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Selective inhibitors of GABA uptake: synthesis and molecular pharmacology of 4-*N*-methylamino-4,5,6,7-tetrahydrobenzo[*d*]isoxazol-3-ol analogues

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Abstract—A series of lipophilic diaromatic derivatives of the glia-selective GABA uptake inhibitor (*R*)-4-amino-4,5,6,7-tetrahydrobenzo[*d*]isoxazol-3-ol [(*R*)-*exo*-THPO, **4**] were synthesized via reductive amination of 3-ethoxy-4,5,6,7-tetrahydrobenzo[*d*]isoxazol-4-one (**9**) or via *N*-alkylation of *O*-alkylatedracemic **4**. The effects of the target compounds on GABA uptake mechanisms in vitro were measured using a rat brain synaptosomal preparation or primary cultures of mouse cortical neurons and glia cells (astrocytes), as well as HEK cells transfected with cloned mouse GABA transporter subtypes (GAT1-4). The activity against isoniazid-induced convulsions in mice after subcutaneous administration of the compounds was determined. All of the compounds were potent inhibitors of synaptosomal uptake the most potent compound being (*RS*)-4-[*N*-(1,1-diphenylbut-1-en-4-yl)amino]-4,5,6,7-tetrahydrobenzo[*d*]isoxazol-3-ol (**17a**, IC_{50} = 0.14 μM). The majority of the compounds showed a weak preference for glial, as compared to neuronal, GABA uptake. The highest degree of selectivity was 10-fold corresponding to the glia selectivity of (*R*)-*N*-methyl-*exo*-THPO (**5**). All derivatives showed a preference for the GAT1 transporter, as compared with GAT2-4, with the exception of (*RS*)-4-[*N*-[1,1-bis(3-methyl-2-thienyl)but-1-en-4-yl]-*N*-methylamino]-4,5,6,7-tetrahydrobenzo[*d*]isoxazol-3-ol (**28d**), which quite surprisingly turned out to be more potent than GABA at both GAT1 and GAT2 subtypes. The GAT1 activity was shown to reside in (*R*)-**28d** whereas (*R*)-**28d** and (*S*)-**28d** contributed equally to GAT2 activity. This makes (*S*)-**28d** a GAT2 selective compound, and (*R*)-**28d** equally effective in inhibition of GAT1 and GAT2 mediated GABA transport. All compounds tested were effective as anticonvulsant reflecting that these compounds have blood–brain barrier permeating ability.

1. Introduction

4-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system (CNS) and has been implicated in a number of CNS disorders, notably epilepsy.[1–7] Although there is no direct proof of impairment of GABA neurotransmission in epileptic phenomena, a large number of in vitro and in vivo pharmacological data are consistent with a major role of GABA in this group of disorders.[6] Thus, obstruction of GABA biosynthesis or administration of GABA antagonists in animal studies can induce convulsions resembling those of epilepsy.[7] Furthermore, enhancement of GABA neurotransmission leads to protection against seizures.[8,9]

Enhancement of GABA transmission by inhibition of GABA uptake has gained much attention as a therapeutic strategy,[10,11] since it functionally increases the effect of GABA in a use-dependent manner, and

Keywords: GABA uptake inhibitor; Anticonvulsant; Gamma-aminobutyric acid; Subtype selective.
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GABA uptake inhibitors have proved effective as anticonvulsants in a variety of experimental models of epilepsy and in epileptic patients.[12–15] Pharmacological intervention along these lines may be beneficial in other CNS disorders and malfunctions, including chronic pain, anxiety, and sleep disorders.

At present, four different subtypes of mammalian GABA transporters have been cloned and given species-dependent nomenclature: GAT1–GAT4 in mouse correspond to GAT-1, BGT-1, GAT-2, GAT-3, respectively, in rat and human.[16–24] The regional and cellular distribution of these subtypes are highly heterogeneous.[24] Early studies disclosed a pharmacological difference between GABA uptake in glial and neuronal cell cultures suggesting that separate transporters on each cell type could account for this difference.[10] It was later shown that there is no simple distinction between the types of GABA transporter expressed at neurons and astrocytes and the reasons for the pharmacological differences observed using these cellular assay systems remain unclear,[24] but may reflect different functional or regulatory mechanisms in these cells. The role of the transporter subtypes and their therapeutic potential as targets will rely on development of potent and subtype selective ligands toward GAT2–GAT4 penetrating the blood–brain barrier (BBB).

Three decades ago the discovery of 4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridine-3-ol (THPO, **3**) as a selective uptake inhibitor without GABA receptor affinity led to the discovery of (*R*)-nipecotic acid (**1**) and guvacine (**2**) as potent and highly selective inhibitors of GABA transport (Fig. 1).[25,26] This discovery subsequently led to the development of more potent derivatives of **1** and **2** as GABA uptake inhibitors, including **6**–**8** with increased potency and showing ability to penetrate the BBB.[12,27–30] Tiagabine (**7**) is now used clinically as an antiepileptic drug.[15]

Like GABA, the cyclic amino acids **1** and **2** are transport substrates with similar affinity for the neuronal and glial GABA transport systems.[25,30–35] The bioisosteric analogue of **1** and **2**, THPO (**3**), however is not a substrate for the GABA transporters and has a 2-fold higher affinity for glial over neuronal transport.[31,33] Although **3** is at least an order of magnitude weaker than **1** as an inhibitor of GABA uptake in vitro, it is capable of increasing extracellular GABA levels more effectively than **1**, and **3** is a more effective anticonvulsant than **1**.[31,36,37] Selective inhibition of glial GABA uptake might improve the therapeutic potential of transport inhibition since the glial transport leads to degradation of GABA, whereas the re-uptake of GABA into neurons, which is coupled to GABA release mechanisms, would be strengthened.[38] The observation that inhibitors selective for neuronal uptake are proconvulsant is supportive evidence of this pharmacological approach.[37]

Some years ago we redesigned the structure of THPO (**3**) to give (*R*)-4-amino-4,5,6,7-tetrahydrobenzo[*d*]isoxazol-3-ol [(*R*)-*exo*-THPO, **4**], which showed increased selectivity for glial uptake.[39] A series of *N*-alkylated derivatives of **4** were synthesized of which the most potent and glia-selective compound turned out to be the *N*-methyl derivative **5**. It was recently demonstrated that **4** and **5** effectively protect against seizures when injected directly into the brain, the protective effect being correlated with the inhibition of glial transport rather than neuronal uptake inhibition.[40] These results further substantiate the strategy of glia-selective intervention, but the therapeutic potential of this strategy can only be elucidated by the design of markedly more potent inhibitors, selective for glial GABA transport, and capable of penetrating the BBB.

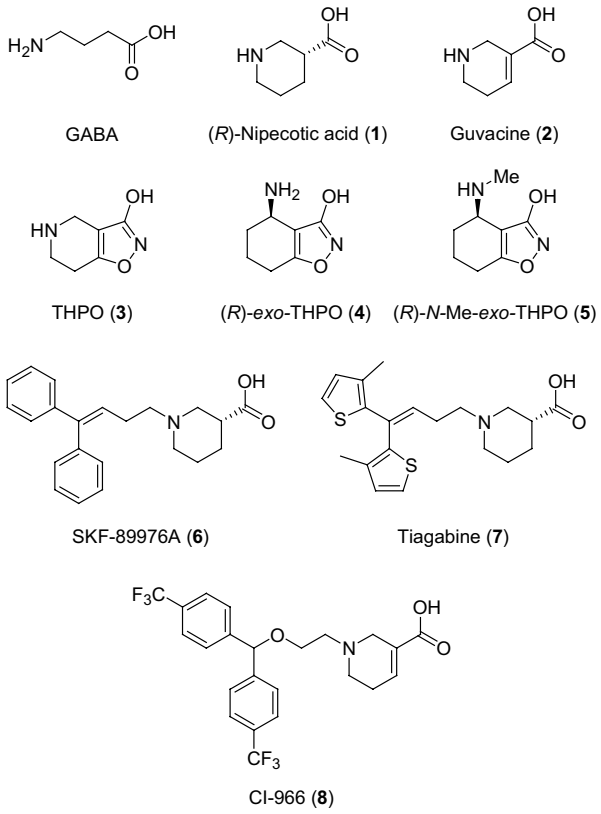


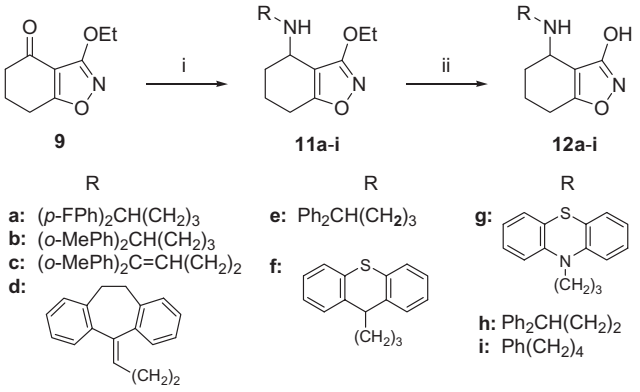
Figure 1. Structure of GABA, the GABA uptake inhibitors (*R*)-nipecotic acid (**1**), guvacine (**2**), (*R*)-4-amino-4,5,6,7-tetrahydrobenzo[*d*]isoxazol-3-ol [(*R*)-*exo*-THPO, **4**], (*R*)-4-methylamino-4,5,6,7-tetrahydrobenzo[*d*]isoxazol-3-ol [(*R*)-*N*-Me-*exo*-THPO, **5**], and lipophilic diaromatic analogues of **1** (**6**–**8**).

These aspects have prompted us to synthesize derivatives of *exo*-THPO containing lipophilic diaromatic side chains with the aim of developing potent and glia-selective GABA uptake inhibitors, sufficiently lipophilic to penetrate the BBB. We here report the results of these efforts.

2. Results

2.1. Chemistry

Two principal routes were employed to obtain the derivatives of (*RS*)-**4**, via reductive amination of ketone **9**[39] (Scheme 1) or via *N*-alkylation of amines **15**, **20**, **23**,

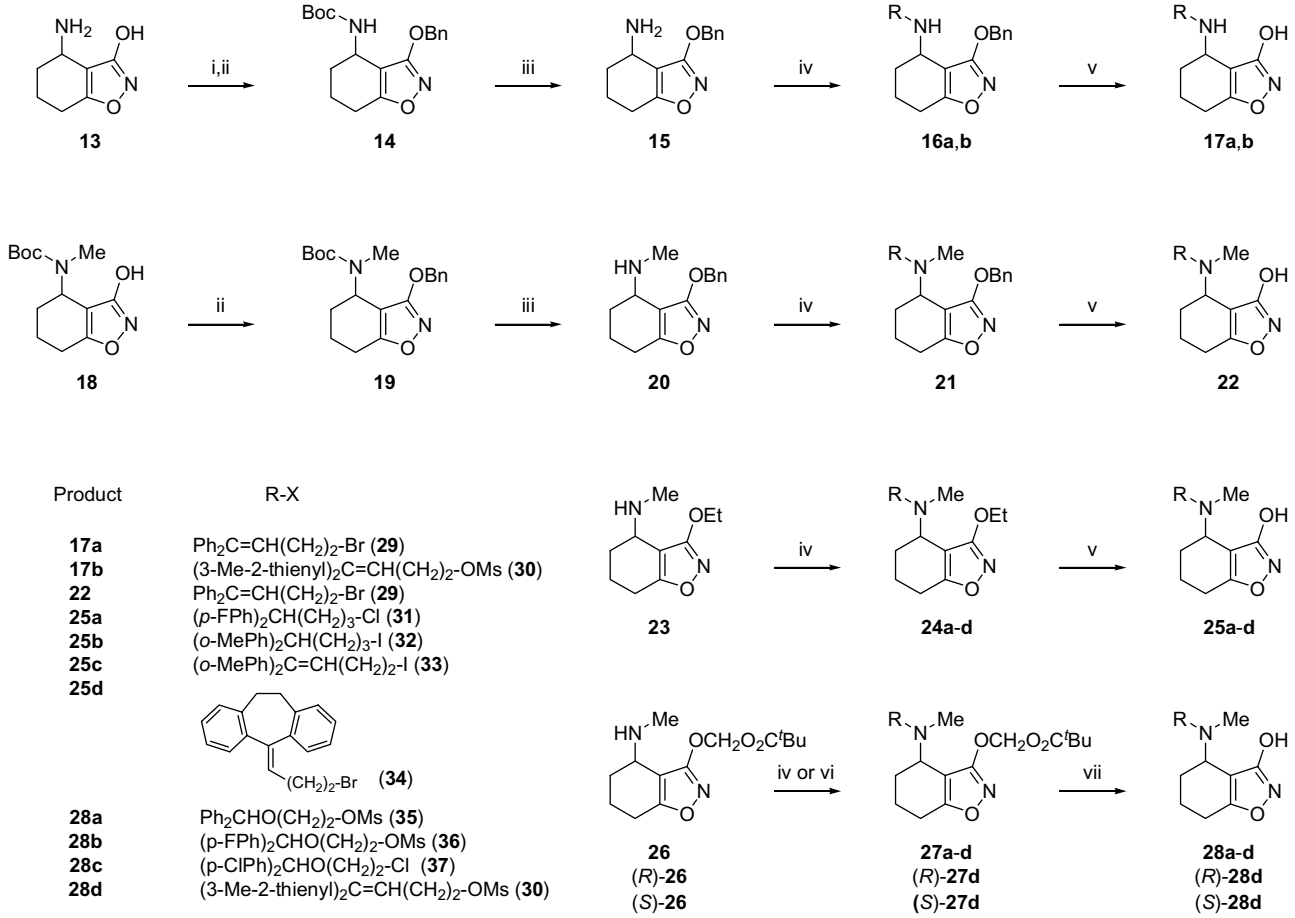


Scheme 1. Reagents and conditions: (i) R–NH_2 (**10a–i**), $NaCNBH_3$; (ii) 33% HBr in HOAc (**a–e**, **g–i**) or 6 M HCl (**f**).

and **26** (Scheme 2). A series of secondary amine derivatives of racemic **4** were synthesized by the reductive amination of **9** with the appropriate amines **10a–i** and $NaCNBH_3$ giving intermediates **11a–i** (Scheme 1). These *O*-ethyl-protected compounds were deprotected with 33% HBr in glacial acetic acid to give the free 3-isoxazolols **12a–i**. Two secondary amine derivatives **17a**,**b** (Scheme 2) were prepared via alkylation of *O*-benzyl protected *exo*-THPO (**15**) obtained in three steps from racemic **13**.[39] Boc-protection of **13** was followed by benzylation of the isoxazolol-ring to give **14**. The Boc-group was then removed with HCl to give **15** and alkylation with **29** and **30** yielded **16a** and **16b**. The benzyl group was removed with hydrochloric acid furnishing **17a** and **17b**. The synthesis of **22** starts from the Boc-protected racemic **18**, which is *O*-benzylated giving **19**, followed by removal of the Boc group with HCl yielding **20**. *N*-Alkylation of *O*-benzyl protected **20** with bromide **29** gave **21** that upon deprotection in hydrochloric acid furnished **22**. Similarly tertiary amine derivatives **25a–d** were obtained by *N*-alkylation of *O*-ethyl-protected racemic *N*-methyl-*exo*-THPO (**23**)[39] with halides **31–34**, but stronger acidic conditions were necessary in the deprotection of *O*-ethyl-protected 3-isoxazolols **24a–d** to provide the desired compounds **25a–d**. A series of tertiary amine derivatives with acid-labile side chains **28a–d** was obtained starting from the pivaloyloxymethyl-protected derivative **26**.[39] *N*-Alkylation with **35–37** and **30** gave **27a–d** and subsequent basic alcoholysis furnished **28a–d**. In the case of (*R*)-**28d** and (*S*)-**28d** the starting materials were (*R*)- and (*S*)-**26**,[39] respectively.

2.2. In vitro pharmacology

The inhibitory effect on GABA uptake in a crude preparation of synaptosomes from rat brain were determined



Scheme 2. Reagents and conditions: (i) $(Boc)_2O$, NaOH; (ii) BnBr, K_2CO_3; (iii) HCl in EtOAc; (iv) R–X (**29–37**), K_2CO_3; (v) HCl in water/EtOH; (vi) R–X (**30**), Li_2CO_3 (**28d**); (vii) NaOH in EtOH.

for all compounds.[41] The compounds were equipotent or more potent inhibitors than the classical inhibitors **1** and **2** in this assay with IC_{50} values below 6 μM (Table 1). Several of the compounds were markedly more potent with IC_{50} values below 1 μM (**12a**,**c**,**e**,**h**, **17b**), the most potent compound being **17a** (IC_{50} = 0.14 μM). Compounds with R_2 = H showed a slightly higher affinity than analogues R_2 = Me.

The compounds were subsequently subjected to further pharmacological characterization using cultured cortical neurons and astrocytes[33] as well as monoclonal cell cultures expressing GABA transporters from mouse (GAT1-4)[40] to probe the glial selectivity and the subtype profile, respectively (Table 1). The majority of the compounds maintained a preference for glial over neuronal GABA uptake, the most selective compound showing 10-fold difference (**12d**), corresponding to the glia selectivity of *N*-methyl-*exo*-THPO. When the affinities for the individual subtypes of the mouse GABA transporter of the compounds were determined, a high preference for GAT1 was evident as observed for the lipophilic derivatives of **1**, compounds **6** and **7**.[15] However, in some cases significant GAT2 activity appeared, as exemplified by **12d**, **17a**,**b**, and **25e**.

Surprisingly, in the case of **28d** the GAT2 potency was higher than GABA itself and at the level of GAT1 potency relative to GABA. This interesting observation prompted us to investigate whether this dual activity was associated with a single enantiomer. We therefore synthesized the enantiomers of **28d** separately proving that while inhibitory effects at GAT1 mainly resides in (*R*)-**28d**, both enantiomers in the racemic mixture contributed to inhibition of GAT2. The affinity in synaptosomal uptake was also determined and found to be at least 200-fold higher for (*R*)-**28d** as compared to (*S*)-**28d** reflecting that GAT1 is the most abundant trans-

Table 1. [^{3}H]GABA uptake inhibition into synaptosomes, cultured cortex astrocytes and neurons, and cells transfected with cloned GABA transporters (GAT1-4) and anticonvulsant effects of GABA uptake inhibitors

Compd			GABA uptake inhibition IC_{50} (μM)							Seizure inhibition ED_{50} (μmol/kg)
			Synaptosomes	Astrocytes	Neurons	GAT1	GAT2	GAT3	GAT4	Isoniazid[a]
	GABA		nd	8[b]	32[b]	17[b]	51[b]	15[b]	17[b]	nd
1	(*R*)-Nipecotic acid (Nip)		5[c]	16[c]	12[c]	24[d]	2350[d]	113[d]	159[d]	nd
2	Guvacine		12[c]	29[c]	32[c]	39[d]	1420[d]	228[d]	378[d]	nd
(*RS*)-**4**	*exo*-THPO		181[c]	208[c]	883[c]	1000[e]	3000[e]	>3000[e]	>3000[e]	nd
(*RS*)-**5**	*N*-Me-*exo*-THPO		63[c]	28[c]	423[c]	450[e]	>3000[e]	>3000[e]	>3000[e]	nd
5	(*R*)-N-Me-*exo*-THPO		39[c]	60[c]	510[c]	nd	nd	nd	nd	nd
7	Tiagabine (Nip-$C_{14}H_{15}S_2$)		0.067[e]	0.18[e]	0.36[e]	0.8[e]	300[e]	>300[e]	800[e]	2.7
	R_1	R_2								
12a	(*p*-FPh)$_2$CH(CH$_2$)$_3$	H	0.24	1	2	4	>100	300	>300	110
12b	(*o*-MePh)$_2$CH(CH$_2$)$_3$	H	0.84	20	7	1.5	>200	>200	>200	44
12c	(*o*-MePh)$_2$C=CH(CH$_2$)$_2$	H	0.3	2	12	10	>300	>300	>300	30
12d	$C_{18}H_{17}$	H	0.76	7	80	7	125	>100	225	68
12e	Ph$_2$CH(CH$_2$)$_3$	H	0.22	0.5	3	4	>100	>100	>100	56
12f	$C_{15}H_{13}S$	H	nd	2.5	6.5	5.5	160	>200	>200	45
12g	$C_{14}H_{12}NS$	H	0.96	3	14	6	>125	>125	>125	71
12h	Ph$_2$CH(CH$_2$)$_2$	H	0.2	1	2	1	>300	>300	>300	310
12i	Ph(CH$_2$)$_4$	H	6.2	15	100	30	>300	>1000	>1000	150
17a	Ph$_2$C=CH(CH$_2$)$_2$	H	0.14	0.6	1.4	6	50	60	90	nd
17b	$C_{14}H_{15}S_2$	H	0.17	2	4	3	130	>100	>100	nd
22	Ph$_2$C=CH(CH$_2$)$_2$	Me	0.37	2	5	2	200	>100	>100	97
25a	(*p*-FPh)$_2$CH(CH$_2$)$_3$	Me	0.73	20	16	11	300	>300	>300	67
25b	(*o*-MePh)$_2$CH(CH$_2$)$_3$	Me	1.5	9	18	11	260	>500	>500	27
25c	(*o*-MePh)$_2$C=CH(CH$_2$)$_2$	Me	0.7	8	5	3	200	>500	>500	50
25d	$C_{18}H_{17}$	Me	1.1	3	7.5	12	125	>500	>500	160[f]
28a	Ph$_2$CHO(CH$_2$)$_2$	Me	0.36	1	4	5	>300	>300	>300	160
28b	(*p*-FPh)$_2$CHO(CH$_2$)$_2$	Me	4.5	15	16	12	>500	>500	>500	55
28c	(*p*-ClPh)$_2$CHO(CH$_2$)$_2$	Me	4.9	13	17	17	>30	nd	nd	81
28d	$C_{14}H_{15}S_2$	Me	0.87	2	2	7	26	>300	>300	63
(*R*)-**28d**	$C_{14}H_{15}S_2$	Me	0.48	0.65	1.5	4	22	>150	>150	24
(*S*)-**28d**	$C_{14}H_{15}S_2$	Me	>100	>100	>100	120	34	>150	>150	>40

nd, not determined.

[a] Anticonvulsant effects in mice after subcutaneous treatment with isoniazid.

[b] K_m value, Ref. 38.

[c] Ref. 39.

[d] Determined at rat GAT-1, human BGT-1, rat GAT-2, and GAT-3 corresponding to mouse GAT1-4, respectively; Ref. 24.

[e] Ref. 40.

[f] Biphasic curve, determined at maximum effect (60%).

porter in this assay. In contrast, Tiagabine (**7**) is only 3-fold more potent than the corresponding (*S*)-form, as an inhibitor of synaptosomal GABA uptake.[28] Also in the cortical glial and neuronal cell assay GABA uptake could only be blocked by (*R*)-**28d**.

2.3. In vivo pharmacology

Most of the compounds were tested as anticonvulsants. The compounds were given subcutaneously to mice treated with isoniazid and all tested compounds turned out to be anticonvulsant, a majority of compounds showing $ED_{50} < 100\,\mu mol/kg$ (Table 1). The most active compound in this assay was **25b** ($ED_{50} = 27\,\mu mol/kg$). This shows that generally the compounds have BBB permeating ability.

3. Discussion

The classical GABA uptake inhibitors, nipecotic acid (**1**) and guvacine (**2**), are the central components of the derivatives containing lipophilic diaromatic side chains on the amino group of these cyclic amino acids (Fig. 1). This substitution has proved to increase potency and to disable these derivatives as substrates for the GABA transporters.[12,30] Numerous derivatives of **1** and **2** have now been synthesized and pharmacologically characterized.[27–29,42–47] Many of these derivatives are highly potent and have ultimately led to a therapeutic agent, Tiagabine (**7**), applicable to the treatment of epilepsy. However, none of the derivatives tested so far have shown significant selectivity for the glial versus the neuronal GABA transport system. Our recent discovery that (*R*)-*exo*-THPO (**4**) and, in particular, (*R*)-*N*-Me-*exo*-THPO (**5**) show selectivity for the glial transport system[39,40] has prompted the synthesis of a series of derivatives of racemic *exo*-THPO, mono- or disubstituted at the amino group. The substituted side chains at the amino group were selected on the basis of those previously employed for the synthesis of nipecotic acid derivatives, such as **6**–**8**. Furthermore some modification was applied to these side chains in order to expand the variation of these substituents.

The new compounds **12a**–**i**, **17a**,**b**, **22**, **25a**–**d**, and **28a**–**e** displayed a broad spectrum of pharmacological profiles. Most of the compounds showed similar potencies at the glial and neuronal transport carriers (Table 1). A notable exception is **12d** which was an order of magnitude more potent as inhibitor of glial GABA uptake and thus comparable in selectivity to (*R*)-**5**. However no correlation between the extent of glia selectivity of the compounds and the anticonvulsant properties in the isoniazid assay could be established. The intraperitonal administration may obscure such correlation due to differences in penetration. Nevertheless, all compounds were anticonvulsant, thus demonstrating BBB permeability.

The inhibitory action of the compounds at the cloned GABA transporter subtypes was evaluated and, like Tiagabine (**7**), most of the lipophilic derivatives of *exo*-THPO or *N*-Me-*exo*-THPO tested showed a high degree of selectivity for GAT1. However, a comparison of the secondary amines **17a** and **17b** with the corresponding *N*-methylated derivatives **22** and **28d** reveals a surprising difference. Like **17a** and **17b**, compound **22** and **28d** showed comparable inhibitory effects in all GABA uptake assay systems but the GAT2 expressing cell assay. Thus, compound **22** is not very potent as inhibitor of GAT2 mediated transport whereas **28d** is more potent than GABA itself at both GAT1 and GAT2. This makes **28d** the first example of a GABA uptake inhibitor showing greater potency than GABA at GAT1 and GAT2 without significant effects at GAT3 and GAT4.

This unique profile of the racemic compound unexpectedly turned out to emerge from very different profiles of the enantiomers. GAT1 activity could be assigned primarily to (*R*)-**28d**, whereas (*S*)-**28d** and (*R*)-**28d** contributes equally to the potency of the racemate at GAT2. Therefore, (*S*)-**28d** displays significant GAT2 selectivity compared to GAT1 and both compounds carry a highly unique pharmacological profile among GABA uptake inhibitors.

Although Tiagabine (**7**), which is a derivative of (*R*)-nipecotic acid (**1**) (Fig. 1), and (*R*)-**28d** are both tertiary amines and contain identical lipophilic side chains, these two compounds show very different relative potencies at GAT1 and GAT2. Both compounds are now key experimental tools in a comprehensive in vivo pharmacological project with the aim of elucidating the physiological role and pharmacological susceptibility of GAT1 and GAT2 in the CNS.

Thus, although our modifications have not increased glia selectivity of (*R*)-**5**, a potent and glia selective compound **12d** penetrating the BBB was obtained and, more importantly, (*R*)-**28d** and (*S*)-**28d** with unique pharmacological profiles was discovered. All three compounds will be relevant in future studies on the therapeutic potential of GABA carriers in CNS disorders. Furthermore these compounds are the starting point for further development of selective GABA uptake inhibitors.

4. Conclusion

A series of lipophilic diaromatic derivatives of the racemic forms of the glia-selective inhibitors *exo*-THPO and *N*-methyl-*exo*-THPO was synthesized and evaluated pharmacologically in vitro and in vivo. In general, the compounds were effective as GABA uptake inhibitors and anticonvulsants. A number of potent compounds were discovered, including the glia-selective derivative **12d**. Compound **28d** appeared to be potent at GAT1 and GAT2, and (*S*)-**28d** turned out to be GAT2 selective. These compounds may be useful experimental tools for investigating the relative pharmacological significance of GABA transporters. In this regard *exo*-THPO and *N*-methyl-*exo*-THPO continue to be useful lead structures for the development of selective inhibitors of GABA uptake.

5. Experimental

5.1. Chemistry

Melting points were determined on a Büchi SMP-20 apparatus and are uncorrected. Mass spectra were obtained on a Quattro MS–MS system from VG Biotech, Fisons Instruments. The MS–MS system was connected to an HP1050 modular HPLC system. A volume of 20–50 μL of the sample (10 mg/mL) dissolved in a mixture of 1% HOAc in acetonitrile/water 1:1 was introduced via the autosampler at a flow of 30 μL/min into the electrospray source. Spectra were obtained at two standard sets of operating conditions: one set to obtain molecular weight information (MH+) (21 eV) and the other set to induce fragmentation patterns (70 eV). The background was subtracted. The relative intensities of the ions are obtained from the fragmentation pattern. When no intensity is indicated for the molecular ion (MH+), this ion was only present under the first set of operating conditions. ^{1}H NMR spectra were recorded on a Bruker AC 200 F (200 MHz), a Bruker Avance DRX 500, a Bruker AC 250F (250 MHz), or a Varian 360L (60 MHz) spectrometer. Deuterated chloroform (99.8% D) dimethylsulfoxide (99.9% D) or D_2O were used as solvents. TMS was used as internal reference standard. Chemical shift values are expressed in ppm values. The following abbreviations are used for multiplicity of NMR signals: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, dd = double doublet, dt = double triplet, m = multiplet, b = broad signal. NMR signals corresponding to exchangeable protons are generally omitted. Content of water in crystalline compounds was determined by Karl Fischer titration. For column chromatography (CC) silica gel of type Kieselgel 60, 230–400 mesh ASTM was used. Optical rotations were measured in thermostated cuvettes on a Perkin Elmer 241 polarimeter. Stereochemical purity were determined on an AGP column (100 × 4.0 mm) eluting with 50 mM AcOH/50 mM ammonium acetate, pH 4.0, H_2O–MeOH (70:30) with a flow of 0.5 mL/min. Standard work-up procedures refer to extraction with the indicated organic solvent from proper aqueous solutions, drying of combined organic extracts over anhydrous $MgSO_4$ or Na_2SO_4, filtering, and evaporation of the solvent in vacuo.

5.1.1. Synthesis of starting materials. 3-Ethoxy-4,5,6,7-tetrahydrobenzo[*d*]isoxazol-4-one (**9**), (*RS*)-4-amino-4,5,6,7-tetrahydrobenzo[*d*]isoxazol-3-ol (**13**), (*RS*)-3-ethoxy-4-methylamino-4,5,6,7-tetrahydrobenzo[*d*]isoxazole (**23**), (*RS*)-4-methylamino-3-pivaloyloxymethyloxy-4,5,6,7-tetrahydrobenzo[*d*]isoxazole (**26**), and the corresponding (*R*)- and (*S*)-forms of **26**, were described previously.[39]

5.1.2. 4,4-Diphenyl-1-butyl iodide. To a suspension of magnesium turnings (15 g, 0.63 mol) in dry THF (75 mL) was added bromobenzene (0.5 g, 0.003 mol). After an initial exothermic reaction had started, the mixture was heated to reflux and a solution of bromobenzene (90 g, 0.57 mol) in dry THF (200 mL) was added dropwise during 30 min. The mixture was heated for additionally 1.5 h. The mixture was cooled to rt and excess magnesium was filtered off in an inert atmosphere. A solution of methyl 4-chlorobutyrate (40 g, 0.29 mol) in dry THF (160 mL) was added dropwise at 15–25 °C. After further stirring for 30 min, the mixture was poured into an aqueous solution of NH_4Cl and ice. Et_2O (500 mL) was added. The organic phase was worked-up according to the standard work-up procedure yielding 65 g of crude 4-chloro-1,1-diphenylbutan-1-ol. The crude alcohol (30 g) was dissolved in a mixture of glacial HOAc (60 mL) and 57% aqueous iodic acid (60 mL). Red phosphorus (5 g) was added and the mixture was refluxed for 6 h. After slowly cooling to rt, the mixture was poured into water and Et_2O. The organic phase was worked-up following the standard procedure above yielding 39 g of the title butyl iodide as a crude oil, which was used without further purification. ^{1}H NMR ($CDCl_3$): δ 7.35–7.15 (m, 10H), 3.95 (t, 1H), 3.20 (t, 2H), 2.15 (p, 2H), 1.70 (p, 2H).

5.1.3. 2,2-Di(2-toluyl)tetrahydrofuran. To a suspension of magnesium turnings (33 g, 1.38 mol) in dry THF (150 mL) was added 2-bromotoluene (4 mL). The reaction mixture was heated to reflux and an exothermic reaction started. The heating mantle was removed and 2-bromotoluene (137 mL) in dry THF (500 mL) was added dropwise over 1 h at reflux temperature (exothermic reaction). The resulting reaction mixture was heated under reflux for additionally 1.5 h. The mixture was cooled to rt and excess magnesium was filtered off in an inert atmosphere. A solution of methyl 4-chlorobutyrate (56.4 g, 0.41 mol) in dry THF (200 mL) was added dropwise at 20 °C. The reaction mixture was stirred at rt for additionally 1 h and was then poured into an aqueous solution of NH_4Cl and ice. The organic phase was worked-up according to the standard work-up procedures. After evaporation of the organic solvent the residue was suspended in a mixture of *n*-heptane/EtOAc (4:1). Filtration of the resulting crystals afforded 2,2-di(2-toluyl)tetrahydrofuran (32.5 g, 31%). ^{1}H NMR ($CDCl_3$): δ 7.65–7.57 (m, 2H), 7.23–7.07 (m, 4H), 7.07–7.00 (m, 2H), 4.02 (t, 2H), 2.57 (t, 2H), 2.10–1.96 (m, 2H), 1.96 (s, 6H).

5.1.4. 4,4-Di(2-toluyl)-1-butylamine (10b). Prepared like 4,4-diphenyl-1-butylamine (**10e**) without final HCl treatment from 4,4-di(2-toluyl)-1-butyl iodide (**32**, 18.7 g, 51 mmol) and isolated as an oil. Yield: 11 g (84%). ^{1}H NMR ($CDCl_3$): δ 7.16–7.05 (m, 8H), 4.23 (t, 1H), 2.70 (t, 2H), 2.27 (s, 6H), 2.03–1.87 (m, 2H), 1.58–1.42 (m, 2H), 1.20 (broad s, 2H).

5.1.5. 4,4-Di(2-toluyl)-3-butenylamine (10c). Prepared like 4,4-diphenyl-1-butylamine (**10e**) without final HCl treatment from 4,4-di(2-toluyl)-3-butenyl iodide (**33**, 34 g, 94 mmol) and isolated as an oil. Yield: 9.9 g (42%). ^{1}H NMR ($CDCl_3$): δ 7.18–7.02 (m, 8H), 5.75 (t, 1H), 2.77 (t, 2H), 2.26 (s, 3H), 2.18 (q, 2H), 2.11 (s, 3H), 1.20 (broad s, 2H).

5.1.6. 4,4-Diphenyl-1-butylamine hydrochloride (10e).[48] To a solution of 4,4-diphenyl-1-butyl iodide (20 g) in dry DMF (150 mL) was added sodium azide (10 g). After

reflux for 1.5 h, the mixture was cooled to rt and subsequently poured into Et_2O and water. The organic phase was worked-up following the standard procedure above. Yield of 4,4-diphenyl-1-butyl azide: 14 g. The crude azide (10 g) was dissolved in EtOH (150 mL), water (10 mL), and glacial HOAc (10 mL). 2% Palladium on activated charcoal was added and the mixture was hydrogenated in a Parr apparatus at 3 atm for 1.5 h. The catalyst was filtered off and the solvents evaporated in vacuo. The remaining viscous oil was dissolved in water and CH_2Cl_2. Aqueous NaOH solution was added to adjust the pH to >11. The organic phase was separated and worked according to the standard procedure above. The hydrochloric salt was prepared by addition of HCl to a solution of the free amino compound in Et_2O. Yield 3.4 g. Mp 172–175 °C.

5.1.7. 3-(Thioxanthen-9-yl)-1-propylamine (10f). A mixture of 3-(thioxanthen-9-yl)-1-propyl iodide[49] (6.9 g, 18.8 mmol) and sodium azide (6.1 g, 94.2 mmol) in dry DMF (100 mL) was heated to reflux for 2 h followed by stirring at rt for 16 h. Water (300 mL) was added followed by extraction with Et_2O (2 × 200 mL). The combined organic phase was washed with water (2 × 250 mL) and brine (2 × 250 mL). Drying of the organic phase over Na_2SO_4, filtration, and removal of solvent in vacuo gave a dark oil of crude 3-(thioxanthen-9-yl)-1-propyl azide (5.0 g, 94%).

The oil was dissolved in EtOH (100 mL) and HOAc (7 mL), water (7 mL), and 2% palladium on activated charcoal (0.55 g) were added. The mixture was hydrogenated in a Parr apparatus at 3 atm for 2 h. Filtration and removal of solvent in vacuo gave an oil, which was subjected to CC (eluent: (1) *n*-heptane/EtOAc 1:1, (2) EtOAc + 2% NEt_3). Evaporation gave the title compound as an oil (1.5 g, 32%). ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.45–7.35 (m, 2H), 7.30–7.10 (m, 6H), 4.00 (t, 1H), 2.60 (broad t, 2H), 1.75 (t, 2H), 1.50–1.25 (m, 4H).

5.1.8. (*RS*)-4-*N*-[4,4-Bis(4-fluorophenyl)but-1-yl]amino-3-ethoxy-4,5,6,7-tetrahydrobenzo[*d*]isoxazole (11a). Prepared according to the procedure for **11e** from **9**[39] (0.40 g, 2.2 mmol) and 4,4-bis(4-fluorophenyl)butylamine,[50] and isolated as an oil. Yield: 0.7 g (75%). ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.15 (dd, 4H), 6.95 (t, 4H), 4.30 (q, 2H), 3.85 (t, 1H), 3.70 (t, 1H), 2.65 (t, 2H), 2.60–2.45 (m, 2H), 2.10–1.90 (m, 3H), 1.85–1.60 (m, 3H), 1.55–1.40 (m, 3H), 1.35 (t, 3H).

5.1.9. (*RS*)-4-*N*-[4,4-Di(2-toluyl)but-1-yl]amino-3-ethoxy-4,5,6,7-tetrahydrobenzo[*d*]isoxazole (11b). The title compound was prepared according to a literature procedure[51] from 4,4-di(2-toluyl)-1-butylamine (**10b**) (1.7 g, 6.7 mmol), **9**[39] (1.0 g, 5.5 mmol), titanium(IV)isopropylate (4.3 mL), $NaCNBH_3$ (0.6 g, 9.5 mmol), and EtOH (20 mL). Yield of the title compound as an oil was 1.0 g (43%). ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.15–7.05 (m, 8H), 4.27 (q, 2H), 3.68 (t, 1H), 2.75–2.45 (m, 4H), 2.27 (s, 6H), 2.05–1.85 (m, 3H), 1.85–1.40 (m, 7H), 1.32 (t, 3H).

5.1.10. (*RS*)-4-*N*-[1,1-Di(2-toluyl)but-1-en-4-yl]amino-3-ethoxy-4,5,6,7-tetrahydrobenzo[*d*]isoxazole (11c). Prepared according to the procedure for **11b** from **9**[39] (2.0 g, 11 mmol) and 4,4-di(2-toluyl)-3-butenylamine (**10c**), and isolated as an oil. Yield: 1.9 g (41%). ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.17–7.03 (m, 8H), 5.80 (t, 1H), 4.27 (q, 2H), 3.67 (t, 1H), 2.76 (t, 2H), 2.70–2.40 (m, 2H), 2.30–2.17 (m, 5H), 2.10 (s, 3H), 2.05–1.40 (m,5H), 1.33 (t, 3H).

5.1.11. (*RS*)-4-*N*-[3-(10,11-Dihydrodibenzo[*a*,*d*]cyclohept-5-ylidene)prop-1-yl]amino-3-ethoxy-4,5,6,7-tetrahydrobenzo[*d*]isoxazole (11d). Prepared according to the procedure for **11e** from **9**[39] (4.0 g, 22 mmol) and 3-(10,11-dihydrodibenzo[*a,d*]cyclohept-5-ylidene)propylamine, hydrochloride,[52] and isolated as an oil. Yield: 6.7 g (74%). ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.33–7.21 (m, 1H), 7.21–7.07 (m, 6H), 7.07–7.00 (m, 1H), 5.90 (t, 1H), 4.25 (q, 2H), 3.65 (t, 1H), 3.50–2.85 (m, 3H), 2.85–2.65 (m, 2H), 2.65–2.40 (m, 2H), 2.35 (q, 2H), 2.05–1.50 (m, 6H), 1.40 (t, 3H).

5.1.12. (*RS*)-4-*N*-(4,4-Diphenylbut-1-yl)amino-3-ethoxy-4,5,6,7-tetrahydrobenzo[*d*]isoxazole (11e). Molecular sieve powder (3 Å) (4 g) was added to a solution of 4,4-diphenylbutylamine hydrochloride (**10e**) (3.0 g, 11.4 mmol) and **9**[39] (1.0 g, 5.52 mmol) in MeOH. $NaCNBH_3$ (1.0 g, 15.9 mmol) was added and the mixture was stirred for 16 h at rt. After filtering off the sieves, water (200 mL) and Et_2O (200 mL) were added and pH adjusted to >11 by addition of aqueous NaOH. The organic phase was worked-up according to the general procedure above leaving an oil, which was purified by CC on silica gel (EtOAc/heptane/NEt_3 50:50:4) and isolated as an oil. Yield: 0.8 g (37%). ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.30–7.10 (m, 10H), 4.30 (q, 2H), 3.85 (t, 1H), 3.65 (t, 1H), 2.65 (t, 2H), 2.60–2.45 (m, 2H), 2.20–2.00 (m, 2H), 2.00–1.85 (m, 1H), 1.85–1.65 (m, 3H), 1.40–1.55 (m, 2H), 1.35 (t, 3H).

5.1.13. (*RS*)-4-*N*-[3-(Thioxanthen-9-yl)prop-1-yl]amino-3-ethoxy-4,5,6,7-tetrahydrobenzo[*d*]isoxazole (11f). Prepared according to the procedure for **11b** from **9**[39] (1.0 g, 5.5 mmol) and 3-(thioxanthen-9-yl)-1-propylamine (**10f**). Yield: 0.58 g (25%) as an oil. ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.45–7.35 (m, 2H), 7.30–7.15 (m, 6H), 4.25 (q, 2H), 3.95 (t, 1H), 3.60 (t, 1H), 2.60–2.50 (m, 4H), 2.05–1.85 (m, 1H), 1.80–1.65 (m, 3H), 1.65–1.50 (m, 3H), 1.50–1.35 (m, 1H), 1.30 (t, 3H).

5.1.14. (*RS*)-4-*N*-[3-(Phenothiazin-10-yl)prop-1-yl]amino-3-ethoxy-4,5,6,7-tetrahydrobenzo[*d*]isoxazole (11g). A solution of **9**[39] (654 mg, 3.61 mmol) and 3-(phenothiazin-10-yl)-propylamine[53] (1.02 g, 3.98 mmol) in toluene (130 mL) was heated under reflux for 6 h. *p*-Toluenesulfonic acid monohydrate (10 mg) was added to the refluxing solution, which was heated under reflux for additionally 16 h. This solution was cooled to 5 °C and was then added to a solution of $NaCNBH_3$ (635 mg, 10.1 mmol) in MeOH (50 mL) at 10 °C. The resulting reaction mixture was stirred for 20 min, before addition of further $NaCNBH_3$ (500 mg, 7.96 mmol). The reaction mixture was stirred for additional 10 min at 10 °C. The

reaction mixture was poured onto water and the phases were separated. The aqueous phase was extracted with EtOAc (3 × 250 mL). The combined organic phases were washed with brine, dried (Na_2SO_4), and evaporated in vacuo. The residue was subjected to CC (*n*-heptane/EtOAc 1:1) to give 640 mg (42%) of the title compound as an oil. ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.18–7.08 (m, 4H), 6.95–6.85 (m, 4H), 4.26 (q, 2H), 3.97 (t, 2H), 3.65 (t, 1H), 2.84–2.70 (m, 2H), 2.60–2.40 (m, 2H), 2.00–1.50 (m,7H), 1.33 (t, 3H).

5.1.15. (*RS*)-4-*N*-(3,3-Diphenylprop-1-yl)amino-3-ethoxy-4,5,6,7-tetrahydrobenzo[*d*]isoxazole (11h). Prepared according to the procedure for **11e** from **9**[39] (3.0 g, 16.6 mmol) and 3,3-diphenylpropylamine.[48] Yield: 5.5 g (89%) as an oil. ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.35–7.15 (m, 10H), 4.25 (q, 2H), 4.10 (t, 1H), 3.65 (t, 1H), 2.65–2.50 (m, 3H), 2.25 (q, 2H), 2.00–1.85 (m, 1H), 1.80–1.60 (m, 4H), 1.60 (s, 1H), 1.40 (t, 3H).

5.1.16. (*RS*)-4-*N*-(4-Phenylbut-1-yl)amino-3-ethoxy-4,5,6,7-tetrahydrobenzo[*d*]isoxazole (11i). Prepared according to the procedure for **11e** from **9**[39] (3.0 g, 16.6 mmol) and 4-phenylbutylamine.[54] Yield: 3.5 g (68%) as an oil. ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.35–7.15 (m, 5H), 4.30 (q, 2H), 3.70 (t, 1H), 2.70–2.50 (m, 6H), 2.00–1.50 (m, 8H), 1.60 (s, 1H), 1.40 (t, 3H).

5.1.17. (*RS*)-4-*N*-[4,4-Bis(4-fluorophenyl)but-1-yl]amino-4,5,6,7-tetrahydrobenzo[*d*]isoxazol-3-ol hydrobromide (12a). Prepared according to the procedure for **12e** from **11a** (0.58 g, 1.4 mmol). Yield: 0.4 g (61%). Mp 205–206 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 7.35 (dd, 4H), 7.15 (t, 4H), 4.20 (broad s, 1H), 4.00 (t, 1H), 3.05 (t, 2H), 2.70–2.55 (m, 2H), 2.10–1.70 (m, 6H), 1.60–1.45 (m, 2H). MS *m*/*z* (%): 399 (MH^+, 4%), 138 (100%), 67 (84%). Anal. Calcd for $C_{23}H_{25}BrF_2N_2O_2$: C, 57.62; H, 5.27; N, 5.84. Found: C, 57.16; H, 5.35; N, 5.86.

5.1.18. (*RS*)-4-*N*-[4,4-Di(2-toluyl)but-1-yl]amino-4,5,6,7-tetrahydrobenzo[*d*]isoxazol-3-ol hydrobromide (12b). Prepared according to the procedure for **12e** from **11b** (1.0 g, 2.4 mmol). Yield: 0.39 g (33%). Mp 217–219 °C (dec). ^{1}H NMR (250 MHz, DMSO-d_6): δ 7.20–7.03 (m, 8H), 4.27–4.13 (m, 2H), 3.06 (t, 2H), 2.75–2.52 (m, 2H), 2.25 (d, 6H), 2.18–1.55 (m, 8H). MS *m*/*z* (%): 391 (MH^+, 7%), 195 (15%), 145 (80%), 138 (92%), 105 (100%). Anal. Calcd for $C_{25}H_{31}BrN_2O_2$: C, 63.68; H, 6.64; N, 5.94. Found: C, 63.33; H, 6.74; N, 6.12.

5.1.19. (*RS*)-4-*N*-[1,1-Di(2-toluyl)but-1-en-4-yl]amino-3-hydroxy-4,5,6,7-tetrahydrobenzo[*d*]isoxazol-3-ol hydrobromide (12c). Prepared according to the procedure for **12e** from **11c** (1.9 g, 4.6 mmol). Yield 1.2 g (57%). Mp 209–211 °C (dec). ^{1}H NMR (250 MHz, DMSO-d_6): δ 7.26–6.96 (m, 8H), 5.73 (t, 1H), 4.24–4.16 (m, 1H), 3.11 (t, 2H), 2.73–2.54 (m, 2H), 2.45–2.27 (m, 2H), 2.22 (s, 3H), 2.15–1.70 (m, 7H), MS *m*/*z* (%): 389 (MH^+, 5%), 143 (33%), 138 (100%), 105 (29%), 67 (44%). Anal. Calcd for $C_{25}H_{29}BrN_2O_2$: C, 63.96; H, 6.24; N, 5.97. Found: C, 63.62; H, 6.29; N, 6.18.

5.1.20. (*RS*)-4-*N*-[3-(10,11-Dihydrodibenzo[*a*,*d*]cyclohept-5-ylidene)propan-1-yl]amino-4,5,6,7-tetrahydrobenzo[*d*]isoxazol-3-ol hydrobromide (12d). Prepared according to the procedure for **12e** from **11d** (4.0 g, 9.7 mmol). Yield 1.8 g (40%). Mp 228–230 °C (dec). ^{1}H NMR (250 MHz, DMSO-d_6): δ 7.28–7.00 (m, 8H), 5.80 (t, 1H), 3.87–3.77 (m, 1H), 3.40–3.10 (m, 2H), 3.00–2.65 (m, 4H), 2.65–2.15 (m, 4H), 2.00–1.55 (m, 4H). MS *m*/*z* (%): 387 (MH^+, 5%), 233 (7%), 138 (41%), 43 (100%). Anal. Calcd for $C_{25}H_{27}BrN_2O_2$: C, 64.23; H, 5.83; N, 5.99. Found: C, 63.99; H, 5.82; N, 6.01.

5.1.21. (*RS*)-4-*N*-(4,4-Diphenylbut-1-yl)amino-4,5,6,7-tetrahydrobenzo[*d*]isoxazol-3-ol hydrobromide (12e). A solution of compound **11e** (0.7 g, 1.79 mmol) in a 33% HBr solution in glacial HOAc (40 mL) was heated at 80–90 °C for 1 h. Excess of solvents were cautiously evaporated in vacuo and ethanol/Et_2O (1:1) was added. The hydrobromide salt of the title compound **12e** was filtered off and dried. Yield 0.6 g (76%). Mp 221–222 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 7.40–7.15 (m, 10H), 4.20 (broad s, 1H), 3.95 (t, 1H), 3.05 (t, 2H), 2.75–2.55 (m, 2H), 2.10–1.70 (m, 6H), 1.65–1.45 (m, 2H). MS *m*/*z* (%): 363 (MH^+, 100%), 138 (89%). Anal. Calcd for $C_{23}H_{27}BrN_2O_2$: C, 62.30; H, 6.15; N, 6.32. Found: C, 62.27; H, 6.17; N, 6.37.

5.1.22. (*RS*)-4-*N*-[3-(Thioxanthen-9-yl)propan-1-yl]-amino-4,5,6,7-tetrahydrobenzo[*d*]isoxazol-3-ol hydrochloride (12f). The ethoxy derivative **11f** (0.42 g, 1.0 mmol) was heated under reflux in 6 M HCl for 66 h. After work-up the hydrochloride salt was isolated by addition and subsequent evaporation of hydrochloric acid and crystallization from EtOH. Yield: 0.37 g (88%). Mp 221–223 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 7.45–7.35 (m, 4H), 7.35–7.25 (m, 4H), 4.20–4.10 (m, 2H), 2.90 (t, 2H), 2.65–2.45 (m, 2H), 2.15–1.90 (m, 2H), 1.85–1.45 (m, 6H). MS *m*/*z* (%): 393 (MH^+, 6%), 239 (37%), 197 (26%), 138 (100%). Anal. Calcd for $C_{23}H_{25}BrN_2O_2S$, 3/4H_2O: C, 62.42; H, 6.05; N, 6.33. Found: C, 62.27; H, 5.75; N, 6.29.

5.1.23. (*RS*)-4-*N*-[3-(Phenothiazin-10-yl)propan-1-yl]-amino-4,5,6,7-tetrahydrobenzo[*d*]isoxazol-3-ol hydrobromide (12g). Prepared according to the procedure for **12e** from **11g** (0.9 g, 2.1 mmol). Yield 0.27 g (27%). Mp 187–189 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 7.26–7.10 (m, 4H), 7.07 (d, 2H), 6.97 (dd, 2H), 4.20–4.09 (m, 1H), 3.95 (t, 2H), 3.16–3.00 (m, 2H), 2.71–2.50 (m, 2H), 2.12–1.65 (m, 6H). MS *m*/*z* (%): 394 (MH^+, 3%), 256 (7%), 138 (18%), 43 (100%). Anal. Calcd for $C_{22}H_{24}BrN_3O_2S$: C, 55.69; H, 5.11; N, 8.86. Found: C, 55.90; H, 5.14; N, 8.90.

5.1.24. (*RS*)-4-*N*-(3,3-Diphenylpropan-1-yl)amino-4,5,6,7-tetrahydrobenzo[*d*]isoxazol-3-ol hydrobromide (12h). Prepared according to the procedure for **12e** from **11h** (1.9 g, 5.0 mmol). Yield: 0.7 g (33%). Mp 218–220 °C (EtOH). ^{1}H NMR (DMSO-d_6): δ 7.40–7.15 (m, 10H), 4.25 (broad s, 1H), 4.05 (t, 1H), 2.95 (t, 2H), 2.70–2.55 (m, 2H), 2.40 (t, 2H), 2.10–1.70 (m, 4H). MS *m*/*z* (%): 349 (MH^+, 50%), 138 (100%), 67 (30%). Anal. Calcd

for $C_{22}H_{25}BrN_2O_2$: C, 61.54; H, 5.88; N, 6.53. Found: C, 61.46; H, 5.88; N, 6.61.

5.1.25. (*RS*)-4-*N*-(4-Phenylbut-1-yl)amino-4,5,6,7-tetrahydrobenzo[*d*]isoxazol-3-ol hydrobromide (12i). Prepared according to the procedure for **12e** from **11i** (3.5 g, 11 mmol). Yield: 2.9 g (71%). Mp 202–204 °C (EtOH). ^{1}H NMR (DMSO-d_6): δ 7.35–7.15 (m, 5H), 4.25 (broad s, 1H), 3.00 (broad t, 2H), 2.70–2.50 (m, 4H), 2.15–1.70 (m, 4H), 1.65–1.55 (m, 4H). MS *m*/*z* (%): 287 (MH^+, 6%), 138 (100%), 91 (42%), 67 (63%). Anal. Calcd for $C_{17}H_{23}BrN_2O_2$: C, 55.58; H, 6.32; N, 7.63. Found: C, 55.74; H, 6.32; N, 7.65.

5.1.26. (*RS*)-4-*N*-(*tert*-Butyloxycarbonyl)amino-3-benzyloxy-4,5,6,7-tetrahydrobenzo[*d*]isoxazole (14). To a solution of **13**[39] (1.0 g, 4.24 mmol) in a mixture of H_2O (10 mL) and THF (5 mL) at 0 °C was added saturated aqueous NaOH until pH ~ 9. Di-*tert*-butyldicarbonate (0.39 g, 9.75 mmol) in THF (5 mL) was added and cooling was removed. A vigorous stirring at rt was continued for 5 h. Et_2O (20 mL) and H_2O (15 mL) was added. The aqueous phase was separated and cooled. Then EtOAc (20 mL) was added followed by $KHSO_4$ (aq) until pH ~ 4. The aqueous phase was extracted with EtOAc (2 × 20 mL) and the combined organic phases was dried and evaporated. Yield of (*RS*)-4-*N*-(*tert*-butyloxycarbonyl)amino-4,5,6,7-tetrahydrobenzo[*d*]isoxazol-3-ol 0.56 g (52%). ^{1}H NMR (60 MHz, $CDCl_3$): δ 5.2–4.9 (m, 1H), 4.5–4.2 (m, 1H), 2.70–2.40 (m, 2H), 1.95–1.65 (m, 4H), 1.50 (s, 9H). A solution of (*RS*)-4-*N*-(*tert*-butyloxycarbonyl)amino-4,5,6,7-tetrahydrobenzo[*d*]isoxazol-3-ol (0.55 g, 2.16 mmol) in DMF (12 mL) was added K_2CO_3 (595 mg, 4.3 mmol) and stirred for 45 min at 40 °C. Benzyl bromide (0.77 mL, 6.5 mmol) was added and stirring continued at 40 °C overnight. The solvent was evaporated and H_2O was added followed by extraction with EtOAc (3 × 25 mL). The combined organic phases was dried and evaporated. The residue was subjected to CC (gradient: tol–EtOAc) to yield 360 mg (48%) of the title compound as an oil. ^{1}H NMR (60 MHz, $CDCl_3$): δ 7.4–7.2 (m, 5H), 5.2 (s, 2H), 4.8–4.5 (m, 2H), 2.6–2.4 (m, 2H), 1.9–1.7 (m, 4H), 1.4 (s, 9H).

5.1.27. (*RS*)-4-Amino-3-benzyloxy-4,5,6,7-tetrahydrobenzo[*d*]isoxazole (15). A suspension of **13** (344 mg, 1.0 mmol) in Et_2O (10 mL) was added 2.5 M HCl in EtOAc (10 mL). A clear solution appeared and stirring was continued for 24 h. Et_2O (15 mL) was added and the precipitate was filtered and washed with Et_2O. Yield: 232 mg (83%). Mp 179–182 °C. ^{1}H NMR (60 MHz, D_2O): δ 7.50 (m, 5H), 5.32 (s, 2H), 4.5–4.2 (m, 1H), 2.8–2.5 (m, 2H), 2.1–1.8 (m, 4H).

5.1.28. (*RS*)-4-[*N*-(1,1-Diphenylbut-1-en-4-yl)amino]-3-benzyloxy-4,5,6,7-tetrahydrobenzo[*d*]isoxazole acetate (16a). A mixture of **15** (375 mg, 1.33 mmol), 4,4-diphenyl-3-butenyl bromide[27] (573 mg, 2.0 mmol), K_2CO_3 (553 mg, 4.0 mmol), and NaI (60 mg, 0.4 mmol) in DMF was heated to 120 °C and stirred for 24 h. Additional 4,4-diphenyl-3-butenyl bromide (573 mg, 2.0 mmol) was added and stirring continued for another 24 h. The solvent was evaporated and H_2O (15 mL) was added followed by extraction with Et_2O (3 × 20 mL). The combined organic phases were dried and evaporated. The remaining oil was purified by gradient CC on silica gel (starting with 1% HOAc in toluene followed by 1% HOAc in MeOH/EtOAc (1:9)). Yield of the title compound as an oil was 0.28 g (41%). ^{1}H NMR (60 MHz, $CDCl_3$): δ 7.5–7.1 (m, 15H), 5.87 (t, 1H), 5.22 (s, 2H), 3.95–3.75 (m, 1H), 3.0–2.7 (m, 2H), 2.55–2.2 (m, 4H), 1.9–1.7 (m, 7H).

5.1.29. (*RS*)-4-[*N*-[1,1-Bis(3-methyl-2-thienyl)but-1-en-4-yl]-amino]-3-benzyloxy-4,5,6,7-tetrahydrobenzo[*d*]isoxazole (16b). A mixture of **17** (182 mg, 0.65 mmol), 1,1-bis(3-methyl-2-thienyl)but-1-en-4-yl methanesulfonate (**30**) (334 mg, 0.98 mmol), K_2CO_3 (269 mg, 1.95 mmol), and NaI (30 mg, 0.2 mmol) in DMF was heated at 100 °C for 24 h. 1,1-Bis(3-methyl-2-thienyl)but-1-en-4-yl methanesulfonate (**30**) (334 mg, 0.98 mmol) was added and stirring continued for 24 h. To the solution was added H_2O (12 mL) by extraction with Et_2O. The combined organic phases were dried and evaporated. The residue was purified by CC (toluene) yielding 0.103 g (33%). ^{1}H NMR (60 MHz, $CDCl_3$): δ 7.45–7.30 (m, 5H), 7.18 (d, 1H), 7.04 (d, 1H), 6.82 (d, 1H), 6.73 (d, 1H), 6.00 (t, 1H), 5.28 (s, 2H), 3.75–3.65 (m, 1H), 2.80–2.15 (m, 6H), 1.98 (s, 3H), 1.92 (s, 3H), 2.05–1.65 (m, 7H).

5.1.30. (*RS*)-4-[*N*-(1,1-Diphenylbut-1-en-4-yl)amino]-4,5,6,7-tetrahydrobenzo[*d*]isoxazol-3-ol hydrochloride (17a). A solution of **16a** (280 mg, 0.62 mmol) in a mixture of 4 M HCl (5 mL) and EtOH (10 mL) was refluxed for 72 h followed by evaporation. EtOH was added and the solution evaporated. The residue was dissolved in MeCN/EtOH (6:1, 3.5 mL) heated and filtered hot. The filtrate was added Et_2O and precipitated at 5 °C for 3 days. The precipitate was filtered off and dried. Yield 77 mg (31%). Mp 134–138 °C. ^{1}H NMR (200 MHz, D_2O/DMSO-d_6 (3:2)): δ 7.4–7.2 (m, 10 H), 6.12 (t, 1H), 4.2–4.1 (m, 1H), 3.2–3.1 (m, 2H), 2.6–2.35 (m, 4H), 1.9–1.7 (m, 4H). Anal. Calcd for $C_{23}H_{25}ClN_2O_2$, 5/4H_2O: C, 65.86; H, 6.61; N, 6.68. Found: C, 65.67; H, 6.17; N, 6.70.

5.1.31. (*RS*)-4-[*N*-[1,1-Bis(3-methyl-2-thienyl)but-1-en-4-yl]amino]-3-hydroxy-4,5,6,7-tetrahydrobenzo[*d*]isoxazole hydrochloride (17b). To a solution of **16b** (103 mg, 0.215 mmol) in EtOH (6 mL) was added 4 M HCl (3 mL) and the solution was heated under reflux for 54 h. Additional EtOH (10 mL) was added followed by activated charcoal treatment. After evaporation the residue was dissolved in EtOH and evaporated. The product was precipitated twice from a mixture MeCN (6 mL) and EtOH (2 mL) by careful addition of Et_2O. Yield 34 mg (36%). Mp 188–191 °C. ^{1}H NMR (200 MHz, D_2O/DMSO-d_6 (3:2)): δ 7.42 (d, 1H), 7.27 (d, 1H), 6.99 (d, 1H), 6.92 (d, 1H), 6.11 (t, 1H), 4.3–4.2 (m, 1H), 3.30–3.15 (m, 2H), 2.75–2.45 (m, 4H), 2.08 (s, 6H), 2.05–1.85 (m, 4H). Anal. Calcd for $C_{21}H_{25}ClN_2O_2S_2$: C, 57.72; H, 5.77; N, 6.41. Found: C, 57.56; H, 5.78; N, 6.67.

5.1.32. (*RS*)-4-[*N*-(*tert*-Butyloxycarbonyl)-*N*-methylamino]-3-benzyloxy-4,5,6,7-tetrahydrobenzo[*d*]isoxazole (19). A mixture of **18**[39] (1.20 g, 4.47 mmol), benzyl bromide (1.59 mL, 13.4 mmol), and K_2CO_3 (1.24 g, 8.94 mmol) in DMF (25 mL) was stirred overnight at 40 °C. The solvent was evaporated and H_2O (25 mL) was added followed by extraction with CH_2Cl_2 (3 × 50 mL). The combined organic phases was dried, evaporated, and the residue purified by CC (gradient: heptane/tol (1:1)–tol–EtOAc) yielding 0.62 g (39%) as an oil. ^{1}H NMR (60 MHz, $CDCl_3$): δ 7.4–7.3 (m, 5H), 5.23 (s, 2H), 5.2–5.1 (m, 1H), 2.7–2.5 (m, 2H), 2.60 (s, 3H), 2.1–1.7 (m, 4H), 1.45 (s, 9H).

5.1.33. (*RS*)-4-*N*-Methylamino-3-benzyloxy-4,5,6,7-tetrahydrobenzo[*d*]isoxazole hydrochloride (20). A solution of **19** (0.62 g, 1.73 mmol) in EtOH (20 mL) was added 0.5 M HCl (aq, 20 mL) and stirred at 45 °C for 2 h followed by evaporation. EtOH was added and evaporated. The residue was dissolved in MeCN (6 mL) and added Et_2O (20 mL) and left at 5 °C for 24 h. The precipitate was filtered and dried, yielding 0.46 g (90%). Mp 156–159 °C. ^{1}H NMR (200 MHz, D_2O): δ 7.5–7.35 (m, 5H), 5.33 (s, 2H), 4.3–4.2 (m, 1H), 2.73 (s, 3H), 2.7–2.6 (m, 2H), 2.05–1.85 (m, 4H). ^{13}C NMR (50 MHz, D_2O): δ 175.4, 135.9, 129.6, 129.5, 129.0, 99.7, 72.9, 50.8, 31.7, 25.9, 22.6, 17.6. Anal. Calcd for $C_{15}H_{19}ClN_2O_2$: C, 61.12; H, 6.50; N, 9.50. Found: C, 60.65; H, 6.52; N, 9.64.

5.1.34. (*RS*)-4-[*N*-(1,1-Diphenylbut-1-en-4-yl)-*N*-methylamino]-3-benzyloxy-4,5,6,7-tetrahydrobenzo[*d*]isoxazole (21). A mixture of **20** (442 mg, 1.50 mmol), 4,4-diphenyl-3-butenyl bromide[27] (646 mg, 2.25 mmol), K_2CO_3 (622 mg, 4.50 mmol), and NaI (75 mg, 0.50 mmol) in DMF (8 mL) was stirred at 120 °C for 24 h. 4,4-Diphenyl-3-butenyl bromide (500 mg, 1.74 mmol) was added and stirring continued for another 24 h. The solvent was evaporated and H_2O (20 mL) added followed by extraction with Et_2O (3 × 25 mL). The combined organic phases was dried and evaporated. The residue was purified by gradient CC (toluene to 10% MeOH in EtOAc) yielding 0.59 g (85%). ^{1}H NMR (60 MHz, $CDCl_3$): δ 7.3–7.1 (m, 15H), 6.0 (t, 1H), 5.23 (s, 2H), 3.6–3.4 (m, 1H), 2.7–2.1 (m, 6H), 2.14 (s, 3H), 1.9–1.7 (m, 4H).

5.1.35. (*RS*)-4-[*N*-(1,1-Diphenylbut-1-en-4-yl)-*N*-methylamino]-4,5,6,7-tetrahydrobenzo[*d*]isoxazol-3-ol hydrochloride (22). To a solution of **21** (0.59 g, 1.27 mmol) in EtOH (15 mL) was added 4 M HCl (7.5 mL) and refluxed for 3 days. The solution was evaporated and the residue was dissolved in EtOH followed by evaporation. The residue was dissolved in acetone (8 mL) and EtOH (2 mL), treated with activated charcoal and precipitated by slow addition of Et_2O at −20 °C. Filtration gave 228 mg (44%). Mp 140–143 °C. ^{1}H NMR (200 MHz, D_2O/DMSO-d_6 (2:1)): δ 7.4–7.0 (m, 10 H), 5.96 (t, 1H), 4.25–4.15 (m, 1H), 3.2–3.05 (m, 2H), 2.6 (s, 3H), 2.55–2.35 (m, 4H), 1.9–1.7 (m, 4H). Anal. Calcd for $C_{24}H_{27}ClN_2O_2$, 3/4EtOH: C, 68.75; H, 7.13; N, 6.29. Found: C, 69.13; H, 7.17; N, 6.25.

5.1.36. (*RS*)-4-[*N*-[4,4-Bis(4-fluorophenyl)but-1-yl]-*N*-methyl-amino]-3-ethoxy-4,5,6,7-tetrahydrobenzo[*d*]isoxazole (24a). To a solution of **23**[39] (1.0 g, 5.10 mmol) in 4-methylpentan-2-one (10 mL) was added bis-4,4-(4-fluorophenyl)-1-butyl chloride[55] (2.0 g, 7.12 mmol), K_2CO_3 (1.0 g, 7.24 mmol), and KI (0.5 g, 3.01 mmol). The mixture was heated under reflux overnight. Inorganic salts were filtered off and the solvent was evaporated. The remaining oil was purified by CC on silica gel (heptane/EtOAc 2:3). Yield of the title compound as an oil was 1.6 g (71%). ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.15 (dd, 4H), 6.95 (t, 4H), 4.25 (q, 2H), 3.85 (t, 1H), 3.60 (t, 1H), 2.60–2.35 (m, 4H), 2.20 (s, 3H), 2.05–1.90 (m, 3H), 1.75–1.60 (m, 3H), 1.50–1.35 (m, 2H), 1.35 (t, 3H).

5.1.37. (*RS*)-4-[*N*-[4,4-Di(2-toluyl)but-1-yl]-*N*-methylamino]-3-ethoxy-4,5,6,7-tetrahydrobenzo[*d*]isoxazole (24b). Prepared by the procedure for **24a** via alkylation of **23**[39] (1.5 g, 7.6 mmol) with 4,4-di(2-toluyl)-1-butyl iodide (**32**) in acetone and isolated as an oil. Yield: 1.8 g (55%). ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.17–7.00 (m, 8H), 4.23 (q, 2H), 3.60 (t, 1H), 2.60–2.35 (m, 4H), 2.26 (dd, 6H), 2.20 (s, 3H), 2.05–1.87 (m, 3H), 1.80–1.60 (m, 4H), 1.56–1.42 (m, 2H), 1.28 (t, 3H).

5.1.38. (*RS*)-4-[*N*-[1,1-Di(2-toluyl)but-1-en-4-yl]-*N*-methylamino]-3-ethoxy-4,5,6,7-tetrahydrobenzo[*d*]isoxazole (24c). Prepared by the procedure for **24a** via alkylation of **23**[39] (1.9 g, 9.7 mmol) with 4,4-di(2-toluyl)-3-butenyl iodide (**33**) in acetone and isolated as an oil. Yield: 2.5 g (60%). ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.17–7.00 (m, 8H), 5.81 (t, 1H), 4.26 (q, 2H), 3.57 (t, 1H), 2.67–2.45 (m, 4H), 2.26 (s, 3H), 2.17 (dd, 6H), 2.07–1.90 (m, 2H), 1.80–1.55 (m, 4H), 1.33 (t, 3H).

5.1.39. (*RS*)-4-[*N*-[3-(10,11-Dihydrodibenzo[*a,d*]cyclohept-5-ylidene)propane-1-yl]-*N*-methylamino]-3-ethoxy-4,5,6,7-tetrahydrobenzo[*d*]isoxazole (24d). Prepared by the procedure for **24a** via alkylation of **23**[39] (1.0 g, 5.1 mmol) with 3-(10,11-dihydrodibenzo[*a,d*]cyclohept-5-ylidene)-1-propyl bromide[52] in acetone and isolated as an oil. Yield: 0.71 g (32%). ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.30–6.98 (m, 8H), 5.88 (t, 1H), 4.24 (q, 2H), 3.63–3.50 (m, 1H), 3.50–2.70 (m, 4H), 2.70–2.45 (m, 4H), 2.35–2.20 (m, 2H), 2.20 (s, 3H), 2.06–1.87 (m, 1H), 1.74–1.60 (m, 3H), 1.30 (t, 3H).

5.1.40. (*RS*)-4-[*N*-[4,4-Bis(4-fluorophenyl)but-1-yl]-*N*-methylamino]-4,5,6,7-tetrahydrobenzo[*d*]isoxazol-3-ol hydrobromide (25a). Prepared according to the procedure for **12e** from **24a** (5.5 g, 13 mmol). Yield: 3.7 g (69%). Mp 78–82 °C. ^{1}H NMR (250 MHz, DMSO-d_6, 60 °C): δ 7.35 (dd, 4H), 7.10 (t, 4H), 4.45 (t, 1H), 4.05 (t, 1H), 3.20 (broad t, 2H), 2.70 (broad s, 3H), 2.70–2.55 (m, 2H), 2.15–1.60 (m, 8H). MS *m/z* (%): 413 (MH^+, 10%), 203 (13%), 138 (100%), 109 (12%), 67 (58%). Anal. Calcd for $C_{24}H_{27}BrF_2N_2O_2$, 1/3H_2O: C, 57.72; H, 5.58; N, 5.61. Found: C, 57.36; H, 5.67; N, 5.66.

5.1.41. (*RS*)-4-[*N*-[4,4-Di(2-toluyl)but-1-yl]-*N*-methylamino]-4,5,6,7-tetrahydrobenzo[*d*]isoxazol-3-ol hydrobromide (25b). Prepared according to the procedure for **12e** from **24b** (1.8 g, 4.2 mmol). Yield: 1.2 g (60%). Mp 193–

195 °C (dec). ^{1}H NMR (250 MHz, DMSO-d_6): δ 7.21–7.03 (m, 8H), 4.47–4.36 (m, 1H), 4.25 (t, 1H), 3.35–3.15 (m, 2H), 2.80–2.55 (m, 5H), 2.26 (s, 6H), 2.20–1.65 (m, 8H). MS *m*/*z* (%): 405 (MH^+, 4%), 268 (27%), 138 (30%), 43 (100%). Anal. Calcd for $C_{26}H_{33}BrN_2O_2$: C, 64.32; H, 6.86; N, 5.77. Found: C, 64.17; H, 6.91; N, 5.79.

5.1.42. (*RS*)-4-[*N*-[4,4-Di(2-toluyl)but-3-en-1-yl]-*N*-methylamino]-4,5,6,7-tetrahydrobenzo[*d*]isoxazol-3-ol hydrobromide (25c). Prepared according to the procedure for **12e** from **24c** (2.5 g, 5.8 mmol). Yield: 1.3 g (46%). Mp 177–179 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 7.30–6.98 (m, 8H), 5.74 (t, 1H), 4.47–4.35 (m, 1H), 3.40–3.15 (m, 2H), 2.80–2.60 (m, 6H), 2.21 (s, 3H), 2.14–1.70 (m, 8H). MS *m*/*z* (%): 403 (MH^+, 19%), 266 (40%), 143 (77%), 138 (100%), 105 (49%), 67 (20%). Anal. Calcd for $C_{26}H_{31}BrN_2O_2$: C, 64.58; H, 6.48; N, 5.80. Found: C, 64.31; H, 6.62; N, 6.02.

5.1.43. (*RS*)-4-[*N*-[3-(10,11-Dihydrodibenzo[*a*,*d*]cyclohept-5-ylidene)prop-1-yl]-*N*-methylamino]-4,5,6,7-tetrahydrobenzo[*d*]isoxazol-3-ol hydrobromide (25d). Prepared according to the procedure for **12e** from **24d** (0.71 g, 1.7 mmol). Yield: 0.18 g (22%). Mp 215–217 °C (dec). ^{1}H NMR (250 MHz, DMSO-d_6): δ 7.30–7.05 (m, 8H), 5.80 (t, 1H), 4.43–4.34 (m, 1H), 3.44–3.05 (m, 7H), 2.95–2.40 (m, 6H), 2.10–1.70 (m, 4H). MS *m*/*z* (%): 401 (MH^+, 26%), 265 (66%), 233 (30%), 138 (84%), 43 (100%). Anal. Calcd for $C_{26}H_{29}BrN_2O_2$, $1/2H_2O$: C, 63.66; H, 6.18; N, 5.71. Found: C, 63.95; H, 6.14; N, 5.87.

5.1.44. (*RS*)-4-[*N*-[2-(Diphenylmethoxy)ethyl]-*N*-methylamino]-3-pivaloyloxymethyloxy-4,5,6,7-tetrahydrobenzo[*d*]isoxazole (27a). Prepared by the procedure below for **27b** via alkylation of **26**[39] (1.3 g, 4.6 mmol) with 2-(diphenylmethoxy)ethyl methanesulfonate (**35**). Yield: 1.2 g (53%) as an oil. ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.40–7.15 (m, 10H), 5.90 (dd, 2H), 5.35 (s, 1H), 3.65 (dt, 1H), 3.50 (dt, 2H), 2.80–2.60 (m, 2H), 2.55 (t, 2H), 2.30 (s, 3H), 2.05–1.95 (m, 1H), 1.80–1.60 (m, 3H), 1.20 (s, 9H).

5.1.45. (*RS*)-4-[*N*-[2-[Bis(4-fluorophenyl)methoxy]ethyl]-*N*-methylamino]-3-pivaloyloxymethyloxy-4,5,6,7-tetrahydrobenzo[*d*]isoxazole (27b). To a solution of **26**[39] (1.1 g, 3.90 mmol) in 4-methylpentan-2-one (18 mL) were added K_2CO_3 (0.7 g, 5.06 mmol) and 2-(bis(4-fluorophenyl)methoxy)ethyl methanesulfonate (**36**) (1.8 g, 5.26 mmol). The mixture was heated under reflux overnight. Inorganic salts were filtered off and the solvent was evaporated. CC afforded pure **27b**. Yield: 1.4 g (68%) as an oil. ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.55 (d, 2H), 7.30 (dd, 4H), 7.00 (t, 4H), 5.90 (dd, 2H), 5.35 (s, 1H), 3.65 (dt, 1H), 3.50 (t, 2H), 2.80–2.60 (m, 2H), 2.55 (broad t, 2H), 2.25 (s, 3H), 2.05–1.95 (m, 1H), 1.80–1.60 (m, 3H), 1.20 (s, 9H).

5.1.46. (*RS*)-4-[*N*-[2-[Bis(4-chlorophenyl)methoxy]ethyl]-*N*-methylamino]-3-pivaloyloxymethyloxy-4,5,6,7-tetrahydrobenzo[*d*]isoxazole (27c). Prepared by the procedure for **27b** via alkylation of **26**[39] (1.2 g, 2.1 mmol) with 2-(bis(4-chlorophenyl)methoxy)ethyl chloride.[42] Yield: 0.5 g (21%) as an oil. ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.35–7.15 (m, 8H), 5.90 (d, 1H), 5.90 (dd, 2H), 3.70 (dt, 1H), 3.50 (t, 2H), 2.85–2.65 (m, 2H), 2.55 (t, 2H), 2.30 (s, 3H), 2.10–1.95 (m, 1H), 1.85–1.60 (m, 3H), 1.20 (s, 9H).

5.1.47. (*RS*)-4-[*N*-[1,1-Bis(3-methyl-2-thienyl)but-1-en-4-yl]-*N*-methylamino]-3-pivaloyloxymethyloxy-4,5,6,7-tetrahydrobenzo[*d*]isoxazole (27d). To a solution of **26**[39] (8.46 g, 30.0 mmol) and 1,1-bis(3-methyl-2-thienyl)but-1-en-4-yl methanesulfonate (**30**) (15 g, 43.8 mmol) in isopropyl acetate (40 mL) was added Li_2CO_3 (2.5 g, 33.8 mmol). The mixture was heated under reflux for 24 h while continuously adding additional Li_2CO_3 in small amounts (4×0.5 g, 27.1 mmol). Et_2O was added and the solution filtered. The organic phase was washed with H_2O. The aqueous phase was extracted and the combined organic phases were washed with brine, dried ($MgSO_4$), and evaporated. CC (Et_3N/EtOAc/heptane 5:10:85) gave **27d** (9.0 g, 56%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.20 (d, 1H), 7.04 (d, 1H), 6.84 (d, 1H), 6.75 (d, 1H), 6.10 (t, 1H), 5.88 (d, 1H), 5.86 (d, 1H), 3.62 (t, 1H), 2.65–2.50 (m, 4H), 2.30–2.24 (m, 2H), 2.20 (s, 3H), 2.04 (s, 3H), 1.99 (s, 3H), 1.75–1.60 (m, 4H), 1.21 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 176.8, 171.4, 168.2, 139.6, 135.3, 135.1, 133.7, 133.3, 131.0, 129.4, 127.9, 124.0, 122.4, 104.8, 84.5, 55.3, 53.6, 38.6, 38.2, 28.7, 26.8, 25.9, 23.0, 19.7, 14.6, 14.2.

5.1.48. (*R*)-4-[*N*-[1,1-Bis(3-methyl-2-thienyl)but-1-en-4-yl]-*N*-methylamino]-3-pivaloyloxymethyloxy-4,5,6,7-tetrahydrobenzo[*d*]isoxazole [(*R*)-27d]. Prepared according to the procedure for **27d** from the (*R*)-**26**[39] (1.40 g, 5.0 mmol). Yield: 1.54 g (59%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.20 (d, 1H), 7.04 (d, 1H), 6.84 (d, 1H), 6.75 (d, 1H), 6.10 (t, 1H), 5.88 (d, 1H), 5.86 (d, 1H), 3.62 (t, 1H), 2.65–2.50 (m, 4H), 2.30–2.24 (m, 2H), 2.20 (s, 3H), 2.04 (s, 3H), 1.99 (s, 3H), 1.75–1.60 (m, 4H), 1.21 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 176.8, 171.4, 168.2, 139.6, 135.3, 135.1, 133.7, 133.3, 131.0, 129.4, 127.9, 124.0, 122.4, 104.8, 84.5, 55.3, 53.6, 38.6, 38.2, 28.7, 26.8, 25.9, 23.0, 19.7, 14.6, 14.2.

5.1.49. (*S*)-4-[*N*-[1,1-Bis(3-methyl-2-thienyl)but-1-en-4-yl]-*N*-methylamino]-3-pivaloyloxymethyloxy-4,5,6,7-tetrahydrobenzo[*d*]isoxazole [(*S*)-27d]. Prepared according to the procedure for **27d** from the (*S*)-**26**[39] (1.37 g, 4.9 mmol). Yield: 1.75 g (68%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.20 (d, 1H), 7.04 (d, 1H), 6.84 (d, 1H), 6.75 (d, 1H), 6.10 (t, 1H), 5.88 (d, 1H), 5.86 (d, 1H), 3.62 (t, 1H), 2.65–2.50 (m, 4H), 2.30–2.24 (m, 2H), 2.20 (s, 3H), 2.04 (s, 3H), 1.99 (s, 3H), 1.75–1.60 (m, 4H), 1.21 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 176.8, 171.4, 168.2, 139.6, 135.3, 135.1, 133.7, 133.3, 131.0, 129.4, 127.9, 124.0, 122.4, 104.8, 84.5, 55.3, 53.6, 38.6, 38.2, 28.7, 26.8, 25.9, 23.0, 19.7, 14.6, 14.2.

5.1.50. (*RS*)-4-[*N*-[2-(Diphenylmethoxy)ethyl]-*N*-methylamino]-4,5,6,7-tetrahydrobenzo[*d*]isoxazol-3-ol hydrochloride (28a). Prepared according to the procedure below for **28b** from **27a** (1.2 g, 2.4 mmol). The obtained sodium salt was converted to the title hydrochloride salt

from CH_2Cl_2 by addition of concentrated hydrochloride acid to pH = 1. Yield: 0.5 g (55%). Mp: 108–113 °C (amorphous) (Et_2O). ^1H NMR (250 MHz, DMSO-d_6): δ 7.45–7.20 (m, 10H), 5.55 (s, 1H), 4.55 (broad s, 1H), 3.80 (broad t, 2H), 3.60–3.40 (m, 2H), 2.80 (broad s, 3H), 2.75–2.60 (m, 2H), 2.30–2.10 (m, 2H), 2.10–1.70 (m, 2H). MS *m/z* (%): 379 (MH+, 4%), 167 (100%), 152 (74%), 138 (36%), 67 (32%). Anal. Calcd for $C_{23}H_{27}ClN_2O_3$: C, 66.57; H, 6.57; N, 6.75. Found: C, 66.21; H, 6.73; N, 6.64.

5.1.51. (*RS*)-4-[*N*-[2-[Bis(4-fluorophenyl)methoxy]ethyl]-*N*-methylamino]-4,5,6,7-tetrahydrobenzo[*d*]isoxazol-3-ol sodium salt (28b). To the pivaloyloxymethyl protected derivative **27b** (0.6 g, 1.13 mmol) in EtOH (7 mL) was added water (1.4 mL) and NaOH powder (0.7 g, 17.5 mmol). The mixture was stirred overnight. EtOH was evaporated in vacuo and water (25 mL) was added. The precipitated crystalline product was filtered off and washed with water. After drying overnight at 70–80 °C in vacuo 0.35 g (71%) of pure title compound remained. Mp: 178–181 °C. ^1H NMR (250 MHz, DMSO-d_6): δ 7.40 (d, 2H), 7.35 (d, 2H), 7.15 (t, 4H), 5.50 (s, 1H), 3.40 (t, 2H), 3.25 (t, 1H), 2.90–2.60 (m, 2H), 2.35–2.10 (m, 2H), 2.20 (s, 3H), 1.90–1.75 (m, 1H), 1.75–1.60 (m, 1H), 1.60–1.45 (m, 1H), 1.45–1.30 (m, 1H). MS *m/z* (%): 415 (MH+, 4%), 203 (100%), 183 (63%), 138 (42%), 67 (30%). Anal. Calcd for $C_{23}H_{23}F_2N_2NaO_3$, $1/2H_2O$: C, 62.01; H, 5.44; N, 6.29. Found: C, 61.55; H, 5.43; N, 5.93.

5.1.52. (*RS*)-4-[*N*-[2-[Bis(4-chlorophenyl)methoxy]ethyl]-*N*-methylamino]-4,5,6,7-tetrahydrobenzo[*d*]isoxazol-3-ol (28c). Prepared according to the procedure for **28b** from **27c** (0.50 g, 0.89 mmol). Yield: 0.38 g (91%). Mp: 201–203 °C (water/EtOH). ^1H NMR (250 MHz, DMSO-d_6): δ 7.45 (s, 8H), 5.55 (s, 1H), 3.50–3.35 (m, 2H), 3.35 (t, 1H), 2.90–2.65 (m, 2H), 2.20 (s, 3H), 2.35–2.20 (m, 2H), 1.85–1.75 (m, 1H), 1.70–1.60 (m, 1H), 1.60–1.40 (m, 2H). MS *m/z* (%): 447 (MH+); 235 (78%), 165 (57%), 138 (100%), 67 (56%). Anal. Calcd for $C_{23}H_{23}Cl_2N_2NaO_3$: C, 58.85; H, 4.95; N, 5.97. Found: C, 58.46; H, 4.99; N, 5.95.

5.1.53. (*RS*)-4-[*N*-[1,1-Bis(3-methyl-2-thienyl)but-1-en-4-yl]-*N*-methylamino]-4,5,6,7-tetrahydrobenzo[*d*]isoxazol-3-ol hydrochloride (28d). To a solution of **27d** (9.0 g, 17.0 mmol) in dry EtOH (150 mL) was added NaOH (9 g, 225 mmol) on an ice bath. The solution was stirred overnight followed by evaporation. Water (100 mL) and EtOAc (100 mL) was added and adjusting to pH 7 with 4 M HCl (~40 mL) enabled extraction with EtOAc. The organic phase was washed with brine, dried ($MgSO_4$), and evaporated. The residue was dissolved in HCl in EtOH then evaporated. Precipitation from EtOH by adding Et_2O gave **28d** (6.3 g, 82%). Mp: 154–157 °C. ^1H NMR (500 MHz, DMSO-d_6): δ 7.49 (d, 1H), 7.32 (d, 1H), 6.94 (d, 1H), 6.83 (d, 1H), 6.03 (br, 1H), 4.44 (br, 1H), 3.45–3.25 (m, 2H), 2.80–2.2.55 (m, 4H), 2.78 (br, 1H), 2.52 (s, 3H), 2.15–2.05 (m, 2H), 2.03 (s, 3H), 1.98 (s, 3H), 2.0–1.9 (m, 1H), 1.8–1.7 (m, 1H). Anal. Calcd for $C_{22}H_{27}ClN_2O_2S_2$: C, 58.58; H, 6.03; N, 6.21. Found: C, 58.16; H, 6.10; N, 6.08.

5.1.54. (*R*)-(−)-4-[*N*-[1,1-Bis(3-methyl-2-thienyl)but-1-en-4-yl]-*N*-methylamino]-4,5,6,7-tetrahydrobenzo[*d*]isoxazol-3-ol hydrochloride [(*R*)-28d]. Prepared according to the procedure for **28d** from (*R*)-**27d** (1.54 g, 2.9 mmol). Yield: 0.88 g (67%). Mp: 160–162 °C. Ee = 98.5% (HPLC), $[\alpha]_D^{20}$ −16.5 (*c* 1.12, $CHCl_3$). Anal. Calcd for $C_{22}H_{27}ClN_2O_2S_2$: C, 58.58; H, 6.03; N, 6.21. Found: C, 58.09; H, 6.14; N, 6.15.

5.1.55. (*S*)-(+)-4-[*N*-[1,1-Bis(3-methyl-2-thienyl)but-1-en-4-yl]-*N*-methylamino]-3-hydroxy-4,5,6,7-tetrahydro-1,2-benzo[*d*]isoxazole hydrochloride [(*S*)-28d]. Prepared according to the procedure for **28d** from (*S*)-**27d** (1.75 g, 3.3 mmol). Yield: 0.94 g (63%). Mp: 161–163 °C. Ee = 99.1% (HPLC), $[\alpha]_D^{20}$ 16.7 (*c* 1.02, $CHCl_3$). Anal. Calcd for $C_{22}H_{27}ClN_2O_2S_2$: C, 58.58; H, 6.03; N, 6.21. Found: C, 58.34; H, 5.96; N, 6.07.

5.1.56. 1,1-Bis(3-methyl-2-thienyl)but-1-en-4-yl methanesulfonate (30). Magnesium (14.0 g, 0.58 mol) was suspended in THF (10 mL). 2-Bromo-3-methylthiophen (100 g, 0.57 mol) in THF (250 mL) was added slowly to maintain gentle reflux. The reaction was heated under reflux for another 15 min. The reaction was cooled to 0 °C and 4-butyrolactone (21.5 g, 0.25 mol) in THF (50 mL) was added dropwise. The reaction was stirred another 30 min at rt upon addition. The reaction was poured on NH_4Cl (100 g) in H_2O (600 mL). The phases were separated and the aqueous phase extracted with THF. The combined organic phases were washed with brine, dried ($MgSO_4$), and evaporated. Recrystallization in THF/*n*-heptane gave the title compound (50.2 g, 63%). Mp: 128–130 °C. ^1H NMR (500 MHz, $CDCl_3$): δ 7.10 (d, 2H), 6.77 (d, 2H), 3.72 (broad s, 1H), 3.66 (t, 2H), 3.38 (broad s, 1H), 2.54 (t, 2H), 1.90 (s, 6H), 1.69 (p, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 142.7, 133.6, 131.7, 121.7, 75.2, 62.9, 39.1, 26.9, 14.4. To a solution of 1,1-bis(3-methyl-2-thienyl)butane-1,4-diol (14.3 g, 50.6 mmol) in MeOH was added 4 M HCl (10 mL) and refluxed for 3 h. Saturated aqueous K_2CO_3 was carefully added. The solution was then extracted with Et_2O. The combined organic phases were washed with brine, dried ($MgSO_4$), and evaporated giving 4,4-bis(3-methyl-2-thienyl)but-3-en-1-ol (13.1 g, 98%) as an oil, which was used without further purification. ^1H NMR (500 MHz, $CDCl_3$): δ 7.20 (d, 1H), 7.04 (d, 1H), 6.84 (d, 1H), 6.75 (d, 1H), 6.10 (t, 1H), 3.68 (t, 2H), 2.39 (q, 2H), 2.04 (s, 3H), 1.99 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 139.2, 135.2, 134.9, 133.5, 131.1, 131.0, 129.4, 124.2, 122.6, 62.0, 33.2, 14.6, 14.2. To a solution of 4,4-bis(3-methyl-2-thienyl)but-3-en-1-ol (13.1 g, 48.4 mmol) in Et_2O (150 mL) was added $MeSO_2Cl$ (5 mL, 7.4 g, 64.6 mmol) and Et_3N (9 mL, 6.55 g, 64.7 mmol). The reaction was stirred for 1 h. The resulting suspension was filtered and the precipitate was washed with Et_2O and the filtrate was evaporated giving 1,1-bis(3-methyl-2-thienyl)but-1-en-4-yl methanesulfonate (17.0 g, 100%) as an oil, which was used without further purification. ^1H NMR (500 MHz, $CDCl_3$): δ 7.22 (d, 1H), 7.06 (d, 1H), 6.86 (d, 1H), 6.77 (d, 1H), 6.03 (t, 1H), 4.28 (t, 2H), 2.97 (s, 3H), 2.58 (q, 2H), 2.04 (s, 3H), 2.00 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 138.6, 135.5, 134.2, 134.0, 131.1, 130.9, 129.6, 128.0, 124.6, 123.1, 68.5, 37.3, 29.6 14.6, 14.1.

5.1.57. 4,4-Di(2-toluyl)-1-butyl iodide (32). 2,2-Di(2-toluyl)tetrahydrofuran (28 g, 0.11 mol) was dissolved in HOAc (250 mL). 5% Palladium on activated charcoal (3 g) was added and the mixture was hydrogenated in a Parr apparatus at 3 atm at 55 °C for 5 h. The catalyst was filtered off and the solvent was evaporated in vacuo. The remaining oil was subjected to CC (*n*-heptane/EtOAc 15:1) to give 4,4-di(2-toluyl)-1-butanol (19 g). A solution of 4,4-di(2-toluyl)-1-butanol (19 g) in HOAc (400 mL) was heated under reflux for 3 h. The cooled solution was evaporated in vacuo to give 4,4-di(2-toluyl)-1-butyl acetate (17 g) as an oil. A solution of 4,4-di(2-toluyl)-1-butyl acetate (9.2 g) in 57% aqueous iodic acid (150 mL) was heated under reflux for 3 h. The cooled solution was poured into a mixture of ice and water and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with water and brine, dried (Na_2SO_4), and evaporated in vacuo to give the crude title compound (11.7 g) as an oil, which was used without further purification. ^{1}H NMR ($CDCl_3$): δ 7.12 (s, 8H), 4.26 (t, 1H), 3.17 (t, 2H), 2.28 (s, 6H), 1.80–2.10 (m, 4H).

5.1.58. 4,4-Di(2-toluyl)-3-butenyl iodide (33). A solution of 2,2-di(2-toluyl)tetrahydrofuran (40 g) in 57% aqueous iodic acid (250 mL) was heated under reflux for 30 min. The cooled solution was extracted with Et_2O. The combined organic phases were washed with water and brine, dried (Na_2SO_4), and evaporated in vacuo. The residue was subjected to CC (*n*-heptane/EtOAc 15:l) to give the title compound as an oil (44 g). ^{1}H NMR ($CDCl_3$): δ 7.22–7.05 (m, 8H), 5.73 (t, 1H), 3.19 (t, 2H), 2.65 (q, 2H), 2.30 (s, 3H), 2.10 (s, 3H).

5.1.59. 2-(Diphenylmethoxy)ethyl methanesulfonate (35). Prepared according to literature procedure[42] from benzhydrol (26 g, 0.14 mol), ethyl bromoacetate (30 g, 0.14 mol) yielding 9.0 g of 2-(diphenylmethoxy)ethanol (0.039 mol, 28%). 4.0 g (0.018 mol) of this was mesylated in continuation of the procedure giving the title compound (5.1 g, 98%). ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.40–7.20 (m, 10H), 5.45 (s, 1H), 4.45 (t, 2H), 3.75 (t, 2H), 3.00 (s, 3H).

5.1.60. 2-[Bis(4-fluorophenyl)methoxy]ethyl methanesulfonate (36). Prepared according to literature procedure[42] from 4,4′-difluorobenzhydrol (6 g, 29 mmol) and ethyl bromoacetate (6 g, 36 mmol). Overall yield: 4.2 g (42%). ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.35 (d, 2H), 7.30 (d, 2H), 7.05 (t, 4H), 5.40 (s, 1H), 4.40 (t, 2H), 3.70 (t, 2H), 3.00 (s, 3H).

5.2. In vitro GABA uptake assays

Determination of the inhibition of GABA uptake into crude synaptosomes, prepared from adult rat brain, were performed using the experimental conditions previously described.[10] The effects on neuronal and glial GABA uptake using neurons and astrocytes cultured from cerebral cortices of 15-day-old mouse embryos and new-born mice, respectively, and the effects on GABA uptake into HEK cells transfected with cloned mouse GABA transporter subtypes (GAT1-4) were determined as described previously.[56] Determinations are means of 2–3 four-fold experiments and variations are less than 15%.

5.3. Determination of anticonvulsant effects

The anticonvulsant effects of the compounds were determined by subcutaneous injection into isoniazid-treated mice using the experimental conditions described previously.[39]

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