DOI: 10.1002/ejoc.200600628

Synthesis of 3-Hydroxy- and 3-Carboxy-Δ^2-isoxazoline Amino Acids and Evaluation of Their Interaction with GABA Receptors and Transporters

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Keywords: Neurotransmitters / Amino acids / Δ^2-Isoxazoline amino acids / GABA receptors and transporters / Binding affinity

A series of 3-hydroxy- and 3-carboxy-Δ^2-isoxazoline amino acids, structurally related to the $GABA_A$ agonist THIP and to the GABA uptake inhibitors THPO and *exo*-THPO, was prepared by means of synthetic strategies involving the 1,3-dipolar cycloaddition of nitrile oxides. All derivatives were submitted to a pharmacological investigation at both GABA receptors and transporters. Unfortunately, all amino acids were devoid of activity except for a very low affinity at $GABA_A$ receptors, displayed by compounds **8** and **17**.

Introduction

γ-Aminobutyric acid (GABA, **1**, Figure 1) is the main inhibitory neurotransmitter in the central nervous system. A decrease in GABAergic neurotransmission seems to be involved in neurological pathologies such as epilepsy,[1,2] anxiety[3] and pain.[4] GABA interacts with three classes of receptors: the ionotropic $GABA_A$ and $GABA_C$ receptors, which are ligand-gated chloride-ion channels and cause inhibition of neuronal firing, and the metabotropic $GABA_B$ receptors, which are G-protein-coupled receptors.[5–7] GABA is removed from the synaptic cleft by high affinity sodium-dependent GABA transporters located both in neurons and in glial cells.

The approaches used to modulate GABAergic transmission include direct agonism at the GABA receptors or inhibition of GABA reuptake into neuronal and glial cell bodies. Among GABA uptake inhibitors, particularly relevant from a therapeutic perspective are those showing selectivity for the glial transporters vs. the neuronal transporters since they increase the amount of GABA taken up by neuronal carriers, consequently, they enhance the release of GABA into the synaptic cleft. Selective $GABA_A$ receptor ligands can be obtained by conformational rigidification of the endogenous neurotransmitter. A typical example is represented by muscimol[8] (**2**, Figure 1), a natural compound found in different species of mushrooms, in which the propanoic acid moiety is bioisosterically replaced by the 3-hydroxyisoxazole group. Besides its good activity as a GABA agonist, muscimol also interacts with the GABA uptake system. A further rigidification of muscimol led to the bicyclic derivative THIP[9] (**3**, Figure 1), which also provides a noticeable $GABA_A$ agonist activity but, at variance with muscimol, is devoid of any activity at GABA transporters. THIP (Gaboxadol) is currently in phase III clinical trials as a hypnotic drug.[10] While THIP behaves as a highly selective $GABA_A$ receptor agonist with functional selectiv-

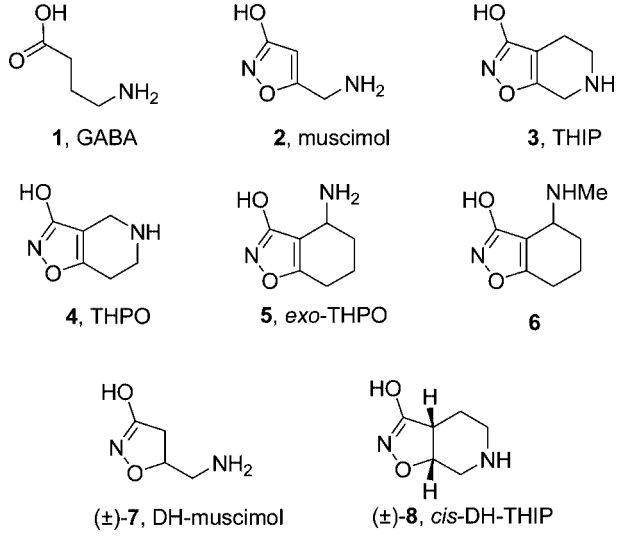

Figure 1. Model compounds.

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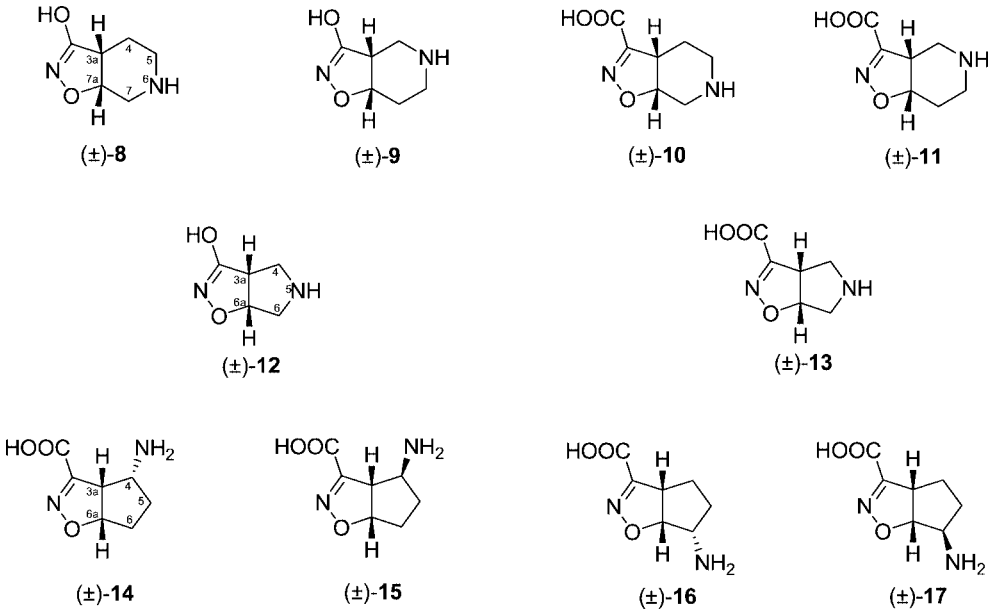


Figure 2. Target compounds.

ity for δ-containing extrasynaptic receptors,[11] its regioisomer 4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridine-3-ol (THPO,[12] **4**, Figure 1) turned out to be a GABA uptake inhibitor, essentially devoid of any activity at GABA receptors. Due to its unexpected selectivity profile, THPO has been used as a model compound for the design of new GABA uptake inhibitors selective for glial transporters (e.g. *exo*-THPO **5** and its *N*-methyl analogue **6**, Figure 1).[13]

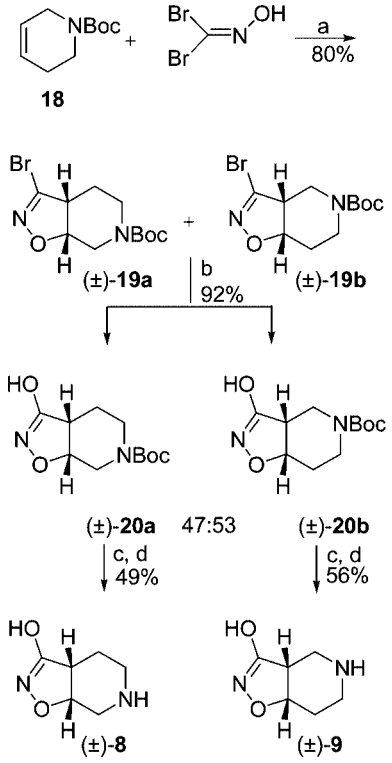
In order to study the structure-activity relationships of THIP, some authors prepared and tested the corresponding isoxazoline derivative **8** (*cis*-DH-THIP, Figure 1).[14] The bioisosteric replacement of the 3-hydroxyisoxazole moiety-with the 3-hydroxyisoxazoline ring was successfully applied to muscimol, in fact, its 4,5-dihydro derivative [(*R*,*S*)-4,5-dihydromuscimol (**7**), Figure 1] turned out to be almost equipotent with the lead compound.[15] Interestingly, while the (*S*)-(+) isomer mainly activated the $GABA_A$ receptors, the inhibition of GABA transporters was exclusively due to the (*R*)-(–) isomer,[15] indicating the influence of the stereochemistry on the pharmacological profile.

The synthetic strategy designed to prepare amino acid **8** yielded also its regioisomer **9**, which is structurally related to THPO (Figure 2).[14] However, due to the low efficiency of the applied synthetic approach, the authors were able to isolate only small amounts of derivative **8**, whereas **9** was not obtained in a pure form and, consequently, its pharmacological activity and selectivity were not evaluated. Since the pharmacological profile of isoxazoline amino acid **9** was not defined and, furthermore, the activity of its regioisomer **8** at GABA transporters was not determined, we devised a new efficient preparation of both amino acids **8** and **9**. As a further development of this research, we also designed, prepared and tested a group of analogues and homologues of amino acids **8** and **9**. In particular, amino acids **10** and **11** (Figure 2) are higher homologues of **8** and **9**, and derivative **11** could also be considered a conformationally constrained GABA-mimetic. Similarly, whereas amino acid **12** has been designed as the lower homologue of both **8** and **9**, derivative **13** can be seen either as a higher homologue of **12** or as another constrained conformer of GABA. Finally, amino acids **14** and **15** were devised as GABA-mimetics, in which the embedded conformations of the amino acid chain were different from that of derivatives **11** and **13**. On the other hand, amino acids **16** and **17** represent new GABA homologues (Figure 2). The new amino acids **8**–**17** were submitted to binding assays at both $GABA_A$ and $GABA_B$ receptors and GABA transporters.

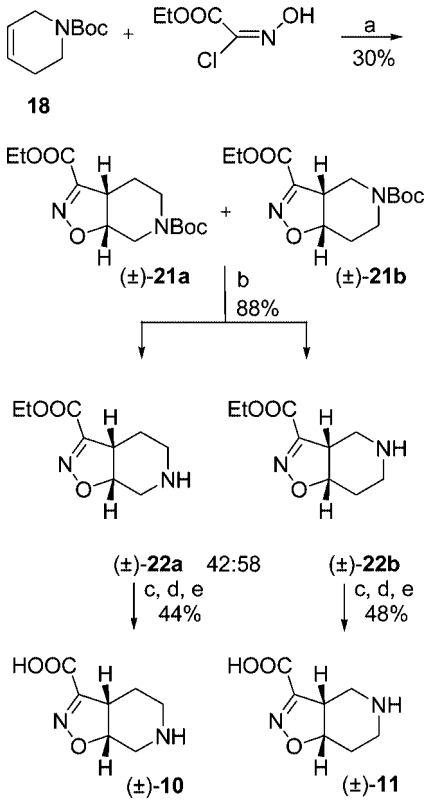
Results and Discussion

The key step of the synthetic pathway used to prepare amino acids **8** and **9** was the 1,3-dipolar cycloaddition of bromonitrile oxide,[16] generated in situ by the treatment of dibromoformaldoxime with solid $NaHCO_3$, to 1-(*tert*-butoxycarbonyl)-1,2,3,6-tetrahydropyridine **18**, in turn obtained from commercially available 1,2,3,6-tetrahydropyridine following a literature procedure.[14] The cycloaddition step (Scheme 1) gave rise to an inseparable mixture of the two regioisomers **19a** and **19b** in an almost equimolar ratio. The subsequent substitution of the 3-bromo group with the 3-hydroxy moiety, achieved with 1 N aqueous NaOH in dioxane at 80 °C, afforded derivatives **20a** and **20b**. These intermediates could be separated by silica gel column chromatography and were then converted into final amino acids **8** and **9** through a standard removal of the *N*-*tert*-butoxy group. Starting from dipolarophile **18**, the synthesis described above allowed us to obtain both isomeric amino acids **8** and **9** in reasonable overall yields of 17% and 22%, respectively. The present synthesis constitutes a significant improvement over the previously reported protocol,[14] which produced the two regioisomers in less than 1% over-

all yield. The structural assignments of intermediate and target derivatives are based on ^{1}H NMR and COSY experiments.



Scheme 1. a: $NaHCO_3$, AcOEt, 3 d, b: 1 N NaOH, dioxane, 80 °C, c: 30% TFA/CH_2Cl_2, d: Amberlite IR-120 (H^+ form), 10% py/H_2O.

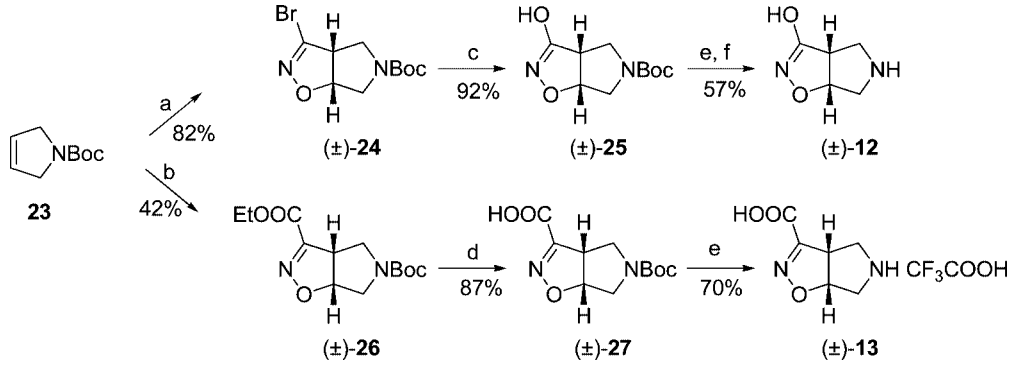


Scheme 2. a: $NaHCO_3$, AcOEt, Δ, 7 d, b: 30% TFA/CH_2Cl_2, c: 1 N NaOH, EtOH, d: 2 N HCl , e: Amberlite IR-120 (H^+ form), 10% py/H_2O.

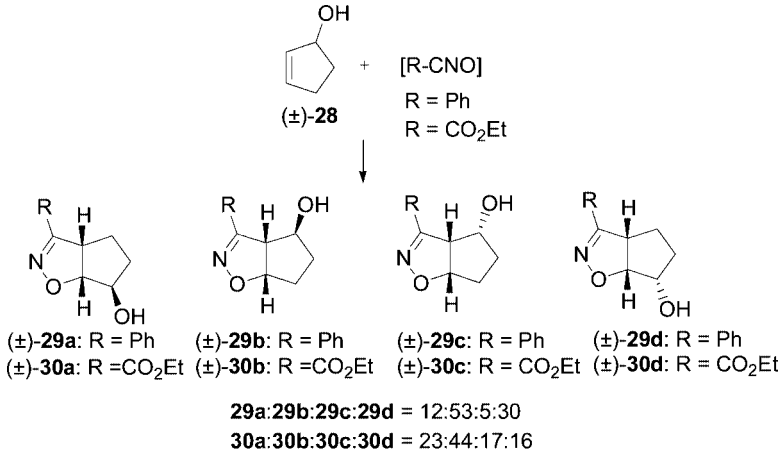
Amino acids **10** and **11** (Scheme 2) were in turn prepared with the same dipolarophile **18** and ethyl 2-chloro-2-(hydroxyimino)acetate[17] as the precursor of the desired 1,3-dipole. Since, in this case, the cycloaddition proceeded very slowly at room temperature, the reaction was carried out in refluxing AcOEt. As in the previous case, cycloadducts **21a** and **21b** were collected as an inseparable mixture in an almost equimolar ratio. The chromatographic separation of the two regioisomers was made possible after removal of the *N*-Boc group at the stage of amino esters **22a** and **22b**. These intermediates were then converted into amino acids **10** and **11** by alkaline hydrolysis of the ester group, followed by acidification and purification by cation-exchange column chromatography. Also, in this case, structural assignments of the regioisomers are based on ^{1}H NMR and COSY experiments.

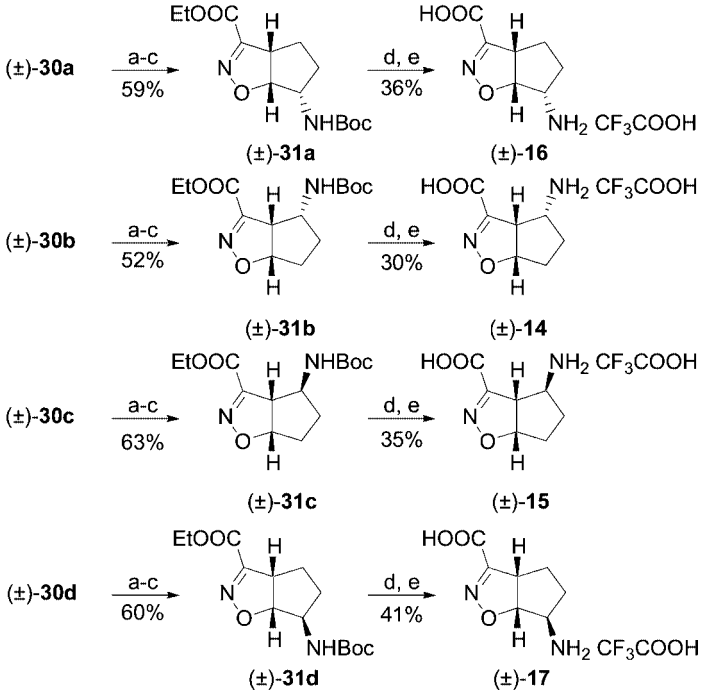
Similarly, amino acids **12** and **13** were prepared with the strategies described above with 1-(*tert*-butoxycarbonyl)-3-pyrroline[18] as the dipolarophile (Scheme 3). The final compound **12** was purified as its zwitterion by ion-exchange column chromatography with Amberlite IR-120 (H^+ form), and 10% aqueous pyridine as the eluent. Conversely, its homologue **13** was isolated as the corresponding crystalline trifluoroacetate.

To prepare amino acids **14**–**17**, we took into account the cycloaddition of ethoxycarbonylformonitrile oxide to *N*-Boc-2-cyclopentenylamine. However, as reported by Curran et al.,[19] the cycloaddition of nitrile oxides to allylic secondary amides proceeds with a high degree of regio- and stereocontrol, leading to the *syn* isomer as the major product (70–90%) with the amide group facing the oxygen of the heterocyclic ring. In most of the reported examples, this stereoisomer was contaminated by marginal amounts of the two *anti* isomers. Similarly, we observed a high degree of stereocontrol when an allylic carbamate (protected as NHBoc) was employed as the dipolarophile in 1,3-dipolar cycloaddition with nitrile oxides.[20] Since we were interested in a strategy able to generate all four stereoisomers in comparable amounts, we employed cyclopenten-1-ol as the dipolarophile. As described by Caramella et al.,[21] benzonitrile oxide (BNO) reacts with cyclopenten-1-ol **28** to yield all four possible stereoisomers **29a**, **29b**, **29c** and **29d** in a 12:53:5:30 ratio (Scheme 4). The ratio among the isomers is the result of a subtle balance of polar, steric and hydrogen-bonding effects. In our hands, the outcome of the cycloaddition of ethoxycarbonylformonitrile oxide to cyclopenten-1-ol turned out to be even more advantageous in terms of stereoisomer distribution, since we isolated the four cycloadducts **30a**–**d** in a relative ratio of 23:44:17:16 (Scheme 4). The structural assignments of the four stereoisomers are based on ^{1}H NMR and COSY experiments. For the 4-substituted derivatives, when H^{3a} and H^4 are in a *trans* relative stereochemistry (e.g. compound **30b**), H^{3a} resonates as a doublet (J = 9.2 Hz), whereas in the corre-



Scheme 3. a: $Br_2CN{=}OH$, $NaHCO_3$, AcOEt, 3 d, b: $EtOOCC(Cl){=}NOH$, $NaHCO_3$, AcOEt, 5 d, c: 1 N NaOH, dioxane, 80 °C, d: 1 N NaOH, EtOH, e: 30% TFA/CH_2Cl_2, f: Amberlite IR-120 (H^+ form), 10% py/H_2O.





Scheme 4. a: $MeSO_2Cl$, TEA, CH_2Cl_2, b: NaN_3, DMSO, 80 °C, c: H_2-Pd/C, Boc_2O, EtOH, d: 20% K_2CO_3 (aq.), EtOH, e: 30% TFA/CH_2Cl_2.

sponding *cis* stereoisomer (e.g. **30c**), H^{3a} appears as a doublet of doublets (J = 8.4 and 8.4 Hz). Analogously, in the case of the 6-substituted derivatives, H^{6a} is a doublet in **30a** (J = 8.8 Hz) and a doublet of doublets in **30d** (J = 5.1 and 9.2 Hz). The four isomers were separated by flash chromatography and then transformed into the desired amino acids following the reaction sequence reported in Scheme 4. After activation of the secondary alcohol as the mesylate, each derivative was submitted to nucleophilic substitution with sodium azide, which proceeded with complete inversion of configuration. The intermediate azides were not characterized but directly submitted to catalytic hydrogenation in the presence of di-*tert*-butyl dicarbonate to give the *N*-Boc-protected compounds **31a**–**d**. As reported for the corresponding hydroxy derivatives, the relative stereochemistry was assigned by means of ^{1}H NMR and COSY experiments. Alkaline hydrolysis of their ester group followed by deprotection of the amino group with trifluoroacetic acid afforded the final amino acids **14**–**17** as trifluoroacetates.

All new compounds **8**–**17** were pharmacologically characterized in receptor binding studies on rat brain membrane preparations. The affinities for $GABA_A$ and $GABA_B$ receptor sites, using [^{3}H]muscimol and [^{3}H]GABA, respectively, were determined with previously described procedures.[22] All the compounds under study displayed K_i and IC_{50} values higher than 100 μM except for amino acids **8** (K_i = 14 μM at $GABA_A$ receptors and IC_{50} = 86 μM at $GABA_B$ receptors) and **17** (K_i = 38 μM at $GABA_A$ receptors). Furthermore, derivatives **8**–**17** were unable to inhibit [^{3}H]GABA uptake when assayed at 1 mM concentration in rat brain synaptosomes, with GABA (1 mM) and Tiagabine (10 μM) as controls. Finally, the amino acids were tested as modulators of spontaneous activity in the rat cortical wedge preparation. All derivatives were assayed at 30 μM alone or in combination with a concentration of GABA capable of eliciting a 20% response. All the compounds were inactive either as direct activators or modulators of GABA responses.

To account for the pharmacological data reported above, we carried out a conformational analysis on selected derivatives **8**, **9** and **11**–**15**, which share the same N–C$^\omega$ through-bond path (where N is the nitrogen of the amino group, and C$^\omega$ corresponds either to the C^3 of the 3-hydroxyisoxazoline (or 3-hydroxyisoxazole) moiety or the carbon of the carboxylate group) with reference compounds **2**–**5** and **7**. Theoretical calculations were performed at the B3LYP/6-31G(d) level, as implemented in GAUSSIAN03.[23] All the compounds showed two populated conformations, tagged as **A** and **B** (Table 1), which were analyzed by examining the inter-atomic distance among the two ionisable centres, N and C$^\omega$.

Inspection of the parameters gathered in Table 1 reveals that amino acid **8**, the only derivative with a detectable binding to GABA receptors, can be compared, in terms of N–C$^\omega$ distance, to the model compound **7**. Consequently, since (*S*)-**7** is the enantiomer of dihydromuscimol acting as a competitive $GABA_A$ agonist (K_i = 0.004 μM),[15] we superimposed its most populated conformer onto the **A** and **B** conformations of (*S*,*S*)-**8**. As reported in Figure 3 (part A), the isoxazoline rings and the amino groups of the two compounds are properly fitted. Therefore, the drop in affinity of **8** compared to **7** might be attributed to a steric repulsion between C^4 and the complementary receptor site. Quite similarly, the low affinity observed for 4-methylmuscimol (IC_{50} > 100 μM)[24] in comparison with muscimol had been mainly attributed to a steric bump of the methyl group located in position 4. Indeed, the C^4 of **8** is located in the space occupied by the 4-methyl group of 4-methylmuscimol, as clearly shown by superimposing their populated conformations (Figure 3, B). As far as the activity at GABA transporters is considered, even though the N–C$^\omega$ distance measured for derivatives **9** and **12** falls in the same range of that of reference compounds **4** and **5**, respectively (Table 1), the two compounds were inactive as GABA uptake inhibitors.

Table 1. Molecular parameters describing the pharmacophoric distance N–C$^\omega$ of reference (**2**–**5** and **7**) and investigated (**8**, **9** and **11**–**15**) compounds.

Compounds[a]	ΔE (kcal/mol)[b]	N–C$^\omega$ distance [Å][c]
2A	0.00	4.52
2B	0.74	4.31
3A	0.00	4.20
3B	1.14	4.12
4A	0.00	3.86
4B	0.96	3.80
5A	0.00	3.34
5B	0.30	3.15
7A	0.00	3.75
7B	1.36	4.29
8A	0.00	3.77
8B	0.18	3.98
9A	0.00	3.73
9B	0.30	3.31
11A	0.00	4.80
11B	0.98	4.75
12A	0.00	3.04
12B	1.90	3.54
13A	0.00	4.08
13B	2.06	4.65
14A	0.00	3.07
14B	1.73	3.16
15A	0.00	4.49
15B	0.98	3.76

[a] **A** corresponds to the most favoured conformation at room temperature whereas **B** is the second most favoured populated conformer. [b] ΔE is the difference in energy between the two optimized conformations, calculated in a polarizable conductor-like solvation model (C-PMC/H_2O). [c] See text for the definition of the N–C$^\omega$ distance.

In summary, we developed a new efficient strategy to synthesize amino acids **8** and **9**, which are structurally related to THIP **3** and THPO **4**, respectively. Furthermore, a group of conformationally rigidified amino acids was prepared, in which the length of the amino acidic chain was modulated to check how critical such a parameter is for biological activity. Our results give evidence that the distance between the two pharmacophoric groups alone is inadequate to rationalize the interaction with both the GABA uptake system and the GABA receptor sites. Crystal structures of suitable ligands inside the transporter or receptor

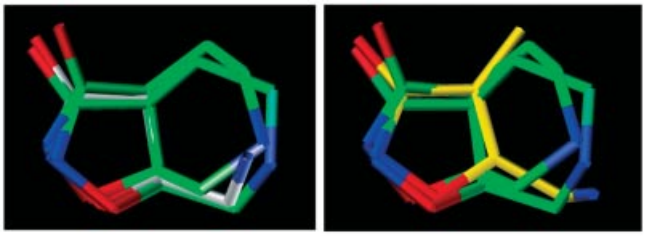


Figure 3. (**A**) Superimposition of (*S*,*S*)-**8A**, (*S*,*S*)-**8B** and (*S*)-**7A**. (**B**) Superimposition of (*S*,*S*)-**8A**, (*S*,*S*)-**8B** and 4-methylmuscimol. Carbon atoms of conformers **8A** and **8B** are depicted in green whereas those of (*S*)-**7** are in white-grey and those of 4-methylmuscimol are in yellow. Pictures were acquired by SYBYL7.2 (Tripos Inc., 1699 South Hanley Rd., St. Louis, Missouri, 63144, USA).

cavities would shed light on the relevant interactions at the molecular level and promote the rational design of novel targeted compounds and are, therefore, highly desirable.

Experimental Section

Material and Methods: ^{1}H NMR spectra were recorded with a Varian 300 (300 MHz) spectrometer at 20 °C. Chemical shifts (δ) are expressed in ppm and coupling constants (J) in Hertz. HPLC analyses were performed with a Jasco PU-980 pump, equipped with a Jasco UV-975 UV/Vis detector and a Lichrosphere Si 60 (Merck) column. TLC analyses were performed with commercial silica gel 60 F_{254} aluminium sheets, and spots were further evidenced by spraying with a dilute alkaline potassium permanganate solution or with ninhydrin. Melting points were determined with a Büchi apparatus and are uncorrected. All reagents were purchased from Aldrich. 1-(*tert*-Butoxycarbonyl)-1,2,3,6-tetrahydropyridine **18**,[14] 1-(*tert*-butoxycarbonyl)-3-pyrroline **23**,[18] (±)-cyclopent-2-en-1-ol (±)-**28**,[25] dibromoformaldoxime[16] and ethyl 2-chloro-2-(hydroxyimino)acetate[17] were prepared according to literature procedures.

***tert*-Butyl (±)-3-Hydroxy-4,5,7,7a-tetrahydroisoxazolo[5,4-*c*]pyridine-6(3a*H*)-carboxylate [(±)-20a] and *tert*-Butyl (±)-3-Hydroxy-3a,6,7,7a-tetrahydroisoxazolo[4,5-*c*]pyridine-5(4*H*)-carboxylate [(±)-20b]:** To a solution of 1-(*tert*-butoxycarbonyl)-1,2,3,6-tetrahydropyridine (**18**) (1.5 g, 8.2 mmol) in AcOEt (30 mL) was added dibromoformaldoxime (3.3 g, 16.4 mmol) and solid $NaHCO_3$ (7 g). The mixture was vigorously stirred for 3 d. The progress of the reaction was monitored by TLC (petroleum ether/AcOEt, 4:1). H_2O (15 mL) was added to the reaction mixture, and the organic layer was separated. The aqueous phase was further extracted with AcOEt (10 mL), and the combined organic layers were dried with anhydrous Na_2SO_4. The crude material, obtained after evaporation of the solvent, was purified by column chromatography (silica gel, petroleum ether/AcOEt, 4:1) to give 2.0 g (80% yield) of an inseparable mixture of cycloadducts (±)-**19a** and **b**, which were directly submitted to the next step.

The mixture of (±)-**19a** and **b** (2.0 g, 6.55 mmol) was dissolved in dioxane (16 mL), and NaOH (1 N, 33 mL) was added. The reaction was stirred and heated at 80 °C for 24 h. The progress of the reaction was monitored by TLC (petroleum ether/AcOEt, 7:3). The aqueous phase was made acidic with 2 N HCl and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic extracts were dried with anhydrous Na_2SO_4 and concentrated under reduced pressure. The two regioisomers were separated by column chromatography (silica gel, petroleum ether/AcOEt, 3:7) to give 0.688 g (43% yield) of (±)-**20a** and 0.776 g (49% yield) of (±)-**20b**.

(±)-20a: Colourless oil. R_f (petroleum ether/AcOEt, 3:7) = 0.22. ^{1}H NMR (300 MHz, [D$_6$]DMSO, 100 °C): δ = 1.42 (s, 9 H), 1.66–1.90 (m, 2 H), 2.86 (ddd, J = 7.5, 7.5, 7.5 Hz, 1 H), 3.16–3.34 (m, 2 H), 3.44 (dd, J = 4.4, 14.5 Hz, 1 H), 3.61 (dd, J = 4.4, 14.5 Hz, 1 H), 4.56 (ddd, J = 4.4, 4.4, 7.5 Hz, 1 H), 10.90 (br. s, 1 H). $C_{11}H_{18}N_2O_4$ (242.27): calcd. C 54.53, H 7.49, N 11.56; found C 54.52, H 7.53, N 11.22.

(±)-20b: Colourless oil. R_f (petroleum ether/AcOEt, 3:7) = 0.35. ^{1}H NMR (300 MHz, [D$_6$]DMSO, 100 °C): δ = 1.42 (s, 9 H), 1.68–1.92 (m, 2 H), 2.85 (ddd, J = 5.8, 5.8, 7.7 Hz, 1 H), 3.18 (ddd, J = 4.8, 6.6, 13.2 Hz, 1 H), 3.36–3.48 (m, 2 H), 3.68 (dd, J = 5.8, 13.5 Hz, 1 H), 4.72 (ddd, J = 5.5, 5.5, 7.7 Hz, 1 H), 11.21 (br. s, 1 H). $C_{11}H_{18}N_2O_4$ (242.27): calcd. C 54.53, H 7.49, N 11.56; found C 54.88, H 7.76, N 11.25.

(±)-3a,4,5,6,7,7a-Hexahydroisoxazolo[5,4-*c*]pyridin-3-ol [(±)-8]: Compound (±)-**20a** (0.688 g, 2.84 mmol) was treated with CF_3COOH (30% CH_2Cl_2 solution, 7.2 mL, 28.4 mmol) at 0 °C. The solution was stirred at room temp. for 3 h. The volatiles were removed under vacuum, and the residue was dissolved in H_2O and purified by cation-exchange column chromatography with Amberlite IR-120 (H^+ form). The aqueous solution was slowly eluted onto the resin, and the column was washed with H_2O until the pH was neutral. The compound was then eluted off the resin with 10% aqueous pyridine, and the product-containing fractions (detected with ninhydrin on a TLC plate) were combined and concentrated under vacuum. The residue was taken up with ethanol, filtered, washed sequentially with ethanol and Et_2O and dried in vacuo at 50 °C to give amino acid (±)-**8** (0.198 g, 49% yield). White prisms, m.p. dec. >197 °C, R_f (*n*BuOH/H$_2$O/CH$_3$COOH, 4:2:1) = 0.37. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 1.52–1.70 (m, 2 H), 2.38–2.58 (m, 2 H), 2.65 (dd, J = 6.2, 13.5 Hz, 1 H), 2.72–2.81 (m, 1 H), 2.88 (dd, J = 5.1, 13.5 Hz, 1 H), 3.30 (br. s, 2 H), 4.31–4.40 (m, 1 H). $C_6H_{10}N_2O_2$ (142.16): calcd. C 50.69, H 7.09, N 19.71; found C 50.76, H 7.32, N 19.49.

(±)-3a,4,5,6,7,7a-Hexahydroisoxazolo[4,5-*c*]pyridin-3-ol [(±)-9]: Compound (±)-**20b** (0.776 g, 3.21 mmol) was treated as described for (±)-**20a** to give amino acid (±)-**9** (0.255 g, 56% yield). White prisms, m.p. dec. 177–179 °C, R_f (*n*BuOH/H$_2$O/CH$_3$COOH, 4:2:1) = 0.42. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 1.52–1.63 (m, 1 H), 1.66–1.77 (m, 1 H), 2.45 (ddd, J = 4.0, 7.7, 12.8 Hz, 1 H), 2.59 (ddd, J = 6.2, 6.2, 6.2 Hz, 1 H), 2.65 (ddd, J = 4.0, 7.3, 12.8 Hz, 1 H), 2.80 (m, 2 H), 3.32 (br. s, 2 H), 4.58 (ddd, J = 6.2, 6.2, 6.2 Hz, 1 H). $C_6H_{10}N_2O_2$ (142.16): calcd. C 50.69, H 7.09, N 19.71; found C 50.39, H 7.15, N 19.51.

Ethyl (±)-3a,4,5,6,7,7a-Hexahydroisoxazolo[5,4-*c*]pyridin-3-carboxylate [(±)-22a] and Ethyl (±)-3a,4,5,6,7,7a-Hexahydroisoxazolo[4,5-*c*]pyridin-3-carboxylate [(±)-22b]: To a solution of 1-(*tert*-butoxycarbonyl)-1,2,3,6-tetrahydropyridine **18** (1.5 g, 8.2 mmol) in AcOEt (30 mL) was added ethyl 2-chloro-2-(hydroxyimino)acetate (2.48 g, 16.4 mmol) and solid $NaHCO_3$ (7 g). The mixture was vigorously stirred and refluxed for 7 d. The progress of the reaction was monitored by TLC (petroleum ether/AcOEt, 4:1). H_2O (10 mL) was added to the reaction mixture, and the organic layer was separated. The aqueous phase was further extracted with AcOEt, and the combined organic layers were dried with anhydrous Na_2SO_4. The crude material, obtained after evaporation of the solvent, was purified by column chromatography (silica gel, petroleum ether/AcOEt, 4:1) to give 0.733 g (30% yield) of an inseparable mixture of cy-

cloadducts (±)-**21a**–**b**, which were directly submitted to the next step.

The mixture of (±)-**21a**–**b** (0.733 g, 2.46 mmol) was treated with CF_3COOH (30% CH_2Cl_2 solution , 6.3 mL, 24.6 mmol) at 0 °C. The solution was stirred at room temp. for 3 h. The disappearance of the starting material was monitored by TLC (petroleum ether/AcOEt, 7:3). The volatiles were removed under vacuum, the residue was dissolved in 5% aqueous $NaHCO_3$ (60 mL) and the aqueous phase was extracted with AcOEt (3 × 20 mL). The combined organic extracts were dried with anhydrous Na_2SO_4 and concentrated under reduced pressure. The two regioisomers were separated by column chromatography (silica gel, CH_2Cl_2/MeOH, 95:5) to give 0.182 g (37% yield) of (±)-**22a** and 0.250 g (51% yield) of (±)-**22b**.

(±)-22a: White prisms from EtOH/Et_2O, m.p. 124–126 °C, R_f (CH_2Cl_2/MeOH, 95:5) = 0.19. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.31 (t, J = 7.1 Hz, 3 H), 1.94–2.12 (m, 2 H), 2.60 (ddd, J = 2.4, 13.2, 13.2 Hz, 1 H), 2.70 (br. s, 1 H), 2.98 (ddd, J = 4.1, 4.1, 13.2 Hz, 1 H), 3.07 (dd, J = 3.2, 15.2 Hz, 1 H), 3.32 (ddd, J = 8.2, 8.2, 8.2 Hz, 1 H), 3.39–3.48 (m, 1 H), 4.24–4.34 (m, 2 H), 4.35–4.42 (m, 1 H). $C_9H_{14}N_2O_3$ (198.22): calcd. C 54.53, H 7.12, N 14.13, found C 54.72, H 7.15, N 13.98.

(±)-22b: Yellow oil. R_f (CH_2Cl_2/MeOH, 95:5) = 0.28. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.36 (t, J = 7.0 Hz, 3 H), 1.92–2.05 (m, 1 H), 2.06–2.16 (m, 1 H), 2.30 (br. s, 1 H), 2.65 (dd, J = 8.4, 11.7 Hz, 1 H), 2.79 (ddd, J = 4.0, 10.6, 12.5 Hz, 1 H), 2.94 (ddd, J = 4.0, 4.0, 12.5 Hz, 1 H), 3.22–3.38 (m, 2 H), 4.26–4.38 (m, 2 H), 4.64–4.72 (m, 1 H). $C_9H_{14}N_2O_3$ (198.22): calcd. C 54.53, H 7.12, N 14.13; found C 54.89, H 7.27, N 13.74.

(±)-3a,4,5,6,7,7a-Hexahydroisoxazolo[5,4-*c*]pyridine-3-carboxylic Acid [(±)-10]: To a solution of (±)-**22a** (0.182 mg, 0.92 mmol) in EtOH (3 mL) was added NaOH (1 N 1.4 mL), and the mixture was stirred at room temp. for 12 h. After removing the solvent under reduced pressure, the solution was made acidic with 2 N HCl and submitted to cation-exchange column chromatography with Amberlite IR-120 (H^+ form) following the procedure described above. The residue was taken up with methanol, filtered, washed sequentially with methanol and Et_2O and dried in vacuo at 50 °C to give amino acid (±)-**10** (0.069 g, 44% yield). White prisms, m.p. dec. 105–110 °C, R_f (*n*BuOH/H_2O/CH_3COOH, 4:2:1) = 0.26. ^{1}H NMR (300 MHz, D_2O): δ = 1.55 (dddd, J = 4.4, 8.4, 9.5, 15.4 Hz, 1 H), 2.05 (dddd, J = 3.7, 6.9, 6.9, 15.4 Hz, 1 H), 2.93 (ddd, J = 3.7, 9.5, 13.2 Hz, 1 H), 3.08 (ddd, J = 4.4, 6.9, 13.2 Hz, 1 H), 3.29 (dd, J = 2.9, 14.7 Hz, 1 H), 3.36–3.45 (m, 1 H), 3.54 (dd, J = 2.2, 14.7 Hz, 1 H), 4.62–4.68 (m, 1 H). $C_7H_{10}N_2O_3$ (170.17): calcd. C 49.41, H 5.92, N 16.46; found C 49.12, H 6.06, N 16.14.

(±)-3a,4,5,6,7,7a-Hexahydroisoxazolo[4,5-*c*]pyridine-3-carboxylic Acid [(±)-11]: Regioisomer (±)-**22b** (0.250 g, 1.26 mmol) was treated as described for (±)-**22a** to give amino acid (±)-**11** (0.102 g, 48% yield). White prisms, m.p. dec. 119–121 °C, R_f (*n*BuOH/H_2O/CH_3COOH, 4:2:1) = 0.30. ^{1}H NMR (300 MHz, D_2O): δ = 2.06–2.14 (m, 2 H), 3.0–3.22 (m, 3 H), 3.37 (dd, J = 6.6, 13.5 Hz, 1 H), 3.60 (ddd, J = 6.6, 8.0, 8.0 Hz, 1 H), 4.75 (ddd, J = 4.0, 4.0, 8.0 Hz, 1 H). $C_7H_{10}N_2O_3$ (170.17): calcd. C 49.41, H 5.92, N 16.46; found C 49.04, H 5.99, N 16.34.

***tert*-Butyl (±)-3-Bromo-3a,4,6,6a-tetrahydro-5*H*-pyrrolo[3,4-*d*]isoxazole-5-carboxylate [(±)-24]:** To a solution of 1-(*tert*-butoxycarbonyl)-3-pyrroline **23** (1 g, 5.9 mmol) in AcOEt (20 mL) was added dibromoformaldoxime (2.4 g, 11.8 mmol) and solid $NaHCO_3$ (5 g). The mixture was vigorously stirred for 3 d. The progress of the reaction was monitored by TLC (petroleum ether/AcOEt, 7:3). H_2O (10 mL) was added to the reaction mixture, and the organic layer was separated. The aqueous phase was further extracted with AcOEt (5 mL), and the combined organic layers were dried with anhydrous Na_2SO_4. The crude material, obtained after evaporation of the solvent, was purified by column chromatography (silica gel, petroleum ether/AcOEt, 7:3) and recrystallization from 2-propanol to give compound (±)-**24** (1.4 g, 82% yield). White prisms, m.p. 50–52 °C, R_f (cyclohexane/AcOEt, 70:30) = 0.35. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.43 (s, 9 H), 3.37 (dd, J = 6.1, 13.5 Hz, 1 H), 3.51 (dd, J = 4.4, 13.5 Hz, 1 H), 3.80–4.10 (m, 3 H), 5.12 (br. dd, J = 5.4, 7.9 Hz, 1 H). $C_{10}H_{15}BrN_2O_3$ (291.14): calcd. C 41.25, H 5.19, N 9.62; found C 41.60, H 5.34, N 9.44.

***tert*-Butyl (±)-3-Hydroxy-3a,4,6,6a-tetrahydro-5*H*-pyrrolo[3,4-*d*]-isoxazole-5-carboxylate [(±)-25]:** To a solution of (±)-**24** (1.4 g, 4.8 mmol) in dioxane (12 mL) was added NaOH (1 N, 24 mL), and the mixture was stirred and heated at 80 °C for 24 h. The aqueous phase was washed twice with Et_2O and then made acidic with 2 N HCl and extracted with AcOEt (4 × 3 mL). The organic phase was dried (Na_2SO_4), the solvent was evaporated under reduced pressure, and the crude material was recrystallized (AcOEt) to give (±)-**25** (1.01 g, 92% yield). White prisms, m.p. 128–130 °C, R_f ($CHCl_3$/MeOH, 9:1 + 1% CF_3COOH) = 0.84. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.43 (s, 9 H), 3.38 (ddd, J = 3.3, 8.4, 8.4 Hz, 1 H), 3.52–79 (m, 3 H), 3.90 (br. d, J = 11.0 Hz, 1 H), 5.19 (ddd, J = 3.3, 6.2, 7.7 Hz, 1 H). $C_{10}H_{16}N_2O_4$ (228.25): calcd. C 52.62, H 7.07, N 12.27; found C 52.69, H 7.14, N 11.82.

(±)-4,5,6,6a-Tetrahydro-3a*H*-pyrrolo[3,4-*d*]isoxazol-3-ol [(±)-12]: Compound (±)-**25** (1.01 g, 4.42 mmol) was treated with CF_3COOH (30% CH_2Cl_2 solution, 11 mL, 44.2 mmol) at 0 °C. The solution was stirred at room temp. for 3 h. The volatiles were removed under vacuum, and the residue was dissolved in H_2O and purified by cation-exchange column chromatography with Amberlite IR-120 (H^+ form), as reported above. The residue was taken up with ethanol, filtered, washed sequentially with ethanol and Et_2O and dried in vacuo at 50 °C to give amino acid (±)-**12** (0.323 g, 57% yield). White prisms, m.p. dec. 180 °C, R_f (*n*BuOH/H_2O/CH_3COOH, 4:2:1) = 0.37. ^{1}H NMR (300 MHz, [D_6]DMSO + 1 drop CF_3COOD): δ = 3.32–3.44 (m, 2 H), 3.48–3.58 (m, 2 H), 3.65 (ddd, J = 1.9, 8.3, 8.3 Hz, 1 H), 5.26 (ddd, J = 1.5, 5.4, 8.3 Hz, 1 H). $C_5H_8N_2O_2$ (128.13): calcd. C 46.87, H 6.29, N 21.86; found C 46.68, H 6.42 N 21.52.

5-*tert*-Butyl 3-Ethyl (±)-3a,4,6,6a-Tetrahydro-5*H*-pyrrolo[3,4-*d*]-isoxazole-3,5-dicarboxylate [(±)-26]: To a solution of 1-(*tert*-butoxycarbonyl)-3-pyrroline **23** (1 g, 5.9 mmol) in AcOEt (20 mL) was added ethyl 2-chloro-2-(hydroxyimino)acetate (1.79 g, 11.8 mmol) and solid $NaHCO_3$ (5 g). The mixture was vigorously stirred for 5 d. The progress of the reaction was monitored by TLC (petroleum ether/AcOEt, 4:1). H_2O (10 mL) was added to the reaction mixture, and the organic layer was separated. The aqueous phase was further extracted with AcOEt (5 mL), and the combined organic layers were dried with anhydrous Na_2SO_4. The crude material, obtained after evaporation of the solvent, was purified by column chromatography (silica gel, petroleum ether/AcOEt, 4:1) to give compound (±)-**26** (0.705 g, 42% yield). Yellow oil, R_f (petroleum ether/AcOEt, 4:1) = 0.18. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.35 (t, J = 7.2 Hz, 3 H), 1.42 (s, 9 H), 3.41–3.54 (m, 2 H), 3.79–4.08 (m, 1 H), 3.80–4.10 (m, 2 H), 4.35 (q, J = 7.2 Hz, 2 H), 5.32 (dd, J = 5.4, 9.6 Hz, 1 H). $C_{13}H_{20}N_2O_5$ (284.31): calcd. C 54.92, H 7.09, N 9.85; found C 54.90, H 7.19, N 9.49.

(±)-5-(*tert*-Butoxycarbonyl)-4,5,6,6a-tetrahydro-3a*H*-pyrrolo[3,4-*d*]-isoxazole-3-carboxylic Acid [(±)-27]: To a solution of (±)-**26** (0.705 g, 2.48 mmol) in EtOH (8 mL) was added NaOH (1 N, 3.7 mL), and the mixture was stirred at room temp. for 3 h. The

volatiles were removed under vacuum, and the aqueous phase was extracted with Et_2O (2 × 3 mL), made acidic with 2 N HCl and extracted with AcOEt (4 × 3 mL). The organic phase was dried (Na_2SO_4), the solvent was evaporated under reduced pressure, and the crude material was recrystallized (AcOEt) to give (±)-**27** (0.55 g, 87% yield). White prisms, m.p. dec. 134–137 °C, R_f ($CHCl_3$/MeOH, 9:1 + 1% CF_3COOH) = 0.34. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.46 (s, 9 H), 3.40–3.59 (m, 2 H), 3.85–4.20 (m, 3 H), 4.40–5.10 (m, 2 H), 4.75 (br. s, 1 H), 5.39 (dd, J = 5.2, 9.4 Hz, 1 H). $C_{11}H_{16}N_2O_5$ (256.26): calcd. C 51.56, H 6.29, N 10.93; found C 51.72, H 6.37, N 10.66.

(±)-3-Carboxy-4,5,6,6a-tetrahydro-3a*H*-pyrrolo[3,4-*d*]isoxazol-5-ium Trifluoroacetate [(±)-13]: Compound (±)-**27** (0.55 g, 1.93 mmol) was treated with CF_3COOH (30% CH_2Cl_2 solution, 4.9 mL, 19.3 mmol) at 0 °C. The solution was stirred at room temp. for 3 h. The volatiles were removed under vacuum, and the residue was taken up with methanol, filtered, washed sequentially with methanol and Et_2O and dried in vacuo at 50 °C to give amino acid (±)-**13** as the corresponding trifluoroacetate (0.365 g, 70% yield). White prisms, m.p. dec. 140–144 °C, R_f (*n*BuOH/H_2O/CH_3COOH, 4:2:1) = 0.27. ^1H NMR (300 MHz, [D6]DMSO + 1 drop CF_3COOD): δ = 3.32–3.46 (m, 2 H), 3.56–3.70 (m, 2 H), 4.30 (m, 1 H), 5.47 (dd, J = 4.8, 9.5 Hz, 1 H). $C_8H_9F_3N_2O_5$ (270.16): calcd. C 35.57, H 3.36, N 10.37; found C 35.21, H 3.39, N 10.09.

Ethyl (±)-(3a*S,6*R**,6a*R**)-6-Hydroxy-4,5,6,6a-tetrahydro-3a*H*-cyclopenta[*d*]isoxazole-3-carboxylate [(±)-30a], Ethyl (±)-(3a*R**,4*S**,6a*S**)-4-Hydroxy-4,5,6,6a-tetrahydro-3a*H*-cyclopenta-[*d*]isoxazole-3-carboxylate [(±)-30b], Ethyl (±)-(3a*R**,4*R**,6a*S**)-4-Hydroxy-4,5,6,6a-tetrahydro-3a*H*-cyclopenta[*d*]isoxazole-3-carboxylate [(±)-30c], and Ethyl (±)-(3a*S**,6*S**,6a*R**)-6-hydroxy-4,5,6,6a-tetrahydro-3a*H*-cyclopenta[*d*]isoxazole-3-carboxylate [(±)-30d]:** To a solution of (±)-cyclopent-2-en-1-ol (±)-**28** (3 g, 35.7 mmol) in AcOEt (60 mL) was added ethyl 2-chloro-2-(hydroxyimino)acetate (5.4 g, 35.7 mmol) and solid $NaHCO_3$ (15 g). The mixture was vigorously stirred for 4 d. Another portion of ethyl 2-chloro-2-(hydroxyimino)acetate (5.4 g, 35.7 mmol) was added, and the mixture was stirred for an additional 3 d. The progress of the reaction was monitored by TLC (petroleum ether/AcOEt, 7:3). H_2O (30 mL) was added to the reaction mixture, and the organic layer was separated. The aqueous phase was further extracted with AcOEt (20 mL), and the combined organic layers were dried with anhydrous Na_2SO_4. The crude material, obtained after evaporation of the solvent, was purified by flash chromatography (silica gel, petroleum ether/AcOEt, 7:3) to give 0.8 g of (±)-**30a**, 2 g of (±)-**30b**, 0.6 g of (±)-**30c**, 0.87 g of (±)-**30d**, 1.1 g of (±)-**30a** + (±)-**30b**, 0.55 g of (±)-**30b** + (±)-**30c**, and 0.2 g of (±)-**30c** + (±)-**30d**. Overall yield: 87%.

(±)-30a: Yellow oil, R_f (cyclohexane/AcOEt, 1:1) = 0.47, HPLC: petroleum ether/AcOEt, 85:15, 1 mL/min, λ = 254 nm, t_R = 8.07 min, ^1H NMR (300 MHz, $CDCl_3$): δ = 1.34 (t, J = 7.3 Hz, 3 H), 1.54 (dddd, J = 4.4, 7.3, 13.2, 13.2 Hz, 1 H), 1.79 (ddd, J = 1.1, 6.2, 13.2 Hz, 1 H), 1.96 (dd, J = 7.3, 13.2 Hz, 1 H), 2.22 (dddd, J = 6.2, 8.8, 13.2, 13.2 Hz, 1 H), 2.31 (br. s, 1 H), 3.96 (dd, J = 8.8, 8.8 Hz, 1 H), 4.26–4.38 (m, 2 H), 4.36 (dd, J = 1.1, 4.4 Hz, 1 H), 4.97 (d, J = 8.8 Hz, 1 H). $C_9H_{13}NO_4$ (199.20): calcd. C 54.26, H 6.58, N 7.03; found C 54.28, H 6.69, N 6.73.

(±)-30b: Recrystallizes from 2-propanol as white prisms, m.p. 111–112 °C, R_f (cyclohexane/AcOEt, 1:1) = 0.40, HPLC: petroleum ether/AcOEt, 85:15, 1 mL/min, λ = 254 nm, t_R = 9.46 min, ^1H NMR (300 MHz, $CDCl_3$): δ = 1.36 (t, J = 7.0 Hz, 3 H), 1.64 (dddd, J = 4.0, 7.0, 13.5, 13.5 Hz, 1 H), 1.81 (dd, J = 6.6, 13.5 Hz, 1 H), 2.13 (dd, J = 7.0, 13.5 Hz, 1 H), 2.20–2.33 (m, 1 H), 2.45 (br. s, 1 H), 3.73 (d, J = 9.2 Hz, 1 H), 4.28–4.40 (m, 2 H), 4.45 (d, J = 4.0 Hz, 1 H), 5.36 (dd, J = 5.5, 9.2 Hz, 1 H). $C_9H_{13}NO_4$ (199.20): calcd. C 54.26, H 6.58, N 7.03; found C 54.38, H 6.73, N 6.85.

(±)-30c: Recrystallizes from 2-propanol as white prisms, m.p. 55 °C, R_f (cyclohexane/AcOEt, 1:1) = 0.36, HPLC: petroleum ether/AcOEt, 85:15, 1 mL/min., λ = 254 nm, t_R = 14.72 min, ^1H NMR (300 MHz, $CDCl_3$): δ = 1.37 (t, J = 7.3 Hz, 3 H), 1.59 (dddd, J = 7.0, 9.2, 12.4, 12.4 Hz, 1 H), 1.78–1.92 (m, 1 H), 2.00 (dddd, J = 2.6, 6.2, 6.2, 12.4 Hz, 1 H), 2.10–2-20 (m, 1 H), 2.54 (br. s, 1 H), 3.92 (dd, J = 7.7, 9.2 Hz, 1 H), 4.34 (q, J = 7.3 Hz, 2 H), 4.49 (ddd, J = 6.2, 7.7, 9.2 Hz, 1 H), 5.23 (ddd, J = 1.1, 5.1, 9.2 Hz, 1 H). $C_9H_{13}NO_4$ (199.20): calcd. C 54.26, H 6.58, N 7.03; found C 54.45, H 6.78, N 6.81.

(±)-30d: Yellow oil, R_f (cyclohexane/AcOEt, 1:1) = 0.33, HPLC: petroleum ether/AcOEt, 85:15, 1 mL/min, λ = 254 nm, t_R = 18.50 min, ^1H NMR (300 MHz, $CDCl_3$): δ = 1.36 (t, J = 7.0 Hz, 3 H), 1.35–1.46 (m, 1 H), 1.76–1.91 (m, 1 H), 1.92–2.02 (m, 2 H), 2.30 (br. s, 1 H), 3.89 (ddd, J = 2.2, 9.2, 9.2 Hz, 1 H), 4.17 (ddd, J = 5.1, 5.1, 10.2 Hz, 1 H), 4.29–4.38 (m, 2 H), 5.02 (dd, J = 5.1, 9.2 Hz, 1 H). $C_9H_{13}NO_4$ (199.20): calcd. C 54.26, H 6.58, N 7.03; found C 54.28, H 6.77, N 6.71.

Ethyl (±)-(3a*S,6*S**,6a*R**)-6-[(*tert*-Butoxycarbonyl)amino]-4,5,6,6a-tetrahydro-3a*H*-cyclopenta[*d*]isoxazole-3-carboxylate [(±)-31a]:** To a solution of (±)-**30a** (500 mg, 2.51 mmol) in CH_2Cl_2 (10 mL), cooled to 0 °C, were added triethylamine (0.524 mL, 3.765 mmol) and methanesulfonyl chloride (0.292 mL, 3.765 mmol). After stirring at room temp. for 30 min, the solution was sequentially washed with 2 N HCl, 5% aqueous $NaHCO_3$ and brine. The organic phase was dried with anhydrous Na_2SO_4, and the solvent was evaporated under reduced pressure. The crude material was dissolved in DMSO (7 mL), and NaN_3 (1.63 g, 25.1 mmol) was added. The mixture was stirred and heated at 80 °C for 5 h. The progress of the reaction was monitored by TLC (petroleum ether/AcOEt, 4:1). The reaction mixture was partitioned between H_2O and Et_2O, and the aqueous phase was further extracted with Et_2O. The combined organic layers were dried with anhydrous Na_2SO_4, and the solvent was evaporated under reduced pressure.

The desired azide was purified by column chromatography (silica gel, petroleum ether/AcOEt, 9:1) to give 430 mg (1.92 mmol) of a yellow oil, which was dissolved in EtOH (20 mL) and submitted to catalytic hydrogenation in the presence of 5% Pd/C (80 mg) and di-*tert*-butyl dicarbonate (628 mg, 2.88 mmol). The progress of the reaction was monitored by TLC (petroleum ether/AcOEt, 4:1). After 24 h, the catalyst was filtered off, and the solvent was evaporated under reduced pressure. The crude material was purified by column chromatography (silica gel, petroleum ether/AcOEt, 4:1) and then recrystallized from 2-propanol to give compound (±)-**31a** (444 mg, 1.49 mmol, 59% overall yield). White prisms, m.p. 93–95 °C, R_f (cyclohexane/AcOEt, 7:3) = 0.40, ^1H NMR (300 MHz, $CDCl_3$): δ = 1.32 (t, J = 7.0 Hz, 3 H), 1.40 (s, 9 H), 1.38–1.50 (m, 1 H), 1.77 (ddd, J = 5.9, 8.0, 13.9 Hz, 1 H), 1.87–2.04 (m, 2 H), 3.85 (dd, J = 8.5, 8.5 Hz, 1 H), 4.00–4.14 (m, 1 H), 4.22–4.34 (m, 2 H), 4.94–5.04 (m, 2 H). $C_{14}H_{22}N_2O_5$ (298.33): calcd. C 56.36, H 7.43, N 9.39; found C 56.49, H 7.64, N 9.06.

Ethyl (±)-(3a*S,4*R**,6a*S**)-4-[(*tert*-Butoxycarbonyl)amino]-4,5,6,6a-tetrahydro-3a*H*-cyclopenta[*d*]isoxazole-3-carboxylate [(±)-31b]:** (±)-**31b** was prepared from (±)-**30b** in a comparable yield to that described for (±)-**31a**. White prisms, m.p. 164–165 °C, R_f (cyclohexane/AcOEt, 7:3) = 0.31, ^1H NMR (300 MHz, $CDCl_3$): δ = 1.37 (t, J = 7.3 Hz, 3 H), 1.44 (s, 9 H), 1.55–1.62 (m, 1 H), 1.76–1.90 (m, 1 H), 2.07 (ddd, J = 6.2, 6.2, 12.4 Hz, 1 H), 2.18 (dd, J = 6.2, 14.3 Hz, 1 H), 4.08(dd, J = 8.4, 8.4 Hz, 1 H), 4.16–4.44 (m, 3

H), 4.70–4.80 (m, 1 H), 5.24 (dd, J = 5.1, 8.4 Hz, 1 H). $C_{14}H_{22}N_2O_5$ (298.33): calcd. C 56.36, H 7.43, N 9.39; found C 56.46, H 7.55, N 9.17.

Ethyl (±)-(3a*S,4*S**,6a*S**)-4-[(*tert*-Butoxycarbonyl)amino]-4,5,6,6a-tetrahydro-3a*H*-cyclopenta[*d*]isoxazole-3-carboxylate [(±)-31c]:** (±)-**31c** was prepared from (±)-**30c** in a comparable yield to that described for (±)-**31a**. White prisms, m.p. 86–88 °C, R_f (cyclohexane/AcOEt, 7:3) = 0.35, ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.36 (t, J = 7.3 Hz, 3 H), 1.44 (s, 9 H), 1.70–1.82 (m, 2 H), 2.06–2.16 (m, 2 H), 3.78 (d, J = 9.2 Hz, 1 H), 4.14–4.21 (m, 1 H), 4.28–4.40 (m, 2 H), 4.60–4.80 (m, 1 H), 5.30 (ddd, J = 2.6, 4.4, 9.2 Hz, 1 H). $C_{14}H_{22}N_2O_5$ (298.33): calcd. C 56.36, H 7.43, N 9.39; found C 56.38, H 7.60, N 9.05.

Ethyl (±)-(3a*S,6*R**,6a*R**)-6-[(*tert*-Butoxycarbonyl)amino]-4,5,6,6a-tetrahydro-3a*H*-cyclopenta[*d*]isoxazole-3-carboxylate [(±)-31d]:** (±)-**31d** was prepared from (±)-**30d** in a similar yield as that described for (±)-**31a**. White prisms, m.p. 108–109 °C, R_f (cyclohexane/AcOEt, 7:3) = 0.37, ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.34 (t, J = 7.3 Hz, 3 H), 1.40 (s, 9 H), 1.60–1.78 (m, 2 H), 1.90–2.15 (m, 2 H), 3.89 (ddd, J = 1.8, 8.8, 8.8 Hz, 1 H), 3.99–4.12 (m, 1 H), 4.20–4.38 (m, 2 H), 4.60–4.80 (m, 1 H), 5.05 (d, J = 8.8 Hz, 1 H). $C_{14}H_{22}N_2O_5$ (298.33): calcd. C 56.36, H 7.43, N 9.39; found C 56.44, H 7.58, N 9.19.

(±)-(3a*S,6*S**,6a*R**)-3-Carboxy-4,5,6,6a-tetrahydro-3a*H*-cyclopenta[*d*]isoxazole-6-ammonium Trifluoroacetate [(±)-16]. Step A:** Compound (±)-**31a** (400 mg, 1.34 mmol) was dissolved in EtOH (10 mL) and treated with aqueous K_2CO_3 (20%, 10 mL). The solution was stirred at room temp. overnight, the volatiles were removed under vacuum, and the aqueous phase was extracted once with CH_2Cl_2 (5 mL). The aqueous phase was then made acidic with 2 N HCl and extracted with CH_2Cl_2 (4 × 5 mL). The combined organic layers were dried with anhydrous Na_2SO_4, and the solvent was evaporated under vacuum to give the crude carboxylic acid, which was directly submitted to the next step.

Step B: The crude carboxylic acid obtained from the previous step (293 mg, 1.085 mmol) was treated with CF_3COOH (30% CH_2Cl_2 solution, 2.8 mL, 10.85 mmol) at 0 °C. The solution was stirred at room temp. for 3 h. The volatiles were removed under vacuum, and the residue was taken up with methanol, filtered, washed sequentially with methanol and Et_2O and dried in vacuo at 50 °C to give amino acid (±)-**16** as the corresponding trifluoroacetate (136 mg, 0.48 mmol, 36% overall yield). White prisms, m.p. 132–137 °C (dec.), R_f (*n*BuOH/H_2O/CH_3COOH, 4:2:1) = 0.21. ^{1}H NMR (300 MHz, D_2O): δ = 1.37 (dddd, J = 7.1, 12.4, 12.4, 12.4 Hz, 1 H), 1.82 (dddd, J = 5.9, 8.4, 12.4, 12.4 Hz, 1 H), 1.95 (dd, J = 7.1, 12.4 Hz, 1 H), 2.04 (ddd, J = 6.2, 6.2, 12.4 Hz, 1 H), 3.58 (ddd, J = 6.2, 6.2, 12.4 Hz, 1 H), 3.99 (dd, J = 8.4, 8.4 Hz, 1 H), 5.06 (dd, J = 6.2, 8.4 Hz, 1 H). $C_9H_{11}F_3N_2O_5$ (284.19): calcd. C 38.04, H 3.90, N 9.86; found C 37.69, H 3.80, N 9.54.

(±)-(3a*S,4*R**,6a*S**)-3-Carboxy-4,5,6,6a-tetrahydro-3a*H*-cyclopenta[*d*]isoxazole-4-ammonium Trifluoroacetate [(±)-14]:** (±)-**14** was prepared from (±)-**31b** in a similar yield as that described for (±)-**16**. White prisms, m.p. dec. 132–138 °C, R_f (*n*BuOH/H_2O/CH_3COOH, 4:2:1) = 0.35. ^{1}H NMR (300 MHz, D_2O): δ = 1.45–1.52 (m, 1 H), 1.76–1.93 (m, 1 H), 2.00–2.14 (m, 2 H), 3.72 (m, 1 H), 4.05 (dd, J = 8.8, 8.8 Hz, 1 H), 5.27 (dd, J = 4.8, 8.8 Hz, 1 H). $C_9H_{11}F_3N_2O_5$ (284.19): calcd. C 38.04, H 3.90, N 9.86, found C 37.74, H 3.99, N 9.51.

(±)-(3a*S,4*S**,6a*S**)-3-Carboxy-4,5,6,6a-tetrahydro-3a*H*-cyclopenta[*d*]isoxazole-4-ammonium Trifluoroacetate [(±)-15]:** (±)-**15** was prepared from (±)-**31c** in a comparable yield to that described for (±)-**16**. White prisms, m.p. dec. 82–89 °C, R_f (*n*BuOH/H_2O/CH_3COOH, 4:2:1) = 0.35. ^{1}H NMR (300 MHz, D_2O): δ = 1.78–1.90 (m, 2 H), 2.00–2.20 (m, 2 H), 3.85 (m, 1 H), 3.93 (d, J = 9.5 Hz, 1 H), 5.32 (ddd, J = 2.2, 5.1, 7.3 Hz, 1 H). $C_9H_{11}F_3N_2O_5$ (284.19): calcd. C 38.04, H 3.90, N 9.86; found C 37.99, H 4.13, N 9.69.

(±)-(3a*S,6*R**,6a*R**)-3-Carboxy-4,5,6,6a-tetrahydro-3a*H*-cyclopenta[*d*]isoxazole-6-ammonium Trifluoroacetate [(±)-17]:** (±)-**17** was prepared from (±)-**31d** in a comparable yield to that described for (±)-**16**. White prisms, m.p. dec. 100–107 °C, R_f (*n*BuOH/H_2O/CH_3COOH, 4:2:1) = 0.25. ^{1}H NMR (300 MHz, D_2O): δ = 1.70–1.98 (m, 3 H), 2.02–2.40 (m, 1 H), 3.67 (m, 1 H), 4.06 (ddd, J = 3.7, 9.2, 9.2 Hz, 1 H), 5.07 (dd, J = 2.2, 9.2 Hz, 1 H). $C_9H_{11}F_3N_2O_5$ (284.19): calcd. C 38.04, H 3.90, N 9.86; found C 37.85, H 4.02, N 9.49.

Binding Assays: The receptor binding technique for determining the affinities for $GABA_A$ and $GABA_B$ receptors was performed on rat brain membrane preparations with either [^{3}H]muscimol or [^{3}H]-GABA as the radioligands, as described previously.[22]

[^{3}H]GABA Uptake Into Cortical Synaptosomes: Fresh cortex from male Wistar rats (150–200 g) was homogenized with 0.4 M sucrose with a glass/teflon homogenizer. The homogenate was centrifuged at 1000 × g for 10 min, the pellet was discarded and the supernatant was centrifuged at 40000 × g for 20 min, and this washing step was repeated once. The final pellet was homogenized in assay buffer (120 mM NaCl, 5 mM KCl, 1.8 mM $CaCl_2$, 1.2 mM $MgSO_4$, 10 mM D-glucose, 1.2 mM NaH_2PO_4, 25 mM $NaHCO_3$, 10 mM HEPES, pH 7.4 and 40 times the pellet volume). Aliquots (50 µL) of uptake buffer/test compound solution and tissue (100 µL, ≈ 2.5 mg original tissue/well) were added to 96-well plates and preincubated 2 min at 25 °C before adding ^{3}H-GABA (50 µL, 1 nM tracer + 25 nM unlabelled GABA) and further incubating for 5 min at 25 °C. Samples were filtered directly onto Whatman GF/B glass fibre filters under vacuum and immediately washed with 0.9% NaCl (3 × 0.5 sec.) with a Packard UniTomTec cell harvester. Bound radioactivity was measured with a liquid scintillation counter (Trilux short, Wallac) with a liquid scintillation cocktail (Optiphase "Supermix", Perkin–Elmer). Non-specific uptake was defined as the uptake in the presence of 1 mM GABA and accounted for 4–5% of total uptake.

GABA Functional Assay in Rat Cortical Wedge: The rat cortical wedge preparation was carried out according to a previously published method.[6] The wedges were left for development of spontaneous activity for 2–3 h. Characterization of effects on the spontaneous activity was initiated when the spontaneous activity was more than 30 spikes per 12 min and stable over a 30 min period. The number of population responses (spikes) per 12 min was counted. Compounds were applied in superfusion buffer, and the wedges were superfused for 20 min. The number of spikes during the last 12 min of drug application were counted, and the frequency (spikes per min) were calculated. The relative frequency, calculated as the ratio between frequencies in the presence and absence of compound, were calculated. Compounds were tested alone or in combination with 20 µM GABA (corresponding to EC_{20} for GABA).

Theoretical Calculations: The conformational space of all the analyzed compounds was explored with the DFT/B3LYP approach at the 6-31G(d) level as implemented in GAUSSIAN03.[23] We fully optimized all the starting geometries deriving from the pseudorotational path of the five- and six-membered carbocyclic rings and, in some instances, from rotation around the exocyclic single bonds. The energies of the conformations were recalculated in a polariz-

able conductor-like solvation model (C-PCM)[26] to obtain values compatible with aqueous solutions.

Acknowledgments

This research has been financially supported by a grant from the University of Milan (FIRST, 2004). Marianne Faber and Charlotte Fredsøe Hundahl are acknowledged for their skilled technical assistance.

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Received: July 20, 2006
Published Online: October 18, 2006

