Organic Syntheses

Enzymatic Saponification of Esters Containing β-Leaving Groups

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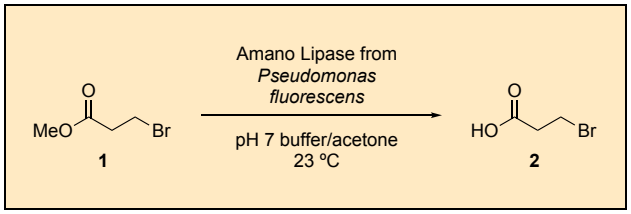
Checked by Ke Zhao and Kevin Campos



Procedure (Note 1)

A. *3-Bromopropionic acid* (**2**). A 500-mL Erlenmeyer flask is equipped with a magnetic stir bar (4.6 cm x 0.75 cm, rod shaped). The reaction flask is then charged with methyl 3-bromopropionate (5.00 g, 29.9 mmol, 1.00 equiv) (Note 2) (**1**) by syringe over 10 seconds (22 G, 7.62 cm needle). The flask containing **1** is then charged with pH 7 buffer (225 mL) (Note 3) and acetone (25 mL) (Note 4) sequentially via graduated cylinder yielding a clear, colorless solution (Figure 1A) (Note 5). The mixture is then stirred at a constant rate of 400 rpm at 23 °C. Once stirred, the flask is then charged with Amano Lipase from *Pseudomonas fluorescens* (0.50 g, 10 wt%) (Note 6) yielding a heterogeneous yellow solution (Figure 1B). The flask is then covered with a 7.6 x 7.6 cm square of aluminum foil and allowed to stir at 23 °C at a rate of 400 rpm for 14.5 h.

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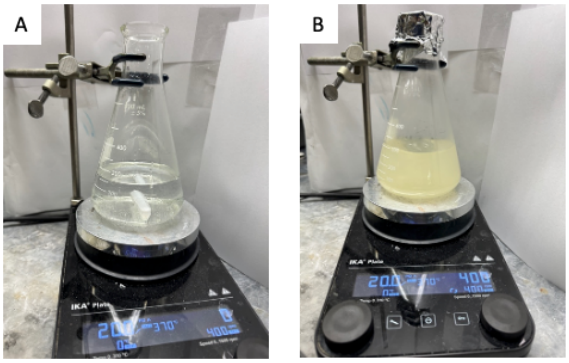


Figure 1. A. Liquid reagents in Erlenmeyer flask; B. Mixture after addition of Amano Lipase

After 14.5 h, the reaction is determined to be complete by TLC analysis (Note 7) and stirring is ceased. This mixture is then acidified by the dropwise addition of aqueous HCl (1.0 M, 20 mL) via syringe over 2 min (18 G, 30.5 cm needle) (Note 8) and stirred for 1 min at 400 rpm. Upon acidification, this mixture is then poured into a 1-L separatory funnel (24/40 joint) (Figure 2A). The reaction vessel is then rinsed with an additional 100 mL of deionized water, and the rinse is transferred to the separatory funnel. The aqueous layer is then extracted with ethyl acetate (4 X 200 mL) (Note 9), and the combined organic layers are collected in a 2-L Erlenmeyer flask (Note 10). The combined organic layers are then added to the 1 L separatory funnel and washed with 75 mL of saturated aqueous sodium chloride (Note 11) (Figure 2B) solution and collected in a separate 2-L Erlenmeyer flask.

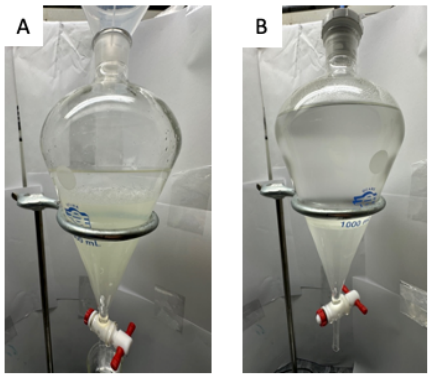


Figure 2. A. First ethyl acetate wash; B. Final brine wash of organic layers

The resulting organic layer is dried over anhydrous Na_2SO_4 (50 g) (Note 12) and vacuum filtered through a medium-porosity glass, fritted funnel (150 mL) eluting with ethyl acetate (50 mL) into a 2-L round-bottomed flask (Figure 3A). This solution is then concentrated by rotary evaporation under reduced pressure (35 °C, 75 mm Hg). The resultant oil is transferred into a tared 20-mL scintillation vial. The 2-L round-bottomed flask is then rinsed with ethyl acetate (3 x 2 mL), and the rinses are transferred into the tared vial. The tared vial is then concentrated by rotary evaporation under reduced pressure (35 °C, 75 mm Hg) (Note 13). The product is then dried under high vacuum (<1.0 mm Hg) for 52 h to provide 3-bromopropionic acid (**2**) as an off-white solid (Figure 3B) (3.21 g, 20.6 mmol, 69% yield, 98.3 wt% purity) (Notes 14, 15, 16 and 17).

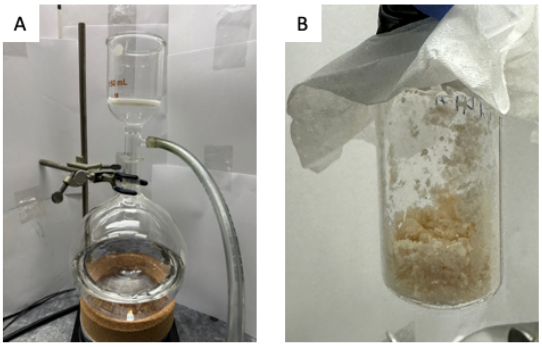


Figure 3. A. Filtration into round bottom flask; B. Pure product

Notes

1. Prior to performing each reaction, a thorough hazard analysis and risk assessment should be carried out with regard to each chemical substance and experimental operation on the scale planned and in the context of the laboratory where the procedures will be carried out. Guidelines for carrying out risk assessments and for analyzing the hazards associated with chemicals can be found in references such as Chapter 4 of "Prudent Practices in the Laboratory" (The National Academies Press, Washington, D.C., 2011; the full text can be accessed free of charge at https://www.nap.edu/catalog/12654/prudent-practices-in-the-laboratory-handling-and-management-of-chemical. See also "Identifying and Evaluating Hazards in Research Laboratories"

(American Chemical Society, 2015) which is available via the associated website "Hazard Assessment in Research Laboratories" at https://www.acs.org/about/governance/committees/chemical-safety.html. In the case of this procedure, the risk assessment should include (but not necessarily be limited to) an evaluation of the potential hazards associated with methyl 3-bromopropionate, pH 7 phosphate buffer, acetone, Amano Lipase from *Pseudomonas fluorescens*, hydrochloric acid, ethyl acetate, sodium chloride, and sodium sulfate.

2. Methyl 3-bromopropionate (98%) was purchased from Ambeed and used as received.
3. pH 7.00 phosphate buffer was purchased from Fisher Scientific and used as received.
4. Acetone (ACS Grade) was purchased from Fisher Scientific and used as received.
5. An oil layer was observed on the bottom of the solution after charging acetone. A subsequent 9 min 400 rpm agitation yielded a clear and homogeneous solution.
6. Amano Lipase from *Pseudomonas fluorescens* (specific activity ≥20,000 units/g) was purchased from Sigma Aldrich and used as received.
7. To monitor reaction progress via TLC, at 14.5 h a 0.5 mL aliquot of the reaction mixture is removed from the reaction vessel and placed into a 20 mL scintillation vial. To this mixture is then added aqueous HCl (1 M, 0.5 mL) and ethyl acetate (0.5 mL). This mixture is then shaken and the organic layer is then spotted onto a silica gel TLC plate against starting material **1**. Using 19:1 hexanes:ethyl acetate as the eluent, the ester starting material **1** has R_f = 0.33 and the carboxylic acid product has R_f = 0.00 using $KMnO_4$ stain as visualization (Figure 4).

Figure 4. TLC of the crude reaction mixture after 14.5 h (S = methyl 3-bromopropionate, C = co-spot of S and R, and R = reaction mixture)

8. Aqueous 12 M HCl was purchased from Sigma Aldrich and the 1 M solution used in the workup was prepared by adding 100 mL of 12 M HCl to 1.1 L of deionized water in a 2-L graduated cylinder.
9. Ethyl acetate (99.5%) was purchased from Fisher Scientific and was used as received.
10. An emulsion forms between the organic and aqueous layers. The emulsion was collected with the organic layer (Figure 5).

Figure 5. Emulsion separated from aqueous layer

11. Sodium chloride (≥99.0%) was purchased from VWR and was used as received.
12. Anhydrous sodium sulfate (99.5%) was purchased from VWR and was used as received.
13. Upon rotary evaporation, the product appears to be a light brown, clear oil (Figure 6). As the vial is dried under high vacuum, the product then solidifies to a crystalline, off-white solid.

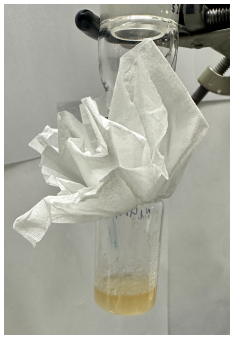


Figure 6. Crude oil after rotary evaporation and drying under reduced pressure

14. The product can be characterized as follows: ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.61 (s, 1H), 3.57 (t, J = 6.8 Hz, 2H), 2.99 (t, J = 6.8 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 177.2, 37.6, 25.1. IR (neat): 2971, 2659, 2568, 1716, 1689, 1428, 1394, 1240, 1142, 951, 917, 889 cm^{-1}. HRMS-APCI (m/z) $[M + H]^+$ calc'd for $C_3H_6O_2{}^{79}Br^+$, 152.9551; found 152.9547; mp 61.9-62.7 °C; R_f 0.2 (3:1; hexanes:ethyl acetate).
15. ^{1}H NMR shifts of the carboxylic acid peak of **2** shift based on concentration of NMR sample.
16. The purity of **2** was determined to be 98.3 wt% by qNMR using 1,3,5-trimethoxybenzene (Sigma Aldrich, >99%) as an internal standard. A second run at the same scale provided **2** in 72% yield with 99.1 wt% purity.
17. The checker suggested that extending high-vacuum (<1 mbar) drying time to 52 h enhanced the purity consistency across runs.

Working with Hazardous Chemicals

The procedures in *Organic Syntheses* are intended for use only by persons with proper training in experimental organic chemistry. All hazardous materials should be handled using the standard procedures for work with chemicals described in references such as "Prudent Practices in the Laboratory" (The National Academies Press, Washington, D.C., 2011; the full text can be accessed free of charge at http://www.nap.edu/catalog.php?record_id=12654). All chemical waste should be disposed of in accordance with local regulations. For general guidelines for the management of chemical waste, see Chapter 8 of Prudent Practices.

In some articles in *Organic Syntheses*, chemical-specific hazards are highlighted in red "Caution Notes" within a procedure. It is important to recognize that the absence of a caution note does not imply that no significant hazards are associated with the chemicals involved in that procedure. Prior to performing a reaction, a thorough risk assessment should be carried out that includes a review of the potential hazards associated with each chemical and experimental operation on the scale that is planned for the procedure. Guidelines for carrying out a risk assessment and for analyzing the hazards associated with chemicals can be found in Chapter 4 of Prudent Practices.

Discussion

The accompanying procedure describes a simple and mild means to access carboxylic acids from methyl esters. Due to their synthetic utility[2] and presence in pharmaceuticals,[3] carboxylic acids and methods to generate them are valuable in organic synthesis. Although carboxylic acids are classically accessed through saponification of esters by hydroxide base,[4] saponification by these means is not tolerant of certain base-labile functionalities. In the case of esters containing β-leaving groups (e.g., **1**, Figure 7), the hydroxide anion can remove the acidic α-proton of the ester, thus affecting an elimination or retro-Michael addition to form an acrylate (e.g., **3**).[5] Therefore, alternative saponification methods under less basic conditions are required to retain the leaving group functionality, while converting esters to acids. Enzymatic hydrolysis to arrive at ester **2** provides an attractive option.

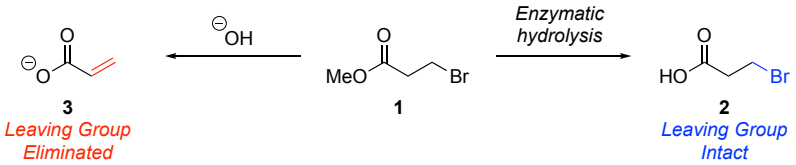


Figure 7. Traditional vs. enzymatic ester hydrolysis

Disclosed in 1993, the Basak group saponified methyl esters bearing various β-leaving groups utilizing Pig Liver Esterase (PLE).[6] This method provided exclusively the desired carboxylic acid products while tolerating a variety of β-leaving groups. Leaving groups including halides, sulfides, selenides, and sulfones were tolerated with no competitive formation of the corresponding acrylate, demonstrating the versatility of this method (Figure 8, **6**–**9**). In addition to leaving group variation, the Basak group also demonstrated that this saponification enabled the synthesis of *N*-benzoyl amino acid derivatives bearing β-leaving groups (**10**–**13**). In these substituted

examples, longer reaction times were observed with moderate yields, however, no undesired product formation was observed.[6]

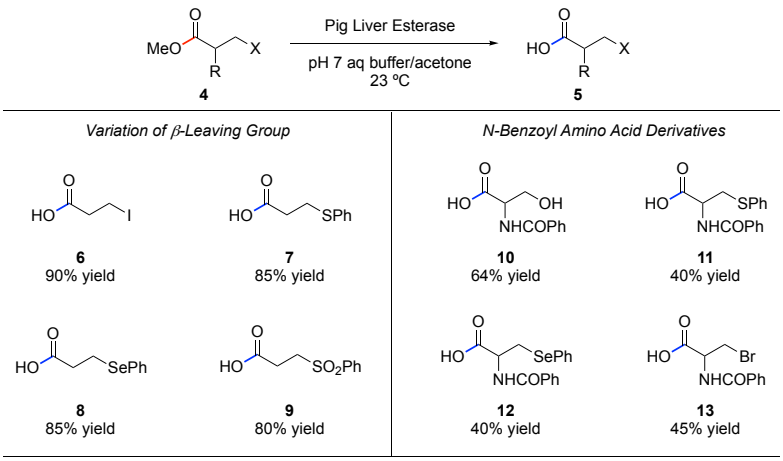
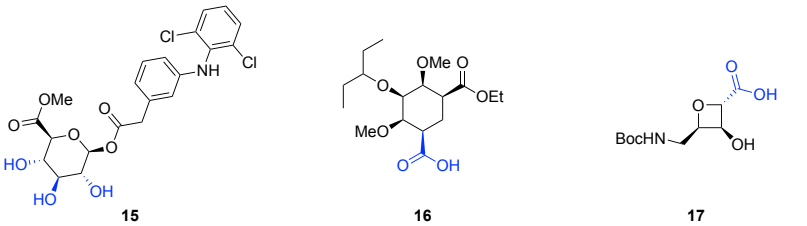


Figure 8. Selected scope of saponification using PLE with respect to leaving group variation and the formation of *N*-benzoyl amino acid derivatives[6]

Whereas the original procedure utilizes PLE, the accompanying procedure uses Amano Lipase from *Pseudomonas fluorescens* instead. Amano Lipase is a more readily available, shelf-stable enzyme that can affect the saponification with high selectivity for the desired product.[7] This enzyme and similar lipases are cultivated from microorganisms that are naturally present in the soil of farms and forests. These processes are overall very environmentally efficient as these saponification reactions require little to no organic solvent.

Furthermore, the saponification of esters mediated by enzymes from microorganisms has been employed by synthetic chemists in complex settings. Yoshioka and coworkers utilized Lipase from *Pseudomonas fluorescens* en route to diclofenac 1-β-*O*-Acyl glucuronide with full saponification of the acetoxy group to afford triol **15** in 90–94% yields (Figure 9).[8] In a synthesis of Oseltamivir Phosphate (Tamiflu) in 2008, the diester precursor to **16** underwent an enzymatic desymmetrization and saponification mediated by Lipase from *Aspergillus oryzae* in quantitative yields and >99.9% enantiomeric excess.[9] Lastly, Wessel and coworkers achieved the synthesis of 2,4-anhydro-5-*N*-(*t*-butoxycarbonyl)amino-D-

lyxonic acid (**17**) in quantitative yields using Lipase L2 from *Candida antarctica*.[10] In this example, several enzymes were screened, including PLE, and Lipase L2 exhibited the best reactivity. All of these processes proceed with high yields and chemoselectivity. In addition, epimerizable stereocenters can be preserved, which highlights the mild nature of enzymatic hydrolysis.



en route to Diclofenac 1-β-O-Acyl Glucuronides	en route to Oseltamivir Phosphate	2,4-Anhydro-5-*N*-(*t*-butoxy-carbonyl)amino-D-lyxonic acid
(Lipase from *Pseudomonas fluorescens)*	(Lipase from *Aspergillus oryzae)*	(Lipase L2 from *Candida antarctica)*

Figure 9. Selected examples of saponifications using lipases isolated from microorganisms

In summary, enzyme-mediated saponification provides a practical means to achieve ester hydrolysis under mild conditions. Specifically, the use of commercially available Amano Lipase from *Pseudomonas fluorescens* enables a simple yet effective method of accomplishing this transformation. Applications in total synthesis have shown this methodology and similar enzyme-mediated approaches are versatile tools to hydrolyze esters in complex settings. Advances in enzymatic saponification would allow for rapid and chemoselective access to carboxylic acids to be leveraged in organic synthesis and in medicinal chemistry.

References

1. Department of Chemistry and Biochemistry, University of California, Los Angeles, California 90095, United States. E-mail: neilgarg@chem.ucla.edu. ORCID 0000-0002-7793-2629. The authors are grateful to the University of California, Los Angeles.
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Appendix
Chemical Abstracts Nomenclature (Registry Number)

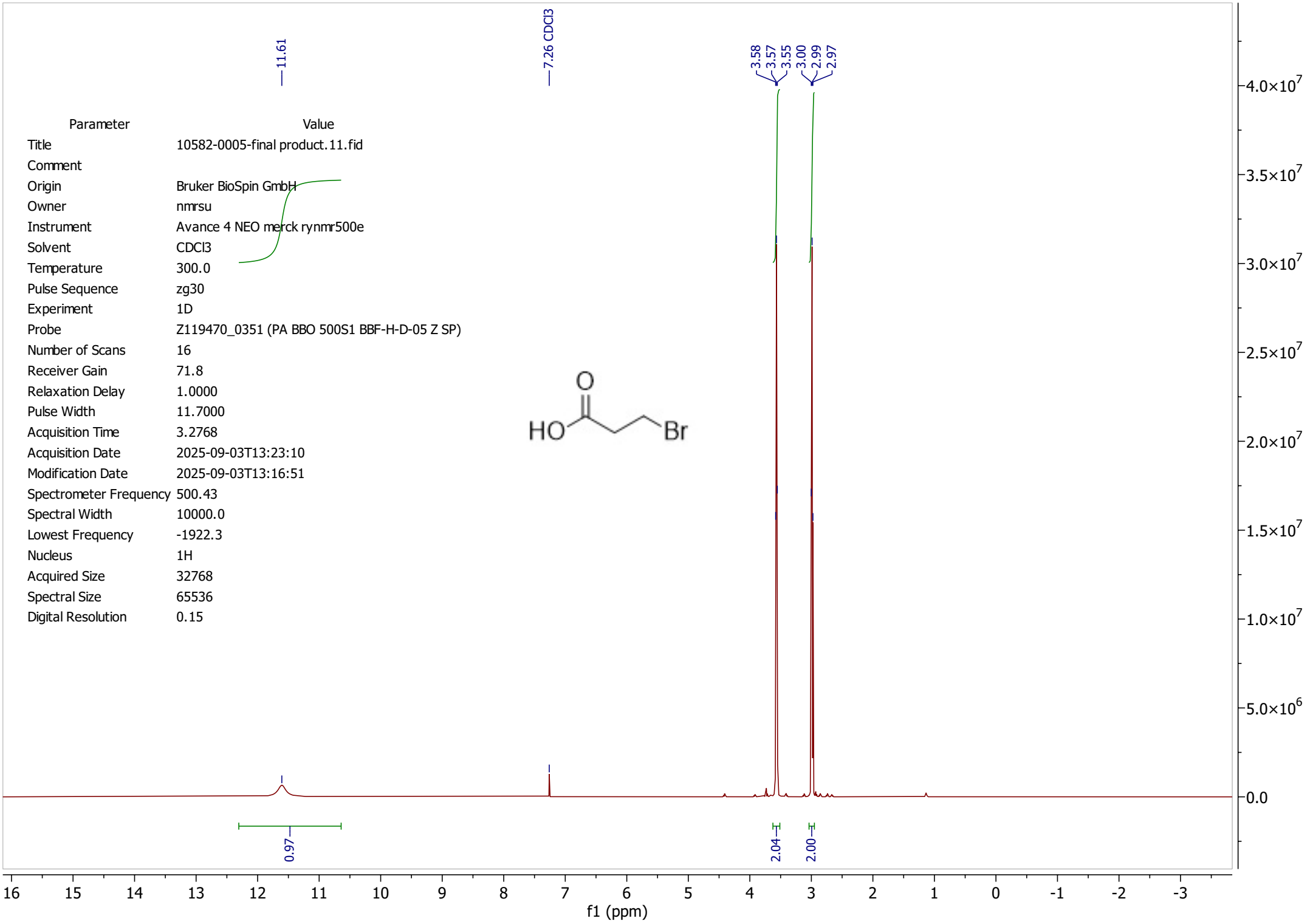
Methyl 3-bromopropionate; (3395-91-3)
Amano Lipase from *Pseudomonas fluorescens*; (9001-62-1)
Hydrochloric acid (12.0 M in water); (7647-01-0)
Sodium Chloride; (7647-14-5)
Sodium Sulfate; (7757-82-6)

Benjamin Janda was born and raised in San Jose, California. In 2023, he received his B.S. in Chemistry from Chapman University where he researched in the laboratory of Professor Allegra Liberman-Martin. In 2023, he began his graduate studies at the University of California, Los Angeles, where he is currently a third-year graduate student in Professor Neil K. Garg's laboratory. His studies focus on synthetic methods leveraging strained intermediates and geometric distortion.

Allison Clark was born in Los Altos, CA and received her B.S. in Chemistry from the University of Southern California where she carried out research under the direction of Professor Megan Fieser. In 2023, she then moved to the University of California, Los Angeles, where she is currently a third-year graduate student in Professor Neil K. Garg's laboratory. Her studies primarily focus on developing synthetic methods utilizing strained intermediates.

Neil Garg is the Distinguished Kenneth N. Trueblood Professor of Chemistry at the University of California, Los Angeles. His laboratory develops practical synthetic methods that challenge long-standing paradigms of reactivity.

Ke Zhao grew up in Zhejiang, China. He earned a B.S. from Lanzhou University and then pursued a Ph.D. in organic chemistry at the University of California, Santa Barbara, where his graduate research focused on bifunctional ligand development and cooperative gold catalysis. He is currently a process chemist at Merck Research Laboratories in Rahway, New Jersey.



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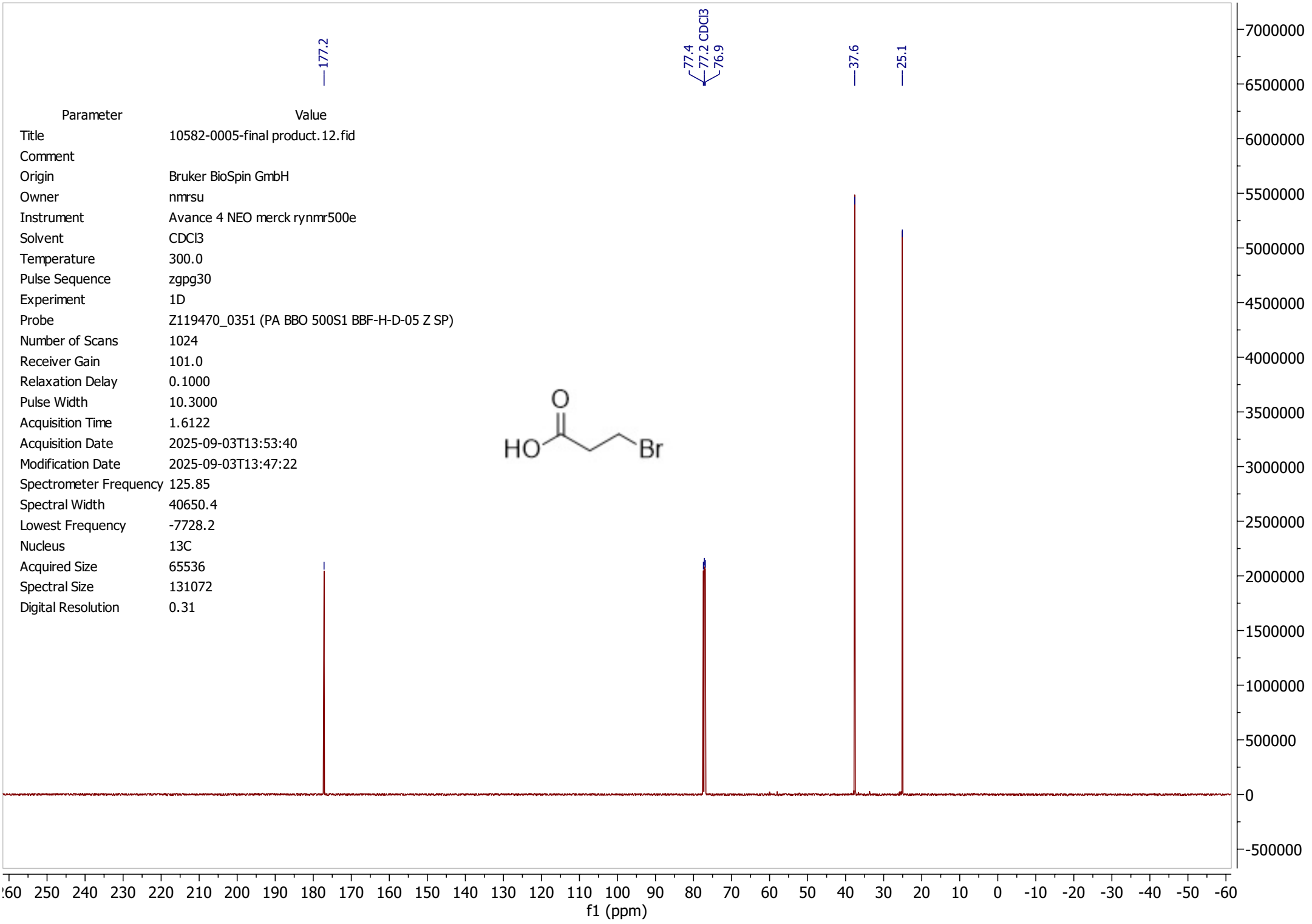
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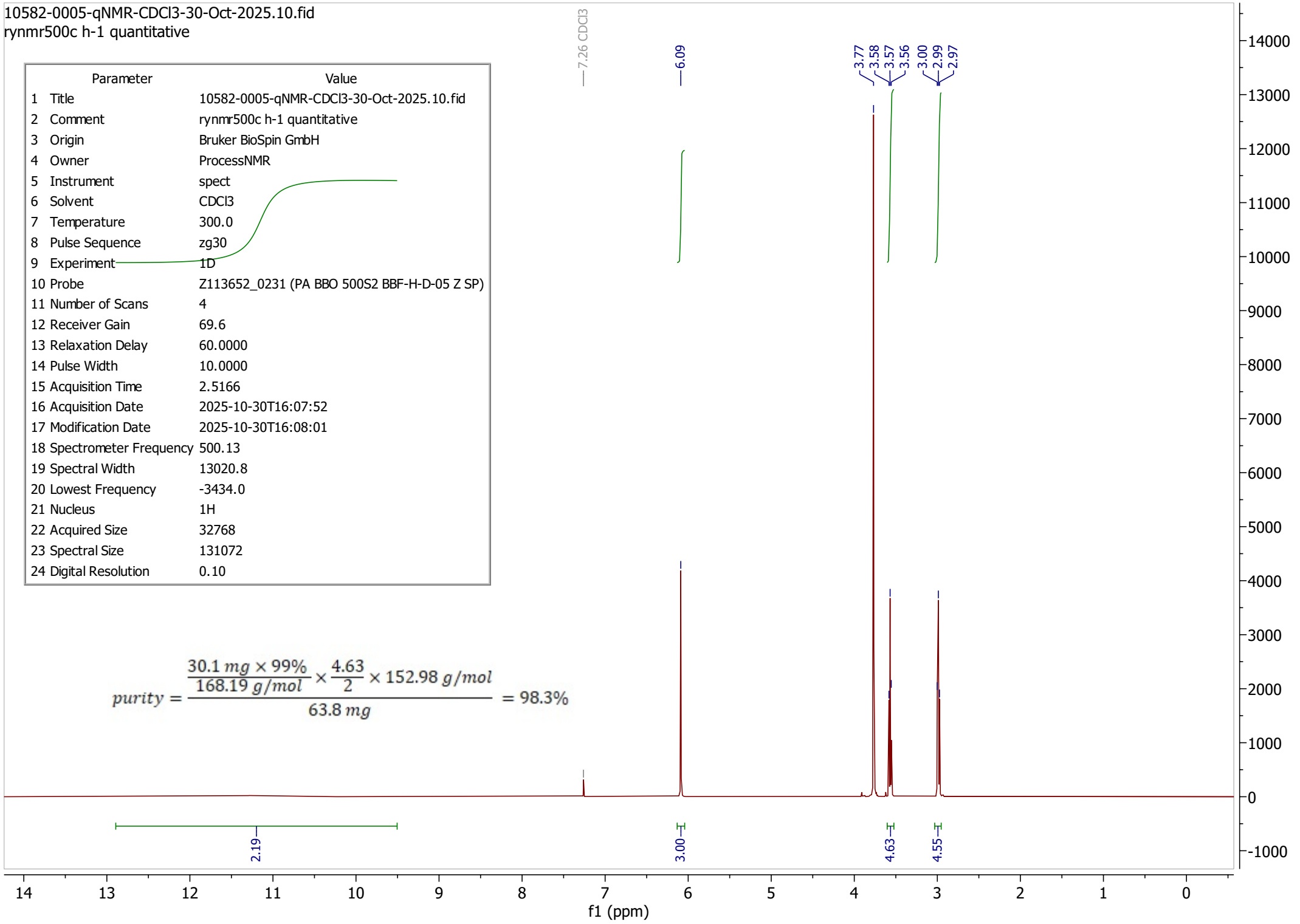

$$purity = \frac{\frac{30.1\ mg \times 99\%}{168.19\ g/mol} \times \frac{4.63}{2} \times 152.98\ g/mol}{63.8\ mg} = 98.3\%$$