Selective Inhibitors of Glial GABA Uptake: Synthesis, Absolute Stereochemistry, and Pharmacology of the Enantiomers of 3-Hydroxy-4-amino-4,5,6,7-tetrahydro-1,2-benzisoxazole (*exo*-THPO) and Analogues

Erik Falch,[†] Jens Perregaard,[‡] Bente Frølund,[†] Birgitte Søkilde,[†] Anders Buur,[‡] Lene M. Hansen,[†] Karla Frydenvang,[†] Lotte Brehm,[†] Tina Bolvig,[§] Orla M. Larsson,[§] Connie Sanchez,[‡] Harold S. White,[▽] Arne Schousboe,[§] and Povl Krogsgaard-Larsen*[,†]

Centre for Drug Design and Transport, Departments of Medicinal Chemistry and Pharmacology, The Royal Danish School of Pharmacy, 2 Universitetsparken, DK-2100 Copenhagen, Denmark, Departments of Medicinal Chemistry and Chemical and Pharmaceutical Research, H. Lundbeck A/S, 9 Ottiliavej, DK-2500 Valby, Denmark, and Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, Utah 84112

Received August 30, 1999

3-Methoxy-4,5,6,7-tetrahydro-1,2-benzisoxazol-4-one (**20a**), or the corresponding 3-ethoxy analogue (**20b**), and 3-chloro-4,5,6,7-tetrahydro-1,2-benzisothiazol-4-one (**51**) were synthesized by regioselective chromic acid oxidation of the respective bicyclic tetrahydrobenzenes **19a,b** and **50**, and they were used as key intermediates for the syntheses of the target zwitterionic 3-isoxazolols **8**−**15** and 3-isothiazolols **16** and **17**, respectively. These reaction sequences involved different reductive processes. Whereas (*RS*)-4-amino-3-hydroxy-4,5,6,7-tetrahydro-1,2-benzisoxazole (**8**, *exo*-THPO) was synthesized via aluminum amalgam reduction of oxime **22a** or **22b**, compounds **9**, **11**−**13**, and **15**−**17** were obtained via reductive aminations. Compound **10** was synthesized via *N*-ethylation of the *N*-Boc-protected primary amine **25**. The enantiomers of **8** were obtained in high enantiomeric purities (ee ≥ 99.1%) via the diastereomeric amides **32** and **33**, synthesized from the primary amine **23b** and (*R*)-α-methoxyphenylacetyl chloride and subsequent separation by preparative HPLC. The enantiomers of **9** were prepared analogously from the secondary amine **27**. On the basis of X-ray crystallographic analyses, the configuration of oxime **22a** was shown to be *E* and the absolute configurations of (−)-**8**·HCl and (+)-**9**·HBr were established to be *R*. The effects of the target compounds on GABA uptake mechanisms in vitro were measured using a rat brain synaptosomal preparation and primary cultures of mouse cortical neurons and glia cells (astrocytes). Whereas the classical GABA uptake inhibitor, (*R*)-nipecotic acid (**2**), nonselectively inhibits neuronal (IC_{50} = 12 μM) and glial (IC_{50} = 16 μM) GABA uptake and 4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridin-3-ol (**1**, THPO) shows some selectivity for glial (IC_{50} = 268 μM) versus neuronal (IC_{50} = 530 μM) GABA uptake, *exo*-THPO (**8**) was shown to be more potent as an inhibitor of glial (IC_{50} = 200 μM) rather than neuronal (IC_{50} = 900 μM) GABA uptake. This selectivity was more pronounced for **9**, which showed IC_{50} values of 40 and 500 μM as an inhibitor of glial and neuronal GABA uptake, respectively. These effects of **8** and **9** proved to be enantioselective, (*R*)-(−)-**8** and (*R*)-(+)-**9** being the active inhibitors of both uptake systems. The selectivity of **9** as a glial GABA uptake inhibitor was largely lost by replacing the *N*-methyl group of **9** by an ethyl group, compound **10** being an almost equipotent inhibitor of glial (IC_{50} = 280 μM) and neuronal (IC_{50} = 400 μM) GABA uptake. The remaining target compounds, **11**−**17**, were very weak or inactive as inhibitors of both uptake systems. Compounds **9**−**13** and **15** were shown to be essentially inactive against isoniazide-induced convulsions in mice after subcutaneous administration. The isomeric pivaloyloxymethyl derivatives of **9**, compounds **43** and **44**, were synthesized and tested as potential prodrugs in the isoniazide animal model. Both **43** (ED_{50} = 150 μmol/kg) and **44** (ED_{50} = 220 μmol/kg) showed anticonvulsant effects, and this effect of **43** was shown to reside in the (*R*)-(+)-enantiomer, **45** (ED_{50} = 44 μmol/kg). Compound **9** also showed anticonvulsant activity when administered intracerebroventricularly (ED_{50} = 59 nmol).

Introduction

The complicity of central neurotransmitters in epilepsy has been the subject of extensive research.[1−3] The possible role of the inhibitory neurotransmitter 4-aminobutyric acid (GABA) has in particular been brought into focus,[4,5] but so far, impairment of GABA-mediated neurotransmission in epilepsy has not been proved. There is, however, indirect evidence of disturbances in GABA neurotransmission in epileptic phenomena. Thus, inhibition of GABA synthesis and administration of GABA antagonists provoke convulsions more or less similar to epileptic seizures.[6] Drug-induced convulsions

* Corresponding author: Prof. Povl Krogsgaard-Larsen. Phone: (+45) 35306511. Fax: (+45) 35306040. E-mail: nordly@medchem.dfh.dk.
[†] Department of Medicinal Chemistry, The Royal Danish School of Pharmacy.
[§] Department of Pharmacology, The Royal Danish School of Pharmacy.
[‡] H. Lundbeck A/S.
[▽] University of Utah.

10.1021/jm9904452 CCC: $18.00
Published on Web 12/04/1999

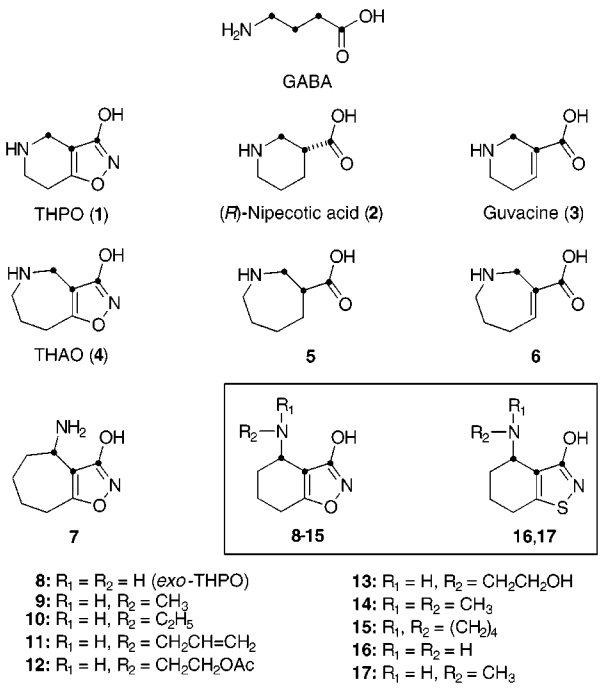


Figure 1. Structures of GABA, the GABA uptake inhibitors 4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridin-3-ol (THPO, **1**), (*R*)-nipecotic acid (**2**), guvacine (**3**), and 5,6,7,8-tetrahydro-4*H*-isoxazolo[4,5-*c*]azepin-3-ol (**4**), a number of structurally related compounds, and the new 3-isoxazolols (**8**−**15**) and 3-isothiazolols (**16**, **17**).

can be counteracted by elevation of brain GABA levels,[7] although there is no simple correlation between the onset of convulsions and brain GABA concentrations.[8]

Inhibitors of GABA transporters, which play a key role in the termination of the GABA neurotransmission process, show anticonvulsant effects in animal models,[9] and members of this group of GABAergic compounds have been clinically useful for the treatment of epilepsy.[10] These anticonvulsant and antiepileptic effects may reflect an enhancement of GABA neurotransmission processes under conditions where GABA is being released physiologically.[10] Thus, the strategies for pharmacological interventions into these transport mechanisms must be (1) effective blockade of both neuronal and glial GABA uptake in order to enhance the inhibitory effect of synaptically released GABA or, preferentially, (2) selective blockade of glial GABA uptake in order to increase the amount of GABA taken up by the neuronal carrier with subsequent elevation of the GABA concentration in nerve terminals and, thus, enhanced release of GABA.

The identification of 4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridin-3-ol (**1**, THPO) as an inhibitor of GABA uptake more than two decades ago[11] prompted the discovery of the structurally related cyclic amino acids, (*R*)-nipecotic acid (**2**)[11] and guvacine (**3**),[12] as GABA uptake inhibitors (Figure 1). Whereas 5,6,7,8-tetrahydro-4*H*-isoxazolo[4,5-*c*]azepin-3-ol (**4**, THAO) was shown to be approximately equipotent with THPO (**1**) as an inhibitor of GABA uptake,[13] the corresponding seven-membered ring amino acids, **5** and **6**, quite surprisingly turned out to be essentially inactive as inhibitors of GABA uptake, and likewise, (*RS*)-4-aminocycloheptano[1,2-*d*]isoxazol-3-ol (**7**) does not detectably interact with GABA transporters.[14]

Like GABA, (*R*)-nipecotic acid (**2**) interacts with the neuronal and glial GABA transport systems with almost equal affinity,[15] and **2** has been shown to be a substrate for the neuronal[16,17] as well as glial[17,18] transporters. Whereas guvacine (**3**) has a cellular pharmacological profile very similar to that of **2**,[12,15,19] THPO (**1**) has been shown not to be a substrate for the glial GABA transporter.[17] Furthermore, **1** has been shown to possess a 2-fold higher affinity for the glial over the neuronal transporter.[15] Although **1** is at least an order of magnitude weaker than **2** as an inhibitor of GABA uptake in vitro,[15] compound **1** is capable of increasing extracellular GABA levels more effectively than **2**,[20] and **1** is much more effective than **2** as an anticonvulsant agent.[21]

We envisage that the very low degree of conformational flexibility of **1**, at least to some extent, can explain its selectivity for the glial GABA transporter and its lack of ability to act as a substrate for the glial transporter[22,23] and that these characteristics underlie the pharmacological profile of **1**, considered to be therapeutically interesting.[22] These considerations prompted us to synthesize and pharmacologically characterize (*RS*)-4-amino-3-hydroxy-4,5,6,7-tetrahydro-1,2-benzisoxazole (**8**, *exo*-THPO) as an analogue of **1** with a comparably low degree of conformational flexibility and with an exocyclic amino group at the ring carbon atom analogous with that linked to the amino group of **1** (Figure 1). Furthermore, (*R*)-**8**, (*S*)-**8,** and the analogues **9**−**17** were synthesized and studied pharmacologically.

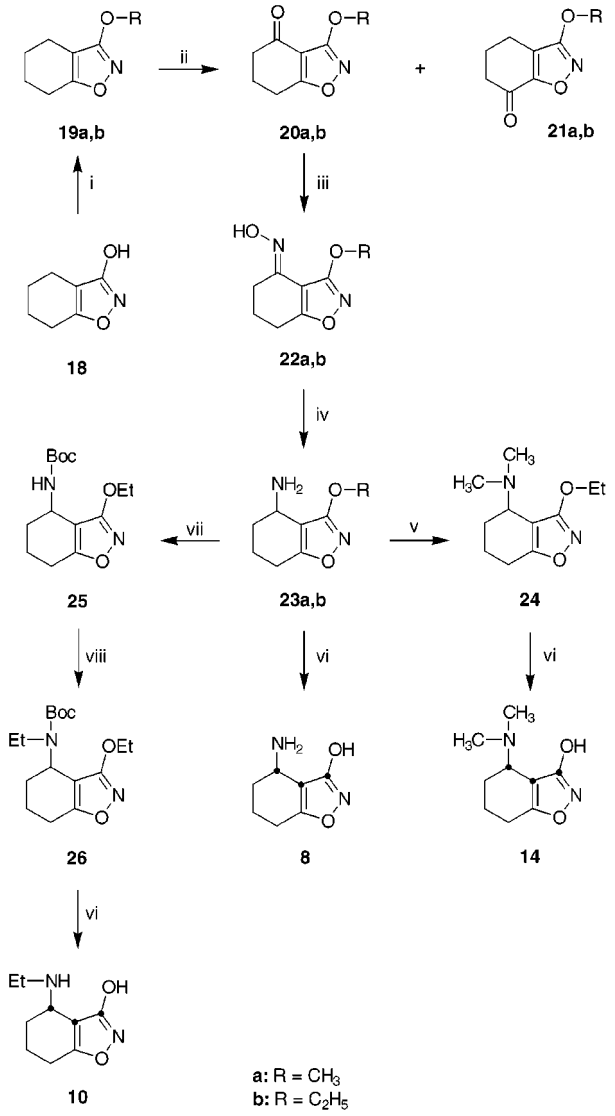
Results

Chemistry. Introduction of oxo groups into the 4-position of compounds **19a,b** (Scheme 1) and **50** (Scheme 5) represents key steps in the sequences for the synthesis of the target compounds. Chromic acid oxidation of **19a** gave the desired ketone **20a** in 55% yield, whereas the undesired isomer **21a** was obtained in 10% yield. In an attempt to increase the yield of the desired ketone and the regioselectivity of this oxidation reaction, **19b**, which contains a 3-ethoxy group, was oxidized under the same condition to give ketone **20b** in a markedly higher yield (71%) and 8% of the isomeric byproduct **21b**. Ketones **20a,b** were converted into the corresponding oximes **22a,b**, both in high yields, but the aluminum amalgam reduction of **22b** gave **23b**·HBr at significantly higher yield (70%) than that of **23a**·HBr (58%) obtained by aluminum reduction of **22a**. Compound **23b** was dimethylated under Eschweiler−Clarke conditions to give **24**, whereas synthesis of the *N*-ethyl analogue of *exo*-THPO (**8**) required Boc protection of **23b** prior to the introduction of the ethyl group.

Compounds **9**, **11**−**13**, and **15** (Scheme 2) and **16** and **17** (Scheme 5) were synthesized via reductive aminations using the appropriate amines and sodium cyanoborohydride. Cleavage of the ethoxy group and acetylation of the primary alcohol group of **30** to give **12** were accomplished in one step by treatment with hydrogen bromide in glacial acetic acid. Pure **11** could only be obtained after Boc protection of crude **11**, purification of intermediate **31,** and subsequent deprotection (Scheme 2).

The enantiomers of *exo*-THPO (**8**) and **9** were synthesized from amines **23b** and **27**, respectively, via forma-

Scheme 1[a]

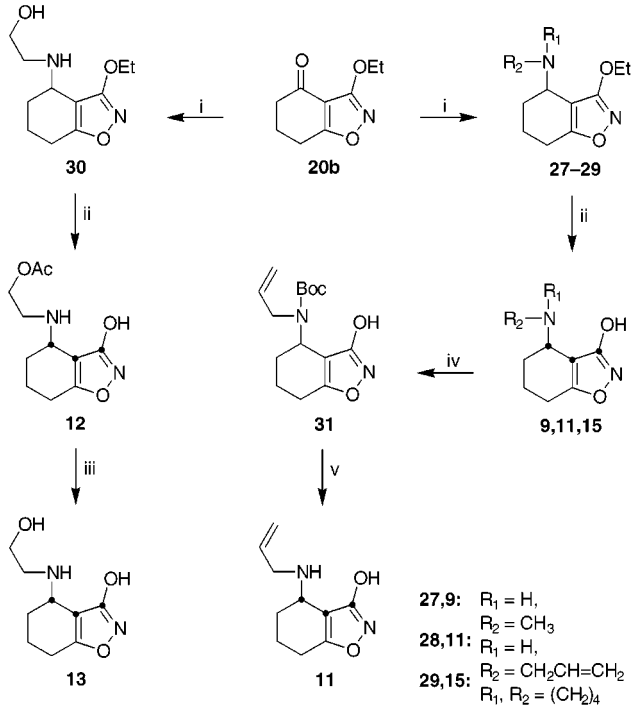


[a] Reagents: (i) CH_2N_2 (**a**) or EtBr, K_2CO_3 (**b**); (ii) $Na_2Cr_2O_7$, H_2SO_4; (iii) NH_2OH; (iv) Al, $HgCl_2$; (v) HCOOH, HCOONa, H_2CO; (vi) HBr in HOAc; (vii) $(Boc)_2O$, NaOH; (viii) EtBr, NaH.

tion of the diastereomeric α-methoxyphenylacetamides **32** and **33**, and **36** and **37**, respectively (Scheme 3). These pairs of diastereomeric amides were separated by preparative HPLC. Acid-catalyzed deprotection of **32** and **33**, chromatographic purification of the Boc-protected intermediates, and, finally, removal of the Boc groups gave the respective enantiomerically pure target compounds (*R*)-(−)-**8** (ee = 99.7%) and (*S*)-(+)-**8** (ee = 99.8%). Reductive cleavage of **36** and **37** using lithium triethylborohydride and subsequent deprotection of the respective secondary amines **38** and **39** by treatment with hydrogen bromide in glacial acetic acid gave enantiomerically pure (*R*)-(+)-**9** (ee = 99.1%) and (*S*)-(−)-**9** (ee > 99.4%), respectively (Scheme 3). The absolute stereochemistry of the enantiomers of **8** and **9** was established by X-ray crystallographic analyses (see the subsequent section).

Boc-protected **9**, compound **40**, was used as intermediate for the synthesis of the isomeric *O*- and *N*-pivaloyloxymethyl prodrugs **43** and **44**, respectively (Scheme 4). The Boc groups of intermediates **41** and **42** were selectively removed by treatment with trifluoro-

Scheme 2[a]



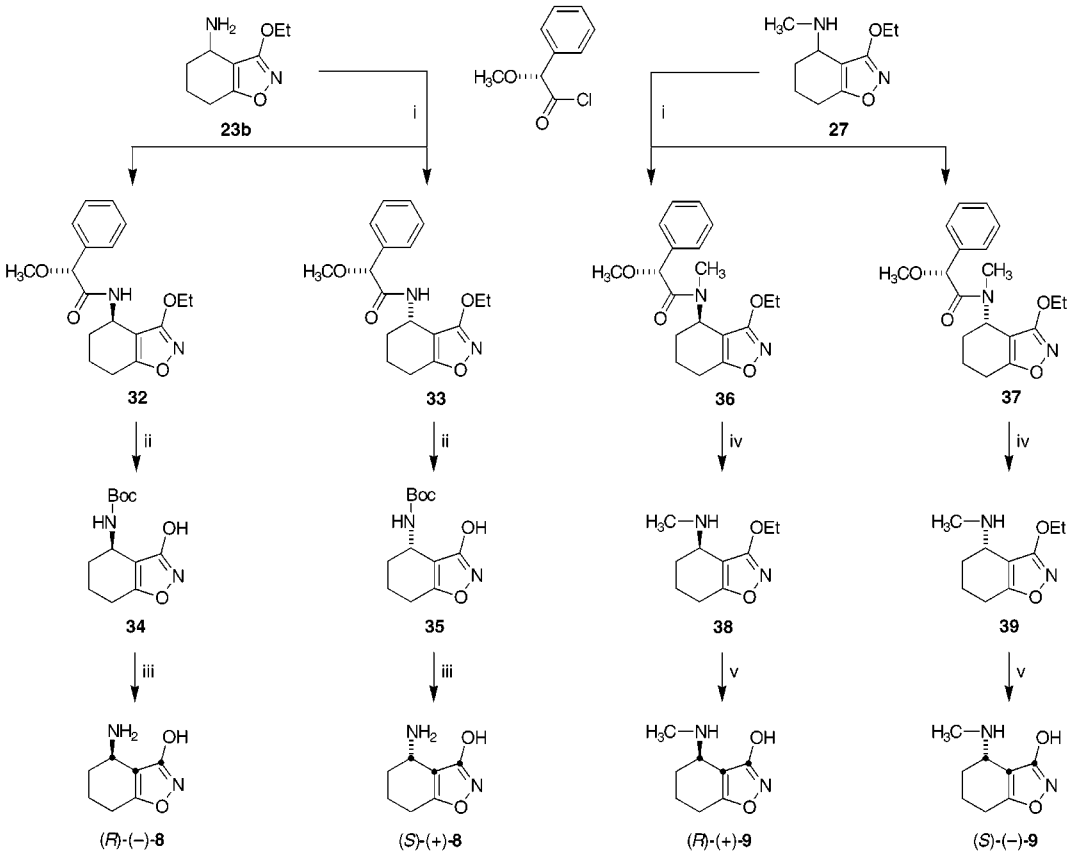
[a] Reagents: (i) R_1NHR_2, $NaCNBH_3$; (ii) HBr in HOAc; (iii) HBr aq (48%); (iv) $(Boc)_2O$, NaOH; (v) CF_3COOH, $(COOH)_2$.

acetic acid in dichloromethane, and the final products **43** and **44** were isolated as oxalate salts. The *O*-pivaloyloxymethyl prodrugs of (*R*)-(+)-**9** and (*S*)-(−)-**9**, compounds **45** and **46**, were synthesized following an identical reaction sequence (Scheme 4), and the final products **45** and **46** were also isolated as oxalate salts.

The 3-isothiazolol analogues of **8** and **9**, compounds **16** and **17,** respectively, were synthesized as outlined in Scheme 5. Treatment of the enamine amide **48** with hydrogen sulfide and subsequent oxidation of the crude reaction product with bromine gave **49** in relatively low yield (33%). Attempted synthesis of a 4-oxo intermediate from **49** for the syntheses of **16** and **17** following a route analogous with that outlined in Scheme 1 for the syntheses of **20a,b** led to extensive decomposition. Chromic acid oxidation of 3-chloro-4,5,6,7-tetrahydro-1,2-benzisothiazole (**50**) did, however, provide **51** but in low yield (27%), but the isomeric byproduct 3-chloro-4,5,6,7-tetrahydro-1,2-benzisothiazol-7-one was formed in 23% yield. Compound **51** was isolated chromatographically and converted into **16** and **17** via ketone **52**.

X-ray Crystallographic Analyses. Perspective drawings[24] of the molecular structures of oxime **22a,** (*R*)-(−)-**8**·HCl, and (*R*)-(+)-**9**·HBr with atomic labeling are depicted in Figure 2. The configuration of **22a** was shown to be *E*, and according to the procedure of Flack,[25,26] the absolute configurations of (−)-**8** and (+)-**9** were established to be *R*. Bond lengths and angles are in agreement with expected values.[27] For all three compounds, the isoxazole rings are planar, and the six-membered rings are intermediates between a C_2[C5, C6] half-chair and a C_s[C6] envelope conformation[28] (Figure 2). The crystal structure of oxime **22a** consists of dimers connected by two hydrogen bonds between the oxime moieties of two molecules related by a center of symmetry. The packing of the dimers is governed by van

Scheme 3[a]



[a] Reagents: (i) PhCH(OMe)COCl, TEA; (ii) HBr aq (48%), $(Boc)_2O$, NaOH; (iii) HCl, Et_2O; (iv) $LiEt_3BH$; (v) HBr in HOAc.

der Waals interactions. Hydrogen bond geometries are listed in Table 1. In the crystal structures of the two salts (*R*)-(−)-**8**·HCl and (*R*)-(+)-**9**·HBr, hydrogen bonds are observed from the ammonium and the hydroxy groups to the halide ions. For (*R*)-(−)-**8**·HCl an additional hydrogen bond is observed between the ammonium group and the nitrogen atom of the isoxazole ring.

In Vitro Pharmacology. The compounds were initially tested for inhibitory effect on GABA uptake using a crude preparation of synaptosomes obtained from rat brain.[30] Compounds showing significant inhibitory effect in this assay (IC_{50} < 300 μM) were subsequently studied for effects on neuronal and glial GABA uptake using cultured cortical neurons and astrocytes, respectively, as test systems.[17]

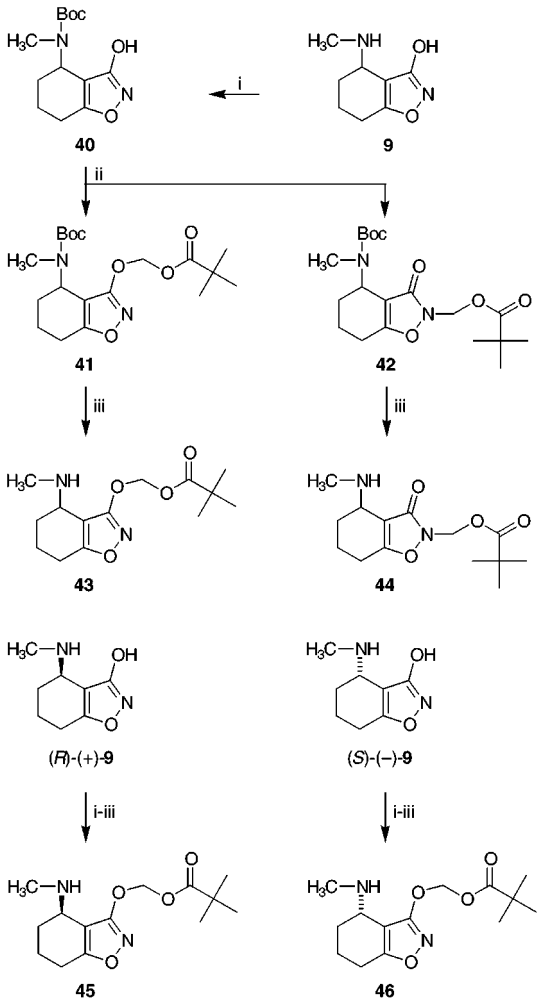
All of the new compounds tested were weaker than the classical monocyclic GABA uptake inhibitors (*R*)-nipecotic acid (**2**)[11,31] and guvacine (**3**),[12] which nonselectively inhibit neuronal and glial GABA uptake[22] (Table 2). THPO (**1**), which is a 3-isoxazolol bioisostere of **2** and **3**,[11] was the first compound showing significant selectivity for the glial GABA uptake system,[32] although **1** is only about 2-fold more potent at the glial than at the neuronal GABA uptake system.[15] *exo*-THPO (**8**), an isomer of **1** containing an exocyclic amino group (Figure 1), was approximately equipotent with **1** but showed a slightly higher degree of glia selectivity than **1**. This selectivity was further increased by conversion of the primary amino group of **8** into a methylamino group to give compound **9** (Table 2). The GABA uptake affinities of **8** as well as **9** were shown to reside in the *(R)*-enantiomers.

The additional glia selectivity of **9** as compared to **1** and **8** was, however, lost by substitution of an ethyl group for the *N*-methyl group of **9**, the resulting compound **10** showing an inhibitor profile very similar to that of **1** (Table 2). Although compounds **11**−**15**, which contain *N*-substituents larger than ethyl or tertiary amino groups, still showed significant affinity in the synaptosomal GABA uptake assay, none of these compounds significantly inhibited the uptake of GABA into cultured neurons or astrocytes. Similarly, the 3-isothiazolol analogues of **8** and **9**, compounds **16** and **17,** respectively, showed negligible effects on neuronal as well as glial GABA uptake.

In Vivo Pharmacology. Some of the new compounds tested in Table 2 were tested for anticonvulsant effects in mice after subcutaneous administration.[33] In agreement with the earlier observation that **1** only to a very limited extent penetrates into the brain after systemic administration in animals with a fully developed blood−brain barrier (BBB),[34] none of the selected compounds, (*R*)-**9** and **10**−**13**, showed significant anticonvulsant effects in mice treated with isoniazide (ED_{50} > 300 μmol/kg).

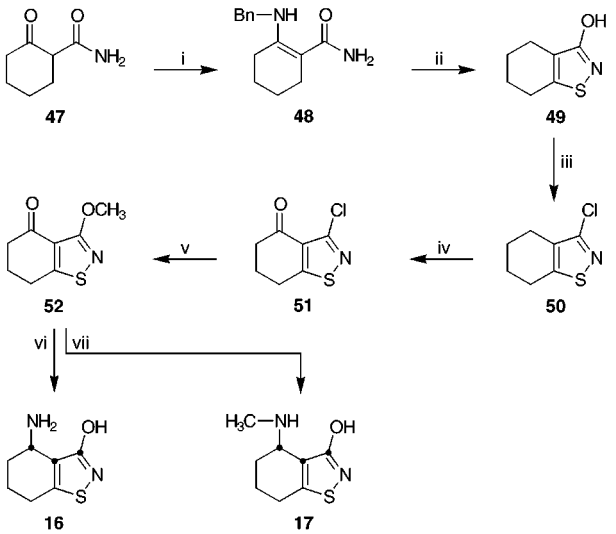
The lack of in vivo anticonvulsant effects of the zwitterionic compounds (*R*)-**9** and **10**−**13**, of which (*R*)-**9** and, to a lesser extent, **10** showed effects in all three in vitro GABA uptake systems used (Table 2), can most likely be attributed to the poor biomembrane permeability characteristics of the compounds as indicated by the low partition coefficient for compound **9** (Table 3).

Scheme 4[a]



[a] Reagents: (i) $(Boc)_2O$, NaOH; (ii) $(CH_3)_3COOCH_2I$, $(CH_3)_3COK$; (iii) CF_3COOH.

Scheme 5[a]



[a] Reagents: (i) $PhCH_2NH_2$; (ii) H_2S, Br_2; (iii) $POCl_3$, H_3PO_4; (iv) $Na_2Cr_2O_7$, H_2SO_4; (v) MeONa; (vi) NH_4OAc, $NaCNBH_3$, HBr in HOAc; (vii) $MeNH_2$, $NaCNBH_3$, HBr in HOAc.

This prompted us to synthesize (Scheme 4) and test the *O*- and *N*-pivaloyloxymethyl derivatives of racemic **9**, compounds **43** and **44**, as potential prodrugs possessing higher lipophilicity at physiological conditions and hence improved permeability across biomembranes. Both compounds **43** and **44** proved stable in aqueous buffer solution ($t_{1/2}$ > 4000 min), whereas in the presence of human plasma they were readily degraded with half-lives of 7 and 15 min, respectively (Table 3). The potential utility of compounds **43** and **44** as prodrugs was further illustrated by systemic administration to mice. Here the compounds were approximately equally effective as anticonvulsants, ED_{50} values being 150 and 220 μmol/kg for **43** and **44**, respectively. Subsequently, the (*R*)- and (*S*)-forms of **43**, compounds **45** and **46**, respectively, were synthesized (Scheme 4) and tested in vivo. These studies disclosed that the anticonvulsant effects of **43** reside in the (*R*)-enantiomer **45** (ED_{50} = 44 μmol/kg), whereas the corresponding (*S*)-enantiomer **46** did not show significant anticonvulsant effects in this in vitro system.

To verify that the anticonvulsant activity of compounds **43** and **44** was the result of hydrolytic production of **9**, it was shown that intracerebroventricular administration[35] of **9** effectively protected mice against audiogenic seizures (ED_{50} = 59 nmol).

Discussion

The heterocyclic amino acids (*R*)-nipecotic acid (**2**)[11,36] and guvacine (**3**)[12] (Figure 1) are inhibitors of GABA uptake showing approximately equal effects on the glial and neuronal GABA transporters (Table 2). Both of these inhibitors have been shown also to be substrates for GABA transporters,[12,16] making analyses of the pharmacology of these compounds difficult. The 3-isoxazolol bioisostere of these amino acids, THPO (**1**),[11] on the other hand, has been shown not to be a substrate for the glial GABA transporter,[37] and although **1** is only a weak inhibitor of GABA uptake, it shows potent anticonvulsant effects after intracerebroventricular administration in animals.[21] These effects of **1** have been associated with its weak but significant selectivity for the glial GABA uptake systems and its lack of ability to act as a substrate for this GABA transporter.[20−23]

Introduction of bulky and lipophilic groups, such as 4,4-diphenyl-3-butenyl and structurally related groups, on the amino groups of **1** and **2** as well as **3** has led to compounds showing markedly more potent effects on GABA uptake than the parent amino acids, and all of these analogues show anticonvulsant effects after systemic administration.[9,10,22,37] The *N*-4,4-diphenyl-3-butenyl analogue of nipecotic acid has been shown not to be a substrate for GABA transporters,[38] and it is unlikely that any of the GABA uptake inhibitors containing this or any of the structurally related *N*-substituents are substrates for GABA transporters.[22] The results of structure−activity studies strongly suggest that it is the amino acid units of these compounds that are recognized by the GABA uptake systems,[22] and it may be proposed that the in vitro pharmacological profiles of these amino acids also determine the pharmacology of the corresponding analogues containing the bulky lipophilic *N*-substituents.

These considerations and the proposal that selective inhibitors of glial GABA uptake may have particular pharmacological and therapeutic interest[22] have prompted us to try to develop cyclic amino acids or bioisosteres thereof showing a degree of glia selectivity

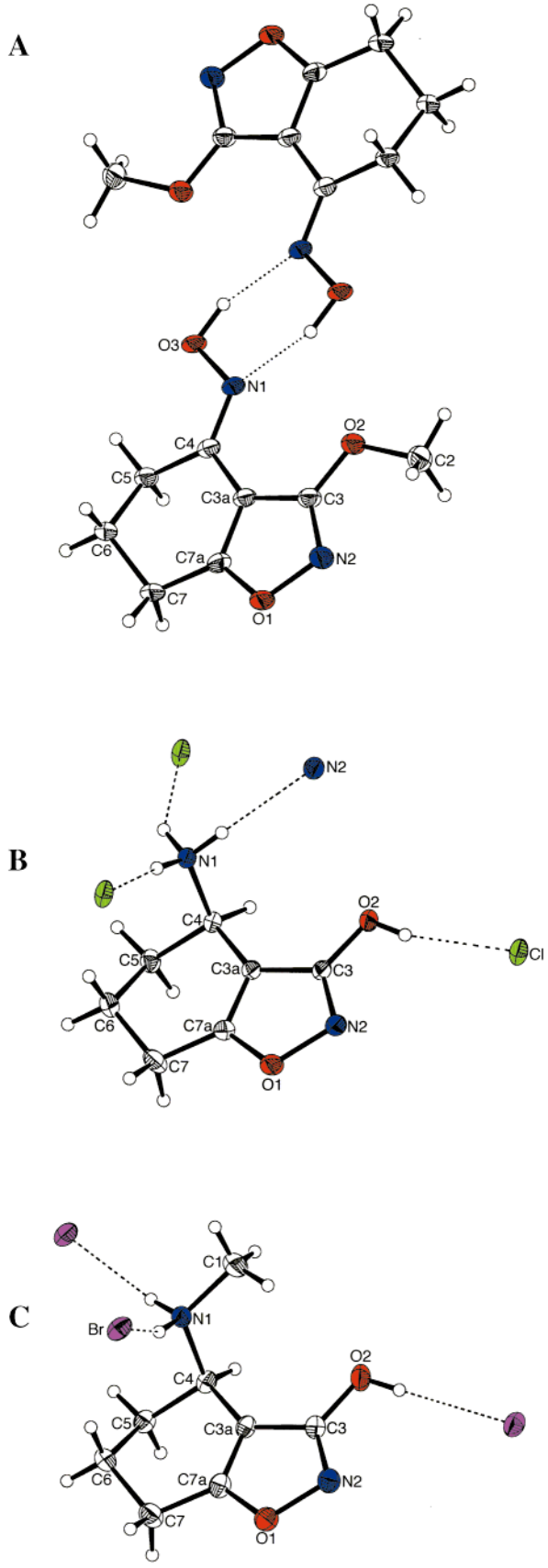


Figure 2. Hydrogen-bonding patterns in the crystals of the oxime **22a** (A), (*R*)-(−)-**8**·HCl (B), and (*R*)-(+)-**9**·**HBr** (C). The molecules are shown in perspective drawings.[24] Displacement ellipsoids enclose 50% probability. Hydrogen atoms are represented by spheres of arbitrary size. Hydrogen bonds are indicated by dashed lines.

Table 1. Hydrogen Bond Geometries (Å, deg) for the Compounds (*R*)-(−)-**8**·HCl, (*R*)-(+)-**9**·HBr, and Oxime **22a**[a]

X−H···Y	X−H	H···Y	X···Y	<XHY
	Compound **8**·HCl			
N1−H1a···Cl^{i}	0.92(4)	2.43(4)	3.208(2)	143(3)
N1−H1b···Cl^{ii}	0.92(4)	2.34(4)	3.237(2)	166(3)
N1−H1c···N2^{i}	0.86(3)	2.16(3)	2.982(2)	162(3)
O2−H2···Cl	0.76(3)	2.20(3)	2.943(1)	166(3)
	Compound **9**·HBr			
N1−H1b···Br	0.93(3)	2.40(3)	3.282(2)	158(3)
N1−H1a···Br^{iii}	0.87(3)	2.42(3)	3.283(2)	173(3)
O2−H2···Br^{iv}	0.84(3)	2.41(3)	3.245(2)	169(3)
	Compound **22a**			
O4−H4···N1^{v}	0.94(2)	1.930(2)	2.808(1)	154(2)

[a] Estimated standard deviations are given in parentheses. Symmetry code: (i) $-x+2, y+0.5, -z+1$; (ii) $-x+2, y-0.5, -z+1$; (iii) $y-1, x, 1-z$; (iv) $1+x-y, 3-y, 0.667-z$; (v) $1-x, 1-y, 2-z$.

Table 2. Inhibition of GABA Uptake into Synaptosomes and Cultured Cortex Neurons and Astrocytes (Values ± SEM, $n = 3-6$)

	uptake inhibition IC_{50} (μM)		
compd	synaptosomes	neurons	astrocytes
2	5 ± 1	12[a]	16[a]
3	12 ± 3	32[a]	29[a]
1	162 ± 15	530[a]	268[a]
8	181 ± 21	883 ± 100	208 ± 38
(*R*)-**8**	118 ± 10	681 ± 87	281 ± 66
(*S*)-**8**	>300	nd	nd
9	63 ± 7	423 ± 57	28 ± 7
(*R*)-**9**	39 ± 3	510 ± 60	60 ± 12
(*S*)-**9**	>300	nd	nd
10	105 ± 9	391 ± 25	278 ± 52
11	98 ± 12	>1000	>500
12	125 ± 17	>1000	>500
13	173 ± 20	>1000	>500
14	291 ± 36	>1000	>500
15	201 ± 19	>1000	>500
16	290 ± 41	>1000	>500
17	242 ± 20	>1000	>500

[a] Compounds **1**−**3** are competitive inhibitors of neuronal and glial GABA uptake,[15] and consequently the IC_{50} values, corresponding to a GABA concentration of 1 μM, were calculated from the respective K_i values[15] using the equation of Cheng and Prusoff.[29] The K_i values were calculated from apparent K_m values with SD values of less than 15%; nd, not dertermined.

Table 3. Physicochemical Characteristics of Compound **9** and Its Pivaloyloxymethyl Prodrugs **43** and **44**

	$t_{1/2}$ (min)		log *D*	
compd	aqueous buffer	human plasma	*n*-octanol	cyclohexane
9	stable	stable	−2.3	nd[a]
43	4500	15	0.65	−0.17
44	16000	7	0.05	−1.4

[a] nd, not determined.

higher than that of **1**. This line of research involved the synthesis of *exo*-THPO (**8**), having an amino group at the same ring carbon atom as in **1,** but with an exocyclic orientation (Figure 1). Compound **8** turned out to be approximately equipotent with **1** as an inhibitor of GABA uptake, but with a marginally higher degree of selectivity for the glial transporter (Table 2). The conversion of the primary amino group of **8** into a secondary amino group by *N*-methylation gave **9**, showing a slight increase in potency but a significantly higher degree of glia selectivity, **9** being about 12 times more potent as an inhibitor of glial than of neuronal GABA uptake (Table 2). Substitution of an ethyl group for the

N-methyl group of **9** to give **10** markedly reduced the effect on glial GABA uptake and thus the glia selectivity of **10**. Further increase in the size of the *N*-alkyl group led to analogues showing little if any effect on neuronal or glial GABA uptake (Table 2).

The effects of **8** as well as **9** on GABA uptake were shown to reside in the (*R*)-enantiomers of the compounds (Table 2, Figure 2). Interestingly, it also is the (*R*)-form of nipecotic acid that is the active GABA uptake inhibitor.[36]

Compound **1** does not easily penetrate the BBB, although the BBB of young animals is more penetrable for **1** than the BBB of adult animals.[34] Like **1**, the bicyclic 3-isoxazolols listed in Table 2 have predominantly zwitterionic structures, and neither (*R*)-**9** nor **10**−**13** showed detectable anticonvulsant effects after subcutaneous administration. However, as expected, compound **9** potently protected Frings mice[35] against audiogenic seizures when administered intracerebroventricularly following a published procedure.[35] These observations together with the low lipophilicity of **9** as described in terms of the distribution coefficient between *n*-octanol and aqueous buffer of pH 7.4 (Table 3) prompted us to synthesize the two isomeric pivaloyloxymethyl derivatives **43** and **44** as potential prodrugs of **9** (Scheme 4). Both these compounds are significantly more lipophilic than the parent compound, and both are quite stable in aqueous buffer but relatively unstable in the presence of human plasma (Table 3). They show approximately equipotent anticonvulsant effects in mice after subcutaneous administration. This effect of **43** was shown to reside in the (*R*)-enantiomer, **45**, whereas the corresponding (*S*)-form, **46**, was inactive. Since neither **45** nor **46** per se showed effects on GABA uptake (data not shown); these observations support the view that the pharmacological effects of **45** are caused by (*R*)-**9,** assumed to be formed in the mouse central nervous system after decomposition of **45**.

Thus, although the selectivity of (*R*)-**9** for the glial GABA uptake system, as compared to the neuronal system, is only 8-fold, compound (*R*)-**9** still is the most glia-selective inhibitor so far described. The conversion of (*R*)-**9** into analogues containing various bulky and lipophilic *N*-substituents, including the 4,4-diphenyl-3-butenyl group, is in progress, and the synthesis and pharmacology of these compounds will be reported separately.

Experimental Section

Chemistry. Melting points were determined in capillary tubes and are uncorrected. Elemental analyses were performed by Mr. G. Cornali, Microanalytical Laboratory, LEO Pharmaceutical Products, Denmark, or by the Analytical Department, H. Lundbeck A/S, Denmark, and are within ±0.4% of the calculated values, unless otherwise stated. Flash chromatography (FC) was performed on Merck silica gel 60 H (5−40 μM) and column chromatography (CC) on Merck silica gel 60 (70−230 mesh, ASTM). Analytical thin-layer chromatography (TLC) was carried out using Merck silica gel F_{254} plates. Compounds containing the 3-isoxazolol moiety were visualized using an $FeCl_3$ spray reagent and compounds containing amino groups were visualized using a ninhydrin spray reagent. ^{1}H NMR spectra were recorded on a Bruker AC 200F (200 MHz), a Bruker AC 250F (250 MHz), or a Varian 360L (60 MHz) spectrometer. Optical rotations were measured in thermostated cuvettes on a Perkin-Elmer 241 polarimeter.

Determination of Stereochemical Purity. The enantiomeric excess (ee) of (*S*)-(+)-**8** was determined at ambient temperature on a 150 × 4 mm Crownpak CR(−) column (Daicel). The column was eluted with 0.4 mL/min of aqueous perchloric acid (pH 1.0). Determination of the ee of (*R*)-(−)-**8** was performed using the same column and eluent, but at 1.5 °C. The instrumentation used consisted of a Jasco 880-PU pump, a Rheodyne 7125 injector, and a Waters M480 UV detector set at 200 nm connected to a Hitachi-Merck D-2000 chromato-integrator.

Determination of ee values for (*R*)-(+)-**9** and (*S*)-(−)-**9** was performed at ambient temperature on a 100 × 4.0 mm CHIRAL-AGP column (ChromTech, Sweden). The column was eluted with 0.5 mL/min of 10 mM potassium phosphate buffer, pH 7.0, using a Waters M510 pump connected to a Waters U6K injector and a Waters 991 photodiode array detector.

Similarly, using the same column and instrumentation as for (*R*)-(+)-**9** and (*S*)-(−)-**9**, the enantiomeric purities of **45** and **46** were determined at ambient temperature. The column was eluted with 0.5 mL/min of 100 mM phosphate buffer (pH 7.0)/MeOH (80:20). The enantiomeric purity was based on peak areas.

Characterization of Prodrugs. The hydrolysis of the *O*- and *N*-pivaloyloxymethyl prodrugs **43** and **44**, respectively, was studied in 0.02 M aqueous phosphate buffer (pH 7.4) at 37 °C (Table 3). The rate of hydrolysis was determined by using a reversed-phase HPLC procedure. The HPLC system consisted of a Hitachi-Merck pump model L6200, a variable wavelength UV detector (type Hitachi-Merck L-4000), and a Hitachi-Merck AS-4000 autosampler. Data acquisition and processing were performed using the Hitachi-Merck HPLC manager (model 6000). The analytical column used was a Lichrospher 100-RP8 (250 × 4 mm) which was kept in a column oven at 40 °C. The mobile phase consisted of MeOH mixed with 0.05 M phosphate (pH 2.1) to which was added 200 μL of TEA/L, in the proportion 40−60% v/v. The flow rate was kept at 1.0 mL/min and the column effluent was monitored at 220 nm. The retention times for the prodrugs were 7.8 and 6.0 min for **43** and **44**, respectively. Quantification of the prodrugs was done by measuring peak area in relation to those of standards chromatographed under the same conditions. The reactions were initiated by adding about 50 μL of a stock solution of a prodrug in MeCN−H_2O (90:10) to 10.0 μL of phosphate buffer, preheated at 37 °C, in screw-capped test tubes, the final prodrug concentration being (2−3) × 10^{-5} M. The solutions were kept in a water bath at 37 ± 0.2 °C. At appropriate times samples were taken and immediately chromatographed. Pseudo-first-order rate constants were determined from the slopes of linear plots of the logarithm of residual prodrug against time.

Hydrolysis of the prodrugs was also studied in 0.02 M phosphate buffer (pH 7.4) containing 80% human plasma at 37 °C. Initial concentrations of the compounds were (2−3) × 10^{-5} M. At appropriate times, samples of 250 μL were withdrawn and deproteinized by mixing with 1000 μL of EtOH. After centrifugation for 3−5 min the clear supernatant fraction was analyzed by HPLC as described above.

The apparent partition coefficients (*D*) of **9** and its prodrugs were determined in two systems, i.e., *n*-octanol−0.05 M phosphate buffer (pH 7.40) and cyclohexane−0.05 M phosphate buffer (pH 7.40) at 21 °C (Table 3). The aqueous and organic phases were mutually saturated at 21 °C before use. The compounds were dissolved in the aqueous buffer phase ((2−3) × 10^{-5} M) and the organic phase−buffer mixtures were shaken for 10−15 min to reach a distribution equilibrium. The volumes of each phase were chosen so that the solute concentration in the aqueous phase, before and after distribution, could be measured readily using the HPLC method described above. At distribution equilibrium the two phases were separated by centrifugation for 3−5 min. During the entire procedure less than 2% of the prodrugs were degraded as determined by HPLC.

3-Methoxy-4,5,6,7-tetrahydro-1,2-benzisoxazole (19a). To a solution of **18**[39] (5.1 g, 37 mmol) in ether (100 mL) was

added a solution of diazomethane (0.3 mol) in ether (100 mL). After stirring for 2 h, HOAc was added and the mixture was evaporated. CC [tol−EtOAc (3:1)] gave 2.94 g (52%) of **19a** as an oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.98 (3H, s), 2.57 (2H, t, J = 6 Hz), 2.29 (2H, t, J = 6 Hz), 1.85−1.65 (4H, m).

3-Ethoxy-4,5,6,7-tetrahydro-1,2-benzisoxazole (19b). To a solution of **18**[39] (100 g, 0.72 mol) in acetone (3 L) was added K_2CO_3 (200 g, 1.45 mol) and the mixture was stirred at 50 °C for 1 h. Ethyl bromide (170 mL, 2.2 mol) in acetone was added during 1.5 h and stirring at 50 °C was continued overnight. Filtration and evaporation followed by CC [EtOAc−heptane (2:3)] afforded 65 g (54%) of **19b** as an oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.26 (2H, q, J = 7 Hz), 2.56 (2H, t, J = 6 Hz), 2.28 (2H, t, J = 6 Hz), 1.85−1.65 (4H, m), 1.38 (3H, t, J = 7 Hz).

3-Methoxy-4,5,6,7-tetrahydro-1,2-benzisoxazol-4-one (20a) and 3-Methoxy-4,5,6,7-tetrahydro-1,2-benzisoxazol-7-one (21a). To a mixture of **19a** (2.70 g, 17.6 mmol), HOAc (55 mL), and concentrated H_2SO_4 (2.1 mL) was added a solution of $Na_2Cr_2O_7 \cdot 2H_2O$ (3.7 g, 12 mmol) in HOAc (30 mL) during 45 min. After stirring for 2 h, the mixture was neutralized by addition of 2 M NaOH. Extraction with Et_2O (4 × 100 mL), drying, evaporation, and CC [tol−EtOAc (10−20%)] gave **21a** (0.28 g, 10%): mp 100−101 °C (tol−light petroleum); ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.06 (3H, s), 2.65 (4H, m), 2.2 (2H, m). Anal. ($C_8H_9NO_3$) C, H, N.

Later fractions contained **20a** (1.55 g, 55%): mp 121−123 °C (tol−light petroleum); ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.05 (3H, s), 2.96 (2H, t, J = 7 Hz), 2.54 (2H, t, J = 7 Hz), 2.25 (2H, m). Anal. ($C_8H_9NO_3$) C, H, N.

3-Ethoxy-4,5,6,7-tetrahydro-1,2-benzisoxazol-4-one (20b) and 3-Ethoxy-4,5,6,7-tetrahydro-1,2-benzisoxazol-7-one (21b). By using a similar procedure as above compounds **20b** and **21b** were synthesized from **19b** (35 g, 0.21 mol).

20b: yield 71%; mp 99−101 °C (EtOAc−Et_2O−light petroleum); ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.38 (2H, q, J = 7 Hz), 2.90 (2H, t, J = 6 Hz), 2.51 (2H, t, J = 6 Hz), 2.19 (2H, m), 1.46 (3H, t, J = 7 Hz). Anal. ($C_9H_{11}NO_3$) C, H, N.

21b: yield 8%; mp 69−71 °C (EtOAc−Et_2O−light petroleum); ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.37 (2H, q, J = 7 Hz), 2.63 (4H, m), 2.18 (2H, m), 1.44 (3H, t, J = 7 Hz). Anal. ($C_9H_{11}NO_3$) C, H, N.

4-(Hydroxyimino)-3-methoxy-4,5,6,7-tetrahydro-1,2-benzisoxazole (22a). A mixture of **20a** (1.84 g, 11 mmol), hydroxylamine hydrochloride (4.6 g, 66 mmol), Na_2CO_3 (3.5 g, 33 mmol), EtOH (80 mL), and water (120 mL) was refluxed for 2.5 h. Evaporation, addition of water (50 mL), and extraction with CH_2Cl_2 (3 × 100 mL) afforded **22a** (1.75 g, 87%): mp 208−210 °C (EtOAc−light petroleum); ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.04 (3H, s), 2.76 (4H, m), 2.01 (2H, m). Anal. ($C_8H_{10}N_2O_3$) C, H, N.

3-Ethoxy-4-(hydroxyimino)-4,5,6,7-tetrahydro-1,2-benzisoxazole (22b). Prepared from **20b** (1.56 g, 8.60 mmol) by the same procedure as above: yield 1.38 g (82%); mp 214−217 °C (EtOAc−light petroleum); ^{1}H NMR (60 MHz, $CDCl_3$−DMSO-d_6 [9:1]) δ 4.30 (2H, q, J = 7 Hz), 2.70 (4H, m), 1.95 (2H, m), 1.35 (3H, t, J = 7 Hz). Anal. ($C_9H_{12}N_2O_3$) C, H, N.

***(RS)*-4-Amino-3-methoxy-4,5,6,7-tetrahydro-1,2-benzisoxazole Hydrobromide (23a).** Aluminum strips (4.32 g, 160 mmol) were treated with an aqueous solution of $HgCl_2$ (5%) for 1 min, filtered, and washed with EtOH. To these pieces was added a solution of **22a** (1.46 g, 8.0 mmol) in aqueous MeOH (75%, 75 mL), and the mixture was stirred at room temperature for 5 days, filtered, and evaporated. The resulting oil (1.20 g, 89%) was dissolved in ether (50 mL) and HBr in HOAc (33%) was added to precipitate **23a.** Recrystallization (EtOH−MeCN−Et_2O) afforded **23a** (1.16 g, 58%): mp 203−205 °C; ^{1}H NMR (200 MHz, D_2O) δ 4.36 (1H, m), 3.95 (3H, s), 2.66 (2H, m), 2.20−1.75 (4H, m). Anal. ($C_8H_{12}N_2O_2 \cdot$HBr) C, H, Br, N.

***(RS)*-4-Amino-3-ethoxy-4,5,6,7-tetrahydro-1,2-benzisoxazole Hydrobromide (23b).** From **22b** (1.30 g, 6.5 mmol): yield 1.19 g (70%); mp 209−211 °C (EtOH−MeCN−Et_2O); ^{1}H NMR (60 MHz, D_2O) δ 4.40 (1H, m), 4.30 (2H, q, J = 7 Hz), 2.65 (2H, m), 2.20−1.75 (4H, m), 1.40 (3H, t, J = 7 Hz). Anal. ($C_9H_{14}N_2O_2 \cdot$HBr) C, H, Br, N.

***(RS)*-4-Amino-3-hydroxy-4,5,6,7-tetrahydro-1,2-benzisoxazole Hydrobromide (8).** A solution of **23a** (2.49 g, 10 mmol) in HBr in HOAc (33%, 20 mL) was stirred at 80 °C for 20 min. Evaporation and treatment of the residue with HBr in HOAc at 80 °C for 20 min gave after evaporation and recrystallization **8** (1.83 g, 78%): mp 187−190 °C (EtOH−Et_2O); ^{1}H NMR (200 MHz, D_2O) δ 4.42 (1H, m), 2.68 (2H, m), 2.2−1.85 (4H, m). Anal. ($C_7H_{10}N_2O_2 \cdot$HBr) C, H, Br, N.

***(RS)*-3-Ethoxy-4-(dimethylamino)-4,5,6,7-tetrahydro-1,2-benzisoxazole Hydrobromide (24).** Extraction with CH_2Cl_2 of a mixture of **23b** (526 mg, 2.0 mmol) and excess 1 M NaOH gave, after drying and evaporation, the free base. Sodium formate (3.0 g, 44 mmol), formic acid (3.0 mL, 78 mmol), and a 30% solution of formaldehyde (3.0 mL) were added and the mixture was heated at 100 °C for 20 h and evaporated. Water (20 mL) and NaOH were added to the residue. Extraction with CH_2Cl_2 (3 × 40 mL), drying, and evaporation gave an oil. To a solution of the oil in ether (20 mL) was added HBr in HOAc and **24** was precipitated. Recrystallization (MeCN−Et_2O) gave pure **24** (422 mg, 72%): mp 158−160 °C; ^{1}H NMR (60 MHz, D_2O) δ 4.53 (1H, m), 4.45 (2H, q, J = 7 Hz), 3.00 (6H, s), 2.75 (2H, m), 2.05 (4H, m), 1.46 (3H, t, J = 7 Hz). Anal. ($C_{11}H_{18}N_2O_2 \cdot$HBr) C, H, Br, N.

***(RS)*-4-(Dimethylamino)-3-hydroxy-4,5,6,7-tetrahydro-1,2-benzisoxazole Hydrobromide (14).** Compound **24** was deprotected as described for **8**: yield 86%; mp 184−186 °C (EtOH−Et_2O); ^{1}H NMR (60 MHz, D_2O) δ 4.50 (1H, m), 3.00 (6H, s), 2.83 (2H, m), 2.05 (4H, m). Anal. ($C_9H_{14}N_2O_2 \cdot$HBr$\cdot$0.33H_2O) C, H, Br, N.

***(RS)*-4-[(*tert*-Butyloxycarbonyl)amino]-3-ethoxy-4,5,6,7-tetrahydro-1,2-benzisoxazole (25).** A solution of **23b** (2.9 g, 11 mmol) in water (30 mL) and dioxane (30 mL) was cooled to 10 °C, and a solution of NaOH (0.88 g, 22 mmol) in water (10 mL) was added. After addition of a solution of di-*tert*-butyl dicarbonate (2.60 g, 12 mmol) in dioxane (12 mL), the mixture was stirred at room temperature for 1.5 h. Water (100 mL) and a small amount of NaOH (to pH > 10) were added and the mixture was stirred for 30 min. The mixture was washed with ether (150 mL) and the ether was discarded. The aqueous phase was adjusted to pH = 3 by addition of $KHSO_4$ and was extracted with ether (2 × 75 mL). Drying, evaporation, and recrystallization (Et_2O−light petroleum) afforded **25** (2.6 g, 84%): mp 115−117 °C; ^{1}H NMR (60 MHz, $CDCl_3$) δ 4.85 (1H, m), 4.45 (2H, q, J = 7 Hz), 2.75 (2H, m), 2.00 (4H, m), 1.65 (9H, s), 1.55 (3H, t, J = 7 Hz). Anal. ($C_{14}H_{22}N_2O_4$) C, H, N.

***(RS)*-4-[(*tert*-Butyloxycarbonyl)-*N*-ethylamino]-3-ethoxy-4,5,6,7-tetrahydro-1,2-benzisoxazole (26).** A solution of **25** (0.57 g, 2.0 mmol) and ethyl iodide (1.6 mL, 20 mmol) in THF (25 mL) was cooled in an ice bath and sodium hydride (60% in mineral oil, 240 mg, 6.0 mmol) was added. After stirring at room temperature overnight, EtOH was added and the mixture was evaporated. Addition of water (5 mL), extraction with EtOAc (2 × 10 mL), and evaporation afforded **26** (0.53 g, 86%) as an oil: ^{1}H NMR (60 MHz, $CDCl_3$) δ 5.1 (1H, br s), 4.15 (2H, q, J = 7 Hz), 3.0 (2H, m), 2.60 (2H, m), 2.1−1.7 (4H, m), 1.45 (9H, s), 1.20 (6H, m).

***(RS)*-4-(Ethylamino)-3-hydroxy-4,5,6,7-tetrahydro-1,2-benzisoxazole (10).** Treatment of **26** with HBr in HOAc as described for **8** gave **10** (66%): mp 189−191 °C (EtOH−MeCN−Et_2O); ^{1}H NMR (60 MHz, D_2O) δ 4.35 (1H, m), 3.30 (2H, q, J = 7 Hz), 2.70 (2H, m), 2.05 (4H, m), 1.30 (3H, t, J = 7 Hz). Anal. ($C_9H_{14}N_2O_2 \cdot$HBr) C, H, Br, N.

***(RS)*-3-Ethoxy-4-(methylamino)-4,5,6,7-tetrahydro-1,2-benzisoxazole (27).** To a mixture of methylamine in EtOH (33%, 5 mL, 40 mmol), methylamine hydrochloride (15 g, 220 mmol), and molecular sieves (3 Å, 25 g) was added a solution of **20b** (4.50 g, 24.8 mmol) in MeOH (100 mL). The mixture was stirred for 30 min at room temperature and sodium cyanoborohydride (7.0 g, 85 mmol) was added portionwise during 20 min. After stirring for 20 h, the mixture was filtered and the filtrate evaporated. Water (50 mL) was added and the pH was adjusted to 10 by addition of NaOH. Extraction with

EtOAc (7 × 50 mL), drying, and evaporation gave crude **27** (4.80 g, 98%) as an oil: ^{1}H NMR (60 MHz, $CDCl_3$) δ 4.35 (2H, q, J = 7 Hz), 3.70 (1H, m), 2.70 (2H, m), 2.50 (3H, s), 1.95 (4H, m), 1.40 (3H, t, J = 7 Hz).

By using the same procedure the following compounds were synthesized.

***(RS)*-3-Ethoxy-4-(2-propen-1-ylamino)-4,5,6,7-tetrahydro-1,2-benzisoxazole (28):** oil; ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.05−5.90 (1H, m), 5.30−5.10 (2H, m), 4.35 (2H, q), 3.75 (1H, t), 3.45−3.30 (2H, m), 2.70−2.45 (2H, m), 2.45 (1H, br s), 2.05−1.95 (1H, m), 1.85−1.70 (3H, m), 1.40 (3H, t).

***(RS)*-3-Ethoxy-4-(1-pyrrolidinyl)-4,5,6,7-tetrahydro-1,2-benzisoxazole (29):** oil; ^{1}H NMR (250 MHz, $CDCl_3$) δ 4.30 (2H, dq), 3.25 (1H, t), 2.80−2.50 (4H, m), 2.25−2.00 (2H, m), 1.90−1.70 (6H, m), 1.55−1.40 (2H, m), 1.40 (3H, t).

***(RS)*-3-Ethoxy-4-(2-hydroxyethylamino)-4,5,6,7-tetrahydro-1,2-benzisoxazole (30):** mp 72−74 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 4.30 (2H, q), 3.75 (1H, t), 3.70−3.60 (2H, m), 2.85 (2H, t), 2.70−2.45 (2H, m), 2.45 (2H, br s), 2.0−1.65 (4H, m), 1.40 (3H, t).

Compounds **27**−**30** were deprotected with HBr in HOAc as described for the synthesis of **8**.

***(RS)*-3-Hydroxy-4-(methylamino)-4,5,6,7-tetrahydro-1,2-benzisoxazole hydrobromide (9):** yield 3.6 g (74%); mp 184−186 °C (EtOH−Et_2O); ^{1}H NMR (250 MHz, DMSO-d_6) δ 8.60 (1H, br s), 4.20 (1H, m), 2.70−2.60 (2H, m), 2.65 (3H, s), 2.15−1.75 (4H, m). Anal. ($C_8H_{12}N_2O_2$·HBr) C, H, Br, N.

***(RS)*-3-Ethoxy-4-(1-pyrrolidinyl)-4,5,6,7-tetrahydro-1,2-benzisoxazole hydrobromide (15):** mp 209−210 °C; ^{1}H NMR (250 MHz, DMSO-d_6) δ 4.35 (1H, br s), 3.70−3.10 (4H, m), 2.75−2.60 (2H, m), 2.30−1.70 (8H, m). Anal. ($C_{11}H_{16}N_2O_2$·HBr) C, H, Br, N.

Compound **11** obtained by this method was impure and was purified by treatment with di-*tert*-butyl dicarbonate by the method described for the synthesis of **25**. CC afforded ***(RS)*-4-[*N*-(*tert*-butyloxycarbonyl)-*N*-(2-propen-1-yl)amino]-3-hydroxy-4,5,6,7-tetrahydro-1,2-benzisoxazole (31)** as an oil: ^{1}H NMR (250 MHz, $CDCl_3$) δ 5.90−5.75 (1H, m), 5.20−5.00 (3H, m), 4.00−3.85 (1H, m), 3.65−3.50 (1H, m), 2.55 (2H, br s), 2.10−1.95 (2H, m), 1.85−1.70 (2H, m), 1.40 (9H, s).

(*RS*)-3-Hydroxy-4-(2-propen-1-ylamino)-4,5,6,7-tetrahydro-1,2-benzisoxazole Oxalate (11). Deprotection of **31** with trifluoroacetic acid and addition of oxalic acid gave **11**: mp 182−183 °C (acetone); ^{1}H NMR (250 MHz, DMSO-d_6) δ 6.00−5.85 (1H, m), 5.45 (1H, d), 5.40 (1H, d), 4.15 (1H, br s), 3.65 (2H, d), 2.75−2.60 (2H, m), 2.20−1.75 (4H, m). Anal. ($C_{10}H_{14}N_2O_2$·$C_2H_2O_4$) C, H, N.

(*RS*)-4-(2-Acetyloxyethylamino)-3-hydroxy-4,5,6,7-tetrahydro-1,2-benzisoxazole Hydrobromide (12). Treatment of **30** with HBr in HOAc resulted in **12**: mp 164−165 °C; ^{1}H NMR (250 MHz, DMSO-d_6) δ 4.40−4.20 (3H, m), 3.30 (2H, t), 2.70−2.55 (2H, m), 2.10 (3H, s), 2.20−2.00 (2H, m), 1.95−1.75 (2H, m). Anal. ($C_{11}H_{16}N_2O_4$·HBr) C, H, Br, N.

***(RS)*-3-Hydroxy-4-(2-hydroxyethylamino)-4,5,6,7-tetrahydro-1,2-benzisoxazole Hydrobromide (13).** A solution of **12** (1.3 g, 4.0 mmol) in water (50 mL) and aqueous HBr (48%, 7 mL) was heated at 100 °C for 1 h. Evaporation and crystallization from EtOH afforded **13** (0.9 g, 80%): mp 172−173 °C; ^{1}H NMR (250 MHz, DMSO-d_6) δ 5.20 (1H, br s), 4.25 (2H, br s), 3.70 (2H, q), 3.10 (2H, t), 2.75−2.55 (2H, m), 2.30−1.95 (2H, m). Anal. ($C_9H_{14}N_2O_3$·HBr) C, H, Br, N.

(*R*)-*N*-(α-Methoxyphenylacetyl) Derivatives of (*S*)-4-Amino-3-ethoxy-4,5,6,7-tetrahydro-1,2-benzisoxazole (33) and (*R*)-4-Amino-3-ethoxy-4,5,6,7-tetrahydro-1,2-benzisoxazole (32). To an ice-cold solution of **23b** (18 g, 100 mmol) in CH_2Cl_2 (500 mL) was added triethylamine (30 mL, 215 mmol) followed by dropwise addition of a solution of (*R*)-(−)-α-methoxyphenylacetyl chloride (from the acid (17 g, 100 mmol) by reaction with $SOCl_2$) in CH_2Cl_2 (80 mL). The mixture was stirred for 2 h at room temperature and water (2 L) was added. The organic phase was separated, washed with dilute HCl, dried, and evaporated. The two diastereomers were separated by preparative HPLC on silical gel [heptane−EtOAc (3:2)]. The first eluted fractions contained **33** (13 g) as an oil. The later fractions contained **32** (11.5 g): mp 96−97 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.45−7.30 (5H, m), 6.85 (1H, br d), 4.95 (1H, dt), 4.60 (1H, s), 4.25 (2H, q), 3.30 (3H, s), 2.75−2.45 (2H, m), 2.05−1.80 (4H, m), 1.30 (3H, t).

***(S)*-4-[(*tert*-Butyloxycarbonyl)amino]-3-hydroxy-4,5,6,7-tetrahydro-1,2-benzisoxazole (35).** A solution of **33** (3.7 g, 11 mmol) in aqueous HBr (48%, 175 mL) and water (175 mL) was refluxed for 1.5 h and evaporated. The residue was dissolved in water, washed with CH_2Cl_2, and evaporated leaving impure (*S*)-(+)-**8** hydrobromide (4.0 g) as an oil. This compound was Boc-protected by the method described for **25**. CC [heptane−EtOAc−EtOH (7:3:1)] gave **35** (0.9 g, 32%): mp 135 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 5.05 (1H, br d), 4.50 (1H, dt), 2.70−2.45 (2H, m), 2.10−1.75 (4H, m), 1.45 (9H, s).

(*R*)-4-[(*tert*-Butyloxycarbonyl)amino]-3-hydroxy-4,5,6,7-tetrahydro-1,2-benzisoxazole (34). The compound was prepared as described for **35**: mp 134 °C; ^{1}H NMR spectrum was identical with that of **35**.

***(S)*-(+)-3-Hydroxy-4-amino-4,5,6,7-tetrahydro-1,2-benzisoxazole Hydrochloride [(*S*)-(+)-8·HCl].** A solution of **35** (0.9 g, 3.5 mmol) in Et_2O (200 mL) saturated with HCl was stirred at room temperature for 1.5 h. Evaporation and crystallization (EtOH−Et_2O) gave (*S*)-(+)-**8**·HCl (0.5 g, 93%): mp 209−210 °C; ^{1}H NMR (250 MHz, DMSO-d_6) δ 8.40 (4H, br s), 4.25 (1H, br t), 2.65−2.55 (2H, m), 2.05−1.75 (4H, m); ee = 99.8%; $[\alpha]_D$ = +19.4 (c = 1.0, MeOH). Anal. ($C_7H_{10}N_2O_2$·HCl) C, H, Cl, N.

(*R*)-(−)-4-Amino-3-hydroxy-4,5,6,7-tetrahydro-1,2-benzisoxazole Hydrochloride [(*R*)-(−)-8·HCl]. Prepared from **34** as described above: mp 209−210 °C; ee = 99.7%; $[\alpha]_D$ = −20.0 (c = 1.0, MeOH); ^{1}H NMR spectrum was identical with that of compound (*S*)-(+)-**8**·HCl. Anal. ($C_7H_{10}N_2O_2$·HCl) C, H, Cl, N.

(*R*)-*N*-(α-Methoxyphenylacetyl) Derivatives of (*R*)-3-Ethoxy-4-(methylamino)-4,5,6,7-tetrahydro-1,2-benzisoxazole (36) and (*S*)-3-Ethoxy-4-(methylamino)-4,5,6,7-tetrahydro-1,2-benzisoxazole (37). The compounds were prepared by the method described for **32** and **33**. The first eluted compound was **37** followed by **36**.

***(R)*-(+)-3-Hydroxy-4-(methylamino)-4,5,6,7-tetrahydro-1,2-benzisoxazole Hydrobromide [(*R*)-(+)-9·HBr].** To a solution of **36** (9.0 g, 26 mmol) in THF was added a solution of lithium triethylborohydride in THF (1 M, 80 mL) dropwise during 20 min at 0−5 °C. After stirring at room temperature overnight, the mixture was poured on ice (500 g) and pH was adjusted to 2 by addition of HCl. THF was removed by evaporation and the aqueous mixture was washed with EtOAc (2 × 50 mL). Concentrated NaOH was added to pH = 11 and extraction with EtOAc (100 mL), drying, and evaporation gave 4.6 g of crude **38**.

A solution of **38** in HBr in HOAc (33%, 150 mL) was heated to 90 °C for 1 h and evaporated. Crystallization of the residue (EtOH−Et_2O) gave 4.2 g (65%) of (*R*)-(+)-**9**·HBr: mp 207−209 °C; ee = 99.1%; $[\alpha]_D$ = +5.6 (c = 1.0, MeOH); ^{1}H NMR spectrum was identical with that of **9**. Anal. ($C_8H_{12}N_2O_2$·HBr) C, H, Br, N.

(*S*)-(−)-3-Hydroxy-4-(methylamino)-4,5,6,7-tetrahydro-1,2-benzisoxazole Hydrobromide [(*S*)-(−)-9·HBr]. The compound was prepared analogously from **37**: mp 208−209 °C; ee > 99.4%; $[\alpha]_D$ = −5.9 (c = 1.0, MeOH); ^{1}H NMR spectrum was identical with that of (*R*)-(+)-**9**. Anal. ($C_8H_{12}N_2O_2$·HBr) C, H, Br, N.

***(RS)*-4-[*N*-(*tert*-Butyloxycarbonyl)methylamino]-3-hydroxy-4,5,6,7-tetrahydro-1,2-benzisoxazole (40).** Compound **9** (7.0 g, 28 mmol) was Boc-protected by the method described for **25**: yield 5.3 g (74%); mp 151−152 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 5.15 (1H, br s), 2.75 (3H, s), 2.65−2.55 (2H, m), 2.10−1.65 (4H, m), 1.50 (9H, s).

(*RS*)-4-[(*tert*-Butyloxycarbonyl)-*N*-methylamino]-3-[(pivaloyloxy)methyloxy]-4,5,6,7-tetrahydro-1,2-benzisoxazole (41) and (*RS*)-4-[(*tert*-Butyloxycarbonyl)-*N*-methylamino]-2-[(pivaloyloxy)methyl]-4,5,6,7-tetrahydro-1,2-benzisoxazol-3-one (42). Potassium *tert*-butoxide (2.5 g, 22 mmol) was cautiously added to a suspension of **40** (5.0 g, 19

mmol) in acetone (50 mL). A solution of iodomethyl pivalate (7.5 g, 31 mmol) in acetone (10 mL) was added and the mixture was stirred overnight. After filtration, the filtrate was evaporated to give a mixture of **41** and **42**. The compounds were separated by CC [heptane−EtOAc (3:2)]. The first fractions contained **41** (oil, 3.9 g, 54%): ^{1}H NMR (250 MHz, $CDCl_3$) δ 5.85 (2H, dd), 5.30−5.00 (1H, m), 2.60−2.50 (3H, m), 2.10−1.50 (6H, m), 1.50 (9H, s), 1.30 (9H, s).

The latter fractions contained **42** (2.1 g, 29%) as an oil: ^{1}H NMR (250 MHz, $CDCl_3$) δ 5.75 (2H, s), 5.10−5.00 (1H, m), 2.65 (3H, s), 2.50−2.40 (2H, m), 2.10−1.60 (4H, m), 1.50 (9H, s), 1.20 (9H, s).

(*RS*)-4-(Methylamino)-3-[(pivaloyloxy)methyloxy]-4,5,6,7-tetrahydro-1,2-benzisoxazole Oxalate (43). To a solution of **41** (3.7 g, 10 mmol) in CH_2Cl_2 was added trifluoroacetic acid (19 mL). The mixture was stirred for 1 h and evaporated. Water (100 mL) and Et_2O (100 mL) were added followed by K_2CO_3 to pH > 9. The organic phase was separated, dried, and evaporated. To a solution of the free base in EtOH was added excess oxalic acid to precipitate **43** (1.6 g, 51%): mp 126−128 °C; ^{1}H NMR (250 MHz, DMSO-d_6) δ 5.90 (2H, dd), 3.85 (1H, t), 2.80−2.55 (2H, m), 2.40 (3H, s), 2.05−1.65 (4H, m), 1.15 (9H, s). Anal. ($C_{14}H_{22}N_2O_4 \cdot C_2H_2O_4$) C, H, N.

(*RS*)-4-(Methylamino)-2-[(pivaloyloxy)methyl]-4,5,6,7-tetrahydro-1,2-benzisoxazol-3-one Hemioxalate (44). Prepared as described for **43**: mp 177−178 °C (acetone); ^{1}H NMR (250 MHz, DMSO-d_6) δ 5.80 (2H, dd), 3.80 (1H, t), 2.60−2.45 (2H, m), 2.50 (3H, s), 2.05−1.60 (4H, m), 1.15 (9H, s). Anal. ($C_{14}H_{22}N_2O_4 \cdot 0.5C_2H_2O_4$) C, H, N.

(*R*)-(+)-4-(Methylamino)-3-[(pivaloyloxy)methyloxy]-4,5,6,7-tetrahydro-1,2-benzisoxazole Hemioxalate (45). The compound was prepared from (*R*)-(+)-**9** by the same procedure as described above for the synthesis of **43**. The hemioxalate salt was crystallized from acetone: mp 210−213 °C; ee = 98.9%; $[\alpha]_D = +5.6$ ($c = 1.0$, MeOH). Anal. ($C_{14}H_{22}N_2O_4 \cdot 0.5C_2H_2O_4$) C, H, N.

(*S*)-(−)-4-(Methylamino)-3-[(pivaloyloxy)methyloxy]-4,5,6,7-tetrahydro-1,2-benzisoxazole Hemioxalate (46). Prepared from (*S*)-(−)-**9** by the same procedure as described above for the synthesis of **43** and crystallized from acetone: mp 211−213 °C; ee = 99.3%; $[\alpha]_D = -5.4$ ($c = 1.0$, MeOH). Anal. ($C_{14}H_{22}N_2O_4 \cdot 0.5C_2H_2O_4$) C, H, N.

2-(Benzylamino)-3,4,5,6-tetrahydrobenzamide (48). A mixture of **47**[40] (10.0 g, 71 mmol), benzylamine (8.4 g, 78 mmol), toluene (35 mL), and molecular sieves (3 Å, 2 g) was refluxed for 2 h, water being removed using a Dean−Stark trap. The reaction mixture was filtered, and the filtrate was evaporated. The residue was crystallized (light petroleum) to give **48** (16 g, 98%): mp 73−74 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 10.16 (1H, t, $J = 6$ Hz), 7.38−7.10 (5H, m), 6.25 (2H, s), 4.31 (2H, d, $J = 6$ Hz), 2.22−1.90 (4H, m), 1.49 (4H, s). Anal. ($C_{14}H_{18}N_2O$) H, N; C: calcd, 72.98; found, 72.45.

3-Hydroxy-4,5,6,7-tetrahydro-1,2-benzisothiazole (49). To a solution of **48** (15 g, 65 mmol) in HOAc (100 mL) was added excess hydrogen sulfide at 80 °C for 4 h. The reaction mixture was evaporated and Et_2O was added to the residue which initiated crystallization. The crystals were dissolved in EtOAc (30 mL) and a solution of Br_2 (8.3 mL, 150 mmol) in EtOAc (30 mL) was dropwise added at room temperature. The mixture was stirred for 20 h at room temperature and evaporated. CC [EtOAc−EtOH (1:1) containing 1% HOAc] gave the title compound (3.3 g, 33%): mp 150−151 °C; ^{1}H NMR (200 MHz, $CDCl_3$−DMSO-d_6 [3:1]) δ 8.5 (1H, br s), 2.83−2.70 (2H, m), 2.48−2.31 (2H, m), 1.96−1.69 (4H, m). Anal. (C_7H_9NOS) C, H, N, S.

3-Chloro-4,5,6,7-tetrahydro-1,2-benzisothiazole (50). A mixture of **49** (4.74 g, 30 mmol), pyridinium hydrochloride (12.7 g, 109 mmol), phosphoric acid (2.1 g, 21 mmol), and phosphorus oxychloride (25 mL, 270 mmol) was stirred at 90 °C for 5 h. The reaction mixture was evaporated and EtOAc (130 mL) was added to the residue. A saturated solution of $NaHCO_3$ (130 mL) was added and after 10 min of stirring the phases were separated. The aqueous phase was extracted with EtOAc (2 × 150 mL) and the combined organic phases were dried and evaporated to give an oil. CC [tol−EtOAc (1:1)] gave **50** (2.8 g, 53%) as a yellow oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.93−2.77 (2H, m), 2.51−2.49 (2H, m), 2.00−1.72 (4H, m). Anal. (C_7H_8ClNS) C, H, N.

3-Chloro-4,5,6,7-tetrahydro-1,2-benzisothiazol-4-one (51). A solution of sodium dichromate (4.4 g, 16 mmol) in HOAc (30 mL) was added dropwise over 1 h to a solution of **50** (2.7 g, 16 mmol) and concentrated H_2SO_4 (1.8 mL) in HOAc (80 mL). The reaction mixture was stirred at room temperature for an additional 2 h and neutralized with a saturated solution of $NaHCO_3$. Extraction with EtO_2 (3 × 150 mL), drying, and evaporation gave an oil. CC [tol−EtOAc (1:1)] eluted first 3-chloro-4,5,6,7-tetrahydro-1,2-benzisothiazol-7-one (680 mg, 23%) as an oil.

The later fractions contained **51** (780 mg, 27%): mp 84−85 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.17 (2H, t, $J = 6.2$ Hz), 2.63 (2H, t, $J = 6.2$ Hz), 2.30−2.15 (2H, m).

3-Methoxy-4,5,6,7-tetrahydro-1,2-benzisothiazol-4-one (52). A mixture of **51** (600 mg, 3.2 mmol) and a solution of Na (506 mg, 22 mmol) in MeOH (22 mL) was stirred at 90 °C for 1 h. The reaction mixture was evaporated and water (20 mL) was added to the residue. Extraction with CH_2Cl_2 (3 × 30 mL), drying, and evaporation gave an oil. CC [tol−EtOAc (4:1)] gave **52** (251 mg, 43%): mp 45−46 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.11 (3H, s), 3.08 (2H, t, $J = 6.1$ Hz), 2.58 (2H, t, $J = 6.1$ Hz), 2.20−2.15 (2H, m). Anal. ($C_8H_9NO_2S$) C, H, N.

(*RS*)-4-Amino-3-hydroxy-4,5,6,7-tetrahydro-1,2-benzisothiazole Hydrobromide (16). To a solution of **52** (185 mg, 1.0 mmol) and NH_4OAc (780 mg, 10.1 mmol) in MeOH (7 mL) was portionwise added sodium cyanoborohydride (44 mg, 0.71 mmol). The mixture was stirred at room temperature for 48 h and acidified with concentrated HCl. The mixture was evaporated and water (3 mL) was added to the residue. The aqueous solution was washed with Et_2O (3 × 15 mL) and solid KOH was added until pH > 10. Extraction with Et_2O (3 × 15 mL), drying, and evaporation gave an oil. The oil was dissolved in EtOH and excess of HCl in EtOAc was added to precipitate (*RS*)-4-amino-3-methoxy-4,5,6,7-tetrahydro-1,2-benzisothiazole hydrochloride (68 mg, 31%): mp 195 °C dec; ^{1}H NMR (200 MHz, D_2O) δ 4.45−4.32 (1H, m), 3.98 (3H, s), 2.90−2.72 (2H, m), 2.28−1.73 (4H, m).

A solution of HBr in HOAc (33%, 3 mL) was added to this compound (60 mg, 0.3 mmol) and the mixture was stirred at room temperature for 48 h. Evaporation and recrystallization of the residue (methanol−ether) gave **16** (28 mg, 41%): mp 160−165 °C; ^{1}H NMR (200 MHz, D_2O) δ 4.39−4.23 (1H), 2.87−2.72 (2H, m), 2.28−2.05 (1H, m), 2.04−1.78 (3H, m). Anal. ($C_7H_{10}N_2OS \cdot HBr \cdot 2.5H_2O$) H, Br, N; C: calcd, 28.39; found, 28.87.

(*RS*)-3-Hydroxy-4-(methylamino)-4,5,6,7-tetrahydro-1,2-benzisothiazole Hydrobromide (17). (*RS*)-3-methoxy-4-(methylamino)-4,5,6,7-tetrahydro-1,2-benzisothiazole hydrochloride was synthesized as described above, using **52** (200 mg, 22 mmol) in MeOH (5 mL), a 33% solution of methylamine (220 μL) in EtOH, methylamine hydrochloride (662 mg, 9.8 mmol), molecular sieves (3 Å), and sodium cyanoborohydride (234 mg, 3.8 mmol). The resulting oil was dissolved in Et_2O and excess of HCl in EtOAc was added to precipitate the compound (172 mg, 68%): mp 146−148 °C; ^{1}H NMR (200 MHz, D_2O) δ 4.42−4.28 (1H, m), 3.98 (3H, s), 3.00−2.78 (2H, m), 2.69 (3H, s), 2.25−1.81 (4H, m).

Deprotection with HBr in HOAc and recrystallization gave **17** (53 mg, 47%): mp 192 °C dec; ^{1}H NMR (200 MHz, D_2O) δ 4.30−4.16 (1H, m), 2.92−2.68 (2H, m), 2.72 (3H, s), 2.24−1.82 (4H, m). Anal. ($C_8H_{12}N_2OS \cdot HBr$) C, H, Br, N.

X-ray Crystallographic Analysis of (*R*)-(−)-4-Amino-3-hydroxy-4,5,6,7-tetrahydro-1,2-benzisoxazole Hydrochloride [(*R*)-(−)-8·HCl]. Crystal data: $[C_7H_{11}N_2O_2]^+$, Cl^-, $M_r = 190.63$, mp 209−210 °C (EtOH−EtOAc), colorless prism, monoclinic, space group $P2_1$ (No. 4), $a = 7.168(2)$ Å, $b = 6.079(1)$ Å, $c = 9.882(2)$ Å, $\beta = 91.44(2)°$, $V = 430.5(2)$ Å^3, $Z = 2$, $D_c = 1.471$ Mg m^{-3}, $F(000) = 200$, μ(Cu Kα) = 3.64 mm^{-1}, $T = 122.0(5)$ K, crystal dimensions = 0.10 × 0.14 × 0.38 mm.

Diffraction data were collected on an Enraf-Nonius CAD-4 diffractometer using graphite monochromated Cu Kα radiation (λ = 1.54184 Å).[41] Intensities were collected using the $\omega/2\theta$ scan mode. Unit cell dimensions were determined by least-squares refinement of 22 reflections (θ range 38.58−43.62°).[41] The reflections were measured in the range $-8 \le h \le 7$, $-7 \le k \le 7$, $-12 \le l \le 12$ ($4.48° < \theta < 74.76°$). Data were reduced using the programs of Blessing (DREADD).[42,43] The intensities of five standard reflections were monitored every 10^4 s (decay 0.8%, not corrected). Absorption correction was applied using the program ABSORB (T_{min} = 0.429; T_{max} = 0.713).[44] A total of 3510 reflections were averaged according to the point group symmetry 2 resulting in 1773 unique reflections (R_{int} = 0.037 on F_o^2).

The structure was solved by the Patterson method using the program SHELXS97[45,46] and refined using the program SHELXL97.[26] Full matrix least-squares refinement on F^2 was performed, minimizing $\sum w(F_o^2 - F_c^2)^2$, with anisotropic displacement parameters for the non-hydrogen atoms. The positions of the hydrogen atoms were located on intermediate difference electron density maps and refined with isotropic displacement parameters fixed at 0.05 Å^2. Extinction correction was applied, extinction coefficient: 0.118(4).[26] The refinement (143 parameters, 1773 reflections) with the molecule having the *(R)*-configuration converged at R_F = 0.026, wR_{F^2} = 0.069 for 1765 reflections with $F_o > 4\sigma(F_o)$; $w = 1/[\sigma(F_o^2) + (0.0481P)^2 + 0.1138P]$, where $P = (F_o^2 + 2F_c^2)/3$; S = 1.069. The Flack absolute structure factor: 0.00(1).[25,26] In the final difference Fourier map maximum and minimum electron densities were 0.23 and −0.26 e Å^{-3}, respectively. Complex atomic scattering factors for neutral atoms were as incorporated in SHELXL97.[26,47]

X-ray Crystallographic Analysis of (*R*)-(+)-3-Hydroxy-4-(methylamino)-4,5,6,7-tetrahydro-1,2-benzisoxazole Hydrobromide [(*R*)-(+)-9·HBr]. Crystal data: $[C_8H_{13}N_2O_2]^+$, Br^-, M_r = 249.11, mp 207−209 °C (CH_2Cl_2−Et_2O), colorless triangles, trigonal, space group $P3_121$ (No. 152), a = 7.807(2) Å, b = 7.807(2) Å, c = 29.078(7) Å, V = 1534.8(7) Å^3, Z = 6, D_c = 1.617 Mg m^{-3}, $F(000)$ = 756, μ(Cu Kα) = 5.27 mm^{-1}, T = 122.0(5) K, crystal dimensions = 0.07 × 0.10 × 0.50 mm.

Diffraction data were collected on an Enraf-Nonius CAD-4 diffractometer using graphite monochromated Cu Kα radiation (λ = 1.54184 Å).[41] Intensities were collected using the $\omega/2\theta$ scan mode. Unit cell dimensions were determined by least-squares refinement of 24 reflections (θ range 39.41−39.84°).[41] The reflections were measured in the range $-9 \le h \le 9$, $-9 \le k \le 9$, $-36 \le l \le 36$ ($4.56° < \theta < 74.75°$). Data were reduced using the programs of Blessing (DREADD).[42,43] The intensities of five standard reflections were monitored every 10^4 s (decay 14.5%). Appropriate scaling was performed using the program SCALE3.[42,43] Absorption correction was applied using the program ABSORB (T_{min} = 0.358; T_{max} = 0.731).[44] A total of 12669 reflections were averaged according to the point group symmetry 321 resulting in 2148 unique reflections (R_{int} = 0.060 on F_o^2).

The structure was solved by the Patterson method using the program SHELXS97[45,46] and refined using the program SHELXL97.[26] Full matrix least-squares refinement on F^2 was performed, minimizing $\sum w(F_o^2 - F_c^2)^2$, with anisotropic displacement parameters for the non-hydrogen atoms. The positions of the hydrogen atoms were located on intermediate difference electron density maps and refined with isotropic displacement parameters fixed at 0.05 Å^2. Extinction correction was applied, extinction coefficient: 0.0017(1).[26] The refinement (158 parameters, 2110 reflections) with the molecule having the *(R)*-configuration converged at R_F = 0.016, wR_{F^2} = 0.041 for 2106 reflections with $F_o > 4\sigma(F_o)$; $w = 1/[\sigma(F_o^2) + 0.8132P]$, where $P = (F_o^2 + 2F_c^2)/3$; S = 1.115. The Flack absolute structure factor: 0.00(2).[25,26] In the final difference Fourier map maximum and minimum electron densities were 0.28 and −0.23 e Å^{-3}, respectively. Complex atomic scattering factors for neutral atoms were as incorporated in SHELXL97.[26,47]

X-ray Crystallographic Analysis of (*E*)-4-(Hydroxyimino)-3-methoxy-4,5,6,7-tetrahydro-1,2-benzisoxazole (22a). Crystal data: $C_8H_{10}N_2O_3$, M_r = 182.18, colorless prism, mp 208−210 °C (H_2O), triclinic, space group $P\bar{1}$ (No. 2), a = 5.0678(3) Å, b = 9.600(1) Å, c = 9.6965(9) Å, α = 116.171(8)°, β = 93.305(7)°, γ = 103.453(8)°, V = 404.76(6) Å^3, Z = 2, D_c = 1.495 Mg m^{-3}, $F(000)$ = 192, μ(Cu Kα) = 0.980 mm^{-1}, T = 122.0(5) K, crystal dimensions = 0.05 × 0.10 × 0.35 mm.

Diffraction data were collected on an Enraf-Nonius CAD-4 diffractometer using graphite monochromated Cu Kα radiation (λ = 1.54184 Å).[41] Intensities were collected using the $\omega/2\theta$ scan mode. Unit cell dimensions were determined by least-squares refinement of 20 reflections (θ range 39.5−48.5°).[41] A total of 1661 unique reflections were measured in the range $-6 \le h \le 6$, $-11 \le k \le 10$, $0 \le l \le 12$ ($5.17° < \theta < 74.91°$). Data were reduced using the programs of Blessing (DREADD).[42,43] Three standard reflections were monitored every 10^4 s (decay 2.2%, not corrected). No absorption correction was applied.

The structure was solved using the program MULTAN11/82 in the Enraf-Nonius Structure Determination Package[48,49] and refined using the program SHELXL93.[50] Full matrix least-squares refinement on F^2 was performed, minimizing $\sum w(F_o^2 - F_c^2)^2$, with anisotropic displacement parameters for the non-hydrogen atoms. The positions of the hydrogen atoms were located on intermediate difference electron density maps and refined with isotropic displacement parameters. Refinement was carried out on all reflections, except for a small number of reflections (010 and 1−12 with $F_c \gg F_o$ and three weak reflections with ΔF^2/esd > 7.5), which were excluded from the final cycles of refinement. The refinement (158 parameters, 1656 reflections) converged at R_F = 0.037, wR_{F^2} = 0.096 for 1604 reflections with $F_o > 4\sigma(F_o)$; $w = 1/[\sigma(F_o^2) + (0.0544P)^2 + 0.1495P]$, where $P = (F_o^2 + 2F_c^2)/3$; S = 1.097. In the final difference Fourier map maximum and minimum electron densities were 0.30 and −0.23 e Å^{-3}, respectively. Complex atomic scattering factors for neutral atoms were as incorporated in SHELXL93.[50]

In Vitro GABA Uptake Assays. In the initial determination of the affinity of compounds for the GABA uptake systems, the effects on GABA uptake in crude synaptosomes, prepared from adult rat brain, were tested.[51] These uptake experiments were performed at a GABA concentration of 0.05 μM with preincubation of the tissue preparation for 10 min at 20 °C in the presence of inhibitor.[22] The effects of compounds on neuronal GABA uptake were measured using neurons cultured from cerebral cortices of 15-day-old mouse embryos, and the uptake experiments were carried out as previously described.[17] Effects of compounds on glial GABA uptake were measured using astrocytes cultured from cerebral cortices of newborn mice, and the uptake experiments were performed as previously described.[17]

Determination of Anticonvulsant Effects. Mice weighing 20−25 g were used. Test compounds were given subcutaneously, and isoniazide (300 μmol/kg) was administered subcutaneously at the same time as two separate injections.[33] Five mice were used per dose, and dose−response relationship was determined in at least two separate trials with overlapping doses. A control group, receiving isoniazide only, was included at each testing. This dose of isoniazide induces intermittent tonic−clonic siezures. The animals were placed individually in Macrolon type II cages, and the time from injection to the first convulsions was recorded. The experiment was terminated at the occurrence of the first convulsions, or after 60 min. The number of animals showing no convulsions within 60 min was recorded, and the ED_{50} values were calculated by log probit analyses.[33]

In another set of experiments, the anticonvulsant activity of compound **9** was tested in Frings mice after intracerebroventricular administration as described previously.[35] The number of animals protected against sound-induced convulsions was recorded and the ED_{50} value for protection calculated.[35]

Acknowledgment. This work was supported by grants from the Lundbeck Foundation, H. Lundbeck A/S, and the Danish State Biotechnology Programme

(1991−1995). The technical assistance of Flemming Hansen, Department of Chemistry, University of Copenhagen, with the collection of X-ray data is gratefully acknowledged. We also express our gratitude to Tina Lindgreen and Anne Nordly for technical and secretarial assistance, respectively.

Supporting Information Available: Tables for the compounds (*R*)-(−)-**8**, (*R*)-(+)-**9,** and **22a** listing final atomic coordinates, equivalent isotropic displacement parameters, anisotropic displacement parameters for non-hydrogen atoms, full lists of bond lengths and bond angles, and lists of structure factors. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Morselli, P. L., Löscher, W., Lloyd, K. G., Meldrum, B., Reynolds, E. H., Eds. *Neurotransmitters, Seizures, and Epilepsy*; Raven Press: New York, 1981.
(2) Fariello, R. G., Morselli, P. L., Lloyd, K. G., Quesney, L. F., Engel, J., Eds. *Neurotransmitters, Seizures, and Epilepsy II*; Raven Press: New York, 1984.
(3) Nistico, G., Morselli, P. L., Lloyd, K. G., Fariello, R. G., Engel, J., Eds. *Neurotransmitters, Seizures, and Epilepsy III*; Raven Press: New York, 1986.
(4) Meldrum, B. S. Epilepsy and γ-aminobutyric acid-mediated inhibition. *Int. Rev. Neurobiol.* **1975**, *17*, 1−36.
(5) Meldrum, B. S. Pharmacology of GABA. *Clin. Neuropharmacol.* **1982**, *5*, 293−316.
(6) Bowery, N. G., Nistico, G., Eds. *GABA: Basic Research and Clinical Applications*; Pythagora Press: Rome, 1989.
(7) Nanavati, S. M.; Silverman, R. B. Design of potential anticonvulsant agents: mechanistic classification of GABA aminotransferase inactivators. *J. Med. Chem.* **1989**, *32*, 2413−2421.
(8) Schechter, P. J. Clinical pharmacology of vigabatrin. *Br. J. Clin. Pharmacol.* **1989**, *27*, 19S−22S.
(9) Yunger, L. M.; Fowler, P. J.; Zarevics, P.; Setler, P. E. Novel inhibitors of γ-aminobutyric acid (GABA) uptake: anticonvulsant actions in rats and mice. *J. Pharmacol. Exp. Ther.* **1984**, *228*, 109−115.
(10) Andersen, K. E.; Braestrup, C.; Grønwald, F. C.; Jørgensen, A. S.; Nielsen, E. B.; Sonnewald, U.; Sørensen, P. O.; Suzdak, P. D.; Knutsen, L. J. S. The synthesis of novel GABA uptake inhibitors. 1. Elucidation of the structure−activity studies leading to the choice of (*R*)-1-[4,4-bis(3-methyl-2-thienyl)-3-butenyl]-3-piperidinecarboxylic acid (tiagabine) as an anticonvulsant drug candidate. *J. Med. Chem.* **1993**, *36*, 1716−1725.
(11) Krogsgaard-Larsen, P.; Johnston, G. A. R. Inhibition of GABA uptake in rat brain slices by nipecotic acid, various isoxazoles and related compounds. *J. Neurochem.* **1975**, *25*, 797−802.
(12) Johnston, G. A. R.; Krogsgaard-Larsen, P.; Stephanson, A. Betel nut constituents as inhibitors of γ-aminobutyric acid uptake. *Nature (London)* **1975**, *258*, 627−628.
(13) Krogsgaard-Larsen, P.; Johnston, G. A. R. Structure−activity studies on the inhibition of GABA binding to rat brain membranes by muscimol and related compounds. *J. Neurochem.* **1978**, *30*, 1377−1382.
(14) Krogsgaard-Larsen, P.; Thyssen, K.; Schaumburg, K. Inhibitors of GABA uptake. Syntheses and proton NMR spectroscopic investigations of guvacine, (3RS,4SR)-4-hydroxypiperidine-3-carboxylic acid, and related compounds. *Acta Chem. Scand., Ser. B* **1978**, *B32*, 327−334.
(15) Larsson, O. M.; Falch, E.; Schousboe, A.; Krogsgaard-Larsen, P. GABA uptake inhibitors: kinetics and molecular pharmacology. *Adv. Biosci. (Oxford)* **1991**, *82*, 197−200.
(16) Johnston, G. A. R.; Stephanson, A. L.; Twitchin, B. Uptake and release of nipecotic acid by rat brain slices. *J. Neurochem.* **1976**, *26*, 83−87.
(17) Larsson, O. M.; Krogsgaard-Larsen, P.; Schousboe, A. Characterization of the uptake of GABA, nipecotic acid and *cis*-4-hydroxynipecotic acid in cultured neurons and astrocytes. *Neurochem. Int.* **1985**, *7*, 853−860.
(18) Larsson, O. M.; Krogsgaard-Larsen, P.; Schousboe, A. High-affinity uptake of (*RS*)-nipecotic acid in astrocytes cultured from mouse brain. Comparison with GABA transport. *J. Neurochem.* **1980**, *34*, 970−977.
(19) Schousboe, A.; Thorbek, P.; Hertz, L.; Krogsgaard-Larsen, P. Effects of GABA analogues of restricted conformation on GABA transport in astrocytes and brain cortex slices and on GABA receptor binding. *J. Neurochem.* **1979**, *33*, 181−189.
(20) Juhász, G.; Kékesi, K. A.; Nyitrai, G.; Dobolyi, A.; Krogsgaard-Larsen, P.; Schousboe, A. Differential effects of nipecotic acid and 4,5,6,7-tetrahydro-isoxazolo[4,5-*c*]pyridin-3-ol on extracellular γ-aminobutyrate levels in rat thalamus. *Eur. J. Pharmacol.* **1997**, *331*, 139−144.
(21) Gonsalves, S. F.; Twitchell, B.; Harbaugh, R. E.; Krogsgaard-Larsen, P.; Schousboe, A. Anticonvulsant activity of intracerebroventricularly administered glial GABA uptake inhibitors and other GABA mimetics in chemical seizure models. *Epilepsy Res.* **1989**, *4*, 34−41.
(22) Krogsgaard-Larsen, P.; Falch, E.; Larsson, O. M.; Schousboe, A. GABA uptake inhibitors: relevance to antiepileptic drug research. *Epilepsy Res.* **1987**, *1*, 77−93.
(23) Falch, E.; Larsson, O. M.; Schousboe, A.; Krogsgaard-Larsen, P. GABA-A agonists and GABA uptake inhibitors: structure−activity relationships. *Drug Dev. Res.* **1990**, *21*, 169−188.
(24) Johnson, C. K. ORTEP II. *A Fortran Thermal-Ellipsoid Plot Programme for Crystal Structure Illustrations;* Report ORNL-5138; Oak Ridge National Laboratory: Oak Ridge, TN, 1976.
(25) Flack, H. D. On enantiomorph-polarity estimation. *Acta Crystallogr.* **1983**, *A39*, 876−881.
(26) Sheldrick, G. M. SHELXL97. *Program for Crystal Structure Refinement*; University of Göttingen: Göttingen, Germany, 1997.
(27) Allen, F. H.; Kennard, O.; Watson, D. G.; Brammer, L.; Orpen, A. G.; Taylor, R. Typical interatomic distances: organic compounds. In *International Tables for Crystallography*; Wilson, A. J. C., Ed.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1995; Vol. C, pp 685−706.
(28) Spek, A. L. PLATON, an integrated tool for the analysis of the results of a single-crystal structure determination. *Acta Crystallogr.* **1990**, *A46*, C34.
(29) Cheng, Y.; Prusoff, W. H. Relationship between the inhibition constant (K_i) and the concentration of inhibitor, which causes 50% inhibition (I_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099−3108.
(30) Krogsgaard-Larsen, P.; Nielsen, L.; Falch, E.; Curtis, D. R. GABA agonists. Resolution, absolute stereochemistry, and enantioselectivity of (*S*)-(+)- and (*R*)-(−)-dihydromuscimol. *J. Med. Chem.* **1986**, *28*, 1612−1617.
(31) Johnston, G. A. R.; Krogsgaard-Larsen, P.; Stephanson, A. L.; Twitchin, B. Inhibition of the uptake of GABA and related amino acids in rat brain slices by the optical isomers of nipecotic acid. *J. Neurochem.* **1976**, *26*, 1029−1032.
(32) Schousboe, A.; Larsson, O. M.; Hertz, L.; Krogsgaard-Larsen, P. Heterocyclic GABA analogues as new selective inhibitors of astroglial GABA transport. *Drug Dev. Res.* **1981**, *1*, 115−127.
(33) Christensen, A. V.; Larsen, J.-J. Antinociceptive and anticonvulsive effect of THIP, a pure GABA agonist. *Pol. J. Pharmacol. Pharm.* **1982**, *34*, 127−134.
(34) Wood, J. D.; Johnson, D. D.; Krogsgaard-Larsen, P.; Schousboe, A. Anticonvulsant activity of the glial-selective GABA uptake inhibitor, THPO. *Neuropharmacology* **1983**, *22*, 139−142.
(35) White, H. S.; Wolf, H. H.; Swinyard, E. A.; Skeen, G. A.; Sofia, R. D. A neuropharmacological evaluation of felbamate as a novel anticonvulsant. *Epilepsia* **1992**, *33*, 564−572.
(36) Johnston, G. A. R.; Krogsgaard-Larsen, P.; Stephanson, A. L.; Twitchin, B. Inhibition of the uptake of GABA and related amino acids in rat brain slices by the optical isomers of nipecotic acid. *J. Neurochem.* **1976**, *26*, 1029−1032.
(37) Knutsen, L. J. S.; Andersen, K. E.; Lau, J.; Lundt, B. F.; Henry, R. F.; Morton, H. E.; Nærum, L.; Petersen, H.; Stephensen, H.; Suzdak, P. D.; Swedberg, M. D. B.; Thomsen, C.; Sørensen, P. O. Synthesis of novel GABA uptake inhibitors. 3. Diaryloxime and diarylvinyl ether derivatives of nipecotic acid and guvacine as anticonvulsant agents. *J. Med. Chem.* **1999**, *42*, 3447−3462.
(38) Larsson, O. M.; Falch, E.; Krogsgaard-Larsen, P.; Schousboe, A. Kinetic characterization of inhibition of GABA uptake into cultured neurons and astrocytes by 4,4-diphenyl-3-butenyl derivatives of nipecotic acid and guvacine. *J. Neurochem.* **1988**, *50*, 818−823.
(39) Jacquier, R.; Petrus, C.; Petrus, F.; Verducci, J. Étude dans la serie des azoles. LXII.- Synthèse univoque d'isoxazolones-3. Étude de leur tautomeric. *Bull. Soc. Chim. Fr.* **1970**, *5*, 1978−1985.
(40) Drewes, H. R. Process for the preparation of cyclohexanone-2-carboxamide. U.S. 4169952 to du Pont de Nemours; *Chem. Abstr.* **1979**, *92*, P 22150a.
(41) Enraf-Nonius. *CAD-4 Software*, version 5.0; Enraf-Nonius: Delft, The Netherlands, 1989.
(42) Blessing, R. H. Data reduction and error analysis for accurate single-crystal diffraction intensities. *Crystallogr. Rev.* **1987**, *1*, 3−58.
(43) Blessing, R. H. DREADD − data reduction and error analysis for single-crystal diffractometer data. *J. Appl. Crystallogr.* **1989**, *22*, 396−397.
(44) DeTitta, G. T. ABSORB: An absorption correction programme for crystals enclosed in capillaries with trapped mother liquor. *J. Appl. Crystallogr.* **1985**, *18*, 75−79.
(45) Sheldrick, G. M. SHELXS97. *Program for the Solution of Crystal Structures;* University of Göttingen: Göttingen, Germany, 1997.

(46) Sheldrick, G. M. Phase annealing in SHELX-90: Direct methods for larger structures. *Acta Crystallogr.* **1990**, *A46*, 467−473.
(47) *International Tables for Crystallography*; Wilson, A. J. C., Ed.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1995; Vol. C, Tables 4.2.6.8 and 6.1.1.4.
(48) Main, P.; Fiske, S. J.; Hull, S. E.; Lessinger, L.; Germain, G.; Declercq, J.-P.; Woolfson, M. M. MULTAN11/82. *A System of Computer Programs for the Automatic Solution of Crystal Structures from X-ray Diffraction Data;* Universities of York: England, and Lovain: Belgium, 1982.
(49) Frenz, B. A.; Associates Inc. *SDP Structure Determination Package*; College Station: Texas, and Enraf-Nonius: Delft, The Netherlands, 1985.
(50) Sheldrick, G. M. SHELXL93. *Program for Crystal Structure Refinement*; University of Göttingen: Göttingen, Germany, 1993.
(51) Fjalland, B. Inhibition by neuroleptics of uptake of [^{3}H]GABA into rat brain synaptosomes. *Acta Pharmacol. Toxicol.* **1987**, *42*, 73−76.

JM9904452