phenylacetic acid, 1798-09-0; *o*-methoxyphenylacetic acid, 93-25-4; *p*-methoxyphenylacetic acid, 104-01-8; 1-bromopentane, 110-53-2; 6-undecyl-4-hydroxy-2-pyrone, 81017-03-0; 4-hydroxy-6-methyl-2-pyrone, 675-10-5; ethyl bromide, 74-96-4; butyl bromide, 109-65-9; pentyl bromide, 110-53-2; heptyl bromide, 629-04-9; undecyl bromide, 693-67-4; (1-butyl) heptyl bromide, 5447-45-0; propionic anhydride, 123-62-6; 6-n-heptyl-4-hydroxy-2-pyrone, 90632-45-4; elastase, 9004-06-2.

Supplementary Material Available: Synthesis and analytical data for compounds 1–13 (4 pages). Ordering information is given on any current masthead page.

Synthesis and Pharmacological Evaluation of *cis*-2,3,3a,4,5,6,7,7a-Octahydro-3-oxoisoxazolo[5,4-*c*]pyridine: A Structural Analogue of the GABA Agonist THIP

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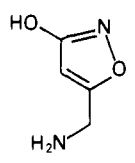
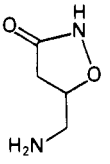
The pharmacological activities of the GABA agonist muscimol (1) and its dihydro analogue (2) have been shown to be almost identical. The closely related 4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol (THIP, 3), although biologically less active than muscimol, was selected for clinical trials. We report the synthesis of the so far elusive *cis*-2,3,3a,4,5,6,7,7a-octahydro-3-oxoisoxazolo[5,4-*c*]pyridine (*cis*-DH-THIP, *cis*-4), which—surprisingly—is devoid of any GABAergic activity.

Muscimol (1), a very active and selective GABA receptor agonist, has served as a model compound for a comprehensive series of heterocyclic GABA analogues.[1] Some



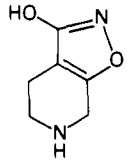
muscimol

1



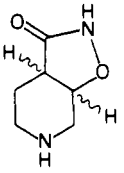
DH-muscimol

2



THIP

3



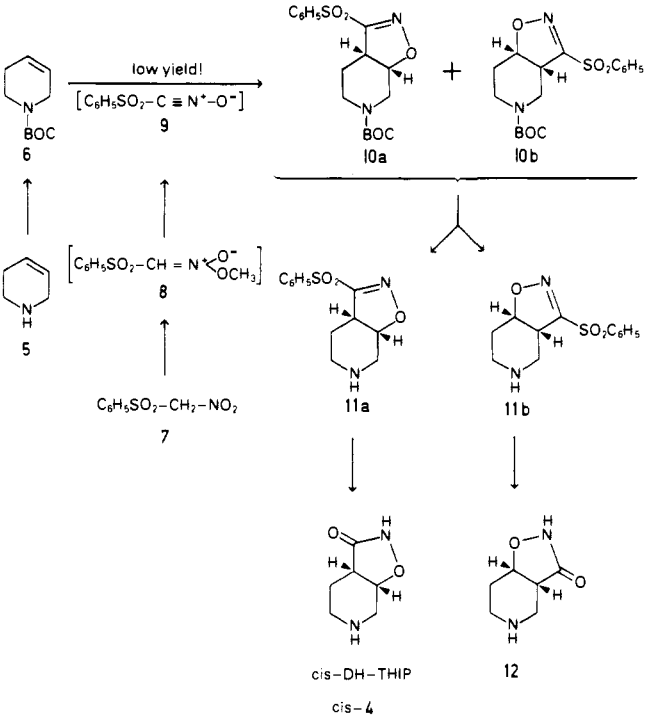
DH-THIP

4

muscimol analogues, including (*R,S*)-4,5-dihydromuscimol (2) and 4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol (THIP), are potent GABA receptor agonists in vivo and in vitro.[2] The relatively nontoxic compound 3 (THIP), which seems to penetrate the blood–brain barrier was selected for clinical trials.[3]

Previous attempts to synthesize 4, a dihydro analogue of 3, have failed.[4] In view of the considerable pharmacological interest in selective GABA receptor agonists, we have decided to develop a synthesis of *cis*-4 (*cis*-DH-THIP).

Scheme I



We report our successful synthesis of *cis*-2,3,3a,4,5,6,7,7a-octahydro-3-oxoisoxazolo[5,4-*c*]pyridine (*cis*-DH-THIP) and compare its pharmacological activity with that of the two well-characterized GABA agonists 3 (THIP) and muscimol (1).

Chemistry. Synthesis of 4 has been approached unsuccessfully[4] in analogy to a reaction sequence developed for the synthesis of 2[5] and following a general procedure for the preparation of 2-isoxazolin-3-ols. In contrast to

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Table I. Biological Activities[a] of *cis*-4 in Comparison to 3 (THIP) and Muscimol (1)

substance	[^{3}H]muscimol IC_{50}, nM	dec of spont firing rate, M	antagonism of bicuculline-induced convulsions (ID_{150}, mg/kg ip)
3 (THIP)	31.5 ± 2.5	$\geq 10^{-5}$ [b]	1.9
cis-4 (cis-DH-THIP)	>1000	no change up to 10^{-4} [c]	>56
muscimol (1)	3 ± 0.3	$\geq 10^{-6}$ [b]	0.76

[a] For details, see the Experimental Section. [b] The agonistic activity of 3 and 1 was antagonized by 10^{-5} M bicuculline. [c] Additionally, *cis*-4 did not show any increase of spontaneous firing as seen with GABA antagonists such as bicuculline.

these approaches we considered 4 as a derivative of a 2-isoxazoline, which can easily be synthesized by use of 1,3-dipolar cycloadditions of nitrile oxides to olefins. The scope of this reaction combined with the ability of 3-(phenylsulfonyl)-2-isoxazolines to undergo nucleophilic substitutions[6] prompted us to undertake the synthesis of *cis*-4 as summarized in Scheme I.

(Phenylsulfonyl)nitromethane (7)[7] was methylated with diazomethane as previously described[8] to yield a solution of nitronic ester 8. *This solution should not be evaporated to dryness, as we have observed a severe explosion after complete removal of solvents and one of us was injured!*[9] Thereafter, solutions of 8 were handled with extreme care, and the published method[8] was modified as exemplified in the Experimental Section to avoid dangerously high concentrations of this reactive intermediate 8. Cycloaddition of nitrile oxide 9, generated in situ from 8, to 1-BOC-1,2,3,6-tetrahydropyridine 6 afforded, although in low yield, a mixture of both isomeric isoxazolines **10a** and **10b**. After cleavage of the protecting group on nitrogen, isoxazolines **11a** and **11b** were separated by medium-pressure column chromatography, the overall yield of **11a** and **11b** from 7 being less than 1% each. Selective decoupling experiments allowed the assignment of all protons in the ^{1}H NMR spectrum of **11a** and **11b**. The coupling constant of 8 Hz between the protons at the ring junction HC(3a) and HC(7a) is in agreement with their expected cis relationship. For completion of the synthesis of *cis*-4, **11a** had to be hydrolyzed. Due to the high water solubility of *cis*-4, a special procedure had to be developed, using barium hydroxide to allow the subsequent removal of inorganic material as salts of low solubility. Finally, *cis*-4 was crystallized from methanol. In contrast to *cis*-4, its isomer 12 could not be separated from inorganic material even by this procedure.

In summary, we have achieved the synthesis of the so far elusive *cis*-4 by using a 1,3-dipolar cycloaddition as key step. Since the resulting compound lacks biological activity, we have not tried to improve the low yield of the cycloaddition.

Pharmacology. GABA agonistic activity of *cis*-4 was determined in three classical tests: (1) displacement of [^{3}H]muscimol in cerebellar tissue of male rats; (2) electrophysiological measurement of spontaneous firing in explants from the cerebellum of 2–4 day old rats; (3) antagonism of bicuculline-induced convulsions in female mice.

In all three tests the two agonists muscimol (1) and 3 (THIP) served as controls. The results, summarized in Table I, show that *cis*-4 (*cis*-DH-THIP) has neither GABA agonistic nor antagonistic activity.

Discussion

(*R*,*S*)-4,5-dihydromuscimol (2) has earlier been characterized as a potent GABA agonist, approximately equipotent to muscimol (1) as a bicuculline-sensitive depressant of neuronal firing and as an inhibitor of [^{3}H]GABA binding.[2] In contrast, the newly synthesized conformationally restricted *cis*-4 (*cis*-DH-THIP) has lost its affinity to the GABA receptor as measured by three classical tests for GABAergic activity. The trans analogue of *cis*-4, if chemically stable, might still show GABAergic activity.

Experimental Section

^{1}H NMR spectra were measured on a Bruker Spectrospin 360-MHz (WH-360) or 90-MHz (HX-90) spectrometer using $(CH_3)_4Si$ as an internal standard. IR and mass spectra were also determined for all new compounds and were consistent with the proposed structures. Solid new compounds were submitted to elemental analysis; these were within ±0.4% of theoretical values, except where noted. Melting points were determined on a Buchi SMP-20 apparatus and are not corrected. All reactions were followed by TLC carried out on Merck F254 silica gel plates. Solutions were dried over Na_2SO_4 and concentrated on a Buchi rotary evaporator at low pressure (water aspirator).

1-(*tert*-Butoxycarbonyl)-1,2,3,6-tetrahydropyridine (6). A solution of **5** (35 mL, 0.38 mol) in dioxane (350 mL) and 1 N Na_2CO_3 (770 mL) was treated with di-*tert*-butyl dicarbonate (78.6 g, 0.36 mol). The resulting emulsion was stirred at room temperature for 5 h, concentrated to half, and extracted thoroughly with ethyl acetate. The organic layers were dried and evaporated. After several hours at high vacuum, 61 g (87%) of **6** remained as a yellow oil. NMR ($CDCl_3$, 90 MHz): δ 1.46 (s, 9 H), 1.96–2.24 (m, 2 H), 3.49 (t, J = 6 Hz, 2 H), 3.91 (dd, J = 5/3 Hz, 2 H), 5.54–6.0 (m, 2 H).

***cis*-6-(*tert*-Butoxycarbonyl)-3a,4,5,6,7,7a-hexahydro-3-(phenylsulfonyl)isoxazolo[5,4-*c*]pyridine (10a) and *cis*-5-(*tert*-Butoxycarbonyl)-3a,4,5,6,7,7a-hexahydro-3-(phenylsulfonyl)isoxazolo[4,5-*c*]pyridine (10b).** A solution of 7 (10 g, 50 mmol) in CH_2Cl_2 (80 mL) was treated in portions with a solution of diazomethane in diethyl ether until the methylation followed by TLC (silica; CH_2Cl_2/CH_3OH, 99:1) was complete. The resulting solution of 8 was diluted with 1,2-dichloroethane followed by concentration at reduced pressure and 0 °C with rigorous safety precautions[9] to about 60 mL. The resulting solution was immediately cooled to −30 °C and added dropwise to a intensively stirred emulsion of **6** (10.1 g, 55 mmol) in 1 M sodium metasilicate (60 mL) at 50 °C. Stirring was continued at 50 °C for 30 min. Then, water was added followed by extraction with CH_2Cl_2. The organic layers were dried and evaporated to yield 21.1 g of a yellow oil. After medium-pressure chromatography on silica (240 g), 1.5 g of an oily mixture of **10a** and **10b** (R_f 0.16) was isolated, accompanied by 7.4 g of recovered **6** (R_f 0.4), 3 g of 3,4-bis(phenylsulfonyl)furoxan (mp 156–157 °C, R_f 0.72), and 1.5 g of phenylsulfonamide (mp 150–151 °C, R_f 0.12). [All R_f values are on silica, CH_2Cl_2/CH_3OH (99:1).] NMR (**10a** and **10b**, $CDCl_3$, 90 MHz): δ 1.43/1.47 (2s, 9 H), 1.8–2.3 (m, 2 H), 2.9–4.25 (m, 5 H), 4.65–5.15 (m, 1 H), 7.45–7.8 (m, 3 H), 7.8–8.15 (m, 2 H). According to the ^{1}H NMR spectrum, there were almost equal amounts of the two isomers **10a** and **10b**.

***cis*-3a,4,5,6,7,7a-Hexahydro-3-(phenylsulfonyl)isoxazolo-[5,4-*c*]pyridine (11a) and *cis*-3a,4,5,6,7,7a-Hexahydro-3-(phenylsulfonyl)isoxazolo[4,5-*c*]pyridine (11b).** A mixture of **10a** and **10b** (21.6 g, produced in several batches from 254 g

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(8) Wade, P. A.; Pillay, M. K. *J. Org. Chem.* **1981**, *46*, 5425.
(9) *Warning!* We strongly recommend avoiding large-scale preparation of nitronic ester 8. Solutions of 8 should be handled with extreme care and stringent safety precautions! Complete removal of solvents may lead to explosive decomposition!

(1.26 mol) of 7) was dissolved at room temperature in neat trifluoroacetic acid (220 mL). After 1 h the reaction mixture was concentrated, and CH_2Cl_2 and 1 N $KHCO_3$ were added to the oily residue. After extraction, the organic layers were dried and evaporated. The residue (11.5 g) was submitted to medium-pressure chromatography on silica to yield 2.3 g (0.7% referring to 7) of **11a** (R_f 0.18) and 2.5 g (0.8% referring to 7) of **11b** (R_f 0.14) [R_f values are on silica, CH_2Cl_2/CH_3OH (95:5).] Both isomers failed to crystallize.

11a: NMR ($CDCl_3$ + D_2O, 360 MHz) δ 1.87–1.98 (m, 1 H), 2.37–2.46 (m, 1 H), 2.96 (ddd, J = 13/10/3 Hz, 1 H), 3.33 (dt, J = 13/5 Hz, 1 H), 3.43 (dd, J = 15/3 Hz, 1 H), 3.71 (dd, J = 15/3 Hz, 1 H), 3.97 (q, J = 8 Hz, 1 H), 4.83 (dt, J = 8/3 Hz, 1 H), 7.5–7.8 (m, 3 H), 7.9–8.1 (m, 2 H).

11b: NMR ($CDCl_3$ + D_2O, 360 MHz) δ 1.88–2.06 (m, 2 H), 2.73–2.90 (m, 3 H), 3.26 (dd, J = 13/7 Hz, 1 H)8 3.48 (dt, J = 7/8 Hz, 1 H), 4.74 (dt, J = 8/4 Hz, 1 H), 7.45–7.78 (m, 3 H), 7.9–8.12 (m, 2 H).

***cis*-2,3,3a,4,5,6,7,7a-Octahydro-3-oxoisoxazolo[5,4-*c*]pyridine (*cis*-4).** Solid barium hydroxide octahydrate (1.76 g) was added to a solution of **11a** (1.5 g, 5.6 mmol) in dioxane/H_2O (1:1) (120 mL). The heterogeneous reaction mixture was stirred at 50 °C for 24 h. By addition of 2 N H_2SO_4, the pH was adjusted to 1 and the precipitated barium sulfate removed by filtration through talcum powder. The filtrate was extracted thoroughly with CH_2Cl_2 to remove phenylsulfonic acid. Barium hydroxide (0.1 N) was added to the water layer to adjust to pH 9, and barium sulfate was filtered off again. The filtrate was extracted once more with CH_2Cl_2 and CO_2 was bubbled through the water layer until pH 6 was reached. Precipitated barium carbonate was filtered off and the filtrate evaporated to dryness at high vacuum. A yellow amorphous residue was the result that crystallized from CH_3OH to yield 542 mg (68%) of *cis*-**4** as a white powder. For final purification this material, combined with analoguous batches, was recrystallized three times from CH_3OH; 203–204 °C. NMR (Me_2SO, 360 MHz) δ 1.55-1.75 (m, 2 H), 2.4–2.6 (m, 2 H), 2.68 (dd, J = 13/6 Hz, 1 H), 2.79 (q, J = 6 Hz, 1 H), 2.90 (dd, J = 13/5 Hz, 1 H), 4.38 (dt, J = 5/6 Hz, 1 H), 5.7–6.7 (b, 2 H). Anal. ($C_6H_{10}N_2O_2$) C, H, N; O: calcd, 22.5; found, 22.0.

***cis*-2,3,3a,4,5,6,7,7a-Octahydro-3-oxoisoxazolo[4,5-*c*]pyridine (12).** Hydrolysis of **11b** and the subsequent workup were done analogously to the hydrolysis of **11a** described above, but **12** could be isolated only as an amorphous powder. The ^1H NMR spectrum showed signals only of **12**, but according to elemental analysis the content of **12** was only about 60%, presumably due to contamination by inorganic material. NMR (D_2O, 90 MHz): δ 1.95–2.3 (m, 2 H), 2.95–3.7 (m, 6 H), 4.5–5.0 (b, 2 H + H_2O). Anal. Calcd for $C_6H_{10}N_2O_2$: C, 50.5; H, 7.1; N, 19.7; O, 22.5. Found: C, 32.1; H, 5.1; N, 10.9; O 36.6; S, 7.3.

Displacment of [^{3}H]Muscimol.[10,11] Tissue. Cerebellar tissue from male rats (Ivanovas, 350–400 g) was homogenized in 20 vol of ice-cold 0.32 M sucrose with a polytron homogenizer and centrifuged at 1000 *g* for 10 min at 4 °C. The supernatant was centrifuged for an additional 20 min at 20000*g* and the resulting pellet resuspended in Tris-citrate buffer (0.05 M, pH 6.7). To further remove endogenous GABA the suspension was dialyzed for 1 day at 4 °C in the resuspension buffer with three buffer changes.

Assay. Triplicate samples were incubated for 30 min at room temperature (2-mL assay volume) in the presence of 2 nM [^{3}H]muscimol (NEN 12.9 Ci/mM) alone or in the presence of varying concentrations of unlabeled displacer. Nonspecific binding was determined in the presence of 10^{-6} M GABA.

Measurement of Spontaneous Firing Rate.[12] Tissue. Explants from the cerebellum of 2–4 day old rats (Wistar) were grown on glass cover slips in a plasma clot in a medium consisting of 25% fetal calf serum, 25% Hanks' BSS, and 50% basal medium Eagle. Cultures were rotated by means of a roller drum, the medium was exchanged weekly and the pH maintained close to 7.0.

Electrophysiology. All electrophysiological experiments were carried out in a temperature-controlled microchamber in Hanks' BSS at 36 °C. Drugs were superfused at a rate between 30 and 60 mL/h.

Antagonism of Bicuculline-Induced Convulsions. Treatment. Female mice (OF1 strain) weighing between 22 and 27 g were given the test treatment intraperitoneally 30 min or orally 60 min before receiving a subcutaneous injection of bicuculline base (5 mg/kg). For each mouse, the time in seconds was recorded from the bicuculline injection to the occurrence of the first clonic convulsions and the mean time calculated for each dose of compound. Four to six doses were used for each test treatment and five to six mice used per dose. The ID_{150}, estimated by regression analysis, was taken to be the dose of drug that prolonged the time to the occurrence of clonic convulsions by 50% compared to the control group.

Registry No. *cis*-**4**, 96666-84-1; **5**, 694-05-3; **6**, 85838-94-4; **7**, 21272-85-5; **8**, 57359-33-8; **10a**, 96666-85-2; **10b**, 96666-86-3; **11a**, 96666-87-4; **11b**, 96666-88-5; **12**, 96666-89-6.

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Stereoselective Antitumor Properties in the Lewis Lung Carcinoma Model Using Bis(morpholinomethyl) Derivatives of Tricyclic Bis(dioxopiperazines)

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Geometric isomers of 2,11-bis(morpholinomethyl)tetrahydrodipyrazino[1,2-*a*:2′,1′-*c*]pyrazine-1,3,10,12-(2*H*,4*H*,9*H*,11*H*)-tetrone (**3** and **4**) and the parent bisimides (**1** and **2**) were studied for their stereoselective antimetastatic activity in the Lewis Lung carcinoma model. The morpholinomethyl cis-syn-trans isomer **4** was more effective as an inhibitor of metastasis than the other three analogues. Using a postamputation protocol, the order of decreasing activity was cis morpholinomethyl analogue **4** > trans morpholinomethyl analogue **3** > parent cis imide **2** > parent trans imide **1**. Increased activity observed for the morpholinomethyl derivatives may reflect differences in solubility and delivery (prodrug) or an intrinsic antitumor activity of the morpholinomethyl–N functionality.

Bis(dioxopiperazines) are of considerable importance owing to their antimetastatic properties and their actions ameliorating anthracycline-induced toxicity in animals.[1] Cyclopropylbis(dioxopiperazines)[2,3] and related tetraazaperhydrophenanthrenes (**1** and **2**)[4] have been employed to assess stereoselective antimetastatic properties. Although

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