World Journal of Chemical Education, 2022, Vol. 10, No. 1, 8-19
Available online at http://pubs.sciepub.com/wjce/10/1/2
Published by Science and Education Publishing
DOI:10.12691/wjce-10-1-2

Introducing NMR to Biomedical Laboratory Scientists through a Laboratory Exercise; Synthesis, Structure Determination and Quantization of Aspirin by Employing an ^{1}H-NMR Bench Top Instrument

Marit Kristin Leiren, Signe Steinkopf*

Western Norway University of Applied Sciences, Department of Safety, Chemistry and Biomedical laboratory Sciences, Campus Kronstad, Inndalsveien 28, N-5063 Bergen, Norway
*Corresponding author: Signe.Lilia.Steinkopf@hvl.no

Received November 11, 2021; Revised December 14, 2021; Accepted December 22, 2021

Abstract In this chemical education research study, NMR was introduced to a group of 3 students with minor chemistry background. A bench top NMR instrument was used to acquire the ^{1}H-NMR spectra. The ^{1}H-NMR spectra were used to monitor the synthesis of **ASA** and product purity. The spectrum of the product confirmed its formation, and the spectra of crude and final product allowed the students to observe the elimination of impurities upon recrystallization of the product. Further, the spectrum of the final product was used to quantify the yield through integration of the proton resonances. The use of integrals of the proton resonances for calculation of the yield of ASA is to our knowledge not described elsewhere in undergraduate experiments. A procedure for the synthesis, recording and processing of ^{1}H-NMR spectra, as well as calculation of the yield is reported. This procedure can be implemented by undergraduate or by high school students and might as well be useful for instructors who wants to introduce NMR spectroscopy early in the curriculum of Chemistry. By including an exercise like this, the students get hands on experience to employ advanced technology that might be commonly used in the future, also in Hospital laboratories. Furthermore, it is useful to introduce one of the most demanding and advanced methods in chemistry as early as possible in the curriculum in Chemistry to promote the chemistry career.

Keywords: *Undergraduate students, synthesis of acetylsalicylic acid, introduction to NMR, Bench Top NMR, integrals, yield, hands-on learning*

Cite This Article: Marit Kristin Leiren, and Signe Steinkopf, "Introducing NMR to Biomedical Laboratory Scientists through a Laboratory Exercise; Synthesis, Structure Determination and Quantization of Aspirin by Employing an ^{1}H-NMR Bench Top Instrument." *World Journal of Chemical Education*, vol. 10, no. 1 (2022): 8-19. doi: 10.12691/wjce-10-1-2.

1. Introduction

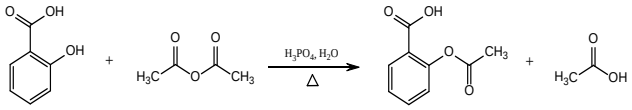
The aim of this study has been to develop a laboratory exercise employing a bench top ^{1}H-NMR instrument as an entry to basic NMR theory for undergraduate students without any former knowledge of ^{1}H- NMR. The laboratory exercise can also be used as an introduction to NMR in chemistry courses at High School, where for some, NMR theory is already a part of the curriculum. In combination with this laboratory exercise, the students will get hands on experience with NMR. The exercise comprises an organic synthesis, and confirmation of structure and determination of the yield by integration of the proton resonances in the ^{1}H-NMR. ^{1}H-NMR spectra of the solvent, the reactants, the crude product, and the final product are included. Further, in Supporting Material, there is a detailed procedure of the synthesis, showing all the spectra and how these are recorded and processed. The latter will be a useful support for teachers and supervisors. Estimates of the total working hour a group of 3 students and the time spent on supervision during the exercise are also included.

In this study, a group of 3 bachelor students in Biomedical Laboratory Sciences with a strong biological bent and minor chemistry background where set to carry out the laboratory exercise. Their chemistry background were 10 credits in General Chemistry, 10 credits in Biochemistry and 5 credits in Organic Chemistry, the latter without any laboratory course.

Based on the idea that the students are motivated by making a drug that is familiar to them, in addition to working with chemicals that are not toxic nor harmful, the choice of synthesis fell on the pain-relieving compound Aspirin, acetylsalicylic acid (ASA). This was based on the facts that the synthesis is straightforward, the reaction is fast, the yield is satisfactory, and the precipitating product from the reaction mixture can easily be collected and purified. In addition, the ^{1}H-NMR spectra of the reactants

and the product shows high resolution of the ^{1}H-NMR signals not suffering of significant overlapping signals and are relatively easy to interpret. Therefore, the reaction step can easily be monitored, and impurities identified, which is the great advantage by using NMR. The synthesis of ASA is integrated in several curriculum of chemistry laboratories [1-5], however, it is not included in the curriculum of most Biomedical Laboratory Sciences.

The synthesis of ASA from salicylic acid (SA) is shown in scheme 1.



Scheme 1. The Formation of acetylsalicylic acid (ASA) from salicylic acid (SA) and acetic acid anhydride

There are several methods that have been used for educational purposes to determine the purity and quantity of ASA. These includes Capillary Electrophoresis [6], Melting Point and Differential Scanning Calorimetry [7] Thin Layer Chromatography, [8] High-Performance Chromatography [9] and ^{1}H-NMR [10]. In recent years, bench top ^{1}H-NMR has been introduced to undergraduate students in Chemistry [11-18]. The use of bench top ^{1}H-NMR early in the chemistry curriculum provides the students hands on access to one of the most important analytical techniques in the chemistry laboratory. The size, maintenance and uncomplicated operation of bench top instruments simplify the use. Further, these bench top ^{1}H-NMR spectrometers can be used in various laboratories, included in Hospitals by Biomedical Laboratory Scientist. Synthesis of ASA is also described by bench top ^{1}H-NMR instrument dealers like Nanolysis [19] and Magritek [20]. Both procedures include structure elucidation, however, none of these procedures includes a method to calculate the yield based on the integrals of the resonances in the spectra. For recording of ^{1}H-NMR spectra, the samples were dissolved in *d*-aceton (Nanolysis) or in *d*-chloroform (Magritek). In these solvents, the acid signal is visible in the spectrum. However, chloroform gives rise to a non-deuterated chloroform resonance at 7.26 ppm, which overlap in the aromatic region of the signals from ASA, and non-deuterated acetone in *d*-acetone will overlap with the methyl signal. This will compromise the accuracy of the integral of the respective peaks and further the yield calculations.

For a more exact determination of the integrals and the chemical shifts, *d*-methanol with 0.03 % TMS was chosen as a solvent. None of the solvent signals of methanol will overlap with the compounds of interest in this experiment. In *d*-methanol with 0.03% TMS, the integrals of the two observable proton groups of ASA, the methyl and the aromatic signals corresponded to three and four protons, respectively [10,21]. The acidic hydrogen will disappear because of deuterium-exchange with *d*-methanol.

The students were told to watch the Youtube video of Lea4sci [22]: "Proton NMR-How to Analyze The peaks of ^{1}H-NMR Spectroscopy", before they came for their first day in the laboratory. For the synthesis of ASA, they used the procedure of John Olmsted III [1] with minor adjustments. Each step of running a ^{1}H-NMR experiment was demonstrated by an instructor. These steps were: how to prepare a ^{1}H-NMR sample and put it into the magnet, and how to shim the sample and select the appropriate ^{1}H-NMR parameters. Further, how to record ^{1}H-NMR spectra and process the data, and, finally, how to determine chemical shifts and the integrals of the resonances appearing in the spectra. Subsequent, the students were shown one whole routine of for recording, processing, and interpreting the ^{1}H-NMR spectra of a pure sample of ASA from Sigma Aldrich. Thereafter they were able to acquire and process the remaining spectra on their own.

2. Experimental

2.1. Conversion of Salicylic Acid to Acetylsalicylic Acid

Minor modifications to the original procedure reported by Olmsted and published in JCE in 1998 [1] were done by the students, and their most successful yield was found when the first crystallization was extended to 1 hour. A wiper fitted to a glass rod can also be used to collect as much product as possible in each transfer to increase the yield, as the crude product and final product are "sticky". The purity of the reactants was verified by ^{1}H-NMR prior to the synthesis. In addition, the ^{1}H-NMR-results showed that water was contaminating the final product, indicating that the product should be dried for some additional time before the final analysis.

The modified procedure is found in Supporting Material.

^{1}H-NMR

The spectra were acquired on a Margitek Spinsolve 60 MHz instrument equipped with MestReNova software. ^{1}H-NMR spectra were recorded of *d*-methanol with 0.03% TMS, and phosphoric acid, acetic anhydride, SA, crude product of ASA and the final product of ASA dissolved in *d*-methanol with 0.03% TMS.

A more detailed procedure for the ^{1}H-NMR experiments and data processing is described further in Supporting Material.

Calculations

Two different equations were used to calculate the yield of the reaction, shown in Table 1.

Table 1. The methods used for calculations of the yield of ASA

Method A (1)eq	$\frac{n_{(mol\ form\ mass\ of\ ASA\ in\ the\ end\ product)}}{n_{(mol\ from\ mass\ SA)}} * 100\ \%$
Method B (2)eq	$\frac{n_{(mol\ calculated\ by\ NMR\ of\ ASA\ in\ the\ end\ product)}}{n_{(mol\ from\ mass\ SA)}} * 100\%$

* By keeping the mol SA constant in both methods they can easily be compared
**In method B, the ^{1}H-NMR spectra were used to eliminate water contamination in the final product.

The integral of water from the solvent (*d*-methanol) had to be subtracted from the integral of the resonance from water content in the final product. This was done by using the ratio of the integral from the water resonance and the methyl group obtained from the ^{1}H-NMR spectra of pure *d*-methanol.

The complete calculations are shown in Supporting Material.

3. Results and Discussions

Overall, this experiment gives the students more through introduction to a synthesis than most regular laboratory exercise by including ^{1}H-NMR. It also represents the more standard procedure to synthetic work in a chemistry laboratory, where the reactions are followed step by step by the NMR, form starting materials to final product.

In most synthesis used early in the curriculum in Chemistry, the yield is calculated by weight only. This might introduce several sources of error from for instance unreacted reactants, intermediates from incomplete reactions, and impure reactants or solvents that will contribute to the weight of the final product. All these possible sources of error can be minimized by recording ^{1}H-NMR spectra of reactants, solvents and product, and subtracting the discoveries in the calculation of the yield.

The ^{1}H-NMR spectrum of ASA in *d*-chloroform is shown in Figure 1, where the resonances were separated in three regions: the acid hydrogen is located at the chemical shift of 11.89 ppm, the aromatic region gives multiple resonances from the four aromatic protons at a chemical shift region of 8.28-7.13 ppm, and the singlet appearing from the methyl group is located at a chemical shift of 2.43 ppm.

The integrals were found by setting the methyl group as the reference peak, to 3.00, corresponding to the three chemical equivalent hydrogens present in the methyl group. However, the integral of the other peaks does not fully add up, caused by the solvent resonance (chloroform, 7.26 ppm) that appears in the aromatic region and overlaps with the aromatic resonances of ASA (8.28 – 7.13 ppm). Therefore, the integral of the aromatic protons in *d*-chloroform will be too high, and other impurities in this region can go undetected. *D*-methanol is the solvent of choice in this experiment because there are no overlapping peaks from the solvent, in addition the working environmental impact is minor compared to chloroform.

Synthesis and ^{1}H-NMR

^{1}H-NMR spectra were recorded of the reactants, the catalyst and the solvent *d*-methanol, in order to investigate if there were contaminants present (Figure 2). These spectra will make it possible to observe the transformation of the starting materials to the final product in the synthesis. Further, it is possible to observe the purity of the final product and decide the origin of appearing contaminants.

In Supporting Material, the full spectra and a table of the relevant shifts are found.

In Figure 3, a cutout of the ^{1}H-NMR spectra, which includes the methyl group and the aromatic region, for the spectra of the starting material, SA, the crude, and the final product of ASA is shown. The alcohol group of SA (Figure 2, spectrum 1 at 5.29 ppm) has disappeared in the spectra of the crude and the final product, and the appearance of a strong singlet at 2.28 ppm is clear. This is in consistence with the chemical shift of the methyl in the acetate group bounded to the alcohol unit in SA in the esterification, confirming that ASA has been formed.

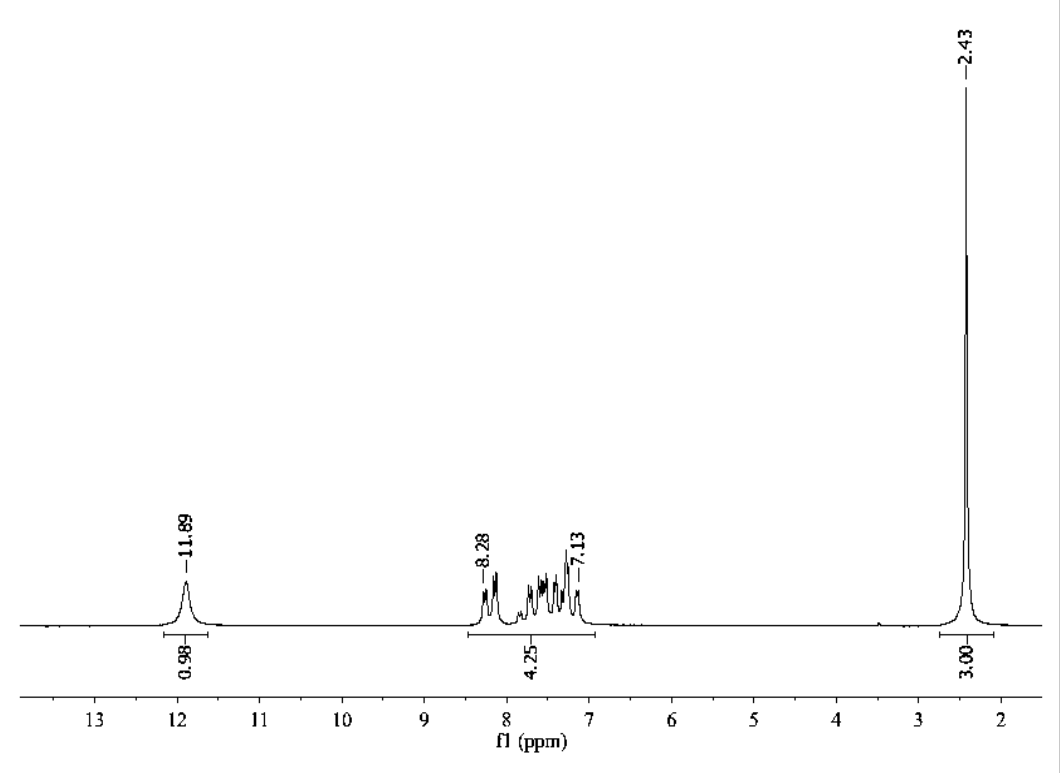


Figure 1. ^{1}H-NMR of ASA in *d*-chloroform, recorded with a 60 MHz instrument from Magritek. The resonance at 11.89 ppm is from the acid proton, the resonance around 7.5 ppm are from the aromatic protons and the resonance at 2.43 is from the methyl group

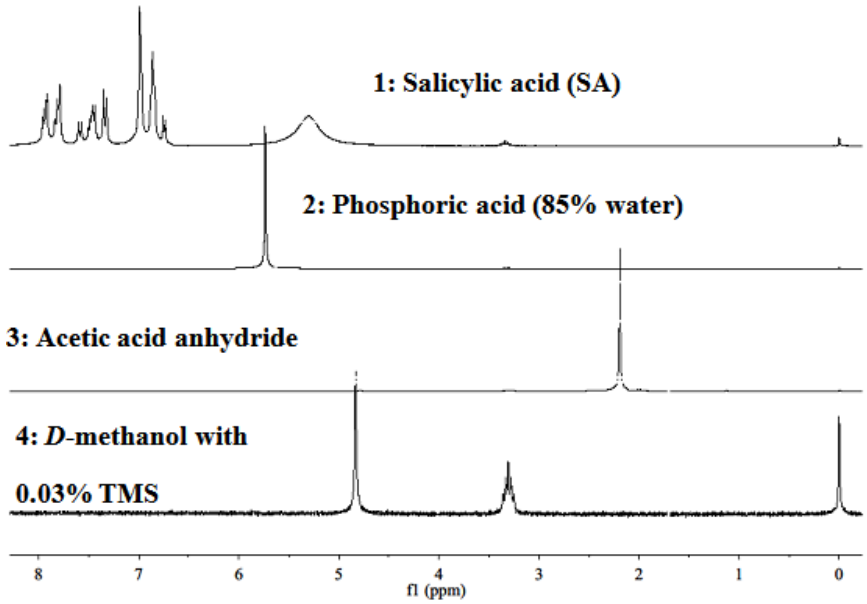


Figure 2. Spectrum 1 shows the ^{1}H-NMR spectra of SA, with the combined resonance from the alcohol group and the water signal at 5.29 ppm, and the resonances that originate from the aromatic hydrogen atoms in the chemical shift region of 6.73-7.95 ppm. Spectrum 2 shows the ^{1}H-NMR spectra of phosphoric acid catalyst (85% water) with the resonance from water at 5.73 ppm. Spectrum 3 shows the ^{1}H-NMR spectra of acetic acid anhydride with a resonance at 2.19 ppm from the methyl groups present. Minor impurities at 2.02 and 1.98 ppm and satellite signals from the methyl group are observable in a cut-out spectra of acetic acid anhydride in Supporting Material (figure 4). Spectrum 4 shows the ^{1}H-NMR spectrum of 99% d-methanol with TMS at 0.00 ppm. The resonance at 3.31 ppm originates from the non-deuterated methyl group and the resonance at 4.83 ppm is appearing from the alcohol group in methanol and water.

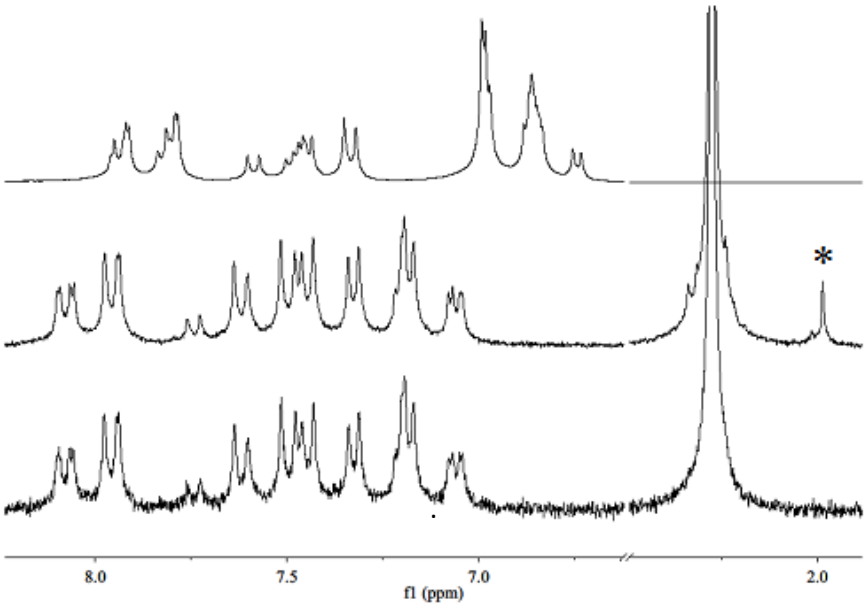


Figure 3. Spectrum top to bottom, show the ^{1}H-NMR spectra of SA (top), the crude product ASA (middle) and the final product, ASA (bottom). The cutout of the ^{1}H-NMR spectra includes the regions where we find the methyl group (1.6-3.00 ppm) and the aromatic region (6.6-8.5 ppm)

The effect of recrystallization can also be observed by comparing the spectra of the crude and the final product. In the spectra of the crude product, the appearance of a resonance up-field from the methyl is observed (1.99 ppm, marked with a star). This is acetic acid that has not been completely removed during work-up. Next to this peak (at 2.02 ppm) traces of methyl acetate form an esterification between acetic acid and *d*-methanol can be seen. In addition, at the methyl resonance of ASA, minor resonances appear and overlaps with the methyl signal. This is not due to contamination of the reagents or solvents proven by the fact that the ^{1}H-NMR spectra of all reagents and solvents were pure. It might be caused of side-reactions, such as formation of dimers or polymers with methyl groups resonating in this area. However, these contaminants, and thus the overlapping signals, disappears after recrystallization.

Minor resonances at 1.13 and 3.26 ppm in the spectra of acetic acid anhydride are assumed to be satellites of the methyl signal, which can be in consistence with the observation of a satellite of the methyl group in ASA with the resonance at 1.21 ppm in both the spectra of the crude and final product of ASA. The other satellite is probably overlapping with the methanol resonance. However, this resonance is so small that it can be neglected in the calculation of the yield.

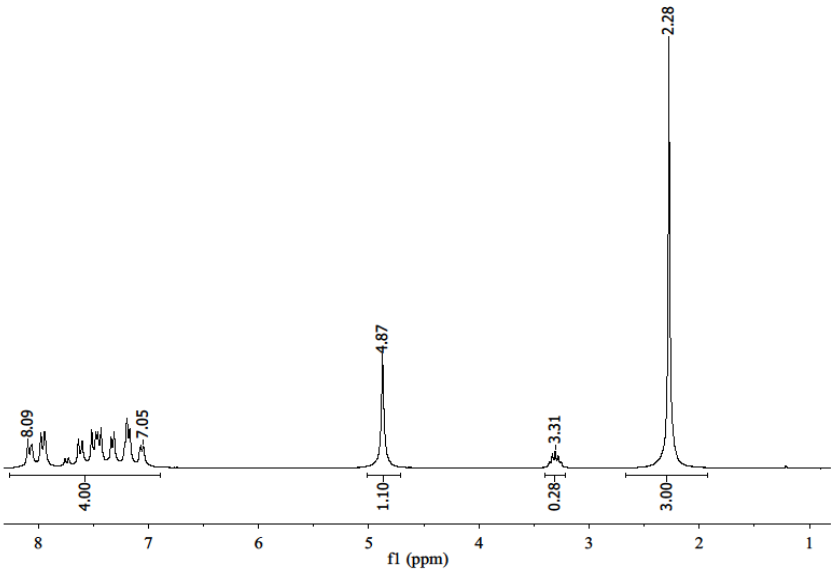


Figure 4. The [1]H-NMR spectrum of the final product of ASA showing the resonances with integrals that were used for quantification of the yield. The integral of the methyl resonance (2.88 ppm) was set to 3.00 and the resonances in the aromatic chemical shift region showed 4.00. In addition, the area of the methyl group in methanol (3.31 ppm, 0.28) and the water signal (4.87 ppm, 1.10) are shown

[1]H-NMR spectrum of the final product in *d*-methanol in Figure 4 is showing the integrals of the resonances that were used to calculate the yield. The integral of the methyl resonance (2.88 ppm) was set to 3.00 and the resonance in the aromatic region automatically was adjusted to 4.00. This correspond to the fact that there are four aromatic hydrogens present in ASA. However, the ratio between the resonance of water (at 4.87 ppm) and the methyl group in methanol (at 3.31 ppm) was too high to originate from the *d*-methanol solvent. This is probably due to water contaminating the final product. Using method B for yield calculation, this error will be eliminated in the reported yield.

The aromatic region of ASA is shifted downfield compared to the aromatic region of the SA (figure 3). This is caused by the electron withdrawing ability of the acetyl group, decreasing the shielding of the aromatic protons.

The resonances in the aromatic area are overlapping doublets and multiples, in total 8 distinct signals. This indicates that the aromatic protons experience different chemical environment that cannot be resolved with a 60 MHz instrument. These signals are caused by the 4 aromatic protons present in ASA.

Yield.

The result form the calculation of the yield is shown in Table 2. By weighing the reactants and the product, in method A, the yield can be either over- or underestimated due to varying water content in both the reactants and the final product.

A spectrum of SA in *d*-chloroform revealed that the crystals were without contaminations. Therefore, it is assumed that the moles of SA can be found directly form the weight. However, method A does not correct for the presence of water or other contaminants in the final product, resulting in an overestimation of the yield. In method B, the mole of ASA is calculated from the integrals obtained from the [1]H-NMR spectrum. Therefore, it is essential that the integrals are correct when using method B. To eliminate errors in the integral, the waiting time between each transient (D1), was set to 10 seconds to let all the resonances be fully relaxed. Method B shows a slightly reduced yield compared to method A because of the elimination of the water present in the final product. Here the students are shown why NMR is superior to other most other analysis techniques used in exercises for undergraduate students.

Table 2. The results of the yield calculations by method A and B

Method	Salicylic acid		Acetylsalicylic acid		Yield (%)
	mass (g)	mol (mmol)	mass (g)	mol (mmol)	
A	1.4004	10.14	1.1315	6.28	61.9
B				6.06	59.8

Method A: Weight of the samples is used to calculate the yield.
Method B: Mol SA is calculated from the weight of the added mass, mol ASA is calculated from the integrals in the [1]H-NMR spectrum.

Complete calculations are shown in Supporting Material.

Time.

Before the group of 3 students entered the laboratory, they spent 2 hours preparing for the synthesis of ASA and 3 hours watching and editing the information from the video of the [1]H-NMR theory. On the synthesis, the students spent 4 hours. For recording and processing the [1]H-NMR spectra, the students spent 4 hours on data recording and 4 hours in processing the data. Further, they spent 8 hours post lab to finish the report. For the students to complete the synthesis of ASA, it was necessary for a supervisor to show the equipment they were using and demonstrate the application of the equipment. This took 2 hours. Guidance hours in connection with recording and processing of [1]H-NMR spectra were 2 hours. This included that the instructor demonstrated how to insert the sample into the magnet, setting the parameters that were used in all experiments, and running one specter. Table 3 sums up the suggested timetable for the exercise, for both students and supervisor/instructor.

Table 3. Overview of the time used for the exercise

Students	Time spent (hours)
preparing for the synthesis	2
watching and editing the information from the video of the ^{1}H-NMR theory	3
the experimental work in the laboratory	4
recording the ^{1}H-NMR data	4
processing the ^{1}H-NMR data	4
Post lab and finishing the report	8
Total	**25**
Supervisor/instructor	
show the equipment they were using and demonstrate the application of the equipment for the laboratory work	2
recording and processing of ^{1}H-NMR spectra	2
go through the most important findings in the NMR spectra	1
correcting the laboratory reports	1
Total	**6**

4. Discussion and Concluding Remarks

The students handled the synthesis and ^{1}H-NMR analysis very well with the necessary guidance. Furthermore, they expressed that they found it motivating to analyze their own product. Since benchtop ^{1}H-NMR will most probably be extensively used in the future, also in medical laboratories, it is important that undergraduate students get an introduction to a simplified ^{1}H-NMR theory and procedure to use the instrument. Further, it is more motivating to get hands on experience with acquiring and interpreting spectra, as opposed to a theoretical approach in a regular classroom setting. The experiment allowed the students to become proficient operator of a benchtop ^{1}H-NMR. They understood the basic experimental parameters involved in the acquisition of the ^{1}H-NMR spectra, and they also gained experience with processing spectra and data analysis using ^{1}H-NMR software.

This experiment, synthesis and NMR analysis can relatively easily be implemented in High Schools and for undergraduate students, and by teachers/instructors. In addition, the students get a nice and informative overview of the possibilities and the advantages of the NMR technique. This work shows that an introduction to one of the most demanding and advanced methods in Chemistry can be introduced at an early stage in the curriculum of Chemistry to promote the chemistry career. The assignment is based on self-study, and with helped by this article and the Supporting Material, the time necessary for the instructor is minimized. This is normally the threshold of a laboratory experiment when advanced instrumentation is used.

Further work

To evaluate the laboratory exercise, both the synthesis and application of ^{1}H-NMR, it will be useful to make a survey on several student groups. Further, to enhance learning, it would be valuable for students to produce videos were both the synthesis and the application of the ^{1}H-NMR instrument are demonstrated [23]. In addition, to reduce the time spent supervising by instructors and technicians during the exercise, it would have been useful to make videos of ^{1}H-NMR theory that covers the theory the students need to perform the exercise.

References

[1] John Olmsted. Synthesis of Aspirin. A general Chemistry Experiment. J. Chem. Educ. 1998, 75, 10, 1261-1263.

[2] Cheli Fossum, Chem-30b, Laboratory Manual, 8-synthesis of Aspirin. Lanely College, Oakland, World press content, 2012. https://laney.edu/cheli-fossum/wp-content/uploads/sites/210/2012/01/8-Synthesis-of-Aspirin.pdf.

[3] Sangeeta Pandita and Sampta Goyal. An efficient Microscale procedure for the synthesis of Aspirin. J. Chem. Educ. 1998,75, 6, 770

[4] Ingrid Montes, David Sanabria, Marilyn García, Joaudimir Castro and Johanna Fajardo. Agreener Approach to Aspirin Synthesis Using Microwave Irradiation. J. Chem. Ed. 2006, 83, 4, 628.

[5] Houston Byrd and Stephen E. O. Donnell. A General Chemistry Laboratory Theme. Spectroscopic Analysis of Aspirin. J. Chem. Educ. 2003, 80, 2, 174. F

[6] F. Welder and C. L. Coleyer. Using Capillary Electrophoresis to Determine the purity of Acetylsalicylic Acid Synthesized in the Undergraduate Laboratory. J. Chem. Educ. 2001, 78, 11, 1525.

[7] Pehlic Ekrem, A. Sapcanin, Mirza Nuhanovic, B. Banjanin. Qualitative methods of identification of Acetylsalicylic Acid by Difference Scanning Calometry and melting point method. Health Med.2011, 5, 6, 1782-1788.

[8] Bochénska. P and Pyka. A. Determination of Acetylsalicylic Acid in Pharmaceutical drugs by TLC with densiometric detection in UV. J. Liq. Chromatogr. Relat. Technol. 2012, 35, 1346-1363.

[9] Sawyer. M and Kumar. V. A rapid High-Performance Liquid Chromatographic Method for the Simultaneous Quantitation of Aspirin, Salicylic Acid and Caffeine in EffervescentTablets. J. Chromatographic Science, 41, 393-397.

[10] Donald P. Hollis. Quantitative Analysis of Aspirin, Phenacetin and Caffein Mixtures by Nuclear Magnetic Resonance Spectrometry. Anal. Chem. 1963, 35, 11, 1682-1684.

[11] Jessica L. Bonjour, Joy M. Pitzer and John A. Frost. Introducing High School Students to NMR Spectroscopy through Percent Composition Determination Using Low-Field Spectrometers. J.Chem. Educ. 2015, 92, 3, 529-533.

[12] Kasey L. Yearty, Joseph T. Sharp, Emme K. Meehan, Doyle R. Wallace, Douglas M. Jackson and Richard W. Morrison. Implementation of picospin Benchtop NMR instruments into Organic Chemistry Teaching Laboratories through spectral Analysis of Ficher Estrification Products. J. Chem. Educ. 2017, 94, 932-935.

[13] Juan F. Araneda, Thaís Mendonçs Barbosa, Paul Hui, Matthew C. Leclerc, Jonathan Ma, Alexander F. G. Maier and Susanne D. Riegel. Incorporating Benchtop NMR Spectrometers in undergraduate Lab: Understanding Resolution and Circumventing Second Order Effects. J. Chem. Educ. 2021, 98, 4, 1227-1232.

[14] Mark Edgar, Benita C. Peraval, Miles Gibson, Init Masania, Ken Beresford, Philippe B. Wilson and Martin Grootveld. Benchtop NMR Spectroscopy and Spectral Analysis of the Cis-and Trans-Stilbene Products of the Witting Reaction. J. Chem. Educ. 2019, 96, 9, 1938-1947.

[15] James E. Kent and Nicholle G. A. Bell. Molecular Properties of Caffeine Explored by NMR: A Benchtop NMR experiment for Undergraduate Physical Chemistry Laboratories. J. Chem. Educ. 2019, 96, 4, 786-791.

[16] Meden F. Isaac-Lam. Incorporation of Benchtop NMR Spektrometer into Organic Chemistry Laboratory: Analysis of an Unknown Liquid. J. Chem. Educ. 2020, 97, 7, 2036-2040.

[17] Jonatan Wong, Houguang Jeremy Chen, Ai-Hua Seow, Susanne D. Riegel and Sumond A. Pullkart. A hands – on Problem based NMR-Experiment using a Benchtop Spectrometer for the Undergraduate Laboratory. World J. Chem. Educ. 2016, 4, 3, 52-63.

[18] Martin Grootveld, Benita Percival, Miles Gibson, Yasan Osman, Mark Edgar, Marco Molinari, Melissa L. Mather, Federico Casanova, Philippe B. Wilson. Progress in low-field benchtop

NMR spectroscopy in chemical and biochemical analysis, Analytica Chimica Acta 1067 (2019) 11-30.
[19] Nanolysis. Synthesis of Aspirin. Benchtop NMR blog. Accession date March 8. 2013.
[20] Spinsolve. Magritik Traditional Undergraduate Experiment. Synthesis of Aspirin. www.magritek.com. Accession date April 7. 2015.
[21] J. L. Jungnichel and J. W. Forbes. Quantitative Measurement of Hydrogen Types by Integrated Nuclear Magnetic Resonance Intensities. Anal. Chem. 1963, 35, 8, 938-924.
[22] NMR teori: Leah4sci. (2013). *Proton NMR - How to Analyze The Peaks Of H-NMR Spectroscopy.* https://www.youtube.com/watch?v=k0eR8YqcA8c Accession date September 27. 2015.
[23] Amy. M. Balija. 1H NMR Spectroscopy Guided – Inquiry Activity Using the NMR Spectrometer: Incorporating Student Generated Videos to Assess Learning. J. Chem. Educ. 2020, 97, 5, 1387-1390.
[24] John Olmsted. Synthesis of Aspirin. A general Chemistry Experiment. J. Chem. Educ. 1998, 75, 10, 1261-1263.

Supporting Material

1. Experimental. The synthesis of Acetylsalicylic acid

This is an experimental procedure based on an article in the Journal of a Chemical Education, 1998, by John Olmsted. [24]

Equipment:

Erlenmeyer flask (125 mL), graduated cylinders (5 mL and 20 mL), spatula, weighing boats, rubber stopper with a ventilating needle, syringe (2 mL), water bath, filter papers, Hirsh funnel fitted to a vacuum flask, glass rods, wiper, beakers (2x 50 mL), a heating plate and an ice bath.

Chemicals:

- Salicylic acid
- Acetic acid anhydride
- Phosphoric acid (85% water)
-d-methanol 99% added 0.03% TMS

Checking the quality of the reagents:

1) Perform an ^{1}H-NMR analysis of the reagents, following the procedure described in chapter 2, to ensure that they are intact before the synthetic experimental work begins.

The synthesis:

2) In an Erlenmeyer flask (125 mL), add salicylic acid (1.4 g), acetic acid anhydride (3.0 mL) and 5 drops of the catalyst, phosphoric acid.

3) Fit a rubber stopper containing a ventilating needle to the top of the Erlenmeyer flask.

4) Swirl the flask with the reaction mixture in a pre-heated water bath (90 °C) till the starting material is dissolved. Then fit the flask to a rack and leave it in the bath for 5 minutes.

5) Remove the flask from the water bath and carefully add 2 mL deionized water through the stopper with a syringe. Water facilitates product formation and decomposes the remaining acetic acid anhydride, both of which are exothermic reactions.

6) When the flask is sufficiently cold, remove the rubber tubing, and add another 20 mL deionized water.

7) After the product mixture has reached room temperature, and precipitation has started, further add 10 mL deionized water, and place the flask in an ice bath for 1 hour.

Vacuum filtration:

8) Collect the crystals by vacuum filtration on a filter paper fitted in a Hirsh funnel. Rinse the Erlenmeyer flask with 15 mL deionized water and pour it into the Hirsh funnel. A wiper fitted to a glass rod should be used to collect most of the remaining crystals on the glassware.

9) Continue vacuum suction for 10 minutes.

10) Transfer the crystals to a pre-weighed beaker (50 mL) and weigh the crude product.

11) A small quantity (ca 10 mg) of the crude product should be saved for ^{1}H-NMR analysis.

Recrystallization:

12) Add 10 mL deionized water, per gram product, to the beaker.

13) Heat the solution to 85-90 °C on a heating plate whilst stirring with a glass rod till all product is dissolved.

14) Then leave the beaker on the bench to slowly reach room temperature before placing it in an ice bath. Slow cooling optimizes for crystal growth.

15) Collect the recrystallized product by vacuum filtration as described above (8-10).

16) Place the beaker with the final product in an oven at 80 °C for at least 1 hour, before it is cooled, weighed, and stored in a sealed container at 4 °C for further analysis by ^{1}H-NMR.

17) Perform an NMR analysis of the crude product and final product following the procedure described in chapter 2.

18) Describe what you observe in all the recorded spectra and compare your result with Table 1 in chapter 2.

19) Calculate the yield by method A and B, as described in chapter 3, and comment on the result.

2. ^{1}H-NMR analysis. Sample preparation and settings of ^{1}H-NMR parameters.

The spectra in this article were acquired on a Margitek Spinsolve 60 MHz instrument equipped with MestReNova software. The samples were added to Norell NMR sample tubes and dissolved in 0.750 ml d-methanol (99%) added 0.03% TMS provided from ChemSupport AS. The ^{1}H-NMR spectra were recorded after running a quick shim to homogenize the magnetic field. All the ^{1}H-NMR spectra were recorded with 32 transients, a repetition time of 10 seconds,

pulse angle of 90° and with an acquisition time of 6.4 seconds. The recorded spectra were saved in MestReNova for further data processing.

Spectra collected in the laboratory exercise.

a) *d*-methanol (or other deuterated solvent).
b) Salicylic acid.
c) Acetic acid anhydride.
d) Phosphoric acid (85% water)
e) Crude product; acetylsalicylicacid.
f) Final product; acetylsalicylicacid.

Data processing.

The spectra were processed with automatic phasing and baseline correction. The reference (TMS) where set to a chemical shift of 0 ppm and the resonances in the spectra were adjusted accordingly. The integral of the methyl resonance and of the aromatic region were manually integrated in MestReNova. The methyl peak was set to 3.00 corresponding to the three hydrogens of the group.

Interpreting the NMR spectra:

The NMR spectra of the reagents gives us information on whether there are any contaminants present and makes it possible to observe disappearing or appearing resonances during the synthesis. The results from the ^{1}H-NMR spectra of the compounds dissolved in *d*-methanol are listed in Table S1. The recorded spectra are found in chapter 4.

Table S1. Results of the NMR spectra of d-methanol, starting materials and product[1]

Compound	Groups visible in ^{1}H-NMR	Peaks (ppm)	Multiplicity[2]	Expected in final product
d-methanol (99% with 0.03% TMS)	Tetramethylsilane (TMS)	0.00	s	Yes. Used as reference peak.
	Methyl group	3.31	m	Yes, this is the NMR solvent.
	Alcohol group and water	4.83[2]	s	Yes, in a ratio of 1.37[3] with the methyl group of *d*-methanol.
Salicylic acid (SA)	Aromatic group	6.73-7.95	m	Yes, the group will also be present in ASA.
	Alcohol group	5.29[4]	s	No, the group is acetylated in the reaction.
Acetic acid anhydride	Methyl groups	2.19	s	No. The excess of anhydride will react with water and decompose to acetic acid, which will be removed by filtration.
Phosphoric acid (85% water)	Water	5.73[2]	s	No, the acid is soluble in water, and will be removed from the product by filtration.
Acetylsalicylicacid (ASA)	Aromatic group	7.04-8.09	m	
	Methyl group	2.28	s	

[1]All the reagents and products were dissolved in *d*-methanol.
[2]Multiplicity: s = singlet, d = doublet, t = triplet, m = multiple
[3]The *d*-methanol used in this article had this ratio. Must be checked by each laboratory.
[4]The resonance of water shifts coincides with the alcohol group and is found downfield compared to the resonance of water in *d*-methanol because of the change in pH. The broad resonance peak is due to extensive hydrogen bonding.

3. Calculations

Method A: The yield of the reaction is found by using equation 1, in Table 1 in the article, after the weight of the starting material (SA) and final product (ASA) has been converted to moles. Raw data is found in Table 2 in the article.

$$Salicylic\ acid\ (SA): \frac{1.4004g}{138.121\frac{g}{mol}} * 1000\frac{mmol}{mol} = 10.14mmol$$

$$Acetylsalicylic\ acid\ (ASA): \frac{1.1315g}{180.158\frac{g}{mol}} * 1000\frac{mmol}{mol} = 6.28mmol$$

$$Yield\ method\ A: \frac{6.28mmol}{10.14mmol} = 61.9\%$$

Method B: The ratio of the integral form the water signal and the methyl group obtained from the ^{1}H-NMR spectra of pure *d*-methanol (Figure 2 in chapter 4) was calculated to 4.11/3 = 1.37. Check your NMR solvent for the correct ratio. These signals also appear in the spectra of the final product, and if the ratio exceeds 1.37, water is contaminating the sample. The area of the water signal in the sample was corrected by formula 3, to eliminate water from the solvent.

$$The\ area\ of\ H_2O\ in\ the\ sample = The\ total\ area\ of\ H_2O - The\ area\ of\ H_2O\ from\ the\ solvent \quad (3)$$

Equation 4 is then solved with respect to moles of ASA, n_{ASA}, where the mole of water in the sample can be expressed by formula 5 and inserted into formula 4, where m is the mass in gram and Mw is the molecular weight.

$$m_{ASA} = n_{ASA} * Mw_{ASA} + n_{H_2O} * Mw_{H_2O} \quad (4)$$

$$n_{H_2O} = n_{ASA} * \frac{\text{the area } H_2O \text{ in the sample}}{2} \quad (5)$$

When the integral of the methyl group is set to 3.00, the methyl in *d*-methanol gave an integral of 0.28. Based on the ratio (1.37) described above, the equation 2 in Table 1 in the manuscript and equations 3-5 is used to find the yield of the final product:

Area of the H_2O resonance in d-methanol: $1.37 * 0.28 = 0.384$

Area of the H_2O resonance in ASA:

$$1.10 - 0.384 = 0.716 \quad (3)$$

$$m_{ASA} = 1.1315g = n_{ASA}\left(180.158\frac{g}{mol} + \frac{0.716}{2} * 18.01\frac{g}{mol}\right) \quad (4 \text{ and } 5)$$

$$n_{ASA} = \frac{180.158\frac{g}{mol} + \frac{0.716}{2} * 18.01\frac{g}{mol}}{1.1315g} = 6.06mmol$$

Yield method B:

$$\frac{6.06mmol}{10.14mmol} * 100\% = 59.8\% \quad (2)$$

4. The ^{1}H-NMR-spectra used in the article.

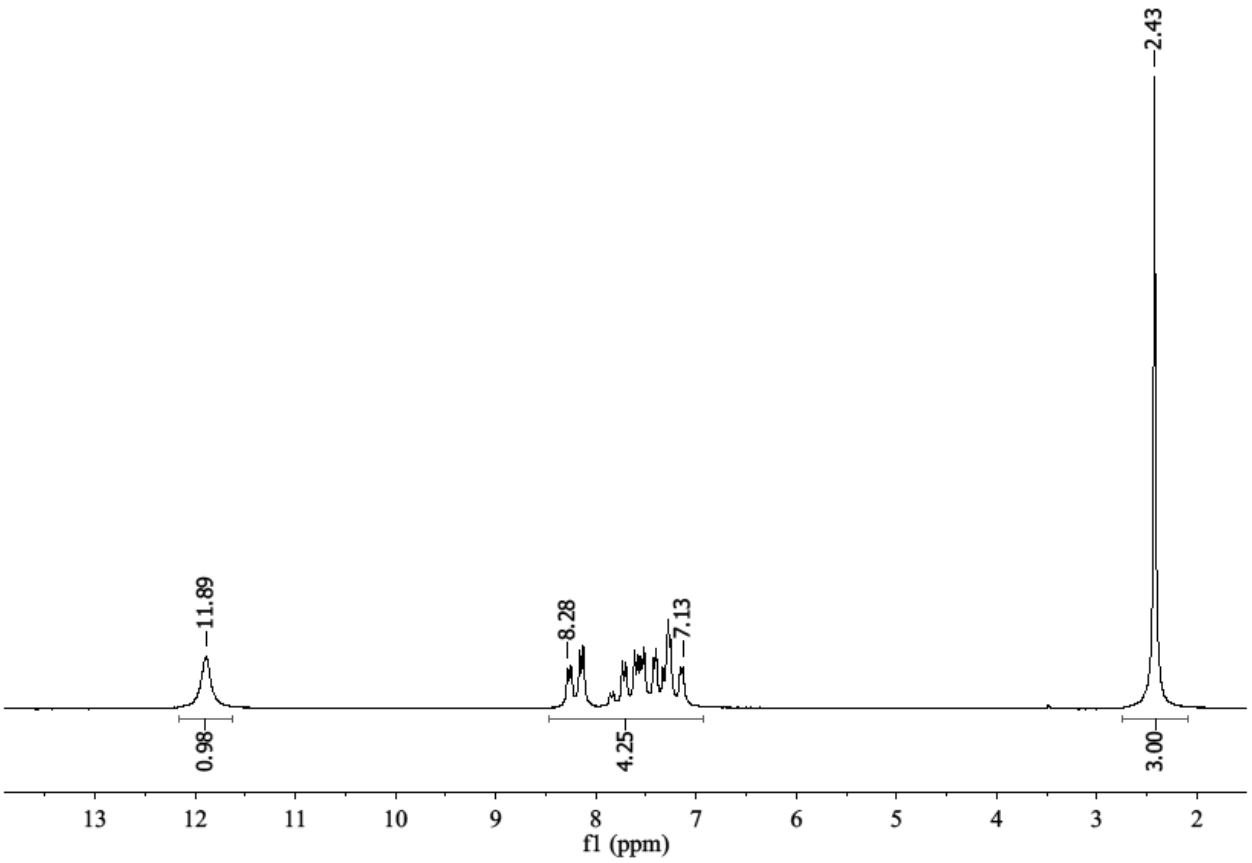


Figure S1. The ^{1}H-NMR spectra of acetylsalicylicacid in *d*-chloroform

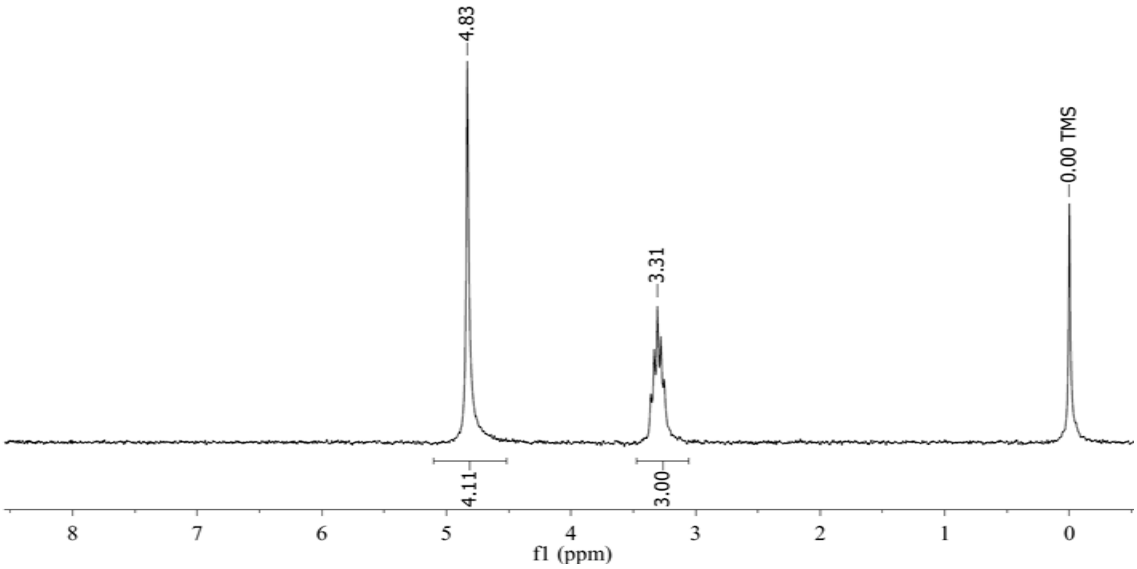


Figure S2. The [1]H-NMR spectra of *d*-methanol with 0.03% TMS

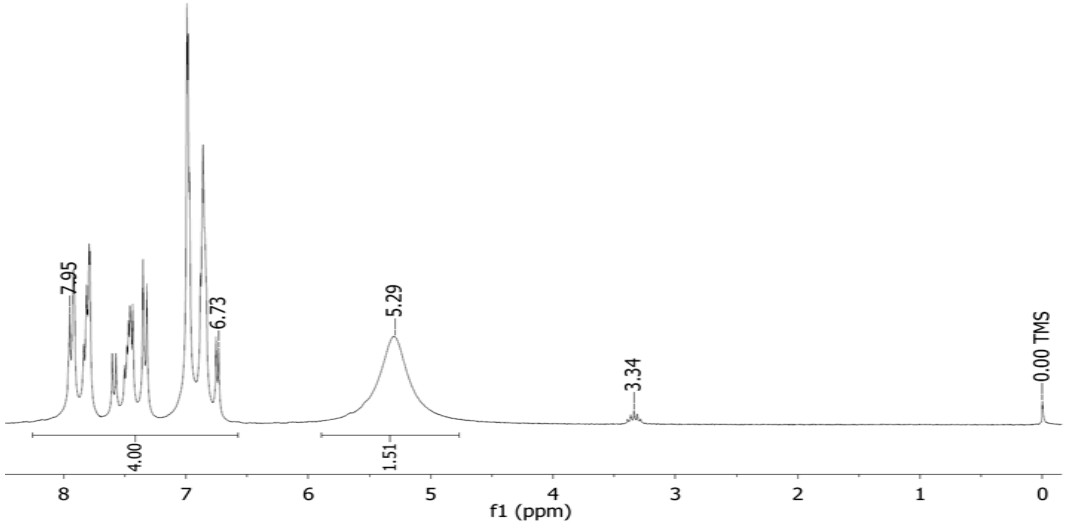


Figure S3. The [1]H-NMR spectra of salicylicacid in *d*-methanol with 0.03% TMS

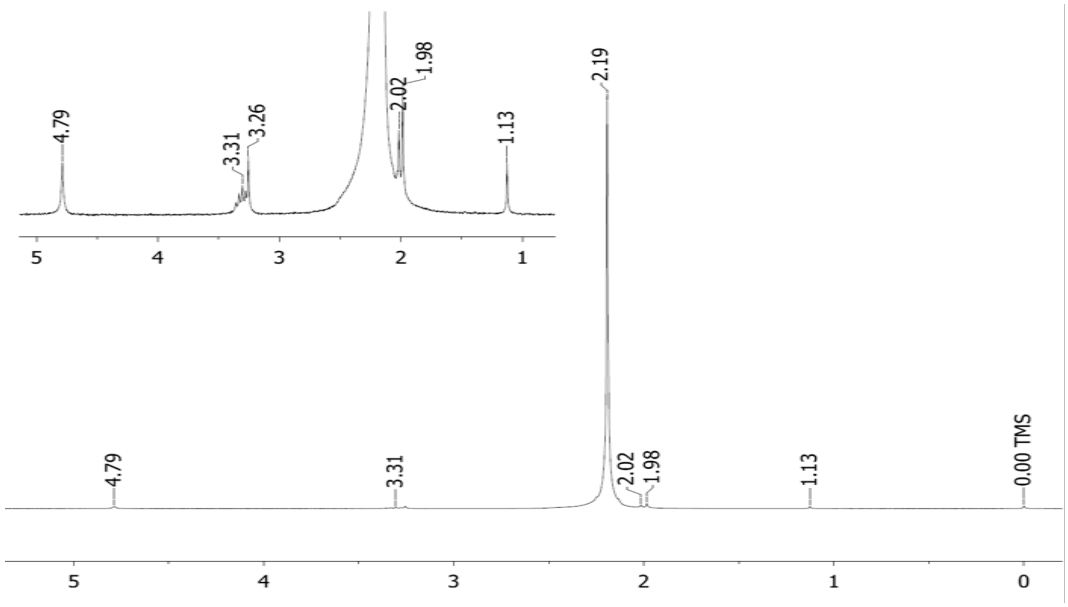


Figure S4. The [1]H-NMR spectra of acetic acid anhydride in *d*-methanol with 0.03 % TMS. The cutout spectra show the area of 1-5 ppm and includes resonances from impurities, solvent, and satellites

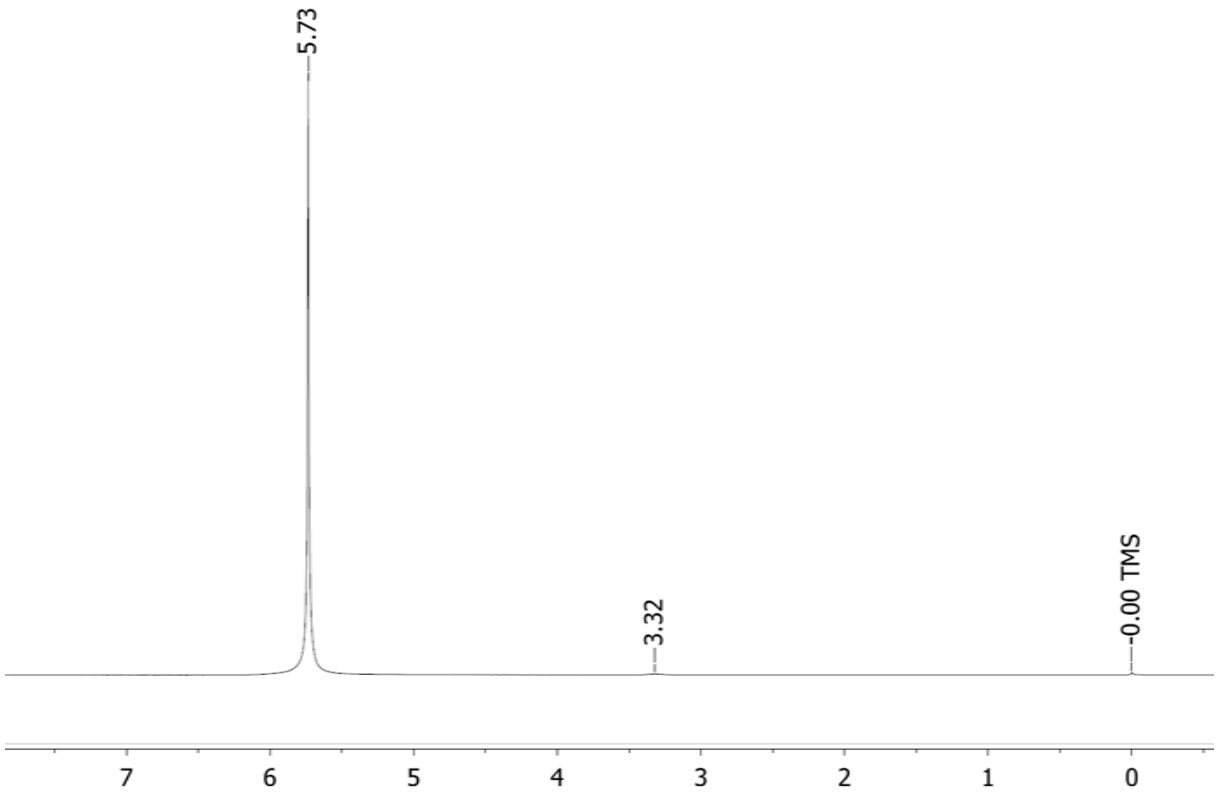


Figure S5. The [1]H-NMR spectra of phosphoric acid (85%) in *d*-methanol with 0.03% TMS. No impurities were found

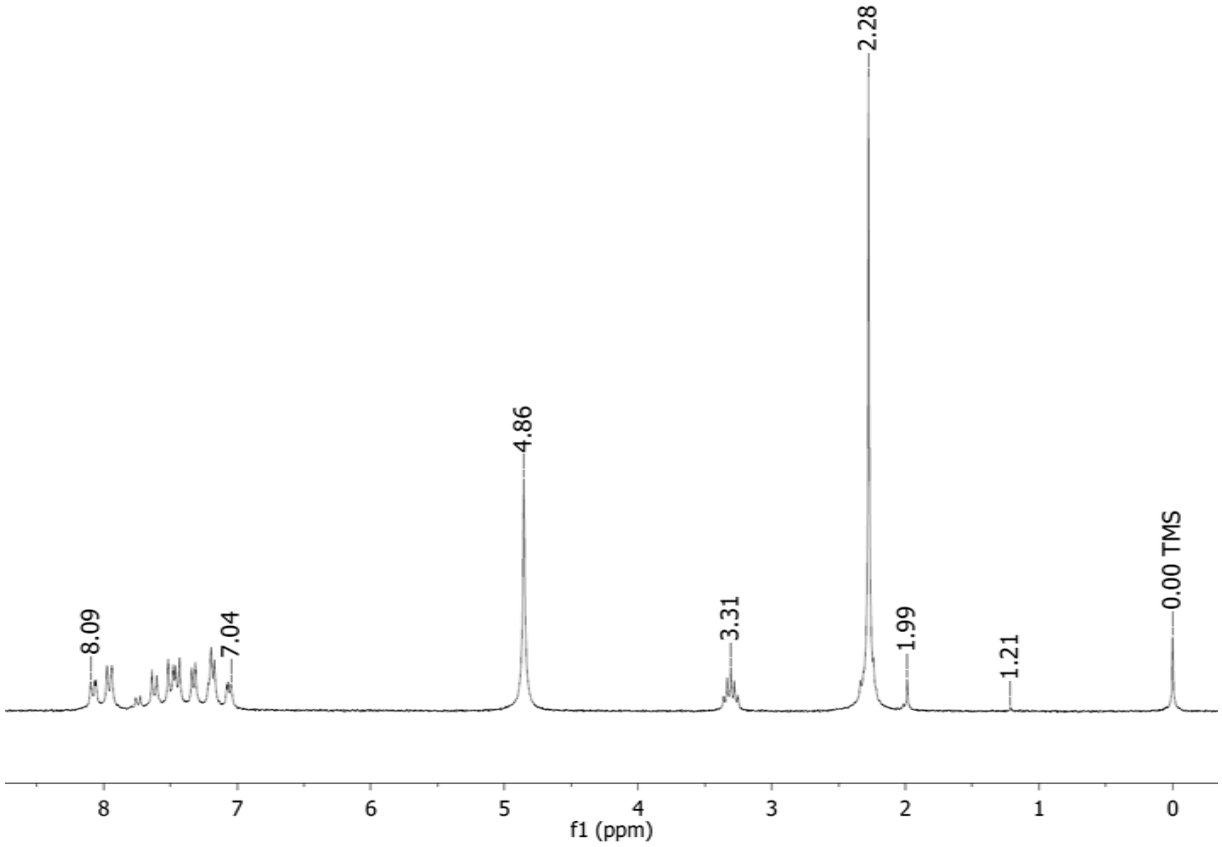


Figure S6. The [1]H-NMR spectra of the crude product acetylsalicylicacid in *d*-methanol with 0.03% TMS

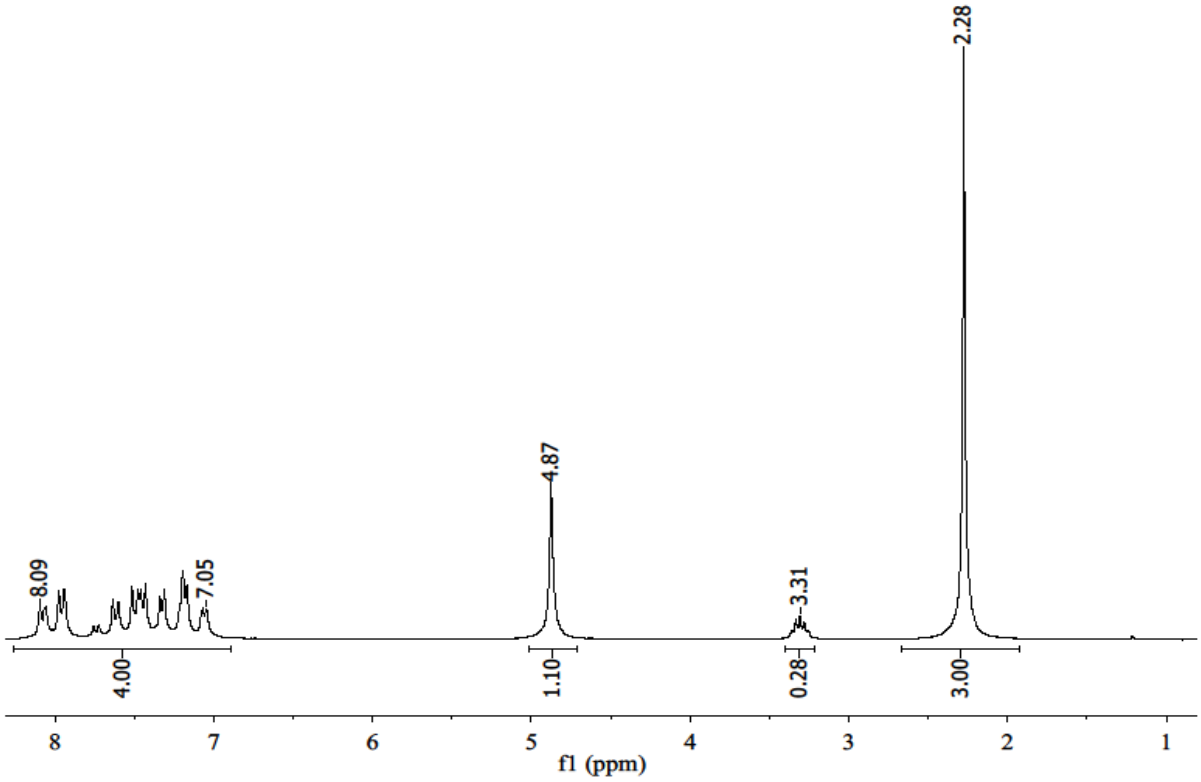


Figure S7. The [1]H-NMR spectrum of the final product, acetylsalicylicacid in *d*-methanol with 0.03% TMS