Synthesis of quinolone antibiotic analogues: a multi-step synthetic chemistry experiment for undergraduates

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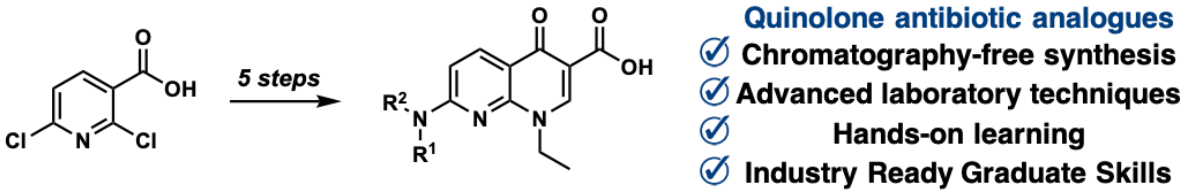
Abstract



A multistep synthesis of quinolone antibiotic analogues was developed as a laboratory experiment for intermediate/advanced undergraduate students. Students can synthesize a range of *des*fluoroenoxacin analogues via a five-step sequence. The experiment includes a range of key practical laboratory techniques including thin-layer chromatography (TLC), liquid-liquid extraction, trituration, recrystallisation and the characterisation of compounds by IR and NMR spectroscopy. The experiment provides an opportunity for students to carry out fundamental organic chemistry transformations from the curriculum such as the preparation of acyl chlorides, 1,4-conjugate addition-elimination, heterocycle synthesis, nucleophilic aromatic substitution and ester hydrolysis. The five-step sequence does not require column chromatography and can be adapted to a range of laboratory settings.

Keywords: Organic chemistry, medicinal chemistry, antibiotics, synthesis, hands-on learning, NMR spectroscopy, IR spectroscopy.

Introduction

The quinolone family of antibiotics are one of the most important group of chemotherapeutics in use for the treatment of community- and hospital-acquired infections.[1] Structurally, they contain a bicyclic core related to 4-quinolone or 4-napthyridone and show varying patterns of substitution (Figure 1).

Initially discovered serendipitously during the synthesis of the antimalarial chloroquine, further investigation led to the first commercial quinolone antibiotic, nalidixic acid (Figure 1) in 1962.[2] In the 1970s – 1980s it was discovered that 6-substitition of the quinoline ring with fluorine, such as in enoxacin and ciprofloxacin (Figure 1), significantly improved the spectrum of activity and the pharmacokinetics.[1] Gemifloxacin (Figure 1) is an example of a fourth-generation quinolone antibiotic that is used for the treatment of community-acquired pneumonia.[3]

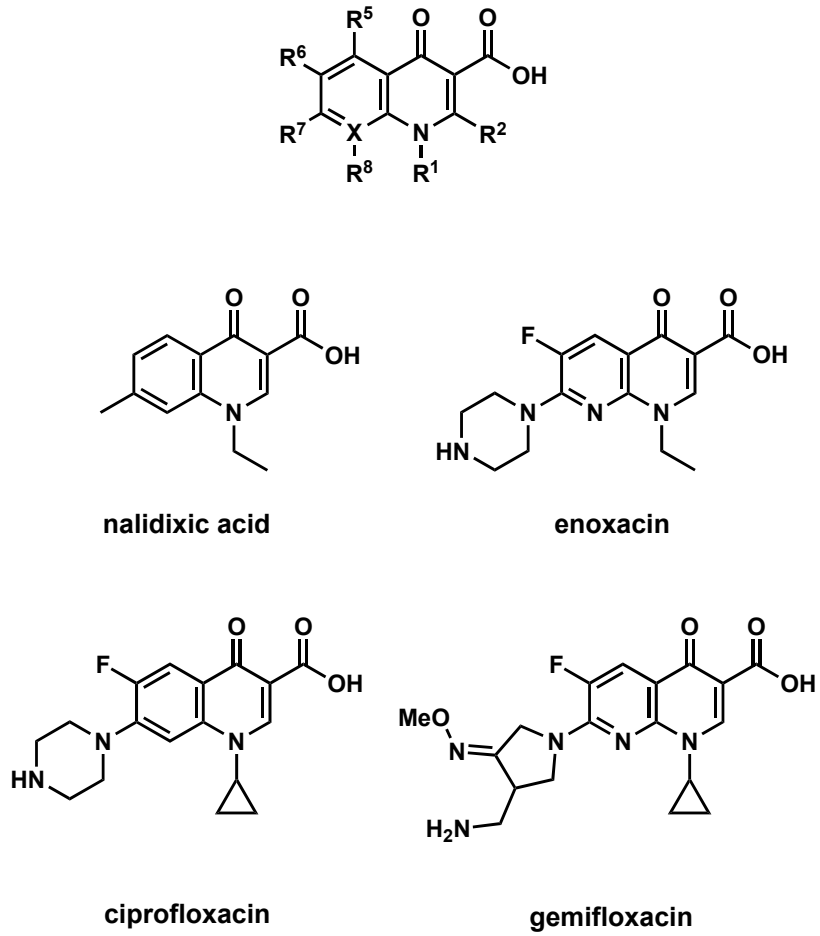


Figure 1: General pharmacophore of quinolone antibiotics and examples of quinolone antibiotics. Positions R^1 and R^{5-8} have been modified to improve potency, selectivity and pharmacokinetics. X = C, quinolone, X = N, napthyridone

The history of antibiotics and the medicinal chemistry around their design and synthesis remains a key learning topic within the undergraduate curriculum in medicinal chemistry.[4] However, incorporating this topic into a synthetic organic chemistry laboratory can be challenging due to the requirements for multi-step synthesis involving column chromatography – a technique not always available in undergraduate teaching laboratories due to demands on equipment, cost and the potential hazards of handing and disposing of free-flowing silica gel.[5] Typically, shorter experiments such as the synthesis of aspirin,[6] acetaminophen[7] and phenacetin[8] are used. These are appropriate for inexperienced students but are not sufficiently challenging for those in advanced laboratory classes.

Herein we report the chromatography-free synthesis of *des*fluoroquinolone analogues of enoxacin. This multi-step experiment allows for a more challenging synthetic organic chemistry experience for undergraduate students and allows them to carry out organic transformations typically used in the pharmaceutical industry and that are taught in the organic chemistry curriculum, which includes the preparation of acyl chlorides, 1,4-conjugate addition-elimination, heterocycle synthesis, nucleophilic aromatic substitution and ester hydrolysis. This experiment provides a guided-inquiry based project that is closely aligned with industrial pharmaceutical chemistry and research and contributes to our ongoing work in training 'industry-ready' graduates in medicinal chemistry.[9–12]

Pedagogical significance

The principal aim of this work is to introduce the concepts of multi-step organic synthesis and conservation of material to undergraduate students. Many experiments in the teaching labs are single step transformations and do not sufficiently communicate to students that many commercial processes are more than one step and require multiple transformations to achieve an end product e.g. a bioactive pharmaceutical. As with our previous work,[1] this study allows students to engage in the design of their own analogues to prepare, leading to a unique drug discovery laboratory experience for each student. The pedagogical goals for this experiment are:

i. To introduce the multistep synthesis concept

ii. To apply the academic knowledge of the aforementioned organic transformations to the laboratory
iii. To utilise common separation techniques in organic chemistry such as the effect of pH on compound solubility within organic/aqueous solvents and how this can be used to separate compounds without the need for chromatography.
iv. To introduce students to compound characterisation by infra-red and ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy.
v. To align the undergraduate academic teaching of antibiotics with the teaching laboratory.

Experimental overview

The laboratory project has been developed to align with a group mini-project approach undertaken by third-year undergraduates at the University of Nottingham. It is split into three components.

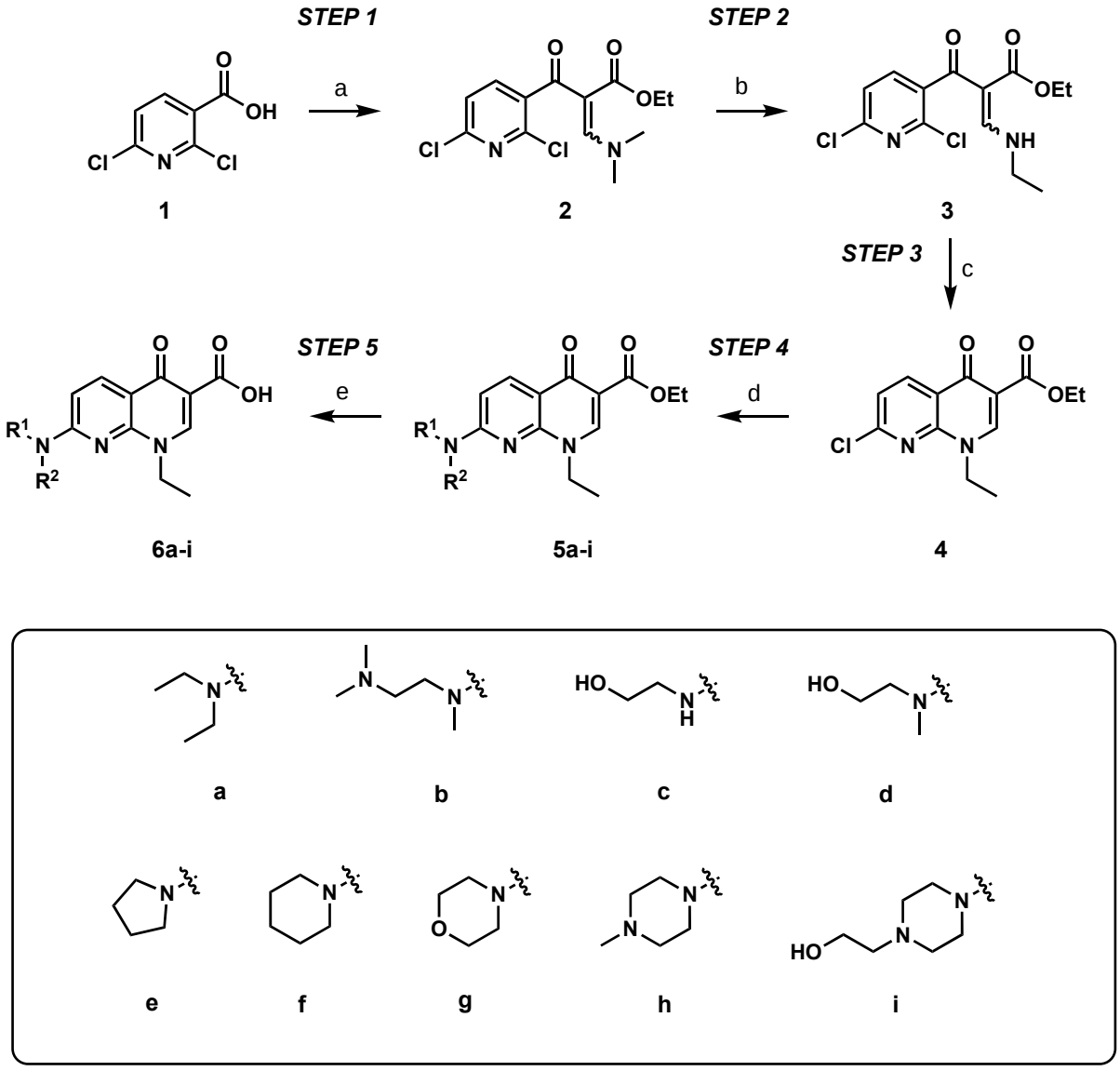
(1) Prelab planning stage to prepare a project proposal
(2) Experimental work
(3) Post-laboratory analysis and write up.

As part of our redesigned laboratory curriculum for third-year undergraduates they undertake pre-lab training in searching the literature using tools such as SciFinder and Reaxys. For mini-projects, students self-select into groups of 5 or 6 students and select projects based on their research interests; as a result up to eight groups of students carry out the project over the course of the year (up to 48 students). As part of their assessment, students are examined on their ability to successfully design a project based on literature revision, effective communication and team working, the identification of hazards, relevant precautions and disposal to ensure safe experimental work, and the use of good chemical laboratory practice (GCLP).

Each group is given two weeks to prepare a project proposal that is formatively assessed. This proposal contains a summary of the literature resources investigated and an experimental plan for their time in the laboratory. It would then be expected that students research three main themes during the first week – the importance of the quinolone family of antibiotics, the synthetic routes to 4-

quinolones/naphthyridones and the reagents required for all procedures. Involving students in this process provides an opportunity for them to become invested in the research, to show that the molecules that they are preparing are not purely an academic exercise but contribute to a wider knowledge on antibiotics. At the start of the second week, students are provided with the specific synthetic sequence they will follow in the lab and appropriate procedures. This semi-orchestrated approach allows for the appropriate chemicals to be in stock for students to start synthetic work the following week. In the second week they will decide on appropriate scale for their reactions, design how to use their time in the laboratory and prepare appropriate Control of Substances Hazardous to Health (COSHH) assessments. These are part of UK Law and are a standard practice for industry. Including these in the laboratory preparation provides a further industry relevant skill for students as they need to research and document the chemical hazards before work can begin. These assessments are approved by a senior lab demonstrator before the laboratory session.

The synthetic sequence (Scheme 1) can be completed in 3 x 2 day laboratory sessions (Figure 2). Students will develop their own laboratory plan as part of the project proposal and this will include details on when students can take breaks from the lab. None of the steps require purification by flash column chromatography and analytically pure material can be obtained by trituration or recrystallisation.



Scheme 1: Synthesis of *des*fluoroenoxacin analogues. *Reagents and conditions*: a. (i) oxalyl chloride, cat. DMF., CH_2Cl_2, 2 h, ice-bath; (ii) ethyl 3,3-dimethylaminoacrylate, Et_3N, toluene, 90 °C, 2.5 h, quant. over two steps; b. $EtNH_2$ (2 M in MeOH), EtOH, Et_2O, 2 h, r.t., 97%; c. K_2CO_3, DMF, 60 °C, 2 h, 98%; d. amine (a-i), Et_3N (where necessary), EtOH, reflux, 2-6 h, 54%-quant.; e. aq. NaOH, 2 h, reflux, 31-92%

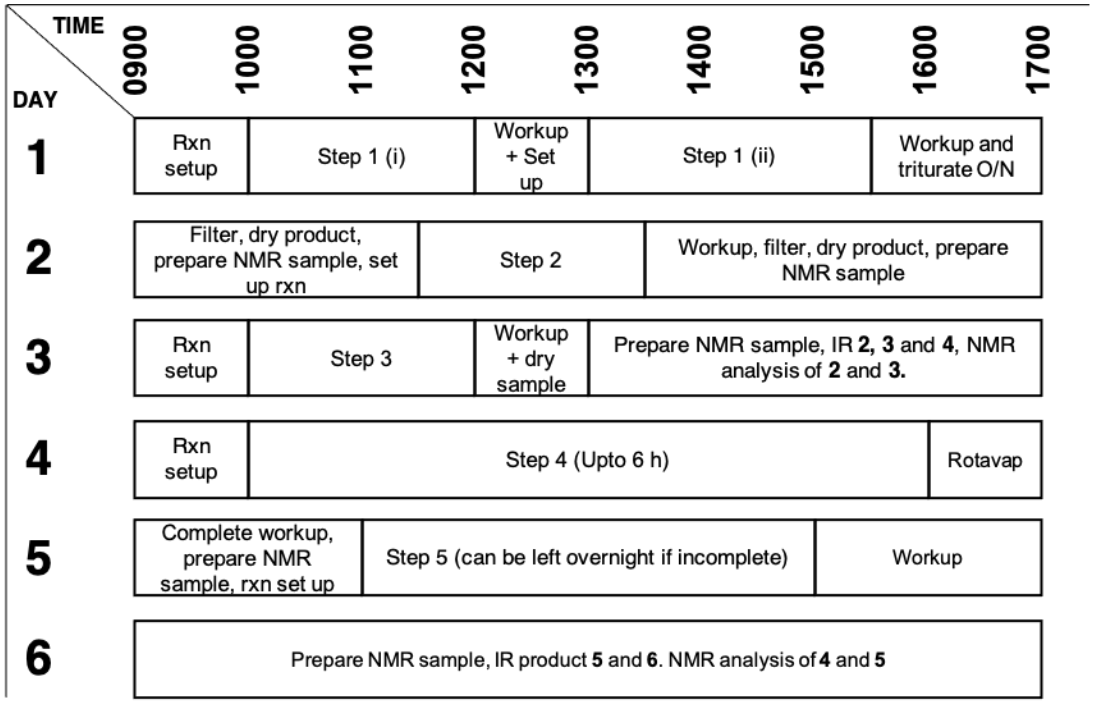


Figure 2: Synthesis timeline

*Day 1: Step 1 – Synthesis of enaminone **2***

In the first session, the students carry out two synthetic steps from carboxylic acid **1** to enaminone **2** (Scheme 1).[13] Step 1(i) is the formation of an acyl chloride using oxalyl chloride catalysed by DMF. Students will generally have studied the mechanism for the formation of acyl chlorides using thionyl chloride and can include a comparison of the use of this to oxalyl chloride in their experimental write up. The reaction is typically completed within 2 hours. The reaction is monitored by thin-layer chromatography (TLC) until the carboxylic acid is consumed. Students are required to investigate appropriate TLC solvent systems; this can take some time and students often require a reminder of the fundamentals of solvent polarity and the affect on TLC spots. To monitor the conversion of the carboxylic acid to the acyl chloride, students will need to quench TLC aliquots of the reaction mixture with methanol, to form the methyl ester, and monitor the progress of the reaction. Isolation by evaporation is then used to give the acyl chloride. Students can confirm the preparation of the intermediate acyl chloride by FT-ATR to show the diagnostic shift in the carbonyl wavenumber (1722 cm^{-1} acid, 1810 cm^{-1} acyl chloride (C=O stretch)), but this is not essential. In Step 1 (ii) the acyl chloride is converted into the enaminone **2** with ethyl 3-(*N*,*N*-dimethylamino)acrylate. The reaction is again

monitored by TLC and is typically completed in 2.5 h. Evaporation of the solvent and liquid-liquid extraction provides the crude enaminone **2** that is purified by trituration with a mixture of diethyl ether and petroleum ether (1:1). In our experience, students are typically familiar with the recrystallisation procedure but are unfamiliar with trituration. To achieve material suitable for easy filtration the crude material should be stirred vigorously in the trituration solvent overnight. This typically provides high quality solid material for filtration. The synthesis of the enaminone is quantitative from the starting carboxylic acid **1** and we report the procedure on a 10 g scale in the Supporting Information. The reaction is roughly mass equivalent (10 g of carboxylic acid providing 11 g of enaminone). It would be more typical, and as effective, for students to carry out this reaction on a 2-3 g scale.

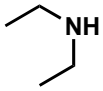
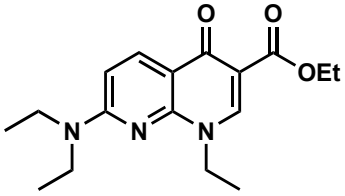
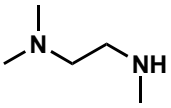
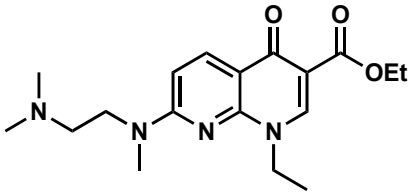
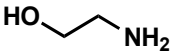
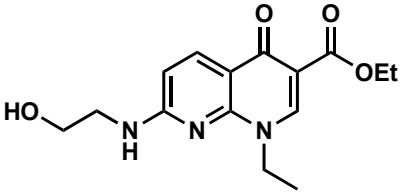
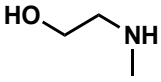
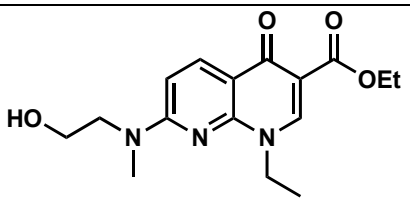
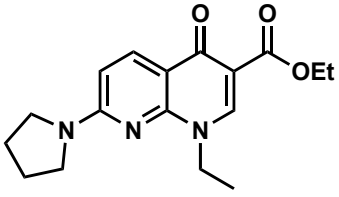
*Day 2 and 3: Steps 2 and 3 – Synthesis of napthyridone **4***

On the next two days, students convert the enaminone **2** to the napthyridone **4** based on a procedure reported by Massari.[14] Initially, the students determine the mass of enaminone **2** and calculate the yield (over two steps). The students also determine the melting point of the product and record the FT-IR spectrum and NMR spectra. We report the ^{1}H and ^{13}C NMR spectra for the enaminone in the Supporting Information. Students then have to calculate the reagent quantities for the next step, complete a COSHH assessment and then set up and run the next step following the procedure provided. The conjugate addition-elimination with ethylamine proceeds within 2 h and in 97% yield on a 10 g scale. The product **3** of the reaction can be purified by trituration with cyclohexane. The conjugate addition provides a mixture of *E* and *Z* isomers in a ratio of 6:1 (*Z*/*E*) that can be determined by ^{1}H NMR (Supporting Information, S13). The conjugate addition-elimination reaction has also been reported with other simple amines such as methylamine, benzylamine and cyclopropylamine and these can be used to introduce further structural diversity into the products.[14,15] The students then characterise the obtained compound by the methods previously detailed. In the following lab session, the third step in the sequence, intramolecular nucleophilic aromatic substitution to prepare the 4-naphthyridone **4** is undertaken. Only the *Z*-enaminone is reactive under the cyclisation conditions, but the enaminone geometry is readily reversible and leads to complete conversion of the enaminone **2** to the napthyridone **4** in 98% yield after 2 h.

The product precipitates by the addition to water and is characterised. Time is given for students for students to collect the IR spectra for the prepared compounds and to review the NMR spectra with a postgraduate demonstrator, if needed.

Day 4-6: Steps 4 and 5 – Library synthesis

At this point, students can prepare a diverse library of *des*fluoroenoxacin analogues by the choice of various amines to carry out the second nucleophilic aromatic substitution reaction (Scheme 1).[14,16] We exemplified the reaction with nine amine nucleophiles to give products **5a-i** undertaken on a 500 mg scale (Table 1).

	Amine	Product	Yield (%)
5a			75
5b			quant.
5c			54
5d			83
5e			75

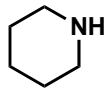
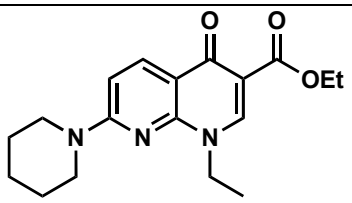
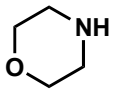
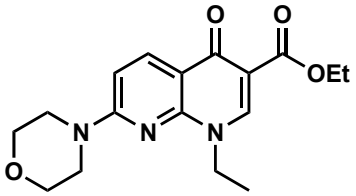
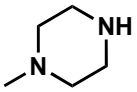
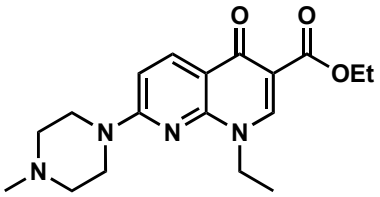
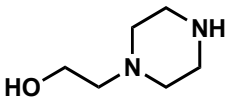
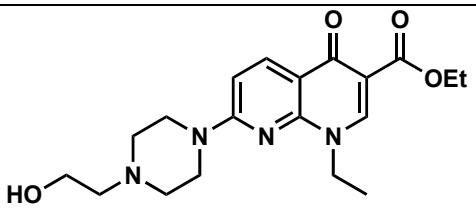
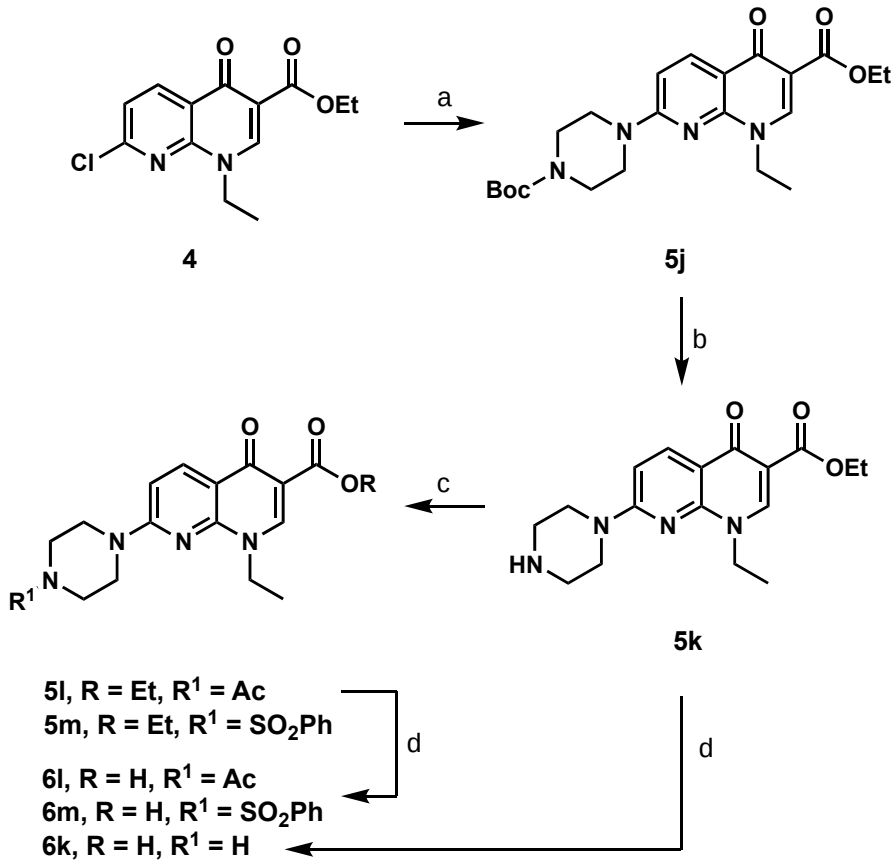
5f			97
5g			59
5h			90
5i			86

Table 1: Summary of *des*fluoroenoxacin aminoesters **5a-i** prepared

These reactions were typically completed within 3-6 h. Students are required to monitor their reactions by TLC to determine the progress of the reaction. The reaction mixtures are easily purified by liquid-liquid extraction and then trituration to give the aminoesters. These esters can be characterised if required, or deprotected using 10% aqueous sodium hydroxide solution to the carboxylic acids **6a-i** (Scheme 1).[16] The deprotection was typically complete within 5 h, but can be left overnight if incomplete. Slow addition of aqueous acetic acid to the reaction mixtures precipitated the final compounds from the reaction mixture. Careful control of pH is needed with substrates with an additional basic nitrogen. In the event the product does not precipitate then the workup can be modified to remove any residual impurities. Evaporation of the extraction mixture to dryness followed by dilution with diethyl ether and adjusting the pH to 7 with aqueous sodium hydroxide provided the zwitterionic products. No synthetic work is planned for the final day of the lab, students have time to collect the analytical data required and review the results with a postgraduate demonstrator, if needed.

If additional time is available, further diversity can be introduced into the library by cleavage of the Boc-group of **5j** with TFA (caution: TFA is highly corrosive and an irritant, it should be used with direct supervision from a postgraduate or academic demonstrator) and subsequent functionalisation of the amine **5k** (Scheme 2). Deprotection of the ester proceeds as with the previous analogues to **6l** and **6m**. *Des*fluoroenoxacin **6k** can be prepared by deprotection of the Boc-group and ester hydrolysis with careful adjustment of the pH following workup.



Scheme 2: *Reagents and conditions*: a. Boc-piperazine, EtOH, reflux, 16 h, quant.; b. TFA, CH_2Cl_2, r.t., 3 h, 86%; c. Ac_2O or $PhSO_2Cl$, Et_3N, CH_2Cl_2, r.t, 6 h, **5l** = 92%, **5m** = 94%; d. aq. NaOH, 2 h, reflux, 67%-quant.

Complete procedures and characterisation of the esters and carboxylic acids can be found in the Supporting Information.

Hazards

A complete list of reagents, CAS numbers and hazard statements for all chemicals used can be found in the Supporting Information. The product naphthyridones should be assumed to have biological activity and direct contact should be avoided. All intermediates should be assumed toxic and handled with care. Standard laboratory personal protective equipment including goggles, lab coats and gloves should be worn and all operations should be undertaken in a fume hood.

Results and discussion

During the project proposal research stage students typically discover a number of classical syntheses of 4-quinolones including the Jacobs-Gould cyclisation (Figure 3, I),[17] reported for an undergraduate synthesis of a similar compound ethyl nalidixate,[18] and the Grohe method to quinolones that was developed at Bayer (Figure 3, II).[19] The research stage also allows students to gain skills in critical analysis of other modern methodologies to quinolones such as transition metal-catalysed transformations (Figure 3, III and IV).[20,21] While many of these transformations are highly effective they require specific bespoke starting materials and do not always provide the desired pharmacophore needed for the quinolone family of antibiotics – these are key discussion points for students to identify during the research process.

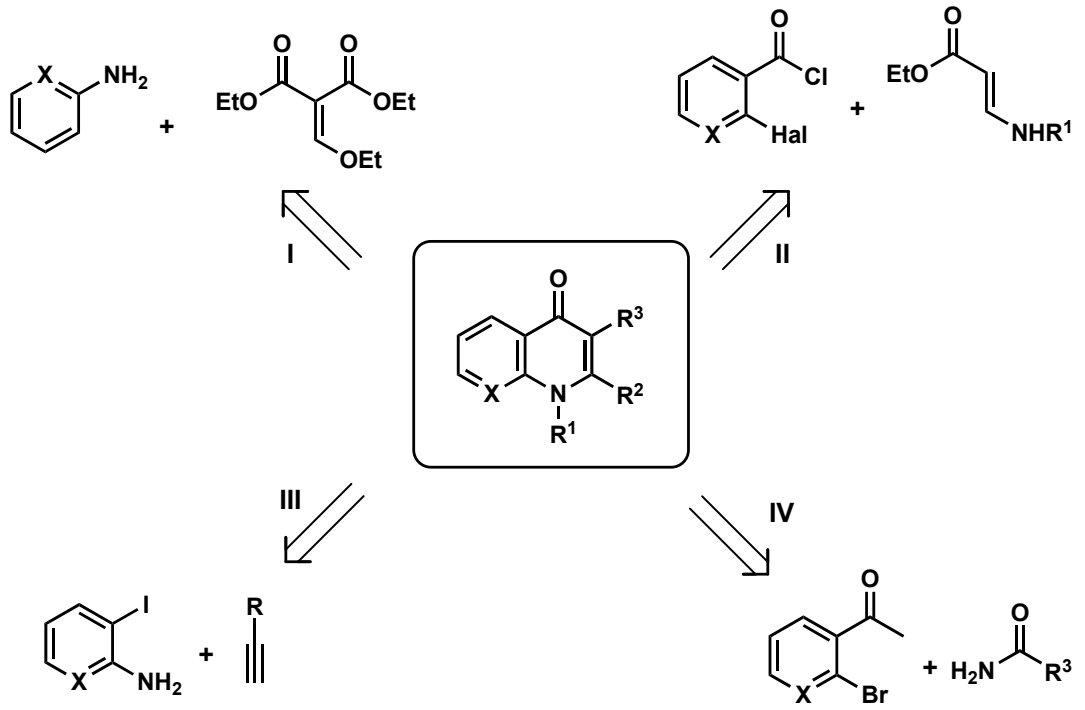


Figure 3: Retrosynthetic routes to the 4-quinolone scaffold

The method described herein is a reliable, high-yielding introduction to multi-step organic synthesis. Each of the six steps of the reaction is a fundamental organic transformation taught to undergraduate students, allowing students to review the basic mechanistic concepts alongside a practical undertaking of the transformation. As flash column chromatography is not required for any of the steps it allows for students to undertake more reactions within the allocated lab time and gain more experience at determining the progress of one reaction to the next by TLC. Additionally, the purification techniques, particularly isolating the final product at a specific pH reinforces the concepts of ionisation and its effect on solubility as well as trituration and filtration. Students will prepare and characterise five compounds throughout the sequence. As the reactions are readily scalable and high yielding it is possible for students to obtain clear ^{13}C NMR spectra of their prepared products without the need for significant spectrometer scans. The outcome of the reactions is not immediately known to the students. They will have to evaluate the yield of each reaction before proceeding to the next step and then they can decide how much of the product they wish to use for each reaction to ensure they have sufficient material. This requires students to be comfortable with stoichiometric calculations to find the quantities required for each component required for the next reaction.

Given the relative ease and scalability in preparing chloronaphthyridine intermediate **4**, the final two steps of the reaction could easily be modified for a lab class that only has a single day in the laboratory. With intermediate **4** provided to students, the nucleophilic aromatic substitution with pyrrolidine to **5e** proceeds to completion in 3 h and subsequent deprotection to **6e** in 0.5 h. This would provide an additional 3.5 h for reaction set up, monitoring, workup and analysis by melting point and IR. This would be suitable for a first- or second-year laboratory experiment and be complementary to other syntheses of bioactive compounds.

Additionally, the final products are likely to have some antibiotic activity and would be suitable for submission for biological assays either in house[22,23] or through initiatives such as the Community for Open Antimicrobial Drug Discovery (Co-ADD) screening panel.[24] This allows for the project to represent a full design-synthesis-test cycle in medicinal chemistry.

Assessment of Learning Outcomes

The primary method of evaluating the achievement learning outcomes of the experiment was through assessment of students written submitted lab reports and laboratory notebook notes. A rubric (Supporting Information) was used to assess five key criteria: (1) team working and time management, (2) safety and good lab practice, (3) technical competence, (4) knowledge and critical thinking, and (5) technical writing and presentation skills. Students use the rubric to self-assess their own communication and team working skills (from Criteria (1)) and this accounts for 5% of the total mark.

Assessors' evaluation of written reports showed that the students were generally successful in achieving the expected learning outcomes and laboratory skills. All of the students were successful in completing the full synthetic route and preparing one compound from **6a-i**. They fully characterised five compounds by melting point, infra-red, ^{1}H NMR and ^{13}C NMR. They were provided high-resolution mass spectra to report on.

Based on the instructor discussions in the laboratory, the most challenging aspect of the project was the monitoring of reactions by thin-layer chromatography (TLC). In previous experimentation work, students were provided appropriate conditions for TLC and the process was generally routine and a confirmatory task at the end of a

reaction. During this experiment, students are tasked with finding their own conditions and using TLC to monitor the progress of the reactions – and for them to decide when a reaction is complete. This often took a lot of time in Day 1 and 2, but students became more accustomed to this by the later steps and their skills improved. Students were reminded during the process that they needed sufficient material to complete each reaction step and were careful to integrate this into their experimental plans, with some students calculating expected quantitative yields ahead of the reactions and estimating mass recovery with different yields to decide on quantities to use for reactions. Some students did require guidance at this point to estimate masses needed. This discussion with students was a valuable opportunity to reinforce the concepts of mass conservation and multistep synthesis, along with the unpredictability of carrying out unfamiliar reactions.

One of the areas of the lab report that students struggled with was the tendency to not properly evidence (in written discussion) the success of the reactions through discussion of the characterisation techniques. Many students wrote that the reactions were successful and linked to the experimental procedure but did not outline how they have come to that conclusion. The ability to describe the relevant results in detail is a new skill presented in the mini-projects and requires more discussion that previous, expository, 'recipe-style' experiments do not afford, and aligns more with academic research projects and research. The rubric for this experiment is general and is standardised for all mini projects but could be tailored by instructors for specific learning outcomes.[25]

Experimental design and time-management have been identified as skill deficits among Chemistry graduates,[26] and this experiment associated assessment of work is designed to encourage students to effectively plan and execute a complex laboratory activity, developing these key industry skills for further study and work.

Conclusions

In this experiment, undergraduate students synthesise *des*fluoroanalogues of the antibiotic enoxacin using an accessible, chromatography-free, multistep synthetic

route with readily available starting materials. The reactions used in the project are basic transformations that are studied in a typical undergraduate curriculum in organic chemistry. The application of these to the synthesis of molecules with potential bioactivity shows the real-life application of organic chemistry in the pharmaceutical industry.

Associated content

The Supporting Information is available online at XXXX.

Complete list of reagents, hazards and CAS numbers. Experimental procedures and appropriate spectroscopic information.

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