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Rapid solid-phase peptide synthesis using thermal and controlled microwave irradiation

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Abstract: A rapid and efficient microwave-assisted solid-phase synthesis method is described for the preparation of the nonapeptide WDTVRISFK, using conventional Fmoc/Bu^t orthogonal protection strategy. The synthesis protocol is based on the use of cycles of pulsed microwave irradiation with intermittent cooling of the reaction during the removal of the Fmoc protecting group and during the coupling. The desired nonapeptide was obtained in highest yield and purity by employing MicroKan technology. The chemical reactions were carried out in a single-mode microwave reactor, equipped with a fiber-optic probe to monitor the reaction temperature continuously. Copyright © 2006 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: solid-phase peptide synthesis; microwave irradiation; microwave-assisted organic synthesis; microwave-assisted peptide synthesis; MicroKan

INTRODUCTION

In the past few years, heating and driving chemical reactions by MW energy has been an increasingly popular theme with the scientific community. This nonclassical heating technique is slowly moving from a laboratory curiosity to an established technique, and is heavily used in both academia and industry. The efficiency of 'microwave flash heating' in dramatically reducing reaction time and increasing product yield/purity is one of the key advantages of this technology [1–7].

Apart from applications in the area of standard solution-phase organic synthesis (SPOS), there are a growing number of publications that report the use of MW heating for SPOS or in conjunction with polymer-supported reagents and catalysts, using a variety of different polymeric materials as insoluble supports [1,2]. Surprisingly, although solid-phase synthesis was originally introduced in the peptide field (solid-phase peptide synthesis (SPPS) [8]), there are only a few reports on the use of MW irradiation for the preparation of peptides and related species on solid phase [9–15]. While some of these studies have discussed the beneficial effects of MW irradiation for SPPS in a qualitative way, not all the reported experiments were conducted in dedicated MW reactors that allow an adequate temperature control of the reaction mixture [9,12,14]. In addition, some of the published peptide sequences were comparatively short (tripeptides), or had amino acid residues that did not require side-chain protection [9,10]. At the same time, a detailed investigation of the solid-phase synthesis of β-peptides was recently published by Murray and Gellman [11]. In this paper, the products obtained by conventional heating and MW heating in a dedicated MW reactor are compared. The authors report that for longer β-peptide report sequences MW irradiation showed a clear superiority over conventional heating in terms of both coupling yield and purity.

We report here the preparation of a nonapeptide containing amino acid residues that require side-chain protection under various conditions by microwave-assisted SPPS, using a precooled reaction vessel in combination with pulsed MW irradiation. For monitoring the reaction temperature during the MW-assisted coupling and Fmoc removal, an internal FO sensor was used.

MATERIALS AND METHODS

General

All commercially available solvents and reagents were used without further purification. DMF, DCM, methanol, TFA, and DIC were purchased from Sigma-Aldrich Kft. (Budapest, Hungary); Wang resin (substitution 0.91 mmol/g), HOBt, and Fmoc-protected amino acids were obtained from Novabiochem (Merck Kft., Budapest, Hungary). Side chains of the amino acids used in the synthesis were protected as follows: Boc (Lys, Trp), Bu^t (Ser, Thr), OBu^t (Asp), and Pbf (Arg).

Abbreviations: As recommended in *J. Pept. Sci.* 2003; **9**: 1–8 and URL: http://www.chem.qmw.ac.uk/iupac/AminoAcid/ with the following additions and variations: SPPS, solid-phase peptide synthesis; SPOS, solid-phase organic synthesis; Pbf, 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl-; PTFE, polytetrafluoroethylene; MW, microwave; FO, fiber optic; MS, mass spectrometry; ESI-MS, electrospray ionization mass spectrometry.

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Microwave-assisted synthesis was performed in a single-mode CEM Discover reactor (CEM Corporation, Matthews, NC, USA) equipped with an external infrared (IR) and an internal FO temperature sensor, using standard 10 ml Pyrex glass vials as reaction vessels. Libra tubes RT3M100 (2-ml polypropylene syringe with frit and a PTFE valve) were obtained from Shimadzu Corp. (Kyoto, Japan). MicroKans were kindly provided by Discovery Partners Ltd. (San Diego, CA, USA). The LC-MS analysis was performed on a Waters Alliance LC (Millford, MA, USA) system, equipped with a Micromass Quadrupole MS detector and operated by a Waters Alliance software. For separation, Cosmosil $5C_{18}$ AR-II (Nacali Tesque Inc., Kyoto, Japan) column (250 mm × 4.6 mm) was used by applying a linear gradient of 5–50% B in 40 min. The mobile phases were 0.1% TFA in H_2O–MeCN, 9 : 1, v/v (eluent A), and 0.1% TFA in H_2O–MeCN, 1 : 9, v/v (eluent B). The flow rate was 1 ml/min. The detection was at 220 nm.

First Residue Loading

A mixture of Fmoc-Lys(Boc)-OH (238 mg, 0.47 mmol), DIC (73 μl, 0.47 mmol), and HOBt (72.6 mg, 0.47 mmol) was preactivated in 1 ml dry DMF and then added to Wang resin (104.2 mg, 0.095 mmol) which was preswollen in 1 ml of DCM for 30 min before use. Subsequently, DMAP (5.8 mg, 0.047 mmol) dissolved in DMF (0.1 ml) was added, and the resin was shaken at ambient temperature for 3 h. This coupling step was repeated twice. The resin was successively washed with DMF, DCM, and methanol (5 times each) and dried under reduced pressure for 3 h. A small portion of the resin (4–6 mg) was subjected to analysis for Fmoc-group content, and this step indicated a substitution level of 0.66 mmol/g (73%). For end-capping, the resin was shaken with a 2 ml mixture of Ac_2O/pyridine/DCM (2 : 3 : 5) at room temperature for 30 min, and was thoroughly washed with DCM, DMF, DCM, and methanol (5 times each).

Preparation of Nonapeptide H-Trp-Asp-Thr-Val-Arg-Ile-Ser-Phe-Lys-OH

Synthesis under standard SPPS conditions (16) (Method A). In a Libra tube, 0.5 ml of 20% piperidine in DMF was added to 30 mg (0.02 mmol) Fmoc-Lys(Boc)-Wang resin and the suspension was shaken at ambient temperature for 20 min. The resin was then washed with DMF, DCM, and methanol (5 times each). In a separate vial, the corresponding Fmoc-amino acids (0.06 mmol in 50 μl DMF), DIC (0.06 mmol, 10 μl), and HOBt (0.06 mmol in 50 μl DMF) were combined. The preactivated coupling cocktail was added to the resin suspended in DCM–DMF (1 : 1, 400 μl), and the reaction mixture was shaken at room temperature for 1 h. The completion of each coupling step was confirmed by the Kaiser test. After the last deprotection step, the peptidyl-resin was dried under reduced pressure.

Synthesis under pulsed microwave irradiation with intermittent cooling to ambient temperature (Method B). The Fmoc-Lys(Boc)-Wang resin (133.8 mg, 0.088 mmol) was transferred to a MW reaction vial, and 1.25 ml of 20% piperidine in DMF was added. The vial was placed in a MW reactor and the mixture was irradiated for 30 s (at constant power, 40 W; temp. monitored with the FO sensor). Then the vial was removed from the MW cavity together with the temperature probe, and cooled down to ambient temperature (*ca* 1 min) with tap water. This process was repeated two more times. After deprotection, the suspension was transferred to a Libra tube and washed with DMF, DCM, and methanol (5 times each). In a separate vial, the corresponding Fmoc-amino acid (0.26 mmol in 200 μl DMF), DIC (0.26 mmol, 38 μl), and HOBt (0.26 mmol in 100 μl DMF) were combined. This preactivated coupling cocktail was added to the resin, preswollen in DCM–DMF (1 : 1, 800 μl), in a MW vial. Then, the sample was irradiated 4 times (30 s each) in a MW reactor (at constant power, 30 W; temp. monitored with the FO sensor). Between each irradiation step, the reaction vessel was removed from the MW cavity, and cooled down to ambient temperature (*ca* 2 min) with tap water. After completing the coupling step (confirmed by the Kaiser test), the suspension was transferred to a Libra tube and washed with DMF, DCM, and methanol (5 times each). After coupling the last amino acid residue, the *N*-terminal Fmoc-group was removed and the side-chain protected peptidyl-resin was dried under reduced pressure.

Synthesis under pulsed microwave irradiation with intermittent cooling to subambient temperature (Method C). The Fmoc-Lys(Boc)-Wang resin (132.4 mg, 0.087 mmol) was transferred to a MW reaction vial and 1.25 ml of 20% piperidine in DMF was added. The vial was cooled down to 0 °C in an ice bath (*ca* 2 min), and then was placed in the MW reactor and irradiated for 30 s (at constant power, 40 W; temp. monitored with the FO sensor). Subsequently, the vial was removed from the MW cavity together with the temperature probe, put in an ice bath, and kept there until the inside temperature reached 0 °C (*ca* 2 min). This process was repeated two more times. After deprotection, the suspension was transferred into a Libra tube and washed with DMF, DCM, and methanol (5 times each). In a separate vial, the corresponding Fmoc-amino acid (0.26 mmol in 200 μl DMF), DIC (0.26 mmol, 38 μl), and HOBt (0.26 mmol in 100 μl DMF) were combined. This preactivated coupling cocktail was added to the resin, preswollen in 800 μl DCM–DMF (1 : 1), in a MW vial. The reaction mixture was put in an ice bath and was kept there until the inside temperature reached 0 °C (*ca* 2 min). Subsequently, the sample was irradiated 4 times (30 s each) in a MW reactor (at constant power, 30 W; temp. monitored with the FO sensor). Between each irradiation step, the vial was removed from the MW cavity, put in an ice bath, and kept there until the inside temperature reached 0 °C (*ca* 2 min). After completing the coupling step (confirmed by the Kaiser test), the resin was transferred into a Libra tube and washed with DMF, DCM, and methanol (5 times each). After coupling the last amino acid residue, the *N*-terminal Fmoc-group was removed and the side-chain protected peptidyl-resin was dried under reduced pressure.

Synthesis in a MicroKan under pulsed microwave irradiation with intermittent cooling to subambient temperature (Method D). The Fmoc-Lys(Boc)-Wang resin (38.4 mg, 0.025 mmol) was placed in a MicroKan capsule. (During all synthesis steps the beads remained in the capsule, and were removed just before the final cleavage!) The MicroKan was transferred to a standard 10 ml MW process vial and 1.25 ml of 20% piperidine in DMF was added to the resin. The vial was put in an ice bath and kept there until the inside temperature reached 0 °C (*ca* 2 min). It was then placed in the MW reactor and irradiated for 30 s (at constant power, 40 W). The vial was

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then removed from the MW cavity and cooled down again to 0 °C (*ca* 2 min). This process was repeated two more times. Subsequently, the MicroKan was transferred to a Libra tube and washed with DMF, DCM, and methanol (5 times each). In a separate vial, Fmoc-amino acid (0.23 mmol in 200 μl DMF), DIC (0.23 mmol, 34 μl), and HOBt (0.23 mmol in 100 μl DMF) were combined. This preactivated coupling cocktail was added to the MicroKan preswollen in 800 μl DCM–DMF (1 : 1) in a MW vial. The vial was placed in an ice bath, and was kept there until the inside temperature reached 0 °C (*ca* 2 min). Subsequently, the sample was irradiated 4 times (30 s each) in a MW reactor (at constant power, 30 W). Between each irradiation step, the vial was removed from the MW cavity, put in an ice bath, and cooled down to 0 °C (*ca* 2 min). After the coupling step, the MicroKan was transferred to a Libra tube and washed with DMF, DCM, and methanol (5 times each). After coupling the last amino acid residue, the *N*-terminal Fmoc-group was removed, and the side-chain protected peptidyl-resin was removed from the MicroKan and dried under reduced pressure. Since it was physically not possible to introduce the FO probe inside the MW vessel in the presence of the MicroKan, the experiments were run under power control, assumed to lead to the same temperature profile.

Synthesis in oil bath at 65 °C (Method E). The Fmoc-Lys(Boc)-Wang resin (30 mg, 0.02 mmol) was transferred to a Libra tube, 0.5 ml of 20% piperidine in DMF was added to the resin, and the reaction vessel was placed in oil bath at 65 °C for 7.5 min. The suspension was then washed with DMF, DCM, and methanol (5 times each). In a separate vial, the corresponding Fmoc-amino acid (0.06 mmol in 50 μl DMF), DIC (0.06 mmol, 10 μl), and HOBt (0.06 mmol in 50 μl DMF) were combined. The preactivated coupling cocktail was added to the resin suspended in 400 μl DCM–DMF (1 : 1) and the reaction vessel was kept at room temperature for 1 h. The completion of each coupling step was confirmed by a ninhydrin test. After the last deprotection step, the peptidyl-resin was dried under reduced pressure.

Final Cleavage from the Resin

The peptide was cleaved from the solid support with a cleavage cocktail (2 ml) of TFA/ethanedithiol/thioanisole/water/phenol (10 ml : 0.25 ml : 0.5 ml : 0.5 ml : 0.75 mg) under vigorous shaking at ambient temperature for 3 h. The resin was filtered, and washed with a small amount of cleavage cocktail. The combined filtrates were concentrated under a stream of nitrogen gas. The residual product was precipitated with ice-cold diethyl ether and the peptide was collected by filtration, dissolved in deionized water, and lyophilized.

RESULTS AND DISCUSSION

Our interest lies in the combinatorial synthesis of oligopeptides having significant inhibitor activity against calmodulin-dependent enzymes. As a model, we have chosen a nonapeptide containing the calmodulin-binding (CaM-binding) octapeptide sequence [17] with an additional Lys residue at the *C*-terminus (H-Trp-Asp-Thr-Val-Arg-Ile-Ser-Phe-Lys-OH). (The ε-amino group was included to offer a binding site for covalent modification with amine-reactive fluorescent probes.) By using standard Fmoc/Bu^t orthogonal protection, the SPPS of H-Trp(Boc)-Asp(OBu^t)-Thr(Bu^t)-Val-Arg(Pbf)-Ile-Ser(Bu^t)-Phe-Lys(Boc)-OH was carried out on polystyrene Wang resin. The first amino acid residue (Fmoc-Lys(Boc)-OH) was attached to the resin by using a standard coupling protocol (triple coupling, fivefold excess reagent cocktail) using DIC, HOBt in DMF, and a catalytic amount of DMAP; the unreacted hydroxyl groups of the resin were blocked with acetic anhydride to avoid the formation of truncated peptides.

To compare, the 4-step synthesis cycles (deprotection, washing, coupling, and washing) – starting from the Fmoc-Lys(Boc)-Wang resin and leading to the side-chain protected model nonapeptide – were carried out under five different reaction conditions: Method A: standard SPPS protocol at room temperature [16]; Method B: pulsed MW irradiation with intermittent cooling of the reaction mixture to ambient temperature (*ca* 20 °C); Method C: pulsed MW irradiation with intermittent cooling of the reaction mixture to subambient temperature (*ca* 0 °C); Method D: pulsed MW irradiation in MicroKan reactors with intermittent cooling of the reaction mixture to subambient temperature (*ca* 0 °C); and Method E: SPPS protocol in oil bath at 65 °C.

For the removal of the Fmoc-group, in all cases, 20% piperidine in DMF was used. In the coupling steps, a reagent cocktail (threefold molar excess of Fmoc-amino acid, DIC, and HOBt) in a DCM–DMF mixture (1 : 1) was used. However, in Method D, the resin particles were encapsulated in IRORI MicroKan made of a Teflon mesh derivative. In order to fully cover the capsule, it was necessary to use a larger reagent volume. In this case, a compromise should be made: use of either the same reagent excess and a diluted reagent cocktail, or the same reagent concentration but a larger reagent excess. In trial experiments, we found that coupling yields are very poor at low reagent concentrations (data not shown). Thus, the latter approach was preferred, i.e. a larger volume of the same concentrated reagent cocktail (corresponding to an overall ninefold reagent excess) was applied.

The final cleavage of the nonapeptide from the resin was performed under identical conditions in all cases. Thus, the major difference between the conventional and MW strategy was in the duration of the reactions, reaction temperature, and in the handling of the resin suspension.

The HPLC/MS analysis of the crude peptides demonstrated that, in all cases, the main chromatographic peak corresponded to the expected nonapeptide (Figure 1). The purity was calculated as the area percent of the main peak over all peaks.

All steps in the standard SPPS (Method A) were carried out in a single polypropylene tube with a frit attached (Shimadzu Libra tube) providing the

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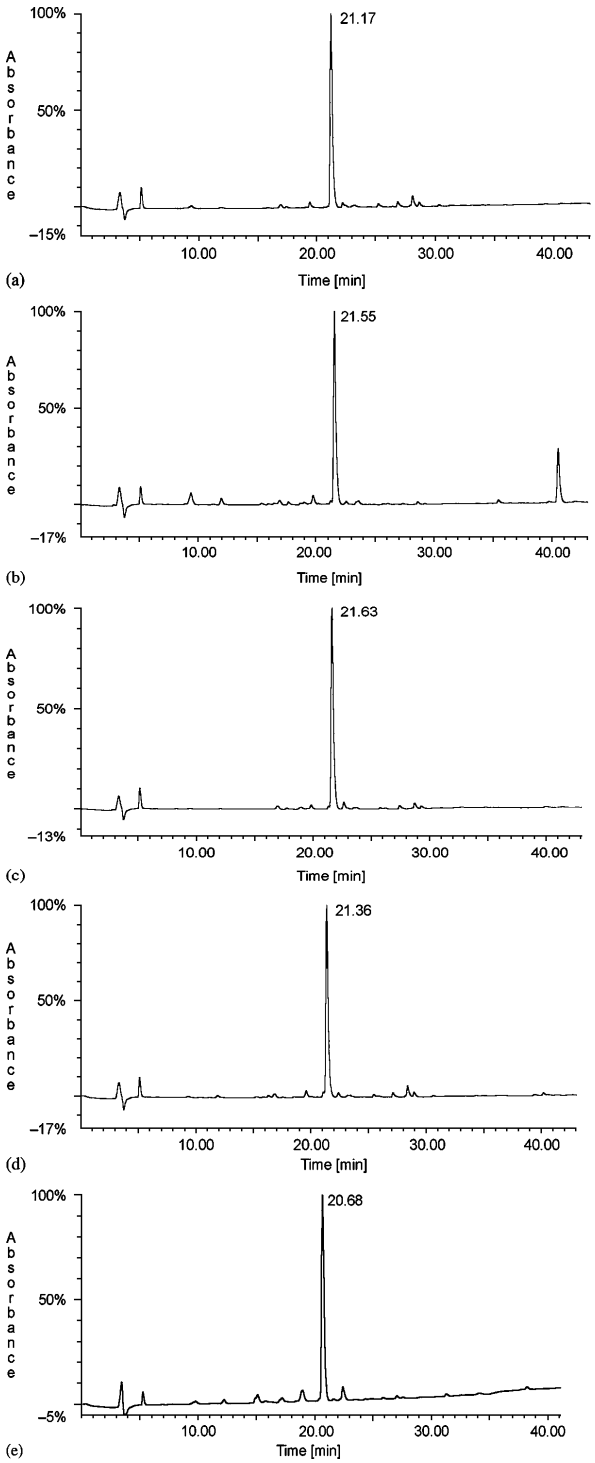

Figure 1 HPLC profile of H-Trp-Asp-Thr-Val-Arg-Ile-Ser-Phe-Lys-OH synthesized: (a) under conventional SPPS method (Method A); (b) under microwave condition with water cooling between the irradiation steps (Method B); (c) under microwave condition with ice-bath cooling between the irradiation steps (Method C); (d) under MW condition in MicroKan, with ice-bath cooling between the irradiation steps (Method D); and (e) in an oil bath (Method E).

nonapeptide (Figure 1(a)) with an overall peptide yield of 82%, and purity of 81% (Table 1).

The microwave-assisted synthesis of the nonapeptide was accomplished in a dedicated single-mode MW reactor under three different reaction conditions (Methods B, C, and D). The deprotection and coupling steps were carried out in a standard 10 ml MW glass reaction vessel. Knowledge of the exact reaction temperature is a must for any fair comparison between MW and conventional heating experiments. In fact, the accurate monitoring of the temperature in a microwave-heated reaction vessel is a nontrivial affair [18–21]. Measurements involving an external IR sensor are known to be problematic, particularly when employing simultaneous cooling of the reaction vessel [20,21]. Eventually, we opted for the use of an internal FO probe for temperature monitoring, rather than the more common external IR sensor. In order to fully control the MW power entering the reaction vessel – instead of using temperature control – we used the less common power-controlled mode involving alternate periods of MW irradiation and cooling. To prevent undesired side reactions (e.g. racemization, inadvertent cleavage of side-chain protecting groups), we decided to keep the reaction temperature as low as possible (65 °C) during all MW irradiation processes. Firstly, in Method B, the reaction mixture was irradiated with constant MW power for a short time, followed by intense cooling with tap water when the temperature inside the vial decreased to *ca* 20 °C. For deprotection, three cycles consisting of 30-s irradiation (at 40 W) and 2-min cooling resulted in complete removal of the Fmoc-group; after washing the resin, the coupling reaction was performed in four cycles consisting of 30-sec irradiation (at 30 W) and 2-min cooling (monitored by the Kaiser test). Thus, the Fmoc removal and the coupling time were 7.5 and 10 min, respectively. Altogether, the time of MW-assisted synthesis of the nonapeptide (Method B) was 2.5 hours (*cf* 11 hours under standard SPPS, Method A); i.e. the synthesis was completed 5 times faster (Figure 1(b)). However, the final yield and purity were less than those of the standard SPPS (Table 1).

We have presumed that the lower purity of the MW peptide product was due to the unusually high temperature during peptide synthesis. In the MW oven, the reaction temperature was permanently monitored in real time (Figure 2) with the aid of the FO sensor immersed in the resin suspension. In the power-controlled mode (set to 30 W), we observed that the actual reaction temperature – when starting from room temperature – rose to almost 100 °C within 30 s. Most probably, this very steep temperature rise can be ascribed to the strong coupling of the polar reaction mixture with MW irradiation. Any special MW effects (critically depending on the MW power used) that have been proposed to be involved in microwave-assisted SPPS [11,15] – in particular, the minimization of intermolecular aggregation, β-sheet formation, and

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Table 1 Preparation of nonapeptide (H-Trp-Asp-Thr-Val-Arg-Ile-Ser-Phe-Lys-OH) by five different solid-phase peptide synthesis (SPPS) protocols

Method	Conditions[b]	Reaction time (h)	Yield[c] (%)	Purity[d] (%)	MS-data[a]	
					$[M+H]^+$	$[M+2H]^{2+}$
A	Standard SPPS 20 °C	11.0	82	81	1151.83	576.50
B	MW SPPS 20 → 96 °C	2.5	64	63	1151.87	576.52
C	MW SPPS 0 → 64 °C	2.5	71	86	1151.83	576.47
D	MW SPPS 0 → 64 °C in MicroKans	2.5	95	83	1152.02	576.63
E	Standard SPPS 64 °C	2.5	80	78	1152.00	576.59

[a] Peptide identified by ESI-MS. Calculated M.W. for $C_{54}H_{82}N_{14}O_{14}$ 1150.61.
[b] See text for details.
[c] Yield of crude peptides after cleavage from resin.
[d] Purity of crude peptides (peak area percent from analytical RP-HPLC/MS monitored via UV at 220 nm).

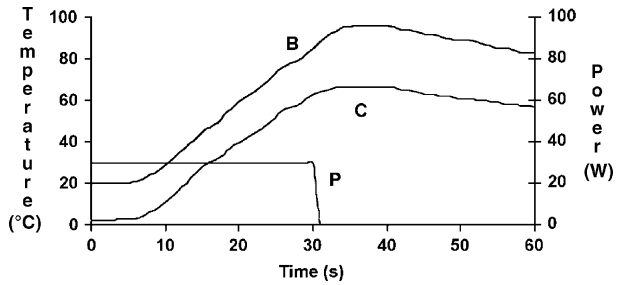

Figure 2 Temperature profiles for a typical microwave-assisted peptide coupling step (DMF, DCM, Fmoc-AA, HOBt, DIC, and resin; *ca* 1.1 ml total reaction volume) using 30 W of constant magnetron power (*P*) for 30 s in a single-mode MW reactor. The temperature was monitored internally with a FO probe immersed in the resin suspension. Starting at room temperature (20 °C) a maximum temperature of 96 °C was observed (profile B, Method B), as compared to only 64 °C using a precooled (0 °C) reaction mixture (profile C, Method C).

steric hindrance – would therefore be difficult to detect under these circumstances.

Thus, to improve the purity of the final product, we modified one crucial factor in the microwave-assisted protocol. We believed that the high value of the observed reaction temperature (*ca* 95 °C) was responsible for increased by-product formation, i.e. for lower purity (no attempts were made to identify the minor impurities). Therefore, more intensive cooling was introduced. The resin suspension in the MW reaction vial was *precooled to 0 °C in an ice bath* before inserting it into the cavity of the reactor. Moreover, the same intensive cooling was applied after all MW irradiation (coupling and deprotection as well) steps (Method C). The FO temperature monitoring clearly demonstrated that the maximum reaction temperature remained below 65 °C (Figure 2) when the irradiation was started at 0 °C. It should be stressed that for both experiments (Methods B and C) *identical amount of microwave power* (as delivered by the magnetron) was used. In Method C the purity of the crude peptide was 86% (Figure 1(c)); this value exceeds the purity of the peptide obtained by the standard protocol (Method A in Table 1).

In conclusion, we ascribe the higher purity obtained with this experimental setup, involving precooling of the reaction mixture to subambient temperature, to the fact that the temperature of the reaction was kept at a comparatively low level (under 65 °C) as compared to experiments starting from room temperature (under 100 °C).

We were sure that the lower yields in Methods B and C were due to the cumbersome manual resin transfer from the glass MW reaction vessel to the polypropylene Libra tube (the loss of resin particles could be even visually recognized). However, because of the design of the available reaction vial provided by the instrument vendor, the resin suspension had to be transferred to a reaction tube with a frit in order to perform the washing steps after the reaction steps. This extra manipulation could be avoided by using a new design of reaction vials suitable for peptide synthesis. To solve this problem, in Method D the resin beads (*ca* 30 mg) were compartmentalized in a MicroKan capsule which was placed in a 10-ml glass MW vessel [22,23]. The capsule has a porous wall that is fully penetrable by small molecules but not by the resin particles, and additionally, it proved stable under the relatively gentle MW irradiation conditions [24]. Thus, by using this MicroKan reactor, the resin transfer became unnecessary, thus the physical loss of the resin particles was completely avoided. Otherwise, Methods C and D were identical, except for the manipulation of the resin beads. The beads were removed from the MicroKan reactor only at the very end, i.e. after coupling and deprotecting the last amino acid residue, for the final cleavage step. As expected, the yield of this peptide was 95%, which was significantly higher than that obtained by conventional SPPS (Table 1). The purity of the final product was 83% (Figure 1(d)), which is practically the same.

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Although there are a few reports on peptide synthesis at higher temperature [25] the well-established, routinely used standard SPPS protocols never recommend elevated temperature. However, to make a fair comparison, we synthesized the test peptide under the same standard conditions as in Method A, but keeping the reaction temperature at 65 °C (the actual temperature in the MW experiment) by using an oil bath. In this case, the rate of heat transfer is significantly lower than under MW conditions, so 10-min coupling and 7.5-min deprotection steps were selected without further optimization. Our simple nonapeptide was synthesized at 65 °C with almost the same yield and purity as obtained under standard conditions at ambient temperature (Figure 1(e)), which was beyond our expectation. However, we have refrained from drawing any conclusion on the use of thermal heating for peptide synthesis.

CONCLUSIONS

We have demonstrated a rapid and efficient method for SPPS, which is based on the use of controlled MW irradiation. Of critical importance for the success of our protocol is the alternation of short pulses of MW irradiation of constant power with cooling of the reaction vessel/mixture to subambient temperatures. In combination with the use of MicroKans for the containment of the resin beads, this method allowed the preparation of a model nonapeptide (WDTVRISFK) in a significantly shorter time, in higher purity, and in better yield than that reached by conventional SPPS approach or a standard MW synthesis method without precooling the reaction mixture. We are currently investigating the underlying effects of the precooling technique in more detail and plan to exploit this method for the synthesis of longer and more difficult peptide sequences on a larger scale in the future.

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REFERENCES

1. Kappe CO, Stadler A. *Microwaves in Organic and Medicinal Chemistry*. Wiley-VCH: Weinheim, Germany, 2005.
2. Lidström P, Tierney JP (eds). *Microwave-Assisted Organic Synthesis*. Blackwell Publishing: Oxford, 2005.
3. Loupy A (ed.). *Microwaves in Organic Synthesis*. Wiley-VCH: Weinheim, Germany, 2002.
4. Hayes BL. *Microwave Synthesis: Chemistry at the Speed of Light*. CEM Publishing: Matthews, 2002.
5. Kappe CO. Controlled microwave heating in modern organic synthesis. *Angew. Chem., Int. Ed. Engl.* 2004; **43**: 6250–6284.
6. Hayes BL. Recent advances in microwave-assisted synthesis. *Aldrichimica Acta* 2004; **37**: 66–77.
7. De La Hoz A, Diaz-Ortiz A, Moreno A. Microwaves in organic synthesis. Thermal and non-thermal microwave effects. *Chem. Soc. Rev.* 2005; **34**: 164–178.
8. Merrifield RB. Solid phase peptide synthesis I. The synthesis of a tetrapeptide. *J. Am. Chem. Soc.* 1963; **85**: 2149–2154.
9. Yu H-M, Chen S-T, Wang K-T. Enhanced coupling efficiency in solid-phase peptide synthesis by microwave irradiation. *Org. Chem.* 1992; **57**: 4781–4784.
10. Erdélyi M, Gogoll A. Rapid microwave-assisted solid-phase peptide synthesis. *Synthesis* 2002; **11**: 1592–1596.
11. Murray JK, Gellman SH. Application of microwave irradiation to the synthesis of 14-helical b-peptides. *Org. Lett.* 2005; **7**: 1517–1520.
12. Matsushita T, Hinou H, Kurogochi M, Shimizu H, Nishimura S-I. Rapid microwave-assisted solid-phase glycopeptide synthesis. *Org. Lett.* 2005; **7**: 877–880.
13. Gorske BC, Jewell SA, Guerard EJ, Blackwell HE. Expedient synthesis and design strategies for new peptoid construction. *Org. Lett.* 2005; **7**: 1521–1524.
14. Olivos HJ, Alluri PG, Reddy MM, Salony D, Kodadek T. Microwave-assisted solid-phase synthesis of peptoids. *Org. Lett.* 2002; **4**: 4057–4059.
15. Collins JM, Collins MJ. Novel method for enhanced solid-phase peptide synthesis using microwave energy. *Biopolymers* 2003; **71**: 361.
16. Weng CC, White PD. Basic procedures. In *Fmoc Solid Phase Peptide Synthesis–A Practical Approach*, Chan WC, White PD (eds). Oxford University Press: Oxford, 2000; 41–76.
17. Nevalainen LT, Aoyama T, Ikura M, Crivici A, Yan H, Chua N-H, Nairn AC. Characterization of novel calmodulin-binding peptides with distinct inhibitory effects on calmodulin-dependent enzymes. *Biochem. J.* 1997; **321**: 107–115.
18. Nüchter M, Ondruschka B, Bonrath W, Gum A. Microwave assisted synthesis – A critical technology overview. *Green Chem.* 2004; **6**: 128–141.
19. Nüchter M, Ondruschka B, Weiß D, Beckert R, Bonrath W, Gum A. Contribution to the qualification of technical microwave systems and to the validation of microwave-assisted reactions and processes. *Chem. Eng. Technol.* 2005; **28**: 871–881.
20. Leadbeater NE, Pillsbury SJ, Shanahan E, Williams VA. An assessment of the technique of simultaneous cooling in conjunction with microwave heating for organic synthesis. *Tetrahedron* 2005; **61**: 3565–3585.
21. Arvela RK, Leadbeater NE. Suzuki coupling of aryl chlorides with phenylboronic acid in water, using microwave heating with simultaneous cooling. *Org. Lett.* 2005; **7**: 2101–2104.
22. Dai W-D, Guo D-S, Sun L-P, Huang X-H. Microwave-assisted solid-phase organic synthesis (MASPOS) as a key step for an indole library construction. *Org. Lett.* 2003; **5**: 2919–2923.
23. Solodenko W, Schön U, Messinger A, Glinschert A, Kirschning A. Microwave-assisted Suzuki–Miyaura reactions with an insoluble pyridine-aldoxime Pd-catalyst. *Synlett* 2004; 1699–1702.
24. Dallinger D, Gorobets N, Kappe CO. Microwave heating of standard polypropylene MicroKans™s in organic solvents to temperatures above 100 °C may lead to deformations and to a partial melt down of the polypropylene meshs. *See:. Mol. Divers.* 2003; **7**: 229–245.
25. Varanda LM, Miranda TM. Solid-phase peptide synthesis at elevated temperatures: a search for an optimized synthesis condition of unsulfated cholecystokinin-12. *J. Pept. Res.* 1997; **50**: 102–108.