# Solid-Phase Synthesis of Polyamine Toxin Analogues: Potent and Selective Antagonists of $Ca^{2+}$-Permeable AMPA Receptors

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The wasp toxin philanthotoxin-433 (PhTX-433) is a nonselective and noncompetitive antagonist of ionotropic receptors, such as ionotropic glutamate receptors and nicotinic acetylcholine receptors. Polyamine toxins are extensively used for the characterization of subtypes of ionotropic glutamate receptors, in particular $Ca^{2+}$-permeable AMPA and kainate receptors. We have previously shown that an analogue of PhTX-433 with one of the amino groups replaced by a methylene group, philanthotoxin-83 (PhTX-83) is a selective and potent antagonist of AMPA receptors. We now describe the solid-phase synthesis of analogues of PhTX-83 and the electrophysiological characterization of these analogues on cloned AMPA and kainate receptors. The polyamine portion of PhTX-83 was modified systematically by changing the position of the secondary amino group along the polyamine chain. In another series of analogues, the acyl moiety of PhTX-83 was replaced by acids of different size and lipophilicity. Using electrophysiological techniques, PhTX-56 was shown to be a highly potent ($K_i$ = 3.3 ± 0.78 nM) and voltage-dependent antagonist of homomeric GluR1 receptors and was more than 1000-fold less potent when tested on heteromeric GluR1+GluR2, as well as homomeric GluR5(Q) receptors, thus being selective for $Ca^{2+}$-permeable AMPA receptors. Variation of the acyl group of PhTX-83 had only minor effect on antagonist potency at homomeric GluR1 receptors but led to a significant decrease in the voltage-dependence. In conclusion, PhTX-56 is a novel, very potent, and selective antagonist of $Ca^{2+}$-permeable AMPA receptors and is a promising tool for structure/function studies of the ion channel of the AMPA receptor.

## Introduction

Glutamate is the major excitatory amino acid neurotransmitter in the mammalian central nervous system (CNS) and acts through two classes of receptors, the ionotropic and metabotropic receptors.[1−3] The ionotropic glutamate receptors (iGluRs) are divided into three subtypes on the basis of the effects of agonists at these receptors, namely, *N*-methyl-D-aspartate (NMDA), (*R*,*S*)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propanoic acid (AMPA), and kainate receptors. The iGluRs are particularly interesting, as there is strong evidence that glutamate-induced excessive $Ca^{2+}$ entry under pathophysiological conditions leads to a wide range of neurological insults including ischemia-induced brain damage, epileptic seizures, and chronic neurodegenerative disorders such as Alzheimer's disease and Huntington's chorea as well as other neurological and psychiatric disorders.[1,4] Moreover, iGluRs are believed to be important for the basic cellular and molecular mechanism by which memories are formed and stored, as characterized by long-term potentiation (LTP)[5,6] and long-term depression (LTD).[7]

AMPA receptors (AMPARs), which are distributed ubiquitously throughout the CNS and mediate fast excitatory neurotransmission, are composed of four subunits designated GluR1−4.[2,3] Although most native AMPARs exhibit low $Ca^{2+}$ permeability, AMPARs with high $Ca^{2+}$ permeability have been reported in a variety of cells in the CNS, a property that is inversely correlated with the relative abundance of the GluR2 subunit.[2,3] AMPARs are important for the generation of LTP[8,9] and recent evidence suggests that the induction of LTP and LTD involves the physical transport of AMPARs into and out of the synaptic membrane,[10−13] which in turn seems to be regulated by the subunit composition of the receptor.[14−16] Moreover, there is increasing evidence that $Ca^{2+}$-permeable AMPARs are important for the pathogenesis of neurological disorders.[17] Thus, use of AMPAR antagonists is believed to be useful in treating neurodegenerative disorders and reducing the amount of neurological damage associated with these disorders.[17−21]

Polyamine toxins form a class of low molecular weight compounds, isolated from venoms of spiders and wasps, which are nonselective antagonists of ionotropic receptors such as iGluRs and nicotinic acetylcholine receptors (nAChRs).[22,23] One such example is the polyamine wasp toxin philanthotoxin-433 [(*S*)-PhTX-433 (**1**)] (Chart 1) isolated from venom of the female digger wasp *Philan-*

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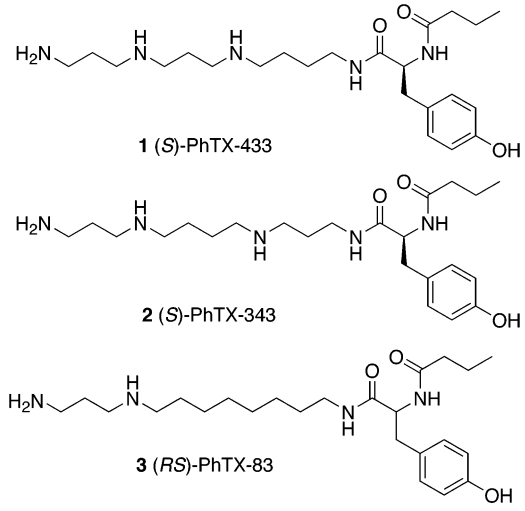
**Chart 1**




*thus triangulum*.[24] Polyamine toxin **1** and its synthetic analogue (*S*)-PhTX-343 (**2**) are antagonists of $Ca^{2+}$-permeable AMPARs and kainate receptors, which make them useful pharmacological tools for determination of the subunit composition of these receptors.[25−28] However, because of the nonspecific action with respect to other ionotropic receptors, the potential of polyamine toxins as drug candidates has so far been considered to be limited.

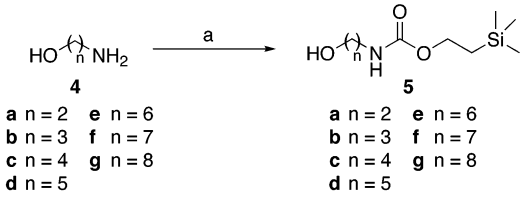
It has been shown that selectivity of **2** as antagonist on various classes of ionotropic receptors could be achieved by modification of the polyamine portion of the molecule.[29−31] In particular, replacements of the secondary amino groups in the polyamine moiety by methylene groups or oxygen atoms result in an enhanced antagonist activity at mammalian muscle type nAChR with concomitant loss of activity at iGluRs sensitive to quisqualate.[29,30] Moreover, removal of one of the inner basic sites in PhTX-343 led to an enhanced potency at non-NMDA receptors (non-NMDARs), whereas the activity at NMDA receptors (NMDARs) and nAChRs remained low.[32] The most striking example was philanthotoxin-83 [(*RS*)-PhTX-83 (**3**)] (Chart 1) which inhibited non-NMDARs with an $IC_{50}$ value of 32 ± 3 nM, while being 100-fold less potent at NMDARs and nAChRs.[32]

We here report the solid-phase synthesis of two series of analogues of (*RS*)-PhTX-83 (**3**). In one series, a systematic variation of the distance between the secondary amino group and the aromatic headgroup moiety was performed, while keeping the total length of the polyamine moiety constant. In another series of analogues, the acyl moiety of (*RS*)-PhTX-83 (**3**) was modified. The compounds were characterized by in vitro electrophysiology on AMPARs comprised of homomeric GluR1 and heteromeric GluR1+GluR2 receptors, as well as kainate receptors consisting of homomeric GluR5-(Q) receptor subunits.

## Results

**Synthesis.** The synthesis of polyamine toxins in general and philanthotoxins in particular has been greatly facilitated by the use of solid-phase synthesis methodologies.[22] Since the philanthotoxin molecule can be divided into three distinct parts (an amino acid moiety, an acyl group, and a long-chain polyamine moiety) linked by amide bonds, solid-phase methodology, similar to that used in peptide synthesis, was employed to synthesize a small library of philanthotoxins.[31] Recently, a general method was described for the solid-phase synthesis of polyamines that allows a sequential construction of polyamines using amino alcohol building blocks of varying lengths, illustrated by the synthesis of eight philanthotoxin analogues with systematic alterations in the polyamine moiety.[33]

**Scheme 1**[a]




[a] Reagents: (a) 2-(trimethylsilyl)ethyl 4-nitrophenyl carbonate, triethylamine.

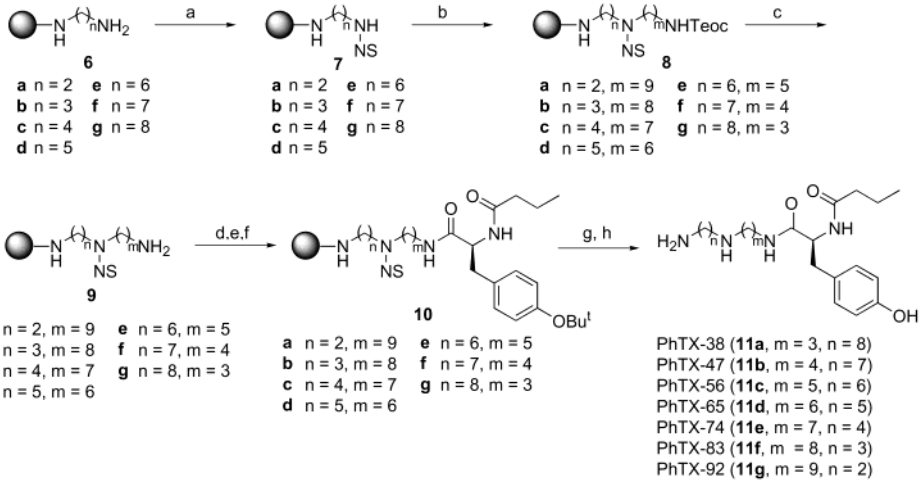
In the present work, the synthesis of two series of analogues of (*RS*)-PhTX-83 (**3**) is described. In the first series of compounds, the chain length between the distal primary amino group and the secondary amino group was systematically varied while keeping the total polyamine length constant. In the second series, the acyl moiety of **3** was replaced by various aliphatic and aromatic acyl groups.

Protected amino alcohols **5a**−**g** used as building blocks for the synthesis of the resin-bound polyamines **9a**−**g** were prepared as outlined in Scheme 1. Amino alcohols **4a**−**e** are commercially available, whereas **4f** and **4g** were synthesized by standard procedures by reacting the appropriate alcohol bromide with potassium phthalimide in DMF and subsequent treatment with hydrazine hydrate.[34] The amino alcohols **4a**−**g** were protected by reaction with 2-(trimethylsilyl)ethyl 4-nitrophenyl carbonate and triethylamine in $CH_2Cl_2$[35] to afford the required chain elongation elements **5a**−**g**, containing a *N*-2-(trimethylsilyl)ethoxycarbonyl (*N*-Teoc) protecting group, as has previously been described for **5b** and **5c**.[33]

The first series of compounds with variation of the polyamine moiety of **3** was synthesized according to Scheme 2, similarly to previously described methods.[33] Commercially available trityl resins derivatized with alkyl diamines (**6a**−**g**) were reacted with *o*-nitrobenzenesulfonyl (NS) chloride in THF/$CH_2Cl_2$ (2:1) to give resin-bound sulfonamides **7a**−**g**. The protected amino alcohols **5a**−**g** were reacted with the sulfonamides **7a**−**g** in a Mitsunobu reaction using 1,1′-(azadicarbonyl)-dipiperidine (ADDP) and tributylphosphine (TBP) in THF/$CH_2Cl_2$ (1:1) to afford resins **8a**−**g**. The *N*-Teoc protection group was selectively removed using tetrabutylammonium fluoride (TBAF) to give resins **9a**−**g**.

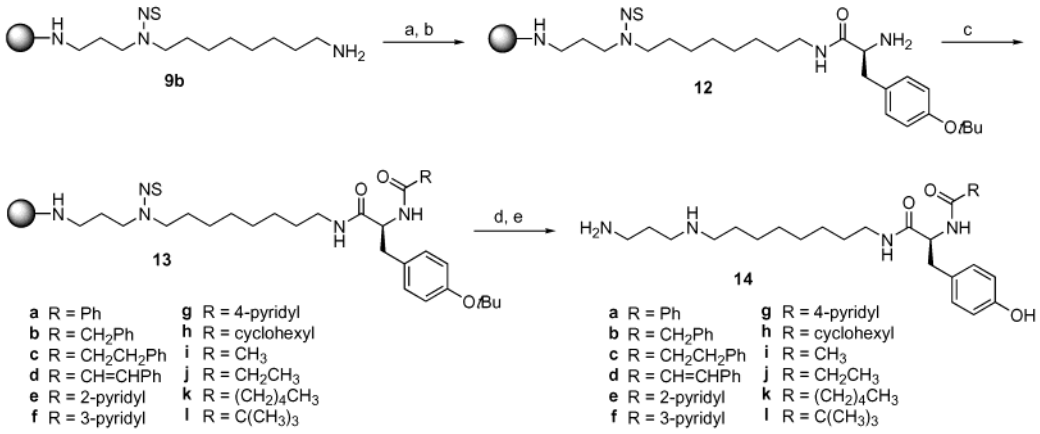
The synthesis of fully protected, resin-bound analogues **10a**−**g** were completed as previously described.[30,31,33] Thus, resins **9a**−**g** were coupled with (*S*)-*N*-Fmoc-*O*-(*tert*-butyl)tyrosine, followed by deprotection of the primary amino group with 20% piperidine in DMF, and subsequent coupling with butyric acid. The NS-amide groups were cleaned by treatment with 2-mercaptoethanol and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU),[36,37] and the final cleavage from the resin and removal of the *tert*-butyl protecting group using $CH_2$-

**Scheme 2**[a]




[a] Reagents: (a) *o*-nitrobenzenesulfonyl chloride, diisopropylethylamine; (b) **5a**−**5g**, ADDP, TBP; (c) TBAF; (d) (*S*)-Fmoc-*O*-(*tert*-butyl)tyrosine, HATU, collidine; (e) 20% piperidine; (f) butyric acid, HATU, collidine; (g) 2-mercaptoethanol, DBU; (h) $CH_2Cl_2$/TFA/triisopropylsilane/$H_2O$ (47.5:47.5:2.5:2.5).

**Scheme 3**[a]




[a] Reagents: (a) (*S*)-Fmoc-*O*-(*tert*-butyl)tyrosine, HATU, collidine; (b) 20% piperidine; (c) appropriate carboxylic acid, HATU, collidine; (d) 2-mercaptoethanol, DBU; (e) $CH_2Cl_2$/TFA/triisopropylsilane/$H_2O$.

$Cl_2$/TFA/triisopropylsilane/$H_2O$ (47.5:47.5:2.5:2.5) gave the desired analogues **11a**−**g** (Scheme 2) as crude products.

The second series of compounds with variation of the butyric acid moiety of (*RS*)-PhTX-83 (**3**) was synthesized as outlined in Scheme 3. Resin **9b** was treated with (*S*)-*N*-Fmoc-*O*-(*tert*-butyl)tyrosine followed by deprotection of the primary amino group with 20% piperidine in DMF to give **12**. Resin **12** was then treated with benzoic acid, phenylacetic acid, 3-phenylpropionic acid, 3-phenylacrylic acid, pyridine-2-carboxylic acid, nicotinic acid, isonicotinic acid, cyclohexanecarboxylic acid, acetic acid, propanoic acid, hexanoic acid, or 2,2-dimethylpropionic acid to give **13a**−**l**, respectively. The final products were obtained by removal of the NS-group and cleavage from the resin with concomitant deprotection of the *tert*-butyl group as described above to give compounds **14a**−**l** as crude products.

All target compounds were subjected to purification by automated preparative HPLC-MS[37] to give overall yields of 10−65% of purified **11a**−**g** and **14a**−**l** as trifluoroacetates on the basis of the loading of functionalized resins **6a**−**g**. All previous attempts to crystallize salts of polyamine toxins of the present type have been unsuccessful.[29−31,33] Purity of the final products was determined by HPLC with evaporative light scattering (ELS) detection and was in the range of 96−100%. This method is capable of detection and quantification of amine impurities, which cannot be detected by conventional UV monitoring, in the final products. All final products were characterized by $^1H$ and $^{13}C$ NMR spectroscopy as well as high-resolution mass spectrometry (HRMS). Since enantiomerically pure amino acids were used as starting materials, the final products were assumed to be the pure (*S*)-forms, as it has been shown previously that the coupling step takes place without racemization.[30]

**In Vitro Electrophysiology.** The analogues of (*RS*)-PhTX-83 (**3**), **11a**−**g** and **14a**−**l**, were tested using two electrode voltage clamped *Xenopus* oocytes expressing homo- or heteromeric AMPARs composed of either GluR1 or GluR1+GluR2 subunits or homomeric kainate receptors composed of GluR5(Q) subunits, essentially as previously described.[39] The analogues were co-applied with 100 $\mu$M glutamate, and the oocytes clamped at holding potentials ($V_H$) of −40 mV or −80 mV. For each of the holding potentials, $IC_{50}$ values were determined and the $K_i$ calculated from these values (Tables 1, 2, and 3).

The results of the pharmacological characterization

**Table 1.** Antagonist Effect of Philanthotoxin Analogues **11a**−**g** on GluR1[a,b]

| compounds | $K_i$ ($\mu$M) −40 mV | $K_i$ ($\mu$M) −80 mV | $V_{H-40}/V_{H-80}$ |
|---|---|---|---|
| PhTX-38 (**11a**) | 8.1 ± 3.0 | 0.35 ± 0.03 | 23 |
| PhTX-47 (**11b**) | 0.135 ± 0.029 | 0.038 ± 0.004 | 3.6 |
| PhTX-56 (**11c**) | 0.287 ± 0.08 | 0.0033 ± 0.78 × $10^{-3}$ | 86 |
| PhTX-65 (**11d**) | >10 | 0.468 ± 0.035 | − |
| PhTX-74 (**11e**) | 3 ± 1.3 | 0.168 ± 0.021 | 18 |
| PhTX-83 (**11f**) | 8.0 ± 3.1 | 0.065 ± 0.020 | 123 |
| PhTX-92 (**11g**) | 6.2 ± 0.7 | 0.41 ± 0.06 | 15 |

[a] Inhibition of the current elicited by 100 $\mu$M glutamate by simultaneous co-application of the antagonist in oocytes injected with GluR1 flop RNA. Values are means of two independent measurements performed in triplicate. [b] $K_i$ for PhTX-343 (**2**) is 170 ± 23 nM (−40 mV) and 22 ± 3 nM (−80 mV).

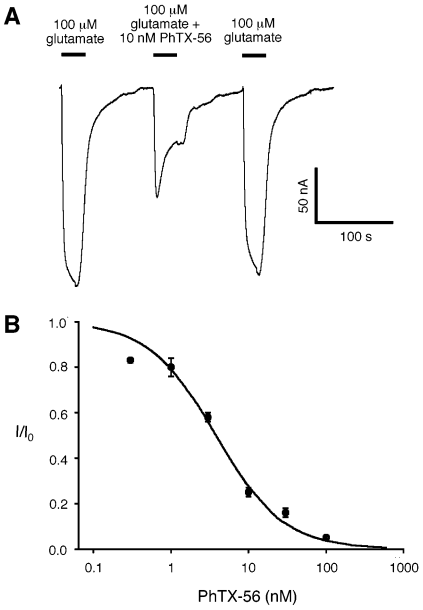



**Figure 1.** (A). Inward currents elicited by 100 $\mu$M glutamate, co-application of 100 $\mu$M glutamate and 10 nM PhTX-56 (**11c**), and application of 100 $\mu$M glutamate after 80 s washout. (B). Concentration-inhibition data for PhTX-56 (**11c**) on homomeric GluR1 flop receptors (holding potential $V_H = -80$ mV). Bars represent standard deviation (SD).

of the series of analogues of (*RS*)-PhTX-83 (**3**) with variation of the polyamine moiety are shown in Table 1. The parent compound, (*S*)-PhTX-83 (**11f**) had a $K_i$ value of 65 ± 20 nM at −80 mV. Moving the secondary amine closer to the aromatic headgroup, as in PhTX-74 (**11e**) and PhTX-65 (**11d**), gradually decreased the antagonistic properties, but further shifting of the amino group toward the aromatic headgroup, as in PhTX-56 (**11c**), led to a dramatic increase in potency. Thus, compound **11c** with a $K_i$ value of 3.3 ± 0.78 nM was the most potent of the compounds tested (Figure 1). PhTX-47 (**11b**) also showed significant potency, with a $K_i$ of 38 ± 4 nM, whereas PhTX-38 (**11a**) was significantly less potent with a $K_i$ of 350 ± 30 nM. All analogues, **11a**−**g**, showed increased potency at more negative holding potentials ($V_{H-40}$ vs $V_{H-80}$), but a large variation in the degree of voltage dependence was observed. The antagonist potency of (*S*)-PhTX-83 (**11f**) and PhTX-56 (**11c**) was highly voltage-dependent with $V_{H-40}/V_{H-80}$ ratios of 123 and 86, respectively, while that of PhTX-47 (**11b**) was almost voltage-independent, with the $V_{H-40}/V_{H-80}$ value of 3.6 (Table 1).

To investigate the selectivity of analogues **11a**−**g** toward other types of AMPARs as well as kainate receptors, the compounds were tested at heteromeric GluR1+GluR2 receptors and at homomeric GluR5(Q) receptors (Table 2). PhTX-56 (**11c**) showed only a weak ability to antagonize GluR1+GluR2 and GluR5(Q) receptors, with $K_i$ values of 5.2 ± 3.0 and 2.2 ± 1.0 $\mu$M, respectively. The compound is thus 1700- and 650-fold, respectively, more potent at homomeric GluR1 AMPARs. PhTX-47 (**11b**) was on the other hand inactive at GluR1+GluR2 receptors in the concentrations tested ($K_i$ > 10 $\mu$M), while being a potent antagonist of GluR5(Q) receptors ($K_i$ = 60 ± 14 nM), and equally potent at GluR1 receptors ($K_i$ = 38 ± 4 nM). The remaining analogues, **11a** and **11d**−**g**, were generally inactive at GluR1+GluR2 receptors, while being equally potent at GluR1 and GluR5(Q) receptors (Tables 1 and 2).

**Table 2.** Antagonist Effect of Philanthotoxin Analogues **11a**−**g** on GluR1+GluR2 and GluR5(Q)[a]

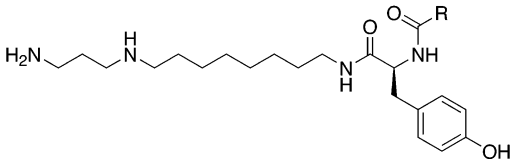
| compounds | $K_i$ ($\mu$M) GluR1+GluR2[b] | $K_i$ ($\mu$M) GluR5(Q)[c] |
|---|---|---|
| PhTX-38 (**11a**) | >10 | 0.9 ± 0.31 |
| PhTX-47 (**11b**) | >10 | 0.060 ± 0.014 |
| PhTX-56 (**11c**) | 5.2 ± 3.0 | 2.2 ± 1.0 |
| PhTX-65 (**11d**) | 6.4 ± 2.0 | 0.8 ± 0.1 |
| PhTX-74 (**11e**) | 1.6 ± 0.5 | 0.29 ± 0.08 |
| PhTX-83 (**11f**) | >10 | 0.25 ± 0.02 |
| PhTX-92 (**11g**) | >10 | 2.1 ± 0.3 |

[a] Inhibition of the current elicited by 100 $\mu$M glutamate by simultaneous co-application of the antagonist in oocytes injected with a 1:1 mixture of GluR1 flop and GluR2 flop RNA, as well as GluR5(Q) flop RNA. Values are means of two independent measurements performed in triplicate. [b] $K_i$ for PhTX-343 (**2**) is > 5 $\mu$M. [c] $K_i$ for PhTX-343 (**2**) is 15 ± 4 nM.

Modification of the acyl part of (*RS*)-PhTX-83 (**3**) generally had little influence on the activity, as shown in Table 3. Substitution of the butyric acid moiety with aromatic acyl groups, as in **14a**−**g**, either decreased or did not affect the activity as compared to (*S*)-PhTX-83 (**11f**), with **14c** being the least potent compound with a $K_i$ value of 250 ± 20 nM. Phenyl and benzyl analogues, **14a** and **14b**, respectively, were 2-fold less potent than **11f**, as was the case for the cinnamoyl analogue **14d**. Compounds **14e**−**g** all contained a pyridyl acyl group instead of the butyric acid, with a nitrogen atom as a potential hydrogen-bond acceptor, at different positions. The $K_i$ values at −80 mV decreased sequentially when moving the nitrogen from the 2- to the 3- and the 4-position with **14g** having a $K_i$ value of 64 ± 20 nM. The opposite relationship was observed at −40 mV, **14e** being the most potent antagonist.

In the analogues **14h**−**l**, the butyric acid part of **11f** was replaced by various aliphatic acyl groups. Substitution with the bulky and flexible cyclohexyl group (**14h**) led to retained activity, whereas the acetyl derivative

**Table 3.** Antagonist Effect of Philanthotoxin Analogues **14a**−**l** on GluR1[a,b]



| compounds | R | $K_i$ (μM) −40 mV | $K_i$ (μM) −80 mV | $V_{H-40}/V_{H-80}$ |
|---|---|---|---|---|
| **11f** | $(CH_2)_2CH_3$ | 8.1 ± 3.0 | 0.065 ± 0.020 | 123 |
| **14a** | Ph | 0.25 ± 0.05 | 0.13 ± 0.01 | 1.9 |
| **14b** | $CH_2Ph$ | 0.25 ± 0.03 | 0.11 ± 0.02 | 2.3 |
| **14c** | $CH_2CH_2Ph$ | 0.254 ± 0.06 | 0.25 ± 0.02 | 1.0 |
| **14d** | CH=CHPh | 0.17 ± 0.05 | 0.11 ± 0.03 | 1.5 |
| **14e** | 2-pyridyl | 0.17 ± 0.04 | 0.130 ± 0.03 | 1.3 |
| **14f** | 3-pyridyl | 0.210 ± 0.04 | 0.088 ± 0.03 | 2.4 |
| **14g** | 4-pyridyl | 0.33 ± 0.08 | 0.064 ± 0.02 | 5.1 |
| **14h** | Cyclohexyl | 0.24 ± 0.04 | 0.078 ± 0.02 | 3.1 |
| **14i** | Me | 0.30 ± 0.05 | 0.22 ± 0.05 | 1.4 |
| **14j** | Et | 0.28 ± 0.05 | 0.10 ± 0.01 | 2.8 |
| **14k** | $(CH_2)_4CH_3$ | 1.0 ± 0.2 | 0.068 ± 0.006 | 14.7 |
| **14l** | $C(CH_3)_3$ | 0.14 ± 0.04 | 0.090 ± 0.007 | 1.6 |

[a] Inhibition of the current elicited by 100 μM glutamate by simultaneous co-application of the antagonist in oocytes injected with GluR1 flop RNA. Values are means of two independent measurements performed in triplicate. [b] $K_i$ for PhTX-343 (**2**) is 170 ± 23 nM (−40 mV) and 22 ± 3 nM (−80 mV).

**14l** showed a reduced activity. Interestingly, increasing the size of the alkyl acyl group to an ethyl (**14j**) and pentyl (**14k**) gradually improved the activity compared to **14i**, with **14k** having a $K_i$ value of 68 ± 6 nM (−80 mV), and showing some voltage dependence. Finally, **14l** with a bulky *tert*-butyl acyl group was equipotent with the cyclohexyl analogue **14h**. Generally, the analogues with variation of the acyl moiety showed only weak voltage dependence, as compared to (*S*)-PhTX-83 (**11f**).

## Discussion

A series of analogues of (*RS*)-PhTX-83 (**3**), a novel potent noncompetitive antagonist of AMPARs, was synthesized using solid-phase methodologies. (*RS*)-PhTX-83 (**3**) was modified by systematic variation of the polyamine portion, while keeping the length and the number of amino groups constant, as well as by changing the acyl portion. The target compounds **11a**−**g** and **14a**−**l** were pharmacologically characterized using electrophysiology, employing AMPARs consisting of homomeric GluR1 and heteromeric GluR1+GluR2 receptors, as well as kainate receptors composed of homomeric GluR5(Q) subunits.

The $K_i$ value of (*S*)-PhTX-83 (**11f**) at homomeric GluR1 receptors was determined to be 65 ± 20 nM (Table 1), while the $IC_{50}$ value of (*RS*)-PhTX-83 (**3**) at non-NMDARs was previously determined as 32 ± 3 nM ($V_H = -80$ mV).[32] In the latter assay, the receptors were activated by kainate that nonselectively activates kainate receptors as well as AMPARs. However, the non-NMDARs were activated in a nondesensitizing manner typical for AMPARs,[40] thus indicating that AMPARs, rather than kainate receptors, are the target for (*RS*)-PhTX-83 (**3**). It has previously been suggested that activation of AMPARs by glutamate and kainate leads to different conformational states of the open channel,[41] and kainate (100 μM) was used as agonist in the non-NMDA receptor assay, whereas glutamate (100 μM) was used in the present study. Since philanthotoxins are believed to be open-channel blockers of AMPAR, it could be expected that antagonism of **3** and **11f** in the two assays would be different, reflecting the two different open states of the receptor channel generated by the two different agonists. However, this is clearly not the case.

Previous studies have shown that modification of the polyamine moiety of (*S*)-PhTX-343 (**2**) had a major impact on the potency, selectivity, and voltage dependence toward iGluRs and nAChRs.[29,32] Thus, replacement of one secondary amino group by a methylene group, as in (*RS*)-PhTX-83 (**3**), led to a significant increase in the potency at AMPARs.[32] We have now shown that changing the position of the secondary amino group in the polyamine moiety of (*S*)-PhTX-83 (**11f**) led to dramatic changes in the potency at GluR1 AMPARs (Table 1), while retaining selectivity and voltage dependence. The most potent analogue was PhTX-56 (**11c**) with a $K_i$ value of 3.3 ± 0.78 nM (Figure 1), thus increasing the potency at AMPARs 20-fold relative to (*S*)-PhTX-83 (**11f**). PhTX-47 (**11b**) was also more potent than **11f** with a $K_i$ value of 38 ± 4 nM, while analogues **11a**, **11d**, **11e**, and **11g** were less potent than **11f** (Table 1).

It is well known, and extensively used, that PhTX-343 (**2**) antagonize $Ca^{2+}$-permeable AMPARs, that is, receptors containing a glutamine (Q) in the Q/R-site, whereas receptors with the GluR2 subunit having arginine (R) at the same position are not antagonized.[2,3] Thus, one or more of the amino groups of (*S*)-PhTX-343 (**2**) is believed to interact with the Q/R-site, and removal of one of these groups, as in (*S*)-PhTX-83 (**11f**) or PhTX-56 (**11c**), might lead to lack of the selectivity for the $Ca^{2+}$-permeble AMPARs. However, as shown in Tables 1 and 2, this is not the case, since both **11f** and **11c** show selectivity for homomeric GluR1 receptors as compared to heteromeric GluR1+GluR2 receptors. These results suggest that it is the primary amino group of (*S*)-PhTX-343 (**2**), (*S*)-PhTX-83 (**11f**), and (*S*)-PhTX-56 (**11c**) that determines the selectivity. Furthermore, (*S*)-PhTX-56 (**11c**) shows an interesting selectivity for AMPAR relative to kainate receptors, being about 650-

fold more potent at GluR1 than at GluR5(Q). This selectivity cannot be explained by differences in the Q/R-site but must be due to other structural differences in the ion channel.

The previously reported enhanced potency of (*RS*)-PhTX-83 (**3**) relative to (*S*)-PhTX-343 (**2**)[32] might, at least in part, be due to increased hydrophobicity of **3**, as it was recently demonstrated that a major component of block by polyamines involves hydrophobic binding.[42] The difference in activity between **3** and (*S*)-PhTX-56 (**11c**), however, cannot be explained by differences in hydrophobicity. It has previously been proposed that the mechanism of the channel blockade by this class of polyamine toxins involves anchoring of the molecules by the aromatic headgroup, while the polyamine portion interacts with polar or charged amino acid residues in the interior of the ion channel, indicating a "stretched-out" conformation.[43] Thus, the increased activity of (*S*)-PhTX-56 (**11c**) as compared to (*S*)-PhTX-83 (**11f**) might reflect the secondary amino group of **11c** being placed in a more favorable position in the ion channel, for example, closer to a charged amino acid residue.

However, recent studies have indicated that the "channel-active" conformation of (*S*)-PhTX-343 (**2**) might be folded; either by hydrogen bonding between the aromatic moiety and the polyamine chain[44] or by folding of the polyamine chain leading to cyclic polyamine structures.[45] In particular, the former hypothesis could explain increased antagonism of **11c**, and the secondary amino group might lead to a more favorable "channel-active" conformation rather than reflecting amino acid positions inside the ion channel. However, further studies are required to elucidate these aspects.

In the series of analogues **14a**−**l**, only minor differences in the antagonistic activity as compared to (*S*)-PhTX-83 (**11f**) was observed, but **14a**−**l** displayed only a weak voltage dependence as compared to **11f**. At $V_H = -80$ mV, the 3-pyridyl (**14f**), 4-pyridyl (**14g**), cyclohexyl (**14h**), and pentyl (**14k**) analogues were the most potent, being equally potent to **11f**, with **14k** showing voltage dependence. Substituting the butyric acid moiety for a phenyl acetyl group, as in **14b**, led to a 2-fold decrease in activity. This is in contrast to what has been demonstrated for (*S*)-PhTX-343 (**2**) when tested at non-NMDA receptors, where replacement of the butyric acid portion of **2** with a phenyl acetyl moiety led to a 50-fold increase in activity relative to **2**,[31] and previous studies have shown that increasing hydrophobicity of **2** enhanced activity at various iGluRs.[46,47] Thus, replacement of the butyric acid moiety with a phenyl acetyl group or replacement of a secondary amino group with a methylene group as in (*RS*)-PhTX-83 (**3**) increases hydrophobicity and concomitantly activity, whereas further increase in the hydrophobicity of (*S*)-PhTX-83 (**11f**) as in **14b** did not affect the activity. This may suggest that **11f** possess an "optimal" hydrophobicity for binding to AMPARs. Another possibility is that increase of hydrophobicity of philanthotoxin analogues also changes the mode by which these analogues bind to AMPARs, similarly to what has been observed for hydrophobic philanthotoxin analogues binding to nAChR.[48]

In conclusion, variation of the polyamine moiety of (*RS*)-PhTX-83 (**3**) had a significant impact on the ability to antagonize AMPARs. (*S*)-PhTX-56 (**11c**), in particular, is a highly potent, selective voltage-dependent antagonist of $Ca^{2+}$-permeable AMPARs, being more than 1000-fold less potent at GluR2-containing AMPARs not permeable to $Ca^{2+}$ and more than 500-fold less potent at kainate receptors. On the other hand, variation of the acyl moiety of (*S*)-PhTX-83 (**11f**), turned out to have a limited effect on the antagonistic activity.

## Experimental Section

**Chemistry. General Procedures.** All starting materials and solvents were used without further purification, except DMF, which was stored over 3 Å molecular sieves, and THF, which was distilled under $N_2$ from sodium/benzophenone. Resin-bound diamines (trityl chloride resin, 1% divinylbenzene, 200−400 mesh) and (*S*)-*N*-Fmoc-*O*-(*tert*-butyl)tyrosine were obtained from Novabiochem (Läufelingen, Switzerland). $^1$H and $^{13}$C spectra were recorded on a Bruker Avance DRX 500 spectrometer operating for $^1$H at 500.13 MHz. Chemical shifts are reported in ppm ($\delta$), using TMS as an internal standard. Coupling constants ($J$) are given in Hz. Multiplicities of $^1$H NMR signals are given as follows: s, singlet; bs, broad singlet; d, doublet; t, triplet; q, quartet; p, pentet; s, sextet; m, multiplet.

Analytical and preparative high-performance liquid chromatography (HPLC-MS) was performed on a Perkin-Elmer API 150 EX instrument equipped with Turbo Ionspray source. The HPLC system consisted of two Shimadzu LC8A pumps. UV trace was obtained with a Shimadzu SPD10A detector operating at 274 nm. Evaporative light scattering (ELS) trace was obtained with Eurosep DDL 31 Light Scattering Detector and was used for estimation of the purity of the final products. Analytical HPLC-MS was performed on a 50 × 4.6 mm YMC RP18 column, using 2 mL/min of water/acetonitrile/TFA 90:10:0.05 raising to 10:90:0.05 during 7 min, with 10 $\mu$L injections. Preparative HPLC-MS (split-flow MS detection) was run with 190 $\mu$L injections (50 mg samples in 1.0 mL MeOH) to a 50 × 20 mm YMC RP18 column eluted with the same solvent gradient at 22.7 mL/min.

Accurate mass determinations (±5 ppm) were performed at the University of Southern Denmark, Odense University, Department of Chemistry, on an IonSpec Fourier Transformer Mass Spectrometer, using matrix-assisted laser desorption ionization (MALDI) with 2,5-dihydroxybenzoic acid as matrix.

**Preparation of *N*-(Trimethylsilyl)ethoxycarbonyl Amino Alcohols 5a−g. General Procedure.** To a solution of an amino alcohol (42.7 mmol) in $CH_2Cl_2$ (70 mL) was added triethylamine (85.4 mmol) and a solution of 2-(trimethylsilyl)-ethyl 4-nitrophenyl carbonate (42.7 mmol) in $CH_2Cl_2$ (20 mL). The reaction mixture was left overnight at room temperature. The solvent was removed in vacuo and the resulting yellow oil was redissolved in $CH_2Cl_2$ and washed several times with brine, saturated $NaHCO_3$, and 2 M NaOH until the organic phase was colorless. The organic phase was dried ($MgSO_4$) and concentrated in vacuo to give a colorless oil. The product was used without further purification.

**3-[*N*-[2-(Trimethylsilyl)ethoxycarbonyl]amino]ethanol (5a).** Yield: 92%. $^1$H NMR ($CDCl_3$): $\delta$ 0.10 [s, $Si(CH_3)_3$], 0.94 (t, $J$ = 8.3 Hz, $SiCH_2$), 2.97 (bs, OH/NH), 3.29 (q, $J$ = 6.1 Hz, $NH-CH_2$), 3.66 (q, $J$ = 5.7 Hz, $HO-CH_2$), 4.12 (t, $J$ = 8.5 Hz, $OCO-CH_2$), 5.25 (bs, OH/NH). $^{13}$C NMR ($CDCl_3$): $\delta$ −1.7 (3 C), 17.6, 43.2, 62.1, 63.1, 157.5.

**3-[*N*-[2-(Trimethylsilyl)ethoxycarbonyl]amino]propanol (5b).**[33] Yield: 89%. $^1$H NMR ($CDCl_3$): $\delta$ 0.16 [s, $Si(CH_3)_3$], 0.98 (t, $J$ = 8.3 Hz, $SiCH_2$), 1.70 (m, $CH_2$), 2.70 (bs, OH/NH), 3.33 (q, $J$ = 6.0 Hz, $NH-CH_2$), 3.68 (q, $J$ = 5.6 Hz, $HO-CH_2$), 4.18 (t, $J$ = 8.4 Hz, $OCO-CH_2$), 4.89 (bs, OH/NH). $^{13}$C NMR ($CDCl_3$): $\delta$ −1.6 (3 C), 17.7, 32.7, 37.3, 59.3, 63.2, 156.5.

**3-[*N*-[2-(Trimethylsilyl)ethoxycarbonyl]amino]-butanol (5c).**[33] Yield: 90%. $^1$H NMR ($CDCl_3$): $\delta$ 0.15 [s, $Si(CH_3)_3$], 0.97 (t, $J$ = 8.2 Hz, $SiCH_2$), 1.60 (m, 4H, $CH_2$), 3.20 (t, $J$ = 7.5 Hz, $NH-CH_2$), 3.67 (q, $J$ = 5.1 Hz, $HO-CH_2$), 4.15 (t,

$J$ = 8.2 Hz, OCO−$CH_2$), 4.72 (bs, OH). $^{13}$C NMR ($CDCl_3$): $\delta$ −1.6 (3 C), 17.7, 26.5, 29.6, 40.5, 62.4, 62.8,156.8.

**3-[*N*-[2-(Trimethylsilyl)ethoxycarbonyl]amino]pentanol (5d).** Yield: 87%. $^1$H NMR ($CDCl_3$): $\delta$ 0.10 [s, Si($CH_3$)$_3$], 0.93 (t, $J$ = 8.5 Hz, Si$CH_2$), 1.34−1.58 (m, 6H, $CH_2$), 1.89 (bs, OH/NH), 3.14 (t, $J$ = 7.5 Hz, NH−$CH_2$), 3.60 (q, $J$ = 5.3 Hz, HO−$CH_2$), 4.10 (t, $J$ = 8.3 Hz, OCO−$CH_2$), 4.71 (bs, OH/NH). $^{13}$C NMR ($CDCl_3$): $\delta$ −1.2 (3 C), 18.1, 23.3, 30.1, 32.5, 41.1, 62.6, 63.1, 156.7.

**3-[*N*-[2-(Trimethylsilyl)ethoxycarbonyl]amino]hexanol (5e).** Yield: 81%. $^1$H NMR ($CDCl_3$): $\delta$ 0.10 [s, Si($CH_3$)$_3$], 0.93 (t, $J$ = 8.5 Hz, Si$CH_2$), 1.34 (bs, 4H, $CH_2$), 1.41−1.56 (m, 4H, $CH_2$), 1.94 (bs, OH/NH), 3.11 (t, $J$ = 7.3 Hz, NH−$CH_2$), 3.59 (t, $J$ = 7.8 Hz, HO−$CH_2$), 4.10 (t, $J$ = 8.3 Hz, OCO−$CH_2$), 4.69 (bs, OH/NH). $^{13}$C NMR ($CDCl_3$): $\delta$ −1.1 (3 C), 18.1, 25.7, 26.8, 30.4, 32.9, 41.1, 63.0, 63.2, 157.3.

**3-[*N*-[2-(Trimethylsilyl)ethoxycarbonyl]amino]heptanol (5f).** Yield: 38%. $^1$H NMR ($CDCl_3$): $\delta$ 0.06 [s, Si($CH_3$)$_3$], 0.87 (t, $J$ = 8.5 Hz, Si$CH_2$), 1.18−1.48 (m, 10H, $CH_2$), 1.68 (bs, OH/NH), 3.09 (t, $J$ = 7.1 Hz, NH−$CH_2$), 3.58 (t, $J$ = 8.5 Hz, HO−$CH_2$), 4.16 (t, $J$ = 7.3 Hz, OCO−$CH_2$), 4.64 (bs, OH/NH). $^{13}$C NMR ($CDCl_3$): $\delta$ −1.6 (3 C), 17.6, 25.5, 26.5, 28.8 (2 C), 29.8, 32.5, 62.6, 70.7, 156.7.

**3-[*N*-[2-(Trimethylsilyl)ethoxycarbonyl]amino]octanol (5g).** Yield: 86%. $^1$H NMR ($CDCl_3$): $\delta$ −0.10 [s, Si($CH_3$)$_3$], 0.93 (t, $J$ = 8.1 Hz, Si$CH_2$), 1.28 (s, 8H, $CH_2$), 1.45 (m, 2H, $CH_2$), 1.52 (m, 2H, $CH_2$), 3.11 (t, $J$ = 6.9 Hz, NH−$CH_2$), 3.59 (t, $J$ = 6.6 Hz, HO−$CH_2$), 4.11 (t, $J$ = 8.1 Hz, OCO−$CH_2$). $^{13}$C NMR ($CDCl_3$): $\delta$ −1.6 (3 C), 17.8, 25.5, 26.5, 29.2, 29.3 (2 C), 30.0, 32.7, 40.9, 62.9, 156.8.

**Synthesis of 11a−g. General Procedure.** A resin-bound diamine (1.0 mmol) was suspended in $CH_2Cl_2$ (25 mL). Diisopropylethylamine (DIEA) (6.0 mmol) and *o*-nitrobenzenesulfonyl chloride (4.0 mmol) were added successively and the reaction mixture was stirred under nitrogen at room temperature for 3 h. The resin was drained, washed with DMF (3 × 5 mL), $CH_2Cl_2$ (3 × 5 mL), MeOH (3 × 5 mL), and $CH_2Cl_2$ (3 × 5 mL) and dried in vacuo.

The above resin (0.92 mmol) was suspended under nitrogen in dry THF/$CH_2Cl_2$ (1:1) (11.0 mL). A solution of a *N*-(trimethylsilyl)ethoxycarbonyl amino alcohol (4.61 mmol) in dry THF/$CH_2Cl_2$ (1:1) (6.0 mL), tributylphosphine (4.61 mmol) and a solution of ADDP (4.61 mmol) in dry THF/$CH_2Cl_2$ (1:1) (6.0 mL) were added successively. The mixture was stirred at room temperature under nitrogen for 3 h. The resin was drained and washed with DMF (3 × 5 mL), $CH_2Cl_2$ (3 × 5 mL), MeOH (3 × 5 mL), and $CH_2Cl_2$ (3 × 5 mL) and dried in vacuo. The procedure was repeated two more times, and the resulting resin was dried in vacuo.

The above resin (0.74 mmol) was suspended in dry THF (30 mL) under nitrogen in a flask equipped with a thermometer. A solution of TBAF (1 M in THF, 3.69 mmol) was added slowly, and the mixture was stirred at 50 °C for 30 min. The reaction mixture was cooled to room temperature; the resin was filtered off and washed with DMF (3 × 5 mL), $CH_2Cl_2$ (3 × 5 mL), MeOH (3 × 5 mL), and $CH_2Cl_2$ (3 × 5 mL) and dried in vacuo.

A solution of (*S*)-*N*-Fmoc-*O*-(*tert*-butyl)tyrosine (1.08 mmol) and HATU (1.08 mmol) in DMF (2 mL), followed by a solution of collidine (1.62 mmol) in DMF (1 mL), was added to the resin (0.27 mmol) placed in a syringe. The mixture was agitated for 2 h at room temperature, and the resin was subsequently washed with DMF (3 × 5 mL), $CH_2Cl_2$ (3 × 5 mL), MeOH (3 × 5 mL), and $CH_2Cl_2$ (3 × 5 mL). The product was treated with 20% piperidine in DMF (v/v, 5.0 mL) and the mixture was agitated for 3 min at room temperature. The resulting resin was washed with DMF (3 × 5 mL), treated again with 20% piperidine in DMF (5.0 mL) for 20 min, and then washed with DMF (3 × 5 mL), $CH_2Cl_2$ (3 × 5 mL), MeOH (3 × 5 mL), and $CH_2Cl_2$ (3 × 5 mL). The resulting resin was treated with a solution of butyric acid (1.08 mmol) and HATU (1.08 mmol) in DMF (2 mL), followed by a solution of collidine (1.62 mmol) in DMF (1 mL). The mixture was agitated for 2 h at room temperature, and the resulting resin was washed with DMF (3 × 5 mL), $CH_2Cl_2$ (3 × 5 mL), MeOH (3 × 5 mL), and $CH_2Cl_2$ (3 × 5 mL).

The resin (0.27 mmol) was treated with DBU (1.35 mmol) in DMF (2 mL) and mercaptoethanol (1.35 mmol) in DMF (2 mL) for 30 min. The resin was drained, washed with DMF (5 × 5 mL), and the procedure was repeated agitating for 5 min. The resin was washed with DMF (3 × 5 mL), $CH_2Cl_2$ (3 × 5 mL), MeOH (3 × 5 mL), and $CH_2Cl_2$ (3 × 5 mL) and then treated with a solution of $CH_2Cl_2$/TFA/triisopropylsilane/$H_2O$ (47.5:47.5:2.5:2.5 v/v, 5 mL) for 2 h. The resin was drained and washed with MeOH (2 × 5 mL) and $CH_2Cl_2$ (2 × 5 mL). The solution of the cleaved product and the washings were combined and evaporated in vacuo to a sticky solid which was triturated with diethyl ether and purified by preparative HPLC to give the final product as a colorless gum.

**(*S*)-*N*-[3-[(8-Aminooctyl)amino]propyl]-4-hydroxy-α-[(1-oxobutyl)amino]benzenepropaneamide bis(trifluoroacetate) (11a).** Yield: 60%. $^1$H NMR and $^{13}$C NMR as previously reported.[29]

**(*S*)-*N*-[4-[(7-Aminoheptyl)amino]butyl]-4-hydroxy-α-[(1-oxobutyl)amino]benzenepropaneamide bis(trifluoroacetate) (11b).** Yield: 65%. $^1$H NMR ($CD_3OD$): $\delta$ 0.84 (t, $J$ = 7.3 Hz, 3H), 1.41−1.69 (m, 16H), 2.16 (t, $J$ = 7.3 Hz, 2H), 2.80 (dd, $J_{AB}$ = 13.8 Hz, $J_{AX}$ = 8.5 Hz, 1H), 2.89−3.19 (m, 9H), 4.43 (t, $J$ = 8.5 Hz, 1H), 6.71 and 7.05 (AA′BB′ system, aromatic H). $^{13}$C NMR ($CD_3OD$): $\delta$ 14.3, 20.6, 24.8, 27.6 (2 C), 27.7 (2 C), 28.8, 30.0, 38.7, 39.1, 39.7, 41.1, 57.3, 116.7 (2 C), 129.5, 131.7 (2 C), 157.7, 174.5, 176.5. HPLC-ELS: 99.7%. HRMS (MALDI): $C_{24}H_{42}N_4O_3$ requires M + 1 at $m/z$ 434.3257; found 434.3263.

**(*S*)-*N*-[5-[(6-Aminohexyl)amino]pentyl]-4-hydroxy-α-[(1-oxobutyl)amino]benzenepropaneamide bis(trifluoroacetate) (11c).** Yield: 16%. $^1$H NMR ($CD_3OD$): $\delta$ 0.85 (t, $J$ = 7.4 Hz, 3H), 1.22−1.69 (m, 16H), 2.16 (t, $J$ = 7.3 Hz, 2H), 2.80 (dd, $J_{AB}$ = 13.6 Hz, $J_{AX}$ = 8.0 Hz, 1H), 2.84−3.23 (m, 9H), 4.45 (t, $J$ = 7.6 Hz, 1H), 6.70 and 7.05 (AA′BB′ system, aromatic H).$^{13}$C NMR ($CD_3OD$): $\delta$ 16.0, 22.3, 26.7, 28.8, 29.0 (2 C), 29.1 (2 C), 30.4, 31.7, 40.4, 40.8, 41.9, 42.6, 58.8, 118.3 (2 C), 131.2, 133.4 (2 C), 159.3, 175.9, 178.0. HPLC-ELS: 99.9%. HRMS (MALDI): $C_{24}H_{42}N_4O_3$ requires M + 1 at $m/z$ 434.3257; found 434.3261.

**(*S*)-*N*-[6-[(5-Aminopentyl)amino]hexyl]-4-hydroxy-α-[(1-oxobutyl)amino]benzenepropaneamide bis(trifluoroacetate) (11d).** Yield: 34%. $^1$H NMR ($CD_3OD$): $\delta$ 0.85 (t, $J$ = 7.5 Hz, 3H), 1.22−1.76 (m, 16H), 2.15 (t, $J$ = 7.6 Hz, 2H), 2.72−3.20 (m, 10H), 4.45 (t, $J$ = 7.7 Hz, 1H), 6.69 and 7.04 (AA′BB′ system, aromatic H).$^{13}$C NMR ($CD_3OD$): $\delta$ 12.3, 18.7, 22.9, 25.2, 25.5, 25.6, 25.6, 26.5 (2 C), 28.4 (2 C), 36.8, 37.2, 38.5, 38.8, 55.1, 114.8 (2 C), 127.7, 129.9 (2 C), 156.0, 172.4, 174.6. HPLC-ELS: 98.9%. HRMS (MALDI): $C_{24}H_{42}N_4O_3$ requires M + 1 at $m/z$ 434.3257; found 434.3272.

**(*S*)-*N*-[7-[(4-Aminobutyl)amino]heptyl]-4-hydroxy-α-[(1-oxobutyl)amino]benzenepropaneamide bis(trifluoroacetate) (11e).** Yield: 31%. $^1$H NMR ($CD_3OD$): $\delta$ 0.85 (t, $J$ = 7.5 Hz, 3H), 1.21−1.76 (m, 16H), 2.15 (t, $J$ = 7.8 Hz, 2H), 2.74−3.24 (m, 10H), 4.45 (t, $J$ = 6.9 Hz, 1H), 6.69 and 7.04 (AA′BB′ system, aromatic H). $^{13}$C NMR ($CD_3OD$): $\delta$ 12.3, 18.7, 22.7, 24.1, 25.6 (2 C), 25.8, 25.9, 28.2 (2 C), 28.5, 36.9, 37.2, 38.5, 38.6, 55.1, 114.8 (2 C), 127.7, 129.9 (2 C), 156.0, 172.4, 174.6. HPLC-ELS: 100.0%. HRMS (MALDI):$C_{24}H_{42}N_4O_3$ requires M + 1 at $m/z$ 434.3257; found 434.3265.

**(*S*)-*N*-[8-[(3-Aminopropyl)amino]octyl]-4-hydroxy-α-[(1-oxobutyl)amino]benzenepropaneamide bis(trifluoroacetate) (11f).** Yield: 27%. $^1$H NMR and $^{13}$C NMR as previously reported.[29]

**(*S*)-*N*-[9-[(2-Aminoethyl)amino]nonyl]-4-hydroxy-α-[(1-oxobutyl)amino]benzenepropaneamide bis(trifluoroacetate) (11g).** Yield: 12%. $^1$H NMR ($CD_3OD$): $\delta$ 0.84 (t, $J$ = 7.5 Hz, 3H), 1.15−1.78 (m, 16H), 2.15 (t, $J$ = 7.6 Hz, 2H), 2.77 (dd, $J_{AX}$ = 8.4 Hz, $J_{AB}$ = 13.0 Hz, 1H), 2.98 (dd, $J_{BX}$ = 6.9 Hz, $J_{AB}$ = 13.0 Hz, 1H), 3.03−3.13 (m, 8H), 4.66 (t, $J$ = 7.3 Hz, 1H), 6.68 and 7.03 (AA′BB′ system, aromatic H).$^{13}$C NMR ($CD_3OD$): $\delta$ 12.3, 18.7, 25.7, 25.8, 26.2, 28.5 (2 C), 28.6, 28.7, 28.7, 35.3, 36.9, 37.2, 38.8, 44.1, 55.0, 114.7 (2 C), 127.7, 129.9 (2

C), 155.0, 172.4, 174.6. HPLC-ELS: 99.3%. HRMS (MALDI): $C_{24}H_{42}N_4O_3$ requires M + 1 at *m/z* 434.3257; found 434.3260.

**Synthesis of 14a−l. General Procedure.** A solution of (*S*)-*N*-Fmoc-*O*-(*tert*-butyl)tyrosine (1.08 mmol) and HATU (1.08 mmol) in DMF (2 mL), followed by a solution of collidine (1.62 mmol) in DMF (1 mL), was added to resin-bound *N*-[(*o*-nitrophenyl)sulfonyl]-*N*-[aminopropyl]-1,8-diamino octane (**9b**, 0.27 mmol), prepared as described above, placed in a syringe. The mixture was agitated for 2 h at room temperature, and the resin was subsequently washed with DMF (3 × 5 mL), $CH_2Cl_2$ (3 × 5 mL), MeOH (3 × 5 mL), and $CH_2Cl_2$ (3 × 5 mL). The product was treated with 20% piperidine in DMF (v/v, 5.0 mL) and the mixture agitated for 3 min at room temperature. The resulting resin was washed with DMF (3 × 5 mL), treated again with 20% piperidine in DMF (5.0 mL) for 20 min, and then washed with DMF (3 × 5 mL), $CH_2Cl_2$ (3 × 5 mL), MeOH (3 × 5 mL), and $CH_2Cl_2$ (3 × 5 mL). The resulting resin was treated with a solution of a carboxylic acid (1.08 mmol) and HATU (1.08 mmol) in DMF (2 mL), followed by a solution of collidine (1.62 mmol) in DMF (1 mL). The mixture was agitated for 2 h at room temperature and the resulting resin washed with DMF (3 × 5 mL), $CH_2Cl_2$ (3 × 5 mL), MeOH (3 × 5 mL), and $CH_2Cl_2$ (3 × 5 mL).

The resin (0.27 mmol) was treated with DBU (1.35 mmol) in DMF (2 mL) and mercaptoethanol (1.35 mmol) in DMF (2 mL) for 30 min. The resin was drained, washed with DMF (5 × 5 mL), and the procedure was repeated agitating for 5 min. The resin was washed with DMF (3 × 5 mL), $CH_2Cl_2$ (3 × 5 mL), MeOH (3 × 5 mL), and $CH_2Cl_2$ (3 × 5 mL) and then treated with a solution of $CH_2Cl_2$/TFA/triisopropylsilane/$H_2O$ (47.5:47.5:2.5:2.5 v/v, 5 mL) for 2 h. The resin was drained and washed with MeOH (2 × 5 mL) and $CH_2Cl_2$ (2 × 5 mL). The solution of the cleaved product and the washings were combined and evaporated in vacuo to a sticky solid which was triturated with diethyl ether and purified by preparative HPLC to give the final product as a clear gum.

**(*S*)-*N*-[8-[(3-Aminopropyl)amino]octyl]-4-hydroxy-α-[(1-oxophenylmethyl)amino]benzenepropaneamide bis-(trifluoroacetate) (14a).** Yield: 25%. $^1$H NMR ($CD_3OD$): δ 1.18−1.44 (m, 10H), 1.68 (t, *J* = 7.8 Hz, 2H), 2.07 (t, *J* = 7.9 Hz, 2H), 2.96−3.12 (m, 10H), 4.69 (t, *J* = 7.0 Hz, 1H), 6.71 and 7.09 (AA′BB′ system, aromatic H), 7.44 (dd, *J* = 7.4 Hz, *J* = 7.2 Hz, 2H), 7.52 (dd, *J* = 7.6 Hz, *J* = 7.6 Hz, 1H), 7.76 (d, *J* = 7.9 Hz, 2H). $^{13}$C NMR ($CD_3OD$): δ 28.3, 30.0, 30.3, 30.6, 32.9, 33.1, 40.8, 41.3, 43.3, 43.4, 48.7, 60.2, 119.2, 131.4, 132.1, 132.5, 134.3, 135.8, 138.3, 160.3, 172.9, 176.7. HPLC-ELS: 98.1%. HRMS (MALDI): $C_{27}H_{40}N_4O_3$ requires M + 1 at *m/z* 468.3100; found 468.3100.

**(*S*)-*N*-[8-[(3-Aminopropyl)amino]octyl]-4-hydroxy-α-[(1-oxo-2-phenylethyl) amino]benzenepropaneamide bis-(trifluoroacetate) (14b).** Yield: 10%. $^1$H NMR ($CD_3OD$): δ 1.30−1.41 (m, 10H), 1.67 (m, 2H), 2.06−2.10 (m, 2H), 2.80 (dd, $J_{AB}$ = 13.6 Hz, $J_{AX}$ = 7.8 Hz, 1H), 2.96−3.13 (m, 10H), 3.48 (d, *J* = 13.5 Hz, 1H), 3.52 (d, *J* = 13.5 Hz, 1H), 4.49 (t, *J* = 7.0 Hz, 1H), 6.67 (d, *J* = 6.5 Hz, 2H), 6.98 (d, *J* = 6.5 Hz, 2H), 7.13 (d, *J* = 6.9 Hz, 2H), 7.21−7.27 (m, 4H). $^{13}$C NMR ($CD_3$-OD): δ 25.7, 27.5, 27.8, 28.0, 30.3, 30.5, 38.3, 38.8, 40.7, 44.0, 46.2, 57.0 116.7, 128.3, 129.3, 130.0, 130.5, 131.7, 137.0, 157.7, 173.9, 174.2. HPLC-ELS: 99.7%. HRMS (MALDI): $C_{28}H_{342}N_4O_3$ requires M + 1 at *m/z* 482.3257; found, 482.3251.

**(*S*)-*N*-[8-[(3-Aminopropyl)amino]octyl]-4-hydroxy-α-[(1-oxo-3-phenylpropyl)amino]benzenepropaneamide bis-(trifluoroacetate) (14c).** Yield: 15%. $^1$H NMR ($CD_3OD$): δ 1.18−1.40 (m, 10H), 1.66−1.71 (m, 2H), 2.06 (m, 2H), 2.47 (t, *J* = 7.2 Hz, 2H), 2.73 (dd, $J_{AX}$ = 7.8 Hz, $J_{AB}$ = 14.0 Hz, 1H), 2.82 (t, *J* = 8.1 Hz, 2H), 2.90 (dd, $J_{AX}$ = 7.2 Hz, $J_{AB}$ = 14.0 Hz, 1H), 3.00−3.13 (m, 7H), 4.41 (t, *J* = 7.2 Hz,1H), 6.68 and 7.00 (AA′BB′ system, aromatic H), 7.14, (d, *J* = 8.1 Hz, 2H), 7.16 (dd, *J* = 7.1 Hz, *J* = 1.2 Hz, 1H), 7.24 (ddd, *J* = 7.5 Hz, *J* = 7.5 Hz, *J* = 1.8 Hz, 2H). $^{13}$C NMR ($CD_3OD$): δ 25.6, 25.9, 26.1, 28.5, 28.5, 28. 6, 31.2, 36.3, 36.9, 37.1, 38.7, 44.2, 55.1, 64.8, 114.8, 125.8, 127.6, 128.0, 128.1, 129.9, 140.8, 155.8, 156.0, 172.2, 173.7. HPLC-ELS: 100.0%. HRMS (MALDI): $C_{29}H_{44}N_4O_3$ requires M + 1 at *m/z* 496.3413; found 496.3405.

**(*S*)-*N*-[8-[(3-Aminopropyl)amino]octyl]-4-hydroxy-α-[(1-oxo-3-phenylprop- 2-enyl)amino]benzenepropaneamide bis(trifluoroacetate) (14d).** Yield: 24%. $^1$H NMR ($CD_3OD$): δ 1.18 (t, *J* = 7.1 Hz, 2H), 1.22−1.40 (m, 8H), 1.68 (t, *J* = 7.5 Hz, 2H), 2.07 (t, *J* = 7.8 Hz, 2H), 2.84−3.17 (m, 10H), 3.61 (q, *J* = 7.1 Hz, 1H), 4.60 (t, *J* = 7.5 Hz, 1H), 6.67 (d, *J* = 15.7 Hz, 1H), 6.71 and 7.07 (AA′BB′ system, aromatic H), 7.39 (d, *J* = 7.0 Hz, 2H), 7.50 (d, *J* = 15.8 Hz, 1H), 7.55 (d, *J* = 7.8 Hz, 1H). $^{13}$C NMR ($CD_3OD$): δ 28.3, 30.1, 30.3, 30.6, 32.9, 33.1, 40.8, 41.5, 43.3, 48.7, 59.8, 61.3, 119.2, 124.4, 131.8, 131.9, 133.8, 134.2, 139.2, 160.3, 171.2, 176.4. HPLC-ELS: 99.8%. HRMS (MALDI): $C_{29}H_{44}N_4O_3$ requires M + 1 at *m/z* 494.3257; found 494.3279.

**(*S*)-*N*-[8-[(3-Aminopropyl)amino]octyl]-4-hydroxy-α-[(1-oxo-1-(pyridin-2-yl)methyl)amino]benzenepropaneamide bis(trifluoroacetate) (14e).** Yield: 32%. $^1$H NMR ($CD_3OD$): δ 1.17−1.46 (m, 10H), 1.69 (m, 2H), 2.04−2.10 (m, 2H), 2.99−3.22 (m, 10H), 4.69 (t, *J* = 7.0 Hz, 1H), 6.69 and 7.06 (AA′BB′ system, aromatic H), 7.55 (m, 1H), 7.95 (m, 1H), 8.05 (d, *J* = 7.5 Hz, 1H), 8.62 (d, *J* = 4.5 Hz, 1H). $^{13}$C NMR ($CD_3OD$): δ 26.3, 28.0, 28.3, 28.6, 30.9, 31.0, 38.8, 39.9, 41.3, 46.7, 57.4, 117.2, 119.7, 128.9, 129.5, 132.4, 130.4, 146.5, 151.4, 158.4, 166.9, 174.1. HPLC-ELS: 99.8%. HRMS (MALDI): $C_{26}H_{39}N_5O_3$ requires M + 1 at *m/z* 469.3053; found, 469.3050.

**(*S*)-*N*-[8-[(3-Aminopropyl)amino]octyl]-4-hydroxy-α-[(1-oxo-1-(pyridin-3-yl)methyl)amino]benzenepropaneamide bis(trifluoroacetate) (14f).** Yield: 34%. $^1$H NMR ($CD_3OD$): δ 1.18−1.45 (m, 10H), 1.68 (m, 2H), 2.04−2.10 (m, 2H), 2.96−3.15 (m, 10H), 4.69 (t, *J* = 7.0 Hz, 1H), 6.70 and 7.10 (AA′BB′ system, aromatic H), 7.74 (dd, *J* = 8.0 Hz, *J* = 5.5 Hz, 1H), 8.45 (d, *J* = 8.5 Hz, 1H), 8.78 (d, *J* = 5.0 Hz, 1H), 9.01 (s, 1H). $^{13}$C NMR ($CD_3OD$): δ 24.4, 25.7, 26.2, 26.5, 26.8, 29.1, 29.2, 36.9, 37.4, 39.6, 47.6, 56.5, 115.4, 115.8, 118.1, 125.1, 128.1, 130.4, 131.9, 138.8, 146.5, 149.5, 156.5, 165.6, 172.4. HPLC-ELS: 99.5%. HRMS (MALDI): $C_{26}H_{39}N_5O_3$ requires M + 1 at *m/z* 469.3053; found, 469.3056.

**(*S*)-*N*-[8-[(3-Aminopropyl)amino]octyl]-4-hydroxy-α-[(1-oxo-1-(pyridin-4-yl)methyl)amino]benzenepropaneamide bis(trifluoroacetate) (14g).** Yield: 29%. $^1$H NMR ($CD_3OD$): δ 1.24−1.43 (m, 10H), 1.65−1.74 (m, 2H), 2.04−2.12 (m, 2H), 2.98−3.19 (m, 10H), 4.74 (t, *J* = 8.0 Hz, 1H), 6.72 and 7.10 (AA′BB′ system, aromatic H), 8.16 (d, *J* = 6.5 Hz, 2H), 8.91 (d, *J* = 6.5 Hz, 2H). $^{13}$C NMR ($CD_3OD$): δ 25.7, 27.5, 27.8, 28.1, 30.4, 30.4, 38.3, 38.7, 40.9, 41.0, 46.2, 57.9, 114.8, 116.7, 125.3, 129.3, 131.8, 147.6, 157.8, 166.5, 173.7. HPLC-ELS: 99.8%. HRMS (MALDI): $C_{26}H_{39}N_5O_3$ requires M + 1 at *m/z* 469.3053; found, 469.3066.

**(*S*)-*N*-[8-[(3-Aminopropyl)amino]octyl]-4-hydroxy-α-[(1-oxo-cyclohexylmethyl)amino]benzenepropaneamide bis(trifluoroacetate) (14h).** Yield: 29%. $^1$H NMR ($CD_3OD$): δ 1.22−1.42 (m, 16H), 1.40−1.71 (m, 7H), 2.07 (t, *J* = 7.6 Hz, 2H), 2.19 (t, *J* = 7.5 Hz, 1H), 2.75 (dd, $J_{AB}$ = 8.3 Hz, $J_{AX}$ = 14.0 Hz, 1H), 2.92 (dd, $J_{AB}$ = 6.9 Hz, $J_{AX}$ = 14.0 Hz, 1H), 2.99−3.14 (m, 8H), 4.46 (t, *J* = 7.8 Hz, 1H), 6.69 and 7.03 (AA′BB′ system, aromatic H). $^{13}$C NMR ($CD_3OD$): δ 26.7, 27.9, 28.1, 28.2, 28.4, 28.7, 29.0, 31.3, 31.5, 31.6, 32.1, 39.1, 39.8, 41.6, 47.1, 47.4, 57.6, 117.5, 130.4, 132.6, 158.6, 175.0, 180.1. HPLC-ELS: 96.1%. HRMS (MALDI): $C_{27}H_{46}N_4O_3$ requires M + 1 at *m/z* 474.3570; found, 474.3573.

**(*S*)-*N*-[8-[(3-Aminopropyl)amino]octyl]-4-hydroxy-α-[(1-oxoethyl)amino]benzenepropaneamide bis(trifluoroacetate) (14i).** Yield: 37%. $^1$H NMR ($CD_3OD$): δ 1.32−1.69 (m, 16H), 1.92 (s, 3H), 2.03−2.08 (m, 6H), 2.80 (dd, $J_{AB}$ = 13.6 Hz, $J_{AX}$ = 8.0 Hz, 1H), 2.98−3.13 (m 9H), 4.45 (t, *J* = 8.0 Hz, 1H), 6.70 and 7.04 (AA′BB′ system, aromatic H). $^{13}$C NMR ($CD_3OD$): δ 22.9, 25.7, 27.0, 27.5, 27.8, 28.0, 30.5, 30.5, 38.2, 38.8, 40.7, 40.9, 46.2, 57.2, 116.6, 129.5, 131.7, 157.7, 173.4, 174.0. HPLC-ELS: 99.7%. HRMS (MALDI): $C_{22}H_{38}N_4O_3$ requires M + 1 at *m/z* 406.2944; found, 406.2947.

**(*S*)-*N*-[8-[(3-Aminopropyl)amino]octyl]-4-hydroxy-α-[(1-oxopropyl)amino]benzenepropaneamide bis(trifluoroacetate) (14j).** Yield: 63%. $^1$H NMR ($CD_3OD$): δ 1.04 (t, *J* = 7.7 Hz, 3H), 1.20−1.47 (m, 10H), 1.69 (m, 2H), 2.07 (m, 2H), 2.19 (q, *J* = 7.7 Hz, 2H), 2.80 (dd, $J_{AB}$ = 13.8 Hz, $J_{AX}$ = 8.0

Hz, 1H), 2.94−3.18 (m, 10H), 4.46 (t, $J$ = 7.3 Hz, 1H), 6.70 and 7.04 (AA′BB′ system, aromatic H). $^{13}$C NMR ($CD_3OD$): $\delta$ 10.7, 25.8, 27.5, 27.8, 28.1, 30.4, 30.5, 38.3, 38.9, 40.7, 46.2, 57.0, 117.0, 129.5, 131.7, 157.7, 174.1, 177.1. HPLC-ELS: 98.5%. HRMS (MALDI): $C_{23}H_{40}N_4O_3$ requires M + 1 at $m/z$ 420.3100; found, 420.3099.

**(*S*)-*N*-[8-[(3-Aminopropyl)amino]octyl]-4-hydroxy-α-[(1-oxohexyl)amino]benzenepropaneamide bis(trifluoroacetate) (14k).** Yield: 10%. $^1$H NMR ($CD_3OD$): $\delta$ 0.88 (t, $J$ = 7.0 Hz, 3H), 1.27−1.41 (m, 16H), 1.52 (m, 2H), 2.08 (m, 2H), 2.17 (t, $J$ = 7.0 Hz, 2H), 2.78 (dd, $J_{AB}$ = 13.6 Hz, $J_{AX}$ = 7.0 Hz, 1H), 2.99−3.31 (m, 8H), 4.48 (t, $J$ = 7.0 Hz, 1H), 6.70 and 7.04 (AA′BB′ system, aromatic H). $^{13}$C NMR ($CD_3OD$): $\delta$ 14.6, 23.8, 25.8, 27.0, 27.5, 27.8, 28.1, 30.4, 30.4, 30.6, 32.8, 37.3, 38.3, 38.8, 40.7, 46.2, 48.9, 56.9, 116.6, 129.5, 131.7, 157.7, 174.1, 176.5. HPLC-ELS: 99.2%. HRMS (MALDI): $C_{26}H_{46}N_4O_3$ requires M + 1 at $m/z$ 462.3570; found, 462.357.

**(*S*)-*N*-[8-[(3-Aminopropyl)amino]octyl]-4-hydroxy-α-[(1-oxo-3-dimethylbutyl)amino]benzenepropaneamide bis(trifluoroacetate) (14l).** Yield: 58%. $^1$H NMR ($CD_3OD$): $\delta$ 1.11 (s, 9H), 1.23−1.44 (m, 10H), 1.69 (m, 2H), 2.08 (m, 2H), 2.84 (dd, $J_{AB}$ = 13.6 Hz, $J_{AX}$ = 7.0 Hz, 1H), 2.96−3.17 (m, 9H), 4.50 (t, $J$ = 7.0 Hz, 1H), 6.70 and 7.05 (AA′BB′ system, aromatic H).$^{13}$C NMR ($CD_3OD$): $\delta$ 24.7, 26.5, 26.8, 27.0, 29.4, 29.6, 37.2, 37.8, 39.1, 39.7, 45.2, 55.7, 116.0, 128.3, 130.8, 156.8, 173.1, 180.2. HPLC-ELS: 99.8%. HRMS (MALDI): $C_{25}H_{44}N_4O_3$ requires M + 1 at $m/z$ 448.3413; found, 448.3418.

**In Vitro Electrophysiology.** 3−5 ovarian lobes were surgically removed from anaesthetized *Xenopus laevis*. The removed ovaries were treated with collagenase type A (1 mg/mL, Boehringer) for 2−3 h at 20 °C in buffer OR-2 (88 mM NaCl, 1.1 mM KCl, 2.4 mM $NaHCO_3$, 0.8 mM $MgCl_2$, and 15 mM HEPES−NaOH pH 7.6) and oocytes at stage V or VI were isolated. Oocytes were maintained in Barth's solution and injected the day after isolation with 5−30 ng cRNA and maintained in Barth's solution (Buffer OR-2 supplemented with 0.3 mM $Ca(NO_3)_2$, 0.3 mM $CaCl_2$, 100 μg/mL gentamycin, 100 IU/mL penicillin, and 100 μg/mL streptomycin). Oocytes were recorded in Ringer solution (115 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$, 0.1 mM $MgCl_2$, and 10 mM HEPES−NaOH pH 7.5) 3−14 days postinjection using a two electrode voltage clamp (Warner OC-725C). The pipets had a resistance of 0.7−2 MΩ and were filled with 3 M KCl. Oocytes were clamped at −100 to −20 mV and for oocytes exhibiting high currents the recordings were performed in low $Ca^{2+}$ Ringer (115 mM NaCl, 2.5 mM, KCl, 0.1 mM $CaCl_2$, 1.8 mM $MgCl_2$, and 10 mM HEPES−NaOH pH 7.5) to avoid activation of the $Ca^{2+}$ activated Cl current.

**cRNA Synthesis.** Fragments containing only the coding regions of the different glutamate receptors were generated by PCR and cloned into pGEMHE. The clones were verified by sequencing. In vitro cRNA transcripts were generated as runoff transcription on 20 μg/mL linerized plasmids by incubation in 40 mM Tris-HCl, pH 7.8, 8 mM $MgCl_2$, 2 mM spermidine, 50 mM NaCl, 3.5 mM DTT, 0.5 mM ATP, 0.5 mM UTP, 0.5 mM CTP, 0.1 mM GTP, 0.5 mM GpppG, and 0.5 U/μL T7 polymerase for 90 min in the presence of trace amounts of [$^{32}$P]UTP for quantification of the cRNA.

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**Note Added after ASAP Posting.** This manuscript was released ASAP on 11/23/2002 with a production error in Scheme 2; the line between the shaded circle and N was missing in **9** and **10**. The correct version was posted on 12/12/2002.

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