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Breaking the New Bottleneck: Our Way into Robotics

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Abstract:
A new robotics system for automated reaction screening and optimization as well as fast analytical characterization is described. The system consists of a parallel synthesis robot capable of performing 12 reactions in parallel and a second robot for analytical sample work-up and dilution. Four HPLC devices enable a high analytical throughput. The successful application of this new system to several examples of automated process optimization is presented.

Introduction

Combinatorial Chemistry[1,2] and High Throughput Screening as enabling technologies in today's drug discovery environment have dramatically influenced the way in which modern process research and development is performed in the pharmaceutical and related industries in at least two ways. First of all, with the extensive use of parallel synthesis and biological testing of large libraries of new compounds in very short periods of time, many more hit structures are found compared to those found by the traditional approach. After some optimization of the lead structures by traditional medicinal chemistry they rapidly enter the development pipeline as potential new drug candidates. As a result, most chemical development departments are facing a dramatic increase in workload to synthesize a larger number of target molecules. There is also increasing pressure to rapidly optimize processes and shorten development timelines to accelerate time to market. As this new trend is usually not connected with additional staffing of process research and development departments, a new bottleneck for the drug development workflow is building up within these departments. Combinatorial chemistry influences process research in the second way, as many departments are now trying to increase their throughput by extensive use of automated parallel synthesizers which became available as an offspring of combinatorial chemistry efforts in medicinal chemistry. These synthesizers have evolved into more and more reliable and sophisticated systems during the past few years.[3−5]

As we faced such a situation with a steadily increasing number of new projects and chemical steps entering chemical development at Schering, a project team was launched in 1997 to evaluate the potential of automation and robotics in supporting fast synthesis optimization with the goal of achieving robust processes for the pilot plant.

The following most important benefits of automated synthesis in chemical process research and development were identified:

- Faster optimization of chemical processes enables a faster supply of drug substance for other development functions (e.g., pharmaceutical development, toxicology, pharmacology, clinical studies).
- A large number of parameters has to be optimized manually in the laboratory with great time efforts for each single reaction. This is often not only time-consuming but also very tedious and always a potential source of errors, whereas a robotic system should work always with the same consistency and reproducibility.
- Reactions which are more thoroughly investigated by means of automated methods will eventually lead to more robust processes and thus decrease the efforts and the costs in the pilot plants as well as in production.
- Increasing the yields by only a few percentage points at each step has a dramatic effect on the overall yield of the whole synthesis sequence and therefore on the cost of the drug substance. Therefore, if a new synthesis can be optimized further by using automated methods, the investment into technical equipment will pay off within a very short period of time.

The optimization of chemical processes can be roughly divided into three phases. *Process Screening* is characterized by a large number of reactions, small reaction volumes, and a large number of variable reaction parameters. During *Process Optimization* a medium number of experiments with larger volumes (up to 50 mL) are performed with a smaller number of reaction variables. In this phase a thorough analytical characterization of the reaction progress becomes crucial. Finally in the *Process Characterization* phase only few reactions with volumes up to 1 L are studied, and only minor changes will be introduced in order to define the final reaction conditions which will give the best results on the lab-scale and also a robust process in the pilot plant. As automated laboratory reactors, for example, LabMax or RC-1, for the characterization of processes were already available at Schering and the bottleneck was seen more in the very time-consuming tasks of process screening and process optimization, we set out to establish a system to support our efforts in these two phases.

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(1) Czarnick, A. W.; Wilson, S. R. *Combinatorial Chemistry: Synthesis and Applications*; Wiley: New York, 1997.
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(3) Emiabata-Smith, D. F.; Crookes, D. L.; Owen, M. R. *Org. Process Res. Dev.* **1999**, *3*, 281.
(4) Armitage, M. A.; Smith, G. E.; Veal, K. *Org. Process Res. Dev.* **1999**, *3*, 189.
(5) Simms, C.; Singh, J. *Org. Process Res. Dev.* **2000**, *4*, 554.

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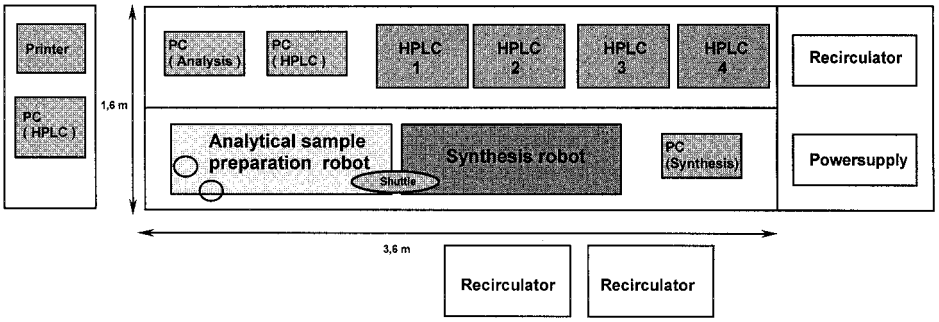
Scheme 1. **Sketch of the robotics system. Top view (doublesized laboratory bench)**



Results and Discussion

As the first step we set up a system specification with the desired properties of the robotics system. Crucial for us was a simple handling of approximately 10−40 experiments with a reaction volume of 10−50 mL each. The system should also be capable of adding different reagents to the reaction mixtures portionwise or continuously. Cross contamination between individual reactions should be prevented by automated washing procedures. A large temperature range (ideally between −70 and +150 °C, including reflux of solvents) should be individually controllable for each reactor. As the perturbation of reaction mixtures, especially in heterogeneous cases, is always a potential source of problems on scale-up, we wanted to stir and not to agitate the reaction mixtures although knowing well that the pilot-plant stirring conditions are complex and cannot be imitated easily on the laboratory scale. To thoroughly characterize the progress of the studied reactions we wanted to collect and work up analytical samples automatically. A direct coupling to an HPLC system or other analytical devices should give the opportunity for a high throughput analytical system. Finally, the controlling software of the robotics system had to be user-friendly and easy to handle.

With this checklist in mind we evaluated the automated synthesizers which were commercially available in 1998/99. Several overviews on these systems were summarized recently in the literature.[6−8] None of the commercially available systems was a complete match to our specifications. A sketch of the robotics system which was established at Schering in 1999 is shown in Scheme 1. It consists of three main parts: a parallel synthesis robot (Bohdan Process Development Workstation), a second robot for the preparation of analytical samples (Bohdan Sample Preparation Workstation, custom-tailored to our needs) (Figure 1), and four Dionex HPLC devices. The synthesis robot is capable of performing 12 parallel reactions with a 25-mL maximum reaction volume each (Figure 2). The temperature range which can be individually controlled for each reaction vessel is between −40 and +140 °C. For all reactions the block- and the inner reaction temperature are measured and reported which can give valuable hints on sometimes unexpected exotherms (Scheme 2, $SOCl_2$ addition to an aromatic acid, in this case the exothermic reaction was, of course, expected). All reactions are stirred by magnetic stir bars and performed under nitrogen or argon as inert gas for sensitive reagents. Both robots have two different types of cannulas for the transfer of individual reagents and solvents to each reaction vessels. The three-channel cannula is capable of piercing septa and is used to transfer air- or moisture-sensitive reagents (e.g., BuLi, DIBAH, $SOCl_2$ were used without deterioration problems), while a steady flow of argon or nitrogen guarantees an inert atmosphere during the transfer step. The second cannula consists of a wider Teflon tubing

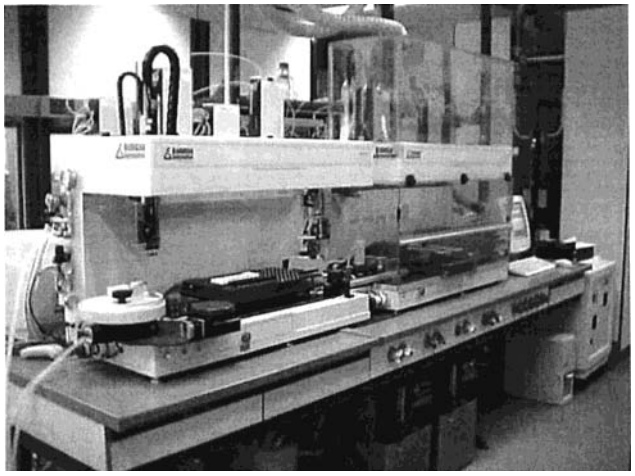


Figure 1. **Bohdan process development workstation and sample preparation workstation.**

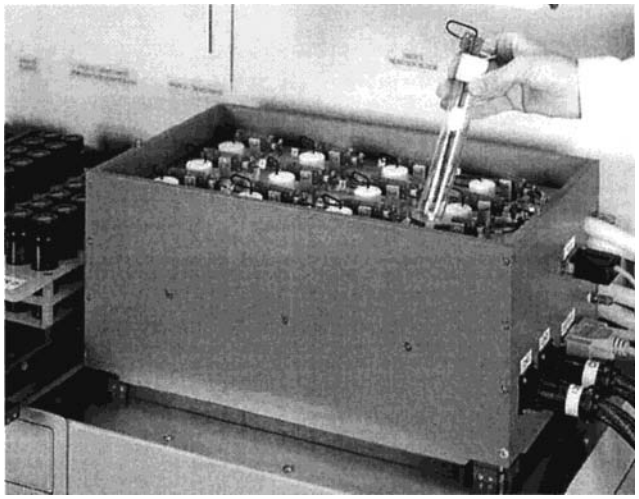
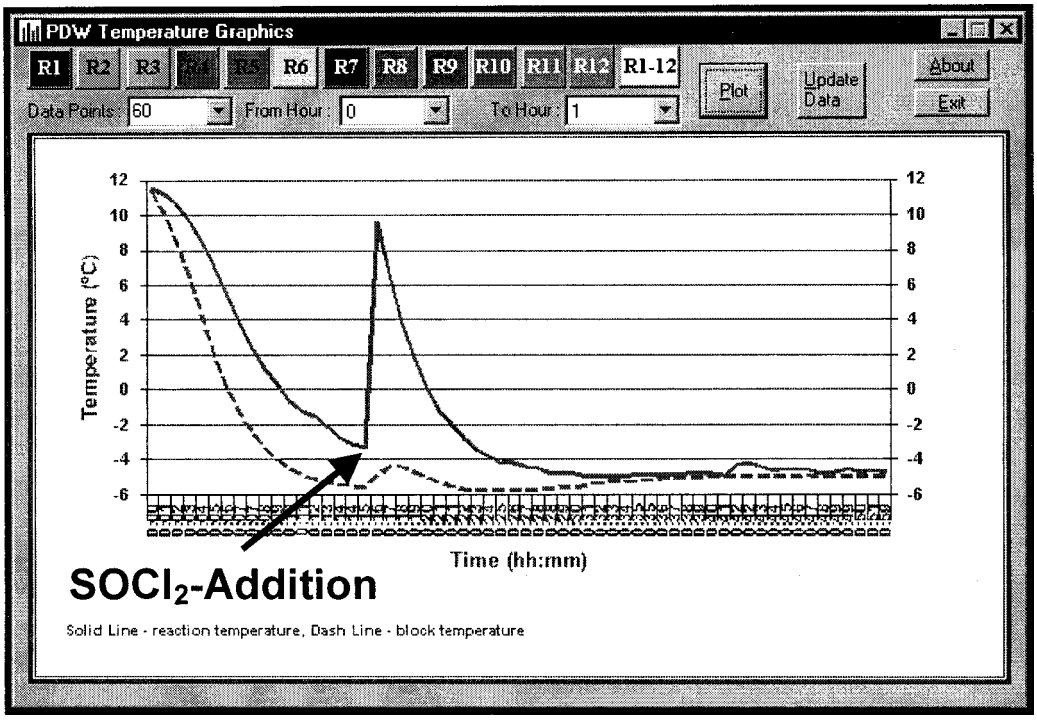


Figure 2. **Reactor assembly.**

(6) Harre, M.; Tilstam, U.; Weinmann, H. *Org. Process Res. Dev.* **1999**, *3*, 304.
(7) Owen, M.; DeWitt, S. Laboratory Automation in Chemical Development. In *Process Chemistry in the Pharmaceutical Industry*; Gadamasetti, K. G., Ed.; Marcel Dekker Inc.: New York, 1999.
(8) Okamoto, H.; Deuchi, K. *Lab. Rob. Autom.* **2000**, *12*, 2.

Scheme 2. **On-line report: temperature curve of an exothermic reaction**



which is stabilized by a widebore metal tube. This cannula enables the system to transfer slurries into the reaction vessels and also to take samples out of the reactions. This is an especially valuable feature as the addition of defined amounts of solid reaction components is always a problem in automated synthesis.

In a typical workflow the 12 reactions are planned and set up in the reaction block. The scheduler software calculates the best way to execute these reactions in the shortest possible time so that the robot arm will not stand idle for longer times. At freely programmable intervals analytical samples can be taken from the individual reaction vessels up to a maximum number of 120 samples per run. The exchange of analytical sample vials between the two robots is achieved by a shuttle system. Whenever a sample has to be taken according to the programmed reaction plan, the synthesis robot sends a signal to the work-up robot. The latter puts a vial with its gripper into the shuttle nest and a quench solution is added to the vial (Figure 3) so that the reaction in the analytical sample will be stopped immediately and a "snapshot" of the reaction mixture will be obtained. The vial is transferred via the shuttle to the synthesis robot, and a small amount of the reaction solution is added with the slurry cannula. After travelling back via the shuttle to the work-up robot the sample is further diluted and finally filled into the HPLC vials directly on autosampler racks. At this point the only manual interference that is necessary is that the autosampler racks have to be transferred to the HPLC devices. In addition to the physical transfer of the analytical samples the data connected with these vials have to be transferred to the HPLC computer. This is achieved via a barcode system. Barcode labels are attached to the autosampler racks, and a barcode reader is used to identify the racks once at the sample preparation workstation and for the second time at the HPLC

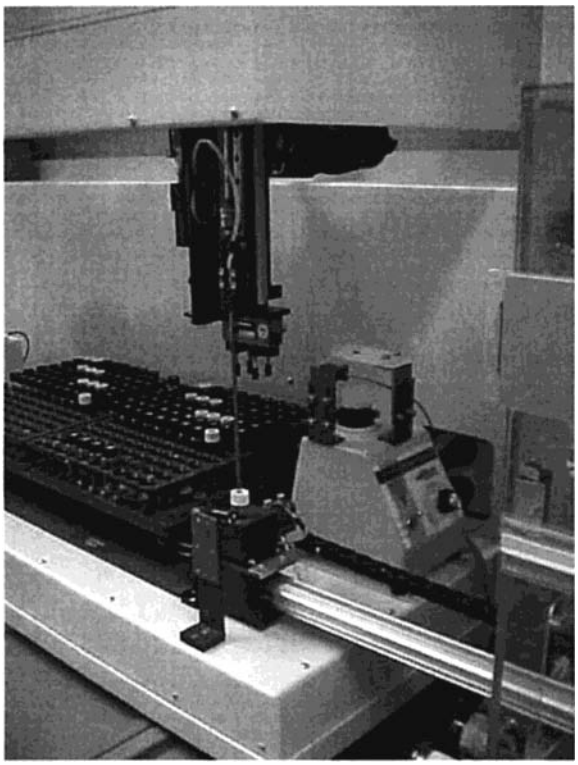


Figure 3. **Shuttle system.**

device as every HPLC is connected to a barcode reader (Figure 4). As the three personal computers controlling the individual parts of the system are connected into a small network, the data connected with these samples and identified by the corresponding barcodes can be easily exchanged in an automated manner. To achieve a high throughput of the analytical samples rapid HPLC analysis methods on short columns are used. Finally the analytical results are transferred into Excel spreadsheets to get a quick overview on the

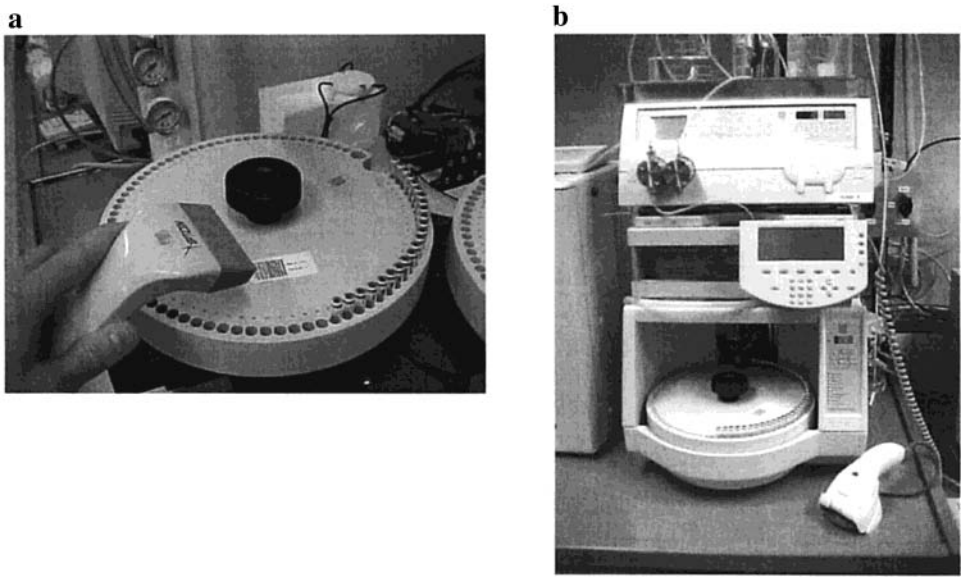
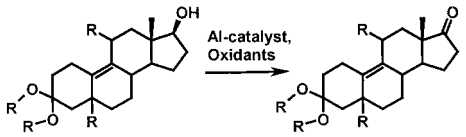
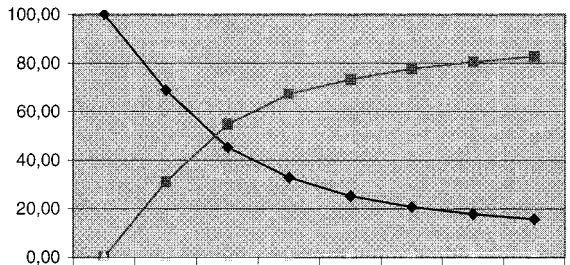


Figure 4. **Analytical sample identification via barcode system. (a) Barcode dionex autosampler rack (Bohdan sample preparation workstation). (b) Barcode reader HPLC.**

Scheme 3. **Determination of reaction kinetics by HPLC data**

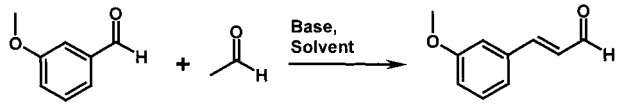




	Start	30 min	60 min	90 min	120 min	150 min	180 min	210 min
Educt	100,00	68,87	45,25	32,79	25,20	20,80	17,92	15,77
Product	0,00	31,13	54,75	67,21	73,30	77,65	80,42	82,60
Impurity	0,00	0,00	0,00	0,00	1,51	1,55	1,66	1,64

progress of the reaction, the formation of the product as well as that of the most important impurities. An example of such a result is shown in Scheme 3 where an Oppenauer oxidation of a steroidal derivative was studied. The progress of the reaction can be followed quickly, and the formation of important impurities over time can also be studied. All vials used during work-up and dilution are septa-capped and can be filled with an inert atmosphere;d therefore, we haven‘t encountered degradation of reaction products as a major problem in the reactions optimized so far. Although this might be a potential source of error, we found that the advantage of a much higher sample throughput and the higher flexibility of our system compared to a direct injection into a single HPLC injection port outweighs this by far. Using this off-line version gives a high reliability of the robotics system as a whole, as an HPLC error during an analytical run will not cause the loss of data or even the stop of the whole robotics run as might be the case in a system with

Scheme 4. **Aldol condensation**



direct injection of the sample into an HPLC injection port. All four HPLC devices are routinely checked and calibrated according to a special protocol to ensure comparability of the analytical results created by the different devices which is especially important when performing statistical experimental design where the level of experimental noise (variation) should be kept to a minimum.

In the following paragraph some examples of the successful implementation of this automated system for reaction optimization are discussed. At first glance the aldol condensation between *m*-methoxy-benzaldehyde and acetaldehyde to *m*-methoxy-cinnamaldehyde (Scheme 4) seems to be a simple reaction. Indeed, this condensation reaction has been known since 1917.[9] The problem with this reaction is the possible formation of many potential side products so that the published yields throughout the whole century used to be in the range of 10−20%. Despite this drawback, using the aldol approach to the reaction product is very attractive compared to other approaches as the starting materials are rather cheap commodities; therefore, we started to study this reaction. A first manual optimization in the laboratory had already improved the isolated yield to 35%. The automated process optimization system was then used to study the effects of different reaction parameters, for example, the kind and amount of base and solvent, and the concentrations, temperature, and the order of addition of the reagents. After only five runs of the synthesis robot (60 experiments) and 480 fast HPLC analyses the yield was nearly doubled (65%). Even more important was that to achieve this result a capacity demand of only 7 working days was needed, and the improved yield was smoothly reproduced on a 65 kg scale

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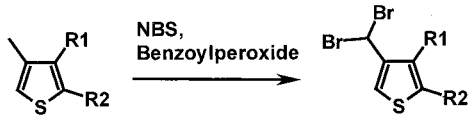
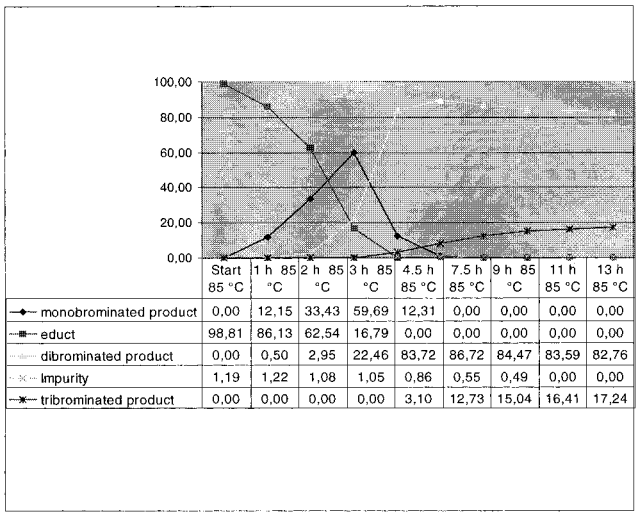
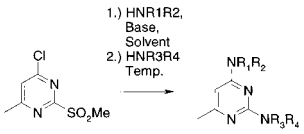
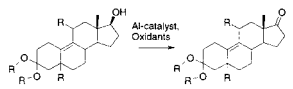
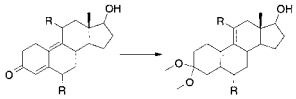
Scheme 5. **Radical dibromination of a thiophene derivative**





	Start 85 °C	1 h 85 °C	2 h 85 °C	3 h 85 °C	4.5 h 85 °C	7.5 h 85 °C	9 h 85 °C	11 h 85 °C	13 h 85 °C
monobrominated product	0,00	12,15	33,43	59,69	12,31	0,00	0,00	0,00	0,00
educt	98,81	86,13	62,54	16,79	0,00	0,00	0,00	0,00	0,00
dibrominated product	0,00	0,50	2,95	22,46	83,72	86,72	84,47	83,59	82,76
Impurity	1,19	1,22	1,08	1,05	0,86	0,55	0,49	0,00	0,00
tribrominated product	0,00	0,00	0,00	0,00	3,10	12,73	15,04	16,41	17,24

Table 1. **Automated process optimization examples**

	Scope of the Study
Formation of an Aromatic Acid Chloride 	• Test reaction for the chemical robustness of the system.
Nucleophilic Aromatic Substitution 	• Base and solvent screening study. New uncommon combinations were detected.
Oppenauer Oxidation of a Steroid 	• Screening of different catalyst, solvents and oxidants combinations. Optimization of the best method.
Ketalization of a Steroidal Ketone 	• Screening of different ketalization conditions (solvents, acids). Optimization of the best method.

in the pilot plant. As this example shows, using automated systems for reaction optimization is not only highly efficient but can also accelerate overall development times.

Another interesting challenge for us was the radical side-chain dibromination of a thiophene derivative (Scheme 5). During this reaction a monobrominated product is formed first, and then this intermediate can react further to the desired dibromo compound or even a tribrominated impurity. Therefore, it is crucial for a successful outcome of the reaction to determine the point during the course of the reaction when the maximum amount of dibromo product is formed as exactly as possible. The necessary reaction time is not always exactly the same since the radical initiator benzoyl peroxide contains varying amounts of water. Therefore, a study with a given batch of benzoyl peroxide was initiated on the robotics system. It was found by HPLC analysis that after 6 h a maximum amount of 89.9% of the dibromo product was formed which then decreases again due to the formation of the tribromo impurity. This information was very valuable for a reproducible reaction in the pilot plant, and it was possible to perform this radical reaction successfully on a scale of 50 kg.

Several other reactions which were optimized successfully by using the automated reaction optimization system are summarized in Table 1. In all cases the results of the experiments were available within a very short time and were reproducible in the laboratory and pilot0plant scale.

Conclusions

In the first 10 months after installation of the automated reaction optimization system over 1200 reactions have been performed during the successful optimization of 26 different synthetic steps. As an analytical support to these automated experiments more than 10 000 rapid HPLC analyses were performed. Automated process research and optimization has become a very efficient and valuable new tool for the rapid development of robust chemical processes in our department. Especially powerful is the combination with design of experiments (DOE) as automated parallel synthesizers enable the organic process research chemist for the first time to perform all of the experiments required by this method without spending too much time and effort. Therefore, a complete picture of the sometimes complex relationship between the different reaction variables can be gained.[3,4] It can be foreseen that automated synthesis systems will change the way that industrial process research and development is performed towards an increased throughput of projects in a manner similar to the way that combinatorial chemistry changed drug discovery. Nevertheless automated systems will not be able to substitute the intellect and intuition of a chemist at the workbench, and therefore, a good cooperation between automation specialists and process chemists will always be crucial for a widespread and successful implementation of this new technology.

Acknowledgment

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