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Laboratory Experiment

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Synthesis of a Parkinson's Disease Treatment Drug, the *R*,*R*-Tartrate Salt *of R*-Rasagiline: A Three Week Introductory Organic Chemistry Lab Sequence

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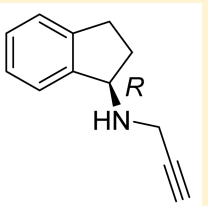
Supporting Information

ABSTRACT: A synthesis of the *R*,*R*-tartrate salt of the popular anti-Parkinson's drug *R*-rasagiline (Azilect) was adapted to introduce the organic laboratory student to a medically relevant synthesis. It makes use of concepts found in the undergraduate organic chemistry curriculum, appropriately fits into three approximately 4 h lab periods, and utilizes readily available equipment and inexpensive commercially available chemicals.



R-rasagiline

KEYWORDS: *Second-Year Undergraduate, Upper-Division Undergraduate, Organic Chemistry, Chirality/Optical Activity, Synthesis, Diastereomers, Drugs/Pharmaceuticals, Asymmetric Synthesis, Hands-On Learning/Manipulatives*

Parkinson's disease (PD) is a degenerative disorder of the central nervous system that often impairs the sufferer's motor skills and speech. Symptoms include muscle rigidity, tremors, and slow physical movement. In PD, decreased stimulation of the motor cortex is caused by a decrease in levels of the neurotransmitter dopamine.[1]

While there is no cure as yet for the disease, drugs such as *R*-rasagiline (Figure 1) alleviate the symptoms[2] by inhibiting the enzyme monoamine oxidase-B (MAO-B),[3] which oxidizes dopamine, thus decreasing its cellular concentration. The result of inhibition of MAO-B by *R*-rasagiline is increased dopamine levels in the brain.[3] *R*-Rasagiline has also been shown to have neuroprotective properties.[3d,4] In comparison with *R*-selegiline (Figure 1), the first MAO inhibitor approved for PD, *R*-rasagiline has reduced side effects and improved efficacy.

This is because *R*-rasagiline is metabolized in vivo to 1-indanamine (Figure 1), which has neuroprotective effects,[5a,b] unlike *R*-selegiline, which is metabolized to the neurotoxic metabolite 1-methamphetamine[5a,c] (Figure 1, street name, "meth"), which causes amphetamine-like cardiac and psychiatric effects.[5d,e] Because of these reasons, *R*-rasagiline has become a popular drug for the treatment of PD.

Illustrations of organic chemistry concepts derived from pharmaceuticals and medicine are inherently valuable but may be particularly interesting to the increasing numbers of organic chemistry students intending to enter the health professions. With this in mind, a known synthesis of *R*/*S*-rasagiline[6] was adapted for the introductory organic chemistry lab. Purification and then optical resolution yield a pure *R*-rasagiline salt[7] (now off-patent; trade name, Azilect). This sequence fits into three approximately 4 h lab periods using standard equipment and commercially available reagents.

In the past 20 years, a number of syntheses of pharmaceutical agents have been published in this journal,[8] but none of them aimed at preparing a drug to treat a neurological disease. Another advantage of the current synthesis is its adaptability toward different curricula. Many syntheses are project-type[9] and do not conveniently fit into blocks of 4 h. This makes it difficult for some schools to conveniently adopt many of the published syntheses for a second-year curriculum. This synthesis can be easily adopted by schools in their traditional second-year organic lab course without modifying lab schedules.

The first goal of this experiment was to experimentally illustrate theoretical concepts commonly found in the second-year or upper-division organic chemistry undergraduate curriculum (e.g., nucleophilic substitution, etc.), while the second goal was to afford students an opportunity to reinforce a range of fundamental laboratory techniques and calculations (e.g., acid−base extraction, etc.). We wanted to achieve these two goals in the context of synthesizing a popular neurological drug.

OVERVIEW OF THE EXPERIMENT

In the first lab (see Student Handout, Experimental Procedure, Supporting Information), students carry out an S_N2 reaction on propargyl benzenesulfonate with indanamine·HCl in aqueous

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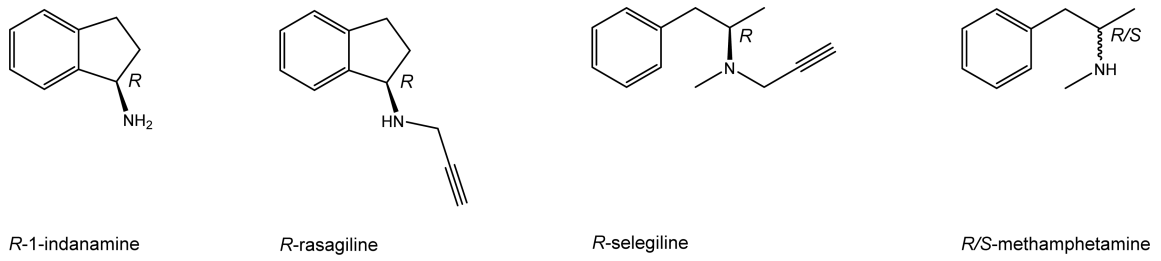
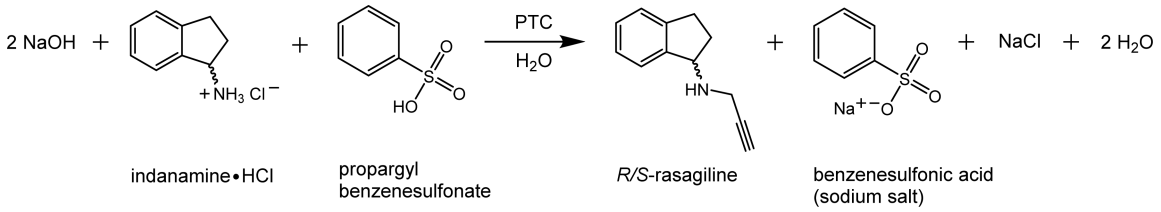
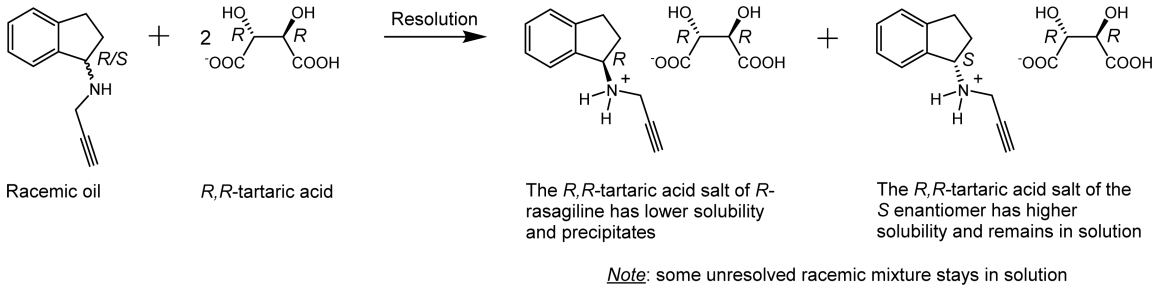


Figure 1. Compared to *R*-rasagiline, *R*-selegiline is similar in structure to *R*/*S*-1-methamphetamine, a neurotoxin.

Scheme 1. Scheme for the Synthesis of *R*/*S*-Rasagiline



Scheme 2. Scheme for the Resolution of *R*/*S*-Rasagiline to *R*-Rasagiline



NaOH using a phase transfer catalyst, tetrabutylammonium bromide (Scheme1).[6] The reaction is monitored by thin-layer chromatography (TLC), and the crude product is extracted into ethyl acetate after NaOH neutralization. The entire procedure takes 4 h. A mechanism for this reaction was provided to the students.

In the second lab, the crude product is partially purified via an acid−base extraction, followed by silica gel chromatography in a 50 mL plastic syringe barrel to remove some dialkylated product (∼10%) to give pure *R*/*S*-rasagiline as a pale yellow oil.

In between the second and third lab, students characterize the initially obtained racemic mixture using ^{1}H, ^{13}C, and infrared (IR) spectroscopy, and gas chromatography−mass spectrometry (GC−MS). The third lab involves the resolution of the racemic secondary amine into *R*-rasagiline salt using *R*,*R*-tartaric acid (Scheme 2).

Recrystallization of the precipitated salt from isopropanol/methanol yields pure *R*-rasagiline/*R*,*R*-tartaric acid salt. The diastereomeric salt is dried overnight in an oven before the students take a melting point and optical rotation of the sample.

In an extension of the experiment, a small cohort of students recovered the free base (pure *R*-rasagiline) using NaOH and characterized it using TLC and ^{1}H and ^{13}C NMR analyses. Table S1 (p S20, Instructor's Notes, Supporting Information) outlines the experiment timelines.

HAZARDS

Ethyl acetate, methanol, toluene, and isopropyl alcohol and their vapors are toxic, can cause skin and eye irritation, and are flammable. Methanol may cause blindness if ingested. Propargyl benzenesulfonate, tetrabutylammonium bromide, 1-indanamine, sodium sulfate, and *R*,*R*-tartaric acid can cause skin irritation, eye irritation, and may cause respiratory irritation. 1-Indanamine may cause vomiting and diarrhea. In view of these hazards, open flames should be avoided, gloves and proper eye protection should be worn, and all manipulations should be carried out in a properly ventilated hood. *R*/*S*-Rasagiline and its *R*,*R*-tartrate salt can have adverse psychotic effects if ingested. Silica gel is carcinogenic. All manipulations involving silica gel should be carried out in a fume hood, and it should be disposed of in appropriately labeled solid waste containers. Standard precautions should be taken when handling all chemicals. Solid and liquid waste should be disposed of in properly labeled containers as halogenated or nonhalogenated waste.

RESULTS AND DISCUSSION

This experiment was performed with 38 students working individually in the second semester organic chemistry lab. They obtained a range of yields (27−63%), with an average of 45% overall yield, from indanamine·HCl to the racemic oil (details can be found in Instructor Notes-Characterization Data and Yields, Supporting Information). Each student carried out hands-on product characterization (^{1}H, ^{13}C, and IR spectroscopy, and GC−MS) on the initially obtained racemic mixture. The resulting data showed that the purity of the students' *R*/*S*-rasagiline product was consistently high, and their ^{1}H and ^{13}C NMR, IR, and GC−MS data agreed with literature values.[10] The racemic mixture was then resolved, and the *R*,*R*-tartaric

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acid salt of *R*-rasagiline was obtained. The average student yield was 91% for the resolution step (based on the *R* isomer only). The salt's purity was excellent as indicated by the expected bright white color of the crystals and, more importantly, by the fact that the average melting point of the salt was 175.5−178.1 °C, in close agreement with reported values (175−177 °C).[11] The literature value for optical rotation is $[\alpha]^{25}_{589}$ = +34.3, *c* = 1.5 g/100 mL of water.[11] Students obtained an average of $[\alpha]^{25}_{589}$ = +31.09, *c* = 1.5 g/100 mL of water. This is an average of 91% enantiomeric excess of the *R*,*R*-tartaric acid salt of *R*-rasagiline. As an extension of the experiment, a cohort of six students (a mixture of second-year and upper-division), working individually, performed the entire experiment with the same results as mentioned above. However, they took the additional step of recovering (average 91% recovery) the free base from the aqueous solution of the *R*,*R*-tartaric acid salt of *R*-rasagiline. TLC and ^{1}H and ^{13}C NMR spectroscopic characterization of *R*-rasagiline as free base showed that a very pure compound was recovered. Further, it demonstrated that an isolated enantiomer has the same NMR spectrum as the racemic mixture.

The complex ^{1}H NMR spectrum of purified *R*/*S*-rasagiline product is very well resolved. Students were given labeled ^{1}H and ^{13}C NMR spectra of propargyl alcohol and 1-indanamine (see Student Handouts—Spectral Data, Supporting Information), and on the basis of this were asked to interpret their proton and carbon NMR spectra of *R*/*S*-rasagiline. In their lab reports, students were also asked to identify homotopic, diastereotopic, and enantiotopic protons in 1-indanamine and in their product *R*/*S*-rasagiline spectra. They were also asked to interpret their IR data and to identify specific fragments for the mass spectrum they obtained.

The vast majority of students (>90%) were successful in their spectral interpretations as judged by their lab reports. Students commented that the 1-indanamine and propargyl alcohol NMR spectra that were provided helped considerably. Since students were also provided with a detailed explanation of homotopic, diastereotopic, and enantiotopic protons, most students were able to identify these protons in their NMR spectrum, though a few needed additional help in clarifying stereochemical concepts such as enantiomers and diastereomers.

Student feedback included comments such as "I plan on med school so I appreciated the chance to synthesize a drug"; "Nice to see organic chemistry concepts employed in the real world"; and "...it takes the mystery out of drug making".

CONCLUSION

The first goal of this experiment was to experimentally illustrate basic organic theoretical concepts such as nucleophilic substitution, acid−base chemistry, and stereochemical concepts such as chirality, enantiomers, diastereomers, and chiral resolution via salt formation. This goal was achieved by integrating these concepts into the experimental framework of the drug synthesis. The experiment also demonstrates that unlike enantiomers, diastereomers may exhibit very different solubility properties, and these differences in solubility can be exploited to separate a racemic mixture in the resolution step. The second goal was to afford students an opportunity to reinforce a range of fundamental laboratory techniques and calculations. Students had to perform mole and weight calculations, optical rotation calculations, monitor reactions via TLC, perform solvent extraction, acid−base extraction, rotary-evaporation, column chromatography, recrystallization, polarimetry, and melting point analysis. In addition, they had individual hands-on use of NMR, IR, and GC−MS instrumentation and then interpreted characterization data. Importantly, these goals were achieved within the framework of the synthesis of a popular drug for a common neurological disease; namely, the synthesis of the *R*,*R*-tartrate salt of *R*-rasagiline, a popular drug used for the treatment of PD.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.5b00357.

Notes for the instructor; detailed experimental procedure; safety information (PDF, DOCX)
Student handout: spectra (PDF, DOCX)
Instructor material: characterization data (PDF, DOCX)

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Notes

The authors declare no competing financial interest.

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