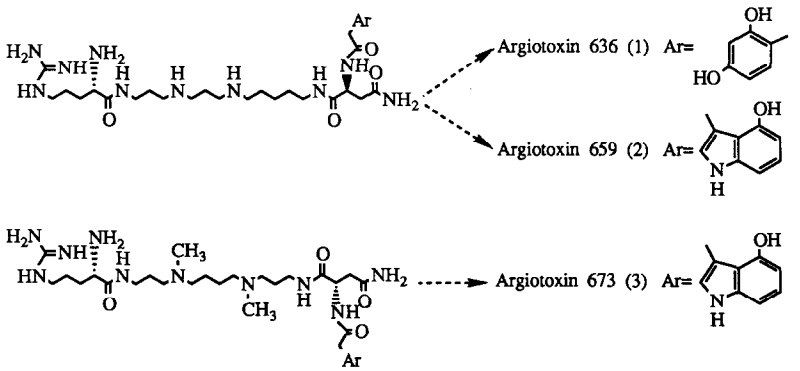
Tetrahedron Letters,Vol.29,No.48,pp 6223-6226,1988 0040-4039/88 $3.00 + .00
Printed in Great Britain Pergamon Press plc

THE TOTAL SYNTHESIS OF ARGIOTOXINS 636, 659 AND 673

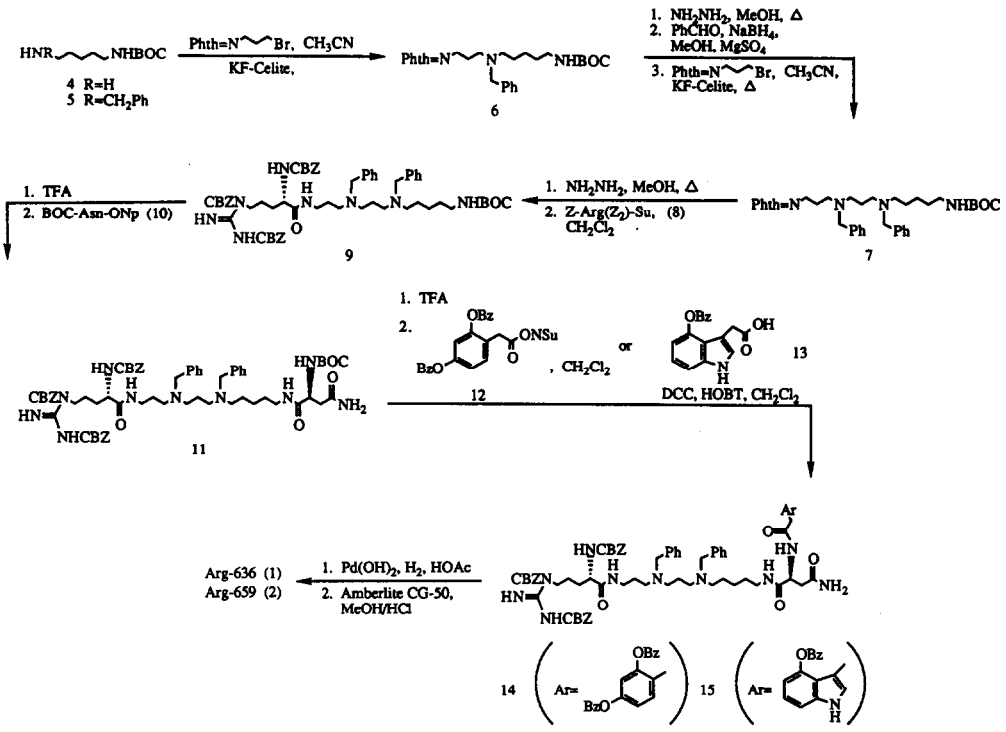
V. John Jasys, Paul R. Kelbaugh, Deane M. Nason, Douglas Phillips, Nicholas A. Saccomano*, Robert A. Volkmann*
Central Research, Pfizer Inc., Groton, CT 06340

Abstract: *Practical syntheses of three polyamine spider toxins isolated from the venom of Argiope aurantia are described.*

The structure and biological activity of three glutamate receptor antagonists, Arg 636(1), 659(2) and 673(3), isolated from the venom of *Argiope aurantia*, an orb weaving spider, have recently been reported.[1] The potential for using these basic low molecular weight "argiotoxins" and other structurally similar non-polypeptide venom constituents as pharmacological tools for studying glutamate receptors has stimulated synthetic interest in the polyamine toxins.[2] Recent reports on the synthesis of Arg 636(1)[1d, 2a] and 659(2)[1d] have prompted us to describe our general strategy, based on methodology developed by Samejima[3] *et al.*, which we have utilized for the preparation of Arg 636(1), 659(2) and 673(3) and other venom constituents.[4]

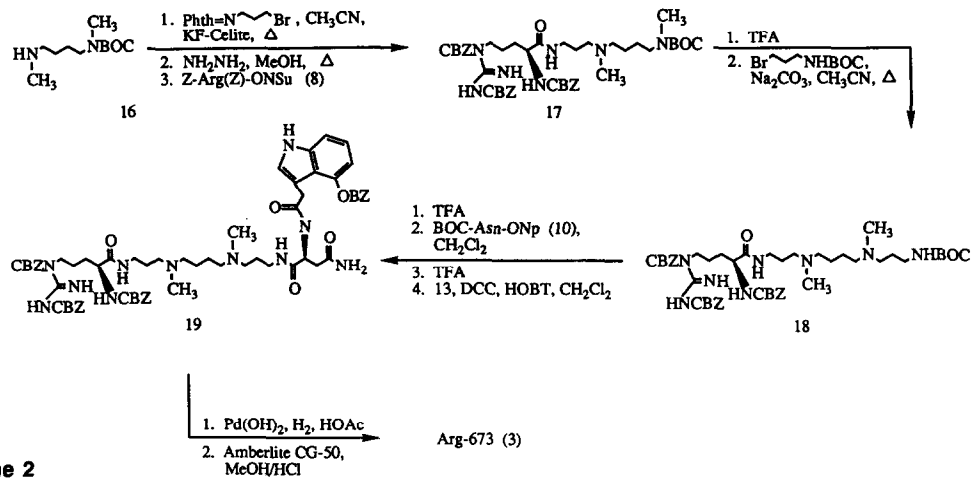


The argiotoxins contain an internal polyamine chain flanked on one side by a N-terminal arginine and on the other by a hydroxyarylacetamide containing terminal asparagine. Both amino acids are linked to the polyamine by amide bonds. With the array of basic sites in the polar argiotoxins, synthetic strategy is largely dictated by the appropriate selection of amine protecting groups. In the case of Arg 636(1) and 659(2), protection of the internal secondary amines with benzyl groups was employed in order that the more labile and versatile BOC or phthalimide groups could be utilized for elaborating either argiotoxin terminus. Neutral potassium fluoride/celite promoted amine alkylations, originally described by Ando and Yamawaki[5] and later utilized by Samejima,[3] *et al.*, were selected for the construction of the polyamine chains since this methodology appeared best suited for analog synthesis.



Scheme 1

Reductive amination ($NaBH_4$/PhCHO/$MgSO_4$/MeOH) of N-BOC-1,5-diaminopentane 4[2b, 6] (Scheme I) cleanly provided benzylamine 5[7] (99%). Alkylation of amine 5 (KF-celite/CH_3CN/82°) with N-(3-bromopropyl)phthalimide generated amine 6[7] (79%). Removal of the phthalimide moiety (NH_2NH_2/MeOH/50°) followed by repetition of the same reductive amination/alkylation protocol smoothly generated dibenzylamine 7[7] (72%). Phthalimide deprotection followed by coupling of the resultant amine (CH_2Cl_2) with N-α-N^G,N^G-Tri-CBZ-L-arginine-N-hydroxysuccinimide ester 8 provided 9[7] (88%) which contains the fully protected N-terminal arginine. The asparagine terminus was also easily accessed by BOC removal (TFA) and treatment of the crude amine with N-BOC-L-asparagine-p-nitrophenyl ester 10 (TEA/CH_2Cl_2) to give 11[7] (79%). The fully protected Arg 636 intermediate 14 was assembled by BOC removal followed by amine coupling (CH_2Cl_2) with hydroxysuccinimide ester 12[2b] (89%). Coupling with indole-acetic acid 13[8] (1.5 equiv. HOBt/1 equiv. DCC/CH_2Cl_2) provided penultimate Arg 659 intermediate 15[7] (71%). Catalytic hydrogenolysis[9] using Pearlman's catalyst ($Pd(OH)_2$/HOAc/2 hr) cleanly removed *every* protecting group in 14 to generate 1 as its acetate salt (>95% crude yield) which was then purified and isolated (Amberlite CG-50/MeOH/HCl) as its HCl salt.[7,10,11] In addition to generating Arg 659 (2), which was isolated as its HCl salt[11] (95%), deprotection of 15 using the aforementioned conditions generated overreduced[12] indole 2 (Ar = ) which along with Arg 659 could be purified as its HCl salt[11] (<5%) by Sephadex (LH-20) chromatography.



Scheme 2

The methodology was easily adapted to the synthesis of Arg 673 (Scheme 2). N,N'-dimethyl-1,4-butane diamine was converted (BOC_2O/dioxane) to monoprotected amine 16[7] (48%). Amine 16 was subjected to the standard N-(3-bromopropyl)phthalimide alkylation (58%) and phthalimide deprotection (87%) conditions, followed by treatment with hydroxysuccinimide ester 8 to generate polyamine 17[7] (85%) containing the arginine terminus. BOC removal (100%) followed by alkylation (Na_2CO_3/DMF) with N-BOC-3-bromo propylamine[13] yielded polyamine 18[7] (65%) which was converted to penultimate Arg 673 intermediate 19[7] via the following four step sequence: (1) BOC removal, (2) amine coupling with p-nitrophenyl ester 10 (68% for two steps), (3) BOC removal, and (4) amine condensation (1.5 equiv. HOBt, DCC, CH_2Cl_2) with indoleacetic acid 13 (82%). Catalytic hydrogenolysis ($Pd(OH)_2$/HOAc/2 hr) smoothly generated Arg 673 (3) which was isolated as its HCl salt (88%).[10] Spectral data[11] (^1H NMR, ^{13}C NMR, FAB M.S.) for 1, 2 and 3 were consistent with the proposed Arg 636, 659 and 673 structures. Biological profile of these and other venom constituents will be reported.

Acknowledgement. We express our gratitude to Ms. D. M. Rescek and Dr. E. B. Whipple for NMR assistance and to Dr. J. S. Stroh and Mr. K. J.Rosnack for FAB M.S. support.

References and Notes

1. (a) P. N. R. Usherwood, I.R. Duce, A. Dell, and G. W. Taylor 1986 European Patent #0208523A2, (b) E. V. Grishin, T. M. Volkova, A. S. Arsen'ev, O. S.Reshetova, V. V. Onoprienko, L. G. Magazanik, S. M.Antorov, and I. M. Fedorova, *Bioorgan. Khim.* 1986, **12**: No. 8, 1121-1124, (c) Y. Aramaki, T. Yasuhara, T. Higashijima, M. Yoshioka, A. Miwa, N. Kawai, and T. Nakajima, *Proc. Japan Acad.* 1986, Ser. B, **62**, 359-362, (d) M. E. Adams, R. L. Carney, F. E. Enderlin, E. T. Fu, M. A. Jarema, J. P. Li, C. A . Miller, D. A.Schooley, M. J. Shapiro, and V. J. Venema, *Bioch. Biophys. Res. Comm.* 1987, **148**, 678-683, (e) T. Budd, P. Clinton, A. Dell, I. R. Duce, S. J. Johnson, D. L. J. Quicke, G. W. Taylor, P. N. R. Usherwood and G. Usoh, *Brain Research* 1988, **448**, 30-39.

2. (a) T. L. Shih, J. Ruiz-Sanchez and H. Mrozik, *Tetrahedron Lett.* 1987, **28**, 6015-6018, (b) Syntheses of related structures NSTX-3 and JSTX-3 have recently been reported: T. Teshima, T. Wakamiya, Y. Aramaki, T. Nakajima, N. Kawai and T. Shiba, *Ibid.* 1987, **28**, 3509-3510; Y. Hashimoto, Y. Endo, K. Shudo, Y. Aramaki, N. Kawai, and T. Nakajima, *Ibid.* 1987, **28**, 3511-3514.

3. K. Samejima, Y. Takeda, M. Kawase, M. Okada and Y. Kyogoku, *Chem. Pharm. Bull.* 1984, **32**, 3428-3435.

4. V. J. Jasys, P. R. Kelbaugh, D. M. Nason, D. Phillips, N. A. Saccomano and R. A. Volkmann, manuscript in press.

5. T. Ando, J. Yamawaki, *Chemistry Letters,* 1979, 45-46.

6. (a) Treatment of excess 1,5-diaminopentane with $(BOC)_2O$/dioxane provided 4. See R. P. Robinson, Ph.D. Dissertation, Yale University, 1984. (b) R. Houssin, J.-L. Bernier and J.-P. Henichart, *Synthesis,* 1988, **3**, 259-261.

7. Intermediates were purified by silica gel chromatography and were fully characterized by ^1H NMR. Structural integrity was confirmed in most cases by ^{13}C NMR and FAB M.S. Yields have not been optimized.

8. G. Poon, Y.-C. Chui and F. C. P. Law, *J. Labelled Compd. Radiopharm.* 1986, **23**, 167-174.

9. (a) K. Yoshida, S. Nakajima, T. Wakamatsu, Y. Ban and M. Shibasaki, *Heterocycles* 1988, **27**, 1167-1168, (b) R. J. Bergeron, K. A. McGovern, M. A. Channing and P. S. Burton, *J. Org. Chem.* 1980, **45**, 1589-1592.

10. The argiotoxins were not stable as their free base and were therefore handled as acid salts. Not having established the number of HCl salts present in each synthetic toxin, we cannot provide an accurate yield. We believe the conversion in each case is high and have assumed in the reported yields the presence of five HCl salts for 1, 2 and 3. In our hands, for example, 1.15 g of 14 provided .638 g (95%) of 1 as its HCl salt. These salts were >95% pure and could be further purified by HPLC (VYDAC C-18; 330 ÅPore; 22 x 250 MM, mobile phase: A = 0.1% TFA in H_2O; B = CH_3CN; Gradient 0-60% B over 1 hour. With the same gradient conditions on an analytical VYDAC C-18 column, retention times for argiotoxins 1, 2 and 3 were 22.1, 25.0 and 23.4 min (1 ml/min).

11. Physical data for the HCl salts of:

1 ^{13}C NMR (125.76 MHz, DMSO d_6): δ 171.73, 171.43, 170.78, 168.54, 157.18, 157.14, 156.03, 130.99, 112.86, 106.21, 102.78, 51.65, 50.09, 46.67, 44.68, 44.02, 43.87, 39.98, 38.29, 37.24, 36.82, 35.93, 28.31, 28.06, 25.56, 24.91, 24.24, 23.19, 22.11. ^1H NMR (500 MHz, DMSO, d_6) δ 1.24 (m), 1.34 (m), 1.51 (m), 1.61 (m), 1.77 (m), 1.84 (m), 2.09 (m), 2.44 (d, J = 6Hz), 2.51 (s), 2.77 (m), 2.91 (m), 3.13-3.39 (m), 3.81 (m), 4.45 (m), 6.17 (dd, J = 8, 2 Hz), 6.40 (d, J = 2 Hz), 6.81 (d, J = 8 Hz), 6.90 (bs), 7.21-7.67 (m), 8.04 (bs), 8.16 (d, J = 8 Hz), 8.90-9.40 (m), 9.70 (bs). MS (FAB) 637 ($M+H^+$). For the TFA salt $[\alpha]_D$ = +10.1°, c = 0.29, MeOH.

2 ^{13}C NMR: δ 172.60, 171.50, 170.50, 168.50, 157.08, 151.05, 138.52, 122.47, 122.12, 117.02, 107.31, 104.06, 103.40, 51.69, 50.12, 46.66, 44.69, 44.01, 43.86, 39.97, 38.27, 37.20, 35.95, 33.79, 28.23, 28.07, 25.60, 24.92, 24.23, 23.13, 22.19. ^1H NMR: δ 1.18 (m), 1.30 (m), 1.52 (m), 1.57 (m), 1.77 (m), 1.85 (m), 2.07 (m), 2.44 (d, J = 6 Hz), 2.51 (s), 2.74 (m), 2.98 (m), 3.20 (m), 3.67 (m), 3.81 (m), 4.48 (m), 6.37 (d, J = 7 Hz), 6.81-6.88 (m), 7.00 (bs); 7.45 (m), 7.70 (bs), 8.00 (bs), 8.3 9-8.44 (m), 9.02-9.27 (m), 10.15 (s), 10.89 (s). MS (FAB) 660 ($M+H^+$).

3 ^{13}C NMR: δ 172.66 , 171.58, 171.06, 168.51*, 157.11, 151.07, 138.54, 122.63, 122.11, 116.99, 107.31,* 103.97, 103.44, 54.08, 53.97, 52.77, 52.44, 51.67, 50.29,* 39.96, 39.11, 39.01, 36.91, 35.98, 35.67, 33.77, 28.05, 24.24, 23.39, 20.53. ^1H NMR: δ 1.51-1.9 6 (m), 2.50 (m), 2.58 (m), 2.71 (bs), 2.75-3.25 (m), 3.71 (m), 3.82 (m), 4.45 (m), 6.41 (d, J = 7 Hz), 6.84-6.91 (m), 7.02 (bs), 7.51 (bs), 7.92 (m), 8.04 (m), 8. 45 (m), 9.06 (m), 10.20 (bs), 10.42 (bs), 10.67 (bs), 10.91 (s). MS (FAB) 674 ($M+H^+$).
*indicates doubling in the ^{13}C NMR.

12. The amount of overreduced product can be minimized by decreasing reaction time.

13. Problems with the removal of the arginine CBZ protecting groups in the KF-celite alkylation in addition to the hydrazine mediated phthalimide deprotection were obviated by using Na_2CO_3/N-BOC-3-bromopropylamine to extend the polyamine chain.

(Received in USA 11 August 1988)