Convergent Synthesis and Pharmacology of Substituted Tetrazolyl-2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid Analogues

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The synthesis and pharmacological characterization of 1- and 2-alkyltetrazolyl analogues of (*RS*)-2-amino-3-[3-hydroxy-5-(2-methyl-2*H*-5-tetrazolyl)-4-isoxazolyl]propionic acid (2-Me-Tet-AMPA), a highly potent and selective agonist at AMPA receptors, are presented. A shorter and more convergent synthetic route than previously described, employing a new method for introducing the amino acid moiety, was developed for these derivatives. The 2-substituted isomers were selective agonists, and their activity correlated inversely with the size of the substituent. Structural explanations of the structure−activity relationship are provided.

Introduction

(*S*)-Glutamic acid (Glu) is the major excitatory neurotransmitter in the central nervous system (CNS). The effects of Glu are mediated by ligand-gated ionotropic receptors (iGluRs) and G-protein-coupled metabotropic receptors (mGluRs). It is generally agreed that GluRs are implicated in a variety of CNS functions, including learning and memory, and that GluRs are potential therapeutic targets in a number of neurological and psychiatric disorders.[1,2] The iGluRs are categorized into three classes according to selective activation by the agonists *N*-methyl-D-aspartic acid (NMDA), (*S*)-2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid [(*S*)-AMPA, **1**] (Figure 1), and kainic acid (KA). Functional iGluRs are believed to assemble as tetramers from homo- or heteromeric combinations of a number of different subunits.[1,3] Thus, seven NMDA subunits (NR1, NR2A−D, NR3A,B), four AMPA subunits (GluR1−4), and five KA subunits (GluR5−7, KA1,2) have been cloned and characterized. In recent years, X-ray crystallographic studies of soluble constructs of the S1S2 ligand-binding domain of GluR2, cocrystallized with agonists and antagonists, have provided detailed insights into the processes of receptor activation, modulation, and inhibition.[4−8]

Enantiomeric resolution and pharmacological characterization of (*RS*)-2-amino-3-[3-hydroxy-5-(2-methyl-2*H*-5-tetrazolyl)-4-isoxazolyl]propionic acid (2-Me-Tet-AMPA, (*RS*)-**2**) has previously revealed (*S*)-**2** as the most potent AMPA receptor agonist yet described,[9,10] whereas the isomeric 1-Me-Tet-AMPA (**3**) shows no significant pharmacological activity.[9] In electrophysiological experiments using cloned homo- or heteromeric receptors in *Xenopus laevis* oocytes consisting of AMPA receptor subunits (GluR1−4), (*S*)-**2** displayed a minor subunit selectivity, showing some preference for cloned homomeric GluR3 or GluR4 receptors over heteromeric GluR1/GluR2 or homomeric GluR2 receptors.[10] More recently, (*S*)-2-Me-Tet-AMPA was cocrystallized with the GluR2S1S2 construct, and it was shown that the 2-methyltetrazolyl substituent is accommodated by a partly hydrophobic pocket in the ligand-binding domain. Interestingly however, the tetrazole ring was not involved in hydrogen bonding, save for a long intramolecular contact, but it merely filled out the pocket.[6]

To further investigate the structural requirements of the AMPA receptor for agonist binding, we then initiated and now describe the synthesis and pharmacologi-

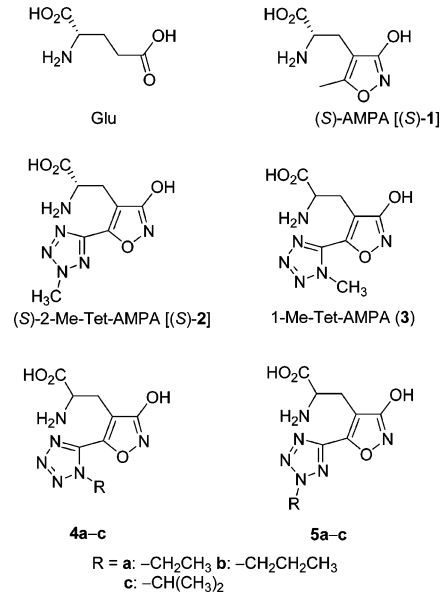

Figure 1. Chemical structures of (*S*)-glutamic acid (Glu), (*S*)-AMPA [(*S*)-**1**], and the AMPA agonist (*S*)-(2)-Me-Tet-AMPA [(*S*)-**2**], 1-Me-Tet-AMPA (**3**) and structures of the new series of alkylated tetrazolyl analogues of AMPA (**4a**−**c**, **5a**−**c**).

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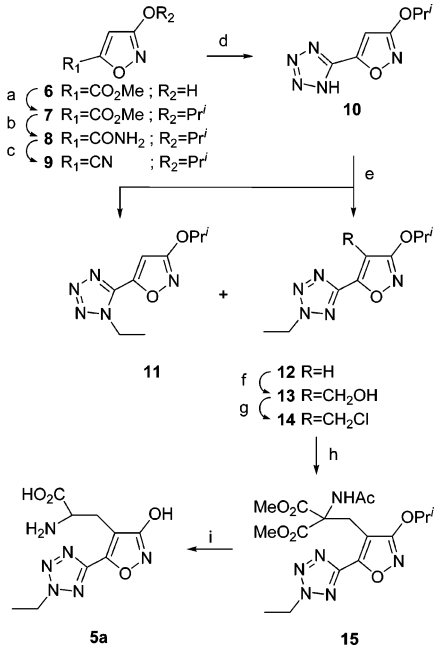
Scheme 1[a]



[a] Reagents and conditions: (a) $BrCH(CH_3)_2$, K_2CO_3, DMF, 60 °C; (b) aqueous NH_3, room temp; (c) $POCl_3$, 70 °C; (d) NaN_3, Et_3NHCl, DME, reflux; (e) ICH_2CH_3, K_2CO_3, acetone, room temp; (f) *n*-BuLi, $(CH_2O)_3$, THF, −78 to 20 °C; (g) $SOCl_2$, reflux; (h) $AcNHCH(CO_2CH_3)_2$, NaH, NaI, DMF, room temp; (i) 6 M HCl/AcOH, reflux.

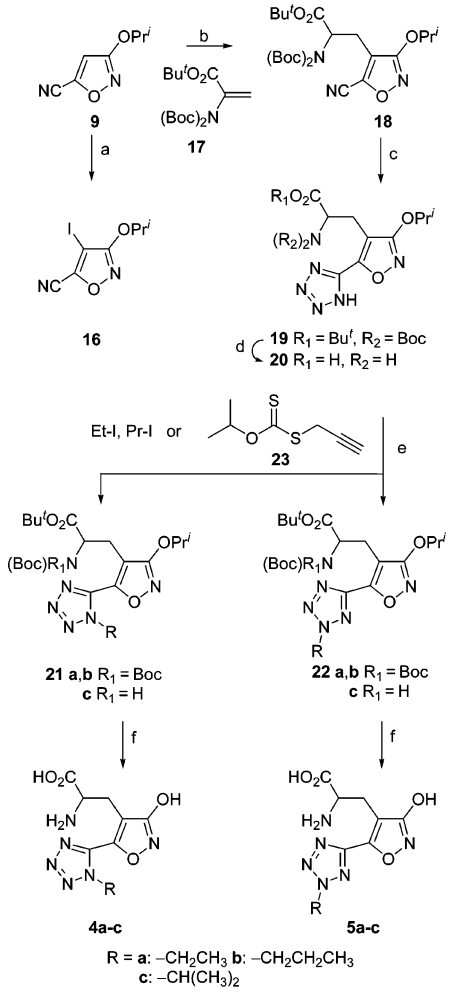
cal characterization of a series of substituted tetrazolyl-AMPA analogues in which the methyl group is replaced by ethyl (**4a**, **5a**), propyl (**4b**, **5b**), or isopropyl (**4c**, **5c**) substituents.

Results

Chemistry. For the synthesis of the target tetrazolyl analogues of AMPA, a more convenient route than that employed in the original synthesis of 2-Me-Tet-AMPA[9] was developed. The 2-ethyl-substituted **5a** was synthesized following the strategy outlined in Scheme 1. The 3-isoxazolol **6** obtained from dimethyl acetylenedicarboxylate[11] was protected as the *O*-isopropyl ether **7**. Aminolysis of the ester **7** to amide **8** was followed by dehydration to nitrile **9**. The reaction of **9** with in situ generated hydrazoic acid afforded tetrazole **10**. Subsequent alkylation with ethyl iodide gave the regioisomers **11** and **12** (ratio 1:3), which were separated by laborious flash column chromatography. Metalation of **12** followed by reaction with trioxane led to the alcohol **13**, albeit in low yield, which was converted to **14** with thionyl chloride. Reaction of chloromethyl **14** with dimethyl acetamidomalonate in the presence of sodium iodide provided **15**, which upon acidic deprotection gave **5a**.

In light of the inconvenience of the route outlined in Scheme 1, there obviously was room for improvement of the synthetic strategy (Scheme 2). Surprisingly, lithiation of the 4-position of the isoxazole ring of **9** at −78 °C was possible without affecting the nitrile group, and the lithium salt in THF solution was stable for several hours at −20 °C. Reaction of the lithiated species with iodine gave **16** in 68% yield (Scheme 2). This observation opens up the possibility of introducing the amino acid moiety at an early stage in the synthesis, but because of the sensitivity of **9** toward hydrolysis,

Scheme 2[a]



[a] Reagents and conditions: (a) *n*-BuLi, −78 °C, THF, I_2; (b) *n*-BuLi, −78 °C, THF, **17**, −50 to −40 °C; (c) NaN_3, Et_3NHCl, DME, 90 °C; (d) 6 M HCl, room temp. (e) **a** and **b**: RI, K_2CO_3, acetone, room temp. (e) **c**: **23**, toluene, reflux. (f) 48% aqueous HBr, reflux.

traditional strategies involving Strecker or Ugi syntheses did not seem feasible. Instead, a Michael addition of **9** to an α-amino acrylate was considered. Various acrylates were tried, and amino acrylate **17**[12] afforded **18** in 45% yield. Employing other metals such as Cu and Mg in this reaction did not improve the yield.

Treatment of **18** with sodium azide and triethylamine hydrochloride gave tetrazole **19** as a key intermediate. Selective deprotection using hydrochloric acid led to amino acid **20** in quantitative yield. Alkylation of **19** with ethyl iodide gave **21a** and **22a** in a 1:2 ratio. Similarly, alkylation with propyl iodide gave the regioisomers **21b** and **22b** in a 1:4 ratio. Alkylation of **19** with isopropyl bromide was very slow, whereas alkylation using *O*-isopropyl-*S*-propargyl xanthate **23**[13] provided the two regioisomers **21c** and **22c** in a 1:2 ratio. In agreement with the literature[14] 2-alkylation of the tetrazole **19** was favored over 1-alkylation because of steric effects. The regioisomers were separated using flash chromatography. Deprotection of **21a**−**c** and **22a**−**c** in one step led to the target products **4a**−**c** and **5a**−**c**, respectively. The regioisomeric assignment was based on a comparison of the tetrazole ^{13}C signal in regioisomers **21a**−**c** and **22a**−**c**. Thus, the tetrazole carbon

Table 1. Receptor Binding Affinities and Electrophysiological Data at Native iGluRs[a]

	receptor binding			
compd	[^{3}H]AMPA IC_{50} (nM)	[^{3}H]KA IC_{50} (nM)	[^{3}H]CGP 39653 K_i (nM)	electrophysiol cortical slice EC_{50} (nM)
(*S*)-**1**	21[b] [19; 22]	24000[c,d] [22000; 27000]	> 100000[b]	3800[d]
3[e]	54000	>100000	>100000	>1000000
(*RS*)-**2**[f]	27	>100000	>100000	330
(*S*)-**2**[f]	9	>100000	>100000	110
4a	27000 [24000; 31000]	>100000	>100000	nd
4b	>100000	>100000	>100000	nd
4c	>100000	>100000	>100000	nd
5a	134 [126; 142]	>100000	>100000	3500 [3200; 3800]
5b	2500 [2300; 2800]	>100000	>100000	32000 [31000; 33000]
5c	6200 [5300; 7200]	>100000	>100000	71000 [67000; 75000]

[a] Values are expressed as the antilog to the log of the mean of at least three individual experiments. The numbers in brackets [min; max] indicate ±SEM according to a logarithmic distribution of IC_{50} or K_i. [b] Reference 27. [c] B_{max1} was 20%. [d] Reference 28. [e] Reference 9. [f] Reference 10.

is more shielded in the 1-isomers (144 ppm) than in the corresponding 2-isomers (155−156 ppm) in accordance with previous reports.[15,16]

In Vitro Pharmacology. The binding affinities of **4a**−**c** and **5a**−**c** for the three classes of native iGluRs were tested using the radioligands [^{3}H]AMPA,[17] [^{3}H]KA,[18] and [^{3}H]CGP 39653[19] (NMDA receptors) in a binding assay using a rat brain synaptosomal preparation[20] (Table 1). Like the 2-methyltetrazolyl isomer (*RS*)-**2**,[9] the 2-isomers of the ethyl, propyl, and isopropyl analogues **5a**−**c** showed affinity for [^{3}H]AMPA binding sites, whereas the corresponding 1-isomers **4a**−**c** had no significant affinity. Affinity for the [^{3}H]KA or [^{3}H]CGP 39653 binding sites was insignificant or could not be detected for any of the substituted tetrazolyl analogues. The affinity for the [^{3}H]AMPA binding site decreased with increasing steric bulk of the tetrazolyl substituent. Thus, the 2-ethyl analogue **5a** showed 5 times weaker affinity than (*RS*)-**2**,[9] whereas the 2-propyl and 2-isopropyl analogues (**5b** and **5c**) displayed a pronounced loss of affinity, being 2 orders of magnitude weaker than (*RS*)-**2**.

A modified version[21] of the previously described rat cortical wedge preparation[22] was used as an in vitro electrophysiological model to evaluate the activity of the compounds at native iGluRs (Table 1). As with (*RS*)-**2**,[9] the 2-substituted alkyltetrazolyl analogues (**5a**−**c**) were AMPA receptor agonists. (*S*)-**2** and compounds **5a**−**c** were further characterized using cloned homomeric AMPA and KA receptors expressed in Sf9 insect cells[23] (Table 2). In general, the order of potencies detected in the binding to native receptors and the cortical wedge assays was also observed in this assay; affinity decreases with increasing substituent size. Although the compounds did not bind detectably to native KA receptor sites (Table 1), they showed affinity for the cloned homomeric GluR5 KA receptor sites (Table 2).

Molecular Modeling. The structural basis for the decreased AMPA receptor affinity with increasing steric bulk of the 2-tetrazolyl substituent was examined by flexibly docking the global minima of **5a**−**c** to the domain-closed extracellular binding construct of GluR2, believed to reflect the agonized state of the receptor. The X-ray structure of (*S*)-**2** bound to GluR2S1S2J (1M5B)[6] was prepared for docking according to the method recommended in the First Discovery package.[24] Usually, it is the *S*-enantiomers of AMPA analogues that possess biological activity, whereas the *R*-enantiomers generally fail to activate the receptor.[10,25] The *S*-enantiomers of **2** and **5a**−**c** were built in tri-ionized form, minimized, conformationally searched using MMFFs/GB-SA, and docked using Glide 3.5.[24] The experimental binding mode of (*S*)-**2** was reproduced (rmsd = 0.4 Å). All four ligands could be docked into the closed binding domain using Glide, although **5b** and **5c** only preferred the binding mode of **2** when docked with scaled van der Waals radii (factor 0.8), indicating that a degree of domain opening would be required to accommodate these ligands. The trends in the unscaled Emodel and Glide scores were in good agreement with the biological data, as follows: **2** (−165.4, −10.9); **5a** (−145.2, −10.2); **5b** (−108.2, −8.5); **5c** (−84.7, −6.0).

Notably, while a rotamer of the propyl analogue **5b** in a **2**-like binding mode is barely tolerated by close approach to the hydrogen bonding network mediated by Y732, the isopropyl analogue **5c** cannot avoid clashing sterically with M708 and E402 (Figure 2). The best ranked complexes corresponding to (*S*)-**2** were refined with constrained minimization using impref in First Discovery[24] to allow for induced fit. The final complexes are depicted in Figure 2. The propyl compound **5b** seems to lose affinity because of the energy penalty associated with folding of the protein side chains into the required conformation and the extra loss of conformational entropy upon ligand binding. The complexes induced by all four ligands are very similar, apart from the effect of the isopropyl group of **5c**. In order for this ligand to bind, E402 would be forced to lengthen its hydrogen bond to T686. This hydrogen bond is known as an interdomain "lock", present in all agonized complexes of the GluR2 construct solved experimentally. Furthermore, M708 is pushed into a less favorable conformation, leading to decreased affinity, overall due to the energetic cost of side chain perturbation. Thus, ligand entropy, steric conflicts, and receptor conformational energy penalties explain the marked loss in affinity over the series, from methyl to ethyl, propyl, and isopropyl analogues.

Discussion

Different structural analogues of AMPA have been synthesized,[1] and introduction of the alanine moiety on the heterocyclic structure has required multiple synthetic steps with low overall yields, involving protecting groups that frequently require harsh conditions for deprotection. Several methods were examined as attempts to overcome these difficulties. For example, various palladium-catalyzed reactions such as the Heck reaction and Negishi and Suzuki coupling reactions employing alanine synthons were tried with limited success. The unexpected observation that lithiation of the 4-position of the heterocycle in **9** was possible encouraged us to attempt conjugate addition of the Grignard reagent of **9** to methyl 2-acetaminoacrylate because similar reactions with alkyl and benzyl Grignard reagents have previously been reported.[26] Since

Table 2. Receptor Binding Affinity at Cloned Subtypes Expressed in Sf9 Cells[a]

	receptor affinity (K_i in nM)					
compd	GluR1	GluR2	GluR3	GluR4	GluR5	GluR6
1	21.9 ± 4.4	16.8 ± 2.9	20.6 ± 2.6	40.0 ± 19.9	1150 ± 114	>1000000
(*S*)-**2**	5.22 ± 0.36	3.89 ± 0.85	2.24 ± 0.09	2.70 ± 0.30	81.0 ± 3.0	18800 ± 3660
5a	145 ± 15	80.2 ± 5.9	44.8 ± 10.3	44.4 ± 3.4	2920 ± 364	>100000
5b	5150 ± 1020	1610 ± 470	1180 ± 192	732 ± 131	5450 ± 670	>100000
5c	10000 ± 2550	2930 ± 960	3710 ± 811	2350 ± 243	34200 ± 1160	>100000

[a] Mean ± SD from at least three experiments.

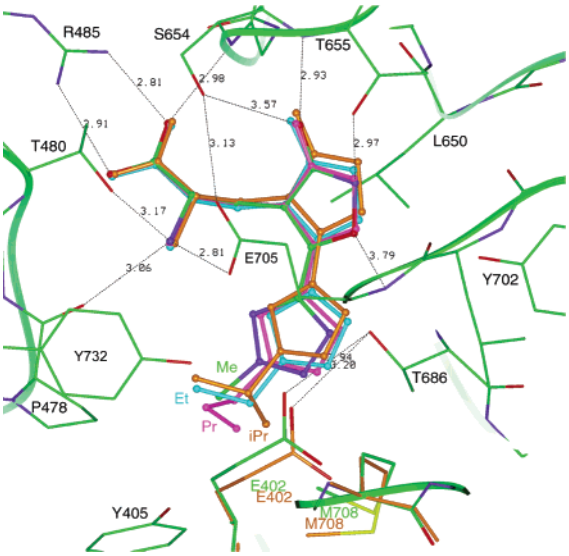


Figure 2. (*S*)-**2** (colored by atom type), (*S*)-**5a** (cyan), (*S*)-**5b** (purple), and (*S*)-**5c** (orange) docked to GluR2S1S2. While 2-ethyl and 2-propyl groups can be accommodated with minor alterations in the binding mode compared to (*S*)-**2**, the isopropyl group of (*S*)-**5c** cannot avoid steric clashes with E402 and M708.

no reaction was observed, we tried different protecting motifs and metals. Dehydroalanine containing two Boc groups and a *tert*-butyl ester (**17**) proved to be an effective substrate for lithiated **9** (Scheme 2). The two Boc groups prevent deprotonation of the amide, enhance the electrophilicity of the alkene, and enable the removal of all protecting groups in one step. This reaction constitutes a method for coupling of the entire alanine moiety to the heterocycle in a single step in gratifying yield. Introduction of the amino acid moiety at this stage made a short and convergent synthesis possible, where the alkyl substituents on the tetrazole ring are introduced immediately before deprotection.

According to the X-ray structure of (*S*)-**2** cocrystallized with the receptor binding pocket of GluR2,[6] the 2-methyltetrazolyl substituent seems to fit perfectly into a partly hydrophobic and partly hydrophilic pocket, and therefore, a loss of affinity could be expected if the pocket has to accommodate larger substituents. Indeed, pharmacological characterization of **5a**−**c** in binding affinity and functional electrophysiological assays shows that affinity and agonist potency of the compounds decrease as the size of the substituent on the tetrazole ring increases. Docking to the receptor illuminates this trend, since the ethyl analogue can be easily docked, whereas propyl and especially isopropyl substitutents can only be accommodated by forcing the side chains in the receptor pocket to rearrange (Figure 2).

In conclusion, we have developed a method for the introduction of an alanine side chain protected with acid-labile groups into the 4-position of the 3-alkoxyisoxazole ring, which greatly facilitates the synthesis of a series of alkyl tetrazolyl-AMPA analogues. 1-Substituted tetrazole derivatives **4a**−**c** were inactive, whereas the agonist potencies of the corresponding 2-isomers **5a**−**c** decreased with increasing size of the alkyl substituent. The structural basis for this order of potency was apparent when the novel ligands were docked into the ligand binding domain of GluR2.

Experimental Section

4-Iodo-3-isopropoxyisoxazole-5-carbonitrile (16). Compound **9** (152 mg, 1.0 mmol) was dissolved in dry THF (4 mL) and cooled to −78 °C. *n*-BuLi (1.6 M, 625 μL) was slowly added, resulting in a dark-red color. The solution was stirred for 20 min, and iodine (305 mg, 1.2 mmol) dissolved in dry THF (0.5 mL) was added. The mixture was stirred and slowly warmed to −20 °C over 4 h, and the reaction was quenched with saturated NH_4Cl. Water (20 mL) was added and extracted with Et_2O (3 × 20 mL). The combined organic phase was washed with aqueous Na_2SO_3 (20%, 2 × 10 mL) and dried ($MgSO_4$), and the solvent was removed in vacuo to give **16** (190 mg, 68%) as white crystals.

***tert*-Butyl (*RS*)-2-(*N*,*N*-Di-*tert*-butoxycarbonylamino)-3-(5-cyano-3-isopropoxy-4-isoxazolyl)propionate (18).** To a stirred solution of **9** (188 mg, 1.24 mmol) in dry THF (3 mL) at −78 °C was added *n*-BuLi (811 μL, 1.6 M) over 5 min. The dark-red solution was stirred for 20 min and slowly warmed to −60 °C. Compound **17** (467 mg, 1.36 mmol) dissolved in dry THF (0.5 mL) was added slowly over 40 min. The solution was stirred at −50 to −40 °C for 5 h. The solution was quenched with saturated NH_4Cl (20 mL), and the aqueous phase was extracted with EtOAc (4 × 20 mL). The combined organic phase was dried ($MgSO_4$), and the solvent was removed in vacuo. Flash chromatography (FC) (pentane/EtOAc 8:1) gave **18** (275 mg, 45%) as white crystals: mp 91−94 °C. Anal. ($C_{24}H_{37}N_3O_8$) C, H, N.

***tert*-Butyl (*RS*)-2-(*N*,*N*-Di-*tert*-butoxycarbonylamino)-3-[3-isopropoxy-5-(1*H*-5-tetrazolyl)-4-isoxazolyl]propionate (19).** Sodium azide (97 mg, 1.49 mmol) and triethylamine hydrochloride (205 mg, 1.49 mmol) were added to a solution of **18** (616 mg, 1.24 mmol) in dry DME (4 mL). The suspension was stirred at 90 °C for 48 h. The reaction mixture was cooled to room temperature (rt), the solvent was removed in vacuo, water (25 mL) was added, and pH was adjusted to 10 with NaOH (2 M). The aqueous phase was extracted with Et_2O (3 × 20 mL). The aqueous phase was acidified to pH = 2 with aqueous $KHSO_4$ (1 M), which resulted in white precipitation, and was then extracted with EtOAc (4 × 25 mL). The combined EtOAc phase was dried ($MgSO_4$) and the solvent was removed in vacuo to give **19** (554 mg, 83%) as white crystals. A sample was recrystallized (toluene): mp 136−138 °C. Anal. ($C_{24}H_{38}N_6O_8$) C, H, N.

***tert*-Butyl (*RS*)-2-(*N*,*N*-Di-*tert*-butoxycarbonylamino)-3-[3-isopropoxy-5-(1-ethyl-1*H*-5-tetrazolyl)-4-isoxazolyl]-propionate (21a) and *tert*-Butyl (*RS*)-2-(*N*,*N*-Di-*tert*-butoxycarbonylamino)-3-[3-isopropoxy-5-(2-ethyl-2*H*-5-tetrazolyl)-4-isoxazolyl]propionate (22a).** Potassium -carbonate (278 mg, 2.00 mmol) was added to a solution of **19** (540 mg, 1.00 mmol) in acetone (2.0 mL). The suspension was stirred for 15 min, and ethyl iodide (244 μL, 3.02 mmol) was added. The suspension was stirred at room temperature for

16 h. The solvent was removed in vacuo, and the residue was dissolved in water (20 mL). The aqueous phase was extracted with EtOAc (4 × 20 mL), the combined organic phases were washed with brine (10 mL) and dried ($MgSO_4$), and the solvent was removed in vacuo to afford a colorless oil. According to ^{1}H NMR a 1:2 mixture of the 1-isomer **21a** and 2-isomer **22a** was obtained. FC (pentane/EtOAc 6:1) gave **21a** (85 mg, 15%) and **22a** (318 mg, 56%) as colorless oils.

(*RS*)-2-Amino-3-[3-hydroxy-5-(2-ethyl-2*H*-5-tetrazolyl)-4-isoxazolyl]propionic Acid (5a). The experimental procedure was according to Scheme 2. A solution of **22a** (333 mg, 0.59 mmol) in aqueous HBr (48%, 2 mL) was refluxed in a preheated 140 °C oil bath for 20 min. The solution was cooled, the solvent was removed in vacuo, and the residue was evaporated with water (3 × 5 mL). The residue was purified by reversed-phase HPLC and recrystallized (water) to give **5a** (115 mg, 73%) as white crystals: mp >220 °C. Anal. ($C_9H_{12}N_6O_4$) C, H, N.

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Supporting Information Available: NMR for all compounds, experimental procedures for **4**, **5**, **7**−**10**, **12**−**15**, **17**, and **20**−**23**, and experimental details for pharmacology and molecular modeling. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Bräuner-Osborne, H.; Egebjerg, J.; Nielsen, E. Ø.; Madsen, U.; Krogsgaard-Larsen, P. Ligands for glutamate receptors: design and therapeutic prospects. *J. Med. Chem.* **2000**, *43*, 2609−2645.
(2) Dingledine, R.; Borges, K.; Bowie, D.; Traynelis, S. F. The glutamate receptor ion channels. *Pharmacol. Rev.* **1999**, *51*, 7−61.
(3) Safferling, M.; Tichelaar, W.; Kümmerle, G.; Juoppila, A.; Kuusinen, A.; Keinänen, K.; Madden, D. R. First images of a glutamate receptor ion channel: oligomeric state and molecular dimensions of GluRB homomers. *Biochemistry* **2001**, *40*, 13948−13953.
(4) Armstrong, N.; Sun, Y.; Chen, G.-Q.; Gouaux, E. Structure of a glutamate-receptor ligand-binding core in complex with kainate. *Nature* **1998**, *395*, 913−917.
(5) Armstrong, N.; Gouaux, E. Mechanisms for activation and antagonism of an AMPA-sensitive glutamate receptor: crystal structures of the GluR2 ligand binding core. *Neuron* **2000**, *28*, 165−181.
(6) Hogner, A.; Kastrup, J. S.; Jin, R.; Liljefors, T.; Mayer, M. L.; Egebjerg, J.; Larsen, I. K.; Gouaux, E. Structural basis for AMPA receptor activation and ligand selectivity: crystal structures of five agonist complexes with the GluR2 ligand-binding core. *J. Mol. Biol.* **2002**, *322*, 93−109.
(7) Sun, Y.; Olson, R.; Horning, M.; Armstrong, N.; Mayer, M.; Gouaux, E. Mechanism of glutamate receptor desensitization. *Nature* **2002**, *417*, 245−253.
(8) Hogner, A.; Greenwood, J. R.; Liljefors, T.; Lunn, M.-L.; Egebjerg, J.; Larsen, I. K.; Gouaux, E.; Kastrup, J. S. Competitive antagonism of AMPA receptors by ligands of different classes: crystal structure of ATPO bound to the GluR2 ligand-binding core, in comparison with DNQX. *J. Med. Chem.* **2003**, *46*, 214−221.
(9) Bang-Andersen, B.; Lenz, S. M.; Skjærbæk, N.; Ebert, B.; Hansen, H. O.; Ebert, B.; Bøgesø, K. P.; Krogsgaard-Larsen, P. Heteroaryl analogues of AMPA. Synthesis and quantitative structure−activity relationships. *J. Med. Chem.* **1997**, *40*, 2831−2842.
(10) Vogensen, S. B.; Jensen, H. S.; Stensbøl, T. B.; Frydenvang, K.; Bang-Andersen, B.; Johansen, T. N.; Egebjerg, J.; Krogsgaard-Larsen, P. Resolution, configurational assignment, and enantiopharmacology of 2-amino-3-[3-hydroxy-5-(2-methyl-2*H*-tetrazol-5-yl)isoxazol-4-yl]propionic acid, a potent GluR3- and GluR4-preferring AMPA receptor agonist. *Chirality* **2000**, *12*, 705−713.
(11) Frey, M.; Jäger, V. Synthesis of N-substituted muscimol derivatives including *N*-glycylmuscimol. *Synthesis* **1985**, *12*, 1100−1104.
(12) Ferreira, P. M. T.; Maia, H. L. S.; Monteiro, L. S. Efficient synthesis of dehydroamino acid derivatives. *Tetrahedron Lett.* **1998**, *39*, 9575−9578.
(13) Boivin, J.; Henriet, E.; Zard, S. Z. A highly efficient reaction for the synthesis of esters and for the inversion of secondary alcohols. *J. Am. Chem. Soc.* **1994**, *116*, 9739−9740.
(14) Butler, R. N. Tetrazoles. In *Comprehensive Heterocyclic Chemistry*; Katritzky, A. R., Rees, C. W., Scriven, E. F. V., Eds.; Pergamon: Oxford, 1996; Vol. II, pp 621−678.
(15) Butler, R. N. Tetrazoles. In *Comprehensive Heterocyclic Chemistry*; Katritzky, A. R., Rees, C. W., Eds.; Pergamon: Oxford, 1984; Vol. I, pp 791−838.
(16) Begtrup, M.; Elguero, J.; Faure, R.; Camps, P.; Estopá, C.; Ilavský, D.; Fruchier, A.; Marzin, C.; de Mendoza, J. Effect of N-substituents on the ^{13}C NMR parameters of azoles. *Magn. Reson. Chem.* **1988**, *26*, 134−151.
(17) Honoré, T.; Nielsen, M. Complex structure of quisqualate-sensitive glutamate receptors in rat cortex. *Neurosci. Lett.* **1985**, *54*, 27−32.
(18) Braitman, D. J.; Coyle, J. T. Inhibition of [^{3}H]kainic acid receptor binding by divalent cations correlates with ion affinity for the calcium channel. *Neuropharmacology* **1987**, *26*, 1247−1251.
(19) Sills, M. A.; Fagg, G.; Pozza, M.; Angst, C.; Brundish, D. E.; Hurt, S. D.; Wilusz, E. J.; Williams, M. [^{3}H]CGP 39653: a new *N*-methyl-D-aspartate antagonist radioligand with low nanomolar affinity in rat brain. *Eur. J. Pharmacol.* **1991**, *192*, 19−24.
(20) Ransom, R. W.; Stec, N. L. Cooperative modulation of [^{3}H]MK801 binding to the *N*-methyl-D-aspartate receptor ion channel complex by L-glutamate, glycine and polyamines. *J. Neurochem.* **1988**, *51*, 830−836.
(21) Madsen, U.; Frølund, B.; Lund, T. M.; Ebert, B.; Krogsgaard-Larsen, P. Design, synthesis and pharmacology of model compounds for indirect elucidation of the topography of AMPA receptor sites. *Eur. J. Med. Chem.* **1993**, *28*, 791−800.
(22) Harrison, N. L.; Simmonds, M. A. Quantitative studies on some antagonists of *N*-methyl-D-aspartate in slices of rat cerebral cortex. *Br. J. Pharmacol.* **1985**, *84*, 381−391.
(23) Bjerrum, E. J.; Kristensen, A. S.; Pickering, D. S.; Greenwood, J. R.; Nielsen, B.; Liljefors, T.; Schousboe, A.; Bräuner-Osborne, H.; Madsen, U. Design, synthesis, and pharmacology of a highly subtype-selective GluR1/2 agonist, (*RS*)-2-amino-3-(4-chloro-3-hydroxy-5-isoxazolyl)propionic acid (Cl-HIBO). *J. Med. Chem.* **2003**, *46*, 2246−2249.
(24) *Macromodel 9.0*, *First Discovery 3.5*, *Glide 3.5*; Schrödinger, Inc. (1500 S. W. First Avenue, Suite 1180, Portland, OR 97201), 2005.
(25) Johansen, T. N.; Greenwood, J. R.; Frydenvang, K.; Madsen, U.; Krogsgaard-Larsen, P. Stereostructure−activity studies on agonists at the AMPA and kainate subtypes of ionotropic glutamate receptors. *Chirality* **2003**, *15*, 167−179.
(26) Cardellicchio, C.; Fiandanese, V.; Marchese, G.; Naso, F.; Ronzini, L. Synthesis of α-amino acid derivatives by copper(I)-catalyzed conjugate addition of Grignard reagents to methyl 2-acetamidoacrylate. *Tetrahedron Lett.* **1985**, *26*, 4387−4390.
(27) Vogensen, S. B.; Greenwood, J. R.; Varming, A. R.; Brehm, L.; Pickering, D. S.; Nielsen, B.; Liljefors, T.; Clausen, R. P.; Johansen, T. N.; Krogsgaard-Larsen, P. A stereochemical anormaly: the cyclised (*R*)-AMPA analogue (*R*)-3-hydroxy-4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridine-5-carboxylic acid [(*R*)-5-HPCA] resembles (*S*)-AMPA at glutamate receptors. *Org. Biomol. Chem.* **2003**, *1*, 1−9.
(28) Nielsen, B.; Fisker, H.; Ebert, B.; Madsen, U.; Curtis, D. R.; Krogsgaard-Larsen, P.; Hansen, J. J. Enzymatic resolution of AMPA by use of α-chymotrypsin. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 107−114.
(29) Lunn, G.; Sansone, E. B. *Destruction of Hazardous Chemicals in the Laboratory*; Wiley: New York, 1994.
(30) Hermit, M. B.; Greenwood, J. R.; Nielsen, B.; Bunch, L.; Jørgensen, C. G.; Vestergaard, H. T.; Stensbøl, T. B.; Sanchez, C.; Krogsgaard-Larsen, P.; Madsen, U.; Bräuner-Osborne, H. Ibotenic acid and thioibotenic acid: a remarkable difference in activity at group III metabotropic glutamate receptors.
(31) Bunch, L.; Johansen, T. H.; Bräuner-Osborne, H.; Stensbøl, T. B.; Johansen, T. N.; Krogsgaard-Larsen, P.; Madsen, U. Synthesis and receptor binding affinity of new selective GluR5 ligands. *Bioorg. Med. Chem.* **2001**, *9*, 875−879.

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