Pergamon

Tetrahedron Letters, Vol. 36, No. 51, pp. 9397-9400, 1995
Elsevier Science Ltd
Printed in Great Britain
0040-4039/95 $9.50+0.00

0040-4039(95)01995-2

Total Synthesis of Modified JSTX Toxins: Reductive Alkylation is a Practical Route to Hexahydropyrimidine Polyamine Amides

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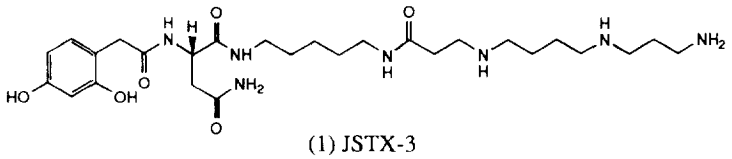
Abstract: Reductive alkylation is a practical route for a total synthesis of regioisomers of spider toxin JSTX-3, a polyamine amide which is a selective glutamate receptor antagonist and may have potential as a neuroprotective agent. The strategy is based upon a reductive alkylation step which enables one free amine to be generated regiospecifically in the new polyamine, or the regiospecific incorporation of a hexahydropyrimidine moiety which conformationally restricts the polyamine amide backbone.

Joro- and Papua New Guinean spider toxins, JSTX and NSTX, are unsymmetrical polyamine amides isolated from the venom of orb-weaver spiders *Nephila clavata* and *Nephila maculata* respectively.[1-4] The structure of JSTX-3 (**1**) was published in 1986 by Nakajima and co-workers.[4] This spider toxin contains an unsymmetrical polyamine, 5.β-Ala.4.3, regioselectively acylated on the primary amino functional group of the cadaverine (1,5-diaminopentane) moiety with 2,4-dihydroxyphenylacetyl-L-asparagine. There is continuing interest in such polyamine amides as channel blockers for glutamic acid and/or nicotinic acetylcholine-gated receptor cation channels, and certain voltage-sensitive calcium channels.[5-10] In this *Letter*, we present a practical synthesis of an iso-JSTX-3 analogue (**18**). There is no published synthesis of iso-JSTX-3, but there have been several recent synthetic studies reported on efficient routes to analogues of these chemically and biologically important spider toxins.[10-12] Comprehensive reviews have been published by Hesse[13] and by Meinwald[14] and their colleagues. We have also assessed the syntheses and neuropharmacological properties of arthropod polyamine amide toxins,[15] and related electrophysiological studies[16] as part of *The Neuropharmacology of Polyamines*.[17] The possibility of selectively photoaffinity labelling JSTX analogues is also reported. Regioselectively acylated polyamine amide (**12**) is a mimic for a diazirine photolabile analogue,[18-20] prepared in order to determine whether this modified spider toxin can still bind as an open-channel blocker of cation channels. This nitrogen atom of the cadaverine moiety was selected for acylation with a photolabile group as it is an amide in JSTX-3 (**1**), but an amine in a related spider toxin, argiotoxin-636.[21] Finally, the first example of a reductive alkylation of a hexahydropyrimidine is reported, building upon the excellent work of Ganem and colleagues.[18,22]



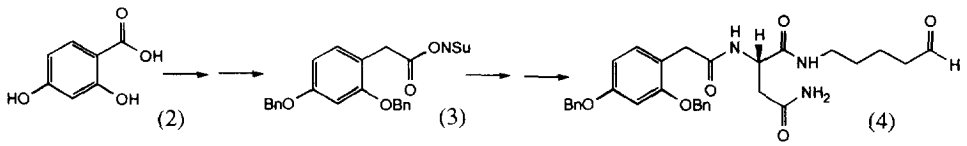
(1) JSTX-3

Deoxy-JSTX-3 (10): Protected chromophore-1,-Asn.5-aldehyde (**4**) was efficiently prepared by an Arndt-Eistert chain homologation strategy and careful Swern oxidation, outlined in the preceding *Letter*.[21] Thus, 2,4-dihydroxybenzoic acid (**2**) was converted into the corresponding ketene and trapped with a large excess of *N*-hydroxysuccinimide to afford activated ester (**3**). We designed the five-methylene moiety of iso-JSTX-3 to be derived from 5-aminopentan-1-ol and incorporated by a reductive amination strategy.

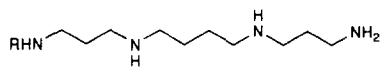
Protected symmetrical polyamine chain, triZ-3.4.3, was prepared in order to carry out our reductive amination to furnish protected deoxy-JSTX-3 analogue (**9**). Spermine (3.4.3) (**5**) was mono-BOC protected on one of the primary amines with $(BOC)_2O$ to afford monoBOC-3.4.3 (**6**). The remaining three amino functional groups were then Z-protected. Fully protected 3.4.3 (**7**) was then treated with TFA to afford tri-Z-protected 3.4.3 (**8**) with a primary amine unmasked (62% overall yield).[23] TriZ-3.4.3 (**8**) was reductively alkylated with aldehyde (**4**) (2 eq. glacial AcOH, 2 eq. $NaCNBH_3$, MeOH, 20°C, 3 h) to afford, after silica chromatography (DCM-MeOH-conc. NH_4OH 150:10:1 v/v/v) fully protected deoxy-JSTX-3 analogue (**9**) as a white solid (93%). This was then deprotected by hydrogenolysis (10% Pd/C, H_2 1 atm, MeOH, 15°C, 3 d) to afford target compound (**10**) as a colourless oil (free base, 92%) after filtering off the catalyst.

N-Acylated-deoxy-JSTX-3 (12): In our synthesis of protected deoxy-JSTX-3 (**9**), we designed the newly formed secondary amine, arising from the reductive alkylation step, to be the sole nucleophilic (unprotected) amine. This then allowed us to *N*-acylate regioselectively this cadaverine nitrogen atom. Thus, standard peptide coupling of protected deoxy-JSTX-3 (**9**) with 4,4,4-trifluorobutanoic acid (3 eq.) (3 eq. DCC, 0.5 eq. HOBt, DCM, 20°C, 16 h) afforded *N*-acylated trifluorobutanoyl derivative (**11**) after purification over silica gel (DCM-MeOH 98:2) as a white solid (53%). *N*-Acylated trifluorobutanoyl JSTX-3 analogue (**11**) was then deprotected by hydrogenolysis using Pearlman's catalyst ($Pd(OH)_2/C$, H_2 1 atm, glacial AcOH, 15°C, 4 h) to afford fully deprotected JSTX-3 analogue (**12**) as its free base (93%).

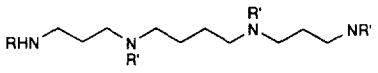
Iso-JSTX-3 (18): We have synthesized iso-JSTX-3 analogue (**18**) *via* a reductive alkylation route using aldehyde (**4**) and a suitably protected 3.4.β-Ala moiety (**15**). Orthogonal protection of spermidine (**13**) (3.4) (0.95 eq. 37% w/w aq. formaldehyde, H_2O, 20°C, 1 h) afforded hexahydropyrimidine protected triamine (**14**) as a colourless oil (94%) after purification by silica column chromatography (DCM-MeOH-conc. NH_4OH 20:4:1 to 10:4:1 v/v/v), following the efficient procedure of Ganem and co-workers.[22] This selectively protected spermidine was regioselectively *N*-acylated on the primary amine with Z-β-Ala (0.9 eq.) (1.5 eq. DCC, 0.05 eq. HOBt, DCM, -78° to 20°C, 16 h) to furnish the suitably protected 3.4.β-Ala unit (**15**) as a white solid after silica gel chromatography (DCM-MeOH-conc. NH_4OH 50:10:1) (58%). When this reaction was performed at higher temperatures (>20°C), the major product resulted from acylation of the secondary amine. The nucleophilicity of this cyclic secondary amine was now used to our advantage in the reductive amination of aldehyde (**4**) under essentially acid-free conditions (1 eq. $NaCNBH_3$, MeOH-DCM 1:1, 20°C, 2 h) to yield the conformationally constrained, hexahydropyrimidine protected iso-JSTX-3 analogue (**16**) (43%). Selective deprotection (3.6 eq. malonic acid, 3.1 eq. pyridine, refluxing MeOH, 2 h) afforded diamine (**17**) as a white solid (66%). Subsequent hydrogenolysis ($Pd(OH)_2/C$, H_2 1 atm, glacial AcOH, 15°C, 3.5 h) afforded the desired iso-JSTX-3 analogue (**18**) as the free amine quantitatively. Final purification of these analogues of polyamine amide spider toxins was by RP-HPLC over C-8 capped silica gel (gradient elution with AcCN-0.1% aq. TFA 5:95 to 25:75, UV detection at 280 nm).



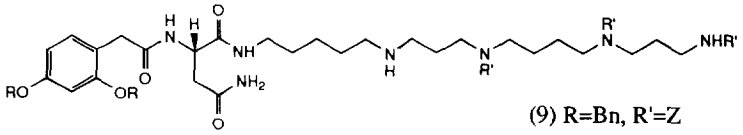
(2) (3) (4)



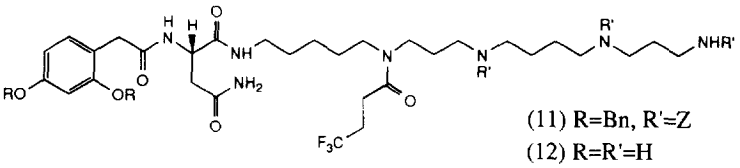
(5) R=H
(6) R=BOC



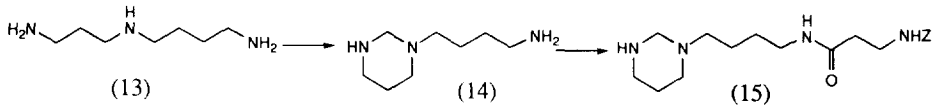
(7) R=BOC, R'=Z
(8) R=H, R'=Z



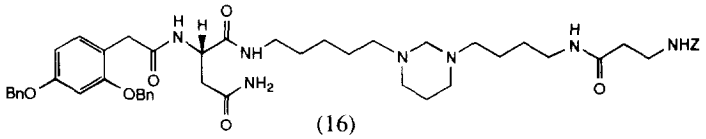
(9) R=Bn, R'=Z
(10) R=R'=H



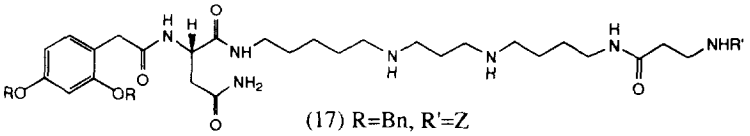
(11) R=Bn, R'=Z
(12) R=R'=H



(13) (14) (15)



(16)



(17) R=Bn, R'=Z
(18) R=R'=H

Acknowledgements: We acknowledge the financial support of MRC (G9219146N), BBSRC/AFRC (AG86/521), and The Wellcome Trust (Project Grant 039750). ISB has a Nuffield Foundation Science Lecturer's award (SCI/180/91/15/G).

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(*Received in UK* 14 *July* 1995; *accepted* 19 *October* 1995)