Synthesis of Philanthotoxin Analogs with a Branched Polyamine Moiety

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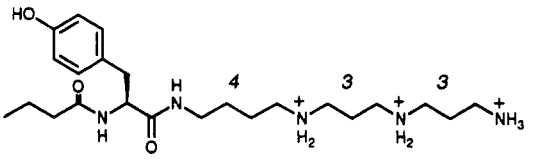
Philanthotoxin (PhTX-433), a potent noncompetitive inhibitor of the L-glutamate receptors and the nicotinic acetylcholine receptors of vertebrates and invertebrates, has a butyryl-tyrosyl-thermospermine structure. Several synthetic analogs of PhTX with hydrophobic alkyl branches in the polyamine chain exhibit 6- to 10-fold enhanced activities to various receptors. Because the branched analogs exhibit such unique activities and because of their importance in tertiary structural studies of ligand/receptor binding, methods for preparing branched PhTX analogs, including photolabile analogs, are presented.

Introduction

L-Glutamate is the major excitatory synaptic transmitter in the central nervous system of vertebrates as well as the peripheral nervous system of insects.[1-4] The L-glutamate receptors (Glu-R) regulate synaptic transmission and neuronal cell degeneration in the vertebrate CNS; they have thus been linked to higher neural functions such as memory and learning, as well as neurological disorders[5] including epilepsy, Huntington's and Alzheimer's diseases, and stroke. The vertebrate Glu-R is classified into two subfamilies: ligand-gated ion channel (ionotropic) and G-protein coupled (metabotropic) receptors. The ionotropic receptors are activated by L-glutamate (Glu) binding and elicit the transport of Na^+ and Ca^{2+} into the cell and K^+ out of the cell.[6] The ionotropic Glu-R are further divided into two subclasses based on their responses to exogenous ligands: (i) *N*-methyl-D-aspartate receptors (NMDA-R) and (ii) kainate (KAIN)/α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors (non-NMDA-R). The invertebrate Glu-R consists of ionotropic receptors classified into four subtypes: (i) quisqualate receptors (qGlu-R, which gate cation channels), (ii) ibotenate receptors (which gate Cl^- channels), (iii) a KAIN-R, and (iv) a purported NMDA-R. Glu-Rs belong to a superfamily of ligand-gated ion channel receptors, including the well-characterized nicotinic acetylcholine (nACh-R)[7] and γ-aminobutyric acid (GABA-R).[7b,8] However, studies on the Glu-Rs have been hampered by their low tissue concentrations, lack of potent antagonists, and difficulty in overexpression.



PhTX-433 1

Figure 1. PhTX-433 (1); 433 indicates the number of methylenes between each N of the polyamine.

The availability of potent agonists and antagonists for these receptors is central for the mode of action studies on a molecular structural basis and design of therapeutic agents. A number of polyamine toxins isolated from spiders[9-13] and the wasp *Philanthus triangulum F.*[14] have been found to be antagonists of Glu-Rs. The major component of the wasp toxin, δ-philanthotoxin or philanthotoxin-433 (PhTX-433), was structurally elucidated as 1 and synthesized by two groups (Figure 1).[15,16] It is a noncompetitive antagonist of Glu-Rs that acts as a channel blocker.[17]

Several groups have reported the preparation of spider and PhTX analogs having higher activity than natural

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(1) Kandel, E. R.; Schwartz, J. H. Principles of Neural Science; Elsevier Science Publishing Co.: New York, 1985.

(2) (a) Foster, A. C.; Fagg, G. E. *Brain Res. Rev.* **1984**, *7*, 103. (b) Watkins, J. C.; Krosgaard-Larsen, P.; Honore, T. *Trends Pharmacol. Sci.* **1990**, *11*, 25.

(3) Cotman, C. W.; Iverson, L. L. *Trends Neurosci.* **1987**, *10*, 263.

(4) Usherwood, P. N. R.; Cull-Candy, S. G. In *Insect Muscle*; Usherwood, P.N.R., Ed.; Academic Press: New York, 1975; p 207.

(5) Choi, D. W. *Neuron* **1988**, *1*, 623.

(6) (a) *Ionic Channels of Excitable Membranes*; Hille, B., Ed.; Sinauer Associates, Inc.: Sunderland, MA, 1992. (b) *Molecular Mechanisms for Sensory Signals*; Kosower, E. M., Ed.; Princeton University Press: Princeton, NJ, 1991.

(7) (a) Noda, M.; Takahashi, H.; Tanabe, T.; Toyosato, M.; Furutani, Y.; Hirose, T.; Asai, M.; Inayama, S.; Miyata, T.; Numa, S. *Nature (London)* **1982**, *299*, 793. (b) Unwin, N. *Neuron* **1993**, *10*, 31.

(8) Schofield, P. R.; Darlison, M. G.; Fujita, N.; Burt, D.; Stephenson, F. A.; Rodriguez, H.; Rhee, L. M.; Ramachandran, J.; Reale, V.; Glencorse, T. A.; Seeburg, P. H.; Barnard, E. A. *Nature (London)* **1987**, *328*, 221.

(9) (a) Grishin, E. V.; Volkova, T. M.; Arseniev, A. S.; Reshetova, V. V.; Onoprienko, L. G.; Magazanik, L. G.; Antonov, S. M.; Federova, I. M. *Bioorg. Khim.* **1986**, *12*, 1121. (b) Grishin, E. V.; Volkova, T. M.; Arseniev, A. S. *Toxicon* **1989**, *27*, 541.

(10) Aramaki, Y.; Yasuhara, T.; Higashijima, T.; Yoshioka, M.; Miwa, A.; Kawai, N.; Nakajima, T. *Proc. Jpn. Acad. (Ser. B)* **1986**, *62*, 359.

(11) Adams, M. E.; Carney, R. L.; Enderlin, F. E.; Fu, E. W.; Jarema, M. A.; Li, J. P.; Miller, C. A.; Schooley, D. A.; Shapiro, M. J.; Venema, V. J. *Biochem. Biophys. Res. Commun.* **1987**, *148*, 678.

(12) Jasys, V. J.; Kelbaugh, P. R.; Nason, D. M.; Phillips, D.; Rosnack, K. J.; Saccomano, N. A.; Stroh, J. G.; Volkman, R. A. *J. Am. Chem. Soc.* **1990**, *112*, 6696 and references cited therein.

(13) Quistad, J. B.; Suwanrumpha, M. A.; Jarema, M. A.; Shapiro, M. J.; Skinner, W.S.; Jamieson, G. C.; Lui, A.; Fu, E. W. *Biochem. Biophys. Res. Commun.* **1990**, *169*, 51.

(14) Piek, T.; Mantel, P.; Engels, E. *Comp. Gen. Pharmacol.* **1971**, *2*, 317.

(15) Piek, T.; Fokkens, R. H.; Karst, H.; Kruk, C.; Lind, A.; van Marle, J.; Nakajima, T.; Nibering, N. M. M.; Shinozaki, H.; Spanner, W.; Tong, T. C. In *Neurotox '88: Molecular Basis of Drug & Pesticide Action*; Lunt, G. G., Ed.; Elsevier: Amsterdam, 1988, p 61.

(16) Eldefrawi, A. T.; Eldefrawi, M. E.; Konno, K.; Mansour, N. A.; Nakanishi, K.;

(17) Ragsdale, D.; Gant, D. B.; Anis, N. A.; Eldefrawi, A. T.; Eldefrawi, M. E. *J. Pharmacol. Exp. Ther.* **1989**, *251*, 156.

0022-3263/93/1958-6596$04.00/0

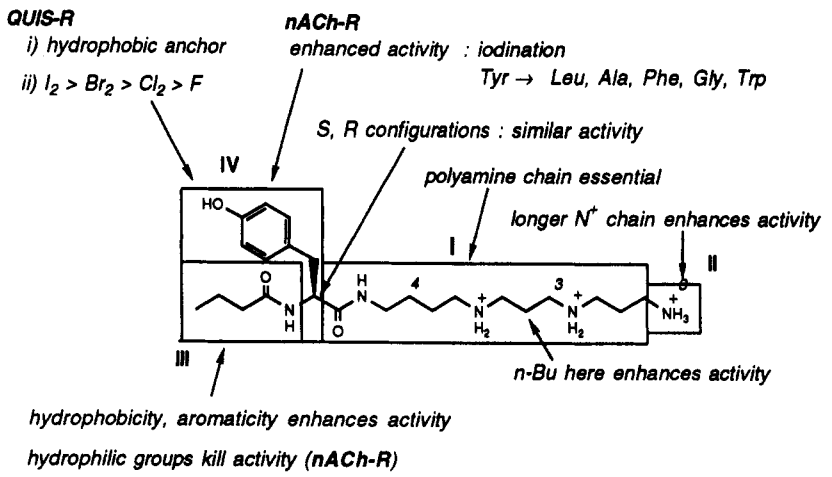


Figure 2. Structure/activity profile.

toxins.[18] We have also synthesized ca. 100 PhTX-433 analogs to study the binding activity of the toxin toward the locust qGlu-R, the vertebrate NMDA-R and non-NMDA-R, as well as the nACh-R of *Torpedo* electric organ.[19,20] The structure of the toxin was dissected into four regions, and various analogs, including those modified in more than two regions, were synthesized[21,22] and submitted to electrophysiological and biochemical assays.[19,20] The results of these structure/activity (SAR) studies are summarized in Figure 2. The extended structures of the PhTX analogs and the availability of many hot and cold analogs containing photoaffinity labels at various sites make them attractive probes to be used as "rulers" in clarifying the tertiary structures of these membrane proteins.

Attachment of the butyl group in the central methylene chain of the polyamine (Figure 2) led to a uniform and several-fold increased antagonistic activity against all three receptors, an unexpected outcome: qGlu-R 5.6-fold,[19] NMDA-R 9.3-fold,[20] and nACh-R 7.8-fold.[20] A recent analysis of the SAR results[23] and preliminary photoaffinity labeling of nACh-R with a PhTX analog[24] have shown that the polyamine moiety inserts itself into the open pore from the cytoplasmic side of the nACh-R, while the hydrophobic regions III and IV (Figure 2) reside in the cytoplasm outside the pore. In this model, the polyamine ammoniums line themselves against the "hydrophilic rings" made up from Ser, Thr, Glu, and Gln residues of the five transmembrane α-helices lining the pore, each α-helix being part of the five subunits constructing the nACh-R. Although Glu-Rs are not homologous with nACh-R, it is believed that the structures of the pore-lining regions are quite similar, and therefore this model should represent a general picture.[7b] Moreover, the model suggests that the increased potency of the butyl analog probably increases the affinity through hydrophobic binding to Leu residues. In order to further check this point for a better understanding of ligand/receptor binding, it is necessary to attach other chains, including those containing photoaffinity labels, at the same and other methylene groups of the polyamine moiety. We report routes for syntheses of branched chain polyamines and PhTXs and the synthesis of a branched chain photolabile PhTX analog.

Results and Discussion

Many methods are available for preparation of polyamines, including amine alkylation, reductive amination, and amide formation followed by reduction to the desired amine. The reported syntheses of PhTX-433 and PhTX-334 were based on the preparation of a polyamine unit using the addition of a primary amine to acrylonitrile followed by LAH reduction to yield the homologated amines.[16] This method was thenadapted for the synthesis of PhTX analogs containing branched polyamines;[21] namely, the appropriate nitrile was prepared, treated with *n*-BuLi, and quenched with an alkyl halide to yield the alkylated product. Unfortunately, the coupled product was obtained as a 1:1 mixture of the desired mono- and undesired bis alkylated products. The resulting chromatographic separation was tedious, and the overall recovery of the desired mono alkylated product was <40%.[21] Rather than optimizing the anion chemistry, a new approach was pursued to provide branched polyamines carrying the alkyl substituent at various positions of the polyamine chain. This procedure is based on reductive amination of an appropriately substituted, protected amino aldehyde with a primary amine.

The initial goals included the preparation of butyl-substituted thermospermine derivatives necessary for the preparation of PhTX-433-Bu3 (**2**), PhTX-334-Bu1 (**3**), and PhTX-433-Bu2 (**4a**) (Figure 3) by known methods.[21] The synthetic sequence for fully protected 433-Bu (**13**) is given in Scheme I.

The aldehyde coupling partner **9** was prepared from hexanenitrile **5**. Alkylation of hexanenitrile by lithiation with LDA, followed by addition of diethyl carbonate,

(18) (a) Blagbrough, I. S.; Bruce, M.; Bycroft, B. W.; Mather, A. J.; Usherwood, P. N. R. *Pestic. Sci.* **1990**, *30*, 397. (b) Blagbrough, I. S.; Brackley, P. T. H.; Bruce, M.; Bycroft, B. W.; Mather, A. J.; Millington, S.; Sudan, H. L.; Usherwood, P. N. R. *Toxicon* **1992**, *30*, 303. (c) Benson, J. A.; Schürmann, F.; Kaufmann, L.; Gsell, L.; Piek, T. *Comp. Biochem. Physiol.* **1992**, *102C*, 267. (d) Asami, T.; Kagechika, H.; Hashimoto, Y.; Shudo, K.; Miwa, A.; Kawai, N.; Nakajima, T. *Biomed. Res.* **1989**, *10*, 185.

(19) Bruce, M.; Eldefrawi, A. T.; Eldefrawi, M. E.; Bukownik, R.; Goodnow, R., Jr.; Kallimopoulos, A.; Konno, K.; Niwa, M.; Nakanishi, K.; Usherwood, P. N. R. *Toxicon* **1990**, *28*, 1333.

(20) (a) Anis, N.; Goodnow, R., Jr.; Konno, K.; Niwa, N.; Kallimopoulos, T.; Bukownik, R.; Nakanishi, K.; Eldefrawi, A.; Eldefrawi, M. *J. Pharmacol. Exp. Ther.* **1990**, *254*, 764.

(21) Goodnow, R., Jr.; Konno, K.; Niwa, M.; Kallimopoulos, T.; Bukownik, R.; Lenares, D.; Nakanishi, K. *Tetrahedron* **1990**, *46*, 3267.

(22) Choi, S.-K.; Goodnow, R. A.; Kalivretenos, A.; Chiles, G. W.; Fushiya, S.; Nakanishi, K. *Tetrahedron* **1992**, *48*, 4793.

(23) Nakanishi, K.; Choi, S.-K.; Hwang, D.; Lerro, K.; Orlando, M.; Kalivretenos, A.G.; Eldefrawi, A.; Eldefrawi, M.; Usherwood, P. N. R. *Pure Appl. Chem.*, in press.

(24) Unpublished results.

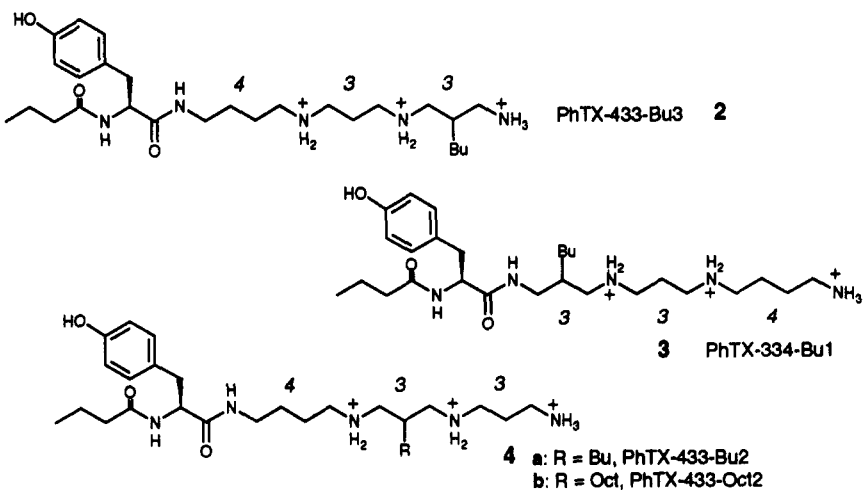
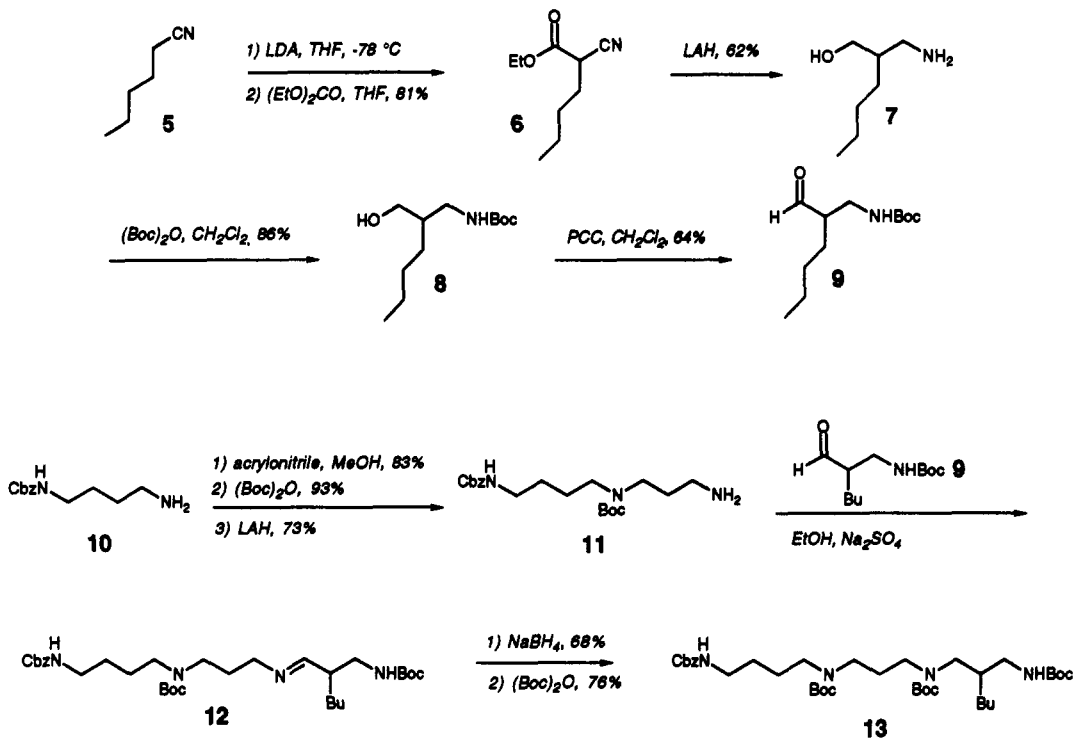


Figure 3. Polyamine-alkylated PhTX analogs.

Scheme I. Synthesis of Polyamine 433-Bu



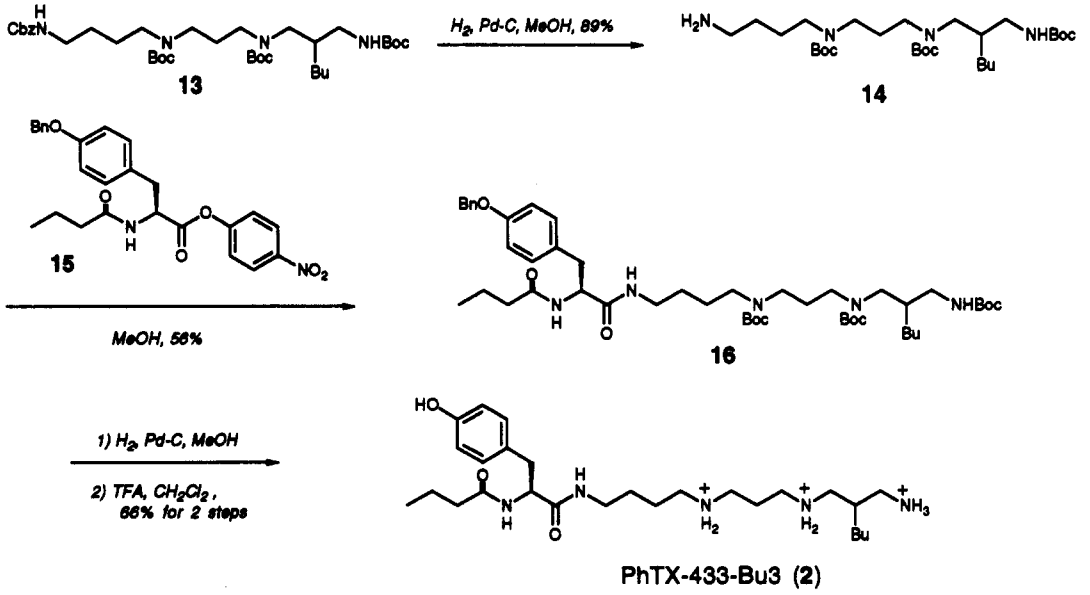
yielded the butyl-substituted cyanoacetate **6** in 81% yield.[25] LAH reduction of the cyanoacetate yielded amino alcohol **7** (62%), which after *N*-Boc protection (86%) and PCC oxidation[26] gave aldehyde **9** (64%). The amine moiety **13** was prepared from mono-Cbz-protected 1,4-butanediamine (**10**) (prepared in four steps from 3-amino-1-propanol, overall yield 52%[27]). Coupling of **10** with acrylonitrile, Boc protection, and LAH reduction of the secondary amine yielded the desired spermidine derivative **11**, 56% overall yield.[21] Coupling of **11** with aldehyde **9** gave imine **12**, which was immediately reduced to the amine with $NaBH_4$ to yield 68% of the desired thermospermine derivative; Boc protection of the amine afforded the fully protected polyamine **13** in 76% yield. This intermediate was then used in the preparation of PhTX-433-Bu3 (**2**) and PhTX-334-Bu1 (**3**) by selective deprotection and further homologation to these analogs.

Polyamine **13** was converted to PhTX-433-Bu3 (**2**) using the known method (Scheme II).[21] Alternatively, the TFA-mediated deprotection of Boc groups of **13** yielded the unprotected polyamine necessary for preparation of PhTX-334-Bu1 (**3**). However, attempts in coupling this reagent with activated ester **15** according to the published scheme[21] only gave the two starting materials and some decomposed activated ester. The primary amine, expected to undergo the coupling reaction, was probably unreactive due to steric hindrance of the butyl group β to the amino group. It was previously noted that the primary amino groups of spermine reacted with the *p*-nitrophenyl ester of tyrosine in preference to the more sterically hindered secondary amino groups in the synthesis of PhTX-343.[21]

In order to overcome this difficulty, an oppositely

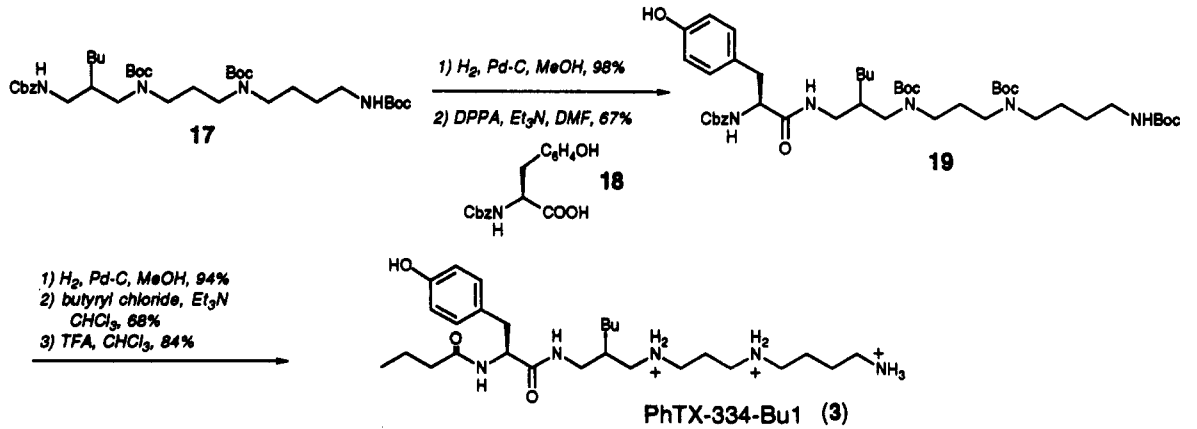
(25) Albarella, J. P. *J. Org. Chem.* **1977**, *42*, 2009.
(26) Corey, E. J.; Suggs, C. *Tetrahedron Lett.* **1975**, 2647.
(27) 3-Amino-1-propanol was protected with the Cbz group in 68% yield, followed by conversion of the free hydroxyl to the mesylate in 91% yield. The mesylate was converted in 97% yield to the nitrile using NaCN, and subsequent LAH reduction afforded the desired monoprotected diamine **10** in 87% yield.

Scheme II. Synthesis of PhTX-433-Bu3 (2)



PhTX-433-Bu3 (2)

Scheme III. Synthesis of PhTX-334-Bu1 (3)



PhTX-334-Bu1 (3)

protected thermospermine analog 17 was prepared according to Scheme I and was converted to PhTX-334-Bu1(3) as shown in Scheme III, using the more reactive DPPA-mediated coupling reaction to form the amide. This type of coupling was found to be less sensitive to the steric environment of the reacting amino group. Instead of beginning with the Boc-protected aldehyde 9 for this synthesis, a Cbz-protected aldehyde corresponding to 9 was prepared. The change in the order of protecting groups allowed for the preparation of a fully Boc-protected polyamine, except for the primary amine function (17). Cbz deprotection of 17 yielded the substituted thermospermine with the correct protection pattern to be converted intoPhTX-334-Bu1 (3). DPPA-mediated coupling of this intermediate with *N*-Cbz-tyrosine 18 yielded the coupled product 19 (67%).[28] Hydrogenation and coupling of the free amine with butyryl chloride followed by deprotection of Boc groups produced PhTX-334-Bu1 (3), all three steps occurring in acceptable yields.

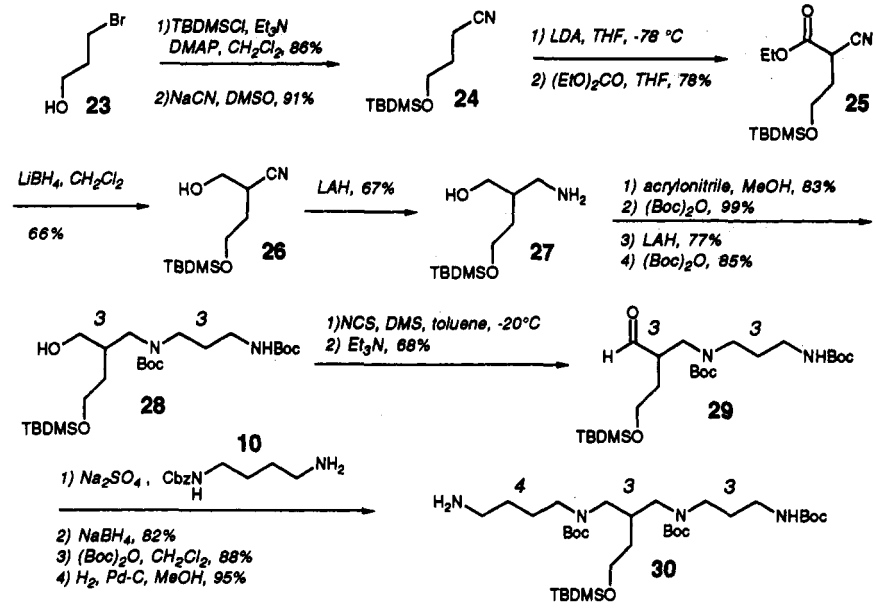
The next goal was to apply this methodology to the synthesis of PhTX-433 analogs containing the alkyl substituent in the second group of methylenes, i.e., PhTX-433-Bu2 (4a) and PhTX-433-Oct2 (4b) (Figure 3). The synthesis of the 433-Bu2 and 433-Oct2 polyamines necessary for these PhTX analogs was based on the synthesis of 433-Bu described in Scheme I. The fully protected alkylated thermospermine analogs of 433-Bu2 and 433-Oct2 were converted to the corresponding PhTX derivatives 4a and 4b, respectively, in good yield by the method described in Scheme II for PhTX-433-Bu3 (2).

Although the synthetic Schemes II and III were efficient for preparation of PhTX analogs with alkyl-substituted polyamines, the preparation of a polyamine with a functionalized side chain had not been attempted. PhTX analogs containing functionalized polyamines are crucial for understanding further details of ligand/receptor binding, e.g., the increased activity of PhTX-433-Bu2 (4a) (Figure 2). A target molecule, PhTX-433-N_3Ph2 (20, Figure 4), contains a photolabile moiety in the polyamine side chain for use in photoaffinity mapping studies. This analog would compliment the previously synthesized photolabile PhTX analogs 21 and 22,[22] which contain the photolabile moiety in regions III and IV (Figure 4).

To facilitate the synthesis of PhTX-433-N_3Ph2 (20), a PhTX-433 analog containing a hydroxyethyl group in the polyamine was prepared, since the use of a hydroxyl group allows the manipulation of the amino groups without difficulty. The synthetic route for the preparation of the hydroxyethyl-substituted polyamine is analogous to that

(28) Shiori, T.; Ninomiya, K.; Yamada, S. *J. Am. Chem. Soc.* **1972**, *94*, 6203.

Scheme IV. Synthesis of Hydroxyethyl-Substituted Polyamine



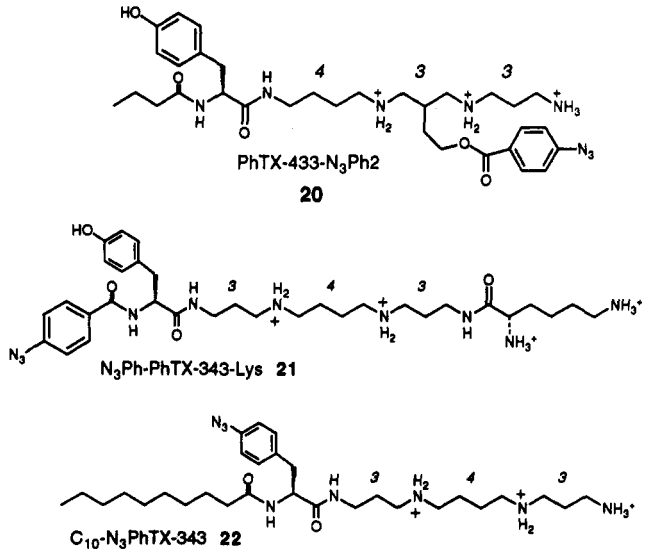


Figure 4. Photolabile PhTX analogs.

used for the alkylated polyamines for **4a** and **4b** and is shown in Scheme IV.

TBDMS protection of 3-bromo-1-propanol (**23**) followed by reaction with NaCN afforded nitrile **24**. Lithiation of **24** and quenching with diethyl carbonate to cyanoacetate **25**, followed by sequential reduction with $LiBH_4$ and LAH gave amino alcohol **27** in 44% yield for the two reduction steps. Compound **27** was coupled with acrylonitrile, protected by Boc, reduced to the amine, and finally Boc-protected to afford the N-protected diamino alcohol **28** in 54% overall yield. Corey–Kim oxidation of **28** gave aldehyde **29** in 68% yield.[29] Reductive coupling of **29** with monoprotected diamine **10** yielded 82% of the desired polyamine. Boc protection and subsequent hydrogenation afforded substituted thermospermine **30**, 84% yield.

Conversion of the hydroxyethyl-substituted polyamine to the desired PhTX analog by the standard method (as for **2**, **4a** and **4b**, Scheme II), however, led to problems in the selective deprotection of the hydroxyethyl group in the latter stages of the synthesis. This difficulty was overcome by use of the modified synthetic route in Scheme V. Compound **30** was thus coupled to the *O*-THP-*N*-Cbz-tyrosine *p*-nitrophenyl ester **31** to afford 68% of the coupled product **32**. Cbz deprotection, followed by coupling with butyryl chloride and subsequent TBDMS deprotection, afforded the activated functionalized PhTX analog **33** in acceptable yield for the three steps.

Conversion of the hydroxyethyl moiety of **33** to the *p*-azidobenzoate ester is shown in Scheme VI. Compound **33** was coupled with *p*-azidobenzoic acid (**34**) in the presence of [1-[3-(dimethylamino)propyl]ethyl]carbodiimide hydrochloride to afford 79% of the ester product **35**.[30] Boc deprotection gave PhTX-433-N_3Ph2 (**20**) in quantitative yield as the TFA salt with a purity of ~90%. Purification of the product using flash silica gel chromatography with *i*-$PrNH_2$/ MeOH/$CHCl_3$ eluent mixtures yielded 77% of the desired analog. A difficulty with this purification procedure was removal of excess *i*-$PrNH_2$ and the generated TFA–*i*-$PrNH_2$ salt. Attempts to purify the crude product by RP flash silica gel chromatography, RP-HPLC, and RP-PTLC after workup of the reaction led to decomposition of the product. The crude TFA salt was therefore used directly for biological assays.

Upon completion of the syntheses of the PhTX analogs containing the substituted polyamine moiety, these compounds were tested for biological activity against qGlu-R as well as the nACh-R. The glutamate activity was tested using the locust muscle assay, whereas the alkylated PhTX analogs were tested against the nACh-R of *Torpedo* electric organ in competitive binding studies with [H^3]perhydrohistrionicotoxin.[31] The photolabile analog showed irreversible blockage of the Glu-Rs in locust muscle upon irradiation, thus showing that the labeled analog had been delivered to the proper binding site and performed the expected function of irreversible binding.[32] Further use

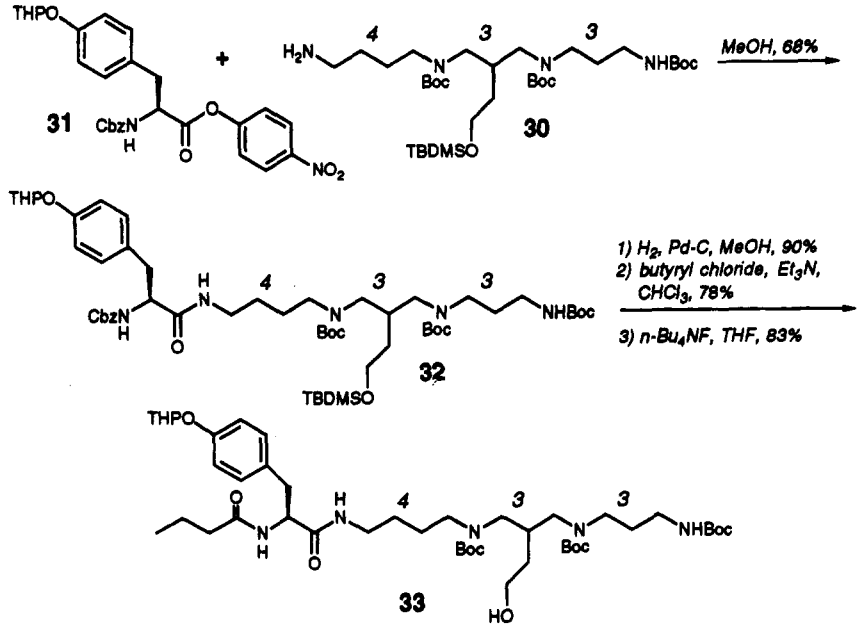
(29) (a) Corey, E. J.; Kim, C. U. *J. Am. Chem. Soc.* **1972**, *94*, 7586. (b) Corey, E. J.; Kim, C. U. *Tetrahedron Lett.* **1974**, 287.

(30) Sheehan, J. C.; Cruickshank, P. A.; Boshart, G. L. *J. Org. Chem.* **1961**, *26*, 2525.

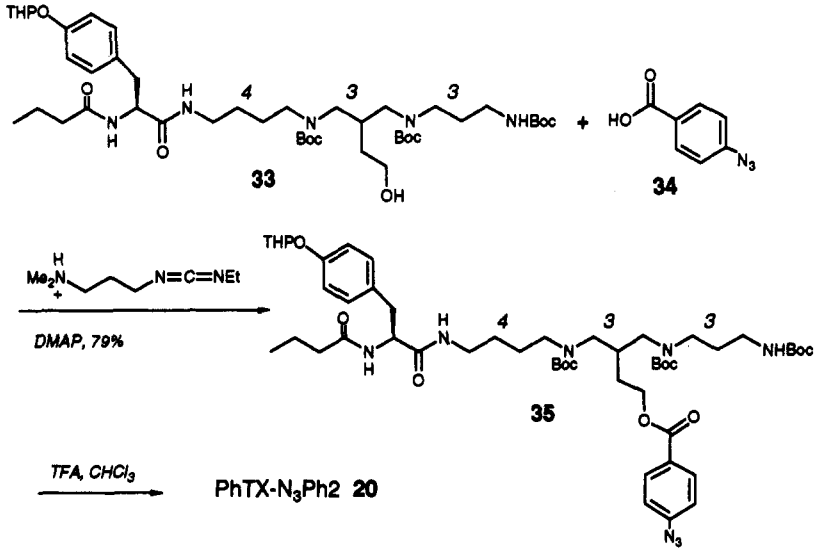
(31) Usherwood, P. N. R.; Nakanishi, K.; Eldefrawi, M.; Kalivretenos, A. Unpublished results.

(32) Usherwood, P. N. R.; Blagbrough, I. S.; Brackley, P. L.; Kerry, K. J.; Sudan, H. L.; Nakanishi, K. In *Neuroreceptors, Ion Channels and Brain*; Kawai, N., Ed.; Elsevier Science Publishing Co.: New York, 1991, in press.

Scheme V. Synthesis of Hydroxyethyl-Substituted PhTX-433



Scheme VI. Synthesis of PhTX-433-N_3Ph2 (20)



of this analog in photoaffinity mapping studies of the nACh-R is ongoing.

In summary, general methods for the preparation of substituted polyamines has been developed. Due to the importance of polyamines in biological systems and, more recently, growing interest in insect polyamine neurotoxins, the schemes outlined above should be useful for the preparation of modified polyamine compounds for use in the study of biological systems. The utility of the routes has been shown by preparation of several analogs of the wasp toxin philanthotoxin in which the alkyl substituents are attached to different segments of the polyamine. The PhTX derivative with the hydroxyethyl group should be useful for further functionalization of the molecule, including photoaffinity labeling.

Experimental Section

General. All solvents were distilled from calcium hydride unless otherwise noted. Dry THF was distilled from sodium–benzophenone ketyl. Ethanol (EtOH) and dioxane were distilled over sodium, and methanol (MeOH) was distilled over magnesium turnings. Starting materials were obtained either from the Aldrich Chemical Co. or from other commercial suppliers and were used as obtained unless otherwise noted. NMR spectra were recorded at 300 or 400 MHz for ^{1}H and at 75.4 MHz for ^{13}C using tetramethylsilane (TMS) as the internal reference. Infrared spectra were recorded on a Perkin-Elmer 1600 spectrometer. Low-resolution mass spectra (LRMS) were obtained on a Nermag-10 spectrometer. High-resolution mass spectra (HRMS) were obtained with a Joel JMS-DX303HF spectrometer. All reactions were carried out under an atmosphere of argon, unless otherwise noted. The usual workup included the use of anhydrous $MgSO_4$ to dry organic extracts, followed by filtration and concentration *in vacuo*. All yields reported are isolated yields of products with purity based on thin-layer chromatography (TLC) and NMR spectroscopy. TLC and preparative TLC (PTLC) were performed on Merck (0.25 mm) glass-backed, precoated silica gel plates (60 F_{254}). Silica

gel flash chromatography was carried out using Universal Scientific 32–63 silica gel.

Ethyl Butylcyanoacetate (6). To a solution of 16 mL (24 mmol, 2.3 equiv) of LDA (1.5 M solution in cyclohexane) and 25 mL of THF at −78 °C was added 1.01 g (1.25 mL, 10.4 mmol) of hexanenitrile in 1.5 mL of THF. The mixture was kept at −78 °C for 30 min, followed by removal of the cold bath for 30 min. At this time, the mixture was recooled to −78 °C, followed by the addition of 1.29 g (1.32 mL, 10.9 mmol, 1.05 equiv) of diethyl carbonate. The reaction mixture was kept at −78 °C for 2.25 h followed by the careful addition of 12.5 mL of a saturated NH_4Cl solution. The mixture was allowed to warm to rt and was then diluted with 75 mL of ether and 25 mL of water. The layers were partitioned, and the organic layer was washed with three 25-mL portions of 1 N HCl, two 25-mL portions of water, and one 25-mL portion of a saturated NaCl solution. The usual workup followed by chromatography (40 g of silica gel, 2–10% EtOAc/hexanes) yielded 1.43 g (81%) of a clear oil. R_f: 0.27 in 10% EtOAc/hexanes. Compound **10** has been previously prepared by this method.[34]

^{1}H NMR ($CDCl_3$): δ 0.94 (t, 3H, J = 7.1 Hz, $(CH_2)_3CH_3$), 1.33 (t, 3H, J = 7.0 Hz, OCH_2CH_3), 1.30–1.55 (m, 4H), 1.95 (m, 2H, CH_2CH), 3.49 (t, 1H, J = 7.0 Hz, $CHCN$), 4.27 (q, 2H, J = 7.1 Hz, CH_3CH_2O). ^{13}C NMR ($CDCl_3$): δ 13.63 (CH_3), 13.95, 21.88, 28.78, 29.52, 37.54 ($CHCN$), 62.68 (CH_2O), 116.58 (CN), 166.20 (C=O). IR (thin film): 3000–2850, 2250 (CN), 1746 (C=O), 1468, 1253, 1210, 1025 cm^{-1}.

2-(Aminomethyl)-1-hexanol (7). To a suspension of 0.0272 g (0.717 mmol, 4.10 equiv) of LAH in 2 mL of ether at 0 °C was added a solution of 0.0297 g (0.175 mmol) of **6** in 0.25 mL of ether. The mixture was kept at 0 °C for 45 min and then at rt for 14.5 h. At this time, the reaction mixture was quenched by the slow addition of 0.050 mL of water (4 equiv based on LAH) and 2 mL of EtOAc. The mixture was stirred for 15 min, filtered through a plug of Celite, eluted with 1 mL of EtOAc, and concentrated to yield a yellow oil. Purification by chromatography (2 g of silica gel, 10% MeOH/5% i-$PrNH_2$/85% $CHCl_3$) afforded 0.0142 g (62%) of a clear oil. R_f: 0.31 in 10% MeOH/5% i-$PrNH_2$/85% $CHCl_3$.

^{1}H NMR ($CDCl_3$): δ 0.89 (t, 3H, J = 6.8 Hz, CH_3), 1.18 (m, 2H, CH_2CH_3), 1.29 (m, 4H, $CHCH_2CH_2$), 1.64 (m, 1H, $CHCH_2OH$), 2.22 (bs, 3H, OH, NH_2), 2.72 (dd, 1H, J = 9.1, 12.2 Hz), 3.10 (ddd, 1H, J = 1.6, 3.5, 12.2 Hz), 3.60 (dd, 1H, J = 8.2, 10.6 Hz), 3.81 (ddd, 1H, J = 1.6, 3.4, 10.7 Hz). ^{13}C NMR ($CDCl_3$): δ 13.95 (CH_3), 22.91 (CH_2CH_3), 28.98, 29.43, 41.06, 47.15 (CH_2NH_2), 68.44 (b, CH_2OH). IR (thin film): 3300 (b), 2956, 2927, 2860, 1573, 1468, 1378, 1328, 1043 cm^{-1}. HRMS: calcd for $C_{17}H_{17}NO$ 131.1310 [M$^+$ + 1], found 131.1309 [M$^+$ + 1].

***N*-Carbo-*tert*-butoxy-2-(hydroxymethyl)-1-aminohexane (8).** A solution of 0.096 g (0.731 mmol) of **7** and 0.167 g (0.765 mmol, 1.05 equiv) of di-*tert*-butyl dicarbonate in 4 mL of CH_2Cl_2 was kept at 23 °C for 19 h. At this time, the reaction was diluted with 10 mL of CH_2Cl_2 and 10 mL of water. The two layers were partitioned, and the aqueous layer was extracted with two 10-mL portions of CH_2Cl_2. The combined organic extracts were washed with one 10-mL portion of a saturated $NaHCO_3$ solution and worked up as usual. The crude product was purified by chromatography (10 g of silica gel, 1% MeOH/$CHCl_3$) to yield 0.145 g (86%) of a faint yellow oil. R_f: 0.34 in 10% MeOH/$CHCl_3$.

^{1}H NMR ($CDCl_3$): δ 0.89 (t, 3H, J = 6.7 Hz, CH_2CH_3), 1.29 (m, 6H), 1.45 (s, 9H, $C(CH_3)_3$), 1.55 (m, 1H, CH), 3.07 (dt, 1H, J = 6.7, 14.4 Hz), 3.27–3.45 (m, 2H), 3.58 (m, 1H), 4.82 (bs, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 14.00 (CH_2CH_3), 22.89, 28.32 ($C(CH_3)_3$), 29.33, 40.70, 41.47, 62.45 (CH_2-OH), 79.80 ($OC(CH_3)_3$), 157.45 (C=O). IR (thin film): 3346 (b), 2958, 2930, 2873, 1688, 1519, 1366, 1284, 1252, 1174 cm^{-1}. HRMS: calcd for $C_{12}H_{25}O_3N$ 232.1913 [M$^+$ + 1], found 232.1806 [M$^+$ + 1].

2-[(Carbo-*tert*-butoxyamino)methyl]hexanal (9). To a suspension of 0.167 g (0.775 mmol, 1.49 equiv) of PCC in 4 mL of CH_2Cl_2 was rapidly added a solution of 0.120 g (0.519 mmol) of **8** in 1 mL of CH_2Cl_2. The orange mixture became darker over time. The reaction was kept at 23 °C for 4 h. At this time, the reaction was diluted by the addition of 10 mL of ether, filtered through a large plug of Florisil, and eluted with 70 mL more of ether. The filtrate was concentrated to yield a yellow oil. Purification by chromatography (5 g of silica gel, $CHCl_3$) yielded 0.0833 g (70%) of a clear yellow oil. R_f: 0.78 in 10% MeOH/$CHCl_3$.

^{1}H NMR ($CDCl_3$): δ 0.91 (t, 3H, J = 7.1 Hz, CH_2CH_3), 1.35 (m, 5H), 1.42 (s, 9H, $C(CH_3)_3$), 1.68 (m, 1H), 2.52 (m, 1H, CH), 3.31 (m, 2H), 4.85 (bs, 1H, NH), 9.67 (d, 1H, J = 1.5 Hz, $HCOCH$). ^{13}C NMR ($CDCl_3$): δ 13.78 (CH_2CH_3), 22.71, 26.24, 28.31 ($C(CH_3)_3$), 29.09, 38.96, 52.58, 79.41 ($OC(CH_3)_3$), 155.85 (NC=O), 204.35 (HC=O). IR (thin film): 3357 (b), 2960, 2932, 2862, 2720, 1714 (b), 1519, 1456, 1392, 1366, 1251, 1172 cm^{-1}. HRMS: calcd for $C_{12}H_{23}O_3N$ 230.1756 [M$^+$ + 1], found 230.1734 [M$^+$ + 1].

***N*-(Carbobenzoxybutyl)-2-cyanoethylamine.** A solution of 0.466 g (2.10 mmol) of 4-carbobenzoxy-1,4-diaminobutane (**10**) and 0.137 g (2.58 mmol, 1.23 equiv) of acrylonitrile in 5 mL of MeOH was stirred at 23 °C for 13 h. At this time, no starting material remained by TLC analysis. The mixture was concentrated, and the residue was purified by chromatography (15 g of silica gel, 0–5% MeOH/$CHCl_3$) to yield 0.479 g (83%) of a faint yellow oil. R_f: 0.35 in 10% MeOH/$CHCl_3$.

^{1}H NMR ($CDCl_3$): δ 1.30 (bs, 1H, CH_2NHCH_2), 1.55 (m, 4H, $CONHCH_2CH_2CH_2$), 2.48 (t, 2H, J = 6.5 Hz, CH_2-CN), 2.64 (t, 2H, J = 6.7 Hz, $CH_2NHCH_2CH_2CN$), 2.89 (t, 2H, J = 6.6 Hz, $NHCH_2CH_2CN$), 3.20 (m, 2H, $CONHCH_2$), 5.09 (bs, 3H, $ArCH_2$, CONH), 7.35 (bs, 5H, ArH). ^{13}C NMR ($CDCl_3$): δ 18.62, 27.11, 27.63, 40.77, 44.91, 48.59, 66.53 ($ArCH_2$), 118.63 (CN), 128.03 (ArC), 128.45 (ArC), 136.58 (ArC), 156.37 ($CONH$). IR (thin film): 3334 (b, NH), 3032, 2934, 2860, 2247 (CN), 1708 (C=O), 1528, 1454, 1252, 1135 cm^{-1}. HRMS: calcd for $C_{15}H_{21}O_2N_3$ 276.1712 [M$^+$ + 1], found 276.1720 [M$^+$ + 1].

***N*-*tert*-Butyl-1-[4-(carbobenzoxyamino)butyl]-1-(2-cyanoethyl)carbamate.** A solution of 0.436 g (1.58 mmol) of *N*-(carbobenzoxybutyl)-2-cyanoethylamine and 0.384 g (1.76 mmol, 1.11 equiv) of di-*tert*-butyl dicarbonate in 7 mL of CH_2Cl_2 was stirred at 23 °C for 6 h. At this time, the solution was concentrated and the residue was purified by chromatography (10 g of silica gel, $CHCl_3$) to yield 0.552 g (93%) of a viscous, clear oil. R_f: 0.69 in 10% MeOH/$CHCl_3$.

^{1}H NMR ($CDCl_3$): δ 1.46 (s, 9H, $(CH_3)_3C$), 1.55 (m, 4H, $CONHCH_2CH_2CH_2$), 2.60 (m, 2H, $CONHCH_2$), 3.25 (m, 4H, CH_2CN, $CbzNH(CH_2)_3CH_2N$), 3.43 (t, 2H, J = 6.7 Hz, CH_2CH_2N), 5.09 (s, 2H, $ArCH_2$), 5.90 (bs, 1H, NH),

7.35 (bs, 5H, Ar*H*). ^{13}C NMR ($CDCl_3$): δ (16.87, 17.44), 25.61(b), 27.12, 28.26 ($(CH_3)_3C$), 40.45, 43.57(b), 47.59(b), 66.53 ($ArCH_2$), 80.46(b, $COC(CH_3)_3$), 118.11(b, *C*N), 128.01 (Ar*C*), 128.44 (Ar*C*), 136.49 (Ar*C*, substituted), 155.15 ($O{=}CC(CH_3)_3$), 156.38 ($ArCH_2C{=}O$). IR (thin film): 3344 (b, NH), 3064, 3033, 3000–2900, 2249 (CN), 1694 (b, C=O), 1531, 1416, 1367, 1251, 1168 cm^{-1}. HRMS: calcd for $C_{20}H_{29}N_3O_4$ 375.2158 [M$^+$], found 375.2173 [M$^+$].

N'-Carbo-*tert*-butoxy-N''-carbobenzoxyspermidine (11). To a suspension of 0.0758 g (2.00 mmol, 3.51 equiv) of LAH in 6 mL of ether at 0 °C was added a solution of 0.214 g (0.570 mmol) of *N*-*tert*-butyl-1-[4-(carbobenzoxyamino)butyl]-1-(2-cyanoethyl)carbamate in 2 mL of ether. The reaction mixture was stirred at 0 °C for 1 h. At this time, 0.15 mL (4 equiv based on LAH) of water and 5 mL of EtOAc were cautiously added to the reaction. The cold bath was removed, and after 15 min the suspension was filtered through a large plug of Celite. The filtrate was concentrated, and the crude product was purified by chromatography (10 g of silica gel, 5% MeOH/5% *i*-$PrNH_2$/90% $CHCl_3$) to afford 0.158 g (73%) of a faint yellow oil. R_f: 0.32 in 5% *i*-$PrNH_2/CHCl_3$.

^{1}H NMR ($CDCl_3$): δ 1.35–1.55 (m, 13H), 1.63 (p, 2H, *J* = 6.9 Hz, $CH_2CH_2NH_2$), 2.68 (t, 2H, *J* = 6.7 Hz), 3.21 (m, 6H), 5.09 (s, 2H, $ArCH_2$), 7.36 (m, 5H, Ar*H*). ^{13}C NMR ($CDCl_3$): δ 25.41, 25.74, 27.24, 28.41, 31.83 (b), 32.55 (b), 39.30 (b), 40.67, 43.85, 44.11, 44.45, 46.34, 66.54, 79.35, 128.04, 128.46, 136.56, 155.64, 156.37. IR (thin film): 3333 (b), 2932, 1689, 1534, 1478, 1419, 1254, 1170 cm^{-1}. HRMS: calcd for $C_{20}H_{34}N_3O_4$ 380.2549 [M$^+$ + 1], found 380.2577 [M$^+$ + 1].

3-Butyl-N'''-carbobenzoxy-N,N''-dicarbo-*tert*-butoxythermospermine. A solution of 0.0601 g (0.262 mmol) of **9** in 0.5 mL of EtOH was added to a suspension of 0.118 g (0.311 mmol, 1.19 equiv) of **11** and 0.224 g (1.58 mmol, 6.03 equiv) of Na_2SO_4 in 1.0 mL of EtOH. The mixture was stirred at 23 °C for 24 h. At this time, the suspension was filtered through a plug of glass wool. To the filtrate was added 0.0794 g (2.10 mmol, 8.01 equiv) of $NaBH_4$, and the mixture was subsequently stirred at 23 °C for 4 h. At this time, 3 mL of water was added to quench the reaction. After addition of the water, the mixture was stirred at 23 °C for 1 h, followed by the addition of 10 mL $CHCl_3$ and 5 mL more water to the reaction mixture. The layers were partitioned, and the aqueous layer was extracted with two 10-mL portions of $CHCl_3$. After the usual workup,the residue was purified by chromatography (10 g of silica gel, 5–10% MeOH/$CHCl_3$) to yield 0.105 g (68%) of a clear oil. R_f: 0.21 in 10% $MeOH/CHCl_3$.

^{1}H NMR ($CDCl_3$): δ 0.88 (m, 3H), 1.27 (m, 6H), 1.30–1.80 (m, 7H), 1.43 (s, 9H), 1.44 (s, 9H), 2.40–2.70 (m, 4H), 3.00 (m, 1H), 3.10–3.40 (m, 7H), 5.09 (s, 2H), 7.35 (m, 5H). ^{13}C NMR ($CDCl_3$): δ 14.00, 22.90, 27.21, 28.45, 29.21, 30.46, 38.09, 40.69, 41.37, 44.04, 46.56, 66.58, 78.82, 128.07, 128.49, 136.56, 156.41. IR (thin film): 3320 (b), 2931 (b), 1694, 1538, 1456, 1418, 1366, 1250, 1171 cm^{-1}. HRMS: calcd for $C_{32}H_{57}N_4O_6$ 593.4278 [M$^+$ + 1], found 593.4277 [M$^+$ + 1].

3-Butyl-N'''-carbobenzoxy-N,N',N''-tricarbo-*tert*-butoxythermospermine (13). A solution of 0.0963 g (0.162 mmol) of 3-butyl-N'''-carbobenzoxy-N,N''-dicarbo-*tert*-butoxythermospermine and 0.0398 g (0.182 mmol, 1.12 equiv) of di-*tert*-butyl dicarbonate in 2.0 mL of CH_2Cl_2 was stirred at 23 °C for 9 h. At this time, the mixture was partitioned with 10 mL of CH_2Cl_2 and 10 mL of water. The aqueous layer was extracted with one 10-mL portion of CH_2Cl_2. The combined organic extracts were washed with one 10-mL portion of a saturated $NaHCO_3$ solution and one 10-mL portion of a saturated brine solution and workedup as usual. The residue was purified by chromatography (10 g of silica gel, $CHCl_3$) to yield 0.0855 g (76%) of a clear oil. R_f: 0.71 in 10% $MeOH/CHCl_3$.

^{1}H NMR ($CDCl_3$): δ 0.89 (t, 3H, *J* = 6.7 Hz, CH_2CH_3), 1.10–1.40 (m, 6H), 1.43 (s, 9H, $C(CH_3)_3$), 1.44 (s, 9H, $C(CH_3)_3$), 1.46 (s, 9H, $C(CH_3)_3$), 1.55 (m, 4H), 1.60-1.80 (m, 3H), 2.80–3.45 (m, 12H), 4.80–5.00 (bs, 1H, N*H*), 5.09 (s, 2H, $ArCH_2$), 5.50 (bs, 1H, N*H*), 7.35 (m, 5H, Ar*H*). ^{13}C NMR ($CDCl_3$): δ 13.93, 22.94, 26.00 (b), 27.20 (b), 28.40, 29.20 (b), 37.76, 40.53, 40.69, 45.00 (b), 46.52, 48.63, 66.54, 79.00 (b), 79.40, 80.00 (b), 128.05, 128.47, 136.56, 155.43, 156.39. IR (thin film): 3341 (b), 2972–2930, 1694, 1522, 1478, 1419, 1366, 1250, 1171 cm^{-1}. HRMS: calcd for $C_{37}H_{65}N_4O_8$ 693.4803 [M$^+$ + 1], found 693.4830 [M$^+$ + 1].

3-Butyl-N,N',N''-tricarbo-*tert*-butoxythermospermine (14). To a solution of 0.0812 g (0.117 mmol) of **13** in 2 mL of MeOH was added ~5 mg of 10% Pd–C. The solution was purged with H_2 gas three times and subsequently kept under an atmosphere of H_2 gas for 2.5 h. At this time, no starting material remained by TLC analysis. The mixture was filtered through a pipette column of Celite, which was eluted with four volumes of MeOH (8 mL total). The filtrate was concentrated, and the residue was purified by chromatography (8 g of silica gel, step gradient of 10% $MeOH/CHCl_3$ to 5% *i*-$PrNH_2$/10% $MeOH/85\%$ $CHCl_3$) to afford 0.0585 g (89%) of a clear oil. R_f: 0.51 in 5% *i*-$PrNH_2$/5% $MeOH/90\%$ $CHCl_3$.

^{1}H NMR ($CDCl_3$): δ 0.89 (t, 3H, *J* = 6.8 Hz, CH_2CH_3), 1.10–1.40 (m, 6H), 1.43 (s, 9H, $C(CH_3)_3$), 1.45 (s, 9H, $C(CH_3)_3$), 1.47 (s, 9H, $C(CH_3)_3$), 1.55 (m, 4H), 1.60-1.75 (m, 2H), 2.72 (m, 2H), 2.80–3.40 (m, 12 H), 5.50 (bs, 1H, N*H*Boc). ^{13}C NMR ($CDCl_3$): δ 13.92, 22.93, 25.94 (b), 27.00 (b), 28.39, 29.28, 30.84, 37.77, 40.50, 41.87, 44.60 (b), 45.31, 46.88, 48.65, 78.50 (b), 79.28, 79.68 (b), 155.43, 156.23, 156.34. IR (thin film): 3367 (b), 3000–2850, 1694, 1504, 1479, 1418, 1391, 1365, 1250, 1169, 867, 774, 733 cm^{-1}.

1-[12-(Carbo-*tert*-butoxyamino)-5,9-diaza-5,9-(carbo-*tert*-butoxy)-11-butyldodecanyl]-N-butyryl-O-benzyltyrosinamide (16). To a solution of 0.0497 g (0.107 mmol, 1.02 equiv) of *N*-carbobenzoxy-*O*-benzyltyrosine 4-nitrophenyl ester (**15**) in 1.5 mL of MeOH was added a solution of 0.0585 g (0.105 mmol) of **14** in 1.0 mL of MeOH. The mixture was stirred at 23 °C for 18.5 h and was then concentrated *in vacuo*. The residue was dissolved in 3 mL of $CHCl_3$. The $CHCl_3$ solution was washed with two 2-mL portions of water, three 2-mL portions of saturated $NaHCO_3$ solution, and two 2-mL portions of saturated brine solution. After the usual workup,the residue was purified by chromatography (10 g of silica gel, 0–10% $MeOH/CHCl_3$) to yield 0.0522 g (56%) of a faint yellow oil. R_f: 0.60 in 10% $MeOH/90\%$ $CHCl_3$.

^{1}H NMR ($CDCl_3$): δ 0.88 (t, 6H, *J* = 7.4 Hz), 1.27 (m, 4H), 1.39 (m, 6H), 1.43 (s, 9H), 1.44 (s, 9H), 1.46 (s, 9H), 1.60 (p, 2H, *J* = 7.4 Hz), 1.73 (bs, 6H), 2.14 (t, 2H, *J* = 7.4 Hz), 2.80–3.30 (2m, 12H), 4.58 (bs, 1H), 5.03 (s, 2H), 6.89 (d, 2H, *J* = 8.6 Hz), 7.12 (d, 2H, *J* = 8.4 Hz), 7.30–7.45 (m, 5H). IR (thin film): 3286 (b), 3067, 2965-2850, 1691, 1641, 1512, 1419, 1366, 1246, 1174 cm^{-1}. HRMS: calcd for $C_{49}H_{80}N_5O_9$ 882.5956 [M$^+$ + 1], found 882.5949 [M$^+$ + 1].

1-(12-Amino-5,9-diaza-11-butyldodecanyl)-N-butyryltyrosinamide, PhTX-433-Bu3 (2). A suspension

of 0.0522 g (0.0591 mmol) of **16** and ~5 mg of 10% Pd–C catalyst in 1.2 mL of MeOH at 23 °C was purged three times with H_2 gas and then kept under 1 atm of H_2 gas for 8 h. The black suspension was filtered through a pipette column of Celite, eluting with 5 mL of MeOH. The filtrate was concentrated, and the residue was dissolved in 1.0 mL of $CHCl_3$ and 0.5 mL of TFA. The mixture was stirred at 23 °C for 15 h. At this time, the reaction mixture was concentrated, and the residue was purified by chromatography (3 g of silica gel, step gradient of 5% *i*-$PrNH_2$/10% MeOH/85% $CHCl_3$ to *i*-$PrNH_2$/MeOH/$CHCl_3$ (1/4/4)) to yield 0.0193 g (66%) of a clear oil.

^1H NMR (CD_3OD): δ 0.84 (t, 3H, J = 7.4 Hz), 0.93 (t, 3H), 1.35 (bs, 6H), 1.55 (m, 7H), 1.95 (m, 3H), 2.17 (t, 2H, J = 7.7 Hz), 2.70–2.90 (m, 8H), 2.96 (m, 6H), 3.05 (m, 4H), 3.16 (m, 2H), 4.43 (t, 1H, J = 9 Hz), 6.70 (d, 2H, J = 8.5 Hz), 7.05 (d, 2H, J = 8.5 Hz). ^{13}C NMR (CD_3OD): δ 13.93, 14.37, 20.29, 23.99, 27.21, 29.16, 30.19, 31.27, 38.47, 38.72, 39.19, 39.96, 45.39, 47.19, 47.42, 47.72, 48.00, 53.86, 56.61, 116.29, 128.94, 131.32, 157.53, 173.74, 175.87. HRMS: calcd for $C_{27}H_{49}N_5O_3$ 492.3913 [M^+ + 1], found 492.3914 [M^+ + 1].

1-[12-(Carbo-*tert*-butoxyamino)-5,9-diaza-5,9-dicarbo-*tert*-butoxy-2-butyldodecanyl]-*N*-carbobenzoxytyrosinamide (19). To a solution of 0.0726 g (0.130 mmol) of 3-butyl-*N'*,*N''*,*N'''*-tricarbo-*tert*-butoxythermospermine, 0.0451 g (0.143 mmol, 1.10 equiv) of *N*-carbobenzoxytyrosine, and 0.040 g (0.031 mL, 0.145 mmol, 1.1 equiv) of DPPA in 2.0 mL of DMF at 23 °C was added 0.0145 g (0.0200 mL, 0.143 mmol, 1.10 equiv) of Et_3N. The mixture was stirred at 23 °C for 25 h, followed by dilution with 15 mL of water. The mixture was extracted with three 20-mL portions of EtOAc. The combined organic extracts were washed with one 20-mL portion of water and one 20-mL portion of brine solution, followed by the usual workup. The residue was purified by chromatography (5 g of silica gel, 0–5% MeOH/$CHCl_3$) to afford 0.0748 g (67%) of a viscous oil which became a white solid over time. R_f: 0.59 in 10% MeOH/90% $CHCl_3$.

^1H NMR ($CDCl_3$): δ 0.87 (t, 3H, J = 7.0 Hz), 1.05–1.30 (m, 6H), 1.38 (bs, 4H), 1.41 (s, 4H), 1.44 (s, 9H), 1.47 (s, 9H), 1.55 (m, 2H), 1.63 (s, 9H), 2.40–3.00 (bm, 5H), 3.00–3.30 (m, 9H), 4.42 (bs, 1H), 4.55–4.70 (bs, 1H, N*H*), 5.10 (m, 2H), 5.65 (bs, 1H, N*H*), 6.76 (bs, 2H), 7.05 (m, 2H), 7.34 (m, 5H). IR (thin film): 3321 (b), 2973, 2930, 1670, 1517, 1478, 1456, 1420, 1366, 1249, 1170 cm^{-1}. HRMS: calcd for $C_{46}H_{74}N_5O_{10}$ 856.5436 [M^+ + 1], found 856.5461 [M^+ + 1].

3-[(*tert*-Butyldimethylsilyl)oxy]-1-bromopropane. To a solution of 6.15 g (4.00 mL, 44.2 mmol) of 3-bromo-1-propanol, 6.68 g (9.20 mL, 66.0 mmol, 1.49 equiv) of Et_3N, and 0.541 g (4.43 mmol, 0.100 equiv) of DMAP in 90 mL of CH_2Cl_2 at 0 °C was added 8.03 g (53.3 mmol, 1.21 equiv) of *tert*-butyldimethylsilyl chloride. After 15 min, the cold bath was removed, and the mixture was subsequently stirred at 23 °C for 16 h. At this time, 75 mL of water was added to the mixture, and the layers were partitioned. The aqueous layer was extracted with one 75-mL portion of CH_2Cl_2. The combined organic extracts were washed with three 75-mL portions of a saturated $CuSO_4$ solution and one 75-mL portion of saturated brine solution and workedup as usual. Distillation of the crude oil under reduced pressure (bp 46–47 °C, 4 mmHg) provided 8.5353 g (76%) of a clear oil. R_f: 0.65 in 5% EtOAc/hexanes.

^1H NMR ($CDCl_3$): δ 0.075 (s, 6H), 0.90 (s, 9H), 2.04 (p, 2H, J = 6.0 Hz), 3.53 (t, 2H, J = 6.4 Hz), 3.74 (t, 2H, J = 5.8 Hz). IR (neat): 2950–2850, 1472, 1257, 1103, 836, 776 cm^{-1}. LRMS: calcd for $C_9H_{22}OBrSi$, 253, 255 [M^+ + 1], found 253, 255 [M^+ + 1].

4-[(*tert*-Butyldimethylsilyl)oxy]butyronitrile (24). A solution of 4.80 g (18.9 mmol) of 3-[(*tert*-butyldimethylsilyl)oxy]-1-bromopropane and 4.64 g (94.7 mmol, 5.01 equiv) of NaCN in 75 mL of DMSO was kept at 23 °C for 25 h. At this time, the mixture was diluted with 75 mL of water and 75 mL of ether. The layers were partitioned, and the aqueous layer was extracted with two 75-mL portions of ether. The combined organic layers were washed with one 75-mL portion of water and one 75-mL portion of a saturated brine solution. After the usual workup, purification by chromatography (15 g of silica gel, hexanes) yielded 3.440 g (91%) of a faint pink oil. R_f: 0.27 in 5% EtOAc/hexanes.

^1H NMR ($CDCl_3$): δ 0.07 (s, 6H, CH_3Si), 0.90 (s, 9H, $(CH_3)_3CSi$), 1.85 (m, 2H, $CH_2CH_2CH_2CN$), 2.46 (t, 2H, J = 7.1 Hz, CH_2CN), 3.72 (t, 2H, J = 5.7 Hz, CH_2OSi). ^{13}C NMR ($CDCl_3$): δ -5.51 (CH_3Si), 13.67 ($CH_2CH_2CH_2CN$), 18.18 ($(CH_3)_3CSi$), 25.80 ($(CH_3)_3CSi$), 28.40 (CH_2CN), 60.49 (CH_2O), 119.68 (*C*N). IR (thin film): 3000–2800, 2249 (CN), 1472, 1256, 1109, 1076, 978, 836, 778 cm^{-1}. HRMS: calcd for $C_{10}H_{22}NOSi$, 200.1470 [M^+ + 1], found 200.1492 [M^+ + 1].

Ethyl 2-[2-[(*tert*-Butyldimethylsilyl)oxy]ethyl]cyanoacetate (25). To a solution of 24.0 mL (36.0 mmol, 2.39 equiv) of LDA (1.5 M solution in cyclohexane) in 45 mL of THF at −78 °C was added a solution of 3.001 g (15.05 mmol) of **24** in 5 mL of THF. The mixture was kept at −78 °C for 30 min, followed by removal of the cold bath for 30 min. The reaction mixture was recooled to −78 °C, followed by the addition of 2.00 g (2.05 mL, 16.9 mmol, 1.12 equiv) of diethyl carbonate. The reaction was kept at −78 °C for 2 h. At this time, 30 mL of a saturated NH_4Cl solution was cautiously added to the reaction with subsequent removal of the cold bath. Upon warming to rt, the reaction mixture was diluted with 200 mL of ether and 50 mL of water. The layers were partitioned, and the organic layer was washed with three 50-mL portions of a saturated $CuSO_4$ solution and one 50-mL portion of a saturated brine solution. Workup, followed by purification by chromatography (50 g of silica gel, 2–5% EtOAc/hexanes) afforded 3.534 g (86%) of a light yellow oil. R_f: 0.26 in 10% EtOAc/hexanes.

^1H NMR ($CDCl_3$): δ 0.07 (s, 3H, CH_3Si), 0.08 (s, 3H, CH_3Si), 0.90 (s, 9H, $(CH_3)_3CSi$), 1.33 (t, 3H, J = 7.1 Hz, CH_3CH_2O), 2.00–2.30 (2 m, 2H, CH_2CH_2CN), 3.80 (m, 3H, CH_2O, *C*HCN), 4.27 (q, 2H, J = 7.1 Hz, CH_3CH_2O). ^{13}C NMR ($CDCl_3$): δ -5.55 (CH_3Si), 13.96 ($CH_2CH_2CH_2CN$), 18.21 ($(CH_3)_3CSi$), 25.80 ($(CH_3)_3CSi$), 32.54, 34.02, 59.03, 62.72, 116.40 (*C*N), 166.30 (C=O). IR (thin film): 3000–2850, 2250 (CN), 1749, 1472, 1256, 1211, 1111, 837, 779 cm^{-1}. HRMS: calcd for $C_{13}H_{26}NO_3Si$ 272.1682 [M^+ + 1], found 272.1694 [M^+ + 1].

2-(Hydroxymethyl)-4-[(*tert*-butyldimethylsilyl)oxy]butanenitrile (26). To a suspension of 0.504 g (23.1 mmol, 2.03 equiv) of $LiBH_4$ in 45 mL of CH_2Cl_2 at 23 °C was added a solution of 3.091 g (11.39 mmol) of **25**. The mixture was stirred at 23 °C for 4 h. At this time, the reaction was cooled to 0 °C, followed by the cautious addition of 15 mL of water. The cold bath was removed and the mixture stirred for 45 min longer. The mixture

was diluted with 80 mL of CH_2Cl_2 and 50 mL of water. The layers were separated, and the aqueous layer was extracted with one 50-mL portion of CH_2Cl_2. The combined organic extracts were washed with two 75-mL portions of water and one 75-mL portion of a saturated brine solution. The usual workup, followed by chromatography (60 g of silica gel, 10–30% EtOAc/hexanes), yielded 1.734 g (66%) of a clear oil. R_f: 0.11 in 20% EtOAc/hexanes.

^{1}H NMR ($CDCl_3$): δ 0.09 (s, 3H, CH_3Si), 0.10 (s, 3H, CH_3Si), 0.91 (s, 9H, $(CH_3)_3CSi$), 1.90 (m, 2H, CH_2CH_2OSi), 3.00 (q, 1H, J = 6.0 Hz, $CHCN$), 3.40 (bs, 1H, OH), 3.82 (m, 4H, $HOCH_2$, $SiOCH_2$). ^{13}C NMR ($CDCl_3$): δ -5.56 (CH_3Si), 18.16 ($(CH_3)_3CSi$), 25.78 ($(CH_3)_3CSi$), 31.86, 32.10, 59.86, 62.45, 120.42 (CN). IR (thin film): 3443 (b), 3000–2850, 2244 (CN), 1472, 1257, 1097, 836, 778 cm^{-1}. HRMS: calcd for $C_{11}H_{24}NO_2Si$ 230.1576 [M^+ + 1], found 230.1567 [M^+ + 1].

2-[1-[2-[(*tert*-Butyldimethylsilyl)oxy]ethyl]]-1-aminopropan-3-ol (27). To a suspension of 0.749 g (19.7 mmol, 4.51 equiv) of LAH in 60 mL of ether at 0 °C was added a solution of 1.001 g (4.363 mmol) of **26** in 5 mL of ether. The suspension was kept at 0 °C for 1 h at which time the cold bath was removed. After 3.5 h at rt, the suspension was cooled to −78 °C followed by the cautious addition of 1.4 mL (4 equiv based on the LAH) of water and 30 mL of EtOAc. After 10 min at -78 °C, the cold bath was removed and the reaction was stirred for an additional 50 min. At this time, the white suspension was filtered through a large plug of Celite, eluting with EtOAc. The filtrate was concentrated, and the residue was purified by chromatography (30 g of gel, step gradient of 10% MeOH/$CHCl_3$ to 5% i-$PrNH_2$/10% MeOH/85% $CHCl_3$) to yield 0.679 g (67%) of a clear oil. R_f: 0.36 in 5% i-$PrNH_2$/10% MeOH/85% $CHCl_3$.

^{1}H NMR ($CDCl_3$): δ 0.06 (s, 6H, CH_3Si), 0.90 (s, 9H, $(CH_3)_3CSi$), 1.50 (m, 2H, $SiOCH_2CH_2$), 1.78 (bs, 1H, $HOCH_2CH$), 2.77 (dd, 1H, J = 6.2, 8.4 Hz, CH_2NH_2), 3.01 (dd, 2H, J = 4.0, 9.5 Hz, CH_2NH_2), 3.68 (m, 3H), 3.77(m, 1H). ^{13}C NMR ($CDCl_3$): δ −5.41 (CH_3Si), 18.25 ($(CH_3)_3CSi$), 25.89 ($(CH_3)_3CSi$), 32.61, 39.32, 46.47, 61.35, 67.65. IR (thin film): 3500–2500 (b, OH, NH_2), 2929, 2857, 1573, 1472, 1255, 1097, 836, 775 cm^{-1}. HRMS: calcd for $C_{11}H_{28}NO_2Si$ 234.1889 [M^+ + 1], found 234.1870 [M^+ + 1].

2-[1-[2-[(*tert*-Butyldimethylsilyl)oxy]ethyl]]-*N*-1-(2-cyanoethyl)-1-aminopropan-3-ol. A solution of 0.242 g (1.04 mmol) of **27** and 0.066 g (0.082 mL, 1.24 mmol, 1.19 equiv) of acrylonitrile in 2.5 mL of MeOH was stirred at 23 °C for 24 h. At this time, no starting material was present by TLC analysis. The mixture was concentrated, and the residue was purified by chromatography (10 g of silica gel, 0–5% MeOH/$CHCl_3$) to yield 0.249 g (83%) of a clear oil. R_f: 0.54 in 10% MeOH/$CHCl_3$.

^{1}H NMR ($CDCl_3$): δ 0.06 (s, 6H, CH_3Si), 0.90 (s, 9H, $(CH_3)_3CSi$), 1.51 (m, 2H, $SiOCH_2CH_2$), 1.89 (bs, 1H, $HOCH_2CH$), 2.54 (t, 2H, J = 6.6 Hz, CH_2CN), 2.69 (dd, 1H, J = 8.6, 12.6 Hz, $CHCH_2N$), 2.80–3.00 (m, 3H, $CHCH_2NHCH_2$), 3.66 (m, 4H, $HOCH_2$, $SiOCH_2$). ^{13}C NMR ($CDCl_3$): δ −5.42 (CH_3Si), 18.51 ($(CH_3)_3CSi$), 25.87 ($(CH_3)_3CSi$), 32.82, 37.57, 45.14, 53.71, 61.16, 67.51, 118.28. HRMS: calcd for $C_{14}H_{31}N_2O_2Si$ 287.2155 [M^+ + 1], found 287.2170 [M^+ + 1].

2-[1-[2-[(*tert*-Butyldimethylsilyl)oxy]ethyl]]-*N*-carbo-*tert*-butoxy-*N*-[1-(2-cyanoethyl)]-1-aminopropan-3-ol. A solution of 0.8481 g (2.960 mmol) of 2-[1-[2-[(*tert*-butyldimethylsilyl)oxy]ethyl]]-*N*-[1-(2-cyanoethyl)]-1-aminopropan-3-ol and 0.739 g (3.38 mmol, 1.14 equiv) of di-*tert*-butyl dicarbonate in 15 mL of CH_2Cl_2 was kept at 23 °C for 3.75 h. The mixture was diluted with 40 mL of CH_2Cl_2 and 25 mL of water and partitioned. The aqueous layer was extracted with one 40-mL portion of CH_2Cl_2. The combined organic layers were washed with one 25-mL portion of a saturated $NaHCO_3$ solution and one 25-mL portion of a saturated brine solution. Usual workup, followed by chromatography (20 g of silica gel, $CHCl_3$), yielded 1.137 g (99%) of a clear oil. R_f: 0.72 in 10% MeOH/$CHCl_3$.

^{1}H NMR ($CDCl_3$): δ 0.07(s, 6H), 0.90 (s, 9H), 1.49 (s, 9H), 1.87 (b, 1H), 2.55-2.70 (m, 3H), 3.03 (m, 2H), 3.30–3.80 (2m, 8H). IR (thin film): 3461 (b), 2950-2850, 2250 (CN), 1694, 1472, 1418, 1368, 1254, 1169, 1093, 836, 776 cm^{-1}. HRMS: calcd for $C_{19}H_{39}N_2O_4Si$ 387.2679 [M^+ + 1], found 387.2688 [M^+ + 1].

2-[1-[2-[(*tert*-Butyldimethylsilyl)oxy]ethyl]]-*N*-carbo-*tert*-butoxy-*N*-[1-(3-aminopropyl)]-1-aminopropan-3-ol. To a suspension of 0.109 g (2.87 mmol, 5.04 equiv) of LAH in 8 mL of ether at 0 °C was added a solution of 0.220 g (0.569 mmol) of 2-[1-[2-[(*tert*-butyldimethylsilyl)oxy]ethyl]]-*N*-carbo-*tert*-butoxy-*N*-[1-(2-cyanoethyl)]-1-aminopropan-3-ol in 1.0 mL of ether. The suspension was stirred at 0 °C for 1.5 h, followed by the cautious, simultaneous addition of 0.210 mL (4 equiv based on the LAH) of water and 5 mL of EtOAc. After 10 min, the cold bath was removed, and the suspension was stirred for 1 h longer. At this time, the mixture was filtered through a large plug of Celite, eluting with EtOAc. The filtrate was concentrated, and the residue was purified by chromatography (10 g of silica gel, step gradient of 10% MeOH/$CHCl_3$ to 5% i-$PrNH_2$/10% MeOH/85% $CHCl_3$) to afford 0.172 g (77%) of a viscous oil. R_f: 0.510 in 5% i-$PrNH_2$/10% MeOH/85% $CHCl_3$.

^{1}H NMR ($CDCl_3$): δ 0.06(s, 6H), 0.89 (s, 9H), 1.47 (s, 9H), 1.67 (m, 4H), 1.85 (m, 1H), 2.70 (t, 2H, J = 2 Hz), 2.90 (m, 1H), 3.12 (m, 1H), 3.31 (m, 1H), 3.47 (m, 4H), 3.70 (m, 4H). ^{13}C NMR ($CDCl_3$): δ −5.42, 18.19, 25.86, 28.36, 32.19, 32.42, 36.40, 39.52, 45.03, 47.39, 60.86, 61.10, 80.15, 157.04. IR (thin film): 3362 (b), 2950–2850, 1682, 1472, 1418, 1366, 1253, 1172, 1093, 836, 775 cm^{-1}. HRMS: calcd for $C_{19}H_{43}N_2O_4Si$ 391.2992 [M^+ + 1], found 391.2974 [M^+ + 1].

2-[1-[2-[(*tert*-Butyldimethylsilyl)oxy]ethyl]-*N*-carbo-*tert*-butoxy-*N*-[1-[3-(carbo-*tert*-butoxyamino)propyl]]-1-aminopropan-3-ol (28). A solution of 0.736 g (1.88 mmol) of 2-[1-[2-[(*tert*-butyldimethylsilyl)oxy]ethyl]]-*N*-carbo-*tert*-butoxy-*N*-[1-(3-aminopropyl)]-1-aminopropan-3-ol and 0.454 g (2.08 mmol, 1.11 equiv) of di-*tert*-butyl dicarbonate in 10 mL of CH_2Cl_2 was stirred at 23 °C for 50 min. At this time, no starting material remained by TLC analysis. The reaction mixture was diluted with 25 mL of CH_2Cl_2 and 15 mL of water, and the layers were partitioned. The aqueous layer was extracted with one 25-mL portion of CH_2Cl_2. The combined organic extracts were washed with one 15-mL portion of saturated $NaHCO_3$ and one 15-mL portion of a saturated brine solution and workedup as usual. Purification by chromatography (30 g of silica gel, $CHCl_3$) afforded 0.787 g (85%) of a clear viscous oil. R_f: 0.68 in 10% MeOH/$CHCl_3$.

^{1}H NMR ($CDCl_3$): δ 0.04 (s, 6H), 0.88 (s, 9H), 1.42 (s, 9H), 1.46 (s, 9H), 1.68 (m, 4H), 1.85 (bs, 1H), 2.88 (m, 1H),

3.08 (m, 4H), 3.28 (m, 2H), 3.45 (bs, 2H), 3.67 (m, 2H). ^{13}C NMR ($CDCl_3$): δ -5.41, 18.21, 25.87, 28.37, 32.19, 36.36, 37.70, 44.68, 47.23, 53.42, 60.89, 61.06, 79.18, 80.57, 155.85, 156.90. IR (thin film): 3360 (b), 2950–2850, 1696, 1473, 1421, 1366, 1252, 1172, 1093, 836, 775 cm^{-1}. HRMS: calcd for $C_{24}H_{51}N_2O_6Si$ 491.3516 [M^+ + 1], found 491.3500 [M^+ + 1].

2-[1-[2-[(*tert*-Butyldimethylsilyl)oxy]ethyl]]-*N*-carbo-*tert*-butoxy-*N*-[1-[3-(carbo-*tert*-butoxyamino)propyl]]-1-aminopropanal (29). To a suspension of 0.0805 g (0.603 mmol, 2.21 equiv) of *N*-chlorosuccinimide in 2.0 mL of toluene at 0°C was added 0.041g (0.048 mL, 0.66 mmol, 2.4 equiv) of dimethyl sulfide. After 5 min, the solution was cooled to -25 °C, followed by the addition of a solution of 0.134 g (0.273 mmol) of **28** in 0.5 mL of toluene. The reaction was held between -25 and -20 °C for 3.5 h. At this time, 0.066 g (0.091 mL, 0.65 mmol, 2.4 equiv) of Et_3N was added to the mixture. The cold bath was removed, and after 5 min, the mixture was diluted with 15 mL of ether. The solution was washed with one 10-mL portion of ice cold 0.1 N HCl solution. The usual workup, followed by chromatography (10 g of silica gel, 10–20% EtOAc/hexanes), yielded 0.0902 g (68%) of a clear oil. R_f : 0.50 in 35% EtOAc/hexanes.

^{1}H NMR ($CDCl_3$): δ 0.09 (s, 6H, CH_3Si), 0.85 (s, 9H, $(CH_3)_3CSi$), 1.42 (s, 9H, $OCCH_3$), 1.43 (s, 9H, $OCCH_3$), 1.65 (m, 4H), 1.87 (bs, 1H), 2.70 (bs, 1H, $CHCH_2$), 3.07 (s, 2H), 3.23 (m, 3H), 3.53 (bs, 1H), 3.64 (m, 2H, $SiOCH_2$), 9.63 (s, 1H, CH_2CHO). ^{13}C NMR ($CDCl_3$): δ -5.56 (CH_3Si), 18.16 ($(CH_3)_3CSi$), 25.62, 25.87 ($(CH_3)_3CSi$), 28.28 ($OCCH_3$), 28.37 ($OCCH_3$), 30.75, 37.34 (b), 37.50(b), 44.11 (b), 44.87, 46.30, 49.47, 60.51, 78.98 (b), 80.33, 155.57, 156.20, 202.90 (CH_2CHO). IR (thin film): 3365 (b), 3000–2850, 1696, 1508, 1472, 1417, 1366, 1252, 1173, 1101, 837, 777 cm^{-1}. HRMS: calcd for $C_{24}H_{49}O_6N_2Si$ 489.3360 [M^+ + 1], found 489.3369 [M^+ + 1].

7-[1-[2-[(*tert*-Butyldimethylsilyl)oxy]ethyl]]-*N*‴-carbobenzoxy-*N*,*N*′-dicarbo-*tert*-butoxythermospermine. To a suspension of 0.052 g (0.23 mmol, 1.3 equiv) of *N*-carbobenzoxy-1,4-diaminobutane (**10**) and 0.153 g (1.08 mmol, 6.10 equiv) of Na_2SO_4 in 2.0 mL of EtOH at 23 °C was added a solution of 0.0867 g (0.177 mmol) of **29** in 1.0 mL of EtOH. The reaction mixture was stirred for 20.5 h at 23 °C, followed by filtration through a plug of glass wool. To the filtrate was added 0.054 g (1.4 mmol, 7.9 equiv) of $NaBH_4$ with subsequent stirring for 10 h. At this time, 2 mL of water was added to the reaction mixture. The mixture was stirred for 2 h longer at 23 °C and then diluted with 15 mL of $CHCl_3$ and 10 mL of water. The layers were separated, and the aqueous layer was extracted with three 10-mL portions of $CHCl_3$. The usual workup and subsequent chromatography (10 g of silica gel, 0–20% MeOH/$CHCl_3$) yielded 0.101 g (82%) of an oil. R_f: 0.51 in 10% MeOH/$CHCl_3$.

^{1}H NMR ($CDCl_3$): δ 0.04 (s, 6H, CH_3Si), 0.88 (s, 9H, $(CH_3)_3CSi$), 1.43 (s, 9H, $OCCH_3$), 1.45 (s, 9H, $OCCH_3$), 1.50–1.70 (2m, 8H), 1.95 (b, 1H), 2.45–2.60 (2m, 4H), 3.00–3.10 (b, 3H), 3.15–3.30 (m, 5H), 3.64 (t, 2H, J = 6.2 Hz, CH_2OSi), 4.75 (bs, 1H), 5.09 (s, 2H, $ArCH_2$), 5.20–5.35 (b, 2H), 7.30–7.40 (b, 5H, Ar*H*). ^{13}C NMR ($CDCl_3$): δ -5.33 (CH_3Si), 18.24 ($(CH_3)_3CSi$), 25.92 ($(CH_3)_3CSi$), 27.83, 28.43 ($OCCH_3$), 33.43, 37.58 (b), 40.99, 43.94 (b), 49.87, 51.05 (b), 61.13, 66.49, 79.01 (b), 79.71, 128.00, 128.07, 128.46, 136.75, 155.82 (b), 156.44. IR (thin film): 3342, 2930, 2857, 1696 (b), 1522, 1473, 1365, 1251, 1172, 1093, 836, 775 cm^{-1}. HRMS: calcd for $C_{36}H_{67}O_7N_4Si$ 695.4778 [M^++ 1], found 695.4800 [M^+ + 1].

7-[1-[2-[(*tert*-Butyldimethylsilyl)oxy]ethyl]]-*N*‴-carbobenzoxy-*N*,*N*′,*N*″-tricarbo-*tert*-butoxythermospermine. A solution of 0.0881 g (0.127 mmol) of 7-[1-[2-[(*tert*-butyldimethylsilyl)oxy]ethyl]]-*N*‴-carbobenzoxy-*N*,*N*′-dicarbo-*tert*-butoxythermospermine and 0.0344 g (0.157 mmol, 1.24 equiv) of $(Boc)_2O$ in 3 mL of CH_2Cl_2 was kept at 23 °C for 11.5 h. At this time, the reaction mixture was concentrated and the residue was purified by chromatography (10 g of silica gel, 0–2% MeOH/$CHCl_3$) to afford 0.0886 g (88%) of a viscous oil. R_f: 0.90 in 10% MeOH/$CHCl_3$.

^{1}H NMR ($CDCl_3$): δ 0.02 (s, 6H, CH_3Si), 0.86 (s, 9H, $(CH_3)_3CSi$), 1.42 (s, 9H, $OCCH_3$), 1.43 (s, 9H, $OCCH_3$), 1.44 (s, 9H, $OCCH_3$), 1.50–1.70 (2m, 8H), 2.12 (b, 1H), 3.00–3.30 (m, 12H), 3.62 (t, 2H, J = 6.4 Hz, OCH_2), 5.08 (s, 2H, $ArCH_2$), 7.30-7.40 (m, 5H, Ar*H*). ^{13}C NMR ($CDCl_3$): δ -5.36 (CH_3Si), 18.14 ($(CH_3)_3CSi$), 25.01, 25.54, 25.84, 27.20, 28.38, 33.07, 33.71, 37.31, 40.65, 43.82, 46.69, 48.93, 49.32, 60.78, 65.51, 78.82, 79.50, 79.74, 128.02, 128.43, 136.56, 155.69, 155.97, 156.36. IR (thin film): 3346, 2931, 1716, 1697, 1540, 1472, 1418, 1365, 1252, 1171, 1092, 836, 775 cm^{-1}. HRMS: calcd for $C_{41}H_{75}O_9N_4Si$ 795.5303 [M^+ + 1], found 795.5281 [M^+ + 1].

7-[1-[2-[(*tert*-Butyldimethylsilyl)oxy]ethyl]]-*N*,*N*′,*N*″-tricarbo-*tert*-butoxythermospermine (30). A suspension of 0.0836 g (0.105 mmol) of 7-[1-[2-[(*tert*-butyldimethylsilyl)oxy]ethyl]]-*N*‴-carbobenzoxy-*N*,*N*′,*N*″-tricarbo-*tert*-butoxythermospermine and ~5 mg of 10% Pd–C catalyst in 1 mL of MeOH at 23 °C was purged three times with H_2 gas and then kept under 1 atm of H_2 gas for 4.5 h. The black suspension was filtered through a pipette column of Celite, eluting with 5 mL of MeOH. The filtrate was concentrated, and the residue was purified by chromatography (5 g of silica gel, step gradient of 10% MeOH/$CHCl_3$ to 5% *i*-$PrNH_2$/10% MeOH/85% $CHCl_3$) to yield 0.0661 g (95%) of a viscous oil. R_f: 0.54 in 5% *i*-$PrNH_2$/10% MeOH/85% $CHCl_3$.

^{1}H NMR ($CDCl_3$): δ 0.03 (s, 6H, CH_3Si), 0.88 (s, 9H, $(CH_3)_3CSi$), 1.44 (s, 9H, $OCCH_3$), 1.45 (s, 9H, $OCCH_3$), 1.46 (s, 9H, $OCCH_3$), 1.55 (m, 2H), 1.65 (m, 4H), 2.15 (bs, 1H, C*H*), 2.72 (bs, 2H), 3.05–3.30 (m, 12H), 3.63 (t, 2H, J = 6.4 Hz, $SiOCH_2$). ^{13}C NMR ($CDCl_3$): δ -5.34 (CH_3Si), 18.17 ($(CH_3)_3CSi$), 25.14, 25.62, 25.87 ($SiC(CH_3)_3$), 27.81, 28.41 ($OC(CH_3)_3$), 30.65 (b), 33.12, 33.64 (b), 37.31 (b), 41.80, 43.79 (b), 46.94 (b), 48.97 (b), 60.79 (CH_2OSi), 78.80 (b), 79.50 (b), 79.76, 156.00 (b). IR (thin film): 3366 (b), 3000–2850, 1692, 1474, 1420, 1366, 1252, 1172, 1092, 836, 775 cm^{-1}. HRMS: calcd for $C_{33}H_{69}O_7N_4Si$ 661.4935 [M^+ + 1], found 661.4915 [M^+ + 1].

1-[12-(Carbo-*tert*-butoxyamino)-5,9-diaza-5,9-dicarbo-*tert*-butoxy-7-[1-[2-[(*tert*-butyldimethylsilyl)oxy]-ethyl]]dodecanyl]-*N*-carbobenzoxy-*O*-(2-tetrahydropyranyl)tyrosinamide (32). A solution of 0.225 g (0.0432 mmol) of *N*-carbobenzoxy-*O*-(2-tetrahydropyranyl)tyrosine 4-nitrophenyl ester (**31**) and 0.0289 g (0.0437 mmol, 1.01 equiv) of **30** in 1.0 mL of MeOH was stirred at 23 °C for 26 h. At this time, the reaction mixture was concentrated, and the residue was dissolved in 4 mL of $CHCl_3$. The $CHCl_3$ solution was washed with two 2-mL portions of water, two 2-mL portions of saturated $NaHCO_3$ solution, and two 2-mL portions of saturated brine solution and workedup as usual. Purification by silica gel chromatog-

raphy (5 g of gel, 0–5% MeOH/$CHCl_3$) yielded 0.0305 g (68%) of a clear oil. R_f: 0.68 in 10% MeOH/$CHCl_3$.

^1H NMR ($CDCl_3$): δ 0.03 (s, 6H, CH_3Si), 0.87 (s, 9H, $(CH_3)_3CSi$), 1.30–1.55 (m, 6H), 1.43 (s, 18H, $OCCH_3$), 1.45 (s, 9H, $OCCH_3$),1.65 (m, 6H), 1.85 (m, 2H), 2.00 (m, 1H), 2.12 (bs, 1H), 2.95–3.30 (m, 12 H), 3.62 (m, 4H), 3.89 (dt, 1H, J = 2.7, 9.0 Hz, CbzNHC*H*), 4.32 (bs, 1H), 5.07 (s, 2H, $ArCH_2O$), 5.36 (t, 1H, J = 3.0 Hz, OC*H*O), 6.95 (d, 2H, J = 8.5 Hz), 7.09 (d, 2H, J = 7.9 Hz), 7.33 (m, 5H). ^{13}C NMR ($CDCl_3$): δ -5.33 (CH_3Si), 18.16 ($(CH_3)_3CSi$), 18.81, 25.15, 25.87 ($SiC(CH_3)_3$), 28.40 ($OC(CH_3)_3$), 30.34, 33.08 (b), 33.90 (b), 37.40, 37.67, 37.92 (b), 38.98, 44.00 (b), 49.00 (b), 49.30 (b), 56.40, 60.80, 62.07, 66.90, 79.00 (b), 79.80, 96.37, 116.57, 127.99, 128.12, 130.22, 155.77, 156.03, 170.80. IR (thin film): 3326 (b), 2935, 1690, 1511, 1472, 1419, 1365, 1246, 1171 cm^{-1}. HRMS: calcd for $C_{55}H_{92}O_{12}N_5Si$ 1042.6510 [M^+ + 1], found 1042.6470 [M^+ + 1].

1-[12-(Carbo-*tert*-butoxyamino)-5,9-diaza-5,9-dicarbo-*tert*-butoxy-7-[1-[2-[(*tert*-butyldimethylsilyl)oxy]ethyl]]dodecanyl]-*O*-(2-tetrahydropyranyl)tyrosinamide. A suspension of 0.0305 g (0.0292 mmol) of **32** and ~5 mg of 10% Pd–C catalyst in 1.0 mL of MeOH at 23 °C was purged three times with H_2 gas and then kept under 1 atm of H_2 gas for 2 h. The black suspension was filtered through a pipette column of Celite, eluting with 5 mL of MeOH. The filtrate was concentrated, and the residue was purified by chromatography (3 g of silica gel, 0–3% MeOH/$CHCl_3$) to yield 0.0239 g (90%) of a cloudy oil. R_f: 0.54 in 5% *i*-$PrNH_2$/10% MeOH/85% $CHCl_3$. R_f: 0.49 in 10% MeOH/$CHCl_3$.

^1H NMR ($CDCl_3$): δ 0.03 (s, 6H, CH_3Si), 0.87 (s, 9H, $(CH_3)_3CSi$), 1.35–1.55 (m, 6H), 1.43 (s, 9H, $OCCH_3$), 1.45 (s, 9H, $OCCH_3$), 1.46 (s, 9H, $OCCH_3$), 1.65 (m, 6H), 1.85 (m, 2H), 2.00 (m, 1H), 2.13 (m, 1H), 2.59 (m, 1H), 3.00–3.30 (m, 12H), 3.54 (m, 2H), 3.62 (m, 2H), 3.91 (m, 1H), 5.30 (bs, 1H), 5.38 (t, 1H, J = 6.0 Hz, OC*H*O), 6.99 (d, 2H, J = 8.5 Hz), 7.13 (d, 2H, J = 8.5 Hz), 7.34 (m, 1H). ^{13}C NMR ($CDCl_3$): δ -5.31 (CH_3Si), 18.19 ($(CH_3)_3CSi$), 18.84, 25.20, 25.90 ($SiC(CH_3)_3$), 26.98, 28.44 ($OC(CH_3)_3$), 30.38, 33.20, 33.75 (b), 37.50, 38.69, 40.27, 44.00 (b), 46.80 (b), 48.97, 56.58, 60.89, 62.12, 79.00 (b), 79.50, 79.77, 96.45 (b), 116.70, 130.16, 130.92, 155.98, 155.74, 174.27. IR (thin film): 3358 (b), 2931, 2858, 1693, 1510, 1472, 1419, 1366, 1250, 1174, 836, 776 cm^{-1}. HRMS: calcd for $C_{47}H_{86}O_{10}N_5Si$ 908.6144 [M^+ + 1], found 908.6154 [M^+ + 1].

1-[12-(Carbo-*tert*-butoxyamino)-5,9-diaza-5,9-dicarbo-*tert*-butoxy-7-[1-[2-[(*tert*-butyldimethylsilyl)oxy]ethyl]]dodecanyl]-*N*-butyryl-*O*-(2-tetrahydropyranyl)tyrosinamide. To a solution of 0.0229 g (0.0251 mmol) of 1-[12-(carbo-*tert*-butoxyamino)-5,9-diaza-5,9-dicarbo-*tert*-butoxy-7-[1-[2-[(*tert*-butyldimethylsilyl)oxy]ethyl]]dodecanyl]-*O*-(2-tetrahydropyranyl)tyrosinamide in 1.0 mL of $CHCl_3$ at 23 °C was added simultaneously 0.0031 g (0.0030 mL, 0.029 mmol, 1.1 equiv) of butyryl chloride and 0.0058 g (0.0080 mL, 0.057 mmol, 2.3 equiv) of Et_3N. After 1 h, another equivalent of butyryl chloride and Et_3N were added to the reaction. After 3 h longer, the reaction mixture was diluted with 10 mL of $CHCl_3$ and 5 mL of water and partitioned. The aqueous layer was extracted with one 10-mL portion of $CHCl_3$, and the combined organic layers were washed with one 10-mL portion of saturated brine solution. The usual workup, followed by silica gel PTLC (two 10 × 20-cm plates, 5% MeOH/$CHCl_3$) yielded 0.0192 g (78%), of a pale yellow, viscous oil. R_f: 0.60 in 10% MeOH/$CHCl_3$.

^1H NMR ($CDCl_3$): δ 0.03 (s, 6H, CH_3Si), 0.88 (s, 9H, $(CH_3)_3CSi$), 0.88 (m, 3H, CH_2CH_3), 1.38 (m, 6H), 1.44 (s, 18H, $OCCH_3$), 1.46 (s, 9H, $OCCH_3$), 1.55–1.70 (m, 8H), 1.84 (m, 2H), 2.00 (bm, 1H), 2.14 (t, 2H, J = 7.1 Hz), 2.05–2.15 (m, 1H), 2.99 (m, 2H), 3.05–3.30 (m, 12H), 3.62 (m, 4H), 3.89 (m, 1H), 4.57 (bs, 1H), 5.32 (bs, 1H), 5.37 (s, 1H, OC*H*O), 6.25 (bs, 2H), 6.96 (d, 2H, J = 8.4 Hz, Ar*H*), 7.10 (d, 2H, J = 8.2 Hz, Ar*H*). ^{13}C NMR ($CDCl_3$): δ -5.32 (CH_3Si), 13.62 (CH_2CH_3), 18.17 ($(CH_3)_3CSi$), 18.83, 18.97, 25.17, 25.88 ($SiC(CH_3)_3$), 30.36, 33.50 (b), 34.00 (b), 37.62, 38.39, 38.97, 43.80 (b), 46.50, 49.30 (b), 54.49, 60.83, 62.07, 79.00 (b), 79.79, 96.45 (b), 116.53, 129.71, 130.17, 155.77, 156.00, 170.97, 172.89. IR (thin film): 3286 (b), 3078, 2932, 2859, 1694, 1644, 1511, 1366, 1250, 1174, 836, 776, 733 cm^{-1}. HRMS: calcd for $C_{51}H_{92}O_{11}N_5Si$ 978.6563 [M^+ + 1], found 978.6533 [M^+ + 1].

1-[12-(Carbo-*tert*-butoxyamino)-5,9-diaza-5,9-dicarbo-*tert*-butoxy-7-[1-(2-hydroxyethyl)]dodecanyl]-*N*-butyryl-*O*-(2-tetrahydropyranyl)tyrosinamide (33). A solution of 0.0174 g (0.0177 mmol) of 1-[12-(carbo-*tert*-butoxyamino)-5,9-diaza-5,9-dicarbo-*tert*-butoxy-7-[1-[2-[(*tert*-butyldimethylsilyl)oxy]ethyl]]dodecanyl]-*N*-butyryl-*O*-(2-tetrahydropyranyl)tyrosinamide and 0.045 mL (0.045 mmol, 2.5 equiv) of *n*-Bu_4NF (1 M solution in THF) in 0.5 mL of THF was kept at 23 °C for 29 h. The reaction mixture was diluted with 5 mL of water and extracted with three 5-mL portions of $CHCl_3$. The combined organic extracts were washed with one 10-mL portion of saturated brine. The usual workup, followed by silica gel PTLC (two 10 × 20-cm plates, 5% MeOH/$CHCl_3$) afforded 0.0127 g (83%) of a faint yellow oil. R_f: 0.54 in 10% MeOH/$CHCl_3$.

^1H NMR ($CDCl_3$): δ 0.87 (t, 3H, J = 7.3 Hz, CH_2CH_3), 1.38 (m, 6H), 1.43 (s, 9H, $OCCH_3$), 1.44 (s, 9H, $OCCH_3$),1.45 (s, 9H, $OCCH_3$), 1.60 (m, 2H), 1.68 (m, 4H), 1.78 (bs, 1H), 1.83 (m, 2H), 2.00 (m, 1H), 2.14 (t, 2H, J = 7.5 Hz), 2.19 (bs, 1H), 2.97 (d, 2H, J = 7.0 Hz), 3.00–3.35 (m, 12H), 3.58 (m, 1H), 3.69 (bs, 2H), 3.89 (m, 1H), 4.56 (m, 1H), 5.25 (b, 1H), 5.36 (s, 1H, OC*H*O), 6.32 (bs, 2H), 6.96 (d, 2H, J = 8.3 Hz, Ar*H*), 7.10 (d, 2H, J = 8.2 Hz, Ar*H*). ^{13}C NMR ($CDCl_3$): δ 13.61 (CH_2CH_3), 18.86, 18.99, 25.17, 26.79, 28.45, 30.37, 32.45, 33.85, 37.56, 37.73, 38.38, 38.65, 45.00 (b), 47.00 (b), 49.37, 54.57, 60.00, 62.13, 79.00, 79.71, 79.93, 96.51, 116.63, 129.70, 130.15, 156.04, 156.10. IR (thin film): 3300 (b, OH, NH), 3100, 3000–2850, 1692, 1510, 1420, 1366, 1238, 1171, 968 cm^{-1}. HRMS: calcd for $C_{45}H_{78}O_{11}N_5$ 864.5698 [M^+ + 1], found 864.5727 [M^+ + 1].

1-[12-(Carbo-*tert*-butoxyamino)-5,9-diaza-5,9-dicarbo-*tert*-butoxy-7-[1-[2-[(4-azidobenzoyl)oxy]ethyl]]dodecanyl]-*N*-butyryl-*O*-(2-tetrahydropyranyl)tyrosinamide (35). A solution of 0.0069 g (0.0079 mmol) of **33**, 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-(dimethylamino)-propyl]-3-ethylcarbodiimide hydrochloride, and 0.1 mg (0.8 μmol, 10 mol %) of DMAP in 1 mL of CH_2Cl_2 was stirred at 23 °C for 5 h. At this time, the mixture was concentrated, and the residue was purified by silica gel PTLC (one 10 × 20-cm plate, 7% MeOH/$CHCl_3$) to yield 0.0063 g (79%) of a colorless oil. R_f: 0.68 in 10% MeOH/$CHCl_3$.

^1H NMR ($CDCl_3$): δ 0.87 (t, 3H, J = 6 Hz, CH_2CH_3), 1.20–1.55 (m, 31H), 1.55–1.90 (m, 12H), 1.97 (m, 1H), 2.04 (t, 2H, J = 6 Hz, $CH_2CH_2CH_3$), 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, OCHO), 6.20 (bs, 2H, NH), 6.95 (d, 2H, J = 8 Hz), 7.08 (m, 4H), 8.00 (d, 2H, J = 9 Hz). LRMS: calcd for $C_{52}H_{80}N_8O_{12}$, 1009 [M^+ + 1], found 1009 [M^+ + 1].

1-[12-amino-5,9-diaza-7-[1-[2-[(4-azidobenzoyl)oxy]-ethyl]]dodecanyl]-*N*-butyryltyrosinamide, PhTX-433-N_3Ph2 (20). To a solution of 0.0020 g (0.0021 mmol) of **35** 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, and the residue was purified by chromatography (2 g of silica gel, step gradient of 1/2/17 to 1/2/6 of i-$PrNH_2$/MeOH/$CHCl_3$) to yield 0.001 g (77%) of a clear oil. R_f: 0.64 in 1/4/4 of i-$PrNH_2$/MeOH/$CHCl_3$.

^{1}H NMR (CD_3OD): δ 0.84 (t, 3H, J = 7.4 Hz, CH_2CH_3), 1.45 (m, 4H), 1.54 (m, 2H, $CH_2CH_2CH_3$), 1.78 (m, 3H), 2.05 (bs, 1H, NCH_2CHCH_2N), 2.15 (t, 2H, J = 7.9 Hz, $CH_2CH_2CH_3$), 2.75 (m, 7H), 2.93 (m, 4H), 3.05–3.20 (m, 3H), 4.40 (m, 3H, NCHCON, CH_2O), 6.68 (d, 2H, J = 8.5 Hz, Tyr ArH), 7.03 (d, 2H, J = 8.5 Hz, Tyr ArH), 7.16 (d, 2H, J = 8.7 Hz, N_3ArH), 8.04 (d, 2H, J = 8.7 Hz, N_3ArH). LRMS: calcd for $C_{32}H_{48}N_8O_5$ 625 [M^+ + 1], found 625 [M^+ + 1].

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Abbreviations: Bn, benzyl; Boc, *tert*-butoxycarbonyl; $(Boc)_2O$, di-*tert*-butyldicarbonate; Cbz, carbobenzoxy; $CHCl_3$, chloroform; CH_2Cl_2, dichloromethane; DCC, dicyclohexylcarbodiimide; DMAP, *N,N*-dimethyl-4-aminopyridine; DMF, dimethyl formamide; DMSO, dimethyl sulfoxide; DPPA, diphenyl phosphorazidate; $(EtO)_2CO$, diethyl carbonate; LAH, lithium aluminum hydride; LDA, lithium diisopropylamide; NaOAc, sodium acetate; PCC, pyridinium chlorochromate; TBDMS, *tert*-butyldimethylsilyl; TFA, trifluoroacetic acid; THF, tetrahydrofuran; THP, 2-tetrahydropyranyl.

Supplementary Material Available: ^{1}H NMR spectra of all new compounds **2, 7–9, 11, 13, 14, 16, 19, 20, 24–30, 32, 33, 35,** N-(carbobenzoxybutyl)-2-cyanoethylamine, *N-tert*-butyl-1-[4-(carbobenzoxyamino)butyl]-1-(2-cyanoethyl)carbamate, 3-butyl-*N‴*-carbobenzoxy-*N,N″*-dicarbo-*tert*-butoxythermospermine, 2-[1-[2-[(*tert*-butyldimethylsilyl)oxy]ethyl]]-*N*-1-(2-cyanoethyl)-1-aminopropan-3-ol, 2-[1-[2-[(*tert*-butyldimethylsilyl)oxy]ethyl]]-*N*-carbo-*tert*-butoxy-*N*-[1-(2-cyanoethyl)]-1-aminopropan-3-ol, 2-[1-[2-[(*tert*-butyldimethylsilyl)oxy]ethyl]]-*N*-[1-(3-aminopropyl)]-1-aminopropan-3-ol, 7-[1-[2-[(*tert*-butyldimethylsilyl)oxy]ethyl]]-*N⁗*-carbobenzoxy-*N,N‴*-dicarbo-*tert*-butoxythermospermine, 7-[1-[2-[(*tert*-butyldimethylsilyl)oxy]ethyl]]-*N‴*-carbobenzoxy-*N,N′,N″*-tricarbo-*tert*-butoxythermospermine, 1-[12-(carbo-*tert*-butoxyamino)-5,9-diaza-5,9-carbo-*tert*-butoxy-7-[1-[2-[(*tert*-butyldimethylsilyl)oxy]ethyl]]dodecanyl]-*O*-(2-tetrahydropyranyl)tyrosinamide, and 1-[12-(carbo-*tert*-butoxyamino)-5,9-diaza-5,9-carbo-*tert*-butoxy-7-[1-[2-[(*tert*-butyldimethylsilyl)oxy]ethyl]]dodecanyl]-*N*-butyryl-*O*-(2-tetrahydropyranyl)tyrosinamide (30 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.