Microreactors

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Microreactor Synthesis of β-Peptides**

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β-Amino acid oligomers (β-peptides) are a unique class of peptides. In contrast to their natural α-amino acid counterparts, β-peptides require few residues to form secondary structures such as turns, helices, or sheets.[1] As a result of these structures and their metabolic stability, β-peptides have attracted considerable interest. However, the secondary structures can also severely hamper the synthesis of these oligomers.[2] Small- to medium-length β-peptides are generally synthesized in solution[1b] or on a solid support,[1c] whereas longer peptides can be prepared by chemical-ligation techniques.[1e] Microwave-assisted solid-phase-peptide-synthesis (SPPS) protocols have also been applied in the preparation of β-peptides.[2,3] Owing to the formation of β-peptidic secondary structures of short chains when synthesized on Merrifield beads, the solid-phase assembly of β-peptides may totally fail, especially when hair-pin turn structures can form. In this case, one has to resort to fragment coupling.[2b,c]

Microreactors (i.e. miniaturized chemical reactors) are attracting attention as an alternative to traditional synthesis in round-bottom flasks.[4] However, in contrast to widely used microanalysis systems, the development of microreactors as a tool for organic synthesis has been slow. A range of chemical transformations has been carried out by using microreactor technologies, whereby most of these reactions only served as a proof of principle.[5]

Herein we describe the first application of a silicon continuous flow microreactor (Figure 1) to the assembly of peptides. The microreactor not only allows for quick "scanning" of reaction conditions, but also for the procurement of synthetically useful amounts of peptides.[6] Our report is also the first demonstration of 1) peptide couplings with *tert*-butyloxycarbonyl (Boc)- and 9-fluorenylmethoxycarbonyl (Fmoc)-protected amino acids in 1–5 minutes at temperatures as high as 120 °C, 2) the use of β^2- and β^3-homoamino acid fluorides for β-peptide couplings, and 3) the advantageous application of a $C_{10}H_4F_{17}$-substituted benzylic ester protecting group in solution-phase peptide synthesis.[7–9]

Microstructured reaction devices hold several advantages for organic synthesis: only small amounts of valuable reagents are required, reaction parameters, such as reaction time and temperature, can be accurately controlled, and various reaction conditions can be scanned in a continuous, time-efficient fashion. The continuous-flow microreactor that we used for our studies is designed to be compatible with a wide range of organic solvents and can be operated over a broad temperature range (−80 °C to +150 °C) in a simple laboratory setting (Figure 1).[10] The reaction volume of the microreactor (78.3 μL) allows microscale reaction scanning (μmol of reagents) and the production of several grams of target material per day.

Initially, we explored the coupling of Fmoc-β^3-homophenylalanine fluoride (Fmoc-β^3hPhe-F, **1**) and H-β^3hPhe-OBn (**2**) in the microreactor (Scheme 1a). Acid fluorides were selected as activated forms of the amino acids as 1) they are readily available from the parent amino acids, 2) they are powerful acylating agents, and 3) the use of a tertiary amine base leads to an ammonium fluoride as the sole soluble by-product.[11] The reaction was monitored at different temperatures (25 °C, 60 °C, and 90 °C) and different reaction times (1, 2, 5, and 10 min) by using Fmoc-β^3hPhe-OBn (**5**) as the internal standard for liquid chromatography LC-MS analysis. Comparing the reaction progress at different temperatures revealed that the maximum yield was obtained after 3 minutes at 90 °C (Scheme 1b). Interestingly, when the coupling of **1** and **2** was conducted at a higher temperature and/or for a prolonged period of time, the amount of dipeptide diminished, as revealed in a second reaction scan (Scheme 1c).[12] Under these conditions, tripeptide **4** was also, not surprisingly, formed owing to Fmoc-cleavage and subsequent peptide coupling with excess acid fluoride.[13] Following reaction screening, dipeptide **3** was prepared on 0.5-mmol

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Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

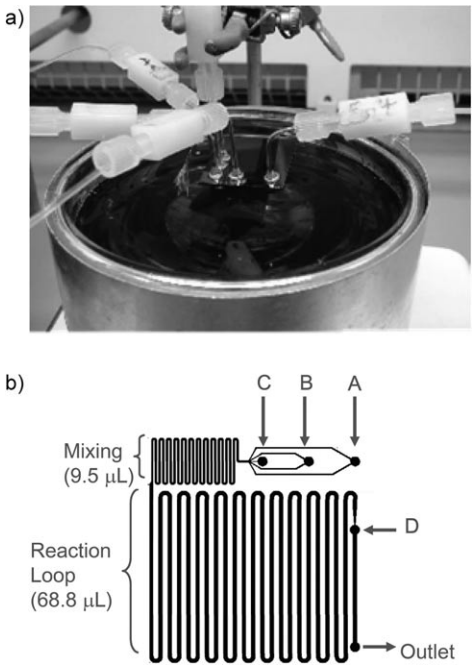
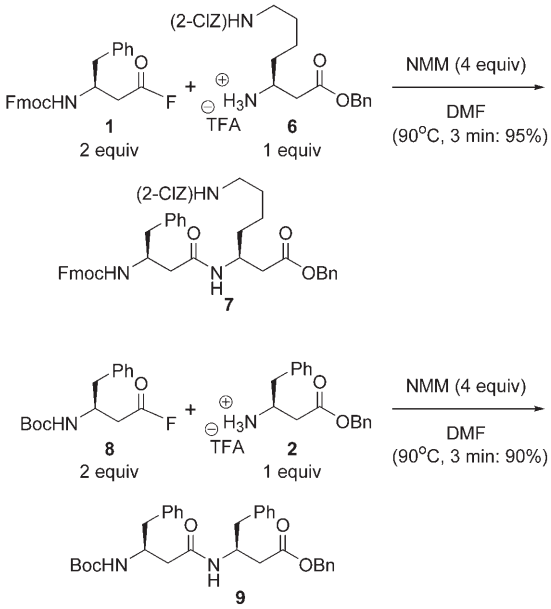


Figure 1. a) Microreactor setup. b) Schematic representation of the microreactor showing the three inlets (acid fluoride (A), amino acid benzyl ester (B) and *N*-methylmorpholine (NMM; C)), the quench port (TFA and the internal standard (D)), the outlet, and the dimensions of the channels (total prequench reactor volume = 78.3 μL).

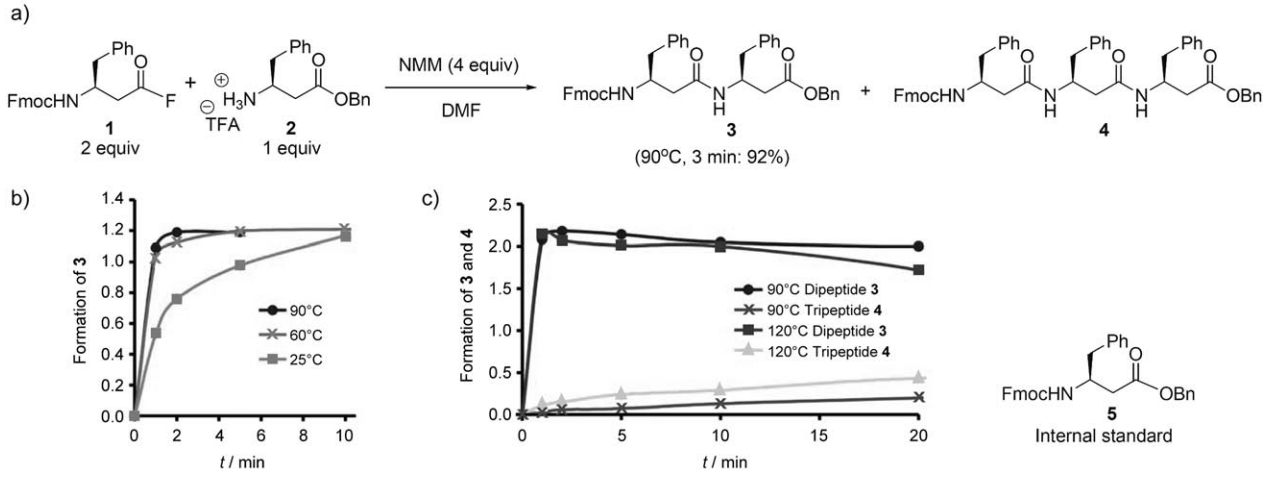


Scheme 2. Synthesis of the β^3-dipeptides **7** and **9**. 2-ClZ = 2-chlorobenzyloxycarbonyl.

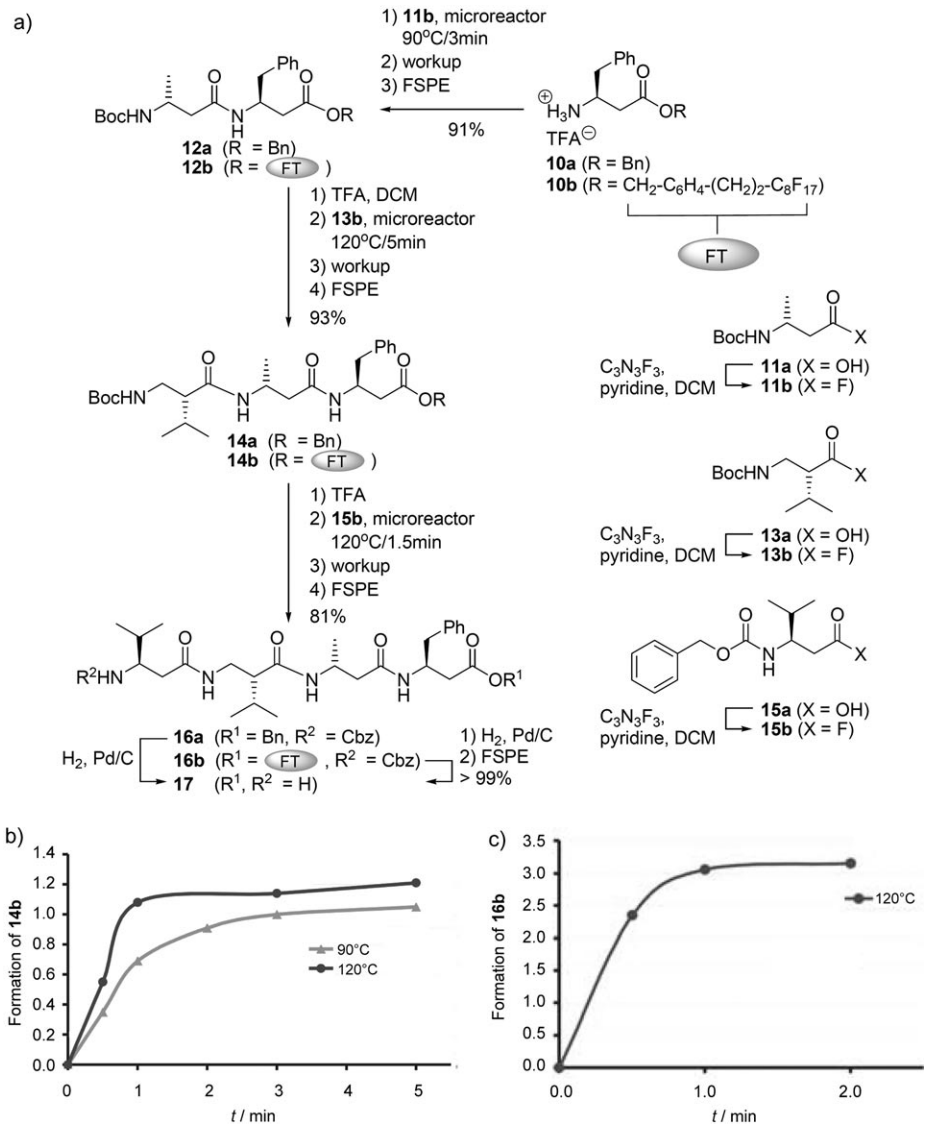
scale (3 min reaction time at 90 °C, 92 % yield after column chromatography). Identical conditions were used for the construction of the β^3-dipeptides **7** (0.5-mmol scale) and **9** (0.3 mmol; Scheme 2).

After having established the utility of our microreactor system for the synthesis of β^3-dipeptides, we set out to assemble tetrapeptide **17** by using the Boc strategy (Scheme 3). The tetrapeptide contains all possible β-peptide bonds, (β^3–β^3)-, (β^3–β^2)-, and (β^2–β^3) as well as a β^3h(*R*)Ala-β^2h(*R*)Val turn-inducing segment.[1f,2b] A fluorous benzyl group was incorporated in the first amino acid to facilitate the purification procedure by fluorous solid-phase extraction (FSPE).[7,8,14] Thus, the assembly of the tetrapeptide **17** commenced with the construction of the first β^3–β^3 peptidic bond by applying the previously established conditions. A reaction time of 3 min at 90 °C provided the Boc-protected dipeptide **12b** in 91 % yield after FSPE. Notably, the product precipitated in the collection flask, which was kept at ambient temperature, indicating the poor solubility of this class of compound.[15]

The formation of the sterically more demanding β^3–β^2 peptide bond required higher temperatures and/or longer residence times as revealed by a reaction scan at 90 °C and 120 °C. Maximum conversion was obtained at 120 °C and 5 min reaction time[16] to give tripeptide **14b** in 93 % yield on a 0.4-mmol scale.[17] The final β^2–β^3 coupling was executed at



Scheme 1. a) Synthesis of dipeptide **3** in the microreactor. b) LC–MS analysis of the formation of **3** as a function of time and temperature. c) LC–MS analysis of the formation of **3** and **4** at higher temperatures and longer reaction times. Bn = benzyl, TFA^- = trifluoroacetate.



Scheme 3. a) Synthesis of the tetrapeptides **16** and **17**. b) Formation of **14b** at different temperatures. c) Formation of **16b** at 120 °C. Cbz = benzyloxycarbonyl, DCM = dichloromethane.

120 °C and a reaction scan revealed the coupling to be complete after 1.5 min. Tetramer **16b** was obtained in 81 % yield. Cleavage of the fluorous tag and removal of the benzyloxycarbonyl function provided the zwitterionic target compound **17**.

To compare the microreactor methodology with traditional assembly strategies, tetrapeptide **17** was also synthesized on a solid support by using the Fmoc strategy and HATU–DIPEA-mediated couplings,[18] as well as in solution by using conventional laboratory glassware. For the solution-phase assembly, two different strategies were investigated: in the first approach we used the above-mentioned fluorous tag in combination with acid fluorides, in the second approach we started from H-β^{3}hPhe-OBn and used HATU–DIPEA coupling conditions. The results of the four assembly methods are collected in Table 1. All procedures provided the tetramer in comparable overall yields. However, purification of the fluorous peptides was much easier when compared with the purification of the nonfluorous peptides. The nonfluorinated tetramer **16a** was particularly difficult to handle owing to its poor solubility. In addition, the microreactor system allowed for precise control over unconventionally high reaction temperatures. This control significantly reduces reaction times and prevents product precipitation during the reaction. Precipitation led to inhomogeneous gel-like reaction mixtures when using traditional laboratory glassware in the solution-phase synthesis.

Table 1: Synthesis of the tetrapeptides **16a/b** and **17** by microreactor technology, by solution-phase couplings, and by solid-phase assembly.[19]

Method	**12a/b** Conditions (Yield)	**14a/b** Conditions (Yield)	**16a/b** Conditions (Yield)	**17** Yield
microreactor	**11b** (2 equiv); NMM (4 equiv), 90 °C, 3 min (91 %)	**13b** (2 equiv); NMM (4 equiv), 120 °C, 5 min (93 %)	**15b** (2 equiv); NMM (4 equiv), 120 °C, 1.5 min (81 %)	> 99 %
solution phase, F tag	**11b** (2 equiv); NMM (4 equiv), RT, 3 h. (94 %)	**13b** (2 equiv); NMM (4 equiv), RT, overnight (93 %)	**15b** (2 equiv); NMM (4 equiv), RT, overnight (93 %)	> 99 %
solution phase[a]	**11a** (2 equiv); HATU (1.8 equiv), NMM (4 equiv), RT, overnight (85 %)	**13a** (2 equiv); HATU (1.8 equiv), NMM (4 equiv), RT, overnight (85 %)	**15a** (2 equiv); HATU (1.8 equiv), NMM (4 equiv) RT, overnight (87 %)	79 %
solid phase[b] (Wang Resin)	Fmoc-**11a** (3 equiv); HATU (2.8 equiv), DIPEA (6 equiv) RT, 1.5 h	Fmoc-**13a** (3 equiv); HATU (2.8 equiv), DIPEA (6 equiv) RT, 1 h	Fmoc-**15a** (3 equiv); HATU (2.8 equiv), DIPEA (6 equiv) RT, 1 h	55 % (after HPLC)[20]

[a] HATU = 2-(1*H*-7-azybenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate. [b] DIPEA = diisopropylethylamine.

In summary, we have demonstrated the use of a silicon-based microreactor for the effective synthesis of peptides. The microstructured reaction device allowed for the detailed investigation and optimization of reaction parameters by using minimal amounts of reagents. Furthermore, it opened the way for the use of unprecedented high reaction temperatures, leading to homogeneous reaction mixtures and of significantly reduced reaction times. Synthesis efficiency was further enhanced by the use of a fluorous benzyl tag that was applied for the first time in the assembly of β-peptides and proved to be particularly useful for the purification of poorly soluble products.[8] Not only will the synthetic strategy outlined herein find applications in the construction of challenging peptides, it will also be applied to the assembly of other biopolymers, including oligosaccharides and oligonucleotides. In general, the continuous-flow process opens many opportunities for multistep syntheses and automation.[21]

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[12] Longer reaction times (30 min) led to an irregular flow as judged from the large error margin in the relative LC–MS UV absorbances of the products and the internal standard.

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[15] The poor solubility dictated the use of a relatively large amount of DMF (30 mg mL^{-1}) to apply it to the fluorous silica column (Fluorchrom, 20 g). The large amount of DMF did not adversely affect the purification. Also see the Supporting information.

[16] The low solubility of the product prohibited lower temperatures and longer reaction times in the microreactor.

[17] a) For this condensation, a ca. 93:7 mixture of enantiomers **(*R*)-13b**/**(*S*)-13b** was used, see [17b]. LC–MS analysis of the product revealed the same diastereomer ratio in the product, indicating that no epimerization had occurred under these reaction conditions. A more detailed epimerization study is currently underway. b) For the synthesis of amino acid fluoride **13**, a 93:7 diasteromeric mixture of Boc-β^2-homovaline ($R/S = 93:7$) was used: T. Hinterman; D. Seebach, *Helv. Chim. Acta* **1998**, *81*, 2093.

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[19] For detailed experimental conditions, see the Supporting Information.

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