Novel 1-Hydroxyazole Bioisosteres of Glutamic Acid. Synthesis, Protolytic Properties, and Pharmacology

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A number of 1-hydroxyazole derivatives were synthesized as bioisosteres of (*S*)-glutamic acid (Glu) and as analogues of the AMPA receptor agonist (*R*,*S*)-2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid (AMPA, **3b**). All compounds were subjected to in vitro pharmacological studies, including a series of Glu receptor binding assays, uptake studies on native as well as cloned Glu uptake systems, and the electrophysiological rat cortical slice model. Compounds **7a**,**b**, analogues of AMPA bearing a 1-hydroxy-5-pyrazolyl moiety as the distal carboxylic functionality, showed only moderate affinity for [^{3}H]AMPA receptor binding sites ($IC_{50} = 2.7 \pm 0.4$ μM and $IC_{50} = 2.6 \pm 0.6$ μM, respectively), correlating with electrophysiological data from the rat cortical wedge model ($EC_{50} = 280 \pm 48$ μM and $EC_{50} = 586 \pm 41$ μM, respectively). 1-Hydroxy-1,2,3-triazol-5-yl analogues of AMPA, compounds **8a**,**b**, showed high affinity for [^{3}H]-AMPA receptor binding sites ($IC_{50} = 0.15 \pm 0.03$ μM and $IC_{50} = 0.13 \pm 0.02$ μM, respectively). Electrophysiological data showed that compound **8a** was devoid of activity in the rat cortical wedge model ($EC_{50} > 1000$ μM), whereas the corresponding 4-methyl analogue **8b** was a potent AMPA receptor agonist ($EC_{50} = 15 \pm 2$ μM). In accordance with this disparity, compound **8a** was found to inhibit synaptosomal [^{3}H]D-aspartic acid uptake ($IC_{50} = 93 \pm 25$ μM), as well as excitatory amino acid transporters (EAATs) EAAT1 ($IC_{50} = 100 \pm 30$ μM) and EAAT2 ($IC_{50} = 300 \pm 80$ μM). By contrast, compound **8b** showed no appreciable affinity for Glu uptake sites, neither synaptosomal nor cloned. Compounds **9a**−**c** and **10a**,**b**, possessing 1-hydroxyimidazole as the terminal acidic function, were devoid of activity in all of the systems tested. Protolytic properties of compounds **7a**,**b**, **8b**, and **9b** were determined by titration, and a correlation between the pK_a values and the activity at AMPA receptors was apparent. Optimized structures of all the synthesized ligands were fitted to the known crystal structure of an AMPA−GluR2 construct. Where substantial reduction or abolition of affinity at AMPA receptors was observed, this could be rationalized on the basis of the ability of the ligand to fit the construct. The results presented in this article point to the utility of 1-hydroxypyrazole and 1,2,3-hydroxytriazole as bioisosteres of carboxylic acids at Glu receptors and transporters. None of the compounds showed significant activity at metabotropic Glu receptors.

Introduction

(*S*)-Glutamic acid (Glu) (**1**) (Figure 1) is the main excitatory neurotransmitter in the central nervous system (CNS). Excitation is mediated by two major classes of receptor systems: the G-protein-coupled metabotropic receptors and the ionotropic receptors.[1−4] The ionotropic Glu (iGlu) receptors are divided into three groups: *N*-methyl-D-aspartic acid (NMDA), (*R*,*S*)-2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid (AMPA), and kainate receptors. For each group of receptors a number of subunits have been cloned. Evidence suggests that receptor complexes are assembled from either four or five subunits.[5] The importance of iGlu receptors in fast synaptic signaling pathways, and thus in learning and memory processes, is well established. Deficits within the glutamatergic signaling pathways in Alzheimer's patients may in part explain loss of cognitive function characteristic of this disease.[6,7]

The actions of Glu in the mammalian brain are carefully regulated, in part by high-efficiency uptake systems that secure the termination of receptor activation such that concentrations of Glu do not become neurotoxic. To date, five different high-affinity sodium-dependent human excitatory amino acid transporters (EAATs) have been cloned (EAAT1−5), of which EAAT1−3 are thought to be responsible for most of the uptake.[8,9] The ability of these transporters to control the concentration of Glu present in the synaptic cleft suggests that they are potential drug targets for treating a number of diseases related to hypo- or hyperactivation of Glu receptors.

We and others have previously demonstrated that a number of 3- and 5-hydroxyazoles serve as useful bioisosteres for the distal carboxylic acid function of Glu.[10−15] The natural products (*S*)-quisqualic acid (**2**)[16]

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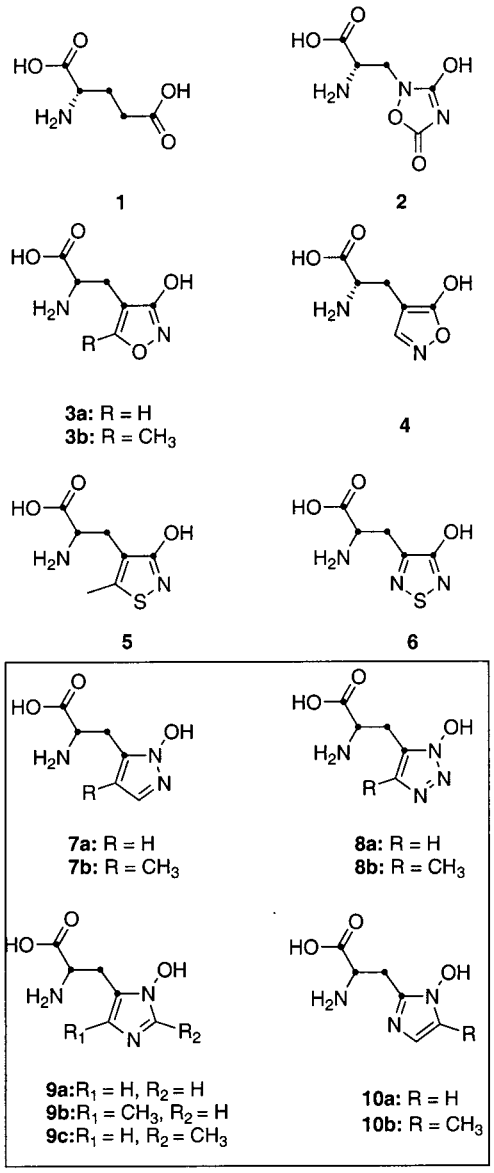
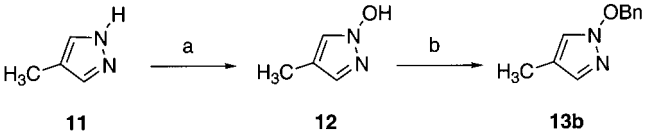


Figure 1. Structures of Glu (**1**) and a number of heterocyclic analogues including the synthesized *N*-hydroxyazoles **7a**,**b**, **8a**,**b**, **9a**−**c**, and **10a**,**b**.

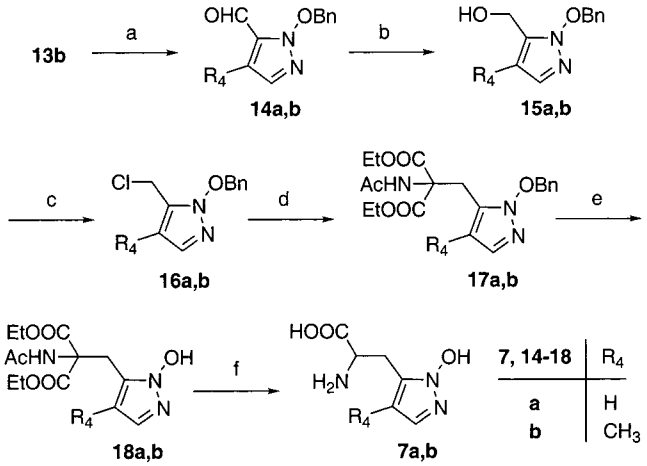
and (*S*)-2-amino-3-(5-hydroxy-4-isoxazolyl)propionic acid [TAN-950A (**4**)][15] bearing the acidic groups 1,2,4-oxadiazolidine-3,5-dione and 5-hydroxyisoxazole, respectively, are both potent Glu receptor agonists. AMPA (**3b**), bearing a 3-hydroxyisoxazole moiety as the distal acidic function, has for long been the standard compound for characterizing these receptors.[17] Furthermore, 3-hydroxyisothiazole (e.g., **5**)[13] and 3-hydroxy-1,2,5-thiadiazole (**6**)[14] analogues of Glu have also been proven to interact with Glu receptors. These results prompted us to search for other azoles that could serve in place of the distal carboxylic acid of Glu. This report describes the synthesis of a series of analogues of AMPA bearing 1-hydroxypyrazole (**7a**,**b**), 1-hydroxy-1,2,3-triazole (**8a**,**b**; in the following 1-hydroxytriazole is used as a synonym for 1-hydroxy-1,2,3-triazole), or 1-hydroxyimidazole (**9a**,**b** and **10a**,**b**) as the distal acid function and the pharmacological evaluation of the target compounds at Glu receptors and uptake sites.

Scheme 1[a]



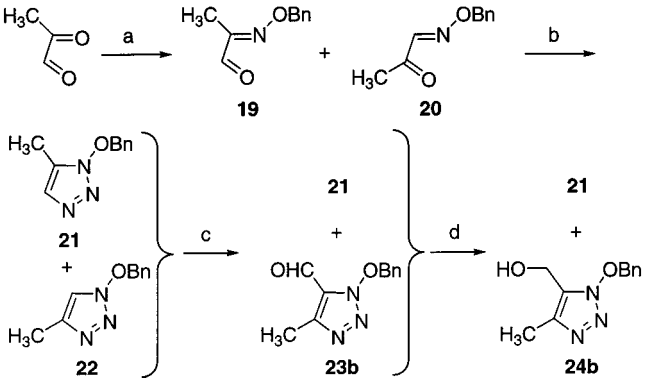
[a] Reagents and conditions: (a) *m*-CPBA, EtOAc, rt; (b) $PhCH_2Br$, $NEtPr^i_2$.

Scheme 2[a]



[a] Reagents and conditions: (a) *n*-BuLi or LDA, THF, DMF, −78 °C to rt; (b) $NaBH_4$, MeOH, 0 °C; (c) $SOCl_2$, rt; (d) $CH_3CONHCH(COOC_2H_5)_2$, NaH, DMF, rt; (e) H_2 (1 atm), Pd/C, MeOH, 0 °C; (f) 4 M HCl or 1 M TFA, reflux, 3h.

Scheme 3[a]

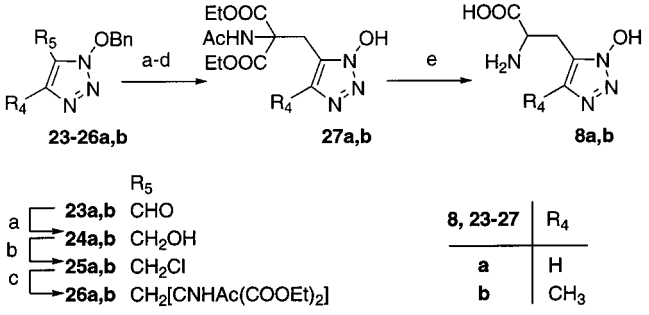


[a] Reagents and conditions: (a) $PhCH_2ONH_2$ (a 2:1 mixture of **19** and **20** was obtained); (b) NH_2NH_2, $Cu(OAc)_2$, pyridine, reflux (2:1 mixture of **21** and **22**); (c) *n*-BuLi, THF, DMF, −78 °C to rt; (d) $NaBH_4$, MeOH, 0 °C.

Results

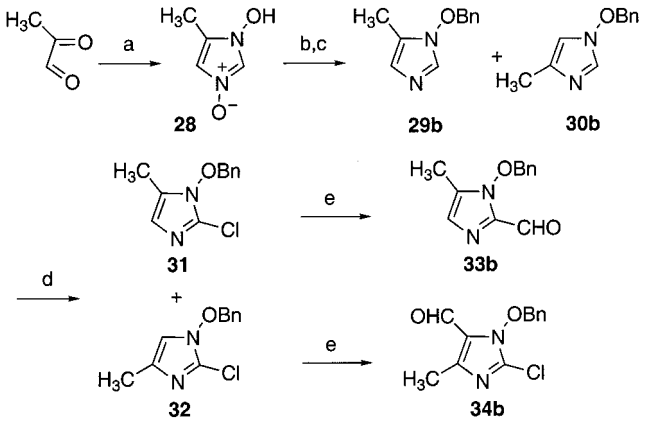
Chemistry. The synthetic approach to the target structures **7a**,**b**, **8a**,**b**, **9a**−**c**, and **10a**,**b** is outlined in Schemes 1−7. The key step in the synthesis of these compounds involves introduction of a formyl substituent at the 5-position of the pyrazole or 1,2,3-triazole ring and at the 2- or 5-position of the imidazole ring. This was accomplished by applying our previously reported method for ortho lithiation of 1-benzyloxypyrazole,[18] 1-benzyloxy-1,2,3-triazole,[19] and 1-benzyloxyimidazole[20,21] and subsequent formylation using DMF. The synthetic sequences used for the transformation of the ortho-formylated 1-benzyloxyazoles **14a**,**b**, **23a**,**b**, **34a**−**c**, and **33a**,**b** into the target compounds **7a**,**b**, **8a**,**b**, **9a**−**c**, and **10a**,**b** were as follows (Schemes 2, 4, 6, and 7): (1) reduction of the formyl group using sodium borohydride,

Scheme 4[a]



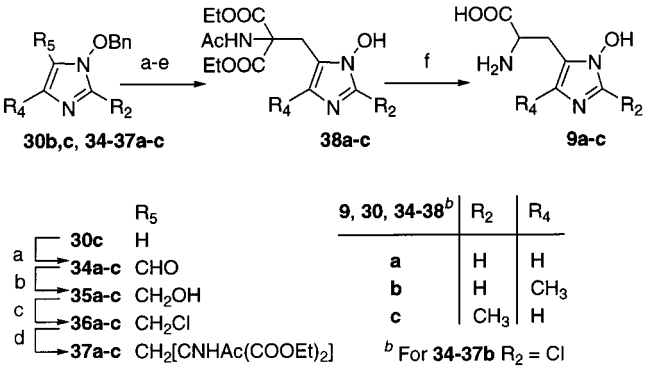
[a] Reagents and conditions: (a) $NaBH_4$, MeOH, 0 °C; (b) $SOCl_2$, CH_2Cl_2, 0 °C; (c) $CH_3CONHCH(COOC_2H_5)_2$, NaH, DMF, rt; (d) H_2 (1 atm), Pd/C, MeOH, 0 °C; (e) 12 M HCl, reflux, 3h.

Scheme 5[a]



[a] Reagents and conditions: (a) $NH_2OH{\cdot}HCl$, aqueous CH_2O, MeOH, rt; (b) $PhCH_2Br$, KOH, MeOH, reflux; (c) PCl_3, $CHCl_3$, reflux (a 1:1 mixture of **29b** and **30b** was obtained); (d) *n*-BuLi, THF, C_2Cl_6, −78 °C to rt (6:5 mixture of **31** and **32**); (e) *n*-BuLi, THF, DMF, −78 °C to rt.

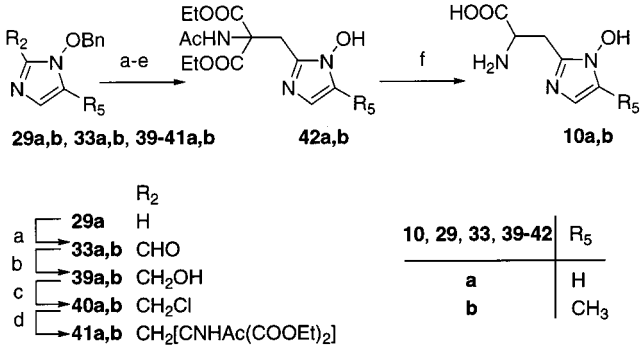
Scheme 6[a]



[a] Reagents and conditions: (a) *n*-BuLi, THF, DMF, −78 °C to rt; (b) $NaBH_4$, MeOH, 0 °C; (c) $SOCl_2$, 0 or 60 °C; (d) $CH_3CONHCH(COOC_2H_5)_2$, NaH, DMF, rt; (e) H_2 (1 atm), Pd/C, MeOH, 0 °C; (f) 4 M HCl, reflux, 3h.

producing the corresponding hydroxymethyl-substituted derivatives (compounds **15a**,**b**, **24a**,**b**, **35a**−**c**, and **39a**,**b**); (2) preparation of the chloromethyl-substituted derivatives by reaction with thionyl chloride (compounds **16a**,**b**, **25a**,**b**, **36a**−**c**, **and 40a**,**b**); (3) introduction of the diethyl acetamidomalonate group (compounds **17a**,**b**, **26a**,**b**, **37a**−**c**, and **41a**,**b**); (4) *O*-debenzylation using hydrogen (1 bar) and palladium on carbon (compounds **18a**,**b**, **27a**,**b**, **38a**−**c**, and **42a**,**b**); and (5) acid deprotection in 4 or 12 M HCl or 1 M TFA. This gave the

Scheme 7[a]



[a] Reagents and conditions: (a) *n*-BuLi, THF, DMF, −78 °C to rt; (b) $NaBH_4$, MeOH, 0 °C; (c) $SOCl_2$, 0 or 60 °C; (d) $CH_3CONHCH(COOC_2H_5)_2$, NaH, DMF, rt; (d) H_2 (1 atm), Pd/C, MeOH, 0 °C; (e) 1 M TFA or 4 M HCl, reflux.

target compounds that were isolated as their hydrochlorides (compounds **7a**, **8**−**10b**, and **9c)** or as the zwitterionic forms following ion exchange chromatography (compounds **7b**, **8**−**10a**). 1-Benzyloxy-4-methylpyrazole (**13b**) required for the synthesis of **7b** was prepared by N-oxidation of 4-methylpyrazole (**11**) followed by O-benzylation in a fashion similar to the previously reported[18] preparation of 1-benzyloxypyrazole (Scheme 1). The 4-methyl-substituted triazole **22** (Scheme 3) required for preparation of **8b** was not readily available, since no existing methodology deals with functionalization of the 4-position of 1-hydroxytriazoles. Thus, **22** was prepared as a 1:2 mixture with the isomeric 1-benzyloxy-5-methyl-1,2,3-triazole (**21**) by treatment of a 1:2 mixture of **19** and **20** with excess hydrazine and by subsequent oxidative cyclization. The isomeric methyl-substituted triazoles **21** and **22** were inseparable by column chromatography. However, only the 4-methyl-substituted triazole **22** underwent ortho lithiation and subsequent formylation, producing a mixture of **23b** and unchanged **21**, which were easily separated after the aldehyde **23b** had been reduced to the corresponding 5-hydroxymethyl derivative **24b** (Scheme 3).

Also, the isomeric 4-methyl- and 5-methyl-1-benzyloxyimidazoles **30b** and **29b** (Scheme 5) were not readily available as separate isomers. However, a 1:1 mixture of **30b** and **29b** was obtained from 4(5)-methyl-1-hydroxyimidazole-3-oxide (**28**) by O-benzylation and subsequent PCl_3-induced deoxygenation. The inseparable mixture of **30b** and **29b** was lithiated at C-2, and subsequent chlorination using hexachloroethane produced the corresponding 2-chloro derivatives **31** and **32**, which could be separated by column chromatography. 1-Benzyloxy-2-chloro-4-methylimidazole (**32**) was selectively ortho-lithiated at C-5. The chlorine at C-2 protects this position from lithiation, and then treatment with DMF produced the aldehyde **34b**, which was converted to the desired imidazole amino acid **9b** using the standard protocol (Scheme 6) with simultaneous and advantageous C-2 dechlorination during the O-debenzylation step. When 1-benzyloxy-2-chloro-5-methylimidazole (**31**) was treated with *n*-BuLi/DMF, the lithiation/formylation occurred at C-2, via chlorine−lithium exchange, and not at C-4 because lithiation at this position is disfavored by the adjacent lone pair at N-3.[22] The resulting 2-formylated imidazole was transformed

Table 1. pK_a Values of Selected Compounds

compound	pK_a values[a]		
	−COOH	bioisostere	$-NH_2$
AMPA (**3b**)[b]	1.94 ± 0.05[b]	5.12 ± 0.03[b]	10.1 ± 0.01[b]
	2.5[c]	4.8[c]	10.0[c]
7a	1.78 ± 0.04	5.78 ± 0.01	9.48 ± 0.01
7b	2.05 ± 0.04	5.89 ± 0.01	9.81 ± 0.01
8b	1.48 ± 0.08	5.07 ± 0.01	9.24 ± 0.01
9b	2.81 ± 0.02	8.82 ± 0.01[d]	10.2 ± 0.01[d]

[a] Determined by potentiometric titration unless otherwise stated. [b] Determined by ^{13}C NMR (ref 13). [c] Reference 23. [d] Assignment may be interchanged.

to the imidazole amino acid **10b** using the standard protocol (Scheme 7).

Protolytic Properties. The pK_a values of compounds **7a**,**b**, **8b**, and **9b** were determined by potentiometric titration. In general the pK_a values of the carboxyl and amino groups of compounds **7a**,**b**, **8b**, and **9b** lay in the same range as those of AMPA (**3b**) (Table 1), although minor differences were seen. The pK_a value of the carboxylic group of the α-amino acid moiety was about one unit higher for compound **9b** than for **7a**,**b**, **8b**, and AMPA (**3b**). The pK_a value of the *N*-hydroxy group of **9b** was found to be at least three pK_a units higher than those of the 1-hydroxypyrazole and the 1-hydroxytriazole groups of analogues **7a**,**b** and **8b.** Thus, **9b** exhibited a much lower degree of acidity than the other azoles.

In Vitro Pharmacology. The affinities of all compounds for AMPA, kainic acid, and NMDA receptor binding sites were determined using [^{3}H]AMPA,[24] [^{3}H]-kainic acid,[25] and [^{3}H][3-(2-carboxypiperazin-4-yl)propyl]phosphonic acid ([^{3}H]CPP),[26] respectively (Table 2). None of the compounds showed detectable affinities for NMDA receptor binding sites (IC_{50} > 100 μM) or appreciable affinities for kainic acid binding sites. The two 1-hydroxypyrazoles **7a**,**b** showed the same affinities for [^{3}H]AMPA binding sites (IC_{50} 2.7 ± 0.4 μM and 2.6 ± 0.6 μM, respectively), being 50-fold weaker than that of AMPA (**3b**). The two 1-hydoxytriazole analogues **8a**,**b** showed affinities that were appreciably higher than those of **7a**,**b** but were similar to each other (IC_{50} = 0.15 ± 0.03 μM and 0.13 ± 0.02 μM, respectively), thus being slightly weaker than AMPA (**3b)** and significantly more potent than **3a**. On the rat cortical wedge preparation, compounds **7a**,**b** were almost equipotent, displaying potencies that correlated with their affinities for AMPA receptors (EC_{50} = 280 ± 48 μM and EC_{50} = 586 ± 41 μM, respectively). Compound **8a** was apparently devoid of activity on the rat cortical wedge preparation (EC_{50} > 1000 μM and IC_{50} > 1000 μM), whereas the 4-methyl analogue **8b** proved to be a potent AMPA receptor agonist (EC_{50} = 15 ± 2 μM). Depolarization evoked by concentrations equivalent to the EC_{50} values of compounds **3a,b**, **7a,b**, and **8b** was not antagonized by 5 μM CPP but fully antagonized by 5 μM NBQX, thus indicating selective actions on AMPA receptors. To further explore the disparity between the affinity and potency of compound **8a**, all compounds were evaluated on both synaptosomal and cloned Glu uptake systems. Whereas the non-methylated analogues **3a** and **8a** showed affinities for both EAAT1 and EAAT2 uptake sites, the corresponding methylated analogues **3b** and **8b** showed no and weaker affinity, respectively, toward these transporters. Likewise, the 1-hydroxypyrazole analogues **7a**,**b** also showed no or low affinity for EAAT1−3. The affinity of **8a** for EAAT1 (IC_{50} = 100 μM) correlated with data gathered from synaptosomal Glu uptake systems using [^{3}H]-D-aspartic acid as a substrate (IC_{50} = 93 μM).

In contrast with compounds **7a**,**b** and **8a**,**b**, the 1-hydroxyimidazole analogues of **3a** and AMPA (**3b**) compounds **9a**−**c** and **10a**,**b** showed no detectable affinities for or agonist or antagonist activity at iGlu receptors.

None of the target compounds showed appreciable agonist or antagonist activity at 1000 μM on $mGlu_1$, $mGlu_2$, or $mGlu_4$ metabotropic glutamate receptor subtypes (data not shown).

Molecular Modeling. Molecular geometries were calculated in solution for **7a**,**b**, **8a**,**b**, **9a**−**c**, and **10a**,**b** in conformations corresponding to that of the bound ligand **3b**, as found in the X-ray crystal structure of its complex with the GluR2−S1S2J construct of Armstrong and Gouaux.[27] The optimized ligand structures were fitted to the crystal structure of the AMPA−GluR2 complex, and **3b** was then removed, as depicted for **7b, 8b**, **9b**, and **10b** in Figure 2. Each ligand was analyzed according to its ability to form hydrogen bonds and favorable or unfavorable van der Waals contacts with the receptor, taking receptor water molecules into account. Critical internuclear distances are noted in Figure 2.

For compounds **7a**,**b** none of the important polar contacts between the ligand and the receptor are interrupted. According to the superposition, the proton at C-3 of the pyrazole ring lies just within the van der Waals contact radius of one of the protons of Met-708 (Figure 2A).

Fitting **8a** and **8b** to the GluR2 crystal structure indicates that these can bind in an AMPA-like mode, making the same favorable contacts with the closed binding domain of the receptor as AMPA, without disrupting hydrogen bonds or entering sterically forbidden zones (Figure 2B).

When compared with **3b** in the GluR2−S1S2 construct, it is apparent that 1-hydroxyimidazoles **9a**−**c** and **10a**,**b** cannot form hydrogen bonds with the tightly bound receptor water W2 or with the backbone NH of Glu-705 (parts C and D of Figure 2). The proton at C-2 in **9a**,**b** and **10a** also collides with Glu-705 according to the superpositions (Figure 2C). Attempting to drive a methyl group into this region on domain closure (**9c**, **10b**) would interfere further with W2 and Glu-705.

Discussion

Recently, Armstrong and Gouaux have elucidated the crystal structure of a construct of the GluR2 binding domain in complex with AMPA, as well as with DNQX, Glu, and kainic acid.[27] Although the side chains of the receptor in the binding site overlap closely between the Glu− and AMPA−GluR2-complexes, as do the α-amino acid portions of the ligands, the distal groups of the ligands are not aligned. However, in combination with water, the distal acidic moieties interact isosterically with the receptor.[27] The aim of the present project has been to elucidate whether one or more *N*-hydroxyazole scaffolds presented here could function in a fashion

Table 2. Receptor Binding, Electrophysiology, and Affinity for Glu Transporters[a]

	IC_{50} (μM)			EC_{50} (μM)	IC_{50} (μM)			
compound	[^{3}H]AMPA	[^{3}H]kainic acid	[^{3}H]CPP	cortical wedge	[^{3}H]D-aspartic acid	EAAT1	EAAT2	EAAT3
AMPA (**3b**)	0.04 ± 0.01	>100	>100	3.5 ± 0.2	>200	>1000	>1000	>1000
3a	0.27 ± 0.06	>100	>100	900 ± 60	>200	270 ± 40	240 ± 20	>1000
7a	2.7 ± 0.4	>100	>100	280 ± 48	>200	>1000	430 ± 80	>1000
7b	2.6 ± 0.6	>100	>100	586 ± 41	>200	>1000	>1000	>1000
8a	0.15 ± 0.03	47 ± 7	>100	>1000	93 ± 25	100 ± 30	300 ± 80	>1000
8b	0.13 ± 0.02	90 ± 3	>100	15 ± 2	>200	>1000	490 ± 30	>1000
9a	>100	>100	>100	>1000	>200	>1000	>1000	>1000
9b	>100	>100	>100	>1000	>200	>1000	>1000	>1000
9c	>100	>100	>100	>1000	>200	>1000	>1000	>1000
10a	>100	>100	>100	>1000	>200	>1000	>1000	>1000
10b	>100	>100	>100	>1000	>200	>1000	>1000	>1000

[a] Data represent mean ± SEM of at least three individual experiments.

similar to the 3-hydroxyisoxazole moiety of AMPA (**3b**), mimicking the action, though not necessarily the binding mode, of the distal carboxylic acid of Glu. Furthermore, we wanted to investigate the relationship among structure, the protolytic properties of these compounds, and their ability to interact with Glu receptors and uptake sites.

The structure−activity relationships described in this paper indicate that the 1-hydroxypyrazole and 1-hydroxytriazole analogues (compounds **7a**,**b** and **8a**,**b**) interact with Glu recognition sites, whereas the less acidic 1-hydroxyimidazole analogues (compounds **9a**−**c** and **10a**,**b**) do not possess the structural and protolytical requirements for activation of Glu receptors or Glu uptake systems.

The two 1-hydroxypyrazoles **7a**,**b** bind to and activate AMPA receptors to a similar extent. Of the 3-hydroxyisoxazoles, only AMPA (**3b**), bearing a 5-methyl substituent, appears to activate AMPA receptors in the rat cortical wedge preparation. This failure of **3a** to depolarize cortical neurones has been suggested to be due to uptake by Glu transporters present in the cortical wedge preparation.[10] This hypothesis is reinforced by the observed affinity of **3a** toward cloned uptake systems (Table 2). Although **7a** is an analogue of **3a**, this compound does not interact with EAAT1, and it retains activity at AMPA receptors in accordance with the proposed role of the uptake systems.

The reduced potency of **7a**,**b** vs **3b** at AMPA receptors (Figure 2A) can be explained by the molecular modeling studies. To accommodate the extra proton at C-3 of **7a** and **7b**, Met-708 and the heterocycle moiety of the ligand must come to occupy positions more distant from one another upon domain closure. Tightening the conformation of the methionine away from the heterocycle will slightly increase the total free energy of the system, as will shifting the ligand from the more optimal AMPA-like position. However, the adjustment required is small (1−2 kcal/mol), in accordance with only losing an order of magnitude of potency.

The similarity of the binding modes and affinities of **8a**,**b** lies in stark contrast to their observed potency on the rat cortical wedge preparation. Compound **8a** apparently does not activate or antagonize AMPA receptors on the cortical wedge, whereas compound **8b** is a potent AMPA receptor agonist. As is the case for the non-methylated 3-hydroxyisoxazol analogue **3a**, **8a** shows affinity for EAAT1 and EAAT2, which again most likely explains its lack of activity on the rat cortical wedge. The affinities of compounds **3a**, **7a**, and **8a** for EAAT 2 are on par with one another, whereas only compounds **3a** and **8a** show affinities for EAAT 1. Taking into account that the affinities of **3a** and **8a** for AMPA receptors do not correlate with the potency found in the rat cortical wedge model, whereas the affinity and potency of **7a** does, these findings could lead to the conclusion that **3a** and **8b** are substrates for EAAT1 in the cortical wedge preparation. However, substrates and blockers cannot be distinguished by the present assay. EAAT2 has been described as the major uptake mechanism in the forebrain, whereas EAAT 1 primarily is localized in the cerebellum.[28] This evidence contradicts the above-mentioned hypothesis that EAAT1 is the primary cause for the lack of potency of **3a** and **8b** observed here and suggests that EAAT2 might be involved. Hence, this issue remains to be clarified.

The lack of activity of **9a**−**c** and **10a**,**b** as AMPA receptor agonists can be rationalized in two ways. First, the pK_a value of the *N*-hydroxy group of the 1-hydroxyimidazole moiety is so high that only a modest proportion of the molecules is expected to be ionized in solution. To date, structures known to function as distal carboxylate bioisosteres at AMPA receptors cover a pK_a range where the ionized form dominates in aqueous solution. A low degree of ionization is expected to disfavor binding by shifting the equilibrium away from the presumably active form carrying three charges. Second, the modeling studies show that the presence of a proton or a methyl group at C-2 of the imidazole ring (**9a**,**b**, **10a**, and **9c**,**10b**, respectively) prohibits two hydrogen bonds with the receptor environment compared with the AMPA binding mode, each corresponding to at least 4 kcal/mol in binding energy. The presence of a methyl group directly interferes with domain closure, thus rendering these imidazole analogues inactive.

The structural requirements for interaction with Glu uptake systems are far from being elucidated, and the results found here do not imply a simple structure−activity relationship. However, these observations lead to the conclusion that the presence of a methyl group neighboring the α-amino acid side chain of the *N*-hydroxyazole hinders uptake by Glu transport mechanisms. Furthermore, iGluRs as well as Glu uptake mechanisms display a preference for hydroxyazoles with pK_a values in the same range as that of the 3-hydroxyisoxazole of AMPA.

In conclusion, the results obtained indicate that 1-hydroxytriazoles and 1-hydroxypyrazoles may be useful as novel carboxylic acids bioisosteres while

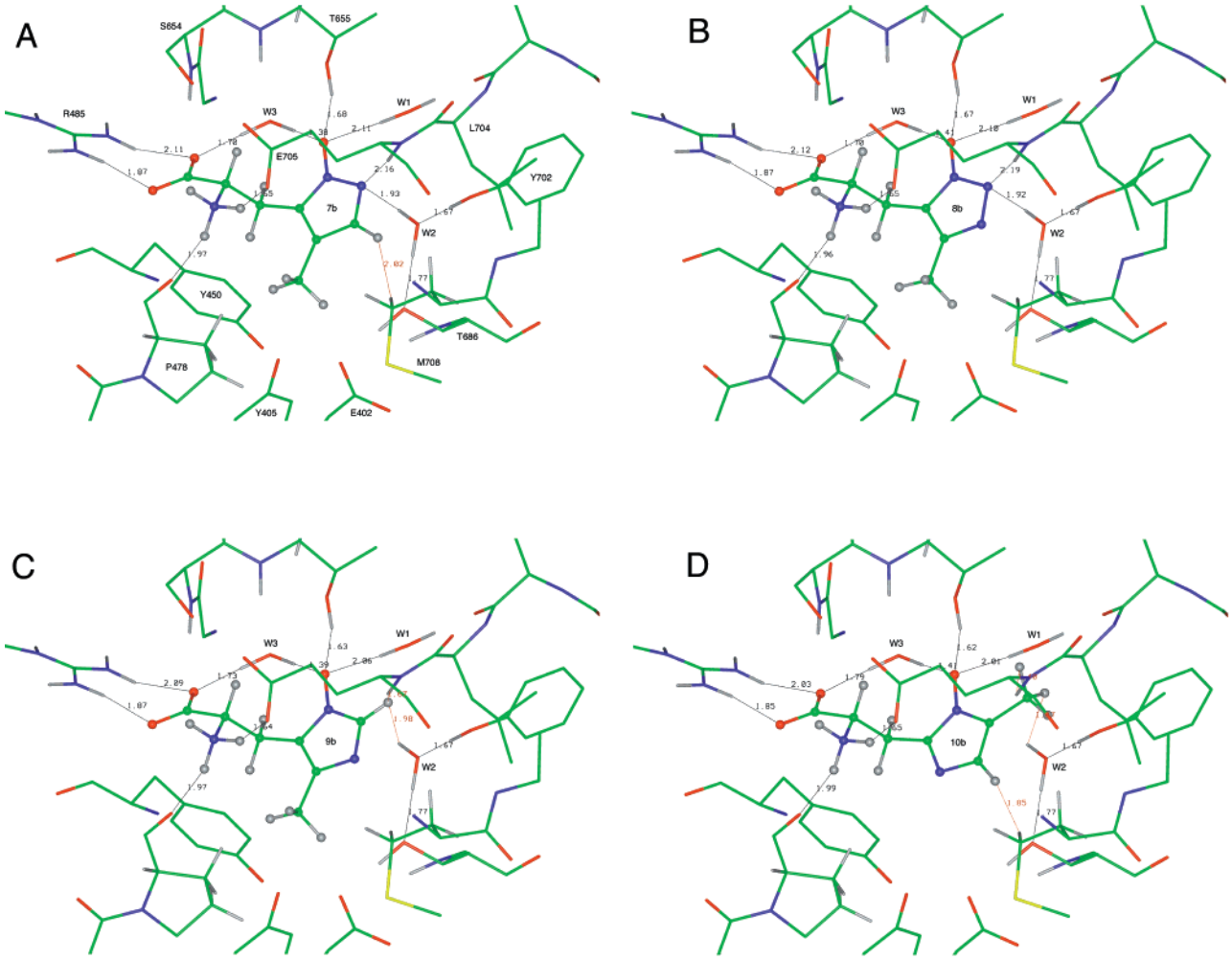


Figure 2. Glu analogues docked into the binding site of GluR2−S1S2J:[27] (A) **7b**; (B) **8b**; (C) **9b**; (D) **10b**. Some residues lining the binding site (Tyr-723 in front and Leu-650 behind) have been removed for clarity. The binding site is highly conserved across AMPA-receptor subunits GluR1−4.[42] Selected hydrogen bonds are shown in black, and unfavorable van der Waals interactions are shown in dark red. W1−3 are binding site water molecules.

N-hydroxyazoles of high pK_a, or lacking a hydrogen bond acceptor α to the acidic hydroxy group, are likely to be poor carboxylic acid bioisosters at iGlu receptors.

Experimental Section

1. Chemistry. All reactions involving air-sensitive reagents were performed under N_2 using syringe−septum cap techniques. All glassware was flame-dried prior to use. Flash column chromatography (FC) was performed using silica gel (Merck, 40−63 mesh). TLC was performed using Merck silica gel 60 F_{254} aluminum sheets. The plates were visualized under UV light (254 nm) and by spraying with ammonium cerium molybdate. Compounds containing amino groups were visualized using a ninhydrin spraying reagent. Melting points are uncorrected. All new compounds were colorless, unless otherwise stated. If not otherwise stated, NMR spectra were recorded on a 200 MHz Bruker or a 300 MHz Varian spectrometer in $CDCl_3$ (tetramethylsilane as internal standard) or D_2O with dioxane as the internal standard at 3.70 ppm (^{1}H spectra) and 67.4 ppm (^{13}C spectra). The multiplicity of ^{13}C NMR signals was assigned from APT (attached proton test) spectra. Microanalyses were within ±0.4% of the theoretical values if not otherwise stated.

All solvents and reagents were obtained from Fluka or Aldrich and used without further purification except for THF, which was distilled from Na/benzophenone under nitrogen, and DMF, which was sequentially dried with and stored over 3 Å molecular sieves.[29]

2. Preparation of Pyrazole Amino Acid 7a. 2.1. 1-Benzyloxy-5-hydroxymethylpyrazole (15a). A solution of 1-benzyloxy-5-formylpyrazole (**14a**)[18] (1.88 g, 9.31 mmol) in MeOH (30 mL) was added with sodium borohydride (0.45 g, 11.9 mmol) and stirred at 0 °C for 30 min. The MeOH was evaporated, and the residue was taken up in CH_2Cl_2 and washed with brine. The organic phase was dried ($MgSO_4$) and concentrated. FC (EtOAc/heptane 1:6 → 1:1) gave 1.86 g (98%) of **15a** as a viscous oil; ^{1}H NMR ($CDCl_3$) δ 7.40−7.26 (m, 5H), 7.22 (d, J = 2.4 Hz, 1H), 6.07 (d, J = 2.4 Hz, 1H), 5.29 (s, 2H), 4.24 (d, J = 4.8 Hz, 2H), 2.35 (br s, 1 H); ^{13}C NMR ($CDCl_3$) δ 135.6 (s), 133.5 (s), 132.7 (d), 130.0 (d), 129.4 (d), 128.7 (d), 102.7 (d), 80.2 (t), 53.8 (t). Anal. ($C_{11}H_{12}N_2O_2$) C, H, N.

2.2. 1-Benzyloxy-5-chloromethylpyrazole (16a). Compound **15a** (1.68 g, 8.2 mmol) was treated with thionyl chloride (3.7 mL, 50 mmol) for 1 h at room temperature. The mixture was concentrated, coevaporated three times with toluene, and dried (room temp (rt), 0.1 mmHg) to give 1.75 g of crude **16a**. FC (EtOAc/heptane 1:10 → 1:2) gave 1.60 g (87%) of **16a** as an oil: ^{1}H NMR ($CDCl_3$) δ 7.42−7.34 (m, 5H), 7.26 (d, J = 2.4 Hz, 1H), 6.16 (d, J = 2.4 Hz, 1H), 5.35 (s, 2H), 4.26 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 133.3 (s), 132.8 (d), 131.8 (s), 129.9 (d), 129.4 (d), 128.7 (d), 103.8 (d), 80.4 (t), 32.9 (t). Anal. ($C_{11}H_{11}ClN_2O$) C, H, N.

2.3. Ethyl 2-Acetamido-3-(1-benzyloxy-5-pyrazolyl)-2-ethoxycarbonylpropionate (17a). Diethyl acetamidomalonate (2.61 g, 12.0 mmol) was slowly added to a suspension of sodium hydride (485 mg, 60% suspension, 12.1 mmol) in dry DMF (30 mL) at 0 °C. The mixture was allowed to warm to

room temperature, and stirring was continued until a clear yellow solution was obtained and no more H_2 evolved (ca. 2 h). Then a solution of **16a** (1.36 g, 6.13 mmol) in dry DMF (12 mL) was added, and the mixture was stirred at room temperature. After 18 h, the reaction was quenched with saturated NH_4Cl (100 mL) and the product was extracted with AcOEt (3 × 100 mL). The combined organic phases were washed twice with an ice-cold 5% solution of NaOH, dried ($MgSO_4$), and concentrated. FC (EtOAc/heptane 1:4 → 1:1) gave 2.22 g (90%) of **17a** as a solid. A small sample was recrystallized from EtOAc/heptane: mp 85−86 °C; ^{1}H NMR ($CDCl_3$) δ 7.39−7.34 (m, 5H), 7.21 (d, J = 2.4 Hz, 1H), 6.57 (br s, NH), 5.78 (d, J = 2.4 Hz, 1H), 5.20 (s, 2H), 4.23 (dq, J = 10.8, 7.2 Hz, 2H), 4.15 (dq, J = 10.8, 7.2 Hz, 2H), 3.46 (s, 2H), 1.86 (s, 3H), 1.21 (t, J = 7.2 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 169.3 (s), 166.7 (s), 132.8 (s), 132.5 (s), 129.9 (d), 129.8 (d), 129.1 (d), 128.3 (d), 103.3 (d), 80.1 (t), 65.4 (s), 62.3 (t), 26.6 (t), 22.2 (q), 13.4 (q). Anal. ($C_{20}H_{25}N_3O_6$) C, H, N.

2.4. Ethyl 2-Acetamido-2-ethoxycarbonyl-3-(1-hydroxy-5-pyrazolyl)propionate (18a). A mixture of **17a** (504 mg, 1.25 mmol), 10% Pd/C (44 mg), and MeOH (25 mL) was stirred under H_2 (1 atm) at 0 °C for 30 min. Filtration through Celite and removal of MeOH gave 377 mg (96%) of **18a** as a solid: mp >210 °C; ^{1}H NMR ($CDCl_3$) δ 7.08 (d, J = 2.4 Hz, 1H), 6.69 (s, 1H), 5.86 (d, J = 2.4 Hz, 1H), 4.31 (dq, J = 10.8, 7.2 Hz, 2H), 4.27 (dq, J = 11.1, 7.1 Hz, 2H), 3.79 (s, 2H), 2.01 (s, 3H), 1.29 (t, J = 7.1 Hz, 6H). The crude product was used in the next step without further purification.

2.5. (*R*,*S*)-2-Amino-3-(1-hydroxy-5-pyrazolyl)propionic Acid Hydrochloride (7a·HCl). A solution of **18a** (373 mg, 1.19 mmol) in 4 M HCl was heated to reflux for 3 h. The mixture was concentrated and evaporated twice with water and twice with toluene to give 267 mg of crude **7a**, which was washed with glacial acetic acid to give 205 mg (83%) of **7a** as the hydrochloride: mp >210 °C; ^{1}H NMR (D_2O) δ 7.16 (d, J = 2.4 Hz, 1H), 6.15 (d, J = 2.4 Hz, 1H), 4.28 (dd, J = 5.4, 7.2 Hz, 1H), 3.34 (dd, J = 5.4, 15.6 Hz, 1H), 3.24 (dd, J = 7.2, 15.6 Hz, 1H); ^{13}C NMR (D_2O) δ 172.1 (s), 133.1 (d), 130.6 (s), 105.4 (d), 53.1 (d), 25.5 (t). Anal. ($C_6H_9N_3O_3$·HCl, 15 mol % H_2O) C, H, N.

3. Preparation of Pyrazole Amino Acid 7b. 3.1. 1-Hydroxy-4-methylpyrazole (12). A mixture of 4-methylpyrazole (**11**) (40 g, 0.49 mol) and *m*-chloroperbenzoic acid (70%, 140 g, 0.57 mol) in EtOAc (3.4 L) was stirred for 5 days. Evaporation of the EtOAc produced a solid that was extracted with water (ca. 200 mL and 4 × 100 mL) on a sintered glass filter. The combined water extracts were extracted with CH_2Cl_2 (4 × 100 mL). The volume of the CH_2Cl_2 phase was reduced to ca. 50 mL and extracted with concentrated HCl (50 mL). The HCl phase was washed with dichloromethane (2 × 50 mL) and combined with the aqueous phase from above. Na_3PO_4·$12H_2O$ (30 g) was added, and the pH was adjusted to 10.5 by the addition of 33% aqueous sodium hydroxide. Continuous extraction with Et_2O for 24 h using a Kutscher−Steudel apparatus gave 16 g (40%) of unchanged starting material (**11**) after removal of the solvents. The pH of the aqueous solution was adjusted to 2 by addition of concentrated HCl. Continuous extraction with Et_2O for 12 h produced 20.2 g of crude 1-hydroxy-4-methylpyrazole (**12**) as brown crystals after removal of the solvents: mp 62−69 °C. Recrystallization from EtOAc/heptane (1:5) gave **12** as colorless crystals (15.5 g, 32%): mp 75−78 °C (lit. 81−82 °C);[30] ^{1}H NMR ($CDCl_3$) δ 11.8 (br s, 1H), 7.13 (q, J = 0.6 Hz, 1H), 6.90 (br s, 1H), 2.02 (d, J = 0.5 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 130.9 (d), 121.5 (d), 113.6 (s), 9.0 (q). Anal. ($C_3H_4N_2O$) C, H, N.

3.2. 1-Benzyloxy-4-methylpyrazole (13b). Benzyl bromide (10.8 mL, 90 mmol) was added to a solution of **12** (8.5 g, 86 mmol) and *N*-ethyldiisopropylamine (15.4 mL, 90 mmol) in 80 mL of dry CH_2Cl_2 at 0 °C. Stirring was continued at room temperature for 16 h. To the mixture was added NaOH (2 M, 100 mL), and the aqueous phase was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic phases were washed with 2 M NaOH and water, dried ($MgSO_4$), and evaporated to dryness. FC (heptane → EtOAc/heptane 1:4) provided 15.3 g (95%) of **13b** as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.35−7.28 (m, 5H), 7.05 (br s, 1H), 6.80 (t, J = 0.9 Hz, 1H), 5.21 (s, 2H), 1.93 (t, J = 0.6 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 133.7, 132.8, 129.2, 128.7, 128.2, 120.9, 113.2, 79.9, 8.7. Anal. ($C_{11}H_{12}N_2O$) C, H, N.

3.3. 1-Benzyloxy-5-formyl-4-methylpyrazole (14b). A freshly prepared solution of LDA (12 mmol) in THF (10 mL) was added at −78 °C over 2 min to a solution of **13b** (1.40 g, 5.2 mmol) in THF (50 mL). After the mixture was stirred at −78 °C for 5 min, DMF (3.1 mL) was added. Stirring was continued for 1 h, and the solution was allowed to warm to room temperature over 10 min and stirred for another 1 h. To the mixture was added 2 M HCl (100 mL), and the mixture was stirred for another 1 h. The organic phase was separated and the aqueous phase extracted with CH_2Cl_2. Drying of the combined organic phases and evaporation followed by FC (EtOAc/heptane 1:4 → 1:1) gave **14b** (1.22 g, 75%) as colorless crystals: mp 48−50 °C. A small sample was recrystallized from heptane: mp 52−53 °C; ^{1}H NMR ($CDCl_3$) δ 9.59 (d, J = 0.6 Hz, 1H), 7.40−7.25 (m, 5H), 7.16 (br s, 1H), 5.37 (s, 2H), 2.21 (d, J = 0.6 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 179.4 (d), 133.1(d), 132.7 (s), 130.5 (s), 129.9 (d), 129.8 (d), 128.7 (d), 119.7 (s), 81.1 (t), 9.4 (q). Anal. ($C_{12}H_{12}N_2O_2$) C, H, N.

3.4. 1-Benzyloxy-5-hydroxymethyl-4-methylpyrazole (15b). Compound **15b** was prepared from **14b** (820 mg, 3.8 mmol) by the method described for compound **15a**. FC (CH_2Cl_2/Et_2O/heptane 1:1:1) gave 740 mg (89%) of **15b**: mp 81−82 °C; ^{1}H NMR ($CDCl_3$) δ 7.40−7.30 (m, 5H), 7.08 (br s, 1H), 5.31 (s, 2H), 4.26 (s, 2H), 2.00 (d, J = 0,5 Hz, 3H), 1.35 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 133.7 S), 132.5 (d), 132.0 (s), 130.0 (d), 129.4 (d), 128.7 (d), 112.6 (s), 80.0 (t), 52.3 (t), 8.5 (q). Anal. ($C_{12}H_{14}N_2O_2$) C, H, N.

3.5. 1-Benzyloxy-5-chloromethyl-4-methylpyrazole (16b). Compound **16b** was prepared from **15b** (943 mg, 4.32 mmol) by the method described for compound **16a**. FC (EtOAc) gave 979 mg (99%) of **16b** as yellow crystals: mp 30−33 °C. A small sample was recrystallized from pentane: mp 36−37 °C; ^{1}H NMR ($CDCl_3$) δ 7.39 (br s, 5H), 7.10 br s, 1H), 5.34 (s,2H), 4.33 (s, 2H), 2.04 (d, J = 0.5 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 133.2 (s), 132.3 (d), 129.8 (d), 129.3 (d), 129.0 (s), 128.6 (d), 113.5 Ss), 80.6 (t), 31.8 (t), 8.5 (q). Anal. ($C_{12}H_{13}ClN_2O$): C, H, N.

3.6. Ethyl 2-Acetamido-3-(1-benzyloxy-4-methyl-5-pyrazolyl)-2-ethoxycarbonylpropionate (17b). Diethyl acetamidomalonate (1.01 g, 4.6 mmol) was slowly added to a suspension of sodium hydride (214 mg, 55% suspension, 4.6 mmol) in dry DMF (1 mL) at room temperature. After the mixture was stirred for 15 min, a solution of **16b** (0.94 g, 4.0 mmol) in dry DMF (0.5 mL) was added, and the mixture was then stirred for 24 h more. The reaction mixture was worked up as described for compound **17a**. FC (EtOAc/heptane 1:1) gave 0.96 g (50%) of **17b** as a solid: mp 84−86 °C. A small sample was recrystallized from EtOAc/heptane: mp 91.5−92 °C; ^{1}H NMR ($CDCl_3$) δ 7.37 (br s, 5H), 7.07 (s, 1H), 6.51 (br s, 1H), 5.13 (s, 2H), 4.24 (dq, J = 10.8, 7.1 Hz, 2H), 4.06 (dq, J = 10.8, 7.1 Hz, 2H), 3.41 (s, 2H), 1.86 (s, 3H), 1.81 (s, 3H), 1.19 (t, J = 7.1 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 169.3 (s), 167.0 (s), 133.0 (s), 132.6 (d), 130.2 (d), 129.3 (d), 128.4 (d), 127.1 (s), 113.3 (s), 80.4 (t), 65.3 (s), 62.5 (t), 26.0 (t), 22.6 (q), 13.6 (q), 8.6 (q). The compound was used in the next step without further purification.

3.7. Ethyl 2-Acetamido-3-(1-hydroxy-4-methyl-5-pyrazolyl)-2-ethoxycarbonylpropionate (18b). Compound **18b** was prepared from **17b** (409 mg, 0.98 mmol) by the method described for compound **18a**. This gave 337 mg (100%) of **18b** as gray crystals: mp 132−134 °C. A small sample was recrystallized from EtOAc/heptane: mp 133−134 °C; ^{1}H NMR (acetone-d_6) δ 7.41 (br s, 1H), 6.87 (s, 1H), 4.24 (dq, J = 10.8, 7.2 Hz, 2H), 4.16 (dq, J = 10.8, 7.2 Hz, 2H), 3.62 (s, 2H), 1.98 (s, 3H), 1.86 (s, 3H), 1.22 (t, J = 7.1 Hz, 6H); ^{13}C NMR (acetone-d_6) δ 168.6 (s), 166.3 (s), 129.8 (d), 125.7 (s), 112.5 (s), 64.6 (s), 61.0 (t), 25.4 (t), 20.8 (q), 12.4 (q), 7.2 (q). Anal. ($C_{14}H_{21}N_3O_6$) C, H, N.

3.8. (*R*,*S*)-2-Amino-3-(1-hydroxy-4-methyl-5-pyrazolyl)-propionic Acid (7b). A solution of **18b** (200 mg, 0.61 mmol)

in 1 M TFA (30 mL) was heated to 120 °C for 12 h. The mixture was concentrated and dried. The brown oil was taken up in water, neutralized to pH = 7 with 2 M NH_3, and applied to an ion exchange column (Amberlite IR-120 (H^+), 5 g). The column was eluted with water to remove TFA and then with 0.5 M NH_3 to liberate the amino acid as its ammonium salt (after evaporation). The ammonium salt of **7b** was taken up in water and applied to an ion exchange column (Amberlite IR-420 (OH^-), 5 g). The column was eluted with water and 0.5 M AcOH, and the fractions containing the product were concentrated and dried to give 103 mg (91%) of the free amino acid **7b** as a solid. A small sample was recrystallized from H_2O: mp >240 °C; ^{1}H NMR (D_2O) δ 6.94 (1H, s), 3.83 (t, J = 6.3 Hz, 1H), 3.13 (dd, J = 15.4, 5.2 Hz, 1H), 3.02 (dd, J = 15.4, 6.6 Hz, 1H), 2.84 (s, 3H); ^{13}C NMR (D_2O) δ 174.05 (s), 131.47 (d), 129.13 (s), 114.68 (s), 54.49 (d), 24.90 (t), 8.57 (q). Anal. ($C_7H_{11}N_3O_3$) C, H, N.

4. Preparation of 1,2,3-Triazole Amino Acid 8a. 4.1. 1-Benzyloxy-5-hydroxymethyl-1,2,3-triazole (24a). Compound **24a** was prepared from 1-benzyloxy-5-formyl-1,2,3-triazole (**23a**)[19] (1.37 g, 6.75 mmol) by the method described for compound **15a**. FC (EtOAc/heptane 1:2) gave 1.29 g (93%) of **24a** as a solid. A small sample was recrystallized from EtOAc/heptane: mp 71−72 °C; ^{1}H NMR ($CDCl_3$) δ 7.48 (s, 1H), 7.42−7.32 (m, 5H), 5.47 (s, 2H), 4.34 (d, J = 4.8 Hz, 2H), 3.08 (br t, $J \approx$ 5 Hz, OH); ^{13}C NMR ($CDCl_3$) δ 132.5 (s), 131.6 (s), 131.2 (d), 130.1 (d), 130.0 (d), 128.9 (d), 82.6 (t), 52.1 (t). Anal. ($C_{10}H_{11}N_3O_2$) C, H, N.

4.2. 1-Benzyloxy-5-chloromethyl-1,2,3-triazole (25a). A solution of **24a** (0.45 g, 2.2 mmol) in CH_2Cl_2 (8 mL) was treated with thionyl chloride (1 mL, 13.7 mmol) and stirred for 1 h at 0 °C. The mixture was concentrated, coevaporated three times with toluene, and dried (rt, 0.1 mmHg) to give 473 mg (97%) of **25a** as a solid, which was used in the next step without further purification: ^{1}H NMR ($CDCl_3$) δ 7.61 (s, 1H), 7.45−7.35 (m, 5H), 5.52 (s, 2H), 4.24 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 132.2 (s), 131.9 (d), 130.1 (d), 130.0 (d), 128.9 (d), 128.4 (s), 82.7 (t), 30.6 (t).

4.3. Ethyl 2-Acetamido-3-(1-benzyloxy-1,2,3-triazol-5-yl)-2-ethoxycarbonylpropionate (26a). Compound **26a** was prepared from **25a** (144 mg, 0.64 mmol) by the method described for compound **17a**. FC (EtOAc/heptane 1:2) gave 246 mg (94%) of **26a** as a solid. A small sample was recrystallized from Et_2O: mp 130−131 °C; ^{1}H NMR ($CDCl_3$) δ 7.42−7.37 (m, 5H), 7.25 (s, 1H), 6.63 (br s, 1H), 5.38 (s, 2H), 4.29−411 (m, 4H), 3.46 (s, 2H), 1.91 (s, 3H), 1.22 (t, J = 7.1 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 169.6 (s), 166.3 (s), 131.9 (s), 131.6 (s), 131.6 (d), 130.1 (d), 129.9 (d), 128.6 (d), 126.3 (s), 82.5 (t), 65.3 (s), 62.8 (t), 25.0 (t), 22.5 (q), 13.7 (q). Anal. ($C_{19}H_{24}N_4O_6$) C, H, N.

4.4. Ethyl 2-Acetamido-2-ethoxycarbonyl-3-(1-hydroxy-1,2,3-triazol-5-yl)propionate (27a). Compound **27a** was prepared from **26a** (457 mg, 1.13 mmol) by the method described for compound **18a**. This gave 331 mg (93%) of **27a** as a solid. Recrystallization (acetone/Et_2O) gave mp 144−145 °C: ^{1}H NMR (D_2O) δ 7.83 (s, 1H), 4.24 (q, J = 7.1 Hz, 4H), 3.64 (s, 2H), 1.99 (s, 3H), 1.19 (t, J = 7.1 Hz, 6H); ^{13}C NMR (D_2O) δ 174.4 (s), 168.7 (s), 127.4 (d), 126.6 (s), 66.3 (s), 65.1 (t), 26.7 (t), 22.3 (q), 13.9 (q). Anal. ($C_{12}H_{18}N_4O_6$) C, H, N.

4.5. (*R*,*S*)-2-Amino-3-(1-hydroxy-1,2,3-triazole-5-yl)propionic Acid (8a). A solution of **27a** (157 mg, 0.50 mmol) in 12 M HCl was heated to reflux for 3 h. The mixture was concentrated and dried (rt, 0.1 mmHg) to give 126 mg of **8a** as the hydrochloride. The free amino acid was obtained by ion exchange chromatography as described for **7b** to give 84 mg (96%) of **8a** as a solid: mp >210 °C; ^{1}H NMR (D_2O) δ 7.94 (s, 1H), 4.13 (dd, J = 5.1, 6,6 Hz, 1H), 3.35 (dd, J = 5.0, 16.0 Hz, 1H), 3.25 (dd, J = 6.6, 15.9 Hz, 1H); ^{13}C NMR (D_2O) δ 173.3 (s), 127.8 (s), 127.0 (d), 54.0 (d), 24.4 (t). Anal. ($C_5H_8N_4O_3$, 130 mol % H_2O) C, H, N.

5. Preparation of 1,2,3-Triazole Amino Acid 8b. 5.1. 1-Benzyloxy-5-hydroxymethyl-4-methyl-1,2,3-triazole (24b). A 2:1 mixture of 2-*O*-benzyloximepropane-1,2-dione (**19**) and 1-*O*-benzyloximepropane-1,2-dione (**20**) (14.9 g, 83.7 mmol), obtained by condensation of methyl glyoxal and *O*-benzylhydroxylamine,[31] was dissolved in MeOH (250 mL), cooled to 0 °C, and treated with hydrazine hydrate (40 mL, 0.84 mol) for 1 h at 0 °C. The mixture was concentrated to give a colorless oil, which was redissolved in pyridine (200 mL) and heated to reflux. Then $Cu(OAc)_2$ hydrate (33 g, 0.17 mol) in pyridine (300 mL) was added, and the reaction mixture was refluxed for 1 h, cooled to room temperature, and evaporated in vacuo. The black slurry was extracted with Et_2O (6 × 150 mL), and the combined organic phases were washed with precooled sulfuric acid (100 mL, 4 M) and then water (200 mL). The Et_2O phase was dried ($MgSO_4$) and evaporated in vacuo. The brown oil (11.5 g) was purified by FC using EtOAc/heptane (1:3 → 1:1), which gave 5.62 g (36%) of an inseparable 2:1 mixture of 1-benzyloxy-5-methyl-1,2,3-triazole (**21**)[19] and 1-benzyloxy-4-methyl-1,2,3-triazole (**22**). A total of 3.04 g (5.78 mmol) of this mixture was dissolved in THF (80 mL), cooled to −78 °C, and treated with *n*-BuLi (1.6 M in hexanes, 4.5 mL, 7.2 mmol). After 2 min, dry DMF (0.67 mL, 9.17 mmol) was added. The reaction mixture was stirred for 30 min at −78 °C and then allowed to warm to room temperature over 30 min. The reaction was quenched with saturated NH_4Cl (50 mL) and extracted with EtOAc (3 × 25 mL). The combined organic phases were dried ($MgSO_4$) and evaporated in vacuo to give crude **23b** and unchanged **21**. Data for **23b**: ^{1}H NMR ($CDCl_3$) δ 9.56 (s, 1H), 7.42−7.20 (m, 5H), 5.56 (s, 2H), 2.47 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 177.8 (s), 146.4 (s), 131.7 (s), 130.4 (d), 130.1 (d), 129.0 (d), 125.8 (s), 83.1 (t), 11.7 (q). The solid was redissolved in MeOH (20 mL), cooled to 0 °C, and treated with $NaBH_4$ (0.33 g, 8.7 mmol) for 30 min at room temperature, then evaporated in vacuo. The crude product was redissolved in CH_2Cl_2 (20 mL) and washed with brine and water. The organic phase was dried ($MgSO_4$) and evaporated in vacuo. FC (EtOAc/heptane 1:3 → 1:2) gave 0.96 g (75%) of **24b** and 1.71 g of unchanged **21**. Data for **24b**: mp 127−129 °C; ^{1}H NMR ($CDCl_3$) δ 7.45−7.30 (m, 5H), 5.47 (s, 2H), 4.34 (s, 2H), 2.33 (br s, 1H), 2.48 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 140.4 (s), 132.8 (s), 130.1 (d), 129.9 (d), 128.9 (d), 127.4 (s), 82.3 (t), 51.2 (t), 10.5 (q). Anal. ($C_{11}H_{13}N_3O_2$) C, H, N.

5.2. Ethyl 2-Acetamido-3-(1-benzyloxy-4-methyl-1,2,3-triazol-5-yl)-2-ethoxycarbonylpropionate (26b). Compound **26b** was prepared from **24b** (0.93 g, 4.25 mmol), using the method described for the preparation of **25a** followed by the method described for the preparation of **17a**. FC (EtOAc/heptane 1:3 → 1:0) gave 1.47 g (83%) of **26b** as a solid: mp 133−135 °C; ^{1}H NMR ($CDCl_3$) δ 7.42−7.32 (m, 5H), 6.50 (br s, 1H), 5.34 (s, 2H), 4.25 (dq, J = 10.8, 7.2 Hz, 2H), 4.07 (dq, J = 10.8, 7.2 Hz, 2H), 3.42 (s, 2H), 2.14 (s, 3H), 1.85 (s, 3H), 1.21 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 169.7 (s), 166.8 (s), 141.0 (s), 132.0 (s), 130.4 (d), 130.0 (d), 128.8 (d), 122.7 (s), 82.5 (t), 64.9 (s), 62.8 (t), 24.8 (t), 22.5 (q), 13.6 (q), 10.5 (q). Anal. ($C_{20}H_{26}N_4O_6$) C, H, N.

5.3. Ethyl 2-Acetamido-3-(1-hydroxy-4-methyl-1,2,3-triazol-5-yl)-2-ethoxycarbonylpropionate (27b). Compound **27b** was prepared from **26b** (292 mg, 0.70 mmol) by the method described for compound **18a**. This gave 227 mg (99%) of **27b** as a solid: mp 144−146 °C; ^{1}H NMR (acetone-d_6) δ 7.62 (s, 1H), 4.20−4.10 (m, 4H), 3.61 (s, 2H), 2.17 (s, 3H), 1.99 (s, 3H), 1.22 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 168.3 (s), 166.0 (s), 136.8 (s), 121.5 (s), 63.9 (s), 60.8 (t), 24.4 (t), 20.4 (q), 12.0 (q), 7.9 (q). Anal. ($C_{13}H_{20}N_4O_6$) C, H, N.

5.4. (*R*,*S*)-2-Amino-3-(1-hydroxy-4-methyl-1,2,3-triazol-5-yl)propionic Acid Hydrochloride (8b·HCl). Compound **27b** (572 mg, 1.74 mmol) was dissolved in 12 M HCl (5 mL) and heated to reflux for 3 h. The reaction mixture was evaporated in vacuo, and the resulting crystals were recrystallized from 2-propanol, which gave 201 mg (62%) of **8b**·HCl: mp >210 °C; ^{1}H NMR (D_2O) δ 4.37 (t, J = 6 Hz, 1H), 3.31 (d, J = 6 Hz, 2H), 2.29 (s, 3H); ^{13}C NMR (D_2O) δ 171.8 (s), 137.2 (s), 124.8 (s), 52.3 (d), 23.3 (t), 8.7 (q). Anal. ($C_6H_{10}N_4O_3$, 115 mol % HCl, 5 mol % Pr^{i}OH) C, H, N.

6. Preparation of Imidazole Amino Acid 9a. 6.1. Ethyl 2-Acetamido-3-(1-benzyloxy-5-imidazolyl)-2-ethoxycarbonylpropionate (37a). A solution of **34a** (814 mg, 2.02 mmol)[20] dissolved in MeOH (20 mL) at 0 °C was treated with

$NaBH_4$ (0.27 g, 3.5 mmol). The mixture was stirred at 0 °C for 3 h and worked up as described for compound **15a** to give crude **35a.** This was stirred with thionyl chloride (10 mL, 140 mmol) at 60 °C for 1.5 h. The mixture was concentrated and evaporated twice with toluene to give 1.1 g of crude **36a** as crystals that were used directly in the following step. The crystals were dissolved in dry DMF (10 mL) and added to a solution of the sodium salt of diethyl acetamidomalonate (12 mmol in 30 mL of DMF), prepared as described for **17a**. The solution was stirred for 16 h at room temperature and worked up as described for **17a** to give 1.1 g of crude **37a**. FC (EtOAc/heptane 1:1 → 1:0, then EtOAc/MeOH 1:0 → 1:5) gave 451 mg (28%) of **37a** as a semicrystalline compound that became an oil when exposed to air: ^{1}H NMR ($CDCl_3$) δ 7.43−7.29 (m, 5H), 6.63 (br s, 1H), 6.68 (br d, J = 0.9 Hz, 1H), 5.00 (s, 2H), 4.25 (dq, J = 10.8, 7.2 Hz, 2H), 4.18 (dq, J = 10.8, 7.2 Hz, 2H), 3.65 (s, 2H), 1.90 (s, 3H), 1,24 (t, J = 7.1 Hz, 6 H); ^{13}C NMR ($CDCl_3$) δ 169.7, 167.2, 132.9, 131.9, 130.1, 129.9, 128.9, 124.8, 122.5, 82.9, 66.1, 62.8, 26.0, 22.7, 13.8. Anal. ($C_{21}H_{26}ClN_3O_3$) C, N, H: calcd, 6.46; found, 5.95.

6.2. (*R,S*)-2-Amino-3-(1-hydroxy-5-imidazolyl)propionic Acid (9a). A mixture of **37a** (422 mg, 1.05 mmol), 10% Pd/C (39 mg), and MeOH (15 mL) was stirred under H_2 (1 atm) at 0 °C for 30 min and at 20 °C for 30 min. Filtration through Celite and removal of MeOH gave 292 mg of crude **38a** as a solid, which was dissolved in 4 M HCl (20 mL) and heated to reflux for 16 h. The mixture was concentrated and evaporated twice with water and twice with toluene to give 272 mg of crude **9a**. The crude product was taken up in water and applied to ion exchange chromatography as described for **7b** to give crude **9a** as a solid, which was dissolved in warm 2-propanol/water and left for 2 days at 5 °C for crystallization. The crystals were filtered off and gave 125 mg (63%) of **9a**: mp >220 °C (dec); ^{1}H NMR (D_2O) δ 8.11 (d, J = 1.8 Hz, 1H), 7.00 (d, J = 1.8 Hz, 1H), 3.99 (dd, J = 3.9, 6.3 Hz, 1H), 3.23 (dd, J = 3.9, 16 Hz, 1H), 3.15 (dd, J = 6.6, 16 Hz, 1H). Anal. ($C_6H_9N_3O_3 \cdot H_2O$) C, H, N.

7. Preparation of Imidazole Amino Acid 9b. 7.1. 4(5)-Methyl-1-hydroxyimidazole-3-oxide (28). A mixture of 40% aqueous methyl glyoxal (42 mL, 0.50 mol), 37% aqueous formaldehyde (27.3 mL, 0.6 mol), and MeOH (15 mL) was cooled to 0 °C, and a solution of hydroxylammonium hydrochloride (70.1 g, 0.5 mol) in water (85 mL) was added over 10 min. Then concentrated HCl (10 mL, 37%) was added over 1 min, and the mixture was stirred for 24 h at room temperature. The reaction mixture was cooled to 0 °C, and the pH was adjusted to 4 by cautious addition of 33% aqueous NaOH. After evaporation of MeOH, the flask was left overnight at 5 °C. The precipitate formed was filtered, washed with water, MeOH, and Et_2O, and dried under vacuum. Recrystallization from water gave 15.7 g (28%) of **28** as a solid: ^{1}H NMR (D_2O) δ 8.24 (d, J = 2.0 Hz, 1H), 6.91 (br s, 1H), 2.13 (s, 3H). The compound was used in the following step without further purification.

7.2. 1-Benzyloxy-2-chloro-5-methylimidazole (31) and 1-Benzyloxy-2-chloro-4-methylimidazole (32). Benzyl bromide (13.5 mL, 0.114 mol) was added to a solution of **28** (13 g, 0.116 mol) and potassium hydroxide (4.78 g, 0.085 mol) in MeOH (40 mL), and the mixture was refluxed for 1 h. After filtration and removal of the solvent, the yellow oil was suspended in $CHCl_3$ (40 mL) and cooled to 0 °C. Then PCl_3 (50 mL) was carefully added at such a rate that the internal temperature was kept under 25 °C. The mixture was allowed to warm to reflux and refluxed for 1.5 h. *Caution: At ca. 40 °C a vigorous exothermic reaction takes place.* The mixture was cooled to room temperature. Solvents and excess PCl_3 were removed in vacuo. The residue was added to toluene (100 mL), and the mixture was evaporated, with repeated addition of toluene and evaporation. Then water (250 mL) and Et_2O (200 mL) were added and the pH was adjusted to 10 by careful addition of 33% aqueous NaOH. Separation of the organic layer, followed by extraction with Et_2O (3 × 50 mL), drying of the combined organic phases ($MgSO_4$), filtration, evaporation, and FC (EtOAc) gave 7.0 g (32%) of a 1:1 mixture of **29b** and **30b**. Attempts to separate the two isomers failed. A total of 2.00 g (10.6 mmol) of this mixture was dissolved in THF (60 mL), cooled to −78 °C, and treated with *n*-BuLi (1.6 M in hexanes, 8.0 mL, 12.8 mmol). After 2 min, hexachloroethane (4.98 g, 21.2 mmol) was added and the reaction mixture was stirred for 1 h at −78 °C and then allowed to warm to room temperature over 1 h. Stirring was continued for 30 min more. The reaction was quenched with saturated $NaHCO_3$ (50 mL) and extracted with CH_2Cl_2. The combined organic phases were dried ($MgSO_4$) and evaoporated in vacuo. FC (heptane/Et_2O) gave 0.59 g (25%) of **32** as an oil: ^{1}H NMR ($CDCl_3$) δ 7.38−7.30 (m, 5H), 6.59 (s, 1H), 5.04 (s, 2H), 2.08 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 132.9 (s), 132.6 (s), 129.4 (d), 129.3 (d), 128.4 (d), 125.5 (s), 113.2 (d), 81.4 (t), 13.9 (q). The crude product was used in the next step without further purification. Further elution gave 0.71 g (30%) of **31** as an oil: ^{1}H NMR ($CDCl_3$) δ 7.40−7.32 (m, 5H), 6.54 (s, 1H), 5.11 (s, 2H), 1.98 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 132.8 (s), 130.02(d), 130.0 (s), 129.7 (d), 128.8 (d), 126.9 (s), 121.2 (d), 81.1 (t), 8.6 (q). The crude product was used in the next step without further purification.

7.3. 1-Benzyloxy-2-chloro-5-hydroxymethyl-4-methylimidazole (35b). At −78 °C a solution of *n*-BuLi (1.6 M in hexanes, 7.2 mL, 11.5 mmol) was added dropwise over 2 min to **32** (2.14 g, 9.6 mmol) dissolved in THF (60 mL). The mixture was stirred for 2 min before DMF (3.7 mL, 48 mmol) was added. Stirring was continued for 1 h at −78 °C, and the mixture was allowed to warm to room temperature over 1 h. Stirring was continued for 30 min more before the reaction was quenched with 2 M HCl (25 mL) and neutralized (pH = 8) by the addition of saturated $NaHCO_3$. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2. The combined organic phases were dried ($MgSO_4$) and evaoporated in vacuo. FC (heptane/Et_2O) gave 2.20 g (92%) of **34b** as an oil, which was used without further purification: ^{1}H NMR ($CDCl_3$) δ 9.68 (s, 1H), 7.45−7.38 (m, 5H), 5.27 (s, 2H), 2.5 (s, 3H). Compound **34b** (2.20 g, 8.78 mmol) was dissolved in MeOH (50 mL), cooled to 0 °C, and treated with $NaBH_4$ (360 mg, 9.5 mmol). The mixture was stirred at 0 °C for 3 h and worked up as described for compound **15a**. FC (EtOAc) gave 1.96 g (89%) of **35b** as a solid. A small sample was recrystallized from EtOAc/heptane: mp 110−111 °C; ^{1}H NMR δ ($CDCl_3$) 7.42 (br s, 5H), 6.60 (br s, 1H), 5.25 (s, 2H), 4.61 (s, 2H), 2.06 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 133.1 (s), 131.9 (s), 130.2 (d), 130.0 (d), 129.0(d), 126.7 (s), 125.5 (s), 81.9 (t), 52.4 (t), 13.0 (q). Anal. ($C_{12}H_{13}ClN_2O_2$) C, H, N.

7.4. Ethyl 2-Acetamido-3-(1-benzyloxy-2-chloro-4-methyl-5-imidazolyl)-2-ethoxycarbonylpropionate (37b). A mixture of **35b** (250 mg, 1.00 mmol) and thionyl chloride (1.0 mL, 14 mmol) in toluene (6 mL) was stirred at 0 °C for 1 h. The mixture was concentrated to give 0.28 g of crude **36b**, which was used directly in the following step. The crude **36b** was dissolved in dry DMF (3 mL) and added to a solution of the sodium salt of diethyl acetamidomalonate (2 mmol in 2 mL of DMF) prepared as described for **17a**. The solution was stirred for 40 h at room temperature and worked up as described for **17a**. FC (EtOAc/heptane 1:1 → 1:0) gave 222 mg (49%) of **37b** as a solid. A small sample was recrystallized from EtOAc/heptane: mp 149−151 °C; ^{1}H NMR ($CDCl_3$) δ 7.48−7.40 (m, 5H), 6.58 (br s, 1H), 4.98 (s, 2H), 4.18−4.06 (m, 4H), 3.57 (s, 2H), 2.01 (s, 3H), 1.74 (s, 3H), 1.18 (t, J = 7.1 Hz, 6 H); ^{13}C NMR ($CDCl_3$) δ 169.62 (s), 167.05 (s), 132.41 (s), 132.14 (s), 130.42 (d), 129.76 (d), 128.60 (d), 125.51 (s), 119.7 (s), 81.69 (t), 62.47 (t), 62.47 (s), 26.37 (t), 22.37 (q), 13.49 (q), 12.83 (q). Anal. ($C_{21}H_{26}ClN_3O_3$) C, H, N.

7.5. Ethyl 2-Acetamido-3-(1-hydroxy-4-methyl-5-imidazolyl)-2-ethoxycarbonylpropionate Hydrochloride (38b·HCl). Compound **38b**·HCl was prepared from **37b** (222 mg, 0.48 mmol) by the method described for compound **18a**, using 2 h at 0 °C for the hydrogenolysis. This gave 112 mg (70%) of **38b**·HCl as a solid. Recrystallization from MeOH/Et_2O afforded crystalline **38b**·HCl: mp 209−211 °C; ^{1}H NMR (D_2O) δ 8.61 (s, 1H), 4.34−4.17 (m, 4H), 3.62 (s, 2H), 2.13 (s, 3H), 1.98 (s, 3H), 1.21 (t, J = 7.2 Hz, 6 H); ^{13}C NMR (D_2O) δ 175.1,

169.3, 129.9, 128.2, 122.7, 66.8, 65.4, 26.2, 22.4, 14.0, 9.8. Anal. ($C_{14}H_{21}N_3O_6$·HCl) C, H, N.

7.6. (*R,S*)-2-Amino-3-(1-hydroxy-4-methyl-5-imidazolyl)propionic Acid Dihydrochloride (9b·2HCl). A solution of **38b**·HCl (100 mg, 0.27 mmol) in 4 M HCl (4 mL) was heated to reflux for 16 h. The mixture was concentrated and coevaporated twice with toluene (25 mL) to give 62 mg (79%) of **9b**·2HCl as a hygroscopic solid: mp 170−172 °C (dec); ^{1}H NMR (D_2O) δ 8.74 (s,1H), 4.36 (t, J = 6.4 Hz, 1H), 3.46−3.31 (m, 2H), 2.25 (s, 3H); ^{13}C NMR (D_2O) δ 171.2 (s), 130.2 (d), 127.8 (s), 122.1 (s), 52.0 (d), 22.9 (t), 9.5 (q). Anal. ($C_7H_{11}N_3O_3$·2HCl, 150 mol % H_2O) C, H, N.

8. Preparation of Imidazole Amino Acid 9c. 8.1. 1-Benzyloxy-5-formyl-2-methylimidazole (34c). At −78 °C a solution of *n*-BuLi (1.57 M in hexanes, 3.5 mL, 5.5 mmol) was added dropwise over 2 min to 1-benzyloxy-2-methylimidazole (**30b**)[21] (1.00 g, 5.50 mmol) dissolved in THF (30 mL). The mixture was stirred for 5 min before DMF (1.95 mL, 25 mmol) was added. Stirring was continued for 1 h at −78 °C, and the mixture was allowed to warm to room temperature over 30 min. The reaction mixture was quenched with 2 M HCl (25 mL) and neutralized (pH 8) by addition of saturated $NaHCO_3$. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (2 × 40 mL). Drying of the combined organic phases and removal of solvents in vacuo, followed by FC (EtOAc/heptane 1:2 → 1:0) gave 567 mg (50%) of **34c** as an oil: ^{1}H NMR δ ($CDCl_3$) 9.67 (s, 1H), 7.64 (s, 1H), 7.50−7.30 (m, 5H), 5.21 (s, 2H), 2.09 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 176.5 (s), 147.6 (s), 138.4 (s), 132.7 (s), 130.2 (d), 129.8 (d), 128.8 (d), 128.0 (d), 81.5 (t), 11.5 (q). The compound was used in the next step without further purification.

8.2. 1-Benzyloxy-5-hydroxymethyl-2-methylimidazole (35c). A solution of **34c** (520 mg, 2.40 mmol) dissolved in MeOH (25 mL) at 0 °C was treated with $NaBH_4$ (0.32 g, 4.2 mmol). The mixture was stirred at 0 °C for 3 h and worked up as described for compound **15a**. FC (EtOAc/MeOH 9:1) gave 400 mg (76%) of **35c** as a solid: mp 91−92 °C; ^{1}H NMR δ ($CDCl_3$) 7.40−7.30 (m, 5H), 6.68 (s, 1H), 5.25 (s, 2H), 4.75 (br s, 1H), 4.56 (s, 2H), 2.11 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 141.0 (s), 133.5(s), 130.0 (d), 129.6 (d), 128.7 (d), 128.4(s), 122.2 (d), 81.4 (t), 52.6 (t), 11.5 (q). The compound was used in the next step without further purification.

8.3. Ethyl 2-Acetamido-3-(1-benzyloxy-2-methyl-5-imidazolyl)-2-ethoxycarbonylpropionate (37c). A mixture of **35c** (369 mg, 1.68 mmol) and thionyl chloride (1.5 mL, 21 mmol) in toluene (9 mL) was stirred at 60 °C for 1 h. The mixture was concentrated to give 386 mg of crude **36c**·HCl as a powder, which was used without further purification: ^{1}H NMR δ (DMSO) 7.71 (s, 1H), 7.58−7.66 (m, 5H), 5.47 (s, 2H), 4.92 (s, 2H), 2.49 (s, 3H); ^{13}C NMR (DMSO) δ 143.0 (s), 132.4 (s), 130.5 (d), 130.2 (d), 129.0 (d), 126.9 (s), 116.4 (d), 82.4 (t), 32.4 (t), 9.8 (q). Crude **36c**·HCl was dissolved in dry DMF (5 mL), and the mixture was added to a solution of the sodium salt of diethyl acetamidomalonate (3.4 mmol in 4 mL of DMF), prepared as described for **17a**. The solution was stirred for 40 h at room temperature and worked up as described for **17a**. FC (EtOAc/heptane 1:1 → 1:0) gave 263 mg (38%) of **37c** as a solid: mp 162−163 °C; ^{1}H NMR ($CDCl_3$) δ 7.41 (br s, 5H), 6.65 (br s, 1H), 6.50 (s, 1H), 4.93 (s, 2H), 4.30−4.03 (m, 4H), 3.69 (s, 2H), 2.24 (s, 3H), 2.05 (s, 3H), 1.24 (t, J = 7.1 Hz, 6 H); ^{13}C NMR ($CDCl_3$) δ 169.5 (s), 167.0 (s), 140.1 (s), 132.6 (s), 130.2 (d), 129.7 (d), 128.9 (d), 123.0 (d), 121.8 (s), 81.5 (t), 66.25 (s), 62.8 (t), 26.7 (t), 22.8 (q), 13.9 (q), 12.1 (q). The compound was used in the next step without further purification.

8.4. (*R,S*)-2-Amino-3-(1-hydroxy-2-methyl-5-imidazolyl)propionic Acid Hydrochloride (9c·2HCl). Compound **38c** was prepared from **37c** (195 mg, 0.48 mmol) by the method described for compound **18a**. After recrystallization from acetone, this provided 124 mg of **38c** as a solid: mp 179 °C; ^{1}H NMR (D_2O) δ 6.81 (s, 1H), 4.20 (q, J = 7.2 Hz, 4H), 3.50 (s, 2H), 2.32 (s, 3H), 1.99 (s, 3H), 1.19 (t, J = 7.1 Hz, 6 H); ^{13}C NMR (D_2O) δ 170.2 (s), 167.6 (s), 137.2 (s), 125.5 (d), 115.8 (s), 66.1 (q), 62.6 (t), 27.0 (t), 22.8 (q), 13.8 (q), 9.5 (q). Compound **38c** was dissolved in 4 M HCl (4 mL) and heated to reflux for 16 h. The mixture was concentrated and coevaporated twice with toluene (25 mL). Recrystallization (twice) from EtOH/Et_2O gave 77 mg (54%) of **9c**·2HCl as a solid. According to ^{1}H NMR, the crystals contained 60 mol % ethanol: mp 142−144 °C (dec); ^{1}H NMR (D_2O) δ 7.19 (s,1H), 4.18 (t, J = 6.4 Hz, 1H), 3.41−3.22 (m, 2H), 2.53 (s, 3H); ^{13}C NMR (D_2O) δ 172.1 (s), 143.7 (s), 126.8 (s), 116.7 (d), 53.4 (d), 25.1 (t), 10.9 (q). Anal. ($C_7H_{11}N_3O_3$·2HCl·H_2O, 60 mol % EtOH) C, H, N.

9. Preparation of Imidazole Amino Acid 10a. 9.1. 1-Benzyloxy-2-hydroxymethylimidazole (39a). At −78 °C a solution of *n*-BuLi (1.6 M in hexanes, 10.8 mL, 17.2 mmol) was added dropwise over 2 min to 1-benzyloxyimidazole (**29a**)[21] (2.48 g, 14.3 mmol) dissolved in THF (80 mL). The mixture was stirred for 5 min before DMF (5.52 mL, 71.4 mmol) was added. The solution was stirred for 1 h at −78 °C and allowed to warm to room temperature over 30 min. The reaction mixture was quenched with 2 M HCl (25 mL) and neutralized (pH 8) by addition of saturated $NaHCO_3$. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (5 × 40 mL). Drying of the combined organic phases and evaporation gave the crude aldehyde **33a**[21] as a yellow oil. This was dissolved in MeOH (75 mL), cooled to 0 °C, and treated with $NaBH_4$ (1.06 g, 28.6 mmol). The mixture was stirred at 0 °C for 2 h and worked up as described for compound **15a**. FC (EtOAc/heptane 1:1) gave 2.91 g (89%) of **39a** as a solid. A small sample was recrystallized from EtOAc/heptane: mp 80−81 °C; ^{1}H NMR δ ($CDCl_3$) 7.40 (s, 5H), 6.79 (s, 2 H), 6.12 (s, 1H), 5.25 (s, 2H), 4,59 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 143.5 (s), 133.4 (s), 129.7 (d), 129.4 (d), 128.7 (d), 123.0 (d), 116.0 (d), 82.5 (t), 53.6 (t). Anal. ($C_{11}H_{12}N_2O_2$) C, H, N.

9.2. 1-Benzyloxy-2-chloromethylimidazole Hydrochloride (40a·HCl). A mixture of compound **39a** (1.49 g, 7.3 mmol), thionyl chloride (2.13 mL, 29.2 mmol), and *N*-ethyldiisopropylamine (2.54 mL, 14.6 mmol) in $CHCl_3$ (35 mL) was refluxed for 2 h. The mixture was concentrated. FC (EtOAc/heptane 1:4) provided pure fractions of unstable **40a**, which were immediately added to 4 M HCl (40 mL). The mixture was coevaporated three times with toluene to give 1.39 g (74%) of **40a**·HCl as a brown crystalline compound. A small sample was recrystallized from EtOAc/MeOH to give light-brown crystals: mp 114−115 °C; ^{1}H NMR (D_2O) δ 7.67 (d, J = 1.5 Hz, 1 H), 7.43−7.40 (m, 6 H), 5.42 (s, 2 H), 4.53 (s, 2 H); ^{13}C NMR (D_2O) δ 134.0 (s), 132.8 (d), 132.5 (d), 131.4 (d), 121.3 (d), 112.0 (d), 85.7 (t), 32.0 (t). Anal. ($C_{11}H_{11}ClN_2O$·HCl) C, H, N.

9.3. Ethyl 2-Acetamido-3-(1-benzyloxy-2-imidazolyl)-2-ethoxycarbonylpropionate (41a). A solution of diethyl acetamidomalonate (1.41 g, 6.48 mmol) in dry DMF (5 mL) was added to a stirred solution of sodium hydride (55%, 389 mg, 16.2 mmol) in dry DMF (5 mL). After 2 h, compound **40a** (1.39 g, 5.4 mmol) in dry DMF (10 mL) was added, and the mixture was stirred for 24 h more at room temperature. The reaction mixture was quenched with saturated $NaHCO_3$ (25 mL) and worked up as described for compound **17a**. FC (EtOAc/MeOH 9:1) provided pure fractions of **41a**, which after evaporation and drying at 0.1 mmHg for several days afforded 2.04 g of a thick oil as a mixture of **41a** and EtOAc (ratio 1:0.57 according to ^{1}H NMR) corresponding to 1.81 g (83%) of **41a**: ^{1}H NMR ($CDCl_3$) δ 7.42−7.35 (m, 5H), 7.0 (s, 1 H), 6.85 (d, J = 1.5 Hz, 1 H), 6.78 (d, J = 1.5 Hz, 1 H), 5.02 (s, 2 H), 4.28 (dq, J = 10.7 and 7.1 Hz, 2 H), 4.22 (dq, J = 10.7 and 7.2 Hz, 2 H), 3.64 (s, 2 H), 1.92 (s, 3 H), 1.25 (t, J = 7.1 Hz, 6 H); ^{13}C NMR ($CDCl_3$) δ 169.4, 167.0, 138.1, 133, 129.8, 129.6, 128.7, 123.7, 115.5, 82.0, 65.2, 62.7, 28.7, 22.7, 13.8. The compound was used in the next step without further purification.

9.4. Ethyl 2-Acetamido-3-(1-hydroxy-2-imidazolyl)-2-ethoxycarbonylpropionate (42a). Compound **42a** was prepared from **41a** (1.01 g, 2.50 mmol) by the method described for compound **18a**. This gave 773 mg (99%) of **42a** as a white foam. Recrystallization from acetone afforded crystals: mp 141−142 °C; ^{1}H NMR (D_2O) δ 7.04 (br s, 2 H), 4.28 (dq, J = 10.7 and 7.1 Hz, 2 H), 4.24 (dq, J = 10.7 and 7.1 Hz, 2 H), 3.73 (s, 2H), 1.98 (s, 3 H), 1.18 (t, J = 7.14 Hz, 6 H); ^{13}C NMR

(D_2O) δ 174.7, 169.0, 133.2, 121.1, 116.3, 66.4, 65.5, 29.0, 22.7, 14.2. Anal. ($C_{13}H_{19}N_3O_6$, 10 mol % H_2O) C, H, N.

9.5. (*R,S*)-2-Amino-3-(1-hydroxy-2-imidazolyl)propionic Acid (10a). A solution of **41a** (252 mg, 0.80 mmol) in 1 M TFA (20 mL) was heated to reflux for 40 h. The mixture was concentrated and coevaporated twice with 2 M HCl (25 mL) and twice with toluene (25 mL). The crude product was taken up in water and applied to ion exchange chromatography as described for **7b** to give 137 mg (100%) of **10a** as a solid. Recrystallization from MeOH gave **10a**: mp 160−161 °C; ^{1}H NMR (D_2O) δ 7.27 (d, J = 2.1 Hz, 1 H), 7.16 (d, J = 2.1 Hz, 1 H), 4.10 (t, J = 6.4 Hz, 1 H), 3.46 (d, J = 6.3 Hz, 2 H); ^{13}C NMR (D_2O) δ 172.6, 136.2, 120.7, 116.5, 53.0, 26.0. An analytical sample was obtained by evaporation of **10a** with 2 M HCl. Anal. ($C_6H_9N_3O_3$·HCl, 20 mol % H_2O) C, H, N.

10. Preparation of Imidazole Amino Acid 10b. 10.1. 1-Benzyloxy-2-hydroxymethyl-5-methylimidazole (39b). At −78 °C a solution of *n*-BuLi (1.6 M in hexanes, 6.7 mL, 10.7 mmol) was added dropwise over 2 min to 1-benzyloxy-2-chloro-5-methylimidazole (**31**) (1.97 g, 8.95 mmol) dissolved in THF (50 mL). The mixture was stirred for 5 min before DMF (3.5 mL, 45 mmol) was added. The solution was stirred for 1 h at −78 °C and was allowed to warm to room temperature over 30 min. The reaction mixture was quenched with 2 M HCl (25 mL) and neutralized (pH 8) by addition of saturated $NaHCO_3$. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (5 × 30 mL). Drying of the combined organic phases and evaporation gave 1.62 g (85%) of the crude aldehyde **33b** as a yellow oil: ^{1}H NMR δ ($CDCl_3$) 9.72 (s, 1H), 7.40 (s, 5H), 6.92 (s, 1H), 5.22 (s, 2H), 1.99 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 178.4 (s), 138.4 (s), 132.5 (s), 131.7 (s), 129.9 (d), 129.4 (d), 128.4 (d), 126.0 (d), 81.5 (t), 7.6 (q). Compound **33b** was dissolved in MeOH (60 mL), cooled to 0 °C, and treated with $NaBH_4$ (0.32 g, 4.2 mmol). The mixture was stirred at 0 °C for 3 h and was worked up as described for compound **15a**. FC (EtOAc) gave 1.40 g (85%) of **39b** as a solid. A small sample was recrystallized from EtOAc/heptane: mp 106−107 °C; ^{1}H NMR δ ($CDCl_3$) 7.46−7.36 (m, 5H), 6.58 (s, 1H), 5.26 (s, 2H), 4.62 (s, 2H), 2.04 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 142.9 (s), 133.5 (s), 130.1 (d), 129.7 (d), 128.9 (d), 125.2 (d), 120.6 (s), 82.1 (t), 54.3 (t), 8.1 (q). Anal. ($C_{12}H_{14}N_2O_2$) C, H, N.

10.2. Ethyl 2-Acetamido-3-(1-benzyloxy-5-methyl-2-imidazolyl)-2-ethoxycarbonylpropionate (41b). Compound **41b** was prepared from **39b** (600 mg, 2.75 mmol) and thionyl chloride (2.0 mL, 28 mmol) in toluene (12 mL) by the method described for **37b**. FC (EtOAc/heptane 1:1 → 1:0) gave 568 mg (50%) of **41b** as an oil: ^{1}H NMR ($CDCl_3$) δ 7.42 (br s, 5H), 6.95 (br s, 1H), 6.55 (s, 1H), 4.97 (s, 2H), 4.29 (dq, J = 10.5, 6.9 Hz, 2H), 4.23 (dq, J = 10.5, 7.2 Hz, 2H), 3.71 (s, 2H), 2.11 (s, 3H), 1.89 (s, 3H), 1.25 (t, J = 7 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 169.4 (s), 167.1 (s), 137.0 (s), 132.7 (s), 129.9 (d), 129.4 (d), 128.6 (d), 124.1 (d), 121.4 (s), 80.9 (t), 65.2 (s), 62.3 (t), 29.1 (t), 22.4 (q), 13.5 (q), 8.0 (q).

10.3. Ethyl 2-Acetamido-3-(1-hydroxy-5-methyl-2-imidazolyl)-2-ethoxycarbonylpropionate (42b). A mixture of **41b** (515 mg, 1.24 mmol), 10% Pd/C (50 mg), and MeOH (5 mL) was stirred under H_2 (1 atm) at 0 °C for 1 h. Filtration through Celite and removal of MeOH gave 367 mg (91%) of **42b** as a solid. A small sample was recrystallized from EtOAc: mp 66−67 °C; ^{1}H NMR (D_2O) δ 6.81 (s, 1H), 4.32−4.18 (m, 4H), 3.71 (s, 2H), 2.07 (s, 3H), 1.97 (s, 3H), 1.18 (t, J = 7 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 174.6(s), 168.9 (s), 132.1 (s), 129.5 (d), 112.4 (s), 66.1 (s), 65.1 (t), 29.0 (t), 22.2 (q), 13.7 (q), 8.2 (q). Anal. ($C_{12}H_{21}N_3O_6$·H_2O) C, H, N.

10.4. (*R,S*)-2-Amino-3-(1-hydroxy-5-methyl-2-imidazolyl)-propionic Acid Dihydrochloride (10b·2HCl). A solution of **42b** (180 mg, 0.55 mmol) in 4 M HCl was heated to reflux for 16 h. The mixture was concentrated and evaporated twice with water and twice with toluene to give 144 mg (92%) of **10b**·2HCl as a hygroscopic solid: mp 150−152 °C (dec); ^{1}H NMR (D_2O) δ 7.09 (s, 1H), 4.42 (t, J = 7.1 Hz, 1H), 3.68−3.53 (m, 2H), 2.24 (s, 3H); ^{13}C NMR (D_2O) δ 171.0 (s), 137.2 (s), 130.5 (d), 114.1 (s), 51.2 (d), 25.3 (t), 7.7 (q). Anal. ($C_7H_{11}N_3O_3$·2HCl·H_2O) C, H, N.

11. Potentiometric Titrations. A titration method was applied for the determination of the pK_a values of the four compounds using a GLpKa (Sirius Analytical Instruments, Ltd.) apparatus. This instrument is specifically designed to determine ionization constants (pK_a values) by titration using potentiometry.

Since the water solubility of the compounds is high, the titrations were performed without use of a cosolvent. Approximately 5 mg of the particular compound was weighed out, 15 mL of 0.15 M KCl was added, and three titrations were performed at 25.1 ± 0.1 °C on the sample using 0.500 N HCl and 0.500 N KOH. The first titration was in the direction of low pH (pH 2) to high pH (pH 12), the second from high pH to low, and the last and third from low to high pH. On the basis of the titration curves and the corresponding blank curve, the three pK_a values were determined for each of the difference curves by numerical refinement using the GLpKa software. A difference curve is defined as the number of protons bound per molecule as a function of pH. The three curves were combined in a multiset, and the refinement was repeated. The final results regarding the pK_a values were from the multiset analysis.

12. Receptor Binding Assays. Affinities for NMDA, AMPA, and kainic acid receptors were determined using [^{3}H]-CPP,[26] [^{3}H]AMPA,[24] and [^{3}H]kainic acid[25] with the following modifications. The membrane preparation used in all the receptor binding experiments were prepared according to the method described by Ransom and Stec.[32] All binding experiments were carried out at 0−4 °C. On the day of experiments, frozen homogenates were quickly thawed and resuspended in 50 volumes of buffer (pH 7.4) (50 mM Tris-HCl + 2.5 mM $CaCl_2$, 30 mM Tris-HCl + 2.5 mM $CaCl_2$, or 50 mM Tris-HCl for [^{3}H]CPP, [^{3}H]AMPA, or [^{3}H]Kainic acid binding, respectively) and centrifuged (48 000*g*, 10 min). This step was repeated four times. In [^{3}H]AMPA experiments 100 mM KSCN was added to the buffer during the final wash and during incubation. The final pellet was resuspended in ice-cold buffer, corresponding to approximately 50, 25, or 25 mg/mL original tissue for [^{3}H]CPP, [^{3}H]AMPA, or [^{3}H]kainic acid binding, respectively. Nonspecific binding was determined using 1 mM Glu. For [^{3}H]CPP binding, aliquots consisted of 50 μL of test, 50 μL of [^{3}H]CPP, and 400 μL of membrane suspension. Following incubation for 30 min, filtration (GF/B) was carried out through a 48-well Brandell cell harvester, followed by washing with 3 × 0.5 mL buffer. To the filters was added a total of 3 mL of OptiFluor, and the amount of bound radioactivity was determined using a TRI-CARB liquid scintillation analyzer. [^{3}H]AMPA and [^{3}H]kainic acid binding were carried out in aliqouts consisting of 25 μL of [^{3}H]ligand, 25 μL of test, and 200 μL of membrane suspension. Binding was terminated by filtration through GF/B filters using a 96-well Packard FilterMate cell harvester and washing with 3 × 250 μL of buffer. Filters were dried, and 25 μL of Microscint 0 was added. The amount of bound radioactivity was determined using a Packard TOPCOUNT microplate scintillation counter. The data were analyzed using Grafit 3.0, Leatherbarrow software. Data were fitted to the equation

$$B = 100 - \frac{100 \times [\text{inhibitor}]^n}{IC_{50}{}^n + [\text{inhibitor}]^n}$$

where B is the binding as a percentage of total specific binding and n is the Hill coefficient.

13. Synaptosomal Glu Uptake. The experiments were carried out essentially as previously described.[33] Rat cortical synaptosomes were prepared from male Sprague−Dawley rats (200−249 g). The final synaptosomal pellet was resuspended in assay buffer containing 128 mM NaCl, 10 mM glucose, 5 mm KCl, 1.5 mM NaH_2PO_4, 1.77 mM $CaCl_2$, 1 mM $MgSO_4$, and 10 mM Tris (pH 7.4) in a final concentration of 2 mg/mL. The experiment was carried out at room temperature following a 5 min preincubation of the synaptosomes with a solution

containing [^{3}H]-(*R*)-aspartic acid (30 nM) and (*R*)-aspartic acid (3 μM). The assay was initiated by adding 800 μL of membrane solution to a mixture of test substance (100 μL) and a solution (100 μL) containing [^{3}H]-(*R*)-aspartic acid (30 nM) and (*R*)-aspartic acid (3 μM). Follwing a 10 min incubation, the reaction was terminated by filtering though a 48-well Brandell cell harvester using GF/B filters. Filters were washed with 3 × 3 mL of 50 mM Tris buffer. Total binding was determined in the presence of buffer, and nonspecific binding was determined in the presence of 1 mM (*R*)-aspartic acid.

14. Testing at metabotropic Glu receptors. Three metabotropic Glu receptor subtypes, $mGlu_{1\alpha}$, $mGlu_2$, and $mGlu_{4a}$, were stably expressed in Chinese hamster ovary cells and used as representatives for groups I, II, and III, respectively. Cell maintenance and pharmacological assays were performed as previously described.[34]

15. In Vitro Electrophysiology. The rat cortical preparation[35] in a modified version[36] was used for the determination of the depolarizing effects of the analogues under study. Compounds that did not show agonist properties were tested as antagonists toward 5 μM AMPA, 5 μM kainic acid, and 10 μM NMDA. Agonists were applied for 90 s. Receptor selectivity was determined by antagonizing agonist responses, evoked with a concentration approximately corresponding to the EC_{50} values of the agonists in question, with 5 μM CPP and 5 μM NBQX for NMDA and AMPA receptors, respectively. Antagonists were applied for 90 s, followed by coapplication of agonist and antagonist for 90 s. The data were fitted to the equation

$$\% \text{ response} = \frac{E_{max}[\text{agonist}]^n}{EC_{50}{}^n + [\text{agonist}]^n}$$

where E_{max} is the relative maximal response and n is the Hill coefficient.

16. EAAT Uptake Procedure. Cell maintenance and EAAT uptake assay was performed as previously described.[37] Cos-7 cells were maintained in Dulbecco's modified eagle's medium (DMEM) containing 10% fetal bovine serum (both GIBCO, Paisley, Scotland) in a humidified incubator containing 5% CO_2 and 95% atmosphere. A total of 1 million cells were split into a 10 cm tissue culture dish, and the following day, cells were transfected with 5 μg of EAAT DNA inserted in pcDNA3 (Invitrogen, La Jolla, CA) using SuperFect (Qiagen, Hilden, Germany) as a DNA carrier. The day after transfection, cells from one 10 cm dish were transferred to 48-well tissue culture plates, and 2 days after transfection, uptake assays were performed.

Each well was washed three times at room temperature with 150 μL of modified phosphate-buffered saline (mPBS, 137 mM NaCl, 8.1 mM Na_2PO_4, 2.7 mM KCl, 1.5 mM KH_2PO_4, 0.9 mM $CaCl_2$, 1.0 mM $MgCl_2$, and 5.6 mM D-glucose, all Sigma Chemicals, St. Louis, MO) over a period of 10 min. Each well was then incubated for 10 min in mPBS containing 100 nM [^{3}H]-(*R*)-aspartic acid (Amersham, Buckinghamshire, U.K.), 900 nM cold (*R*)-aspartic acid (Sigma Chemicals, St. Louis, MO), and test ligand. Washing the wells three times with 150 μL of ice-cold mPBS terminated uptake, and cells were lysed by addition of 200 μL of 0.1% sodium dodecyl sulfate (SDS, Sigma Chemicals, St. Louis, MO). The lysate was transferred to scintillation vials containing 2 mL of OptiFluor scintillation fluid (Packard, Groningen, The Netherlands) and counted on a scintillation counter.

17. Molecular Modeling. Anionic deprotonated heterocyclic fragments corresponding to **7a,b**, **8a,b**, **9a**−**c**, and **10a,b** were constructed by removing the common glycine unit and replacing it with a proton, leaving a ring-methyl group in place of the side chain. These nine substructures were then geometry-optimized in water without constraints, using finite difference solution of the Poisson−Boltzmann equations in a self-consistent reaction field (PB-SCRF method[38]). Density functional theory (B3LYP) was used to approximate the wave function, using the 6-311+G(d,p) basis set in Jaguar.[39] In the case of 1-hydroxypyrazole, this method has been shown to produce aqueous-phase geometries that are close to, and of intermediate character between, high-level gas-phase ab initio structures and X-ray crystal structures.[38] The appropriate ring-methyls of the optimized heterocycles were resubstituted with the glycine moiety in zwitterionic form, and the three dihedral angles defining the α-amino acid conformation were adjusted to match those recorded by Armstrong and Gouaux[27] for AMPA bound to GluR2−S1S2J. The structures were reoptimized in solution, loosely constraining these dihedrals and freezing the ring atoms plus those atoms directly bound to the ring. This optimization was performed using the Cramer−Truhlar AM1-SM2 semiempirical method implemented in Spartan 5.1.[40] The result was aqueous 3D geometries for the negatively charged tri-ionized forms of **7a,b**, **8a,b**, **9a**−**c**, and **10a,b** in solution, corresponding to the bound conformation of **3b** and close to the aqueous local energy minima.

The nine structures were docked into the binding site of the centrally located protein of the three almost identical subunit constructs found in the crystal structure of the GluR2−S1S2J−AMPA complex of Armstrong and Gouaux.[27] This procedure involved superimposing each structure in turn on **3b**, using least-squares fitting. Five atoms of each structure were used: both of the oxygens and the nitrogen of the α-amino acids, the distal phenolate-like oxygen, and the ring atom attached to it. Protons were added to the proximal residues using the builder in InsightII.[41] Crucial water molecules were protonated, and the waters were rotated to maximize hydrogen bonding. The dihedral angles of rotatable side chain hydroxyls were also adjusted to complete the hydrogen bond network.

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Supporting Information Available: Detailed description of the molecular modeling. This material is available free of charge via the Internet at http://pubs.acs.org.

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