



Published in final edited form as:

Bioorg Med Chem Lett. 2020 November 01; 30(21): 127509. doi:10.1016/j.bmcl.2020.127509.

Synthesis and biological evaluation of semi-synthetic albocycline analogs

Samer S. Daher^a, Kevin P. Franklin^a, Tyler Scherzi^b, Paul M. Dunman^b, Rodrigo B. Andrade^{a,*}

^aDepartment of Chemistry, Temple University, Philadelphia, PA 19122

^bDepartment of Microbiology and Immunology, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642

Abstract

Albocycline (ALB) is a unique macrolactone natural product with potent, narrow-spectrum activity against methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-intermediate (VISA), and vancomycin-resistant *S. aureus* (VRSA) strains (MIC = 0.5–1.0 µg/mL). Described herein is the synthesis and evaluation of a novel series analogs derived from albocycline by functionalization at three specific sites: the C2-C3 enone, the tertiary carbinol at C4, and the allylic C16 methyl group. Exploration of the structure-activity relationships (SAR) by means of minimum inhibitory concentration assays (MICs) revealed that C4 ester analog **6** was twice as potent as ALB, which represents a class of lead compound that can be further studied to address multi-drug resistant pathogens.

Keywords

albocycline; MIC; SAR; antibiotic

Antibacterial drug discovery has benefited greatly from the systematic investigation of natural products (NPs). In fact, approximately 60% of all the FDA-approved antibiotics are either NPs, NP-derived, or NP-inspired small molecules.^{1,2} However, the rapid development of bacterial resistance to antibiotics coupled with marked changes in the Pharmaceutical Sector has resulted in a global health crisis.³ The structural modification of natural product derived antibiotics (e.g., β-lactams, aminoglycosides, macrolides, tetracyclines) by either semi- or total synthesis is a highly effective tactic for exploring novel drugs to address bacterial resistance. As part of a program directed at bioactive natural products synthesis, we

* Corresponding author: tel: +1 215 204 7155; fax: +1 215 204 9851; randrade@temple.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Supplementary Material

General methods, isolation protocol, minimum inhibitory concentration (MIC) method, synthetic methods for the synthesis and characterization of **6**, **7**, **9–12**, and **15** Spectral data (¹H and ¹³C NMR) for **6**, **7**, **9–12**, and **15**, and 2D-NMR for ALB (**1**) and analog **9**.

were drawn to albocycline (ALB, **1**) due to its unique structure—a 14-membered macrolactone with four stereogenic centers and three alkenes—in addition to its antibacterial activity (Figure 1).⁴

Significantly, Tomoda reported in 2013 that ALB was equipotent with vancomycin (**2**) against methicillin-resistant *Staphylococci aureus* (MRSA) and more potent against vancomycin-resistant *S. aureus* (VRSA).⁴ A recent report on a spike in MRSA incidences, in addition to a pressing need for drugs to address VISA and VRSA, was further motivation to carry SAR studies on this validated antibacterial natural product.⁵

Studies by Zucchi and co-workers further reported that ALB could be deployed as an anti-mold agent.⁶ Agricultural losses resulting from “white mold” disease caused by *Sclerotinia sclerotiorum* in the United States can exceed \$200M annually.⁷ Significantly, *in vivo* studies suggested further interest in ALB for future therapeutic applications due to the absence of toxicity in mice as well as in humans.^{8,9} In 2018, we demonstrated ALB was not toxic to human cells at a final concentration 64 µg/mL in colorimetric MTT Cell Proliferation Assays using human HepG2 hepatocellular liver carcinoma cells.¹⁰ Accordingly, exploring the ALB framework by preparing novel analogs and evaluating them in MIC assays could assist in addressing the aforementioned threats to public health and society.

Two total syntheses of albocycline (i.e., ingramycin) have been reported to date. The first was from Tanner and Somfai in 1987 and required 40 total steps (21 in the longest linear sequence).¹¹ In 2017, we achieved the second synthesis in 14 overall steps.¹² Alternatively, ALB can be readily accessed by culturing the producing organism, *S. maizeus*, as was reported in the Upjohn patent.¹³ The latter (i.e., semi-synthetic) approach allows access to multi-milligram amounts of material that be employed in the synthesis of novel ALB analogs through judicious modifications that modulate potency, physicochemical, and ADMET properties that have been enormously successful across a wide range of antibiotic classes.¹⁴

Previous reports indicated that 2,3-dihydro- (**3**) and 5,6-dihydroalbocycline (**4**) did not possess antibacterial activity, thus highlighting the importance of unsaturation at those sites (Figure 2).¹⁵ On the other hand, cineromycin B (**5**) maintains biological activity against MRSA, suggesting that the C7 site is amenable to modification by either semi- or total synthesis.¹⁶ Sites that had not been explored included the tertiary C4 carbinol, the *O*-methyl ether at C7, and the allylic C16 methyl group.

Straightforward C4 carbinol functionalization includes both esterification and ether formation by acylation and alkylation. With respect to the former, initial attempts based on Yamaguchi or Steglich (i.e., DMAP) conditions did not deliver any ester adduct resulting in recovery of ALB.^{12,17} However, previous work performed by Upjohn revealed that prolonged heating under pyridine and catalytic DMAP delivered the 4-bromobenzoate adduct, which was subjected to single x-ray crystallography in order to confirm the structure of ALB (**1**).¹⁸ By employing the same reaction conditions, albeit with 4-fluorobenzoyl chloride, which was selected to reflect the increasing trend in medicinal chemistry of

incorporating fluorine substituents in FDA-approved drugs, benzoate analog **6** was obtained in 42% yield (Scheme 1).¹⁹

We reasoned that the installation of a propargylic ether at C6 could allow for the rapid generation of a library of ALB congeners by using the venerable copper(I)-catalyzed azide-alkyne cycloaddition (i.e., Click) reaction.²⁰ In addition, an alkyne-functionalized analog of ALB could serve as a chemical probe for use in affinity-based protein profiling (ABPP)^{21,22} to help identify the target of ALB.¹⁰ To this end, we reacted ALB (**1**) with NaH and propargyl bromide in presence of catalytic tetrabutylammonium iodide (TBAI), which afforded 4-O-propargyl albocycline (**7**) in 55% yield after column chromatography, in addition 15% of recovered starting material. It is noteworthy that owing to the hindered nature of the tertiary C4 carbinol, the reaction mixture required heating in analogy to the acylation reaction.

Inspection of albocycline's structure reveals a non-obvious site of functionalization, namely the allylic C16 methyl group. We were inspired by Undheim and co-workers who successfully functionalized the allylic C10 methyl group on the erythromycin framework by employing *N*-bromosuccinimide (NBS) in the presence of peroxides.²³ Accordingly, we attempted to generate a similar allylic bromide derivative on ALB (**1**) that would allow ready access to a variety of analogs (e.g., amines, ethers, esters, and carbamates) using simple substitution reactions. In the event, we obtained an inseparable 1:1 mixture of allylic bromide regioisomers **8** and **8'** in low yield (Scheme 2). Alternatively, site selective Riley oxidation of **1** at C16 with selenium dioxide (SeO₂) proved to be a superior approach allowing access of allylic alcohol **9** in a modest 35% yield.

Establishing a synthetic route to allylic alcohol **9** from ALB allowed ready access to several functional groups such as aldehyde, ether, and azide through simple functional group interconversions. The rationale behind accessing the oxidized derivative of allylic alcohol **9**, namely the C16 aldehyde, was the known presence of this moiety in established macrolides as typified by tylosin.²⁴ Accordingly, reaction of allylic alcohol **9** with the Dess-Martin periodinane (DMP) furnished aldehyde **10** in 55% yield (Scheme 3). In order to test the influence of the hydroxyl group at C16 on biological activity enabled by potential hydrogen bonding interactions, we synthesized the 16-methoxyalbocycline (**11**) using by alkylating the hydroxyl group of **9** with Meerwein's salt and Proton Sponge in 55% yield.

The abundance of the azide functionality in medicinal chemistry due to its kinetic stability under physiological conditions, coupled with their unique reactivity patterns make them promising candidates to explore. Initial attempts at activating the allylic alcohol in **9** by preparing sulfonate (e.g., mesylate or tosylate) derivatives for subsequent displacement with NaN₃ were unsuccessful. Alternatively, the use of diphenyl phosphoryl azide (DPPA) and DBU allowed the formation of diphenyl phosphate intermediate **12** within the first 20 minutes; however, displacement with azide was observed after stirring an additional 3–4 hours to furnish an inseparable mixture of azide regioisomers **13** and **14**, in which the latter is the product of S_N2' displacement and is often observed in allylic substrates according to literature precedent (Scheme 4).²⁵

Recent reports from Hergenrother and co-workers suggested that compounds exhibiting primary amine functionalities tend to be highly accumulating inside the cell due to their amphiphilic nature, rigidity, and low globularity.²⁶ This in return enhances the activity of the small molecules, in which the rate of compounds crossing porins will be higher than the rate at which they are pumped out. Hence, considering this approach may lead to better efficacy especially against Gram-negative bacteria, as was shown for modified compounds towards *E. coli*, as a result of overcoming their outer membrane by favoring diffusion and prolonged accumulation inside the bacteria similar to what is typically encountered with Gram-positives.²⁶ Accordingly, we were motivated to incorporate amine functionality on the framework of ALB in order to test if there is an increased spectrum of activity. The presence of the α,β -unsaturated enone motif in ALB between C2 and C3 is a potential site for incorporating primary or secondary amines by conjugate (Michael) addition. Moreover, previous work conducted by Colby and co-workers showed that *aza*-Michael addition of amines to α -methylene- γ -lactones resulted in enhancing solubility, biological activity, and pharmacokinetic profile of drugs.²⁷ Consequently, initial efforts focused on reacting ALB with secondary amines such as pyrrolidine. However, ALB was recovered and no desired adduct was detected. Alternatively, thiols are potentially another class of nucleophiles that can undergo *thia*-Michael addition with unsaturated enones.²⁸ Smith and co-workers previously reported the 1,4-addition of thiophenol to the α,β -unsaturated lactone of ALB during the course of a semi-synthetic campaign toward of melanogenesis inhibitors.²⁹ Accordingly, we were inspired to incorporate a fragment bearing both amine and thiol functionalities to ensure the incorporation of amine analog within the ALB scaffold. To this end, 2-mercaptoethylamine was reacted with **1** in CH_2Cl_2 at rt for 4–6 h, which afforded β -amino-sulfide adduct **15** as a 2:1 ratio of diastereomers at C3 (LCMS) in 40% yield (Scheme 5). Both diastereomers were readily subsequently separated by reverse-phase column chromatography.

To evaluate the potency of ALB and its semi-synthetic analogs, MIC assays were employed against *S. aureus* (UAMS-1) and (USA300) strains. The results of those assays are shown in Table 1. Against resistant *S. aureus* strains, only two analogs **5** and **6** displayed promising biological activity compared to ALB. The *O*-desmethyl congener of ALB, cineromycin B (**5**), was 4–8 fold less potent than ALB against (UAMS-1) and two-fold less potent than ALB against USA-300. The fact that C7 modification didn't destroy bioactivity suggest this site should be further explored. Notably, the *p*-fluorobenzoyl C4 ester of albocycline **6** was nearly equipotent with ALB against MSSA strain (UAMS-1) and 2-fold more potent than ALB against the MRSA strain (USA-300). While analogs **9** and **10** showed moderate potency, activity was completely destroyed for analogs **7**, **11**, **12**, and **15**. On the other hand, testing the biological activity of major and the minor diastereomers of β -amino-sulfide adduct **15** against various Gram-negative strains (i.e., *A. baumannii*, *E. coli*, and *P. aeruginosa*) using kanamycin as positive control revealed poor biological activity, suggesting that inability of **15** to targeting Gram-negative pathogens (see supporting information).

In conclusion, the natural product macrolactone albocycline (ALB, **1**) has been cultured from *S. maizeus* and synthetically modified to access analogs at the C2–3 enone, C4 tertiary alcohol, and C16 allylic methyl group. The *O*-desmethyl congener of **1**, cineromycin B (**5**)

was isolated during the culturing of *S. maizeus*. All compounds were subjected to MIC evaluation against Gram-positive MSSA and MRSA. While most modifications resulted in loss of activity, cineromycin B (**5**) and the C4 benzoate derivative **6** were the most promising with the latter analog being two-fold more potent than ALB against MRSA. Future work aimed at determining the target of albocycline will assist in guiding structure-activity relationships in order to discover novel analogs that address the need for drugs that treat resistant pathogens including MRSA.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The NIH (GM126221) and Temple University supported this research. Support for the NMR facility at Temple University by a CURE grant from the Pennsylvania Department of Health is gratefully acknowledged. We thank Dr. David P. Labeda (National Center for Agricultural Utilization Research, United States Department of Agriculture) for kindly providing *S. maizeus*. We thank Prof. Ann Valentine for optimizing the protocol for culturing *S. maizeus*, as well as Drs. Robert Stanley and Carol Manhart for kindly providing autoclave facilities. We thank Dr. Bettina Buttaro for assistance with MIC evaluation against Gram-negative pathogens. Finally, we thank Dr. Charles DeBrosse, Director of the Temple University NMR Facility in the Department of Chemistry, for kind assistance with NMR experiments.

Abbreviations

MRSA	methicillin-resistant <i>Staphylococcus aureus</i>
VISA	vancomycin-intermediate <i>Staphylococcus aureus</i>
VRSA	vancomycin-resistant <i>Staphylococcus aureus</i>
SAR	structure-activity relationship
MIC	Minimum Inhibitory Concentration
NPs	natural products
ALB	albocycline
ABPP	affinity-based protein profiling
TBAI	tetrabutylammonium iodide
NBS	N-bromosuccinimide
DPPA	diphenyl phosphoryl azide

References:

- (1). Newman DJ; Cragg GM J. Nat. Prod 2012, 75, 311. [PubMed: 22316239]
- (2). Newman DJ; Cragg GM Future Med. Chem 2009, 1, 1415. [PubMed: 21426057]
- (3). Solomon SL; Oliver KB Am. Fam. Physician 2014, 89, 938. [PubMed: 25162160]
- (4). Koyama N; Yotsumoto M; Onaka H; Tomoda H J. Antibiot 2013, 66, 303.
- (5). Hassoun A; Linden PK; Friedman B Crit. Care 2017, 21, 211. [PubMed: 28807042]

- (6). Zucchi TD; Almeida LG; Moraes LAB; Consoli FL *Ind. Crops. Prod* 2014, 52, 264.
- (7). Bolton MD; Thomma BP; Nelson BD *Mol. Plant Pathol* 2006, 7, 1. [PubMed: 20507424]
- (8). Reusser F *J Bacteriol* 1969, 100, 11. [PubMed: 4241976]
- (9). Miyairi N; Takashima M; Shimizu K; Sakai H *J Antibiot (Tokyo)* 1966, 19, 56. [PubMed: 5952892]
- (10). Liang H; Zhou GF; Ge YH; D'Ambrosio EA; Eidem TM; Blanchard C; Shehatou C; Chatare VK; Dunman PM; Valentine AM; Voelz VA; Grimes CL; Andrade RB *Bioorg. Med. Chem* 2018, 26, 3453. [PubMed: 29805074]
- (11). Tanner D; Somfai P *Tetrahedron* 1987, 43, 4395.
- (12). Chatare VKA, R. B *Angew. Chem. Int. Ed* 2017, 56, 5909.
- (13). Bergy ME; Hoeksema H; Johnson LRE; Kinch DG; Google Patents: 1972.
- (14). Wright PM; Seiple IB; Myers AG *Angew. Chem. Int. Ed* 2014, 53, 8840.
- (15). Slechta L; Cialdella J; Hoeksema H *J. Antibiot* 1978, 31, 319.
- (16). Terekhova LP; Galatenko OA; Kulyaeva VV; Malkina ND; Boikova YV; Katrukha GS; Shashkov AS; Gerbst AG; Nifantiev NE *Russ. Chem. Bull* 2007, 56, 815.
- (17). Johnson TC; Chin MR; Han TX; Shen JP; Rana T; Siegel D *J. Am. Chem. Soc* 2016, 138, 6068. [PubMed: 27096704]
- (18). Thomas RC; Chidester CG *J. Antibiot* 1982, 35, 1658.
- (19). Shah P; Westwell AD *J Enzyme Inhib Med Chem* 2007, 22, 527. [PubMed: 18035820]
- (20). Kolb HC; Sharpless KB *Drug Discov. Today* 2003, 8, 1128. [PubMed: 14678739]
- (21). Wright MH; Sieber SA *Nat. Prod. Rep* 2016, 33, 681. [PubMed: 27098809]
- (22). Gersch M; Kreuzer J; Sieber SA *Nat. Prod. Rep* 2012, 29, 659. [PubMed: 22504336]
- (23). Gunnes S; Undheim K *Bioorg. Med. Chem* 2007, 15, 119. [PubMed: 17056262]
- (24). Suchodolski JS; Dowd SE; Westermarck E; Steiner JM; Wolcott RD; Spillmann T; Harmoinen JA *BMC Microbiol* 2009, 9, 210. [PubMed: 19799792]
- (25). Torikai K; Suga H *J. Am. Chem. Soc* 2014, 136, 17359. [PubMed: 25454254]
- (26). Richter MF; Drown BS; Riley AP; Garcia A; Shirai T; Svec RL; Hergenrother PJ *Nature* 2017, 545, 299. [PubMed: 28489819]
- (27). Woods JR; Mo H; Bieberich AA; Alavanja T; Colby DA *J. Med. Chem* 2011, 54, 7934. [PubMed: 22029741]
- (28). Woods JR; Mo H; Bieberich AA; Alavanja T; Colby DA *MedChemComm* 2013, 4, 27.
- (29). Sunazuka T; Obata R; Zhuorong L; Takamatsu S; Komiyama K; Omura S; Smith AB *Tetrahedron Lett* 1994, 35, 2635.

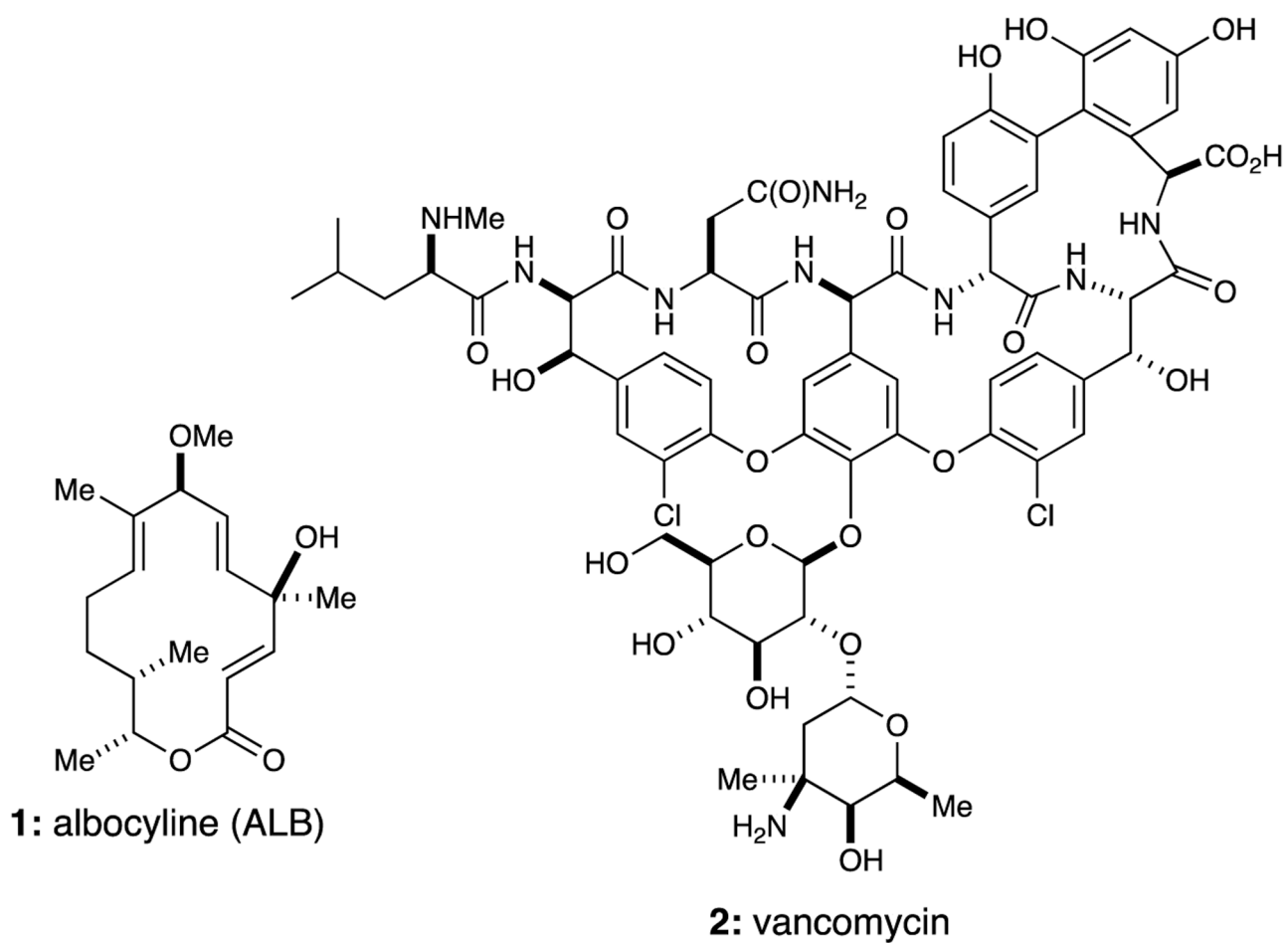


Figure 1.
Structures of albocycline (ALB, **1**) and vancomycin (**2**).

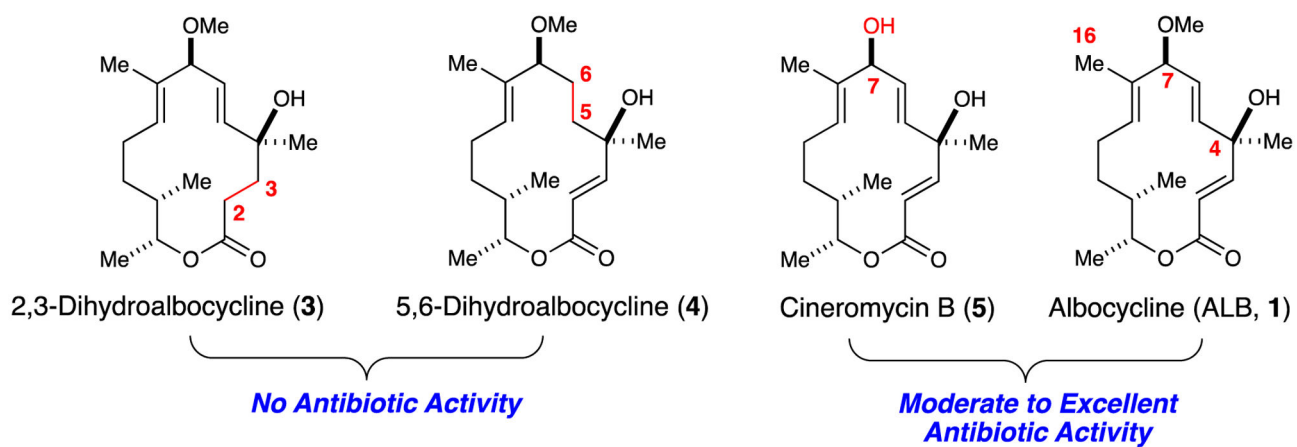
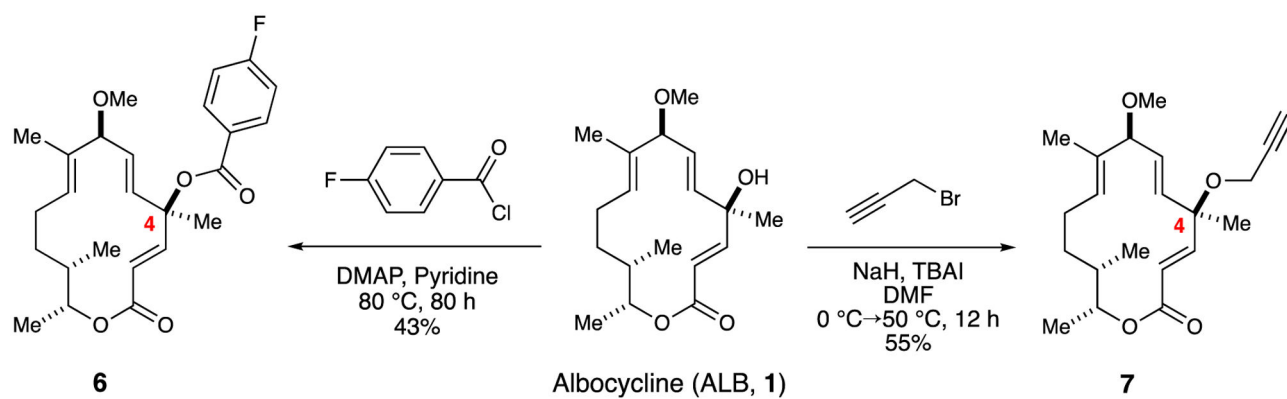
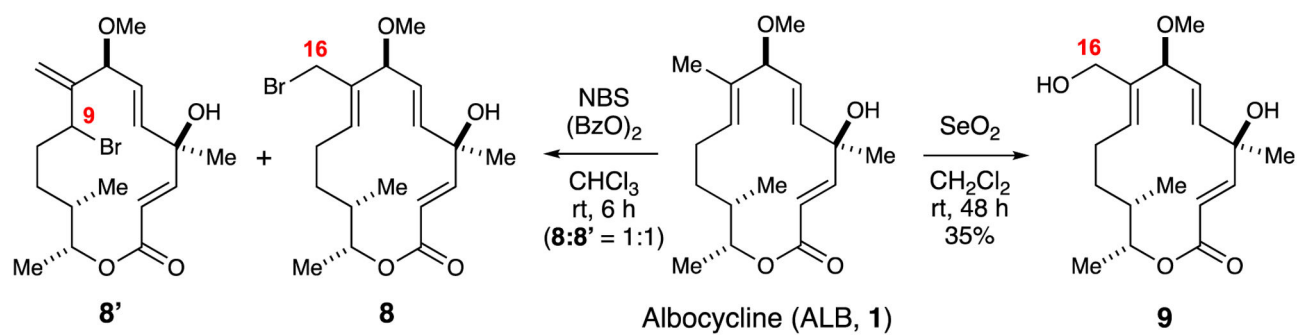


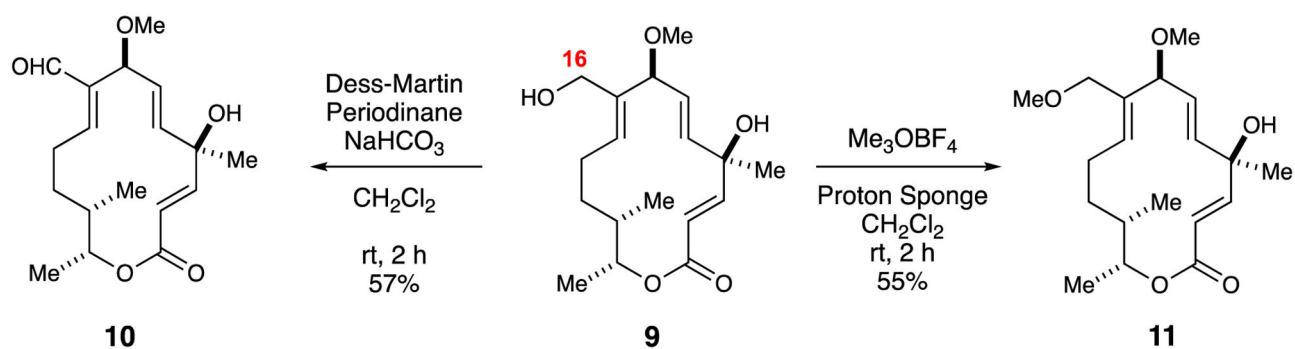
Figure 2.
Studying antibiotic activity across albocycline and its analogs.

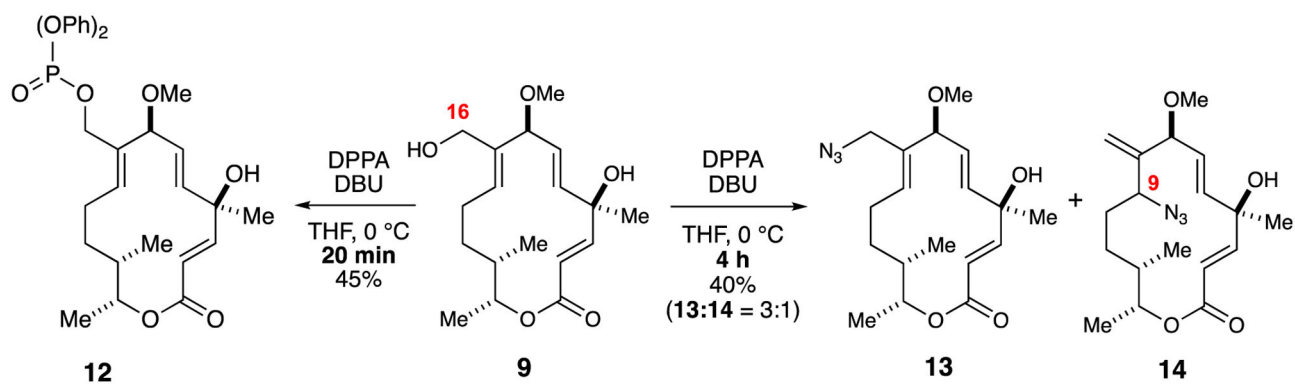
**Scheme 1.**

Functionalization of ALB at the C4 carbinol position by acylation to access ester analog 6 or by alkylation to access ether analog 7.

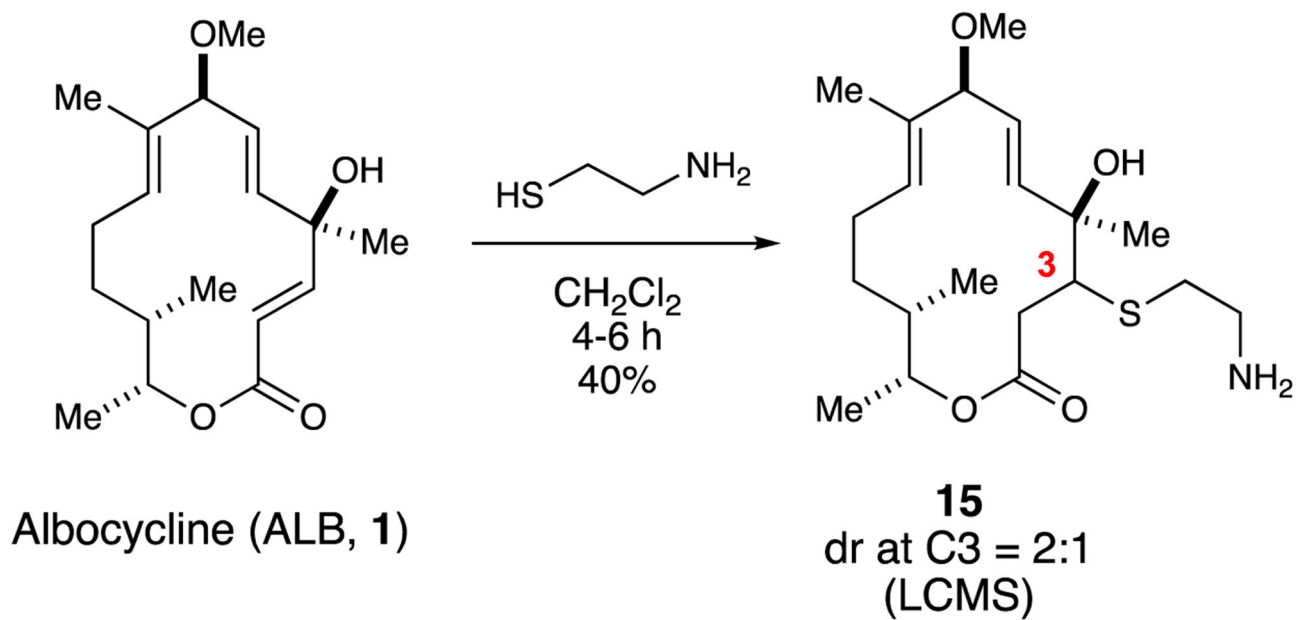
**Scheme 2.**

Allylic functionalization at C16 with NBS or SeO₂ to generate regioisomeric bromides **8** and **8'** or allylic alcohol **9**, respectively.

**Scheme 3.**Allylic functionalization at C16 to generate aldehyde **10** and ether **11**

**Scheme 4.**

Allylic functionalization at C16 to generate azides **13** and **14** from isolable phosphonate intermediate **12**.





Scheme 5.
Synthesis of P-amino-sulfide adduct **15** (dr = 2:1) of ALB.

Table 1.

MIC ($\mu\text{g/mL}$) values for ALB (**1**) and its corresponding analogs against *S. aureus* (UAMS-1) and (USA-300).

Compounds	MIC ($\mu\text{g/mL}$) <i>S. aureus</i>	
	UAMS-1 (MSSA)	USA300 (MRSA)
ALB (1)	2	2
Cineromycin (5)	8–16	4
6	1–4	1
7	>256	ND
9	32	ND
10	32	ND
11	128	ND
12	>256	>256
15 (major)	128	256

 Equipotent with ALB (**1**)

 More potent than ALB (**1**)

* ND = Not Determined