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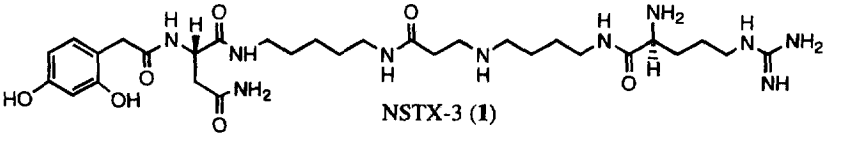
Practical, Convergent Total Synthesis of Polyamine Amide Spider Toxin NSTX-3

Ian S. Blagbrough,* Eduardo Moya, and Steven P. Walford

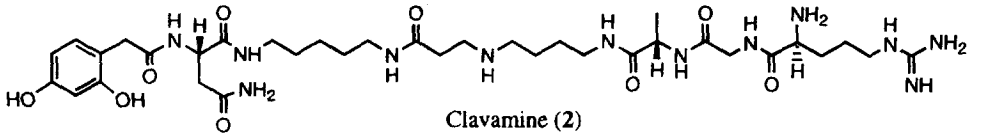
Department of Medicinal Chemistry, School of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY, U.K.

Abstract: A practical, total synthesis of polyamine amide spider toxin NSTX-3, a potent glutamate receptor antagonist with potential as a neuroprotective agent, is reported. The unsymmetrical polyamine moiety was built by a conjugate addition to afford putreanine and regioselective acylation of L-asparaginyl-cadaverine.

Nephila clavata is an orb-weaver spider which envenomates with essentially millimolar glutamate and a complex mixture of unsymmetrical polyamine amides toxins e.g. NSTX-3 (**1**) [1] and peptide based toxins, NPTXs.[2,3] The polyamine amide components are open-channel glutamate receptor blockers.[1] The unusual structure of NSTX-3 (**1**) was solved and published by Nakajima, Kawai, and their co-workers.[1,4] This spider toxin contains an unsymmetrical polyamine (5.β-Ala.4.Arg), regioselectively acylated on the primary amino functional group of the cadaverine (1,5-diaminopentane) moiety with 2,4-dihydroxyphenyl-acetyl-L-asparagine. The terminal amine of the putreanine (β-Ala.4) moiety is acylated with L-arginine. Polyamine amide β-Ala.4.Arg carries up to three positive charges, at physiological pH. Confirmation of the structure of this regioselectively diacylated unsymmetrical polyamine came with the total syntheses of NSTX-3 (**1**) [5,6] and the closely related tripeptide (Ala.Gly.Arg) containing spider toxin clavamine (**2**).[7-9] There is continuing interest in polyamine amides as channel blockers for glutamic acid and/or nicotinic acetylcholine-gated cation channels, and certain voltage-sensitive calcium channels[1,10-16] and as novel natural products containing polyamine amides.[17] In this *Letter*, we present a convergent total synthesis of NSTX-3 (**1**) [18] based upon a strategy which allows the putreanine moiety (β-Ala.4) to be incorporated first. This practical route ensures sufficient material is available for pharmacological evaluation.



NSTX-3 (**1**)



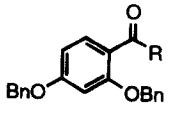
Clavamine (**2**)

NSTX-3 (1) synthesis: 2,4-Dibenzyloxyphenylacetic acid activated as its *N*-hydroxysuccinimide ester (**3**) was prepared by an Arndt-Eistert chain homologation strategy from 2,4-dibenzyloxybenzoic acid (**4**). After conversion of acid (**4**) into the corresponding acid chloride (**5**) (oxalyl chloride, 1.2 eq., pyr., 1.1 eq., PhMe, 0 to 20°C, 30 mins), reaction with an ethereal (EtOH free) solution of diazomethane (10 eq., 0 to 20°C, 2 h) gave diazoketone (**6**) as a yellow solid mp 98-99°C dec., in 81 % yield from acid (**4**). Arndt-Eistert reaction (anhydrous DMF, 20°C, PhCOOAg 0.2 eq., 75 mins) gave the desired activated ester (**3**) as a white solid (80 %) mp 145-146°C (lit. [5] mp 143-143.5°C), after silica gel chromatography, *via* trapping of the presumed ketene intermediate (**7**) *in situ* with *N*-hydroxysuccinimide (10 eq.).

The cadaverine moiety of NSTX-3 (**1**) was incorporated in (2,4-dibenzyloxy)phenylacetyl-L-Asn-mono-BOC cadaverine (**8**) which was designed for selective deprotection to afford free primary amine (**9**) for coupling with a putreanine containing polyamine amide moiety.[6] Thus, mono-BOC cadaverine was prepared by reacting cadaverine (1,5-diaminopentane) (3.0 eq.) with BOC_2O (1.0 eq., THF, 0°C, 16 h, 62 %).[19] *N*-BOC-1, 5-Diaminopentane was then acylated with Z-L-AsnOpNP (**10**) (1.1 eq., DCM, 20°C, 16 h) which efficiently gave orthogonally protected Asn-cadaverine (**11**) (69 %). Hydrogenolysis (H_2, 1 atm, 10 % Pd/C, MeOH, 15°C, 16 h) of Z-protected Asn (**11**) gave free amine (**12**) (86 %) which was then *N*-acylated with activated chromophore (**3**) (DCM, NEt_3 1.1 eq., 20°C, 16 h) affording BOC protected amine (**8**) (79 %). Free amine (**9**),[20] incorporating the required protected chromophore-L-Asn-cadaverine moiety, was obtained by brief treatment (1 h) of BOC protected amine (**8**) with TFA in DCM (1:1) at 0°C (79 % as the free base after silica gel chromatography DCM:MeOH:conc. NH_4OH 75:10:1 v/v/v).[19]

The β-Ala.4.Arg moiety of NSTX-3 (**1**) was designed to be incorporated by acylation of primary amine (**9**). Therefore, polyamine-Arg (**13**) was prepared from putrescine (1,4-diaminobutane) (**14**). Mono-Z protection of putrescine (**14**) to afford carbamate (**15**)[21] was not found to be a practical strategy, yields were typically <3% using Z-Cl in aq. NaOH/THF at 0°C where the di-Z protected diamine predominated.[21] A convenient way around this problem was *via* mono-BOC-mono-Z-putrescine. Mono-BOC protection of putrescine (**14**) (3.0 eq.) (BOC_2O 1.0 eq., THF, 0°C, 16 h) afforded carbamate (**16**) (76 %) which was then reacted with Z-Cl (1.1 eq.) under Schotten-Baumann conditions (1 M aq. NaOH, 1.1 eq., 0 to 20°C, 16 h) to give the required unsymmetrical dicarbamate (82 %) which was selectively deprotected with TFA in DCM (1:1) (0°C, 1 h) to yield mono-Z-putrescine (**15**)[21] (76 %). Amine (**15**) underwent 1,4-Michael addition with t-butyl acrylate (1.1 eq.) (MeOH, 20°C, 16 h) to afford the desired conjugate (**17**) (39 %). The protected terminal amine of conjugate (**17**) was hydrogenolysed (H_2, 1 atm, 10 % Pd/C, MeOH, 15°C, 16 h) to yield primary amine t-butyl ester (**18**) (96 %) which was acylated with Z_3ArgOH (1.1 eq.) (DCM, DCC 1.5 eq., HOBt 0.05 eq., 20°C, 16 h) to afford the desired amide (**19**) (75 %). The secondary amine functional group of the amide ester (**19**) was protected by carbamoylation with Z-Cl (1.1 eq.), in DCM, using NaOEt (1.1 eq. in EtOH) to yield the fully protected polyamine amide (**20**) (73 %) whose t-butyl ester was deprotected with TFA in DCM (1:1) (20°C, 16 h) to afford the desired protected β-Ala.4.Arg as the free acid (**13**) (91 %).

Putreanine moiety (**13**) was acylated with primary amine (**9**) (1.0 eq., DMF, 7 d, 20°C) after the acid had been activated as its pentafluorophenyl ester [free acid (**13**) activated with pentafluorophenol (1.1 eq.) (THF, DCC 1.5 eq., 16 h, 20°C)]. The activated ester was not isolated, but the solution was used directly in the next step, to afford protected NSTX-3 (**21**), in 65 % overall yield from primary amine (**9**).



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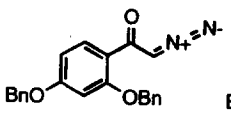
(5) R=Cl



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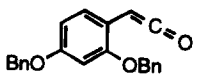

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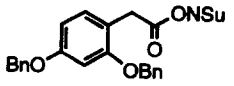

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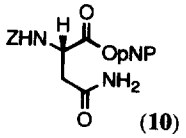

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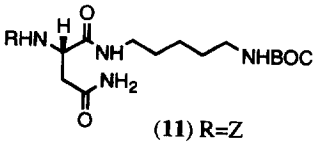
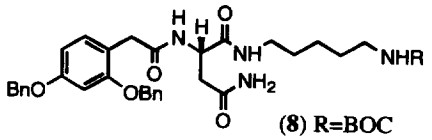
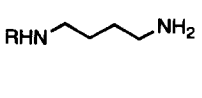


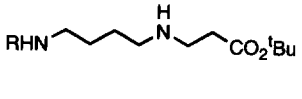
(11) R=Z
(12) R=H



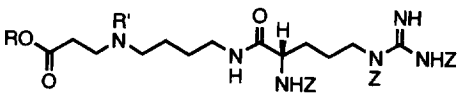
(8) R=BOC
(9) R=H



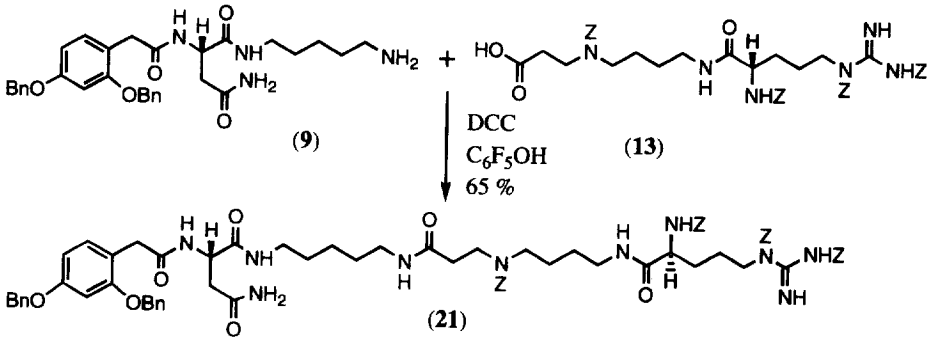
(14) R=H
(15) R=Z
(16) R=BOC



(17) R=Z
(18) R=H



(19) R=t-Bu; R'=H
(20) R=t-Bu; R'=Z
(13) R=H; R'=Z



NSTX-3 (1)

Efficient deprotection was accomplished by hydrogenolysis (H_2, 1 atm, 20°C, 4 h) of the polyamine amide (**21**), in the presence of Pearlman's catalyst, $Pd(OH)_2$ on carbon in glacial acetic acid, to afford spider toxin polyamine amide NSTX-3 (**1**) as the corresponding polyacetate salt. Crude NSTX-3 (**1**) was purified by RP-HPLC, linear gradient elution with A = H_2O, 0.1 % TFA; B = MeCN; 0 to 20 min, 95 to 85 % A; 20 to 25 min, 85 to 10 % A; 25 to 30 min, 10 % A; 30 to 35 min, 10 to 95 % A; λ = 280 nm, C8 column, 25 cm x 10 mm i.d., 4 ml/min, to afford NSTX-3 (**1**) polytrifluoroacetate salt, a cream coloured foam (98 % after RP-HPLC). FAB mass spectroscopy, in 3-nitrobenzyl alcohol matrix, displayed FAB +ve ion 665, FAB -ve ion 663, $C_{30}H_{52}N_{10}O_7$ requires M = 664; FAB -ve ion (M+1TFA) 777, $C_{32}H_{53}N_{10}F_3O_9$ requires M = 778.

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