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Laboratory Experiment

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Recruiting the Students To Fight Cancer: Total Synthesis of Goniothalamin

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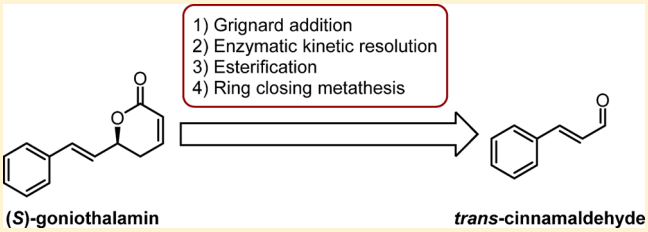
Supporting Information

ABSTRACT: A modified total synthesis of (*S*)-goniothalamin is described for an advanced course in organic chemistry. This experiment gives students an opportunity to handle organometallic reagents and perform an enzymatic kinetic resolution and a metathesis reaction, all in the same synthesis. Furthermore, students learn flame-drying techniques for the manipulation of air-sensitive materials and high-performance liquid chromatography handling and analysis, thus strengthening their experimental knowledge.



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Nature provides us with an unlimited source of natural products that exhibit interesting biological activities. The development of new compounds bearing various structural arrangements and remarkable properties stems mainly from these natural products. For example, it has been shown that styryl lactones, largely found in the genus *Goniothalamus*, exhibited remarkable antiproliferative activity against cancer cells (Figure 1). The *Goniothalamus* family consists of about

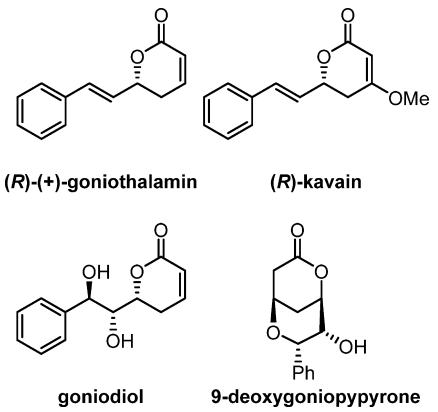

Figure 1. Examples of styryl lactones found in nature.

115 species of shrubs and trees that grow mainly in Asia. Many of these plants are used as a source of fiber, as a repellent against mosquitoes, and especially in traditional medicine. For example, extracts of *Goniothalamus amuyon* seeds are used to treat edema and rheumatism near the southern coast of Taiwan. The insignificant effects that these styryl lactones have on normal cells is well documented, which suggests that the cytotoxicity of the latter seems to specifically target cancer cells.[1] A substantial amount of evidence suggests that the antiproliferative activity of styryl lactones is linked to the induction of apoptosis in target cells.[1]

The goniothalamin styryl is a lactone that was isolated for the first time from the bark of *Cryptocarya caloneura* in 1967 and was assigned to have (*S*)-stereochemistry.[2] However, the stereochemistry was later revised to the (*R*)-configuration.[3] Later on, several derivatives of goniothalamin were discovered from a variety of tropical/subtropical plants, including *Cryptocarya moschata*, *Bryonopsis laciniosa*, and various *Goniothalamus* species.

In 1972, Geran et al.[4] demonstrated during an *in vivo* screening that ethanolic extracts from the bark of *Goniothalamus giganteus* were highly toxic to P-388 cancer cell lines in mice. (*R*)-Goniothalamin exhibits a cytotoxic activity *in vitro* against various strains of cancer cells, such as MDA-MB-231 (breast cancer), HeLa cells (cervical carcinoma), HGC-27 (gastric carcinoma), HL-60 (leukemia), and Caov-3 (ovarian carcinoma). Measured inhibitory activities (IC_{50}) are of the order of micromolar, and it has been shown that the cytotoxic activity comes from different phenomena, such as DNA damage or apoptosis induction or reduction of the migration ability of cells.[5] Recently, it has been shown that the unnatural (*S*)-enantiomer of goniothalamin showed strong cytotoxic activity (IC_{50} = 4 nM) against cell line 786-0 (kidney cancer).[6]

The synthetic chemist today has a primary role to play in the development of new treatment by implementing efficient syntheses of these compounds and preparing unnatural analogues to optimize the biological activity. This highlights the parts of the molecule necessary for biological activity in order to optimize them. Many efficient syntheses of

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goniothalamin and its analogues have been reported in the literature.[7−9] Some natural product total syntheses have appeared in this Journal.[10−12] However, to our knowledge, no analogous experimental procedure has appeared in this Journal. The aim of this experiment is to synthesize enantiomerically enriched goniothalamin in a fast and efficient manner. For that purpose, a modified procedure that allows students to access the latter in fewer steps and higher efficiency has been developed using several key organic reactions that can be found in any research laboratory.

This experiment is designed for an advanced organic course and gives students an opportunity to use organometallic reagents and perform an enzymatic kinetic resolution and a metathesis reaction, all in the same synthesis. These key organic reactions are discussed in most lecture courses but rarely implemented in laboratory experiments. In addition, students learn flame-drying techniques for the manipulation of air-sensitive materials and high-performance liquid chromatography (HPLC) handling and analysis to observe the separated enantiomers. This experiment has been used in an advanced organic course taken by first-year Master's degree students. Students are given 5 days over the course of the semester to complete the synthesis individually; however, only three full laboratory periods (8 h/period) are required. At the end of the semester, each student is asked to submit a full report detailing the entire synthesis (procedures, mechanisms, and NMR and HPLC analyses, etc.), which constitutes a major part of their final grade. For more details on the logistics of scheduling this experiment, see the Supporting Information.

■ PROCEDURE DESCRIPTION

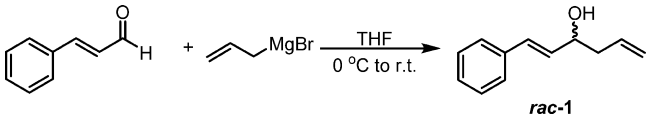
Equipment and Chemicals

A detailed list of all of the equipment and chemicals needed for this experiment can be found in the Supporting Information. A vacuum pump capable of delivering pressures of less than 1 Torr and a compressed argon cylinder are needed for this experiment. All other chemicals can be purchased from Aldrich, Fisher Chemicals, VWR, or Acros; a detailed list of chemicals and their suppliers are given in the Supporting Information.

Step 1: Addition of the Grignard Reagent

Commercially available allylmagnesium bromide is added to *trans*-cinnamaldehyde to form the desired racemic alcohol **1** (Scheme 1). This reaction is fast and, if done properly, highly

Scheme 1. Grignard's Addition to *trans*-Cinnamaldehyde



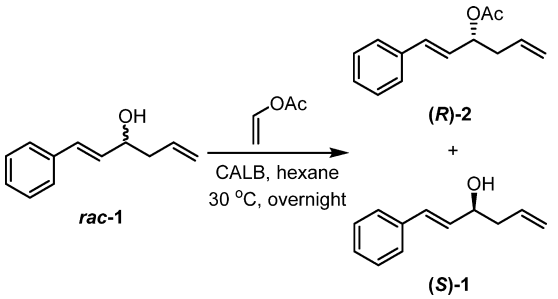
selective. Slow addition of the Grignard reagent is necessary to avoid secondary products and thus avoid a column chromatography of the crude mixture. This reaction serves as a good indicator of a student's manipulation skills.

Generally, no column chromatography is necessary and the crude product is directly used in the next step.

Step 2a: Enzymatic Kinetic Resolution

Lipase CALB (*Candida antarctica* (*C. antarctica*) lipase B or Novozyme 435) is a hydrolase isolated from *C. antarctica* and immobilized in the form of cross-linked aggregates (cross-linked copolymers). This lipase performs the enzymatic transesterification of racemic alcohols by selecting one of the two enantiomers. Vinyl acetate is an irreversible acyl donor (the formation of acetaldehyde in the transesterification reaction prevents the reverse reaction). The advantage of this method is that it can isolate both enantiomerically enriched compounds (unconsumed alcohol (*S*)-**1** and newly formed acetate (*R*)-**2**) by chromatography on silica gel (Scheme 2). The enantiomeric

Scheme 2. Enzymatic Kinetic Resolution of *rac*-1



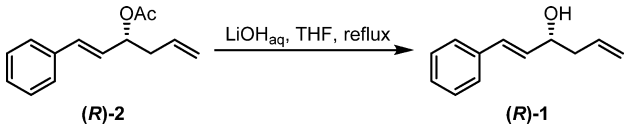
excesses are dependent on the structure of the substrate, the nature of the acylating agent, the reaction parameters, and the time of reaction (and, therefore, the conversion of the substrate).[13]

The crude mixture is analyzed by chiral HPLC to determine the enantiomeric excess of the alcohol and ^{1}H NMR analysis to measure the conversion rate. The alcohol (*S*)-**1** and acetate (*R*)-**2** are separated by chromatography on silica gel and used in subsequent steps.

Step 2b (Side Reaction): Hydrolysis of the Acetate

The acetate (*R*)-**2** is hydrolyzed in standard conditions to generate the other enantiomer of the alcohol (*R*)-**1** (Scheme 3).

Scheme 3. Hydrolysis of Acetate (*R*)-2

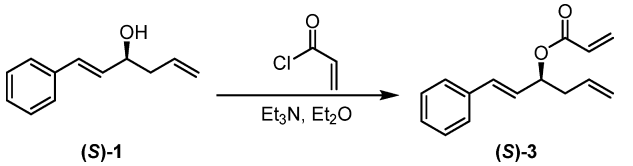


This enantiomer will not be used in subsequent steps but it demonstrates to students that the enantiomers, once mixed, are now completely separated. This enantiomer, if needed, could be converted to the desired (*S*)-**1** via a Mitsunobu reaction.[14]

Step 3: Esterification of Alcohol (*S*)-1

Esterification of the separated alcohol (*S*)-**1** from step 2a in typical conditions furnishes the precursor for the ring closing metathesis (RCM) reaction, generally in high yields (Scheme 4).

Scheme 4. Esterification of Alcohol (*S*)-1



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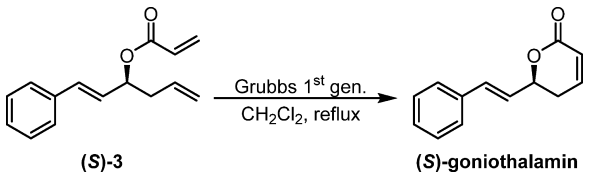
Step 4: Ring Closing Metathesis

The importance of the RCM reaction is demonstrated by its high efficiency to close a wide range of rings and its wide use in the literature as a key step in the synthesis of numerous natural products.[15] In this experiment, first generation Grubbs catalyst is used to cyclize (*S*)-**3** and complete the synthesis of (*S*)-goniothalamin (Scheme 5).

Scheme 5. RCM Reaction To Form (*S*)-Goniothalamin



Although the first generation Grubbs catalyst is not as active as the more recent Grubbs catalysts (second generation and Hoveyda–Grubbs catalysts), the former was found to be cheaper and to catalyze the desired reaction readily in acceptable yields.

HAZARDS

All reactions should be carried out in a fume hood. Triethylamine and allyl magnesium bromide are corrosive. Ethyl acetate, tetrahydrofuran, diethyl ether, triethylamine, acryloyl chloride, allyl magnesium bromide, acetone, and petroleum ether are flammable. Dichloromethane, *trans*-cinnamaldehyde, tetrahydrofuran, ethyl acetate, acetone, and silica gel are irritants. Hexane, dichloromethane, acryloyl chloride, petroleum ether, and diethyl ether are toxic. *n*-Hexane is a neurotoxin. Standard precautions should be employed when working with compressed gases; cylinders should be chained to a wall, and should be opened only with the regulator securely attached. All reaction products should be considered as irritant.

RESULTS

Students have successfully carried out all steps of the experiment. As a result, they were exposed to state of the art chemistry and a wide range of crucial experimental techniques that students will later on need when they take a job or begin a Ph.D. program in organic chemistry. Yields and enantiomeric excess (ee; for (*S*)-**1** and (*R*)-**1**) values obtained by the students for the synthesis of all compounds (**1**–**4**) in this experiment are in Table 1 and Table 2, respectively. The reaction was repeated four times over 4 years (33 students overall) and proved to be highly successful with students and instructors alike.

Table 1. Student Results: Yields

step	av of student yields[a] (%)	range of student yields (%)
1	92.6	80–100
2a[b]	47	36–50
2b	76.1	67–81
3	77.1	53–95
4	63.4	43–72

[a]Average yields of 8 students (N = 8) per step. [b]The maximum theoretical yield for this step is 50%.

Table 2. Student Results: Enantiomeric Excess

alcohol	av of student ee[a] (%)	range of student ee (%)
(*S*)-**1**[b]	93.5	88–99
(*R*)-**1**[c]	87.2	76–92

[a]Average ee of 8 students (N = 8) per alcohol, determined by chiral HPLC. [b]Alcohol from step 2a. [c]Alcohol from step 2b.

ASSOCIATED CONTENT

Supporting Information

Student handout, detailed notes including details on the logistics of scheduling this experiment, list of all of the equipment and chemicals needed for this experiment, and NMR spectra. This material is available via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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