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Synthesis of Neurotoxic Nephila Spider Venoms: NSTX-3 and JSTX-3

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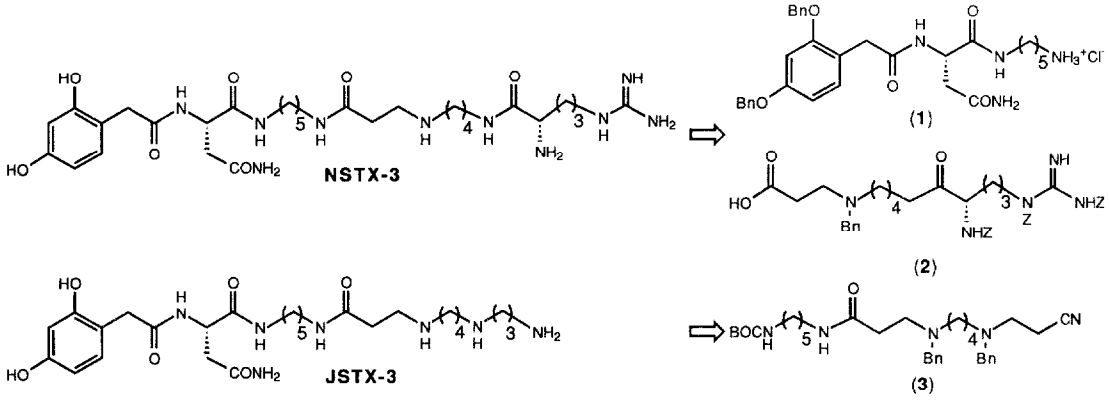
Abstract: Efficient and practical synthetic routes to the polyamine spider venom principles, NSTX-3 and JSTX-3 are described.

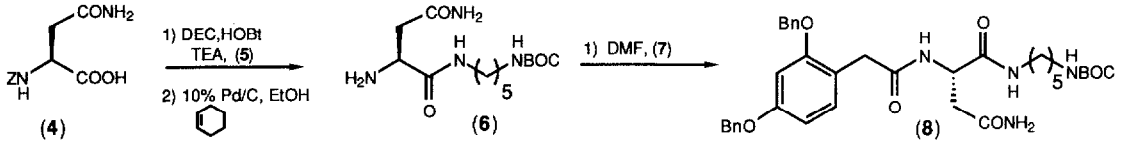
The interruption of glutaminergic neuromuscular transmission in invertebrates is a strategy which spiders have develpoed for predation. Kawai and coworkers have demonstrated that the insect neurotoxin, NSTX-3, which was isolated from the Papua New Guinean spider (Nephila maculata)[1] and a chemically related toxin, JSTX-3, isolated from the Joro spider (Nephila clavata)[1] irreversibly suppress the excitatory post synaptic potential (EPSP) and glutamate induced potential in certain invertebrate neuromuscular preparations.[2a-e] Other studies have begun to describe the effect of these and other spider toxins at the mammalian glutamate synapse.[2e-g,5] Chemical synthesis of these toxins have provided limited quantities for pharmacological evaluation.[3,4] Ongoing biological studies in several laboratories will hopefully clarify the molecular mode of action of these interesting materials.[5]

Both toxins possess a 2,4-dihydroxyphenylacetyl-L-asparaginyl-cadaverine-β-alanyl-putrescine substructure but differ in that NSTX terminates with an arginine residue and JSTX ends with a propylamine. In our synthetic approach to NSTX-3, (Scheme 1) the suitably masked left and right halves of the molecule, (**1**) and (**2**) are joined through the formation of β-alanine peptide bond. An alternate and equally satisfying strategy was adopted for JSTX-3 in which the polyamine-β-alanine portion of the toxin (**3**), constructed in an appropriately protected form was subsequently capped with asparagine and dihydroxyphenylacetic acid units.



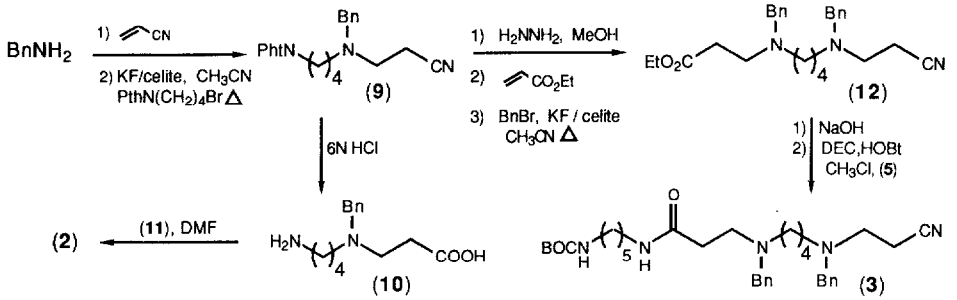
Scheme 1.

The condensation of carbobenzyloxy-L-asparagine (**4**) (Scheme 2) with mono-N-BOC-cadaverine (**5**)[6] followed by catalytic transfer hydrogenolysis of the N-CBZ group[7] provides (**6**) in 83% yield from (**4**).[16a] Coupling of the free amine (**6**) with hydroxysuccinimide-2,4-dibenzyloxyphenylacetate (**7**)[8] afforded (**8**) (81%), which was deprotected in dioxane/HCl and gave (**1**) as the hydrochloride salt.[9]



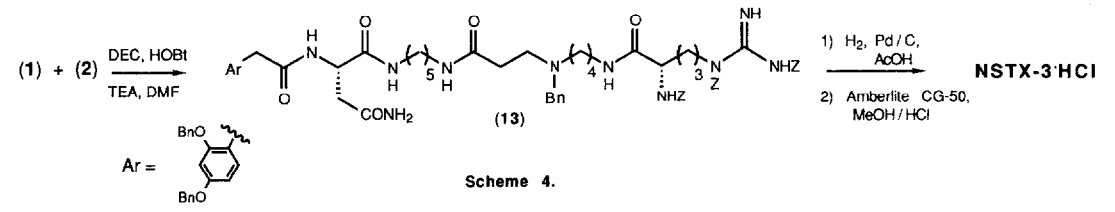
Scheme 2.

Benzylamine served as a starting point for the synthesis of (**2**) and (**3**)(Scheme 3). Therefore, selective mono-cyanoethylation[10] followed by alkylation of the resulting N-β-cyanoethylbenzylamine with N-(4-bromobutyl)phthalimide[11] furnished (**9**) in 85.8% yield. Hydrolysis of (**9**) in 6N HCl gives rise to amino acid (**10**)[12](64%) which was treated with N-α-N^G,$N^{G'}$-tri-CBZ-L-arginine-N-hydroxysuccinimide ester (**11**)[13] in DMF to yield acid (**2**)(83%). Cyanoethylamine (**9**) is also employed as an intermediate in the preparation of (**3**) in the route to JSTX. Deprotection of (**9**) with hydrazine in methanol (96%), selective mono-carboethoxyethylation[14] (99%) and alkylation[11] with benzyl bromide (81%) made available diaminocyanoester (**12**). Base hydrolysis (71%) and coupling of the resulting half acid nitrile with amine (**5**) affords polyamine substrate (**3**) in 84% yield.

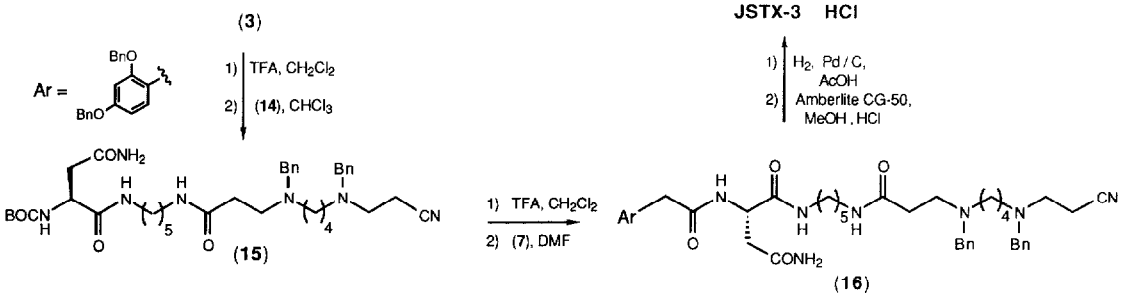


Scheme 3.

The synthesis of NSTX was completed (Scheme 4) by peptide bond formation between (**1**) and (**2**)(54.9%) which provided the completely protected NSTX substrate (**13**). Hydrogenolysis of the six protecting groups with Pearlman's catalyst[15] and subsequent purification on Amberlite CG-50(MeOH/HCl) provided NSTX-3 (53%) as the hydrochloride salt.[16,17a] Completion of the JSTX synthesis starting from (**3**)(Scheme 5) was accomplished in the following manner. The N-BOC group present in polyamine (**3**) was removed with TFA/CH_2Cl_2 and the resulting amine was treated with N-α-BOC-L-asparagine-p-nitrophenyl ester (**14**) which in turn gave rise to (**15**)(53.5% from (**3**)). Removal of the N-BOC group of the asparagine residue of (**15**) followed by treatment with N-hydroxysuccinimide ester (**7**) afforded the protected JSTX derivative (**16**)(67% from (**15**)). Hydrogenolysis of the four benzyl groups with concomitant reduction of the nitrile allowed for the formation of JSTX-3, which was purified on Amberlite CG-50(MeOH/HCl) and was isolated in 95% yield as its HCl salt.[16,17c]



Scheme 4.



Scheme 5.

In conclusion, practical syntheses of the polyamine spider toxins NSTX and JSTX are described. As spider toxins are now recognized as important tools for the study of excitatory amino acid neurochemical transmission and related pharmacology[2], efficient syntheses of these materials, related toxins[18] and analogues are needed to provide the generous supplies necessary for continued biological investigations.

Acknowledgements: We thank Dr. J. Stroh and Mr. K. Rosnack for MS data and Dr. E. Whipple and Ms. D. Rescek for ^1H and ^{13}C NMR spectra.

References and Notes

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8 The preparation of hydroxysuccinimide-2,4-dibenzyloxyphenylacetate is outlined in references 3 and 4.

9 The hydrochloride salt of (**1**) is generated as needed and used with no further purification. However, this HCl salt can be isolated in 71%.

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12 The purification of amino acid (**10**) was accomplished on Dowex-50 with 95% MeOH/5% aq NH_4OH as the eluent.

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16 For reasons of stability, the synthetic toxins are isolated as their HCl salts. The stoichiometry of HCl to toxin molecules is undetermined ; therefore, the exact yield of the final product is uncertain. The assumption is made that all the basic nitrogens exist as the corresponding HCl salts (3 for JSTX, 3 for NSTX) and the yields reported reflect this stoichiometry. Purity of the final toxins was ascertained by HPLC and ^{13}C NMR

17 (a) Intermediates were characterized by ^{1}H NMR and in most cases ^{13}C NMR and by FAB M.S..Yields are not optimized.

(b) Spectral Data NSTX-3-HCl: **^{1}H NMR**(300MHz, d_3-MeOD) ∂ 6.95(d,1H,J=8.1Hz), 6.38(d,1H,2.3Hz), 6.31 (dd,1H,J=8.1Hz,J=2.3Hz), 3.95(m,1H),3.56(d,1H,J=10.5Hz), 3.46(d1H,,J=10.5Hz),3.4-3.0(m,13H), 2.72(m,4H), 1.94(m,2H),1.81-1.60(m,6H),1.51(m,4H),1.33(m,2H). **^{13}C NMR**(75.43MHz,d_3-MeOD) ∂ 174.35, 172.21, 171.51, 70.00 (2 lines) ,159.07,158.62, 157.32,132.93, 114.21, 108.10, 103.92, 54.81, 54.16, 51.86, 45.06,41.81,40.91, 40.25, 39.67, 38.69, 37.82, 32.05, 29.81, 29.74, 29.69, 27.19, 26.66, 24.87, 24.46. **MS**(FAB): 665(M+1) ; **IR**(KBr): 3536,3451,2925,1635,1555, 591,564 cm^{-1}

(c) Spectral Data JSTX-3-HCl **^{1}H NMR**(250.13,d_6-DMSO) ∂ 9.8-9.1(bm,6H), 8.45(bs,3H), 8.25(bm,1H), 8.17(m,1H), 7.71(bm,1H),7.51(bs,1H),6.90(bs,1H), 6.82(d,1H,J=8.2Hz), 6.42(d,1H,J=2.2Hz), 6.15(dd,1H, J=8.2Hz, J=2.2Hz), 4.44(m,1H), 3.27(m,2H),3.16(s,2H),3.14-2.72(m,14H), 2.45(m,2H), 2.06(m,2H), 1.73(m,4H),1.38(m,4H),1.20(m,2H). **^{13}C NMR**(62.896MHz, d_6-DMSO) ∂ 171.66, 171.38, 170.61, 168.6, 157.10, 155.97, 130.80, 112.73, 106,20, 102.83, 50.04, 45.84, 43.70, 42.91, 40.48, 38.80, 38.42,37.29, 36.74,36.06 ,30.90,28.45,23.50, 23.45,23.37, 22.39; **MS**(FAB):565 (M+1). **IR**(KBr): 3448, 3428, 2973, 2853, 1656, 1549, 1520,1462,1306,1267, 1222,1174,1102,977, 599, 575 cm^{-1}.

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