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Practical Synthesis of the Putative Polyamine Spider Toxin FTX: a Proposed Blocker of Voltage-Sensitive Calcium Channels

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Abstract: FTX (FTX-3.3), a putative polyamine toxin from American funnel-web spiders, has been synthesized from tri-CBZ-L-arginine. Raney nickel mediated desulfurization of a thioamide, generated selectively with Lawesson's reagent in the presence of the benzyloxycarbamates, allowed ready access to the *S*-enantiomer. The synthetic sample with the claimed structure for FTX-3.3 differs spectroscopically from the data published for the isolated toxin. The published structure requires a reappraisal of the spectroscopic data.

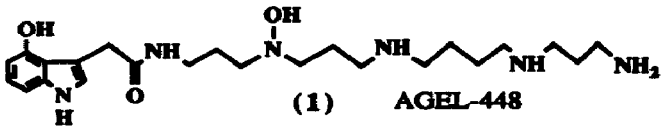
In recent years, there has been considerable interest in isolating and identifying the constituents of arthropod venoms [1, 2]. In particular, the non-proteinaceous polyamine and polyamine amide toxins from funnel- [3] and orb-web spiders [4], from parasitic solitary digger wasp *Philanthus triangulum* [5, 6], and their synthetic analogues [7] have aroused intense activity amongst biologists and chemists due to their potent and selective pharmacological activity at subclasses of excitatory amino acid receptors and certain voltage-sensitive calcium channels [8, 9]. Thus, these polyamine amide low molecular weight toxins and analogues of these arthropod natural products are lead compounds for excitatory amino acid receptor, non-competitive antagonists useful in the design of selective pharmacological tools, pharmaceutical and agrochemical agents [10, 11].

The low molecular weight toxins from the American funnel-web spider, *Agelenopsis aperta*, have been found [12] to contain, in a generalized sense, a hydroxyaromatic moiety bound *via* a one-to-three carbon atom spacer through an amide functional group to an unsymmetrical polyamine chain [1]. Typically, *A. aperta* toxins include Agel-448 (1), Agel-452 (2), and Agel-505a (3), differentiated as α-agatoxins from the peptide toxins (μ- and ω-agatoxins) according to their retention times on reverse phase HPLC [12, 13]. Recently, Llinás and co-workers isolated an atypical polyamine toxin from a preparation of this funnel-web spider toxin venom which they called FTX (4) [14, 15, 16] and which we have named FTX-3.3 in order to delineate the number of carbon atoms between the nitrogen atoms along the polyamine chain [17], consistent with the nomenclature for other synthetic polyamine amide toxins [5, 7]. In this *Letter*, we report the first synthesis of this unusual structure, but synthetic (4) does not display the reported ir and uv spectroscopic data [14, 15]. Therefore, our unambiguous synthesis of the published structure (4) requires a reappraisal of the spectroscopic data for the polyamine fraction obtained by hydrolysis of the crude venom [16] and necessitates that a different structure for the *A. aperta* toxin FTX is deduced. We have prepared (4) by a practical route which ensures that homogeneous material is available for pharmacological assessment.

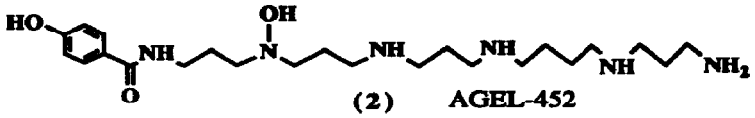
Initially, we decided to synthesize the novel natural product (4) by forming an amide bond between an appropriately protected symmetrical polyamine and an α-amino acid (L-ornithine or L-arginine). The amide carbonyl group would then need reduction to the required secondary amine. Such a convergent route from L-arginine could be biomimetic with respect to (4), as it is likely that the guanidine group is derived from arginine and the 1,3-diaminopropane moiety from L-methionine (as observed in spermine biosynthesis) or from the reduction of β-alanine. In order to remove all of the amino protecting groups concomitantly, we elected to use CBZ and hydrogenolysis. Thus, *N*, *N'*-di-CBZ-3,3'-iminobispropylamine (5) and tri-CBZ-L-arginine (9) were required. The dicarbamate (5) was prepared [9] from *N*-mono-BOC-3,3'-iminobispropylamine (6) by modification of the literature procedures [18, 19]. 3,3'-Iminobispropylamine (7) (3 equiv.) was mono-BOC protected with di-*tert*-butyldicarbonate (81%). CBZ protecting groups for the remaining primary and the secondary amines were added under typical Schotten-Baumann acylation conditions, in aqueous NaOH solution at 25°C, to yield the fully protected amine (8) (100%). Mono-BOC-di-CBZ (8) was treated with TFA (0°C, 1 h) and, after silica gel column chromatography under basic and polar conditions (eluant CH_2Cl_2/ MeOH/conc NH_4OH, 100:10:1), the desired protected polyamine (5) was obtained (86%) and was homogeneous by tlc.

The amide bond was formed between (5) and tri-CBZ-L-arginine (9) (CH_2Cl_2, DCC, HOBt) to afford poly-CBZ protected sFTX-3.3 (10) (83%). We attempted a hydride reduction of amide (10) to amine (15) under a variety of conditions, but we were unsuccessful. Although there are literature conditions for such a transformation using *e.g.* $LiAlH_4$ [20], $NaBH_4$ [21], and B_2H_6 [22] we found only loss of the guanidine group, formation of metal (aluminium/boron) complexes which were inert to subsequent reduction, or extensive polymerization and decomposition under more forcing conditions. In attempts to overcome this reduction of the guanidine functional group, we examined the reduction of selectively protected L-ornithine amide (11) which would require a subsequent guanadinylation of the γ-primary amine. The α-BOC-γ-CBZ-L-Orn-3.3(BOC) (11) was prepared, but, under similar hydride reducing conditions, no reduction of the amide carbonyl group was observed, only some BOC deprotection and metal complexation. Possibly, an α-carbamate (BOC or CBZ) may prevent facile hydride reduction of the amide. Therefore, we prepared α-FMOC-γ-BOC-L-Orn-3(CBZ).3(CBZ) (12) from (5) (CH_2Cl_2, DCC, HOBt) (97%). Prior to attempted reduction of the amide group, regioselective deprotection was achieved with 20% piperidine in DMF (25°C, 2 h) to yield amine (13) (97%). Hydride reduction of (13) also afforded only metal complexes with no detectable reduction of the amide carbonyl group.

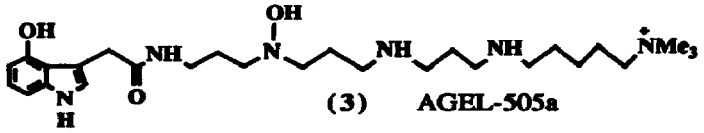
The amide functional group was reduced to the desired secondary amine by regiochemically selective conversion of (10) into the corresponding thioamide (14) (Lawesson's reagent, PhMe, 1.5 h, 80°C, 63%) [23] and desulfurization with Raney nickel (in a large excess), after washing with anhydrous diethyl ether (6 x 5 ml) and then stirring at 25°C for 1 h (25%) [24]. The penta-CBZ-FTX-3.3 (15) was deprotected by hydrogenolysis (H_2/10% Pd/C in MeOH, 25°C, 1 atm, 100%) to afford FTX-3.3 (4), the desired free amine which displayed satisfactory NMR spectra. Finally, (4) was lyophilized from aqueous HCl solution and the resultant pentahydrochloride salt was characterized. This gave satisfactory ^1H and ^{13}C NMR spectral data, FAB +ve ion 274, $C_{12}H_{31}N_7$ requires 273, and also appropriate, but significantly different ir ($\upsilon = 1665\ cm^{-1}$) and uv ($\lambda_{max} = 198$ nm, pH = 2; $\lambda_{max} = 200$ nm, pH = 7; $\lambda_{max} = 203$ nm, pH = 12) spectral data from those reported [15].



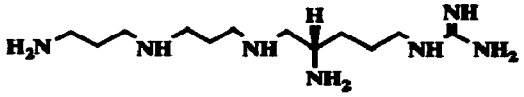
(1) AGEL-448



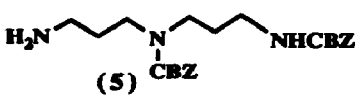
(2) AGEL-452



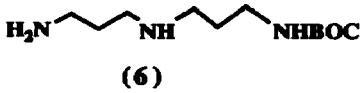
(3) AGEL-505a



(4) FTX-3.3 (FTX)



(5)



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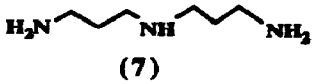
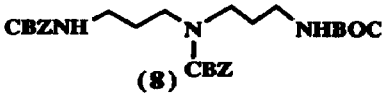


(7)



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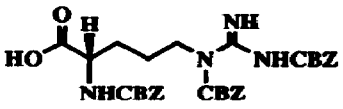
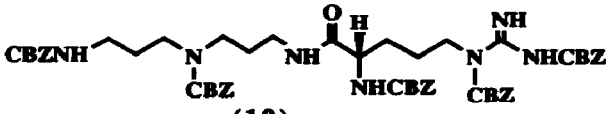


(9)

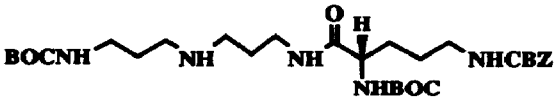
CBZ = $PhCH_2OCO$

BOC = Me_3COCO

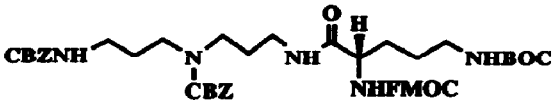
FMOC = 9-Fluorenyl-CH_2OCO



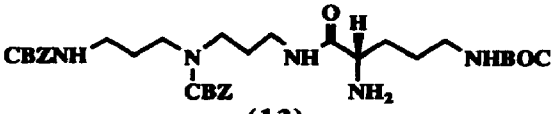
(10)



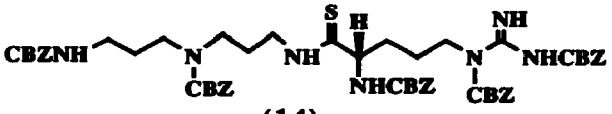
(11)



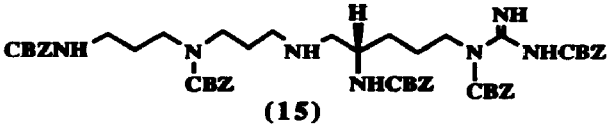
(12)



(13)



(14)



(15)

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