Journal of Medicinal Chemistry

Article

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J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/jm4000165 • Publication Date (Web): 15 May 2013

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Journal of Medicinal Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Synthesis and Pharmacological Characterization of 4-Substituted-2-Aminobicyclo[3.1.0]hexane-2,6-Dicarboxylates: Identification of New Potent and Selective Metabotropic Glutamate 2/3 Receptor Agonists.[+]

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[+]Dedicated to Professor Gary L. Grunewald, Ph.D. on the occasion of his 75th birthday.

Abstract

As part of our ongoing interest in identifying novel agonists acting at metabotropic glutamate (mGlu) 2/3 receptors, we have explored the effect of structural modifications of 1*S*,2*S*,5*R*,6*S*-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylate (LY354740), a potent and pharmacologically balanced mGlu2/3 receptor agonist. Incorporation of relatively small substituents (e.g. F, O) at the C4 position of this molecule resulted in additional highly potent mGlu2/3 agonists that demonstrate excellent selectivity over the other mGlu receptor subtypes, while addition of larger C4-substituents (e.g. SPh) led to a loss of agonist potency and/or the appearance of weak mGlu2/3 receptor antagonist activity. Further characterization of the α-fluoro-substituted analog (LY459477) in vivo revealed

that this molecule possesses good oral bioavailability in rats and effectively suppresses phencyclidine-evoked locomotor activity at doses that do not impair neuromuscular coordination. This molecule therefore represents a valuable new addition to the arsenal of pharmacological tools competent to investigate mGlu2/3 receptor function both in vitro and in vivo.

Introduction

Metabotropic glutamate (mGlu) receptors constitute an important class of cell-surface-localized, glutamate-sensing proteins which have drawn considerable attention as targets for novel therapeutic agents.[1] Of the eight known mGlu receptor clones, mGlu2 and mGlu3 receptors are among the most-well studied owing in part the availability of potent and selective small molecule agonist, antagonist and allosteric modulator tools that have been used in preclinical models to validate the role of these receptors in a diverse array of psychiatric,[2] neurologic[3] and proliferative[4] disorders. Identifying molecules capable of increasing mGlu2/3 receptor signaling (orthosteric agonists and/or positive allosteric modulators) has been the subject of particular interest owing to clinical evidence that oral prodrugs LY544344 (**1**) and LY2140023.H_2O (**2**) of mGlu2/3 receptor agonists LY354740 (**3**) and LY404039 (**4**) (Figure 1) were effective in alleviating core symptoms associated with generalized anxiety disorder (**1**) and schizophrenia (**2**).[5,6]

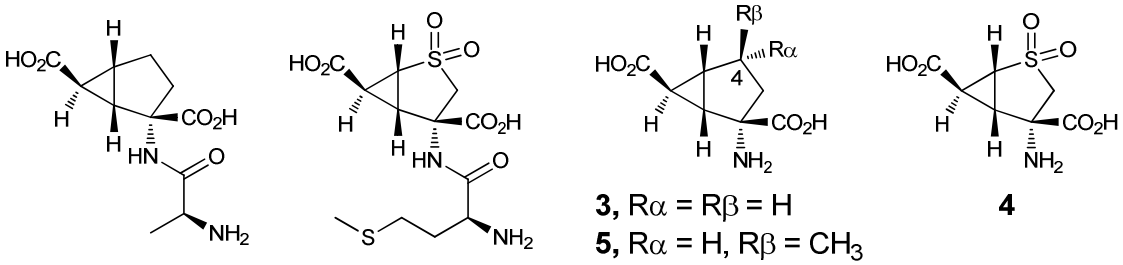
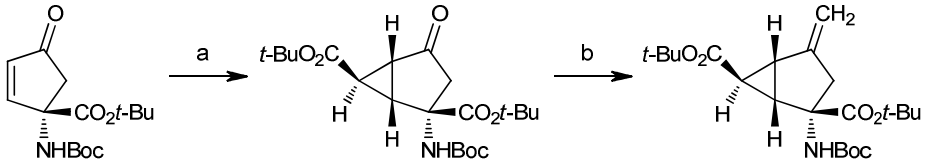


Figure 1. Metabotropic glutamate 2/3 receptor agonists (**3**-**6**) and clinically assessed oral prodrugs (**1**, **2**).

We have previously disclosed that constrained bicyclic amino acids such as those represented by compounds **3** and **4** (Figure 1) possessed highly potent and selective agonist effects at mGlu2/3 receptors.[7] In addition, we found that substitution at the C4-position of **3** with a methyl group led to **5**, a balanced mGlu2/3 agonist and **6** (LY541850), a molecule that exhibits unprecedented mGlu2 agonist/mGlu3 antagonist pharmacology both in cells expressing recombinant human receptors and in native rat brain preparations.[8] In this account, we have extended our investigation of the effect of C4-substituent structure and stereochemistry on mGlu2/3 receptor function. This effort has led to the identification of multiple new highly potent and selective mGlu2/3 receptor agonists. Since methyl substitution at the C4-position demonstrated a dramatic influence of stereochemistry on mGlu2/3 receptor functional responses, we sought to further explore small substitutions at C4 on either the α-face (side opposite of ring fusion hydrogens) or β-face (same side as ring fusion hydrogens) of the constrained bicyclohexane framework.

Chemistry: Our synthetic strategy was enabled by versatile intermediate **8**, which by virtue of the ketone functionality present at the C4 position of the bicyclic nucleus allowed us to incorporate a variety of substituents at this site with a high degree of stereochemical control. The synthesis started with the known enantiomerically pure enone **7**.[9] Carboxy-cyclopropanation of **7** provided ketone **8** in high yield and diastereoselectivity (*ds* >95:5).[10,11] Further treatment of **8** with methyltriphenylphosphonium ylide gave exocyclic olefin **9** (Scheme 1).

Scheme 1. Preparation of versatile intermediate **8**.

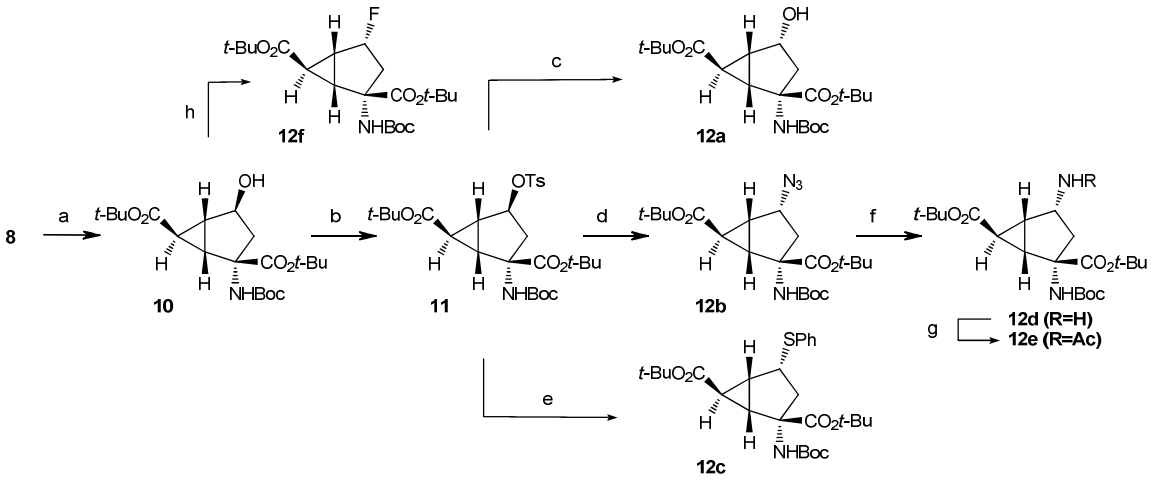


7 **8** **9**

Reagents and conditions. (a) *t*-butyl 2-(2,3,4,5-tetrahydrothiophen-1-yl)acetate, F_3CCH_2OH (64%); (b) Ph_3PCH_3Br, NaHMDS, THF (99%).

Preparation of analogs possessing substituents oriented on the α-face of **3** is depicted in Scheme 2. Reduction of ketone **8** with L-selectride proceeded with hydride attack exclusively from the concave face of the bicyclic ring system to give β-carbinol **10** which was then smoothly converted to tosylate **11** under standard conditions. Nucleophilic displacement of the tosylate proceeded with complete inversion of stereochemistry to yield intermediates **12a-c**. Azide **12b** was reduced to primary amine **12d**, which was in turn acetylated to provide **12e**. Finally, alcohol **10** was treated with DAST to provide C4α-fluoro intermediate **12f**. Analogs possessing substituents oriented on the β-face of **3** were prepared as shown in Scheme 3. Stereoselective conversion of **10** to C4α-bromide derivative **13** was achieved in high yield under standard conditions. Displacement of the bromide with either azide or thiophenol nucleophiles proceeded with inversion of stereochemistry to give intermediates **14a** and **14b**, respectively. Azide **14a** was reduced to amine derivative **14c** which was then acetylated to give **14d**. Unlike the smooth conversion of carbinol **10** to C4α-fluoro analog **12f**, attempts to prepare useful quantities of C4β-fluoro analog **14e** were largely unsuccessful.

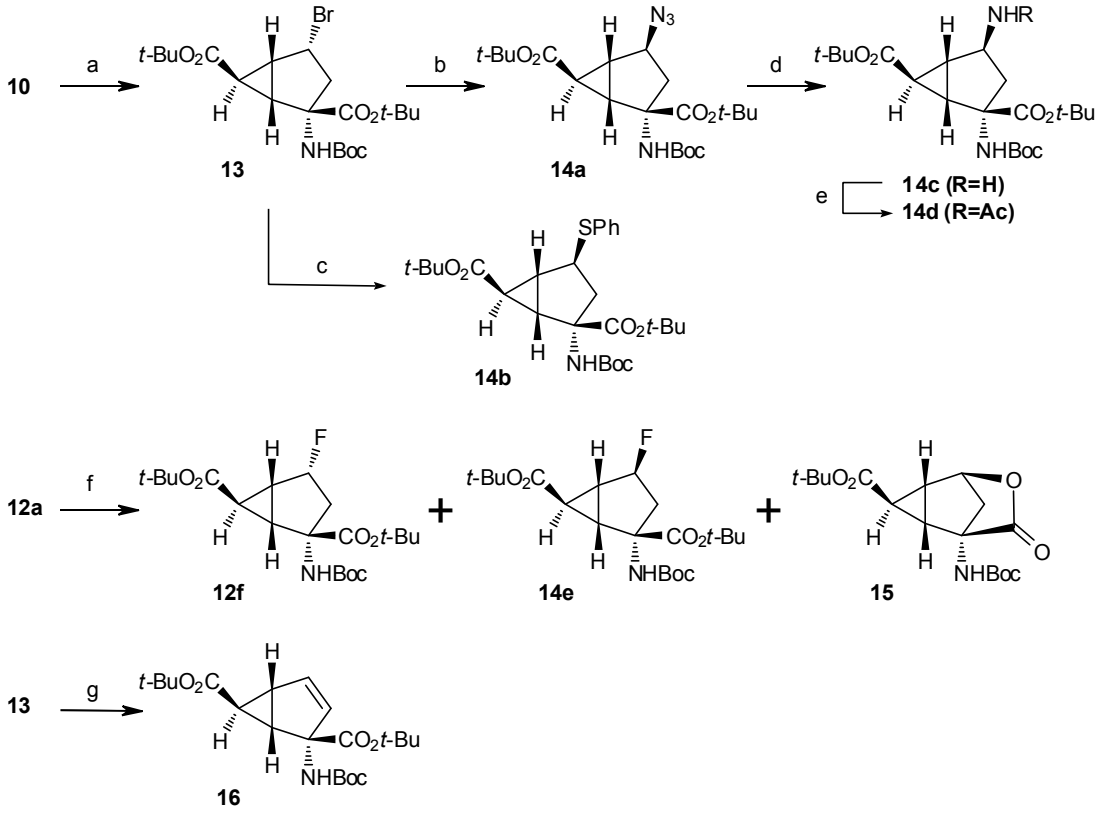
Scheme 2. Preparation of C4α amino acid precursors.

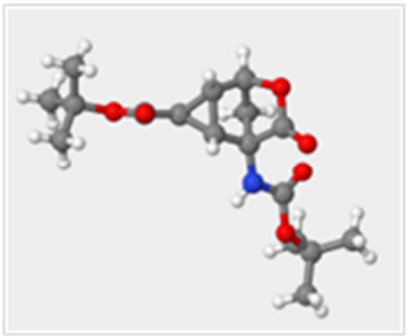


Reagents and conditions. (a) L-selectride, THF (92%); (b) TsCl, NEt_3, DMAP, CH_2Cl_2 (70%); (c) CF_3CO_3Na, DMF; then aq. $NaHCO_3$ (62%); (d) $TMSN_3$, TBAF, THF (92%); (e) PhSH, K_2CO_3, DMF (89%); (f) H_2, Pd/C, EtOH (97%); (g) Ac_2O, NEt_3, DMAP, CH_2Cl_2 (94%); (h) DAST, CH_2Cl_2 (71%).

Reaction of alcohol **12a** with DAST led to the isolation lactone **15** as the primary product (88%), whose structure was unambiguously determined by single crystal X-ray analysis.[12] This product may have been produced via intramolecular trapping of a transient C4-carbocation by the C2-ester with concomitant loss of the *tert*-butyl group, though this proposed mechanistic path has not been rigorously established. Most of the remaining mass was comprised of previously prepared α-F intermediate **12f** (8%), with only trace quantities (2%) of desired β-fluoro analog **14e** being isolated. Attempts to displace bromide **13** with fluoride were unsuccessful, leading exclusively to elimination product **16**.

Scheme 3. Preparation of C4β amino acid precurosors

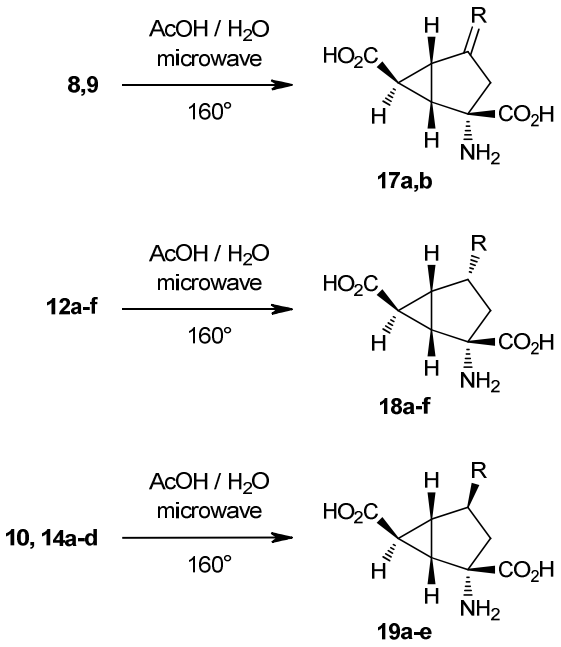




Reagents and conditions. (a) PPh_3, Br_2, PhMe (67%); (b) $TMSN_3$, TBAF, THF (91%); (c) PhSH, K_2CO_3, DMF (95%); (d) H_2, Pd/C, EtOH (97%); (e) Ac_2O, NEt_3, DMAP, CH_2Cl_2 (90%); (f) DAST, CH_2Cl_2 (88% of **15**); (g) KHF_2 / TBADHF, dodecane or TBAF, ACN (48%). Inset: X-ray crystal structure of compound **15**.

Finally, fully protected amino acids were converted to their corresponding zwitterions through our recently reported microwave-assisted solvolysis with AcOH and water (Scheme 4).[13] In the case of **12d** and **14c**, hydrolysis under these conditions provided the corresponding acetate salts.

Scheme 4. Deprotection of N-Boc, di-*tert*-butyl ester intermediates.

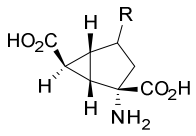


Results: Compounds **3, 5, 6, 17a,b, 18a-f** and **19a-e** were assessed for their functional effects in recombinant human mGlu2 and mGlu3-expressing cells (Table 1).[14] Each compound was also assessed for functional activity in cells expressing each of the other six human mGlu receptor subtypes. Except for relatively weak potency responses in hmGlu6 and hmGlu8-receptor expressing cells[15] as noted in Table 1, no discernable

activity was observed for these molecules up to the highest test concentration (25 μM for agonist, 12.5 μM for antagonist) in cells expressing the other mGlu receptor subtypes (mGlu1, mGlu4, mGlu5 and mGlu7; Table S2). Introduction of a carbonyl group at the C4-position of **3** led to a dramatic enhancement in agonist potency for compound **17a** in both mGlu2 (EC_{50} = 0.16 nM) and mGlu3 (EC_{50} = 0.11 nM) expressing cells. In contrast, enhanced potency at mGlu2 (EC_{50} = 1.3 nM) but not mGlu3 (EC_{50} = 15.5 nM) was observed for exocyclic methylene analog **17b** compared to **3**, and this molecule was found to be considerably more potent than either of the corresponding single-bond linked methyl analogs **5** or **6**.

Substitution at C4 with either hydroxyl (**18a** and **19a**) or azide (**18b** and **19b**) groups did not substantially enhance or diminished agonist potency compared to unsubstituted **3**, though these substitutions did lead to enhanced potency when compared to the corresponding methyl-substituted variants. Introduction of the more polar amino substituent in either α- or β- configurations resulted in a modest loss of agonist potency relative to **3** (α-NH_2, **18c**, EC_{50} = 47.9 nM and 57.1 nM at mGlu2 and mGlu3; β-NH_2, **19c**, EC_{50} = 21.2 nM and 24.9 nM at mGlu2 and mGlu3). Interestingly, acetylated variants of **18c** and **19c** led to differential functional outcomes. Acetylation of β-amino derivative **19c** led to diminished potency, but maintained mGlu2 and mGlu3 receptor agonist activity for **19d** (EC_{50} = 257 nM and 193 nM at mGlu2 and mGlu3, respectively), whereas acetylation of α-amino analog **18c** afforded **18d**, a relatively low potency antagonist at both mGlu2 (IC_{50} = 4990 nM) and mGlu3 (IC_{50} = 3390 nM).

Table 1. Functional Agonist or *Antagonist* Responses of C4-Substituted Analogs in Cells Expressing Recombinant Human mGlu Receptor Subtypes.[10]

			Functional responses in hmGlu receptor-expressing cells (inhibition of forskolin-stimulated cAMP)[14,15] EC$_{50}$ (nM) ± SEM; (% max effect)[a] or *IC$_{50}$ (nM)*± SEM*; (% max effect)*[b]			
No.	R	Face	mGlu2	mGlu3	mGlu6	mGlu8
3	H	n/a	5.6 ± 0.97 (100)	27.1 ± 5.7 (98)	3360 ± 706 (96)	>25000
5	CH_3	β	28.6 ± 7.01 (98)	166 ± 66.3 (88)	9280 ± 3730 (94	>25000
6	CH_3	α	63.6 ± 11.8 (98)	*3420 ± 2100 (100)*	7540 ± 1570 (94)	>25000
17a	=O	n/a	0.16 ± 0.03 (98)	0.11 ± 0.03 (99)	1380 ± 167 (100)	6530 ± 1990 (98)
17b	$=CH_2$	n/a	1.3 ± 0.14 (99)	15.5 ± 4.25 (97)	267 ± 11 (100)	1590 ± 172 (96)
18a	OH	α	7.6 ± 0.52 (99)	14.2 ± 3.34 (99)	4370 ± 348 (98)	>25000
19a	OH	β	7.5 ± 0.95 (99)	6.6 ± 1.42 (98)	3040 ± 298 (100)	>25000
18b	N_3	α	8.2 ± 1.03 (97)	12.3 ± 2.94 (96)	780 ± 38.9 (99)	12400 ± 5200 (82)
19b	N_3	β	12.2 ± 2.26 (99)	23.5 ± 2.92 (98)	1890 ± 428 (100)	12000 ± 1560 (91)
18c	NH_2	α	47.9 ± 6.90 (98)	57.1 ± 10.5 (99)	11800 ± 588 (96)	>25000
19c	NH_2	β	21.2 ± 2.17 (99)	24.9 ± 3.73 (98)	9370 ± 1420 (97)	>25000
18d	NHAc	α	*4990 ± 1070 (92)*	*3390 ± 543 (100)*	6690 ± 824 (87)	>25000
19d	NHAc	β	257 ± 16.2 (93)	193 ± 43.2 (81)	709 ± 69.1 (99)	4300 ± 776 (85)
18e	SPh	α	*7020± 770*[c] *(100)*	*895± 350 (100)*	1270 ± 254 (98)	>25000
19e	SPh	β	1170 ± 239 (49) *15300± 5400 (53)*	*3370± 1210 (100)*	3250 ± 535 (98)	>25000
18f	F	α	0.56 ± 0.13 (98)	0.24 ± 0.04 (99)	1410 ± 129 (99)	11500 ± 4090 (92)

[a]Maximal response achieved compared to glutamate (for mGlu2, mGlu3, mGlu8) or L-AP4 (mGlu6). [b]Maximal % inhibition achieved in reversing a 100% glutamate (mGlu2, mGlu3, mGlu8) or L-AP4 (mGlu6) agonist response. [c]In two out of eight agonist format assays, low potency and submaximal (~30%) efficacy agonist responses were observed. The other six agonist format assays failed to demonstrate an agonist response above baseline. In comparison, four out of four antagonist format assays displayed consistent antagonist effects with 100% maximal inhibition of glutamate.

Incorporation of the larger and more lipophilic phenylthioether substituent in either stereo-configuration at C4 led to a loss of agonist efficacy, with both **18e** and **19e** producing relatively low potency antagonist effects in both mGlu2 and mGlu3 receptor-expressing cells. These latter findings pertaining to aminoacetyl and thiophenyl substituents suggest that the region of the agonist-receptor complex adjacent to the C4 position, especially in the area occupied by C4α substituents, has limited tolerance for steric bulk. Finally, introduction of fluorine at the C4α position of **3** led to a significant enhancement of agonist potency at both mGlu2 and mGlu3 receptors, with **18f** (mGlu2 EC_{50} = 0.56 nM; mGlu3 EC_{50} = 0.24 nM) being particularly noteworthy owing to its sub-nanomolar potencies at these receptors.

Molecular modeling. In an effort to rationalize the observed structure-activity relationships of C4 substituted variants of **3**, we investigated possible mGlu3 receptor binding modes for selected compounds. A published X-ray crystal structure of the reference mGlu2/3 receptor agonist (1R,2R)-3-[(1S)-1-amino-2-hydroxy-2-oxo-ethyl]cyclopropane-1,2-dicarboxylic acid (DCG-IV, **20**) bound within the amino terminal domain (ATD) of mGlu3 (PDB ID = 2e4v[16] served as a useful template for generating proposed binding modes for compounds exhibiting agonist behavior. All modeling was carried out in the Schrodinger Maestro program, version 9.2.[17] Images depicting proposed binding modes were generated using PyMOL, version 1.2r3.[18]

As can be seen in Figure 2, compound **20** is involved in a network of hydrogen-binding interactions at the mGlu3 glutamate binding site. These hydrogen-bonds include

interactions with the backbone and with the side chains of nearby polar residues. For example, the carboxyl group of the α-amino acid portion of **20** interacts with the backbone of Ser-151 and Thr-174, while side chains Lys-389 and Arg-68 interact with the C2' carboxyl group. Of particular note are the bound water molecule which appears to form H-bonds with both C2' and C3' carboxylate functionalities and the backbone N-H of Ser-278, which also acts as an H-bond donor to the C3'-carboxylate.

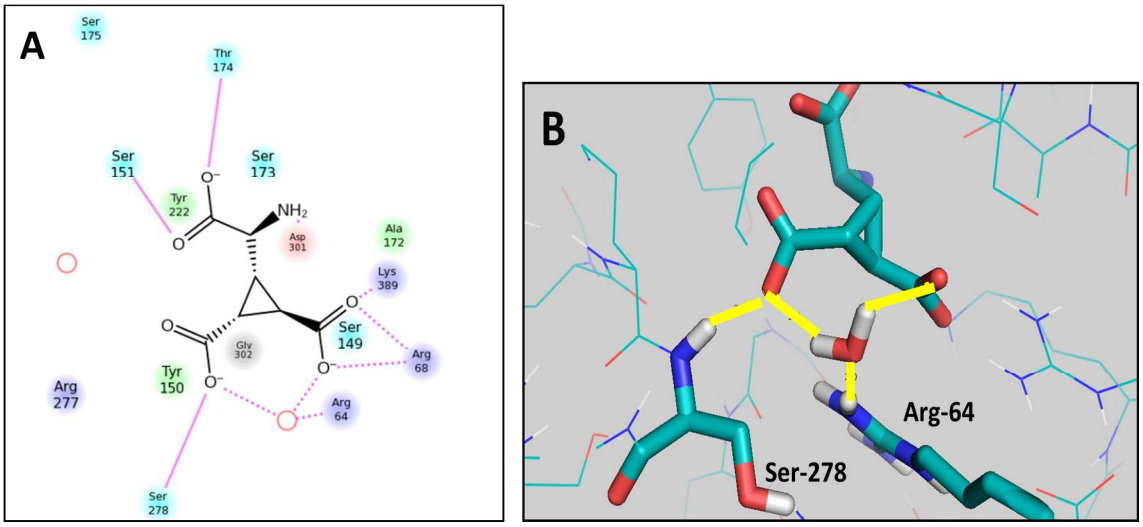


Figure 2. X-ray crystal structure of **20** bound to mGlu3 (PDB ID = 2e4v)[16] showing the hydrogen-bonding network (panel A) and a special representation depicting the location of a bound water molecule interacting with the C2' and C3' carboxylates present in **20** as well as a likely H-bond between the C3'-carboxylate and a backbone N-H arising from Ser-278 (panel B).

We used the structure of mGlu3 complexed with **20** to construct a binding mode for **3** and C4-substituted analogs of **3** that maintained the analogous key hydrogen-bonding interactions with the embedded glutamate backbone in these molecules (Figure 3). Thus, the glutamate pharmacophore present in **3** is predicted to form H-bond interactions with most of the mGlu3 residues that directly interact with **20**, but is capable

of only a single H-bond contact to the bound water molecule and no H-bond interactions with Ser-278 (Figure 3A). Based on this proposed binding mode, it is plausible that small polar hydrogen bond-acceptor substituents suitably positioned at the C4 carbon of **3** might result in more tightly bound compounds owing to the formation of additional H-bond interactions. This is illustrated for analogs **17a** and **18f** in Figures 3B and 3C, respectively. Introduction of these substituents places the carbonyl oxygen of **17a** and fluorine of **18f** in very close proximity of both the bound water molecule and backbone N-H of Ser-278. It is hypothesized that these additional binding interactions might in turn result in a more highly stabilized closed (G-protein-activating) form of the ATD leading to the observed increases in agonist potencies compared to **3**. Though speculative, the lack of a potency increase for analog **18a** compared to that seen with **18f** may be the result of an inability of the hydroxyl functionality of **18a** to achieve a rotomer that directs the lone pair electrons on the oxygen in such a way that would form a productive H-bond with Ser-278 and the crystal water. Similarly, the reduced potency of other analogs containing hydrogen bond donating substituents at the C4 position, such as **18c**, may be due in part to the lack of a productive interaction with the backbone N-H of Ser-278 (Figure 3D).

We also examined the impact of C4 thiophenyl substitution (e.g. **18e** and **19e)** on predicted docking to the mGlu3 ATD in either closed or open forms. Based on the observed antagonist pharmacology for these molecules and the close proximity of receptor residues (e.g. Ser-278) to the C4-position of bound agonists (Figures 2 and 3), we anticipated severe steric clashes arising from C4-SPh substitution with the closed form of the protein, and this was indeed observed for both **18e** (Figure 4, panel A) and

19e (not shown). Conversely, each of these molecules could be readily accommodated when docked to the published antagonist bound form of the mGlu3 ATD (PDB ID = 3sm9)[19] as exemplified by **18e** (Figure 4, panel B).

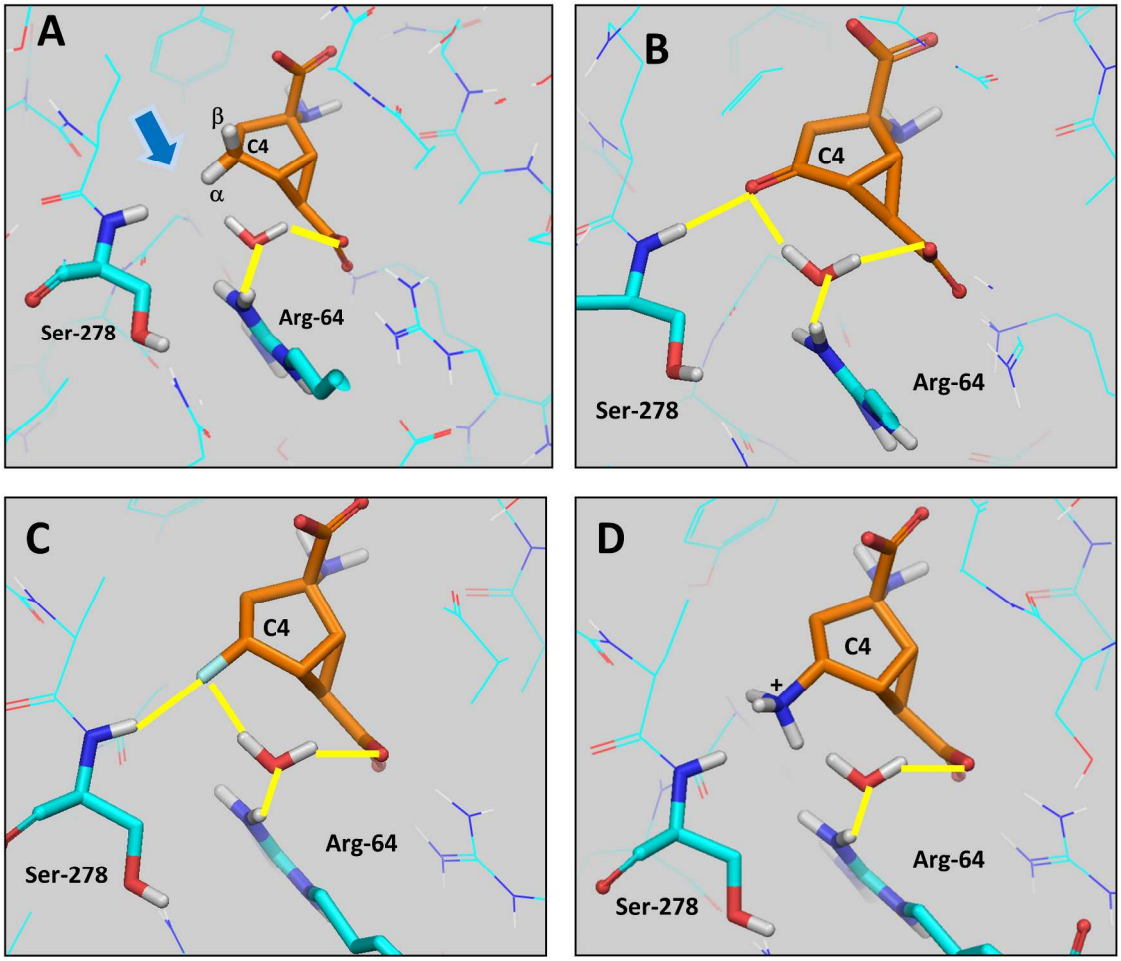


Figure 3. Proposed binding mode for compounds **3** (panel A), **17a** (panel B), **18f** (panel C) and **18c** (panel D) docked in the ATD of mGlu3 (2e4v).[16] Arrow in panel A indicates ligand-associated space into which appropriate polar substituents capable of hydrogen-bond acceptor interactions with Ser-278 and the bound water molecule. These possible interactions are evident in the docked structures of **17a** (panel B) and **18f** (panel C). Hydrogen bond donating substituents at C4 make fewer productive interactions (panel D) resulting in a loss of potency relative to **17a** and **18f**.

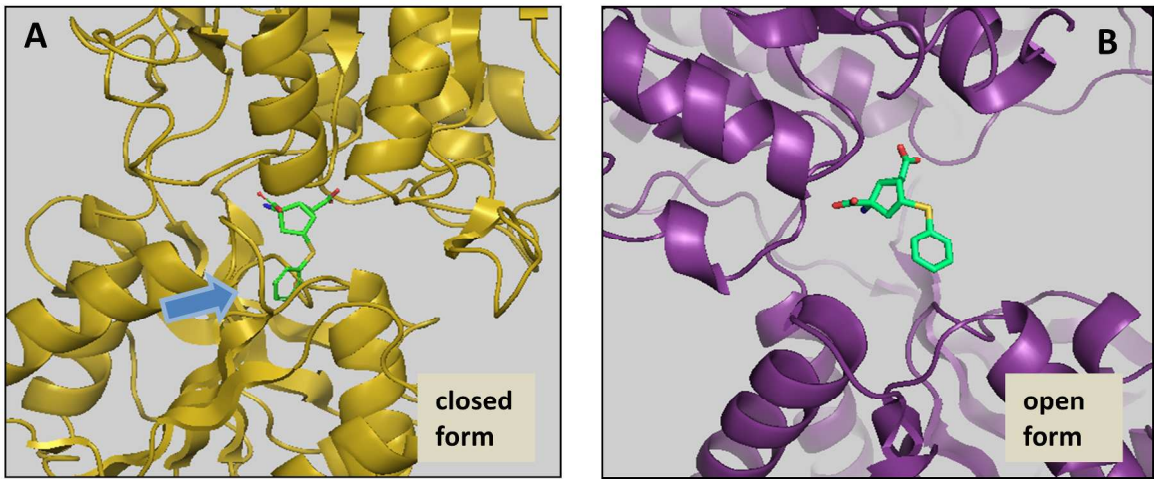


Figure 4. The closed, agonist-bound, form of the mGlu3 ATD (2e4v)[16] cannot accommodate steric bulk at C4 as evident by significant steric clashes between a portion of the protein chain in the lower lobe and the thiophenyl group in **18e** (green), indicated by the arrow in Panel A. Compound **18e**, which exhibits antagonist activity, is more likely to form productive interactions with the open form of the mGlu3 ATD (3sm9)[19] as depicted in Panel B.

Rat Pharmacokinetics. Compounds of this structural class (constrained glutamate analogs) possess negative cLogP / cLogD values. They typically display very high aqueous solubility, low passive permeability and, with one known exception,[20] are not metabolized. In spite of their low passive permeability, several members of this class have been reported to exhibit good to excellent oral absorption in preclinical species[4c,21a,b] Intestinal absorption in the rat has been suggested to be transporter-mediated owing to the observation of enantioselective absorption[7c], however, the identity of this putative transporter remains unknown. Owing to its sub-nanomolar

mGlu2/3 receptor potency and excellent selectivity profile, compound **18f** was further assessed in vivo.[10,22,23] The pharmacokinetic attributes of **18f** were evaluated in male Sprague-Dawley rats (Figure 5) following oral administration at 1, 3 and 10 mg/kg and intravenously at 3 mg/kg and plasma drug concentrations were monitored over 24 hours post-dosing

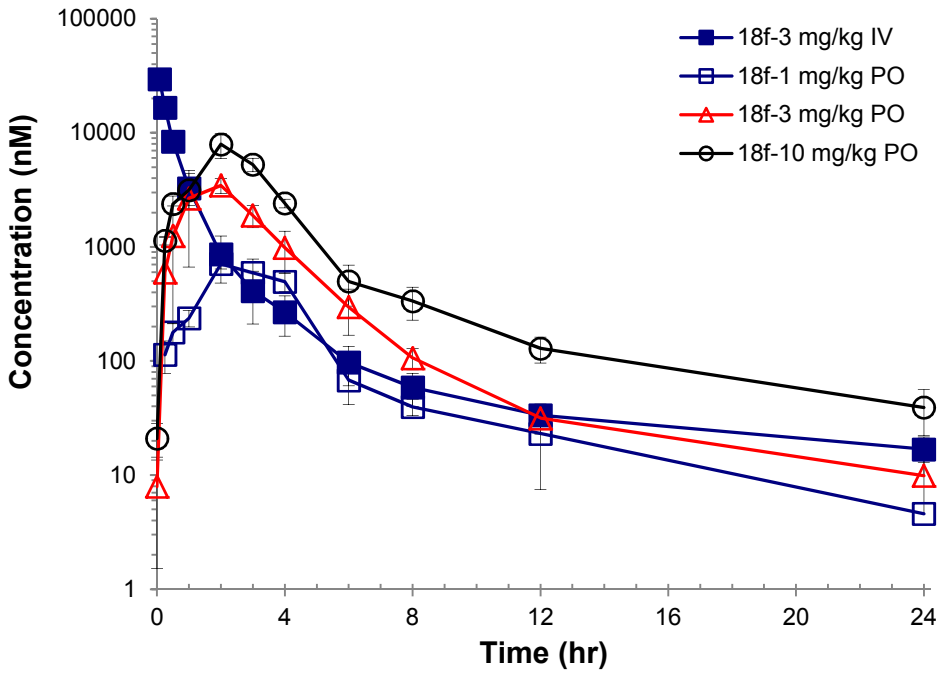


Figure 5. Plasma pharmacokinetics for compound **18f** following single oral or intravenous doses in male Sprague-Dawley rats.

Table 2. Mean pharmacokinetic parameters associated with **18f** dosed by either oral or intraveneous route of administration in rats[10,15]

Parameter	Units	3 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg
Route		IV	PO	PO	PO
AUC_{0-24}	nM*Hours	16700±2030	2790±233	10700±1680	23600±3850
C_o or C_{max}	nM	36660±3040	713±73	3940±1230	7940±2010
T_{max}	Hours	0±0	2.5±0.6	1.8±0.5	2.0±0
CL	mL/min/kg	14.8±1.8	n.c.	n.c.	n.c.
$V_{d\ ss}$	L/kg	1.25±0.25	n.c.	n.c.	n.c.
$T_{1/2}$	Hours	7.3±2.5	2.7±0.6	3.5±1.2	5.9±2.3
F	%	n.c.	n.c.	64.4±11.1	n.c.

AUC_{0-24} Area under the plasma concentration time curve from 0-24 hours post-dosing
C_o Plasma concentration after intravenous dosing extrapolated to time 0
C_{max} Peak plasma concentration after oral dosing
CL Intravenous clearance
$V_{d\ ss}$ Volume of distribution at steady state
$T_{1/2}$ Plasma disappearance half-life
F% Oral bioavailability

Mean plasma pharmacokinetic parameters are shown in Table 2 and plasma concentration-time curves in Figure 5. Oral absorption of **18f** was relatively slow with peak plasma concentrations occurring two hours post-dosing. Plasma AUC and Cmax values increased with dose in an approximately linear manner over the oral dose range of 1 to 10 mg/kg. Clearance was low at about 27% of liver blood flow with a volume of distribution of about twice total body water.[24] Plasma concentrations declined with a half-life of 2.7-7.3 hours. Despite the polar nature of this zwitterionic compound, **18f** was extensively absorbed after oral administration based upon the calculated oral bioavailability of 64%.

Behavioral pharmacology. Given that the mGlu2/3 receptor agonist potencies as well as plasma exposures for **18f** compare favorably to those reported for **4**,[7c] a molecule known to produce potent effects in rodent models of psychosis and has demonstrated efficacy in schizophrenic patients,[6a] the effect of **18f** in the phencyclidine (PCP) model of psychosis was evaluated. We employed the same dose range in this study as was assessed in the aforementioned pharmacokinetic studies (Figure 6A). As can be seen, a dose-dependent decrease in PCP-elicited ambulations was observed when **18f** was administered orally two hours prior to testing (90 minutes prior to 5 mg/kg PCP), leading to a calculated ED_{50} of 2.08 mg/kg in this assay. A one-way between-groups ANOVA was calculated on the ambulations [$F(4,35) = 14.75$, $p < 0.0001$] and a Dunnett Multiple Comparison Test (PCP alone as comparison control; alpha set at $p < 0.05$) indicated that only the 1 mg/kg dose group failed to statistically separate from PCP challenge. In order to rule out potentially confounding effects of motor impairment, **18f** was assessed using the rotorod test (Figure 6B). Oral doses of 3, 10 and 30 mg/kg of **18f** were again given two hours prior to testing. Evidence of motor impairment was observed at 30 mg/kg, this dose reducing the mean amount of time spent on the rotating barrel by nearly 70% [$F(3,28) = 59.68$, $p < 0.0001$; Dunnett Multiple Comparison Test with Vehicle as comparison control; alpha set at $p < 0.05$]. However, at doses associated with efficacy in the PCP model (3 and 10 mg/kg), no disruption in motor coordination was apparent, indicating the pharmacologic specificity of this antipsychotic-like effect.

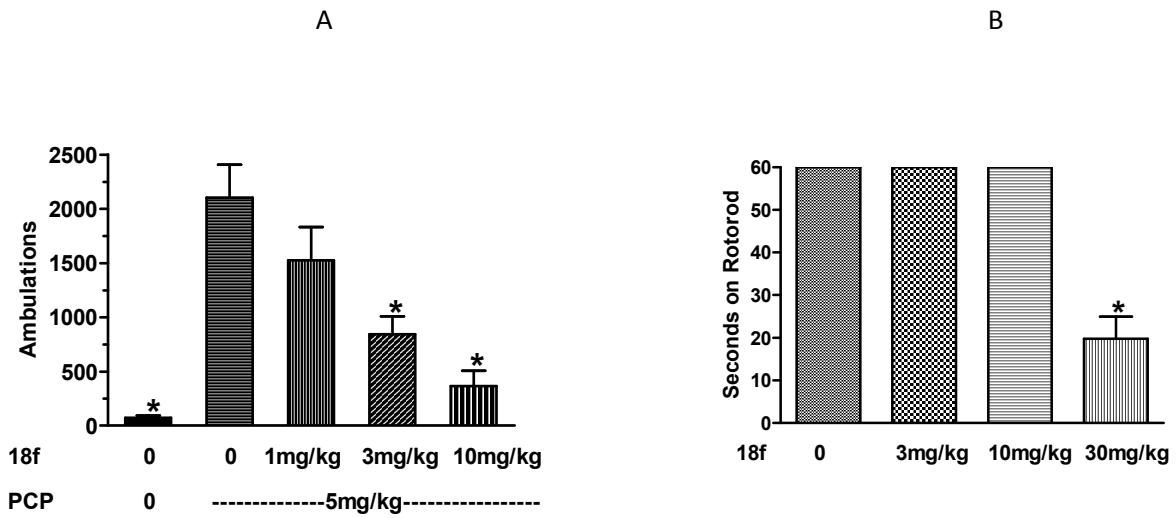


Figure 6. Effect of oral doses of **18f** on PCP-evoked ambulations (A) and rotorod performance (B) in rats. Compound **18f** was administered as an aqueous solution two hours prior to evaluation in each test. PCP-LMA (n=8 / group); Rotorod (n=8 / group). * $p < 0.05$ versus PCP (panel A) or Vehicle (panel B) using Dunnett Multiple Comparison Test.

Summary: Investigation of substituents attached to the C4-position of 2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylate **3** has led to the identification of multiple additional highly potent and selective mGlu2/3 receptor agonists, including two (**17a**, **18f**) displaying remarkable sub-nanomolar potency at these targets. Results from this study have also revealed apparent steric limits adjacent to the C4-position of this bicyclic scaffold which, when exceeded (e.g. **18e** and **19e**), abolish agonist pharmacology at both mGlu2 and mGlu3 receptors. Interestingly, of the compounds prepared thus far in this series, only the previously described C4α-methyl analog **6** was found to possess mixed mGlu2 agonist / mGlu3 antagonist pharmacology. The molecular basis for this highly unusual and unexpected functional selectivity remains

elusive. While attempts to explain this functional selectivity using molecular modeling have been made,[8a] it is unlikely that anything short of solved crystal structures of this molecule with the mGlu2 and mGlu3 proteins will prove helpful. Even then, it is entirely possible that the reason(s) underlying this functional selectivity will remain enigmatic. Finally, **18f**, one of the more highly potent analogs derived from this work, was found to be orally bioavailable in rats and to produce potent antipsychotic-like effects in a rodent model of psychosis at doses that do not impair motor function. Hence, **18f** (LY459477) represents a valuable new pharmacological tool for studying mGlu2/3 receptors both in vitro and in vivo.[25]

Experimental Section.

Synthesis. ^{1}H- and ^{13}C-NMR spectra were obtained on a Varian Unity INOVA 400 at 400 MHz and 125 MHz respectively, unless noted otherwise. TMS was used as an internal standard. LCMS data were obtained using an Agilent 1100 Series HPLC on a Gemini-NX C18 110A, 50 X 2.00 mm column. HRMS were obtained using an Agilent 1100 Series LC and TOF mass spectrometer, on a Gemini-NX C18 110A, 50 X 2.00 mm column. Optical rotations were obtained on a Perkin Elmer Polarimeter 341. Melting points were obtained on an OptiMelt Automated Melting Point System. Microwave reactions were run in a Biotage Initiator Microwave. Reactions were monitored by thin layer chromatography using silica gel 60 F_{254} plates from EMD and staining with ninhydrin. Unless otherwise noted normal phase purifications were performed using RediSep® pre-packed columns from Teledyne Isco. On the basis of

combustion analysis or HPLC data all final compounds were >95% pure. Numbering convention used in NMR assignments is provided in Figure 7. A table is provided with detailed elemental analysis (C, H, N) as Supporting Information.

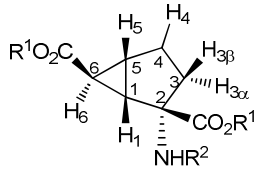


Figure 7. Numbering convention used in NMR peak assignments.

tert-Butyl-2-(thiophene)-acetate sulfonium bromide. To a solution of tetrahydrothiophene (216 mL, 216 g, 1.63 mol) in 325 mL of acetone was added *t*-butyl bromoacetate (160 mL, 197 g, 1.08 mol). Precipitate formed within 1 h. After stirring overnight, the solid was filtered, washed with acetone, and vacuum dried to obtain the sulfonium bromide salt (298 g, 1.05 mol, 97% yield). ^{1}H NMR (500 MHz, DMSO-d_6, δ): 4.40 (s, 1H), 3.51 (m, 2H), 3.48 (m, 2H), 2.23 (m, 2H), 2.13 (m, 2H), 1.42 (s, 9H).

Sulfonium ylide. To a 0 °C solution of sulfonium bromide salt (757 g, 2.67 mol) in 2.2 L of CH_2Cl_2 was added 1.7 L of a saturated aqueous K_2CO_3 solution keeping the temperature below 10 °C. After stirring the biphasic mixture 1.5 h, 223 mL of 50% aq. NaOH was added portion-wise keeping the temperature below 5 °C. The mixture was stirred for 3 h and filtered. The salts were rinsed with CH_2Cl_2. The layers were separated and the aqueous layer was extracted with 600 mL of CH_2Cl_2. The combined organic layers were dried over solid K_2CO_3 and concentrated under vacuum to give the sulfonium ylide (533. g, 2.64 mol, 98% yield) as a clear, yellow oil. Upon storage in the freezer, the oil crystallized giving an off-white solid, mp 48-50 °C. ^{1}H NMR (500 MHz, $CDCl_3$, δ): 3.12 (m, 2H), 3.00 (m, 2H), 2.85 (s, 1H), 2.41 (m, 2H), 1.84 (m, 2H), 1.38 (s, 9H).

(1S,2S,5R,6R)-Di-*tert*-butyl 2-[(*tert*-butoxycarbonyl)amino]-4-oxobicyclo[3.1.0]hexane-2,6-dicarboxylate, (8). To a 0 °C solution of enone **7** (194 g, 653 mmol) in 650 mL of CH_2Cl_2 was added trifluoroethanol (474 mL, 6.5 mol, 10 equiv). A solution of the sulfonium ylide, prepared above, (396 g, 1.96 mol) in 325 mL CH_2Cl_2 was added drop-wise over 40 min keeping the temperature below 10 °C. After 1 h, the ice bath was removed. After 2.5 h, HPLC analysis showed the reaction to be complete. (The ratio of desired exo to endo diastereomer was > 95:5 by HPLC). Deionized water (680 mL) was added to the reaction mixture and the layers were separated. The aqueous layer was extracted with 400 mL CH_2Cl_2. The combined organic layers were washed with 500 mL of brine, dried with Na_2SO_4, and concentrated under vacuum to obtain 587 g of an amber solid. The solid was dissolved in 400 mL of CH_2Cl_2 and eluted through a 1.6 kg silica gel plug using 5:1:1 hexanes:MTBE: CH_2Cl_2 as the eluent. A total of 13.2 L of eluent was collected and concentrated to give 398.7 g of white solid. The solid was dissolved in 3 L of refluxing 70:30 hexanes:MTBE. The solution was allowed to cool to rt overnight (precipitate formed at 50 °C) and then cooled in an ice bath for 1 h. The solid was filtered, rinsed with cold solvent (approximately 700 mL), and vacuum dried at 35 °C to obtain ketone **8** (173 g, 420 mmol, 64%) as a white solid. mp 144-46 °C. $[\alpha]_D^{20}$ +30.5 (c = 1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$, δ): 5.36 (bs, 1H), 2.88 (m, 1H), 2.64 (dd, J = 5.2, 3.2 Hz, 1H), 2.37 (d, J = 2.7 Hz, 1H), 2.23 (bs, 1H), 1.45 (s, 9H), 1.43 (s, 18H). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 206.2, 171.2, 168.5, 155.3, 83.4, 82.7, 80.7, 61.2, 43.2, 36.0, 34.3 28.4, 28.2, 28.0, 25.3. IR ($CHCl_3$): 2982, 1744, 1719, 1485, 1394, 1309 cm-1. MS (ES+) m/e (%

relative intensity) 412.2 (M+ + 1, 79), 356.2 (50), 300.1 (97), 276.1 (68), 244.1 (100). Anal. Calcd. for $C_{21}H_{33}NO_7$: C, H, N..

(1S,2S,5R,6S)-Di-*tert*-butyl 2-[(*tert*-butoxycarbonyl)amino]-4-methylenebicyclo[3.1.0]hexane-2,6-dicarboxylate, (9). Methyltriphenylphosphonium bromide (11.5 g, 31.59 mmol, 1.3 equiv.) was dissolved in anhydrous THF (243 mL) in a 1 L round-bottom flask under nitrogen and cooled to 0 °C. Sodium bis(trimethylsilyl)amide (1 M in THF, 31.6 mL, 31.59 mmol, 1.3 equiv.) was added drop-wise over 13 min to the stirred suspension. The resultant bright-yellow mixture was stirred at 0 °C for approximately 20 min at which time a solution of ketone **8** (10.0 g, 24.30 mmol, 1.0 equiv.) in THF (16 mL) and added to the ylide via cannula. The reaction was slowly warmed to rt and allowed to stir overnight. The reaction was diluted with ethyl acetate (500 mL) and washed with water (200 mL) and brine (150 mL). The organic was dried over Na_2SO_4, filtered, and concentrated in vacuo to give a crude oil that was purified on a 400 g silica column eluting with 85/15 hexanes/ethyl acetate. Fractions containing product were combined and concentrated in vacuo to give exocyclic olefin **9** (9.95 g, 24.3 mmol, 99%) as a white solid. mp 75-77 °C. $[\alpha]_D^{20}$ -47.45 (c = 1.054, EtOH). MS (ES+) 432.2 $[M+Na]^+$. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 5.19 (bs, 1H), 5.02 (d, J = 2.5 Hz, 1H), 4.85 (s, 1H), 3.06 (bm, 1H), 2.42 (m, 2H), 1.96 (m, 1H), 1.85 (t, J = 3.0 Hz, 1H), 1.44 (s, 9H), 1.43 (s, 18H). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 171.5, 170.5, 155.1, 147.2, 129.5, 120.4, 115.3, 107.2, 81.8, 81.2, 65.0, 33.9, 28.2, 28.1, 27.8, 24.9. HRMS ESI (m/z): calcd. for $C_{22}H_{35}NO_6Na$ $[M+Na]^+$: 432.2368. Found: 432.2378.

(1S,2S,4S,5R,6R)-di-*tert*-butyl 2-[(*tert*-butoxycarbonyl)amino]-4-hydroxybicyclo[3.1.0]hexane-2,6-dicarboxylate, (10). To a solution of the ketone **8** (20.0 g, 48.60 mmol, 1.0 equiv.) in THF (240 mL) at -5 to -10 °C was added a 1M solution of lithium tri(sec-butyl)borohydride in THF (60.0 mL, 60.0 mmol, 1.23 equiv.) in a drop-wise manner maintaining the internal temperature between -7 and -3 °C. After 3.5 h at -5 to 0 °C, no starting material remained as determined by TLC (70/30 hexanes/ethyl acetate). 2M Aqueous sodium carbonate (88.0 mL) was added drop-wise followed by 35% aqueous hydrogen peroxide (15.0 mL, 182.13 mmol) in water (70.0 mL, 3.89 mol) which was also added drop-wise over about 10 min. MTBE (200 mL) was added, mixed for 15 min and the layers were separated. The organic layer was washed with 150 mL each of 40% $NaHSO_3$, water, and brine. The organic layer was concentrated in vacuo to a white solid which was dissolved in 400 mL hot heptane:THF (65:35). The solution was concentrated in vacuo until solid began to precipitate. The mixture was removed from the rotary evaporator and allowed to stand at rt for 1 h. The white solid was filtered, washed with heptane and dried in vacuo to give a white solid. The filtrate was concentrated then dissolved in 200 mL hot heptane:THF (65:35). The solution was concentrated in vacuo until solid began to precipitate. The mixture was removed from the rotary evaporator and allowed to stand at rt for 1 h. The white solid was filtered, washed with heptane and dried in vacuo to give a white solid. Both lots were combined to give **10** (18.5 g, 44.7 mmol, 92%) as a white crystalline solid. mp 187-188 °C. $[\alpha]_D^{20}$ -28.6 (c = 1, MeOH). MS (ES+) 414.2 $[M+H]^+$. ^1H NMR (500 MHz, $CDCl_3$, δ): 5.31 (bs, 1H), 4.38 (d, J = 10.5 Hz, 1H), 4.30 (dd, J = 11.0, 6.0 Hz, 1H), 2.68 (d, J = 15.3 Hz, 1H), 2.17 (m, 1H), 2.07 (m, 1H), 1.58

(m, 1H), 1.45 (s, 9H), 1.44 (s, 9H), 1.43 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 175.1, 170.4, 155.5, 83.15, 81.31, 80.09, 73.68, 66.80, 34.33, 28.28, 28.04, 22.02. HRMS ESI (m/z): calcd. for $C_{21}H_{35}NO_7Na$ $[M+Na]^+$: 436.2306. Found: 436.2307.

(1S,2S,4S,5R,6R)-Di-*tert*-butyl 2-[(*tert*-butoxycarbonyl)amino]-4-(p-tolylsulfonyloxy)-bicyclo[3.1.0]hexane-2,6-dicarboxylate, (11). A solution of the alcohol **10** (5.54 g, 13.4 mmol, 1.00 equiv.) in CH_2Cl_2 (54 mL), was treated with p-toluenesulfonyl chloride (2.84 g, 14.8 mmol, 1.10 equiv.) followed by triethylamine (1.90 mL, 13.6 mmol, 1.00 equiv.). The mixture was cooled in an ice-water bath and 4-DMAP (2.79 g, 22.8 mmol, 1.70 equiv.) was added. After 10 min the ice bath was removed and stirring was continued at rt overnight. The reaction was washed with 30% $NaHSO_4$, water and brine. The organic was dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude orange oil was purified on a 1.5 kg silica column eluting with 80/20 hexanes/acetone. Product fractions were combined and concentrated to give **11** (5.33 g, 9.39 mmol, 70%) as a white crystalline solid. mp 144-146 °C. $[\alpha]_D^{20}$ -39.00 (c = 1.00, DMSO). MS (ES+) 590.2 $[M+Na]^+$. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 4.97 (d, J = 5.9 Hz, 1H), 4.95 (bs, 1H), 2.64 (dd, J = 5.8, 3.3 Hz, 1H), 2.58 Hz (d, J = 15.6 Hz, 1H), 2.43 (s, 3H), 2.15 (m, 1H), 1.56 (dd, J = 16.4, 5.9 Hz, 1H), 1.47 (s, 9H), 1.40 (s, 18H), 1.38 (t, J = 3.3 Hz, 1H). HRMS ESI (m/z): calcd. for $C_{28}H_{41}NO_9SNa$ $[M+Na]^+$: 590.2394. Found: 590.2402.

(1S,2S,4R,5R,6R)-Di-*tert*-butyl 2-[(*tert*-butoxycarbonyl)amino]-4-hydroxybicyclo[3.1.0]hexane-2,6-dicarboxylate, (12a). A 5 mL microwave vessel was charged with **11** (703 mg, 1.24 mmol, 1.00 equiv.) and sodium trifluoroacetate (680

mg, 4.94 mmol, 3.99 equiv.). Dimethylformamide (5.0 mL) was added and the reaction heated to 100 C for 10 h at approximately 30 watts in a microwave. No tosylate was visible by TLC (80/20 hexanes/acetone; ninhydrin stain). Saturated aqueous $NaHCO_3$ (1 mL) was added and the reaction was stirred at rt for 2 h. The mixture was diluted with ethyl acetate and washed with 20% saturated aqueous NaCl (3X). The organic was dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude material was purified on an 80 g silica column eluting with a gradient from 80/20 (hold for 10 column volumes) to 70/30 hexanes/acetone. Pure fractions were combined and concentrated to give **12a** as white solid (315 mg, 62%). $[\alpha]_D^{20}$ 2.70 (c = 1.00, EtOH). MS (ES+) 436.2 $[M+Na]^+$. ^{1}H NMR (400 MHz, CDCl3, δ): 5.26 (bs, 1H, NH), 4.76 (m, 1H, H4), 2.83 (bm, 1H, H3β), 2.15 (m, 1H, H5), 2.11 (dd, J = 6.5, 2.8 Hz, 1H, H1), 2.03 (bs, 1H, OH), 1.97 (t, J = 3.2 Hz, 1H, H6), 1.45 (s, 9H, *t*-Bu), 1.43 (s, 9H, *t*-Bu), 1.42 (s, 9H, *t*-Bu), 1.12 (dd, J = 14, 8.2 Hz, 1H, H3α). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 171.9 (CO ester), 171.2 (CO ester), 155.0 (CO Boc), 81.86 [*C*$(CH_3)_3$], 81.22 [C(*C*$H_3)_3$], 72.18 (C4), 65.01 (C2), 28.26 (C3), 28.07 (CH_3), 27.83 (C1, C5), 19.72 (C6). HRMS ESI (m/z): calcd. for $C_{21}H_{35}NO_7Na$ $[M+Na]^+$: 436.2306. Found: 436.2295.

(1S,2S,4R,5R,6S)-Di-*tert*-butyl 4-azido-2-[(*tert*-butoxycarbonyl)amino]bicyclo[3.1.0]hexane-2,6-dicarboxylate, (12b). To a flask containing **11** (8.04 g, 14.2 mmol, 1.00 equiv.) and azidotrimethylsilane (3.77 mL, 28.3 mmol, 2.0 equiv.) was added a 1M solution of tetrabutylammonium fluoride in THF (28 mL, 28 mmol, 2.0 equiv.) at rt. The mixture was heated to 75 °C. After 17 h the reaction was cooled to rt and concentrated in vacuo to an oil. The oil was purified on silica eluting with a gradient from 95/5 (hold for 10 column volumes) to 80/20 hexanes/ethyl

acetate over 10 column volumes to give **12b** (5.7 g, 92%) as a viscous oil. $[\alpha]_D^{20}$ 70.74 (c = 1.00, $CHCl_3$). MS (ES+) 461.4 $[M+Na]^+$. ^{1}H NMR (400 MHz, CDCl3, δ): 5.23 (bs, 1H, NH), 4.44 (m, 1H, H4), 2.92 (bm, 1H, H3β), 2.15 (m, 1H, H5), 2.10 (dd, J = 6.3, 3.1 Hz, 1H, H1), 1.92 (t, J = 3.1 Hz, 1H, H6), 1.46 (s, 9H, *t*-Bu), 1.44 (s, 9H, *t*-Bu), 1.43 (s, 9H, *t*-Bu), 1.17 (dd, J = 14, 9.2 Hz, 1H, H3α). ^{13}C NMR (125 MHz, DMSO, δ): 172.1 (CO ester), 170.9 (CO ester), 155.5 (CO Boc), 81.16 [*C*$(CH_3)_3$], 80.75 [C(*C*$H_3)_3$], 78.75 [*C*$(CH_3)_3$], 64.84 (C2), 60.93 (C4) 36.89 (C3), 33.12 (C1), 29.76 (C5), 28.58 [-$(CH_3)_3$], 28.15 [-$(CH_3)_3$], 27.90 [-$(CH_3)_3$], 20.39 (C6). HRMS ESI (m/z): calcd. for $C_{21}H_{34}N_4O_6Na$ $[M+Na]^+$: 461.2371. Found: 461.2372. Anal. calcd. for $C_{21}H_{34}N_4O_6$: C, H, N.

(1R,2S,4R,5R,6R)-Di-*tert*-butyl 2-[(*tert*-butoxycarbonyl)amino]-4-(phenylthio)bicyclo[3.1.0]hexane-2,6-dicarboxylate, (12c). A solution of **11** (400 mg, 0.704 mmol, 1.00 equiv.) in dimethylformamide (3 mL) was treated with potassium carbonate (200 mg, 1.4 mmol, 2.0 equiv.) and benzenethiol (0.14 mL, 1.4 mmol, 2.0 equiv.). The reaction was heated to 70 °C in a microwave for 40 min. The reaction was diluted with ethyl acetate and washed with water (2X) and brine. The organic was dried over Na_2SO_4, filtered, and concentrated. The crude material was purified on a 40 g silica column eluting with a gradient from 90/10 hexanes/ethyl acetate (hold for 5 column volumes) to 80/20 hexanes/ethyl acetate over 10 column volumes to give **12c** (317 mg, 89%) as a colorless oil. $[\alpha]_D^{20}$ 81.60 (c = 1.00, $CHCl_3$). MS (ES+) 528.2 $[M+Na]^+$. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.42 (m, 2H, ArCH), 7.25 (m, 3H, ArCH), 5.25 (bs, 1H, NH), 4.09 (m, 1H, H4), 3.01 (bm, 1H, H3β), 2.15 (dd, J = 5.8, 3.0 Hz, 1H, H1), 2.07 (m, 1H, H5), 1.84 (m, 1H, H6), 1.45 (s, 9H, *t*-Bu), 1.44 (s, 9H, *t*-Bu), 1.42 (s, 9H, *t*-Bu), 1.21 (dd, J = 14, 10 Hz, 1H, H3α). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 171.6 (CO

ester), 170.9 (CO ester), 155.0 (CO Boc), 134.7 (ArC), 131.9 (ArCH), 128.9 (Ar CH), 127.1 (ArCH), 81.91 [*C*$(CH_3)_3$], 81.20 [*C*$(CH_3)_3$], 80.05 [*C*$(CH_3)_3$], 66.39 (C2), 49.43 (C3), 47.28 (C4), 31.35 (C1), 28.26 [-$(CH_3)_3$], 28.09 [-$(CH_3)_3$], 27.84 [-$(CH_3)_3$], 26.96 (C5), 21.44 (C6).

(1S,2S,4R,5R,6S)-Di-*tert*-butyl 4-amino-2-[(*tert*-butoxycarbonyl)amino)]bicyclo[3.1.0]hexane-2,6-dicarboxylate, (12d). A 500 mL Parr bottle was charged with 5% Pd/C (1.499 g, 0.70 mmol, 0.08 equiv.) was and purged with N_2. The catalyst was wetted with absolute ethanol (25 ml). The azide **12b** (4.02 g, 9.17 mmol, 1.0 equiv.) and absolute ethanol (100 ml) were then added to the slurry. The tube was sealed, purged with N_2 (4X), purged with H_2 (4X) and pressurized to 20 psi with H_2. After 2h the reaction mixture was vented, purged with N_2 (4X) filtered and the clear darkly colored filtrate was concentrated to give 4.5 g of a dark brown oil. The crude oil was dissolved in about 80 ml of 7:1 ethyl acetate:MeOH. *Si*-DMT and *Si*-Triamine (both available from Silicycle) were added (4 g each) and the mixture stirred at rt overnight. The mixture was filtered to remove the resin. The colorless filtrate was concentrated in vacuo to give **12d** (3.66 g, 97%) as a white solid. $[\alpha]_D^{20}$ 28.02 (c = 0.99, $CHCl_3$). MS (ES+) 413.0 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 5.19 (bs, 1H, NHBoc), 3.90 (m, 1H, H4), 2.73 (bm, 1H,H3β), 2.12 (dd, J = 6.4, 2.5, Hz, 1H, H1), 2.06 (m, 1H, H5), 1.78 (t, J = 2.6 Hz, 1H, H6), 1.46 (s, 9H, *t*-Bu), 1.43 (s, 18H, *t*-Bu), 1.38 (bs, 2H, NH_2), 0.89 (dd, J = 14, 9.2 Hz, H3α). ^{13}C NMR (125 MHz, DMSO, δ): 172.7 (CO ester), 172.2 (CO ester), 155.5 (CO Boc), 80.39 [*C*$(CH_3)_3$], 80.05 [*C*$(CH_3)_3$], 78.36 [*C*$(CH_3)_3$], 65.00 (C2), 52.01 (C4), 34.48 (C3), 33.75 (C1), 28.63 [-$(CH_3)_3$], 28.24 [-$(CH_3)_3$], 27.96 [-

$(CH_3)_3$], 21.21 (C5), 19.27 (C6). HRMS ESI (m/z): calcd. for $C_{21}H_{37}N_2O_6$ $[M+H]^+$: 413.2646. Found: 413.2652.

(1S,2S,4R,5R,6S)-Di-*tert*-butyl 4-acetamido-2-[(*tert*-butoxycarbonyl)amino]bicyclo[3.1.0]hexane-2,6-dicarboxylate, (12e). To a stirred solution of **12d** (250 mg, 0.606 mmol, 1.00 equiv.) in dichloromethane (3 mL) were added triethylamine (0.17 mL, 1.2 mmol, 2.0 equiv.), N,N-dimethyl-4-pyridinamine (7.4 mg, 0.061 mmol, 0.10 equiv.), and acetic anhydride (0.06 mL, 0.7 mmol, 1.1 equiv.) at rt. After stirring the mixture over the weekend, the solvent was evaporated in vacuo. The crude material was purified on 40 g of silica eluting with a gradient from 1/1 hexanes/ethyl acetate to 100% ethyl acetate to give **12e** (258 mg, 94%). $[\alpha]_D^{20}$ 30.50 (c = 1.000, EtOH). MS (ES+) 477.2 $[M+Na]^+$. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 6.0 (bs, 1H, NH), 5.38 (bs, 1H, NH), 4.83 (m, 1H, H4), 2.73 (bm, 1H, H3β), 2.2 (m, 2H, H1/5), 1.94 (s, 3H, CH_3), 1.80 (m, 1H, H6), 1.45 (s, 9H, *t*-Bu), 1.40 (s, 9H, *t*-Bu), 1.39 (s, 9H, *t*-Bu), 1.00 (bm, 1H, H3α). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 171.4 (CO ester), 170.8 (CO ester), 155.1 (CO Boc), 82.12 [*C*$(CH_3)_3$], 65.07 (C2), 50.02 (C4), 34.60 (C3), 33.12 (C1), 28.23 [-$(CH_3)_3$], 28.06 [-$(CH_3)_3$], 27.82 [-$(CH_3)_3$], 25.21 (C5), 23.18 (CO*C*H_3), 20.21 (C6) HRMS ESI (m/z): calcd. for $C_{23}H_{38}N_2O_7Na$ $[M+Na]^+$: 477.2571. Found: 477.2562.

(1R,2S,4R,5R,6R)-Di-*tert*-butyl 2-[(*tert*-butoxycarbonyl)amino]-4-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylate, (12f). A solution of **10** (177 mg, 0.428 mmol, 1.00 equiv.) in dichloromethane (6 mL) was cooled to 0 °C and treated with diethylaminosulfur trifluoride (0.06 mL, 0.453 mmol, 1.1 equiv.). After 2 h the cooling bath was removed and the reaction was allowed to stir at rt overnight. The reaction was

diluted with ethyl acetate and washed with water and brine. The organic was dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude material was purified on a 24 g silica column eluting with a gradient from 95/5 (hold for 5 column volumes) to 85/15 hexanes/acetone over 20 column volumes to give **12f** (126 mg, 71%) as a white solid. $[\alpha]_D^{20}$ 18.82 (c = 0.99, $CHCl_3$). MS (ES+) 438.0 $[M+Na]^+$. ^1H NMR (400 MHz, $CDCl_3$, δ): 5.56-5.38 (m, 1H, H4), 5.26 (bs, 1H, NH), 2.99 (bm, 1H, H3β), 2.24 (m, 1H, H1), 2.13 (ddd, J = 6.5, 3.3, 3.3 Hz, 1H, H5), 2.08 (t, J = 2.9 Hz, 1H, H6), 1.46 (s, 9H, *t*-Bu), 1.45 (s, 9H, *t*-Bu), 1.44 (s, 9H, *t*-Bu), 1.37 (m, 1H, H3α) ^{13}C NMR (125 MHz, DMSO, δ): 172.3 (CO ester), 170.7 (CO ester), 155.5 (CO Boc), 93.04 (d, J = 181 Hz, C4), 81.25 [*C*$(CH_3)_3$], 80.85 [*C*$(CH_3)_3$], 78.76 [*C*$(CH_3)_3$], 64.20 (C2), 36.91 (d, J = 22 Hz, C3), 33.16 (C1), 29.65 (d, J = 22 Hz, C5), 28.57 [-$(CH_3)_3$], 28.13 [-$(CH_3)_3$], 27.85 [-$(CH_3)_3$], 20.52 (C6). HRMS ESI (m/z): calcd. for $C_{21}H_{34}FNO_6Na$ $[M+Na]^+$: 438.2262. Found: 438.2264. $C_{21}H_{34}FNO_6$: 461.2371. Found: 461.2372. Anal. calcd. for $C_{21}H_{34}FNO_6$: C, H, N.

(1R,2S,4R,5R,6R)-Di-*tert*-butyl 4-bromo-2-[(*tert*-butoxycarbonyl)amino]bicyclo[3.1.0]hexane-2,6-dicarboxylate, (13). A solution of triphenylphosphine (25.0 g, 94.40 mmol, 2.0 equiv.) in toluene (400 mL) was treated with bromine (4.8 mL, 93 mmol, 2.0 equiv.) until a yellow color persisted. Just enough Ph_3P was added until the color disappeared. Within 1 min a suspension formed. A solution of **10** (19.5 g, 47.2 mmol, 1.00 equiv.) in toluene (100 mL) and pyridine (300 mL) was added via cannulation over approximately 40 min. The reaction was heated to 75 °C and stirred overnight. The reaction was cooled to rt, diluted with ethyl acetate, filtered, and concentrated in vacuo. The material was slurried in MTBE, filtered, and

concentrated in vacuo. The crude material was passed through a plug of silica eluting with 86/7/7 hexanes/MTBE/DCM to give **13** (15.1 g, 67%) as a white crystalline solid. $[\alpha]_D^{20}$ 70.02 (c = 1.003, $CHCl_3$). MS (ES+) 497.8, 499.8 $[M+Na]^+$. ^1H NMR (400 MHz, $CDCl_3$, δ): 5.25 (bs, 1H, NH), 4.73 (m, 1H, H4), 3.15 (bm, 1H, H3β), 2.34 (m, 1H, H5), 2.23 (dd, J = 6.2, 3.1 Hz, H1), 1.93 (t, J = 3.1 Hz, 1H, H6), 1.56 (dd, J = 14, 9.4 Hz, 1H, H3α), 1.46 (s, 9H, *t*-Bu), 1.45 (s, 9H, *t*-Bu), 1.43 (s, 9H, *t*-Bu). ^{13}C NMR (125 MHz, DMSO, δ): 171.9 (CO ester), 170.6 (CO ester), 155.6 (CO Boc), 81.37 [*C*$(CH_3)_3$], 80.97 [*C*$(CH_3)_3$], 78.85 [*C*$(CH_3)_3$], 65.66 (C2), 49.82 (C4), 42.34 (C3), 37.25 (C1), 34.11 (C5), 28.56 [-$(CH_3)_3$], 28.15 [-$(CH_3)_3$], 27.87 [-$(CH_3)_3$], 23.69 (C6). HRMS ESI (m/z): calcd. for $C_{21}H_{34}BrNO_6Na$ $[M+Na]^+$: 498.1462. Found: 498.1464. Anal. calcd. for $C_{21}H_{34}BrNO_6$: C, H, N.

(1S,2S,4S,5R,6S)-Di-*tert*-butyl 4-azido-2-[(*tert*-butoxycarbonyl)amino]bicyclo[3.1.0]hexane-2,6-dicarboxylate, (14a). The title compound was prepared from **13** (18.5 g, 38.8 mmol, 1.00 equiv.) following the general procedure used to prepare **12b** providing **14a** (15.5 g, 91%) as a viscous oil. $[\alpha]_D^{20}$ -22.01 (c = 0.999, $CHCl_3$). MS (ES+) 461.2 $[M+Na]^+$. ^1H NMR (400 MHz, CDCl3, δ): 5.06 (bs, 1H, NH), 4.17 (d, J = 6.7 Hz, 1H, H4), 2.60 (bm, 1H, H3β), 2.55 (dd, J = 6.0, 3.1 Hz, 1H, H1/5), 2.10 (dd, J = 6.0, 3.1 Hz, 1H, H1/5),1.56 (m, 1H, H3α), 1.50 (t, J = 3.2 Hz, 1H, H6), 1.49 (s, 9H, *t*-Bu), 1.425 (s, 9H, *t*-Bu), 1.424 (s, 9H, *t*-Bu). ^{13}C NMR (125 MHz, DMSO, δ): 171.5 (CO ester), 170.9 (CO ester), 155.4 (CO Boc), 80.83 [*C*$(CH_3)_3$], 80.70 [*C*$(CH_3)_3$], 78.84 [*C*$(CH_3)_3$], 65.79 (C2), 61.07 (C4), 38.91 (C3), 33.78 (C1), 30.99 (C5), 28.53 [-$(CH_3)_3$], 28.13 [-$(CH_3)_3$], 27.86 [-$(CH_3)_3$], 21.82 (C6). HRMS

ESI (m/z): calcd. for $C_{21}H_{34}N4O_6$: 461.2371. Found: 461.2373. Anal. calcd. for $C_{21}H_{34}N_4O_6$: C, H, N.

(1R,2S,4S,5R,6R)-Di-*tert*-butyl 2-[(*tert*-butoxycarbonyl)amino]-4-(phenylthio)bicyclo[3.1.0]hexane-2,6-dicarboxylate, (14b). The title compound was prepared from **13** (200 mg, 0.420 mmol, 1.00 equiv.) following the general procedure used to prepare **12c** giving **14b** (202 mg, 95%) as a white foam. $[\alpha]_D^{20}$ -63.80 (c = 1.000, EtOH). MS (ES-) 504.0 $[M-H]^-$. ^{1}H NMR (400 MHz, CDCl3, δ): 7.37 (m, 2H, ArCH), 7.30–7.24 (m, 2H, ArCH), 7.20 (m, 1H, ArCH), 5.02 (bs, 1H, NH), 3.76 (d, J = 7.9 Hz, 1H, H4), 2.70 (bm, 1H, H3β), 2.66 (dd, J = 6.2, 3.2 Hz, 1H, H1/5), 2.18 (dd, J = 6.2, 3.2 Hz, 1H, H1/5), 1.77 (dd, J = 15, 7.9 Hz, 1H, H3α), 1.56 (t, J = 3.1 Hz, 1H, H6), 1.54 (s, 9H, *t*-Bu), 1.43 (s, 9H, *t*-Bu), 1.41 (s, 9H, *t*-Bu). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 171.4 (CO ester), 170.6 (CO ester), 155.0 (CO Boc), 136.3 (ArC), 130.9 (ArCH), 130.7 (ArCH), 129.1 (ArCH), 128.9 (ArCH), 126.8 (ArCH), 82.16 [*C*$(CH_3)_3$], 81.07 [*C*$(CH_3)_3$], 80.25 [*C*$(CH_3)_3$], 66.74 (C2), 48.63 (C3/C4), 48.46 (C3/C4), 35.04 (C1), 33.86 (C5), 28.29 [-$(CH_3)_3$], 28.09 [-$(CH_3)_3$], 27.84 [-$(CH_3)_3$], 24.27 (C6). HRMS ESI (m/z): calcd. for $C_{27}H_{39}NO_6SNa$ $[M+Na]^+$: 528.2390. Found: 528.2401.

(1S,2S,4S,5R,6S)-Di-*tert*-butyl 4-amino-2-[(*tert*-butoxycarbonyl)amino]bicyclo[3.1.0]hexane-2,6-dicarboxylate, (14c). The title compound was prepared from **14a** (5.0 g, 11.4 mmol, 1.0 equiv.) following the general procedure used to prepare **12d** affording **14c** (4.5 g, 97%) as a white solid. $[\alpha]_D^{20}$ -20.99 (c = 1.001, $CHCl_3$). MS (ES+) 413.4 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 5.15 (bs, 1H, NH), 3.50 (d, J = 7.1 Hz, 1H, H4), 2.40 (bm, 1H, H3β), 2.35 (dd, J = 6.2, 3.1 Hz,

1H, H1/5), 1.90 (dd, J = 6.1, 3.1 Hz, 1H, H1/5), 1.74 (bs, 2H, NH_2), 1.59 (dd, J = 15, 7.4 Hz, 1H, H3α), 1.48 (s, 9H, *t*-Bu), 1.45 (t, J = 3.3 Hz, 1H, H6), 1.43 (s, 9H, *t*-Bu), 1.42 (s, 9H, *t*-Bu). ^{13}C NMR (125 MHz, DMSO, δ): 174.2 (CO ester), 171.5 (CO ester), 155.6 (CO Boc), 80.77 [*C*$(CH_3)_3$], 80.20 [*C*$(CH_3)_3$], 78.52 [*C*$(CH_3)_3$], 66.04 (C2), 53.26 (C4), 43.03 (C3), 36.65 (C1), 34.52 (C5), 28.58 [-$(CH_3)_3$], 28.17 [-$(CH_3)_3$], 27.91 [-$(CH_3)_3$], 22.56 (C6). HRMS ESI (m/z): calcd. for $C_{21}H_{37}N2O_6$ $[M+H]^+$: 413.2646. Found: 413.2651. Anal. calcd. for $C_{21}H_{36}N_2O_6$: C, H, N.

(1S,2S,4S,5R,6S)-Di-*t*-butyl 4-acetamido-2-[(*t*-butoxycarbonyl)amino]bicyclo[3.1.0]hexane-2,6-dicarboxylate, (14d). The title compound was prepared from **14c** (250 mg, 0.61 mmol, 1.00 equiv.) following the general procedure used to prepare **12e** affording **14d** (251 mg, 90%) as a white solid. MS (ES+) 477.2 $[M+Na]^+$. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.30 (bs, 1H, NH), 5.30 (bs, 1H, NH), 4.58 (t, J = 7.6 Hz, 1H, H4), 2.55 (bd, J = 15 Hz, 1H, H3β), 2.16 (dd, J = 5.9, 2.7 Hz, 1H, H1/5), 1.97 (s, 3H, CH_3), 1.94 (dd, J = 5.9, 3.0 Hz, 1H, H1/5), 1.60 (dd, J = 16, 8.0 Hz, 1H, H3α), 1.56 (t, J = 3.1 Hz, 1H, H6), 1.48 (s, 9H, *t*-Bu), 1.425 (s, 9H, *t*-Bu), 1.418 (s, 9H, *t*-Bu). HRMS ESI (m/z): calcd. for $C_{23}H_{38}N_2O_7Na$ $[M+Na]^+$: 477.2571. Found: 477.2573.

(1S,2S,3R,4R,5S)-*t*-butyl 1-((tert-butoxycarbonyl)amino)-7-oxo-6-oxatricyclo[3.2.1.02,4]octane-3-carboxylate, (15). A solution of **12a** (180 mg, 0.435 mmol, 1.0 equiv) in dichloromethane (6.0 mL) was cooled to 0 °C and treated with DAST (0.060 mL, 0.45 mmol, 1.05 equiv). After 3 h the reaction was diluted with ethyl acetate and washed with water and brine. The organic layer was dried over Na_2SO_4,

filtered, and concentrated in vacuo. The crude material was purified on a 24 g silica column eluting with a gradient from 90/10 (hold for 5 column volumes) to 80/20 hexanes/acetone over 15 column volumes. Fractions containing the major component were combined and concentrated to give **15** (130 mg, 88%) as a white solid. MS (ES+) 362.2 $[M+Na]^+$. ^1H NMR (400 MHz, $CDCl_3$, δ): 7.83 (bs, 1H, NH), 4.96 (t, J = 1.7 Hz, 1H, H4), 2.60 (t, J = 2.1 Hz, 1H, H6), 2.28 (bm, 1H, H3β), 2.10 (m, 1H, H1/5), 1.84 (m, 1H, H1/5), 1.74 (m, 1H, H3α), 1.38 (s, 9H, *t*-Bu), 1.36 (s, 9H, *t*-Bu). X-Ray.[12]

(1S,2S,5R,6S)-di-t-butyl 2-((t-butoxycarbonyl)amino)bicyclo[3.1.0]hex-3-ene-2,6-dicarboxylate, (16). A solution of **13** (200 mg, 0.420 mmol, 1.0 equiv) in acetonitrile (0.85 mL) was treated with a 1 M solution of TBAF in THF (0.85 mL, 0.85 mmol, 2.0 equiv) and heated in a Biotage Initiator microwave at 100 °C for 2 h. The reaction was diluted with ethyl acetate and washed with water and brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude material was purified on a 24 g silica column eluting with a gradient from 95/5 to 85/15 hexanes/acetone over 25 column volumes. Fractions containing the major component were combined and concentrated to give **16** (80 mg, 48%) as a white foam. $[\alpha]_D^{20}$ -207.86 (c = 1.001, $CHCl_3$).MS (ES+) 418.2 $[M+Na]^+$. ^1H NMR (400 MHz, $CDCl_3$, δ): 6.14 (dd, J = 4.9, 1.7 Hz, 1H, H4), 5.49 (bm, 1H, H3), 5.06 (bm, 1H, NH), 2.69 (m, 1H, H1/5), 2.46 (m, 1H, H1/5), 1.47 (s, 9H, *t*-Bu), 1.45 (s, 9H, *t*-Bu), 1.45 (1H, H6, buried under *t*-Bu signals), 1.44 (s, 9H, *t*-Bu). Anal. calcd. for $C_{21}H_{33}NO_6$: C, H, N.

(1S,2S,5R,6R)-2-Amino-4-oxobicyclo[3.1.0]hexane-2,6-dicarboxylic acid, (17a). A mixture of **8** (200 mg, 0.486 mmol, 1.00 equiv.) in 50% aqueous acetic acid (3.0 mL)

was heated to 160 °C in a microwave for 5 min resulting in a light brown solution. The vessel was scratched with a glass rod and allowed to stand at rt overnight. The solid was collected by vacuum filtration washing with isopropyl alcohol. The solid was taken up in water and concentrated in vacuo three times to remove excess acetic acid providing zwitterion **17a** as a white powder (19 mg, 20%). $[\alpha]_D^{20}$ 59.88 (c = 1.002, 0.1 N NaOH). MS (ES+) 200.2 $[M+H]^+$. ^{1}H NMR (400 MHz, D_2O, δ): 2.77 (m, 1H, H5), 2.58 (m, 1H, H1), 2.49, 2.40 (ABq, J = 18 Hz, 2H, H3α/β), 2.42 (s, 1H, H6). ^{13}C NMR (125 MHz, D_2O/KOD, δ): 208.0 (C4 ketone), 173.8 (CO_2H), 173.12 (CO_2H), 60.47 (C2), 39.94 (C3), 36.70 (C6), 33.15 (C5), 24.65 (C1). HRMS ESI (m/z): calcd. for $C_8H_{10}NO_5$ $[M+H]^+$: 200.0553. Found: 200.0559. Anal. calcd. for $C_8H_9NO_5$: C, H, N.

(1S,2S,5R,6S)-2-Amino-4-methylenebicyclo[3.1.0]hexane-2,6-dicarboxylic acid, (17b). A mixture of **9** (62 mg, 0.151 mmol, 1.00 equiv.) in 50% aq acetic acid (1.1 mL) was heated to 160 °C in a microwave for 5 min. The vessel was scratched with a glass rod and allowed to stand at rt overnight. The solid was collected by vacuum filtration washing with isopropyl alcohol. The solid was taken up in water and concentrated in vacuo three times to remove excess acetic acid providing zwitterion **17b** as a white powder (13 mg, 44%). $[\alpha]_D^{20}$ -30.00 (c = 1.000, 0.1 N NaOH). MS (ES-) 198.2 $[M+H]^+$. ^{1}H NMR (400 MHz, D_2O/KOD, δ): 5.04 (s, 1H, CH sp^2), 4.85 (s, 1H, CH sp^2), 2.59 (m, 1H, H1/5), 2.58 (d, J = 16 Hz, 1H, H3β), 2.37 (m, 1H, H1/5), 2.16 (d, J = 17 Hz, 1H, H3α), 1.98 (m, 1H, H6). ^{13}C NMR (125 MHz, D_2O/KOD, δ): 178.5 (CO_2H), 175.5 (CO_2H), 146.6 (sp^2 CH_2), 107.4 (C4), 65.06 (C2), 37.40 (C3), 34.25 (C5), 33.63 (C1), 26.57 (C6). HRMS ESI (m/z): calcd. for $C_9H_{12}NO_4$ $[M+H]^+$: 198.0761. Found: 198.0763. Anal.for $C_9H_{11}NO_4 \cdot 0.25H_2O$: C, H, N.

(1S,2S,4R,5R,6R)-2-Amino-4-hydroxybicyclo[3.1.0]hexane-2,6-dicarboxylic acid, (18a). A mixture of **12a** (100 mg, 0.242 mmol, 1 equiv) in 50% aq. acetic acid (1.8 mL) was heated to 160 °C in a microwave for 5 min. The vessel was scratched with a glass rod and allowed to stand at rt overnight. The solid was collected by vacuum filtration washing with isopropyl alcohol. The solid was taken up in water and concentrated in vacuo three times to remove excess acetic acid providing zwitterion **18a** as a white powder (34 mg, 70%). MS (ES+) 202.2 $[M+H]^+$. ^{1}H NMR (400 MHz, D_2O/KOD, δ): 4.47 (m, 1H, H4), 2.05 (dd, J = 13, 8.2 Hz, 1H, H3β), 1.88 (m, 1H, H5), 1.76 (m, 1H, H1), 1.67 (m, 1H, H6), 0.91 (dd, J = 14, 8.5 Hz, H3α). ^{13}C NMR (100 MHz, D_2O/KOD, δ): 177.7 (CO_2), 175.1 (CO_2), 71.01 (C4), 64.86 (C2), 36.53 (C3), 32.61 (C5), 31.43 (C1), 20.26 (C6). HRMS ESI (m/z): calcd. for $C_8H_{12}NO_5$ $[M+H]^+$: 202.0710. Found: 202.0709. Anal. calcd. for $C_8H_{11}NO_5$: C, H, N.

(1S,2S,4R,5R,6S)-2-Amino-4-azidobicyclo[3.1.0]hexane-2,6-dicarboxylic acid, (18b). A mixture of **12b** (203 mg, 0.463 mmol, 1 equiv) in 50% aq. acetic acid (4.0 mL) was heated to 160 °C in a microwave for 6 min. The suspension was concentrated in vacuo. Water was added and removed in vacuo twice to remove excess AcOH and afford zwitterion **18b** (76 mg, 72%) as a white solid. $[\alpha]_D^{20}$ 115.77 (c = 1.002, 0.1 N NaOH). MS (ES-) 225.0 $[M+H]^+$. ^{1}H NMR (400 MHz, D_2O/KOD, δ): 4.23 (m, 1H, H4), 2.11 (dd, J = 14, 8.0 Hz, 1H, H3β), 2.00 (m, 1H, H5), 1.83 (dd, J = 6.3, 2.7 Hz, 1H, H1), 1.69 (m, 1H, H6), 1.02 (dd, J = 14, 9.3 Hz, 1H, H3α). ^{13}C NMR (125 MHz, D_2O/KOD, δ): 179.0 (CO_2), 175.9 (CO_2), 65.18 (C2), 61.26 (C4), 35.46 (C3), 31.73 (C1), 29.91 (C5), 21.98 (C6). HRMS ESI (m/z): calcd. for $C_8H_{11}N_4O_4$ $[M+H]^+$: 227.0775. Found: 227.0775. Anal. calcd. for $C_8H_{10}N_4O_4$: C, H, N.

(1S,2S,4R,5R,6S)-2,4-Diaminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid acetic acid salt, (18c). A mixture of **12d** (129 mg, 0.313 mmol, 1 equiv) in 50% aq. acetic acid (3.2 mL) was heated to 160 °C in a microwave for 6 min. The suspension was concentrated in vacuo. Water was added and removed in vacuo four times to remove excess AcOH. The solid was slurried in water and collected via vacuum filtration to give zwitterion **18c** (62 mg, 99%) as a white solid. $[\alpha]_D^{20}$ 14.01 (c = 0.999, 0.1 N NaOH). MS (ES+) 201.0 $[M+H]^+$. ^{1}H NMR (400 MHz, D_2O, δ): 4.17 (ddd, J = 8.9, 8.9, 4.7 Hz, 1H), 2.36 (dd, J = 14, 8.0 Hz, 1H), 2.20 (ddd, J = 7.0, 4.0, 4.0 Hz, 1H), 2.10 (dd, J = 6.7, 3.1 Hz, 1H), 1.97 (s, trace acetic acid, 0.3 mol%), 1.93 (t, J = 3.1 Hz, 1H), 1.90 (s, 3H), 1.42 (dd, J = 14, 9.1 Hz, 1H). ^{13}C NMR (125 MHz, D_2O/KOD, δ): 177.7, 177.3, 174.2, 64.95, 51.34, 33.19, 31.51, 28.26, 21.40, 21.01. HRMS ESI (m/z): calcd. for $C_8H_{13}N_2O_4$ $[M+H]^+$: 201.0870. Found: 201.0868. Anal. calcd. for $C_8H_{12}N_2O_4 \cdot 0.5H_2O \cdot 0.3C_2H_4O_2$: C, H, N.

(1S,2S,4R,5R,6S)-4-Acetamido-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid, (18d). A mixture of **12e** (258 mg, 0.568 mmol, 1 equiv) in 50% aq. acetic acid (5.2 mL) was heated to 160 °C in a microwave for 5 min. The suspension was cooled to 0 °C and filtered. The filter cake was washed with isopropyl alcohol and dried under vacuum to give zwitterion **18d** (121 mg, 88%) as a white solid. $[\alpha]_D^{20}$ 63.84 (c = 1.003, 0.1 N NaOH). MS (ES+) 201.0 $[M+H]^+$. ^{1}H NMR (400 MHz, D_2O/ pyridine-d_5, δ): 4.17 (ddd, J = 9.2, 9.2, 4.7 Hz, 1H), 2.28 (dd, J = 14, 8.4 Hz, 1H), 2.06 (m, 1H), 1.95 (dd, J = 6.5, 3.0 Hz, 1H), 1.82 (s, 3H), 1.74 (t, J = 3.0 Hz, 1H), 1.15 (dd, J = 14, 9.1 Hz, 1H). ^{13}C NMR (125 MHz, D_2O/KOD, δ): 180.2 (CO_2), 178.9 (CO), 173.9 (CO_2), 65.01 (C2), 50.55 (C4), 37.00 (C3), 33.06 (C1), 30.34 (C5), 21.78 (C6), 21.69 (CH_3). HRMS ESI (m/z): calcd.

for $C_{10}H_{15}N_2O_5$ $[M+H]^+$: 243.0975. Found: 243.0980. Anal. calcd. for $C_{10}H_{14}N_2O_5 \cdot 0.75H_2O$: C, H, N.

(1R,2S,4R,5R,6R)-2-Amino-4-(phenylthio)bicyclo[3.1.0]hexane-2,6-dicarboxylic acid, (18e). A mixture of **12c** (315 mg, 0.623 mmol, 1 equiv) in 50% aq. acetic acid (6.2 mL) was heated to 160 °C in a microwave for 7 min. The suspension was concentrated in vacuo. Water was added and removed in vacuo twice to remove excess AcOH. The solid was slurried in water and collected via vacuum filtration. The filter cake was washed with isopropyl alcohol and dried under vacuum to give zwitterion **18e** (143 mg, 78%) as a white solid. $[\alpha]_D^{20}$ 109.78 (c = 1.002, 0.1 N NaOH). MS (ES+) 294.2 $[M+H]^+$. ^{1}H NMR (400 MHz, D_2O/KOD, δ): 7.36 (d, J = 7.5 Hz, 1H), 7.21 (m, 3H), 3.89 (m, 1H), 2.13 (dd, J = 14, 7.9 Hz, 1H), 1.82 (m, 2H), 1.48 (m, 1H), 1.00 (m, 1H). ^{13}C NMR (125 MHz, D_2O/KOD, δ): 180.2 (CO_2), 179.4 (CO_2), 133.4 (Ph), 132.1(Ph), 129.2 (Ph), 127.6 (Ph), 66.09 (C2), 47.04 (C3), 39.36 (C4), 35.57 (C1), 31.82 (C5), 23.16 (C6). HRMS ESI (m/z): calcd. for $C_{14}H_{16}NO_4S$ $[M+H]^+$: 294.0795. Found: 294.0799. Anal. calcd. for $C_{14}H_{15}NO_4S \cdot 1.25H_2O$: C, H, N.

(1R,2S,4R,5R,6R)-2-Amino-4-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid, (18f). A mixture of **12f** (46 mg, 0.110 mmol, 1 equiv) in 50% aq. acetic acid (0.8 mL) was heated to 160 °C in a microwave for 5 min. The reaction was concentrated in vacuo. Water was added and removed in vacuo three times to give zwitterion **18f** (17 mg, 76%) as a white solid. $[\alpha]_D^{20}$ 18.00 (c = 1.000, 0.1 N NaOH). MS (ES+) 204.0 $[M+H]^+$. ^{1}H NMR (400 MHz, D_2O, δ): 5.53–5.34 (m, 1H), 2.36 (dd, J = 14, 8.0 Hz, 1H), 2.19 (m, 1H), 1.99 (m, 1H), 1.93 (t, J = 3.0 Hz, 1H), 1.47 (ddd, J = 22, 14, 7.0 Hz, 1H).

^{13}C NMR (100 MHz, D_2O/pyridine-d_5, δ): 177.8 (CO_2), 174.9 (CO_2), 93.65 (d, J = 144 Hz, C4), 64.50 (C2), 34.74 (d, J = 21 Hz, C3), 31.52 (d, J = 7 Hz, C1), 30.22 (d, J = 19 Hz, C5), 21.28 (C6). HRMS ESI (m/z): calcd. for $C_8H_{11}FNO_4$ $[M+H]^+$: 204.0667. Found: 204.0668. Anal. calcd. for $C_8H_{10}FNO_4$: C, H, N.

(1S,2S,4S,5R,6R)-2-Amino-4-hydroxybicyclo[3.1.0]hexane-2,6-dicarboxylic acid, (19a). A mixture of **10** (100 mg, 0.242 mmol, 1 equiv) in 50% aq. acetic acid (1.8 mL) was heated to 160 °C in a microwave for 5 min. The vessel was scratched with a glass rod and allowed to stand at rt overnight. The solid was collected by vacuum filtration washing with isopropyl alcohol. The solid was taken up in water and concentrated in vacuo three times to remove excess acetic acid providing zwitterion **19a** as a white powder (25 mg, 51%). $[\alpha]_D^{20}$ 66.16 (c = 0.701, 0.1 N NaOH). MS (ES-) 202.2 $[M+H]^+$. ^{1}H NMR (400 MHz, D_2O/pyridine-d_5, δ): 4.18 (d, J = 5.4 Hz, 1H), 2.03 (m, 1H), 1.96 (d, J = 16 Hz, 1H), 1.94 (s, 1H), 1.71 (dd, J = 15, 5.2 Hz, 1H), 1.43 (m, 1H). ^{13}C NMR (100 MHz, D_2O/pyridine-d_5, δ): 178.8 (CO_2), 176.0 (CO_2), 73.12 (C4), 66.41 (C2), 39.03 (C3), 33.93 (C1), 32.08 (C5), 23.54 (C6). HRMS ESI (m/z): calcd. for $C_8H_{12}NO_5$ $[M+H]^+$: 202.0710. Found: 202.0714 Anal. calcd. for $C_8H_{11}NO_5$: C, H, N.

(1S,2S,4S,5R,6S)-2-Amino-4-azidobicyclo[3.1.0]hexane-2,6-dicarboxylic acid, (19b). A mixture of **14a** (100 mg, 0.228 mmol, 1 equiv) in 50% aq. acetic acid (2.0 mL) was heated to 160 °C in a microwave for 6 min. The suspension was concentrated in vacuo. Water was added and removed in vacuo twice to remove excess AcOH. The material was slurried in IPA and collected via vacuum filtration. The filter cake was washed with isopropyl alcohol and dried under vacuum to give zwitterion **19b** (38 mg,

74%) as a white solid. MS (ES-) 227.0 $[M+H]^+$. ^{1}H NMR (400 MHz, D_2O/KOD, δ): 4.04 (d, J = 5.6 Hz, 1H), 2.07 (m, 1H), 2.02 (m, 1H), 1.78 (m, 1H), 1.35 (m, 2H). ^{13}C NMR (100 MHz, D_2O/pyridine-d_5, δ): 178.4, 174.7, 66.64, 62.14, 38.52, 31.93, 31.48, 24.08. HRMS ESI (m/z): calcd. for $C_8H_{11}N_4O_4$ $[M+H]^+$: 227.0775. Found: 227.0778. Anal. calcd. for $C_8H_{10}N_4O_4$: C, H, N.

(1S,2S,4S,5R,6S)-2,4-Diaminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid acetic acid salt, (19c). A mixture of **14c** (129 mg, 0.313 mmol, 1 equiv) in 50% aq. acetic acid (3.2 mL) was heated to 160 °C in a microwave for 6 min. The suspension was concentrated in vacuo. Water was added and removed in vacuo four times to remove excess AcOH. The solid was slurried in water and collected via vacuum filtration to give the acetate salt **19c** (62 mg, 99%) as a white solid. $[\alpha]_D^{20}$ -4.00 (c = 0.999, 0.1 N NaOH). MS (ES-) 201.2 $[M+H]^+$. ^{1}H NMR (400 MHz, D_2O, δ): 3.95 (d, J = 6.8 Hz, 1H), 2.18 (m, 2H), 2.10 (m, 1H), 1.91 (s, 3H), 1.88 (dd, J = 15, 6.8 Hz, 1H), 1.70 (m, 1H). ^{13}C NMR (100 MHz, D_2O/pyridine-d_5, δ): 177.5 (HOAc), 177.0 (CO_2), 174.9 (CO_2), 65.87 (C2), 52.68 (C4), 34.72 (C3), 32.67 (C1), 29.37 (C5), 23.21 (HOAc), 20.82 (C6). HRMS ESI (m/z): calcd. for $C_8H_{13}N_2O_4$ $[M+H]^+$: 201.0870. Found: 201.0875. Anal. calcd. for $C_8H_{12}N_2O_4 \cdot 0.9C_2H_4O_2$: C, H, N.

(1S,2S,4S,5R,6S)-4-Acetamido-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid, (19d). A mixture of **14d** (200 mg, 0.440 mmol, 1 equiv) in 50% aq. acetic acid (4.0 mL) was heated to 160 °C in a microwave for 6 min. The suspension was concentrated in vacuo. Water was added and removed in vacuo four times to give zwitterion **19d** (87 mg, 82%) as a white solid. $[\alpha]_D^{20}$ -46.08 (c = 0.998, 0.1 N NaOH). MS (ES-) 243.2

[M+H]$^+$. ^{1}H NMR (400 MHz, D_2O, δ): 4.42 (d, J = 7.4 Hz, 1H), 2.28 (m, 1H), 2.08 (m, 1H), 2.01 (d, J = 15 Hz, 1H), 1.86 (s, 3H), 1.83 (dd, J = 15, 7.4 Hz, 1H), 1.76 (t, J = 2.9 Hz, 1H). ^{13}C NMR (100 MHz, D_2O/pyridine-d_5, δ): 175.1 (CO_2), 174.8 (CO_2), 172.7 (CO), 66.35 (C2), 51.13 (C4), 37.39 (C3), 33.58 (C1), 33.21 (C5), 22.25 (CH_3), 21.13 (C6). HRMS ESI (m/z): calcd. for $C_{10}H_{15}N_2O_5$ [M+H]$^+$: 243.0975. Found: 243.0981. Anal. calcd. for $C_{10}H_{14}N_2O_5 \cdot 1.5H_2O$: C, H, N.

(1R,2S,4S,5R,6R)-2-Amino-4-(phenylthio)bicyclo[3.1.0]hexane-2,6-dicarboxylic acid, (19e). A mixture of **14b** (181 mg, 0.358 mmol, 1 equiv) in 50% aq. acetic acid (3.6 mL) was heated to 160 °C in a microwave for 7 min. The suspension was concentrated in vacuo. Water was added and removed in vacuo twice to remove excess AcOH. The solid was slurried in water and collected via vacuum filtration. The filter cake was washed with isopropyl alcohol and dried under vacuum to give zwitterion **19e** (85 mg, 81%) as a white solid. $[\alpha]_D^{20}$ -102.9 (c = 0.797, 0.1 N NaOH). MS (ES-) 294.0 [M+H]$^+$. ^{1}H NMR (400 MHz, D_2O/KOD, δ): 7.32 (d, J = 7.5 Hz, 2H), 7.24 (t, J = 7.5 Hz, 2H), 7.16 (t, J = 7.5 Hz, 1H), 3.75 (d, J = 7.0 Hz, 1H), 2.14 (d, J = 15 Hz, 1H), 2.10 (m, 1H), 1.82 (m, 1H), 1.56 (dd, J = 15, 7.0 Hz, 1H), 1.46 (m, 1H). ^{13}C NMR (100 MHz, D_2O/KOD, δ): 180.1 (CO_2), 179.0 (CO_2), 135.4 (Ph), 130.2 (Ph), 129.2 (Ph), 126.8 (Ph), 66.45 (C2), 48.17 (C3), 40.55 (C4), 33.71(C1), 32.63 (C5), 25.64 (C6). HRMS ESI (m/z): calcd. for $C_{14}H_{16}NO_4S$ [M+H]$^+$: 294.0795. Found: 294.0799. Purity >98% based on HPLC (Gemini-NX C18 110A, 50 X 2.00 mm column; 2 μL injection; 5-100% water/acetonitrile + 0.1% formic acid gradient over 7.0 min; flow rate = 1.0 mL/min; UV detection at 214 nm).

In Vitro Pharmacology: Compounds were solubilized in 0.1N NaOH as 10 mM stocks, stored at -20 °C, and serially diluted in assay buffer supplemented with 20 mM HEPES buffer at the start of each experiment. Inhibition of forskolin-stimulated cAMP production in recombinant mGlu2 and mGlu3 cells was assessed using HTRF: AV12 cells stably expressing rat glutamate transporter EAAT1 and mGlu2 or mGlu3. Twenty-four hours before the assay, cells were plated at a density of 8,000-10,000 cells per well (mGlu2) or 6,000-8,000 cells per well (mGlu3) in tissue culture treated, 96-well, half-area black plates and incubated in medium containing 250 μM (mGlu2) or 125 μM (mGlu3) of glutamine. Compounds were tested in 10-point concentration response curves using three-fold serial dilution. The final reaction mixture contained 1 μM forskolin (Sigma F6886) and up to 25 μM of compound. Reactions were incubated at 37 ºC for 20 minutes. Positive control = DCG-IV. After lysis, Cisbio™ detection reagents were incubated at room temp for 1 hour. The HTRF signal (ratio of fluorescence at 665 to 620 nM) was detected with an EnVision plate reader. Raw data were converted to pmole / well of cAMP with a cAMP standard curve generated for each experiment. Relative EC_{50} values were calculated from the top-bottom range of the concentration response curve using a four-parameter logistic curve fitting program (ActivityBase v5.3.1.22). The activity of test compounds for the other human mGlu receptors were assessed in either FLIPR or cAMP modes using methods analogous to those developed for mGlu2 and mGlu3. Antagonist assays (other than mGlu6) used glutamate as the agonist at a concentration that produced an EC_{90} response. Antagonist effects were quantified by calculating the % inhibition of the EC_{90} response. The mGlu6 cAMP assays used L-AP4 (Tocris) as the reference agonist. All data were

calculated as relative IC_{50} or EC_{50} values using a four-parameter logistic curve fitting program (ActivityBase v5.3.1.22).

Rat Pharmacokinetics: Compound **18f** was dissolved in water, adjusted to pH of 7-7.5 with NaOH, and administered to fasted male Sprague Dawley rats (approximately 250g, Harlan Industries) at dosages of 1, 3 and 10 mg/kg by oral gavage and intravenously at 3 mg/kg. Serial blood samples (11-12 samples per rat) were collected from a jugular vein catheter into EDTA tubes, centrifuged, and stored frozen until analyzed. Twenty-five µl aliquots of thawed plasma were mixed with an equal volume of methanol:water (1:1) containing an analog internal standard. The mixture was diluted with 300 µl water and added to a Waters MAX 10 mg SPE plate. The plate was washed with 300 µl water followed by 300 µl methanol and the analyte eluted with 400 µl methanol/formic acid (96:4). Extracts were concentrated, reconstituted in 50 µl of water, and analyzed by LC-MS/MS using two Shimadzu LC-20AD pumps (Kyoto, Japan), a Leap PAL autosampler (Carrboro, NC), and a Sciex API 4000 triple quadrupole mass spectrometer (Applied Biosystems/MDS; Foster City, CA) equipped with a TurboIonSpray interface, and operated in positive ion mode. Chromatographic separation was accomplished on a 2.1 x 50 mm, 5 µm BioBasic AX HPLC column (Thermo Scientific, Pittsburgh, PA) using a binary step gradient. The initial mobile phase system was composed of methanol/water, (40:60, v/v; mobile phase A), and glacial acetic acid/water (25:75, v/v; mobile phase B). The step gradient profile changed from 1% B for 0.2 minutes, 35% B at 0.30 minutes to 0.40 minutes, 60% B at 0.50 minutes, and returning to 1% B at 0.76 minutes. The flow rate was 1.0 mL/minute and the column was at ambient temperature, with flow directed

to the mass spectrometer between 0.27 and 0.5 min. The selected reaction monitoring $(M+H)^+$ transition was m/z 204.1 > 77.1. Pharmacokinetic parameters were calculated by noncompartmental analysis using Watson 7.4 (Thermo Fischer Scientific).

Rat PCP-locomotor assay: Male Sprague Dawley rats (250–300 g) were group-housed (maximum of seven rats per cage) under standard laboratory conditions with ad libitum access to food and water (12 h light/dark cycle). However, rats were fasted during the night prior to oral drug administration. Following a 30-min acclimation period, rats were orally administered test agent (1 ml/kg) or sterile water (1 ml/kg), and then returned to transparent, plastic shoe-box cages of dimensions 45 cm x 25 cm x 20 cm, with 1 cm depth of wood chips as bedding and a metal grill on top of the cage. After the designated time (1-3 hours), the rats were given a s.c. injection of PCP (Sigma Chemicals, St. Louis, MO, USA) or sterile water (1 ml/kg) and once again returned to the shoe-box cages for immediate locomotor activity monitoring over an additional 60 minutes. Motor monitors (Hamilton Kinder, San Diego, CA, USA) consisted of a rectangular rack of 12 photo beams arranged in an 8 x 4 formation. Shoe-box cages were placed inside this rack, enabling the activity of the rat to be monitored.

Rat Rotorod assay: The ROTOR-ROD™ System (San Diego Instruments, San Diego CA) was used to measure motor function in rats .The rotor rod apparatus is adjusted for a fall height (onto a foam padded platform), of 24 - 30 inches to utilize the 'fear of falling' instinct as a natural motivator. Rats (Harlan Sprague Dawley, Harlan Industries 175-250 grams) were trained to stay on the rotorod (4 rpm) for a period of one minute. Each animal was given four chances to pass, and those animals that failed were not retested. Rats that passed the first training test were then re-tested for one minute about 30 to 60

minutes later. The re-trained testing is pass fail only with failed rats excluded from study. Trained rats (8 animals / group) were then dosed with test compound or vehicle and re-tested on the rotorod 120 minutes later. Statistical significance was determined using a one way ANOVA analysis of variance with a post-hoc Dunnetts multiple comparison test.

Associated Content

Supporting Information: Elemental Analysis; functional responses at mGlu1, mGlu4, mGlu5, and mGlu7; and x-ray data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes: The authors declare no competing financial interest.

Abbreviations Used

ATD, amino terminal domain; C_0, plasma concentration after iv dosing extrapolated to *t* = 0; C_{max}, peak plasma concentration after oral dosing; CL, iv clearance; DAST, diethylaminosulfur trifluoride; HTRF, Homogenous time resolved fluorescence; mGlu, metabotropic glutamate; PCP, phencyclidine; *Si*-DMT, SiliaMetS® Dimercaptotriazine; Si-Triamine, SiliaMetS® Triamine; $V_{d\ ss}$, volume of distribution at steady state.

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