that did not ovulate at a dose of x μg of analogue. The in vitro histamine-release activity of each analogue was determined by using peritoneal cells from male Sprague–Dawley rats in a standard assay,[14] and the results are given as the ED_{50} values expressed in micrograms/milliliter (standard compound 48/80 has an ED_{50} of 0.58 in this assay system). The results are given in Table IV.

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Synthesis and Pharmacological Evaluation of γ-Aminobutyric Acid Analogues. New Ligand for $GABA_B$ Sites[1]

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Baclofen (β-*p*-chlorophenyl-GABA) is the only selective agonist for the bicuculline-insensitive $GABA_B$ receptor. We report the synthesis of new GABA analogues and baclofen analogues. In vitro, two compounds, 4-amino-3-benzo[*b*]furan-2-ylbutanoic acid (**9g**) and 4-amino-3-(5-methoxybenzo[*b*]furan-2-yl)butanoic acid (**9h**), showed an affinity for the $GABA_B$ receptor. The results obtained with racemic compounds of benzofuran structure, new for this series, and the surprising inactivity of compound **3a** (4-amino-3-(4-hydroxyphenyl)butanoic acid) permit the proposal of an hypothesis for the structure–activity relationships with regard to $GABA_B$ receptor.

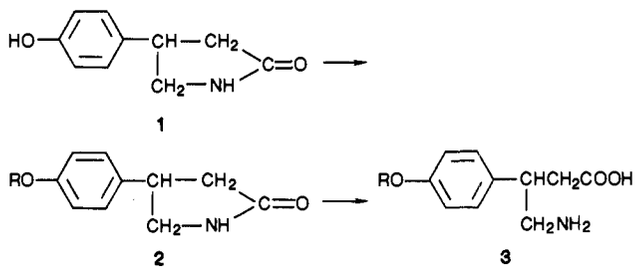
γ-Aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the central nervous system.[2,3] GABA is involved in the regulation of a variety of physiological mechanisms[4,5] and implicated in the pathophysiology of several central nervous system diseases.[6] Therefore, a variety of compounds with properties of GABA have been investigated,[7-9] essentially GABA agonists, GABA antagonists, and GABA uptake inhibitors. Two subclasses of receptors for GABA have been defined and designated $GABA_A$ and $GABA_B$ receptors.[10,11] $GABA_A$ receptors are selectively activated by the GABA analogue muscimol and blocked by the convulsants such as bicuculline or picrotoxin. A selective agonist for the $GABA_B$ receptor is β-*p*-chlorophenyl-GABA (baclofen).[11] Until now, recent papers have investigated essentially agonists and antagonists of $GABA_A$ receptor. In contrast for $GABA_B$ receptor, few compounds were studied and activities and consequently structure–activity relationships were practically unknown.[12] The present paper describes the synthesis of new baclofen racemic analogues and the binding studies at $GABA_A$ and $GABA_B$ receptors.

Chemistry

Scheme I illustrates the procedure used for the synthesis of compounds **3a–e**. Lactam **1** (prepared according to a procedure described elsewhere[13]) was treated with alkyl chloride or alkylaryl chloride in absolute alcohol with sodium to give ethers **2**. The hydrolysis of **2a–e** in alkaline condition furnished the GABA analogues **3a–e**. The compounds are characterized as free base or hydrochloride.

The analogues of GABA with benzofuran or benzoxazol structure were synthesized according to Scheme II. A Reformatsky reaction of compounds **4** gave the α,β-unsaturated esters **5**. Esters **5** were treated with NBS in dry CCl_4 to furnish the bromo esters **6**, which were treated with a large excess of liquid ammonia in THF to give the unsaturated lactams **7**. The hydrogenation of **7g–h** at atmospheric pressure lead to compounds **8g,h**. Compounds **8f,i,j** were prepared by hydrogenation in an autoclave of

Scheme I



a, R = H; **b**, R = *i*-Pr; **c**, R = $CH_2C_6H_5$; **d**, R = CH_2-4-FC_6H_4; **e**, R = CH_2-5-Cl-2-thienyl

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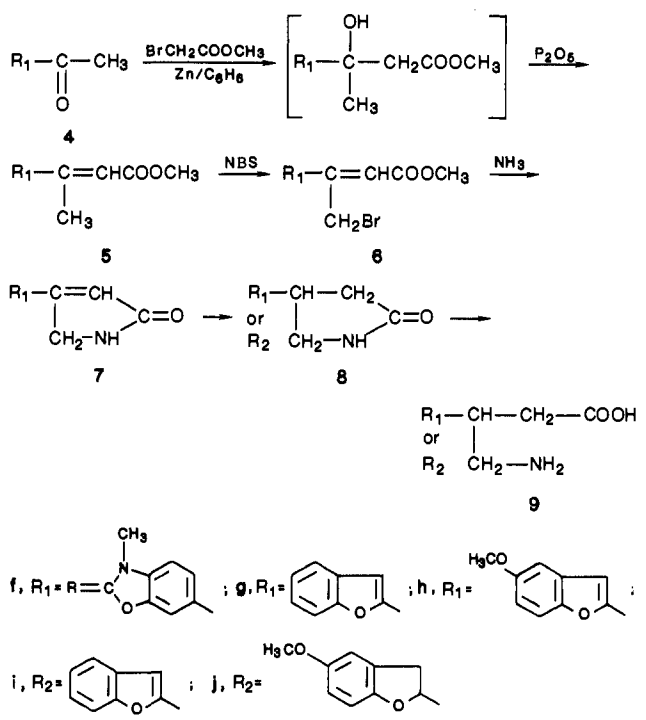
Table I. Physical Data

compd	R, R_1, R_2	yield, %	mp, °C	recryst solvent	formula	anal.	method
2b	$(CH_3)_2CH$	45	110	H_2O	$C_{13}H_{17}NO_2$	C, H, N, O	A
2c	$PhCH_2$	85	151	EtOH (95°)	$C_{17}H_{17}NO_2$	C, H, N, O	A
2d	4-$FPhCH_2$	85	142	EtOH (95°)	$C_{17}H_{16}NO_2F$	C, H, N, F	A
2e	5-chloro-2-thienylmethyl	85	118	EtOH (95°)	$C_{15}H_{14}NO_2SCl$	C, H, N, O, S, Cl	A
3b	$(CH_3)_2CH$	80	210–213 dec	H_2O	$C_{13}H_{19}NO_3$	C, H, N, O	B_2
3c	$PhCH_2$	70	220–224 dec	EtOH (95°)	$C_{17}H_{20}NO_3Cl$	C, H, N, O, Cl	B_1
3d	4-$FPhCH_2$	74	215–218 dec	EtOH (90°)	$C_{17}H_{19}NO_3ClF$	C, H, N, Cl, F	B_1
3e	5-chloro-2-thienylmethyl	53	188–193 dec	EtOH (95°)	$C_{15}H_{16}NO_3SCl$	C, H, N, O, S, Cl	B_2
5f	3-methyl-2-oxobenzoxazol-6-yl	70	139–140	EtOH	$C_{13}H_{13}NO_4$	C, H, N, O	C
5g	benzo[*b*]furan-2-yl	75	86	petroleum ether	$C_{13}H_{12}O_3$	C, H, O	C
5h	5-methoxybenzo[*b*]furan-2-yl	80	128–129	EtOH (95°)	$C_{14}H_{14}O_4$	C, H, O	C
6f	3-methyl-2-oxobenzoxazol-6-yl	55	148–149	EtOH	$C_{13}H_{11}O_3Br$	C, H, N, O, Br	D
6g	benzo[*b*]furan-2-yl	70	110	$CHCl_3$	$C_{13}H_{11}O_3Br$	C, H, O, Br	D
6h	5-methoxybenzo[*b*]furan-2-yl	45	99–100	EtOH (95°)	$C_{14}H_{13}O_4Br$	C, H, O, Br	D
7f	3-methyl-2-oxobenzoxazol-6-yl	45	300 dec	DMF	$C_{12}H_{10}N_2O_3$	C, H, N, O	E
7g	benzo[*b*]furan-2-yl	60	220–225 dec	EtOH (95°)	$C_{12}H_9NO_2$	C, H, N, O	E
7h	5-methoxybenzo[*b*]furan-2-yl	65	248–255 dec	EtOH	$C_{13}H_{11}NO_3$	C, H, N, O	E
8f	3-methyl-2-oxobenzoxazol-6-yl	80	225	EtOH	$C_{12}H_{12}N_2O_3$	C, H, N, O	F
8g	ref 19						
8h	ref 19						
8i	2,3-dihydrobenzo[*b*]furan-2-yl	80	151	H_2O	$C_{12}H_{13}NO_2$	C, H, N, O	F
8j	2,3-dihydro-5-methoxybenzo[*b*]furan-2-yl	65	148–150	EtOH (95°)	$C_{13}H_{15}NO_3$	C, H, N, O	F
9f	3-methyl-2-oxobenzoxazol-6-yl	30	252	EtOH	$C_{13}H_{15}N_2O_4Cl$	C, H, N, O, Cl	H
9g	benzo[*b*]furan-2-yl	90	210–214 dec	H_2O	$C_{12}H_{13}NO_3$	C, H, N, O	I
9h	5-methoxybenzo[*b*]furan-2-yl	70	185–190 dec	EtOH (95°)	$C_{13}H_{15}NO_4$	C, H, N, O	I
9i	2,3-dihydrobenzo[*b*]furan-2-yl	80	228–232 dec	H_2O	$C_{12}H_{15}NO_3$	C, H, N, O	I
9j	2,3-dihydro-5-methoxybenzo[*b*]furan-2-yl	95	180–185 dec	EtOH (95°)	$C_{13}H_{17}NO_4$	C, H, N, O	I

Scheme II



the derivatives **7g,h**. Amino acids **9f–j** were obtained by refluxing in either acidic or alkaline conditions. Table I lists the physical data of the synthesized compounds.

Biological Results

All the compounds were tested for their ability to displace [^{3}H]GABA from rat brain membranes ($GABA_A$ sites) and also to displace [^{3}H]baclofen ($GABA_B$ sites) from rat brain membranes. The results of these experiments were shown in Table II.

$GABA_A$ Sites. In Tris-citrate buffer, pH 7.1, all the compounds tested (up to 100 μM) failed to displace more than 20% of the tritium specifically bound to $GABA_A$ receptors. The addition of increasing concentrations of

Table II. Binding Results

compound	IC_{50},[a] μM [^{3}H]GABA binding ($GABA_A$)	IC_{50},[a] μM [^{3}H]baclofen binding ($GABA_B$)
GABA	0.03	0.03
muscimol	0.01	
baclofen		0.2
3a[c]	>100	>100
3b[c]	>100	
3c[c]	>100	>100
3d[c]	>100	>100
3e[c]	>100	
9f[b]	>100	>100
9g[b]	>100	18
9h[b]	>100	5.6
9i[c]	>100	>100
9j[b]	>100	>100

[a] Results were means of two experiments done in triplicate. [b] Compounds (10 mM) were dissolved in 50 mM Tris buffer (pH 10.5) before further dilution in buffer of binding (less than 1% v/v of 50 mM Tris or 50 mM Tris buffer, pH 10.5). [c] Compounds (10 mM) were dissolved in Me_2SO before further dilution in buffer of binding (less than 1% v/v of Me_2SO).

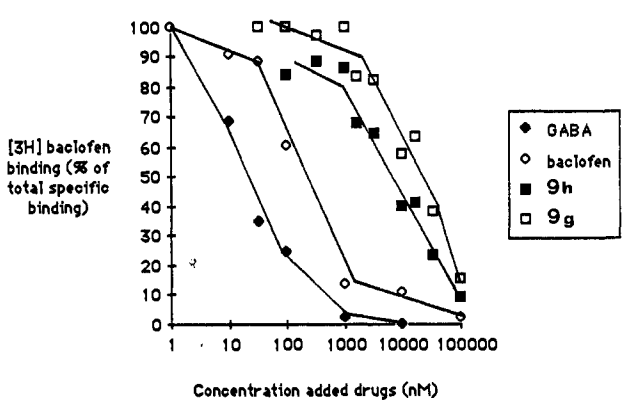


Figure 1. Displacement of [^{3}H]baclofen binding to rat crude synaptic membranes by drugs.

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unlabeled GABA and muscimol produced a dose-dependent reduction in binding. The IC_{50} values for GABA and muscimol were 0.03 and 0.01 μM, respectively.

$GABA_B$ Sites. In contrast, the two products **9g** and **9h** displaced binding of [^{3}H]baclofen to $GABA_B$ sites (Figure 1) on rat whole brain synaptic membranes. The degree of displacement was dependent on the concentration of compounds **9g** and **9h**. The IC_{50} values for **9g** and **9h** were 18 and 5.5 μM, respectively.

Discussion

The present data prove the specificity of baclofen analogues for the bicuculline-insensitive $GABA_B$ receptor. Indeed, as baclofen, none of the tested compounds displace [^{3}H]GABA from the $GABA_A$ receptor, even with higher concentrations.

For the $GABA_B$ receptor, compounds **9g** and **9h** displace [^{3}H]baclofen respectively with IC_{50} values of 18 and 5.6 μM. As baclofen, these compounds discriminate $GABA_A$ and $GABA_B$ receptors. Until now, because few products were studied, the structure–activity relationships at the $GABA_B$ receptor site are still unclear,[11,12] although some studies have been published.[14] The binding studies do not permit the discrimination between agonist and antagonist activities. However, from the present results, the following hypothesis can be proposed.

The higher activities of **9g** and **9h** compared with those of **9i** and **9j** establish the necessity that the C_3 atom of the GABA chain should be in the plane of the benzofuran ring. This is confirmed by the crystal structure of baclofen.[15] Likewise, the structure of the aromatic side chain of potential $GABA_B$ ligands should be planar (**9g** and **9h** are active, **9i** and **9j** are inactive).

Moreover, the $GABA_B$ receptor could accommodate molecules larger than baclofen.

Furthermore, with regard to the substitution possibilities, it is surprising to observe the whole inactivity of **3a**. Therefore, for the binding studies, the lipophilic feature and the steric hindrance are not the only features: **3a** is inactive (IC_{50} > 100 μM) while **9g** (IC_{50} = 18 μM) and **9h** (IC_{50} = 5.6 μM) are active. For **3a**, because of electronic properties, the hydroxyl group could hinder binding to the $GABA_B$ receptor site.

Experimental Section

Chemistry. Melting points were determined on a Büchi SMP 20 apparatus and are not corrected. IR spectra were recorded on a Beckman Acculab IV spectrometer. ^{1}H NMR were recorded with a Bruker WP 80 pulsed Fourier transform spectrometer using $(CH_3)_4$ Si as an internal standard, except for the compound dissolved in D_2O, where sodium 3-(trimethylsilyl)propanesulfonate was used. Elemental analyses were performed by CNRS–Vernaison and were in agreement with the proposed structures.

General Procedures for the Syntheses of Phenol Ethers 2b–e. Method A. Compound **1**[13] (1.77 g, 0.01 mol) was dissolved in 50 mL of ethanol containing 0.23 g of sodium. Alkyl chloride or arylalkyl chloride (0.01 mol) was added and the mixture was refluxed for 30 min. After reaction, the mixture was filtered, and after cooling, the crude product was precipitated. The precipitate was recrystallized in appropriate solvent.

Compound **2b** displayed the following: ^{1}H NMR ($CDCl_3$) δ 1.3 (d, 6 H, J = 9 Hz), 2.4–2.6 (m, 2 H), 3.3–3.8 (m, 3 H), 4.5 (m, 1 H, J = 9 Hz), 6.0 (s, 1 H), 6.85 (d, 2 H, J = 9 Hz), 7.2 (d, 2 H, J = 9 Hz).

General Procedures for the Syntheses of 4-Amino-3-substituted-butanoic Acids 3a–e. Lactams **2a–e** (0.005 mol) were refluxed for 1 h in 20 mL of alcohol (95°) and 5 mL of 10 N NaOH. After cooling, the mixture was acidified to pH 3 with 10% HCl solution (method B1) or neutralized to pH 7 with CH_3COOH solution (method B2). The amino acid hydrochloride or amino acid was filtered and recrystallized from the appropriate solvent.

Compound **3b** displayed the following: ^{1}H NMR (CF_3COOD) δ 1.5 (d, 6 H, J = 9 Hz), 2.9–3.1 (m, 2 H), 3.5–3.9 (m, 3 H), 4.75 (m, 1 H, J = 9 Hz), 7.2 (d, 2 H, J = 9 Hz), 7.4 (d, 2 H, J = 9 Hz).

General Procedures for the Syntheses of Methyl 3-Substituted But-2-enoate Derivatives 5f–h. Method C. Addition of methyl bromoacetate (45.9 g, 0.3 mol) to a stirred suspension of zinc (19.5 g, 0.3 mol) and acetyl derivatives **4f–h** (0.1 mol)[16–18] in anhydrous benzene (300 mL) at reflux temperature gave a complex which was hydrolyzed with 2 M H_2SO_4 (150 mL) and yielded crude methyl 3-substituted but-2-enoate derivatives **5f–h**, which was used for the next step without purification. A solution of **5f–h** (0.1 mol) and P_2O_5 (10 g) in 200 mL of toluene was refluxed for 3 h. The mixture was filtered after cooling and toluene was removed under vacuum. The residue was recrystallized from appropriate solvent.

Compound **5g** displayed the following: ^{1}H NMR ($CDCl_3$) δ 2.55 (d, 3 H, J = 1 Hz), 3.8 (s, 3 H), 6.7 (q, 1 H, J = 1 Hz), 7.0–7.7 (m, 5 H).

General Procedures for the Allylic Bromination of Methyl 3-Substituted But-2-enoate Derivatives 6f–h. Method D. The unsaturated esters **5f–h** (0.02 mol) were refluxed with NBS (3.92 g, 0.022 mol) in CCl_4 (150 mL) for 48 h. After cooling, the solution was filtered and the filtrate evaporated to dryness under vacuum. The crude product was recrystallized.

Compound **6g** displayed the following: ^{1}H NMR ($CDCl_3$) δ 3.75 (s, 3 H), 4.9 (s, 2 H), 6.7 (s, 1 H), 7.1–7.8 (m, 5 H).

General Procedures for the Syntheses of 4-Substituted 1,5-Dihydro-2*H*-pyrrol-2-one Derivatives 7f–h. Method E. Bromo esters **6f–h** (0.01 mol) were dissolved in 20 mL of THF, and the solution was added with stirring to 50 mL of liquid ammonia. After 3 h the solution was filtered and the precipitate washed with water and recrystallized.

Compound **7g** displayed the following: ^{1}H NMR (Me_2SO-d_6) δ 4.4 (s, 2 H), 6.4 (s, 1 H), 7.25–7.75 (m, 5 H), 8.25 (s, 1 H).

General Procedures for the Syntheses of Lactams 8f,i,j. Method F. The unsaturated lactams **7f–h** (0.01 mol) in 200 mL of ethanol were shaken at 45 °C for 4 h in an autoclave with freshly prepared Raney nickel catalyst under a pressure of 30 atm of hydrogen. The mixture was filtered, the filtrate evaporated, and the product, recrystallized from appropriate solvent.

Compound **8i** displayed the following: ^{1}H NMR ($CDCl_3$) δ 2.4 (m, 2 H), 2.7–3.6 (m, 5 H), 4.75 (m, 1 H), 6.3 (br s, 1 H), 6.7–7.3 (m, 4 H).

General Procedures for the Hydrogenation of 7g,h to 8g,h. Method G. The unsaturated lactams **7g,h** (0.01 mol) were shaken in 200 mL of ethanol with Raney nickel catalyst at room temperature under atmospheric pressure of hydrogen. The mixture was filtered, the filtrate evaporated, and the product recrystallized. Physical data of **8g–h** are in accordance with the literature.[19]

Hydrolysis of 8f to 4-Amino-3-(3-methyl-2-oxobenzoxazol-6-yl)butanoic Acid Hydrochloride (9f). Method H. A solution of **8f** (1.16 g, 0.005 mol) in 6 M HCl (50 mL) was refluxed for 1 h. The mixture was evaporated under vacuum and the residue recrystallized from alcohol (95°) affording **9f**: ^{1}H NMR (Me_2SO-d_6) δ 2.6–2.9 (m, 2 H), 2.9–3.7 (m, 3 H), 3.3 (s, 3 H), 7.2–7.5 (m, 3 H).

General Procedures for the Syntheses of 4-Amino-3-benzo[*b*]furan-2-ylbutanoic Acids 9g–j. Method I. A solution of **8g–j** (0.005 mol) in 20 mL of alcohol (95°) and 5 mL of 10 N NaOH was refluxed for 1 h. After reaction, the alcohol was

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evaporated under reduced pressure, and the solution was neutralized to pH 7 with diluted CH_3COOH. The precipitate was filtered and recrystallized in appropriate solvent.

Compound **9g** displayed the following: ^1H NMR (CF_3COOD) δ 3.2 (d, 2 H), 3.7-4 (m, 3 H), 6.8 (s, 1 H), 7.25-7.7 (m, 4 H).

Biochemical Assays. Crude synaptic membranes (CSM) were prepared from whole rat brain according to the method of Enna and Snyder.[20] Male Wistar rats (250 g) were killed by decapitation. Membranes from rat cerebral cortex were homogenized in ice-cold 0.32 M sucrose (20 mL for one brain) with a laboratory mixer (Silverson) for 60 s. The crude nuclear pellet (P_1) was isolated by centrifugation (4 °C) at 1000*g* for 10 min and discarded. The supernatant was recentrifuged (4 °C) for 20 min at 20000*g*. The crude mitochondrial pellet (P_2) from this centrifugation was lysed by resuspension in ice-cold water (20 mL for one brain). After homogenization with a laboratory mixer, the mixture was centrifugated (4 °C) for 20 min at 8000*g*. The supernatant and soft upper "buffy coat" of the pellet were collected and centrifuged (4 °C) at 48000*g* for 10 min to yield the crude synaptic membranes.

[^{3}H]GABA Binding Assay ($GABA_A$ Assay). CSM (P_4) were stored at -20 °C for at least 18 h before use (up to 2 months). After thawing, the membranes were resuspended in 50 mM Tris-citrate buffer, pH 7.1, containing Triton X-100 (0.05% v/v), and the homogenate was incubated at 37 °C for 30 min. The suspension was centrifuged (4 °C) for 10 min at 48000*g*. The resultant pellet was homogenized in ice-cold 50 mM Tris-citrate buffer, pH 7.1 (4.5 mL for one brain), with a Potter-Elvehjem homogenizer fitted with a Teflon pestle.

For the binding assay procedures, aliquots of synaptic membranes (0.5 mg of protein) were incubated at 4 °C for 5 min in 2 mL of Tris-citrate buffer containing 0.4 μCi of [^{3}H]GABA (4-amino-*n*-[2,3-^{3}H]butyric acid, Amersham) with a specific activity of 78 Ci/mM. Various concentrations of compounds to be tested were added. At the end of the incubation, the mixture was quickly filtered under vacuum through premoistened Whatman GF/C filters and washed with 10 mL of ice-cold Tris-citrate buffer. Filters were transfered to a scintillation vial containing 5 mL of HP/b Beckman scintillation fluid. The tritium content of each sample was estimated by liquid scintillation spectrometry. Nonspecific binding determined in the presence of 100 μM of muscimol represented less than 10.8% of the total binding and was substracted from the total binding to give specific binding.

The IC_{50} values for tested compounds were estimated by measuring the inhibition of different concentrations and performing log prohibit analyses of the results.

[^{3}H]Baclofen Binding Assay ($GABA_B$ Assay). Interaction with the $GABA_B$ receptors was examined with [^{3}H]baclofen as described by Hill and Bowery.[11] CSM (P_4) were washed with ice-cold distilled water (20 mL for one brain) by centrifugation (4 °C) for 10 min at 48000*g*. The resulting pellet was stored frozen at -20 °C for at least 18 h prior to use (up to 2 months). After decongelation for 15 min at 20 °C, membranes were resuspended in 50 mM Tris·HCl, pH 7.4, buffer with 2.5 mM $CaCl_2$ (10 mL for one brain) and incubated for 45 min at 20 °C. This suspension was centrifuged (4 °C) at 7000*g* for 10 min and the resultant pellet incubated in Tris·HCl buffer (10 mL for one brain). These centrifugations and incubations were started against three times. In a final time, the suspension was centrifuged (4 °C) at 7000*g* for 10 min and the pellet resuspended in Tris·HCl buffer (4.5 mL for one brain).

Membranes equivalent to 0.5 mg of protein were incubated in triplicate in 1 mL of 50 mM Tris·HCl, pH 7.4, buffer with 2.5 mM $CaCl_2$ containing the drugs to be tested and 0.6 μCi of [^{3}H]baclofen (DL-[*butyl*-4-^{3}H(N)]baclofen, NEN) with a specific activity of 45 Ci/mM. These homogenates were incubated for 30 min at room temperature in conical microcentrifuge tubes and the assay terminated by centrifugation at 7000*g* for 10 min. The supernatant was discarded, the pellet was carefully rinsed two times with 1 mL of Tris·HCl buffer, and remaining fluid blotted from the surface of the pellet was aspirated under vacuum. The pellet was solubilized with ultrasonic bath for 10 min in 1 mL of HP/b Beckman scintillation fluid. The radioactivity was measured 12 h later in a liquid scintillation counter. Nonspecific binding was determined with 1 mM GABA and presented 66.6% of total binding. IC_{50} values were estimated as described elsewhere.

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Resolution of Racemic Carbocyclic Analogues of Purine Nucleosides through the Action of Adenosine Deaminase. Antiviral Activity of the Carbocyclic 2′-Deoxyguanosine Enantiomers

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The action of adenosine deaminase on racemic carbocyclic analogues of 6-aminopurine nucleosides was investigated. When either racemic carbocyclic adenosine [(±)-*C*-Ado] or the racemic carbocyclic analogue [(±)-*C*-2,6-DAP-2′-dR] of 2,6-diaminopurine 2′-deoxyribofuranoside was incubated with this enzyme, approximately half of the material was deaminated rapidly. From the resulting solution, the D isomers of the deaminated carbocyclic analogues (D-carbocyclic inosine, D-*C*-Ino, or D-carbocyclic 2′-deoxyguanosine, D-2′-CDG) and the L isomers of the undeaminated carbocyclic analogues were isolated. At higher concentrations of the enzyme, deamination of L-*C*-Ado and L-*C*-2,6-DAP-2′-dR proceeded slowly, thus also making the other enantiomers accessible. In tests in vitro against herpes simplex virus, types 1 and 2, D-2′-CDG was as active and potent as (±)-2′-CDG, whereas L-2′-CDG displayed only modest activity. In contrast to the previously reported high activity and potency of (±)-*C*-2,6-DAP-2′-dR against these two viruses, L-*C*-2,6-DAP-2′-dR was inactive.

The general synthetic routes[1-9] to the requisite cyclopentane precursors of carbocyclic analogues of nucleosides lead to the racemic forms of the target nucleoside analogues. It has been assumed that the various biological

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