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# Potential Neuroprotective Drugs in Cerebral Ischemia: New Saturated and Polyunsaturated Lipids Coupled to Hydrophilic Moieties: Synthesis and Biological Activity

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The ganglioside GM1 has neuroprotective effects but is not of therapeutic value because of its lack of bioavailability. Thus, molecules that mimic GM1 represent a novel approach to neuroprotection. We have synthesized 19 small GM1-like analogues whose simplified structure includes a hydrophobic saturated or unsaturated moiety linked to a hydrophilic moiety. We report their neuroprotective effects in two distinct models of nerve cell death using hippocampus-derived HT22 cells. We found that several analogues protected the HT22 cells from death at concentrations ranging from 2 to 5 $\mu$M. Additional neuroprotective assays using cortical slices injured by glutamate confirmed these results. Since members of the MAP kinase family are known to be key players in nerve cell survival and death, we characterized the role of these kinases in the neuroprotective mechanisms of the GM1-like analogues. Interestingly, the results indicate that the compounds provide neuroprotection through distinct mechanisms of action.

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## Introduction

Stroke is the third leading cause of mortality and the primary cause of disability in adults.[1] Therefore, it is critical to identify new, efficacious pharmacological treatments. One pharmacological approach for treatment of stroke is called neuroprotective therapy. Its goal is to lower the activation of toxic pathways and enhance the activity of endogenous neuroprotective mechanisms. Numerous compounds have been proposed to protect the brain from cerebral ischemia-induced damage caused by pathogenic mechanisms which include excitotoxicity, overproduction of free radicals, inflammation, and apoptosis. These compounds can act as glutamate receptor antagonists, antioxidants, anti-inflammatory agents[2−4] or antiapoptotic agents as well as by mimicking neurotrophic factors[5] to initiate neuroprotection.[6]

It has been reported that the amphiphilic, monosialotetrahexosylganglioside (GM1[a]) ($II^3$NeuAc-GgOs$c_4$Cer) has anti-neurotoxic, neuroprotective, and neurorestorative effects on various central neurotransmitter systems.[7] Since GM1 is not of therapeutic value because of its lack of bioavailability and its low blood−brain barrier (BBB) permeation,[7] the search for small molecules that can mimic the effects of GM1 and thereby alleviate the consequences of neuroinjury is a novel approach to the maintenance of neuronal integrity.

GM1 consists of a hydrophobic ceramide region containing a saturated and an unsaturated lipid chain and a carbohydrate region consisting of an α(2−3)-linked, inner core sialic acid (Figure 1a). We designed new small GM1-like analogues whose structure includes a hydrophobic saturated or unsaturated moiety linked to a hydrophilic moiety through an amide bond (Figure 1b).

The aim of this present work is to report the synthesis of these new GM1-like analogues, to study the structure−anti-ischemia activity relationships of this series of analogues and to examine their possible mechanism of action.

Two series of analogues, whose general structure is outlined in Figure 1b, have been synthesized. In the first series, various lipophilic saturated, unsaturated, or cyclic polyunsaturated moieties have been introduced, while in the second series, the carboxylic acid function was replaced by different hydrophilic groups including bioisosters of the carboxylate, such as a phosphonic acid (Scheme 2), a tetrazole (Scheme 3), or an ascorbic acid moiety (Scheme 4). Introduction of ascorbic acid was supported by several reports that showed that ascorbic acid conjugates can improve BBB permeation properties.[8−10] In order for the new analogues to be effective as anti-ischemic agents, they must pass the BBB to reach the brain, so such a modification could significantly improve their therapeutic value.

All of the newly synthesized analogues were first screened for their neuroprotective effects in a cell culture-based assay, and then some representative analogues were screened in a brain slice assay to confirm the cell culture-based assay results. In an attempt to understand their mechanisms of action, a limited series of biochemical studies were carried out as well.

## Results

**Chemistry.** As shown in Figure 1b, three structural parameters can be modulated in order to elicit possible

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[a] Abbreviations: BBB, blood−brain barrier; DCC, 1,3-dicyclohexylcarbodiimide; DCU, dicyclohexylurea; DHAA, dihydroascorbic acid; ERK, extracellular signal regulated kinase; GM1, monosialotetrahexosylganglioside; GLUT1, glucose transporter 1; HOBt, dicyclohexylcarbodiimide 1-hydroxybenzotriazole; IAA, iodoacetic acid; JNK, c-jun N-terminal kinase; MAPK, mitogen-activated protein kinase; SDS−PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SVTC2, sodium-dependent vitamin C transporter; TEAC, Trolox equivalent antioxidant capacity; TTC, 2,3,5-triphenyltetrazolium chloride.

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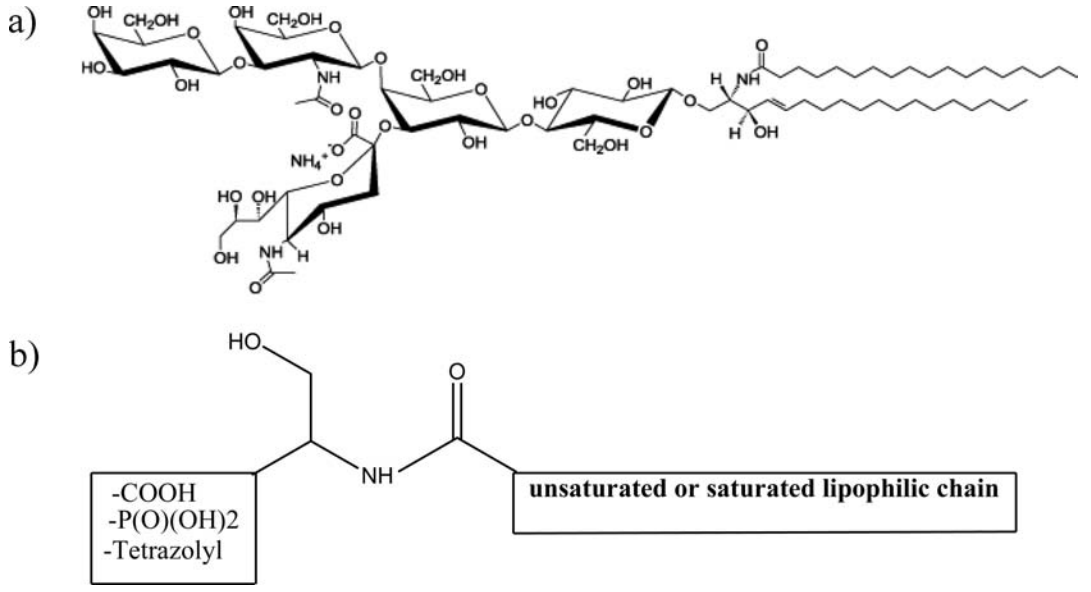
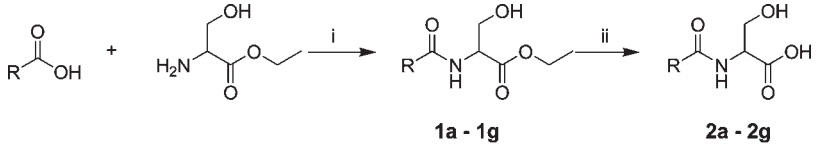



**Figure 1.** (a) Structure of GM1 (monosialotetrahexosylganglioside). (b) General simplified structure of GM1-like analogues.

**Scheme 1**[a]




**1a.2a.** R = stearyl
**1b.2b.** R = linoleyl
**1c. 2c.** R = linolenyl
**1d.2d.** R = retinoyl
**1e. 2e.** R = arachidonyl
**1f. 2f.** R = abietyl
**1g.2g.** R = abscisyl

[a] Reagents and conditions: (i) ROOH, fatty acid, DCC/HOBt, $CH_2Cl_2$, room temp; (ii) $LiOH \cdot H_2O$, ethanol/$H_2O$, 3:1 (v/v).

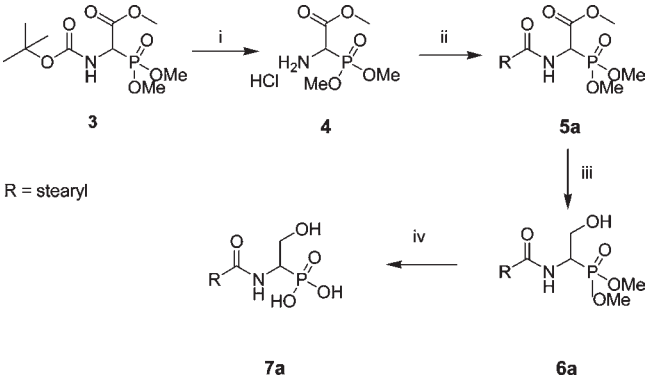
neuroprotective effects. Variation of the lipophilic moiety is represented by the first series, in which various hydrophobic saturated or unsaturated moieties linked to a serine residue through an amide bond have been introduced. The corresponding compounds were synthesized as depicted in Scheme 1 (compounds **2g**, **2f**, **2b**, **1b**, **2c**, **2e**, **2a**).

The key intermediates **1a**−**g** were obtained in good yields by acylation of commercially available L-serine ethyl ester hydrochloride with different fatty acids using 1,3-dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt) as coupling reagents. The corresponding carboxylic acid analogues **2a**−**g** were simply obtained through a smooth hydrolysis in basic conditions of the ester derivatives **1a**−**g**.

Variation of the hydrophilic moiety is represented by the second series of analogues in which the lipophilic moieties (stearyl or linoleyl) are conserved but the carboxylic function has been replaced by the bioisosteric groups: phosphonic acid (→**7a**) or ascorbic acid (→**17a**,**b**).

For the synthesis of the phosphonic analogues **6a** and **7a**, the synthetic scheme (Scheme 2) was as follows. After Boc deprotection of the commercially available (±)-Boc-α-phosphonoglycine trimethyl ester (**3**) in acidic conditions, the corresponding HCl in ether salt **4** was quantitatively isolated. The resulting HCl in ether salt was then N-acylated by stearic acid using DCC/HOBt as a coupling agent, leading to the desired acylated compound **5a**. Compound **6a** was obtained by reduction of the serine ester using the $NaBH_4$ reagent. Finally, treatment of **6a** with TMSI under $N_2$ gives the desired compound **7a.**

**Scheme 2**[a]




[a] Reagents and conditions: (i) HCl in ether; (ii) R = stearylic acid, DCC/HOBt, $CH_2Cl_2$, room temp; (iii) $NaBH_4$, LiCl, THF/EtOH, room temp; (iv) TMSI, $CH_2Cl_2$, room temp.

For the synthesis of the linoleyltetrazole analogue the synthetic scheme (Scheme 3) was the following. Commercially available ethyl cyanoglyoxylate oxime (**8**) was converted into the corresponding 2-tetrazol-5-yl-2-oximinoacetic acid (**9**)

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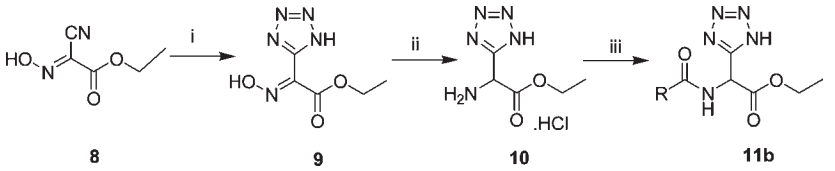
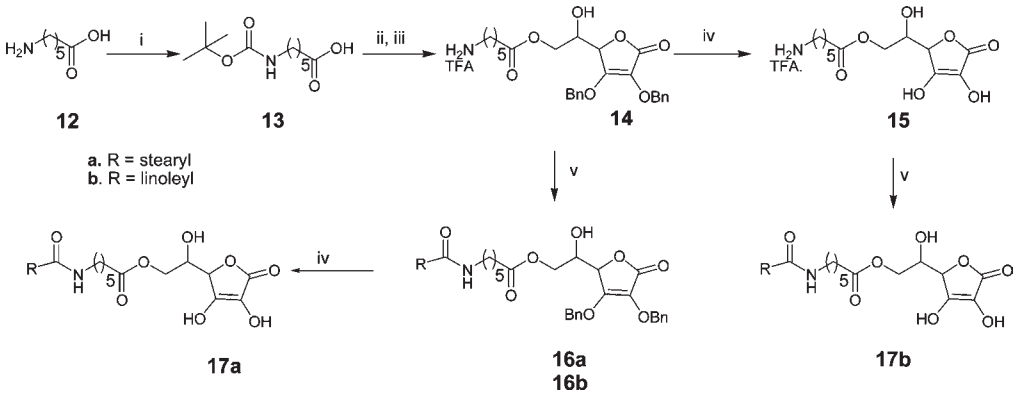
**Scheme 3**[a]




[a] Reagents and conditions: (i) $NaN_3$ (10 equiv), DMF, 70°C; (ii) $Pd(OH)_2/C$, $H_2$ (1 atm), MeOH, 3 N HCl, room temp; (iii) linoleic acid, ethyl chloroformate, pyridine, $CH_2Cl_2$, room temp.

**Scheme 4**[a]




[a] Reagents and conditions: (i) *t*-$Boc_2O$, 1 M NaOH, 1:1 dioxane−$H_2O$; (ii) 2,3-di-*O*-benzyl-L-ascorbic acid, BOP, DIEA, $CH_2Cl_2$, room temp; (iii) TFA, $CH_2Cl_2$, room temp; (iv) $H_2$, $Pd(OH)_2/C$ MeOH, room temp; (v) ROOH, BOP, DIEA, $CH_2Cl_2$.

using $NaN_3$ in DMF for 72 h at 70 °C. This intermediate was reduced to the corresponding ethyl α-amino α-tetrazolacetate **10** in MeOH, using Pd/C as the catalyst. Next, treatment of **10** with linoleic acid in the presence of ethyl chloroformate in pyridine and dichloromethane led to compound **11b**.[11]

The ascorbic acid analogues were synthesized according to the following synthetic scheme (Scheme 4). It is noted that in this case a linker, 6-aminohexanoic acid (**12**), was introduced between the fatty acid and ascorbic acid moieties. The two desired analogues, saturated **17a** and unsaturated **17b**, were synthesized according to two different procedures because a selective reducing step was required. The analogue bearing a stearyl substituent (**17a**) was obtained through the synthesis of its dibenzyl protected ascorbic acid intermediate (**14**), which was accomplished through the following sequence. 6-Aminocaproic acid (**12**) was first N-protected (→**13**) before esterification of the terminal carboxylic acid function with 2,3-di-*O*-benzyl-L-ascorbic acid. This esterification was achieved using BOP as a coupling agent. The dibenzyl protected ascorbic acid was synthesized according to a reported three-step synthetic procedure in a moderate yield of 57%. The *N*-Boc protecting group was removed in acidic conditions, and the resulting TFA salt **14** was quantitatively isolated and then acylated by stearic and linoleic acids using BOP as a coupling agent, leading to the dibenzyl protected analogues **16a** and **16b**. The stearyl analogue was directly deprotected by catalytic hydrogenolysis leading to the final desired analogue **17a**. Since deprotection of the unsaturated linoleyl analogue **16b** cannot be directly achieved by hydrogenolysis, analogue **17b** was obtained directly from intermediate **14** by a direct catalytic hydrogenolysis leading to deprotected analogue **15** and followed by a coupling reaction with linoleic acid using BOP reagent, leading to the desired analogue **17b**.

**Biology.** In order to test the neuroprotective properties of the new GM1-like compounds, we used cell culture-based assays that reproduce a number of the pathophysiological changes associated with ischemia as a screening tool. The primary assay has been recently described by Maher et al.[14] and utilizes the mouse HT22 hippocampal nerve cell line in combination with iodoacetic acid (IAA) to induce ischemia in vitro. This cell culture-based assay was used as a screen for the identification of the most potent and efficacious neuroprotective compounds in the series of the newly synthesized GM1-like analogues. Tissue slice assays were performed only on several representative GM1-like analogues in order to confirm the cell-culture based assay results. In order to begin to understand the mechanism of action by which the new analogues act as neuroprotective agents against ischemia, biochemical assays for glutathione, ATP, and activation of MAPK pathways were also performed.

**Cell Culture-Based Neuroprotection Studies.** The new GM1-like analogues were tested for their neuroprotective effects in two different models of nerve cell death. In the first model, IAA, a known, irreversible glyceraldehyde 3-phosphate dehydrogenase inhibitor[15] was used to induce chemical ischemia.[14] The pathophysiological changes observed in nerve cells following treatment with IAA are very similar to changes that have been seen in the brain in animal models of ischemic stroke.[14] Treatment of HT22 cells with IAA induced a dose dependent increase in cell death 20 h later with $< 5\%$ survival at 20 $\mu$M. This toxic dose of IAA was found to be highly reproducible from assay to assay. The flavonoid fisetin[16] that was previously reported to be neuroprotective in this assay[14] was used as reference compound for comparison purposes with the new GM1-like analogues. The results obtained from this HT22/IAA assay are given in Table 1 and shown graphically in Figure 2, allowing a direct comparison between the different obtained $EC_{50}$ values for all of the tested compounds. Half-maximal effective concentrations ($EC_{50} \pm SD$) for protection were determined by exposing the HT22 cells to different doses of each analogue in the presence

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**Table 1.** Protection of HT22 Cells from IAA and Glutamate Toxicity by GM1-like Analogues

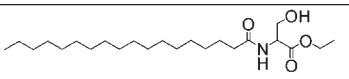
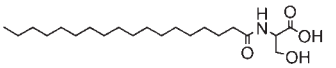
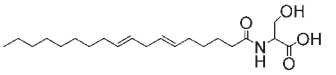
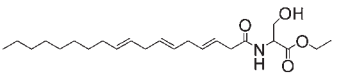
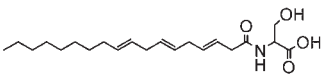
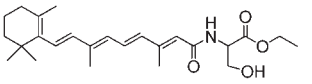
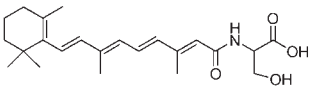
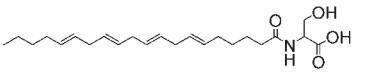
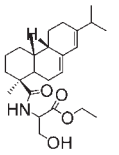
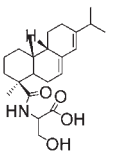
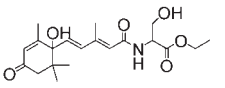
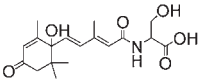
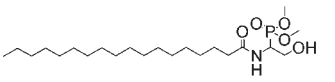
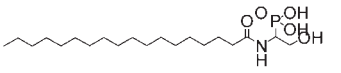
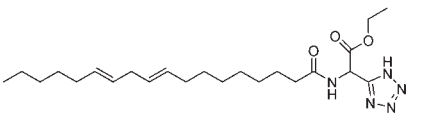
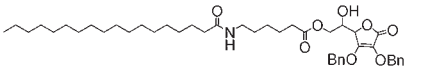
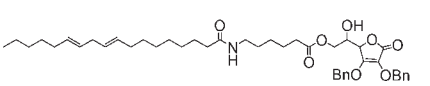
| N° | STRUCTURE | $EC_{50}$ (μM) HT22/IAA[a] | % max. protection[b] | Toxicity at 10μM (MTT Assay)[c] | HT22/ glutamate[d] (% max protection) |
|---|---|---|---|---|---|
| **1a** |  | 9.8 ± 1.3 | 56% | 53% | 16% |
| **2a** |  | 3.2 ± 1.4 | 65% | 28% | 16% |
| **2b** |  | 2.9 ± 1.3 | 70% | 17% | 12% |
| **1c** |  | 17.1 ± 1.5 | 40% | 25% | 11% |
| **2c** |  | 30.2 ± 1.2 | 38% | 32% | 18% |
| **1d** |  | 11.6 ± 1.4 | 45% | 15% | 14% |
| **2d** |  | 4.1 ± 1.4 | 63% | 20% | 16% |
| **2e** |  | 2.6 ± 1.1 | 83% | 40% | 15% |
| **1f** |  | 1.05 ± 1.1 | 85% | 25% | 55% |
| **2f** |  | - | 27% | 26% | 10% |
| **1g** |  | 13.6 ± 1.4 | 39% | 1% | 21% |
| **2g** |  | 2.7 ± 1.3 | 70% | 40% | 14% |
| **6a** |  | 4.1 ± 1.2 | 64% | 11% | 17% |
| **7a** |  | 2.1 ± 1.4 | 74% | 50% | 20% |
| **11b** |  | 11.3 ± 1.5 | 41% | 4% | 8% |
| **16a** |  | 2.5 ± 1.4 | 68% | 48% | 19% |
| **16b** |  | 8.2 ± 1.1 | 59% | 7% | 13% |

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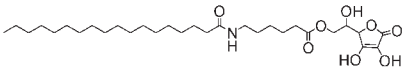
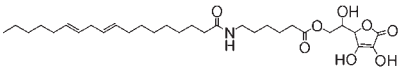
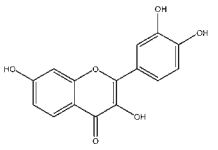
**Table 1.** Continued

| N° | STRUCTURE | $EC_{50}$ (μM) HT22/IAA[a] | % max. protection[b] | Toxicity at 10μM (MTT Assay)[c] | HT22/ glutamate[d] (% max protection) |
|---|---|---|---|---|---|
| **17a** |  | 1.8 ± 1.3 | 92% | 40% | 65% |
| **17b** |  | 6.4 ± 1.3 | 55% | 19% | 32% |
| **Fisetin** |  | 2.8 ± 0.5 | 95% | 15% | 95% |

[a] Half maximal effective concentrations ($EC_{50}$ ± SD) for protection from IAA toxicity were determined by exposing HT22 cells to different doses of each analogue in the presence of 20 μM IAA for 2 h (HT22/IAA). Cell viability was determined after 24 h by the MTT assay. [b] The maximal percent of survival at the most effective dose (which varied from 1 to 10 μM depending on the analogue) is also indicated. [c] To estimate toxicity, HT22 cells were exposed to 10 μM of each analogue and cell viability was determined after 24 h by the MTT assay. [d] For HT22/glutamate, the HT22 cells were exposed to different doses of each analogue in the presence of 5 mM glutamate for 24 h. Cell viability was determined after 24 h by the MTT assay, and the maximal percent of survival at the most effective dose (which varied from 1 to 10 μM depending on the analogue) was determined. The average % survival in cells treated with glutamate alone was 15 ± 5%.

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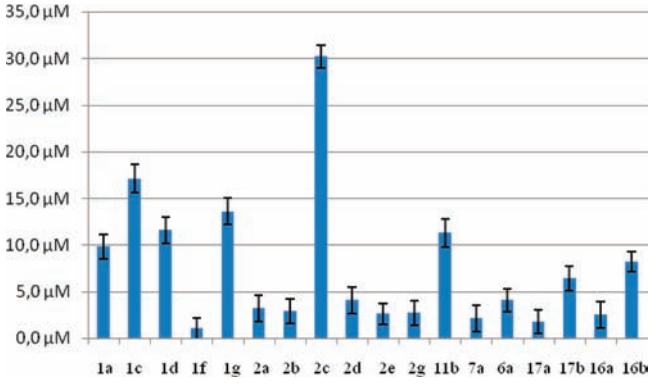


**Figure 2.** Comparative representation of $EC_{50}$ values for the protection of HT22 cells from IAA toxicity by the GM1-like analogues.

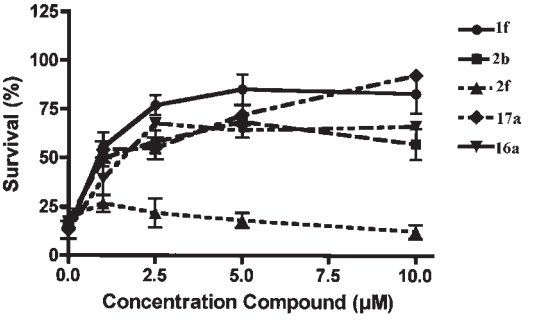


**Figure 3.** Dose−response curves for neuroprotection from IAA toxicity: representative HT22 cell protection curves for the GM1-like analogues after IAA injury. HT22 cells were treated with 20 μM IAA for 2 h alone or in the presence of increasing doses of compounds **1f**, **2b**, **2f**, **16a**, or **17a**. The same concentrations of GM1-like compounds were also included in the fresh medium added after the 2 h treatment with IAA. The % survival was measured after 24 h by the MTT assay. Similar results were obtained in three to five independent experiments.

of 20 μM IAA for 2 h. Cell viability was determined after 24 h by the MTT assay (see ref 29 in the Experimental Section). Figure 3 shows the dose−response curves for five of the GM1-like analogues. As can be seen, the maximal effects of most of the analogues were seen at concentrations between 2 and 5 μM.

The second model used to test the neuroprotective effects of the GM1-like analogues is oxidative glutamate toxicity (Table 1). In this widely used model of oxidative stress-induced death, treatment of the HT22 cells with 5 mM glutamate induced cell death within 24 h via a well characterized pathway involving glutathione depletion and reactive oxygen species production.[17] Surprisingly, in this assay, only **1f** and **17a** showed a highly significant reduction in cell death (> 50% survival) while **17b** modestly reduced cell death (> 30% survival).

**In Vitro Injury Models of Brain Slices.** 2,3,5-Triphenyltetrazolium chloride (TTC) has been widely used in the assessment of brain ischemia.[18] This method of quantitative measurement of extracted red formazan and colorimetry on brain slices incubated with TTC solutions allows the evaluation of neuroprotective agents in a more complex system. Two representative compounds of this new series of GM1-like analogues were assayed on brain slices injured with 1 mM glutamate, and the results are summarized in Figure 4. Although both compounds provided significant protection, **2e** was more effective than **2a**, which is consistent with the results from the HT22 cell culture-based assays.

**Biochemical Studies.** Having identified several new GM1-like compounds with neuroprotective effects, the question of their mechanism of action was an important issue. Previously, it was shown that some compounds that protect from IAA toxicity do so by preventing the loss of glutathione and/or ATP.[14] However, none of the GM1-like compounds (data not shown) had any effect on either glutathione or ATP levels, so we considered other mechanisms of protection. We next investigated the p38 MAPK and JNK pathways because these kinase pathways can be activated during the

IAA-induced cell death process in the HT22 cells (unpublished results) as well as in other in vitro and animal models of ischemia and their activation contributes to nerve cell death.[19] Thus, it was possible that either or both of these pathways could be specifically altered by the GM1-like compounds, thereby reducing the observed cell death. Using specific antibodies to the phosphorylated forms of p38 MAPK and JNK as well as their specific substrates, MAPKAP kinase 2 and c-jun, respectively, the effects of the GM1-like compounds on these pathways were examined. All of the GM1-like drugs with $EC_{50}$ values below 10 $\mu$M were tested in these assays, and the results for **17a**, **7a**, **1f**, **2c**, and **2e** are shown in Figure 5.

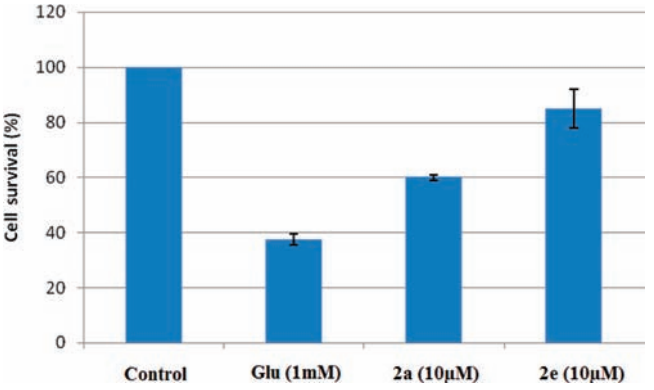


**Figure 4.** Neuroprotective effect of selected GM1-like analogues **2a** and **2e** on glutamate-injured brain slices. Brain slices were cultured with inhibitors 1 h prior to and during glutamate application. The viability of the cortical slices was evaluated by the TTC method, $n = 6$. Data are mean ± SD: $P < 0.05$ vs control, $p > 0.05$ vs injury group.

We next examined the Ras-ERK cascade which has been implicated in nerve cell survival[20−23] and might be activated by the new GM1-like compounds. For these studies, we again tested all of the GM1-like compounds with $EC_{50}$ values less than 10 $\mu$M using SDS−PAGE and immunoblotting in combination with antibodies to the phosphorylated and therefore activated form of ERK. The results for **2e**, **2c**, **1f**, **7a**, and **17a** are shown in Figure 6. These results suggest that the GM1-like compounds are not selective inhibitors or activators of the Ras-ERK cascade signaling pathway.

## Discussion and Conclusions

The neuroprotective effects at relevant concentrations (1−10 $\mu$M) of this new class of GM1-like analogues were first established using two cell culture-based assays with death being an end point. Both of these assays have been shown to be effective at identifying drugs that are neuroprotective in animal models of neurological disorders.[3,14,17] It was further shown that the new GM1-like analogues can protect rat brain slices against glutamate injury, confirming the results obtained with the cell culture based-assays.

From these results, it can be seen that the majority of this new group of compounds present neuroprotective effects at concentrations ranging from 1 to 30 $\mu$M. The most active analogues in this screening model are **1f** ($EC_{50} = 1.05$ $\mu$M) and **17a** ($EC_{50} = 1.8$ $\mu$M). **1f** contains in its structure an abietyl lipophilic moiety, while **17a** includes a stearyl moiety coupled to an ascorbic acid moiety through a $\omega$-aminohexanoic acid linker. On the basis of the results with the entire group of

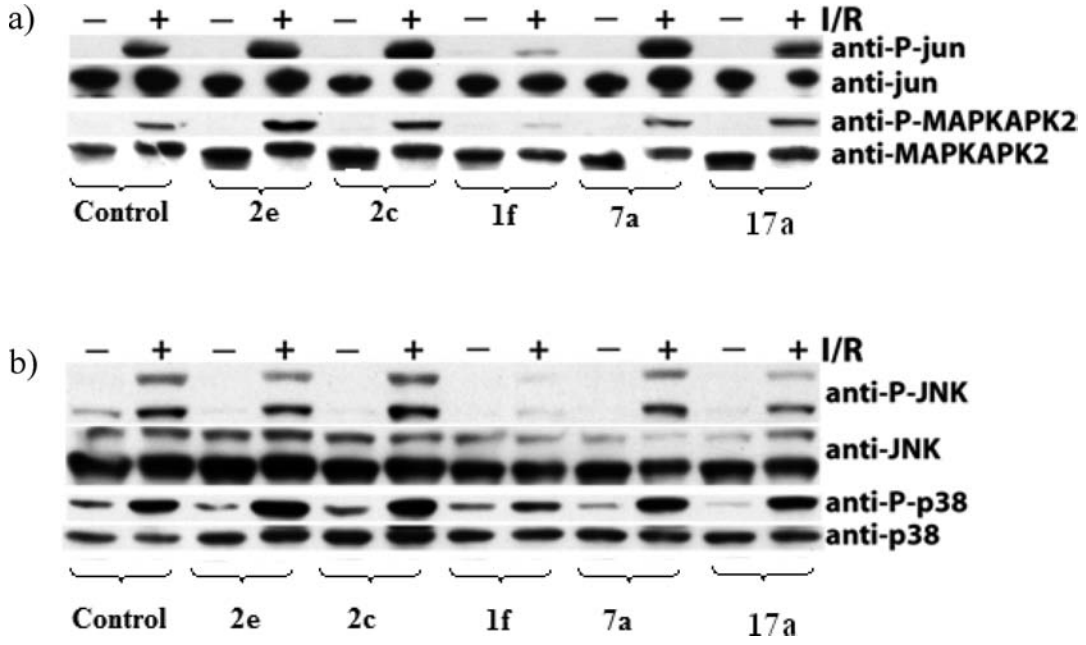


**Figure 5.** (a) Effects of GM1-like compounds on c-Jun and MAPAPK2 phosphorylation. HT22 cells were untreated (control) or treated with 10 $\mu$M **2e**, **2c**, **1f**, **7a**, and **17a** in the absence (−) or presence (+) of 20 $\mu$M IAA for 2 h. Following 2 h of recovery, cell lysates were prepared and equal amounts of protein were analyzed by SDS−PAGE and immunoblotting with antibodies to phospho and total Jun and phospho and total MAPKAPK2. (b) Effects of GM1-like compounds on JNK and p38 MAPK phosphorylation. HT22 cells were untreated (control) or treated with 10 $\mu$M **2e**, **2c**, **1f**, **7a**, and **17a** in in the absence (−) or presence (+) of 20 $\mu$M IAA for 2 h. Following 2 h of recovery, cell lysates were prepared and equal amounts of protein were analyzed by SDS−PAGE and immunoblotting with antibodies to phospho and total JNK and phospho and total p38 MAP kinase. From these studies, it appears that only one compound, **1f**, reduced both p38 MAPK and JNK activation and completely inhibited phosphorylation of their respective substrates MAPKAP kinase 2 and c-jun. In contrast, **2e**, **7a**, **2c**, and **17a** along with the other GM1-like compounds tested had no effect on the activation of these kinases or on the phosphorylation of their substrates. These results suggest that **1f**, **2e**, and **17a**, as well as the other GM1-like compounds, exert their neuroprotective effects by distinct mechanisms.

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compounds, it can be observed that both lipophilic and hydrophilic moieties influence their neuroprotective effects. As exemplified by the homologous compounds **2a** and **2c**, **16a** and **16b**, or **17a** and **17b** bearing stearyl or linoleyl lipophilic chains, these studies showed differing neuroprotective effects for analogues bearing saturated or unsaturated side chains. Despite the recent results from Stronkin[4] showing that polyunsaturated fatty acids such as docosahexanoic acid provide potent protection against neurodegeneration after hypoxia/hypoglycemia, our results clearly show that compounds including a saturated stearyl chain are more active than their corresponding unsaturated analogues. Surprisingly, the protection of the carboxylic function by an ethyl ester (**1c**, **1f**, and **11b**) did not necessarily impede the observed neuroprotective effects. Importantly, some of the analogues in which the serine moiety was replaced by an ascorbic acid were found to be highly active (e.g., **17a** with an $EC_{50} = 1.8\ \mu M$). This result is quite encouraging because, as already mentioned in the Introduction, ascorbic acid could help anti-ischemic agents reach the brain after BBB permeation via active transport. Ascorbic acid is metabolized in vivo into its oxidized form (dihydroascorbic acid (DHAA)) which is then transported across the BBB via either the glucose transporter GLUT1[24] or the $Na^+$-dependent ascorbate transporter SVTC2.[1025] Thus, it can be concluded that both lipoyl and hydrophilic moieties induce or modulate the observed neuroprotective effects.

On the basis of the results shown in Figure 2 using the HT22 cell-based assay, it appears that the efficacies of the new GM1-like compounds are similar to those observed for flavonoids[14] of which fisetin, is a representative example.[5] A major difference between the neuroprotective effects observed for fisetin and for the new GM1-like analogues is that some of these latter compounds (e.g., **2b**, **7a**, **1c**) show a decrease in their neuroprotective efficacy at higher concentrations (10 $\mu$M) that is not associated with toxicity. This result suggests that some of these compounds could interact with specific cell surface receptors, inducing cooperativity between the receptors and leading to receptor clustering as has often been observed for immunological responses.[26] This observation coupled with the data on MAP kinase signaling pathways strongly suggests that the new GM1-like analogues are not acting as antioxidants but rather as inducers of antioxidant and/or neuroprotective protein synthesis. This conclusion is supported by their complete lack of activity in the TEAC assay, a test tube assay for antioxidant activity[27] (data not shown). Although their mechanism of action remains to be fully elucidated, our preliminary experiments clearly indicate that the whole group of newly designed compounds does not provide neuroprotection through the same mechanisms. For example, both **2e**, bearing an arachidonyl moiety, and **17a**, with a stearyl moiety in which the serine moiety was substituted by an ascorbic acid, had no effect on p38 MAPK and JNK activity while **1f**, bearing an abietyl moiety, reduced both p38 MAPK and JNK activation and completely inhibited phosphorylation of their respective substrates, MAPKAP kinase 2 and c-Jun, although all three compounds showed strong neuroprotective effects on HT22 cells treated with IAA. In addition, we observed that none of the GM1-like compounds activated ERK kinase, while the reference compound, fisetin, which also has a strong neuroprotective effect in this ischemia model, did activate ERK.

In summary, we have successfully synthesized a series of new GM1-like analogues that are structurally simplified molecules compared to GM1, since they consist of a lipophilic chain or more hindered backbone linked to hydrophilic moieties. Some of the new analogues exhibit potent neuroprotective effects on nerve cells and brain tissue slices exposed to ischemia-like insults. Both analogues **1f** and **17a** are of particular interest, since they are very efficacious with $EC_{50}$ values ranging between 1 and 2 $\mu$M. Given these potent effects in cell- and tissue-based assays, further exploration of the neuroprotective effects of these GM1-like analogues in animal models of ischemia is clearly warranted.

## Experimental Section

**Chemistry.** Starting materials and reagents were obtained from commercial suppliers and were used without purification. Tetrahydrofuran (THF) was distilled over sodium benzophenone ketyl immediately prior to use. Methylene dichloride ($CH_2Cl_2$) was distilled over $P_2O_5$ just prior to use. Nuclear magnetic resonance spectra were recorded at 250 MHz for $^1H$ on a Bruker AC-250 spectrometer. Elemental analyses were within $\pm 0.4\%$ of theorical values for all compounds. Chemical shifts are expressed as ppm units (part per million) downfield from TMS (tetramethylsilane). Electrospray mass spectra were obtained on a Waters Micromass ZMD spectrometer by direct injection of the sample solubilized in $CH_3CN$. Analytical thin layer chromatographies (TLC) and preparative thin layers chromatographies (PLC) were performed using silica gel plates 0.2 mm thick and 1 mm thick, respectively (60F254 Merck). Preparative flash column chromatographies were carried out on silica gel (230−240 mesh, G60 Merck). Purity of the compounds have been controlled by centesimal analysis (see Supporting Information).

Intermediates compounds 2,3-di-*O*-benzyl-L-ascorbic acid, which have been already reported,[12,13] are not described.

Unless specified, all the tested compounds described in the manuscript present >95% purity established through combustion analysis.

**Procedure A (Coupling Reaction by DCC/HOBt). Ethyl 3-Hydroxy-2-stearamidopropanoate (1a).** To a solution of stearic acid (500 mg, 1.75 mmol) in methylene dichloride (DCM) (15 mL), distilled and under inert atmosphere, were added *N*-methylmorpholine (0.576 mL, 5.27 mmol), 1,3 dicyclohexylcarbodiimide

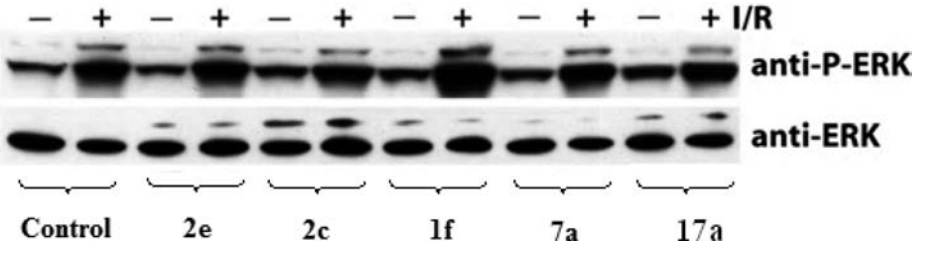


**Figure 6.** Effects of GM1-like compounds on ERK phosphorylation. HT22 cells were untreated (control) or treated with 10 $\mu$M **2e**, **2c**, **1f**, **7a**, and **17a** in the absence of (−) or presence (+) of 20 $\mu$M IAA for 2 h. Following 2 h of recovery, cell lysates were prepared and equal amounts of protein were analyzed by SDS−PAGE and immunoblotting with antibodies to phospho and total ERK.

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(DCC) (361 mg, 1.75 mmol), and 1-hydroxybenzotriazole (HOBt) (237 mg, 1.75 mmol). The reaction mixture was stirred at 0 °C for 1 h. Then L-serine ethyl ester hydrochloride (296 mg, 1.75 mmol) was added. The reaction mixture was stirred at room temperature under nitrogen for 24 h. After filtration, 10 mL of DCM was added at 0 °C to the filtrate to precipitate the DCU. The DCM was evaporated under reduced pressure. The residue was washed with 5% citric acid (3 × 15 mL), 5% $NaHCO_3$ (3 × 15 mL), and brine (2 × 15 mL). The organic phase was dried over $MgSO_4$. After filtration and evaporation under reduced pressure, silica gel chromatography of the residue (cyclohexane/EtoAc (1:1)) gave **1a** (300 mg, 42% yield) as a white solid. $R_f$ = 0.5 (cyclohexane/EtoAc (1:1)). $^1$H NMR (250 MHz, $CDCl_3$) $\delta$: 6.54 (d, $J$ = 6.8 Hz, 1H, −(O)CN*H*−); 4.65 (m, 1H, −HNC*H*COO−); 4.23 (q, $J$ = 7.10 Hz, 2H, −COOC$H_2$CH$_3$); 3.95 (m, 2H, −C$H_2$OH); 2.25 (t, $J$ = 7.6 Hz, 2H, −C$H_2$C(O)−); 1.61 (m, 2H, C$H_2$CH$_2$C(O)−); 1.23 (m, 31H, −(C$H_2$)$_{14}$− and CH$_3$C$H_2$O−); 0.85 (m, 3H, −C$H_3$). $^{13}$C NMR (250 MHz, CDCl3) $\delta$: 173.8, 171.0, 63.7, 62.1, 55.9, 36.5, 31.9, 29.7, 29.5, 29.3, 29.2, 25.5, 22.7, 14.1. MS (ESI): $m/z$ 400 ([M + H]$^+$), 100%. Anal. ($C_{23}H_{45}NO_4$) C, H, N.

**Procedure B (Ester Hydrolysis). 3-Hydroxy-2-stearamidopropanoic Acid (2a).** To a solution of compound **1a** (263 mg, 0.65 mmol) in EtOH/$H_2O$ (3:1) was added lithium hydroxide monohydrate (82 mg, 0.45 mmol) at room temperature, and then the mixture was stirred for 1 h. The progress of the reaction was followed by TLC. After an overnight reaction followed by evaporation of the solvent, the residue was acidified with 5% $KHSO_4$ to pH 3. After extraction with methylene dichloride (DCM) (3 × 20 mL), the residue was dried and the solvent evaporated. A solid white compound was obtained (198 mg, 81% yield). $^1$H NMR (250 MHz, $CDCl_3$) $\delta$: 6.54 (d, $J$ = 6.8 Hz, 1H, −(O)CN*H*−); 4.67 (m, 1H, −HNC*H*COOH); 3.94 (m, 2H, −C$H_2$OH); 2.28 (t, $J$ = 7.6 Hz, 2H, −C$H_2$C(O)−); 1.66 (m, 2H, −C$H_2$CH$_2$C(O)−); 1.21(m, 28H, −(C$H_2$)$_{14}$−); 0.89 (3H, m, −C$H_3$). $^{13}$C NMR (250 MHz, MeOH) $\delta$: 173.8; 171.0; 63.7; 55.9; 36.5; 31.9; 29.7; 29.5; 29.3; 29.2; 25.5; 22.7; 14.1. MS (ESI): $m/z$ 372 ([M + H]$^+$), 100%. Anal. ($C_{21}H_{41}NO_4$) C, H, N.

**Ethyl 3-Hydroxy-2-(9*E*,12*E*)-octadeca-9, 12-dienamidopropanoate (1b).** According to general procedure A, the reaction of linoleic acid (200 mg, 0.71 mmol) with L-serine ethyl ester chlorohydrate (120 mg, 0.71 mmol) afforded the title compound **1b** as a transparent oil (80 mg, 30% yield). $R_f$ = 0.125 ($CH_2Cl_2$/MeOH, 98:2). $^1$H NMR (250 MHz, $CDCl_3$) $\delta$: 6.52 (s, 1H, −N*H*); 5.34 (m, 4H, −C*H*=C−); 4.64 (m, 1H, −C*H*NCO); 4.21 (m, 2H, O−C$H_2$CH$_3$); 3.9 (m, 2H, −C$H_2$OH); 2.75 (m, 2H, −HC=CHC$H_2$CH=CH−); 2.25 (m, 4H, 2 −HC=CH−C$H_2$−); 2.02 (m, 2H, −C$H_2$C(O)NH 1.63 (m, 2H, −C$H_2$CH$_2$C(O)); 1.29 (m, 18H, (−C$H_2$)$_9$); 0.87 (m, 3H, −C$H_3$). $^{13}$C NMR (250 MHz, $CDCl_3$) $\delta$: 174.0; 170.7; 130.3; 130.1; 128.1; 128.0; 63.8; 62.6; 54.9; 54.4; 36.6; 31.6; 29.7; 29.5; 29.4; 29.3(2C); 28.6; 27.4; 25.7; 25.2; 22.7; 14.2. MS (ESI): $m/z$ 396 ([M+H]$^+$), 100%. Anal. ($C_{23}H_{41}NO_4$) C, H, N.

**3-Hydroxy-2-(9*E*,12*E*)-octadeca-9,12-dienamidopropanoic Acid (2b).** According to general procedure B, the reaction of ethyl 3-hydroxy-2-(9*E*,12*E*)-octadeca-9,12-dienamidopropanoate **1b** (100 mg, 0.25 mmol) with lithium hydroxide monohydrate (19 mg, 0.45 mmol) afforded the title compound **2b** as a yellow solid. A solid white compound was obtained (54 mg, 64% yield). $^1$H NMR (250 MHz, $CDCl_3$) $\delta$: 5.32 (m, 4H, −C*H*=C*H*−); 4.49 (m, 1H, C*H*NCO); 3.77 (m, 2H, −C$H_2$OH); 2.75 (m, 2H, −CH=CHC$H_2$CH=CH−); 2.21 (m, 2H, −C$H_2$NC(O)); 2.02 (m, 4H, −CH=CHC$H_2$−); 1.59 (m, 2H, −C$H_2$CH$_2$NC(O)); 1.28 (m, 18H, −(C$H_2$)$_9$−); 0.87 (m, 3H, −C$H_3$). $^{13}$C NMR (250 MHz, $CDCl_3$) $\delta$:181.6; 181.4; 130.2; 129.9; 128.0; 127.8; 60.8; 56.9; 37.6; 36.5; 33.8 (2C); 31.5; 29.7; 29.3; 29.3; 28.6; 27.1; 25.6; 22.6; 14.1. MS (ESI): $m/z$ 368 ([M + H]$^+$), 100%. Anal. ($C_{21}H_{37}NO_4$) C, H, N.

**Ethyl 3-Hydroxy-2-(3*E*,6*E*,9*E*)-octadeca-3,6,9-trienamidopropanoate (1c).** According to general procedure A, the reaction of linolenic acid (200 mg, 0.71 mmol) with L-serine ethyl hydrochloride (121 mg, 0.71 mmol) afforded the title compound **1c** as a white oil (120 mg, 43% yield). $^1$H NMR (250 MHz, $CDCl_3$) $\delta$: 6.39 (d, $J$ = 6.7 Hz, 1H, −N*H*−); 5.35 (m, 6H, −C*H*=C*H*−); 4.67 (m, 1H, −(O)CNC*H*COO−); 4.23 (q, $J$ =7.1 Hz, 2H, −COOC$H_2$CH$_3$); 3.94 (d, $J$ = 3.9 Hz, 2H, −C$H_2$OH); 2.80 (m, 4H, −CH=CHC$H_2$CH=CH−); 2.26 (t, $J$ = 7.3 Hz, 2H, −C$H_2$NC(O)); 2.07 (m, 4H, −CH=CHC$H_2$−); 1.64 (m, 2H, −C$H_2$CH$_2$NC(O)); 1.33 (m, 14H, −(C$H_2$)$_7$−); 0.97 (t, $J$ = 7.51 Hz, 3H, −C$H_3$). $^{13}$C NMR (250 MHz, $CDCl_3$) $\delta$: 174.3; 170.9; 132.8; 130.7; 128.8; 128.7; 128.2; 127.7; 64.2; 62.5; 55.5; 36.9; 30.0; 29.8; 29.7; 29.6; 27.7; 26.1; 26.0; 21.0; 20.9; 15.0; 14.2. MS (ESI): $m/z$ 394 ([M + H]$^+$), 100%. Anal. ($C_{23}H_{39}NO_4$) C, H, N.

**3-Hydroxy-2-(3*E*,6*E*,9*E*)-octadeca-3,6,9-trienamidopropanoic Acid (2c).** According to general procedure B, the reaction of ethyl 3-hydroxy-2-(9*Z*,12*Z*,15*Z*)-octadeca-9,12,15-trienamidopropanoate (101 mg, 0.25 mmol) with lithium hydroxide monohydrate (32 mg, 0.76 mmol) afforded the title compound **2c** as a yellow solid (70 mg, 75% yield). $^1$H NMR (250 MHz, $CDCl_3$) $\delta$: 5.34 (m, 6H, −C*H*=C*H*−); 4.47 (s, 1H, −C*H*NC(O)); 3.91 (m, 2H, −C$H_2$OH); 2.78 (t, $J$ = 5.1 Hz, 4H, −C$H_2$−C=CH−); 2.22 (s, 2H, −C$H_2$NC(O)); 2.03 (m, 4H, 2 −HC=CHC$H_2$−); 1.57 (s, 2H, −C$H_2$CH$_2$NC(O)); 1.28 (m, 10H, −(C$H_2$)$_5$−); 0.96 (t, $J$ = 7.5 Hz, 3H, −C$H_3$). $^{13}$C NMR (250 MHz, $CDCl_3$) $\delta$: 174.3; 170.9; 132.0; 130.2; 128.8; 127.9; 127.4; 60.8; 56.9; 37.7; 36.5; 33.8; 29.9; 29.7; 29.4; 28.6; 26.9; 25.2; 13.9. MS (ESI): $m/z$ 366 ([M+H]$^+$), 100%. Anal. ($C_{21}H_{35}NO_4$) C, H, N.

**Ethyl 2-((2*Z*,4*E*,6*E*,8*E*)-3,7-Dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,4,6,8-tetraenamido)- 3-hydroxypropanoate (1d).** According to general procedure A, the reaction of retinoic acid (300 mg, 0.99 mmol) with L-serine ethyl ester chlorhydrate (169 mg, 1 mmol) afforded the title compound **1d** as a yellow solid (215 mg, 54%, yield). $^1$H NMR (250 MHz, $CDCl_3$) $\delta$: 6.72 (m, 2H, −C*H*=C*H*−); 6.13 (m, 4H, −C*H*=C*H*−); 4.66 (m, 1H, −C*H*NC(O)); 4.20 (q, $J$ = 6.9 Hz, 2H, −C$H_2$OH); 3.92 (t, $J$ = 4.5 Hz, 2H, −COOC$H_2$CH$_3$); 1.96 (m, 2H, −C$H_2$); 1.68 (s, 3H, −HC=HCC$H_3$); 1.39 (m, 4H, 2 −C$H_2$−); 1.26 (m, 3H, −C$H_3$); 0.99 (m, 6H, C−(C$H_3$)$_2$). $^{13}$C NMR (250 MHz, $CDCl_3$) $\delta$: 170.7; 167.3; 149.8; 139.0; 137.5; 137.2; 135.2; 130.2; 129.8; 1 29.4; 128.3; 1 20.4; 63.4; 61.8; 54.7; 39.4; 34.1; 33.0; 28.8; 26.8; 21.6; 19.1; 14.0; 13.5; 12.8. MS (ESI): $m/z$ 416 ([M+H]$^+$) 100%. Anal. ($C_{25}H_{37}NO_4$) C, H, N.

**2-((2*E*,4*E*,6*E*,8*E*)-3,7-Dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,4,6,8-tetraenamido)-3-hydroxypropanoic Acid (2d).** According to general procedure B, the reaction of ethyl 2-((2*Z*,4*E*,6*Z*,8*E*)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexa-1-enyl)-nona-2,4,6,8-tetraenamido)-3-hydroxypropanoate (150 mg, 0.24 mmol) with lithium hydroxide monohydrate (45 mg, 0.72 mmol) afforded the title compound **2d** as a yellow solid (130 mg, 98% yield). $^1$H NMR ($CDCl_3$, 250 MHz) $\delta$: 6.95 (d, $J$=11 Hz, 1H, −(O)-CN*H*−); 6.29−6.14 (m, 4H, −C*H*=C*H*−), 5.75 (s, 2H, −(O)-CC*H*=CH− and −C*H*=CH−), 4.67 (m, 1H, −CH$_2$O*H*), 4.17 (d, 1H, (O)CNC*H*−), 3.82 (d, $J$ = 9 Hz, 2H, −C$H_2$OH), 2.36 (m, 2H, −C$H_2$−), 2.06 (m, 2H, −C$H_2$−), 1.99 (s, 3H, −CH=CC$H_3$), 1.74 (s, 4H, −C$H_2$−), 1.61 (m, 2H, −C$H_2$−), 1.48 (m, 3H, −C$H_3$), 1.03 (s, 6H, −C(C$H_3$)$_2$). MS (ESI): $m/z$ 388 ([M + H]$^+$),100%. Anal. ($C_{23}H_{33}NO_4$) C, H, N.

**Ethyl 3-Hydroxy-2-(5*E*,8*E*,11*E*,14*E*)-icosa-5,8,11,14-tetraenamidopropanoate (1e).** According to general procedure A, the reaction of arachidonic acid (50 mg, 0.16 mmol) with L-serine ethyl ester chlorhydrate (19 mg, 0.16 mmol) afforded the title compound **1e** as a yellow oil (20 mg, 30% yield). $^1$H NMR ($CDCl_3$, 250 MHz) $\delta$: 5.40 (m, 8H, 4 −C*H*=C*H*−), 4.70 (m, 1H, −(O)CN−C*H*−COO−), 4.25 (m, 2H, −C$H_2$OCH$_3$); 3.95(m, 2H, −C$H_2$OH), 2.80 (m, 6H, −CH=CHC$H_2$CH=CH−), 2.40 (m, 2H, −C$H_2$NC(O)−), 2.15 (m, 4H, 2 −HC=CH−C$H_2$−), 1.70 (m, 2H, −C$H_2$CH$_2$NC(O)−), 1.30 (m, 6H, −C$H_2$−), 0.85 (m, 3H, −C$H_3$). $^{13}$C NMR (250 MHz, $CDCl_3$) $\delta$: 174.2, 171.0, 130.5, 129.0, 128.7, 128.5, 127.8, 127.5, 63.4, 63.0, 61.7, 54.3, 32.7, 31.5, 29.7, 27.2, 26.4, 25.6, 24.5, 22.5, 14.0. MS (ESI): $m/z$ 420 ([M + H]$^+$), 100%.

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**3-Hydroxy-2-(5*E*,8*E*,11*E*,14*E*)-icosa-5,8,11,14-tetraenamidopropanoic Acid (2e).** According to general procedure B, the reaction of ethyl 3-hydroxy-2-(5*E*,8*E*,11*E*,14*E*)-icosa-5,8,11,14-tetraenamidopropanoate (**1e**) (20 mg, 0.047 mmol) with lithium hydroxide monohydrate (5 mg, 0.14 mmol) afforded the title compound **2e** as a yellow oil with a quantitative yield. $^1$H NMR ($CDCl_3$, 250 MHz) $\delta$: 5.40 (m, 8H, 4 −C*H*═C*H*−), 4.70 (s, 1H, −(O)CNC*H*C(O)−), 3.95 (m, 2H, −C$H_2$OH); 2.80 (m, 6H, 3 −CH═CHC$H_2$CH═CH−), 2.40 (m, 2H, −C$H_2$NC(O)−), 2.15 (m, H, 4H, 2 −CH═CHC$H_2$−), 1.70 (m, 2H, −C$H_2$CH$_2$NC(O)−), 1.30 (m, 6H, 3 −C$H_2$−), 0.85 (m, 3H, −C$H_3$). $^{13}$C NMR (250 MHz, $CDCl_3$) $\delta$: 174.2, 171.0, 130.51, 129.0, 128.7, 128.6, 127.8, 127.5, 63.4, 63.07, 54.3, 32.7, 31.5, 29.7, 27.2, 26.4, 25.6, 24.5, 22.5, 14.0. MS (ESI): *m*/*z* 392 ([M + H]$^+$), 100%. Anal. ($C_{23}H_{37}NO_4$) C, H, N.

**Ethyl 3-Hydroxy-2-((1*R*,4a*R*,4b*R*,10a*R*)-7-isopropyl-1,4a-dimethyl-1,2,3,4a,4b,5,6,10,10a-decahydrophenanthrene-1-carboxamido)-propanoate (1f).** According to general procedure A, the reaction of abietic acid (1 g, 3.3 mmol) with L-serine ethyl ester chlorhydrate (559 mg, 3.3 mmol) afforded the title compound **1f** as a white solid ($R_f$ = 0.62, cHex/AcOEt, 1/1; 500 mg, 50% yield). $^1$H NMR (250 MHz, $CDCl_3$) $\delta$: 6.76 (m, 1H, −(O)CN*H*−); 5.72 (s, 1H, −C*H*═CH−); 5.31 (m, 1H, −HC═C*H*−); 4.59 (m, 1H, −HNC-*H*COO−); 4.21 (m, 2H, −COOC$H_2$CH$_3$); 3.89 (m, 2H, −C$H_2$OH); 1.97 (m, 4H, 4 −CH$_2$C*H*CH$_2$−); 1.56 (m, 3H, −CC$H_3$); 1.40 (s, 5H, −C$H_2$CH$_2$C*H*H−); 1.26 (m, 8H, 4 −C$H_2$−); 0.962 (m, 6H, −C(C$H_3$)$_2$); 0.816 (s, 3H, −C$H_3$). $^{13}$C NMR (250 MHz, $CDCl_3$) $\delta$: 179.0; 170.4; 144.9; 139.0; 134.9; 122.0; 120.0; 63.3; 61.6; 54.6; 50.5; 45.5; 45.3; 37.8; 37.0; 34.5; 34.2; 29.8; 27.0; 26.5; 24.9; 22.1; 21.0; 20.5; 17.9; 16.5; 13.8. MS (ESI): *m*/*z* 418 ([M+H]$^+$), 100%. Anal. ($C_{25}$-$H_{39}NO_4$) C, H, N.

**3-Hydroxy-2-((1*R*,4a*R*,4b*R*,10a*R*)-7-isopropyl-1,4a-dimethyl-1,2,3,4a,4b,5,6,10,10a-decahydrophenanthrene-1-carboxamido)-propanoic Acid (2f).** According to general procedure B, the reaction of ethyl 3-hydroxy-2-((1*R*,4a*R*,4b*R*,10a*R*)-7-isopropyl-1,4a-dimethyl-1,2,3,4a,4b,5,6,10,10a decahydrophenanthrene-1-carboxamido)propanoate (**1f**) (300 mg, 0.71 mmol) with lithium hydroxide monohydrate (90 mg, 2,1 mmol) afforded the title compound **2f** (200 mg, 71% yield). $^1$H NMR (250 MHz, $CDCl_3$) $\delta$: 5.72 (s, 1H, −C*H*═CH−); 5.28 (m, 1H, −CH═C*H*−); 4.54 (m, 1H, −HNC-*H*COO−); 3.98 (m, 2H, −C$H_2$OH); 2.16 (m, 5H, 4 −CH$_2$-CHCH$_2$−); 1.56 (m, 3H, −CC$H_3$); 1.29 (m, 8H, 4 −C$H_2$−); 0.99 (m, 6H, −CH$_2$(C$H_3$)$_2$); 0,811 (s, 3H, −C$H_3$). $^{13}$C NMR (250 MHz, $CDCl_3$) $\delta$: 180.0; 173.6; 146.0; 136.0; 122.9; 120.9; 63.3; 55.5; 53.9; 51.4; 47.1; 46.0; 38.7; 35.4; 35.1; 28.0; 25.3; 21.9 ; 21.4; 18.8; 17.3; 14.7. MS (ESI): *m*/*z* 390 ([M+H]$^+$), 100%. Anal. ($C_{23}$-$H_{35}NO_4$) C, H, N.

**Ethyl 3-Hydroxy-2-((2*E*,4*E*)-5-(1-hydroxy-2,6,6-trimethyl-4-oxocyclohex-2-enyl)-3-methylpenta-2,4-dienamido)propanoate (1g).** According to general procedure A, the reaction of abscisic acid (200 mg, 0.75 mmol) with L-serine ethyl ester chlorhydrate (128.2 mg, 0.75 mmol) afforded the title compound **1g** as a white solid ($R_f$ = 0.13, cHex/AcOEt, 1/1; 170 mg, 60% yield). $^1$H NMR (250 MHz, $CDCl_3$) $\delta$: 7.73 (m, 1H, −(O)C−N*H*−); 6.80 (m, 1H, −C*H*═CH−C−OH); 5.85 (s, 1H, −CH═C*H*−C(O)−); 5.74 (s, 1H, −CH═C*H*C(O)−); 4.67 (m, 1H, −HN−C*H*−COO−); 4.21 (m, 2H, −COO−C$H_2$CH$_3$); 3.94 (m, 2H, −C$H_2$OH); 2.28 (m, 2H, −C$H_2$−C(O)−); 2.01 (m, 6H, −C(C$H_3$)$_2$); 1.71 (m, 6H, 2 CH-═CC$H_3$−); 1.05 (m, 3H, −C$H_3$); 0.953 (s, 3H, −C$H_3$). $^{13}$C NMR (250 MHz, $CDCl_3$) $\delta$: 198.3; 170.8; 166.5; 163.7; 145.7; 135.6; 128.1; 126.6; 121.2; 79.6; 63.2; 62.1; 60.5; 54.6; 49.9; 41.6; 24.5; 23.3; 21.2; 19.2; 14.2. MS (ESI): *m*/*z* 380 ([M + H]$^+$), 100%. Anal. ($C_{20}$-$H_{29}NO_6$) C, H, N.

**3-Hydroxy-2-((2*E*,4*E*)-5-(1-hydroxy-2,6,6-trimethyl-4-oxocyclohex-2-enyl)-3-methylpenta-2,4-dienamido)propanoic Acid (2g).** According to general procedure B, the reaction of ethyl 3-hydroxy-2-((2*E*,4*E*)-5-(1-hydroxy-2,6,6-trimethyl-4-oxocyclohex-2-enyl)-3-methylpenta-2,4-dienamido)propanoate (**1g**) (300 mg, 0.71 mmol) with lithium hydroxide monohydrate (90 mg, 2.1 mmol) afforded the title compound **2g** (200 mg, 71%, yield). $^1$H NMR (250 MHz, $CDCl_3$) $\delta$: 7.79 (d, 1H, −(O)CN*H*−); 6.23 (d, 1H, −C*H*═CHCOH); 5.95 (s, 2H, 2 −HC═C*H*−); 4.47 (m, 1H, −HNC*H*COO−; 3.90 (m, 2H, −C$H_2$OH); 2.62 (m, 1H, (O)CC$H_2$C); 1.97 (m, 6H, C$H_3$-C═CH−); 1.33 (m, 3H, −C$H_3$); 1.09 (m, 6H, −C(C*H*3)$_2$). MS (ESI): *m*/*z* 352 ([M + H]$^+$),100%. Anal. ($C_{18}H_{25}NO_6$) C, H, N.

**Methyl 2-Amino-2-(dimethoxyphosphoryl)acetate (4).** HCl in ether (2 mL) was added to a solution of Boc-α-phosphonoglycine trimethyl ester (1 g, 3.6 mmol) in diethyl ether, and the solution was stirred for 24 h at room temperature. Evaporation of the solvent gave the desired product **4** as a white solid in quantitative yield. $^1$H NMR (250 MHz, $CDCl_3$) $\delta$: 4.65 (m, 1H, −HNC*H*COO−); 4.24 (m, 6H, (C$H_3$O)$_2$P(O)); 4.04 (m, 3H, −COO−C$H_3$). MS (ESI): *m*/*z* 198 ([M + H]$^+$), 100%.

**Methyl 2-(Dimethoxyphosphoryl)-2-stearamidoacetate (5a).** According to general procedure A, the reaction of stearic acid (545 mg, 1.9 mmol) with methyl 2-amino-2-(dimethoxyphosphoryl)acetate (384 mg, 1.61 mmol) afforded the title compound **5a** as a yellow oil (358 mg, 48% yield). $^1$H NMR (250 MHz, $CDCl_3$) $\delta$: 4.40 (m, 1H, −HNC*H*COO−); 3.80 (m, 3H, −COOC$H_3$); 3.64 (m, 6H, (C$H_3$O)$_2$P(O)); 1.83 (m, 2H, −CH$_2$-C$H_2$C(O)); 1.62 (m, 2H, −C$H_2$CH$_2$C(O); 1.23 (m, 28H, (−C$H_2$−)$_{14}$); 0.87 (m, 3H, −C$H_3$). $^{13}$C NMR (250 MHz, $CDCl_3$) $\delta$: 181.3; 172.9; 54.1; 53.4; 49.5; 25.4; 36.3; 33.7; 32.0; 29.7; 29.4; 29.2; 25.6; 24.9; 22.8; 14.2. MS (ESI): *m*/*z* 436 ([M + H]$^+$), 100%.

**Dimethyl 2-Hydroxy-1-stearamidoethylphosphonate (6a).** Methyl 2-(dimethoxyphosphoryl)-2-steramidoacetate (**5a**) (150 mg, 0.32 mmol) was dissolved in THF (5.5 mL), and anhydrous lithium chloride (273 mg, 6.4 mmol) and $NaBH_4$ (244 mg, 6.4 mmol) were added. After addition of ethanol (11 mL), the mixture was stirred at room temperature overnight. The mixture was cooled with ice−water, adjusted to pH 4 by the gradual addition of 10% citric acid (3.8 mL), and concentrated in vacuo. Water (70 mL) was added to the residue, which was extracted with methylene chloride three times and dried over $MgSO_4$. Removal of the solvent and purification by column chromatography over silica gel, eluting with $CH_2Cl_2$−ether−MeOH (4:4:0.5) gave **6a** (30 mg, 22%, yield) ($R_f$ = 0.5). $^1$H NMR (250 MHz, $CDCl_3$) $\delta$: 6.54 (m, 1H, −(O)CN*H*−); 4.50 (m, 1H, NHC*H*COO−); 3.99 (m, 1H, −C*H*HOH); 3.80 (m, 2H, −CH*H*OH and −C*H*NC(O)); 3.43 (m, 6H, (C$H_3$O)$_2$P(O)); 2.25 (m, 2H, −C$H_2$C(O)−); 1.94 (m, 2H, −C$H_2$CH$_2$C(O)−), 1.24 (m, 28H. −(C$H_2$)$_{14}$−); 0.87 (m, 3H, −C$H_3$). $^{13}$C NMR (250 MHz, $CDCl_3$) $\delta$: 181.3; 36.3; 31.7; 29.5; 29.1; 25.4; 25.2; 24.5; 22.5; 22.2; 13.9. MS (ESI): *m*/*z* 436 ([M + H]$^+$), 100%. Anal. ($C_{22}H_{46}NO_5P$) C, H, N.

**2-Hydroxy-1-stearamidoethylphosphonic Acid (7a).** Ester dimethyl 2-hydroxyl-1-stearamidoethylphosphonate was dissolved in methylene chloride. Iodotrimethylsilane (5.0 mol equiv) was added, and the mixture was stirred overnight at 50 °C under $N_2$. Evaporation in vacuo gave **7a** as a yellow oil which was crystallized from 1MeOH−1$H_2$O (32 mg, 22%) ($R_f$ = 0.3, $CH_2$-$Cl_2$−ether−MeOH (4:4:0.5). $^1$H NMR (250 MHz, $CDCl_3$) $\delta$: 4.50 (m, 1H, −(O*H*)P(O); 3.95 (m, 1H, (O*H*)P(O)); 3.74 (m, 2H, −C$H_2$OH); 3.54 (m, 1H, −(O)CNHC*H*P); 2.31 (m, 2H, −C$H_2$C-(O)−); 1.88 (m, 2H, −CH$_2$C$H_2$C(O)−); 1.67 (m, 2H, −C$H_2$CH$_2$C-(O)−); 1.24 (m, 28H. −C$H_2$−); 0.87 (m, 3H, −C$H_3$). MS (ESI): *m*/*z* 408 ([M+H]$^+$), 100%. Anal. ($C_{20}H_{42}NO_5P$) C, H, N.

**Ethyl 2-Tetrazol -5-yl-2-oximinoacetate (9).** A mixture of (3 g, 21.12 mmol) of ethyl 2-cyano-2-oximinoacetate and (1.5 g, 0.22 mmol) of sodium azide ($NaN_3$) was stirred in 20 mL of dry DMF in an oil bath maintained at 70 °C for 30 h. The reaction mixture was then concentrated under reduced pressure which was subjected to rotary evaporation for 2 h to remove most of the DMF. Then 70 mL of ethyl acetate and 20 mL of water were added, and the mixture was stirred until solution was complete. The pH was adjusted to pH 1 by the addition of 5 mL of 5 N HCl. The phases were separated, and the ethyl acetate was washed with 20 mL of $H_2O$, dried, and evaporated under reduced pressure. The residue was purified by column chromatography, eluting with cyclohexane/EtOAc (1: 1) to give a solid.

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$R_f$ = 0.23 (1.5 g, 39% yield). $^1$H NMR (250 MHz, DMSO-$d_6$) $\delta$: 4.28 (q, $J$ = 7.1 Hz, 2H, $CH_3CH_2COO-$); 1.25 (m, 3H, $CH_3$-$CH_2COO-$). MS (ESI): $m/z$ 186 ([M + H]$^+$), 100%.

**Ethyl 2-Amino-2-(1*H*-tetrazol-5-yl)acetate (10).** A solution of 5-ethyl 2-tetrazol-5-yl-2-oximinoacetate (**9**) was dissolved in 10 mL of MeOH. Then 40 mg of 10% in weight of Pearlman's catalyst ($Pd(OH)_2$ over activated charcoal) was added to the solution and the resulting suspension was stirred at room temperature under $H_2$ atmosphere for 2 h. An amount of 6 mL of a solution of 3 N HCl/MeOH was added, and the mixture was stirred for 18 h at room temperature. The solution was filtered and concentrated under reduced pressure to give compound **10** as a yellow solid with a quantitative yield. $^1$H NMR (250 MHz, DMSO-$d_6$) $\delta$: 4.78 (m, 1H, $NH_2-CH$-C(O); 4.24 (m, 2H, $CH_3CH_2COO-$; 1.15 (m, 3H, $CH_3CH_2COO-$). MS (ESI): $m/z$ 172 ([M + H]$^+$), 100%.

**Ethyl 2-(9*Z*,12*Z*)-Heptadeca-9,12-dienamido-2-(1*H*-tetrazol-5-yl)acetate (11b).** A mixture of linoleic acid (229 mg, 0.82 mmol), ethyl chloroformate (98.28 mg, 0.91 mmol), and pyridine in $CH_2Cl_2$ was stirred at 0 °C for 20 min, and then a solution of ethyl 2-amino-2-(1*H*-tetrazol-5-yl)acetate (**10**) (150 mg, 0.70 mmol) in $CH_2Cl_2$ was added dropwise at 0 °C. The reaction mixture was stirred for 2 h at room temperature. The solution was evaporated under reduced pressure, 5 mL of ethyl acetate was added, and the residue was washed with 3 × 10 mL of $H_2O$. The organic phase was dried over $MgSO_4$. After filtration and evaporation under reduced pressure, silica gel chromatography (cyclohexane/EtOAc (1:1)) of the residue gave a transparent oil (140 mg, 47% yield). $^1$H NMR (250 MHz, DMSO-$d_6$) $\delta$: 5.30 (m, 4H, 2 $-CH=CH-$); 4.78 (m, 1H, $NH_2$-CHC(O)); 4.03 (q, $J$ = 7.1 Hz, 2H, $CH_3CH_2OC(O)$); 2.63 (m, 2H, $-CH=CHCH_2CH=CH-$); 2.28 (m, 2H, $-CH_2CH_2C(O)-$); 2.07 (m, 6H, $-CH_2C(O)-$ and 2 $-CH_2C=CH-$); 1.49 (m, 2H, $CH_2CH_2C(O)$); 1.24 (m, 17H, $-CH_2-$); 0.84 (m, 3H, $-CH_3$). MS (ESI): $m/z$ 420 ([M + H]$^+$). 100%.Anal. ($C_{23}H_{45}N_5O_3$) C, H, N.

**6-*N*-(*tert*-Butyloxycarbonyl)aminocaproic Acid (13).** 6-Aminocaproic acid (2.62 g, 20.0 mmol) was dissolved in a dioxane−$H_2O$ (2:1) solution. The reaction mixture was cooled to 0 °C, and a 1 M aqueous solution of NaOH (20 mL, 20.0 mmol) was added, followed by $Boc_2O$ (4.80 g, 22.0 mmol) which was added as a solid. The reaction mixture was stirred at room temperature for 3 h. The solutions were concentrated under reduced pressure. The basic aqueous residue was washed once with EtOAc (50 mL). The aqueous layer was acidified by aqueous 1 M HCl until pH 1 and then extracted with EtOAc (3 × 50 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to afford the desired compound **13** as colorless oil which slowly crystallized (4.01 g, quantitative). $R_f$ = 0.71 (EtOAc). $^1$H NMR (250 MHz, $CDCl_3$) $\delta$: 3.63 (s, 1H, broad s, N*H*); 3.01 (m, 2H, $-NHCH_2-$); 2.25 (t, $J$ = 7.5 Hz, 2H, $-CH_2COOH$); 1.56−1.17 (m, 15H, $-CH_2(CH_2)_3CH_2-$ and $CH_3$ Boc). $^{13}$C NMR (250 MHz, $CDCl_3$) $\delta$: 178.4; 66.7; 60.2; 53.2; 40.0; 29.4; 28.1; 26.0; 24.5; 20.6; 13.9. MS (ESI): $m/z$ 232 ([M + H]$^+$, 100%.

**Generale Procedure C (Coupling Reaction Procedure DIEA/BOP). 6-Aminocaproyl-2,3-di-*o*-benzyl-L-Ascorbic Acid Trifluoroacetic Acid Salt (14).** 6-*N*-(*tert*-Butyloxycarbonyl)aminocaproic acid (0.47 g, 2.07 mmol) was dissolved in freshly distilled $CH_2Cl_2$ (10 mL) with 1 equiv (1.14 g, 2.59 mmol) of BOP reagent. The reaction mixture was cooled to 0 °C, and then 1.0 equiv of DIEA (443 $\mu$L, 2.59 mmol) was added dropwise. The reaction mixture was stirred for 1 h at room temperature and then once again cooled to 0 °C. A $CH_2Cl_2$ solution (30 mL) of alcohol 2,3-di-*O*-benzyl-L-ascorbic acid (0.92 g, 2.59 mmol) and of DIEA (886 $\mu$L, 5.18 mmol) was added dropwise. The solution was allowed to warm and was stirred overnight at room temperature. The solvent was removed under reduced pressure, and the residue was dissolved in EtOAc (100 mL). The organic layer was washed successively with 5% aqueous citric acid (3 × 50 mL), brine (50 mL), 5% aqueous $NaHCO_3$ (3 × 50 mL), and brine (50 mL), was dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The crude residue was purified by flash chromatography (EtOAc−hexane, 2:3 and then 1: 1) to give the desired compound (0.88 g, 71% yield) as a white solid. $^1$H NMR ($CDCl_3$, 250 MHz) $\delta$: 7.41 (m, 10H, Ar*H*), 5.20 (s, 4H, $-OCH_2C_6H_5$), 4.72 (d, 1H, $-OCH_2CH$ (OH)$-CH-$), 4.61 (s, 1 H, $-CH$ (O*H*)−), 4.35 (d, 2J = 11.5 Hz, 3$J$=7.0 Hz 1 H, $-O-CHHCH-$), 4.23 (d, 2J=11.5 Hz, 3$J$= 4.8 Hz, 1 H, $-OCHHCHCH-$), 4.14 (s, 1 H, $-OCH_2CH$(OH)-CH−), 3.19−3.02 (m, 2 H, $-NHCH_2-$), 2.36 (t, 3$J$ = 6.3 Hz, 2 H, $-CH_2C(O)O-$), 1.87−1.36 (m, 15H, $CH_3$ Boc and $-NHCH_2(CH_2)_3CH2C(O)O-$). MS (ESI): $m/z$ 570 ([M+H]$^+$, 100%. The *N*-Boc derivative (0.8 g, 2.0 mmol) was dissolved in $CH_2Cl_2$ (15 mL). The solution was cooled to 0 °C, and trifluoroacetic acid (1 mL, 20.0 mmol) was added dropwise. The resulting reaction mixture was stirred for 2 h at room temperature. The solvent and excess of TFA were removed under reduced pressure. The residue was triturated in $Et_2O$, and the title compound was quantitatively isolated as a white solid (0.84 g). $R_f$ = 0.16 1c-Hex/1AcOEt. $^1$H NMR ($CDCl_3$, 250 MHz) $\delta$: 7.41 (m, 10H, Ar*H*), 5.22 (s, 4H, $-O-CH_2-C_6H_5$), 4.71 (d, 1H, $-OCH_2-CH$ (OH)$-CH-$), 4.61 (s, 1 H, $-CH$ (O*H*)−), 4.39 (d, 2$J$ = 11.5 Hz, 3$J$ = 7.0 Hz 1 H, $-O-CHH-CH$(OH)$-CH-$), 4.23 (d, 2$J$ = 11.5 Hz, 3$J$ = 4.8 Hz, 2 H, $-O-CHH-CH$(OH)−CH−), 3.18−3.04 (m, 2 H, $-NH_2-CH_2-$), 2.36 (t, 3$J$ = 6.3 Hz, 2 H, $-CH_2-C(O)-O-$), 1.71−1.26 (m, 6H, $NH_2-CH_2-(CH_2)_3-CH_2-C(O)-O-$). MS (ESI): $m/z$ 583 ([M + H]$^+$,100%.

**6-Aminocaproyl-2,3-L-Ascorbic Acid Trifluoroacetic Acid Salt (15).** The protected ascorbic acid derivative (848 mg, 1.4 mmol) was dissolved in 3 mL of MeOH. Then 10% in weight of Pearlman's catalyst ($Pd(OH)_2$ over activated charcoal) was added to the previous solution and the resulting suspension was stirred at room temperature under $H_2$ atmosphere for 2 h. The solution was filtered and concentrated under reduced pressure to yield the deprotected ascorbic acid derivative (460 mg, 85% yield) as a white solid. $^1$H NMR ($CDCl_3$, 250 MHz) $\delta$: 4.72 (d, 1H, $-OCH_2-CH$ (OH)$-CH-$), 4.61 (s, 1 H, $-CH$ (O*H*)−), 4.39 (d, 2$J$=11.5 Hz, 3$J$=7.0 Hz 1 H, $-O-CHH-CH$ (OH)$-CH-$), 4.23 (d, 2$J$ = 11.5 Hz, 3$J$=4.8 Hz, 2 H, $-O-CHH-CH$ (OH)−CH−), 3.04−3.18 (m, 2 H, $-NH_2-CH_2-$), 2.36 (t, 3$J$=6.3 Hz, 2 H, $-CH_2-C(O)-O-$), 1.71−1.40 (m, 6H, $NH_2-CH_2-(CH_2)_3-CH_2-C(O)-O-$). MS (ESI): $m/z$ 403 ([M+H]$^+$,100%.

***N*-(9-(3,4-Bis(benzyloxy)-5-oxo-2,5-dihydrofuran-2-yl)-9-hydroxy-6-oxononyl)stearamide (16a). 16a** was synthesized from stearic acid (0.106 g, 0.53 mmol) with 6-aminocaproyl-2,3-di-*o*-benzyl-L-ascorbic acid trifluoroacetic acid salt (**14**) (0.25 g, 0.53 mmol) following the general procedure C (100 mg, 26% yield). $^1$H NMR (250 MHz, $CDCl_3$) $\delta$: 7.31 (m, 10H, Ar*H*); 5.28 (m, 4H, $-OCH_2-C_6H_5$); 5.02 (m, 1H, $-COOCH_2CH(OH)CH-$); 4.33 (m, 1H, $-COOCH_2CHOH$); 4.33 (m, 1H, $-COOCH_2CHOH$); 3.24 (m, 2H, $-(O)CNHCH_2-$); 2.25 (m, 2H, $-CH_2COO-$); 2.18 (m, 2H, $-CH_2CH_2C(O)-$); 1.61 (m, 6H, $-(O)CNH(CH_2)_3COO-$); 1.29 (m, 30H, $-(CH_2)_{15}-$); 0.88 (m, 3H, $-CH_3$). MS (ESI): $m/z$ 736 ([M+H]$^+$,100%. Anal. ($C_{44}H_{65}NO_8$) C, H, N.

**2-(3,4-Bis(benzyloxy)-5-oxo-2,5-dihydrofuran-2-yl)-2-hydroxyethyl 6-(9*E*,12*E*)-Octadeca-9,12-dienamidohexanoate (16b). 16b** was synthesized from linoleic acid (0.148 g, 0.53 mmol) with 6-aminocaproyl-2,3-di-*o*-benzyl-L-ascorbic acid trifluoroacetic acid salt (**14**) (0.25 g, 0.53 mmol) following the general procedure C (100 mg, 26% yield). $^1$H NMR (250 MHz, $CDCl_3$) $\delta$: 7.37 (m, 10H, Ar*H*); 5.33 (m, 4H, 2-$CH=CH-$); 5.29 (m, 2H, $-OCH_2C_6H_5$); 5.19 (m, 2H, $-O-CH_4-C_6H_5$); 5.08 (m, 1H, $-O-CH_2-CH(OH)-CH-$); 4.33 (m, 1H, $-OCH_2CH(OH)-CH-$); 4.29 (m, 1H, $-OCHHCH-$); 4.22 (m, 1H, $-OCHH-CH-$); 3.24 (m, 2H, $-(O)CNHCH_2-$); 2.76 (m, 2H, $-CH=CHCH_2CH=CH-$); 2.34 (t, d, $J$=7.2 Hz, 2H, $-CH_2C(O)-NH-$); 2.14 (t, d, $J$=7.2 Hz, 2H, $-CH_2C(O)O-$); 2.02 (m, 4H, $CH_2CH_2C(O)-$ and $(O)CNHCH_2CH_2-$); 1.96 (m, 4H, 2 $-CH_2-CH=CH-$); 1.28 (m, 18 H$-(CH_2)_9-$); 0.88 (m, 3H, $-CH_3$). $^{13}$C NMR (250 MHz, $CDCl_3$) $\delta$: 173.7; 169.4; 157.0; 135.9; 135.3;

127.8; 31.4; 29.6; 29.3; 27.1; 25.8; 25.6; 24.2; 23.9; 22.4; 19.3; 14.0. MS (ESI): $m/z$ 734 ($[M+H]^+$), 100%. Anal. ($C_{44}H_{61}NO_8$) C, H, N.

***N*-(9-(3,4-Dihydroxy-5-oxo-2.5-dihydrofuran-2-yl)-9-hydroxy-6-oxononyl)stearamide (17a).** The protected ascorbic acid derivative **16a (**100 mg, 0.14 mmol) was dissolved in 3 mL of MeOH. Then 10% in weight of Pearlman's catalyst ($Pd(OH)_2$ over activated charcoal) was added to the previous solution and the resulting suspension was stirred at room temperature under $H_2$ atmosphere for 2 h. The solution was filtered and concentrated under reduced pressure to give the deprotected ascorbic acid derivative **17a** as a white solid in quantitative yield. $^1$H NMR (250 MHz, $CDCl_3$) $\delta$: 5.10 (m, 1H, $-COOCH_2CH(OH)CH-$)); 4.44 (m, 1H, $-COOCH_2CH(OH)CH-$); 4.30 (m, 2H, $-COOCH_2CH$-OH); 3.20 (m, 2H, $(O)CNHCH_2-$); 2.25 (m, 2H, $-CH_2COO-$); 2.18 (m, 2H, $-CH_2C(O)N$); 1.68 (m, 4H, $-NHCH_2CH_2CH_2C$-$(O)-$); 1.30 (m, 32H, $-CH_2-$); 0.88 (m, 3H, $-CH_3$). MS (ESI): $m/z$ 556 ($[M+H]^+$, 100%. Anal. ($C_{30}H_{53}NO_8$) C, H, N.

**2-(3,4-Dihydroxy-5-oxo-2,5-dihydrofuran-2-yl)-2-hydroxyethyl 6-(9*Z*,12*Z*)-Octadeca-9,12-dienamidohexanoate (17b). 17b** was synthesized from linoleic acid (0.23 g, 0.82 mmol) with 6-aminocaproyl-2,3-L-ascorbic acid trifluoroacetic acid salt (**15**) (0.237 g, 0.82 mmol) following the general procedure C (0, 2 g, 44% yield) as a white oil. $^1$H NMR (250 MHz, $CDCl_3$) $\delta$: 6.06 (m, 1H, $-NH-$); 5.35 (m, 4H, 2 $-CH=CH-$); 5.05 (m, 1H, OHC$=$COHCHOC(O)); 4.74 (m, 1H, $-COOCH_2CHOH$); 4.34 (m, 2H, $-COOCH_2CHOH$); 3.21 (m, 2H, $-(O)CNHCH_2CH_2-$); 2.75 (t, d, $J$ = 5.5 Hz, 2H, $-CH=CHCH_2CH=CH-$); 2.48 (m, 2H, $-CH_2C(O)NH$); 2.32 (m, 2H, $-CH_2C(O)O-$); 2.05 (m, 4H, 2 $-CH_2CH=CH-$); 1.59 (m, 4H, $-CH_2CH_2C(O)-$ and $(O)CNH$-$CH_2CH_2-$); 1.28 (m, 18H, $-(CH_2)_9-$); 0.87 (d, $J$ = 6.5 Hz, 3H, $CH_3-$). $^{13}$C NMR (250 MHz, $CDCl_3$) $\delta$: 181.6; 147.0; 130.2; 129.9; 128.0; 127.8; 31.4; 29.6; 29.3; 27.1; 25.8; 25.6; 24.2; 23.9; 22.4; 19.3; 14.0. MS (ESI): $m/z$ 554 ($[M+H]^+$), 100%. Anal. ($C_{30}$-$H_{49}NO_8$) C, H, N.

**Biology. Cell Culture.** Fetal calf serum (FCS) and dialyzed FCS (DFCS) were from Hyclone (Logan, UT). Dulbecco's modified Eagle's medium (DMEM) was purchased from Invitrogen (Carlsbad, CA). HT22 cells[28] were grown in DMEM supplemented with 10% FCS and antibiotics.

**Cytotoxicity Assay.** Cell viability was determined by a modified version of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay based on the standard procedure.[29] Cells were seeded onto 96-well microtiter plates at a density of $5 \times 10^3$ cells per well. For the in vitro ischemia assay, the next day, the medium was replaced with DMEM supplemented with 7.5% DFCS and the cells were treated with 20 $\mu$M iodoacetic acid (IAA) alone or in the presence of the different GM1-like compounds. After 2 h the medium in each well was aspirated and replaced with fresh medium without IAA but containing the GM1-like compounds. After 20 h, the medium in each well was aspirated and replaced with fresh medium containing 2.5 $\mu$g/mL MTT. After 4 h of incubation at 37 °C, the cells were solubilized with 100 $\mu$L of a solution containing 50% dimethylformamide and 20% SDS (pH 4.7). For the oxidative glutamate toxicity assay, the next day, the medium was replaced with DMEM supplemented with 7.5% DFCS and the cells were treated with the different GM1-like compounds alone or in the presence of 5 mM glutamate. After 24 h, the medium in each well was aspirated and replaced with fresh medium containing 2.5 $\mu$g/mL MTT. After 4 h of incubation at 37 °C, the cells were solubilized with 100 $\mu$L of a solution containing 50% dimethylformamide and 20% SDS (pH 4.7). For both assays, the absorbance at 570 nm was measured on the following day with a microplate reader (Molecular Devices). Results were confirmed by visual inspection of the wells. Controls included compound alone to test for toxicity and compound with no cells to test for interference with the assay chemistry.

**Total Glutathione and ATP.** Total intracellular glutathione and ATP were determined by chemical and chemiluminescent assays as described.[14]

**SDS−PAGE and Immunoblotting.** HT22 cells from the same density cultures as used for the cell death assays were untreated or treated with the GM1-like compounds alone or in the presence of 20 $\mu$M IAA for 2 h followed by 2 h of recovery in the presence of the GM1-like compounds. The cells were washed twice in phosphate buffered saline and solubilized in SDS−sample buffer containing 0.1 mM $Na_3VO_4$ and 1 mM phenylmethylsulfonyl fluoride (PMSF), boiled for 5 min, and either analyzed immediately or stored frozen at −70 °C. Proteins were separated on 10% SDS−polyacrylamide gels and transferred to nitrocellulose. Equal loading and transfer of the samples were confirmed by staining the nitrocellulose with Ponceau-S. Transfers were blocked for 1 h at room temperature with 5% nonfat milk in TBS/0.1% Tween-20 and then incubated overnight at 4 °C in the primary antibody diluted in 5% BSA in TBS/0.05% Tween-20. The primary antibodies used were phospho-p44/42 MAP kinase antibody (no. 9101, 1/1000), phospho-p38 MAP kinase (no. 9211, 1/1000), phospho-SAPK/JNK (no. 9255, 1/1000), phospho c-Jun (no. 9261, 1/1000), phospho-MAPKAPK-2 antibody (no. 3041, 1/1000), and total MAPKAPK-2 (no. 3042, 1/1000) from Cell Signaling (Beverly, MA); total p38 antibody (no. sc-728, 1/500) and c-Jun/AP-1 antibody (no. sc-44-G) from Santa Cruz Biotechnology (Santa Cruz, CA); JNK1 antibody (no. 15701A, 1/500) from Pharmingen and pan ERK antibody (no. E17120, 1/10000) from Transduction Laboratories (San Diego, CA). The transfers were rinsed with TBS/0.05% Tween-20 and incubated for 1 h at room temperature in horseradish peroxidase−goat antirabbit or goat antimouse (Biorad, Hercules, CA) diluted 1/5000 in 5% nonfat milk in TBS/0.1% Tween-20. The immunoblots were developed with the Super Signal reagent (Pierce, Rockford, IL).

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**Supporting Information Available:** Elemental analysis results for compounds **1a−g**, **2a−2**, **6a**, **7a**, **11**, **16a**, **16b**, **17a**, and **17b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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