# Synthesis and ^{1}H NMR Spectroscopic Elucidation of Five- and Six-Membered D-Ribonolactone Derivatives

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**S** *Supporting Information*

**ABSTRACT:** Lactone-size identification of D-ribonolactone derivatives has been debated for four decades due to complex lactone-ring rearrangements and acetal migration. This laboratory experiment for an upper-division undergraduate organic chemistry laboratory course describes a fast and reliable assignment of lactone-size derivatives from D-ribose using ^{1}H and ^{1}H–^{1}H NOESY NMR spectroscopy. In addition, the further derivatization of these lactones via *O*-acylation is described, followed by a theoretical study that allows conformational analysis of these molecules in solution using JANOCCHIO software. Overall, this experiment uses simple, inexpensive, and commercially available reagents, and the reactions are performed in mild conditions. The products are solid and can be either purified by crystallization or used directly in the next step of the synthesis. ^{13}C, 2D ^{1}H–^{1}H COSY, and ^{1}H–^{13}C HMQC or HSQC spectra are also analyzed. Therefore, this laboratory experiment allows students to explore several NMR techniques and relate them with basic NMR concepts, such as scalar coupling constants related to dihedral angles and the NOE effect, to predict lactone-size and conformation of the synthesized molecules in solution.

**KEYWORDS:** *Upper-Division Undergraduate, Laboratory Instruction, Organic Chemistry, Synthesis, Computer-Based Learning, Carbohydrates, Chirality/Optical Activity, Equilibrium, IR Spectroscopy, NMR Spectroscopy*
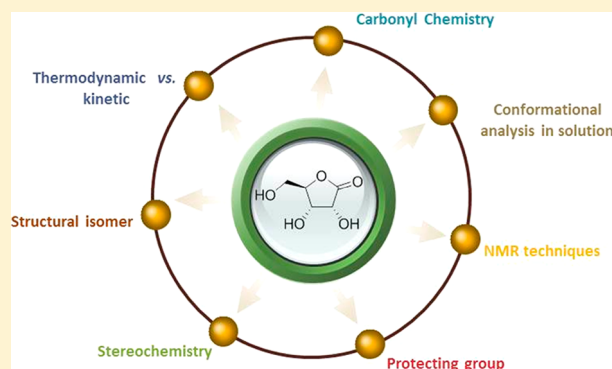
## ■ INTRODUCTION

Carbohydrates constitute a major class of building blocks for the synthesis of natural products and biologically active compounds. Ribose is an important natural saccharide and acts as the glue that cements the genetic code.[1] However, this class of compounds has generally been neglected at the undergraduate level because the characterization of pentose sugars is considered complicated and often requires the synthesis of derivatives, during which unexpected rearrangements can occur.[2] Several experiments have been reported in this *Journal* that focus on the synthetic transformations of carbohydrates,[3] including one D-ribose glycosylation with methanol in an acidic medium.[4] However, a key limitation in the latter experiment is the need for ^{1}H NMR analysis of the six possible tautomers of ribose (**1**)[2,4] (Scheme 1) in aqueous solution, which requires complex ^{1}H NMR analysis at the undergraduate level.

Oxidation of the anomeric center of an aldose can simplify the characterization by transforming the hemiacetal functional group into a carboxylic acid group that can form an intramolecular cyclic ester (lactone). In the case of D-ribose, a five-membered lactone (**2**) and a six-membered lactone may result (Scheme 2).[5] Although these two lactones can be differentiated using ^{1}H NMR spectroscopy,[6] the analysis is not straightforward, which is generally true for saccharide-derivative

**Scheme 1. Six Tautomers (Percent Abundance) for D-Ribose (1) in Aqueous Solution**
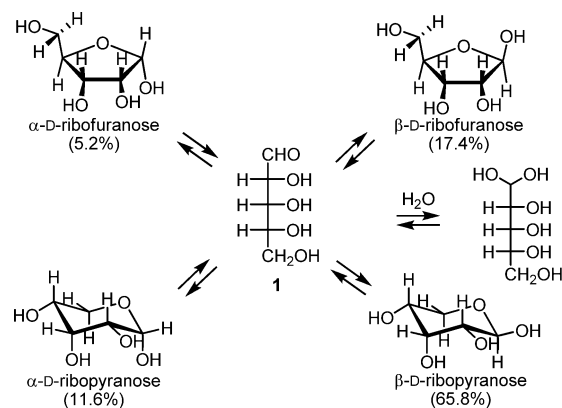


lactones.[1] This laboratory experiment describes the preparation of five- and six-membered lactones from D-ribose (**1**) using protocols for 1,2-diol protection.[1,7] Structural assignments of these two lactones are accomplished by two-dimensional [1]H–[1]H correlation spectroscopy (COSY), [1]H–[13]C heteronuclear correlation through quantum coherence (HMQC) or

## Scheme 2. Syntheses of Five- and Six-Membered D-Ribolactones
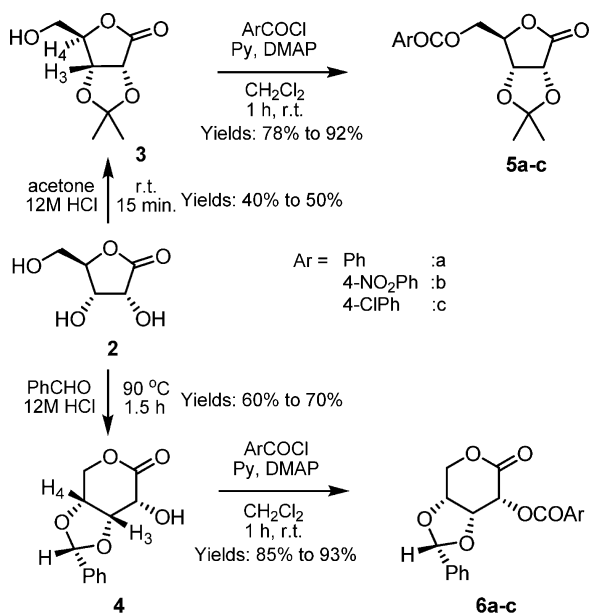


heteronuclear single quantum coherence (HSQC), and $^1$H−$^1$H nuclear Overhauser effect spectroscopy (NOESY). The experiments require an NMR spectrometer with a minimum of a 300 MHz field strength and are organized in two ways: (1) a single, 3-h lab session for preparing the kinetic product (isopropylidene-D-ribolactone, **3**) and the thermodynamic adduct (benzylidene-D-ribonolactone, **4**), followed by NMR analysis; and (2) two, 3-h lab sessions for the preparation of **3** and **4**, as well as the syntheses of the *O*-acyl derivatives of these lactones (**5** and **6**), and NMR studies to verify whether unexpected products are formed as a result of undesired rearrangements. In addition, a theoretical study is described using the open source software JANOCCHIO.[8] This software allows for comparisons of experimental *J* and NOE values for structures modeled with computational programs, which permits conformational analysis of the molecules in solution. Thus, this laboratory experiment provides an excellent opportunity for upper-division undergraduate majors in chemistry to enhance their knowledge of organic synthesis and NMR spectroscopy. The main pedagogic goal of this laboratory experiment is to teach students the difference between scalar couplings (*J*) and the dipolar interaction of spins (NOE effect) and how these NMR principals can be used to assign the lactone ring-size of D-ribonolactone derivatives correctly.

## ■ EXPERIMENT

### Lab Session 1

Preparation of isopropylidene-D-ribonolactone **3** and Zinner-lactone **4**. Students work in groups of two. D-Ribonolactone (**2**) is supplied to students. Detailed procedures are in the Supporting Information.

**2,3-*O*-Isopropylidene-D-ribonolactone (3).** HCl (12 M, 0.1 mL) is added to D-ribonolactone (**2**) (150 mg) and acetone (1.5 mL) in a 10 mL round-bottomed flask (RBF). After 15 min at room temperature, CH$_2$Cl$_2$ (0.5 mL) and NaHCO$_3$ (0.3 g) are added. After workup, **3** is obtained and characterized by melting point, IR spectroscopy, and NMR spectroscopy without further purification.

**3,4-*O*-Benzylidene-D-ribonolactone (4).** D-Ribolactone (**2**) (85 mg) is added to benzaldehyde (0.58 mL). The mixture is warmed to 50 °C, HCl (12 M, 0.05 mL) is added, and the mixture is heated at 90 °C over 1.5 h. After workup, **4** is obtained and characterized by melting point, IR spectroscopy, and NMR spectroscopy without further purification.

### Lab Session 2

**Extension Project: 5-*O*-Acyl-2,3-*O*-isopropylidene-D-ribonolactone (5) and 2-*O*-Acyl-3,4-*O*-benzylidene-D-ribonolactone (6).** **3** or **4** (0.40 mmol) and CH$_2$Cl$_2$ (2.5 mL) are added to a 25 mL RBF. An acyl chloride (benzoyl chloride, 4-nitrobenzoyl chloride, or 4-chlorobenzoyl chloride) (0.48 mmol) is added, followed by pyridine (0.5 mL) and a catalytic amount of DMAP, and the mixture reacts at room temperature for 1 h. After workup, a white solid is obtained, which can be crystallized from a mix of acetone and hexanes or purified by flash chromatography. **5** and **6** are characterized by melting point, IR spectroscopy,[1,7] and NMR spectroscopy.

## ■ HAZARDS

Chloroform-*d* is carcinogen (LD$_{50}$ 0.908 g/kg). Dichloromethane can be narcotic at high concentrations and is a suspected carcinogen. Acyl chlorides are lachrymators and must be manipulated in a fume hood. Long-term exposure to pyridine can cause sterility in males. Hydrochloric acid is a gas that is dissolved in water, and the concentrated acid fumes in air. The vapors are extremely corrosive and irritating. It is not expected that the carbohydrate derivatives herein synthesized present toxicity risks in the quantities manipulated. However, to address the main safety risks during this experiment, it is strongly advised that students wear gloves and use a fume hood when working with the aforementioned chemicals and substrates.

## ■ RESULTS AND DISCUSSION

This experiment has been performed four times in two different institutions (Brazil and Uruguay) with a total of 16 students. Thirteen students performed both sessions, and 12 students were able to synthesize all compounds. Lactone **3** was obtained in 40−60% yield; the Zinner-lactone **4** was prepared in 60−70% yield. Carbonyl stretching on IR and the melting point (all agreed with literature) determination were used to confirm the synthesis of the desired products **3** and **4**. The *O*-acylation of **3** and **4** was conducted using benzoyl chloride, 4-nitrobenzoyl chloride, and 4-chlorobenzoyl chloride to give **5a−c** (78−92% yield) and **6a−c** (85−93% yield), respectively. Products **5** and **6** were confirmed by observing an extra carbonyl stretching in the IR (two carbonyls) and by melting point (all agreed with literature) determination, followed by a comparison with published values.[2,7] All compounds synthesized are solids at room temperature and were fully characterized after crystallization.

The structure of the product of the reaction of D-ribonolactone (**2**) with benzaldehyde in acidic media was initially proposed by Zinner et al.[9] and confirmed by NMR spectroscopy as 3,5-*O*-benzylidene-1,4-lactone,[10] a five-membered lactone. Surprisingly, the X-ray crystal structure of its *O*-acetyl derivative was the six-membered lactone.[11] A complex study involving acetal migration and ring expansion was performed to explain this unexpected result.[5] Thus, the use of D-ribonolactone (**2**) and its *O*-acyl derivatives as chiral building blocks relies on the development of a facile method

that allows for the correct assignment of the lactone ring-size.[1] $^{13}$C NMR spectroscopy was proposed as a method to assign the lactone ring-size, supported by considerable differences in the C-3 and C-4 chemical shifts.[1] $^{13}$C NMR data (available in the Supporting Information) compiled for the five- and six-membered lactones prepared in this experiment are shown in Table 1. Six-membered lactones generally have shielded C-3

**Table 1. C-3 and C-4 $^{13}$C NMR Chemical Shifts for Five- and Six-Membered D-Ribonolactone Derivatives (DMSO-$d_6$)$^a$**

| Lactone | C-3 | C-4 | Ring Size |
|---|---|---|---|
| **6a** | 74.4 | 73.4 | 6 |
| **6b** | 74.2 | 73.4 | 6 |
| **6c** | 74.5 | 73.5 | 6 |
| **4** | 76.7 | 67.8 | 6 |
| **2** | 68.6 | 85.4 | 5 |
| **3** | 78.0 | 82.2 | 5 |
| **5a** | 77.3 | 79.6 | 5 |
| **5b** | 77.1 | 79.5 | 5 |
| **5c** | 77.2 | 79.5 | 5 |

$^a$All data presented in this table were collected from samples prepared by students.

and C-4 (lactones **4**, **6** vs **3**, **5**). On the other hand, D-ribonolactone (**2**), a five-membered lactone, has the C-3 at 68.6 ppm, which is farther upfield than the C-3 chemical shifts found in the six-membered lactones **6**. Additionally, C-4 in the six-membered lactones **6** is approximately 6 ppm farther upfield than the five-membered lactones **5**. Thus, structural determination based solely on $^{13}$C NMR spectroscopy for these lactones can be misleading.

Because H3 and H4 are trans in the five-membered derivatives and cis in the six-membered lactones (Scheme 2), the nuclear Overhauser effect (NOE) was proposed as a reliable method to identify the lactone ring-size for these compounds.

However, the chemical shifts of the hydrogens on some of these molecules are too close to one another in the $^1$H NMR spectra to use 1D NOE. Instead, the 2D version ($^1$H–$^1$H 2D NOESY) was chosen.[1] In the $^1$H–$^1$H NOESY spectrum (Figure 1) for the kinetic isopropylidene-lactone **3** and 5–10% of the six-membered thermodynamic lactone, the diastereotopic CH$_2$ (3.95 ppm) gives a strong NOE with H-4 (4.65 ppm) and, as expected, there is no cross peak or only a very weak signal between H-3 (4.80 ppm) and H-4. (See the Supporting Information for further discussion.)

The Zinner-lactone **4** is insoluble in most organic solvents. Therefore, the NMR analysis was performed in DMSO-$d_6$. A drop of D$_2$O was added to the sample, which resulted in a proton/deuterium exchange that shifted the hydroxyl hydrogen from 5.87 to 5.97 ppm. Consequently, the coupling ($^3J$) between OH (5.87 ppm) and H-2 (4.65 ppm) decreased, which resulted in a better solved hydrogen spectrum (Figure 2).

The $^1$H–$^1$H NOESY spectrum for the Zinner lactone **4** (Figure 3) showed a medium intensity H3–H4 cross peak, which indicated a six-membered lactone.

The same trend was observed for the H3–H4 NOE contacts in the 1,4- and 1,5-D-ribolactones synthesized in session 2 (Table 2). The H3–H4 contact was of moderate intensity for the six-membered lactones, and the H3–H4 interaction of the five-membered lactones was not present or was very weak. Thus, the H3–H4 interaction gave practical information about the lactone ring-size.

What can be inferred about ring rearrangements during the acylation of **3** and **4**? As seen in Table 2, rearrangement did not occur during the acylation reaction because there was no change in the lactone ring-size from **3** to **5** or from **4** to **6**.

Next, students were instructed to check and interpret the differences in the cross peaks found in the $^1$H–$^1$H NOESY and $^1$H–$^1$H COSY spectra. For example, the singlet at 5.72 ppm assigned to the benzylidene hydrogen of **4** gives an NOE to H-3 and H-4 (Figure 3). The same interaction was not observed
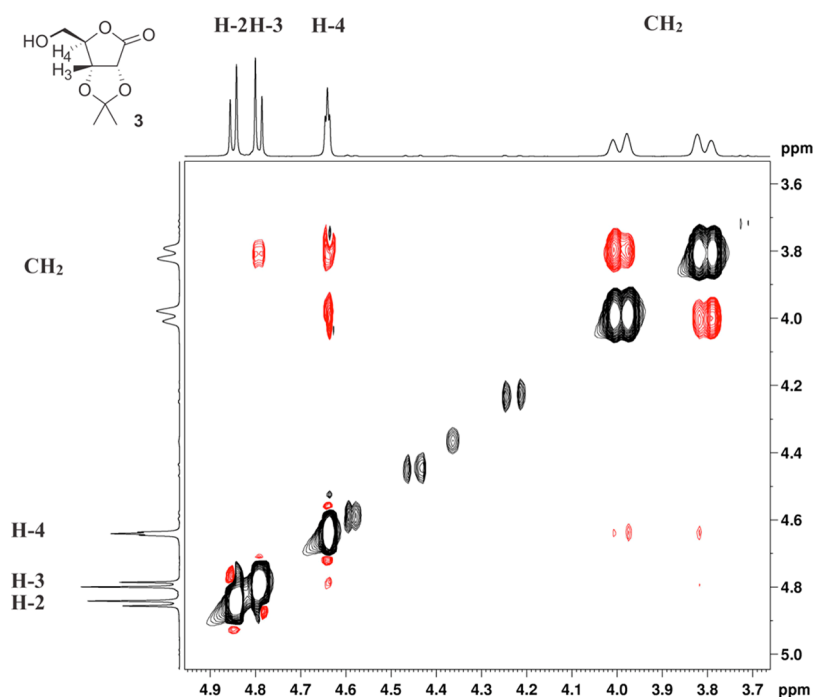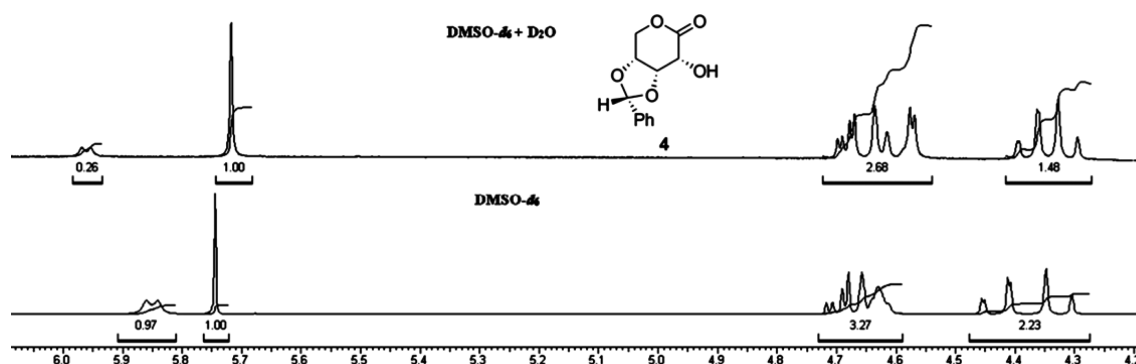


**Figure 1.** $^1$H–$^1$H NOESY for lactone **3** in CDCl$_3$.

**Figure 2.** $^1$H NMR spectra for lactone **4** in DMSO-$d_6$ (bottom) and after the addition of drops of D$_2$O (top).
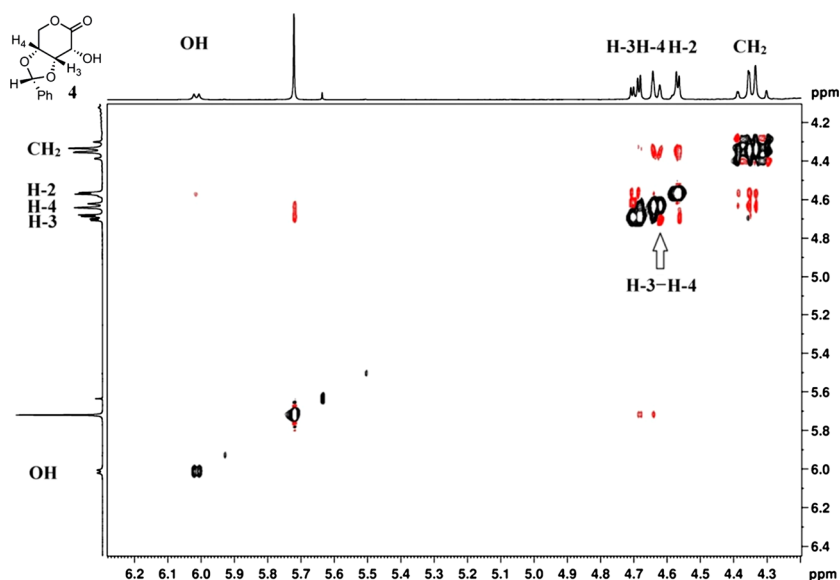


**Figure 3.** $^1$H–$^1$H NOESY spectrum for lactone **4** in DMSO-$d_6$ + D$_2$O.

**Table 2. NOE Intensities for 1,4- and 1,5-D-Ribolactones[a]**

| Lactone | H-2, H-3 | H-2, H-5a | H-3, H-4[b] | H-4, H-5a/b | Ring Size |
|---------|----------|-----------|-------------|-------------|-----------|
| **3** | M | W | W | M | 5 |
| **4** | X | M | M | M | 6 |
| **5a** | X | W | W | M | 5 |
| **5b** | X | W | W | M | 5 |
| **5c** | X | W | W | M | 5 |
| **6a** | M | M | M | M | 6 |
| **6b** | M | M | M | W | 6 |
| **6c** | M | M | M | W | 6 |

[a]M, medium intensity; W, weak intensity or nonobserved contact; X, cross peaks are too close. [b]H-3, H-4 NOE contact allows for lactone ring-size identification.

in the $^1$H–$^1$H COSY spectrum (Figure 4). This behavior is related to NOE (hydrogens close in space but not necessarily adjacent) and the $J$ (scalar couplings) concepts, respectively.

Finally, $^1$H–$^{13}$C heteronuclear correlation HMQC or HSQC was explored to confirm the hydrogen assignments (Figure 5). The advantages, such as phase sensitivity, of using HSQC over HMQC were discussed with students.

### Additional Discussion

Experimental $J$ and NOE values for the synthesized compounds were compared with those obtained by computational modeling using the program JANOCCHIO.[8] Students were

instructed to draw the structure of each synthesized lactone and have its energy minimized using MM2 dynamics (using ChemBio3D software). Then, the minimized structure was uploaded to JANOCCHIO, which allowed for comparisons of the experimental/theoretical $J$ and NOE values. Thus, conformational analyses of each molecule in solution and the dihedral angles were determined (see Supporting Information), allowing students to postulate in their reports the conformation of each lactone in solution. Conformational analysis of pyranosyl and furanosyl rings in solution is the basis for understanding the function of carbohydrate derivatives of biological interest, such as the furanosyl ring in DNA and RNA.

Additionally, explanations for the major and minor diastereoisomers of isopropylidene- and benzylidene-D-ribonolactones can also be found (see Supporting Information).[8]

To assess the achievement of the pedagogic goals, post-lab questions were designed to further explore the pedagogic aims of this laboratory experiment (see Supporting Information). A survey of the students' lab reports revealed that students were able to understand $J$ and NOE concepts and relate them to the obtained results. Students were also stimulated to discuss in class (typically during reaction time and solvent evaporation) the conformations that each molecule would assume in solution based on the comparison of calculated/experimental $J$ and NOE.
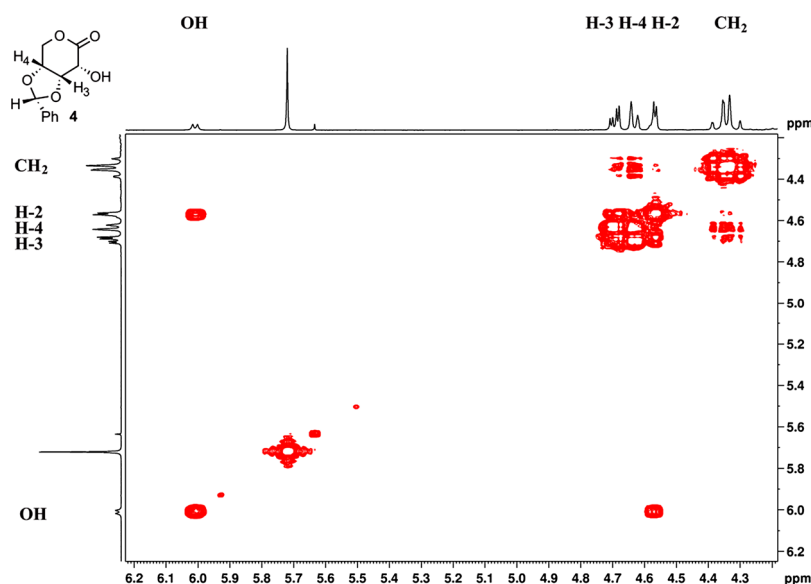
**Figure 4.** $^1H-^1H$ COSY spectrum for lactone **4** in DMSO-$d_6$.
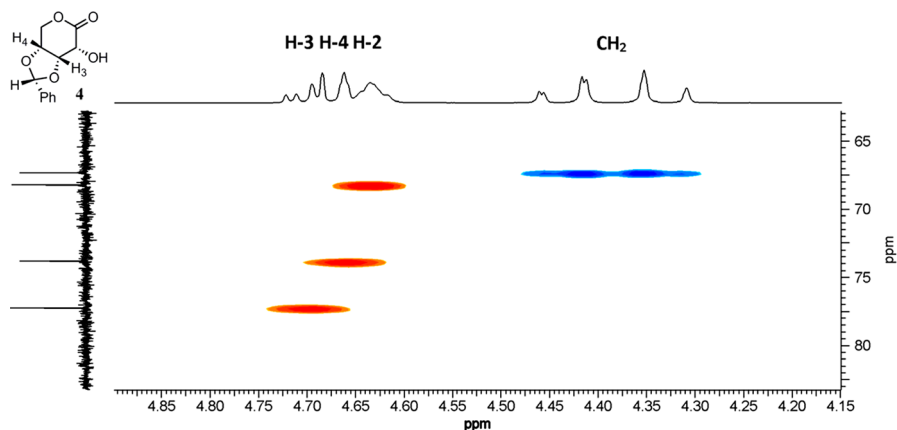


**Figure 5.** $^1H-^{13}C$ HSQC spectrum for lactone **4** in DMSO-$d_6$.

## CONCLUSION

This laboratory experiment addressed important concepts of organic chemistry, such as the oxidation of D-ribose (**2**) to produce the respective D-ribonolactone (**1**), isopropylidene and benzylidene protecting groups, kinetic (five-membered lactone) versus thermodynamic (six-membered lactone) products, and conformational analysis (geometry of the synthesized molecules in solution) associated with carbohydrate derivatives using NMR techniques. These concepts coincided with carbonyl chemistry lectures taught in the second organic chemistry course while providing a refresher of many earlier theories, thus making the experiment an excellent end-of-semester choice. The compounds were solids and were easily purified by crystallization or used in the next step of synthesis without further purification. The NOE between H3−H4 allowed for the identification of five- and six-membered D-ribonolactones and their possible rearrangements during its *O*-acylations. The experiments were successfully tested in four semesters by 14 upper-division undergraduate students majoring in chemistry at UFRGS, Brazil and 2 students at Universidad de la Republica, Uruguay. Students were evaluated by post-lab questions and lab reports.

## ASSOCIATED CONTENT

**S** **Supporting Information**

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/ed500845c.

> Information for instructors, detailed experimental procedures, additional discussion for students, tips for NMR assignment, answers to questions, IR, and NMR spectra (PDF, DOCX)

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**Notes**

The authors declare no competing financial interest.

## ACKNOWLEDGMENTS

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