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Total Synthesis of Polyamine Amide Spider Toxin Argiotoxin-636 by a Practical Reductive Alkylation Strategy

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Abstract: Reductive alkylation is a practical strategy for a total synthesis of spider toxin argiotoxin-636, a polyamine amide which is a selective glutamate receptor antagonist and may have potential as a neuroprotective agent. Central to this synthesis are a Swern oxidation and a reductive alkylation.

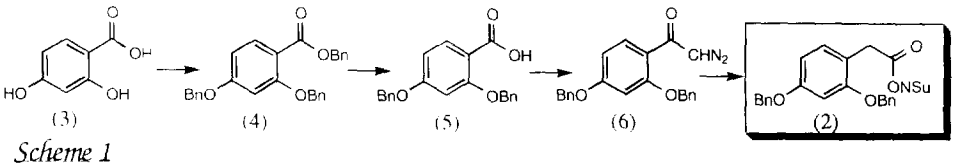
Argiotoxin-636 [ArgTX-636, (**1**), argiopine] is an unsymmetrical polyamine amide isolated from the venom of certain orb-weaver spiders.[1] The unusual structure of ArgTX-636 (**1**) was solved and published independently by Grishin (1986),[2] and by Adams (1987)[3] and their co-workers. This spider toxin contains an unsymmetrical polyamine (5.3.3), regioselectively acylated on the primary amino functional group of the cadaverine (1,5-diaminopentane) moiety with 2,4-dihydroxyphenylacetyl-L-asparagine. The terminal amine of the triamine (3.3) moiety is acylated with L-arginine. This polyamine amide (3.3-Arg, sFTX-3.3)[4] carries up to four positive charges, at physiological pH. There is continuing interest in such polyamine amides as channel blockers for glutamic acid and/or nicotinic acetylcholine-gated cation channels, and certain voltage-sensitive calcium channels.[1,5-11] In this *Letter*, we present a synthesis of ArgTX-636 (**1**) based upon a practical, reductive alkylation strategy which allows the 3.3-triamine moiety to be incorporated intact.[12]

Argiotoxin-636 (1) synthesis: Protected, activated chromophore (**2**) was efficiently prepared by an Arndt-Eistert chain homologation strategy (*Scheme 1*). Thus, 2,4-dihydroxybenzoic acid (**3**) (Aldrich) was poly-*O*-benzylated (3.3 eq. BnBr, 3.3 eq. NaH, anhydrous DMF, 0 to 20°C, 16 h, 77%), and the resulting cream-coloured crystalline ester (**4**) (EtOAc-hexane) was saponified (aq. NaOH 1 M, dioxan, heated under reflux, 90 mins), cooled, and acidified (aq. HCl 2 M) to afford protected benzoic acid (**5**), as cream-coloured crystals (EtOAc-hexane, 89%). After conversion of acid (**5**) into the corresponding acid chloride (1.2 eq. oxalyl chloride, 1.1 eq. pyridine, PhMe, 0 to 20°C, 30 mins), diazoketone formation was effected with an ethereal (ethanol free) solution of diazomethane (10 eq., 0 to 20°C, 2 h) which gave diazoketone (**6**) as a yellow solid mp 98-99°C dec., in 81% from acid (**5**). Arndt-Eistert reaction (0.2 eq. PhCOOAg, anhydrous DMF, 20°C, 75 mins) gave the presumed ketene intermediate which was not isolated, but was reacted *in situ* with a large excess of *N*-hydroxysuccinimide (10 eq.) to afford, after silica gel chromatography, activated ester (**2**) as a white solid (80%) mp 145-146°C (lit.[13] mp 143-143.5°C).

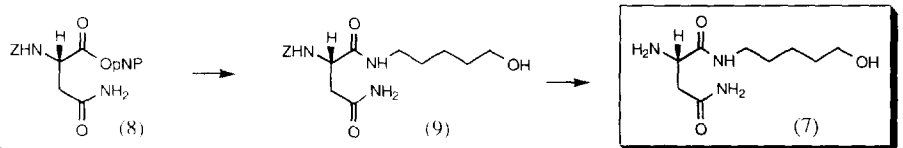
We designed the cadaverine moiety of ArgTX-636 (**1**) to be derived from 5-aminopentan-1-ol. This is incorporated, in this total synthesis, in Asn diamide (**7**) (see: *Scheme 2*). Thus, 5-aminopentan-1-ol was *N*-acylated with Z-L-Asn.OpNP (**8**) (NovaBiochem) (DCM, 20°C, 16 h) which gave primary alcohol (**9**) as a white solid (85%) mp 172-173°C. Hydrogenolysis (10% Pd/C, H_2 1 atm, MeOH, 15°C, 16 h) of Z-Asn derivative (**9**) gave aminoalcohol (**7**) as a white solid (100%) mp 107-109°C. Amino alcohol (**7**) was *N*-acylated with activated chromophore (**2**) (1.2 eq. Et_3N, DMF, 20°C, 16 h) which gave, after chromatography, primary alcohol (**10**) (*Scheme 3*) as a white solid (96%) mp 186-190°C. Key aldehyde (**11**), incorporating protected chromophore and L-Asn, together with the precursor of the 5-methylene unit, was prepared by careful Swern oxidation[14] of alcohol (**10**) (3 eq. DMSO, 2 eq. oxalyl chloride, DCM, -78°C, then (**10**) in DCM-DMSO 3:10 v/v, -78°C, quenched at -78°C with 5 eq. Et_3N, and then warmed to 20°C over 90 mins). After silica chromatography, aldehyde (**11**) was obtained as a white solid (63%) mp 168-170°C; none of the desired product (**11**) was obtained by a PCC[15] or pyridine-SO_3-DMSO[16-18] based oxidation of alcohol (**10**).

We designed the polyamine-Arg moiety of ArgTX-636 (**1**) to be incorporated by reductive alkylation of primary amine (**12**) which was prepared (*Scheme 4*) from triamine (**13**). Mono-BOC protection of *N*-(3-aminopropyl)-1,3-propanediamine (**13**), followed by acylation of the remaining free primary amine in (**14**) with L-Arg(Z_3) (NovaBiochem) gave the required polyamine-Arg moiety (**15**) (1.5 eq. DCC, 0.05 eq. HOBt, DCM, 20°C, 16 h, 88%) as a white solid, mp 111-112°C, after silica gel chromatography. We established that it was expeditious to protect the free secondary amino functional group at this point, as there were complications in the reductive alkylation reaction if this nucleophilic nitrogen was left unprotected. It is likely that a hexahydropyrimidine was formed, incorporating both nucleophilic nitrogen atoms which are separated by three methylenes, and tlc analysis showed that many products were formed on reductive alkylation of (**15**) after removal of the BOC protecting group. We elected to protect (**15**) by incorporating another Z group which will be removed concomitantly with the protecting groups on the chromophore and L-Arg residues. Therefore, the secondary amine (**15**) was reacted with Z-Cl under Schotten-Baumann conditions (1.5 eq. aq. NaOH 2 M, 20°C, 16 h), extraction (DCM) and silica gel chromatography afforded fully protected (**16**) as a white solid (96%). The essential primary amino functional group, in polyamine-Arg moiety (**12**), was then unmasked (TFA-DCM 1:1, 0°C, 30 mins, 95%).

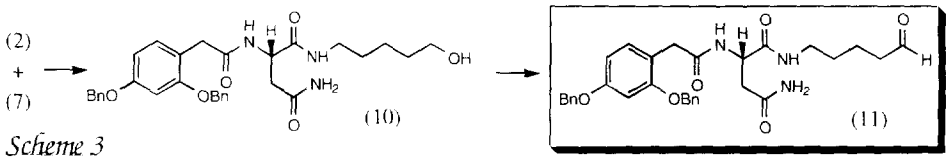
Polyamine-Arg moiety (**12**) was reductively alkylated with aldehyde (**11**) to afford protected ArgTX-636 (**17**) (see: *Scheme 5*). Thus, to a methanolic solution of aldehyde (**11**), primary amine (**12**) (1.2 eq. in MeOH) was added, followed by glacial HOAc (2 eq.) and $NaCNBH_3$ (1.5 eq.), and the colourless reaction mixture stirred at 20°C for 2 h, after this time the reaction was quenched with an excess of glacial HOAc. Preadsorbtion on silica gel and column chromatography (DCM-MeOH-conc. NH_4OH 300:10:1 to 200:10:1 v/v/v) gave the desired protected polyamine amide (**17**) as a white solid (48%). Efficient deprotection was accomplished by hydrogenolysis, but we found that the use of 10% Pd/C in MeOH required extended reaction times (96 h) and afforded three major products, in low yields. However, hydrogenolysis (H_2 1 atm, 15°C, 4 h) of polyamine amide (**17**), in the presence of Pearlman's catalyst ($Pd(OH)_2$/C, glacial HOAc), afforded spider toxin ArgTX-636 (**1**) as the corresponding polyacetate salt, a white foam (80%).



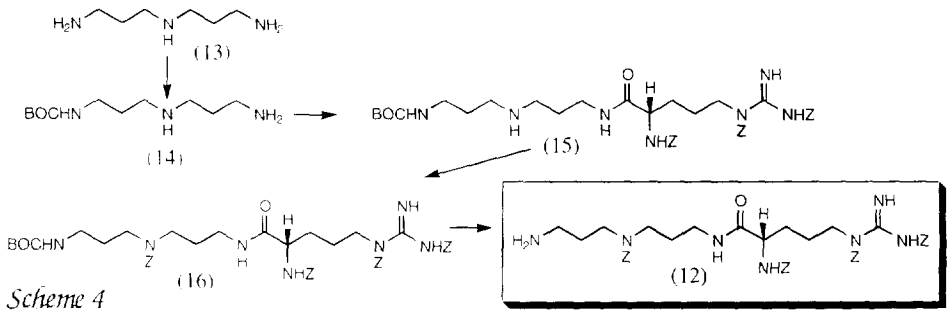
Scheme 1



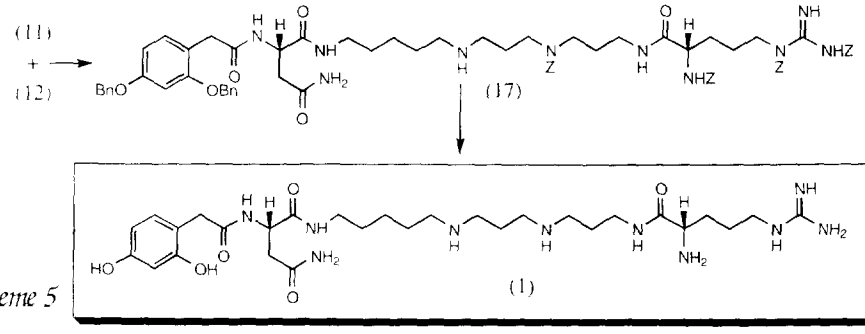
Scheme 2



Scheme 3



Scheme 4



Scheme 5

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