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SHORT REVIEW

Parallel microwave chemistry in silicon carbide microtiter platforms: a review

C. Oliver Kappe · Markus Damm

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Abstract In this review, applications of silicon carbide-based microtiter platforms designed for use in combination with dedicated multimode microwave reactors are described. These platforms are employed not only for the efficient parallel synthesis of compound libraries, but also in the context of high-throughput reaction screening/optimization and a number of other (bio)analytical and biomedical applications. Since the semiconducting plate material (silicon carbide) is strongly microwave absorbing and possesses high thermal conductivity, no temperature gradients across the microtiter plate exist. Therefore, many of the disadvantages experienced in attempting to perform microtiter plate chemistry under conventional microwave conditions can be eliminated. In general, the silicon carbide-based microtiter platforms allow sealed vessel processing (either directly in the well or in glass vials placed into the wells) of volumes ranging from 0.02–3.0 mL at a maximum temperature/pressure limit of 200 °C/20 bar. Depending on the specific plate and rotor configuration, a maximum of 80–192 transformations can be carried out in parallel in a single microwave irradiation experiment under strict temperature control. A platform type utilizing HPLC/GC vials as reaction vessels allows analysis directly from the reaction vessel eliminating the need for a transfer step from the reaction to the analysis vial. The latter system is particularly useful for analytical applications as well as reaction optimization/screening.

Keywords High-throughput experimentation · Silicon carbide · Microwave heating · Microtiter plates · Parallel sample treatment

C. O. Kappe (✉) · M. Damm
Christian Doppler Laboratory for Microwave Chemistry (CDLMC) and Institute of Chemistry, Karl-Franzens-University Graz, Heinrichstrasse 28, 8010 Graz, Austria
e-mail: oliver.kappe@uni-graz.at

Introduction

High-speed synthesis using microwave heating technology has attracted a considerable amount of attention in the past two decades. More than 6,000 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 [1–4]. 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 [4,5]. 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 with experiments carried out at the boiling point of the solvent (Arrhenius-type thermal acceleration). In addition, this type of high-temperature processing can facilitate the discovery of novel reaction pathways since the extreme reaction conditions attainable by microwave heating sometimes lead to unusual reactivity which is not easily duplicated by conventional heating [1–5]. This serves to expand "chemical space" in general, and "biologically-relevant, medicinal chemistry space", in particular. These advantages have not only been exploited for classical organic synthesis [1–5], but also in the context of library synthesis in medicinal chemistry/drug discovery projects where speed and efficiency are often critical factors [3,6].

Apart from the use of microwave technology for the actual synthesis of a single compound or a compound library,

high-speed microwave processing has also been proven extremely valuable for reaction optimization, since 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 [1–3]. In order to increase throughput and efficiency, both reaction optimization and library synthesis today are generally performed on a small scale in single-mode microwave reactors with incorporated robotic vial handling capabilities [7,8]. However, this 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, or in the context of a large library synthesis project (>100 compounds). In those instances the time-saving 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 [9].

Several attempts have therefore been made to perform microwave chemistry in microtiter plates using multimode microwave heating technology, combining the benefits of parallel and microwave processing. These earlier studies have been extensively reviewed in 2007 [9], and clearly highlight the problems associated with the use of this concept, namely the (i) thermal instability of standard polypropylene plates under comparatively high-temperature microwave conditions, and (ii) the formation of significant temperature gradients between individual wells, leading to a non-uniform temperature distribution across the microwave transparent plates. While the issue of temperature stability can be resolved in part by utilizing PTFE (Teflon®) or high-temperature polyethylene (HTPE) as plate materials, dealing with transient and static temperature gradients in a set-up of this type is a non-trivial affair. Typically, a microwave-absorbing solvent/reaction mixture in a well located on the outside region of the plate will show a significantly lower temperature than the same material located in a well in the middle of the plate when irradiated in a microwave field [9]. Additionally, temperature gradients may result from hot and cold spots that invariably exist in a multimode microwave cavity. These temperature differences may lead to significantly reduced conversions or product purities in some of the wells of the plate [9].

To address these problems, custom-built variations of PTFE microtiter plates were developed that contain strongly microwave absorbing materials, such as graphite pellets or high absorbing liquids located on the outside perimeter of the plate [9]. In a related strategy, deep-well plates that are made out of a strongly microwave-absorbing material (carbon-doped Teflon®) were recently commercialized [9]. Here, the polymeric material used for the construction of the plates—and not the specific solvent/reaction mixture contained in a well—absorbs the microwave energy. This means that the individual wells will be heated by microwave irradiation regardless of the dielectric properties of the reaction mixture. This system has been used successfully for several microwave-assisted parallel processes [9].

However, a significant limitation of all of the early microtiter plate systems is the fact that none of these parallel set ups allows MAOS to be performed under sealed vessel conditions in a pressure range similar to what can be attained with single-mode reactors (ca. 20–30 bar) [7]. Therefore, microwave chemistry in microtiter plates has so far been limited to the use of high boiling solvents under open vessel conditions or to sealed vessel reaction conditions that will cause only a small overpressurization (2–4 bar) [9]. This means that one of the key advantages of controlled microwave heating, namely the ability to superheat low-boiling solvents far above their boiling point, is lost. Furthermore, in the context of library synthesis, optimized protocols that are often obtained with a single mode microwave reactor in a sequential iterative format cannot be directly adapted to a multimode parallel plate format.

Since 2007, our group has introduced a series of sealed microtiter plates made out of silicon carbide (SiC) for use in a dedicated multimode microwave instrument [10]. Since the semiconducting plate material (SiC) is strongly microwave absorbing and possesses high thermal conductivity, no temperature gradients across the microtiter plate exist. Therefore, many of the previous problems experienced in attempting to perform microtiter plate chemistry under microwave conditions [9] have been eliminated. In combination with a proper sealing mechanism these systems allow to perform high-speed microwave chemistry in a highly parallelized and miniaturized format (0.02–3.0 mL) at a maximum temperature/pressure limit of 200 °C/20 bar. Depending on the specific plate and rotor configuration up to 80–192 reactions can be carried out in parallel in a single microwave irradiation experiment under strict temperature control. Over the past few years several modifications and novel applications of the silicon carbide plate technology have been described, which are summarized in this review.

Silicon carbide plate/rotor design

In recent years, the use of sintered silicon carbide (SiC) has become increasingly popular for a variety of applications in microwave chemistry. SiC is a strongly microwave absorbing chemically inert ceramic material that can be utilized at extremely high temperatures due to its high melting point (~2,700 °C) and very low thermal expansion coefficient (Table 1) [11–13]. Microwave irradiation induces a flow of electrons in the semiconducting SiC that heats the

Table 1 Comparison of material data for sintered silicon carbide (SiC) and borosilicate (Pyrex®) glass

	SiC	Pyrex®
Melting point mp (°C)	~ 2, 700	~ 800
Thermal conductivity λ (W m^{-1} K^{-1})	115	1.2
Thermal coeff. of expansion α (K^{-1})	3.0×10^{-6}	3.3×10^{-6}
Specific heat capacity C_p (J g^{-1} K^{-1})	0.6	0.7
Density ρ (g mL^{-1})	3.15	2.23
Thermal effusivity[a] e (J $s^{-1/2}$ m^{-2} K^{-1})	15,000	1,400
Porosity (closed) P (%)	2	–
Vickers Hardness HV 0.5	2300	–

Data from Ref. [14]
[a] The thermal effusivity e of a material is defined as the square root of the product of its thermal conductivity and volumetric heat capacity [$e = (k\rho C_p)^{0.5}$]

material very efficiently through resistance (ohmic) heating mechanisms. A variety of SiC materials in the form of powders, granules, and vessels (crucibles) have been used for some time in high-temperature microwave processing applications taking advantage of the extremely good microwave absorption properties, chemical resistance, and high thermal conductivity [11–14].

More related to the field of synthetic chemistry, our group has recently developed the so-called passive heating elements (PHEs) made out of sintered SiC that aid in the microwave heating of weakly absorbing or transparent reaction mixtures [15]. The cylindrical shape of the PHEs allows the use of these materials in reaction vials designed for both single-mode [15–17] and multimode [18] microwave reactors. In addition, we have developed reaction vials made completely out of SiC for use in single-mode microwave reactors [14,19]. These reaction vials now allow the heating of completely microwave transparent solvents or reaction mixtures [14]. Moreover, due to the high chemical resistance of SiC [20], reactions involving corrosive reagents can be performed without degradation of the vessel material. Examples include high-temperature fluorine–chlorine exchange reactions using triethylamine trihydrofluoride, and the hydrolysis of nitriles with aqueous potassium hydroxide [14]. Notably, the use of SiC carbide reaction vessels provides an almost complete shielding of the contents inside from the electromagnetic field [21]. Therefore, these "microwave" experiments do not involve electromagnetic field effects on the chemistry since the semiconducting ceramic vial is effectively preventing microwave irradiation to penetrate to the reaction mixture. The involvement of electromagnetic field effects (specific/nonthermal microwave effects) can therefore easily be evaluated by comparing the results obtained in microwave-transparent Pyrex® vials with experiments performed in SiC vials at the same reaction temperature [14,19].

Our originally (2007) reported SiC microtiter platform consisted of a $82 \times 62.5 \times 18$ mm SiC plate and was designed to perform reactions directly inside the bore holes of the SiC platform [10]. The upper surface of the plate contained a standard 8×6 matrix of 48 wells with a total filling volume of 410 μL (SiC plate A, Fig. 1a). The wells were shaped in classical round-bottom design, dedicated for a maximum working volume of 300 μL. In order to allow runs under closed vessel conditions an appropriate sealing mechanism was utilized consisting of a 10-mm alumina top plate equipped with adequate conical bore holes for sample withdrawal and an attached polymer sealing mat. Using this set-up it was possible to superheat a range of common solvents (ethanol, water, acetonitrile, THF, toluene) far above their boiling point without any loss of material. In a representative test run, 300 μL ethanol contained in each of the 48 wells were heated to a temperature of 180 °C which corresponds to a pressure of ca. 25 bar inside the well. Control experiments confirmed that cross-contamination between wells does not occur.

As sample withdrawal/cleaning of this prototype proved somewhat impractical for library synthesis, variations of the original microtiter plate format were subsequently developed that involved blocks/rotors of SiC containing cylindrical wells of appropriate dimensions to accommodate disposable 5 mL Wheaton glass vials [22]. This led to the commercialization of a 6×4 matrix SiC plate for use with 24 disposable glass vials equipped with a polytetrafluoroethylene (PTFE) seal and a polyetheretherketone (PEEK) screw cap (SiC Plate B, Fig. 1b), operable up to similar temperature/pressure limits (200 °C/20 bar) compared with the original design. This platform can be operated without an additional aluminum sealing top plate, since the glass vials withstand the high temperatures/pressures.

Recently, we have described a related system involving a 5×4 deep-well matrix in which 20 standard aluminum crimp top or screw-capped HPLC/GC autosampler vials are placed (SiC Plates C, Fig. 1c) [23]. In combination with an aluminum top plate and an appropriate sealing mechanism, microwave processing at temperatures up to 200 °C and pressures of up to 20 bar is possible (SiC Plate D, Fig. 1d) [23]. In order to confirm a safe operation limit of 200 °C and/or 20 bar for this platform a variety of solvents were exposed

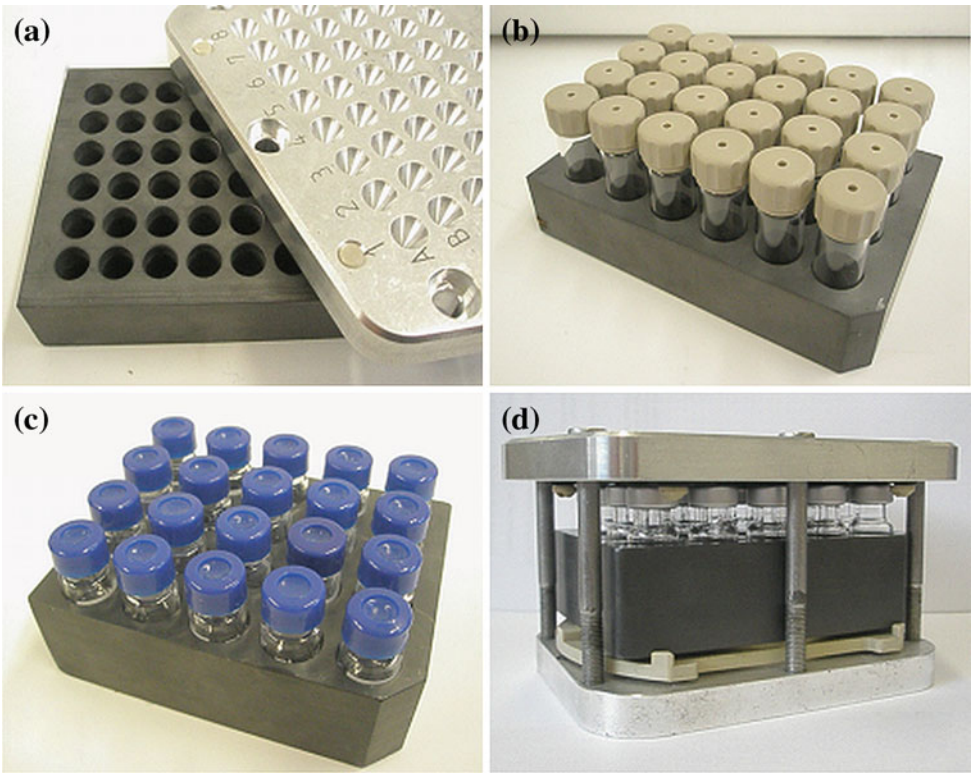


Fig. 1 Available SiC plate formats for high-throughput experimentation. **a** SiC Plate A (8 × 6 matrix): reactions are performed directly inside the bore holes of the SiC block (20–300 μL, max. 200 °C/20 bar). **b** SiC Plate B (6 × 4 matrix): reactions are performed in disposable 5 mL Wheaton glass vials sealed with PEEK screw caps (0.3–3.0 mL, max. 200 °C/20 bar). **c** SiC Plate C (5 × 4 matrix): reactions are performed in standard HPLC/GC autosampler vials fitted with polypropylene screw caps (0.5–1.5 mL, max. 200 °C/8 bar). **d** SiC Plate D (5 × 4 matrix): reactions are performed in standard HPLC/GC autosampler vials fitted with aluminum crimp tops; the set-up is additionally equipped with a sealing top plate (0.5–1.5 mL, max. 200 °C/20 bar)

Table 2 Evaluation of different solvents at a calculated maximum ~20 bar autogenic pressure in the HPLC/GC vial microwave platform (SiC Plate D, Fig. 1d)

Solvent/bp (°C)	tan δ[a]	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

Data from Ref. [23]. Experiments at 250 °C were performed in a prototype reactor, therefore higher temperatures could be reached
[a] Data from Ref. [5]

to the appropriate temperature corresponding to a calculated autogenic solvent pressure of ~20 bar for an extended time period (Table 2). 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 2 no destruction/vessel failure of the HPLC/GC glass vials was experienced. A particular advantage of this approach is that both synthesis and HPLC/UV, LC/MS, or GC/MS analysis can be performed in the same vial without any need of sample transfer.

All SiC rotor systems allow the use of inert gases (by flushing the vials with gas prior to sealing or loading of the rotors/vessels in a glove box for performing sensitive chemistries), and magnetic stirring with the aid of small stir bars (see below) [10,22–24].

For the performance of parallel microwave chemistry using the set ups shown in Fig. 1a,b,c,d, the corresponding plate systems have to be mounted on a dedicated turntable inside the cavity of a Synthos 3000 multimode microwave reactor (Anton Paar GmbH), with up to four plates being processed simultaneously (Fig. 2a) [25].[1] For all SiC platforms (Fig. 1a,b,c,d) temperature monitoring is accomplished by an external IR sensor integrated into the bottom of the multimode microwave instrument, measuring the outer surface temperature of the corresponding SiC platform which can be correlated with the actual internal reaction temperature [26].

Achieving homogeneity with respect to the temperature distribution in the individual wells/vials is of critical importance for the success, general applicability and the reproducibility of parallel microwave chemistry experiments. By using strongly microwave absorbing SiC as plate material, the microwave absorption characteristics of the individual

[1] All SiC microtiter platforms described in this review are commercially available from Anton Paar GmbH, Graz, Austria. In general, the plates are made by sintering a corresponding green compact of silicon carbide. See http://www.anton-paar.com for further details.

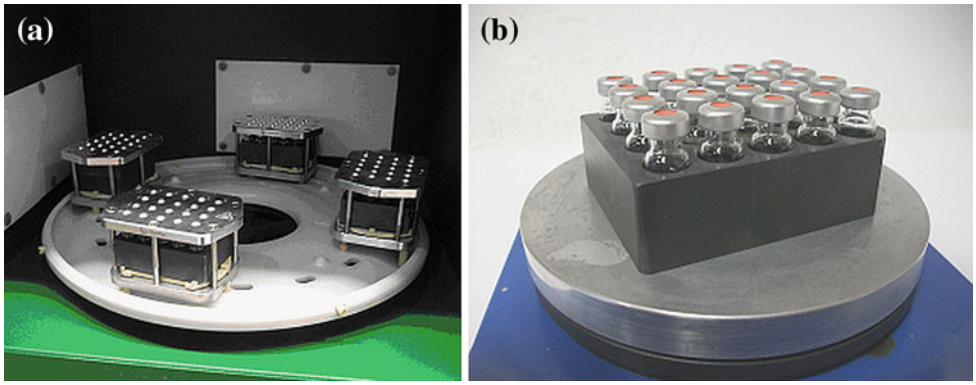


Fig. 2 **a** Four SiC Plates D mounted on a turntable inside the Synthos 3000 multimode microwave reactor. **b** SiC plate C placed on a standard hotplate/stirrer for low-temperature/pressure applications

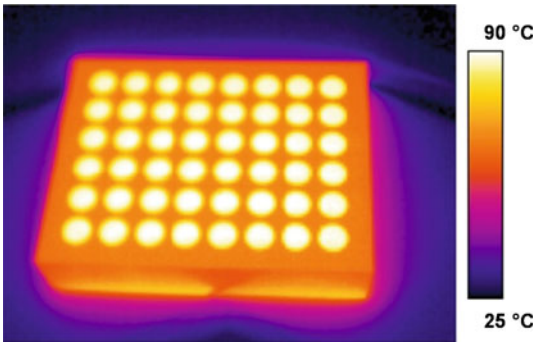


Fig. 3 IR thermal image of a silicon carbide microtiter plate (System A, Fig. 1a) containing 300 µL of water in each of the 48 wells exposed to 2.45 GHz microwave irradiation inside a multimode microwave cavity. The recorded average temperature inside the 48 wells after 1 min of irradiation at 300 W was 89.0 °C with a maximum measured deviation between individual wells of 0.3 °C

reaction mixtures contained in the 48 wells are practically irrelevant, since the semiconducting plate itself will absorb microwave energy much stronger than any organic material contained inside the wells. As shown in Fig. 3, exposing the SiC Plate A filled with 300 µL of water in each of the 48 wells to 300 W of microwave irradiation for 60 s leads to a very homogeneous heating of the entire plate, with minimal deviations (<0.3 °C) in the temperatures recorded in the individual water-filled wells [10]. These results have been confirmed by direct on-line monitoring of the temperature increase across the SiC plate using an IR thermovision camera installed on a microwave instrument possessing a cylindrical opening on its top [26]. The SiC blocks can be heated to temperatures >250 °C in only 1 min, confirming the strong microwave absorption characteristics of this semiconducting ceramic material [21,26].

A series of control experiments confirmed that the excellent *temperature* homogeneity observed for heating the "naked" SiC plate also translates to *reaction* homogeneity for a chemical transformation using the fully sealed plate set-up shown in Fig. 1a, employing a temperature sensitive esterification process as a model reaction. By performing the esterification reaction in the sealed microtiter plate (Fig. 1a) at an internal temperature of 145 °C for 20 min the conversions in all 48 wells were virtually identical (57–59%, average conversion 57.7%, SD = 0.6) [10]. Similar control experiments were also performed for the other SiC platforms [22–24].

Apart from recording the heating of the SiC platform with an IR camera, additional proof of temperature homogeneity was obtained by inserting multiple fiber optic temperature probes (Fig. 4, inset) directly into the HPLC/GC vials containing solvents with different microwave absorption characteristics [26]. Again, the result of this experiment—leading to virtually identical heating profiles (Fig. 4)—demonstrates that solvents having vastly different microwave absorption characteristics (and boiling points, see Table 2) can be readily heated to identical reaction temperatures.

It has to be emphasized that since SiC is an extremely strongly microwave absorbing material, the microwave absorption characteristics of the individual reaction mixtures contained inside the wells/vials are practically irrelevant. As demonstrated in a series of carefully conducted control experiments [23,26], the heating of reaction mixtures contained in the individual wells or reaction vials essentially occurs by conventional heat transfer from the SiC blocks to the liquid directly in contact with the SiC (SiC Plate A, Fig. 1a) or via initial heat transfer to the glass vessels that ultimately conduct the heat to the contents inside the glass vials (SiC Plates B–D, Fig. 1b,c,d). Due to the high thermal effusivity of this material (a measure for the ability to exchange thermal energy with its surroundings), heat transfer through the glass wall of the vial to the reaction mixture is reasonably fast. The main benefit resulting from this conventional conductive heat transfer is the possibility to heat reaction mixtures with vastly different microwave absorption characteristics (tan δ, cf. Table 2) in the same sealed vessel microwave experiment. Quite unlike conventional parallel microwave synthesis using multivessel rotors or microwave transparent microtiter plates [9], the contents of the wells/vials inside the SiC plate have no influence on the final reaction temperature and even weakly absorbing reaction mixtures can be easily heated to the desired temperature [26].

Because of the high thermal conductivity of SiC (Table 1) it is apparent that instead of using microwave irradiation, the

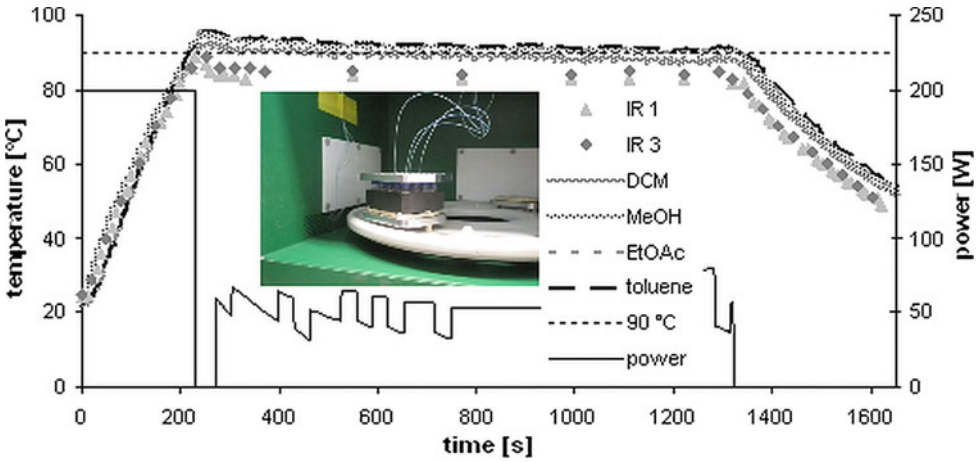
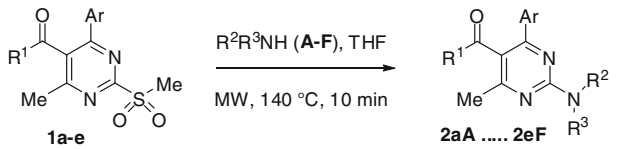


Fig. 4 Temperature (surface IR/internal fiber optic) and power profiles for temperature-controlled heating experiments (set temperature 90 °C, maximum initial microwave power 200 W) using four different solvents (DCM, bp 40 °C, tan δ 0.042; MeOH, bp 65 °C, tan δ 0.659; EtOAc, bp 77 °C, tan δ 0.059; toluene, bp 110 °C, δ 0.040) contained in HPLC/GC vials fitted in a 5 × 4 SiC plate (SiC Plate D) [27]. The *inset* shows the fiber optic probes introduced into the multimode microwave cavity. The HPLC/GC vials are equipped with immersion tubes which allow online internal temperature monitoring [26]

SiC blocks can also be heated by simply placing the platforms on a pre-heated conventional hotplate/stirrer (Fig. 2b). For example, using a pre-set hotplate temperature of 200 °C, a maximum temperature of 196 °C inside the wells of the SiC material was reached within ∼5 min after placing the block on the hotplate. No temperature inhomogeneities in any of the wells or on the surface were detectable using an IR thermovision camera [26]. In particular for low-temperature applications using the SiC Plates B and C (Fig. 1) this has been a viable strategy (see below). For high-temperature/pressure applications (Plates A and D, Fig. 1) the use of a dedicated microwave instrument which also presents some form of protection in case of vessel failure is the preferred option.



a: R^1 = OEt, Ar = Ph
b: R^1 = OEt, Ar = 4-Me-C_6H_4
c: R^1 = OEt, Ar = 3-Cl-C_6H_4
d: R^1 = NEt_2, Ar = Ph
e: R^1 = NEt_2, Ar = 4-F-C_6H_4

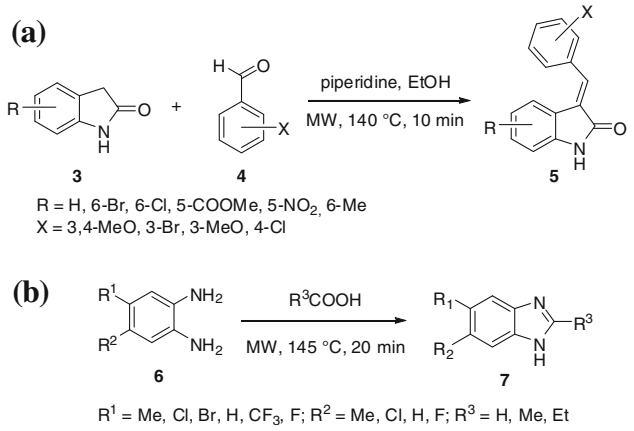
A: pyrrolidine
B: piperidine
C: ethanolamine
D: benzylamine
E: 4-fluorophenethylamine
F: 2-methoxyphenethylamine

Scheme 1 Synthesis of a 30-member library of 2-aminopyrimidines using SiC Plate A

Library synthesis

One of the most obvious applications of the SiC microtiter platforms described herein is for compound library synthesis. Here, the benefits of high-speed microwave chemistry and parallel processing can be combined. In this context, the original 2007 SiC platform (SiC Plate A, Fig 1a) where reactions are performed directly inside the bore holes of the SiC block (8 × 6 matrix) was utilized for the generation of a 30-member library of 2-aminopyrimidines performing solution-phase chemistry [10]. Using this technology a set of five sulfones (**1a**–**e**) was rapidly generated from the appropriate building blocks [28] and subsequently reacted with six primary and secondary aliphatic amines **A**–**F** to furnish the desired 2-aminopyrimidine library **2** on a 0.01-mmol scale (150 μL reaction volume) (Scheme 1). For synthesizing the 5 × 6 combinatorial matrix, columns 1–5 of the plate were loaded with the five sulfones **1a**–**e**, and rows A–F with the six amines **A**–**F**. The chosen reaction conditions for the nucleophilic displacements were essentially the same as those obtained by initial reaction optimization using single-mode microwave experiments (1.2 equiv of the amine, THF, 145 °C, 10 min) [28]. All 30 reactions were successful, with one exception (**1cC**), and cleanly generated the expected 2-aminopyrimidine products **2** as confirmed by LC–MS analysis [10].

Utilizing the 6 × 4 SiC Plate B (Fig. 1b) a team from Boehringer-Ingelheim has described the synthesis of 24-member libraries of oxindole derivatives and substituted benzimidazoles, respectively [22]. By combination of six oxindoles (**3**) with four benzaldehydes (**4**) in ethanol with substoichiometric amounts of piperidine using microwave heating to 140 °C for 10 min the 24 desired benzylidine oxindoles **5** were successfully synthesized in good to high yields (50–98%) and



Scheme 2 Synthesis of a 24-member library of oxindole and benzimidazole derivatives using SiC Plate B

excellent purities (Scheme 2a). For the benzimidazole synthesis, eight phenylene diamines **6** and three carboxylic acids were reacted at 145 °C for 20 min. The carboxylic acids were used in considerable excess, acting simultaneously as substrate and solvent. Even in this extreme case, where large variations in temperature between individual reaction vessels can be expected (different microwave absorptivity of the carboxylic acid solvents) [9], the SiC block ensured homogeneous temperatures across all reaction vessels leading to high isolated product yields (40–95%) and excellent purities for 22 of the desired 24 benzimidazoles **7** (Scheme 2b). For both library syntheses, the obtained yields were in good agreement with control experiments using single-mode microwave technology [22].

The Leadbeater group has employed SiC Plate A (Fig. 1a) for the preparation of a 12-member library of *N*-aryl functionalized β-amino esters **8** on a 1-mmol scale via an aza-Michael reaction between four anilines and three Michael acceptors using a reaction temperature of 200 °C and a reaction time of 20 min (Scheme 3a) [24]. In this particular case, the temperature homogeneity provided by the SiC plate is of crucial importance as demonstrated in earlier parallel microwave synthesis attempts that were severely complicated by the different microwave absorbance characteristics of the individual building blocks, leading to different reaction temperatures when microwave transparent parallel rotors systems were used [29].

Similarly, SiC Plate B (Fig. 1b) was used to prepare a library of 24 biaryls of type **9** via a Suzuki–Miyaura cross-coupling methodology (Scheme 3b), and a library of 1,4-dihydropyridines **10** via Hantzsch multicomponent synthesis (Scheme 3c), respectively [24]. In the Suzuki–Miyaura coupling protocol, four aryl bromides and six arylboronic acids were selected, performing each reaction on a 0.25-mmol scale inside the disposable 5 mL glass vials (160 °C for 20 min). Good yields were obtained with the exception of the couplings with 2,5-difluorophenylboronic acid and 2,6-dichlorophenylboronic acid, presumably because these two substrates are electron-poor and sterically congested, thus impeding the transmetalation. Yields with 2-bromoanisole were generally lower than with the 4-bromo analog, an expected result based on steric arguments [24].

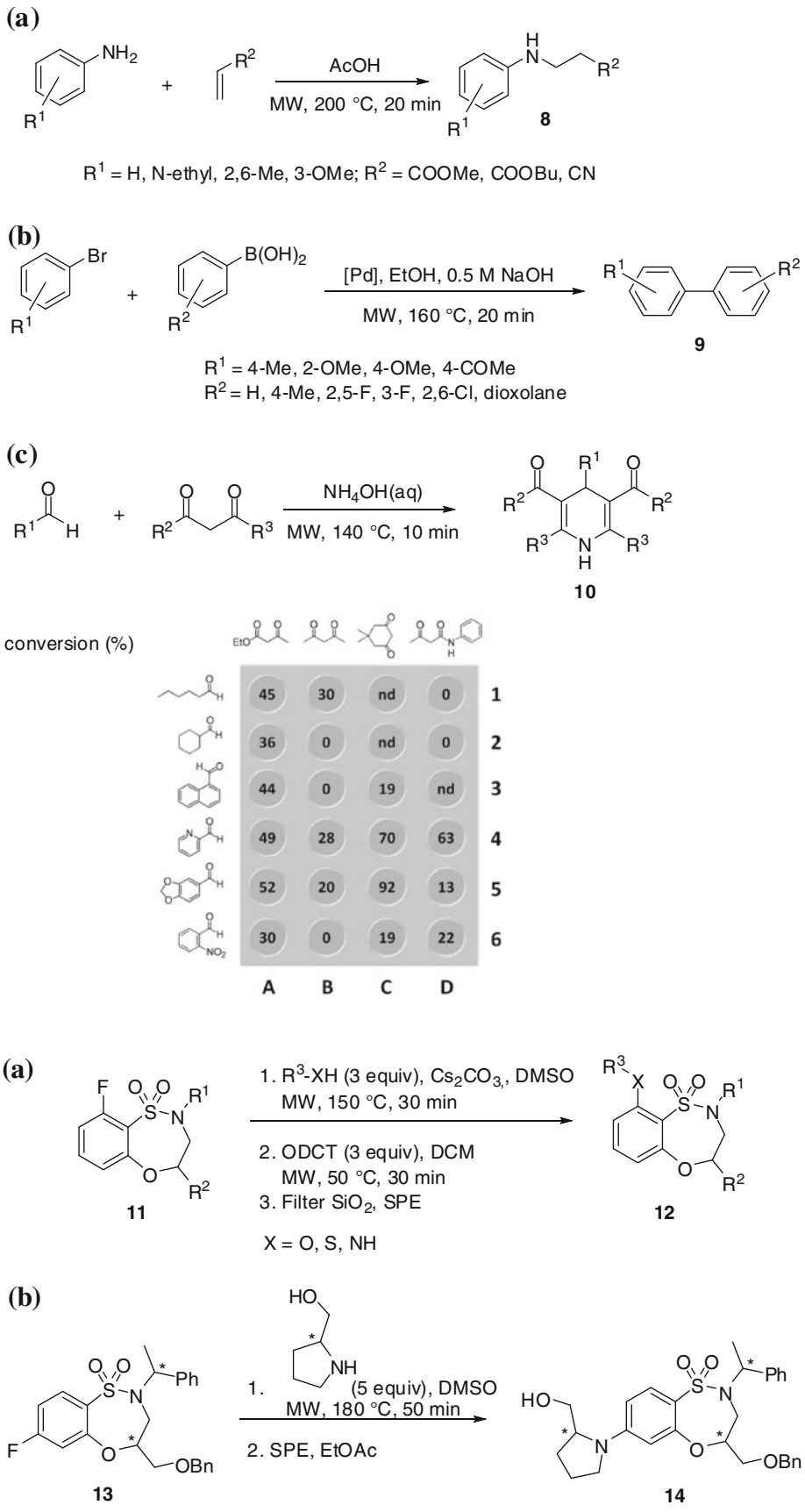
For the Hantzsch synthesis, six aldehyde substrates and four β-dicarbonyl compounds using a stoichiometric ratio of 1:5 mmol were screened, aqueous ammonium hydroxide (35%) was used both as the ammonia source and the solvent for the reaction, and the reaction mixtures were heated for 10 min at 140 °C. The results varied considerably for this library (Scheme 3c), the best conversions being obtained with ethyl acetoacetate as the dicarbonyl component. Of the aldehydes screened, pyridine-2-carboxaldehyde and 3,4-methylenedioxybenzaldehyde gave the best results.

In a library generation project described by Hanson and coworkers, SiC Plate B (Fig. 1b) was utilized for an S_NAr-based benzothiaoxazepine-1,1′-dioxide library synthesis (**12**, 78 members), employing an efficient microwave protocol for the diversification of benzothiaoxazepine-1,1′-dioxides **11** (8 derivatives) at the aromatic fluoride position with a variety of 21 nucleophilic species (Scheme 4a) [30]. Initial reaction optimization in a single-mode microwave reactor was followed by parallel library synthesis utilizing the SiC microtiter plate system. For the synthesis of the 72-member library a single microwave irradiation experiment was carried out using three 6×4 SiC blocks at a reaction temperature of 150 °C for 30 min (Scheme 4a). After cooling the SiC reaction blocks to ambient conditions, a stock solution of oligomeric dichlorotriazine ($ODCT_{50}$) scavenger was added directly to the 5 mL reaction vessels to scavenge excess nucleophilic species. Subsequently, the 72 vessels were heated again to 50 °C for an additional 30 min using microwave heating. After workup and purification most of the desired structures of type **12** were obtained in satisfying yield and purity, and were submitted for an evaluation of biological activity [30].

In related work by Organ and Hanson, the parallel synthesis of a collection of all eight optically pure, stereoisomeric sultams **14** described via an S_NAr reaction starting from the corresponding enantiomerically pure fluoro precursors **13** and aminoalcohols was described [31]. Reactions were carried out on a 100 mg scale in DMSO at 180 °C for 50 min, followed by workup and analysis to produce the desired products in high yield.

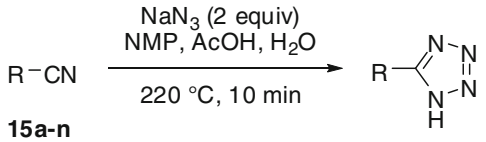
Using the SiC Plate B in combination with a standard hotplate, a collection of fourteen 5-substituted 1*H*-tetrazoles **16** was synthesized in parallel from the corresponding aryl nitriles and sodium azide at 220 °C (Table 3) [32]. After analyzing the results from more than 200 independent screening experiments performed in the microtiter plate using 2,2-diphenylacetonitrile (**15a**) as a model compound,

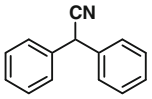
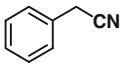
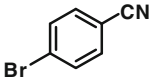
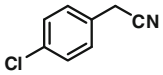
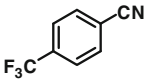
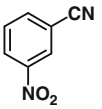
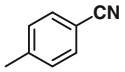
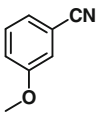
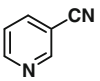
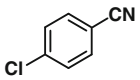
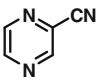
Scheme 3 Synthesis/reaction screening of *N*-aryl functionalized β-amino esters **8**, biaryls **9** and dihydropyridines **10** using SiC plate systems A and B. The *number* in the *wells* corresponds to the product conversions in the Hantzsch synthesis



Scheme 4 **a** Synthesis of a 72-member library of benzothiaoxazepine-1,1′-dioxides **12**, and **b** synthesis of a collection of eight benzothiaoxazepine-1,1′-dioxides **14** using SiC Plate B

Table 3 Parallel synthesis of 5-substituted-1*H*-tetrazoles **16** utilizing SiC Plate B on a hotplate (Fig. 2b)



Entry	Substrate	Time (min)	Yield (%)	Entry	Substrate	Time (min)	Yield (%)
15a		15	83	**15h**		10	77
15b		10	97	**15i**		10	94
15c		10	88	**15j**		10	93
15d		10	84	**15k**		10	91
15e		10	92	**15l**		10	96
15f		10	94	**15m**		10	79
15g		10	97	**15n**		10	69

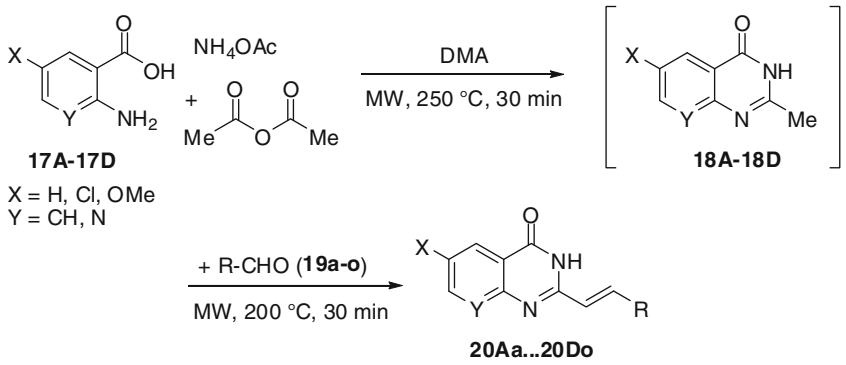
Data from Ref. [32]

an optimum set of conditions was found that provided clean and complete nitrile into tetrazole conversion and involved the use of NMP as solvent, AcOH as Brønsted acid, and H_2O as cosolvent. Applying two equivalents of NaN_3, a 7:2:1 ratio of NMP/AcOH/H_2O, and 220 °C as reaction temperature provided full conversion into the desired tetrazole **16a** within 10 min and furnished a 85% yield of isolated product. The optimized conditions could be applied to a series of nitriles providing the desired tetrazoles **16b–n** in equally high yields (Table 3). As this protocol is inherently unsafe due to the formation of hydrazoic acid, the batch protocol was readily transferred to a continuous flow protocol with minor modifications, allowing the safe preparation of tetrazoles on a gram scale [32].

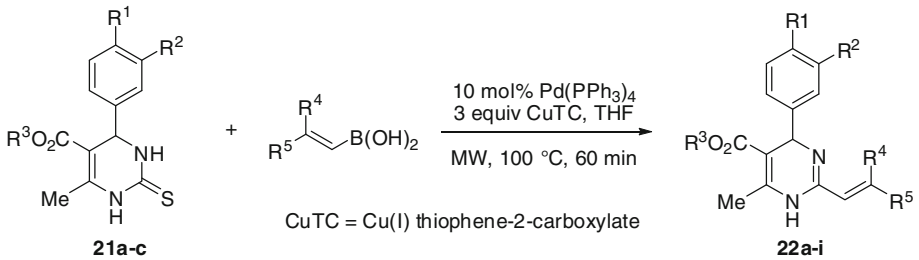
The SiC Plate D employing sealed HPLC/GC vials (Fig. 1d) was utilized to generate a 40-member library of 2-styrylquinazolin-4(3*H*)-ones **20** (Scheme 5) via a two-step condensation strategy [33]. For the initial three-component condensation step **17** → **18**, solutions of the corresponding anthranilic acids **17A–D** (0.5 mmol) in DMA containing the appropriate amounts of Ac_2O (2.0 mmol) and NH_4OAc (1.5 mmol) were filled into the corresponding 40 HPLC/GC vials (2 plates of 20 each). After sealing and microwave processing at 250 °C for 30 min using a prototype setup, the plates were cooled down to ambient conditions. For the second reaction step (**18** → **20**), 0.5 mmol of the appropriate aldehyde building blocks **19a–o** were added neat to the HPLC/GC vials. Subsequent microwave processing at 200 °C for 30 min provided the desired 2-styrylquinazolinones **20**. Work-up of the reaction mixtures in all cases involved addition of water and filtration of the precipitated solids, followed by washing with water. In the majority of cases, the crude purity of the obtained heterocycles (25–83% yield) was >90% as judged from HPLC–UV (215 nm) and/or ^{1}H NMR analysis [33].

Similarly, the palladium(0)-catalyzed, copper(I)-mediated cross-coupling of dihydropyrimidine-2-thiones **21a–c** with three commercially available alkenylboronic acids was

Scheme 5 Generation of a 40-member library of 2-styrylquinazolin-4(3*H*)-ones **20** using a two-step strategy employing SiC microtiter plate system D



Scheme 6 Generation of a collection of nine 2-alkenyl-1, 4-dihydropyrimidines **22a–i** employing SiC Plate D



investigated in the same SiC plate system (Scheme 6) [34]. Initially, the reaction optimization of this Liebeskind–Srogl-type desulfitative C–C coupling was performed using single-mode microwave conditions. The highest isolated yields for the nine 2-alkenyl-1,4-dihydropyrimidines **22a–i** (55–71%) were obtained by a single microwave irradiation cycle at 100 °C for 60 min, applying 10 mol% of $Pd(PPh_3)_4$ as a catalyst and 3 equiv of CuTC as an additive (THF, Ar atmosphere). Almost identical results regarding product yield and purity were obtained using the SiC plate system (60-69%) and the same reaction temperature/time (Scheme 6). In addition, the effectiveness of magnetic stirring of the strongly heterogeneous reaction mixture (3 equiv of CuTC) and the possibility of performing chemistry under inert conditions (Ar) in the HPLC/GC has been demonstrated using this chemistry.

Reaction optimization and screening

As already mentioned in "Introduction" section, apart from the use of microwave chemistry for the synthesis of a single compound or a compound library, high-speed microwave processing has also been proven extremely valuable for reaction optimization, since 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 [1–3]. Using sequential microwave processing in single-mode reactors, 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 have to be 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 2009, we have described a high-throughput SiC platform involving a 5 × 4 deep-well matrix in which 20 standard aluminum crimp top or screw-capped HPLC/GC autosampler vials are placed (SiC Plate C, Fig. 1c) [23]. In combination with an aluminum top plate and an appropriate sealing mechanism, microwave processing at temperatures up to 200 °C and pressures of up to 20 bar is possible (SiC Plate D, Fig. 1d) [23]. A particular advantage of this platform is that both synthesis and HPLC/UV, LC/MS, or GC/MS analysis can be performed in the same vial without any need of sample transfer.

As an example to highlight the usefulness of this platform for catalyst screening, the esterification of 3-phenylpropionic acid (**23a**) with methanol using both Brønsted (H_2SO_4, HCl) and Lewis acids [BF_3, $Yb(OTf)_3$] in different concentrations was investigated (Fig. 5) [23]. Preliminary experiments using single-mode microwave instrumentation demonstrated that a temperature range of 120–150 °C and a timeframe of 10 min were suitable for these esterification processes. Utilizing the 5 × 4 SiC Plate D (Fig. 1d) the effect of catalyst

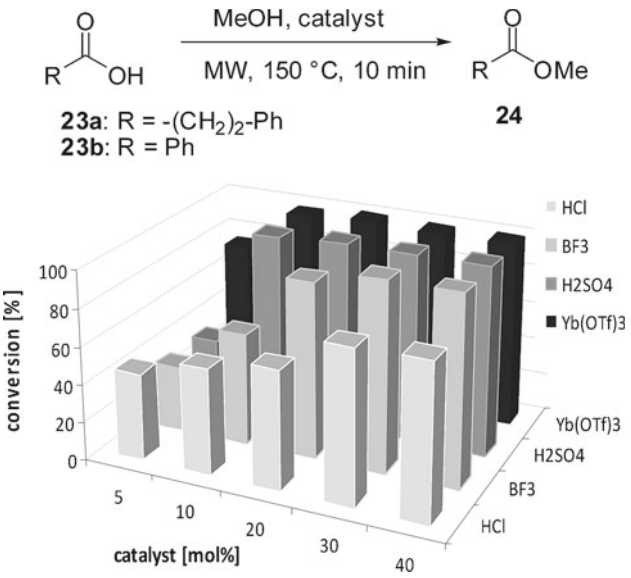


Fig. 5 HPLC-UV conversions (215 nm, peak area %) for the microwave-assisted (150 °C, 10 min) esterification of 3-phenylpropionic acid (**23a**) with methanol catalyzed by different acids in varying concentration using the 5 × 4 SiC Plate platform D (Fig. 1d). For BF_3 the concentration values need to be multiplied by 10 (i.e., 50–400 mol%)

type and concentration on the esterification process at 150 °C (10 min) was established in one single irradiation experiment in a time frame of ∼25 min (including the ramp times for heating and cooling). In comparison, performing all 20 esterifications by automated sequential microwave processing [8] 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 Fig. 5, quantitative conversion after 10 min was achieved with $Yb(OTf)_3$ (20 mol%), H_2SO_4 (30 mol%) and BF_3 (3.0 equiv).

An identical optimization study was subsequently also performed for benzoic acid (**23b**) [23]. With the aim of performing a rapid substrate screening campaign, the optimum esterification conditions with methanol identified for benzoic acid (20 mol% $Yb(OTf)_3$, 150 °C, 30 min) were then applied to a diverse set of 20 aliphatic, aromatic and heteroaromatic carboxylic acids. As shown in Fig. 6, for the majority of the selected acids (**13**) a high degree of esterification (>95%) was achieved using the previously optimized conditions [23]. In order 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. This technology has therefore obvious applications for optimization studies in library synthesis.

In order to demonstrate the ability of the SiC platform to screen different catalysts and solvents in parallel for their reactivity and therefore to rapidly identify an optimum solvent/catalyst combination for a specific transformation, the coupling reaction between diphenylmethanol (**25**) and dibenzoylmethane (**26**) was optimized [23]. Employing the 5 × 4 SiC Plate D (Fig. 1d) five solvents (DCM, $MeNO_2$, MeCN, THF, and toluene) were initially screened against

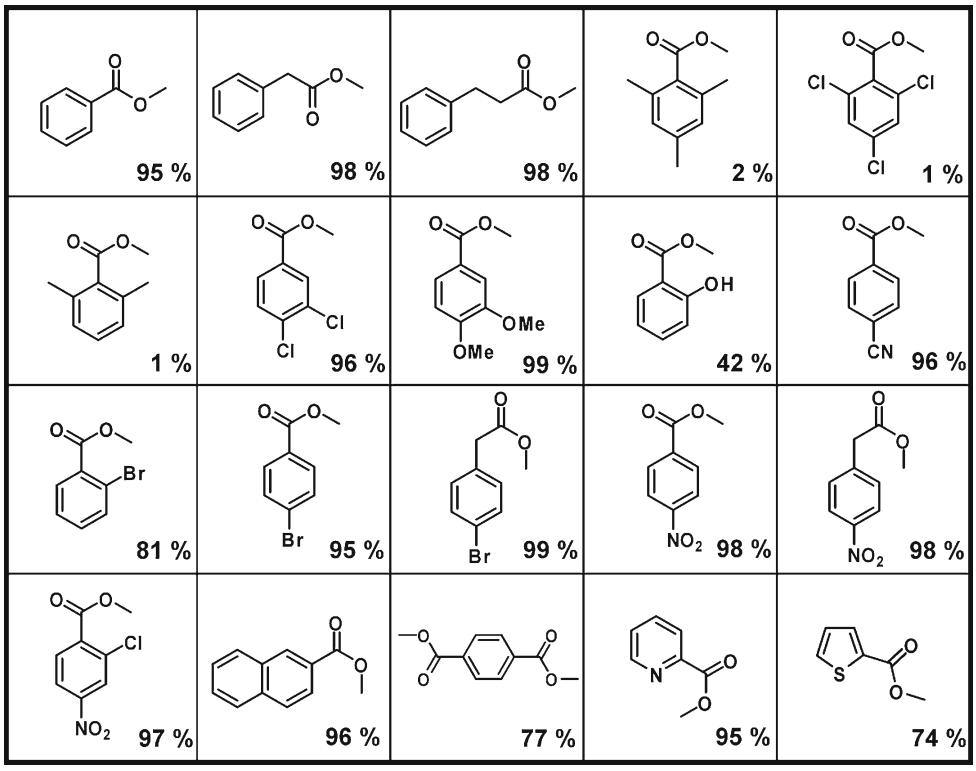
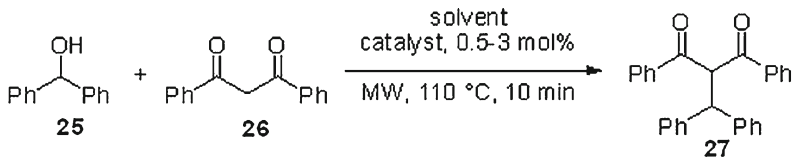


Fig. 6 Conversions for the esterification of carboxylic acids with methanol using SiC Plate D. Reaction conditions: 20 mol% $Yb(OTf)_3$, MeOH, 150 °C, 30 min. Conversions were determined by HPLC/UV at 215 nm (peak area %)



(a)

catalyst	DCM	$MeNO_2$	MeCN	THF	toluene
$Sc(OTf)_3$	95	98	82	84	94
$Cu(OTf)_2$	94	98	71	94	93
$Zn(OTf)_2$	53	97	89	37	25
$FeCl_3$	97	95	82	30	69

solvent

(b)

catalyst-concentration	$MeNO_2$		DCM		
	$Sc(OTf)_3$	$FeCl_3$	$Sc(OTf)_3$	$FeCl_3$	$Cu(OTf)_2$
3 mol%	96	92	99	99	97
2 mol%	95	82	96	95	96
1 mol%	89	29	90	83	91
0.5 mol%	82	26	92	51	92

solvent/catalyst

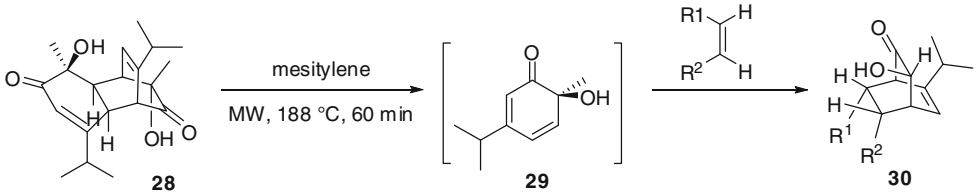
Fig. 7 HPLC-UV purities (215 nm, peak area %) for a solvent/catalyst screen in the C–C coupling of diphenylmethanol (**25**) with dibenzoylmethane (**26**) using the 5 × 4 SiC parallel reaction platform (SiC Plate D, Fig. 1d). **a** Reaction conditions: 3 mol% catalyst, 110 °C, 10 min. **b** Reaction conditions: 0.5–3 mol% catalyst, 110 °C, 10 min

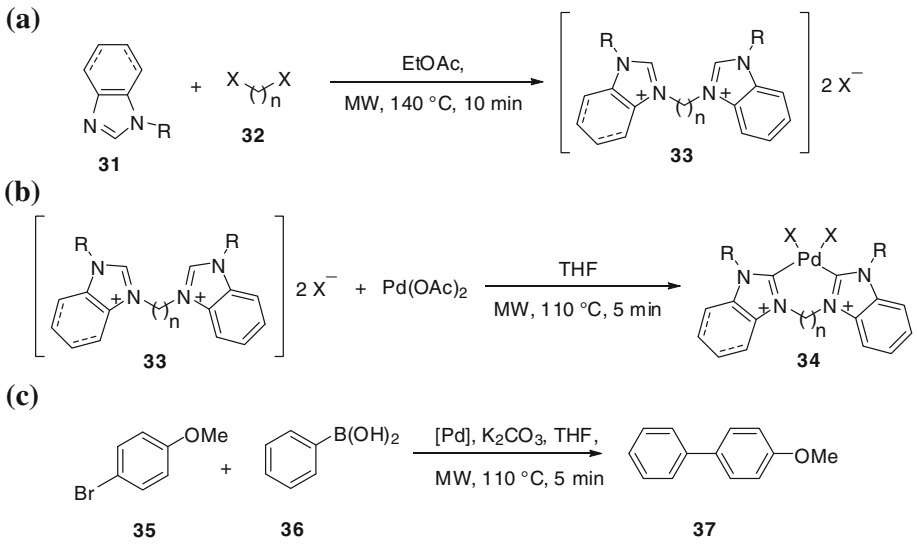
four metal catalysts [$Sc(OTf_3)$, $Cu(OTf)_2$, $Zn(OTf)_2$, $FeCl_3$] employing a 3 mol% catalyst concentration at 110 °C (10 min). The crude product purities are shown in Fig. 7a. In a follow-up plate experiment, the best solvent/catalyst combinations were re-run at lower catalyst concentrations (Fig. 7b). The optimum conditions derived from the two plate experiments shown in Fig. 7a and b therefore utilized DCM as solvent, 3 mol% of $FeCl_3$ as catalyst and 110 °C reaction temperature for 10 min.

Such SiC plate optimization studies thus allow the reactivity screening of solvents with different microwave absorption characteristics (tan δ, see Table 2) in a parallel microwave experiment. A similar experiment would not be feasible in conventional (polypropylene or PTFE) microtiter plates since the differences in solvent tan δ would lead to different reaction temperatures in the individual reaction wells/vials upon exposure to microwave irradiation [9,26]. In these parallel runs using different solvents in the HLPC/GC SiC Plate D the maximum safe operational temperature is dictated by the solvent with the lowest boiling point. In the case of the experiment described in Fig. 7b, the use of DCM (bp 40 °C) limits the safe use of the platform to 150 °C (see Table 2).

In a recent investigation by Porco, a very efficient parallel screen of suitable cycloaddition partners for *ortho*-quinols utilizing the SiC Plate A (Fig. 1a) was reported [35]. In this study, a broad panel of reaction partners in the retro-[4+2]/[4+2] cascade of *o*-quinol dimers of type **28** have been evaluated in an effort to study the scope and limitations of this methodology, to obtain both bicyclo[2.2.2]octenone and *cis*-decalin frameworks, and to understand the factors determining regio- and diastereoselectivity (Scheme 7). Initial reaction optimization was performed in a single-mode microwave reactor using dimer **28** and *N*-phenylmaleimide as model substrates, demonstrating that mesitylene afforded clean reactions within 15–60 min at 188 °C. Utilizing the optimized microwave conditions, 57 reaction partners were then evaluated in a parallel reaction screen. Reactions were conducted on a ca. 0.01 mmol scale using 3.0 mg of dimer **28** as substrate, 10 equiv of the corresponding reaction partner, and 50 µL of mesitylene under microwave heating at 188 °C. After 60 min reaction time, reaction mixtures were filtered through a silica gel plug, concentrated, and evaluated using ultra performance liquid chromatography (UPLC)–MS. Based on UPLC–MS data, reactions showing major peaks in the HPLC trace were then scaled up (0.06 mmol, 20 mg substrate) using single-mode microwave technology. The results of the reaction screening indicated that dimer **28** was completely consumed in all cases. Of the 57 reactions, 12 afforded products of type **30** corresponding to [4+2] adducts

Scheme 7 Reactivity screening in retro-[4+2]/[4+2] reactions to afford bicyclo[2.2.2]-octenones (SiC Plate A)





Scheme 8 **a** Preparation of 20 bis-imidazolium salts; **b** followed by the preparation of *cis* and *trans* Pd-NHC complexes; and **c** reactivity screening of the generated palladium catalysts in a Suzuki–Miyaura cross-coupling. SiC Plate B (Fig. 1b) was used for all steps

between the *o*-quinol (**29**) and alkene reaction partners, while some of the other reactions afforded undesired byproducts. These studies clearly demonstrate that the SiC platforms can be employed to quickly screen for reactivity, or even discover new reactivities, as the high-temperature/pressure conditions available in these reactors can otherwise not easily be accessed.

The Leadbeater group has employed SiC Plate B (Fig. 1b) for the rapid preparation of a range of *N*-heterocyclic carbene (NHC) ligands and their palladium complexes (Scheme 8) [36]. Ultimately, the generated catalysts were screened for their performance in a Suzuki–Miyaura cross-coupling reaction (Scheme 8c). Here, several reaction steps were performed in sequence in the SiC microtiter system. Initially, the generation of 20 bis-imidazolium salts **33**, choosing five imidazole-based starting materials (4 mmol, **31**) and four dihaloalkanes (2 mmol, **32**), was performed in ethyl acetate (1 mL) at 140 °C for 10 min (Scheme 8a). Of the 20 reactions performed, 19 bis-imidazolium salts were isolated in 9–99% yield; one reaction yielding no product. In the subsequent reaction step the previously isolated bis-imidazolium salts **33** (1 mmol) were mixed with palladium acetate (1 mmol) and THF (1 mL) and heated for 5 min at a temperature of 110 °C (Scheme 8b). After cooling the plate to ambient temperature, potassium carbonate (2 mmol) as well as a THF solution containing 1 mmol of 4-bromoanisole (**35**) and 1.1 mmol of phenylboronic acid (**36**) as reaction partners for the Suzuki coupling (Scheme 8c) were added directly to the glass vials. Thereafter, the SiC plate setup was re-heated to 110 °C and the target temperature was maintained for 5 min. The reaction was stopped before completion after 5 min, to assess the activity of each catalyst in a more meaningful manner, bearing in mind that running the reaction longer, many of the assays could show higher final conversion, thus not allowing to differentiate between the complexes screened.

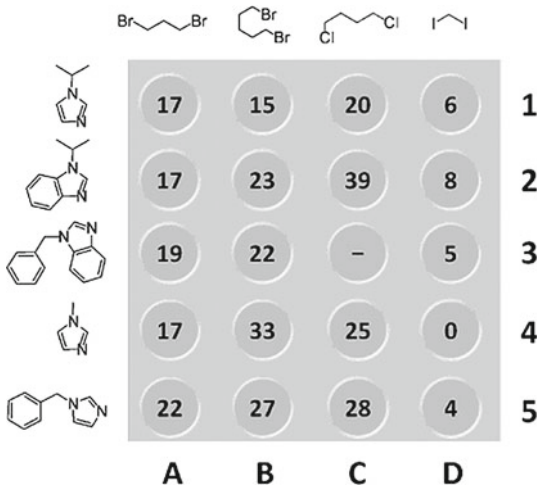


Fig. 8 Screening the library of Pd catalysts **34** in the Suzuki–Miyaura coupling protocol shown in Scheme 8c. For ease of reference, the components making up the bisimidazolium Pd catalysts **34** are shown. The *number* in the *wells* corresponds to the isolated yields from the coupling reaction

Figure 8 shows the results of the catalyst screen. The highest yield for biaryl **37** was obtained using the palladium complex originating from the bis-imidazolium salt formed from 1,4-dichlorobutane and *N*-isopropylbenzimidazole (C2).

Bioanalytical and biomedical applications

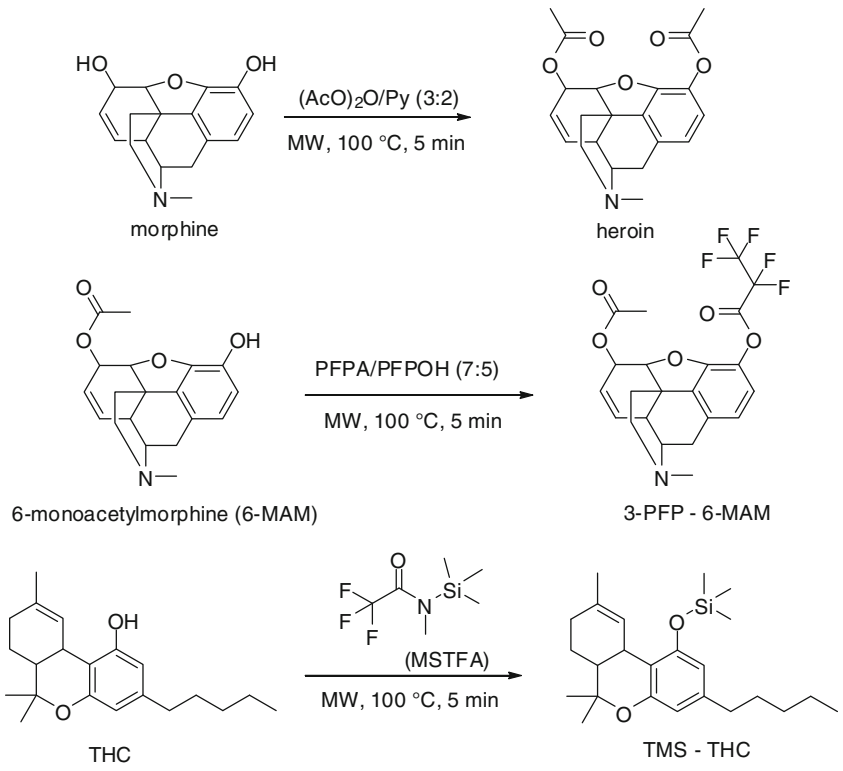
In this section, various other applications of SiC microtiter plate technology are summarized. For use in analytical chemistry, most studies have utilized the SiC plate system equipped with HPLC/GC vials (SiC Plate C and D, Fig. 1c,d), since here microwave treatment and analysis can be conducted from the same vial.

Derivatization reactions

The use of microwave heating for performing GC-derivatization protocols in forensic/clinical toxicology and doping control is steadily increasing [37]. Early work from the mid 1990s has demonstrated the potential usefulness of microwave irradiation for several standard GC–MS derivatization reactions, such as trimethylsilylation, acetylation, and pentafluorobenzoylation [37]. Subsequent studies have validated the use of microwave-assisted derivatization techniques for several case studies in the area of clinical and forensic toxicology [37]. In recent years a range of derivatization protocols based on microwave technology has been disclosed in the literature, including not only examples associated with forensic/clinical toxicology and drug monitoring/doping control, but also involving food- and environmental analysis, in addition to other disciplines [37]. In most of the published examples on microwave-assisted derivatization protocols the time required for complete derivatization could be significantly reduced compared with conventional procedures, typically to less than 5 min [37].

The potential of microwave-assisted derivatization techniques applying a dedicated single-mode microwave reactor with online temperature and pressure control was recently thoroughly investigated [38]. The use of this equipment has allowed a detailed analysis of several microwave-assisted derivatization protocols comparing the efficiency of microwave and conventional heating methods utilizing a combination of GC–MS and liquid chromatography coupled with mass detection (LC–MS) techniques [38]. These studies revealed that for standard derivatization protocols, such as acetylation, pentafluoropropionylation, and trimethylsilylation a reaction time of 5 min at 100 °C in a microwave reactor was sufficient to allow an effective derivatization (Scheme 9). Control experiments using standard operating procedures (30 min at 60 °C conventional heating) have indicated that faster derivatization under microwave irradiation is a consequence of the higher reaction temperatures that can rapidly be attained in a sealed vessel and the more efficient heat transfer to the reaction mixture applying direct in core microwave dielectric heating [38].

Employing SiC Plate C (Fig. 1c) in combination with standard low volume (maximum filling volume 200 µL, conical design) screw-cap GC vials, the microwave-assisted GC derivatization protocols shown in Scheme 9 could be efficiently parallelized and miniaturized, allowing the simultaneous execution of up to 80 derivatization protocols (5 min at 100 °C) including acetylation (exemplified for morphine), pentafluoropropionylation (for 6-monoacetylmorphine), and trimethylsilylation (for Δ^9-tetrahydrocannabinol) in the GC vial/SiC plate format using a 50 µL reaction volume [39]. Since the heat transfer occurs mostly by conventional conductive heating and in these experiments the pressure build up in the vial is not very high at 100 °C reaction tempera-



Scheme 9 GC derivatization reactions performed in SiC Plate C (Fig. 1c)

ture (max 3 bar), identical results can also be obtained in a standard hotplate experiment at 100 °C (cf. Fig. 2b) using the same SiC set-up [39].

In contrast to the low-temperature derivatization processes described in Scheme 9, the high-temperature derivatization of a set of 19 fatty acids was performed at 150 °C for 10 min using BF_3/MeOH as derivatization/esterification reagent [23]. Heating MeOH to a reaction temperature of 150 °C, a pressure of up to 13 bar is generated, implicating the necessity of using the SiC Plate D set-up inside a multimode microwave reactor (including the aluminum sealing plate to increase pressure resistance, Fig. 1d). In a single microwave irradiation experiment, 19 fatty acids (plus 3-phenylpropionic acid as reference compound) were derivatized within only 10 min at 150 °C, delivering quantitative esterification of the fatty acids to the corresponding methyl esters as confirmed by GC–MS analysis [23].

Forced degradation

Forced degradation or stress studies of drug substances play an integral role in the development of pharmaceuticals [40]. Results from forced degradation studies reveal important data on the stability of a given drug molecule and on the generation of "pharmaceutical impurities" resulting from these degradation processes. As these impurities may have pharmacological or toxicological relevance, the presence of these impurities must be carefully monitored. Long-term storage tests performed to investigate the stability of a developed drug are expensive due to the time involved. Therefore, the method of forced degradation uses external stress conditions like acids or bases (typically ~1 M), oxidative stress (hydrogen peroxide up to 3%), temperature increase, and exposure to light, to enforce the degradation of a drug candidate [40]. Traditionally, forced degradation studies in solution are performed using reaction volumes between 10 and 100 mL (drug concentration 1–10 mg/mL), applying comparatively moderate temperatures ranging from room temperature up to ~100 °C (reflux conditions) which implicates long reaction times (from hours to days), low sample throughput, and a time-consuming sample handling/analytical regime [40].

Applying single-mode microwave technology and the anti-inflammatory drug indomethacin (Fig. 9) as reference sample, a stepwise increase of the reaction temperature from 100 °C to 160 °C, testing the degradation of indomethacin in a 0.1 M HCl solution revealed that similar decomposition rates can be obtained in 1 min at 160 °C compared with 1 h at 100 °C without additional decomposition products observed due to the harsher reaction conditions [41]. By utilizing the SiC Plate D (Fig. 1d) a set of five detergents (H_2O_2, AcOH, HCl, $NaHCO_3$, NaOH) in four different concentrations (0.001–15 M) was evaluated in parallel for the ability to decompose indomethacin. Thus, a single

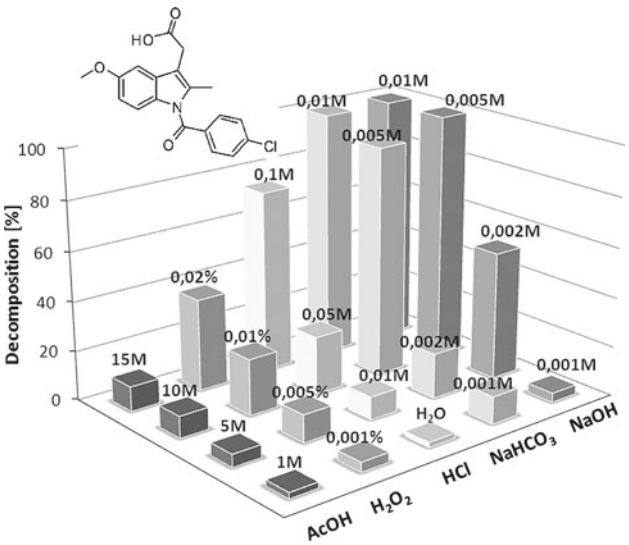


Fig. 9 Parallel degradation of indomethacin (*inset*) at 150 °C/5 min evaluating different stress conditions (HPLC-UV analysis at 230 nm). The SiC Plate D (Fig. 1d) was used

5 min microwave irradiation experiment at 150 °C allowed the efficient screening of 20 decomposition conditions for indomethacin (Fig. 9).

In addition, solvent stability tests exposing indomethacin to 20 different, mostly organic, solvents at 150 °C and 160 °C for 30 min and the exposure of the solid drug to various gases (N_2, Ar, O_2, NH_3, air), applying high temperatures were also performed using the same platform [41].

Protein hydrolysis and enzymatic digestion

Amino acid composition analysis in proteins and peptides is a classical but rather complex analytical method [42]. Prior to analysis, the proteins/peptides have to be completely hydrolyzed involving an acid-catalyzed cleavage of the peptide bonds to yield the free amino acids. The resulting hydrolyzates can then be analyzed by well-established amino acid analysis techniques employing pre- or post-column derivatization in combination with ion exchange or reversed-phase high-performance liquid chromatography to determine the amino acid composition [42]. The critical hydrolysis step is usually performed by heating the samples to be analyzed at elevated temperatures in the presence of high concentrations of acids for extended time periods. Traditional liquid- or gas-phase hydrolysis is generally performed in sealed tubes using 6 N HCl at 110 °C for 24 h, although methanesulfonic acid (MSA) has also been employed under similar conditions in order to determine sensitive amino acids, such as methionine and tryptophan [42].

As the acid hydrolysis step is rather time-consuming, several reports over the past 20 years have advocated the use of microwave irradiation as a tool to speed-up the hydrolysis of

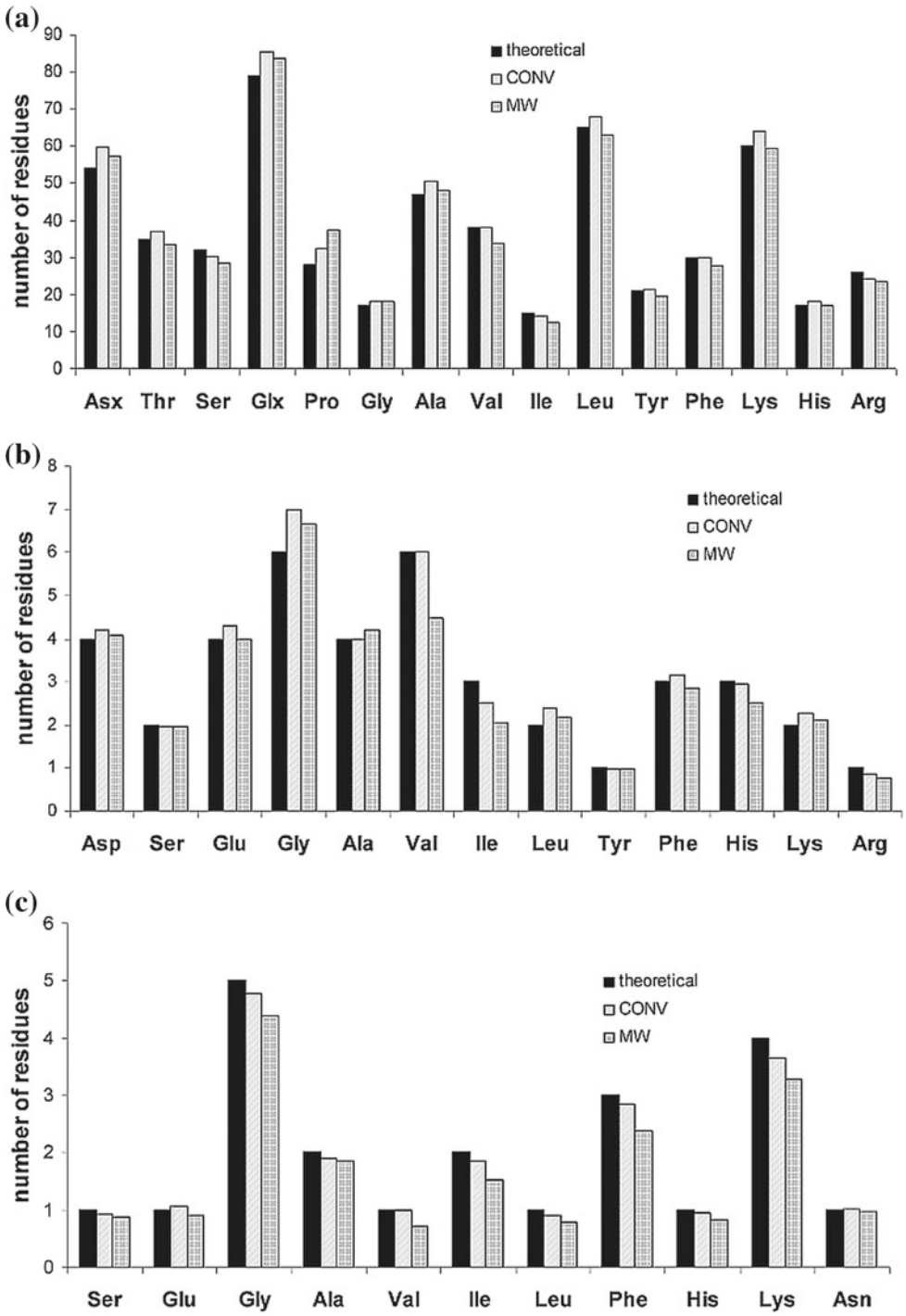


Fig. 10 Comparison of amino acid compositions of **a** BSA, **b** β-amyloid, and **c** MAGAININ peptide following liquid-phase hydrolysis with 6 M HCl at 160 °C for 5 min performed in SiC microtiter plate system D (Fig. 1d). The proteins/peptides were subjected to conventional sealed vessel hydrolysis at 110 °C for 24 h (CONV) and to microwave-assisted hydrolysis (MW). The theoretical number of residues is shown in *black*

proteins and peptides under acidic conditions [43]. Indeed, using sealed vessel microwave irradiation in a higher temperature range, hydrolysis times could often be reduced to a few minutes retaining the recovery rates attained by conventional methods [43]. Using the hydrolysis of bovine serum albumin (BSA) with 6 N HCl as a model transformation, initial optimization studies were performed in a single-mode microwave reactor applying temperatures from 110–200 °C and hydrolysis times from 1 to 10 min. At a reaction temperature of 160 °C for 5 min similar results compared with the conventional method (110 °C, 24 h) were obtained [43]. These optimized reaction conditions (160 °C, 5 min) were then applied to a miniaturization/downscaling approach ultimately reducing the reaction volume from 3 mL, required in the conventional and single-mode microwave experiments, to a total reaction volume as low as 100 µL. The hydrolysis

reactions were performed directly inside standard HPLC/GC vials equipped with 200 µL glass inlets having the advantage of eliminating the required transfer step from the reaction to the analysis vial. As shown in Fig. 10, this protocol was applied successfully to the hydrolysis of BSA protein, β-amyloid and MAGAININ peptides, obtaining similar recovery rates for the single amino acids in all cases when the results are compared with conventional hydrolysis procedures.

An additional advantage of the low-volume hydrolysis approach is that the traditional evaporation step required for larger volumes of acid can be eliminated and replaced by the use of a fast parallel sample concentration method (for example, using a Speedvac or a sample concentrator) and/or by simply placing the HPLC/GC vials directly into a dessicator filled with KOH for ∼3 h. Since the sample preparation step for the chromatographic amino acid determination involving the addition of 100 µL of Li-Citrat buffer can be performed directly from the HPLC/GC vials any potential contamination or loss of material usually caused by transferring samples can be eliminated.

In a related approach, Leadbeater and coworkers have investigated the proteolytic digestion of insulin chain B by trypsin using SiC Plate A (Fig. 1a) [24]. Various protease-to-protein ratios were tested, heating the platform for 30 min at ∼ 50 °C. Not unexpectedly, the level of digestion decreased with increasing protease-to-protein ratio (1:5, 1:10, 1:25 were tested). Similarly, SiC Plates B and C were evaluated for a low-volume enzymatic extraction method for the selenomethionine (SeMet) determination in selenized yeast samples [44]. In contrast to traditional methods which generally utilize large sample volumes consuming significant amounts of costly enzymes, the improved protocol describes a reduction of required sample volumes from originally 5 to 1 mL per extract without observing any chances regarding the amount of extracted SeMet. Since the temperature applied for the enzymatic hydrolysis of selenium-enriched yeast with a combination of protease/lipase did not exceed 37 °C (higher temperature are not suitable for these enzymes), the extractions were performed heating the SiC platform on a standard hotplate/stirrer (cf. Fig. 2b). This setup is preferable compared with the traditionally used method involving a water bath and 50 mL polyethylene tubes [44].

Extraction studies

In the last decade, microwave dielectric heating principles have penetrated many different research areas, including analytical scale extraction protocols. The ability to rapidly heat sample–solvent mixtures was found to be beneficial for performing microwave-assisted extractions (MAE). The main advantage of this technique is, particularly in combination with sealed vessels where extractions can be performed at elevated temperatures, an accelerated release of target compounds from the sample matrix into the solvent [45]. Shortened extraction times in addition to an improved recovery of analytes compared with conventional extraction techniques have been reported for most published examples of high-temperature MAEs [45].

Table 4 Caffeine quantification investigating 9 Nespresso grand crus coffees and a sample of Hornig coffee (arabica)

Coffee	Caffeine (µg)	Coffee	Caffeine (µg)
Decaffeinato	5 ± 2	Roma	160 ± 6
Cosi	141 ± 1	Arpeggio	142 ± 13
Volluto	144 ± 2	Vivalto	136 ± 4
Cappricio	165 ± 10	Ristretto	151 ± 4
Livanto	144 ± 6	Arabica	129 ± 10

Microwave-assisted extractions (in triplicate) were performed in MeOH in SiC Plate C using the previously optimized conditions (90 °C, 10 min)

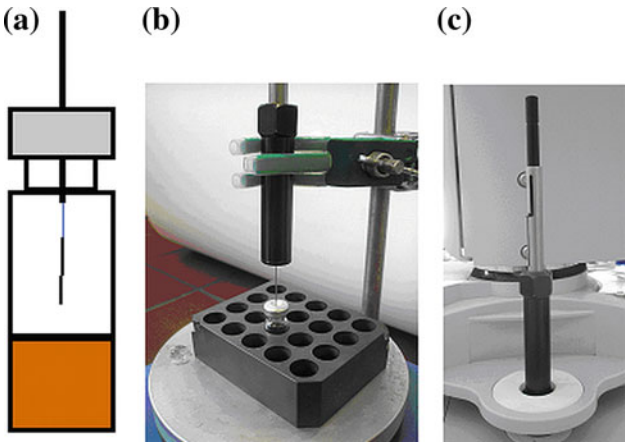


Fig. 11 Manual SPME analysis in standard 3 mL HPLC/GC vials. **a** Schematic drawing of the HPLC vessel showing the glass vessel filled with 0.5 mL water + coffee powder and SPME fiber inserted into the headspace. **b** The fiber is exposed to the headspace of the vial for 15 min at 60 °C to allow adsorption of the volatile compounds to the fiber core. **c** Subsequently, the manual fiber assembly is introduced to the GC injection port and desorption of the compounds at 250 °C for 5 min was conducted

The SiC Plates C and D (Fig. 1c, d) were recently evaluated for the extraction and quantification of caffeine from commercial coffee powders assessing different solvent types, extraction temperatures and times [27]. The miniaturized parallel extraction technique allows solvent extractions to be performed at significantly expanded temperature/pressure limits and shortened extraction times, using standard HPLC autosampler vials as reaction vessels. For these investigations the SiC heating platforms were utilized either in combination with a standard hotplate (conventional heating, < 200 °C, <8 bar) or a multimode microwave reactor (microwave-assisted heating of the SiC blocks) for high-temperature/pressure

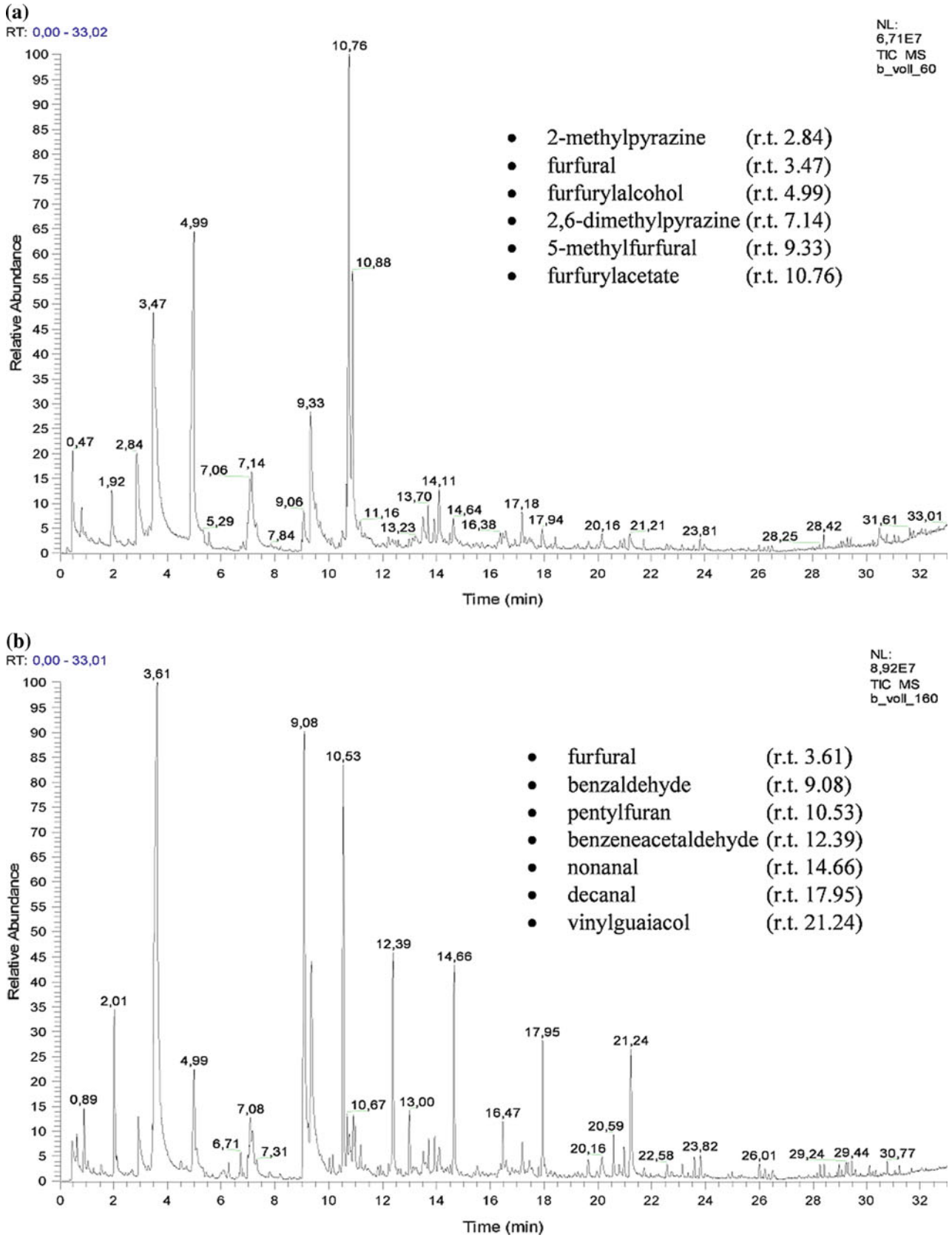


Fig. 12 Comparison of low-temperature (60 °C) and high-temperature (160 °C) extraction of "volluto" coffee applying headspace-SPME–GC–MS analysis

applications (<200 °C, <20 bar). For example, 141 ± 11 μg caffeine (5 mg coffee powder) were extracted during a single extraction cycle using methanol as extraction solvent, whereas only 90 ± 11 were obtained performing the extraction in DCM, applying the same reaction conditions (90 °C, 10 min). In multiple extraction experiments a total of ∼ 150 μg caffeine was extracted from 5 mg commercial coffee powder.

In addition, the caffeine quantification of different coffee samples (9 Nespresso grand crus + arabica coffee from Hornig) was performed using two SiC heating platforms in the multimode microwave system to perform the 30 extractions simultaneously (experiments performed in triplicates) [27]. A 5 mg sample of each selected coffee powder was extracted in MeOH applying the previously optimized standard extraction conditions (90 °C, 10 min) resulting in detected amounts of caffeine ranging between 129 and 165 μg, except for the decaffeinated samples containing only small amounts of caffeine (5 ± 2 μg caffeine/5 mg decaffeinato coffee, Table 4).

Further to the quantitative caffeine determination, a comparative qualitative analysis of the liquid phase coffee extracts and the headspace volatiles was performed, placing special emphasis on headspace analysis using solid-phase microextraction (SPME) techniques. Remarkable differences regarding peak pattern and main peaks were observed when low-temperature extraction (60 °C) and high-temperature extraction (160 °C) are compared prior to headspace-SPME–GC–MS performed in the same HPLC/GC vials. The SPME fibers were introduced into the vials directly after heating to 60 °C and 160 °C for 30 min, respectively, without opening the vials, preventing loss of volatile material (Fig. 11). Different main components and peak patterns were obtained applying headspace-SPME–GC–MS analysis when low-temperature (conventional heating of the samples on a standard hotplate/stirrer, Fig. 1c) and high-temperature extraction (microwave-assisted heating of the SiC platform, Fig. 1d) are compared. The results from the headspace-SPME–GC–MS experiments are shown in Fig. 12).

Conclusion

The SiC microtiter platforms developed since 2007 have proven to be very valuable tools for performing high-throughput experimentation, combining the benefits of both high-speed sealed vessel microwave chemistry and parallel processing. Different modifications (Fig. 1) have allowed their use not only for traditional library synthesis, but also for efficient reaction optimization/screening and in a variety of (bio)analytical applications. Reaction volumes can be as small as 50 μL and can reach up to 3 mL at a maximum temperature/pressure limit of 200 °C/20 bar.

An important difference to traditional parallel microwave chemistry using microtiter plates is the fact that the plate material itself (SiC) is strongly microwave absorbing, and therefore absorbs most of the microwave energy and will be heated to the desired target temperature very rapidly. Due to the high thermal conductivity and effusivity of the semiconducting SiC material no temperature gradients across the microtiter plate exist, and heat will be transferred efficiently to the contents inside the reaction wells/vials. By using strongly microwave absorbing silicon carbide as plate material, the microwave absorption characteristics of the individual reaction mixtures contained in the individual wells/vials are practically irrelevant, since the semiconducting plate itself will absorb microwave energy much stronger than any organic material contained inside the wells. This means that solvents/reaction mixtures having vastly different microwave absorption characteristics and boiling points can be readily heated to identical reaction temperatures, and can therefore be processed in parallel in the same microwave irradiation experiment.

Depending on the specific plate and rotor configuration up to 80–192 transformations can be carried out in parallel in a single microwave irradiation experiment using a dedicated multimode microwave instrument. Because of the high thermal conductivity of SiC, instead of using microwave irradiation, the SiC blocks can also be heated with a conventional hotplate/stirrer. In particular for low-temperature/pressure applications this has been a viable strategy.

It can be expected that these high-throughput platforms will be used for many different applications in the future, in all those instances where operating in a high-temperature/pressure regime under strict temperature control, and miniaturization and parallelization is important. Compared with the more traditional automated sequential processing mode involving single-mode microwave technology, the use of the SiC parallel platforms does not only allow a significant saving in time and efficiency, but also represents a considerable cost-saving aspect, as the used vials are standard disposable glass vials which cost a small fraction compared with the dedicated microwave vials used in combination with commercially available single-mode microwave reactors.

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