CHEMISTRY
A European Journal

A Journal of
ChemPubSoc Europe

## Accepted Article

**Title:** Exploiting the MeDbz Linker to Generate Protected or Unprotected C-Terminally Modified Peptides

**Authors:** Christine A Arbour, Hasina Y Saraha, Timothy F McMillan, and Jennifer Stockdill



**To be cited as:** *Chem. Eur. J.* 10.1002/chem.201703380

**Link to VoR:** http://dx.doi.org/10.1002/chem.201703380

Supported by
ACES

WILEY-VCH

10.1002/chem.201703380

COMMUNICATION

WILEY-VCH

# Exploiting the MeDbz Linker to Generate Protected or Unprotected C-Terminally Modified Peptides

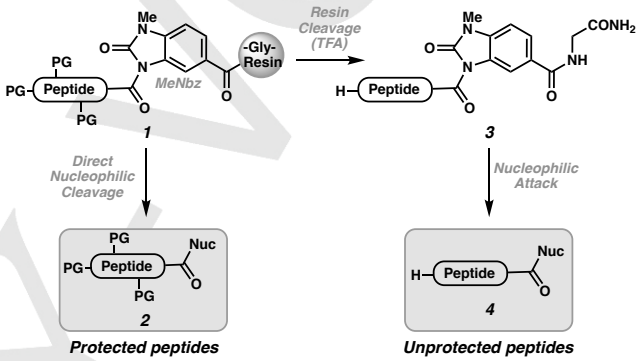
Christine A. Arbour, Hasina Y. Saraha, Timothy F. McMillan, and Jennifer L. Stockdill*

**Abstract:** C-terminally modified peptides are important targets for pharmaceutical and biochemical applications. Known methods for C-terminal diversification are limited mainly in terms of the scope of accessible modifications or by epimerization of the C-terminal amino acid. In this work, we present a broadly applicable approach that enables access to a variety of C-terminally functionalized peptides in either protected or unprotected form. This chemistry proceeds without epimerization of C-terminal Ala and tolerates nucleophiles of varying nucleophilicity. Finally, unprotected peptides bearing nucleophilic side chain groups can be selectively functionalized by strong nucleophiles, while macrocyclization is observed for weaker nucleophiles. The potential utility of this method is demonstrated through the divergent synthesis of the conotoxin conopressin G and GLP-1(7-36) and analogs.

C-Terminally modified peptides are important for the development and delivery of peptide based pharmaceuticals because they improve peptide activity,[1] stability,[2] hydrophobicity, and membrane permeability.[3] Additionally, the vulnerability of C-terminal esters to cleavage by endogenous esterases makes them excellent pro-drugs.[3] Meanwhile, C-terminal thioesters[4] and hydrazides[5] are critical to the synthesis of larger peptide targets via native chemical ligation (NCL)[6] and non-cysteine NCL.[7] Despite the demand for C-terminally modified peptides, there remain significant limitations in the available strategies to access them by chemical synthesis.[8,9] Variations at the C-terminus traditionally require repetition of the solid-phase peptide synthesis (SPPS) on a different linker for each desired C-terminal moiety[2a,2c,10] or the use of a C-terminal glycine.[11] Solution-phase activation of protected C-terminal acids risks epimerization.[12] Side-chain anchoring strategies are limited by the need for particular amino acids at the C-terminus and by epimerization during the activation of the C-terminal carboxylic acid.[13] Recent efforts to diversify the C-terminus from a single SPPS effort suffer from epimerization,[14] require extended reaction times or heating,[15] are incompatible with common cysteine protecting groups,[15,16] or require pre-functionalization of the peptide.[17]

For broad utility, the ideal functionalization method should employ a commercially available resin/linker, use convenient reagents for activation, undergo reaction with a variety of nucleophiles of varying steric and nucleophilic properties, and proceed at ambient temperature without epimerization of the C-terminal residue. Furthermore, the approach should enable the user to select between the production of protected peptides and the solution-phase diversification of unprotected peptides. To date, no report has demonstrated the achievement of this set of objectives. In this manuscript, we commandeer the commercially available MeDbz linker[18] to realize these goals for the first time.

Our strategy for divergent C-terminal functionalization is outlined in **Scheme 1**. MeNbz-Linked resin-bound peptides **1** are accessible upon activation of the MeDbz linker.[18] We envisioned that these peptides could be directly cleaved to afford protected C-terminally modified peptides **2** or treated with trifluoroacetic acid (TFA) to afford side-chain-deprotected MeNbz peptides **3**. Upon exposure to various nucleophiles, unprotected peptides **3** would be diversified at the C terminus (**4**). The susceptibility of this linker to attack by weaker nucleophiles than thiols[4] and hydrazine[19] remains under-explored.[20] Additionally, the direct nucleophilic cleavage of this linker from resin has not been reported.




**Scheme 1.** Proposed strategy for C-terminal peptide modification.

To establish the reactivity of the resin-bound MeNbz group, we treated MeNbz-linked tripeptides (**5a-c**) with a variety of nucleophiles (**Table 1**). A Gly residue was installed at the C-terminus (**5a**) to avoid concerns related to epimerization at this stage. The generation of thioesters from MeNbz is well established,[18b] so we began our investigation with nitrogen nucleophiles. As the smallest nucleophile, we expected that bubbling with ammonia would readily induce cleavage from the resin.[21] Indeed, we observed complete removal of the peptide from the resin after bubbling with a balloon of $NH_3$ for 1.5 h. The corresponding tripeptide carboxamide was isolated in 41% yield. Primary amines of varying nucleophilicity were evaluated.[22] Butylamine and 3-azidopropylamine[23] proceeded with complete conversion. Propargyl amine and benzyl amine are slightly less nucleophilic than primary alkyl amines, but both are efficient in displacing MeNbz. Importantly, azide and propargyl containing products can be further diversified via azide-alkyne cycloaddition.[24] The least nucleophilic amine tested was aniline, which led to minimal product formation. However, in the presence of 5 equiv Hünig's base, aniline displacement proceeded with 53% conversion, allowing access to peptide N-aryl amides via this strategy.

Because of the unique biological properties of C-terminal esters,[3] we were interested in the ability of oxygen nucleophiles to displace MeNbz. To maximize the amount of $RO^-$ in solution, alcohols were combined with KO*t*Bu and added to the swelled resin.[14] Primary alkoxides reacted with complete conversion to afford the corresponding esters. Steric hindrance in the nucleophile slows the reaction

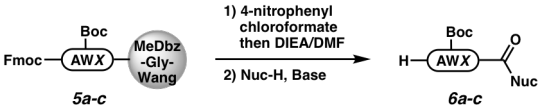
[a] C. A. Arbour, H. Y. Saraha, T. F. McMillan, Prof., Dr. J. L. Stockdill
Department of Chemistry
Wayne State University, Detroit, MI 48202
stockdill@wayne.edu

Electronic Supplementary Information (ESI) available: See
DOI: 10.1039/x0xx00000x

considerably, with isopropoxide proceeding to only 62% conversion. Although benzyl oxide was also less efficient than the alkoxides, phenoxide led to complete conversion. Finally, treatment with aqueous NaOH resulted in >99% conversion to the corresponding carboxylic acid. Overall, a variety of strong, weak, and even branched *N*- and *O*-nucleophiles are effective in cleaving MeNbz from the resin. Additionally, hindered C-terminal amino acids and bulky protecting groups are tolerated. Tripeptide **5b** (X=Ile) and **5c** (X=Arg(Pbf)) reacted with excellent conversion when treated with ammonia, butylamine, or MeOH/KO*t*Bu.[25]

To demonstrate the convenience of this approach, we synthesized conopressin G (**7**), a C-terminal carboxamide-containing vasopressin homolog isolated from the venom of piscivorous *Conus* snails, and 2 analogs (**Scheme 2**).[26] This neuroactive peptide contains a single disulfide bridge and a C-terminal carboxamide. Following SPPS, the disulfide bond was accessed via iodine-mediated Trt cleavage/oxidation. The MeDbz linker was activated in the presence of the disulfide linkage. The resin was divided into 3 vessels and treated with ammonia, propargyl-amine, or MeOH/KO*t*Bu, cleaving the peptide from the resin and generating the fully protected conopressins. The remaining protecting groups were removed, affording native conopressin (**7-NH$_2$**) in 29% isolated yield, propargyl conopressin (**7-NHpropargyl**) in 7% yield, and conopressin methyl ester (**7-OMe**) in 11% isolated yield. Additionally, we synthesized the active portion of glucagon-like peptide-1, GLP-1(7-36). GLP-1 receptor agonists are state-of-the-art pharmaceutical agents, with 16 different GLP-1 agonists in clinical trials as of 2015.[27] GLP-1(7-36)–*MeNbz-Gly-Resin* was treated with ammonia in DMF for 1.5 h to afford native GLP-1(7-36) in >99% conversion and 4% isolated yield (27% crude).

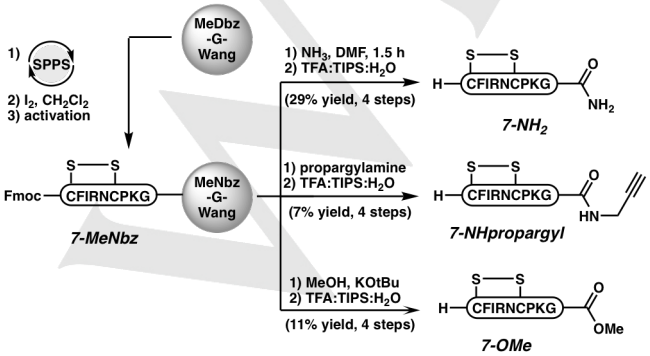
**Table 1.** Access to C-terminally modified protected peptides by direct cleavage of MeNbz-linked peptides on resin.




*5a-c* *6a-c*

| entry[a] | amino acid (*X*) | Nuc-H | base (5 equiv) | % conversion[b] (% isolated yield) |
|---|---|---|---|---|
| 1 | Gly | $NH_3$ | – | >99 (41) |
| 2 | Gly | $H_2N$ | – | >99 |
| 3[c] | Gly | $H_2N$ $N_3$ | – | >99 (30) |
| 4 | Gly | $H_2N$ | – | >99 |
| 5 | Gly | $H_2N$ | – | 98 |
| 6 | Gly | $H_2N$ | DIEA | 53[d] |
| 7[e] | Gly | MeOH | KO*t*Bu | >99 (41) |
| 8[e] | Gly | EtOH | KO*t*Bu | >99 |
| 9 | Gly | *i*-PrOH | KO*t*Bu | 62 |
| 10 | Gly | BnOH | KO*t*Bu | 63 |
| 11[f] | Gly | PhOH | KO*t*Bu | 98 |
| 12 | Gly | $H_2O$ | NaOH | >99 |
| 13 | Ile | $NH_3$ | – | >99 (68) |
| 14 | Ile | $H_2N$ | – | >99 |
| 15[e] | Ile | MeOH | KO*t*Bu | 96 |
| 16 | Arg(Pbf) | $NH_3$ | – | >99 |
| 17 | Arg(Pbf) | $H_2N$ | – | >99 |
| 18[e] | Arg(Pbf) | MeOH | KO*t*Bu | >99 |

[a] All reactions were conducted on 100 mg of resin at rt (24 ± 1 °C), [b] Conversion based on MS ratio of MeNbz-Gly and AWG-MeNbz-Gly, [c] A 0.7 M solution of Nuc-H in DMF (500 μL) was used, [d] N-terminus is Fmoc protected, [e] Incomplete removal of Fmoc was observed, [f] Reaction was conducted in MeCN.




**Scheme 2.** Divergent synthesis of conopressin G and analogs.

With the viability of the method established, our next focus was evaluating the extent of epimerization under these conditions using Fmoc-AW(Boc)A–*MeNbz-Gly-Wang* (**8**).[25] To our delight, no epimerization was observed upon displacement of MeNbz by butylamine (**Table SI-1**). Given the extent of epimerization observed by Meldal and co-workers in the presence of KO*t*-Bu (50%),[14, 28] we expected to observe epimerization during treatment with KO*t*-Bu/MeOH. Indeed, under these conditions, 15% epimerization was observed. Dawson reported <2% epimerization during the activation of C-terminal Tyr-Dbz in the presence of Hünig's base.[18] Thus, we hypothesized that under similar conditions, an epimerization-free ester modification might be feasible. Indeed, treatment with 5 equiv Hünig's base in MeOH led to complete conversion with no observable epimerization. Analogously, employing Hünig's base in water led to the carboxylic acid with no epimerization. Additionally, H-AW(Boc)H(Trt)-*MeNbz-Gly-Wang* was prepared and cleaved with ammonia. No epimerization was observed.[25]

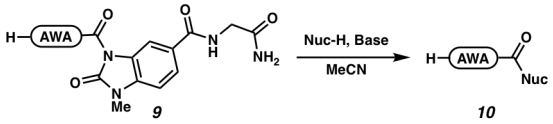
An advantage of the MeDbz linker is that the activated linker (MeNbz) is stable to typical post-SPPS manipulations including resin cleavage, purification,[18] and storage.[29] Thus, we imagined that solution-phase modifications of unprotected peptides would be feasible. The ability to diversify the C-terminus of an unprotected peptide in solution would be ideal for situations where the SPPS itself led to multiple close-eluting products. Rather than diversification during resin cleavage followed by several challenging purifications, a single purification could be executed, followed by solution-phase diversification of the pure MeNbz peptide. For simplicity during evaluation of the nucleophile scope, we generated the tripeptide H-AWA-*MeNbz-Gly-NH$_2$* (**9**), which does not have any nucleophilic side chains. The crude peptide was dissolved in MeCN then treated with the nucleophile. To avoid epimerization, Hünig's base was employed when a stoichiometric base was needed. A variety of primary amines were tolerated, leading to complete conversion in 30 min (**Table 3**). In contrast to the resin-bound approach, complete conversion was also observed for less nucleophilic amines such as aniline. Hydrazine and hydroxylamine were excellent nucleophiles,[30] affording the corresponding hydrazide and hydroxamide[31] with >99% conversion. The reaction has some sensitivity to steric effects, as demonstrated by the low conversion observed with Weinreb amine. Primary alkyl, benzyl, and phenyl alcohols were competent nucleophiles in the presence of Hünig's base, whereas *i*-PrOH was more sluggish (61% conversion, 5 h). In the presence of aqueous NaOH, the carboxylic acid was observed. Finally, the β-amino alcohol could be generated by $NaBH_4$ addition.

The solution-phase C-terminal diversification of MeNbz-linked peptides is notable in the ready accessibility of the

10.1002/chem.201703380




**Table 2.** Scope of nucleophilic substitution of MeNbz in solution.



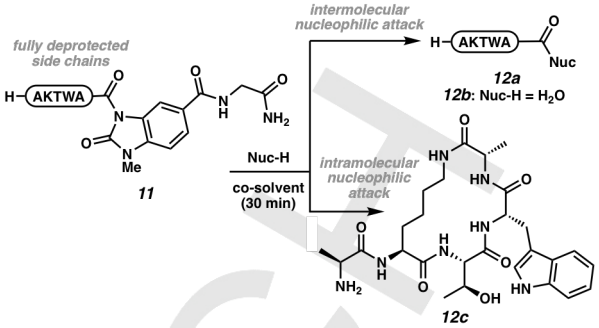

| entry[a] | Nuc-H | base[b] | time (h) | conversion (%)[c] |
|---|---|---|---|---|
| 1 |  | – | 0.5 | >99 |
| 2[d] |  | – | 0.5 | >99 |
| 3 |  | – | 0.5 | >99 |
| 4 |  | – | 0.5 | >99 |
| 5 |  | DIEA | 6 | >99 |
| 6 | $H_2N–NH_2$ | – | 0.5 | >99 |
| 7[e] | $H_2N–OH$ | DIEA | 3 | >99 |
| 8[e] |  | DIEA | 6 | 48 |
| 9 | MeOH | DIEA | 0.5 | >99 |
| 10 | EtOH | DIEA | 5 | >99 |
| 11 | BnOH | DIEA | 0.5 | >99 |
| 12[f] | PhOH | DIEA | 1 | >99 |
| 13 | *i*-PrOH | DIEA | 5 | 61 |
| 14 | $H_2O$ | NaOH | 0.5 | >99 |
| 15[g] | $NaBH_4$ | – | 0.5 | >99 |

[a] All reactions were performed on 20 mg crude peptide in 200 μL MeCN at rt (24 ± 1 °C), [b] 5 equiv, unless noted. [c] Conversion based on MS data, [d] 10 equiv Nuc-H was first dissolved in 100 μL MeCN then added to the peptide mixture, [e] 5 equiv of the Nuc•HCl salt was used with 10 equiv base, [f] 10 equiv of PhOH was added directly to the peptide mixture, [g] Reaction was conducted in 200 μL THF, giving the C-terminal amino alcohol.

activated peptide, the mild conditions, the short reaction times, and the scope of nucleophiles demonstrated. For maximum utility, the functionalization of unprotected peptides should be compatible with residues bearing nucleophilic side chains.[17] Thus, we synthesized H-AKTWA-*MeNbz*-*Gly* (**11**) and subjected it to various nucleophiles for 30 min (**Table 3**). The reactions were quenched by dilution with 1:1 MeCN:$H_2O$, and the product distribution (**12a**:**12b**:**12c**) was analyzed by HPLC integration. The functionalized acyclic peptide (**12a**) was the desired target. Unreacted peptide **11** was hydrolyzed to the corresponding acid (**12b**) during the quench and apart from entry 2, this product represents unreacted starting material. Attack of either the N-terminus or the side chain of Lys or Thr would afford a cyclic peptide. The sole macrocyclic product was assigned as **12c** based on the reactivity of the primary amine relative to an α-branched amine or alcohol and by independent synthesis of the head-to-tail cyclized lactam.[25]

In 1:1 MeCN:$BuNH_2$, only the intermolecular amide product was observed. Repeating this reaction with 1:1:1 MeCN:$H_2O$:$BuNH_2$ still led primarily to the amide with 7% hydrolysis occurring during the reaction. Thus, the modification has reasonable tolerance to aqueous conditions when an excess of nucleophile is employed. Propargylamine was similarly effective, while the reduced nucleophilicity of aniline resulted in an 8:92 ratio of amide to macrolactam. Peptide hydrazides[19, 32] and hydroxamides[31] were generated with no macrocycle formation. In contrast, treatment with Weinreb amine led to 89% conversion to the macrolactam. Functionalization with MeOH proceeded with complete conversion to a 42:58 ratio of methyl ester to lactam. In the presence of a non-nucleophilic base, the lactam was formed with 91% conversion. Finally, sodium borohydride reduction was slower, likely because of the MeCN co-solvent, but no macrocycle was observed.

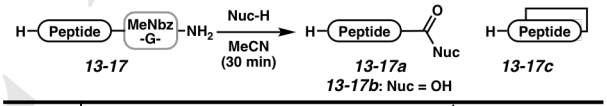
**Table 3.** Selectivity for intermolecular attack vs intramolecular attack.




| entry[a] | Nuc-H | base | co-solvent | 12a:12b:12c (%)[b] | | |
|---|---|---|---|---|---|---|
| 1 |  | – | MeCN | >99 | – | – |
| 2[c] |  | – | MeCN/$H_2O$ | 93 | 7 | – |
| 3 |  | – | MeCN | >99 | – | – |
| 4 |  | DIEA (5 equiv) | MeCN | 8 | – | 92 |
| 5 | $H_2N–NH_2$ | – | MeCN | >99 | – | – |
| 6[d] | $H_2N–OH$ | DIEA (10 equiv) | MeCN | 82 | 18 | – |
| 7[d] |  | DIEA (10 equiv) | MeCN | – | 11 | 89 |
| 8 | MeOH | DIEA (5 equiv) | MeCN | 42 | – | 58 |
| 9[e] | – | DIEA (10 equiv) | MeCN | – | 9 | 91 |
| 10[f] | $NaBH_4$ | – | THF/MeCN | 77 | 23 | – |

[a]Unless noted, all reactions were performed on 20 mg crude peptide in 200 μL MeCN with 200 μL nucleophile at rt (24 ± 1 °C), [b]Relative ratio of MS data, [c]Performed in 200 μL each MeCN, $H_2O$, $BuNH_2$, [d]5 equiv of the Nuc•HCl salt was used and Hünig's base was increased accordingly, [e]Performed on 60 mg crude peptide in 600 μL MeCN, [f]Performed in 100 μL THF and 200 μL MeCN using 5 equiv $NaBH_4$.

**Table 4.** Selectivity for cyclization-prone substrates and evaluation of othe nucleophilic side chains.




| entry[a] | Peptide (*13-17*) | Nuc-H | base | solvent | *a* : *b* : *c* (%)[b] | | |
|---|---|---|---|---|---|---|---|
| 1 | AKT*P*WA (*13*) |  | – | MeCN | >99 | – | – |
| 2 | AKT*P*WA | MeOH | DIEA (5 equiv) | MeCN | 39 | – | 61 |
| 3 | AKT*P*WA | – | DIEA (11 equiv) | MeCN | – | – | >99 |
| 4 | AKT*G*WA (*14*) |  | – | MeCN | 97 | – | 3 |
| 5 | AKT*G*WA | MeOH | DIEA (5 equiv) | MeCN | 9 | – | 88 |
| 6 | AKT*G*WA | – | DIEA (11 equiv) | MeCN | – | – | >99 |
| 7 | A*ST*GWA (*15*) |  | – | MeCN | >99 | – | – |
| 8 | A*ST*GWA | MeOH | DIEA (5 equiv) | MeCN | >99 | – | – |
| 9 | A*ST*GWA | – | DIEA (11 equiv) | MeCN | – | 65 | 35 |
| 10 | A*CT*GWA (*16*) |  | – | MeCN | >99 | – | – |
| 11 | A*CT*GWA | MeOH | DIEA (5 equiv) | MeCN | >99 | – | – |
| 12 | A*CT*GWA | – | DIEA (11 equiv) | MeCN | – | 34 | 66 |
| 13 | A*YT*GWA (*17*) |  | – | MeCN | >99 | – | – |
| 14 | A*YT*GWA | MeOH | DIEA (5 equiv) | MeCN | >99 | – | – |
| 15 | A*YT*GWA | – | DIEA (11 equiv) | MeCN | – | 19 | 81 |

[a]Unless noted, all reactions were performed on 5 mg crude peptide in 100 μL MeCN:$H_2O$ (95:5) with 100 μL nucleophile at rt (24 ± 1 °C), [b]Relative ratio of MS data.

Peptides containing Pro and Gly are generally more prone to macrocyclization.[33] Peptides **13** and **14** were evaluated to determine whether more cyclization-prone substrates could be efficiently functionalized (**Table 4**). The proclivity of these peptides towards macrocyclization was validated by control reactions with MeOH/Hünig's base and Hünig's base alone

(entry 2,3,5,6). Peptides **13** and **14** were more prone to cyclization than AKTWA. Yet, in the presence of a 50:47.5:2.5 ratio of butylamine/MeCN/$H_2O$, excellent conversion to the butyl amide (**13a**, **14a**) was observed for both peptides (entry 1,4). All remaining nucleophilic side chains were evaluated by replacing Lys with Ser (**15**), Cys (**16**), and Tyr (**17**). In all cases, the butyl amide and the methyl ester could be accessed with no hydrolysis or macrocyclization. Omission of the exogenous nucleophile confirmed that these peptides form macrocycles during the short reaction (entry 9, 12, 15). Overall, intermolecular C-terminal functionalization with strong, unhindered nucleophiles occurs with excellent selectivity. Finally, to probe the utility of the in-solution chemistry, Fmoc-GLP-1(7-36)–*MeDbz-Gly-Rink* was activated and cleaved from the resin to afford unprotected GLP-1(7-36)–*MeNbz-Gly-*$NH_2$ in 45% crude yield. Subsequent displacement with butylamine in MeCN/$H_2O$ led to GLP-1(7-36)-NHBu with >99% conversion and 22% isolated yield.

In summary, we have developed a versatile method for the C-terminal functionalization of peptides. Our approach is tolerant of a variety of nucleophiles, yielding carboxamides, alkyl and aryl amides and esters, hydrazides, hydroxamides, acids, and amino alcohols from a single SPPS effort. Either protected or unprotected peptides can be used. When employing a large excess of a strong nucleophile, both water and unprotected nucleophilic side chains are tolerated. We have demonstrated the utility of this approach via the divergent synthesis of 3 conopressin G derivatives and 2 GLP-1(7-36) derivatives. This convenient method will facilitate the synthesis of important bioactive peptides with diverse C-terminal functionalities, enabling investigation of their potential as pharmaceutical agents.

## Acknowledgements

The authors thank the National Institutes of Health (R00-GM097095) and Wayne State University (WSU) for generous financial support (startup funds to JLS, Rumble-Schaap Fellowship to CAA, Knoller Fellowship to HYS). We acknowledge Dr. Johnna A. Birbeck and Dr. Bashar Ksebati of the WSU Lumigen Instrument Center for spectroscopic support and Shimadzu for a grant supporting the MS.

**Keywords:** peptide • C-terminal functionalization • MeDbz linker • N-acyl urea • epimerization

## Notes and references

[1] B. A. Eipper, D. A. Stoffers, R. E. Mains, *Annu. Rev. Neurosci.* **1992**, *15*, 57–85.
[2] (a) J. Alsina, F. Albericio, *Biopolymers* **2003**, *71*, 454–477; (b) M. F Songster, G. Barany, *Methods Enzymol* **1997**, *289*, 126–174; (c) W.-J. Fang, T. Yakovleva, J. V. Aldrich, *Biopolymers (Pept. Sci.)* **2011**, *96*, 715–722.
[3] (a) J. A. Zablocki, F. S. Tjoeng, P. R. Bovy, M. Miyano, R. B. Garland, K. Williams, L. Schretzman, M. E. Zupec, J. G. Rico, R. J. Lindmark, M. V. Toth, D. E. McMackins, S. P. Adams, S. G. Panzer-Knodle, N. S. Nicholson, B. B. Taite, A. K. Salyers, L. W. King, J. G. Campion, L. P. Feigen, *Bioorg. Med. Chem.* **1995**, *3*, 539–551; (b) H.-J. Doh, W.-J. Cho, C.-S. Yong, H.-G. Choi, J. S. Kim, C.-H. Lee, D.-D. Kim, *J. Pharm. Sci.* **2003**, *92*, 1008–1017.
[4] (a) F. Thomas, *J. Pept. Sci.* **2013**, *19*, 141–147; (b) Z. P. Gates, B. Dhayalan, S. B. H. Kent, *Chem. Commun.* **2016**, *52*, 13979–13982; (c) H. Li, S. Dong, *Sci. China Chem.* **2017**, *60*, 201–213; and references therein.
[5] (a) J.-S. Zheng, S. Tang, Y.-K. Qi, Z.-P. Wang, L. Liu, *Nat. Protoc.* **2013**, *8*, 2483–2495; (b) J. Li, Y. Li, Q. He, Y. Li, H. Li, L. Liu, *Org. Biomol. Chem.* **2014**, *12*, 5435–5441; (c) S.-S. Wang, *J. Org. Chem.* **1975**, *40*, 1235–1239.
[6] (a) P. E. Dawson, T. W. Muir, I. Clark-Lewis, S. B. Kent, *Science*, **1994**, *266*, 776−779; (b) E. C. B. Johnson, S. B. H. Kent, *J. Am. Chem. Soc.* **2006**, *128*, 6640–6646.
[7] S. Shang, Z. Tan, S. J. Danishefsky, *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 5986–5989.
[8] Enzymatic approaches have also been reported. See: (a) R. Kulathila, K. A. Merkler, D. J. Merkler, *Nat. Prod. Rep.* **1999**, *16*, 145–154; (b) T. Nuijens, C. Cusan, J. A. W. Kruijtzer, D. T. S. Rijkers, R. M. J. Liskamp, P. J. L. M. Quaedflieg, *J. Org. Chem.* **2009**, *74*, 5145–5150; (c) J. M. Antos, G.-L. Chew, C. P. Guimaraes, N. C. Yoder, G. M. Grotenbreg, M. W.-L. Popp, H. D. Ploegh, *J. Am. Chem. Soc.* **2009**, *131*, 10800–10801, (d) T. Nuijens, E. Piva, J. A. W. Kruijtzer, D. T. S. Rijkers, R. M. J. Liskamp, P. J. L. M. Quaedflieg, *Tetrahedron Lett.* **2012**, *53*, 3777–3779; (e) C. G. Boeriu, A. E. Frissen, E. Boer, K. Kekem, D.-J. van Zoelen, I. F. Eggen, *J. Mol. Catal. B: Enzym.* **2010**, *66*, 33–42; (f) B. Wu, H. J. Wijma, L. Song, H. J. Rozeboom, C. Poloni, Y. Tian, M. I. Arif, T. Nuijens, P. J. L. M. Quaedflieg, W. Szymanski, B. L. Feringa, D. B. Janssen, *ACS Catal.* **2016**, *6*, 5405–5414.
[9] E. Nicolás, J. Clemente, M. Perelló, F. Albericio, E. Pedroso, E. Giralt, *Tetrahedron Lett.* **1992**, *33*, 2183–2186.
[10] M. Góngora-Benítez, J. Tulla-Puche, F. Albericio, *ACS Comb. Sci.* **2013**, *15* 217–228; (b) Qvortrup, K.; V. V. Komnatnyy, T. E. Nielsen, *Org. Lett.* **2014**, *16*, 4782–4785, (c) H. T. Ten Brink, J. T. Meijer, R. V. Geel, M. Damen, D. W. P. M Löwik, J. C. M. Van Hest, *J. Pept. Sci.* **2006**, *12*, 686–692.
[11] J. A. Camarero, A. Adeva, T. W. Muir *Lett. Pept. Sci.* **2000**, *7*, 17–21.
[12] B. Henkel, L. Zhang, E. Bayer, *Liebigs Ann. Recl.* **1997**, 2161–2168.
[13] (a) Z. Huang, D. J. Derksen, J. C. Vederas, *Org. Lett.* **2010**, *12*, 2282–2285; (b) V. Diaz-Rodriguez, E. Ganusova, T. M. Rappe, J. M. Becker, M. D. Distefano, *J. Org. Chem.* **2015**, *80*, 11266–11274.
[14] J. Hansen, F. Diness, M. Meldal, *Org. Biomol. Chem.* **2016**, *14*, 3238–3245.
[15] (a) H. E. Elashal, Y. E. Sim, M. Raj, *Chem. Sci*. **2017**, *8,* 117–123; (b) H. E. Elashel, R. D. Cohen, M. Raj, *Chem. Commun.* **2016**, *52*, 9699–9702.
[16] (a) J. A. Camarero, B. J. Hackel, J. J. de Yoreo, A. R. Mitchell, *J. Org. Chem.* **2004**, *69*, 4145–4151; (b) Y. Kwon, K. Welsh, A. R. Mitchell, J. A. Camarero, *Org. Lett.* **2004**, *6*, 3801–3804; (c) R. A. Turner, R. J. Weber, R. S. Lokey, *Org. Lett.* **2010**, *12*, 1852–1855.
[17] (a) P. Wang, K. T. Shaw, B. Wingham, R. Ramage, *Tetrahedron Lett.* **1998**, *39*, 8719–8720, (b) A. A. Vinogradov, M. D. Simon, B. L. Pentelute, *Org. Lett.* **2016**, *18*, 1222–1225.
[18] (a) J. B. Blanco-Canosa, P. E. Dawson, *Angew. Chem. Int. Ed.* **2008**, *47*, 6851–6855; (b) S. K. Mahto, C. J. Howard, J. C. Shimko, J. J. Ottesen *ChemBioChem*, **2011**, *12*, 2488–2494; (c) J. B. Blanco-Canosa, B. Nardone, F. Albericio, P. E. Dawson, *J. Am. Chem. Soc.* **2015**, *137*, 7197–7209.
[19] (a) P. Siman, V. Karthikean, M. Nikolav, W. Fischle, A. Brik, *Angew. Chem. Int. Ed.* **2013**, *52*, 8059–8063; (b) H. P. Hemantha, S. D. Bavikar, Y. Herman-Bachinsky, N. Haj-Yahya, S. Bondalapati, A. Ciechanover, A. Brik *J. Am. Chem. Soc.* **2014**, *136*, 2665–2673; (c) S. Buhler, J. H. Akkerdaas, T. A. Pertinhez, R. Van Ree, A. Dossena, S. Sforza, T. Tedeschi, *J. Pept. Sci.* **2017**, *23*, 282–293.
[20] (a) R. Pascal, D. Chauvey, R. Sola, *Tetrahedron Lett.* **1994**, *34*, 6291–6294; (b) B. Zacharie, G. Sauvé, C. Penney, *Tetrahedron* **1993**, *49*, 10489–10500.
[21] (a) A. M. Bray, N. J. Maeji, A. G. Jhingran, R. M. Valerio, *Tetrahedron Lett.* **1991**, *32*, 6163–6166, (b) A. M. Bray, R. M. Valerio, N. J. Maeji, *Tetrahedron Lett.* **1993**, *34*, 4411–4414, (c) R. Sola, P. Saguer, M.-L. David, R. Pascal, *J. Chem. Soc., Chem. Commun.* **1993**, 1786–1788.
[22] In Table 1, non-gaseous nucleophiles were left overnight (19 h) to ensure complete conversion. However, complete conversion is observed after as little as 1.5 h (see SI).
[23] J.-P. Ebran, N. Dendane, O. Melnyk, *Org. Lett.* **2011**, *13*, 4336–4339.
[24] (a) R. Huisgen, *Proc. Chem. Soc. Lond.* **1961**, 357–396; (b) V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angew. Chem. Int. Ed.* **2002**, *41*, 2596–2599; (c) C. W. Tornøe, C. Christensen, M. Meldal, *J. Org. Chem.* **2002**, *67*, 3057–3064; (d) A. A. H. A. Fuaad, F. Azmi, M. Skwarczynski, I. Toth, *Molecules* **2013**, *18*, 13148–13174; (e) W. Tang, M. L. Becker, *Chem. Soc. Rev*. **2014**, *43*, 7013–7039.
[25] See the supporting information for details.
[26] (a) L. J. Cruz, V. de Santos, G. C. Zafaralla, C. A. Ramilo, R. Zeikus, W. R. Gray, B. M. Olivera, *J. Biol. Chem.* **1987**, *262*, 15821–15824; (b) M. Martínez-Padrón, J. Edstrom, M. Wickham, K. Lukowiak, *J. Exp. Biol.* **1992**, *172*, 79–105; (c) M. Salzet, P. Bulet, A. Van Dorsselaer, J. Malecha, *Eur. J. Biochem.* **1994**, *217*, 897–903.
[27] K. Fosgerau, T. Hoffmann, *Drug Disc. Today* **2015**, *20*, 122–128.
[28] Meldal and co-workers observed 1:1 D:L-Ala after exposure to $C_3H_7$-SH and 10 equiv KO*t*-Bu for 24 h.[14]
[29] Our samples stored as a solid or a solution in MeCN at –20 ºC remained free of any hydrolyzed peptides for at least 5 weeks.
[30] G. Klopman, *Tetrahedron* **1970**, *26*, 4549–4554.
[31] W. Zhang, L. Zhang, X. Li, J. A. Weigel, S. E. Hall, J. P. Mayer, *J. Comb. Chem.* **2001**, *3*, 151–153.
[32] C. Bello, F. Kikul, C. F. W. Becker *J. Pept. Sci.* **2015**, *21*, 201–207.
[33] (a) H. Kessler, B. Haase, *Int. J. Pept. Protein Res.* **1992**, *39*, 36–40; (b) T. Jeremic, A. Linden, H. Heimgartner, *Helv. Chim. Acta* **2004**, *87*, 3056–3079.