Journal of Peptide Science
J. Peptide Sci. 2006; **12**: 213–219
Published online 27 September 2005 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/psc.711

JOURNAL OF PEPTIDE SCIENCE

Optimized selective *N*-methylation of peptides on solid support

ERIC BIRON, JAYANTA CHATTERJEE and HORST KESSLER*

Department Chemie, Lehrstuhl II für Organische Chemie, Technische Universität München, Germany

Received 10 June 2005; Accepted 28 June 2005

Abstract: Peptides containing N^{α}-methylamino acids exhibit interesting therapeutic profiles and are increasingly recognized as potentially useful therapeutics. Unfortunately, their synthesis is hampered by the high price and nonavailability of many N^{α}-methylamino acids. An efficient and practical three-step procedure for selective *N*-methylation of peptides on solid support is described. The procedure was based on the well known solid-phase *N*-methylation of N^{α}-arylsulfonyl peptides, which was improved by using dimethylsulfate and the less expensive DBU as base. Every step of the procedure, amine activation by an *o*-nitrobenzenesulfonyl group, selective *N*-methylation and removal of the sulfonamide group, was optimized in respect of time and economy. The described optimized three-step procedure is performed in 35 min without solvent changes, instead of 3 h. Tripeptides (Fmoc-Phe-MeXaa-Leu-OH) containing *N*-methylated common amino acids were also prepared using the optimized procedure to demonstrate its compatibility with these amino acids. The described procedure allows an efficient synthesis of N^{α}-methylamino acid containing peptides in a very short time using Fmoc solid-phase peptide synthesis.

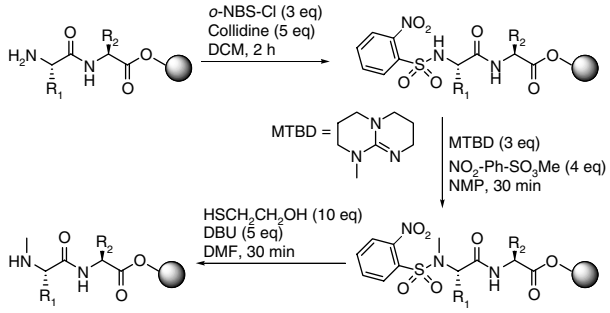
Keywords: *N*-methylation; *N*-methylated peptides; *N*-methylpeptides; *N*-methylamino acids; *o*-nitrobenzenesulfonyl protecting group; solid-phase synthesis; Mitsunobu

INTRODUCTION

N-Methylamino acids are constituents of several biologically active natural products [1–3] such as cyclosporine [4,5], dolastatins [6–8] and didemnins [9–11] exhibiting highly interesting therapeutic profiles. Incorporation of *N*-methylamino acids into biologically active peptides is useful for obtaining information about backbone conformation [12–14] and has been shown to improve pharmacological parameters such as lipophilicity [15,16], proteolytic stability [16,17], bioavailability [18,19] and conformational rigidity [12,15]. It may also result in enhanced potency [20–23], new receptor subtype selectivity [24–27] and conversion of an agonist into an antagonist [28]. Therefore *N*-methylamino acid–containing peptides are increasingly recognized as potentially useful therapeutics [15,23,29,30].

Unfortunately, only a small number of protected *N*-methylamino acids, most with aliphatic side chains, are commercially available and affordable. Therefore a general procedure for *N*-methylation of amino acids containing functionalized side chains is needed. Most existing solution-phase methodologies are limited to aliphatic amino acids or are characterized by harsh reaction conditions [31]. We recently described a convenient general procedure to prepare N^{α}-methylamino acids in solution based on the well-known *N*-alkylation of N^{α}-arylsulfonylamino esters [32]. On the other hand, when only small amounts of N^{α}-methylamino acids are needed, e.g. during N^{α}-methyl scanning of bioactive peptides, the simplest strategy for *N*-methylation would involve direct *N*-methylation of the desired amino acid on solid-phase during peptide synthesis. In that case, the *N*-methylation procedure has to be compatible with Fmoc solid-phase peptide synthesis. Methods for selective *N*-methylation of peptides on solid support have already been reported [33–37]. The most efficient solid-phase procedure, first described by Miller and Scanlan (Scheme 1) [33], is a three-step sequence involving amine activation by an *o*-nitrobenzenesulfonyl group (*o*-NBS), followed by direct alkylation [33,34] or Mitsunobu reaction [35,36] on the activated nitrogen and then removal of the sulfonamide group. A total of 3 h is added to the normal coupling/deprotection sequence with this three-step procedure and can be time-consuming when *N*-methylated peptide libraries are synthesized. In order to reduce the time and cost of the *N*-methylation step during Fmoc–SPPS, we aimed at optimizing every step of the procedure described by Miller and Scanlan [33]. On the other hand, compatibility of the procedure with every common amino acid is still unclear. Therefore we were also interested in demonstrating the compatibility of the optimized *N*-methylation procedure with different side chain–functionalized amino acids.

*Correspondence to: H. Kessler, Department Chemie, Lehrstuhl II für Organische Chemie, Technische Universität München, Lichtenbergstrasse 4, Garching D-85747, Germany;
e-mail: Horst.Kessler@ch.tum.de

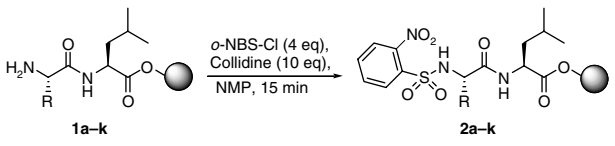


Scheme 1 Three-step procedure for site-selective *N*-methylation of peptide on solid support described by Miller and Scanlan [34].

RESULTS AND DISCUSSION

The first step uses the method developed by Fukuyama for the preparation of secondary amines [38]. In the procedure described by Miller and Scanlan [33], protection of the resin-bound free amine peptide with *o*-nitrobenzenesulfonyl chloride (*o*-NBS-Cl) is completed in DCM at room temperature in 2 h (Scheme 1), while protection is completed in 30 min in DMF in the procedure described by Reichwein and Liskamp [35]. Time needed for complete protection was investigated with the trityl resin-bound amine-free dipeptide **1a** by treatment with *o*-NBS-Cl and collidine in dichloromethane, tetrahydrofuran or *N*–methylpyrrolidone (NMP). The investigation revealed that protection in THF needed more than 2 h, while complete conversion was observed in 1 h in DCM and in only 15 min in NMP. NMP has also the advantage that no solvent changes have to be done after Fmoc deprotection.

Trityl resin (Tritylchloride polystyrene (TCP))-bound N^{α}-*o*-NBS-protected dipeptides **2b**–**k** were prepared following the optimized procedure (Scheme 2), i.e. by treatment of the corresponding resin-bound amine-free dipeptides **1b**–**k** with *o*-nitrobenzenesulfonyl chloride (4 eq) in the presence of collidine (10 eq) in NMP for 15 min. Monitoring of the reaction by HPLC revealed that protection was completed in 15 min for every tested dipeptide (purity >99%). Analysis of the N^{α}-*o*-NBS-Xaa-Leu-OH dipeptides **2a**–**k** was accomplished by HPLC and ESI-MS (Table 1). Protection of dipeptides **1a**–**k** with *o*-NBS-Cl did not involve racemization as shown by HPLC of the crude product and by comparing with the corresponding N^{α}-*o*-NBS-D-Xaa-Leu-OH dipeptides.



Scheme 2 Synthesis of *o*-NBS protected resin-bound dipeptides **2a**–**k**.

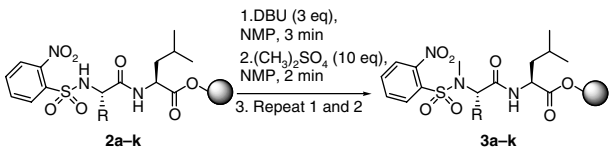
Table 1 Synthesis of N^{α}-*o*-NBS-Xaa-Leu-OH **2a–k**

Peptide	Xaa	HPLC[a] [t_R] (min)	ESI-MS $[M + H]^+$
2a	Phe	20.9	464.2
2b	Lys(Boc)	21.4	545.3
2c	Arg(Pbf)	23.3	725.4
2d	Ser(*t*Bu)	21.7	460.3
2e	Trp	20.7	503.2
2f	Tyr(*t*Bu)	23.1	536.2
2g	Glu(*t*Bu)	21.7	502.3
2h	Gln(Trt)	25.6	687.2
2i	Met	19.6	448.2
2j	Cys(Trt)	26.9	662.2
2k	His(Trt)	22.2	695.1

[a] RP-HPLC on C18 column, MeCN 10 to 100% in 30 min.

In the procedure described by Miller and Scanlan, *N*-methylation of the resulting resin-bound sulfonamides is achieved by using 4 eq of methyl *p*-nitrobenzenesulfonate in combination with 3 eq of the hindered, nonionic guanidium base MTBD (7-methyl-1,5,7-triazabicyclo[4.4.0]dec-5-ene) in DMF and is completed in 30 min (Scheme 1) [33,34]. The high price of MTBD led us to consider the possibility of using other less expensive, structurally related hindered bases like TBD (1,5,7-triazabicyclo[4.4.0]dec-5-ene) or DBU (1,8-diazabicyclo [5,4,0]undec-7-ene). We have recently reported the successful *N*-methylation of *o*-NBS amino acid methyl esters in DMF using DBU as base [32]. A first investigation of the reaction on the resin-bound N^{α}-*o*-NBS-dipeptide **2a** with DBU and dimethylsulfate in DMF revealed a very important side reaction coming from the reaction of DBU with dimethylsulfate and full conversion into N^{α}-*o*-NBS-N^{α}-methyl dipeptide **3a** could not be achieved. The same problem has been observed when TBD is used as base. To overcome this problem, DBU was first reacted with the resin-bound N^{α}-*o*-NBS-protected dipeptide **2a** to ensure complete deprotonation of the sulfonamide and was characterized by the yellow coloration of the resin. After 5 min of reaction, dimethylsulfate was added to the resin for *N*-methylation and the yellow coloration completely disappeared. Monitoring of the reaction showed that 91% of the N^{α}-*o*-NBS dipeptide **2a** is *N*-methylated after 3 min of reaction with DBU and 2 min with dimethylsulfate. Full conversion (>99%) into N^{α}-methyl-N^{α}-*o*-NBS dipeptide **3a** is achieved when the procedure is repeated once more.

The newly optimized *N*-methylation procedure with the less expensive DBU was tested on the other resin-bound N^{α}-*o*-NBS dipeptides **2b–k**. *N*-Methylation was then performed in NMP using 3 eq of DBU for 3 min followed by 10 eq of dimethylsulfate for 2 min (Scheme 3). The sequence was repeated once more



Scheme 3 Solid-phase synthesis of N^{α}-methyl-N^{α}-*o*-NBS-dipeptides **3a**–**k** using DBU and dimethylsulfate.

and gave, in most cases, the corresponding N^{α}-*o*-NBS-N^{α}-methyl dipeptides **3b–j** in >99% purity (HPLC). Unfortunately, a considerable amount of impurity was observed during *N*-methylation of His(Trt)-containing dipeptide **2k.** HPLC–MS analysis revealed that *N*-methylation also occurs on the side chain of histidine with loss of the trityl protecting group. Side chain *N*-methylation of histidine was also observed in solution [32]. Analysis of the N^{α}-*o*-NBS-MeXaa-Leu-OH dipeptides **3a–k** was accomplished by HPLC and ESI-MS (Table 2). *N*-methylation of the dipeptides **2a–j** with DBU and dimethylsulfate did not involve racemization as shown by HPLC of the crude product and by comparing with the corresponding N^{α}-*o*-NBS-D-MeXaa-Leu-OH dipeptides.

To overcome the problem of side-chain methylation, *N*-methylation via Mitsunobu reaction was investigated on the N^{α}-*o*-NBS-dipeptide **2k**. In the procedure described by Yang and Chiu [36], *N*-methylation of resin-bound N^{α}-*o*-NBS-amino acids under Mitsunobu conditions is performed with 5 eq of triphenylphosphine, 10 eq of methanol and 5 eq of diethyl azodicarboxylate (DEAD) in THF for 1 h. The same conditions were applied for *N*-methylation of the resin-bound N^{α}-*o*-NBS-dipeptide **2k** and full conversion without side-chain methylation was observed. Monitoring of the reaction over time revealed that *N*-methylation of the resin-bound N^{α}-*o*-NBS-dipeptide **2k** under Mitsunobu conditions is completed after only 5 min. *N*-Methylation under Mitsunobu conditions would be therefore the method of choice for *N*-methylation of His(Trt) on solid support.

N-methylation under Mitsunobu conditions was performed on the other resin-bound N^{α}-*o*-NBS-dipeptides **2a**–**j** and complete conversion to corresponding N^{α}-methyl-N^{α}-*o*-NBS-dipeptides **3a**–**j** was observed in 10 min in every case (Scheme 4). *N*-Methylation of resin-bound dipeptides **2a**–**k** by Mitsunobu reaction did not involve racemization as shown by HPLC of the crude products and by comparing with the corresponding N^{α}-*o*-NBS-D-MeXaa-Leu-OH dipeptides. Analysis of the N^{α}-*o*-NBS-MeXaa-Leu-OH dipeptides **3a**–**k** from the Mitsunobu procedure was accomplished by HPLC and ESI-MS (Table 2) and showed exactly the same profile as the dipeptides from the DBU procedure.

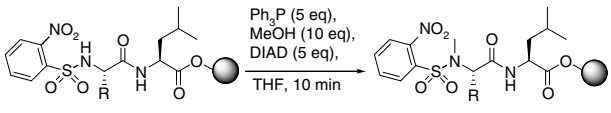
Both *N*-methylation procedures are very efficient and are completed in only ten min. One positive point about the *N*-methylation procedure with DBU is that no solvent changes have to be made, since the reaction is done in NMP. On the other side, *N*-methylation under Mitsunobu conditions needs dry THF, and solvent changes have to be made.

In the procedure described by Miller and Scanlan, removal of the *o*-NBS protecting group is achieved in 30 min with 10 eq of mercaptoethanol and 5 eq of DBU in DMF (Scheme 1) [33,34]. Time needed for complete *o*-NBS deprotection was investigated on resin-bound N^{α}-methyl-N^{α}-*o*-NBS-dipeptide **3a** in NMP and revealed that the reaction is completed in only 5 min. The shortened deprotection procedure was performed on the other resin-bound N^{α}-methyl-N^{α}-*o*-NBS-dipeptides **3b**–**k** and repeated once more for 5 min to ensure complete deprotection in every case (Scheme 5). Removal of the *o*-NBS protection was monitored by HPLC and complete deprotection was observed after repeating two times, 5 min for every studied dipeptide. A major advantage of the *o*-NBS protecting group is that deprotection with mercaptoethanol is selective for *N*-methylated derivatives and does not proceed when the protected amine is not alkylated.

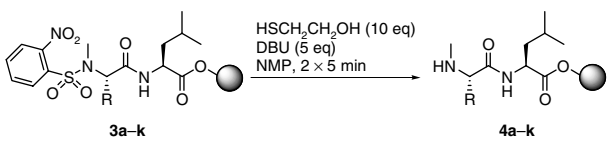
Table 2 Synthesis of N^{α}-*o*-NBS-MeXaa-Leu-OH **3a–k**

Peptide	MeXaa	HPLC[a] [t_R] (min)	ESI-MS $[M + H]^+$
3a	MePhe	22.3	464.2
3b	MeLys(Boc)	22.5	545.3
3c	MeArg(Pbf)	23.9	725.4
3d	MeSer(*t*Bu)	23.0	460.3
3e	MeTrp	22.3	503.2
3f	MeTyr(*t*Bu)	24.4	536.2
3g	MeGlu(*t*Bu)	23.1	502.3
3h	MeGln(Trt)	26.3	687.2
3i	MeMet	21.1	448.2
3j	MeCys(Trt)	28.0	662.2
3k	MeHis(Trt)	22.9	695.1

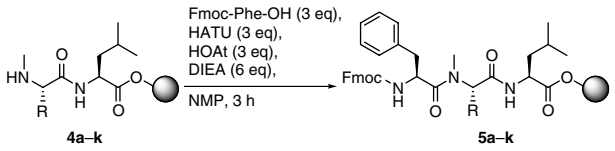
[a] RP-HPLC on C18 column, MeCN 10 to 100% in 30 min.



Scheme 4 Solid-phase synthesis of N^{α}-methyl-N^{α}-*o*-NBS-dipeptides **3a**–**k** under Mitsunobu conditions.



Scheme 5 Synthesis of resin-bound N^{α}-methylamine free dipeptide **4a**–**k**.



Scheme 6 Solid-phase synthesis of N^{α}-methylamino acid containing tripeptides **5a**–**k**.

Couplings on N^{α}-methylamino acids are known to be more challenging than normal couplings [39]. Coupling of Fmoc-Phe-OH to the resin-bound N^{α}-methylamino dipeptide **4a** could not be achieved when *N*-[(1*H*-benzotriazol-1-yl)-dimethylaminomethylene]-*N*-methylmethanaminium tetrafluoroborate *N*-oxide (TBTU) and 1-hydroxybenzotriazole (HOBt) were used as coupling mixture. The use of triphosgene has been recently described for couplings on resin-bound *N*-alkylated peptide [40–43], but the use *N*-[(dimethylamino)-1H-1,2,3-triazolo[4,5 – *b*]pyridine-1-yl-methylene]-*N*-methylmethanaminium hexafluorophosphate (HATU) as coupling reagent was preferred, because no solvent changes are needed and the handling is easier and safer. A combination of HATU with 1-hydroxy-7-azabenzotriazole (HOAt) and diisopropylethylamine (DIEA) in NMP was used to couple Fmoc-Phe-OH to resin-bound N^{α}-methylamine free dipeptides **4a**–**k** (Scheme 6). Every coupling was completed after 3 h (>99% purity) except coupling on the resin-bound dipeptide **5j** containing MeCys(Trt) where only 53% yield was observed. Therefore, coupling on *N*-methyl cysteine–containing dipeptide **4j** had to be performed for 12 h until complete conversion was observed. The tripeptide Fmoc-MePhe-MePhe-Leu-OH **6a** was efficiently prepared (>99% purity) by coupling Fmoc-MePhe-OH to the resin-bound dipeptide **4a** using HATU and HOAt for 3 h. Analysis of the tripeptides **5a–k** and **6a** was accomplished by HPLC (Figure 1) and ESI-MS (Table 3). Couplings using HATU/HOAt did not involve racemization as shown by HPLC of the crude products. The optimized three-step procedure and coupling with HATU/HOAt on the N^{α}-methyl peptides did not cause any detectable racemization of the peptide stereocenters.

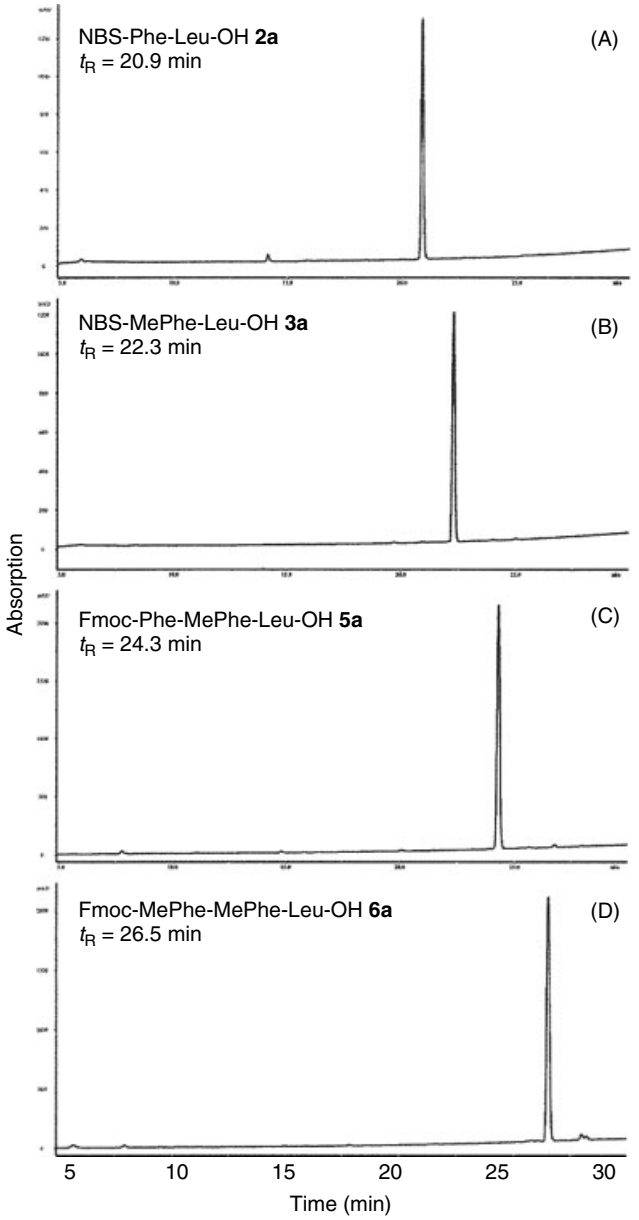


Figure 1 HPLC-chromatograms (λ = 220 nM) of: (A) NBS-Phe-Leu-OH **2a** (MeCN 10–100%); (B) NBS-MePhe-Leu-OH **3a** (MeCN 10–100%); (C) Fmoc-Phe-MePhe-Leu-OH **5a** (MeCN 30–100%); (D) Fmoc-MePhe-MePhe-Leu-OH **6a** (MeCN 30–100%).

CONCLUSION

A simple and convenient optimized three-step procedure is described for the preparation of N^{α}-methylpeptides on solid support. Every step of the procedure for the site-selective *N*-methylation of peptides on solid support described by Miller and Scanlan [33] was optimized in respect of time and economy. Protection and activation of the amino function of the resin-bound amino-free peptide with *o*-NBS-Cl is performed in NMP and is completed in 15 min. Afterwards, the *N*-methylation step can be performed by direct *N*-alkylation using DBU and dimethylsulfate in NMP or under Mitsunobu conditions in THF. Both *N*-methylation reactions are completed in 10 min. Finally the *o*-NBS protecting group is selectively removed by using mercaptoethanol and DBU in NMP twice for 5 min. The optimized procedure is completed in 35 min instead of 3 h for the three steps and no solvent changes are necessary. Compatibility of the procedure with every commonly used amino acid was demonstrated by using the optimized sequence to prepare tripeptides containing different N^{α}-methylamino acids. The *o*-NBS procedure on solid support is also an easy and inexpensive

Table 3 Synthesis of Fmoc-Phe-MeXaa-Leu-OH **5a–k** and Fmoc-MePhe-MePhe-Leu-OH[b] **6a**

Entry	-MeXaa-	HPLC[a] [t_R] (min)	ESI-MS $[M + H]^+$
5a	MePhe	24.3	662.2
5b	MeLys(Boc)	24.5	743.3
5c	MeArg(Pbf)	25.3	923.6
5d	MeSer(*t*Bu)	25.1	658.3
5e	MeTrp	23.9	702.3
5f	MeTyr(*t*Bu)	26.5	734.2
5g	MeGlu(*t*Bu)	25.0	700.2
5h	MeGln(Trt)	28.2	885.4
5i	MeMet	23.4	646.2
5j	MeCys(Trt)	30.4	860.3
5k	MeHis(Trt)	23.7	894.4
6a	Fmoc-MePhe-MePhe-Leu-OH	26,5	676.3

[a] RP-HPLC on C18 column, MeCN 30 to 100% in 30 min.
[b] Prepared similarly.

way to prepare peptides containing N^{α}-methyl-D- and unnatural amino acids. Coupling to N^{α}-methylamino acids on solid support can be efficiently performed with HATU and HOAt. The entire procedure and coupling with HATU/HOAt on the N^{α}-methyl peptides did not cause any detectable racemization of the peptide stereocenters. The optimized procedure is compatible with Fmoc–SPPS conditions and allows a rapid and efficient synthesis of *N*-methyl peptide analogs on solid support.

MATERIALS AND METHODS

General

Tritylchloride polystyrene (TCP) resin (0.94 mmol/g) was purchased from PepChem (Tübingen Germany). Coupling reagents and amino acid derivatives were purchased from Merck Biosciences (Läufelfingen, Switzerland), Perseptive Biosystems GmbH (Hambourg, Germany) and Neosystem (Strasbourg, France). All other reagents and solvents were purchased from Merck (Darmstadt, Germany), Aldrich (Steinheim, Germany) and Fluka (Neu-Ulm, Germany) and were used as received. Standard syringe techniques were applied for transferring dry solvents. Reactions on solid support were performed in filter columns (2 ml) from Abimed. RP-HPLC analyses were conducted on Amersham Pharmacia Biotech instruments using Omnicrom YMC columns (analytical: 2 mm × 250 mm, 5 μm C18, 1 ml/min) with different 30-min linear gradients from water (0.1% TFA) and MeCN (0.1% TFA) and detection at 220 nM. Mass spectra (ESI) were performed on a LCQ Finnigan instrument.

Loading of TCP Resin (General Procedure)

Peptide synthesis was carried out using TCP resin (1 mmol/g) following standard Fmoc strategy [44]. Fmoc-Leu-OH (1.2 eq) was attached to the TCP resin with DIEA (2.5 eq) in anhydrous DCM (2 ml) at room temperature for 1.5 h. After filtration, the remaining trityl chloride groups were capped by a solution of DCM, MeOH, DIEA (17 : 2 : 1; v : v : v) for 15 min. The resin was filtered and washed thoroughly with DCM (2×), DMF (3×) and MeOH (5×). The loading capacity was determined by weight after drying the resin under vacuum, and ranged from 0.75 to 0.8 mmol/g.

Fmoc Deprotection (General Procedure)

The resin-bound Fmoc peptide was treated with 20% piperidine in NMP (v/v) for 15 min and a second time for 10 min. The resin was washed with NMP (5×).

TBTU/HOBt Coupling (General Procedure)

A solution of Fmoc-Xaa-OH (3 eq), TBTU (3 eq), HOBt (3 eq), DIEA (6 eq) in NMP was added to the resin-bound free amine peptide and shaken for 30 min at room temperature. The coupling was repeated another time and the resin was washed with NMP (5×).

o-NBS Protection (Optimized Procedure)

A solution of *o*-NBS-Cl (4 eq) and collidine (10 eq) in NMP was added to the resin-bound free amine peptides **1a–k** and shaken for 15 min at room temperature. The resin was washed with NMP (5×). *o*-NBS-peptides **2a–k** were cleaved from resin by treatment of a small amount of resin with 20% HFIP in DCM (v/v) for 10 min and analyzed by RP-HPLC (MeCN 10–100%) and ESI-MS (Table 1).

N-Methylation with DBU (Optimized Procedure)

A solution of DBU (3 eq) in NMP was added to the resin-bound *o*-NBS-protected peptides **2a–k** and shaken for 3 min. A solution of dimethylsulfate (10 eq) in NMP was then added to the reaction mixture and shaken for 2 min. The resin was filtered off, washed once with NMP and the *N*-methylation procedure repeated once more. The resin was washed with NMP (5×). N^{α}-Methyl-N^{α}-*o*-NBS-peptides **3a–k** were cleaved from resin by treatment of a small amount of resin with 20% HFIP in DCM (v/v) for 10 min and analyzed by RP-HPLC (MeCN 10–100%) and ESI-MS (Table 2).

N-Methylation under Mitsunobu Conditions (Optimized Procedure)

A solution of triphenylphosphine (5 eq) and MeOH (10 eq) in dry THF was added to the resin-bound *o*-NBS-protected peptides **2a–k** and shaken for 1 min. A solution of DIAD (5 eq) in dry THF was then added portionwise to the reaction mixture and shaken for 10 min at room temperature. The resin was filtered off, and washed with NMP (5×). N^{α}-Methyl-N^{α}-*o*-NBS-peptides **3a–k** were cleaved from resin by treatment of a small amount of resin with 20% HFIP in DCM (v/v) for 10 min and analyzed by RP-HPLC (MeCN 10–100%) and ESI-MS (Table 2).

o-NBS Deprotection (Optimized Procedure)

For *o*-NBS deprotection, the resin-bound N^{α}-methyl-N^{α}-*o*-NBS-peptides **3a–k** was treated with a solution of mercaptoethanol (10 eq) and DBU (5 eq) in NMP for 5 min. The

deprotection procedure was repeated once more and the resin was washed with NMP (5×).

HATU/HOAt Coupling (General Procedure)

A solution of Fmoc-Phe-OH (3 eq) or Fmoc-MePhe-OH, HATU (3 eq), HOAt (3 eq), DIEA (6 eq) in NMP was added to the resin-bound N^{α}-methylamine free dipeptides **4a**–**k** and shaken for 3 h at room temperature. The coupling was repeated another time and the resin was washed with NMP (5×). The tripeptides **5a**–**k** and **6a** were cleaved from resin by treatment of a small amount of resin with 20% HFIP in DCM (v/v) for 10 min and analyzed by RP-HPLC (MeCN 30–100%) and ESI-MS (Table 3).

Acknowledgements

The authors gratefully acknowledge the technical assistance from B. Cordes. E.B. thanks the Alexander von Humboldt Foundation for Postdoctoral Fellowships. Financial support was provided by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie.

REFERENCES

1. Humphrey JM, Chamberlin RA. Chemical synthesis of natural product peptides: coupling methods for the incorporation of noncoded amino acids into peptides. *Chem. Rev.* 1997; **97**: 2243–2266. DOI: 10.1021/cr950005s
2. Wipf P. Synthetic studies of biologically active marine cyclopeptides. *Chem. Rev.* 1995; **95**: 2115–2134.
3. Fusetani N, Matsunaga S. Bioactive sponge peptides. *Chem. Rev.* 1993; **93**: 1771–1791.
4. Wenger RM. Synthesis of cyclosporine. 3. Total synthesis of cyclosporine-A and cyclosporine-H, 2 fungal metabolites isolated from the species *Tolypocladium-inflatum gams*. *Helv. Chim. Acta* 1984; **67**: 502–525. DOI: 10.1002/hlca.19840670220
5. Wenger RM. Synthesis of cyclosporine and analogs: structural requirements for immunosuppressive activity. *Angew. Chem., Int. Ed. Engl.* 1985; **24**: 77–85. DOI: 10.1002/anie.198500773
6. Pettit GR, Kamano Y, Herald CL, Dufresne C, Bates RB, Schmidt JM, Cerny RL, Kizu H. Antineoplastic agents. 190. Isolation and structure of the cyclodepsipeptide dolastatin-14. *J. Org. Chem.* 1990; **55**: 2989–2990.
7. Pettit GR, Kamano Y, Herald CL, Fujii Y, Kizu H, Boyd MR, Boettner FE, Doubek DL, Schmidt JM, Chapuis JC, Michel C. Isolation of dolastatins 10–15 from the marine mollusk *Dolabella-Auricularia*. *Tetrahedron* 1993; **49**: 9151–9170.
8. Bates RB, Brusoe KG, Burns JJ, Caldera S, Cui W, Gangwar S, Gramme MR, McClure KJ, Rouen GP, Schadow H, Stessman CC, Taylor SR, Vu VH, Yarick GV, Zhang J, Pettit GR, Bontems R. Dolastatins. 26. Synthesis and stereochemistry of dolastatin 11. *J. Am. Chem. Soc.* 1977; **119**: 2111–2113.
9. Jouin P, Poncet J, Dufour MN, Pantaloni A, Castro B. Synthesis of the cyclodepsipeptide nordidemnin-B, a cytotoxic minor product isolated from the sea tunicate *Tridemnum-cyanophorum*. *J. Org. Chem.* 1989; **54**: 617–627.
10. Li W-R, Joullié MM. The didemnins: biological properties, chemistry and total synthesis. In *Studies in Natural Products Chemistry, Vol. 10, Stereoselective Synthesis (Part F)*, Atta-ur-Rahman (ed). Elsevier: Amsterdam, 1992; 241–302.
11. Ramanjulu IM, Ding X, Joullié MM. Synthesis of a reduced ring analog of didemnin B. *J. Org. Chem.* 1997; **62**: 4961–4969. DOI: 10.1021/jo9623696
12. Vitoux B, Aubry A, Cung MT, Marraud M. *N*-Methyl peptides. 7. Conformational perturbations induced by *N*-methylation of model dipeptides. *Int. J. Pept. Protein Res.* 1986; **27**: 617–632.
13. Goodfellow VS, Marathe MV, Kuhlman KG, Fitzpatrick TD, Cuarado D, Hanson W, Zuzack JS, Ross SE, Wieczorek M, Burkard M, Whalley ET. Bradykinin receptor antagonists containing *N*-substituted amino acids: In vitro and in vivo B-2 and B-1 receptor antagonist activity. *J. Med. Chem.* 1996; **39**: 1472–1484. DOI: 10.1021/jm950716i
14. Takeuchi Y, Marshall GR. Conformational analysis of reverse-turn constraints by *N*-methylation and *N*-hydroxylation of amide bonds in peptides and non-peptide mimetics. *J. Am. Chem. Soc.* 1998; **120**: 5363–5372. DOI: 10.1021/ja970855k
15. Fairlie DP, Abbenante G, March DR. Macrocyclic peptidomimetics: forcing peptides into bioactive conformations. *Curr. Med. Chem.* 1995; **2**: 654–686.
16. Cody WL, He JX, Reily MD, Haleen SJ, Walker DM, Reyner EL, Stewart BH, Doherty AM. Design of a potent combined pseudopeptide endothelin-A/endothelin-B receptor antagonist, Ac-DBhg(16)-Leu-Asp-Ile-[NMe]Ile-Trp(21) (PD 156252): examination of its pharmacokinetic and spectral properties. *J. Med. Chem.* 1997; **40**: 2228–2240. DOI: 10.1021/jm970161m
17. Haviv F, Fitzpatrick TD, Swenson RE, Nichols CJ, Mort NA, Bush EU, Diaz G, Bammert G, Nguyen A, Nellans HN, Hoffman DJ, Johnson ES, Greer J. Effect of *N*-methyl substitution of the peptide-bonds in luteinizing-hormone-releasing hormone agonists. *J. Med. Chem.* 1993; **36**: 363–369.
18. Ali FE, Bennet DB, Calvo RR, Elliot JD, Hwang S, Ku TW, Lago MA, Nichols AJ, Romoff TT, Shah DH, Vasko JA, Wong AS, Yellin TO, Yuan C, Samanen JM. Conformationally constrained peptides and semipeptides derived from RGD as potent inhibitors of the platelet fibrinogen receptor and platelet-aggregation. *J. Med. Chem.* 1994; **37**: 769–780.
19. Mazur RH, James PA, Tyner DA, Hallinan EA, Sanner JH, Schulze R. Bradykinin analogs containing N^{α}-methyl amino acids. *J. Med. Chem.* 1980; **23**: 758–763.
20. Tonelli AE. Effects of isolated *N*-methylated residues on conformational characteristics of polypeptides. *Biopolymers* 1976; **16**: 1615–1622. DOI: 10.1002/bip.1976.360150814
21. Manavalan P, Momany FA. Conformational energy studies on *N*-methylated analogs of thyrotropin releasing hormone, enkephalin, and luteinizing-hormone-releasing hormone. *Biopolymers* 1980; **19**: 1943–1973. DOI: 10.1002/bip.1980.360191103
22. Ron R, Gilon C, Hanani M, Vromen A, Selinger Z, Chorev M. *N*-Methylated analogs of Ac[Nle28,31]CCK(26–33) – synthesis, activity, and receptor selectivity. *J. Med. Chem.* 1992; **35**: 2806–2811.
23. Dechantsreiter MA, Planker E, Mathä B, Lohof E, Hölzemann G, Jonczyk A, Goodman SL, Kessler H. *N*-Methylated cyclic RGD peptides as highly active and selective alpha(v)beta(3) integrin antagonists. *J. Med. Chem.* 1999; **42**: 3033–3040. DOI: 10.1021/jm970832g
24. Laufer R, Wormser U, Friedman ZY, Gilon C, Chorev M, Selinger Z. Neurokinin-B is a preferred agonist for a neuronal substance-P receptor and its action is antagonized by enkephalin. *Proc. Natl. Acad. Sci. U.S.A.* 1985; **82**: 7444–7448.
25. Laufer R, Gilon C, Chorev M, Selinger Z. Characterization of a neurokinin-B receptor-site in rat-brain using a highly selective radioligand. *J. Biol. Chem.* 1986; **261**: 257–263.
26. Rajeswaran WJ, Hocart SJ, Murphy WA. Highly potent and subtype selective ligands derived by *N*-methyl scan of a somatostatin antagonist. *J. Med. Chem.* 2001; **44**: 1305–1311. DOI: 10.1021/jm0005048
27. Rajeswaran WJ, Hocart SJ, Murphy WA. *N*-Methyl scan of somatostatin octapeptide agonists produces interesting effects on receptor subtype specificity. *J. Med. Chem.* 2001; **44**: 1416–1421. DOI: 10.1021/jm000361p

28. Mazur RH, James PA, Tyner DA, Hallinan EA, Sanner JH, Schulze R. Bradykinin analogs containing *N*-alpha-methyl amino-acids. *J. Med. Chem.* 1980; **23**: 758–763.
29. Schmidt R, Kalman A, Chung NN, Lemieux C, Horvath C, Schiller PW. Structure-activity-relationships of dermorphin analogs containing *N*-substituted amino-acids in the 2-position of the peptide sequence. *Int. J. Pept. Protein Res.* 1995; **46**: 47–55.
30. Angell YM, Thomas TL, Flentke GR, Rich DH. Solid-phase synthesis of cyclosporine peptides. *J. Am. Chem. Soc.* 1995; **117**: 7279–7280 and references cited therein.
31. Gilon C, Dechantsreiter MA, Burkhart F, Friedler A, Kessler H. Synthesis of *N*-alkylated peptides. In *Houben-Weyl Methods of Organic Chemistry, Vol E22C, Synthesis of Peptides and Peptidomimetics*, Goodman M, Felix A, Moroder L, Toniolo C (eds). Georg Thieme Verlag: Stutgart, 2002; 215–271 and references cited therein.
32. Biron E, Kessler H. Convenient synthesis of *N*-methylamino acids compatible with Fmoc solid-phase peptide synthesis. *J. Org. Chem.* 2005; **70**: 5183–5189, published ASAP on-line May 20th 2005: DOI: 10.1021/jo050477z
33. Miller SC, Scanlan TS. Site-selective *N*-methylation of peptides on solid support. *J. Am. Chem. Soc.* 1997; **119**: 2301–2302. DOI: 10.1021/ja9635443
34. Miller SC, Scanlan TS. oNBS-SPPS: a new method for solid-phase peptide synthesis. *J. Am. Chem. Soc.* 1998; **120**: 2690–2691. DOI: 10.1021/ja974252k
35. Reichwein JF, Liskamp MJ. Site-specific *N*-alkylation of peptides on the solid phase. *Tetrahedron Lett.* 1998; **39**: 1243–1246. DOI: 10.1016/S0040-4039(97)10766-3
36. Yang L, Chiu K. Solid phase synthesis of Fmoc *N*-methyl amino acids: application of the fukuyama amine synthesis. *Tetrahedron Lett.* 1997; **38**: 7307–7310. DOI: 10.1016/S0040-4039(97)01774-7
37. Laplante C, Hall DG. Direct mono-*N*-methylation of solid supported amino acids: a useful application of the matteson rearrangement of α-aminoalkylboronic esters. *Org. Lett.* 2001; **3**: 1487–1490. DOI: 10.1021/ol0158031
38. Fukuyama T, Jow C-K, Cheung M. 2- and 4-Nitrobenzenesulfonamides: exceptionally versatile means for preparation of secondary amines and protection of amines. *Tetrahedron Lett.* 1995; **36**: 6373–6374. DOI: 10.1016/0040-4039(95)01316-A
39. Teixidó M, Albericio F, Giralt E. Solid-phase synthesis and characterization of *N*-methyl-rich peptides. *J. Pept. Res.* 2005; **65**: 153–166. DOI: 10.1111/j.1399–3011.2004.00213.x
40. Falb E, Yechezkel T, Salitra Y, Gilon C. *In situ* generation of Fmoc-amino acid chlorides using *bis*-(trichloromethyl)carbonate and its utilization for difficult couplings in solid-phase peptide synthesis. *J. Pept. Res.* 1999; **53**: 507–517. DOI: 10.1034/j.1399–3011.1999.00049.x
41. Thern B, Rudolph J, Jung G. Total synthesis of the nematicidal cyclododecapeptide omphalotin A by using racemization-free triphosgene-mediated couplings in the solid phase. *Angew. Chem., Int. Ed. Engl.* 2002; **41**: 2307–2309. DOI: 10.1002/1521-3773(20020703)41 : 13⟨2307::AID-ANIE2307⟩3.0.CO;2-Y
42. Thern B, Rudolph J, Jung G. Triphosgene as highly efficient reagent for the solid-phase coupling of *N*-alkylated amino acids – total synthesis of cyclosporin O. *Tetrahedron Lett.* 2002; **43**: 5013–5016. DOI: 10.1016/S0040-4039(02)00657-3
43. Sewald N. Efficient, racemization-free peptide coupling of *N*-alkyl amino acids by using amino acid chlorides generated in situ – total syntheses of the cyclopeptides cyclosporin O and omphalotin A. *Angew. Chem., Int. Ed. Engl.* 2002; **41**: 4661–4663. DOI: 10.1002/anie.200290008
44. Fields GB, Noble RL. Solid phase peptide synthesis utilizing 9-fluorenylmethoxycarbonyl amino acids. *Int. J. Pept. Protein Res.* 1990; **35**: 161–214.