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Total Syntheses of Polyamine Amides PhTX-4.3.3 and PhTX-3.4.3: Reductive Alkylation is a Rapid, Practical Route to Philanthotoxins

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Abstract: Reductive alkylation allows a rapid and practical entry to the polyamine amide wasp toxin philanthotoxin-4.3.3. Philanthotoxin-3.4.3 has been prepared, in two steps, by the monoacylation of spermine. These polyamine amides are selective molecular pharmacological tools for cation channels gated by glutamic acid and acetylcholine, and may have potential as neuroprotective agents.

Philanthotoxin-4.3.3 [PhTX-4.3.3, δ-philanthotoxin, (**1**)] is an unsymmetrical polyamine amide isolated from the venom of the solitary beewolf wasp *Philanthus triangulum* which preys on honey bees to support the next generation of wasps, and has been studied extensively by Piek and co-workers.[1,2] The structure of PhTX-4.3.3 (**1**) was solved and published simultaneously, but independently, in 1988, by Piek and co-workers,[2] Tong,[3] and by Eldefrawi, Nakanishi, Usherwood, and co-workers.[4] This wasp toxin contains the unsymmetrical polyamine thermospermine (4.3.3) (**2**), regioselectively monoacylated on the primary amino functional group of the putrescine (1,4-diaminobutane) moiety with *N*-n-butanoyl-L-tyrosine. There is continuing interest in polyamine amides as channel blockers of glutamic acid and/or nicotinic acetylcholine-gated receptor cation channels, and certain voltage-sensitive calcium channels.[1,5-8] In this *Letter*, we present rapid, practical syntheses of PhTX-4.3.3 (**1**) together with its regioisomer PhTX-3.4.3 (**3**) which contains the corresponding symmetrical polyamine, spermine (3.4.3).

There have been two published syntheses of PhTX-4.3.3 (**1**). One by Tong,[3] in which the key nitrogen-to-carbon bond of the unsymmetrical polyamine (between 4 and 3.3) was formed by alkylation of a secondary *N*-benzylamine with a substituted primary butyl bromide. In the synthesis by Nakanishi and co-workers,[4] the *N*-(3-aminopropyl)-1,3-propanediamine (3.3) moiety was introduced by two sequential cyanoethylations of putrescine (4) (acrylonitrile, BOC protection, and LAH reduction of the nitrile to the corresponding primary amine). We report a reductive alkylation route to PhTX-4.3.3 (**1**) which is rapid, efficient, and may be described as biomimetic as polyamines 5.3.3 (**4**) and 4.3.3 (**2**) can be biosynthesized *via* the corresponding imine obtained from a primary amine and an aldehyde,[9] in addition to the biosynthetic pathway for spermidine (4.3) which is alkylation of putrescine with an *S*-adenosyl-L-methionine unit.[10]

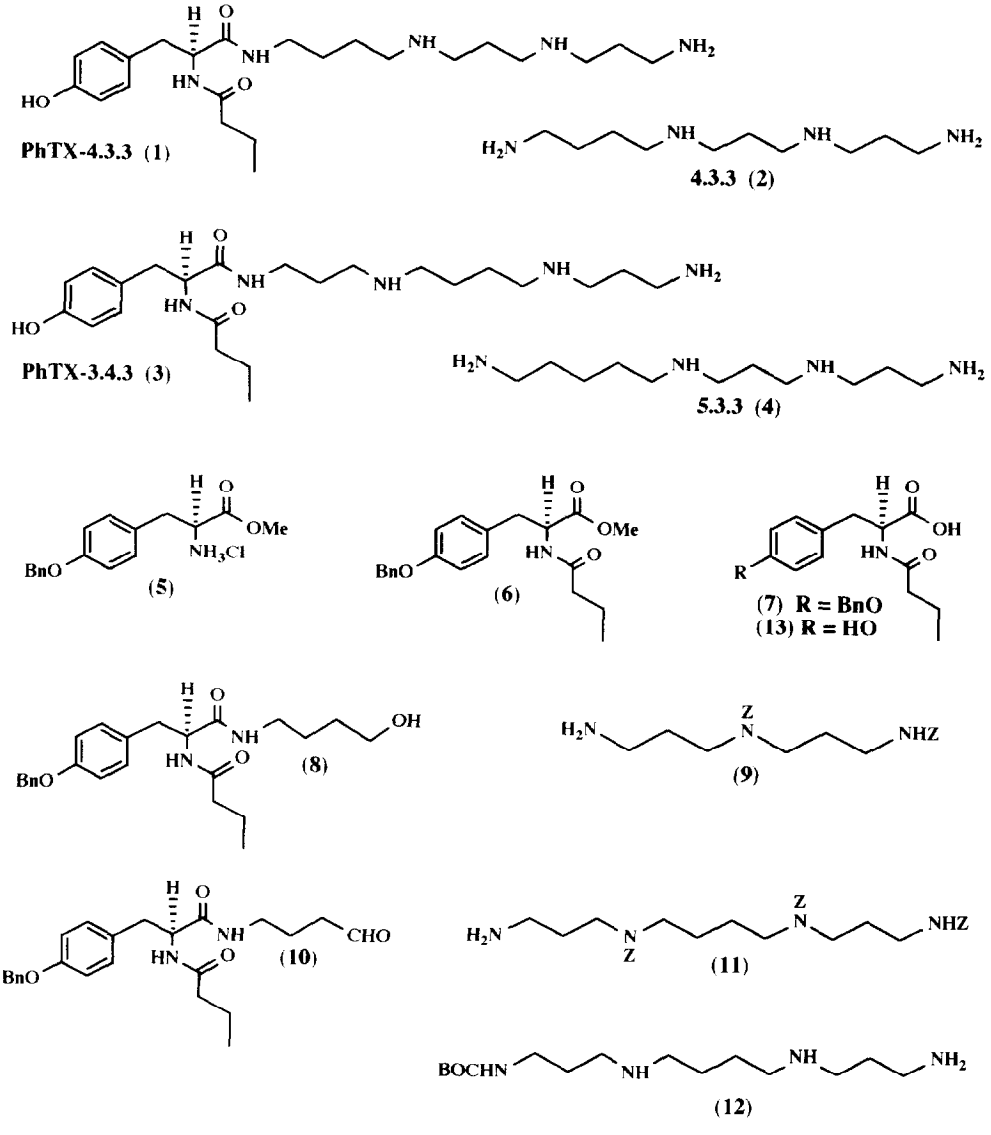
Philanthotoxin-4.3.3 (1) synthesis: L-Tyrosine methyl ester.HCl *O*-benzyl ether (**5**) is readily available (Novabiochem). The benzyl protecting group will lessen the hydrophilicity of the Tyr-polyamine conjugate which is always problematical during the final stages of a polyamine preparation and purification.

Furthermore, the *O*-benzyl ether will be removed with the carbobenzyloxy (Z) carbamate functional groups which we have chosen for the protection of the unsymmetrical polyamine. Therefore, there will only be the requirement for one deblocking procedure, hydrogenolysis. *N*-Acylation of the protected L-Tyr methyl ester (**5**) was smoothly and efficiently achieved with n-butanoyl chloride, in DCM containing triethylamine (2.2 eq.), at 25°C over 2 h, to afford the amide chromophore (**6**)(83%). Methyl ester (**6**) was saponified (3 eq. aq. NaOH 1 M, MeOH, 25°C, 4 h then acidified with aq. HCl 3 M, 96%) to afford free carboxylic acid (**7**). Selective monoacylation of 4-aminobutan-1-ol with acid (**7**), mediated by DCC (1.5 eq.) and a catalytic amount of 1-hydroxybenzotriazole (HOBt) (0.05 eq.), occurred, not unexpectedly, at the nitrogen atom which is more nucleophilic than the oxygen atom. This acylation afforded primary alcohol (**8**) (DCM, 25°C, 16 h) in excellent yield (90%). Conversion of the primary alcohol into the triflate (2.2 eq. Tf_2O, 2.0 eq. pyridine, DCM, 0°C) and attempted alkylation with *N*,*N*'-diZ-3-aminopropyl-1,3-propanediamine (diZ-3.3) (**9**) gave a multi-component mixture, with low recovery. Therefore, we decided to functionalize alcohol (**8**) as the corresponding aldehyde (**10**) for a reductive alkylation approach to the desired wasp toxin (**1**).

Oxidation of primary alcohol (**8**) with PCC (2.0 eq.), in DCM containing Celite equivalent to the mass of PCC,[11] at 25°C for 2 h, afforded an inhomogeneous mixture. This was somewhat surprising as smooth oxidation[11] of *N*-acetyl-4-aminobutan-1-ol and 4-phthalimidobutan-1-ol had been achieved under similar conditions with PCC. We attribute the formation of many products to oxidation of the *C*- and *O*-benzyl functionalities. DMSO activated with oxalyl chloride, in DCM at -78°C (Swern oxidation),[12, 13] efficiently afforded the desired aldehyde (**10**) (91%). Reductive alkylation of the primary amine in diZ-3.3 (**9**) with aldehyde (**10**), in MeOH at 25°C, using $NaCNBH_3$ and controlling the pH between 6.0 and 7.5 by the controlled addition of glacial AcOH, formed the unsymmetrical polyamine moiety, in 45% yield. This unoptimized yield is comparable with literature yields for *N*-alkylation using primary iodides and for reductive alkylation of an aldehyde with a primary amino functional group. Efficient deprotection of the *O*-benzyl and the Z carbamates was accomplished by hydrogenolysis (10% Pd/C, H_2 1 atm, MeOH, 15°C, 16 h) which gave PhTX-4.3.3 (**1**) as its free base (80%). Subsequent lyophilization from aq. HCl (2 M) afforded the corresponding trihydrochloride salt as a freely water soluble white powder. The six steps in this route to PhTX-4.3.3 (**1**) have been accomplished in 25% overall yield from the starting L-Tyr.OMe.HCl (**5**) and 24% overall yield from 3.3, the starting material for diZ-3.3 (**9**) synthesis.

Philanthotoxin-3.4.3 (3) syntheses: Protected L-Tyr methyl ester.HCl salt (**5**) was converted into n-butanoyl amide (**7**), as above, prior to acylation of triZ-spermine (**11**). This was prepared by mono-BOC protection of spermine (3.4.3) with $(BOC)_2O$, in THF at 25°C (16 h), affording monoBOC-3.4.3 (**12**) as a colourless, hygroscopic glass (62%). In any mono-functionalization of a symmetrical di- or polyamine there is a low yielding initial protection step, generally followed by chromatographic purification. We used 3 eq. of polyamine to carbamoylating reagent and purified by extensive column chromatography (eluting with DCM-MeOH-conc. NH_4OH (20:5:1 to 4:2:1, v/v/v). We also determined that significantly better yields and more efficient recovery of the desired product are achieved when the majority of the basic nitrogen atoms are protected throughout a polyamine synthesis. Therefore, instead of acylating monoBOC-3.4.3 (**12**), we prepared monoBOC-triZ-3.4.3 (3.3 eq. Z-Cl, aq. NaOH 1 M, 0 to 25°C, 16 h, 100%). Brief treatment (30 mins) with an excess of TFA, at 0°C, afforded triZ-3.4.3 (**11**) as its TFA salt (99%).

Free primary amine (**11**), from the TFA salt of triZ-protected spermine (triZ-3.4.3), was liberated and isolated by flash column chromatography over silica gel eluting with DCM-MeOH-conc NH_4OH (200:10:1 to 100:10:1, v/v/v). Acylation of protected spermine (**11**) with *O*-benzyl-protected L-Tyr carboxylic acid (**7**) proceeded smoothly (1.5 eq. DCC, 0.05 eq. HOBt, DCM, 25°C, 16 h, 79%). Finally, the desired wasp toxin analogue (**3**) was deprotected by hydrogenolysis to remove the *O*-benzyl and the three carbamate functional groups (10% Pd/C, H_2 1 atm, MeOH, 15°C, 16 h, 70%). Lyophilization of this monoacylated spermine from aq. HCl (2 M) afforded the corresponding trihydrochloride salt as a white hygroscopic solid. In our hands, the polyamine polyhydrochloride salt was more stable than the free base which scavenged carbonic acid from the atmosphere. This five step synthesis, from spermine, was accomplished in 34% overall yield.



We also report that a two step, practical synthesis of PhTX-3.4.3 (**3**) can be achieved from L-Tyr and spermine. *N*-n-Butanoylation of L-Tyr with pentafluorophenyl butanoate, in DMF and triethylamine (1.2 eq.) was smoothly accomplished at 25°C (48 h, 32%). The desired amide (**13**) was separated from *O*-acylated (12%) and *N,O*-diacylated (20%) products by silica column chromatography. n-Butanoyl chloride also afforded this mixture of *N*- (34%) (**13**) and *O*-acylated (10%), and *N,O*-diacylated (16%) L-Tyr. Monoacylation of spermine (3 eq.) with n-butanoyl-L-Tyr (**13**) (1.5 eq. DCC, EtOAc, 25°C, 16 h) afforded PhTX-3.4.3 (**3**) (51%). This two step synthesis, from spermine, was accomplished in 17% overall yield. These synthetic sequences are rapid, practical routes to PhTX-4.3.3 (**1**) and its analogues e.g. PhTX-3.4.3 (**3**). Clearly, they are amenable to the incorporation of a variety of chromophores replacing the L-Tyr residue, and to the inclusion of alternative protected-unsymmetrical polyamines or symmetrical free di- and polyamines.[8]

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