A chemoenzymatic synthesis of Baclofen

Looking for an effective way of concluding a basic course of organic chemistry

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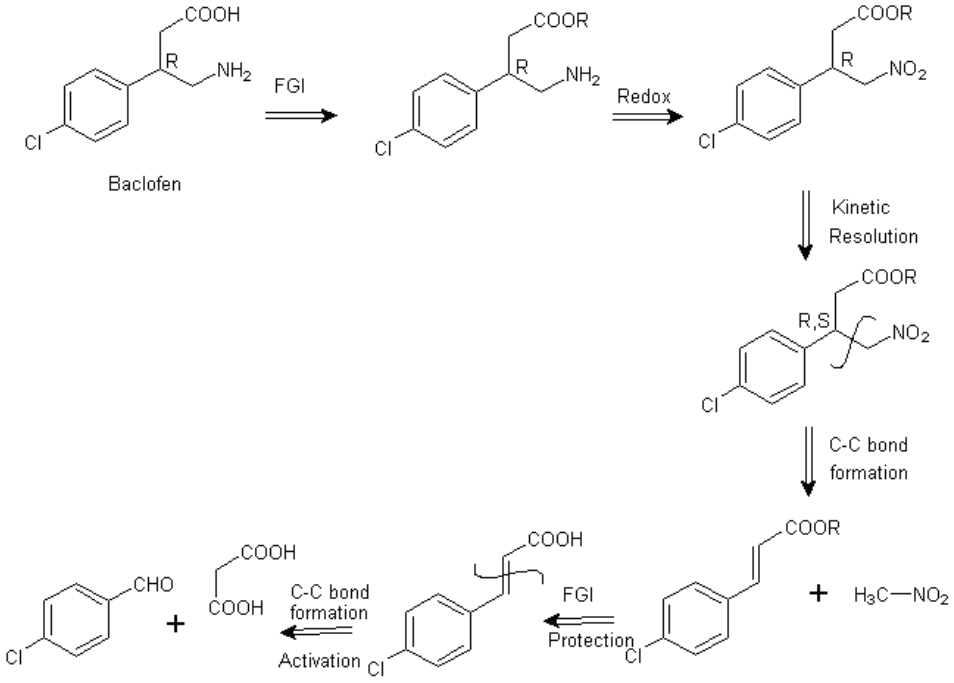
Abstract
The chemoenzymatic synthesis of an antispastic drug [4-amino-3-(4-chlorophenyl)butanoic acid, also known as Baclofen] is described. The synthesis consists of six steps and was carried out by a group of 18-year old students at the end of a two-year introductory organic chemistry course. The main purposes of this experience were to show how a few important organic reactions can be connected in order to prepare a biologically active molecule and to check the students' experimental skills in a particularly motivating context. After a short comment on the experimental results, the article discusses thoroughly the pedagogical benefits of such a challenging project, emphasizing the effectiveness of a close cooperation between organic and analytical chemistry.

Keywords
Second-Year Undergraduate; Organic Chemistry; Hands-On Learning/Manipulatives; Synthesis; Drugs/Pharmaceuticals; Enzymes; Chromatography; Spectroscopy; Chirality/Optical isomers.

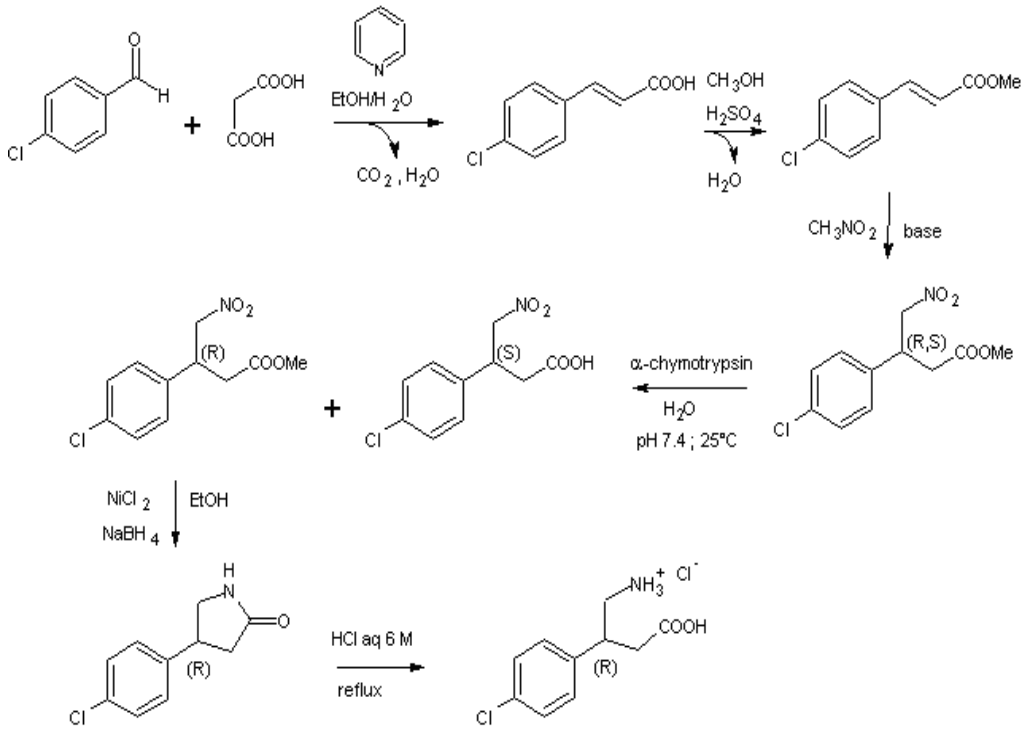
Teachers of a basic organic chemistry course usually do not have time to illustrate meaningful applications of organic reactions. As a consequence, even good students end up learning a list of reactions that they cannot put together in a logical way so as to produce a target-oriented process. They remind us of people who know a lot of words of a foreign language, but are not able to make a sentence and say what they want to say. This was more or less the situation of the students of our technical high school at the end of the fourth class after a two-year introductory organic chemistry course.
In an attempt to solve this problem we thought that the best way to reply to annoying questions such as "What are all these reactions for?" would be to recall that the job of an organic chemist is basically to make molecules. So we decided to synthesize Baclofen, a drug widely used to treat muscle spasms associated with multiple sclerosis, Lou Gehrig's disease and spinal cord injuries [1].
The main purposes of our project were: to apply both various laboratory techniques and important organic reactions to the synthesis of a biologically active molecule; to help students to integrate the theory of organic chemistry with practical experience; to give them the opportunity to face one of the basic problems in modern organic synthesis: reaction control, which means to reach the highest possible level in chemoselectivity, regioselectivity and stereoselectivity.

Overview and theoretical background of the synthesis
The chemical name of Baclofen is 4-amino-3-(4-chlorophenyl)butanoic acid (scheme I). Despite its apparent simplicity, this molecule shows several structural aspects which deserve attention. It contains both a carboxylic and an amino group in an unusual 1,4-relationship, and a 1,4-disubstituted benzenic ring. Last but not least, there is a stereogenic centre. This feature is of particular interest, because the enantiomers of this compound differ in their pharmacodynamic and toxicological properties: the (R)(-)-enantiomer is much more active but also more toxic than the (S)(+)-enantiomer [2].
Because of its biological and pharmacological importance, there are several reports in the literature concerning the total synthesis of Baclofen [3]. Among these, we focused our attention on the work of Felluga and coworkers [4], which seemed more suitable for our purposes, albeit with some additions and the necessary appropriate modifications.
According to our retrosynthetic approach (scheme I)[5], the starting compound is 4-chlorobenzaldehyde, a simple and inexpensive aromatic compound having the correct substitution pattern.



Scheme I. Retrosynthetic analysis of Baclofen.

The synthetic path that corresponds to this retrosynthesis is illustrated in scheme II. The first step is a Knoevenagel reaction, an aldol-type condensation widely used to prepare cinnamic acids, where 4-chlorobenzaldehyde is the acceptor whereas malonic acid plays the role of an activated donor [6]. The presence of the C=C bond conjugated with the carbonyl is a key factor here, because it paves the way for the following Michael reaction, which allows the formation of the second new C-C bond and the required 1,4-relationship between the two final functional groups. Before the Michael reaction, however, it is necessary to protect the carboxylic group through esterification.



Scheme II. Baclofen synthesis.

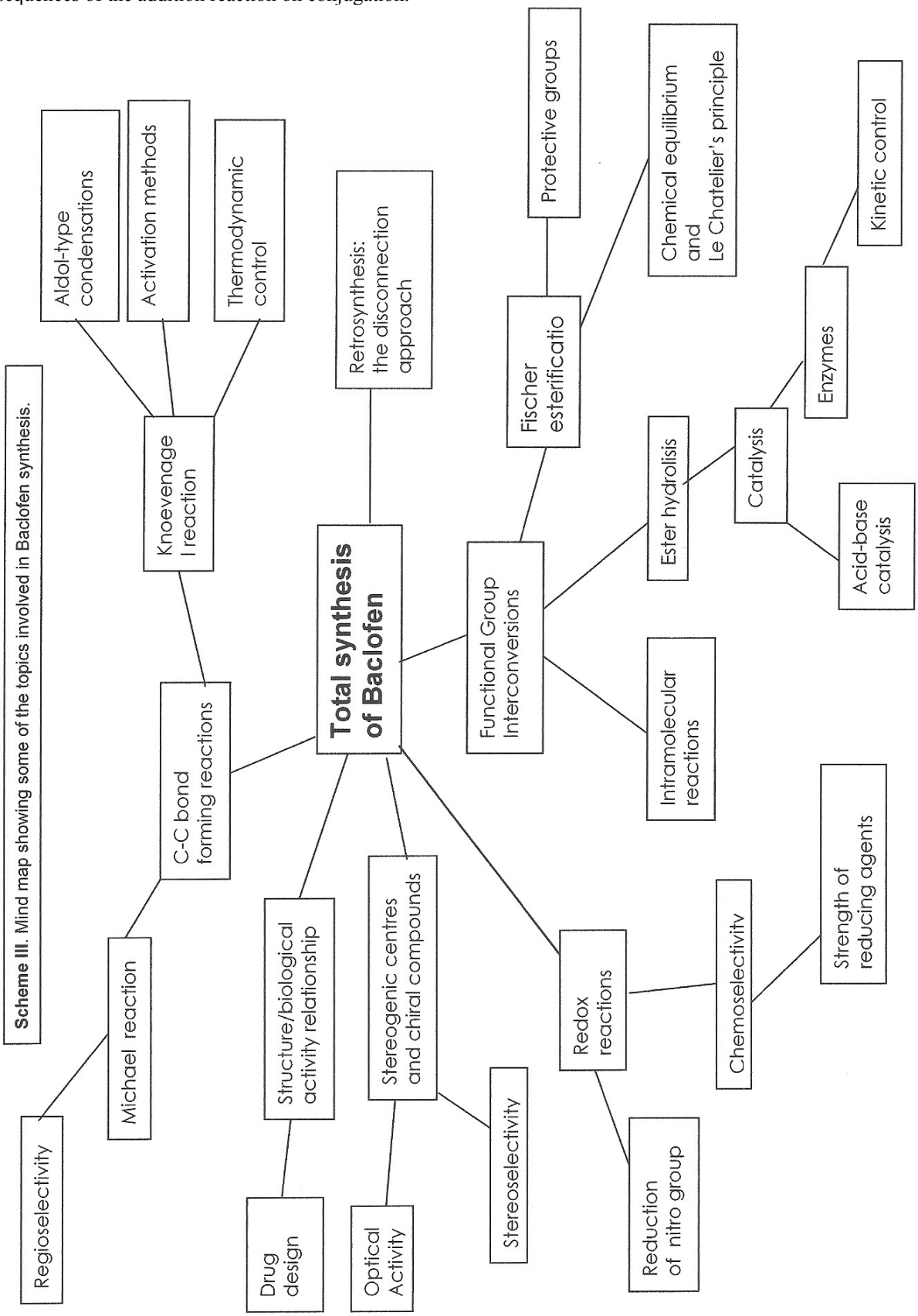
In the nucleophilic attack of nitromethane on the cinnamate ester a problem of regioselectivity may arise. But the competition between direct addition and conjugate addition is easily won by the latter, thanks to the use of a α,β-unsaturated ester as acceptor and of nitromethane as donor, both of which favour the conjugate addition mechanism [7].
In the last step the sp^2-hybridized prochiral β-carbon atom turns into a stereogenic centre. However, because the two enantiotopic faces of the planar conjugated system show the same reactivity towards the nucleophile, the reaction produces a racemic mixture of (R) and (S) γ-nitroesters. Then the mixture reacts with α-chymotrypsin, an enzyme which stereoselectively hydrolizes the (S)-nitroester faster than it does with the (R)-enantiomer [4]. A simple extraction follows, which separates the (S)-nitroacid from a nitroester sample enriched in the (R)-enantiomer.
As the nitro group has already performed its task, it can be reduced to an amino group. If we used for this purpose a strong reducing agent like $LiAlH_4$ it would reduce the ester group as well. It is therefore necessary to employ a milder, more chemoselective reagent, like H_2 and Pd/C [4] or $NaBH_4$ in combination with a transition metal [8-9]. Actually, the resulting aminoester is not isolated, because of an intramolecular acylic nucleophilic substitution leading to the formation of a lactam [4-(4-chlorophenyl)pyrrolidin-2-one]. After a chromatographic purification step the optical activity of the lactam is checked by polarimetric measures. Eventually, the acid hydrolysis of the lactam affords Baclofen in the form of its hydrochloride.

Hazards
All the reactions and the purification steps were carried out in a fume hood. 4-chlorobenzaldehyde, malonic acid, 4-chlorocinnamic acid, methyl 4-chlorocinnamate, α-chymotrypsin and Baclofen itself are irritant to skin, eyes and respiratory system, and so is 6 M hydrochloric acid. Methanol is flammable and toxic. Sodium borohydride is toxic by contact and by ingestion. It forms hydrogen by reaction with water and can cause burns. 1,1,3,3-Tetramethylguanidine, the base used in the Michael reaction, is flammable and can cause burns. Pyridine is flammable and harmful by inhalation and by contact. Concentrated sulphuric acid can cause severe burns. Nickel chloride is a cancer suspect agent and n can cause sensitization to skin. Nitromethane and all the solvents used (see the experimental procedure) are highly flammable. The hazards of some intermediates (γ-nitroester and lactam) are unknown.

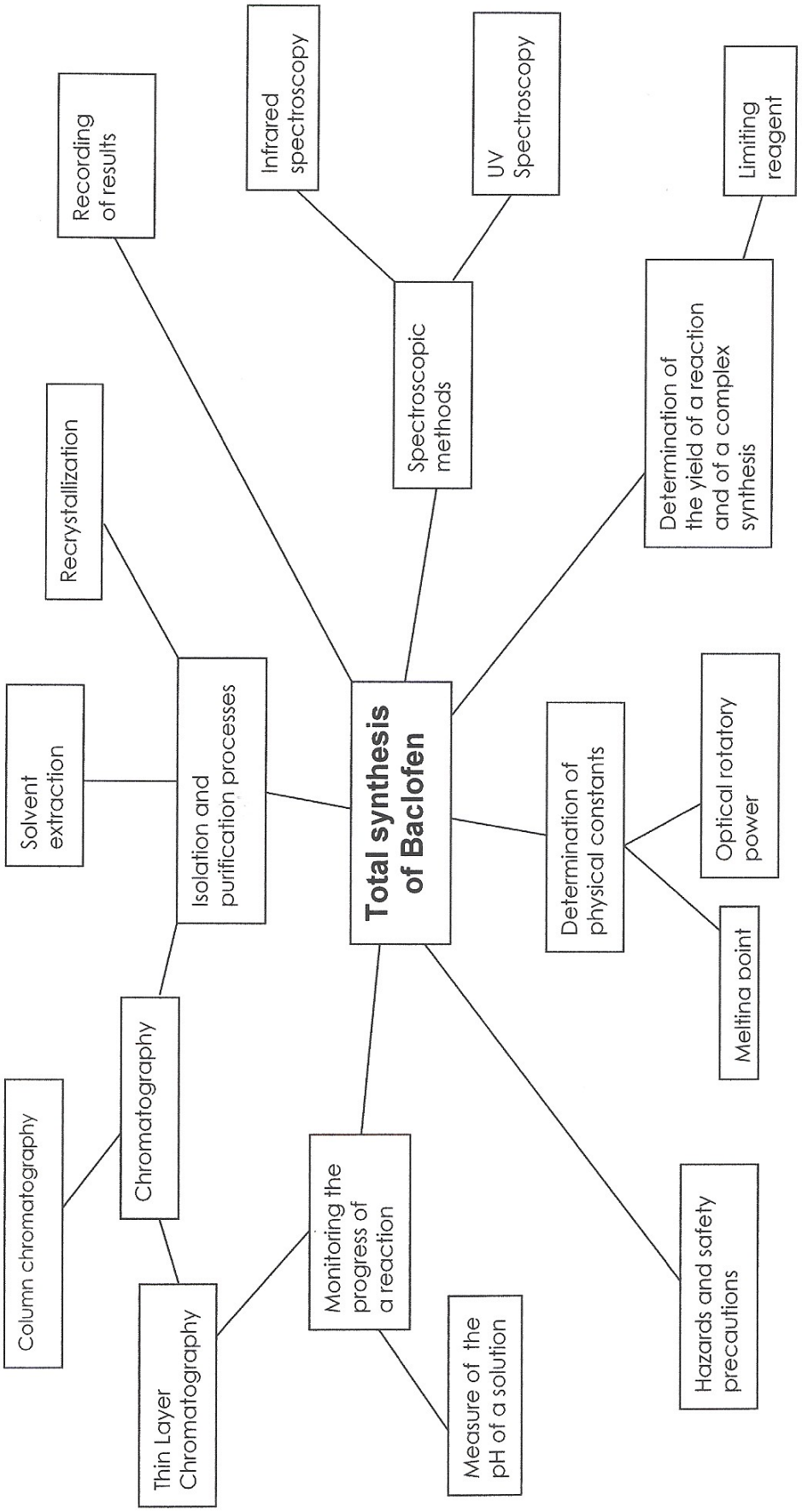
Results
The project required a 42 hour lab period for its completion. Throughout the synthesis students worked in pairs. The average overall yield obtained by the seven groups of our class was 12%. The physical properties of the final product were in accordance with those reported in the literature. Its optical purity (about 30%) was indirectly evaluated through polarimetric analysis of the lactam, taking advantage of its higher specific optical rotatory power.
We found two steps particularly challenging: the enzymatic resolution (conversion lower than expected, despite the long reaction time) and the γ-nitroester reduction (high conversion, but rather low yield). See the experimental part for details. These steps should be optimized through the identification of the nature of by-products and an accurate evaluation of the influence of experimental conditions on the outcome of the reaction.
Students were regularly asked to record their observations and experimental data, in order to write a final laboratory report. Some of them noticed that resolution of racemic mixtures drastically lowers the overall yield, resulting in a waste of reagents. This observation led to an interesting discussion, highlighting the recent development of asymmetric synthesis.

Pedagogical benefits for students
The map of scheme III illustrates how many topics can be involved and dealt with in planning and carrying out a total synthesis. This makes the project particularly suitable for revising some important reactions and concepts of a standard organic chemistry course and for creating useful links between them.
It should be kept in mind that in a target synthesis each chemical transformation is part of a logical scheme, where it plays a specific role, and even the order of steps is closely related to the chemical properties of the intermediate compounds. For example, even a trivial reaction like the Fischer esterification can become significant, once employed in the Baclofen synthesis. Not only did it protect the carboxylic group during the Michael reaction, it also allowed the enzymatic resolution to take place and favoured the cyclization step, which resulted in the formation of a very useful lactam. In this sense we think that such an activity may support a better understanding of metabolic pathways, an important topic in the biochemistry course.
As scheme IV clearly shows, throughout the synthesis our students could appreciate the benefits from a close cooperation between organic and analytical chemistry. TLC analysis of some reaction mixtures and product purification by column chromatography were effective approaches to the theory and practice of chromatography. In addition, at each step of the synthesis students were asked to compare the IR spectra of the starting compound and of the product. This "dynamic" interpretation of spectra is particularly useful for developing the ability of relating spectroscopic similarities and differences to the structural changes occurring during a reaction. Something similar happened after the Michael

step, when the striking difference between UV spectra of methyl cinnamate and γ-nitroester highlighted the consequences of the addition reaction on conjugation.



Scheme III. Mind map showing some of the topics involved in Baclofen synthesis.



Scheme IV. Mind map of the analytical techniques and other laboratory operations involved in Baclofen synthesis.

Conclusion

Target synthesis calls for diverse skills in the organic chemist. For this reason, we think that a total synthesis is the ideal activity to conclude an introductory course, provided that a well equipped laboratory is available. Due to its complexity, however, such a project requires accurate planning and should be supported by a close cooperation between organic and analytical chemistry teachers. Some slow reactions and some time-consuming purification procedures make it hard to meet the experimental needs, particularly when the organization of the school timetable is rather rigid.

Acknowledgements

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