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Solid-phase synthesis of neuroactive spider–wasp hybrid toxin analogues using a backbone amide linker

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Abstract—Polyamine toxins isolated from the venoms of spiders and wasps and their synthetic analogues are uncompetitive antagonists of ligand-gated ionotropic receptors in the central- and peripheral nervous systems, and have proved valuable as tools for the investigation of receptor structure and function. In the present letter we describe the efficient solid-phase synthesis (SPS) of novel hybrid toxins using a BAL resin. This strategy enables the bidirectional construction of toxin molecules and has a potential in SPS of chemically diverse libraries of toxin analogues for structure–activity relationship (SAR) studies.

Polyamine toxins isolated from natural sources such as venoms from spiders (e.g., argiotoxin-636, **1**, Fig. 1)[1] and wasps (PhTX-433, **2**)[2] are uncompetitive inhibitors of a variety of ligand-gated ion channels in humans as well as invertebrates.[3] Natural and synthetic polyamine derivatives have subsequently proved valuable as probes in the structural and functional studies of ligand-gated receptors, including *N*-methyl-D-aspartic acid (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and nicotinic acetylcholine (nACh) receptors.[4] Furthermore, the recent launch of the drug memantine,[5] a selective uncompetitive NMDA receptor antagonist, for the management of moderate to severe Alzheimers disease, underlines the potential of compounds with this mode of action in drug discovery.

The lack of selectivity towards single types of ionotropic receptors displayed by these natural products is probably linked to their natural role as prey suppression tools,[6] but this feature is undesirable from a drug development perspective. Attempts to improve selectivity towards certain classes of human receptors, by performing SAR studies have been successful. Thus, we have reported a structural modification of the tyrosine head-group of the synthetic wasp toxin analogue PhTX-343 (**3a**) to give the cyclohexylalanine analogue PhTX(Cha)-343 (**3b**), which proved to be the most potent nAChR antagonist reported to date.[7] Furthermore, analogues with altered polyamine moieties have been reported to selectively antagonize subtypes of glutamate receptors.[8]

In order to obtain pure natural toxins as well as unnatural analogues in useful quantities for further biological studies, efficient synthetic strategies are of great importance.[9] Recent development of SPS methods has improved access to polyamine toxins considerably. Thus, a variety of SPS methods based on alkylation or reductive methods have been developed for the sequential assembly of polyamine moieties,[10] as have linker strategies for the preparation of toxin analogues containing diamines or commercially available polyamines (e.g., spermine).[11–14]

In short, the latter approaches towards polyamine toxin targets have employed trityl resins loaded with a di- or polyamine as the starting point for diversification of the toxin head group. N-terminal elongated PhTX analogues have been prepared by loading ω-amino acids onto a trityl linker via the nitrogen functionality, which was followed by on-resin carboxy group activation for the attachment of the polyamine moiety.[12] Also, two different side chain anchoring strategies that allow a bidirectional SPS of spider toxins containing a primary amide functionality (glutamine)[13] or hybrid toxins containing a phenol functionality (tyrosine) such as in

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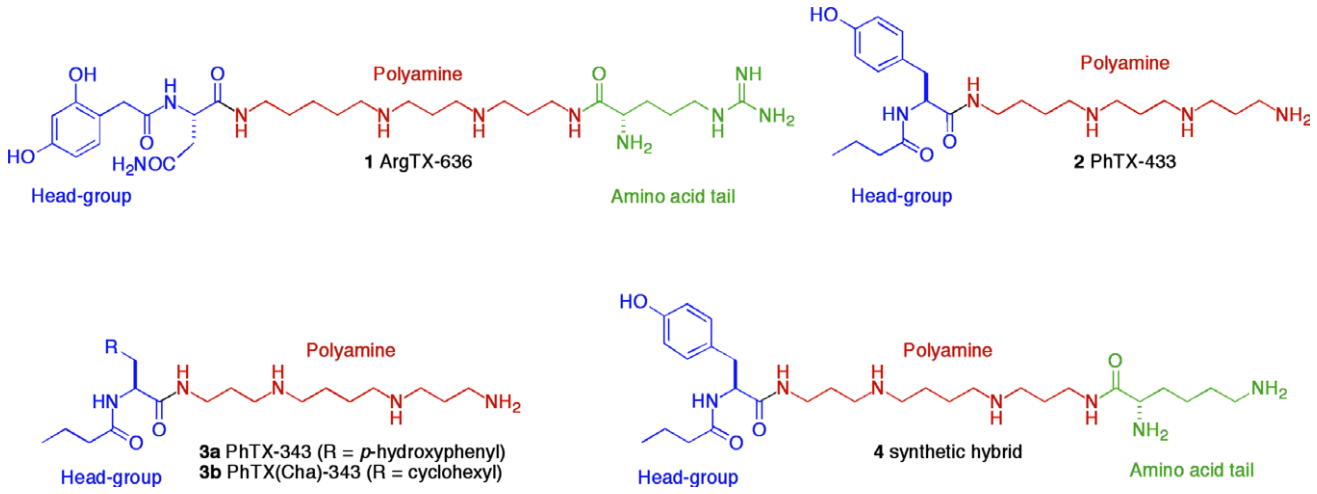
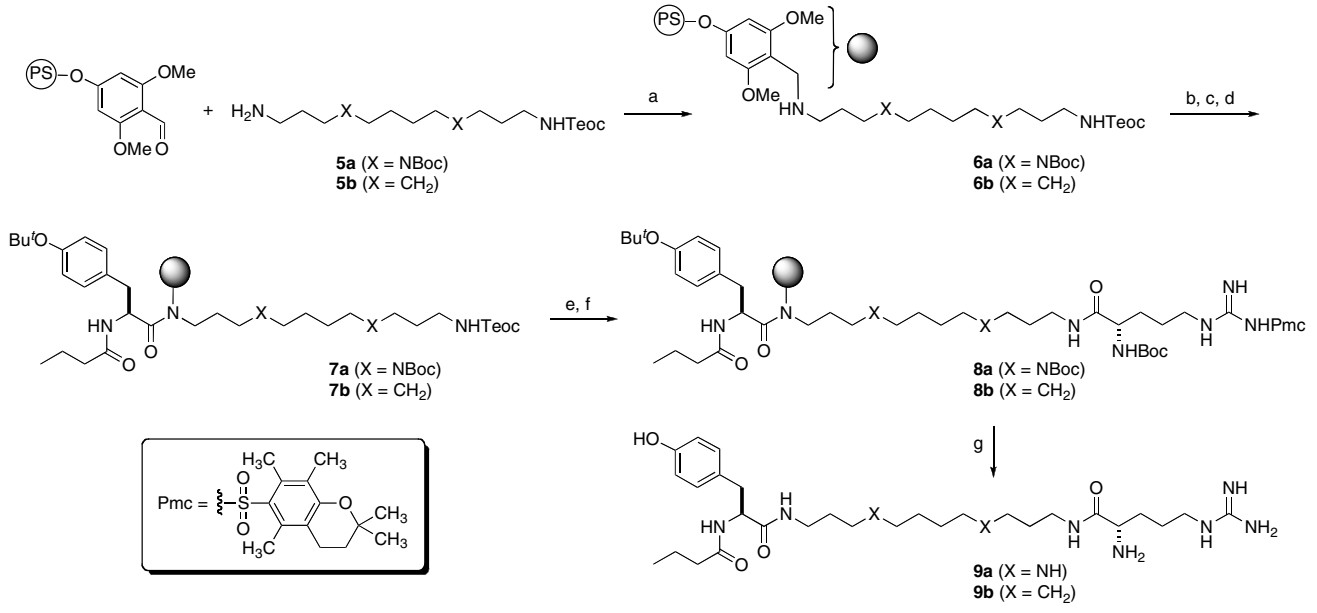


Figure 1. Structures of natural acylpolyamine neurotoxins isolated from the venom mixtures of spiders **1** and wasps **2** as well as synthetic analogues **3a**, **3b**, and **4**. The numerals in the name of **1** denote the molecular mass. In philanthotoxin (PhTX) analogues **2**–**4** the digits denote the number of methylene groups that separate the amino functionalities, starting from the 'head-group'.

compound **4**,[14] have been reported. Finally, the sequential method developed by Bienz and co-workers[15] for SPS of curtatoxin analogues should in principle be applicable for bidirectional functionalization of polyamine toxins.

In the present letter, we report on a versatile and simple bidirectional SPS method, which allows for the synthesis of combinatorial libraries where a head-group, a polyamine moiety, and an amino acid tail may be introduced and varied independently. As a backbone amide linker (BAL)[16] strategy is applied, this process does not require any specific side chain functionality for anchoring.

A protected tetraamine **5a** and a diamine **5b** were chosen as building blocks for the initial on-resin immobilization (Scheme 1).[17] The reductive amination in the loading step was performed with $NaBH(OAc)_3$ in a solvent mixture of DMF–HOAc (9:1).[18] In a preliminary sequence, the relatively difficult peptide coupling step that derivatizes the anchoring secondary amino group was attempted using benzotriazol-1-yl-oxy-tris(pyrrol-



Scheme 1. Synthesis of hybrid toxins **9a** and **9b** on a BAL resin. Reagents and conditions: (a) $NaBH(OAc)_3$ (10 equiv), DMF–HOAc (9:1), 16 h; (b) Fmoc-Tyr(Bu^t)-OH (5 equiv), TFFH (5 equiv), $i Pr_2EtN$ (10 equiv), DMF, 16 h; (c) 20% piperidine–DMF, 2 × 10 min; (d) Pfp butanoate (3 equiv), Dhbt-OH (1 equiv), $i Pr_2EtN$ (3 equiv), DMF, 4 h; (e) Bu_4NF (5 equiv), DMF, 50 °C, 30 min; (f) Boc-Arg(Pmc)-OH (5 equiv), TBTU (5 equiv), $i Pr_2EtN$ (10 equiv), DMF, 16 h; (g) TFA–CH_2Cl_2 (95:5), 3 h.

idino)phosphonium hexafluorophosphate (PyBOP)[19] as the coupling reagent. This method proved unsuccessful, however, and fluoro-*N*,*N*,*N*,*N*-tetramethylformamidinium hexafluorophosphate (TFFH)[20] was used instead to generate the acid fluoride in situ, which also parallels the results of Barany, Albericio, Jensen, and co-workers.[16] After introduction of the tyrosine moiety, the butyryl moiety was installed as previously described[21] to give resins **7a** and **7b**. Subsequently, the Teoc group was removed with Bu_4NF,[21] and the arginine tail was introduced via the commercially available Boc-Arg(Pmc)-OH building block, prior to cleavage from the resins which gave crude **9a** and **9b**.

The toxin analogues were obtained in high overall yields after purification by preparative reversed-phase HPLC: **9a** (36%, 86% per step) and **9b** (33%, 85% per step).[22,23] Compared to the overall yields obtained in our previous work using phenol-anchoring on a trityl bromide resin (10–21%),[14] the yields obtained using the BAL strategy are higher. Furthermore, this strategy is suitable for synthesis and diversification of toxin analogues containing aliphatic amino acid residues, such as the highly potent analogues containing cyclohexylalanine.[7]

In conclusion, we have developed an efficient and highly versatile strategy for the assembly of N-terminally elongated wasp toxin analogues using a backbone amide linker on solid phase. These 'spider–wasp hybrid' toxins are so far largely unexplored with respect to their inhibitory effects against various classes of ionotropic receptors. The spermine analogue **9a**, however, has previously been prepared by solution-phase chemistry,[24] and was shown to be almost four times as potent as PhTX-343 in a locust muscle twitch assay.[25] Finally, we envision that this linker strategy will be particularly suitable for the total synthesis of natural spider toxins on solid phase.

Acknowledgments

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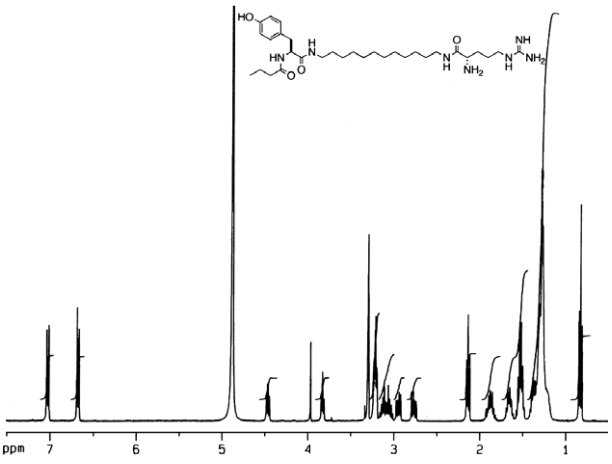
References and notes

1. Adams, M. E.; Carney, R. L.; Enderlin, F. E.; Fu, E. T.; Jarema, M. A.; Li, J. P.; Miller, C. A.; Schooley, D. A.; Shapiro, M. J.; Venema, V. J. *Biochem. Biophys. Res. Commun.* **1987**, *148*, 678–683.
2. (a) Eldefrawi, A. T.; Eldefrawi, M. E.; Konno, K.; Mansour, N. A.; Nakanishi, K.; Eugene, O.; Usherwood, P. N. R. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 4910–4913; (b) Piek, T.; Fokkens, R. H.; Karst, H.; Kruk, C.; Lind, A.; van Marle, J.; Tong, Y. C. In *Neurotox 8: Molecular Basis of Drug and Pesticide Action*; Lunt, G. G., Ed.; Elsevier: Amsterdam, 1988; pp 61–76.
3. (a) Scott, R. H.; Sutton, K. G.; Dolphin, A. C. *Trends Neurosci.* **1993**, *16*, 153–160; (b) Mueller, A. L.; Roeloffs, R.; Jackson, H. *Alkaloids* **1995**, *46*, 63–94.
4. For reviews, see: (a) Strømgaard, K.; Andersen, K.; Krogsgaard-Larsen, P.; Jaroszewski, J. W. *Mini Rev. Med. Chem.* **2001**, *1*, 317–338; (b) Strømgaard, K.; Mellor, I. R. *Med. Res. Rev.* **2004**, *24*, 589–620; (c) Mellor, I. R.; Usherwood, P. N. R. *Toxicon* **2004**, *43*, 493–508.
5. Lipton, S. A. *Nat. Rev. Drug Discov.* **2006**, *5*, 160–170.
6. Cohen, S. S. *A Guide to Polyamines*; Oxford University Press: New York, 1998.
7. Olsen, C. A.; Mellor, I. R.; Wellendorph, P.; Usherwood, P. N. R.; Witt, M.; Franzyk, H.; Jaroszewski, J. W. *ChemMedChem* **2006**, *1*, 303–305.
8. Mellor, I. R.; Brier, T. J.; Pluteanu, F.; Strømgaard, K.; Saghyan, A.; Eldursi, N.; Brierley, M. J.; Andersen, K.; Jaroszewski, J. W.; Krogsgaard-Larsen, P.; Usherwood, P. N. R. *Neuropharmacology* **2002**, *44*, 70–80.
9. For reviews, see: (a) Karigiannis, G.; Papaioannou, D. *Eur. J. Org. Chem.* **2000**, 1841–1863; (b) Kuksa, V.; Buchan, R.; Lin, P. K. T. *Synthesis-Stuttgart* **2000**, 1189–1207.
10. For a comprehensive review of available methods for formation of the amino functionality on solid phase, see: Olsen, C. A.; Franzyk, H.; Jaroszewski, J. W. *Synthesis-Stuttgart* **2005**, 2631–2653.
11. (a) Strømgaard, K.; Brier, T. J.; Andersen, K.; Mellor, I. R.; Saghyan, A.; Tikhonov, D.; Usherwood, P. N. R.; Krogsgaard-Larsen, P.; Jaroszewski, J. W. *J. Med. Chem.* **2000**, *43*, 4526–4533; (b) Jørgensen, M. R.; Olsen, C. A.; Mellor, I. R.; Usherwood, P. N. R.; Witt, M.; Franzyk, H.; Jaroszewski, J. W. *J. Med. Chem.* **2005**, *48*, 56–70.
12. Jørgensen, M. R.; Jaroszewski, J. W.; Witt, M.; Franzyk, H. *Synthesis-Stuttgart* **2005**, 2687–2694.
13. Bycroft, B. W.; Chan, W. C.; Hone, N. D.; Millington, S.; Nash, I. A. *J. Am. Chem. Soc.* **1994**, *116*, 7415–7416.
14. Olsen, C. A.; Jørgensen, M. R.; Hansen, S. H.; Witt, M.; Jaroszewski, J. W.; Franzyk, H. *Org. Lett.* **2005**, *7*, 1703–1706.
15. (a) Manov, N.; Bienz, S. *Tetrahedron* **2001**, *57*, 7893–7898; (b) Manov, N.; Tzouros, M.; Chesnov, S.; Bigler, L.; Bienz, S. *Helv. Chim. Acta* **2002**, *85*, 2827–2846.
16. Jensen, K. J.; Alsina, J.; Songster, M. F.; Vágner, J.; Albericio, F.; Barany, G. *J. Am. Chem. Soc.* **1998**, *120*, 5441–5452.
17. For preparation and characterization of **5a** and **5b**, see Ref. 12.
18. Conditions were inspired by Korínek, M.; Dudic, M.; Havránek, M., poster presented at PacifiChem 2005, Honolulu, Hawaii, USA.
19. Coste, J.; Le-Nguyen, D.; Castro, B. *Tetrahedron Lett.* **1990**, *31*, 205–208.
20. Carpino, L. A.; El-Faham, A. *J. Am. Chem. Soc.* **1995**, *117*, 5401–5402.
21. Olsen, C. A.; Witt, M.; Jaroszewski, J. W.; Franzyk, H. *Org. Lett.* **2003**, *5*, 4183–4185.
22. Characterization data for compound **9a**: Yield = 35 mg (36%, 86% per step). RP-HPLC$_{215\,nm}$: >99%. ESI-MS: $C_{29}H_{54}N_9O_4^+$ requires $[M+H]^+$ at *m*/*z* 592.4; found, 592.8. ^{1}H NMR data as previously reported.[24] ^{13}C NMR (100 MHz, CD_3OD): δ 176.4, 175.2, 170.8, 162.2 (q, J = 35 Hz, TFA), 158.9, 157.5, 131.4 (2C), 129.1, 117.8 (q, J = 290 Hz, TFA), 116.4 (2C), 57.1, 54.3, 49.4, 48.4, 48.3, 46.7, 46.3, 41.8, 38.8, 38.0, 37.6, 36.9, 29.8, 27.5 (2C), 25.6, 24.4, 20.4, 14.0. Characterization data for compound **9b**: Yield = 25 mg (33%, 85% per step).



^{1}H NMR (400 MHz, CD_3OD): δ 7.03 (d, $J = 8.5$ Hz, 2H), 6.69 (d, $J = 8.5$ Hz, 2H), 4.48 (br t, $J = 7.1$ Hz, 1H, H-α-Tyr), 3.84 (br t, $J = 6.6$ Hz, 1H, H-α-Arg), 3.31 (m, 4H, $2 \times CH_2NH$), 3.15 (ddt, $J = 20.0$ Hz, $J = 6.8$ Hz, $J = 6.4$ Hz, 1H, H-β-Arg), 3.08 (ddt, $J = 20.0$ Hz, $J = 6.8$ Hz, $J = 6.4$ Hz, 1H, H-β-Arg), 2.96 (dd, $J = 13.6$ Hz, $J = 6.9$ Hz, 1H, H-β-Tyr), 2.78 (dd, $J = 13.6$ Hz, $J = 8.3$ Hz, 1H, H-β-Tyr), 2.15 (d, $J = 7.4$ Hz, 2H, CH_2CO-But), 1.89 (m, 2H), 1.68 (m, 2H), 1.54 (sextet, $J = 7.4$ Hz, 2H, CH_2-But), 1.28 (m, 18H), 0.84 (t, $J = 7.4$ Hz, 3H, CH_3-But). ^{13}C NMR (100 MHz, CD_3OD): δ 175.9, 173.7, 169.7, 161.1 (q, $J = 35$ Hz, TFA), 158.8, 157.4, 131.3 (2C), 129.1, 116.2 (2C), 56.5, 54.1, 41.8, 40.8, 40.4, 38.8, 38.5, 30.7 (2C), 30.6 (2C), 30.4 (2C), 30.3 (2C), 29.8, 28.0, 27.9, 25.5, 20.3, 13.9. RP-HPLC$_{215\,nm}$: >99%. ESI-MS: $C_{31}H_{56}N_7O_4^+$ requires $[M+H]^+$ at m/z 590.4; found, 590.5. HRMS: $C_{31}H_{56}N_7O_4^+$ requires $[M+H]^+$ at m/z 590.43883; found, 590.43869; $\Delta M = 0.24$ ppm.

23. Attempted purification of toxin **9a** by simple reversed-phase vacuum liquid chromatography (VLC) as previously reported[7,12,21] did not lead to a satisfactory purity, as judged by analytical RP-HPLC$_{215\,nm}$ (~80%), which is in contrast to the excellent purities (>99%) obtained after isolation by preparative RP-HPLC.
24. Goodnow, R., Jr.; Konno, K.; Niwa, M.; Kallimopoulos, T.; Bukownik, R.; Lenares, D.; Nakanishi, K. *Tetrahedron* **1990**, *46*, 3267–3286.
25. Nakanishi, K.; Goodnow, R., Jr.; Konno, K.; Niwa, M.; Bukownik, R.; Kallimopoulos, T.; Usherwood, P. N. R.; Eldefrawi, A. T.; Eldefrawi, M. E. *Pure Appl. Chem.* **1990**, *62*, 1223–1230.