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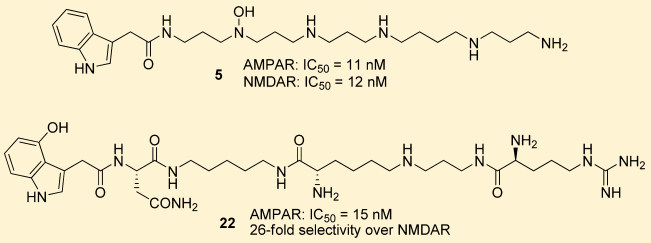
Structure–Activity Relationship Studies of *N*-Methylated and *N*-Hydroxylated Spider Polyamine Toxins as Inhibitors of Ionotropic Glutamate Receptors

Niels G. Nørager,[†] Mette H. Poulsen, Anna G. Jensen, Nanna S. Jeppesen, Anders S. Kristensen, and Kristian Strømgaard*

Department of Drug Design and Pharmacology, University of Copenhagen, Universitetsparken 2, DK-2100 Copenhagen, Denmark

Supporting Information

ABSTRACT: Polyamine toxins from spiders and wasps are potent open-channel blockers of ionotropic glutamate (iGlu) receptors. It is well-established that secondary amino groups in the polyamine moiety of these toxins are key to both selectivity and potency at iGlu receptors, still some native spider polyamine toxins comprise both *N*-methyl and *N*-hydroxy functionalities. Here, we investigate the effect of both *N*-methylation and *N*-hydroxylation of spider polyamine toxins by the synthesis and biological evaluation of the naturally occurring *N*-methylated argiopinines and pseudoargiopinines I and II, *N*-hydroxylated Agel-489 and Agel-505, as well as *N*-methylated analogues of the NMDA and AMPA iGlu receptor subtype selective antagonists ArgTX-93 and ArgTX-48. Efficient synthetic strategies for the synthesis of target compounds were developed, and evaluation of biological activity at AMPA and NMDA receptors identified highly potent and in some cases very selective ligands.



INTRODUCTION

The ionotropic glutamate (iGlu) receptors are a family of ligand-gated cation channels that mediate the majority of excitatory synaptic transmission in the vertebrate brain. The family comprises three subfamilies, the *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and kainate receptors.[1] These receptors are considered as promising drug targets, as they play a crucial role in normal brain function and abnormal activity is believed to play a critical role in a range of neurological and psychiatric disorders.[1,2]

Polyamine toxins are a group of small molecules isolated from spiders and wasps, which have been shown to be use- and voltage-dependent open-channel blockers of iGlu receptors.[3,4] They have been found to be valuable pharmacological tools for studying the iGlu receptors due to their high potency and selectivity.[3−5] Most importantly, polyamine toxins are unique tools to distinguish among AMPA and KA receptor subtypes on the basis of their Ca^{2+}-permeability, as polyamine toxins only block Ca^{2+}-permeable iGlu receptors, which is related to the nature of the amino acid in the so-called Q/R-site.[6]

A large number of structure–activity relationship (SAR) studies of polyamine toxins and their iGlu receptor inhibition have been conducted[3,4,7] which have focused on the functional roles of the four general components that comprise the polyamine toxin scaffold (Figure 1). Modifications of head-groups, amino acid linkers, and tails have been performed, while the polyamine chain has been modified in its length and the number and positioning of secondary amines. However, only very limited investigation into the effect of *N*-functionalization of the secondary amino groups in the polyamine has been conducted despite SAR studies having shown the importance of these groups for both selectivity and potency.[8,9]

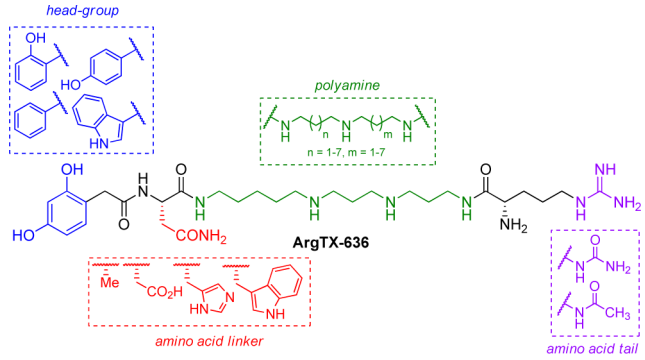


Figure 1. Overview of modifications made to argiotoxin-636 from the orb-weaver spider, *Argiope lobata*, in recent SAR studies at iGlu receptors.

Two classes of *N*-functionalized naturally occurring polyamine toxins have been identified containing *N*-methylation or -hydroxylation of the polyamine chain. Notable examples of these compounds are the family of *N*-methylated argiopinines (**1** and **2**) and pseudoargiopinines (**3** and **4**) isolated from the *Argiope lobata* spider[10] and the *N*-hydroxylated α agatoxins

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from the *Agelenopsis aperta* spider (**5** and **6**, Figure 2).[11,12] To investigate the influence of the *N*-functionalization on iGlu

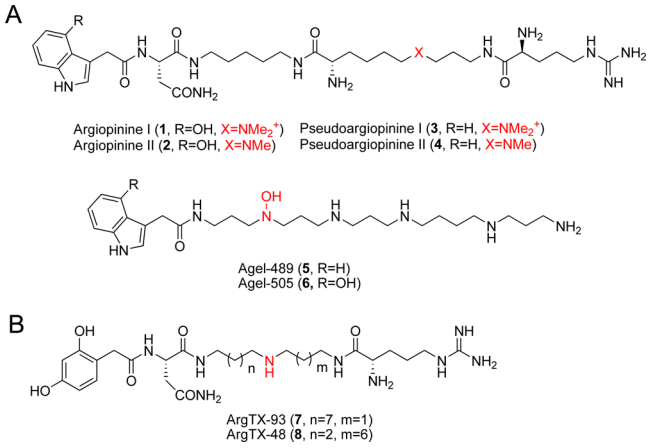


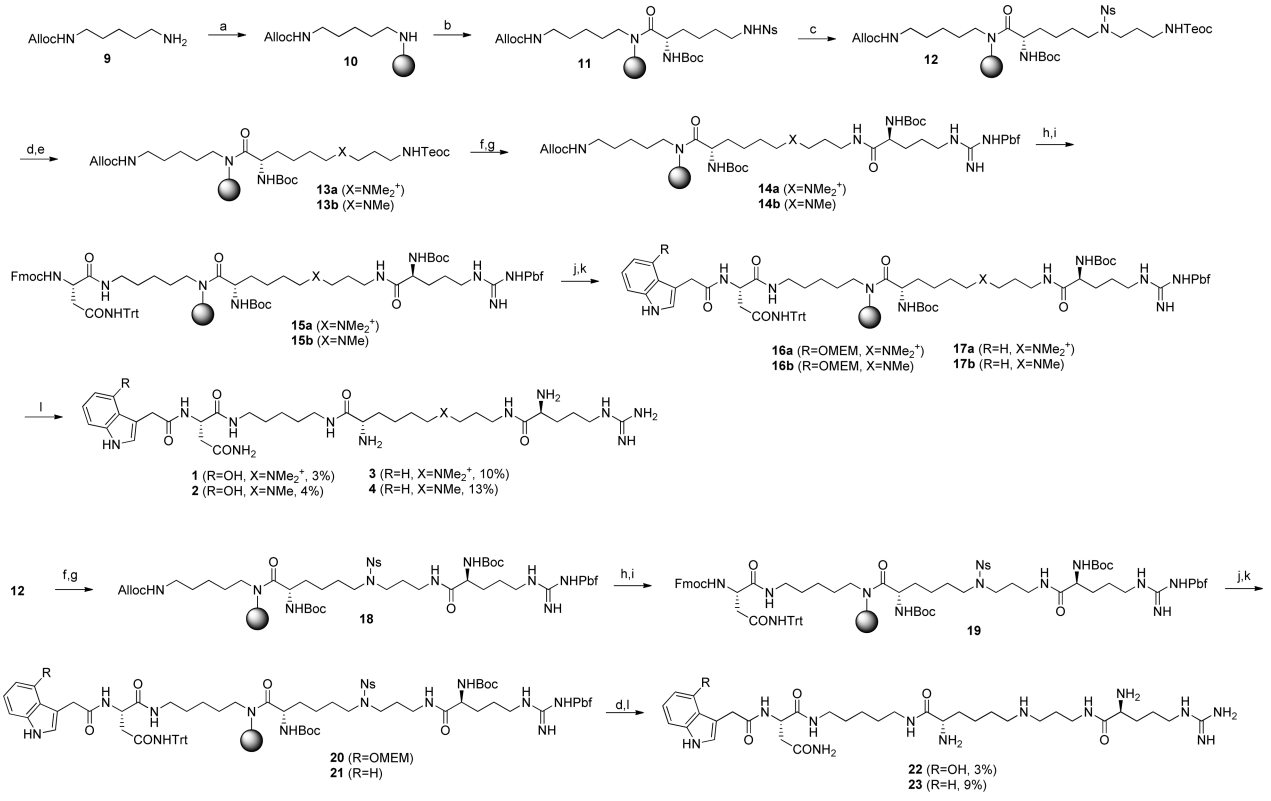
Figure 2. (A) Structures of natural spider polyamine toxins containing *N*-hydroxyl and *N*-methyl functionalities in the polyamine moiety. (B) Argiotoxin derivatives, **7** and **8**, which have shown to be potent and selective inhibitors of NMDA and AMPA receptors, respectively.

receptor inhibition, we designed, synthesized, and evaluated these naturally occurring polyamine toxins as well as their nonfunctionalized analogues. Furthermore, we synthesized *N*-methylated analogues of two polyamine toxin-based NMDA and AMPA selective iGlu receptor antagonists, ArgTX-93 (**7**) and ArgTX-48 (**8**).[9] A recent study has suggested that an intramolecular hydrogen bond between the polyamine chain and the headgroup carbonyl is the cause of the selectivity difference between **7** and **8**,[8] thus we envisioned that blocking this hydrogen bond through *N*-methylation could provide useful SAR information toward elucidating the active conformation of this class of compounds.

RESULTS AND DISCUSSION

Design and Synthesis. Currently, no synthetic procedures for preparation of compounds **1**–**4** have been developed. These compounds are highly polar and contain several nucleophilic moieties requiring extensive use of protection groups, thus we envisioned that a solid-phase organic synthesis (SPOS) approach would be advantageous. The synthesis was attempted using a backbone amide linker (BAL),[13] which has previously been extensively used in the SPOS of polyamine toxins.[8,14−16] A mono *N*-allyloxycarbonyl (*N*-Alloc) protected 1,5-diaminopentane (**9**) was loaded onto a polystyrene resin through reductive amination of a BAL handle (Scheme 1). Boc-L-Lys(Ns)-OH was subsequently coupled with 2-(1*H*-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) and diisopropylethyl amine (DIPEA) to the resin-bound secondary amine (**10**), which provided resin-

Scheme 1. Synthesis of 1−4 and Nonmethylated Analogues 22 and 23[a]



[a]Reagents and conditions: (a) BAL polystyrene resin (loading: 0.87 mmol/g), $NaBH(OAc)_3$, DMF/AcOH(9:1), rt; (b) Boc-L-Lys(Ns)-OH, HATU, DIPEA, DCM/DMF (9:1), rt; (c) *N*-Teoc 3-amino-1-propanol, Bu_3P, ADDP, DCM/THF (1:1), rt; (d) DBU, 2-mercaptoethanol, DMF, rt; (e) MeI, TEA, DMF, rt (compound **13a**) or CH_2O, TEA, $NaBH(OAc)_3$, DMF, rt (compound **13b**); (f) TBAF, THF, 55 °C; (g) Boc-L-Arg(Pbf)-OH, HATU, DIPEA, DCM/DMF (9:1), rt; (h) borane dimethylamine, $Pd(PPh_3)_4$, DCM, rt; (i) Fmoc-L-Asn(Tr)-OH, HATU, DIPEA, DCM/DMF (9:1), rt; (j) piperidine, DMF, rt; (k) 2-(4-((2-methoxyethoxy)methoxy)-1*H*-indol-3-yl)acetic acid (compounds **16a**−**b**) or 2-(1*H*-indol-3-yl)acetic acid (compounds **17a**−**b**), HATU, DIPEA, DCM/DMF (9:1), rt; (l) TFA/DCM/EDT/H_2O (75:20:2.5:2.5), rt.

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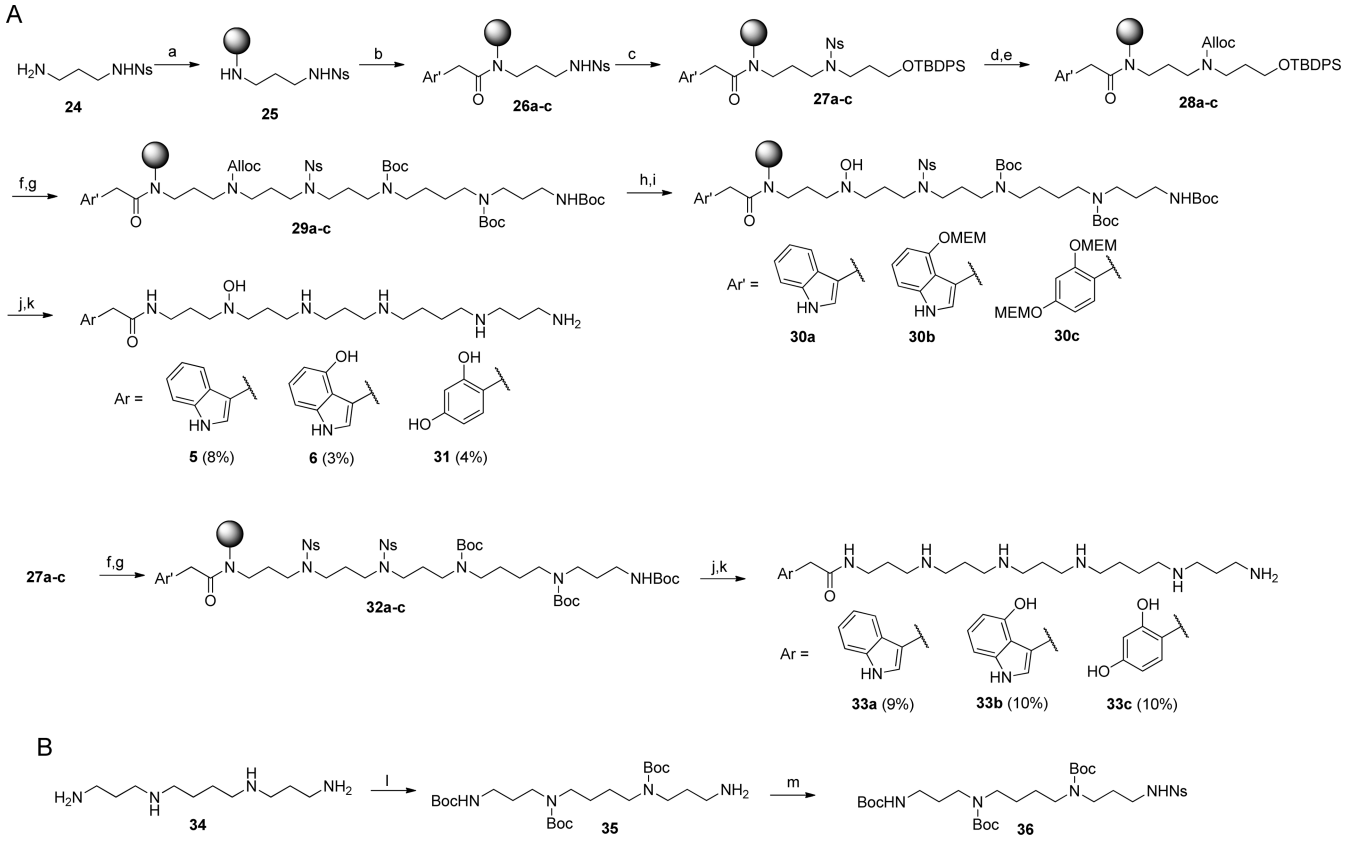
Scheme 2. Synthesis of *N*-Hydroxylated Polyamine Toxins 5, 6, and 31 and Nonhydroxylated Analogues 33a–c[a]



[a]Reagents and conditions: (a) BAL polystyrene resin (loading: 0.87 mmol/g), $NaBH(OAc)_3$, DMF/AcOH(9:1), rt; (b) 2-(1*H*-indol-3-yl)acetic acid (compound **26a**), 2-(4-((2-methoxyethoxy)methoxy)-1*H*-indol-3-yl)acetic acid (compound **26b**), or 2-(2,4-bis((2-methoxyethoxy)methoxy)-phenyl)acetic acid (compound **26c**), HATU, DIPEA, DCM/DMF (9:1), rt; (c) *O*-TBDPS 1,3-propanediol, Me_3P, ADDP, DCM/THF (1:1), rt; (d) DBU, 2-mercaptoethanol, DMF, rt; (e) allyl chloroformate, collidine, DCM, rt; (f) TBAF, THF, 55 °C; (g) **36**, Bu_3P, ADDP, DCM/THF (1:1), rt; (h) borane dimethylamine, $Pd(PPh_3)_4$, DCM, rt; (i) Davis' oxaziridine, DCM, rt; (j) DBU, 2-mercaptoethanol, DMF, rt; (k) TFA/DCM/EDT/H_2O (75:20:2.5:2.5), rt; (l) ethyl trifluoroacetate, MeOH, −78 °C → 0 °C, then $(Boc)_2O$, MeOH 0 °C → rt; then concd aq NH_3 to pH > 11 (55%); (m) 4-nitrobenzenesulfonyl chloride, TEA, DCM, 0 °C → rt (80%).

bound intermediate **11** that was further elongated through a Fukuyama–Mitsunobu reaction with *N*-2-(trimethylsilyl)-ethyloxycarbonyl (*N*-Teoc) protected 1-amino-3-propanol and using 1,1-(azodicarbonyl)dipiperidine (ADDP) and Bu_3P as redox pair.[17] This yielded the key intermediate **12** (Scheme 1), where the polyamine chain had been assembled with orthogonal protection groups that allows independent chain elongation in three directions. The synthesis proceeded with deprotection of the *N*-Ns group followed by either mono-methylation in a one-pot reductive amination procedure with methanol, triethylamine (TEA), and sodium triacetoxyborohydride or dimethylations with iodomethane and TEA to provide intermediates **13a–b** (Scheme 1). The *N*-Teoc protection group of these intermediates was removed with tetra-*n*-butylammonium fluoride (TBAF), and Boc-L-Arg(Pbf)-OH was coupled to the resulting primary amine. Palladium catalyzed deprotection of the *N*-Alloc group, followed by coupling with Fmoc-L-Asn(Tr)-OH, yielded resin-bound **15a–b**, which were subsequently coupled to either *O*-2-methoxyethoxymethyl (*O*-MEM) protected 4-hydroxyindolyl (**16a–b**) or indolyl (**17a–b**) acetic acids (Scheme 1). Final cleavage of the compounds from the resin and concomitant removal of the acid labile protection groups provided the desired compounds **1–4** (Scheme 1, 3–13% yield, 12 steps, 75–84% average yields per step). The yields of compounds **1** and **2** were lower, as side product formation was observed during the resin cleavage and deprotection step, likely due to alkylations by electrophiles formed during the removal of the *O*-MEM group. In an attempt to limit these side reactions addition of four different scavengers during cleavage from the resin were examined: triisopropylsilane (TIPS), 1,2-ethanedithiol (EDT), thioanisole, and thiophenol, with EDT resulting in formation of less side products. The corresponding nonalkylated analogues (**22** and **23**) were synthesized following a similar approach but omitting the above-described *N*-methylation step (Scheme 1, 3–9% yield, 11 steps, 71–80% average yields per step).

The naturally occurring *N*-hydroxylated polyamine toxins **5** and **6** have previously been synthesized in solution.[18,19] However, to circumvent the tedious purification steps and enable parallel synthesis of the target compounds and their analogues, we developed a solid-phase procedure (Scheme 2). The synthesis commenced with loading of mono *N*-Ns protected 1,3-diaminopropane (**24**) onto a polystyrene resin through reductive amination of a BAL handle (Scheme 2A). Three phenyl and indolyl acetyl headgroups were subsequently coupled to the resin-bound secondary amine (**25**), providing intermediates **26a–c**, which were reacted with mono *O*-*tert*-butyldiphenylsilyl (*O*-TBPDS) protected 1,3-propanediol in a Fukuyama–Mitsunobu reaction (Scheme 2). Use of the less hindered PMe_3 phosphine source was found to be advanta-

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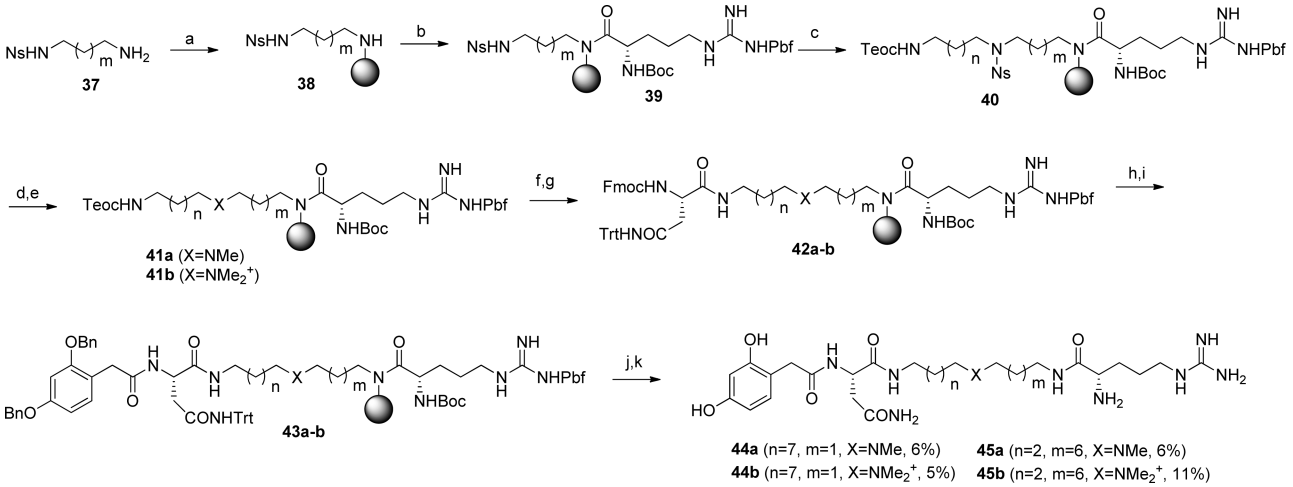
Scheme 3. Synthesis of *N*-Methylated Analogues of 7 and 8[a]



[a]Reagents and conditions: (a) BAL polystyrene resin (loading: 0.87 mmol/g), $NaBH(OAc)_3$, DMF/AcOH(9:1), rt; (b) Boc-L-Arg(Pbf)-OH, HATU, DIPEA, DCM/DMF (9:1), rt; (c) *N*-Teoc 9-amino-1-nonanol or *N*-Teoc 4-amino-1-butanol, Bu_3P, ADDP, DCM/THF (1:1), rt; (d) DBU, 2-mercaptoethanol, DMF, rt; (e) CH_2O, TEA, $NaBH(OAc)_3$, DMF, rt (compound **41a**) or MeI, TEA, DMF, rt (compound **41b**); (f) TBAF, THF, 55 °C; (g) Fmoc-L-Asn(Tr)-OH, HATU, DIPEA, DCM/DMF (9:1), rt; (h) piperidine, DMF, rt; (i) 2-(2,4-bis(benzyloxy)phenyl)acetic acid, HATU, DIPEA, DCM/DMF (9:1), rt; (j) TFA/CH_2Cl_2/TIPS/H_2O (75:20:2.5:2.5), rt; (k) H_2, $Pd(OH)_2$/C, AcOH, rt.

geous compared to PBu_3, which is generally used in the Tsunoda protocol, as previously reported when employing diols in the Fukuyama–Mitsunobu reaction.[20] Protection group substitution of **27a–c**, through sequential *N*-Ns removal and *N*-Alloc protection yielding intermediates **28a–c**, was conducted, as it was envisioned that the *N*-Alloc protection group could be orthogonally removed prior to an *N*-hydroxylation step, thus ensuring regioselectivity at this secondary amino group. The remaining part of the polyamine chain was synthesized through deprotection of the *O*-TBDPS group with TBAF, followed by an "inverse" Fukuyama–Mitsunobu reaction with an appropriately protected spermine analogue (**36**),[20] which was readily synthesized from commercially available spermine (Scheme 2B).[21] In the subsequent key steps, *N*-Alloc deprotection of resin-bound **29a–c** was followed by selective oxidation of the free secondary amine to provide intermediates **30a–c** (Scheme 2A). The oxidants *m*-chloroperoxybenzoic acid (*m*CPBA), hydrogen peroxide, and Davis' oxaziridine were examined for *N*-hydroxylation, and it was found that the latter reagent provided the best result in terms of yield and purity.[22] Repetitive treatments with Davis' oxaziridine were required to drive the reaction to completion, and only minor side product formation, such as overoxidation to the nitrone as previously reported when oxidizing secondary amines with this reagent, was observed.[18,23] Furthermore, NMR analysis showed no sign of indole oxidation. Finally, *N*-Ns deprotection, followed by cleavage from the resin and concomitant removal of the remaining acid labile protection groups, provided the desired *N*-hydroxylated compounds **5**, **6**, and **31** (Scheme 2A, 3–8%, 11 steps, 74–78% average yields per step). In comparison to previously published synthetic procedures,[18,19] lower absolute yields were obtained. However, the present procedure required fewer synthetic steps and circumvented cumbersome purifications, thus resulting in an efficient procedure suitable for SAR studies of this class of compounds. The corresponding nonhydroxylated analogues were synthesized using the same procedure but omitting the protection group substitution and oxidation steps, yielding **33a–b** (Scheme 2A, 9–10%, 7 steps, 71–72% average yields per step).

Finally, mono- and di-*N*-methylated analogues of **7** and **8**, respectively, were synthesized following a revised version of a previously reported solid-phase synthesis procedure (Scheme 3).[14] The initial loading of a *N*-Ns protected diamine onto the resin, coupling of Boc-L-Arg(Pbf)-OH to the primary amine, and Fukuyama–Mitsunobu elongation with an *N*-Teoc protected amino alcohol, yielding intermediate **40**, were performed as described previously.[14] At this point, the *N*-Ns protecting group was selectively removed and subsequent *N*-methylation of the resulting secondary amine provided intermediates **41a–b** (Scheme 3). Subsequent *N*-Teoc deprotection and coupling with Fmoc-L-Asn(Tr)-OH were performed following standard procedures, and the desired products **44a–b** and **45a–b** were isolated after resin cleavage and concomitant protection group removal (Scheme 3, 5–11% yield, 11 steps, 76–82% average yields per step).

Pharmacology. To address the biological activity at iGlu receptors of the compounds prepared, their inhibitory potencies were evaluated at NMDA (GluN1/2A) and AMPA ($GluA1_i$) receptors recombinantly expressed in *Xenopus laevis* oocytes, using two-electrode voltage-clamp electrophysiology. Compound potencies were assessed by measuring their degree of inhibition of agonist-evoked currents at a concentration of 100 nM (Figure 3), and for selected compounds full concentration–inhibition relationships were determined at these receptor subtypes at a membrane potential of −80 mV (Table 1).

Compounds **1–4** were previously investigated at native iGlu receptors from rat pyramidal neurons, which suggested that these compounds are inhibitors of AMPA and kainate receptors.[24] Here we observed that all compounds **1–4** showed >40% inhibition of the AMPA receptor at a concentration of 100 nM (Figure 3A) and a propensity toward the mono-*N*-methylated analogues (**2** and **4**) being more potent than their di-*N*-methylated counterparts (**1** and **3**). The nonmethylated analogues **22** and **23** showed inhibition of AMPA receptors in the same range (84% and 81% inhibition, respectively). All six compounds showed significantly lower inhibition at the NMDA receptor (<20%), and as observed for the AMPA receptor, *N*-

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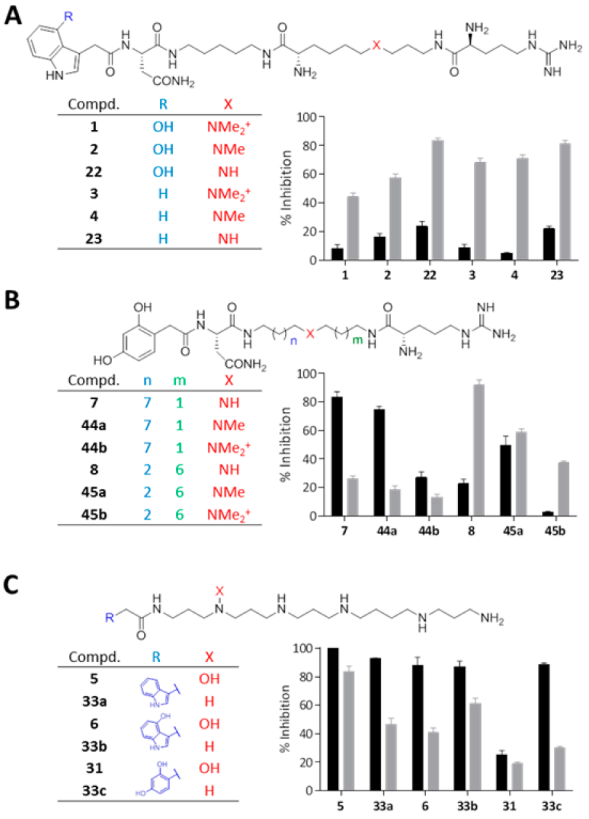

Figure 3. Screening of % inhibition of agonist-evoked currents (L-glutamate (100 μM) and glycine (100 μM) for GluN1/2A and L-glutamate (300 μM) for GluA1$_i$) by 100 nM *N*-functionalized polyamine toxins at NMDA (black bars) and AMPA (gray bars) receptors expressed in oocytes and held at a membrane potential of −80 mV.

Table 1. IC_{50} Values of Selected Compounds

	IC_{50} (nM)[a]	
compd	GluN1/2A[b]	GluA1$_i$[c]
1	3852 [3120−4755]	123 [103−146]
2	1634 [1276−2094]	52 [43−63]
22	387 [225−665]	15 [11−19]
5	12 [7−20]	11 [7−16]

[a]Mean IC_{50} values (95% confidence interval in brackets) determined from 4−18 different oocytes at a membrane potential of −80 mV. [b]Inhibition of the current elicited by 100 μM L-glutamate and 100 μM glycine by simultaneous coapplication of the antagonist at oocytes injected with a 1:10 ratio of GluN1/N2A. [c]Inhibition of the current elicited by 300 μM L-glutamate by simultaneous coapplication of the antagonist at oocytes injected with GluA1$_i$.

methylation led to a lower degree of inhibition. We then performed full concentration−inhibition correlations for compounds **1**, **2**, and **22** at AMPA and NMDA receptors (Figure 4), which allow a precise measure of the effect of different degrees of *N*-methylation on inhibitory potency. In conformity with the single concentration determination, we observed that increasing *N*-methylation led to decreased potency at both AMPA and NMDA receptors (Table 1), with a strong preference for AMPA receptors. In fact, compound **22** is a selective (26-fold vs NMDA receptors) and very potent (IC_{50} = 15 nM) inhibitor of AMPA receptors, while **1** and **2** show similar selectivity but lower receptor inhibition (Table 1).

Similarly, the four *N*-methylated analogues of **7** and **8** were examined for their inhibitory potency at AMPA and NMDA receptors (Figure 3B). Initially, the native compounds **7** and **8** were confirmed as potent and selective inhibitors of the NMDA and AMPA receptors, respectively, as demonstrated recently.[9] In a previous study combing synthesis, biological testing, and molecular modeling, we suggested such differences in AMPA versus NMDA receptor activity is caused by different active compound conformations due to intramolecular hydrogen bonds between the secondary amines in the polyamine chain and the carbonyl groups in the headgroups.[8] Examination of the inhibitory potencies of *N*-methylated analogues **44a−b** and **45a−b** furthermore support this hypothesis for selectivity. Specifically, di-*N*-methylation of the secondary amine in the NMDA receptor selective **7**, compound **44b**, which removes hydrogen bond donor capacity, led to remarkable reduction in inhibition at the NMDA receptor, while mono-*N*-methylation, as in **44a**, preserves hydrogen bond donor capacity, although adding steric bulk around the secondary amine, did not change the inhibition significantly compared to **7** (Figure 3B). A similar, but less pronounced, tendency was observed for mono- and di-*N*-methylation of the AMPA receptor selective **8**, as di-*N*-methylation (**45b**) led to a relatively larger reduction in inhibition of the AMPA receptor compared to the corresponding mono-*N*-methylated analogue (**45a**) (Figure 3B). Collectively, this indicates that hydrogen bond donation by the secondary amines is central for determining activity of **7** and **8** at AMPA and NMDA receptors. The observed reduced degree of inhibition upon *N*-methylation could in principle also be caused by unfavorable steric clash from the introduced methyl groups, however, this was not observed for the mono-*N*-methylated analogues.

Finally, the *N*-hydroxylated compounds **5−6** and **31** and their nonhydroxylated analogues (**33a−c**) were tested for activity at AMPA and NMDA receptors (Figure 3C). The naturally occurring *N*-hydroxylated polyamine toxins **5** and **6** were highly potent inhibitors of the NMDA receptors with >90% inhibition of agonist-induced currents from a 100 nM concentration, as previously suggested.[25−27] Interestingly, the indole headgroups seemed to be critical for these effects, as substitution of the native headgroup with a phenyl acetyl group (**31**), as found for example in argiotoxin-636, reduced inhibition dramatically. In contrast, replacement of the *N*-hydroxylate group by a secondary amine did not affect NMDA receptor inhibition, and both *N*-hydroxylated compounds **5** and **6** as well as their nonhydroxylated analogues **33a−b** showed inhibition >90% (Figure 3C). Finally, we observed that removal of the *N*-hydroxyl group in **31**, yielding **33c**, led to a significant increase in NMDA receptor inhibition. For all six compounds, the degree of inhibition at AMPA receptors was reduced compared to their NMDA receptor activity and no distinct correlation between the presence of the *N*-hydroxylation and the degree of inhibition was observed (Figure 3C). Finally, IC_{50} values were obtained from full concentration−inhibition curves for **5** at AMPA and NMDA receptors and verified that **5** is indeed a very potent, but also nonselective, inhibitor of iGlu receptors, with IC_{50} values of 12 and 11 nM at AMPA and NMDA receptors, respectively (Table 1).

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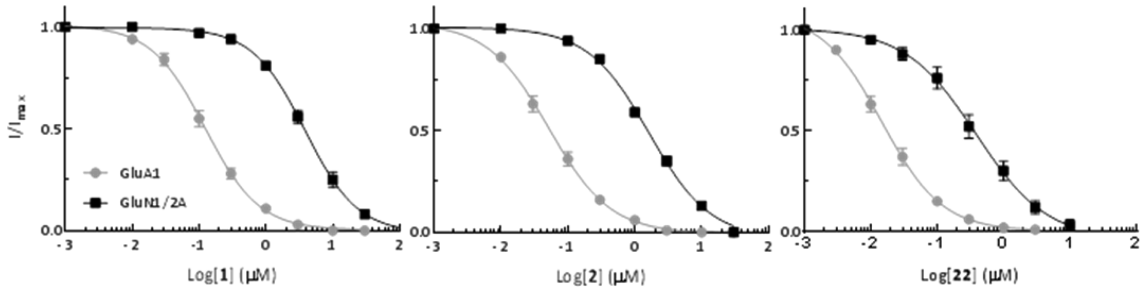

Figure 4. Determination of compound inhibitory potency (IC_{50}) at GluA1 and GluN1/2A receptors. Composite concentration−inhibition curves for inhibition by **1** (left), **2** (middle), and **22** (right) at AMPA (gray circles) and NMDA (black squares) receptors at a membrane potential of −80 mV. Data points represent the means ± SEM of experiments with >4 oocytes per curve.

CONCLUSION

In summary, we have developed solid-phase synthetic methodologies for selective *N*-methylation and *N*-hydroxylation of secondary amines in the polyamine moiety of polyamine toxins. We have then exploited these methodologies for the synthesis of three groups of polyamine toxins: the naturally occurring *N*-methylated **1**−**4**, *N*-hydroxylated **5** and **6**, *N*-methylated analogues of **7** and **8**, as well as their corresponding nonfunctionalized analogues. These compounds were used to investigate the influence of *N*-methylation and *N*-hydroxylations in the polyamine part of polyamine toxins for inhibition of iGlu receptor subtypes.

Examination of the **1**−**4** resulted in the discovery of a new group of potent and selective AMPA receptor antagonists. We found that *N*-methylations of polyamine toxins generally did not affect selectivity between AMPA and NMDA receptors while the inhibitory potency was gradually reduced upon *N*-methylation. In particular, di-*N*-methylation of **7** and **8** resulted in loss of inhibitory potency, indicating that the hydrogen bond donating property of these secondary amines is important for iGlu receptor inhibition of this class of compounds. Finally, *N*-hydroxylated α agatoxins **5** and **6** were found to be highly potent NMDA receptor antagonists, with **5** also having high potency for AMPA receptor antagonism. Furthermore, their nonhydroxylated analogues (**33a**−**b**) showed comparable inhibition of NMDA receptors, thus indicating that the *N*-hydroxylation is not crucial to iGlu receptor inhibition, which would simplify future SAR studies of this class of polyamine toxins.

EXPERIMENTAL SECTION

Chemistry: General Procedures. Unless otherwise stated, starting materials were obtained from commercial suppliers and were used without further purification. The BAL-resin used was 2-(3,5-dimethoxy-4-formylphenoxy)ethyl polystyrene, with a loading of 0.87 mmol/g, which was purchased from Novabiochem. 2-(Trimethylsilyl)-ethyl 3-hydroxypropylcarbamate,[28] 2-(trimethylsilyl)ethyl (4-hydroxybutyl)carbamate,[29] 2-(trimethylsilyl)ethyl (9-hydroxynonyl)-carbamate,[14] *N*-(3-aminopropyl)-2-nitrobenzenesulfonamide,[30] *N*-(8-aminooctyl)-2-nitrobenzenesulfonamide,[14] 3-((*tert*-butyldiphenylsilyl)-oxy)propan-1-ol,[31] 2-(2,4-bis(benzyloxy)phenyl)acetic acid,[32] and allyl (5-aminopentyl)carbamate[33] were prepared according to literature procedures. THF, DMF, and DCM were dried, degassed, and scrubbed using a Glass Contour solvent purification system immediately before use. Preparative HPLC was performed on Agilent 1100 system using a C18 reverse phase column (Zorbax 300 SB-C18, 21.2 mm × 250 mm) with a linear gradient of the binary solvent system of water/acetonitrile/formic acid (A, 95/5/0.1%; and B, 5/95/0.1%) with a flow rate of 20 mL/min and UV detection at 215 and 254 nm. ^{1}H NMR and ^{13}C NMR spectra were recorded on an Avance 400 spectrometer (at 400 or 100 MHz, respectively) or an Avance 600 spectrometer (at 600 or 150 MHz, respectively), using $CDCl_3$, CD_3OD, or DMSO-d_6 as solvent. Chemical shifts are reported in ppm (δ) using residual solvent as an internal standard; $CDCl_3$, 7.26 (^{1}H), 77.16 (^{13}C) ppm; CD_3OD. 3.31, 49.00 ppm; DMSO-d_6, 2.50, 39.52 ppm. Coupling constants (J) are given in Hz. The purity of the compounds was determined by LC-MS. LC-MS analysis was performed on an Agilent 6410 triple quadrupole mass spectrometer instrument using electron spray coupled to an Agilent 1200 HPLC system (ESI-LC/MS) with autosampler and diode array detector using a gradient (0%−100% B over 3 min, then 100% B for 3 min) of the binary solvent system of water/acetonitrile/TFA (A, 95/5/0.1%, and B, 5/95/0.1%) with a flow rate of 1 mL/min. During ESI-LC/MS analysis, evaporative light scattering (ELS) traces were obtained with a Sedere Sedex 85 light scattering ELS detector (ELSD), which was used for estimation of the purity of the final products. Analyses at intermediate stages of the synthesis were performed by cleaving samples from the resin as follows: a 1 mL screwcap vial was charged with a 1 mg sample of the resin and 0.4 mL TFA/DCM/EDT (75:20:5). The vial was agitated periodically over 1 h and filtered using a syringe filter and disposable syringe. The crude product was analyzed by LC-MS. High resolution mass spectra (HRMS) were obtained using a Micromass Q-Tof II instrument.

Mono-*N*-methylation: General Procedure. The resin was swelled in DMF (4 mL) for 30 min. The solvent was drained, 37% formaldehyde in water (0.12 mL, 1.5 mmol), triethylamine (0.11 mL, 0.75 mmol), and sodium triacetoxyborohydride (0.16 g, 0.75 mmol) were added, and the mixture was agitated for 2 h at rt. The solvent was drained, and the resin was washed with DMF, DCM, MeOH, and DCM (3 × 4 mL each) and dried in vacuo.

Di-*N*-methylation: General Procedure. The resin was swelled in DMF (4 mL) for 30 min. The solvent was drained, methyl iodide (0.19 mL, 3.0 mmol) and triethylamine (0.22 mL, 1.50 mmol) were added, and the mixture was agitated for 2 h at rt. The solvent was drained, and the resin was washed with DMF, DCM, MeOH, and DCM (3 × 4 mL each) and dried in vacuo.

***N*-Hydroxylation: General Procedure.** The resin was swelled in dry DCM (4 mL) for 30 min. The resin was drained, and a solution of (+)-(8,8-dichlorocamphorylsulfonyl)oxaziridine (0.22 g, 0.75 mmol) in dry DCM (4 mL) was added. The mixture was agitated for 3 h at rt, drained, washed with DMF, DCM, MeOH, and DCM (3 × 4 mL each), and the resin dried in vacuo. The procedure was repeated three times.

Preparation of 1−4. The BAL resin (0.18 g, 0.15 mmol) was swelled in DMF (4 mL) for 1 h. The resin was drained, and a solution of allyl (5-aminopentyl)carbamate (**9**, 0.28 g, 1.50 mmol) in dry DMF/glacial acetic acid (9:1, 4 mL) was added. After agitation for 15 min, sodium triacetoxyborohydride (0.32 g, 1.5 mmol) was added. The reaction mixture was agitated overnight at rt. The solvent was drained, and the resin was washed with DMF, DIPEA (10% in DMF), DMF, DCM, MeOH, and DCM (3 × 4 mL each) and dried in vacuo. The resin (**10**) was swelled in DCM (4 mL) for 30 min and then drained. A solution of Boc-L-Lys(Ns)-OH (0.32 g, 0.75 mmol) and HATU (0.28 g, 0.75 mmol) in DCM/DMF (9:1, 4 mL) was added, followed

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by DIPEA (0.27 mL, 1.5 mmol). The reaction mixture was agitated overnight at rt. The resin was drained, washed with DMF, DCM, MeOH, and DCM (3 × 4 mL each), and dried in vacuo. The resin (**11**) was swelled in dry THF/DCM (1:1, 2 mL) for 30 min. The mixture was flushed with nitrogen and kept under an atmosphere of nitrogen throughout the reaction. A solution of 2-(trimethylsilyl)ethyl 3-hydroxypropylcarbamate (0.17 g, 0.75 mmol) in THF/DCM (1:1, 1 mL) was added, followed by tributylphosphine (0.19 mL, 0.75 mmol). After agitation for 15 min, a solution of ADDP (0.19 g, 0.75 mmol) in THF/DCM (1:1, 1 mL) was added. The mixture was agitated for 3 h, then drained, washed with DMF, DCM, MeOH, and DCM, and dried in vacuo. The procedure was repeated twice. The resin (**12**) was swelled in DMF (4 mL) for 30 min. After draining, solutions of DBU (0.09 mL, 0.60 mmol) in DMF (2 mL) and 2-mercaptoethanol (0.04 mL) in DMF (2 mL) were added. The mixture was agitated for 30 min, drained, and washed with DMF (3 × 4 mL). The procedure was repeated until the solution was colorless (three times). The resin was washed with DMF, DCM, MeOH, and DCM (3 × 4 mL each) and dried in vacuo. The resin was subsequently methylated using the two general procedures. The resin (**13**) was swelled in dry THF (4 mL) for 30 min and heated to 55 °C. A 1.0 M solution of TBAF in THF (0.75 mL, 0.75 mmol) was added slowly, and the mixture was agitated for 30 min at 55 °C. The mixture was cooled to rt, drained, and washed with THF (3 × 4 mL), and the procedure was repeated once. The resin was washed with DMF, DCM, MeOH, and DCM (3 × 4 mL each) and dried in vacuo. Boc-L-Arg(Pbf)-OH (0.40 g, 0.75 mmol) was coupled to the resin using the same procedure as for **10**. The resin (**14**) was swelled in dry DCM (2 mL). The mixture was flushed with nitrogen and kept under an atmosphere of nitrogen throughout the reaction. A solution of borane dimethylamine complex (0.35 g, 6.0 mmol) in dry DCM (1 mL) was added, and after 5 min of agitation a solution of tetrakis(triphenylphosphine)palladium(0) (0.017 g, 0.015 mmol) in dry DCM (1 mL) was added. The mixture was agitated at rt for 2 h, drained, washed with sodium diethyldithiocarbamate trihydrate (0.02 M in DMF), DMF, DCM, MeOH, and DCM (3 × 4 mL each), and the resin dried in vacuo. Fmoc-L-Asn(Tr)-OH (0.45 g, 0.75 mmol) was coupled to the resin using the same procedure as for **10**. The resin (**15**) was then swelled in DMF (4 mL) for 30 min, drained, and a solution of piperidine (20% in DMF, 4 mL) was added. After agitation for 20 min, the resin was drained and washed with DMF (3 × 4 mL). The treatment was repeated once. The resin was washed with DMF, DCM, MeOH, and DCM (3 × 4 mL each) and dried in vacuo. The resin was swelled in DCM (4 mL) for 30 min and then drained. 2-(1*H*-Indol-3-yl)acetic acid (0.13 g, 0.75 mmol) or 2-(4-((2-methoxyethoxy)methoxy)-1*H*-indol-3-yl)acetic acid (**54**, 0.21 g, 0.75 mmol) were coupled to the resin using the same procedure as for **10**. The resin (**16** and **17**) was swelled in DCM (4 mL) for 30 min and then drained. A solution of TFA/DCM/EDT/H_2O (75:20:2.5:2.5, 4 mL) was added. The mixture was agitated for 3 h, drained, and the solution collected. The resin was washed with DCM (2 mL) and MeOH (2 mL), and the washings were collected. The solutions were combined and evaporated by a stream of nitrogen. The residue was purified by preparative HPLC, yielding compounds **1**–**4**.

*(S)-5-Amino-6-((5-((S)-4-amino-2-(2-(4-hydroxy-1H-indol-3-yl)-acetamido)-4-oxobutanamido)pentyl)amino)-N-(3-((S)-2-amino-5-guanidinopentanamido)propyl)-N,N-dimethyl-6-oxohexan-1-aminium Tetrakis(2,2,2-trifluoroacetate) (**1**).* Prepared with **54** and dimethylation. Yield: 8.0 mg, 4.4% (77% per step over 12 steps). ^{1}H NMR (600 MHz, CD_3OD) δ 7.07 (s, 1H), 6.98–6.93 (m, 2H), 6.45 (d, J = 7.0 Hz, 1H), 4.67 (t, J = 6.2 Hz, 1H), 3.94–3.80 (m, 3H), 3.76 (t, J = 6.6 Hz, 1H), 3.49–3.43 (m, 1H), 3.32–3.21 (m, 7H), 3.16–3.07 (m, 4H), 3.04 (m, 6H), 2.75–2.69 (m, 2H), 2.02–1.78 (m, 8H), 1.74–1.67 (m, 2H), 1.48–1.30 (m, 6H), 1.20–1.10 (m, 2H). ^{13}C NMR (150 MHz, CD_3OD) δ 174.8, 173.6, 171.8, 169.0, 168.3, 157.3, 150.5, 139.2, 122.8, 122.5, 116.7, 107.3, 103.8, 52.8, 50.6, 49.7, 40.3, 38.7, 36.2, 36.0, 34.0, 30.7, 28.3, 28.2, 28.0, 24.1, 23.2, 22.4, 21.8, 21.5. HRMS (EI) exact mass calculated for $C_{36}H_{63}N_{12}O_6^+$ [M^+] 759.4994; found 759.4999. Purity (ELSD): 98%.

*(S)-2-(2-(4-Hydroxy-1H-indol-3-yl)acetamido)-N^1-((6S,17S)-1,6,17-triamino-1-imino-12-methyl-7,18-dioxo-2,8,12,19-tetraaza-tetracosan-24-yl)succinamide Tetrakis(2,2,2-trifluoroacetate) (**2**).* Prepared with **54** and monomethylation. Yield: 5.8 mg, 3.2% (85% per step over 12 steps). ^{1}H NMR (600 MHz, CD_3OD) δ 7.07 (s, 1H), 6.97–6.92 (m, 2H), 6.45 (d, J = 7.0 Hz, 1H), 4.68 (t, J = 6.4 Hz, 1H), 3.92–3.79 (m, 3H), 3.74 (t, J = 6.6 Hz, 1H), 3.46–3.41 (m, 1H), 3.28–2.99 (m, 11H), 2.91–2.83 (m, 2H), 2.82 (s, 3H), 2.75–2.67 (m, 2H), 2.00–1.65 (m, 10H), 1.57–1.29 (m, 6H), 1.20–1.12 (m, 2H). ^{13}C NMR (150 MHz, CD_3OD) δ 174.8, 173.6, 171.8, 168.3, 161.8, 161.6, 157.3, 150.5, 139.2, 122.8, 122.5, 116.7, 107.3, 103.8, 53.8, 52.8, 50.6, 40.3, 38.7, 36.0, 34.0, 30.6, 28.3, 28.0, 24.1, 24.0, 23.4, 23.2, 21.6. HRMS (EI) exact mass calculated for $C_{36}H_{60}N_{12}O_6$ [MH^+] 745.4837; found 745.4811. Purity (ELSD): 99%.

*(S)-6-((5-((S)-2-(2-(1H-Indol-3-yl)acetamido)-4-amino-4-oxobutanamido)pentyl)amino)-5-amino-N-(3-((S)-2-amino-5-guanidinopentanamido)propyl)-N,N-dimethyl-6-oxohexan-1-aminium Tetrakis(2,2,2-trifluoroacetate) (**3**).* Prepared with 2-(1*H*-indol-3-yl)acetic acid and dimethylation. Yield: 17.8 mg, 10.0% (83% per step over 12 steps). ^{1}H NMR (400 MHz, CD_3OD) δ 7.56 (dt, J = 8.0, 1.3 Hz, 1H), 7.38 (dt, J = 8.0, 0.8 Hz, 1H), 7.22 (s, 1H), 7.13 (ddd, J = 8.0, 7.0, 1.0 Hz, 1H), 7.04 (ddd, J = 8.0, 7.0, 1.3 Hz, 1H), 4.68 (dd, J = 7.3, 6.0 Hz, 1H), 3.90 (t, J = 6.8 Hz, 1H), 3.79 (t, J = 6.5 Hz, 1H), 3.75–3.73 (m, 2H), 3.45–3.38 (m, 1H), 3.29–3.11 (m, 10H), 3.09–3.03 (m, 1H), 2.99 (s, 3H), 2.98 (s, 3H), 2.72–2.59 (m, 2H), 1.98–1.82 (m, 6H), 1.81–1.62 (m, 4H), 1.51–1.34 (m, 6H), 1.26–1.18 (m, 2H). ^{13}C NMR (100 MHz, CD_3OD) δ 174.9, 173.2, 170.4, 169.9, 163.2, 162.9, 158.8, 138.2, 128.5, 125.2, 122.8, 120.2, 119.5, 112.6, 109.4, 65.2, 63.5, 54.2, 52.0, 51.1, 41.7, 40.3, 40.1, 37.8, 37.6, 33.9, 32.1, 29.7, 29.6, 29.5, 25.5, 24.7, 23.8, 23.2, 22.9. HRMS (EI) exact mass calculated for $C_{36}H_{63}N_{12}O_5^+$ [M^+] 743.5044; found 743.5029. Purity (ELSD): 99%.

*(S)-2-(2-(1H-Indol-3-yl)acetamido)-N^1-((6S,17S)-1,6,17-triamino-1-imino-12-methyl-7,18-dioxo-2,8,12,19-tetraazatetracosan-24-yl)-succinamide Tetrakis(2,2,2-trifluoroacetate) (**4**).* Prepared with 2-(1*H*-indol-3-yl)acetic acid and monomethylation. Yield: 23.2 mg, 13.0% (84% per step over 12 steps). ^{1}H NMR (400 MHz, CD_3OD) δ 7.55 (dt, J = 7.5, 1.0 Hz, 1H), 7.38 (dt, J = 8.0, 1.0 Hz, 1H), 7.22 (s, 1H), 7.13 (ddd, J = 8.0, 7.0, 1.3 Hz, 1H), 7.04 (ddd, J = 8.0, 7.0, 1.0 Hz, 1H), 4.70–4.67 (m, 1H), 3.88 (t, J = 6.8 Hz, 1H), 3.79–3.73 (m, 3H), 3.44–3.35 (m, 1H), 3.25–2.98 (m, 11H), 2.76 (s, 3H), 2.73–2.59 (m, 2H), 1.98–1.81 (m, 6H), 1.80–1.62 (m, 4H), 1.50–1.33 (m, 6H), 1.26–1.18 (m, 2H). ^{13}C NMR (100 MHz, CD_3OD) δ 174.9, 173.2, 170.5, 169.9, 163.2, 162.9, 158.8, 138.2, 128.5, 125.2, 122.8, 120.2, 119.5, 116.7, 112.6, 109.4, 55.1, 54.2, 52.0, 41.7, 40.3, 40.1, 37.7, 33.9, 32.1, 29.7, 29.6, 25.5, 24.7, 23.0. HRMS (EI) exact mass calculated for $C_{35}H_{61}N_{12}O_5$ [MH^+] 729.4888; found 729.4871. Purity (ELSD): 99%.

Preparation of 22 and 23. Resin **12** was Teoc-deprotected and coupled to Boc-L-Arg(Pbf)-OH (0.40 g, 0.75 mmol) using the same procedure as **13**, and the formed intermediate (**18**) was Alloc-deprotected and coupled to Fmoc-L-Asn(Tr)-OH (0.45 g, 0.75 mmol) in the same manner as **14**. The resin (**19**) was Fmoc-deprotected and coupled to 2-(1*H*-indol-3-yl)acetic acid (0.13 g, 0.75 mmol) or 2-(4-((2-methoxyethoxy)methoxy)-1*H*-indol-3-yl)acetic acid (**54**, 0.21 g, 0.75 mmol) using the same procedure as for **15**, and the intermediates **20**–**21** were Ns-deprotected using the same procedure as for **12**, cleaved from the resin, and purified by preparative HPLC, yielding compounds **22** and **23.**

*(S)-2-(2-(4-Hydroxy-1H-indol-3-yl)acetamido)-N^1-((6S,17S)-1,6,17-triamino-1-imino-7,18-dioxo-2,8,12,19-tetraazatetracosan-24-yl)succinamide Tetrakis(2,2,2-trifluoroacetate) (**22**).* Yield: 4.4 mg, 2.5% (71% per step over 11 steps). ^{1}H NMR (600 MHz, CD_3OD) δ 7.07 (s, 1H), 6.97–6.92 (m, 2H), 6.45 (d, J = 7.0 Hz, 1H), 4.68 (t, J = 6.2 Hz, 1H), 3.92–3.78 (m, 3H), 3.73 (t, J = 6.6 Hz, 1H), 3.47–3.42 (m, 1H), 3.29–3.20 (m, 4H), 3.14–2.96 (m, 7H), 2.75–2.67 (m, 2H), 1.98–1.80 (m, 6H), 1.78–1.66 (m, 4H), 1.49–1.27 (m, 6H), 1.20–1.11 (m, 2H). ^{13}C NMR (150 MHz, CD_3OD) δ 174.8, 173.6, 171.8, 169.3, 168.3, 157.3, 150.5, 139.2, 122.8, 122.5, 116.6, 107.3, 103.8, 52.7, 50.6, 45.1, 40.3, 38.8, 36.1, 35.9, 34.0, 30.6, 28.3, 28.2, 28.1, 26.0, 25.5, 24.1, 23.2, 21.5. HRMS (EI) exact mass calculated for $C_{34}H_{59}N_{12}O_6$ [MH^+] 731.4681; found 731.4663. Purity (ELSD): 99%.

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*(S)-2-(2-(1H-Indol-3-yl)acetamido)-N¹-((6S,17S)-1,6,17-triamino-1-imino-7,18-dioxo-2,8,12,19-tetraazatetracosan-24-yl)-succinamide Tetrakis(2,2,2-trifluoroacetate) (**23**).* Yield: 15.3 mg, 8.7% (80% per step over 11 steps). ^{1}H NMR (400 MHz, CD_3OD) δ 7.55 (d, J = 7.8 Hz, 1H), 7.37 (d, J = 8.3 Hz, 1H), 7.22 (s, 1H), 7.13 (dt, J = 7.6, 1.0 Hz, 1H), 7.04 (dt, J = 7.6, 1.0 Hz, 1H), 4.69 (t, J = 6.7 Hz, 1H), 3.88 (t, J = 6.8 Hz, 1H), 3.77−3.73 (m, 3H), 3.44−3.35 (m, 1H), 3.26−3.10 (m, 6H), 3.08−2.92 (m, 5H), 2.72−2.60 (m, 2H), 1.95−1.79 (m, 6H), 1.77−1.62 (m, 4H), 1.50−1.32 (m, 6H), 1.25−1.17 (m, 2H). ^{13}C NMR (100 MHz, CD_3OD) δ 175.0, 173.2, 170.7, 169.9, 163.3, 162.9, 158.8, 138.2, 128.5, 125.2, 122.8, 120.2, 119.4, 116.7, 112.6, 109.3, 54.2, 52.0, 46.5, 41.7, 40.3, 40.2, 37.7, 37.4, 33.9, 32.0, 29.7, 29.6, 27.4, 26.8, 25.5, 24.8, 22.9, 20.8. HRMS (EI) exact mass calculated for $C_{34}H_{59}N_{12}O_5$ [MH^+] 715.4731; found 715.4710. Purity (ELSD): 99%.

Preparation of 5, 6, and 31. *N*-(3-Aminopropyl)-2-nitrobenzenesulfonamide (**24**, 0.39 g, 1.50 mmol) was loaded onto a BAL resin using the standard procedure as shown for **9**. The resin (**25**) was coupled to 2-(1*H*-indol-3-yl)acetic acid (0.13 g, 0.75 mmol), 2-(4-((2-methoxyethoxy)methoxy)-1*H*-indol-3-yl)acetic acid (**54**, 0.21 g, 0.75 mmol), or 2-(2,4-bis((2-methoxyethoxy)methoxy)phenyl)acetic acid (**48**, 0.18 g, 0.75 mmol) in a similar manner to **10**. The resin (**26**) was coupled to 3-((*tert*-butyldiphenylsilyl)oxy)propan-1-ol (0.24 g, 0.75 mmol) using the same procedure as for **11**, using 1 M trimethylphosphine in THF (0.75 mL, 0.75 mmol) as the phosphine source. The resin (**27**) was Ns-deprotected as described for **12**, and the resin was then swelled in dry DCM (2 mL) for 30 min. Collidine (0.12 mL, 0.90 mmol) was added, followed by a solution of allyl chloroformate (0.15 mL, 1.35 mmol) in DCM (2 mL). The mixture was agitated for 1 h at rt, drained, washed with DMF, DCM, MeOH, and DCM, and the resin dried in vacuo. The resin (**28**) was TBDPS-deprotected using the same protocol as **13** and coupled to *tert*-butyl (4-((*tert*-butoxycarbonyl)(3-((*tert*-butoxycarbonyl)amino)propyl)-amino)butyl)(3-(2-nitrophenylsulfonamido)propyl)carbamate (**36**, 0.31 g, 0.45 mmol) as described for **11**. The resin (**29**) was Alloc-deprotected using the same procedure as for **14**, followed by hydroxylation using the general procedure. The resin (**30**) was Ns-deprotected following the protocol used for **12**, cleaved, and purified by preparative HPLC, yielding compounds **5**, **6**, and **31**.

*N-(20-Amino-4-hydroxy-4,8,12,17-tetraazaicosyl)-2-(indolin-3-yl)acetamide Tetrakis(2,2,2-trifluoroacetate) (**5**).* Prepared using 2-(1*H*-indol-3-yl)acetic acid. Yield: 9.8 mg, 6.9% (78% per step over 11 steps). ^{1}H NMR (400 MHz, CD_3OD) δ 7.57 (dt, J = 8.0, 1.3 Hz, 1H), 7.38 (dt, J = 8.0, 1.0 Hz, 1H), 7.22 (s, 1H), 7.15−7.11 (m, 1H), 7.07−7.02 (m, 1H), 3.67 (s, 2H), 3.14−2.93 (m, 14H), 2.76−2.67 (m, 4H), 2.12−2.03 (m, 4H), 1.96−1.86 (m, 2H), 1.84−1.72 (m, 6H). ^{13}C NMR (100 MHz, CD_3OD) δ 175.6, 138.2, 128.6, 125.0, 122.8, 120.1, 119.6, 112.6, 110.0, 57.9, 45.8, 37.8, 37.1, 34.0, 25.4, 24.2. HRMS (EI) exact mass calculated for $C_{26}H_{48}N_7O_2$ [MH^+] 490.3869; found 490.3850. Purity (ELSD): 99%.

*N-(20-Amino-4-hydroxy-4,8,12,17-tetraazaicosyl)-2-(4-hydroxyindolin-3-yl)acetamide Tetrakis(2,2,2-trifluoroacetate) (**6**).* Prepared using **54**. Yield: 4.9 mg, 3.4% (74% per step over 11 steps). ^{1}H NMR (400 MHz, CD_3OD) δ 7.04 (s, 1H), 6.96−6.90 (m, 2H), 6.41 (dd, J = 7.0, 1.3 Hz, 2H), 3.82 (s, 2H), 3.37−3.32 (m, 2H), 3.14−2.90 (m, 18H), 2.12−1.99 (m, 6H), 1.83−1.76 (m, 6H). ^{13}C NMR (100 MHz, CD_3OD) δ 177.9, 151.9, 140.9, 124.3, 118.5, 108.5, 105.5, 45.8, 37.6, 36.4, 35.0, 30.6, 27.9, 25.0, 24.0. HRMS (EI) exact mass calculated for $C_{26}H_{48}N_7O_3$ [MH^+] 506.3819; found 506.3822. Purity (ELSD): 98%.

*N-(20-Amino-4-hydroxy-4,8,12,17-tetraazaicosyl)-2-(2,4-dihydroxyphenyl)acetamide Tetrakis(2,2,2-trifluoroacetate) (**31**).* Prepared using **48**. Yield: 5.1 mg, 3.5% (74% per step over 11 steps). ^{1}H NMR (400 MHz, CD_3OD) δ 6.95 (d, J = 8.2 Hz, 1H), 6.34 (d, J = 2.3 Hz, 1H), 6.29 (dd, J = 8.0, 2.3 Hz, 1H), 3.45 (s, 2H), 3.36−3.31 (m, 2H), 3.18−2.96 (m, 18H), 2.19−2.06 (m, 6H), 1.92−1.79 (m, 6H). ^{13}C NMR (100 MHz, CD_3OD) δ 177.6, 159.5, 157.7, 133.0, 119.8, 114.2, 107.8, 103.6, 45.8, 38.2, 37.8, 36.5, 27.7, 25.4, 24.1. HRMS (EI) exact mass calculated for $C_{24}H_{47}N_6O_4$ [MH^+] 483.3659; found 483.3648. Purity (ELSD): 98%.

Preparation of 33a−c. The resin (**27**) was coupled to *tert*-butyl (4-((*tert*-butoxycarbonyl)(3-((*tert*-butoxycarbonyl)amino)propyl)-amino)butyl)(3-(2-nitrophenylsulfonamido)propyl)carbamate (**36**, 0.31 g, 0.45 mmol) using the same procedure as for **11**. The resin (**32**) was Ns-deprotected using the standard procedure shown for **12**, cleaved from the resin, and purified by preparative HPLC, yielding compounds **33a−c**.

*N-(20-Amino-4,8,12,17-tetraazaicosyl)-2-(indolin-3-yl)acetamide Pentakis(2,2,2-trifluoroacetate) (**33a**).* Prepared using 2-(1*H*-indol-3-yl)acetic acid. Yield: 9.8 mg, 6.9% (78% per step over 11 steps). Yield: 14.2 mg, 9.1% (71% per step over 7 steps). ^{1}H NMR (400 MHz, CD_3OD) δ 7.55 (d, J = 7.7 Hz, 1H), 7.37 (d, J = 8.0 Hz, 1H), 7.21 (s, 1H), 7.12 (dt, J = 7.2, 1.1 Hz, 1H), 7.03 (dt, J = 7.7, 1.1 Hz, 1H), 3.70 (s, 2H), 3.15−2.99 (m, 14H), 2.92−2.83 (m, 4H), 2.16−1.96 (m, 6H), 1.86−1.76 (m, 6H). ^{13}C NMR (100 MHz, CD_3OD) δ 175.2, 162.1, 161.6, 136.9, 127.2, 123.9, 121.4, 118.8, 118.1, 114.9, 111.3, 108.1, 44.7, 36.7, 35.5, 32.7, 26.5, 24.3, 23.1. HRMS (EI) exact mass calculated for $C_{26}H_{48}N_7O$ [MH^+] 474.3920; found 474.3913. Purity (ELSD): 99%.

*N-(20-Amino-4,8,12,17-tetraazaicosyl)-2-(4-hydroxyindolin-3-yl)-acetamide Pentakis(2,2,2-trifluoroacetate) (**33b**).* Prepared using **54**. Yield: 15.2 mg, 9.6% (72% per step over 7 steps). ^{1}H NMR (400 MHz, CD_3OD) δ 7.03 (s, 1H), 6.95−6.88 (m, 2H), 6.40 (dd, J = 7.0, 1.5 Hz, 1H), 3.81 (s, 2H), 3.37−3.32 (m, 2H), 3.15−2.91 (m, 18H), 2.14−1.97 (m, 6H), 1.85−1.77 (m, 6H). ^{13}C NMR (100 MHz, CD_3OD) δ 178.2, 152.2, 140.7, 124.1, 123.9, 118.2, 108.7, 105.0, 45.9, 37.8, 36.4, 35.1, 30.7, 27.7, 25.4, 24.2, 24.0. HRMS (EI) exact mass calculated for $C_{26}H_{48}N_7O_2$ [MH^+] 490.3869; found 490.3861. Purity (ELSD): 99%.

*N-(20-Amino-4,8,12,17-tetraazaicosyl)-2-(2,4-dihydroxyphenyl)-acetamide Pentakis(2,2,2-trifluoroacetate) (**33c**).* Prepared using **48**. Yield: 14.8 mg, 9.5% (71% per step over 7 steps). ^{1}H NMR (400 MHz, CD_3OD) δ 6.94 (d, J = 8.3 Hz, 1H), 6.34 (d, J = 2.5 Hz, 1H), 6.28 (dd, J = 8.3, 2.5 Hz, 1H), 3.44 (s, 2H), 3.36−3.31 (m, 2H), 3.16−2.97 (m, 18H), 2.16−2.04 (m, 6H), 1.90−1.77 (m, 6H). ^{13}C NMR (100 MHz, CD_3OD) δ 177.2, 159.1, 157.6, 132.9, 119.6, 116.7, 114.3, 107.9, 103.8, 45.9, 38.4, 37.8, 36.4, 27.7, 25.4, 24.2. HRMS (EI) exact mass calculated for $C_{24}H_{47}N_6O_3$ [MH^+] 467.3709; found 467.3703. Purity (ELSD): 99%.

Preparation of 44a−b and 45a−b. *N*-(3-Aminopropyl)-2-nitrobenzenesulfonamide (0.19 g, 0.75 mmol) or *N*-(8-aminooctyl)-2-nitrobenzenesulfonamide (0.25 g, 0.75 mmol) was loaded onto a BAL resin using the standard procedure as shown for **9**. The resin (**38**) was coupled to Boc-L-Arg(Pbf)-OH (0.40 g, 0.75 mmol) using the same procedure as for **10**, and 2-(trimethylsilyl)ethyl 4-hydroxybutylcarbamate (0.18 g, 0.75 mmol) or 2-(trimethylsilyl)ethyl 9-hydroxynonylcarbamate (0.23 g, 0.75 mmol) was coupled onto the resin as described for **11**. The resin (**40**) was Ns-deprotected using the same procedure as for **12**, followed by methylation using the two general procedures. The resin (**41**) was Teoc-deprotected and coupled to Fmoc-L-Asn(Tr)-OH (0.45 g, 0.75 mmol) using the same procedure as for **13**, and the intermediate **42** was Fmoc-deprotected and coupled to 2-(2,4-bis(benzyloxy)phenyl)acetic acid (0.26 g, 0.75 mmol) using the same procedure as for **15**. **43** was cleaved from the resin following the standard protocol as described for **16**, using TIPS instead of EDT as scavenger, and the residue was purified by preparative HPLC. The benzyl-protected intermediate was dissolved in glacial acetic acid (8 mL) under an atmosphere of nitrogen. $Pd(OH)_2/C$ (10% w/w) was added, and hydrogen was bubbled through the solution for 5 min. The mixture was then stirred for 3 h under a static atmosphere of hydrogen. The mixture was filtered and the solid washed with MeOH (4 mL). The organic phases were collected, water (10 mL) was added, and the solvent was removed by lyophilization. The crude product was purified by preparative HPLC, yielding compounds **44a−b** and **45a−b**.

*(S)-N¹-(9-((3-((S)-2-Amino-5-guanidinopentanamido)propyl)-(methyl)amino)nonyl)-2-(2-(2,4-dihydroxyphenyl)acetamido)-succinamide Tris(2,2,2-trifluoroacetate) (**44a**).* Prepared using *N*-(3-aminopropyl)-2-nitrobenzenesulfonamide, 2-(trimethylsilyl)ethyl 9-hydroxynonylcarbamate, and monomethylation. Yield: 9.0 mg, 6.0%

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(77% per step over 11 steps). ^{1}H NMR (600 MHz, CD_3OD) δ 6.96 (d, J = 8.1 Hz, 1H), 6.36 (d, J = 2.2 Hz, 1H), 6.30 (dd, J = 8.1, 2.2 Hz, 1H), 4.69 (t, J = 6.2 Hz, 1H), 3.91 (t, J = 6.8 Hz, 1H), 3.52−3.41 (m, 3H), 3.31−3.05 (9H), 2.86 (s, 3H), 2.73−2.68 (m, 2H), 2.03−1.86 (m, 4H), 1.76−1.66 (m, 4H), 1.51−1.45 (m, 2H), 1.41−1.25 (m, 10H). ^{13}C NMR (150 MHz, CD_3OD) δ 173.7, 171.7, 169.1, 161.8, 161.6, 157.7, 157.3, 155.9, 131.4, 117.8, 115.8, 112.8, 106.7, 102.5, 53.5, 52.7, 50.3, 40.3, 39.0, 37.4, 36.1, 28.7, 28.6, 28.5, 28.3, 26.0, 25.9, 24.1, 23.6. HRMS (EI) exact mass calculated for $C_{31}H_{56}N_9O_6$ [MH^+] 650.4354; found 650.4337. Purity (ELSD): 99%.

*N-(9-((S)-4-Amino-2-(2-(2,4-dihydroxyphenyl)acetamido)-4-oxobutanamido)-N-(3-((S)-2-amino-5-guanidinopentanamido)propyl)-N,N-dimethylnonan-1-aminium Tris(2,2,2-trifluoroacetate) (**44b**).* Prepared using *N*-(3-aminopropyl)-2-nitrobenzenesulfonamide, 2-(trimethylsilyl)ethyl 9-hydroxynonylcarbamate, and dimethylation. Yield: 7.4 mg, 4.9% (76% per step over 11 steps). ^{1}H NMR (600 MHz, CD_3OD) δ 6.96 (d, J = 8.1 Hz, 1H), 6.36 (d, J = 2.6 Hz, 1H), 6.30 (dd, J = 8.1, 2.6 Hz, 1H), 4.68 (t, J = 6.2 Hz, 1H), 3.93 (t, J = 6.6 Hz, 1H), 3.53−3.42 (m, 3H), 3.38−3.34 (m, 2H), 3.31−3.24 (m, 6H), 3.14−3.10 (m, 1H), 3.09 (s, 6H), 2.70 (d, J = 6.6 Hz, 2H), 2.05−1.86 (m, 4H), 1.79−1.67 (m, 4H), 1.51−1.45 (m, 2H), 1.39−1.25 (m, 10H). ^{13}C NMR (150 MHz, CD_3OD) δ 173.7, 171.7, 169.0, 161.6, 157.7, 157.3, 155.9, 131.4, 117.8, 115.8, 112.9, 106.7, 102.5, 64.3, 61.6, 52.7, 50.4, 49.9, 40.4, 39.0, 37.4, 36.2, 36.1, 28.7, 28.4, 28.3, 26.0, 25.7, 24.1, 22.5, 22.0. HRMS (EI) exact mass calculated for $C_{32}H_{58}N_9O_6^+$ [M^+] 664.4510; found 664.4498. Purity (ELSD): 99%.

*(S)-N^1-(4-((8-((S)-2-Amino-5-guanidinopentanamido)octyl)(methyl)amino)butyl)-2-(2-(2,4-dihydroxyphenyl)acetamido)succinamide Tris(2,2,2-trifluoroacetate) (**45a**).* Prepared using *N*-(8-aminooctyl)-2-nitrobenzenesulfonamide, 2-(trimethylsilyl)ethyl 4-hydroxybutylcarbamate, and monomethylation. Yield: 8.8 mg, 5.9% (77% per step over 11 steps). ^{1}H NMR (600 MHz, CD_3OD) δ 6.98 (d, J = 8.1 Hz, 1H), 6.37 (d, J = 2.2 Hz, 1H), 6.30 (dd, J = 8.3, 2.1 Hz, 1H), 4.60 (t, J = 6.1 Hz, 1H), 3.86 (t, J = 6.6 Hz, 1H), 3.53 (d, J = 15.0 Hz, 1H), 3.41 (d, J = 15.0 Hz, 1H), 3.28−3.22 (m, 5H), 3.12−2.91 (m, 4H), 2.79−2.69 (m, 5H), 1.95−1.85 (m, 2H), 1.73−1.51 (m, 10H), 1.44−1.36 (m, 8H). ^{13}C NMR (150 MHz, CD_3OD) δ 173.8, 173.6, 172.2, 168.3, 161.6, 161.4, 157.7, 157.3, 155.9, 131.7, 113.0, 106.7, 102.4, 55.4, 52.7, 50.7, 40.4, 39.3, 38.9, 37.3, 35.6, 28.8, 28.7, 28.4, 26.5, 26.1, 24.1. HRMS (EI) exact mass calculated for $C_{31}H_{56}N_9O_6$ [MH^+] 650.4354; found 650.4338. Purity (ELSD): 99%.

*N-(4-((S)-4-Amino-2-(2-(2,4-dihydroxyphenyl)acetamido)-4-oxobutanamido)butyl)-8-((S)-2-amino-5-guanidinopentanamido)-N,N-dimethyloctan-1-aminium tris(2,2,2-trifluoroacetate) (**45b**).* Prepared using *N*-(8-aminooctyl)-2-nitrobenzenesulfonamide, 2-(trimethylsilyl)ethyl 4-hydroxybutylcarbamate, and dimethylation. Yield: 16.8 mg, 11.1% (82% per step over 11 steps). ^{1}H NMR (600 MHz, CD_3OD) δ 6.98 (d, J = 8.4 Hz, 1H), 6.37 (d, J = 2.6 Hz, 1H), 6.31 (dd, J = 8.4, 2.6 Hz, 1H), 4.60 (t, J = 6.1 Hz, 1H), 3.87 (t, J = 6.6 Hz, 1H), 3.53 (d, J = 15.0 Hz, 1H), 3.41 (d, J = 15.0 Hz, 1H), 3.40−3.35 (m, 1H), 3.28−3.23 (m, 5H), 3.19−3.14 (m, 4H), 2.93 (m, 6H), 2.77 (dd, J = 15.8, 7.0 Hz, 1H), 2.71 (dd, J = 15.8, 5.1 Hz, 1H), 1.96−1.85 (m, 2H), 1.74−1.49 (m, 10H), 1.45−1.36 (m, 8H). ^{13}C NMR (150 MHz, CD_3OD) δ 173.5, 172.3, 168.3, 157.7, 157.4, 131.8, 113.3, 106.7, 102.4, 64.2, 63.6, 52.7, 50.8, 49.7, 40.3, 39.3, 37.6, 35.6, 28.8, 28.7, 28.4, 26.5, 25.9, 25.6, 24.1, 22.1, 18.9. HRMS (EI) exact mass calculated for $C_{32}H_{58}N_9O_6^+$ [M^+] 664.4510; found 664.4515. Purity (ELSD): 99%.

Electrophysiology: In Vitro cRNA Transcription. cDNA encoding rat GluA1$_i$ or GluN1/2A subunits inserted into the vectors pGEM-HE or pCIneo, respectively, were used for preparation of cRNA transcripts. Plasmid DNA were grown in Top10 *Escherichia coli* bacteria (Invitrogen, Carlsbad, CA) and purified using the NucleoSpin Midi purification kit (Macherey-Nagel GmbH, Düren, Germany) according to the protocol supplied by the manufacturer and linearized by restriction enzyme digestion. cRNA was synthesized from linearized cDNAs by in vitro transcription using the mMESSAGE mMACHINE T7 mRNA-capping kit (Ambion, Austin, TX) according to the protocol supplied by the manufacturer.

Oocyte Electrophysiology. Mature female *Xenopus laevis* (Nasco, Modesto, CA) were anesthetized using 0.1% ethyl 3-aminobenzoate and their ovaries surgically removed using approved animal protocols. Ovarian tissue was dissected and treated with collagenase (1 mg/mL) in Ca^{2+}-free Barth's medium (in mM: 82.5 NaCl, 2 KCl, 5 HEPES, 0.82 $MgCl_2$) for 1−2 h at rt. For expression of recombinant AMPA and NMDA receptors, ocytes were injected with 25 nL of cRNA (1 ng/nL for GluA1$_i$ or 0.5 ng/nL for GluN1/2A) and incubated at 17 °C in Barth's medium (in mM: 88 NaCl, 1 KCl, 0.33 $Ca(NO_3)_2$, 0.41 $CaC1_2$, 0.82 $MgSO_4$, 2.4 $NaHCO_3$, 10 HEPES; pH 7.4) with gentamicin (0.10 mg/mL). Oocytes were used for recordings 2−4 days postinjection and were voltage clamped with the use of a OC-725C two-electrode voltage clamp amplifier (Warner Instruments, Hamden, CT) with both microelectrodes filled with 3 M KCl. Recordings were made while the oocytes were continuously superfused with frog Ringer's solution (in mM: 115 NaCl, 2 KCl, 1.8 $BaCl_2$, 5 HEPES; pH 7.6). Test compounds were dissolved in frog Ringer's solution and added by bath application. Recordings were made at rt at a holding potential of −80 mV. Antagonist concentration−inhibition curves were constructed by measuring the maximal current induced by the saturating concentration of agonist (300 μM glutamate for GluA1$_i$; 100 μM glutamate and 100 μM glycine for GluN1/2A) and then applying increasing concentrations of antagonist in the presence of the appropriate agonist. Composite concentration−inhibition data from experiments at 3−8 individual oocytes were plotted using GraphPad Prism software (GraphPad Software, Inc., San Diego, CA) and fitted by an iterative least-squares routine to the equation $I = I_{min} + [(I_{max} - I_{min})/(1 + ([antagonist]/IC_{50})^{n_H})]$, where I is the agonist-evoked current at a given antagonist concentration, I_{max} is the maximum current, I_{min} is the minimum current, [antagonist] is the concentration of antagonist, and n_H is the Hill slope. The IC_{50} is the concentration of antagonist producing 50% of I_{max}.

■ ASSOCIATED CONTENT

Supporting Information

Preparation of building blocks **36**, **48**, and **54**, as well as ^{1}H and ^{13}C NMR spectra of final compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +45 3533 6114. Fax: +45 3533 6040. E-mail: kristian.stromgaard@sund.ku.dk.

Present Address

†For N.G.N.: Nuevolution A/S, Rønnegade 8, DK-2100 Copenhagen, Denmark.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

ADDP, 1,1-(azodicarbonyl)dipiperidine; Alloc, allyloxycarbonyl; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; ArgTX, argiotoxin; DIPEA, diisopropylamine; EDT, 1,2-ethanedithiol; HATU, 2-(1*H*-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; *m*CPBA, *m*-chloroperoxybenzoic acid; MEM, 2-methoxyethoxymethyl; NMDA, *N*-methyl-D-aspartate; Ns, 2-nitrobenzenesulfonamide; Pbf, 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl; TBAF, tetra-*n*-butylammonium fluoride; TBDPS, *tert*-butyldiphenylsil-

yl; TEA, triethylamine; Teoc, 2-(trimethylsilyl)-ethyloxycarbonyl; TIPS, triisopropylsilane

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