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Preliminary communication

Synthesis and biochemical studies of spirocyclic amino acids. II. Activity of 2-azaspiro[5.5]undecane-7-carboxylates as GABA-uptake inhibitors

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Summary — Novel GABA analogous spirocyclic amino acids were prepared and investigated for interaction with GABA-A and GABA-B receptors as well as the GABA uptake system. Starting from known bromopropyl lactones and arylalkylamines, spirocyclic hydroxyalkyl lactams were obtained, which were reduced by $LiAlH_4$ to yield spirocyclic hydroxymethyl piperidines. Oxidation by Jones' reagent followed by subsequent esterification gave the title compounds which represent conformationally restricted analogues of GABA. Whereas the new spirocyclic amino acids showed no activity at GABA receptors they proved to be active as GABA uptake inhibitors. An examination of the relationship between structure and GABA uptake inhibition revealed a strong dependence of activity on the length of the alkyl chain in *N*-arylalkyl substituents.

spirocyclic amino acid / GABA uptake inhibitor / structure–activity relationship

Introduction

GABA (γ-aminobutyric acid) is one of the most important inhibitory neurotransmitters in the central nervous system [1–3]. The development of GABA-mimetic drugs is a rewarding aim, because the impairment of the central GABA system seems to be involved in certain psychiatric and neurological disorders [4], such as Huntington's chorea [5], Parkinson's disease [6] and epilepsy [7].

Extensive studies on the different mechanisms involved in the course of GABA synaptic transmission, revealed the most important targets for GABA-mimetic drugs to be binding to GABA-A and GABA-B receptors, GABA synthesis by L-glutamate-1-decarboxylase (GAD), GABA degradation by GABA-2-oxoglutarate aminotransferase (GABA-T) and GABA uptake [3]. GABA uptake inhibitors enhance the concentration of GABA in the synaptic cleft and as a result they potentiate the GABAergic neurotransmission [3]. Therefore, the development of new GABA uptake inhibitors is a valuable objective in drug research because they act as GABA-mimetic drugs where GABA is physiologically released.

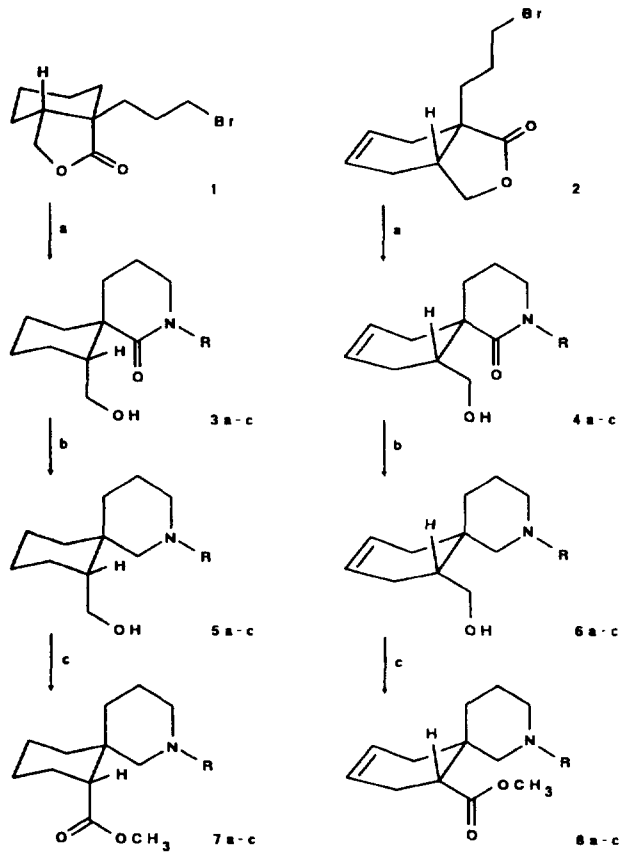
GABA can exist in a wide variety of conformations due to comparative freedom of rotation about the single bonds [2]. Extensive structure–activity studies on conformationally restricted analogues of GABA demonstrate that incorporation of bulky substituents, unsaturation, carbocyclic rings, heterocyclic rings or a combination of these into the GABA skeleton may lead to analogs which are specific inhibitors of the GABA uptake system [3, 8].

In the last years many amino acids have been synthesized. These act as GABA uptake inhibitors, but most are unable to cross the blood–brain barrier [9, 10]. A recent strategy to develop a systemically active GABA-mimetic compound was to attach an arylalkyl group to the nitrogen of a GABA analogous amino acid, such as nipecotic acid [11].

In conjunction with a program concerning with the synthetic properties of non-aromatic isobenzofuranones [12–14], we recently published a rearrangement of aminoalkyl lactones to spirocyclic hydroxymethyl lactams [15], which could be easily transformed into spirocyclic amino acids with a GABA analogous structure. We now want to present the synthesis and binding studies of these novel, arylalkyl-substituted, spirocyclic amino acids, which have shown activity as GABA uptake inhibitors.

Chemistry

The synthesis of the new spirocyclic amino acids (scheme 1) started from bromopropyl lactones **1** and **2** respectively, which were readily accessible by α-lithiation of unsubstituted isobenzofuranones [12–14] followed by electrophilic substitution with 1,3-dibromopropane as described previously [15]. Reaction of the bromopropyl lactones **1** and **2** with excess arylalkylamine ($BzNH_2$ [15]; $Ph_2CH(CH_2)_nNH_2$, n = 1 or 2) led to formation of aminoalkyl lactones, which rearranged under substitution conditions (toluene/reflux/16 h) to spirocyclic hydroxyalkyl lactams **3a–c** and **4a–c**. After separation of excess arylalkylamine by washing with dilute acid and crystallization we isolated **3a–c** and **4a–c** in moderate to excellent yields (see table I). Structure of the spirocyclic lactams **3a–c** and **4a–c** was ensured by spectroscopic methods. The



Scheme 1. (a) RNH_2/toluene, reflux, 16 h; (b) $LiAlH_4$/THF, reflux, 2 h; (c) CrO_3/H_2SO_4, 20°C, 3 h then $MeOH/H_2SO_4$, reflux, 16 h.

interpretation of the ^{1}H-NMR spectra, which showed well-separated signals of carbinol, *N*-aralkyl and *N*-methylene protons (H-3) at 400 MHz, allowed the assignment of the structures. Mass spectrometric fragmentation agreed with the postulated structures of **3a–c** and **4a–c**. IR absorption bands of δ-lactams (1600–1625 cm^{-1}) at low wavenumbers indicated the presence of an intramolecular hydrogen bridge between the lactam carbonyls and hydroxymethyl groups in **3a–c** and **4a–c**.

Reduction of spirocyclic lactams **3a–c** and **4a–c** with solutions of $LiAlH_4$ in THF gave spirocyclic amines **5a–c** and **6a–c** in good yields (table I). Comparing the ^{1}H-NMR spectral data of spirocyclic amines **5a–c** and **6a–c** with the corresponding spiro lactams **3a–c** and **4a–c** showed typical shifts of *N*-aralkyl and *N*-methylene protons (H-3) to high fields, due to lost of inductive and anisotropic effects of the lactam carbonyl group. Large shift increments (≈ 1.4 ppm) were observed between the geminal protons at C-1 in **5a–c** and **6a–c**.

Looking ahead we assumed that the final oxidation step of the spirocyclic amines **5a–c** and **6a–c** to the desired spirocyclic amino acids could be problematic; first because the oxidation reagent should only attack at the carbinol-C but not at the nitrogen or the double bond, and second because we had doubts about finding an isolation procedure for the zwitterionic reaction products. The method of choice was the use of Jones' reagent which specifically oxidized the carbinol function to the carboxyl group. Subsequent esterification of the resulting amino acids to their methyl esters **7a–c** and **8a–c** allowed isolation from acidic solution of the chromium salts by extraction with dichloromethane. Purification of crude **7a–c** and **8a–c** by flash chromatography and formation of the well-crystallizable hydrochlorides yielded the final products (table I).

The diastereomeric purity of esters **7a–c** and **8a–c** was outlined by homogeneity in TLC and single appearance of the very sharp methyl ester signals (≈ 3.60 ppm) in all ^{1}H-NMR spectra at 400 MHz. No epimerization at C-7 took place during the transformation to spirocyclic esters **7a–c** and **8a–c** because we isolated diastereomerically pure products. This is consistent with epimerization experiments we have recently published [19]. IR absorption bands of the estercarbonyl group (1725–1730 cm^{-1}) and mass spectrometric fragmentation agreed with the postulated structures of **7a–c** and **8a–c**.

Biochemistry

The affinities of the spirocyclic amino acid esters **7a–c** and **8a–c** to GABA-A and GABA-B receptors were determined *in vitro* by using the specific agonists

Table I. Structure and physical properties of **3–8**.

Compound	*R*	*Synthesis method*	*Recrystallization solvent*	*Yield (%)*	*Empirical formula*	*Anal*	*Mp (°C)*
3a	$PhCH_2$-	A	Methanol	95	$C_{18}H_{25}NO_2$	C, H, N	20
3b	Ph_2CHCH_2-	A	Toluene	85	$C_{25}H_{31}NO_2$	C, H, N	55
3c	$Ph_2CHCH_2CH_2$-	A	Toluene	65	$C_{26}H_{33}NO_2$	C, H, N	110
4a	$PhCH_2$-	A	Methanol	53	$C_{18}H_{23}NO_2$	C, H, N	91–92
4b	Ph_2CHCH_2-	A	Toluene	85	$C_{25}H_{29}NO_2$	C, H, N	147
4c	$Ph_2CHCH_2CH_2$-	A	Toluene	93	$C_{26}H_{31}NO_2$	C, H, N	132
5a	$PhCH_2$-	B	Ether	75	$C_{18}H_{27}NO{\cdot}HCl$	C, H, N, Cl	40
5b	Ph_2CHCH_2-	B	Ether	70	$C_{25}H_{33}NO{\cdot}HCl$	C, H, N, Cl	118–120
5c	$Ph_2CHCH_2CH_2$-	B	Ether	64	$C_{26}H_{35}NO{\cdot}HCl$	C, H, N, Cl	113
6a	$PhCH_2$-	B	Ether	57	$C_{18}H_{25}NO{\cdot}HCl$	C, H, N, Cl	54
6b	Ph_2CHCH_2-	B	Ether	68	$C_{25}H_{31}NO{\cdot}HCl$	C, H, N, Cl	165–167
6c	$Ph_2CHCH_2CH_2$-	B	Ether	64	$C_{26}H_{33}NO{\cdot}HCl$	C, H, N, Cl	85–86
7a	$PhCH_2$-	C	Ether	40	$C_{19}H_{27}NO_2{\cdot}HCl$	C, H, N, Cl	200–202
7b	Ph_2CHCH_2-	C	Ether	41	$C_{26}H_{33}NO_2{\cdot}HCl$	C, H, N, Cl	119–120
7c	$Ph_2CHCH_2CH_2$-	C	Ether	56	$C_{27}H_{35}NO_2{\cdot}HCl$	C, H, N, Cl	202–204
8a	$PhCH_2$-	C	2-Propanol	39	$C_{19}H_{25}NO_2{\cdot}HCl$	C, H, N, Cl	185
8b	Ph_2CHCH_2-	C	Ether	74	$C_{26}H_{31}NO_2{\cdot}HCl$	C, H, N, Cl	160
8c	$Ph_2CHCH_2CH_2$-	C	Ether	59	$C_{27}H_{33}NO_2{\cdot}HCl$	C, H, N, Cl	79

[^{3}H]-muscimol and [^{3}H]-CGP 27492 as radioligands. There were no interactions at GABA-A or GABA-B receptors up to concentrations of 10^{-4} M.

Inhibition of the neuronal uptake of [^{3}H]-GABA by **7a–c** and **8a–c** was examined at a concentration of 10^{-4} M and compared with the activity of nipecotic acid, a known potent inhibitor of GABA uptake (table II). In contrast to the negative results in receptor binding **7a–c** and **8a–c** showed activity as GABA uptake inhibitors. The IC_{50} values were determined as 3.36 μM for nipecotic acid and 42.21 μM for **8c**, which was the most active compound of this series.

An examination of the relationship between structure and GABA uptake inhibition potency in our series of spirocycles revealed an increasing activity going from simple *N*-benzyl-substituted compounds (**7a**, **8a**) to substances bearing a long chain *N*-arylalkyl substituent (**7c**, **8c**). On the other hand, the influence of saturation (**7a–c**) or unsaturation (**8a–c**) in the carbocyclic ring seemed to be rather small.

Results and discussion

Compounds **7a–c** and **8a–c** did not show affinity to GABA-A and GABA-B receptors but moderate activity as GABA uptake inhibitors. Our finding that **7a–c** and **8a–c** act as specific inhibitors of GABA re-uptake agrees with the view that the structural element of the system that binds the amino group of GABA at the GABA transport carrier shows a higher degree of steric and stereochemical tolerance than the GABA receptor sites [8].

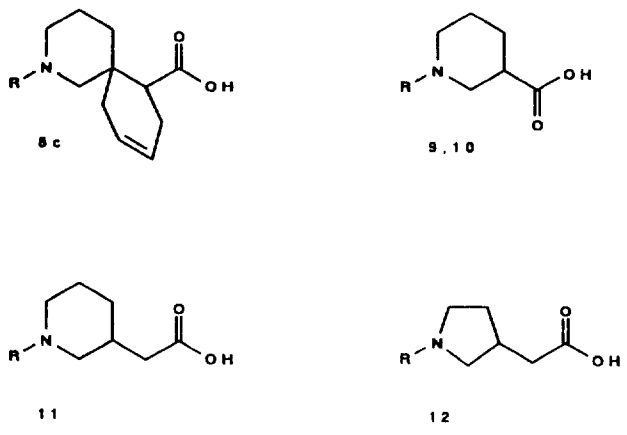
A comparison of our most active compound **8c** with related conformationally restricted analogues of

Table II. [^{3}H]-GABA uptake inhibition of spirocyclic undecanes **7a–c** and undecenes **8a–c** in comparison to nipecotic acid **9**.

Compound	*R*	*[^{3}H]-GABA uptake (%)*[a]
7a	$PhCH_2$-	85
7b	Ph_2CHCH_2-	42
7c	$Ph_2CHCH_2CH_2$-	26
8a	$PhCH_2$-	79
8b	Ph_2CHCH_2-	47
8c	$Ph_2CHCH_2CH_2$-	22
9	–	4

[a]Concentration of the inhibitor was 10^{-4} M; 100% uptake corresponds to no inhibition.

GABA (scheme 2) outlines particular information on structure–activity relationships in this series. The spirocyclic amino acid **8c** and nipecotic acid **9** are specific uptake inhibitors and homo-β-proline **12** is an inhibitor of GABA-A and GABA-B receptor binding and GABA uptake [8], whereas piperidin-3-acetic acid **11** does not show significant effects in these systems [8]. Thus specificity of interaction at the GABAergic system seems to depend mainly on the underlying ring system of the heterocyclic GABA analogues. Comparing uptake inhibition of GABA analogues, which are based on the same heterocyclic skeleton as spirocycles **7a–c** or **8a–c** (table II), piperidines **9** and **10** [11] (table III) show a significant effect on activity depending on the substituents attached at the nitrogen.



Scheme 2.

Table III. [^{3}H]-GABA uptake inhibition of **8c** in comparison to known inhibitors of related structure.

Compound	*R*	*IC_{50}*
8c	$Ph_2CH(CH_2)_2$-	42.21
9	H	3.36
10	$Ph_2C{=}CH(CH_2)_2$-	0.20
11	H	Inactive
12	H	2.50

The fact that even highly restricted spirocyclic amino acids like **7a–c** and **8a–c** exhibit activity as GABA uptake inhibitors increases our hopes of finding specific GABA uptake inhibitors with higher activity by modification of the spirocyclic ring system. Targets of current research are GABA analogues which are homologous to **7a–c** and **8a–c** and which are based on spirocycles with five-membered heterocyclic and/or carbocyclic rings. Although the diphenylpropyl-substituted derivative **8c** proved to be the most active in our series, we are hopeful that derivatives bearing a diphenylbutyl or diphenylpentyl substituent, which are presently in preparation, may be more active than **8c**.

Experimental protocols

Chemistry

All melting points were determined on a Kofler melting point apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on a Bruker WM-250 or AM-400 WB, using tetramethylsilane as an internal standard. Infrared spectra were measured on a Perkin Elmer 298 spectrophotometer. Mass spectra were detected on a MAT CH-7 by L Jirovetz. Microanalyses were determined by J Theiner (Institute of Physical Chemistry).

General method A. Preparation of lactams 3 and 4
The corresponding bromoalkyl lactone (20 mmol) [15] was dissolved in toluene (100 ml). The appropriate amine (100 mmol) was added and the mixture was refluxed for 16 h. The organic layer was washed with 2 M HCl (3 x 100 ml), dried (Na_2SO_4) and the solvent was evaporated at reduced pressure. The residue was purified by crystallization to give lactams **3** or **4**.

General method B. Preparation of amines 5 and 6
To the corresponding spirolactam (20 mmol) dissolved in dry THF (150 ml), was added $LiAlH_4$ (1 M in THF, 50 ml) at 0°C. The mixture was refluxed for 2 h, cooled to 0°C and hydrolysed by addition of H_2O (9 ml). After stirring for 2 h the slurry was filtered off and washed with ethyl acetate (50 ml). Evaporation of the solvent gave spirocyclic amines **5** or **6** (free

base), which was dissolved in ether (150 ml) and treated with HCl (1 M in ether, 25 ml). After evaporation of the solvent at reduced pressure the residue was recrystallized to yield spirocyclic amine hydrochlorides **5**•HCl or **6**•HCl.

General method C. Preparation of esters 7 and 8
The corresponding spirocyclic amine (free base, 20 mmol) was dissolved in acetone (100 ml), conc H_2SO_4 (20 mmol) was added at 0°C and the mixture was treated with Jones' reagent, which was prepared from CrO_3 (2.9 g, 29 mmol), conc H_2SO_4 (4.3 g, 44 mmol) and water (8.3 ml). After stirring for 3 h at room temperature isopropanol (20 ml) was added, the solvent was evaporated, the residue was suspended in toluene and the solvent was removed *in vacuo*. The residue was dissolved in methanol (100 ml), conc H_2SO_4 (2.9 g, 30 mmol) was added and the mixture was refluxed for 16 h. After removal of the solvent *in vacuo* the residue was dissolved in H_2SO_4 (1 M, 100 ml) and extracted with CH_2Cl_2 (3 x 100 ml). The organic layer was evaporated, the residue was suspended in NaOH (2 M, 100 ml) and extracted with ethyl acetate (3 x 100 ml). The organic layer was dried (Na_2SO_4) and the solvent was removed *in vacuo*. The residue was dissolved in a mixture of CH_2Cl_2 and ethyl acetate (9:1) and passed through a short column filled with silica gel (50 g). Combined eluates were evaporated to give the spirocyclic amino acid esters **7** or **8** (free base), which was dissolved in ether (150 ml) and treated with HCl (1 M in ether, 25 ml). After evaporation of the solvent the residue was recrystallized to give spirocyclic ester hydrochlorides **7**•HCl or **8**•HCl.

*(6RS, 7RS)-N-Benzyl-7-hydroxymethyl-2-azaspiro[5.5]undecanone **3a***
Preparation from **1** and benzylamine as described previously [15].

*(6RS, 7RS)-N-(2',2'-Diphenylethyl)-7-hydroxymethyl-2-azaspiro[5.5]undecanone **3b***
Preparation from **1** (5.2 g) and 2,2-diphenylethylamine (19.7 g), *Method A*. Crystallization from toluene gave **3b** (6.42 g, 85%, colourless crystals), mp 55°C. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.32–7.16 (m, 10H, aromatic-H), 4.40 (t, *J* = 8.0 Hz, 1H, H-2'), 4.08 (dd, *J* = 13.0 and 8.0 Hz, 1H, H-1'/l), 3.83 (dd, *J* = 13.0 and 8.0 Hz, 1H, H-1'/2), 3.77 (dd, *J* = 11.0 and 5.5 Hz, 1H, H-12/1), 3.58 (dd, *J* = 11.0 and 4.5 Hz, 1H, H-12/2), 2.95 (ddd, *J* = 12.0, 8.0 and 6.0 Hz, 1H, H-3/1), 2.89 (dt, *J* = 12.0 and 6.0 Hz, 1H, H-3/2), 2.08 (ddd, *J* = 13.0, 8.0 and 3.5 Hz, 1H, H-5/1), 2.02 (ddd, *J* = 13.0, 9.0 and 4.0 Hz, 1H, H-5/2), 1.70–1.50 (m, 5H), 1.48–1.27 (m, 5H), 1.08 (m, 1H). IR (KBr) 1605 cm^{-1} (delta-lactam). MS (70 eV) *m/z* (relative intensity) 377 (8%, M$^+$), 210 (100%, M$^+$ – 167), 167 (39%, Ph_2CH^+). Anal $C_{25}H_{31}NO_2$ (C, H, N).

*(6RS, 7RS)-N-(3',3'-Diphenylpropyl)-7-hydroxymethyl-2-azaspiro[5.5]undecanone **3c***
Preparation from **1** (5.2 g) and 3,3-diphenylpropylamine (21.1 g), *Method A*. Crystallization from toluene gave **3c** (5.09 g, 65%, colourless crystals), mp 110°C. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.31–7.08 (m, 10H, aromatic-H), 3.95–3.87 (m, H-3', H-12/1), 3.64 (dd, *J* = 12.0 and 4.0 Hz, 1H, H-12/2), 3.32–3.10 (m, 4H, H-3/1, H-3/2, H-1'/1, H-1'/2), 3.02 (m, 1H, OH), 2.33–2.20 (m, 3H, H-5/1, H-2'/1, H-2'/2), 2.15 (ddd, *J* = 13.0, 9.0 and 4.0 Hz, 1H, H-5/2), 1.94–1.50 (m, 7H), 1.50–1.24 (m, 3H), 1.18 (m, 1H). IR (KBr) 1615 cm^{-1} (delta-lactam). MS (70 eV) *m/z* (relative intensity) 391 (27%, M$^+$), 211 (100%, M$^+$ – $Ph_2C{=}CH_2$), 167 (36%, Ph_2CH^+). Anal $C_{26}H_{33}NO_2$ (C, H, N).

*(6RS, 7SR)-N-Benzyl-7-hydroxymethyl-2-azaspiro[5.5]undec-9-enone **4a***
Preparation from **2** and benzylamine as described previously [15].

*(6RS, 7SR)-N-(2',2'-Diphenylethyl)-7-hydroxymethyl-2-azaspiro[5.5]undec-9-enone **4b***
Preparation from **2** (5.18 g) and 2,2-diphenylethylamine (19.7 g), *Method A*. Crystallization from toluene gave **4b** (6.38 g, 85%, colourless crystals), mp 147°C. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.30–7.20 (m, 10H, aromatic-H), 5.57 (m, 1H, H-10), 5.52 (m, 1H, H-9), 4.43 (t, *J* = 8.0 Hz, 1H, H-2'), 4.17 (dd, *J* = 12.0 and 8.0 Hz, 1H, H-1'/1), 3.94 (ddd, *J* = 11.0, 8.0 and 3.0 Hz, 1H, H-12/1), 3.76 (dd, *J* = 12.0 and 8.0 Hz, 1H, H-1'/2), 3.49 (dd, *J* = 11.0 and 3.0 Hz, 1H, OH), 3.37 (dt, *J* = 4.0 and 11.0 Hz, 1H, H-12/2), 2.91 (m, 1H), 2.91 (t, *J* = 6.0 Hz, 2H, H-3), 2.19 (m, 1H), 1.93–1.38 (m, 7H). IR (KBr) 1600 cm^{-1} (delta-lactam). MS (70 eV) *m/z* (relative intensity) 375 (9%, M$^+$), 208 (100%, M$^+$ – 167), 167 (43%, Ph_2CH^+). Anal $C_{25}H_{29}NO_2$ (C, H, N).

*(6RS, 7SR)-N-(3',3'-Diphenylpropyl)-7-hydroxymethyl-2-azaspiro[5.5]undec-9-enone **4c***
Preparation from **2** (5.18 g) and 3,3-diphenylpropylamine (21.1 g), *Method A*. Crystallization from toluene gave **4c** (7.25 g, 93%, colourless crystals), mp 132°C. ^{1}H-NMR ($CDCl_3$, 250 MHz) δ 7.28 (m, 10H, aromatic-H), 5.55 (m, 2H, H-9, H-10), 4.03 (dd, *J* = 12.0 and 8.0 Hz, 1H, H-12/1), 3.93 (t, *J* = 7.5 Hz, 1H, H-3'), 3.45–3.10 (m, 5H, H-3/1, H-3/2, H-12/2, H-1'/1, H-1'/2), 2.99 (m, 1H), 2.4–2.15 (m, 3H), 2.00–1.40 (m, 7H). IR (KBr) 1615 cm^{-1} (delta-lactam). MS (70 eV) *m/z* (relative intensity) 389 (1%, M$^+$), 209 (11%, M$^+$ – $Ph_2C{=}CH_2$), 167 (100%, Ph_2CH^+). Anal $C_{26}H_{31}NO_2$ (C, H, N).

*(6RS, 7RS)-N-Benzyl-7-hydroxymethyl-2-azaspiro[5.5]undecane **5a***
Prepared by reduction of **3a** with $LiAlH_4$ as described previously [15].

*(6RS, 7RS)-N-(2',2'-Diphenylethyl)-7-hydroxymethyl-2-azaspiro[5.5]undecane **5b***
Starting material **3b** (7.55 g), *Method B*, gave **5b** (6.76 g, 93%, yellow oil) and **5b**•HCl by crystallization from ether (5.6 g, 70%, discoloured crystals), mp 118–120°C. ^{1}H-NMR (free base, $CDCl_3$, 400 MHz) δ 7.32–7.13 (m, 10H, aromatic-H), 5.12 (m, 1H, OH), 4.23 (dd, *J* = 8.0 and 6.0 Hz, 1H, H-2'), 3.77 (dd, *J* = 12.0 and 3.0 Hz, 1H, H-12/1), 3.44 (dd, *J* = 12.0 and 4.0 Hz, 1H, H-12/2), 3.10 (dd, *J* = 13.0 and 8.0 Hz, 1H, H-1'/1), 2.93 (dd, *J* = 13.0 and 6.0 Hz, 1H, H-1'/2), 2.92 (m, 1H, H-3/1), 2.85 (d, *J* = 10.0 Hz, 1H, H-1/1), 1.93 (m, 1H), 1.75–1.00 (m, 14H). IR (KBr) 3370 cm^{-1} (NH, OH). MS (70 eV) *m/z* (relative intensity) = 363 (1%, M$^+$), 196 (100%, M$^+$ – Ph_2CH), 167 (4%, Ph_2CH^+). Anal $C_{25}H_{33}NO$•HCl (C, H, N, Cl).

*(6RS, 7RS)-N-(3',3'-Diphenylpropyl)-7-hydroxymethyl-2-azaspiro[5.5]undecane **5c***
Starting material **3c** (7.83 g), *Method B*, gave **5c** (6.65 g, 88%, yellow oil) and **5c**•HCl by crystallization from ether (5.3 g, 64%, discoloured crystals), mp 113°C. ^{1}H-NMR (free base, $CDCl_3$, 400 MHz) δ 7.31–7.12 (m, 10H, aromatic-H), 3.99 (dd, *J* = 12.0 and 2.0 Hz, 1H, H-12/1), 3.89 (m, 1H, H-3'), 3.46 (dd, *J* = 12.0 and 4.0 Hz, 1H, H-12/2), 3.10 (d, *J* = 11.0 Hz, 1H, H-1/1), 2.91 (m, 1H, H-3/1), 2.35–2.25 (m, 4H, H-1', H-2'), 1.96–1.70 (m, 3H), 1.70–1.55 (m, 3H), 1.55 (d, *J* = 11.0 Hz, 1H, H-1/2), 1.45–1.25 (m, 6H), 1.20–1.07 (m, 2H). IR (KBr) 3360 cm^{-1} (NH, OH). MS (70 eV) *m/z* (relative intensity) 377 (35%, M$^+$), 196 (100%, M$^+$ – $Ph_2C{=}CH_2$ – H), 167 (11%, Ph_2CH^+). Anal $C_{26}H_{35}NO$•HCl (C, H, N, Cl).

(6RS, 7SR)-N-Benzyl-7-hydroxymethyl-2-azaspiro[5.5]undec-9-ene ***6a***
Prepared by reduction of **4a** with $LiAlH_4$ as described previously [15].

(6RS, 7SR)-N-(2',2'-Diphenylethyl)-7-hydroxymethyl-2-azaspiro[5.5]undec-9-ene ***6b***
Starting material **4b** (7.51 g), *Method B*, gave **6b** (7.01 g, 97%, yellow oil) and **6b**•HCl by crystallization from ether (5.41 g, 68%, discoloured crystals), mp 165–167°C. ^{1}H-NMR (free base, $CDCl_3$, 250 MHz) δ 7.35–7.15 (m, 10H, aromatic-H), 5.6 (m, 2H, H-9, H-10), 4.12 (m, 1H, H-2'), 3.71 (dd, *J* = 13.0 and 2.0 Hz, 1H, H-12/1), 3.30–2.95 (m, 5H, H-1/1, H-3/1, H-12/2, H-1'), 2.60–2.10 (m, 3H), 2.00–1.80 (m, 5H), 1.60–1.40 (m, 3H). IR (KBr) 3310 cm^{-1} (NH, OH). MS (70 eV) *m/z* (relative intensity) 361 (1%, M$^+$), 194 (100%, M$^+$ – Ph_2CH), 167 (2%, Ph_2CH^+). Anal $C_{25}H_{31}NO$•HCl (C, H, N, Cl).

(6RS, 7SR)-N-(3',3'-Diphenylpropyl)-7-hydroxymethyl-2-azaspiro[5.5]undec-9-ene ***6c***
Starting material **4c** (7.79 g), *Method B*, gave **6c** (6.39 g, 85%, yellow oil) and **6c**•HCl by crystallization from ether (5.27 g, 64%, discoloured crystals), mp 85–86°C. ^{1}H-NMR (free base, $CDCl_3$, 250 MHz) δ 7.31–7.11 (m, 10H, aromatic-H), 5.64 (m, 1H, H-10), 5.48 (m, 1H, H-9), 4.01 (dd, *J* = 12.0 and 2.0 Hz, 1H, H-12/1), 3.89 (m, 1H, H-3'), 3.45 (dd, *J* = 12.0 and 1.0 Hz, 1H, H-12/2), 2.95 (d, *J* = 12.0 Hz, 1H, H-1/1), 2.60–2.13 (m, 5H), 2.02–1.81 (m, 4H), 1.72–1.56 (m, 4H), 1.48 (d, *J* = 12.0 Hz, 1H, H-1/2), 1.23 (m, 1H). IR (KBr) 3380 cm^{-1} (NH, OH). MS (70 eV) *m/z* (relative intensity) 375 (51%, M$^+$), 194 (100%, M$^+$ – $Ph_2C{=}CH_2$ – H), 167 (11%, Ph_2CH^+). Anal $C_{26}H_{33}NO$•HCl (C, H, N, Cl).

(6RS, 7RS)-Methyl-N-benzyl-2-azaspiro[5.5]undecane-7-carboxylate ***7a***
Starting material **5a** (5.47 g), *Method C*, gave **7a** (2.83 g, 47%, yellow oil) and **7a**•HCl by crystallization from ether (2.7 g, 40%, discoloured crystals), mp 202–205°C. ^{1}H-NMR (free base, $CDCl_3$, 400 MHz) δ 7.35–7.18 (m, 5H, aromatic-H), 3.60 (s, 3H, OCH_3), 3.44 (d, *J* = 14.0 Hz, 1H, H-1'/1), 3.36 (d, *J* = 14.0 Hz, 1H, H-1'/2), 2.65–2.30 (m, 3H), 2.20–2.00 (m, 2H), 1.75–1.40 (m, 7H), 1.36–1.20 (m, 3H), 1.05–0.75 (m, 2H). IR (KBr) 1730 cm^{-1} (ester). MS (70 eV) *m/z* (relative intensity) 301 (22%, M$^+$), 270 (5%, M$^+$ – CH_3O), 210 (40%, M$^+$ – $PhCH_2$), 91 (100%, $PhCH_2^+$). Anal $C_{19}H_{27}NO_2$•HCl (C, H, N, Cl).

(6RS, 7RS)-Methyl-N-(2',2'-diphenylethyl)-2-azaspiro[5.5]undecane-7-carboxylate ***7b***
Starting material **5b** (7.27 g), *Method C*, gave **7b** (6.19 g, 79%, yellow oil) and **7b**•HCl by crystallization from ether (3.51 g, 41%, discoloured crystals), mp 119–120°C. ^{1}H-NMR (free base, $CDCl_3$, 400 MHz) δ 7.35–7.10 (m, 10H, aromatic-H), 4.16 (t, *J* = 8.0 Hz, 1H, H-2'), 3.60 (s, 3H, OCH_3), 2.84 (d, *J* = 8.0 Hz, 2H, H-1'), 2.74–2.60 (m, 2H), 2.18–1.97 (m, 2H), 1.70–1.05 (m, 11H), 1.05–0.75 (m, 2H). IR (KBr) 1730 cm^{-1} (ester). MS (70 eV) *m/z* (relative intensity) 391 (0.5%, M$^+$), 360 (2%, M$^+$ – CH_3O), 224 (100%, M$^+$ – Ph_2CH), 167 (3%, Ph_2CH^+). Anal $C_{26}H_{33}NO_2$•HCl (C, H, N, Cl).

(6RS, 7RS)-Methyl-N-(3',3'-diphenylpropyl)-2-azaspiro[5.5]undecane-7-carboxylate ***7c***
Starting material **5c** (7.55 g), *Method C*, gave **7c** (6.49 g, 80%, yellow oil) and **7c**•HCl by crystallization from ether (4.95 g, 56%, discoloured crystals), mp 202–204°C. ^{1}H-NMR (free base, $CDCl_3$, 400 MHz) δ 7.30–7.12 (m, 10H, aromatic-H), 4.04 (t, *J* = 7.0 Hz, 1H, H-3'), 3.58 (s, 3H, OCH_3), 2.65–2.30 (m, 3H), 2.23–2.06 (m, 3H), 2.05–1.90 (m, 2H), 1.76–1.57 (m, 4H), 1.54–1.38 (m, 3H), 1.37–1.20 (m, 3H), 0.99 (m, 1H), 0.87 (m, 1H). IR (KBr) 1725 cm^{-1} (ester). MS (70 eV) *m/z* (relative intensity) 405 (32%, M$^+$), 374 (3%, M$^+$ – CH_3O), 224 (100%, M$^+$ – $Ph_2C{=}CH_2$ – H), 167 (7%, Ph_2CH^+). Anal $C_{27}H_{35}NO_2$•HCl (C, H, N, Cl).

(6RS, 7SR)-Methyl-N-benzyl-2-azaspiro[5.5]undec-9-ene-7-carboxylate ***8a***
Starting material **6a** (5.43 g), *Method C*, gave **8a** (3.95 g, 66%, yellow oil) and **8a**•HCl by crystallization from 2-propanol (2.62 g, 39%, yellow crystals), mp 185°C. ^{1}H-NMR (free base, $CDCl_3$, 400 MHz): δ 7.33–7.16 (m, 5H, aromatic-H), 5.65 (m, 1H, H-10), 5.56 (m, 1H, H-9), 3.58 (s, 3H, OCH_3), 3.46 (d, *J* = 14.0 Hz, 1H, H-1'/1), 3.39 (d, *J* = 14.0 Hz, 1H, H-1'/2), 2.77–2.60 (m, 2H), 2.53 (m, 1H), 2.42 (m, 1H), 2.30–2.10 (m, 4H), 1.80–1.47 (m, 3H), 1.40–1.20 (m, 2H). IR (KBr) 1730 cm^{-1} (ester). MS (70 eV) *m/z* (relative intensity) = 299 (61%, M$^+$), 268 (5%, M$^+$ – CH_3O), 208 (19%, M$^+$ – $PhCH_2$), 91 (100%, $PhCH_2^+$). Anal $C_{19}H_{25}NO_2$•HCl (C, H, N, Cl).

(6RS, 7SR)-Methyl-N-(2',2'-diphenylethyl)-2-azaspiro[5.5]-undec-9-ene-7-carboxylate ***8b***
Starting material **6b** (7.23 g), *Method C*, gave **8b** (6.54 g, 84%, yellow oil) and **8b**•HCl by crystallization from ether (6.31 g, 74%, discoloured crystals), mp 160°C. ^{1}H-NMR (free base, $CDCl_3$, 400 MHz) δ 7.30–7.12 (m, 10H, aromatic-H), 5.56 (m, 1H, H-10), 5.47 (m, 1H, H-9), 4.15 (t, *J* = 8.0 Hz, 1H, H-2'), 3.63 (s, 3H, OCH_3), 2.85 (d, *J* = 8.0 Hz, 2H, H-1'), 2.70–2.52 (m, 2H), 2.42 (m, 1H), 2.30–2.00 (m, 4H), 1.60–1.46 (m, 2H), 1.45–1.35 (m, 2H), 1.34–1.20 (m, 2H). IR (KBr) 1730 cm^{-1} (ester). MS (70 eV) *m/z* (relative intensity) 389 (0.2%, M$^+$), 358 (1%, M$^+$ – CH_3O), 222 (100%, M$^+$ – Ph_2CH), 167 (4%, Ph_2CH^+). Anal $C_{26}H_{31}NO_2$•HCl (C, H, N, Cl).

(6RS, 7SR)-Methyl-N-(3',3'-diphenylpropyl)-2-azaspiro[5.5]-undec-9-ene-7-carboxylate ***8c***
Starting material **6c** (7.51 g), *Method C*, gave **8c** (7.26 g, 90%, yellow oil) and **8c**•HCl by crystallization from ether (5.19 g, 59%, discoloured crystals), mp 79°C. ^{1}H-NMR (free base, $CDCl_3$, 400 MHz) δ 7.30–7.12 (m, 10H, aromatic-H), 5.69 (m, 1H, H-10), 5.62 (m, 1H, H-9), 4.05 (t, *J* = 7.0 Hz, 1H, H-3'), 3.59 (s, 3H, OCH_3), 2.70 (m, 1H), 2.57–1.91 (m, 10H), 1.80–1.45 (m, 3H), 1.42–1.20 (m, 3H). IR (KBr) 1730 cm^{-1} (ester). MS (70 eV) *m/z* (relative intensity) 403 (44%, M$^+$), 372 (2%, M$^+$ – CH_3O), 222 (100%, M$^+$ – $Ph_2C{=}CH_2$ – H), 167 (9%, Ph_2CH^+). Anal $C_{27}H_{33}NO_2$•HCl (C, H, N, Cl).

Biochemistry

GABA-A receptor assay
The [^{3}H]-muscimol radioreceptor assay was a modification of the method of Beaumont *et al* [16].

Brain membranes. A bovine brain was obtained from a local slaughter house. The cerebral cortex was dissected and homogenized in 6 volumes of 0.32 M sucrose containing 1 mM K_2HPO_4 and 1 mM $MgCl_2$ with a glass-Teflon homogenizer. The suspension was sedimented at 1000 *g* for 10 min and the resulting supernatant was centrifuged at 8000 *g* for 15 min at 4°C. The pellets were resuspended in the same volume of 1 mM Tris-HCl buffer (pH 7.0) containing 2 mM EDTA and left for 40 min at 4°C. The material was centrifuged twice at 30 000 *g* for 20 min and the pellets were suspended in 80 ml of 50 mM Tris-citrate buffer (pH 7.2) at 20°C. Aliquots (2 ml)

were distributed into cryovials, frozen in liquid propane and stored in liquid nitrogen. Just before use, the needed amount of membranes were thawed quickly in a water-bath at 37°C.

Radioreceptor assay. The thawed membranes were centrifuged at 37 000 *g* for 15 min and the pellet resuspended in the same buffer. This step was repeated. The radioreceptor assay was performed in 1 ml 50 mM Tris-citrate buffer (pH 7.2) containing 300–500 μg membrane protein, 5 nM [^{3}H]muscimol (10.3 Ci/mmol, NEN) and compounds to be tested. Incubation was performed at 22°C for 15 min. The reaction was terminated by rapid filtration through Whatman GF/B filters (presoaked in 0.2% polyethyleneimine) and buffer. The filter-bound radioactivity was counted in Irgascint A300 (Ciba-Geigy) in an Intertechnique scintillation counter. Non-specific binding was defined by 10^{-5} M GABA. Assays were performed in triplicate. IC_{50} values were obtained by computer-aided curve fitting, according to a single-site model.

GABA-B receptor assay

The [^{3}H]-CGP 27492 radioreceptor assay described by Bittiger *et al* [17] was used on male rats (Tif: RAI f (SPF)) of about 200 g body weight.

Brain membranes. The animals were decapitated, the brains removed, the cerebral cortices dissected and homogenized in 10 volumes of ice-cold 0.32 M sucrose, containing $MgCl_2$ (1 mM) and K_2HPO_4 (1 nM), with a glass/teflon homogenizer. The membranes were centrifuged at 750 *g*, the pellets resuspended and the centrifugation repeated. The supernatants were pooled and centrifuged at 18 000 *g* for 15 min. The pellet was osmotically shocked in 5 ml H_2O and kept on ice for 30 min. The suspension was centrifuged at 39 000 *g*, resuspended in Krebs–Henseleit buffer (pH 7.4) containing 20 mM Tris, and kept for 2 d at –20°C. The membranes were thawed at room temperature, washed three times with Krebs–Hanseleit buffer by centrifugation at 18 000 *g* for 15 min, left overnight at 4°C and washed again three times. The final pellet was resuspended with a glass/teflon homogenizer in 20 ml of the same buffer. Aliquots (2 ml) were frozen and stored in liquid nitrogen. Just before use membranes were thawed quickly in a water bath at 37°C and again washed by centrifugation at 18 000 *g* for 15 min with the same buffer three times.

Radioreceptor assay. The radioreceptor assay was performed in 2 ml Krebs–Hanseleit buffer (pH 7.4) containing 20 mM Tris, 200–300 μg membrane protein, 2 nM [^{3}H]CGP 27492 (15.0 Ci/mmol, Ciba-Geigy, Horsham, UK) and the compound to be tested. The incubation was performed at 20°C for 40 min and terminated by rapid filtration on Whatman GF/B glass fiber filters, which were washed twice with 5 ml ice-cold buffer. Filter-bound radioactivity was counted in Irgascint A300 (Ciba-Geigy). Incubation were performed in triplicate and non-specific binding was determined in the presence of 10 μM (–)-lioresal. IC_{50} values were obtained by computer-aided curve fitting, according to single-site model.

GABA reuptake assay

The uptake of [^{3}H]-GABA into rat midbrain synaptosomes was studied. Mesencephali and diencephali of male Tif: RAIf (SPF) rats (Tierfarm Sisseln, Switzerland) weighing 200 g were homogenized in ice-cold 0.32 M sucrose (tissue/sucrose 1:10) with a teflon grinder according to the method of Yunger *et al* [18]. Nuclei and cell debris were removed by centrifugation at 1000 *g* for 10 min at 4°C. The supernatant homogenate was centrifuged at 16 500 *g* for 20 min at 4°C, and the resulting pellet was washed with 0.32 M sucrose and then resuspended in one half of the original volume of 0.32 M glucose (final concentration of glucose in the incubation sample: 8 mM). The homogenate was diluted 1:20 with Krebs–Ringer bicarbonate buffer (KRB; composition (in mmol/l): NaCl, 118; KCl, 4.8; $CaCl_2$, 2.7; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25; γ-aminoxyacetic acid, 0.01). The buffer was equilibrated with 5% CO_2 in O_2. Portions of the synaptosomal solution (250 μl) was pipetted into Beckman Biovials. The compounds to be tested were solved in KRB and added in a volume of 200 μl. The samples were preincubated at 37°C for 15 min under an atmosphere of 5% CO_2 in O_2. After cooling to 0°C 50 μ [2,3-^{3}H(N)]-GABA (diluted with unlabeled GABA to a final specific concentration of 0.25 Ci/mmol, New England Nuclear, Boston, USA) was added to yield a final concentration of 0.7 μM and the incubation was continued for another 3 min. Blanks were incubated at 0°C in the presence of 1 mM nipecotic acid. The incubation was stopped by cooling to 0°C. The synaptosomes were collected on 'Millipore'-filters (0.45 μm). The dried filters were counted in 10 ml toluene containing 0.6% butyl-PBD.

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References

1 Roberts E, Chase TN, Tower DB (1976) *In: Kroc Foundation Series*, Vol 5 Raven Press, New York, USA
2 Allan RD, Johnston GAR (1983) *Med Res Rev* 3, 91–118
3 Krogsgaard-Larsen P, Lenicque P, Jacobsen P (1984) *In: CRC Handbook of Stereoisomers: Drugs in Psychopharmacology* (Smith DF, ed) CRC Press, Boca Raton, USA, 369–398
4 Krogsgaard-Larsen P, Scheel-Krüger J, Kofod H (1979) *GABA-Neurotransmitters: Pharmacochemical, Biochemical and Pharmacological Aspects*. Munksgaard, Copenhagen, Denmark
5 Chase TN, Wexler NS, Barbeau A (1988) *Huntington's Disease*. Raven Press, New York, USA
6 Avoli M (1988) *In: Neurotransmitters and Cortical Functions: From Molecules to Mind* (Avoli M, Reader TA, Dykes RW, Gloor P, eds) Plenum Press, New York, USA, p 187
7 Nistico G, Engel J, Fariello RG, Lloyd KG, Morselli PL (1986) *Neurotransmitters, Seizures, and Epilepsy III*. Raven Press, New York, USA
8 Nielsen L, Brehm L, Krogsgaard-Larsen P (1990) *J Med Chem* 33, 71–77
9 Croucher MJ, Meldrum BS, Krogsgaard-Larsen P (1983) *Eur J Pharmacol* 89, 217–228
10 Löscher W (1982) *Neuropharmacol* 21, 803–810
11 Yunger LM, Fowler PJ, Zarevics P, Setler PE (1984) *J Pharmacol Exp Ther* 228, 109–115
12 Mostler U, Urban E (1989) *Monatsh Chem* 120, 349–355
13 Stejskal R, Urban E, Völlenkle H (1991) *Monatsch Chem* 122, 145–156
14 Rachenzentner A, Urban E (1992) *Arch Pharm (Weinheim)* 325, 101–105
15 Dostal C, Lauritz S, Urban E (1992) *Heterocycles* 34, 135–148
16 Beaumont K, Chilton WS, Yamamura HI, Enna SJ (1978) *Brain Res* 148, 153–162
17 Bittiger H, Reymann N, Hull R, Kane P (1988) *Eur J Neurosci* Suppl, Abstr 16.10
18 Yunger LM, Moonsammy GI, Rush JA (1983) *Neurochem Res* 8, 757–769
19 Hollauf G, Urban E (1994) *Heterocycles* 38, 2295–2308