J. Pharm. Pharmacol. 1996, 48:179–182
Received April 21, 1995
Accepted April 28, 1995

Efficient Syntheses of Polyamine and Polyamine Amide Voltage-Sensitive Calcium Channel Blockers: FTX-3.3 and sFTX-3.3

EDUARDO MOYA AND IAN S. BLAGBROUGH

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

Abstract

Efficient syntheses of FTX-3.3 and sFTX-3.3, voltage-sensitive calcium channel blockers are described. These modified polyamines were prepared from selectively protected polyamines and purified on a practical scale.

There is continuing interest in isolating and identifying the constituents of arthropod venoms. In particular, the non-proteinaceous polyamine containing toxins from certain spiders and a solitary wasp *Philanthus triangulum* (Usherwood et al 1991; Blagbrough & Usherwood 1992) are of industrial and academic interest. These polyamines or polyamine amide toxins are important because of their potency, selectivity, and novel modes of action as potentiators and, at higher concentrations, as non-competitive antagonists of ligand-gated ion channels, use-dependent channel blockers (Usherwood et al 1991; Blagbrough & Usherwood 1992). These polyamine amides generally contain a hydroxy-aromatic group bound via a short spacer, often an amino acid residue, to an unsymmetrical polyamine chain terminating in a primary amino or guanidino (from the amino acid L-arginine) functional group (Usherwood et al 1991). These arthropod toxins and their many synthetic analogues have a paralysing effect on insects and can be made selective for ion channels which are important in cellular signalling phenomena (Usherwood et al 1991). Therefore, they have potential as lead compounds in both pharmaceutical (Blagbrough & Usherwood 1992) and pesticide research (Blagbrough et al 1992).

Argiotoxin-636 (ArgTX-636) (**1**), a low molecular weight spider toxin, has been isolated from *Argiope aurantia* (Adams et al 1987) and *A. lobata* (Grishin et al. 1986). This polyamine amide is accompanied, in these spider venoms, by the toxins ArgTX-659 (**2**) and ArgTX-673 (**3**) (Grishin et al 1986; Adams et al 1987; Usherwood et al 1991; Blagbrough & Usherwood 1992; Blagbrough et al 1992). These are open-channel (use-dependent) blockers of invertebrate (and vertebrate) glutamic acid gated ion channels; they are co-injected, by the spider, with up to 1 mM glutamic acid. Over fifty related polyamine amide toxins have now been isolated from a variety of spiders, characterized by spectroscopy and pharmacology, and many of them synthesized (McCormick & Meinwald 1993; Schäfer et al 1994).

Correspondence: I. S. Blagbrough, Department of Medicinal Chemistry, School of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY, UK.

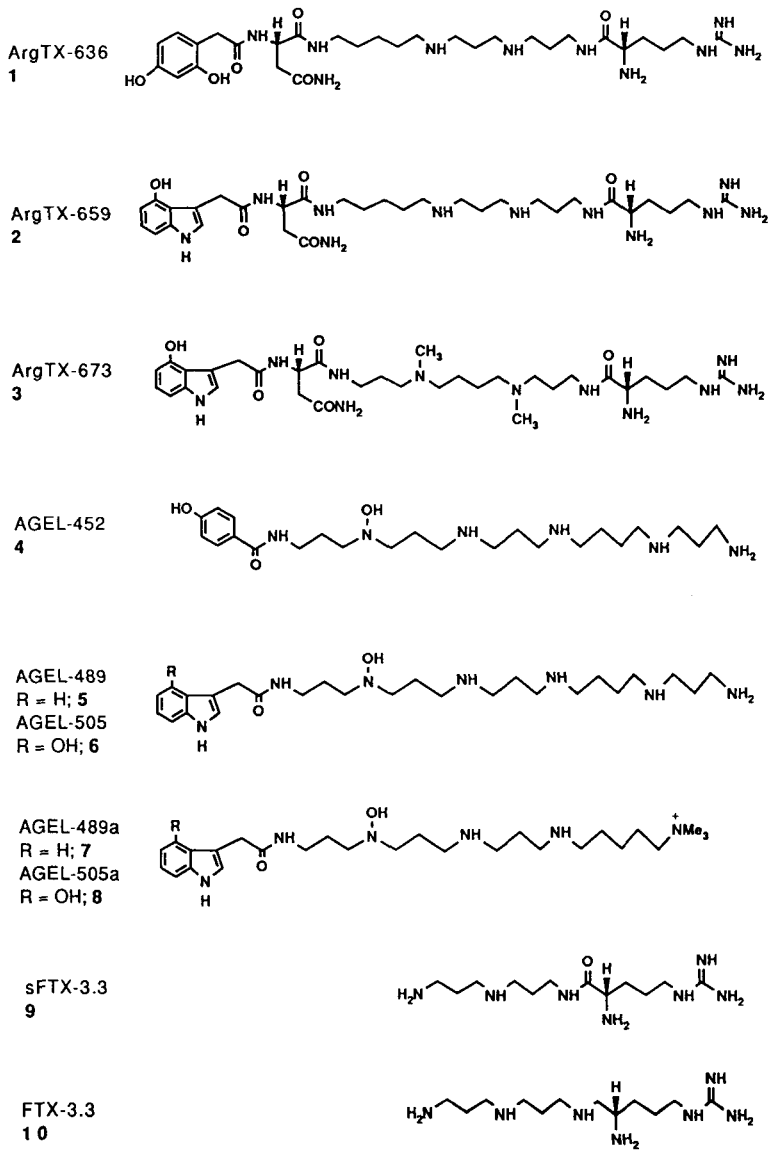
Some of the toxins from *Agelenopsis aperta*, the American funnel web spider, have been prepared (Jasys et al 1990, 1991). These polyamine amides, purified from *A. aperta*, include AGEL-452 (**4**), (containing a 4-hydroxybenzamide chromophore and a regioselectively *N*-hydroxylated 3.3.3.4.3 polyamine chain), AGEL-489 (**5**), (containing an indole-3-acetamide chromophore and a similarly *N*-hydroxylated 3.3.4.3 polyamine chain), AGEL-505 (**6**), (containing a 4-hydroxyindole-3-acetamide chromophore on this *N*-hydroxylated polyamine), AGEL-489a (**7**), (containing an indole-3-acetamide chromophore), and AGEL-505a (**8**), (containing a 4-hydroxyindole-3-acetamide chromophore). Both (**7**) and (**8**) contain an unusual unsymmetrical polyamine chain, *N*-hydroxylated 3.3.3.5, terminating with a quaternized trimethylammonium moiety.

We have recently reported preliminary details (Blagbrough et al 1994; Moya et al 1994a) of our practical synthesis of sFTX-3.3 (**9**), a polyamine amide, and an unambiguous route to the proposed structure for FTX-3.3 (**10**) (Cherksey et al 1991; Scott et al 1993) from *A. aperta*. The proposed FTX-3.3 (**10**) structure is remarkable, containing an arginine-derived peptide bond which has been reduced to the corresponding secondary amine. Efficient regiochemical control of unsymmetrical polyamines is a prerequisite for these studies (Moya et al 1994b). In the synthesis of 5.3.3-Arg, found in the spider toxins ArgTX-636 (**1**) and ArgTX-659 (**2**), we have prepared sFTX-3.3 (**9**), (Arg-3.3), an analogue of FTX (**10**), (FTX-3.3) (Blagbrough et al 1994; Moya et al 1994a) from *A. aperta* (Cherksey et al 1991; Scott et al 1993). The voltage-sensitive calcium channel blockers FTX-3.3 (**10**) and sFTX-3.3 (**9**) have been prepared from protected L-arginine.

Materials and Methods

General details

Thin layer chromatography was performed on pre-coated plates (Merck TLC aluminium sheets silica 60 F_{254}). The plates were visualized with either ninhydrin in *n*-butanol, or potassium permanganate in aqueous sodium hydroxide



Structure 1.

solution with heating. Column chromatography was performed as described by Still et al (1978) using Sorbsil C60-H flash chromatography silica gel (40–60 μm) purchased from Prolabo, Eccles, Manchester. Low resolution mass spectra were recorded on a VG Analytical 7070E with a VG 2000 data system. EI spectra were produced at 70 eV, CI was employed using *iso*-butane as the reagent gas, and +ve and −ve FAB was performed using 3-nitrobenzyl alcohol as the matrix. IR spectra were obtained using thin discs (KBr) on a Perkin-Elmer 782 infrared spectrophotometer and UV spectra were obtained in aqueous solution with a Perkin-Elmer Lambda 3 UV/Vis spectrophotometer. All chemicals and reagents were purchased from Aldrich or Novabiochem. Solvents were purchased from Fisons and were either HPLC grade or purified according to Perrin & Amarego (1988).

Synthesis of sFTX-3.3 (**9**)

Mono-BOC-[3-(*N*-aminopropyl)]-1,3-propanediamine (**11**) (mono-BOC-3.3) was prepared by treating [3-(*N*-aminopropyl)]-1,3-propanediamine (**12**) (3 equiv.) with di-*tert*-butyl dicarbonate (BOC anhydride), in anhydrous THF, at 0°C (1 h). The reaction mixture was then allowed to warm to 25°C over 16 h (81%). The homogeneous product was acylated with tri-CBZ-L-arginine (**13**) and deprotected (TFA and then Pd/C, 10%, H_2 1 atm, 25°C) to afford the desired product (100%). Other synthetic routes were used, including the coupling of [3-(*N*-aminopropyl)]-1,3-propanediamine (**12**) with tri-*Z*-L-arginine *N*-succinimide ester (dichloromethane (DCM), 25°C, 18 h, 48%) to afford partially protected sFTX-3.3. Subsequent hydrogenolysis (Pd/C, 10%, H_2 1 atm, 25°C, 18 h, 100%) yielded sFTX-3.3 (**9**) as the free base. An alternative, higher yielding route to the title compound, was to couple tri-*Z*-L-arginine (**13**) with di-*N*,*N*′-Z-[3-(*N*-aminopropyl)]-1,3-propanediamine (**14**) (prepared by terminal mono BOC protection of [3-(*N*-aminopropyl)]-1,3-propanediamine (**12**), Z protecting the remaining primary and secondary amines and finally removing the terminal BOC group with TFA, 0°C, 1 h, 86%)

under standard peptide coupling conditions (DCC (?), HOBt cat, DCM, 83%). The fully protected sFTX-3.3 (**15**) was hydrogenolysed (Pd/C, 10%, H_2 1 atm, 25°C, 18 h, 100%) to the desired sFTX-3.3 (**9**) in 58% overall yield.

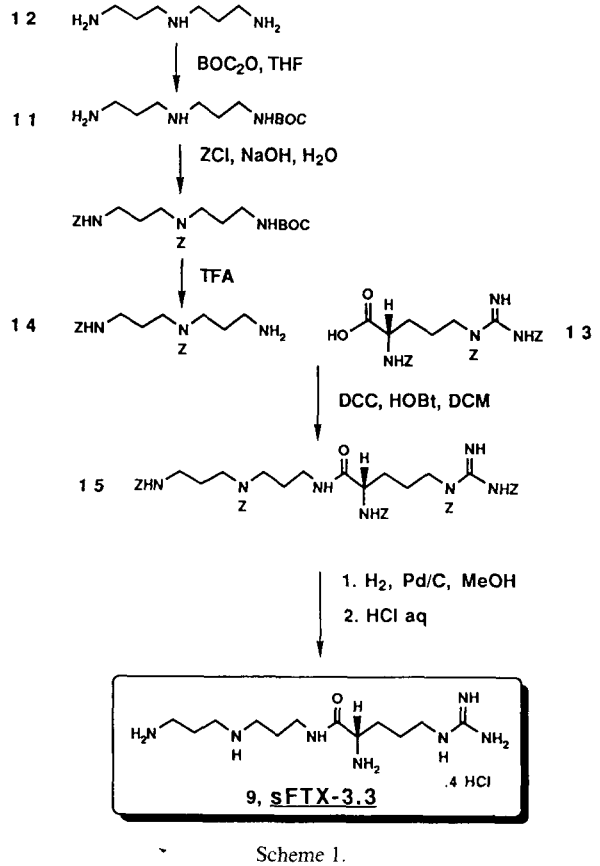
Synthesis of FTX-3.3 (**10**)

FTX-3.3 was synthesized by reacting poly-Z-protected 3.3-Arg (**15**) with Lawesson's reagent, in toluene at 80°C, (1·5 h) to yield thioamide (**16**) (63%). The thioamide was desulphurized with an excess of Raney nickel (Et_2O, 25°C, 1 h, 25%) and the product was hydrogenolysed to yield FTX-3.3 (**10**) as the free base (100%).

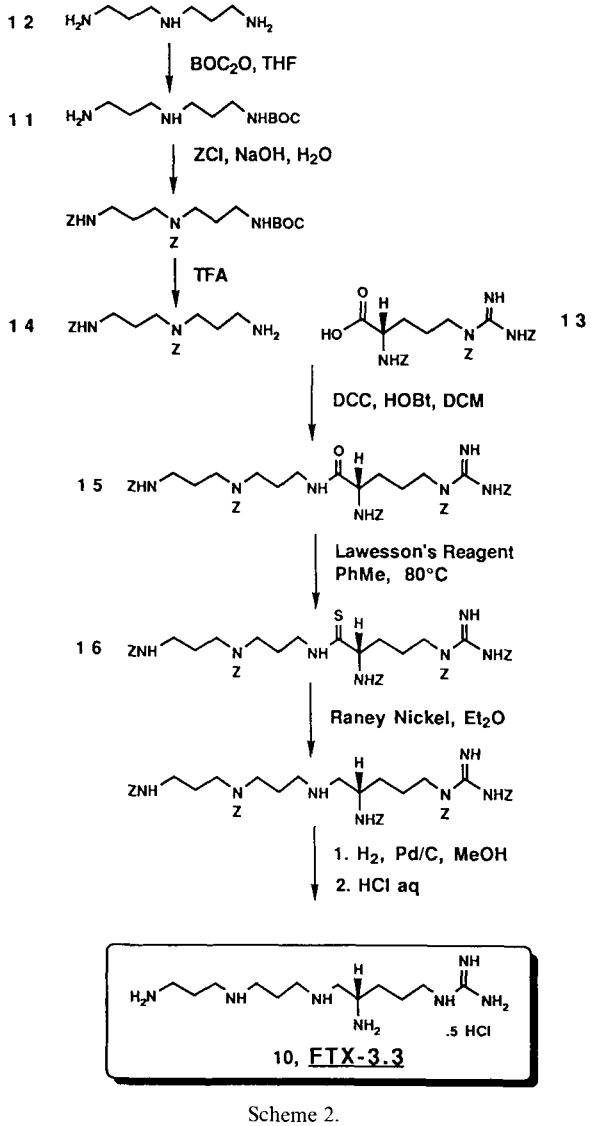
Results and Discussion

Our aims are regioselective control in the mono-acylation of unsymmetrical polyamines. This problem in practical synthetic organic chemistry can be effectively overcome by using the *tert*-butyloxycarbonyl (BOC) protecting group. Mono-BOC-[3-(*N*-aminopropyl)]-1,3-propanediamine (**11**) (mono-BOC-3.3) was efficiently prepared (81%) by treating [3-(*N*-aminopropyl)]-1,3-propanediamine (**12**) (3 equiv.) with di-*tert*-butyl dicarbonate, in anhydrous THF. The homogeneous product was acylated with tri-CBZ-L-arginine (**13**) and deprotected under standard peptide conditions (TFA and then Pd/C) to afford the desired product quantitatively. Several other routes were also used, the quickest being coupling [3-(*N*-aminopropyl)]-1,3-propanediamine (**12**) with tri-Z-L-arginine *N*-succinimide ester to afford partially protected sFTX-3.3. Subsequent hydrogenolysis, under standard conditions, quantitatively yielded sFTX-3.3 (**9**) as the free base. A higher yielding route, though with a greater number of steps, was to couple tri-Z-L-arginine (**13**) with di-*N*,*N*′-Z-[3-(*N*-aminopropyl)]-1,3-propanediamine (**14**) (prepared by terminally mono BOC protecting 3.3-triamine (**10**), Z protecting the remaining primary and secondary amines and finally removing the terminal BOC group with TFA at ice temperatures, in 86% yield) under standard peptide coupling conditions (DCC, HOBt cat, DCM, 83%). The fully protected sFTX-3.3 (**15**) was quantitatively hydrogenolysed (10% Pd/C, H_2 1 atm) to the desired sFTX-3.3 (**9**) in 58% overall yield. The most efficient route we have developed for the preparation of sFTX-3.3 (**9**) is depicted in Scheme 1.

Several attempted routes to FTX-3.3 (**10**) were considered, but were found to be impractical e.g. lithium aluminium hydride or diborane reduction of the poly-Z-protected amide. FTX-3.3 (**10**) was synthesized, on a practical scale, by treating poly-Z-protected 3.3-Arg (**15**) with Lawesson's reagent (PhMe, 80°C, 1·5 h) to yield thioamide (16) (63%)



Scheme 1.



Scheme 2.

regiochemically selectively in the presence of the carbamates. Thioamide (**16**) was desulphurized with excess Raney nickel (25%) and the product was hydrogenolysed to yield FTX-3.3 (**10**) quantitatively as the free base (see Scheme 2).

Typical organic purification techniques are generally unsuitable for these polar, freely aqueous soluble toxin analogues, unless they are *N*-protected. For an example of these problems, the necessity of a Z-protection step, entirely for the purpose of purifying a secondary amine, in a synthesis of squalamine, a steroidal-spermidine antibiotic from tissues of the dogfish shark *Squalus acanthias*, has recently been published by Moriarty et al (1994). These highly basic compounds also react with atmospheric carbonic acid to afford ammonium carbonate salts. However, these salts can be readily decomposed by treatment with aqueous dilute hydrochloric acid solution to yield the corresponding (poly)-hydrochloride salt of the polyamine. This hydrochloride salt formation also serves to remove any residual carbamic acid functional groups, resulting from incomplete decarboxylation during the hydrogenolysis of the Z-protecting group (evidenced by δ Å 156 ppm signals in the ^{13}C NMR spectra).

FTX-3.3 (**10**) displayed FAB +ve ion 274, $C_{12}H_{31}N_7$ requires 273; IR n = 3400 cm^{-1}, NH stretch; UV, lmax = 198 nm, pH = 2; lmax = 200 nm, pH = 7; lmax = 203 nm, pH 12. These spectroscopic data are not compatible with the results published by Cherksey et al (1991) which include IR n = 3450 cm and 1100 cm^{-1}; UV lmax = 208 nm, pH = 2; lmax = 225 nm, pH = 8; lmax = 230 nm, pH 12. Therefore, as the spectroscopic data do not correlate, the structure published by Cherksey and colleagues (1991) cannot be correct for the natural product.

Conclusions

In this paper, we have described the total syntheses of sFTX-3.3 (**9**) and FTX-3.3 (**10**). These polyamines and the analogous polyamine amides are now being examined in detailed electrophysiological studies (Usherwood et al 1994) in vertebrates and invertebrates. The synthetic medicinal chemistry detailed above highlights some of the difficulties (McCormick et al 1993) of working with polyamines and polyamine amides on a practical scale, in synthetic medicinal chemistry. Nevertheless, we have established a number of practical routes to sFTX-3.3 (**9**), an important ion channel modulator (Scott et al 1993). We have demonstrated that the published structure of FTX-3.3 (**10**) (Cherksey et al 1991) is incompatible with its spectroscopic properties, and therefore, this putative spider toxin remains uncharacterized chemically and spectroscopically. The possibility of obtaining FTX from *A. aperta* venom is intriguing, and we are currently examining the isolation of this natural product.

Acknowledgements

These studies were supported by the BBSRC (fellowship AG 86/521 to E. Moya). Ian S. Blagbrough is the recipient of a Nuffield Foundation science lecturer's award (SCI/180/91/15/G).

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