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Development of 2'-substituted (2*S*,1'*R*,2'*S*)-2-(carboxycyclopropyl)glycine analogues as potent *N*-methyl-D-aspartic acid receptor agonists

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Abstract

A series of 2'-substituted analogues of the selective NMDA receptor ligand (2*S*,1'*R*,2'*S*)-2-(carboxycyclopropyl)glycine ((*S*)-CCG-IV) have been designed, synthesized and pharmacologically characterized. The design was based on a docking study hypothesizing that substituents in the 2'-position would protrude into a region where differences among the NMDA receptor GluN2 subunits exist. Various synthetic routes were explored, and two different routes provided a series of alkyl-substituted analogues. Pharmacological characterization revealed that these compounds are NMDA receptor agonists and that potency decreases with increasing size of the alkyl groups. Variations in agonist activity are observed at the different recombinant NMDA receptor subtypes. This study demonstrates that it is possible to introduce substituents in the 2'-position of (*S*)-CCG-IV while maintaining agonist activity and that variation among NMDA receptor subtypes may be achieved by probing this region of the receptor.

Keywords

NMDA receptor; CCG; glutamate; molecular modeling; subtype selectivity; electrophysiology

Introduction

The glutamate receptors (GluRs) are a group of receptors with a prominent role in neurotransmission, since most of the excitatory signals in the brain are mediated by glutamate (Figure 1).¹⁻³ These receptors are involved in key processes of the central nervous system (CNS) such as learning and memory,⁴ but are also implicated in several neuropathological conditions, such as ischemia, epilepsy, schizophrenia, chronic pain, and Alzheimer's disease.⁵ Therefore, the GluRs have been the target of extensive research for more than three decades, and one of the goals has been to develop compounds that can alter glutamatergic neurotransmission in a selective manner. Such compounds have been and continue to be important tools in discovering new therapeutic possibilities and to gain a functional understanding of this group of receptors.⁶

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Two different signaling pathways divide the GluRs: metabotropic glutamate receptors (mGluRs) are G-protein-coupled receptors that mediate slow modulatory responses, and ionotropic glutamate receptors (iGluRs) are cation-conducting ion channels that mediate fast neurotransmission via depolarization of the membrane potential. Based on activation by selective ligands and sequence homology, the iGluRs are divided into *N*-methyl-D-aspartic acid (NMDA), (*S*)-2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid (AMPA) and kainic acid (KA) receptors. The iGluRs are homo- or heteromeric assemblies of four subunits. Seven NMDA (GluN1, GluN2A-D, GluN3A-B), four AMPA (GluA1-4) and five KA (GluK1-5) receptor subunits have been identified.³ The majority of native NMDA receptors require assembly of two GluN1 subunits together with two GluN2 subunits. GluN3 subunits can also assemble with GluN1 and GluN2 subunits, but the function and physiological role of these triheteromeric GluN1/GluN2/GluN3 receptors are less well understood. NMDA receptors composed of GluN1 and GluN2 subunits (i.e. GluN1/GluN2) are activated by the simultaneous binding of the endogenous agonists glycine or (*R*)-serine to GluN1 and glutamate to GluN2. Over the last decade, an increasing number of crystal structures of the agonist binding domains (ABDs) of the iGluRs have appeared (86 available structures to date, covering all the major mammalian GluR groups as well as bacterial homologues) providing detailed structural knowledge of agonist binding and the molecular pharmacology of this group of receptors.^{2, 7-11} These crystallographic structures have shown that iGluR ABDs are clamshell-like structures, where the agonist binds in the cleft formed by two lobes. Upon agonist binding, the two lobes close around the agonist, whereas binding of antagonists stabilizes an open domain structure.

We have previously reported the synthesis and pharmacological characterization of a series of 4-substituted (*RS*)-2-(*N*-hydroxypyrazol-5-yl)glycine ((*RS*)-NHP5G, **3a**) analogues as NMDA receptor ligands.¹²⁻¹⁴ This series demonstrated that introduction of substituents at the 4-position of **3a** led to compounds that displayed different efficacies on different GluN2 subtypes. Thus, 4-propyl substituted **3b** appears to be a functional antagonist at the GluN1/GluN2A subtype, but a partial agonist at the GluN1/GluN2D subtype. However, the affinities and potencies of the compounds in this series were weak.¹⁵ Furthermore, we have previously shown that the highly potent (2*S*,4*R*)-4-methyl glutamate (SYM2081, **1b**) shows a significant difference in potency at GluN2 subunits.¹⁶ In order to improve potency we exploited docking studies in the ABD of the GluN2A receptor subunit, to design, synthesize, and characterize a series of compounds based on the most potent NMDA receptor agonist (2*S*,1'*R*,2'*S*)-2-(carboxycyclopropyl)glycine ((*S*)-CCG-IV, **4a**).^{16, 17} CCGs are naturally occurring glutamate analogues isolated from seeds of *Aesculus parviflora* and *Blighia sapida*, belonging to the family Sapindaceae¹⁸ and seeds of *Ephedra altissima* belonging to the family Ephedraceae.¹⁹ These have been important lead structures for the development of a range of selective GluR agonists and antagonists. Three of the stereoisomers are very potent NMDA receptor agonists and may serve as lead structures for obtaining subtype selective NMDA receptor ligands.

While a large number of 2-, 1' - and 3' substituted CCG analogues have been synthesized, there is no analogues described with substituents in the 2' -position, and therefore synthetic routes are unknown. Herein, we describe our design of the analogues, development of synthetic routes to the first 2' -substituted **4a** analogues, as well as the pharmacological characterization at iGluRs.

Results

Design

Initially, the triionized state of **4d** was minimized using macromodel and docked using GLIDE. The grid was prepared with GLIDE from the crystal structure of glutamate in the

ABD of GluN2A (2A5T:chain B) that had been prepared and minimized following the standard settings in PRIME. This gave a binding mode of **4d** shown in Figure 2 overlapping nicely with glutamate in the crystal structure. Comparing with the presumed binding mode of **3b** in the same model we saw that substituents in the 2'-position of **4a** would overlap with the 4-position of **3a** (Figure 2). The substituents in this position is protruding into a region where non-conserved residues are present. We therefore decided to probe the same region based on the **4a** scaffold as we have previously done with **3a** by synthesizing previously unreported 2'-substituted analogues of **4a**.

Chemistry

Two distinct routes to the alkyl substituted analogs of **4a** were employed starting from commercially available (*R*)-Garners aldehyde (GA) (Scheme 1 and 2). Initially, Still-Genari reactions between GA and **5a-d** were explored (Scheme 1). The α -substituted bis(trifluoroethyl)phosphonoester **5a** is commercially available and **5b-d** were synthesized as previously described.^{20, 21} The *cis*- α,β -unsaturated esters **6a** and **6b** were obtained in good yields under the Still-Genari conditions but the α -substituted bis(trifluoroethyl)phosphonoesters **5c** and **5d** resulted in no reaction at -78°C . Increasing temperature ($-78 - 0^\circ\text{C}$) resulted in decomposition and isolation of the desired products in very low yields ($< 15\%$). The intermediates **6a** and **6b** was cyclized into the lactones **7a** and **7b** using TFA in DCM or *p*-TsOH in MeOH as previously reported.^{22, 23}

In order to introduce larger alkyl substituents the unsubstituted lactone **7a** was attempted α -halogenated. Selective α -iodination was performed using I_2 in a mixture of CCl_4 and pyridine to afford **8**.²⁴

Negishi and Stille cross-couplings on this α -halo-lactone **8** introduced ethyl, *n*-propyl, *n*-butyl, benzyl, vinyl and phenyl substituents (Scheme 2). Increasing bulk of the Negishi reagent resulted in diminished yield in the cross-coupling. The method for preparation of the Negishi reagents was crucial for the yield in the cross-coupling. Transmetalation with ZnBr_2 from the corresponding Grignard reagents resulted in very low yields in the coupling compared to oxidative addition of highly reactive Rieke zinc to alkyl halides.²⁵ This was presumed to occur since MgBr_2 has been shown to inhibit cross coupling in some instances.²⁶ Compound **7c-e** and **7g** were prepared by Negishi cross-couplings whereas compound **7f** and **7h** was prepared by Stille cross-couplings (Scheme 2).

Shimamoto et al. have previously reported cyclopropanation of **7a** to obtain the two diastereoisomers in a 6:1 ratio (Scheme 2).²⁷ In an attempt to improve the stereo-selectivity and suppress insertion of diazomethane into the NH bond, additional bulk was introduced on the nitrogen by protection with a second Boc group to afford the imide **9a**. Cyclopropanation on this substrate resulted in isolation of **10a** as a single diastereoisomer with concomitant increased yield compared to cyclopropanation of **7a**. Several attempts were performed to displace the highly toxic and potentially dangerous diazomethane with other cyclopropanation reagents. Among them the Simmon-Smith and Corey Chaykovsky cyclopropanation were attempted, however without success.^{28, 29} Compound **7b-f** were similarly bis-Boc protected and subsequently underwent cyclopropanation to provide **10b-e** in low yield (Scheme 3).

Compound **7h** bearing a vinyl substituent decomposed under the protection conditions and was only isolated in 21 % yield. Therefore cyclopropanation was performed directly on **7h** to obtain **11** in 40 % yield (Scheme 4). Interestingly only cyclopropanation of the pendant double bond was observed in an excess of diazomethane and none of the endocyclic double bond was cyclopropanated, emphasizing how sensitive the cyclopropanation is to steric bulk

in this system. In case of the phenyl substituted compound **9f** no reaction was observed in the cyclopropanation. The difficulty of cyclopropanation of highly substituted C-C double bonds or C-C double bonds with *cis*-unsaturated esters or amides has been noted by others.^{30, 31} The low yields observed in the cyclopropanation in this small series were not surprising since these compounds both contain a tri-substituted double bond and are α,β -unsaturated lactones. The cyclopropanated products **10b-e** were Boc-protected prior to hydrolysis to obtain **12b-e** in high yields (Scheme 5). Hydrolysis was performed with NaOH in a mixture of water and THF to obtain **13b-e**. Subsequent oxidation of the alcohol with KMnO₄ in basic solution, deprotection and precipitation gave the desired analogs **4b-e** as HCl salts.

Pharmacology

The affinities of the compounds at the major iGluR subgroups were investigated using radioligand binding to rat cortical synaptosomes. As shown in Table 1, all the analogues are selective NMDA receptor ligands and the affinity decreases with increasing substituent size. Introducing a methyl group in the 2'-position of **4a** decreases the affinity at AMPA receptors by more than an order of magnitude, while the affinity at KA and NMDA receptors are maintained. Extending the alkyl chain length to ethyl and *n*-propyl an order of magnitude decrease in KA and NMDA receptor affinity is observed for each additional carbon, and AMPA receptor affinity is abolished. The *n*-butyl substituted analog **4e** is not binding to AMPA and KA receptors at a concentration of 100 μ M., but displays weak affinity at NMDA receptors equipotent to the propyl substituted analogue.

The affinity at native NMDA receptors prompted functional characterization at recombinant NMDA receptors expressed in *Xenopus laevis* oocytes to determine the activity at individual receptor subtypes (Table 2, Figures 3 and 4).

The potencies of compounds **4b-e** are decreasing at all four subtypes with increasing substituent size as seen in the synaptosomal binding assay (Figure 3 and Table 2). When looking at the development in relative agonist efficacies of compounds **4b-e** differences among the subtypes can be observed. Thus, agonist efficacy relative to Glu increases from 83 % to 94 % at GluN2D when going from the methyl substituted **4b** to the *n*-butyl substituted **4e** whereas the relative efficacy at GluN2A decreases from 94 % to 65 % (Figure 4).

Discussion

We have designed and synthesized a series of 2'-alkyl substituted analogues of the highly potent NMDA receptor agonist **4a** in order to obtain NMDA receptor agonists that display differences among the cloned GluN2 subtypes. The design of this series was based on previous observations with a series of 4-alkyl substituted analogues of the moderately potent NMDA receptor agonist **3a** that discriminated subtypes in terms of agonist efficacy and that **1b** discriminate subtypes based on potency.¹⁴⁻¹⁶ Thus, we wanted to explore the possibility of achieving more potent compounds with the ability to discriminate the different subtypes either by potency or efficacy by adding substituents to **4a** that would protrude into the same area as the 4-alkyl groups in **3a** and **1b**. Two different synthetic routes were developed to yield this series of alkyl substituted analogues of **4a**.

Pharmacological characterization of the new analogues revealed that although some potency is lost when introducing larger alkyl groups in the 2'-postion, the analogues are indeed more potent than the corresponding 4-substituted **3a** and previously published glutamate analogues.^{15, 16} The increased potency of 2'-substituted **4a** compared to the corresponding 4-substituted **3a** may be due to a better orientation and thereby receptor interaction of the

distal acid group of the **4a** analogues. The variation in agonist efficacy of the new analogues of **4a** at the different subtypes is less pronounced than seen with the series of **3a** analogues. For example, 4-Et-NHP5G activates GluN2A and GluN2D with 5% and 70 % relative to Glu, respectively, whereas ethyl-substituted **4c** activates 83% and 91%, respectively. Nevertheless, propyl substituted **4d** activates GluN2A and GluN2B with 67 % and 105 %, respectively, thus significant differences are achievable. Furthermore, differences in potency is also observed, in example compound **4c** is 15-fold more potent at GluN2D compared to GluN2A. While **1b** is slightly more selective compared to **4c**, **1b** is also a highly potent agonist at kainate receptors and is less potent at GluN2D³², and thus **4c** is one of the most potent and selective agonists towards the GluN2D. This study shows that it is possible to introduce substituents in the 2' position of (*S*)-CCG-IV, and we believe that more variation and introduction of more polar substituents will lead to larger differences among subtypes, however other synthetic routes needs to be explored in order to facilitate the synthesis of such compounds.

Experimental

Chemistry

General Methods. All reactions involving dry solvents or air sensitive reagents were performed under an argon atmosphere and glassware was flame-dried prior to use under vacuum. Flash chromatography (FC) was performed using silica Merck 60A (35-70 μ m). TLC was carried out using Merck silica gel 60 F₂₅₄ aluminum sheets. The compounds were detected as single spots on TLC plates and visualised using UV light (254 nm) and different spraying reagents such as potassium permanganate, anisaldehyde and ninhydrin. All solvents and reagents were analytical grade purchased from Aldrich and used without further purification. Dry THF and DMF were obtained from a solvent purification system (SPS). ¹H (400 MHz) and ¹³C (100 MHz) NMR were recorded on a Bruker instrument using TMS as internal standard. Chemical shifts (δ) are given in parts per million (ppm), and coupling constants (*J*) are given in Hertz (Hz). The following abbreviations are used: bs = broad singlet, s = singlet, d = doublet, dd = doublet of doublet, ddd = doublet of doublet of doublet, dddd = doublet of doublet of doublet of doublet, t = triplet, dt = doublet of triplet, dddt = doublet of doublet of doublet of triplet, tt = triplet of triplet, q = quartet, dq = doublet of quartet, m = multiplet. LC/MS(A) and LC/MS(B) were performed with an Agilent 6410 Triple Quadrupole Mass Spectrometer instrument using electron spray coupled to an Agilent 1200 HPLC system (ESI-LC/MS) with a C₁₈ reverse phase column (Zorbax Eclipse XBD-C₁₈, 4.6 mm $\times$ 50 mm), autosampler and diode array detector using a linear gradient of the binary solvent system of H₂O/ACN/formic acid (A: 95/5/0.1, and B: 5/95/0.086) with a flow rate of 1 mL/min. During ESI-LC/MS analysis, evaporative light scattering (ELS) traces were obtained with a Sedex Sedex 85 Light Scattering Detector. LC/MS(A): linear gradient 0 - 90 % B over 4 min, then 0.5 min 100 % B. LC/MS(B): linear gradient 0 - 90 % B over 3 min, then 1.5 min 100 % B. Products were more than 95% pure according to LC/MS.

General procedure for alkylation methyl bis(2,2,2 trifluoroethyl)phosphonoacetate

Methyl 2 (bis(2,2,2 trifluoroethoxy)phosphoryl)propanoate (5b)—To a solution of *t*-BuOK (1.27 g, 11.3 mmol, 1.2 equiv.) in THF (13 mL) was slowly added methyl bis(2,2,2-trifluoroethyl)phosphonoacetate (2.0 mL, 3.0 g, 9.46 mmol, 1 equiv.) at 0 °C under argon. The mixture was stirred at 0 °C for 30 min, and methyl iodide (2.96 mL, 47.3 mmol, 5 equiv.) was slowly added at 0 °C. The solution was allowed to warm up to room temperature, stirred for 24 h and treated with a saturated aq solution of NH₄Cl (20 mL). The reaction mixture was extracted with EtOAc (3 $\times$ 50 mL), dried over Na₂SO₄, filtered and evaporated to dryness. Purification by silica gel chromatography (Eluent: 30 % EtOAc in heptane) afforded **5b** (2.30 g, 73 %) as a colorless oil. ¹H-NMR (300 MHz; CDCl₃) δ :

4.49-4.38 (m, 4H), 3.78 (s, 3H), 3.20 (dq, $J = 22.8, 7.4$ Hz, 1H), 1.52 (dd, $J = 19.3, 7.4$ Hz, 3H). NMR identical to literature.³³

Methyl 2-(bis(2,2,2-trifluoroethoxy)phosphoryl)butanoate (5c)—5c (2.69 g, 52 %) as a colorless oil. ¹H-NMR (300 MHz; CDCl₃): δ 4.53-4.34 (m, 4H), 3.78 (s, 3H), 3.06 (m, 1H), 2.08-1.90 (m, 2H), 1.04 (t, $J = 6.9$ Hz, 3H). NMR identical to literature.³⁴

Methyl 2-(bis(2,2,2-trifluoroethoxy)phosphoryl)pentanoate (5d)—5d (1.73 g, 32 %) ¹H-NMR (300 MHz; CDCl₃): δ 4.50-4.35 (m, 4H), 3.79 (s, 3H), 3.14 (ddd, $J = 21.8, 10.3, 4.3$ Hz, 1H), 2.09-1.78 (m, 2H), 1.53-1.27 (m, 2H), 0.96 (t, $J = 7.3$ Hz, 3H).

General procedure for Still-Genari reaction

(S,Z)-tert-Butyl 4-(3-methoxy-3-oxoprop-1-en-1-yl)-2,2-dimethyloxazolidine-3-carboxylate (6a)—18-Crown-6 (44 g, 123.3 mmol, 8.5 equiv.) and **5a** (8.1 g, 25.5 mmol, 1.3 equiv.) were dissolved in dry THF (240 mL) and cooled to -78 °C under an atmosphere of argon. 0.5 M KHMDS (47.1 mL, 23.6 mmol, 1.2 equiv.) was added dropwise and the solution was aged for 1 h. Garner's aldehyde (4.5 g, 19.6 mmol, 1 equiv.) in dry THF (13 mL) was slowly added dropwise. The solution was stirred for 3 h at -78 °C. The reaction was quenched with saturated aq NH₄Cl (100 mL). The mixture was extracted with EtOAc (3 $\times$ 150 mL) and the combined organic phases, were dried over Na₂SO₄, filtered and evaporated to dryness. The crude product was purified by silica gel chromatography (Eluent: 20 % EtOAc in heptane) to afford **6a** (4.0 g, 72 %) as colorless oil. ¹H-NMR (400 MHz; CDCl₃): δ 6.36, 6.27 (dd, $J = 10.7, 9.2$ Hz, 1H, rotamers), 5.85 (m, 1H), 5.41 (bs, 1H), 4.33-4.25 (m, 1H), 3.79 (dd, $J = 9.2, 3.1$ Hz, 1H), 3.73 (s, 3H), 1.66-1.41 (m, 15H, rotamers). NMR identical to literature.³⁵

(S,Z)-tert-Butyl 4-(3-methoxy-2-methyl-3-oxoprop-1-en-1-yl)-2,2-dimethyloxazolidine-3-carboxylate (6b)—6b (2.5 g, 89 %). ¹H-NMR (300 MHz; CDCl₃): δ 6.10-5.95 (m, 1H), 5.14-5.12 (bs, 1H), 4.22 (m, 1H), 3.79-3.73 (m, 4H), 1.92 (s, 3H), 1.64-1.38 (m, 15H). NMR identical to literature.²³

(S,Z)-tert-Butyl 4-(2-(methoxycarbonyl)but-1-en-1-yl)-2,2-dimethyloxazolidine-3-carboxylate (6c)—6c (247 mg, 15.7 %) as a pale yellow oil. ¹H-NMR (300 MHz; CD₃OD): δ 5.94 (m, 1H), 5.03 (m, 1H), 4.20 (m, 1H), 3.73 (s, 3H), 2.42-2.21 (m, 2H), 1.48 (m, 15H), 1.05 (t, $J = 7.1$ Hz, 3H).

Methyl 2-(bis(2,2,2-trifluoroethoxy)phosphoryl)pentanoate (6d)—6d (100 mg, 10.6 %) as a pale yellow oil. ¹H-NMR (300 MHz; CD₃OD): δ 5.94-5.91 (m, 1H), 5.06-5.00 (m, 1H), 4.22-4.17 (m, 1H), 3.72 (m, 4H), 2.37-2.15 (m, 2H), 1.58-1.37 (m, 17H), 0.91 (t, $J = 7.1$ Hz, 3H).

(S)-tert-Butyl (6-oxo-3,6-dihydro-2H-pyran-3-yl)carbamate (7a)—To a solution of 6a (5.1 g, 17.9 mmol) in DCM (100 mL), was added TFA (2.87 mL, 38.6 mmol, 2.1 equiv.) and the solution was stirred for 24 h at room temperature. The solvent was evaporated and the crude product was purified by silica gel chromatography (Eluent: 60 % EtOAc in heptane) to afford **7a** (2.5 g, 65 %) as a white solid. ¹H-NMR (400 MHz; CDCl₃): δ 6.88 (bs, 1H), 6.10 (dd, $J = 9.8, 1.3$ Hz, 1H), 4.94 (bs, 1H), 4.50-4.36 (m, 3H), 1.45 (s, 9H). NMR identical to literature.²⁷

(S)-tert Butyl (5-methyl-6-oxo-3,6-dihydro-2H-pyran-3-yl)carbamate (7b)—6b (2.36 g, 7.9 mmol, 1 equiv.) was dissolved in MeOH (43 mL). p-TsOH (75 mg, 0.40 mmol, 0.05 equiv.) was added and the solution stirred at room temperature for 24 h. The solvent

was evaporated and the crude product was purified by silica gel chromatography (Eluent: 20-50 % EtOAc in heptane) to afford **7b** (1.1 g, 61 %) as white solid. ((460 mg, 1.54 mmol) starting material, and (100 mg, 0.41 mmol) of the amination opened intermediate recovered). (76 % yield based on recovered starting material). ¹H-NMR (300 MHz; CDCl₃): δ 6.59 (d, *J* = 4.8 Hz, 1H), 4.78 (d, *J* = 7.9 Hz, 1H), 4.48-4.34 (m, 3H), 1.96 (s, 3H), 1.46 (s, 9H). NMR identical to literature.²³

(S)-tert-butyl (5-iodo-6-oxo-3,6-dihydro-2H-pyran-3-yl)carbamate (8)—7a (1.4 g, 6.6 mmol, 1 equiv.) was dissolved in CCl₄ (10 mL) and pyridine (10 mL). A solution of iodine (8.38 g, 33 mmol, 5.0 equiv.) dissolved in pyridine and CCl₄ (1:1, 45 mL) was added at room temperature. The solution was heated at 40 °C for 30 min. The reaction was quenched by addition of 1M aq Na₂S₂O₃ (50 mL) and stirred for 10 min. The phases were separated and the water phase extracted with DCM (2 × 50 mL). The combined organic phases were dried over Na₂SO₄, filtered and evaporated to dryness. The crude product was purified by silica gel chromatography (Eluent: 50 % EtOAc in heptane) to afford **8** (1.8 g, 80 %) as off-white solid. ¹H-NMR (300 MHz; CD₃OD): δ 7.62 (d, *J* = 4.9 Hz, 1H), 4.54 (dd, *J* = 10.7, 3.8 Hz, 1H), 4.36 (m, 2H), 1.47 (s, 9H). ¹³C-NMR (75 MHz; CD₃OD): δ 159.83 (s, 1C), 156.06 (s, 1C), 154.71 (s, 1C), 90.24 (s, 1C), 79.89 (s, 1C), 70.73 (s, 1C), 46.58 (s, 1C), 27.57 (s, 3C). LC/MS(A): RT 3.42, ELSD: 100 %, UV_{254nm} 100 %, (M-Boc)H⁺ 240.0.

General procedure for Negishi cross-coupling

(S)-tert-Butyl (5-ethyl-6-oxo-3,6-dihydro-2H-pyran-3-yl)carbamate (7c)—A solution of **8** (310 mg, 0.91 mmol, 1 equiv.) and Pd(PPh₃)₂Cl₂ (63.8 mg, 0.091 mmol, 0.1 equiv.) in dry degassed DMF (3.8 mL) was added Et₂Zn (0.59 mL, 0.59 mmol, 1M in hexanes, 0.65 equiv.) at room temperature and stirred for 1.5 h. The reaction was quenched with saturated aq NH₄Cl (10 mL). Et₂O (25 mL) was added and the phases separated. The water phase was extracted with Et₂O (2 × 25 mL) and the combined organic phases were washed with brine (10 mL), dried over Na₂SO₄, filtered and evaporated to dryness. The crude product was purified by silica gel chromatography (Eluent: 5 % Acetone in DCM) to afford **7c** (130 mg, 60 %) as white solid. ¹H-NMR (300 MHz; CD₃OD): δ 6.62 (d, *J* = 2.2 Hz, 1H), 4.45-4.38 (m, 2H), 4.23 (m, 1H), 2.33 (q, *J* = 7.4 Hz, 2H), 1.48 (s, 9H), 1.11 (t, *J* = 7.4 Hz, 3H). ¹³C-NMR (75 MHz; CD₃OD): δ 166.05 (s, 1C), 157.36 (s, 1C), 139.54 (s, 1C), 135.94 (s, 1C), 80.69 (s, 1C), 71.16 (s, 1C), 44.88 (s, 1C), 28.69 (s, 3C), 24.86 (s, 1C), 12.71 (s, 1C). LC/MS(A): RT 3.43, ELSD: 0 %, UV_{254nm} 100 %, (M-Boc)H⁺ 142.1.

(S)-tert-Butyl (6-oxo-5-propyl-3,6-dihydro-2H-pyran-3-yl)carbamate (7d)—7d (415 mg, 46 %) as white solid. ¹H-NMR (400 MHz; MeOD): δ 6.63 (d, *J* = 4.1 Hz, 1H), 4.45-4.38 (m, 2H), 4.20 (dd, *J* = 10.2, 4.8 Hz, 1H), 2.34-2.21 (m, 2H), 1.58-1.45 (m, 11H), 0.96 (t, *J* = 7.4 Hz, 3H). ¹³C-NMR (101 MHz; MeOD): δ 166.28 (s, 1C), 157.61 (s, 1C), 140.98 (s, 1C), 134.54 (s, 1C), 80.80 (s, 1C), 71.23 (s, 1C), 44.97 (s, 1C), 33.84 (s, 1C), 28.65 (s, 3C), 22.35 (s, 1C), 13.95 (s, 1C). LC/MS(A): RT 3.74, ELSD: 0 %, UV 100 %, (M-Boc)H⁺ 156.1.

Preparation of ~ 0.7 M propylzinc(II) iodide: A dry flask was charged with a highly reactive Rieke Zn suspension in THF (12.1 mL, 0.76 M, 9.2 mmol) under an atmosphere of argon and covered with alumina foil. A solution of *n*-propyl iodide (0.90 mL, 1.56 g, 9.2 mmol) was added at rt, and the mixture was stirred for 24 h.

(S)-tert-Butyl (5-butyl-6-oxo-3,6-dihydro-2H-pyran-3-yl)carbamate (7e)—7e (273 mg, 35 %) as a off white solid. ¹H-NMR (400 MHz; CDCl₃): δ 6.61 (dd, *J* = 4.2, 1.0 Hz, 1H), 4.43-4.36 (m, 2H), 4.22-4.18 (m, 1H), 2.33-2.22 (m, 2H), 1.50-1.31 (m, 13H), 0.94 (t, *J*

= 7.2 Hz, 3H). ^{13}C -NMR (101 MHz; CDCl_3): δ 166.29 (s, 1C), 157.63 (s, 1C), 140.79 (s, 1C), 134.78 (s, 1C), 80.80 (s, 1C), 71.23 (s, 1C), 44.98 (s, 1C), 31.50 (s, 1C), 31.38 (s, 1C), 28.66 (s, 3C), 23.30 (s, 1C), 14.17 (s, 1C). LC/MS(A): RT 4.05, ELSD: 0 %, UV 100 %, (M-Boc) H^+ 170.1.

Preparation of ~ 0.7 M butylzinc(II) iodide: A dry flask was charged with a highly reactive Rieke Zn suspension in THF (9.92 mL, 0.76 M, 7.54 mmol) under an atmosphere of argon and covered with alumina foil. A solution of butyl iodide (0.85 mL, 1.39 g, 7.54 mmol) was added slowly at rt, and the mixture was stirred for 24 h.

(S)-tert-Butyl (5-benzyl-6-oxo-3,6-dihydro-2H-pyran-3-yl)carbamate (7g)—7g (90 mg, 20 %) as a colorless oil. ^1H -NMR (400 MHz; CD_3OD): δ 7.36-7.19 (m, 5H), 6.48-6.46 (m, 1H), 4.39-4.35 (m, 2H), 4.21-4.16 (m, 1H), 3.59 (s, 2H), 1.43 (s, 9H). ^{13}C -NMR (101 MHz; CD_3OD): δ 166.03 (s, 1C), 157.58 (s, 1C), 142.04 (s, 1C), 139.44 (s, 1C), 134.53 (s, 1C), 130.26 (s, 2C), 129.60 (s, 2C), 127.63 (s, 1C), 79.87 (s, 1C), 71.23 (s, 1C), 45.06 (s, 1C), 37.69 (s, 1C), 28.72 (s, 3C). LC/MS(A): RT 3.99, ELSD: 0 %, UV 95.6 %, MH^+ 204.1.

Preparation of ~ 0.5 M benzylzinc(II) bromide: A dry flask was charged with a highly reactive Rieke Zn suspension (4.96 mL, 0.76 M, 3.77 mmol). This suspension was cooled to 0 °C. A solution of benzyl bromide (0.45 mL, 0.65 g, 3.77 mmol) in THF (2.13 mL) was added dropwise, and the mixture was stirred at 0 °C for 3 h.

General procedure for Stille cross-coupling

(S)-tert-Butyl (6-oxo-5-phenyl-3,6-dihydro-2H-pyran-3-yl)carbamate (7f)—A flask charged with ZnBr_2 (36 mg, 0.16 mmol, 0.16 equiv.) was flame dried and put under argon atmosphere. The flask was charged with $\text{Pd}[(\text{o-tolyl})_3\text{P}]_2\text{Cl}_2$ (40 mg, 0.05 mmol, 0.05 equiv.), **8** (339 mg, 1.0 mmol, 1 equiv.) and DMF (2 mL) was added. Tributylphenyltin (0.35 mL, 400 mg, 1.1 mmol, 1.1 equiv.) was added slowly at room temperature and the solution stirred at 65 °C for 1.5 h. The reaction was quenched by addition of saturated aq NH_4Cl (10 mL) and Et_2O (20 mL) was added. The phases were separated and the organic phase washed with brine, dried over Na_2SO_4 , filtered and evaporated to dryness. The crude product was dissolved in ACN (30 mL) and washed with heptane (4×20 mL). The ACN phase was evaporated to dryness and the crude product was purified by silica gel chromatography (Eluent: 30 % EtOAc in heptane) to afford **7f** (258 mg, 89 %) as white solid. ^1H -NMR (400 MHz; CD_3OD): δ 7.47-7.45 (m, 2H), 7.35 (m, 3H), 6.95 (d, J = 4.4 Hz, 1H), 4.54-4.52 (m, 2H), 4.34-4.30 (m, 1H), 1.47 (s, 9H). ^{13}C -NMR (101 MHz; CD_3OD): δ 165.15 (s, 1C), 157.60 (s, 1C), 143.06 (s, 1C), 136.51 (s, 1C), 134.54 (s, 1C), 129.57 (s, 2C), 129.48 (s, 1C), 129.12 (s, 2C), 80.91 (s, 1C), 71.08 (s, 1C), 45.33 (s, 1C), 28.67 (s, 3C). LC/MS(A): RT 3.84, ELSD: 100 %, UV 100 %, (M-Boc) H^+ 190.1.

(S)-tert-Butyl (6-oxo-5-vinyl-3,6-dihydro-2H-pyran-3-yl)carbamate (7h)—7h (1.17 g, 56 %) as off-white solid. ^1H -NMR (400 MHz; CDCl_3): δ 6.83 (d, J = 5.2 Hz, 1H), 6.53-6.46 (m, 1H), 5.87 (dd, J = 17.6, 1.2 Hz, 1H), 5.36 (dd, J = 11.2, 1.2 Hz, 1H), 4.94 (d, J = 4.6 Hz, 1H), 4.54 (dd, J = 7.7, 3.9 Hz, 1H), 4.46 (dd, J = 11.5, 4.0 Hz, 1H), 4.34 (dd, J = 11.4, 3.5 Hz, 1H), 1.47 (s, 9H). ^{13}C -NMR (101 MHz; CDCl_3): δ 162.62 (s, 1C), 154.83 (d, J = 2.8 Hz, 1C), 137.19 (s, 1C), 131.03 (s, 1C), 130.27 (s, 1C), 119.34 (s, 1C), 80.66 (s, 1C), 70.03 (s, 1C), 43.48 (s, 1C), 28.27 (s, 3C). LC/MS(A): RT 2.87, ELSD: 0 %, $\text{UV}_{254\text{nm}}$ 100 %, MNa^+ 262.1.

tert-Butyl N-[(tert-butoxy)carbonyl]-N-[(3S)-6-oxo-3,6-dihydro-2H-pyran-3-yl]carbamate (9a)—To a solution of 7a (260 mg, 1.22 mmol, 1 equiv.) in ACN (5.6 mL), (Boc) $_2\text{O}$ (799 mg, 3.66 mmol, 3 equiv.) and DMAP (29 mg, 0.24 mmol, 0.2 equiv.) was

added successively. The solution was stirred for 2 h at room temperature, the solvent evaporated off and the crude product purified by silica gel chromatography (Eluent: 20 % EtOAc in heptane) to afford **9a** (375 mg, 98 %) as white solid. ¹H-NMR (400 MHz; MeOD): δ 7.02 (ddd, *J* = 10.1, 2.6, 1.6 Hz, 1H), 5.96 (dd, *J* = 10.1, 2.6 Hz, 1H), 5.28 (dddd, *J* = 9.9, 6.1, 2.7, 2.7 Hz, 1H), 4.59 (dd, *J* = 10.4, 10.1 Hz, 1H), 4.44 (ddd, *J* = 10.6, 6.1, 1.6 Hz, 1H), 1.53 (s, 18H). ¹³C-NMR (101 MHz; MeOD): δ 165.07 (s, 1C), 153.49 (s, 2C), 150.45 (s, 1C), 119.74 (s, 1C), 85.39 (s, 2C), 69.12 (s, 1C), 50.93 (s, 1C), 28.40 (s, 6C). LC/MS(A): RT 4.03, ELSD: 0 %, UV 100 %, MNa⁺ 336.1

tert-Butyl N-[(tert-butoxy)carbonyl]-N-[(1S,5S,6R)-2-oxo-3-oxabicyclo[4.1.0]heptan-5-yl]carbamate (10a)—Pd(OAc)₂ (27 mg, 0.12 mmol, 0.05 equiv.) was suspended in a solution of **9a** (0.750 g, 2.4 mmol, 1 equiv.) in Et₂O (50 mL). A solution of CH₂N₂ (approx. 5.0 equiv.) in Et₂O was added dropwise over approximately 15 min. The solution was stirred for another 15 min and quenched by addition of glacial acetic acid (0.69 mL, 721 mg, 12 mmol) and the solvent was evaporated off. The crude product was purified by silica gel chromatography (Eluent: 20 % EtOAc in heptane) to afford **10a** (628 mg, 80 %) as white solid. ¹H-NMR (400 MHz; CDCl₃): δ 4.67 (m, 1H), 4.40 (ddd, *J* = 13.3, 1.9, 1.6 Hz, 1H), 4.27 (dd, *J* = 13.3, 4.0 Hz, 1H), 2.03 (dddd, *J* = 9.4, 7.4, 4.9, 0.7 Hz, 1H), 1.91-1.85 (m, 1H), 1.53 (s, 18H), 1.32-1.23 (m, 2H). ¹³C-NMR (101 MHz; CDCl₃): δ 169.78 (s, 1C), 152.74 (s, 2C), 83.26 (s, 2C), 68.06 (s, 1C), 47.20 (s, 1C), 27.83 (s, 6C), 17.64 (s, 1C), 16.53 (s, 1C), 8.65 (s, 1C). LC/MS(A): RT 3.91, ELSD: 0 %, UV_{210nm} 0 %, (2M)Na⁺ 677.4.

General procedure for Boc-protection

tert-Butyl N-[(tert-butoxy)carbonyl]-N-[(3S)-5-methyl-6-oxo-3,6-dihydro-2H-pyran-3-yl]carbamate (9b)—A solution of **7b** (1.1 g, 4.8 mmol, 1 equiv.) in ACN (22 mL) was added (Boc)₂O (3.14 g, 14.4 mmol, 3 equiv.) and DMAP (117 mg, 0.96 mmol, 0.2 equiv.) successively. The solution was stirred for 2 h at room temperature, the solvent evaporated off and the crude product purified by silica gel chromatography (Eluent: 20 % EtOAc in heptane) to afford **9b** (1.54 g, 98 %) as white solid. ¹H-NMR (300 MHz; CDCl₃): δ 6.53 (dq, *J* = 2.7, 1.4 Hz, 1H), 5.27-5.18 (m, 1H), 5.27-5.18 (m, 1H), 4.57 (td, *J* = 10.3, 1.2 Hz, 1H), 4.34 (ddt, *J* = 10.2, 6.0, 1.4 Hz, 1H), 1.95 (dt, *J* = 2.4, 1.3 Hz, 3H), 1.51 (s, 18H). ¹³C-NMR (151 MHz; CDCl₃): δ 164.39 (s, 1C), 151.95 (s, 2C), 142.00 (s, 1C), 126.42 (s, 1C), 83.98 (s, 2C), 67.80 (s, 1C), 49.80 (s, 1C), 28.08 (s, 6C), 17.38 (s, 1C). LC/MS(A): RT 4.28, ELSD: 0 %, UV 100 %, (M-2Boc)H⁺ 128.1.

tert-Butyl N-[(tert-butoxy)carbonyl]-N-[(3S)-5-ethyl-6-oxo-3,6-dihydro-2H-pyran-3-yl]carbamate (9c)—**9c** (587 mg, 100 %) as white solid. ¹H-NMR (300 MHz; CD₃OD): δ 6.65 (s, 1H), 5.24-5.19 (m, 1H), 4.54 (t, *J* = 9.8 Hz, 1H), 4.40 (dd, *J* = 10.6, 5.9 Hz, 1H), 2.33 (q, *J* = 7.3 Hz, 2H), 1.53 (s, 18H), 1.13 (t, *J* = 7.4 Hz, 3H). LC/MS(A): RT 4.51, ELSD: 100 %, UV 100 %, (M-2Boc)H⁺ 142.1.

tert-Butyl N-[(tert-butoxy)carbonyl]-N-[(3S)-6-oxo-5-propyl-3,6-dihydro-2H-pyran-3-yl]carbamate (9d)—**9d** (500 mg, 100 %) as white solid. ¹H-NMR (400 MHz; CD₃OD): δ 6.62 (m, 1H), 5.18 (dddt, *J* = 9.1, 5.9, 3.2, 1.6 Hz, 1H), 4.53 (dd, *J* = 10.7, 9.0 Hz, 1H), 4.38 (ddd, *J* = 10.7, 5.9, 1.3 Hz, 1H), 2.32-2.19 (m, 2H), 1.55-1.46 (m, 20H), 0.96 (t, *J* = 7.4 Hz, 3H). ¹³C-NMR (101 MHz; CD₃OD): δ 166.19 (s, 1C), 153.69 (s, 2C), 142.61 (s, 1C), 132.43 (s, 1C), 85.06 (s, 2C), 69.31 (s, 1C), 51.02 (s, 1C), 34.04 (s, 1C), 28.39 (s, 6C), 22.62 (s, 1C), 14.17 (s, 1C). LC/MS(A): RT 4.78, ELSD: 0 %, UV 93 %, (M-2Boc)H⁺ 156.1.

tert-Butyl N-[(tert-butoxy)carbonyl]-N-[(3S)-5-butyl-6-oxo-3,6-dihydro-2H-pyran-3-yl]carbamate (9e)—9e (340 mg, 100 %). ¹H-NMR (400 MHz; CD₃OD): δ 6.62 (m, 1H), 5.17 (dddt, *J* = 9.1, 5.9, 3.2, 1.6 Hz, 1H), 4.53 (dd, *J* = 10.7, 9.0 Hz, 1H), 4.38 (ddd, *J* = 10.7, 5.9, 1.3 Hz, 1H), 2.35–2.22 (m, 2H), 1.50–1.34 (m, 22H), 0.93 (t, *J* = 7.2 Hz, 3H). ¹³C-NMR (101 MHz; CD₃OD): δ 165.97 (s, 1C), 153.47 (s, 2C), 142.20 (s, 1C), 132.44 (s, 1C), 84.83 (s, 2C), 69.10 (s, 1C), 50.80 (s, 1C), 31.44 (s, 1C), 31.41 (s, 1C), 28.19 (s, 6C), 23.23 (s, 1C), 14.18 (s, 1C). LC/MS(A): RT 4.95, ELSD: 0 %, UV 100 %, (M-2Boc)H⁺ 170.1.

tert-Butyl N-[(tert-butoxy)carbonyl]-N-[(3S)-6-oxo-5-phenyl-3,6-dihydro-2H-pyran-3-yl]carbamate (9f)—9f (300 mg, 88 %). ¹H-NMR (400 MHz; CDCl₃): δ 7.47–7.45 (m, 2H), 7.37–7.29 (m, 3H), 6.89 (dd, *J* = 2.6, 1.8 Hz, 1H), 5.41 (ddd, *J* = 10.7, 6.0, 2.6 Hz, 1H), 4.69 (dd, *J* = 10.7, 10.1 Hz, 1H), 4.42 (ddd, *J* = 10.1, 6.0, 1.8 Hz, 1H), 1.49 (s, 18H). ¹³C-NMR (101 MHz; CDCl₃): δ 162.69 (s, 1C), 151.90 (s, 2C), 144.06 (s, 1C), 135.07 (s, 1C), 130.23 (s, 1C), 128.39 (s, 1C), 128.35 (s, 2C), 128.26 (s, 2C), 84.03 (s, 2C), 67.40 (s, 1C), 50.22 (s, 1C), 28.00 (s, 6C). LC/MS(A): RT 4.73, ELSD: 100 %, UV 89 %, MH⁺ 190.0.

General procedure for cyclopropanation (Method A)

tert-Butyl N-[(tert-butoxy)carbonyl]-N-[(1S,5S,6R)-1-methyl-2-oxo-3-oxabicyclo[4.1.0]heptan-5-yl]carbamate (10b)—Pd(OAc)₂ (11.2 mg, 0.05 mmol, 0.05 equiv.) was suspended in a solution of **9b** (330 mg, 1 mmol, 1 equiv.) in Et₂O (20 mL). A solution of CH₂N₂ (approx. 5 equiv.) in Et₂O was added dropwise over approximately 15 min. The solution was stirred for another 15 min and quenched by addition of glacial acetic acid (0.29 mL, 300 mg, 5 mmol) and the solvent was evaporated off. The crude product was purified by silica gel chromatography (Eluent: 0–50 % EtOAc in heptane) to afford **10b** (157 mg, 46 %, 72 % based on recovered starting material). ¹H-NMR (300 MHz; CDCl₃): δ 4.71 (m, 1H), 4.31–4.29 (m, 2H), 1.51 (s, 19H), 1.39 (s, 3H), 1.34 (dd, *J* = 5.8 Hz, 1H), 1.03 (dd, *J* = 8.2, 6.2 Hz, 1H). ¹³C-NMR (151 MHz; CDCl₃): δ 172.52 (s, 1C), 152.85 (s, 2C), 83.11 (s, 2C), 69.09 (s, 1C), 48.34 (s, 1C), 27.92 (s, 6C), 24.79 (s, 1C), 21.41 (s, 1C), 20.87 (s, 1C), 15.27 (s, 1C). LC/MS(A): RT 4.26, ELSD: 0 %, UV 0 %, (M-2Boc)H⁺ 142.1.

tert-Butyl N-[(tert-butoxy)carbonyl]-N-[(1S,5S,6R)-1-ethyl-2-oxo-3-oxabicyclo[4.1.0]heptan-5-yl]carbamate (10c)—10c (70 mg, 31 %, 57 % based on recovered starting material). ¹H-NMR (300 MHz; CD₃OD): δ 4.67 (m, 1H), 4.45 (dd, *J* = 13.3, 4.6 Hz, 1H), 4.24 (ddd, *J* = 13.4, 1.9, 1.2 Hz, 1H), 1.80 (dq, *J* = 14.3, 7.2 Hz, 1H), 1.64 (m, 1H), 1.46–1.36 (m, 20H), 1.10 (dd, *J* = 8.4, 6.2 Hz, 1H), 1.00 (t, *J* = 7.4 Hz, 3H). ¹³C-NMR (75 MHz; CD₃OD): δ 174.99 (s, 1C), 154.02 (s, 2C), 84.05 (s, 2C), 69.94 (s, 1C), 50.04 (s, 1C), 28.72 (s, 1C), 28.25 (s, 6C), 27.45 (s, 1C), 26.00 (s, 1C), 14.10 (s, 1C), 11.65 (s, 1C). LC/MS(A): RT 4.55, ELSD: 100 %, UV 0 %, (M-2Boc)H⁺ 156.1.

tert-Butyl N-[(tert-butoxy)carbonyl]-N-[(1S,5S,6R)-2-oxo-1-propyl-3-oxabicyclo[4.1.0]heptan-5-yl]carbamate (10d)—10d (105 mg, 15 %, 48 % based on recovered starting material) as white solid. ¹H-NMR (400 MHz; MeOD): δ 4.68 (m, 1H), 4.46 (dd, *J* = 13.3, 4.7 Hz, 1H), 4.27 (ddd, *J* = 13.3, 2.1, 1.4 Hz, 1H), 1.72–1.35 (m, 24H), 1.12 (dd, *J* = 8.4, 6.2 Hz, 1H), 0.90 (t, *J* = 7.3 Hz, 3H). ¹³C-NMR (101 MHz; MeOD): δ 175.22 (s, 1C), 154.26 (s, 2C), 84.20 (s, 2C), 69.93 (s, 1C), 50.20 (s, 1C), 38.14 (s, 1C), 28.22 (s, 6C), 26.51 (s, 1C), 26.19 (s, 1C), 21.22 (s, 1C), 14.74 (s, 1C), 14.58 (s, 1C). LC/MS(A): RT 4.69, ELSD: 0 %, UV_{210nm} 100 %, (M-2Boc)H⁺ 170.1.

General procedure for cyclopropanation (Method B)

$\text{Pd}_2(\text{dba})_3$ (52 mg, 0.057 mmol, 0.05 equiv.) was suspended in a solution of **9d** (400 mg, 1.13 mmol, 1 equiv.) in Et_2O (22 mL). A solution of CH_2N_2 (approx. 5 equiv.) in Et_2O was added dropwise over approximately 15 min. The solution was stirred for another 15 min and quenched by addition of glacial acetic acid (0.32 mL, 3.39 mmol) and the solvent was evaporated off. The crude product was purified by silica gel chromatography (Eluent: 20 % EtOAc in heptane) to afford **10d** (155 mg, 37 %, 63 % based on recovered starting material) as white solid. NMR and LC/MS identical to Method A.

tert-Butyl N-[(tert-butoxy)carbonyl]-N-[(1S,5S,6R)-1-butyl-2-oxo-3-oxabicyclo[4.1.0]heptan-5-yl]carbamate (10e)—10e (72 mg, 29 %, 48 % based on recovered starting material). $^1\text{H-NMR}$ (400 MHz; MeOD): δ 4.67 (m, 1H), 4.46 (dd, J = 13.3, 4.7 Hz, 1H), 4.27 (ddd, J = 13.3, 2.1, 1.4 Hz, 1H), 1.69-1.63 (m, 2H), 1.59-1.26 (m, 24H), 1.11 (dd, J = 8.5, 6.2 Hz, 1H), 0.91 (t, J = 7.3 Hz, 3H). $^{13}\text{C-NMR}$ (101 MHz; MeOD): δ 175.24 (s, 1C), 154.25 (s, 2C), 84.20 (s, 2C), 69.91 (s, 1C), 50.23 (s, 1C), 35.65 (s, 1C), 30.20 (s, 1C), 28.24 (s, 6C), 26.53 (s, 1C), 26.18 (s, 1C), 23.96 (s, 1C), 14.82 (s, 1C), 14.39 (s, 1C). LC/MS(B): RT 4.19, ELSD: 100 %, UV 0 %, (M-2Boc) H^+ 184.1.

(S)-tert-butyl (5-cyclopropyl-6-oxo-3,6-dihydro-2H-pyran-3-yl)carbamate (11)—11 (440 mg, 41 %). $^1\text{H-NMR}$ (400 MHz; CDCl_3): δ 6.27 (d, J = 5.2 Hz, 1H), 4.79 (bs, 1H), 4.38 (d, J = 3.5 Hz, 1H), 4.36 (d, J = 3.8 Hz, 1H), 4.30-4.26 (m, 1H), 1.84-1.77 (m, 1H), 1.43 (s, 9H), 0.85 (m, 1H), 0.84-0.82 (m, 1H), 0.59-0.55 (m, 1H), 0.52-0.48 (m, 1H).

General procedure for deprotection of a single Boc-group

tert-Butyl ((1S,5S,6R)-1-methyl-2-oxo-3-oxabicyclo[4.1.0]heptan-5-yl)carbamate (12b)—10b (300 mg, 0.88 mmol, 1 equiv.) was dissolved in DCM (5.85 mL) and TFA (67 μL , 0.88 mmol, 1 equiv.) was added. The solution was stirred for 10 min and the solvent was evaporated off. The compound was purified by silica gel chromatography (Eluent: 40-70 % EtOAc in heptane) to afford **12b** (207 mg, 95 %) as a white solid. $^1\text{H-NMR}$ (300 MHz; CD_3OD): δ 4.29-4.15 (m, 2H), 4.06 (s, 1H), 1.69-1.59 (m, 2H), 1.47 (s, 9H), 1.34 (s, 3H), 1.07 (dd, J = 7.7, 5.9 Hz, 1H). LC/MS(A): RT 3.04, ELSD: 0 %, UV 0 %, (M-Boc) H^+ 142.1.

tert-Butyl ((1S,5S,6R)-1-ethyl-2-oxo-3-oxabicyclo[4.1.0]heptan-5-yl)carbamate (12c)—12c (170 mg, 95 %) of the desired product as a white foam. $^1\text{H-NMR}$ (300 MHz; CD_3OD): δ 4.32 (dd, J = 12.6, 2.9 Hz, 1H), 4.18 (ddd, J = 12.6, 1.6, 1.6 Hz, 1H), 4.08 (bs, 1H), 1.85-1.69 (m, 2H), 1.60 (dd, J = 5.8, 5.8 Hz, 1H), 1.51-1.32 (m, 10H), 1.14 (dd, J = 8.1, 6.2 Hz, 1H), 0.98 (t, J = 7.0 Hz, 3H). $^{13}\text{C-NMR}$ (75 MHz; CD_3OD): δ 174.74 (s, 1C), 157.60 (s, 1C), 80.43 (s, 1C), 69.83 (s, 1C), 45.01 (s, 1C), 28.85 (s, 1C), 28.75 (s, 3C), 27.36 (s, 1C), 27.01 (s, 1C), 15.66 (s, 1C), 11.92 (s, 1C). LC/MS(A): RT 3.87, ELSD: 100 %, UV 0 %, (M-Boc) H^+ 156.0.

tert-Butyl ((1S,5S,6R)-2-oxo-1-propyl-3-oxabicyclo[4.1.0]heptan-5-yl)carbamate (12d)—12d (130 mg, 95 %) as white foam. $^1\text{H-NMR}$ (300 MHz; CD_3OD): δ 4.29 (dd, J = 12.6, 3.0 Hz, 1H), 4.15 (ddd, J = 12.6, 1.6, 1.6 Hz, 1H), 4.06 (ddd, J = 2.7, 2.7, 1.2 Hz, 1H), 1.82-1.71 (m, 2H), 1.58 (dd, J = 5.8, 5.8 Hz, 1H), 1.53-1.41 (m, 11H), 1.31 (ddd, J = 13.2, 9.5, 6.4 Hz, 1H), 1.12 (dd, J = 8.2, 6.1 Hz, 1H), 0.92 (t, J = 7.3 Hz, 3H). $^{13}\text{C-NMR}$ (75 MHz; CD_3OD): δ 174.78 (s, 1C), 157.61 (s, 1C), 80.44 (s, 1C), 69.82 (s, 1C), 45.04 (s, 1C), 38.14 (s, 1C), 28.76 (s, 3C), 27.54 (s, 1C), 25.98 (s, 1C), 21.60 (s, 1C), 16.07 (s, 1C), 14.55 (s, 1C). LC/MS(A): RT 4.03, ELSD: 100 %, UV 0 %, (M-Boc) H^+ 170.1.

tert-Butyl ((1S,5S,6R)-1-butyl-2-oxo-3-oxabicyclo[4.1.0]heptan-5-yl)carbamate (12e)—12e (90 mg, 94 %) as white foam. ¹H-NMR (300 MHz; CD₃OD): δ 4.30 (dd, *J* = 12.5, 3.0 Hz, 1H), 4.16 (ddd, *J* = 12.6, 1.9, 1.5 Hz, 1H), 4.06 (bs, 1H), 1.81 (m, *J* = 4.7 Hz, 2H), 1.58 (dd, *J* = 5.8, 5.8 Hz, 1H), 1.47-1.26 (m, 14H), 1.12 (dd, *J* = 8.1, 6.1 Hz, 1H), 0.92 (t, *J* = 7.2 Hz, 3H). ¹³C-NMR (75 MHz; CD₃OD): δ 174.79 (s, 1C), 157.69 (s, 1C), 80.44 (s, 1C), 69.81 (s, 1C), 45.05 (s, 1C), 35.69 (s, 1C), 30.65 (s, 1C), 28.79 (s, 3C), 27.54 (s, 1C), 26.05 (s, 1C), 23.83 (s, 1C), 16.19 (s, 1C), 14.50 (s, 1C). LC/MS(A): RT 4.04, ELSD: 98.4 %, UV 0 %, (M-Boc)H⁺ 184.1.

General procedure for hydrolysis

(1S,2R)-2-((S)-1-((tert-Butoxycarbonyl)amino)-2-hydroxyethyl)-1-methylcyclopropanecarboxylic acid (13b)—To a solution **12b** (207 mg, 0.86 mmol, 1 equiv.) in THF (1.6 mL) was added 1M aq NaOH (1.54 mL, 1.54 mmol, 1.8 equiv.) at room temperature and stirred at 40 °C for 30 min. EtOAc (50 mL) was added then 1M KHSO₄ (10 mL). The phases were separated and the water phase extracted with additional EtOAc (2 × 50 mL). The combined organic phases were dried over Na₂SO₄, filtered and evaporated to dryness. The crude product was purified by silica gel chromatography (Eluent: 1% AcOH in EtOAc) to afford **13b** (167 mg, 75 %) as a white solid. ¹H-NMR (300 MHz; CD₃OD): δ 3.70 (ddd, *J* = 10.2, 5.8, 4.2 Hz, 1H), 3.51 (dd, *J* = 11.1, 4.2 Hz, 1H), 3.44 (dd, *J* = 11.1, 5.8 Hz, 1H), 1.44-1.37 (m, 10H), 1.27 (s, 3H), 1.24-1.15 (m, 1H), 0.86 (dd, *J* = 8.5, 4.1 Hz, 1H). ¹³C-NMR (75 MHz; CD₃OD): δ 177.68 (s, 1C), 158.06 (s, 1C), 79.95 (s, 1C), 65.33 (s, 1C), 53.25 (s, 1C), 32.10 (s, 1C), 28.88 (s, 3C), 25.04 (s, 1C), 21.69 (s, 1C), 20.87 (s, 1C). LC/MS(A): RT 2.64, ELSD: 100 %, UV 0 %, (M-Boc)H⁺ 160.2.

(1S,2R)-2-((S)-1-((tert-Butoxycarbonyl)amino)-2-hydroxyethyl)-1-ethylcyclopropanecarboxylic acid (13c)—**13c** (145 mg, 79 %) as white foam. ¹H-NMR (300 MHz; CD₃OD): δ 3.67 (ddd, *J* = 10.1, 6.0, 4.0 Hz, 1H), 3.56 (dd, *J* = 11.0, 3.8 Hz, 1H), 3.48 (dd, *J* = 11.1, 6.1 Hz, 1H), 1.86 (dq, *J* = 14.0, 7.1 Hz, 1H), 1.47 (s, 9H), 1.38 (dd, *J* = 5.9, 4.2 Hz, 1H), 1.32-1.19 (m, 2H), 1.02 (t, *J* = 7.2 Hz, 3H), 0.86 (dd, *J* = 8.5, 4.1 Hz, 1H). ¹³C-NMR (75 MHz; CD₃OD): δ 176.91 (s, 1C), 157.90 (s, 1C), 79.81 (s, 1C), 65.16 (s, 1C), 53.24 (s, 1C), 31.56 (s, 1C), 30.79 (s, 1C), 29.92 (s, 1C), 28.80 (s, 3C), 19.22 (s, 1C), 12.08 (s, 1C). LC/MS(A): RT 2.91, ELSD: 100 %, UV 0 %, (M-Boc)H⁺ 174.1

(1S,2R)-2-((S)-1-((tert-Butoxycarbonyl)amino)-2-hydroxyethyl)-1-propylcyclopropanecarboxylic acid (13d)—**13d** (92 mg, 67 %). ¹H-NMR (300 MHz; CD₃OD): δ 3.61 (ddd, *J* = 10.0, 6.1, 3.8 Hz, 1H), 3.53 (dd, *J* = 11.0, 3.8 Hz, 1H), 3.44 (dd, *J* = 11.0, 6.1 Hz, 1H), 1.82 (ddd, *J* = 13.5, 9.5, 6.1 Hz, 1H), 1.53-1.35 (m, 12H), 1.28-1.10 (m, 2H), 0.91-0.83 (m, 4H). ¹³C-NMR (75 MHz; CD₃OD): δ 176.82 (s, 1C), 157.88 (s, 1C), 79.80 (s, 1C), 65.14 (s, 1C), 53.34 (s, 1C), 39.24 (s, 1C), 30.73 (s, 1C), 30.48 (s, 1C), 28.81 (s, 3C), 21.77 (s, 1C), 19.36 (s, 1C), 14.62 (s, 1C). LC/MS(A): RT 3.22, ELSD: 100 %, UV 0 %, (M-Boc)H⁺ 188.1.

(1S,2R)-2-((S)-1-((tert-Butoxycarbonyl)amino)-2-hydroxyethyl)-1-butylcyclopropanecarboxylic acid (13e)—**13e** (52 mg, 62 %) as white solid. ¹H-NMR (400 MHz; MeOD): δ 3.64 (ddd, *J* = 10.1, 6.1, 3.9 Hz, 1H), 3.57 (dd, *J* = 11.1, 3.9 Hz, 1H), 3.48 (dd, *J* = 11.1, 6.1 Hz, 1H), 1.87 (ddd, *J* = 13.8, 9.2, 5.1 Hz, 1H), 1.50-1.17 (m, 16H), 0.92 (t, *J* = 7.3 Hz, 3H), 0.87 (dd, *J* = 8.6, 4.3 Hz, 1H). ¹³C-NMR (101 MHz; CD₃OD): δ 177.30 (s, 1C), 158.15 (s, 1C), 79.96 (s, 1C), 65.30 (s, 1C), 53.58 (s, 1C), 47.70 (s, 1C), 36.93 (s, 1C), 30.84 (s, 1C), 28.84 (s, 3C), 23.92 (s, 1C), 19.22 (s, 1C), 14.42 (s, 1C), 9.23 (s, 1C). LC/MS(A): RT 4.04, ELSD: 100 %, UV 0 %, (M-Boc)H⁺ 202.0.

General procedure for oxidation (Method A)

(1*S*,2*R*)-2-((*S*)-amino(carboxy)methyl)-1-methylcyclopropanecarboxylic acid hydrochloride (14b and 4b-HCl)—13b (167 mg, 0.64 mmol, 1 equiv.) was dissolved in 1M aq NaOH (3.2 mL) and KMnO₄ (458 mg, 2.90 mmol, 4.5 equiv.) was added. The mixture was stirred for 4 h at room temperature. 2-propanol (4 mL) was added and the solution stirred for 40 min. Mn₂O was filtered off and the filter washed with 2-propanol. The solvent was evaporated off and EtOAc (30 mL) and 1M KHSO₄ (10 mL) were added. The water phase was extracted with EtOAc (3 × 30 mL). The combined organic phases were dried twice over Na₂SO₄, filtered and evaporated to dryness to afford **14b** (123 mg, 71 %) as a white solid. LC/MS(A): RT 2.67, ELSD: 100 %, UV 0 %, (M-Boc)H⁺ 174.0. **14b** (123 mg, 0.45 mmol, 1 equiv.) was dissolved in a minimum amount of dioxane (~ 1 mL). 4M HCl in dioxane (1 mL) was added dropwise. After 1 h the precipitate was centrifuged and the solvent was decanted off. The precipitate that was washed with Et₂O (4 mL), the mixture centrifuged and the solvent decanted off. The white solid was dried to afford **4b-HCl** (81 mg, 86 % (71 % when corrected from 1,4-dioxane)) as white solid. ¹H-NMR (300 MHz; CD₃OD): δ 4.22 (d, *J* = 10.2 Hz, 1H), 1.50-1.42 (m, 2H), 1.37 (s, 3H), 1.19-1.12 (m, 1H). ¹³C-NMR (75 MHz; CD₃OD): δ 176.71 (s, 1C), 171.24 (s, 1C), 52.54 (s, 1C), 29.54 (s, 1C), 25.61 (s, 1C), 21.35 (s, 1C), 20.92 (s, 1C). LC/MS(A): RT 0.57, ELSD: 0 %, UV 0 %, MH⁺ 174.0.

General procedure for oxidation (Method B)

(1*S*,2*R*)-2-((*S*)-amino(carboxy)methyl)-1-ethylcyclopropanecarboxylic acid hydrochloride (14c and 4c-HCl)—13c (145 mg, 0.53 mmol, 1 equiv.) was dissolved in 1M NaOH (2.7 mL) and KMnO₄ (376 mg, 2.39 mmol, 4.5 equiv.) was added. The mixture was stirred for 4 h at room temperature. 2-propanol (4 mL) was added and the solution stirred for 40 min. Mn₂O was filtered off and the filter washed with 2-propanol. The solvent was evaporated off and EtOAc (30 mL) and 1M KHSO₄ (10 mL) were added. The water phase was extracted with EtOAc (3 × 30 mL). The combined organic phases were dried over Na₂SO₄, filtered and evaporated to dryness. The compound was purified by silica gel chromatography (Eluent: 1% CH₃COOH in EtOAc) to afford **14c** (125 mg, 0.44 mmol, 83 %). ¹H-NMR (400 MHz; MeOD): δ 4.33 (d, *J* = 9.9 Hz, 1H), 1.72 (dq, *J* = 14.3, 7.1 Hz, 1H), 1.46-1.33 (m, 12H), 1.03 (t, *J* = 7.3 Hz, 3H), 0.93 (dd, *J* = 7.9, 3.8 Hz, 1H). LC/MS(A): RT 2.90, ELSD: 100 %, UV 0 %, (M-Boc)H⁺ 188.1. **14c** (125 mg, 0.44 mmol) was dissolved in a minimum amount of Et₂O (0.3 mL). 2M HCl in Et₂O (2 mL) was added dropwise. The solution was stirred for 1 h, the mixture centrifuged and the solvent removed by decantation. The precipitate was washed with Et₂O (4 mL), centrifuged and the solvent removed by decantation. The white solid was dried on high vacuum to afford **4c-HCl** (91 mg, 94 %). ¹H-NMR (300 MHz; CD₃OD): δ 4.21 (d, *J* = 10.1 Hz, 1H), 1.70 (dq, *J* = 14.2, 7.2 Hz, 1H), 1.59 (dq, *J* = 14.2, 7.2 Hz, 1H), 1.49-1.40 (m, 2H), 1.15 (dd, *J* = 6.2, 2.4 Hz, 1H), 1.08 (t, *J* = 7.3 Hz, 3H). ¹³C-NMR (101 MHz; CD₃OD): δ 176.15 (s, 1C), 171.30 (s, 1C), 52.78 (s, 1C), 31.49 (s, 1C), 29.20 (s, 1C), 28.68 (s, 1C), 20.42 (s, 1C), 11.87 (s, 1C). LC/MS(A): RT 0.89, ELSD: 100 %, UV 0 %, MH⁺ 188.1

(1*S*,2*R*)-2-((*S*)-amino(carboxy)methyl)-1-propylcyclopropanecarboxylic acid hydrochloride (14d and 4d-HCl)—14d (92 mg, 0.31 mmol, 95 %) as a white foam/solid. ¹H-NMR (300 MHz; CD₃OD): δ 4.28 (d, *J* = 10.0 Hz, 1H), 1.73-1.22 (m, 15H), 0.92 (m, 4H). ¹³C-NMR (75 MHz; CD₃OD): δ 176.64 (s, 1C), 175.30 (s, 1C), 157.70 (s, 1C), 80.29 (s, 1C), 53.51 (s, 1C), 39.02 (s, 1C), 31.41 (s, 1C), 30.48 (s, 1C), 28.77 (s, 3C), 21.66 (s, 1C), 20.23 (s, 1C), 14.67 (s, 1C). LC/MS(A): RT 3.20, ELSD: 100 %, UV 0 %, (M-Boc)H⁺ 202.1.

4d·HCl (52 mg, 71 %). ¹H-NMR (400 MHz; CD₃OD): δ 4.18 (d, *J* = 9.8 Hz, 1H), 1.67-1.40 (m, 6H), 1.16-1.11 (m, 1H), 0.92 (t, *J* = 6.9 Hz, 3H). ¹³C-NMR (101 MHz; CD₃OD): δ 176.19 (s, 1C), 171.26 (s, 1C), 52.77 (s, 1C), 38.50 (s, 1C), 30.49 (s, 1C), 28.73 (s, 1C), 21.59 (s, 1C), 20.53 (s, 1C), 14.53 (s, 1C). LC/MS(A): RT 1.51, ELSD: 100 %, UV 0 %, MH⁺ 202.1.

(1*S*,2*R*)-2-((*S*)-amino(carboxy)methyl)-1-butylcyclopropanecarboxylic acid hydrochloride (14e and 4e·HCl)—14e (40 mg, 0.13 mmol, 75 %). ¹H-NMR (400 MHz; MeOD): δ 4.28 (d, *J* = 9.8 Hz, 1H), 1.69 (ddd, *J* = 12.6, 10.6, 4.3 Hz, 1H), 1.52-1.25 (m, 16H), 0.91 (bs, 1H), 0.89 (t, *J* = 7.3 Hz, 3H). ¹³C-NMR (101 MHz; CD₃OD): δ 176.90 (s, 1C), 175.53 (s, 1C), 157.9 (s, 1C), 80.36 (s, 1C), 53.59 (s, 1C), 36.52 (s, 1C), 31.43 (s, 1C), 30.66 (s, 1C), 30.55 (s, 1C), 28.73 (s, 3C), 23.93 (s, 1C), 20.17 (s, 1C), 14.37 (s, 1C).

4e·HCl (26 mg, 82 %). ¹H-NMR (400 MHz; CD₃OD): δ 4.16 (d, *J* = 9.8 Hz, 1H), 1.65-1.40 (m, 6H), 1.32 (m, 2H), 1.11 (m, 1H), 0.91 (t, *J* = 7.3 Hz, 3H). ¹³C-NMR (101 MHz; CD₃OD): δ 176.22 (s, 1C), 171.32 (s, 1C), 52.77 (s, 1C), 36.06 (s, 1C), 30.66 (s, 1C), 30.57 (s, 1C), 28.80 (s, 1C), 23.90 (s, 1C), 20.58 (s, 1C), 14.34 (s, 1C). LC/MS(A): RT 1.73, ELSD: 100 %, UV 0 %, (M-Boc)H⁺ 216.1.

Molecular Modeling and Ligand—Protein Docking: The Schrödinger 2011 suite was used for modeling and docking studies (Schrödinger, Portland, OR). The crystal structure of the soluble GluN2A-ABD construct in complex with glutamate (PDB code 2A5T)³⁶ was employed in the protein preparation wizard in Maestro (Schrödinger, Portland, OR) with standard parameters for assigning charges and protons and performing a minimization. None of the missing amino acid side chains were modeled. This model was then used to generate Van der Waals and electrostatic grids with the docking module Glide using default parameters (Schrödinger, Portland, OR). These grids were then used for ligand docking.

The CCG analogues **4a-e** and (*R*)-Pr-NHP5G (**3b**) were submitted to minimization in macromodel (Schrödinger, Portland, OR) in tri-ionized forms using default parameters. The best scoring poses according to G-score were used. Figures were created using Pymol (ver. 0.99, Delano Scientific).

In Vitro Pharmacology

Receptor Binding Assays—Affinities for native AMPA, KA and NMDA receptors in rat cortical synaptosomes were determined using 5 nM [³H]AMPA³⁷ (*K*_d = 35 nM, 53.4 Ci/mmol), 5 nM [³H]KA (*K*_d = 5.6 nM, 49.9 Ci/mmol)³⁸ and 2 nM [³H]CGP 39653 (*K*_d = 6 nM, 40.5 Ci/mmol)³⁹ respectively, with minor modifications as previously described.⁴⁰ Rat brain membrane preparations used in these receptor binding experiments were prepared according to a method previously described.⁴¹

Two-Electrode Voltage-Clamp Electrophysiology

Wild-type cDNAs for rat GluN1 (GluN1-1a; GenBank accession numbers U11418), rat GluN2A (D13211), rat GluN2B (U11419), rat GluN2C (M91563), and rat GluN2D (L31611) were provided by Drs. S. Heinemann (Salk Institute, San Diego, CA), S. Nakanishi (Kyoto University, Kyoto, Japan), and P. Seeburg (University of Heidelberg, Heidelberg, Germany). For expression in *Xenopus* oocytes, DNA constructs were linearized by restriction enzymes and used as templates to synthesize cRNAs using the mMessage mMachine kit (Ambion). *Xenopus* oocytes for injection were prepared as previously described.⁴² Two-electrode voltage-clamp recordings were performed essentially as previously described.⁴³ During recordings, the oocytes were voltage-clamped at −60 mV to −40 mV and continuously perfused with extracellular solution containing (in mM) 90 NaCl,

0.5, BaCl₂, 1 KCl, 0.01 EDTA, and 10 HEPES (pH 7.4). 50 μM glycine was included in the extracellular solution at all times.

Agonist concentration–response data for individual oocytes were fitted to the Hill equation. The logEC₅₀ and n_H from the individual oocytes were used to calculate the mean ± SEM. For graphical presentation, datasets from individual oocytes were normalized to the fitted maximal response and the mean ± SEM was calculated for each of the normalized data points. These averaged data points were then fitted to the Hill equation and plotted together with the resulting curve. Relative I_{max} was calculated from a full concentration–response measurement as $I_{\text{max, agonist}}/I_{\text{max, glutamate}}$, where I_{max, agonist} is the fitted value according to the Hill equation and I_{max, glutamate} is the maximal response evoked by glutamate in the same recording.

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Nonstandard abbreviations

NHP5G	<i>N</i> -hydroxypyrazol-5-ylglycine
NMDA	<i>N</i> -methyl-D-aspartic acid
CCG	carboxycyclopropylglycine
GluRs	glutamate receptors
Glu, CNS	central nervous system
mGluRs	metabotropic glutamate receptors
iGluRs	ionotropic glutamate receptors
AMPA	(<i>S</i>)-2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid
KA	kainic acid
ABD	agonist binding domain

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Figure 1.

Structures of glutamic acid (Glu), (2*S*,4*R*)-4-methylglutamate (**1b**) and *N*-methyl-D-aspartic acid (NMDA, **2**), and structures of the NMDA receptor selective ligands **3a** and **4a** and a series of alkyl substituted analogues.

Figure 2.

A) X-ray crystal structure (PDB:2A5T) of the ABD of GluN2A (blue) containing Glu (**1**, blue), and **4a** (pink) docked in a model of GluN2A ABD created from the crystal structure using the protein preparation module and Glide in the Schrodinger Suite. B) **3b** (light blue) and **4d** (green) docked in GluN2A ABD demonstrating the overlap of the alkyl groups in the 2' position of **4a**. C) Broader view of GluN2A ABD showing the orientation of **4d** in the clamshell-like structure formed by the upper (S1) and lower (S2) lobes of the ABD.

Figure 3.

Mean concentration–response curves for compounds Me-, Et-, *n*Pr- and *n*Bu-CCG (**4b-e**) determined using two-electrode voltage-clamp recordings on *Xenopus* oocytes co-expressing GluN1 and GluN2A-D. The curves are normalized to the maximal current response ($I_{\max}$) to the agonist obtained by fitting the full concentration–response data to the Hill equation (See Experimental). Data points are represented as mean $\pm$ SEM from 3-6 oocytes. All EC_{50} -values are listed in Table 2.

Figure 4.

A) Comparison of maximal currents induced by **4a-e** relative to Glu at NR1/NR2A-D subtypes. The relative efficacies are the maximal current responses to the indicated agonists obtained by fitting the full concentration-response data normalized to the maximal response activated by glutamate in the same recording (See Experimental). B) Comparison of agonist potencies (pLogEC₅₀) for **4a-e**. Values are listed in Table 2. Data for (S)-CCG-IV (**4a**) are from Erreger et al., 2007.¹⁶

Scheme 1.

Reagents and conditions: a) KHMDS, **5a-d**, 18-crown-6, THF, -78°C , 3 h. (for **6a**: 72 %; for **6b**: 89 %; for **6c**: 16 %; for **6d**: 10 %); b) TFA, DCM, rt, 24 h for **7a**; c) *p*-TsOH, MeOH, rt, 24 h for **7b**.

Scheme 2.

Reagents and conditions: a) I_2 , Pyridine, CCl_4 , $40^\circ C$, 30 min; b) Et_2Zn , $Pd(PPh_3)_2Cl_2$, DMF, rt, 1.5 h. (**7c**: 60 %); c) $RZnX$, $Pd(PPh_3)_2Cl_2$, DMF, rt, 1.5 h. (X = Br or I, for **7d**: 46 %; for **7e**: 35 %; for **7g**: 20 %); d) $ZnBr_2$, $Pd[(\alpha\text{-tolyl})_3P]_2Cl_2$, Bu_3SnPh or tributyl(vinyl)tin, DMF, $65^\circ C$, 1.5 h. (for **7f**: 89 %, for **7h**: 56 %); e) DMAP, Boc_2O , ACN, rt, 2 h; f) $Pd(OAc)_2$, CH_2N_2 , Et_2O , rt, 30 min.

Scheme 3.

Reagents and conditions: a) DMAP, Boc_2O , ACN, rt, 2 h; b) $\text{Pd}(\text{OAc})_2$, CH_2N_2 , Et_2O , rt, 30 min. (for **10b**: 46 %; for **10c**: 31 %; for **10d**: 15 %); c) $\text{Pd}_2(\text{dba})_3$, CH_2N_2 , Et_2O , rt, 30 min. (for **10d**: 37 %; for **10e**: 29 %; for **10f**: no reaction).

Scheme 4.

Reagents and conditions: a) $\text{Pd}_2(\text{dba})_3$, CH_2N_2 , Et_2O , rt, 30 min.

Scheme 5.

Reagents and conditions: a) TFA, DCM, rt, 10 min. (for **12b**: 98 %; for **12c**: 95 %; for **12d**: 95 %; for **12e**: 94 %); b) 1M aq NaOH, THF, 40°C, 30 min. (for **13b**: 75 %; for **13c**: 79 %; for **13d**: 67 %; for **13e**: 62 %); c) 1M aq NaOH, KMnO₄, rt, 4 h. (for **14b**: 71 %; for **14c**: 83 %; for **14d**: 95 %; for **14e**: 75 %); d) 2M HCl in Et₂O. (for **4b**: 71 %; for **4c**: 94 %; for **4d**: 71 %; for **4e**: 82 %).

Table 1

Receptor binding affinities of compounds **4a-e** at three major groups of iGluRs in rat cortical synaptosome assay. The numbers in brackets [min, max] indicate mean $\pm$ SEM according to a logarithmic distribution.⁴⁴ CGP39653 is a competitive NMDA receptor antagonist and AMPA and KA are agonists at AMPA and KA receptors, respectively. K_i values are calculated using the Cheng-Prusoff relationship. Data for **3a** and **3b** is from Clausen et al¹⁴ ND means not determined and NA means no activity.

Compd	R	K_i (μ M)		
		[³ H]AMPA	[³ H]KA	[³ H]CGP39653
3a	H	>100	>100	10
3b	n-Pr	>100	>100	14
4a	H	1.1 [1.0;1.3]	0.31 [0.27;0.36]	0.0065 [0.0057;0.0074]
4b	Me	29 [26;32]	0.11 [0.08;0.14]	0.0080 [0.0066;0.0098]
4c	Et	> 100	0.61 [0.58;0.63]	0.061 [0.053;0.070]
4d	n-Pr	> 100	21 [19;22]	1.2 [1.1;1.4]
4e	n-Bu	> 100	> 100	1.3 [1.1;1.5]

Table 2

Potency and efficacy relative to Glu of compounds **4a-e** at recombinant GluN1/GluN2A-D receptors expressed in *Xenopus* oocytes. Data are from 3-12 oocytes and the numbers in brackets [min, max] indicate mean $\pm$ SEM according to a logarithmic distribution.⁴⁴ The relative maximal currents (relative $I_{\max}$) are the maximal responses to the indicated agonists obtained by fitting the full concentration-response data normalized to the maximal response activated by glutamate in the same recording (See Experimental). Data for **3a** and **3b** is from Clausen et al¹⁴ and for **4a** from Erreger et al.¹⁶ ND means not determined and NA means no activity.

Compound	R	Subtype	EC ₅₀ (μ M)	Rel. $I_{\max}$	n _{Hill}
3a	H	GluN1/GluN2A	82	0.45	1.3
		GluN1/GluN2B	48	0.58	1.6
		GluN1/GluN2C	54	0.52	1.4
		GluN1/GluN2D	ND	ND	ND
3b	n-Pr	GluN1/GluN2A	NA	NA	NA
		GluN1/GluN2B	105	0.06	1.9
		GluN1/GluN2C	429	0.22	1.1
		GluN1/GluN2D	153	0.37	1.6
4a	H	GluN1/GluN2A	0.262	0.99	1.1
		GluN1/GluN2B	0.083	1.23	1.1
		GluN1/GluN2C	0.110	0.90	1.1
		GluN1/GluN2D	0.036	1.11	1.3
4b	Me	GluN1/GluN2A	0.277[0.261;0.292]	0.94 $\pm$ 0.01	1.2
		GluN1/GluN2B	0.045[0.043;0.047]	1.14 $\pm$ 0.02	1.7
		GluN1/GluN2C	0.124[0.120;0.129]	0.81 $\pm$ 0.04	1.3
		GluN1/GluN2D	0.026[0.025;0.026]	0.83 $\pm$ 0.02	1.9
4c	Et	GluN1/GluN2A	1.18[0.673;1.40]	0.83 $\pm$ 0.03	1.3
		GluN1/GluN2B	0.272[0.257;0.286]	1.02 $\pm$ 0.04	1.5
		GluN1/GluN2C	0.540[0.519;0.559]	0.81 $\pm$ 0.02	1.5
		GluN1/GluN2D	0.080[0.077;0.083]	0.91 $\pm$ 0.01	1.6
4d	n-Pr	GluN1/GluN2A	46.3[45.2;47.4]	0.67 $\pm$ 0.01	1.1
		GluN1/GluN2B	15.8[15.2;16.3]	1.05 $\pm$ 0.02	1.3
		GluN1/GluN2C	29.9[29.3;30.5]	0.70 $\pm$ 0.02	1.4
		GluN1/GluN2D	6.56 [6.15;6.90]	0.89 $\pm$ 0.01	1.6
4e	n-Bu	GluN1/GluN2A	28.8[28.4;29.2]	0.65 $\pm$ 0.02	1.4
		GluN1/GluN2B	10.2[9.82;10.5]	1.07 $\pm$ 0.01	1.6
		GluN1/GluN2C	16.0[15.6;16.5]	0.85 $\pm$ 0.02	1.5
		GluN1/GluN2D	3.30 [3.21;3.39]	0.94 $\pm$ 0.01	1.7