# Structural Requirements for a Lipoamino Acid in Modulating the Anticonvulsant Activities of Systemically Active Galanin Analogues

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Introduction of lipoamino acid (LAA), Lys-palmitoyl, and cationization into a series of galanin analogues yielded systemically active anticonvulsant compounds. To study the relationship between the LAA structure and anticonvulsant activity, orthogonally protected LAAs were synthesized in which the Lys side chain was coupled to fatty acids varying in length from $C_8$ to $C_{18}$ or was coupled to a monodispersed polyethylene glycol, $PEG_4$. Galanin receptor affinity, serum stability, lipophilicity (log *D*), and activity in the 6 Hz mouse model of epilepsy of each of the newly synthesized analogues were determined following systemic administration. The presence of various LAAs or Lys($MPEG_4$) did not affect the receptor binding properties of the modified peptides, but their anticonvulsant activities varied substantially and were generally correlated with their lipophilicity. Our results suggest that varying the length or polarity of the LAA residue adjacent to positively charged amino acid residues may effectively modulate the antiepileptic activity of the galanin analogues.

## Introduction

When delivered directly into the brain, many neuropeptides, including galanin, neuropeptide Y, somatostatin, neurotensin, or opioid peptides can modulate excitatory or inhibitory circuits and suppress seizures and/or pain sensation.[1−4] Despite a considerable interest in generating blood−brain barrier (BBB[a]) permeable analogues of neuropeptides, only a few successful examples have been reported to date.[4−12] To improve central nervous system (CNS) bioavailability of neuroactive peptides, various strategies have been explored, including lipidization, cationization, and glycosylation.[13−15] Banks and Kastin showed that the lipophilicity of peptides improved their permeability through the BBB.[16] Despite the finding that only small amounts of the peptides entered the brain, the authors noted a direct correlation between the log *D* values and the blood-to-brain ratio. Reversible lipidization of opioid peptides also improved centrally mediated analgesic effects.[17] It is also important to note that oral bioavailability of the somatostatin analogue TT-232 was achieved by coupling various LAAs to either N- or C-terminal components of the analogue.[18] Although lipophilicity has been acknowledged to play an important role in structure−bioavailability relationships,[16,19] very few examples are available where lipidization of neuropeptides has been found to improve their activity in the brain.[17,20,21] As pointed out in a review by Witt et al.,[13] "lipidization of peptides may increase plasma protein binding, systemic elimination, and intracellular sequestration, thus hampering their efficient penetration into the CNS." Indeed, when just the lipidization strategy was applied to galanin, the truncated galanin analogue containing a Lys-palmitoyl residue was inactive as an anticonvulsant, despite displaying high affinity toward galanin receptors and a high log *D*.[22]

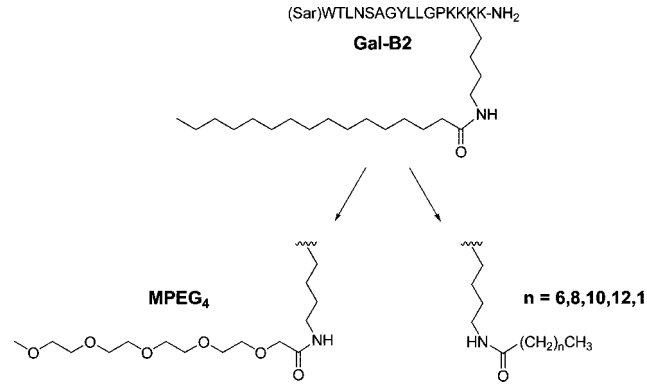


**Figure 1.** Structures of Gal-B2 and LAAs used in this SAR study. Note that $MPEG_4$ has an identical number of atoms as the $C_{16}$ (palmitoyl) but is significantly less lipophilic.

In contrast, lipidization appeared very effective in improving the CNS bioavailability of the truncated galanin analogues when the Lys(palmitoyl) residue was introduced in concert with cationization.[22] The most active analogue, Gal-B2, contained the C-terminal "-Lys-Lys-Lys(palmitoyl)-Lys-$NH_2$" motif (Figure 1). The combined lipidization and cationization resulted in very potent antiepileptic compounds; e.g., the anticonvulsant $ED_{50}$ of Gal-B2 was found to be 0.8 mg/kg when tested intraperitoneally (ip) in the 6 Hz pharmacoresistant model of epilepsy. Since galanin suppresses seizures by activating GalR1 and GalR2 located in the hippocampus and other limbic structures, our data suggest that the combination of cationization and lipidization is effective in improving the BBB penetration of Gal-B2 without negatively affecting receptor affinity. Furthermore, our results suggest that the sequence position of LAAs

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[a] Abbreviations: AUC, area under the curve; BBB, blood−brain barrier; CD, circular dichroism; CNS, central nervous system; DCC, *N*,*N*′-dicyclohexylcarbodiimide; DIPEA, *N*,*N*-diisopropylethylamine; Fmoc, *N*-(9-fluorenyl)methoxycarbonyl; GalR1, galanin receptors subtype 1; GalR2, galanin receptors subtype 2; GPCRs, G-protein-coupled receptors; ip, intraperitoneally; LAA, lipoamino acid; $MPEG_4$, monodispersed polyethylene glycol $PEG_4$; PyBop, (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate; SAR, structure−activity relationship; SPPS, solid phase peptide synthesis; TFA, trifluoroacetic acid; TFE, 2,2,2-trifluoroethanol.

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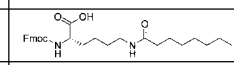
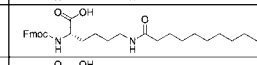
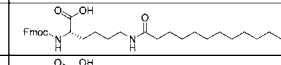
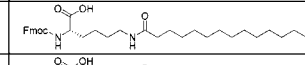
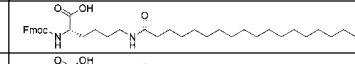
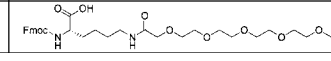
**Table 1.** Structures of LAAs Used for SPPS of the Systemically Active Galanin Analogues

| LAA | Name | Structure |
|---|---|---|
| 1 | Fmoc-Lys(octanoyl)-OH |  |
| 2 | Fmoc-Lys(decanoyl)-OH |  |
| 3 | Fmoc-Lys(lauroyl)-OH |  |
| 4 | Fmoc-Lys(myristoyl)-OH |  |
| 5 | Fmoc-Lys(stearoyl)-OH |  |
| 6 | Fmoc-Lys(MPEG$_4$)-OH |  |

is important for the systemic activity of Gal-B2, suggesting that the lipidization/cationization motif was not simply producing the additive effect by combining hydrophobicity and positive charges.

In order to define the structural requirements of the LAA residue for improving the anticonvulsant activity of Gal-B2, we synthesized a series of LAAs in which the Lys side chain was either coupled to fatty acids, varying in length from $C_8$ to $C_{18}$, or to a monodispersed $PEG_4$ ($MPEG_4$). These LAAs were used to replace the Lys-palmitoyl residue in position 16 of Gal-B2 (Figure 1). The binding affinity, octanol/water partitioning coefficient, and ability to suppress seizures in the in vivo 6 Hz model of epilepsy was determined following ip administration. Our findings suggest that the length and polarity of the LAAs are important for maintaining antiepileptic activity of the galanin analogues.

## Results

**Design and Chemical Synthesis.** To study the role of Lys-palmitoyl, residue 16, in the activity of Gal-B2, we designed a series of analogues where we systematically changed the length of the fatty acid or replaced the palmitoyl motif with a $MPEG_4$ (Figure 1). The length of the LAAs varied from $C_8$ to $C_{18}$ by two carbon atom increments (Table 1). The $MPEG_4$-containing Gal-B2 analogue was designed to dissect the lipophilic contribution of the Lys-palmitoyl residue, since $MPEG_4$ is significantly more polar than $C_{16}$ despite having an identical number of atoms. To synthesize these new structure−activity relationship (SAR) analogues of Gal-B2, we applied a direct coupling of the orthrogonally protected $N^{\varepsilon}$-lipolysine intermediates ($N^{\varepsilon}$-palmitoyl-$N^{\alpha}$-Fmoc-lysine is commercially available). For the synthesis of $N^{\varepsilon}$-octanoyl **1** ($C_8$), -decanoyl **2** ($C_{10}$), -lauryl **3** ($C_{12}$), -myristoyl **4** ($C_{14}$), and -stearoyl **5** ($C_{18}$) substituted $N^{\alpha}$-Fmoc-lysine, a convenient method was applied using $Na_2CO_3$ as a coupling reagent with a corresponding acid halide in dioxane/water solvent system (Scheme 1). Synthesis of Fmoc-Lys-($MPEG_4$)-OH **6** was achieved by coupling Fmoc-Lys-OH with perfluorophenol activated $MPEG_4$-acetic acid **7**. Compound **7** was synthesized from the substitution reaction of $MPEG_4$-OH with ethyl bromoacetate followed by ester hydrolysis (Scheme 2). The modified amino acids were purified by flash chromatography and used for solid-phase peptide synthesis (SPPS).

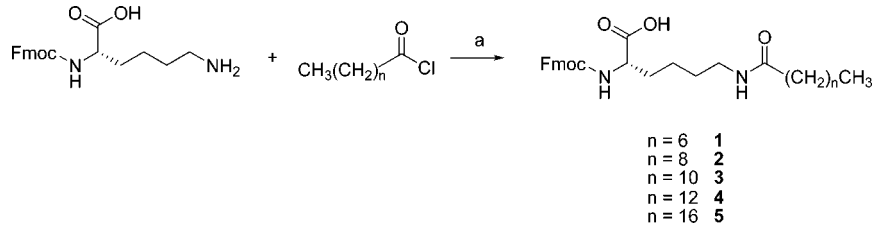
The SPPS was carried out using an automated peptide synthesizer at 25 $\mu$mol scale on preloaded Fmoc-Lys(Boc)-Rink amide AM resin. The 5-fold Fmoc-amino acids/PyBop/DIPEA (1:1:2) were used in peptide synthesis. A 2-fold of the Fmoc protected LAAs or $MPEG_4$-lysine was used in manual coupling synthesis. The synthesized peptides were cleaved from the resin using reagent K (trifluoroacetic acid (TFA)/water/thioanisole/phenol/ethanedithiol, 90/5/5/7.5/2.5 v/v), followed by precipitation and washing with ice cold methyl *tert*-butyl ether (MTBE). Crude peptides were purified by preparative reversed-phase HPLC using a diphenyl column with a linear gradient of water/acetonitrile (both buffered with 0.1% TFA). The purities of peptides were greater than 95% by analytical HPLC analyses (Supporting Information Figure S1 and Table S1). Purified analogues were quantified by UV absorbance ($\lambda$ = 279.8 nm, $\varepsilon$ = 7000 $cm^{-1}$ $M^{-1}$), and their monoisotopic mass was confirmed by MALDI-TOF mass spectrometry (Table 2).

**Physicochemical and Structural Properties.** To determine the lipophilicity of the analogues, log *D* values were calculated using the combination of the HPLC retention times and the log *D* determined from the classical shake-flask method, as previously described.[22] Calculations of log *D* values are provided in Supporting Information Table S2. For the LAA containing analogues, the log *D* values ranged from 0.78 for Gal-B2-$C_8$ to 1.35 for Gal-B2-$C_{18}$. As shown in Figure 2, the log *D* values linearly correlated with the carbon chain length of the LAA (linear fit yielded $R^2 = 0.98$). Since the log *D* values increased 4-fold by replacing one Lys residue with Lys-stearoyl (compare log *D* of Gal-(K)4 (0.34)[22] with that of Gal-B2-$C_{18}$ (1.35)) and almost doubled by changing the LAA from $C_8$ to $C_{18}$, these results emphasize the effectiveness of the LAAs to increase the lipophilicity of short peptides. The analogue with the $MPEG_4$-amino acid isostere had approximately 2-fold lower log *D* (0.57), compared to the Lys-palmitoyl residue (1.24).

Gal-B2 is largely unstructured in water but contains 23% α-helical content in the presence of 50% trifluoroethanol (TFE).[22] In our previous work,[22] we hypothesized that the presence of LAA could stabilize the helical conformation of the systemically active galanin analogues by hydrophobic interactions with Tyr9 and/or Trp2. To test this hypothesis, the conformational properties of the Gal-B2 analogues were investigated by circular dichroism (CD). In the presence of 5 mM potassium phosphate containing 150 mM NaF (pH 7.5), the helical content varied among the analogues from 1% to 7% (only Gal-B2-$C_8$ had 15%). The helical content in 50% TFE increased for most analogues compared to that determined in "buffer only" conditions (Table 2). No apparent correlations were observed between the length of the LAA and percentage of helical content in the analogues.

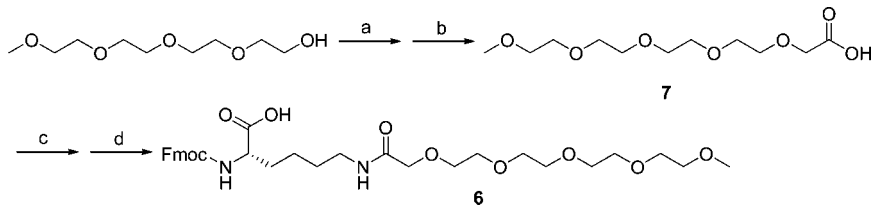
**Metabolic Stability.** To investigate how the length of the LAA residue might affect resistance of the galanin analogues to proteolytic degradation, we employed the in vitro metabolic stability assay. The half-life of the analogues was determined in buffered 25% rat blood serum incubated at 37 °C. The amount of each analogue remaining after an appropriate time interval was quantified by HPLC, and the time-course of disappearance of the analogues is shown in Figure 3A. Calculated half-lives for the analogues are summarized in Figure 3B. All analogues exhibited pronounced resistance to proteolytic degradation (for comparison, the unmodified Gal(1−16) analogue had 7.8 min half-time under these conditions).[22] Interestingly, the galanin analogues containing the shortest or the longest aliphatic chain were the least stable whereas the analogue containing $C_{14}$ had the longest half-life of 12 h (Figure 3B). This correlation suggested a rather complex mechanism by which the LAAs might increase the stability of galanin analogues in plasma. We hypothesized that the metabolic stability of the galanin analogues might be correlated with their serum albumin binding properties (e.g., binding properties of long-chain carboxylic acids to albumin are dependent on aliphatic chain length).[23] However,

**Scheme 1.** Synthesis of the Fmoc-Protected LAAs[a]




[a] Reagents and conditions: (a) $Na_2CO_3$, $H_2O$/dioxane, room temp, overnight, 64−87%.

**Scheme 2.** Synthesis of Fmoc-Lys(MPEG$_4$)-OH[a]




[a] Reagents and conditions: (a) NaH, 0 °C, 1 h; ethyl bromoacetate, 50 °C, 16 h; (b) 1 M LiOH/$CH_3OH$, 6 h, 59% (from two steps); (c) perfluorophenol, DCC, overnight; (d) Fmoc-L-lysine, DIPEA, 0 °C, 60% (from two steps).

**Table 2.** Structure and Properties of the Galanin Analogues[a]

| analog | structure | HPLC retention time[b] | α-helix[c] (%) | MS (MALDI, $MH^+$) calcd/found |
|---|---|---|---|---|
| Gal-(K)4[d] | (Sar)WTLNSAGYLLGP**KKKK** | 15.13±0.42 | 14 | 1873.09/1873.14 |
| Gal-B2-$C_8$ | (Sar)WTLNSAGYLLGP**KK(Lys-octanoyl)K** | 20.13±0.17 | 9 | 2001.21/2001.17 |
| Gal-B2-$C_{10}$ | (Sar)WTLNSAGYLLGP**KK(Lys-decanoyl)K** | 21.49±0.05 | 6 | 2029.24/2029.45 |
| Gal-B2-$C_{12}$ | (Sar)WTLNSAGYLLGP**KK(Lys-lauroyl)K** | 22.51±0.03 | 16 | 2057.28/2057.26 |
| Gal-B2-$C_{14}$ | (Sar)WTLNSAGYLLGP**KK(Lys-myristoyl)K** | 23.76±0.18 | 16 | 2085.31/2085.33 |
| Gal-B2[d] | (Sar)WTLNSAGYLLGP**KK(Lys-palmitoyl)K** | 25.84±0.10 | 23 | 2112.31/2112.33 |
| Gal-B2-$C_{18}$ | (Sar)WTLNSAGYLLGP**KK(Lys-stearoyl)K** | 26.73±0.18 | 5 | 2141.33/2141.48 |
| Gal-B2-MPEG$_4$ | (Sar)WTLNSAGYLLGP**KK(Lys-MPEG$_4$)K** | 17.52±0.03 | 18 | 2123.23/2123.29 |

[a] All analogues are amidated at the C-terminus. [b] A linear gradient of water/90% acetonitrile (buffered with 0.1% TFA) on a Vydac diphenyl column, starting from 20% acetonitrile to 100% acetonitrile in 40 min. [c] Determined in the presence of 50% v/v 2,2,2-trifluoroethanol. [d] Data from ref 22.

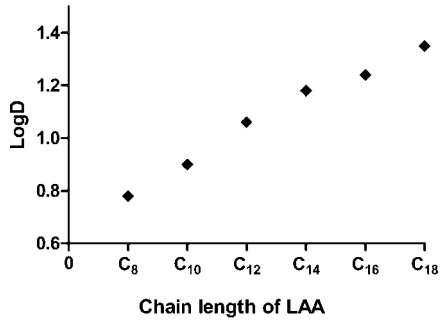



**Figure 2.** Relationships between the aliphatic chain length of the LAAs in Gal-B2 analogues and the octanol/water partition coefficient (log *D*). Linear fit yielded $R^2 = 0.9839$, $P < 0.0001$.

our efforts in determining the protein binding properties of Gal-B2 by microdialysis failed because of possible micelle formation.[22] The Gal-B2-MPEG$_4$ analogue exhibited a very long half-life (12.4 ± 0.65 h), suggesting that the extended amino acid residue might provide steric hindrance for access of proteinases.

**In Vitro and in Vivo Pharmacology.** Since two galanin receptors, GalR1 and GalR2, are known to be involved in controlling seizures in the brain,[24,25] we investigated the interactions of the galanin analogues with both receptor subtypes using a competitive binding assay. As described in our previous work, $K_i$ values were determined using a time-resolved fluorescence binding assay with a europium-labeled galanin.[22] The GPCR membrane preparations used in the assay were commercially available (Perkin-Elmer or Millipore) and were derived from recombinant human GalR1 or GalR2 gene sequences. As shown in Table 3, when compared to each other, none of the analogues studied here exhibited significantly different affinities toward either receptor subtype. This suggested that the relatively large LAA moiety did not affect the affinity of the analogues toward the galanin receptors. All analogues maintained several-fold preference in binding to GalR1 subtype, compared to GalR2, similar to that of unmodified Gal(1−16).[22]

The anticonvulsant activity of the analogues was studied in the 6 Hz (32 mA) model of pharmacoresistant epilepsy following ip administration of a bolus dose of 4 mg/kg. The analogues were evaluated for their ability to suppress seizures at various times (0.25−4 h) after peptide administration. The time−response curves were then integrated to provide a qualitative measurement of efficacy, i.e., area under the curve (AUC) values (Table 3, Figure 4). The analogues differed significantly in their ability to protect mice from seizures. On the basis of the AUC values, the most active galanin analogues contained Gal-B2-$C_{16}$ and Gal-B2-$C_{18}$, whereas the least active analogues included $C_8$ and $C_{10}$. Interestingly, the Lys-MPEG$_4$-containing analogue was also active as an anticonvulsant, albeit significantly less when compared to Gal-B2.

## Discussion

To study the structural requirements for the LAA residue 16 in mediating the antiepileptic activity of the systemically active galanin analogues,[22] we synthesized and characterized a new series of analogues that differed in their length/polarity of the fatty acid moiety coupled to the Lys16 residue. The major finding of this work was that the six galanin analogues (despite

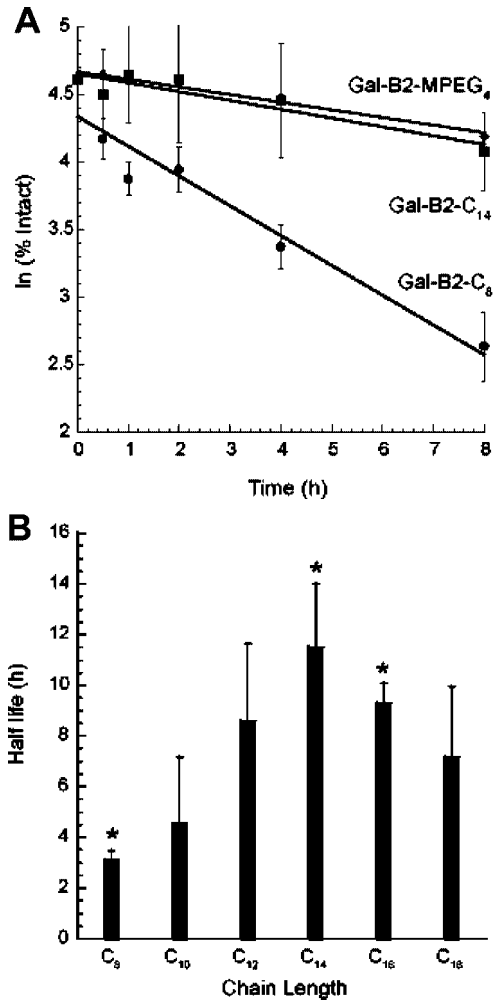



**Figure 3.** (A) In vitro serum stability assay for the galanin analogues. Representative plots showing a time-course of degradation for $C_8$- (●), $C_{14}$- (■), and $MPEG_4$ (◆)-containing galanin analogues in the presence of 25% rat serum at 37 °C. Degradation of analogues was monitored by analytical HPLC. Data points were obtained at 0, 0.5, 1, 2, 4, and 8 h from the average of at least three independent experiments. (B) Relationships between the length of the LAAs and the in vitro metabolic stability of the galanin analogues. The half-lives were determined by incubating the peptides in 25% diluted rat serum at 37 °C. The concentrations of the remaining analogues were determined by HPLC. Data were obtained from at least three independent experiments. ANOVA single factor analysis yielded a *P*-value of 0.004 for $C_8$-, $C_{14}$-, and $C_{16}$-containing species (*).

having comparable affinities toward the galanin receptors and differing in the length of the fatty acid from $C_8$ to $C_{18}$) exhibited pronounced differences in their anticonvulsant activities following ip administration. The analogues containing shorter fatty acids were less active compared to those with longer chains. These results suggest that the increased lipophilicity provided by the longer fatty acids is an important factor in improving the anticonvulsant activity of galanin analogues following systemic administration. Further work will be required to determine whether the resulting compounds are agonists, partial agonists, or antagonists. Interestingly, both Gal-B2 and Gal-B2-$C_8$ showed comparable analgesic activities in a mouse pain assay following ip administration (E. Adkins-Scholl, H. S. White, G. Bulaj, unpublished results), suggesting that systemically active galanin analogues varying in the length of a LAA may control seizures or pain via galanin receptors in the CNS or peripheral nerves, respectively.[26−28] Our previous data indicated that the position of the LAA residue appeared to be important for the anticonvulsant activity of Gal-B2,[22] thus implying that lipophilicity alone (which would be expected to increase passive diffusion) is not the sole important factor for improving seizure suppression by this analogue. Although the "-Lys-Lys-Lys(palmitoyl)-Lys-$NH_2$" motif appeared to be the most effective in increasing the anticonvulsant activity of the galanin analogues following ip administration, clearly, more SAR studies, or perhaps even a combinatorial approach, will be needed to further improve the potency of the Gal-B2 related analogues.[22]

Introduction of the LAA to the galanin analogues significantly improved their in vitro metabolic stability; this effect may be, at least in part, accounted for by steric effects of the long side chain in position 16 of the analogues. This finding is not surprising in light of previous reports with lipopeptides, in which prolonged in vitro and/or in vivo half-lives were observed,[29] including gonadotropin-releasing hormone analogues,[30] glucagon-like peptide 1 analogues,[31] insulin,[32] and octreotide.[20] The metabolic stability of lipopeptides may also be affected by their interactions with fatty acid binding sites in serum albumin.[32] As shown in Figure 3B, the longest serum half-lives were observed for Gal-B2-$C_{14}$ and Gal-B2-$C_{16}$ whereas the shortest were observed for Gal-B2-$C_8$. Interestingly, the $C_8$ (octanoic acid) moiety binds with 11-fold higher affinity to human serum albumin compared to the $C_{14}$ (myristic acid).[23]

Another aspect of this work is our less conventional approach of using orthogonally protected LAAs and $MPEG_4$-amino acids as building blocks for SPPS. Introduction of LAA into peptides have been reported using three distinct strategies: (1) on-resin fatty acid acylation of $N^{\alpha}$-amino acids[33,34] or $N^{\varepsilon}$-lysine,[35] (2) esterification of fatty acids,[36] and (3) conjugation at the C-terminus of peptide.[18,37] Our strategy of using the Fmoc protected LAAs directly coupled during the solid-phase peptide synthesis offers apparent advantages in applications such as routine synthesis of lipopeptide analogues for SAR studies or synthesis of peptide-based combinatorial libraries directed toward optimization of the chain length of the LAAs in the cationic/lipidic motif.

To what extent the systemically active galanin analogues may have improved the BBB permeability remains unanswered. However, since some of the modified galanin analogues possess pronounced antiepileptic activity believed to be mediated by the galanin receptors located in the limbic system and, thus, behind the blood−brain barrier, these data suggest that sufficient quantities of the analogues must penetrate into the brain. As we have suggested previously,[22] lipidization may improve CNS bioavailability of Gal-B2 via a combination of adsorptive-mediated endocytosis and passive diffusion.[38,39] More mechanistic studies assessing the mechanism of transport of galanin analogues across the cell-based models of BBB are underway. Regardless of the mechanism by which these analogues penetrate into the brain, our data suggest that changing the length and/or polarity of the LAA residue in the context of cationization may offer a useful strategy to modulate CNS bioavailability of galanin analogues. In summary, we believe that the variations in lipidization and cationization appear to provide a useful strategy for modulating CNS bioavailability of not only the galanin analogues described here but perhaps even other neuropeptides.

## Experimental Section

**General Synthetic Procedures.** Reagent chemicals were obtained from Aldrich Chemical Corporation and were used without prior purification. Reactions were performed under $N_2$ atmosphere,

**Table 3.** In Vitro and in Vivo Pharmacological Properties of the Galanin Analogues

| | in vitro assay (receptor binding), $K_i$ (nM) | | in vivo assay (anticonvulsant activity) (6 Hz, 32 mA, 4 mg/kg) | | | | |
|---|---|---|---|---|---|---|---|
| analogue | GalR1 | GalR2 | 15 min | 30 min | 1 h | 2 h | 4 h |
| Gal-(K)4[a] | 0.4±0.1 | 24.0±9.9 | 0/4 | 3/4 | 1/3 | 0/3 | 0/4 |
| Gal-B2-$C_8$ | 0.7±0.1 | 14.9±0.6 | 0/4 | 0/4 | 0/4 | 0/4 | 1/4 |
| Gal-B2-$C_{10}$ | 1.3±0.7 | 14.4±0.6 | 2/4 | 2/4 | 0/4 | 0/4 | 0/4 |
| Gal-B2-$C_{12}$ | 1.4±0.4 | 16.1±8.5 | 1/4 | 3/4 | 2/4 | 0/4 | 0/4 |
| Gal-B2-$C_{14}$ | 2.6±0.1 | 18.2±1.6 | 2/4 | 3/4 | 2/4 | 0/4 | 0/4 |
| Gal-B2[a] | 3.5±1.0 | 51.5±34.4 | 3/4 | 4/4 | 4/4 | 4/4 | 0/4 |
| Gal-B2-$C_{18}$ | 4.0±2.0 | 15.0±1.0 | 4/4 | 3/4 | 4/4 | 2/4 | 0/4 |
| Gal-B2-$MPEG_4$ | 0.5±0.1 | 20.5±6.5 | 1/4 | 1/4 | 1/4 | 2/4 | 0/4 |

[a] Data from ref 22.

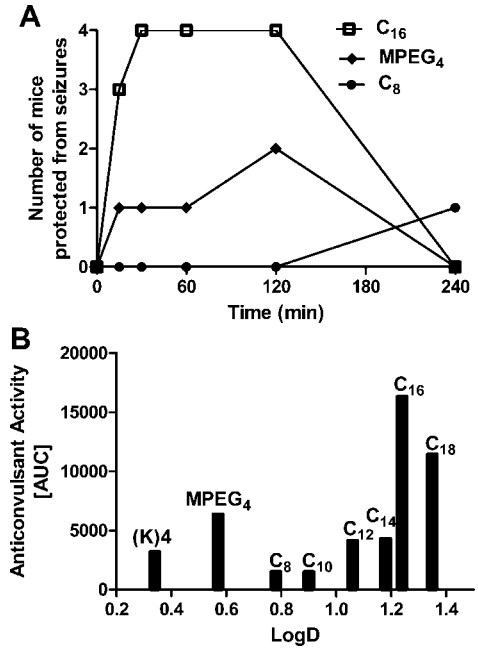



**Figure 4.** Anticonvulsant activity of the galanin analogues. The analogues were administered ip as a bolus dose of 4 mg/kg: (A) time−response studies ranging from 15 min to 4 h were used to calculate area under the curve values (AUC); (B) relationships between log *D* and the anticonvulsant activity (AUC values) of the galanin analogues in the 6 Hz model of epilepsy.

unless otherwise indicated. Chromatography refers to flash chromatography on silica gel (Whatman 230−400 mesh ASTM silica gel). Analytical thin layer chromatography was performed using EMD Aluminium TLC silica gel 60 PF254. Preparative HPLC was performed on a Waters 600 pump system equipped with a Waters 2487 dual wavelength detector ($\lambda_1 = 220$ nm, $\lambda_2 = 280$ nm) and a preparative Vydac diphenyl column (219TP101522). Analytical HPLC used an analytical Vydac diphenyl column (219TP54). The HPLC mobile phases are buffer A, 100% water (0.1% TFA), and buffer B, 90% acetonitrile (0.1% TFA). Metabolic stability used a Waters Alliance 2695 system equipped with an autosampler, dual wavelength detector, and a Waters YMC ODS-A diphenyl column (AA125052503WT). NMR spectra were recorded at 400 MHz ($^1$H), 101 MHz ($^{13}$C) at 25 °C. Proton and carbon chemical shifts are given in ppm relative to TMS internal standard. MALDI-TOF MS was conducted at the University of Utah Core Facility. CD spectra were obtained on an Aviv 62DS CD spectropolarimeter at room temperature. Optical rotations were measured on a Perkin-Elmer polarimeter (model 343) using a 1 mL capacity quartz cell with a 10 cm path length.

**Fmoc-Lys(octanoyl)-OH (1).** Fmoc-L-lysine (0.737 g, 2.0 mmol) was added to dioxane (10 mL), and then a solution of $Na_2CO_3$ (0.636 g, 6.0 mmol) in $H_2O$ (12.6 mL) was added dropwise at 0 °C. After 5 min, a solution of octanoyl chloride (0.325 g, 2.0 mmol) in dioxane (10 mL) was added to the mixture. The solution was allowed to warm to room temperature and stirred overnight. The solvents were extracted with $CH_2Cl_2$ (250 mL), washed with saturated $NaHCO_3$ (20 mL), 1 M HCl (2 mL), and brine (20 mL), dried over $Na_2SO_4$, and concentrated. Flash chromatography ($CH_2Cl_2/CH_3OH$, 6:1) gave a white amorphous solid **1** (0.86 g, 87%). TLC $R_f = 0.29$ ($CH_2Cl_2/CH_3OH$, 10:1). $[\alpha]_D^{20}$ +10.2° (*c* 1, $CHCl_3$). $^1$H NMR ($CDCl_3$): $\delta$ (ppm) 7.68 (d, $J = 7.6$ Hz, 2H), 7.53 (m, 2H), 7.32 (m, 2H), 7.23 (m, 2H), 5.67 (m, 1H), 4.29 (m, 2H), 4.13 (m, 1H), 3.19 (m, 2H), 2.10 (tm, 2H), 1.84−1.17 (m, 16H), 0.78 (m, 3H). $^{13}$C NMR ($CDCl_3$): $\delta$ (ppm) 174.40, 143.95, 141.40, 127.90, 127.29, 125.39, 120.12, 67.15, 47.30, 39.42, 36.84, 31.94, 29.52, 29.27, 26.07, 22.84, 14.31. HRMS (MALDI) (*m*/*z*) ($MNa^+$): found 517.2673. Calcd for $C_{29}H_{38}N_2O_5Na$ 517.2678.

**Fmoc-Lys(decanoyl)-OH (2).** Compound **2** was submitted to the same procedure described above for the preparation of **1** and gave a white amorphous solid (yield, 78%). TLC $R_f = 0.40$ ($CH_2Cl_2/CH_3OH$, 8:1). $[\alpha]_D^{20}$ +12.0° (*c* 1, $CHCl_3$). $^1$H NMR ($CDCl_3$): $\delta$ (ppm) 7.67 (d, $J = 7.6$ Hz, 2H), 7.52 (m, 2H), 7.30 (m, 2H), 7.21 (m, 2H), 5.78 (d, $J = 7.6$ Hz,1H), 4.27 (m, 2H), 4.12 (m, 1H), 3.17 (m, 1H), 2.10 (tm, 2H), 1.83−1.14 (m, 20H), 0.78 (t, $J = 7.2$ Hz, 3H). $^{13}$C NMR ($CDCl_3$): $\delta$ (ppm) 174.69, 156.64, 144.14, 143.91, 141.45, 127.94, 127.31, 125.38, 120.17, 98.99, 67.27, 66.36, 61.17, 54.04, 47.31, 39.41, 36.87, 32.26, 32.09, 29.72, 29.60, 29.53, 29.04, 26.08, 22.90, 22.54, 14.36. HRMS (MALDI) (*m*/*z*) ($MNa^+$): found 545.2978. Calcd for $C_{31}H_{42}N_2O_5Na$ 545.2991.

**Fmoc-Lys(lauroyl)-OH (3).** Compound **3** was submitted to the same procedure described above for the preparation of **1** and gave a white solid (yield, 64%), mp 126.0−128.0 °C. TLC $R_f = 0.76$ ($CH_2Cl_2/CH_3OH$, 4:1). $[\alpha]_D^{20}$ +14.3° (*c* 1, $CHCl_3$). $^1$H NMR ($CDCl_3$): $\delta$ (ppm) 7.67 (d, $J = 7.6$ Hz, 2H), 7.52 (m, 2H), 7.30 (m, 2H), 7.21 (m, 2H), 5.82−5.77 (m, 2H), 4.26−4.39 (m, 2H), 4.12 (t, $J = 7.2$ Hz, 1H), 3.17 (m, 2H), 2.09 (m, 2H), 1.83−1.35 (m, 6H), 1.48 (m, 17H), 0.79 (t, $J = 7.2$ Hz, 3H). $^{13}$C NMR ($CDCl_3$): $\delta$ (ppm) 175.11, 174.81, 156.56, 143.93, 141.49, 127.93, 127.31, 125.38, 120.18, 67.32, 53.76, 47.34, 39.36, 36.94, 32.13, 29.84, 29.72, 29.56, 29.14, 26.03, 22.91, 22.34, 14.35. HRMS (MALDI) (*m*/*z*) ($MNa^+$): found 573.3299. Calcd for $C_{33}H_{46}N_2O_5Na$ 573.3304.

**Fmoc-Lys(myristoyl)-OH (4).** Compound **4** was submitted to the same procedure described above for the preparation of **1** and gave a white solid (yield, 64%), mp 132.0−134.0 °C. TLC $R_f = 0.81$ ($CH_2Cl_2/CH_3OH$, 3:1). $[\alpha]_D^{20}$ +13.4° (*c* 1, $CHCl_3$). $^1$H NMR ($CDCl_3$): $\delta$ (ppm) 7.73 (d, $J = 7.6$ Hz, 2H), 7.59 (m, 2H), 7.37 (m, 2H), 7.27 (m, 2H), 5.97 (br, 1H), 5.79 (d, $J = 7.6$ Hz, 1H), 4.41−4.33 (m, 2H), 4.18 (t, $J = 7.6$ Hz, 1H), 3.23 (m, 2H), 2.15 (m, 2H), 1.89−1.41 (m, 6H), 1.21 (m, 22H), 0.87 (t, $J = 6.8$ Hz, 3H). $^{13}$C NMR ($CDCl_3$): $\delta$ (ppm) 175.27, 174.86, 156.58, 144.11, 143.92, 141.48, 127.94, 127.32, 125.39, 120.18, 67.37, 53.78, 47.33, 39.39, 36.91, 32.16, 29.93, 29.89, 29.75, 29.59, 29.53, 29.08, 26.06, 22.93, 22.40, 14.36. HRMS (MALDI) (*m*/*z*) ($MNa^+$): found 601.3623. Calcd for $C_{35}H_{50}N_2O_5Na$ 601.3617.

**Fmoc-Lys(stearoyl)-OH (5).** Compound **5** was submitted to the same procedure described above for the preparation of **1** and gave a white solid (yield, 68%), mp 133.0−135.0 °C. TLC $R_f = 0.62$ ($CH_2Cl_2/CH_3OH$, 5:1). $[\alpha]_D^{20}$ +11.8 ° (*c* 1, $CHCl_3$). $^1$H NMR ($CDCl_3$): $\delta$ (ppm) 7.68 (d, $J = 7.6$ Hz, 2H), 7.52 (m, 2H), 7.31 (m, 2H), 7.22 (m, 2H), 5.73 (d, $J = 7.2$, Hz, 1H), 4.28 (m, 2H), 4.12 (m, 1H), 3.19 (m, 2H), 2.09 (m, 2H), 1.84−1.18 (m, 37H), 0.80 (m, 3H). $^{13}$C NMR ($CDCl_3$): $\delta$ (ppm) 175.27, 174.69, 156.54,

144.13, 143.94, 141.41, 127.94, 127.31, 125.40, 120.19, 67.32, 53.75, 47.35, 39.29, 36.97, 32.15, 29.93, 29.58, 29.10, 29.19, 26.03, 22.92, 22.33, 14.35. HRMS (MALDI) (*m*/*z*) ($MNa^+$): found 657.4240. Calcd for $C_{39}H_{58}N_2O_5$ Na 657.4243.

**$MPEG_4$-acetic Acid (7).** To a solution of $MPEG_4$-OH (2.20 g, 10.6 mmol) in THF (20 mL) was added NaH (60%, 0.635 g, 15.9 mmol) at 0 °C. The solution was stirred for 1 h at room temperature, and then ethyl bromoacetate (1.4 mL, 12.6 mmol) was added dropwise. After 16 h at 50 °C, the solution was concentrated in vacuo. The residue was hydrolyzed with 1 M LiOH (8 mL) and $CH_3OH$ (8 mL) for 6 h at room temperature. The solution was acidified with 1 M HCl to pH 1, and then the solvents were removed under reduced pressure. The residue was purified by chromatography ($CH_2Cl_2$/$CH_3OH$, 10:1) to afford **7** (1.66 g, 59%) as a colorless oil. TLC $R_f$ = 0.15 ($CH_2Cl_2$/$CH_3OH$, 4:1). $^1$H NMR ($CDCl_3$): $\delta$ (ppm) 9.10 (br, 1H), 4.03 (s, 2H), 73.57−3.44 (m, 16H), 3.25 (s, 3H). $^{13}$C NMR ($CDCl_3$): $\delta$ (ppm) 172.94, 71.93, 71.09, 70.64, 70.58, 70.55, 70.51, 70.45, 68.61, 61.61, 59.02. HRMS (MALDI) (*m*/*z*) ($MNa^+$): found 289.1248. Calcd for $C_{11}H_{22}O_7Na$ 289.1263.

**Fmoc-Lys($MPEG_4$)-OH (6).** To a solution of $MPEG_4$-acetic acid **7** (0.266 g, 1.0 mmol) and perfluorophenol (0.202 g, 1.1 mmol) in $CH_2Cl_2$ (5 mL) was added DCC (0.247 g, 1.2 mmol) at room temperature. After being stirred overnight, the mixture was diluted with acetone (50 mL) and filtered. The residue was dried in vacuo to give the activated ester, which was used directly in the next step. The crude oil in DMF (5 mL) was added to a solution of Fmoc-L-lysine (0.442 g, 1.2 mmol) and DIPEA (0.7 mL, 4 mmol) in DMF (10 mL) at 0 °C. The solution was stirred overnight at room temperature and then poured into brine (50 mL), extracted with $CH_2Cl_2$ (150 mL), and washed with 1 M HCl, brine, dried, and purified by flash chromatography ($CH_2Cl_2$/$CH_3OH$, 5:1) to afford an amorphous solid **6** (0.37 g, 60%). TLC $R_f$ = 0.21 ($CH_2Cl_2$/$CH_3OH$, 5:1). $[\alpha]_D^{20}$ +19.5 ° (*c* 3, $CHCl_3$). $^1$H NMR ($CDCl_3$): $\delta$ (ppm) 7.67 (d, $J$ = 7.2 Hz, 2H), 7.52 (m, 2H), 7.29 (m, 2H), 7.21 (m, 2H), 5.78 (d, $J$ = 6.8 Hz, 1H), 4.29 (d, $J$ = 6.8 Hz, 2H), 4.11 (m, 1H), 3.91 (s, 2H), 3.54−3.52 (m, 14H), 3.45−3.43 (m, 2H), 3.27 (s, 3H), 1.81−1.18 (m, 6H). $^{13}$C NMR ($CDCl_3$): $\delta$ (ppm) 171.04, 156.36, 144.18, 144.03, 141.48, 127.90, 127.30, 125.39, 120.15, 72.07, 71.09, 70.69, 70.61, 70.27, 67.05, 59.15, 53.92, 47.38, 38.54, 31.78, 29.91, 29.00, 22.27. HRMS (MALDI) (*m*/*z*) ($MNa^+$): found 639.2917. Calcd for $C_{32}H_{44}N_2O_{10}Na$ 639.2893.

**Peptide Synthesis.** All galanin analogues were synthesized on a Symphony peptide synthesizer (Protein Technologies Inc.) using Fmoc-based coupling protocols as previously described.[22] Cleaved peptides were purified by reversed-phase HPLC using preparative HPLC column (Vydac diphenyl, 219TP1011522) and eluted with a linear gradient of acetonitrile (0.1% TFA). The flow rate was 10 mL/min, and the elution was monitored by UV detection at 220 and 280 nm. Buffer A (0.1% TFA in water) and buffer B (0.1% TFA, v/v, in 90% aqueous acetonitrile) were used to produce a linear gradient from 20% to 100% of buffer B over 40 min. Purified analogues were quantified by measuring UV absorbance at 279.8 nm (molar absorbance coefficient $\varepsilon$ = 7000 $cm^{-1}$ $M^{-1}$).

**Partitioning Coefficient, log *D*.** The log *D* values were determined on the basis of calculated capacity factors derived from HPLC retention times as previously described.[22] The log *D* for five galanin analogues were determined using conventional shake-flask methods, using 400 $\mu$g of peptide reconstituted in 1 mL of phosphate buffered saline. The aqueous peptide solution was shaken with an equal volume of water-saturated octanol for 24 h. Concentration of the aqueous layer was determined by HPLC. Retention times for the analogues were determine on a linear gradient of 20−100% buffer B in 40 min, with immediate return to initial conditions and a 20 min re-equilibration. A linear correlation between log *D* and HPLC retention times gave calculated log *D* values.

**Circular Dichroism.** α-Helical conformation of each analogue was studied using CD as previously described.[22] Peptides were reconstituted in NaF buffer to a concentration of 0.1 mg/mL. The peptides were scanned from 250 to 200 nm, with a 1 nm step size and 1.0 s dwell time. Data were averaged from five scans.

**Metabolic Stability Assay.** Peptide stability was assessed in a buffered 25% rat blood serum assay as previously described.[22] To 200 $\mu$L of buffered serum, 2.5 $\mu$g of peptide was added and the solution was incubated at 37 °C. Reactions were quenched at select time points upon the addition of "quenching solution" (15% trichloroacetic acid in 40% isopropanol), and the remaining amount of intact peptide was determined by HPLC.

**Pharmacological Characterization.** Galanin receptor binding assay and the anticonvulsant screening were carried out as previously described in detail.[22] Briefly, the competitive binding assay was performed using human GalR1 and GalR2 receptor membrane preparations and europium-labeled galanin (Perkin-Elmer). Samples were incubated at room temperature for 90 min, followed by four washings with wash buffer (50 mM Tris-HCl, pH 7.5, and 5 mM $MgCl_2$) using a vacuum manifold. Enhancement solution (200 $\mu$L) was added, and the plates were incubated at room temperature for 30 min. The plates were read on a VICTOR$^3$ spectrofluorometer. Competition binding curves were analyzed using GraphPad Prism software, using a nonlinear regression, sigmoidal dose−response (variable slope) curve with no constraints or weights, seen below:

$$y = \text{Bottom} + \frac{\text{Top} - \text{Bottom}}{1 + 10^{(x - \log \text{EC}_{50})}}; \quad \text{EC}_{50} = 10\left[\log K_i\left(1 + \frac{[\text{Eu-galanin}]}{K_d}\right)\right]$$

Top and Bottom are the plateaus on the *y*-axis. With this model, we hold the $K_d$ and concentration of europium-labeled galanin constant (4.3 and 2 nM, respectively) and assume one binding site with reversible binding at equilibrium. Anticonvulsant efficacy was assessed following ip administration to five groups of CF-1 mice ($n$ = 4 mice) at a dose of 4 mg/kg. At various times (i.e., 0.25, 0.5, 1, 2, and 4 h) after administration, mice were challenged with a 6 Hz corneal stimulation (32 mA, 3 s, 6 Hz). Mice not displaying a characteristic limbic seizure were considered protected. The percent of animals protected at each time point was plotted against time, and the AUC values were calculated with GraphPad Prism.

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**Supporting Information Available:** Tables S1 and S2 listing the purity and log *D* calculations, respectively, and Figure S1 showing the HPLC chromatograms of the galanin analogues. This material is available free of charge via the Internet at http://pubs.acs.org.

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