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3-Substituted phenylalanines as selective AMPA- and kainate receptor ligands

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ABSTRACT

On the basis of X-ray structures of ionotropic glutamate receptor constructs in complex with amino acid-based AMPA and kainate receptor antagonists, a series of rigid as well as flexible biaromatic alanine derivatives carrying selected hydrogen bond acceptors and donors have been synthesized in order to investigate the structural determinants for receptor selectivity between AMPA and the GluR5 subtype of kainate receptors. Compounds selective for either GluR5 or AMPA receptors were identified. One particular substituent position appeared to be of special importance for control of ligand selectivity. Using molecular modeling the observed structure–activity relationships at AMPA and GluR5 receptors were deduced.

1. Introduction

Ionotropic glutamate receptors (iGluRs) are the major excitatory amino acid neurotransmitter receptors in the vertebrate central nervous system and are separated into three functionally distinct subclasses: *N*-methyl-D-aspartic acid (NMDA), (*RS*)-2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid (AMPA) and (2*S*,3*S*,4*S*)-3-carboxymethyl-4-isopropenyl-pyrrolidine-2-carboxylic acid (kainate, KA) receptors. All three classes play an important role in controlling synaptic plasticity, underlying learning and memory processes and other central nervous system (CNS) functions, such as synaptogenesis and the development of functional neurons.[1–4] AMPA receptors, which mediate the majority of the fast excitatory amino acid synaptic transmission in the CNS and affect short-term changes in synaptic strength, are cation-selective tetrameric heterooligomers formed by combinations of the subunits GluR1-4, whereas kainate receptors are tetrameric assemblies of GluR5-7, KA1 and KA2 subunits. Their role is not fully understood; however, studies suggest that both pre- and postsynaptic kainate receptors can regulate neurotransmission at many synapses and seem to be involved in short- and long-term plastic phenomena.[5] Recently GluR5 receptors were reported to play an important role in nociception and central sensitization.[3,6–8]

There is a clear pharmacological distinction between NMDA and other iGluRs. However, most of AMPA receptor agonists and antagonists bind also kainate receptors and only few ligands discriminate between homomeric AMPA and kainate receptors. Only recently the available crystal structures of the iGluR binding core in complex with agonists or antagonists have provided more detailed information on ligands' binding modes and insight into the molecular mechanisms of ligand selectivity. Well known examples are unselective AMPA/GluR5 antagonist (*S*)-2-amino-3-[5-*tert*-butyl-3-(phosphono-methoxy)-4-isoxazolyl]-propionic acid [(*S*)-ATPO (1)], co-crystallized with GluR2 as well as GluR5 binding cores[9–11] and complexes of the selective GluR5 antagonists (*S*)-1-(2-amino-2-carboxyethyl)-3-(2-carboxybenzyl)-pyrimidine-2,4-dione (UBP302, **3**) and (*S*)-1-(2-amino-2-carboxyethyl)-3-(2-carboxythiophene-3-yl)methyl-5-methyl-pyrimidine-2,4-dione (UBP310, **4**)[12–15] co-crystallized with the GluR5 construct (Fig. 1). UBP302 and UBP310 belong to the group of willardiine-based antagonists and show nanomolar antagonist potency at human recombinant GluR5 receptors with high selectivity against cloned human GluR1-4 and GluR6 receptors.[15,16]

Competitive non-NMDA iGluR amino acid-based antagonists typically consist of an α-amino part and a distal acidic part linked together through a heterocyclic ring system. Analysis of the crystal structures of the (*S*)-ATPO, UBP302 and UBP310 complexes[9,11,15] revealed that the spacer helps stabilize the receptor in an open conformation.

In the present study we have set out to replace the linker parts of the UBP compounds with two aromatic ring systems and to investigate the influence of a carboxylic acid substituent in the distal phenyl ring on iGluR affinity (Fig. 1b). With replacement of uracil by phenyl it is possible to introduce substituents (R_1) in the proximal phenyl ring exploiting various regions of the binding

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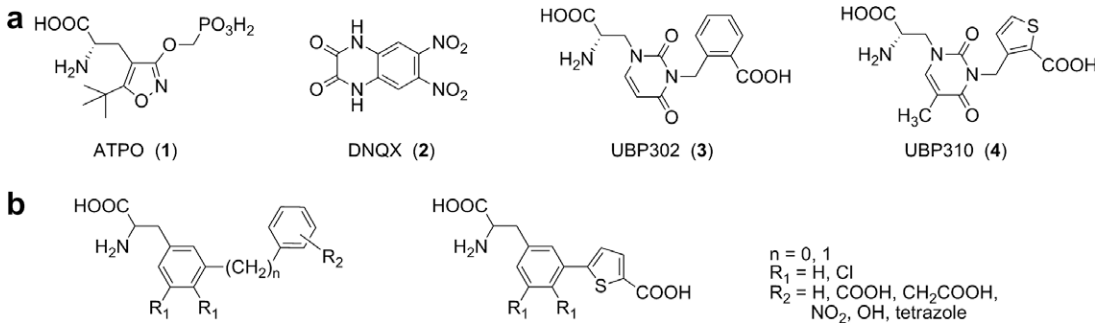


Figure 1. (a) Structures of selected AMPA and kainate receptor antagonists. (b) Structures of target compounds.

pocket and potentially increasing the affinity to the receptor. Based on the analysis of the crystal structures, introduction of a phenyl ring instead of the heterocyclic parts of the ligands is expected to be accepted both by AMPA as well as GluR5 receptors. In analogy with the UBP compounds a second phenyl/benzyl/thiophenyl fragment bearing carboxyl or methyl carboxyl groups has been placed in position 3 of the phenylalanine ring, to some extent mimicking the substitution pattern of the UBP compounds. The position of the carboxylic substituent R_2 in the distal phenyl has also been examined.

In addition, we also decided to investigate analogues in which the terminal acidic group was replaced by a series of hydrogen bond acceptors or donors, such as nitro, tetrazolyl and hydroxyl substituents (R_2). All new target compounds were pharmacologically characterized at native (NMDA, AMPA, KA) and at recombinant (GluR5, GluR6, GluR7) receptors. Finally, in attempt to explain the observed selectivity towards either AMPA-R or GluR5, molecular docking of selected target compounds was performed, using as templates the (*S*)-ATPO-GluR2 and UBP302-GluR5 binding core complexes (PDB ID: 1N0T and 2F35), respectively.

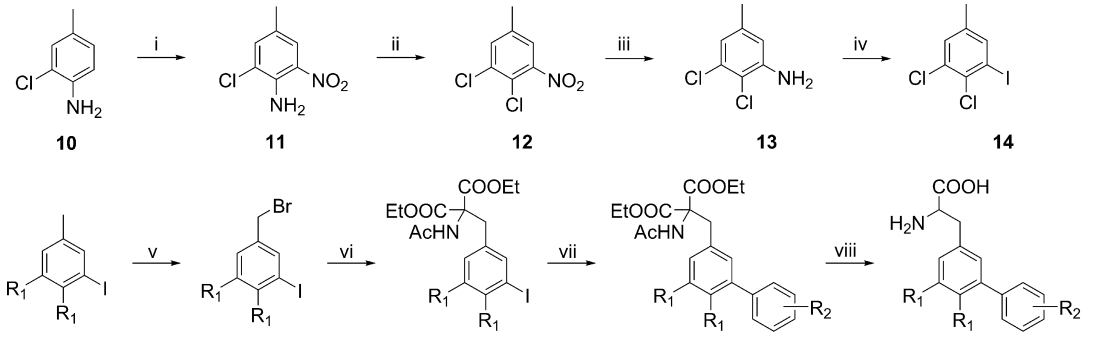
2. Results and discussion

2.1. Chemistry

The synthetic pathways for the new phenylalanine derivatives are illustrated on the Schemes 1–5.

Most of the target amino acids were prepared as a result of a number of Suzuki cross-coupling reactions of aryl halides with boronic acids. Hence, synthesis of the key intermediates **7**, **16**, **24**, **29** and **30** containing iodo-, bromo- or methylbromo- substituents, was crucial for the strategy.

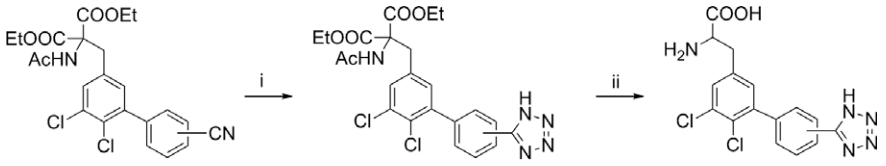
Compounds **6** and **15** were obtained as a result of benzylic bromination of **5** and **14**, respectively, with NBS under free radical conditions (Scheme 1). 3-Iodotoluene (**5**) is commercially available; 1,2-dichloro-3-iodo-5-methyl-benzene (**14**) was synthesized according to the literature methods from 2-chloro-4-methylaniline (**10**). Nitration into the position 6 of the phenyl ring with 96% nitric acid, following the Sandmayer reaction using $CuCl_2$ gave 1,2-dichloro-5-methyl-3-nitrobenzene (**12**). Reduction of the nitro group with Fe in the presence of hydrochloric acid afforded **13**, which



	R_1	R_2
8a	H	2-CN
8b	H	3-CN
8c	H	4-CN
8d	H	3-CH_2CN
8e	H	4-CH_2CN
17a	Cl	2-CN
17b	Cl	3-CN
17c	Cl	4-CN
17d	Cl	2-OCH_3
17e	Cl	3-OCH_3
17f	Cl	4-OCH_3
17g	Cl	3-NO_2
17h	Cl	4-NO_2

	R_1	R_2
9a	H	2-COOH
9b	H	3-COOH
9c	H	4-COOH
9d	H	3-CH_2COOH
9e	H	4-CH_2COOH
18a	Cl	2-COOH
18b	Cl	3-COOH
18c	Cl	4-COOH
18d	Cl	2-OH
18e	Cl	3-OH
18f	Cl	4-OH
18g	Cl	3-NO_2
18h	Cl	4-NO_2

Scheme 1. Reagents and conditions: (i) (1) $(CH_3COO)_2O$, 96% HNO_3, <15 °C, (2) HCl, reflux; (ii) (1) $CuCl_2$, *tert*-$BuNO_2$, acetonitrile, 65 °C, (2) 20% HCl; (iii) Fe, HCl, ethanol, reflux; (iv) (1) $NaNO_2$, H_2SO_4, acetic acid, 25 °C → 75 °C, (2) urea, KI; (v) NBS, benzoyl peroxide, CCl_4, reflux; (vi) NaH 1.5 equiv, diethyl acetamidomalonate 1.5 equiv, DMF, rt; (vii) substituted phenylboronic acid 1.5 equiv, $PdCl_2(PPh_3)_2$ 5 mol %, triethylamine, DME:water (1:1), rt or 50 °C; (viii) 48% HBr, reflux.

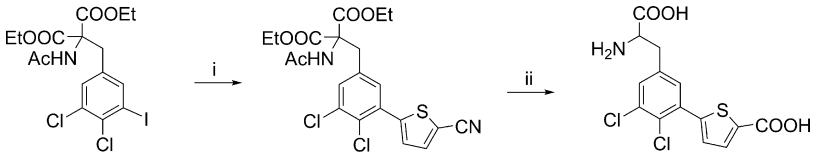


17a, R=2-CN
17b, R=3-CN
17c, R=4-CN

19a, R=2-tetrazolyl
19b, R=3-tetrazolyl
19c, R=4-tetrazolyl

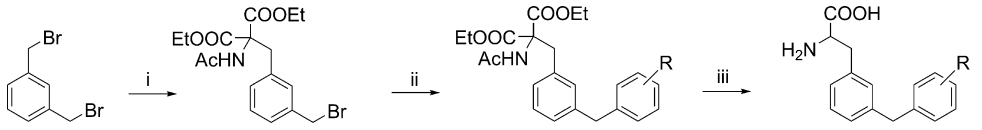
20a, R=2-tetrazolyl
20b, R=3-tetrazolyl
20c, R=4-tetrazolyl

Scheme 2. Reagents and conditions: (i) NaN_3, TEA hydrochloride, DME, reflux; (ii) $HCl/H_2O/AcOH$ (1:1:1), reflux.



16 **21** **22**

Scheme 3. Reagents and conditions: (i) 5-cyanothiophene-2-boronic acid 1.5 equiv, $Pd(PPh_3)_4$ 5 mol %, K_2CO_3 3 equiv, toluene/ethanol/water (10:3:3), rt → 50 °C; (ii) $HCl/H_2O/AcOH$ (1:1:1), reflux.



23 **24**

25a, R=2-COOEt
25b, R=3-CN
25c, R=4-CN

26a, R=2-COOH
26b, R=3-COOH
26c, R=4-COOH

Scheme 4. Reagents and conditions: (i) NaH 1.1 equiv, diethyl acetamidomalonate 1.1 equiv, DMF, rt; (ii) phenylboronic acid 1.5 equiv, $[1,1'\text{-}(PPh_2)_2Fe]PdCl_2$ 5 mol %, 3 M NaOH, TBAB, THF:water (5:1), 50 °C; (iii) 48% HBr, reflux.

was converted into the iodide **14** through the corresponding diazonium salt. The key intermediates **7** and **16** were prepared from the benzyl bromides **6** and **15**, respectively, through substitutions with the sodium salt of diethyl acetamidomalonate.

Treatment of **7** and **16** with phenylboronic acids in the presence of palladium complexes afforded the biphenyl derivatives **8a–e** and **17a–h**. (Scheme 1). A short optimization of Suzuki cross-coupling conditions was performed: various palladium sources, solvents and bases were examined; however, as most of the reactions were conducted at elevated temperature and because of the basic conditions, partial deprotection of the diethyl acetamidomalonate moiety was observed, which led to problems with isolation of the product and low yield. Eventually, use of a mixture of dimethoxyethane (DME) and water (1:1) as solvent, $PdCl_2(PPh_3)$ and triethylamine as base turned out to produce the most effective conditions, under which most reactions could be completed at room temperature.

To introduce the tetrazolyl ring system into the distal phenyl ring, the corresponding nitriles **17a–c** were heated for 48 h with sodium azide in DME in the presence of triethylammonium hydrochloride (Scheme 2).

In the case of 5-cyanothiophene-2-boronic acid however, the Suzuki cross-coupling did not lead to the coupled product. Instead, de-iodination was observed. After optimizing the conditions, compound **21** was obtained—only in low yield though—using a mixture of toluene–ethanol–water (in ratio 10:3:3) as solvent and $Pd(PPh_3)_4$ as catalyst (Scheme 3).

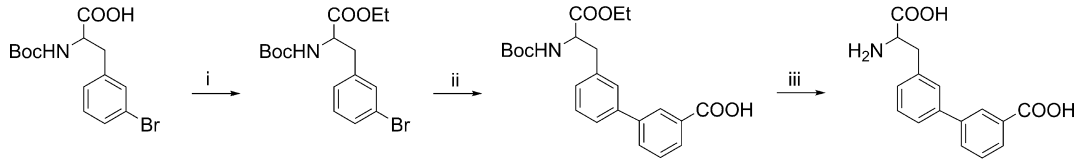
In the case of benzylbromide **24** (Scheme 4) the Suzuki coupling reaction conditions used for the preparation of **8a–e** turned out to be ineffective. However, using an electron-rich and hindered $PdCl_2$(dppf) complex ([1,1′-bis(diphenylphosphino)ferrocene] dichloropalladium(II)) in a biphasic solvent system (THF and water 5:1) at 50 °C and under the presence of tetrabutylammonium bromide (TBAB), the conversions were completed within 2 h to give **25a–c** in good yields. The phase transfer catalyst, TBAB, has recently been used successfully in Suzuki couplings performed under biphasic conditions,[17–19] enhancing the rate of the coupling reaction by activating the boronic acid through formation of a boronate complex $[ArB(OH)_3]^-$ $[R_4N]^+$.

Deprotection of the prepared biphenyl-, thiophenyl- and benzylphenyl derivatives in 48% aqueous hydrobromic acid or—under milder conditions—in a mixture of acetic acid and hydrochloric acid followed by reverse-phase HPLC purification afforded the target amino acids **9a–e**, **18a–h**, **20a–c**, **22** and **26a–c**.

In order to prepare the *S*- and *R*-enantiomers of **9b** (Scheme 5) commercially available (*S*)- and (*R*)-*N*-Boc-3-bromo-phenylalanine, respectively, were treated with ethyl iodide in the presence of sodium bicarbonate in DMF,[20] followed by reaction with 3-cyano-phenylboronic acid under Suzuki coupling conditions as described for **8b**. Deprotection in 48% aqueous hydrobromic acid gave the desired amino acids **33** (*S*, ee = 99.2%) and **34** (*R*, ee = 98.4%).

2.2. Pharmacology

All target compounds were tested for in vitro pharmacological activity at recombinant rat GluR5(Q)$_{1b}$, GluR6(*V,C,R*) and GluR7A homomeric kainate receptors expressed in *Sf*9 insect cell mem-



27, (*S*)
28, (*R*)
29, (*S*)
30, (*R*)
31, (*S*)
32, (*R*)
33, (*S*)
34, (*R*)

Scheme 5. Reagents and conditions: (i) C_2H_5I 5 equiv, $NaHCO_3$ 2 equiv, DMF, rt; (ii) 3-cyanophenylboronic acid 1.5 equiv, $PdCl_2(PPh_3)_2$ 5 mol %, triethylamine, DME:water (1:1), rt; (iii) 48% HBr, reflux.

branes as well as at the native AMPA, kainate and NMDA receptors in rat cortical membranes (Table 1).

In the AMPA receptor binding assay to native receptors, all target compounds containing carboxyl groups showed either no or very low affinity (IC_{50} higher than or close to 100 μM). However, only analogues containing a hydroxyl substituent showed measurable affinities, **18e** (the dichloro-substituted biphenyl analogue possessing an hydroxyl group in position 3 of the distal phenyl ring) being the most active (IC_{50} = 4.6 μM); more potent than (*S*)-ATPO. Change of substituent position resulted in a decrease of affinity; the 4-hydroxy analogue (**18f**) was approximately sevenfold less potent than the parent compound, while the 2-hydroxy analogue (**18d**) was almost inactive in this assay. Weak micromolar affinity towards AMPA receptors was also expressed by 4-carboxy substituted biphenyl analogues **9c** and **18c** and carboxythiophene analogue **22**. None of the amino acids active in the AMPA receptor binding assay displayed measurable affinity to GluR5s.

At homomeric GluR5, compounds with structures based on the biphenyl core with a carboxylic acid group attached to the distal phenyl ring in position 3 (**9b** and **18b**) showed the highest affinity (7.9 μM and 2.8 μM, respectively); comparable to that of (*S*)-ATPO. Adding two chlorines as R_1 substituents to the phenylalanine ring improved GluR5 potency, making analogue **18b** the most active GluR5 ligand among the obtained compounds. Interestingly, neither **9b** nor **18b** showed measurable affinity towards native iGluR: AMPA, kainate or NMDA receptors.

To examine, which isomer of the active target 3-carboxy substituted biphenyl compounds is responsible for GluR5 kainate receptor activity, both the *S*- and *R*-enantiomers of **9b** (compounds **33** and **34**, respectively) were synthesized and tested. As expected on the basis of similarity of structures **9b** and UBP302, the GluR5-subtype activity was located in the *S*-isomer of **9b** (**33**), while the corresponding *R*-enantiomer (34) showed no measurable affinity (K_i > 1 mM).

The position of the carboxyl substituent seems to be imperative for high affinity, as none of the positional isomers of **9b** and **18b** displayed any significant affinity. In addition, replacement of the acidic group with an hydroxy group (**18d–f**), nitro group (**18g**, **h**) or tetrazole ring (**20a-c**), or prolongation of the terminal acidic chain (**9d,e**) or exchange of the whole carboxybenzene fragment into 2-carboxythiophene-5-yl resulted in a marked drop of affinity at GluR5 compared to **9b**/**18b**.

In addition, the three analogues possessing the methylene linker between the two phenyl rings (**26a–c**) showed almost no affinity at GluR5, regardless of the position of the carboxylic group. None of the target amino acids expressed measurable affinity to native NMDA or KA receptors, GluR6 or GluR7 rat homomeric recombinant receptors.

Table 1
Binding affinities at native AMPA receptors and homomeric GluR5 kainate receptors[a]

	IC_{50} (μM) [^{3}H]AMPA	K_i (μM) GluR5
(*S*)-ATPO	16 ± 1[b]	2.90 ± 0.26
UBP302[c]	nd	nd
9a	>100	>100
9b	>100	7.9 ± 0.8
9c	41 ± 2	>100
9d	>100	⩾100
9e	>100	>100
18a	>100	>100
18b	>100	2.80 ± 0.78
18c	42 ± 6	>100
18d	85 ± 1	>100
18e	4.6 ± 0.2	>100
18f	33 ± 4	>100
18g	>100	>100
18h	>100	>100
20a	>100	>100
20b	>100	⩾100
20c	>100	>100
22	61 ± 8	⩾100
26a	>100	⩾100
26b	>100	50–100
26c	>100	⩾100
33	nd	4.08 ± 0.45
34	nd	>1000

nd = Not determined.

[a] Values come from three independent experiments and are expressed as the mean ± SEM.

[b] (*S*)-ATPO affinity data at AMPA receptors taken from Ref. 10; affinity at GluR5 homomeric receptors determined in current study.

[c] UBP302 binding affinity at GluR2 S1S2 ligand-binding core K_d = 146 ± 33 μM and at GluR5 S1S2 ligand-binding core K_d = 3.94 ± 0.7 μM.[15]

2.3. Molecular modeling study

To understand recombinant homomeric GluR5 affinity data obtained for the new compounds, molecular docking of selected compounds was undertaken. The series of structures with biphenyl or phenylbenzyl core acting as a spacer between amino acid part and the distal carboxyl group (**9a–c**, and **18a–c**) were docked into the ligand-binding domain of UBP302-GluR5 complex (PDB ID: 2F35).

Molecular modeling was performed using Schrödinger Suite 2007 environment.[21] The initial ligand structures were prepared with MacroModel v. 9.5 (MMFFs forcefield including water solvation, carboxy and amino groups were ionized). Only *S*-enantiomers were taken into account. Protein preparation and docking of ligands was performed using Glide 4.5 (all water molecules were removed from the protein before docking). The obtained poses, in which amino acid chain was bound in the way corresponding to the binding mode of UBP302, were ranked according to highest scores (GlideScore function) and lowest energies.

The most effective poses were observed for the structures **9b** and **18b** (the top pose of **18b** is presented in Fig. 2). As expected, apart from contacts between α-amino acid and residues Arg508, Thr503, Pro501; **18b** forms also hydrogen bond interactions between the distal carboxylic acid group and residue Thr675 of domain 2, resembling the binding mode of UBP302. The

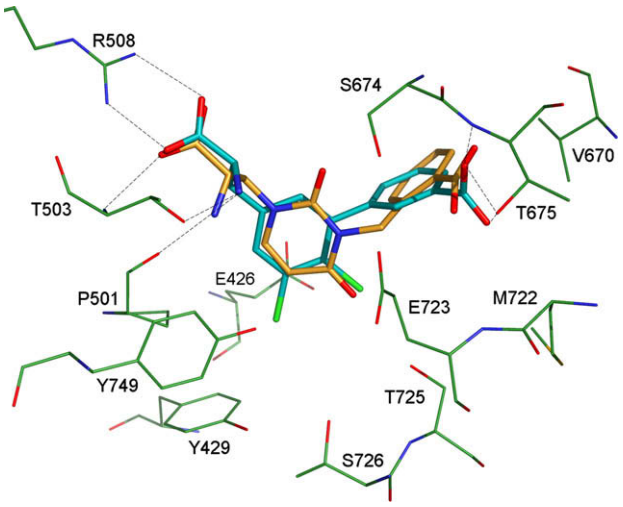


Figure 2. Compound **18b** (blue) docked to UBP302-GluR5 S1S2 complex. Residues of UBP302-GluR5 binding core—green, UBP302 ligand—orange. Ion pair interactions and hydrogen bonds within the distance lower than 3.5 Å between **18b** and the protein are represented by dashes. Prepared by Pymol.[22]

phenylalanine ring is almost coplanar with the uracil ring of the willardiine antagonist; position 5 in **18b**, substituted with a chlorine atom, overlaps with position 5 of uracil. It has recently been reported for willardiine antagonists series that substitution in this position of the uracil ring with methyl (i.e., in UBP310 compound) or halogen improved both potency and selectivity for kainate receptors.[13,16] The comparison of UBP310-GluR5 with other crystal structures of antagonists with either the GluR2 or GluR5 binding core reveals that the methyl group of UBP310, *t*-butyl group in ATPO structures, and one of the nitro groups in DNQX (Fig. 1) occupy a similar space of the GluR2/GluR5 binding pocket. The chlorine atom in position 5 of phenylalanine in **18b** seems to fill this partly polar and partly hydrophobic cavity, which likely increases the affinity to GluR5 compared to the non-substituted analogue **9b**. On the other hand, the second chlorine atom in position 4 is located in a cavity formed by Ser726, Thr725, hydroxy groups of Tyr749 and Tyr429 and methylene carbons of the Glu723 side chain; polar residues surrounding this space tolerate better the oxygen atom of the carbonyl group in UBP302 than hydrophobic chlorine—this effect most likely contributes to lower the affinity of the phenylalanine amino acids at GluR5.

Docking of 2- and 4-carboxy isomers of **9b**/**18b** resulted in lower scored poses with the distal acidic group interacting with Ser674 and/or Thr675. However, no clear connection between docking scores and the binding affinity of compounds can be observed. Low or no activity of **9a**, **c** and **26a**–**c** in the GluR5 assay is most likely connected to the absence of substituents in positions 4 and/or 5 of phenylalanine.

To explain the AMPA receptor affinity data, molecular docking of compound **18e** (3-hydroxy-substituted analogue) was attempted, using (*S*)-ATPO-GluR2 binding core complex (PDB ID: 1N0T) as a template. Preparation of the protein and ligands (both *S*- and *R*-enantiomers) as well as the docking procedure was performed in analogy to the experiment described above for the GluR5 binding core (water molecules were removed). The results were clear-cut, with ligands given highest scores and lowest energies in binding poses with amino acid chain bound in the way corresponding to the binding mode of (*S*)-ATPO antagonist.

Even though certain reservations should be made, docking experiments indicated that both enantiomers of **18e** are likely to be active at AMPA receptors. In the top ranked poses (Fig. 3a and b), despite the difference in chiral center configuration, the amino acidic part of both isomers makes contacts with the protein in a way resembling the ATPO binding mode and involving Arg485, Thr480, Pro478 and Glu705 residues. The rigid biphenyl linker between the amino acidic moiety and the 3-hydroxy group, interacting with Thr655 of domain 2, stabilizes, like in case of ATPO, the open conformation of the receptor. Chlorine atoms of phenylalanine are located in partly polar, partly hydrophobic cavities; the exchange of Ser726 in GluR5 into lipophilic Met708 in GluR2 receptor has a big influence on accommodation of **18e** by GluR2, while in the case of **18b** in the GluR5 binding core, a polar substituent in position 4 seems to be more suitable. The presence of chlorine in this position probably also contributes to higher affinity of **18e** at AMPA receptors compared to ATPO.

3. Conclusion

In efforts to investigate the structural determinants for receptor selectivity between AMPA and the GluR5 receptors, a series of rigid as well as flexible biaromatic alanine derivatives carrying selected hydrogen bond acceptors and donors have been synthesized. The

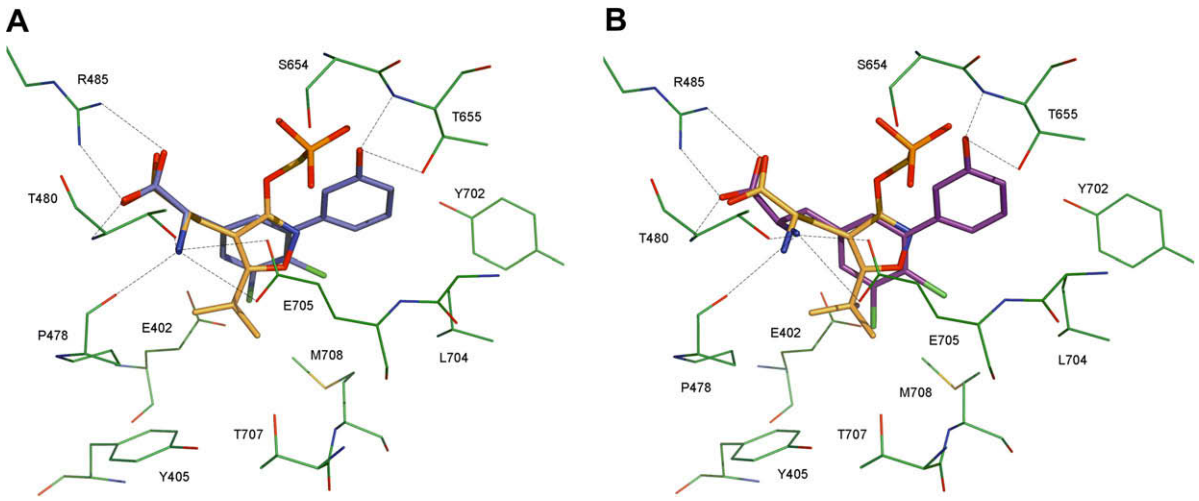


Figure 3. Compound **18e** docked to (S)-ATPO-GluR2 S1S2 complex. Residues of ATPO-GluR2 binding core—green, (*S*)-ATPO ligand—orange. (a) Top ranked pose of the *S*-enantiomer (navy blue); (b) top ranked pose of the *R*-enantiomer (purple). Ion pair interactions and hydrogen bonds within the distance lower than 3.5 Å between **18e** and the protein are represented by dashes. Prepared by Pymol.[22]

established structure–activity relationships revealed that among the series one particular substituent position appeared to be essential for control of ligand selectivity. Among the new series of compounds both GluR5-selective (**18b**) as well as AMPA-selective (**18e**) compounds were identified. For compound **18b** and for the corresponding positional isomers **18a, c** docking to the UBP302-GluR5 complex was in good agreement with the observed GluR5 receptor affinity. Likewise for compound **18e,** good agreement between AMPA receptor affinity and docking to the (*S*)-ATPO-GluR2 complex was observed. For further improvements on potency and subtype selectivity at AMPA/kainate receptors, compounds **18b, e** provide good starting points for the design of ligands.

4. Experimental

4.1. Chemistry. General procedures

Analytical thin-layer chromatography (TLC) was performed on Silica Gel 60 F_{254} aluminum plates (Merck). All compounds were detected using UV light and a $KMnO_4$ spraying reagent, the target amino acids were also visualized using a ninhydrin spraying reagent. Flash column chromatography (FC) was performed using CombiFlash Companion System (Teledyne Isco, Inc.) on Redi*Sep* columns with silica gel (average particle size 35–70 μm). The mixture of heptane fraction and ethyl acetate (both HPLC purity) were used as eluents, unless otherwise stated. ^{1}H NMR spectra were recorded on a 300 MHz Varian Mercury 300BB or 300 MHz Varian Gemini 2000BB spectrometer. ^{13}C NMR spectra were recorded on the Varian Gemini spectrometer. $CDCl_3$ (with TMS as internal standard), D_2O and DMSO-d_6 (dimethyl-d_6-sulfoxide) were used as solvents. Chemical shifts (δ) are given in parts per million (ppm), and coupling constants (J) are given in hertz (Hz). GC–MS were recorded on a Shimadzu QP 5050A mounted with a Supelco MDN-5S column and using chemical ionization.

Elemental analyses were performed by J. Theiner, Microanalytical Laboratory, Department of Physical Chemistry, University of Vienna, Austria, and are within ±0.4% of the theoretical values.

Unless otherwise stated, preparative chromatography was performed using HPLC system consisting of a Jasco 880-PU pump, a Rheodyne 7125 injector equipped with a 5.0 mL sample loop, a Shimadzu SPD-6A detector (220 nm or 250 nm) and a Merck-Hitachi D-2000 Chromato-Integrator. Purification of the target amino acids was performed on a reverse-phase XTerra MS C_{18} column (10 × 300 mm 10 μm, Waters). The column was eluted at 8 mL/min with 15 mM acetic acid containing methanol in varying concentrations depending on the structure of the target amino acid. The enantiomeric purity expressed by the enantiomeric excess (ee) was determined by chiral HPLC using an analytical Chirobiotic T column (4.6 × 150 mm, ASTEC) equipped with a Chirobiotic T guard column (4.6 × 50 mm, ASTEC) and connected to the HPLC system described above (detection at 250 nm). Elution was performed with 60% (v/v) ethanol in water adjusted with acetic acid to pH 4 (0.5 mL/min). Optical rotation was measured on a Perkin–Elmer 241 polarimeter. CD spectra were recorded at ambient temperature in 1.0-cm cuvettes on an OLIS DSM (Digital Subtractive Method) 10 CD spectrophotometer.

4.2. 1-(Bromomethyl)-3-iodobenzene (6)

Compounds **6**[23] and **7** were prepared according to the literature procedure.[24] A mixture of commercially available 1-iodo-3-methylbenzene **5** (4.36 g, 20 mmol), NBS (N-bromosuccinimide 5.34 g, 30 mmol) and benzoyl peroxide (0.727 g, 3 mmol) in tetrachloromethane (100 mL) was refluxed for 4 h. NBS and benzoyl peroxide were added in adequate amounts each hour. The progress of the reaction was followed by ^{1}H NMR. After cooling and filtration, dichloromethane (50 mL) was added; the organic phase was washed with 2 M NaOH, dried ($MgSO_4$) and evaporated. Purification by flash column chromatography (heptane) afforded a white solid, which consisted of a mixture of **6** and the corresponding dibromo derivative in ratio 4.3:1. Yield of **6**, calculated on the basis of ^{1}H NMR spectra, 4.13 g, 70%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.40 (s, 2H, CH_2Br), 7.08 (t, J = 7.6 Hz, 1H, Ar), 7.35 (dd, J_1 = 7.6 Hz, J_2 = 1.2 Hz, 1H, Ar), 7.63 (d, 1H, J = 7.9 Hz, Ar), 7.75 (s, 1H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 32.2, 94.4, 128.3, 130.5, 137.4, 137.9, 138.8.

4.3. Diethyl 2-acetamido-2-(3-iodobenzyl)malonate (7)

A 60% suspension of sodium hydride in mineral oil (0.78 g, 19.5 mmol) in dry DMF (10 mL) was cooled to 5 °C, and a solution of diethyl acetamidomalonate (4.23 g, 19.5 mmol) in dry DMF (20 mL) was added under nitrogen atmosphere. After stirring for 30 min the solution of **6** (mixed with 1,1-(dibromomethyl)-3-iodobenzene in ratio 4.3:1, recalculated amount of **6**—3.86 g, 13.0 mmol) in DMF (20 mL) was added. The mixture was stirred for 16 h at ambient temperature under nitrogen atmosphere, 3 mL of glacial acetic acid was added and the solvent was evaporated under reduced pressure. The residue was suspended in toluene (80 mL), stirred for 10 min, filtered and washed with water (80 mL) and brine (80 mL). After drying ($MgSO_4$), evaporation and flash column chromatography, **7** was isolated as a white solid. Yield 3.87 g, 69%; MS: m/z 433 [M$^+$]; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.30 (t, J = 7.1 Hz, 6H, OCH_2CH_3), 2.05 (s, 3H, $NHCOCH_3$), 3.59 (s, 2H, $ArCH_2$), 4.27 (q, J = 7.1 Hz, 4H, OCH_2CH_3), 6.54 (s, 1H, NH), 6.90–7.03 (m, 2H, Ar), 7.37 (s, 1H, Ar), 7.57 (d, J = 7.1 Hz, 1H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.3, 23.2, 37.4, 63.0, 67.2, 94.3, 129.1, 130.0, 136.2, 137.7, 139.0, 167.2 169.2.

4.4. General procedure for diethyl 2-acetamido-2-(biphenyl-3-ylmethyl)malonates (8a–e)

Compounds **8a**–**e** were prepared in analogy to a literature method.[25] Suspension of **7** (433 mg, 1 mmol) and bis(triphenylphosphine)palladium(II) dichloride $PdCl_2(PPh_3)_2$ (35 mg, 0.05 mmol) was stirred in DME (25 mL) under nitrogen atmosphere at room temperature for 15 min. Phenylboronic acid (1.5 mmol), triethylamine (5.58 mL, 40 mmol) and water (25 mL) were added and the mixture was stirred under nitrogen at room temperature for several hours, controlled by GC–MS. If the starting material was not converted after 24 h of stirring, the temperature was raised to 50 °C. When the reaction was finished, the mixture was filtrated and water (30 mL) was added. After extraction with ethyl acetate (3 × 50 mL) the combined extracts were washed with 2 M NaOH (50 mL) following water (50 mL), dried ($MgSO_4$) and evaporated. The raw product was purified by flash column chromatography.

4.4.1. Diethyl 2-acetamido-2-((2′-cyanobiphenyl-3-yl)methyl)-malonate (8a)

Yield 183 mg, 45%; MS: m/z 408 [M$^+$]; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.28 (t, J = 7.2 Hz, 6H, OCH_2CH_3), 2.12 (s, 3H, $NHCOCH_3$), 3.73 (s, 2H, $ArCH_2$), 4.20–4.30 (m, 4H, OCH_2CH_3), 6.99 (s, 1H, NH), 7.11–7.20 (m, 1H, Ar), 7.25 (s, 1H, Ar), 7.25–7.47 (m, 4H, Ar), 7.64 (dt, J_1 = 7.7 Hz, J_2 = 1.4 Hz, 1H, Ar), 7.76 (dd, J_1 = 8.3 Hz, J_2 = 1.4 Hz, 1H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.3, 23.4, 38.2, 62.8, 67.5, 111.3, 118.8, 127.7, 128.8, 129.9, 130.3, 130.5, 132.9, 133.6, 135.9, 138.3, 145.4, 167.3, 169.5.

4.4.2. Diethyl 2-acetamido-2-((3′-cyanobiphenyl-3-yl)methyl)-malonate (8b)

Yield 325 mg, 80%; MS: m/z 408 [M$^+$]; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.30 (t, J = 7.1 Hz, 6H, OCH_2CH_3), 2.03 (s, 3H, $NHCOCH_3$),

3.74 (s, 2H, $ArCH_2$), 4.29 (dq, J_1 = 7.1 Hz, J_2 = 2.4 Hz, 4H, OCH_2CH_3), 6.56 (s, 1H, NH), 7.06 (d, J = 7.3 Hz, 1H, Ar), 7.18 (s, 1H, Ar), 7.37 (t, J = 7.3 Hz, 1H, Ar), 7.44 (d, J = 7.6 Hz, 1H, Ar), 7.54 (t, J = 7.6 Hz, 1H, Ar), 7.63 (d, J = 7.9 Hz, 1H, Ar), 7.72–7.78 (m, 2H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.3, 23.3, 38.0, 63.0, 67.3, 113.1, 118.8, 126.1, 128.7, 129.2, 129.7, 129.9, 130.6, 130.9, 131.4, 136.4, 139.0, 142.2, 167.5, 169.1.

4.4.3. Diethyl 2-acetamido-2-((4′-cyanobiphenyl-3-yl)methyl)-malonate (8c)

Yield 263 mg, 64%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.29 (t, J = 7.1 Hz, 6H, OCH_2CH_3), 2.01 (s, 3H, $NHCOCH_3$), 3.74 (s, 2H, $ArCH_2$), 4.28 (dq, J_1 = 7.1 Hz, J_2 = 2.4 Hz, 4H, OCH_2CH_3), 6.56 (s, 1H, NH), 7.06 (d, J = 7.6 Hz, 1H, Ar), 7.21 (s, 1H, Ar), 7.37 (t, J = 7.6 Hz, 1H, Ar), 7.46 (d, J = 7.9 Hz, 1H, Ar), 7.61 (d, J = 8.2 Hz, 2H, Ar), 7.71 (d, J = 8.2 Hz, 2H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.3, 23.3, 38.0, 63.0, 67.3, 111.2, 118.9, 126.2, 127.7, 128.8, 129.2, 130.1, 132.7, 136.4, 139.3, 145.4, 167.4, 169.1.

4.4.4. Diethyl 2-acetamido-2-((3′-(cyanomethyl)biphenyl-3-yl)-methyl)malonate (8d)

Yield 355 mg, 84%; MS: m/z 422 [M^+]; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.31 (t, J = 7.2 Hz, 6H, OCH_2CH_3), 2.05 (s, 3H, $NHCOCH_3$), 3.74 (s, 2H, $ArCH_2$), 3.82 (s, 2H, CH_2CN), 4.30 (dq, J_1 = 7.2 Hz, J_2 = 1.7 Hz, 4H, OCH_2CH_3), 6.58 (s, 1H, NH), 7.02 (d, J = 7.7 Hz, 1H, Ar), 7.21 (s, 1H, Ar), 7.28–7.32 (m, 1H, Ar), 7.35 (d, J = 7.7 Hz, 1H, Ar), 7.41–7.48 (m, 4H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.3, 23.4, 23.9, 38.1, 63.0, 67.4, 118.9, 126.2, 126.6, 126.8, 126.9, 128.8, 128.9, 129.3, 129.7, 130.5, 136.0, 140.4, 142.0, 167.5, 169.2.

4.4.5. Diethyl 2-acetamido-2-((4′-(cyanomethyl)biphenyl-3-yl)-methyl)malonate (8e)

Yield 329 mg, 78%; MS: m/z 422 [M^+]; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.29 (t, J = 7.1 Hz, 6H, OCH_2CH_3), 2.02 (s, 3H, $NHCOCH_3$), 3.72 (s, 2H, $ArCH_2$), 3.79 (s, 2H, CH_2CN), 4.28 (dq, J_1 = 7.1 Hz, J_2 = 2.1 Hz, 4H, OCH_2CH_3), 6.57 (s, 1H, NH), 7.01 (d, J = 7.6 Hz, 1H, Ar), 7.20 (s, 1H, Ar), 7.32 (d, J = 7.6 Hz, 1H, Ar), 7.36–7.40 (m, 2H, Ar), 7.45 (d, J = 7.6 Hz, 1H, Ar), 7.52 (d, J = 8.2 Hz, 2H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.5, 23.5, 23.7, 38.2, 63.1, 67.5, 119.0, 126.2, 127.9, 128.6, 128.9, 129.1, 129.2, 129.3, 136.2, 140.4, 141.0, 167.7, 169.3.

4.5. (*RS*)-3′-(2-Amino-2-carboxyethyl)biphenyl-2-carboxylic acid (9a)

A mixture of **8a** (102 mg, 0.25 mmol), acetic acid (2 mL), 12 M HCl (2 mL), and water (2 mL) was refluxed for 20 h. The reaction mixture was evaporated and purified by HPLC followed by recrystallization from water to give **9a** as white crystals. Yield 34 mg, 48%; Anal. Calcd for $C_{16}H_{15}NO_4 \cdot H_2O$: C, 63.36; H, 5.65; N, 4.62. Found: C, 63.33; H, 5.39; N, 4.45; ^{1}H NMR (300 MHz, DMSO-d_6 + 50 μL D_2O): δ 2.91 (dd, J_1 = 14.0 Hz, J_2 = 8.5 Hz, 1H, $ArCH_2$), 3.18 (dd, J_1 = 14.3 Hz, J_2 = 3.9 Hz, 1H, $ArCH_2$), 3.53–3.57 (m, 1H, $ArCH_2CH$), 7.14–7.26 (m, 3H, Ar), 7.30 (t, J = 7.7 Hz, 1H, Ar), 7.38–7.43 (m, 2H, Ar), 7.53 (t, J = 7.4 Hz, 1H, Ar), 7.64 (d, J = 8.3 Hz, 1H, Ar).

4.6. General procedure for compounds 9b, c

Compounds **8b**, **c** (204 mg, 0.5 mmol) were deprotected using the procedure described for **9a**. The reaction mixture was evaporated and purified by HPLC using a gradient mobile phase consisting of aqueous trifluoroacetic acid and acetonitrile.

4.6.1. (*RS*)-3′-(2-Amino-2-carboxyethyl)biphenyl-3-carboxylic acid (9b)

Yield 45 mg, 32%; Anal. Calcd for $C_{16}H_{15}NO_4 \cdot 0.3\ C_2HF_3O_2 \cdot 0.4H_2O$: C, 61.03; H, 4.97; N, 4.29. Found: C, 61.20; H, 5.24; N, 4.34; ^{1}H NMR (300 MHz, DMSO-d_6 + 50 μL D_2O): δ 2.99 (dd, J_1 = 14.3 Hz, J_2 = 8.3 Hz, 1H, $ArCH_2$), 3.21 (dd, J_1 = 14.3 Hz, J_2 = 4.7 Hz, 1H, $ArCH_2$), 3.61–3.65 (m, 1H, $ArCH_2CH$), 7.27 (d, J = 7.7 Hz, 1H, Ar), 7.40 (t, J = 7.4 Hz, 1H, Ar), 7.51–7.59 (m, 3H, Ar), 7.86–7.90 (m, 2H, Ar), 8.14 (s, 1H, Ar).

4.6.2. (*RS*)-3′-(2-Amino-2-carboxyethyl)biphenyl-4-carboxylic acid (9c)

Yield 37 mg, 26%; Anal. Calcd for $C_{16}H_{15}NO_4 \cdot 0.3\ C_2HF_3O_2$: C, 62.40; H, 4.83; N, 4.38. Found: C, 62.11; H, 4.92; N, 4.22; ^{1}H NMR (300 MHz, DMSO-d_6 + 50 μL D_2O): δ 2.98 (dd, J_1 = 14.3 Hz, J_2 = 8.0 Hz, 1H, $ArCH_2$), 3.21 (dd, J_1 = 14.3 Hz, J_2 = 4.4 Hz, 1H, $ArCH_2$), 3.61–3.65 (m, 1H, $ArCH_2CH$), 7.28 (d, J = 7.7 Hz, 1H, Ar), 7.41 (t, J = 7.4 Hz, 1H, Ar), 7.56–7.59 (m, 2H, Ar), 7.77 (d, J = 8.3 Hz, 2H, Ar), 8.03 (d, J = 8.3 Hz, 2H, Ar).

4.7. General procedure for compounds 9d,e

A mixture of **8d**, **e** (297 mg, 0.7 mmol), acetic acid (2 mL), 12 M HCl (2 mL), and water (2 mL) was refluxed for 16 h. The mixture of acids was removed in vacuo; to the solid residue water (10 mL) was added and evaporated again. The raw product was refluxed with water (10 mL) for 5 h. After cooling the mixture was evaporated under reduced pressure and purified by reverse-phase flash column chromatography using R-18 Silica Gel and a gradient mobile phase consisting of 15 mM aqueous acetic acid and methanol.

4.7.1. (*RS*)-2-Amino-3-(3′-(carboxymethyl)biphenyl-3-yl)propanoic acid (9d)

Yield 191 mg, 91%; Anal. Calcd for $C_{17}H_{17}NO_4\ 0.7H_2O$: C, 65.46; H, 5.95; N, 4.49. Found: C, 65.35; H, 5.77; N, 4.38; ^{1}H NMR (300 MHz, DMSO-d_6 + 50 μL D_2O): δ 2.96 (dd, J_1 = 14.3 Hz, J_2 = 8.0 Hz, 1H, $ArCH_2$), 3.21 (dd, J_1 = 14.3 Hz, J_2 = 4.4 Hz, 1H, $ArCH_2$), 3.60 (s, 2H, CH_2COOH), 3.62–3.67 (m, 1H, $ArCH_2CH$), 7.19–7.23 (m, 2H, Ar), 7.35–7.40 (m, 2H, Ar), 7.46–7.51 (m, 4H, Ar).

4.7.2. (*RS*)-2-Amino-3-(4′-(carboxymethyl)biphenyl-3-yl)propanoic acid (9e)

Yield 186 mg, 88%; Anal. Calcd for $C_{17}H_{17}NO_4\ H_2O$: C, 64.34; H, 6.03; N, 4.41. Found: C, 64.35; H, 5.72; N, 4.36; ^{1}H NMR (300 MHz, DMSO-d_6 + 50 μL D_2O): δ 2.99 (dd, J_1 = 14.3 Hz, J_2 = 7.7 Hz, 1H, $ArCH_2$), 3.18 (dd, J_1 = 14.3 Hz, J_2 = 4.7 Hz, 1H, $ArCH_2$), 3.57 (s, 2H, CH_2COOH), 3.71–3.75 (m, 1H, $ArCH_2CH$), 7.20 (d, J = 7.4 Hz, 1H, Ar), 7.29 (d, J = 8.0 Hz, 2H, Ar), 7.37 (t, J = 7.7 Hz, 1H, Ar), 7.48–7.59 (m, 2H, Ar), 7.56 (d, J = 8.0 Hz, 2H, Ar).

4.8. 2-Chloro-4-methyl-6-nitroaniline (11)

Compound **11**[26] was prepared according to the literature procedure.[27] While stirring, 2-chloro-4-methylaniline **10** (14.16 g, 100 mmol) was slowly added to acetic anhydride (110 mL, 1.16 mol) and cooled to 5–7 °C. To this mixture 96% nitric acid (9.63 mL, 0.22 mol) was added dropwise over a period of 1 h, keeping the temperature below 15 °C. The reaction mixture was stirred for additional 10 min, and then poured into ice-water (400 mL). The resulting yellowish solid was filtered; the wet product was treated with 12 M HCl (20 mL) and EtOH (40 mL). The mixture was refluxed for 4 h. Upon cooling ethanol was removed by evaporation, and the residue was poured into the mixture of ammonia (40 mL) and water (200 mL). The orange solid was filtered, washed with water (3 × 100 mL) and dried to afford **11**, which was directly used in next step. Yield 10.82 g, 58%; MS: m/z 186 [M^+]; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.27 (s, 3H, CH_3), 6.41 (br s, 2H, NH_2), 7.38 (s, 1H, Ar), 7.90 (s, 1H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.4, 121.9, 124.9, 125.9, 127.4, 136.7, 139.6.

4.9. 1,2-Dichloro-5-methyl-3-nitrobenzene (12)

Compound **12**[28] was prepared according to the literature procedure.[29] To a mixture of *tert*-butyl nitrite (7.75 g, 75 mmol) and anhydrous copper(II) chloride (8.0 g, 60 mmol) in dry acetonitrile (40 mL) at 65 °C, a solution of **11** (9.30 g, 50 mmol) in dry acetonitrile (60 mL) was added dropwise under vigorous stirring during 30 min. Then stirring was continued for 1 h. Upon cooling, the mixture was poured into 20% hydrochloric acid (200 mL) and extracted with diethyl ether (3 × 120 mL). The combined organic phases were dried ($MgSO_4$) and evaporated. Based on ^{1}H NMR and GC–MS analyses, the resulting yellow crystals proved to be **12**, and could be used in the following step without further purification. Yield 8.85 g, 89%; MS: *m*/*z* 205 [M^+]; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.57 (s, 3H, CH_3), 7.67 (s, 2H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.9, 123.9, 134.2, 134.9, 135.0, 138.8, 149.2.

4.10. 2,3-Dichloro-5-methylaniline (13)

Compound **13**[28] was prepared according to the literature procedure.[30] Iron powder (48 g, 86 mmol), water (80 mL) and 12 M hydrochloric acid (1.57 mL, 19 mmol) were added consecutively to a stirred solution of **12** (8.24 g, 40 mmol) in ethanol (310 mL). The reaction mixture was refluxed for 90 min, filtered hot and ethanol was removed under reduced pressure. The residue was washed with dichloromethane (80 mL). To the aqueous phase 2 M NaOH (50 mL) was added, then the solution was extracted with dichloromethane (3 × 80 mL), the combined organic extracts were dried ($MgSO_4$), and the solvent was removed in vacuo. Obtained brown oil without further purification could be used in the next step. Yield 7.04 g, 86%; MS: *m*/*z* 175 [M^+]; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.21 (s, 3H, CH_3), 4.08 (br s, 2H, NH_2), 6.47 (s, 1H, Ar), 6.67 (s, 1H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.1, 114.4, 114.5, 120.6, 132.6, 138.0, 144.3.

4.11. 1,2-Dichloro-3-iodo-5-methylbenzene (14)

Compound **14** was prepared according to the known literature procedure.[27] The suspension of **13** (5.28 g, 30 mmol) in glacial acetic acid (30 mL) was heated to form a clear solution, then cooled to 25 °C. A pre-cooled solution of sodium nitrite (3.0 g, 48 mmol) in cold concentrated sulfuric acid (16.5 mL) was slowly added over a period of 30 min, stirred for another 30 min, then heated to 75 °C, stirred for 1 h and cooled to 25 °C. The mixture was poured into ice-water (130 mL) while stirring and successively treated with of urea (3.0 g, 50 mmol) and aqueous KI solution (7.47 g, 45 mmol, in 35 mL of water). After KI addition was completed, solid $NaHSO_3$ was added until the color of the mixture obviously changed. The reaction mixture was extracted with dichloromethane (3 × 120 mL), the organic phases were dried over $MgSO_4$, and the solvent was removed under reduced pressure. Purification by flash column chromatography (heptane) afforded **14** as a white low melting solid. Yield 8.61 g, 54%; MS: *m*/*z* 286 [M^+]; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.27 (s, 3H, CH_3), 7.25 (s, 1H, Ar), 7.59 (s, 1H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.4, 99.1, 131.1, 131.7, 133.5, 139.1, 139.2.

4.12. 5-(Bromomethyl)-1,2-dichloro-3-iodobenzene (15)

Compound **15** was prepared according to the modified literature method described for **6**. A mixture of **14** (7.47 g, 26 mmol), NBS (6.946 g, 38 mmol) and benzoyl peroxide (0.945 g, 3.9 mmol) in tetrachloromethane (150 mL) was refluxed for 10 h. The progress of the reaction was controlled by ^{1}H NMR. After cooling and filtration, dichloromethane (80 mL) was added and the liquid was washed with 2 M NaOH, dried ($MgSO_4$) and evaporated under reduced pressure. Purification with column chromatography (heptane) afforded a white solid, the mixture of **15** and dibromo derivative in ratio 2.5:1, respectively. Yield of **15**, calculated on the basis of the proton NMR spectra, 6.06 g, 64%; MS: *m*/*z* 364 [M^+]; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.33 (s, 2H, CH_2Br), 7.48 (d, *J* = 2.1 Hz, 1H, Ar), 7.70 (d, 1H, *J* = 2.1 Hz, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 30.2, 99.3, 128.7, 130.9, 132.5, 136.2, 138.8.

4.13. Diethyl 2-acetamido-2-(3,4-dichloro-5-iodobenzyl)-malonate (16)

Compound **16** was prepared from **15** (5.49 g, 15 mmol) by the method described for compound **7**. After purification by flash column chromatography compound **16** was isolated as a white solid. Yield 5.88 g, 78%; MS: *m*/*z* 501 [M^+]; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.32 (t, *J* = 7.2 Hz, 6H, OCH_2CH_3), 2.08 (s, 3H, $NHCOCH_3$), 3.57 (s, 2H, Ar), 4.28 (q, *J* = 7.2 Hz, 4H, OCH_2CH_3), 6.57 (s, 1H, NH), 7.08 (d, *J* = 1.8 Hz, 1H, Ar), 7.43 (d, *J* = 1.8 Hz,1H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.3, 23.2, 36.5, 63.3, 67.0, 99.1, 131.7, 132.1, 135.7, 136.7, 139.9, 166.9, 169.4.

4.14. General procedure for compounds 17a–h

Compounds **17a**–**h** were prepared from **16** (502 mg, 1 mmol) and the relevant substituted phenylboronic acids according to the procedure described for compounds **8a**–**e**.

4.14.1. Diethyl 2-acetamido-2-((5,6-dichloro-2′-cyanobiphenyl-3-yl)methyl)malonate (17a)

Yield 0.234 g, 49%; MS: *m*/*z* 476 [M^+−14]; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.29 (t, *J* = 7.2 Hz, 6H, OCH_2CH_3), 2.08 (s, 3H, $NHCOCH_3$), 3.65 (s, 2H, $ArCH_2$), 4.26 (q, *J* = 7.2 Hz, 4H, OCH_2CH_3), 6.89 (s, 1H, NH), 6.93 (d, *J* = 1.9 Hz, 1H, Ar), 7.22 (d, *J* = 1.9 Hz, 1H, Ar), 7.40 (dt, J_1 = 7.7 Hz, J_2 = 1.4 Hz, 1H, Ar), 7.51 (dt, J_1 = 7.7 Hz, J_2 = 1.4 Hz, 1H, Ar), 7.65 (dt, J_1 = 7.7 Hz, J_2 = 1.4 Hz, 1H, Ar), 7.76 (dt, J_1 = 7.7 Hz, J_2 = 1.4 Hz, 1H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.4, 23.3, 37.2, 63.2, 67.2, 112.5, 117.7, 128.7, 130.4, 130.7, 131.0, 132.1, 132.5, 132.9, 133.6, 135.4, 138.6, 142.5, 166.9, 169.6.

4.14.2. Diethyl 2-acetamido-2-((5,6-dichloro-3′-cyanobiphenyl-3-yl)methyl)malonate (17b)

Yield 0.343 g, 72%; MS: *m*/*z* 476 [M^+−14]; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.28 (t, *J* = 7.2 Hz, 6H, OCH_2CH_3), 2.03 (s, 3H, $NHCOCH_3$), 3.66 (s, 2H, $ArCH_2$), 4.26 (dq, J_1 = 7.2 Hz, J_2 = 1.7 Hz, 4H, OCH_2CH_3), 6.58 (s, 1H, NH), 6.81 (d, *J* = 1.9 Hz, 1H, Ar), 7.16 (d, *J* = 1.9 Hz, 1H, Ar), 7.53–7.58 (m, 2H, Ar), 7.61 (s, 1H, Ar), 7.66–7.69 (m, 1H, Ar); ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.9, 22.1, 36.8, 62.0, 66.7, 111.5, 118.3, 128.3, 129.6, 131.4, 131.8, 132.0, 132.6, 133.9, 135.9, 139.3, 139.4, 166.6, 169.3.

4.14.3. Diethyl 2-acetamido-2-((5,6-dichloro-4′-cyanobiphenyl-3-yl)methyl)malonate (17c)

Yield 0.329 g, 69%; MS: *m*/*z* 476 [M^+−14]; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.28 (t, *J* = 7.2 Hz, 6H, OCH_2CH_3), 2.02 (s, 3H, $NHCOCH_3$), 3.66 (s, 2H, $ArCH_2$), 4.22–4.28 (m, 4H, OCH_2CH_3), 6.58 (s, 1H, NH), 6.81 (d, *J* = 2.1 Hz, 1H, Ar), 7.16 (d, *J* = 2.1 Hz, 1H, Ar), 7.44 (d, *J* = 8.5 Hz, 2H, Ar), 7.71 (d, *J* = 8.5 Hz, 2H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.2, 23.2, 37.0, 63.1, 67.0, 112.1, 118.5, 129.7, 129.9, 130.5, 131.7, 132.1, 133.7, 135.4, 140.5, 143.4, 167.0, 169.3.

4.14.4. Diethyl 2-acetamido-2-((5,6-dichloro-2′-methoxybiphenyl-3-yl)methyl)malonate (17d)

Yield 0.299 g, 62%; MS: *m*/*z* 481 [M^+−14]; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.27 (t, *J* = 7.2 Hz, 6H, OCH_2CH_3), 2.02 (s, 3H, $NHCOCH_3$), 3.62 (s, 2H, $ArCH_2$), 3.76 (s, 3H, OCH_3), 4.25 (dq, J_1 = 7.2 Hz,

J_2 = 1.4 Hz, 4H, OCH_2CH_3), 6.59 (s, 1H, NH), 6.81 (d, J = 2.2 Hz, 1H, Ar), 6.93–7.08 (m, 4H, Ar), 7.36 (dt, J_1 = 7.7 Hz, J_2 = 1.9 Hz, 1H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.3, 23.2, 37.1, 55.7, 63.1, 67.1, 111.1, 120.4, 128.1, 129.9, 130.5, 130.6, 131.4, 132.8, 134.5, 136.8, 139.8, 156.4, 167.2, 169.2.

4.14.5. Diethyl 2-acetamido-2-((5,6-dichloro-3′-methoxybiphenyl-3-yl)methyl)malonate (17e)

Yield 0.357 g, 74%; MS: m/z 481 [M^+−14]; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.28 (t, J = 7.2 Hz, 6H, OCH$_2CH_3$), 2.03 (s, 3H, NHCOCH_3), 3.63 (s, 2H, ArCH_2), 3.82 (s, 3H, OCH_3), 4.26 (dq, J_1 = 7.2 Hz, J_2 = 1.4 Hz, 4H, OCH_2CH_3), 6.58 (s, 1H, NH), 6.85–6.93 (m, 3H, Ar), 7.10 (d, J = 1.9 Hz, 1H, Ar), 7.24 (s, 1H, Ar), 7.32 (t, J = 7.7 Hz, 1H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.3, 23.3, 37.1, 55.5, 63.1, 67.1, 113.6, 114.9, 121.5, 129.3, 130.1, 130.7, 130.9, 133.5, 134.9, 140.3, 142.4, 159.2, 167.2, 169.3.

4.14.6. Diethyl 2-acetamido-2-((5,6-dichloro-4′-methoxybiphenyl-3-yl)methyl)malonate (17f)

Yield 0.328 g, 68%; MS: m/z 481 [M^+−14]; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.28 (t, J = 7.2 Hz, 6H, OCH$_2CH_3$), 2.03 (s, 3H, NHCOCH_3), 3.63 (s, 2H, ArCH_2), 3.85 (s, 3H, OCH_3), 4.26 (dq, J_1 = 7.2 Hz, J_2 = 1.4 Hz, 4H, OCH_2CH_3), 6.57 (s, 1H, NH), 6.83 (d, J = 2.2 Hz, 1H, Ar), 6.93 (d, J = 8.8 Hz, 2H, Ar), 7.07 (d, J = 2.2 Hz, 1H, Ar), 7.25 (d, J = 8.8 Hz, 2H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.3, 23.3, 37.1, 55.5, 63.1, 67.1, 113.6, 130.2, 130.3, 130.5, 131.1, 131.4, 133.3, 134.8, 142.2, 159.3, 167.2, 169.3.

4.14.7. Diethyl 2-acetamido-2-((5,6-dichloro-3′-nitrobiphenyl-3-yl)methyl)malonate (17g)

Yield 0.258 g, 52%; MS: m/z 496 [M^+−14]; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.30 (t, J = 7.2 Hz, 6H, OCH$_2CH_3$), 2.06 (s, 3H, NHCOCH_3), 3.68 (s, 2H, ArCH_2), 4.26 (dq, J_1 = 7.2 Hz, J_2 = 1.9 Hz, 4H, OCH_2CH_3), 6.62 (s, 1H, NH), 6.88 (d, J = 2.2 Hz, 1H, Ar), 7.19 (d, J = 2.2 Hz, 1H, Ar), 7.61 (t, J = 8.0 Hz, 1H, Ar), 7.72 (dt, J_1 = 7.7 Hz, J_2 = 1.7 Hz, 1H, Ar), 8.19 (t, J = 1.7 Hz, 1H, Ar), 8.24–8.28 (m, 1H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.3, 23.3, 37.1, 63.3, 67.1, 123.1, 124.2, 129.3, 130.0, 131.8, 133.9, 135.4, 135.6, 139.9, 140.4, 148.1, 167.1, 169.4.

4.14.8. Diethyl 2-acetamido-2-((5,6-dichloro-4′-nitrobiphenyl-3-yl)methyl)malonate (17h)

Yield 0.278 g, 56%; MS: m/z 496 [M^+−14]; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.28 (t, J = 7.2 Hz, 6H, OCH$_2CH_3$), 2.03 (s, 3H, NHCOCH_3), 3.67 (s, 2H, ArCH_2), 4.23–4.32 (m, 4H, OCH_2CH_3), 6.59 (s, 1H, NH), 6.84 (d, J = 1.9 Hz, 1H, Ar), 7.18 (d, J = 1.9 Hz, 1H, Ar), 7.51 (dt, J_1 = 8.8 Hz, J_2 = 1.9 Hz, 2H, Ar), 8.28 (dt, J_1 = 8.8 Hz, J_2 = 1.9 Hz, 2H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.3, 23.3, 37.1, 63.2, 67.1, 123.6, 129.8, 130.2, 130.5, 131.9, 133.9, 135.5, 140.2, 145.3, 147.6, 167.1, 169.3.

4.15. General procedure for compounds 18a–h

A mixture of **17a–h** (0.5 mmol) in 48% aq HBr (6 mL) was refluxed for 2 h. The reaction mixture was evaporated under reduced pressure and purified by reverse-phase HPLC (mobile phase: a mixture of methanol in 15 mM acetic acid) to give white crystals of the target amino acid.

4.15.1. (*RS*)-5′-(2-Amino-2-carboxyethyl)-2′,3′-dichlorobiphenyl-2-carboxylic acid (18a)

Yield 92 mg, 52%; Anal. Calcd for $C_{16}H_{13}Cl_2NO_4$ 2.25H_2O: C, 48.69; H, 4.47; N, 3.55. Found: C, 48.78; H, 4.61; N, 3.45; ^{1}H NMR (300 MHz, DMSO-d_6 + 50 μL D_2O): δ 2.91–3.00 (m, 1H, ArCH_2), 3.06–3.18 (m, 1H, ArCH_2), 3.59–3.63 (m, 1H, ArCH_2CH), 7.10 (d, J = 1.9 Hz, 1H, Ar), 7.24–7.31 (m, 1H, Ar), 7.43–7.53 (m, 2H, Ar), 7.62 (t, J = 8.5 Hz, 1H, Ar), 7.89 (d, J = 8.5 Hz, 1H, Ar).

4.15.2. (*RS*)-5′-(2-Amino-2-carboxyethyl)-2′,3′-dichlorobiphenyl-3-carboxylic acid (18b)

Yield 129 mg, 73%; Anal. Calcd for $C_{16}H_{13}Cl_2NO_4$ 1.75H_2O: C, 49.82; H, 4.31; N, 3.63. Found: C, 49.53; H, 3.94; N, 3.64; ^{1}H NMR (300 MHz, DMSO-d_6 + 50 μL D_2O): δ 2.99 (dd, J_1 = 14.3 Hz, J_2 = 7.4 Hz, 1H, ArCH_2), 3.14 (dd, J_1 = 14.3 Hz, J_2 = 4.4 Hz, 1H, ArCH_2), 3.75–3.79 (m, 1H, ArCH_2CH), 7.23 (d, J = 1.9 Hz, 1H, Ar), 7.52 (d, J = 1.9 Hz, 1H, Ar), 7.59 (t, J = 7.4 Hz, 1H, Ar), 7.99 (d, J = 7.7 Hz, 1H, Ar), 7.95–7.98 (m, 2H, Ar).

4.15.3. (*RS*)-5′-(2-Amino-2-carboxyethyl)-2′,3′-dichlorobiphenyl-4-carboxylic acid (18c)

Yield 140 mg, 79%; Anal. Calcd for $C_{16}H_{13}Cl_2NO_4$·0.5H_2O: C, 52.91; H, 3.89; N, 3.86. Found: C, 53.07; H, 3.66; N, 3.71; ^{1}H NMR (300 MHz, DMSO-d_6 + 50 μL D_2O): δ 2.96 (dd, J_1 = 14.4 Hz, J_2 = 7.7 Hz, 1H, ArCH_2), 3.14 (dd, J_1 = 14.4 Hz, J_2 = 4.5 Hz, 1H, ArCH_2), 3.52–3.56 (m, 1H, ArCH_2CH), 7.25 (d, J = 1.9 Hz, 1H, Ar), 7.54 (d, J = 8.5 Hz, 2H, Ar), 7.57 (d, J = 1.9 Hz, 1H, Ar), 7.99 (d, J = 8.5 Hz, 2H, Ar).

4.15.4. (*RS*)-2-Amino-3-(5,6-dichloro-2′-hydroxybiphenyl-3-yl)propanoic acid (18d)

Yield 108 mg, 67%; Anal. Calcd for $C_{15}H_{13}Cl_2NO_3$·0.5H_2O: C, 53.75; H, 4.21; N, 4.18. Found: C, 53.72; H, 3.88; N, 4.04; ^{1}H NMR (300 MHz, DMSO-d_6 + 50 μL D_2O): δ 2.91 (dd, J_1 = 14.3 Hz, J_2 = 8.0 Hz, 1H, ArCH_2), 3.12 (dd, J_1 = 14.3 Hz, J_2 = 5.5 Hz, 1H, ArCH_2), 3.47–3.50 (m, 1H, ArCH_2CH), 6.82–6.89 (m, 2H, Ar), 7.07 (d, J = 6.6 Hz, 1H, Ar), 7.11 (s, 1H, Ar), 7.20 (dt, J_1 = 7.7 Hz, J_2 = 1.7 Hz, 1H, Ar), 7.46 (s, 1H, Ar).

4.15.5. (*RS*)-2-Amino-3-(5,6-dichloro-3′-hydroxybiphenyl-3-yl)-propanoic acid (18e)

Yield 143 mg, 88%; Anal. Calcd for $C_{15}H_{13}Cl_2NO_3$·0.25H_2O: C, 54.48; H, 4.11; N, 4.24. Found: C, 54.27; H, 3.89; N, 4.12; ^{1}H NMR (300 MHz, DMSO-d_6 + 50 μL D_2O): δ 2.92 (dd, J_1 = 14.0 Hz, J_2 = 7.7 Hz, 1H, ArCH_2), 3.10 (dd, J_1 = 14.0 Hz, J_2 = 4.1 Hz, 1H, ArCH_2), 3.50–3.54 (m, 1H, ArCH_2CH), 6.77–6.82 (m, 3H, Ar), 7.18 (d, J = 1.9 Hz, 1H, Ar), 7.23 (t, J = 8.8 Hz, 1H, Ar), 7.47 (d, J = 1.9 Hz, 1H, Ar).

4.15.6. (*RS*)-2-Amino-3-(5,6-dichloro-4′-hydroxybiphenyl-3-yl)-propanoic acid (18f)

Yield 127 mg, 78%; Anal. Calcd for $C_{15}H_{13}Cl_2NO_3$·H_2O: C, 52.34; H, 4.39; N, 4.07. Found: C, 52.08; H, 4.13; N, 3.93; ^{1}H NMR (300 MHz, DMSO-d_6 + 50 μL D_2O): δ 2.93 (dd, J_1 = 14.3 Hz, J_2 = 7.7 Hz, 1H, ArCH_2), 3.10 (dd, J_1 = 14.3 Hz, J_2 = 4.1 Hz, 1H, ArCH_2), 3.56–3.60 (m, 1H, ArCH_2CH), 6.81 (d, J = 8.5 Hz, 2H, Ar), 7.17 (d, J = 1.9 Hz, 1H, Ar), 7.23 (d, J = 8.5 Hz, 2H, Ar), 7.40 (d, J = 1.9 Hz, 1H, Ar).

4.15.7. (*RS*)-2-Amino-3-(5,6-dichloro-3′-nitrobiphenyl-3-yl)-propanoic acid (18g)

Yield 130 mg, 73%; Anal. Calcd for $C_{15}H_{12}Cl_2N_2O_4$·1.2H_2O: C, 47.82; H, 3.85; N, 7.43. Found: C, 48.05; H, 3.70; N, 7.14; ^{1}H NMR (300 MHz, DMSO-d_6 + 50 μL D_2O): δ 2.95 (dd, J_1 = 14.3 Hz, J_2 = 8.0 Hz, 1H, ArCH_2), 3.14 (dd, J_1 = 14.3 Hz, J_2 = 4.7 Hz, 1H, ArCH_2), 3.52–3.56 (m, 1H, ArCH_2CH), 7.30 (d, J = 1.9 Hz, 1H, Ar), 7.57 (d, J = 1.9 Hz, 1H, Ar), 7.75 (t, J = 8.8 Hz, 1H, Ar), 7.89 (d, J = 8.0 Hz, 1H, Ar), 8.23–8.27 (m, 2H, Ar).

4.15.8. (*RS*)-2-Amino-3-(5,6-dichloro-4′-nitrobiphenyl-3-yl)-propanoic acid (18h)

Yield 138 mg, 78%; Anal. Calcd for $C_{15}H_{12}Cl_2N_2O_4$·0.5H_2O: C, 49.47; H, 3.60; N, 7.69. Found: C, 49.19; H, 3.24; N, 7.44; ^{1}H NMR (300 MHz, DMSO-d_6 + 50 μL D_2O): δ 2.96 (dd, J_1 = 14.3 Hz, J_2 = 7.4 Hz, 1H, ArCH_2), 3.10 (dd, J_1 = 14.3 Hz, J_2 = 5.0 Hz, 1H, ArCH_2),

3.52–3.56 (m, 1H, $ArCH_2CH$), 7.28 (d, J = 1.9 Hz, 1H, Ar), 7.57 (d, J = 1.9 Hz, 1H, Ar), 7.7 (d, J = 8.5 Hz, 2H, Ar), 8.28 (d, J = 8.5 Hz, 2H, Ar).

4.16. General procedure for compounds 19a–c

Compounds **19a–c** were prepared according to the known literature procedure.[31] A suspension of **17a–c** (239 mg, 0.5 mmol), NaN_3 (65 mg, 1 mmol) and TEA hydrochloride (138 mg, 1 mmol) in 1,2-dimethoxyethane (5 mL) was boiled under reflux for 48 h. The mixture was cooled and poured into water (4 mL). pH of the aqueous phase was adjusted to ca. 1 by addition of 37% HCl (aq) and the aqueous phase was extracted with diethyl ether. The organic extracts were dried ($MgSO_4$) and the solvent was removed in vacuo. Flash column chromatography gave crystals of the tetrazolyl derivatives **19a–c**.

4.16.1. Diethyl 2-acetamido-2-((5,6-dichloro-2′-(1H-tetrazol-5-yl)biphenyl-3-yl)methyl)malonate (19a)

Yield 159 mg, 61%; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.11–1.22 (m, 6H, OCH_2CH_3), 1.99 (s, 3H, $NHCOCH_3$), 3.44 (s, 2H, $ArCH_2$), 4.14 (q, J = 7.1 Hz, 4H, OCH_2CH_3), 6.86 (s, 1H, Ar), 7.21 (s, 1H, Ar), 7.42 (d, J = 7.6 Hz, 1H, Ar), 7.66–7.74 (m, 2H, Ar), 7.89 (d, J = 6.5 Hz, 1H, Ar), 8.24 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.9, 22.1, 36.8, 62.1, 66.7, 121.8, 128.3, 128.8, 129.0, 129.2, 130.8, 130.9, 131.7, 135.3, 137.9, 140.6, 144.1, 155.2, 166.6, 169.3.

4.16.2. Diethyl 2-acetamido-2-((5,6-dichloro-3′-(1H-tetrazol-5-yl)biphenyl-3-yl)methyl)malonate (19b)

Yield 196 mg, 75%; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.14 (t, J = 7.1 Hz, 6H, OCH_2CH_3), 1.9 (s, 3H, $NHCOCH_3$), 3.47 (s, 2H, $ArCH_2$), 4.15 (q, J = 7.1 Hz, 4H, OCH_2CH_3), 7.00 (s, 1H, Ar), 7.26 (s, 1H, Ar), 7.62 (d, J = 7.6 Hz, 1H, Ar), 7.73 (t, J = 7.6 Hz, 1H, Ar), 8.07–8.10 (m, 2H, Ar), 8.35 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.9, 22.1, 36.9, 62.0, 66.7, 124.2, 126.6, 127.3, 128.3, 129.5, 129.8, 131.2, 131.7, 131.9, 135.9, 139.3, 140.4, 154.8, 166.6, 169.4.

4.16.3. Diethyl 2-acetamido-2-((5,6-dichloro-4′-(1H-tetrazol-5-yl)biphenyl-3-yl)methyl)malonate (19c)

Yield 207 mg, 80%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.27 (t, J = 7.1 Hz, 6H, OCH_2CH_3), 2.11 (s, 3H, $NHCOCH_3$), 3.22 (br s, 1H, NH tetrazole), 3.67 (s, 2H, $ArCH_2$), 4.28 (q, J = 7.1 Hz, 4H, OCH_2CH_3), 6.86 (s, 1H, NH), 6.88 (d, J = 2.1 Hz, 1H, Ar), 7.14 (d, J = 2.1 Hz, 1H, Ar), 7.49 (d, J = 8.2 Hz, 2H, Ar), 8.18 (d, J = 8.2 Hz, 2H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.3, 23.3, 37.2, 63.4, 67.2, 123.9, 127.4, 130.1, 130.8, 131.1, 132.1, 133.7, 134.9, 141.4, 141.8, 156.3, 167.0, 170.6.

4.17. General procedure for compounds 20a–c

The target amino acids **20a–c** were obtained from **19a–c** (156 mg, 0.3 mmol) according to the procedure described for compounds **18a–h**.

4.17.1. (*RS*)-2-Amino-3-(5,6-dichloro-2′-(1H-tetrazol-5-yl)-biphenyl-3-yl)propanoic acid (20a)

Yield 58 mg, 51%; Anal. Calcd for $C_{16}H_{13}Cl_2N_5O_2 \cdot 1.5H_2O$: C, 47.42; H, 3.98; N, 17.28. Found: C, 47.13; H, 4.04; N, 17.14; ^{1}H NMR (300 MHz, DMSO-d_6 + 50 μL D_2O): δ 2.96 (dd, J_1 = 14.3 Hz, J_2 = 8.0 Hz, 1H, $ArCH_2$), 3.16 (dd, J_1 = 14.3 Hz, J_2 = 4.4 Hz, 1H, $ArCH_2$), 3.74–3.80 (m, 1H, $ArCH_2CH$), 7.24 (d, J = 2.2 Hz, 1H, Ar), 7.34–7.40 (m, 2H, Ar), 7.46 (dd, J_1 = 7.7 Hz, J_2 = 1.9 Hz, 1H, Ar), 7.54–7.61 (m, 3H, Ar), 7.78–7.81 (m, 1H, Ar).

4.17.2. (*RS*)-2-Amino-3-(5,6-dichloro-3′-(1H-tetrazol-5-yl)-biphenyl-3-yl)propanoic acid (20b)

Yield 88 mg, 78%; Anal. Calcd for $C_{16}H_{13}Cl_2N_5O_2 \cdot 1.35H_2O$: C, 47.74; H, 3.93; N, 17.40. Found: C, 47.65; H, 4.08; N, 17.34; ^{1}H NMR (300 MHz, DMSO-d_6 + 50 μL D_2O): δ 2.93 (dd, J_1 = 14.3 Hz, J_2 = 7.7 Hz, 1H, $ArCH_2$), 3.10 (dd, J_1 = 14.3 Hz, J_2 = 4.7 Hz, 1H, $ArCH_2$), 3.89–3.97 (m, 1H, $ArCH_2CH$), 7.32 (d, J = 1.7 Hz, 1H, Ar), 7.48–7.51 (m, 2H, Ar), 7.58 (t, J = 7.4 Hz, 1H, Ar), 8.05 (d, J = 7.7 Hz, 1H, Ar), 8.11 (s, 1H, Ar).

4.17.3. (*RS*)-2-Amino-3-(5,6-dichloro-4′-(1H-tetrazol-5-yl)biphenyl-3-yl)propanoic acid (20c)

Yield 93 mg, 82%; Anal. Calcd for $C_{16}H_{13}Cl_2N_5O_2 \cdot 0.65H_2O$: C, 49.29; H, 3.70; N, 17.96. Found: C, 49.48; H, 3.87; N, 17.69; ^{1}H NMR (300 MHz, DMSO-d_6 + 50 μL D_2O): δ 3.01 (dd, J_1 = 14.3 Hz, J_2 = 7.7 Hz, 1H, $ArCH_2$), 3.15 (dd, J_1 = 14.3 Hz, J_2 = 4.7 Hz, 1H, $ArCH_2$), 3.78–3.82 (m, 1H, $ArCH_2CH$), 7.27 (d, J = 1.9 Hz, 1H, Ar), 7.51 (d, J = 1.9 Hz, 1H, Ar), 7.56 (d, J = 8.3 Hz, 2H, Ar), 8.06 (d, J = 8.3 Hz, 2H, Ar).

4.18. Diethyl 2-acetamido-2-(3,4-dichloro-5-(5-cyanothiophen-2-yl)benzyl)malonate (21)

Compound **21** was prepared in analogy to the literature procedure.[32] Compound **16** (602 mg, 1.2 mmol), 5-cyanothiophene-2-boronic acid (275 mg, 1.8 mmol), $Pd(PPh_3)_4$ (70 mg, 0.06 mmol) and potassium carbonate (498 mg, 3.6 mmol) were suspended in the mixture of toluene (15 mL), ethanol (4.5 mL) and water (4.5 mL). The mixture was stirred under nitrogen atmosphere for 2 days at room temperature and then for next 3 days at 50 °C. The reaction mixture was then partitioned into water (50 mL) and ethyl acetate (50 mL). The organic phase was dried ($MgSO_4$), filtered and concentrated under reduced pressure. The crude material was purified by flash column chromatography to give a yellowish oil **21**. Yield 140 mg, 24%; MS: m/z 482 [M^+]; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.29 (t, J = 7.1 Hz, 6H, OCH_2CH_3), 2.04 (s, 3H, $NHCOCH_3$), 3.61 (s, 2H, $ArCH_2$), 4.29 (q, J = 7.1 Hz, 4H, OCH_2CH_3), 6.60 (s, 1H, NH), 7.02 (d, J = 2.1 Hz, 1H, Ar), 7.18 (d, J = 2.1 Hz, 1H, Ar), 7.27 (d, J = 3.8 Hz, 1H, thiophene), 7.61 (d, J = 3.8 Hz, 1H, thiophene); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.3, 23.3, 37.0, 63.3, 67.1, 110.6, 113.9, 129.2, 130.3, 131.1, 131.8, 132.4, 132.8, 134.5, 135.7, 146.5, 167.0, 169.4.

4.19. (*RS*)-5-(5-(2-Amino-2-carboxyethyl)-2,3-dichlorophenyl)thiophene-2-carboxylic acid (22)

Compound **21** (102 mg, 0.2 mmol) was deprotected and purified by the method described for compounds **18a–h**. Yield 33 mg, 43%; Anal. Calcd for $C_{14}H_{11}Cl_2NO_4S \cdot 2H_2O$: C, 42.44; H, 3.82; N, 3.53. Found: C, 42.75; H, 3.54; N, 3.55; ^{1}H NMR (300 MHz, DMSO-d_6 + 50 μL D_2O): δ 2.96 (dd, J_1 = 14.8 Hz, J_2 = 7.7 Hz, 1H, $ArCH_2$), 3.16 (dd, J_1 = 14.8 Hz, J_2 = 4.7 Hz, 1H, $ArCH_2$), 3.75–3.80 (m, 1H, $ArCH_2CH$), 7.42 (d, J = 3.9 Hz, 1H, thiophene), 7.51 (s, 1H, Ar), 7.56 (s, 1H, Ar), 7.68 (d, J = 3.9 Hz, 1H, thiophene).

4.20. Diethyl 2-acetamido-2-(3-(bromomethyl)benzyl) malonate (24)

Compound **24** was prepared from **23** (2.11 g, 8 mmol) by the method described for the compound **7**. Purification by flash column chromatography afforded white solid of **24** (1.16 g, 36%); MS: m/z 399 [M^+]; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.23 (t, J = 7.2 Hz, 6H, OCH_2CH_3), 1.97 (s, 3H, $NHCOCH_3$), 3.57 (s, 2H, $ArCH_2$), 4.20 (q, J = 7.2 Hz, 4H, OCH_2CH_3), 4.35 (s, 2H, CH_2Br), 6.43 (s, 1H, NH), 6.84 (dt, J_1 = 6.6 Hz, J_2 = 1.7 Hz, 1H, Ar), 6.97 (s, 1H, Ar), 7.13–7.17 (m, 2H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.3, 23.3, 33.5, 37.7, 62.9, 67.3, 94.3, 127.8, 128.7, 129.8, 130.9, 136.0, 137.9, 167.3, 169.6.

4.21. General procedure for compounds 25a–c

Compounds **25a**–**c** were prepared according to the modified literature procedure.[17] [1,1′-bis(diphenylphosphino) ferrocene]dichloropalladium(II) (complex with dichloromethane) (41 mg, 0.05 mmol) and boronic acid (or boronic acid pinacol ester) (1.5 mmol) were charged into a 10 mL Schlenk flask. The flask was evacuated and backfilled with nitrogen before adding THF (5 mL), 3 M NaOH solution (1 mL), tetrabutylammonium bromide (TBAB, 65 mg, 0.2 mmol) and **24** (400 mg, 1 mmol). The mixture was stirred at 50 °C under nitrogen atmosphere for 3 h, then washed with 1 M NaOH and the aqueous phase was extracted with diethyl ether (30 mL). The combined organic layers were washed with brine (20 mL), dried ($MgSO_4$) and concentrated in vacuo. The crude material was purified by flash column chromatography.

4.21.1. Diethyl 2-acetamido-2-(3-(2-(ethoxycarbonyl)benzyl)-benzyl)malonate (25a)

Yield 390 mg, 83%; MS: *m*/*z* 469 [M^+]; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.26 (t, J = 7.2 Hz, 6H, OCH_2CH_3), 1.31 (t, J = 7.2 Hz, 3H, OCH_2CH_3), 1.94 (s, 3H, $NHCOCH_3$), 3.56 (s, 2H, CH_2), 4.11–4.30 (m, 6H, OCH_2CH_3), 4.34 (s, 2H, $ArCH_2Ar$), 6.50 (s, 1H, NH), 6.72 (s, 1H, Ar), 6.82 (d, J = 7.4 Hz, 1H, Ar), 7.06 (d, J = 7.7 Hz, 1H, Ar), 7.13–7.18 (m, 2H, Ar), 7.28 (dt, J_1 = 7.7 Hz, J_2 = 1.4 Hz, 1H, Ar), 7.42 (dt, J_1 = 7.7 Hz, J_2 = 1.4 Hz, 1H, Ar), 7.88 (dd, J_1 = 7.7 Hz, J_2 = 1.7 Hz, 1H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.2, 14.4, 23.2, 38.0, 39.7, 61.1, 62.7, 67.3, 126.4, 127.6, 128.0, 128.2, 130.3, 130.5, 130.7, 131.7, 131.9, 135.3, 141.1, 141.7, 167.4, 167.6, 169.0.

4.21.2. Diethyl 2-acetamido-2-(3-(3-cyanobenzyl)benzyl)-malonate (25b)

Yield 271 mg, 64%; MS: *m*/*z* 422 [M^+]; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.25 (t, J = 7.1, 6H, OCH_2CH_3), 1.94 (s, 3H, $NHCOCH_3$), 3.61 (s, 2H, CH_2), 3.95 (s, 2H, $ArCH_2Ar$), 4.13–4.26 (m, 4H, OCH_2CH_3), 6.48 (s, 1H, NH), 6.76 (s, 1H, Ar), 6.89 (d, J = 7.6 Hz, 1H, Ar), 7.06 (d, J = 7.6 Hz, 1H, Ar), 7.22 (t, J = 7.3 Hz, 1H, Ar), 7.38–7.41 (m, 3H, Ar), 7.49–7.52 (m, 1H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.3, 23.2, 37.9, 41.5, 62.8, 67.3, 112.6, 118.8, 127.9, 128.3, 128.8, 129.4, 130.0, 130.5, 132.3, 133.5, 135.9, 139.4, 142.5, 167.4, 169.0.

4.21.3. Diethyl 2-acetamido-2-(3-(4-cyanobenzyl)benzyl)-malonate (25c)

Yield 266 mg, 63%; MS: *m*/*z* 422 [M^+]; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.25 (t, J = 7.1, 6H, OCH_2CH_3), 1.92 (s, 3H, $NHCOCH_3$), 3.60 (s, 2H, CH_2), 3.97 (s, 2H, $ArCH_2Ar$), 4.12–4.25 (m, 4H, OCH_2CH_3), 6.45 (s, 1H, NH), 6.75 (s, 1H, Ar), 6.88 (d, J = 7.3 Hz, 1H, Ar), 7.06 (d, J = 7.6 Hz, 1H, Ar), 7.19–7.27 (m, 3H, Ar), 7.58 (d, J = 7.9 Hz, 2H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.3, 23.2, 37.9, 42.0, 62.8, 67.3, 110.2, 118.9, 128.0, 128.3, 128.8, 129.7, 130.6, 132.4, 135.9, 139.4, 146.6, 167.4, 168.9.

4.22. (*RS*)-2-(3-(2-Amino-2-carboxyethyl)benzyl)benzoic acid (26a)

A mixture of **25a** (352 mg, 0.75 mmol), acetic acid (3 mL), 12 M HCl (3 mL), and water (3 mL) was refluxed for 20 h. After cooling the mixture of acids was removed under reduced pressure; the solid residue was dissolved in water (8 mL) and purified by a reverse-phase flash column chromatography using R-18 Silica Gel (mobile phase: mixture of methanol and 15 mM acetic acid with the gradient of concentration), followed by recrystallization from water. Yield 205 mg, 91%; Anal. Calcd for $C_{17}H_{17}NO_4 \cdot H_2O$: C, 64.34; H, 6.03; N, 4.41. Found: C, 64.36; H, 6.15; N, 4.22; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.79 (dd, J_1 = 14.3 Hz, J_2 = 8.3 Hz, 1H, $ArCH_2$), 3.10 (dd, J_1 = 14.3 Hz, J_2 = 4.4 Hz, 1H, $ArCH_2$), 3.44 (dd, J_1 = 4.7 Hz, J_2 = 8.3 Hz, 1H, $ArCH_2$), 4.28 (s, 2H, $ArCH_2Ar$), 6.93 (d, J = 7.4 Hz, 1H, Ar), 7.03 (d, J = 7.7 Hz, 1H, Ar), 7.11–7.16 (m, 2H, Ar), 7.24–7.28 (m, 2H, Ar), 7.41 (dt, J_1 = 7.6 Hz, J_2 = 1.7 Hz, 1H, Ar), 7.71 (dd, J_1 = 7.7 Hz, J_2 = 1.7 Hz, 1H, Ar).

4.23. General procedure for compounds 26b, c

The target amino acid **26b, c** were obtained from **25b, c** (210 mg, 0.5 mmol) and purified according to the procedure described for compounds **18a**–**h**.

4.23.1. (*RS*)-3-(3-(2-Amino-2-carboxyethyl)benzyl)benzoic acid (26b)

Yield 98 mg, 66%; Anal. Calcd for $C_{17}H_{17}NO_4 \cdot 0.5H_2O$: C, 66.22; H, 5.88 N, 4.54. Found: C, 66.13; H, 5.51; N, 4.39; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.87 (dd, J_1 = 14.0 Hz, J_2 = 7.4 Hz, 1H, $ArCH_2$), 3.09 (dd, J_1 = 14.0 Hz, J_2 = 4.4 Hz, 1H, $ArCH_2$), 3.46–3.49 (m, 1H, $ArCH_2$), 3.93 (s, 2H, $ArCH_2Ar$), 7.03 (d, J = 7.4 Hz, 1H, Ar), 7.08 (d, J = 7.4 Hz, 1H, Ar), 7.15–7.20 (m, 2H, Ar), 7.34 (t, J = 7.4 Hz, 1H, Ar), 7.42 (d, J = 7.7 Hz, 1H, Ar), 7. 71 (d, J = 7.4 Hz, 1H, Ar), 7.75 (s, 1H, Ar).

4.23.2. (*RS*)-4-(3-(2-Amino-2-carboxyethyl)benzyl)benzoic acid (26c)

Yield 129 mg, 87%; Anal. Calcd for $C_{17}H_{17}NO_4 \cdot 0.2H_2O$: C, 67.4; H, 5.79 N, 4.62. Found: C, 67.1; H, 5.39; N, 4.42; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.79 (dd, J_1 = 14.3 Hz, J_2 = 8.5 Hz, 1H, $ArCH_2$), 3.13 (dd, J_1 = 14.3 Hz, J_2 = 4.1 Hz, 1H, $ArCH_2$), 3.39 (dd, J_1 = 4.3 Hz, J_2 = 8.5 Hz, 1H, $ArCH_2$), 3.95 (s, $ArCH_2Ar$), 7.03 (d, J = 7.4 Hz, 1H, Ar), 7.09 (d, J = 7.7 Hz, 1H, Ar), 7.15–7.21 (m, 2H, Ar), 7.31 (d, J = 8.0 Hz, 2H, Ar), 7. 71 (d, J = 8.0 Hz, 2H, Ar).

4.24. General procedure for compounds 29 and 30

Compounds **29** and **30** were prepared in analogy to the literature procedure.[20] To a suspension of *N*-Boc-(*S*)-3-bromophenylalanine **27** (or *N*-Boc-(*R*)-3-bromophenylalanine **28**) (344 mg, 1 mmol) and sodium hydrogencarbonate (168 mg, 2 mmol) in DMF (5 mL), ethyl iodide (0.4 mL, 5 mmol) was added and the mixture was stirred at room temperature for 24 h. After this time the mixture was partitioned into water and ethyl acetate. The organic phase was washed with water, dried over Na_2SO_4 and evaporated. Crude product was purified by flash column chromatography.

4.24.1. Ethyl (*S*)-3-(3-bromophenyl)-2-(pivaloyloxyamino)-propanoate (29)

Yield 331 mg, 89%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.25 (t, J = 7.2 Hz, 3H, OCH_2CH_3), 1.44 (s, 9H, CH_3), 2.97–3.14 (m, 2H, $ArCH_2$), 4.17 (q, J = 7.2 Hz, 2H, OCH_2CH_3), 4.53 (m, 1H, *CH*NH), 5.02 (d, J = 6.9 Hz, 1H, NH), 7.07 (d, J = 7.4 Hz, 1H, Ar), 7.15 (t, J = 7.7 Hz, 1H, Ar), 7.28 (s, 1H, Ar), 7.36 (d, J = 8.0 Hz, 1H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.4, 28.5, 38.2, 54.5, 61.7, 80.2, 122.5, 128.0, 130.0, 130.1, 132.5, 138.5, 155.0, 171.5.

4.24.2. Ethyl (*R*)-3-(3-bromophenyl)-2-(pivaloyloxyamino)-propanoate (30)

Yield 320 mg, 86%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.26 (t, J = 7.2 Hz, 3H, OCH_2CH_3), 1.44 (s, 9H, CH_3), 2.96–3.14 (m, 2H, $ArCH_2$), 4.17 (q, J = 7.2 Hz, 2H, OCH_2CH_3), 4.53 (m, 1H, *CH*NH), 5.02 (d, J = 6.9 Hz, 1H, NH), 7.07 (d, J = 7.4 Hz, 1H, Ar), 7.15 (t, J = 7.7 Hz, 1H, Ar), 7.28 (s, 1H, Ar), 7.36 (d, J = 8.0 Hz, 1H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.4, 28.5, 38.2, 54.5, 61.7, 80.2, 122.5, 128.0, 130.0, 130.1, 132.5, 138.5, 155.0, 171.5.

4.25. General procedure for compounds 31 and 32

Compounds **31** and **32** were prepared from **29** and **30**, respectively (242 mg, 0.65 mmol), and 3-cyano-phenylboronic acid according to the procedure described for compounds **8a**–**e**.

4.25.1. Ethyl (*S*)-3-(3′-cyanobiphenyl-3-yl)-2-(pivaloyloxy-amino)propanoate (31)

Yield 228 mg, 89%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.22 (t, J = 7.2 Hz, 3H, OCH_2CH_3), 1.41 (s, 9H, CH_3), 3.09–3.21 (m, 2H, $ArCH_2$), 4.16 (q, J = 7.2 Hz, 2H, OCH_2CH_3), 4.61 (m, 1H, *CH*NH), 5.03 (d, J = 7.1 Hz, 1H, NH), 7.06 (d, J = 7.6 Hz, 1H, Ar), 7.20 (s, 1H, Ar), 7.37 (t, J = 7.6 Hz, 1H, Ar), 7.46 (d, J = 7.6 Hz, 1H, Ar), 7.61 (d, J = 8.0 Hz, 2H, Ar), 7.70 (d, J = 8.0 Hz, 2H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.4, 28.5, 38.6, 54.6, 61.6, 80.1, 111.1, 118.6, 126.1, 128.3, 129.4, 129.7, 129.9, 130.6, 130.9, 131.4, 136.4, 140.2, 143.4, 159.9, 171.8.

4.25.2. Ethyl (*R*)-3-(3′-cyanobiphenyl-3-yl)-2-(pivaloyloxy-amino)propanoate (32)

Yield 221 mg, 86%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.22 (t, J = 7.2 Hz, 3H, OCH_2CH_3), 1.41 (s, 9H, CH_3), 3.09–3.21 (m, 2H, $ArCH_2$), 4.16 (q, J = 7.2 Hz, 2H, OCH_2CH_3), 4.61 (m, 1H, *CH*NH), 5.03 (d, J = 7.1 Hz, 1H, NH), 7.06 (d, J = 7.6 Hz, 1H, Ar), 7.20 (s, 1H, Ar), 7.37 (t, J = 7.6 Hz, 1H, Ar), 7.46 (d, J = 7.6 Hz, 1H, Ar), 7.61 (d, J = 8.0 Hz, 2H, Ar), 7.70 (d, J = 8.0 Hz, 2H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.4, 28.5, 38.6, 54.6, 61.6, 80.1, 111.1, 118.6, 126.1, 128.3, 129.4, 129.7, 129.9, 130.6, 130.9, 131.4, 136.4, 140.2, 143.4, 159.9, 171.8.

4.26. General procedure for compounds 33 and 34

The target amino acids **33** and **34** were obtained from **31** and **32** (197 mg, 0.5 mmol) according to the procedure described for compounds **18a**–**h**, followed by two re-crystallizations from water.

4.26.1. (*S*)-3′-(2-Amino-2-carboxyethyl)biphenyl-3-carboxylic acid (33)

Yield 53 mg, 37%; ee = 99.2%; $[\alpha]_D^{22}$ +26.2° (*c* 0.014, 1 M HCl:EtOH 1:1); $\Delta\varepsilon$ (214 nm) = +0.031 m^2/mol; Anal. Calcd for $C_{16}H_{15}NO_4 \cdot 1.1H_2O$: C, 62.98; H, 6.68; N, 4.59. Found: C, 62.94; H, 5.40; N, 4.63; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.97 (dd, J_1 = 14.3 Hz, J_2 = 8.3 Hz, 1H, $ArCH_2$), 3.23 (dd, J_1 = 14.3 Hz, J_2 = 4.1 Hz, 1H, $ArCH_2$), 3.55 (dd, J_1 = 8.3 Hz, J_2 = 4.1 Hz, 1H, $ArCH_2CH$), 7.28 (d, J = 7.7 Hz, 1H, Ar), 7.38 (t, J = 7.7 Hz, 1H, Ar), 7.51–7.59 (m, 3H, Ar), 7.86–7.91 (m, 2H, Ar), 8.19 (s, 1H, Ar).

4.26.2. (*R*)-3′-(2-Amino-2-carboxyethyl)biphenyl-3-carboxylic acid (34)

Yield 34 mg, 24%; ee = 98.4%; $[\alpha]_D^{22}$ −23.7° (*c* 0.014, 1 M HCl:EtOH 1:1); $\Delta\varepsilon$ (219 nm) = −0.027 m^2/mol; Anal. Calcd for $C_{16}H_{15}NO_4 \cdot 1.2H_2O$: C, 62.61; H, 5.71; N, 4.56. Found: C, 62.68; H, 5.43; N, 4.60; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.97 (dd, J_1 = 14.3 Hz, J_2 = 8.3 Hz, 1H, $ArCH_2$), 3.23 (dd, J_1 = 14.3 Hz, J_2 = 4.1 Hz, 1H, $ArCH_2$), 3.55 (dd, J_1 = 8.3 Hz, J_2 = 4.1 Hz, 1H, $ArCH_2CH$), 7.28 (d, J = 7.7 Hz, 1H, Ar), 7.38 (t, J = 7.7 Hz, 1H, Ar), 7.51–7.59 (m, 3H, Ar), 7.86–7.91 (m, 2H, Ar), 8.19 (s, 1H, Ar).

4.27. Pharmacology

Rat brain membrane preparations used in the native receptor binding experiments were prepared according to the method described by Ransom and Stec.[33] Affinity for AMPA,[34] KA[35] and NMDA[36] receptor sites was determined using 5 nM [^{3}H]AMPA, 5 nM [^{3}H]KA, and 2 nM [^{3}H]CGP 39653 with some modifications as previously described.[37]

Recombinant rat iGluR5(Q)$_{1b}$, iGluR6(*V,C,R*) and iGluR7A were expressed in *Sf*9 insect cells by baculoviral infection and receptor binding assays carried out using [^{3}H]-SYM 2081 radioligand as previously detailed.[38]

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