High-Throughput Experimentation Platform: Parallel Microwave Chemistry in HPLC/GC Vials

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A high-throughput reaction platform for performing parallel microwave chemistry in sealed HPLC/GC vials is described. The system consists of a strongly microwave-absorbing silicon carbide plate with 20 cylindrical wells of appropriate dimensions to be fitted with standard HPLC/GC autosampler vials serving as reaction vessels. In combination with an aluminum sealing plate the setup can be used for microwave processing reaction volumes from 0.5−1.5 mL at a maximum temperature/pressure limit of 250 °C/20 bar. The parallel reaction platform displays excellent temperature and reaction homogeneity and has been used for high-throughput reaction optimization studies involving the parallel screening of catalyst, solvent and substrate reactivity for esterification reactions and metal-catalyzed dehydrative C−C couplings.

Introduction

High-speed synthesis using microwave heating technology has attracted a considerable amount of attention in recent years. More than 4000 articles have been published in the area of microwave-assisted organic synthesis (MAOS) since the first reports on the use of microwave heating to accelerate organic chemical transformations were published in 1986.[1,2] The efficiency of "microwave flash heating" in dramatically reducing reaction times and increasing product yields/purities is one of the key advantages of this enabling technology. Most of the published applications today involve the use of sealed vessel technology in combination with dedicated single-mode microwave reactors.[1,2] Here the advantages of rapid and direct volumetric microwave heating are combined with the capability to superheat solvents far above their boiling points in a sealed vessel. This method allows significantly higher reaction temperatures to be reached than using conventional reflux conditions, and therefore often results in considerable rate enhancements when compared to experiments carried out at the boiling point of the solvent. These advantages have not only been exploited for classical organic synthesis,[1,2] but also in the context of library synthesis in medicinal chemistry/drug discovery projects where speed and efficiency are often critical factors.[3]

Apart from the use of microwave technology for the actual synthesis of a single compound or a compound library,[1−3] high-speed microwave processing has also been proven extremely valuable for reaction optimization because many of the key parameters in a chemical transformation such as reaction temperature and time, variations in solvents, additives, and catalysts, or the molar ratios/concentrations of the substrates can be evaluated in a comparatively short time frame.[1−4] To increase throughput and efficiency, such reaction optimization studies are generally performed on a small scale in single-mode microwave reactors with incorporated robotic vial handling capabilities.[5] Here, the specialized Pyrex microwave reaction vials provided by the instrument manufacturers are inserted in an automated sequential fashion into the microwave reactor. After processing, the vials are removed from the cavity, and the degree of conversion in each of the vials is monitored by standard analytical methods such as LC/MS, HPLC/UV, or GC/MS. This generally requires the transfer of aliquots of the crude reaction mixture from each of the processed microwave reaction vessels into appropriate HPLC/GC autosampler vials. This manual transfer step not only bears the risk of material loss by human error and contamination, but also requires a considerable amount of time and effort. In addition, the above-mentioned automated sequential processing strategy becomes impractical when a large number of optimization experiments need to be performed, as for example in the context of a statistical "Design of Experiment" (DoE) campaign.[6] In those instances, the timesaving aspect associated with microwave chemistry may be compromised by having to irradiate each reaction vial individually, and the utilization of a parallel microwave processing technique will clearly be advantageous.[7]

Herein, we describe a high-throughput experimentation platform for performing parallel microwave chemistry that enables the use of standard HPLC/GC autosampler vials as reaction vessels. The platform consists of a block of silicon carbide (SiC) with a 5 × 4 deep well matrix in which the HPLC/GC vials are placed. The sealing mechanism of the platform allows microwave processing at temperatures up to 250 °C and pressures of up to 20 bar.

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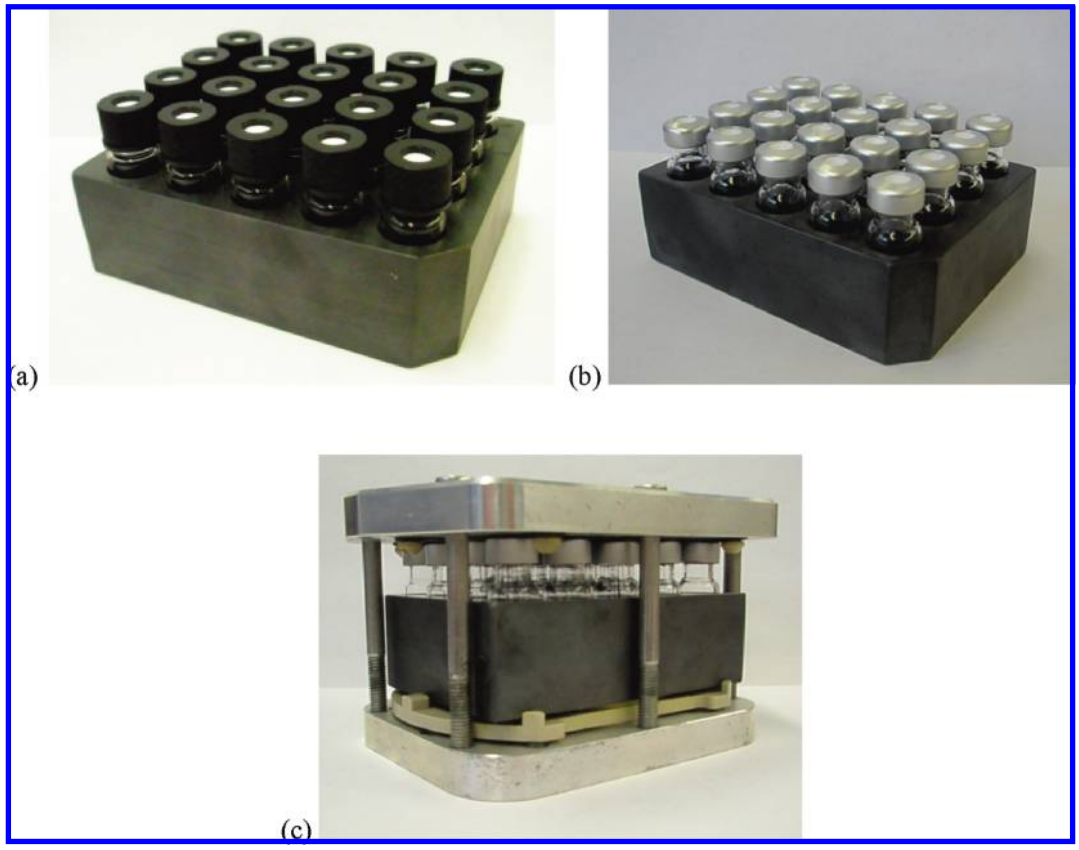


Figure 1. High-throughput experimentation rotors (82 × 62.5 × 25 mm) for parallel microwave chemistry made out of sintered SiC fitted with HPLC/GC vials. (a) Experimental setup for utilizing vials with screw threads and the corresponding screw caps made of polypropylene fitted with PTFE-coated silicone septa for reaction temperatures of ~150 °C (max pressure ~8 bar). (b) Experimental setup for utilizing vials with aluminum crimp tops with PTFE-coated silicone septa for reaction temperatures of ~250 °C (max pressure ~8 bar). (c) Experimental setup for utilizing vials with aluminum crimp tops with PTFE-coated silicone septa for achieving reaction temperatures of 250 °C (max pressure 20 bar). The sealed rotor setup consisting of the SiC plate on an aluminum base (10 mm) with PEEK spacer, covered with an aluminum top plate (10 mm) and fixed with six stainless steel hex bolts for use in a multimode microwave reactor (Synthos 3000, Anton Paar GmbH). For an image of the setup inside the microwave reactor, see Figure S1 in the Supporting Information.

The use of this setup for high-speed parallel reaction optimization is reported.

Results and Discussion

Silicon Carbide Plate/Rotor Development. In 2007, we reported the development of a microtiter plate made out of sintered silicon carbide (SiC) for performing sealed vessel parallel library synthesis in a multimode microwave reactor (200 °C, 20 bar).[8] In this prototype, reactions were performed directly inside the wells of the 6 × 8 matrix with a maximum working volume of 300 μL. Sintered SiC is chemically completely inert, strongly microwave absorbing, and because of its high melting point (~2700 °C) and very low thermal expansion coefficient, can be employed at extremely high temperatures.[9] By using microwave absorbing SiC as plate material, the microwave absorption characteristics of the individual reaction mixtures contained in the wells are practically irrelevant because the semiconducting plate itself will absorb microwave energy much stronger than any organic material contained inside the wells. Importantly, because of the large heat capacity and high thermal conductivity of SiC no temperature gradients across the microtiter plate exist.[8] This material has therefore proven ideal for developing accessories for parallel microwave chemistry applications. In a recent publication, a SiC rotor system for the parallel synthesis of compound libraries employing 5 mL glass reaction vials inside a 6 × 4 matrix has been described.[10]

Our current prototype designed for high-throughput reaction optimization in standard HPLC/GC vials consists of a 82 × 62.5 × 25 mm SiC plate made by sintering a corresponding green compact of SiC. The upper surface of the plate contains a 5 × 4 matrix of 20 cylindrical wells of appropriate dimensions (12 mm diameter and 20 mm depth) to be fitted with standard HPLC/GC autosampler vials. For different applications and temperature/pressure limits (see below) the use of two vial types has been evaluated. Vials with screw threads and the corresponding screw caps made of polypropylene are suitable for reaction temperatures below 150 °C and pressures of ~8 bar (Figure 1a). In contrast, aluminum crimp top vials can be used at significantly higher temperatures (250 °C) because the metal caps do not melt but are also limited to a maximum pressure of ~8 bar (Figure 1b). However, using the aluminum crimp top vials a temperature/pressure limit of 250 °C and 20 bar can be achieved by placing the SiC plate fitted with the HPLC/GC vials between two aluminum plates, fixed with 6 stainless steel hex bolts to increase pressure resistance (Figure 1c).[8] By using vials with polypropylene screw caps a limit of 150 °C/20 bar can be obtained under these conditions.

Table 1. Evaluation of Different Solvents at a Calculated Maximum ~20 bar Autogenic Pressure in the HPLC/GC Vial Microwave Platform (Figure 1c)[a]

solvent/bp (°C)	tan δ [b]	temperature (°C)	pressure (bar)
DCM/40	0.042	150	20
acetone/56	0.054	180	20
chloroform/61	0.091	190	20
MeOH/65	0.659	170	20
THF/66	0.047	190	20
MeCN/81	0.062	220	20
water/100	0.123	210	20
$MeNO_2$/101	0.064	230	20
toluene/110	0.040	250	17
NMP/204	0.275	250	3

[a] Irradiation experiments were performed in a Synthos 3000 multimode microwave unit with IR temperature control (surface temperature of SiC plate) for 10 min. [b] See ref 11 for details.

To ensure pressure tightness and stability at elevated temperatures using the setup shown in Figure 1c, three different septa were tested under a variety of experimental conditions (see Figures S2 and S3 in the Supporting Information). Conventional rubber septa showed deformations when exposed to temperatures >200 °C, in particular in combination with solvents such as chloroform or toluene. In contrast, PTFE septa showed resistance to both solvents and high temperature but could not be penetrated smoothly by standard HPLC/GC autosampler needles and were therefore not further considered. Best results were ultimately achieved with PTFE coated silicone septa (Macherey-Nagel, sealing disks N 11 Silicone/PTFE, thickness 0.15 mm), which were therefore used for all subsequent experiments described in this work. To confirm a safe operation limit of 250 °C or 20 bar for the HPLC/GC vial reaction platform a variety of solvents were exposed to the appropriate temperature corresponding to a calculated autogenic solvent pressure of ~20 bar for an extended time period (Table 1). For all cases the results were satisfactory, in the sense that no loss/evaporation of solvent or deformation of the septa occurred. Importantly, under the conditions shown in Table 1, no destruction/vessel failure of the HPLC/GC glass vials was experienced.

For the performance of parallel microwave chemistry in the set-ups shown in Figure 1a or 1b, the plate system is mounted on a dedicated turntable inside a multimode microwave cavity.[8,10] Up to four SiC plates ($4 \times 20 = 80$ reaction vials) can be mounted on the currently available turntable (Figure S1 in the Supporting Information). The reaction temperature is monitored and controlled by using the feedback from an IR temperature sensor integrated into the bottom of the cavity that records the bottom surface temperature of the silicon carbide plate. Similar to the situation with our original SiC plate prototype,[8] control experiments using fiber-optic probes inserted directly into the HPLC/GC vials indicated that the actual reaction temperature inside the HPLC/GC vials is consistently ~15% higher than the measured surface temperature of the SiC block (Figures S4 and S5 in the Supporting Information). This discrepancy is likely to be the consequence of inadvertent cooling of the SiC surface by the comparatively cold air inside the microwave cavity. In those instances where an accurate absolute reaction temperature is required, the use of a calibration factor is therefore suggested.[8] In the current manuscript, all stated reaction temperatures refer to the monitored SiC block surface temperature.

Temperature and Reaction Homogeneity. As already demonstrated in previous studies using IR thermography, irradiation of SiC plates in multimode microwave cavities leads to a very homogeneous heating of the entire plate, with minimal deviations in temperature recorded at different positions of the plate.[8,10] In a new series of experiments, we were interested in determining if the temperature homogeneity observed for heating the "naked" SiC plate would also translate to reaction homogeneity for a chemical transformation performed directly in the HPLC/GC vials in the SiC plate. For this purpose, we have chosen the BF_3-mediated esterification of benzoic acid (**1a**) with methanol as model reaction.[12] As anticipated, when the esterification reaction was performed in the HPLC/GC vial platform (Figure 1c, 1.0 mL reaction volume) at a measured SiC block surface temperature of 150 °C for 10 min, the conversions in all 20 vials were virtually identical (37.0−37.8%, average conversion 37.4%, SD = 0.25; see Figure S6 in the Supporting Information) using 3.0 equiv of BF_3. Because of the temperature sensitivity of this reaction any significant temperature variations between individual HPLC/GC vials in the SiC plate would have led to differences in conversions.[8]

In an additional set of experiments, we have demonstrated that not all the vial positions in the 5×4 SiC plate matrix need to be filled and that the presence of "empty" neighboring wells has no influence on the outcome of the reaction (Figure S7, Supporting Information). It is therefore apparent that the SiC reaction platform can be utilized to perform synthetic transformations using solvents with vastly different microwave absorption characteristics (tan δ values, see Table 1) in parallel.[11] Quite unlike conventional parallel microwave synthesis using multivessel rotors or microtiter plates,[7] the contents of the HPLC/GC vials inside the SiC plate have no influence on the final reaction temperature and even weakly absorbing reaction mixtures can easily be heated to the desired temperatures.[8,10] Furthermore, these experiments have also shown that the position of the HPLC/GC vials in the plate is irrelevant for the observed conversion (Figure S7, Supporting Information). However, we have noticed that the filling volume can in some cases influence the progress of a reaction. For the BF_3-mediated esterification reaction at 150 °C using methanol as solvent, for example, reaction vials with a filling volume of 0.3 mL did show slightly lower conversions compared to vials filled with 1.0 or 1.5 mL reaction mixture (Figure S7, Supporting Information). A likely explanation for this observation is that using a low filling volume will result in a comparatively large head space in the reaction vial which in turn may cause a significant amount of the volatile reaction mixture being in the gas phase at high temperatures where almost no reaction occurs. It should be emphasized that the aluminum top plate in the setup shown in Figure 1c will shield the contents of the vials from the microwave field, since most of the microwave irradiation will be reflected by the aluminum.[8] However, when experiments are performed without the aluminum plate (Figure 1a, b), the microwave field may contribute to some

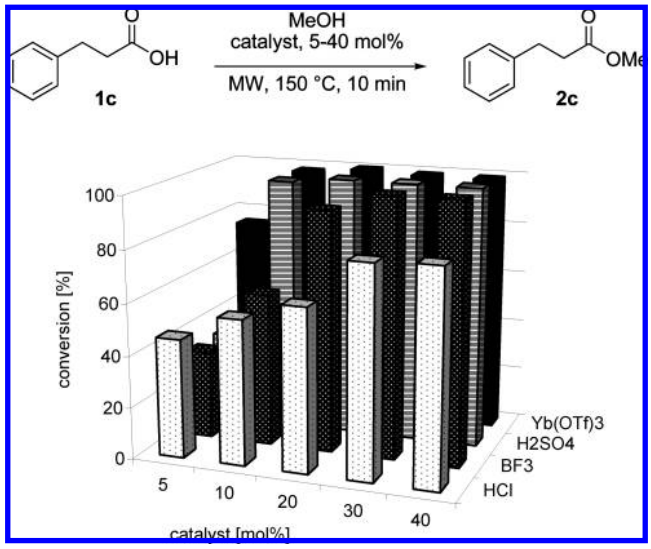


Figure 2. HPLC-UV conversions (215 nm, peak area %) for the microwave-assisted (150 °C, 10 min) esterification of 3-phenylpropionic acid (**1c**) with methanol catalyzed by different acids in varying concentration using the 5 × 4 SiC parallel reaction platform (Figure 1c). For BF_3 the concentration values need to be multiplied by 10 (i.e., 50−400 mol%).

extent on the heating of the reaction mixtures in the reaction vials (dielectric heating). The recommended optimum filling volume for the use of HPLC/GC vials in the SiC platform therefore lies in the range of 0.5−1.5 mL.

Application Toward Reaction Optimization and Screening. The validation studies described above have confirmed the heating homogeneity across the SiC plate. At the same time it was demonstrated that the HPLC/GC vial platform shown in Figure 1c could be used at similar temperature and pressure limits as the currently available single-mode microwave reactors (250 °C and 20 bar) employing a wide range of solvents. Consequently, we were interested to evaluate the performance of this high-throughput experimentation platform for a variety of reaction optimization and screening studies. The transformations described below were performed in parallel directly in the HPLC/GC autosampler vials (∼1 mL reaction volume) in concentrations (5−10 mM) suitable for subsequent direct injection of a 2 μL fraction into a HPLC-UV or 1 μL fraction into a GC-MS instrument for reaction monitoring. For this purpose, the vials were transferred manually from the SiC plate into the appropriate HPLC or GC autosampler racks, although future versions of the platform may employ SiC plates of suitable geometry that can be directly fitted into an HPLC, LC or GC autosampler system.

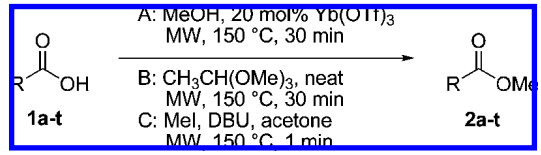
Catalyst Screening. As a first example, we investigated the esterification of 3-phenylpropionic acid (**1c**) with methanol using both Bronsted (H_2SO_4, HCl) and Lewis acids [BF_3, $Yb(OTf)_3$] in different concentrations (Figure 2).[13] 3-Phenylpropionic acid was chosen as a representative of an aliphatic carboxylic acid having an aryl chromophor that allows easy monitoring of conversion by standard HPLC-UV (215 nm) measurements. Preliminary experiments using single-mode microwave instrumentation demonstrated that a temperature range of 120−150 °C and a time frame of 10 min were suitable for these esterification processes. The effect of catalyst type and concentration on the esterification process at 150 °C (10 min) was established, utilizing the 5 × 4 SiC plate matrix, in one single irradiation experiment in a time frame of ∼25 min (including the ramp times for heating and cooling, see Figure S4, Supporting Information). In comparison, the performance of all 20 esterifications by automated sequential microwave processing would require an overall processing time of at least 5 h (20 × 15 min) again including the heating/cooling times and the time required for automated vessel transfer. As can be seen in Figure 2, quantitative conversion after 10 min was achieved with $Yb(OTf)_3$ (20 mol %), H_2SO_4 (30 mol %), and BF_3 (3.0 equiv).

Substrate Screening. In an effort to extend the esterification method shown above also to aromatic carboxylic acids, we have subsequently performed the same catalyst screen with benzoic acid (**1a**) and 2,4,6-trimethylbenzoic acid (**1d**), the latter substrate being an example of a sterically encumbered carboxylic acid. Because of the expected lower reactivity of aromatic carboxylic acids compared to their aliphatic analogs the reaction time was extended from 10 to 30 min. For benzoic acid (**1a**), satisfactory results were obtained with $Yb(OTf)_3$ and H_2SO_4 where virtually complete conversion to the corresponding methyl esters could be achieved using 20 and 40 mol% of catalyst, respectively (Figure S8a, Supporting Information). In the case of the sterically hindered 2,4,6-trimethylbenzoic acid (**1d**), none of the catalysts used provided any significant level of esterification (Figure S8b, Supporting Information).

With the aim of performing a substrate screening campaign, we then applied the optimum esterification conditions identified for benzoic acid [20 mol% $Yb(OTf)_3$, 150 °C, 30 min] to a diverse set of 20 aliphatic, aromatic, and heteroaromatic carboxylic acids **1a**−**t**. As shown in Table 2 (method A), for the majority of the selected acids (13) a high degree of esterification (>95%) was achieved using the previously optimized conditions. As expected, sterically hindered acids such as **1d**−**f** gave very low conversions (<2%), whereas in four cases (**1i**, **1k**, **1r**, **1t**), moderate levels of esterification (40−80%) were obtained. To acquire the same information on substrate reactivity using a sequential microwave method, an instrument processing time of ∼12 h (20 × 35 min) would be required.

Method Screening. Since the conversions for the esterification **1** → **2** using the $Yb(OTf)_3$/MeOH system (method A) were not complete in several cases, in particular for sterically demanding substrates, alternative esterification methods were explored. Preliminary studies with 2,4,6-trimethylbenzoic acid (**1d**) applying a single-mode microwave reactor indicated that complete esterification of this sterically hindered acid could be obtained within 30 min at 150 °C using trimethyl orthoacetate (TMOA) under solvent- and catalyst-free conditions (Figure S10, Supporting Information).[14] Gratifyingly, application of this protocol to the diverse set of 20 carboxylic acids **1a**−**t** led to virtually complete esterifications in all cases (Table 2, method B). Only in a few instances, trace amounts of the corresponding carboxylic acids could be detected in the crude reaction mixture by HPLC-UV monitoring. An even more efficient

Table 2. Conversions for the Microwave-Assisted Esterification of Carboxylic Acids **1a**−**t** Using the 5 × 4 SiC Parallel Reaction Platform (Figure 1c)



carboxylic acid **1**[a]	method A conversion[b] (%)	method B conversion[b] (%)	method C conversion[b] (%)	measured M^+ of esters **2**[c]
benzoic acid (**1a**)	95	>99	>99	136
phenylacetic acid (**1b**)	98	>99	>99	150
3-phenylpropionic acid (**1c**)	98	>99	99	164
2,4,6-trimethylbenzoic acid (**1d**)	2	99	>99	178
2,4,6-trichlorobenzoic acid (**1e**)	1	99	98	238
2,6-dimethylbenzoic acid (**1f**)	1	99	>99	164
3,4-dichlorobenzoic acid (**1g**)	96	>99	>99	204
3,4-dimethoxybenzoic acid (**1h**)	99	>99	>99	196
2-hydroxybenzoic acid (**1i**)	42	>99	>99	152
4-cyanobenzoic acid (**1j**)	96	99	>99	161
2-bromobenzoic acid (**1k**)	81	99	>99	214
4-bromobenzoic acid (**1l**)	95	99	>99	214
4-bromophenylacetic acid (**1m**)	99	99	>99	228
4-nitrobenzoic acid (**1n**)	98	98	99	181
4-nitrophenylacetic acid (**1o**)	98	99	>99	195
2-chloro-4-nitrobenzoic acid (**1p**)	97	99	98	215
napthalene-2-carboxylic acid (**1q**)	96	99	99	186
benzene-1,4-dicarboxylic acid (**1r**)	77	99	>99	180
pyridine-2-carboxylic acid (**1s**)	95	>99	>99	137
thiophene-2-carboxylic (**1t**)	74	96	>99	142

[a] For a graphical representation of esters and conversions (method A), see Figure S9 in the Supporting Information. [b] Conversion determined by HPLC/UV at 215 nm (peak area %). [c] The identity of the corresponding methyl esters **2a**−**t** was established by GC-MS (EI) measurements and comparison of MS fragmentation patterns with the NIST library.

method for preparing esters **2** from the corresponding acids **1** was found to be the base-catalyzed alkylation with methyl iodide (~5 equiv) using acetone as solvent.[15] Typically, potassium carbonate is used as a base for this transformation but since homogeneous reaction conditions for direct injection of the crude reaction mixture into an HPLC system were required, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) was employed with equal efficiency. Full conversion was obtained within 1 min at 150 °C in a single-mode cavity and translation to the SiC parallel platform similarly resulted in complete esterification for all examples (Table 2, method C).[16]

Solvent Screening. Finally, we wanted to investigate the possibility to screen different solvents for the same chemical transformation in parallel using a single SiC plate. As a synthetically interesting model reaction we have chosen the metal-catalyzed direct C−C bond formation between alcohols and active methylene compounds. Recent work by Baba and co-workers has demonstrated that dehydrative C−C coupling of allylic/benzylic alcohols with active methylenes can be achieved at elevated temperatures (80 °C) using $InCl_3$ as a catalyst.[17] In 2008, the same group has published an extensive reoptimization of this coupling protocol using single-mode microwave technology.[18] A number of transition metal catalysts [$Sc(OTf)_3$, $Cu(OTf)_2$, $FeCl_3$] and solvents (toluene, MeCN, 1,2-dichloroethane, chlorobenzene) were identified that provided high yields of the desired coupling products.[18] To fine-tune the solvent/catalyst selection and to rapidly identify an optimum solvent/catalyst combination we have reoptimized the coupling reaction between diphenylmethanol (**3**) and dibenzoylmethane (**4**) using the HPLC/GC reaction vial platform (Figure 3).[17,18] In agreement with the results reported by Baba and co-workers,[18] preliminary experiments in a single-mode microwave instrument confirmed that at 110 °C full conversion to product **5** could be achieved within 10 min, using, for example, 3 mol % of $Sc(OTf)_3$ as a catalyst in combination with toluene as solvent. Moving to the 5 × 4 SiC platform, we have initially screened five solvents (DCM, $MeNO_2$, MeCN, THF, and toluene) against four metal catalysts [$Sc(OTf_3)$, $Cu(OTf)_2$, $Zn(OTf)_2$, $FeCl_3$] employing a 3 mol % catalyst concentration at 110 °C (10 min). The crude product purities are shown in Figure 3a. Despite the fact that good conversions were achieved in nearly all cases, high product purities were mainly obtained for coupling experiments using DCM or $MeNO_2$ as solvent. In runs that employed MeCN, THF or toluene the formation of undesired byproducts, mainly resulting from the self-condensation of diphenylmethanol (**3**) to the corresponding bis(diphenylmethyl) ether, were observed in the HPLC-UV traces.[17] In a follow-up plate experiment, the best solvent/catalyst combinations were rerun at lower catalyst concentrations (Figure 3b). However, significant reductions in purities/conversions were typically experienced using a lower than 3 mol % catalyst loading. The optimum conditions derived from the two plate experiments shown in Figure 3a and b, therefore, utilized DCM as solvent, 3 mol% of $FeCl_3$ as catalyst, and 110 °C reaction temperature for 10 min. A final plate run involving only five HPLC/GC vials validated that the optimum coupling conditions identified for the small-scale/high-dilution experiments described above (0.01 mmol substrates per vial) could be translated to a preparatively useful C−C coupling (0.15 mmol **3**/**4** per vial) allowing the synthesis of C-alkylated dibenzoylmethane **5** in 96% isolated yield on a ~60 mg scale (Figure 3c).

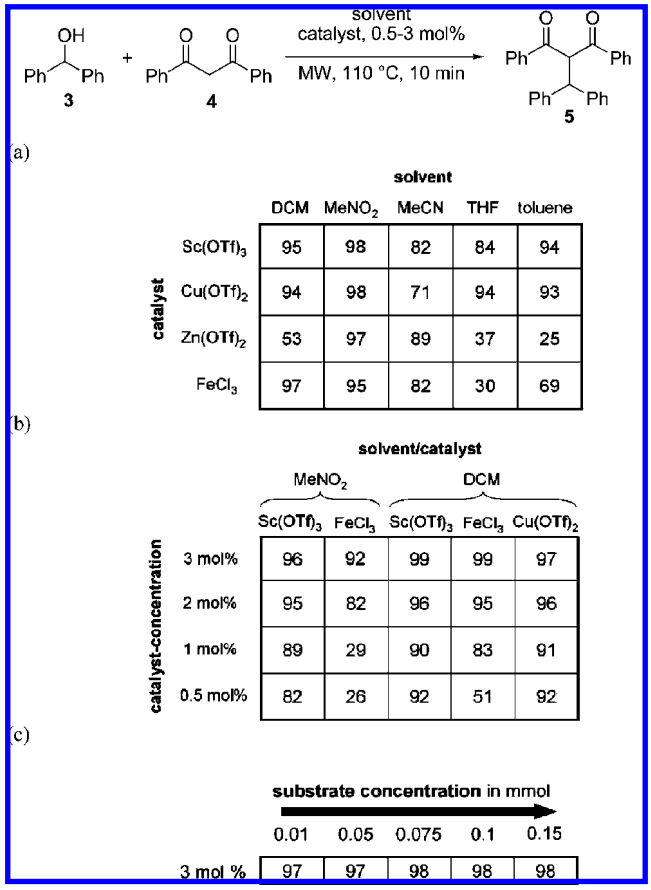


Figure 3. HPLC-UV purities (215 nm, peak area %) for a solvent/catalyst screen in the C−C coupling of diphenylmethanol (**3**) with dibenzoylmethane (**4**) using the 5 × 4 SiC parallel reaction platform (Figure 1c). (a) Reaction conditions: 3 mol% catalyst, 110 °C, 10 min. (b) Reaction conditions: 0.5−3 mol% catalyst, 110 °C, 10 min. (c) Reaction conditions: Substrate concentration **3/4** 0.01−0.15 mmol per vial, DCM, 3 mol% $FeCl_3$, 110 °C, 10 min. Given purities refer to integration (area %) of product, substrate, and side product peak.

The optimization studies described above allow the reactivity screening of solvents with different microwave absorption characteristics (tan δ, see Table 1) in a parallel microwave experiment. It should be emphasized that a similar experiment would not be feasible in conventional (polypropylene or PTFE) microtiter plates because the differences in solvent tan δ would lead to different reaction temperatures in the individual reaction wells/vials upon exposure to microwave irradiation.[7] It should also be stressed that in parallel runs using different solvents in the HLPC/GC reaction vial platform the maximum safe operational temperature is dictated by the solvent with the lowest boiling point. In the case of the experiment described in Figure 3b, the use of DCM (bp 40 °C) limits the safe use of the platform to 150 °C (see Table 1).

Application in Microwave-Assisted Derivatization Techniques. Bioanalytical applications for the identification and quantification of drugs and their metabolites in various biological matrices generally rely on hyphenated detection techniques that provide both high levels of specificity and sensitivity. Among the broad range of analytical techniques available GC/MS still is the method of choice for most screening procedures in forensic and clinical toxicology, and in doping control.[19] Despite the many advantages of the GC/MS method, time-consuming derivatization steps are often required to obtain desirable chromatographic characteristics or to improve the stability and detectability of the target analytes.[19] These derivatization processes typically require reaction times from 30 min up to several hours. As a consequence, sample derivatization often is the rate limiting step in the overall GC/MS based analysis procedure. In this context, the microwave-assisted, acid-catalyzed esterifications of carboxylic acids with methanol introduced above are of potential interest as high-speed GC-derivatization protocols.[19] In particular, the BF_3/MeOH procedure (see Figure 2) has been used extensively as esterification/derivatization method for carboxylic acids of bioanalytical interest to allow detection/quantification by GC/MS.[20] Typically, these derivatizations are performed at 60 °C for 30 min in a drying oven,[19,20] with only a small number or published reports advocating the use of microwave technology for this esterification step.[21] To evaluate the performance of the HPLC/GC reaction vial platform shown in Figure 1c for GC-derivatization processes, we have studied the esterification of selected fatty acids of bioanalytical interest using the BF_3-mediated method optimized for 3-phenylpropionic acid (**1c**) (Figure 2). Thus, 19 fatty acids **6a**−**s** (Figure 4) together with reference compound **1c** were treated with 3.0 equiv BF_3 in MeOH and heated for 10 min at 150 °C in the setup shown in Figure 1c. In all cases, GC/MS monitoring confirmed that quantitative esterification to the corresponding methyl esters had occurred.

Conclusions

In conclusion, we have demonstrated that parallel microwave chemistry can be carried out efficiently in a SiC plate fitted with standard disposable HPLC/GC autosampler vials. Because the SiC plate material itself is strongly microwave absorbing, the absorbance characteristics of the reaction mixtures contained in the vials are irrelevant.[22] As confirmed by reactivity measurements, all 20 HPLC/GC vials in the setup are heated uniformly when exposing the SiC plate to microwave irradiation in a multimode reactor. The use of aluminum crimp caps with PTFE coated silicon septa in combination with an appropriate plate sealing mechanism allows processing of reaction volumes from 0.5−1.5 mL at temperatures of ∼250 °C and pressures of up to ∼20 bar. After microwave processing, the reaction vials are transferred into the appropriate HPLC or GC autosampler racks for direct analysis via hyphenated chromatographic/spectroscopic techniques, eliminating the necessity of reaction mixture transfer from a "reaction" into an "analysis" vial. The high-throughput experimentation platform introduced herein allows a rapid optimization of reaction conditions by screening the influence of solvents, additives, catalysts, or of the molar ratios/concentrations of substrates in parallel in a single microwave irradiation experiment. Compared to conventional optimization campaigns using automated sequential microwave processing this constitutes a considerable increase in efficiency. In addition, the usefulness of the parallel reaction setup for performing high-speed GC-derivatization reactions for biomedical analysis has been demonstrated. With the

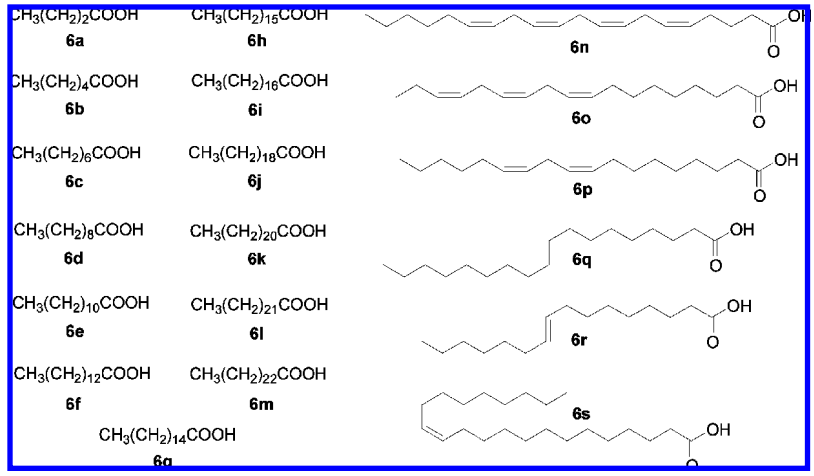


Figure 4. Fatty acids **6a**−**s** employed in the microwave-assisted derivatization with 3.0 equiv BF_3/MeOH. Reaction conditions: 150 °C, 10 min, 5 × 4 SiC parallel reaction platform (Figure 1c). The identity of the corresponding methyl esters was established by GC-MS (EI) measurements and comparison of MS fragmentation patterns with the NIST library.

possibility of performing 80 reactions in parallel in the current design, the HPLC/GC microwave reaction vial platform has obvious additional applications in combinatorial chemistry and library synthesis by either rapidly validating the reactivity of building blocks (scope and limitation studies) or by synthesizing compound libraries in parallel on small scale (10−100 mg).[23]

Experimental Section

General and Materials. All chemicals were purchased from commercial sources and used without further purification; 1.5 mL HPLC/GC crimp vials with the corresponding crimp cap tops were purchased from VWR International (Cat. No. 548-0003 and 548-0008, respectively, Vienna, Austria). Screw cap vials with the corresponding screw caps were purchased from Roth GmbH (Cat. No. E159 and E161 respectively, Karlsruhe, Germany). PTFE-coated silicon sealing disks (N 11 and N 8) were purchased from Macherey-Nagel (Cat. No. 70263 and 70248 respectively, Düren, Germany). Silicon carbide plates (Ekasic F SSiC) were custom manufactured by ESK Ceramics GmbH (Kempten, Germany).

HPLC-UV Analysis. Analytical HPLC analysis (Shimadzu LC 20 AD) was carried out on a C 18 reversed-phase analytical column (150 × 4.6 mm, particle size 5 μm) using mobile phases A (water/acetonitrile 90:10 (v/v) + 0.1% TFA) and B (acetonitrile + 0.1% TFA) at a flow rate of 0.5 mL/min. The following gradient was applied: linear increase from solution 30% B to 100% B in 9 min, hold at 100% solution B for 2 min.

GC-MS Analysis. GC-MS (FOCUS-GC - DSQ II MS, ThermoFisher) monitoring was based on electron impact ionization (70 eV) using a HP-5MS column (30 m × 0.250 mm × 0.025 μm). After 1 min at 50 °C the temperature was increased in 10 °C/min steps up to 300 °C and kept at 300 °C for 2 min. The carrier gas was helium and the flow rate 1.0 mL/min in constant flow mode. The identity of the peaks in the chromatograms was confirmed by computerized comparison with the NIST library.

Microwave Irradiation Experiments. Microwave-assisted synthesis using the high-throughput experimentation platforms shown in Figure 1 was carried out in a Synthos 3000 multimode microwave reactor (Anton Paar GmbH).[24] The individual HPLC/GC vials fitted inside the plate were filled with appropriate amounts (0.3−1.5 mL) of reaction mixture and sealed either by a standard screw cap (Figure 1a) or with an aluminum crimp top (Figure 1b) employing PTFE-coated silicone septa in both cases. For the assembly shown in Figure 1c, the SiC plate was covered with an aluminum top plate and was fixed finger tight with the six hex bolts.[8] The whole assembly was placed on a dedicated plate rotor inside the microwave reactor (see Figure S1 in the Supporting Information). During irradiation, the surface temperature of the plate was monitored and controlled by an IR sensor and suitable maximum microwave power levels are employed to avoid overheating (Figure S4 in the Supporting Information). After it was cooled to about 50 °C with forced air cooling, the plate can be removed from the cavity. For a typical experiment at 150 °C maximum reaction temperature, a ramp time of ∼5 min and a cooling time to 50 °C of ∼10 min were experienced (Figure S4, Supporting Information). All time specifications in this manuscript refer to hold times at the maximum temperature and not to total irradiation/experiment time. Subsequent to the irradiation step, the sealed HPLC/GC vials are transferred manually from the SiC plate into the appropriate HPLC or GC autosampler racks for direct reaction analysis using HPLC/UV or GC/MS (injection volume 2 and 1 μL, respectively).

Determination of Operation Limits for HPLC/GC Vials (Table 1). For the evaluation of the maximum attainable temperature and pressure parameters the HPLC/GC vials were filled with solvent (1.0 mL) and heated in the SiC plate (Figure 1c) to either 250 °C or to a temperature value corresponding to a calculated autogenic pressure of 20 bar. The temperature was regulated by modifying the magnetron microwave output power manually. An experiment was considered successful when no vial damage occurred, solvent did not evaporate during the heating and neither the septa or screw caps were molten or visually harmed after the cooling process was finished.

Reaction Homogeneity. Esterification of Benzoic Acid (Figure S6, Supporting Information). For the evaluation of reaction homogeneity across the SiC plate shown in Figure 1c, a 3.0 equiv BF_3 stock solution (based on benzoic acid **1b**) was prepared from 45 mL of MeOH, 45 mg (0.37 mmol) of acid **1a** and 855 μL of 10% (w/w) (1.3 M) BF_3/MeOH solution (1.11 mmol BF_3). Each HPLC/GC vial was filled with 1.0 mL of this mixture, and the plate was irradiated for 10 min at 150 °C. Microwave processing and handling of the plate under microwave conditions was performed as described above and the conversions for the individual runs were measured by HPLC-UV (215 nm). Before the individual vials were put into the autosampler of the HPLC-UV the reactions were quenched with 300 μL of a 0.1 M $NaHCO_3$ solution. The same stock solution was prepared for testing different filling volumes (Figure S7, Supporting Information) using identical microwave and monitoring protocols.

Catalyst Screening for the Esterification of 3-Phenylpropionic Acid (Figure 2). A stock solution of 3-phenylpropionic acid (**1b**) (2.0 mg/mL in MeOH, 13 mM) was dispensed in each of the HPLC/GC vials (Figure 1c) and filled up with the corresponding catalyst stock solutions in MeOH to a total filling volume of 1.0 mL leading to final catalyst concentrations of 5, 10, 20, 30, and 40 mol %, respectively, and 50−400 mol% for BF_3. Microwave processing at 150 °C for 10 min and subsequent analysis by HPLC-UV (215 nm) provided the data shown in Figure 2. Analogous experiments with benzoic acid (**1a**) and 2,4,6-trimethylbenzoic acid (**1d**) using 30 min reaction time at 150 °C led to the results given in Figure S8, Supporting Information.

Parallel Reactivity/Substrate Screening of 20 Carboxylic Acids (Table 2). Method A. The optimum conditions from the catalyst screening for benzoic acid (**1a**) [20 mol% $Yb(OTf)_3$, 150 °C, 30 min] were utilized to form the esters of carboxylic acids **1a**−**t** (Table 2, method A). For this purpose, to 1.0 mg (∼4−8 μmol) of each of the acids **1a**−**t** contained in individual HPLC/GC vials was added a $Yb(OTf)_3$ catalyst stock solution (3.3 mM) in MeOH of appropriate concentration/quantity. The total filling volume was again 1.0 mL and the conversions were analyzed by HPLC-UV (215 nm) directly after irradiation.

Method B. Similarly, 1.0 mg (∼4−8 μmol) samples of carboxylic acids 1a-t were dissolved in 1.0 mL (0.96 g, 8 mmol) of trimethylorthoacetate (TMOA) and irradiated at 150 °C for 30 min. HPLC-UV (215 nm) analysis provided the data shown in Table 2.

Method C. One milligram (4−8 μmol) samples of carboxylic acids **1a**−**t** were dissolved in a mixture of acetone (1 mL), DBU (12 μmol, 1.85 mg), and MeI (30 μmol, 4.26 mg) irradiated at 150 °C for one min (hold time). HPLC-UV (215 nm) analysis provided the data shown in Table 2.

Dehydrative C−C Coupling Reaction Using Metal Catalysts (Figure 3). Figure 3a. Diphenylmethanol (**3**, 0.01 mmol, 1.84 mg) and dibenzoylmethane (**4**, 2.24 mg) were weighed into 20 individual HPLC/GC vials. After the addition of 1.0 mL of an 1.5 mM stock solution of the corresponding metal catalyst salts [$Sc(OTf_3)$, $Cu(OTf)_2$, $Zn(OTf)_2$, $FeCl_3$] in the appropriate solvents (DCM, $MeNO_2$, MeCN, THF and toluene) the SiC plate was heated at 110 °C for 10 min and subsequently analyzed by HPLC-UV (215 nm) (Figure 3a).

Figure 3b. In a similar fashion, experiments were repeated using only DCM and $MeNO_2$ as solvents albeit using different catalyst concentrations (3, 2, 1, 0.5 mol %) under otherwise identical conditions.

Figure 3c. In a final experiment, the optimum reaction conditions (DCM as solvent, 3 mol% of $FeCl_3$ as catalyst and 110 °C reaction temperature for 10 min) were run in higher concentrations using 0.01, 0.05, 0.075, 0.1, and 0.15 mmol substrate/vial keeping the catalyst concentration at 3 mol % and the total reaction volume at 1.0 mL. From the experiment utilizing 0.15 mmol of substrates **3** and **4**, a 56.2 mg (96%) sample of C-alkylated dibenzoylmethane **5** was isolated by precipitation as a colorless solid: mp 226−227 °C; lit.[25g] mp 227 °C; MS (negative APCI, *m*/*z*) 389 [100, (M − 1)]; ^{13}C NMR (75 MHz, $CDCl_3$) δ 194.1, 141.7, 136.7, 133.2, 128.61, 128.58, 128.54, 128.3, 126.6, 62.3, 52.4; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (4H, d, J = 7.8 Hz), 7.48 (2H, t, J = 7.3 Hz), 7.37−7.05 (14H, m), 6.37 (1H, d, J = 11.5 Hz), 5.35 (1H, d, J = 11.5 Hz).

Fatty Acid Esterification (GC-Derivatization, Figure 4). The set of 19 fatty acids **6a**−**s** together with reference compound **1c** (1.0 mg, 2.7−11 μmol) was treated with 3.0 equiv BF_3 in MeOH (total volume 1.0 mL) and heated for 10 min at 150 °C in the HPLC/GC reaction vial platform shown in Figure 1c. For analyzing purposes the reaction mixtures were analyzed by GC-MS immediately after irradiation. The identity of the corresponding methyl esters was established by GC-MS (EI) measurements and comparison of MS fragmentation patterns with the NIST library.

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Supporting Information Available. Additional pictures of SiC plate set-ups and reaction homogeneity and optimization studies. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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