

Cite this: *Chem. Sci.*, 2018, 9, 350Received 14th August 2017
Accepted 7th November 2017

DOI: 10.1039/c7sc03553e

rsc.li/chemical-science

Epimerization-free access to C-terminal cysteine peptide acids, carboxamides, secondary amides, and esters *via* complimentary strategies†

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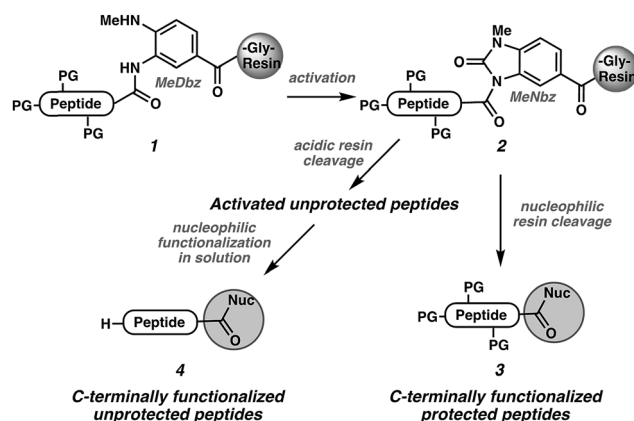
C-Terminal cysteine peptide acids are difficult to access without epimerization of the cysteine α -stereocenter. Diversification of the C-terminus after solid-phase peptide synthesis poses an even greater challenge because of the proclivity of the cysteine α -stereocenter to undergo deprotonation upon activation of the C-terminal carboxylic acid. We present herein two general strategies to access C-terminal cysteine peptide derivatives without detectable epimerization, diketopiperazine formation, or piperidinyalanine side products.

C-Terminal cysteine peptides, including prenylated and farnesylated peptides,¹ disulfide linked peptide toxins,² and insulinotropic peptides,^{3,4} comprise an important but synthetically challenging class of biologically active peptides. Many of these peptides are modified at the C-terminus. C-terminal modifications such as esters and amides can be critical to maintaining a peptide's active conformation,⁵ *in vivo* activity, and pharmacokinetics;⁶ therefore, the ability to vary the peptide structure in this location is crucial to drug development efforts.⁷ Although several methods have been reported for C-terminal functionalization after solid-phase peptide synthesis (SPPS) is complete,⁸ these approaches either result in epimerization when applied to C-terminal Cys peptides⁹ or the applicability of the method to C-terminal Cys peptides is not addressed.^{10,11} While activation of the C-terminal carboxylic acid can induce epimerization *via* oxazolone formation in most amino acids,¹² cysteine is also prone to epimerization *via* direct deprotonation during its attachment to the resin¹³ and upon prolonged or repeated exposure to base (*i.e.*, during peptide elongation *via* Fmoc SPPS).¹⁴ Therefore, even the preparation of simple carboxylic acids or carboxamides of C-terminal cysteine peptides can be fraught with contamination by epimerized products,^{1f,g,13a,15} reducing the overall yield and complicating the purification of the target peptides. A method for the epimerization-free synthesis and subsequent C-terminal modification of C-terminal Cys peptides would be highly impactful.

In this work, we report the first mild and convenient method for the epimerization-free diversification of peptides bearing a C-terminal cysteine.¹⁶ Carboxylic acids, primary and secondary

amides, and esters are accessed without epimerization or formation of diketopiperazine and piperidinyalanine side products.¹⁷ We apply this strategy to the total synthesis of the nicotinic acetylcholine receptor (nAChR) antagonist α -conotoxin ImI.¹⁸ Additionally, we include an alternate strategy employing N-deprotected cysteine derivatives as nucleophiles, and we demonstrate its utility *via* the synthesis of the insect pheromone α -factor.¹

In the context of our ongoing efforts toward the synthesis of disulfide-linked α - and μ -conotoxins,^{19,20} we were concerned about possible epimerization of the C-terminal cysteine during the SPPS. We recently reported a strategy for C-terminal functionalization of non-cysteine peptides involving activation of the methyl-diaminobenzoyl (MeDbz) linker (**1** $\rightarrow$ **2**)²¹ followed by nucleophilic cleavage of the *N*-acyl urea (MeNbz) group²² to yield various protected (**3**) or unprotected (**4**) peptides (Scheme 1).²³ If this approach were to prove mild enough to enable

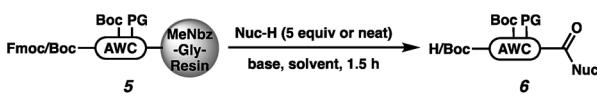


Scheme 1 Our strategy for C-terminal functionalization of non-Cys terminated peptides.

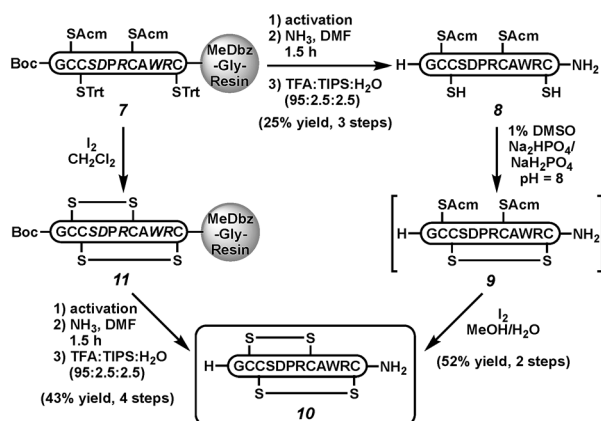
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† Electronic supplementary information (ESI) available. See DOI: 10.1039/c7sc03553e

Table 1 Evaluation of epimerization during nucleophilic cleavage of the MeNbz group in C-terminal cysteine peptides

						
Entry ^a	PG	Nuc-H	Base (5 equiv.)	Solvent	% conversion	Epimerization ^b (% D-X)
1 ^c	Trt	NH ₃	—	DMF	>99	<1
2	Trt	PhCH ₂ NH ₂	—	PhCH ₂ NH ₂	>99	16
3	Trt	PhCH ₂ NH ₂	—	MeCN	>99	<1
4	Trt	BuNH ₂	—	BuNH ₂	>99	8
5	Trt	BuNH ₂	—	DMF	>99	10
6	Trt	BuNH ₂	—	MeCN	>99	9
7 ^d	Trt	BuNH ₂	—	MeCN	>99	<1
8 ^c	Acm	BuNH ₂	—	MeCN	>99	<1
9 ^c	Mob	BuNH ₂	—	MeCN	>99	<1
10 ^c	Bn	BuNH ₂	—	MeCN	>99	<1
11	StBu	BuNH ₂	—	MeCN	>99	<1
12 ^c	<i>t</i> Bu	BuNH ₂	—	MeCN	>99	<1
13 ^e	Trt	MeOH	KOtBu	MeOH	>99	42
14 ^f	Trt	MeOH	DIEA	MeOH	>99	<1
15 ^g	Trt	MeOH	—	MeOH/Na ₂ HPO ₄ (aq)	>99	<1
16	Trt	H ₂ O	DIEA	H ₂ O/MeCN	56	<1

^a All reactions were performed on 20 mg of resin containing all L amino acids in 200 μ L of solvent at ambient temperature (24 ± 1 °C). ^b All PGs were removed prior to epimerization assay unless otherwise noted. ^c Cys(PG) was intact during epimerization assay. ^d 1.1 equiv. of BuNH₂ was used. ^e 0.7 equiv. KOtBu. ^f Reaction was conducted for 3 h. ^g Na₂HPO₄/NaH₂PO₄ buffer at pH = 8.

Scheme 2 Synthesis of conotoxin α -lml (10).

H-Cys-OEt, H-Cys-NH₂, or H-Cys-NHBu in the presence of Hünig's base (Table 2). Protected peptides Boc-AW(Boc)AC-OH (14a), Boc-AW(Boc)AC-OEt (14b), and Boc-AW(Boc)AC-NH₂ (14c) were formed with complete conversion (entries 1–3), while Boc-AW(Boc)AC-NHBu (14d) was formed with 38% conversion. The elongation was more efficient in solution,²¹ and unprotected peptides 14e–h were accessed with quantitative conversion (entries 5–8). We assumed that the mildly basic reaction conditions would result in rapid S to N acyl transfer upon cysteine thiol addition either on resin or in solution. *In situ* generation of the backbone amide was confirmed by independent synthesis of H-AWAC-OH followed by co-injection with 14e.²⁶ The extent of product peptide epimerization was

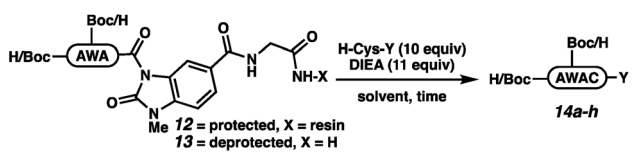
evaluated for the ethyl ester (14f), which is the most epimerization-prone derivative. Comparison to a co-injection of H-AWA(D-Cys)-OEt confirmed that the product peptides are not epimerized to any observable extent under the reaction conditions (Fig. SI-057†).

We next executed the cysteine elongation of a series of peptides varying in length and hydrophobicity both on the resin and in solution (Table 3). The unprotected peptide H-AKTWA-MeNbz-Gly (15b) was functionalized in solution to afford H-AKTWAC-OH (15c) with complete conversion in 30 min with no observed side-chain macrocyclization.²³ To enable comparison with the crypto-thioester approach,²⁷ C-terminal proline-containing peptide 16a was cleaved from resin using H-Cys-OH to afford protected H-AKTWPC-OH (16c) with 10% conversion over 4 h.³⁸ Repeating this reaction in solution on unprotected peptide (16b) led to complete conversion after 1 h at ambient temperature. Elongation of Boc-LYRAGLAY (17a) proceeded with resin cleavage and complete conversion in the presence of DMF and NCL buffer. Hydrophobic peptide 18, a fragment of amyloid β ($\text{A}\beta$ (36–42)),³⁹ was elongated both on resin (entry 5) and in solution (entry 6). On-resin elongation proved challenging for this substrate (10% conversion), while complete conversion was observed in solution. Overall, for shorter or non-hydrophobic peptides, this chemistry could be executed on resin and in the absence of added thiol. In challenging cases, resin cleavage and then in solution native chemical ligation⁴⁰ afforded the target peptides.

To confirm the viability of this approach in the context of a complex natural product, we executed the total synthesis of the insect pheromone α -factor (21, Scheme 3), which requires



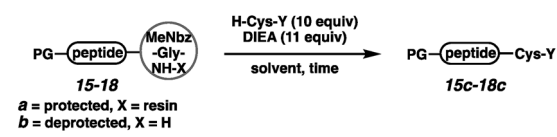
Table 2 C-terminal elongation by nucleophilic attack of cysteine on MeNbz



Entry ^a	Substrate	Y	Solvent	Time (h)	Conversion ^b (%)
1	12	OH (14a)	(5 : 1) DMF : H ₂ O	4	>99
2	12	OEt (14b)	DMF	4	>99
3	12	NH ₂ (14c)	DMF	4	>99
4 ^c	12	NHBu (14d)	DMF	4	>38
5	13	OH (14e)	(2 : 1) MeCN : H ₂ O	0.5	>99
6	13	OEt (14f)	MeCN	0.5	>99
7	13	NH ₂ (14g)	MeCN	0.5	>99
8 ^{c,d}	13	NHBu (14h)	(25 : 1) MeCN : H ₂ O	0.5	>99

^a Unless noted, on resin reactions were performed on 20 mg resin in 500 μ L solvent, solution-phase reactions were performed on 20 mg crude peptide in 200 μ L solvent, 100 μ L of H₂O was added as indicated, rt = 24 $\pm$ 1 $^{\circ}$ C. ^b Conversion based on integration of relevant peaks in HPLC/MS data. ^c Cysteine formation was observed. ^d Performed on 3.8 mg of 13 using 520 μ L MeCN:H₂O.

Table 3 Cysteine elongation to generate C-terminal acids, carboxamides, and esters



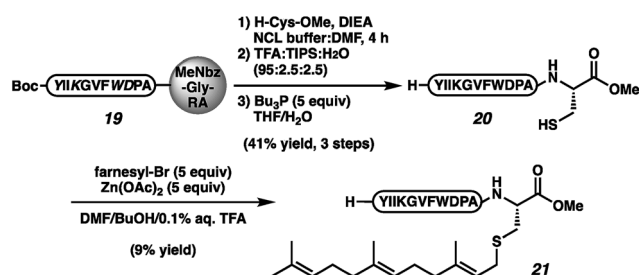
Entry ^a	PG	Substrate	Peptide	Y	Solvent	Time (h)	Conversion ^b (%)
1 ^c	H	15b	AKTWA (15)	OH	(5 : 1) MeCN : H ₂ O	0.5	>99
2	Boc	16a	AKTWAP (16)	OH	(1 : 1) DMF : NCL buffer	4	10
3 ^d	H	16b	AKTWAP (16)	OH	NCL buffer	1	>99
4	Boc	17a	LYRAGLAY (17)	NH ₂	(1 : 1) DMF : NCL buffer	4	>99
5	Boc	18a	VGGVVI (18)	OMe	(1 : 1) DMF : NCL buffer	4	10
6 ^e	H	18b	VGGVVI (18)	OMe	NCL buffer	0.5	>99

^a Unless noted, on-resin reaction were performed on 20 mg peptide/resin in 500 μ L solvent, rt = 24 $\pm$ 1 $^{\circ}$ C, NCL buffer at pH 7.2. ^b Based on integration of relevant peaks in HPLC/MS data. ^c Used 600 μ L solvent. ^d Performed on 10 mg of 16b using 250 μ L solvent. ^e Performed on 67 mg of 18b using 250 μ L solvent.

both the C-terminal ester and the prenyl moiety for bio-activity.^{1c-h,41} Protected des-farnesyl α -factor was generated by displacement of peptide 19 with cysteine methyl ester. The elongation was conducted in NCL buffer in an effort to reduce cysteine-functionalized by-products. However, even under these

reducing conditions, cystine-appended α -factor was still observed. Side-chain deprotection, cystine reduction, and HPLC purification afforded peptide 20 in 41% yield over 3 steps. Alkylation per the reported conditions afforded α -factor in 9% yield (21).⁴¹

In summary, we have developed two broadly applicable strategies for the epimerization-free preparation of C-terminal cysteine peptides. The first approach exploits the exceptionally mild activating nature of the *N*-acyl urea group for the direct diversification of the C terminus. Additionally, an alternative strategy wherein cysteine derivatives serve as the nucleophile in a resin-cleaving elongation reaction was also effective. For both strategies, the target peptides are prepared without observation of either diketopiperazine or piperidinylalanine side products. The utility of these methods was demonstrated in the preparation of the disulfide-linked conotoxin α -ImI, bearing a C-terminal cysteine carboxamide and the insect pheromone

Scheme 3 Synthesis of α -factor by cysteine elongation.

α -factor, which possesses a C-terminal cysteine methyl ester. Notably, no previous report has demonstrated successful functionalization of C-terminal cysteine peptides to access carboxylic acids, carboxamides, and other C-terminal derivatives without detectable epimerization of the α -stereocenter.

Conflicts of Interest

The authors declare no conflicts of interest.

Acknowledgements

The authors thank the National Institutes of Health (R00-GM097095), the National Science Foundation (CAREER Award supporting undergrad researcher TLV: CHE-1554752), and Wayne State University for generous financial support (startup funds to JLS, Rumble-Schaap Fellowships to CAA and TDK, Knoller Fellowship to HYS). We thank Alomone Labs for providing an analytical sample of α -conotoxin ImI free of charge. We also gratefully acknowledge the staff of the WSU Lumigen Instrument Center and Shimadzu for a grant supporting the mass spectrometer.

References

- (a) S. Clarke, *Annu. Rev. Biochem.*, 1992, **61**, 355–386; (b) J. A. Glomset, M. H. Gelb and C. C. Farnsworth, *Trends Biochem. Sci.*, 1990, **15**, 139–142; (c) C. A. Hrycyna and S. Clarke, *Mol. Cell. Biol.*, 1990, **10**, 5071–5076; (d) F. L. Zhang and P. J. Casey, *Annu. Rev. Biochem.*, 1992, **65**, 241–269; (e) D. G. Mullen, K. Kyro, M. Hauser, M. Gustavsson, G. Veglia, J. M. Becker, F. Naider and M. D. Distefano, *Bioorg. Med. Chem.*, 2011, **19**, 490–497; (f) V. Diaz-Rodriguez, D. G. Mullen, E. Ganusova, J. M. Becker and M. D. Distefano, *Org. Lett.*, 2012, **14**, 5648–5651; (g) V. Diaz-Rodriguez, E. Ganusova, T. M. Rappe, J. M. Becker and M. D. Distefano, *J. Org. Chem.*, 2015, **80**, 11266–11274; (h) F. R. Naider and J. M. Becker, *Pept. Sci.: Present Future, Proc. Int. Pept. Symp.*, 1st, 1997, 3–14.
- (a) D. J. Craik and D. J. Adams, *ACS Chem. Biol.*, 2007, **2**, 457–468; (b) M. C. Inserra, S. N. Kompella, S. N. Vetter, A. Brust, N. L. Daly, H. Cuny, D. J. Craik, P. F. Alewood, D. J. Adams and R. J. Lewis, *Biochem. Pharmacol.*, 2013, **86**, 791–799; (c) A. Gould and J. A. Camarero, *ChemBioChem*, 2017, **18**, 1350–1363.
- W. Shang, X. Yang, X. Ju, Y. Xie, Y. Zhang and W.-H. Lee, *J. Pept. Sci.*, 2017, **23**, 707–715.
- J. M. Conlon, V. Musale, S. Attoub, M. L. Mangoni, J. Leprince, L. Coquet, T. Jouenne, Y. H. A. Abdel-Wahab, P. R. Flatt and A. C. Rinaldi, *J. Pept. Sci.*, 2017, **23**, 769–776.
- T. S. Kang, S. Vivekanandan, S. D. S. Jois and R. M. Kini, *Angew. Chem., Int. Ed.*, 2005, **44**, 6333–6337.
- (a) D. J. Merkler, *Enzyme Microb. Technol.*, 1994, **16**, 450–456; (b) H. Bultmann, J. Teuton and C. R. Brandt, *Antimicrob. Agents Chemother.*, 2007, **51**, 1596–1607.
- D. Goodwin, P. Simerska and I. Toth, *Curr. Med. Chem.*, 2012, **19**, 4451–4461.
- J. Alsina and F. Albericio, *Biopolymers*, 2003, **71**, 454–477.
- J. Hansen, F. Diness and M. Meldal, *Org. Biomol. Chem.*, 2016, **14**, 3238–3245.
- (a) H. E. Elashal, Y. E. Sim and M. Raj, *Chem. Sci.*, 2017, **8**, 117–123; (b) H. E. Elashel, R. D. Cohen and M. Raj, *Chem. Commun.*, 2016, **52**, 9699–9702.
- (a) J. A. Camarero, B. J. Hackel, J. J. de Yoreo and A. R. Mitchell, *J. Org. Chem.*, 2004, **69**, 4145–4151; (b) Y. Kwon, K. Welsh, A. R. Mitchell and J. A. Camarero, *Org. Lett.*, 2004, **6**, 3801–3804; (c) R. A. Turner, R. J. Weber and R. S. Lokey, *Org. Lett.*, 2010, **12**, 1852–1855; (d) E. Nicolás, J. Clemente, M. Perelló, F. Albericio, E. Pederroso and E. Giralt, *Tetrahedron Lett.*, 1992, **33**, 2183–2186; (e) A. A. Vinogradov, M. D. Simon and B. L. Pentelute, *Org. Lett.*, 2016, **18**, 1222–1225.
- Oxazolone formation: B. Henkel, L. Zhang and E. Bayer, *Liebigs Ann./Recl.*, 1997, **10**, 2161–2186.
- (a) Y. Fujiwara, K. Akaji and Y. Kiso, *Chem. Pharm. Bull.*, 1994, **42**, 724–726; (b) Y. M. Angell, J. Alsina, F. Albericio and G. Barany, *J. Pept. Res.*, 2002, **60**, 292–299; (c) H. Hibino, Y. Miki and Y. Nishiuchi, *J. Pept. Sci.*, 2014, **20**, 30–35.
- (a) Y. Han, F. Albericio and G. Barany, *J. Org. Chem.*, 1997, **62**, 4307–4312; (b) I. Ramos-Tomillero, H. Rodríguez and F. Albericio, *Org. Lett.*, 2015, **17**, 1680–1683; (c) A. Isidro-Llobet, M. Álvarez and F. Albericio, *Chem. Rev.*, 2009, **109**, 2455–2504; (d) H. Hibino and Y. Nishiuchi, *Org. Lett.*, 2012, **14**, 1926–1929; (e) H. Hibino, Y. Miki and Y. Nishiuchi, *J. Pept. Sci.*, 2014, **20**, 30–35; (f) J. Lukszo, D. Patterson, F. Albericio and S. Kates, *Lett. Pept. Sci.*, 1996, **3**, 157–166.
- (a) U. Boas, J. Brask and K. J. Jensen, *Chem. Rev.*, 2009, **109**, 2092–2118 and references therein; (b) G. Barany, Y. Han, B. Hargittai, R.-Q. Liu and J. T. Varkey, *Biopolymers*, 2003, **71**, 652–666; (c) V. Juvekar and Y. D. Gong, *Org. Lett.*, 2016, **18**, 836–839; (d) Z. Huang, D. J. Derksen and J. C. Vederas, *Org. Lett.*, 2010, **12**, 2282–2285.
- (a) A. Selvaraj, H.-T. Chen, A. Y. Huang and C.-L. Kao, *Chem. Sci.*, DOI: 10.1039/c7sc03229c; (b) B. H. Gless, P. Peng, K. D. Pedersen, C. H. Gotfredsen, H. Ingmer and C. A. Olsen, *Org. Lett.*, 2017, **19**, 5276–5279.
- (a) P. Cherkupally, G. A. Acosta, S. Ramesh, B. G. de la Torre, T. Govender, H. G. Kruger and F. Albericio, *Amino Acids*, 2014, **46**, 1827–1838; (b) J. Lukszo, D. Patterson, F. Albericio and S. A. Kates, *Lett. Pept. Sci.*, 1996, **3**, 157–166; (c) V. Juvekar, K.-T. Kim and Y.-D. Gong, *Bull. Korean Chem. Soc.*, 2017, **38**, 54–62.
- (a) D. S. Johnson, J. Martinez, A. B. Elgoyhen, S. F. Heinemann and J. M. McIntosh, *Mol. Pharmacol.*, 1995, **48**, 194–199; (b) E. F. R. Pereira, M. Alkondon, J. M. McIntosh and E. X. Albuquerque, *J. Pharmacol. Exp. Ther.*, 1996, **278**(3), 1472–1483; (c) S. Luo, T. A. Nguyen, G. E. Cartier, B. M. Olivera, D. Yoshikami and J. M. McIntosh, *Biochemistry*, 1999, **38**, 14542–14548; (d) M. Ellison, J. M. McIntosh and B. M. Olivera, *J. Biol. Chem.*, 2003, **278**, 757–764; (e) M. Ellison, F. Gao, H.-L. Wang, S. M. Sine, J. M. McIntosh and B. M. Olivera, *Biochemistry*, 2004, **43**, 16019–16026.



- 19 (a) B. M. Olivera, *Mol. Biol. Cell*, 1997, **8**, 2101–2109; (b) H. Terlau and B. M. Olivera, *Physiol. Rev.*, 2004, **84**, 4–68; (c) B. M. Olivera, J. S. Imperial and G. Bulaj, in *Perspectives in Molecular Toxinology*, 2002, pp. 143–158; (d) O. Buczek, G. Bulaj and B. M. Olivera, *Cell. Mol. Life Sci.*, 2005, **62**, 3067–3079; (e) M. C. Inserra, S. N. Kompella, I. Vetter, A. Brust, N. L. Daly, H. Cuny, D. J. Craik, P. F. Alewood, D. J. Adams and R. J. Lewis, *Biochem. Pharmacol.*, 2013, **86**, 791–799.
- 20 (a) K. B. Akondi, M. Muttenthaler, S. Dutertre, Q. Kaas, D. J. Craik, R. J. Lewis and P. F. Alewood, *Chem. Rev.*, 2014, **114**, 5815–5847; (b) M. Goñgora-Benítez, J. Tulla-Puche and F. Albericio, *Chem. Rev.*, 2013, **114**, 901–926.
- 21 (a) J. B. Blanco-Canosa and P. E. Dawson, *Angew. Chem., Int. Ed.*, 2008, **47**, 6851–6855; (b) J. B. Blanco-Canosa, B. Nardone, F. Albericio and P. E. Dawson, *J. Am. Chem. Soc.*, 2015, **137**, 7197–7209; (c) S. K. Mahto, C. J. Howard, J. C. Shimko and J. J. Ottesen, *ChemBioChem*, 2011, **12**, 2488–2494.
- 22 R. Pascal, D. Chauvey and R. Sola, *Tetrahedron Lett.*, 1994, **35**, 6291–6294.
- 23 C. A. Arbour, H. Y. Saraha, T. F. McMillan and J. L. Stockdill, *Chem.–Eur. J.*, 2017, **23**, 12484–12488.
- 24 A. El-Faham and F. Albericio, *Chem. Rev.*, 2011, **111**, 6557–6602.
- 25 Y. M. Angell, J. Alsina, F. Albericio and G. Barany, *J. Pept. Res.*, 2002, **60**, 292–299.
- 26 See the ESI for details.†
- 27 D. Lelièvre, V. P. Terrier, A. F. Delmas and V. Aucagne, *Org. Lett.*, 2016, **18**, 920–923.
- 28 Extended exposure to H₂O/DIEA (18 h) increased conversion to 82%; however, 3% epimerization of Cys(Trt) was observed.
- 29 T. D. Kondasinghe, H. Y. Saraha, S. B. Odeesho and J. L. Stockdill, *Org. Biomol. Chem.*, 2017, **15**, 2914–2918.
- 30 (a) J. M. McIntosh, D. Yoshikami, E. Mahe, D. B. Nielsen, J. E. Rivier, W. R. Gray and B. M. Olivera, *J. Biol. Chem.*, 1994, **269**, 16733–16739; (b) D. S. Johnson, J. Martinez, A. B. Elgoyhen, S. F. Heinemann and J. M. McIntosh, *Mol. Pharm.*, 1995, **48**, 194–199; (c) M. Ellison, F. Gao, H.-L. Wang, S. M. Sine, J. M. McIntosh and B. M. Olivera, *Biochemistry*, 2004, **43**, 16019–16026.
- 31 (a) I. V. Maslennikov, Z. O. Shenkarev, M. N. Zhmak, V. T. Ivanov, C. Methfessel and A. S. Arseniev, *FEBS Lett.*, 1999, **444**, 275–280; (b) J. Gehrmann, N. L. Daly, P. F. Alewood and D. J. Craik, *J. Med. Chem.*, 1999, **42**, 2364–2372; (c) J. S. Nielsen, P. Buczek and G. Bulaj, *J. Pept. Sci.*, 2004, **10**, 249–256.
- 32 (a) J. M. McIntosh, D. Yoshikami, E. Mahe, D. B. Nielsen, J. E. Rivier, W. R. Gray and B. M. Olivera, *J. Biol. Chem.*, 1994, **269**, 16733–16739; (b) K. B. Akondi, M. Muttenthaler, S. Dutertre, Q. Kaas, D. J. Craik, R. J. Lewis and P. F. Alewood, *Chem. Rev.*, 2014, **114**, 5815–5847.
- 33 C. J. Armishaw, N. L. Daly, S. T. Nevin, D. J. Adams, D. J. Craik and P. F. Alewood, *J. Biol. Chem.*, 2006, **281**, 14136–14143.
- 34 70% crude yield, 86% HPLC purity.
- 35 R. Söll and A. G. Beck-Sickinger, *J. Pept. Sci.*, 2000, **6**, 387–397.
- 36 (a) B. Dang, T. Kubota, A. M. Correa, F. Bezanilla and S. B. H. Kent, *Angew. Chem., Int. Ed.*, 2014, **53**, 8970–8974; (b) C. Sun, G. Luo, S. Neravelta, S. S. Ghosh and B. Forood, *Bioorg. Med. Chem. Lett.*, 2013, **23**, 5203–5208.
- 37 G. A. Acosta, M. Royo, B. G. de la Torre and F. Albericio, *Tetrahedron Lett.*, 2017, **58**, 2788–2791.
- 38 (a) L. Raibaut, P. Seeberger and O. McInyk, *Org. Lett.*, 2013, **15**, 5516–5519; (b) L. Raibaut, M. Cargoët, N. Ollivier, Y. M. Chang, H. Drobecq, E. Boll, R. Desmet, J.-C. M. Monbaliu and O. McInyk, *Chem. Sci.*, 2016, **7**, 2657–2665.
- 39 (a) B. Bacsá, S. Bösze and C. O. Kappe, *J. Org. Chem.*, 2010, **75**, 2103–2106; (b) M. Quibell, W. G. Turnell and T. Johnson, *J. Org. Chem.*, 1994, **59**, 1745–1750; (c) M. M. Condron, B. H. Monien and G. Bitan, *Open Biotechnol. J.*, 2008, **2**, 87–93.
- 40 (a) P. E. Dawson, T. W. Muir, I. Clark-Lewis and S. B. Kent, *Science*, 1994, **266**, 776–779; (b) E. C. B. Johnson and S. B. H. Kent, *J. Am. Chem. Soc.*, 2006, **128**, 6640–6646.
- 41 D. G. Mullen, K. Kyro, M. Hauser, M. Gustavsson, G. Veglia, J. M. Becker, F. Naider and M. D. Distefano, *Bioorg. Med. Chem.*, 2007, **15**, 931–938.

