praise for being a challenge that was defeated in total synthesis. Besides, most of the time, total synthesis is not extremely successful.

**Scheme 4.** Coupling the Glycan to the Alycone: Creating Landomycin A

The molecular structure of landomycin A is indeed difficult to recreate. Preserving yield and stereochemistry at the same time is not synthetically easy to do. With a sugar, there are many stereogenic centers. The synthesis that the Yu group invented proved to be innovative and inclusive of other researchers’ studies—a real conquer in total synthesis. With landomycin A’s defined stereochemistry and orientation of the glycan, the assembly of this molecule written by the Yu group is elegant. Being able to achieve 0.34% yield towards the end of the 63 step synthesis of the naturally occurring potent antitumor drug is an accomplishment in itself and is fully deserving of its place in the Journal of the American Chemical Society.
REFERENCES


The Yu group gave a total synthesis of landomycin A. The report is mainly based on their synthetic pathways.


In this paper, Yang and Yu summarize their 2011 publication on their total synthesis of Landomycin A. This paper includes their extensive research into the literature. They characterize landomycin A syntheses based on other people’s research. Although worth mentioning in this report, the Yu group did not include most of the synthetic pathways mentioned in their paper. The extensive research into the saccharides (i.e. the glycan formation from Sulikowski’s group) demonstrated the urgency of uncovering a different method of attaching the glycan to the aglycone – rather than a traditional method of coupling. One synthesis pathway introduced in their own synthesis of landomycin A was the Roush group’s intramolecular Michael addition for which they modified (Figure 2). For this reason, it is the only synthesis laid out in this report.


Roush and Neitz introduce their synthesis of the aglycone of landomycin A (i.e., landomycinone). Using X-ray crystallography, Roush and Neitz proved successful synthesis of the derivative. Spectroscopic data including 1HNMR spectra, both Roush and Neitz confirmed that their synthesis is not the aglycone found in the bacterial culture strain. Under careful studies into the literature of the landomycinone formation, the Yu group was able to achieve a successful total synthesis of landomycinone A that is identical to the natural product.


While their research was exceedingly important to the development of saccharide, Sulikowski and Guo’s formation of the glycan was not used in the Yu group’s total synthesis of the landomycin A. However, it did point them in the direction they wanted to take during retrosynthetic analysis of possible disconnections.

(5) Data was taken off analysis on Chem-Draw, further proven to be correct using (2)


Protocol for performing the synthesis of this scheme.