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Solid Phase Syntheses of Polyamine Toxins HO-416b and PhTX-433. Use of an Efficient Polyamide Reduction Strategy That Facilitates Access to Branched Analogues

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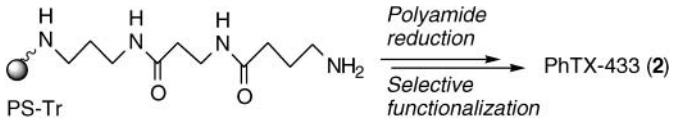
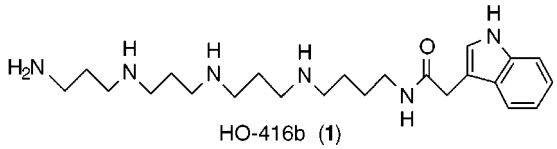
ABSTRACT

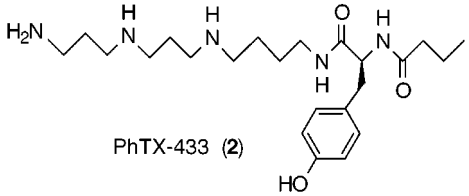


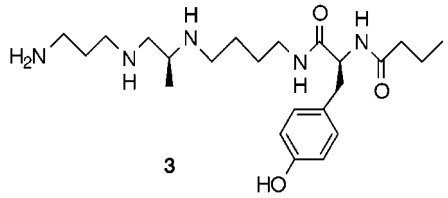
Polyamine toxins HO-416b (1) and PhTX-433 (2) isolated from the venom of insects are important lead compounds in neuropharmacology. Their total synthesis has been achieved on a trityl derivatized resin in good yield and purity using a mild borane reduction protocol to access the polyamine chains from polyamide precursors. The synthesis of PhTX isomer 3 demonstrates the potential of this strategy for the generation of branched analogues.

The polyamine natural toxins HO-416b (**1**) and philanthotoxin-433 (PhTX-433, **2**) are isolated respectively from the venoms of funnel web spider *Hololena curta*[1] and female digger wasp *Philanthus triangulum* (bee wolf).[2] The latter, for instance, produces **2** for paralyzing insect preys by a mechanism of action that antagonizes ionotropic glutamate[2] and nicotinic acetylcholine[3] receptors present in neuromuscular junction. Several acylpolyamines including PhTX-433 and a number of its reported analogues[4] are known to inhibit signal transmission in the central nervous system (CNS) of mammalians.[5] Consequently, these molecules generate considerable interest as a promising class of lead compounds for therapeutic applications[6] such as neuroprotection in cases of strokes,[7] as well as probes for receptor studies.[8]

The isolation of polyamine toxins from the venom glands of the insects, however, is troublesome and provides limited amounts of material. Therefore, there is a need for versatile synthetic routes that will allow access to practical quantities







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(2) Eldefrawi, A. T.; Eldefrawi, M. E.; Konno, K.; Mansour, N. A.; Nakanishi, K.; Oltz, E.; Usherwood, P. N. R. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 4910−4913.
(3) Piek, T.; Hue, B. *Comput. Biochem. Physiol.* **1989**, *93C*, 403−406.

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of the natural toxins and a wide variety of analogues thereof. Ideally, solid phase approaches would be preferable in order to accelerate and simplify these efforts.

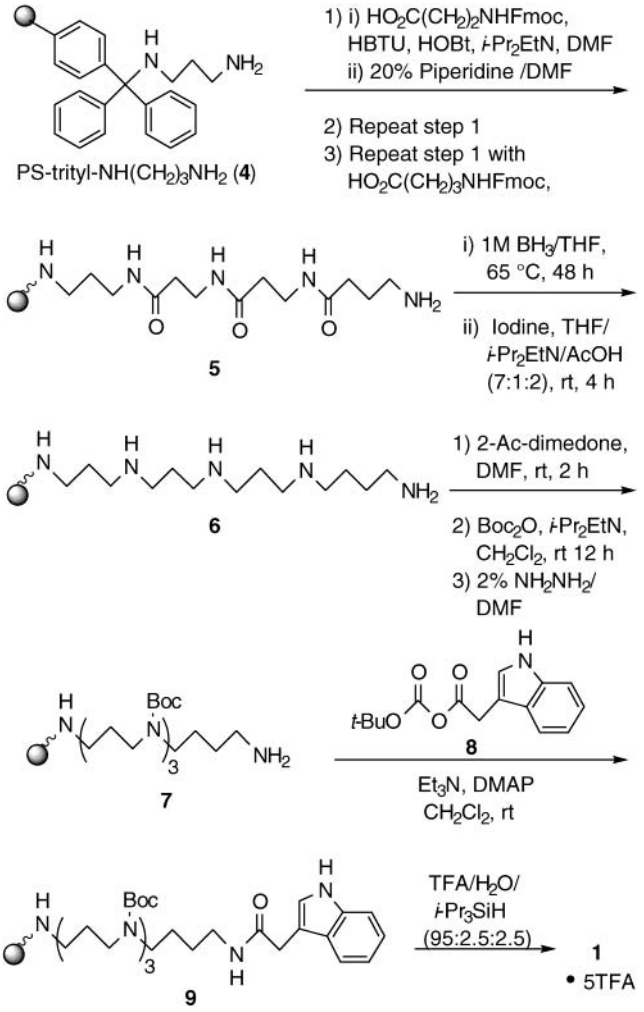
Natural polyamines **1** and **2** have been synthesized previously using multistep solution phase strategies to construct the unsymmetrical secondary amines by iterative modifications of nitrogen functionalities.[9] These strategies are not easily amenable to the preparation of certain classes of analogues. For instance, the synthesis of polyamines with chiral spacing units remains challenging. Indeed, few examples of such branched analogues have been reported.[10]

Herein we report on a very efficient solid phase strategy for the total syntheses of **1** and **2** that relies on the reduction of polyamide precursors to provide the required polyamine chains. In addition, we demonstrate with the synthesis of **3**—a structural isomer of **2** embodying a reduced alanine in place of the central *n*-propyl polyamine spacer—that the use of polyamide precursors is extremely advantageous for the generation of branched analogues.

Typical procedures for the reduction of amides by excess borane require a strongly acidic workup to cleave the resulting borane−amine intermediates and isolate the free amines.[11] These conditions are not compatible with most supports used in solid phase synthesis. In contrast, the mild oxidative workup recently reported by our group allows the use of practical resins with acid-sensitive trityl linkers.[12] Its use for the synthesis of polyamine toxins was first demonstrated for toxin HO-416b (**1**) (Scheme 1).

From the diaminopropyl resin **4**,[12] three successive steps of amino acid coupling and deprotection, twice with Fmoc-protected β-alanine then followed with Fmoc-γ-aminobutyric acid, provided βAla-βAla-γAbu triamide **5**. The latter was reduced with borane and then treated with a buffered solution of iodine to afford tetramine **6**. Aliquots of this polyamine

Scheme 1. Synthesis of HO-416b (**1**)



product, a polyacetylated derivative, and the triamide precursor were cleaved from the resin and analyzed (NMR, MS) to ascertain the efficiency of this operation. To functionalize **6** selectively, we employed a sequence described by Nash et al. in their hemisynthesis of PhTX-343 from spermine.[13] Selective protection of the primary amine of **6** as a bis-*N*-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl] (Dde) derivative[14] was followed by blocking of the secondary amines with Boc_2O. Then, the orthogonally protected terminal amine was liberated with dilute hydrazine, and the resulting intermediate **7** was coupled with mixed anhydride **8**[9a] to afford resin **9**. Treatment of **9** with a solution of trifluoroacetic acid (TFA)/water/triisopropylsilane provided crude toxin **1** as a penta(trifluoracetate) ammonium salt in 57% yield and 81% purity according to HPLC analysis.[15] Two rounds of precipitation with ether afforded material of 90% purity with a 37% overall yield from tripeptide **5**. This work, performed in nine chemical steps from resin **4**, constitutes the first solid phase synthesis of HO-416b (**1**).

(4) For a selection of recent reports on the synthesis of philanthotoxin analogues, see: (a) Strømgaard, K.; Brierley, M. J.; Andersen, K.; Sløk, F. A.; Mellor, I. R.; Usherwood, P. N. R.; Krogsgaard-Larsen, P.; Jaroszewski, J. W. *J. Med. Chem.* **1999**, *42*, 5224−5234. (b) Rosini, M.; Budriesi, R.; Bixel, M. G.; Bolognesi, M. L.; Chiarini, A.; Hucho, F.; Krogsgaard-Larsen, P.; Mellor, I. R.; Minarini, A.; Tumiatti, V.; Usherwood, P. N. R.; Melchiorre, C. *J. Med. Chem.* **1999**, *42*, 5212−5223. (c) Nakanishi, K.; Huang, X.; Jiang, H.; Liu, Y.; Fang, K.; Huang, D.; Choi, S.-K.; Katz, E.; Eldefrawi, M. *Bioorg. Med. Chem. Lett.* **1997**, *5*, 11969-1988. (d) Huang, D.; Jiang, H.; Nakanishi, K.; Usherwood, P. N. R. *Tetrahedron* **1997**, *53*, 12391−12404.

(5) For a recent report involving PhTX-343, see: Bähring, R.; Mayer, M. L. *J. Physiol.* **1998**, *509*, 635−650.

(6) For reviews on the pharmacology of polyamines, see: (a) Mueller, A. L.; Roeloffs, R.; Jackson, H. *The Alkaloids*; Academic Press: New York, 1995; Vol. 46, Chapt. 2, pp 63−94. (b) Carter, C., Ed. *The Neuropharmacology of Polyamines*; Academic Press: San Diego, 1994.

(7) Green, A. C.; Nakanishi, K.; Usherwood, P. N. R. *Brain Res.* **1996**, *717*, 135−147.

(8) Choi, S.-K.; Kalivretenos, A.; Usherwood, P. N. R.; Nakanishi, K. *Chem. Biol.* **1995**, *2*, 23−32.

(9) HO-416b (**1**): (a) Hidai, Y.; Kan, T.; Fukuyama, T. *Tetrahedron Lett.* **1999**, *40*, 4711−4714. PhTX-433 (**2**): (b) Piek, T.; Fokkens, R. H.; Karst, H.; Kruk, C.; Lind, A.; van Marie, J.; Nakajima, T.; Nibbering, N. M. M.; Shinozaki, H.; Spanier, W.; Tong, Y. C. In *Neurotox '88L Molecular Basis of Drug and Pesticide Action*; 1988; Abstract, p 61. (c) Reference 2.

(10) For one example, see: Kalivretenos, A. G.; Nakanishi, K. *J. Org. Chem.* **1993**, *58*, 6596−6608.

(11) For an example on a peptide derivative, see: Northrop, R. C., Jr.; Russ, P. L. *J. Org. Chem.* **1977**, *42*, 4148−4150.

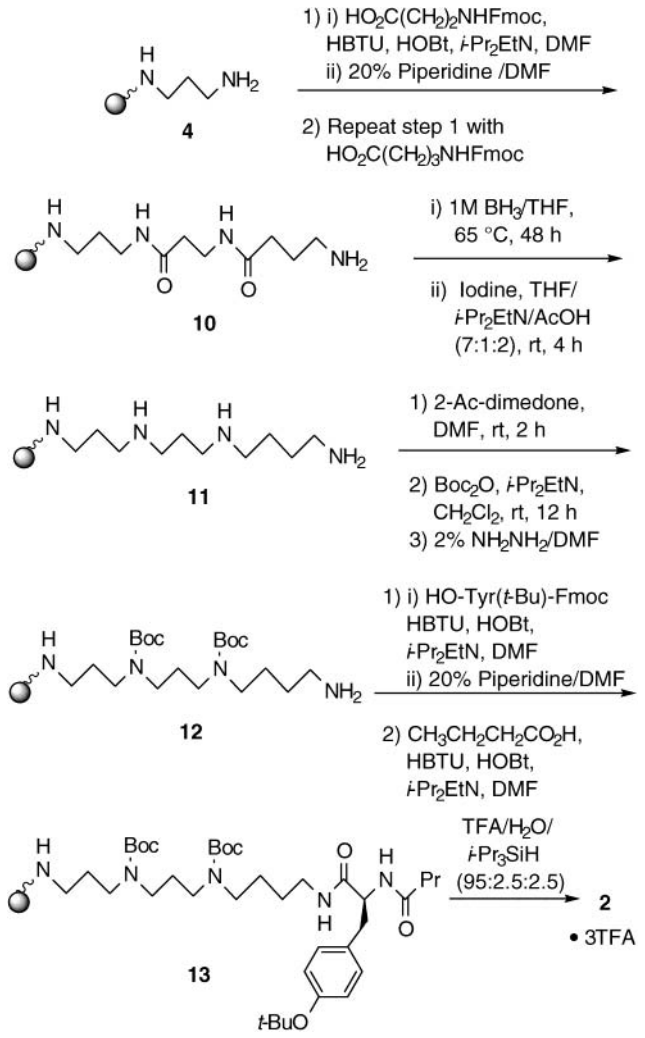
(12) Hall, D. G.; Laplante, C.; Manku, S.; Nagendran, J. *J. Org. Chem.* **1999**, *64*, 668−669.

(13) Nash, I. A.; Bycroft, B. W.; Chan, W. C. *Tetrahedron Lett.* **1996**, *37*, 2625−2628.

(14) Bycroft, B. W.; Chan, W. C.; Chhabra, S. R.; Hone, N. D. *J. Chem. Soc., Chem. Commun.* **1993**, 778−779.

The synthesis of PhTX-433 was achieved using a similar sequence (Scheme 2). Thus from resin **4**, two successive

Scheme 2. Synthesis of PhTX-433 (**2**)



amide coupling and deprotection steps provided βAla-γAbu diamide **10**. Reduction of the latter followed by oxidative workup gave triamine **11**. Assessing reduction efficiency as well as selectively protecting the internal secondary amines of **11** were carried out as described above for **6**. Then, the resulting intermediate **12** was coupled with Fmoc-L-Tyr(*t*-Bu) and treated with piperidine, and the final amide coupling step with butyric acid afforded **13**.[13] Its cleavage from the resin provided crude toxin **2** as a tris(trifluoracetate) ammonium salt in 77% yield and 80% purity according to HPLC analysis. Finally, two rounds of precipitation with ether increased purity to >90% with a 55% overall yield from dipeptide **10**.[15] Our synthetic product was compared satisfactorily by HPLC, MS, and ^{1}H NMR with an authentic sample of natural PhTX-433 (**2**) (see Supporting Information). This solid-supported total synthesis of PhTX-433 was also completed in nine steps.[16] Since several protected natural and unnatural amino acids are available, our strategy is very advantageous for analogue generation. To demonstrate this we have synthesized isomer **3**, a branched analogue of **2** containing a reduced alanine as a chiral two-carbon unit instead of the central *n*-propyl group. Analogues with two-carbon spacers cannot be made using standard methods for diamine synthesis like the conjugate addition of amines on acrylonitriles followed by reduction.[2] Except for the use of L-Ala as first amino acid, the synthetic sequences for **2** and **3** are identical.[17] We obtained analogue **3** in 88% purity and 75% yield from the corresponding dipeptide.[15]

With the total solid-supported syntheses of neuroactive polyamine toxins **1**, **2**, and analogue **3** we have further demonstrated the usefulness of our mild oxidative workup protocol for the borane-promoted reduction of polyamides. This approach shows potential for the generation of libraries of analogues displaying a branched polyamine chain made from diverse amino acid building blocks.

Acknowledgment. This work was supported by the Natural Science and Engineering Research Council (NSERC) of Canada, the Alberta Heritage Foundation for Medical Research (AHFMR), and the University of Alberta. S.M. thanks AHFMR for a Graduate Studentship. The authors are thankful to Prof. K. Nakanishi (Columbia University) for providing a sample of natural PhTX-433.

Supporting Information Available: Experimental details for the syntheses of **1**, **2**, and **3** with selected NMR, MS, and HPLC conditions and data, including a comparison between synthetic and natural PhTX-433. This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) NMR and MS data are fully consistent with the assigned structures (see Supporting Information). RP-HPLC analyses were performed on a Zorbax SB-C18 column (4.6 × 150 mm, 5 μm) using 0.1% TFA water/acetonitrile mobile phases and UV detection. Should higher homogeneity be required toward biological testing, products could be further purified by semipreparative HPLC.

(16) Another solid-supported synthesis of PhTX-433 was recently reported. See: Chhabra, S. R.; Khan, A. N.; Bycroft, B. W. *Tetrahedron Lett.* **2000**, *41*, 1099−1102.

(17) This borane reduction protocol was shown to be racemization free (see ref 12).