Two-Step Synthesis of Paracetamol (Acetaminophen), a Practical Illustration of Carbonyl Reactivity for Year-One Biosciences Students

Shah, Ifat; Rose, Michael; Phillips, Helen; Flower, Stephen E.; Woodman, Timothy J.; Garty, Cameron; Threadgill, Michael D.

Published in:
Journal of Chemical Education

DOI:
10.1021/acs.jchemed.3c00549

Publication date:
2023

Citation for published version (APA):
Shah, I., Rose, M., Phillips, H., Flower, S. E., Woodman, T. J., Garty, C., & Threadgill, M. D. (2023). Two-Step Synthesis of Paracetamol (Acetaminophen), a Practical Illustration of Carbonyl Reactivity for Year-One Biosciences Students. *Journal of Chemical Education*, *100*(10), 3955-3959. https://doi.org/10.1021/acs.jchemed.3c00549

JOURNAL OF CHEMICAL EDUCATION

pubs.acs.org/jchemeduc

Article

Two-Step Synthesis of Paracetamol (Acetaminophen), a Practical Illustration of Carbonyl Reactivity for Year-One Biosciences Students

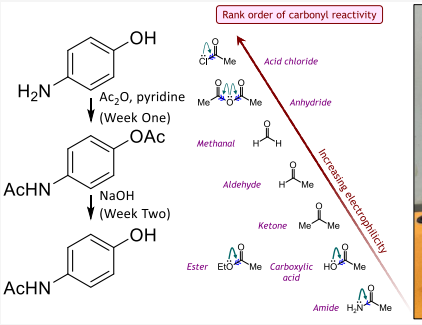
Ifat Parveen,* Michael Rose, Helen C. Phillips, Stephen E. Flower, Timothy J. Woodman, Cameron A. Garty, and Michael D. Threadgill

Cite This: https://doi.org/10.1021/acs.jchemed.3c00549

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: An understanding of basic organic chemical reactivity is key for many biosciences students. The reactivities of different carbonyl groups affect their function in many biomolecules. A practical class, the two-step synthesis of paracetamol, has been devised to illustrate the electrophilic reactivities of carbonyls, which was covered in the accompanying lecture program. Students also examine the UV, IR, NMR, and mass spectra of the esters and amides, building further on the understanding gained in lectures. The practical work itself has been devised to be able to be run in bioscience laboratories with limited chemical facilities. The class has been enhanced during four academic years with strong support from the students.



KEYWORDS: *First-Year Undergraduate/General, Organic Chemistry, Hands-On Learning/Manipulatives, Amides, Esters, Paracetamol, Reinforcement of Chemical Reactivity, Integration of Spectroscopy, Adaptation to Limited Laboratory Facilities*

INTRODUCTION

A significant issue in the teaching of chemistry to early year undergraduate biosciences students has been reinforcing the fundamental concepts of organic reactivity that the students encounter in the lecture program. Students taking undergraduate programs in biochemistry, pharmacology, pharmacy, and related disciplines need to have a basic understanding of organic reactivity to be able to rationalize enzyme-catalyzed processes and anabolism, catabolism, and metabolism at a molecular level. For example, knowledge and understanding of the different electrophilic reactivities of different types of carbonyl groups are key to understanding the stability of the amides in the backbones of proteins, the mechanisms of the proteases, and the acylating power of acyl phosphates in biological systems. Master of Pharmacy and Master of Pharmacology students at the University of Bath and BSc (Biochemistry) students at Aberystwyth University receive lectures setting out the rank order of electrophilic reactivity of the different types of carbonyl groups along with the underlying rationale for this ranking. There is a risk that students regard this as purely theoretical; therefore, this practical synthesis of paracetamol was devised to reinforce their understanding and place it in a "real world" context. These students also receive theoretical training in solving simple NMR, MS, and IR spectra, and the practical experiment allows them to relate their structure elucidation skills to a real problem in the laboratory. The practical serves to integrate students' knowledge and understanding as well as provide opportunities for laboratory experience, teamwork, and time management.[1]

Students taking chemistry as a foundation or subsidiary topic to support their core biosciences will often work in a department where provision of chemical laboratory facilities, equipment, and instrumentation is very limited, unlike those in a mainline chemistry department. Thus, this practical experiment is designed for maximum gain in reinforcing lecture content while being capable of being run for inexperienced students in a 30-person laboratory with two fume cupboards.

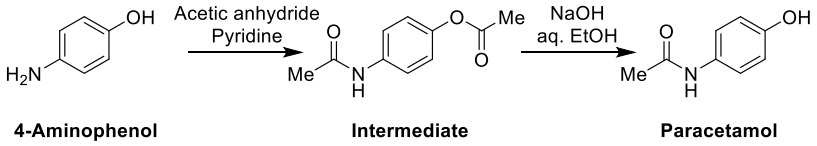
EXPERIMENTAL OVERVIEW

Choice of Route and Target Molecule

In choosing a target molecule for synthesis, it was important to select one with which these non-chemistry students were familiar in their daily lives to promote engagement. In addition, the synthetic work had to be feasible to be completed within two 4 h practical classes. As this was the only organic chemistry laboratory time available to the students in their programs, it was important to design the synthesis to illustrate the

Received: June 8, 2023
Revised: September 6, 2023

Scheme 1. Reaction Scheme for the Two-Step Synthesis of Paracetamol from 4-Aminophenol



maximum number of points from the parallel lectures. Also important was that, in view of the limited facilities, the practical work involved relatively safe reagents and solvents and could be carried out in large test tubes ("boiling tubes") using only four magnetic stirrer units at room temperature.

Paracetamol fulfills many requirements as a target molecule. It has been used very widely for over 60 years for the symptomatic relief of mild and moderate pain both alone and in combinations with other analgesic drugs. It is also used to treat fever, although it appears to be devoid of anti-inflammatory activity. It is available as 500 mg tablets as an over-the-counter medicine in the UK and is inexpensive. Thus, many of the students will be familiar with its use. The recommended maximum daily dose is 4.0 g, with hepatotoxicity as the dose-limiting toxicity. It is not considered to be carcinogenic.[2] "Paracetamol" is the International Nonproprietary Name (INN) but "Acetaminophen" is used in the USA as the United States Adopted Name (USAN).

This choice of target molecule was also apposite, as there is available a synthetic route that illustrates many points and concepts from the parallel lectures in basic spectroscopy and electrophilic reactivity of carbonyl groups. Paracetamol has been reported to be synthesized by various routes, including Beckmann rearrangement of 4-hydroxyacetophenone oxime with a Vilsmeier reagent[3] or a boronic acid,[4] demethylation of 4-methoxyacetanilide with BBr_3,[5] regioselective oxidation of acetanilide,[6] and oxidation of N-(4-hydroxyphenyl)-acetaldehyde imine.[7] However, none of the above fulfills the design requirements of illustrating the lecture material on reactivity of carbonyl groups, safe reagents, and simple apparatus. Paracetamol has also been prepared by direct monoacetylation of 4-aminophenol with acetic anhydride in aqueous solution in the presence of detergent[8] or with other mild acetylating agents. However, to give the students more experience of a two-step synthesis, along with isolation and characterization of an intermediate, deacetylation of 4-aminophenol, followed by chemoselective hydrolysis of the intermediate ester (Scheme 1), was selected. Ellis has produced a monograph describing a synthesis of paracetamol which obviates the intermediate[9] but therefore loses the understanding of differential reactivity of amides and esters which this present practical provides.

Development and Optimization

The initial setup of the experiment was as follows: 4-aminophenol was suspended in dichloromethane and treated with various excesses of acetic anhydride and catalytic pyridine to provide 4-acetamidophenyl acetate. This procedure was low-yielding and unreliable, owing to the poor solubility of 4-aminophenol in this solvent, and gave mixtures of starting material, paracetamol, and the desired intermediate. Changing the solvent to ethyl acetate reduced the toxicity of the solvent and effectively dissolved the 4-aminophenol, giving quantitative yields of the isolated intermediate 4-acetamidophenyl acetate. To reduce the exposure of the students to the aerosol dust of 4-aminophenol, this compound was supplied to students as a preweighed and predissolved solution of 4-aminophenol (200 mg) in ethyl acetate (2.0 mL). This approach eliminated the need for each student to accurately weigh the fine powder in the two small fume cupboards available. The reaction vessels were changed from conventional round-bottomed Quickfit flasks to boiling tubes to save cost of equipment for the 40 students and to allow 20 reactions to be stirred simultaneously with one magnetic hot plate stirrer in a fume cupboard. The diacylation reaction was optimized to be complete in *ca.* 30 min using five equivalents of acetic anhydride and two drops of pyridine; this time was most convenient for scheduling of the laboratory class. The mobile phase for the TLC monitoring of the reaction was optimized as ethyl acetate/petroleum ether (60−80) (2:1).

Initially, the workup involved simple wash with aq. $NaHCO_3$ (to hydrolyze excess acetic anhydride and remove acetic acid), and wash with hydrochloric acid (2 M) to remove pyridine. However, the pyridine was not effectively removed under these conditions and an extra wash with aq. $CuSO_4$ was added. A wash with saturated aq. NaCl was added to minimize the use of anhydrous $MgSO_4$ in the drying step with a concomitant loss of yield.

In the second step, the hydrolysis required ethanol (4.0 mL) for complete dissolution and for the substrate to remain in solution when the aq. NaOH (2.0 M, 2.0 mL) was added. Students were supplied with the aq. NaOH solution to avoid having to manipulate hygroscopic and corrosive NaOH pellets. Again, the conditions were optimized so that the reaction was complete within 40 min. In the first run-through of the hydrolysis reaction, the first step of workup was to add ethyl acetate and wash with water; this led to considerable loss of yield as the phenolate anion from the product paracetamol partitioned into the highly basic aqueous phase. The first wash was changed to aq. HCl, which obviated this problem by lowering the pH of the aqueous phase and retaining paracetamol in the organic phase.

HAZARDS

At the beginning of each class, students were given a safety briefing, including the use of Personal Protective Equipment. Students were warned that they would be excluded if they breached the safety requirements.

4-Aminophenol is harmful if swallowed or inhaled; the risk is minimized by presenting this compound as a premeasured solution in EtOAc. Acetic anhydride is harmful if swallowed and can cause chemical burns; the risk is minimized by students manipulating the liquid in the fume cupboard only and using eye protection and disposable gloves. Pyridine is harmful if swallowed and is flammable; the risk is minimized by students manipulating the liquid only in the fume cupboard and using eye protection and disposable gloves, in the absence of sources of ignition. Ethyl acetate, petroleum, and ethanol are flammable; no sources of ignition are present. Hydrochloric acid and sodium hydroxide cause chemical burns; only dilute

https://doi.org/10.1021/acs.jchemed.3c00549
J. Chem. Educ. XXXX, XXX, XXX−XXX

solutions are used by the students, who wear eye and hand protection.

RESULTS AND DISCUSSION

Synthesis

As the students had different educational backgrounds and previous experience, it was important that detailed protocols were provided in clear English (Supporting Information). These protocols not only gave instructions of the experimental work to be carried out but also asked the students specific questions on why they were conducting individual steps and made the students think about underlying concepts. Example of these questions include "*Why is a pencil used, rather than a pen?*" [referring to marking a TLC plate], "*Why is this washing with aq. $NaHCO_3$necessary? What is the gas produced?*" [referring to a washing step], "*Why is the solution washed immediately with acid? What is the function of hydrochloric acid? Why not wash with water first?*" [referring to another washing step], and "*What are the Rf value(s)? Which spot(s) are colored with $FeCl_3$? Why?*". The laboratory protocols also outlined the reports that the students would have to write and how they would be assessed. Students formed working pairs for the class. All students were given a briefing on laboratory safety at the start of each session, followed by an introduction to the practical.

Students added a magnetic stirrer bead to the solution of 4-aminophenol (200 mg, 1.83 mmol) in ethyl acetate (2.0 mL) in a boiling tube and placed it in a beaker over a stirrer hot plate, by which it was stirred slowly. Acetic anhydride (1.00 mL, 10.6 mmol) was added using a glass pipet, followed by 2−4 drops of catalyst pyridine from a glass Pasteur pipet. The progress of the reaction was monitored by TLC on Al-backed F254 silica gel plates (approximately 8.0 × 2.5 cm^2). The TLC technique was demonstrated by the class leader (Figure 1). Students made their own capillary tubes for spotting the plates by heating soda-glass Pasteur pipets in a Bunsen flame in a separate room and drawing them out; this gave the students confidence in safely manipulating hot and softened glass. TLC spots for the starting 4-aminophenol and the intermediate 4-acetamidophenyl acetate were visualized using a UV lamp and by dipping the plates (using forceps) into $FeCl_3$ in MeOH to reveal unmasked phenols as red−purple spots. Students noted the 4-aminophenol spot colored with Fe(III) but did not see the 4-acetamidophenyl acetate, confirming that both the ester and the amide had formed. When the reaction was shown to be complete, the class leader demonstrated the use of a separating funnel. Students then carried out the washing procedure (aq. $NaHCO_3$, aq. HCl, aq. $CuSO_4$, saturated aq. NaCl) and dried the solution with anhydrous $MgSO_4$. As only two rotary evaporators were available, the solvents were evaporated by the class technician during the week between the two practical sessions.

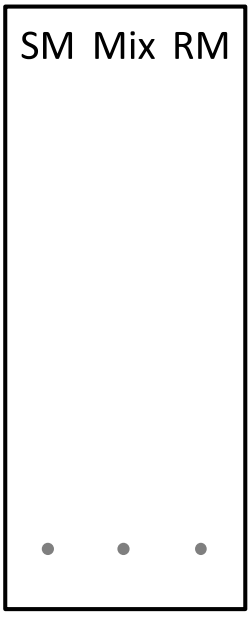


Figure 1. Example of diagram given to students to illustrate a good TLC technique.

In the second practical class (in Week Two), students weighed their intermediate 4-acetamidophenyl acetate and calculated the percentage yields (reinforcing the mole concept). Small samples were retained to run UV and IR spectra ("Spectroscopy in a Suitcase"). The remaining intermediate material (in small round-bottomed flasks) was then dissolved in 95% ethanol (4.0 mL) and aq. NaOH (2.0 M, 2.0 mL) was added using a glass pipet. These reaction mixtures were stirred, using one magnetic stirrer hot plate for each five flasks, and progress was again monitored by TLC. In this step, students were expecting the final product paracetamol to color with Fe(III), with the intermediate ester not reacting with this visualizing reagent. When the hydrolysis was shown to be complete (absence of the ester spot on TLC), the reaction mixture was diluted with ethyl acetate as a water-immiscible solvent to facilitate washings. Using a separating funnel and the skills learned in Week One, students washed the reaction mixture with hydrochloric acid and saturated brine. The protocol quizzed the students as to why each of these washes was applied. The solution was then dried ($MgSO_4$) and the solvent was evaporated. Students then calculated the percentage yields.

Spectroscopy

Students ran their own IR spectra for the intermediate acetamidophenyl acetate and paracetamol using a simple "Spectroscopy in a Suitcase" instrument. They used the IR bands for the two carbonyls in the former to reinforce their understanding of the "Rank Order of Carbonyl Reactivity" concept from the lecture program. This concept ranks the different types of carbonyl group by polarization of the C=O, with explanation in terms of inductive and mesomeric/resonance effects of substituents (Figure 2). This rank order is also a rank order of the electrophilic reactivity of the carbonyls and is broadly reflected in their IR stretching frequencies. Thus, the ester carbonyl absorbs at 1749 cm^{-1} and the amide (lower in the rank order) absorbs at 1663 cm^{-1} (Figure 3).

Copies of the ^1H and ^{13}C NMR spectra for 4-aminophenol, the intermediate 4-acetamidophenyl acetate, and the paracetamol were provided to the students to avoid using excessive instrument time. Students were expected to assign the signals in these spectra, paying particular attention to the downfield shift of the aromatic proton signals (and corresponding carbon signals) when the amine and phenolic OH were acylated. Similarly, they observed upfield shifts of the signals for ^1H and ^{13}C *ortho* to oxygen when the ester was hydrolyzed to form paracetamol (Figure 3). These data were used to reinforce the

https://doi.org/10.1021/acs.jchemed.3c00549
J. Chem. Educ. XXXX, XXX, XXX−XXX

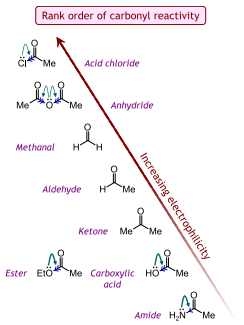


Figure 2. Still of part of the animated PowerPoint slide used in lectures on the reactivity of different carbonyl compounds, developing the rank order of reactivity.

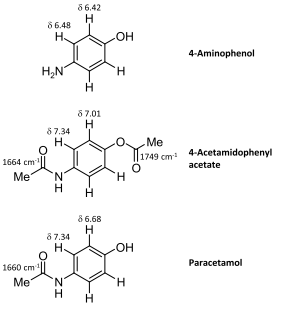


Figure 3. IR absorption bands for the C=O stretches of the amide and ester carbonyls (illustrating the rank order of carbonyls) and ^{1}H NMR chemical shifts for Ar−H (illustrating inductive and mesomeric effects on electron-density).

concepts of mesomeric electron-donation into aromatic rings that were learned in the accompanying lectures.

Low-resolution mass spectra for the intermediate 4-acetamidophenyl acetate and for the product paracetamol were provided. The spectrum of paracetamol shows a strong pseudomolecular ion at m/z 152, corresponding to $[M + H]^+$. Students were expected to calculate the MW of paracetamol and relate this to the observed mass. The spectrum of the intermediate ester showed $[M + H]^+$ at m/z 194 and a fragment ion at m/z 152, showing a loss of ketene and confirming the acetate ester. Calculating the MWs gave students confidence in considering chemical formulas.

Outcomes and Relevance to Lecture Material

The Learning Outcomes for this class were *(i)* confidence and skills in laboratory manipulation of chemicals, *(ii)* reinforcement of lecture material on structure and reactivity of carbonyl compounds (important in understanding biochemical processes), *(iii)* reinforcement of lecture material on interpretation of spectroscopic data, and *(iv)* integration and application of knowledge and understanding in chemical structure and reactivity.

The students taking this module were in their first year of undergraduate study and had a wide range of previous experience in a laboratory setting. Thus, this experiment was designed with simple equipment and relatively simple manipulations. Each laboratory class of 40 students was supported by an academic class leader, a senior teaching technician, and a postgraduate demonstrator, who provided demonstrations of the use of equipment and of techniques.

The carbonyl group is a key functional group in many biochemical processes including the biosynthesis of fatty acids, carbohydrates (glycosidic bonds), peptides, and the urea cycle. Understanding its reactivity is important to these biosciences students. The lecture program has three lectures in which the reactivities of the different types of carbonyl group are discussed and explained in terms of inductive and mesomeric effects of substituents (Figure 3). This practical class reinforces the powerful electrophilic reactivity of acid anhydrides in the first step and the greater electrophilicity of esters (compared with amides) in the hydrolysis step.

Students receive four lectures on interpretation of IR, NMR, and MS, which are supported by a problem-solving workshop class. This practical class enables the students to place this classroom learning in the context of real spectra from a real chemical synthesis that they carried out. Concepts of electron density are also reinforced by the consideration of NMR chemical shifts, while the rank order of carbonyls is illustrated by the IR spectra.

This practical class was designed carefully to map closely onto the lecture and workshop materials and to explore amides and ester carbonyl groups, which are important in biochemistry.

Written Report and Assessment

Students were required to submit a written report on their experimental work, on the underlying chemistry and mechanisms, and on their interpretation of the spectra. This report formed a summative assessment for the module, contributing 30% of the total module mark (half of the coursework element). During Week One, students were advised to start preparing for writing this report by considering and revising the chemical mechanisms, including curly arrows. In a parallel lecture class, students were given guidance on writing scientific reports and using concise scientific language. Reports, capped at 3000 words, were submitted as hard copies and were assessed independently by two academics. The mean mark in the 2019−2020 assessment was 56.5% (range 42%−72%) with a bimodal distribution. The lower peak of percentages indicated those students who found general difficulty in writing cogent scientific English. To address this latter problem, students in subsequent years were provided with a model report describing synthesis of a different drug (aspirin) to help them to organize their material and to understand scientific writing style.

Feedback from Students

Students were surveyed anonymously following the practical classes in early 2019 and early 2022 (classes in 2020 and 2021 were virtual owing to Covid-19 regulations). The responses are given in detail in the Supporting Information. Students were generally supportive and appreciated the integration of the practical and lecture material. Some minor suggestions from students were acted upon to refine the practical. They were particularly appreciative of the specimen written report in guiding them in writing scientific English.

■ CONCLUSION

In conclusion, a simple practical class has been developed which gives students experience in chemical transformations and handling chemicals and equipment. It was designed carefully to illustrate many of the points raised in the lectures on carbonyl (bio)chemistry and spectroscopy, enhancing the integration of the students' knowledge and understanding. This practical class has been designed for a biosciences department with minimal chemical laboratory facilities.

https://doi.org/10.1021/acs.jchemed.3c00549
J. Chem. Educ. XXXX, XXX, XXX−XXX

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available at https://pubs.acs.org/doi/10.1021/acs.jchemed.3c00549.

Protocols, specimen scientific report given to students, feedback from students, image of the experimental setup for Week One (PDF)

Spectra (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Ifat Parveen − *Department of Life Sciences, Aberystwyth University, Aberystwyth SY23 3DA, United Kingdom;* orcid.org/0000-0001-7455-2914; Email: ifp@aber.ac.uk

Authors

Michael Rose − *Tasmanian Institute of Agriculture, University of Tasmania, Sandy Bay TAS 7005, Australia*

Helen C. Phillips − *Department of Life Sciences, Aberystwyth University, Aberystwyth SY23 3DA, United Kingdom*

Stephen E. Flower − *Department of Chemistry, University of Bath, Bath BA2 7AY, United Kingdom*

Timothy J. Woodman − *Department of Life Sciences, University of Bath, Bath BA2 7AY, United Kingdom*

Cameron A. Garty − *Department of Life Sciences, Aberystwyth University, Aberystwyth SY23 3DA, United Kingdom*

Michael D. Threadgill − *Department of Life Sciences, Aberystwyth University, Aberystwyth SY23 3DA, United Kingdom; Department of Life Sciences, University of Bath, Bath BA2 7AY, United Kingdom*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jchemed.3c00549

Author Contributions

All authors contributed to the preparation of the manuscript. I.P., M.R., and M.D.T. devised the experiment. H.C.P. and C.A.G. carried out optimization runs and supported the classes. T.J.W. supplied the NMR spectra. H.C.P. supplied the mass spectra. S.E.F. contributed to the pedagogical aspects of the work.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The development work was supported by Aberystwyth University. We thank Prof. Hazel Davey (Aberystwyth University) for the helpful discussions. We thank the undergraduate students for their helpful and constructive comments which helped to refine the practical class.

■ REFERENCES

(1) Reid, N.; Shah, I. The role of laboratory work in university chemistry. *Chem. Ed. Res. Practice* **2007**, *8*, 172−185.

(2) Bergman, K.; Müller, L.; Weberg Teigen, S. The genotoxicity and carcinogenicity of paracetamol: a regulatory (re)view. *Mutat. Res.* **1996**, *349*, 263−288.

(3) Mo, X.; Morgan, T. D. R.; Ang, H. T.; Hall, D. G. Scope and mechanism of a true organocatalytic Beckmann rearrangement with a boronic acid/perfluoropinacol system under ambient conditions. *J. Am. Chem. Soc.* **2018**, *140*, 5264−5271.

(4) Narahari, S. R.; Reguri, B. R.; Mukkanti, K. Beckmann rearrangement of oximes using pivaloyl chloride/DMF complex. *Tetrahedron Lett.* **2011**, *52*, 4888−4891.

(5) Vodnala, N.; Gujjarappa, R.; Hazra, C. K.; Kaldhi, D.; Kabi, A. K.; Beifuss, U.; Malakar, C. C. Copper-catalyzed site-selective oxidative C——C bond cleavage of simple ketones for the synthesis of anilides and paracetamol. *Adv. Synth. Catal.* **2019**, *361*, 135−145.

(6) Liu, H.; Wang, X.; Gu, Y. Direct acetoxylation and etherification of anilides using phenyliodine bis(trifluoroacetate). *Org. Biomol. Chem.* **2011**, *9*, 1614−1620.

(7) Deshidi, R.; Rizvi, M. A.; Shah, B. A. Highly efficient dehydrogenative cross-coupling of aldehydes with amines and alcohols. *RSC Adv.* **2015**, *5*, 90521−90524.

(8) Naik, S.; Bhattacharjya, G.; Talukdar, B.; Patel, B. K. Chemoselective acylation of amines in aqueous media. *Eur. J. Org. Chem.* **2004**, *2004*, 1254−1260.

(9) Ellis, F. *Paracetamol − a curriculum resource*; Royal Society of Chemistry: London, U.K., 2002.