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Design and Synthesis of 2,3-*trans*-Proline Analogues as Ligands for Ionotropic Glutamate Receptors and Excitatory Amino Acid Transporters

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Abstract

Development of pharmacological tools for the ionotropic glutamate receptors (iGluRs) is imperative for the study and understanding of the role and function of these receptors in the central nervous system. We report the synthesis of 18 analogues of (2*S*,3*R*)-2-carboxy-3-pyrrolidine acetic acid (**3a**), which explores the effect of introducing a substituent on the ε -carbon (**3c–q**). A new synthetic method was developed for the efficient synthesis of racemic **3a** and applied to give expedited access to 13 racemic analogues of **3a**. Pharmacological characterization was carried out at native iGluRs, cloned homomeric kainate receptors (GluK1–3), NMDA receptors (GluN1/GluN2A–D), and excitatory amino acid transporters (EAAT1–3). From the structure–activity relationship studies, several new ligands emerged, exemplified by triazole **3p–d1**, GluK3-preferring (GluK1/GluK3 K_i ratio of 15), and the structurally closely related tetrazole **3q–s3–4** that displayed 4.4–100-fold preference as an antagonist for the GluN1/GluN2A receptor ($K_i = 0.61 \mu\text{M}$) over GluN1/GluN2B–D ($K_i = 2.7–62 \mu\text{M}$).

Graphical Abstract

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Author Contributions

C.B.M.P., medicinal chemistry and manuscript preparation; A.A., medicinal chemistry and manuscript preparation; M.K.-J., medicinal chemistry; Y.L., pharmacology; E.A., medicinal chemistry; W.E.B.-Y., pharmacology; F.Y., pharmacology; J.T.S., pharmacology; M.S., medicinal chemistry; B.N., pharmacology; K.A.F., X-ray structure; A.A.J., pharmacology; K.B.H., pharmacology; D.S.P., pharmacology and manuscript preparation; L.B., medicinal chemistry and manuscript preparation.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acscchemneuro.9b00205. Experimental details for crystal structure determination of compound **9c–s1** and compound **9d–s2** (PDF)

The authors declare no competing financial interest.

Keywords

Glutamate receptors; NMDA receptors; kainate; proline analogues; CNS

INTRODUCTION

Most of the fast excitatory neurotransmission in the central nervous system (CNS) is mediated by the endogenous α -amino acid (*S*)-glutamate (Glu) through the ionotropic glutamate receptors (iGluRs).¹ Based on pharmacological studies of synthetic ligands and natural products, these receptors were early on divided into three groups, named the α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), the kainic acid (KA) and the *N*-methyl-D-aspartate (NMDA) receptors.² For the AMPA receptors, four subunits have been identified (GluA1–4),³ while five KA receptor subunits have been cloned (GluK1–5).⁴ For the NMDA receptors, four Glu binding subunits have been identified (GluN2A–D) as well as three glycine (Gly) binding subunits (GluN1 and GluN3A–B).⁵ The subunits GluA1–4 can all be freely combined in vitro, to form homomeric as well as heteromeric receptors complexes. However, it remains unknown to which extent the conceivable combinations are expressed in vivo.⁶ The KA receptors may form functional receptors in vitro, which are homomers of one of the three GluK1–3 subunits, but also combinations thereof. The GluK4 and GluK5 subunits only form functional receptors in vitro upon the combination with one of the three subunits GluK1–3.⁷ Likewise, the NMDA receptors only form Glu-sensitive functional receptors in vitro from two Gly-binding subunits (GluN1) and two Glu-binding subunits (GluN2A–D).^{8,9} The extracellular domains of the functional receptors are arranged as a dimer of dimers, whereas the transmembrane domain is arranged as a symmetrical tetramer, which forms the ion channel.¹⁰

The natural product KA, and other GluK agonists, are known to be potent neuronal excitants and neurotoxins as well as potent convulsants.¹¹ KA receptor knockout animal models have shown that these animals have a higher threshold in the perception of pain, indicating a potential role for antagonists in the treatment of pain.¹² Furthermore, GluK antagonists might have a beneficial effect on neurodegenerative diseases such as multiple sclerosis,¹³ Alzheimer's disease,¹⁴ and Huntington's disease,¹⁵ as well as in several psychiatric disorders including schizophrenia,¹⁶ major depression,¹⁷ autism,¹⁸ bipolar disorder,¹⁹ and obsessive-compulsive disorder.²⁰

In the search for subtype selective ligands for iGluRs, a substantial number of Glu analogues have been prepared and characterized pharmacologically, including >200 different 4-substituted Glu analogues by us^{37–42} and others^{35,43–47} (see Table 1). Also a number of analogues of the natural product KA have been prepared, including the structurally simplified KA analogue **3a** (CPAA) as well as the conformationally restricted analogues **1b**²⁷ and **1c**²³ (Table 1). While several competitive agonists and antagonists are able to

differentiate between the three iGluR groups,^{25,48} the success rate when it comes to the development of subtype-selective iGluR ligands targeting the orthosteric binding site has been low. In fact, only subtype-selective agonists and antagonists for the homomeric GluK1 receptor as well as the selective GluK3 agonist **1c** (Table 1) have been disclosed to this date.

Glutamate is a flexible molecule which can rotate around its C(2)–C(3) and C(3)–C(4) bonds. Nine distinct staggered conformations can be adapted through this rotation.⁴⁹ The energy differences of these conformations are all in the same order of magnitude.⁵⁰ From the extensive number of X-ray crystallographic studies of agonists bound in the agonist binding domains of GluA2 and GluK1, it has been shown that a “folded” conformation of the Glu backbone favors iGluR agonist activity over metabotropic Glu receptor (mGluR) agonist activity. Whereas an “extended” conformation is favored by the mGluRs.^{51,52}

3a (CPAA) is a conformational restricted analogue of Glu, locked in the C(1)–C(3) anti conformation, but it allows for different C(2)–C(5) conformations, by rotation around the C(3)–C(4) bond. This implies that **3a** can still adopt both the “folded” as well as the “extended” conformations. Conformational flexibility is reduced in the Glu backbone when substituted on the 4-position, favoring a “folded” conformation for compounds with a *syn* relative stereochemical relationship.⁵³ In this study, we fused structures of series **2a–d** with proline analogue **3a** and present the synthesis and pharmacological evaluation of these new conformationally restricted Glu analogues.

RESULTS AND DISCUSSIONS

Chemistry.

Compound **3a** and the ethyl and phenyl analogues **3c** and **3d**, respectively, were prepared following our previous work for the synthesis of methyl analogue **3b**.²⁶ The key step is a stereoselective 1,4-addition of the appropriate cuprate to enone **4** (Scheme 1).⁵⁴ For **3a**, the cuprate addition with vinyl bromide gave intermediate **6a** in 65% yield as a single *trans*-diastereomer, determined by ¹H NMR and in agreement with literature values for optical rotation.⁵⁵ Simultaneous hydroboration of the olefin and reduction of the lactam functionality with borane gave alcohol **7a** in 52%. Cleavage of the TBS-ether was accomplished with TBAF to yield diol **8a**, which was oxidized to diacid **9a**, by in situ generated ruthenium(VIII) oxide.⁵⁶ Finally, removal of the Boc-group by TFA in DCM gave crude **3a**, which was purified by preparative HPLC to yield **3a** as the HCl salt.

The ethyl **3c** and phenyl **3d** analogues (Scheme 1) were prepared, starting from enone **4** and butenyl-2-bromide (**5c**) or α -bromostyrene (**5d**), respectively. After the one-pot reduction/hydroboration reaction both intermediate alcohols **7c** and **7d** were separated into their respective diastereomers **7c-s1**, **7c-s2**, and **7d-s2**. The stereoisomer **7d-s1** could not be purified to more than >90% by dry column vacuum chromatography (DCVC), but it led directly to the free diol **8d-s1** when purified by preparative HPLC.

After cleavage of the silyl ether, oxidation, and Boc-group removal, the optically pure compounds **3c-s1**, **3c-s2**, **3d-s2**, and **3d-s1** were obtained as their corresponding HCl salts. The absolute stereochemistry could be determined for (2*S*,3*R*,*eS*)-**9c-s1** and (2*S*,3*R*,*eS*)-**9d-**

s2 by single-crystal X-ray diffraction, as they gave monocrystalline solids from EtOAc/heptane (Figure 1).

Due to the limited availability of commercial vinyl bromides and the lengthy synthesis, we set out to find a more general and convenient procedure. Several other methods for the preparation of **3a** have been reported, however none of these would allow for readily modification of the substituent on the α -carbon.^{57–63} We envisioned enone **10** as a suitable key intermediate for conjugate additions of different substituted malonates. Clearly, this strategy comes with the loss of absolute stereochemical control, which we accepted as the cost for a more feasible access to new analogues for a more comprehensive study of the structure–activity relationship (SAR).

Enone **10** was prepared according to literature procedure, starting from proline.⁶⁴ The feasibility of this route was demonstrated by the synthesis of ($\pm$)-**3a** in 87% overall yield from 2.0 g/7.65 mmol of enone **10**. After a short optimization, the most optimal conditions for the conjugated addition were found to be 1 equiv of NaH with 2.5 equiv of the corresponding malonate **11a**, **11e–11m**, stirred for 3–24h in THF at 50 °C, which yielded the desired Michael products **12a**, **12e–12m**, in 35–99% isolated yield (Scheme 2). The subsequent global deprotection and decarboxylation was achieved by reflux in 6 M HCl for 4–9 days. The crude of all final products **3a**, **3e–3m** were purified by preparative HPLC, and the d.r. determined by ¹H NMR (Table 2). Exempt to this: For analogue **3i** the diastereomeric mixture could be separated into its corresponding two diastereomers **3i-d1** and **3i-d2**.

For 2-hydroxyethyl analogue **3n** (Scheme 3), conjugated addition to enone **10** with cyclic malonate **11n** gave **12n** in 71% yield. Aqueous acidic global hydrolysis yielded the corresponding γ -lactone **13**, and a second step with basic hydrolysis was required to obtain the disodium salt of **3n**.

The homologue of **3n**, analogue **3o** (Scheme 4), was prepared via the standard protocol for the conjugated addition of **11o** to enone **10**, followed by hydroboration of the olefin with borane to give **12o**. Hydrolysis and subsequent decarboxylation was done in aq. 6 M HCl to give **3o** in 28% after purification by preparative HPLC.

The introduction of simple five-membered heterocycles was also pursued and analogues **3p** (Scheme 5) and **3q** (Scheme 6, Table 2) were designed. For the synthesis of triazole **3p**, conjugated addition of **11p** to enone **10** yielded **15** in 89%. The propargyl functionality in **15** was then converted to the corresponding triazole **12p** by reaction with azidotrimethylsilane.⁶⁵ Global deprotection of **12p** was readily achieved and gave **3p** in 78%.

The synthesis of the tetrazole **3q** (Scheme 6, Table 2) commenced with an alkylation of ($\pm$)-*trans*-**12a** with sodium hydride and bromoacetonitrile to yield nitrile ($\pm$)-*trans*-**16** (Scheme 6). Subsequent reaction with sodium azide and triethylammonium chloride converted the nitrile functionality into tetrazole ($\pm$)-*trans*-**17**.⁶⁶ At this stage, the enantiomers were separated by chiral preparative HPLC to afford the two optically pure 2,3-*trans* enantiomers **17-s1** and **17-s2**. Hydrogenation over a Pd/C catalyst afforded the free aminetetrazoles **18-s1**

and **18-s2**, respectively, in quantitative yields. Each enantiomer was subjected to a global deprotection and decarboxylation under aqueous acidic conditions which afforded a mixture of diastereomers at the ϵ -position. At this stage, the two diastereomers **3q-s1** and **3q-s2** could be separated on chiral preparative HPLC to afford the two optically active enantiomers. Unfortunately, the diastereomeric mixture **3q-s3-4** could not be separated using the HPLC columns available to us.

Pharmacological Characterization.

Binding affinities of **3a,c-q** were determined at native iGluRs (rat synaptosomes) (Table 2; Figure 2), and recombinant rat homomeric GluK1–3 receptors expressed in sf9 cells (Table 3) and at recombinant EAAT1–3 expressed in HEK293 cells in a conventional [^3H]-D-Asp uptake assay (Table 4). Furthermore, functional studies on recombinant NMDA receptors expressed in *Xenopus laevis* oocytes were carried out using two-electrode voltage-clamp electrophysiology (Table 5).

Extension of the methyl group of **3b** to the corresponding ethyl **3c-s2** and **3c-s1** resulted in an overall decrease of binding affinity for iGluRs, however not all receptor subtypes were equally affected, resulting in a 7-fold selectivity for GluK3 over GluK1. Extending the alkyl chain further to *n*-propyl, analogue **3e**, and *i*-propyl, analogue **3f**, abolishes all affinities for iGluRs. Interestingly, inhibition of the glutamate transporters (EAATs) is restored, with an *i*-propyl group in the ϵ -position, making it a selective inhibitor of these transporters. Introduction of a phenyl ring, compound **3d**, results in loss of affinity for the iGluRs and inhibitory activity at EAAT1–3. Insertion of a tethering methylene group provides the corresponding racemic benzyl **3g**, which then shows affinity for KA receptors, with a selectivity of 9-fold for GluK3 over GluK1. This compound also moderately inhibited EAAT1 and EAAT2, displaying a 10-fold selectivity for these subtypes over EAAT3. Extending the spacer to an ethylene group gives compound **3h**, which again shows a decrease in affinity for KA receptors, compared to benzyl analogue **3g**.

Introduction of a fluoride atom in to the ϵ -position of **3a** provides analogue **3i**, which was isolated as its two separate diastereomers **3i-d1** and **3i-d2**. For both, the binding affinities for the iGluRs were improved, compared to **3a**, most prominently reflected in the nanomolar affinity exhibited by **3i-d2** at GluK3. Although the GluK1/GluK3 selectivity ratio for **3i-d1** is slightly higher than for **3i-d2** (7 vs 5), both analogues are interesting as chemical tools as they are completely devoid of inhibitory activity at EAAT1–3.

For the methoxy analogue **3j**, only one of the two diastereomers could be isolated, and it proved to be without significant binding affinity for iGluRs or functional activity at EAAT1–3. Analogue **3k** is distinct from the other analogues in the series, as it comprises two α -amino acid functionalities. Thus, two generally distinct binding modes can be envisaged. While this speculation was not investigated in further detail herein, **3k** shows midrange micromolar affinity for both AMPA and NMDA receptors as well as the GluK3 subunit.

The two analogues **3l** and **3m** both contain additional carboxylic acid functionalities in the ϵ -position. While **3l** displays high-nanomolar to mid-micromolar affinity to all iGluRs

tested, **3m** is a selective albeit weak NMDA receptor ligand ($K_i = 53 \mu\text{M}$). Both **3l** and **3m** display no functional activity at EAAT1–3. The 2'-hydroxyethyl analogue **3n** is a low affinity ligand with midrange micromolar affinity for native NMDA receptors as well as GluK3. Extending the side chain by a methylene group, alcohol **3o**, leads to an inactive compound in all assays tested here.

The diastereomeric mixture of triazole analogue **3p** showed selectivity for native KA receptors as well as for the cloned homomeric GluK1–3 receptors. Most interestingly, upon separation of the two diastereomers, the broad-range KA receptor affinity profile was found to be retained in **3p-d2**, while the diastereomer **3p-d1** displays low micromolar binding affinity for the GluK3 subtype ($K_i = 6.6 \mu\text{M}$) with a GluK1/GluK3 selectivity ratio of 15 and negligible binding affinity for other iGluR subtypes and EAAT1–3. This profile makes it an attractive chemical tool compound for studying the biological role of the GluK3 subtype.

Lastly, the diastereomeric mixture of tetrazole analogue **3q** displays high-nanomolar to mid-micromolar affinities for cloned homomeric GluK1–3 receptors. However, this mixture exhibits midnanomolar affinity ($K_i = 0.21 \mu\text{M}$) for native NMDA receptors, while having no significant binding affinity for the other native iGluRs. Enantiomer **3q-s1** displayed a significant loss in affinity for native NMDA receptors and was not further characterized (Table 5). Enantiomer **3q-s2** retained the affinity for native NMDA receptors, and functional characterization showed this compound to be a low to midrange micromolar antagonist for GluN1/GluN2A–D receptors. A most interesting result was obtained with the diastereomeric mixture **3q-s3–4**, which also acted as an antagonist at GluN1/GluN2A–D receptors, showing preference for GluN1/GluN2A receptors ($K_i = 0.61 \mu\text{M}$) over GluN1/GluN2B–D receptors ($K_i = 2.7\text{--}62 \mu\text{M}$).

CONCLUSION

In conclusion, two synthetic strategies were taken for the synthesis of the herein reported 2,3-*trans* proline analogues **3a,b–q**. Five analogues, including **3a** (CPAA), were prepared by a linear five-step enantioselective strategy from common intermediate enone **4**. A more efficient racemic route was developed, and starting from common intermediate enone **10** final compounds were obtained in only two or three steps, in overall yields of up to 87%. Furthermore, this procedure allowed for expedited gram scale synthesis of ($\pm$)-**3a**.

Pharmacological characterization at the iGluRs and EAATs show that the added substituents on the ϵ -carbon of **3a** follow a different SAR compared to the 4-substituted Glu analogues and are generally unfavorable, with the following exceptions: (1) Introduction of a triazolyl-CH₂- group gave **3p** as a mixture of four stereoisomers, with a pharmacological profile of low-to-mid micromolar affinity for GluK1–3. Diastereomers were separated and notably **3p-d1** showed reduced affinity for GluK1,2, i.e., enhanced 15-fold selectivity for GluK3 ($K_i = 6.6 \mu\text{M}$) with no significant affinity for any of the native Glu receptors and EAAT transporters tested. In comparison with other known GluK3-preferring ligands, the general profile of **3p** makes it an attractive tool compound for further exploring and understanding of the structural requirements for GluK3 selectivity and studying the biological role of the GluK3 subtype. (2) Exchanging the triazolyl for a tetrazolyl gave **3q**, as a mixture of four

stereoisomers, which displayed selective midrange nanomolar binding affinity at native NMDA receptors over native AMPA and KA receptors. (3) The diastereomeric mixture **3q-s3,4** was shown to be an antagonist at the GluN1/GluN2A receptor ($K_i = 0.61 \mu\text{M}$) with a 4–10-fold preference over GluN1/GluN2B–D, and may serve as a starting point for a fully GluN2A subtype selective antagonist.

METHODS

Chemistry.

All reactions involving dry solvents or sensitive agents were performed under a nitrogen atmosphere and glassware was dried prior to use. Commercially available chemicals were used without further purification. Solvents were dried prior to use with an SG water solvent purification system or dried by standard procedures, and reactions were monitored by analytical thin-layer chromatography (TLC, Merck silica gel 60 F₂₅₄ aluminum sheets). Dry-column vacuum chromatography (DCVC) was carried out using Merck silica gel 60A (15–40 μm),⁶⁸ and flash chromatography was carried out using Merck silica gel 60A (35–70 μm). ¹H NMR spectra were recorded on a 400 MHz Bruker Avance III or 600 MHz Bruker Avance III HD, and ¹³C NMR spectra were recorded on a 101 MHz Bruker Avance III or 151 MHz Bruker Avance III HD. Chemical shifts are reported in δ (ppm) relative to the singlet at $\delta = 7.26$ ppm of CDCl₃ and to the singlet at 4.79 ppm of D₂O for ¹H NMR, and to the center line of the triplet at $\delta = 77.16$ ppm of CDCl₃ and to the singlet at 67.19 ppm of 1,4-dioxane used as external reference in D₂O for ¹³C NMR. Analytical HPLC was performed using an UltiMate HPLC system consisting of an LPG-3400A pump (1 mL/min), a WPS-3000SL autosampler, and a 3000 Diode Array Detector installed with a Gemini-NX C18 (250 × 4.60 mm, 3 μm) column. Solvent A: H₂O + 0.1% TFA; Solvent B: MeCN-H₂O 9:1 + 0.1% TFA. For HPLC control, data collection and data handling, Chromeleon software v. 6.80 was used. Preparative HPLC was carried out on an Ultimate HPLC system with an LPG-3200BX pump, a Rheodyne 9721i injector, a 10 mL loop, an MWD-3000SD detector (200, 210, 225, and 254 nm), and a Gemini-NX C18 (250 × 21.2 mm, 5 μm) column for preparative purifications or a Gemini-NX C18 (250 × 10.00 mm, 5 μm) column for semipreparative purifications. Solvent A: H₂O + 0.1% TFA; Solvent B: MeCN-H₂O 9:1 + 0.1% TFA. For HPLC control, data collection and data handling, Chromeleon software v. 6.80 was used. Chiral preparative HPLC was performed using the same instrumentation as mentioned above. UPLC-MS spectra were recorded using an Acquity UPLC H-Class Waters series solvent delivery system equipped with an autoinjector coupled to an Acquity QDa and TUV detectors installed with an Acquity UPLCBECH C18 (50 × 2.1 mm, 1.7 μm) column. Solvent A: MeCN-H₂O 95:5 + 0.1% HCO₂H; Solvent B: MeCN + 0.1% HCO₂H. For data collection and data handling, MassLynx software was used. Optical rotations were determined in a thermostated cuvette on an Anton Paar MCP300 Modular Circular Polarimeter. Compounds were dried under high vacuum or freeze-dried using a ScanVac Cool Safe Freeze Drier. The purity of compounds submitted for pharmacological characterization was determined by ¹H NMR and HPLC to be >95%.

General Procedure I.—NaH (60% in mineral oil) (1.0 equiv) was suspended in THF (4 mL), to which was added dropwise the corresponding malonate (2.5 equiv) and stirred for 15

min at rt. Enone **10**, which was prepared according to literature procedure, starting from proline,⁶⁴ was dissolved in THF (2 mL) and added dropwise to the reaction. The reaction was heated to 50 °C and stirred for 3–24 h. The crude was extracted with EtOAc, and the combined organic fractions were washed with brine and dried over MgSO₄. The crude was purified by DCVC or flash chromatography.

General Procedure II.—12e–p (mixture of *trans* enantiomers) was suspended in excess 6 M HCl_(aq.) and stirred under reflux for 4–9 days. The excess of HCl and H₂O was evaporated under reduced pressure to give a residue which was dissolved in water or DMSO (2 mL) and without any further purification submitted to preparative HPLC. The isolated fractions were treated with 3 × 1 M HCl_(aq.), and the volatiles were evaporated under reduced pressure. The final product was freeze-dried to afford the mixture of *trans* diastereomers.

(2S,3R)-tert-Butyl 2-(((tert-Butyldimethylsilyl)oxy)methyl)-5-oxo-3-vinylpyrrolidine-1-carboxylate (6a).—Vinyl bromide (1.0 M in THF) (14.05 mL, 14.05 mmol, 2.3 equiv) was dissolved in dry THF (26 mL). The solution was cooled to –78 °C. *t*-Butyllithium (1.7 M in THF) (17.39 mL, 28.70 mmol, 4.7 equiv) was added dropwise, and the reaction was stirred for 30 min at –78 °C. A suspension of CuCN (656 mg, 7.33 mmol, 1.2 equiv) in dry THF (10 mL) was added dropwise, and the reaction mixture was stirred at –42 °C for 15 min. The reaction mixture was cooled to –78 °C, and a solution of **4** (2.0 g, 6.11 mmol, 1 equiv) in dry THF (24 mL) was added dropwise, followed by chlorotrimethylsilane (1.78 mL, 14.05 mmol, 2.3 equiv). The reaction mixture was stirred at –42 °C for 1 h and was quenched with sat. NH₄Cl (20 mL). Brine (40 mL) was added, and the product was extracted with EtOAc (4 × 40 mL). The organic layers were combined, washed with brine (60 mL), and dried over Na₂SO₄. The crude product was purified by DCVC (0 → 10% EtOAc in heptane), which afforded the product as a colorless oil (1.41 g, 3.97 mmol, 65%). LCMS (ESI) *m/z* = 356.2 [M + H]⁺; *R*_f = 0.45 (3:1 heptane/EtOAc); [*α*]_D²⁵₅₈₉ = –37.3° (*c* = 0.31, EtOH); ¹H NMR (300 MHz, CDCl₃) δ 5.91–5.78 (m, 1H), 5.11 (dt, *J* = 17.10, 1.10 Hz, 1H), 5.08 (dt, *J* = 10.20, 0.80 Hz, 1H), 3.94 (dd, *J* = 10.45, 3.85, 1H), 3.90–3.86 (m, 1 H), 3.72 (dd, *J* = 10.18, 1.93 Hz, 1H), 2.97–2.84 (m, 2H), 2.32–2.19 (m, 1H), 1.52 (s, 9H), 0.87 (s, 9H), 0.05 (s, 3H) 0.03 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 173.6, 149.7, 139.1, 114.8, 82.8, 64.4, 63.3, 38.1, 37.1, 28.0, 25.8, 18.2, 5.5.

tert-Butyl (2S,3R)-3-(But-1-en-2-yl)-2-(((tert-butyldimethylsilyl)oxy)methyl)-5-oxopyrrolidine-1-carboxylate (6c).—2-Bromobut-1-ene (1.89 g, 14.05 mmol, 2.3 equiv) was dissolved in dry THF (40 mL). The solution was cooled to –78 °C. *tert*-Butyllithium (1.45 M in THF) (19.79 mL, 28.70 mmol, 4.7 equiv) was added dropwise, and the reaction was stirred for 30 min at –78 °C. A suspension of CuCN (656 mg, 7.33 mmol, 1.2 equiv) in dry THF (10 mL) was added dropwise, and the reaction mixture was stirred at –42 °C for 15 min. The reaction mixture was cooled to –78 °C, and a solution of **4** (2.0 g, 6.11 mmol, 1 equiv) in dry THF (25 mL) was added dropwise, followed by chlorotrimethylsilane (1.78 mL, 14.05 mmol, 2.3 equiv). The reaction mixture was warmed to –42 °C and stirred for 1 h. The reaction mixture was quenched with sat. NH₄Cl (40 mL). Brine (80 mL) was added, and the product was extracted with EtOAc (4 × 80 mL). The

organic layers were combined, washed with brine (60 mL), and dried over MgSO₄. The crude product was purified by DCVC (0 → 4% EtOAc in heptane), which afforded the product as a colorless oil (1.43 g, 3.73 mmol, 61%). LCMS (ESI) m/z = 384.3 [M + H]⁺; R_f = 0.52 (3:1 heptane/EtOAc); $[\alpha]^{25}_{589}$ = −45.4° (c = 0.41, EtOH); ¹H NMR (400 MHz, CDCl₃) δ 4.82 (t, J = 1.38 Hz, 1H), 4.81 (s, 1H), 3.96 (ddd, J = 4.20, 2.40, 1.50 Hz, 1H), 3.90 (dd, J = 10.30, 4.30 Hz, 1H), 3.73 (dd, J = 10.29, 2.26 Hz, 1H), 2.93 (dd, J = 17.30, 9.54 Hz, 1H), 2.83 (d, J = 9.50 Hz, 1H), 2.34 (dd, J = 17.32, 1.51 Hz, 1H), 2.05 (ttq, J = 15.50, 15.50, 8.80, 8.80, 1.30, 1.30, 1.30 Hz, 2H), 1.54 (s, 9H), 1.07 (t, J = 7.40 Hz, 3H), 0.89 (s, 9H), 0.06 (s, 3H), 0.05 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 174.1, 152.0, 150.1, 108.0, 82.8, 64.0, 63.9, 38.8, 38.0, 28.1, 27.0, 25.8, 18.2, 12.1, −5.5.

tert-Butyl (2S,3R)-2-(((tert-Butyldimethylsilyl)oxy)methyl)-5-oxo-3-(1-phenylvinyl)pyrrolidine-1-carboxylate (6d).— α -Bromostyrene (2.57 g, 14.05 mmol, 2.3 equiv) was dissolved in dry THF (40 mL). The solution was cooled to −78 °C. *t*-Butyllithium (1.45 M in THF) (19.79 mL, 28.70 mmol, 4.7 equiv) was added dropwise, and the reaction was stirred for 30 min at −78 °C. A suspension of CuCN (656 mg, 7.33 mmol, 1.2 equiv) in dry THF (10 mL) was added dropwise, and the reaction was stirred at −42 °C for 15 min. The reaction mixture was cooled to −78 °C, and a solution of **4** (2.0 g, 6.11 mmol, 1 equiv) in dry THF (25 mL) was added dropwise, followed by chlorotrimethylsilane (1.78 mL, 14.05 mmol, 2.3 equiv). The reaction mixture was warmed to −42 °C and stirred for 1 h. The reaction mixture was quenched with sat. NH₄Cl (40 mL). Brine (80 mL) was added, and the product was extracted with EtOAc (4 × 80 mL). The organic layers were combined, washed with brine (60 mL), and dried over MgSO₄. The crude product was purified by DCVC (0 → 4% EtOAc in heptane), which yielded the product as a colorless oil (2.16 g, 5.01 mmol, 82%). LCMS (ESI) m/z = 432.3 [M + H]⁺; R_f = 0.48 (3:1 heptane/EtOAc); ¹H NMR (400 MHz, CDCl₃) δ 7.41–7.27 (m, 5H), 5.36 (s, 1H), 5.11 (d, J = 1.0 Hz, 1H), 4.10–4.06 (m, 1H), 3.91 (dd, J = 10.5, 4.5 Hz, 1H), 3.75 (dd, J = 10.5, 2.4 Hz, 1H), 3.42 (dd, J = 9.0, 1.2 Hz, 1H), 3.05 (dd, J = 17.7, 9.2 Hz, 1H), 2.46 (dd, J = 17.6, 1.6 Hz, 1H), 1.51 (s, 9H), 0.89 (s, 8H), 0.05 (s, 3H), 0.05 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 173.9, 150.2, 150.0, 140.7, 128.7, 128.1, 126.7, 112.3, 83.1, 64.1, 64.1, 38.4, 37.4, 28.2, 26.0, 18.4, −5.4.

(2S,3R)-tert-Butyl 2-(((tert-Butyldimethylsilyl)oxy)methyl)-3-(2-hydroxyethyl)pyrrolidine-1-carboxylate (7a).—**6a** (600 mg, 1.69 mmol, 1 equiv) was dissolved in dry THF (25 mL). BH₃–THF complex (1 M in THF) (10.12 mL, 10.12 mmol, 6 equiv) was added dropwise, and the reaction was stirred for 4 h under reflux. The reaction was allowed to cool down to rt, THF (20 mL) was added, and the reaction was cooled to 0 °C. H₂O (1.2 mL) was added carefully, followed by dropwise addition of 2 M NaOH_(aq.) (24 mL, 48 mmol, 28.40 equiv) and H₂O₂ in H₂O (30% w/w) (4.8 mL, 47.0 mmol, 28 equiv). The reaction was stirred vigorously for 1 h at rt, it was quenched with sat. NaHCO₃ solution (42 mL), and then it was stirred for an additional 30 min. The product was extracted with Et₂O/THF 1:1 (3 × 50 mL), and the combined organic layers were washed with brine (120 mL) and dried over MgSO₄. The crude product was purified by DCVC (0 → 30% EtOAc in heptane), which afforded the product as a colorless oil (315 mg, 0.88 mmol, 52%). LCMS (ESI) m/z = 260.3 [M + H]⁺; R_f = 0.58 (2:1 EtOAc/heptane); $[\alpha]^{25}_{589}$ = −37.7° (c =

0.26, EtOH); ^1H NMR (300 MHz, CDCl_3) δ 5.01 (d, J = 7.43 Hz, 1H), 3.83–3.50 (m, 5H), 3.25 (ddd, J = 11.00, 8.66, 6.74 Hz, 1H), 2.11–1.90 (m, 2H), 1.90–1.71 (m, 2H), 1.71–1.53 (m, 1H), 1.49 (s, 9H); ^{13}C NMR (75 MHz, CDCl_3) δ 154.3, 79.4, 63.3, 61.2, 46.1, 38.1, 36.8, 30.2, 28.6, 25.9, 18.3, –3.5, –5.3.

tert-Butyl (2S,3R)-2-(((tert-Butyldimethylsilyl)oxy)methyl)-3-((S)-1-hydroxybutan-2-yl)pyrrolidine-1-carboxylate (7c-s1).—6c (1.85 g, 4.82 mmol, 1 equiv) was dissolved in dry THF (75 mL). BH_3 –THF complex (1 M in THF) (24.1 mL, 24.1 mmol, 5 equiv) was added dropwise, and the reaction was stirred for 20 h at rt. The reaction was cooled to 0 °C, and THF (37 mL) was added. H_2O (3.7 mL) was added carefully, followed by dropwise addition of 2 M NaOH (72.3 mL, 144.68 mmol, 30 equiv) and H_2O_2 in H_2O (30% w/w) (13.72 mL, 135.04 mmol, 28 equiv). The reaction was stirred vigorously for 1 h at rt, it was quenched with sat. NaHCO_3 solution (150 mL), and then it was stirred for an additional 1 h. The product was extracted with EtOAc (3×150 mL), and the combined organic layers were washed with brine (200 mL) and dried over MgSO_4 . The diastereomers were separated and purified by DCVC (10 $\rightarrow$ 22% EtOAc in heptane), which afforded the product as a colorless oil (515 mg, 1.33 mmol, 28%). LCMS (ESI) m/z = 388.3 $[\text{M} + \text{H}]^+$; R_f = 0.61 (2:1 EtOAc/heptane); $[\alpha]_D^{25} = -32.3^\circ$ (c = 1.035, EtOH); ^1H NMR (400 MHz, CDCl_3) δ 3.71 (br s, 2H), 3.68–3.49 (m, 3H), 3.48–3.39 (m, 1H), 3.26–3.15 (m, 1H), 2.46 (br s, 1H), 2.05–1.94 (m, 2H), 1.74–1.59 (m, 1H), 1.45 (s, 9H), 1.42–1.27 (m, 2H), 0.92 (t, J = 7.30 Hz, 4H), 0.89 (s, 9H), 0.05 (br s, 6H); ^{13}C NMR (100 MHz, CDCl_3) (rotamers in brackets) δ 154.2, 79.4 [79.0], 64.4 [63.4], 63.3 [63.1], 60.7, [60.4], 46.6 [45.9], 44.9 [44.8], 41.7 [40.2], 28.5, 27.5 [26.6], 25.9, 21.0 [20.7], 18.3, 11.9 [11.7], –5.5.

tert-Butyl (2S,3R)-2-(((tert-Butyldimethylsilyl)oxy)methyl)-3-((R)-1-hydroxybutan-2-yl)pyrrolidine-1-carboxylate (7c-s2).—From the same reaction as 7c-s1 which afforded the product as a colorless oil (524 mg, 1.35 mmol, 28%). LCMS (ESI) m/z = 388.3 $[\text{M} + \text{H}]^+$; R_f = 0.65 (2:1 EtOAc/heptane); $[\alpha]_D^{25} = -35.1^\circ$ (c = 0.75, EtOH); ^1H NMR (400 MHz, CDCl_3) δ 3.79 (dd, J = 9.30, 3.50 Hz, 1H), 3.76–3.66 (m, 2H), 3.62 (dd, J = 11.50, 4.00 Hz, 1H), 3.59–3.50 (m, 1H), 3.50–3.38 (m, 1H), 3.19 (dd, J = 17.60, 7.30 Hz, 1H), 2.30 (br s, 1H), 1.99 (dtd, J = 13.40, 7.40, 7.40, 5.90 Hz, 1H), 1.71–1.59 (m, 1H), 1.46 (s, 9H), 1.44–1.36 (m, 2H), 1.35–1.27 (m, 1H), 0.93 (t, J = 7.40 Hz, 3H), 0.90 (s, 9H), 0.07 (s, 6H); ^{13}C NMR (100 MHz, CDCl_3) (rotamers in brackets) δ 154.2, 79.5 [79.1], 64.8 [63.7], 62.5, 61.1 [61.0], 46.2 [45.5], 45.1 [44.9], 42.6 [41.0], 28.5, 27.9 [27.3], 25.9, 21.4 [21.4], 18.3, 11.7, –5.5.

tert-Butyl (2S,3R)-2-(((tert-Butyldimethylsilyl)oxy)methyl)-3-((S)-2-hydroxy-1-phenylethyl)pyrrolidine-1-carboxylate (7d-s2).—6d (4.21 g, 9.75 mmol, 1 equiv) was dissolved in dry THF (160 mL). BH_3 –THF complex (1 M in THF) (48.8 mL, 48.8 mmol, 5 equiv) was added dropwise, and the reaction was stirred for 20 h at rt. The reaction was cooled to 0 °C, and THF (55 mL) was added. H_2O (7.6 mL) was added carefully, followed by dropwise addition of 2 M $\text{NaOH}_{(\text{aq})}$ (146 mL, 292.61 mmol, 30 equiv) and H_2O_2 in H_2O (30% w/w) (27.9 mL, 273.10 mmol, 28 equiv). The reaction was stirred vigorously for 1 h at rt, it was quenched with sat. NaHCO_3 solution (320 mL), and it was stirred for an additional hour. The product was extracted with EtOAc (3×150 mL), and the combined organic layers

were washed with brine (200 mL) and dried over MgSO₄. The diastereomers were separated and purified by DCVC (10 → 26% EtOAc in heptane), which afforded the product as a colorless oil (723 mg, 1.66 mmol, 17%). LCMS (ESI) m/z = 436.3 [M + H]⁺; R_f = 0.68 (1:1 EtOAc/heptane); $[\alpha]^{25}_{589}$ = -35.1° (c = 0.75, EtOH); ¹H NMR (400 MHz, CDCl₃) δ 7.37–7.27 (m, 2H), 7.26–7.19 (m, 3H), 3.96–3.79 (m, 4H), 3.68–3.40 (m, 1H), 3.31 (dt, J = 8.64, 35.37 Hz, 1H), 3.22–3.09 (m, 1H), 2.68 (dt, J = 5.54, 11.06 Hz, 1H), 2.65–2.54 (m, 1H), 2.18–1.91 (m, 1H), 1.77 (q, J = 7.06, 7.50, 14.04 Hz, 1H), 1.47 (d, J = 12.55 Hz, 9H), 1.39–1.30 (m, 1H), 0.93 (s, 9H), 0.10 (s, 6H). ¹³C NMR (101 MHz, CDCl₃) δ 154.5, 128.9, 128.8, 128.6, 127.1, 62.2, 51.2, 28.7, 26.1, 25.9, 21.2, 18.5, 14.3, 14.2, -5.2.

(2S,3R)-tert-Butyl 3-(2-Hydroxyethyl)-2-(hydroxymethyl)-pyrrolidine-1-carboxylate (8a).—

7a (440 mg, 1.23 mmol, 1 equiv) was dissolved in dry THF (12 mL), and TBAF (1 M in THF) (2.09 mL, 2.09 mmol, 1.7 equiv) was added dropwise. The reaction was stirred for 1 h at rt. The reaction was quenched with sat. NaHCO₃ (6 mL), and H₂O (6 mL) was added. The aqueous phase was extracted with EtOAc (3 × 30 mL). The combined organic fractions were washed with brine (100 mL) and dried over MgSO₄. The crude product was purified by DCVC (100% EtOAc), which afforded the product as a colorless oil (253 mg, 1.03 mmol, 84%). LCMS (ESI) m/z = 246.2 [M + H]⁺; R_f = 0.21 (100% EtOAc); $[\alpha]^{25}_{589}$ = -38.4° (c = 0.19, EtOH); ¹H NMR (300 MHz, CDCl₃) δ 5.01 (d, J = 7.43 Hz, 1H), 3.83–3.50 (m, 5H), 3.25 (ddd, J = 11.00, 8.66, 6.74 Hz, 1H), 2.11–1.90 (m, 2H), 1.90–1.71 (m, 2H), 1.71–1.53 (m, 1H), 1.49 (s, 9H); ¹³C NMR (100 MHz, CDCl₃) δ 156.8, 80.3, 66.7, 65.6, 60.9, 46.5, 38.2, 36.1, 30.2, 28.4

tert-Butyl (2S,3R)-3-((S)-1-Hydroxybutan-2-yl)-2-(hydroxymethyl)pyrrolidine-1-carboxylate (8c-s1).—

7c-s1 (533 mg, 1.38 mmol, 1 equiv) was dissolved in dry THF (15 mL), and TBAF (1 M in THF) (2.52 mL, 2.34 mmol, 1.7 equiv) was added dropwise. The reaction was stirred for 1 h at rt. The reaction was quenched with sat. NaHCO₃ (8 mL), and H₂O (8 mL) was added. The aqueous phase was extracted with EtOAc (3 × 40 mL), and the combined organic fractions were washed with brine (75 mL) and dried over MgSO₄. The crude product was purified by DCVC (80 → 94% EtOAc in heptane), which yielded the product as a colorless oil (303 mg, 1.11 mmol, 80%). LCMS (ESI) m/z = 274.2 [M + H]⁺; R_f = 0.30 (2:1 EtOAc/heptane); $[\alpha]^{25}_{589}$ = -93.0° (c = 0.115, EtOH); ¹H NMR (400 MHz, CDCl₃) δ 4.82 (br s, 1H) 3.89–3.76 (m, 1H) 3.72–3.51 (m, 5H) 3.19 (ddd, J = 11.00, 8.50, 7.03 Hz, 1H) 2.12 (br s, 1H) 2.02–1.81 (m, 2H) 1.65 (ddd, J = 16.60, 12.30, 8.30 Hz, 1H) 1.54–1.33 (m, 12H) 0.94 (t, J = 7.50 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 156.8, 80.3, 67.6, 63.0, 62.5, 46.4, 44.1, 42.3, 28.5, 27.4, 22.2, 11.8.

tert-Butyl (2S,3R)-3-((R)-1-Hydroxybutan-2-yl)-2-(hydroxymethyl)pyrrolidine-1-carboxylate (8c-s2).—

7c-s2 (575.7 mg, 1.49 mmol, 1 equiv) was dissolved in dry THF (15 mL), and TBAF (1 M in THF) (2.52 mL, 2.52 mmol, 1.7 equiv) was added dropwise. The reaction was stirred for 1 h at rt. The reaction was quenched with sat. NaHCO₃ (8 mL), and H₂O (8 mL) was added. The aqueous phase was extracted with EtOAc (3 × 40 mL). The combined organic fractions were washed with brine (75 mL) and dried over MgSO₄. The crude product was purified by DCVC (84 → 92% EtOAc in heptane), which afforded the product as a colorless oil (328 mg, 1.20 mmol, 81%). LCMS (ESI) m/z = 274.2 [M + H]⁺;

$R_f = 0.42$ (100% EtOAc); $[\alpha]_{589}^{25} = -26.2^\circ$ ($c = 0.625$, EtOH); ^1H NMR (400 MHz, CDCl_3) δ 4.82 (br s, 1H), 3.82–3.52 (m, 6H), 3.17 (ddd, $J = 11.04, 9.41, 6.65$ Hz, 1H), 2.18–1.96 (m, 2H), 1.96–1.68 (m, 2H), 1.66–1.51 (m, 1H), 1.47 (s, 9H), 1.45–1.39 (m, 1H), 1.38–1.24 (m, 1H), 0.95 (t, $J = 7.28$ Hz, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ 156.6, 80.2, 67.2, 63.0, 62.8, 46.4, 44.3, 42.2, 28.4, 27.3, 20.5, 11.9.

tert-Butyl (2S,3R)-3-((R)-2-Hydroxy-1-phenylethyl)-2-(hydroxymethyl)pyrrolidine-1-carboxylate (8d-s1).—The compound **8d-s1** was

obtained as a side-product from the synthesis of **7d-s2**. After isolation of **7d-s2** by DCVC, the remaining fractions were combined and submitted to preparative HPLC, which afforded **8d-s1** as a colorless oil (157 mg, 0.49 mmol, 5%). LCMS (ESI) $m/z = 322.2$ $[\text{M} + \text{H}]^+$; ^1H NMR (400 MHz, CDCl_3) δ 7.35–7.24 (m, 3H), 7.21–7.13 (m, 2H), 3.92–3.75 (m, 2H), 3.75–3.65 (m, 1H), 3.42–3.09 (m, 4H), 2.84–2.67 (m, 1H), 2.30–2.15 (m, 1H), 2.15–1.96 (m, 1H), 1.74 (dp, $J = 7.4, 13.9$ Hz, 1H), 1.40 (s, 9H); ^{13}C NMR (101 MHz, CDCl_3) δ 156.6, 140.6, 129.0, 128.5, 127.5, 80.4, 66.3, 64.9, 62.9, 51.4, 46.2, 43.0, 29.5, 28.5.

tert-Butyl (2S, 3R)-3-((S)-2-Hydroxy-1-phenylethyl)-2-(hydroxymethyl)pyrrolidine-1-carboxylate (8d-s2).—**7d-s2** (700 mg, 1.61 mmol, 1

equiv) was dissolved in dry THF (20 mL), and TBAF (1 M in THF) (2.73 mL, 2.72 mmol, 1.7 equiv) was added dropwise. The reaction was stirred for 1 h at rt. The reaction was quenched with sat. NaHCO_3 (10 mL), and H_2O (8 mL) was added. The aqueous phase was extracted with EtOAc (3×50 mL). The combined organic fractions were washed with brine (100 mL) and dried over MgSO_4 . The crude product was purified by DCVC (54 $\rightarrow$ 70% EtOAc in heptane), which afforded the product as a colorless oil (290 mg, 0.90 mmol, 56%). LCMS (ESI) $m/z = 322.2$ $[\text{M} + \text{H}]^+$; $R_f = 0.26$ (1:1 EtOAc/heptane); ^1H NMR (400 MHz, CDCl_3) δ 7.35–7.29 (m, 2H), 7.27–7.19 (m, 3H), 3.96–3.83 (m, 3H), 3.79–3.67 (m, 2H), 3.37 (br. s, 1H), 3.14 (dt, $J = 7.2, 11$ Hz, 1H), 2.74 (br. s, 1H), 2.32 (br. s, 1H), 1.70 (dq, $J = 6.8, 13.0$ Hz, 1H), 1.45 (s, 9H), 1.36 (dq, $J = 7.2, 14.2$ Hz, 1H); ^{13}C NMR (101 MHz, CDCl_3) δ 156.5, 141.0, 128.8, 128.7, 127.1, 80.4, 66.7, 65.6, 63.4, 51.0, 45.8, 42.8, 28.4, 14.3.

(2S,3R)-1-(tert-Butoxycarbonyl)-3-(carboxymethyl)pyrrolidine-2-carboxylic Acid (9a).—**8a** (275 mg, 1.12 mmol, 1.0 equiv) was dissolved in EtOAc (9.3 mL), and

MeCN (9.3 mL) was added. NaIO_4 (1960 mg, 9.19 mmol, 8.2 equiv) was suspended in H_2O (8.5 mL), followed by addition of $\text{RuCl}_3 \cdot \text{H}_2\text{O}$ (4.65 mg, 0.02 mmol, 0.02 equiv). The aqueous phase was transferred to the organic phase, and H_2O (4 mL) was added. The reaction was vigorously stirred for 4 h at rt. The reaction mixture was filtered through Celite, and the residue was washed with EtOAc (30 mL). The layers were separated, and the aqueous phase was extracted with EtOAc (3×30 mL). The organic fractions were combined, washed with brine (75 mL), and dried over MgSO_4 . The crude product was purified by DCVC (10 $\rightarrow$ 50% EtOAc in heptane % AcOH). All fractions were combined and evaporated. The residue was dissolved in a minimum volume of warm EtOAc (approximately 1.5 mL). Heptane (approximately 7 mL) was added until the solution stayed turbid, and the mixture was placed at 5°C overnight, which afforded a crystalline white solid (228 mg, 0.83 mmol, 75%); LCMS (ESI) $m/z = 274.1$ $[\text{M} + \text{H}]^+$; ^1H NMR (300 MHz,

CDCl₃) δ 10.21 (br. s, 2H), 4.01 (dd, J = 50.90, 6.30 Hz, 1H), 3.71–3.39 (m, 2 H), 2.92–2.64 (m, 2H), 2.51 (dt, J = 15.75, 7.67 Hz, 1H), 2.24 (dq, J = 11.86, 6.04 Hz, 1H), 1.78–1.59 (m, 1H), 1.47 (d, J = 15.68 Hz, 9H); ¹³C NMR (100 MHz, CDCl₃) δ 176.2, 153.8, 80.8, 64.1, 45.9, 40.7, 38.9, 37.3, 30.3, 28.2.

(2S,3R)-1-(tert-Butoxycarbonyl)-3-((S)-1-carboxypropyl)-pyrrolidine-2-carboxylic Acid (9c-s1).—8c-s1 (325 mg, 1.19 mmol, 1.0 equiv) was dissolved in EtOAc (10.0 mL), and MeCN (10.0 mL) was added. NaIO₄ (2085 mg, 9.75 mmol, 8.2 equiv) was suspended in H₂O (9.0 mL), followed by the addition of RuCl₃·H₂O (4.93 mg, 0.02 mmol, 0.02 equiv). The aqueous phase was transferred to the organic phase, and H₂O (4.3 mL) was added. The reaction was vigorously stirred for 7 h at rt. The reaction mixture was filtered through Celite, and the residue was washed with EtOAc (35 mL). The layers were separated, and the aqueous phase was extracted with EtOAc (3 × 25 mL). The organic fractions were combined, washed with brine (75 mL), and dried over MgSO₄. The crude product was purified by DCVC (34 → 50% EtOAc in heptane + 1% AcOH), which afforded the product as a white solid (110 mg). The impure fractions were combined and together crystallized in minimum volume of warm EtOAc (approximately 1 mL). Heptane (approximately 6 mL) was added until the solution stayed turbid, and the mixture was placed at 5 °C and was left overnight, which afforded additional product as a crystalline white solid (27 mg). This was combined with the already obtained product (138 mg, 0.46 mmol 38%). LCMS (ESI) m/z = 302.1 [M + H]⁺; mp: 133.7–135.3 °C (decomposition); [α]_D²⁵₅₈₉ = +5.1° (c = 0.33, EtOH); ¹H NMR (400 MHz, CDCl₃) (rotamers in brackets) δ 11.21 (br s, 2H), 4.22 [4.08] (d, J = 5.27 Hz, 1H), 3.66–3.37 (m, 2H), 2.71–2.54 (m, 1H), 2.53–2.37 (m, 1H), 2.17–2.06 (m, 1H), 1.85–1.68 (m, 3H), 1.47 [1.42] (s, 9H), 0.98 (t, J = 7.28 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) (rotamers in brackets) δ 179.5 [179.4], 178.6 [175.8], 155.7 [153.8], 81.4 [80.9], 62.0, 49.3 [49.2], 45.9 [45.7], 45.3 [44.0], 28.4, 28.2, 23.8 [23.4], 11.8 [11.7].

(2S,3R)-1-(tert-Butoxycarbonyl)-3-((R)-1-carboxypropyl)-pyrrolidine-2-carboxylic Acid (9c-s2).—8c-s2 (325 mg, 1.19 mmol, 1.0 equiv) was dissolved in EtOAc (10.0 mL), and MeCN (10.0 mL) was added. NaIO₄ (2085 mg, 9.75 mmol, 8.2 equiv) was suspended in H₂O (9.0 mL), followed by the addition of RuCl₃·H₂O (4.93 mg, 0.02 mmol, 0.02 equiv). The aqueous phase was transferred to the organic phase, and H₂O (4.3 mL) was added. The reaction was vigorously stirred for 7 h at rt. The reaction mixture was filtered through Celite, and the residue was washed with EtOAc (35 mL). The layers were separated, and the aqueous phase was extracted with EtOAc (3 × 25 mL). The organic fractions were combined, washed with brine (75 mL), and dried over MgSO₄. The crude product was purified by DCVC (34 → 50% EtOAc in heptane + 1% AcOH), which afforded the product as a white solid (110 mg, 31%). The impure fractions were combined and together crystallized in minimum volume of warm EtOAc (approximately 1 mL). Heptane (approximately 6 mL) was added until the solution stayed turbid, and the mixture was placed at 5 °C and was left overnight, which afforded the product as a white solid (27 mg). This was combined with the already obtained product (138 mg, 0.46 mmol, 38%). LCMS (ESI) m/z = 302.1 [M + H]⁺; mp: 128.0–129.4 °C (decomposition); [α]_D²⁵₅₈₉ = –3.0° (c = 0.32, EtOH); ¹H NMR (400 MHz, CDCl₃) (rotamers in brackets) δ 9.21 (br s, 2H), 4.14 [3.93] (d, J = 7.53 Hz, 1H), 3.70–3.62 [3.59–3.51] (m, 1H), 3.47–3.33 (m, 1H), 2.74–2.59 (m, 1H),

2.42–2.29 (m, 1H), 2.18–2.06 (m, 1H), 1.79–1.55 (m, 3H), 1.45 [1.41] (s, 9H) 0.97 (t, J = 7.28 Hz, 3H); ^{13}C NMR (100 MHz, CDCl_3) (rotamers in brackets) δ 179.7 [179.4], 178.0 [176.1], 155.0 [153.7], 81.1 [81.0], 63.7 [63.2], 50.2, 46.0 [45.8], 45.5 [44.5], 28.4, 28.2, 23.6 [23.4], 11.5 [11.4].

(2S,3R)-1-(tert-Butoxycarbonyl)-3-((R)-Carboxy(phenyl)methyl)-pyrrolidine-2-carboxylic Acid (9d-s1).—**8d-s1** (130 mg, 0.40 mmol, 1.0 equiv) was dissolved in EtOAc (3.4 mL), and MeCN (3.4 mL) was added. NaIO_4 (709 mg, 3.32 mmol, 8.2 equiv) was suspended in H_2O (3.0 mL), followed by the addition of $\text{RuCl}_3 \cdot \text{H}_2\text{O}$ (1.68 mg, 0.02 mmol, 0.01 equiv). The aqueous phase was transferred to the organic phase, and H_2O (1.5 mL) was added. The reaction was vigorously stirred for 4 h at rt. The reaction mixture was filtered through Celite, and the residue was washed with EtOAc (10 mL). The layers were separated, and the aqueous phase was extracted with EtOAc (3×10 mL). The organic fractions were combined, washed with brine (25 mL), and dried over MgSO_4 . The crude product was purified by DCVC (40 $\rightarrow$ 55% EtOAc in heptane + 1% AcOH), which afforded the product as a white solid. The product was crystallized in minimum volume of warm i -POH (approximately 4 mL). H_2O (approximately 1 mL) was added until the solution stayed turbid, and the mixture was placed at rt and was left overnight, which afforded the product as a crystalline white solid (32 mg, 0.09 mmol, 23%). LCMS (ESI) m/z = 350.2 [$\text{M} + \text{H}$] $^+$; ^1H NMR (400 MHz, acetone) δ 7.43–7.38 (m, 2H), 7.38–7.32 (m, 2H), 7.32–7.27 (m, 1H), 3.86 (dd, J = 2.3, 36.8 Hz, 1H), 3.60 (dd, J = 6.6, 11.2 Hz, 1H), 3.57–3.47 (m, 2H), 3.10 (ddd, J = 3.0, 6.6, 10.9 Hz, 1H), 2.29–2.13 (m, 1H), 1.93–1.79 (m, 1H), 1.37 (d, J = 43.6 Hz, 9H).

(2S,3R)-1-(tert-Butoxycarbonyl)-3-((S)-carboxy(phenyl)methyl)-pyrrolidine-2-carboxylic Acid (9d-s2).—**8d-s2** (290 mg, 0.90 mmol, 1.0 equiv) was dissolved in EtOAc (7.6 mL), and MeCN (7.6 mL) was added. NaIO_4 (1583 mg, 7.40 mmol, 8.2 equiv) was suspended in H_2O (6.8 mL), followed by the addition of $\text{RuCl}_3 \cdot \text{H}_2\text{O}$ (3.74 mg, 0.02 mmol, 0.02 equiv). The aqueous phase was transferred to the organic phase, and H_2O (3.3 mL) was added. The reaction was vigorously stirred for 4 h at rt. The reaction mixture was filtered through Celite, and the residue was washed with EtOAc (20 mL). The layers were separated, and the aqueous phase was extracted with EtOAc (3×15 mL). The organic fractions were combined, washed with brine (50 mL), and dried over MgSO_4 . The crude product was purified by DCVC (40 $\rightarrow$ 55% EtOAc in heptane + 1% AcOH), which afforded the product as a white solid (129 mg, 0.37 mmol, 41%). LCMS (ESI) m/z = 350.2 [$\text{M} + \text{H}$] $^+$; ^1H NMR (400 MHz, acetone) δ 11.13 (br. s, 2H) 7.47–7.41 (m, 2H), 7.41–7.35 (m, 2H), 7.35–7.29 (m, 1H), 4.11 (dd, J = 3.6, 28.2 Hz, 1H), 3.68 (dd, J = 4.0, 10.6 Hz, 1H), 3.49–3.32 (m, 2H), 3.14–3.03 (m, 1H), 1.85 (ddq, J = 7.5, 12.7, 25.3 Hz, 1H), 1.45–1.36 (m, 10H).

(2S,3R)-3-(Carboxymethyl)pyrrolidine-2-carboxylic Acid Hydrochloride (3a).—**9a** (205 mg, 0.75 mmol, 1 equiv) was dissolved in DCM (25 mL), and TFA (25 mL) was added dropwise. The reaction was stirred for 2 h. The solvents were removed under reduced pressure. The crude was dissolved in 1 M $\text{HCl}_{(\text{aq})}$ (3×30 mL), and the solvent was evaporated. The crude was purified by preparative HPLC. The combined fractions were

evaporated and redissolved in 1 M HCl_(aq.) (3 × 30 mL), and the solvent was evaporated. The final compound was freeze-dried, which afforded the product as a hygroscopic solid (21 mg, 0.10 mmol, 13%). LCMS (ESI) m/z = 174.1 [M + H]⁺; [α]₂₅⁵⁸⁹ = +18.3° (c = 0.61, H₂O); ¹H NMR (300 MHz, D₂O) δ 3.81 (d, J = 7.70 Hz, 1H), 3.50–3.34 (m, 2H), 2.85 (dd, J = 15.70, 4.70 Hz, 1H), 2.79–2.64 (m, 1H), 2.54 (dd, J = 15.70, 8.80 Hz, 1H), 2.38–2.25 (m, 1H), 1.81 (dq, J = 13.34, 8.30 Hz, 1H); ¹³C NMR (100 MHz, D₂O) δ 177.1, 174.0, 65.4, 45.5, 39.9, 38.1, 30.5.

(2S,3R)-3-((S)-1-Carboxypropyl)pyrrolidine-2-carboxylic Acid Hydrochloride (3c-s1).—**9c-s1** (105 mg, 0.34 mmol, 1 equiv) was dissolved in DCM (6.8 mL), and the

solution was cooled to 0 °C. TFA (6.8 mL) was added dropwise, and the reaction was allowed to warm to rt and stirred for 2 h. The solvents were removed under reduced pressure. The crude was purified by preparative HPLC. The purified compound was dissolved in 1 M HCl in H₂O (3 × 30 mL), and solvent was evaporated. The final compound was freeze-dried, which afforded the product as a white solid (38 mg, 0.16 mmol, 47%). LCMS (ESI) m/z = 202.1 [M + H]⁺; mp: 184.1–188.7 °C (decomposition); [α]₂₅⁵⁸⁹ = +18.3° (c = 0.57, H₂O); ¹H NMR (300 MHz, D₂O) δ 4.31 (d, J = 7.28 Hz, 1H), 3.50 (ddd, J = 11.92, 7.40, 4.77 Hz, 1H), 3.40 (ddd, J = 11.50, 8.80, 7.00 Hz, 1H), 2.82 (quin, J = 7.65 Hz, 1H), 2.69 (dd, J = 14.30, 7.50 Hz, 1H), 2.34–2.24 (m, 1H), 1.93 (dq, J = 13.55, 8.28 Hz, 1H), 1.78–1.68 (m, 2H), 0.95 (t, J = 7.40 Hz, 3H); ¹³C NMR (100 MHz, D₂O) δ 178.5, 171.9, 62.0, 49.3, 46.2, 44.0, 28.0, 24.2, 11.6.

(2S,3R)-3-((R)-1-Carboxypropyl)pyrrolidine-2-carboxylic Acid Hydrochloride (3c-s2).—**9c-s2** (89 mg, 0.29 mmol, 1 equiv) was dissolved in DCM (5.8 mL), and the

solution was cooled to 0 °C. TFA (5.8 mL) was added dropwise, and the reaction was allowed to warm to rt and stirred for 2 h. The solvents were removed under reduced pressure. The crude was purified by preparative HPLC. The combined fractions were evaporated and redissolved in 1 M HCl (3 × 30 mL), and the solvent was evaporated. The final compound was freeze-dried, which afforded the product as a white solid (27 mg, 0.11 mmol, 39%). LCMS (ESI) m/z = 202.1 [M + H]⁺; mp: 68.7–71.2 °C (decomposition); [α]₂₅⁵⁸⁹ = +12.9° (c = 0.23, H₂O); ¹H NMR (300 MHz, D₂O) δ 4.24 (d, J = 7.28 Hz, 1H), 3.51 (ddd, J = 11.73, 7.84, 4.02 Hz, 1H), 3.39 (ddd, J = 11.54, 9.91, 7.15 Hz, 1H), 2.82 (quin, J = 8.09 Hz, 1H), 2.57 (td, J = 9.16, 4.27 Hz, 1H), 2.42–2.28 (m, 1H), 1.90 (dtd, J = 13.50, 9.00, 9.00, 8.20 Hz, 1H), 1.80–1.56 (m, 2H), 0.94 (t, J = 7.40 Hz, 3H); ¹³C NMR (100 MHz, D₂O) δ 179.0, 171.8, 62.6, 49.9, 46.1, 44.3, 28.6, 23.5, 11.3.

(2S,3R)-3-((R)-Carboxy(phenyl)methyl)pyrrolidine-2-carboxylic Acid

Hydrochloride (3d-s1).—**9d-s1** (25 mg, 0.07 mmol, 1 equiv) was dissolved in DCM (1.4 mL), and the solution was cooled to 0 °C. TFA (1.4 mL) was added dropwise, and the reaction was allowed to warm to rt and stirred for 2 h. The solvents were removed under reduced pressure. The crude was purified by preparative HPLC. The combined fractions were evaporated and redissolved in 1 M HCl (3 × 30 mL), and the solvent was evaporated. The final compound was freeze-dried, which afforded the product as a white solid (12 mg, 0.04 mmol, 60%). LCMS (ESI) m/z = 250.1 [M + H]⁺; ¹H NMR (600 MHz, D₂O) δ 7.49–7.42 (m, 5H), 3.96 (d, J = 5.4 Hz, 1H), 3.88 (d, J = 10.1 Hz, 1H), 3.53 (td, J = 2.8, 7.3

Hz, 2H), 3.34 (ddt, J = 5.8, 7.92, 10.5 Hz, 1H), 2.36 (dq, J = 7.6, 14.9 Hz, 1H), 2.08 (dq, J = 7.1, 13.6 Hz, 1H); ^{13}C NMR (151 MHz, D_2O) δ 175.6, 170.8, 135.7, 129.2, 128.8, 128.5, 61.4, 53.4, 45.3, 44.9, 28.3.

(2S,3R)-3-((S)-Carboxy(phenyl)methyl)pyrrolidine-2-carboxylic Acid

Hydrochloride (3d-s2).—9d-s2 (25 mg, 0.07 mmol, 1 equiv) was dissolved in DCM (1.4 mL), and the solution was cooled to 0 °C. TFA (1.4 mL) was added dropwise, and the reaction was allowed to warm to rt and stirred for 2 h. The solvents were removed under reduced pressure. The crude was purified by preparative HPLC. The combined fractions were evaporated and redissolved in 1 M HCl (3 × 30 mL), and the solvent was evaporated. The final compound was freeze-dried, which afforded the product as a white solid (10 mg, 0.035 mmol, 50%). LCMS (ESI) m/z = 250.1 $[\text{M} + \text{H}]^+$; ^1H NMR (600 MHz, D_2O) δ 7.48–7.44 (m, 2H), 7.44–7.40 (m, 3H), 4.18 (d, J = 7.4 Hz, 1H), 3.93 (d, J = 9.3 Hz, 1H), 3.41–3.36 (m, 2H), 3.32 (p, J = 7.8 Hz, 1H), 2.03 (dq, J = 6.8, 13.5 Hz, 1H), 1.69 (dq, J = 8.4, 13.7 Hz, 1H). ^{13}C NMR (151 MHz, D_2O) δ 175.9, 171.2, 135.8, 129.2, 128.7, 128.4, 62.8, 52.9, 44.7, 44.1, 27.4.

trans-1-Benzyl 2-Methyl 3-(1,3-Dimethoxy-1,3-dioxopropan-2-yl)pyrrolidine-1,2-dicarboxylate (12a).—The title compound was prepared according to general procedure I,

starting from **10** (2.0 g, 7.65 mmol) and dimethyl malonate (2.53 g, 2.19 mL, 19.14 mmol), which afforded the title compound as a colorless oil (2.98 g, 7.57 mmol, 99%). LCMS (ESI) m/z = 394.1 $[\text{M} + \text{H}]^+$; R_f = 0.41 (1:1 heptane/EtOAc); ^1H NMR (600 MHz, CDCl_3) δ 7.38–7.28 (m, 5H), 5.18 (dd, J = 12.4, 15.5 Hz, 1H), 5.05 (dd, J = 12.4, 56.3 Hz, 1H), 4.24 (dd, J = 4.8, 25.0 Hz, 1H), 3.77–3.73 (m, 7.5H), 3.69–3.63 (m, 1H), 3.60–3.58 (m, 1H), 3.57 (s, 1.5H), 3.51 (dd, J = 1.4, 8.9 Hz, 1H), 3.05–2.97 (m, 1H), 2.20 (tdd, J = 6.4, 10.2, 12.9 Hz, 1H), 1.78 (dp, J = 7.0, 21.1 Hz, 1H); ^{13}C NMR (151 MHz, CDCl_3) (rotamers) δ 172.1, 172.0, 168.0, 168.0, 167.9, 167.9, 154.7, 154.1, 136.5, 136.4, 128.5, 128.4, 128.06, 128.04, 128.00, 127.96, 127.90, 67.24, 67.18, 62.5, 62.1, 53.8, 53.7, 52.85, 52.81, 52.79, 52.5, 52.3, 45.6, 45.1, 43.3, 42.3, 28.2, 27.6.

trans-1-Benzyl 2-Methyl 3-(1-Ethoxy-2-(ethoxycarbonyl)-1-oxopentan-2-yl)pyrrolidine-1,2-dicarboxylate (12e).—The title compound was prepared according

to general procedure I, starting from **10** (200 mg, 0.77 mmol) and diethyl 2-propylmalonate (387 mg, 0.39 mL, 1.91 mmol), which afforded the title compound as a colorless oil (146 mg, 0.31 mmol, 41%). LCMS (ESI) m/z = 464.2 $[\text{M} + \text{H}]^+$; R_f = 0.31 (2:1 heptane/EtOAc); ^1H NMR (400 MHz, CDCl_3) δ 7.39–7.27 (m, 6H), 5.24–4.96 (m, 2H), 4.55 (dd, J = 3.6, 18.8 Hz, 1H), 4.26–3.94 (m, 4H), 3.77 (s, 1.5H), 3.58 (s, 1.5H), 3.51 (ddd, J = 6.4, 8.8, 10.5 Hz, 2H), 3.06–2.93 (m, 1H), 2.30–2.18 (m, 1H), 2.06–1.87 (m, 3H), 1.28–1.12 (m, 8H), 0.91 (t, J = 7.2 Hz, 3H); ^{13}C NMR (101 MHz, CDCl_3) (rotamers) δ 173.4, 173.3, 170.7, 170.62, 170.57, 170.5, 154.6, 154.2, 136.8, 136.7, 128.7, 128.5, 128.2, 128.1, 128.0, 127.9, 67.2, 61.7, 61.6, 61.4, 60.9, 59.9, 59.8, 52.5, 52.3, 46.9, 46.0, 45.7, 45.4, 36.4, 26.5, 25.8, 17.74, 17.72, 14.5, 14.12, 14.07, 14.05, 13.99.

trans-1-Benzyl 2-Methyl 3-(1-Ethoxy-2-(ethoxycarbonyl)-3-methyl-1-

oxobutan-2-yl)pyrrolidine-1,2-dicarboxylate (12f).—The title compound was

prepared according to general procedure I, starting from **10** (200 mg, 0.77 mmol) and diethyl 2-isopropylmalonate (387 mg, 0.39 mL, 1.91 mmol), which afforded the title compound as a colorless oil (125 mg, 0.27 mmol, 35%). LCMS (ESI) m/z = 464.2 $[M + H]^+$; R_f = 0.32 (2:1 heptane/EtOAc); 1H NMR (400 MHz, $CDCl_3$) δ 7.37–7.27 (m, 5H), 5.18 (dd, J = 5.9, 12.5 Hz, 1H), 5.02 (dd, J = 12.4, 14.2 Hz, 1H), 4.51 (dd, J = 3.4, 19.3 Hz, 1H), 4.26–3.95 (m, 4H), 3.76 (s, 1.5H), 3.58 (s, 1.5H), 3.55–3.38 (m, 2H), 3.25–3.12 (m, 1H), 2.54 (dp, J = 6.6, 10.0 Hz, 1H), 2.31–2.17 (m, 1H), 2.07–1.90 (m, 1H), 1.31–1.10 (m, 6H), 1.03–0.91 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) (rotamers) δ 172.9, 169.8, 169.60, 169.57, 169.5, 154.6, 154.2, 136.8, 136.7, 128.55, 128.49, 128.23, 128.19, 128.12, 128.05, 67.2, 67.1, 64.3, 64.2, 61.5, 61.5, 61.4, 61.3, 60.8, 46.8, 46.0, 45.7, 45.3, 31.9, 31.8, 26.8, 26.1, 18.9, 18.7, 18.5, 18.45, 14.3, 14.2, 14.1, 14.0.

trans-1-Benzyl 2-Methyl 3-(2-Benzyl-1,3-diethoxy-1,3-dioxopropan-2-yl)pyrrolidine-1,2-dicarboxylate (12g).—

The title compound was prepared according to general procedure I (5 h reaction time), starting from **10** (180 mg, 0.69 mmol) and diethyl benzylmalonate (431 mg, 0.41 mL, 1.72 mmol), which afforded the title compound as a colorless oil (210 mg, 0.41 mmol, 60%). LCMS (ESI) m/z = 512.2 $[M + H]^+$; R_f = 0.10 (8:2 heptane/EtOAc); 1H NMR (400 MHz, $CDCl_3$) δ 7.37–7.28 (m, 5H), 7.25–7.18 (m, 3H), 7.12–7.07 (m, 2H), 5.20 (d, J = 12.3 Hz, 1H), 5.01 (d, J = 12.9 Hz, 1H), 4.67 (br d, J = 21.1 Hz, 1H), 4.20–3.79 (m, 4H), 3.73 (s, 1.5H), 3.57 (s, 1.5H), 3.56–3.41 (m, 2H), 3.32 (s, 2H), 2.94–2.83 (m, 1H), 2.34–2.11 (m, 2H), 1.17 (t, J = 7.2 Hz, 3H), 1.08 (t, J = 7.2 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) (rotamers) δ 173.2, 173.1, 170.3, 170.2, 170.13, 170.10, 154.5, 154.2, 136.7, 136.6, 135.4, 130.1, 128.6, 128.54, 128.49, 128.3, 128.2, 128.1, 127.4, 67.2, 61.94, 61.89, 61.8, 61.7, 61.4, 61.3, 61.2, 52.5, 52.3, 46.7, 45.9, 45.5, 45.2, 40.0, 26.2, 25.5, 14.0, 13.9, 13.83, 13.79.

trans-1-Benzyl 2-Methyl 3-(1-Ethoxy-2-(ethoxycarbonyl)-1-oxo-4-phenylbutan-2-yl)pyrrolidine-1,2-dicarboxylate (12h).—

The title compound was prepared according to general procedure I, starting from **10** (200 mg, 0.77 mmol) and diethyl 2-phenethylmalonate (506 mg, 1.91 mmol), which afforded the title compound as a colorless oil (242 mg, 0.46 mmol, 60%). LCMS (ESI) m/z = 526.3 $[M + H]^+$; R_f = 0.39 (2:1 heptane/EtOAc); 1H NMR (600 MHz, $CDCl_3$) δ 7.36–7.26 (m, 7H), 7.22–7.18 (m, 1H), 7.18–7.14 (m, 2H), 5.23–5.15 (m, 1H), 5.02 (dd, J = 12.4, 29.2 Hz, 1H), 4.60 (dd, J = 3.5, 33.1 Hz, 1H), 4.25–4.16 (m, 2H), 4.16–4.00 (m, 2H), 3.77 (s, 1.5H), 3.59 (s, 1.5H), 3.58–3.45 (m, 2H), 3.09 (ddq, J = 3.8, 8.3, 12.3 Hz, 1H), 2.63–2.54 (m, 1H), 2.50–2.43 (m, 1H), 2.32–2.20 (m, 3H), 2.13–1.99 (m, 1H), 1.28–1.18 (m, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) (rotamers) δ 173.3, 173.2, 172.7, 170.5, 170.4, 170.32, 170.30, 170.27, 170.25, 170.22, 170.18, 154.59, 154.57, 154.23, 154.18, 141.18, 141.15, 141.11, 136.8, 136.71, 136.66, 128.67, 128.65, 128.62, 128.57, 128.52, 128.51, 128.46, 128.45, 128.20, 128.16, 128.13, 128.10, 128.08, 128.06, 126.37, 126.35, 67.22, 67.18, 67.16, 61.90, 61.88, 61.86, 61.83, 61.80, 61.78, 61.6, 61.5, 61.44, 61.41, 61.0, 60.9, 59.9, 59.81, 59.80, 52.6, 52.4, 47.22, 47.14, 46.05, 46.01, 45.98, 45.4, 36.5, 36.3, 30.89, 30.87, 26.7, 26.6, 26.0, 25.9, 14.3, 14.16, 14.14, 14.12, 14.10, 14.08.

trans-1-Benzyl 2-Methyl 3-(2-Fluoro-1,3-dimethoxy-1,3-dioxopropan-2-yl)pyrrolidine-1,2-dicarboxylate (12i).—The title compound was prepared according to general procedure I, starting from **10** (200 mg, 0.77 mmol) and dimethyl 2-fluoromalonate (287 mg, 1.91 mmol), which afforded the title compound as a colorless oil (198 mg, 0.48 mmol, 63%). LCMS (ESI) m/z = 412.1 $[M + H]^+$; R_f = 0.32 (2:1 heptane/EtOAc); 1H NMR (600 MHz, $CDCl_3$) δ 7.37–7.27 (m, 5H), 5.18 (dd, J = 12.3, 34.5 Hz, 1H), 5.03 (dd, J = 12.4, 67.1 Hz, 1H), 4.37 (dd, J = 5.5, 40.4 Hz, 1H), 3.84–3.80 (m, 6H), 3.81–3.72 (m, 1H), 3.74 (s, 1.5H), 3.57–3.52 (m, 1H), 3.51 (s, 1.5H), 3.39–3.26 (m, 1H), 2.22–2.08 (m, 1H), 2.03–1.90 (m, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) (rotamers) δ 172.1, 165.5, 165.4, 165.33, 165.31, 165.27, 165.23, 165.16, 165.1, 154.5, 153.9, 136.5, 136.4, 128.6, 128.5, 128.2, 128.14, 128.07, 128.0, 95.4, 95.1, 94.1, 93.7, 67.4, 67.3, 59.52, 59.50, 58.85, 58.83, 53.84, 53.81, 53.7, 52.7, 52.4, 48.3, 48.1, 47.1, 46.9, 46.2, 45.8, 32.0, 26.03, 26.01, 25.3, 25.2, 22.8, 14.3, 14.2.

trans-1-Benzyl 2-Methyl 3-(1,2,3-Trimethoxy-1,3-dioxopropan-2-yl)pyrrolidine-1,2-dicarboxylate (12j).—The title compound was prepared according to general procedure I (24 h reaction time), starting from **10** (135 mg, 0.52 mmol) and dimethyl methoxymalonate (209 mg, 0.18 mL, 1.29 mmol), which afforded the title compound as a colorless oil (149 mg, 0.35 mmol, 68%). LCMS (ESI) m/z = 424.2 $[M + H]^+$; R_f = 0.40 (1:1 heptane/EtOAc); 1H NMR (400 MHz, $CDCl_3$) (rotamers) δ 7.38–7.27 (m, 5H), 5.18 (d, J = 12.5 Hz, 1H), 5.05 (d, J = 12.5 Hz, 1H), 4.61 (br d, J = 23.3, 1H), 3.77–3.71 (4s, 7.5H), 3.61–3.45 (m, 2H), 3.57 (s, 1.5H), 3.48 (br s, 3H), 3.17–3.09 (m, 1H), 2.17–2.05 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) (rotamers) δ 172.93, 172.91, 168.3, 168.14, 168.09, 154.6, 154.2, 136.7, 128.50, 128.47, 128.1, 128.0, 85.3, 85.2, 67.24, 67.21, 60.3, 59.7, 55.4, 53.0, 52.9, 52.2, 52.80, 52.6, 52.4, 48.9, 47.8, 46.2, 45.7, 29.8, 26.0, 25.2.

trans-1-Benzyl 2-Methyl 3-(2-((tert-Butoxycarbonyl)amino)-1,3-diethoxy-1,3-dioxopropan-2-yl)pyrrolidine-1,2-dicarboxylate (12k).—The title compound was prepared according to general procedure I, starting from **10** (200 mg, 0.77 mmol) and diethyl 2-((tert-butoxycarbonyl)amino)malonate (527 mg, 1.91 mmol), which afforded the title compound as a colorless oil (347 mg, 0.65 mmol, 84%). LCMS (ESI) m/z = 537.2 $[M + H]^+$; R_f = 0.28 (2:1 heptane/EtOAc); 1H NMR (400 MHz, $CDCl_3$) δ 7.35–7.27 (m, 5H), 5.99 (d, J = 6.17 Hz, 1H), 5.18 (dd, J = 12.3, 23.3 Hz, 1H), 5.00 (dd, J = 12.5, 47.7 Hz, 1H), 4.58 (dd, J = 2.77, 22.91 Hz, 1H), 4.33–4.14 (m, 3H), 4.14–3.99 (m, 1H), 3.73 (s, 1.5H), 3.56 (s, 1.5H), 3.59–3.49 (m, 2H), 3.44–3.34 (m, 1H), 2.32–2.18 (m, 1H), 2.12–2.03 (m, 1H), 1.40 (s, 9H), 1.22 (dt, J = 7.10, 15.46 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) (rotamers) δ 172.8, 172.7, 167.5, 167.4, 167.3, 167.1, 154.6, 154.3, 154.2, 136.7, 136.7, 128.54, 128.47, 128.08, 128.03, 128.00, 80.9, 80.8, 67.2, 67.13, 67.07, 63.1, 62.9, 62.8, 60.9, 60.5, 52.5, 52.3, 48.4, 47.3, 46.3, 45.6, 31.0, 28.23, 28.20, 26.1, 25.2, 14.04, 14.00, 13.90, 13.86.

trans-Triethyl 1-(1-((Benzyloxy)carbonyl)-2-(methoxycarbonyl)-pyrrolidin-3-yl)ethane-1,1,2-tricarboxylate (12l).—The title compound was prepared according to general procedure I, starting from **10** (200 mg, 0.77 mmol) and triethylethane-1,1,2-tricarboxylate (471 mg, 0.44 mL, 1.91 mmol), which afforded the title compound as a colorless oil (285 mg, 0.56 mmol, 73%). LCMS (ESI) m/z = 508.2 $[M + H]^+$; R_f = 0.23 (2:1

heptane/EtOAc); ^1H NMR (400 MHz, CDCl_3) δ 7.37–7.27 (m, 5H), 5.18 (dd, J = 12.41, 15.72 Hz, 1H), 5.03 (dd, J = 12.44, 30.17 Hz, 1H), 4.60 (dd, J = 4.21, 10.41 Hz, 1H), 4.26–4.14 (m, 3H), 4.14–4.03 (m, 3H), 3.74 (s, 1.5H), 3.67–3.59 (m, 1H), 3.55 (s, 1.5H), 3.54–3.46 (m, 1H), 3.19–3.10 (m, 1H), 3.02 (d, J = 7.95 Hz, 2H), 2.29–2.17 (m, 1H), 2.08–1.88 (m, 1H), 1.29–1.13 (m, 9H); ^{13}C NMR (101 MHz, CDCl_3) δ 173.0, 170.10, 170.08, 169.4, 169.3, 154.6, 154.1, 136.7, 136.6, 128.6, 128.5, 128.14, 128.09, 67.2, 62.19, 62.16, 61.4, 61.0, 60.8, 57.5, 57.49, 52.5, 52.3, 47.4, 46.3, 46.2, 45.6, 38.5, 38.3, 27.1, 26.4, 14.2, 14.00, 13.98, 13.93, 13.91.

trans-1-Benzyl 2-Methyl 3-(4-Cyano-1-ethoxy-2-(ethoxycarbonyl)-1-oxobutan-2-yl)pyrrolidine-1,2-dicarboxylate (12m).—The title compound was prepared according to general procedure I, starting from **10** (200 mg, 0.77 mmol) and diethyl 2-(2-cyanoethyl)malonate (408 mg, 0.38 mL, 1.91 mmol), which afforded the title compound as a colorless oil (354 mg, 0.74 mmol, 97%). LCMS (ESI) m/z = 475.2 $[\text{M} + \text{H}]^+$; R_f = 0.39 (2:1 heptane/EtOAc); ^1H NMR (600 MHz, CDCl_3) δ 7.37–7.28 (m, 5H), 5.19 (dd, J = 12.3, 16.8 Hz, 1H), 5.03 (dd, J = 12.4, 33.4 Hz, 1H), 4.49 (dd, J = 3.8, 36.7 Hz, 1H), 4.26–4.05 (m, 4H), 3.77 (s, 1.5H), 3.58 (s, 1.5H), 3.64–3.48 (m, 2H), 2.97–2.89 (m, 1H), 2.54–2.46 (m, 1H), 2.46–2.39 (m, 1H), 2.33–2.26 (m, 2H), 2.25–2.15 (m, 1H), 2.04–1.92 (m, 1H), 1.27–1.19 (m, 6H); ^{13}C NMR (151 MHz, CDCl_3) (rotamers) δ 172.81, 172.78, 169.2, 169.1, 169.04, 169.03, 154.5, 154.0, 136.55, 136.47, 128.60, 128.57, 128.24, 128.21, 128.1, 118.9, 67.37, 67.36, 62.50, 62.47, 62.4, 61.2, 60.7, 59.1, 59.0, 52.7, 52.5, 48.0, 46.8, 46.0, 45.4, 30.2, 30.1, 26.8, 26.2, 14.01, 13.99, 13.9, 13.4.

trans-3-(Carboxymethyl)pyrrolidine-2-carboxylic Acid Hydrochloride (3a).—The title compound was prepared according to general procedure II, starting from **12a** (1.50 g, 4.13 mmol) and stirred for 5 days, which afforded the title compound as a white solid (753 mg, 3.59 mmol, 87%). LCMS (ESI) m/z = 174.1 $[\text{M} + \text{H}]^+$; ^1H NMR (600 MHz, D_2O) δ 4.15 (d, J = 8.46 Hz, 1H), 3.51 (ddd, J = 4.48, 8.29, 11.84 Hz, 1H), 3.43 (ddd, J = 7.34, 9.03, 11.80 Hz, 1H), 2.91–2.81 (m, 2H), 2.72–2.65 (m, 1H), 2.39 (dtd, J = 4.43, 7.27, 13.26 Hz, 1H), 1.87 (dq, J = 8.74, 13.48 Hz, 1H); ^{13}C NMR (151 MHz, D_2O) δ 175.4, 171.0, 62.9, 45.2, 38.5, 36.2, 29.7.

trans-3-(1-Carboxybutyl)pyrrolidine-2-carboxylic Acid Hydrochloride (3e).—The title compound was prepared according to general procedure II, starting from **12e** (140 mg, 0.30 mmol) and stirred for 6 days. The diastereomers could not be separated on preparative HPLC, which afforded a mixture of the *trans*-diastereomers **3e** as a white solid (21 mg, 0.08 mmol, 28%). d.r. 63:37; LCMS (ESI) m/z = 216.1 $[\text{M} + \text{H}]^+$; ^1H NMR (600 MHz, D_2O) (minor *trans*-diastereomer in brackets) δ 4.21 (d, J = 6.8 Hz, 1H), [4.16 (d, J = 7.34 Hz, 1H)], 3.45–3.37 (m, 1H), [3.45–3.37 (m, 1H)], 3.35–3.26 (m, 1H), [3.35–3.26 (m, 1H)], 2.76–2.65 (m, 2H), [2.76–2.65 (m, 1H)], [2.71–2.65 (m, 1H)], [2.26 (dtd, J = 3.92, 7.35, 14.31 Hz, 1H)], 2.23–2.16 (m, 1H), [1.90–1.77 (m, 1H)], 1.90–1.77 (m, 1H), 1.62 (tdd, J = 5.1, 9.7, 14.0 Hz, 1H), [1.58–1.51 (m, 2H)], 1.58–1.51 (m, 1H), [1.34–1.17 (m, 2H)], 1.34–1.17 (m, 2H), [0.82 (t, J = 7.33 Hz, 3H)], 0.82 (t, J = 7.4 Hz, 3H); ^{13}C NMR (151 MHz, D_2O) (minor *trans*-diastereomer in brackets) δ [178.5], 178.0, 171.4, [171.3], [61.9],

61.6, [47.6], 46.8, 45.6, [45.5], [43.9], 43.6, 32.3, [31.6], [28.0], 27.3, 19.9, [19.8], [13.1], 13.0.

trans-3-(1-Carboxy-2-methylpropyl)pyrrolidine-2-carboxylic Acid

Hydrochloride (3f).—The title compound was prepared according to general procedure II, starting from 12f (120 mg, 0.25 mmol) and stirred for 8 days. The diastereomers could not be separated on preparative HPLC, which afforded a mixture of the *trans*-diastereomers **3f** as a white solid (27 mg, 0.11 mmol, 43%). d.r. 67:33; LCMS (ESI) m/z = 216.1 $[M + H]^+$; 1H NMR (600 MHz, D₂O) (minor *trans*-diastereomer in brackets) δ [4.25 (d, J = 7.2 Hz, 1H)], 4.17 (d, J = 8.2 Hz, 1H), [3.56–3.44 (m, 1H)], 3.56–3.44 (m, 1H), [3.44–3.36 (m, 1H)], 3.44–3.36 (m, 1H), [2.97–2.86 (m, 1H)], 2.97–2.86 (m, 1H), 2.61 (t, J = 7.3 Hz, 1H), [2.49 (dd, J = 56.0, 8.8 Hz, 1H)], [2.37 (dtd, J = 4.1, 7.3, 13.6 Hz, 1H)], 2.34–2.25 (m, 1H), [2.09–2.00 (m, 1H)], 2.09–2.00 (m, 2H), [1.94–1.84 (m, 1H)], 0.99 (dd, J = 6.7, 24.9 Hz, 6H), [0.98 (dd, J = 6.9, 51.2 Hz, 6H)]; ^{13}C NMR (151 MHz, D₂O) (minor *trans*-diastereomer in brackets) δ [177.2], 177.1, [171.3], 171.1, [62.2], 61.6, [53.6], 53.1, 45.5, [45.2], [42.2], 41.5, 30.2, [28.4], [28.1], 26.7, [20.5], 19.8, 19.1, [17.3].

trans-3-(1-Carboxy-2-phenylethyl)pyrrolidine-2-carboxylic Acid Hydrochloride

(3g).—The title compound was prepared according to general procedure II, starting from 12g (184 mg, 0.36 mmol) and stirred for 7 days. The diastereomers could not be separated on preparative HPLC, which afforded a mixture of the *trans*-diastereomers **3g** as a white solid (24 mg, 0.080 mmol, 22%). d.r. 58:42; LCMS (ESI) m/z = 264.1 $[M + H]^+$; 1H NMR (400 MHz, D₂O) δ 7.43–7.38 (m, 2H), 7.36–7.31 (m, 3H), 4.34 (d, J = 7.5 Hz, 1H), 3.60–3.37 (m, 2H), 3.22–2.93 (m, 3H), 2.83 (p, J = 7.6 Hz, 1H), 2.49–2.29 (2m, 1H), 2.10–1.96 (m, 1H); ^{13}C NMR (151 MHz, D₂O) δ 178.4, 178.2, 173.9, 173.6, 139.44, 139.41, 129.72, 129.69, 129.65, 129.61, 127.7, 127.6, 64.0, 63.9, 51.1, 50.4, 46.0, 45.9, 45.0, 44.8, 37.3, 36.4, 28.8, 28.1.

trans-3-(1-Carboxy-3-phenylpropyl)pyrrolidine-2-carboxylic Acid

Hydrochloride (3h).—The title compound was prepared according to general procedure II, starting from 12h (225 mg, 0.43 mmol) and stirred for 5 days. The diastereomers could not be separated on preparative HPLC, which afforded a mixture of the *trans*-diastereomers **3h** as a white solid (48 mg, 0.15 mmol, 36%). LCMS (ESI) m/z = 278.1 $[M + H]^+$; 1H NMR (600 MHz, D₂O) δ 7.39 (t, J = 7.5 Hz, 2H), 7.33–7.28 (m, 3H), 4.21 (d, J = 7.5 Hz, 1H), 3.50–3.44 (m, 1H), 3.42–3.33 (m, 1H), 2.86–2.72 (m, 3H), 2.72–2.60 (m, 1H), 2.34–2.24 (m, 1H), 2.13–1.83 (m, 3H); ^{13}C NMR (151 MHz, D₂O) δ 178.0, 171.2, 141.4, 128.7, 128.6, 126.3, 61.7, 47.1, 45.4, 43.7, 32.6, 31.1, 28.0.

trans-3-(Carboxyfluoromethyl)pyrrolidine-2-carboxylic Acid Hydrochloride (3i-d1).

—The title compound was prepared according to general procedure II, starting from 12i (150 mg, 0.36 mmol) and stirred for 4 days. The diastereomers were separated on preparative HPLC, which afforded the *trans*-diastereomer **3i-d1** as a white solid (4 mg, 0.02 mmol, 5%). d.r. 95:5; LCMS (ESI) m/z = 192.1 $[M + H]^+$; 1H NMR (600 MHz, D₂O) δ 5.29 (dd, J = 2.8, 47.5 Hz, 1H), 4.41 (d, J = 6.6 Hz, 1H), 3.50 (ddd, J = 5.4, 7.7, 12.6 Hz, 1H), 3.41 (dt, J = 7.9, 11.5 Hz, 1H), 3.26 (dq, J = 2.7, 8.9, 31.7 Hz, 1H), 2.43 (dtd, J = 5.5, 7.5,

13.8 Hz, 1H), 2.14 (dq, $J = 7.9, 13.7$ Hz, 1H); ^{13}C NMR (151 MHz, D_2O) δ 171.8, 171.6, 170.5, 88.8, 87.6, 58.3, 58.2, 45.9, 43.8, 43.7, 27.12, 27.10.

And *trans*-diastereomer **3i-d2** as a white solid (35 mg, 0.15 mmol, 43%). d.r. 1:99; LCMS (ESI) $m/z = 192.1$ $[\text{M} + \text{H}]^+$; ^1H NMR (600 MHz, D_2O) δ 5.41 (dd, $J = 2.6, 49.0$ Hz, 1H), 4.41 (d, $J = 7.9$ Hz, 1H), 3.53 (ddd, $J = 5.2, 8.2, 11.7$ Hz, 1H), 3.46 (dt, $J = 8.0, 11.7$ Hz, 1H), 3.21 (dq, $J = 2.7, 8.2, 31.9$ Hz, 1H), 2.21 (dtd, $J = 5.1, 7.8, 13.2$ Hz, 1H), 2.10 (dq, $J = 8.3, 13.6$ Hz, 1H); ^{13}C NMR (151 MHz, D_2O) δ 172.5, 172.4, 171.1, 88.5, 87.3, 60.22, 60.19, 45.6, 44.1, 44.0, 23.82, 23.80.

trans-3-(Carboxy(methoxy)methyl)pyrrolidine-2-carboxylic Acid Hydrochloride (3j-d1).—The title compound was prepared according to general procedure II, starting from **12j** (189 mg, 0.45 mmol) and stirred for 5 days. One diastereomer was purified on preparative HPLC, which afforded the *trans*-diastereomer **3j-d1** as a white solid (6 mg, 0.025 mmol, 6%). d.r. 1:99; LCMS (ESI) $m/z = 204.1$ $[\text{M} + \text{H}]^+$; ^1H NMR (600 MHz, D_2O) δ 4.41 (d, $J = 5.7$ Hz, 1H), 4.12 (d, $J = 3.5$ Hz, 1H), 3.55–3.50 (m, 1H), 3.48 (s, 3H), 3.46–3.35 (m, 1H), 3.14–3.09 (m, 1H), 2.40–2.33 (m, 1H), 2.13–2.06 (m, 1H); ^{13}C NMR (151 MHz, D_2O) δ 175.6, 172.1, 80.7, 60.3, 59.6, 46.8, 45.4, 28.3.

trans-3-(Amino(carboxy)methyl)pyrrolidine-2-carboxylic Acid Dihydrochloride (3k).—The title compound was prepared according to general procedure II, starting from **12k** (300 mg, 0.59 mmol) and stirred for 5 days. The diastereomers could not be separated on preparative HPLC, which afforded a mixture of the *trans*-diastereomers **3k** as a white solid (57 mg, 0.22 mmol, 37%). d.r. 50:50; LCMS (ESI) $m/z = 189.1$ $[\text{M} + \text{H}]^+$; ^1H NMR (600 MHz, D_2O) (two diastereomers) δ 4.53 (d, $J = 9.2$ Hz, 1H), 4.32 (d, $J = 5.7$ Hz, 1H), 4.30–4.24 (m, 1H), 3.48 (tdd, $J = 3.6, 7.9, 12.0$ Hz, 1H), 3.38–3.29 (m, 1H), 2.99 (qd, $J = 4.8, 8.9$ Hz, 1H), 2.95–2.85 (m, 1H), 2.42–2.30 (m, 1H), 2.24–2.15 (m, 1H), 1.98–1.88 (m, 1H); ^{13}C NMR (151 MHz, D_2O) (two diastereomers) δ 170.79, 170.75, 169.9, 169.8, 61.8, 60.5, 54.1, 53.6, 45.3, 44.9, 42.1, 41.6, 27.8, 27.0.

trans-2-(2-Carboxypyrrolidin-3-yl)succinic Acid Hydrochloride (3l).—The title compound was prepared according to general procedure II, starting from **12l** (275 mg, 0.54 mmol) and stirred for 9 days. The diastereomers could not be separated on preparative HPLC, which afforded a mixture of the *trans*-diastereomers **3l** as a white solid (48 mg, 0.18 mmol, 33%). d.r. 63:37; LCMS (ESI) $m/z = 232.1$ $[\text{M} + \text{H}]^+$; ^1H NMR (400 MHz, D_2O) (minor *trans*-diastereomer in brackets) δ 4.36 (d, $J = 8.5$ Hz, 1H), [4.33 (d, $J = 7.4$ Hz, 1H)], 3.49–3.39 (m, 1H), [3.49–3.39 (m, 1H)], 3.35–3.25 (m, 1H), [3.35–3.25 (m, 1H)], 3.22 (ddd, $J = 4.6, 5.6, 10.1$ Hz, 1H), [3.02 (ddd, $J = 4.8, 7.4, 9.7$ Hz, 1H)], 2.86–2.62 (m, 3H), [2.86–2.62 (m, 3H)], 2.31–2.19 (m, 1H), [2.31–2.19 (m, 1H)], 1.90–1.76 (m, 1H), [1.90–1.76 (m, 1H)]; ^{13}C NMR (151 MHz, D_2O) (minor *trans*-diastereomer in brackets) δ [176.7], 176.0, 175.53, [175.51], 171.0, [170.9], 61.2, [61.0], 45.6, [45.5], [43.2], 43.0, [42.8], 42.2, 34.6, [33.7], [28.1], 27.1.

trans-2-(2-Carboxypyrrolidin-3-yl)pentanedioic Acid Hydrochloride (3m).—The title compound was prepared according to general procedure II, starting from **12m** (300 mg, 0.63 mmol) and stirred for 9 days. The diastereomers could not be separated on preparative

HPLC, which afforded a mixture of the *trans*-diastereomers **3m** as a white solid (52 mg, 0.18 mmol, 29%). d.r. 67:33; LCMS (ESI) m/z = 246.1 $[M + H]^+$; 1H NMR (600 MHz, D_2O) (minor *trans*-diastereomer in brackets) δ 4.33 (d, J = 6.9 Hz, 1H), [4.28 (d, J = 7.4 Hz, 1H)], 3.56–3.48 (m, 1H), [3.56–3.48 (m, 1H)], 3.45–3.36 (m, 1H), [3.45–3.36 (m, 1H)], 2.90–2.80 (m, 2H), [2.90–2.80 (m, 1H)], [2.72–2.69 (m, 1H)], 2.58–2.43 (m, 2H), [2.58–2.43 (m, 2H)], [2.40–2.35 (m, 1H)], 2.32 (dtd, J = 4.5, 7.3, 13.8 Hz, 1H), 2.08–1.89 (m, 3H), [2.08–1.89 (m, 3H)]; ^{13}C NMR (151 MHz, D_2O) (minor *trans*-diastereomer in brackets) δ [177.4], 177.33, [177.27], 176.9, 171.6, [171.4], [61.9], 61.7, [46.6], 46.1, 45.5, [45.4], [43.7], 43.6, 31.34, [31.29], [27.8], 27.3, 25.2, [24.4].

trans-1-Benzyl 2-Methyl 3-(3-(Ethoxycarbonyl)-2-oxotetrahydrofuran-3-yl)pyrrolidine-1,2-dicarboxylate (12n).—The title compound was prepared according to general procedure I, starting from **10** (200 mg, 0.77 mmol) and ethyl 2-oxotetrahydrofuran-3-carboxylate (303 mg, 1.91 mmol), which afforded the title compound as a colorless oil (228 mg, 0.54 mmol, 71%). LCMS (ESI) m/z = 420.2 $[M + H]^+$; R_f = 0.25 (1:1 heptane/EtOAc); 1H NMR (400 MHz, $CDCl_3$) δ 7.38–7.27 (m, 5H), 5.27–4.93 (m, 2H), 4.44–4.11 (m, 5H), 3.75 (d, J = 5.95 Hz, 1.5H), 3.72–3.64 (m, 1H), 3.64–3.55 (m, 1H), 3.53 (d, J = 4.05 Hz, 1.5H), 3.32 (td, J = 5.79, 7.84 Hz, 0.5H), 3.15 (dtd, J = 5.42, 7.66, 13.28 Hz, 0.5H), 2.74 (dq, J = 2.21, 6.87, 14.18 Hz, 1H), 2.37–2.17 (m, 2H), 1.89–1.71 (m, 1H), 1.33–1.21 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) (rotamers) δ 173.0, 172.93, 172.87, 172.6, 172.3, 172.2, 168.3, 168.2, 168.11, 168.07, 154.7, 154.6, 154.0, 136.5, 136.35, 136.31, 128.60, 128.56, 128.54, 128.21, 128.15, 128.08, 128.05, 67.5, 67.4, 66.5, 66.4, 66.1, 63.03, 62.97, 62.9, 61.6, 61.22, 61.19, 60.7, 56.89, 56.87, 56.77, 56.69, 52.66, 52.5, 52.4, 52.3, 46.9, 46.6, 46.3, 46.1, 46.0, 45.9, 45.7, 45.5, 31.0, 29.8, 29.8, 28.71, 28.66, 27.7, 27.1, 26.9, 26.3, 14.1, 14.0.

Disodium trans-3-(1-Carboxylato-3-hydroxypropyl)pyrrolidine-2-carboxylate (3n).—The title compound was prepared according to general procedure II, starting from **12n** (200 mg, 0.48 mmol) and stirred for 4 days. The diastereomers could not be separated on preparative HPLC, but afforded a mixture of the *trans*-diastereomers of the corresponding lactone-HCl salt **13**, which was submitted to 3 equiv of 0.5 M NaOH(_{aq}) and afforded a mixture of the *trans*-diastereomers **3n** as a white solid (52 mg, 0.20 mmol, 42%). d.r. 53:47; 1H NMR (600 MHz, D_2O) (minor *trans*-diastereomer in brackets) δ [4.05 (d, J = 5.2 Hz, 1H)], 3.98 (d, J = 4.6 Hz, 1H), [3.74–3.64 (m, 2H)], 3.65–3.55 (m, 2H), [3.49–3.38 (m, 2H)], 3.49–3.38 (m, 2H), [2.73 (p, J = 6.3 Hz, 1H)], [2.62–2.55 (m, 1H)], 2.53 (ddd, J = 3.7, 6.6, 10.7 Hz, 1H), 2.41 (td, J = 3.8, 10.4 Hz, 1H), [2.19–2.09 (m, 1H)], 2.19–2.09 (m, 1H), [2.01–1.91 (m, 1H)], 2.01–1.91 (m, 2H), [1.91–1.82 (m, 1H)], 1.91–1.82 (m, 1H), [1.82–1.74 (m, 1H)]; ^{13}C NMR (151 MHz, D_2O) (minor *trans*-diastereomer in brackets) δ [181.9], 181.7, [174.5], 174.4, [64.1], 63.4, [60.2], 60.1, 48.8, [46.9], 45.4, [45.10], 45.08, [45.0], 33.9, [31.8], [28.2], 27.0.

trans-1-Benzyl 2-Methyl 3-(1-Ethoxy-2-(ethoxycarbonyl)-1-oxopent-4-en-2-yl)pyrrolidine-1,2-dicarboxylate (14).—The title compound was prepared according to general procedure I (3 h reaction time), starting from **10** (270 mg, 1.03 mmol) and diethyl allylmalonate (517 mg, 0.54 mL, 2.58 mmol), which afforded the title compound as a

colorless oil (444 mg, 0.96 mmol, 93%). LCMS (ESI) m/z = 462.2 $[M + H]^+$; R_f = 0.18 (7:3 heptane/EtOAc); 1H NMR (400 MHz, $CDCl_3$) (rotamers) δ 7.38–7.28 (m, 5H), 5.71–5.57 (m, 1H), 5.19 (d, J = 12.3 Hz, 1H), 5.15–5.07 (m, 2H), 5.01 (d, J = 12.5 Hz, 1H), 4.58 (br d, J = 16.2 Hz, 1H), 4.21–3.93 (m, 4H), 3.77 (s, 1.5H), 3.59 (s, 1.5H), 3.57–3.45 (m, 2H), 3.02–2.92 (m, 1H), 2.74 (br d, J = 6.1 Hz, 2H), 2.28–2.14 (m, 1H), 2.09–1.94 (m, 1H), 1.21 (t, J = 7.2 Hz, 3H), 1.17 (t, J = 7.3 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) (rotamers) δ 173.3, 173.2, 170.1, 170.0, 169.9, 154.5, 154.2, 136.7, 136.6, 131.9, 128.6, 128.5, 128.2, 128.14, 128.11, 128.08, 119.8, 67.2, 61.8, 61.5, 61.0, 59.8, 52.5, 52.3, 46.7, 46.0, 45.6, 45.4, 38.6, 26.3, 25.6, 14.13, 14.11, 14.01, 13.98.

trans-1-Benzyl 2-Methyl 3-(1-Ethoxy-2-(ethoxycarbonyl)-5-hydroxy-1-oxopentan-2-yl)pyrrolidine-1,2-dicarboxylate (12o).—To a solution of *trans*-enantiomers **14** (410 mg, 0.89 mmol, 1 equiv) in anhydrous THF (9 mL) at 0 °C, 1 M borane tetrahydrofuran complex solution (1.3 mL, 1.33 mmol, 1.5 equiv) was added and the resulting solution was stirred at this temperature for 2 h. Then, water (80 μ L, 4.44 mmol, 5 equiv), 4 M aq NaOH (933 μ L, 3.73 mmol, 4.2 equiv), and 30% aq peroxide solution (483 μ L, 4.26 mmol, 4.8 equiv) were added to the previous mixture, which was stirred at 0 °C for an additional hour. After that, the reaction mixture was diluted with EtOAc (60 mL) and water (60 mL). The aqueous layer was extracted with EtOAc (3 $\times$ 60 mL), and the collected organic layers were washed with brine (60 mL), dried over $MgSO_4$, and filtered. The solvent of the filtrate was removed under reduced pressure to give a residue that was purified by flash chromatography (silica gel, heptane/ EtOAc 1:1, isocratic) to afford the desired mixture of *trans*-enantiomers as a colorless oil (211 mg, 0.44 mmol, 50%). LCMS (ESI) m/z = 480.2 $[M + H]^+$; R_f = 0.10 (1:1 heptane/EtOAc); 1H NMR (400 MHz, $CDCl_3$) (rotamers) δ 7.37–7.27 (m, 5H), 5.19 (d, J = 12.7 Hz, 1H), 5.02 (d, J = 12.4 Hz, 1H), 4.56 (br d, J = 19.0 Hz, 1H), 4.23–3.96 (m, 4H), 3.76 (s, 1.5H), 3.62 (t, J = 6.2 Hz, 2H), 3.58 (s, 1.5H), 3.55–3.44 (m, 2H), 3.05–2.95 (m, 1H), 2.29–2.15 (m, 1H), 2.05 (t, J = 8.2 Hz, 2H), 2.03–1.95 (m, 1H), 1.52–1.42 (m, 2H), 1.26–1.16 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) (rotamers) δ 173.31, 173.27, 170.5, 170.44, 170.42, 170.3, 154.5, 154.2, 136.7, 136.6, 129.1, 128.51, 128.47, 128.3, 128.15, 128.13, 128.10, 127.99, 125.4, 67.2, 62.5, 61.8, 61.8, 61.4, 60.9, 59.51, 59.50, 52.6, 52.3, 47.1, 46.0, 45.4, 30.7, 30.6, 27.6, 26.5, 25.8, 14.1, 14.03, 14.00.

trans-3-(1-Carboxy-4-hydroxybutyl)pyrrolidine-2-carboxylic Acid Hydrochloride (3o).—The title compound was prepared according to general procedure II, starting from **12o** (119 mg, 0.25 mmol) and stirred for 3 days. The diastereomers could not be separated on preparative HPLC, which afforded a mixture of the *trans*-diastereomers **3o** as a white solid (19 mg, 0.071 mmol, 29%). d.r. 56:44; LCMS (ESI) m/z = 250.1 $[M + H]^+$; 1H NMR (600 MHz, D_2O) (two diastereomers) δ 4.33–4.20 (2m, 1H), 3.71–3.61 (m, 2H), 3.54–3.48 (m, 1H), 3.44–3.38 (m, 1H), 2.88–2.68 (2m, 2H), 2.40–2.28 (m, 1H), 1.98–1.90 (m, 1H), 1.90–1.76 (m, 4H); ^{13}C NMR (151 MHz, D_2O) (two diastereomers) δ 178.6, 178.1, 172.4, 172.1, 62.7, 62.5, 47.6, 47.0, 46.1, 46.0, 45.5, 44.4, 44.3, 30.2, 30.1, 28.5, 28.2, 28.0, 27.4.

trans-1-Benzyl 2-Methyl 3-(1-Methoxy-2-(methoxycarbonyl)-1-oxopent-4-yn-2-yl)pyrrolidine-1,2-dicarboxylate (15).—The title compound was prepared according to general procedure I, starting from **10** (180 mg, 0.69 mmol) and dimethyl propargylmalonate (293 mg, 0.28 mL 1.72 mmol), which afforded the title compound as a colorless oil (264 mg, 0.61 mmol, 89%). LCMS (ESI) m/z = 432.2 $[M + H]^+$; R_f = 0.17 (7:3 heptane/EtOAc); 1H NMR (400 MHz, $CDCl_3$) (rotamers) δ 7.39–7.27 (m, 5H), 5.18 (d, J = 12.5 Hz, 1H), 5.05 (d, J = 12.0 Hz, 1H), 4.58–4.49 (br d, J = 11.4 Hz, 1H), 3.78 (s, 1.5H), 3.74 (s, 1.5H), 3.72 (s, 1.5H), 3.66 (s, 1.5H), 3.63 (s, 1.5H), 3.61 (s, 1.5H), 3.60–3.49 (m, 2H), 3.32–3.23 (m, 1H), 3.01–2.86 (m, 2H), 2.37–2.23 (m, 1H), 2.10–1.94 (m, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) (rotamers) δ 173.0, 172.9, 169.7, 169.63, 169.61, 169.5, 154.6, 154.2, 136.65, 136.63, 128.60, 128.5, 128.21, 128.18, 78.10, 78.07, 72.4, 67.34, 67.30, 61.3, 60.8, 58.9, 53.2, 53.1, 53.0, 52.7, 52.5, 46.5, 46.0, 45.5, 45.4, 26.6, 25.9, 24.34, 24.32.

trans-1-Benzyl 2-Methyl 3-(2-((1H-1,2,3-Triazol-4-yl)methyl)-1,3-dimethoxy-1,3-dioxopropan-2-yl)pyrrolidine-1,2-dicarboxylate (12p).—In a 9 mL vessel, a solution of **15** (391 mg, 0.91 mmol), copper(I) iodide (17 mg, 0.091 mmol), and azidotrimethylsilane (95%, 380 μ L, 2.72 mmol) in DMF·MeOH 1:1 (5 mL) was prepared under argon atmosphere. The vessel was sealed with a septum, heated to reflux, and stirred overnight. Next day, the reaction mixture was allowed to reach rt and then diluted with water (50 mL) and EtOAc (50 mL). The aqueous layer was extracted with EtOAc (3 $\times$ 50 mL), and the collected organic layers were washed with water (3 $\times$ 50 mL), dried over $MgSO_4$, and filtered. After that, the solvent of the filtrate was removed under reduced pressure to give a brown oil that was purified by flash chromatography (silica gel, heptane/EtOAc 1:1 to 2:8, gradient) to afford the desired mixture of trans-enantiomers as a white solid (307 mg, 0.65 mmol, 71%). LCMS (ESI) m/z = 475.2 $[M + H]^+$; 1H NMR (400 MHz, $CDCl_3$) (rotamers) δ 7.48 (s, 1H), 7.39–7.26 (m, 5H), 5.19 (d, J = 12.5 Hz, 1H), 5.04 (br t, J = 11.9 Hz, 1H), 4.61 (br d, J = 25.0, 1H), 3.74–3.72 (2s, 1.5H), 3.69 (s, 1.5H), 3.66 (s, 1.5H), 3.63 (s, 1.5H), 3.60 (s, 1.5H), 3.56 (s, 1.5H), 3.55–3.38 (m, 4H), 3.01–2.91 (m, 1H), 2.32–2.18 (m, 1H), 2.17–2.01 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) (rotamers) δ 173.2, 173.1, 170.4, 170.3, 170.1, 154.8, 154.4, 140.8, 136.6, 132.1, 128.72, 128.70, 128.34, 128.29, 128.26, 67.5, 61.6, 61.1, 60.3, 60.2, 53.2, 53.14, 53.10, 53.0, 52.8, 52.6, 47.0, 46.0, 45.9, 45.4, 30.03, 30.00, 26.7, 25.9.

trans-3-(1-Carboxy-2-(1H-1,2,3-triazol-4-yl)ethyl)pyrrolidine-2-carboxylic Acid Hydrochloride (3p).—The title compound was prepared according to general procedure II, starting from **12p** (289 mg, 0.61 mmol) and stirred for 3 days. The two epsilon diastereomers were separated on preparative HPLC. For the *trans*-diastereomer **3p-d1**: as a white solid (18 mg, 0.06 mmol, 10%). d.r. 99:1; LCMS (ESI) m/z = 255.1 $[M + H]^+$; 1H NMR (600 MHz, D_2O) δ 8.02 (br s, 1H), 4.49 (br d, J = 8.1 Hz, 1H), 3.56–3.50 (m, 1H), 3.43–3.36 (m, 1H), 3.34–3.28 (m, 2H), 3.22–3.15 (m, 1H), 2.87–2.80 (m, 1H), 2.37–2.31 (m, 1H), 2.00–1.92 (m, 1H); ^{13}C NMR (151 MHz, D_2O) δ 176.1, 171.6, 141.9, 127.3, 62.0, 46.8, 46.3, 43.7, 27.6, 25.5. For the *trans*-diastereomer **3p-d2**: as a white solid (8 mg, 0.03 mmol, 5%). d.r. 10:90; LCMS (ESI) m/z = 255.1 $[M + H]^+$; 1H NMR (400 MHz, D_2O) δ 7.92 (br s, 1H), 4.39 (br d, J = 7.4 Hz, 1H), 3.64–3.53 (m, 1H), 3.49–3.42 (m, 1H), 3.26–3.19 (m, 2H), 3.15–3.07 (m, 1H), 2.98–2.88 (m, 1H), 2.51–2.41 (m, 1H), 2.11–2.00

(m, 1H); ^{13}C NMR (151 MHz, D_2O) δ 177.1, 171.9, 142.2, 127.4, 62.3, 48.3, 46.1, 44.0, 28.8, 25.2. In addition, a mixture of the *trans*-diastereomers **3p-d1**/**3p-d2** was isolated as a white solid (113 mg, 0.39 mmol, 64%). LCMS (ESI) m/z = 255.1 $[\text{M} + \text{H}]^+$.

trans-1-Benzyl 2-Methyl 3-(2-(Cyanomethyl)-1,3-dimethoxy-1,3-dioxopropan-2-yl)pyrrolidine-1,2-dicarboxylate (16).—To a solution of **12a** (5.23 g, 13.3 mmol, 1 equiv) in THF (150 mL) was added NaH (532 mg, 13.3 mmol, 1 equiv) at 0 °C, and the reaction mixture was stirred for 15 min. Then bromoacetonitrile (7.92 g, 66.6 mmol, 5 equiv) was added, and the mixture was stirred at 50 °C for 23 h. The reaction was quenched with NH_4Cl (100 mL). The mixture was concentrated under reduced pressure, and following purified by DCVC (0 $\rightarrow$ 50% EtOAc in heptane), to afford the title compound as a colorless oil (4.80 g, 11.11 mmol, 84%). LCMS (ESI) m/z = 433.2 $[\text{M} + \text{H}]^+$; R_f = 0.27 (2:1 heptane/EtOAc); ^1H NMR (600 MHz, CDCl_3) δ 7.37–7.29 (m, 5H), 5.19 (dd, J = 12.4, 19.0 Hz, 1H), 5.05 (dd, J = 12.3, 41.0 Hz, 1H), 4.46 (dd, J = 4.4, 25.1 Hz, 1H), 3.80–3.73 (m, 6H), 3.72 (s, 1.5H), 3.71–3.61 (m, 1H), 3.60 (s, 1.5H), 3.53 (dt, J = 7.8, 10.8 Hz, 1H), 3.20–3.14 (m, 1H), 3.12–2.97 (m, 2H), 2.35–2.23 (m, 1H), 2.04 (ddt, J = 7.4, 14.5, 47.9 Hz, 1H); ^{13}C NMR (151 MHz, CDCl_3) (rotamers) δ 172.3, 172.2, 168.3, 168.2, 168.13, 168.08, 154.6, 154.0, 136.5, 136.4, 128.64, 128.61, 128.34, 128.29, 128.25, 128.21, 115.9, 115.8, 67.5, 67.4, 60.6, 60.5, 57.54, 57.50, 53.8, 53.72, 53.68, 52.9, 52.7, 47.3, 46.2, 46.0, 45.5, 27.0, 26.3, 22.92, 22.90, 21.2.

trans-1-Benzyl 2-Methyl 3-(2-((2H-Tetrazol-5-yl)methyl)-1,3-dimethoxy-1,3-dioxopropan-2-yl)pyrrolidine-1,2-dicarboxylate (17-s1 and 17-s2).—To a vial with a solution of **16** (1.31 g, 3.03 mmol, 1 equiv) in dry toluene (17.5 mL) were added sodium azide (1.77 g, 27.3 mmol, 9 equiv) and triethylammonium chloride (3.76 g, 27.3 mmol, 9 equiv) under N_2 atmosphere. The vial was capped and stirred for 19 h at 115 °C using a sand bath. The reaction mixture was quenched using sat. Na_2CO_3 (0.30 mL) with H_2O (15 mL) added in addition. The separated aqueous phase was acidified using 1 M HCl to about pH 2 and extracted with EtOAc (3 $\times$ 20 mL). The combined organic phases were concentrated in vacuo and purified using preparative HPLC to give the racemic product (0.796 mg, 55%). The enantiomers were obtained using a ChiralPak AD (250 $\times$ 20 mm, 10 μm) column (isocratic elution: 70:30:0.1 *n*-heptane/EtOH/TFA, 20 mL/min) after four injections of the racemic mixture. The fractions of first eluting enantiomer were pooled, concentrated in vacuo, and freeze-dried to afford pure enantiomer **17-s1** as a clear oil (419 mg, 0.88 mmol, 29%). LCMS (ESI) m/z = 476.2 $[\text{M} + \text{H}]^+$; ^1H NMR (600 MHz, CDCl_3) δ 7.40–7.27 (m, 5H), 5.21 (dd, J = 19.2, 12.3 Hz, 1H), 5.07 (dd, J = 53.9, 12.3 Hz, 1H), 4.59 (dd, J = 33.4, 3.7 Hz, 1H), 3.74–3.65 (m, 8H), 3.64–3.61 (m, 2H), 3.60–3.54 (m, 2H), 3.54–3.46 (m, 1H), 3.10–2.99 (m, 1H), 2.38–2.22 (m, 1H), 2.18–1.98 (m, 1H); ^{13}C NMR (151 MHz, CDCl_3) (rotamers) δ 173.2, 173.1, 173.0, 172.93, 172.92, 169.6, 169.54, 169.51, 169.4, 155.1, 154.4, 136.13, 136.09, 136.06, 128.7, 128.6, 128.44, 128.42, 128.2, 128.1, 67.9, 67.7, 61.2, 60.91, 60.87, 59.7, 59.6, 59.63, 59.61, 53.6, 53.55, 53.53, 53.47, 53.12, 53.11, 52.9, 47.53, 47.50, 46.48, 46.46, 46.0, 45.5, 28.2, 27.2, 26.2. The fractions of the second eluting enantiomer were pooled, concentrated in vacuo, and freeze-dried to afford the pure enantiomer **17-s2** as a clear oil (370 mg, 0.78 mmol, 26%). LCMS (ESI) m/z = 476.2 $[\text{M} + \text{H}]^+$; ^1H NMR (600 MHz, CDCl_3) δ 7.38–7.27 (m, 5H), 5.20 (dd, J = 17.4, 12.4 Hz, 1H), 5.06 (dd, J = 52.2, 12.4

Hz, 1H), 4.60 (dd, J = 29.6, 3.8 Hz, 1H), 3.72–3.64 (m, 7H), 3.64–3.58 (m, 3H), 3.56 (s, 2H), 3.53–3.46 (m, 1H), 3.10–3.00 (m, 1H), 2.29 (m, 1H), 2.18–1.96 (m, 1H); ^{13}C NMR (151 MHz, CDCl_3) (rotamers) δ 173.1, 172.9, 169.6, 169.5, 169.4, 154.9, 154.3, 136.23, 136.22, 128.65, 128.61, 128.33, 128.29, 128.14, 128.10, 67.7, 67.6, 61.3, 60.9, 59.53, 59.49, 53.52, 53.50, 53.4, 53.0, 52.8, 47.4, 46.4, 45.9, 45.5, 28.4, 28.3, 27.1, 26.1, 25.3.

trans-Dimethyl 2-((2H-Tetrazol-5-yl)methyl)-2-(2-(methoxycarbonyl)pyrrolidin-3-yl)malonate (18-s1).—To a round-bottomed flask with a three-way valve connected were added **17-s1** (419 mg, 0.88 mmol, 1 equiv) and 10 mol % Pd/C (9.3 mg, 0.1 equiv). The flask was evacuated and refilled with nitrogen three times, and MeOH (5 mL) was added. The flask was evacuated and refilled once with nitrogen and three times with hydrogen. The reaction was stirred for 1 day at rt. The reaction flask was evacuated and refilled with nitrogen three times. The reaction mixture was filtered over a pad of Celite with MeOH (25 mL) under a flow of nitrogen. The catalyst was washed with water and disposed, while the MeOH solution was concentrated in vacuo to give the crude of the desired enantiomer **18-s1** as a clear oil (313 mg, 0.92 mmol). The crude product was used in next reaction without further purification. LCMS (ESI) m/z = 342.1 $[\text{M} + \text{H}]^+$.

trans-Dimethyl 2-((2H-Tetrazol-5-yl)methyl)-2-(2-(methoxycarbonyl)pyrrolidin-3-yl)malonate (18-s2).—To a round-bottomed flask with a three-way valve connected were added **17-s2** (370 mg, 0.78 mmol, 1 equiv) and 10 mol % Pd/C (8.2 mg, 0.1 equiv). The flask was evacuated and refilled with nitrogen three times, and MeOH (4 mL) was added. The flask was evacuated and refilled once with nitrogen and three times with hydrogen. The reaction was stirred for 1 day at rt. The reaction flask was evacuated and refilled with nitrogen three times. The reaction mixture was filtered over a pad of Celite with MeOH (25 mL) under a flow of nitrogen. The catalyst was washed with water and disposed, while the MeOH solution was concentrated in vacuo to give the crude of the desired enantiomer **18-s2** as a clear oil (267 mg, 0.78 mmol). Product used in next reaction without further purification. LCMS (ESI) m/z = 342.1 $[\text{M} + \text{H}]^+$.

trans-3-(1-Carboxy-2-(2H-tetrazol-5-yl)ethyl)pyrrolidine-2-carboxylic Acid (3q-s1 and 3q-s2).—A stirring solution of crude **18-s1** (301 mg, 0.88 mmol, 1 equiv) in excess 6 M HCl was stirred under reflux for 4 days, under an argon atmosphere. The acidic solution was concentrated in vacuo, and the crude mixture of diastereomers was resolved using a chiral Chirobiotic T column (500 $\times$ 10 mm) (Astec) (isocratic 20% B; solvent A: NH_4OAc (15 mM) + AcOH to pH 4; solvent B: EtOH, 5 mL/min) to afford **3q-s1** (contains 2.5 equiv of AcONH_4) (the first eluting diastereomer) as semisolid (hygroscopic), after removal of the solvent in vacuo (181 mg, 0.40 mmol, 46% over two steps). d.r. 96:4; LCMS (ESI) m/z 256.1 $[\text{M} + \text{H}]^+$; $[\alpha]^{25}_{589} = -20.6^\circ$ (c = 0.369, 0.1 M HCl); ^1H NMR (600 MHz, D_2O) δ 4.17 (d, J = 5.7 Hz, 1H), 3.45 (dt, J = 12.9, 6.6 Hz, 1H), 3.42–3.36 (m, 1H), 3.28 (d, J = 8.2 Hz, 2H), 2.91 (q, J = 7.9 Hz, 1H), 2.60 (p, J = 7.3 Hz, 1H), 2.20 (dt, J = 13.9, 7.0 Hz, 1H), 1.91 (dt, J = 14.5, 7.2 Hz, 1H); ^{13}C NMR (151 MHz, D_2O) δ 179.1, 173.8, 157.3, 63.8, 50.2, 45.2, 44.7, 27.7, 25.9. The second eluting diastereomer, **3q-s2** (contains 0.14 of equiv AcONH_4), was obtained as a white solid after removal of the solvent in vacuo (41 mg, 0.15 mmol, 18% over two steps). d.r. 4:96; LCMS (ESI) m/z 256.1 $[\text{M} + \text{H}]^+$; $[\alpha]^{25}_{589} = -28.6^\circ$

(*c* = 0.273, 0.1 M HCl); ¹H NMR (600 MHz, D₂O) δ 4.08 (d, *J* = 4.9 Hz, 1H), 3.43 (t, *J* = 7.5 Hz, 2H), 3.25 (dd, *J* = 15.0, 9.6 Hz, 1H), 3.06 (dd, *J* = 14.9, 5.0 Hz, 1H), 2.93 (ddd, *J* = 9.6, 6.9, 5.0 Hz, 1H), 2.87–2.79 (m, 1H), 2.14 (dt, *J* = 14.8, 7.5 Hz, 1H), 1.96 (dt, *J* = 13.6, 7.0 Hz, 1H); ¹³C NMR (151 MHz, D₂O) δ 180.4, 173.8, 160.6, 63.4, 49.3, 45.0, 44.9, 26.9, 25.2.

trans-3-(1-Carboxy-2-(1H-tetrazol-5-yl)ethyl)pyrrolidine-2-carboxylic Acid

Hydrochloride (3q-s3–4).—A stirring solution of crude 18-s2 (265 mg, 0.78 mmol, 1 equiv) in excess 6 M HCl_(aq.) was stirred under reflux for 4 days, under argon. The acidic solution was concentrated in vacuo to afford a mixture of the *trans*-diastereomers **3q-s3/3q-s4** as a white solid (226 mg, 0.77 mmol, 99%). d.r. 2:1. LCMS (ESI) *m/z* 256.1 [M + H]⁺; ¹H NMR (600 MHz, D₂O) (two diastereomers) δ 4.48 (dd, *J* = 38.3, 7.9 Hz, 1H), 3.64–3.24 (m, 5H), 3.01–2.81 (m, 1H), 2.49–2.29 (m, 1H), 2.10–1.90 (m, 1H); ¹³C NMR (151 MHz, D₂O) (two diastereomers) δ 175.5, 174.9, 171.1, 171.0, 154.6, 154.5, 61.4, 61.2, 45.54, 45.51, 45.4, 44.5, 43.2, 43.1, 28.2, 26.9, 24.0, 23.3.

Pharmacological Characterization.

Ligand binding affinities for native AMPA, KA, and NMDA receptors in rat cortical synaptosomes were determined as previously described using [³H]-AMPA, [³H]-KA, and [³H]-CGP 39653, respectively.⁴⁰ CPM values were fitted by nonlinear regression using GraphPad Prism 7.0 (GraphPad Software, San Diego, CA). Ligand affinities at recombinant rat homomeric GluK1–3 were determined as previously described^{21,52} using [³H]-KA and the newly developed radioligand [³H]-NF608 for GluK1.⁶⁹ Competition curves (*n* = 3) were conducted in triplicate at 12–16 ligand concentrations. Data were analyzed using GraphPad Prism v6 (GraphPad Software, San Diego, CA) to determine ligand affinity (*K_i* one site equation) and Hill slope (four-parameter logistic equation) values. The inhibitory concentrations of the compounds were determined at human EAAT1, EAAT2, and EAAT3 stably expressed in HEK293 cells in a conventional [³H]-D-Asp uptake assay essentially as described previously.⁷⁰ The cell lines were cultured at 37 °C in a humidified 5% CO₂ atmosphere in Dulbecco's modified Eagle's medium Glutamax-I supplemented with 5% dialyzed fetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin, and 1 mg/mL G-418. The day before the assay, cells were split into poly-D-lysine-coated white 96-well plates. At 16–24 h later, the culture medium was aspirated and cells were washed twice with 100 μL of assay buffer (Hank's buffered saline solution supplemented with 20 mM HEPES, 1 mM CaCl₂ and 1 mM MgCl₂, pH 7.4). Then 50 μL of assay buffer supplemented with 100 nM [³H]-D-Asp (PerkinElmer, Boston, MA) and various concentrations of test compounds were added to the wells, and the plate was incubated at 37 °C for 3 min. Nonspecific [³H]-D-Asp uptake/binding in the cells was determined in the presence of 3 mM (S)-Glu. The assay mixtures were quickly removed from the wells, and the wells were washed with 2 × 100 μL ice-cold assay buffer, after which 150 μL of Microscint-20 scintillation fluid (PerkinElmer, Boston, MA) was added to each well. Then the plate was shaken for at least 1 h and counted in a TopCounter (PerkinElmer, Boston, MA). The experiments were performed in duplicate at least three times for each compound. Functional characterization at cloned GluN1/GluN2A-D receptors was done using rat cDNAs for GluN1–1a (GenBank U08261; hereafter GluN1), GluN2A (D13211), GluN2B (U11419), GluN2C (M91563), and GluN2D (L31611)

were provided by S. Heinemann (Salk Institute), S. Nakanishi (Osaka Bioscience Institute), and P. Seeburg (University of Heidelberg). The cDNA encoding rat GluN2B was modified by removing a T7 RNA polymerase termination site located in the C-terminal domain, without changing the amino acid sequence.⁷¹ DNA constructs were first linearized by restriction enzymes and then used as templates for in vitro cRNA transcription. The cRNA encoding GluN1 was coinjected with different GluN2 subunit cRNA at a volume of 50 nL (0.5–5 ng) into *Xenopus* oocytes (purchased from Rob Weymouth at Xenopus 1, Dexter, MI). The injected oocytes were incubated at 17 °C for at least 2 days before recording in modified Barth's solution containing (in mM) 88 NaCl, 1.0 KCl, 15 HEPES, 2.4 NaHCO₃, 0.41 CaCl₂, 0.82 MgSO₄, and 0.3 Ca(NO₃)₂ (pH 7.5; osmolarity 0.22 Osm/kg) supplemented with 0.1 U/L penicillin and 0.1 mg/L streptomycin. Two-electrode voltage-clamp recordings were performed essentially as previously described.⁷¹ Briefly, a gravity-driven perfusion system was used with extracellular recording solution containing (in mM) 90 NaCl, 1 KCl, 10 HEPES, 0.5 BaCl₂, and 0.01 EDTA (pH 7.4; osmolarity 0.20 Osm/kg) at a flow rate of 5 mL/min. Oocytes expressing GluN1/2A or GluN1/2B were injected with 50 nL or 30 nL of 50 mM BAPTA at least 10 min before recording to prevent activity-dependent increases in response amplitude.^{72,73} For antagonist concentration–inhibition experiments, 100 μM glycine and 1 μM glutamate were coapplied to elicit current response followed by increasing concentrations of compound to inhibit current the response. Concentration–response data were analyzed using GraphPad Prism (GraphPad Software). Antagonist concentration–inhibition data were fitted to

$$I = I_{\min} + (I_{\max} - I_{\min}) / (1 + 10^{((\log[A] - \log IC_{50})nH)})$$

, where $I_{\min}$ is the minimum current in

response to the agonist plus a saturating concentration of antagonist. For some combinations of antagonist and NMDA receptor subtype, $I_{\min}$ was constrained to be 0. For graphical presentation, the data from individual oocytes were normalized to the current response to glutamate plus glycine in the same recording and averaged. Antagonist binding affinities were estimated using published glutamate EC₅₀ values for GluN1/2A–D,⁷⁴ IC₅₀ values for individual oocytes, the agonist concentration $[A]$, and the Cheng–Prusoff relationship: $K_i = IC_{50} / (1 + [A] / EC_{50})$.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

AMPA

α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

CPAA	(2 <i>S</i> ,3 <i>R</i>)-2-carboxy-3-pyrrolidine acetic acid
DCVC	dry column vacuum chromatography
DHK	dihydrokainic acid
EAATs	excitatory amino acid transporters
Glu	(<i>S</i>)-glutamic acid
GluK1–3	Kainate receptor subunits 1–3
iGluRs	ionotropic Glu receptors
KA	kainic acid
NMDA	<i>N</i> -methyl-D-aspartate
SAR	structure–activity relationship

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Figure 1.

Crystal structures of Boc-protected **3c-s1** (**9c-s1**, A) and **3d-s2** (**9d-s2**, B). Displacement ellipsoids of the non-hydrogen atoms are shown at the 50% probability level. Hydrogen atoms are represented by spheres of arbitrary size. Oxygen atoms are in red and nitrogen atoms in blue.

Figure 2.

Concentration–inhibition data of **3q-s1–4** and **3q-s2** at NMDA receptor subtypes. (A) Representative two-electrode voltage-clamp recordings showing inhibition of GluN1/2A and GluN1/2D by **3q-s1–4**. Receptors were activated by 100 μM glycine and 1 μM glutamate. (B) Concentration–inhibition data for **3q-s1–4** at different NMDA receptor subtypes (GluN1/2A–D). (C) Concentration–inhibition data for **3q-s2** at different NMDA receptor subtypes (GluN1/2A–D). Data points are shown as mean $\pm$ SEM from six oocytes. The K_i values were derived from the IC_{50} values using the Cheng–Prusoff relationship (Table 1).

Scheme 1. Synthesis of 3a, 3c-s1, 3c-s2, 3d-s1, and 3d-s2^a

^aReagents and conditions: (a) *t*-butyllithium, CuCN, THF, −78 to −50 °C, followed by 4, TMSCl, −50 °C, 1 h, 61–82%; (b) BH₃·THF, THF, rt, 20h, followed by H₂O, NaOH, H₂O₂, 0 °C to rt, 1 h, separation of the two diastereomers by DCVC, 17–52%; (c) TBAF, THF, rt, 56–84%; (d) RuCl₃, NaIO₄, H₂O, MeCN, EtOAc, rt, 7 h, 23–75%; (e) TFA, DCM, rt, 2 h, followed by 1 M HCl, 13–60%.

Scheme 2. Synthesis of 3a and 3e–m by Conjugate Addition of Substituted Malonic Ester 11a and 11e–m to Enone 10^a

^aReagents and conditions: (a) NaH, THF, 50 °C, 3–24 h, 35–99%; (b) 6 M HCl, reflux, 4–9 days, 6–87%.

Scheme 3. Synthesis of 3n from Enone 10^a

^aReagents and conditions: (a) NaH, THF, 50 °C, 4 h, 71%; (b) 6 M HCl, reflux, 4 days. (c) NaOH (42% over two steps).

Scheme 4. Synthesis of 3o from Intermediate 14^a

^aReagents and conditions: (a) NaH, THF, 50 °C, 4 h, 93%; (b) BH₃·THF, THF, 0 °C, 2 h, followed by H₂O, NaOH, H₂O₂, 0 °C, 1 h, 49%; (c) 6 M HCl, reflux, 3 days, 28%.

Scheme 5. Synthesis of 3p from Alkyne 15^a

^aReagents and conditions: (a) NaH, THF, 50 °C, 4 h, 89%; (b) TMSN₃, CuI, DMF, MeOH, reflux, 18 h, 71%; (c) 6 M HCl, reflux, 3 days, 78%.

Scheme 6. Synthesis of 3q and Separation of Stereoisomers^a

^aReagents and conditions: (a) NaH, BrCH₂CN, THF, 50 °C, 23 h, 83%; (b) NaN₃, Et₃NHCl, toluene, 115 °C, 19 h, 55% (c) separation of the 2,3-*trans* enantiomers by preparative HPLC using a ChiralPak AD column, 26–29%; (d) Pd/C, H₂, MeOH, r.t. 24 h, quant.; (e) 6 M HCl, reflux, 4 days, 99%; (f) separation of diastereoisomers by preparative HPLC using Chirobiotic T column, 18–80%.

Table 1.

Chemical Structures and Affinities of AMPA, NMDA, KA, Selected Published Glu Analogues 1a–e and 2a–d, and New Series of C ϵ -Substituted Analogues of 3a (CPAA)^a

compd ^b	IC ₅₀				K _i			
	AMPA	KA	NMDA	GluK1	GluK2	GluK3	GluK1/GluK3	GluK2/GluK3
AMPA (a)	0.04	>100	>100	1.2	>1000	43	0.03	>23
KA (b)	4	0.007	>100	0.08	0.01	0.03	3	0.3
NMDA (c)	>100	>100	35	n.d.	n.d.	n.d.	n.a.	n.a.
1a (d)	>100	6	>100	8.18	12.3	0.38	21	32
1b (e)	>100	14	3	0.67	2.1	0.62	1.1	3
1c (f)	0.54	0.23	>100	0.66	0.16	0.006	110	27
1d (g)	2	23	>100	0.002	>1000	2.53	0.001	>400
1e (h)	10	24	n.d.	0.03	0.02	0.002	15	10
2a (i)	0.43	0.36	0.33	0.14	0.33	0.49	0.3	0.7
2b (j)	27	0.03	6	0.0007	0.02	0.006	0.1	3
2c (k)	16	0.12	28	0.002	0.07	0.03	0.07	2
2d (l)	2	>100	>100	0.01	>100	2.25	0.004	>45

^a All values in μ M.

^b Data taken from (a) refs 21–23; (b) refs 23, 24; (c) ref 22; (d) refs 24, 25; (e) refs 26, 27; (f) refs 23, 28; (g) refs 29, 30; (h) refs 31, 32; (i) ref 33; (j) refs 24, 34, 35; (k) refs 36, 37; (l) refs 37, 38. n.d. = not determined; n.a. = not applicable.

Table 2.

Binding Affinities of 3a–q at Native iGluRs^a

compd	R-group	2,3-transconfiguration	ϵ -carbon; Abs stereochem or d.r.	IC ₅₀			K _i	
				AMPA	KA	NMDA		
3a^b	H	absolute (2 <i>S</i> , 3 <i>R</i>)	n.a.	106	1.6	0.5		
3b-d1^c	Me-	absolute (2 <i>S</i> , 3 <i>R</i>)	99:1	>100	2.4	22		
3b-d2^c	Me-	absolute (2 <i>S</i> , 3 <i>R</i>)	1:99	>100	1.8	>100		
3c-s1	Et-	absolute (2 <i>S</i> , 3 <i>R</i>)	<i>S</i>	>100	>100	13 [4.88 ± 0.02]		
3c-s2	Et-	absolute (2 <i>S</i> , 3 <i>R</i>)	<i>R</i>	>100	>100	>100		
3d-s1	Ph-	absolute (2 <i>S</i> , 3 <i>R</i>)	<i>R</i>	>100	>100	>100		
3d-s2	Ph-	absolute (2 <i>S</i> , 3 <i>R</i>)	<i>S</i>	>100	>100	>100		
3e	<i>n</i> Pr-	racemic	63:37	>100	>100	>100		
3f	<i>i</i> Pr-	racemic	67:33	>100	>100	>100		
3g	Bn-	racemic	58:42	>100	71 [4.15 ± 0.04]	>100		
3h	Ph(CH ₂) ₂ -	racemic	n.d.	>100	>100	>100		
3i-d1	F-	racemic	95:5	>100	48 [4.32 ± 0.03]	20 [4.71 ± 0.04]		
3i-d2	F-	racemic	1:99	>100	9.4 [5.04 ± 0.07]	6.0 [5.23 ± 0.05]		
3j-d1	MeO-	racemic	1:99	>100	>100	>100		
3k	H ₂ N-	racemic	50:50	31 [4.51 ± 0.03]	>100	8.9 [5.06 ± 0.02]		
3l	HOOCCH ₂ -	racemic	63:37	>100	23 [4.63 ± 0.02]	2.7 [5.58 ± 0.03]

compd	R-group	2,3-trans configuration	ϵ -carbon; Abs stereochem or d.r.	IC ₅₀			K _i	
				AMPA	KA	NMDA		
3m	HOOC(CH ₂) ₂ -	racemic	67:33	>100	>100	53 [4.28 ± 0.01]		
3n	HO(CH ₂) ₂ -	racemic	53:47	>100 ^c	>100	61 [4.23 ± 0.06]		
3o	HO(CH ₂) ₃ -	racemic	56:44	>100	>100	>100		
3p	triazolyl-CH ₂ -	racemic	60:40	>100	59 [4.23 ± 0.03]	>100		
3p-d1	triazolyl-CH ₂ -	racemic	99:1	>100	>100	>100		
3p-d2	triazolyl-CH ₂ -	racemic	10:90	>100	58 [4.24 ± 0.01]	51 [4.30 ± 0.03]		
3q^d	tetrazolyl-CH ₂ -	racemic	56:34	>100	93 [4.04 ± 0.06]	0.21 [6.69 ± 0.07]		

^a All values in μ M. Data are mean values of at least three individual experiments performed in triplicate. For AMPA and KA: IC₅₀ values with mean pIC₅₀ ± SEM in brackets. For NMDA: K_i values with mean pK_i ± SEM in brackets.

^b Data for native receptors taken from ref 67.

^c Data taken from ref 26.

^d K_i = > 1000 μ M at cloned homomeric GluA2 receptors; -: not tested; n.d.: not determined; n.a.: not applicable.

Table 3.

Binding Affinities of 3a–q at Cloned Homomeric Kainate Receptors^a

compd	R-group	2,3- <i>trans</i> configuration	<i>e</i> -carbon; Abs stereochem or d.r.	<i>K_i</i>			GluK1/K3
				GluK1	GluK2	GluK3	
3a	H	absolute (2 <i>S</i> , 3 <i>R</i>)	n.a.	2.8	2.2	0.51	6
3b-d1 ^b	Me-	absolute (2 <i>S</i> , 3 <i>R</i>)	99:1	0.23	3.8	0.33	1
3b-d2 ^b	Me-	absolute (2 <i>S</i> , 3 <i>R</i>)	1:99	0.39	0.51	0.10	4
3c-s1	Et-	absolute (2 <i>S</i> , 3 <i>R</i>)	<i>S</i>	18 ± 3.8	>100	11 ± 2.4	2
3c-s2	Et-	absolute (2 <i>S</i> , 3 <i>R</i>)	<i>R</i>	41 ± 14	70 ± 14	6.3 ± 1.3	7
3d-s1	Ph-	absolute (2 <i>S</i> , 3 <i>R</i>)	<i>R</i>	>100	>100	>100	n.a.
3d-s2	Ph-	absolute (2 <i>S</i> , 3 <i>R</i>)	<i>S</i>	>100	>100	>100	n.a.
3e	<i>n</i> Pr-	racemic	63:37	>100	>100	87 ± 16	n.a.
3f	<i>P</i> Pr-	racemic	67:33	>100	>100	>100	n.a.
3g	Bn-	racemic	58:42	46 ± 2.2	67 ± 4.6	5.4 ± 0.42	9
3h	Ph(CH ₂) ₂ -	racemic	n.d.	>100	>100	86 ± 7.3	n.a.
3i-d1	F-	racemic	95:5	35 ± 0.26	66 ± 20.2	5.3 ± 0.13	7
3i-d2	F-	racemic	1:99	3.3 ± 0.18	2.6 ± 0.15	0.71 ± 0.08	5
3j-d1	MeO-	racemic	1:99	>100	>100	82 ± 11.2	n.a.
3k	H ₂ N-	racemic	50:50	>100	>100	33 ± 2.4	>3

compd	R-group	2,3- <i>trans</i> configuration	ε-carbon; Abs stereochem or d.r.	K_i				GluK1/K3
				GluK1	GluK2	GluK3		
3l	HOOCCH ₂ -	racemic	63:37	2.0 ± 0.16	33 ± 1.1	6.4 ± 1.2		0
3m	HOOC(CH ₂) ₂ -	racemic	67:33	>100	>100	>100		n.a.
3n	HO(CH ₂) ₂ -	racemic	53:47	>100	>100	52 ± 3.0		>2
3o	HO(CH ₂) ₃ -	racemic	56:44	>100	>100	>100		n.a.
3p	triazolyl-CH ₂ -	racemic	60:40	14 ± 2.0	55 ± 9.0	3.1 ± 0.14		5
3p-d1	triazolyl-CH ₂ -	racemic	99:1	~100	>100	6.6 ± 0.09		~15
3p-d2	triazolyl-CH ₂ -	racemic	10:90	-	43 ± 5.2	2.7 ± 0.32		n.a.
3q ^c	tetrazolyl-CH ₂ -	racemic	56:34	2.1 ± 0.1	61 ^d	4.9 ± 0.5		0

^aAll values in μ M. Data are mean values of at least three individual experiments performed in triplicate. K_i values reported are the mean ± SEM

^bData taken from ref 26.

^c $K_i = >1000 \mu$ M at cloned homomeric GluA2 receptors

^dTested once; -: not tested; n.d.: not determined; n.a.: not applicable.

Table 4.

Pharmacological Characterization of 3a,c–q at EAAT1, EAAT2, and EAAT3 Stably Expressed in HEK293 Cells^a

compd	R-group	2,3- <i>trans</i> configuration	ϵ -carbon; Abs stereochem or d.r.	IC ₅₀		
				EAAT1	EAAT2	EAAT3
3a	H	absolute (2 <i>S</i> , 3 <i>R</i>)	n.a.	>300	>300	>300
3c-s1	Et–	absolute (2 <i>S</i> , 3 <i>R</i>)	<i>S</i>	~100 [~4.0]	~30 [~4.5]	~50 [~4.3]
3c-s2	Et–	absolute (2 <i>S</i> , 3 <i>R</i>)	<i>R</i>	>300 [<3.5]	>300 [<3.5]	>300 [<3.5]
3d-s1	Ph–	absolute (2 <i>S</i> , 3 <i>R</i>)	<i>R</i>	>300 [<3.5]	~50 [~4.3]	>300 [<3.5]
3d-s2	Ph–	absolute (2 <i>S</i> , 3 <i>R</i>)	<i>S</i>	>300 [<3.5]	>300 [<3.5]	>300 [<3.5]
3e	<i>n</i> Pr–	racemic	63:37	~500 [~3.3]	~100 [~4.0]	~1000 [~3.0]
3f	<i>i</i> Pr–	racemic	67:33	45 [4.34 ± 0.13]	16 [4.80 ± 0.07]	35 [4.45 ± 0.06]
3g	Bn–	racemic	58:42	34 [4.46 ± 0.05]	20 [4.70 ± 0.04]	~200 [~4.4]
3h	Ph(CH ₂) ₂ –	racemic	n.d.	>300 [<3.5]	>300 [<3.5]	>300 [<3.5]
3i-d1	F–	racemic	95:5	>300 [<3.5]	>300 [<3.5]	>300 [<3.5]
3i-d2	F–	racemic	1:99	>300 [<3.5]	>300 [<3.5]	>300 [<3.5]
3j-d1	MeO–	racemic	1:99	>1000 [<3.0]	~300 [~3.5]	>1000 [<3.0]
3k	H ₂ N–	racemic	50:50	>300 [<3.5]	>300 [<3.5]	>300 [<3.5]
3l	HOOCCH ₂ –	racemic	63:37	>1000 [<3.0]	>1000 [<3.0]	>1000 [<3.0]
3m	HOOC(CH ₂) ₂ –	racemic	67:33	>300 [<3.5]	>300 [<3.5]	>300 [<3.5]
3n	HO(CH ₂) ₂ –	racemic	53:47	>300 [<3.5]	>300 [<3.5]	>300 [<3.5]

compd	R-group	2,3- <i>trans</i> configuration	ϵ -carbon; Abs stereochem or d.r.	IC ₅₀		
				EAAT1	EAAT2	EAAT3
3o	HO(CH ₂) ₃ -	racemic	56:44	>300 [<3.5]	>300 [<3.5]	>300 [<3.5]
3p	triazolyl-CH ₂ -	racemic	60:40	>300 [<3.5]	>300 [<3.5]	>300 [<3.5]
3p-d1	triazolyl-CH ₂ -	racemic	99:1	>300 [<3.5]	>300 [<3.5]	>300 [<3.5]
3p-d2	triazolyl-CH ₂ -	racemic	10:90	-	-	-
3q	tetrazolyl-CH ₂ -	racemic	56:34	>300 [<3.5]	>300 [<3.5]	>300 [<3.5]

^aIC₅₀ values are given in μ M with mean pIC₅₀ $\pm$ SEM in brackets. Data are mean values of at least three individual experiments performed in triplicate. -; not tested; n.d.: not determined.

Table 5.

Binding Affinities of 3q-s1–4 at Native NMDA Receptors and Functional Characterization as Antagonists at Cloned GluN1/GluN2A–D Receptors^a

compd	2,3- <i>trans</i> configuration	Abs stereochem or d.r. at α -carbon	NMDA (binding affinity)	K_i			
				GluN1/GluN2A	GluN1/GluN2B	GluN1/GluN2C	GluN1/GluN2D
3q-s1	(-)-Abs (n.d.)	96:4	28 [4.56 $\pm$ 0.06]	–	–	–	–
3q-s2	(-)-Abs (n.d.)	4:96	1.3 [5.89 $\pm$ 0.09]	1.2 $\pm$ 0.1	2.8 $\pm$ 0.1	6.5 $\pm$ 0.6	15 $\pm$ 2
3q-s3,4	(+)-Abs (n.d.)	1:2	0.25 [6.61 $\pm$ 0.07]	0.61 $\pm$ 0.02	2.7 $\pm$ 0.1	12 $\pm$ 1	62 $\pm$ 9

^a All values in μ M. Binding data are mean values of at least three individual experiments performed in triplicate. For NMDA: K_i values with mean $pK_i \pm$ SEM in brackets. For GluN1/GluN2A–D, K_i values are reported as the mean $\pm$ SEM from six oocytes and were estimated from IC₅₀ values using the Cheng–Prusoff relationship (see Methods). –: not tested; n.d.: not determined.