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Identification of Selective Norbornane-Type Aspartate Analogue Inhibitors of the Glutamate Transporter 1 (GLT-1) from the Chemical Universe Generated Database (GDB)

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A variety of conformationally constrained aspartate and glutamate analogues inhibit the glutamate transporter 1 (GLT-1, also known as EAAT2). To expand the search for such analogues, a virtual library of aliphatic aspartate and glutamate analogues was generated starting from the chemical universe database GDB-11, which contains 26.4 million possible molecules up to 11 atoms of C, N, O, F, resulting in 101026 aspartate analogues and 151285 glutamate analogues. Virtual screening was realized by high-throughput docking to the glutamate binding site of the glutamate transporter homologue from *Pyrococcus horikoshii* (PDB code: 1XFH) using Autodock. Norbornane-type aspartate analogues were selected from the top-scoring virtual hits and synthesized. Testing and optimization led to the identification of (1*R**,2*R**,3*S**,4*R**,6*R**)-2-amino-6-phenethyl-bicyclo[2.2.1]heptane-2,3-dicarboxylic acid as a new inhibitor of GLT-1 with IC_{50} = 1.4 μM against GLT-1 and no inhibition of the related transporter EAAC1. The systematic diversification of known ligands by enumeration with help of GDB followed by virtual screening, synthesis, and testing as exemplified here provides a general strategy for drug discovery.

Introduction

Small molecule drug discovery forms an essential part of modern medicine.[1] New discoveries are however increasingly difficult, in part because a very large number of small molecules have already been described in the scientific and patent literature. Recently, we reported the chemical universe generated database up to 11 atoms (GDB-11[a]) and up to 13 atoms (GDB-13), enumerating all organic molecules virtually possible under simple chemical stability and synthetic feasibility rules.[2−4] The databases contain at least 1000-fold more structures than data sets of known molecules of similar size and thus provide a very large reservoir of yet unexploited chemical diversity for drug discovery in the context of de novo drug design approaches.[5,6]

In our effort to demonstrate the use of GDB for identifying new drugs,[7−9] we became interested in the case of aspartate and glutamate analogues as ligands of various targets in the central nervous system (CNS). Glutamate is the major excitatory neurotransmitter in the mammalian CNS and is linked to higher brain functions such as memory and learning. Concomitantly, glutamate acts as an excitotoxin at higher extracellular concentrations triggering neuronal cell death. One family of proteins that glutamate and aspartate analogues act on are Na-dependent high-affinity glutamate transporters. These transporters, which exist in five different subtypes, remove glutamate from the synaptic cleft to terminate signal transmission and to prevent neurotoxic effects.[10,11] Dysfunction of glutamate transporters has been identified in a number of neurodegenerative diseases (e.g., Alzheimer's disease, amyotrophic lateral sclerosis), ischemic stroke injury, or epilepsy, which makes them interesting targets for biology and medicine.[11−13] There is a need for more, especially subtype-selective ligands of glutamate transporters, which might serve for further elucidation of their physiological roles and eventually as therapeutics.[11,14,15] Various medicinal chemistry programs have identified analogues of aspartate and glutamate as favorable structures for binding the glutamate transporter 1 (GLT-1), such as **1** (*trans*-2,3-PDC),[16] **2** (3-Me-L-2,3-PDC),[17] **3** (L-CCG-III),[18] **4** (L-CBG-IV),[19] **5** (L-3,4-MPDC),[20] **6** (azabicyclo-ODD),[21] and **7** (WAY-855)[22] (Figure 1).

Herein we report the identification of inhibitors of GLT-1 by virtual screening of a library of aspartate and glutamate analogues derived from GDB-11, followed by synthesis, testing, and optimization of selected hits. The study led to a new class of norbornane-type aspartate analogues, including in particular *rac*-**23a** and its optimized derivative *rac*-**25a**, which exhibit enhanced selectivity between GLT-1 and the excitatory amino acid carrier 1 (EAAC1) compared to previously known ligands.

Results and Discussion

Virtual Screening. We set out to explore the chemical space of glutamate and aspartate analogues for new ligands by exhaustive enumeration from the chemical universe database GDB, followed by high-throughput virtual screening, synthesis,

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[a] Abbreviations: GDB: generated database; GLT-1: glutamate transporter 1; EAAC1: excitatory amino acid carrier 1.

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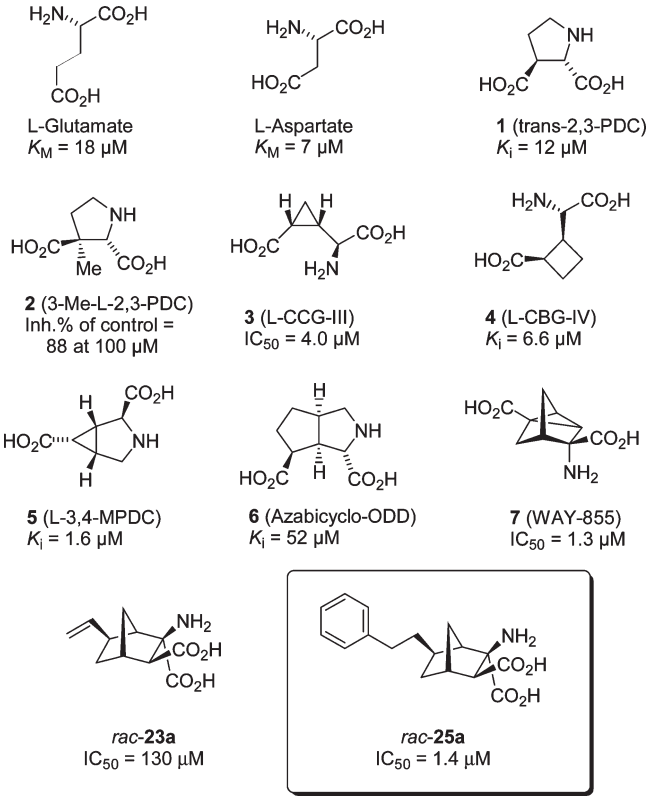


Figure 1. Native ligands L-glutamate and L-aspartate[23] and ligands of glutamate transporters. The inhibitory activities at GLT-1 are indicated.

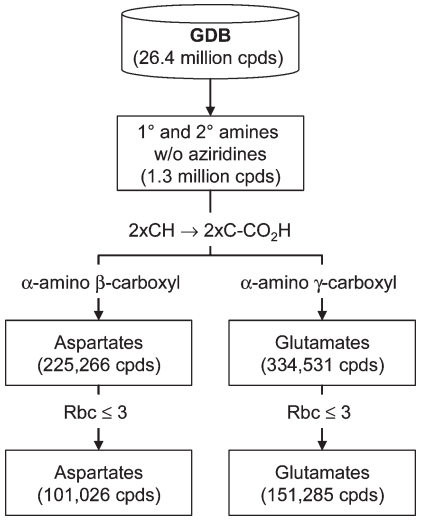


Figure 2. Construction of aspartate and glutamate libraries from the chemical universe database GDB. See Supporting Information Table S1 and Figure S1 for details on the database composition.

and testing. The enumeration was realized from GDB-11,[24] which contains 26.4 million possible molecules up to 11 atoms of C, N, O, and F. Two virtual libraries of aspartate and glutamate analogues were constructed from GDB-11 as shown in Figure 2. Thus, the subset of 1.3 million primary and secondary monoamines ($C_xH_yNH_z$, $x \leq 10$, $z = 1$ or 2, excluding aziridines) was extracted and expanded by replacing all possible pairs of nongeminal hydrogens on carbons by a pair of carboxyl groups, resulting in a library of 13.7 million aminodicarboxylic acids. The aspartate analogues were obtained by considering only

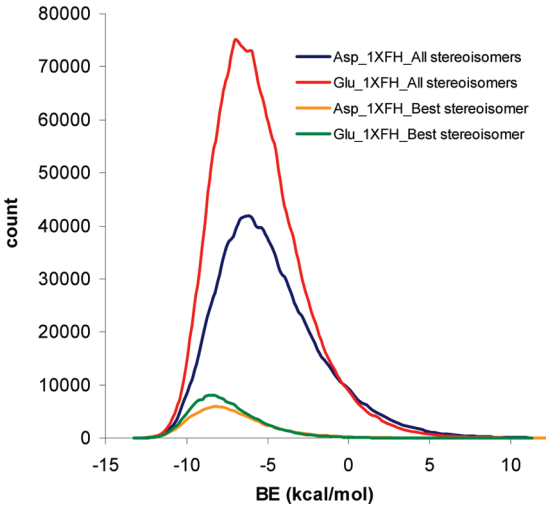
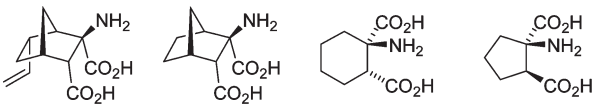


Figure 3. Distribution of estimated binding energies of glutamate and aspartate analogues. Docking studies were performed with Autodock on the crystal structure of the glutamate transporter homologue from *Pyrococcus horikoshii* 1XFH.

α-amino acids with a β-carboxyl group, corresponding to 225266 structures. Similarly, the glutamate analogues were obtained by considering all α-amino acids with a γ-carboxyl group, resulting in 334531 structures. These virtual libraries were further reduced to conformationally constrained structures (rotatable bond count Rbc ≤3), leaving 101026 aspartate analogues and 151285 glutamate analogues (Figure 2, Supporting Information Table S1, Figure S1).

Virtual screening of the analogue libraries was realized by high-throughput docking to the glutamate binding site of the glutamate transporter homologue from *Pyrococcus horikoshii*, for which a crystal structure has recently been reported with an electron density compatible with bound glutamate at the active site (1XFH).[25] This bacterial homologue shares 37% amino acid sequence identity with the human transporter GLT-1. The libraries were expanded to 3D using CORINA,[26] resulting in 810702 (aspartates) and 1253354 (glutamates) stereoisomers, which were docked using Autodock 3.0.5.[27] This docking program is suitable for proper positioning of ligands as well as for delivering good estimates of binding energies for various ligands.[28] Docking positioned the natural ligand glutamate in a pose compatible with the crystallographically observed electron density, and with good estimated binding free energy score (BE = −9.10 kcal/mol). For each molecule docked, the lowest estimated binding energy for 15 docking attempts was taken as the reference value. Gaussian curves were observed for the distribution of binding energies (Figure 3). The known GLT-1 ligands **1**−**7** (Figure 1) had estimated binding energies in the upper 6% of all docked stereoisomers, with **4** and **7** being even among the top 0.54% of all stereoisomers.

The best scoring ligands of each library were inspected visually to select compounds for synthesis. The top-scoring glutamate analogues were tri- and tetracyclic structures similar to **7** which were judged too challenging for synthesis. The aspartate library by contrast contained many bicyclic structures clearly amenable to chemical synthesis. We focused our attention on norbornane-type aspartate analogues, including the best docking and yet unknown aspartate analogue **8** (rank 1, BE = −13.34 kcal/mol) and the parent norbornane lacking the vinyl appendage **9d** (rank 19, BE = −12.52 kcal/mol), for



8 (rank 1) -13.34 kcal/mol | **9d** (rank 19) -12.52 kcal/mol | **10** (rank 4888) -10.46 kcal/mol | **11** (rank 5176) -10.42 kcal/mol

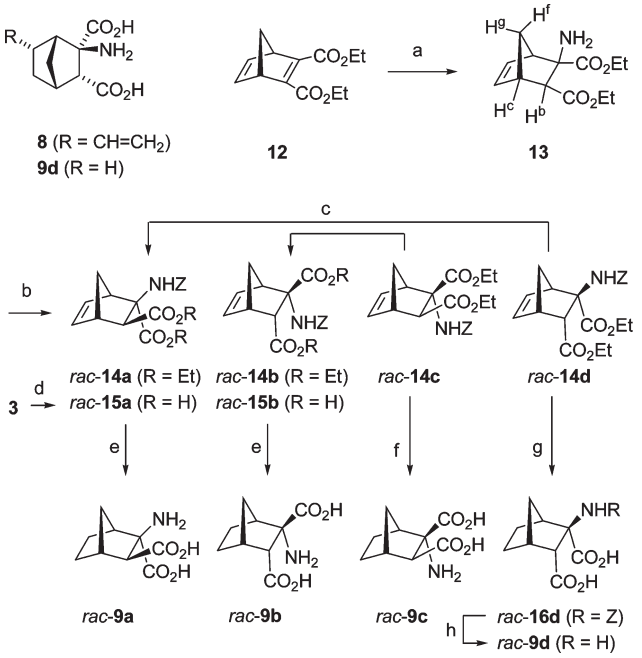
Figure 4. Structure of selected virtual hits. The binding energy estimated by Autodock to the glutamate binding site in GLT-1 is indicated in kcal/mol.

which only two of the eight stereoisomers had been described previously[29,30] (Figure 4). The simplified monocyclic analogues **10** (rank 5176, BE = −10.42 kcal/mol) and **11** (rank 4888, BE = −10.46 kcal/mol) were also considered because their synthesis has already been described[31−33] in connection with biological assays for vesicular glutamate transporters,[34] tumor-growth inhibition (cyclopentane derivative),[32,35] herpes simplex virus ribonucleotide reductase inhibition,[33] and uptake in tumor cells (cyclohexane derivative).[36] Their activity on EAATs had not been determined but 1*S*,2*R*-amino-cyclopentanedicarboxylic acid is an agonist of metabotropic glutamate receptors.[37]

Synthesis. The norbornane aspartate analogue **9** was prepared starting with the Diels−Alder reaction of cyclopentadiene with diethyl acetylene dicarboxylate to provide **12**.[38] The crude norbornadiene **12** was subjected to Michael addition of ammonia by heating in a saturated ethanolic solution of ammonium acetate to give **13** as a mixture of diastereoisomers, which were separated by column chromatography as the benzyloxycarbonyl (*Z*) protected derivatives **14a**−**d** (Scheme 1). The relative stereochemistry was assigned by 2D-NMR spectroscopy (COSY, ROESY). An NOE-effect between H^b and H^f indicated the *exo*-position of H^b in *rac*-**14b** and *rac*-**14d**. On the other hand, the *endo*-position of H^b in *rac*-**14a** and *rac*-**14c** was indicated by a strong W-coupling between H^b and H^g in COSY-NMR and no NOE-effect between H^b and H^f. Additionally, the $^3J(H^b, H^c)$ coupling constants of the *rac*-**14b** and *rac*-**14d** diastereoisomers displaying H^b in the *exo*-position (both 3.21 Hz) were larger than that of the H^b-*endo* diastereoisomers *rac*-**14a** (1.5 Hz) and *rac*-**14c** (1.95 Hz), consistent with the Karplus-relation predicting that $^3J(H^b, H^c)$ with H^b in *exo*-position is larger than $^3J(H^b, H^c)$ with H^b in the *endo*-position because the dihedral angle for the latter is closer to 90°.

Partial epimerization of the ester function of *rac*-**14** was observed under basic conditions and allowed the pairwise assignment of diastereoisomers possessing the same relative configuration at the amino acid α-carbon atom as *rac*-**14a**/*rac*-**14d** and *rac*-**14b**/*rac*-**14c**. Complete epimerization was observed upon hydrolysis of the diastereoisomeric mixture *rac*-**14** with LiOH in THF/MeOH/water at 70 °C for 24 h to afford the *Z*-protected *trans*-dicarboxylates *rac*-**15a** and *rac*-**15b**. The products were separated by preparative RP-HPLC and subjected to hydrogenolysis to provide the free amino acids *rac*-**9a** and *rac*-**9b**. Hydrogenation of the *cis*-dicarboxylic ester *rac*-**14c** followed by acidic hydrolysis in 3 N HCl under reflux gave the *cis*-dicarboxylic amino acid *rac*-**9c** in good yields without any epimerization. The same sequence applied to *rac*-**14d** however resulted in a mixture of *cis*- and *trans*-dicarboxylates. Therefore *rac*-**14d** was reprotected to the benzyl carbamate *rac*-**16d**, separated from its *trans*-dicarboxylate diastereoisomer *rac*-**16a** by preparative RP-HPLC, and subjected to hydrogenation to yield *rac*-**9d**.

Scheme 1. Structure of **8** and **9d** and Synthesis of All Diastereoisomers *rac*-**9a**−**d**[a]



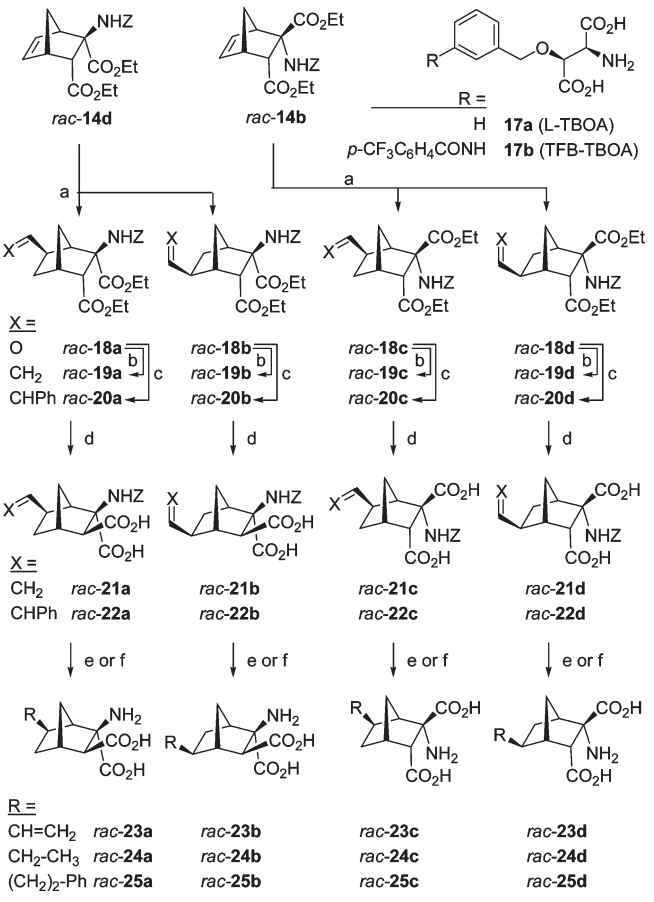
[a] Conditions: (a) NH_4OAc, EtOH, 80 °C, 18 h, then (b) BnOCOCl, Na_2CO_3, CH_2Cl_2/H_2O (1/1), rt, 12 h (43% over 3 steps from cyclopentadiene and diethyl acetylene dicarboxylate; **14a**, 10%; **14b**, 4%; **14c**, 10%; **14d**, 19%); (c) Na, EtOH, rt, 40 h; (d) LiOH, THF/MeOH/H_2O, 70 °C, 24 h, then separation/purification by RP-HPLC (*rac*-**15a**, 47%; *rac*-**15b**, 64%); (e) H_2, Pd/C, MeOH, rt, 90 min (*rac*-**9a**, 89%; *rac*-**9b**, 84%); (f) H_2, Pd/C, MeOH, rt, 90 min, then 3 N HCl, reflux, 30 h (88%); (g) H_2, Pd/C, MeOH, rt, 90 min, then 6 N HCl, reflux, 3 days, then BnOCOCl, NaOH, H_2O, 0 °C → rt, 5 h, then separation/purification by RP-HPLC (3%); (h) H_2, Pd/C, MeOH, rt, 90 min (quant). All compounds were prepared as racemates.

The assignment of the relative configuration of the amino acid in this series was made possible by the crystal structure determination of *rac*-**16d** (Scheme 1).

Because of the strong tendency of the *cis*-dicarboxylate diastereoisomers to epimerize to the *trans*-dicarboxylate under a variety of conditions, the synthesis of **8** was envisioned only for the case of the more stable *trans*-dicarboxylate diastereoisomers (Scheme 2). The synthesis started from *rac*-**14d** and *rac*-**14b**, which were most easily obtained in pure form as the first and last eluting fraction during the chromatographic separation. Hydroformylation under 40 bar of syngas and unmodified $Rh(CO)_2$acac as catalyst at 60 °C in toluene[39] gave exclusively the *exo*-formylated products *rac*-**18a**,**b** and *rac*-**18c**,**d**, a selectivity well documented for similar cases.[39,40] These aldehydes proved rather unstable and formed insoluble precipitates when kept in solution at room temperature or upon storage in pure form at −18 °C, presumably through the formation of cyclic trioxanes,[31] although their formation could not be confirmed. All attempts to epimerize the aldehyde to the corresponding *endo*-diastereoisomer in any of *rac*-**18a**−**d** under basic conditions resulted in slow decomposition.

Wittig olefination of *rac*-**18a**−**d** with triphenyl methylene phosphorane gave the corresponding vinyl products *rac*-**19a**−**d**. Ester hydrolysis, accompanied by epimerization to the *trans*-dicarboxylate in the case of *rac*-**19a**,**b**, provided the *Z*-protected diacids *rac*-**21a**−**d**, which were finally deprotected by acidic hydrolysis to yield amino acids *rac*-**23a**−**d**

Scheme 2. Synthesis of *rac*-**23a**−**d** (Diastereoisomers of **8** with *exo*-Vinyl Substituent) and Analogues, and Structure of the Known GLT-1 Inhibitors **17a**−**b**[a]

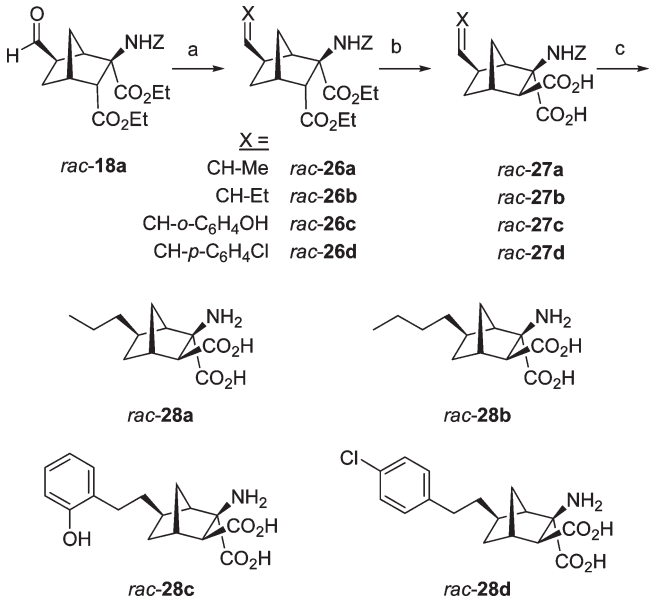


[a] Conditions: (a) CO/H_2 (1/1), $Rh(CO)_2acac$, toluene, 60 °C, 24 h (*rac*-**18a**, 35%; *rac*-**18b**, 30%; *rac*-**18c**, 30%; *rac*-**18d**, 50%); (b) $MePPh_3Br$, *n*BuLi, THF, −18 °C, 70−90 min (*rac*-**19a**, 72%; *rac*-**19b**, 64%; *rac*-**19c**, 43%; *rac*-**19d**, 65%); (c) $BnPPh_3Cl$, *n*BuLi, THF, −18 °C, 60−90 min (*rac*-**20a**, quant; *rac*-**20b**, 70%; *rac*-**20c**, 52%; *rac*-**20d**, 72%); (d) LiOH, THF/MeOH/H_2O, 70 °C, 48 h (*rac*-**21a**, 59%; *rac*-**21b**, 41%; *rac*-**21c**, 67%; *rac*-**21d**, 64%; *rac*-**22a**, 43%; *rac*-**22b**, 45%; *rac*-**22c**, 68%; *rac*-**22d**, 57%); (e) AcOH/conc HCl (4/1), 80 °C, 2 h (*rac*-**23a**, 60%; *rac*-**23b**, 65%; *rac*-**23c**, 56%; *rac*-**23d**, 62%); (f) H_2, Pd/C, MeOH, rt, 90 min (*rac*-**24a**−**d**, quant; *rac*-**25a**, 44%; *rac*-**25b**, 75%; *rac*-**25c**, 46%; *rac*-**25d**, quant).

after preparative RP-HPLC purification. Alternatively, hydrogenation of *rac*-**21a**−**d** provided the corresponding saturated ethyl analogues *rac*-**24a**−**d**. With the aim of probing substitution of the vinyl group for activity improvements in analogy to the known benzyl-sustituted GLT-1 inhibitor **17a** (L-TBOA),[41] olefination of *rac*-**18a**−**d** with benzylidene triphenyl phosphorane yielded *rac*-**20a**−**d** as *E*/*Z* mixtures and finally the phenethyl substituted norbornanes *rac*-**25a**−**d** upon hydrogenation. The assignment of the position and *exo*-configuration of the aldehyde group and its derivatives in the different products was obtained from COSY-NMR data and confirmed by the X-ray crystal structure of *rac*-**21b**.

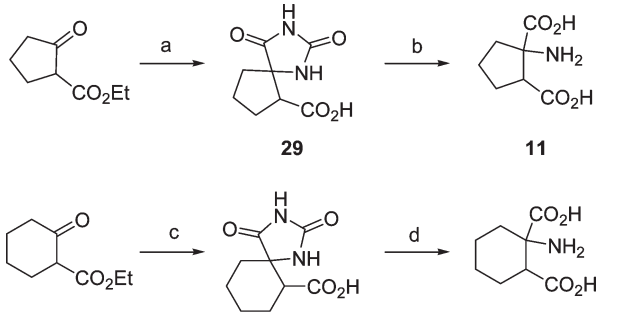
In view of the higher GLT-1 inhibitory activity of *rac*-**9a**, *rac*-**23a**, and in particular *rac*-**25a**, indicating favorable effects of added substituents on inhibition in these diastereoisomers (see below), additional derivatives were prepared for this diastereoisomer only (Scheme 3). Thus Wittig olefination of *rac*-**18a** with triphenyl (ethylidene)phosphorane,

Scheme 3. Synthesis of Additional Analogues of *rac*-**23a**[a]



[a] Conditions: (a) $EtPPh_3Br$ (*rac*-**26a**), $nPrPPh_3Br$ (*rac*-**26b**), (2-hydroxybenzyl)triphenylphosphonium bromide (*rac*-**26c**) or (4-chlorobenzyl)-triphenylphosphonium chloride (*rac*-**26d**), *n*BuLi, THF, −18 °C, 15 min (*rac*-**26a**, 88%; *rac*-**26c**, 76%; *rac*-**26d**, 51%); (b) LiOH, THF/MeOH/H_2O, 70 °C, 48 h (*rac*-**27a**, 46%; *rac*-**27b**, 39% over 2 steps; *rac*-**27d**, 43%); (c) H_2, Pd/C, MeOH, rt, 90 min, (*rac*-**28a**, 96%; *rac*-**28b**, quant; *rac*-**28c**, 27% over 2 steps) or H_2, Pt/C, EtOH, rt, 4 h, then AcOH/HCl (2/1), 80 °C, 90 min (*rac*-**28d**, 67%).

Scheme 4. Synthesis of Monocyclic Aspartate Analogues **10** and **11**[a]



[a] Conditions: (a) (i) KCN, $(NH_4)_2CO_3$, EtOH/H_2O (1/1), 60 °C, 28 h, (ii) 4 N HCl, 40 °C, 24 h; (b) 130 °C, 5 h, sealed tube (19% overall, dr: 58/42); (c) (i) $(NH_4)_2CO_3$, KCN, EtOH/H_2O (1/1), 50 °C, 8 h, (ii) 4 N HCl, 50 °C, 2 d; (d) 6 N HCl, reflux, 7 days (31% overall, dr: 81/19).

triphenyl (propylidene)phosphorane, triphenyl (2-hydroxybenzylidene)phosphorane, and triphenyl (4-chlorobenzylidene)phosphorane gave intermediates *rac*-**26a**−**d** as *E*/*Z* mixtures, respectively. Ester hydrolysis under basic conditions accompanied by epimerization to the *trans*-dicarboxylates then gave *rac*-**27a**−**d**, which were finally subjected to hydrogenation to yield the free amino acids *rac*-**28a**−**d**. The structures were confirmed by the crystal structure determination of *rac*-*E*-**26c**.

The monocyclic aspartate analogues **10** and **11** were prepared as mixtures of diastereoisomers by a simple two-step procedure (Scheme 4). Bucherer−Bergs reaction of 2-oxocyclopentane carboxylic acid ethyl ester, followed by acidic ester hydrolysis in 4 N HCl at 40 °C, gave hydantoin **29**

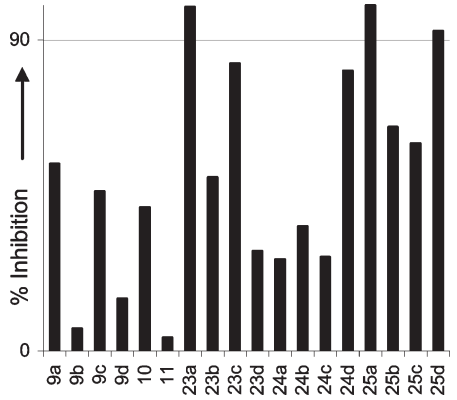


Figure 5. Inhibition of [^{3}H]-glutamate uptake in GLT-1-transfected HEK293 cells measured at 500 μM ligand concentration. GLT-1-HEK293 cells were incubated for 10 min at room temperature in HBSS buffer containing 40 nM [^{3}H]-glutamate and 10 μM cold glutamate. % indicates the inhibition of glutamate uptake compared to control uptakes without compounds.

as a nonseparable mixture of diastereoisomers, which was treated with 6 N HCl at 130 °C for 5 h and purified by anion-exchange chromatography to yield **11**. Similarly, the Bucherer–Bergs reaction with 2-oxocyclohexane carboxylic acid ethyl ester and acidic hydrolysis provided hydantoin **30**, which was further processed to amino acid **10**.

Inhibition of Glutamate Transporters. The inhibitory potential of the aspartate analogues *rac*-**9a**–**d**, *rac*-**23a**–**d**, *rac*-**24a**–**d**, *rac*-**25a**–**d**, **10**, and **11** was first tested at 500 μM ligand concentration for inhibition of [^{3}H]-glutamate uptake in GLT-1-transfected HEK293 cells or oocytes (Figure 5).[42] The uptake of [^{3}H]-glutamate was measured in presence and absence of ligand and the activities are given in percent inhibition, corresponding to the percentage of glutamate blocked from being transported into the cell compared to the control measurement without ligand. In the case of the unsubstituted norbornane aspartates *rac*-**9a**–**d**, significant inhibition was observed with *rac*-**9a** (*exo*-amino *trans*-dicarboxylate) and *rac*-**9c** (*endo*-amino *cis*-dicarboxylate). The higher epimerization tendency of *cis*-dicarboxylate diastereoisomers and the higher activity of the *exo*-amino *trans*-dicarboxylate *rac*-**9a** led to the choice to investigate further analogues in the latter case only, which also seemed reasonable considering the *trans*-stereochemistry of other known aspartate analogue inhibitors of GLT-1 (Figure 1). The most active stereoisomer in the vinyl-substituted norbornanes *rac*-**23a**–**d** was again the *exo*-amino *trans*-dicarboxylate, ligand *rac*-**23a**, which carried the vinyl substituent *meta* to the amino acid function similarly to the second most active ligand in this series *rac*-**23c**. A similar activity pattern was observed in the benzyl appended analogues *rac*-**25a**–**d**, which showed 93% inhibition as an equimolar mixture of diastereoisomers. The *rac*-**25a** diastereoisomer showed essentially complete inhibition of glutamate uptake. The ethyl substituted series *rac*-**24a**–**d** behaved differently, with *rac*-**24d** showing the strongest activity in this assay. In the monocyclic analogues, only the cyclohexane compound **10** showed significant inhibition, while the cyclopentane compound **11** was inactive.

A detailed characterization was carried out by determination of the IC_{50} values for the three ligands showing over 90% inhibition at 500 μM, *rac*-**23a**, *rac*-**25a**, and *rac*-**25d**. The study was extended to analogues *rac*-**28a**–**d** bearing

Table 1. IC_{50} Values for Inhibition of [^{3}H]-Glutamate Uptake by GLT-1 and EAAC1 Expressed in *Xenopus laevis* Oocytes

	IC_{50} (GLT-1) (μM)	IC_{50} (EAAC1) (μM)
rac-**23a** (vinyl)	130 ± 70	no inhibition
rac-**25a** (phenethyl)	1.4 ± 0.7	no inhibition
rac-**25d** (phenethyl)	19 ± 5	no inhibition
rac-**28a** (propyl)	25 ± 3	no inhibition
rac-**28b** (butyl)	14 ± 8	nd
rac-**28c** (*o*-HOPh)	21 ± 11	no inhibition
rac-**28d** (*p*-ClPh)	17 ± 11	no inhibition
7[22]	1.3	53
17a[41]	3.8	7.0
17b[43]	0.017	0.3

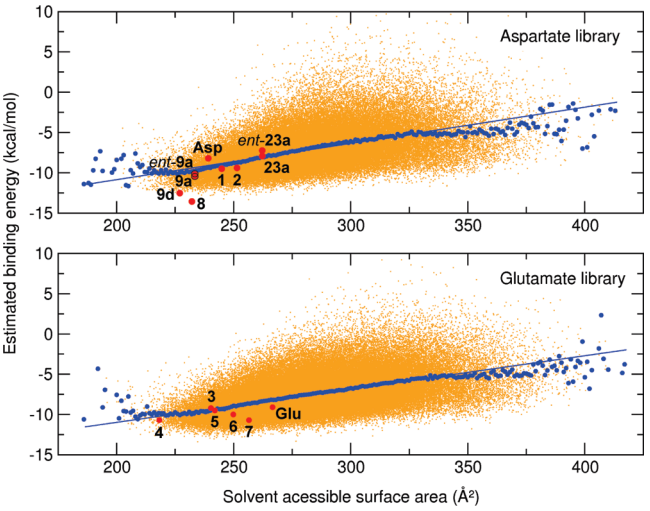


Figure 6. Estimated binding energy by Autodock as a function of the calculated solvent accessible surface area (sasa). Orange dots: all best-docking stereoisomers from the libraries of virtual aspartate respectively glutamate analogues. Blue line: linear fits for the orange dots, for aspartates: $y = -19.86 + 0.045x$, $r^2 = 0.24$, for glutamates: $y = -19.25 + 0.041x$, $r^2 = 0.26$. Blue dots: average estimated binding energy as a function of sasa. Red dots: values for selected ligands (structures in Figure 1 and 4, *ent*-**9a** is the enantiomer of **9a**, *ent*-**23a** is the enantiomer of **23a**).

various *exo*-alkyl substituents (Scheme 3) and the known GLT-1 inhibitor **17a** (Scheme 2) as a positive control. The values were measured for inhibition of [^{3}H]-glutamate uptake by GLT-1 and EAAC1 expressed in *Xenopus laevis* oocytes. The results confirmed the initial activity assay with the exception of the vinyl substituted derivative *rac*-**23a**, which had much weaker activity. The compounds showed inhibition in the micromolar range, with the phenethyl derivative *rac*-**25a** confirming its strong activity with an IC_{50} = 1.4 μM (Table 1). This value is comparable to the values reported for the polycyclic amino acid **7** and for **17a**, which contains a phenyl group like *rac*-**25a**. Interestingly, all of the norbornane inhibitors showed complete selectivity toward GLT-1 and no measurable inhibition of EAAC1, while the positive control **17a** inhibited both GLT-1 and EAAC1. It should be noted that the **17a** analogue **17b** (TFB-TBOA),[43] in which the position 3 of the benzyl group bears a *p*-(trifluoromethyl)benzamido group, is 100-fold more potent than **17a** but also significantly cross-reacts with EAAC1.

Molecular Modeling. The libraries of virtual aspartate and glutamate analogues derived from GDB provided an essential source of molecular diversity from which the norbornane aspartic acid skeleton was suggested by docking as a possible

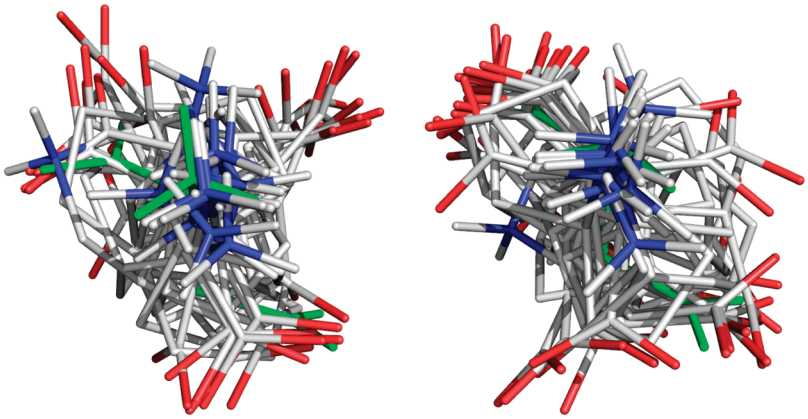


Figure 7. Lowest energy poses predicted by Autodock in the glutamate binding pocket of 1XFH (left) and the corresponding aspartate binding pocket of 2NWL (right) shown from the same viewpoint for all compounds from Figure 1 and all diastereoisomers of the norbornane aspartate **9**. Aspartate is shown in green. The α-amino acid carboxyl group is at upper left.

lead structure. Docking was used for ranking because the scores consistently placed the known aspartate and glutamate analogue inhibitors among the top-scoring structures. However, in the case of **9** where all diastereisomers were tested, the experimentally tested inhibitory activity on GLT-1 was not found in the virtual hit itself (*rac*-**9d**) but rather in its diastereoisomers *rac*-**9a** and *rac*-**9c**. A reanalysis of the docking results was therefore carried out to better understand the role played by docking in the compound selection process, including a comparison of docking results with the more recently reported crystal structures of the glutamate transporter homologue from *Pyrococcus horikoshii* 2NWL and 2NWW,[44] which contain aspartate, respectively, the inhibitor **17a** at the glutamate binding site.

One striking feature of the glutamate binding site in both 1XFH and 2NWL is that the binding pocket is quite small. While there was no significant correlation with the molecular weight or the number of cycles in the molecules, the docking scores of the virtual aspartate and glutamate analogue library in 1XFH were strongly influenced by the solvent accessible surface area (sasa), which measures the overall compactness of the structure (Figure 6). This measure was also consistent with the high ranking obtained for the known reference ligands. This suggests that the docking scores guided compound selection mainly toward structurally compact analogues.

Analysis of the docking poses of the various aspartate and glutamate analogues within the glutamate binding site in 1XFH showed a conserved pattern for the position of the α-amino carboxylic acid, while the side chain carboxyl group occupied one of two possible positions (Figure 7). A similar pattern was observed for redocking into 2NWL, with the pose of aspartate corresponding to its crystallographic position. These positions were compatible with a variety of backbone stereochemistries such as the various diastereoisomers of the norbornane aspartate **9**. It is therefore hardly surprising that the observed stronger inhibition of the *trans*-dicarboxylic ligands *rac*-**9a** and *rac*-**9c** was not predicted by docking of **9** to 1XFH. Although in the case of 2NWL the strongest docking diastereoisomer of **9** indeed corresponded to one of the enantiomers of the most active diastereoisomer *rac*-**9c**, the analysis of stereochemical preferences within such a tight binding pocket probably goes beyond the predicting capability of docking, in particular considering that modeling was performed on the structure of glutamate receptor analogues and not the actual GLT-1.

The increased potency of the phenethyl substituted ligand *rac*-**25a** compared to the nonsubstituted analogues is probably due to hydrophobic contacts between the benzyl group and the protein. This increased binding could not be rationalized by docking because the ligand did not dock properly into 1XFH, 2NWL, or even 2NWW, which contains a binding site occupied by the benzylether reference **17a**. In the latter case, even **17a** itself did not redock in its crystallographic position using either Autodock or Glide. These programs also failed to produce satifactory docking poses for the various *exo*-substituted norbornane aspartates (**23a**−**d**, **24a**−**d**, **25a**−**d**, **28a**−**d**), which either reflects significant structural differences between these proteins and GLT-1 or indicates induced-fit upon binding. It is therefore apparent that exploratory synthetic chemistry played an essential role in the optimization process once the initial norbornane aspartate hits had been identified.

Conclusion

In summary, the experiments above demonstrate the use of the chemical universe database GDB for exploring systematically the small molecule chemical space for drug discovery in the case of aspartate and glutamate analogues. Virtual screening by high-throughput docking identified norbornane aspartates among the highest scoring hits. Synthesis and testing led to the identification of the new GLT-1 inhibitors *rac*-**9a** and *rac*-**23a** and, through exploratory chemical synthesis, the optimized analogue *rac*-**25a**, which compares favorably with previously known GLT-1 inhibitors in terms of activity and selectivity. Further activity optimization might include the synthesis of pure enantiomers and more extended variations of the aromatic group in *rac*-**25a** because these may allow to increase the affinity.

The systematic diversification of known ligands by enumeration with help of GDB followed by synthesis and testing as exemplified here provides a general strategy for drug discovery. Similarly to our recent report on scaffold enumeration from GDB to discover α7-nicotinic receptor ligands,[9] the present library design preserved the pharmacophore of the reference ligands while varying only the carbon scaffold. This approach produced a focused library with a strong bias toward the desired activity, thus increasing the probability of a reward from the synthetic resources invested independent of the quality of the scoring function used. This conservative

design, although limiting the exploratory chemistry to relatively modest variations of the reference ligands, also allowed us to capitalize on well-established synthetic methods for amino acids for the synthetic part of the study.

In the perspective of using GDB as a source for drug discovery, one can speculate that more general searches of the entire GDB, or of a biogenically biased subset of GDB,[45] might lead to innovative bioactive chemotypes otherwise difficult to discover. Such general searches will require enrichment schemes with less constraining ligand-based filters than those used here, combined with reliable scoring functions compatible with the size of GDB (millions to billions of structures) and larger laboratory resources to tackle more diverse and probably more challenging syntheses.

Experimental Section

Database Generation. The aspartate and glutamate analogues libraries were constructed by selecting and modifying GDB molecules (available from www.gdb.unibe.ch) using the ChemAxon JChem package.

Docking Studies. The protonation state of all the compounds was set at pH = 7.4, and the compounds were expanded to 3D structures using CORINA. AutoDock Tools (ADT3) were applied to prepare and parametrize the receptor protein and ligands in batch mode. All structures were then docked into the target receptor protein using a set of precalculated AutoDock grid maps of interactions between each atom type of the ligands and each atom type of amino acid residues in the binding pocket. Fifteen Larmackian genetic algorithm runs were set to output 15 best docking conformations and 15 best estimated binding energies for each structure. The most favorable docking conformation and its estimated binding energy value was taken to be the final docking result for that input structure.

Synthesis. All reagents were obtained from commercial sources (Sigma-Aldrich, Fluka, Acros Organics) and used without further purification. For reactions, HPLC grade solvents or dry solvents (obtained from a solvent drying system) were used. Solvents used for extraction and flash chromatography were distilled from technical quality. Milli-Q water was used for reactions, ion-exchange chromatography, and crystallization, and deionized water was used for extractions. Sensitive reactions were carried out under argon. Flash chromatography was performed on silica gel (Fluka) with a particle size of 0.04−0.063 mm if not otherwise noted. For thin-layer chromatography (TLC), Macherey-Nagel SIL G-25 UV_{254} precoated plates were used. Preparative RP-HPLC was performed with a Waters Delta Prep 4000 system with a Waters Prepak cartridge (500 g) as column and Waters 486 tunable absorbance detector. Eluents were: A, Milli-Q water with 0.1% TFA; B, Milli-Q water/acetonitrile (40/60) with 0.1% TFA; C, Milli-Q water/acetonitrile (10/90) with 0.1% TFA. NMR experiments were conducted on Bruker AC 300 or Bruker DRX 400 instruments. Chemical shifts (δ) are reported in ppm relative to $CDCl_3$ (^{1}H, δ = 7.26 ppm; ^{13}C, δ = 77.16 ppm), MeOD (^{1}H, δ = 3.31 ppm; ^{13}C,: δ = 49.00 ppm), DMSO-d_6 (^{1}H, δ = 2.50 ppm; ^{13}C, δ = 39.52 ppm) or D_2O (^{1}H, δ = 4.79 ppm). External calibration of ^{13}C-spectra in D_2O was done with MeOH. MS spectra were provided by the service of mass spectrometry of the Department of Chemistry and Biochemistry, University of Berne. Crystallographic measurements were conducted by Dr. Jürg Hauser, University of Bern, and Dr. Antonia Neels, CSEM Neuchâtel. All compounds tested had >95% purity as determined by analytical RP-HPLC.

2-Benzyloxycarbonylaminobicyclo[2.2.1]hept-5-ene-2,3-dicarboxylic Acid Diethyl Ester (*rac*-14a−d). Freshly distilled cyclopentadiene (4.66 g, 70.52 mmol) was added dropwise to diethyl acetylenedicarboxylate (12.00 g, 70.52 mmol) cooled to 0 °C in an ice−water bath under stirring. The mixture was stirred at 0 °C for 1 h and for 5 h at room temperature to yield the crude Diels−Alder adduct (16.67 g), which was used without further purification. A saturated solution of NH_4OAc (20 g) in absolute ethanol (24 mL) was added to the crude (6 g) in a sealable reactor. After stirring at 80 °C for 18 h in the closed vessel, the mixture was evaporated to dryness and partitioned between 0.1 M aq Na_2CO_3 (100 mL) and EtOAc (100 mL). The aqueous phase was extracted with EtOAc (3 × 100 mL) and the combined organic phases were washed with brine, dried over $MgSO_4$, and evaporated to give the crude amine as a brown oil. The residue was dissolved in CH_2Cl_2 (130 mL) and Na_2CO_3 (2.96 g, 27.94 mmol) in H_2O (130 mL) followed by benzylchloroformate were added. After stirring at room temperature under an N_2 atmosphere for 12 h, the reaction was partitioned. The aqueous phase was extracted with CH_2Cl_2 (3 × 80 mL), and the combined organic phases were washed with brine, dried over $MgSO_4$, and evaporated. Purification by flash chromatography (hexane/EtOAc: 85/15 to 70/30) gave the products as slightly yellow oils in the order *rac*-**14b** (0.381 g, 0.983 mmol, 4%), *rac*-**14a**, and *rac*-**14c** (1.99 g, 5.14 mmol, 20%, ratio 1/1 by ^{1}H NMR)), *rac*-**14d** (1.831 g, 4.73 mmol, 19%), and recovered Diels−Alder adduct (1.547 g, 26%). *rac*-**14a** and *rac*-**14c** (100 mg, 0.26 mmol) were separated by RP-HPLC (A/B: 50/50 to 38/62 in 12 min, then 38/62 in isocratic mode while eluting the products, λ = 214 nm, t_R (*rac*-**14a**) = 15.8 min, t_R (*rac*-**14c**) = 17 min) to give *rac*-**14a** (34 mg, 0.088 mmol) and *rac*-**14c** (20 mg, 0.052 mmol) as transparent oils.

(1*S,2*R**,3*S**,4*R**)-2-Benzyloxycarbonylamino-bicyclo[2.2.1]-hept-5-ene-2,3-dicarboxylic Acid Diethyl Ester (*rac*-14a).** ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35−7.28 (m, 5H), 6.32 (dd, J = 5.6, 3.0 Hz, 1H), 6.07 (br s, 1H), 5.67 (br s, 1H), 5.04 (s, 2H), 4.30−3.84 (m, 4H), 3.23 (d, J = 1.9 Hz, 1H), 3.05 (s, 1H), 3.01 (s, 1H), 2.25 (d, J = 9.2 Hz, 1H), 1.64 (dd, J = 9.4, 1.6 Hz, 1H), 1.28−1.10 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.06, 170.93, 155.20, 139.61, 136.39, 134.76, 128.54, 128.31, 128.22, 67.84, 67.02, 61.62, 60.98, 52.49, 49.99, 47.62, 46.86, 14.19. HRMS: calcd for $C_{21}H_{25}NNaO_6$, 410.1574; found, 410.1576.

(1*S,2*S**,3*R**,4*R**)-2-Benzyloxycarbonylamino-bicyclo[2.2.1]-hept-5-ene-2,3-dicarboxylic Acid Diethyl Ester (*rac*-14b).** ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34−7.28 (m, 5H), 6.37 (dd, J = 5.6, 2.9 Hz, 1H), 6.19 (dd, J = 5.6, 3.2 Hz, 2H), 5.04 (s, 2H), 4.24−4.18 (m, 2H), 4.12−4.01 (m, 2H), 3.62 (s, 1H), 3.48 (d, J = 3.1 Hz, 1H), 3.17 (s, 1H), 1.79 (d, J = 9.3 Hz, 1H), 1.54 (d, J = 9.4 Hz, 1H), 1.25−1.19 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.91, 172.32, 155.60, 138.44, 136.76, 135.03, 128.53, 128.17, 128.13, 77.36, 67.73, 66.74, 61.85, 61.11, 51.80, 51.39, 46.94, 14.22, 14.17. HRMS: calcd for $C_{21}H_{25}NNaO_6$, 410.1574; found, 410.1577.

(1*S,2*S**,3*S**,4*R**)-2-Benzyloxycarbonylamino-bicyclo[2.2.1]-hept-5-ene-2,3-dicarboxylic Acid Diethyl Ester (*rac*-14c).** ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38−7.28 (m, 5H), 6.34 (dd, J = 5.6, 3.1 Hz, 1H), 6.16 (dd, J = 5.4, 3.1 Hz, 1H), 5.19−4.94 (m, 3H), 4.18−4.02 (m, 4H), 3.74 (s, 1H), 2.99 (s, 1H), 2.44 (d, J = 9.7 Hz, 1H), 2.16 (d, J = 2.6 Hz, 1H), 1.69 (d, J = 9.7 Hz, 1H), 1.27−1.16 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.04, 171.66, 155.27, 138.60, 137.28, 136.38, 128.65, 128.34, 128.26, 69.11, 67.02, 61.78, 61.23, 58.61, 49.97, 47.65, 46.49, 14.23, 14.03. HRMS: calcd for $C_{21}H_{25}NNaO_6$, 410.1574; found, 410.1576.

(1*S,2*R**,3*R**,4*R**)-2-Benzyloxycarbonylamino-bicyclo[2.2.1]-hept-5-ene-2,3-dicarboxylic Acid Diethyl Ester (*rac*-14d).** ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45−7.28 (m, 5H), 6.33 (dd, J = 5.1, 3.3 Hz, 1H), 6.26 (dd, J = 5.5, 2.9 Hz, 1H), 5.42 (s, 1H), 5.26−4.93 (m, 2H), 4.16−3.93 (m, 4H), 3.44 (s, 1H), 3.11 (s, 1H), 2.97 (d, J = 3.4 Hz, 1H), 1.75 (d, J = 9.1 Hz, 1H), 1.59 (dt, J = 9.0, 1.65 Hz, 1H), 1.21 (t, J = 7.1 Hz, 3H), 1.13 (t, J = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.04, 170.32, 155.11, 136.30, 135.40, 134.92, 128.34, 128.10, 128.02, 69.96, 66.64, 60.97, 60.51, 58.53, 51.99, 47.16, 46.01, 14.03, 13.79. HRMS: calcd for $C_{21}H_{25}NNaO_6$, 410.1574; found, 410.1577.

Procedure A. Epimerization of (1*S,2*S**,3*R**,4*R**)-2-Benzyloxycarbonylamino-bicyclo[2.2.1]hept-5-ene-2,3-dicarboxylic Acid Diethyl Ester (*rac*-14b).** Sodium (35 mg, 1.50 mmol) was dissolved in

absolute ethanol (2 mL) over 3 Å molecular sieves under an Ar atmosphere and cooled to 0 °C in an ice−water bath. *rac*-**14b** (58 mg, 0.150 mmol) in absolute ethanol (2 mL) was slowly added, and the reaction mixture was warmed to room temperature and stirred for 40 h. After cooling back to 0 °C, the reaction was neutralized with 1 N HCl and partitioned between water (15 mL) and ethyl acetate (15 mL). The aqueous phase was washed with ethyl acetate (2 times), and the combined organic phases were washed with brine, dried over Na_2SO_4, and evaporated. The mixture of diastereomers was separated by flash chromatography (hexane/EtOAc: 85/15) to yield *rac*-**14b** (18 mg, 0.046 mmol, 31%) and *rac*-**14c** (20 mg, 0.052 mmol, 34%).

Epimerization of (1*S,2*R**,3*R**,4*R**)-2-Benzyloxycarbonylamino-bicyclo[2.2.1]hept-5-ene-2,3-dicarboxylic Acid Diethyl Ester (*rac*-14d).** *rac*-**14d** (140 mg, 0.361 mmol) was treated following Procedure A to yield after flash chromatography (hexane/EtOAc: 85/15 to 80/20) *rac*-**14a** (87 mg, 0.225 mmol, 62%) and *rac*-**14d** (22 mg, 0.0565 mmol, 16%) in separate form.

Procedure B. (1*R,2*R**,3*S**,4*S**)-2-Benzyloxycarbonylamino-bicyclo[2.2.1]hept-5-ene-2,3-dicarboxylic Acid (*rac*-15a).** The compound *rac*-**14d** (415 mg, 1.07 mmol) in THF/MeOH (16.7 mL/5.6 mL) was treated with LiOH H_2O (270 mg, 6.43 mmol) in H_2O (5.6 mL). The reaction was stirred at 70 °C for 24 h. After evaporation of the organic solvents, the reaction mixture was cooled to 0 °C in an ice−water bath, acidified to pH 2 with 1 N HCl, and extracted with EtOAc (4 times). The organic phases were combined, washed with brine, dried over $MgSO_4$, and evaporated. The crude was purified by RP-HPLC (t_R = 18.0 min (A/B = 85/15 to A/B = 55/45 in 30 min)) to give *rac*-**15a** as a white solid (167 mg, 0.504 mmol, 47%). ^{1}H NMR (300 MHz, MeOD) δ 7.52−7.14 (m, 5H), 6.39 (dd, J = 5.6, 3.0 Hz, 1H), 6.06 (br s, 1H), 5.06 (s, 2H), 3.36 (d, J = 2.0 Hz, 1H, overlaid with MeOD), 3.07 (s, 1H), 3.03 (s, 1H), 2.44 (d, J = 8.9 Hz, 1H), 1.62 (dd, J = 9.2, 1.5 Hz, 1H). ^{13}C NMR (75 MHz, MeOD) δ 176.23, 174.68, 157.88, 141.30, 138.10, 135.05, 129.33, 128.80, 128.63, 68.81, 67.48, 53.50, 50.17, 49.29, 48.15. HRMS: calcd for $C_{17}H_{18}NO_6$, 332.1129; found, 332.1137.

(1*R,2*S**,3*R**,4*S**)-2-Benzyloxycarbonylamino-bicyclo[2.2.1]-hept-5-ene-2,3-dicarboxylic Acid (*rac*-15b).** The compound *rac*-**14b** (183 mg, 0.472 mmol) was treated following Procedure B (RP-HPLC: t_R = 11.8 min (A/B: 75/25 to 55/45 in 20 min)) to give *rac*-**15b** as a white solid (100 mg, 0.302 mmol, 64%). Alternatively, a mixture of *rac*-**14a** and *rac*-**14c** (569 mg, 1.469 mmol) was treated following Procedure B to give *rac*-**15a** (67 mg, 0.202 mmol, 14%) and *rac*-**15b** (99 mg, 0.299 mmol, 20%). (RP-HPLC: t_R (*rac*-**15a**) = 20 min, t_R (*rac*-**15b**) = 29.2 min with A/B = 85/15 to A/B = 45/55 in 40 min). ^{1}H NMR (300 MHz, MeOD) δ 7.38−7.25 (m, 5H), 6.32 (dd, J = 5.6, 2.9 Hz, 1H), 6.17 (dd, J = 5.4, 3.1 Hz, 1H), 3.56 (s, 1H), 3.49 (d, J = 3.2 Hz, 1H), 3.19 (s, 1H), 1.85 (d, J = 9.1 Hz, 1H), 1.49 (d, J = 9.1 Hz, 1H). ^{13}C NMR (75 MHz, MeOD) δ 176.45, 175.60, 157.62, 138.66, 138.13, 136.36, 129.41, 128.93, 128.69, 68.27, 67.46, 52.22, 51.85, 47.49. HRMS: calcd for $C_{17}H_{17}NNaO_6$, 354.0953; found, 354.0955.

Procedure C. (1*S,2*R**,3*S**,4*R**)-2-Amino-bicyclo[2.2.1]heptane-2,3-dicarboxylic Acid (*rac*-9a).** A solution of *rac*-**15a** (20 mg, 0.060 mmol) and 10% palladium on charcoal (7 mg) in MeOH (3 mL) was stirred under 1 atm H_2 at room temperature for 90 min. The reaction mixture was filtered through a pad of Celite, washing with MeOH, evaporated to dryness, and redissolved in 0.05 N HCl and re-evaporated to dryness (3×) to yield *rac*-**9a** as the HCl salt (13 mg, 0.054 mmol, 89%) as a white solid. ^{1}H NMR (300 MHz, D_2O) δ 3.43 (d, J = 1.2 Hz, 1H), 2.71 (d, J = 2.3 Hz, 1H), 2.59 (d, J = 2.3 Hz, 1H), 2.01 (d, J = 11.4 Hz, 1H), 1.80−1.51 (m, 3H), 1.49−1.35 (m, 2H). ^{13}C NMR (75 MHz, D_2O) δ 175.50, 172.76, 68.68, 52.54, 47.15, 43.18, 37.04, 27.60, 24.16. HRMS: calcd for $C_9H_{14}NO_4$, 200.0922; found, 200.0917.

(1*S,2*S**,3*R**,4*R**)-2-Amino-bicyclo[2.2.1]heptane-2,3-dicarboxylic Acid (*rac*-9b).** The compound *rac*-**15b** (20 mg, 0.060 mmol) was reacted following Procedure C to yield the HCl salt of *rac*-**9b** (12 mg, 0.051 mmol, 84%) as a white solid. ^{1}H NMR (300 MHz, D_2O) δ 3.61 (d, J = 3.3 Hz, 1H), 2.76 (s, 1H), 2.71 (s, 1H), 1.98 (d, J = 10.9 Hz, 1H), 1.80−1.37 (m, 5H). ^{13}C NMR (75 MHz, D_2O) δ 175.35, 175.20, 64.45, 49.32, 46.82, 40.68, 36.70, 22.77, 22.62. HRMS: calcd for $C_9H_{14}NO_4$, 200.0917; found, 200.0920.

(1*S,2*S**,3*S**,4*R**)-2-Aminobicyclo[2.2.1]heptane-2,3-dicarboxylic Acid (*rac*-9c).** The compound *rac*-**14c** (32 mg, 0.083 mmol) was reacted following Procedure C to give the crude aminodiester, which was further heated with 3 N HCl (3 mL) under reflux for 30 h. Evaporation to dryness under vacuum afforded *rac*-**9c** (17 mg, 0.072 mmol, 88%) as a white solid. ^{1}H NMR (300 MHz, D_2O) δ 2.68 (s, 3H), 2.33 (d, J = 10.3 Hz, 1H), 1.86−1.62 (m, 2H), 1.61−1.44 (m, 2H), 1.43−1.31 (m, 1H). ^{13}C NMR (75 MHz, D_2O) δ 174.97, 172.95, 68.60, 57.44, 46.35, 38.76, 37.99, 27.36, 22.97. HRMS: calcd for $C_9H_{14}NO_4$, 200.0917; found, 200.0920.

(1*S,2*R**,3*R**,4*R**)-2-Benzyloxycarbonylaminobicyclo[2.2.1]-heptane-2,3-dicarboxylic Acid (*rac*-16d) and (1*S**,2*R**,3*S**,4*R**)-2-Benzyloxycarbonylaminobicyclo[2.2.1]heptane-2,3-dicarboxylic Acid (*rac*-16a).** The compound *rac*-**14d** (505 mg, 1.30 mmol) was reacted following Procedure C to give the crude aminodiester, which was further heated with 6 N HCl (12 mL) under reflux for 3 days. After evaporation under vacuum, the partly epimerized amino acid was dissolved in H_2O (4.8 mL), cooled to 0 °C in an ice−water bath, and 1 N NaOH (4.8 mL) followed by benzyl-chloroformate (0.6 mL, 4.09 mmol) were added. After stirring at 0 °C for 1 h and at room temperature for 4 h, the reaction was cooled to 0 °C, then was adjusted to pH 7 by dropwise addition of 1 N HCl, and lyophilized to dryness. The crude was purified by RP-HPLC (A/B = 68/32 to A/B = 38/62 in 30 min, λ = 214 nm) to yield *rac*-**16a** (t_R = 8.4 min, 103 mg, 0.309 mmol, 24%) and *rac*-**16d** (t_R = 18 min, 12 mg, 0.036 mmol, 3%) as white solids. *rac*-**16a**: ^{1}H NMR (300 MHz, MeOD) δ 7.41−7.19 (m, 5H), 5.10−4.94 (m, 2H), 3.54 (d, J = 1.6 Hz, 1H), 2.40 (dd, J = 14.9, 3.0 Hz, 3H), 1.69−1.23 (m, 5H). ^{13}C NMR (75 MHz, MeOD) δ 176.01, 175.06, 138.26, 129.36, 128.81, 128.66, 70.60, 67.38, 54.75, 47.55, 44.06, 38.59, 28.64, 25.11. HRMS: calcd for $C_{17}H_{20}NO_6$, 334.1285; found, 334.1293. *rac*-**16d**: ^{1}H NMR (300 MHz, MeOD) δ 7.45−7.25 (m, 5H), 5.17 (q, J = 12.4 Hz, 2H), 2.84 (d, J = 3.2 Hz, 1H), 2.59 (s, 1H), 2.50 (s, 1H), 2.21−2.06 (m, 1H), 1.81 (d, J = 10.1 Hz, 1H), 1.58−1.34 (m, 4H). ^{13}C NMR (75 MHz, MeOD) δ 174.37, 173.60, 137.56, 129.53, 129.25, 129.02, 68.92, 68.60, 60.29, 49.39, 41.89, 38.29, 25.65, 22.90; HRMS: calcd for $C_{17}H_{19}NNaO_6$, 356.1105; found, 356.1112.

(1*S,2*R**,3*R**,4*R**)-2-Aminobicyclo[2.2.1]heptane-2,3-dicarboxylic Acid (*rac*-9d).** The compound *rac*-**16d** (9 mg, 0.027 mmol) was treated following Procedure C to give *rac*-**9d** (5.4 mg, 0.027 mmol, quant) as a white solid. ^{1}H NMR (300 MHz, D_2O) δ 2.72 (d, J = 3.4 Hz, 1H), 2.57 (s, 1H), 2.44 (s, 1H), 2.08−2.00 (m, 1H), 1.81−1.63 (m, 2H), 1.62−1.43 (m, 3H). ^{13}C NMR (75 MHz, D_2O) δ 176.35, 172.91, 69.11, 55.57, 46.98, 39.61, 37.05, 25.42, 21.19. HRMS: calcd for $C_9H_{14}NO_4$, 200.0917; found, 200.0920.

(1*R,2*R**,3*R**,4*S**,6*R**)-2-Benzyloxycarbonylamino-6-formyl-bicyclo[2.2.1]heptane-2,3-dicarboxylic Acid Diethyl Ester (*rac*-18a) and (1*R**,2*R**,3*R**,4*S**,5*S**)-2-Benzyloxycarbonylamino-5-formyl-bicyclo[2.2.1]heptane-2,3-dicarboxylic Acid Diethyl Ester (*rac*-18b).** The compound *rac*-**14d** (1.085 g, 2.801 mmol) in toluene (11 mL) was transferred to a 50 mL autoclave and (acetylacetonato)dicarbonylrhodium(I) (9.4 mg, 0.036 mmol, 1.3 mol %) was added. A CO/H_2 (1/1) gas pressure of 40 bar was applied, and the reaction was stirred at 60 °C for 24 h. After cooling the reaction, the solvent was evaporated and the oily residue was purified by flash chromatography (hexane/EtOAc: 80/20 to 70/30) to yield *rac*-**18a** (406 mg, 0.973 mmol, 35%) and *rac*-**18b** (345 mg, 0.826 mmol, 30%) as colorless oils in separated form. *rac*-**18a**: ^{1}H NMR (400 MHz, C_6D_6) δ 9.34 (s, 1H), 7.29−7.21 (m, 2H), 7.14−7.01 (m, 3H), 5.10 (d, J = 12.3 Hz, 1H), 4.99 (d, J = 12.3 Hz, 1H), 4.78 (br s, 1H), 4.17−3.96 (m, 2H), 3.96−3.78 (m, 2H), 3.70−3.62 (m, 1H), 3.40 (s, 1H), 2.25−2.17 (m, 2H),

1.95−1.69 (m, 2H), 1.20 (d, J = 11.0 Hz, 1H), 1.03−0.88 (m, 6H), 0.84 (d, J = 10.7 Hz, 1H). ^{13}C NMR (101 MHz, C_6D_6) δ 200.87, 170.38, 169.89, 154.79, 137.14, 128.70, 128.59, 128.34, 66.92, 66.90, 61.30, 60.60, 57.45, 48.45, 46.95, 40.56, 35.11, 24.26, 14.21, 14.01. HRMS: calcd for $C_{22}H_{27}NNaO_7$, 440.1680; found, 440.1686. *rac*-**18b**: ^{1}H NMR (300 MHz, C_6D_6) δ 9.36 (s, 1H), 7.29−7.21 (m, 2H), 7.15−7.01 (m, 3H), 5.06 (dd, J = 28.2, 12.3 Hz, 2H), 4.83 (s, 1H), 4.13−3.80 (m, 4H), 3.44−3.23 (m, 1H), 2.76 (br s, 1H), 2.60 (dd, J = 3.9, 1.4 Hz, 1H), 2.44 (d, J = 4.1 Hz, 1H), 1.91−1.78 (m, 1H), 1.71 (ddd, J = 13.4, 6.2, 4.3 Hz, 1H), 1.25−1.16 (m, 1H), 1.03 (t, J = 7.1 Hz, 3H), 0.99−0.86 (m, 4H). ^{13}C NMR (101 MHz, C_6D_6) δ 201.25, 170.26, 170.16, 154.86, 137.27, 128.67, 128.59, 67.92, 66.78, 61.44, 60.47, 57.07, 47.92, 47.82, 41.16, 35.51, 25.08, 14.22, 13.98. HRMS: calcd for $C_{22}H_{27}NNaO_7$, 440.1680; found, 440.1692.

(1*R,2*S**,3*R**,4*S**,6*R**)-2-Benzyloxycarbonylamino-6-formyl-bicyclo[2.2.1]heptane-2,3-dicarboxylic Acid Diethyl Ester (*rac*-18c) and (1*R**,2*S**,3*R**,4*S**,5*S**)-2-Benzyloxycarbonylamino-5-formyl-bicyclo[2.2.1]heptane-2,3-dicarboxylic Acid Diethyl Ester (*rac*-18d).** The compound *rac*-**14b** (272 mg, 0.702 mmol) and (acetylacetonato)dicarbonylrhodium(I) (2.4 mg, 0.0093 mmol, 1.3 mol %) in toluene (3 mL) were treated according to the procedure for hydroformylation above. Purification by flash chromatography (hexane/EtOAc: 80/20) afforded *rac*-**18c** (89 mg, 0.214 mmol, 30%) and *rac*-**18d** (146 mg, 0.350 mmol, 50%) as colorless oils in separated form. *rac*-**18c**: ^{1}H NMR (400 MHz, C_6D_6) δ 9.23 (s, 1H), 8.03 (br s, 1H), 7.27−7.21 (m, 2H), 7.11−6.94 (m, 3H), 5.11 (d, J = 12.4 Hz, 1H), 5.04 (d, J = 12.4 Hz, 1H), 4.12−3.82 (m, 2H), 3.79−3.61 (m, 2H), 2.76 (dd, J = 3.7, 1.7 Hz, 1H), 2.59 (d, J = 3.3 Hz, 1H), 2.21 (br s, 1H), 1.96 (d, J = 12.6 Hz, 1H), 1.70 (d, J = 11.0 Hz, 1H), 1.31 (ddd, J = 13.3, 8.8, 2.5 Hz, 1H), 1.04 (d, J = 11.1 Hz, 1H), 0.97−0.84 (m, 3H), 0.79 (t, J = 7.1 Hz, 3H), 0.59 (br s, 1H). ^{13}C NMR (101 MHz, C_6D_6) δ 200.62, 172.62, 172.40, 137.28, 128.65, 128.50, 128.19, 66.93, 64.18, 61.58, 61.15, 50.98, 48.66, 46.47, 40.49, 35.13, 23.16, 14.02, 13.88. HRMS: calcd for $C_{22}H_{27}NNaO_7$, 440.1680; found, 440.1684. *rac*-**18d**: ^{1}H NMR (300 MHz, C_6D_6) δ 9.06 (s, 1H), 7.40 (br s, 1H), 7.30−7.21 (m, 2H), 7.13−6.97 (m, 3H), 5.07 (s, 2H), 4.09−3.87 (m, 2H), 3.81−3.65 (m, 2H), 3.34 (br s, 1H), 2.97 (d, J = 3.9 Hz, 1H), 2.59 (d, J = 2.4 Hz, 1H), 2.50−2.33 (m, 1H), 1.85−1.59 (m, 2H), 1.56−1.39 (m, 1H), 1.08 (d, J = 10.9 Hz, 1H), 0.91 (t, J = 7.0 Hz, 4H), 0.81 (t, J = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, C_6D_6) δ 200.50, 172.88, 171.79, 156.15, 137.37, 128.63, 128.46, 128.14, 66.82, 64.19, 61.54, 61.18, 50.69, 48.01, 45.69, 41.65, 35.75, 24.82, 14.03, 13.92. HRMS: calcd for $C_{22}H_{27}NNaO_7$, 440.1680; found, 440.1686.

Procedure D. (1*R,2*R**,3*R**,4*R**,6*S**)-2-Benzyloxycarbonylamino-6-vinyl-bicyclo[2.2.1]heptane-2,3-dicarboxylic Acid Diethyl Ester (*rac*-19a).** Methyltriphenylphosphonium bromide (428 mg, 1.20 mmol) was suspended in anhydrous THF (12 mL) in an oven-dried flask over 4 Å molecular sieves and under an Ar atmosphere. After cooling to −18 °C in a salt−ice bath, 2.5 M *n*-butyllithium in hexanes (0.43 mL, 1.08 mmol) was added dropwise under stirring. The strongly yellow solution was stirred at 0 °C for 1 h and at room temperature for 30 min. A solution of *rac*-**18a** (200 mg, 0.479 mmol) in anhydrous THF (3.6 mL) was transferred dropwise to the ylid at −78 °C. The reaction was stirred at −18 °C, monitored by TLC, and quenched with glacial acetic acid (0.5 mL) after 70 min. The suspension was filtered, evaporated, and coevaporated with toluene to dryness under vacuum. The crude was purified by flash chromatography (hexane/EtOAc: 80/20) to obtain *rac*-**19a** (143 mg, 0.344 mmol, 72%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40−7.29 (m, 5H), 5.77 (ddd, J = 17.1, 10.3, 6.8 Hz, 1H), 5.30 (s, 1H), 5.19−4.84 (m, 4H), 4.29−3.95 (m, 4H), 2.95−2.84 (m, 1H), 2.83−2.74 (m, 2H), 2.61 (br s, 1H), 2.14−1.96 (m, 1H), 1.70 (d, J = 10.8 Hz, 1H), 1.62−1.45 (m, 1H), 1.38−1.26 (m, 1H), 1.26−1.12 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.83, 170.15, 154.68, 142.35, 136.43, 128.50, 128.19, 128.16, 113.28, 67.60, 66.72, 61.20, 60.50, 56.98, 52.12, 40.59, 38.13, 34.65, 30.31, 14.20, 13.98. HRMS: calcd for $C_{23}H_{29}NNaO_6$, 438.1887; found, 438.1870.

(1*S,2*R**,3*R**,4*S**,5*R**)-2-Benzyloxycarbonylamino-5-vinyl-bicyclo[2.2.1]heptane-2,3-dicarboxylic Acid Diethyl Ester (*rac*-19b).** The compound *rac*-**18b** (120 mg, 0.287 mmol) was treated following Procedure D to yield after purification by flash chromatography (hexane/EtOAc: 85/15) *rac*-**19b** (76 mg, 0.183 mmol, 64%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41−7.27 (m, 5H), 5.78 (ddd, J = 17.3, 10.2, 7.2 Hz, 1H), 5.19 (br s, 1H), 5.16−4.88 (m, 4H), 4.27−3.93 (m, 4H), 2.96 (br s, 1H), 2.93−2.81 (m, 2H), 2.46 (dd, J = 3.8, 1.4 Hz, 1H), 1.98 (ddd, J = 13.2, 8.4, 2.6 Hz, 1H), 1.72 (dd, J = 10.4, 1.3 Hz, 1H), 1.52 (d, J = 10.4 Hz, 1H), 1.37−1.26 (m, 1H), 1.26−1.10 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.53, 170.43, 154.92, 142.77, 136.53, 128.58, 128.27, 128.23, 113.14, 67.51, 66.73, 61.49, 60.48, 57.77, 48.03, 45.68, 37.94, 35.18, 31.65, 14.25, 14.04. HRMS: calcd for $C_{23}H_{29}NNaO_6$, 438.1887; found, 438.1872.

(1*R,2*S**,3*R**,4*R**,6*S**)-2-Benzyloxycarbonylamino-6-vinyl-bicyclo[2.2.1]heptane-2,3-dicarboxylic Acid Diethyl Ester (*rac*-19c).** The compound *rac*-**18c** (245 mg, 0.586 mmol) was treated following Procedure D to yield after purification by flash chromatography (hexane/EtOAc: 85/15) *rac*-**19c** (106 mg, 0.255 mmol, 43%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51 (s, 1H), 7.41−7.26 (m, 5H), 5.76 (ddd, J = 16.7, 10.1, 6.8 Hz, 1H), 5.08 (s, 2H), 5.03−4.85 (m, 2H), 4.31−3.99 (m, 4H), 3.01 (s, 1H), 2.94 (d, J = 2.2 Hz, 1H), 2.58 (br s, 2H), 1.85−1.50 (m, 4H), 1.41−1.30 (m, 1H), 1.26 (t, J = 7.1 Hz, 3H), 1.17 (t, J = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.16, 172.47, 155.79, 142.23, 128.53, 128.18, 128.09, 113.65, 66.69, 64.18, 61.59, 61.20, 50.26, 40.99, 37.82, 34.81, 30.07, 14.24, 14.12. HRMS: calcd for $C_{23}H_{29}NNaO_6$, 438.1887; found, 438.1880.

(1*S,2*S**,3*R**,4*S**,5*R**)-2-Benzyloxycarbonylamino-5-vinyl-bicyclo[2.2.1]heptane-2,3-dicarboxylic Acid Diethyl Ester (*rac*-19d).** The compound *rac*-**18d** (134 mg, 0.321 mmol) was treated following Procedure D to yield after purification by flash chromatography (hexane/EtOAc: 90/10 to 80/20) *rac*-**19d** (87 mg, 0.209 mmol, 65%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.59 (s, 1H), 7.44−7.24 (m, 5H), 5.72 (ddd, J = 17.3, 10.3, 7.3 Hz, 1H), 5.19−5.02 (m, 2H), 5.00−4.86 (m, 2H), 4.33−4.02 (m, 4H), 3.22 (s, 1H), 2.93 (d, J = 3.8 Hz, 1H), 2.45 (d, J = 2.3 Hz, 1H), 2.40−2.28 (d, J = 6.6 Hz, 1H), 2.02−1.90 (m, 1H), 1.84 (d, J = 8.8 Hz, 1H), 1.62 (d, J = 10.7 Hz, 1H), 1.28 (t, J = 7.1 Hz, 5H), 1.19 (t, J = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.28, 172.16, 155.97, 142.29, 136.77, 128.44, 128.04, 128.00, 113.22, 66.57, 63.51, 61.46, 61.17, 51.04, 46.02, 45.62, 38.88, 34.93, 31.34, 14.18, 14.04. HRMS: calcd for $C_{23}H_{29}NNaO_6$, 438.1887; found, 438.1904.

(1*R,2*R**,3*R**,4*R**,6*S**)-2-Benzyloxycarbonylamino-6-styryl-bicyclo[2.2.1]heptane-2,3-dicarboxylic Acid Diethyl Ester (*rac*-20a).** The compound *rac*-**18a** (150 mg, 0.359 mmol) and benzyltriphenylphosphonium chloride (349 mg, 0.898 mmol) were treated following Procedure D to yield after purification by flash chromatography (hexane/EtOAc: 85/15 to 70/30) a mixture of *E*/*Z*-isomers (ratio: 54/46) of *rac*-**20a** (177 mg, 0.359 mmol, quant) as a colorless oil. ^{1}H NMR (300 MHz, C_6D_6) δ 7.39 (d, J = 7.3 Hz, 1H), 7.31−6.96 (m, 10H, overlaid with solvent peak), 6.36 (dd, J = 28.5, 13.7 Hz, 1H), 6.07 (dd, J = 15.8, 7.3 Hz, 1H × 0.46), 5.39 (dd, J = 11.5, 9.6 Hz, 1H × 0.54), 5.14−4.97 (m, 2H), 4.92 (br s, 1H × 0.54), 4.85 (br s, 1H × 0.46), 4.23−3.77 (m, 4H), 3.69−3.57 (m, 1H × 0.54), 3.53 (d, J = 5.9 Hz, 1H × 0.46), 3.02 (s, 1H × 0.46), 2.82 (s, 1H × 0.54), 2.66−2.50 (m, 1H), 2.48−2.23 (m, 2H), 1.49−1.13 (m, 4H), 1.10−0.94 (m, 4H), 0.80 (t, J = 7.1 Hz, 3H × 0.54). ^{13}C NMR (75 MHz, C_6D_6) δ 170.72, 170.61, 170.40, 170.08, 154.78, 154.71, 138.27, 137.80, 137.33, 137.31, 137.05, 134.93, 129.39, 129.25, 128.70, 128.67, 128.64, 128.59, 128.56, 128.50, 128.25, 128.19, 127.16, 126.93, 126.59, 67.95, 67.71, 66.79, 66.72, 61.36, 61.18, 60.45, 60.30, 57.12, 56.98, 53.71, 52.63, 41.12, 40.64, 37.94, 35.29, 34.99, 34.28, 33.33, 31.74, 14.31, 14.26, 14.13, 13.75. HRMS: calcd for $C_{29}H_{33}NNaO_6$, 514.2205; found, 514.2205.

(1*S,2*R**,3*R**,4*S**,5*R**)-2-Benzyloxycarbonylamino-5-styryl-bicyclo[2.2.1]heptane-2,3-dicarboxylic Acid Diethyl Ester (*rac*-20b).** The compound *rac*-**18b** (350 mg, 0.838 mmol) and benzyltriphenylphosphonium chloride (815 mg, 2.09 mmol) were treated

following Procedure D to yield after purification by flash chromatography (hexane/EtOAc: 85/15) a mixture of *E*/*Z*-isomers (ratio: 57/43) of *rac*-**20b** (287 mg, 0.584 mmol, 70%) as a colorless oil. ^{1}H NMR (300 MHz, C_6D_6) δ 7.51−7.39 (m, 2H), 7.27 (s, 3H), 7.33−6.95 (m, 8H), 6.37 (dd, J = 26.7, 13.6 Hz, 1H), 6.07 (dd, J = 15.8, 7.9 Hz, 1H × 0.43), 5.40 (dd, J = 11.6, 10.1 Hz, 1H × 0.57), 5.14 (dd, J = 12.3, 7.5 Hz, 1H), 5.03 (dd, J = 12.3, 8.7 Hz, 1H), 4.87−4.68 (m, 1H), 4.19−3.91 (m, 3H), 3.84 (q, J = 7.1 Hz, 1H), 3.60 (m, 1H × 0.57), 3.35 (m, 1H × 0.43), 3.09 (dd, J = 20.4, 1.9 Hz, 1H), 2.66−2.23 (m, 2H), 1.52−1.12 (m, 4H), 0.95 (ddt, J = 40.5, 20.8, 7.1 Hz, 6H). ^{13}C NMR (75 MHz, C_6D_6) δ 170.43, 170.40, 170.27, 170.16, 155.17, 155.08, 138.24, 137.85, 137.38, 137.35, 135.21, 128.72, 128.67, 128.64, 128.60, 128.57, 128.46, 128.24, 128.19, 127.18, 126.88, 126.56, 67.56, 67.29, 66.75, 66.74, 61.33, 61.25, 60.40, 60.37, 58.04, 57.77, 48.21, 47.66, 46.72, 46.60, 37.97, 35.56, 35.42, 34.20, 33.80, 32.65, 14.32, 14.22, 14.01, 13.98. HRMS: calcd for $C_{29}H_{33}NNaO_6$, 514.2200; found, 514.2198.

(1*R,2*S**,3*R**,4*R**,6*S**)-2-Benzyloxycarbonylamino-6-styryl-bicyclo[2.2.1]heptane-2,3-dicarboxylic Acid Diethyl Ester (*rac*-20c).** The compound *rac*-**18c** (70 mg, 0.168 mmol) and benzyltriphenylphosphonium chloride (163 mg, 0.419 mmol) were treated following Procedure D to yield after purification by flash chromatography (hexane/EtOAc: 85/15) a mixture of *E*/*Z*-isomers (ratio: 53/47) of *rac*-**20c** (43 mg, 52%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 (d, J = 8.3 Hz, 1H), 7.34−6.93 (m, 10H), 6.20 (dd, J = 19.4, 13.8 Hz, 1H), 6.01 (dd, J = 15.8, 6.2 Hz, 0.51H), 5.45 (dd, J = 11.2, 10.0 Hz, 0.46H), 5.10−4.92 (m, 1.48H), 4.79 (d, J = 12.3 Hz, 0.43H), 4.25−3.83 (m, 4H), 3.08 (br s, 0.45H), 2.98 (d, J = 13.7 Hz, 1H), 2.86 (d, J = 2.5 Hz, 0.53H), 2.80 (d, J = 2.3 Hz, 0.52H), 2.67 (br s, 0.54H), 2.56−2.42 (m, 1H), 1.83−1.62 (m, 2H), 1.56 (d, J = 8.1 Hz, 0.69H), 1.38−1.24 (m, 0.62H), 1.22−0.95 (m, 7H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.12, 173.01, 172.47, 155.80, 137.68, 136.43, 134.33, 129.18, 128.83, 128.58, 128.54, 128.46, 128.21, 128.11, 128.00, 127.10, 126.71, 126.16, 66.72, 66.64, 64.16, 61.62, 61.52, 61.24, 50.25, 50.19, 41.01, 40.92, 37.40, 35.52, 35.12, 33.11, 32.97, 30.99, 14.24, 14.20, 14.12, 14.08. HRMS: calcd for $C_{29}H_{33}NNaO_6$, 514.2200; found, 514.2209.

(1*S,2*S**,3*R**,4*S**,5*R**)-2-Benzyloxycarbonylamino-5-styryl-bicyclo[2.2.1]heptane-2,3-dicarboxylic Acid Diethyl Ester (*rac*-20d).** The compound *rac*-**18d** (80 mg, 0.192 mmol) and benzyltriphenylphosphonium chloride (186 mg, 0.479 mmol) were treated following Procedure D to yield after purification by flash chromatography (hexane/EtOAc: 80/20) a mixture of *E*/*Z*-isomers (ratio: 63/37) of *rac*-**20d** (68 mg, 0.138 mmol, 72%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51 (d, J = 11.5 Hz, 1H), 7.34−7.03 (m, 10H), 6.23 (dd, J = 13.7, 7.8 Hz, 1H), 5.99 (dd, J = 15.8, 7.8 Hz, 0.58H), 5.41 (dd, J = 11.2, 10.3 Hz, 0.34H), 5.16−4.87 (m, 2H), 4.30−3.73 (m, 4H), 3.16 (s, 1H), 2.92−2.75 (m, 1H), 2.50−2.29 (m, 1H), 2.05−1.89 (m, 1H), 1.87−1.72 (m, 1H), 1.64 (t, J = 12.5 Hz, 1H), 1.34−0.88 (m, 8H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.32, 172.19, 156.03, 137.43, 136.52, 134.24, 128.92, 128.62, 128.50, 128.24, 128.10, 128.07, 127.23, 126.80, 126.11, 66.65, 63.52, 61.56, 61.29, 51.17, 50.77, 46.87, 46.44, 45.71, 45.46, 41.28, 38.52, 35.26, 33.70, 33.28, 32.20, 29.78, 14.27, 14.09, 13.85. HRMS: calcd for $C_{29}H_{33}NNaO_6$, 514.2200; found, 514.2217.

Procedure E. (1*R,2*R**,3*S**,4*R**,6*S**)-2-Benzyloxycarbonylamino-6-vinyl-bicyclo[2.2.1]heptane-2,3-dicarboxylic Acid (*rac*-21a).** The compound *rac*-**19a** (110 mg, 0.265 mmol) was dissolved in THF/MeOH (7.2 mL/1.8 mL) and LiOH monohydrate (44 mg, 1.06 mmol) in H_2O (1.8 mL) was added. The solution was stirred at 70 °C for 48 h. After evaporation of the organic solvents, the reaction mixture was cooled to 0 °C and acidified to pH 2 with 1 N HCl. The suspension was extracted with EtOAc (4 × 15 mL), the combined organic phases were washed with brine, dried over $MgSO_4$, and evaporated. Purification by flash chromatography (CH_2Cl_2/MeOH: 98/2 with 0.5% acetic acid) yielded *rac*-**21a** (56 mg, 0.156 mmol, 59%) as a white solid. ^{1}H NMR (300 MHz, MeOD) δ 7.39−7.22 (m, 5H), 5.75 (ddd, J = 17.4, 10.4, 7.1 Hz, 1H), 5.09−4.91 (m, 4H), 3.54 (s, 1H), 2.45 (d, J = 2.8 Hz, 1H), 2.36−2.15 (m, 3H), 1.69−1.51 (m, 2H), 1.42 (dt, J = 12.6, 4.5 Hz, 1H). ^{13}C NMR (75 MHz, MeOD) δ 176.02, 174.89, 157.80, 143.26, 138.22, 129.37, 128.82, 128.66, 113.90, 70.64, 67.41, 54.23, 53.07, 44.15, 41.02, 36.28, 35.80. HRMS: calcd for $C_{19}H_{21}NNaO_6$, 382.1266; found, 382.1278.

(1*S,2*R**,3*S**,4*S**,5*R**)-2-Benzyloxycarbonylamino-5-vinyl-bicyclo[2.2.1]heptane-2,3-dicarboxylic Acid (*rac*-21b).** The compound *rac*-**19b** (99 mg, 0.238 mmol) was treated following Procedure E to yield after purification by recrystallization in EtOH/H_2O *rac*-**21b** (35 mg, 0.097 mmol, 41%) as a white solid. ^{1}H NMR (300 MHz, MeOD) δ 7.66−7.02 (m, 5H), 5.78 (ddd, J = 17.4, 10.3, 7.4 Hz, 1H), 5.25−4.91 (m, 4H), 3.60 (d, J = 1.4 Hz, 1H), 2.40 (d, J = 3.5 Hz, 1H), 2.27 (s, 3H), 1.71−1.49 (m, 2H), 1.32 (dt, J = 9.5, 4.4 Hz, 1H). ^{13}C NMR (75 MHz, MeOD) δ 175.79, 174.98, 157.86, 143.57, 138.23, 129.36, 128.81, 128.65, 113.58, 70.30, 67.40, 54.84, 49.72, 47.85, 45.19, 35.95, 32.62. HRMS: calcd for $C_{19}H_{22}NO_6$, 360.1442; found, 360.1446.

(1*R,2*S**,3*R**,4*R**,6*S**)-2-Benzyloxycarbonylamino-6-vinyl-bicyclo[2.2.1]heptane-2,3-dicarboxylic Acid (*rac*-21c).** The compound *rac*-**19c** (26 mg, 0.062 mmol) was treated following Procedure E to yield *rac*-**21c** (15 mg, 0.042 mmol, 67%) as a white solid after precipitating it from a slowly evaporating solution in EtOH/H_2O. ^{1}H NMR (300 MHz, MeOD) δ 7.69−6.99 (m, 5H), 5.78 (ddd, J = 17.1, 8.7, 6.9 Hz, 1H), 5.27−4.75 (m, 4H), 3.06 (d, J = 2.5 Hz, 1H), 2.88 (s, 1H), 2.68−2.46 (m, 2H), 1.87−1.66 (m, 2H), 1.56 (d, J = 10.5 Hz, 1H), 1.44−1.26 (m, 1H). ^{13}C NMR (75 MHz, MeOD) δ 176.42, 175.68, 157.76, 143.59, 138.28, 129.45, 128.96, 128.70, 113.83, 67.47, 65.04, 51.44, 50.77, 42.15, 39.19, 35.21, 31.21. HRMS: calcd for $C_{19}H_{22}NO_6$, 360.1442; found, 360.1455.

(1*S,2*S**,3*R**,4*S**,5*R**)-2-Benzyloxycarbonylamino-5-vinyl-bicyclo[2.2.1]heptane-2,3-dicarboxylic Acid (*rac*-21d).** The compound *rac*-**19d** (181 mg, 0.436 mmol) was treated following Procedure E to yield after purification by flash chromatography (CH_2Cl_2/MeOH: 98/2 with 0.5% acetic acid) *rac*-**21d** (100 mg, 0.278 mmol, 64%) as a white solid. ^{1}H NMR (300 MHz, MeOD) δ 7.52−7.08 (m, 5H), 5.76 (ddd, J = 17.4, 10.2, 7.3 Hz, 1H), 5.07 (s, 2H), 5.00−4.89 (m, 2H), 3.11−3.01 (m, 2H), 2.49−2.30 (m, 2H), 1.97−1.77 (m, 2H), 1.59 (d, J = 10.4 Hz, 1H), 1.38−1.15 (m, 1H). ^{13}C NMR (75 MHz, MeOD) δ 176.71, 175.49, 157.97, 143.66, 138.21, 129.50, 129.02, 128.74, 113.60, 67.56, 64.56, 51.67, 47.41, 46.75, 40.28, 35.47, 32.21. HRMS: calcd for $C_{19}H_{22}NO_6$, 360.1442; found, 360.1449.

(1*R,2*R**,3*S**,4*R**,6*S**)-2-Benzyloxycarbonylamino-6-styryl-bicyclo[2.2.1]heptane-2,3-dicarboxylic Acid (*rac*-22a).** The compound *rac*-**20a** (294 mg, 0.598 mmol) was treated following Procedure E to yield after purification by preparative RP-HPLC (A/C: 50/50 to 20/80 in 30 min, λ = 214 nm, t_R = 12.0 min) a mixture of *E*/*Z*-isomers (ratio: 56/44) of *rac*-**22a** (112 mg, 0.257 mmol, 43%) as a white solid. ^{1}H NMR (300 MHz, MeOD) δ 7.44−7.08 (m, 10H), 6.40−6.26 (m, 1H), 6.15 (dd, J = 15.8, 7.5 Hz, 1H × 0.56), 5.55 (dd, J = 11.5, 9.8 Hz, 1H × 0.44), 5.11−4.95 (m, 2H), 3.65−3.49 (m, 1H), 2.88 (br s, 1H × 0.44), 2.57−2.26 (m, 4H), 1.83−1.63 (m, 2H), 1.51 (dt, J = 12.6, 4.4 Hz, 1H × 0.56), 1.38 (dt, J = 12.6, 4.3 Hz, 1H × 0.44). ^{13}C NMR (75 MHz, MeOD) δ 176.22, 175.29, 157.81, 138.85, 138.32, 137.08, 134.93, 130.35, 129.77, 129.50, 129.36, 129.27, 128.81, 128.68, 128.65, 128.09, 127.76, 127.09, 70.96, 70.59, 67.42, 54.52, 53.58, 44.11, 40.61, 39.13, 37.05, 36.38, 36.04, 35.80. HRMS: calcd for $C_{25}H_{25}NNaO_6$, 458.1579; found, 458.1595.

(1*S,2*R**,3*S**,4*S**,5*R**)-2-Benzyloxycarbonylamino-5-styryl-bicyclo[2.2.1]heptane-2,3-dicarboxylic Acid (*rac*-22b).** The compound *rac*-**20b** (0.263 mg, 0.535 mmol) was treated following Procedure E to yield after purification by preparative RP-HPLC (A/C: 50/50 to 20/80 in 30 min, λ = 214 nm, t_R = 4.4 min) a mixture of *E*/*Z*-isomers (ratio: 55/45) of *rac*-**22b** (104 mg, 0.239 mmol, 45%) as a white solid. ^{1}H NMR (300 MHz, MeOD) δ 7.49−7.00 (m, 10H), 6.46−6.27 (m, 1H), 6.17 (dd, J = 15.8,

7.9 Hz, 1H × 0.45), 5.58 (dd, J = 11.4, 10.0 Hz, 1H × 0.55), 5.10−4.95 (m, 2H), 3.70−3.50 (m, 1H), 2.83−2.69 (m, 1H × 0.55), 2.56−2.21 (m, 4H), 1.86−1.60 (m, 2H), 1.49−1.24 (m, 1H). ^{13}C NMR (75 MHz, MeOD) δ 175.58, 174.87, 157.88, 138.95, 138.55, 138.22, 137.66, 135.15, 130.17, 129.73, 129.47, 129.36, 129.25, 129.19, 128.82, 128.66, 128.03, 127.82, 127.12, 70.27, 67.43, 54.86, 54.59, 50.77, 50.21, 47.93, 44.77, 40.13, 36.56, 36.23, 35.33, 33.36. HRMS: calcd for $C_{25}H_{25}NNaO_6$, 458.1579; found, 458.1578.

(1*R,2*S**,3*R**,4*R**,6*S**)-2-Benzyloxycarbonylamino-6-styryl-bicyclo[2.2.1]heptane-2,3-dicarboxylic Acid (*rac*-22c).** The compound *rac*-**20c** (42 mg, 0.085 mmol) was treated following Procedure E to yield after purification by preparative RP-HPLC (A/C: 50/50 to 20/80 in 30 min, λ = 214 nm, t_R = 7.1 min) a mixture of *E*/*Z*-isomers (ratio: approximately 50/50) of *rac*-**22c** (25 mg, 0.057 mmol, 68%) as a white solid. ^{1}H NMR (300 MHz, MeOD) δ 7.55−6.98 (m, 10H), 6.42−6.08 (m, 1H + 1H × 0.5), 5.63 (t, J = 10.8 Hz, 1H × 0.5), 5.10 (dd, J = 35.8, 12.8 Hz, 2H), 3.23−3.03 (m, 1H), 2.95 (br s, 1H), 2.81−2.55 (m, 2H), 1.99−1.75 (m, 2H), 1.69 (d, J = 10.3 Hz, 1H), 1.43 (d, J = 13.7 Hz, 1H), 1.36−1.22 (m, 1H). ^{13}C NMR (75 MHz, MeOD) δ 176.29, 175.73, 157.59, 139.05, 138.72, 138.41, 137.97, 137.47, 135.32, 130.34, 129.73, 129.49, 129.46, 129.42, 129.15, 129.01, 128.89, 128.76, 128.53, 127.97, 127.68, 127.09, 67.54, 65.09, 52.00, 51.78, 51.06, 50.82, 42.23, 42.02, 38.86, 36.01, 35.49, 34.27, 32.17. HRMS: calcd for $C_{25}H_{25}NNaO_6$, 458.1574; found, 458.1575.

(1*S,2*S**,3*R**,4*S**,5*R**)-2-Benzyloxycarbonylamino-5-styryl-bicyclo[2.2.1]heptane-2,3-dicarboxylic Acid (*rac*-22d).** The compound *rac*-**20d** (59 mg, 0.120 mmol) was treated following Procedure E to yield after purification by preparative RP-HPLC (A/C: 50/50 to 20/80 in 30 min, λ = 214 nm, t_R = 7.0 min) a mixture of *E*/*Z*-isomers (ratio: 57/43) of *rac*-**22d** (30 mg, 0.069 mmol, 57%) as a white solid. ^{1}H NMR (300 MHz, MeOD) δ 7.50−7.07 (m, 10H), 6.42−6.28 (m, 1H), 6.17 (dd, J = 15.8, 7.7 Hz, 1H × 0.57), 5.66−5.48 (m, 1H × 0.43), 5.21−5.00 (m, 2H), 3.17−3.03 (m, 2H), 3.02−2.88 (m, 1H × 0.43), 2.73−2.31 (m, 2H), 2.09−1.84 (m, 2H), 1.74 (t, J = 11.6 Hz, 1H), 1.41−1.15 (m, 1H). ^{13}C NMR (75 MHz, MeOD) δ 176.69, 175.45, 157.98, 138.95, 138.49, 137.62, 135.27, 130.06, 129.71, 129.47, 129.19, 128.99, 128.71, 128.02, 127.72, 127.10, 67.53, 64.55, 51.75, 51.30, 47.82, 46.83, 46.62, 39.86, 35.90, 35.68, 35.05, 34.57, 32.93. HRMS: calcd for $C_{25}H_{25}NNaO_6$, 458.1574; found, 458.1584.

Procedure F. (1*R,2*R**,3*S**,4*R**,6*S**)-2-Amino-6-vinyl-bicyclo-[2.2.1]heptane-2,3-dicarboxylic Acid Trifluoroacetate (*rac*-23a).** The compound *rac*-**21a** (12 mg, 0.033 mmol) was heated to 80 °C in glacial acetic acid/36% HCl (2 mL/0.5 mL) for 2 h under stirring. Cooling to room temperature and evaporation of the solvent under vacuum gave the crude product. Purification by RP-HPLC (A/B: 90/10 to 60/40 in 20 min, t_R (RP-HPLC) = 13.4 min) afforded the TFA-salt of *rac*-**23a** (6.8 mg, 0.020 mmol, 60%) as a white solid. ^{1}H NMR (300 MHz, MeOD) δ 5.77 (ddd, J = 17.2, 10.3, 7.0 Hz, 1H), 5.11−4.94 (m, 2H), 3.50 (d, J = 1.6 Hz, 1H), 2.69 (d, J = 2.6 Hz, 1H), 2.58 (br s, 1H), 2.33 (s, 1H), 1.97 (d, J = 11.4 Hz, 1H), 1.85−1.72 (m, 1H), 1.65 (d, J = 11.3 Hz, 1H), 1.50 (dt, J = 12.7, 4.4 Hz, 1H). ^{13}C NMR (75 MHz, DMSO) δ 173.35, 171.95, 142.59, 113.00, 67.32, 52.07, 51.73, 41.06, 34.83, 33.75. HRMS: calcd for $C_{11}H_{16}NO_4$, 226.1079; found, 226.1082.

(1*S,2*R**,3*S**,4*S**,5*R**)-2-Amino-5-vinyl-bicyclo[2.2.1]heptane-2,3-dicarboxylic acid trifluoroacetate (*rac*-23b).** The compound *rac*-**21b** (14 mg, 0.039 mmol) was treated following Procedure F to yield the TFA-salt of *rac*-**23b** (8.6 mg, 0.025 mmol, 65%) as a white solid (t_R (RP-HPLC) = 12.2 min). ^{1}H NMR (300 MHz, D_2O) δ 5.80 (ddd, J = 17.4, 10.3, 7.2 Hz, 1H), 5.14−4.93 (m, 2H), 3.47 (d, J = 1.3 Hz, 1H), 2.64−2.50 (m, 2H), 2.45−2.28 (m, 1H), 1.90 (d, J = 11.4 Hz, 1H), 1.78−1.58 (m, 2H), 1.56−1.40 (m, 1H). ^{13}C NMR (75 MHz, DMSO) δ 173.17, 172.02, 142.73, 112.99, 66.91, 52.54, 46.81, 46.70, 43.50, 34.01, 30.99. HRMS: calcd for $C_{11}H_{16}NO_4$, 226.1079; found, 226.1080.

(1*R,2*S**,3*R**,4*R**,6*S**)-2-Amino-6-vinyl-bicyclo[2.2.1]heptane-2,3-dicarboxylic Acid Trifluoroacetate (*rac*-23c).** The compound *rac*-**21c** (12 mg, 0.033 mmol) was treated following Procedure F to yield the TFA-salt of *rac*-**23c** (6.3 mg, 0.019 mmol, 56%) as a white solid (t_R (RP-HPLC) = 12.8 min). ^{1}H NMR (300 MHz, DMSO) δ 5.75 (ddd, J = 17.0, 10.3, 6.4 Hz, 1H), 4.99 (dd, J = 13.6, 12.2 Hz, 2H), 3.36 (d, J = 2.7 Hz, 1H), 2.59 (br s, 1H), 2.29 (s, 1H), 1.85−1.65 (m, 2H), 1.36−1.17 (m, 3H). ^{13}C NMR (101 MHz, DMSO) δ 173.22, 172.49, 142.22, 113.75, 62.63, 51.30, 47.51, 36.49, 32.83, 29.72. HRMS: calcd for $C_{11}H_{16}NO_4$, 226.1079; found, 226.1076.

(1*S,2*S**,3*R**,4*S**,5*R**)-2-Amino-5-vinyl-bicyclo[2.2.1]heptane-2,3-dicarboxylic Acid Trifluoroacetate (*rac*-23d).** The compound *rac*-**21d** (39 mg, 0.109 mmol) was treated following Procedure F to yield the TFA-salt of *rac*-**23d** (23 mg, 0.068 mmol, 62%) as a white solid (t_R (RP-HPLC) = 9.9 min). ^{1}H NMR (300 MHz, DMSO) δ 5.76 (ddd, J = 17.5, 10.6, 7.0 Hz, 1H), 5.10−4.80 (m, 2H), 3.44 (d, J = 4.0 Hz, 1H), 2.50−2.42 (m, 2H, overlaid with solvent peak), 2.35 (d, J = 3.3 Hz, 1H), 2.05−1.86 (m, 1H), 1.79 (d, J = 9.9 Hz, 1H), 1.41−1.15 (m, 2H). ^{13}C NMR (75 MHz, DMSO) δ 172.59, 172.46, 142.70, 113.06, 62.35, 48.93, 46.34, 45.01, 38.06, 33.31, 29.70. HRMS: calcd for $C_{11}H_{16}NO_4$, 226.1079; found, 226.1081.

(1*R,2*R**,3*S**,4*R**,6*R**)-2-Amino-6-ethyl-bicyclo[2.2.1]heptane-2,3-dicarboxylic Acid Trifluoroacetate (*rac*-24a).** The compound *rac*-**21a** (12 mg, 0.033 mmol) was treated following Procedure C (using CH_3CN/H_2O (1/1) + 0.5% TFA for washing) to yield the TFA salt of *rac*-**24a** (12 mg, quant) as a white solid. ^{1}H NMR (300 MHz, MeOD) δ 3.44 (s, 1H), 2.64 (s, 1H), 2.29 (s, 1H), 1.92 (d, J = 8.8 Hz, 1H), 1.81−1.52 (m, 3H), 1.25 (ddd, J = 20.5, 14.0, 7.3 Hz, 3H), 0.87 (t, J = 7.3 Hz, 3H). ^{13}C NMR (75 MHz, MeOD) δ 173.45, 171.85, 67.39, 50.78, 41.21, 38.07, 37.24, 35.66, 33.42, 29.77, 12.05. HRMS: calcd for $C_{11}H_{18}NO_4$, 228.1230; found, 228.1237.

(1*S,2*R**,3*S**,4*S**,5*S**)-2-Amino-5-ethyl-bicyclo[2.2.1]heptane-2,3-dicarboxylic Acid Trifluoroacetate (*rac*-24b).** The compound *rac*-**21b** (13 mg, 0.036 mmol) was treated following Procedure C (using CH_3CN/H_2O (1/1) + 0.5% TFA for washing) to yield the TFA salt of *rac*-**24b** (13 mg, quant) as a white solid. ^{1}H NMR (300 MHz, MeOD) δ 3.43 (s, 1H), 2.46 (s, 1H), 1.95 (d, J = 10.0 Hz, 1H), 1.87−1.75 (m, 1H), 1.72−1.52 (m, 2H), 1.49−1.01 (m, 4H), 0.91 (t, J = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, MeOD) δ 175.52, 172.88, 163.37 (TFA), 162.91 (TFA), 120.15 (TFA), 116.27 (TFA), 68.72, 53.71, 48.63, 43.85, 34.88, 33.28, 29.90, 12.48. HRMS: calcd for $C_{11}H_{18}NO_4$, 228.1230; found, 228.1235.

(1*R,2*S**,3*R**,4*R**,6*R**)-2-Amino-6-ethyl-bicyclo[2.2.1]heptane-2,3-dicarboxylic Acid Trifluoroacetate (*rac*-24c).** The compound *rac*-**21c** (10 mg, 0.028 mmol) was treated following Procedure C (using CH_3CN/H_2O (1/1) + 0.5% TFA for washing) to yield the TFA salt of *rac*-**24c** (10 mg, quant) as a white solid. ^{1}H NMR (300 MHz, MeOD) δ 3.61 (s, 1H), 2.68 (s, 1H), 2.42 (s, 1H), 2.04−1.67 (m, 3H), 1.62−1.07 (m, 4H), 0.93 (t, J = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 173.73, 172.81, 62.91, 50.37, 47.05, 40.47, 34.82, 32.64, 31.20, 28.17, 11.76. HRMS: calcd for $C_{11}H_{18}NO_4$, 228.1230; found, 228.1235.

(1*S,2*S**,3*R**,4*S**,5*S**)-2-Amino-5-ethyl-bicyclo[2.2.1]heptane-2,3-dicarboxylic Acid Trifluoroacetate (*rac*-24d).** The compound *rac*-**21d** (20 mg, 0.056 mmol) was treated following Procedure C (using CH_3CN/H_2O (1/1) + 0.5% TFA for washing) to yield the TFA salt of *rac*-**24d** (19 mg, quant) as a white solid. ^{1}H NMR (300 MHz, MeOD) δ 3.63 (d, J = 3.4 Hz, 1H), 2.63 (s, 1H), 2.51 (s, 1H), 2.05−1.85 (m, 2H), 1.79−1.63 (m, 1H), 1.52 (d, J = 10.3 Hz, 1H), 1.45−1.07 (m, 3H), 0.92 (t, J = 7.3 Hz, 3H). ^{13}C NMR (75 MHz, MeOD) δ 174.11, 64.59, 50.43, 48.50, 45.90, 38.33, 34.34, 32.13, 30.01, 12.33. HRMS: calcd for $C_{11}H_{18}NO_4$, 228.1230; found, 228.1235.

(1*R,2*R**,3*S**,4*R**,6*R**)-2-Amino-6-phenethyl-bicyclo[2.2.1]-heptane-2,3-dicarboxylic Acid Trifluoroacetate (*rac*-25a).** The compound *rac*-**22a** (19 mg, 0.044 mmol) was treated following Procedure C using CH_3CN/H_2O (1/1) + 0.5% TFA for washing. The crude product (16 mg) was purified by RP-HPLC (A/B: 60/40 to 40/60 in 20 min, λ = 254 nm, t_R = 9.0 min) to give the TFA-salt of *rac*-**25a** (8 mg, 0.019 mmol, 44%) as a white solid. ^{1}H NMR (300 MHz, MeOD) δ 7.37−7.00 (m, 5H), 3.46 (s, 1H), 2.64 (s, 1H), 2.56 (t, J = 7.9 Hz, 2H), 2.31 (s, 1H), 2.03−1.85

(m, 2H), 1.82−1.40 (m, 4H), 1.36−1.21 (m, 1H). ^{13}C NMR (75 MHz, DMSO) δ 173.46, 172. 37, 142.09, 128.24, 128.18, 125.55, 67.57, 51.79, 51.25, 40.98, 37.79, 35.95, 35.15, 33.50. HRMS: calcd for $C_{17}H_{22}NO_4$, 304.1548; found, 304.1543.

(1*S,2*R**,3*S**,4*S**,5*S**)-2-Amino-5-phenethyl-bicyclo[2.2.1]-heptane-2,3-dicarboxylic Acid (*rac*-25b).** The compound *rac*-**22b** (20 mg, 0.046 mmol) was treated following Procedure C to afford *rac*-**25b** (11 mg, 0.035 mmol, 75%) as a white solid. ^{1}H NMR (300 MHz, DMSO) δ 7.39−7.03 (m, 5H), 3.18 (s, 1H), 2.58−2.52 (m, 2H, overlaid with solvent peak), 2.41 (s, 1H), 2.01 (d, *J* = 11.2 Hz, 1H), 1.69−1.00 (m, 7H). ^{13}C NMR (75 MHz, DMSO) δ 173.39, 172.04, 142.19, 128.25, 128.18, 125.55, 66.83, 52.89, 46.72, 45.85, 37.65, 33.86, 33.46, 32.08. HRMS: calcd for $C_{17}H_{22}NO_4$, 304.1543; found, 304.1553.

(1*R,2*S**,3*R**,4*R**,6*R**)-2-Amino-6-phenethyl-bicyclo[2.2.1]-heptane-2,3-dicarboxylic Acid Trifluoroacetate (*rac*-25c).** The compound *rac*-**22c** (9 mg, 0.021 mmol) was treated following Procedure C to afford after purification by RP-HPLC (A/B: 60/40 to 40/60 in 20 min, λ = 254 nm, t_R = 11.2 min) the TFA salt of *rac*-**25c** (4 mg, 0.010 mmol, 46%) as a white solid. ^{1}H NMR (300 MHz, MeOD) δ 7.37−7.04 (m, 5H), 3.22 (d, *J* = 3.0 Hz, 1H), 2.73−2.46 (m, 3H), 2.40−2.25 (m, 2H), 1.97−1.58 (m, 3H), 1.46 (d, *J* = 10.6 Hz, 2H), 1.36−1.26 (m, 1H), 1.15 (d, *J* = 11.2 Hz, 1H). ^{13}C NMR (75 MHz, DMSO) δ 173.33, 172.75, 158.05 (TFA), 157.64 (TFA), 142.29, 128.25, 128.18, 125.61, 119.21 (TFA), 115.24 (TFA), 62.96, 50.69, 47.12, 37.75, 33.34, 32.75, 31.37. HRMS: calcd for $C_{17}H_{22}NO_4$, 304.1543; found, 304.1555.

(1*S,2*S**,3*R**,4*S**,5*S**)-2-Amino-5-phenethyl-bicyclo[2.2.1]-heptane-2,3-dicarboxylic Acid (*rac*-25d).** The compound *rac*-**22d** (10 mg, 0.023 mmol) was treated following Procedure C to afford *rac*-**25d** (7 mg, 0.023 mmol, quant) as a white solid. ^{1}H NMR (300 MHz, MeOD) δ 7.37−7.01 (m, 5H), 3.52 (s, 1H), 2.74−2.44 (m, 4H), 2.19 (d, *J* = 10.1 Hz, 1H), 1.98−1.40 (m, 5H), 1.17 (d, *J* = 12.0 Hz, 1H). ^{13}C NMR (75 MHz, DMSO) δ 173.86, 173.53, 142.35, 128.28, 128.15, 125.48, 62.00, 49.23, 46.20, 44.56, 38.69, 38.31, 34.66, 33.50, 33.24, 31.40. HRMS: calcd for $C_{17}H_{22}NO_4$, 304.1543; found, 304.1551.

(1*R,2*R**,3*S**,4*R**,6*S**)-2-Benzyloxycarbonylamino-6-propenyl-bicyclo[2.2.1]heptane-2,3-dicarboxylic Acid Diethyl Ester (*rac*-26a).** The compound *rac*-**18a** (150 mg, 0.359 mmol) and ethyltriphenylphosphonium bromide (334 mg, 0.898 mmol) were treated following Procedure D to yield after purification by flash chromatography (hexane/EtOAc: 80/20) a mixture of *E*/*Z*-isomers of *rac*-**26a** (136 mg, 0.317 mmol, 88%, purity ∼93% (NMR)) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42−7.28 (m, 5H), 5.43−5.17 (m, 2H), 5.16−4.92 (m, 3H), 4.23−4.01 (m, 4H), 3.14−3.00 (m, 1H), 2.79 (dd, *J* = 4.2, 1.5 Hz, 1H), 2.70−2.53 (m, 2H), 2.22 (ddd, *J* = 12.8, 8.6, 2.4 Hz, 1H), 1.79−1.48 (m, 6H), 1.22 (dt, *J* = 17.8, 7.1 Hz, 7H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.94, 170.26, 154.68, 136.52, 135.82, 128.65, 128.55, 128.31, 123.98, 67.77, 66.88, 61.41, 60.60, 57.16, 53.31, 40.69, 35.18, 32.69, 32.48, 14.33, 14.15, 13.42. HRMS: calcd for $C_{24}H_{31}NNaO_6$, 452.2044; found, 452.2049.

(1*R,2*R**,3*S**,4*R**,6*S**)-2-Benzyloxycarbonylamino-6-[2-(2-hydroxy-phenyl)-vinyl]-bicyclo[2.2.1]heptane-2,3-dicarboxylic Acid Diethyl Ester (*rac*-26c).** The compound *rac*-**18a** (100 mg, 0.240 mmol), (2-hydroxybenzyl)triphenylphosphonium bromide (270 mg, 0.598 mmol), and 2.5 M *n*-butyllithium in hexane (0.48 mL, 1.198 mmol) were treated following Procedure D to yield after purification by flash chromatography (hexane/EtOAc: 80/20 to 50/50) a mixture of *E*/*Z*-isomers of *rac*-**26c** (92 mg, 0.181 mmol, 76%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39−7.26 (m, 6H), 7.07 (ddd, *J* = 9.6, 5.6, 1.7 Hz, 1H), 6.88−6.76 (m, 2H), 6.60 (d, *J* = 16.0 Hz, 0.74H), 6.11 (dd, *J* = 15.9, 7.4 Hz, 0.77H), 5.25−4.95 (m, 3H), 4.33−3.95 (m, 4H), 3.16−2.99 (m, *J* = 12.7, 7.8 Hz, 0.71H), 2.88 (s, 0.77H), 2.81 (d, *J* = 3.2 Hz, 0.68H), 2.65 (s, 0.87H), 2.34−2.22 (m, 0.73H), 2.16 (ddd, *J* = 12.8, 8.6, 2.0 Hz, 0.77H), 1.69 (dd, *J* = 38.2, 10.7 Hz, 2H), 1.49−1.36 (m, 1H), 1.32−1.10 (m, 7H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.98, 170.33, 154.87, 153.12, 136.38, 135.96, 128.60, 128.50, 128.36, 128.30, 128.24, 128.12, 127.19, 124.85, 123.63, 120.61, 115.94, 67.74, 66.95, 61.48, 60.72, 60.58, 57.15, 52.72, 40.74, 38.20, 35.02, 31.35, 29.78, 14.27, 14.06. HRMS: calcd for $C_{29}H_{33}NNaO_7$, 530.2149; found, 530.2154.

(1*R,2*R**,3*S**,4*R**,6*S**)-2-Benzyloxycarbonylamino-6-[2-(4-chloro-phenyl)-vinyl]-bicyclo[2.2.1]heptane-2,3-dicarboxylic Acid Diethyl Ester (*rac*-26d).** The compound *rac*-**18a** (457 mg, 1.10 mmol) and (4-chlorobenzyl)triphenylphosphonium chloride (1.159 g, 2.737 mmol) were treated following Procedure D to yield after purification by flash chromatography (hexane/EtOAc: 80/20) a mixture of *E*/*Z*-isomers (ratio: 64/36) of *rac*-**26d** (294 mg, 0.559 mmol, 51%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55−7.28 (m, 9H), 6.52−6.33 (m, 1H), 6.24 (dd, *J* = 15.8, 7.4 Hz, 0.66H), 5.67 (dd, *J* = 11.5, 9.6 Hz, 0.37H), 5.39 (d, *J* = 5.1 Hz, 1H), 5.32−5.10 (m, 2H), 4.42−3.99 (m, 4H), 3.41−3.19 (m, 1H), 3.05 (s, 0.51H), 3.01−2.86 (m, 1H), 2.79 (br s, 1H), 2.52−2.37 (m, 0.49H), 2.30−2.21 (m, 0.51H), 2.03−1.68 (m, 2H), 1.59−1.21 (m, 7H), 1.12 (t, *J* = 7.1 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.73, 170.26, 154.83, 137.28, 136.48, 135.82, 132.56, 130.16, 128.67, 128.43, 128.32, 127.38, 127.10, 67.91, 66.91, 61.66, 60.61, 57.14, 53.26, 40.29, 35.36, 33.95, 32.85, 14.30, 13.77. HRMS: calcd for $C_{29}H_{32}ClNNaO_6$, 548.1810; found, 548.1819.

(1*R,2*R**,3*S**,4*R**,6*S**)-2-Benzyloxycarbonylamino-6-propenyl-bicyclo[2.2.1]heptane-2,3-dicarboxylic Acid (*rac*-27a).** The compound *rac*-**26a** (123 mg, 0.317 mmol) was treated following Procedure E to yield after purification by preparative RP-HPLC (A/C: 60/40 to 30/70 in 30 min, λ = 214 nm, t_R = 17.8 min) a mixture of *E*/*Z*-isomers of *rac*-**27a** (49 mg, 0.131 mmol, 46%) as a white solid. ^{1}H NMR (300 MHz, MeOD) δ 7.68−7.00 (m, 5H), 5.51−5.19 (m, 2H), 5.08−4.96 (m, 2H), 3.55 (s, 1H), 2.66−2.53 (m, 1H), 2.46 (d, *J* = 2.8 Hz, 1H), 2.31 (d, *J* = 10.7 Hz, 1H), 2.15 (s, 1H), 1.79−1.65 (m, 1H), 1.59 (d, *J* = 5.9 Hz, 4H), 1.29 (dt, *J* = 12.4, 4.4 Hz, 1H). ^{13}C NMR (75 MHz, MeOD) δ 176.06, 174.97, 157.77, 138.20, 136.42, 129.35, 128.80, 128.64, 124.67, 70.60, 67.40, 54.08, 53.87, 44.17, 38.32, 36.10, 34.59, 13.19. HRMS: calcd for $C_{20}H_{23}NNaO_6$, 396.1418; found, 396.1421.

(1*R,2*R**,3*S**,4*R**,6*S**)-2-Benzyloxycarbonylamino-6-but-1-enyl-bicyclo[2.2.1]heptane-2,3-dicarboxylic Acid (*rac*-27b).** The compound *rac*-**18a** (200 mg, 0.479 mmol) and propyltriphenylphosphonium bromide (461 mg, 1.198 mmol) were treated following Procedure D to yield after purification by flash chromatography *rac*-**26b** as a colorless oil (169 mg, purity 65% (NMR)). This product (140 mg) was treated as described above for the synthesis of *rac*-**21a** to yield after purification by preparative RP-HPLC (A/C: 60/40 to 40/60 in 20 min, λ = 214 nm, t_R = 12.0 min) a mixture of *E*/*Z*-isomers of *rac*-**27b** (60 mg, 0.155 mmol, 39%) as a white solid. ^{1}H NMR (300 MHz, MeOD) δ 7.48−7.16 (m, 5H), 5.38−5.16 (m, 2H), 5.10−4.94 (m, 2H), 3.54 (d, *J* = 1.4 Hz, 1H), 2.64−2.51 (m, 1H), 2.45 (d, *J* = 2.7 Hz, 1H), 2.42−2.25 (m, 1H), 2.14 (s, 1H), 2.11−1.95 (m, 2H), 1.69 (ddd, *J* = 12 Hz, 7.5 Hz, 2.2 Hz, 1H), 1.59 (dd, *J* = 10.7, 0.9 Hz, 1H), 1.34−1.23 (m, 1H), 0.94 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (75 MHz, MeOD) δ 176.05, 174.93, 157.74, 138.16, 134.83, 132.38, 129.34, 128.79, 128.62, 70.59, 67.39, 54.04, 44.14, 38.45, 36.05, 34.70, 21.74, 14.56. HRMS: calcd for $C_{13}H_{22}NO_4$, 256.1543; found, 256.1549.

(1*R,2*R**,3*S**,4*R**,6*S**)-2-Benzyloxycarbonylamino-6-[2-(4-chloro-phenyl)-vinyl]-bicyclo[2.2.1]heptane-2,3-dicarboxylic Acid (*rac*-27d).** The compound *rac*-**26d** (96 mg, 0.182 mmol) was treated following Procedure E to yield after purification by preparative RP-HPLC (A/C: 50/50 to 30/70 in 20 min, λ = 214 nm, t_R = 14.0 min) a mixture of *E*/*Z*-isomers (ratio: 51/49) of *rac*-**27d** (37 mg, 0.079 mmol, 43%) as a white solid. ^{1}H NMR (300 MHz, MeOD) δ 7.46−7.07 (m, 9H), 6.38−6.25 (m, 1H), 6.18 (dd, *J* = 15.8, 7.4 Hz, 0.49H), 5.59 (dd, *J* = 11.5, 10.0 Hz, 0.48H), 5.09−4.95 (m, 2H), 3.62−3.47 (m, 1H), 2.88−2.74 (m, 1H), 2.56−2.25 (m, 3.47H), 1.83−1.62 (dd, *J* = 20.4, 10.0 Hz, 2H), 1.51 (dt, *J* = 12.6, 4.4 Hz, 0.53H), 1.38 (dt, *J* = 12.5, 4.3 Hz, 0.64H). ^{13}C NMR (75 MHz, MeOD) δ 175.99, 174.84, 157.80,

138.22, 137.87, 137.60, 136.98, 135.89, 133.66, 133.57, 131.26, 129.59, 129.41, 129.37, 129.09, 128.82, 128.68, 128.65, 128.55, 127.90, 70.71, 70.46, 67.42, 54.32, 53.48, 44.17, 40.62, 38.90, 36.94, 36.37, 36.05, 35.77. HRMS: calcd for $C_{25}H_{24}ClNNaO_6$, 492.1184; found, 492.1186.

(1*R,2*R**,3*S**,4*R**,6*R**)-2-Amino-6-propyl-bicyclo[2.2.1]-heptane-2,3-dicarboxylic Acid Trifluoroacetate (*rac*-28a).** The compound *rac*-**27a** (22 mg, 0.059 mmol) was treated following Procedure C (using CH_3CN/H_2O (1/1) + 0.5% TFA for washing) to yield *rac*-**28a** (20 mg, 0.056 mmol, 96%) as a pale-yellow solid. ^{1}H NMR (300 MHz, MeOD) δ 3.41 (s, 1H), 2.69 (s, 1H), 2.37 (s, 1H), 2.09−1.90 (m, 1H), 1.81−1.58 (m, 3H), 1.45−1.15 (m, 5H), 0.90 (t, J = 6.7 Hz, 3H). ^{13}C NMR (75 MHz, DMSO) δ 172.42, 170.48, 67.40, 51.26, 50.35, 41.27, 37.28, 35.84, 34.77, 33.26, 20.21, 13.72. HRMS: calcd for $C_{12}H_{20}NO_4$, 242.1387; found, 242.1391.

(1*R,2*R**,3*S**,4*R**,6*R**)-2-Amino-6-butyl-bicyclo[2.2.1]heptane-2,3-dicarboxylic Acid Trifluoroacetate (*rac*-28b).** The compound *rac*-**27b** (35 mg, 0.090 mmol) was treated following Procedure C (using CH_3CN/H_2O (1/1) + 0.5% TFA for washing) to yield *rac*-**28b** (33 mg, 0.089 mmol, quant) as a white solid. ^{1}H NMR (300 MHz, MeOD) δ 2.61 (s, 1H), 2.16 (s, 1H), 1.98 (s, 1H), 1.77 (d, J = 11.4 Hz, 2H), 1.56 (d, J = 10.7 Hz, 1H), 1.46−1.03 (m, 8H), 0.88 (t, J = 6.8 Hz, 3H). ^{13}C NMR (75 MHz, DMSO) δ 172.51, 158.04 (TFA), 115.81 (TFA), 115.34 (TFA), 67.61, 51.55, 50.84, 41.15, 36.02, 35.12, 34.98, 33.33, 29.44, 21.97, 13.92. HRMS: calcd for $C_{13}H_{22}NO_4$, 256.1543; found, 256.1549.

(1*R,2*R**,3*S**,4*R**,6*R**)-2-Amino-6-[2-(2-hydroxy-phenyl)-ethyl]-bicyclo[2.2.1]heptane-2,3-dicarboxylic Acid Trifluoroacetate (*rac*-28c).** The compound *rac*-**26c** (90 mg, 0.177 mmol) was treated following Procedure E to yield after purification by preparative RP-HPLC (A/C: 65/35 to 35/65 in 30 min, λ = 214 nm, t_R = 16.2 min) *rac*-**27c** as a white solid (45 mg, dicarboxylic acid with side product, purity ~80%). The dicarboxylic acid (30 mg) was treated following Procedure C (using CH_3CN/H_2O (1/1) + 0.5% TFA for washing) to afford after purification by RP-HPLC (A/B: 70/30 to 40/60 in 30 min, λ = 254 nm, t_R = 26.2 min) the TFA salt of *rac*-**28c** (14 mg, 0.032 mmol, 27%) as a white solid. ^{1}H NMR (300 MHz, MeOD) δ 6.94−6.80 (m, 2H), 6.72−6.48 (m, 2H), 3.35 (s, 1H), 2.61−2.51 (m, 1H), 2.44 (ddd, J = 8.7, 6.8, 2.2 Hz, 2H), 2.26 (s, 1H), 1.92−1.73 (m, 2H), 1.70−1.29 (m, 4H), 1.27−1.12 (m, 2H). ^{13}C NMR (75 MHz, DMSO) δ 172.91, 171.40, 154.90, 129.63, 128.09, 126.64, 118.80, 114.81, 67.47, 51.54, 50.88, 41.09, 35.92, 35.81, 35.47, 33.45, 28.01. HRMS: calcd for $C_{17}H_{22}NO_5$, 320.1492; found, 320.1499.

(1*R,2*R**,3*S**,4*R**,6*R**)-2-Amino-6-[2-(4-chloro-phenyl)-ethyl]-bicyclo[2.2.1]heptane-2,3-dicarboxylic Acid Trifluoroacetate (*rac*-28d).** The compound *rac*-**27d** (20 mg, 0.043 mmol) and 10% Pt on charcoal in EtOH (2 mL) were stirred under a 1 atm H_2 atmosphere for 4 h. The reaction was filtered through a pad of Celite, the residue was washed with CH_3CN/H_2O (1/1) + 0.5% TFA, and the filtrate was evaporated to dryness under vacuum. AcOH/HCl (2/1, 5 mL) was added, and the reaction was stirred at 80 °C for 90 min. After evaporation of the solvent, the crude product was purified by RP-HPLC (A/B: 50/50 to 30/70 in 20 min, λ = 254 nm, t_R = 5.2 min) to afford the TFA salt of *rac*-**28d** (13 mg, 0.013 mmol, 67%) as a white solid. ^{1}H NMR (300 MHz, MeOD) δ 7.37−7.04 (m, 4H), 3.45 (s, 1H), 2.71−2.62 (m, 1H), 2.56 (t, J = 7.7 Hz, 2H), 2.31 (s, 1H), 2.02−1.80 (m, 2H), 1.80−1.38 (m, 4H), 1.28−1.15 (m, 1H). ^{13}C NMR (75 MHz, DMSO) δ 173.51, 141.07, 130.16, 128.07, 67.55, 51.87, 51.19, 40.93, 37.46, 35.90, 34.89, 33.53, 32.68. HRMS: calcd for $C_{17}H_{20}ClNO_4$, 337.1132; found, 337.1138.

2,4-Dioxo-1,3-diaza-spiro[4.4]nonane-6-carboxylic Acid (29). The synthesis was adapted from literature.[31] 2-Oxo-cyclopentanecarboxylic acid ethyl ester (1.39 mL, 9.60 mmol) was added to a suspension of ammonium carbonate (3.97 g, 41.37 mmol) and KCN (1.50 g, 23.06 mmol) in EtOH/H_2O (10.2 mL/10.2 mL). The mixture was heated to 60 °C and stirred for 2 days. After evaporation of EtOH, the aqueous residue was acidified to pH 3 with 1 N HCl and extracted with EtOAc (4×). The organic phases were combined, washed with brine, dried over Na_2SO_4, and evaporated to provide 1.57 g of a white solid. Purification of 300 mg of this product by flash chromatography (EtOAc/hexane: 5/5 to 8/2) gave 2-cyano-2-hydroxycyclopentanecarboxylic acid (66 mg) and a mixture of **29** and its ethyl ester (161 mg). A 505 mg portion of the 1.57 above was stirred in 4 N HCl (10 mL) at 40 °C for 24 h. The solvent was evaporated under reduced pressure to give crude **29** (376 mg) as a white solid, which was used without further purification in the next step. Purification of an 80 mg portion by RP-HPLC (A/B: 100/0 to 70/30 in 30 min) gave pure **29** (50 mg, 0.252 mmol, 38%, diastereomeric ratio: 84/16) as a white solid. ^{1}H NMR (300 MHz, MeOD) δ 3.23 (dd, J = 10.4, 8.9 Hz, 1H × 0.84), 3.11 (dd, J = 10.3, 9.3 Hz, 1H × 0.16), 2.34−1.69 (m, 6H). ^{13}C NMR (75 MHz, DMSO) δ 177.47, 172.58, 171.62, 156.73, 69.87, 68.72, 52.18, 37.39, 36.90, 27.25, 25.78, 22.14, 21.85. HRMS: calcd for $C_8H_{10}N_2NaO_4$, 221.0538; found, 221.0539.

1-Amino-cyclopentane-1,2-dicarboxylic Acid Hydrochloride Salt (11). Crude **29** (70 mg, 0.353 mmol) was stirred in a sealed tube at 130 °C for 5 h. The solvent was removed under reduced pressure, and the yellow solid was purified by anion exchange chromatography (Dowex 1 × 8, 100−200 mesh, AcO form). The crude was dissolved in a minimum amount of H_2O, adjusted to pH 9 with 1 N NaOH and deposited on the column. After washing the resin with H_2O, the amino acid was eluted with 0.5 M AcOH and the ninhydrine positive fractions were evaporated to dryness. After treatment with diluted HCl (3×), **11** (23 mg, 0.133 mmol, 19% overall yield, diastereomeric ratio: 58/42) was obtained as a white solid. ^{1}H NMR (300 MHz, D_2O) δ 3.43 (t, J = 9.0 Hz, 1H × 0.58), 3.10 (t, J = 9.4 Hz, 1H × 0.42), 2.43−1.68 (m, 6H). ^{13}C NMR (75 MHz, D_2O) δ 174.82, 174.26, 173.49, 173.36, 66.38, 66.26, 52.96, 51.01, 36.21, 27.78, 26.63, 22.39, 21.64. HRMS: calcd for $C_7H_{12}NO_4$, 174.0766; found, 174.0771.

2,4-Dioxo-1,3-diaza-spiro[4.5]decane-6-carboxylic Acid (30). The synthesis was adapted from literature.[32,33] Ammonium carbonate (1.86 g, 19.4 mmol) and KCN (459 mg, 7.05 mmol) were added to ethyl-2-oxocyclohexane carboxylate (0.94 mL, 5.9 mmol) in EtOH/H_2O (6.3 mL/6.3 mL). The reaction was stirred at 50 °C for 8 h. After evaporation of EtOH, the suspension was extracted with EtOAc (3 times). The combined organic phases were washed with H_2O and brine, dried over $MgSO_4$, and evaporated to yield crude ethyl ester (1.20 g) as a white solid. The crude product (150 mg) was stirred in 4 N HCl (3 mL) at 50 °C for 2 days. Evaporation of the solvent and purification of the residue by RP-HPLC (A/B: 90/10 to 60/40 in 30 min) yielded **30** (81 mg, 0.382 mmol, 52%, diastereomeric ratio: 84/16) as a white solid. ^{1}H NMR (300 MHz, MeOD) δ 2.83 (dd, J = 13.0, 4.2 Hz, 1H × 0.84), 2.62 (dd, J = 12.9, 4.0 Hz, 1H × 0.16), 2.33−1.19 (m, 8H). ^{13}C NMR (75 MHz, MeOD) δ 180.73, 179.21, 175.63, 174.40, 159.75, 159.51, 65.44, 62.59, 52.20, 47.43, 36.75, 36.62, 27.09, 25.83, 25.53, 25.33, 22.01, 21.94. HRMS: calcd for $C_9H_{12}N_2NaO_4$, 235.0695; found, 235.0696.

1-Amino-cyclohexane-1,2-dicarboxylic Acid Hydrochloride Salt (10). 30 (69 mg, 0.289 mmol) was stirred in 6 N HCl (2 mL) at 110 °C for 7 days. After removal of the solvent under vacuum, the crude was purified by cation exchange chromatography (Dowex 50 × 8, 20−50 mesh, H^+ form). The yellow solid was loaded on the column at pH 2 in a minimum amount of H_2O, the resin was washed with MeOH and H_2O, and the product was eluted with 0.5 N NaOH. The ninhydrine active fractions were combined and evaporated to dryness. The residue was purified by anion exchange chromatography (Dowex 1 × 8, 100−200 mesh, AcO form). It was loaded at pH 10 on the column, the resin was washed with MeOH and H_2O, and the product was eluted with 1 N AcOH. The ninhydrine active fractions were evaporated and the product was treated with 1 N HCl (3 times) to yield the HCl salt of **10** (23 mg, 0.104 mmol, 36%, diastereomeric ratio 81/19) as a white solid. ^{1}H NMR (300 MHz,

D_2O) δ 3.27 (dd, J = 12.9, 4.2 Hz, 1H × 0.81), 2.83 (dd, J = 12.1, 5.2 Hz, 1H × 0.19), 2.31−1.28 (m, 8H). ^{13}C NMR (75 MHz, D_2O) δ 176.03, 175.93, 174.25, 172.67, 60.89, 60.21, 47.03, 45.49, 33.32, 32.42, 31.10, 25.42, 23.56, 23.34, 20.66, 18.93. HRMS: calcd for $C_8H_{14}NO_4$, 188.0923; found, 188.0918.

Activity Assays. All compounds tested had ≥95% purity and were conditioned as 8−100 mM stock solutions in DMSO.

Glutamate Transporter Assays.[46] **[^{3}H]-Glutamate Uptake in Human GLT-1-Transfected HEK293 Cells.** The human embryonic kidney cell line (HEK293) was purchased from American Type Culture Collection (ATCC) and was cultured following the instruction of ATCC. Cells were placed in 12-well plates (1.5×10^5 cells/well) with poly-D-lysine coating. The human GLT-1 cDNA in pcDNA3.1 mammalian expression vector (Invitrogen) was introduced into the cells using FuGENE6 transfection reagent (Roche) following the manufacturer's instruction. After 1 day of transfection, the medium was removed and the cells were treated with 450 μL of HEPES-buffered saline (HBSS: 140 mM NaCl, 2.5 mM KCl, 1.2 mM $MgCl_2$, 1.2 mM $CaCl_2$, 1.2 mM K_2HPO_4, 10 mM glucose, 5 mM Tris, 10 mM HEPES (pH 7.4)) with or without glutamate or aspartate analogue. The uptake assay was started by adding 50 μL glutamate solution (100 μM) containing 0.2 μCi/well of [^{3}H]-glutamate (PerkinElmer). After 10 min uptake at room temperature, the medium was removed and the cells were washed with ice-cold HBSS twice then lysed with 0.5 M NaOH overnight. The radioactivity was measured by a β-counter using scintillation solution. To eliminate a possibility of nonspecific effect of the compound to the endogenous transporters in the HEK293 cells, HEK293 cells without vector transfection were analyzed with or without compound as well.

[^{3}H]-Glutamate Uptake in Human GLT-1-Expressed *Xenopus* Oocytes. *Xenopus laevis* oocytes were prepared as described previously. (1) The capped RNA was synthesized using SP6 polymerase (mMessage mMachine SP6 kit, Ambion) and human GLT-1 cDNA in pTLNII *Xenopus* expression vector linealized by Mlu I. The synthesized cRNA was injected into the oocytes (5 ng/oocyte) and were incubated with MBM (88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 0.82 mM $MgSO_4$, 0.66 mM $NaNO_3$, 0.75 mM $CaCl_2$, 10 mM HEPES (pH 7.5 with Tris base), 100 units/mL penicillin, 0.1 mg/mL streptomycin) for 2 days at 17 °C. The uptake assays of radiolabeled glutamate ([^{3}H]-glutamate, PerkinElmer) were carried out as described before (2) in Na^+ transport medium (100 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM HEPES (pH 7.5 with Tris base), 10 μM cold glutamate) in the presence of 0.5 μCi of [^{3}H]-glutamate for each group with or without the glutamate or aspartate analogue. To eliminate a possibility of nonspecific effect of the compound to the endogenous transporters in the oocytes, H_2O-injected oocytes were analyzed with or without compound as well. After 10 min uptake at room temperature, the oocytes were washed with ice-cold Na^+ transport medium, and 200 μL of this solution containing a single oocyte was placed into a new vial and treated with 200 μL of 10% SDS overnight. The radioactivity of this lysate was measured by a β-counter after the treatment of 5 mL of scintillation liquid overnight.

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Supporting Information Available: Statistics of virtual aspartate and glutamate library composition, images of ^1H and ^{13}C NMR spectra for all compounds, crystallography. This material is available free of charge via the Internet at http://pubs.acs.org.

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