Tetrahedron Letters,Vol.28,No.48,pp 6015-6018,1987 0040-4039/87 $3.00 + .00
Printed in Great Britain Pergamon Journals Ltd.

The Total Synthesis of Argiopine (Argiotoxin-636)

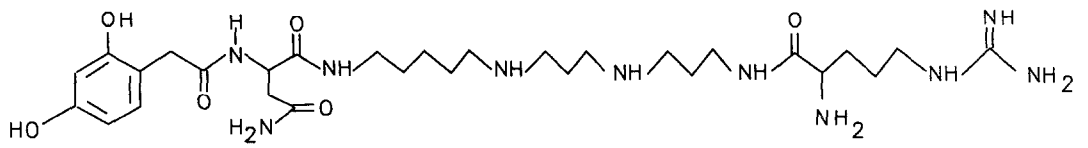
Thomas L. Shih, Jose Ruiz-Sanchez, Helmut Mrozik

Merck Sharp & Dohme Research Laboratories, Rahway NJ 07065

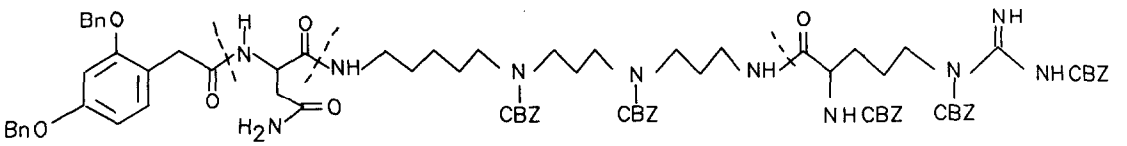
Abstract: The total synthesis of a constituent of spider venom with reported glutamate receptor channel activity is described.

Recent reports[1] of the isolation of low molecular weight non-polypeptide components from the venom of spiders of the genera Argiope, Araneus, and Nephila with amino-acid receptor activity provided the impetus to synthesize argiopine (argiotoxin-636) 1. This toxin has been assigned the structure[1b] shown in Scheme 1. It contains an unusual arrangement of two amino-acids linked *via* a polyamine and terminating as a dihydroxyphenylacetamide. Since a definitive X-ray structure was not available, and since the amino-acid stereochemistry does not appear to have been defined, we set out to synthesize the proposed structure, incorporating the L-forms of asparagine and arginine. As will be evident, the strategy employed permits the preparation of any stereoisomer of 1 for SAR correlations. These studies are now underway.

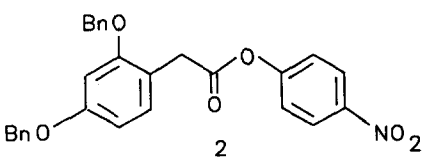
SCHEME 1



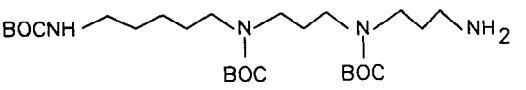
1 Argiopine



7



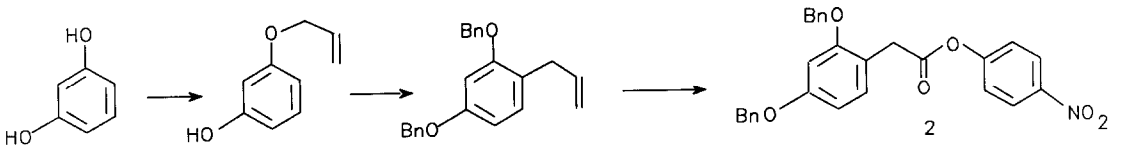
2



Our approach to 1, a structure characterized by an impressive array of highly polar functional groups (free phenolic hydroxyl, amine, and guanidine residues), makes use chiefly of the standard carbobenzyloxy (CBZ) and t-butyloxycarbonyl (BOC) peptide protecting groups. The key penultimate intermediate, 7 (Scheme 1), was the fully benzyl-protected toxin which could be deblocked in one step to provide the desired product. While the necessary amino-acids are commercially available, the aromatic acid and the polyamine moieties are not. The aromatic portion is an isomer of homogentisic acid. While its synthesis has been reported[2] we chose a different route for its preparation. We obtained it in multi-gram quantity following Scheme 2. Thus, resorcinol was allylated (allyl bromide, K_2CO_3, acetone, 50%)[3] and Claisen rearrangement of the product (170°C, 3 h, 45%), followed by benzylation (benzyl bromide, DMF, NaH, 90%) provided the olefin precursor which was degraded (OsO_4, $NaIO_4$, Jones oxidation, 30% overall)[4] to the aromatic acid[2b,5]. Activation as the *p*-nitrophenyl ester (NaH, DMF, CH_2Cl_2, oxalyl chloride, *p*-nitrophenol, 90%) enables the formation of the final amide bond.

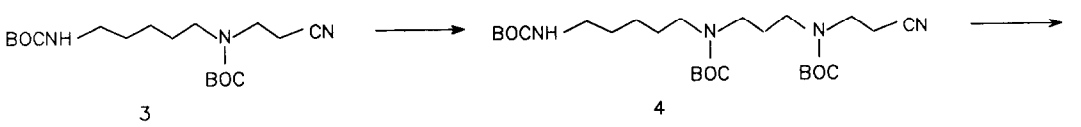
The preparation of the polyamine portion of argiopine was modeled after a published approach to spermine analogs[6] (Scheme 3). Cadaverine was monoprotected with 2-(tert-butoxy-carbonyloximino)-2-phenylacetonitrile (BOC-ON) and treated with acrylonitrile, followed by BOC protection of the product in one pot to afford nitrile 3 (30%)[5]. Subsequent hydrogenation (40 psi, 20°C) in ammonia-saturated ethanol with sponge RaNi followed by Michael addition to acrylonitrile and BOC protection yields nitrile 4 (85% overall without isolation of intermediates). Treatment of 4 under previously described hydrogenation conditions and addition of tri-CBZ-arginine *p*-nitrophenyl ester in THF afforded intermediate 5 in 95% yield[5]. Removal of the three BOC groups was readily accomplished in trifluoroacetic acid (TFA) at 20°C for 1 h[7]. The resulting amine salt was treated *in situ* with excess triethylamine (TEA) and N-BOC-asparagine *p*-nitrophenyl ester (THF, 20°C, 5h) followed by addition of CBZ-chloride (3 eq., 0°C-20°C, 16 h) to yield 6 (74%)[8]. The reaction appears to be regiospecific, as no other isomer was detected by TLC analysis. Proof of this structure was accomplished by independent synthesis *via* Scheme 4 of 6 from the asparagine end. The success of this route (15% overall yield from N-BOC-asparagine *p*-nitrophenyl ester) depended on the anticipated resistance of the CBZ group towards hydrogenolytic cleavage during the reduction of the nitrile function with sponge Raney nickel. The products (6) from the two schemes were indistinguishable when compared by C13 and proton NMR (400 MHz) spectroscopy, TLC, specific rotation, mass spectrometry, and infrared spectroscopy. Treatment of 6 with TFA (20°C, 1 h) followed by TEA and 2 eq. of active ester 2 in THF provided the fully protected toxin[5] 7 in 80% yield. Final removal of all the benzyl functions was best achieved in liquid HF (-5°C, 2-3 h) containing *circa* 10% (v/v) anisole (85%)[9]. The toxin was purified by HPLC utilizing a Whatman partisil 5 ODS-3 RAC column eluting with 97:3:0.05 (v:v:v) water:acetonitrile:TFA (retention time=6-8 min at 1.5 mL/min). The purified product has a proton NMR spectrum which conforms to structure 1 and is in agreement with that reported for argiopine[1a]. Other measured physical properties are: IR($CHCl_3$) 3100(broad), 1713, 1694, 1682, 1670, 1660, 1651, 1645, 1634, 1555, 1502, 1200, 1130, 1010, 977, 836, 796, 719 cm^{-1}; $[a]_D$= +8.7°, c= 0.27, methanol; M S (FAB) 637 ($M+H^+$)[10]. The biological evaluation of the synthetic argiopine is underway and will be reported elsewhere.

SCHEME 2

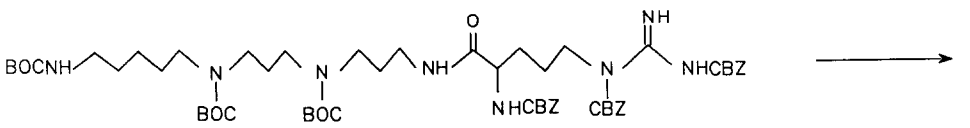


2

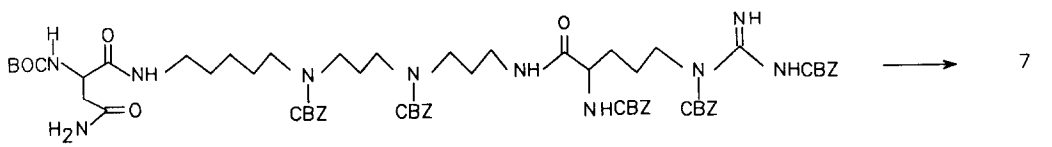
SCHEME 3



3 4

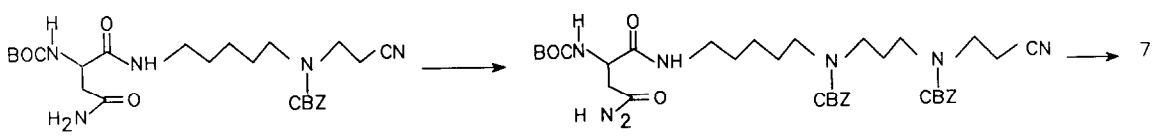


5



6 7

SCHEME 4



7

References and Notes

1. (a). P.N.R. Usherwood, I.R. Duce, A. Dell, and G.W. Taylor 1986 European Patent #0208523A2, NMR trace in CD_3OD given; (b). E.V. Grishin, T.M.Volkova, A.S. Arsen'ev, O.S. Reshetova, V.V. Onoprienko, I.G. Magazanik, S.M. Antorov, and I.M. Fedorova, Bioorgan. Khim. 12: No.8, 1121-1124 (1986); (c). Y. Aramaki, T. Yasuhara, T. Higashijima, M. Yoshioka, A. Miwa, N. Kawai, and T. Nakajima, Proc. Japan Acad. 62 Ser. B 359-362 (1986). (d) 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, Tetrahedron Lett. 28, 3509-3510 (1987); Y. Hashimoto, Y. Endo, K. Shudo, Y. Aramaki, N. Kawai, and T. Nakajima, Ibid., 3511-3514 (1987).

2. (a). F. Wessely and W. Metlesics, Monatsh. 85, 637-653 (1954); T. Kametani and S. Kano, Yakugaku Zasshi 85, 256-261 (1965). (b). The desired acid is a solid: m.p. 137-137.5°C; IR ($CHCl_3$) 3025, 1714, 1614, 1589, 1508, 1215, 1167, 1023 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, TMS) d 3.64(s,2H), 5.03(s,4H), 6.55(dd,1H,J=2,8Hz), 6.61(d,1H,J=2Hz), 7.12(d,1H,J=8Hz), 7.36(m,10H), 13.5(br s,1H).

3. M.M. Ponpipom, B.Z. Yue, R.L. Bugianesi, D.R. Brooker, M.N. Chang, and T.Y. Shen, Tetrahedron Lett. 27, 309-312 (1986).

4. R. Pappo, D.S. Allen, Jr., R.U. Lemieux, and W.S. Johnson, J. Org. Chem. 21, 478-479 (1956).

5. All intermediate compounds were fully characterized by ^1H NMR, IR, and MS. Selected NMR: 3 (300 MHz, $CDCl_3$) d 1.2-1.38(m), 1.44(s), 1.47(s), 1.40-1.60(m), 2.60(m), 3.11(br q,J=6Hz), 3.25(t,J=7Hz), 3.45(t,J=7Hz), 4.53(br s); 4 (300 MHz, $CDCl_3$) d 1.30(m), 1.32-1.56(m), 1.60-1.80(m), 1.75(t,J=7Hz), 2.60(m), 3.00-3.20(m), 3.25(m,J=7Hz), 3.45(t,J=7Hz), 4.55(br s); 7 (300 MHz, $CDCl_3$) d 1.0-1.90(m), 2.24(br dd), 2.70(br dd), 2.75-3.35(m), 3.57(s), 3.65-4.30(m), 4.60(br s), 4.9-5.17(m), 5.20(s), 5.50(br m), 5.80-6.20(m), 6.55(dd,J=2,8Hz), 6.64(d,J=2Hz), 6.80(br s), 7.13(d,J=8Hz), 7.20-7.45(m), 9.25-9.50(m).

6. M. Israel, J.S. Rosenfield, and E.J. Modest, J. Med. Chem. 7, 710-716 (1964).

7. T.W. Greene, "Protective Groups in Organic Synthesis", Wiley-Interscience, U.S.A., 1981, p. 232.

8. Physical data of 6: IR($CHCl_3$) 3400, 3020, 2750, 1725, 1715, 1694, 1683, 1670, 1655, 1612, 1512, 1504, 1252, 1217, 1097, 750, 697, 665 cm^{-1}; ^1H NMR (400 MHz, 55°C probe temp., $CDCl_3$, TMS) d 0.86(br m), 1.24(m), 1.36(s), 1.45(s), 1.50(m), 1.60-1.85(m), 2.50(dd,J=6,15Hz), 2.81(dd,J=4,15Hz), 3.00(br m), 3.15(m), 3.41(s), 3.89(m), 3.99(m), 4.15(br s), 4.38(br t), 5.0-5.15(m), 5.22(s), 6.00(m), 6.89(m), 7.22-7.40(m), 9.28(m); $[a]_D$= +11°, c=1.16, chloroform; MS (FAB) 1257($M+H^+$).

9. The authors wish to thank Mr. David A. Boulton for his assistance in running the HF reaction. A.R. Mitchell and R.B. Merrifield, J. Org. Chem. 41, 2015-2019 (1976).

10. The authors wish to thank Dr. Byron Arison and Mr. Herman Flynn for 400 MHz NMR spectra of the synthetic argiopine and Dr. Lawrence Colwell and Mr. Jack Smith for MS determinations.

(Received in USA 14 August 1987)