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# γ-Aminobutyric Acid Esters. 1. Synthesis, Brain Uptake, and Pharmacological Studies of Aliphatic and Steroid Esters of γ-Aminobutyric Acid

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Labeled and unlabeled aliphatic and steroid esters of γ-amino[U-$^{14}$C]butyric acid (GABA) were synthesized and tested for their capacity to penetrate the blood–brain barrier and for evidence of central neuropharmacological activity in rodents. The uptake of the labeled 9,12,15-octadecatrienyl (linolenyl), 3-cholesteryl, 1-butyl, and the 9-fluoro-11β,17-dihydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-21-yl (dexamethasone) esters of GABA into mouse brain increased 2-, 25-, 74-, and 81-fold over GABA, respectively. The cholesteryl ester of GABA depressed the general motor activity of mice and rats in a dose-dependent manner, whereas the 1-butyl, linolenyl, and dexamethasone esters were inactive by this test. Studies of the rates of hydrolysis, GABA receptor binding capacity, and octanol/water partition coefficients indicated that pharmacological activity of the esters after entry into the central nervous system (CNS) was dependent on their capacity to release GABA by enzymatic hydrolysis and their lipid solubility.

Several investigations report lower than normal levels of γ-aminobutyric acid (GABA) in postmortem tissues of the central nervous system (CNS) of patients with Huntington's disease[1-3] and epilepsy[4-7] and suggest that such deficiencies may contribute to the pathophysiology of these neuropsychiatric disorders. These observations raise the possibility that "replacement" procedures that increase CNS levels of GABA may be useful in the treatment of such neuropsychiatric disorders. GABA, however, crosses the "blood–brain" barrier very poorly,[8] so that methods that alter this property are of interest. Previous investigations have reported that lipophilic esters[9,10] and Schiff's base derivatives[11] can facilitate the uptake of GABA into the brain. With these observations in mind, we have synthesized U-$^{14}$C-labeled and unlabeled aliphatic and steroid esters of GABA and evaluated the penetration of the labeled compounds through the blood–brain diffusion barrier. In addition, some neuropharmacological properties of the unlabeled products were evaluated as "prodrugs" that release GABA after hydrolysis within the CNS.

**Chemistry.** The butyl ester of GABA was prepared in good yield by direct esterification with 1-butanol at 100 °C in the presence of anhydrous HCl as the catalyst as shown in Scheme I. The 9,12,15-octadecatrienyl alcohol (linolenol), cholesterol, and dexamethasone esters of GABA were synthesized according to Scheme II. The amino group of GABA was first protected by formation of its *tert*-butoxycarbonyl (*t*-Boc) derivative (**2**) by reaction with *t*-Boc-ON reagent.[12] This product was converted to the symmetrical anhydride (**3**) by using dicyclohexylcarbodiimide[13] (DCC). Each alcohol was esterified by reaction with **3** to yield products **4**, which, after treatment with trifluoroacetic acid to remove the *t*-Boc group, gave the desired compounds **5–7**. Similar methods on a microscale were used to prepare 1 and **5–7** as radioactive derivatives, with all the radioactivity present at $^{14}$C in the GABA moiety of the molecules. The IR and NMR spectra and elemental analyses of the compounds were consistent with their proposed structures.

**Scheme I**

$$\underset{\text{GABA}}{H_2N(CH_2)_3COOH} \xrightarrow[HCl]{CH_3(CH_2)_3OH} \underset{\mathbf{1}}{[H_3N(CH_2)_3CO_2(CH_2)_3CH_3]^+Cl^-}$$

## Results and Discussion

**Brain Uptake Studies.** Initially, a double-labeling method was used to compare the simultaneous uptake of each $^{14}$C-labeled GABA ester with [$^3$H]GABA in the brain

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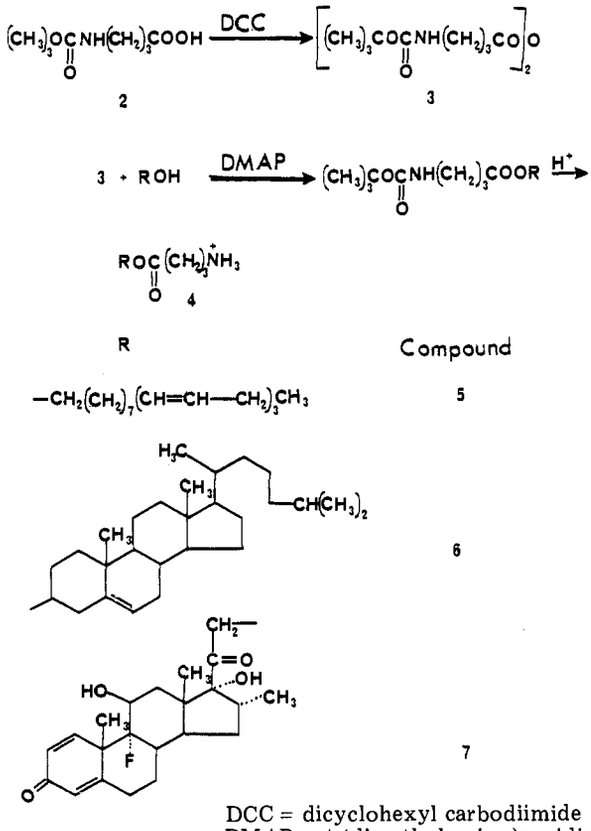
Table I. Uptake of [$^{14}$C]GABA by Mouse Tissues[a]

| dose, μmol/kg | injection route[b] | brain concn, nmol/g | liver concn, nmol/g | BPI[c] (5 min), % |
|---|---|---|---|---|
| 30 | iv | 0.04 | 4.2 | 0.95 |
| 60 | ip | 0.23 | 29 | 0.80 |
| 120 | ip | 0.45 | 46 | 1.00 |
| 160 | ip | 0.66 | 71 | 0.93 |
| 210 | ip | 0.83 | 83 | 1.00 |
| 270 | sc | 1.03 | 104 | 1.00 |
| 380 | sc | 1.81 | 165 | 1.10 |
| mean ± SEM | | | | 0.96 ± 0.09 |

[a] All determinations were carried out at 5 min after injection. The volumes of the injected doses into individual mice (body weight 18-24 g) for the iv, ip, and sc doses were 0.15, 0.3, and 0.5 mL, respectively. The measured concentrations in brain and liver represent the actual amounts of GABA found by thin-layer chromatographic (TLC) analysis of the tissue homogenates. Each tissue was homogenized in 8 mL of pyridine in a glass homogenizer. The precipitated material was removed by centrifugation for 10 min at 12000*g*, and the supernatant was evaporated to dryness under $N_2$ and analyzed by TLC in solvent A (see Experimental Section). This procedure ensured that only labeled GABA, and not its metabolites, was being measured. The mean BPI for labeled GABA was 0.96 ± 0.09% ($n$ = 7) and was apparently not dependent on the route of injection and the dose (between 30 and 380 mol/kg). [b] iv, intravenous; ip, intraperitoneal; sc, subcutaneous injections. [c] BPI (brain penetration index) = ([brain]/[liver]) × 100.

Scheme II




of the same animal after a systemic injection. This approach, however, was unsuccessful; within 5 min the [$^3$H]GABA was found to yield large amounts of tritiated water, so that uptake measurements (as the total radioactivity associated with brain) were a reflection of a tritium exchange process rather than the uptake of GABA. We therefore developed a procedure in which the uptake of a $^{14}$C-labeled compound into the brain was compared with the uptake into the liver of the same animal. The liver was chosen as a reference, since it is an organ that has no barrier to diffusible molecules present in the blood. Measurements of the quantity of a compound present in brain and liver at 5 min after a subcutaneous injection were used to calculate a "brain penetration index" (BPI), the amount present per gram of brain tissue as a percent of the amount per gram of liver tissue. Thus, a compound for which there is no blood–brain barrier would have a BPI of 100%, and a compound that preferentially accumulates in the brain would have a BPI value of greater than 100%. This procedure has the advantage of applicability even for a sparingly soluble lipid ester that tends to remain largely at the site of the injection and slowly diffuse into the circulation. Table I summarizes the results of the application of this method to $^{14}$C-labeled GABA. The data indicate that the BPI values for labeled GABA (mean ± SEM = 0.96 ± 0.09%) were low and independent of the route of administration [intravenous (ip), subcutaneous (sc), or intraperitoneal (ip)] of the compound and the dose (30–380 μmol/kg, 3–39 mg/kg) used. They are comparable to those obtained by the "brain uptake index" (BUI) method of Oldendorf[14] (i.e., a BUI value of 2% at 30 s after administration of the compound).

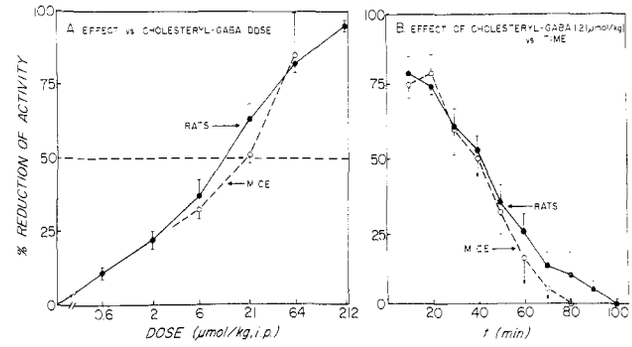



**Figure** 1. Effects of cholesteryl-GABA ester (**6**) on general motor activity. A: Effect of ip dose of compound **6** on the motor activity of rats and mice ($n$ = 6 per dose) as percent of vehicle control activity. Cholesterol itself had no significant behavioral effect over that of a vehicle control injection. B: Time course of recovery from reduced motor activity of the animals at a dose of 21 μmol/kg, ip ($n$ = 6 per group).

Table II gives BPI results for the GABA esters. These data indicate that the uptake of the dexamethasone, *n*-butyl, cholesteryl, and 1-linolenyl esters were, respectively, increased by a factor of 81-, 74-, and 2-fold over GABA. Table III gives data on uptake for compounds **5**–**7** at several times after injection. The radioactivity was found to accumulate in brain and to persist for at least 180 min postinjection in the case of the cholesteryl ester (**6**) (Table III), whereas the concentrations in liver were more variable over time. These measurements suggest that the GABA esters can enter the CNS and provide a source for a steady supply of GABA, possibly by slow hydrolysis. TLC analysis of brain homogenates at 1 h after administration indicated that about 70% of the radioactivity corresponded to the intact cholesteryl-GABA molecule and that the remaining radioactivity consisted of unidentified volatile products (presumably metabolites of GABA, such as acetic acid).

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Table II. BPI[a] for $^{14}$C-Labeled GABA Esters in Mouse Brain

| compd | dose, mg/kg (μmol/kg) | brain concn, nmol/kg | liver concn, nmol/kg | BPI,[a] % | partition coefficient, $K$[b] |
|---|---|---|---|---|---|
| 1 | 74 (380) | 19.2 ± 0.3 | 28.0 ± 1.6 | 74 ± 3.4 | nd |
| 5 | 90 (260) | 3.5 ± 0.4 | 141.7 ± 8 | 2 ± 0.6 | nd |
| 6 | 24 (50) | 0.17 ± 0.03 | 0.69 ± 0.08 | 25 ± 7 | 110 |
| 7 | 41 (70) | 8.7 ± 1 | 10.8 ± 2 | 81 ± 20 | 0.9 |
| GABA[c] | | | | 1 | 0.004 |

[a] BPI = brain penetration index = [brain]/[liver] × 100. Each $^{14}$C-labeled compound was injected sc into mice in 0.5 mL of 25% propylene glycol in water. Time is 5 min, and the data represent means plus or minus SEM ($n$ = 3). [b] $K$ (partition coefficient) = 1-octanol/water partition coefficient, measured by using the radioactive compounds. About 1–10 μg of compounds was shaken overnight at 25 °C in a mixture of 5 mL each of octanol and water. The two layers were separated, and aliquots were used for radioactivity measurements to determine the distribution in each solvent. $K = C_{octanol}/C_{water}$ ($C$ = molar concentration); nd, not determined. [c] See Table I.

Table III. Time Course of Uptake in Mouse Brain[a]

| compd | dose, mg/kg | time, min | brain concn, nmol/g |
|---|---|---|---|
| 5 | 90 | 5 | 3.5 |
| | 90 | 15 | 11.0 |
| | 90 | 45 | 24.9 |
| 6 | 24 | 5 | 0.17 |
| | 24 | 15 | 0.67 |
| | 24 | 60 | 0.79 |
| | 24 | 180 | 0.60 |
| 7 | 41 | 5 | 8.7 |
| | 41 | 15 | 21.5 |

[a] See Tables I and II for methods.

**Pharmacological Properties.** Two test methods were used to evaluate the neuropharmacological activity of the GABA esters. First, the effect of intraperitoneal injections of test compounds on the general motor activity of mice during a 60-min period was determined in a Stoelting electronic activity monitor.[15] Dose–response curves were obtained, from which the half-maximally effective values were calculated (Figure 1). GABA itself did not have a significant effect as expected because of its poor uptake (Table I) and rapid decomposition, presumably by GABA-α-oxoglutarate aminotransferase (GABA-T). This observation is similar to previous experiments in which systemically injected doses of up to 1 g/kg (9.7 mmol/kg) had little effect on motor activity in rats.[16] Studies of the behavioral effects in rats and mice of the esters in comparison with the effects of the dexamethasone, butyl, and linolenyl esters (Table IV), as well as the corresponding alcohol moiety of each compound (administered at equivalent or higher doses) (data not shown), were not active in changing the motor activity of tests rats. Only the cholesteryl ester was active (Table IV). The BPI (±SEM) of free [$^{14}$C]cholesterol was 205 ± 14 in three mice at 5 min following sc injection. Thus, this compound accumulated in brain at a considerably higher rate than the cholesterol ester of GABA (**6**) when administered at equivalent doses but had no behavioral effect; the activity of test rats was equal to that of controls (vehicle-injected and uninjected rats). The dose–response data for this ester (**6**) (see Figure 1), however, indicate that a 50% reduction of motor activity occurred for rats receiving ip doses of 8 μmol/kg (3.5 mg/kg) and for mice injected with 17 μmol/kg (7.4 mg/kg). The amount of compound **6** present in the mouse brain at 5 min after a considerably larger dose (50 μmol/kg; 24 mg/kg) than the $ED_{50}$ of 17 μmol/kg was only 0.17 nmol/g (74 ng/g) (see Table II). This observation suggests that the dose of cholesteryl-GABA required for behavioral effects is extremely small and comparable to that of other GABA agonists.[16]

In a second behavioral test, the ability of a substance to delay the onset of bicuculline-induced epileptic seizures in the mouse was measured (Table V). There were no significant effects at 1 or 6 h. At 24 h after ip injection

Table IV. Effects of GABA Esters on General Motor Activity in the Mouse

| compd | dose mg/kg | dose μmol/kg, ip | general motor act.[b] |
|---|---|---|---|
| GABA[b] | 200 | 1942 | 100 ± 15 |
| 1 | 60 | 307 | 89 ± 9 |
| | 128 | 654 | 83 ± 15 |
| 5 | 12 | 34 | 90 ± 2 |
| | 36 | 103 | 74 ± 3* |
| 6 | 3 | 6 | 67 ± 3* |
| | 10 | 21 | 49 ± 4** |
| | 33 | 65 | 15 ± 14** |
| 7 | 16 | 27 | 94 ± 10 |

[a] Cumulative activity scores over 60-min postinjection of the drug are expressed as the mean percentage of appropriate vehicle control for each drug. [$\bar{X}$ ± SEM, $n$ = 6]; * = $p < 0.01$; ** = $p < 0.001$ by $t$ test. The effects of 1-butanol, 1-linolenol, cholesterol, and dexamethasone on general motor activity at doses equal to those used for their corresponding GABA esters 1 and **5**–**7**, respectively, were found to be equivalent to the vehicle control and, thus, not significant determinants of the behavioral effects. [b] GABA = γ-aminobutyric acid.

Table V. Anticonvulsant Properties in the Mouse

| compd | dose, mg/kg | onset of seizures: % saline control[a] | survival time: % saline control |
|---|---|---|---|
| saline | | 100 | 100 |
| vehicle | | 109 ± 7 | 123 ± 10 |
| cholesterol | 84 | 118 ± 6 | 110 ± 7 |
| 6 | 3 | 120 ± 6* | 95 ± 15 |
| 6 | 16 | 129 ± 4** | 101 ± 20 |
| 6 | 30 | 130 ± 8** | 104 ± 18 |
| 6 | 84 | 137 ± 7*** | 109 ± 9 |
| 5 | 12 | 102 ± 4 | 118 ± 10 |
| 5 | 17 | 73 ± 15** | 111 ± 14 |
| 5 | 25 | 78 ± 9** | 68 ± 9*** |
| 5 | 36 | 50 ± 5*** | 62 ± 6*** |
| 5 | 50 | 40 ± 11*** | 63 ± 8*** |

[a] Anticonvulsant action at 24 h after pretreatment with the test compound. The seizure onset time was measured in seconds following an injection of bicuculline (0.3 mg/kg). Data for saline controls were 184 ± 19 and 253 ± 28 s, respectively, for onset of seizures and death with respect to **5**. Data are means plus or minus SEM, $n \geq 6$ in each group. * = $p < 0.5$; ** = $p < 0.05$; *** = $p < 0.005$ by $t$ test of difference between drug and saline controls.

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Table VI. Inhibition of [$^3$H]GABA Binding to Synaptic Membranes from Rat Cerebellum

| compd | $IC_{50}$,[a] nM |
|---|---|
| GABA | 70 |
| 5 | 40 000 |
| 6 | 10 000 |

[a] The $IC_{50}$ value represents the dose of each compound required for 50% inhibition of the binding of [$^3$H]GABA (15 nM) to synaptic membrane preparations (from rat cerebellum) in a receptor-binding assay.[21,22]

of the cholesteryl derivative (6) at a dose of 30 mg/kg (64 μmol/kg), the onset of seizures was delayed by 30% (as compared to saline controls) after an acute ip injection of bicuculline at 0.3 mg/kg. Although this difference was not as significant when compared with vehicle or cholesterol controls, this type of delay in the effect of the compound on seizure activity has been reported previously for γ-vinyl-GABA.[17] In contrast, the linolenyl ester of GABA had the surprising property of *accelerating* the seizure onset time by 60% at a dose of 50 mg/kg, even though it had no significant effect on the general motor activity of mice (Table IV). The dexamethasone and butyl esters had no effect on seizures in mice.

The fact that the bicuculline-induced seizures could be influences at 24 h after the administration of two GABA esters (5 and 6) but not at earlier times indicates that no significant direct, acute anticonvulsant activity can be ascribed to the compounds.

These results demonstrate that a compound such as cholesterol, which readily passes through the blood–brain barrier, can transport covalently bound GABA across the barrier. Once such a compound enters the CNS, its pharmacological activity could be due to the properties of the intact molecule or to its capacity to release GABA after hydrolysis. To test for the possibility that the intact molecules might be active, we measured the capacity of compounds 5 and 6 to inhibit the binding of [$^3$H]GABA to GABA "receptors" using membrane preparations of rat cerebellum.[18,19] $IC_{50}$ values were determined by fitting data to linearized transformations, based on including at least two concentrations of antagonists above and below the $IC_{50}$, with at least triplicate replication per concentration. The results (see Table VI) indicate that the concentrations of 5 and 6 required for 50% inhibition ($IC_{50}$) of binding were much greater than for GABA. Both 6 and 7 had low binding affinities to the receptors in comparison with potent GABA agonists, which can displace [$^3$H]GABA at low nanomolar concentrations.[20] These results suggest that the pharmacological activity of the cholesteryl ester of GABA (6) is probably not due to its direct interactions at GABA–receptor binding sites; its activity might, however, be related to release of GABA by hydrolysis of the ester as a "prodrug".

Cholesterol hydrolases[21] and esterases[22] are present in mammalian brain tissue. That such enzymes can release

Table VII. Enzymatic Hydrolysis of Cholesteryl [$^{14}$C]GABA Ester by Rat Brain Homogenate

| time, min | amount of unreacted[a] substrate (6), nmol |
|---|---|
| 10 | 53.0 ± 7.0 |
| 30 | 43.5 ± 6.3 |
| 60 | 34.5 ± 4.9 |
| control[b] | 55.6 ± 2.6 |

[a] Cholesteryl-[$^{14}$C]GABA ester (6) (3 mg/mL) was incubated at 37 °C in the presence of $S_1$ fractions of rat brain homogenates[23] (0.8 mg/mL of protein). Analysis of 40-μL aliquots removed at various time intervals was carried out, after quenching the reaction with 0.5 mL of ethanol, by thin-layer chromatography (TLC) on silica gel in $CHCl_3$/MeOH/HOAc (18:6:1). [b] Control, the initial amount of substrate used, which did not change after 1 h at 37 °C.

GABA from 6 was demonstrated (see Table VII) with the crude supernatant (10000*g*, $S_1$) fraction of rat brain homogenates[23] as a source of hydrolases. Both 6 and 7 released [$^{14}$C]GABA at approximately equivalent rates ($k_{obsd}$ = 8.45 and 6.48 × $10^{-3}$ $min^{-1}$, respectively). This finding, however, cannot explain differences in the physiological activity of 6 and 7. Both compounds readily entered the brain (high BPI values; Table II), yet only 6 was active behaviorally (Table IV), even though its uptake into brain was less than that of 7. Clearly, additional factors must be important for the observed differences in the pharmacological activity. We found that the octanol/water partition coefficient for 6 was 110, while that for 7 was only 0.9 (Table II). This result suggests that the property of high lipid solubility, in addition to a capacity for entry into the CNS, may be necessary for physiological activity. Perhaps it is essential for the compound to become associated with the lipid bilayer of neural membranes to function as a "prodrug" for the steady release of GABA by hydrolysis.

In summary, we have found an effective GABA delivery system that can produce apparent central GABA-like behaviorally depresseant activity, possibly by sustained and slow release of GABA by local hydrolysis in the brain. Depending on lipid solubility, this release may include GABA–receptor-accessible membrane sites, as well as the cytoplasmic medium where it is rapidly destroyed before reaching GABA receptor sites. Such delivery systems may also be useful for other hydrophilic neurotransmitters.

## Experimental Section

**Chemistry.** Melting points were determined on a hot-stage apparatus and are uncorrected. IR spectra were obtained in a Perkin-Elmer Infracord spectrophotometer. NMR spectra were determined in a CFT 20 with tetramethylsilane as an internal reference. Elemental analyses for C, H, and N were carried out by Midwest Micro Labs Ltd., Indianapolis, IN: all compounds analyzed within ±0.4% of their theoretical values. Thin-layer chromatographic (TLC) determinations were carried out on silica gel coated plastic Eastman Kodak TLC sheets with the following solvent systems: (A) chloroform/acetic acid, 9:1; (B) chloroform/methanol, 9:3; (C) propanol/acetic acid/water, 4:1:1; (D) chloroform/methanol/acetic acid, 90:30:3; (E) chloroform/methanol/acetic acid, 18:6:1; (F) ethyl acetate/acetic acid/ethanol, 9:1:1; (G) benzene/acetone, 9:1; (H) ethyl acetate/hexane, 1:1.

***n*-Butyl 4-Aminobutyrate (1).** A solution of 100 mg (0.97 mmol) of γ-aminobutyric acid in 30 mL of anhydrous 1-butanol (dried over calcium hydride and distilled) was saturated with anhydrous HCl gas and sealed in a glass ampule. The mixture was heated over a steam bath for 16 h and then evaporated to dryness to yield a white solid, which was crystallized from an ethanol/ether mixture as previously reported.[24] The yield was

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92% based on GABA: mp 72 °C; TLC in solvent A gave single-spot, $R_f$ 0.44; IR (Neat) 3550, 1730 $cm^{-1}$. The radioactive *n*-butyl ester of GABA was prepared as above with 4 mg of GABA, 50 μCi of [U-$^{14}$C]GABA (New England Nuclear Corp., Boston, MA), and 3 mL of anhydrous butanol as starting materials. The product was obtained in 82% yield with a specific activity of 186 μCi/mmol. On TLC plates, all (>99%) of the radioactivity was found to be associated with a single spot, which comigrated with the unlabeled ester at $R_f$ 0.44 in solvent A. After hydrolysis of the ester in 6 N HCl for 15 min at room temperature, all the radioactivity was transferred to the position of GABA on the TLC plates.

**4-[(*tert*-Butoxycarbonyl)amino]butyric acid (2)** was prepared by the reaction of GABA with Boc-ON [2-[(*tert*-butoxycarbonyl)oximino]-2-phenylacetonitrile]. This was obtained in 96% yield. The product had a melting point of 52–54 °C and migrated as a single spot on TLC plates: $R_f$ values were 0.84 and 0.71 in solvents E and F, respectively. The U-$^{14}$C analogue of **2** was also prepared from 30-mg quantities GABA and 100 μCi of uniformly labeled [$^{14}$C]GABA (New England Nuclear Corp.).

**4-[(*tert*-Butoxycarbonyl)amino]butyric Anhydride (3).** A cold solution (0 °C) of 558 mg (2.7 mmol) dicyclohexylcarbodiimide in 8 mL of dry methylene chloride was added to a cold solution of *t*-Boc-GABA (1.05 g, 5.1 mmol) in 7 mL of dry methylene chloride. An immediate precipitation of dicyclohexylurea was formed. The mixture was stirred for an additional 2 h at room temperature and then filtered to remove the urea derivative. The filtrate was evaporated on a rotary evaporator. The solid product so obtained was redissolved in 20 mL of anhydrous ethyl acetate and kept overnight at 4 °C to allow further precipitation of any remaining dicyclohexylurea. The resulting solution was then filtered, the filtrate was concentrated to 8 mL in vacuo, and dry petroleum ether was added to precipitate a white compound. This was isolated by filtration, washed with a mixture of ethyl acetate/petroleum ether (1:4), dried in vacuo over $P_2O_5$, and then crystallized from the solvent mixture to yield 915 mg (2.36 mmol) of the anhydride (87%). The compound had a melting point of 106 °C and migrated with an $R_f$ value of 0.74 in solvent F on TLC plates: IR (Nujol) 3480, 3400, 1810, 1730, 1680 $cm^{-1}$. Anal. ($C_{18}H_{32}N_2O_7$) C, H, N. The radioactive anhydride was synthesized as above in 84% yield by using 15 mg of $^{14}$C-labeled *t*-Boc-GABA as a starting material. The product had a specific activity of 2 mCi/mmol and an identical $R_f$ value to the unlabeled compound.

**3-Cholesteryl 4-[(*tert*-Butoxycarbonyl)amino]butyrate (8).** A solution of 1.63 g 4.20 mmol) of *t*-Boc-GABA anhydride in 25 mL of tetrahydrofuran (distilled just before use from calcium hydride) was added to a solution of 1.35 g (3.49 mmol) of cholesterol and dry tetrahydrofuran, followed by 427 mg (3.5 mmol) of dry 4-(dimethylamino)pyridine. The mixture was stirred under nitrogen for 48 h at room temperature and then evaporated to dryness to give a waxy solid. The product was dissolved in 7 mL of ethyl acetate and washed with 15 mL of 5% (w/v) sodium bicarbonate (3 times) and then with 15 mL of distilled water (3 times). The ethyl acetate solution was separated and dried over anhydrous sodium sulfate and filtered, and then the filtrate was evaporated to dryness. The product was recrystallized from acetone/water to yield 1.95 g of compound **8** with a melting point of 122.5 °C. TLC $R_f$ values of 0.98 and 0.52 were obtained in solvents D and G, respectively. The $^{14}$C-labeled compound was synthesized as above with 300 mg of cholesterol as a starting material: yield 83%; specific activity 13.6 μCi/mmol; IR 3450, 1730, 1690, 1700 $cm^{-1}$; NMR ($CDCl_3$) δ 5.35 (br m, 1 H), 4.55 (m, 2 H), 3.15 (m, 2 H), 2.10–2.40 (m, 4 H), 1.45 (s, 9 H). Anal. ($C_{36}H_{61}NO_4$) C, H, N.

**3-Cholesteryl 4-Aminobutyrate (6).** A solution of **8** (100 mg, 175 μmol) in 0.53 mL of 4 M HCl in dioxane and 2.0 mL of dry dioxane was stirred at room temperature for 70 min. The solution was concentrated in vacuo at room temperature, and the white solid thus obtained was washed repeatedly with ethyl acetate and dried under vacuum over $P_2O_5$ to give 50 mg of compound **6** (56%): TLC (solvent E) $R_f$ 0.38; TLC (solvent B) $R_f$ 0.33. As the amine hydrochloride was very hygroscopic, good IR and NMR spectra could be obtained only by making the *N*-acetyl derivative by acetylation with acetic anhydride in pyridine: IR (Neat) 3350, 1720, 1630 (*N*-acetyl derivative); NMR (*N*-acetyl derivative) ($CDCl_3$) δ 5.3 (br m, 1 H), 3.2 (m, 2 H), 2.34 (m, 2 H), 2.3–1.0 (br m), 1.96 (s, 3 H), 1.26 (s, 3 H), 0.9 (s, 3 H). Anal. ($C_{31}H_{54}NO_2Cl·H_2O$) C, H, N.

**9,12,15-Octadecatrienyl 4-[(*tert*-Butoxycarbonyl)amino]butyrate (Linolenyl Ester of *t*-Boc-GABA) (9).** A solution of linolenyl alcohol (285 mg, 1.05 mmol) in 35 mL of benzene under an atmosphere of nitrogen was added to γ-[(*tert*-butyloxycarbonyl)amino]butyric acid anhydride (480 mg, 1.24 mmol) and 4-(dimethylamino)pyridine (150 mg, 1.23 mmol). The mixture was stirred at room temperature for 4 h. The solution was washed with 5% sodium bicarbonate, ice-cold 0.1 N HCl solution, and finally with ice-cold water. The organic layer was then dried over anhydrous sodium sulfate and concentrated to yield 370 mg (76%) of the required ester (**9**): TLC (solvent system H) $R_f$ 0.74; IR (Neat) 3500, 1720, 1160 $cm^{-1}$; NMR ($CDCl_3$) δ 5.36 (m, 6 H), 4.55 (br m, 1 H), 4.06 (t, $J$ = 6.5 Hz, 2 H), 3.15 (m, 2 H), 2.8 (m, 4 H), 2.29 (m, 2 H), 2.0–1.3 (br m, 18 H), 1.43 (s, 9 H), 0.97 (t, $J$ = 7.5 Hz, 3 H). A similar procedure was used to prepare the $^{14}$C-labeled derivative with *t*-Boc-[$^{14}$C]GABA anhydride and unlabeled linolenyl alcohol (10 mg): yield 70%; TLC (solvent system H) $R_f$ 0.73.

**9,12,15-Octadecatrienyl 4-Aminobutyrate (5).** A solution of the *t*-Boc-GABA ester of linolenyl alcohol (**9**; 330 mg, 0.74 mmol) in 20 mL of methylene chloride was cooled with ice under nitrogen atmosphere. Trifluoroacetic acid (0.5 mL) was added to the solution, and the mixture was stirred at 0 °C for 1 h and at room temperature for an additional 2 h. The solution was concentrated under aspirator vacuum, and the residue was taken up in 20 mL of chloroform and washed with 5% (w/v) sodium bicarbonate solution and with water. The chloroform layer was dried over anhydrous magnesium sulfate and concentrated under vacuum to yield a light yellow liquid; 200 mg (0.58 mmol, 78%). Because of its hygroscopic properties, the product (**5**) was converted to the *N*-acetyl derivative (**10**) with acetic anhydride in pyridine prior to its characterization and elemental analysis: TLC (solvent B) $R_f$ 0.43; IR (Neat) 3300, 1730, 1650, 1550 $cm^{-1}$; NMR ($CDCl_3$) δ 5.36 (m, 6 H), 4.05 (t, $J$ = 4.8 Hz, 2 H), 3.28 (m, 2 H), 2.8 (m, 4 H), 2.35 (t, $J$ = 6.6 Hz, 2 H), 2.0–1.30 (br m, 18 H), 1.95 (s, 3 H), 1.59 (s, 3 H), 0.97 (t, $J$ = 7.5 Hz, 3 H). Anal. ($C_{24}H_{41}NO_3$) C, H, N. The radioactive derivative was prepared from $^{14}$C-labeled *t*-Boc-GABA linolenyl ester (7 mg) as above. The specific activity of the final compound was 120 μCi/mol.

**9-Fluoro-11β,17-dihydroxy-21-[4-[(*tert*-butoxycarbonyl)amino]butyryl]-16α-methylpregna-1,4-diene-3,20-dione [21-[γ-[(*tert*-Butoxycarbonyl)amino]butyryl]dexamethasone] (11).** A solution of dexamethasone (Sigma Chemical Co.; 120 mg, 0.31 mmol), *t*-Boc-GABA anhydride **3** (144 mg, 0.37 mmol), and 4-(dimethylamino)pyridine (45 mg, 0.37 mmol) in 20 mL of dry methylene chloride was stirred at room temperature for 70 min. The solution was washed serially with 5% (w/v) sodium bicarbonate solution, 0.1 N HCl, and brine. The organic layer was dried over anhydrous $Na_2SO_4$ and concentrated in vacuo to obtain 0.164 g (0.28 mmol, 92%) of the ester **11**: mp 177–179 °C (ethyl acetate); TLC (ethyl acetate) $R_f$ 0.74 (dexamethasone had $R_f$ 0.38); IR ($CHCl_3$) 3500, 1710, 1660 $cm^{-1}$; NMR ($CDCl_3$) δ 7.27 (d, $J$ = 10.5 Hz, 1 H), 6.37–6.1 (m, 2 H), 4.89 (s, 2 H), 4.75–4.05 (m, 2 H), 3.19 (m, 2 H), 2.48 (m, 2 H), 1.63 (s, 3 H), 1.54 (s, 2 H), 1.44 (s, 9 H), 1.05 (s, 3 H), 0.91 (d, $J$ = 7.3 Hz, 3 H). Anal. ($C_{31}H_{44}FNO_8$) C, H, N. The radioactive derivative was prepared as above with $^{14}$C-labeled *t*-Boc-GABA anhydride and 10 mg of dexamethasone.

**Dexamethasone 21-(γ-Aminobutyrate) (7).** A solution of dexamethasone 21-[γ-[(*tert*-butoxycarbonyl)amino]butyrate] (**11**; 400 mg, 0.69 mmol) and trifluoroacetic acid, 0.2 mL in 20 mL of methylene chloride, was stirred at room temperature for 1 h, and the mixture was concentrated in vacuo. The residue was taken up in 9 mL of 0.1 N HCl and lyophilized. The crude product was purified in a silica gel column (20 × 1 cm) by eluting with $CHCl_3/CH_3OH$ (40:10). The residue was taken up in 10 mL of water and freeze-dried to obtain **7** as the trifluoroacetate monohydrate as a fluffy solid: yield 207 mg (58%); TLC (solvent

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B) $R_f$ 0.76; TLC (solvent C) $R_f$ 0.52; mp 185–188 °C; IR (Neat) 3500, 1720, 1670, 1650, 1620 $cm^{-1}$; NMR (acetone-$d_6$) $\delta$ 7.26 (m, 1 H), 6.0–6.24 (m, 2 H), 4.99 (d, $J$ = 1.9 Hz, 2 H), 4.1–4.7 (br m, 2 H), 3.84 (m, 2 H), 3.30 (s, 3 H), 2.40–2.86 (m, 14 H), 1.61 (s, 3 H), 0.98 (m, 3 H). Anal. of the trifluoroacetate ($C_{28}H_{37}F_4NO_8 \cdot H_2O$) C, H, N. The $^{14}C$-labeled dexamethasone 21-($\gamma$-aminobutyrate) was obtained from the $t$-Boc derivative as above with 6 mg of starting material: yield 75%; specific activity 34 $\mu$Ci/mmol.

**Biochemistry. Brain Uptake Studies.** Each labeled test compound was injected as a solution in 0.5 mL of saline for compound 7 or in 25% propylene glycol in water for compounds 1, 5, and 6 subcutaneously (sc) into young adult male BALB-c mice (18–24 g body weight). After 5 min (or later), animals were sacrificed, and the brain and liver were removed, weighed, and homogenized, respectively, in 8 and 10 mL of "brain protein solvent"[25] (1%, w/v, sodium dodecyl sulfate in 6 M urea and 19 mM EDTA, pH 7.4, 0.03 M phosphate). Aliquots (0.5 mL) of the homogenates were mixed with 10 mL of Aquasol 2 (New England Nuclear Corp.) and counted in a Beckman liquid scintillation counter. The total uptake of each compound per gram of brain and liver tissue was calculated. The proportion of uptake into brain as compared to liver (100%) of the same animal was designated as the "Brain Penetration Index" (BPI), expressed as a percentage typically at 5 min after injection. The TLC methods used to identify $^{14}C$-labeled material are described in the legend for Table I.

**Pharmacology. General Locomotor Activity Tests.** The compounds were dissolved in 25% propylene glycol in water and injected ip into male BALB-c mice (18–24 g) and rats (200 g). Dose–response measurements were obtained for six animals per dose or time point; animals were monitored singly for 60 min with a Stoelting electronic activity monitor (EAM) apparatus as previously described[15] to assess general motor activity of the animals. The dose at which a compound reduced such spontaneous motor activity by 50% in comparison to the control (vehicle-injected and uninjected) animals was used as a measure of pharmacological activity.

**Anticonvulsant Activity in Mice.** The compounds were injected ip at 1, 6, and 24 h before the administration of bicuculline (0.3 mg/kg sc). The latency to the onset of generalized tonic–clonic epileptic seizures[26,27] and the protection against lethality[28] of bicuculline were measured and evaluated as a percent of the data for vehicle-injected controls (Table V).

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**Registry No.** 1, 75319-75-4; 2, 57294-38-9; 2 ($^{14}C$ labeled), 89210-65-1; 3, 89231-63-0; 3 ($^{14}C$ labeled), 89231-64-1; 5, 89210-66-2; 5 ($^{14}C$ labeled), 89210-67-3; 6, 89210-68-4; 6·HCl, 89210-69-5; 6 ($^{14}C$ labeled), 89210-70-8; 6 (*N*-acetyl derivative), 89210-71-9; 7, 89231-65-2; 7 ($^{14}C$ labeled), 89231-66-3; 8, 89210-72-0; 8 ($^{14}C$ labeled), 89210-73-1; 9, 89210-74-2; 9 ($^{14}C$ labeled), 89210-75-3; 10, 89231-67-4; 11, 89231-68-5; 11 ($^{14}C$ labeled), 89231-69-6; $\gamma$-aminobutyric acid, 56-12-2; 2-[(*tert*-butoxycarbonyl)oximino]-2-phenylacetonitrile, 58632-95-4; cholesterol, 57-88-5; linolenyl alcohol, 506-44-5; dexamethasone, 50-02-2; [U-$^{14}C$]GABA, 29315-46-6; [U-$^{14}C$]GABA butyl ester, 89210-76-4.

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# 2-Chloroethyl (Methylsulfonyl)methanesulfonate and Related (Methylsulfonyl)methanesulfonates. Antineoplastic Activity in Vivo

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2-Haloethyl and ethyl (methylsulfonyl)methanesulfonates were prepared via sulfene intermediates. 2-Chloroethyl (methylsulfonyl)methanesulfonate is highly active against P388 leukemia in vivo; the majority of leukemic mice treated with this compound at 50 mg/kg per day, qd 1–5, survived more than 30 days and about 37% survived for more than 60 days. 2-Fluoroethyl (methylsulfonyl)methanesulfonate is also highly effective against P388 cells in vivo, but it is more toxic. Other (methylsulfonyl)methanesulfonate esters are more active than the analogous methanesulfonates and chloromethanesulfonates.

The most active of the *N*-nitrosoureas against experimental neoplasma are the *N*-(2-chloroethyl)-*N*-nitrosoureas (1, X = Cl; Chart I) and the *N*-(2-fluoroethyl)-*N*-nitrosoureas (1, X = F).[1-4] Similarly, the most active triazenylimidazoles or triazenylbenzenes against murine L1210 leukemia are 3-(2-chloroethyl)-1-triazenyl derivatives (2); i.e., the most active triazenes have at least one 2-chloroethyl group attached to the terminal nitrogen atom.[5,6] Studies of the decomposition of *N*-(2-chloroethyl)-*N*-nitrosoureas (1, X = Cl) in aqueous media[7-16]

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