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SYNTHESIS OF GLUTAMATE RECEPTOR ANTAGONIST PHILANTHOTOXIN-433 (PhTX-433) AND ITS ANALOGS*

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ABSTRACT

The synthesis of the non-competative glutamate receptor inhibitor, philanthotoxin 433 (PhTX-433) is described. Furthermore, two synthetic routes are described for the preparation of a variety of structural analogs.

INTRODUCTION

Glutamate receptors are known to serve as the principal receptors for excitory neurotransmitters in mammalian brain and central nervous system. These receptors are grouped into three sub-groups based on their selective response to exogenous agonists: N-methyl-D-aspartate (NMDA), kainic acid, and quisqualic acid. The receptors are believed to be part of the processes involved in memory and learning as well as in neurodegenerative diseases such as Huntington's and Alzheimer's diseases.[1] In the past the pharmacological study of glutamate receptors[2,3] has been somewhat limited by the unavailability of selective glutamate receptor antagonists, especially for quisqualate and kainate sub-type receptors. The recent discovery that the venoms of certain spiders[4,5,6] and wasps[7] exert inhibitory effects on vertebrate and invertebrate neurons and muscle fibers has focused a great deal of attention on the isolation and synthesis of the active components of these toxins. In the case of the Egyptian desert solitary digger wasp, *Philanthus triangulum* the venom mixture was characterized by Piek[8,9] before isolation, structure determination and synthesis of philanthotoxin 433 (PhTX-433) **1**.[10,11] Here we report the synthesis of PhTX-433, as well as two general methods for the synthesis of a variety of analogs. The analogs are required for structure-activity studies and for securing radioactive compounds that can be used for isolating the glutamate receptor by (photo)affinity labelling.[12]

*Dedicated to Prof. Yu Wang on the occasion of his 80th birthday.

Synthesis of PhTX-433

Efforts toward structure determination of the most active component of the vemon mixture using UV, ^1H NMR, and FAB-fragment MS clarified the general structure of the butyryl / tyrosyl / polyamine sequence in which the polyamine fragment consisted of 10 methylene groups. However, because of the relatively limited amount of the toxin available (ca. 1 mg) these spectroscopic methods did not unambiguously determine the sequence of the methylene groups, thus leaving three possible structural isomers shown in Fig. 1. As a result all three isomers, PhTX-433 **1**, PhTX-343 **2** and PhTX-334 **3** were synthesized as shown in Fig. 2. Only recently, a mass spectral technique of B / E linked scanning has made this type of differentiation possible.[13]

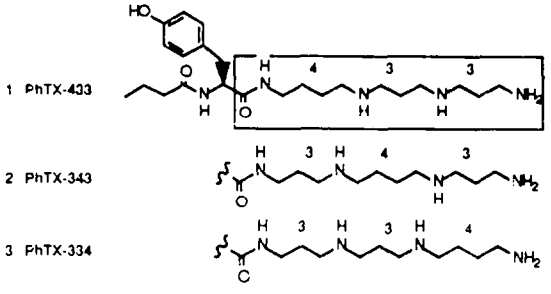


Fig. 1

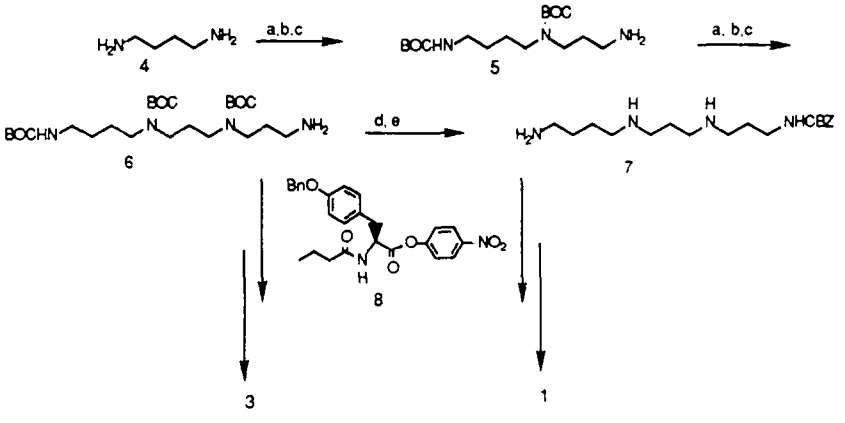


Fig. 2 a acrylonitrile, CH_3OH b. $(BOC)_2O$, CH_2Cl_2; c. $LiAlH_4$, Et_2O, d. CBZCl, Et_3N, $CHCl_3$; e. TFA, $CHCl_3$

PhTX-343 **2** was obtained by coupling of commercially available spermine (343) and N-butyryl-O-benzyl-L-tyrosine *p*-nitrophenol ester **8** and subsequent hydrogenolysis of the tyrosyl-hydroxy protecting group. For the syntheses of PhTX-433 **1** and PhTX-334 **3**, the assymetric polyamine moieties were first synthesized as shown by repetition of the three step sequence: (i) Michael addition to acrylonitrile; (ii) tert-butoxycarbonyl (BOC) protection; and (iii) lithium aluminum hydride reduction. Coupling of the resulting amine **6** to the tyrosyl ester **8** followed by deprotection gave PhTX-334 **3**. Further protection of the free amino terminal of **6** with carbobenzyloxy (CBZ) and removal of the BOC groups with trifluoroacetic acid yielded polyamine **7** necessary for PhTX-433 **1**. Synthetic PhTX-433 was found to be identical with the natural product in all respects, i.e., ^{1}H NMR, MS, CD, HPLC reverse phase elution, and biological activity. The physiological activity of PhTX-343 **2** and PhTX-334 **3** were, respectively, 80% and 125% that of natural PhTX-433 **1** as measured by the locust muscle twitch inhibition concentration. The philanthotoxins have also been shown to exert allosteric inhibition of nicotinic acetycholing receptors of vertebrates and insects,[14] and glutamate receptors of rat brain.[15]

Synthesis of PhTX-343 Type Analogs

A structure-activity study was undertaken in order to increase the inhibitory effect of an analog at a particular concentration (IC_{50}) in the locust muscle assay, operating on the assumption that an increase in activity would be observed as inhibition of muscle contraction at lower ligand concentrations. Spermine was used for the synthesess of most analogs because the biological activity of PhTX-343 was similar to that of natural PhTX-433 (80%) and because of its commercial availability. Furthermore, its symmetric structure makes it unnecessary to differentiate the two terminal amino groups when coupling to the *p*-nitrophenol activated esters; apparently only primary amimes were reactive in this coupling since no product arising from the reaction of secondary amines could be detected.

The majority of analogs were synthesized according to Method A shown in Fig. 3 with only slight modifications if necessary. Taking into account the structural similarity between PhTX-433 and other neurologically active spider toxins, the molecule was divided into four moieties, A, B, C and D (Fig. 4) in order to assess the structure activity relation in a systematic manner. In Moiety A the butyryl moiety of the natural PhTX-433 suggests the possible necessity of a hydophobic region in the molecule. Thus, analogs **15** - **20** were synthsized by exchanging butyryl chloride with the appropriate acyl chloride. In order to investigate the affect of the tyrosyl moiety in Moiety B, analogs **21** - **27** were prepared by the coupling of spermine and the p-nitrophenol esters of the corresponding N-butyryl amino acids. Analog **22**[16] was obtained by treating N-BOC-tyrosine with acetic anhydride and triethyl amine before proceeding with Method A. Analogs **28** and

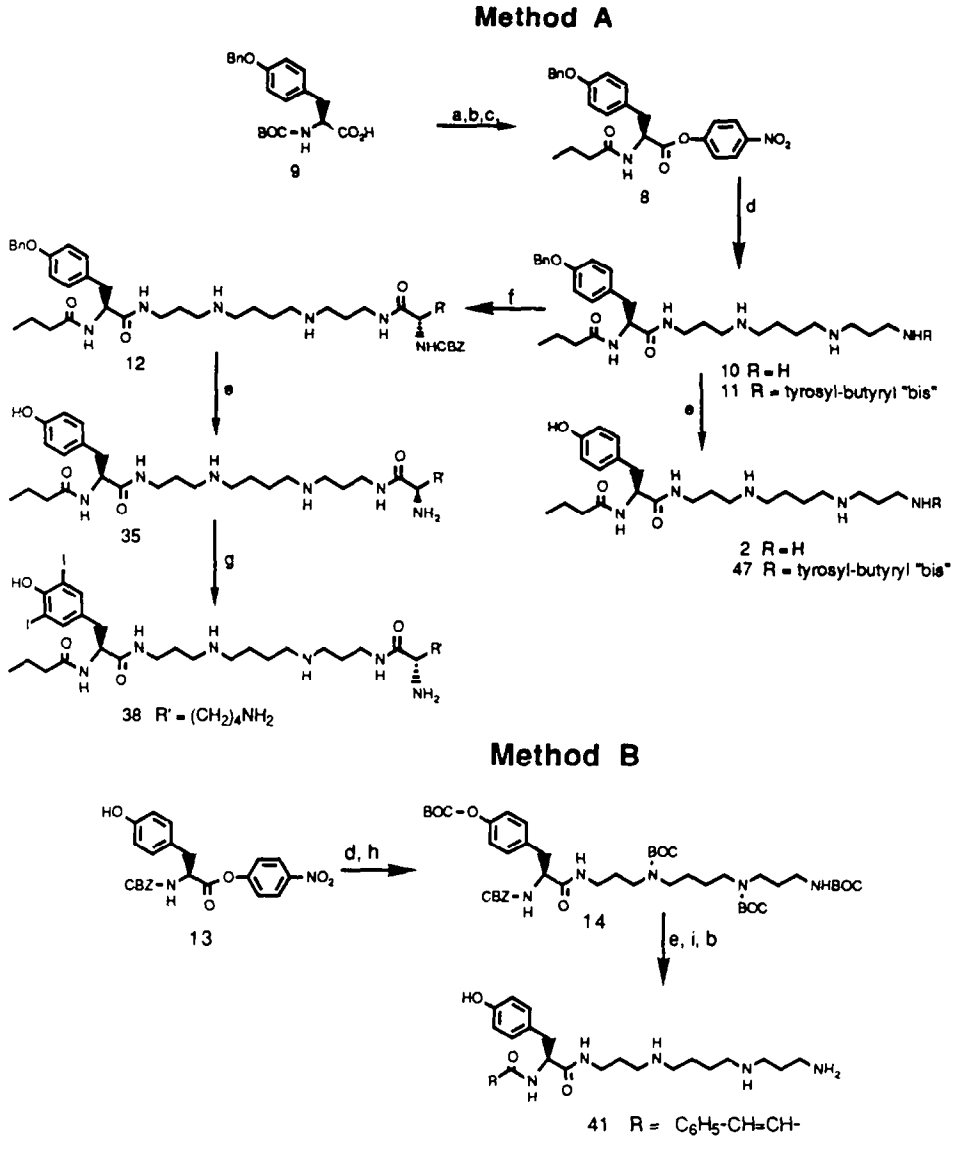


Fig 3

a. *p*- nitrophenol, DCC, EtOAc; b. TFA, $CHCl_3$; c. Et_3N, butyryl chloride, $CHCl_3$; d spermine, CH_3OH;
e. H_2, 5% Pd / C, CH_3OH f.Nα,ε-di-CBZ-L-lysine *p*-nitrophenol ester , DMF;
g NBS, KI, K_2HPO_4, CH_3OH/H_2O (5:1); h. $(BOC)_2O$, CH_3OH, pyridine (cat.); i. cinnamoyl chloride, Et_3N, $CHCl_3$

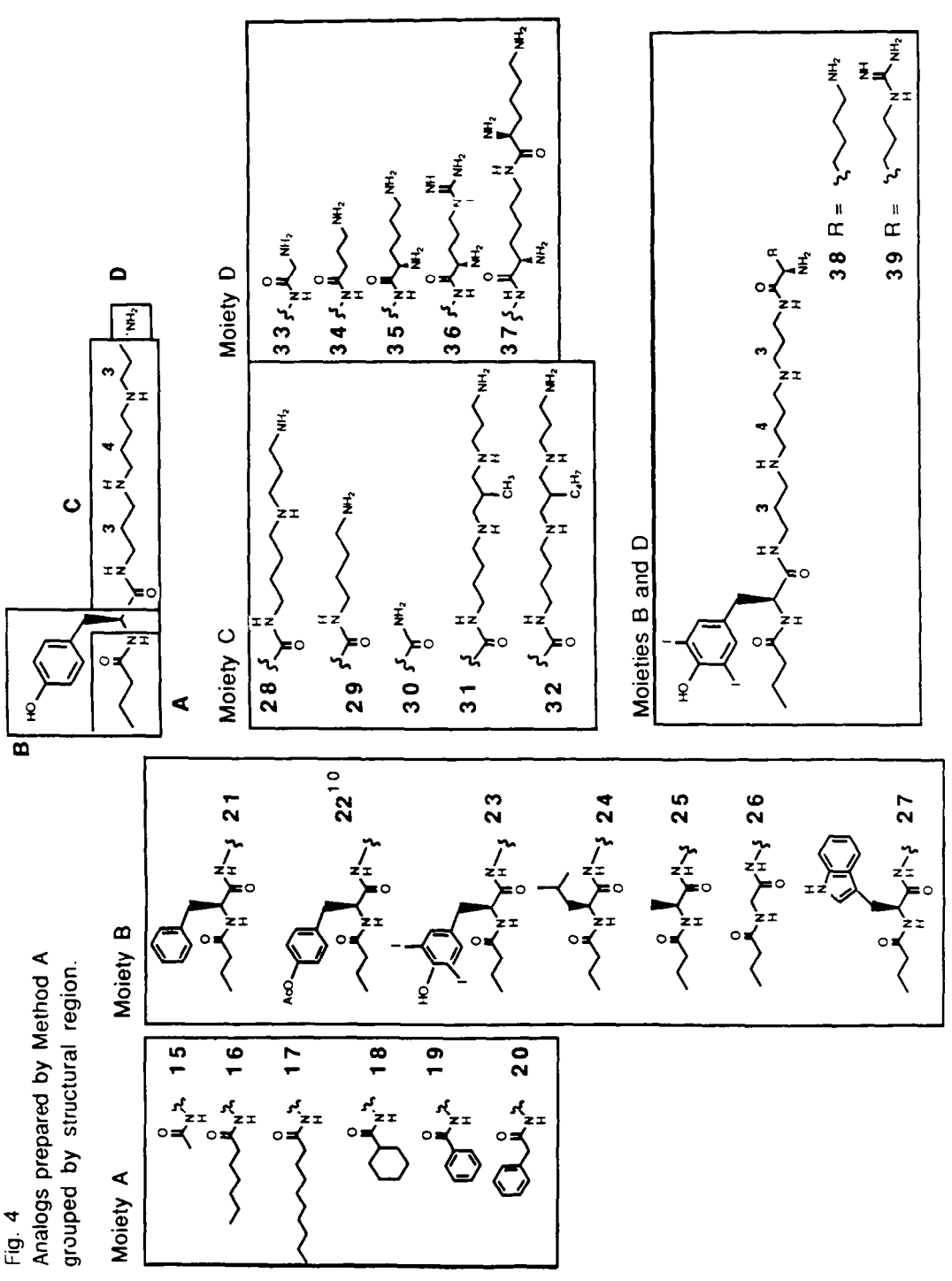


Fig. 4
Analogs prepared by Method A grouped by structural region.

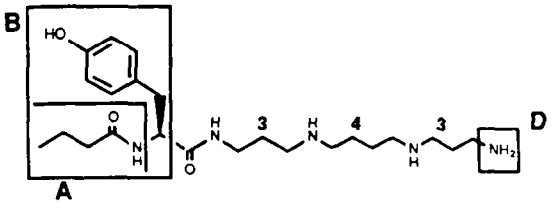
29 of Moiety C were obtained through coupling of polyamine intermediates with **8** and hydrogenolysis; mixing of **8** with ammonium acetate and hydrogenolysis yielded **30**. Analogs **31** and **32** with branchings were prepared in order to examine the effects of alternating hydrophobic and hydrophilic regions and branching in the polyamine moiety; if active, a group suited for affinity binding to a solid support could be linked to the terminal of the branching. They were made by coupling of ester **8** and an alkylated 433-type polyamine; such alkyl polyamines were synthesized in the same manner as thermospermine (433) except that the an alkyl group was introduced by bromo-alkyl quenching of the lithiated anion α to the nitrile of the BOC-protected Michael adduct of diamino butane and acrylonitrile. These intermediates were then transformed into polyamines analogous to polyamine **7**.

Arginine and other amino acids were linked to Moiety D, since in the spider toxins argiopine,[4] NSTX-3,[5] argiotoxin 659,[6] and argiotoxin 673,[6] which show similar inhibition of locust muscle contraction,[3] the polyamine moiety contains an additional arginine residue. Thus analogs **33** - **37** were synthesized by coupling O-benzyl-PhTX-343 with the p-nitro-phenol esters of the corresponding amino acids, or in the case of **36**, with commercially available $(CBZ)_3$-arginine N-hydroxysuccinimide ester followed by deprotection under hydrogenolysis conditions.

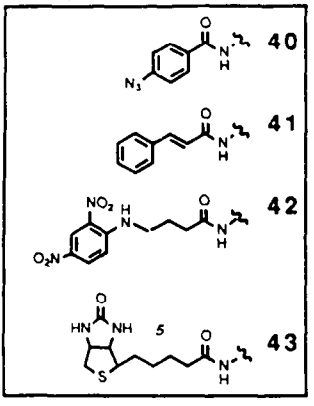
Analogs **40** - **45** in Fig. 5 were prepared to check the possibility of converting Moiety A into groups that could be used for photoaffinity labelling (**40**, **44**, **45**) or affinity labelling (**42**, **43**). As these functionalities are sensitive to the hydrogenolysis conditions employed for O-benzyl deprotection, they were synthesized according to Method B shown in Fig. 3. Thus N-carbobenzyloxylation (Method B, **13**) instead of N-butoxycarbonylation (Method A, **9**) allowed hydrogenolysis to be performed prior to attachment of the functionality sensitive to reduction. N-tyrosyl acylation was achieved with either the free acid and diphenylphosphoryl azide or with the N-hydoxysuccinimide ester, depending on availability; in all cases the N-tyrosyl acylation proceeded the BOC deprotection step.

The coupling reaction of *p*-nitrophenol esters with spermine (Method A, Fig. 3) invariably led to some formation of bis adducts **11**, i.e., spermines acylated on both amino terminals. These bis analogs were easily separated, and upon hydrogenolysis yielded a series of bis-type PhTX analogs (**46** - **50**), Fig. 6. These anaolgs were used in oder to determine whether the effect of PhTX-type molecules on receptor/membrane complexes is a channel-blocking mechanism or a membrane stabilization mechanism. It was also of interest to investigate the biological activities of simple mono- and bis-acylated spermine-343 molecules. Thus three sets of mono- and bis-acyl spermine analogs (**51** - **56**), Fig. 6, were similarly synthesized by reacting the appropriate *p*-nitrophenol esters with excess spermine.

Fig. 5
Analogs prepared by Method B grouped by structural region.



Moiety A



Moieties A, B, and D

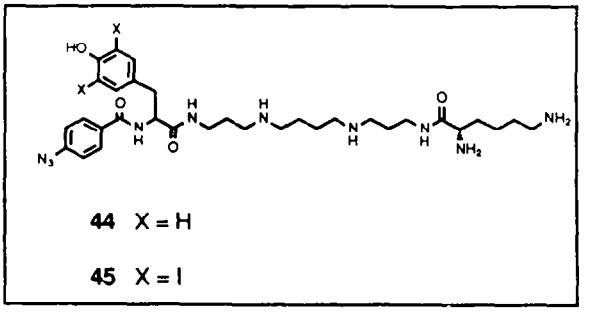
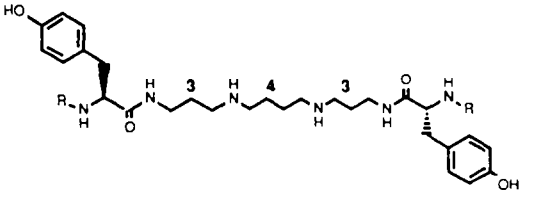
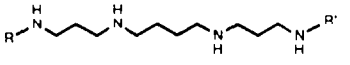


Fig. 6



46 R = CH_3CO, 47 R = n-C_3H_7CO, 48 R = n-$C_6H_{13}CO$
49 R = n-$C_9H_{19}CO$, 50 R = C_6H_5CO



51 R = n-C_3H_7CO, R' = H 52 R = n-$C_6H_{13}CO$, R' = H 53 R = n-$C_9H_{19}CO$, R' = H
54 R = R' = n-C_3H_7CO 55 R = R' = n-$C_6H_{13}CO$ 56 R = R' = n-$C_9H_{19}CO$

Finally, radio-labelled analogs are necessary both for use in direct pharmacological characterization of receptors as well as for isolation of the glutamate receptor by photoaffinity labelling or affinity labelling. It was fortunate that introduction of iodine, which we had hoped to use for radio-labelling of the tyrosyl moiety, also increased the biological activity approximately ten-fold. Cold iodinated analogs (**23, 38, 39, 45**) were prepared by use of NBS and KI on a milligram scale, while radioactive ^{125}I analogs were prepared with $Na^{125}I$ and chloramine T in buffered solution on a micro-scale and purified by reverse phase HPLC.[17]

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Experimental

CI-MS (NH_3) spectra were obtained on a Nermag-10 while FAB-MS (3-nitrobenzyl alcohol matrix) spectra were obtained with a JOEL DX-303. Proton NMR spectra were recorded on a Bruker WM-250 instrument using residual proton solvent peaks of either $CDCl_3$ at 7.24 ppm or CD_3OD at 4.68 ppm as an internal standard. NMR spectra were measured in CD_3OD and as free bases unless specified. The solvents DMF and *i*-$PrNH_2$ and the reagents Et_3N and pyridine were distilled at atmospheric pressure over CaH_2. Acrylonitrile was distilled neat at atmospheric pressure. HPLC was used to identify the correct isomer of the natural product with the following column and conditions. Column: YMC-ODS, 4.6 x 250 mm; solvent: (12.5%CH_3CN, 0.1% TFA)/H_2O; flow rate: 1mL / min.; dectection: 274 nm.

2-(Diaminobutyl)-ethylnitrile

Acrylonitrile (3.1 g, 58.4 mmol) in 1.5 mL CH_3OH solution was added to a 1.5 mL CH_3OH solution of diaminobutane (4.3 g, 48.8 mmol) at 0°C and was stirred for 12 hr. The reaction was terminated by evaporation of the solvent and the oil was applied directly to a silica gel flash column, eluting with 3:1 $CHCl_3$:CH_3OH and15:5:1, $CHCl_3$ / CH_3OH / *i*-$PrNH_2$. The product was obtained as a clear oil in 65 % yield. CI-MS ($C_7H_{15}N_3$): *m/z*

142 $(M+1)^+$; NMR: δ 1.32 (4H, complex) 1.53 (2H, br s), 1.65 (1H, s), 1.82 (1H, s), 2.35 (2H, t, J = 6.6 Hz), 2.46 (3H, complex), 2.75 (2H, t, J = 6.6Hz).

2-(N,N'-di-BOC-diaminobutyl)-ethylnitrile

A solution of 2.82 g (20.0 mmol) of the above ethylnitrile and 4.8 g (22 mmol) of BOC anhydride in 70 mL of CH_2Cl_2 was stirred at room temperature for 12 hr. The reaction was worked up by pouring the mixture into water and extracting with EtOAC three times. The combined organic layers were washed with aqueous $NaHCO_3$ and saturated NaCl solutions. After drying the solution over $MgSO_4$ and evaporating the solvent, the crude oil was chromatographed on silica with $CHCl_3$, followed by 1% CH_3OH / $CHCl_3$, yielding 4.2 g (75%) of the desired product. CI-MS ($C_{17}H_{31}N_3O_4$): *m/z* 342 $(M+1)^+$; NMR: δ 1.40 (9H, s) 1.43 (9H, s) 2.57 (2H, br s), 3.08 (2H, q, J = 6.1Hz), 3.24 (2H, t, J = 7.4Hz), 3.42 (2H, t, J = 6.7Hz), 4.58 (1H, br s).

N',N"-di-BOC-polyamine-34 (5)

To a suspension of 0.062 g (1.63 mmol) of lithium aluminum hydride (LAH) in 10 mL of Et_2O was added 0.158 g (0.463 mmol) of the above nitrile at 0°C, and the mixture was stirred at 0°C for 30 min. The excess LAH was quenched with 1 N NaOH at 0°C and the resulting white suspension was filter through celite and washed with Et_2O. The filtrate was washed with water and the water layers were extracted with Et_2O. The combined Et_2O layers were washed with brine, dried over $MgSO_4$, and evaporated to yield the 0.108 g (68%) of the crude oil that was carried on to the next reaction without further purification. CI-MS ($C_{17}H_{35}N_3O_4$): *m/z* 346 $(M+1)^+$; NMR: δ 1.37 (9H, s), 1.38 (9H, s), 2.61 (2H, t, J = 6.7Hz), 3.06 (4H, t, J = 6.7Hz), 3.18 (2H, br s), 4.65 (1H, br, s).

Di-BOC-polyamine-34-ethylnitrile

A mixture of 2.20 g (6.38 mmol) of (**5**) and 0.63 mL (9.57 mmol) of acrylonitrile in 10 mL of CH_3OH was stirred at room temperature for 12 hr. The reaction was worked up by evaporation of solvent and chromatographed on silica gel with 1 % then to 2 % CH_3OH / $CHCl_3$ from which was obtained 2.47 g (97 %) of the desired product. CI-MS ($C_{20}H_{38}N_4O_4$): *m/z* 399 $(M+1)^+$; NMR: δ 1.39 (9H, s), 1.40 (9H, s), 1.65 (2H, quintet, J = 6.9Hz), 2.47 (2H, t, J = 6.8Hz), 2.58 (2H, t, J = 6.8Hz), 2.87 (2H, t, J = 6.8Hz), 3.10 (4H, t, J = 6.3Hz), 3.20 (1H, br s), 4.58 (1H, br s).

Tri-BOC-polyamine-34-ethylnitrile

To a 20 mL CH_3OH solution of the above di-BOC-nitrile 2.47 g (6.21 mmol) was added 1.62 g (7.45 mmol) of BOC anhydride. This mixture was stirred for 12 hr. The reaction was worked up in the same manner as for 2-(Di-N,N'-BOC-diaminobutyl)-ethylnitrile yielding 3.06 g (98%). CI-MS ($C_{25}H_{46}N_4O_6$): *m/z* 499 $(M+1)^+$; NMR: δ 1.39 (9H, s), 1.40 (9H, s),

1.42 (9H, s), 1.71 (2H, quintet, J = 7.4Hz), 2.57 (2H, br s), 3.12 (4H, complex), 3.22 (2H, t, J = 7.3Hz), 3.43 (2H, t , J = 6.7Hz), 4.61 (1H, br s).

2',3',4'-tri-N-BOC-thermospermine (6)

A $CHCl_3$ solution of the above tri-BOC-nitrile, 5.2 g (10.1 mmol) was treated with 2.4 g of LAH as described in the procedures for synthesis of (**5**). Polyamine (**6**) was obtained after silica column chromotography, 1:10 CH_3OH / $CHCl_3$ in 91% yield (4.61 g).

4'-N-CBz-1',2',3'-tri-N-BOC-thermospermine (7)

To a 10 mL $CHCl_3$ solution of 1 g (2.0 mmol) of (**6**) and Et_3N (0.33 mL, 2.4 mmol) was added 0.34 mL (2.4 mmol) of CBZ-Cl and this mixture was stirred for 30 min at room temperature. The mixture after evaporation of the solvent was directly chromatographed on silica with 1% CH_3OH / $CHCl_3$. The desired product was obtained in 94% (1.12 g) yield. CI-MS ($C_{33}H_{56}N_4O_8$): *m/z* 637 $(M+1)^+$; NMR: δ 0.79 (27H, s), 1.06 (4H, quintet, J = 6.8Hz), 2.46 (12H, complex), 4.41 (2H, s), 6.68 (5H, m).

Method A

N-BOC-O-benzyl-L-tyrosine-*p*-nitrophenol ester

To a solution of 3.33 g (9.0 mmol) of N-BOC-O-benzyl-L-tyrosine (**9**) (Sigma) in 35 mL of EtOAc was added 1.25 g (9.0 mmol) of *p*-nitrophenol and 1.95 g (9.45 mmol) dicyclodicarbodiimide. The solution was stirred at room temperature for 1.5 hr and then filtered through celite. The resulting filtrate was extracted with water and sat. $NaHCO_3$. The aqueous extracts were then extracted three times with EtOAc. The combined organic layers were shaken 3 times with saturated NaCl solution, dried over $MgSO_4$, filtered, and evaporated to a slightly yellow, white powder. The powder was recrystallized from EtOH to yield after filtration and washing with cold EtOH 3.44 g (78%) of a white powder. EI-MS ($C_{27}H_{28}N_2O_7$): *m/z* 492 (M^+); NMR: δ 1.44 (9H, s), 3.15 (2H, d, J = 5Hz), 4.73 (3H, t, J = 5Hz), 5.10 (2H, s), 6.94 (2H, d, J = 10.4Hz), 7.12 (4H, d, J = 10.4Hz), 7.38 (5H, m), 8.22 (2H, d, J = 10.4Hz).

N-butyryl-O-benzyl-L-tyrosine-*p*-nitrophenol ester (8)

To a solution of 2.95 g (6.0 mmol) of N-BOC-O-benzyl-L-tyrosine-*p*-nitrophenol ester in 30 mL of $CHCl_3$ was added 15 mL of trifluoroacetic acid (TFA) and this mixture was stirred at room temperature. After roughly 2 hr when all of the starting material was consumed according to TLC (silica, 35% EtOAc/hexane), the solution was evaporated to dryness. The resulting solid was suspended in 10 mL of $CHCl_3$ with stirring and to this suspension was

added simultaneously 0.75 mL (7.20 mmol) of butyryl chloride and 2.50 mL (18.0 mmol) of Et_3N. The slightly yellow solution was stirred at room temperature. After 90 min the solution was evaporated to a slightly yellow solid and recrystallized with EtOH or chromatographed on silica with $CHCl_3$ yielding 2.0g (72%) of the desired product. CI-MS ($C_{26}H_{26}N_2O_6$): *m/z* 463 $(M+1)^+$; NMR: δ 1.05 (3H, t, J = 7.8Hz), 1.80 (2H, m, J = 5.7Hz), 2.58 (2H, t, J = 5.7Hz), 3.3 (2H, m), 5.15 (1H, m), 5.18(2H, s), 6.94 (2H, d, J = 10.4Hz), 7.12 (4H, d, J = 10.4Hz), 7.38 (5H, m), 8.25 (2H, d, J = 10.4Hz).

N-butyryl-O-benzyl-L-tyrosine-spermineamide (10) and Bis[N-butyryl-O-benzyl-L-tyrosine]-spermineamide (11)

To a 10 mL CH_3OH solution of 0.36 g (0.78 mmol) of (**8**) was added dropwise a 10 mL CH_3OH solution of 0.19 g (0.94 mmol) of spermine with stirring at room temperature. After 1 hour, the reaction mixture was evaporated to a yellow, semi-crystalline oil and 10 mL of $CHCl_3$ / CH_3OH (1:1) was added to enhance crystallization of the *p*-nitrophenol. This suspension was filtered through celite and washed with 10 mL of $CHCl_3$ / CH_3OH (1:1) solution. The filtrate was evaporated to a clear yellow oil and then chromatographed on 25 g of silica with a step gradient system of 9:1 $CHCl_3$ / CH_3OH, 15:5:1 $CHCl_3$ / CH_3OH / *i*-$PrNH_2$ eluting the "Bis" adduct (**11**) 0.003 g (20 %). CI-MS ($C_{50}H_{68}N_6O_4$): *m/z* 849 $(M+1)^+$; NMR: δ 0.63 (6H, t, J = 5.2Hz), 1.32 (4H, q, J = 6.8 Hz), 1.94 (4H, t, J = 7.8Hz), 4.25 (2H, t, J = 7.8Hz), 4.83 (4H, s), 6.70 (4H, d, J = 8.3Hz), 6.93 (4H, d , J = 8.3Hz), 7.19 (10H, m). The column elution was continued with 4:4:1 $CHCl_3$ / CH_3OH / *i*-$PrNH_2$ yielding 0.127 g (38.4%) of a clear, light yellow oil (**10**). CI-MS ($C_{30}H_{47}N_5O_3$): *m/z* 526 $(M+1)^+$; NMR: δ 0.72 (3H, t, J = 5.2Hz), 1.50 (10H, complex), 2.01 (2H, t, J = 5.2Hz), 2.55 (12H, complex), 4.48 (1H, t, J = 7.8Hz), 4.92 (2H, s), 6.88 (2H, d, J = 8.3Hz), 7.04 (2H, d, J = 8.3Hz), 7.27 (5H, m).

PhTX-343 (2)

To a 15 mL CH_3OH solution containing 0.20 g of (**10**) was added 0.2 g of 5% Pd/C. This solution was purged several times with hydrogen. The starting material was usually consumed after 2 to 3 hr. The reaction was terminated by filtration through celite and careful washing of the carbon with copious volumes of CH_3OH. After evaporation of the solvent, the clear oil was chromatographed on 10 g of silica with 10:1 $CHCl_3$ / CH_3OH and 4:4:1 $CHCl_3$ / CH_3OH / *i*-$PrNH_2$. The desired product, 0.164 g (99%) was obtained as a clear oil. CI-MS ($C_{23}H_{41}N_5O_3$): *m/z* 436 $(M+1)^+$; NMR: δ 0.74 (3H, t, J = 5.2Hz), 2.05 (2H, t, J = 5.2Hz), 4.33 (1H, t, J = 5.2Hz), 6.58 (2H, d, J = 7.8Hz), 6.94 (2H, d, J = 7.8Hz); HPLC retention time: 8.30 min, nautral product 9.63 min.

Bis-PhTX-343 (47)

A 10 mL CH_3OH solution of 0.208 g (0.245 mmol) of (**11**) was treated in the same manner as for the synthesis of (**2**) above with 0.05 g of 5% Pd/C. The reaction mixture was purified

on a silica flash column with 15:5:1 $CHCl_3$ / CH_3OH / *i*-$PrNH_2$ yielding 0.124 g (76%) of the desired product. CI-MS ($C_{36}H_{56}N_6O_6$): *m/z* 669 $(M+1)^+$; NMR: δ 0.68 (6H, t, J = 5.2Hz), 1.98 (4H, t, J = 5.2Hz), 4.52 (2H, t, J = 5.5Hz), 6.77 (4H, d, J = 8.8Hz), 7.12 (4H, d, J = 8.8Hz).

O-benzyl-PhTX-di-2',3'-N,N-BOC-334

To a stirred solution of 0.022 g (0.44 mmol) of (**6**) in 0.4 mL of CH_3OH was added 0.018 g (0.04 mmol) of (**8**) and the mixture was stirred for 15 min. After evaporation of the solvent, the mixture was loaded onto a silica flash column and eluted with 1% CH_3OH / $CHCl_3$ yielding 13 mg (39%) of the desired product.

PhTX-334 (3)

To a stirred solution of.195 g (0.25 mmol) of O-benzyl-PhTX-di-2',3'-N,N-BOC-334 in 3 mL of $CHCl_3$ was added 3 mL of TFA and this mixture was stirred at room temperature for 15 min. After evaporation of the solvent, the crude oil was loaded onto a silica flash column and eluted with a step gradient of 15:5:1 and 3:3:1 $CHCl_3$ / CH_3OH / *i*-$PrNH_2$ which yielded 0.072 g (67%) of the desired product. This pure free amine was dissolved in 3 mL of CH_3OH and this solution was stirred with 0.07 g of 5% Pd / C under hydrogen atmosphere at room temperature for 12 hr. The reaction was terminated by filtration and washing through celite with CH_3OH followed by removal of solvent *in vacuo* and then loading onto a silica flash column, eluting with a step gradient of 15:5:1 and 3:3:1 $CHCl_3$ / CH_3OH / *i*-$PrNH_2$ yielding 0.045 g (75%) of the desired product as a clear oil. CI-MS ($C_{23}H_{41}N_5O_3$): *m/z* 436 $(M+1)^+$; NMR: δ 0.67 (3H, t, J = 7.4Hz), 1.35 (2H, sextet, J = 7.4Hz), 1.99 (2H, t, J = 7.3Hz), 4.20 (1H, t, J = 7.5Hz), 6.52 (2H, d, J = 8.3Hz), 6.86 (2H, d, J = 8.3Hz); HPLC retention time: 8.43 min, natural product 9.63 min.

O-benzyl-PhTX-4'-N-CBZ-433

To a stirred solution of 244 mg (0.52 mmol) of (**8**) in 1 mL of CH_3OH was added 200 mg (0.65 mmol) of (**7**) in 1 mL of CH_3OH. This mixture was stirred for 15 min at room temperature. After evaporation of the solvent, the crude yellow oil was loaded onto a silica gel column and the desired product was eluted with a step gradient of 2% CH_3OH / $CHCl_3$ and 15:5:1 $CHCl_3$ / CH_3OH / *i*-$PrNH_2$ which yielded 5 mg (23%).

PhTX-433 (1)

A mixture of 310 mg (0.47 mmol) of O-benzyl-PhTX-4'-N-CBZ-433 and 310 mg of 5% Pd/C in 1 mL of CH_3OH was stirred under hydrogen atmosphere for 12 hr. The mixture was then filtered and washed through celite with CH_3OH before loading onto a silica flash column and eluting with a step gradient of 15:5:1 and 3:3:1 $CHCl_3$ / CH_3OH / *i*-$PrNH_2$. Thus 100 mg (49%) of the desired compound was obtained in the form of a clear oil. CI-MS ($C_{23}H_{41}N_5O_3$): *m/z* 436 $(M+1)^+$; NMR: δ 0.58 (3H, t, J = 7.4Hz), 1.90 (2H, t, J = 7.2Hz),

4.16 (1H, t, J = 8.4Hz), 6.44 (2H, d, J = 8.4Hz), 6.78 (2H, d, J = 8.4Hz); HPLC retention time: 9.63 min, natural product 9.63 min, co-injection of synthetic and natural products eluted as one peak at 9.63 min.

Analog Synthetic Procedures

Moiety D

(i) O-benzyl-PhTX-343-N-α-N^G,$N^{G'}$-tri-CBZ-L-arginine-amide (12)

To a 5 mL DMF solution of 0.452 g (0.816 mmol) of (**10**) was added 0.58 g (0.86 mmol) of N-α-N^G,$N^{G'}$-tri-CBZ-L-arginine-N-hydroxysuccinimide ester (Bachem), and this solution was stirred overnight at room temperature. The reaction was worked up by evaporation of the solvent and extraction of the slightly yellow oil with $CHCl_3$ and washing the oraganic extracts with aqueous $NaHCO_3$, water, and brine. The crude product was chromatographed on silica with 9:1 $CHCl_3$ / CH_3OH and 15:5:1 $CHCl_3$ / CH_3OH / *i*-$PrNH_2$ to yield 0.817 g (91%) of the desired product. NMR: δ 0.82 (3H, dt*, J = 1.5, 7.5Hz), 2.11 (2H, complex*), 4.25 (1H, br s*), 4.52 (1H, br s*), 4.52 (1H, br s*), 5.0 - 5.2 (8H, complex*), 6.85 (2H, d, J = 8.6Hz), 7.10 (2H, d, J = 8.6 Hz), 7.30 (20H, complex). *Complex couplings were due to a mixture of conformational isomers.

(ii) PhTX-343-L-arginine-amide (13)

To a 10 mL CH_3OH solution of 0.81 g (0.74 mmol) of (**12**) was added 0.05 g of 5% Pd/C followed by hydrogenolysis at room temperature overnight. The reaction mixture was filtered and washed through celite with approximately 20 mL of CH_3OH. The crude oil was passed through a column of 12 g of silica eluting with a step gradient of 1:1 CH_3OH / $CHCl_3$, 2:2:1 $CHCl_3$ / CH_3OH / *i*-$PrNH_2$ and 2:2:1:1 $CHCl_3$ / CH_3OH / *i*-$PrNH_2$ / H_2O. After evaporationg of the eluant solvent, the precipitated silica was filtered off and washed thoroughly with 1:1 CH_3OH / $CHCl_3$. The desired product was obtained as a clear foam, 0.25 g (56%). CI-MS ($C_{29}H_{53}N_9O_4$): *m/z* 592 $(M+1)^+$; NMR: δ 0.53 (3H, t, J = 7.4Hz), 4.15 (1H, t, J = 7.5Hz), 6.37 (2H, d, J = 8.2Hz), 6.71 (2H, d, J = 8.2Hz).

Moiety A

(i) N-decanoyl-O-benzyl-L-tyrosine-*p*-nitrophenol ester

To a solution of 1.5 g (3.05 mmol) of N-BOC-O-benzyl-L-tyrosine-*p*-nitrophenol in 15 mL of $CHCl_3$ was added 8 mL of TFA and this mixture was stirred at room temperature. After roughly 2 hr when all of the starting material was consumed according to TLC (silica, 35% EtOAc/hexane), the solution was evaporated to dryness. The resulting solid was suspended in 15 mL of $CHCl_3$ with stirring and to this suspension was added simultaneously 0.697 g (3.66 mmol) of decanoyl chloride and 1.27 mL (9.15 mmol) of Et_3N. The slightly yellow solution was stirred at room temperature. After 90 min the solution was evaporated to a

slightly yellow solid and recrystallized with EtOH or chromatographed on silica with $CHCl_3$ yielding 1.48 g (89%) of the desired product. NMR: δ 0.87 (3H, t, J = 7.7Hz), 2.22 (2H, t, J = 7.8 Hz), 3.20 (2H, d, J = 6.8Hz), 5.01 (1H, t, J = 6.8Hz), 5.06 (2H, s), 6.96 (2H, d, J = 8.1Hz), 7.12 (2H, d, J = 8.1Hz), 7.17 (2H, d, J = 8.1Hz), 7.41 (5H, complex), 8.24 (2H, d, J = 8.1Hz).

(ii) N-decanoyl-O-benzyl-L-tyrosine-spermine-amide and Bis[N-decanoyl-O-benzyl-L-tyrosine]-spermine-amide

To a 10 mL CH_3OH solution of 1.45 g (2.38 mmol) of N-decanoyl-O-benzyl-L-tyrosine-*p*-nitrophenol ester was added dropwise a 10 mL CH_3OH solution of 0.58 g (2.85 mmol) of spermine with stirring at room temperature. After 1 hr, the reaction mixture was concentrated to a yellow, semi-crystalline oil and 10 mL of $CHCl_3$ / CH_3OH (1:1) was added to enhance crystallization of the *p*-nitrophenol. This suspension was filtered through celite and washed with 10 mL of $CHCl_3$ / CH_3OH (1:1) solution. The filtrate was concentrated to a clear yellow oil and then chromatographed on 25 g of silica with a step gradient of 9:1 $CHCl_3$ / CH_3OH and 15:5:1 $CHCl_3$ / CH_3OH / *i*-$PrNH_2$ eluting the "Bis" adduct 0.322 g (27%). NMR: d 0.70 (6H, t, J = 6.5Hz), 1.98 (4H, t, J = 6.5Hz), 4.29 (2H, t, J = 8.0Hz), 4.85 (4H, s), 6.73 (4H, d, J = 8.7Hz), 6.95 (4H, d, J = 8.7Hz), 7.16 (10H, complex). The column elution was continued with 4:4:1 $CHCl_3$ / CH_3OH / *i*-$PrNH_2$ which yielded 0.322 g (58%) of a clear, light yellow oil. NMR: δ 0.70 (3H, t, J = 5.2Hz), 1.97 (2H, t, J = 7.3Hz), 4.30 (1H, t, J = 7.2Hz), 4.86 (2H, s), 6.72 (2H, d, J = 8.7Hz), 6.97 (2H, d, J = 8.7Hz), 7.18 (5H, complex).

(iii) C_{10}-PhTX-343 (17)

To a 2mL CH_3OH solution containing 0.055 g (0.090 mmol) of N-decanoyl-O-benzyl-L-tyrosine-spermine-amide was added roughly 0.02 g of 5% Pd/C. This solution was purged several times with hydrogen and then stirred for 12 hr. The reaction was terminated by filtration through celite and careful washing of the carbon with copious volumes of CH_3OH. After evaporation of the solvent, the clear oil was chromatographed on 10 g of silica with 10:1 $CHCl_3$ / CH_3OH and 4:4:1 $CHCl_3$ / CH_3OH / *i*-$PrNH_2$. The desired product, 0.26 g (55%) was obtained as a clear oil. CI-MS ($C_{29}H_{53}N_5O_3$): *m/z* 520 $(M+i)^+$; NMR: δ 0.70 (3H, t, J = 6.6Hz), 2.97 (2H, t, J = 6.3Hz), 4.26 (1H, t, J = 7.6Hz), 6.48 (2H, d, J = 7.9Hz), 6.83 (2H, d, J = 7.9Hz).

(iv) Bis-C_{10}-PhTX-343 (49)

A 3 mL CH_3OH solution of 0.110 g (0.108 mmol) **(11)** was treated in the same manner as for the synthesis of **(2)** above with roughly 0.05 g of 5% Pd/C. The product was purified on a silica flash column with 15:5:1 $CHCl_3$ / CH_3OH / *i*-$PrNH_2$ yielding 0.072 g (80%) of the desired product. CI-MS ($C_{48}H_{80}N_6O_6$): *m/z* 859 $(M+Na)^+$, 837 $(M+1)^+$; NMR: δ 0.67 (6H, t, J

= 6.9Hz), 2.97 (4H, br t, J = 6.3Hz), 4.21 (2H, t, J = 7.6Hz), 6.48 (4H, d, J = 8.4Hz), 6.82 (4H, d, J = 8.4Hz).

Moiety B

(i) N-butyryl-L-glycine-*p*-nitrophenol ester

To a solution of N-BOC-L-gylcine-*p*-nitrophenol ester (Sigma), 0.25 g (0.834 mmol) in 3 mL of $CHCl_3$ was added 2 mL of TFA at room temperature with stirring. This solution was stirred for 30 min before the solvent was evaporated. The white powder was suspended in 3 mL of $CHCl_3$ and to this solution was added simultaneously 0.35 mL (2.5 mmol) of Et_3N and 0.10 mL (1.00 mmol) of butyryl chloride. This solution was stirred for 30 min before evaporation of the solvent and loading of the crude oil onto a silica flash column from which the pure product was eluted with $CHCl_3$ in 85% yield (0.188 g). NMR ($CDCl_3$): δ 1.54 (3H, t, J = 7.4Hz), 2.25 (2H, m), 2.82 (2H, t, J = 7.5Hz), 4.78 (2H, s) 7.95 (2H, d, J = 9.6Hz), 8.84 (2H, d, J = 9.6Hz).

(ii) N-butyryl-L-glycine-spermine-amide (26)

To a 7 mL CH_3OH solution of spermine 0.171 g (0.848 mmol) was added dropwise a 7 mL CH_3OH solution of N-butyryl-L-glycine-*p*-nitrophenol ester 0.188 g (0.71 mmol) with stirring at room temperature. This mixture was stirred for 30 min before evaporation of the solvent to a yellow, semi-crystalline oil. Roughly 10 mL of $CHCl_3$ / CH_3OH (1:1) was added to enhance crystallization of the *p*-nitrophenol. This suspension was filtered and washed through celite with 10 mL of $CHCL_3$ / CH_3OH (1:1) solution. The filtrate was evaporated to a clear yellow oil and then chromatographed on 6.8 g of silica with a step gradient system of 9:1 $CHCl_3$ / CH_3OH, 15:5:1 $CHCl_3$ / CH_3OH / *i*-$PrNH_2$ and 4:4:1 $CHCl_3$ / CH_3OH / *i*-$PrNH_2$ yielding 0.118 g (51%) of a clear, light yellow oil. FAB-MS ($C_{16}H_{35}N_5O_2$): *m/z* 352 $(M+Na)^+$, 330 $(M+1)^+$; NMR: δ 0.90 (3H, t, J = 7.4Hz), 2.17 (2H, t, J = 7.5Hz), 3.74 (2H, s).

Moiety C

(i) PhTX-43 (28)

The corresponding O-benzyl-tyrosyl-amine was deprotected in the same manner as for (**2**) in 89% yield from 0.189 g of starting material. CI-MS ($C_{20}H_{34}N_4O_3$): *m/z* 379 $(M+1)^+$; NMR: δ 0.64 (3H, t, J = 7.4Hz), 1.94 (2H, t, J = 7.6Hz), 4.26 (1H, t, J = 7.6Hz), 6.47 (2H, d, J = 8.4Hz), 6.82 (2H, d, J = 8.4Hz).

(ii) PhTX-4 (29)

The corresponding O-benzyl-tyrosyl-amine, 0.220 g (0.535 mmol) was deprotected in the same manner as above in 88% yield. CI-MS ($C_{17}H_{27}N_3O_3$): *m/z* 322 $(M+1)^+$; NMR: δ 0.66 (3H,

t, J = 7.5Hz), 1.96 (2H, t, J = 7.5Hz), 4.28 (1H, t, J = 7.5Hz), 6.49 (2H, d, J = 8.5Hz), 6.84 (2H, d, J = 8.5Hz).

(iii) PhTX-0 (30)

To a stirred solution of NH_4OAc 0.166 g (2.16 mmol) in 2 mL of DMF was added 0.200 g (0.433 mmol) of (**8**) dissolved in 3 mL of DMF; this mixture was stirred for 5 min before terminating the reaction by pouring it into 0.1 N NaOH aq. and extracting with EtOAc. The combined organic extracts were washed with brine and dried over $MgSO_4$ before evaporation of the solvent and elution from a silica flash column with 2 % CH_3OH / $CHCl_3$ yeilding 0.129 g (88%) of pure product. This clear oil was then dissolved in 15 mL of CH_3OH and treated with 0.130 g of 5% Pd / C and hydrogen for 1 hr. The reaction mixture was purified first by filtration and washing through celite, followed by evaporation of the solvent and recrystallization from 1:2 CH_3OH / $CHCl_3$, yielding 0.036 g (38%) of pure product. The mother liquid was re-evaporated and recrystallized from the 1:1:2 CH_3OH / Et_2O / $CHCl_3$ to give another 0.02 g, a total yield of 50%. CI-MS ($C_{13}H_{18}N_2O_3$): *m/z* 251 $(M+1)^+$; NMR: δ 0.64 (3H, t, J = 7.4Hz), 1.33 (2H, m), 1.94 (2H, t, J = 7.6Hz), 2.58 (1H, dd, J = 13.9, 9.0Hz), 2.86 (1H, dd, J = 13.9, 5.7Hz), 4.37 (1H, dd, J = 9.0, 5.7Hz), 6.50 (2H, d, J = 8.4Hz), 6.87 (2H, d, J = 8.4Hz).

(iv) N-[2-(1-(butyl)cyanoethyl)]-butane-1,4-diamine

To a 10 mL dry THF solution at -78°C of 0.44 g (3.15 mmol) of 2-(diaminobutyl)-ethylnitrile, was added 1.38 mL of 2.5 molar n-butyl lithium in hexane (Aldrich) (3.45 mmol) and this mixture was stirred for 5 min. To this suspension was added dropwise 0.33 mL of 1-bromobutane (3.1 mmol) and then the reaction temperature was raised to 0°C. After stirring for another 5 min, the reaction temperature was allowed to rise to room temperature. After quenching by addition of H_2O, the solvent was evaporated and the residue suspended in water was extracted 3 times with $CHCl_3$. The combined organic layers were washed with brine, dried over $MgSO_4$, and then evaporated to yield a mixture of mono- and di-alkylation products (*ca* 1:1) in 74 % yield. This mixture was purified on silica gel with 10 % CH_3OH / $CHCl_3$. NMR ($CDCl_3$): δ 0.95 (3H, t, J = 6Hz), 1.1 - 1.7 (11H, complex), 2.6 - 3.9 (5H, complex), 3.75 (1H, t, J = 6.3Hz).

(v) PhTX-(butyl)433 (32)

N-butyryl-O-benzyl-L-tyrosyl-butyl-thermospermine (433) was deprotected in the same manner as for (**2**) yielding 0.081 g (28%) of pure product. FAB-MS ($C_{27}H_{49}N_5O_3$): *m/z* 492 $(M+1)^+$; NMR: δ 0.84 (3H, t, J = 7.5 Hz), 0.87 (3H, t, J = 5.9Hz), 2.14 (4H, t, J = 7.7Hz), 4.44 (1H, t, J = 7.0Hz), 6.72 (2H, d, J = 8.4Hz), 7.00 (2H, d, J = 8.4Hz).

Method B

(i) N-CBZ-L-tyrosyl-spermine-amide

To a 3 mL DMF solution of 0.56 g (2.75 mmol) of spermine was added dropwise a 3 mL DMF solution of 1.0 g (2.29 mmol) of N-CBZ-L-tyrosine *p*-nitrophenol ester resulting in the instant formation of a bright yellow color. After completion of the ester addition, the solution was stirred for another 30 min. The desired product was obtained by evaporating the solvent, adding 20 mL of $CHCl_3$ and evaporating again. The bright yellow oily suspension was suspended in 10 mL of CH_3OH / $CHCl_3$ (1:1) and filtered through celite followed by rinsing with the same solution. Upon evaporation of the clear yellow solution, the yellowish crude oil was purified on 15 g of silica eluting the desired product with a gradient of 9:1 $CHCl_3$ / CH_3OH, 15:5:1 $CHCl_3$ / CH_3OH / *i*-$PrNH_2$, and 4:4:1 $CHCl_3$ / CH_3OH / *i*-$PrNH_2$. This purification yielded 0.58 g (51%) of the desired product. FAB-MS ($C_{27}H_{41}N_5O_4$): *m/z* 500 $(M+1)^+$; NMR: δ 4.21 (H, br, s), 5.02 (2H, s), 6.67 (2H, d, J = 8.4Hz), 6.96 (2H, d, J = 8.4Hz), 7.25 (5H, s).

(ii) N-CBZ-L-tyrosyl-spermine-N_b,Ne-di-BOC-L-lysine-diamide

To a 5 mL DMF solution of 0.62 g (1.2 mmol) of N-CBZ-L-tyrosyl-spermine amide was added dropwise a 5 mL DMF solution of 0.39 g (1.2 mmol) of N_a,Ne-di-BOC-L-lysine *p*-nitrophenol ester. This solution was stirred at room temperature for 30 min. The reaction was worked up by evaporation of DMF under high vacuum followed by addition of 6 mL of CH_3OH / $CHCl_3$ (1:1); this suspension was filtered and washed through celite with the same (1:1) solution. The clear yellow oil obtained after filtration and evaporation of the solvent was chromatographed on 32 g of silica and eluted with 9:1 $CHCl_3$ / CH_3OH and 15:5:1 $CHCl_3$ / CH_3OH / *i*-$PrNH_2$ by which 0.59 g (71%) of the desired product was obtained as a white foam. FAB-MS ($C_{43}H_{69}N_7O_9$): *m/z* 828 $(M+1)^+$; NMR: δ 1.33 (18H, s), 3.84 (1H, dd, J = 8.1, 4.7Hz), 4.12 (1H, t, J = 7.5Hz), 4.88 (1H, d, J = 13.0Hz), 4.97 (1H, d, J = 13.0Hz), 6.59 (2H, d, J = 8.2Hz), 7.03 (2H, d, J = 8.2Hz), 7.30 (5H, m).

(iii) N-CBZ-O-BOC-L-tyrosyl-di-BOC-spermine-N_a,Ne-di-BOC-L-lysine-diamide

To a 10 mL CH_3OH solution containing 0.59 g (0.88 mmol) of the above diamide was added 0.81 mL (3.51 mmol) of BOC anhydride and 0.07 mL (0.88 mmol) of pyridine, and this mixture was stirred at room temperature for 12 hr. The clear oil was purified by evaporation of the solvent and by elution from 10.5 g of silica with 2% CH_3OH / $CHCl_3$. The resulting product was obtained as a clear oil in 82% yield, (0.08 g). NMR: δ 1.38 and 1.45 (each s, total 45H), 3.87 (1H, br m), 4.24 (1H, br m), 4.92 (1H, d, J = 12Hz), 5.00 (1H, d, J = 12Hz), 6.95 (2H, d, J = 8.6Hz), 7.18 (2H, d, J = 8.6Hz), 7.20 (5H, m).

(iv) O-BOC-L-tyrosyl-di-BOC-spermine-N_a,Ne-di-BOC-L-lysine-diamide

In a 10 mL of CH_3OH, 0.48 g (0.44 mmol) of the above N-CBZ-O-BOC-diamide was dissolved and roughly 0.150 g of 5 % Pd / C was added. This suspension was stirred under hydrogen atmosphere at room temperature for 12 hr. The reaction was worked up by filtration

through celite and washing with copious volumes of CH_3OH. The filtrate was concentrated leaving a clear oil which was chromatographed on silica with a 1 to 5% CH_3OH / $CHCl_3$ step gradient. The desired product was obtained in pure form weighing 0.32 g (77%). NMR: δ 1.35 and 1.41 (each s, total 45H), 3.86 (2H, br m), 6.98 (2H, d, J = 8.5Hz), 7.17 (2H, d, J =8.5Hz).

(v) N-(*p*-azidobenzamide)-O-BOC-L-tyrosyl-di-2',3'-N,N-BOC-spermine-N_a,Ne-di-BOC-L-lysine-triamide

In 6 mL of DMF containing 0.32 g (0.34 mmol) of the above *per*-BOC-diamide was added with stirring 0.06 g (0.37 mmol) of *p*-azidobenzoic acid and 0.08 mL (0.37 mmol) of diphenylphosphoryl azide. Finally, 0.08 mL (0.37 mmol) of Et_3N was added and this mixture was stirred overnight. The reaction was worked up by pouring the reaction mixture into 15 mL of water and extracting 3 times with EtOAc. The combined organic layers were washed twice with brine and dried over $MgSO_4$. After evaporation of the solvent, the crude oil was chromatographed on silica with $CHCl_3$ followed by 2.5% CH_3OH / $CHCl_3$. The product was obtained in 69% yield (0.25 g). NMR: δ 1.37 and 1.44 (each s, total 45H), 3.88 (1H, br s), 4.68 (1H, br s), 6.96 (2H, d, J = 8.6Hz), 7.05 (2H, d, J = 8.6Hz), 7.24 (2H, d, J = 8.6Hz), 7.75 (2H, d, J = 8.6Hz).

(vi) N-(*p*-azidobenzamide)-L-tyrosyl-spermine-L-lysine-triamide (44)

BOC deprotection of the above *per*-BOC-azido-triamide, 0.12 g (0.129 mmol) was effected in 4 mL of $CHCL_3$ with 3 mL of TFA for 1 hr at room temperature with stirring. After 3 repetitive evaporations of $CHCl_3$, the crude oil was chromatographed on silica with 15:5:1 $CHCl_3$ / CH_3OH / *i*-$PrNH_2$, and 5:5:1 $CHCl_3$ / CH_3OH / *i*-$PrNH_2$. The desired product was obtained in 70% yield. FAB-MS ($C_{32}H_{50}N_{10}O_4$): *m/z* 661 $(M+Na)^+$, 639 $(M+1)^+$; NMR (TFA salt): δ 3.58 (1H, t, J = 7.6Hz), 4.28 (1H, t, J = 7.6Hz), 6.43 (2H, d, J = 8.6Hz), 6.80 (2H, d, J = 8.6Hz), 6.84 (2H, d, J = 8.6Hz), 7.53 (2H, d, J = 8.6Hz).

(vii) N-(*p*-azidobenzamide)-di-iodo-L-tyrosyl-spermine-L-lysine-triamide (46)

To a 1.3 mL solution H_2O / CH_3OH (5:1) containing 27.4 mg (0.025 mmol) of (**44**), 10.8 mg (0.065 mmol) of KI, and 17.4 mg (0.10 mmol) of K_2HPO_4 was added dropwise by pipette with rapid stirring 9.8 mg (0.065 mmol) of N-bromo succinimide dissolved in 1 mL of CH_3OH / H_2O (1:1). All solution were degassed with argon. The reaction mixture was stirred for 30 min and then lyophilized to dryness. The brownish powder was loaded onto a silica pipette column and eluted with 15:5:1 $CHCl_3$ / CH_3OH / *i*-$PrNH_2$, and 4:4:1 $CHCl_3$ / CH_3OH / *i*-$PrNH_2$. FAB-MS ($C_{32}H_{48}N_{10}O_4I_2$): *m/z* 891 $(M+1)^+$; NMR (D_2O, TFA salt): δ 3.72 (1H, br, s), 4.38 (1H, br s), 6.98 (2H, d, J = 7.5Hz), 7.52 (2H, s), 7.70 (2H, d, J = 7.5Hz).

Alternate Method

Heptanoylspermine-amide (52) and Bis-heptanoylspermine-amide (55)

To a solution of 0.126 g (0.50 mmol) of *p*-nitrophenol heptanoate in 3.0 mL of CH_3OH was added a 3 mL CH_3OH solution of 0.145 g (0.717 mmol) of spermine. This solution was stirred at room temperature for 2 hr before evaporation of solvent, followed by filtration and washing of the cloudy, yellow suspension through celite with 1:1 CH_3OH / $CHCl_3$. The resulting filtrate was evaporated and the clear yellow oil loaded onto a silica flash column from which was eluted first a mixtrue of *p*-nitrophenol and the "Bis" product, (**55**), with 15:5:1 $CHCl_3$ / CH_3OH /*i*-$PrNH_2$. A $CHCl_3$ solution of the impure "Bis" product was washed with sat. $NaHCO_3$ and brine, dried over $MgSO_4$ before evaporation and re-elution of 20 mg (19%) of pure "Bis" product from a second silica flash column with 3:1:0 and 4:4:1 $CHCl_3$ / CH_3OH / *i*-$PrNH_2$. CI-MS ($C_{24}H_{50}N_4O_2$): *m/z* 427 $(M+1)^+$; NMR: d 0.72 (6H, t, J = 6.8Hz), 1.12 (10H, br s), 1.43 (12H, complex), 1.98 (4H, t, J = 7.7Hz), 2.40 (8H, m), 3.03 (4H, t, J = 6.8Hz). The original flash column elution was continued with 4:4:1 and 1:1:1 $CHCl_3$ / CH_3OH / *i*-$PrNH_2$ yielding pure mono-acylated product (**52**), 0.099 g (63%). CI-MS ($C_{17}H_{38}N_4O$): *m/z* 315 $(M+1)^+$; NMR: δ 0.73 (3H, t, J = 6.8Hz), 1.13 (8H, br s), 1.3 -1.6 (10H, complex), 1.99 (2H, t, J = 7.6Hz), 2.3 - 2.6 (4H, complex), 3.04 (2H, t, J = 6.8Hz).

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11 Numerals denote the number of methylene groups in the polyamine moiety. Thus 433 stands for $HN(CH_2)_4NH(CH_2)_3NH(CH_2)_3NH_2$.

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16 The polyamine structure for **22** is 433.

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