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Design and Synthesis of a Series of L-*trans*-4-Substituted Prolines as Selective Antagonists for the Ionotropic Glutamate Receptors Including Functional and X-ray Crystallographic Studies of New Subtype Selective Kainic Acid Receptor Subtype 1 (GluK1) Antagonist (2*S*,4*R*)-4-(2-Carboxyphenoxy)pyrrolidine-2-carboxylic Acid

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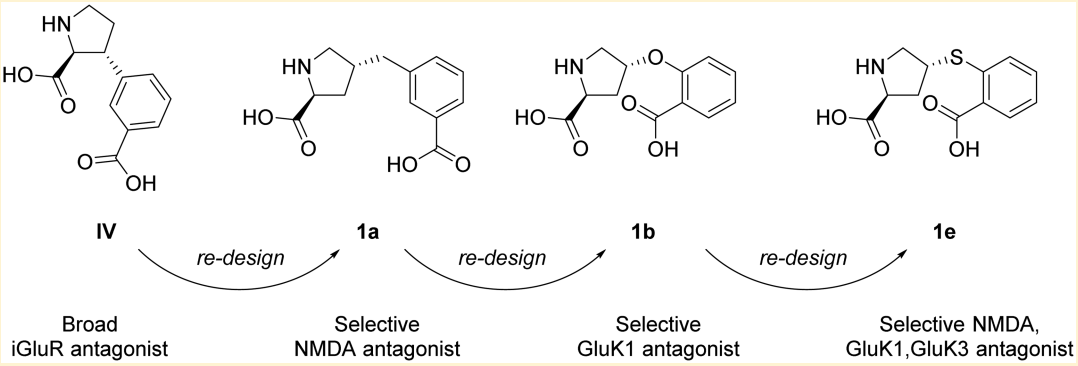
Supporting Information



ABSTRACT: Ionotropic glutamate receptor antagonists are valuable tool compounds for studies of neurological pathways in the central nervous system. On the basis of rational ligand design, a new class of selective antagonists, represented by (2*S*,4*R*)-4-(2-carboxyphenoxy)pyrrolidine-2-carboxylic acid (**1b**), for cloned homomeric kainic acid receptors subtype 1 (GluK1) was attained (K_i = 4 μM). In a functional assay, **1b** displayed full antagonist activity with IC_{50} = 6 ± 2 μM. A crystal structure was obtained of **1b** when bound in the ligand binding domain of GluK1. A domain opening of 13−14° was seen compared to the structure with glutamate, consistent with **1b** being an antagonist. A structure−activity relationship study showed that the chemical nature of the tethering atom (C, O, or S) linking the pyrrolidine ring and the phenyl ring plays a key role in the receptor selectivity profile and that substituents on the phenyl ring are well accommodated by the GluK1 receptor.

INTRODUCTION

(*S*)-Glutamic acid (Glu, Figure 1) functions as the major excitatory neurotransmitter in the mammalian central nervous system (CNS). The Glu receptors are divided into two major classes based on of their structural nature: the ionotropic Glu receptors (iGluRs)[1] and the G-protein coupled metabotropic Glu receptors (mGluRs).[2] Lastly, uptake of Glu from the synapse is mediated by the excitatory amino acid transporters (EAATs).[3−5]

The Glu receptors are involved in basic neurophysiological processes such as motor function, cognition, and plasticity. This, however, is a delicate balance as excessive Glu signaling has been shown to be neurotoxic,[6,7] ultimately leading to neuronal death. Several psychiatric diseases and neurological disorders have been proposed to have an origin in a malfunctioning Glu neurotransmitter system(s) which are therefore considered as promising therapeutic targets. Diseases span over depression,[8] anxiety,[9] and schizophrenia,[10] while neurological disorders comprise neurodegenerative diseases such as Alzheimer's,[11] Parkinson's,[12] and epilepsy.[13] The iGluRs are divided into three groups (Figure 1): the α-

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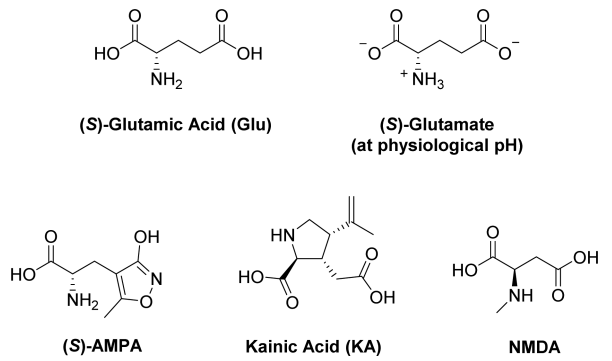

Figure 1. Chemical structures of Glu and iGluR group-defining agonists AMPA, KA, and NMDA.

amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (subunits GluA1−4), the kainic acid (KA) receptors (subunits GluK1−5), and the *N*-methyl-D-aspartic acid (NMDA) receptors (subunits GluN1, GluN2A-D, and GluN3A,B).

Upon extending the distance between the α-amino acid moiety and the distal γ-carboxylic acid group of the endogenous agonist Glu, the ligand binding domain (LBD) is prevented from closing, leading to antagonist function.[14] This design strategy has led to a number of chemically distinct parental scaffolds (Figure 2): 3′-functionalized 2-amino-3-(4′-isoxazolyl)propionic acids (e.g., 2-amino-3-[5-*tert*-butyl-3-(phosphonomethoxy)-1,2-oxazol-4-yl]propanoic acid (ATPO)), amino acid bioisosteric quinoxalinediones (e.g., 6,7-dinitroquinoxaline-2,3-dione (DNQX) and 7-nitro-2,3-dioxo-1,4-dihydroquinoxaline-6-carbonitrile (CNQX)),[15] 6-functionalized decahydroisoquinoline-3-carboxylic acids (**I**),[16,17] substituted phenylalanines (**II**),[18] *N*-functionalized analogues of the natural product willardiine (e.g., α-amino-3-[(4-carboxyphenyl)methyl]-3,4-dihydro-2,4-dioxo-1(2*H*)-pyrimidinepropanoic acid (**III**)),[19] and recently 3-(3-carboxyphenyl)pyrrolidine-2-carboxylic acids (**IV**)[20,21] Figure 2). By SAR studies of these parental scaffolds, group-selective iGluR antagonists have been disclosed, but only subtype selective antagonists for the KA receptor subtype GluK1 has been reported: (3*S*,4a*R*,6*S*,8a*R*)-6-(((*S*)-2-carboxy-4,4-difluoropyrrolidin-1-yl)methyl)decahydroisoquinoline-3-carboxylic acid (LY466195)[22] and (*S*)-3-((3-(2-amino-2-carboxyethyl)-5-methyl-2,6-dioxo-3,6-dihydropyrimidin-1(2*H*)-yl)methyl)-thiophene-2-carboxylic acid (UBP310)[23] (Figure 2). In our search for novel parental scaffolds for subsequent discovery of subtype selective iGluR antagonists, we here present our work on a successful scaffold-jump of **IV**.

RESULTS AND DISCUSSION

Inspired by our previous work on the *trans*-3-(3-carboxyphenyl)proline scaffold **IV**,[20,21] we were intrigued by the thought of repositioning the 3-phenyl ring to the *trans*-4-position. This affords compound **V** (purple), which is linear in shape with respect to the α-amino acid and the distal carboxylic acid functionalities (Figure 3). In silico energy minimization of **V** followed by superimposition onto the binding conformation of **IVa** in GluA2 (pdb code: 4yma)[21] by aligning the two α-amino acid functionalities shows that the two distal carboxylic acid functionalities align poorly and are perpendicular to each other (Figure 3B). For compound **V** to attain a more convex shape as **IVa**, a sp^3-tethering carbon atom between the pyrrolidine ring and the phenyl group was introduced (compound **1a**). Superimposition of a low-energy conformation of **1a** with the X-ray diffraction determined binding conformation of **IVa** (pdb code: 4yma) (Figure 3B) confirms that **1a** has a convex shape similar to **IVa**, placing its phenyl ring in parallel to the phenyl ring of **IVa**.

The synthesis of **1a** (Scheme 1) commenced with benzylation of readily available protected (*S*)-pyroglutaminol **2** to give **3** in acceptable yield as the single *trans*-diastereomer. Reduction of the *endo*-cyclic carbonyl group with borane provided the expected pyrrolidine ring but also the benzylic alcohol from reduction of the arylic ester (compound **4**). Cleavage of the silyl ether with TBAF gave diol **5**, which was oxidized to the diacid **6** using TEMPO. Removal of the BOC group was done by HCl(g)/AcOH in diethyl ether to provide target compound **1a** as the HCl salt.

The binding affinity of **1a** toward the plethora of ionotropic Glu receptors was evaluated at native AMPA, KA, and NMDA receptors (rat synaptosomes) and cloned rat homomeric subtypes GluK1−3 (Table 1). **1a** displayed negligible affinity

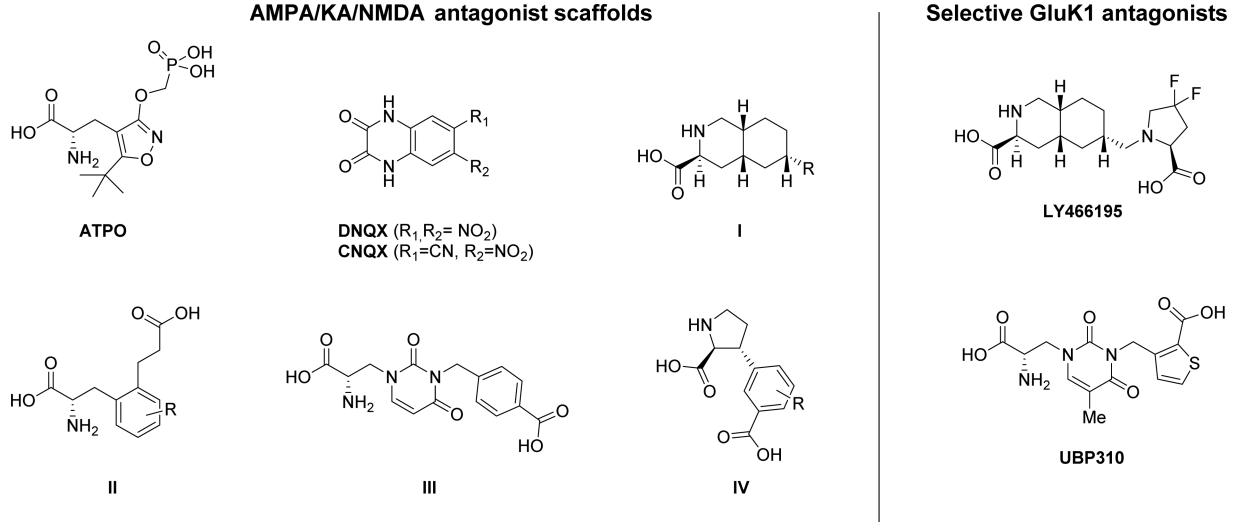

Figure 2. Chemical structures of iGluR antagonists and subtype selective GluK1 antagonists.

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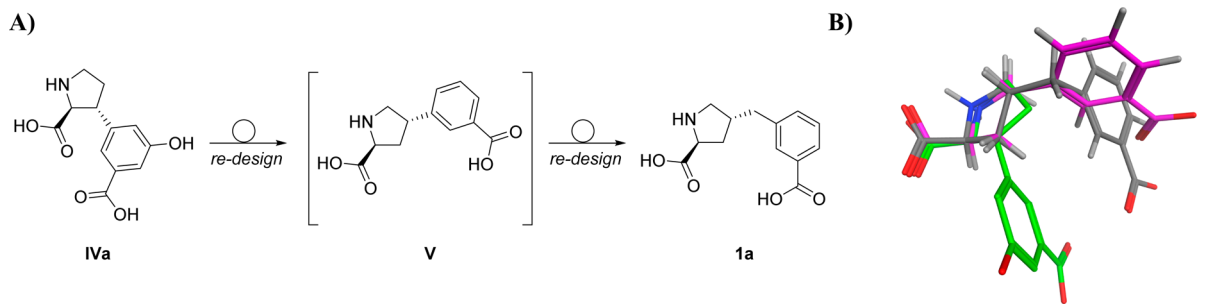
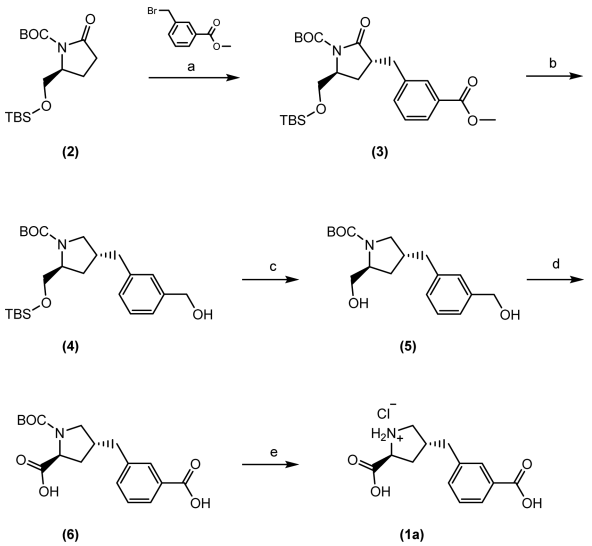


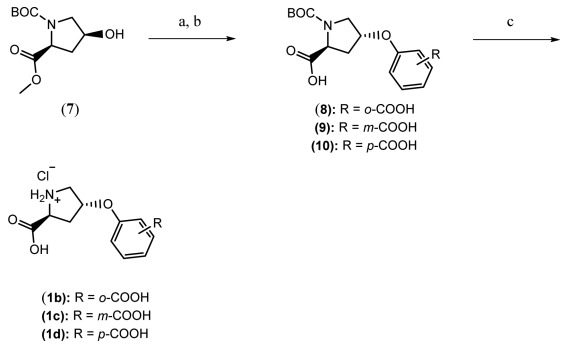
Figure 3. (A) Rational design of 4-substituted proline **1a** starting from 3-substituted proline **IV** (e.g., the 5-hydroxy analogue **IVa**[21]). (B) Superimposition of binding mode of **IVa** in GluA2-LBD (pdb code: 4yma) (green), **V** (purple), and **1a** (gray) by alignment of the α-amino acid moieties.

Scheme 1. Stereo-Controlled Synthesis of 1a Starting from Readily Available Protected (*S*)-Pyroglutaminol 2[a]



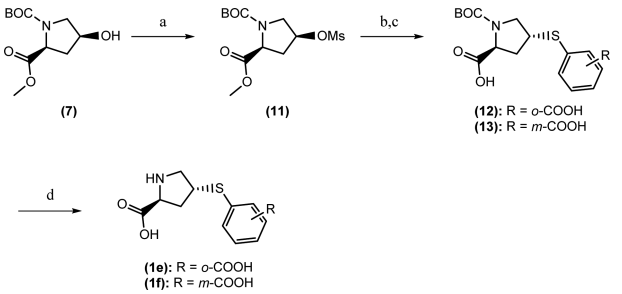
[a]Reagents and conditions: (a) LHMDS, THF, −78 °C to rt (64%); (b) BH_3, THF, reflux (56%); (c) TBAF, THF, rt (52%); (d) TEMPO, $NaClO_2$, NaOCl, MeCN, H_2O, 0 °C (62%); (e) HCl(g)/AcOH in Et_2O rt (99%).

Scheme 2. Stereo-Controlled Synthesis of 1b−d Starting from Readily Available Protected L-*cis*-4-Hydroxy-proline 7[a]



[a]Reagents and conditions: (a) appropriately substituted phenol, DIAD, PPh_3, THF, rt (53−91%); (b) LiOH, THF, 0 °C to rt (53−91%, two steps; (c) **1b**,**d**:TFA in DCM, then 1 M HCl, **1c**:HCl/AcOH in Et_2O (89−96%).

Scheme 3. Stereo-Controlled Synthesis of 1e,f Starting from Readily Available Protected L-*cis*-4-Hydroxy-proline 7[a]



[a]Reagents and conditions: (a) MsCl, TEA, THF, 0 °C (98%); (b) appropriately substituted thiophenol, K_2CO_3, DMF, 50 °C; (c) LiOH, THF, 0 °C to rt (78% for **12**); (d) TFA in DCM (94% and 40%, **1e** and **1f**, respectively).

for AMPA and KA receptors but midrange micromolar affinity for the NMDA receptors (K_i = 69 μM). At cloned homomeric GluK1−3 receptors, **1a** was also without appreciable affinity. The observed selectivity for the NMDA receptors prompted us to explore the influence of the chemical nature of the tethering carbon atom on iGluRs group and subtype binding affinity.[24] Furthermore, we decided to investigate the position of the carboxylate group on the phenyl ring and challenge the *trans*-stereochemistry by synthesizing selected *cis*-stereoisomers. In all, syntheses of three *trans*-ether analogues **1b−d** (Scheme 2), two *trans*-thioether analogues **1e**,**f** (Scheme 3), three *cis*-ethers **1g−i** (Scheme 4), and two *cis*-thioethers **1j**,**k** (Scheme 5) were carried out.

All 10 analogues were synthesized by an efficient strategy starting from commercially available suitably protected (*S*)-*cis*-4-hydroxy-proline (**7**) (Scheme 2 and 3) or (*S*)-*trans*-4-hydroxy-proline (**14**) (Scheme 4 and 5). Generally, the *cis*/*trans*-ether analogues were obtained directly by a Mitsunobu protocol (Scheme 2 and 4), while the *cis*/*trans*-thioethers were obtained by a two-step procedure with mesylates **11** and **18** as intermediates (Scheme 3 and 5).

Binding Affinity of 1b−k. Pharmacological characterization of **1b−k** at native ionotropic Glu receptors (rat synaptosomes) and rat homomeric cloned GluK1−3 receptors revealed both *expected* as well as *unexpected* binding affinity profiles (Table 1). None of the compounds showed appreciable affinity for AMPA receptors. By substituting the carbon tether for an oxygen atom (compound **1c**), an unexpected shift in pharmacological profile from selective midrange micromolar affinity for the NMDA receptors to selective midrange micromolar affinity for GluK1 was observed. However, even more interestingly, repositioning the arylic carboxylic acid

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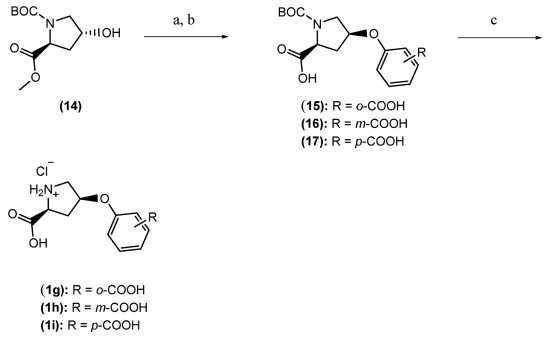
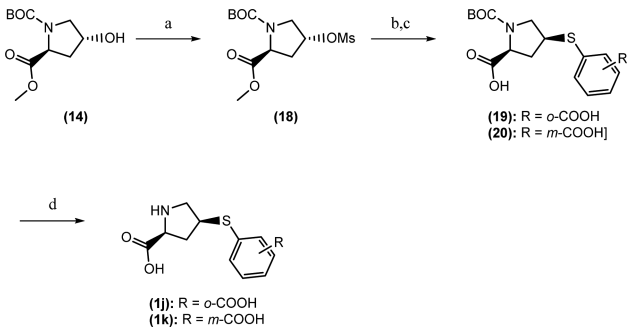
Scheme 4. Stereo-Controlled Synthesis of 1g−i Starting from Readily Available Protected L-*trans*-4-Hydroxyproline 14[a]



[a]Reagents and conditions: (a) Appropriately substituted phenol, DIAD, PPh_3, THF, rt; (b) LiOH, THF, 0 °C to rt (53−91%, two steps); (c) TFA in DCM, then 1 M HCl or HCl/AcOH in Et_2O (85−93%).

Scheme 5. Stereo-Controlled Synthesis of 1j,k Starting from Readily Available Protected L-*trans*-4-Hydroxy-proline 14[a]



[a]Reagents and conditions: (a) MsCl, TEA, THF, 0 °C (90%); (b) Appropriately substituted thiophenol, K_2CO_3, DMF, 50 °C; (c) LiOH, THF, 0 °C to rt (67%, two steps for **19**); (d) TFA in DCM (74% for **1j** and 38% for **1k**, three steps).

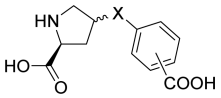
group to the *ortho*-position increased the affinity by 9-fold (compound **1b**). In contrast, when the carboxylic acid group was placed in the *para*-position, compound **1d**, the pharmacological profile was shifted back to selectivity for the NMDA receptors.

The thioether analogue of **1b**, compound **1e**, was no longer selective for GluK1 but displayed midrange micromolar affinity for native NMDA receptors, GluK1, and GluK3 receptors (Table 1). This was unexpected and underlines how subtle chemical changes to a ligand may modify its pharmacological profile. Thioether **1f** displays midrange micromolar affinity for native NMDA receptors which is in contrast to its oxygen analogue **1c**, showing highest binding affinity for homomeric GluK1 receptors. Finally, all the synthesized *cis*-stereoisomers **1g−k** displayed negligible affinity for any of the iGluRs. The four analogues **1b−d,g** were characterized in a conventional [^{3}H]-D-Asp uptake assay and displayed no inhibitory activity at EAAT1−3 (IC_{50} > 1000 μM).[25]

Functional Characterization of 1b at Recombinant Homomeric GluK1 and GluK2 Receptors. Given the GluK1-selective binding affinity profile of **1b** (Table 1), we next characterized its function at recombinantly expressed KA receptors. Outside-out patches containing either GluK1 or GluK2 homomers were subjected to fast Glu applications (250 ms, 1 mM). An inhibition curve was constructed by subjecting the patches to various concentrations of **1b** (Figure 4). As expected from our previous results, GluK2 responses were unaffected by concentrations of **1b** up to 100 μM, while GluK1 responses were almost completely blocked at this concentration (Figure 4). The IC_{50} was determined to 5.7 ± 2.0 μM for GluK1 receptors and >100 μM for GluK2 receptors (Figure 4C).

X-ray Structure of 1b in GluK1 LBD. As **1b** turned out to be a selective GluK1 antagonist, we crystallized **1b** with a soluble construct of the GluK1 LBD. The binding affinity (K_i = 2.27 ± 0.12 μM) at GluK1 LBD was found to be similar to that of full-length GluK1 (4.0 μM; Table 1). The complex crystallizes with two molecules in the asymmetric unit of the

Table 1. Binding Affinities of 4-Substituted Prolines at Native iGluRs (Rat Synaptosomes) and Homomeric Cloned Rat GluK1−3 Receptors[a]



compd	2,4 stereochemistry	-X-	COOH	AMPA K_i (μM)	KA K_i (μM)	NMDA K_i (μM)	GluK1 K_i (μM)	GluK2 K_i (μM)	GluK3 K_i (μM)
1a	*trans*	CH_2	*meta*	>100	>100	69 [4.18 ± 0.05]	>100	>100	>100
1b	*trans*	O	*ortho*	>100	>100	>100	4.0 [5.40 ± 0.03]	>100	>100
1c	*trans*	O	*meta*	>100	>100	>100	36 [4.48 ± 0.12]	>100	>1000
1d	*trans*	O	*para*	>100	>100	81 [4.11 ± 0.09]	>100	>100	>100
1e	*trans*	S	*ortho*	>100	>100	19 [4.73 ± 0.05]	8.4 [5.10 ± 0.11]	>100	22 [4.66 ± 0.02]
1f	*trans*	S	*meta*	>100	>100	78 [4.11 ± 0.03]	>100	>100	>100
1g	*cis*	O	*meta*	>100	>100	>100	>100	>100	>100
1h	*cis*	O	*ortho*	>100	>100	>100	>100	>100	>100
1i	*cis*	O	*para*	>100	>100	>100	>100	>100	>100
1j	*cis*	S	*ortho*	>100	>100	>100	>100	>100	>100
1k	*cis*	S	*meta*	>100	>100	>100	>100	>100	>100
LY466195[b]							0.05		8.9
UBP310[c]							0.022	>100	0.93

[a]Radioligands: AMPA, [^{3}H]AMPA; KA, [^{3}H]KA; NMDA, [^{3}H]CGP-39653; GluK1, [^{3}H]NF608; GluK2 and GluK3, [^{3}H]KA. For all, pK_i ± SEM (M) in square brackets. [b]From ref 22. [c]From ref 21.

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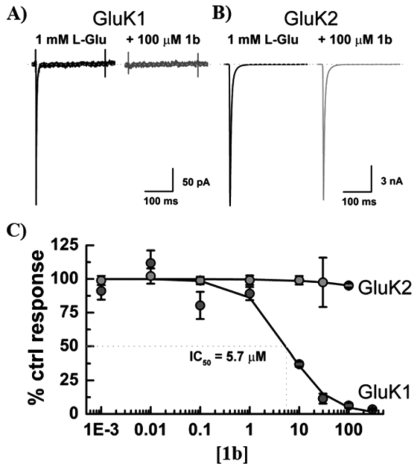

Figure 4. Compound **1b** inhibits recombinant GluK1 but not GluK2 in HEK293T cells. (A) Example traces of GluK1 responses evoked by 1 mM Glu (250 ms) in outside-out patches at a holding potential of −100 mV, in the absence (left) or presence (right, gray) of 100 μM **1b**. (B) Example traces of GluK2 responses evoked by 1 mM Glu (250 ms) in outside-out patches at a holding potential of −100 mV, in the absence (left) or presence (right, light gray) of 100 μM **1b**. (C) Inhibition plot for **1b** for 1 mM Glu. Data are represented as a percentage of the control response in the absence of **1b** (mean ± SEM); 3−5 responses were obtained for each concentration of **1b** tested.

crystal, forming a dimer (Figure 5A, Table 2). Despite the low resolution of the structure (3.2 Å), the electron density was well-defined for most of the amino acid residues, and **1b** could be modeled into the density in both binding sites (Figure 5B). After molecular replacement (only protein atoms included), the electron density revealed the position of **1b**. However, the conformation of the pyrrolidine ring could not be determined unambiguously at this resolution. **1b** was inserted in a low energy conformation, allowing a hydrogen bond to be formed between the pyrrolidine nitrogen of **1b** to the side chain carboxylate of Glu738 (GluK1−2 numbering including signal peptide). Unfortunately, it has not been possible to improve the resolution beyond 3 Å.

The amino acid part of **1b** interacts with binding site residues in the same way as previously observed for agonists and antagonists.[26] The pyrrolidine carboxylate group of **1b** makes salt bridges with the side chain of the essential Arg523 and furthermore hydrogen bonds to the main chain nitrogen of Thr518 and the side chain hydroxyl group of Ser689 (Figure 5C). The pyrrolidine nitrogen forms salt bridges with the side chain carboxylate of Glu738 as well as a hydrogen bond to the backbone oxygen of Pro516 and to the side chain hydroxyl group of Thr518. The distal carboxylate group of **1b** interacts with Thr690 through a hydrogen bond to the side chain hydroxyl group and to the backbone nitrogen of Glu738. The aromatic ring is placed in a binding pocket surrounded within 4

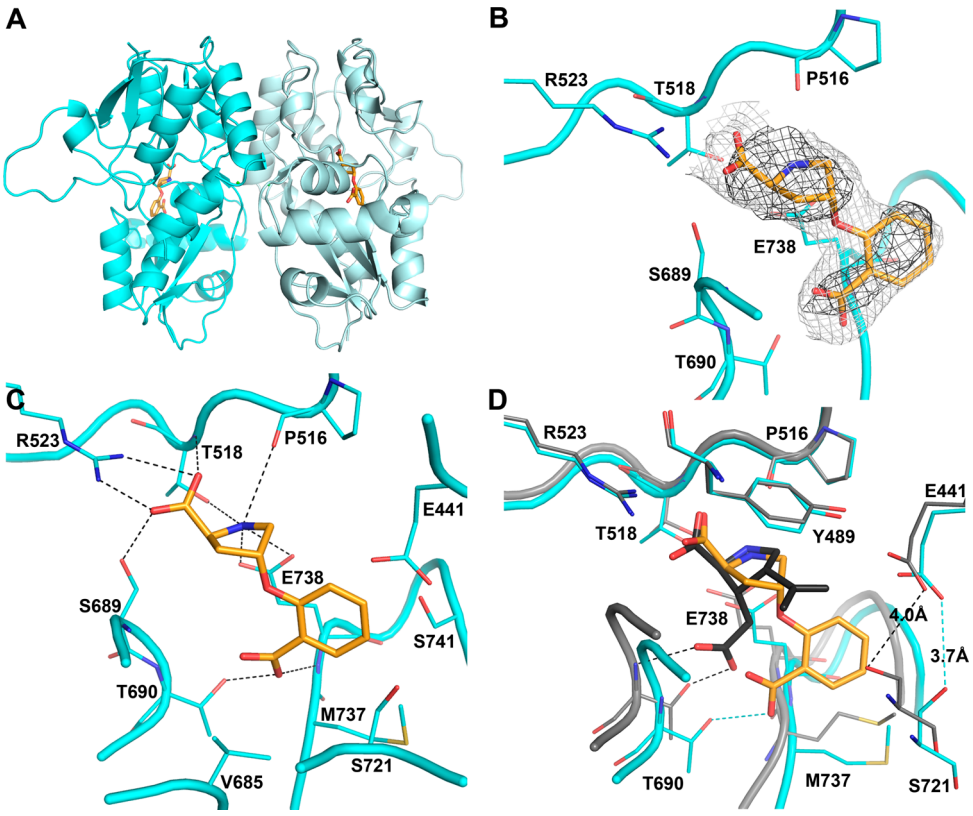

Figure 5. Structure of GluK1 LBD with the antagonist **1b** bound. (A) GluK1 LBD (molA, cyan; molB, pale cyan) in complex with **1b** (orange) crystallizes as a dimer. (B) $2F_o - F_c$ electron density for **1b** after molecular replacement (black), at which point the ligand was not introduced into the model, as well as the final electron density (white). Both maps are contoured at 1 σ. (C) Binding site interactions with **1b**. Hydrogen bonds are shown as stippled black lines (up to 3.4 Å). Residues forming the pocket (within 4 Å) for the aromatic ring of **1b** are shown. (D) Comparison of the binding mode of **1b** and kainate (black; pdb code: 4e0x, gray) in GluK1 LBD. GluK1 structures were superimposed on residues of D1. Interactions of the ligands with Thr690 are shown as well as the D1−D2 interlobe hydrogen bond. The tube illustrates the different domain openings through movement of D2 residues. Hydrogen bonds are colored cyan for **1b** and black for kainate.

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Table 2. Crystal Data, Data Collection, And Refinement Statistics of GluK1 LBD in Complex with 1b

Crystal Data	
beamline	I911−3
space group	$P4_12_12$
unit cell dimensions (Å)	
	$a = b = 71.99$
	$c = 233.36$
molecules in a.u.[a]	2
Data collection and Processing	
resolution	29.75−3.18 (3.29−3.18)[b]
unique reflections	10993
average multiplicity	6.5 (6.7)
completeness (%)	99.8 (100)
Wilson B-factor (Å^2)	56
R_{merge} (%)	14.0 (40.6)
$I/\sigma I$	4.4 (1.9)
Refinement	
number of:	
amino acid residues	504
1b molecules	2
sulfate ions	6
R_{work}[c] (%)/R_{free}[d] (%)	21.9/27.1
average B-values (Å^2) for:	
amino acid residues/**1b**	50/42
sulfate ions	106
RMS deviation:	
bond length (Å)/angles (deg)	0.002/0.46
residues in allowed regions of Ramachandran plot (%)[e]	100.0

[a]a.u.: Asymmetric unit of the crystal. [b]Values in parentheses correspond to the outermost resolution shell. [c]$R_{work} = \sum_{hkl}(||F_{o,hkl}| - |F_{c,hkl}||)/|F_{o,hkl}|$, where $|F_{o,hkl}|$ and $|F_{c,hkl}|$ are the observed and calcdulated structure factor amplitudes, respectively. [d]R_{free} is equivalent to R_{work}, but calcdulated with 5% of the reflections omitted from the refinement process. [e]The Ramachandran plot was calcdulated according to MolProbity.[28]

Å by Ser721, Met737, Glu738, and Ser741 from D2 as well as Glu441 from D1 (Figure 5C).

When an antagonist binds to the LBD, it stabilizes the LBD in an open conformation[14,27] whereas an agonist stabilizes a closed conformation of the LBD. We have calculated the lobes D1−D2 opening of **1b** relative to the structure of GluK1 LBD with glutamate (pdb code: 2f36, molA), in which the LBD is in a fully closed conformation. The domain opening was found to be 14.2° for molA and 12.8° for molB, which are both in the lower end of observed domain openings induced by antagonists when crystallized with GluK1 LBD (10−49°).[26] This suggested that it might be possible to introduce even larger substituents into the **1b** scaffold. Interestingly, the domain opening observed for the partial agonist kainate is 8.4° (pdb code: 4e0x, molA), which leaves the question open as to where the exact cutoff is for domain opening of GluK1 antagonism vs partial agonism.

Comparing the binding modes of **1b** and kainate in GluK1 LBD reveals that the aromatic ring of **1b** is positioned in the binding site between the isopropenyl group and the distal carboxylate of kainate (Figure 5D). Because of the larger aromatic moiety in **1b**, the LBD is stabilized in the more open conformation, illustrated by difference in the positions of residues in lobe D2 (Figure 5D). This change in domain opening makes it possible for the distal carboxylate of both kainate and **1b** to interact with the same D2 residue, Thr690. Furthermore, the isopropenyl group in kainate and the aromatic ring in **1b** both interfere with the important interlobe hydrogen bond (between Glu441 in D1 and Ser721 in D2). The interaction is longer than observed for full agonists, e.g., 3.9 Å for **1b** versus 3.0 Å in the structure with glutamate bound (pdb code: 2f36, molA).

Compound **1b** possesses a 9-fold higher affinity for GluK1 compared with compound **1c** (carboxylate group in *ortho* vs *meta*-position, respectively) and complete loss of binding affinity (compound **1d**, K_i > 100 μM) when placed in the *para*-position. A structural analysis thereof suggests that for the *meta*-position, the carboxylic acid would be located at a hydrophobic site (Leu735, Met737, and Val685). A conformational change in the side chain of Met737 would be required to accommodate the carboxylic acid, which might contribute to the lower affinity of **1c**. The carboxylic acid moiety in the *para*-position in **1d** would lead to clash with Ser721 and Met737, consistent with the observed loss of affinity.

To address the selectivity of **1b** toward GluK1 over GluK2,3, we compared the amino residues of difference among the orthosteric binding sites. It seems that the most likely explanation for the loss of affinity at GluK2 and GluK3 is the presence of the larger Asn residue in GluK2 and GluK3 compared to Ser721 in GluK1, which may result in unfavorable steric contacts to the benzene moiety of **1b**.

Design, Synthesis, and Pharmacological Evaluation of 1l−t. With this new class of selective GluK1 antagonists in hand, we set to explore the SAR of **1b** (Table 3). We were curious to explore the insertion of a methylene group between the aryl ring and the carboxylic acid functionality (**1l**) for enhancement of ligand flexibility and thereby possibility for hydrogen bonding interactions with the receptor and to reposition this substituent to the *meta*-position (**1m**), as an analogue of **1c**. In analogues **1n−p**, the phenyl ring was replaced with a naphthyl group to increase lipophilicity and thereby possibly enhance binding affinity. Alternatively, incorporation of a 4-carboxylic acid group (**1q**), a 4-methyl group (**1r**), a 4-acetyl group (**1s**), and a 6-methyl group (**1t**) were planned.

The synthesis of analogues **1l-t** followed the strategy outlined for **1b−d** (Scheme 2). Starting from *cis*-alcohol **7**, the appropriate phenolic esters **21−28** were reacted to give *trans*-ethers **29−37** in good to high yields and stereochemically pure. Subsequent global deprotection by reflux in 4 M HCl gave target structures **1l−t**. All analogues were characterized pharmacologically in binding assays at native iGluRs and homomeric GluA2 and GluK1−3 receptor subtypes (Table 3). Insertion of a methylene group between the aryl ring and the carboxylic acid **1l** results in a 5-fold decrease in GluK1 affinity but maintaining the subtype selectivity profile. Reposition of the very same group to the *meta*-position, compound **1m**, leads to complete loss of binding affinity for GluK1 and any of the iGluRs. Of the three naphthyl analogues **1n−p**, both **1n** and **1p** maintain low micromolar affinity for the GluK1 receptor. The flexible nature of the side chains of Ser741 and Met737, respectively, allow for the additional bulkiness of the naphthyl group. On the other hand, **1o** is without affinity for the GluK1 receptor, which can be ascribed to a clear steric clash with residues Tyr489 and Glu441.

The 4-position was probed with chemically diverse substituents (Table 3): A carboxylic acid (**1q**) led to complete loss of binding affinity analogous to **1d**, while a 4-methyl group

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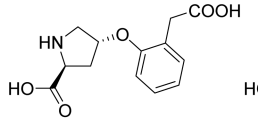
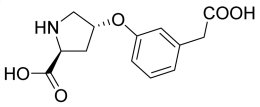
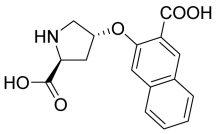
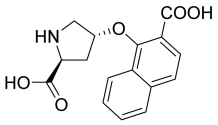
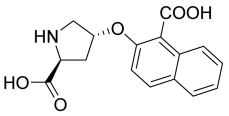
Table 3. Binding Affinities of L-*trans*-4-Substituted Prolines 1l−t at Native iGluRs and Homomeric Cloned GluK1−3 Receptors[a]

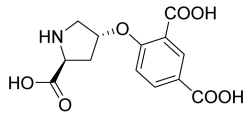

1l

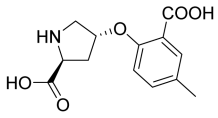

1m

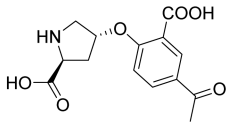

1n

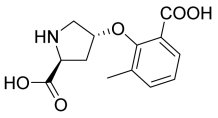

1o


1p


1q


1r


1s


1t

compd	AMPA K_i (μM)	KA K_i (μM)	NMDA K_i (μM)	GluA2 K_i (μM)	GluK1 K_i (μM)	GluK2 K_i (μM)	GluK3 K_i (μM)
1l	>100	>100	>100	>100	24 [4.62 ± 0.01]	>1000	>100
1m	>100	>100	>100	>100	>100	>100	>100
1n	>100	>100	>100	>100	3.5 [5.455 ± 0.005]	>1000	>100
1o	>100	>100	>100	>100	>100	>100	>100
1p	>100	>100	>100	>100	4.5 [5.35 ± 0.08]	>100	137 [3.88 ± 0.07]
1q	>100	>100	>100	>100	≈100	>1000	>100
1r	>100	>100	>100	>100	24 [4.63 ± 0.06]	>100	>100
1s	>100	>100	>100	>100	4.7 [5.33 ± 0.03]	>100	30 [4.53 ± 0.06]
1t	>100	>100	>100	>100	5.4 [5.27 ± 0.03]	>1000	>100

[a]Radioligands: AMPA, [^{3}H]AMPA; KA, [^{3}H]KA; NMDA, [^{3}H]CGP-39653; GluK1, [^{3}H]NF608; GluK2 and GluK3, [^{3}H]KA. For all, pK_i ± SEM (M) in square brackets.

(**1r**) led to a 6-fold reduction in binding affinity. Only a 4-acetyl group (**1s**) showed the same affinity as the lead structure (**1b**), consistent with the acetyl group being positioned in a partly hydrophobic pocket (Val685, Leu735, and Met737). Finally, a 6-methyl group (**1t**) was well accommodated by the GluK1 receptor and showed similar binding affinity compared with **1b** (Table 3).

CONCLUSION

In conclusion, the L-*trans*-4-substituted proline scaffold was disclosed as a new scaffold acting as iGluR antagonists. It was shown that the chemical nature of the tethering atom linking together the proline functionality and the substituted aryl ring was of key importance for the observed iGluR profile. The position of the aryl carboxylate functionality together with the carbon, oxygen, and sulfur tethers resulted in differentiated pharmacological profiles. Most notably, analogues **1b**,**n**,**p**,**t** were shown to be selective GluK1 antagonists in the low micromolar range. An X-ray structure of **1b** in complex with GluK1 LBD revealed that **1b** interacts with binding site amino acid residues in a similar way to KA but induces a ∼5° larger domain opening. A SAR study of **1b** showed that introduction of substituents were allowed for on the aryl ring, compounds **1n**,**p**,**s**,**t**, but limitations also existed (loss of binding affinity) when large bulkiness was oriented in the direction of residues Glu441 and Tyr489, compounds **1o**,**q**.

EXPERIMENTAL SECTION

General. All reagents were obtained from commercial suppliers and used without further purification. Dry solvents were obtained differently. THF was distilled over sodium/benzophenone. Et_2O was dried over neatly cut sodium. All solvents were tested for water content using a Carl Fisher apparatus. Water- or air-sensitive reactions were conducted in flame-dried glassware under nitrogen with syringe−septum cap technique. Purification was performed by dry column vacuum chromatography (DCVC) with silica gel size 25−40 μm (Merck, Silica gel 60). For TLC, Merck TLC silica gel F_{254} plates were used with appropriate spray reagents: $KMnO_4$ or molybdenum blue. ^{1}H NMR and ^{13}C NMR spectra were obtained on a Varian Mercury Plus (300 MHz) and a Varian Gemini 2000 instrument (75 MHz), respectively. Preparative HPLC was either done using an Agilent HPLC system with a 1100 series pump (20 mL/min), a 1200 series multiple wavelength detector, and a Zorbax, 300 SB-C18 column (21.2 mm × 250 mm, 7 μm), or using a TSP UV100 detector, a JASCO 880-PU pump (10 mL/min), a Merck Hitachi D-2000 Chromato-Integrator, and an XTerraPrep MS C18, 10 mm × 300 mm, 10 μm) column. LC-MS was performed using an Agilent 1200 series solvent delivery system equipped with an autoinjector coupled to an Agilent 6400 series triple quadrupole mass spectrometer and an electrospray ionization source. The XBridge column (4.6 mm × 100 mm, 3.5 μm) was eluted at 1.0 mL/min with a linear gradient of H_2O/CH_3CN/HCOOH (A, 90:10:0.05; and B, 10:90:0.046). Optical rotation was measured using a PerkinElmer 241 spectrometer, with Na lamp at 589 nm. Melting points were measured using an automated melting point apparatus, MPA100 OptiMelt (SRS) and are stated uncorrected. Compounds were dry either under high vacuum or freeze-dried using a Holm & Halby, Heto LyoPro 6000 freeze-drier.

*For the Experimental Work Leading to **1l**−**t**, the Following Apparatuses Were Used.* ^{1}H NMR spectra were recorded on a 400 MHz Avance Bruker and ^{13}C NMR spectra on a 100 MHz Avance Bruker, respectively, using $CDCl_3$, D_2O, or CD_3OD as solvent with $CDCl_3$ (δ 7.26/77.0), D_2O (δ 4.79), or CD_3OD (δ 3.31/49.0) as internal standards, respectively. Analytical HPLC was performed using a LaChrom Merck Hitachi L-7100 pump, autosampler L-7200, and UV detector L-7400 (254 nm) installed with a ChromolithSpeedROD RP-18 column (4.6 mm × 50 mm) using a 5 → 95% CH_3CN gradient in H_2O containing 0.1% TFA with a flow-rate of 4 mL/min. Preparative HPLC was done using Dionex Ultimate 3000 pump, Dionex Ultimate 3000 multiple wavelength detector, and Dionex Ultimate 3000 fraction collector and Gemini-NX C18 column (10 mm × 250 mm, 5 μm). The loop size was 10 mL, the mobile phase was 0.1% TFA in different

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mixtures of H_2O and CH_3CN, and the flow was 5 mL/min. LC-MS was performed using a Agilent 1100 HPLC systems with a XBridge 3.5 μm C-18 column (100 mm × 4.60 mm) using gradient elution from buffer A (H_2O:CH_3CN:HCOOH, 95:5:0.1) to buffer B (H_2O:CH_3CN: HCOOH, 5:95:0.05) over 10 min flow rate: 1.0 mL/min, coupled to an Hewlett-Packard 1100 series mass spectrometer with an electrospray ionization source. Melting points were measured using a Stanford Research Systems Optimelt automatic melting point system and are stated uncorrected. Optical rotation was measured using a Bellingham-Stanley ADP410 polarimeter with Na lamp (589 nm). Compounds were dried under high vacuum. The purity of all tested compounds **1a**−**t** was determined by HPLC to be >95% (254 nm).

*(2S,4R)-4-(3-Carboxybenzyl)-proline Hydrochloride (**1a**).* Diacid **6** (121 mg, 0.35 mmol, 1.00 equiv) was dissolved in dry Et_2O (2 mL), cooled to 0 °C, and 1 M HCl in Et_2O (4.18 mL, 4.18 mmol, 11.9 equiv) added under an atmosphere of nitrogen. The mixture was stirred at rt for 18 h and concentrated in vacuo to yield the deprotected amino acid **1a** (98 mg, 99%) as a yellow foam. The mixture was triturated with CH_3CN twice to yield a white, colloid precipitate that was isolated by decantation and drying of the semisolid in vacuo. ^{1}H NMR (300 MHz, D_2O): δ 2.05−2.18 (1H, m), 2.18−2.34 (1H, m), 2.57−2.75 (1H, m), 2.75−2.88 (2H, m), 2.98−3.10 (1H, m), 3.52 (1H, dd, *J* = 11, 7 Hz), 4.40 (1H, dd, *J* = 9, 4 Hz), 7.38−7.53 (2H, m), 7.78−7.88 (2H, m). ^{13}C NMR (75 MHz, $CDCl_3$): δ 34.5, 37.6, 39.1, 50.8, 60.5, 128.4, 129.5, 130.3, 130.4, 134.6, 140.4, 171.0, 173.3. LCMS: *m*/*z* [M + H]$^+$: calcd 250.1, found 250.1. OR: $[\alpha]^{22}_D$ −17 (*c* = 0.34; 2 M NaOH). Melting point: decomposition.

*(2S,4R)-4-(2-Carboxyphenoxy)pyrrolidine-2-carboxylic Acid Trifluoroacetic Acid (**1b**).* Diacid **8** (70 mg, 0.20 mmol, 1.00 equiv) was suspended in DCM (1.5 mL) and dissolved by addition of TFA (2 mL). The mixture was left to stir at rt under an atmosphere of nitrogen for 18 h. The mixture was concentrated in vacuo, suspended in H_2O, and freeze-dried to yield amino acid **1b** (65 mg, 89%) as a white solid. ^{1}H NMR (300 MHz, D_2O): δ 2.33 (1H, ddd, *J* = 15, 11, 5 Hz), 2.62 (1H, dd, *J* = 15, 8 Hz), 3.63 (2H, m), 4.53 (1H, dd, *J* = 11, 8 Hz), 5.20 (1H, m), 6.97−7.05 (2H, m), 7.46 (1H, ddd, *J* = 8, 8, 2 Hz), 7.64 (1H, dd, *J* = 8, 2 Hz). ^{13}C NMR (75 MHz, D_2O): δ 35.2, 51.4, 59.7, 77.6, 115.7, 121.3, 122.6, 132.1, 134.8, 155.2, 170.4, 172.4. LCMS: *m*/*z* [M + H]$^+$: calcd 252.1, found 252.2. OR: $[\alpha]^{22}_D$ −6.7 (*c* = 0.37; H_2O). Melting point: decomposition.

*(2S,4R)-4-(3-Carboxyphenoxy)pyrrolidine-2-carboxylic Acid Hydrochloride (**1c**).* Diacid **9** (120 mg, 0.34 mmol, 1.00 equiv) was dissolved in AcOH (3 mL) and 1 M HCl in Et_2O (2 mL) added. The mixture was left to stir at rt under an atmosphere of nitrogen for 18 h. The white precipitate formed was isolated by decantation of the solvents, washing with Et_2O, suspension in Et_2O, and filtration. The solid was dried in vacuo to yield amino acid **1c** (94 mg, 96%) as a white solid. ^{1}H NMR (300 MHz, D_2O): δ 2.43 (1H, ddd, *J* = 14, 10, 5 Hz), 2.74 (1H, dd, *J* = 14, 8 Hz), 3.59−3.80 (2H, m), 4.61 (1H, dd, *J* = 11, 8 Hz), 5.31 (1H, bs), 7.22 (1H, dd, *J* = 8, 2 Hz), 7.44 (1H, t, *J* = 8 Hz), 7.51 (1H, bs), 7.64 (1H, d, *J* = 8 Hz). ^{13}C NMR (75 MHz, D_2O): δ 35.1, 51.6, 59.4, 76.6, 116.9, 121.9, 124.0, 130.7, 131.8, 156.2, 170.4, 172.4. LCMS: *m*/*z* [M + H]$^+$: calcd 252.1, found 252.0. HPLC: >99% (254 nm). OR: $[\alpha]^{22}_D$ −9.5 (*c* = 0.57; 99.9% EtOH). Melting point: decomposition.

*(2S,4R)-4-(4-Carboxyphenoxy)pyrrolidine-2-carboxylic Acid Hydrochloride (**1d**).* Diacid **10** (89 mg, 0.25 mmol, 1.00 equiv) was suspended in DCM (4.5 mL) and TFA (4.5 mL) added. The mixture was left to stir at rt under an atmosphere of nitrogen for 18 h. The mixture was concentrated in vacuo, dissolved in H_2O, and freeze-dried. The salt was dissolved in 2 M HCl (5 mL), concentrated in vacuo, dissolved in H_2O (3 × 5 mL), and concentrated in vacuo after each addition. The salt was then dissolved in H_2O and freeze-dried to yield amino acid **1d** (65 mg, 90%) as a reddish foam. ^{1}H NMR (300 MHz, D_2O): δ 1.36 (1H, m), 1.63 (1H, dd, *J* = 14, 8 Hz), 2.24 (1H, d, *J* = 13 Hz), 2.66 (1H, dd, *J* = 14, 5 Hz), 3.07 (1H, dd, *J* = 9, 8 Hz), 4.18 (1H, m), 6.23 (2H, dm, *J* = 9 Hz), 7.19 (2H, dm, *J* = 9 Hz). ^{13}C NMR (75 MHz, D_2O): δ 34.4, 48.6, 57.7, 75.5, 111.9, 125.7, 128.0, 156.0, 171.6, 178.3. LCMS: *m*/*z* [M + H]$^+$: calcd 252.1, found 252.1. OR: $[\alpha]^{22}_D$ −81.18 (*c* = 0.11; 2 M NaOH). Melting point: decomposition.

*(2S,4R)-4-((2-Carboxyphenyl)thio)pyrrolidine-2-carboxylic Acid Trifluoroacetic Acid (**1e**).* Protected thioether **12** (196 mg, 0.53 mmol) was dissolved in DCM (5 mL) and TFA (4 mL) added. The mixture was stirred at rt for 18 h under an atmosphere of nitrogen and subsequently concentrated in vacuo. The product was dissolved in H_2O (15 mL) and freeze-dried to yield amino acid **1e** (191 mg, 94%) as a pale-brown solid. ^{1}H NMR (400 MHz, D_2O): δ 2.41−2.48 (1H, m), 2.60−2.68 (1H, m), 3.37 (1H, dd, *J* = 12, 4 Hz), 3.87 (1H, dd, *J* = 12, 4 Hz), 4.25 (1H, s), 4.63 (1H, dd, *J* = 12, 8 Hz), 7.34 (1H, t, *J* = 8 Hz), 7.45 (1H, d, *J* = 8 Hz), 7.54 (1H, t, *J* = 8, Hz), 7.88 (1H, d, *J* = 8 Hz). ^{13}C NMR (400 MHz, D_2O): δ 34.3, 41.9, 50.8, 59.0, 126.7, 129.3, 130.4, 131.1, 133.2, 135.3, 163.0, 171.2. LCMS: *m*/*z* [M + H]$^+$: calcd 268.1, found 268.1. OR: $[\alpha]^{22}_D$ −11.78 (*c* = 0.44; H_2O and 2% TFA). Melting point: decomposition.

*(2S,4R)-4-((3-Carboxyphenyl)thio)pyrrolidine-2-carboxylic Acid (**1f**).* Methyl 3-mercaptobenzoate (286 mg, 1.70 mmol, 1.44 equiv) was dissolved in DMF (12 mL) and K_2CO_3 (243 mg, 1.76 mmol, 1.49 equiv) added. Mesylate **11** (365 mg, 1.18 mmol, 1.00 equiv) was added to the mixture, which was subsequently left to stir at rt under an atmosphere of nitrogen for 18 h. To the mixture was added 10% brine (100 mL) and extracted with Et_2O (3 × 60 mL). The combined organic phases was washed with 0.5 M NaOH (2 × 80 mL) and brine (60 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude oil was dissolved in THF (10 mL), cooled to 0 °C, and 2 M NaOH (5 mL) added dropwise over the course of 5 min. After additional 15 min at 0 °C, the mixture was left to stir at rt for 19 h. The mixture was added H_2O (20 mL) and washed with Et_2O (2 × 20 mL). The aqueous phase was acidified (pH ≈ 1) using conc HCl, extracted with a combination of Et_2O and THF (3:4) (20 mL), and concentrated in vacuo to give crude, protected diacid **13**. The crude diacid was suspended in DCM (2 mL) and dissolved by addition of TFA (2 mL). The mixture was left to stir at rt under an atmosphere of nitrogen for 22 h. The mixture was concentrated in vacuo, dissolved in H_2O (30 mL), and washed with DCM (3 × 30 mL). The water phase was freeze-dried to yield amino acid **1f** (121 mg, 40%) as an off-white solid. ^{1}H NMR (400 MHz, D_2O): δ 2.33−2.40 (1H, m), 2.49−2.57 (1H, m), 3.32 (1H, dd, *J* = 12, 4 Hz), 3.77 (1H, dd, *J* = 12, 4 Hz), 4.07 (1H, pentet, *J* = 4 Hz), 4.60 (1H, t, *J* = 8 Hz), 7.37 (1H, t, *J* = 8 Hz), 7.56 (1H, d, *J* = 8 Hz), 7.78 (1H, d, *J* = 8 Hz), 7.85 (1H, s). ^{13}C NMR (400 MHz, D_2O): δ 34.4, 43.5, 50.7, 58.9, 129.1, 129.66, 130.7, 132.4, 133.0, 136.4, 169.2, 171.4. LCMS: *m*/*z* [M + H]$^+$: calcd 268.1, found 268.1. OR: $[\alpha]^{22}_D$ −6.6 (*c* = 0.29; H_2O). Melting point: decomposition.

*(2S,4S)-4-(1-Carboxyphenoxy)pyrrolidine-2-carboxylic Acid Trifluoroacetic Acid (**1g**).* Protected proline **15** (288 mg, 0.82 mmol, 1.00 equiv) was suspended in DCM (5 mL) and dissolved by addition of TFA (5 mL). The mixture was left to stir at rt under an atmosphere of nitrogen for 18 h. The mixture was concentrated in vacuo, dissolved in H_2O (5 mL), and freeze-dried to yield proline salt **1g** (260 mg, 87%) as a brownish foam. ^{1}H NMR (300 MHz, D_2O): δ 2.46−2.62 (2H, m), 3.47 (1H, dd, *J* = 13, 4 Hz), 3.69 (1H, d, *J* = 13 Hz), 4.29 (1H, dd, *J* = 10, 4 Hz), 5.14 (1H, m), 6.95−7.03 (2H, m), 7.40−7.46 (1H, m), 7.61 (1H, dd, *J* = 8, 2 Hz). ^{13}C NMR (400 MHz, D_2O): δ 34.3, 51.1, 59.5, 76.6, 115.2, 121.3, 122.2, 131.5, 134.2, 154.5, 170.4, 172.7. LCMS: *m*/*z* [M + H]$^+$: calcd 252.1, found 252.1. OR: $[\alpha]^{22}_D$ −7.5 (*c* = 0.31; H_2O with 1% TFA). Melting point: decomposition.

*(2S,4S)-4-(3-Carboxyphenoxy)pyrrolidine-2-carboxylic Acid Hydrochloride (**1h**).* Protected proline **16** (204 mg, 0.58 mmol, 1.00 equiv) was suspended in DCM (5 mL) and dissolved by addition of TFA (5 mL). The mixture was left to stir at rt under an atmosphere of nitrogen for 18 h. The mixture was concentrated in vacuo, dissolved in H_2O (5 mL), and freeze-dried. The salt was dissolved in 2 M HCl (3 mL), concentrated in vacuo, redissolved in H_2O (3 × 5 mL), and concentrated in vacuo to give **1h** (156 mg, 93%) as a reddish foam. ^{1}H NMR (300 MHz, D_2O): δ 2.50−2.67 (2H, m), 3.59 (1H, dd, *J* = 13, 4 Hz), 3.70 (1H, d, *J* = 13 Hz), 4.59 (1H, t, *J* = 6 Hz), 5.14 (1H, m), 7.03 (1H, ddm, *J* = 8, 3 Hz), 7.25−7.34 (2H, m), 7.47 (1H, dm, *J* = 8 Hz). ^{13}C NMR (75 MHz, D_2O): δ 34.3, 51.4, 58.8, 75.2, 116.3, 121.3,

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123.4, 130.1, 131.0, 155.1, 169.6, 171.7. LCMS: m/z $[M + H]^+$: calcd 252.1, found 252.1. OR: $[\alpha]^{22}_D$ + 8.0 (c = 0.56; H_2O). Melting point: decomposition.

*(2S,4S)-4-(1-Carboxyphenoxy)pyrrolidine-2-carboxylic Acid Trifluoroacetic Acid (**1i**).* Protected proline **17** (262 mg, 0.75 mmol, 1.00 equiv) was suspended in DCM (5 mL) and TFA (5 mL) added. The mixture was left to stir at rt under an atmosphere of nitrogen for 18 h. The mixture was concentrated in vacuo, dissolved in H_2O (5 mL), and freeze-dried to yield proline salt **1i** (231 mg, 85%) as a brownish foam. ^{1}H NMR (300 MHz, D_2O): δ 2.51−2.66 (2H, m), 3.57 (1H, dd, J = 13, 4 Hz), 3.70 (1H, d, J = 13 Hz), 4.53 (1H, dd, J = 9, 4 Hz), 5.25 (1H, m), 6.87−6.92 (2H, m), 7.84−7.90 (2H, m). ^{13}C NMR (400 MHz, $((CD_3)_2SO)$: δ 34.3, 50.7, 57.8, 75.0, 113.7, 115.2, 123.9, 131.4, 158.5, 166.8, 170.2. LCMS: m/z $[M + H]^+$: calcd 252.1, found 252.0. OR: $[\alpha]^{22}_D$ + 28 (c = 0.25; H_2O and 1% TFA). Melting point: decomposition.

*(2S,4S)-4-((2-Carboxyphenyl)thio)pyrrolidine-2-carboxylic Acid (**1j**).* Protected proline **19** (100 mg, 0.27 mmol) was suspended in DCM (1 mL) and dissolved by addition of TFA (1 mL). The mixture was left to stir at rt under nitrogen for 1 h. The mixture was concentrated in vacuo, dissolved in H_2O (30 mL), and washed with DCM (3 × 20 mL). The aqueous phase was freeze-dried to yield **1j** as its zwitterion (54 mg, 74%) as an off-white solid. ^{1}H NMR (400 MHz, D_2O): δ 2.18−2.25 (1H, m), 2.77−2.85 (1H, m), 3.34 (0.5H, d, J = 4 Hz), 3.39 (0.5H, d, J = 4 Hz), 3.76 (1H, dd, J = 12, 8 Hz), 4.15 (1H, m), 4.50 (1H, dd, J = 8, 4 Hz), 7.26 (1H, t, J = 8 Hz), 7.35 (1H, t, J = 8 Hz), 7.47 (2H, t, J = 8 Hz). ^{13}C NMR (400 MHz, D_2O): δ 34.0, 41.6, 50.8, 58.9, 126.6, 129.5, 130.4, 131.0, 133.1, 135.3, 162.8, 170.9. LCMS: m/z $[M + H]^+$: calcd 268.1, found 268.1. OR: $[\alpha]^{22}_D$ −4.7 (c = 0.19; H_2O and 5% TFA). Melting point: decomposition.

*(2S,4S)-4-((3-Carboxyphenyl)thio)pyrrolidine-2-carboxylic Acid (**1k**).* Methyl 3-mercaptobenzoate (473 mg, 2.81 mmol, 1.51 equiv) was dissolved in DMF (22 mL) and K_2CO_3 (397 mg, 2.87 mmol, 1.54 equiv) added. Mesylate **18** (604 mg, 1.87 mmol, 1.00 equiv) was dropwise added to the mixture, which was subsequently left to stir at rt under nitrogen for 18 h. The mixture was added 1% brine (400 mL) and extracted with Et_2O (3 × 100 mL). The combined organic phases was washed with 0.5 M NaOH (2 × 100 mL) and brine (80 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The oil was dissolved in THF (10 mL), cooled to 0 °C, and 2 M NaOH (5 mL) added dropwise over the course of 5 min. After an additional 15 min at 0 °C, the mixture was left to stir at rt for 20 h. The mixture was added H_2O (30 mL) and washed with Et_2O (2 × 30 mL). The aqueous phase was acidified (pH ≈ 1) using conc HCl, extracted with a combination of Et_2O and THF (3:4) (40 mL), and concentrated in vacuo to give crude diacid **20**. The crude diacid **20** was suspended in DCM (4 mL) and dissolved by addition of TFA (4 mL). The mixture was left to stir at rt under nitrogen for 21 h. The mixture was concentrated in vacuo, dissolved in H_2O (30 mL), and washed with DCM (3 × 30 mL). The aqueous phase was freeze-dried to yield **1k** as its zwitterion (188 mg, 38%) as an off-white solid. ^{1}H NMR (400 MHz, D_2O): δ 2.25−2.32 (1H, m), 2.79−2.87 (1H, m), 3.48 (1H, dd, J = 12, 4 Hz), 3.76 (1H, dd, J = 12, 4 Hz), 4.20 (1H, p, J = 4 Hz), 4.44 (1H, dd, J = 12, 8 Hz), 7.55 (1H, t, J = 8 Hz), 7.76 (1H, d, J = 8 Hz), 7.78 (1H, dd, J = 8, 4 Hz), 8.01 (1H, s). ^{13}C NMR (400 MHz, D_2O): δ 34.3, 43.7, 50.7, 59.8, 129.3, 129.7, 130.9, 132.9, 133.2, 137.0, 169.7, 171.8. LCMS: m/z $[M + H]^+$: calcd 268.1, found 268.1. OR: $[\alpha]^{22}_D$ −18 (c = 0.24; H_2O). Melting point: decomposition.

*(2S,4R)-4-(2-(Carboxymethyl)phenoxy)pyrrolidine-2-carboxylic Acid Hydrochloride (**1l**).* To a flask containing protected proline **29** (97 mg, 0.25 mmol, 1.00 equiv) was added 4 M HCl (5 mL), and the mixture was heated to reflux for 19 h. The mixture was concentrated in vacuo (water aspirator). Water (2 × 20 mL) was added, and the mixture was concentrated in vacuo after each addition. The mixture was purified using prep HPLC. The HCl salt was obtained by concentrating the product in vacuo from 1 M HCl (3 × 10 mL), yielding **1l** (39 mg, 53%) as a beige semisolid. ^{1}H NMR (400 MHz, D_2O) δ 2.30−2.45 (m, 1H), 2.59−2.69 (m, 1H), 3.53−3.66 (m, 3H), 3.67−3.78 (m, 1H), 4.48−4.60 (m, 1H), 5.16−5.26 (m, 1H), 6.87−7.03 (m, 2H), 7.16−7.24 (m, 1H), 7.24−7.34 (m, 1H). ^{13}C NMR (100 MHz, D_2O) δ 34.6, 35.9, 51.1, 58.8, 75.4, 112.3, 121.9, 123.6, 129.2, 131.8, 153.9, 171.1, 176.9. LCMS: m/z $(M + H)^+$: calcd 266.1, found 266.0. OR: $[\alpha]^{25}_D$ + 8.0 (c = 0.5; H_2O). Melting point: decomposition.

*(2S,4R)-4-(3-(Carboxymethyl)phenoxy)pyrrolidine-2-carboxylic Acid Hydrochloride (**1m**).* Carried out as described for **1l** with **30** (142 mg, 0.361 mmol, 1.00 equiv), 4 M HCl (5 mL), and heating to reflux for 2 h. The mixture was purified using prep HPLC to yield **1m** as the TFA salt. The HCl salt was obtained by concentrating the product in vacuo from 1 M HCl (3 × 10 mL) to yield **1m** (62 mg, 57%) as a white solid. ^{1}H NMR (400 MHz, D_2O) δ 2.45 (ddd, J = 4, 10, 14 Hz, 1H), 2.72−2.85 (m, 1H), 3.69−3.78 (m, 4H), 4.67 (dd, J = 8, 10 Hz, 1H), 5.27−5.34 (m, 1H), 6.93−7.06 (m, 3H), 7.39 (dd, J = 8 Hz, 1H). ^{13}C NMR (100 MHz, D_2O) δ 34.4, 40.3, 51.0, 58.6, 75.8, 114.8, 117.1, 123.4, 130.2, 136.0, 155.9, 171.7, 176.4. LCMS: m/z $(M + H)^+$: calcd 266.1, found 266.0. OR: $[\alpha]^{25}_D$ + 80 (c = 0.25; H_2O). TLC: R_f 0.46 (heptane:EtOAc, 1:1). HPLC: >99% (254 nm). Melting point: 203.2−211.0 °C.

*(2S,4R)-4-((3-Carboxynaphthalen-2-yl)oxy)pyrrolidine-2-carboxylic Acid Hydrochloride (**1n**).* Carried out as described for **1l** with **31** (97 mg, 0.25 mmol), 4 M HCl (5 mL), and heating to reflux for 19 h. The mixture was purified using prep HPLC to yield **1n** as the TFA salt. The HCl salt was obtained by concentrating the product in vacuo from 1 M HCl (3 × 10 mL) to yield **1n** (40 mg, 63%) as a beige semisolid. ^{1}H NMR (400 MHz, D_2O) δ 2.44 (ddd, J = 4, 10, 15 Hz, 1H), 2.78 (dd, J = 8, 14 Hz, 1H), 3.67 (s, 2H), 4.67 (t, J = 9 Hz, 1H), 5.39 (br s, 1H), 7.35−7.46 (m, 2H), 7.52 (t, J = 8 Hz, 1H), 7.77 (d, J = 8 Hz, 1H), 7.85 (d, J = 8 Hz, 1H), 8.35 (s, 1H). ^{13}C NMR (100 MHz, D_2O) δ 36.2, 52.2, 60.2, 78.6, 112.2, 124.0, 126.5, 127.8, 129.8, 129.8, 129.9, 129.9, 134.4, 137.3, 153.4, 169.7, 171.4. LCMS: m/z $(M + H)^+$: calcd 302.1, found 302.0. OR: $[\alpha]^{25}_D$ + 1.6 (c = 1.25; abs EtOH). Melting point: decomposition.

*(2S,4R)-4-((2-Carboxynaphthalen-1-yl)oxy)pyrrolidine-2-carboxylic Acid Hydrochloride (**1o**).* Carried out as described for **1l** with **32** (276 mg, 0.643 mmol, 1.00 equiv), 4 M HCl (7.5 mL), and heated to reflux for 39 h. The mixture was purified using prep HPLC to yield the TFA salt of **1o**. The HCl salt was obtained by concentrating the product in vacuo from 1 M HCl (3 × 10 mL) to yield **1o** (88 mg, 41%) as an off-white solid. ^{1}H NMR (400 MHz, D_2O) δ 2.22−2.29 (m, 1H), 2.76 (dd, J = 8, 14 Hz, 1H), 3.69−3.74 (m, 1H), 3.90 (d, J = 13 Hz, 1H), 5.18 (br s, 1H), 7.69−7.76 (m, 2H), 7.83 (d, J = 7 Hz, 1H), 7.86−7.90 (m, 1H), 8.02 (d, J = 6 Hz, 1H), 8.22 (s, 1H). ^{13}C NMR (100 MHz, D_2O) δ 35.0, 51.4, 59.8, 84.1, 119.9, 122.8, 124.9, 126.2, 127.4, 127.8, 128.2, 129.0, 136.7, 153.5, 170.2, 172.4. LCMS: m/z $(M + H)^+$: calcd 302.1, found 302.0. Melting point: 218.5−229.8 °C.

*(2S,4R)-4-((1-Carboxynaphthalen-2-yl)oxy)pyrrolidine-2-carboxylic Acid Hydrochloride (**1p**).* To a flask containing protected proline **33** (168 mg, 0.391 mmol, 1.00 equiv), 4 M HCl (5 mL) was added, and the mixture was heated to reflux for 23 h. The mixture was concentrated in vacuo, H_2O (10 mL) added, and concentrated in vacuo again. Then 2 M NaOH (5 mL) was added and the mixture heated to reflux for 2.5 h. H_2O (10 mL) was added and the mixture washed with DCM (3 × 10 mL). The aqueous phase was acidified using 6 M HCl, the precipitate retained on a filter, and dried in vacuo to yield the HCl salt of **1p** (67 mg, 51%) as a beige/brown solid. ^{1}H NMR (400 MHz, CD_3OD) δ 2.42 (ddd, J = 4, 11, 14 Hz, 1H), 2.72−2.83 (m, 1H), 3.63−3.73 (m, 1H), 3.76 (dd, J = 4, 13 Hz, 1H), 4.72 (dd, J = 7, 11 Hz, 1H), 5.48 (t, J = 4 Hz, 1H), 7.42−7.52 (m, 2H), 7.56 (ddd, J = 1, 7, 8 Hz, 1H), 7.84−7.93 (m, 2H), 8.02 (d, J = 9 Hz, 1H). ^{13}C NMR (101 MHz, CD_3OD) δ 36.2, 52.5, 60.0, 79.6, 117.2, 122.5, 125.2, 126.3, 128.9, 129.4, 131.0, 131.8, 133.1, 151.9, 170.7, 171.2. LCMS: m/z $(M + H)^+$: calcd 302.1, found 302.0. Melting point: decomposition.

*4-(((3R,5S)-5-Carboxypyrrolidin-3-yl)oxy)isophthalic Acid (**1q**).* To a flask with protected proline **34** (189 mg, 0.432 mmol, 1.00 equiv) was added 4 M HCl (4 mL), and the mixture was heated to reflux for 21 h. The mixture was concentrated in vacuo, H_2O (10 mL) added, and the mixture was concentrated in vacuo again to yield the HCl salt of **1q** (136 mg, 95%) as an off-white solid. ^{1}H NMR (400

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MHz, CD_3OD) δ 2.51 (ddd, J = 4, 10, 15 Hz, 1H), 2.73−2.82 (m, 1H), 3.65−3.78 (m, 2H), 4.77 (dd, J = 8, 10 Hz, 1H), 5.43−5.48 (br s, 1H), 7.29 (d, J = 9 Hz, 1H), 8.21 (dd, J = 2, 9 Hz, 1H), 8.51 (d, J = 2 Hz, 1H). ^{13}C NMR (101 MHz, CD_3OD) δ 34.7, 50.9, 58.4, 77.4, 114.9, 121.7, 124.2, 133.6, 134.9, 159.2, 166.9, 167.2, 169.3. LCMS: m/z $(M + H)^+$: calcd 296.1, found 296.0. OR: $[\alpha]^{25}_D$ −109 (c = 0.055; 99.9% EtOH). Melting point: 184.2−198.0 °C.

*(2S,4R)-4-(2-Carboxy-4-methylphenoxy)pyrrolidine-2-carboxylic Acid Hydrochloride (**1r**).* Carried out as described for **1l** with **35** (90 mg, 0.237 mmol, 1.00 equiv), 4 M HCl (5 mL), and heated to reflux for 24 h. The mixture was purified using prep HPLC to yield the TFA salt of **1r.** The HCl salt was obtained by concentrating the product in vacuo from 1 M HCl (3 × 10 mL) to yield the HCl salt of **1r** (50 mg, 70%) as a beige semisolid. ^{1}H NMR (400 MHz, CD_3OD) δ 0.99 (s, 3H), 2.41−2.48 (m, 2H), 3.40 (dd, J = 8, 11 Hz, 1H), 3.50−3.56 (m, 2H), 3.97−4.04 (m, 1H), 5.74 (d, J = 8 Hz, 1H), 6.07−6.15 (m, 1H), 6.31 (d, J = 2 Hz, 1H). ^{13}C NMR (101 MHz, CD_3OD) δ 18.0, 33.1, 49.6, 57.5, 76.0, 114.4, 119.3, 130.4, 131.0, 133.5, 151.4, 168.7, 170.1. LCMS: m/z $(M + H)^+$: calcd 266.1, found 266.0. OR: $[\alpha]^{25}_D$ −2 (c = 1; H_2O). Melting point: 173.3−207.9 °C.

*(2S,4R)-4-(4-Acetyl-2-carboxyphenoxy)pyrrolidine-2-carboxylic Acid Hydrochloride (**1s**).* Carried out as described for **1l** with **36** (152 mg, 0.373 mmol, 1.00 equiv), 4 M HCl (5 mL), and heated to reflux for 19 h. The mixture was purified using prep HPLC to yield the TFA salt of **1s.** The HCl salt was obtained by concentrating the product in vacuo from 1 M HCl (3 × 10 mL) to yield the HCl salt of **1s** (76 mg, 62%) as a beige semisolid. ^{1}H NMR (400 MHz, D_2O) δ 2.50−2.59 (m, 1H), 2.60 (s, 3H), 2.81 (ddt, J = 2, 8, 15 Hz, 1H), 3.74−3.88 (m, 2H), 4.71 (dd, J = 8, 10 Hz, 1H), 5.47 (t, J = 4 Hz, 1H), 7.18 (d, J = 8.85 Hz, 1H), 8.10 (dd, J = 2.39, 8.85 Hz, 1H), 8.27 (d, J = 2.33 Hz, 1H). ^{13}C NMR (101 MHz, D_2O) δ 25.9, 34.6, 50.9, 58.9, 77.1, 114.3, 114.8, 117.7, 120.7, 129.8, 132.6, 134.6, 159.1, 162.7, 163.0, 168.9, 171.4, 201.0. LCMS: m/z $(M + H)^+$: calcd 294.1, found 294.0. OR: $[\alpha]^{25}_D$ −36 (c = 0.055; H_2O). Melting point: 140.1−212.1 °C.

*(2S,4R)-4-(2-Carboxy-6-methylphenoxy)pyrrolidine-2-carboxylic Acid Hydrochloride (**1t**).* Carried out as described for **1l** with **37** (59 mg, 0.16 mmol, 1.00 equiv), 4 M HCl (2 mL), and heated to reflux for 19 h. The mixture was purified using prep HPLC to yield the TFA salt of **1t.** The HCl salt was obtained by concentrating the product in vacuo from 1 M HCl (3 × 10 mL) to yield the HCl salt of **1t** (40 mg, 86%) as a reddish semisolid. ^{1}H NMR (400 MHz, D_2O) δ 2.09−2.23 (m, 1H), 2.10 (s, 3H), 2.47−2.61 (m, 1H), 3.52−3.71 (m, 2H), 4.67 (d, J = 8 Hz, 1H), 4.77−4.84 (m, 1H), 6.99−7.12 (m, 1H), 7.39 (dd, J = 2, 8 Hz, 1H), 7.55 (dd, J = 2, 8 Hz, 1H). ^{13}C NMR (101 MHz, D_2O) δ 16.1, 34.6, 51.5, 58.9, 81.9, 124.1, 124.7, 129.3, 132.5, 136.5, 153.7, 170.1, 171.0. LCMS: m/z $(M + H)^+$: calcd 266.1, found 266.0; Melting point: decomposition.

*(3R,5S)-(tert-Butoxycarbonyl)-5-(((tert-butyl)dimethylsilyloxy)-methyl)-3-(3-(methoxycarbo-nyl)benzyl)-pyrrolidin-2-one (**3**).* Protected pyroglutaminol **2** (500 mg, 1.52 mmol, 1.00 equiv) was weighed out in a dry flask that was afterward purged with and placed under an atmosphere of nitrogen. Dry THF (2 mL) was added and the solution cooled to −78 °C. Then 1 M LHMDS (1.8 mL, 1.8 mmol, 1.2 equiv) was dropwise added over the course of 10 min and stirred for 2 h prior to use. Methyl 3-bromomethylbenzoate (454 mg, 1.98 mmol, 1.31 equiv) was dissolved in dry THF (2 mL) in a dry flask and dropwise added to the mixture of compound **2** over the course of 15 min. The mixture was stirred for 3 h before being removed from the cooling bath and allowed to warm up to rt. The mixture was quenched by addition of saturated NH_4Cl (4 mL) and transferred to a separatory funnel containing EtOAc (5 mL). The aqueous phase was reextracted with EtOAc (2 × 5 mL), and the combined organic phases was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The product was purified using DCVC (0−28% EtOAc in heptanes) to afford phenyl ester **3** as a dark-orange oil (467 mg, 64%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.00 (6H, s), 0.85 (9H, s), 1.55 (9H, s), 1.75−1.93 (2H, m), 1.95−2.07 (1H, m), 3.05−3.20 (1H, m), 3.30−3.40 (1H, m), 3.56−3.64 (1H, m), 3.83−3.93 (4H, m), 4.00−4.09 (1H, m), 7.33−7.40 (2H, m), 7.81−7.85 (1H, m), 7.85−7.90 (1H, m). ^{13}C NMR (75 MHz, $CDCl_3$): δ −5.39, −5.35, 18.3, 26.0, 28.3, 28.5, 37.1, 44.5, 52.2, 57.0, 64.2, 82.9, 127.7, 128.6, 129.8, 130.3, 133.4, 139.6, 150.0, 166.9, 175.4. LCMS: m/z $[M + H]^+$: calcd 378.2, found 378.1. TLC: R_f 0.37 (EtOAc:heptanes, 1:4). OR: $[\alpha]^{20.5}_D$ −31.55 (c = 0.37 g/100 mL; Abs EtOH).

*(2S,4R)-(tert-Butoxycarbonyl)-2-(((tert-butyl)dimethylsilyloxy)-methyl)-4-(3-(hydroxymethyl)benzyl)-pyrrolidine (**4**).* Compound **3** (1.31 g, 2.75 mmol, 1.00 equiv) was weighed out in a dry flask and dissolved in dry THF (10 mL) under an atmosphere of nitrogen. Then 1 M BH_3·THF complex (14.6 mL, 14.6 mmol, 5.31 equiv) was added and the mixture heated to reflux for 21 h. The mixture was cooled to 0 °C and extra THF (35 mL) added. H_2O (4.2 mL) was carefully added dropwise over the course of 30 min. Then 2 M NaOH (22.3 mL) was carefully added dropwise over the course of 30 min followed by addition of 30% H_2O_2 (7 mL) over the course of 15 min. After stirring for 5 min at 0 °C, the mixture was removed from the ice-bath and left to stir for 1.5 h at rt. The mixture was transferred to a separation funnel containing sat. $NaHCO_3$ (75 mL) and EtOAc (75 mL). The aqueous phase was extracted with EtOAc (2 × 75 mL). The pooled organic phases were washed with brine (100 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The mixture was purified using DVCV (0−100% EtOAc in heptanes) to yield benzyl alcohol **4** (0.68 g, 56%) as a thick, colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.03 (6H, s), 0.88 (9H, s), 1.46 (9H, s), 1.55−1.85 (2H, m), 1.95−2.15 (1H, m), 2.50−2.75 (3H, m), 2.95−3.15 (1H, m), 3.35−3.55 (1.5H, m), 3.55−3.85 (2H, m), 3.85−3.95 (0.5H, m), 4.68 (2H, s), 7.08 (1H, d, J = 7 Hz), 7.12−7.25 (3H, m). ^{13}C NMR (75 MHz, $CDCl_3$): δ −5.1, 18.5, 26.1, 28.8, 34.1, 34.7, 37.9, 39.0, 39.9, 52.1, 52.5, 58.6, 58.8, 63.6, 64.0, 65.5, 79.2, 79.5, 124.8, 127.3, 128.0, 128.6, 140.7, 141.1, 154.5. LCMS: m/z $[M + H]^+$: calcd 380.2, found 380.2 (-t-Bu). TLC: R_f 0.46 (EtOAc:heptanes, 1:1). OR: $[\alpha]^{20.5}_D$ −28.41 (c = 0.35 g/100 mL; Abs EtOH).

*(2S,4R)-tert-butyl 2-(hydroxymethyl)-4-(3-(hydroxymethyl)-benzyl)pyrrolidine-1-carboxylate (**5**).* Alcohol **4** (0.67 g, 1.55 mmol, 1.00 equiv) was dissolved in dry THF (11 mL) and 1 M TBAF in THF (4.66 mL, 4.66 mmol, 3.01 equiv) added under an atmosphere of nitrogen. The mixture was left to stir at rt for 1 h before adding satd $NaHCO_3$ (40 mL). The mixture was transferred to a separatory funnel containing EtOAc (30 mL). The aqueous phase was extracted with EtOAc (2 × 50 mL), and the pooled organic phases were washed with brine (50 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The mixture was purified using DVCV (0−100% EtOAc in toluene) to yield diol **5** (0.26 g, 52%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.48 (9H, s), 1.60−2.05 (3H, m), 2.35−2.55 (1H, m), 2.55−2.70 (2H, m), 3.10−3.20 (1H, m), 3.42 (1H, dd, J = 10, 7 Hz), 3.52−3.65 (2H, m), 4.08 (1H, br s), 4.46 (1H, br s), 4.69 (2H, s), 7.07 (1H, d, J = 7 Hz), 7.16 (1H, s), 7.18−7.33 (2H, m). ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.7, 34.3, 39.1, 39.4, 52.6, 59.6, 65.4, 68.0, 80.6, 125.0, 127.3, 128.0, 128.7, 140.4, 141.2. LCMS: m/z $[M + H]^+$: calcd 266.1, found 266.1 (-t-Bu). TLC: R_f 0.10 (EtOAc:heptanes, 1:1). OR: $[\alpha]^{20.5}_D$ −14.11 (c = 0.25 g/100 mL; Abs EtOH).

*(2S,4R)-1-(tert-Butoxycarbonyl)-4-(3-carboxybenzyl)pyrrolidine-2-carboxylic Acid (**6**).* Diol **5** (180 mg, 0.56 mmol, 1.00 equiv) was dissolved in CH_3CN (2.4 mL) and a sodium phosphate buffer (1.8 mL, pH = 6.7 (670 mM)) and TEMPO (12 mg, 0.08 mmol, 0.14 equiv) added. The reaction mixture was heated to 35 °C and a solution of $NaClO_2$ (200 mg, 2.24 mmol, 4.00 equiv) in H_2O (0.48 mL) added dropwise and a solution of NaOCl (8 μL 10−13% aqueous solution, approximately 1 mg, approximately 0.013 mmol, 0.023 equiv) in H_2O (0.24 mL) simultaneously over the course of 2 h. The lack of characteristic intermediate coloring of mixture facilitated the addition of additional TEMPO (10 mg, 0.06 mmol, 0.11 equiv) and NaOCl (2 drops). This resulted in a dark coloring of the mixture that disappeared shortly after. The mixture was stirred for 18 h at 35 °C. The mixture was cooled to 0 °C, added H_2O (2 mL) followed by addition of 2 M NaOH (dropwise until pH = 10). The mixture was dropwise added a solution of Na_2SO_3 (300 mg, 2.38 mmol, 4.25 equiv) in H_2O (2 mL). The mixture was stirred at rt for 30 min. The mixture was transferred to a separatory funnel and washed with Et_2O (2 × 20 mL). The aqueous mixture was made acidic using 4 M HCl (a few drops, pH = 2) and extracted with Et_2O (3 × 20 mL). The pooled organic phases

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was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to yield diacid **6** (122 mg, 62%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.44 (4H, s), 1.50 (5H, s), 1.75−2.10 (1.46H, m), 2.25−2.40 (0.57H, m), 2.55−2.95 (3H, m), 3.00−3.20 (1H, m), 3.58 (0.57H, m), 3.80 (0.46H, m), 4.32 (0.46H, m), 4.42 (0.57H, d, J = 8 Hz), 7.33−7.44 (2H, m), 7.87−7.99 (2H, m). ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.4, 28.5, 35.0, 36.2, 38.4, 38.8, 39.2, 51.5, 51.8, 59.0, 80.7, 81.2, 128.3, 128.8, 129.7, 130.2, 134.0, 140.1, 153.8, 155.4, 171.6, 176.6, 178.7. LCMS: m/z $[M + H]^+$: calcd 350.2, found 350.3. TLC: R_f 0.46 (EtOAc:heptanes, 2:1 + 2% AcOH). OR: $[\alpha]^{20}_D$ −3.4 (c = 0.38; Abs EtOH).

*(2S,4R)-1-(tert-Butoxycarbonyl)-4-(2-carboxyphenoxy)-pyrrolidine-2-carboxylic Acid (**8**).* Commercially available **7** (140 mg, 0.57 mmol, 1.00 equiv), methyl 2-hydroxybenzoate (108 mg, 0.71 mmol, 1.24 equiv), PPh_3 (140 mg, 0.69 mmol, 1.21 equiv), and DIAD (181 mg, 0.69 mmol, 1.21 equiv) were weighed out in a dry flask and dissolved in dry THF (4 mL) under an atmosphere of nitrogen. The mixture was left to stir at rt for 18 h. The mixture was added Et_2O/THF (1:1, 20 mL) and washed with 2 M NaOH (2 × 10 mL), brine (15 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The mixture was purified using DCVC (0−24% EtOAc in toluene). The mixture was dissolved in THF (6 mL), cooled to 0 °C, and 1 M LiOH (6 mL) added dropwise over the course of 2 min followed by addition of 4 M NaOH (1 mL) over the course of 2 min. The mixture was left to stir at 0 °C for 15 min and at rt for 20 h. The mixture was added H_2O (20 mL) and the aqueous phase washed with Et_2O (2 × 20 mL). To the aqueous phase was added conc HCl (pH = 1−2) and extracted with a mixture of Et_2O and THF (1:1, 3 × 20 mL). The pooled organic phases was washed with brine (30 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The mixture was purified using DCVC (0−100% EtOAc in heptanes containing 2% AcOH) to yield diacid **8** (123 mg, 61%) as a colorless oil. ^{1}H NMR (300 MHz, $(CDCl_3)$: δ 1.42/1.43 (9H, 2s), 2.30−2.50 (1H, m), 2.55−2.75 (1H, m), 3.67−3.95 (2H, m), 4.49 (0.5H, t, J = 8 Hz), 4.59 (0.5H, t, J = 8 Hz), 5.09 (1H, bs), 6.96 (1H, dd, J = 8, 5 Hz), 7.07 (1H, td, J = 8, 4 Hz), 7.50 (1H, m), 7.98 (1H, dm, J = 8 Hz). ^{13}C NMR (75 MHz, $(CDCl_3)$: δ 28.3, 28.4, 35.3, 36.6, 51.7, 52.1, 57.8, 57.9, 76.8, 77.2, 81.5, 81.7, 114.8, 115.1, 119.7, 120.1, 122.1, 133.2, 133.4, 134.6, 134.7, 153.9, 155.3, 156.3, 168.6, 168.7, 177.1, 177.5. LCMS: m/z $[M + H]^+$: calcd 252.1, found 252.2. HPLC: >99% (254 nm). TLC: R_f 0.13 (EtOAc:heptanes, 2:1 + 2% AcOH). OR: $[\alpha]^{25}_D$ −42 (c = 0.52; Abs EtOH).

*(2S,4R)-1-(tert-Butoxycarbonyl)-4-(3-carboxyphenoxy)-pyrrolidine-2-carboxylic Acid (**9**).* Commercially available **7** (300 mg, 1.22 mmol, 1.00 equiv), methyl 3-hydroxybenzoate (230 mg, 1.51 mmol, 1.24 equiv), and PPh_3 (374 mg, 1.43 mmol, 1.17 equiv) were weighed out in a dry flask, dissolved in dry THF (8 mL), and cooled to 0 °C. DIAD (385 mg, 1.47 mmol, 1.20 equiv) was dropwise added over 15 min under an atmosphere of nitrogen, and the mixture was left to stir at rt for 18 h. The mixture was added Et_2O (20 mL) and washed with 2 M NaOH (2 × 10 mL), brine (15 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The mixture was purified using DCVC ((1) column. 0−25% EtOAc in toluene; (2) column. 0−35% in heptanes). The mixture was dissolved in THF (10 mL), cooled to 0 °C, and 2 M NaOH (5 mL) added dropwise over the course of 2 min. The mixture was left to stir at 0 °C for 15 min and at rt for 20 h. To the mixture was added H_2O (20 mL) and the aqueous phase washed with Et_2O (2 × 20 mL). The aqueous phase was added conc HCl (pH = 1−2) and extracted with a mixture of Et_2O and THF (1:1, 3 × 20 mL). The pooled organic phases was washed with brine (30 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo to yield diacid **9** (336 mg, 91%) as a white foam. ^{1}H NMR (300 MHz, $(CD_3)_2SO$): δ 1.36 (9H, s), 2.10−2.30 (1H, m), 2.35−2.52 (1H, m), 3.50−3.70 (2H, m), 4.21 (1H, q, J = 8 Hz), 5.08 (1H, br s), 7.20 (1H, dd, J = 8, 2 Hz), 7.36−7.46 (2H, m), 7.54 (1H, d, J = 7 Hz). ^{13}C NMR (75 MHz, $(CD_3)_2SO$): δ 27.9, 28.1, 35.0, 35.8, 51.6, 51.8, 57.4, 57.6, 74.8, 75.7, 79.3, 115.5, 115.8, 120.2, 120.3, 122.0, 122.1, 129.9, 132.2, 153.0, 153.4, 156.4, 166.8, 173.2, 173.6. LCMS: m/z $[M + H]^+$: calcd 252.1, found 252.0. TLC: R_f 0.18 (EtOAc:heptanes, 2:1 + 2% AcOH). OR: $[\alpha]^{25}_D$ −23 (c = 0.45; Abs EtOH).

*(2S,4R)-1-(tert-Butoxycarbonyl)-4-(4-carboxyphenoxy)-pyrrolidine-2-carboxylic Acid (**10**).* Commercially available **7** (140 mg, 0.57 mmol, 1.00 equiv), methyl 2-hydroxybenzoate (108 mg, 0.71 mmol, 1.24 equiv), PPh_3 (140 mg, 0.69 mmol, 1.21 equiv), and DIAD (181 mg, 0.69 mmol, 1.21 equiv) were weighed out in a dry flask and dissolved in dry THF (4 mL) under an atmosphere of nitrogen. The mixture was left to stir at rt for 18 h. To the mixture was added Et_2O/THF (1:1, 20 mL) and washed with 2 M NaOH (2 × 10 mL) and brine (15 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The mixture was purified using DCVC (0−24% EtOAc in toluene). The mixture was dissolved in THF (6 mL), cooled to 0 °C, and 1 M LiOH (6 mL) added dropwise over the course of 2 min followed by addition of 4 M NaOH (1 mL) over the course of 2 min. The mixture was left to stir at 0 °C for 15 min and at rt for 20 h. The mixture was added H_2O (20 mL) and the aqueous phase washed with Et_2O (2 × 20 mL). To the aqueous phase was added conc HCl (pH = 1−2) and extracted with a mixture of Et_2O and THF (1:1, 3 × 20 mL). The pooled organic phases was washed with brine (30 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The mixture was purified using DCVC (0−100% EtOAc in heptanes containing 2% AcOH) to yield diacid **10** (106 mg, 53%) as a colorless oil. ^{1}H NMR (300 MHz, $((CD_3)_2SO)$: δ 1.34/1.35 (9H, 2 × s), 2.15−2.30 (1H, m), 2.35−2.54 (1H, m), 3.54 (1H, d, J = 13 Hz), 3.64 (1H, td, J = 11, 3 Hz), 4.19 (1H, q, J = 8 Hz), 5.08 (1H, br s), 6.99 (2H, d, J = 9 Hz), 7.86 (2H, d, J = 9 Hz). ^{13}C NMR (75 MHz, $((CD_3)_2SO)$: δ 27.9, 28.1, 35.1, 35.9, 51.7, 52.0, 57.4, 57.6, 74.9, 75.7, 79.3, 115.0, 123.3, 131.4, 153.0, 153.4, 160.1, 166.8, 173.2, 173.7. LCMS: m/z $[M + H]^+$: calcd 252.1, found 252.1. HPLC: >99% (254 nm). TLC: R_f 0.19 (EtOAc:heptanes, 2:1 + 2% AcOH). OR: $[\alpha]^{25}_D$ −22 (c = 0.45; Abs EtOH).

*(2S,4S)-1-tert-Butyl 2-methyl 4-((methylsulfonyl)oxy)pyrrolidine-1,2-dicarboxylate (**11**).* Commercially available alcohol **7** (550 mg, 2.24 mmol, 1.00 equiv) was dissolved in dry THF (5 mL) and added TEA (1.70 mL, 3.95 mmol, 1.76 equiv) under an atmosphere of nitrogen. The mixture was cooled to 0 °C, dropwise added mesyl chloride (0.30 mL, 3.88 mmol, 1.73 equiv) over the course of 10 min, and the mixture was stirred at 0 °C for an additional 5 h. The mixture was added H_2O (10 mL) and extracted with EtOAc (3 × 10 mL). The combined organic phases were washed with 1 M HCl (10 mL), sat. $NaHCO_3$ (15 mL), brine (10 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo to yield mesylate **11** (714 mg, 98%) as a slightly, white solid. ^{1}H NMR (300 MHz, $(CDCl_3)$: δ 1.44, 1.49 (9H, 2s), 2.46−2.58 (2H, m), 3.02 (3H, s), 3.75−3.84 (2H, m), 3.76 (3H, s), 4.42 (0.53H, dd, J = 8, 3 Hz), 4.53 (0.46H, dd, J = 7, 5 Hz), 5.24 (1H, m).

*(2S,4R)-1-(tert-Butoxycarbonyl)-4-((2-carboxyphenyl)thio)-pyrrolidine-2-carboxylic Acid (**12**).* Methyl 2-mercaptobenzoate (307 mg, 183 mmol, 1.63 equiv) was dissolved in DMF (7 mL) and K_2CO_3 (265 mg, 1.92 mmol, 1.71 equiv) added. Mesylate **11** (345 mg, 1.12 mmol, 1.00 equiv) was dropwise added to the mixture over the course of 5 min, which was subsequently heated to 50 °C and stirred under an atmosphere of nitrogen for 18 h. The mixture was added 10% brine (75 mL) and extracted with Et_2O (2 × 75 mL). The combined organic phases were washed with 0.5 M NaOH (2 × 50 mL) and brine (50 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude oil was dissolved in THF (7.5 mL), cooled to 0 °C, and 2 M LiOH (20 mL) added dropwise over the course of 5 min. After an additional 15 min at 0 °C, the mixture was left to stir for 18 h at rt. To the mixture was added H_2O (75 mL) and was washed with Et_2O (2 × 50 mL). The aqueous phase was acidified (pH ≈ 1) using conc HCl and extracted with a combination of Et_2O and THF (1:1) (3 × 70 mL). The combined organic phases was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified using DCVC (0−75% EtOAc in toluene and 2% AcOH), yielding thioether **12** (304 mg, 78%) as a clear, colorless oil. ^{1}H NMR (300 MHz, $(CDCl_3)$: δ 1.43/1.47 (9H, 2s), 2.26−2.68 (2H, m), 3.40−3.56 (1H, m), 3.96−4.06 (1.5H, m), 4.17 (0.5H, dd, J = 11, 7 Hz), 4.46 (0.5H, dd, J = 8, 5 Hz), 4.57 (0.5H, dd, J = 8, 3 Hz), 7.13−7.25 (1H, m), 7.33 (1H, t, J = 10 Hz), 7.48 (1H, q, J = 8 Hz), 8.07 (1H, dd, J = 8, 3 Hz), 12.0 (2H, bs). ^{13}C NMR (75 MHz, $(CDCl_3)$: δ 28.4, 28.5, 35.5, 36.5, 40.5, 41.0, 52.4, 52.5, 58.4, 58.5, 81.3, 81.7, 124.7,

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124.8, 126.6, 126.8, 126.9, 132.5, 133.5, 140.7, 140.8, 153.5, 155.0, 171.3, 176.5, 178.4. LCMS: m/z $[M + H]^+$: calcd 368.1, found 268.1 (-Boc). HPLC: >99% (254 nm). TLC: R_f 0.19 (EtOAc:heptanes, 2:1 + 2% AcOH). OR: $[\alpha]^{22}_D$ −23 (c = 0.49; abs EtOH).

*(2S,4S)-1-(tert-Butoxycarbonyl)-4-(2-carboxyphenoxy)-pyrrolidine-2-carboxylic Acid (**15**).* Methyl ester **14** (303 mg, 1.24 mmol, 1.00 equiv), methyl 2-hydroxybenzoate (236 mg, 1.55 mmol, 1.26 equiv), PPh_3 (395 mg, 1.51 mmol, 1.22 equiv), and DIAD (325 mg, 1.61 mmol, 1.30 equiv) were weighed out in a dry flask and dissolved in dry THF (6 mL) under an atmosphere of nitrogen. The mixture was left to stir at rt for 18 h. To the mixture was added a combination of Et_2O and THF (1:1, 40 mL) and washed with 2 M NaOH (2 × 20 mL) and brine (20 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The mixture was purified using DCVC (0−30% EtOAc in toluene). The diester was dissolved in THF (6 mL), cooled to 0 °C, and 2 M LiOH (12 mL) added dropwise over the course of 5 min. The mixture was left to stir at 0 °C for 15 min and at rt for 20 h. The mixture was added H_2O (40 mL) and the aqueous phase washed with Et_2O (2 × 40 mL). The aqueous phase was added conc HCl (pH ≈ 0) and extracted with a mixture of Et_2O and THF (1:1, 3 × 40 mL). The combined organic phases was washed with brine (50 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo to yield diacid **15** (333 mg, 77%) as a clear, colorless oil. ^{1}H NMR (300 MHz, $((CD_3)_2SO)$: δ 1.36/1.41 (9H, 2s), 2.10−2.18 (1H, m), 2.59−2.74 (1H, m), 3.38 (1H, dd, J = 11, 4 Hz), 3.79−3.87 (1H, m), 4.19−4.27 (1H, m), 4.93−5.01 (1H, m), 7.00−7.09 (2H, m), 7.44−7.50 (1H, m), 7.62 (1H, dd, J = 8, 2 Hz). ^{13}C NMR (75 MHz, $((CD_3)_2SO)$: δ 28.0, 28.2, 34.7, 35.5, 51.3, 51.7, 57.2, 57.4, 75.8, 76.7, 78.9, 79.1, 115.5, 121.0, 122.5, 130.9, 132.8, 152.7, 153.2, 155.7, 166.8, 172.7, 173.0. LCMS: m/z $[M + H]^+$: calcd 352.1, found 252.1 (-Boc). HPLC: >99% (254 nm). TLC: R_f 0.20 (EtOAc:heptanes, 2:1 + 2% AcOH).

*(2S,4S)-1-(tert-Butoxycarbonyl)-4-(3-carboxyphenoxy)-pyrrolidine-2-carboxylic Acid (**16**).* Methyl ester **14** (350 mg, 1.43 mmol, 1.00 equiv), methyl 3-hydroxybenzoate (270 mg, 1.77 mmol, 1.24 equiv), DIAD (351 mg, 1.74 mmol, 1.22 equiv), and PPh_3 (452 mg, 1.72 mmol, 1.20 equiv) were weight out in a dry flask and dissolved in dry THF (8 mL) under an atmosphere of nitrogen. The mixture was left to stir at rt for 18 h. To the mixture was added a combination of Et_2O and THF (1:1, 40 mL) and washed with 2 M NaOH (2 × 20 mL) and brine (25 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The mixture was purified using DCVC (0−24%, EtOAc in toluene). The diester was dissolved in THF (12 mL), cooled to 0 °C, and dropwise added 1 M LiOH (12 mL) over the course of 2 min and subsequently 4 M NaOH (2 mL) over the course of 4 min. The mixture was left to stir at 0 °C for 15 min and at rt for 20 h. The mixture was added H_2O (40 mL) and the aqueous phase washed with Et_2O (2 × 40 mL). The aqueous phase was added conc HCl (pH ≈ 1) and extracted with a mixture of Et_2O and THF (1:1, 3 × 40 mL). The pooled organic phases were washed with brine (50 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The mixture was purified using DCVC (0−100% EtOAc in heptanes containing 2% AcOH) to yield diacid **16** (343 mg, 68%) as a clear, colorless oil. ^{1}H NMR (300 MHz, $((CD_3)_2SO)$: δ 1.36/1.40 (9H, 2s), 2.15−2.26 (1H, m), 2.50−2.65 (1H, m), 3.41 (1H, dd, J = 12, 6 Hz), 3.65−3.77 (1H, m), 4.28 (1H, m), 5.05−5.15 (1H, m), 7.07−7.14 (1H, m), 7.32−7.35 (1H, m), 7.40 (1H, t, J = 8 Hz), 7.52 (1H, dm, J = 8 Hz). ^{13}C NMR (75 MHz, $((CD_3)_2SO)$: δ 28.0, 28.2, 34.7, 35.5, 51.4, 51.8, 57.2, 57.4, 74.5, 75.6, 78.8, 79.0, 116.0, 119.8, 121.8, 129.8, 132.2, 153.1, 153.2, 156.3, 166.8, 172.7, 172.9. LCMS: m/z $[M + H]^+$: calcd 352.1, found 252.1 (-Boc). HPLC: >99% (254 nm). TLC: R_f 0.27 (EtOAc:heptanes, 2:1 + 2% AcOH). OR: $[\alpha]^{25}_D$ −17 (c = 0.49; abs EtOH).

*(2S,4S)-1-(tert-Butoxycarbonyl)-4-(4-carboxyphenoxy)-pyrrolidine-2-carboxylic Acid (**17**).* Methyl ester **14** (305 mg, 1.24 mmol, 1.00 equiv), methyl 4-hydroxybenzoate (246 mg, 1.62 mmol, 1.30 equiv), PPh_3 (395 mg, 1.51 mmol, 1.21 equiv), and DIAD (317 mg, 1.57 mmol, 1.26 equiv) were weighed out in a dry flask and dissolved in dry THF (6 mL) under an atmosphere of nitrogen. The mixture was left to stir at rt for 18 h. To the mixture was added a combination of Et_2O and THF (1:1, 40 mL) and washed with 2 M NaOH (2 × 20 mL) and brine (25 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The mixture was purified using DCVC (0−30% EtOAc in toluene). The diester was dissolved in THF (6 mL), cooled to 0 °C, and 2 M LiOH (12 mL) added dropwise over the course of 5 min. The mixture was left to stir at 0 °C for 15 min and at rt for 20 h. To the mixture was added H_2O (40 mL) and the aqueous phase washed with Et_2O (2 × 40 mL). The aqueous phase was added conc HCl (pH = 0) and extracted with a mixture of Et_2O and THF (1:1, 3 × 40 mL). The combined organic phases was washed with brine (50 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo to yield diacid **17** (311 mg, 71%) as a clear, colorless oil. ^{1}H NMR (300 MHz, $((CD_3)_2SO)$: δ 1.36/1.40 (9H, 2s), 2.17−2.22 (2H, m), 2.51−2.66 (1H, m), 3.41 (1H, dd, J = 12, 6 Hz), 3.69−3.78 (1H, m), 4.24−4.32 (1H, m), 5.08−5.11 (1H, m), 6.92 (2H, dd, J = 9, 3 Hz), 7.86 (2H, d, J = 9 Hz). ^{13}C NMR (75 MHz, $((CD_3)_2SO)$: δ 28.0, 28.2, 30.5, 34.7, 35.5, 51.5, 51.8, 57.2, 57.4, 74.6, 75.7, 78.9, 79.0, 115.0, 123.2, 131.3, 153.0, 153.1, 166.8, 172.6, 172.9. LCMS: m/z $[M + H]^+$: calcd 352.1, found 252.1 (-Boc). HPLC: >99% (254 nm). TLC: R_f 0.23 (EtOAc:heptanes, 2:1 + 2% AcOH).

*(2S,4R)-1-tert-Butyl 2-Methyl 4-((methylsulfonyl)oxy)pyrrolidine-1,2-dicarboxylate (**18**).* Alcohol **14** (2.11 g, 8.60 mmol, 1.00 equiv) was dissolved in dry THF (15 mL) and TEA (1.70 mL, 12.2 mmol, 1.42 equiv) added under nitrogen. The mixture was cooled to 0 °C and mesyl chloride (1.00 mL, 12.9 mmol, 1.50 equiv) added dropwise over the course of 15 min, and the mixture was stirred for additional 5 h. To the mixture was added H_2O (30 mL) and extracted with EtOAc (3 × 25 mL). The combined organic phases was washed with 1 M HCl (30 mL), satd $NaHCO_3$ (40 mL), and brine (30 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo to yield mesylate **18** (2.50 g, 90%) as a white solid. ^{1}H NMR (300 MHz, $(CDCl_3)$: 1.43, 1.47 (9H, 2s), 2.25 (0.40H, dd, J = 14, 5 Hz), 2.28 (0.60, dd, J = 14, 5 Hz), 2.58 (0.40, ddd, J = 14, 8, 3 Hz), 2.67 (0.60, tdd, J = 14, 8, 2 Hz) 3.06 (3H, s), 3.72−3.89 (5H, m), 4.40 (0.60H, t, J = 8 Hz), 4.46 (0.40H, t, J = 8 Hz), 5.26 (1H, m).

*(2S,4S)-1-(tert-Butoxycarbonyl)-4-((2-carboxyphenyl)thio)-pyrrolidine-2-carboxylic Acid (**19**).* Methyl 2-mercaptobenzoate (180 mg, 1.07 mmol, 1.66 equiv) was dissolved in DMF (4 mL) and K_2CO_3 (149 mg, 1.08 mmol, 1.67 equiv) added. Mesylate **18** (200 mg, 0.65 mmol, 1.00 equiv) was dropwise added to the mixture, which was subsequently heated to 50 °C and stirred under nitrogen for 18 h. To the mixture was added 10% brine (40 mL) and extracted with Et_2O (2 × 50 mL). The combined organic phases was washed with 0.5 M NaOH (2 × 30 mL) and brine (50 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The oil was purified using DCVC (0−22% EtOAc in toluene). The oil was dissolved in THF (5 mL), cooled to 0 °C, and 2 M LiOH (10 mL) added dropwise over the course of 5 min. After an additional 15 min at 0 °C, the mixture was left to stir for 18 h at rt. To the mixture was added H_2O (50 mL) and was washed with Et_2O (2 × 25 mL). The aqueous phase was acidified (pH ≈ 1) using conc HCl and extracted with a combination of Et_2O and THF (1:1) (3 × 25 mL). The combined organic phases was washed with brine (25 mL), dried over $MgSO_4$, filtered and concentrated in vacuo. The crude product was purified using DCVC (0−75% EtOAc in toluene added 2% AcOH), yielding thioether **19** (153 mg, 67%) as a white solid. ^{1}H NMR (300 MHz, $(CDCl_3)$: δ 1.47/1.54 (9H, 2s), 2.44−2.66 (2H, m), 3.60−3.78 (1H, m), 3.89−4.03 (1H, m), 4.09−4.16 (1H, m), 4.44 (0.5H, d, J = 8 Hz), 4.55 (0.5H, d, J = 8 Hz), 7.20 (1H, d, J = 8 Hz), 7.33 (1H, d, J = 8 Hz), 7.48 (2H, t, J = 8 Hz). ^{13}C NMR (400 MHz, $(CDCl_3)$: δ 28.3, 28.5, 34.9, 36.0, 42.0, 50.9, 57.8, 58.0, 80.7, 81.0, 124.7, 124.8, 126.9, 127.1, 128.3, 128.5, 132.5, 132.6, 132.9, 133.1, 135.8, 140.3, 140.8, 153.9, 154.7, 171.7, 179.1, 179.7. LCMS: m/z $[M + H]^+$: calcd 368.1, found 268.1 (-Boc). HPLC: >99% (254 nm). TLC: R_f 0.18 (EtOAc:heptanes, 2:1 + 2% AcOH). OR: $[\alpha]^{22}_D$ −28 (c = 0.29; abs EtOH).

*Methyl 2-(2-Hydroxyphenyl) Acetate (**21**).* 2-Hydroxyphenylacetic acid (1.00 g, 6.57 mmol, 1.00 equiv) was dissolved in MeOH (20 mL). H_2SO_4 (0.55 mL, 10.3 mmol, 1.57 equiv) was added dropwise, and the reaction mixture was heated to reflux (3 h) under an atmosphere of nitrogen. The mixture was concentrated in vacuo and the residue

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dissolved in water (68 mL) and extracted with EtOAc (3 × 10 mL). The combined organic fractions were dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified using flash chromatography (heptane:EtOAc, 1:1) to give ester **21** as a white solid (745 mg, 4.48 mmol, 68%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.69 (s, 2H), 3.75 (s, 3H), 6.88 (ddd, J = 2, 7 Hz, 1H), 6.94 (dd, J = 1, 8 Hz, 1H), 7.10 (dd, J = 2, 8 Hz, 1H), 7.20 (ddd, J = 2, 8 Hz, 1H), 7.30 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 37.7, 52.7, 117.7, 120.5, 120.9, 129.3, 131.0, 155.2, 174.3. LC-MS: m/z (M + H$^+$) calcd 167.1, found 167.1.TLC: R_f 0.65 (heptane/EtOAc, 1:1). HPLC: >99% (254 nm). Melting point: 72.4−73.7 °C. Characterization is consistent with the literature.[29]

*Methyl 2-(3-Hydroxyphenyl) Acetate (**22**).* 3-Hydroxyphenyl acetic acid (3.00 g, 19.7 mmol, 1.00 equiv) was dissolved in MeOH (66 mL). H_2SO_4 (1.6 mL, 30.02 mmol, 1.52 equiv) was added slowly, and the reaction mixture was heated to reflux overnight (18 h). The mixture was concentrated in vacuo and the residue dissolved in water (68 mL) and extracted with EtOAc (4 × 33 mL). The combined organic fractions were dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified using flash chromatography (heptane:EtOAc, 1:1) to yield ester **22** as a brown/orange oil (2.7 g, 82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.58 (s, 2H), 3.70 (s, 3H), 6.70−6.80 (m, 2H), 6.82 (ddd, J = 1, 2, 7 Hz, 1H), 7.18 (dd, J = 8 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) 41.1, 52.2, 114.3, 116.2, 121.5, 129.8, 135.4, 155.9, 172.4. LCMS: m/z (M + H)$^+$: calcd 167.1, found 167.1. TLC: R_f 0.46 (heptane:EtOAc, 1:1). Characterization is consistent with the literature.[29]

*Methyl 3-Hydroxy-2-naphthoate (**23**).* Carried out as described for **22**, starting from 3-hydroxy-2-naphthoic acid (1.00 g, 5.33 mmol, 1.00 equiv), MeOH (20 mL), conc H_2SO_4 (0.40 mL, 7.50 mmol, 1.41 equiv), and reflux for 44 h. The mixture was purified using flash chromatography (heptane:EtOAc, 4:1) to yield ester **12** as a yellow solid (821 mg, 77%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.03 (s, 3H), 7.32 (s, 1H), 7.32 (ddd, J = 1.20, 6.76, 8.13 Hz, 1H), 7.50 (ddd, J = 1, 7, 8 Hz, 1H), 7.69 (dt, J = 1, 8 Hz, 1H), 7.80 (dd, J = 1, 8 Hz, 1H), 8.50 (d, J = 1 Hz, 1H), 10.42 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 52.7, 78.3, 111.9, 124.1, 126.5, 127.2, 129.3, 129.4, 132.6, 138.1, 156.5, 170.4. LCMS: m/z (M + H)$^+$: calcd 203.1, found 203.0. HPLC: 99% (254 nm). Melting point: 71.4−73.4 °C.

*Methyl 1-Hydroxy-2-naphthoate (**24**).* Carried out as described for **22**, starting from 1-hydroxy-2-naphthoic acid (1.01 g, 5.31 mmol, 1.00 equiv), MeOH (20 mL), conc H_2SO_4 (0.40 mL, 7.50 mmol, 1.41 equiv), and heated to reflux for 44 h. Mixture was purified using flash chromatography (heptane:EtOAc, 99:1) to give the ester **24** as a white solid (342 mg, 32%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.00 (s, 3H), 7.29 (dd, J = 1, 9 Hz, 1H), 7.53 (ddd, J = 1, 7, 8 Hz, 1H), 7.61 (ddd, J = 1, 7, 8 Hz, 1H), 7.77 (dd, J = 2, 8 Hz, 1H), 7.78 (dd, J = 2, 8 Hz, 1H), 8.42 (ddt, J = 1, 2, 8 Hz, 1H), 11.98 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 52.4, 105.7, 118.8, 124.0, 124.4, 124.9, 125.9, 127.6, 129.5, 137.4, 161.1, 171.6. LCMS: m/z (M + H)$^+$: calcd 203.1, found 203.0. HPLC: >99% (254 nm). TLC: R_f 0.37 (heptane:EtOAc, 1:1). Melting point: 78.3−79.4 °C.

*Methyl 2-Hydroxy-1-naphthoate (**25**).* 2-Hydroxy-1-naphthoic acid (1.01 g, 5.39 mmol, 1.00 equiv) and $KHCO_3$ (640 mg, 6.39 mmol, 1.19 equiv) were weighed out and dissolved in anhydrous DMF (3.5 mL). CH_3I (0.50 mL, 1.13 g, 7.96 mmol, 1.48 equiv) was added dropwise, while the reaction mixture was stirred under an atmosphere of nitrogen. The reaction mixture was left for 21 h at rt, after which it was quenched using H_2O (10 mL). The mixture was extracted using EtOAc (2 × 10 mL), and the combined organic phases were washed with satd $NaHCO_3$ (0.5 mL), H_2O (10 mL), and brine (0.5 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated in vacuo. The mixture was purified using flash chromatography (heptane:EtOAc, 1:1 + 2% AcOH) to yield ester **25** as a white solid (444 mg, 41%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.12 (s, 3H), 7.17 (d, J = 9 Hz, 1H), 7.37 (ddd, J = 1, 7, 8 Hz, 1H), 7.56 (ddd, J = 1, 7, 9 Hz, 1H), 7.72−7.79 (m, 1H), 7.90 (d, J = 9 Hz, 1H), 8.74 (d, J = 8 Hz, 1H), 12.25 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 52.4, 104.7, 119.3, 123.7, 125.3, 128.5, 128.7, 129.1, 131.8, 136.9, 164.4, 172.8. LCMS: m/z (M + H)$^+$: calcd 203.1, found 203.0. HPLC: 87% (254 nm). TLC: R_f 0.76 (heptane:EtOAc, 1:1 + 2% AcOH). Melting point: 79.4−80.5 °C.

*Dimethyl 4-Hydroxyisophthalate (**26**).* Carried out as described for **27** starting from 4-hydroxyisophthalic acid (1.01 mg, 5.52 mmol, 1.00 equiv), MeOH (15 mL), and conc H_2SO_4 (0.2 mL, 3.75 mmol, 0.68 equiv) and heated to reflux for 18 h. The mixture was purified using flash chromatography (heptane:EtOAc, 1:1 + 2% AcOH) to yield ester **26** as a white semisolid (504 mg, 44%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.91 (s, 3H), 3.99 (s, 3H), 7.02 (d, J = 9 Hz, 1H), 8.12 (dd, J = 2, 9 Hz, 1H), 8.57 (d, J = 2 Hz, 1H), 11.19 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 52.3, 52.8, 112.3, 118.0, 121.6, 132.7, 136.8, 150.1, 165.3, 166.2. LCMS: m/z (M + H)$^+$: calcd 211.1, found 211.0. HPLC: >99% (254 nm). TLC: R_f 0.70 (heptane:EtOAc, 1:1 + 2% AcOH).

*Methyl 5-Methylsalicylate (**27**).* 5-Methylsalicylic acid (1.00 g, 6.59 mmol, 1.00 equiv) was dissolved in MeOH (15 mL). H_2SO_4 (0.2 mL, 3.75 mmol, 0.57 equiv) was added dropwise, and the reaction mixture was heated to reflux for 24 h. The mixture was concentrated in vacuo. The residue was dissolved in EtOAc (10 mL) and washed with satd $NaHCO_3$ (2 × 10 mL) and brine (10 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The mixture was purified using flash chromatography (heptane:EtOAc, 1:1 + 2.5% AcOH) to yield ester **27** as a clear yellow/orange oil (621 mg, 57%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.28 (s, 3H), 3.94 (s, 3H), 6.88 (d, J = 8 Hz, 1H), 7.23−7.30 (m, 1H), 7.63 (d, J = 2 Hz, 1H), 10.54 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.4, 52.2, 111.9, 117.3, 128.3, 129.6, 136.7, 159.5, 170.6. LCMS: m/z (M + H)$^+$: calcd 167.1, found 167.1. HPLC: >99% (254 nm). TLC: R_f 0.76 (heptane:EtOAc, 1:1 + 2% AcOH).

*Methyl 3-Methylsalicylate (**28**).* Carried out as described for **27** with 3-methylsalicylic acid (**8**) (1.03 g, 6.74 mmol, 1.00 equiv), MeOH (20 mL), and conc H_2SO_4 (0.4 mL, 7.50 mmol, 1.11 equiv) and heated to reflux for 4 days. The mixture was purified by flash chromatography (heptane:EtOAc, 4:1) to yield ester **28** as a clear oil (534 mg, 49%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.27 (s, 3H), 3.94 (s, 3H), 6.78 (d, J = 8 Hz, 1H), 7.32 (dd, J = 2, 7 Hz, 1H), 7.69 (dd, J = 2, 8 Hz, 1H), 11.01 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.8, 52.4, 111.8, 118.6, 126.8, 127.5, 136.6, 160.2, 171.2. LCMS: m/z (M + H)$^+$: calcd 167.1, found 167.1. TLC: R_f 0.46 (heptane:EtOAc, 1:1).

*(2S,4R)-1-tert-Butyl 2-Methyl 4-(2-(2-Methoxy-2-oxoethyl)-phenoxy)pyrrolidine-1,2-dicarboxylate (**29**).* In a dry flask *N*-Boc-*cis*-4-hydroxy-L-proline methyl ester (201 mg, 0.82 mmol, 1.00 equiv), methyl 2- hydroxy-phenylacetate (**21**) (280 mg, 1.69 mmol, 1.00 equiv), and Ph_3P (590 mg, 2.25 mmol, 1.33 eqiuv) were dissolved in anhydrous THF (2.7 mL) under an atmosphere of argon and cooled to 0 °C. DIAD (0.32 mL, 1.63 mmol, 0.96 equiv) was added dropwise, and the mixture was left to stir at rt over the course of 20 h. The reaction mixture was diluted with Et_2O:THF (1:1, 40 mL) and quenched/washed with satd Na_2CO_3:H_2O (1:1, 2 × 20 mL) and brine (25 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated in vacuo. The mixture was purified using flash chromatography (heptane:EtOAc, 1:1 + 2% AcOH) to yield proline **29** (97 mg, 30%) as a clear, off-white semisolid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.31−1.44 (br s, 9H), 2.14 (ddd, J = 5, 8, 13 Hz, 1H), 2.39−2.53 (m, 1H), 3.42−3.58 (m, 1H), 3.61 (s, 3H), 3.64−3.75 (m, 3H), 3.68 (s, 3H), 4.29−4.44 (m, 1H), 4.84−4.98 (m, 1H), 6.29 (s, 1H), 6.72 (d, J = 8 Hz, 1H), 6.83−6.93 (m, 1H), 7.08−7.21 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) (rotamers) δ 22.1, 28.4, 28.5, 35.9, 36.3, 36.8, 37.8, 52.0, 52.3, 57.8, 58.2, 70.3, 74.8, 75.4, 77.3, 80.6, 112.1, 112.3, 117.7, 121.5, 124.3, 128.7, 129.3, 131.2, 131.6, 154.9, 156.5, 172.2, 173.4, 173.6. LCMS: m/z (M + H)$^+$: calcd 294.1, found 294.0. TLC: R_f 0.48 (heptane:EtOAc, 1:1 + 2% AcOH).

*(2S,4R)-1-tert-Butyl 2-Methyl 4-(3-(2-Methoxy-2-oxoethyl)-phenoxy)pyrrolidine-1,2-dicarboxylate (**30**).* Carried out as described for **29** with *N*-Boc-*cis*-4-hydroxy-(*S*)-proline methyl ester (500 mg, 2.04 mmol, 1.00 equiv), Ph_3P (644 mg, 2.45 mmol, 1.20 equiv), methyl-3-hydroxy-phenylacetate (**22**) (407 mg, 2.45 mmol, 1.20 equiv), DIAD (507 mg, 2.51 mmol, 1.23 equiv), and anhydous THF (14.5 mL) and stirring for 43 h under an atmosphere of argon. The mixture was concentrated on Celite and purified using flash chromatography (0−100% EtOAc in heptanes) to yield proline **30** as a white semisolid (142 mg, 18% yield). ^{1}H NMR (400 MHz,

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$CDCl_3$) δ 1.44 (s, 9H), 2.14−2.28 (m, 1H), 2.48−2.53 (m, 1H), 3.59 (br s, 2H), 3.70 (s, 3H), 3.75 (s, 3H), 3.79 (d, J = 3 Hz, 2H), 4.42 (t, J = 7.83 Hz, 0.5H), 4.48 (t, J = 7.83 Hz, 0.5H), 4.90 (br s, 1H), 6.75 (d, J = 8 Hz, 1H), 6.83 (s, 1H), 6.89 (d, J = 7 Hz, 1H), 7.21−7.25 (dd, J = 7 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.8, 28.1, 28.2, 35.5, 36.4, 41.0, 51.8, 51.9, 52.2, 57.5, 57.9, 74.5, 80.3, 113.9, 116.5, 122.2, 129.6, 135.5, 156.9, 171.6, 173.2. LCMS: m/z $(M + H)^+$: calcd 294.1, found 294.0 (-Boc). TLC: R_f 0.35 (heptane:EtOAc, 1:1 + 2% AcOH).

*(2S,4R)-1-tert-Butyl 2-Methyl 4-((3-(Methoxycarbonyl) naphthalen-2-yl)oxy) pyrrolidine-1,2-dicarboxylate (**31**).* Carried out as described for **29** with *N*-Boc-*cis*-4-hydroxy-(*S*)-proline methyl ester (201 mg, 0.82 mmol, 1.00 equiv), methyl 3-hydroxy-2-naphthoate (**23**) (198 mg, 0.98 mmol, 1,20 equiv), Ph_3P (257 mg, 0.98 mmol, 1.20 equiv), anhydrous THF (7 mL), and DIAD (0.2 mL, 205 mg, 1.02 mmol, 1.24 equiv) and stirring for 26 h under an atmosphere of argon at rt. The mixture was concentrated on Celite and purified using flash chromatography (heptane:EtOAc, 2:1) to yield proline **31** (81 mg, 23% yield) as a semisolid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.43 (s, 9H), 2.25−2.32 (m, 1H), 2.60−2.72 (m, 1H), 3.76 (br s, 3H), 3.78−3.87 (m, 1H), 3.92 (s, 3H), 4.51 (t, J = 8 Hz, 0.5H), 4.55 (t, J = 8 Hz, 0.5H), 5.09 (br d, J = 14 Hz, 1H), 7.15 (s, 1H), 7.34−7.44 (m, 1H), 7.52 (m, 1H), 7.71 (t, J = 9 Hz, 1H), 7.82 (dd, J = 5, 8 Hz, 1H), 8.30 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) (rotamers) δ 14.2, 14.3, 21.1, 22.1, 22.8, 28.4, 28.5, 29.1, 32.0, 35.7, 36.8, 51.8, 52.2, 52.2, 52.3, 52.4, 57.9, 58.3, 60.5, 70.1, 76.1, 80.5, 110.3, 110.4, 123.0, 125.0, 126.6, 128.2, 128.7, 128.8, 133.3, 135.8, 152.9, 153.9, 156.5, 166.8, 171.3, 173.6. LCMS: m/z $(M + H)^+$: calcd 330.1, found 330.1. TLC: R_f 0.26 (heptane:EtOAc, 2:1).

*(2S,4R)-1-tert-Butyl 2-Methyl 4-((2-(Methoxycarbonyl)-naphthalen-1-yl)oxy)pyrrolidine-1,2-dicarboxylate (**32**).* Carried out as described for **29** with *N*-Boc-*cis*-4-hydroxy-(*S*)-proline methyl ester (203 mg, 0.83 mmol, 1.00 equiv), methyl 1-hydroxy-2-naphthoate (**24**) (199 mg, 0.98 mmol, 1.18 equiv), Ph_3P (257 mg, 0.98 mmol, 1.18 equiv), and anhydrous THF (6 mL), DIAD (0.2 mL, 205 mg, 1.02 mmol, 1.23 equiv) and stirring for 26 h under an atmosphere of argon at rt. The mixture was purified using flash chromatography (heptane/EtOAc, 1:1) to yield a sticky oil (276 mg, 79% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.45 (s, 9H), 2.08−2.23 (m, 1H), 2.68−2.82 (m, 1H), 3.54 (ddd, J = 4, 7, 12 Hz, 1H), 3.71 (s, 3H), 3.73−3.86 (m, 1H), 3.96 (br s, 3H), 4.64 (t, J = 8 Hz, 0.5H), 4.72 (t, J = 8 Hz, 0.5H), 4.95−4.99 (m, 1H), 7.48−7.67 (m, 3H), 7.80−7.89 (m, 2H), 8.15 (d, J = 8 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) (rotamers) δ 14.1, 21.9, 22.7, 28.3, 28.4, 31.9, 36.5, 37.4, 51.7, 52.1, 52.2, 52.3, 58.0, 58.4, 70.1, 77.2, 80.3, 82.9, 83.9, 123.4, 123.6, 124.0, 126.7, 126.8, 127.7, 128.6, 136.6, 166.5, 173.3, 173.4. LCMS: m/z $(M + H)^+$: calcd 330.1, found 330.1. TLC: R_f 0.62 (heptane:EtOAc, 1:1).

*(2S,4R)-1-tert-Butyl 2-Methyl 4-(3-(2-Methoxy-2-oxoethyl)-phenoxy)pyrrolidine-1,2-dicarboxylate (**33**).* Carried out as described for **29** with *N*-Boc-*cis*-4-hydroxy-(*S*)-proline methyl ester (208 mg, 0.85 mmol, 1.00 equiv), methyl 2-hydroxy-1-naphthoate (**25**) (203 mg, 1.00 mmol, 1.18 equiv), Ph_3P (258 mg, 0.98 mmol, 1.15 equiv), anhydrous THF (6 mL), and DIAD (0.20 mL, 205 mg, 1.02 mmol, 1.20 equiv) and stirring for 18 h under an atmosphere of argon at rt. The mixture was purified using flash chromatography (heptane:EtOAc, 1:1 + 2% AcOH) to yield proline **33** (168 mg, 48% yield) as a sticky solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.44 (s, 9H), 2.19 (m, 1H), 2.53−2.63 (m, 1H), 3.72 (s, 3H), 3.75−3.80 (m, 1H), 4.00 (s, 3H), 4.45 (t, J = 8 Hz, 0.5H), 4.50 (t, J = 8 Hz, 0.5H), 5.06−5.13 (m, 1H), 7.20 (dd, J = 2, 9 Hz, 1H), 7.41 (ddt, J = 2, 7, 8 Hz, 1H), 7.51 (ddt, J = 2, 7, 8 Hz, 1H), 7.78 (m, 2H), 7.89 (dd, J = 4, 9 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) (rotamers) δ 14.1, 20.5, 21.9, 22.7, 28.3, 28.3, 29.0, 31.9, 35.8, 36.7, 52.0, 52.1, 52.3, 52.3, 52.5, 57.6, 58.0, 80.5, 115.5, 115.5, 120.1, 124.0, 124.9, 127.8, 127.9, 128.1, 129.2, 131.0, 131.7, 151.7, 153.6, 154.3, 168.1, 173.4, 175.5. LCMS: m/z $(M + H)^+$: calcd 330.1, found 330.1 (- Boc). TLC: R_f 0.49 (heptane:EtOAc, 1:1 + 2% AcOH).

*(2S,4R)-1-tert-Butyl 2-Methyl 4-(2,4-Bis(methoxycarbonyl)-phenoxy)pyrrolidine-1,2-dicarboxylate (**34**).* Carried out as described for **29** with *N*-Boc-*cis*-4-hydroxy-(*S*)-proline methyl ester (200 mg, 0.82 mmol, 1.00 equiv), dimethyl 4-hydroxy-isophthalate (**26**) (195 mg, 0.93 mmol, 1.22 equiv), Ph_3P (262 mg, 1.00 mmol, 1.22 equiv), anhydrous THF (6 mL), and DIAD (0.20 mL, 1.02 mmol, 1.24 equiv) and stirring for 4 h under an atmosphere of argon at rt. The mixture was purified using flash chromatography (heptane/EtOAc, 1:1) to yield proline **34** (189 mg, 53% yield) as a solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.38−1.45 (s, 9H), 2.26 (ddd, J = 5, 8, 14 Hz, 1H), 2.51−2.67 (m, 1H), 3.66 (s, 3H), 3.75 (br s, 2H), 3.92 (s, 3H), 3.94 (s, 3H), 4.48 (t, J = 8 Hz, 0.5H), 4.52 (t, J = 8 Hz, 0.5H), 5.00−5.10 (m, 1H), 6.93 (dd, J = 3, 9 Hz, 1H), 8.06−8.15 (m, 1H), 8.45 (d, J = 3 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) (rotamers) δ 28.2, 28.3, 35.6, 36.7, 51.7, 51.9, 52.2, 52.3, 52.4, 57.6, 58.0, 76.1, 76.8, 80.6, 113.9, 121.6, 123.2, 133.9, 134.7, 153.6, 159.7, 165.6, 165.8, 173.1, 173.2. LCMS: m/z $(M + H)^+$: calcd 338.1, found 338.0. TLC: R_f 0.40 (heptane:EtOAc, 1:1).

*(2S,4R)-1-tert-Butyl 2-Methyl-4 (2-(Methoxycarbonyl)-4-methylphenoxy)-pyrrolidine-1,2-dicarboxylate (**35**).* Carried out as described for **29** with *N*-Boc-*cis*-4-hydroxy-(*S*)-proline methyl ester (201 mg, 0.82 mmol, 1.00 equiv), **27** (165 mg, 0.99 mmol, 1.21 equiv), Ph_3P (260 mg, 0.99 mmol, 1.21 equiv), anhydrous THF (6 mL), and DIAD (0.2 mL, 205 mg, 1.02 mmol, 1.24 equiv) and stirring for 4 h under an atmosphere of argon at rt. The mixture was purified using flash chromatography (heptane/EtOAc, 1:1) to yield proline **35** (91 mg, 29%) as a sticky solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.43 (s, 9H), 2.17−2.22 (m, 1H), 2.31 (d, J = 3 Hz, 3H), 2.50−2.66 (m, 1H), 3.70−3.75 (m, 1H), 3.75 (s, 3H), 3.86 (s, 3H), 4.45 (t, J = 8 Hz, 0.5H), 4.53 (t, J = 8 Hz, 0.5H), 4.98 (m, 1H), 6.82 (d, J = 8 Hz, 1H), 7.23 (d, J = 7 Hz, 1H), 7.60 (s, 1H). LCMS: m/z $(M + H)^+$: calcd 294.1, found 294.1. TLC: R_f 0.49 (heptane:EtOAc 1:1).

*(2S,4R)-1-tert-Butyl 2-Methyl 4-(4-Acetyl-2-(methoxycarbonyl)-phenoxy)pyrrolidine-1,2-dicarboxylate (**36**).* Carried out as described for **29** with *N*-Boc-*cis*-4-hydroxy-(*S*)-proline methyl ester (204 mg, 0.83 mmol, 1.00 equiv), methyl 5-acetylsalicylate (**27**) (199 mg, 1.03 mmol, 1.24 equiv), Ph_3P (258 mg, 0.98 mmol, 1.18 equiv), anhydrous THF (6 mL), and DIAD (0.20 mL, 1.02 mmol, 1.23 equiv) and stirring for 15 h under an atmosphere of argon at rt. The mixture was purified using flash chromatography (heptane:EtOAc, 1:1) to yield proline **36** (152 mg, 46% yield) as a semisolid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.43 (s, 9H), 2.26 (m, 1H), 2.50 (s, 3H), 2.51−2.66 (m, 1H), 3.66 (s, 3H), 3.70−3.80 (m, 1H), 3.90 (s, 3H), 4.23−4.38 (m, 1H), 4.41−4.55 (m, 1H), 5.06 (d, J = 9 Hz, 1H), 6.94 (d, J = 8 Hz, 1H), 8.01−8.10 (m, 1H), 8.36 (s, 1H). LCMS: m/z $(M + H)^+$: calcd 322.1 found 322.1. TLC: R_f 0.21 (heptane:EtOAc, 1:1).

*(2S,4R)-1-tert-Butyl 2-Methyl 4-(2-(methoxycarbonyl)-6-methyl-phenoxy) pyrrolidine-1,2-dicarboxylate (**37**).* Carried out as described for **29** with *N*-Boc-*cis*-4-hydroxy-(*S*)-proline methyl ester (202 mg, 0.82 mmol, 1.00 equiv), methyl 3-methylsalicylate (**28**) (221 mg, 1.33 mmol, 1.62 equiv), Ph_3P (267 mg, 1.02 mmol, 1.24 equiv), anhydrous THF (6 mL), and DIAD (0.20 mL, 1.02 mmol, 1.24 equiv) and stirring 29 h under an atmosphere of argon at rt. The mixture was purified using flash chromatography (heptane:EtOAc, 1:1) to yield proline **37** (59 mg, 19% yield) as a semisolid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.44 (s, 9H), 2.05−2.15 (m, 1H), 2.25 (s, 3H), 3.48−3.53 (m, 1H), 3.63−3.72 (m, 1H), 3.74 (s, 3H), 3.89 (s, 3H), 4.59 (t, J = 8 Hz, 0.5H), 4.58 (t, J = 8 Hz, 0.5H), 4.66 (br s, 1H), 7.04−7.08 (m, 1H), 7.35 (t, J = 7 Hz, 1H), 7.66 (d, J = 8 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) (rotamers) δ 16.5, 16.7, 22.1, 28.4, 28.5, 36.5, 37.5, 51.6, 52.2, 52.4, 58.0, 58.5, 70.2, 80.5, 81.4, 82.4, 123.9, 124.5, 129.8, 133.1, 135.5, 135.6, 153.8, 154.4, 155.1, 156.5, 166.9, 172.4, 173.4, 173.6. LCMS: m/z $(M + H)^+$: calcd 294.1, found 294.1. TLC: R_f 0.21 (heptane:EtOAc, 1:1).

Cell Culture and Transfection. HEK293*T*/17 cells (ATCC) were maintained in minimal essential medium (MEM) containing GlutaMAX and supplemented with 10% fetal bovine serum (Invitrogen). Cells were plated at a low density on poly-D-lysine-coated 35 mm plastic dishes and were transiently transfected 24 h later using the calcdium phosphate method. Rat cDNA encoding for GluK1−2a and GluK2a subunits was cotransfected with cDNA encoding for eGFP to identify transfected cells.

Radioligand Binding Pharmacology. Binding at native iGluR in rat brain synaptosomal membranes was carried out as previously

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detailed.[30] Ligand affinities at recombinant rat GluA2 and GluK1−3 were determined as previously described,[30] applying [^{3}H]KA or [^{3}H]NF608[31] in GluK1 binding experiments. Data were analyzed using GraphPad Prism 6 (GraphPad Software, San Diego, CA) to determine ligand K_i values.

Electrophysiological Recordings. Experiments were performed 36−48 h after transfection. Agonist solutions were rapidly applied to outside-out patches excised from transfected cells using a piezoelectric stack (Physik Instrumente). Solution exchange (10−90% rise time of 250−350 μs) was determined in a separate experiment by measuring the liquid junction current. All recordings were performed using an Axopatch 200B (Molecular Devices) using thick-walled borosilicate glass pipettes (3−6 MΩ) coated with dental wax to reduce electrical noise. Current records were filtered at 5 kHz, digitized at 25 kHz, and series resistance (3−12 MΩ) compensated by 95%. Recordings were performed at a holding potential of −100 mV. All experiments were performed at rt. All chemicals used for electrophysiology were purchased from Sigma-Aldrich unless otherwise indicated. The external solution contained (in mM) 150 NaCl, 5 HEPES, 0.1 $MgCl_2$, and 0.1 $CaCl_2$, pH 7.3−7.4. The internal solution contained (in mM) 115 NaCl, 10 NaF, 5 HEPES, 5 Na_4BAPTA (Life Technologies), 1 $MgCl_2$, 0.5 $CaCl_2$, and 10 Na_2ATP, pH 7.3−7.4. The osmotic pressure of all solutions was adjusted to 295−300 mOsm with sucrose. Concentrated (100×) Glu stocks were prepared and stored at −20 °C; stocks were thawed and diluted on the day of the experiment. Concentrated (100 mM in 10 mM NaOH) stocks of **1b** were prepared and stored at −20 °C; stocks were thawed on the day of the experiment and added to the control and agonist external solutions for the following final concentrations (μM): 0.001, 0.01, 0.1, 1, 10, 30, 100, and 300. As a control, an equivalent amount of NaOH was added to the external solutions not containing **1b**. Data acquisition was performed using pClamp10 software (Molecular Devices), tabulated using Excel (Microsoft Corp) and illustrated using Adobe Illustrator. Inhibition curves were fit using Origin 7.0 (OriginLab) using the following equation:

$$I = \frac{I_{max}}{1 + \left(\frac{[\text{antagonist}]}{IC_{50}}\right)^n}$$

Where I_{max} is the peak current in the absence of antagonist, I is the peak current at ([antagonist]), IC_{50} is the concentration required for 50% inhibition, and n is the slope.

X-ray Structure Determination. Rat GluK1 LBD (segment S1 residues 430−544 and segment S2 residues 667−805, numbering according to UNP P22756) was expressed and purified in the presence of glutamate as previously described.[32] Crystallization of GluK1 LBD in complex with **1b** was performed using the hanging drop vapor diffusion method at 6 °C. A protein solution consisting of 5.1 mg/mL GluK1 LBD and 5.16 mM **1b** in 10 mM HEPES, 20 mM NaCl, and 1 mM EDTA, pH 7.0, was prepared and used for crystallization experiments. Crystals for diffraction studies were obtained with the following reservoir conditions: 15.2% PEG4000, 0.3 M lithium sulfate, and 0.1 M cacodylate buffer pH 6.5 (1 μL of protein−ligand complex and 1 μL of reservoir). The crystals grew within a few days, and they were flash-cooled with liquid nitrogen after immersion in cryo-buffer (1 μL of reservoir solution added to 1 μL of 8% glycerol, 8% ethylene glycol, 9% sucrose, and 2% glucose).

Diffraction data were collected on beamline I911-3[33] at MAX-lab, Lund, Sweden, to a final resolution of 3.18 Å. The data were processed with XDS[34] and scaled using SCALA[35] in CCP4i.[36] The structure was solved by molecular replacement using PHASER[37] within CCP4i and with the structure of GluK1 LBD in complex with the antagonist (*S*)-ATPO as a search model (pdb code, 1vso; molA, protein atoms only).[27] The search model was defined into two lobes: D1 and D2. Two molecules were found in the asymmetric unit of the crystal. Visual inspection of the structure in COOT[38] revealed well-defined electron density for the residues in most areas and density corresponding to the ligand **1b**. The ligand coordinates were created in Maestro [Maestro version 9.3.5, Schrödinger, LLC, New York, NY, 2012] and fitted into the electron density. Topology and parameter files for **1b** were obtained using eLBOW,[39] keeping ligand geometry from Maestro. The Phaser solution was used for model building manually in COOT. The structure was refined in PHENIX[40] with individual isotropic *B* factors, NCS, secondary structural restraints as well as optimizing weights (X-ray/stereochemistry and X-ray/ADP). Finally, the structure was validated using tools in COOT and PHENIX as well as the PDB ADIT validation server. Statistics of data collection and refinement can be found in Table 1. D1−D2 domain openings were calcdulated using the DynDom server.[41]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.6b01516.

Molecular formula strings (CSV)

Accession Codes

The structure coordinates and corresponding structure factor file of GluK1 LBD with **1b** has been deposited in the Protein Data Bank under the accession code 5m2v. Authors will release the atomic coordinates and experimental data upon article publication.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; CNS, central nervous system; EAATs, excitatory amino acid transporters; Glu, (*S*)-glutamic acid; mGluRs, G-protein coupled metabotropic Glu receptors; iGluRs, ionotropic Glu receptors; KA, kainic acid; LBD, ligand binding domain; LHMDS, lithium hexamethyldisilazide; NMDA, *N*-methyl-D-aspartic acid; rt, room temperature

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