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Combination of a Customized Robotic System with a TLC Scanner for High-Throughput Reaction Screening

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Abstract:

Combination of a new and highly efficient robotic system for high-throughput reaction screening with a thin-layer chromatography (TLC) scanner is described. The system consists of a parallel synthesis robot capable of performing up to several hundred reactions in parallel and a second robot for analytical sample workup, dilution, and TLC-spotting. The automatically prepared TLC plates are analyzed with a modern TLC scanner which gives rapidly semi-quantitative analytical results on the outcome of the single reactions. Several automated chemistry examples clearly demonstrate the high efficiency of this combined system and its superiority compared to more classical approaches, e.g., HPLC analyses.

Introduction

The way in which chemical process research and development is performed has been changing dramatically over the past decade. This is due to the high pressure on chemical development departments arising from a steadily increasing output of new candidates from drug discovery units as well as increased competition on the market which makes the rapid development of innovative drugs crucial for economic success. To cope with these challenges many pharmaceutical companies heavily invested in robotic systems to accelerate process R & D. Most of these early systems were focusing on reaction optimization,[1−3] and a lot of successful examples of automated reaction optimization have been published in the past few years.

However, in the early stages of process research when a new drug candidate has just been handed over from the Medicinal Chemistry department, a lot of experiments have to be performed to find the best starting points for a further and more detailed optimization. Route scouting for improved alternative synthetic approaches requires a high number of reactions to be performed rapidly. A drawback in this early development phase is that usually only very limited quantities of starting materials and core intermediates are available. This means that the screening experiments have to be performed using very small volumes; however, the results have to be reliable and reproducible also on a larger scale.

An additional problem is the rapid analytical characterization of a high number of reactions, e.g. in catalytic reaction screening, where a lot of different parameters such as catalyst, ligands, additives, solvents, temperatures, etc. have to be tested. By using traditional analytical testing methods, such as, e.g., HPLC, relatively long overall analyses times have to be accepted even if short columns and gradient methods are used. Therefore, this creates a new bottleneck in the overall process of rapid reaction screening. To overcome this problem several approaches using color or fluorescence assays for reaction high-throughput screening were published recently.[4−6] However, these assay systems usually give more or less qualitative hints on the reaction outcome and are therefore not always sufficient for reaction screening in an industrial chemical process R & D. To fill this gap we developed a new, rapid and reliable method for determination of reaction outcomes based on automated spotting of analytical samples on thin-layer chromatography (TLC) plates in combination with a modern TLC scanner.

Results and Discussion

After the successful establishment of a first automated system for process optimization (Bohdan Process Development Workstation and Sample Preparation Workstation)[7] within our Automated Process Optimization group we set out to expand the concept of laboratory automation to small-scale reaction screening, route scouting, and polymorphism studies.

On the basis of our specifications we made the decision to establish for these purposes a custom-tailored system of Zinsser Analytic.[8] The whole robotics system consists of three parts (see Figures 1 and 2).

The first robot (CALLI) is responsible for the preparation of the individual reaction vials and the dispensing and weighing of solid starting materials. It is capable of dosing solids into reaction vials of different sizes (1−25 mL) with two variable solid-dispensing pipets "VARIX" (see Figure 3). Six different reagents and starting materials can be kept under inert atmosphere in powder storage containers in an argon box. The reaction vials are also kept in an argon box. Solids can be dosed in amounts between 1 mg and 2 g and

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(3) Okamoto H.; Deuchi, K. *Lab. Rob. Autom.* **2000**, *12*, 2−12.
(4) Stambuli, J. P.; Hartwig, J. F. *Current Opinion in Chemical Biology* **2003**, *7*, 420−426.
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(8) See http://www.zinsser-analytic.com.

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Figure 1. **Overview of the high-throughput robotic system.**

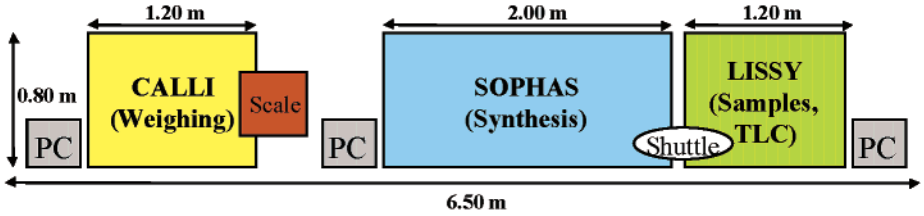


Figure 2. **CALLI, LISSY, and SOPHAS.**

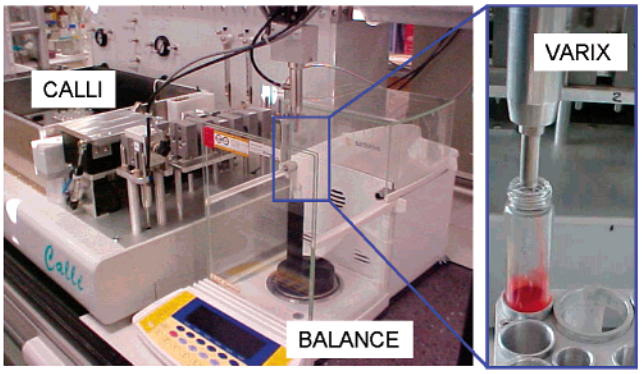


Figure 3. **Solid dispensing with CALLI on the balance.**

the exact weight that was transferred to the individual vial is determined by weighing on an integrated balance. Depending on the physical properties of the solid reactants, the accuracy is about ±5%. Solvents can be added automatically by using two cannulas. The amount of solvent is modified to the actual amount of dispensed solid by software calculation.

On the synthesis robot SOPHAS it is possible to handle up to seven reaction blocks with reaction volumes varying between 1 mL (96-well format) and 20 mL (8-well format). Reactions can be performed in a temperature range between −50 °C (limited by the cooling unit) and +140 °C. Extensive temperature studies revealed an interaction of the block format and the real internal solution temperature. For each block and vial format we measured external and internal temperature and detected an overall deviation of about 3−5 °C. Within one reaction block the temperature distribution is quite homogeneous, varying around ±1 °C. For rapid dispensing of solvents and liquid reagents four parallel stainless steel three-channel-cannulas are used. Depending on the type of solvent, our liquid handling parameters were developed to add the desired solvents and solutions with an accuracy of approximately ±4%. Especially the bigger errors were obtained by handling very small amounts of solvents (about 30 μL) such as dichloromethane. Two slurry cannulas are available as gripper tools. As a new tool on SOPHAS an additional ceramic needle was integrated for handling corrosive reagents such as $POCl_3$, $SOCl_2$, or SO_2Cl_2. Looking for an easy evaluation reaction for the suitability of the ceramic needle we tested the formation of benzoic acid chloride using a solution of $SOCl_2$ in ethyl acetate as well as neat $SOCl_2$. In both cases the benzoic acid (dissolved in ethyl acetate) was transformed quantitatively into the acid chloride using 2 equiv of $SOCl_2$ without any damage to the ceramic needle. A quick analytical TLC showed no starting material left.

Solid dispensing can be achieved on the SOPHAS robot in a way similar to that for the CALLI robot by using solid-dispensing pipets. Independent addition of two reactive gases to the reaction vials is possible. Agitation is done by shaking the reactor blocks which is also possible under complete inert atmosphere (nitrogen or argon). Reaction products can be isolated on a filtration unit by applying either vacuum filtration or positive pressure on the individual filters either on a 24-filter cartridge rack or a 96-well filter plate. Solvents can be changed during longer synthesis sequences by evaporation on a custom-tailored evaporation unit (see Figure 4). Finally analytical samples can be automatically transferred to the third robot LISSY via a shuttle system.

The third robot LISSY is responsible for sample workup (quenching and dilution) and reformatting into various analytical sample vials for HPLC or HPLC−MS systems. This robot takes over the sample racks from the shuttle to SOPHAS and can store them in a stacker for three to five sample racks. On top of this, LISSY is also capable of spotting 1-μL samples automatically in a parallel fashion on TLC plates (see Figure 5). Direct spraying with detection

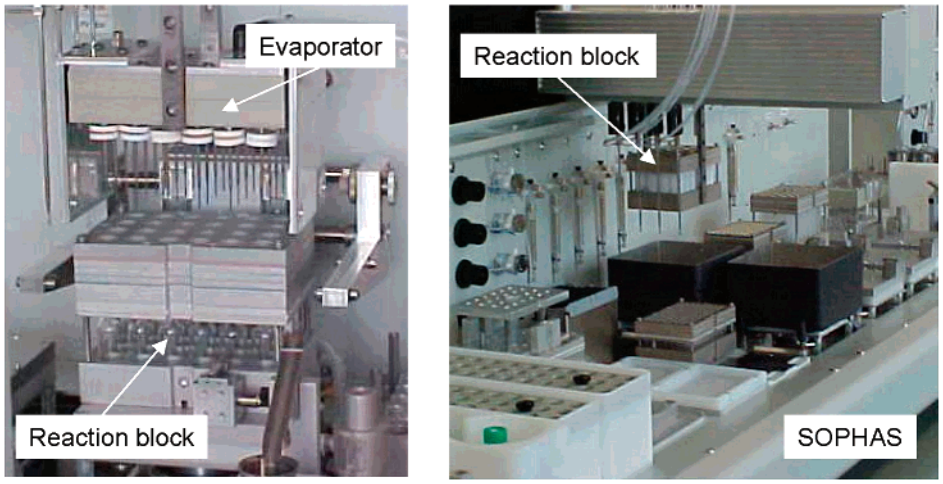


Figure 4. **Evaporation unit and reaction-block handling on SOPHAS.**

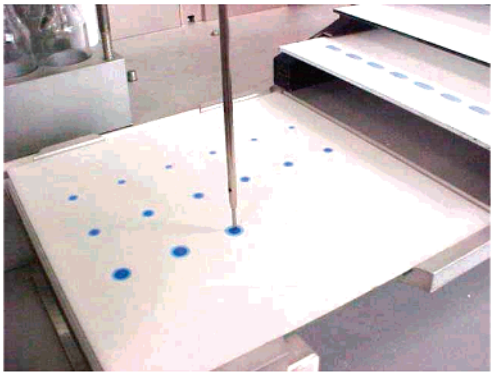


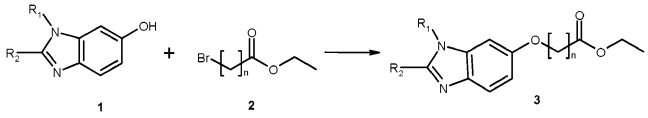
Figure 5. **Automated sample spotting on TLC plates with LISSY.**

agents is possible and can be used to easily detect certain reaction products by color assays.

Thin-layer chromatography as an analytical method for high-throughput determination of reaction outcome is currently a hot topic in industrial and academic synthesis laboratories. After we had performed the studies described in this article, two other papers appeared which reported about the use of high performance thin-layer chromatography for the assessment of the quality of combinatorial libraries[9] and for the detection of unexpected reactions in organometallic combinatorial catalysis.[10] For our initial studies we used the commercially available CAMAG TLC-SCANNER 3 which allowed measurements via adsorption and fluorescence in a wavelength area from 190 to 800 nm (see Figure 6).[11] The scanner was integrated in the sample preparation workflow of the TLC spotting robot LISSY.

Our first investigation was focused on the synthesis of an alkoxy-benzimidazole derivative **3** (Scheme 1) which was used as an early intermediate in the synthesis of a potential development candidate.[12] First, the analytical TLC assay was developed by screening wavelength and mobile solvent phase (also mixed-solvent systems) for optimal product detection

Scheme 1. **Fast Optimization of Aryl Ether Synthesis 3**



and separation. With the use of the experimental procedures from the Medicinal Chemistry protocol, the optimization of TLC condition succeeded very fast. After obtaining the best TLC scanning conditions the reaction was screened on the SOPHAS robot. In a single screening run we varied four different parameters: temperature, base, solvent, and additive.

Two reaction blocks (each with 24 glass reaction vials of 4-mL volume) were filled with 1.0 equiv of phenol **1** (about 300 mg) and 1.1 equiv of bromoethylester **2**. After adding different solvents (acetone, toluene, acetonitrile, DMF, butanone, ethanol), bases (Cs_2CO_3, K_2CO_3, KO^{t}Bu, NaOEt all added as solids), and additives (different iodides to promote in situ Finkelstein reaction) one reactor block was shaken at ambient temperature and another one at 55 °C. Our reaction array was a selection of 48, taking solubilty of substrate and reagents into account. Aliquots of the reaction vials were taken after 2, 6, and 16 h, quenched, diluted, and delivered to our automated TLC-spotting system LISSY via the shuttle system. Preparation of the TLC plates with the optimal mobile-phase system and subsequent analysis of the plates in the TLC-scanning apparatus showed directly qualitatively and semi-quantitatively the reaction outcomes.

Figure 7 shows two different reaction conditions of the screening. It is fairly easy to notice that condition B is superior to condition A. Best conditions for this transformation in this first screening were 1.0 equiv of phenol, 1.1 equiv of K_2CO_3, 0.1 equiv of NaI in acetone. Relative area integration of the sample TLC revealed a conversion of more than 87%. The experimental results were transferred back to the laboratory and were reproduced sucessfully on a 10-g scale (85% isolated yield) using classical mechanical stirring for optimal agitation. The whole reaction screening and analysis sequence (48 reactions, 154 TLC lanes) took only 2 days plus 1 day for reaction preparation and planning processes.

(9) Salo, P. K.; Pertovaara, A. M.; Salo, V.-M. A.; Salomies, H. E. M.; Kostiainen, R. K. *J. Comb. Chem.* **2003**, *5*, 223−232.
(10) Lavastre, O.; Touzani, R.; Garbacia, S. *Adv. Synth. Catal.* **2003**, *345*, 974−977.
(11) See http://www.camag.com.
(12) For related compounds, see: Ezquerra, J.; Lamas, C. *Tetrahedron* **1997**, *37*, 12755−12764.

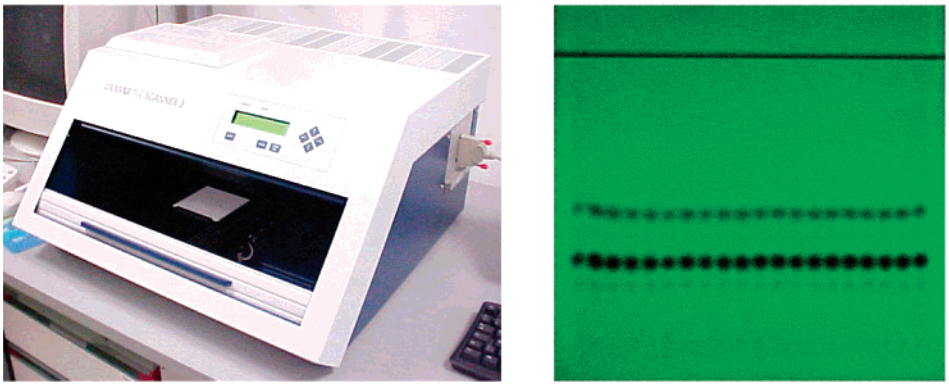


Figure 6. **CAMAG TLC-Scanner 3 and a typical HPTLC plate (20 spots per 10 cm × 10 cm developed plate, spotted by LISSY).**

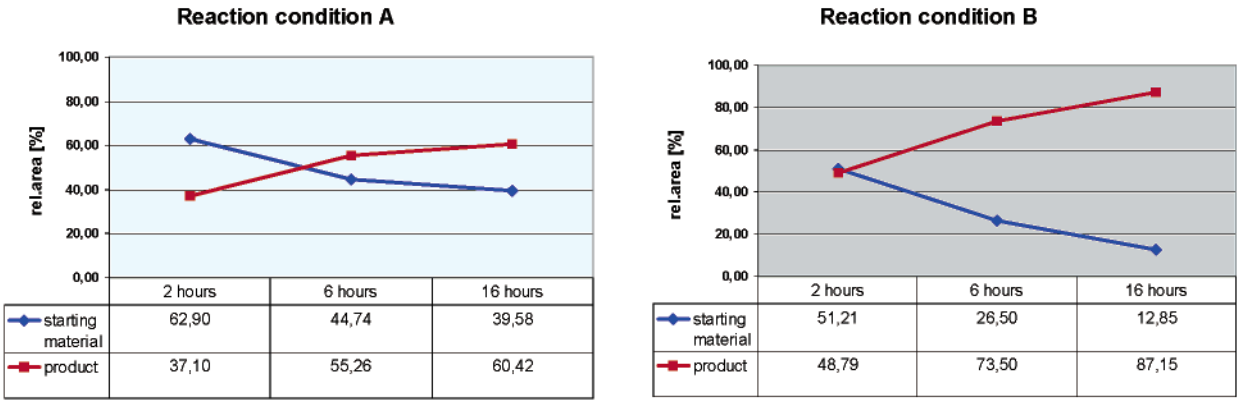
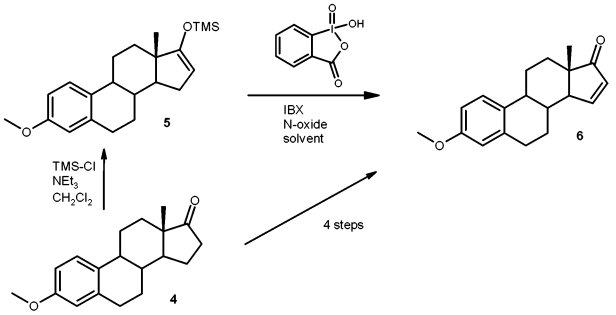


Figure 7. **Time-conversion plots of two representative reaction conditions for the aryl ether synthesis 3.**

Scheme 2. **Reaction Screening for Optimization of Δ15-Estrone Methyl Ether-15-ene 6**



Another detailed analysis of the TLC-scanning methodology was performed on the oxidation of estrone methyl ether **4** to Δ15-estrone methyl ether **6** (Scheme 2). In this case emphasis was put on the comparison of the analytical TLC results with classical HPLC analysis. The classical preparation of enone **6** involved four chemical steps including ketalization, bromination, dehydrobromination, and deketalization chemistry. Due to this lengthy synthesis we were attracted by a recent report from K. C. Nicolaou et al. who had developed a mild and selective method for this type of transformation.[13]

Oxidation of silyl enol ether **5** by using IBX·MPO[14] in a fast proof-of-concept experiment yielded the desired enone **6** in low yield. We set up a broad reaction screening by varying solvents (DMSO, dichloromethane), temperatures (room temperature, 40 and 70 °C), type and equivalents of *N*-oxide (MPO, NMO, and TEMPO), type of base (seven organic bases, three different buffered waters, pH 6.5−7.5, inorganic salts such as $NaHCO_3$, $NaCO_3$), and amount of additional base. The amount of IBX was dispensed with the CALLI robot, and the reproducibility was very good (nearly all samples within a range of ±2 mg). More than 100 single experiments in five robotic runs were performed on SOPHAS, taking just a fraction of the full array into account. Due to the number of experiments and analytical samples (four samples per reaction) we used the SOPHAS robotic system combined with the TLC scanner for fast reaction analysis. Additionally, we measured all samples again by traditional HPLC analysis and compared the obained results of both analytical methods.[15] Figure 8 shows the two independent analytics (TLC and HPLC) for the same reaction conditions. The correlation is very good.

Good conditions for this reaction were found: 1.0 equiv of silyl enol ether **5**, 1.5 equiv of IBX, and 1.5 equiv of MPO 1.0 equiv of Hünigs base in a solvent mixture of methylene chloride and DMSO at room temperature gave about 80% conversion. Methylene chloride proved to be necessary for solubility of the starting material, thus increas-

(13) Nicolaou, K. C.; Gray, D. L. F.; Montagnon, T.; Harrison, S. T. *Angew. Chem.* **2002**, *114*, 1038−1042; *Angew. Chem., Int. Ed.* **2002**, *41*, 996−1000.

(14) IBX = *o*-iodoxybenzoic acid; MPO = *p*-methoxy pyridine *N*-oxide (hydrate); A 1:1 complex is formed in situ.

(15) For correct monitoring of the reaction products by TLC due to different scanning wavelengths between TLC and HPLC the absolute area values had to be corrected. The silyl enol ether **5** hydrolyzes on silica plates, thus yielding the ketone **4**. For easier comparison of TLC and HPLC results we took the sum of these two compounds, due to the very similar responding factors in HPLC.

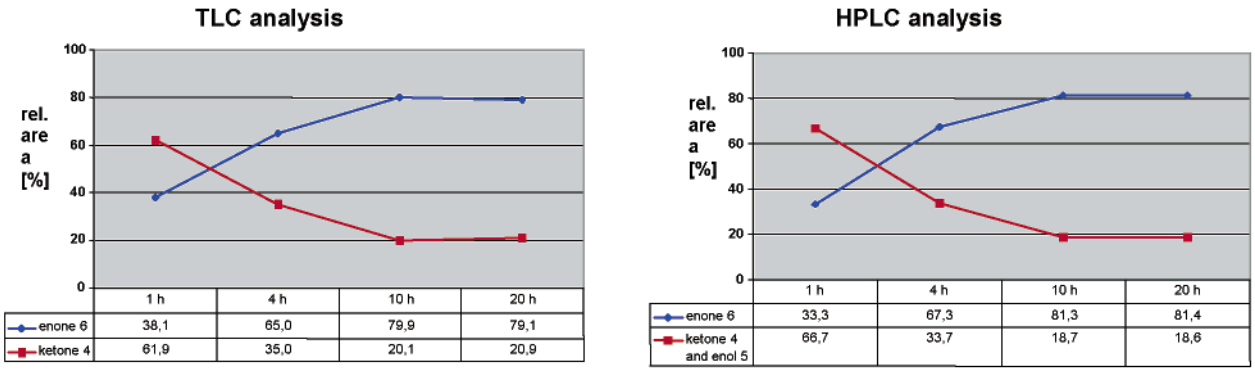


Figure 8. **Correlation of TLC and HPLC chromatograms of the same reaction sample.**[15]

ing yield and decreasing reaction temperature. Overall, the TLC scanner results show the same trends of each reaction as does the corresponding HPLC analysis. The major advantage of TLC is the enormous reduction of time for the analytical data collection. In this study we analyzed more than 420 reaction samples via TLC and HPLC. TLC was about 15−20-fold faster than HPLC (TLC: 8 h versus HPLC: 140 h) giving the same qualitative and semi-quantitative information. Additionally, the consumption of pure reagent grade solvents for the analytics was dramatically reduced, resulting in cost savings and a lower amount of waste solvent.

Currently, the implementation of an automated enzyme screening methodology and the integration of catalyst libraries for transition metal catalysis are ongoing.[16]

Conclusions

A new and highly efficient robotic system for high-throughput reaction screening was established. Automated TLC preparation in combination with a modern TLC scanner was developed as a powerful tool for high-throughput semi-quantitative determination of reaction outcome. Several automated chemistry examples clearly demonstrate the high efficiency of this combined system and its superiority compared to more classical approaches, e.g., HPLC analyses. The new high-throughput TLC method is thus far only limited by the accuracy of the spotting of small sample amounts to the TLC plate and by the detection limit of the scanner. Further miniaturization holds a great potential for even higher sample throughput with this analytical method.

Experimental Section

Solvents and reagents were used as received from commercial and internal suppliers. HPLC was performed on a DIONEX system (P580 pump, Gina 50 Autosampler, and HP1100 UV detector). All analytical TLC plates were scanned on a CAMAG TLC Scanner 3.

General Procedure for Alkylation of Alkoxy-benzimidazole Derivative 3. Each vial of a 24-vial reflux reaction block was filled with 1.0 equiv of starting material and 1.1 equiv of solid base (Cs_2CO_3, K_2CO_3, KO^{t}Bu, NaOEt) on the SOPHAS robot. Additionally, 0.1 equiv of NaI was added to reaction vials 13−24. Then, 700 μL of solvent (DMF, acetonitrile, acetone, butanone, ethanol) and 1.1 equiv of bromethylester (neat) were added, using the four stainless steel needles. The reaction block was shaken with a speed of 650 rpm at room temperature or at 55 °C. Samples were taken out of the reaction mixture after 2, 8, and 20 h and quenched with ethyl acetate (containing 1% of AcOH). Finally, the samples were diluted to a concentration of 1 mg/mL with the LISSY robot, and 2 μL were spotted on glas TLC plates (Merck HPTLC plates 10 cm × 10 cm, silica gel 60 F_{254}). The development of the plates was performed with a mixture of hexane/ethanol (80:20 vol/vol), and the plates were scanned with the TLC Scanner at a wavelength of 450 nm (tungsten lamp) (see Table 1).

Table 1. **Some representative reaction conversions of the alkylation (8 of 48) after 20 h**

reaction	solvent	base	NaI additive	T [°C]	conversion [%]
1	DMF	Cs_2CO_3	−	20	51
2	DMF	Cs_2CO_3	+	20	61
3	DMF	K_2CO_3	−	55	75
4	DMF	K_2CO_3	+	55	83
5	acetonitrile	Cs_2CO_3	−	55	77
6	acetonitrile	Cs_2CO_3	+	55	84
7	acetone	K_2CO_3	−	55	73
8	acetone	K_2CO_3	+	55	87

General Procedure for Oxidation of Estrone Methyl Ether 4. Each vial of a 24-vial reflux reaction block was filled with 1.5 equiv of solid IBX with the CALLI robot. Then, the addition of the 1.5 equiv of *N*-oxide (MPO, NMO, and TEMPO) and 1.0 equiv of silyl enol ether **5** was performed manually. One milliliter of DMSO (or DMSO/dichloromethane as 5:2 mixture) was added with the four stainless steel needles an the reaction was shaken at 75 °C or room temperature. Reaction samples were taken after 1, 4, 10, and 20 h, hydrolyzed with saturated $NaHCO_3$ solution, and extracted with dichloromethane. A sample of the organic layer was diluted with acetonitrile to a concentration of 0.5 mg/mL for HPLC (column: Kromasil C8, 5 μm; mobile phase: acetonitrile/water, gradient: 60−100% acetonitrile in 15 min; flow rate: 1 mL/min; UV detector 220 nm; retention time of **5** = 12.70 min, of **4** = 3.23 min) and analytical TLC. One microliter of the samples was spotted on glass TLC plates (Merck HPTLC plates 10 cm × 10 cm, silica gel 60 F_{254}) with the LISSY robot. The development of the plates was performed with a mixture of hexane/ethanol

(16) A recent publication on automated enzyme-screening methodology: Yazbeck, D. R.; Tao, J.; Martinez, C. A.; Kline, B. J.; Hu, S. *Adv. Synth. Catal.* **2003**, *345*, 524−532.

Table 2. **Some represenative reaction conversions (8 of 120) of the IBX oxidation after 20 hours**

reaction	solvent	*N*-oxide [1.5 equiv]	base additive [1.5 equiv] (more than 10 screened)	*T* [°C]	conversion HPLC [%]	conversion TLC [%]
1	DMSO	-	-	75	42	43
2	DMSO	MPO	-	75	73	72
3	DMSO	NMO	-	75	50	49
4	DMSO	TEMPO	-	75	54	54
5	DMSO	MPO	NEt_3	75	81	80
6	DMSO/CH_2Cl_2	MPO	NEt_3	20	69	69
7	DMSO/ CH_2Cl_2	MPO	Hunigs base	20	71	70
8	DMSO/ CH_2Cl_2	MPO	Hunigs base	20	82	81

(80/20 vol/vol), and the plates were scanned with the TLC scanner at a wavelength of 200 nm (deuterium lamp) (see Table 2). See also reference 15.

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