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What's that optical isomer?

Problem-solving using
^{31}P benchtop NMR spectroscopy

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INTRODUCTION

At first glance, it might appear that molecules are two-dimensional in nature, especially when they are presented on paper or slides in a presentation. In reality, most molecules are three-dimensional as a result of the spatial arrangements which their atoms and bonds occupy.[1] The 3D orientation of molecules in space is known as stereochemistry and is taught to students in introductory and advanced chemistry and biology courses. One of the core concepts of stereochemistry is chirality. A chiral center, in its simplest and most common case, is an atom with four different groups bonded to it. This concept is introduced to help students gain a better understanding of the differences between optical isomers (*i.e.*, enantiomers and diastereomers). Enantiomers are defined as optical isomers that have a non-superimposable mirror image (**Figure 1**), while diastereomers are defined as all optical isomers that are not enantiomers.

While enantiomers differ only by their respective configurations (*R*/*S*), this small distinction has enormous implications with respect to biological activity and metabolism. Perhaps the most infamous example of these implications is the case of thalidomide.

In the 1950s, thalidomide was a pharmaceutical sold to help reduce nausea during pregnancy. Unknown at the time were the different effects of the (*R*) and (*S*) enantiomers on the body. Later studies revealed that (*R*)-thalidomide has sedative effects, while its enantiomer (*S*)-thalidomide has teratogenic effects.[2] Thus, being able to distinguish between enantiomers is crucial.

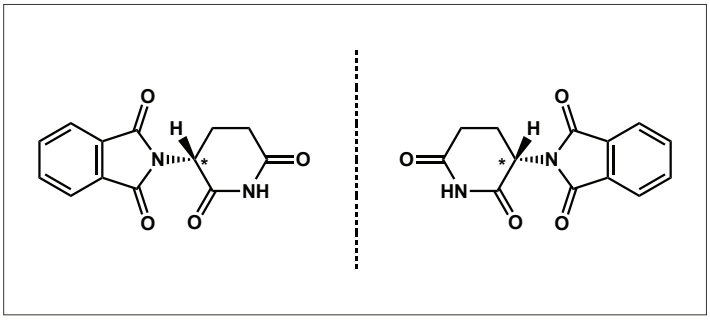


Figure 1. The chemical structures depicting the (R, left) and (S, right) enantiomers of thalidomide.

Nuclear magnetic resonance (NMR) spectroscopy is an analytical chemistry technique with a unique ability for structural elucidation, as well as the unique advantage of being inherently quantitative. As a result, NMR spectroscopy has been used to determine the enantiomeric excess and absolute configuration of substrates. Since enantiomers are magnetically equivalent by nature, the NMR spectra of enantiomers show one distinct set of signals, and as a result, the two species are indistinguishable. However, chiral derivatizing agents (CDA) can be used to convert a mixture of enantiomers into diastereomers, allowing for the amount of each enantiomer to be determined.

Although the topic of stereochemistry is taught throughout the undergraduate curriculum, the spatial relationships between the atoms and bonds in a molecule still elude many students. Dry labs have been implemented to help students with the visualization of these concepts using 3D molecular kits; however, more hands-on and experimental exposure is needed to help students better understand these concepts. Herein, we report a wet lab approach to help complement the learning of stereochemistry using ^{31}P benchtop NMR spectroscopy. In this experiment, adapted from an article published by Sculimbrene and Fenton,[3] ^{31}P NMR spectroscopy was utilized to determine different stereochemical outcomes when enantiopure or racemic alcohols are coupled to an achiral phosphorus center.

PROCEDURE

Racemic, (*R*), or, (*S*)-*sec*-phenethyl alcohol (24.6 mmol, 3.00 equiv.) was dissolved in toluene (30 mL) in a flask. Dimethylaniline (DMA) (16.4 mmol, 2.00 equiv.) and phosphorus trichloride (8.19 mmol, 1.00 equiv.) were added sequentially. The reaction was allowed to stir for 2 hours. The reaction mixture was then washed with 30 mL of distilled H_2O, 30 mL of 5M NH_4OH and 30 mL of a saturated brine solution. The organic layer was dried with $MgSO_4$, filtered, and concentrated. This procedure was repeated for racemic, (*R*), and (*S*)-*sec*-phenethyl alcohol to yield the respective phosphonate compounds.

Di-*sec*-phenethyl phosphonate (120 mg, 0.413 mmol) was dissolved in 0.6 mL of $CDCl_3$. The ^{31}P NMR spectrum was then acquired for each of the final products using the Benchtop NMR 60 instrument (spectral width: 100 ppm, spectral center: 10, number of points: 8192, number of scans: 256, scan delay: 0 seconds).

RESULTS AND DISCUSSION

Figure 2 depicts two ^{31}P NMR spectra of di-*sec*-phenethyl phosphonate synthesized with: a) racemic *sec*-phenethyl alcohol and b) *(R)* or *(S)* enantiopure *sec*-phenethyl alcohol. A quick analysis of the obtained spectra reveals that the racemic mixture results in three signals in a 1:2:1 ratio while the *(R)* or the *(S)* enantiomer results in a single resonance in the ^{31}P NMR spectrum.

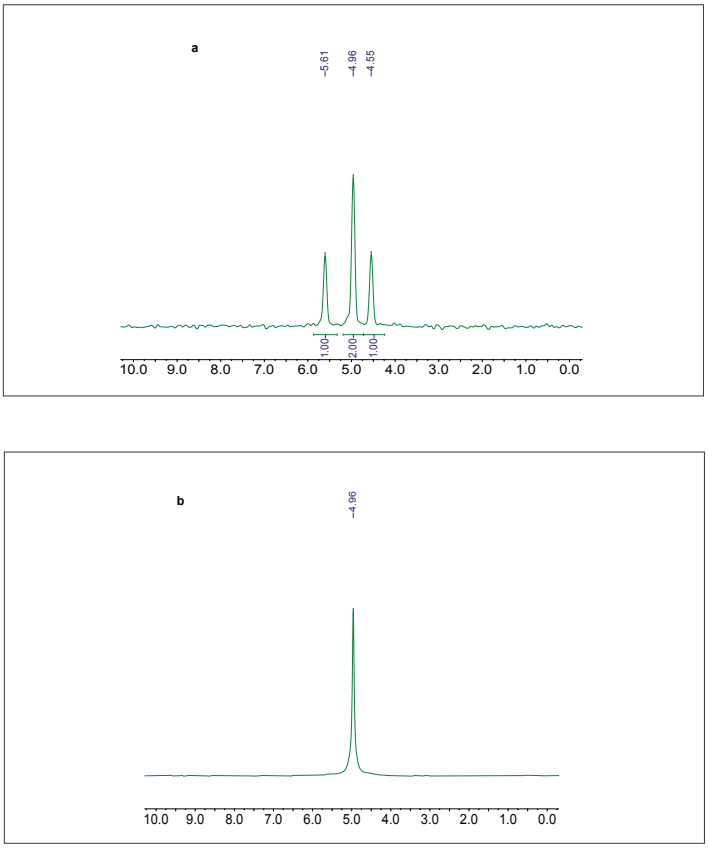
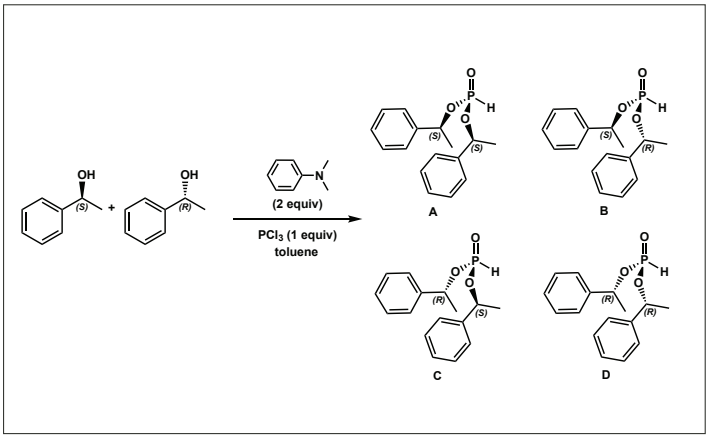


Figure 2. a) $^{31}P\{^{1}H\}$ NMR spectrum of racemic di-sec-phenethyl phosphonate (top). b) $^{31}P\{^{1}H\}$ NMR spectrum of optically pure (R) or (S) di-sec-phenethyl phosphonate (bottom).

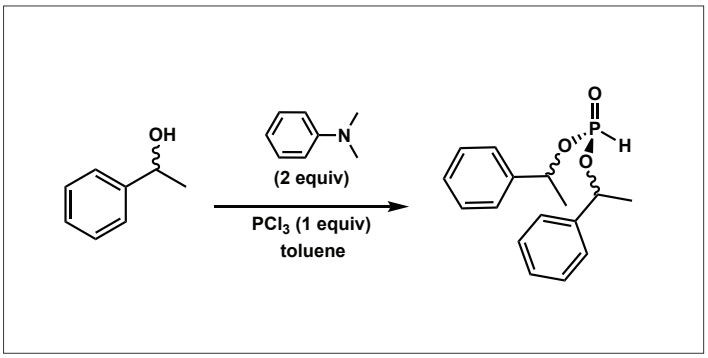
The differences in the ^{31}P NMR spectra can be explained by considering the starting material used. In the scenario where racemic *sec*-phenethyl alcohol was used, half of this substrate was composed of the (*R*)-enantiomer while the other half was composed of the (*S*)-enantiomer. If one takes into account the different kinds of dialkyl phosphonate stereoisomers that can be formed using a combination both of (*R*) and (*S*) *sec*-phenethyl alcohol, it is clear that four final products will be synthesized.



Scheme 1. Reaction scheme for the synthesis of the four stereoisomers of di-sec-phenethyl phosphonate produced when using racemic sec-phenethyl alcohol as the starting reagent.

As shown in Scheme 1, when racemic *sec*-phenethyl alcohol is used as a starting material, four products are synthesized (A, B, C, D). Products A and D are enantiomers while products B and C are diastereomers of one another as well as of products A and D. Since enantiomers are magnetically equivalent, products A and D give rise to the same chemical shift, at 4.96 ppm. Products B and C are diastereomers of each other as well as products A and D, and thus, provide signals at two different chemical shifts, 5.61 and 4.55 ppm. As a result, three distinct signals are observed in the ^{31}P NMR spectrum with a 1:2:1 ratio as shown in **Figure 2a**.

Conversely, if only (*R*) or (*S*) enantiopure *sec*-phenethyl alcohol is used for the synthesis as shown in **Scheme 2**, only one product is formed, D or A, respectively. This leads to the observation of a single signal in the ^{31}P NMR spectrum (**Figure 2b**).



Scheme 2. Reaction scheme for the synthesis of one stereoisomer of di-sec-phenethyl phosphonate using (R) or (S) enantiopure sec-phenethyl alcohol.

CONCLUSION

In this experiment, di-*sec*-phenethyl phosphonate was synthesized and analyzed using ^{31}P NMR spectroscopy. The resulting spectra varied depending on which substrate was used. If starting from the racemic mixture, multiple stereoisomers were formed, as opposed to only one stereoisomer when starting from optically pure substrates. Furthermore, students can benefit from a wet lab approach to stereochemistry, which also incorporates heteroatom NMR benchtop spectroscopy.

References

[1] Hunt, I .Ch7:Stereochemistry.http://www.chem.ucalgary.ca/courses/351/Carey5th/Ch07/ch7-0.html (accessed Mar 20, 2020).

[2] Vargesson, N. *Birth Defects Research Part C: Embryo Today: Reviews* **2015**, *105* (2), 140-156.

[3] Fenton, O.; Sculimbrene, B. *Journal of Chemical Education* **2011**, *88* (5), 662-664.

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