The Effects of Conformational Constraints and Steric Bulk in the Amino Acid Moiety of Philanthotoxins on AMPAR Antagonism

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Philanthotoxin-343 (PhTX-343), a synthetic analogue of wasp toxin PhTX-433, is a noncompetitive antagonist at ionotropic receptors (e.g., AChR or iGluR). To determine possible effects of variations of the amino acid side chain, a library consisting of seventeen PhTX-343 analogues was prepared. Thus, tyrosine was replaced by either apolar, conformationally constrained, or bulky amino acids, whereas the acyl unit and the polyamine moiety were kept unchanged. Analogues with tertiary amide groups were prepared for the first time. Pentafluorophenyl esters were employed for amide bond formation, establishing general protocols for philanthotoxin solution- and solid-phase synthesis (39−90% and 42−54% overall yields, respectively). The analogues were tested for their ability to antagonize kainate-induced currents of 2-amino-3-(3-hydroxy-5-methyl-4-isoxazoyl)propanoic acid receptors (AMPAR) expressed in *Xenopus* oocytes from rat brain mRNA. This showed that steric bulk in the amino acid moiety is well tolerated and suggests that binding to AMPAR does not involve the α-NHCO group as a donor in hydrogen bonding.

Introduction

Polyamine toxins comprise several subtypes of compounds isolated from the venoms of spiders and wasps that are used for paralysis of prey. Philanthotoxin-433 (PhTX-433, **1**) was isolated from the Egyptian digger wasp *Philanthus triangulum* (Figure 1).[1]

PhTX-433 consists of three subunits: a polyamine moiety containing four amine functionalities separated by three or four methylene groups (region 1), a butyryl group (region 2), and a residue of the L-amino acid tyrosine (region 3). PhTX-433 and its synthetic analogue PhTX-343 (**2**) have been shown to be noncompetitive inhibitors at mammalian ligand-gated cation channels such as nicotinic acetylcholine receptors (nAChR) and ionotropic glutamate receptors (iGluR), including 2-amino-3-(3-hydroxy-5-methyl-4-isoxazoyl)propanoic acid receptors (AMPAR).[2−11] In a preliminary model for the receptor binding of these toxins it was envisaged that the positively charged polyamine chain interacts electrostatically with ionized carboxylic acid groups of aspartic or glutamic acid residues present in the interior of the ion channel.[7,12] More recent models propose interactions of amino groups with main-chain carbonyl oxygen atoms that line the narrowest part of the AMPAR pore.[13] Hydrophobic interactions involving the toxin headgroup (regions 2 and 3) contribute decisively to the binding, since the biological activities of philanthotoxins are qualitatively and quantitatively different from those of the parent polyamines.[14−16] Also, the potency of philanthotoxins is enhanced with increased hydrophobicity of the *N*-acyl group.[10,11] The antagonism on calcium-permeable iGluR[4,17] is potentially important because excessive influx of Ca^{2+} ions into the neurons is associated with various neurodegenerative disorders and acute brain seizures.[18] In addition to serving as potential drug leads, PhTX-433 and PhTX-343 analogues are of interest as probes in the studies of receptor structure and function.[6,12,19]

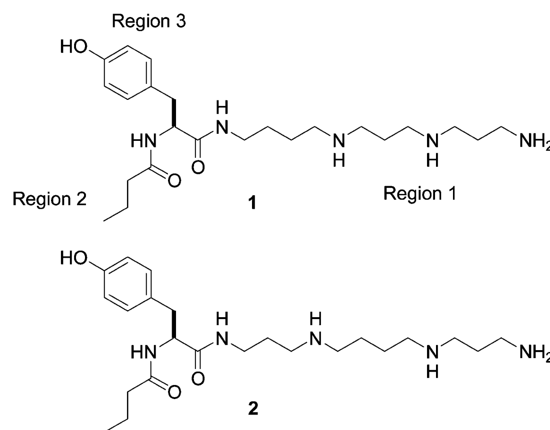

Figure 1. Natural polyamine toxin PhTX-433 (**1**) and the synthetic analogue PhTX-343 (**2**).

Recently, philanthotoxin analogues exhibiting variations in the polyamine chain have been synthesized by solution- and solid-phase methods,[4,5,20−23] and their antagonistic effects at various subtypes of iGluR and nAChR were investigated. Although substitution of the asymmetric polyamine of the natural PhTX-433 with the symmetric spermine to give PhTX-343 has an insignificant influence on biological activity,[1,8−11] changes in the number and position of the secondary amino functionalities can cause pronounced effects in the

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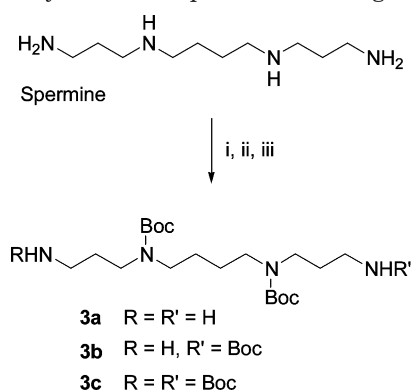
Scheme 1. Synthesis of Spermine Building Blocks[a]



[a] Reagents: (i) CF_3COOEt, MeOH, −50 °C; (ii) Boc_2O; (iii) NaOH, H_2O.

pharmacological profiles.[2−5,20] In contrast to these studies, only a few analogues representing systematic variations of the amino acid part of the molecule have been synthesized and investigated. These comprise PhTX-343 analogues with the *p*-hydroxy group replaced by a *m*-hydroxy group,[5] as well as analogues in which tyrosine is replaced with phenylalanine or its halogenated, alkoxylated, or azido-substituted analogues.[7−11] The overall trend in this series is that lack of the phenolic functionality in PhTX-343 analogues may result in a decrease or an increase of potency on iGluR depending on the receptor system studied,[9−11] while the antagonism of nACh was occasionally enhanced.[8,11] Likewise, replacement of tyrosine in PhTX-343 with nonpolar aliphatic amino acids (e.g., glycine, alanine, or leucine) gave rise to comparable potency at some ionotropic receptors and to decreased potency at other receptors.[10,11] The analogues of PhTX-433 and PhTX-343 with altered amino acid moieties have been tested mostly on insect ionotropic receptors.[8−10] Hence, there is a need for a more comprehensive exploration of structure−activity relationships for analogues of PhTX-343 altered in the amino acid moiety on mammalian ion channels. Synthesis of these analogues and determination of their antagonist potencies on rat brain AMPAR is the subject of the present work. As the three subunits of the PhTX-343 molecule are interconnected by amide bonds, strategies from peptide synthesis may be employed for sequential synthesis of analogues.[5] A range of bulky and conformationally restricted amino acids was investigated as substitutes for tyrosine. Here, the chosen amino acid protection−activation strategy is based on the fluorenylmethoxycarbonyl (Fmoc) group and the pentafluorophenyl (Pfp) ester group, both for synthesis in solution[22,23] and, for the first time, in solid-phase philanthotoxin synthesis. *tert*-Butoxycarbonyl (Boc) group was applied for protection of amino functionalities in the polyamine. All synthesized analogues contain the residue of spermine and a butyryl group as in PhTX-343, to enable a more straightforward interpretation of the biological results.

Results and Discussion

Synthesis of Building Blocks. The polyamine building block, tri-Boc-protected spermine (**3b**), was synthesized by a modification of a previously reported procedure (Scheme 1).[22,24]

Thus, treatment of spermine with 1 equiv of ethyl trifluoroacetate, followed by Boc protection and removal of the *N*-trifluoroacetyl group, afforded a mixture of di-, tri-, and tetra-Boc-protected spermine derivatives (**3a**, **3b**, and **3c**, respectively) in the ratio 1:2:1. The substantial by-product **3a** was used for solid-phase synthesis of PhTX-343 analogues (see below). The reaction conditions were also modified to obtain **3a** as the main product (87% yield) by employing 2.3 equiv of ethyl trifluoroacetate. The building block for introduction of the *N*-acyl moiety, pentafluorophenyl butyrate, was obtained from butyryl chloride and pentafluorophenol.[22]

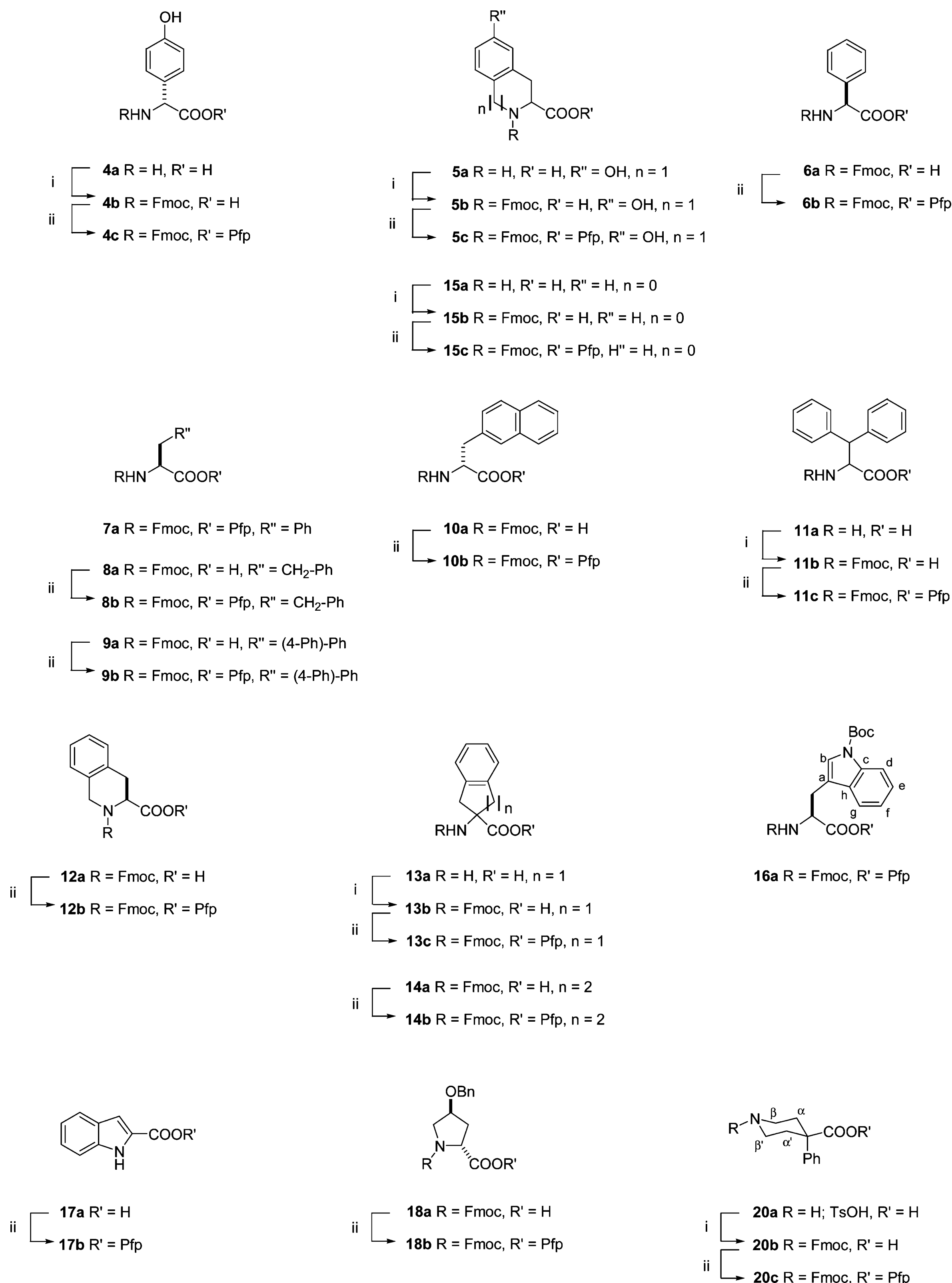
The majority of amino acids used for synthesis of philanthotoxins **4**−**20** were commercially available, but most of these required N^{α} protection and carboxy activation. The Fmoc group was introduced by conventional treatment[25] with fluorenylmethoxycarbonyl chloride (Fmoc-Cl) in dioxane−10% aqueous Na_2CO_3 (Scheme 2) generally giving high yields (77−91%), except for 2-[(9*H*-fluoren-9-ylmethoxycarbonyl)amino]indan-2-carboxylic acid (**13b**), obtained in only 17% yield, probably due to a steric hindrance of the amino group at the spiro position. The resulting Fmoc-protected amino acids were subsequently activated as Pfp esters by base-catalyzed tandem transesterification with pentafluorophenyl trifluoroacetate in pyridine−DMF.[26]

The active Pfp esters were chosen due to their ease of formation, handling, stability, and appropriate reactivity in amide formation with primary amino groups. Hence, Pfp esters are still a useful and inexpensive alternative to more recently developed and highly efficient coupling agents such as PyBOP[27] or HATU.[28] Moreover, the Pfp ester couplings proceed without any detectable racemization of the amino acid chiral center when used in solution-phase as well as in solid-phase couplings.[22,29,30] In general the Pfp esters were obtained in high yields (76−100%). Only the Pfp ester of N^{α}-Fmoc-protected 6-hydroxy-1,2,3,4,-tetrahydroisoquinoline (**5c**) was isolated in a lower yield (58%), most likely caused by the lack of phenol protection. The prepared novel N^{α}-Fmoc-protected amino acid Pfp esters were characterized by ^{1}H and ^{13}C NMR, and by ion-cyclotron resonance HRMS. The compounds containing a tertiary amide group (**5**, **12**, **15**, and **17**−**20**) exhibited two sets of ^{1}H and ^{13}C signals originating from the presence of rotamers of amide bonds (see Experimental Section). For this reason, some of the spectra were recorded at elevated temperatures.

The hydroxyproline-derived building block **19f** was synthesized as depicted in Scheme 3. In the first step, the carboxy group in N^{α}-benzyloxycarbonyl-4-hydroxyproline (**19a**) was esterified to give methyl ester **19b**.[31] Subsequently, a Mitsunobu reaction between the partially protected hydroxyproline derivative **19b** and 2-naphthol using diethyl azodicarboxylate (DEAD) and Ph_3P in the presence of Et_3N afforded the C-4 inverted compound in 74% yield.[32] Removal of the Z-group by hydrogenolysis employing 10% Pd/C as catalyst gave **19d** in 86% yield. Alkaline hydrolysis of **19d** in MeCN−10% aqueous Na_2CO_3 was followed by evaporation of MeCN, and subsequently the Fmoc group was introduced by addition of Fmoc-Cl in dioxane. This one-pot procedure afforded **19e** in 68% yield. Finally, the Pfp ester (**19f**) was prepared in quantitative yield as described above.

Solution-Phase Synthesis of Philanthotoxins. In the solution-phase synthetic route (Scheme 4), Fmoc-pro-

Scheme 2. Synthesis of Amino Acid Building Blocks[a]

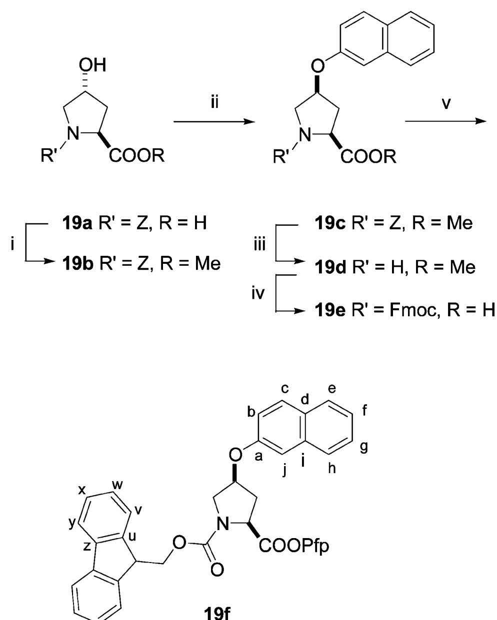


[a] Reagents: (i) Fmoc-Cl, 10% Na_2CO_3, dioxane; (ii) $CF_3COOPfp$, pyridine, DMF. Fmoc: fluorenylmethoxycarbonyl. Pfp: pentafluorophenyl. Ph: phenyl. Bn: benzyl.

tected and Pfp ester-activated amino acids were coupled to the free primary amino group of spermine derivative **3b**.

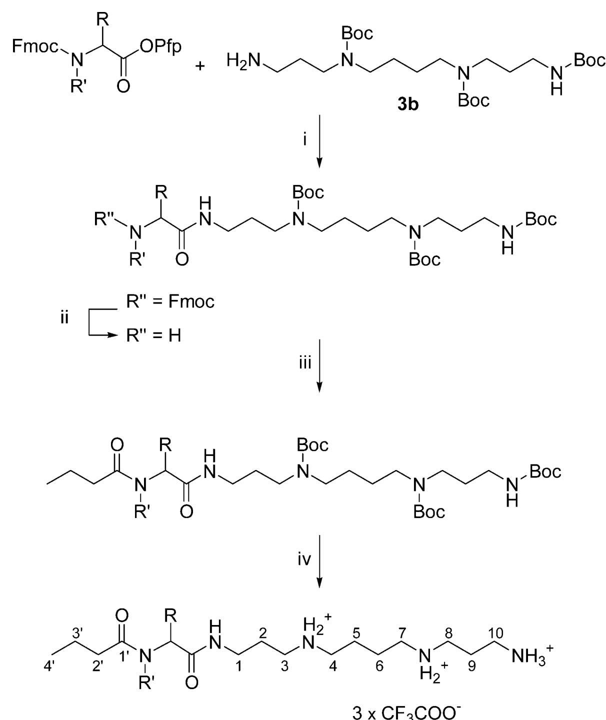
The resulting intermediates were isolated in high yields (72−97%) after purification by VLC. Simplification of the procedure by omitting the VLC purification step was not advantageous, as it caused purification of the subsequent Fmoc-deprotected intermediates to be more tedious. Removal of the Fmoc group was performed with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in dry THF with 1-octanethiol as dibenzofulvene scavenger,[33] giving intermediate N^{α}-deprotected polyamine

Scheme 3. Synthesis of the Building Block **19f**[a]



[a] Reagents: (i) MeI, Et_3N, MeCN; (ii) 2-naphthol (1.5 equiv), DEAD (1.5 equiv), Ph_3P (1.5 equiv), dry THF; (iii) H_2, 10% Pd/C, EtOH; (iv) 10% aqueous Na_2CO_3, MeCN; then removal of MeCN and addition of Fmoc-Cl in dioxane; (v) $CF_3COOPfp$, pyridine, DMF. Z: benzyloxycarbonyl.

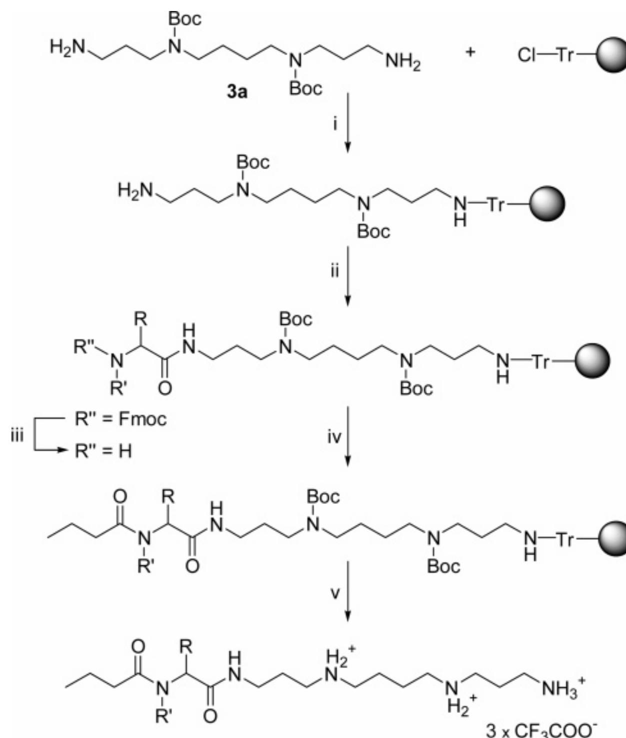
Scheme 4. Solution-Phase Synthesis of Philanthotoxins[a]



[a] Reagents: (i) Et_3N, CH_2Cl_2, room temperature, 3 h; (ii) DBU, 1-octanethiol, THF, room temperature, 2 h; (iii) $C_3H_7COOPfp$, CH_2Cl_2, room temperature, 3 h; (iv) 20% TFA−CH_2Cl_2, room temperature, 3 h.

amides in high yields (88−95%). In a second N-acylation step, the butyryl group was attached using pentafluorophenyl butyrate, to yield Boc-protected philanthotoxins in good to excellent yields (69−96%). Finally, the Boc groups in the polyamine moiety and any other acid-labile protecting groups present in the side chain of the amino acid moiety were removed by treatment with trifluoroacetic acid (TFA). The resulting target compounds were isolated by reversed-phase VLC as tris-(TFA) salts in 85−100% yield.[22] By this method (Scheme 4), philanthotoxins **4**−**8**, **12**−**16**, **18**, and **20** were synthesized. Most of the analogues were obtained in high to excellent overall yield (70−90%). The only exceptions were the D-4-hydroxyphenylglycine derivative **4** and the 6-hydroxy-1,2,3,4-tetrahydroisoquinoline derivative **5**, obtained in 45% and 39% overall yield, respectively. This is probably due to the presence of a free phenolic functionality in the amino acid side chain, which may lead to intermolecular esterification giving phenyl esters. The analogue **17**, lacking the *N*-butyryl group, was obtained in three steps by conversion of 1*H*-indole-2-carboxylic acid (**17a**) to its Pfp ester **17b**, coupling with **3a**, and Boc deprotection, with an overall yield of 72%.

Scheme 5. Solid-Phase Synthesis of Philanthotoxins[a]



[a] Reagents: (i) THF, 50 °C, 4 h, then 10% DIPEA−MeOH; (ii) Fmoc-NR′(CHR)-OPfp (2.5 equiv), DIPEA (2.5 equiv), HODhbt (1 equiv), DMF, room temperature, overnight; (iii) 20% piperidine−DMF, room temperature, 2 × 10 min; (iv) $C_3H_7COOPfp$ (2.5 equiv), DIPEA (2.5 equiv), HODhbt (1 equiv), DMF, room temperature, 3 h or overnight; (v) CH_2Cl_2−TFA−triisopropylsilane (78:20:2), room temperature, 1 h.

Solid-Phase Synthesis (SPS) of Philanthotoxins. A conventional polystyrene-based 2-chlorotrityl chloride resin was used as solid support. Although this type of linker is quite acid-labile, its high stability toward bases makes it ideal for reverse synthesis of N-terminal amino peptides as well as for acylation of resin-bound amines using the Fmoc strategy, the product being readily cleaved from the resin with acid (e.g., TFA−CH_2Cl_2). The coupling reactions were followed by using the Kaiser test.[34] The synthetic strategy is outlined in Scheme 5.

In the resin-loading step, di-Boc-protected spermine (**3a**) was attached to the 2-chlorotrityl linker using a

Table 1. IC_{50} Values (μM) and Yields of Philanthotoxins Tested in the AMPAR Assay (But = C_3H_7CO-; Sp = $H_2N(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH-$)

No.	Philanthotoxin	Yield	IC_{50}	No.	Philanthotoxin	Yield	IC_{50}	No.	Philanthotoxin	Yield	IC_{50}
2		-	0.13 ± 0.03	4		45%[a]	0.07 ± 0.005	5		39%[a]	0.22 ± 0.03
6		86%[a]	0.32 ± 0.07	7		86%[a]	0.43 ± 0.14	8		80%[a]	0.17 ± 0.05
9		54%[b]	0.08 ± 0.01	10		44%[b]	0.10 ± 0.02	11		42%[b]	0.30 ± 0.03
12		90%[a]	0.37 ± 0.14	13		71%[a]	0.19 ± 0.07	14		72%[a]	0.25 ± 0.04
15		70%[a]	1.39 ± 0.89	16		54%[a]	0.30 ± 0.13	17		72%[c]	not active
18		83%[a]	0.26 ± 0.10	19		52%[b]	1.04 ± 0.21	20		80%[a]	1.32 ± 0.88

[a] Four-step synthetic route in solution. [b] Four-step solid-phase synthesis. [c] Two-step synthetic route in solution.

large excess of **3a** in order to minimize cross-linking. The excess **3a** used in the reaction was recovered and employed for loading of subsequent portions of the resin. The free primary amino group of the resin-bound **3a** was acylated with Fmoc-protected amino acid Pfp esters using 3-hydroxy-3,4-dihydro-1,2,3-benzotriazin-4-one (HODhbt) as an additive to enhance coupling efficiency, while diisopropylethylamine (DIPEA) was employed as the tertiary amine.[35] Removal of the Fmoc group was performed with 20% piperidine in DMF as in peptide SPS, followed by N-acylation with pentafluorophenyl butyrate.[22] The product was cleaved from the resin with CH_2Cl_2−TFA−triisopropylsilane (78:20:2) with concomitant *N*-Boc deprotection. The resulting philanthotoxins **9**−**11** and **19** were isolated as tris(TFA) salts by reversed-phase VLC in 42−54% overall yield.

Pharmacology. For AMPAR assays, toad (*Xenopus laevis*) oocytes injected with mRNA isolated from whole rat brain 1−3 days after they were removed from a toad[3] expressed high levels of AMPAR 3−5 days later. A two-electrode voltage clamp was used to measure the effect of the philanthotoxins on currents evoked by application of 10^{-4} M kainic acid to these oocytes. All measurements were performed at a holding potential of −80 mV. The oocytes were successively exposed to five concentrations of each toxin, and the resulting reductions in the amplitude of the kainate-induced current were used to derive IC_{50} values. In Table 1 the IC_{50} values (μM) of the novel philanthotoxins **4**−**6** and **8**−**20** synthesized in this work are listed together with the values obtained for PhTX-343 (**2**) and its phenylalanine analogue **7**.

Two of the synthesized PhTX-343 analogues, **4** and **5**, still contain a phenolic functionality as in the parent compound. Shortening of the distance between the amino acid α-carbon and the aromatic ring (compound **4**) improved the potency by a factor of 2, whereas the

constrained analogue **5** was 2-fold less active. Compound **4** has the opposite absolute configuration compared to compound **2**, whereas compound **5** is racemic and has the hydroxy group shifted from para to meta position with respect to the amino acid side chain. The observed potencies for analogues **4** and **5** are in accordance with previous studies which showed that the enantiomers of PhTX-343 (**2**) have similar potencies at several types of ionotropic receptors.[36] However, the change to a meta-hydroxylated benzene ring only led to a moderate decrease in activity, as compared to the complete loss of antagonistic activity at iGluR, which was seen for the *m*-hydroxy analogue of compound **2** in the nAChR assay.[5] Moreover, due to the minor differences observed between **2** and **5**, other conformationally constrained analogues of PhTX-343, including other hydroxylated 1,2,3,4-tetrahydroisoquinoline derivatives, may be of interest for future studies.

Removal of the hydroxy group in PhTX-343 (**2**) to give the phenylalanine analogue **7** represents a known modification,[8−11] which resulted in a 3-fold decrease on AMPAR (Table 1). Next, an array of nonphenolic philanthotoxins (i.e. **6**−**20**) representing variations of the basic structure **7** was considered. These compounds may be subdivided into three groups: (i) simple homologues **6**−**8**; (ii) compounds containing two benzene rings **9**−**11**; and (iii) cyclic analogues **12**−**15** and **17**−**20**. The selection of amino acids in the latter series followed their commercial availability.

Homologues **6** and **7** displayed similar activity with IC_{50} values 0.32 μM and 0.43 μM, respectively, whereas the phenethyl analogue **8** is 2-fold more potent (IC_{50} = 0.17 μM) and essentially equivalent to PhTX-343 (**2**). Replacement of the benzene ring of **7** with an indole ring (compound **16**) resulted in a practically equipotent derivative. For philanthotoxins containing two aromatic rings, the linearly extended aromatic structures **9** and **10** gave rise to a higher potency than the branched analogue **11**, which is equipotent with phenylalanine derivative **7**. Introduction of steric constraints by cyclization involving the aromatic ring and either the α-carbon (compounds **13** and **14**) or the α-nitrogen (**5**, **12**, and **15**) of the amino acid moiety resulted in potencies comparable to that of **7**. In addition, the absence of a phenolic functionality resulted in slightly lowered activity while a more hydrophobic but still relatively flexible aromatic amino acid side chain is favorable.

Compounds **5**, **12**, **15**, and **17**−**20** are to our knowledge the first tertiary amide philanthotoxins prepared. While the loss of activity of **17** is readily explainable by the lack of the *N*-butanoyl group, the data for the remaining compounds support that a secondary amide (NHCO) is not required for antagonist activity at rat brain AMPAR, since tertiary amides **5** and **12** have activities equivalent to that of the secondary amide **7**. Thus, it can be concluded that the α-NHCO portion of the amino acid moiety does not participate as a hydrogen-bond donor upon interaction with AMPAR.

Of the two analogues (**18** and **19**) based on an L-hydroxyproline scaffold but differing in stereochemistry at C-4, the benzyl derivative **18** was somewhat more active than the naphthyl derivative **19**. The retained activity of derivative **20** shows that the shift of the amino acid nitrogen from the α-position to the γ-position is tolerated, supporting the conclusion that the α-nitrogen is not involved in the receptor binding. Moreover, the present results demonstrate that cyclic aliphatic substructures in the amino acid (compounds **5**, **12**−**14**, **18**, **19**, and in particular **20**) are compatible with AMPAR antagonism.

Conclusion

The present work has demonstrated the following: (i) The Fmoc-Pfp methodology[22] is equally applicable in the solution-phase and the solid-phase synthesis of philanthotoxins containing a range of amino acids representing a high structural diversity. The protocol is compatible with the presence of unprotected phenol groups, although at a penalty of somewhat diminished yield. (ii) Simple purification by reversed-phase VLC[22] proved extendable to all synthesized analogues. Reversed-phase VLC is therefore an attractive, rapid, and broad-scope alternative to reversed-phase HPLC[4,5,20,21,36] for large-scale purification of philanthotoxins. (iii) All 18 philanthotoxins (except **17**) investigated (Table 1) proved to have potencies roughly within 1 order of magnitude, in spite of strongly dissimilar amino acid residues. Thus, this relatively modest effect of the amino acid structure on the antagonistic potency on AMPAR seems to suggest that the headgroups of philanthotoxins occupy a nonpolar binding site with no strongly direction-dependent binding interactions, resulting in a broad-scope toleration of the headgroup structure. (iv) The presence of cyclized amino acid structures appears to be of minor importance for the activity. The relative insensitivity of the antagonism of philanthotoxins on AMPAR to amino acid structure is similar to the results previously obtained with a limited number of analogues tested on insect iGluR sensitive to quisqualate (qGluR)[10] and contrasts the pronounced dependency on the structure of the polyamine chain.[2−5,20] (v) The vast majority of philanthotoxins so far described in the literature contain a benzene ring. However, the present results together with an earlier report[10] showing a leucine analogue to be equipotent with PhTX-343 at qGluR and indicate that philanthotoxins containing bulky aliphatic amino acid side chains are attractive targets for future studies. (vi) Finally, the present observation that hydrogen-bond donor capacity of the amino acid α-nitrogen appears to be unessential for the activity is an important guidance for future SAR investigations.

Experimental Section

General Procedures. Unless otherwise stated, starting materials and solvents were purchased from commercial suppliers (amino acid derivatives from Bachem or Novabiochem, reagents and solvents from Sigma-Aldrich, Fluka, or Lancaster, resins for solid-phase synthesis from Novabiochem) and used as received. CH_2Cl_2 was distilled from P_2O_5 and stored over 4 Å molecular sieves. THF was distilled from Na/benzophenone immediately before use. MeCN was dried over 3 Å molecular sieves. Dried DMF for solid-phase synthesis was obtained from Fluka. ^{1}H NMR and ^{13}C NMR spectra were recorded at 400.14 and 100.62 MHz, respectively, on a Bruker AMX 400 spectrometer, or at 300.06 and 75.45 MHz, respectively, on a Varian Mercury Plus spectrometer, using $CDCl_3$ or CD_3OD as solvents and tetramethylsilane (TMS) as internal standard. Coupling constants (*J* values) are given in Hertz (Hz), and multiplicities of ^{1}H NMR signals are reported as follows: s, singlet; d, doublet; t, triplet; q, quartet; p, pentet,

sx, sextet; m, multiplet; br, broad. Assignments are based on 2D NMR experiments (COSY, HMBC, and HSQC). High-resolution mass spectrometry (HRMS) measurements were performed on a Bruker APEX III Q Fourier transform mass spectrometer equipped with a 7 T superconducting magnet and an external electrospray ion source (Apollo source). The spectra were calibrated with a capillary skimmer dissociation spectrum of LHRH (luteinizing hormone-releasing hormone). The samples were introduced into the elctrospray ion source using a 250 μL syringe with a syringe pump flow of 2 μL/min. Vacuum liquid chromatography (VLC) was performed using Merck silica gel 60H (particle size < 45 μm) or Merck Lichroprep RP-18 (40−63 μm) for normal-phase and reversed-phase VLC, respectively. Analytical RP-HPLC was performed on a Shimadzu system consisting of an SCL-10A VP controller, an SIL-10AD VP autosampler, an LC-10AT VP pump, an SPD-M10A VP diode array detector, and a CTO-10AC VP column oven, using a 150 × 4.6 mm i.d. Phenomenex Luna C18(2) 3μ column. The HPLC system was controlled by Class VP 6 software; elution was performed with two different solvent systems (total flow of 0.8 mL/min). System I: solvent A = MeCN−H_2O−TFA 10:90:0.1 and solvent B = MeCN−H_2O−TFA 90:10:0.1; t = 0−5 min 0% B, t = 5−30 min 0−40% B, t = 30−35 min 40−100% B). System II: solvent A = MeOH−H_2O−TFA 10:90:0.1 and solvent B = MeOH−H_2O−TFA 90:10:0.1; t = 0−5 min 0% B, t = 5−35 min 0−100% B. The purities of target compounds **2** and **4**−**20** were determined (from UV absorption intergration at λ = 215 nm) in both system I and II, showing purities within the ranges 97.0−99.8% and 95.9−99.5%, respectively.

General Procedure A: Fmoc Protection of Amino Acids. A solution of Fmoc-Cl (1 equiv) in dioxane (2.6 mL/mmol) was added to a suspension of the amino acid in dioxane (1.3 mL/mmol) and 10% aqueous Na_2CO_3 (2.6 mL/mmol) at 0 °C. The mixture was stirred for 1 h at 0 °C and then for 1 h at room temperature. The reaction mixture was poured into water and washed with Et_2O. The aqueous phase was acidified with concentrated aqueous HCl, and the precipitated product was isolated by filtration and dried in vacuo.

General Procedure B: Fmoc Protection of Amino Acids. General procedure B was the same as general procedure A, except that upon acidification with concentrated HCl the reaction mixture was extracted with EtOAc (3 times). The combined EtOAc phases were dried (Na_2SO_4), filtered, and concentrated in vacuo. The product was either used without further purification or purified by VLC (hexanes−EtOAc 2:1 to 1:4 with 0.1% AcOH added).

General Procedure C: Pfp Ester Formation. The Fmoc-protected amino acid was dissolved in dry DMF (2 mL/mmol). Pyridine (1.1 equiv) and pentafluorophenyl trifluoroacetate (2.0 equiv) were added. The mixture was stirred for 1 h at room temperature under N_2, when it was diluted with EtOAc, and then washed with 0.1 M aqueous HCl, saturated aqueous $NaHCO_3$, and water. The organic phase was dried (Na_2SO_4), filtered, and concentrated. The product was either used without further purification or purified by VLC (hexanes−EtOAc 20:1 to 5:1).

General Procedure D: Coupling of Pfp Esters with 3b. The amino acid Pfp ester (1.0 equiv) and **3b** (1.0 equiv) were dissolved in dry CH_2Cl_2 (1 mL/100 mg of Pfp ester), Et_3N (1.0 equiv) was added, and the mixture was stirred under N_2 for 3 h at room temperature. The reaction mixture was concentrated, and the product was isolated by VLC (hexanes−EtOAc 3:1 to 1:2).

General Procedure E: Fmoc Deprotection. The Fmoc-protected compound was dissolved in dry THF (1 mL/100 mg), and 1-octanethiol (10 equiv) and DBU (0.2 equiv) were added. The mixture was stirred under N_2 for 2 h at room temperature, when it was concentrated in vacuo. The product was isolated by VLC (CH_2Cl_2−MeOH−32% aqueous NH_3 400:4:1 to 400:40:1).

General Procedure F: Introduction of *N*-Butyryl Group. The partially protected spermine amino acid conjugate and pentafluorophenyl butyrate (1.1 equiv) were dissolved in dry CH_2Cl_2 (1 mL/100 mg of conjugate), and Et_3N (1.0 equiv) was then added. The mixture was stirred under N_2 at room temperature for 3 h. The reaction mixture was concentrated, and the crude product was purified by VLC (hexanes−EtOAc 2:1 to 1:4).

General Procedure G: Boc Deprotection. The tri-Boc-protected philanthotoxin was treated with 10% TFA (100 equiv) in dry CH_2Cl_2 for 3 h at room temperature. The reaction mixture was concentrated in vacuo, and the resulting philanthotoxin was isolated by reversed-phase VLC (0.1% TFA in H_2O to MeCN−H_2O−TFA 300:700:1).

General Procedure H: Solid-Phase Synthesis of Philanthotoxins. 2-Chlorotrityl resin preloaded with **3a**[36] was swelled in dry DMF (12 mL/mmol of resin) for 30 min. The solvent was removed by suction, and the resin was treated with a Pfp-activated Fmoc-protected amino acid (2.0 equiv), HODhbt (1.0 equiv), and DIPEA (2.0 equiv) in dry DMF (12 mL/mmol of resin). The mixture was left overnight under N_2 at a shaking table. The solvent was removed by suction, and the resin was washed with DMF (3 × 12 mL/mmol of resin), CH_2Cl_2 (3 × 12 mL/mmol of resin), and DMF (3 × 12 mL/mmol of resin). The resin was treated with 20% piperidine in DMF (12 mL/mmol of resin) for 2 × 10 min at room temperature and was then washed with DMF (3 × 12 mL/mmol of resin). The resulting resin was treated with pentafluorophenyl butyrate (2.5 equiv), HODhbt (1.0 equiv), and DIPEA (2.5 equiv) in dry DMF (12 mL/mmol of resin) under N_2 for 3 h at room temperature. The solvent was removed by suction, and the resin was washed successively with DMF (3 × 12 mL/mmol of resin), CH_2Cl_2 (3 × 12 mL/mmol of resin), and MeOH (3 × 12 mL/mmol of resin) and then dried overnight in vacuo. The dried resin was treated with CH_2Cl_2−TFA−triisopropylsilane (78:20:2, 12 mL/mmol of resin) for 1 h at room temperature. The filtrate was collected by suction, and the resin was treated with 20% TFA in CH_2Cl_2 (12 mL/mmol of resin) and then with CH_2Cl_2 (12 mL/mmol of resin). The combined filtrates were concentrated, and the product was isolated by reversed-phase VLC (0.1% TFA in H_2O to MeCN−H_2O−TFA 300:700:1).

4,9-Diaza-4,9-di-(*tert*-butoxycarbonyl)dodecane-1,12-diamine (3a). Spermine (5.19 g, 0.026 mol) was dissolved in MeOH (350 mL) under N_2. Ethyl trifluoroacetate (7.0 mL, 0.059 mol) was added during 0.5 h at −50 °C. An additional amount of MeOH (100 mL) was added to dissolve the precipitate formed, and the mixture was stirred under N_2 for an additional 0.5 h at 0 °C. Di(*tert*-butyl) pyrocarbonate (16.78 g, 0.077 mol) in MeOH (30 mL) was added during 10 min, and the mixture was stirred overnight at room temperature. The reaction mixture was treated with 2 M aqueous NaOH (115 mL) for 4 h at room temperature and then overnight at 5 °C. MeOH was removed in vacuo, and the residue was partitioned between CH_2Cl_2 (350 mL) and water (200 mL). The CH_2Cl_2 phase was washed with brine (100 mL), whereas the aqueous phase was extracted with CH_2Cl_2 (5 × 100 mL). The combined organic phases were dried (Na_2SO_4) and filtered, and the solvent was evaporated. Purification by VLC (CH_2Cl_2−MeOH 100:1 to CH_2Cl_2−MeOH−32% aqueous NH_3 40:10:1) gave **3a** (9.02 g, 87%) as a yellow syrup. TLC: R_f 0.14 (CH_2Cl_2−MeOH−32% aqueous NH_3 50:10:1). ^1H NMR and ^{13}C NMR spectra were as earlier reported.[36]

Loading of 2-Chlorotrityl Chloride Resin with 3a. Compound **3a** (4.25 g, 10.56 mmol) was dissolved in dry THF (15 mL), and 2-chlorotrityl chloride resin (0.892 g, loading 1.14 mmol/g, 1.02 mmol) was added in 4 portions during 1 h at 50 °C. The mixture was stirred for 3 h at 50 °C and, upon cooling, was transferred to a syringe fitted with a Teflon filter and a Teflon stopcock. The solvent was removed by suction, and the resin was treated with 10% DIPEA in MeOH (15 mL) for 10 min to trap remaining 2-chlorotrityl chloride groups. The resin was washed with DMF (3 × 10 mL), CH_2Cl_2 (3 × 10 mL), and MeOH (3 × 10 mL) and then dried in vacuo to give the resin loaded with **3a** (1.123 g, loading approximately 0.80 mmol/g based on the increase in weight).

Pentafluorophenyl D-α-[(9*H*-fluoren-9-ylmethoxycarbonyl)amino]-4-hydroxybenzeneethanoate (4c). The Fmoc group was introduced to D-(4-hydroxyphenyl)glycine (**4a**, 506

mg, 3.027 mmol) according to general procedure B. The resulting **4b** (1.211 g, 79%) was used without further purification. The carboxylic acid **4b** (730 mg, 1.875 mmol) was subjected to general procedure C, which gave the Pfp ester **4c** (786 mg, 76%) as a white foam. TLC: R_f 0.11 (hexanes−EtOAc 4:1). $[\alpha]_D^{25}$: −49.2° (*c* 0.52, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.74 (2H, d, J = 7.3 Hz, H-y), 7.56 (2H, d, J = 6.6 Hz, H-v), 7.38 (2H, t, J = 7.3 Hz, H-x), 7.32−7.26 (4H, m, H-d and H-w), 6.85 (2H, d, J = 7.3 Hz, H-c), 5.63 (1H, s, H-α), 4.39−4.50 (2H, m, Fmoc-CH_2), 4.21 (1H, t, J = 7.3 Hz, Fmoc-CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 167.5 (C=O, amino acid), 156.5 (2C, C=O Fmoc and C-d), 143.4 (C-u), 141.2 (2C, C-z), 140.5 (2C, C-2′/C-6′, Pfp), 139.0 (C-4′, Pfp), 137.5 (2C, C-3′/C-5′, Pfp), 129.0 (2C, C-b), 127.8, 127.1 (each 2C, C-w and C-x), 125.0 (2C, C-v), 124.9 (C-1′, Pfp), 120.0 (2C, C-y), 116.3 (2C, C-c), 67.9 (Fmoc-CH$_2$), 57.9 (C-α), 47.3 (Fmoc-CH). HRMS: calcd for $C_{29}H_{18}F_5NO_5Na$ [M + Na]$^+$ 578.09973, found 578.09926.

D-*N*-[3-[[4-[(3-Aminopropyl)amino]butyl]amino]propyl]-4-hydroxy-α-[(1-oxobutyl)amino]benzeneethanamide Tris(trifluoroacetate) (4). Compounds **4c** (97 mg, 0.175 mmol) and **3b** (88 mg, 0.175 mmol) were coupled by using general procedure D to give the conjugate as a foam (110 mg, 72%). TLC: R_f 0.45 (hexanes−EtOAc 1:2). Fmoc deprotection of the conjugate (422 mg, 0.483 mmol) was performed according to general procedure E to give the amine intermediate (284 mg, 90%) as a syrup. TLC: R_f 0.2 (CH_2Cl_2−MeOH−32% aqueous NH_3 50:10:1). The *N*-butyryl group was introduced to the amine intermediate (209 mg, 0.321 mmol) according to general procedure F to give the protected analogue (160 mg, 69%) as an oil. TLC: R_f 0.44 (hexanes−EtOAc 1:9). Deprotection of the latter (110 mg, 0.152 mmol) was performed according to general procedure G to give **4d** (116 mg, 100%; overall yield: 45%) as a syrup. ^{1}H NMR (300 MHz, CD_3OD): δ 7.24 (2H, dt, J = 8.8 and 2.5 Hz, H-b), 6.77 (2H, dt, J = 8.8 and 2.5 Hz, H-c), 5.12 (1H, s, H-α), 3.40−3.20 (2H, m, H-1), 3.18−2.90 (10H, m, H-3, H-4, H-7, H-8, and H-10), 2.25 (2H, t, J = 7.5 Hz, H-2′), 2.09 (2H, m, H-9), 1.91−1.80 (2H, p, J = 6.8 Hz, H-2), 1.83−1.73 (4H, m, H-5 and H-6), 1.63 (2H, sx, J = 7.5 Hz, H-3′), 0.95 (3H, t, J = 7.5 Hz, H-4′). ^{13}C NMR (75 MHz, CD_3OD): δ 175.8 (C-1′), 174.3 (C=O, amino acid), 158.7 (C-d), 130.0 (C-b), 128.2 (C-a), 116.4 (C-c), 59.4 (C-α), 48.2, 48.1 (C-4 and C-7), 46.1 (C-3), 45.8 (C-8), 38.4 (C-2′), 37.8 (C-10), 36.7 (C-1), 27.6 (C-2), 25.4 (C-9), 24.3 (2C, C-5 and C-6), 20.3 (C-3′), 14.1 (C-4′). HRMS: calcd for $C_{22}H_{40}N_5O_3$ [M + H]$^+$ 422.31257, found: 422.31218.

Pentafluorophenyl DL-2-(9*H*-Fluoren-9-ylmethyl)-6-hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (5c). 6-Hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (**5a**, 250 mg, 1.18 mmol) was Fmoc protected as described in general procedure A to give **5b** (394 mg, 80%). Compound **5b** (200 mg, 0.481 mmol) was esterified by using general procedure C to give **5c** (162 mg, 58%) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.77 (2H, d, J = 7.9 Hz, H-y), 7.65−7.42 (2H, m, H-v), 7.45−7.35 (2H, m, H-x), 7.34−7.24 (2H, m, H-w), 7.00 (1H, d, J = 7.9 Hz, H-b), 6.74−6.69 (2H, m, H-c, H-e), 5.42 (1H, dd, J = 3.8 and 5.5 Hz, H-α), 5.17 (1H, br s, OH), 4.74−4.42 (4H, m, Fmoc-CH_2, H-γ), 4.34*, 4.28* (1H, t, J = 6.6 Hz, Fmoc-CH), 3.37−3.20 (2H, m, H-β). ^{13}C NMR (75 MHz, $CDCl_3$): δ 167.7*, 167.6* (C=O, amino acid), 156.1*, 155.3*, 155.0*, 154.9* (2C, C=O Fmoc, C-d), 144.0*, 143.9*, 143.7*, 143.5* (2C, C-u), 141.5*, 141.4* (2C, C-z), 140.0 (2C, C-2′/C-6′, Pfp), 139.0 (C-4′, Pfp), 137.5 (2C, C-3′/C-5′, Pfp), 132.7*, 132.5* (C-f), 128.0*, 127.9*, 127.8* (2C, C-x and C-b), 127.2 (2C, C-w), 125.2*, 125.1*, 125.1*, 125.0*, (2C, C-v), 124.9 (C-1′ Pfp), 124.4 (C-a), 120.2 (2C, C-y), 115.0, 114.8 (C-c, and C-e), 68.6*, 68.4* (Fmoc-CH$_2$), 54.0*, 53.6* (C-α), 47.4*, 47.3* (Fmoc-CH), 44.4*, 44.3* (C-γ), 32.2*, 31.7* (C-β); (*) denotes double signals originating from two amide rotamers present. HRMS: calcd for $C_{31}H_{21}F_5NO_5$ [M + H]$^+$ 582.13344, found 582.13318.

DL-*N*-[3-[[4-[(3-Aminopropyl)amino]butyl]amino]propyl]-6-hydroxy-2-(1-oxobutyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylamide Tris(trifluoroacetate) (5). Pfp ester **5c** (65 mg, 0.11 mmol) and **3b** (61 mg, 0.12 mmol) were coupled according to general procedure D to give the conjugate (90 mg, 89%) as a foam. TLC: R_f 0.32 (hexanes−EtOAc 1:2). Fmoc deprotection of the conjugate (80 mg, 0.089 mmol) was performed by general procedure E to give the amine intermediate (52 mg, 87%) as a syrup. TLC: R_f 0.21 (CH_2Cl_2−MeOH−32% aqueous NH_3 150:10:1). The *N*-butyryl group was introduced to the amine intermediate (44 mg, 0.065 mmol) by using general procedure F to give the Boc-protected **5** (25 mg, 51%) as a syrup. Deprotection of the latter (25 mg, 0.033 mmol) was performed according to general procedure G to give **5** (26 mg, 100%, overall yield: 39%) as a syrup. ^{1}H NMR (300 MHz, CD_3OD): δ 7.09 (1H, d, J = 8 Hz, H-b), 6.72−6.63 (2H, m, H-c and H-e), 4.56 (1H, t, J = 5.0 Hz, H-α), 4.65 (1H, d, J = 14.6 Hz, H_A-γ, major rotamer), 4.64 (1H, d, J = 16.0 Hz, H_A-γ, minor rotamer), 4.57 (1H, d, J = 14.6 Hz, H_B-γ, major rotamer), 4.47 (1H, d, J = 16.0 Hz, H_B-γ, minor rotamer), 3.32−3.22 (1H, m, H_A-1), 3.20−3.00 (9H, m, H-β, H_B-1, H-4, H-8, and H-10), 2.86 (2H, t, J = 7.4 Hz, H-7), 2.78−2.18 (4H, m, H-2′ and H-3), 2.27−2.04 (2H, m, H-9), 1.88−1.62 (8H, m, H-2, H-5, H-6, and H-3′), 1.03 (3H, t, J = 7.4 Hz, H-4′, major rotamer), 0.99 (3H, t, J = 7.4 Hz, H-4′, minor rotamer). ^{13}C NMR (75 MHz, CD_3OD): δ 175.8 (C-1′), 174.8 (C=O, amino acid), 157.8 (C-d), 136.4 (C-f), 128.4 (C-b), 125.4 (C-a), 115.3, 114.8 (C-c and C-e), 56.2 (C-α), 48.3, 48.1 (C-4 and C-7), 46.9 (C-γ), 45.9 (C-8), 45.7 (C-3), 37.8 (2C, C-10 and C-2′), 36.4 (C-1), 33.2 (C-β), 27.5 (C-2), 25.5 (C-9), 24.3 (2C, C-5 and C-6), 19.3 (C-3′), 14.3 (C-4′); only the signals corresponding to the major rotamer are given. HRMS: calcd for $C_{24}H_{42}N_5O_3$ [M + H]$^+$ 448.32822, found 448.32767.

Pentafluorophenyl L-α-[(9*H*-Fluoren-9-ylmethoxycarbonyl)amino]-α-benzeneethanoate (6b). Carboxylic acid **6a** (1.08 g, 2.89 mmol) was esterified by using general procedure C to give **6b** (1.38 g, 89%) as a white foam. TLC: R_f 0.60 (petroleum ether−EtOAc 2:1). $[\alpha]_D^{25}$: +36.6° (*c* 0.56, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.73 (2H, d, J = 7.7 Hz, H-y), 7.56 (2H, d, J = 7.3 Hz, H-v), 7.45−7.24 (9H, m, Ph-H, H-w, and H-x), 5.54 (1H, s, H-α), 4.50−4.40 (2H, m, Fmoc-CH_2), 4.21 (1H, t, J = 7.0 Hz, Fmoc-CH). ^{13}C NMR (75 MHz, $CDCl_3$): 168.3 (C=O, amino acid), 155.2 (C=O, Fmoc), 143.6, 143.4 (2C, C-u), 141.2 (2C, C-z), 140.9 (2C, C-2′/C-6′, Pfp), 139.3 (C-4′, Pfp), 137.7 (2C, C-3′/C-5′, Pfp), 134.4 (C-a), 129.4 (5C, Ph-C), 127.7 (2C, C-w or C-x), 127.5 (Ph-C), 127.1 (2C, C-w or C-x), 124.9 (2C, C-v), 124.8 (C-1′, Pfp), 120.0 (2C, C-y), 67.5 (Fmoc-CH$_2$), 58.2 (C-α), 47.2 (Fmoc-CH). Anal. Calcd for $C_{29}H_{18}F_5NO_4$: C, 64.57; H, 3.36; F, 17.61; N, 2.60. Found: C, 64.55; H, 3.61; F, 17.61; N, 2.51.

L-*N*-[3-[[4-[(3-Aminopropyl)amino]butyl]amino]propyl]-α-[(1-oxobutyl)amino]benzeneethanamide Tris(trifluoroacetate) (6). Pfp ester **6b** (242 mg, 0.448 mmol) and compound **3b** (198 mg, 0.395 mmol) were dissolved in dry CH_2Cl_2 (2.0 mL), Et_3N (55 μL, 0.40 mmol) was added, and the mixture was stirred for 2 h at room temperature. The reaction mixture was diluted with CH_2Cl_2 (20 mL) and washed with water (2 × 15 mL). The organic phase was dried (Na_2SO_4) and filtered, and the solvent was evaporated. The crude conjugate (0.395 mmol) was dissolved in dry THF (3.5 mL), and DBU (79 μL, 0.079 mmol) and 1-octanethiol (10 equiv) were added. After 2 h at room temperature the reaction mixture was concentrated, and the product was purified by VLC to give quantitatively the Fmoc-deprotected product (240 mg, 0.377 mmol). TLC: R_f 0.16 (CH_2Cl_2−MeOH−32% aqueous NH_3 200:10:1). Coupling with pentafluorophenyl butyrate according to general procedure F gave the Boc-protected intermediate (249 mg, 94%). Removal of the Boc groups from the latter (211 mg, 0.300 mmol) was performed as described in general procedure G to give **6** (215 mg, 92%; overall yield: 86%). ^{1}H NMR (400 MHz, CD_3OD): δ 7.46−7.32 (5H, m, H-b, H-c and H-d), 5.26 (1H, s, H-α), 3.41−3.22 (2H, m, H-1), 3.18−2.91 (10H, m, H-3, H-4, H-7, H-8, and H-10), 2.27 (2H, t, J = 7.5 Hz, H-2′), 2.09 (2H, p, J = 7.8 Hz, H-9), 1.87 (2H, p, J = 6.7 Hz, H-2), 1.82−1.74 (4H, m, H-5 and H-6), 1.64 (2H, sx, J = 7.5 Hz, H-3′), 0.94 (3H, t, J = 7.5 Hz, H-4′). ^{13}C NMR (100 MHz, CD_3OD): δ 176.2 (C-1′), 174.2 (C=O, amino acid), 138.0 (C-a), 129.9 and 116.4 (2C, C-b and C-c), 129.6 (C-d), 59.8 (C-α), 48.2 and 48.1

(C-4 and C-7), 46.1 and 45.8 (C-3 and C-8), 38.4 (C-2′), 37.8 (C-10), 36.8 (C-1), 27.5 (C-2), 25.4 (C-9), 24.2 (2C, C-5 and C-6), 20.2 (C-3′), 14.0 (C-4′). HRMS: calcd for $C_{22}H_{40}N_5O_2$ $[M + H]^+$ 406.31765, found 406.31724.

L-*N*-[3-[[4-[(3-Aminopropyl)amino]butyl]amino]propyl]-4-α-[(1-oxobutyl)amino]benzenepropanamide Tris(trifluoroacetate) (7). Compounds **7a** (200 mg, 0.361 mmol) and **3b** (181 mg, 0.361 mmol) were coupled by using general procedure D to yield the conjugate (296 mg, 94%) as a foam. TLC: R_f 0.22 (hexanes−EtOAc 1:1). Fmoc deprotection of the conjugate (275 mg, 0.315 mmol) was performed according to general procedure E to give the product (106 mg, 100%) as a colorless syrup. TLC: R_f 0.10 (CH_2Cl_2−MeOH−32% aqueous NH_3 200:10:1). The *N*-butyryl group was introduced to the latter (178 mg, 0.274 mmol) using general procedure F to give the Boc-protected intermediate (191 mg, 97%) as a foam. TLC: R_f 0.30 (hexanes−EtOAc 1:4). Deprotection of the latter (161 mg, 0.226 mmol) was performed according to general procedure G to give **7** (161 mg, 94%; overall yield: 86%) as a syrup. ^{1}H NMR (400 MHz, CD_3OD): δ 7.33−7.18 (5H, m, ArH), 4.47 (1H, dd, *J* = 6.8 Hz and *J* = 8.7 Hz, H-α), 3.33−2.87 (14H, m, H-1, H-3, H-4, H-7, H-8, H-10, and H-β), 2.16 (2H, t, *J* = 7.4 Hz, H-2′), 2.14−2.03 (2H, m, H-9), 1.86−1.76 (6H, m, H-2, H-5, and H-6), 1.53 (2H, sx, *J* = 7.4 Hz, H-3′), 0.83 (3H, t, *J* = 7.4 Hz, H-4′). ^{13}C NMR (100 MHz, CD_3OD): δ 176.2 (C-1′), 175.0 (C=O, amino acid), 138.4 (C-a), 130.2 and 129.5 (each 2C, C-b and C-c), 127.9 (C-d), 56.7 (C-α), 48.4 and 48.1 (C-4 and C-7), 46.2 and 45.8 (C-3 and C-8), 38.6 (2C, C-2′ and C-β), 37.8 (C-10), 36.8 (C-1), 27.4 (C-2), 25.4 (C-9), 24.3 and 24.2 (C-5 and C-6), 20.2 (C-3′), 13.9 (C-4′). HRMS: calcd for $C_{23}H_{42}N_5O_2$ $[M + H]^+$ 420.33330, found 420.33290.

Pentafluorophenyl L-α-[(9*H*-Fluoren-9-ylmethoxycarbonyl)amino]benzenebutanoate (8b). Carboxylic acid **8a** (500 mg, 1.25 mmol) was esterified by using general procedure C to give **8b** (679 mg, 96%) as a white foam. TLC: R_f 0.50 (hexanes − EtOAc 7:1). $[\alpha]_D^{25}$: +2.4° (*c* 3.2, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.74 (2H, d, *J* = 8.5 Hz, H-y), 7.56 (2H, d, *J* = 7.5 Hz, H-v), 7.42−7.14 (9H, m, Ph-H, H-w, and H-x), 5.28 (1H, d, *J* = 8.8 Hz, NH), 4.75 (1H, m, H-α), 4.46 (2H, d, *J* = 7.0 Hz, Fmoc-C*H*$_2$), 4.22 (1H, t, *J* = 7.0 Hz, Fmoc-C*H*), 2.76 (2H, t, *J* = 7.9 Hz, H-γ), 2.42−2.25, 2.25−2.10 (each 1H, m, H-β). ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.7 (C=O, amino acid), 155.6 (C=O, Fmoc), 143.6 and 143.5 (2C, C-u), 141.3 (2C, C-z), 140.7 (2C, C-2′/C-6′, Pfp), 139.8 (C-a), 139.5 (C-4′, Pfp), 137.8 (2C, C-3′/C-5′, Pfp), 128.7, 128.4 (4C, C-b and C-c), 128.3 (C-1′, Pfp), 127.7 (2C, C-x), 127.1 (2C, C-w), 126.5 (C-d), 125.0 (2C, C-v), 120.0 (2C, C-y), 67.3 (Fmoc-*C*H$_2$), 53.7 (C-α), 47.3 (Fmoc-*C*H), 34.3 (C-β), 31.7 (C-γ). Anal. Calcd for $C_{31}H_{22}F_5NO_4$: C, 65.61; H, 3.91; F, 16.71; N, 2.47, found: C, 65.24; H, 4.04; F, 17.69; N, 2.39.

L-*N*-[3-[[4-[(3-Aminopropyl)amino]butyl]amino]propyl]-α-[(1-oxobutyl)amino]benzenebutanamide Tris(trifluoroacetate) (8). Pfp ester **8b** (400 mg, 0.705 mmol) and compound **3b** (354 mg, 0.705 mmol) were coupled by using general procedure D to yield the conjugate (83 mg, 93%). TLC: R_f 0.22 (hexanes−EtOAc 1:1). Fmoc deprotection of the conjugate (403 mg, 0.455 mmol) was performed by using general procedure E to give the amine intermediate (287 mg, 95%) as a syrup. TLC: R_f 0.18 (CH_2Cl_2−MeOH−32% aqueous NH_3 200:10:1). The *N*-butyryl group was introduced to the amine intermediate (219 mg, 0.330 mmol) using general procedure F to give the Boc-protected intermediate (222 mg, 92%). TLC: R_f 0.08 (hexanes − EtOAc 1:2). Deprotection of the latter (183 mg, 0.249 mmol) was performed according to general procedure G to give **8** (191 mg, 99%; overall yield: 80%) as a syrup. ^{1}H NMR (300 MHz, CD_3OD): δ 7.28−7.12 (5H, m, Ph-H), 4.14 (1H, dd, *J* = 9.1 and 5.2 Hz, H-α), 3.40−3.20 (2H, m, H-1), 3.17−2.94 (10H, m, H-3, H-4, H-7, H-8, and H-10), 2.80−2.60 (2H, m, H-γ), 2.35−2.19 (2H, m, H-2′), 2.14−2.02 (2H, m, H-9), 2.10−1.90 (2H, m, H-β), 1.87 (2H, p, *J* = 6.8 Hz, H-2), 1.84−1.74 (4H, m, H-5 and H-6), 1.66 (2H, sx, *J* = 7.5 Hz, H-3′), 0.98 (3H, t, *J* = 7.5 Hz, H-4′). ^{13}C NMR (75 MHz, CD_3OD): δ 176.2 (C-1′), 175.5 (C=O, amino acid), 141.8 (C-a), 129.3 and 129.2 (each 2C, C-b and C-c), 126.9 (C-d), 55.1 (C-α), 48.2 and 48.1 (C-4 and C-7), 46.2 (C-3), 45.8 (C-8), 38.6 (C-2′), 37.8 (C-10), 36.8 (C-1), 34.6 (C-β), 33.2 (C-γ), 27.5 (C-2), 25.4 (C-9), 24.3 (2C, C-5 and C-6), 20.4 (C-3′), 14.2 (C-4′). HRMS: calcd for $C_{24}H_{44}N_5O_2$ $[M + H]^+$ 434.34895, found 434.34848.

Pentafluorophenyl L-α-[(9*H*-Fluoren-9-ylmethoxycarbonyl)amino]-4-biphenylpropanoate (9b). Compound **9a** (500 mg, 1.079 mmol) was esterified using general procedure C to give **9b** (493 mg, 73%) as a white foam. TLC: R_f 0.29 (hexanes−EtOAc 4:1). $[\alpha]_D^{25}$: −8.0° (*c* 0.64, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.74 (2H, d, *J* = 7.4 Hz, H-y), 7.57−7.50 (6H, m, H-v, H-b, and H-c′), 7.45−7.32 (5H, m, H-d′, H-w, and H-x), 7.32−7.22 (4H, m, H-c and H-b′), 5.25 (1H, d, *J* = 8.3 Hz, NH), 5.04 (1H, q, *J* = 8.3 Hz, H-α), 4.52−4.36 (2H, m, Fmoc-C*H*$_2$), 4.21 (1H, t, *J* = 6.9 Hz, Fmoc-C*H*), 3.36 (1H, dd, *J* = 14.0 and 8.3 Hz, H_A-β), 3.27 (1H, dd, *J* = 14.0 and 8.3 Hz, H_B-β). ^{13}C NMR (75 MHz, $CDCl_3$): δ 167.9 (C=O, amino acid), 155.4 (C=O, Fmoc), 143.6 and 143.5 (2C, C-u), 141.3 (2 C, C-z), 141.0 (2C, C-2′/C-6′, Pfp), 140.5 and 140.4 (C-d, and C-a′), 139.5 (C-4′, Pfp), 137.8 (2C, C-3′/C-5′, Pfp), 133.5 (C-a), 129.7†, 128.8†, 127.6†, 127.4, 127.1†, 127.0†, 125.1, 125.0 (13C, C-b, C-c, C-b, C-c′, C-d′, C-v, C-w, and C-x), 124.9 (C-1′, Pfp), 120.0 (2C, C-y), 67.4 (Fmoc-*C*H$_2$), 54.7 (C-α), 47.3 (Fmoc-*C*H), 37.7 (C-β); (†) denotes two-carbon signals. HRMS: calcd for $C_{36}H_{24}F_5NO_4Na$ $[M + Na]^+$ 652.15177, found 652.15176.

L-*N*-[3-[[4-[(3-Aminopropyl)amino]butyl]amino]propyl]-α-[(1-oxobutyl)amino]-4-biphenylpropenamide Tris(trifluoroacetate) (9). Using general procedure H, resin loaded with **3a** (250 mg, loading approximately0.80 mmol/g, 0.20 mmol) was elongated successively with **9b** and pentafluorophenyl butyrate to give, after deprotection and cleavage, philanthotoxin **9** (91 mg, 54%). ^{1}H NMR (300 MHz, CD_3OD): δ 7.50−7.48 (4H, m, H-b′ and H-c), 7.39−7.33 (2H, ddt, *J* = 7.7, 7.1, and 1.1 Hz, H-c′), 7.29−7.20 (3H, m, H-b and H-d′), 4.45 (1H, dd, *J* = 8.5 and 6.9 Hz, H-α), 3.30−2.80 (14H, m, H-1, H-8, H-β, H-10, H-3, H-4, and H-7), 2.14 (2H, t, 7.4 Hz, H-2′), 2.14−1.98 (2H, m, H-9), 1.84−1.68 (6H, m, H-2, H-5, and H-6), 1.51 (2H, sx, *J* = 7.4 Hz, H-3′), 0.80 (3H, t, *J* = 7.4 Hz, H-4′). ^{13}C NMR (75 MHz, CD_3OD): δ 175.9 (C-1′), 174.6 (C=O, amino acid), 141.7 and 140.8 (C-d and C-a′), 137.2 (C-a), 130.5 (2C, C-b), 129.7 (2C, C-c′), 128.1 (C-d′), 127.8, 127.6 (C-c and C-b′), 56.7 (C-α), 48.2 (C-4), 48.1 (C-7), 46.2 (C-3), 45.9 (C-8), 38.6 (C-2′), 38.3 (C-β), 37.8 (C-10), 36.8 (C-1), 27.5 (C-2), 25.5 (C-9), 24.4 and 24.3 (C-5 and C-6), 20.3 (C-3′), 14.0 (C-4′). HRMS: calcd for $C_{29}H_{46}N_5O_2$ $[M + H]^+$ 496.36460, found 496.36401.

Pentaflouorophenyl D-α-[(9*H*-Fluoren-9-ylmethoxycarbonyl)amino]-2-naphthalenepropanoate (10b). Compound **10a** (500 mg, 1.14 mmol) was esterified using general procedure C give **10b** (686 mg, 100%) as a white solid. TLC: R_f 0.47 (hexanes−EtOAc 3:1). $[\alpha]_D^{25}$: +10.3° (*c* 1.2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.84−7.70 (6H, m, H-y, H-c, H-e, H-h, and H-j), 7.53−7.48 (4H, m, H-v, H-f, and H-g), 7.40−7.24 (5H, m, H-b, H-w, and H-x), 5.24 (1H, d, *J* = 8.4 Hz, NH), 5.14 (1H, m, H-α), 4.52−4.36 (2H, m, Fmoc-C*H*$_2$), 4.21 (1H, t, *J* = 6.9 Hz, Fmoc-C*H*), 3.50 (1H, dd, *J* = 14.1 and 5.8 Hz, H_A-β), 3.44 (1H, dd, *J* = 14.1 and 6.5 Hz, H_B-β). ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.2 (C=O, amino acid), 155.7 (C=O, Fmoc), 143.8 and 143.7 (C-u), 141.5 (2C, C-z), 141.0 (2C, C-2′/C-6′, Pfp), 139.5 (C-4′, Pfp), 137.8 (2C, C-3′/C-5′, Pfp), 133.6, 132.8, 132.2 (C-a, C-d, and C-i), 128.9, 128.4, 128.0†, 127.9† (6C, C-c, C-e, C-h, C-j, and C-x,), 127.2 (C-w), 127.1 (C-b), 126.6 and 126.3 (C-f and C-g), 125.1 (2C, C-v), 124.8 (C-1′, Pfp), 120.1 (2C, C-y), 67.5 (Fmoc-*C*H$_2$), 54.8 (C-α), 47.2 (Fmoc-*C*H), 38.2 (C-β); (†) denotes two-carbon signals. HRMS: calcd for $C_{34}H_{22}F_5NO_4Na$ $[M + Na]^+$ 626.13612, found 626.13627.

D-*N*-[3-[[4-[(3-Aminopropyl)amino]butyl]amino]propyl]-α-[(1-oxobutyl)amino]-2-naphthalenepropanamide Tris(trifluoroacetate) (10). Using general procedure H, resin loaded with **3a** (250 mg, loading approximately 0.80 mmol/g, 0.20 mmol) was elongated successively with **10b** and pentafluorophenyl butyrate to give, after deprotection and cleavage, philanthotoxin **10** (71 mg, 44%) as a syrup. ^{1}H NMR (300 MHz, CD_3OD): δ 7.84−7.75 (3H, m, H-c, H-e, and H-h), 7.70

(1H, s, H-j), 7.49−7.36 (3H, m, H-b, H-f, and H-g), 4.59 (1H, dd, J = 8.4 and 7.0 Hz, H-α), 3.33−3.01 (10H, m, H-1, H-3, H-8, H-10, and H-β), 2.90−2.75 (4H, m, H-4 and H-7), 2.17 (2H, t, J = 7.4 Hz, H-2′), 2.09 (2H, m, H-9), 1.84−1.69 (6H, m, H-2, H-5, and H-6), 1.51 (2H, sx, J = 7.4 Hz, H-3′), 0.80 (3H, t, J = 7.4 Hz, H-4′). ^{13}C NMR (75 MHz, CD_3OD): δ 176.2 (C-1′), 174.8 (C═O, amino acid), 135.9, 134.9, 133.9 (C-a, C-d, and C-i), 129.2, 128.9, 128.7 and 128.6 (C-c, C-e, C-h, and C-j), 128.4 (C-b), 127.2 and 126.8 (C-f and C-g), 56.9 (C-α), 48.5 and 48.3 (C-4 and C-7), 46.4 (C-3), 46.1 (C-8), 39.1 (C-2′), 38.9 (C-10), 38.1 (C-1), 37.1 (C-β), 27.7 (C-2), 25.7 (C-9), 24.6 (2C, C-5 and C-6), 20.6 (C-3′), 14.2 (C-4′). HRMS: calcd for $C_{27}H_{44}N_5O_2$ [M + H]$^+$ 470.34895, found 470.34851.

Pentafluorophenyl DL-2-[(9*H*-Fluoren-9-ylmethoxycarbonyl)amino]-3,3-diphenylpropanoate (11c). The Fmoc group was attached to DL-β,β-diphenylalanine (**15a**, 500 mg, 2.07 mmol) using general procedure B, which afforded **11b** (937 mg, 98%) as a foam. TLC: R_f 0.28 (hexanes−EtOAc−AcOH 20:10:1). The latter compound (779 mg, 1.68 mmol) was esterified using general procedure C to give **11c** (855 mg, 81%). TLC: R_f 0.18 (hexanes−EtOAc 7:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.68 (2H, d, J = 7.2 Hz, H-y), 7.41 (2H, d, J = 7.2 Hz, H-v), 7.35−7.15 (14H, m, H-w, H-x, and 2 × Ph), 5.47 (1H, t, J = 8.5 Hz, NH), 5.10 (1H, d, J = 8.5 Hz, H-α), 4.53 (1H, d, J = 8.5 Hz, H-β), 4.52−4.36 (2H, m, Fmoc-C*H*$_2$), 4.21 (1H, t, J = 6.9 Hz, Fmoc-C*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.0 (C═O, amino acid), 155.7 (C═O, Fmoc), 143.7 (2C, C-u), 141.5 (2C, C-z), 141.0 (2C, C-2′/C-6′, Pfp), 139.5 (C-4′, Pfp), 139.3 and 138.8 (2C, C-a), 137.5 (2C, C-3′/C-5′, Pfp), 129.3 and 129.1 (each 4C, C-b and C-c), 128.6 and 128.2 (2C, C-x), 127.9 and 127.8 (2C, C-d), 127.2 (2C, C-w), 125.2 (2C, C-v), 125.1 (C-1′ Pfp), 120.1 (2C, C-y), 67.7 (Fmoc-*C*H$_2$), 57.2 (C-α), 53.2 (C-β), 47.1 (Fmoc-*C*H). HRMS: calcd for $C_{36}H_{25}F_5NO_4Na$ [M + Na]$^+$ 652.15177, found 652.15103.

DL-*N*-[3-[[4-[(3-Aminopropyl)amino]butyl]amino]propyl]-3,3-diphenyl-2-[(1-oxobutyl)amino]propanamide Tris(trifluoroacetate) (11). Using general procedure H, resin loaded with **3a** (250 mg, loading approximately 0.80 mmol/g, 0.20 mmol) was elongated successively with **11c** and with pentafluorophenyl butyrate to give, after deprotection and cleavage, philanthotoxin **11** (71 mg, 42%) as a syrup. ^{1}H NMR (300 MHz, CD_3OD): δ 7.39−7.12 (10H, m, Ph−H), 5.24 (1H, d, J = 12.0 Hz, H-α), 4.37 (1H, d, J = 12.0 Hz, H-β), 3.20−2.96 (8H, m, H-1, H-7, H-8, and H-10), 2.87−2.73 (2H, m, H-4), 2.90−2.75 (1H, m, H$_A$-3), 2.37−2.25 (1H, m, H$_B$-3), 2.10 (2H, p, J = 7.7 Hz, H-9), 2.01 (2H, t, J = 7.3 Hz, H-2′), 1.87−1.67 (4H, m, H-5 and H-6), 1.67−1.52 (2H, m, H-2), 1.45−132 (2H, sx, J = 7.3 Hz, H-3′), 0.70 (3H, t, J = 7.3 Hz, H-4′). ^{13}C NMR (75 MHz, CD_3OD): δ 175.3 (C-1′), 173.6 (C═O, amino acid), 142.3 and 141.7 (2C, C-a), 129.5, 129.3 and 129.0 (8C, C-b and C-c), 127.9 (2C, C-d), 57.6 (C-α), 54.5 (C-β), 48.2 and 47.1 (C-7 and C-4), 45.8 (2C, C-3 and C-8), 39.6 (C-2′), 37.8 (C-10), 36.5 (C-1), 27.2 (C-2), 25.4 (C-9), 24.3 (2 C, C-5 and C-6), 20.3 (C-3′), 13.8 (C-4′). HRMS: calcd for $C_{29}H_{46}N_5O_2$ [M + H]$^+$ 496.36460, found 496.36428.

Pentafluorophenyl L-2-(9*H*-Fluoren-9-ylmethoxycarbonyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (12). Compound **6a** (507 mg, 1.27 mmol) was esterified using general procedure C to give **6b** (544 mg, 76%) as a white foam. TLC: R_f 0.56 (hexanes−EtOAc 2:1). $[\alpha]_D^{25}$: 0° (c 0.68, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.77*, 7.76 (each 2H, d, J = 7.5 Hz, H-y), 7.62, 7.57* (each 2H, t, J = 7.8 Hz, H-v), 7.44−7.37 (2H, m, H-x), 7.34−7.12 (6H, m, H-w, H-b, H-c, H-d, and H-e), 5.42 (1H, dd, J = 5.8 and 3.6 Hz, H-α), 5.22* (1H, t, J = 4.9 Hz, H-α), 4.82−4.45 (4H, m, Fmoc-C*H*$_2$, H-γ), 4.35, 4.28* (each 1H, t, J = 6.8 Hz, Fmoc-C*H*), 3.43−3.29 (2H, m, H-β). ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.3 (C═O, amino acid), 143.8 (2C, C-u), 141.5 (2C, C-z), 133.0, 132.4, 131.2, 128.6, 128.1, 127.9, 127.7, 127.5, 127.3, 126.7, 126.5, 125.1 and 120.2 (C-v, C-w, C-x, C-y, C-b. C-c, C-d, C-e, mixture of rotamers), 68.5* and 68.3 (Fmoc-*C*H$_2$), 54.1* and 53.7 (C-α), 47.3 (Fmoc-*C*H), 44.8* and 44.7 (C-γ), 32.5* and 31.7 (C-β); (*) denotes signals arising from a minor amide bond rotamer. Anal. Calcd for $C_{31}H_{20}F_5NO_4$: C, 65.84; H, 3.56; F, 16.80; N, 2.51. Found: C, 65.91; H, 3.44; F, 16.93; N, 2.39.

L-*N*-[3-[[4-[(3-Aminopropyl)amino]butyl]amino]propyl]-2-(1-oxobutyl)isoquinoline-3-carboxylamide Tris(trifluoroacetate) (12). Pfp ester **12b** (346 mg, 0.435 mmol) and compound **3b** (199 mg, 0.386 mmol) were dissolved in dry CH_2Cl_2 (2.0 mL), Et_3N (55 μL, 0.40 mmol) was added, and the mixture was stirred for 2 h at room temperature, when it was diluted with CH_2Cl_2 (20 mL) and washed with water (2 × 15 mL). The organic phase was dried (Na_2SO_4), filtered, and concentrated. The crude product (0.398 mmol) was dissolved in dry THF (3.5 mL), DBU (79 μL, 0.079 mmol) and 1-octanethiol (10 equiv) were added, and then the mixture was stirred for 2 h at room temperature. The reaction mixture was concentrated, and the Fmoc-deprotected compound was isolated in quantitative yield by VLC. TLC: R_f 0.20 (CH_2Cl_2−MeOH−32% aqueous NH_3 200:10:1). The *N*-butyryl group was attached to the latter product (255 mg, 0.386 mmol) using general procedure F to yield the Boc-protected intermediate (267 mg, 95%). Deprotection of the latter (221 mg, 0.302 mmol) was performed according to general procedure G to give **12** (221 mg, 95%; overall yield: 90%) as a syrup. ^{1}H NMR (400 MHz, CD_3OD, T = 328 K): δ 7.29−7.18 (4H, m, 4 Ar−H), 4.56 (1H, t, J = 5.5 Hz, H-α), 4.72 (2H, br s, H-γ), 3.30−3.13 (6H, m, H-1, H-3, and H-8), 3.09 (4H, t, J = 7.5 Hz, H-4 and H-7), 2.88 (2H, t, J = 7.5 Hz, H-10), 2.75 (2H, m, H-β), 2.78−2.18 (2H, t, J = 7.4 Hz, H-2′), 2.27−2.04 (2H, p, J = 7.5 Hz, H-9), 1.88−1.63 (8H, m, H-2, H-5, H-6, and H-3′), 1.06−0.95 (3H, t, J = 7.4 Hz, H-4′). ^{13}C NMR (100 MHz, CD_3OD): δ 176.1 (C-1′), 175.4 (C═O, amino acid), 135.2−127.4 (6C, Ph-C, one major and several minor rotamers), 56.4 (C-α), 48.3 and 48.2 (C-4 and C-7), 47.4 (C-γ), 45.9 and 45.8 (C-3 and C-8), 37.8 (C-10), 36.7 (C-2′), 36.4 (C-1), 32.9 (C-β), 27.5 (C-2), 25.4 (C-9), 24.3 and 24.2 (C-5 and C-6), 19.3 (C-3′), 14.2 (C-4′); only signals for the major rotamer have been assigned. HRMS: calcd for $C_{23}H_{42}N_5O_2$ [M + H]$^+$ 432.33330, found 432.33308.

Pentafluorophenyl 2-[(9*H*-Fluoren-9-ylmethoxycarbonyl)amino]indan-2-carboxylate (13c). The Fmoc group was introduced to **13a** (hydrochloride; 255 mg, 1.19 mmol) using general procedure B to give **13b** (82 mg, 17%). TLC: R_f 0.09 (hexanes−EtOAc−AcOH 20:10:1). The carboxylic acid **13b** (131 mg, 0.33 mmol) was esterified using general procedure C to give **13c** (147 mg, 79%). TLC: R_f 0.64 (hexanes−EtOAc 2:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.74 (2H, br d, J = 7.4 Hz, H-y), 7.56 (2H, br d, J = 7.4 Hz, H-v), 7.38 (2H, br t, J = 7.4 Hz, H-x), 7.29 (2H, br t, J = 7.4 Hz, H-w), 7.26−7.24 (4H, m, H-b and H-c), 5.49 (1H, s, NH), 4.60 (2H, d, J = 6.3 Hz, Fmoc-C*H$_2$*) 4.21 (1H, t, J = 6.3 Hz, Fmoc-C*H*), 3.85 (2H, d, J = 16.5 Hz, H$_A$-β), 3.40 (2H, d, J = 16.5 Hz, H$_B$-β). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.1 (C═O, amino acid), 155.4 (C═O, Fmoc), 143.5 (2C, C-u), 141.2 (2C, C-z), 140.7 (2C, C-2′/C-6′, Pfp), 139.5 (C-4′, Pfp), 138.7 (2C, C-a), 137.8 (2C, C-3′/C-5′, Pfp), 127.7 and 127.0 (2C, C-x and C-w), 127.4 (2C, C-b), 124.9 (2C, C-v), 124.7 (2C, C-c), 124.5 (C-1′, Pfp), 120.0 (2C, C-y), 67.4 (Fmoc-*C*H$_2$), 66.3 (C-α), 47.3 (Fmoc-*C*H), 44.1 (2C, C-β). HRMS: calcd for $C_{31}H_{20}F_5NO_4Na$ [M + Na]$^+$ 588.12047, found 588.12006.

DL-*N*-[3-[[4-[(3-Aminopropyl)amino]butyl]amino]propyl]-2-[(1-oxobutyl)amino]indan-2-carboxylamide Tris(trifluoroacetate) (13). Pfp ester **13c** (100 mg, 0.177 mmol) and compound **3b** (89 mg, 0.177 mmol) were coupled using general procedure D to yield the conjugate (159 mg, 100%) as a foam. TLC: R_f 0.13 (hexanes−EtOAc 1:1). Fmoc deprotection of the conjugate (127 mg, 0.141 mmol) was performed using general procedure E to give the amine intermediate (92 mg, 96%) as an oil. TLC: R_f 0.21 (CH_2Cl_2−MeOH−32% aqueous NH_3 200:10:1). The *N*-butyryl group was introduced to the latter (76 mg, 0.112 mmol) according to general procedure F to give the Boc-protected product (62 mg, 44%) as an oil. TLC: R_f 0.25 (hexanes−EtOAc 1:4). Deprotection of the latter (36 mg, 0.048 mmol) as described in general procedure G yielded **13** (40 mg, 100%; overall yield: 71%) as a syrup. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.74 (2H, d, J = 7.3 Hz, H-y), 7.55 (2H, d, J = 7.3 Hz, H-v), 7.38 (2H, t, J = 7.3 Hz, H-x), 7.29 (2H, t, J = 7.3 Hz, H-w), 7.24−7.18 (4H, m, H-b and H-c), 5.36 (1H, s,

NH), 4.60 (2H, d, $J = 6.0$ Hz, Fmoc-CH_2), 4.20 (1H, t, $J = 6.0$ Hz, Fmoc-CH), 3.85 (2H, d, $J = 16.3$ Hz, H_A-β), 3.40 (2H, d, $J = 16.3$ Hz, H_B-β). ^{13}C NMR (75 MHz, $CDCl_3$): δ 177.3 (C-1′), 176.7 (C=O, amino acid), 142.2 (2C, C-a), 128.0 (2C, C-b), 125.6 (2C, C-c), 68.1 (C-α), 48.3 and 48.2 (C-4 and C-7), 46.0 and 45.8 (C-3 and C-8), 44.2 (2C, C-β), 38.6 (C-2′), 37.8 (C-10), 36.8 (C-1), 27.8 (C-2), 25.4 (C-9), 24.3 and 24.2 (C-5 and C-6), 20.1 (C-3′), 14.0 (C-4′). HRMS: calcd for $C_{24}H_{42}N_5O_2$ $[M + H]^+$ 432.33330, found 432.33318.

Pentafluorophenyl DL-2-[(9*H*-Fluoren-9-ylmethoxycarbonyl)amino]-1,2,3,4-tetrahydronaphthalene-2-carboxylate (14b). Compound **14a** (150 mg, 0.363 mmol) was esterified using general procedure C to give **14b** (202 mg, 96%) as a white foam. TLC: R_f 0.38 (hexanes−EtOAc 7:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.72 (2H, br d, $J = 7.5$ Hz, H-y), 7.52 (2H, br d, $J = 7.5$ Hz, H-v), 7.35 (2H, br t, $J = 7.5$ Hz, H-x), 7.24 (2H, br t, $J = 7.5$ Hz, H-w), 7.20−7.06 (4H, br m, side chain Ar−H), 5.49 (1H, s, NH), 4.45 (2H, br d, $J = 6.5$ Hz, Fmoc-CH_2), 4.21 (1H, t, $J = 6.5$ Hz, Fmoc-CH), 3.59 (1H, br d, $J = 17.0$ Hz, H_A-β'), 3.17 (1H, br d, $J = 17.0$ Hz, H_B-β'), 3.00−2.75 (2H, m, H-γ), 2.75−2.59 (1H, m, H_A-β), 2.36−2.20 (1H, m, H_B-β). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.5 (C=O, amino acid), 155.2 (C=O, Fmoc), 143.5 (2C, C-u), 141.3 (2C, C-z), 140.9 (2C, C-2′/C-6′, Pfp), 139.5 (C-4′, Pfp), 138.0 (2C, C-3′/C-5′, Pfp), 134.4 and 130.9 (C-a and C-f), 129.4 and 128.9 (C-b and C-e), 127.7 (2C, C-x), 127.0 (2C, C-w), 126.9 and 126.4 (4C, C-c and C-d), 124.9 (2C, C-v), 124.8 (C-1′, Pfp), 120.0 (2C, C-y), 67.1 (Fmoc-CH_2), 58.7 (C-α), 47.3 (Fmoc-CH), 38.1 (C-β'), 28.6 (C-β), 25.1 (C-γ). HRMS: calcd for $C_{32}H_{22}F_5NO_4Na$ $[M + Na]^+$ 602.13612, found 602.13541.

DL-*N*-[3-[[4-[(3-Aminopropyl)amino]butyl]amino]propyl]-2-[(1-oxobutyl)amino]-1,2,3,4-tetrahydronaphthalene-2-carboxylamide Tris(trifluoroacetate) (14). Pfp ester **14b** (175 mg, 0.302 mmol) and compound **3b** (153 mg, 0.302 mmol) were coupled using general procedure D to yield the conjugate (243 mg, 90%) as a foam. TLC: R_f 0.17 (hexanes−EtOAc 1:1). Fmoc deprotection of the conjugate (206 mg, 0.229 mmol) was performed as described in general procedure E to give the amine intermediate (150 mg, 97%) as an oil. TLC: R_f 0.70 (CH_2Cl_2−MeOH−32% aqueous NH_3 200:10:1). The *N*-butyryl group was introduced to the amine intermediate (109 mg, 0.161 mmol) according to general procedure F but with the reaction time prolonged to 7 h. An additional amount of pentafluorophenyl butyrate (45 mg, 0.177 mmol) in dry CH_2Cl_2 (0.5 mL) was added, and the mixture was stirred overnight. Dry THF (2 mL) was added, and the mixture was stirred overnight at 40 °C. DBU (5 μL, 0.032 mmol) was added, and then the mixture was stirred at 40 °C for 5 days, when the solvents were removed in vacuo. Purification of the residue by VLC gave unreacted starting material (18 mg, 16%) and the Boc-protected product (98 mg, 82%). TLC: R_f 0.21 (hexanes−EtOAc 1:4). Deprotection of the latter (70 mg, 0.094 mmol) as described in general procedure G yielded **14** (75 mg, 100%; overall yield: 72%) as a syrup. ^{1}H NMR (300 MHz, CD_3OD): δ 7.10−6.90 (4H, m, Ar−H), 3.32−3.26 (2H, m, H-1), 3.19 (1H, d, $J = 17.0$ Hz, H_A-β'), 3.11−2.94 (10H, m, H-3, H-4, H-7, H-8, and H-10), 2.92 (1H, d, $J = 17.0$ Hz, H_B-β'), 2.75 (2H, dd, $J = 8.1$ and 5.1 Hz, H-γ), 2.43−2.32 (1H, m, $J = 13.2$ and 5.1 Hz, H_A-β), 2.11 (2H, t, 7.3 Hz, H-2′), 2.08−1.95 (3H, m, H-9 and H_B-β), 1.86−1.70 (6H, m, H-2, H-5, and H-6), 1.50 (2H, sx, $J = 7.3$ Hz, H-3′), 0.82 (3H, t, $J = 7.3$ Hz, H-4′). ^{13}C NMR (75 MHz, CD_3OD): δ 177.8 (C-1′), 176.1 (C=O, amino acid), 135.8 and 133.4 (C-a and C-f), 130.1 and 129.1 (C-b and C-e), 127.0 and 126.9 (C-c and C-d), 124.8 (C-1′, Pfp), 59.6 (C-α), 48.3 and 48.1 (C-4 and C-7), 46.0 and 45.8 (C-3 and C-8), 38.8 (C-β'), 38.7 (C-2′), 37.8 (C-10), 36.8 (C-1), 29.4 (C-β), 27.8 (C-2), 26.2 (C-γ), 25.4 (C-9), 24.4 and 24.3 (C-5 and C-6), 20.4 (C-3′), 14.2 (C-4′). HRMS: calcd for $C_{25}H_{44}N_5O_2$ $[M + H]^+$ 446.34895, found 446.34849.

DL-Indoline-2-carboxylic Acid (15a). A solution of indole-2-carboxylic acid (1.00 g, 6.21 mmol) and concentrated H_2SO_4 (0.56 mL) in MeOH (34 mL) was heated under reflux for 6 h.[37] The reaction mixture was concentrated in vacuo, and the residue was dissolved in CH_2Cl_2 (200 mL) and washed with saturated aqueous $NaHCO_3$ (2 × 80 mL). The organic phase was dried (Na_2SO_4) and filtered, and the solvent was evaporated. Purification by VLC (petroleum ether to petroleum ether−EtOAc 4:1) yielded methyl indole-2-carboxylate (0.984 g, 91%) as a powder. TLC: R_f 0.28 (petroleum ether−EtOAc 4:1). ^{1}H NMR data: as earlier reported.[38] ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.8 (C=O), 137.1, 127.6, 127.2, 125.5, 122.7 and 120.9 (6C, Ar−C), 52.2 (OCH_3). The methyl ester (395 mg, 2.25 mmol) was suspended in MeOH (12 mL), Mg (225 mg, 9.0 mmol) was added, and the mixture was stirred under N_2 at room temperature overnight. The reaction mixture was poured into cold 2 M aqueous HCl (12 mL), and then the mixture was stirred until it became clear, when pH was adjusted to 8−9 with 2 M aqueous NH_3. MeOH was removed in vacuo, the residue was suspended in water (50 mL), and the solution was extracted with EtOAc (3 × 100 mL). The combined EtOAc phases were dried (Na_2SO_4) and filtered, and the solvent was evaporated. Purification by VLC (hexanes−EtOAc 20:1 to 10:1) afforded methyl indoline-2-carboxylate[39] (295 mg, 74%) as a yellowish solid. TLC: R_f 0.34 (hexanes−EtOAc 4:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.07 (2H, m, Ar−H), 6.70 (2H, m, Ar−H), 4.39 (1H, dd, $J = 11.0$ and 5.5 Hz, H-α), 4.74 (3H, s, CH_3O), 3.39 (1H, dd, $J = 18.0$ and 11.0 Hz, H-2), 3.31 (1H, dd, $J = 18.0$ and 5.5 Hz). The methyl ester (295 mg, 1.66 mmol) was suspended in water (12.5 mL), a solution of KOH (95 mg, 1 equiv) in MeOH (0.5 mL) was added, and the mixture was stirred at room temperature overnight. The reaction mixture was concentrated, and then the residue was suspended in water (50 mL), acidified with 4 M aqueous HCl, and extracted with EtOAc (3 × 50 mL). The combined EtOAc phases were dried (Na_2SO_4) and filtered, and then the solvent was removed to give crude **15a** (237 mg, 87%), which was used in the next step without further purification. TLC: R_f 0.36 (hexanes−EtOAc 4:1). NMR data: essentially as earlier reported.[40]

Pentafluorophenyl DL-1-(9*H*-Fluoren-9-ylmethoxycarbonyl)indoline-2-carboxylate (15c). Compound **15a** (233 mg, 1.43 mmol) was converted to the Fmoc derivative using general procedure B to give **15b** (422 mg, 77%) as a foam. TLC: R_f 0.18 (hexanes−EtOAc 2:1). Compound **15b** (407 mg, 1.06 mmol) was esterified according to general procedure C to give **15c** (542 mg, 93%) as a white foam. TLC: R_f 0.37 (hexanes−EtOAc 7:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.77 (2H, d, $J = 7.3$ Hz, H-y), 7.61 (2H, d, $J = 7.3$ Hz, H-v), 7.41 (2H, t, $J = 7.3$ Hz, H-x), 7.33−7.29 (2H, m, H-w), 7.29−6.80 (4H, m, indoline-ArH), 5.30−5.20 (1H, br m, H-α), 4.83 and 4.58 (each 1H, br m, Fmoc-CH_2), 4.40−4.28 (1H, m, Fmoc-CH), 3.88−3.68 (1H, br m, H-β'), 3.49−3.25 (1H, br m, H-β'). ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.7*, 167.6* (C=O, amino acid), 153.6*, 152.1* (C=O, Fmoc), 144.0−136.0 (Pfp *C*-F, C-u, C-z, C-a, C-f), 128.5−127.2 (6C, C-x, C-w, C-b, C-d), 125.0−123.3 (C-v, C-c, C-1′ Pfp), 120.2 (C-y), 115.1 and 114.9 (C-e), 68.4 (Fmoc-CH_2), 59.9* and 59.8* (C-α), 47.2 (Fmoc-CH), 33.3* and 32.2* (C-β); (*) denotes doubling of signals originating from two amide rotamers present. HRMS: calcd for $C_{30}H_{19}F_5NO_4$ $[M + H]^+$ 552.12288, found 552.12269.

DL-*N*-[3-[[4-[(3-Aminopropyl)amino]butyl]amino]propyl]-1-(1-oxobutyl)indoline-2-carboxylamide Tris(trifluoroacetate) (15). Pfp ester **15c** (199 mg, 0.360 mmol) and compound **3b** (181 mg, 0.360 mmol) were coupled according to general procedure D to give the conjugate (297 mg, 95%) as a foam. TLC: R_f 0.26 (hexanes−EtOAc 4:1). Fmoc deprotection of the conjugate (200 mg, 0.230 mmol) according to general procedure E afforded the amine intermediate (142 mg, 95%) as a syrup. TLC: R_f 0.38 (CH_2Cl_2−MeOH−32% aqueous NH_3 200:10:1). The amine intermediate (111 mg, 0.171 mmol) was dissolved in dry CH_2Cl_2 (1 mL) and a solution of pentafluorophenyl butyrate (48 mg, 0.188 mmol) in dry CH_2Cl_2 (1 mL) and Et_3N (24 μL, 0.171 mmol) were added. The mixture was stirred for 3 h at room temperature, when it was concentrated in vacuo. The residue was dissolved in dry CH_2Cl_2 (1 mL), additional amounts of pentafluorophenyl butyrate (48 mg, 0.188 mmol) and Et_3N (24 μL, 0.171 mmol) were added, and the mixture was stirred at room temperature overnight. Butyryl chloride (35.5 μL, 0.342 mmol) and Et_3N (120 μL, 0.855

mmol) were added, and the stirring was continued for 2 h at room temperature. The solvent was evaporated and the residue was purified by VLC to give the Boc-protected product (112 mg, 91%) as a syrup. TLC: R_f 0.28 (hexanes−EtOAc 1:2). Deprotection of the latter (71 mg, 0.10 mmol) was performed according to general procedure G to give **15** (64 mg, 85%; overall yield: 70%) as a syrup. ^{1}H NMR (400 MHz, CD_3OD, T = 328 K): δ 7.23−7.21 (3H, m, H-b, H-c, and H-d), 7.04 (1H, t, J = 7.9 Hz, H-e), 5.04 (1H, dd, J = 10.9 and 3.6 Hz, H-α), 3.32−3.31 (2H, m, H-1), 3.16−2.99 (12H, m, H-β, H-3, H-4, H-7, H-8, and H-10), 2.15−2.04 (2H, p, J = 7.4 Hz, H-2′), 1.90 (2H, p, J = 6.9 Hz, H-9), 1.85−1.67 (8H, m, H-2, H-5, H-6, and H-3′), 1.01 (3H, t, J = 7.4 Hz, H-4′). ^{13}C NMR (75 MHz, CD_3OD, T = 328 K): δ 174.2, (C=O, amino acid), 128.6 (Ar−C), 125.1 (Ar−C), 63.2 (C-α), 48.3 and 48.2 (C-4 and C-7), 46.4 (C-8), 45.9 (C-3), 37.9, 37.8, and 36.4 (C-2′, C-1, and C-10), 27.5 (C-2), 25.3 (C-9), 24.2 (2C, C-5 and C-6), 19.1 (C-3′), 14.0 (C-4′); several signals are missing due to overlapping and extensively broadened lines. HRMS: calcd for $C_{23}H_{40}N_5O_2$ [M + H]$^+$ 418.31765, found 418.31718.

L-*N*-[3-[[4-[(3-Aminopropyl)amino]butyl]amino]propyl]-α-[(1-oxobutyl)amino]-1*H*-indole-3-propanamide Tris(trifluoroacetate) (16). Compound **16a** (402 mg, 0.581 mmol) and compound **3b** (292 mg, 0.581 mmol) were coupled using general procedure D to give the conjugate (572 mg, 97%) as a foam. TLC: R_f 0.47 (hexanes−EtOAc 1:2). Fmoc deprotection of the conjugate (382 mg, 0.378 mmol) was performed using general procedure E to give the amine intermediate (279 mg, 94%) as an oil. TLC: R_f 0.11 (CH_2Cl_2−MeOH−32% aqueous NH_3 200:10:1). The *N*-butyryl group was introduced to the amine intermediate (382 mg, 0.378 mmol) according to general procedure F to give the Boc-protected product (194 mg, 70%). TLC: R_f 0.13 (hexanes−EtOAc 1:2). Deprotection as described in general procedure G yielded **16** (141 mg, 85%; overall yield: 54%). ^{1}H NMR (300 MHz, CD_3OD): δ 7.56 (1H, br d, J = 8.0 Hz, H-g), 7.33 (1H, br d, J = 8.0 Hz, H-d), 7.13 (1H, s, H-b), 7.09 and 7.01 (each 1H, dt, J = 2 × 8.0 and 1.1 Hz, H-e and H-f), 4.50 (1H, t, J = 7.6 Hz, H-α), 3.30−3.00 (10H, m, H-1, H-4, H-8, H-10, and H-β), 2.90−2.75 (4H, m, H-3 and H-7), 2.17 (2H, t, J = 7.4 Hz, H-2′), 2.09 (2H, p, H-9), 1.84−1.69 (6H, m, H-2, H-5, and H-6), 1.51 (2H, sx, J = 7.4 Hz, H-3′), 0.80 (3H, t, J = 7.4 Hz, H-4′). ^{13}C NMR (75 MHz, CD_3OD): δ 175.9 (C-1′), 175.3 (C=O, amino acid), 137.7 (C-c), 128.4 (C-h), 124.3 (C-b), 122.3 and 119.6 (C-e and C-f), 119.1 (C-g), 112.2 (C-d), 110.6 (C-a), 56.3 (C-α), 48.2 and 48.0 (C-4 and C-7), 45.8 (2C, C-3 and C-8), 38.6 (C-2′), 37.8 (C-10), 36.6 (C-1), 28.7 (C-β), 27.4 (C-2), 25.5 (C-9), 24.3 (2C, C-5 and C-6), 20.3 (C-3′), 14.0 (C-4′). HRMS: calcd for $C_{25}H_{43}N_6O_2$ [M + H]$^+$ 459.34420, found 459.34377.

Pentafluorophenyl 1*H*-Indole-2-carboxylate (17b). Indole-2-carboxylic acid (**17a**, 100 mg, 0.624 mmol) was esterified according to general procedure C to give **17b** (195 mg, 96%) as a white foam. TLC: R_f 0.61 (hexanes−EtOAc 5:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.74 (1H, s, NH), 7.50 (1H, d, J = 8.1 Hz, H-7), 7.30 (1H, s, H-3), 7.18 (2H, m, H-5 and H-6), 6.97 (1H, m, H-4). ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.5 (C=O), 141.0 (2C, C-2′/C-6′, Pfp), 139.3 (C-4′, Pfp), 137.8 (2C, C-3′/C-5′, Pfp), 138.1, 127.4, 127.1, 124.8, 123.3†, 121.8 (C-α, C-1′ pfp, C-a, C-b, C-c, C-d, and C-f), 112.8 and 112.2 (C-β and C-e); (†) denotes two-carbon signal. HRMS: calcd for $C_{15}H_6F_5NO_2$ [M + H]$^+$ 328.03915, found 328.03949.

***N*-[3-[[4-[(3-Aminopropyl)amino]butyl]amino]propyl]-α-[(1-oxobutyl)amino]-1*H*-indole-3-carboxylamide Tris(trifluoroacetate) (17).** Pfp ester **17b** (79 mg, 0.241 mol) and compound **3b** (121 mg, 0.241 mmol) were coupled using general procedure D to give the conjugate (124 mg, 79%) as a foam. TLC: R_f 0.25 (petroleum ether−EtOAc 4:1). Deprotection was performed according to general procedure G to give **17** (36 mg, 92%; overall yield: 72%) as a syrup. ^{1}H NMR (400 MHz, CD_3OD): δ 7.60 (1H, br d, J = 8.2 Hz, H-b), 7.45 (1H, br d, J = 8.2 Hz, H-e), 7.23 (1H, br t, J = 8.2 Hz, H-c), 7.09 (1H, s, H-β), 7.07 (1H, br t, 8.2 Hz, H-d), 3.53 (2H, t, J = 6.5 Hz, H-1), 3.15−3.03 (10H, m, H-3, H-4, H-7, H-8, and H-10), 2.11−2.00 (4H, m, H-2 and H-9), 1.85−1.80 (4H, m, H-5 and H-6). ^{13}C NMR (100 MHz, CD_3OD): δ 165.2 (C=O), 136.4, 131.6, and 129.0 (C-f, C-a, and C-α), 125.3, 122.8, and 121.3 (C-b, C-c, and C-d), 113.1 and 104.8 (C-β and C-e), 48.3 and 48.1 (C-4 and C-7), 46.4 and 45.8 (C-8 and C-3), 37.8 (C-10), 37.0 (C-1), 27.9 (C-2), 25.4 (C-9), 24.3 and 24.2 (C-5 and C-6). HRMS: calcd for $C_{19}H_{32}N_5O$ [M + H]$^+$ 346.26014, found 346.25988.

Pentafluorophenyl (2*R*,4*S*)-1-(9*H*-Fluoren-9-ylmethoxycarbonyl)-4-phenylmethyl-pyrrolidine-2-carboxylate (18b). Compound **18a** (200 mg, 0.45 mmol) was esterified using general procedure C to give **18b** (274 mg, 100%) as a white foam. TLC: R_f 0.39 (hexanes−EtOAc 4:1). $[\alpha]^{25}_D$: −42.6° (c 0.65, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.72 (2H, dd, J = 7.3 and 3.3 Hz, H-y), 7.56 (2H, m, H-v), 7.37−7.21 (9H, m, H-x, H-w, and Ph), 4.82 (1H, dt, J = 2 × 7.7 and 4.0 Hz, H-α), 4.60−4.48 (2H, m, PhC*H*$_2$−O), 4.46−4.34 (2H, m, Fmoc-C*H*$_2$), 4.33−4.19 (2H, m, Fmoc-C*H* and H-γ), 3.92 (1H, br d, J = 12.1 Hz, H_A-δ minor rotamer), 3.78−3.65 (2H, m, H_A-δ and H_B-δ major rotamer, and H_B-δ minor rotamer), 2.75−2.57 (1H, m, H_A-β), 2.39−2.20 (1H, m, H_B-β). ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.9* and 168.5* (C=O, amino acid), 154.9* and 154.2* (C=O, Fmoc), 144.0*, 143.7*, 143.6*, and 143.2* (2C, C-u), 141.2 and 141.1 (2C, C-z), 141.0 (2C, C-2′/C-6′, Pfp), 139.3 (C-4′, Pfp), 137.8 (2C, C-3′/C-5′, Pfp), 137.2* and 137.2* (C-a), 128.5, 128.0, 127.7, and 127.6 (6C, C-b, C-c, C-d, and C-x), 127.0 and 126.9 (2 × C-w), 125.1*, 125.0*, 124.9*, and 124.8* (3C, C-v, C-1′ Pfp), 120.0 (2C, C-y), 76.7* and 75.7* (CH-γ), 71.4 (Ph*C*H$_2$−O), 68.5* and 67.9* (Fmoc-*C*H$_2$), 57.9* and 57.7* (C-α), 52.2* and 51.9* (C-δ), 47.2 (Fmoc-*C*H), 37.5* and 36.0* (C-β); (*) denotes double signals originating from two amide rotamers present. HRMS: calcd for $C_{33}H_{25}F_5NO_5$ [M + H]$^+$ 610.16475, found 610.16451.

(2*R*,4*S*)-*N*-[3-[[4-[(3-Aminopropyl)amino]butyl]amino]-propyl]-4-phenylmethyl-1-(1-oxobutyl)pyrrolidine-2-carboxylamide Tris(trifluoroacetate) (18). Pfp ester **18b** (111 mg, 0.182 mmol) and compound **3b** (92 mg, 0.182 mmol) were dissolved in dry CH_2Cl_2 (1 mL), Et_3N (25 μL, 0.182 mmol) was added, and the mixture was stirred at room temperature for 1.5 h, when the solvent was removed in vacuo. The crude conjugate (0.182 mmol) was dissolved in dry THF (1.5 mL), and 1-octanethiol (320 μL, 1.82 mmol) and DBU (12 μL) were added. After stirring of the mixture for 3 h at room temperature, TLC showed incomplete conversion. The mixture was then concentrated and redissolved in THF (1.5 mL), additional amounts of 1-octanethiol (320 μL, 1.82 mmol) and DBU (18 μL) were added, and the mixture was stirred for 0.5 h at room temperature. Purification by VLC yielded the amine intermediate (113 mg, 98%) as a syrup. TLC: R_f 0.22 (CH_2Cl_2−MeOH−32% aqueous NH_3 400:20:1). The amine intermediate (103 mg, 0.162 mmol) was coupled with pentafluorophenyl butyrate according to general procedure F to yield the Boc-protected product (99 mg, 87%) as an oil. Deprotection of the latter (94 mg, 0.135 mmol) was performed as described in general procedure G to give **18** (103 mg, 94%; overall yield: 83%) as a syrup. ^{1}H NMR (300 MHz, CD_3OD): δ 7.26−7.13 (5H, Ph), 4.49 (1H, d, J = 12.0 Hz, PhC*H*$_2$−O), 4.43 (1H, d, J = 12.0 Hz, PhC*H*$_2$−O), 4.25 (1H, dd, J = 9.2 and 7.7 Hz, H-α), 4.22−4.17 (1H, m, H-γ), 3.67 (1H, br d, J = 11.5 Hz, H_A-δ), 3.07 (1H, dd, J = 11.5 and 3.7 Hz, H_B-δ), 3.41−3.30 (2H, m, H-1), 3.16−2.87 (10H, m, H-3, H-4, H-7, H-8, and H-10), 2.49−2.38 (2H, m, H_A-β), 2.38−2.22 (2H, m, H_B-β), 2.19 (2H, t, J = 7.3 Hz, H-2′), 2.04−1.90 (2H, m, H-9), 1.86−1.73 (2H, m, H-2), 1.73−1.64 (4H, m, H-5 and H-6), 1.51 (2H, sx, J = 7.3 Hz, H-3′), 0.86 (3H, t, J = 7.3 Hz, H-4′). ^{13}C NMR (100 MHz, CD_3OD): δ 175.8, 174.8 (C-1′ and C=O, amino acid), 139.5 (C-a), 129.5 and 128.7 (5C, C-b, C-c, and C-d), 78.7 (Ph*C*H$_2$−O), 71.9 (C-γ), 60.7 (C-α), 54.2 (C-δ), 48.2 and 48.1 (C-4 and C-7), 46.2 and 45.8 (C-3 and C-8), 37.8 (C-10), 37.3 (C-2′), 36.7 (C-1), 36.5 (C-β), 27.5 (C-2), 25.3 (C-9), 24.2 (2C, C-5 and C-6), 19.3 (C-3′), 14.1 (C-4′). HRMS: calcd for $C_{26}H_{46}N_5O_3$ [M + H]$^+$ 476.35958, found 476.35891.

Methyl (2*S*,4*R*)-4-Hydroxy-1-[(phenylmethoxy)carbonyl]pyrrolidine-2-carboxylate (19b). Compound **19a** (2.00 g, 7.54 mmol) and Et_3N (1.6 mL, 11.3 mmol) were dissolved in MeCN (19.0 mL), and MeI (0.9 mL, 15.08 mmol) in MeCN (4.0

mL) were added at 0 °C. The mixture was stirred at 55 °C overnight, when additional amounts of MeI (0.9 mL, 15.08 mmol) and Et_3N (3.3 mL, 22.62 mmol) were added, and then the mixture was stirred at 55 °C for 4 h. The reaction mixture was distributed between Et_2O (100 mL) and 0.1 M aqueous HCl (100 mL), and the aqueous phase was extracted with Et_2O (2 × 100 mL). The combined organic phases were washed with 0.1 M aqueous NaOH (100 mL), dried (Na_2SO_4), and filtered, and the solvent was removed to give crude **19b** (1.35 g, 64%), which was used without further purification. TLC: R_f 0.18 (hexanes−EtOAc 1:1). NMR data: as earlier reported.[41]

Pentafluorophenyl (2*S*,4*S*)-1-(9*H*-Fluoren-9-ylmethoxycarbonyl)-4-(2-naphthyloxy)pyrrolidine-2-carboxylate (19f). Alcohol **19b** (123 mg, 0.44 mmol), 2-naphthol (95 mg, 0.66 mmol), and Ph_3P (173 mg, 0.66 mol) were dissolved in dry THF (1.5 mL), and then a solution of DEAD (104 μL, 0.66 mmol) in dry THF (0.5 mL) was added dropwise. The mixture was stirred at room temperature under N_2 overnight, and then the solvent was removed in vacuo. Purification by VLC (hexanes to hexanes−acetone 5:1) afforded **19c** (133 mg, 74%) as a yellowish solid contaminated with a small amount of hydrazine-*N*,*N*′-dicarboxylic acid diethyl ester according to ^1H NMR. TLC: R_f 0.33 (petroleum ether−EtOAc 4:1). A solution of **19c** (552 mg, 1.36 mmol) in absolute EtOH (10 mL) and glacial HOAc (0.3 mL) was hydrogenated in the presence of 10% Pd/C (75 mg) with vigorous stirring at room temperature overnight. The reaction mixture was filtered through a bed of Celite and concentrated, and the residue was dissolved in CH_2Cl_2 (50 mL) and washed with 1 M aqueous NaOH (20 mL) and brine (20 mL). The organic phase was dried (Na_2SO_4), filtered, and concentrated and the residue subjected to purification by VLC (CH_2Cl_2−MeOH−32% aqueous NH_3, 400:4:1 to 400:10:1) to give **19d** (317 mg, 86%) as a syrup. TLC: R_f 0.29 (CH_2Cl_2−MeOH−32% aqueous NH_3 400:10:1). ^1H NMR (400 MHz, $CDCl_3$): δ 7.77−7.69 (3H, m, H-c, H-e and H-h), 7.41 and 7.35 (each 1H, br t, *J* = 8.2 Hz, H-f and H-g), 7.06−7.03, (2H, m, H-b, H-j), 4.96 (1H, m, H-α), 3.88 (1H, dd, *J* = 9.3 and 4.2 Hz, H-γ), 3.50 (3H, s, CH_3−O), 3.40 (1H, dt, *J* = 12.4 and 1.4 Hz, H_A-δ), 3.10 (1H, dd, *J* = 12.4 and 4.2 Hz, H_B-δ), 2.50 (1H, m, H_A-β), 2.40 (1H, m, H_B-β). ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.9 (C═O), 155.0 (C-a), 134.5 and 129.2 (C-d and C-i), 129.8 (C-c), 127.8, 126.9 and 126.5 (3C, C-e, C-g and C-h), 119.5 (C-b), 108.3 (C-j), 77.1 (C-γ), 59.5 (C-α), 53.0 and 52.4 (CH_3−O and C-δ), 36.8 (C-β).

Compound **19d** (197 mg, 0.726 mmol) was suspended in a mixture of MeCN (10 mL) and 10% aqueous Na_2CO_3 (15 mL), and the mixture was stirred overnight at room temperature, followed by stirring for 16 h at 40 °C. MeCN was removed in vacuo, and dioxane (20 mL) and Fmoc-Cl (188 mg, 0.726 mmol) in dioxane (3 mL) were added successively at 0 °C. The mixture was stirred at 0 °C for 1 h and was then poured into water (100 mL). The resulting mixture was washed with hexane (50 mL), and the aqueous phase was acidified with 4 M aqueous HCl and extracted with EtOAc (4 × 50 mL). The combined EtOAc phases were dried (Na_2SO_4), filtered, and concentrated. Purification by VLC (hexanes−EtOAc 2:1 to hexanes−EtOAc−HOAc 1000:1000:1) afforded **19e** (237 mg, 68%) as a syrup. TLC: R_f 0.20 (hexanes−EtOAc−HOAc 1000:1000:1). Compound **19e** (286 mg, 0.596 mmol) was esterified according to general procedure C to give **19f** (392 mg, 100%). TLC: R_f 0.40 (hexanes−EtOAc 2:1). $[\alpha]^{25}_D$: −52.2° (*c* 0.55, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 7.81−7.64 (5H, m, H-y and 3 × Ar−H, naphthyl), 7.58−7.50 (2H, m, H-v), 7.49−7.20 (5H, m, H-x, H-w, and Ar−H, naphthyl), 7.17−7.09 (1H, dt, *J* = 8.0 and 1.1 Hz, Ar−H, naphthyl), 7.08−6.98 (2H, m, H-b and H-j), 5.16 and 5.12* (1H, m, H-α), 4.97 and 4.87* (2H, dd, *J* = 9.5 and 1.8 Hz, H-γ), 4.59−4.49 and 4.42−4.31* (each 1H, m, Fmoc-C*H₂*), 4.29−4.19 (1H, m, Fmoc-C*H*), 4.00−3.80 (2H, m, H-δ), 2.92−2.79 (1H, m, H_A-β), 2.79−2.62 (1H, m, H_B-β). ^{13}C NMR (75 MHz, $CDCl_3$): 167.4* and 167.1 (C═O, amino acid), 154.5* and 154.1 (C═O, Fmoc), 154.2 (C-a), 144.1*, 143.8, 143.5, and 143.3* (2C, C-u), 141.3 and 141.1* (2C, C-z), 134.1, (2C, C-i), 141.0 (2C, C-2′/C-6′, Pfp), 139.3 (C-4′, Pfp), 137.8 (2C, C-3′/C-5′, Pfp), 130.0 and 129.9* (Ar−C, naphthyl), 129.4 and 129.3* (Ar−C, naphthyl), 127.8, 127.7, 127.1, 127.0, 126.7, 126.6, 126.5, 125.0, 124.9, 124.8, 124.2, and 124.1 (C-x, C-w, C-v, 8 × Ar−C naphthyl, C-1′ Pfp), 120.0 (2C, C-y), 119.3 and 119.2* (C-b), 108.1 and 108.0* (C-j), 75.4 and 74.4* (C-γ), 68.3 and 68.0* (Fmoc-*C*H₂), 58.0 and 57.8* (C-α), 52.6* and 52.1 (C-δ), 47.4* and 47.3 (Fmoc-*C*H), 37.1* and 35.9 (C-β); (*) denotes additional signals originating from a minor amide rotamer. HRMS: calcd for $C_{36}H_{25}F_5NO_5$ $[M + H]^+$ 646.16474, found 646.16439.

(2*S*,4*S*)-*N*-[3-[[4-[(3-Aminopropyl)amino]butyl]amino]propyl]-4-(2-naphthyloxy)-1-(1-oxobutyl)pyrrolidine-2-carboxylamide Tris(trifluoroacetate) (19). Using general procedure H, resin loaded with **3a** (250 mg, loading approximately 0.80 mmol/g, 0.20 mmol) was elongated successively with **19f** and pentafluorophenyl butyrate to give, after deprotection and cleavage, **19** (89 mg, 52%) as a syrup. ^1H NMR (300 MHz, CD_3OD): δ 7.76 (3H, m, H-c, H-e and H-h), 7.43 (1H, t, *J* = 7.4 Hz, H-g), 7.33 (1H, t, *J* = 7.4 Hz, H-f), 7.22 (1H, s, H-j), 7.04 (1H, dd, *J* = 8.8 and 2.2 Hz, H-b), 5.22 (1H, m, H-γ), 4.56 (1H, d, *J* = 8.0 Hz, H-α), 4.08−3.82 (2H, m, H-δ), 3.46−3.24 (2H, m, H-1), 3.15−2.80 (10H, m, H-3, H-4, H-7, H-8 and H-10), 2.70−2.56 (1H, m, H_A-β), 2.51−2.40 (3H, m, H-2′ and H_B-β), 2.08 (2H, p, *J* = 7.4 Hz, H-9), 1.98−1.82 (2H, m, H-2), 1.84−1.69 (6H, m, H-5, H-6 and H-3′), 1.00 (3H, t, *J* = 7.2 Hz, H-4′). ^{13}C NMR (75 MHz, CD_3OD): δ 175.3 and 175.2 (C-1′ and C═O, amino acid), 155.5 (C-a), 135.6 (C-i), 130.6, 128.4, and 127.7 (3C, C-c, C-e and C-h), 130.4 (C-d), 127.4 (C-g), 124.9 (C-f), 119.8 (C-b), 109.2 (C-j), 77.1 (C-γ), 60.8 (C-α), 54.2 (C-δ), 48.2 and 48.1 (C-4 and C-7), 46.1 (C-3), 45.8 (C-8), 37.8 (C-10), 37.3 (C-2′), 36.8 (C-1), 35.8 (C-β), 27.6 (C-2), 25.4 (C-9), 24.3 (2C, C-5 and C-6), 18.9 (C-3′), 14.3 (C-4′). HRMS: calcd for $C_{29}H_{46}N_5O_3$ $[M + H]^+$ 512.35952, found 512.35990.

Pentafluorophenyl DL-1-(9*H*-Fluoren-9-ylmethoxycarbonyl)-4-phenylpiperidine-4-carboxylate (20c). Compound **20a** (*p*-toluenesulfonate; 500 mg, 1.33 mmol) was Fmoc protected using general procedure A to give **20b** (517 mg, 91%) as a white solid. The latter compound (510 mg, 1.19 mmol) was esterified according to general procedure C to give **20c** (613 mg, 87%) as a white foam. ^1H NMR (300 MHz, $CDCl_3$): δ 7.73 (2H, d, *J* = 7.4 Hz, H-y), 7.56 (2H, br d, *J* = 7.4 Hz, H-v), 7.43−7.25 (9H, m, 4 × Ar−H, and Ph), 4.49 (2H, br d, *J* = 6.5 Hz, Fmoc-C*H₂*), 4.23 (1H, t, *J* = 6.5 Hz, Fmoc-C*H*), 4.17 (1H, br d, *J* = 11.6 Hz, H_{eq}-α), 3.96 (1H, br d, *J* = 11.6 Hz, H_{eq}-α′), 3.08 (2H, br t, *J* = 11.6 Hz, H_{ax}-α and H_{ax}-α′), 2.60 (2H, m, H_{eq}-β and H_{eq}-β′), 1.99 (1H, m, H_{ax}-β), 1.83 (1H, m, H_{ax}-β′). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.4 (C═O, amino acid), 155.0 (C═O, Fmoc), 143.8 (2C, C-u), 141.3 (2C, C-z), 141.0 (2C, C-2′/C-6′, Pfp), 140.1 (C-a), 139.3 (C-4′, Pfp), 137.8 (2C, C-3′/C-5′, Pfp), 129.0 and 125.7 (2C, C-b and C-c), 128.1 (C-d), 127.7 (2C, C-x), 127.0 (2C, C-w), 124.9 (2C, C-v), 124.8 (C-1′, Pfp), 120.0 (2C, C-y), 67.3 (Fmoc-*C*H₂), 50.4 (C-γ), 47.7 (Fmoc-*C*H), 41.7 (2C, C-α/C-α′), 33.8/33.5 (C-β/C-β′). Anal. Calcd for $C_{33}H_{24}F_5NO_4$: C, 66.78; H, 4.08; F, 16.00; N, 2.36. Found: C, 66.57; H, 3.82; F, 16.13; N, 2.28.

DL-*N*-[3-[[4-[(3-Aminopropyl)amino]butyl]amino]propyl]-1-(1-oxobutyl)-4-phenyl-piperidine-4-carboxylamide Tris(trifluoroacetate) (20). Pfp ester **20c** (400 mg, 0.674 mmol) and compound **3b** (339 mg, 0.674 mmol) were coupled using general procedure D to yield the conjugate (546 mg, 89%) as a foam. TLC: R_f 0.17 (hexanes−EtOAc 1:1). Fmoc deprotection of the conjugate (403 mg, 0.442 mmol) was performed using general procedure E to give the amine intermediate (294 mg, 96%) as a syrup. TLC: R_f 0.23 (CH_2Cl_2−MeOH−32% aqueous NH_3 200:20:1). The *N*-butyryl group was introduced to the latter (217 mg, 0.315 mmol) using general procedure F to give the Boc-protected product (208 mg, 87%) as a syrup. TLC: R_f 0.20 (hexanes−EtOAc; 1:2). Deprotection of the Boc-protected product (158 mg, 0.208 mmol) was performed according to general procedure G to give **20** (164 mg, 98%; overall yield: 80%) as a syrup. ^1H NMR (300 MHz, CD_3OD): δ 7.46−7.32 (4H, m, H-b and H-c), 7.30−7.22 (1H, m, H-d), 4.08 (1H, dt, *J* = 14.0 and 4.1 Hz, H_{eq}-α), 3.76 (1H, dt, *J* = 14.0 and 4.1 Hz, H_{eq}-α′), 3.44 (1H, m, H_{ax}-α′), 3.20 (1H,

m, H_{ax}-α), 3.32−3.02 (8H, m, H-1, H-4, H-8, and H-10), 2.90 (2H, t, J = 7.0 Hz, H-7), 2.72 (2H, t, J = 7.3 Hz, H-3), 2.61−2.43 (2H, m, H_{eq}-β and H_{eq}-β'), 2.39 (2H, t, J = 7.4 Hz, H-2′), 2.10 (2H, p, J = 7.4 Hz, H-9), 2.07−1.92 (2H, m, H_{ax}-β and H_{ax}-β'), 1.86−1.68 (6H, m, H-2, H-5 and H-6), 1.62 (2H, sx, J = 7.4 Hz, H-3′), 0.97 (3H, t, J = 7.4 Hz, H-4′). ^{13}C NMR (75 MHz, CD_3OD): δ 177.1 (C-1′), 173.5 (C=O, amino acid), 143.5 (C-d), 129.7 (C-f), 128.2 (C-b), 126.7 (C-a), 50.3 (C-γ), 48.2 (C-4), 48.1 (C-7), 46.2 (C-3), 45.8 (C-8), 44.6 and 40.4 (C-α and C-α′), 37.8 (C-10), 37.1 (C-1), 36.0 (C-2′), 35.2 and 34.1 (C-β and C-β'), 27.5 (C-2), 25.4 (C-9), 24.3 (2C, C-5 and C-6), 20.0 (C-3′), 14.3 (C-4′). HRMS: calcd for $C_{26}H_{46}N_5O_2$ [M + H]$^+$ 460.36460, found 460.36413.

Electrophysiology. AMPAR Assay. A two-electrode voltage clamp (TEVC) was used to record responses to 100 μM kainite of *X. laevis* oocytes expressing AMPAR. *X. laevis* oocytes were injected with rat brain RNA and incubated at 18 °C for at least 3 days.[3] Single oocytes were transferred to a perfusion bath and continuously washed with saline containing 120 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, and 5 mM HEPES (pH adjusted 7.5 with NaOH). Microelectrodes were pulled from borosilicate glass capillaries (GC150TF-10, Clark Electromedical Instruments) using a Sutter P-97 programmable puller and had resistances of ~0.5 MΩ when filled with 3.0 M KCl. The oocytes were voltage clamped at −80 mV using an Axoclamp (Axon Instruments), and output currents were digitized with Sony PCM and recorded to videotape with a Sony VCR. Responses of AMPAR were elicited by perfusion of 100 μM kainic acid for 120 s. Philanthotoxins (**2** and **4**−**20**) were coapplied from 40 to 80 s of this kainic acid application.

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