

Assessment and use of two silicon carbide multi-well plates for library synthesis and proteolytic digests using microwave heating

Lauren M. Stencel, Chad M. Kormos, Keri B. Avery and Nicholas E. Leadbeater*

Received 2nd February 2009, Accepted 24th March 2009

First published as an Advance Article on the web 21st April 2009

DOI: 10.1039/b902112d

The use of two silicon carbide plates is reported for the preparation of three libraries of organic molecules using microwave heating. In addition, a preliminary study has been carried out, showing that one of the plates can also be used in a proteomics setting. Both the 24-position and 48-position plates heated evenly when irradiated with microwave energy. The 48-position plate was used to prepare a library of *N*-aryl functionalized β -amino esters *via* an aza-Michael reaction between anilines and Michael acceptors. The 24-position plate was used to prepare a library of biaryls *via* a Suzuki coupling methodology and a library of 1,4-dihydropyridines *via* a Hantzsch synthesis. The 48-position plate was also used to perform the proteolytic digestion of insulin chain B by trypsin.

Introduction

Microwave heating offers an alternative to conventional heating methods and proves to be very useful in preparative organic chemistry.^{1,2} Although microwave heating of reactions may not always offer a direct rate enhancement over conventional heating under strictly comparable conditions, it is a very versatile tool because it offers reproducible non-contact heating as well as precise temperature monitoring and data recording. Taking advantage of the benefits of microwave heating and parallel processing, chemists have become interested in performing reactions in well plates.^{3,4} Although an attractive proposition, the use of well plates in a microwave unit has some significant problems. The most important issue to overcome is uneven heating across the plate. Many of the early reports using polypropylene, Teflon or high-temperature polyethylene plates showed that wells located on the periphery were at a significantly lower temperature than those on the inside due to radiative heat loss as well as lower microwave coupling. In an attempt to overcome this problem, plates doped with strongly microwave absorbing materials such as graphite have been used. More recently a 48-position plate made of silicon carbide has been used for parallel synthesis in a microwave unit with success.⁵ Silicon carbide is an inert, highly microwave absorbing material and has been previously used as a heating insert for reaction mixtures containing non-absorbing reagents or solvents.⁶ The use of a silicon carbide plate allows for equal heating of the wells. This was shown using IR thermal imaging. The plate was used for preparing a library of 2-aminopyrimidines.⁵ While it proves useful for rapid library preparation, a drawback of using the plate is that each well has a working volume of only 0.1–0.3 mL. In a recent development, a silicon carbide plate capable of holding up to 24 standard glass vials has become available.⁷ Each vial is capable of holding from 0.3–3 mL of reaction mixture. Again

uniform heating has been shown by IR thermography. The plate has been used for the preparation of a library of oxindoles and substituted benzimidazoles.

In our laboratory, we have performed a study on the heating characteristics of both silicon carbide plates (Fig. 1) using organic reactions as probes in addition to using the plates for the preparation of three libraries of compounds. In addition, we have used the 48-position plate to perform the digestion of insulin chain B using trypsin. We report our results here.



Fig. 1 (a) A 48-position silicon carbide plate; (b) A 24-position silicon carbide plate capable of holding standard glass vials.

Department of Chemistry, University of Connecticut, 55 North Eagleville Rd, Storrs, CT 06269-3060, USA. E-mail: nicholas.leadbeater@uconn.edu; Fax: +1 860 486 2981; Tel: +1 860 486 5076

Results and discussion

Organic chemistry

Our first objective was to determine how even the heating was across the plates as well as, in the case of the 24-position plate, the applicability of standard glass vials as reaction vessels. Starting with the 48-position plate, the previous literature report showed uniformity in heating for an esterification reaction. We decided to use the aza-Michael reaction between aniline and methyl acrylate catalyzed by acetic acid to yield an *N*-aryl functionalized β -amino ester as a probe for the heating characteristics of the block. This reaction was chosen because we have studied it extensively before.^{8,9} It is performed neat at 200 °C for 20 min. The pressure of the reaction mixture during the run peaks at around 200 psi. The chemistry is temperature dependent. Insufficient heating leads to poor yields; too much heating leads to side-product formation and decomposition. We loaded twelve representative wells in the plate with reaction mixture, placed a PFA foil across all the wells and then sealed the plate by attaching the Viton cushion and alumina top plate. The microwave unit is capable of holding four of the silicon carbide plates in a rotor. Although we performed all of our tests in one plate, for even rotor distribution we placed an empty plate in the vacant diagonally-opposite position. The plates were heated over a 5 min period to an external temperature of 180 °C as monitored using an IR sensor located on the bottom of the microwave unit. This corresponds to a temperature inside the wells of 200 °C since a calibration factor of 1.11 has to be used to equate the temperature read remotely by the infrared sensor to the actual temperature of the silicon carbide block.¹⁰ The plates were held at this temperature for 20 min before allowing them to cool to 50 °C, this taking approximately 15 min. We then determined the product conversion of the contents of each loaded well. The results are shown schematically in Fig. 2. With one exception (well A7) all the conversions laid within the range of 71–75%, and no by-product formation was observed in the NMR spectra of the product mixtures. This suggests that the heating

across the plate is uniform and the calibration factor accurately gauges the reaction temperature.

To probe the heating characteristics of the 24-position plate we turned our attention to the Suzuki coupling reaction between 4-bromoanisole and phenylboronic acid. We chose to use a 1:1 water/ethanol (v:v) mix as solvent, sodium hydroxide as base and ligandless palladium (0.002 mol%) as catalyst.^{11,12} We loaded eight vials with reaction mixture. We also introduced an additional variable, namely stirring. Given the relative size of the glass vials used in the 24-position plate, adequate mixing becomes necessary in order to ensure reaction-to-reaction reproducibility. The Synthos 3000 microwave unit is equipped with a magnetic stirrer under the rotor. To probe the efficiency of stirring in the plate, we decided to place small stir-bars in half the vials used. The loaded plates were heated over a 5 min period to an external temperature of 140 °C (internal temperature of ~156 °C) and held at this temperature for 20 min. Product conversions are shown schematically in Fig. 3. We know these Suzuki conditions to be fairly robust, so we were not surprised to see near complete conversion in six of the eight vessels. Interestingly, the two reactions that did not reach complete conversion were also reactions not containing stir-bars. Based on the higher overall yields of the stirred reactions as compared to the unstirred reactions, we concluded that the stirring mechanism is efficacious in increasing reaction-to-reaction reproducibility.

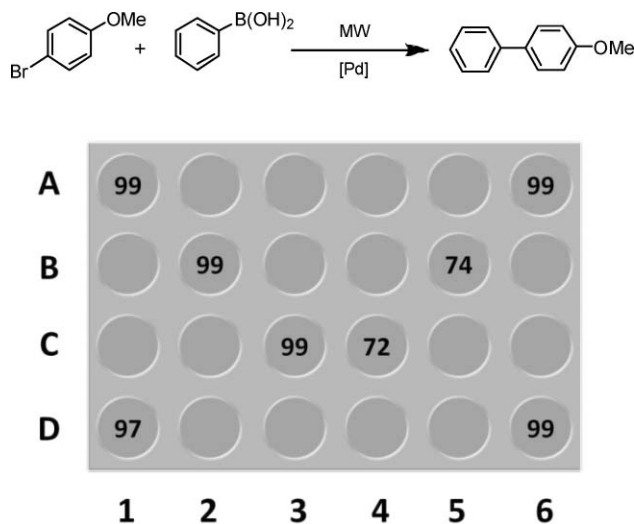


Fig. 3 Testing the temperature uniformity and stirring efficiency of the 24-position plate. Values shown represent conversions (%).

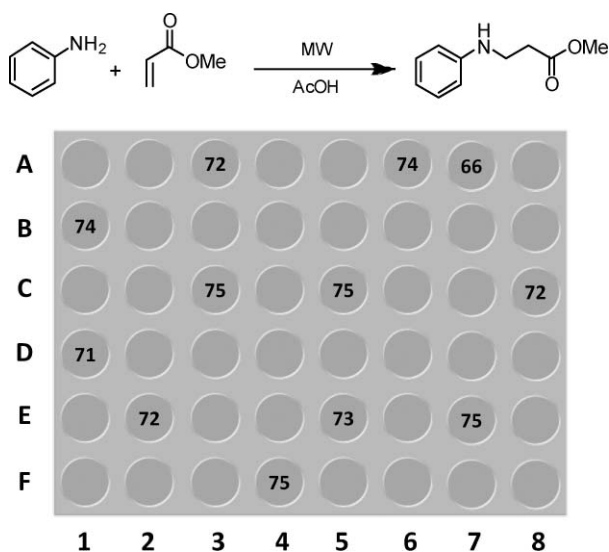


Fig. 2 Testing the temperature uniformity of the 48-position plate. Values shown represent product conversions (%).

We next turned our attention back to the 48-position plate with the objective of preparing a library of twelve *N*-aryl functionalized β -amino esters on the 1 mmol scale. We chose four anilines and three Michael acceptors. We knew from previous attempts to prepare a library of *N*-aryl functionalized β -amino esters using another set-up that homogeneity of temperature across each reaction vessel was essential.⁹ In our previous work we had initially attempted to prepare the library using a carousel of twelve sealed glass tubes, measuring the internal temperature of one using a fiber-optic probe and the external temperature of all twelve using an external IR sensor. We found significant temperature variation across the vessels. As a result the outcome of the reaction was dependent on which vessel was used as the temperature control during the course of the run. The effects could be mollified

by adding a small quantity of a highly microwave absorbing quaternary ammonium salt to each reaction vessel. Using the 48-position plate we believed that the highly microwave absorbing silicon carbide should ensure homogeneity of temperature across all the reaction mixtures. The results are shown pictorially in Fig. 4. The product yields obtained in the reactions of methyl acrylate with the four aniline substrates are comparable to those from our previous report using a monomode microwave apparatus in which we ran the reactions one at a time. This shows that the silicon carbide plate absorbs the majority of the microwave energy and thus facilitates uniform heating across the wells. The trend in reactivity across the aniline substrates is reflected in the product yields: 2,6-dimethylaniline is least reactive and 3-methoxyaniline (*m*-anisidine) most reactive.

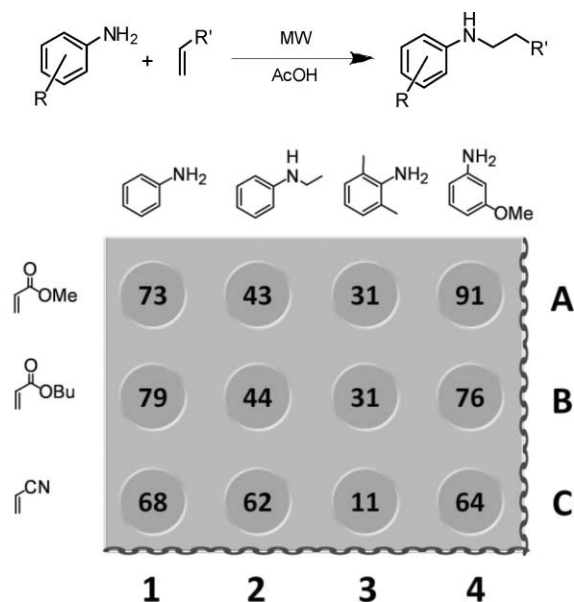


Fig. 4 Preparing a library of twelve *N*-aryl functionalized β -amino esters in the 48-position plate. Values shown represent product conversions (%).

Moving to the 24-position plate we prepared a library of twenty-four biaryls employing the Suzuki coupling protocol previously used. We screened four aryl bromides and six arylboronic acids, performing each reaction on the 0.25 mmol scale in glass vials. The results are shown in Fig. 5. 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.

Our final organic chemistry library as part of this study was a range of twenty-four 1,4-dihydropyridines, prepared in a multicomponent reaction between an aldehyde, a β -dicarbonyl compound and ammonia (Hantzsch synthesis). Our motivation behind choosing this reaction is that the dihydropyridine products have attracted considerable attention due to their biological activity.¹³ In addition, microwave heating has been used as a tool for preparing dihydropyridines.^{14–16} We screened six aldehyde substrates and four β -dicarbonyl compounds using a stoichiometric ratio of 1 mmol:5 mmol. A volume of 0.33 mL aqueous ammonium

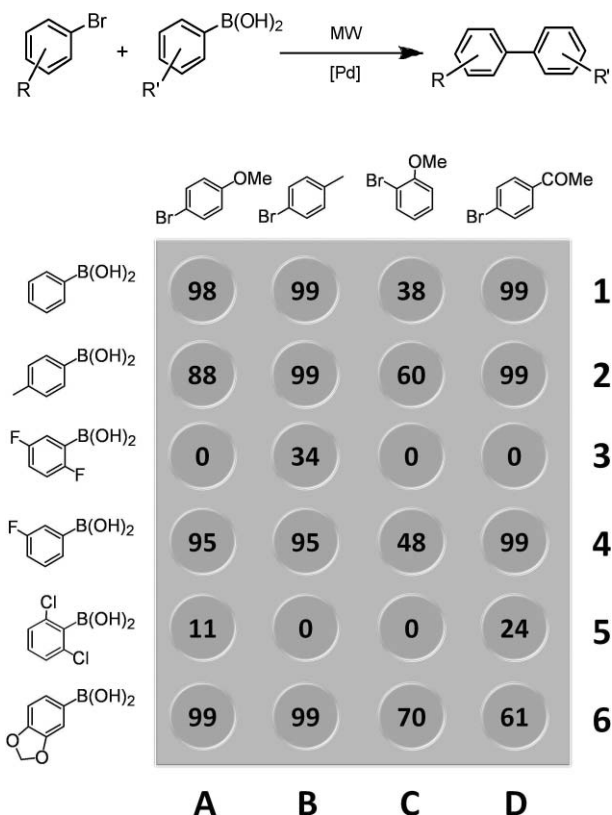


Fig. 5 Preparing a library of twenty-four biaryls in the 24-position plate. Values shown represent product conversions (%).

hydroxide (35%) was used both as the ammonia source and the solvent for the reaction. Vials were loaded, sealed and placed into the plate which was then heated to a target external temperature of 120 °C (internal temperature of ~140 °C). The plate was then held at this temperature for 10 min before allowing it to cool. The product conversions are shown in Fig. 6. The results varied considerably for this library; the best conversions being obtained with ethyl acetoacetate as the dicarbonyl component. Of the aldehydes screened, pyridine-2-carboxaldehyde and 3,4-methylene dioxylbenzaldehyde gave the best results. Our motivation for screening 2-nitrobenzaldehyde as a substrate comes from the fact that it is used in the synthesis of nifedipine (brand names Adalat, Nifedical, and Procardia), a 1,4-dihydropyridine that serves as a calcium channel inhibitor.¹⁷ Our library allowed us to prepare three analogs of this compound.

Proteolytic digestion

Scientists working in the biosciences have found increasingly that microwave heating can be an enabling technology in their research fields. One area that has seen considerable activity is the application of controlled microwave heating in proteolytic digestion. Reports have suggested that microwave heating can increase the efficiency of tryptic digestions compared to conventional heating.^{18–26} Since only very small volumes of material are generally used, it is difficult to know with certainty how much of the microwave energy actually couples with the sample and thus how evenly they are heated. A commercially available accessory is available for use with a monomode microwave unit. It comprises

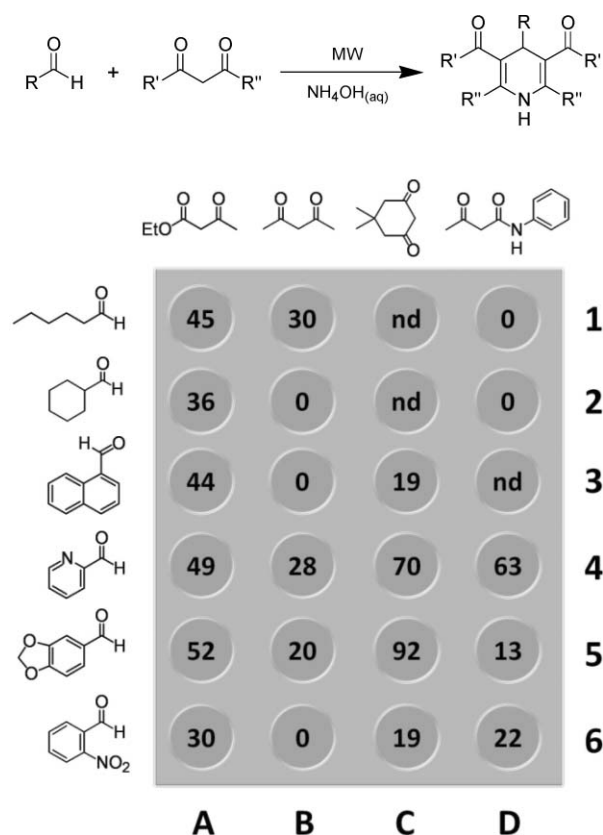


Fig. 6 Preparing a library of twenty-four 1,4-dihydropyridines in the 24-position plate. Values shown represent product conversions (%). [n.d. = not determinable due to complex mixture of product, starting material and by-products.]

a holder capable of containing up to 14 Eppendorf tubes.²⁷ It holds the tubes in a bath of moderating fluid (usually water) and IR thermography shows that, when irradiated with microwave energy, the tubes can be heated uniformly. For higher throughput, it would be useful to be able to use standard microtiter plates in conjunction with microwave heating. This way they could be interfaced with peripheral robotic instrumentation for loading, analyzing and cataloging. It occurred to us that the 48-position silicon carbide plate could be ideal for this and so decided to perform a preliminary study as part of our investigations.

Our first objective was to determine how uniform the results from a proteolytic digest were across the plate. We chose to perform the digestion of insulin chain B using trypsin as a test. Using an insulin chain B concentration of 1 mg/mL and a protease-to-protein ratio of 1:5 (w/w), we loaded twelve wells in the 48-position plate with 100 μ L aliquots of protein and trypsin solution. The plate was heated over a 5 min period to an external temperature of 45 °C (internal temperature of ~50 °C) where it was then held for a further 30 min. We then removed the plate and quenched the reactions by injecting a small volume (20 μ L) of 2 M hydrochloric acid into the filled wells. We then analyzed the contents of each well using HPLC to determine the level of digestion. We performed the heating study in triplicate and our results are shown schematically in Fig. 7. We find that the level of digestion is not highly dependent on the location on the plate, suggesting the heating is even across the wells.

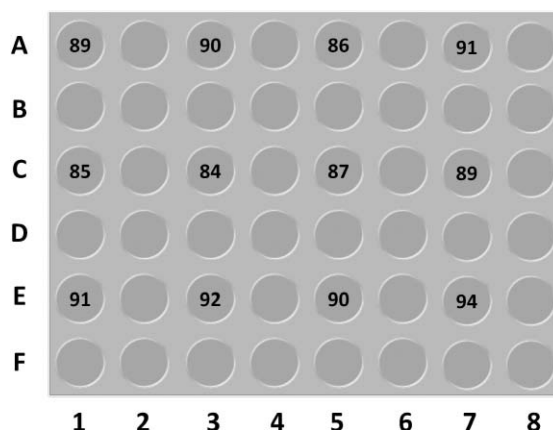


Fig. 7 Testing the uniformity of the 48-position plate for the digestion of insulin chain B using trypsin. Values shown represent amount of substrate digested (%).

We next wanted to use the plate to probe the effects of varying the protease-to-protein ratio on the extent of digestion. We filled the wells in the plate with solutions of protease-to-protein ratio 1:5, 1:10 and 1:25. We loaded four wells at each different ratio, thus filling a total of twelve wells. We sealed the plate and then heated it using an identical protocol as in our first trial (50 °C internal temp for 5 min ramp and 30 min hold) and upon completion analyzed the contents of each well. The results are shown schematically in Fig. 8. Not unexpectedly, the level of digestion decreases with increasing protease-to-protein ratio. Our main objective was to see whether, again, there was uniformity in results across the plate. We found this was indeed the case, the values for the four wells at each protease-to-protein ratio being comparable to each other.

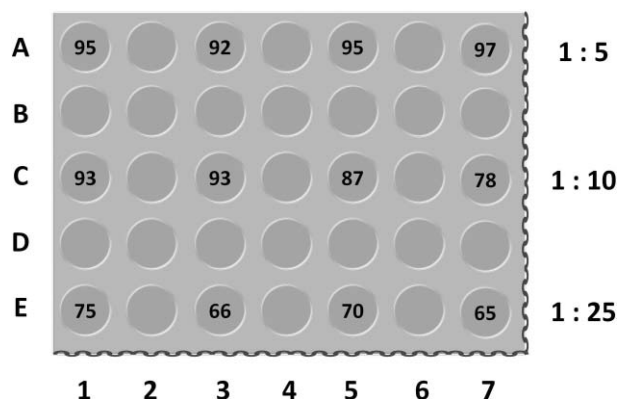


Fig. 8 Using the 48-position plate to probe the effects of varying the protease-to-protein ratio on the extent of digestion of insulin chain B using trypsin. Values shown represent amount of substrate digested (%) using 1:5, 1:10 and 1:25 ratios of protease-to-protein.

Conclusion

In summary, we have used two silicon carbide plates for the successful preparation of three libraries of organic molecules using microwave heating. In addition, we have performed a preliminary study that shows the 48-position plate can also be used in a proteomics setting. We first confirmed that both the 24- and 48-position plates heated evenly upon irradiation with

microwave energy. We then used the 48-position plate to prepare a library of *N*-aryl functionalized β -amino esters using an aza-Michael reaction between anilines and Michael acceptors. We used the 24-position plate to prepare libraries of biaryls and 1,4-dihydropyridines using Suzuki coupling and Hantzsch synthesis methodologies respectively. We used the 48-position plate to perform the digestion of insulin chain B using trypsin.

Experimental

General experimental

For the organic chemistry, all materials were obtained from commercial suppliers and used without further purification. ^1H - and ^{13}C -NMR spectra were recorded at 293 K on a 400 MHz spectrometer. For the proteolytic digestions, Trizma hydrochloride and insulin chain B oxidized from bovine pancreas were purchased from Sigma and TPCK trypsin was purchased and used as obtained from Pierce. HPLC analysis was performed using a Hewlett Packard Series 1100 HPLC.

Description and use of the microwave apparatus

A commercially available multimode microwave unit (Anton Paar Synthos 3000) was used for the reactions. The instrument is equipped with two magnetrons, with combined continuous microwave output power from 0 to 1400 W. Reactions were performed using either a ROTOR 4 $\times$ 48 MC Wellplate or a ROTOR 4 $\times$ 24 MG5. When using the 48-position plate, the individual wells of the plate are filled using a micropipettor. After filling, a PFA foil is used to cover the entire plate, the Viton cushion put in place followed by the alumina top plate and this held in place by six hexagonal bolts. The whole assembly is then placed on a dedicated plate rotor and a protective top cover locked in place. When using the 24-position plate, sealed glass vials are put into the wells, the plate placed on a dedicated plate rotor and a protective top cover locked in place. When using either plate, for weight-balance purposes either two or four plates need to be placed onto the rotor. The temperature of the plates is monitored using an IR sensor located on the bottom of the microwave unit. To ensure the proper internal reaction temperature, a calibration factor of 1.11 is applied (the temperature of the inside of the wells being 1.11 times that measured on the outside of the plate). After cooling, the vials in the 24 position rotor can be removed and the contents easily accessed by puncturing the PTFE seal with a syringe or else removing the vial top entirely. In the case of the 48-position rotor, the contents of each well can be removed using a syringe. The wells can be rinsed with solvent to obtain the product quantitatively, again this operation can be performed on each individual well with a syringe. When needed, reaction mixtures were stirred by means of a rotating magnetic plate located below the floor of the microwave cavity and a Teflon-coated magnetic stir bar in the wells.

Experimental procedures

Library of twelve *N*-aryl functionalized β -amino esters. The substituted-aniline (1.0 mmol), Michael acceptor (1.0 mmol), and acetic acid (0.35 mmol, 20 μL) were combined directly in a well of the 48-position silicon carbide block. The block was sealed, placed

onto the rotor together with an empty plate on the diagonally opposite position, the protective top cover locked in place and then the entire rotor assembly placed into the microwave unit. The plates were heated to an external temperature of 180 $^\circ\text{C}$ as measured by the IR sensor (~ 200 $^\circ\text{C}$ internal temperature) over a 5 min period ramping up to a microwave power of 1200 W. The plate was then held at this temperature for an additional 20 min. Upon cooling, an aliquot from each occupied well was removed, analyzed using ^1H -NMR spectroscopy and the product conversion determined. Anilines used: aniline (90 μL), *N*-ethyl aniline (126 μL), 2,6-dimethylaniline (123 μL), 3-methoxyaniline (112 μL). Michael acceptors used: methyl acrylate (90 μL), *n*-butyl acrylate (143 μL), acrylonitrile (66 μL).

Library of twenty-four biaryls. Aqueous sodium hydroxide (1.00 mL, 0.5 M), a solution of aryl bromide (0.500 mL, 0.50 M in ethanol), a solution of aryl boronic acid (0.500 mL, 0.65 M in ethanol), and palladium (50 μL , 10 $\mu\text{g}/\text{mL}$ in 0.05 wt.% aq. HCl, prepared by dilution of an ICP standard solution) were combined in a Wheaton[®] 15 $\times$ 46 mm screw cap vial equipped with a small Teflon-coated magnetic stir-bar. The vial was sealed, capped and placed into a well of the 24-position silicon carbide plate. Once all the vials had been loaded, the filled plate was placed onto the rotor together with an empty plate on the diagonally opposite position, the protective top cover locked in place and then the entire rotor assembly placed into the microwave unit. The plates were heated to an external temperature of 140 $^\circ\text{C}$ as measured by the IR sensor (~ 156 $^\circ\text{C}$ internal temperature) over a 5 min period ramping up to a microwave power of 1200 W. The plate was then held at this temperature for an additional 20 min. Upon cooling, the contents of each vial were each rapidly acidified with 2 M HCl to stop any further reaction. Each product mixture was then individually worked up. This involved emptying the contents of the vial into a separatory funnel, extracting with diethyl ether twice, combining the organics, drying with magnesium sulfate and finally evaporating the solvent under reduced pressure. Product conversions were determined by using ^1H -NMR spectroscopy in the presence of an internal standard.

Library of twenty-four 1,4-dihydropyridines. Aldehyde (1 mmol), β -ketoester (5 mmol), and ammonium hydroxide (0.33 mL) were combined in a Wheaton[®] 15 $\times$ 46 mm screw cap vial equipped with a small Teflon-coated magnetic stir-bar. The vial was sealed, capped and placed into a well of the 24-position silicon carbide plate. Once all the vials had been loaded, the filled plate was placed onto the rotor together with an empty plate on the diagonally opposite position, the protective top cover locked in place and then the entire rotor assembly placed into the microwave unit. The plates were heated to an external temperature of 125 $^\circ\text{C}$ as measured by the IR sensor (~ 140 $^\circ\text{C}$ internal temperature) over a 5 min period ramping up to a microwave power of 1000 W. The plate was then held at this temperature for an additional 10 min. Upon cooling, methanol was added to the contents of each vial and crude NMR conversions obtained.

Digestion of insulin chain B to test the heating characteristics of the SiC plate. Two stock solutions were prepared, one containing insulin chain B (~ 1 mg) in 1 mL of 100 μM Trizma hydrochloride solution, the other trypsin (~ 1 mg) in 5 mL of 100 μM Trizma

hydrochloride solution. A 100 μL of the trypsin solution and 100 μL of the protein solution were combined directly in each required well of the 48-position silicon carbide block, a total of 12 wells being filled. The block was sealed, placed onto the rotor together with an empty plate on the diagonally opposite position, the protective top cover locked in place and then the entire rotor assembly placed into the microwave unit. The plates were heated to an external temperature of 45 $^{\circ}\text{C}$ as measured by the IR sensor (~ 50 $^{\circ}\text{C}$ internal temperature) over a 5 min period ramping up to a microwave power of 600 W. The plate was then held at this temperature for an additional 30 min. Upon cooling, the contents of each occupied well was quenched by the addition 20 μL of 2M HCl. This was followed by immediate HPLC analysis. A 15 μL aliquot of the reaction was separated on a reverse phase C_{18} column with a 25 minute linear gradient from 5 to 55% of 0.1% trifluoroacetic acid in acetonitrile at a 1 min/mL flow rate with UV detection at 210 nm. On the chromatogram, the area under each peak was integrated (one peak at 15.6 min for undigested insulin chain B [FVNQHLC $_{\alpha}$ GSHLVEALYLVC $_{\alpha}$ GERGFFYTPKA, and two peaks at 14.1 min [FVNQHLC $_{\alpha}$ GSHLVEALYLVC $_{\alpha}$ GER] and 11.6 min [GFFYTPK] corresponding to digested fragments). The relative areas of undigested and digested protein were used to determine the level of digestion.

Using the 48-position plate to probe the effects of varying the protease-to-protein ratio on the extent of digestion of insulin chain B using trypsin. Two stock solutions were prepared, one containing insulin chain B (~ 1 mg) in 1 mL of 100 μM Trizma hydrochloride solution, the other trypsin (~ 1 mg) in 1 mL of 100 μM Trizma hydrochloride solution. From the trypsin stock solution, three dilutions were prepared, one a 5-fold, one a 10-fold and one a 25-fold. A 100 μL of the requisite trypsin solution and 100 μL of the protein solution were combined directly in each required well of the 48-position silicon carbide block, a total of 12 wells being filled (4 at a protease-to-protein ratio of 1:5, 4 at 1:10 and 4 at 1:25). The block was heated and the products analysed in an identical protocol to that used for assessing the heating characteristics of the SiC plate.

Acknowledgements

Anton Paar USA are thanked for access to the silicon carbide plates and assistance from Michael Canavan and Reinhardt Klopfer is acknowledged. Funding from the American Chemical Society Petroleum Research Foundation (45433-AC1) and the National Science Foundation REU program at the University of Connecticut is acknowledged.

Notes and references

- For books on the use of microwave heating in organic synthesis, see: (a) A. Loupy, Ed., *Microwaves in Organic Synthesis*, Wiley-VCH, Weinheim, 2006; (b) C. O. Kappe and A. Stadler, *Microwaves in Organic and Medicinal Chemistry*, Wiley-VCH, Weinheim, 2005; (c) P. Lidström and J. P. Tierney, Eds., *Microwave-Assisted Organic Synthesis*, Blackwell, Oxford, 2005.
- For recent reviews see: (a) C. O. Kappe, *Chem. Soc. Rev.*, 2008, **37**, 1127; (b) V. Polshettiwar and R. S. Varma, *Chem. Soc. Rev.*, 2008, **37**, 1546; (c) C. O. Kappe, *Angew. Chem., Int. Ed.*, 2004, **43**, 6250.
- For reviews see: (a) C. O. Kappe and M. Matloobi, *Comb. Chem. High Throughput Screening*, 2007, **10**, 735; (b) W. M. Dai and J. Y. Shi, *Comb. Chem. High Throughput Screening*, 2007, **10**, 837; (c) M. Nüchter and B. Ondruschka, *Mol. Divers.*, 2003, **7**, 253.
- For examples see: (a) L. Pisani, H. Prokopcová, J. M. Kremsner and C. O. Kappe, *J. Comb. Chem.*, 2007, **9**, 415; (b) J. Alcázar, *J. Comb. Chem.*, 2005, **7**, 353; (c) M.-D. H. De Bas and D. F. O'Shea, *J. Comb. Chem.*, 2005, **7**, 947; (d) G. A. Strohmeier and C. O. Kappe, *J. Comb. Chem.*, 2002, **4**, 154; (e) M. C. Coleman, J. M. D. MacElroy, J. F. Gallagher and D. F. O'Shea, *J. Comb. Chem.*, 2002, **4**, 87.
- J. M. Kremsner, A. Stadler and C. O. Kappe, *J. Comb. Chem.*, 2007, **9**, 285.
- J. M. Kremsner and C. O. Kappe, *J. Org. Chem.*, 2006, **71**, 4651.
- M. Treu, T. Karner, R. Kousek, H. Berger, M. Mayer, D. B. McConnell and A. Stadler, *J. Comb. Chem.*, 2008, **10**, 863.
- K. M. Amore, N. E. Leadbeater, T. A. Miller and J. R. Schmink, *Tetrahedron Lett.*, 2006, **47**, 8583.
- N. E. Leadbeater and J. R. Schmink, *Tetrahedron*, 2007, **63**, 6764.
- The difference between internal and external temperature arises due to air flow through the rotor. This flow actively maintains an appropriate temperature for rotor parts that may otherwise overheat during extended runs.
- For a review of the Suzuki reaction using water as a solvent in conjunction with microwave heating see: N. E. Leadbeater, *Chem. Commun.*, 2005, 2881.
- (a) N. E. Leadbeater, V. A. Williams, T. M. Barnard and M. J. Collins, *Org. Proc. Res. Dev.*, 2006, **10**, 833; (b) R. K. Arvela, N. E. Leadbeater and M. J. Collins, *Tetrahedron*, 2005, **61**, 9349; (c) R. K. Arvela and N. E. Leadbeater, *Org. Lett.*, 2005, **7**, 2101; (d) R. K. Arvela, N. E. Leadbeater, M. S. Sangi, V. A. Williams, P. Granados and R. D. Singer, *J. Org. Chem.*, 2005, **70**, 1786.
- See for example: (a) A. E. Thompson and J. E. Pope, *Rheumatology*, 2005, **44**, 145; (b) P. A. Poole-Wilson, B. A. Kirwan, Z. Vokó, S. de Brouwer, F. J. van Dalen and J. Lubsen, *Cardiovas. Drugs Ther.*, 2006, **20**, 45; (c) A. Di Stilo, S. Visentin, C. Clara, A. M. Gasco, G. Ermondi and A. Gasco, *J. Med. Chem.*, 1998, **41**, 5393; (d) R. A. Coburn, M. Wierzbica, M. J. Suto, A. J. Solo, A. M. Triggles and D. J. Triggles, *J. Med. Chem.*, 1988, **31**, 2103.
- For a review see: J. J. Vanden Eynde and A. Mayence, *Molecules*, 2003, **8**, 381.
- (a) S. Torchy, G. Cordonnier, D. Barbry and J. J. Vanden Eynde, *Molecules*, 2002, **7**, 528; (b) L. Ohberg and J. Westman, *Synlett*, 2001, 1296; (c) R. Alajarin, P. Jordan, J. J. Vaquero and J. Alvarez-Builla, *Synthesis*, 1995, 389; (d) Y.-W. Zhang, Z.-X. Shan, B. Pan, X.-H. Lu and M.-H. Chen, *Synth. Commun.*, 1995, **25**, 857; (e) B. M. Khadilkar, V. G. Gaikar and A. A. Chitnavis, *Tetrahedron Lett.*, 1995, **36**, 8083; (f) M. Suarez, A. Loupy, E. Perez, L. Moran, G. Gerona, A. Morales and M. Autié, *Heterocycl. Commun.*, 1996, **2**, 275; (g) V. Sivamurugan, A. Vinu, M. Palanichamy and V. Murugesan, *Heteroatom Chem.*, 2006, **17**, 267; (h) R. Alajarin, J. J. Vaquero, J. L. Garcia Navio and J. Alvarez-Builla, *Synlett*, 1992, 297.
- M. D. Bowman, J. L. Holcomb, C. M. Kormos, N. E. Leadbeater and V. A. Williams, *Org. Proc. Res. Dev.*, 2008, **12**, 41.
- For a review see: R. Pontremoli, G. Leoncini and A. Parodi, *Expert Rev. Cardiovasc. Ther.*, 2005, **3**, 43.
- W. N. Sandoval, V. Pham, E. S. Ingle, P. S. Liu and J. R. Lill, *Comb. Chem. High Throughput Screening*, 2007, **10**, 751.
- J. R. Lill, E. S. Ingle, P. S. Liu, V. Pham and W. N. Sandoval, *Mass Spectrom. Rev.*, 2007, **26**, 657.
- J. M. Collins and N. E. Leadbeater, *Org. Biomol. Chem.*, 2007, **5**, 1141.
- X. Liu, K. Chan, I. K. Chu and J. J. Li, *Carbohydrate Res.*, 2008, **343**, 2870.
- S. Lin, G. P. Yao, D. W. Qi, Y. Li, C. H. Deng, P. Y. Yang and X. M. Zhang, *Anal. Chem.*, 2008, **80**, 3655.
- B. N. Pramanik, U. A. Mirza, Y. H. Ing, Y.-H. Liu, P. L. Bartner, P. C. Weber and A. K. Bose, *Protein Sci.*, 2002, **11**, 2676.
- W. Sun, S. Gao, L. Wang, Y. Chen, S. Wu, X. Wang, D. Zheng and Y. Gao, *Mol. Cell Proteomics*, 2006, **5**, 769.
- H. W. Vesper, L. Mi, A. Enada and G. L. Myers, *Rapid Commun. Mass Spectrom.*, 2005, **19**, 2865.
- H.-F. Juan, S.-C. Chang, H.-C. Huang and S.-T. Chen, *Proteomics*, 2005, **5**, 840.
- CEM Corp. <http://www.cem.com/biosciences/>.