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Solid-phase synthesis of PhTX-3.2.4 and PhTX-2.3.3 derivatives

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Abstract—A new solid-phase method for the synthesis of derivatives of the philanthotoxins is described. Diamines are attached as carbamates to hydroxymethyl polystyrene resin. Selective mono-alkylations by acid-labile, substituted benzhydryl chlorides, followed by reductive alkylations with Fmoc-protected amino aldehydes are employed to assemble the polyamine backbone. Different acid-stability of the benzhydrylic protective groups allows the selective removal from secondary amines for subsequent branching.

Polyamines terminally conjugated with different amino acids such as tryptophan, tyrosine or arginine have been found to be constituents in several arthropod venom mixtures.[1,2] The most extensively investigated group of naturally occurring polyamine toxins is the philanthotoxins (PhTXs), where the compound PhTX-4.3.3 (Fig. 1) has been isolated from the venom of the Egyptian digger wasp *Philanthus triangulum*.[3,4] The polyamine toxins have been found to interact with cation selective ion channels and to have non-competitive antagonistic properties towards ionotropic glutamate receptors[5] and towards the open pore of muscular and neuronal nicotinergic acetylcholine receptors.[6] The PhTXs are low molecular weight polyamines, with one terminus as a primary amine and the other terminus linked to a relatively non-polar residue via an amide bond. SAR studies of PhTXs as ligands to receptors have shown that significant changes in affinity and selectivity for different receptors can be observed by altering the terminal amino group, the polyamine backbone, the aromatic residue or the hydrophobic terminus of the ligand.[7]

A variety of structural analogues of PhTX-4.3.3 have been synthesized, and several different routes for solution synthesis have been presented.[3,4,8] However, the synthesis of acylpolyamine compounds has shifted towards a solid-phase approach, largely as a result of the difficulties of working with polyamines in solution. A few solid-phase protocols for the synthesis of PhTX analogues and other acylpolyamine toxins have been published recently.[9–11] The presented solid-phase approaches use a polyamine part that is either a pre-constructed polyamine[9b–c] selectively protected with orthogonal protecting groups before introduction to the solid support, or constructed sequentially using reductive alkylation,[9d] modified Mitsunobu reactions[10] or polypeptide reduction.[11] In this communication, the versatility of our previously published method for polyamine synthesis,[12] as an alternative route to acylpolyamine toxins, is presented by the synthesis of derivatives of the previously unpublished PhTX-3.2.4 and PhTX-2.3.3. The assembly of the polyamine backbones is performed by reductive alkylations of amines, protected with acid-labile benzhydryl-type amino-protecting groups, with *N*-protected amino aldehydes according to our previously reported protocol.[12] This method allows primary amines, attached to solid-phase, to be selectively mono-alkylated with 4-methoxy dityl (Mmd)[13] and 4,4′-dimethoxy dityl (Dod)[14,15] groups. The Mmd and the Dod groups are used as semi-permanent or temporary protecting groups, respectively, and cleavage of the temporary Dod group introduces the possibility of further derivatizing the secondary amines.

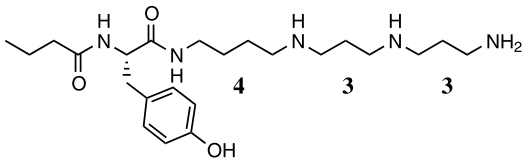

Figure 1. Structure of PhTX-4.3.3.

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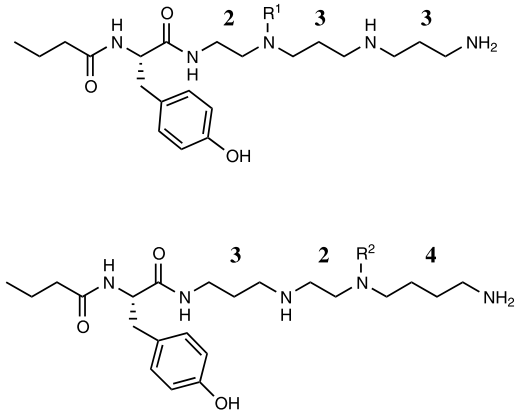
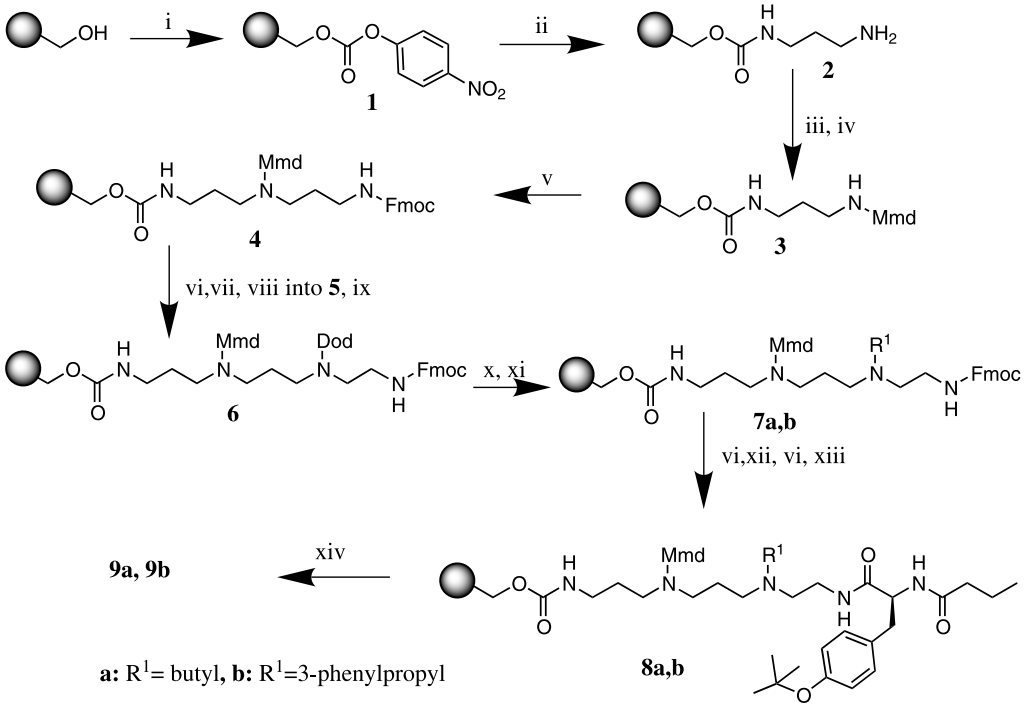


Figure 2. **9a**: R^1 = butyl, $(M+H)^+$ = 464.3601; **9b**: R^1 = 3-phenylpropyl; **16**: R^2 = isobutyl, $(M+H)^+$ = 478.3757; **17**: R^2 = H. (Calculated monoisotopic molecular weights.)

The PhTX analogues **9a** and **9b** (Fig. 2) with the 2.3.3 backbone were synthesized as follows (Scheme 1). Conventional polystyrene-based hydroxymethyl resin was converted to the 4-nitrophenyl-carbonate resin **1**.[16] The N-terminal 1,3-diaminopropane group was attached to the solid support by incubating resin **1** with 1,3-diaminopropane forming **2**. Alkylation with an excess of 4-methoxydityl chloride and subsequent washes with 10% trifluoroacetic acid (TFA) gave the mono-Mmd protected **3**.[12] The secondary amino group was then reductively alkylated for 3×1 h with *N*-Fmoc-3-aminopropionaldehyde to give **4**.[17] After Fmoc-deprotection, the resin-bound primary amine was alkylated with excess 4,4′-dimethoxydityl chloride, treated with 5% TFA forming **5** and then reductively alkylated with *N*-Fmoc-2-aminoacetaldehyde to give **6**. Cleavage of the tertiary Dod-group makes it possible to introduce branching on the nitrogen and reductive alkylation with butyraldehyde or 3-phenylpropionaldehyde gave **7a** and **7b**, respectively. Fmoc-deprotection and acylation with activated Fmoc-Tyr (O*t*Bu)-OH and butyric acid employing standard Fmoc-peptide protocol resulted in **8a** and **8b**. Cleavage from the resin was accomplished with neat TFA for 2.5 h at 50°C. After filtration from the resin and evaporation of the TFA solution, the crude products were dissolved in 0.5 M aqueous HCl and extracted with EtOAc. Analysis of the aqueous phase by RP-HPLC[18] (Fig. 3) and MALDI-TOF[19] showed the expected products **9a** (at 10.7 min) and **9b** (at 12.0 min) in >80% purity and 75–80% overall yield.[20] In addition, small fractions of resins **4**, **6** and **7** were withdrawn, cleaved with neat TFA and analyzed by RP-HPLC in order to monitor the progress of the reaction and for characterization of the intermediate products by MALDI-TOF.



Scheme 1. (i) 10 equiv. of 4-nitrophenyl chloroformate, 10 equiv. of *N*-methylmorpholine (NMM), dichloromethane (DCM), 2 h, rt; (ii) 10 equiv. of 1,3-diaminopropane, *N*,*N*-dimethylformamide (DMF), 1.5 h, rt; (iii) 4 equiv. of Mmd-Cl, 10 equiv. of *N*,*N*-diisopropylethylamine (DIEA), DCM, 1.5 h; (iv) 10×10 s with 10% TFA in DCM; (v) 3 equiv. of *N*-Fmoc-3-aminopropionaldehyde, 3 equiv. of $NaCNBH_3$, 1-methyl-2-pyrrolidinone (NMP) with 3% AcOH, 40°C, 3×1 h; (vi) 20% piperidine in DMF, 30 min; (vii) 4 equiv. of Dod-Cl, 10 equiv. of DIEA, DCM, 1.5 h; (viii) 6×10 s with 5% TFA in DCM; (ix) 3 equiv. of *N*-Fmoc-2-aminoacetaldehyde, 3 equiv. of $NaCNBH_3$, NMP with 3% AcOH, 40°C, 3×1 h; (x) 5×10 s+1×15 min with 5% TFA in DCM, (xi) **a**: 3 equiv. of butyraldehyde or **b**: 3-phenylpropionaldehyde, 3 equiv. of $NaCNBH_3$, NMP with 3% AcOH, 40°C, 3×1 h; (xii) 1.5 equiv. of Fmoc-Tyr(O*t*Bu)-OH, 1.5 equiv. of TBTU, 1.5 equiv. of HOBt, 3 equiv. of DIEA, DCM/DMF (1:1), 20 min, rt; (xiii) 1.5 equiv. of butyric acid, 1.5 equiv. of TBTU, 1.5 equiv. of HOBt, 3 equiv. of DIEA, DCM/DMF (1:1), 20 min, rt; (xiv) TFA, 50°C, 2.5 h.

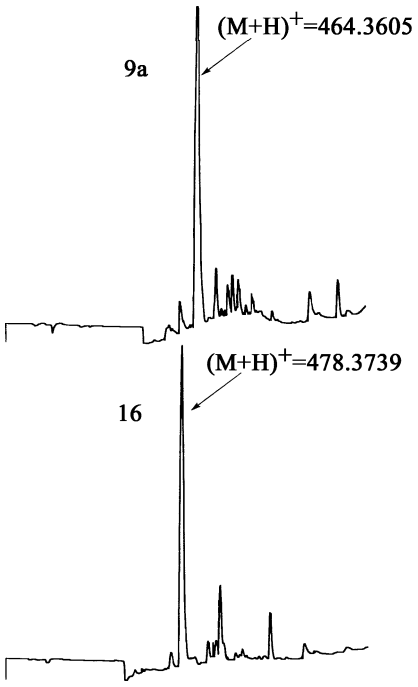
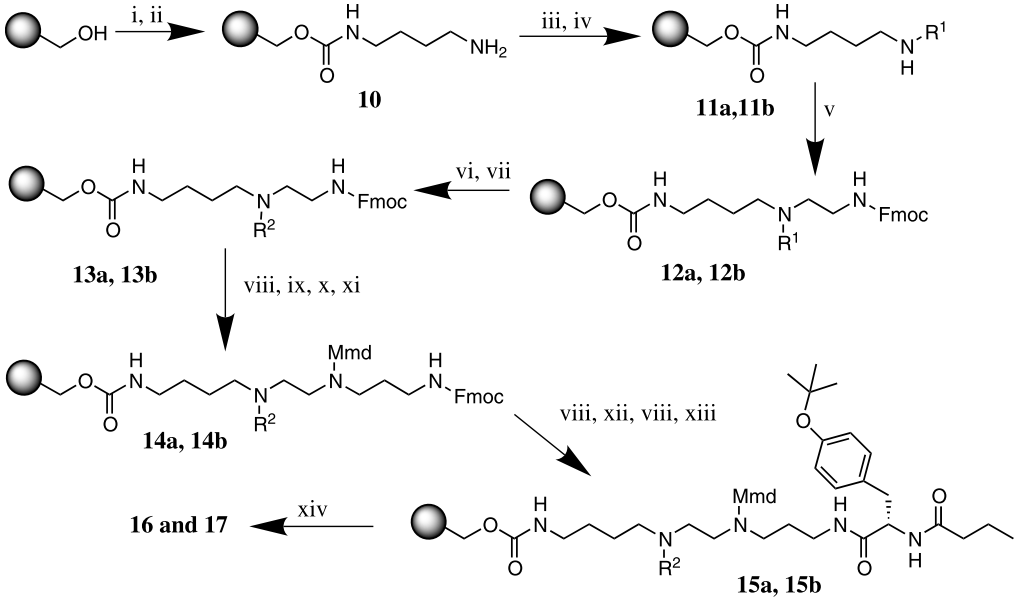


Figure 3. HPLC chromatograms of **9a** and **16**, displaying absorbance at 279 nm and the molecular weights of main peaks.

The PhTX analogues **16** and **17** (Fig. 2) were synthesized using the same Dod/Mmd method as for **9a** and **9b**. However, the intermediate alkyl chains are altered and both the branched and the unbranched analogue possessing the same polyamine backbone are synthesized according to Scheme 2. Resin bound 1,4-diaminobutane, **10** was alkylated with either Dod-Cl or Mmd-Cl and treated with 5% TFA to give **11a** and **11b**, respectively. Subsequent reductive alkylation with *N*-Fmoc-2-aminoacetaldehyde gave **12a** and **12b**. Washing of resin **12a** with 5% TFA and a subsequent reductive alkylation with isobutyraldehyde gave **13a**. Cleavage of the Fmoc group and repetition of the protection with Mmd-Cl and the reductive alkylation of the terminal amine with *N*-Fmoc-3-aminopropionaldehyde gave **14a** and **14b**. Acylation as above with activated Fmoc-Tyr (O*t*Bu)-OH and butyric acid formed **15a** and **15b**, which were cleaved from the resin in neat TFA for 2.5 h at 50°C. The products **16** and **17** (Fig. 2) were treated and analyzed as above. The main peaks at 9.5 or 8.8 min in the RP-HPLC analyses were of satisfying purity (>75%) (Fig. 3) and displayed the correct molecular weights, with an overall yield of 50%.

In conclusion, analogues of the polyamine toxins of the philanthotoxin group can be conveniently and rapidly synthesized on solid support. Sequential elongation and



a: R^1= Dod, R^2=isobutyl, **b**: R^1=R^2=Mmd

Scheme 2. (i) 10 equiv. of 4-nitrophenyl chloroformate, 10 equiv. of NMM, DCM, 2 h, rt; (ii) 10 equiv. of 1,4-diaminobutane, DMF, 1.5 h, rt; (iii) **a**: 4 equiv. of Dod-Cl or **b**: 4 equiv. of Mmd-Cl, 10 equiv. of DIEA, DCM, 1.5 h; (iv) **a**: 6×10 s with 5% TFA in DCM, **b**: 10×10 s with 10% TFA in DCM; (v) 3 equiv. of *N*-Fmoc-2-aminoacetaldehyde, 3 equiv. of $NaCNBH_3$, NMP with 3% AcOH, 40°C, 3×1 h; (vi) only **12a**: 5×10 s+1×15 min with 5% TFA in DCM; (vii) only **12a**: 3 equiv. of isobutyraldehyde, 3 equiv. of $NaCNBH_3$, NMP with 3% AcOH, 40°C, 3×1 h; (viii) 20% piperidine in DMF, 30 min; (ix) 4 equiv. of Mmd-Cl, 10 equiv. of DIEA, DCM, 1.5 h; (x) 10×10 s with 10% TFA in DCM; (xi) 3 equiv. of *N*-Fmoc-3-aminopropionaldehyde, 3 equiv. of $NaCNBH_3$, NMP with 3% AcOH, 40°C, 3×1 h; (xii) 1.5 equiv. of Fmoc-Tyr(O*t*Bu)-OH, 1.5 equiv. of TBTU, 1.5 equiv. of HOBt, 3 equiv. of DIEA, DCM/DMF (1:1), 20 min, rt; (xiii) 1.5 equiv. of butyric acid, 1.5 equiv. of TBTU, 1.5 equiv. of HOBt, 3 equiv. of DIEA, DCM/DMF (1:1), 20 min, rt; (xiv) TFA, 50°C, 2.5 h.

selective branching of the polyamine backbones, using reductive alkylations with repetitive protocols, can be used in order to obtain a large number of derivatives of acylpolyamine toxins.

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13. 4-Methoxy dityl chloride (Mmd-Cl): 4-methoxy benzophenone (10 g, 47.1 mmol) was dissolved in EtOH (250 ml). $NaBH_4$ (0.9 g, 23.6 mmol, 0.5 equiv.) was added slowly and the reduction was left to stir overnight. TLC (petroleum ether/EtOAc, 9/1) indicated completeness of the reduction and the mixture was poured onto water (250 ml), stirred for 1 h and 4-methoxy benzhydrol could be filtered off and dried. 4-Methoxy benzhydrol (5 g, 23.3 mmol) was dissolved in dried DCM (50 ml), oxalyl chloride (2.25 ml, 25.7 mmol, 1.1 equiv.) was added dropwise and the reaction was left stirring for 2 h at room temperature. The solvent was evaporated and 4-methoxydityl chloride was crystallized and recrystallized from warm petroleum ether. Yield: 5.4 g, 99%.
14. 4,4′-Dimethoxy dityl chloride (Dod-Cl): 4,4′-dimethoxy benzhydrol (6.0 g, 24.6 mmol) was dissolved in dried DCM (60 ml), oxalyl chloride (2.36 ml, 27 mmol, 1.1 equiv.) was added dropwise and the reaction was left with stirring for 1.5 h at room temperature. The solvent was evaporated and the 4,4′-dimethoxydityl chloride was crystallized and recrystallized from warm petroleum ether. Yield: 5.8 g, 90%.
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17. Synthesis of *N*-Fmoc-amino aldehydes: 3-aminopropanol or 2-aminoethanol (40 mmol) was dissolved in dried DCM (150 ml) and cooled in an ice bath. Fmoc-Cl (20 mmol) dissolved in DCM (120 ml) was added through a funnel over 30 min. After removal of cooling, the mixtures were stirred for another 1.5 h followed by extraction, three times with 0.5 M HCl (aq.), drying and evaporation. *N*-Fmoc-protected amino alcohols were obtained in almost quantitative yields and were converted to *N*-Fmoc-protected amino aldehydes under Swern conditions.[21]
18. HPLC analyses were performed on a Machery–Nagel KS 100/4 Nucleosil® 120-3 C_{18}. Elution gradient was 10–70% of B in 15 min with a flow of 0.8 ml/min. Solvent A was 0.1% TFA/H_2O and solvent B was 0.1% TFA/acetonitrile. Detection was performed at 279 nm, and the purity was measured by integration of the absorbance of the Fmoc group of intermediate products or the tyrosine of the final products.
19. MALDI-TOF analyses were performed on a Voyager-DE STR (Applied Biosystems, USA) in reflector mode with α-cyano-hydroxy cinnamic acid (Fluka) as matrix. Internal calibration was made against the resolved matrix peak at 379.0930 and all obtained molecular weights are monoisotopic.
20. Overall yields are the isolated yield compared to the theoretical maximum yield on initial resin loading (0.6 mmol/g).
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