# γ-Aminobutyric Acid Esters. 2. Synthesis, Brain Uptake, and Pharmacological Properties of Lipid Esters of γ-Aminobutyric Acid

James N. Jacob, Victor E. Shashoua,* Alexander Campbell, and Ross J. Baldessarini

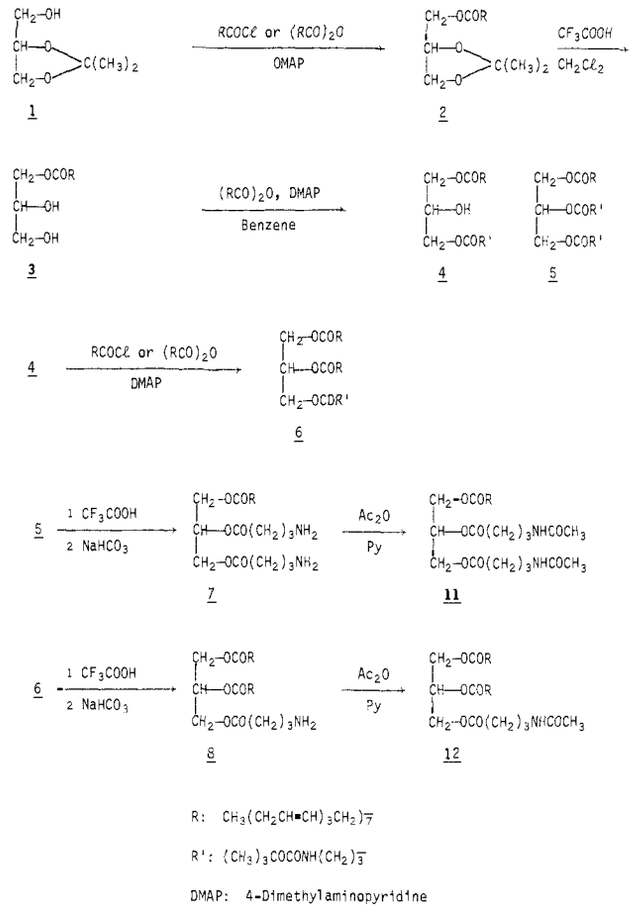
*Mailman Research Center, McLean Hospital, and Departments of Biological Chemistry and Psychiatry, and Neuroscience Program, Harvard Medical School, Belmont, Massachusetts 02178.* *Received April 2, 1984*

Two lipid esters of U-$^{14}$C-labeled and unlabeled γ-aminobutyric acid (GABA) were synthesized to test the possibility that natural lipid analogues, which resemble normal components of lipid bilayer membranes, can penetrate the blood–brain barrier and transport exogenous GABA to the brain. The uptake of 1-linolenoyl-2,3-bis(4-aminobutyryl)propane-1,2,3-triol and 1,2-dilinolenoyl-3-(4-aminobutyryl)propane-1,2,3-triol into mouse brain relative to liver was found to be, respectively, 75- and 127-fold greater than that of free GABA. The results indicate that there is little or no blood–brain barrier for the GABA ester molecules at doses up to 0.36 mmol/kg. Both ester compounds, but neither free GABA nor the lipid components delivered systemically, demonstrated central nervous system depressant properties by inhibiting the general motor activity of mice. Brain tissue has esterase activity which can release GABA from these compounds. This suggests that these compounds function as "prodrugs" to release GABA in the CNS.

A variety of compounds with properties of a γ-aminobutyric acid (GABA) agonist have been investigated[1] in an effort to improve the poor uptake of GABA through the blood–brain diffusion barrier[2] into the CNS and to provide potential new treatments for neuropsychiatric disorders such as epilepsy[3] or Huntington's disease.[4,5] We found in an earlier study[6] that several aliphatic and steroid ester derivatives of GABA can penetrate the blood–brain barrier by as much as 80-fold more than GABA to produce behaviorally depressant neuropharmacological effects expected of GABA agonists. We have extended this approach and now report testing the possibility that esters of natural lipids resembling those already present in brain lipid bilayer membranes might be useful as carriers for transporting GABA into the CNS. Thus $^{14}$C-labeled and unlabeled GABA esters of mono- and dilinolenoylglycerol were synthesized, and their uptake into mammalian brain and neuropharmacologic actions were investigated, using previously reported methods.[6] The results suggest that there is little or no blood–brain barrier for these lipid GABA esters, at doses up to 0.36 mmol/kg.

**Chemistry.** Scheme I illustrates the procedure used for the synthesis of esters of GABA (**7** and **8**). Two of the hydroxyl groups of glycerol were first protected by forming the acetonide **1** (commercially available as solketal, Aldrich Chemical Co.); the remaining hydroxyl was then esterified with linolenic anhydride in the presence of 4-(dimethylamino)pyridine (DMAP) in dry benzene to give the ester **2**, which was purified by chromatography on a silica gel column. This ester **2** was then treated with trifluoroacetic acid at 0–5 °C in methylene chloride to give the diol ester **3**, which was separated on a silica gel column from the unconverted starting material. Treatment of **3** with 1 molar equiv of 4-[(*tert*-butoxycarbonyl)amino]butyric anhydride[6] (*t*-Boc-GABA anhydride) in the presence of 4-(dimethylamino)pyridine gave a mixture of two products separated on thin-layer chromatography (TLC) with $R_f$ 0.38 and 0.67 (ethyl acetate:petroleum ether, 1:1). These were identified by NMR as the triester **5** containing two *t*-Boc-GABA groups (higher $R_f$) and the diester **4** with one *t*-Boc-GABA group (lower $R_f$). Compound **4** was preferentially synthesized when compound **3** was treated with less than 0.5 molar equiv of *t*-Boc-GABA anhydride. The unreacted excess compound **3** remaining in the reaction mixture was separated from **4** by silica gel column chromatography. The diester **4** was converted to compound **6** with linolenic anhydride or linolenoyl chloride. Compound **5** was the only product obtained when **3** was treated with 2 molar equiv of *t*-Boc-GABA anhydride.

**Scheme I**




*Address all correspondence to Dr. Victor E. Shashoua, Mailman Research Center, McLean Hospital, Harvard Medical School, Belmont, MA 02178.

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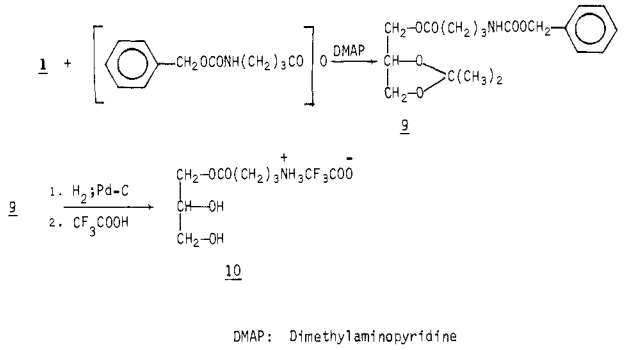
**Table I.** BPI[a] Values of Lipid Esters of GABA

| compd | dose, mg/kg | [brain], nmol/g | [liver], nmol/g | BPI | partition coefficient,[b] $K$ |
|---|---|---|---|---|---|
| GABA[c] | 3–39 | 0.04–1.81 | 4.2–165 | 0.96 ± 0.09 | 0.004 |
| **10** | 67 | 4.8 ± 0.2 | 157 ± 12 | 3 ± 0.35 | 0.01 |
| **7** | 100 | 2.81 ± 0.36 | 3.79 ± 0.58 | 75 ± 13 | 2.13 |
| **8** | 47 | 5.9 ± 0.4 | 4.7 ± 0.4 | 127 ± 8.3 | 6.60 |

[a] BPI (brain penetration index) = ([brain]/[liver]) × 100, at 5 min after sc administration of labeled test compound. BPI is independent of the initial dose, and the initial dose was selected depending on the specific radioactivity of the compound to yield detectable amounts of $^{14}C$. Test compounds were given to Balb-c mice (20 ± 2 g) as a solution in 0.5 mL of water (GABA, **10**) or 0.5 mL of 25% (vol) propylene glycol in water for **7** and **8**. The values represent the mean ± SEM for three measurements. [b] Octanol:water partition coefficient. [c] The value is for a range of doses (ref 6).

**Scheme II**




Both of the triesters **5** and **6** were converted to their corresponding amines **7** and **8** by treatment with trifluoroacetic acid at 0 °C under a nitrogen atmosphere to remove the *t*-Boc protecting groups. The trifluoroacetate salts formed at this step were then converted to the corresponding free amines by extraction of the chloroform solutions of the compounds with dilute aqueous sodium bicarbonate. The free amines and the intermediates were stored in chloroform solution below 0 °C to prevent oxidation of the lipid moiety of the molecules.

The mono-GABA ester of glycerol (**10**) was synthesized according to Scheme II by reaction of the monoacetonide of glycerol with *N*-carbobenzoxy-GABA anhydride to obtain **9**. This product was converted to compound **10** by hydrogenolysis and treatment with trifluoroacetic acid.

The three compounds, **7**, **8**, and **10**, were also synthesized on a microscale as radioactive derivatives with use of U-$^{14}C$-labeled Boc-GABA anhydride with all the radioactivity present in the GABA moiety of the molecule.

## Results and Discussion

**Brain Uptake Studies.** The capacity of the test compounds to penetrate the blood–brain barrier of mice was evaluated by measurements of their brain penetration index (BPI).[6] This index is defined as the quantity of compound present in brain as a percent of the amount in liver, per gram of tissue, typically at 5 min after a subcutaneous (sc) administration. Table I summarizes the results obtained.

This procedure presumably reflects the absorption and systemic distribution of a test agent injected into a peripheral tissue as well as its ability to penetrate lipid membrane bilayers to reach brain tissue. The liver was chosen as a reference since it has little or no barrier for most substances present in blood. The BPI of even sparingly soluble lipophilic compounds, which tend to remain largely at the site of injection, reflects the relative availability to the CNS of the circulating dose. The BPI value for GABA was 1.0%, and this value was increased by 75-, 127-, and 3-fold, respectively, for compounds **7**, **8**, and **10** (Table I).

Thus, in contrast to GABA itself, there is evidently little or no blood–brain barrier to the relatively nonpolar lipid derivatives **7** and **8**; in fact, the uptake into brain for compound **8** was higher than in liver, suggesting its preferential accumulation in the CNS. Brain uptake of the more polar derivative **10** was less than that of **7** and **8**, paralleling the octanol:water partitioning of all of these compounds (Table I). Mouse brain continued to accumulate compounds **7**, **8**, and **10** after systemic injection for at least 30–60 min and as long as 4 h in the case of compound **8** (Table II). With increased doses there was a corresponding increase in the uptake of compounds **7** and **8** (Table II), presumably reflecting their greater availability in the systemic circulation. This pattern of slow distribution and elimination probably reflects slow absorption from the site of injection or slow secondary release of the highly insoluble molecules into the bloodstream from lipoid tissue stores. Due to the similarity of the test compounds (especially the dilipid ester compound **8**) to natural components of membrane lipids, it seems probable that the compounds may become associated with brain membrane lipid bilayers to provide a reservoir of the esters which may subsequently release GABA following hydrolysis by the esterases present in the CSF and brain membranes.[7] Table III shows the results of a test of this hypothesis.

**Table II.** Effects of Time and Dose on Uptake of the Drug in Mouse Brain

| compd[a] | time, min | dose, mg/kg | mode of injection | brain uptake,[b] nmol/g |
|---|---|---|---|---|
| **10** | 5 | 78 | ip | 4.8 |
| **10** | 15 | 78 | ip | 14.0 |
| **10** | 30 | 78 | ip | 16.4 |
| **7** | 5 | 100 | sc | 2.81 |
| **7** | 60 | 9.4 | sc | 3.48 |
| **7** | 60 | 104 | sc | 27.64 |
| **8** | 5 | 50 | ip | 1.94 |
| **8** | 15 | 50 | ip | 1.65 |
| **8** | 45 | 50 | ip | 4.52 |
| **8** | 60 | 14 | sc | 1.37 |
| **8** | 60 | 120 | sc | 5.97 |
| **8** | 240 | 33 | ip | 7.07 |

[a] Each dose of the $^{14}C$-labeled compound was administered systemically to a mouse in either 0.5 mL of 0.14 N saline (for compound **10**) or 0.5 mL of 25% propylene glycol and water (for compounds **7** and **8**). [b] Brain was homogenized in 8 mL of brain protein solvent[11] [1% w/v) sodium dodecyl sulfate in 6 M urea and 19 nM EDTA, pH 7.4, 0.03 M phosphate]. Aliquots (0.5–1.5 mL) of the homogenate were mixed with 10 mL of Aquasol-2 (New England Nuclear Co.) and counted for radioactivity.

**Table III.** Hydrolysis of **7** and **8** by Rat Brain Homogenate[a]

| compd | rate of hydrolysis, nmol $min^{-1}$ (mg of protein)$^{-1}$ |
|---|---|
| **7** | 1.20 ± 0.50 |
| **8** | 0.44 ± 0.18 |

[a] For details, see the Experimental Section.

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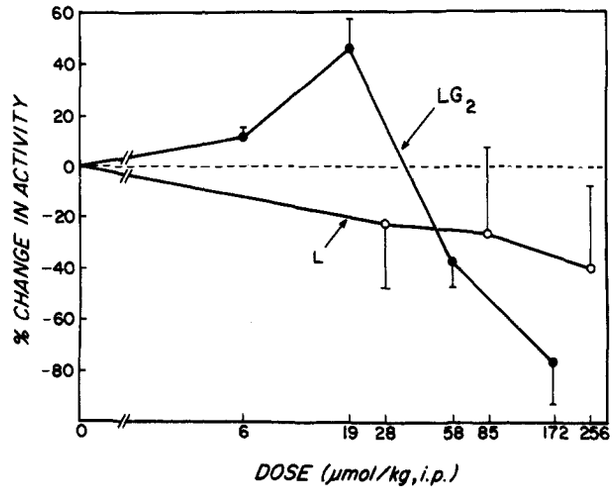


**Figure 1.** Dose–response data for the effect of 1-linolenoyl-2,3-bis(4-aminobutyryl)propane-1,2,3-triol ($LG_2$; compound 7) on the general motor activity of test mice. The compound enhanced motor activity at low doses (50% at 10 μmol/kg) and reduced activity at higher doses (50% at approximately 85 μmol/kg). The data for linoleic acid (L) as a control show a slight but not significant ($p > 0.05$) tendency to depress activity. Each point represents data for the mean ± SEM for six test mice in comparison to vehicle control (25% propylene glycol in water).

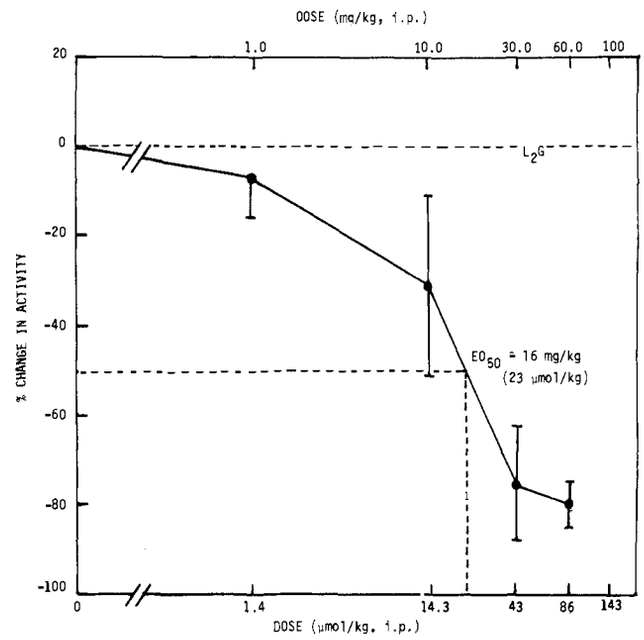


**Figure 2.** Dose–response curve for the effect of 1,2-dilinolenoyl-3-(4-aminobutyryl)propane-1,2,3-triol ($L_2G$; compound 8) on the general motor activity of test mice. Each point represents data for the mean ± SEM for six mice in comparison to vehicle control (25% (vol) propylene glycol in water).

Measurements of the rates of hydrolysis of compounds 7 and 8 by esterases present in the crude supernatant fraction of rat brain homogenates[8] indicate that hydrolysis by CNS tissue can occur in vitro, since the $^{14}C$-labeled molecules were found to release [$^{14}C$]GABA. Evidence indicating that the same reactions can also occur in vivo was obtained by analysis of the fate of the molecules, following uptake into mouse brain. $^{14}C$-labeled compound 7 was injected at a dose of 98 mg/kg sc. After 1 h the animal was sacrificed, and its brain and liver were homogenized in pyridine and centrifuged to yield a supernatant. An aliquot of the extract from each tissue was evaporated to dryness and analyzed by TLC with use of butanol, acetic acid, pyridine, water (5:1:3.3:4) as the solvent system. In the brain 40% of the total radioactivity present comigrated with the unhydrolyzed compound 7, while 60% corresponded to GABA. In the liver the total labeled products were distributed as 31% compound 7, 13% GABA, and 57% as volatile products (probably [$^{14}C$]acetic acid). Such results with brain tissue in vitro and in vivo suggest that the compound behaves as a "prodrug" which releases its GABA activity in the CNS following hydrolysis. In contrast, liver evidently yields a more rapid and complex metabolic breakdown of these compounds.

**Pharmacological Properties.** The general motor activity of albino mice was measured in a Stoelting electronic activity monitor (EAM) apparatus[9] for 60 min after an intraperitoneal (ip) injection of a test compound. Dose–response curves were obtained for the di-GABA linolenoyl ester of glycerol ($LG_2$ or compound 7) (Figure 1) and for the mono-GABA ester ($L_2G$ or compound 8) (Figure 2). GABA itself had no significant effect even at a dose of about 500 mmol/kg (52 g/kg, ip), because of its poor uptake into brain and rapid decomposition, presumably by GABA:2-oxoglutarate aminotransferase (GABA-T), whereas compounds 7 and 8 were active at much lower doses (Figures 1 and 2). Even though the general motor activity was depressed, the animals were quite alert, and the effect lasted for about 40–50 min.

TLC separation to permit estimates of the actual concentrations of intact molecules present after 5 min in the brain after sc injections of 98 and 47 mg/kg doses (188 and 67 μmol/kg, respectively) of compounds 7 and 8 indicated that only 3.0 and 5.9 nmol, respectively, were present per gram of brain tissue, indicating that even such minute quantities yielded significant pharmacologic activity. The di-GABA lipid analogue ($LG_2$ or compound 7) also had the curious property of enhancing locomotor activity at low doses, below 10 mg/kg (20 μmol/kg), but depressing such behavior at higher doses. Figure 1 illustrates the biphasic dose–response curve for compound 7. This type of behavioral effect was not observed for the dilipid mono-GABA glyceride ester (compound 8, see Figure 2); here a monophasic dose-related response, with only depression of locomotor activity ($ED_{50}$ = 16 mg/kg), was obtained for the compound. The change in the structure of the molecule from one containing a single GABA to a di-GABA substituent apparently changes its pharmacological properties. We do not know the reason for this type of pharmacological activity, observed for compound 7; one explanation among many possibilities is that the intact molecule may have a slight preference for $(GABA)_B$ receptors. A similar biphasic action has been reported for *p*-chlorophenyl-GABA (Baclofen),[10] which has been found to preferentially bind to type B GABA receptors.[11]

The octanol:water partition coefficients (Table I) for 7 and 8 were 2.13 and 6.60, respectively. The highly lipophilic nature of 7 and 8 helps these compounds to reach brain tissue and may contribute to their prolonged availability (Table I). The lipid solubility seems to be an important factor for the neuropharmacological activity since the glyceryl ester of GABA (compound 10), which had an octanol:water partition coefficient of only 0.01, was found not to be behaviorally active (data not shown). This

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is similar to our previous finding with a water-soluble steroid (dexamethasone) derivative of GABA.[6]

## Conclusion

In the present study we found that lipids similar to natural components of brain tissue can be used to transport a polar, neuropharmacologically active compound such as GABA into the brain. Its BPI value was increased by 75- to 127-fold by the use of the highly lipophilic "transporter" molecules. These compounds can enter the brain, where they behave as "prodrugs" to slowly release GABA by hydrolysis, delivering the neurotransmitter probably to membrane-bound GABA receptor sites. These studies illustrate a procedure for penetrating the blood–brain barrier and may represent a general method to deliver highly hydrophilic, neuropharmacologically active substancess (such as amino acids, peptides, or monoamines) to active sites in the CNS.

## Experimental Section

**Chemistry.** Melting points were determined on a hot-stage Fisher Jones apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer infracord spectrophotometer and are reported in reciprocal centimeters. NMR spectra were recorded on a CFT 20 spectrometer. The NMR and IR spectra were characteristic for the compounds. Elemental analyses were performed by the Midwest Microlab Ltd., Indianapolis, IN, and were in agreement (within ±0.4%) with the proposed structures. Thin-layer chromatography (TLC) was performed on 100-μm-thick precoated silica gel chromatogram sheets by Eastman. For column chromatography, silica gel (230–400 mesh) was used as the adsorbant.

**1-Linolenoyl-2,3-*O*-isopropylideneglycerol (2). Using Linolenic Anhydride.** A solution of glycerol acetonide (1) (470 mg, 3.55 mmol), linolenic anhydride (Sigma Chemical Co., St. Louis) (1.90 g, 3.5 mmol), and 4-(dimethylamino)pyridine (460 mg, 3.8 μmol) in 45 mL of dry benzene was stirred at room temperature under $N_2$ atmosphere for 8 h. The benzene solution was washed with 5% (w/v) sodium bicarbonate solution (2 × 20 mL), 0.1 N HCl solution (2 × 20 mL), and brine (2 × 20 mL). The organic layer was dried over anhydrous magnesium sulfate and concentrated under vacuum to provide a crude yield of 2.18 g. This material was purified by passage through a silica gel column by eluting with 5% (vol) ethyl acetate in petroleum ether to give 953 mg (2.4 mmol) of the pure compound **2** (yield 69.5%). TLC: $R_f$ 0.66 [ethyl acetate:petroleum ether (1:1)]. Anal. ($C_{24}H_{40}O_4$) C, H.

**Symmetrical *t*-Boc-GABA Anhydride.** The compound was prepared by reacting *t*-Boc-GABA or *t*-Boc-[$^{14}$C]GABA with dicyclohexylcarbodiimide as previously described.[6]

**1-Glyceryl Linolenoate (3).** A solution of 1.20 g (3.1 mmol) of the linolenoyl ester of glycerol acetonide (**2**) in 50 mL of dry methylene chloride was stirred with 2.2 mL of trifluoroacetic acid at 0 °C for 12 h under $N_2$. The solution was concentrated in a rotary evaporator, and traces of the trifluoroacetic acid were removed under high vacuum. The crude material was purified by chromatography on a silica gel column, eluting with ethyl acetate (30%) in petroleum ether to obtain 730 mg. This material, upon keeping at −20 °C as a solution in 30% (vol) ethyl acetate–petroleum ether, yielded 98.0 mg of a colorless solid, mp 62–62.5 °C. The IR, NMR, and mass spectra indicated that it was the ester of saturated fatty acids present as impurities from the reagent, linolenic anhydride, which escaped the earlier purification process. MS: 330.27706 ($M^+$, calcd for glyceryl palmitate, $C_{19}H_{38}O_4$, 330.27701); 299.26243 ($M^+ - CH_2OH$). After removal of the crystallized solid, the filtrate was concentrated to yield 470 mg (1.34 mmol) of the product (**3**) (43% yield), which migrated as a single spot on TLC and had NMR and IR spectra consistent with the proposed structure. TLC: $R_f$ 0.12 [ethyl acetate:petroleum ether (1:1)].

**1-Linolenoyl-3-[4-[(*tert*-butoxycarbonyl)amino]-butyryl]propane-1,2,3-triol (4).** *t*-Boc-GABA anhydride (250 mg, 640 μmol) and 4-(dimethylamino)pyridine (80.0 mg, 660 μmol) were added to a solution of 1-linolenoylglycerol (472 mg, 1.34 mmol) in 50 mL of dry benzene. The mixture was stirred overnight under $N_2$ atmosphere. The benzene solution was then washed with 0.1 N HCl solution, 5% (w/v) aqueous $NaHCO_3$, and water. The organic layer was removed and dried over anhydrous $MgSO_4$ and concentrated in vacuo to yield 504 mg of crude product. This material was then purified by chromatography on a silica gel column, eluting with 30% ethyl acetate in petroleum ether to get 234 mg (440 μmol) of compound 4 (TLC pure) as a viscous liquid (68.0% yield). The same procedures were used on a microscale (20 mg) to synthesize compound 4 as a radioactive derivative with U-$^{14}$C-labeled *t*-Boc-GABA anhydride. TLC: $R_f$ 0.45 [ethyl acetate:petroleum ether (1:1)]. Anal. ($C_{30}H_{51}NO_7$) C, H, N.

**1-Linolenoyl-2,3-bis[4-[(*tert*-butoxycarbonyl)amino]-butyryl]propane-1,2,3-triol (5).** The symmetrical anhydride of *t*-Boc-GABA (567 mg, 1.45 mmol) and 4-(dimethylamino)-pyridine (178 mg, 1.45 mmol) were added to a solution of 1-linolenoylglycerol (256 mg, 730 μmol) in 50 mL of dry benzene. The mixture was stirred for 6 h under $N_2$ atmosphere. The reaction mixture was worked up as before and the organic layer was concentrated in vacuo to yield 1.23 g of the crude product (**5**). The material was then passed through a silica gel column. Elution with petroleum ether:ethyl acetate (80:20) yielded the pure product **5**, 356 mg, 0.49 mmol (68.0% yield). TLC: $R_f$ 0.67 [ethyl acetate:petroleum ether (1:1)]. Anal. ($C_{39}H_{66}N_2O_{10}$) C, H, N.

**1,2-Dilinolenoyl-3-[4-[(*tert*-butoxycarbonyl)amino]-butyryl]propane-1,2,3-triol (6).** A solution of linolenoyl chloride (11.0 mg, 0.38 mmol) in 10 mL of dry benzene was added dropwise from a pressure-equalizing funnel to a solution of 1-linolenoyl-3-[4-[(*tert*-butoxycarbonyl)amino]butyryl]glycerol (**4**) (193 mg, 0.36 μmol) and 4-(dimethylamino)pyridine (55.0 mg, 0.47 mmol) in 15 mL of dry benzene at 0 °C under $N_2$. The mixture was stirred for 4 h at about 10 °C, after which the benzene solution was washed with 0.1 N HCl, 5% (w/v) $NaHCO_3$, and brine. The organic layer was separated, dried over anhydrous $MgSO_4$, and concentrated in vacuo to yield 223.7 mg of crude material, which was purified by passing through a silica gel column to yield 186.4 mg (65.0% yield) of the purified compound **6**. This material was unstable at room temperature; it could be stored as a chloroform solution at −20 °C. TLC: $R_f$ 0.40 (ethyl acetate:petroleum ether (1:9)]. Anal. ($C_{48}H_{70}NO_8$) H, N; C: calcd, 72.22; found, 71.69.

**1-Linolenoyl-2,3-bis(4-aminobutyryl)propane-1,2,3-triol (7).** The 1-linolenoyl-2,3-bis[4-[(*tert*-butoxycarbonyl)amino]-butyryl] ester **5** (200 mg, 277 μmol) in 50 mL of dry methylene chloride containing 2.2 mL of trifluoroacetic acid was stirred at 0 °C under $N_2$ for 6 h. The solution was concentrated under vacuum and was taken in 15 mL of ethyl acetate. The ethyl acetate solution was treated with saturated NaCl solution adjusted to pH 8.5 with $NaHCO_3$. The organic layer was separated, dried over anhydrous $Na_2SO_4$, and concentrated under vacuum to obtain 98 mg (188 μmol) of the diamine (compound **7**) (68%). TLC: $R_f$ 0.77 (BuOH:AcOH:pyridine:water, 5:1:3.3:4, vol). Since the free amine was unstable, the *N*-acetyl derivative **11** was used for analysis, and it was obtained by acetylation with acetic anhydride in pyridine. Anal. ($C_{33}H_{54}N_2O_8$) C, H, N.

Compound **7** was also synthesized by the above methods as a radioactive derivative on a microscale (20 mg). All the radioactivity in the compound was present as U-$^{14}$C in the GABA moiety of the compound. The product had a specific activity of 139 μCi/mmol and migrated with a $R_f$ value of 0.77 (BuOH:AcOH:pyridine:$H_2O$, 5:1:3.3:4, vol) on silica gel TLC plates.

**1,2-Dilinolenoyl-3-(4-aminobutyryl)propane-1,2,3-triol (8).** A solution of 1,2-dilinolenoyl-3-[4-[(*tert*-butoxycarbonyl)-amino]butyryl]propane-1,2,3-triol (**6**) (40 mg, 50.0 μmol) in 15 mL of methylene chloride containing 0.25 mL of trifluoroacetic acid at 0 °C was stirred overnight under $N_2$. The solution was then concentrated under vacuum. The viscous residue was passed through a silica gel (8 g) column, eluting first with ethyl acetate:petroleum ether (1:1, vol) and then with chloroform:methanol (4:1, vol). The ninhydrin positive (TLC) fractions of the chloroform:methanol eluents were combined and concentrated to obtain 62 mg of material. This was dissolved in 15 mL of chloroform and treated with 10 mL of $NaHCO_3$ solution containing sodium chloride. The chloroform layer was separated, dried over anhydrous $Na_2SO_4$, and concentrated to yield 29.5 mg (45 μmol) (84.0%) of pure product (compound **8**). TLC: $R_f$ 0.58 (chloro-

form:methanol, 9:3, vol). Since the free amine was not stable, the *N*-acetyl derivative (compound **12**) was prepared for analysis by reacting the free amine with acetic anhydride and 4-(dimethylamino)pyridine.

Compound **8** was also synthesized as a radioactive derivative by using the above methods on a microscale. The product had all its label as $^{14}C$ in the GABA substituent of the molecule. It migrated at an $R_f$ of 0.58 ($CHCl_3$:$CH_3OH$ 9:3) on TLC and had a specific activity of 41 $\mu$Ci/mmol.

***N*-Carbobenzoxy-γ-aminobutyric Acid (Cbz-GABA) (13).** GABA (5.0 g, 48.5 mmol) was dissolved in 2 M NaOH solution (24.3 mL) and the solution was cooled to 0 °C. Carbobenzoxy chloride (9.11 g, 53.4 mmol) and 2 M NaOH solution (29.1 mL) were then added in seven portions each over 1 h. After the additions were completed, the mixture was stirred vigorously at room temperature overnight. The mixture was extracted with ethyl acetate (25 mL × 3) and the aqueous phase was acidified with 3 M HCl to pH 2.3. The acidified mixture was extracted with ethyl acetate (3 × 40 mL). The combined ethyl acetate extracts were then washed with brine, dried over anhydrous $MgSO_4$, and concentrated to a colorless oil, which crystallized from ethyl acetate:petroleum ether as a white solid, 6.12 g (53.2%), mp 66.5–68 °C. TLC: $R_f$ 0.84 (chloroform:methanol:acetic acid, 18:6:1), $R_f$ 0.75 (ethyl acetate:acetic acid:ethanol, 9:1:1). IR (Nujol): 1670 (C=O).

**Symmetrical Anhydride of 4-(Carbobenzoxyamino)-butyric Acid (14).** An ice-cold solution of dicyclohexylcarbodiimide (DCCI) (216 mg, 1.05 mmol) in dry methylene chloride (6 mL) was added to an ice-cold solution of Cbz-GABA (500 mg, 2.10 mmol) in dry methylene chloride (2 mL). Dicyclohexylurea precipitated as a white solid. The mixture was stored at room temperature for 3 h and then the precipitate was removed by filtration and the filtrate was concentrated and crystallized from ethyl acetate; yield 354 mg (74.0%), mp 85–87 °C. IR: 1795 $cm^{-1}$. Anal. ($C_{24}H_{28}N_2O_7$) C, H, N.

**1-[[4-(Carbobenzoxyamino)butyryl]oxy]-2,3-*O*-(isopropylidenedioxy)propane.** Isopropylideneglycerol (**1**) (370 mg, 2.8 mmol), 4-(carbobenzoxyamino)butyric acid anhydride (**14**) (1.28 g, 2.8 mmol), and 4-(dimethylamino)pyridine (360 mg, 3 mmol) were dissolved in 20 mL of dry benzene, and the solution was stirred overnight at room temperature under anhydrous conditions. The solution was washed with 5% (w/v) sodium bicarbonate solution, 0.1 N HCl, and water. The organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuum to yield 849 mg (2.4 mmol) (86.0%) of product (compound **9**). TLC (ethyl acetate:hexane, 1:1, v): $R_f$ 0.57. Anal. ($C_{18}H_{25}NO_6$) C, H, N.

**1-(4-Aminobutyryl)propane-1,2,3-triol (10).** A solution of the carbobenzoxy derivative **9** (see Scheme II) (200 mg, 570 $\mu$mol) and 10% (wt) palladium on charcoal (30 mg) in 10 mL of ethanol was stirred overnight under hydrogen atmosphere. The solution was filtered to remove the catalyst and was concentrated under vacuum to get the product (compound **10**) as a viscous liquid (104 mg, 432 $\mu$mol) (76%) which had a single ninhydrin positive spot on analytical thin-layer chromatography ($R_f$ 0.35 in chloroform:methanol, 9:3, vol).

The product obtained after hydrogenolysis (150 mg, 690 $\mu$mol) was taken in 20 mL of methylene chloride, and 0.2 mL of trifluoroacetic acid was added and the mixture was stirred at room temperature for 3 h. The solvent was removed under vacuum, and the residue was passed through a silica gel column, eluting with a chloroform:methanol gradient to obtain 77.7 mg (267 $\mu$mol) of the trifluoroacetate (39.0% yield). This material was not stable at room temperature as a neat sample. TLC: $R_f$ 0.45 (BuOH:AcOH:$H_2O$, 4:1:1). Anal. ($C_9H_{16}F_3NO_6 \cdot 2H_2O$) C, N, H: calcd, 6.12; found, 5.17.

A radioactive derivative (specific activity 64.5 $\mu$Ci/mmol) was synthesized by using the above procedures with carbobenzoxy-[U-$^{14}C$]GABA anhydride.

**Measurements of Brain Penetration Index.** Each of the $^{14}C$-labeled compounds **7**, **8**, and **10** was dissolved in 125 $\mu$L of propylene glycol, diluted with water up to 0.5 mL, and then injected subcutaneously (sc) into test Balb-c mice (20 ± 2 g weight). After 5 min the animals were sacrificed, and the brain and liver were removed, weighed, and homogenized, respectively, in 8 and 10 mL of brain protein solvent[12] (phosphate buffer containing urea, sodium dodecyl sulfate, and EDTA at pH 7.6). Next, 0.5-mL aliquots of the tissue extracts were mixed with 10 mL of Aquasol 2 (New England Nuclear Corp., Boston, MA) and counted for $^{14}C$ in a Beckman liquid scintillation counter at ca. 50% efficiency. The counts of $^{14}C$ radioactivity in each tissue were used to calculate the total quantity of the compound present in brain and liver per gram of tissue. The ratio of the amount in brain as a percent of that present in liver at 5 min was taken as the brain penetration index (BPI)[6] (Table I). BPI values of 100% would be obtained for molecules for which there is no blood–brain barrier; those with values >100% are preferentially taken up by brain tissue in comparison to liver.[6] Measurements at longer uptake times were also obtained (Table II) to evaluate the accumulation and disappearance of the compounds in brain.

**Pharmacology.** All the compounds were stored at −15 °C as a chloroform solution to avoid air oxidation. For pharmacological testing, aliquots were evaporated under $N_2$ and dissolved in 25% propylene glycol in water.

Alteration of general motor activity[9] following an intraperitoneal (ip) injection of each test compound in comparison to vehicle control was used to determine neuropharmacological properties as prevously described. Also vehicle-injected animals showed no significant behavioral differences from uninjected animals.[6]

**In Vitro Hydrolysis of 7 and 8 Using Brain Homogenate.** Rat brain was homogenized in 0.32 M sucrose containing 0.1 M $CaAc_2$. This crude brain homogenate was centrifuged (10000*g*) and the supernatant ($S_1$) was used as the source of hydrolytic enzyme activity.[8] Protein concentration was determined by the method of Lowry et al.[13] A mixture of brain homogenate (0.2 mL) and either $^{14}C$-labeled **7** (2–8 mg/mL) or $^{14}C$-labeled **8** (1–4 mg/mL) in Tris A buffer (0.2 mL, pH 7.2) was incubated at 37 °C. At various times, 50-$\mu$L aliquots were removed, quenched with 0.5 mL of ethanol, and analyzed by thin-layer chromatography (TLC). TLC was carried out on silica gel in either chloroform:methanol:acetic acid (18:6:1; for compound **7**) or chloroform:methanol (9:3; for compound **8**). Fractions corresponding to the authentic samples of unlabeled **7** and **8** and GABA were analyzed for radioactivity (Table III).

**In Vivo Breakdown of Compound 7.** The $^{14}C$-labeled compound **7** (98 mg/kg, sc) was administered to a mouse. After 1 h the animal was sacrificed, and brain and liver were each homogenized in 3 mL of pyridine. The homogenates were centrifuged, and the clear pyridine supernatant fraction was removed for analysis: 0.5-mL aliquots were concentrated under a stream of $N_2$ and separated by TLC on silica gel on plastic sheets in butanol:acetic acid:pyridine:waer (5:1:3.3:4). The plates were cut into segments and analyzed for $^{14}C$. In the brain 40% of total radioactivity was found to be cochromatographic with compound **7**, and the remaining label comigrated with authentic GABA. In the liver only 31% of the label corresponded to compound **7**, and only 13% to GABA. The remaining radioactivity present in the sample was due to volatile products.

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