Tetrahedron Vol. 49, No. 26, pp. 5777-5790, 1993
Printed in Great Britain

0040-4020/93 $6.00+.00

Synthesis and Assay of Hybrid Analogs of Argiotoxin-636 and Philanthotoxin-433: Glutamate Receptor Antagonists

Seok-Ki Choi, Koji Nakanishi*

Department of Chemistry, Columbia University, New York, NY 10027, USA

Peter N. R. Usherwood

Department of Life Science, University of Nottingham, Nottingham NG7 2RD, UK

(*Received in USA* 19 *April* 1993)

Key words: *Argiotoxin-636; Philanthotoxin-433; Glutamate receptor antagonists; Structure-activity relationship; Selective antagonism; Photoaffinity label; Polyamine-amide toxins*

Abstract: *The synthesis of the non-competitive glutamate receptor antagonist, argiotoxin-636 is described. Furthermore, synthetic routes are presented for the preparation of structural analogs including argiotoxin-philanthotoxin hybrids. Biological activities on glutamate receptors are discussed.*

INTRODUCTION

Glutamate receptors(Glu-R) comprise a class of excitatory amino acid receptors involved in signal transduction in the central nervous systems (CNS) of vertebrates and some invertebrate animals.[1] Glu-R have been the focus of intense research because of their participation in the process of learning, long-term potentiation (memory) and their possible involvement in neuronal pathologies such as Alzheimer disease, Huntington's chorea, ischemia, and epilepsy.[2] The Glu-R is classified into two classes, the G-protein coupled metarbotropic class and the ionotropic class. The latter ionotropic Glu-R is further divided into two subtypes based on their responses to exogenous ligands: (i) N-methyl-D-aspartate receptor (NMDA-R) and (ii) kainate (KAIN)/quisqualate (QUIS)/α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptor (KAIN or non-NMDA-R).[1] The ionotropic Glu-R are multi-subunit protein complexes with external, glutamate binding site(s) and an internal ion channel. Nicotinic acetylcholine receptors (nACh-R) are also widely distributed. For example, nACh-R are the major ion channels of the excitable membrane of the mammalian skeletal neuromuscular junction.[3] Here, it is a membrane-bound, 270 kilodalton glycoprotein with two agonist binding sites and an internal ion channel. Recently, several polyamine amides have been reported as potent inhibitors of ionotropic Glu-R as well as nACh-R in both vertebrates and invertebrates.[4]

Philanthotoxin-433 (numerals denote the number of methylene groups in the polyamine) has been identified as the most active component of the venom of the solitary digger wasp, *Philanthus triangulum F.*[5] and its structure has been determined as **1a** by both spectroscopy and synthesis (Figure 1).[6] Argiotoxin-636 (636 indicates the molecular weight) is one of a number of the polyamine-amides isolated from the venom of the spider, *Argiope* (**2** in Figure 1). It strongly antagonizes Glu-R.[7] Electrophysiological and biochemical studies suggest that these polyamine toxins are non-competitive antagonists, blocking the ion channels of Glu-R and nACh-R allosterically or directly at submicromolar concentrations.[8]

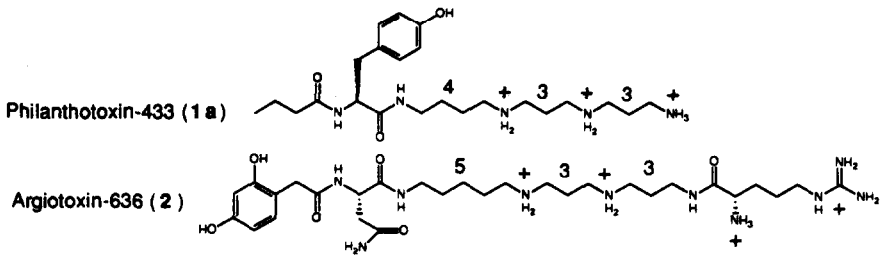


Figure 1. Structures and Philanthotoxin-433 and Argiotoxin-636

Although the genes encoding several Glu-R subtypes have been cloned and sequenced,[9] isolation of receptor proteins is still rare due to the lack of abundant natural sources and selective ligands. Since polyamine-amides such as philanthotoxin (PhTX) and argiotoxin (ArgTX) have high binding affinities for Glu-R, coupled with affinity chromatography, they could be used to isolate natural Glu-R from a relatively pure source of the receptor. Moreover, since the elongated structures can be modified at various sites,[10] they provide unique probes for tertiary structural studies of nACh-R and Glu-R ion channels via usage of photolabile analogs. For example, preliminary photoaffinity labeling studies have shown that PhTX analogs block the nACh-R gate by having the aromatic moiety in the cytoplasmic side and inserting the polyamine chain into the channel.[11]

We recently reported that ArgTX-636 is about 65 times more potent than PhTX-343 (**1b** in Figure 2) as an antagonist of the NMDA subtype of mammalian Glu-R (IC_{50} = 0.04 μM for ArgTX-636), whereas ArgTX-636 and PhTX-343 have similar potencies on the KAIN subtype of mammalian Glu-R (IC_{50}s; 0.07μM and 0.12μM respectively).[12] These results indicate that the structural variations such as the sequence and length of polyamine sequences, and the non-polyamine structures are major factors involved in the selective bindings to these two highly homologous Glu-R subtypes. This finding led us to prepare further analogs of ArgTX-636, structural hybrids of PhTX/ArgTX and several photoaffinity labeled analogs. The syntheses of these analogs are presented together with preliminary assays results on the quisqualate-sensitive (non-NMDA subtype) Glu-R; comparative studies of assays on different Glu-R subtypes and photoaffinity labeling studies are subjects of ongoing and future studies.

SYNTHETIC ANALOGS OF ArgTX-636 AND PhTX-343

In Figure 2 are shown structures of analogs derived from ArgTX-636 and/or PhTX-343; most ArgTXs are characterized by the presence of an asparagine moiety. In **3** to **5**, the 2,4-dihydroxyphenylacetyl group of ArgTX-636 is replaced with 2,4-dimethoxyphenylacetyl, 3,4-dimethoxycinnamoyl, or p-azidophenylacetyl (photoaffinity labeled), respectively. In the ArgTX-PhTX hybrid analogs, the 2,4-dihydroxyphenylacetyl group from ArgTX-636 was replaced with the non-polyamine portion of PhTX-343. Analog **6** is half PhTX-343 and half ArgTX-636; it is composed of butyryl-L-tyrosyl (from PhTX-343) and L-asparagine-533-L-arginine (from ArgTX-636). In **7** to **8**, a long aliphatic chain (C10) or trans-diene is used instead of a butyryl group, since previous structure-activity

relationship studies of PhTX-343 analogs showed highly enhanced activities with C10 and dienoyl analogs.[10, 13] Compounds **5**, **7** and **8** have the photosensitive azide group, which upon irradiation has been

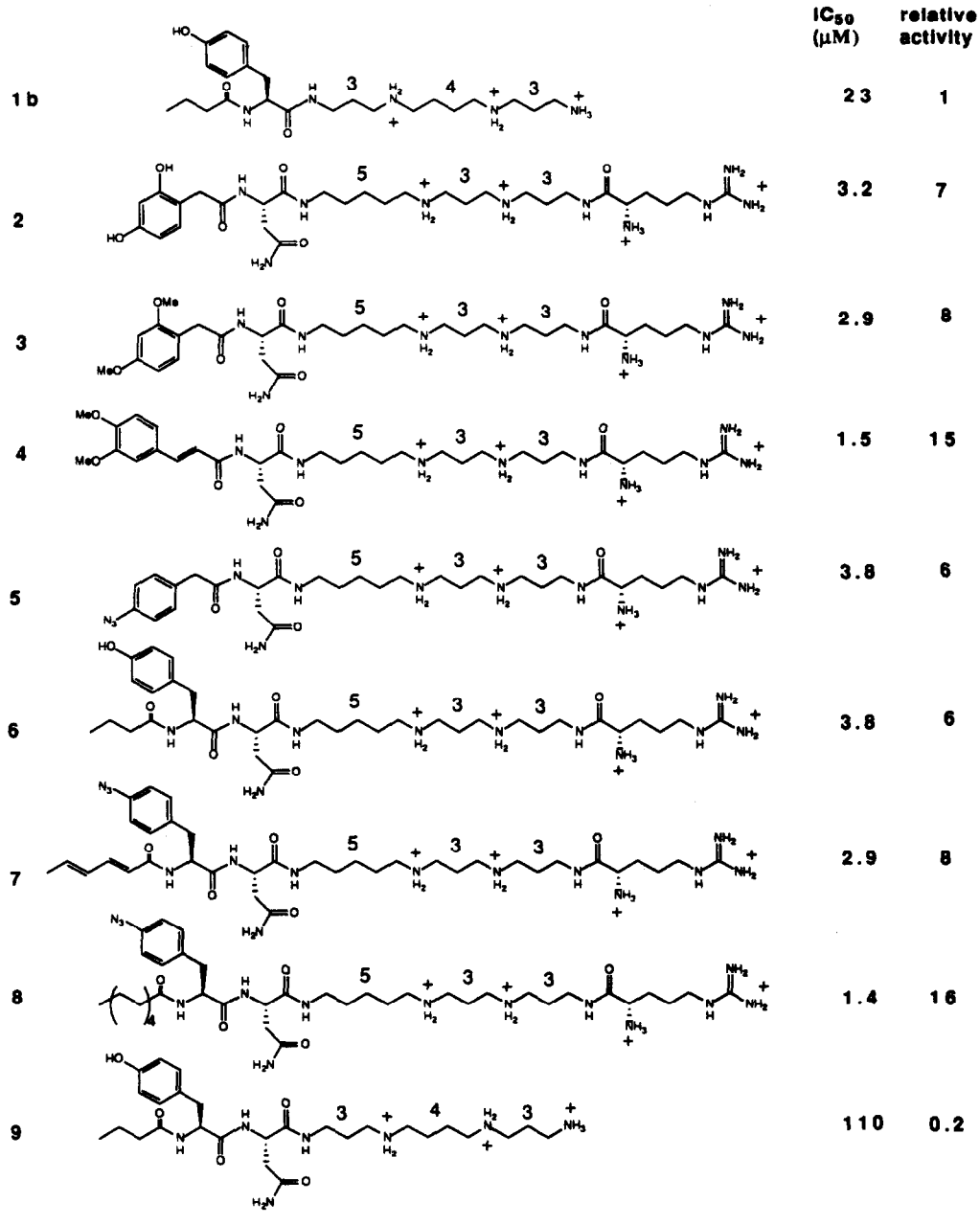
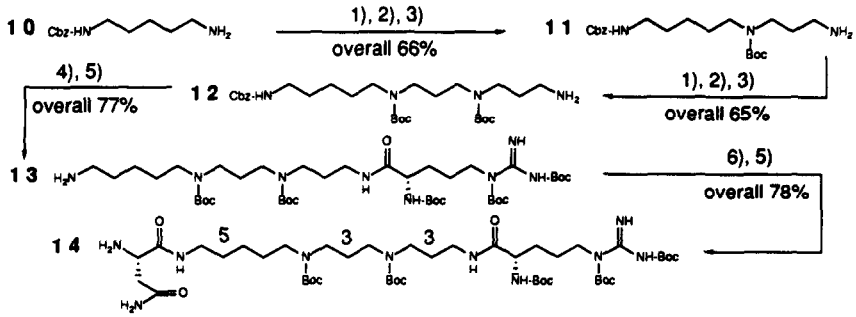


Figure 2. Structures and Activities of ArgTX-636 / PhTX-343 Analogs

shown to irreversibly inactivate the locust QUIS-R.[14] In analog **9**, L-asparagine has been inserted into the region between L-tyrosine and the polyamine of PhTX-343. These synthetic analogs have been assayed for activity on QUIS-R of locust skeletal muscle, the results of which are shown in Figure 2.

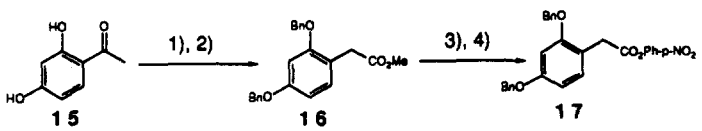
Although synthesis of ArgTX-636 has been reported earlier,[15] we have developed an alternative synthetic route for easy preparation of ArgTX-636 and other related analogs. The synthesis of ArgTX-636 is shown in three separate Schemes 1, 2, and 3. For the polyamine portion of ArgTX-636, three consecutive steps involving addition, protection and reduction reactions are reiterated (Scheme 1). The N-Cbz protected cadaverine (**10**)[16] was added to acrylonitrile, a synthetic unit for propylamine. The generated secondary amine was protected as an N-Boc group and then the nitrile was reduced with $LiAlH_4$ to afford **11**. These procedures were repeated to prepare **12**. The free terminal amine of **12** was condensed with an activated ester of N-Boc protected L-arginine and the N-Cbz group was hydrogenolysed using 10% Pd/C and H_2 (1 atm). The primary amine of **13** was reacted with an activated ester of N-Cbz protected L-asparagine. After deprotection of N-Cbz group, the polyamine structure of ArgTX-636 (**14**) was obtained.



Scheme 1. Preparation of Polyamine Portion of ArgTX-636

reagents:1) acrylonitrile, MeOH, rt; 2) $(Boc)_2O$, MeOH, rt; 3) $LiAlH_4$, ether, 0°C; 4) N^{α}, N^{G}, $N^{G'}$-tri-Boc-L-arginine N-hydroxysuccinimide ester, DMF, 85%; 5) H_2, 10% Pd/C, MeOH, rt; 6) N-Cbz-L-asparagine p-nitrophenyl ester, DMF, rt, 88%

The 2,4-dibenzyloxyphenylacetic acid for the aromatic portion of ArgTX-636 was prepared in three steps starting from a commercially available 2,4-dihydroxyacetophenone (Scheme 2). After benzylation of the dihydroxy groups of **15**, the acetophenone derivative was treated with Tl(III) and $HClO_4$ in MeOH (Willgerodt-Kindler rearrangement condition[17]) to get **16** in 91% yield. After hydrolysis of the methyl

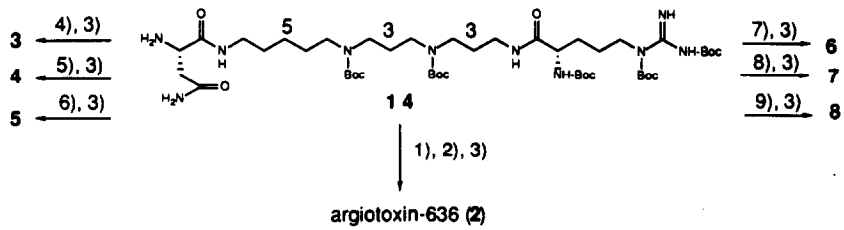


Scheme 2. Preparation of Aromatic Portion of ArgTX-636

reagents:1) BnBr, K_2CO_3, acetone, reflux, 92%; 2) $Tl(NO_3)_3$, 70% $HClO_4$, MeOH, rt, 91%; 3) LiOH, MeOH-DME-H_2O (3:2:1), rt, 98%; 4) p-nitrophenol, DCC, acetone-EtOAc (1:3), 86%

ester, dibenzyloxyphenylacetic acid was converted to p-nitrophenyl ester (**17**) by conventional DCC coupling. For the methylated analog of ArgTX-636 (**3**), 2,4-dimethoxyacetophenone was similarly treated to afford 2,4-dimethoxyphenylacetic acid in overall 84% yield (not shown).

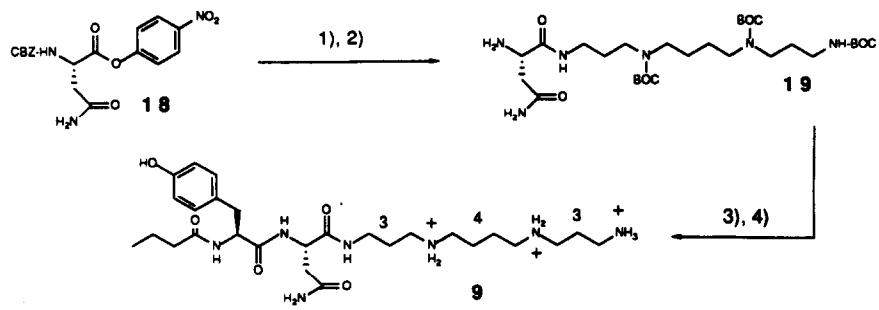
The synthesis of ArgTX-636 and its analogs is summarized in Scheme 3. The Boc-protected polyamine moiety (**14**) was coupled with the activated ester (**17**) in DMF. The benzyl and Boc-protected argiotoxin-636 was treated with H_2 gas (1 atm) on 10% Pd/C and then trifluoroacetic acid. Argiotoxin-636 was obtained as a trifluoroacetic acid salt (**2**)[18]. ArgTX-636 analogs (**3** to **5**) and ArgTX-PhTX hybrids (**6** to **8**)[19] were prepared similarly by coupling of **14** with one of the activated esters of corresponding acids and by deprotection of Boc groups with trifluoroacetic acid.



Scheme 3. Synthesis of ArgTX-636 and ArgTX-PhTX Analogs

reagents:1) **17**, DMAP, DMF, 94%; 2) H_2, 10% Pd/C, MeOH, 95%; 3) CF_3CO_2H-CH_2Cl_2 (1:1), 0°C, quantitative; 4) 2,4-dimethoxyphenylacetic acid p-nitrophenyl ester, 93%; 5) 3,4-dimethoxycinnamic acid p-nitrophenyl ester, 74%; 6) 4-azidophenyl acetic acid p-nitrophenyl ester, 85%; 7) butyryl-L-tyrosine p-nitrophenyl ester, 75%; 8) 2,4-hexadienoyl-p-azido-L-phenylalanine p-nitrophenyl ester, 79%; 9) decanoyl-p-azido-L-phenylalanine p-nitrophenyl ester, 84%

Analog (**9**) was synthesized as shown in Scheme 4. The commercially available N-Cbz-L-asparagine p-nitrophenyl ester was reacted with spermine followed by Boc-protection of the free amines. The amino group of L-asparagine obtained after hydrogenolysis (**19**) was coupled with butyryl-L-tyrosine p-nitrophenyl ester. Trifluoroacetic acid treatment gave butyryl-L-tyrosine-L-asparagine-343 in overall 43% yield.



Scheme 4. Synthesis of PhTX-Asn-343

reagents:1) a. spermine, MeOH ; b. $(Boc)_2O$, MeOH, overall 54% ; 2) H_2, 10% Pd/C, MeOH, 87% ; 3) N-butyryl-L-tyrosine p-nitrophenyl ester, DMF, 92% ; 4) TFA- CH_2Cl_2 (1:1), 0°C, 4 hrs, quantitative

The inhibitory activities of ArgTX-636 and PhTX-343 analogs were assayed on locust skeletal muscle,[13] where Glu-R sensitive to quisqualate (QUIS-R)[1] are highly abundant. Although it is premature to discuss any structure-activity relationships with the limited data, a few comments are as follows. Argiotoxin-636 (**2**, IC_{50} = 3.2 μM) has relative activity of 7 compared to PhTX-343 (**1b**, relative activity =1, IC_{50} = 23 μM). As noted earlier in the assays on PhTX analogs for the QUIS-R, NMDA-R and nACh-R assays,[8, 10, 13] the hydroxyl function of the Tyr moiety plays no significant role, while increasing the hydrophobicity or bulk in the west end of the molecule increases the activity. This is noted in the similar activities of Argiotoxin-636 **2** and its O-methylated analog **3**, and the enhanced activities seen in analogs **4** and **8**. It is encouraging to note the potent activities of the photoaffinity labeled analogs, **5**, **7** and **8**. Insertion of the asparagine moiety into PhTX-343 as in **9** leads to greatly diminished activity; this reduction is unique and could not be predicted from past structure-activity studies. All of the compounds reversibly antagonized QUIS-R, and when muscle preparations were irradiated with U.V., the azido analogs became irreversible antagonists, showing that photolabile polyamine-amides (**5**, **7** and **8**) could serve as potent reagents for the identification and characterization of Glu-R.

In summary, a synthetic scheme has been developed for the synthesis of argiotoxin analogs and argiotoxin-636/philanthotoxin-343 hybrids. These and other synthetic polyamine-amide toxins will be used in future studies directed towards clarifying the differences in the NMDA and KAIN subtypes and designing specific antagonists of mammalian Glu-R.

ACKNOWLEDGEMENTS

The studies have been suported by NIH grant *AI 10187* (KN) and the U.K. SERC (PNRU).

EXPERIMENTAL

DCI-MS spectra were obtained on a Nermag-10 instrument while FAB-MS (3-nitrobenzyl alcohol or thioglycerol matrix) were obtained with a JEOL JMS-DX303HF mass spectrometer. Proton NMR spectra were measured with any one of three instruments: a Varian-200 MHz, Varian-300 MHz, or Varian-400 MHz. Carbon-13 spectra were recorded with a Varian-300 (75.4 MHz for carbon-13 resonance). The proton and carbon spectra were measured in any of the following deuterated solvents: deuterochloroform ($CDCl_3$) with 0.03% tetramethylsilane (TMS) and CD_3OD with 0.015% TMS. Proton chemical shifts are reported in parts per million (ppm) downfield from TMS (0.00ppm) as referenced accordingly for each compound. Spectra were referenced when not otherwise specified on the basis of residual solvent peaks (CD_3OD, 4.78 ppm or 3.30 ppm; $CDCl_3$, 7.24 ppm). Carbon-13 spectra were measured in $CDCl_3$ or CD_3OD and were referenced with respect to the carbon-13 signal of $CDCl_3$ (δ = 77.0 ppm) or CD_3OD (δ = 49.0 ppm).

The solvents DMF and *i*-$PrNH_2$ were distilled at atmospheric pressure over CaH_2, while CH_3OH was distilled over magnesium turnings. Hydrogenolysis of N-Cbz or O-Benzyl group was carried out under H_2 gas (1 atm) in the presence of a catalytic amount of 10% Pd/C.

2-(N^5'-Cbz-1',5'-diaminopentyl)-ethylnitrile: Acrylonitrile (0.68g, 12.82 mmol) in 5 mL CH_3OH was added to a 12 mL CH_3OH solution containing 2.88g (12.20 mmol) of N-Cbz-1,5-diaminopentane[16](**10**) at room temperature and was stirred for 24 hr. The reaction was terminated by evaporation of the solvent and the oil was applied to a silica gel flash column, eluted with 5% CH_3OH/CH_2Cl_2. The product was obtained as an oil in 92% (3.25g) yield. DCI-MS (CH_4, $C_{16}H_{23}N_3O_2$): m/z 290$(M+1)^+$, 159; ^{1}H-NMR (200 MHz, CD_3OD, TMS) δ: 1.33-1.55 (m, 6H), 2.53-2.61 (t, J = 6.8Hz, 4H), 2.81-2.87 (t, J = 6.8Hz, 2H), 3.08-3.15 (t, J = 6.8Hz, 2H), 5.06 (s, 2H), 7.30-7.35 (m, 5H)

2-($N^{5'}$-Cbz-$N^{1'}$-Boc-1',5'-diaminopentyl)-ethylnitrile: To 15 mL of a CH_3OH solution containing the above nitrile (3.25g, 11.24 mmol) was added di-*tert* -butyl dicarbonate (2.94g, 13.47 mmol) dissolved in 10 mL of CH_3OH. The mixture was stirred at room temperature for 24 hr. The reaction mixture was concentrated in vacuo and the oily residue was taken up in 150 mL of CH_2Cl_2. The organic layer was washed with 0.5 M citric acid, sat. $NaHCO_3$ and brine solutions. After drying the solution over $MgSO_4$ and evaporating the solvent, the crude oil was chromatographed on a flash silica gel column with CH_2Cl_2, followed by 5% CH_3OH/CH_2Cl_2, yielding 4.18g (95%) of the desired product. DCI-MS (CH_4, $C_{21}H_{31}N_3O_4$): m/z 390$(M+1)^+$, 334, 290, 279; ^{1}H-NMR (400 MHz, $CDCl_3$, TMS) δ: 1.29-1.34 (quintet, J = 7.0Hz, 2H), 1.46 (s, 9H), 1.53-1.54 (m, 4H), 2.55-2.61 (broad d, 2H), 3.18-3.26 (m, 2H), 3.42-3.45 (t, J = 6.7Hz, 2H), 5.09 (s,2H), 7.31-7.36 (m, 5H)

N^1-Cbz-N^7-Boc-polyamine53 (11): To a suspension of 1.42g (37.42 mmol) of lithium aluminum hydride (LAH) in 200 mL of dry ether was added 4.16g (10.70 mmol) of 2-($N^{5'}$-Cbz-$N^{1'}$-Boc-1',5'-diaminopentyl)-ethylnitrile dissolved in 70 mL of ether at 0°C, and the mixture was stirred under Ar atmosphere at 0°C for 3 hr. The excess LAH was quenched with 1N NaOH at 0°C and the resulting white precipitate was filtered through Celite and washed with 50 mL of Et_2O. The combined ethereal layers were washed with brine, dried over $MgSO_4$, and evaporated in vacuo. The crude oil was flash chromatographed on silica gel with 5% CH_3OH/CH_2Cl_2, and 16:2:1 $CH_2Cl_2/CH_3OH/i$-$PrNH_2$. The product was obtained in 75% (3.15g) yield. DCI-MS (CH_4, $C_{21}H_{35}N_3O_4$): m/z 394$(M+1)^+$, 366, 338, 322, 294; ^{1}H-NMR (300 MHz, CD_3OD, TMS) δ: 1.28-1.32 (quintet, J = 7.0Hz, 2H), 1.45 (s, 9H), 1.50-1.55 (m, 4H), 1.64-1.68 (quintet, J = 6.9Hz, 2H), 2.58-2.62 (t, J = 6.9Hz, 2H), 3.09-3.25 (m, 6H), 5.06 (s, 2H), 7.31-7.35 (m, 5H); ^{13}C-NMR (75.43 MHz, CD_3OD) δ: 25.0, 28.7, 29.1, 30.6, 32.1, 39.9, 40.0, 41.6, 45.0, 45.9, 67.3, 80.8, 128.7, 128.9, 129.4, 138.5, 157.5, 158.9.

N^1-Cbz-N^7-Boc-polyamine53-ethylnitrile: A mixture of 2.8g (7.12 mmol) of **11** and 0.39g (7.35 mmol) of acrylonitrile in 25 mL of CH_3OH was stirred at room temperature for 20 hr. The reaction mixture was worked up as for 2-($N^{5'}$-Cbz-1',5'-diaminopentyl)-ethylnitrile. The desired product was obtained in 89% (2.83g) yield. R_f = 0.64 in 10% CH_3OH/CH_2Cl_2. ^{1}H-NMR (300 MHz, CD_3OD, TMS) δ: 1.26-1.33 (quintet, J = 6.9Hz, 2H), 1.46 (s, 9H), 1.50-1.58 (m, 4H), 1.70-1.75 (quintet, J = 7.0Hz, 2H), 2.57-2.61 (t, J = 7.0Hz, 4H), 2,83-2.88 (t, J = 7.0Hz, 2H), 3.10-3.28 (m, 6H), 5.07 (s, 2H), 7.34-7.36 (m, 5H)

N^1-Cbz-N^7,N^{11}-di-Boc-polyamine53-ethylnitrile: To a 20 mL CH_3OH solution containing 2.70g (6.05 mmol) of the above nitrile was added 1.58g (7.24 mmol) of di-*tert* -butyl dicarbonate in 10 mL of CH_3OH . The mixture was stirred for 20 hr. The reaction was worked up in the same manner as 2-($N^{5'}$-Cbz-$N^{1'}$-Boc-1',5'-diaminopentyl)-ethylnitrile yielding 3.24g (98%) of the product. R_f = 0.59 in 5% CH_3OH/CH_2Cl_2. ^{1}H-NMR (400 MHz, $CDCl_3$, TMS) δ: 1.28-1.35 (quintet, J =7.2Hz, 2H), 1.46 (s, 18H), 1.52-1.53 (m, 4H), 1.73-1.79 (quintet, J = 7.2Hz, 2H), 2.58-2.63 (broad d, 2H), 3.17-3.22 (m, 6H), 3.25-3.29 (t, J = 7.2Hz, 2H), 3.46-3.48 (broad s, 4H), 5.10 (s, 2H), 7.30-7.37 (m, 5H)

N^1-Cbz-N^7,N^{11}-di-Boc-polyamine533 (12): A dry ether solution of 2.5g (4.58 mmol) of N^1-Cbz-N^7,N^{11}-di-Boc-polyamine53-ethylnitrile was treated with 0.63g (16.60 mmol) of LAH as described in the synthesis of **11**. The product was obtained after a flash silica column chromatography in 74% yield (1.86g). FAB-MS (thioglycerol matrix, $C_{29}H_{50}N_4O_6$): m/z 551$(M+1)^+$, 443, 343; ^{1}H-NMR (400 MHz, CD_3OD, TMS) δ: 1.29-1.32 (quintet, J = 7.1Hz, 2H), 1.46 (s, 18H), 1.50-1.54 (m, 4H), 1.65-1.70 (quintet, J = 6.8Hz, 2H), 1.72-1.80 (quintet, J = 6.8Hz, 2H), 2.60-2.63 (t, J = 6.8Hz, 2H), 3.10-3.13 (t, J = 6.8Hz, 2H), 3.17-3.21 (t, J = 6.8Hz, 4H), 3.26-3.31 (m, 4H), 5.06 (s, 2H), 7.34-7.35 (m, 5H).

N^1-Cbz-N^7, N^{11}-di-Boc-polyamine533-N^α, N^G, $N^{G'}$-tri-Boc-L-arg-amide: To a 5 mL solution of 1.33g (2.42 mmol) of **12** was added 1.45g (2.54 mmol) of N^α, N^G, $N^{G'}$-tri-Boc-L-arginine N-hydroxysuccinimide ester (Bachem) dissolved in 5 mL of DMF. The reaction was worked up by evaporation of the solvent and extraction of the pale yellow oil with CH_2Cl_2 and by washing the organic extracts with sat. $NaHCO_3$ and brine. After drying over $MgSO_4$ and evaporation, the crude product was chromatographed on a flash silica column with 5% CH_3OH/CH_2Cl_2 to yield 2.07g (85%) of the desired product. R_f = 0.64 in 10% CH_3OH/CH_2Cl_2. FAB-MS (3-nitrobenzyl alcohol matrix, $C_{50}H_{86}N_8O_{13}$):

m/z 1007(M)$^+$, 908, 808, 752, 507; ^{1}H-NMR (400 MHz, CD_3OD, TMS) δ: 1.31-1.36 (m, 4H), 1.45-1.48 (four s, 45H), 1.50-1.85 (m, 10H), 3.10-3.29 (m, 12H), 3.80-3.95 (m, 2H), 3.95-4.01 (broad s, 1H), 5.01 (s, 2H), 7.30-7.35 (m, 5H); ^{13}C-NMR (75.43 MHz, CD_3OD) δ: 25.0, 26.1, 26.4, 26.7, 28.6, 28.8, 29.2, 29.4, 30.5, 30.6, 34.7, 41.6, 45.4, 45.8, 45.9, 46.0, 46.1, 46.2, 56.1, 67.3, 79.9, 80.9, 81.0, 85.2, 128.7, 128.9, 129,4, 138.5, 156.0, 157.3, 157.4, 157.7, 162.0, 164.6, 174.9.

N^7, N^{11}-di-Boc-polyamine533-N^α, N^G, $N^{G'}$-tri-Boc-L-arg-amide (13): To a 50 mL CH_3OH solution of 1.61g (1.60 mmol) of N^1-Cbz-N^7, N^{11}-di-Boc-polyamine533-N^α, N^G, $N^{G'}$-tri-Boc-L-arg-amide was added 0.3g of 10% Pd/C. This solution was purged several times with H_2 gas. The mixture was stirred overnight at room temperature under H_2 (1 atm) gas. The reaction was terminated by filtration through a pad of Celite and by careful washing of the catalyst with copious volumes of CH_3OH. After evaporation of the solvent, the clear oil was chromatographed on a silica gel with 10:1 CH_2Cl_2/CH_3OH and 20:2:1 CH_2Cl_2/CH_3OH/*i*-$PrNH_2$. The desired product, 1.27g (91%) was obtained as a clear oil. R_f = 0.5 in 20:2:1 CH_2Cl_2/CH_3OH/*i*-$PrNH_2$. FAB-MS (3-nitrobenzyl alcohol matrix, C42H80N8O11): m/z 873(M)$^+$, 773, 673; ^{1}H-NMR (400 MHz, CD_3OD, TMS) δ: 1.29-1.38 (m, 4H), 1.44-1.48 (three s, 45H), 1.51-1.65 (m, 4H), 1.70-1.77 (m, 6H), 2.61-2.64 (t, J = 7.2Hz, 2H), 3.14-3.31 (m, 10H), 3.83-3.86 (m, 2H), 3.98 (broad s, 1H); ^{13}C-NMR (75.43 MHz, CD_3OD) δ: 25.2, 25.6, 26.3, 28.0, 28.3, 28.7, 28.8, 29.5, 30.0, 30.5, 33.6, 36.2, 37.6, 37.8, 38.1, 42.5, 45.4, 46.1, 48.1, 52.6, 56.0, 79.9, 80.4, 80.7, 80.9, 85.1, 156.0, 157.1, 157.6, 161.7, 174.7.

N^α-Cbz-L-asn-N^7,N^{11}-di-Boc-polyamine533-N^α, N^G, $N^{G'}$-tri-Boc-L-arg-amide: To a 15 mL DMF solution of 0.95g (1.09 mmol) of **13** was added 0.45g (1.16 mmol) of N-Cbz-L-asparagine p-nitrophenyl ester, and this solution was stirred overnight at room temperature. The reaction was worked up by evaporation of the solvent and extraction of the oily residue with CH_2Cl_2 (200 mL) and by washing the organic extract with sat. $NaHCO_3$ and brine. After drying over $MgSO_4$ and evaporation, the oily residue was flash chromatographed on a silica gel column with 10% CH_3OH/CH_2Cl_2 to yield 1.07g (88%) of the desired product. R_f = 0.48 in 10% CH_3OH/CH_2Cl_2. FAB-MS (3-nitrobenzyl alcohol matrix, C54H92N10O15): m/z 1121(M)$^+$, 1072, 1021, 935, 921, 865, 721, 621; ^{1}H-NMR (400 MHz, CD_3OD, TMS) δ: 1.25-1.40 (m, 4H), 1.44-1.48 (five s, 45H), 1.51-1.64 (m, 4H), 1.70-1.80 (m, 6H), 2.58-2.71 (two dd, J = 6.0, 7.6Hz, 2H), 3.29-3.10 (broad s, 12H), 3.80-3.90 (m, 2H), 3.92-4.02 (broad s, 1H), 4.46-4.48 (dd, J = 6.0, 7.6Hz, 1H), 5.09 (s, 2H), 7.30-7.35 (m, 5H); ^{13}C-NMR (75.43 MHz, CD_3OD) δ: 25.0, 26.4, 28.1, 28.3, 28.7, 28.8, 29.7, 29.8, 30.0, 30.5, 37.6, 37.7, 38.0, 38.2, 38.3, 38.7, 40.3, 45.2, 45.5, 45.7, 45.9, 46.2, 53.4, 56.1, 67.8, 79.9, 80.5, 80.8, 81.0, 85.2, 128.9, 129.0, 129.5, 138.0, 156.0, 157.2, 157.7, 162.0, 164.6, 173.4, 174.8.

H_2N-L-asn-N^7, N^{11}-di-Boc-polyamine533-N^α,N^G,$N^{G'}$-tri-Boc-L-arg-amide (14): To a 50 mL CH_3OH solution of 1.06g (0.95 mmol) N^α-Cbz-L-asn-N^7, N^{11}-di-Boc-polyamine533-N^α, N^G, $N^{G'}$-tri-Boc-L-arg-amide was added 0.3g of 10% Pd/C followed by hydrogenolysis at room temperature overnight. The reaction mixture was filtered and washed through Celite with 50 mL of CH_3OH. After evaporation of the solvent, the crude oil was passed through a silica column with 10% CH_3OH/CH_2Cl_2. The product was obtained as a clear foam, 0.83g (89%). R_f = 0.40 in 10% CH_3OH/CH_2Cl_2. FAB-MS (3-nitrobenzyl alcohol matrix, C46H86N10O13): m/z 987(M)$^+$, 887, 801, 787, 727, 687, 487, 343; ^{1}H-NMR (400 MHz, CD_3OD, TMS) δ: 1.31-1.41 (m, 4H), 1.46-1.56 (four s, 45H), 1.61-1.70 (m, 4H), 1.72-1.78 (m, 6H), 2.42-2.48 (dd, J = 8.0, 15.0Hz, 1H), 2.60-2.65 (dd, J = 5.0, 15.0Hz, 1H), 3.14-3.30 (broad s, 12H), 3.63-3.67 (dd, J = 5.0, 8.0Hz, 1H), 3.85-3.95 (m, 2H), 4.00 (broad s, 1H); ^{13}C-NMR (75.43 MHz, CD_3OD) δ: 25.2, 26.4, 28.3, 28.7, 28.8, 29.1, 29.2, 29.4, 30.1, 30.5, 30.6, 40.2, 41.3, 46.0, 46.2, 53.4, 56.1, 79.9, 80.5, 80.9, 81.0, 85.2, 156.1, 157.2, 157.3, 162.0, 164.6, 174.9, 175.8, 176.3.

2,4-Dibenzyloxyphenylacetic acid methyl ester (15): To a solution of 70% $HClO_4$-CH_3OH (0.8 mL : 3 mL) containing 0.74g (1.67 mmol) of $Tl(NO_3)_3 \cdot 3H_2O$ was added 0.5g (1.50 mmol) of 2,4-dibenzyloxyacetophenone[20] in 5 mL CH_3OH. This solution was stirred for 80 min at room temperature, forming a white precipitate which was filtered and washed through a pad of Celite with 5 mL of CH_3OH. The filtrate was diluted with 20 mL water and extracted with 60 mL of CH_2Cl_2. The organic extract was washed with sat. $NaHCO_3$ and brine. After drying with $MgSO_4$ and evaporation, the crude oil was purified

with a flash silica column eluted with 1:2 EtOAc/hexane. The desired product was obtained as a clear oil in 91% (0.495g) yield. R_f = 0.65 in 1:2 EtOAc/hexane. ^{1}H-NMR (300 MHz, $CDCl_3$, TMS) δ: 3.58 (s, 3H), 4.96 (s, 2H), 4.97 (s, 2H), 6.49-6.52 (dd, J = 2.1, 8.1Hz, 1H), 6.57-6.58 (d, J = 2.1Hz, 1H), 7.05-7.08 (d, J = 8.1Hz, 1H), 7.26-7.39 (m, 10H) ; ^{13}C-NMR (75.43 MHz, $CDCl_3$) δ: 35.4, 51.7, 69.9, 70.1, 100.6, 105.5, 116.1, 127.1, 127.5, 127.8, 127.9, 128.5, 128.6, 131.2, 136.9, 157.5, 159.3, 172.5.

2,4-Dibenzyloxyphenylacetic acid: To 4 mL of DME containing 0.38g (1.05 mmole) of 2,4-dibenzyloxyphenylacetic acid methyl ester was added 0.13g (3.15 mmol) of $LiOH{\bullet}H_2O$ in 8 mL of CH_3OH-H_2O (3:1). The solution was stirred overnight at room temperature and was worked up by evaporation of the solvent and addition of 4 mL of 1.0 M HCl to the residue. The white precipitates were collected and washed with 10 mL of water, yielding 0.358g of the desired product (98%) which was used without further purification. DCI-MS (CH_4, $C_{22}H_{20}O_4$): m/z 349(M+1)$^+$, 331, 181; ^{1}H-NMR (400 MHz, CD_3OD, TMS) δ: 3.52 (s, 2H), 5.03 (s, 2H), 5.06 (s, 2H), 6.51-6.54 (dd, J = 2.4, 8.4Hz, 1H), 6.61 (d, J = 2.4Hz, 1H), 7.08-7.09 (d, J = 8.4Hz, 1H), 7.27-7.45 (m, 10H).

2,4-Dibenzyloxyphenylacetic acid p-nitrophenyl ester (16): To a 3 mL solution of acetone-EtOAc (1:2) containing 0.1g (0.2 mmol) of 2,4-dibenzyloxyphenylacetic acid and 0.044g (0.32 mmol) of p-nitrophenol was added 0.062g (0.30 mmol) of dicycloheylcarbodiimide (DCC) in 1 mL of EtOAc at 0°C under Ar atmosphere. This solution was stirred for 12 hr, while the bath temperature was slowly increased to room temperature. The white precipitate was filtered through a pad of Celite and the filtrate was concentrated to dryness. The crude product was chromatographed on a flash silica gel column with 1:2 EtOAc/hexane. The desired product was obtained as a white solid in 86% (0.116 mg) yield. R_f = 0.60 in 1:2 EtOAc/hexane. DCI-MS (CH_4, $C_{28}H_{23}N_1O_6$): m/z 470(M)$^+$, 331, 230; ^{1}H-NMR (200 MHz, $CDCl_3$, TMS) δ: 3.84 (s, 2H), 5.04 (s, 4H), 6.54-6.59 (dd, J = 2.4, 8.2Hz, 1H), 6.65-6.66 (d, J = 2.4Hz, 1H), 6.99-7.02 (d, J =9.2Hz, 2H), 7.14-7.18 (d, J = 8.2Hz, 1H), 7.32-7.40 (m, 10H), 8.12-8.16 (d, J = 9.2Hz, 2H).

argiotoxin-636 (2): To a 2 mL DMF solution containing 92 mg (0.093 mmol) of **14** and 6 mg (0.049 mmol) of N,N-dimethyl-4-aminopyridine (DMAP) was added 53 mg (0.11 mmol) of **16** dissolved in 1 mL of DMF. This solution was stirred overnight at room temperature. After evaporation of the solvent, the residue was dissolved in 50 mL CH_2Cl_2 and washed 3 times with sat. $NaHCO_3$. The organic layer was further washed with brine and then dried with $MgSO_4$ before filtration through a pad of Celite. The filtrate was concentrated in vacuo to yield a pale yellow oil which was purified by flash chromatography on a silica gel column using 10% CH_3OH/CH_2Cl_2. The desired product (Rf = 0.7-0.6 in 10% CH_3OH/CH_2Cl_2) was obtained in 94% yield (116 mg). FAB-MS (3-nitrobenzyl alcohol matrix, $C_{68}H_{104}N_1O_{16}$): m/z 1339(M+Na)$^+$, 1317(M)$^+$, 1249, 1217, 1117, 1061, 1027, 817; ^{1}H-NMR (400 MHz, CD_3OD, TMS) δ: 1.19-1.41 (m, 4H), 1.44-1.53 (five s, 45H), 1.60-1.80 (m, 10H), 2.48-2.54 (dd, J = 6.0, 15.6Hz, 1H), 2.57-2.62 (dd, J = 6.0, 15.6Hz, 1H), 3.12-3.25 (m, 12H), 3.55 (s, 2H), 3.82-3.87 (m, 2H), 3.99 (broad s, 1H), 4.63-4.65 (t, J = 6.0Hz, 1H), 5.04 (s, 2H), 5.10 (s, 2H), 6.56-6.60 (dd, J = 2.4, 8.4HZ, 1H), 6.69 (d, J =2.4Hz, 1H), 7.11-7.14 (d, J = 8.4Hz, 1H), 7.28-7.44 (m, 10H); ^{13}C-NMR (75.43 MHz, CD_3OD) δ: 24.9, 26.3, 28.3, 28.6, 28.8, 29.9, 30.4, 34.7, 37.6, 38.4, 40.2, 45.4, 45.9, 46.1, 51.4, 55.9, 71.0, 71.1, 80.5, 80.7, 80.9, 85.1, 101.8, 107.4, 117.3, 128.2, 128.5, 128.8, 129.5, 132.6, 138.4, 155.9, 157.1, 158.6, 160.8, 172.6, 174.2, 174.8.

To a 5 mL CH_3OH solution of 0.1 g (0.076 mmol) di-O-benzyl-penta-N-Boc-argiotoxin-636 (above) was added 50 mg of 10% Pd/C followed by hydrogenolysis at room temperature overnight. The reaction mixture was filtered and washed through a pad of Celite with 10 mL CH_3OH. The crude product was passed through a column of silica gel and eluted with 10% CH_3OH/CH_2Cl_2. The desired product was obtained as a clear foam, 82 mg (95%). FAB-MS (3-nitrobenzyl alcohol matrix, $C_{54}H_{92}N_1O_{16}$): m/z 1159(M+Na)$^+$, 1137(M)$^+$, 1037, 937, 923, 837, 637; ^{1}H-NMR (400 MHz, CD_3OD, TMS) δ: 1.22-1.42 (m, 4H), 1.44-1.54 (five s, 45H), 1.58-1.80 (m, 10H), 2.59-2.65 (dd, J =6.0, 15.6Hz, 1H), 2.69-2.75 (dd, J =6.0, 15.6Hz, 1H), 3.12-3.20 (m, 12H), 3.44-3.45 (d, J =4.8Hz, 2H), 3.82-3.89 (m, 2H), 3.98 (broad s, 1H), 4.63-4.66 (t, J = 6.0Hz, 1H), 6.26-6.29 (dd, J =2.4, 8.4Hz, 1H), 6.34 (d, 2.4Hz, 1H), 6.93-6.95 (d, J =8.4Hz, 1H); ^{13}C-NMR (75.43 MHz, CD_3OD) δ: 25.1, 26.5, 28.3, 28.5, 28.6, 28.7, 29.3, 29.5, 29.6, 30.0, 30.1, 30.5, 30.8, 37.4, 37.7, 38.9, 40.4, 45.4, 45.5, 45.9, 46.1, 46.2, 46.3, 51.6, 56.2, 80.0, 80.6, 80.9, 81.1, 85.3, 103.9, 108.1, 114.1, 132.8, 156.1, 157.4, 159.2, 172.98, 174.96, 175.05, 175.13.

To 2 mL of dry CH_2Cl_2 containing 0.073 g (0.064 mmol) of penta-N-Boc-argiotoxin-636 was added 2 mL of TFA in ice bath. This mixture was stirred for 3 hr under Ar atmosphere at 0°C. The solvent was removed in vacuo yielding an oily residue. The free TFA was removed by repeatedly dissolving the oily residue in 2ml of CH_3OH and evaporating, and finally by lyophilization of a frozen aqueous solution of the residue. Argiotoxin-636 as a TFA salt was obtained in quantitative yield. FAB-MS (3-nitrobenzyl alcohol matrix, $C_{29}H_{52}N_{10}O_6$): m/z 659$(M+Na)^+$, 637$(M)^+$, 623, 509, 487, 329; ^{1}H-NMR (400 MHz, CD_3OD, TMS; TFA salt) δ: 1.28-1.49 (m, 2H), 1.51-1.69 (m, 6H), 1.87-1.96 (m, 4H), 2.06-2.11 (m, 2H), 2.70-2.71 (d, J = 6.0Hz, 2H), 2.81-3.43 (m, 16H), 3.87-3.90 (t, J = 6.4Hz, 1H), 4.60-4.63 (t, J = 6.0Hz, 1H), 6.28-6.31 (dd, J =2.4, 8.4Hz, 1H), 6.35 (d, J = 2.4Hz, 1H), 6.95-6.97 (d, J = 8.4Hz, 1H); ^{13}C-NMR (75.43 MHz, CD_3OD; TFA salt) δ: 24.2, 24.3, 25.6, 26.4, 27.4, 28.1, 29.3, 29.7, 37.2, 37.4, 38.7, 39.7, 41.7, 45.6, 46.0, 46.7, 52.1, 54.2, 103.9, 108.1, 114.4, 133.0, 157.4, 159.2, 162.1, 170.7, 173.4, 175.5.

2,4-Di-methoxyphenylacetyl-L-asn-polyamine533-L-arg (3): To a 1 mL DMF solution of 50 mg (0.051 mmol) **14** and 4 mg (0.033 mmol) of DMAP was added 18 mg (0.057 mmol) of 2,4-dimethoxyphenylacetic acid p-nitrophenyl ester dissolved in 1 mL of DMF. The solution was stirred overnight at room temperature. The reaction mixture was worked up and purified in the same manner as for synthesis of argiotoxin-636. The desired product was obtained as a white foam in 93% (55mg) yield. R_f = 0.58 in 10% CH_3OH/CH_2Cl_2. FAB-MS (3-nitrobenzyl alcohol matrix, $C_{56}H_{96}N_{10}O_{16}$): m/z 1187$(M+Na)^+$, 1165$(M)^+$, 1065, 979, 965, 909, 803, 687, 665, 604, 457; ^{1}H-NMR (400 MHz, CD_3OD, TMS) δ: 1.20-1.32 (m, 4H), 1.44-1.54 (four s, 45H), 1.55-1.80 (m, 10H), 2.56-2.63 (dd, J =6.0, 15.0Hz, 1H), 2.65-2.75 (dd, J =6.0, 15.0Hz, 1H), 3.10-3.30 (broad s, 12H), 3.47-3.48 (d, J =4.0Hz, 2H), 3.76-3.88 (m, 8H), 3.99 (broad s, 1H), 4.62-4.65 (t, J = 6.0Hz, 1H), 6.46-6.49 (dd, J =2.0, 8.0Hz, 1H), 6.53-6.54 (d, 2.0Hz, 1H), 7.09-7.11 (d, J =8.0Hz, 1H); ^{13}C-NMR (75.43 MHz, CD_3OD) δ:25.2, 26.4, 28.3, 28.7, 28.8, 29.1, 29.2, 29.4, 30.2, 30.5, 30.6, 40.2, 41.3, 46.0, 46.2, 53.4, 56.1, 57.0, 79.9, 80.5, 80.8, 81.0, 85.2, 99.8, 106.0, 116.9, 133.4, 156.0, 157.2, 157.7, 159.7, 162.0, 172.9, 174.4, 174.9.

The N-Boc groups were removed as described for synthesis of argiotoxin-636. FAB-MS (3-nitrobenzyl alcohol matrix, $C_{31}H_{56}N_{10}O_6$): m/z 687$(M+Na)^+$, 665$(M)^+$, 373, 329; ^{1}H-NMR (400 MHz, CD_3OD, TMS; TFA salt) δ: 1.33-1.39 (m, 2H), 1.48-1.53 (quintet, J =7.0Hz, 2H), 1.62-1.71 (m, 4H), 1.87-1.96 (m, 4H), 2.09-2.13 (quintet, J = 7Hz, 2H), 2.61-2.72 (two dd, J = 6.0, 15.0 Hz, 2H), 2.92-3.47 (m, 14H), 3.49-3.50 (d, J = 4.0Hz, 2H), 3.78 (s, 3H), 3.82 (s, 3H), 3.88-3.91 (t, J = 6.4 Hz, 1H), 4.61-4.65 (t, J = 6.0Hz, 1H), 6.48-6.50 (dd, J = 2.4, 8.4Hz, 1H), 6.54 (d, J = 2.4Hz, 1H), 7.10-7.12 (d, J = 8.4Hz, 1H) ; ^{13}C-NMR (75.43 MHz, CD_3OD; TFA salt) δ: 24.2, 24.4, 25.5, 25.6, 27.4, 29.5, 29.7, 30.5, 37.4, 37.7, 38.3, 39.9, 41.7, 45.7, 46.0, 46.6, 51.9, 54.2, 55.9, 56.2, 99.6, 105.9, 117.0, 132.0, 158.8, 159.8, 162.1, 170.8, 173.3, 174.8.

3.4-Di-methoxycinnamoyl-L-asn-polyamine533-L-arg (4): To a 1 mL DMF solution of 40 mg (0.041mmol) **14** and 4 mg (0.033 mmol) DMAP was added 15 mg (0.046 mmol) 3,4-di-methoxycinnamic acid p-nitrophenyl ester dissolved in 1 mL DMF. The solution was stirred overnight at room temperature. The reaction mixture was worked up and purified in the same manner as for synthesis of argiotoxin-636. The desired product was obtained as a white foam in 74% (35.8 mg) yield. R_f = 0.72 in 10% CH_3OH/CH_2Cl_2. FAB-MS (3-nitrobenzyl alcohol matrix, $C_{57}H_{96}N_{10}O_{16}$): m/z 1199$(M+Na)^+$, 1177$(M)^+$, 1077, 977, 877, 699; ^{1}H-NMR (300 MHz, CD_3OD, TMS) δ: 1.28-1.40 (m, 4H), 1.43-1.53 (five s, 45H), 1.58-1.73 (m, 10H), 2.65-2.80 (two dd, J = 6.3, 15.6Hz, 2H), 3.10-3.22 (m, 12H), 3.81-3.86 (broad s, 8H), 3.98 (broad s, 1H), 4.80-4.85 (t, J = 6.3Hz, 1H), 6.52-6.58 (d, J = 15.5Hz, 1H), 6.94-6.97 (d, J = 8.2Hz, 1H), 7.13-7.14 (dd, J = 2.0, 8.2Hz, 1H), 7.15-7.16 (d, J = 2.0Hz, 1H), 7.46-7.51 (d, J = 15.5Hz, 1H) ; ^{13}C-NMR (75.43 MHz, CD_3OD) δ: 25.1, 26.4, 28.3, 28.7, 28.8, 29.3, 30.1, 30.5, 38.2, 40.4, 45.5, 46.0, 46.2, 51.9, 56.1, 56.4, 56.5, 80.5, 80.8, 81.0, 85.2, 114.4, 112.7, 119.3, 123.4, 129.2, 142.4, 150.7, 152.4, 156.0, 157.2, 157.7, 162.0, 168.6, 173.1, 174.8.

The N-Boc groups were removed as for the synthesis of argiotoxin-636. FAB-MS (3-nitrobenzyl alcohol matrix, $C_{32}H_{56}N_{10}O_6$): m/z 699$(M+Na)^+$, 677$(M)^+$, 427; ^{1}H-NMR (400 MHz, CD_3OD, TMS; TFA salt) δ: 1.42-1.46 (quintet, J = 7.6Hz, 2H), 1.53-1.59 (quintet, J = 7.2Hz, 2H), 1.67-1.75 (m, 4H), 1.87-1.99 (m, 4H), 2.12-2.17 (quintet, J = 8.0Hz, 2H), 2.70-2.82 (two dd, J = 6.0, 15.6Hz, 2H), 3.00-3.04 (t, J = 7.6Hz, 2H), 3.06-3.09 (t, J = 7.6Hz, 2H), 3.10-3.12 (t, J = 7.2Hz, 2H), 3.20-3.30 (m, 4H), 3.41-3.48 (q, J = 7.2Hz, 2H), 3.86 (s, 3H), 3.88-3.92 (t, J = 7.6Hz, 1H), 3.98 (s, 3H), 4.81-4.82 (t, J = 6.0Hz, 1H), 6.56-6.60 (d, J = 15.6Hz, 1H), 6.95-6.98 (d, J = 8.0Hz, 1H), 7.12-7.15 (dd, J = 2.0,

8.0Hz, 1H), 7.17 (d, J = 2.0Hz, 1H), 7.47-7.51 (d, J = 15.6Hz, 1H); ^{13}C-NMR (75.43 MHz, CD_3OD; TFA salt) δ: 24.1, 24.4, 25.5, 26.5, 27.3, 29.5, 29.7, 37.4, 39.8, 41.7, 45.7, 45.9, 46.6, 52.1, 54.1, 55.1, 55.4, 111.4, 112.7, 119.2, 123.5, 129.2, 142.5, 150.7, 152.5, 158.7, 168.9, 170.7, 173.4.

4-Azidophenylacetyl-L-asn-polyamine533-L-arg (5): To a 1 mL DMF solution of 40 mg (0.041mmol) **14** and 4 mg (0.033 mmol) DMAP was added 13 mg (0.044 mmol) of 4-azidophenylacetic acid p-nitrophenyl ester dissolved in 1 mL DMF. The solution was stirred overnight while being protected from direct, strong light at room temperature. The reaction mixture was worked up and purified in the same manner as for synthesis of argiotoxin-636. The desired product was obtained as a white foam in 85% (39.5 mg) yield. R_f = 0.47 in 10% CH_3OH/CH_2Cl_2. FAB-MS (3-nitrobenzyl alcohol matrix, $C_{54}H_{91}N_{13}O_{14}$): m/z 1168$(M+Na)^+$, 1146$(M)^+$, 1120, 1046, 1020, 960, 946, 920; ^{1}H-NMR (400 MHz, CD_3OD, TMS) δ: 1.20-1.41 (m, 4H), 1.44-1.54 (five s, 45H), 1.58-1.80 (m, 10H), 2.59-2.64 (dd, J = 7.0,15.0Hz, 1H), 2.66-2.71 (dd, J = 7.0, 15.0Hz, 1H), 3.12-3.25 (m, 12H), 3.55 (s, 2H), 3.80-3.95 (m, 2H), 3.99 (broad s, 1H), 4.65-4.69 (t, J = 7.0Hz, 1H), 6.99-7.02 (d, J = 8.4Hz, 2H), 7.30-7.33 (d, J = 8.4Hz, 2H); ^{13}C-NMR (75.43 MHz, CD_3OD) δ: 25.1, 26.4, 28.3, 28.7, 28.8, 30.0, 30.5, 38.0, 40.4, 42.8, 45.5, 46.0, 46.1, 46.2, 46.3, 51.8, 56.1, 80.9, 81.1, 85.2, 120.1, 131.8, 133.6, 140.1, 156.1, 157.3, 162.0, 172.9, 173.5, 174.8, 174.9.

The N-Boc groups were removed as for the synthesis of argiotoxin-636. FAB-MS (3-nitrobenzyl alcohol matrix, $C_{29}H_{51}N_{13}O_4$): m/z 646$(M)^+$, 620, 399, 367, 237; ^{1}H-NMR (400 MHz, CD_3OD, TMS; TFA salt) δ: 1.34-1.40 (quintet, J = 7.2Hz, 2H), 1.50-1.56 (m, 2H), 1.64-1.73 (m, 4H), 1.87-1.98 (m, 4H), 2.10-2.14 (quintet, J = 7.0Hz, 2H), 2.61-2.67 (dd, J = 7.0, 15.6Hz, 1H), 2.69-2.74 (dd, J = 7.0, 15.6Hz, 1H), 2.96-3.28 (m, 12H), 3.41-3.52 (q, J = 6.8Hz, 2H), 3.57 (s, 2H), 3.88-3.91 (t, J = 6.4Hz, 1H), 4.63-4.67 (t, J = 7.0Hz, 1H), 7.00-7.03 (d, J = 8.4Hz, 2H), 7.30-7.33 (d, J = 8.4Hz, 1H) ; ^{13}C-NMR (75.43 MHz, CD_3OD; TFA salt) δ: 24.2, 24.4, 25.5, 26.5, 27.4, 29.5, 29.7, 37.4, 37.8, 39.7, 41.7, 42.7, 45.6, 45.9, 46.6, 52.1, 54.1, 120.1, 131.8, 133.6, 140.2, 170.7, 173.2, 173.8, 174.8.

Butyryl-L-tyr-L-asn-polyamine533-L-arg (6): To a 1 mL DMF solution of 50 mg (0.05mmol) **14** and 4 mg (0.033 mmol) DMAP was added 21 mg (0.056 mmol) butyryl-L-tyrosine p-nitrophenyl ester[21] dissolved in 1 mL DMF. The solution was stirred overnight at room temperature. The reaction mixture was worked up and purified in the same manner as for the synthesis of argiotoxin-636. The desired product was obtained as a white foam in 75% (46.3 mg) yield. R_f = 0.59 in 12% CH_3OH/CH_2Cl_2. FAB-MS (3-nitrobenzyl alcohol matrix, $C_{59}H_{101}N_{11}O_{16}$): m/z 1243$(M+Na)^+$, 1221$(M)^+$, 1121, 1021, 965; ^{1}H-NMR (400 MHz, CD_3OD, TMS) δ: 0.83-0.90 (t, J =10.0Hz, 3H), 1.27-1.40 (m, 6H), 1.44-1.54 (five s, 45H), 1.56-1.75 (m, 10H), 2.16-2.19 (t, J = 7.2Hz, 2H), 2.44-2.72 (m, 2H), 2.78-2.89 (m, 1H), 2.93-3.06 (m, 1H), 3.12-3.28 (broad s, 12H), 3.81-3.88 (m, 2H), 3.98 (broad s, 1H), 4.31-4.46 (two* t, J = 7.0Hz, 1H), 4.58-4.64 (two* t, J = 6.0Hz, 1H), 6.70-6.72 (d, J = 8.0Hz, 2H),,7.03-7.05 (d, J = 8.0Hz, 1H), 7.06-7.08 (d, J = 8.0Hz, 1H); ^{13}C-NMR (75.43 MHz, CD_3OD) δ: 14.0, 14.1, 20.2, 25.1, 26.4, 28.3, 28.6, 28.8, 30.0, 30.5, 37.2, 37.3, 37.4, 37.5, 38.4, 38.6, 40.5, 45.5, 46.0, 46.2, 48.1, 48.4, 48.7, 49.0, 49.3, 49.6, 49.9, 51.3, 51.5, 56.1, 57.0, 57.6, 79.9, 80.5, 80.8, 81.0, 85.2, 116.2, 128.8, 131.3, 156.0, 157.2, 157.3, 157.4, 157.7, 162.0, 172.6, 173.9, 174.9, 175.1, 176.5.

The N-Boc groups were removed as in the synthesis of argiotoxin-636. FAB-MS (3-nitrobenzyl alcohol matrix, $C_{34}H_{61}N_{11}O_6$): m/z 742$(M+Na)^+$,720$(M)^+$; ^{1}H-NMR (400 MHz, CD_3OD, TMS; TFA salt) δ: 0.84-0.90 (m, 3H), 1.33-1.40 (m, 2H), 1.53-1.58 (m, 2H), 1.60-1.70 (m, 2H), 1.84-1.97 (m, 2H), 2.13-2.19 (two* t, J = 6.5Hz, 2H), 2.55 (broad s, 1H), 2.71 (broad s, 1H), 2.81-3.47 (m, 16H), 3.89-3.92 (t, J = 6.0Hz, 1H), 4.29-4.45 (two* t, J = 7.0Hz, 1H), 4.60-4.63 (t, J = 5.2Hz, 1H), 6.71-6.73 (d, J = 7.0Hz, 2H), 7.04-7.09 (two* d, J =7.2Hz, 2H); ^{13}C-NMR (75.43 MHz, CD_3OD; TFA salt) δ: 14.0, 20.2, 24.1, 24.4, 25.5, 26.5, 27.3, 29.5, 29.6, 37.3, 38.4, 38.6, 39.8, 41.6, 45.6, 45.9, 46.6, 51.4, 51.6, 54.1, 57.2, 57.7, 116.3, 128.8, 131.2, 157.3, 158.7, 170.7, 172.9, 174.1, 176.7. *1:1 conformational isomers[19]

2,4-Hexadienoyl-p-azido-L-phe-L-asn-polyamine533-L-arg (7): To a 1 mL DMF solution of 50 mg (0.05mmol) **14** and 4 mg (0.033 mmol) DMAP was added 23 mg (0.055 mmol) 2,4-hexadienoyl-p-azido-L-phenylalanine p-nitrophenyl ester[22] dissolved in 1 mL DMF. The solution was stirred overnight protected from direct, strong light at room temperature. The reaction mixture was worked up and purified in the same manner as for the synthesis of argiotoxin-636. The desired product was obtained as a white foam in 79% (51 mg) yield. R_f = 0.53 in 12% CH_3OH/CH_2Cl_2. FAB-MS (3-nitrobenzyl alcohol matrix, $C_{61}H_{100}N_{14}O_{15}$): m/z 1270$(M)^+$, 1243, 1285, 1170, 1098, 1084, 1070; ^{1}H-NMR (300 MHz, CD_3OD)

δ: 1.23-1.39 (m, 4H), 1.44-1.54 (five s, 45H), 1.61-1.75 (m, 10H), 1.82-1.84 (d, J = 6.3Hz, 3H), 2.65-2.75 (m, 2H), 2.85-2.93 (dd, J = 8.0, 15.0Hz, 1H), 2.95-3.04 (dd, J = 8.0, 12.0Hz, 1H), 3.10-3.20 (m, 12H), 3.80-3.90 (m, 2H), 3.99 (broad s, 1H), 4.54-4.62 (m, 2H), 5.92-5.97 (d, J = 15.0Hz, 1H), 6.12-6.26 (m, 2H), 6.96-6.99 (d, J = 8.4Hz, 2H), 7.06-7.14 (dd, J = 10.0, 15.0Hz, 1H), 7.26-7.29 (d, J = 8.4Hz, 1H); ^{13}C-NMR (75.43 MHz, CD_3OD) δ: 18.7, 25.1, 26.4, 28.3, 28.7, 28.8, 29.3, 30.0, 30.5, 37.4, 37.8, 40.5, 45.5, 45.9, 46.1, 46.2, 51.6, 56.9, 79.9, 80.5, 80.8, 81.0, 85.2, 120.1, 122.0, 131.1, 131.9, 135.2, 139.5, 140.0, 143.1, 156.0, 157.3, 157.7, 164.7, 169.3, 172.5, 173.3, 174.9, 175.1.

The N-Boc groups were removed as in the synthesis of argiotoxin-636. FAB-MS (3-nitrobenzyl alcohol matrix, $C_{36}H_{60}N_{14}O_5$): m/z 791(M+Na)$^+$, 769(M)$^+$, 741, 487; ^{1}H-NMR (400 MHz, CD_3OD, TMS; TFA salt) δ: 1.36-1.43 (m, 2H), 1.53-1.57 (m, 2H), 1.66-1.76 (m, 4H), 1.83-1.84 (d, J = 5.6Hz, 3H), 1.86-1.99 (m, 4H), 2.12-2.15 (quintet, J = 6.0Hz, 2H), 2.70-2.71 (d, J = 5.0Hz, 2H), 2.93-3.39 (m, 16H), 3.89-3.92 (t, J = 6.0Hz, 1H), 4.52-4.55 (dd, J = 6.0, 8.4Hz, 1H), 4.59-4.61 (t, J = 5.0Hz, 1H), 5.94-5.97 (d, J = 15.2Hz, 1H), 6.12-6.25 (m, 2H), 6.98-7.00 (d, J = 8.4Hz, 2H), 7.07-7.13 (dd, J = 10.4, 15.2Hz, 1H), 7.27-7.29 (d, J = 8.4Hz, 2H); ^{13}C-NMR (75.43 MHz, CD_3OD; TFA salt) δ: 18.7, 24.2, 24.4, 25.5, 26.6, 27.4, 29.5, 29.7, 37.4, 37.7, 39.9, 41.7, 45.7, 46.0, 46.7, 47.5, 47.8, 51.8, 54.2, 57.2, 120.1, 122.0, 131.1, 131.9, 135.2, 139.7, 140.1, 143.3, 158.8, 169.7, 170.8, 172.8, 173.6.

Decanoyl-p-azido-L-phe-L-asn-polyamine533-L-arg (8): To a 1 mL DMF solution of 40 mg (0.041mmol) **14** and 4 mg (0.033 mmol) DMAP was added 22 mg (0.046 mmol) decanoyl-p-azido-L-phenylalanine p-nitrophenyl ester[23] dissolved in 1 mL DMF. The solution was stirred overnight while being protected from direct, strong light at room temperature. The reaction mixture was worked up and purified in the same manner as for the synthesis of argiotoxin-636. The desired product was obtained as a white foam in 84% (45 mg) yield. R_f = 0.55 in 12% CH_3OH/CH_2Cl_2. FAB-MS (3-nitrobenzyl alcohol matrix, $C_{65}H_{112}N_{14}O_{15}$): m/z 1351(M+Na)$^+$, 1329(M)$^+$, 1277, 1251, 1229, 1143, 1129, 1101, 1073; ^{1}H-NMR (400 MHz, CD_3OD, TMS) δ: 0.88-0.91 (t, J = 5.0Hz, 3H), 1.14-1.38 (broad s, 18H), 1.44-1.54 (five s, 45H), 1.59-1.75 (m, 10H), 2.16-2.20(t, J = 5.7Hz, 2H), 2.60-2.75 (m, 2H), 2.83-2.88 (dd, J =6.0, 10.5Hz, 1H), 2.89-2.95 (dd, J =6.0, 10.5Hz,1H), 3.04-3.18 (m, 12H), 3.81-3.87 (m, 2H), 3.98 (broad s, 1H), 4.60-4.66 (m, 2H), 6.97-6.99 (d, J = 6.3Hz, 2H), 7.25-7.29 (d, J = 6.3Hz, 2H); ^{13}C-NMR (75.43 MHz, CD_3OD) δ: 14.5, 23.8, 25.1, 26.4, 28.3, 28.7, 28.8, 30.0, 30.2, 30.5, 30.6, 33.0, 36.6, 36.8, 37.3, 37.4, 37.5, 37.6, 40.5, 45.5, 45.9, 46.1, 46.2, 51.3, 51.6, 56.0, 56.1, 56.4, 57.1, 80.0, 80.5, 80.8, 81.0, 85.2, 120.1, 131.9, 135.4, 139.9, 156.0, 157.3, 162.1, 172.6, 173.5, 174.9, 175.0, 176.6.

The N-Boc groups were removed as in the synthesis of argiotoxin-636. FAB-MS (3-nitrobenzyl alcohol matrix, $C_{40}H_{72}N_{14}O_5$): m/z 851(M+Na)$^+$, 829(M)$^+$, 801, 427, 399, 354; ^{1}H-NMR (400 MHz, CD_3OD, TMS; TFA salt) δ: 0.88-0.92 (t, J =6.8Hz, 3H), 1.13-1.59 (m, 13H), 1.66-1.72 (m, 4H), 1.87-1.97 (m, 4H), 2.11-2.16(m, 2H), 2.17-2.21 (t, J = 7.6Hz, 2H), 2.57-2.72 (two* dd, J = 6.0, 11.0Hz, and d, J = 6.0Hz, 1H), 2.85-2.91 (two* dd, J =6.0, 11.0Hz, 1H), 3.02-3.28 (m, 13H), 3.43-3.46 (quintet, J = 7.6Hz, 2H), 3.88-3.91 (t, J = 6.0Hz,1H), 4.40-4.53 (dd, J = 6.0, 10.0Hz, and t, J =6.0Hz, 1H), 4.59-4.65 (two* t, J =6.0Hz, 1H), 6.97-7.00 (d, J = 8.4Hz, 2H), 7.25-7.29 (two* d, J = 8.4Hz, 2H); ^{13}C-NMR (75.43 MHz, CD_3OD; TFA salt) δ:14.5, 23.8, 24.2, 24.4, 25.5, 26.5, 26.9, 27.4, 29.5, 30.0, 30.2, 30.5, 30.6, 33.1, 36.6, 36.8, 37.3, 37.5, 39.9, 41.7, 45.7, 45.9, 46.6, 51.4, 51.7, 54.1, 56.7, 57.1, 120.1, 131.8, 135.3, 140.0, 170.7, 172.9, 173.8, 175.0, 176.8. *1:1 conformational isomers[19]

N-Cbz-L-asn-N,N',N''-tri-Boc-polyamine 343: To a 10 mL solution containing 0.43 g (2.13 mmol) of spermine was added 0.55g (1.42 mmol) of N-Cbz-L-asparagine p-nitrophenyl ester (**18**) while stirring. After 10 hr stirring at room temperature, the reaction mixture was concentrated to a yellow oil and 10 mL CH_3OH/CH_2Cl_2(1:1) was added to enhance crystallization of undesired yellow precipitates. The resulting suspension was filtered and washed through a pad of Celite with 10 mL of the same solution. The filtrate was concentrated to yield crude product as a clear yellow oil. The crude product containing momeric as well as bis-meric adducts was dissolved in 6 mL CH_3OH followed by addition of 1.86g (8.52 mmol) di-*tert*-butyl dicarbonate in 4 mL CH_3OH. The reaction mixture was stirred overnight at room temperature. The clear oil obtained after evaporation of solvent was chromatographed on a flash silica column and eluted with 1% CH_3OH/CH_2Cl_2 and 10% CH_3OH/CH_2Cl_2 yielding 0.58g (54%) of the desired product as a colorless oil. R_f = 0.60 in 10% CH_3OH/CH_2Cl_2. FAB-MS (3-nitrobenzyl alcohol matrix, $C_{37}H_{62}N_6O_{10}$): m/z 773(M+Na)$^+$, 751(M)$^+$, 651, 595, 451; ^{1}H-NMR (300 MHz, CD_3OD) δ: 1.43-1.45 (two s, 27H), 1.51-1.58 (broad s, 4H), 1.60-1.69 (broad s, 4H), 2.65-2.70 (m, 2H), 3.07-3.19 (m, 12H), 4.48-4.52 (t, J = 6.9Hz, 1H), 5.09 (s, 2H), 7.26-7.35 (m, 5H); ^{13}C-NMR (75.43 MHz, CD_3OD) δ: 26.5,

26.8, 26.9, 28.8, 29.4, 29.6, 38.1, 38.9, 45.3, 45.6, 45.9, 47.7, 47. 9, 48.1, 53.4, 67.7, 79.7, 80.7, 128.8, 128.9, 129.4, 137.9, 157.1, 157.9, 158.2, 173.3, 174.7.

H2N-L-asn-tri-Boc-polyamine343 (19): To a 15 mL CH_3OH solution of 0.55g (0.73 mmol) N-Cbz-L-asn-N,N',N"-tri-Boc-polyamine 343 was added 100 mg of 10% Pd/C followed by hydrogenolysis at room temperature overnight. The reaction mixture was filtered and washed through a pad of Celite. The solvent was evaporated in vacuo and the residue was purified by chromatography on a flash silica column using 5% CH_3OH/CH_2Cl_2. This yielded 0.39g (87%) of desired product as a white foam. R_f = 0.4 in 10% CH_3OH/CH_2Cl_2. FAB-MS (3-nitrobenzyl alcohol matrix, $C_{29}H_{56}N_6O_8$): m/z 617$(M)^+$, 517, 417, 317; ^{1}H-NMR (300 MHz, CD_3OD) δ: 1.51-1.53 (two s, 27H), 1.55-1.59 (m, 4H), 1.72-1.81 (m, 4H), 2.41-2.58 (dd, J = 8.0, 15.3Hz, 1H), 2.68-2.74 (dd, J = 4.8, 15.3Hz, 1H), 3.09-3.13 (t, J= 6.6Hz, 2H), 3.20-3.29 (broad s, 10H), 3.70-3.74 (dd, J = 4.8, 8.0Hz, 1H); ^{13}C-NMR (75.43 MHz, CD_3OD) δ: 37.8, 38.8, 41.2, 45.6, 45.9, 47.9, 48.1, 48.2, 53.4, 79.7, 80.7, 157.2, 158.2, 175.6, 176.3.

Butyryl-L-tyr-L-asn-tri-Boc-polyamine343: In 1 mL DMF containing 0.142g (0.23 mmol) of **19** was added while stirring 90 mg (0.24 mmol) of butyryl-L-tyrosine p-nitrophenyl ester[21] dissolved in 1 mL DMF. This solution was stirred at room temperature for 24 hr before evaporation of the solvent. The slightly yellow oily residue was dissolved in 50 mL CH_2Cl_2. The organic solution was washed with sat. $NaHCO_3$ and brine. The organic layer was dried with $MgSO_4$ before filtration through a pad of Celite. The filtrate was concentrated in vacuo to yield the oily residue, which was chromatographed on a flash silica gel column with 9:1 CH_2Cl_2/CH_3OH, eluting 0.18g (92%) of the desired product as a clear oil. R_f = 0.48 in 10% CH_3OH/CH_2Cl_2. FAB-MS (3-nitrobenzyl alcohol matrix, $C_{42}H_{71}N_7O_{11}$): m/z 872$(M+Na)^+$, 850$(M)^+$, 750, 694, 650, 576, 550; ^{1}H-NMR (400 MHz, CD_3OD) δ: 0.82-0.89 (t, J = 7.2Hz, 3H), 1.40-1.44 (three s, 27H), 1.49-1.57 (m, 6H), 1.60-1.67 (broad s, 4H), 2.14-2.18 (t, J = 7.2Hz, 2H), 2.50-2.70 (m, 1H), 2.79-2.88 (two* dd, J = 8.0, 13.2Hz, 1H), 3.00-3.04 (m, 3H), 3.10-3.20 (broad s, 10H), 4.31-4.47 (m, 1H), 4.61-4.62 (m, 1H), 6.69-6.71 (d, J = 8.4Hz, 2H), 7.02-7.04 (d, J = 8.4Hz, 1H), 7.05-7.07 (d, J = 8.4Hz, 1H); ^{13}C-NMR (75.43 MHz, CD_3OD) δ: 14.0, 20.1, 26.7, 27.1, 28.5, 28.8, 29.3, 29.4, 29.6, 37.1, 37.4, 37.5, 38.5, 38.6, 38.9, 45.7, 46.0, 46.1, 47.9, 48.0, 48.1, 51.3, 51.5, 56.8, 57.6, 79.9, 80.9, 116.3, 128.9, 131.3, 157.4, 158.4, 172.7, 173.8, 175.0, 176.3. *1:1 conformational isomers[19]

Butyryl-L-tyr-L-asn-polyamine343 (9): The deprotection of N-Boc groups of butyryl-L-tyr-L-asn-tri-Boc-polyamine343 (0.13g, 0.15 mmol) was effected in 2 mL dry CH_2Cl_2 and 2 mL TFA in the same manner as described in the synthesis of argiotoxin-636. FAB-MS (3-nitrobenzyl alcohol matrix, $C_{27}H_{47}N_7O_5$): m/z 572$(M+Na)^+$, 550$(M)^+$, 476; ^{1}H-NMR (300 MHz, CD_3OD; TFA salt) δ: 0.81-0.89 (t, J =7.5Hz, 3H), 1.50-1.59 (quintet, J = 6.0Hz, 2H), 1.77-1.89 (m, 6H), 2.03-2.14(m, 2H), 2.15-2.20 (t, J = 7.8Hz, 2H), 2.48-2.67 (dd*, J = 6.0, 15.0Hz, and dd*, J = 8.0, 15.0Hz, 1H), 2.69-2.73 (t, J =8.0Hz, 1H), 2.80-2.94 (two* dd, J = 5.7, 13.8Hz, 1H), 2.95-3.15 (m, 13H), 3.20-3.34 (m, 2H), 4.28-4.45 (dd*, J = 6.0, 8.0Hz, and t*, J =8.0Hz, 1H), 4.57-4.64 (two* t, J =5.7Hz, 1H), 6.70-6.73 (two* d, J = 8.4Hz, 2H), 7.02-7.04 (d, J = 8.7Hz, 1H), 7.05-7.08 (d, J = 8.7Hz, 1H); ^{13}C-NMR (75.43 MHz, CD_3OD; TFA salt) δ: 13.9, 20.1, 20.2, 24.1, 25.3, 27.3, 36.9, 37.0, 37.2, 37.4, 37.8, 38.4, 38.6, 45.8, 46.0, 51.2, 51.5, 57.2, 57.7, 116.3, 128.8, 131.3, 157.3, 174.0, 174.3, 174.9, 176.7. *1:1 conformational isomers[19]

REFERENCES AND NOTES

1. (a) Kandel, E. R.; Schwartz, J. H. *Principles of Neural Science* ; Elsevier Science Publishing Co.: New York, 1985. (b) Braestrup, C.; Krogsgaard-Larsen, P. In *A Textbook of Drug Design and Development* , Krogsgaard-Larsen, P.; Bundgaard, H., Eds.; Harwood Academic Publishers: Chur, Switzerland, 1991; pp. 335-433. (c) In *Excitatory Amino Acid Receptors* , Krogsgaard-Larsen, P.; Hansen, J. J., Eds.; Ellis Horwood Limited: Chichester, England, 1992.
2. (a) Greenamyre, J. T.; Penny, J. B.; Young, A. B.; D'Amato, C. J.; Hicks, S. P.; Shoulson, I. *Science* **1985**, *227*, 1496-1499. (b) Simon, R. P.; Swan, J. H.; Griffiths, I. Meldrum, B. S. *Science* **1984**, *226*, 850-852.
3. (a) Brisson, A.; Unwin, P. N. T. *Nature* **1985**, *315*, 474-477. (b) Gaizi, J.- L.; Revah, F.; Bessis, A. Changeux, J.-P. *Annu. Rev. Pharmacol.* **1991**, *31*, 37-72.

4. (a) Aramaki, Y.; Yasuhara, T.; Higashijima, T.; Yoshioka, M.; Miwa, A.; Kawai, N.; Nakajima,T. *Proc. Japan. Acad. (Ser. B)* **1986**, *62*, 359-362. (b) Adams, M. E.; Carney, R. L.; Enderlin, F. E.; Fu, E. W.; Jarema, M. A.; Li, J. P.; Miller, C. A.; Schooley, D. A.; Shapiro, M. j.; Venema, V. J. *Biochem. Biophys. Res. Commun.* **1987**, *148*, 678-683. (c) Jasys, V. J.; Kelbaugh, P. R.; Nason, D. M.; Phillips, D.; Rosnack, K. J.; Saccomano, N. A.; Stroh, J. G.; Volkmann, R. A.*J. Am. Chem. Soc.* **1990**, *112*, 6696-6704, and references cited therein. (d) Quistad, G. B.; Suwanrumpha, S.; Jarema, M. A.; Shapiro, M. J.; Skinner, W. S.; Jamieson, G. C.; Lui, A.;Fu, E. W. *Biochem. Biophys. Res. Commun.* **1990**, *169*, 51-56.
5. Piek, T.; Mantel, P.; Engels, E. *Comp. Gen. Pharmacol.* **1971**, *2*,317.
6. (a) Eldefrawi, A. T.; Eldefrawi, M. E.; Konno, K.; Mansour, N. A.; Nakanishi, K.; Oltz, E.; Usherwood, P. N. R. *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 4910-4913. (b) Piek, T.; Fokkens, R. H.; Karst, H.; Kruk, C.; Lind, A.; Van Marle, J.; Nakajima, T.; Nibbering, N. M. M.; Shinozaki, H.; Spanjer, W.; Tong, Y. C. In *Neurotox '88: Molecular Basis of Drug & Pesticide Action* , Lunt, G. G., Ed.; Elsevier: Amsterdam, 1988; pp. 61-76. (c) Kenny, P. T. M.; Goodnow Jr., R. A.; Konno, K.; Nakanishi, K. *Rapid Commun. In Mass Spectrometry* **1989**, *3*, 295-297.
7. (a) Grishin, E. Y.; Volkova, T. M.; Arseniev, A. S.; Reshetova, O. S.; Onoprienko, V. V.; Magazanik, L. G.; Antonov, S. M.; Federova, I. M. *Bioorg. Khim.* **1986**, *12*, 1121. (b) Grishin, E. Y.; Volkova, T. M.; Arseniev, A. S. *Toxicon* **1989**, *27*, 541-549. (c) Adams, M. E.; Carney, R. L.; Enderlin, F. E.; Fu, E. W.; Jarema, M. A.; Li, J. P.; Miller, C. A.; Schooley, D. A.; Shapiro, M. j.; Venema, V. J. *Biochem. Biophys. Res. Commun.* **1987**, *148*, 678-683.
8. Chiles, G.; Choi, S.-K.; Elderfrawi, A.; Elderfrawi, M.; Fushiya, S.; Goodnow, Jr, R.; Kalivretenos, A.; Nakanishi, K.; Usherwood, P. In *Neurotox '91: Molecular Basis of Drug and Pesticide Action* , Duice, I. R., Ed.; Elsevier Applied Science: London, 1992; pp. 3-17.
9. (a) Nakanishi, N.; Shneider, N. A.; Axel, R. *Neuron* **1990**, *5*, 569-581. (b) Hollman, M.; Óshea-Greenfield, A.; Rogers, S. W.; Heineman, S. *Nature* **1989**, *342*, 643-648. (c) Moriyoshi, K.; Masayuki, m.; Takahiro, I.; Shigemoto, R.; Mizuno, N.; Nakanishi, S. *Nature* **1991**, *354*, 31-37.
10. Anis, N.S.; Goodnow, Jr., R.; Niwa, M.; Konno, K.; Kallimopoulos, T.; Bukownik, R.; Nakanishi, K.; Usherwood, P.; Eldefrawi, A.; Eldefrawi, M. *J. Pharm. Exp. Therapeutics* **1990**, *254*, 764-773.
11. Choi, S.-K.; Kalivretenos, A. G.; Nakanishi, K. manuscript in preparation.
12. Brackley, P. T. H.; Bell, D. R.; Choi, S.-K.; Nakanishi, K.; Usherwood, P. N. R. *J. Pharmacol. Exp. Ther.* **1992**, submitted.
13. Bruce, M.; Bukownik, R.; Eldefrawi, A. T.; Eldefrawi, M. E.; Goodnow, R.; Kallimopoulos, T.; Konno, K.; Nakanishi, K.; Niwa, M.; Usherwood, P. N. R. *Toxicon* **1990**, *28*, 1333-1346.
14. Goodnow Jr., R. A.; Nakanishi, K.; Sudan, H. L.; Usherwood, P. N. R. *Neuroscience Lett.* **1991**, *125*, 62-64.
15. (a) Jays, V. J.; Kelbaugh, P. R.; Nason, D. M.; Phillips, D.; Saccomano, N. A.; Volkman, R. A. *Tetrahedron Letters* **1988**, *29* , 6223-6226. (b) Shih, T. L.; Ruiz-Sanchez, J.; Mrozik, H. *Tetrahedron Letters* **1987**, *28*, 6015-6018.
16. Atwell, G. J.; Denny, W. A. *Synthesis* **1984**, 1032-1033.
17. Mckillop, A.; Swan, B. P.; Taylor, E. C. *J. Am. Chem. Soc.* **1971**, *93* , 4919-4920.
18. The synthetic ArgTX-636 (TFA salt) was confirmed by FAB-MS, and ^{1}H- and ^{13}C-NMR spectra (CD_3OD) which were consistent with those (HCl salt, DMSO-d_6) of ref. 14 (a), although slight variations were observed possibly due to differences in NMR solvents and types of polyamine salts.
19. The α-methine NMR signal (in CD_3OD, asterisked signal in Experimental) of the asn moiety in analogs **6**, **8** and **9** with the alkanoyl-tyr-asn-polyamine structure showed that they existed as a 1:1 mixture of conformers. This was not the case with analog **7** with the alkenoyl-tyr-asn-polyamine strucutre. We cannot account for this difference in the NMR signals.
20. 2,4-Dibenzyloxyacetophenone was obtained after 24 hr reflux of the mixture containing 2,4-dihydroxyacetophenone (1.0 eq), benzylbromide (2.1 eq) and K_2CO_3(2.5 eq) in acetone. R_f = 0.63 in 1:2 EtOAc/hexane.
21. Goodnow, Jr., R.; Konno, K.; Niwa, M.; Kallimopoulos, T. A.; Bukownik, R.; Lenare, D.; Nakanishi, K. *Tetrahedron* **1990**, *46* , 3267-3286.
22. 2,4-Hexadienoyl-p-azido-L-phenylalanine p-nitrophenylester was prepared from N-Boc-p-azido-L-phenylalanine p-nitrophenyl ester as in the synthesis of butyryl-L-tyrosine p-nitrophenyl ester.
23. Choi, S.-K.; Goodnow, Jr., R.; Kalivretenos, A.; Chiles, G. W.; Fushiya, S.; Nakanishi, K. *Tetrahedron* **1992**, *48* , 4793-4822.