Tetrahedron 69 (2013) 2555–2559

Contents lists available at SciVerse ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Total synthesis of α-conotoxin MII using a soluble-tag-assisted method

Shingo Kitada [a], Shuji Fujita [b], Yohei Okada [a], Shokaku Kim [a], Kazuhiro Chiba [a,*]

[a] Department of Applied Biological Science, Tokyo University of Agriculture and Technology, 3-5-8 Saiwai-cho, Fuchu, Tokyo 183-8509, Japan
[b] Research and Development Division, JITSUBO Co., Ltd., 2-24-16 Naka-cho, Koganei, Tokyo 184-0012, Japan

ARTICLE INFO

Article history:
Received 1 December 2012
Received in revised form 16 January 2013
Accepted 21 January 2013
Available online 1 February 2013

Keywords:
α-Conotoxin MII
Soluble tag
Liquid-phase peptide synthesis
Hydrophobic benzyl amine
Disulfide bond

ABSTRACT

Oxidative disulfide bond formations have been applied to soluble-tag-assisted method successfully to realize the total synthesis of α-conotoxin MII (**4**) that comprises 16 amino acid residues and possesses 2 disulfide bonds. Orthogonal peptide folding using DEAD and iodine oxidations in combination with Mmt and Acm groups for the side chain protection of cysteines could be carried out with no peptide aggregation.

1. Introduction

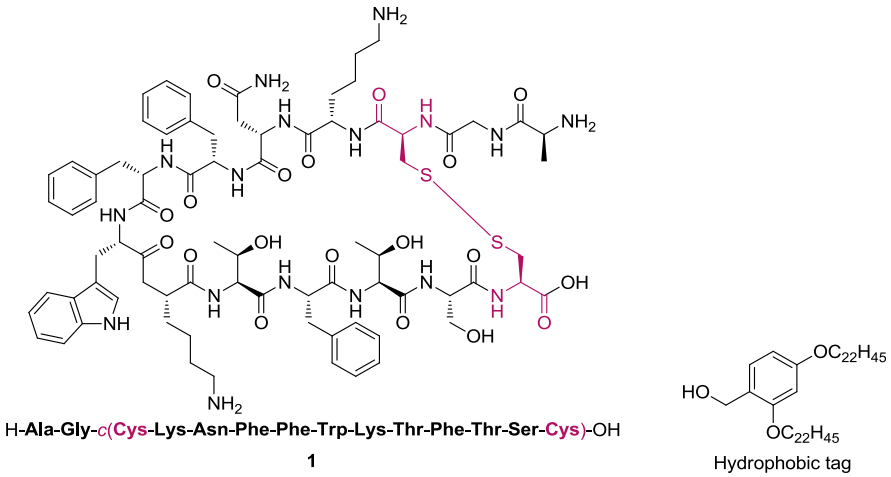
Conotoxins are disulfide-rich peptides comprising 10–30 amino acid residues, originally isolated from the venom of marine snails belonging to the genus *Conus*.[1] In recent years, based on their neurotoxic potentials, conotoxins have received much attention as novel analgesics that modulate the functions of various ion channels.[2] In particular, α-conotoxins, which are characterized by potent and specific inhibition of nicotinicacetylcholine receptors, constitute one of the largest families of conotoxins[3] and their structures and activities have been investigated in detail both practically and theoretically.[4] However, the restricted amount of naturally occurring α-conotoxins has hampered further investigations of these promising medicinal candidates. In this context, several chemical syntheses of α-conotoxins have been accomplished; some of these have even resulted in the production of artificial variants of the native forms, realizing improvements in bioavailability and/or enhancements in bioactivity.[5]

In order for chemical synthesis of peptides to be successful, their aggregation behavior must be taken into account, especially in liquid-phase approaches. For example, amyloid β-peptides and collagens have a well-known tendency to form insoluble fibrils through intermolecular hydrogen bonding; these are associated with various diseases. In addition, a high content of hydrophobic residues tends to cause peptide aggregation in aqueous media, impeding the use of standard peptide purification procedures. Such hydrophobicity also hinders peptide folding, which could be a crucial drawback in the chemical synthesis of disulfide-rich peptides, because their linear precursors are required to adopt folded conformations. Therefore, effective chemical synthesis of α-conotoxins remains challenging.

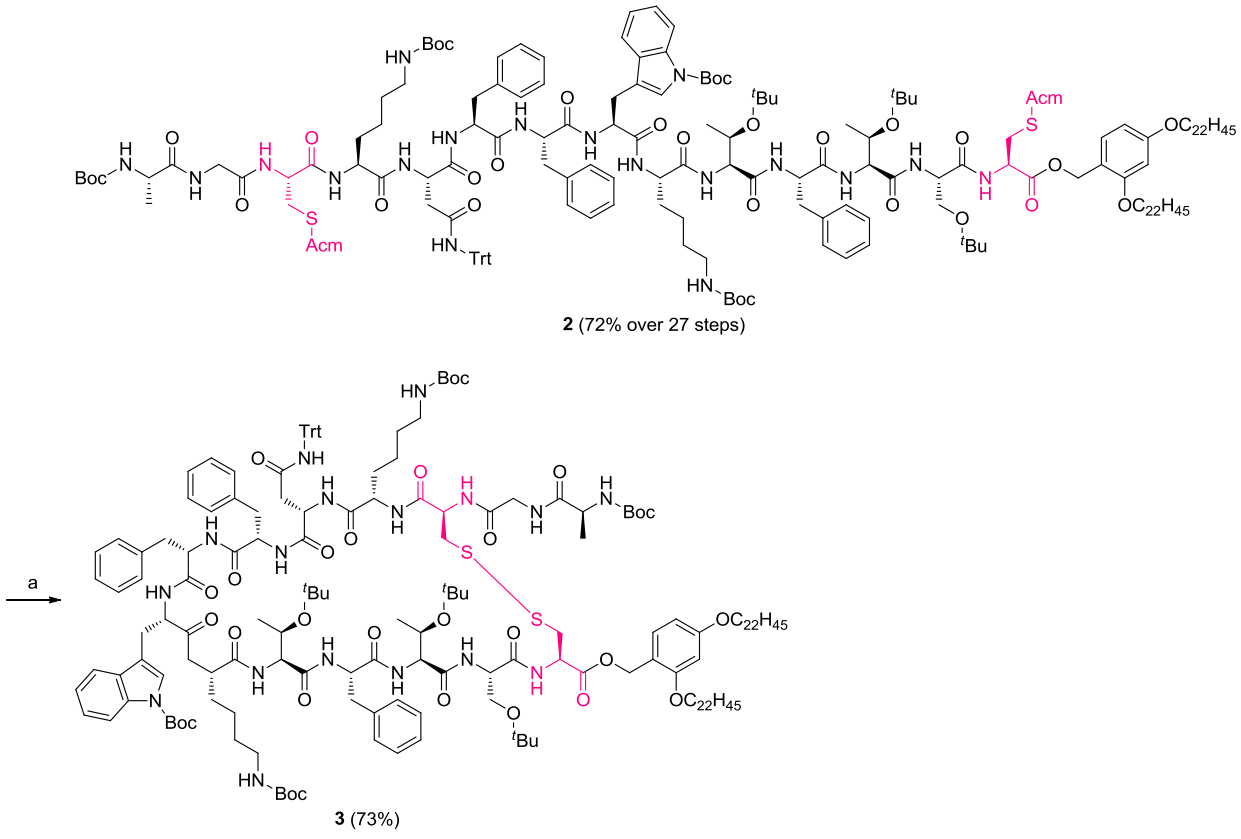
One approach to address peptide aggregation is through the use of soluble tags;[6] this method enables significant control of peptide solubility, with unique product isolation methodologies to ease reaction work-up. When hydrophobic supports[7] are employed in combination with less polar solvents, peptide aggregation based on high contents of hydrophobic residues should be inhibited. We have developed hydrophobic soluble-tag-assisted methods using benzyl alcohols bearing long alkyl chains as supports,[8] which have led to versatile preparation of bioactive peptides.[9] Using this method, tagged peptides are highly soluble in less polar solvents such as THF or CH_2Cl_2. We have also applied our method to an existing oxidative strategy in which iodine is used in combination with acetamidomethyl (Acm) groups for side chain protection of cysteines, leading to the synthesis of somatostatin (**1**), a growth-hormone-inhibiting peptide possessing one disulfide bond (Scheme 1).[10] The linear precursor (**2**) of somatostatin (**1**) was prepared using general Fmoc chemistry, followed by the iodine oxidation to form the tagged cyclic peptide (**3**) (Scheme 2). All protective groups could be removed under acidic conditions in one

* Corresponding author. Tel./fax: +81 42 367 5667; e-mail address: chiba@cc.tuat.ac.jp (K. Chiba).

0040-4020/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tet.2013.01.068



Scheme 1. Structure of somatostatin (**1**) and hydrophobic tag.



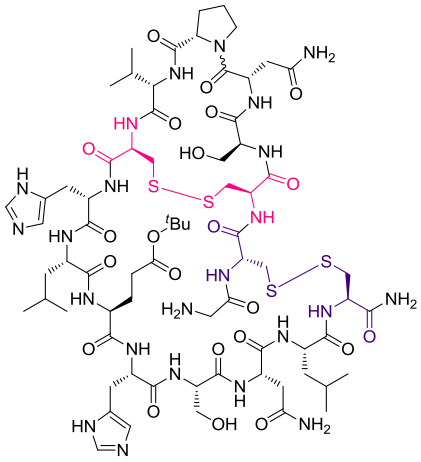
Scheme 2. Synthesis of somatostatin (**1**).

step to give somatostatin (**1**), and the couplings and deprotections were carried out efficiently in the liquid phase. Described herein is the application of the soluble-tag-assisted method toward the synthesis of disulfide-rich α-conotoxin.

2. Results and discussion

The present work began with the selection of α-conotoxin MII (**4**), which comprises 16 amino acid residues and possesses 2 disulfide bonds, as a model (Scheme 3). In order to realize orthogonal two-step oxidative disulfide bond formation, a monomethoxytrityl (Mmt) group was chosen in combination with an Acm group for side chain protection of cysteines, because the Mmt group can be removed under weakly acidic conditions. Moreover, the *C*-terminus of α-conotoxin MII is the amide form; thus, tag modification is also required.

Based on the existing linker strategy of solid-phase approaches, we simply designed a hydrophobic benzyl amine (**5**) that could be prepared through reductive amination (Scheme 4). With this tag in hand, the linear precursor (**6**) of α-conotoxin MII (**4**) was prepared

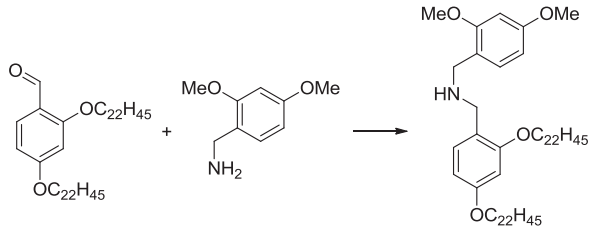


H-Gly-c(Cys-c(Cys-Ser-Asn-Pro-Val-Cys)-His-Leu-Glu-His-Ser-Asn-Leu-Cys)-NH_2

4

Scheme 3. Structure of α-conotoxin MII (**4**).

using general Fmoc chemistry (Scheme 5). In this case, the hydrophobic benzyl amine (**5**) was found to act as an effective soluble tag, and the resulting (**6**) was then subjected to weakly acidic conditions to remove the Mmt groups selectively, followed by DEAD oxidation. To our satisfaction, formation of the tagged cyclic peptide (**7**) took place efficiently, with little deprotection of the side chains. Formation of the second disulfide bond was carried out via iodine oxidation, and all protective groups were removed under acidic conditions in one step to give α-conotoxin MII (**4**). The tagged peptide was highly soluble in less polar solvents, and aggregation posed little problem throughout the overall procedure, enabling efficient orthogonal two-step oxidative disulfide bond formation.



5 (93%)

Reagents and condition: $NaBH(OAc)_3$, Toluene/DMF

Scheme 4. Preparation of hydrophobic benzyl amine (**5**).

3. Conclusion

In conclusion, we successfully applied the soluble-tag-assisted method to oxidative disulfide bond formation, leading to the total synthesis of α-conotoxin MII (**4**). DEAD and iodine oxidations were employed in combination with the use of Mmt and Acm groups for side chain protection of cysteines to achieve orthogonal peptide folding with no peptide aggregation. This strategy is expected to be a considerable aid to further investigation of the therapeutic possibilities of α-conotoxins.

4. Experimental section

4.1. Preparation of the hydrophobic benzyl amine (5)

2,4-Bis(docosyloxy)benzaldehyde (4.53 g, 6.0 mmol) was dissolved in toluene (60 ml). 2,4-Dimethoxybenzylamine (3.01 g, 18.0 mmol), $NaBH(OAc)_3$ (3.81 g, 18.0 mmol), and DMF (60 ml) were then added to the solution. The reaction mixture was stirred at 40 °C until the reaction completed. After completion, MeOH was added to the reaction mixture to give the product as a precipitate. The precipitate was dissolved in CH_2Cl_2, and CH_2Cl_2 solution was washed with $NaHCO_3$(aq) and brine. The organic layer was dried over anhydrous Na_2SO_4. After filtration and evaporation under reduced pressure, the product was obtained in 5.06 g, 93% yield.

*4.1.1. Compound (**5**).* Hydrophobic benzyl amine (**5**), amorphous solid.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.17 (2H, d, *J*=7.8 Hz), 7.15 (2H, d, *J*=7.8 Hz), 6.46–6.36 (4H, m), 3.92 (2H, t, *J*=6.6 Hz), 3.91 (2H, t, *J*=6.6 Hz), 3.79 (3H, s), 3.78–3.73 (4H, m) 3.77 (3H, s), 1.82–1.68 (4H, m), 1.50–1.17 (76H, m), 0.88 (6H, t, *J*=6.9 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.2, 159.7, 158.5, 158.1, 130.6, 130.6, 120.0, 119.7, 104.3, 103.7, 99.7, 98.4, 68.1, 67.3, 55.3, 55.2, 48.2, 47.6, 31.9, 29.7, 29.6, 29.6, 29.6, 29.4, 29.4, 29.3, 29.3, 29.2, 26.1, 26.0, 22.7, 14.1. HRMS $[M+H]^+$ calcd for $C_{60}H_{108}NO_4$906.8278, found 906.8275.

4.2. General method for the introduction of amino acid into the hydrophobic tag

The hydrophobic tag (906 mg, 1.0 mmol) was dissolved in CH_2Cl_2 (20 mL). Amino acid (1.5 mol equiv), DIC (189 mg, 1.5 mmol), and DMAP (12.2 mg, 0.1 mmol) were then added to the solution. The reaction mixture was stirred at room temperature until the reaction completed. After the completion, MeCN was added to the reaction mixture to give the product as a precipitate.

4.3. General method for the deprotection of Fmoc group of the tagged peptide

The tagged peptide was dissolved in 1% DBU and 1% piperidine in THF (20 mL). The reaction mixture was stirred at room temperature until the reaction completed. After the completion, 12 M HCl was added to the reaction mixture to neutralize, and then MeCN was added to give the deprotected product as a precipitate.

4.4. General method for the coupling of amino acid to the tagged peptide

The tagged peptide was dissolved in THF (20 mL). Amino acid (1.2 mol equiv), HBTU (1.2 mol equiv), HOBt (1.2 mol equiv), and DIPEA (2.4 mol equiv) were then added to the solution. The reaction mixture was stirred at room temperature until the reaction completed. After the completion, MeCN was added to the reaction mixture to give the coupled product as a precipitate.

Boc-Gly-Cys(Acm)-Cys(Mmt)-Ser(^{t}Bu)-Asn(Trt)-Pro-Val-Cys-(Acm)-His(Trt)-Leu-Glu(^{t}Bu)-His(Trt)-Ser(^{t}Bu)-Asn(Trt)-Leu-Cys-(Mmt)-N(Dmb)-TAG (**6**), amorphous solid.

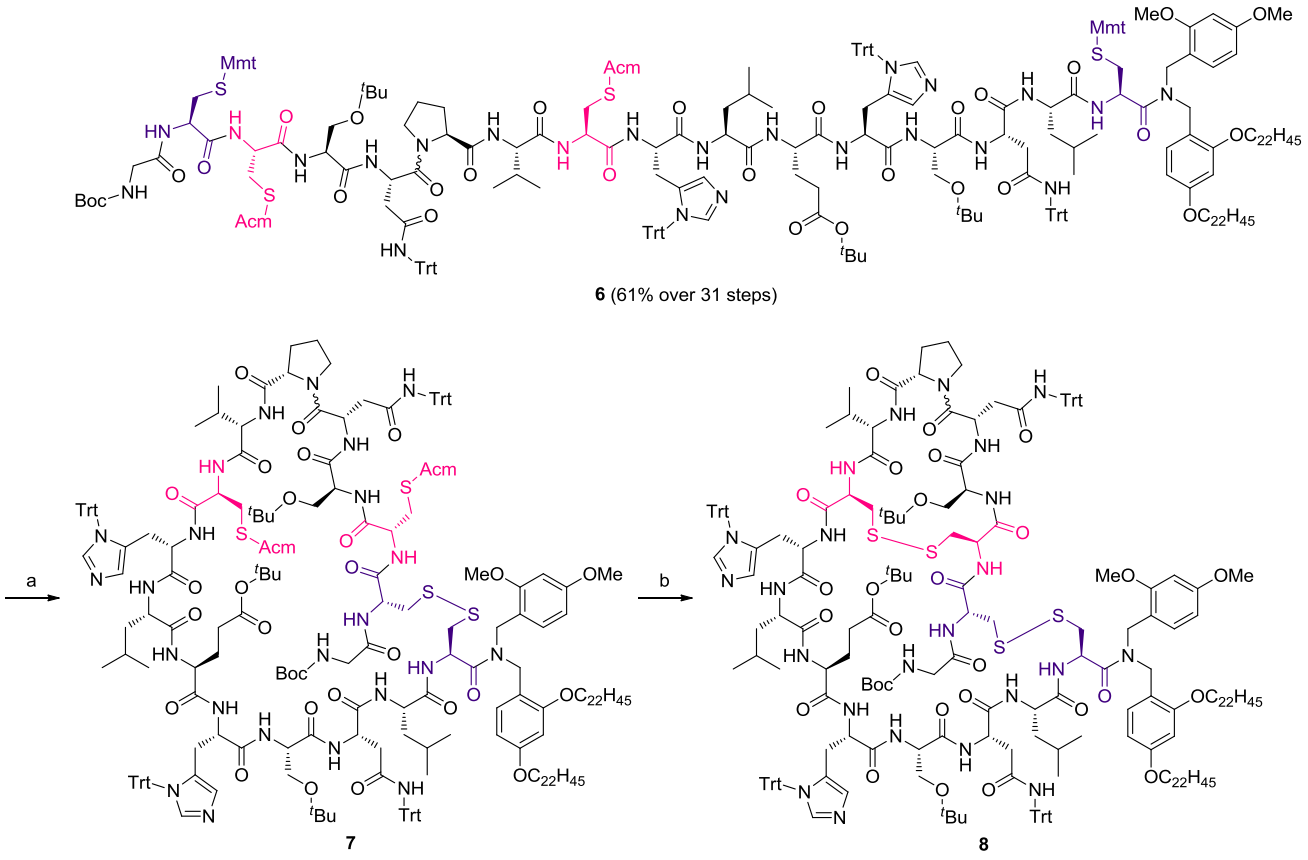
HRMS $[M+2Na]^{2+}$ calcd for $C_{266}H_{341}N_{25}O_{32}S_4Na_2$2287.2293, found 2287.2295.

4.5. Acidic deprotection of Mmt groups

Tagged peptide (**6**) (35.0 mg, 0.010 mmol) was dissolved in 1% TFA and 5% triisopropylsilane (TIS) in CH_2Cl_2 (5 mL). The reaction mixture was stirred at room temperature until the reaction completed. After the completion, DIPEA was added to the reaction mixture to neutralize, which was then poured into CH_2Cl_2, and the CH_2Cl_2 solution was washed with brine. The deprotected product was obtained through evaporation under reduced pressure.

4.6. Oxidative disulfide bond formation using DEAD

Tagged peptide was dissolved in THF (10 mL). DEAD (10 mol equiv) was then added to the solution. The reaction



Scheme 5. Synthesis of α-conotoxin MII (**4**).

mixture was stirred at room temperature until the reaction completed. After the completion, the cyclized product was obtained through evaporation under reduced pressure.

Boc-Gly-Cys(Acm)-*c*(Cys-Ser(^{t}Bu)-Asn(Trt)-Pro-Val-Cys(Acm)-His(Trt)-Leu-Glu(^{t}Bu)-His(Trt)-Ser(^{t}Bu)-Asn(Trt)-Leu-Cys)-N(Dmb)-TAG (**7**), amorphous solid.

HRMS $[M+1+2Na]^{2+}$ calcd for $C_{226}H_{307}N_{25}O_{30}S_4Na_2$2014.1017, found 2014.1017.

4.7. Oxidative disulfide bond formation using iodine

The tagged peptide (**7**) was dissolved in THF (9 mL). I_2 (10 mol equiv) in THF (1 mL) was then added to the solution. The reaction mixture was stirred at room temperature until the reaction completed. After the completion, 1 M ascorbic acid (1 mL) was added and product was extracted by CH_2Cl_2. MeCN was then added to the reaction mixture to give the cyclized product as a precipitate.

Boc-Gly-*c*(Cys-*c*(Cys-Ser(^{t}Bu)-Asn(Trt)-Pro-Val-Cys)-His(Trt)-Leu-Glu(^{t}Bu)-His(Trt)-Ser(^{t}Bu)-Asn(Trt)-Leu-Cys)-N(Dmb)-TAG (**8**), amorphous solid.

HRMS $[M+2Na]^{2+}$ calcd for $C_{220}H_{295}N_{23}O_{28}S_4Na_2$1941.5551, found 1941.5553.

4.8. Acidic deprotection of all protective groups

Tagged peptide was dissolved in 2.5% TIS and 2.5% H_2O in TFA (5 mL). The reaction mixture was stirred at room temperature until the reaction completed. After the completion, the reaction mixture was filtrated by hydrophilic PTFE filter, and then diisopropyl ether (DIPE) was added to the filtrate to give the product as a precipitate in 12.1 mg, 71% yield over four steps.

*4.8.1. α-Conotoxin MII (**4**).* HRMS $[M+Na]^+$ calcd for $C_{67}H_{103}N_{23}O_{22}S_4Na$ 1732.6429, found 1732.6432.

Acknowledgements

This work was partially supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Supplementary data

Additional schemes, general information, spectra information, and copies of ^{1}H and ^{13}C NMR spectra. Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.tet.2013.01.068.

References and notes

1. Terlau, H.; Olivera, B. M. *Physiol. Rev.* **2004**, *84*, 41–68.
2. (a) Carstens, B. B.; Clark, R. J.; Daly, N. L.; Harvey, P. J.; Kaas, Q.; Craik, D. J. *Curr. Pharm. Des.* **2011**, *17*, 4242–4253; (b) Halai, R.; Craik, D. J. *Nat. Prod. Rep.* **2009**, *26*, 526–536; (c) Craik, D. J.; Adams, D. J. *ACS Chem. Biol.* **2007**, *2*, 457–468; (d) Nicke, A.; Loughnan, M. L.; Millard, E. L.; Alewood, P. F.; Adams, D. J.; Daly, N. L.; Craik, D. J.; Lewis, R. J. *J. Biol. Chem.* **2003**, *278*, 3137–3144.
3. (a) Azam, L.; McIntosh, J. M. *Acta Pharmacol. Sin.* **2009**, *30*, 771–783; (b) Ellison, M.; Gao, F.; Wang, H.-L.; Sine, S. M.; McIntosh, J. M.; Olivera, B. M. *Biochemistry*

2004, *43*, 16019–16026; (c) Dutton, J. L.; Craik, D. J. *Curr. Med. Chem.* **2001**, *8*, 327–344; (d) Harvey, S. C.; McIntosh, J. M.; Cartier, G. E.; Maddox, F. N.; Luetje, C. W. *Mol. Pharmacol.* **1997**, *51*, 336–342; (e) Cartier, G. E.; Yoshikami, D.; Gray, W. R.; Luo, S.; Olivera, B. M.; McIntosh, J. M. *J. Biol. Chem.* **1996**, *271*, 7522–7528; (f) Johnson, D. S.; Martinez, J.; Elgoyhen, A. B.; Heinemann, S. F.; McIntosh, J. M. *Mol. Pharmacol.* **1995**, *48*, 194–199; (g) McIntosh, J. M.; Yoshikami, D.; Mahe, E.; Nielsen, D. B.; Rivier, J. E.; Gray, W. R.; Olivera, B. M. *J. Biol. Chem.* **1994**, *269*, 16733–16739.

4. (a) Yu, R.; Kaas, Q.; David, J.; Craik, D. J. *J. Phys. Chem. B* **2012**, *116*, 6097–6105; (b) Yu, R.; Craik, D. J.; Kaas, Q. *PLoS Comput. Biol.* **2011**, *7*, e1002011; (c) Armishaw, C.; Jensen, A. A.; Balle, T.; Clark, R. J.; Harpsøe, K.; Skonberg, C.; Liljefors, T.; Strømgaard, K. *J. Biol. Chem.* **2009**, *284*, 9498–9512; (d) Maslennikov, I. V.; Shenkarev, Z. O.; Zhmak, M. N.; Ivanov, V. T.; Methfessel, C.; Tsetlin, V. I.; Arseniev, A. S. *FEBS Lett.* **1999**, *444*, 275–280; (e) Gehrmann, J.; Daly, N. L.; Alewood, P. F.; Craik, D. J. *J. Med. Chem.* **1999**, *42*, 2364–2372; (f) Rogers, J. P.; Luginbühl, P.; Shen, G. S.; McCabe, R. T.; Stevens, R. C.; Wemmer, D. E. *Biochemistry* **1999**, *38*, 3874–3882; (g) Quiram, P. A.; Jones, J. J.; Sine, S. M. *J. Biol. Chem.* **1999**, *274*, 19517–19524; (h) Lamthanh, H.; Jegou-Matheron, C.; Servent, D.; Ménez, A.; Lancelin, J. M. *FEBS Lett.* **1999**, *454*, 293–298; (i) Quiram, P. A.; Sine, S. M. *J. Biol. Chem.* **1998**, *273*, 11007–11011.
5. (a) de Araujo, A. D.; Callaghan, B.; Nevin, S. T.; Daly, N. L.; Craik, D. J.; Moretta, M.; Hopping, G.; Christie, M. J.; Adams, D. J.; Alewood, P. F. *Angew. Chem., Int. Ed.* **2011**, *50*, 6527–6529; (b) Muttenthaler, M.; Nevin, S. T.; Grishin, A. A.; Ngo, S. T.; Choy, P. T.; Daly, N. L.; Hu, S. H.; Armishaw, C. J.; Wang, C. I.; Lewis, R. J.; Martin, J. L.; Noakes, P. G.; Craik, D. J.; Adams, D. J.; Alewood, P. F. *J. Am. Chem. Soc.* **2010**, *132*, 3514–3522; (c) Armishaw, C. J.; Dutton, J. L.; Craik, D. J.; Alewood, P. F. *Biopolymers* **2010**, *94*, 307–313; (d) MacRaild, C. A.; Illesinghe, J.; van Lierop, B. J.; Townsend, A. L.; Chebib, M.; Livett, B. G.; Robinson, A. J.; Norton, R. S. *J. Med. Chem.* **2009**, *52*, 755–762; (e) Walewska, A.; Zhang, M.; Skalicky, J. J.; Yoshikami, D.; Olivera, B. M.; Bulaj, G. *Angew.Chem., Int. Ed.* **2009**, *48*, 2221–2224; (f) Galanis, A. S.; Albericio, F.; Grotli, M. *Biopolymers* **2009**, *92*, 23–34; (g) Whiteaker, P.; Marks, M. J.; Christensen, S.; Dowell, C.; Collins, A. C.; McIntosh, J. M. *J. Pharmacol. Exp. Ther.* **2008**, *325*, 910–919; (h) Armishaw, C. J.; Daly, N. L.; Nevin, S. T.; Adams, D. J.; Craik, D. J.; Alewood, P. F. *J. Biol. Chem.* **2006**, *281*, 14136–14143; (i) Vishwanath, V. A.; McIntosh, J. M. *Bioconjugate Chem.* **2006**, *17*, 1612–1617; (j) Clark, R. J.; Fischer, H.; Nevin, S. T.; Adams, D. J.; Craik, D. J. *J. Biol. Chem.* **2006**, *281*, 23254–23263.
6. (a) Shemyakin, M. N.; Ovchinnikov, Y. A.; Kiryushkin, A. A.; Kozhevnikovd, I. V. *Tetrahedron Lett.* **1965**, *6*, 2323–2327; (b) Geckeler, K.; Pillai, V. N. R.; Mutter, M. *Adv. Polym. Sci.* **1981**, *39*, 65–94; (c) Narita, M.; Hirata, M.; Kusano, K.; Itsuno, S.; Ue, M.; Okawara, M. *Pept. Chem.* **1980**, *17*, 107–112; (d) Mutter, M.; Hagenmaier, H.; Bayer, E. *Angew. Chem., Int. Ed. Engl.* **1971**, *10*, 811–812; (e) Bayer, E.; Mutter, M. *Nature* **1972**, *237*, 512–513; (f) Bayer, E. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 113–129; (g) Gravert, D. J.; Janda, K. D. *Chem. Rev.* **1997**, *97*, 489–510; (h) Dickerson, T. J.; Reed, N. N.; Janda, K. D. *Chem. Rev.* **2002**, *102*, 3325–3344; (i) Bergbreiter, D. E. *Chem. Rev.* **2002**, *102*, 3345–3384; (j) Bergbreiter, D. E.; Tian, J.; Hongfa, C. *Chem. Rev.* **2009**, *109*, 530–582.
7. (a) Palcic, M. M.; Heerze, L. D.; Pierce, M.; Hindsgaul, O. *Glycoconjugate J.* **1988**, *5*, 49–63; (b) Stangier, P.; Palcic, M. M.; Bundle, D. R. *Carbohydr. Res.* **1995**, *267*, 153–159; (c) Kanie, O.; Barresi, F.; Ding, Y. L.; Labbe, J.; Otter, A.; Forsberg, L. S.; Ernst, B.; Hindsgaul, O. *Angew. Chem., Int. Ed.* **1996**, *34*, 2720–2722; (d) Ding, Y.; Labbe, J.; Kanie, O.; Hindsgaul, O. *Bioorg. Med. Chem.* **1996**, *4*, 683–692; (e) Nilsson, U. J. *J. Chromatogr., A* **2000**, *885*, 305–319; (f) Nilsson, U. J.; Fournier, E. J. L.; Hindsgaul, O. *Bioorg. Med. Chem.* **1998**, *6*, 1563–1575; (g) Sato, R.; Toma, K.; Nomura, K.; Takagi, M.; Yoshida, T.; Azefu, Y.; Tamiaki, H. *J. Carbohydr. Chem.* **2004**, *23*, 375–388; (h) Kanie, O.; Ohtsuka, I.; Ako, T.; Daikoku, S.; Kame, Y.; Kato, R. *Angew. Chem., Int. Ed.* **2006**, *45*, 3851–3854; (i) Pozsgay, V. *Org. Lett.* **1999**, *1*, 477–479; (j) Pozsgay, V. *Tetrahedron: Asymmetry* **2000**, *11*, 151–172; (k) Bauer, J.; Rademann, J. *J. Am. Chem. Soc.* **2005**, *127*, 7296–7297; (l) Encinas, L.; Chiara, J. L. *Eur. J. Org. Chem.* **2009**, 2163–2173; (m) Li, J.; Sung, S.; Tian, J.; Bergbreiter, D. E. *Tetrahedron* **2005**, *61*, 12081–12092; (n) Guo, J.; Lu, Y.-J.; Zhang, L.; Ye, X.-S. *Synlett* **2012**, 1696–1700.
8. (a) Chiba, K.; Kono, Y.; Kim, S.; Nishimoto, K.; Kitano, Y.; Tada, M. *Chem. Commun.* **2002**, 1766–1767; (b) Kim, S.; Tsuruyama, A.; Ohmori, A.; Chiba, K. *Chem. Commun.* **2008**, 1816–1818; (c) Iijima, M.; Mikami, Y.; Yoshioka, T.; Kim, S.; Kamiya, H.; Chiba, K. *Langmuir* **2009**, *25*, 11043–11047.
9. Tana, G.; Kitada, S.; Fujita, S.; Okada, Y.; Kim, S.; Chiba, K. *Chem. Commun.* **2010**, 8219–8221.
10. Kitada, S.; Fujita, S.; Okada, Y.; Kim, S.; Chiba, K. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 4476–4479.