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SHORT COMMUNICATION

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Fluorenylmethoxycarbonyl-*N*-methylamino Acids Synthesized in a Flow Tube-in-Tube Reactor with a Liquid–Liquid Semipermeable Membrane

Annette E. Buba,[a] Stefan Koch,*[a] Horst Kunz,*[a] and Holger Löwe[a]

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Both steps of the *N*-methylation of 9-fluorenylmethoxycarbonyl (Fmoc) amino acids were carried out in a microstructured tube-in-tube reactor equipped with a semipermeable Teflon® AF 2400 membrane as the inner tubing. In the first step, gaseous formaldehyde was passed through the inner membrane to effect the acid-catalyzed conversion of the Fmoc amino acids into the corresponding *N*-Fmoc oxazolidinones. In the second step, liquid–liquid transfer of trifluoroacetic acid was used for the first time in such a reactor for the reductive opening of these oxazolidinones to give Fmoc *N*-methylamino acids in high yields.

Introduction

N-Methylated amino acids (NMAs) are known constituents of important biologically active peptides, as for example the immunosuppressant cyclosporine (**1**)[1] and dolastatine D10 (**2**),[2] which is a promising anticancer drug (Figure 1). *N*-Methylamino acid residues lack the ability to be hydrogen donors and significantly change the physical properties of peptides. They influence the conformation of NMA-containing peptides[3] and cause an increased proteolytic resistance that is useful for medical applications.[4]

N-Methylamino acids have been synthesized by nucleophilic substitution of α-bromo acids[5] and α-hydroxy acid derivatives,[6] by methylation of sulfonamides,[7] carbamates or amides,[8] as well as through reductive amination.[9,10] Selective access to NMAs by avoiding hazardous chemicals, racemization, and harsh reaction conditions was introduced by Freidinger et al.[11] in a two-step process via oxazolidinones **4** (Scheme 1). However, these syntheses suffer from inconvenient up-scaling and require careful purification procedures. In particular, long reaction times make them less attractive for industrial applications. Flow chemistry[12] using special mixers that are more efficient than stirrers with regard to concentration and temperature gradient[13] can eliminate most of these drawbacks. In addition, up-scaling can easily be facilitated in a microreactor by continuous processing, and the purification can be performed during the process, for example, with integrated columns.

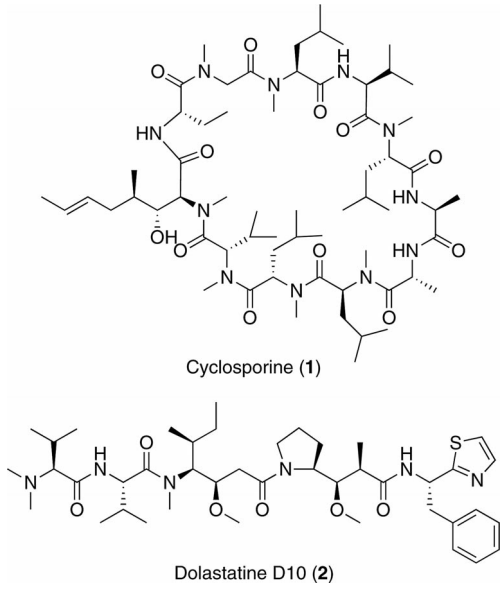
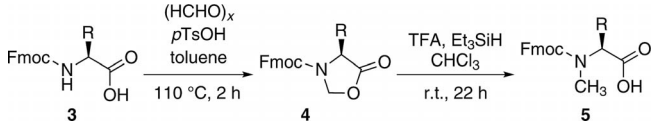


Figure 1. *N*-Methylamino acids in natural peptide drugs.



Scheme 1. *N*-Methylation of amino acids in two steps according to Freidinger et al. Fmoc = 9-fluorenylmethoxycarbonyl, TFA = trifluoroacetic acid.

Results and Discussion

To demonstrate these advantages, we developed a continuous flow version of the second step of Freidinger's[11a] two-step synthesis by using a tube-in-tube reactor. A semipermeable membrane, such as Teflon® AF 2400, was ap-

[a] Institut für Organische Chemie, Johannes Gutenberg-Universität Mainz,
Duesbergweg 10–14, 55128 Mainz, Deutschland
Fax: +49-6131-39-24786
E-mail: schtefkoch@hotmail.com
hokunz@uni-mainz.de
Homepage: http://www.ak-kunz.chemie.uni-mainz.de/

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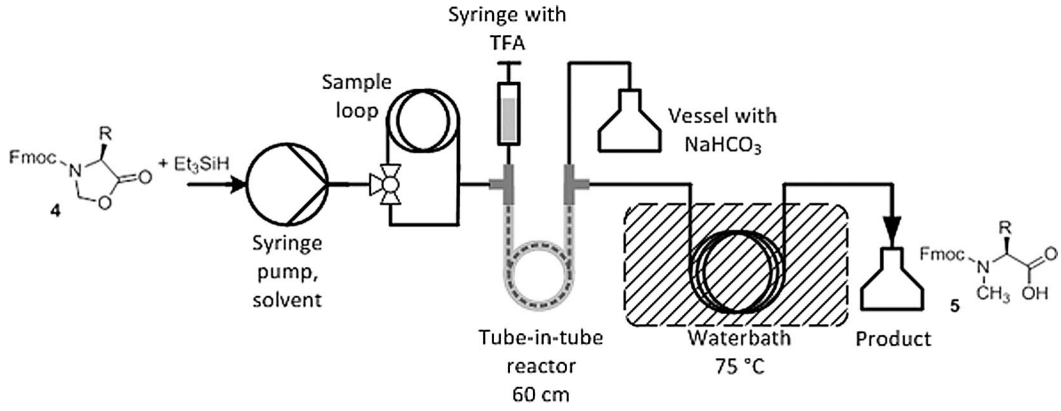


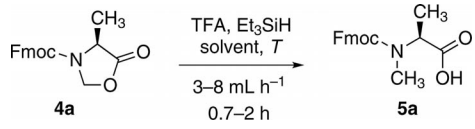
Figure 2. Flow reactor used for the second step of the NMA synthesis.

plied for the first time as a mixing system with optimal liquid–liquid contact over a large exchange surface, which would prevent early undesired side reactions, for example, Mannich-type reactions, and the need for a Lewis acid support.[11f] Teflon® AF is a stable amorphous copolymer consisting of 2,2-bis(trifluoromethyl)-4,5-difluoro-1,3-dioxolane and tetrafluoroethylene.[14] It is highly permeable to gases[15] and, to a limited extent, to solutes.[16] AF membranes were successfully applied in pioneering work by Ley[17] in gas–liquid transfers in syntheses.

To demonstrate the liquid–liquid transfer through a Teflon® AF 2400 membrane in synthesis, oxazolidinones **4** as substrates were prepared by heating Fmoc amino acids **3** with paraformaldehyde under acid catalysis (Scheme 1).[11a] The reductive ring opening of the oxazolidinones was performed in a flow reactor with a tube-in-tube configuration[17] (Figure 2), which consisted of an inner Teflon® AF 2400 membrane (60 cm) and an outer polytetrafluoroethylene (PTFE) tubing. Connection through a T-piece ensured independent liquid flow into the reactor. Trifluoroacetic acid (TFA) was chosen as the liquid acid catalyst that was injected into the outer tubing of the tube-in-tube system. It penetrated the inner membrane at room temperature without affecting the material, as demonstrated by repeated use of the reactor.

The dissolved substrates, Fmoc amino acid oxazolidinone **4**, and triethylsilane were placed into the sample loop and the solvent was pumped by using a syringe pump through the reactor. During solvent flow, the level of TFA in the outer tubing significantly declined. Transfer of the substrates into the outer tube was never observed. No amino acid derivatives were detectable in the trapping hydrogen carbonate solution. The high yields of the products also give evidence that substrates and products do not pass the inner tube. In a subsequent coil, the temperature was elevated to accelerate the reaction. For optimization of the conditions, Fmoc-protected alanine oxazolidinone **4a** was chosen as a model substrate (Table 1). By using chloroform as the solvent according to Freidinger's protocol at 50 °C with a flow rate of 6 mL h^{-1}, a conversion of 33% was observed within 1 h (Table 1, entry 1). A lower flow rate resulted in a higher conversion (Table 1, entry 2). At higher temperatures, acetonitrile and chlorobenzene proved to be the most effective solvents, and conversions up to 100% within 2 h were achieved (Table 1, entries 4–6). A further increase in the temperature allowed a flow rate up to 8 mL h^{-1}, a reaction time of 1 h (Table 1, entries 7–9), and a reduction in the amount of triethylsilane (Table 1, entry 10).

Table 1. Optimization of the reaction conditions for NMA synthesis.

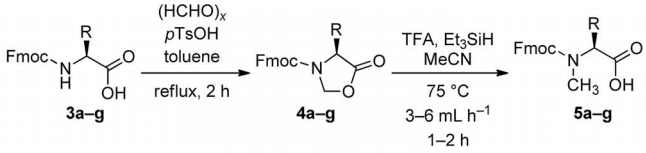


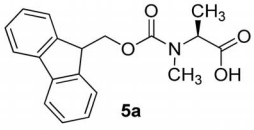
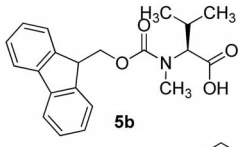
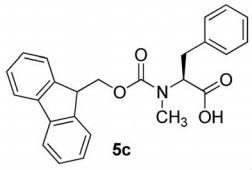
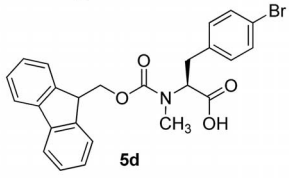
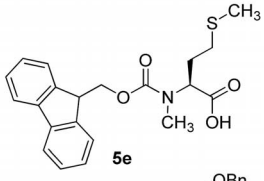
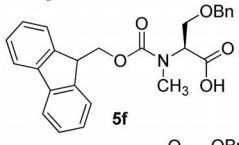
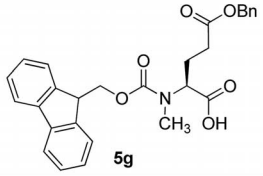
Entry	Solvent	T [°C]	Flow rate [mL h^{-1}]	Et_3SiH [equiv.]	Conversion[a] [%]
1	chloroform	50	6	3	33
2	chloroform	50	3	3	40
3	dioxane	70	3	3	2
4	toluene	70	3	3	71
5	chlorobenzene	70	3	3	100
6	acetonitrile	70	3	3	100
7	acetonitrile	70	6	3	>95
8	acetonitrile	75	6	3	100
9	acetonitrile	75	8	3	100
10	acetonitrile	75	8	2	100

[a] Fmoc-L-alanine oxazolidinone (97.0 mg, 0.30 mmol), solvent (1.5 mL), triethylsilane, trifluoroacetic acid. All reactions were performed in a solvent-free apparatus. Conversion was determined by ^{1}H NMR spectroscopy.

After optimization of the conditions, the general applicability of this flow-through synthesis to Fmoc α-amino acids **3a**–**g** was studied. The results for amino acids with aliphatic (i.e., **5a**,**b**), aromatic (i.e., **5c**,**d**), and heterofunctional side chains (i.e., **5e**–**g**) are given in Table 2. Although each amino acid required an optimized flow rate, as determined by monitoring by ^{1}H NMR spectroscopy, all *N*-methylamino acids except for methionine (i.e., product **5e**) were obtained in high yields. With methionine, side products were formed, which resulted in difficult purification. Thus, the yield was determined by NMR spectroscopy.

Table 2. Fmoc *N*-methylamino acids from Fmoc amino acids.



Entry	Yield [%][a] (product)	Final product	Yield [%][b]
1	99 (**4a**)	 **5a**	97
2	90 (**4b**)	 **5b**	94[c,d]
3	81 (**4c**)	 **5c**	90[c,d]
4	95 (**4d**)	 **5d**	91[c]
5	94 (**4e**)	 **5e**	56[c,e]
6	99 (**4f**)	 **5f**	95[c,d]
7	61 (**4g**)	 **5g**	80[c]

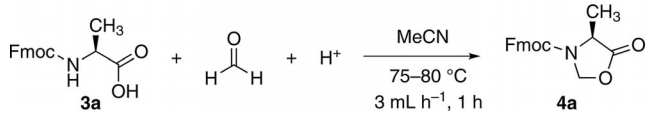
[a] First step, batch reaction: Fmoc amino acid, *p*-toluenesulfonic acid, paraformaldehyde, toluene, reflux, 2 h; isolated yield. [b] Second step: Fmoc-oxazolidinone (0.30 mmol), triethylsilane (0.10 mL, 0.63 mmol, 2.0 equiv.), trifluoroacetic acid (1.5 mL, 19.5 mmol, 65 equiv.), acetonitrile (1.5 mL), flow rate = 6 mL h^{-1}, reaction time = 1 h; isolated yield. [c] Flow rate = 3 mL h^{-1}; reaction time = 2 h. [d] 0.15 mL (0.94 mmol, 3.0 equiv.) of triethylsilane. [e] Yield was determined by ^{1}H NMR spectroscopy.

Further investigations showed that the first step of Freidinger's synthesis, the formation of oxazolidinone **4a**, can also be performed in a tube-in-tube reactor by exploiting the high gas permeability of Teflon® AF 2400 membranes. Instead of solid paraformaldehyde, gaseous formaldehyde was used, which was generated by heating paraformaldehyde in a separate pressure-resistant vessel. The formaldehyde permeability of the Teflon® AF 2400 membrane was tested by using a Schiff reagent.[18] Gaseous formaldehyde is stable at 80–100 °C. Below 75 °C, it polymerizes and paraformaldehyde precipitates on the membrane. In processes with gaseous reactants, outgassing is often observed.[17d] Performance of these conversions in a closed reactor under pressure avoids this problem and enables a reaction in a homogeneous liquid–gas solution. To maintain a constant and high pressure (1–2 bar), an HPLC pump was used instead of a syringe pump, as shown in Figure 3.

Fmoc-alanine (**3a**) and catalytic *p*-toluenesulfonic acid were dissolved in acetonitrile and injected into the sample loop. The reactor was flushed with gaseous formaldehyde through the membrane. Subsequently, the solvent was pumped into the reactor with a flow rate of 3 mL h^{-1}. A pressure of 0.5–1.0 bar was sufficient to avoid outgassing of formaldehyde.

The model reaction yielded 91% of *N*-Fmoc-L-4-methyloxazolidin-5-one (**4a**, 100% conversion; Table 3, entry 1) within 1 h. No azeotropic distillation of water was necessary. Trifluoroacetic acid could be used as a substitute for *p*-toluenesulfonic acid as the catalyst, and its use also led to complete conversion. However, some side products were observed under these conditions (Table 3, entry 2).

Table 3. Optimization of the reaction conditions for oxazolidinone syntheses.



Entry	Acid	Conversion[a] [%]
1	*p*TsOH	100
2	TFA	100

[a] Fmoc-L-alanine (96.5 mg, 0.31 mmol), acid in catalytic amounts, gaseous formaldehyde from paraformaldehyde, acetonitrile (1.5 mL). Conversion was determined by ^{1}H NMR spectroscopy.

Notably, performing both steps, that is, the formation of the oxazolidinone and reductive ring opening, in a single combined two tube-in-tube flow reactor at a flow rate of 3 mL h^{-1} and an elevated reaction temperature (80 °C) was not successful, because the excess amount of formaldehyde from the first step preferably reacted with triethylsilane, which is required for the reductive opening of the oxazolidinone ring.

Conclusions

Teflon® AF 2400 membranes in tube-in-tube flow reactors were demonstrated to be permeable to gaseous reactants and also to liquid organic acids. The liquid–liquid transfer of trifluoroacetic acid in a flow reactor enabled the highly efficient synthesis of Fmoc *N*-methylamino acids, which are interesting components used in the syntheses of

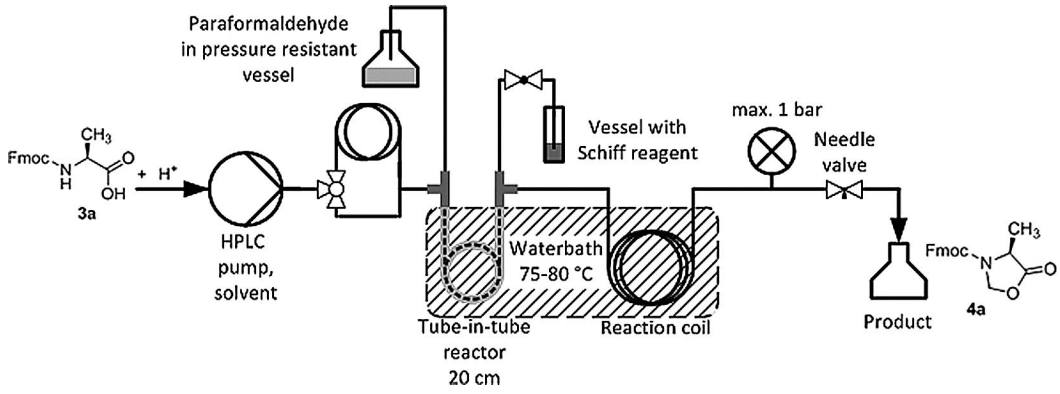


Figure 3. Flow tube-in-tube reactor used for the synthesis of Fmoc-oxazolidinones.

natural products and drugs. Liquid transfer through semipermeable membranes, in general, should open up attractive perspectives for processes performed in flow reactors, for example, if trapping of a desired but unstable product with a liquid reactant is demanded.

Experimental Section

General Procedure for the Flow-Through Synthesis of 9*H*-Fluoren-9-ylmethyl-(*S*)-4-methyl-5-oxo-1,3-oxazolidine-3-carboxylate (4a) in a Tube-in-Tube Reactor: A solution of Fmoc-L-alanine (**3a**; 100 mg, 0.32 mmol) and *p*-toluenesulfonic acid (23.7 mg, 138 μmol) in acetonitrile (1.5 mL) was injected into the sample loop. The tube-in-tube reactor was subsequently flooded with gaseous formaldehyde generated by heating paraformaldehyde in a separate vessel. Acetonitrile was passed through the reactor at a flow rate of 3 mL h^{-1} and 0.5 bar. The temperature of the coils was set to 75–80 °C. The product was collected at the output stream. The solution was concentrated in vacuo. Codistillation with dichloromethane gave the product as a colorless solid, yield 95.2 mg (294 μmol, 91%).

General Procedure for Flow-Through Syntheses of *N*-Methyl Amino Acids 5a–g: The reactor set up is shown in Figure 2. The Fmoc-protected α-amino acid derived oxazolidinone was dissolved in acetonitrile (1.50 mL) and triethylsilane was added. The temperature of the coils was set to 75 °C, and the outer tube of the tube-in-tube reactor was filled with trifluoroacetic acid (1.50 mL, 19.5 mmol). Acetonitrile was passed through the reactor by a syringe pump with a set flow rate. The product was collected at the output stream. To record the NMR spectra, the obtained solution was concentrated in vacuo and codistilled with dichloromethane twice. For isolation, the product solution was diluted with a saturated aqueous solution of $NaHCO_3$ (15 mL). The aqueous layer was washed with dichloromethane and then acidified with 1 M HCl (pH ≈ 2). The acidic layer was then extracted with dichloromethane (2× 15 mL), and the combined organic layer was dried with $MgSO_4$. The solvent was removed under reduced pressure. The obtained solids were recrystallized from diethyl ether. Oils were purified by flash chromatography on silica (cyclohexane/ethyl acetate, 3:1).

Supporting Information (see footnote on the first page of this article): Experimental description of the batch and flow-through syntheses, characterization data, and ^1H NMR and ^{13}C NMR spectra for all key intermediates and products.

Acknowledgments

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