

The Introduction of High-Throughput Experimentation Methods for Suzuki–Miyaura Coupling Reactions in University Education

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During the last decade, combinatorial and high-throughput techniques have revolutionized pharmaceutical research (1–3). Hundreds to thousands of times more compounds can be synthesized and screened than with traditional approaches. This can, in principle, decrease the time-to-market for new drugs tremendously (4, 5). Moreover, combinatorial and high-throughput techniques are increasingly being applied in fields such as inorganic materials (6–8) catalysis (6, 9–11), and polymer science (6, 12, 13). Although these activities are significantly expanding within companies, high-throughput experimentation (HTE) is not (yet) an important issue in academic chemical research, even though students will most likely use those techniques after their education. Therefore, we have initiated small research projects for second-year students that include the use of fully automated synthesis ro-

bots. In those projects they learn and discover the new way of thinking that is required for combinatorial and high-throughput research.

In the student project, an automated synthesizer was used for the optimization of the Suzuki–Miyaura coupling. This C–C coupling reaction of aryl boronic acids (or boronic esters) with aryl halides was first reported by Suzuki and Miyaura in 1981 (14). Ever since, it has been applied to many different cross-coupling reactions in organic synthesis (15, 16) and polymer synthesis (17). Recently, the application of such a coupling reaction in a practical course has been reported (18). Moreover, in the last several years some examples of high-throughput optimization of the Suzuki–Miyaura coupling reaction appeared in literature utilizing standard offline reactions with automated workup (19), semi-automated parallel synthesis with manual loading of the reactors (20), and utilizing fully automated serial (21) and parallel (22) synthesis robots.

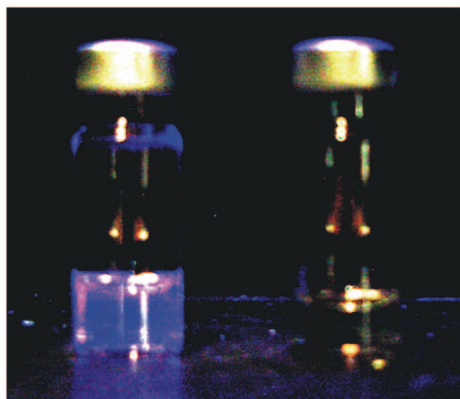
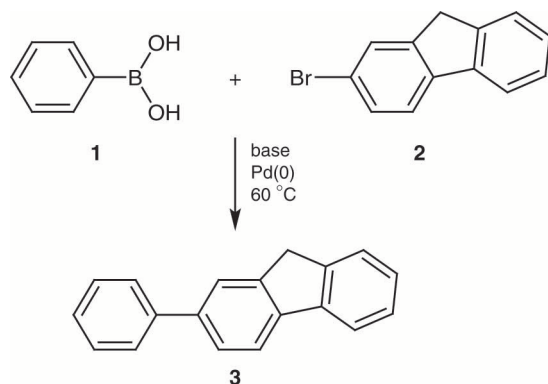


Figure 1. (Top) Schematic representation of the investigated Suzuki–Miyaura coupling. (Bottom) Photograph showing both a successful coupling reaction (left vial) and an unsuccessful reaction (right vial) under UV irradiation demonstrating the fluorescence of the 2-phenylfluorene product.

The Practical Course

The practical course is commonly performed by chemistry majors in their fourth semester. Previously to this course, students have had two theoretical courses in organic chemistry and one practical course on organic chemistry that included performing “standard” laboratory protocols. This practical course is organized as a research project that runs during half a semester for one and a half days per week. Groups of four students are divided over eight different state-of-the-art research projects in three different research groups. The goal of those projects is to provide the first contact with chemical research to the students. This implies that the students get a general manual that encourages them to search the scientific literature in a specific direction. From the literature, a more specific project is generated together with the members from the research group and the course instructors.

In this article the HTE-project is described in more detail. As a consequence of the organization of these research projects, only four students have performed this project. However, currently a second group of four students is performing the research project, which is slightly modified to keep it state-of-the-art.

The Project

The project consists of the optimization of the Suzuki–Miyaura coupling reaction utilizing a fully automated parallel synthesizer. The optimization of the coupling reaction was performed by investigating the coupling of phenyl boronic acid 1 with 2-bromofluorene 2 (Figure 1, top). This reac-

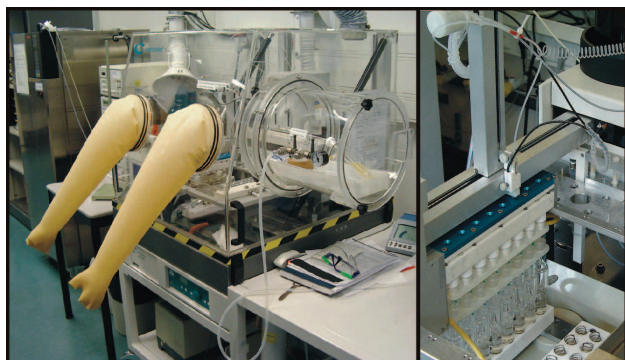


Figure 2. (Left) Picture of the Chemspeed ASW2000 automated synthesizer with its hood. (Right) Close-up of the workspace with the xyz-liquid handling and a reactor block.

tion was chosen because the resulting 2-phenylfluorene **3** (**23**) is fluorescent providing a visual indication that the reaction is proceeding. In addition, the fluorescence of the product makes the project more application directed (e.g., organic light-emitting diodes) and at the same time also more interesting for the students. Figure 1 shows reaction mixtures under UV irradiation of both successful (left) and unsuccessful (right) coupling attempts, demonstrating the fluorescence of

the desired product. During the project, a Chemspeed ASW2000 synthesis robot was utilized as depicted in Figure 2. This robot system is built in a modular way, whereby different (up to five) reactor blocks can be positioned inside the working area of the xyz-liquid handling system. An inert atmosphere is provided by a small argon overpressure over the reactors and a large argon flow through the hood covering the robot.

After a literature search to investigate the mechanism of this coupling reaction and to compare the Suzuki–Miyaura coupling with other available C–C coupling reactions like Grignard (**24**) or Stille (**25**), all four students performed a coupling reaction utilizing “classical” lab experimentation with different conditions to become accustomed to this reaction. These lab experiments were performed in tetrahydrofuran (THF) with potassium hydroxide as base dissolved in water and palladium(II) acetate or tetrakis(triphenylphosphine)palladium(0), Pd(PPh₃)₄, as catalyst. The resulting two-phase reaction mixtures were heated to 60 °C for 16 hours. The progress of the reactions was investigated both by thin-layer chromatography (TLC) and gas chromatography (GC). The students had to understand the principles of GC to optimize the GC method to separate the signals of the reagents and solvents. Moreover, for quantification of the conversion, *n*-butylbenzene was found to be a suitable standard. With palladium(II) acetate no reaction took place,

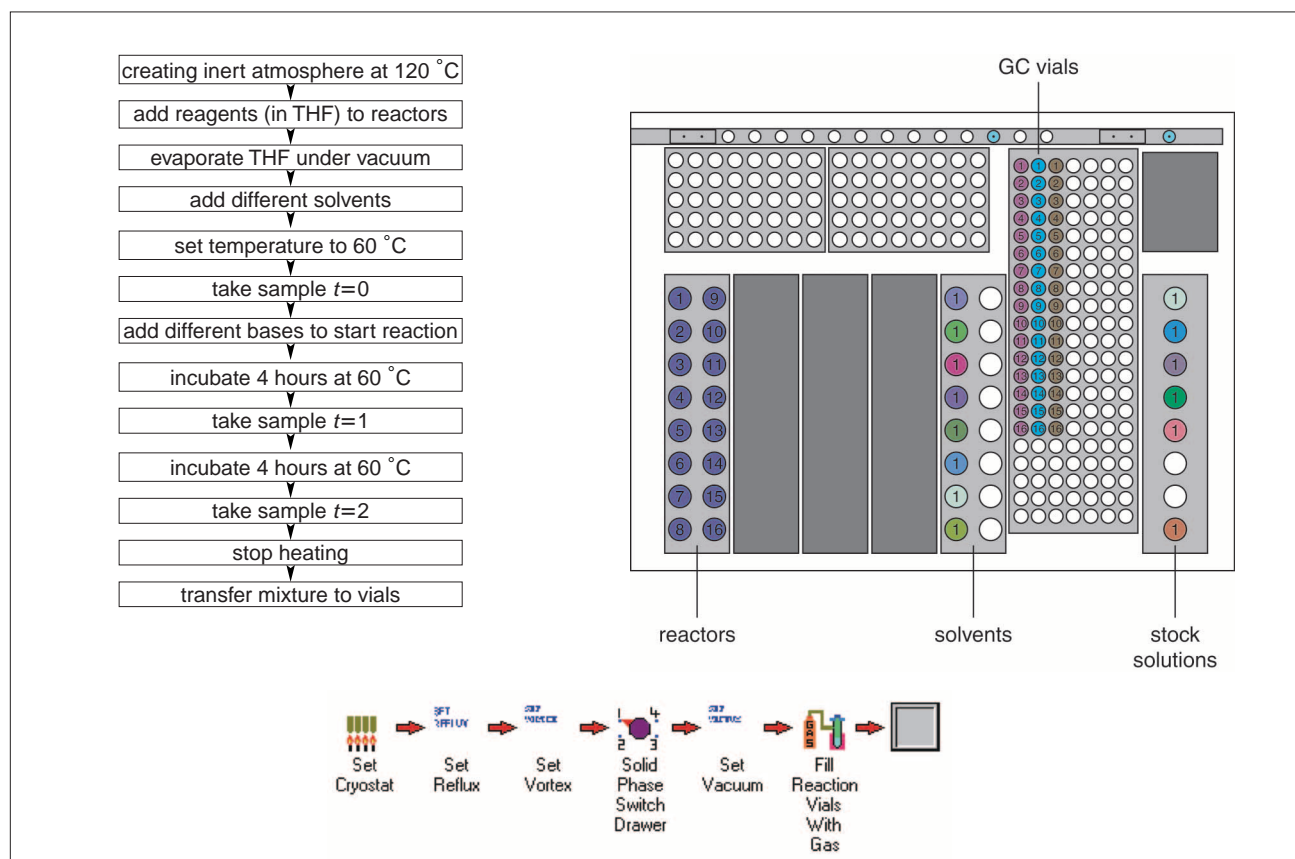


Figure 3. (Top left) Flow scheme of the automated Suzuki–Miyaura coupling. (Top right) Schematic layout of the workspace from the automated synthesizer. (Bottom) Screenshot from building a method while programming the automated synthesizer.

which is due to inactivation of the catalyst by oxygen as indicated by the black color of the reaction. With the less oxygen-sensitive $\text{Pd}(\text{PPh}_3)_4$ catalyst, the Suzuki–Miyaura coupling was performed successfully and the product was purified by preparative column chromatography. Finally, the resulting 2-phenylfluorene was characterized by ^1H -NMR spectroscopy (including the complete assignment utilizing the known spectra of the reagents).

After those “classical” reactions, the students investigated combinations of eight different organic solvents and two bases (dissolved in water) for the Suzuki–Miyaura coupling using the automated synthesizer. This library space resulted in a total number of 16 parallel reactions. Utilizing their laboratory experience, the experimental details were converted into a step-by-step flow scheme (Figure 3, top left) for the synthesis robot. The steps include, for example, creating inert atmosphere (set high temperature, apply vacuum, fill with argon), switch on reflux and stirring, and regularly rinsing the tubings and needle to prevent cross-contamination and inaccuracy due to small air bubbles in the tubings. After an introduction to the controlling software of the automated synthesizer the students were able to program the synthesis robot independently. The programming consists of three main steps: (i) designing a tray with reactors, stock solutions, and sample vials (Figure 3, top right); (ii) programming different methods like creating inert atmosphere (Figure 3, bottom) or sampling and (iii) building an application from the different methods.

The programmed application was utilized for the automated reactions. After dispensing the different stock solutions, 100- μL zero-time samples were taken from the reaction mixtures for GC analysis and subsequently the bases were added

to start the reactions. The two reactions in pyridine were performed without additional base to investigate whether pyridine could act as base in the reaction itself. Samples (100 μL) were taken from the reaction mixtures for GC analysis after four and eight hours of vortexing at 60 °C. For sampling of the two-phase reaction mixtures, the vortexing was interrupted so that a sample could be taken from the upper organic layer. The conversions determined from GC are depicted in Figure 4 for all combinations of solvent and base. The reactions in pyridine did not show any conversion, demonstrating that additional base is necessary to catalyze the reaction. Moreover, the reactions in cyclohexane, *n*-pentane, and *n*-butanol did not succeed because the reagents were not sufficiently soluble. All other solvents revealed coupling of the phenyl boronic acid with the 2-bromofluorene utilizing both potassium carbonate and tetraethylammonium hydroxide (TEAH) as base. In general, potassium carbonate proved to be more active in the reaction than TEAH and the reactions in THF showed the highest conversion.

Hazards

All reagents are irritants and thus lab coats, gloves, and safety glasses should be worn at all times. The organic solvents used are flammable, so heating steps should be performed with an oil bath or heating mantle instead of a Bunsen burner. Where possible, fume hoods should be utilized.

Summary

The laboratory project allows for the discussion of the reaction mechanism of the Suzuki–Miyaura coupling reaction. In addition, discussion of the literature search could lead to a better understanding of research in general and the discussion should lead to generation of the research goals for this project. The practical part of the project makes the students familiar with working under inert atmosphere. However, if the appropriate equipment for working under inert atmosphere is not available in a laboratory, novel catalysts that do not require inert atmosphere (26) might be utilized to adapt the project. To characterize and monitor the reaction, TLC, ^1H -NMR spectroscopy and GC were applied, which allowed discussion of the techniques as well as interpretation of the results. But (in our opinion) most importantly, the students became familiar with high-throughput experimentation, which will probably be an important part of their future operating environment. The authors do realize that not many student laboratories are equipped with fully automated Chemspeed (27) robots. However, manual parallel reactions could also be performed utilizing, for example, carousel reactors (28).

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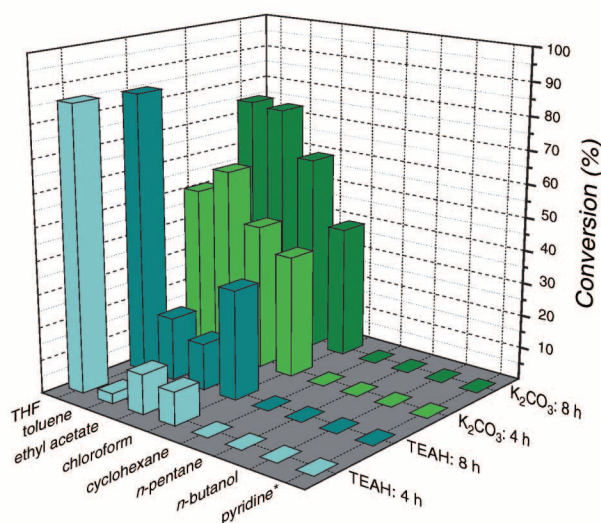


Figure 4. Conversions obtained with GC for the Suzuki–Miyaura coupling of phenyl boronic acid with 2-bromofluorene in eight different solvents with two bases. (*The reactions in pyridine were performed without additional base. TEAH is tetraethylammonium hydroxide.)

Supplemental Material

Instructions for the students and notes for the instructor are available in this issue of *JCE Online*.

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