Four Step Total Synthesis of an H_3 Receptor Antagonist using only tools found in a typical teaching laboratory.

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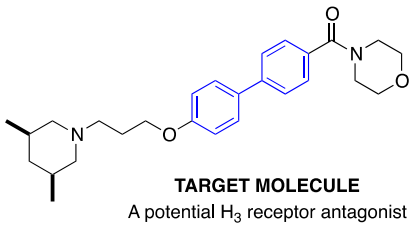
ABSTRACT

Organic synthesis in a modern research laboratory uses state-of-the-art equipment to provide inert atmospheres, track reaction progress and facilitate the purification of intermediates. Such facilities are not available in a high school laboratory. In this paper, synthetic organic chemistry is taken back to basics: A four step total synthesis of a potential histamine H_3 receptor antagonist is completed by A-level students in a school laboratory using only basic equipment. In addition to its bioactivity, the target molecule was selected based on its potentially crystalline nature, allowing for the purification of intermediates by recrystallization. A synthetic route was developed that could be conducted without the need for inert conditions and with intermediates stable enough to be left from one week to the next, with characterization of intermediates and the target compound carried out by collaboration with researchers and analytical facilities at the University of Oxford (U.K.). By working on the project, students not only developed their practical chemistry and problem solving skills, but also experienced the thrill of discovery that inspires people to be scientists.

GRAPHICAL ABSTRACT



TARGET MOLECULE
A potential H_3 receptor antagonist

KEYWORDS

High School/Introductory Chemistry, First-Year Undergraduate/General, Organic Chemistry, Laboratory Instruction, Hands-On Learning/Manipulatives, Aromatic Compounds, Medicinal Chemistry, Synthesis

INTRODUCTION

The practical application of chemistry has long been a core element of the teaching of the subject at both high school and university levels. However, within the constraints of the school curriculum, timetable, and class sizes, the opportunity for high school students in the U.K. to carry out extended practical work (lasting more than 1-2 hours) has become increasingly rare. A synthetic chemistry project was designed to provide high school students with an opportunity to apply their understanding of organic chemistry to complete a multistep organic synthesis, and in the process discover what it feels like to work and think as a scientist.

While a number of papers describing laboratory experiments involving multistep organic syntheses have been published in recent years,[1-5] these have universally formed part of an undergraduate course. To our knowledge, this is the first report describing a multistep organic synthesis completed solely by high school students, demonstrating the value and educational benefits of collaboration between schools and universities.

EXPERIMENTAL OVERVIEW

The total synthesis of a target molecule is described using only the tools found in a typical teaching laboratory. The work was completed by groups of high school students (aged 16-18, U.K. 'A-level') as part of an extra-curricular science club. A collaboration was established with chemists at the University of Oxford which enabled students to synthesize and purify intermediates at school, and then submit them to Oxford for analysis. The latter activities stimulated discussions with students on the shapes and structures of organic molecules, which provided a real-life setting of concepts such as VSEPR theory and isomerism.

The synthesis is suitable for an advanced high school class, given appropriate teaching support, access to the required laboratory equipment, and to analytical facilities – most likely through a similar

collaboration with an academic or industrial partner. Equally, it would be highly appropriate for an undergraduate university organic chemistry laboratory course.

Choice of target molecule

The first part of the project involved the choice of a suitable target molecule. Any target molecule chosen would require a synthetic route that could be carried out in air using only basic laboratory equipment, and preferably proceeding through crystalline intermediates that could be purified by standard recrystallization techniques.

Biaryl compounds often exhibit this latter property, and can also be readily synthesized by Suzuki-Miyaura coupling between an aryl halide and an arylboronic acid in the presence of a palladium catalyst.[6,7] This reaction – one of the most important in contemporary organic chemistry – has been widely investigated: it is well known to be compatible with a broad range of functional groups, proceed in high yields, and can be performed on the open bench. In the context of this project, it also has the benefit of allowing high school students to put Nobel Prize-winning chemistry into practice. With this disconnection in mind, an initial literature search for molecules that feature a biaryl ring system revealed **1** ((4'-(3-((3*R**,5*S**)-3,5-dimethylpiperidin-1-yl)propoxy)-[1,1'-biphenyl]-4-yl)(morpholino)methanone, Figure 1, biaryl ring system highlighted in blue) to be a suitable target molecule.[8]

Aside from cross-coupling, retrosynthetic analysis of **1** suggested a potential synthetic route that had the benefit of containing chemistry familiar to A-level students, namely amide formation via a nucleophilic addition-elimination reaction between an acyl chloride and an amine, and nucleophilic substitution reactions to produce both C–O and C–N covalent bonds (see Figure 1).

The target molecule has the potential to act as a histamine H_3 receptor antagonist. The histamine H_3 receptor is an important presynaptic autoreceptor that inhibits the release of histamine. More recently it has been recognized as a heteroreceptor that regulates the release of other important neurotransmitters. H_3 receptor antagonists are, therefore, sought for the potential treatment of a variety of disorders affecting cognition (e.g. attention deficit and hyperactivity disorder (ADHD), Alzheimer's disease and schizophrenia), sleep (e.g. hypersomnia and narcolepsy), and energy

homeostasis (e.g. obesity).[9] This potential use for the target molecule added a real-life connection to the project, and gave students an opportunity to research the biochemistry of histamine interactions.

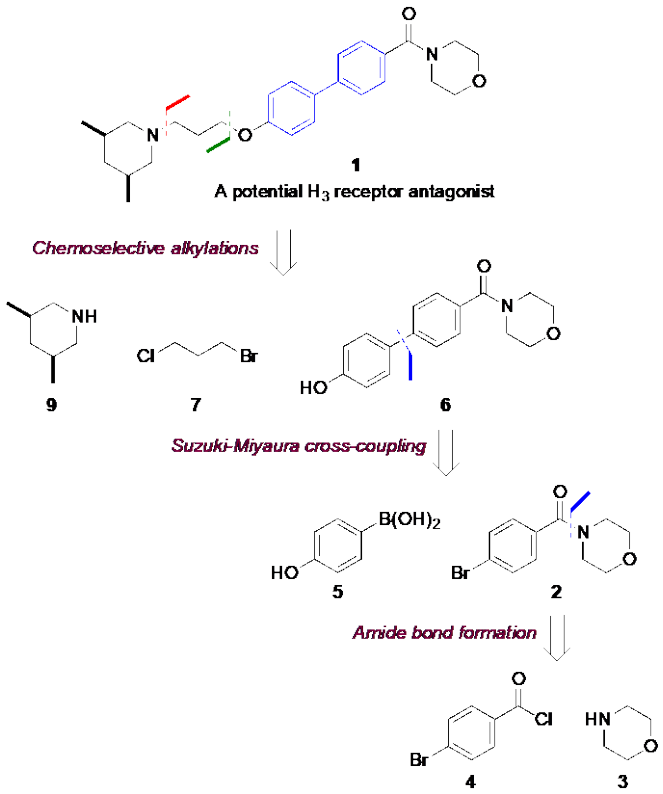


Figure 1. Retrosynthetic analysis of the potential histamine H_3 receptor antagonist **1**

HAZARDS

Students were required to complete a full risk assessment for each experiment using the Hazard and Precautionary statements for each chemical provided on the MSDS. The amide formation step was carried out in a fumehood owing to the volatility of diethyl ether, and 4-bromobenzoyl chloride being a known lachrymator. All other reactions were performed on the open bench. Overnight heating was carried out using a stirrer-hotplate with a sand bath. Organic waste was disposed of following standard guidelines. Intermediates and products are unknown and so were handled with care throughout. Full hazards for individual chemicals are provided in the Supporting Information (pp S3-S6).

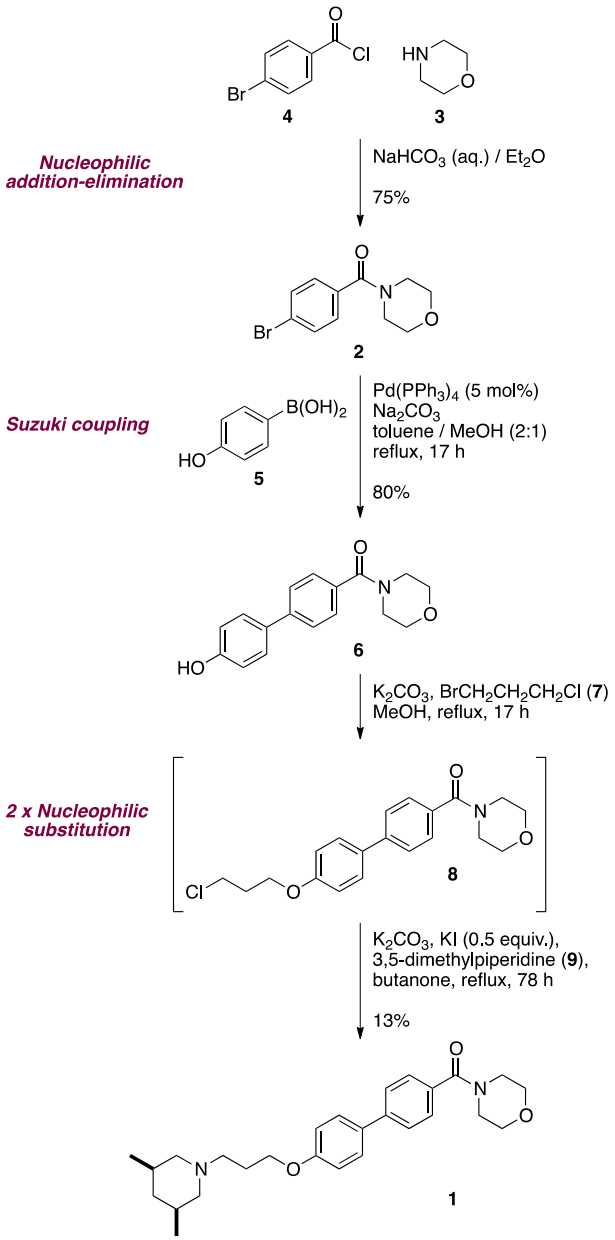
RESULTS AND DISCUSSION

Synthesis

The first step in the synthesis was the formation of the (4-bromophenyl)(morpholino)methanone (**2**, Scheme 1), for which Schotten-Baumann conditions were chosen owing to the straightforward and high yielding nature of this reaction.[10] Morpholine **3** was added to a vigorously stirred solution of 4-bromobenzoyl chloride **4** in a biphasic mixture of diethyl ether and saturated aqueous $NaHCO_3$. After stirring for 10 minutes, an aqueous work up afforded the morpholine amide **2**, which was purified by recrystallization from hexane/ethyl ethanoate to yield the pure amide as a white crystalline solid (63-75%, average 72%).

The next step involved Suzuki-Miyaura coupling between 4-hydroxyphenylboronic acid (**5**) and amide **2** to create (4'-hydroxy-[1,1'-biphenyl]-4-yl)(morpholino)methanone (**6**).[6,7] Initial attempts tested the encapsulated palladium methodology developed by the Ley group, a modern, 'green' take on this classic reaction in which the palladium(II) acetate pre-catalyst is encapsulated in polyurea microcapsules.[11] This method was selected due to the stability to storage (and use) in air of the Pd-EnCat system, and the ease of work-up – the insoluble polyurea-encapsulated palladium catalyst being removed from the reaction mixture by simple filtration.[12] In practice, while the reaction seemed to proceed to completion as judged by TLC, students struggled to isolate the product biaryl phenol **6**. Eventually, this was found to be due to the highly insoluble nature of **6**; in removing the insoluble catalyst from the reaction mixture by filtration, students were inadvertently also filtering off, and disposing of, the desired product.

Recourse to a more 'traditional' catalyst system for the Suzuki reaction solved this problem, and indeed took advantage of the poor solubility of **6**.[13] After heating (overnight) a solution of **2** and **5** in a toluene-methanol mix, with aqueous Na_2CO_3 as base and $Pd(PPh_3)_4$ as catalyst, simple filtration of the diluted reaction mixture afforded the desired biaryl phenol **6** as a cream-colored solid, which could be purified by recystallization from hot methanol (16-80%, average 45%).

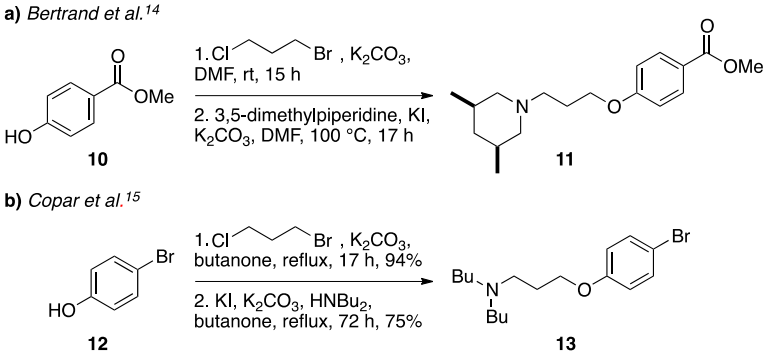


125 **Scheme 1.** Total synthesis of target molecule **1**.

The final step of the synthesis involved installation of the amine sidechain by a chemoselective alkylation, whereby 1-bromo-3-chloropropane **7** undergoes a nucleophilic substitution reaction with **6** under refluxing alkaline conditions to substitute the bromide, giving intermediate chloride **8**.

130 Subsequent alkylation with 3,5-dimethylpiperidine **9** via a Finkelstein reaction[10] utilizing potassium iodide as a catalyst would then yield the final target molecule **1**.

Research showed that Bertrand *et al.* had performed a similar alkylation of methyl 4-hydroxybenzoate **10** (Scheme 2a) using potassium carbonate as base.[14] However, this procedure uses dimethylformamide as solvent, and was therefore ruled out for replication in a school laboratory owing to the associated health hazards. Copar *et al.* had shown that a similar sequence could be carried out using butanone as solvent (Scheme 2b).[15] Chemoselective substitution of 1-bromo-3-chloropropane at the 1-position by the phenolate anion was followed by alkylation at the 3-position with dibutylamine.



Scheme 2. Literature precedent for chemoselective phenol / amine alkylations using 1-bromo-3-chloropropane.

In the hands of the students, initial attempts to react biaryl phenol **6** with 1-bromo-3-chloropropane were successful but low yielding, which we suspected was due to the low solubility of **6** in butanone. The reaction was therefore repeated using methanol as solvent, and, after heating overnight at reflux with **7** and K_2CO_3, the crude phenol alkylation product **8** was obtained after a base wash to remove residual phenol (Scheme 1). Without further purification, **8** was reacted directly with 3,5-dimethylpiperidine (which was purchased as a 1:0.3 mixture of *cis* and *trans* diastereomers) according to the Copar conditions. Once the reaction was deemed complete by TLC, the solution was concentrated and the solid residue extracted into dichloromethane. Residual 3,5-dimethylpiperidine could be removed by trituration with hexane, which afforded a small amount of a white solid. Recrystallization from hexane/ethyl ethanoate gave the final target molecule **1** (1:0.3 mixture of cis:trans isomers) as a colorless crystalline solid.

Analysis of intermediate and target structures

Once pure samples of **2**, **6** and **1** had been obtained, these were sent for spectroscopic analysis

(^{1}H/^{13}C NMR, IR, HRMS) at the Department of Chemistry, University of Oxford. As all intermediates were crystalline, X-ray crystal structures could also be obtained.[16] At each stage students were required to carefully analyze the spectra to confirm the identity of their products. In addition to providing the students with an opportunity to practice the analytical skills learnt as part of their A-level course, the analysis revealed a number of interesting points which led to informal discussions about molecular motion, and the numerous factors that can affect the three dimensional shape of a molecule in a crystal structure. Spectra and full analysis are provided in the Supporting Information.

Firstly, it was noted that compounds **1**, **2** and **6** feature broad signals in both their ^{1}H and ^{13}C NMR spectra (Supporting Information, pp S10-S18), corresponding to the methylene (CH_2) groups of the morpholine ring. This is due to conjugation of the nitrogen lone pair with the carbonyl π*-orbital, which results in a significant energy barrier to C–N bond rotation (Figure 2), and leads to slow interconversion of proton and carbon environments on the NMR timescale (e.g. H_a/H_b in **2**), leading to broad, unresolved signals. This conformational effect is clearly evidenced in the X-ray structure of **2**, which shows a trigonal planar arrangement of atoms at the amide nitrogen, and a C–N bond shorter than those in the ring. It also reveals that the arene ring in **2** is twisted relative to the amide carbonyl (torsion angle C14/C9–C7/N4 = 76.4°): if the arene and morpholine rings of **2** were coplanar, the indicated hydrogen atoms would necessarily occupy the same space, resulting in a steric clash.

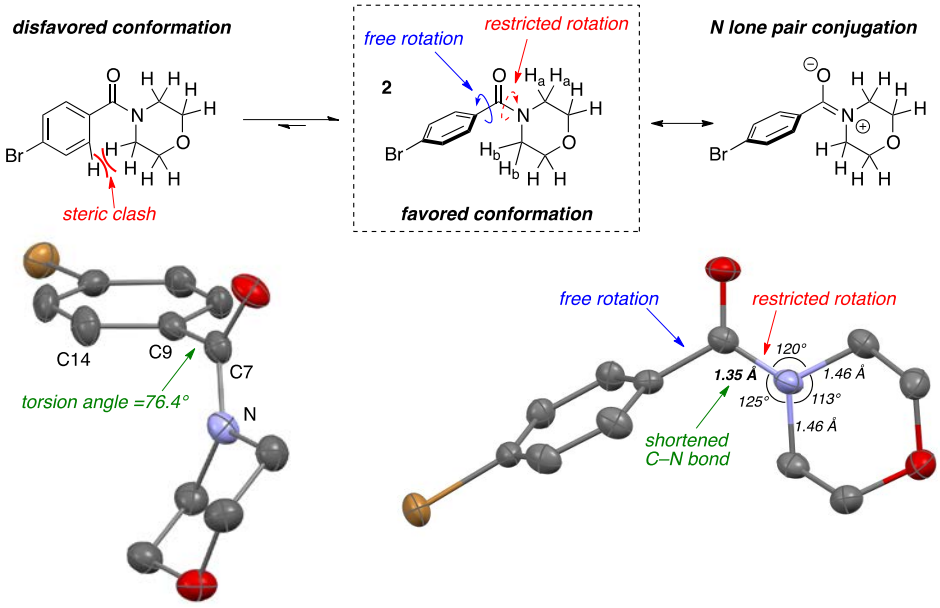


Figure 2. X-ray crystal structure of morpholine amide **2** showing a trigonal planar arrangement of atoms about the amide nitrogen, a shortened amide C–N bond which reflects N lone pair conjugation with the carbonyl π^* orbital, and twisting of the arene-carbonyl bond to avoid steric clash with the morpholine ring.[17]

This behaviour was also seen with compound **6**, where a further steric clash would occur if the aryl groups of the biaryl ring system were co-planar. The X-ray structure of **6** (Figure 3) reveals the twisted nature of the biaryl axis to minimize these steric interactions which, combined with the amide conformation discussed above, results in a helical conformation along the arene–arene–amide axis.

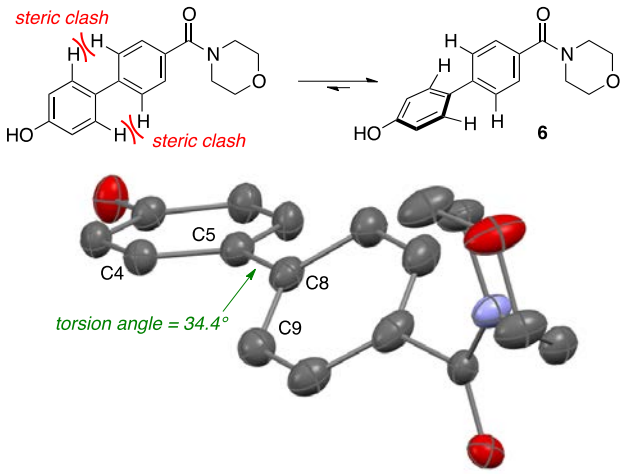


Figure 3. X-ray structure of **6** showing the helical disposition of the aryl groups and amide to minimize unfavorable steric interactions.[17]

Whilst aromatic rings are planar, the six-membered morpholine and piperidine rings of **1** are sp^3-rich and could thus adopt a range of conformations, the lowest energy of which is likely to be the (all-staggered) chair form. The X-ray crystal structure of ***cis*-1** (Figure 4), a crystal of which could be isolated by recrystallization, clearly revealed not only the chair conformations of both of these rings, but again also the twisted nature of the biaryl-amide core. Taken together, **1** thus exhibits a highly specific solid state 3D-conformation based on a number of conformation-controlling effects, which are likely also important for its bioactivity.

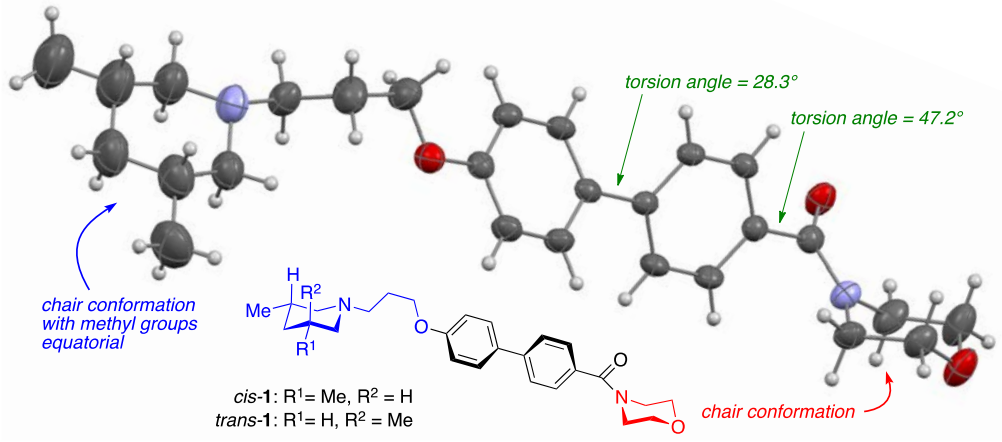


Figure 4. X-ray crystal structure of *cis*-**1**, showing the chair conformation adopted by the piperidine and morpholine rings, and torsion angles through the molecule.[17]

As noted above, compound **1** was in fact prepared as a 1:0.3 mixture of diastereomers, due to the use of a commercial mixture of *cis*- and *trans*-3,5-dimethylpiperidine in the final amination step. Although differing only in the positioning of a single methyl substitutent on the piperidine ring, these diastereomers were found to possess distinct physical properties; for instance, their ^{1}H and ^{13}C NMR spectra (Supporting Information, pp S16) showed significant differences in chemical shifts, in particular in the highlighted 'blue' region of **1** (Figure 4). Specifically, the chemical shifts of the methyl groups in compound **1** were diagnostic of its two isomers: for the major (*cis*) isomer, which features a plane of symmetry through the piperidine ring, the methyl protons appeared at 0.86 ppm, while for the minor (*trans*) isomer, which exhibits an axis of symmetry, the methyl protons were now found at 0.95 ppm. The two methyl groups are oriented in different environments in *trans*-**1**, and might be expected to appear as two separate doublets. However, as the rate of piperidine ring flipping is rapid on the NMR timescale at room temperature, a single, time-averaged chemical shift is seen, with these protons appearing to be equivalent. Integration of these peaks allowed us to determine the ratio of the two isomers. *Trans*-**1** also displayed a higher retention factor on TLC (R_f 0.12 (*cis*) and 0.25 (*trans*) in 10% MeOH/EtOAc). In addition, the crystalline mixture of *cis*-**1** and *trans*-**1** appeared to melt in two stages, which would be consistent with different melting points of the two isomers.

Whilst such physical distinctions are not readily predicted, these observations afforded students a real-life insight into the significant effect of seemingly slight configurational differences on bulk

material properties. Although not an initial aim of the project, by obtaining full spectral analysis and crystal structures for the intermediates and final molecule in the synthesis, the high school students developed a deeper understanding of the effects controlling the 3D structure of organic molecules. The collaboration with Oxford University was invaluable in this aspect of the project.

Summary

Work on this project was undertaken by students for one hour each week during science club time. Students were required to plan each experiment fully and to apply their understanding of mole calculations and densities to calculate quantities of starting materials and reagents based on the literature methods. After overnight heating, mixtures were analyzed by thin layer chromatography (TLC) and, if the reaction was judged complete, simply allowed to cool, placed in a cupboard, and then worked up the following week. Once purified by recrystallization, samples were sent off to Oxford for spectral analysis. This meant that, including analysis of spectra, a single experiment could take 7-8 weeks to complete, and over their two year period attending the club each group of students may only complete 6 or 7 experiments.

Each year, a particular group of students would therefore make a small step forward, and then hand the project over to the next group. They in turn would start by learning the practical techniques required (heating under reflux, TLC, recrystallization) on experiments that were known to work, before turning to the total synthesis. As a result, the completion of the total synthesis has involved the work of five groups of students (18 students in total) working over a nine year period, with the total synthesis being achieved by the final two groups (3 students). The extended period of time throughout which this synthesis was developed reflects the ability of the students involved, the developmental nature of the work and the constraints of the science club hours. This could be substantially shortened depending on the scenario in which it was to be adopted.

At the end of the project all students wrote a short report, which was successfully submitted for a Gold CREST award.[17] In addition, a number of the groups presented the project at the Big Bang UK Young Scientists and Engineers Competition, where they were able to discuss their work both with the public and with leading scientists in the field.

CONCLUSION

The total synthesis of a histamine H_3 receptor antagonist was completed by high school students using solely tools found in a typical teaching laboratory. When starting the project, a key aim was to introduce pure science to high-achieving high school students as an alternative career path to medicine – a subject that arguably attracts the majority of talented scientists at high school level. It was hoped that by experiencing the excitement of discovering something new, students would be inspired to consider pure science as a future career path. Although a small number of students involved in the project went on to non-scientific careers, and two into medicine, a significant number indeed pursued careers in pure science, and pleasingly three students from the early years of the project are now undertaking PhDs in prominent research groups around the U.K. The close collaboration with scientists at the University of Oxford and the NMR spectra and crystal structures obtained, enabled students to appreciate the 3D shape of molecules, rather than regarding them simply as 2D drawings on paper.

ASSOCIATED CONTENT

Supporting Information

Health and safety information
Experimental procedures and characterization data for all compounds.
NMR, IR and HRMS spectra and X-ray crystallographic data for all compounds
Exemplar pages from student's lab book.

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