Enantioselective Synthesis of Cyclothiazide Analogues: Novel Probes of the Stereospecific Actions of Benzothiadiazines at AMPA-Type Glutamate Receptors

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Abstract: The stereospecific interactions of the eight stereoisomers of dihydromethylcyclothiazide, an analogue of cyclothiazide, with AMPA-type glutamate receptors was investigated using electrophysiological methods that measured the ability of each stereoisomer to inhibit AMPA receptor desensitization. The eight stereoisomers were obtained by HPLC separation of four pairs of enantiomerically pure (>95% ee) diastereomers prepared from (1*R-exo*)-, (1*R-endo*)-, (1*S-exo*)-, and (1*S-endo*)-2-methylbicyclo[2.2.1]heptane-2-carboxaldehyde intermediates. The desensitization process was blocked most potently by [1*S*-[1α,2α(*R**),4α]]-dihydromethylcyclothiazide, one of the stereoisomers prepared from the (1*S-endo*)-carboxaldehyde. The smallest effects on the desensitization process were found for the four stereoisomers prepared from the (1*R-exo*)- and (1*R-endo*)-carboxaldehydes. Significant differences in the ability to inhibit desensitization were observed between all diastereomer pairs except those prepared from the (1*S-exo*)-carboxaldehyde.

Introduction

L-Glutamate is an important excitatory neurotransmitter in the mammalian central nervous system. Multiple categories of glutamate receptors have been identified, and each receptor is composed of combinations of individual protein subunits. The receptors subserve fast, glutamatergic synaptic signaling between central neurons and belong in the category of α-amino-2,3-dihydro-5-methyl-3-oxo-4-isoxazolepropanoic acid or AMPA-type glutamate receptors, which are glutamate-gated ion channels composed of subunits designated GluRA-D[1] or GluR1-4.[2−4] Functional properties such as agonist selectivity, calcium permeability, desensitization kinetics, and sensitivity to gating modifiers have been characterized for individual subunits by combining electrophysiology, pharmacology, and recombinant receptor expression systems. The differences between these functional properties suggest that functional diversity among native AMPA receptors may in part be determined by expression of various combinations of individual subunits.

For several years there has been sustained interest in the physiological importance of AMPA receptor desensitization in synaptic transmission.[5,6] Recently some potent and reversible 2*H*-1,2,4-benzothiadiazine 1,1-dioxide derivatives (Chart 1) have been used to study this phenomenon,[7,8] and its possible involvement in the processes of neuronal excitotoxicity[9−12] and learning.[13] From among the benzothiadiazine derivatives investigated thus far, the drug cyclothiazide [3-(bicyclo[2.2.1]-hept-5-en-2-yl)-6-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide, Chart 1] is of particular interest because of its high potency and receptor specificity. This drug reduces the desensitization of AMPA but not kainate receptors.[14] Moreover, cyclothiazide's effect on AMPA receptor desensitization is strongly influenced by alternative splicing of a 38 amino acid external domain of individual AMPA receptor subunits called the flip/flop region. Site directed mutation studies indicate that a single amino acid replacement within the flip/flop region eliminates cyclothiazide's effects.[15,16] Finally, cyclothiazide not only affects the desensitization properties of AMPA receptors but also allosterically reduces the antagonist actions of competitive and noncompetitive antagonists of AMPA receptors.[17]

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(1) Keinanen, K.; Wisden, W.; Sommer, B.; Werner, P.; Herb, A.; Verdoorn, T. A.; Sakmann, B.; Seeburg, P. H. *Science* **1990**, *249*, 556−560.
(2) Hollmann, M.; O'Shea-Greenfield, A.; Rogers, S. W.; Heinemann, S. *Nature* **1989**, *342*, 643−648.
(3) Bettler, B.; Boulter, J.; Hermans-Borgmeyer, I.; O'Shea-Greenfield, A.; Deneris, E. S.; Moll, C.; Borgmeyer, U.; Hollmann, M.; Heinemann, S. *Neuron* **1990**, *5*, 583−595.
(4) Boulter, J.; Hollmann, M.; O'Shea-Greenfield, A.; Hartley, M.; Deneris, E.; Maron, C.; Heinemann, S. *Science* **1990**, *249*, 1033−1037.
(5) Trussell, L. O.; Fischbach, G. D. *Neuron* **1989** *3*, 209−218.
(6) Zorumski, C. F.; Mennerick, S. J. In *Direct and Allosteric Control of Glutamate Receptors*; Palfreyman, M. G., Reynolds, I. J., Skolnick, P., Eds.; CRC Press: Boca Raton, 1994; pp 57−75.
(7) Yamada, K. A.; Rothman, S. M. *J. Physiol.* **1992**, *458*, 385−407.
(8) Yamada, K. A.; Tang, C.-M. *J. Neurosci.* **1993**, *13*, 3904−3915.
(9) Moudy, A. M.; Yamada, K. A.; Rothman, S. M. *Neuropharmacology* **1994**, *33*, 953−962.
(10) May, P. C.; Robison, P. M. *J. Neurochem.* **1993**, *60*, 1171−1174.
(11) David, J. C.; Rosengarten, A. M.; Yamada, K. A.; Goldberg, M. P. *Neurology* **1995**, *45*(Suppl), Abstract 527P, p A309.
(12) Bateman, M. C.; Bagwe, M. R.; Yamada, K. A.; Goldberg, M. P. *Soc. Neurosci. Abstr.* **1993**, *19*, Abstract 673.9, p 1643.
(13) Zivkovic, I.; Thompson, D. M.; Bertolino, M.; Uzunov, D.; DiBella, M.; Costa, E.; Guidotti, A. *J. Pharmacol. Exp. Ther.* **1995**, *272*, 300−309.
(14) Partin, K. M.; Patneau, D. K.; Winters, C. A.; Mayer, M. L.; Buonanno, A. *Neuron* **1993**, *11*, 1069−1082.
(15) Partin, K. M.; Patneau, D. K.; Mayer, M. L. *Mol. Pharmacol.* **1994**, *46*, 129−138.
(16) Partin, K. M.; Bowie, D.; Mayer, M. L. *Neuron* **1995**, *14*, 833−843.
(17) (a) Zorumski, C. F.; Yamada, K. A.; Price, M. T.; Olney, J. W. *Neuron* **1993**, *10*, 61−67. (b) Johansen, T. H.; Chaudhary, A.; Verdoorn, T. A. *Mol. Pharmacol.* **1995**, *48*, 946−955. (c) Partin, K. M.; Mayer, M. L. *Mol. Pharmacol.* **1996**, *49*, 142−148. (d) Yamada, K. A.; Turetsky, D. M. *Br. J. Pharmacol.* **1996**, in press.

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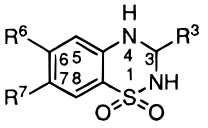
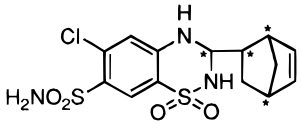
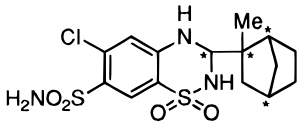
Chart 1



2*H*-1,2,4-BENZOTHIADIAZINE 1,1-DIOXIDE



CYCLOTHIAZIDE



DIHYDROMETHYLCYCLOTHIAZIDE

Since commercial preparations of this drug contain a mixture of eight stereoisomers (four racemic diastereomers),[18] it is necessary to first separate the four racemic diastereomers from each other by chromatography and then to separate each racemate into the enantiomer components using chiral chromatography if one is to obtain each stereoisomer for biological studies. Thus far, using separation procedures and NMR spectroscopy, it has been possible to separate, to make structural assignments, and to identify which racemic diastereomer is most active as a potentiatior of AMPA-mediated current.[19] In addition, the most active racemic diastereomer has been separated further into the [1*S*-[1α,2α(*S**),4α]]- and [1*R*-[1α,2α-(*R**),4α]]-stereoisomers, and it has been shown that there is a 100-fold difference in activity for these enantiomers.[19] The absolute configuration of the most active enantiomer has not been determined.

Further progress in understanding the specific actions of cyclothiazide or cyclothiazide-like drugs on AMPA receptor function requires that the absolute configuration of the most active stereoisomer of cyclothiazide, or a close structural analogue, be determined. Moreover, additional biological studies requiring large amounts of each stereoisomer necessitate the development of new synthetic methods that avoid the need for extensive separation (particularly chiral chromatography) techniques. To this end, we report a synthetic strategy that provides a practical route to each of the eight stereoisomers of dihydromethylcyclothiazide, a close structural analogue of cyclothiazide (see Charts 1 and 2). In addition, the results of electrophysiological studies that establish the absolute configuration of the dihydromethylcyclothiazide stereoisomer having the highest potency as an inhibitor of AMPA receptor desensitization as well as other structure−activity relationships are described.

Results and Discussion

The stereoisomeric composition of cyclothiazide results from the coupling, under strongly acidic conditions, of a mixture of racemic *exo*- and *endo*-bicyclo[2.2.1]hept-5-ene-2-carboxaldehyde to 4-amino-6-chloro-1,3-benzenedisulfonamide.[20] While it is possible to prepare both *exo*- and *endo*-bicyclo[2.2.1]hept-5-ene-2-carboxaldehyde in optically enriched form using enantioselective Diels−Alder reactions (see below for references), the potential for enolization of the carboxaldehyde group and carbonium ion rearrangements of this compound under the acidic conditions of the coupling reaction suggested to us that the stereochemical integrity of these carboxaldehydes might not be maintained in the cyclothiazide stereoisomer products.[21] Hence, we concluded that modifying the structure of the bicyclo[2.2.1]-hept-5-ene-2-carboxaldehyde to preclude the potential epimerization and racemization of the stereoisomer products during the coupling reaction was prudent. Thus, we chose to prepare by an enantiospecific (>95% ee) synthetic route the four stereoisomers of 2-methylbicyclo[2.2.1]heptane-2-carboxaldehyde. These stereoisomers were then subsequently utilized for the preparation of the four diastereomeric pairs of enantiomerically pure dihydromethylcyclothiazide stereoisomers (Chart 2).

From among the enantioselective Diels−Alder reactions catalyzed by chiral Lewis acids,[22−26] the best enantioselectivities are obtained, in most cases, using α,β-unsaturated aldehydes as dienophiles.[23,24] Several chiral catalysts can effect the Diels−Alder reaction between cyclopentadiene and methacrolein to yield 2-methylbicyclo[2.2.1]hept-5-ene-2-carboxaldehyde (**12** or **25**) as a mixture of *exo*- and *endo*-isomers with high yields and excellent enantioselectivities (90−99% ee) under very mild conditions.[24,26] Tartaric acid-derived chiral (acyloxy)borane (CAB) catalysts were chosen for enantioselective synthesis of compounds **12** and **25** because they can be prepared conveniently from commercially available 99% ee L-tartaric acid ([*R*-(*R**,*R**)]-2,3-dihydroxybutanedioic acid, **5**) and 98% ee D-tartaric acid ([*S*-(*R**,*R**)]-2,3-dihydroxybutanedioic acid, **6**).

Synthesis of Chiral Ligands 9 and 10. The asymmetric Diels−Alder reactions catalyzed by CAB catalysts have been developed by Yamamato and colleagues.[24c,25,26] CAB catalysts with [*R*-(*R**,*R**)]- and [*S*-(*R**,*R**)]-2-[(2,6-dimethoxybenzoyl)-

(18) Nusser, E.; Banerjee, A.; Gal, G. *Chirality* **1991**, *3*, 2−13.

(19) Cordi, A. A.; Serkiz, B.; Hennig, P.; Mahieu, J.-P.; Bobichon, C.; de Nanteuil, G.; Lepagnol, J. M. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1957−1960.

(20) Whitehead, C. W.; Traverso, J. J.; Sullivan, H. R.; Marshall, F. J. *J. Org. Chem.* **1961**, *26*, 2814−2818.

(21) A preliminary experiment showed that some epimerization of the carboxaldehyde group of *exo*-2-methylbicyclo[2.2.1]hept-5-ene-2-carboxaldehyde occurred during the reaction of this compound with 4-amino-6-chloro-1,3-benzenedisulfonamide.

(22) (a) Hashimoto, S. I.; Komeshima, N.; Koga, K. *J. Chem. Soc., Chem. Commun.* **1979**, 437−438. (b) Kelly, T. R.; Whiting, A.; Chandrakumar, N. S. *J. Am. Chem. Soc.* **1986**, *108*, 3510−3512. (c) Takemura, H.; Komeshima, N.; Takahashi, I.; Hashimoto, S. I.; Ikota, N.; Tomiota, K.; Koga, K. *Tetrahedron Lett.* **1987**, *28*, 5687−5690. (d) Bir, G.; Kaufmann, D. *Tetrahedron Lett.* **1987**, *28*, 777−780. (e) Corey, E. J.; Imwinkelried, R.; Pikul, S.; Xiang, Y. B. *J. Am. Chem. Soc.* **1989**, *111*, 5493−5495. (f) Sartor, D.; Saffrich, J.; Helmchen, G.; Richards, C. J.; Lambert, H. *Tetrahedron: Asymmetry* **1991**, *2*, 639−642. (g) Corey, E. J.; Imai, N.; Zhang, H. Y. *J. Am. Chem. Soc.* **1991**, *113*, 728−729. (h) Devine, P. N.; Oh, T. *J. Org. Chem.* **1992**, *57*, 396−399. (i) Itsuno, S.; Katsuhiro, K.; Watanabe, K.; Koizumi, T.; Ito, K. *Tetrahedron: Asymmetry* **1994**, *5*, 523−526. (j) Odenkirk, W.; Bosnich, B. *J. Chem. Soc., Chem. Commun.* **1995**, 1181−1182.

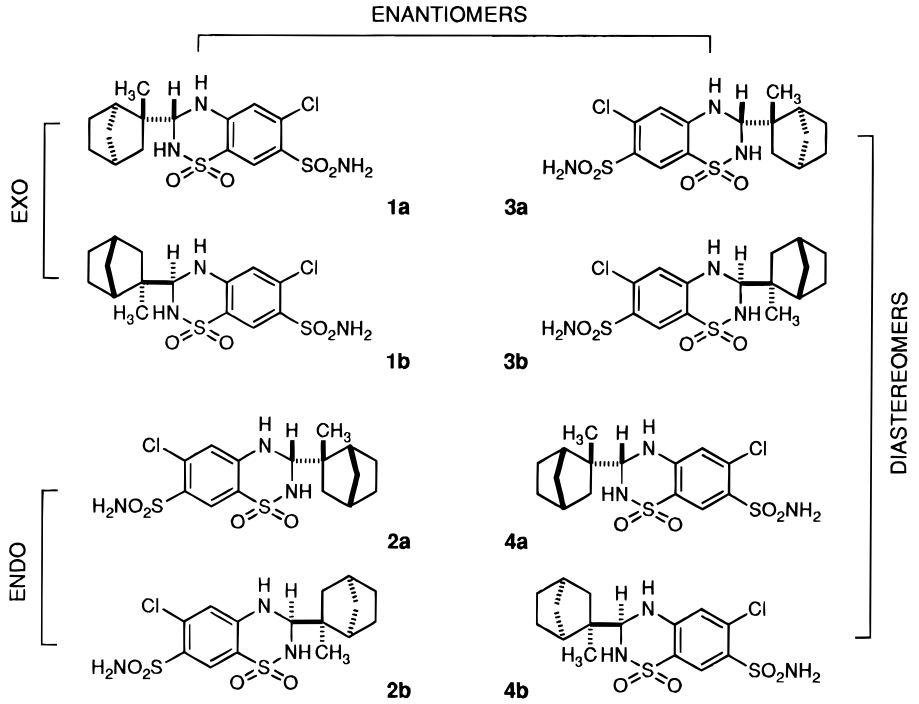
(23) (a) Corey, E. J.; Loh, T. P.; *J. Am. Chem. Soc.* **1991**, *113*, 8966−8967. (b) Corey, E. J.; Loh, T. P.; Roper, T. D.; Azimioara, M. D.; Noe, M. C. *J. Am. Chem. Soc.* **1992**, *114*, 8290−8292. (c) Corey, E. J.; Roper, T. D.; Ishihara, K.; Sarakinos, G. *Tetrahedron Lett.* **1993**, *34*, 8399−8402. (d) Maruoka, K; Murase, N.; Yamamoto, H. *J. Org. Chem.* **1993**, *58*, 2938−2939.

(24) (a) Bao, J.; Wulff, W. D. *J. Am. Chem. Soc.* **1993**, *115*, 3814−3815. (b) Kundig, P.; Bourdin, B.; Bernardinelli, G. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1856−1858. (c) Ishihara, K.; Yamamoto, H. *J. Am. Chem. Soc.* **1994**, *116*, 1561−1562.

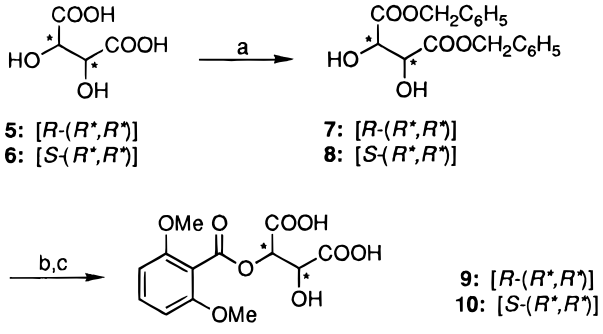
(25) (a) Furuta, K.; Miwa, Y.; Iwanaga, K.; Yamamoto, H. *J. Am. Chem. Soc.* **1988**, *110*, 6254−6255. (b) Furuta, K.; Kanematsu, A.; Yamamoto, H.; Takaoka, S. *Tetrahedron Lett.* **1989**, *30*, 7231−7232. (c) Ishihara, K.; Gao, Q.; Yamamoto, H. *J. Org. Chem.* **1993**, *58*, 6917−6919.

(26) (a) Furuta, K.; Shimizu, S.; Miwa, Y.; Yamamoto, H. *J. Org. Chem.* **1989**, *54*, 1481−1483. (b) Ishihara, K.; Gao, Q.; Yamamoto, H. *J. Am. Chem. Soc.* **1993**, *115*, 10412−10413.

Chart 2. The Eight Stereoisomers of Dihydromethylcyclothiazide

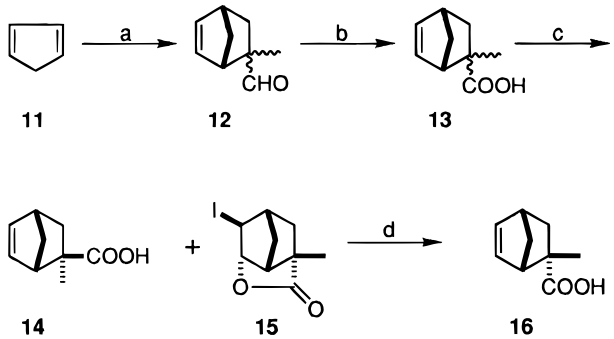


Scheme 1[a]



[a] Reagents: (a) benzyl alcohol, reflux, 2 h; (b) 2,6-dimethoxybenzoic acid, trifluoroacetic anhydride, room temperature, 1 h; (c) 10% Pd−C, H_2, 100 psi, 3 h.

Scheme 2[a]



[a] Reagents: (a) methacrolein, **9**/BH_3·THF, −78 °C, 6 h; (b) Jones reagent, 0 °C, ∼1 h; (c) I_2−KI−$NaHCO_3$−KOH, room temperature, 30 min; (d) Zn−HOAc, room temperature, 3 h.

oxy-3-hydroxybutanedioic acid (Scheme 1, **9** and **10**, respectively) as ligands are optimal for the preparation of compounds **12** and **25**, respectively (*exo*:*endo* ∼9:1, 87% yield and 96% ee, respectively).[26] Compounds **9** and **10** were prepared by a variation of the method reported for the synthesis of compound **9**.[27] Heating a mixture of acid **5** (1 equiv) with benzyl alcohol (4 equiv) in the absence of solvent and acidic catalyst at 200 °C for 1 h and then at 225 °C for another 1 h while removing water using a Dean-Stark apparatus gave, after chromatographic purification, a 93% yield of diester **7** as fine white crystals. Diester **8** was obtained in 95% isolated yield from acid **6** by using the same procedure. Monoacyloxylation of diesters **7** and **8** with 2,6-dimethylbenzoic acid in the presence of trifluoroacetic anhydride gave intermediate triesters (55% and 65%, respectively, structures not shown) which were then hydrogenolyzed on a Pd−C catalyst to give products **9** (99%) and **10** (96%), respectively.[25c]

Synthesis of Dihydromethylcyclothiazide Stereoisomer Pairs 1a,1b and 2a,2b. As indicated in Scheme 2, the Diels−Alder reaction between cyclopentadiene (**11**) and methacrolein catalyzed at −78 °C by CAB (prepared from compound **9** and BH_3·THF) yielded a mixture of (1*S*-*exo*)- and (1*S*-*endo*)-2-methylbicyclo[2.2.1]hept-5-ene-2-carboxaldehydes (**12**) which was immediately oxidized (Jones reagent) without purification or characterization to the corresponding acids **13** (74% yield overall for the two steps). Reaction of acids **13** with an aqueous solution of I_2−KI−$NaHCO_3$ (1:4:6 mol ratio) in aqueous NaOH solution converted acids **13** into a readily separated mixture of (1*S*-*exo*)-acid **14** (77%) and iodolactone **15** (10%).[28] Iodolactone **15** was then converted smoothly into (1*S*-*endo*)-acid **16** using zinc dust in HOAc at room temperature (92%).[29]

Acid **14** was found to have $[\alpha]^{22}_D = -66.2°$, which indicated an enantiomeric purity of 98.3% ee.[30] Although acid **16** is expected to have the same optical purity as acid **14**, this conclusion was verified by a ^{1}H NMR experiment (Figure 1) utilizing iodolactone **15** and the chiral alcohol (*R*)-2,2,2-trifluoro-

(27) Furuta, K.; Gao, Q.-z.; Yamamoto, H. *Org. Synth.* **1992**, *71*, 86−94.

(28) (a) Rondestvedt, C. S., Jr.; Ver Nooy, C. D. *J. Am. Chem. Soc.* **1955**, *77*, 4878−4883. (b) Meek, J. S.; Trapp, M. B. *J. Am. Chem. Soc.* **1957**, *79*, 3909−3912. (c) Berson, J. A.; Remanick, A. *J. Am. Chem. Soc.* **1961**, *83*, 4947−4956.

(29) (a) Berson, J. A.; Ben-Efraim, D. A. *J. Am. Chem. Soc.* **1959**, *81*, 4083−4087. (b) Kirmse, W.; Siegfried, R. *J. Am. Chem. Soc.* **1983**, *105*, 950−956.

(30) Berson, J. A.; Walla, J. S.; Remanick, A.; Suzuki, S.; Reynolds-Warnhoff, P.; Willner, D. *J. Am. Chem. Soc.* **1961**, *83*, 3986−3997.

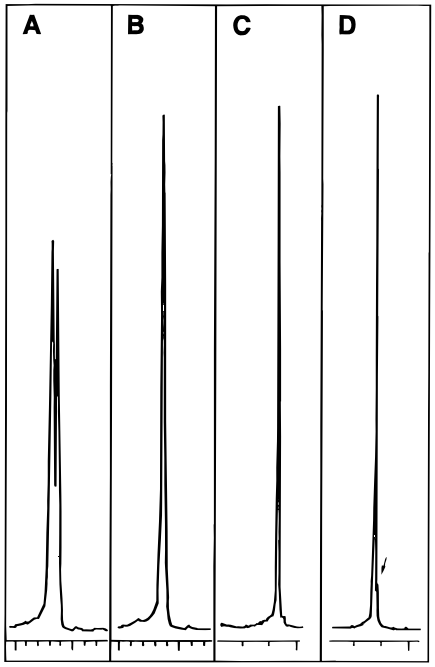
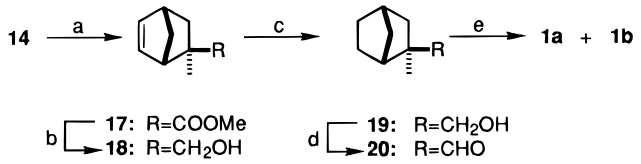


Figure 1. Digitized NMR spectra of the methyl groups of iodolactones **15** and **28** recorded in the presence of the chiral alcohol (*R*)-2,2,2-trifluoro-1-(9-anthryl)ethanol in $CDCl_3$. (A) Racemic mixture of idodolactones **15** and **28** derived from the Diels−Alder reaction of cyclopentadiene and methacrolein carried out in the absence of a CAB catalyst. The resonances are at δ 1.14 and δ 1.35. (B) Iodolactone **15** ($[\alpha]^{22}_D = -103.5°$) derived from the same Diels−Alder reaction carried out in the presence of CAB catalyst prepared from ligand **9**. (C) Iodolactone **28** ($[\alpha]^{22}_D = +104.8°$) derived from the same Diels−Alder reaction carried out in the presence of CAB catalyst prepared from ligand **10**. (D) Iodolactone **28** containg 10 mol % of a racemic mixture of iodolactones **15** and **28**. A small peak (indicated by the arrow) for iodolactone **15** is detectable upfield of the large peak recorded for iodolactone **28**. The scale of the *x*-axis in panels A and B is expanded by a factor of 2.3 relative to that shown in panels C and D.

Scheme 3[a]

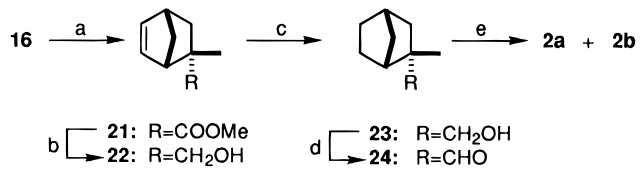


[a] Reagents: (a) CH_2N_2, room temperature; (b) DIBALH, room temperature, 4 h; (c) 10% Pd−C, H_2, 45 psi, room temperature, overnight; (d) PCC−NaOAc, room temperature, 2 h; (e) 4-amino-6-chloro-1,3-benzenedisulfonamide, EtOH−6 N HCl (1:1), room temperature, overnight.

1-(9-anthryl)ethanol in $CDCl_3$.[31] Control experiments indicated that 5% of the enantiomeric iodolactone **28** (vide infra) was readily detected by this method.

Acid **14** (Scheme 3) was methylated with diazomethane to give ester **17** (95%) which was then reduced with DIBALH to give olefinic alcohol **18** (84%). Hydrogenation of the double bond in alcohol **18** using a Pd−C catalyst yielded saturated alcohol **19** (83%). Without purification, crude aldehyde **20**, obtained by NaOAc buffered PCC oxidation of compound **19**, was reacted with 4-amino-6-chloro-1,3-benzenedisulfonamide[20] to give an ∼1:1 ratio of the first pair of enantiomerically pure (i.e., >95% ee) diastereomer products **1a** and **1b**. Similarly, an ∼1:1 ratio of the second pair of enantiomerically pure diastereomer products **2a** and **2b** was obtained from acid **16** utilizing the analogous reaction sequence outlined in Scheme 4.

Scheme 4[a]



[a] Reagents: As described in the legend to Scheme 3.

Chart 3

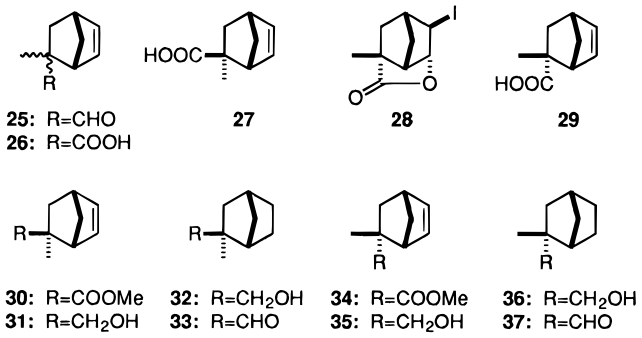
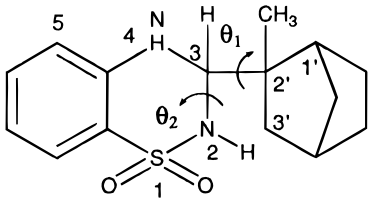


Chart 4



Synthesis of Dihydromethylcyclothiazide Stereoisomer Pairs 3a,3b and 4a,4b. Under the same reaction conditions, but using the catalyst made from ligand **10** and BH_3·THF, a mixture of (1*R-exo*) and (1*R-endo*)-2-methylbicyclo[2.2.1]hept-5-ene-2-carboxaldehydes (**25**, Chart 3) was prepared. Oxidation of carboxaldehydes **25** with Jones reagent followed by iodolactone separation gave (1*R-exo*)-acid **27** (76% yield, $[\alpha]^{22}_D = +65.6°$, indicating 97.5% ee[30]) and iodolactone **28** (12% yield, >95% ee as determined by the ^{1}H NMR experiment summarized in Figure 1). The (1*R-exo*)-acid **27** was converted in four steps (**27** → **30** → **31** → **32** → **33**) into (1*S-exo*)-carboxaldehyde **33**, and compound **33** was converted into an ∼1:1 ratio of the third pair of enantiomerically pure diastereomer products **3a** and **3b**. Finally, (1*R-endo*)-acid **29** was converted in four steps (**29** → **34** → **35** → **36** → **37**) into (1*S-endo*)-carboxaldehyde **37**, and compound **37** was converted into an ∼1:1 ratio of the fourth pair of enantiomerically pure diastereomer products **4a** and **4b**.

Conformational Analysis and Assignments of Absolute Configuration

A Monte Carlo conformational search[32] was performed on each of the diastereomers using the simplified model compound shown in Chart 4. Because high quality parameters were not available for aryl sulfonamides, the low energy conformations were reminimized in vacuo using AM1. Figure 2 shows the minimum energy conformations of compounds **38a** and **38b** which are the model compounds with stereochemistries corresponding to those of the dihydromethylcyclothiazide diastereomer pair **4a** (the most active stereoisomer, *vide infra*) and **4b**.

(31) (a) Parker, D. *Chem. Rev.* **1991**, *91*, 1441−1457. (b) Pirkle, W. H.; Sikkenga, D. L.; Pavlin, M. S. *J. Org. Chem.*, **1977**, *42*, 384−387. (c) Pirkle, W. H.; Sikkenga, D. L. *J. Org. Chem.*, **1977**, *42*, 1370−1374.

(32) Goodman, J. M.; Still, W. C. *J. Comput. Chem.* **1991**, *12*, 1110−1117.

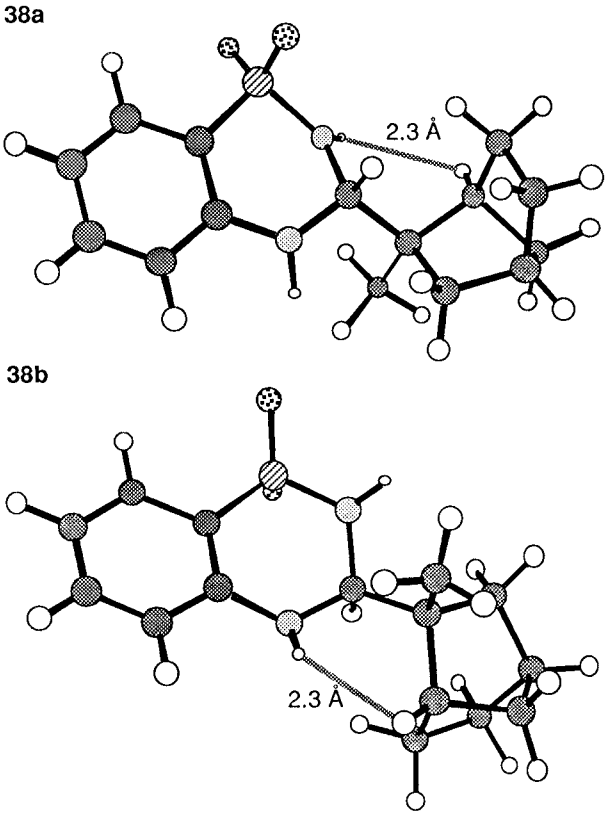


Figure 2. The global minima, calculated using AM1, of model compounds **38a** and **38b**. The diagnostic NOEs used to identify the chirality of C-3 in the corresponding dihydromethylcyclothiazides **4a** and **4b**, respectively, are shown. In acetone-d_6 compound **4a** shows an NOE between the sulfonamide hydrogen H-2 and the bridgehead hydrogen H-1′. Compound **4b** shows an NOE between the amino hydrogen H-4 and the bridgehead hydrogen H-1′. Similary, but not shown, in the structures assigned to diasteromer pair **3a** and **3b** an NOE between H-1′ and H-4 is observed for compound **3a**, and an NOE between H-1′ and H-2 is observed for compound **3b**.

Using the conformational search procedure, several low energy conformations were identified for each compound. The structural similarity of the eight compounds results in similar minima being observed for each compound. Rotation of the bicyclo[2.2.1]heptyl moiety relative to the heterocyclic ring results in three low energy, staggered conformations which can be characterized using the dihedral angle θ_1 (H-3, C-3, C-2′, methyl C).[33] The low energy conformations were also found to include two different ring puckers which can be characterized by the angle θ_2 (H-2, N-2, C-3, H-3). When θ_2 is near $\pm 130°$ (the sign of the angle depends on the chirality of C-3) the bicyclo[2.2.1]heptyl moiety is in a pseudoequatorial orientation and near $\pm 35°$ this moiety is in a pseudoaxial orientation. The energy difference between the pseudoaxial and pseudoequatorial conformations is between 1.9 and 3.4 kcal/mol.

Table 1 lists the energy differences between the low energy states. For compounds **1a**−**4a** and **1b**−**4b** the minimum energy conformation of each stereoisomer contained the bicyclo[2.2.1]-heptyl moiety in a pseudoequatorial orientation with θ_1 near 180°. In each case, the energy difference between the global minimum and the next lowest conformer is between 1.9 and 2.8 kcal/mol as calculated using AM1.

(33) The ring carbons in the bicyclo[2.2.1]heptyl group are represented as primed numbers to avoid confusion. The names given for the dihydromethylcyclothiazide stereoisomers in the Experimental Section do not contain the primed numbering of these carbons because labeling of the carbons in this manner is not in conformance with correct rules of nomenclature.

Table 1. Energies of Dihydromethylcyclothiazide Conformers Determined in Vacuum Using AM1

compd[a]	conformer	ΔH_f (kcal/mol)	θ_1	θ_2
1a	1	0	174	128[b]
	2	2.8	145	145[b]
	3	3.4	−54	23[c]
	4	3.7	40	128[b]
1b	1	0	177	−130[b]
	2	2.5	−175	−23[c]
	3	3.5	−51	−129[b]
	4	3.8	46	−132[b]
2a	1	0	−172	−126[b]
	2	1.9	−171	−33[c]
	3	3.0	−38	−136[b]
	4	5.9	83	−136[b]
2b	1	0	−174	128[b]
	2	2.2	−174	41[c]
	3	3.1	−41	135[b]
	4	5.8	69	135[b]

[a] Data are given for compounds **1a**,**b** and **2a**,**b**. For the enantiomers, **3a**,**b** and **4a**,**b**, the angles are of the opposite sign. [b] Pseudoaxial. [c] Pseudoequatorial.

Because the molecular modeling studies show that each dihydromethylcylothiazide stereoisomer exists substantially as a single conformer in solution, the assignment of chirality at C-3 for the separated diastereomer pairs was based on a correlation of the calculated minimum energy conformations with NOE data from 2D NOESY experiments performed on separated diastereomer pairs **3a** and **3b** and **4a** and **4b**. From modeling each pair of diastereomers, it was observed that altering the chirality at C-3 alters the relationship between the atoms in the heterocyclic ring and those in the bicyclo[2.2.1]-heptyl moiety. In one diastereomer of each pair the short distance between the bridgehead hydrogen H-1′ and the sulfonamide hydrogen H-2 indicates that an NOE between these two hydrogens is expected, while in the other diastereomer of the pair an NOE between bridgehead hydrogen H-1′ and the proximate amino hydrogen H-4 is expected (Figure 2). These expected NOEs were observed in 2D NOESY experiments wherein one diastereomer in each pair shows an NOE between H-1′ and H-2, while the other diastereomer in the pair shows an NOE between H-1′ and H-4. Accordingly, this NOE data together with the assignment of the H-2 and H-4 resonances (made possible by the fact that only H-4 has an expected, and observed, NOE with aromatic hydrogen H-5) enables the unambiguous assignment of structure to each component of a diastereomer pair. In several cases, additional NOEs were also evident between the amino and sulfonamide hydrogens and the hydrogens attached to C-3′. These additional NOEs are consistent with the structural assignments made for the diastereomer pairs. Thus, the absolute configurations of the dihydromethylcyclothiazide stereoisomers shown in Chart 2 are based on the combination of the results from this NMR analysis, which was used for the assignment of chirality at C-3, and the chiralities of the stereocenters established by the enantioselective synthesis of the bicyclo[2.2.1]heptyl portion of the molecules.

Electrophysiology. Voltage clamp recordings were obtained from cultured postnatal rat hippocampal neurons using the patch clamp technique in the whole-cell configuration.[34] Pairs of diastereomers and individual diastereomers >95% pure were tested for the ability to reduce AMPA receptor desensitization (Figure 3). AMPA receptor gated currents were elicited by application of 1 mM L-glutamate for 300 ms, which typically resulted in a large inward current that desensitized to a much smaller amplitude (usually about 10% or less of the peak

(34) Hamill, O. P.; Marty, A.; Neher, E.; Sakmann, B.; Sigworth, F. J. *Pflüegers Arch.* **1981**, *391*, 85−100.

current), reaching a steady-state well within the first 100 ms of glutamate application. Reduction of desensitization was manifested by slowing the rate of desensitization and by potentiation of the steady-state current amplitude. The steady-state current was defined as the average current measured over the last 30 ms of the 300 ms application of glutamate, and potentiation by a compound was quantitated as a percentage of the control response without compound. The results demonstrate that the absolute configuration of the bicyclo[2.2.1]heptyl portion of the molecules had the largest effect upon pharmacological activity. Thus, diastereomer pairs **1a** and **1b** and **2a** and **2b** prepared from (1*R-exo*)-carboxaldehyde **20** and (1*R-endo*)-carboxaldehyde **24**, respectively, were less potent inhibitors of desensitization than diastereomer pairs **3a** and **3b** and **4a** and **4b** prepared from (1*S-exo*)-carboxaldehyde **33** and (1*S-endo*)-carboxaldehyde **37**, respectively (Figure 3A,B). Moreover, 1 μM diastereomer pair **4a** and **4b** had a potency comparable to that of 1 μM of a commercial preparation of cyclothiazide (Figure 3B), suggesting that the absence of the double bond and the presence of the 2-methyl substituent in the bicyclo[2.2.1]heptyl group of the dihydromethylcyclothiazide diastereomers does not markedly diminish their potency as inhibitors of AMPA receptor desensitization.

The diastereomer pairs were also tested at 10 μM, and greater reduction of desensitization was observed than at 1 μM. Notably, the superiority of pair **4a** and **4b** as seen at 1 μM was much more obvious at the higher concentration (Figure 3C, open bars). Then, each diastereomer pair was separated into its components (>95% purity by NMR criteria for each HPLC purified diastereomer), and the individual diastereomers were tested at 10 μM. For each diastereomer pair, each diastereomer component was evaluated on the same cells to reduce variability in what were relatively small, but significant differences in activity between the components of the **1a** and **1b** pair and the **2a** and **2b** pair. In contrast, **3a** and **3b** were not significantly different in activity, while the largest difference was between **4a** and **4b** (Figure 3C, hatched bars). The most active compound was **4a**, and representative traces comparing **4a** to its enantiomer **2a** emphasize the dramatic difference in activity between these compounds at low micromolar concentrations (Figure 3D,E).

At 10 μM, the potentiation of steady-state currents by the diastereomers was quickly and completely reversible, except for compound **4a**. The average halftime to recovery to the control steady-state to peak ratio (usually about 0.10 for control responses) was 163 $\pm$ 29 s for compound **4a** (five cells) compared to 62 $\pm$ 10 s for **4b** (four cells, $P < 0.01$). The slow recovery from potentiation is a characteristic of commercially available cyclothiazide[8,17a,35] and suggests that the activity of cyclothiazide is dominated by the stereoisomer component having a structure analogous to that of compound **4a**. For cyclothiazide, this stereoisomer is 1*R*-[1α,2α(*R**),4α]]-3-bicyclo-[2.2.1]hept-5-en-2-yl-6-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide. As observed with pair **4a** and **4b**, the slow off rate for compound **4a** may explain why the activity of the pair is similar to that of compound **4a** alone, instead of giving activity intermediate between compounds **4a** and **4b**. In fact, some of the activity observed for compound **4b** may be accounted for by the presence of a few percent (<5%) of compound **4a**.

The availability of these pure dihydromethylcyclothiazide stereoisomers will make it possible to address many additional issues related to glutamate physiology and pharmacology. For example, these compounds will be useful pharmacological probes of AMPA receptor subtype specificity. Studies of the stereospecific effects of the compounds on glutamate excitotoxicity and the memory enhancing effects of each stereoisomer are also warranted. It is also likely that the dihydromethylcyclothiazide stereoisomers will be of use in pharmacological studies of the stereospecificity of benzothiadiazine actions on the sodium−chloride cotransporter found in mammalian kidney. Historically, this cotransporter was the original therapeutic target of benzothiadiazine or "thiazide-like" diuretics, and the inhibi-

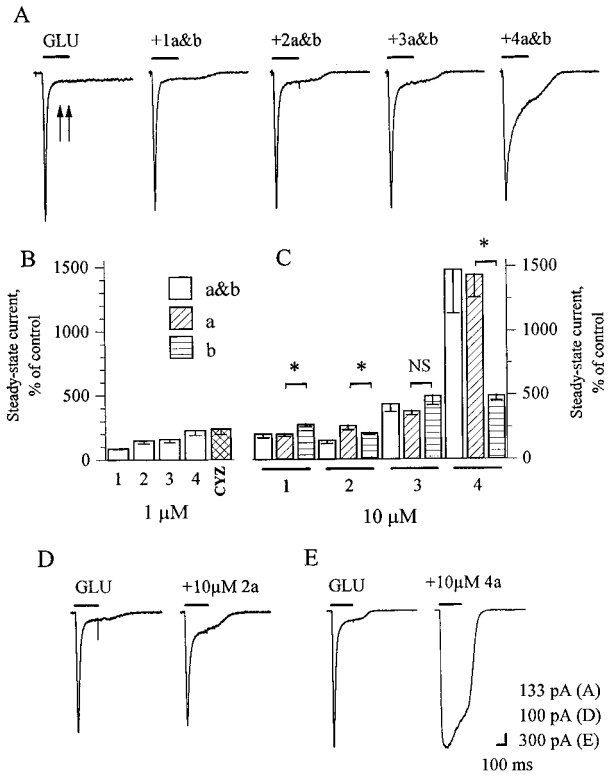


Figure 3. Stereoisomers of dihydromethylcyclothiazide differentially affect AMPA receptor desensitization. (A) Recordings from a representative voltage-clamped neuron at −60 mV. L-Glutamate (GLU, 1 mM) applied for the duration of the bar produces a large inward current that diminishes (desensitizes) to a much smaller steady-state amplitude (defined as the average measured between the arrows) in spite of the continued presence of glutamate. Successive traces from left to right are GLU currents after preapplication of 1 μM of the diastereomer pairs **1a** and **b**, **2a** and **b**, **3a** and **b**, and **4a** and **b**. Current desensitization was slowest and thus most obvious with **4a** and **b**, but potentiation of the steady-state current also reflects reduction of desensitization and was evident for pairs **2a** and **b** and **3a** and **b**. (B) Cumulative data from 13 cells upon which all four pairs were tested showed that **4a** and **b** potentiates the steady-state current the most, comparable to cyclothiazide (CYZ, 9 of 13 cells, trace not shown in A). (C) In a different set of cells, 10 μM of each of the diastereomer pairs potentiated GLU currents (open bars), and the superiority of **4a** and **b** over the other three pairs was more obvious. Each pair of hatched bars in (C) represent comparisons between 10 μM of the separated ***a*** and ***b*** diastereomer components. The four diastereomer pairs were evaluated on four separate sets of cells ($n = 11-14$), with each ***a*** and ***b*** *pair* tested on the same set of cells. Except for **3a** versus **3b**, a significantly different (*$P < 0.05$) potentiation of the GLU current was observed for the separated stereoisomers of each diastereomer pair. (D and E) Representative tracings from two different cells exemplifying the stereospecific reduction of desensitization by dihydromethylcyclothiazide isomers. Compounds **2a** and **4a** are an enantiomeric pair. Stereoisomer **2a** (10 μM) produced modest potentiation of the steady-state current (D), while stereoisomer **4a** (10 μM) produced marked steady-state current potentiation and obvious reduction of desensitization (E).

(35) Patneau, D. K.; Vyklickly, L. J.; Mayer, M. L. *J. Neurosci.* **1993**, *13*, 3496−3509.

tion of this cotransporter explains the clinical use of these compounds for the treatment of hypertension and edematous states resulting from heart, kidney, and liver disease.[36] The recent cloning and characterization of this cotransporter[37−39] greatly enhances the attractiveness of the suggested stereochemical studies.

Experimental Section

General Methods. All melting points were determined with a capillary melting point apparatus and are uncorrected. NMR spectra were recorded at ambient temperature in $CDCl_3$ (unless noted otherwise) with a 5 mm probe on either a Varian Gemini-300 operating at 300 MHz (^{1}H) or 75 MHz (^{13}C). For ^{1}H NMR and ^{13}C NMR spectra the internal references were TMS (δ 0.00) and $CDCl_3$ (δ 77.00), respectively. IR spectra were recorded as films on a NaCl plate with a Perkin-Elmer 1710 FT-IR spectrophotometer. Optical rotations were recorded on a Perkin-Elmer Model 241 polarimeter. Elemental analyses were carried out by M-H-W Laboratories, Phoenix, AZ. Solvents were used either as purchased or dried and purified by standard methodology. The 4-amino-6-chloro-1,3-benzenedisulfonamide was purchased from Aldrich Chemical Co., Milwaukee, WI. Flash chromatography was performed using silica gel (32−63 microns) purchased from Scientific Adsorbants, Atlanta, GA.

Computer Modeling and 2D NOESY. Monte-Carlo conformational searches[32] were performed using Macromodel 5.0[40] with the MM3* derivative of the MM3 forcefield.[41] The low energy conformers were reminimized with Mopac 93[42] using the AM1 method.[43] The 2D NOESY experiments were performed at 600 MHz in acetone-d_6.

[*R*-(*R,*R**)]-2,3-Dihydroxybutanedioic Acid Bis(phenylmethyl) Ester (7).** A mixture of [*R*-(*R**,*R**)]-2,3-dihydroxybutanedioic acid (**5**, 10 g, 66.6 mmol) and benzyl alcohol (28.8 g, 266.4 mmol) was heated at 200 °C (oil bath temperature) in a flask equipped with a Dean-Stark apparatus for 1 h under nitrogen. Then, the temperature was raised to 225 °C (oil bath temperature) for another 1 h. The mixture was cooled to room temperature and purified by chromatography (silica gel, hexane−Et_2O−CH_2Cl_2 = 6:2.5:1.5) to give dibenzyl ester **7** (20.5 g, 93%) as a white solid: mp 62−64 °C (lit.[27,44] mp 49−50 °C; mp 67−69 °C); $[\alpha]^{22}_D = +8.3°$ (EtOH); $[\alpha]^{22}_D = -10.1°$ ($CHCl_3$); IR 3483, 3032, 2961, 1747, 1498, 1456, 1378, 1265, 1128, 1090, 738, 698 cm^{-1}; ^{1}H NMR δ 7.37 (s, 10H), 5.28 (dd, J = 12.3 Hz, J = 20.3 Hz, 4H), 4.63 (d, J = 6.9 Hz, 2H), 3.34−3.30 (m, 2H); ^{13}C NMR δ 171.33, 134.71, 128.64, 128.36, 72.08, 68.02. Anal. Calcd for $C_{18}H_{18}O_6$: C, 65.45; H, 5.49. Found: C, 65.69; H, 5.32.

[*S*-(*R,*R**)]-2,3-Dihydroxybutanedioic Acid Bis(phenylmethyl) Ester (8).** Using the same procedure described immediately above, [*S*-(*R**,*R**)]-2,3-dihydroxybutanedioic acid (**6**, 10 g, 66.6 mmol) gave dibenzyl ester **8** (20.8 g, 95%) as a white solid with the same IR, ^{1}H NMR, and ^{13}C NMR data as **7**. Compound **8** had mp 66−68 °C (lit.[44] mp 67.5−69 °C); $[\alpha]^{22}_D = -8.0°$ (EtOH). Anal. Calcd for $C_{18}H_{18}O_6$: C, 65.45; H, 5.49. Found: C, 65.60; H, 5.55.

[*R*-(*R,*R**)]-2-[(2,6-Dimethoxybenzoyl)oxy-3-hydroxybutanedioic Acid (9).** A stirred solution of compound **7** (18 g, 50 mmol) and 2,6-dimethoxybenzoic acid (9.3 g, 51 mmol) in dry dichloromethane (150 mL) was treated with trifloroacetic anhydride (11.6 g, 55 mmol) added dropwise within 10 min at 0 °C under nitrogen. After an additional 60 min at room temperature, the mixture was poured into saturated aqueous $NaHCO_3$ and extracted with dichloromethane (2 × 300 mL). The combined organic layers were washed with water (300 mL) and dried over Na_2SO_4. The solvent was removed to give an oil which was purified by chromatography (silica gel, hexane−Et_2O−CH_2Cl_2 = 6:2.5:1.5) to give an intermediate triester (16.5 g, 63%) as a colorless oil. This triester (16 g, 30 mmol) in EtOAc (150 mL) was hydrogenolyzed over a 10% Pd−C catalyst (1.6 g) under hydrogen (100 psi, room temperature) for 2 h. The catalyst was filtered and washed with EtOAc (30 mL). The solvent was removed to give compound **9** as a colorless oil which, after the addition of EtOAc (30 mL), became a white solid (9.5 g, 99%): mp 178−181 °C (lit.[27] mp 184−186 °C); $[\alpha]^{22}_D = -75.1°$ (EtOH); IR 3402 (br), 3031, 1790, 1741, 1723, 1601, 1478, 1259, 1103 cm^{-1}; ^{1}H NMR (acetone-d_6) δ 7.36 (t, J = 8.5 Hz, 1H), 6.67 (d, J = 8.6 Hz, 2H), 5.41 (d, J = 2.1 Hz, 1H), 4.57 (s, 1H), 3.72 (s, 6H); ^{13}C NMR (DMSO-d_6) δ 171.62, 168.27, 164.78, 157.29, 131.77, 111.74, 104.23, 73.77, 70.12, 55.97. Anal. Calcd for $C_{13}H_{14}O_9$: C, 49.69; H, 4.49. Found: C, 49.49; H, 4.75.

[*S*-(*R,*R**)]-2-[(2,6-Dimethoxybenzoyl)oxy-3-hydroxybutanedioic Acid (10).** Using the same procedure described immediately above, dibenzyl ester **8** (14.9 g, 41.1 mmol) gave an intermediate triester (14.0 g, 65%) as a colorless oil which was hydrogenolyzed to give compound **10** (8.0 g, 96%) as a white solid: mp 173−176 °C; $[\alpha]^{22}_D = +72.2°$ (EtOH). The IR, ^{1}H NMR, and ^{13}C NMR spectra were identical to those described for compound **9**. Anal. Calcd for $C_{13}H_{14}O_9$: C, 49.69; H, 4.49. Found: C, 49.65; H, 4.31.

(1*S*-*exo*)-2-Methylbicyclo[2.2.1]hept-5-ene-2-carboxylic Acid (14) and [3*S*-(3α,3a*β*,5α,6*β*,6a*β*)]-Hexahydro-6-iodo-3-methyl-3,5-methano-2*H*-cyclopenta[*b*]furan-2-one (15). To a stirred solution of crude product **12** (a mixture of (1*S*-*endo*)- and (1*S*-*exo*)-diastereomers) in acetone (150 mL), which was obtained by a literature procedure[26a] from methacrolein (3.3 mL, 40 mmol) and cyclopentadiene (**11**, 9.8 mL, 120 mmol) in the presence of CAB catalyst (10% by mol) made from compound **9** (1.3 g, 4 mmol) and BH_3·THF (1.0 M solution in THF, 4.0 mL, 4.0 mmol), was added dropwise Jones reagent until an orange color persisted at 0 °C. Isopropyl alcohol (2 mL) was added to destroy any excess reagent. After most solvent was removed, the residue was diluted with water (100 mL) and extracted with EtOAc (2 × 150 mL). The combined organic layers were washed with brine (150 mL) and dried over Na_2SO_4. The solvent was removed to give an oil which was dissolved in 10% aqueous K_2CO_3 (30 mL) and extracted with ether (2 × 30 mL). The aqueous layer was acidified with 6 N aqueous HCl and extracted with EtOAc (2 × 100 mL). The combined organic layers were washed with brine (100 mL) and dried over Na_2SO_4. The solvent was removed to give a mixture of acids **13** (4.5 g, 74% overall for the two steps) as a yellowish solid.

To a solution of acids **13** (4.5 g, 30 mmol) and NaOH (1.2 g, 30 mmol) dissolved in water (10 mL) was added a solution of I_2−KI−$NaHCO_3$ (1:4:6 by mol) in water (60 mL/10 mmol of I_2) at 0 °C until a deep orange color persisted. After stirring for another 40 min, the mixture was extracted with Et_2O (2 × 50 mL). The aqueous layer was acidifed with 6 N aqueous HCl at 0 °C and then was extracted with Et_2O (2 × 100 mL). The combined organic layers were washed with water (100 mL), 10% $Na_2S_2O_3$ (20 mL), and brine (100 mL) and dried over Na_2SO_4. The solvent was removed to give crude product as a yellow semisolid which was purified by recrystallization from hexane to give product **14** (3.4 g, 76%) as white crystals: mp 39−41 °C; $[\alpha]^{22}_D = -66.2°$ (95% EtOH); IR 2978, 2877, 1695, 1578, 1458, 1405 cm^{-1}; ^{1}H NMR δ 6.26−6.23 (m, 1H), 6.12−6.09 (m, 1H), 3.06 (s, 1H), 2.85 (s, 1H), 2.46 (dd, J = 3.9 Hz, J = 12.1 Hz, 1H), 1.47 (s, 2H), 1.17 (s, 3H), 0.88 (d, J = 12.1 Hz, 1H); ^{13}C NMR δ 185.84, 138.74, 133.53, 50.41, 49.50, 49.04, 42.84, 37.35, 24.21. Anal. Calcd for $C_9H_{12}O_2$: C, 71.03; H, 7.95. Found: C, 70.88; H, 8.13.

The combined organic layers separated from the above acidified aqueous layer were washed with 10% aqueous $Na_2S_2O_3$ (20 mL) and brine (50 mL) and dried over Na_2SO_4. The solvent was removed to give a solid which was purified by chromatography (silica gel, 10% EtOAc in hexane) to give iodolactone **15** (0.8 g, 9.7%) as colorless needles (from Et_2O−hexane): mp 81−82 °C; $[\alpha]^{22}_D = -103.5°$ ($CHCl_3$); IR 2968, 2881, 1776, 1443, 1380, 1084, 1043 cm^{-1}; ^{1}H NMR δ 5.04 (d, J = 5.5 Hz, 1H), 3.82 (d, J = 2.5 Hz, 1H), 2.77 (d, J = 4.4 Hz, 1H), 2.64 (s, 1H), 2.34 (dd, J = 1.8 Hz, J = 11.6 Hz, 1H), 1.92

(36) Stokes, J. B. *Kidney Int.* **1989**, *36*, 427−433.
(37) Gamba, G.; Saltzberg, S. N.; Lombardi, M.; Miyanoshita, A.; Lytton, J.; Hediger, M. A.; Brenner, B. M.; Hebert, S. C. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 2749−2753.
(38) Gamba, G.; Miyanoshita, A.; Lombardi, M.; Lytton, J.; Lee, W.-S.; Hediger, M. A.; Hebert, S. C. *J. Biol. Chem.* **1994**, *269*, 17713−17722.
(39) Hebert, S. C.; Gamba, G. *Clin. Investig.* **1994**, *72*, 692−694.
(40) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440−467.
(41) Allinger, N. L.; Yuh, Y. H.; Lii, J.-H. *J. Am. Chem. Soc.* **1989**, *111*, 8551−8556.
(42) Mopac 93. Fujitsu Limited obtained through QCPE, Creative Arts Building 181, Bloomington, IN, 47405.
(43) Stewart, J. J. P. *J. Comput. Aided Mol. Design* **1990**, *4*, 1−105.
(44) Smith, H. L.; Brown, E. S.; Smith, J. D.; Andrako, J. *J. Pharm. Sci.* **1965**, *54*, 1269−1273.

(dd, $J = 2.2$ Hz, $J = 13.5$ Hz, 1H), 1.86 (dd, $J = 1.7$ Hz, $J = 9.9$ Hz, 1H), 1.54 (dd, $J = 4.2$ Hz, $J = 13.6$ Hz, 1H), 1.15 (s, 3H); ^{13}C NMR δ 180.85, 86.90, 51.55, 47.39, 42.67, 41.75, 36.21, 29.86, 19.29. Anal. Calcd for $C_9H_{11}IO_2$: C, 38.87; H, 3.99; I, 45.63. Found: C, 39.06; H, 3.77; I, 45.49.

(1*R*-*exo*)-2-Methylbicyclo[2.2.1]hept-5-ene-2-carboxylic Acid (27) and [3*R*-(3α,3a*β*,5α,6*β*,6a*β*)]-Hexahydro-6-iodo-3-methyl-3,5-methano-2*H*-cyclopenta[*b*]furan-2-one (28). Using the same procedure described immediately above, acids **26** were converted into compounds **27** and **28**.

Compound **27** (76% yield) was obtained as white crystals: mp 38−41 °C; $[\alpha]^{22}_D = +65.6°$ (95% EtOH, lit.[30] $[\alpha]^{22}_D = +67.3°$ in 95% EtOH). The IR, ^{1}H NMR, and ^{13}C NMR spectra were identical to those described for compound **14**. Anal. Calcd for $C_9H_{12}O_2$: C, 71.03; H, 7.95. Found: C, 70.88; H, 7.76.

Compound **28** (12% yield) was obtained as colorless needles: mp 81−82 °C; $[\alpha]^{22}_D = +104.8°$ ($CHCl_3$). The IR, ^{1}H NMR, and ^{13}C NMR spectra were identical to those described for compound **15**. Anal. Calcd for $C_9H_{11}IO_2$: C, 38.87; H, 3.99; I, 45.63. Found: C, 39.00; H, 4.04; I, 45.45.

(1*S*-*endo*)-2-Methylbicyclo[2.2.1]hept-5-ene-2-carboxylic Acid (16). To a solution of iodolactone **15** (6.0 g, 21.6 mmol) in HOAc (25 mL) was added portions of zinc dust (2.1 g, 32 mmol) with stirring in a water bath (22 °C). After stirring at room temperature for another 3 h, the mixture was filtered and washed with Et_2O (2 × 30 mL). The combined organic layers were washed with water (3 × 50 mL) and dried over Na_2SO_4. The solvent was removed to give a solid which was purifed by recrystallization from 25% aqueous EtOH to give acid **16** (3.0 g, 91%) as white crystals: mp 96−100 °C; $[\alpha]^{22}_D = +109.7°$ ($CHCl_3$); IR 2983, 1696, 1573, 1470, 1374 cm^{-1}; ^{1}H NMR δ 6.18−6.11 (m, 2H), 2.84 (s, 1H), 2.79 (s, 1H), 1.88 (dd, $J = 2.6$ Hz, $J = 12.0$ Hz, 1H), 1.57 (d, $J = 8.7$ Hz, 1H), 1.48 (s, 3H), 1.46−1.15 (m, 2H); ^{13}C NMR δ 184.45, 137.88, 135.45, 50.72, 49.98, 46.87, 42.55, 37.62, 26.47. Anal. Calcd for $C_9H_{12}O_2$: C, 71.03; H, 7.95. Found: C, 71.10; H, 7.76.

(1*R*-*endo*)-2-Methylbicyclo[2.2.1]hept-5-ene-2-carboxylic Acid (29). Compound **29** (96% yield) was obtained from iodolactone **28** using the same procedure described immediately above as white crystals (hexane): mp 95−99 °C; $[\alpha]^{22}_D = -107.1°$ ($CHCl_3$). The IR, ^{1}H NMR, and ^{13}C NMR spectra were identical to those described for compound **16**. Anal. Calcd for $C_9H_{12}O_2$: C, 71.03; H, 7.95. Found: C, 70.79; H, 7.83.

(1*S*-*exo*)-2-Methylbicyclo[2.2.1]hept-5-ene-2-carboxylic Acid Methyl Ester (17). Acid **14** (3.2 g, 21 mmol) was treated with diazomethane in Et_2O to give the corresponding methyl ester as a yellowish oil. Purification by chromatography (silica gel, 10% EtOAc in hexane) gave ester **17** (3.3 g, 94.6%) as a colorless oil: $[\alpha]^{22}_D = -63.2°$ ($CHCl_3$); IR 3062, 2973, 2876, 1731, 1576 cm^{-1}; ^{1}H NMR δ 6.10−6.01 (m, 1H), 5.96−5.94 (m, 1H), 3.57 (s, 3H), 2.90 (s, 1H), 2.69 (s, 1H), 2.32 (dd, $J = 3.9$ Hz, $J = 12.0$ Hz, 1H), 1.33−1.23 (m, 2H), 0.97 (s, 3H), 0.72 (dd, $J = 2.5$ Hz, $J = 11.5$ Hz, 1H); ^{13}C NMR δ 178.77, 138.32, 133.25, 51.60, 50.11, 49.26, 48.76, 42.55, 37.33, 23.96. Anal. Calcd for $C_{10}H_{14}O_2$: C, 72.26; H, 8.49. Found: C, 72.41; H, 8.33.

(1*R*-*exo*)-2-Methylbicyclo[2.2.1]hept-5-ene-2-carboxylic Acid Methyl Ester (30). This compound was prepared from acid **27** using the same procedure described immediately above**.** Compound **30** (92% yield) was obtained as a colorless oil: $[\alpha]^{22}_D = +59.9°$ ($CHCl_3$). The IR, ^{1}H NMR, and ^{13}C NMR spectra were identical to those described for compound **17**. Anal. Calcd for $C_{10}H_{14}O_2$: C, 72.26; H, 8.49. Found: C, 72.24; H, 8.59.

(1*S*-*exo*)-2-Methylbicyclo[2.2.1]hept-5-ene-2-methanol (18). To a solution of compound **17** (2.0 g, 12 mmol) in dichloromethane (100 mL) was added a solution of DIBALH in THF (1.0 solution in THF, 30 mL, 30 mmol) at 0 °C under nitrogen. After the mixture was stirred for another 4 h at room temperature (monitored by TLC, 30% EtOAc in hexane), isopropyl alcohol (2 mL) was added at 0 °C to destroy any excess reagent, and then 10% aqueous HCl (30 mL) was added. The organic layer was separated, and the aqueous layer was extracted with dichloromethane (2 × 30 mL). The combined organic layers were washed with brine (2 × 50 mL) and dried over Na_2SO_4. The solvent was removed to give a solid, which was purified by recrystallization from hexane to give product **18** (1.4 g, 84%) as colorless crystals: mp 56−57 °C; $[\alpha]^{22}_D = -63.5°$ ($CHCl_3$); IR 3250, 3060, 2960, 2925, 2869, 1572, 1451, 1039, 1012 cm^{-1}; ^{1}H NMR δ 6.15−6.08 (m, 2H), 3.57 (s, 2H), 2.77 (s, 1H), 2.56 (s, 1H), 1.55 (d, $J = 8.6$ Hz, 1H), 1.44 (dd, $J = 3.6$ Hz, $J = 11.7$ Hz, 1H), 1.36 (d, $J = 8.5$ Hz, 1H), 0.92 (s, 3H), 0.78 (dd, $J = 2.6$ Hz, $J = 11.7$ Hz, 1H); ^{13}C NMR δ 136.76, 135.37, 72.21, 47.76, 47.51, 43.61, 43.05, 37.24, 22.75. Anal. Calcd for $C_9H_{14}O$: C, 78.21; H, 10.21. Found: C, 78.15; H, 10.15.

(1*R*-*exo*)-2-Methylbicyclo[2.2.1]hept-5-ene-2-methanol (31). Compound **31** (86% yield) was obtained from ester **30** using the same procedure described immediately above as colorless crystals (from hexane): mp 56−57 °C; $[\alpha]^{22}_D = +63.0°$ ($CHCl_3$). The IR, ^{1}H NMR, and ^{13}C NMR spectra were identical to those described for compound **18**. Anal. Calcd for $C_9H_{14}O$: C, 78.21; H, 10.21. Found: C, 78.00; H, 10.42.

(1*R*-*exo*)-2-Methylbicyclo[2.2.1]heptane-2-methanol (19). A solution of compound **18** (1.3 g, 9.4 mmol) in EtOAc (30 mL) was hydrogenated (45 psi, room temperature, overnight) over 10% Pd−C catalyst (130 mg). The catalyst was filtered and washed with EtOAc (30 mL). The combined organic layers were evaporated to give a colorless oil which was purified by chromatography (silica gel, 20% EtOAc in hexane) to give compound **19** (1.1 g, 83%) as colorless crystals: mp 28−30 °C (from hexane); $[\alpha]^{22}_D = -27.9°$ ($CHCl_3$); IR 3350, 2950, 2869, 1451, 1048, 1027 cm^{-1}; ^{1}H NMR δ 3.40 (d, $J = 10.6$ Hz, 1H), 3.21 (d, $J = 10.6$ Hz, 1H), 2.18 (t, $J = 4.3$ Hz, 1H), 1.98 (d, $J = 3.9$ Hz, 1H), 1.65−1.13 (m, 7H), 1.04 (s, 3H), 0.82 (dd, $J = 2.7$ Hz, $J = 12.2$ Hz, 1H); ^{13}C NMR δ 70.94, 41.70, 42.34, 41.85, 37.64, 37.51, 28.47, 24.46, 21.58. Anal. Calcd for $C_9H_{16}O$: C, 77.09; H, 11.50. Found: C, 77.14; H, 11.41.

(1*S*-*exo*)-2-Methylbicyclo[2.2.1]heptane-2-methanol (32). Compound **32** (96% yield) was obtained from alcohol **31** using the same procedure described immediately above as colorless crystals (from hexane): mp 28−30 °C; $[\alpha]^{22}_D = +28.2°$ ($CHCl_3$). The IR, ^{1}H NMR, and ^{13}C NMR spectra were identical to those described for compound **19**. Anal. Calcd for $C_9H_{16}O$: C, 77.09; H, 11.50. Found: C, 77.02; H, 11.66.

[1*R*-[1α,2*β*(*R),4α]]-6-Chloro-3,4-dihydro-3-(2-methylbicyclo-[2.2.1]hept-2-yl)-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-Dioxide (1a) and [1*R*-[1α,2*β*(*S**),4α]]-6-Chloro-3,4-dihydro-3-(2-methylbicyclo[2.2.1]hept-2-yl)-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-Dioxide (1b).** To a stirred suspension of PCC (6.5 g, 30 mmol) and NaOAc (2.5 g, 30 mmol) in dichloromethane (125 mL) was added a solution of alcohol **19** (2.8 g, 20 mmol) in dichloromethane (25 mL) at 10 °C under nitrogen. After the mixture was stirred for another 2 h at room temperature, it was filtered through a short column filled with silica gel and washed with Et_2O (2 × 25 mL). The combined organic layers were washed with brine (2 × 50 mL) and dried over Na_2SO_4. The solvent was removed to give (1*R*-*exo*)-2-methylbicyclo[2.2.1]heptane-2-carboxaldehyde (**20**, 2.2 g, 80%) as an oil which was used immediately without further purification or characterization.

To a stirred suspension of 4-amino-6-chloro-1,3-benzenedisulfonamide (4.7 g, 16.5 mmol) in 6 N aqueous HCl (50 mL) and EtOH (50 mL) at 50 °C was added carboxaldehyde **20** (2.2 g, 16 mmol). After 30 min at 50 °C, the reaction mixture was stirred at room temperature overnight. The solid product mixture was diluted with water (100 mL), cooled in an ice−water bath for 1 h, filtered, and washed with water until the solid was free of mineral acid. The solid was purifed by recrystallization from 50% aqueous EtOH to give a mixture of **1a** and **1b** (∼1:1, 4.0 g, 60%) as white crystals: mp 288−291 °C. Anal. Calcd for $C_{15}H_{20}ClN_3O_4S_2$: C, 44.38; H, 4.97; Cl, 8.73; N, 10.35; S, 15.80. Found: C, 44.68; H, 5.25; Cl, 8.95; N, 10.40; S, 15.66.

This mixture was separated by reversed phase HPLC (Alltech Econosil C18 10U column, 250-mm × 10-mm, 1:1 acetone in water, 1.5 mL/min). Compound **1a** was obtained as white crystals: mp 285−286.5 °C; $[\alpha]^{22}_D = -120.5°$ (EtOH); IR 3371, 3261, 2954, 2873, 1597, 1555, 1496, 1334, 1272, 1161, 1069, 1033, 952 cm^{-1}; ^{1}H NMR (CD_3OD) δ 8.16 (s, 1H), 7.12 (s, 1H), 4.72 (s, 1H) 1.12 (s, 3H); ^{13}C NMR (CD_3OD) δ 149.29, 136.40, 129.38, 127.78, 120.60, 118.87, 73.51, 44.76, 44.42, 44.05, 39.62, 39.03, 28.88, 25.35, 17.98.

Compound **1b** was obtained as white crystals: mp 279−281 °C; $[\alpha]^{22}_D = +93.9°$ (EtOH); IR 3381, 3282, 2953, 2874, 1597, 1555, 1497, 1458, 1344, 1167, 1069, 960 cm^{-1}; ^{1}H NMR (CD_3OD) δ 8.17 (s, 1H),

7.10 (s, 1H), 4.65 (s, 1H) 1.10 (s, 3H); ^{13}C NMR (CD_3OD) δ 148.84, 136.46, 129.49, 127.76, 120.75, 118.70, 73.39, 44.58, 44.10, 43.70, 39.03, 38.67, 28.88, 25.55, 17.70.

[1*S*-[1α,2*β*(*S),4α]]-6-Chloro-3,4-dihydro-3-(2-methylbicyclo-[2.2.1]hept-2-yl)-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-Dioxide (3a) and [1*S*-[1α,2*β*(*R**),4α]]-6-Chloro-3,4-dihydro-3-(2-methylbicyclo[2.2.1]hept-2-yl)-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-Dioxide (3b).** Using the same two step procedure described immediately above**,** alcohol **32** gave a mixture of diastereomers **3a** and **3b** (∼1:1, 47% yield) as white crystals (from 50% aqueous EtOH): mp 287−289 °C. Anal. Calcd for $C_{15}H_{20}ClN_3O_4S_2$: C, 44.38; H, 4.97; Cl, 8.73; N, 10.35; S, 15.80. Found: C, 44.28; H, 5.18; Cl, 8.49; N, 10.32; S, 15.65.

This mixture was separated by reversed phase HPLC (Alltech Econosil C18 10U column, 250-mm × 10-mm, 1:1 acetone in water, 1.5 mL/min). Compound **3a** was obtained as white crystals and had: mp 279−280 °C; $[\alpha]^{22}_D = +116.8°$ (EtOH). The IR, ^{1}H NMR, and ^{13}C NMR spectra were identical to those described for compound **1a**.

Compound **3b** was obtained as white crystals and had mp 277−278 °C; $[\alpha]^{22}_D = -90.2°$ (EtOH). The IR, ^{1}H NMR, and ^{13}C NMR spectra were identical to those described for compound **1b**.

(1*S*-*endo*)-2-Methylbicyclo[2.2.1]hept-5-ene-2-methanol (22). Acid **16** (3.0 g, 23.9 mmol) was treated with diazomethane in Et_2O to give a yellowish oil which was purified by chromatography (silica gel, 10% EtOAc in hexane) to give the corresponding methyl ester **21** (3.3 g, 67%) as a colorless oil which was used without characterization.

To a solution of ester **21** (2.8 g, 16.8 mmol) in dichloromethane (100 mL) was added a solution of DIBALH (1.0 M solution in THF, 54 mL, 54 mmol) at 0 °C under nitrogen. After stirring at room temperature for another 3 h, the reaction was quenched by the addition of 6 N aqueous HCl at 0 °C. The organic layer was separated, and the aqueous layer was extracted with dichloromethane (2 × 50 mL). The combined organic layers were washed with water (100 mL) and brine (100 mL) and dried over Na_2SO_4. The solvent was removed to give a solid which was recrystallized from hexane to give alcohol **22** (1.78 g, 75%) as white crystals: mp 90−92 °C; $[\alpha]^{22}_D = +31.6°$ ($CHCl_3$); IR 3277, 3066, 2967, 2933, 2870, 1572, 1464, 1443, 1034 cm^{-1}; ^{1}H NMR δ 6.13−6.07 (m, 2H), 3.27 (q, J = 10.5 Hz, 2H), 2.79 (s, 1H), 2.50 (d, J = 1.2 Hz, 1H), 1.25 (s, 3H); ^{13}C NMR δ 136.40, 135.22, 71.01, 49.56, 47.57, 43.69, 42.53, 36.91, 24.87. Anal. Calcd for $C_9H_{14}O$: C, 78.21; H, 10.21. Found: C, 78.31; H, 10.36.

(1*R*-*endo*)-2-Methylbicyclo[2.2.1]hept-5-ene-2-methanol (35). Using the same two step procedure described immediately above, acid **29** was converted to methyl ester **34** (67% yield, colorless oil) and then reduced to give alcohol **35** (77% yield) as white crystals (from hexane): mp 90−92 °C; $[\alpha]^{22}_D = -31.3°$ ($CHCl_3$). The IR, ^{1}H NMR, and ^{13}C NMR spectra were identical to those reported for compound **22**. Anal. Calcd for $C_9H_{14}O$: C, 78.21; H, 10.21. Found: C, 78.43; H, 10.12.

(1*R*-*endo*)-2-Methylbicyclo[2.2.1]heptane-2-methanol (23). A solution of compound **22** (1.7 g, 12.3 mmol) in EtOAc (50 mL) was hydrogenated (45 psi, room temperature, overnight) over 10% Pd−C catalyst (170 mg). The catalyst was filtered and washed with EtOAc (2 × 50 mL). The combined organic layers were evaporated to give a colorless oil which was purified by chromatography (20% EtOAc in hexane) to give compound **23** (1.4 g, 78%) as colorless crystals (from hexane): mp 70−72 °C; $[\alpha]^{22}_D = +12.9°$ ($CHCl_3$); IR 3304, 2954, 2870, 1472, 1454, 1033 cm^{-1}; ^{1}H NMR 3.52−3.37 (m, 2H), 2.20 (t, J = 4.3 Hz, 1H), 1.94 (d, J = 3.2 Hz, 1H), 1.67−1.00 (m, 7H), 1.04 (s, 3H), 0.85 (dd, J = 2.5 Hz, J = 12.0 Hz, 1H); ^{13}C NMR δ 69.90, 43.85, 42.75, 42.26, 37.63, 37.10, 28.36, 25.38, 24.41. Anal. Calcd for $C_9H_{16}O$: C, 77.09; H, 11.50. Found: C, 77.32; H, 11.30.

(1*S*-*endo*)-2-Methylbicyclo[2.2.1]heptane-2-methanol (36). Using the same procedure described immediately above, alcohol **35** gave compound **36** (87% yield) as colorless crystals (from hexane): mp 70−72 °C; $[\alpha]^{22}_D = -12.5°$ ($CHCl_3$). The IR, ^{1}H NMR, and ^{13}C NMR spectra were identical to those reported for compound **23**. Anal. Calcd for $C_9H_{16}O$: C, 77.09; H, 11.50. Found: C, 76.88; H, 11.44.

[1*R*-[1α,2α(*S),4α]]-6-Chloro-3,4-dihydro-3-(2-methylbicyclo-[2.2.1]hept-2-yl)-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-Dioxide (2a) and [1*R*-[1α,2α(*R**),4α]]-6-Chloro-3,4-dihydro-3-(2-methylbicyclo[2.2.1]hept-2-yl)-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-Dioxide (2b).** Using the same procedure described for preparation of diastereomers **1a** and **1b**, alcohol **23** (1.4 g, 9.8 mmol) was first converted into crude (1*R*-*endo*)-2-methylbicyclo[2.2.1]heptane-2-carboxaldehyde (**24**, 1.34 g, 10 mmol containing traces of pyridine), which was reacted with 4-amino-6-chloro-1,3-benzenedisulfonamide (2.9 g, 10 mmol) to give diastereomers **2a** and **2b** (∼1:1, 2.0 g, 46%) as white crystals (from 50% aqueous EtOH): mp 284−286 °C. Anal. Calcd for $C_{15}H_{20}ClN_3O_4S_2$: C, 44.38; H, 4.97; Cl, 8.73; N, 10.35; S, 15.80. Found: C, 44.19; H, 5.23; Cl, 8.62; N, 10.51; S, 15.90.

This mixture was separated by reversed phase HPLC (Alltech Econosil C18 10U column, 250-mm × 10-mm, 47% acetone in water, 1.2 mL/min). Compound **2a** was obtained as white crystals: mp 284−286 °C; $[\alpha]^{22}_D = -109.1°$ (EtOH); IR 3273, 2955, 2873, 1597, 1535, 1479, 1426, 1338, 1256, 1175, 1064, 946, 731 cm^{-1}; ^{1}H NMR (CD_3OD) δ 8.17 (s, 1H), 7.09 (s, 1H), 4.75 (s, 1H) 1.08 (s, 3H); ^{13}C NMR (CD_3OD) δ 149.21, 136.45, 129.28, 127.75, 120.54, 118.67, 73.14, 47.34, 44.83, 44.68, 39.02, 38.12, 28.96, 24.51, 21.17.

Compound **2b** was obtained as white crystals: mp 281−283 °C; $[\alpha]^{22}_D = +118.6°$ (EtOH); IR 3366, 2960, 1598, 1554, 1497, 1337, 1173, 1067, 1039, 952 cm^{-1}; ^{1}H NMR (CD_3OD) δ 8.19 (s, 1H), 7.17 (s, 1H), 4.62 (s, 1H) 1.06 (s, 3H); ^{13}C NMR (CD_3OD) δ 148.75, 136.50, 129.71, 127.76, 121.03, 118.87, 73.05, 45.49, 44.92, 44.58, 38.72, 38.31, 29.19, 25.22, 21.06.

[1*S*-[1α,2α(*R),4α]]-6-Chloro-3,4-dihydro-3-(2-methylbicyclo-[2.2.1]hept-2-yl)-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-Dioxide (4a) and [1*S*-[1α,2α(*S**),4α]]-6-Chloro-3,4-dihydro-3-(2-methylbicyclo[2.2.1]hept-2-yl)-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-Dioxide (4b).** Using the same two step procedure described immediately above, alcohol **36** gave a mixture of diasteromers **4a** and **4b** (∼1:1, 39% yield) as white crystals (from 50% aqueous EtOH): mp 278−281 °C. Anal. Calcd for $C_{15}H_{20}ClN_3O_4S_2$: C, 44.38; H, 4.97; Cl, 8.73; N, 10.35; S, 15.80. Found: C, 44.58; H, 4.78; Cl, 8.87; N, 10.35; S, 16.06.

This mixture was separated by reversed phase HPLC (Alltech Econosil C18 10U column, 250-mm × 10-mm, 47% acetone in water, 1.2 mL/min). Compound **4a** was obtained as white crystals: mp 277−278.5 °C; $[\alpha]^{22}_D = +108.4°$ (EtOH). The IR, ^{1}H NMR, and ^{13}C NMR spectra were identical to those described for compound **2a**.

Compound **4b** was obtained as white crystals: mp 276−278 °C; $[\alpha]^{22}_D = -118.3°$ (EtOH). The IR, ^{1}H NMR, and ^{13}C NMR spectra were identical to those described for compound **2b**.

Cell Cultures. Primary cultures of dissociated hippocampal neurons from P1 neonatal rat pups were prepared via methods originally described by Huettner and Baughman,[45] with slight modifications.[8] In brief, hippocampi from Sprague-Dawley P1 neonatal rat pups were removed, minced, and incubated with papain. The tissue was then mechanically dissociated by gentle trituration, centrifuged through a solution of albumin/trypsin inhibitor, and resuspended in growth medium. Neurons were plated on a layer of glial cells in 35 mm plastic dishes, which were prepared by preplating cortical glia from P1−4 rats 4 days earlier and allowing them to grow to confluence. 5-Fluoro-2-deoxyuridine and uridine were added 2 days after the neurons were plated to prevent glial overgrowth. Cells were used 1−7 days after plating.

Electrophysiology. Growth medium was replaced by an external recording solution which contained (in mM) 140 NaCl, 3.0 KCl, 1.0 $MgCl_2$, 2.0 $CaCl_2$, 10 HEPES, Phenol Red 10 mg/L, 0.6 μM tetrodotoxin, and 20 μM MK-801; pH 7.3. The internal recording solution contained (in mM) CsCl 130, 10 TEACl, 10 HEPES, 1.1 EGTA, 5.5 glucose; pH 7.2. Aqueous L-glutamate stock solutions (50 mM) were diluted in external recording solution to a final L-glutamate concentration of 1 mM. Stock solutions of dihydromethylcyclothiazide stereoisomers were made in DMSO and diluted in external recording solution; the final DMSO concentration was 0.1% by volume. Recordings were performed at room temperature on the stage of an inverted microscope. Patch electrodes were pulled in three stages (Sutter Instruments, Novato, California) and had DC resistances of 5−10 MΩ. These electrodes were connected to a patch amplifier in voltage-clamp mode using the whole-cell configuration.[34] Compensation was made for ∼60−80% of the series resistance. The holding potential in all experiments was −60 mV.

(45) Huettner, J. E.; Baughman, R. W. *J. Neurosci.* **1986**, *6*, 3044−3060.

Drug Application. Continuous perfusion of the dish with drug-free recording solution was accomplished via gravity flow at a rate of 2−3 mL/min. The cell recorded from was also locally perfused via a large-bore fused silica-quartz tube (internal diameter 320 μm) connected to four separate reservoirs, each with a separate solenoid valve to control its flow. Only one valve could be opened at a time, allowing control buffer, or various stereoisomers to bathe the cell from this first tube. L-Glutamate (1 mM) was applied via a second identical tube by opening its solenoid valve while simultaneously closing the valve to the first tube. Flow occurred via gravity.

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