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Synthesis Of Novel And Photolabile Philanthotoxin Analogs: Glutamate Receptor Antagonists†

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Key words: philanthotoxin; glutamate receptor antagonists; photoaffinity label; structure-activity relationship; polyamine.

Abstract: *The synthetic methods for 27 novel and photolabile philanthotoxin analogs are described. Most analogs were synthesized by two general methods with modifications of these methods where necessary.*

INTRODUCTION

Glutamate receptors (Glu-R) are the principal excitatory receptors mediating synaptic transmission in many vertebrate and invertebrate neurons. Recently, Glu-R have been the focus of intense research because of their involvement in the processes of memory and learning, as well as in neurodegenerative diseases such as Alzheimer's disease, Huntington's Chorea,[1] ischemia,[2] and epilepsy. Despite such an important role, the structures of Glu-R are not yet known due to a lack of methods for the purification and isolation of these membrane bound receptors. Glu-R, which are multi-subunit protein complexes, have been classified according to their selective activation by exogenous agonists into several groups: in vertebrates, the quisqualic acid (metabotropic receptor), the α-amino-3-hydroxy-5-methylisoxazole-propionic acid (AMPA), the kainic acid, the N-methyl-D-aspartatic acid (NMDA), the α-amino-4-phosphonobutyric acid subtypes;[3] in invertebrates, the quisqualic acid, the ibotenic acid, and the kainic acid subtypes.[4] Selective antagonists of Glu-R are also of great importance for the further classification and study of these receptors, but such compounds are rare. During the past 15 years, the venoms of insects and other arthropods have proved to be valuable sources of Glu-R antagonists.[5,6] In particular, the venom of the solitary digger wasp, *Philanthus triangulum F.*, has been shown to contain at least four components, each of which antagonizes insect quisqualic acid subtype Glu-R (quis-R).[7] Insect quis-R innervate insect muscle and are thought to be structurally and functionally related to several Glu-R subtypes in mammalian central nervous system (CNS).[8] The structure of the most potent component of the wasp venom, philanthotoxin-433 (PhTX-433),[9] shown in Fig. 1, was determined and synthesized by two

† Dedicated to Professor Gabor Fodor on the occasion of his 75th birthday.

groups independently in 1988.[10,11] Since that time, two general synthetic methods were used to synthesize more than 50 analogs.[12] The analogs were assayed for activity against insect quis-R,[13,14] vertebrate NMDA

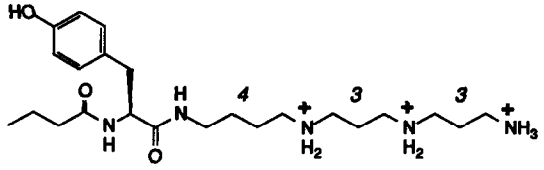
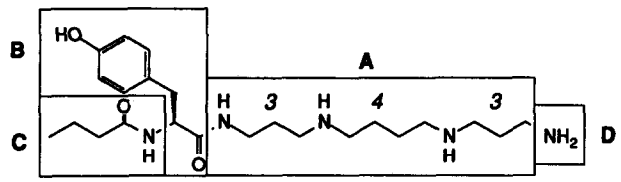


Fig. 1. Philanthotoxin-433 (1)

subtype Glu-R (NMDA-R) and vertebrate nicotinic acetylcholine receptors (nACh-R)[15] resulting in the elucidation of separate structure-activity relationship (SAR) profiles for each of these three ligand-gated ion channel receptors. In addition to their inhibitory potency in micromolar concentration ranges, philanthotoxins also display a potentiating action on Glu-R in nanomolar concentration ranges.[16] Furthermore, four radiolabelled philanthotoxin analogs were shown to bind with a high degree of non-specific binding to rat brain preparations.[17] Three photolabile philanthotoxin analogs have been used to photolytically induce irreversible antagonism of quis-R in insect muscle.[18] Here we describe the synthetic methods that have been used to synthesize a new series of analogs for SAR studies some of which have also been used for photoaffinity labelling of the philanthotoxin binding site in Glu-R. The physiological assay results of these molecules will be reported elsewhere.

Synthesis of philanthotoxin analogs for Structure-Activity Relationship Studies

SAR studies were organized by the division of the philanthotoxin molecule into four regions, (A, B, C, and D as shown below in Fig. 2) followed by the synthesis of analogs containing modifications in each region.



PhTX-343 (2)

Fig. 2. Four regions of PhTX-343

The polyamine spermine (343) was used for the synthesis of most of the analogs due to its commercial availability and its symmetry; acylation at either primary amino terminal gives the same product, unlike that for thermospermine (433), the polyamine of the natural product. Physiological and pharmacological assays indicated that the greater potency of PhTX-433 (1) over PhTX-343 (2) though significant, was small enough to sacrifice in favor of synthetic convenience. Single modifications of substituting a decanoyl moiety for a butyryl moiety in Region C, tryptophan or di-iodo-tyrosine for tyrosine in Region B, and extending the polyamine chain with arginine in Region D all significantly enhanced the antagonistic potency of the analogs as measured against insect quis-R.[18] In order to systematically probe the effect of combining these modifications,

doubly and triply modified molecules (**3-7**) shown in Fig. 3 were synthesized by Method A, shown in Scheme 1.

Analogs **8-10**, containing modifications in the tyrosine ring, were synthesized from the common intermediate PhTX-343 (**2**), the preparation of which was reported previously.[12] Structure-activity studies had indicated that di-iodination of PhTX-343 (**2**) significantly enhanced the antagonistic activity of the molecule against insect quis-R. This was advantageous for the purpose of introducing a radiolabel[17] while enhancing the physiological potency of a particular ligand. It was also of interest to explore the effect on physiological potency modifying the tyrosyl ring, such as by halogenation or nitration. Treatment of PhTX-343 (**2**) with Br_2 in several solvents and under a variety of different conditions resulted in complex mixtures of reaction products, the ^{1}H-NMR spectra of which indicated multiple sites of bromination. However, selective *meta* dibromination was achieved in acceptable yield by separate adsorption of bromine and polyamine (**2**) on silica gel, followed by mixing of the impregnated silica gels.[19] A similar method was applied again to PhTX-343 and a CCl_4 solution of Cl_2 of known molarity both adsorbed to silica gel resulting in the dichloro product **9**. Nitrated polyamine **10** was made by treatment of a phosphate buffered (pH = 8.1) solution of PhTX-343 (**2**) with a 10-fold mol excess of tetranitromethane (TNM); treatment with a single equivalent of TNM resulted in no reaction product.[20]

Safety considerations precluded the direct fluorination of PhTX-343;[21] instead the commercially available 3-fluoro-DL-tyrosine was transformed in 5 steps to 3-fluoro-DL-PhTX-343 (**11**) according to a shortened Method A procedure in which the use of a benzyl protection group for the tyrosyl hydroxyl group was omitted. It was found that the lack of benzyl protection posed no problems and even enhanced the overall yield due to the exclusion of one synthetic step. The D-epimer, D-PhTX-343 (**12**), was made by the same methodology as that for 3-fluoro-DL-PhTX-343 (**11**) in 4 steps from N-Boc-D-tyrosine, again without benzyl protection of the tyrosine hydroxyl. D-PhTX-343 was iodinated in the same manner as for C10-I_2-PhTX-343-arginine (**4**) to give I_2-D-PhTX-343 (**13**).

SAR results had indicated that polar, neutral moieties in Region C had a deleterious effect on physiological potency. However, in order to test the effect on physiological potency by ligands which contain negatively charged moieties in Region C, N-([N-glutamoyl]-γ-aminobutanoyl)-PhTX-343 (**15**) and N-glutamoyl-PhTX-343 (**16**) were synthesized by Method B intermediate (**14**), shown in Fig. 4. Furthermore, it was found in a previous physiological assay work that biotinylated analogs were generally insoluble. It was felt that the solubility of such analogs could be improved by introducing a negatively charged link between the biotin and tyrosyl moieties. Biotinamoyl-PhTX-343 (**17**) was also synthesized via the same intermediate as for analogs **15** and **16**.

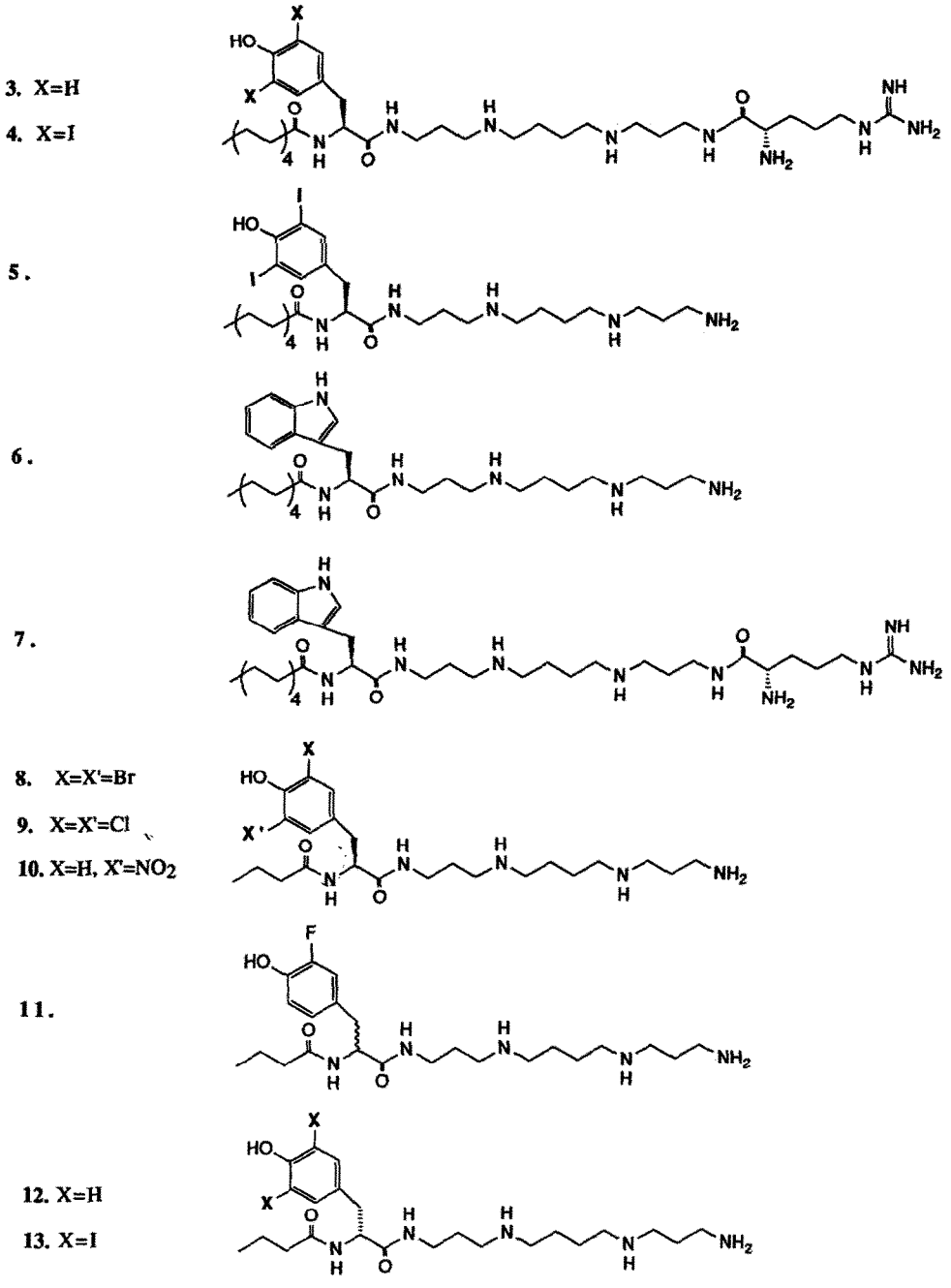
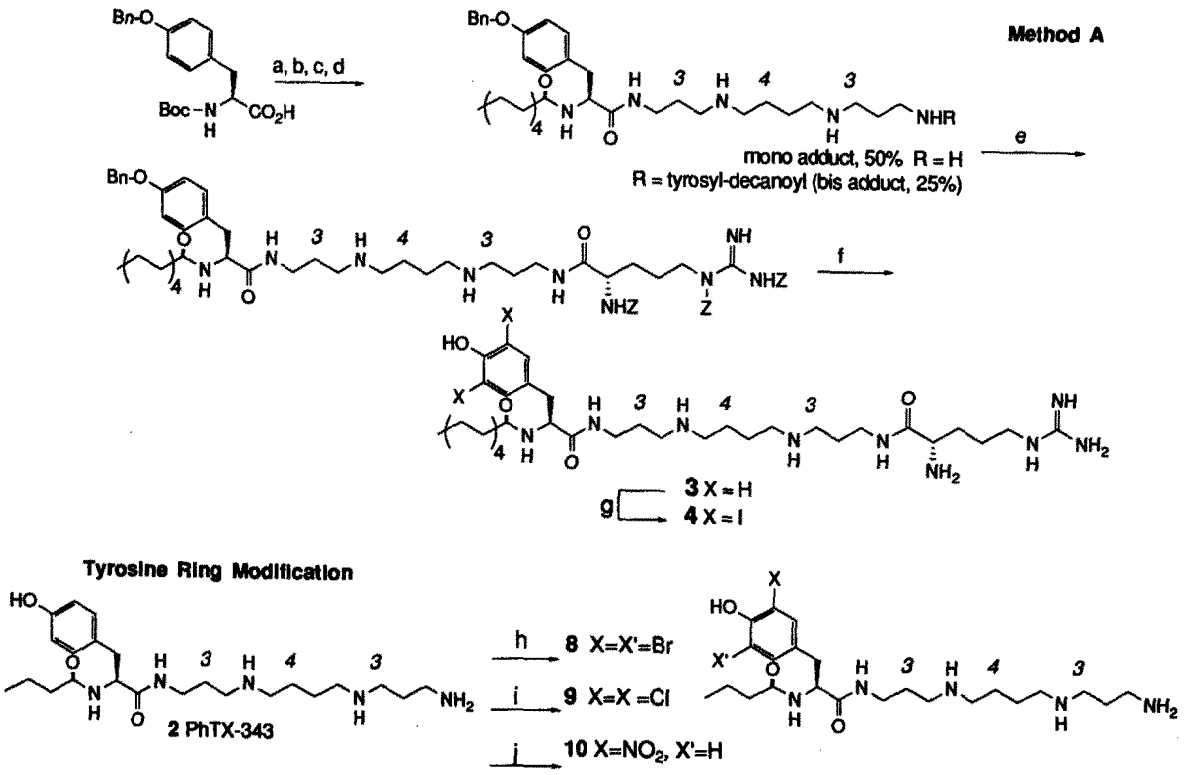


Fig. 3. Philanthoxin analogs synthesized by Method A procedures



Scheme 1. a) DCC, *p*-nitrophenol, EtOAc, 80%; b) TFA, CH_2Cl_2; c) decanoyl chloride, Et_3N, CH_2Cl_2, 80%; d) spermine, CH_3OH, mono-adduct 50%; e) N-α-N^G,$N^{G'}$-tri-Cbz-L-arginine N-hydroxysuccinimide ester, DMF, 56%; f) H_2, 10% Pd/carbon, CH_3OH, 73%; g) KI, NBS, K_2HPO_4, H_2O/CH_3OH, 29%; h) Br_2 adsorbed on silica gel, 33%; i) Cl_2/CCl_4 adsorbed on silica gel, 64%; i) TNM, 0.1 M K_2HPO_4 buffer, 60%.

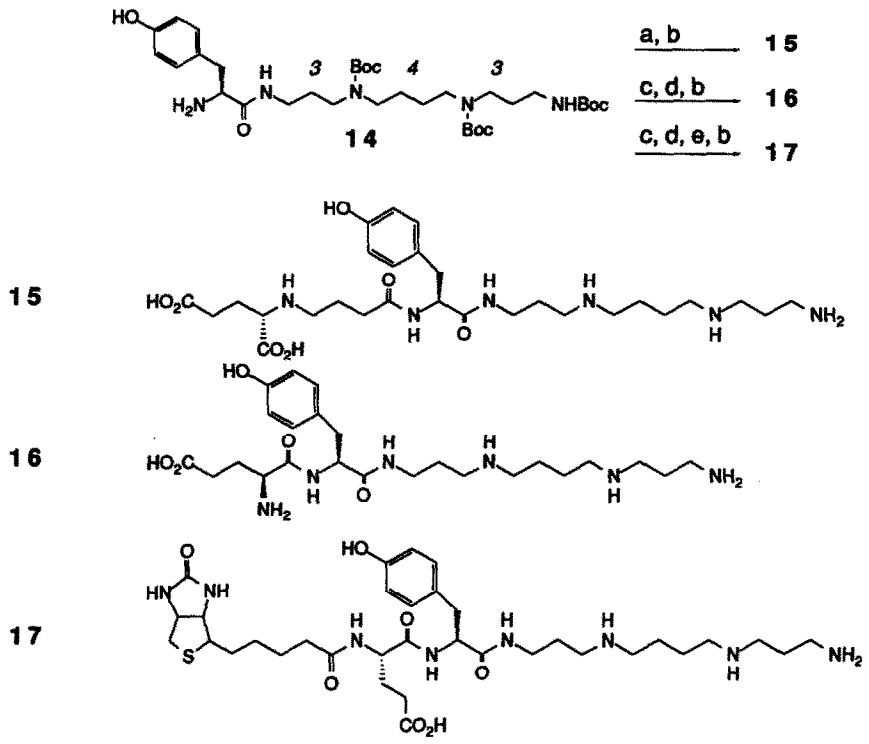


Fig. 4. Method B intermediate (**14**) used to synthesize Region C modified philanthotoxin analogs.

a) N-(3-carboxypropane), N-Boc-L-glutamate di-*t* -butyl ester, DCC, CH_2Cl_2, 77% b) TFA, CH_2Cl_2; c)N-Cbz-γ-*t*-butyl-L-glutamic acid, DCC, CH_2Cl_2, 93%; d) H_2, 10% Pd/carbon, CH_3OH 80% e) biotin N-hydroxysuccinimide ester, DMF, 76%.

Design and synthesis of photoaffinity analogs

Unlike the proposed structurally and functionally similar nicotinic acetylcholine receptors (nACh-R), Glu-R are still targets for isolation and characterization. One of the problems in the isolation of Glu-R from various sources has been a lack of potent and selective antagonists. Recently, it has been shown that photolabile philanthotoxin analogs can irreversibly inhibit a critical site for neurotransmission in insect muscle, putatively identified as quis-R.[18] Therefore, it appears that philanthotoxin analogs may be useful in the photoaffinity labelling approach for isolation of Glu-R from partially purified sources as well as in identifying the sites of activity of Glu-R. Since philanthotoxin analogs have an extended structure, they may also be useful in mapping the tertiary structure of Glu-R in the event that these receptors become available in partially or fully purified form. For these reasons, several philanthotoxin analogs containing photolabile moieties in various locations were synthesized.

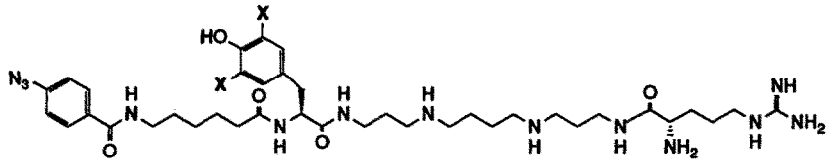
A previously reported photoaffinity ligand, I_2-PhTX-343-lysine *p*-azidobenzamide had shown disappointingly low antagonistic activity;[13] given the three SAR profiles that had been elucidated (insect quis-R, mammalian NMDA-R, and vertebrate nACh-R), it was felt that it would be possible to design and synthesize more potent photoaffinity labels. I_2-PhTX-343-arginine and I_2-PhTX-343-lysine were shown to be two of the more potent analogs active against insect quis-R.[13] Replacement of the butyryl moiety with more hydrophobic or rigid aromatic groups produced more potent analogs. For the choice of a hydrophobic moiety in Region C, solubility factors also required consideration; it had been shown that if a ligand were too hydrophobic in Region C, the overall solubility of the molecule would be low. The combination of a C6 spacer attached to a *p*-azidobenzamide group in analog **18** and **19** seemed to strike the necessary balance. The choice of the photolabile aromatic azido group is largely due to the convenience of introducing and handling this group. The use of this functionality has been quite popular in the synthesis of many other photoaffinity labels.[22]

Ligands containing photolabile groups attached to flat aromatic or unsaturated moieties were also of interest. It had been shown that limitations of the degrees of rotation of moieties in Region C appeared to enhance potency when assayed against insect quis-R, perhaps due to Region C moieties binding into a hydrophobic pocket with limited steric and rotational freedom. The presence or absence of such steric and rotational constraints would have a direct bearing on the site specific nature of covalent binding of a photolabile functionality at a particular position. Since the substitution of the butyryl group with a cinnamoyl group had significantly increased the antagonistic potency, *p*-azidocinnamide analog **20** was synthesized in order to assay the effect of this modification. The mono- and diiodo forms, **21** and **22** were also synthesized to provide several SAR data points for the *p*-azidocinnamide modification.

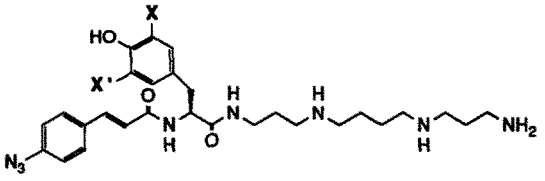
Analogs **20-22** were synthesized from the Method B intermediate **14** shown in Fig. 4. Unlike Method A, in which amino deprotection is effected late in the synthesis via catalytic hydrogenolysis conditions, in Method B hydrogenolytic conditions are applied early in the synthesis to a *per*-Boc protected polyamine to distinguish the tyrosyl amino terminal. Once the azido-containing functionality is attached, deprotection of the Boc groups is achieved with TFA, conditions to which the azido group is stable. In the same manner, intermediate **30**, common to targets **18**, **19**, and **23-25** was synthesized as shown in Scheme 2. Analog **26** was synthesized via a similar route except that the $N\alpha$, $N\varepsilon$-di-Boc-L-lysine *p*-nitrophenyl ester was used instead N-α-N^G,$N^{G'}$-tri-Boc-L-arginine N-hydroxysuccinimide ester to extend the polyamine chain. N-(*p*-azidobenzamide) caproic acid was prepared by treating N-hydroxysuccinimide *p*-azidobenzoate with excess caproic acid. *p*-Azidobenzoic acid was prepared from *p*-aminobenzoic acid according to a reported method;[23] *p*-azidocinnamic acid was synthesized in the same manner from *p*-aminocinnamic acid.

SAR assays had indicated that a diene containing philanthotoxin analog was highly active. In an attempt to take advantage of this active functionality, an azidobenzyl diene ether analog was synthesized as shown in Scheme 2. The ether linkage was included between the diene and the *p*-azidobenzyl group in order to counter the problem of low solubility of the ligand that was shown for ligands with highly hydrophobic groups in Region C. penta-Boc protected precursor to diene **25** was generated by coupling intermediate **30** with the N-hydroxysuccinimide ester of diene **35**. The synthesis of the diene ether is shown in Scheme 2. All analogs synthesized by Method B were obtained as TFA (trifluoroacetic acid) salts by Boc deprotection with TFA, followed by reversed phase flash column chromatography or reversed phase HPLC.

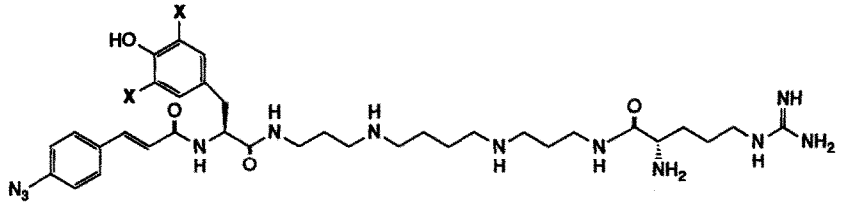
18. X=H
19. X=I



20. X'=X=H
21. X =H, X'=I
22. X=X'=I



23. X=H
24. X=I



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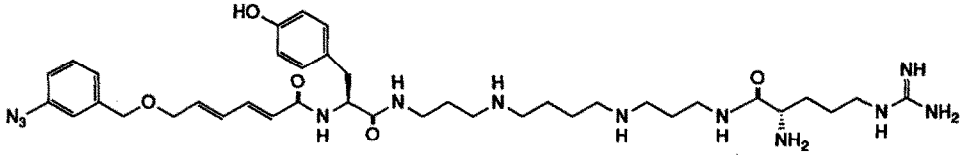

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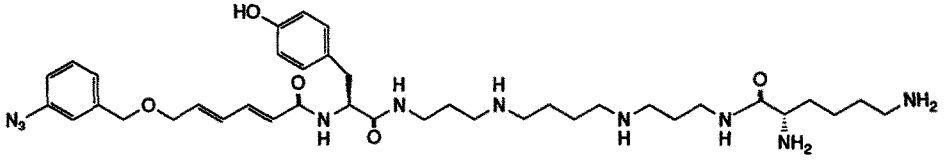

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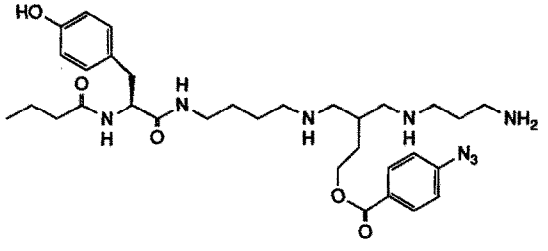

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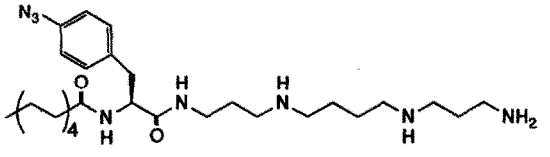
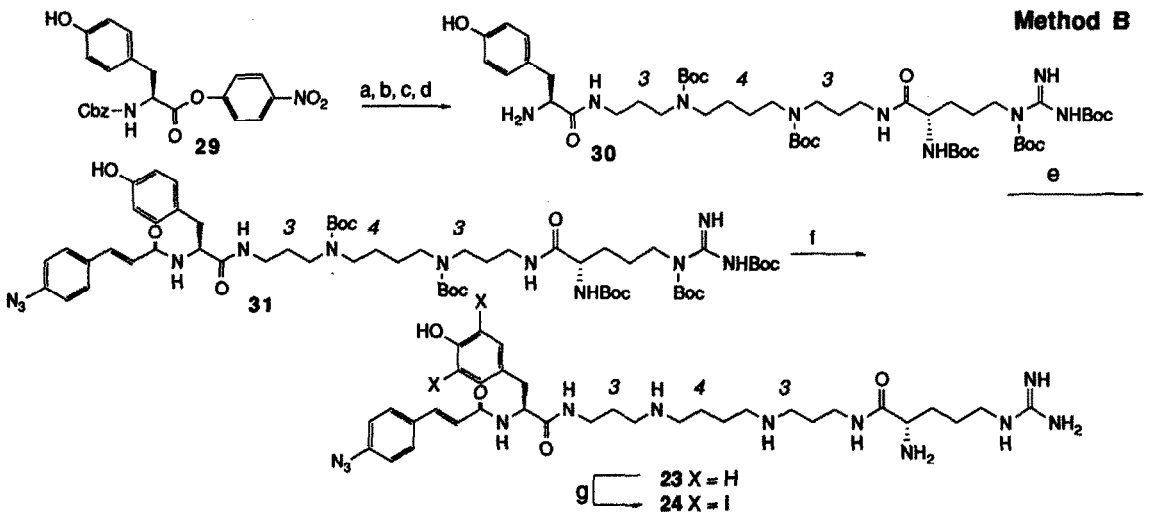
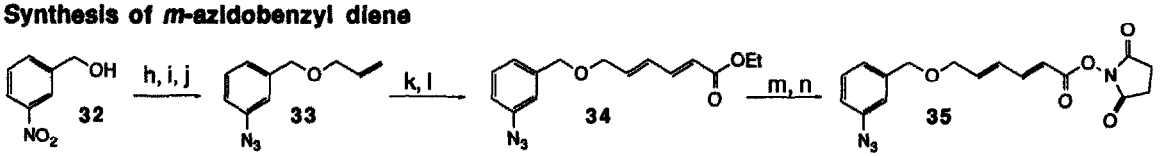


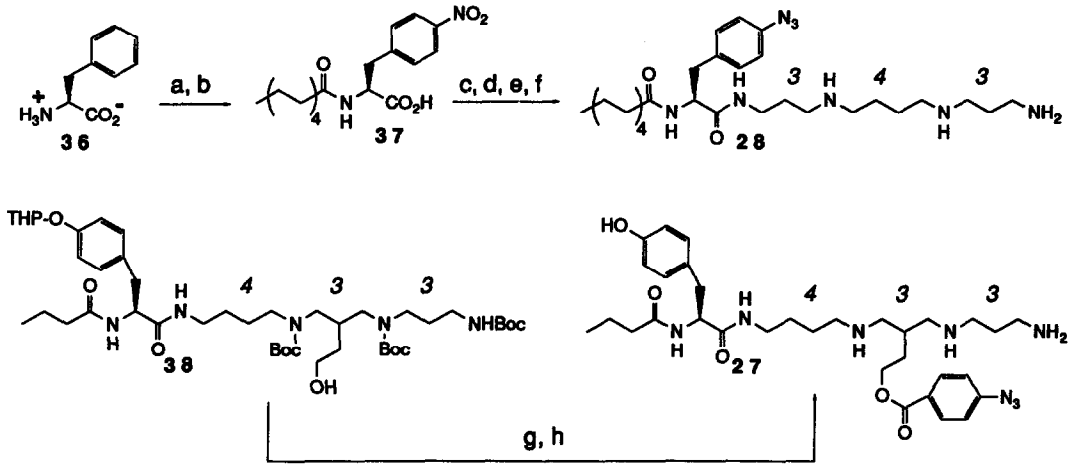
Fig. 5 Photoaffinity analogs synthesized by Method B



Synthesis of *m*-azidobenzyl diene



Scheme 2. a) spermine, CH_3OH, 45%; b) N-α-N^G, $N^{G'}$-tri-Boc-L-arginine-N-hydroxysuccinimide ester, 90 %; c) $(Boc)_2O$, CH_2Cl_2; d) H_2, 10% Pd/carbon, CH_3OH, 67%; e) *p*-azidocinnamic acid, DPPA, DMF, 82%; f) TFA, CH_2Cl_2, 83% g) KI, NBS, K_2HPO_4, H_2O/CH_3OH, 85%; h) allyl bromide KO*t*-Bu/THF, 80%; i) $SnCl_2$, EtOH, 94%; j) 1. $NaNO_2$, conc. HCl, 2. NaN_3, H_2O, 89%; k) cat. OsO_4, $NaIO_4$/dioxane-H_2O, 75%; l) triethyl 4-phosphonocrotonate, *n*-BuLi, THF, -78°C, 40%; m) LiOH/DME-CH_3OH-H_2O, 88%; n) DCC, CH_2Cl_2, N-hydroxysuccinimide, 92%.



Scheme 3. a) conc. HNO_3/H_2SO_4, 38%; b) decanoic acid, EtOCOCl, Et_3N, THF, 61%; c) H_2, 10% Pd/C, AcOH-MeOH, 98%; d) 1. $NaNO_2$, HCl-AcOH, 2. NaN_3, H_2O, 95%; e) DCC, p-nitrophenol, EtOAc, 60%; f) spermine, MeOH/CH_2Cl_2, 50%; g) p-azidobenzoic acid, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, DMAP, CH_2Cl_2, 79%; h) $CHCl_3$, TFA, 77%.

Since it had been shown that an alkyl branch in the polyamine chain (Region A) enhanced antagonistic potency, it was of interest to attach a photolabile moiety in the same region in order to examine photoaffinity labelling to that binding site in the receptor. Thus, analog **27** was synthesized as shown in Scheme 3 by condensing the amino-protected polyamine alcohol **38** with *p*-azidobenzoic acid with N-ethyl, N'-dimethylaminopropyl carbodiimide hydrochloride, followed by amino-deprotection and purification. The synthesis of this branched polyamine philanthotoxin analog along with the syntheses of other branched polyamine analogs will be published elsewhere.[24]

Finally, it was also of interest to install a photoaffinity label into Region B of the philanthotoxin structure in order to provide ligands which would covalently cross-link different regions of the philanthotoxin ligand with the Glu-R protein. For this reason, *p*-azidophenyl alanine philanthotoxin analog **28** was synthesized as shown in Scheme 3. The methodology is similar to the shortened version of Method A discussed above using L-*p*-azidophenylalanine instead of L-tyrosine.

In conclusion, 27 novel philanthotoxin analogs have been synthesized to further define the structure / activity profile of this toxin, based on two general synthetic methods. Modification of these methods were utilized where necessary. The main purpose of the present studies was aimed toward the preparation of radioactive philanthotoxin analogs carrying photolabile groups at various sites for use as probes for tertiary structural studies of Glu-R proteins. The photolabile moiety has been located at several positions in the toxin to take advantage of its extended linear structure. Photoaffinity mapping studies utilizing these analogs are currently in progress.

Experimental

DCI-MS spectra were obtained on a Nermag-10 instrument. High resolution FAB-MS were obtained with a JOEL JMS-DX303HF mass spectrometer. Proton spectra were measured with any one of three instruments: a Bruker WM-250 (250 MHZ), Varian VXR-300 MHz (300 MHz), or Varian VXR-400 (400 MHz). Carbon-13 spectra were recorded with a Varian VXR-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 $(CH_3)_4Si$ (TMS), CD_3OD, and $CDCl_3/CD_3OD$ (1:1) mixture with 0.015% TMS, and 99.9% D_2O. Proton chemical shifts are reported in parts per million (ppm) downfield from Me_4Si (0.00 ppm) or DSS (3-trimethylsilyl)-1-propanesulfonic acid, sodium salt, 0.00 ppm) as referenced accordingly for each compound. Spectra were referenced when not otherwise specified on the basis of residual solvent peaks (D_2O, 4.65 ppm; CD_3OD, 4.78 ppm or 3.30 ppm; $CDCl_3$, 7.24 ppm). Carbon-13 NMR spectra were measured in $CDCl_3$ and were referenced with respect to the ^{13}C signal ($\delta = 77$ ppm) of $CDCl_3$ or in D_2O and referenced with respect to the ^{13}C signal of dioxane ($\delta = 66.0$ ppm) added as an internal standard. In some cases, ^{13}C-NMR spectra were referenced externally with respect to DSS (0.00 ppm).

HPLC purifications were performed with a Perkin-Elmer Series 4 Liquid Chromatograph interfaced with a Perkin-Elmer 7500 Professional Computer linked to a Kratos Spectroflow 773 UV/visible wavelength detector. A YMC-ODS (20 x 280 mm) column was used in all cases. HPLC grade solvents (Aldrich) were used for all HPLC purifications.

FT-IR spectra were obtained on a Perkin-Elmer 1600 FTIR spectrophotometer. CD spectra were measured with a JASCO 500A Spectropolarimeter interfaced with a JASCO DP 500N data system computer, and the data was processed on an IBM-PC.

Triethylamine (Et_3N), dimethylformamide (DMF) and isopropylamine (i-$PrNH_2$) were distilled at atmospheric pressure over CaH_2, while methanol (CH_3OH) was distilled over magnesium turnings.

Compounds were judged to be pure on the basis of silica gel TLC using several different solvent systems and on the basis of the proton and carbon NMR by which no impurities were detectable.

The reference to a "pre-washed ODS pipette column" refers to the following procedure. To a pasteur pipette (14 cm) plugged with a small piece of cotton was added ≈1.5 cm of Baker octadecyl (C18) reversed phase gel (40 μ). The gel was shaken with CH_3CN to effect thorough wetting and even settling of the gel in the pipette. Once settled, the gel was washed successively with several pipette volumnes each of CH_3CN, CH_3CN/H_2O (1:1) and H_2O.

Method A

N-Decanoyl-O-benzyl-L-tyrosyl spermine amide and bis[N-decanoyl-O-benzyl-L-tyrosyl] spermine diamide

To a 10 mL CH_3OH solution of 1.45 g (2.66 mmol) of N-decanoyl-O-benzyl-L-tyrosine p-nitrophenyl ester was added dropwise a 10 mL CH_3OH solution of 0.65 g (3.2 mmol, 1.2 eq) of spermine with stirring at room temperature. After 1 h, the reaction mixture was concentrated to a yellow oil and 10 mL of $CH_3OH/CHCl_3$ (1:1) was added to enhance crystallization of p-nitrophenol. The resulting suspension was

filtered and washed through Celite with 10 mL of the same solution. The filtrate was concentrated to a clear yellow oil and then purified by silica gel flash column chromatography using a step gradient of 9:1 ($CHCl_3/CH_3OH$) and 15:5:1 ($CHCl_3/CH_3OH/i$-$PrNH_2$) eluting bis[N-decanoyl-O-benzyl-L-tyrosyl] spermine di-amide in 25% yield (0.33 g). FAB-MS (glycerol, $C_{62}H_{92}N_6O_6$): m/z 1016 $(M)^+$; ^{1}H-NMR (250 MHz, $CDCl_3$) δ: 0.88 (t, J = 6Hz, 6H), 1.14-1.35 (broad s, 24H), 1.40-1.65 (broad s, 12H), 2.15 (t, J = 6Hz, 4H), 2.50-3.00 (m 12H), 3.22 (m, 4H), 4.50 (m, 2H), 5.00 (s, 4H), 6.89 (d, J = 8Hz, 4H), 7.11 (d, J = 8Hz, 4H), 7.30-7.50 (m, 10H). The column elution was continued with 4:4:1 ($CHCl_3/CD_3OD/i$-$PrNH_2$) which yielded 0.81 g (50%) of N-decanoyl-O-benzyl-L-tyrosyl spermine amide as a light yellow oil. FAB-MS (glycerol matrix, $C_{36}H_{59}N_5O_3$): m/z 610 $(M)^+$; ^{1}H-NMR (250 MHz, $CDCl_3$) δ: 0.89 (t, J = 6Hz, 3H), 1.15-1.35 (broad s, 12H), 1.45-1.80 (m 10H), 2.12 (t, J = 6Hz, 3H), 2.45-3.00 (m 12H), 3.25 (m, 2H), 4.50 (m, 1H), 5.0 (s, 2H), 6.30 (d, J = 6Hz, 1H), 6.88 (d, J = 8Hz, 2H), 7.12 (d, J = 8Hz, 2H), 7.35 (m, 5H).

C10-PhTX-343

To a 10 mL solution containing 0.25 g (0.41 mmol) of N-decanoyl-O-benzyl-L-tyrosine spermine amide was added roughly 0.15 g of 10% Pd/carbon catalyst. This solution was purged several times with hydrogen and then stirred under an atmosphere of hydrogen at room temperature for 12 h. The reaction was terminated by filtration of the suspension through Celite followed by careful washing with copious volumes of CH_3OH. After evaporation of the solvent, the clear oil was chromatographed on 25 g of silica gel with 10:1 ($CHCl_3/CD_3OD$) and 15:5:1 ($CHCl_3/CD_3OD/i$-$PrNH_2$) eluting 0.13 g (61%) of C10-PhTX-343 as a clear oil. DCI-MS (NH_3, $C_{29}H_{53}N_5O_3$): m/z 520 $(M+1)^+$; ^{1}H-NMR (250 MHz, CD_3OD) δ: 0.85 (t, J = 6Hz, 3H), 1.20 (broad s, 12H), 1.55 (m, 10H), 2.15 (t, J = 5Hz, 2H), 3.20 (t, J = 6Hz, 2H), 4.42 (t, J = 7Hz, 1H), 6.68 (d, J = 8Hz, 2H), 7.02 (d, J = 8Hz, 2H).

O-Benzyl-C10-tyrosyl-spermine-N-α-N^G,$N^{G'}$-tri-Cbz-L-arginine

To 6 mL DMF a solution of 0.34 g (0.56 mmol) of O-benzyl-C10-tyrosyl-spermine amide was added 0.43 g (0.63 mmol, 1.1 eq) of N-α-N^G,$N^{G'}$-tri-Cbz-L-arginine-N-hydroxysuccinimide ester (BACHEM Bioscience, Inc.) dissolved in 6 mL of dry CH_2Cl_2.. This solution was stirred overnight at room temperature. After evaporation of the solvent, the residue was dissolved in 50 mL $CHCl_3$ and extracted 3 times with sat. $NaHCO_3$ solution. The $CHCl_3$ layer was washed twice with brine and then dried with $MgSO_4$ before filtration through a pad of Celite . The filtrate was concentrated and the oily residue was chromatographed on a silica gel flash column with a step gradient of 9:1 ($CHCl_3/CD_3OD$) and 15:5:1 ($CHCl_3/CD_3OD/i$-$PrNH_2$) eluting 0.37 g (56%) of the desired product as a clear oil. FAB-MS (glycerol, $C_{66}H_{89}N_9O_{10}$): m/z 1168 $(M)^+$; ^{1}H-NMR (250 MHz, $CDCl_3$) δ: 0.81 (t, J = 7Hz, 3H), 1.15 (broad s, 12H), 1.54 (broad m, 14H), 2.12 (t, J = 6Hz, 2H), 2.40-3.20 (m 14H), 3.50 (t, J = 7Hz, 2H), 4.15 (broad m, 1H), 4.45 (broad m, 1H), 4.95-5.20 (m, 8H), 6.85 (d, J = 9Hz, 2H), 7.10 (d, J = 9Hz, 2H), 7.28 (m, 20H).

C10-PhTX-343-L-arginine

To a 25 mL solution of 0.56 g (0.48 mmol) of O-Benzyl-C10-tyrosyl-spermine-N-α-N^G,$N^{G'}$-tri-Cbz-arginine was added 0.46 g of 10% palladium on carbon catalyst. This suspension was purged several times

with hydrogen gas and left to stir for 24 h at room temperature under 1 atm of hydrogen gas. The reaction mixture was filtered and washed through Celite followed by evaporation of the solvent. The resulting crude oil was purified by silica gel flash column chromatography, eluting with a step gradient of 1:1 CH_2Cl_2/CD_3OD, 2:2:1 $CHCl_3/CD_3OD/i$-$PrNH_2$ and 2:2:1:1 ($CHCl_3/CD_3OD/i$-$PrNH_2/H_2O$). After evaporation of the eluent solvent, the precipitated silica gel was filtered off and washed thoroughly with 1:1 (CD_3OD/CH_2Cl_2). Evaporation of the filtrate yielded 0.24 g (73%) of the desired product. FAB-MS (glycerol, $C_{35}H_{65}N_9O_4$): m/z 676 (M+1)+; ^{1}H-NMR (250 MHz, CD_3OD) δ: 0.79 (t, J = 6Hz, 3H), 1.16 (s, 12H), 1.5 (m 14H), 2.09 (t, J = 6Hz, 2H), 2.60-3.45 (m 16H), 3.73 (t, J = 7Hz, 1H), 4.31 (J = 7Hz, 1H), 6.60 (d, J = 8Hz, 2H), 6.95 (d, J = 8Hz, 2H).

C10-diiodo-PhTX-343-L-arginine

To a 10 mL solution of H_2O/CH_3OH (4:1) containing 0.15 g (0.22 mmol) of C10-PhTX-343-L-arginine, 0.092 g (0.55 mmol, 2.5 eq) of KI and 0.19 g (1.11 mmol 5 eq) of K_2HPO_4 was added dropwise with rapid stirring 0.098 g (0.55 mmol, 2.50 eq) of N-bromosuccinimide dissolved in 10 mL of CH_3OH/H_2O (1:1). After completion of the dropwise addition of NBS, the reaction mixture was stirred for 30 min at room temperature under an atmosphere of argon. The reaction mixture was then filtered through cotton and the filtrate was frozen and lyophilized to dryness. The resulting solid was loaded onto a silica gel flash column and eluted with a step gradient composed of 1:1 (CH_2Cl_2/CH_3OH), 2:2:1 ($CH_2Cl_2/CH_3OH/i$-$PrNH_2$), 2:2:1:1 ($CH_2Cl_2/CH_3OH/i$-$PrNH_2/H_2O$). After evaporation of the eluent solvent, the residue was taken up in 2 mL of (CH_2Cl_2/CH_3OH) (1:1) and the insoluble silica gel was filtered off and washed with 1:1 (CH_2Cl_2/CH_3OH). The desired product was obtained as a clear foam, 0.060 g (29%). FAB-MS (glycerol, $C_{35}H_{63}N_9O_4I_2$): 928 $(M+1)^+$; ^{1}H-NMR (250 MHz, CD_3OD) δ: 0.82 (t, J = 6Hz, 3H), 1.05-2.00 (m 26H), 2.10 (m, 2H), 2.68-3.55 (m 16H), 3.78 (t, J = 7Hz, 1H), 4.35 (t, J = 7Hz, 1H), 7.52 (s, 2H).

Dibromo-PhTX-343-(TFA)3

A 3 mL CH_3OH solution of 0.030 g (0.038 mmol) of PhTX-343-(TFA)3 was added to 0.25 g of silica gel and the solvent was removed in vacuo. To a separate quantity of 0.25 g of silica gel was added 4.0 mL (0.077 mmol, 2.0 eq) of bromine. The silica gel was agitated to evenly distribute the brown color of the bromine throughout the silica gel. The bromine-impregnated silica gel was added to that containing PhTX-343-(TFA)3 and the test tube containing this mixture was rotated horizontally for 2 h at room temperature under an atmosphere of argon. The silica gel was transferred to a small column and washed several times with 1:20 (CH_3OH/i-$PrNH_2$). Evaporation in vacuo of the column extracts yielded the crude di-bromo-PhTX-343, which was purified by reversed phase HPLC (solvent: CH_3CN [(15-30%)/20 min, 30% for 10 min]/0.5% TFA in H_2O; flow rate: 7.0 mL/min; detection: 274 nm). The expected product, dibromo-PhTX-343-(TFA)3, eluted at 22.5 min in 33% yield (0.012 g). FAB-MS (thioglycerol, $C_{23}H_{39}N_5O_3Br_2$): m/z 594 $(M+1)^+$; ^{1}H-NMR (300 MHz, CD_3OD) δ: 0.74 (t, 3H, J = 7.5Hz), 1.44 (sextet, J = 7.2Hz, 2H), 1.71 (broad s, 6H), 1.98 (t, J = 7.8Hz, 2H), 2.07 (t, J = 7.2Hz, 2H), 2.80-3.10 (m, 14H), 4.30 (t, J = 7Hz, 1H), 7.28 (s, 2H); ^{13}C-NMR (75.4 MHz, D_2O, DSS as external reference) δ: 15.2, 17.7, 21.6, 21.8, 25.5, 26.5, 28.1, 38.1, 38.7, 39.2, 39.9, 47.3, 47.6, 49.7, 49.7, 57.1, 58.0, 113.7, 134.3, 135.7, 151.2, 176.1, 179.9.

Dichloro-PhTX-343-(TFA)3

In 5 mL of CCl_4 in a round bottom flask of known total mass cooled to -78°C chlorine gas was bubbled for 10 min until a pronounced yellow color was visible. The flask was stoppered and allowed to come to room temperature before weighing in order to determine the quantity of chlorine gas contained in solution: 43.0 mg/mL. To a separate round bottom flask containing 0.50 g of silica gel was added 5 mL of CH_3OH containing 0.026 g (0.060 mmol) of PhTX-343-(TFA)3. The solvent of this mixture was removed in vacuo. To another quantity of silica gel (0.25 g) was added 200 μL (0.12 mmol, 2.0 eq) of the CCl_4 solution. The Cl_2 containing silica gel was then agitated in order to effect uniform distribution of the chlorine gas before adding the PhTX-343-(TFA)3-impregnated silica gel. The flask containing this mixture of silica gels was rotated horizontally for 7 h under an argon atmosphere at room temperature before transferring the silica gel mixture to an empty chromatography column which was washed several times with 1:20 (CH_3OH/*i*-$PrNH_2$). The eluted solvent was evaporated in vacuo leaving the crude dichloro-PhTX-343, which was purified by reversed HPLC (solvent: CH_3OH [(15-50%)/60 min,]/0.5% TFA in H_2O; flow rate: 7.0 mL/min; detection: 274 nm). Dichloro-PhTX-343-(TFA)3, eluting at 56 min, was obtained in 64% yield (0.019 g) as a clear oil. FAB-MS (glycerol/thioglycerol, $C_{23}H_{39}N_5O_3Cl_2$): m/z calcd 504.2508, found 504.2467 $(M+1)^+$; ^{1}H-NMR (300 MHz, D_2O) δ: 0.77 (t, J = 7Hz, 3H), 1.48 (sextet, J = 7Hz, 2H), 1.73 (m, 6H), 2.20 (m, 2H), 2.12 (t, J = 7Hz, 2H), 2.75-3.30 (bm, 14H), 4.32 (t, J = 7Hz, 1H), 7.10 (s, 2H).

3-Nitro-PhTX-343-(TFA)3

PhTX-343-(TFA)3, 0.030 g (0.038 mmol) was dissolved in 3 mL of 0.1 M sodium phosphate, pH 8.1. To this solution was added 0.074 g (0.38 mmol, 10 eq) of $C(NO_2)_4$. This solution was stirred at room temperature under argon atmosphere. After 5 h, this reaction mixture was lyophilized to dryness. The residue was dissolved in 0.25 mL of H_2O and loaded onto a small column containing 6 mL of pre-washed ODS gel. This column was then eluted with H_2O followed by 69:30:1 (H_2O/CH_3CN/TFA). This product was further purified by reversed phased HPLC (solvent: CH_3OH [(15-50%)/20 min,]/0.5% TFA in H_2O; flow rate: 7.0 mL/min; detection: 274 nm) eluting at 26 min and yielding 0.021 g (66%) of 3-nitro-PhTX-343-(TFA)3. FAB-MS (nitrobenzyl alcohol, $C_{23}H_{40}N_6O_5$): m/z 481 $(M+1)^+$; ^{1}H-NMR (250 MHz, D_2O) δ: 0.81 (t, J = 7.4Hz, 3H), 1.53 (sextet, J = 7.3Hz, 2H), 1.80 (m, 6H), 2.14 (m, 2H), 2.24 (t, J = 7.2Hz, 2H), 2.83 (t, J = 7.6Hz, 2H), 3.01 (m, 4H), 3.12 (m, 10H), 4.45 (t, J = 7Hz, 1H), 6.89 (d, J = 9Hz, 1H), 7.42 (dd, J = 9, 2Hz, 1H), 7.86 (d, J = 2Hz, 1H).

N-Boc-3-fluoro-DL-tyrosine *p*-nitrophenyl ester

To a 2 mL solution of 1:1 (dioxane/H_2O) containing 0.10 g (0.50 mmol) of 3-fluoro-DL-tyrosine (Sigma) and 0.14 ml (1.00 mmol, 2.0 eq) of Et_3N was added 0.16 g (0.53 mmol, 1.00 eq) of 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile (Boc-ON) and this mixture was stirred for 12 h at room temperature under an atmosphere of argon. The reaction was worked up by pouring it into 20 mL of dilute, aqueous $NaHCO_3$ solution and extracting the aqueous layer 3 times with EtOAc. The combined EtOAc layers were washed twice with brine before drying over $MgSO_4$. The slurry was filtered through a cotton plug and the filtrate was concentrated in vacuo to a light yellow oil. This oil was carried on to the next step without further purification.

The above oil was dissolved in 6.5 mL of dry EtOAc and to this clear solution was added 0.068 g (0.49 mmol, 1.0 eq) of *p*-nitrophenol and 0.10 g (0.49 mmol, 1.0 eq) of DCC. The mixture was then stirred at room temperature for 2 h under an argon atmosphere during which time a white precipitate formed. The reaction was terminated by filtering and washing the mixture through Celite with EtOAc. The filtrate was extracted twice with H_2O and once with brine before being dried over $MgSO_4$ and filtered again, followed by concentration in vacuo. The resultant crude oil was purified by silica gel column chromatography with 9:5 (hexanes/EtOAc) yielding 0.11 g (52%) of the desired product. Rf: 0.3 in 9:5 (hexanes/EtOAc). DCI-MS (NH_3, $C_{20}H_{21}N_2O_7F$): m/z 420 $(M)^+$, 363, 277, 261, 139; ^{1}H-NMR (250 MHz, $CDCl_3$) δ: 1.35 (s, 9H), 3.03 (d, J = 6Hz, 2H), 4.59 (t, J = 6Hz, 1H), 6.8 (m, 2H), 7.10 (d, J = 10Hz, 2H), 8.03 (d, J = 9Hz, 1H), 8.17 (d, J = 9Hz, 2H).

N-butyryl-3-fluoro-DL-tyrosine *p*-nitrophenyl ester

To a 1 mL solution of dry CH_2Cl_2 containing 0.11 g (0.25 mmol) of N-Boc-3-fluoro-DL-tyrosine *p*-nitrophenyl ester was added 1 mL of TFA. This mixture was stirred at room temperature under argon for 2 h before evaporation of the solvent in vacuo. The resultant white solid was then suspended in 2 mL of CH_2Cl_2 followed by the simultaneous addition of 28 µL (0.27 mmol, 1.0 eq) of butyryl chloride and 78 µL (0.56 mmol, 2.2 eq) of Et_3N. This reaction mixture was stirred under argon at room temperature for 90 min before evaporation of the solvent in vacuo. The resultant crude solid was purified on a silica gel flash column, eluting with $CHCl_3$. The desired product was obtained in 73% yield (0.073 g). Rf: 0.2 in $CHCl_3$. DCI-MS (NH_3, $C_{19}H_{19}N_2O_6F$): m/z 391 $(M+1)^+$; ^{1}H-NMR (250 MHz, $CDCl_3$) δ: 0.91 (t, J = 8Hz, 3H), 1.63 (sextet, J = 7Hz, 2H), 2.24 (t, J = 7Hz, 2H), 3.14 (m, 2H), 5.01 (m, 2H), 6.16 (m, 1H), 6.91 (m, 2H), 7.17 (d, J = 7Hz, 2H), 8.10 (d, J = 9Hz, 1H), 8.22 (d, J = 9Hz, 2H).

3-fluoro-DL-PhTX-343

A 5 mL solution 4:1 (CH_3OH/THF) containing 0.73 g (0.19 mmol) of N-butyryl-3-fluoro-DL-tyrosine *p*-nitrophenyl ester was added dropwise to 5 mL of CH_3OH containing 0.075 g (0.37 mmol, 2.00 eq) of spermine resulting in the instant formation of a bright yellow color. Upon completion of the dropwise addition, the reaction mixture was stirred at room temperature for 3 h under an argon atmosphere before evaporation of the solvent yielding a thick yellow oil. This oil was purified on a silica gel column, eluting with a step gradient of 9:1 ($CHCl_3$/CH_3OH) followed by 15:5:1 and 4:4:1 ($CHCl_3$/CH_3OH/*i*-$PrNH_2$). The desired product was obtained in 55% yield (0.047 g) as a clear, slightly yellow oil. Rf: 0.2 in 4:4:1 ($CHCl_3$/CH_3OH/*i*-$PrNH_2$). DCI-MS (NH_3, $C_{23}H_{40}N_5O_3F$): m/z 454 $(M+1)^+$; ^{1}H-NMR (300 MHz, CD_3OD) δ: 0.85 (t, J = 8Hz, 3H), 1.55 (sextet, J = 8Hz, 2H), 1.81 (m, 6H), 2.08 (t, J = 8Hz, 2H), 2.17 (t, J = 7Hz, 2H), 2.75-3.3 (complex, 14H), 4.39 (t, J = 8Hz, 2H), 6.85 (d, J = 12Hz, 1H).

D-PhTX-343

N-butyryl-D-tyrosine *p*-nitrophenyl ester, 0.60 g (1.6 mmol) was obtained in the same manner as for N-butyryl 3-fluoro-DL-tyrosine *p*-nitrophenyl ester from the commercially available D-tyrosine (Sigma). The

ester was reacted with 0.34 g (1.7 mmol, 1.1 eq) of spermine in 20 mL of CH_3OH in the same manner as for 3-fluoro-PhTX-343 yielding 0.24 g (35%) of a clear oil. R_f: 0.52 in 4:4:1 ($CHCl_3/CH_3OH/i$-$PrNH_2$). FAB-MS (thioglycerol, $C_{23}H_{41}N_5O_3$): m/z 436 $(M+1)^+$; ^{1}H-NMR (400 MHz, D_2O) δ: 0.59 (t, J = 6Hz, 3H), 1.31 (t, J = 5Hz, 2H), 1.55 (m, 6H), 1.90 (m, 2H), 2.01 (t, J = 5Hz, 2H), 2.57 (t, J = 6Hz, 2H), 2.75-3.05 (m, 12H), 4.19 (t, J = 6Hz, 1H), 6.65 (d, J = 6Hz, 2H), 6.95 (d, J = 6Hz, 2H); ^{13}C-NMR (75.4 MHz, $CDCl_3$) δ: 13.4, 18.8, 26.7, 28.1, 31.1, 37.4, 37.8, 39.6, 46.5, 47.0, 54.8, 115.3, 127.0, 130.1, 156.0, 171.3, 173.4.

Diiodo-D-PhTX-343

Diiodo-D-PhTX-343 0.060 g (0.15 mmol) was iodinated in the same manner as for C10-diiodo-PhTX-343-L-arginine. The resultant crude diiodo-D-PhTX-343 was purified on a silica gel column eluted with a step gradient of 8:2 ($CHCl_3/CH_3OH$) followed by 15:5:1, and 4:4:1 ($CHCl_3/CH_3OH/i$-$PrNH_2$). Pure I_2-D-PhTX-343 was obtained in 61% yield (0.061 g) as a slightly yellow oil. R_f: 0.70 in 4:4:1 ($CHCl_3/CH_3OH/i$-$PrNH_2$). FAB-MS (thioglycerol, $C_{23}H_{39}N_5O_3I_2$): m/z 687 $(M)^+$; ^{1}H-NMR (400 MHz, D_2O) δ: 0.71 (t, J = 8Hz, 3H), 1.44 (m, 8H), 1.74 (m, 2H), 2.09 (t, J = 6Hz, 2H), 2.47 (m, 2H), 2.70-3.30 (m, 12H), 4.14 (t, J = 3Hz, 1H), 7.41 (s, 2H); ^{13}C-NMR(75 MHz, D_2O, DSS as external reference) δ: 15.5, 21.7, 22.5, 25.5, 25.8, 26.7, 28.2, 37.7, 38.6, 39.4, 40.0, 46.8, 47.4, 49.7, 49.8, 58.6, 91.3, 127.7, 142.3, 165.4, 176.3, 179.8.

Method B

***p*-Azidocinnamic acid**

To a 10 mL solution of H_2O and 2 mL of H_2SO_4 at 0°C containing 1.00 g (5.00 mmol) of *p*-aminocinnamic acid-hydrochloride was added 0.41 g (6.0 mmol, 1.2 eq) of $NaNO_2$.. This solution was stirred at 0°C for 30 min resulting in the solubilization of the mixture. After 30 min, excess urea (≈30 mg) was added to destroy any excess $NaNO_2$. This solution was stirred for another 15 min before 0.65 g (10.00 mmol, 2.00 eq) of NaN_3 in 2 mL of H_2O was added dropwise resulting in immediate formation of a white precipitate and the evolution of nitrogen. After completion of the addition, the mixture was stirred for 15 min at 0°C. The mixture was then filtered and washed with cold water. The remaining solid was dried for 48 h under vacuum before recrystallization from boiling ethanol. The desired product was obtained as a light yellow powder in 80% yield (0.76 g). Rf = 0.74 in 5:1 ($CHCl_3/CH_3OH$). DCI-MS (NH_3,$C_9H_7O_2N_3$): 207 $(M+18)^+$; ^{1}H-NMR (300 MHz, $CD_3OD/CDCl_3$ (5:1), TMS) δ: 6.41 (d, J = 16Hz, 1H), 7.08 (d, J = 9Hz, 2H), 7.60 (d, J = 8Hz, 2H), 7.63 (d, J = 16Hz, 1H); ^{13}C-NMR (75.4 MHz, $CD_3OD/CDCl_3$ (5:1) $CDCl_3$) δ: 116.8, 118.4, 128.7, 130.5, 141.2, 143.0, 168.2.

N-(*p*-azidocinnamoyl)-L-tyrosyl-N, N', N"- tri-Boc-spermine amide

To 5 mL of DMF containing 0.51 g (0.67 mmol) of L-tyrosyl-N, N', N"- tri-Boc-spermine amide[25] was added 0.14 g (0.74 mmol, 1.1 eq) of *p*-azidocinnamic acid, 0.16 mL (0.72 mmol, 1.1 eq) of diphenylphosphoryl azide (Aldrich), and 0.10 mL (0.72 mmol, 1.1 eq) of Et_3N. The mixture was stirred for 12 h under argon atmosphere. The solvent was removed in vacuo and the residual oil was partitioned between 30 mL of H_2O and 50 mL of EtOAc. The aqueous layer was separated and extracted with EtOAc (2 x 50 mL).

The combined organic layers were washed three times with brine followed by concentration of the organic layers in vacuo leaving an oil. The oil was purified on a silica gel flash chromatography column eluted with a step gradient of 99:1 and 9:1 ($CHCl_3/CH_3OH$) yielding the desired product in 85% yield (0.53 g). ^{1}H-NMR (300 MHz, $CDCl_3$, TMS) δ: 1.35 (s, 9H), 1.45 (s, 18H), 1.50 (broad m, 8H), 3.00 (broad m, 14H), 4.74 (broad s, 1H), 6.38 (d, J = 15Hz, 1H), 6.85 (d, J = 8Hz, 2H), 6.93 (d, J = 9Hz, 2H), 7.12 (d, J = 9Hz, 2H), 7.31 (d, J = 8Hz, 7.55 (d, J = 15Hz1H).

N-(*p*-azidocinnamoyl)-L-tyrosyl-spermine amide

To 10 mL of dry CH_2Cl_2 containing 0.53 g (0.56 mmol) of N-(*p*-azidocinnamoyl)-L-tyrosyl-N, N', N"- tri-Boc-spermine amide was added 1.0 mL of TFA. This solution was stirred under argon atmosphere at room temperature for 2 h. The reaction was terminated by evaporation of the solvent leaving a brown oil. The oil was purified by silica gel flash column chromatography, eluting with a step gradient of 9:1 ($CHCl_3/CH_3OH$), 15:5:1 and 4:4:1 ($CHCl_3/CH_3OH/i\text{-}PrNH_2$). The desired product was obtained as a clear oil in 33% yield (0.16 g). DCI-MS (NH_3, $C_{28}H_{40}N_8O_3$): 537 $(M+1)^+$; ^{1}H-NMR (300MHz, CD_3OD, TMS) δ: 1.50-2.00 (m, 8), 2.50-3.10 (m, 12H), 4.56 (t, J = 8Hz, 1H), 6.67 (d, J = 15Hz, 1H), 6.71 (d, J = 8Hz, 1H), 7.05 (d, J = 8Hz, 2H), 7.07 (d, J = 9Hz, 2H), 7.46 (d, J = 15Hz, 1H), 7.58 (d, J = 9Hz, 2H).

N-(*p*-azidocinnamoyl)-mono-and di-iodo-L-tyrosyl-spermine amide

To a 0.2 mL CH_3OH solution of 0.016 g (0.018 mmol) of N-(*p*-azidocinnamoyl)-L-tyrosyl-spermine amide was added 1 mL of H_2O containing 0.009 g (0.05 mmol, 3 eq) of KI and 0.014 g (0.078 mmol, 4.2 eq) of K_2HPO_4. To this mixture was added dropwise 1 mL of CH_3OH/H_2O (1:1) containing 0.009 g (0.05 mmol, 3 eq) of N-bromosuccinimide. The reaction mixture was stirred under argon atmosphere at room temperature for 30 min. The reaction was terminated by removal of the solvent by lyophilization. The residue was dissolved in 0.5% TFA/H_2O and applied to a pre-washed ODS reversed phase pipette column. The column was eluted with H_2O, CH_3CN/H_2O (1:1), followed by elution with CH_3CN/H_2O/TFA (89:10:1), CH_3CN/H_2O/TFA (79:20:1), and CH_3CN/H_2O/TFA (50:50:1). This resulted in the elution of two products, mono- and diiodo compouds which were separated by reversed phase HPLC (Rainin Dynamax HPLC system, YMC-Pack ODS semi-preparative reversed phase column, [(15-50% CH_3CN)/30 min]/0.5% TFA/H_2O, 1.0 mL/min, 274 nm detection) resulting in the isolation of 0.012 g (65%) of N-(*p*-azidocinnamoyl)- monoiodo-L-tyrosyl-spermine amide and 0.0092 g (45%) of N-(*p*-azidocinnamoyl)-diiodo-L-tyrosyl-spermine amide. N-(*p*-azidocinnamoyl)-diiodo-L-tyrosyl-spermine amide: FAB-MS (nitrobenzyl alcohol, $C_{28}H_{39}N_8O_3I$): m/z 663 $(M+1)^+$; ^{1}H-NMR (300 MHz, CD_3OD, TMS) δ: 3.00 (broad m, 14H), 4.48 (t, J = 6Hz, 1H), 6.64 (d, J = 15Hz, 1H), 6.78 (d, J = 8Hz, 1H), 7.09 (d, J = 10Hz, 1H), 7.09 (d, J = 8Hz, 2H), 7.49 (d, J = 15Hz, 1H), 7.66 (s, 1H), 7.62 (d, J = 10Hz, 2H). N-(*p*-Azidocinnamoyl)-diiodo-L-tyrosyl-spermine amide: FAB-MS (nitrobenzyl alcohol, $C_{28}H_{38}N_8O_3I_2$): m/z 789 $(M+1)^+$; ^{1}H-NMR (300 MHz, CD_3OD, TMS) δ: 3.00 (broad m, 14H), 4.46 (t, J = 9Hz, 1H), 6.62 (d, J = 15Hz, 1H), 7.10 (d, J = 6Hz, 2H), 7.49 (d, J = 15Hz, 1H), 7.67 (s, 2H), 7.60 (d, J = 8Hz, 2H).

N-α-N^G,N$^{G'}$-tri-Boc-L-arginine-N-hydroxysuccinimide ester

A 1 mL EtOAc solution of 0.380 g (0.421 mmol, 1.00 eq) of N-hydroxysuccinimide was added to a 3 mL EtOAc solution containing 0.200 g (0.421 mmol) of N-α-N^G,$N^{G'}$-tri-Boc-L-arginine (BACHEM Bioscience, Inc., Philadelphia, PA). To this solution was added 0.206 g (0.421 mmol, 1.00 eq) of DCC. This solution was stirred for 1 h under an argon atmosphere at room temperature. After 1 h, the reaction mixture was filtered through Celite and the filtrate was evaporated in vacuo. The white residue was dissolved in 1 mL of 9:5 (hexanes/EtOAc) and washed through a 2 cm plug of silica gel. The filtrate was evaporated leaving 0.344 g (76%) of a white foam. R_f: 0.28 in 9:5 (hexanes/EtOAc). FAB-MS (thioglycerol, $C_{25}H_{41}N_5O_{10}$): calcd 572.2917, found 572.2918 $(M+1)^+$; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.46 (s, 9H), 1.50 (s, 9H), 1.53 (s, 9H), 1.79 (broad s, 2H), 1.95 (broad s, 2H), 2.84 (s, 4H), 3.81 (t, J = 7Hz, 2H), 4.02 (q, J = 7Hz, 1H), 6.01 (d, J = 8.6Hz, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 14.1, 20.9, 24.6, 25.5, 27.7, 28.1, 28.2, 43.8, 51.8, 60.3, 78.8, 79.8, 83.9, 154.7, 155.0, 160.4, 163.4, 168.0, 168.6, 171.0; FT-IR (KBr pellet): 3388, 2981, 1745, 1715, 1611, 1369, 1277, 1252, 1149 cm^{-1}.

N-Cbz-tyrosyl-spermine-N-α-N^G,$N^{G'}$-tri-Boc-L-arginine di-amide

To a 5 mL DMF solution containing 0.265 g (0.532 mmol) of N-Cbz-tyrosyl-spermine amide was added 0.304 g (0.532 mmol, 1.00 eq) of N-α-N^G,$N^{G'}$-tri-Boc-L-arginine-N-hydroxysuccinimide ester. This mixture was stirred under argon at room temperature for 12 h. The reaction was worked up by evaporation of the solvent in vacuo. The residue was dissolved in $CHCl_3$ and this solution was extracted with H_2O. The combined aqueous layers were extracted twice with $CHCl_3$. The $CHCl_3$ layers were combined and washed once with brine before being dried over $MgSO_4$. The slurry was filtered through cotton and the filtrate was concentrated in vacuo to yield a slightly yellowish oil. This oil was purified by chromatography on silica gel using a step gradient of 9:1 ($CHCl_3$/CH_3OH) and 15:5:1 ($CHCl_3$/CH_3OH/*i*-$PrNH_2$). The product was obtained as a clear oil in 90% yield (0.457 g). R_f: 0.79 in 15:5:1 ($CHCl_3$/CH_3OH/*i*-$PrNH_2$). FAB-MS (glycerol, $C_{48}H_{77}O_{11}N_9$): m/z 956 $(M+1)^+$; ^{1}H-NMR (300 MHZ, CD_3OD, TMS): δ 1.10-1.17 (m, 4H), 1.43 (s, 9H), 1.47 (s, 9H), 1.53 (s, 9H), 1.61-1.68 (m, 10H), 2.46-2.58 (m, 8H), 2.28 (1H), 2.95 ((m. 1H), 3.11-3.24 (m, 7H), 3.86 (m, 2H), 4.00 (s, 1H), 4.25 (m. 1H), 5.03 (m, 2H), 6.70 (d, J = 8Hz, 2H), 7.04 (d, J = 8Hz), 2H), 7.27 (m, 5H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 24.5, 24.9, 25.1, 25.9, 26.2, 27.2, 28.1, 28.2, 28.4, 28.7, 36.8, 37.8, 38.3, 42.7, 43.8, 46.0, 46.6, 48.4, 48.7, 49.9, 53.9, 56.7, 66.6, 79.1, 79.5, 84.0, 115.6, 126.8, 127.8, 127.9, 128.3, 130.3, 136.2, 154.6, 155.6, 156.3, 160.7, 163.1, 170.8, 172.7, 175.8; FT-IR (thin film): 2977, 1713, 1652, 1609, 1515, 1455, 1272, 1250, 1148 cm^{-1}.

N-Cbz-tyrosyl-N,N'-di-Boc-spermine-N-α-N^G,$N^{G'}$-tri-Boc-L-arginine di-amide

N-Cbz-tyrosyl-spermine-N-α-N^G,$N^{G'}$-tri-Boc-L-arginine di-amide, 0.279 g (0.292 mmol), was dissolved in 3 mL of CH_2Cl_2 and to this solution was added 0.134 g (0.613 mmol, 2.10 eq) of di-*tert*-butyl dicarbonate ($(Boc)_2O$). This mixture was stirred under argon atmosphere at room temperature for 12 h. The reaction was worked up by evaporation of the solvent in vacuo followed by silica gel flash column chromatography, eluting with $CHCl_3$ followed by 50:1 ($CHCl_3$/CH_3OH). This yielded 0.274 g (81%) of a white foam. R_f: 0.45 in 20:1 ($CHCl_3$/CH_3OH). FAB-MS (thioglycerol, $C_{58}H_{93}N_9O_{15}$): m/z 1156 $(M+1)^+$; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.24 (m, 2H), 1.30-2.90 (bm, 35H), 2.90-3.55 (bm, 17H), 3.71 (q, J = 7.02, 2H), 3.90 (broad s, 2H), 4.20-4.40 (broad s, 2H), 5.07 (m, 2H), 6.76 (d, J = 7Hz, 2H), 7.01 (d, J =

7Hz, 2H), 7.32 (broad s, 5H), 9.35 (broad s, 2H); ^{13}C-NMR (75.4 MHz, $CDCl_3$) δ: 18.1, 24.4, 24.5, 25.3, 25.5, 25.6, 25.7, 25.8, 25.9, 27.3, 27.6, 27.8, 28.1, 28.2, 28.7, 28.8, 31.0, 43.8, 43.9, 46.6, 46.8, 50.2, 56.5, 57.9, 66.7, 79.1, 79.1, 79.5, 79.8, 83.9, 84.0, 115.4, 127.0, 127.8, 127.9, 128.3, 130.2, 136.1, 154.7, 155.5, 155.8, 155.9, 160.7, 171.0, 172.4; FT-IR (KBr pellet): 3898, 2979, 1716, 1612, 1517, 1454, 1367, 1274, 1252, 1148 cm^{-1}.

H_2N-tyrosyl-N,N'-di-Boc-spermine-N-α-N^G,$N^{G'}$-tri-Boc-L-arginine di-amide

To a 3.5 mL CH_3OH solution of 0.39 g (0.34 mmol) of N-Cbz-tyrosyl-N,N'-di-Boc-spermine-N-α-N^G,$N^{G'}$-tri-Boc-L-arginine di-amide was added approximately 0.050 g of 10% Pd/C. This solution was purged with hydrogen gas several times using an aspirator. The solution was then stirred at room temperature for 4 h under a hydrogen atmosphere before the mixture was filtered and washed through a pad of Celite. The solvent was evaporated in vacuo and the residue was purified by chromatography on silica gel using a step gradient of $CHCl_3$, 100:1, 50:1, and 20:1 ($CHCl_3/CH_3OH$). This yielded 0.23 g (67%) of a white foam. R_f: 0.30 in 10:1 ($CHCl_3/CH_3OH$). ^{1}H-NMR (250 MHz, CD_3OD, TMS) δ: 1.25-1.55 (broad s, 45H), 1.58-1.80 (bm, 10H), (ABX system: δ_a = 2.73, δ_b = 2.88, δ_x = 3.98, J_{ab} = 15Hz, $J_{ax/bx}$ = 8Hz, 1H, 1H, 1H), 2.95-3.30 (bm, 16H), 3.44 (broad s, 1H), 3.86 (d, J = 5.7Hz, 2H), 6.71 (d, J = 8Hz, 2H), 7.01 (d, J = 8Hz, 2H); ^{13}C-NMR (75.4 MHz, $CDCl_3$) δ: 24.5, 25.4, 25.5, 25.6, 25.8, 25.9, 27.9, 28.2, 28.4, 28.8, 28.9, 35.7, 36.4, 37.4, 40.2, 40.6, 43.7, 43.9, 46.7, 50.5, 53.8, 54.1, 56.4, 56.7, 79.1, 79.5, 84.0, 84.2, 115.5, 127.5, 128.2, 128.7, 130.3, 130.9, 154.8, 155.5, 155.9, 160.8, 163.0, 163.3, 172.3, 174.3, 174.7; FT-IR (KBr pellet): 3377, 2980, 1708, 1673, 1610, 1571, 1367, 1273, 1252, 1149 cm^{-1}.

N-(*p*-azidocinnamoyl)-tyrosyl-N,N'-di-Boc-spermine-N-α-N^G,$N^{G'}$-tri-Boc-L-arginine di-amide

To a dry round bottom flask containing 0.18 g (0.095 mmol, 1.0 eq) of *p*-azidocinnamic acid was added 1 mL of oxalyl chloride. This mixture was stirred for 90 min at room temperature under an argon atmosphere. At this time, the excess oxalyl chloride was completely removed in vacuo. The residue was dissolved in 1.0 mL of dry CH_2Cl_2 and this solution was added to a 1 mL CH_2Cl_2 solution of 0.97 g (0.095 mmol) of H_2N-tyrosyl-N,N'di-Boc-spermine-N-α-N^G,$N^{G'}$-tri-Boc-L-arginine di-amide and 15 μl (0.11 mmol, 1.1 eq) of Et_3N. This reaction mixture was stirred at room temperature under argon atmosphere for 12 h protected from strong, direct light. The reaction was worked up by adding 30 mL of CH_2Cl_2 and extracting this solution with diluted aqueous $NaHCO_3$ solution 3 times. The combined aqueous layers were extracted 2 times with CH_2Cl_2. The combined organic layers were washed once with brine solution, dried over $MgSO_4$ and then filtered through a plug of cotton. The filtrate was concentrated in vacuo leaving a clear, slightly yellow oil. This oil was purified by chromatography on silica gel using a step gradient of $CHCl_3$, 100:1, and 50:1 ($CHCl_3/CH_3OH$). This purification yielded 0.92 g (82%) of a white foam. R_f: 0.61 in 9:1 ($CHCl_3/CH_3OH$). FAB-MS (thioglycerol, $C_{59}H_{92}N_{12}O_{14}$): m/z 1193 $(M+1)^+$; ^{1}H-NMR (300 MHz, CD_3OD, TMS) δ: 1.78 (t, J = 7Hz, 2H), 1.35-1.52 (broad s, 36H), 1.53 (s, 9H), 1.59-1.78 (bm, 11H), (ABX system: δ_a = 2.88, δ_b = 3.10, δ_x = 4.59, J_{ab} = 13Hz, $J_{ax/bx}$ = 7Hz, 1H, 1H, 1H), 3.07-3.28 (bm, 15H), 3.60 (q, J = 7Hz, 2H), 3.87 (bm, 2H), 3.98 (broad s, 1H), 6.63 (d, J = 16Hz, 1H), 6.70 (d, J = 8Hz, 2H), 7.08 (d, J = 7Hz, 4H), 7.46 (d, J = 16Hz, 1H), 7.57 (d, J = 8Hz, 2H); ^{13}C-NMR (75.4 MHz, $CDCl_3$)

δ: 20.7, 24.5, 25.5, 25.81, 25.9, 27.3, 27.4, 27.9, 28.2, 28.3, 30.0, 36.0, 36.6, 37.4, 37.9, 38.2, 43.0, 43.7, 43.9, 44.2, 44.7, 46.7, 47.0, 53.8, 54.1, 54.9, 55.3, 79.3, 79.7, 80.0, 84.1, 115.5, 119.3, 119.4, 119.5, 120.1, 121.6, 127.2, 129.3, 129.6, 129.8, 130.4, 131.1, 131.5, 140.0, 141.2, 145.3, 154.7, 155.6, 156.1, 160.7, 163.1, 165.4, 170.9, 172.5; FT-IR (KBr pellet): 3291, 2976, 2360, 2122, 1652, 1611, 1516, 1367, 1274, 1252, 1149 cm^{-1}.

N-(*p*-azidocinnamoyl)-tyrosyl-spermine-L-arginine di-amide-(TFA)$_4$

In 1 mL of dry CH_2Cl_2 was dissolved 0.12 g (0.090 mmol) of N-(p-azidocinnamoyl)-tyrosyl-N,N'-di-Boc-spermine-N-α-N^G,$N^{G'}$-tri-Boc-L-arginine di-amide and to this stirred solution was added 1 mL of TFA. This solution was stirred under argon atmosphere at room temperature protected from direct, strong light for 1.5 h. The reaction was worked up by complete evaporation in vacuo of the solvent which left a light yellow oil. This oil was purified by loading it onto a pipette column containing 2 g of silica gel and eluting the TFA salt of the product with a step gradient of $CHCl_3$, 9:1 and 5:1 of ($CHCl_3$/CH_3OH). This resulted in 0.094 g (83%) of a light yellow oil, the TFA salt of the desired product. Rf: 0.20 in 2:2:1:1 (CH_3OH/$CHCl_3$/H_2O/*i*-$PrNH_2$). FAB-MS (glycerol, $C_{34}H_{52}N_{12}O_4$): calcd 693.4313, found 693.4313 $(M+1)^+$; ^{1}H-NMR (300 MHz, D_2O, dioxane as external reference) δ: 1.10-1.20 (m 1H), 1.40-1.65 (bm, 5H), 1.65-1.82 (bm, 7H), 2.61 (broad s, 3H), 2.85 (bm, 9H), 3.05 (bm, 7H), 3.80 (t, J = 6Hz, 1H), 4.29 (t, J = 8Hz, 1H), 6.50 (d, J = 16Hz, 1H), 6.68 (d, J = 8Hz, 2H), 6.88 (d, J = 8Hz, 2H), 6.98 (d, J = 16Hz, 2H), 7.22 (d, 16Hz, 1H), 7.35 (d, 8Hz, 2H); ^{13}C-NMR (75.4 MHz, D_2O, dioxane as external reference) δ: 22.6, 22.7, 23.6, 25.3, 27.9, 35.8, 36.2, 36.4, 40.2, 44.7, 45.0, 46.8, 46.9, 52.8, 56.1,.115.4, 118.4, 119.3, 128.0, 129.6, 130.5, 130.8, 141.0, 141.6, 154.5, 168.3, 169.5, 173.8; FT-IR (thin film): 3070, 2360, 2123, 1670, 1516, 1200, 1132, 1044 cm^{-1}.

N-(*p*-azidocinnamoyl)-diiodo-tyrosyl-spermine-L-arginine di-amide-(TFA)$_4$

To a 10-mL round bottom flask containing 0.029 g (0.023 mmol) of N-(p-azidocinnamoyl)-tyrosyl-spermine-L-arginine diamide-(TFA)$_4$ was added 2 mL of H_2O/CH_3OH (1:1) solvent containing 0.076 g (0.046 mmol, 2.0 eq) of KI and 0.024 g (0.13 mmol, 6.0 eq) of K_2HPO_4. This mixture was stirred to effect complete solubilization of all reagents before adding dropwise a 1.5 mL CH_3OH solution containing 0.082 g (0.046 mmol, 2 eq) of NBS. This addition caused a temporary darkening of the solution as well as the formation of a dark precipitate. Upon completion of the addition of the NBS, the reaction mixture was stirred under a nitrogen atmosphere at room temperature protected from direct, strong light for 30 min. The reaction was terminated by filtering the mixture through a plug of cotton and adjusting the solution to pH 1 by addition of TFA. The solvent was removed in vacuo leaving a light brown residue. This residue was dissolved in 5 mL of CH_3OH and 1 g of silica gel was added followed by complete evaporation in vacuo of the solvent, leaving the silica gel impregnated with the crude product. The impregnated silica gel was loaded dry to a pipette column containing 1.0 g of fresh silica gel. This column was eluted with 9:1 ($CHCl_3$/CH_3OH) followed by elution with 5:1 ($CHCl_3$/CH_3OH) at which time was obtained the TFA salt of the desired product in 85% yield (0.030 g). Rf: 0.15 in 2:2:1:1 (CH_3OH/$CHCl_3$/H_2O/*i*-$PrNH_2$). FAB-MS (glycerol, $C_{34}H_{50}I_2N_{12}O_4$): m/z 945 $(M+1)^+$; ^{1}H-NMR (250 MHz, CD_3OD, TMS) δ: 1.29 (s, 2H), 1.51-2.05 (bm, 12H), 2.80-3.15 (bm, 10H), 3.15-3.38 (bm, 4H), 3.92 (t, J = 6Hz, 1H), 4.48 (t, J = 8Hz, 1H), 6.64 (d, J =

16Hz, 1H), 7.10 (d, J = 8Hz, 2H), 7.49 (d, J = 16Hz, 1H), 7.60 (d, J = 8Hz, 2H), 7.66 (s, 2H); ^{13}C-NMR (75.4 MHz, D_2O dioxane) δ: 22.6, 22.7, 23.6, 25.4, 27.9, 29.8, 30.1, 35.0, 35.9, 36.5, 40.3, 44.7, 45.0, 46.9, 52.8, 55.6, 118.3, 119.4, 129.6, 130.8, 133.0, 140.1, 141.2, 163.1, 165.7, 168.4; FT-IR (thin film): 2928 (br), 2123, 1679, 1203, 1100, 673 cm^{-1}.

6-(*p*-azidobenzamide)-caproic acid

To a 10 mL dioxane solution containing 0.50 g (3.1 mmol) of *p*-azidobenzoic acid was added 0.35 g (3.06 mmol, 1.0 eq) of N-hydroxysuccinimide and 0.63 g (3.06 mmol, 1.0 eq) of DCC. This solution was stirred under an argon atmosphere at room temperature protected from direct, strong light for 12 h. A white precipitate formed during this time. The reaction was terminated by filtration and washing of the suspension through a pad of Celite with dioxane. The filtrate was evaporated leaving a white foam. This white solid was dissolved in 10 mL of DMF, and to this solution was added 0.51 g (3.9 mmol, 1.3 eq) of 6-aminocaproic acid (Aldrich). This solution was stirred for 12 h at room temperature under an argon atmosphere again protected from direct, strong light. The reaction was worked up by evaporating the solvent in vacuo, leaving a clear oil. The oil was dissolved in 50 mL of $CHCl_3$ was extracted twice with H_2O. The combined aqueous layers were back extracted twice with $CHCl_3$. The combined $CHCl_3$ layers were washed once with brine and dried over $MgSO_4$. The slurry was filtered through a plug of cotton, followed by evaporation in vacuo of the solvent leaving a beige solid. The solid was recrystallized from dioxane/pet. ether yielding 0.43 g (51%) of the desired coupled product. R_f: 0.31 in 64:35:1 (hexanes/EtOAc/TFA). EI-MS ($C_{13}H_{16}N_4O_3$): m/z calcd 276.1222, found 276.1222 $(M)^+$; ^{1}H-NMR (300 MHz, CD_3OD, TMS) δ: 1.42 (m, 2H), 1.65 (m, 4H), 2.35 (t, J = 8Hz, 2H), 3.40 (t, J = 8Hz, 2H), 7.09 (d, J = 8Hz, 2H), 7.83 (d, J = 8Hz, 2H); ^{13}C-NMR (75.4 MHz, CD_3OD) δ: 25.0, 26.9, 29.4, 34.3, 40.3, 119.3, 129.4, 131.4, 143.7, 168.3, 176.9.

N-[6-(p-azidobenzamide)-caproyl]-tyrosyl-N,N'-di-Boc-spermine-N-α-N^G,N$^{G'}$-tri-Boc-L-arginine di-amide

In 2 mL of DMF was dissolved 0.235 g (0.229 mmol) of N-Cbz-tyrosyl-N,N'-di-Boc-spermine-N-α-N^G,N$^{G'}$-tri-Boc-L-arginine di-amide, 0.0630 g (0.229 mmol, 1.0 eq) of 6-(*p*-azidobenzamide)-caproic acid, 36.5 μL (0.24 mmol, 1.05 eq) of DEPC. To this stirred mixture was added 33.4 μL of Et_3N (0.24 mmol, 1.05 eq). This mixture was stirred at room temperature under an argon atmosphere protected from strong, direct light for 18 h. The reaction was terminated by evaporation of the solvent in vacuo. The residue was dissolved in $CHCl_3$ and extracted 3 times with dilute $NaHCO_3$. The combined aqueous layers were extracted twice with $CHCl_3$. The combined organic layers were washed once with brine and dried further over $MgSO_4$. The $MgSO_4$ slurry was filtered through a plug of cotton and the filtrate was evaporated in vacuo. The residual oil was purified by chromatography on 15 g of silica gel, eluting with a step gradient of $CHCl_3$, 100:1, 25:1, and 20:1 ($CHCl_3$/CH_3OH). The desired product was obtained as a clear oil in 58% yield (0.17 g). R_f: 0.38 in 7.5% (CH_3OH/$CHCl_3$). ^{1}H-NMR (300 MHz, CD_3OD, TMS) δ: 1.28 (m, 2H), 1.32-1.80 (bm, 62H), 2.21 (t, J = 7.5Hz, 2H), (ABX system: δ_a = 2.76, δ_b = 3.06, δ_x = 4.48, J_{ab} = 13Hz, $J_{ax/bx}$ = 5Hz, 1H, 1H, 1H), 3.00-3.28 (bm, 18H), 3.85 (bm, 2H), 3.99 (bm, 1H), 6.70 (d, J = 8Hz, 2H), 7.04 (d, J = 8Hz, 2H), 7.12 (d, J = 8Hz, 2H), 7.53 (d, J = 8Hz, 2H); FT-IR (KBr pellet): 3388, 2978, 2936, 2124, 1652, 1606, 1516, 1278, 1252, 1148 cm^{-1}.

N-[6-(p-azidobenzamide)-caproyl]-tyrosyl-spermine-L-arginine di-amide-(TFA)$_4$

To a 1 mL solution of dry CH_2Cl_2 containing 0.170 g (0.133 mmol) of N-[6-(*p*-azidobenzamide)-caproyl]-tyrosyl-N,N'-di-Boc-spermine-N-α-N^G,$N^{G'}$-tri-Boc-L-arginine di-amide was added 1 mL of TFA. This mixture was stirred at room temperature under an argon atmosphere, protected from direct, strong light for 1 h. The solvent was then removed in vacuo leaving a slightly yellow oil. The resultant residue was dissolved in 0.5 mL of H_2O, and this aqueous solution was loaded onto a pipette column of pre-washed ODS. The column was eluted with a step gradient of H_2O, 20:1 (H_2O/CH_3CN), and 100:20:1 (H_2O/CH_3CN/TFA). The product was obtained as a slightly yellow oil in 76% yield (0.126 g). R_f: 0.25 in 2:2:1:1 ($CH_3OH/CHCl_3/H_2O/i$-$PrNH_2$). ^{1}H-NMR (300 MHz, D_2O, DSS as external standard) δ: 1.15 (m, J = 7.5Hz, 2H), 1.49 (m, J = 7Hz, 4H), 1.57-1.80 (bm, 9H), 1.90 (m, J = 7.5Hz, 5H), 2.20 (t, J = 7.5Hz, 2H), 2.70 (t, J = 8.7Hz, 2H), (AX of ABX system: δ_a = 2.82, δ_X = 4.35, J_{ab} = 13Hz, $J_{ax/bx}$ = 5Hz, 1H, 1H), 2.94 (m, 2H), 3.04 (m, 6H), 3.18 (t, J = 7.2Hz, 5H), 3.32-3.40 (m, 13H), 3.95 (t, J = 7Hz, 1H), 6.76 (d, J = 9Hz, 2H), 6.99 (d, J = 9Hz, 2H), 7.00 (d, J = 9Hz, 2H), 7.65 (d, J = 8Hz, 2H); ^{13}C-NMR (75.4 MHz, D_2O, DSS as external standard) δ: 25.5, 26.4, 28.0, 28.1, 30.7, 30.8, 37.9, 38.6, 39.1, 39.2, 42.4, 43.0, 47.6, 47.9, 49.6, 51.6, 55.6, 58.2, 118.0, 121.7, 130.8, 131.4, 132.6, 133.1, 146.1, 157.1, 172.1, 172.4, 176.2, 179.4; FT-IR (thin film): 3298, 2950, 2127, 1677, 1204, 1135, 1024 cm^{-1}.

N-[6-(p-azidobenzamide)-caproyl]-di-iodo-tyrosyl-spermine-L-arginine di-amide-(TFA)$_4$

To a 10 mL round bottom flask containing 0.042 g (0.033 mmol) N-[6-(*p*-azidobenzamide)-caproyl]-tyrosyl-spermine-L-arginine diamide-(TFA)$_4$ was added 2.5 mL of H_2O containing 0.011 g (0.066 mmol, 2.0 eq) of KI and 0.035 g (0.20 mmol, 3.0 eq) of K_2HPO_4. This mixture was stirred to effect complete solubilization of all reagents before adding dropwise a 1.0 mL CH_3OH solution of 0.012 g (0.066 mmol, 2.0 eq) of NBS. This addition caused a temporary darkening of the solution as well as the formation of a dark precipitate. Upon completion of the addition of NBS, the reaction mixture was stirred under a nitrogen atmosphere at room temperature protected from direct, strong light for 15 min. The reaction was terminated by filtration of the mixture through a plug of cotton; the filtrate was lyophilized to dryness, leaving a light brown residue. The residue was dissolved in 0.5 mL of H_2O and loaded to a small flash column containing 2.5 g of pre-washed ODS gel. The column was eluted with a step gradient of H_2O, 20:1 (H_2O/CH_3CN), 79:20:1 and 50:50:1 (H_2O/CH_3CN/TFA) which yielded 0.042 g (86%) of a clear, slightly yellow oil. FAB-MS (glycerol, $C_{38}H_{59}I_2N_{13}O_5$): m/z 1031 $(M)^+$; ^{1}H-NMR (250 MHz, CD_3OD, TMS) δ: 1.29 (broad s, 4H), 1.45-1.75 (m, 6H), 1.75-2.05 (m, 9H), 2.23 (t, J = 5Hz, 2H), (AX of ABX system: δ_a = 2.76, δ_X = 4.31, J_{ab} = 13Hz, $J_{ax/bx}$ = 5Hz, 1H, 1H), 2.90-3.16 (bm, 9H), 3.16-3.40 (bm, 5H), 3.90 (t, J = 7Hz, 1H), 7.14 (d, J = 8Hz, 2H), 7.62 (s, 2H), 7.85 (d, J = 8Hz, 2H); ^{13}C-NMR (75.4 MHz, D_2O, DSS as external reference) δ: 23.3, 25.5, 26.3, 27.9, 28.1, 30.9, 37.9, 38.2, 38.3, 42.4, 43.1, 47.9, 49.7, 51.6, 55.7, 58.8, 118.9, 121.7, 131.5, 136.1, 162.9, 172.6, 182.8; FT-IR (thin film): 3222, 2125, 1686, 1208, 1140 cm^{-1}.

***m*-nitrobenzyl allyl ether**

To a stirred suspension of 1.83 g of KOtBu (16.3 mmol) in 100 mL of anhydrous THF cooled in an ice bath was added dropwise *m*-nitrobenzyl alcohol 2.5 g (16 mmol, 1.0 eq) with stirring under an argon

atmosphere. After completion of the addition, the mixture was stirred for 10 min at 0°C. At this time, a 25 mL THF solution of 2.37 g of allylbromide (19.6 mmol, 1.20 eq) was added dropwise to the stirred solution. The mixture was then stirred for 80 min at 0°C. The ice bath was then removed and the temperature of the solution was allowed to come to room temperature for 1 h before gradually increasing the temperature to 50 °C while stirring for 6 h. The reaction was worked up by filtration through Celite to remove a precipitate that had formed followed by removal of the solvent in vacuo leaving a yellow oil. The oil was purified by silica gel flash column chromatography, eluting with hexanes/CH_2Cl_2 (2:3) to afford a colorless oil in 80% yield (2.53 g). Rf: 0.40 in hexanes/CH_2Cl_2 (2:3). ^{1}H-NMR (300 MHz, $CDCl_3$, TMS) δ: 4.10 (dt, J = 10Hz, 5Hz, 2H), 4.61 (s, 2H), 5.25 (dd, J = 2Hz, 11Hz, 1H), 5.34 (dd, J = 2Hz, 17Hz, 1H), 5.96 (ddd, J = 5Hz, 11Hz, 17Hz, 1H), 7.52 (t, J = 8Hz, 1H), 7.69 (d, J = 8Hz, 1H), 8.13 (dd, J = 2Hz, 8Hz, 1H), 8.22 (broad s, 1H); ^{13}C-NMR (75.4 MHz, $CDCl_3$) δ: 70.6, 71.6, 117.6, 122.1, 122.4, 129.2, 133.3, 134.0, 140.5, 148.2.

_m_-aminobenzyl allyl ether

_m_-Nitrobenzyl allyl ether (2.5 g, 12 mmol) was dissolved in 25 mL of 95% ethanol and to this solution was added 13.88 g (61.52 mmol, 5.5 eq) of $SnCl_2 \cdot 2H_2O$. The reaction mixture was stirred at 70°C for 30 min under an argon atmosphere resulting in the formation of a bright yellow solution. The solution was then poured into 10 g of ice containing 10 mL of saturated $NaHCO_3$ and the pH was carefully adjusted to pH 8 by addition of solid $NaHCO_3$. The aqueous suspension was extracted with EtOAc (2x100 mL). The combined organic extracts were washed with brine and dried over $MgSO_4$ followed by filtration. The filtrate was concentrated in vacuo leaving 1.94 g (94% crude yield) of a slightly yellow oil. This product was carried onto the next step without further purification. ^{1}H-NMR (250 MHz, $CDCl_3$, TMS) δ: 3.66 (broad s, NH_2), 4.02 (d, J = 6Hz, 2H), 4.43 (s, 2H), 5.30 (dd, J = 2Hz, 10Hz, 1H), 5.30 (dd, J = 2Hz, 18Hz, 1H), 5.95 (ddd, J = 6Hz, 10Hz, 18Hz, 1H), 6.58 (dd, J = 3Hz, 8Hz, 1H), 6.70 (m, 2H), 7.10 (t, J = 8Hz, 1H).

_m_-azidobenzyl allyl ether.

In a solution of 3 mL of conc. HCl and 5 mL of H_2O was dissolved 1.90 g (11 mmol) of _m_-aminobenzyl allyl ether and to this solution was added dropwise 5 mL of cold aqueous $NaNO_2$ (0.78 g, 11.38 mmol, 1.05 eq) The resulting mixture was stirred for 1 h at 0°C. At this time, a cold 5 mL aqueous solution of 0.775 g (11.9 mmol, 1.1 eq) of NaN_3 was added dropwise. After completion of the addition, 10 mL of Et_2O was added and the mixture was stirred for 30 min at 0°C. At this time, the reaction mixture was extracted with 100 mL of Et_2O. The combined Et_2O layers were then washed once with cold, saturated $NaHCO_3$ solution, and once with brine. The ether layers were dried over $MgSO_4$, followed by filtration and concentration in vacuo leaving 0.775 g (89% crude yield) of a slightly brown oil. This oil was used without further purification. ^{1}H-NMR (300 MHz, $CDCl_3$, TMS) δ: 4.02 (d, J = 6Hz, 2H), 4.52 (s, 2H), 5.20 (dd, J = 1Hz, 10Hz, 1H), 5.30 (dd, J = 1Hz, 17Hz, 1H), 5.93 (ddd, J = 6Hz, 10Hz, 17Hz, 1H), 6.91 (dd, J = 2Hz, 8Hz, 1H), 7.02 (broad s, 1H), 7.08 (d, J = 8Hz, 1H), 7.28 (t, J = 8Hz, 1H); ^{13}C-NMR (75.4 MHz, $CDCl_3$) δ: 71.1, 71.2, 117.1, 117.8, 118.0, 123.8, 129.5, 134.3, 140.0, 140.3.

2-(_m_-azidobenzyloxy)-acetaldehyde

To 50 mL of a dioxane/H_2O (1:1) solution at 0°C containing 1.0 g (5.0 mmol) of *m*-azidobenzyl allyl ether was added 0.15 mL of a 2.5% (wt/vol) solution of OsO_4 in *tert*-butanol. The solution was stirred for 5 min at 0°C after which time was added slowly 3.19 g (14.9 mmol, 3.00 eq) of $NaIO_4$ powder. After completion of the addition, the mixture was stirred for 2 h at 0°C and for another 7 h at room temperature during which time a white precipitate formed. The suspension was filtered and washed with 10 mL of CH_2Cl_2 through Celite . The filtrate was extracted with CH_2Cl_2 (2x80mL). The organic layers were combined and washed once with cold, saturated $NaHCO_3$ and once with brine. The organic layer was dried over $MgSO_4$ and filtered. The filtrate was evaporated in vacuo leaving a brown oil which was purified by silica gel column chromatography, eluting with a step gradient of hexanes/CH_2Cl_2 (2:3) and ether/CH_2Cl_2 (1:3). The desired product was obtained in 75% yield (0.76 g) as a dark oil. ^{1}H-NMR (250 MHz, $CDCl_3$, TMS) δ: 4.08 (s, 2H), 4.57 (s, 2H), 6.94 (dd, J = 2Hz, 8Hz, 1H), 7.02 (broad s, 1H), 7.09 (d, J = 8Hz, 1H), 7.30 (t, J = 8Hz, 1H), 9.70 (s, 1H); ^{13}C-NMR (75.4 MHz, $CDCl_3$) δ: 72.9, 75.4, 118.2, 118.7, 124.2, 129.9, 138.9, 140.4, 199.9.

Ethyl 6-(*m*-azidobenzyloxy)-E,E-2,4-hexadienoate

To a 7.0 mL solution of dry THF at -78°C containing 0.63 g (2.5 mmol, 1.1 eq) of triethyl 4-phosphonocrotonate was added dropwise under an argon atmosphere 1.10 mL of n-BuLi 2.5 M in hexane (2.75 mmol, 1.1 eq). The solution was stirred for 80 min at -78°C before a 5 mL THF solution containing 0.50 g (2.46 mmol) of 2-(*m*-azidobenzyloxy)-acetaldehyde was added dropwise. The resulting solution was stirred for 8 h at -78°C and then for 1 h at room temperature. The reaction was terminated by quenching with 10 mL of saturated NH_4Cl solution. The mixture was then extracted with 100 mL of Et_2O. The organic extracts were washed with brine, dried over $MgSO_4$ and filtered. The filtrate was evaporated in vacuo leaving a yellow oil which was purified by silica gel flash column chromatography, eluting with Et_2O/hexanes (1:3). The desired product was obtained in 40% yield (0.28 g) as a slightly yellow oil. R_f: 0.32 in Et_2O/hexanes (1:3). DCI-MS (NH_3, $C_{15}H_{17}N_3O_3$): m/z 287 $(M)^+$, 259 $(M-N_2)^+$; ^{1}H-NMR (250 MHz, $CDCl_3$, TMS) δ: 1.26 (t, J = 4Hz, 3H), 4.07-4.21 (m, 4H), 4.51 (s, 2H), 5.87 (d, J = 15Hz, 1H), 6.15 (dt, J = 5Hz, 15Hz, 1H), 6.35-6.45 (dd, J = 11Hz, 15Hz, 1H), 6.93 (dd, J = 2Hz, 8Hz, 1H), 7.00 (broad s, 1H), 7.09 (d, J = 8Hz, 1H), 7.21-7.34 (m, 2H); ^{13}C-NMR (75.4 MHz, $CDCl_3$) δ: 14.2, 60.3, 69.8, 71.9, 117.9, 118.3, 129.3, 129.8, 138.1, 140.0, 140.2, 143.5, 166.8.

6-(*m*-Azidobenzyloxy)-E,E-2,4-hexadienoic acid

To 0.10 g (2.5 mmol) of LiOH·H_2O dissolved in 4 mL of DME/CH_3OH/H_2O (3:2:1) was added 0.23 g (0.82 mmol) ethyl 6-(*m*-azidobenzyloxy)-E, E-2,4-hexadienoate in 2 mL of DME/CH_3OH/H_2O (3:2:1). This solution was stirred at room temperature for 24 h under an argon atmosphere while protected from strong, direct light. The reaction mixture was acidified by addition of 6 mL of cold 1.0 M HCl and extracted with CH_2Cl_2 (2x30 mL). The combined organic extracts were washed with brine solution and dried over $MgSO_4$. This suspension was filtered and the filtrate was evaporated in vacuo,.leaving the desired product in crude form in 88% yield (0.187 g) which crystallized upon standing. This product was not purified further. ^{1}H-NMR (250 MHz, $CDCl_3$, TMS) δ: 4.14 (d, J = 5Hz, 2H), 4.48 (s, 2H), 5.88 (d, J = 15Hz, 1H), 6.20 (dt, J =

5Hz, 15Hz, 1H), 6.45 (dd, J = 11Hz, 15Hz, 1H), 6.95 (dd, J = 2Hz, 8Hz, 1H), 7.01 (s, 1H), 7.10 (d, J = 8Hz, 1H), 7.29-7.41 (m, 2H), 8.87 (broad s, 1H); ^{13}C-NMR (75.4 MHz, $CDCl_3$) δ: 69.7, 71.9, 118.0, 118.4, 124.1, 128.9, 129.8, 139.6, 139.9, 140.2, 145.9, 172.4.

6–(*m*-Azidobenzyloxy)-E,E-2,4-hexadienoic acid N-hydroxysuccinimide ester

To 6–(*m*-azidobenzyloxy)-E,E-2,4-hexadienoic acid (0.18 g, 0.71 mmol) dissolved in 5 mL of dry CH_2Cl_2 was added 0.082 g (0.71 mmol, 1.0 eq) of N-hydroxysuccinimide. To the resulting solution cooled to 0°C was added 0.16 g (0.78 mmol, 1.1 eq) of DCC dissolved in 3 mL of CH_2Cl_2. The reaction mixture was stirred under an argon atmosphere for 2 h at 0°C and then for 12 h at room temperature while protected from strong, direct light. At this time, the white suspension was filtered and washed through Celite with 10 mL of CH_2Cl_2. The filtrate was diluted with CH_2Cl_2 and washed with cold, saturated $NaHCO_3$ solution (2x10 mL). The organic layer was dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo leaving a pale yellow oil which was purified by silica gel flash column chromatography, eluting with 1:1 (EtOAc/hexanes). The desired product was obtained as a thick oil in 92% yield (0.23 g). Rf = 0.37 in 1:1 (EtOAc/hexanes). ^{1}H-NMR (250 MHz, $CDCl_3$, TMS) δ: 2.81 (s, 4H), 4.15 (d, J = 4Hz, 2H), 4.49 (s, 2H), 6.00 (d, J = 15Hz, 1H), 6.28 (dt, J = 4Hz, 15Hz, 1H), 6.48 (dd, J = 11Hz, 15Hz, 1H), 6.65 (dd, J = 2Hz, 8Hz, 1H), 7.00 (s, 1H), 7.08 (d, J = 8Hz, 1H), 7.31 (t, J = 8Hz, 1H), 7.48 (dd, J = 11Hz, 15Hz, 1H).

N-[(6-*m*-azidobenzyloxy)-E,E-2,4-hexadienoamide]-L-tyrosyl-N, N'-di-Boc-spermine-N-α-N^G, $N^{G'}$-tri-Boc-L-arginine di-amide

A 4 mL solution of anhydrous DMF containing 0.19 g (0.19 mmol, 1.0 eq) H_2N-L-tyrosyl-N, N'-di-Boc-spermine-N-α-N^G, $N^{G'}$-tri-Boc-L-arginine di-amide and 0.067 g (0.19 mmol) 6-(*m*-azidobenzyloxy)-E,E-2,4-hexadienoic acid N-hydroxysuccinimide ester was stirred at room temperature under argon atmosphere for 48 h while protecting the mixture from strong, direct light. The reaction was terminated by concentration in vacuo leaving a slightly yellow oil which was taken up in CH_2Cl_2 (30 mL) and extracted with saturated $NaHCO_3$ (3x10 mL) and with brine. The organic layer was dried over $MgSO_4$ and filtered; the filtrate was concentrated in vacuo and the residue was purified by silica gel flash column chromatography, eluting with 9:2 (CH_2Cl_2/CH_3OH). The desired product was obtained as a white foam in 40% yield (0.094 g). FAB-MS (glycerol, $C_{63}H_{98}N_{12}O_{15}$): m/z 1262 $(M)^+$; ^{1}H-NMR (400 MHz, CD_3OD, TMS) δ: 1.44-1.70 (m, 57 H), 2.85 (dd, AA'B system, $J_{aa'}$=8Hz, J_{ab}=15Hz, benzylic 1H), 3.05 (broad s, 1H), 3.11-3.27 (m, 12H), 3.86 (m, 2H), 3.98 (broad s, 1H), 4.15 (d, J = 5Hz, 2H), 4.50-4.60 (m, 3H), 6.08 (d, J = 15Hz, 1H), 6.16 (dt, J = 15Hz, 5Hz, 1H), 6.09 (d, J = 15Hz, 1H), 6.43 (dd, ABB' system, J_{ab}=11Hz, $J_{ab'}$=15Hz, 1H), 6.70 (d, J = 8Hz, 1H), 6.99 (dd, J = 2Hz, 8Hz, 1H), 7.05 (m, 3H), 7.10-7.17 (m, 2H), 7.36 (t, J = 8Hz, 1H); ^{13}C-NMR (75.4 MHz, CD_3OD) δ: 26.5, 26.8, 27.2, 28.3, 28.5, 28.7, 28.9, 29.1, 29.2, 30.5, 30.7, 37.6, 38.3, 38.4, 45.5, 46.3, 56.2, 56.9, 71.0, 72.8, 80.0, 80.6, 81.0, 85.2, 116.3, 119.0, 119.3, 125.1, 125.3, 129.0, 130.7, 131.0, 131.3, 138.7, 141.5, 141.6, 142.0, 156.1, 157.5, 157.6, 157.8, 162.1, 164.3, 168.3, 173.5, 175.0.

N-[(6-*m*-azidobenzyloxy)-E,E-2,4-hexadienoamide]-L-tyrosyl-spermine-L-arginine di-amide- $(TFA)_4$

In 3 mL of dry CH_2Cl_2 was dissolved 0.090 g (71 μmol) of N-[(6-*m*-azidobenzyloxy)-E,E-2,4-hexadienoamoyl]-L-tyrosyl-N, N'-di-Boc-spermine-N-α-N^G, $N^{G'}$-tri-Boc-L-arginine di-amide and to this stirred solution was added 2 mL of TFA. This solution was stirred under an argon atmosphere at room temperature protected from direct, strong light for 2 h. At this time, the solvent was removed in vacuo leaving a slightly yellow oil. The resultant oil was dissolved in 0.5 mL of $H_2O/CH_3OH/TFA$ (80:20:1) and loaded onto a pre-washed ODS pipette column. The column was eluted with $H_2O/CH_3OH/TFA$ (80:20:1) to afford the desired product as a slightly yellow oil in 88% yield (0.077 g). FAB-MS (glycerol, $C_{38}H_{58}N_{12}O_5$): m/z 785 $(M+Na)^+$, 763 $(M+1)^+$; ^{1}H-NMR (300 MHz, CD_3OD, TMS) δ: 1.64-1.99 (m, 12H), 2.82-3.49 (m, 16), 3.92 (t, J = 7Hz, 1H), 4.16 (d, J = 5.0Hz, 2H), 4.45-4.50 (t, J = 7Hz, 1H), 4.55 (s, 2H), 6.10 (d, J = 15Hz, 1H), 6.20 (dd, J = 5Hz, 15Hz, 1H), 6.46 (dd, J = 11Hz, 15Hz, 1H), 6.73 (d, J = 8Hz, 2H), 7.01-7.20 (m, 6H), 7.38 (t, J = 8Hz, 1H), ^{13}C-NMR (75.4 MHz, CD_3OD) δ: 24.4, 25.6, 27.4, 29.7, 36.9, 37.5, 38.1, 41.7, 46.2, 46.6, 54.2, 57.4, 71.0, 72.9, 116.4, 119.1, 119.3, 124.8, 125.4, 128.9, 130.7, 131.1, 131.4, 139.0, 141.7, 141.9, 157.5, 158.8, 168.6, 170.8, 175.0.

N-(6-*m*-Azidobenzyloxy)-E,E-hexadienoamide)-L-tyrosyl-N-N'-di-Boc-spermine-N_α, N_ε-di-Boc-L-lysine diamide

A 2 mL solution of anhydrous DMF containing H_2N-tyrosyl-N, N'-di-Boc-spermine-N_α, N_ε-di-Boc-L-lysine diamide (0.12 g, 0.14 mmol, 1.1 eq) and 0.049 g (0.13 mmol) of 6-(*m*-azidobenzyloxy)-E,E-2,4-hexadienoic acid N-hydroxysuccinimide ester was stirred at room temperature under an argon atmosphere for 48 h while protecting the reaction mixture from strong, direct light. At this time, the reaction mixture was concentrated in vacuo leaving a slightly yellow oil. The oil was taken up in 20 mL of CH_2Cl_2 and extracted with saturated $NaHCO_3$ (3x10 mL). The organic layer was washed with brine and dried over $MgSO_4$. The suspension was filtered and the filtrate was concentrated in vacuo leaving an oily residue which was purified by prep-TLC (3x1000μ, 20x20 cm) eluted with .9:1 (CH_2Cl_2/CH_3OH). The desired product was obtained in 50% yield (0.077 g) as a slightly yellow foam. FAB-MS (glycerol, $C_{58}H_{90}N_{10}O_{13}$): m/z 1158 $(M+Na)^+$, 1036 $(M+1\text{-Boc})^+$; ^{1}H-NMR (400 MHz, CD_3OD, TMS) δ: 1.30-1.73 (m, 50H), 2.85 (dd, J = 7Hz, 13Hz, 1H), 2.95-3.29 (m, 15H) 3.97 (dd, J = 5Hz, 8Hz, 1H), 4.16 (d, J = 5Hz, 2H), 4.55-4.60 (m, 3H), 6.10 (d, J = 15Hz, 1H), 6.18 (dt, J = 15Hz, 5Hz, 1H), 6.45 (dd, J = 11Hz, 15Hz, 1H), 6.71 (d, J = 8Hz, 2H), 7.00 (dd, J = 2Hz, 8Hz, 1H), 7.06 (m, 3H), 7.15 (m, 2H), 7.38 (t, J = 8Hz, 1H); ^{13}C-NMR (75.4 MHz, CD_3OD) δ: 24.2, 26.9, 27.0, 27.1, 27.2, 28.8, 29.3, 29.8, 30.6, 33.0, 33.1, 37.5, 37.6, 38.2, 38.4, 41.0, 45.3, 45.5, 46.2, 56.3, 56.9, 71.0, 72.7, 79.8, 80.5, 80.9, 116.2, 119.0, 119.2, 125.0, 125.2, 129.0, 130.7, 131.0, 131.3, 138.6, 141.4, 141.5, 141.9, 157.3, 157.5, 157.6, 157.8, 158.5, 168.2, 173.4, 175.2.

N-[(6-(*m*-azidobenzyloxy)-E,E-2,4-hexadienoamide]-L-tyrosyl-spermine-L-lysine diamide-$(TFA)_4$

In 2 mL of dry CH_2Cl_2 was dissolved 0.070 g (62 μmol) of N-[(6-*m*-azidobenzyloxy)-E,E-hexadienoamide]-tyrosyl-N-N'-di-Boc-spermine-N_α, N_ε-di-Boc-L-lysine diamide and to this stirred solution was added 2 mL of TFA. This solution was stirred under an argon atmosphere at room temperature protected from strong, direct light for 2 h. At this time, the solvent was removed in vacuo leaving a slightly yellowish oil. The residue was dissolved in 0.5 mL of 80:20:1 ($H_2O/CH_3OH/TFA$) and loaded onto a pre-washed ODS

pipette column and eluted with 80:20:1 (H_2O/CH_3OH/TFA) to afford the desired product as a slightly yellow oil in 65% yield (0.048 g). FAB-MS (glycerol, $C_{38}H_{58}N_{10}O_5$): m/z 735 $(M)^+$; ^{1}H-NMR (300 MHz, CD_3OD, TMS) δ: 1.45-1.53 (m, 2H), 1.68-1.96 (m, 12H), 2.84-3.10 (m, 12H), 3.14-3.48 (m, 4H), 3.87 (t, J = 7Hz, 1H), 4.14 (d, J = 5Hz, 2H), 4.44-4.59 (m, 3H), 6.09 (d, J = 15Hz, 1H), 6.13-6.21 (m, 1H), 6.45 (dd, J = 11Hz, 15Hz, 1H), 6.72 (d, J = 8Hz, 2H), 6.95-7.18 (m, 6H), 7.35 (t, J = 8Hz, 1H); ^{13}C-NMR (75.4 MHz, CD_3OD) δ: 22.0, 24.3, 27.3, 28.0, 32.0, 36.8, 37.4, 40.2, 46.2, 46.5, 54.2, 57.3, 71.0, 72.8, 116.3, 119.0, 119.2, 124.7, 125.3, 128.8, 130.6, 131.0, 131.3, 138.9, 168.5, 170.8, 174.9.

L-4-Nitrophenylalanine

L-phenylalanine was nitrated and isolated in 38% yield according to a literature procedure.[26] ^{1}H-NMR (300 MHz, CD_3OD-NaOD, TMS) δ: 2.93 (dd, J = 8Hz, 13Hz, 1H), 3.19 (dd, J = 5Hz, 13Hz, 1H), 3.32-3.55 (dd, J = 5Hz, 8Hz, 1H), 7.51 (d, J = 9Hz, 2H), 8.14 (d, J = 9Hz, 2H).

N-(decanoyl)-4-nitro-L-phenylalanine

Decanoic acid (0.861 g, 5.00 mmol, 1.00 eq) and 0.70 mL (5.0 mmol, 1.0 eq) of Et_3N were dissolved in 15 mL of THF and the solution was cooled to -10°C. To this solution was added 0.48 mL (5.0 mmol, 1.00 eq) of EtOCOCl. This solution was stirred for 30 min at -10°C. At this time, to the cooled solution was added with vigorous stirring 1.05 g (5.00 mmol) of 4-nitro-L-phenylalanine dissolved in 5 mL of 1.0 M NaOH. The ice bath was removed and the mixture was stirred at room temperature for 45 min. The reaction was terminated by pouring the mixture into 100 mL of ice-water, followed by acidification with 7.5 mL of 1.0 M HCl, resulting in the formation of a white precipitate. The precipitate was collected by filtration, washed with 10 mL of H_2O, and dried in vacuo. The white solid was recrystallized from benzene yielding 1.11 g (61%) of the desired product as white crystals. ^{1}H-NMR (300 MHz, CD_3OD, TMS) δ: 0.89 (t, J = 6Hz, 3H), 1.10-1.31 (m, 12H), 1.45 (quintet, J = 7Hz, 2H), 2.14 (t, J = 7Hz, 2H), 3.06 (dd, J = 10Hz, 14Hz, 1H), 3.38 (dd, J = 5Hz, 14Hz, 1H), 4.77 (dd, J = 5Hz, 10Hz, 1H), 7.48 (d, J = 9Hz, 2H), 8.15 (d, J = 9Hz, 2H).

N-(decanoyl)-4-amino-L-phenylalanine

N-(decanoyl)-4-nitro-L-phenylalanine (1.00 g, 2.70 mmol) was dissolved in 1.5 mL of CH_3CO_2H and 40 mL of CH_3OH and to this solution was added 0.075 g of 10% Pd/carbon catalyst. The reaction mixture was cooled in an ice bath and was purged several times with hydrogen. The suspension was stirred under a hydrogen atmosphere at 0°C for 6 h. At this time, the reaction mixture was vented to the atmosphere and allowed to warm to room temperature in order to dissolve the precipitated product. The reaction mixture was filtered and washed through Celite with 10 mL of CH_3OH. Concentration of the filtrate in vacuo afforded 0.90 g (98% crude yield) of the desired product as a white powder. DCI-MS (CH_4, $C_{19}H_{30}N_2O_3$): m/z 335 $(M+1)^+$; ^{1}H-NMR (400 MHz, NaOD/CD_3OD, TMS) δ: 0.90 (t, J = 7Hz, 3H), 1.30 (broad s, 12H), 1.52 (quintet, J = 7Hz, 2H), 2.16 (t, J = 7Hz, 2H), 2.85 (dd, J = 8Hz, 14Hz, 1H), 3.08 (dd, J = 5Hz, 14Hz, 1H), 4.42 (dd, J = 5Hz, 8Hz, 1H), 6.66 (d, J = 8Hz, 2H), 6.99 (d, J = 8Hz, 2H); ^{13}C-NMR (75.4 MHz, D_2SO_4/CD_3OD) δ: 14.3, 23.5, 26.9, 29.9, 30.1, 30.2, 30.3, 32.8, 35.9, 37.3, 55.3, 124.2, 130.5, 131.8, 139.4, 172.3, 177.5.

N-(decanoyl)-4-azido-L-phenylalanine

N-(decanoyl)-4-amino-L-phenylalanine (0.80 g, 2.4 mmol) was dissolved in 7.1 mL of 1.0 M HCl/CH_3CO_2H (3.1:4), and the resulting solution was cooled in an ice bath. To this solution was added 0.17 g (2.5 mmol, 1.0 eq) of $NaNO_2$ dissolved in 2 mL of H_2O. This mixture was stirred at 0°C for 30 min. At this time a 2 mL H_2O solution containing 0.16 g (2.5 mmol 1.0 eq) of NaN_3 was added dropwise to this mixture. The reaction mixture was then stirred at 0°C for 1 h during which time a white precipitate formed. The white precipitate was collected by filtration and washed with 20 mL of H_2O. The solid was dried in vacuo to afford 0.82 g (95%) of a pale yellow solid. DCI-MS (CH_4, $C_{19}H_{28}N_4O_3$): m/z 361 $(M+1)^+$, 343 $(M-H_2O)^+$; ^{1}H-NMR (400 MHz, CD_3OD, TMS) δ: 0.90 (t, J = 6Hz, 3H), 1.13-1.32 (m, 12H), 1.46 (quintet, J = 7Hz, 2H), 2.14 (t, J = 7Hz, 2H), 2.90 (dd, J = 10Hz, 14Hz, 1H), 3.21 (dd, J = 5Hz, 14Hz, 1H), 4.66 (dd, J = 5Hz, 10Hz, 1H), 6.98 (d, J = 7Hz, 2H), 7.26 (d, J = 7Hz, 2H); ^{13}C-NMR (75.4 MHz, $CDCl_3$) δ: 14.1, 22.6, 25.6, 29.1, 29.26, 29.30, 29.4, 31.8, 36.4, 36.7, 53.2, 119.1, 130.7, 132.5, 138.9, 174.1, 174.3.

N-(decanoyl)-4-azido-L-phenylalanine *p*-nitrophenyl ester

To a suspension of 0.70 g (1.9 mmol) of N-(decanoyl)-4-azido-L-phenylalanine and 0.28 g (2.0 mmol, 1.1 eq) of *p*-nitrophenol in 15 mL of EtOAc at 0°C was added 0.42 g (2.0 mmol, 1.1 eq) of DCC dissolved in 3 mL of EtOAc. The mixture was stirred for 2 h at 0°C and then for 22 h at room temperature while protecting the mixture from strong, direct light. The mixture was filtered and washed through Celite with 5 mL of EtOAc. The filtrate was diluted with 60 mL of EtOAc and washed once with cold, saturated $NaHCO_3$ and once with brine. The organic layer was dried over $MgSO_4$ followed by filtration. The filtrate was concentrated in vacuo leaving a slightly yellow oil which solidified on standing. This solid was purified by silica gel flash column chromatography, eluting with hexanes/EtOAc (1:1). The desired product was obtained as a pale yellow solid in 60% yield (0.56 g). FAB-MS (nitrobenzyl alcohol, $C_{25}H_{31}N_5O_5$): m/z 482 $(M+1)^+$, 453 $(M-N_2)^+$; ^{1}H-NMR (400 MHz, $CDCl_3$, TMS) δ: 0.88 (t, J = 7Hz, 3H), 1.26 (m, 12H), 1.62 (m, 2H), 2.23 (t, J = 7Hz, 2H), 3.25 (heptet, 2H), 5.06 (dd, J = 7Hz, 14Hz, 1H), 5.90 (d, J = 8Hz, 1H), 7.02 (d, J = 8Hz, 2H), 7.20 (m, 4H), 8.28 (d, J = 9Hz, 2H); ^{13}C-NMR (75.4 MHz, $CDCl_3$) δ: 14.1, 22.6, 25.5, 29.1, 29.2, 29.3, 29.4, 31.8, 36.3, 37.1, 53.3, 119.4, 122.2, 125.3, 130.6, 131.9, 145.5, 154.8, 169.6, 173.1.

N-(decanoyl)-4-azido-L-phenylalanine-spermine amide

To 0.24 g (0.50 mmol) of N-(decanoyl)-4-azido-L-phenylalanine *p*-nitrophenyl ester in 4 mL of CH_3OH/CH_2Cl_2 (3:5) was added at room temperature with stirring under an argon atmosphere 0.12 g (0.60 mmol, 1.2 eq) of spermine dissolved in 3 mL of CH_3OH. The reaction mixture was stirred for 5 h at room temperature while protected from strong, direct light. At this time, the solvent was removed in vacuo and to the residue was added 8 mL of CH_2Cl_2/CH_3OH (1:1). The resulting suspension was then filtered and washed through Celite with 5 mL of CH_2Cl_2/CH_3OH (1:1). The filtrate was concentrated in vacuo leaving a clear yellow oil which was purified by silica gel flash column chromatography, eluting with a step gradient of CH_2Cl_2/CH_3OH (9:1) and $CH_2Cl_2/CH_3OH/i$-$PrNH_2$ (15:5:1). This resulted in the elution of the bis-acylated spermine adduct (0.049 g). The column elution was continued with $CH_2Cl_2/CH_3OH/i$-$PrNH_2$ (4:4:1) which gave the desired product as a light yellow, clear oil in 50% yield (0.14 g). FAB-MS (nitrobenzyl alcohol, $C_{29}H_{52}N_8O_2$): m/z 545 $(M+1)^+$, 517 $(M-N_2)^+$; ^{1}H-NMR (300 MHz, CD_3OD, TMS) δ: 0.92 (t, J = 6Hz, 3H),

1.13-1.37 (broad s, 12H), 1.46-1.73 (m, 10H), 2.17 (t, J = 7Hz, 2H), 2.48-2.72 (m, 10H), 2.86 (dd, J = 9Hz, 14Hz, 2H), 3.10 (dd, J = 7Hz, 14Hz, 1H), 3.21 (t, J = 7Hz, 2H), 4.57 (dd, J = 7Hz, 9Hz, 1H), 6.99 (d, J = 8Hz, 2H), 7.29 (d, J = 8Hz, 2H); ^{13}C-NMR (75.4 MHz, TFA salt in CD_3OD, TMS) δ: 14.4, 23.7, 24.1, 24.2, 25.3, 26.9, 27.3, 30.1, 30.4, 30.5, 33.0, 36.8, 36.9, 37.8, 38.0, 45.8, 46.2, 50.4, 120.0, 131.8, 135.5, 139.9, 174.7, 176.4.

1-[(12-*tert*-butylcarbamoyl)-5,9-diaza-5,9-diBoc-7-[1-(2-(4-azidobenzoyl)oxy)ethyl]]dodecanyl N-butyryl-O-(2-tetrahydropyranyl) tyrosine amide

A solution of 0.0069 g (0.0079 mmol) of 1-[12-(*tert* -butylcarbamoyl)-5,9-diaza-5,9-(diBoc)-7-[1-(2-(hydroxy)ethyl]]dodecanyl N-butyryl-O-(2-tetrahydropyranyl) tyrosine amide, 0.0019 g (0.012 mmol, 1.5 equiv) of 4-azidobenzoic acid, 0.0025 g (0.013 mmol, 1.6 equiv) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and 0.1 mg (0.8 mmol, 10 mole%) of 4-dimethylaminopyridine was prepared in 1 mL of CH_2Cl_2. The reaction mixture was stirred at 23 °C for 5 h. At this time, the mixture was concentrated in vacuo and the residue was purified by silica gel PTLC (1-10 x 20 cm plate, 7% $CH_3OH/CHCl_3$) to yield 0.0063 g (79%) of a colorless oil. R_f: 0.68 in 9:1 ($CH_3OH/CHCl_3$). FAB-MS (nitrobenzyl alcohol, $C_{52}H_{80}N_8O_{12}$): m/z 1009 $(M+1)^+$; ^{1}H-NMR (300 MHz, $CDCl_3$,TMS) δ: 0.87 (t, J = 6Hz, 3H), 1.20-1.55 (m, 31H), 1.55-1.90 (m, 12H), 1.97 (m, 1H), 2.04 (t, J = 6Hz, 2H), 2.90-3.30 (m, 14H), 3.58 (m, 1H), 3.88 (m, 1H), 4.35 (m, 2H), 4.55 (m, 1H), 5.25 (bs, 1H, NH), 5.35 (s, 1H), 6.20 (bs, 2H,), 6.95 (d, J = 8Hz, 2H), 7.08 (m, 4H), 8.00 (d, J = 9Hz, 2H).

1-[(12-amino)-5,9-diaza-7-[1-(2-(4-azidobenzoyl)oxy)ethyl]]dodecanyl N-butyryl tyrosine amide

To a solution of 0.0020 g (0.0021 mmol) of 1-[12-(*tert* -butylcarbamoyl)-5,9-diaza-5,9-diBoc-7-[1-(2-(4-azidobenzoyl)oxy)ethyl]] dodecanyl N-butyryl-O-(2-tetrahydropyranyl) tyrosine amide in 0.5 mL of $CHCl_3$ was added 0.25 mL of TFA. The mixture was stirred at 23 °C for 5 h. At this time, the mixture was concentrated in vacuo. The residue was purified by silica gel column chromatography (2 g of silica gel, step gradient of 1:2:17 to 1:2:6 of (i-$PrNH_2/CH_3OH/CHCl_3$) to yield 0.001 g (77%) of a clear oil. R_f: 0.64 in 1:4:4 of (i-$PrNH_2/CH_3OH/CHCl_3$). FAB-MS (nitrobenzyl alcohol, $C_{32}H_{48}N_8O_5$): m/z 625 $(M+1)^+$; ^{1}H NMR: (400 MHz, CD_3OD, residual solvent peak: 3.30 ppm) δ: 0.84 (t, J = 7.4Hz, 3H), 1.45 (m, 4H), 1.54 (m, 2H), 1.78 (m, 3H), 2.05 (bs, 1H), 2.15 (t, J = 7.9Hz, 2H), 2.75 (m, 7H), 2.93 (m, 4H), 3.05-3.20 (m, 3H), 4.40 (m, 3H), 6.68 (d, J = 8.5Hz, 2H), 7.03 (d, J = 8.5Hz, 2H), 7.16 (d, J = 8.7Hz, 2H), 8.04 (d, J = 8.7Hz, 2H).

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