Tetrahydrolipstatin Analogues as Modulators of Endocannabinoid 2-Arachidonoylglycerol Metabolism

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A series of 21 analogues of tetrahydrolipstatin (THL, **1**) were synthesized and tested as inhibitors of the formation or hydrolysis of the endocannabinoid 2-arachidonoylglycerol (2-AG). Three of the novel compounds, i.e., **11**, **13**, and **15**, inhibited 2-AG formation via the diacylglycerol lipase α (DAGLα) with IC_{50} values lower than 50 nM (IC_{50} of THL = 1 μM) and were between 23- and 375-fold selective vs 2-AG hydrolysis by monoacylglycerol lipase (MAGL) as well as vs cannabinoid CB_1 and CB_2 receptors and anandamide hydrolysis by fatty acid amide hydrolase (FAAH). Three other THL analogues, i.e., **14**, **16**, and **18**, were slightly more potent than THL against DAGLα and appreciably selective vs MAGL, CB receptors, and FAAH (15−26-fold). One compound, i.e., **8**, was a potent inhibitor of MAGL-like activity (IC_{50} = 0.41 μM), and relatively (∼7-fold) selective vs the other targets tested.

Introduction

Of the two most studied endogenous agonists of CB_1 and CB_2[a] cannabinoid receptors (endocannabinoids), anandamide (AEA),[1] and 2-arachidonoylglycerol (2-AG),[2] this latter exhibits the highest abundance in tissues[3] and selectivity for CB receptors.[4] As a consequence, pharmacological manipulation of the metabolism of 2-AG would be expected to have a major impact on cannabinergic signaling. As with other monoglycerides, 2-AG takes part in several pathways of lipid metabolism and many biochemical routes may lead to and/or involve it. In most of the cases when 2-AG exerts its role as a signaling molecule, 2-arachidonate-containing diacylglycerols (DAGs) seem to act as direct precursors for 2-AG biosynthesis (Figure 1).[5] DAGs are in turn produced, depending on the type of stimulus, from the hydrolysis of either phosphoinositides (PI), catalyzed by a PI-selective phospholipase C, or arachidonate-containing phosphatidic acid (PA), catalyzed by a PA phosphohydrolase. DAGs are eventually converted into 2-AG by the action of two *sn*-1 selective DAG lipases α and β (DAGLs).[6] 2-AG is inactivated by cellular reuptake and subsequent intracellular enzymatic hydrolytic degradation. Although fatty amide hydrolase (FAAH), the enzyme responsible of the hydrolysis of AEA, catalyzes also 2-AG hydrolysis, a monoacylglycerol lipase (MAGL) is suggested to play a more important role in this respect.[5] Additional metabolic pathways that may be important both in terminating the effects of 2-AG and in the production of biologically active metabolites involve oxidative mechanisms catalyzed by lipoxygenases, cyclooxygenases, and P-450 monooxygenases.[5]

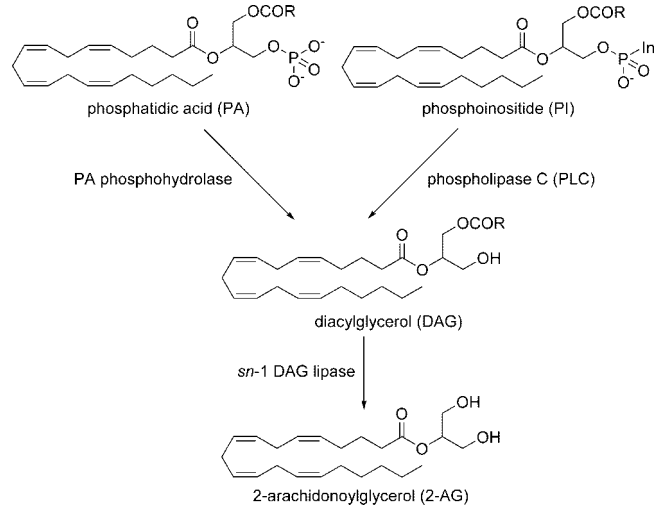

Figure 1. Main biosynthetic pathways for 2-AG. Abbreviations: R, acyl chain of a fatty acid; In, inositol.

Inhibitors of DAGLs might produce a blockade of 2-AG signaling with pharmacological consequences parallel (but not identical) to those of cannabinoid receptor antagonists, while inhibitors of MAGL could offer a rational therapeutic approach to a variety of diseases in which elevation of endocannabinoid levels represents an adaptive reaction to re-establish normal homeostasis when this is pathologically perturbed.[7]

Several compounds have been screened as possible MAGL inhibitors, but they either lack selectivity over FAAH as in the case of α-methyl-1-arachidonoylglycerol, O-2203, and O-2204[8] or, even when selective vs FAAH, as in the case of arachidonoylmaleimide and URB602,[9] they have not been tested on DAGLs. On the other hand, only recently, two potent and selective inhibitors of the 2-AG biosynthesis, fluorophosphonates O-3841 and O-3640, have been described.[10] They showed an excellent selectivity for DAGLα over a series of other proteins of the endocannabinoid system, but were less potent than tetrahydrolipstatin (THL). This is a mammalian lipase inhibitor derived from lipstatin, a lipid produced by *Streptomyces*

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[a] Abbrevations: THL, tetrahydrolipstatin; 2-AG, 2-arachidonoylglycerol; AEA, anandamide; CB_1, cannabinoid receptor 1; CB_2, cannabinoid receptor 2; DAGLα, diacylglycerol lipase α; MAGL, monoacylglycerol lipase; FAAH, fatty acid amide hydrolase; DDC, *N*,*N*′-dicyclohexylcarbodiimide; DMAP, 4-dimethylaminopyridine; COS-7, transformed African green monkey kidney fibroblast cells; HEK293, human embryonic kidney cells.

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toxytricini.[11] Irreversible inhibition with THL as a consequence of the formation of a stable covalent binding of the β-lactone moiety of the molecule with a catalytic serine hydroxyl group has been initially reported for pancreatic and gastric lipases,[12a,d,e] while, in the case of pancreatic carboxylester lipase, the inhibition was found to be reversible due to hydrolysis of the enzyme−THL adduct.[12c] By virtue of these biological activities, THL has been widely investigated since the early 1990s as an antiobesity drug and is currently marketed under the trade name of Xenical. In 2003, THL was found to potently inhibit also DAGLα and β[6] and has since been used, despite its nonspecificity, in most studies investigating the biological roles of 2-AG and of DAGLs, in particular their involvement in depolarization-induced suppression inhibition (DSI) or excitation (DSE), two well-known forms of endocannabinoid-mediated short-term synaptic plasticity.[13] Finally, THL was recently found also to inhibit one of the minor enzymes catalyzing 2-AG hydrolysis in the brain, the α/β hydrolase 12 (ABDH12), although at concentrations ∼100-fold higher than those required to inhibit DAGLα.[14] By contrast, controversial results have been very recently reported as to whether THL exerts an antagonistic effect on CB_1 receptors.[13a,15]

In view of the considerable interest in the development of selective and efficacious pharmacological tools for the study of the functions of all the endocannabinoid enzymes cloned so far and their targeting in various disorders, we have synthesized a series of 21 analogues of THL by varying the nature of the aminoacidic moiety and of the *N*-substitution and have investigated the effects of these variations on the activity on MAGL and DAGLα and their selectivity vs cannabinoid CB_1 and CB_2 receptors and FAAH.

Chemistry. The THL analogues in Table 1 were prepared as detailed below and as summarized in Scheme 1. Because of its biological properties, THL has been the subject of much synthetic activity and several total syntheses have been reported.[16] However, such known processes require generally more than 10 steps, utilize and/or form unstable intermediates, and require sometimes complicated purification procedures of stereoisomers. It was therefore envisioned that the key intermediate for our analogues, the hydroxy lactone **22**, could be most conveniently obtained from the readily available and rather inexpensive THL itself.[17]

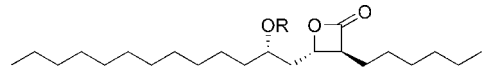
Saponification of THL (**1**) with KOH in MeOH, followed by the immediate treatment of the resulting dihydroxy acid potassium salt with benzyl bromide, afforded benzyl ester **23**. Selective protection of the more accessible C-5 hydroxy group was carried out with triisopropylsilyl trifluoromethanesulfonate (TIPSOTf) and *sym*-collidine.[16j] Hydrogenolysis of the benzyl group of **24** and lactonization of the acid **25** with *N*-phenylbis(trifluoromethanesulfonimide) ($PhNTf_2$) furnished the β-lactone **26**. It is to be noted that our attempts to achieve β-lactone ring formation under Adam's conditions ($PhSO_2Cl$/pyridine) used in the majority of approaches to the total synthesis of THL resulted in a rather poor yield (22%) of **26**. The removal of the TIPS protecting group with n-$Bu_4N^+F^-$ and AcOH in THF provided the hydroxy lactone **22**, whose physical and spectral data were in good agreement with those reported.[16i,p,q,r] Esterification of **22** with *N*-Boc-L-leucine, 4-methylpentanoic acid, *N*-acetylglycine, and pyroglutamic acid using DCC and DMAP afforded compounds **3**,[18] **7**, **9**, and **18**,[19] respectively. Similar coupling of **22** with *N*-Cbz-protected L-leucine, D-leucine, glycine, L-phenylalanine, L-alanine, β-alanine, L-valine, L-isoleucine, and L-proline gave compounds **4**,[16h,i,n,q,20] **6**, **10**, **20**, **27**−**31**, respectively. Hydrogenolysis of the Cbz protecting group furnished the corresponding amines **5**,[16i,n,p,20] **21**, and **32**−**38**, which were converted, with the exception of **5**, into their *N*-formyl derivatives **2**,[21] **8**,[21] **11**, **12**,[21]and **13**,[21] **15**, **17**, and **19** with formic acetic anhydride, while treatment of amines **36** and **37** with acetyl chloride afforded the corresponding *N*-acetyl derivatives **14** and **16**. Attempts to obtain the above *N*-formyl and *N*-acetyl derivatives by direct coupling of **22** with the corresponding *N*-formyl or *N*-acetyl amino acids resulted in lower yields.[16q]

Biological Evaluation. Well established protocols were used to assess the activity of the novel compounds on MAGL and DAGLα. The membrane fraction of COS-7 cells overexpressing DAGLα was used to assess the activity against the hydrolysis of *sn*-1-[^{3}H]oleoyl-2-arachidonoylglycerol to [^{3}H]oleic acid and 2-AG. The formation of [^{3}H]oleic acid, measured by scintillation counting after thin layer chromatography separation of the reaction products, was used as a measure of DAGL activity. The cytosolic fraction of COS-7 cells, which contains MAGL as opposed as the other 2-AG hydrolysing enzymes,[14] was used to measure the rate of 2-[^{3}H]arachidonoylglycerol hydrolysis to [^{3}H]arachidonic acid and glycerol. The formation of the tritiated fatty acid, measured by scintillation counting after thin layer chromatography separation of the reaction products, was used as a measure of MAGL activity. The inhibitory activity of the most potent DAGL inhibitor was also evaluated on 2-AG biosynthesis in intact neuronal cells in order to gain an idea of its degree of permeation through the cell plasma membrane. Finally, the specificity of the most active compounds against either 2-AG formation or hydrolysis vs cannabinoid receptors was evaluated by binding assays carried out with membranes prepared from HEK-293 cells overexpressing either the human recombinant CB_1 or CB_2 receptors, whereas specificity vs FAAH was evaluated by using rat brain membranes and [^{14}C]anandamide as substrate.

Results and Discussion

The results of the pharmacological assays shown in Table 1, and the salient aspects of SARs can be summarized as follows. Three of the compounds examined, i.e., **11**, **13**, and **15**, inhibited 2-AG formation via the diacylglycerol lipase α (DAGLα) with IC_{50} values lower than 50 nM. Under the same assay conditions, the IC_{50} of THL was 1 μM, i.e., ∼17-fold higher than previously published.[6] This might be due to the stronger degree of expression of recombinant DAGLα observed in the transfected cells used for these experiments with respect to those used for previous experiments (data not shown). In fact, DAGLα activity increases with the amounts of protein[6] and, when using the same amount of substrate, higher concentrations of inhibitors might be required to exert a 50% inhibition in the presence of higher amounts of enzyme. The three compounds were between 23- and 375-fold selective vs 2-AG hydrolysis by MAGL as well as vs cannabinoid CB_1 and CB_2 receptors and AEA hydrolysis by FAAH in rat brain membranes. Three other THL analogues, i.e., **14**, **16**, and **18**, were slightly more potent than THL against DAGLα and appreciably selective vs MAGL, CB receptors, and FAAH (15−26-fold). Three other compounds, i.e. **9**, **12**, and **17**, were about as potent as THL against DAGLα but were not selective vs the other targets tested. Finally, one compound, i.e., **8**, was a potent inhibitor of MAGL-like activity (IC_{50} = 0.41 μM) and relatively (∼7-fold) selective vs the other targets tested. The amino acidic side chain is essentially for DAGLα inhibitory activity, as demonstrated by the lack of activity of the hydroxy lactone **22**. The *N*-formyl group is also important for activity. Its removal or replacement with the more bulky Boc or Cbz

Table 1. Structures and Results of DAGL, MAGL, CB_1, CB_2, and FAAH Assays of THL (**1**) and THL Analogues (**2**−**22**)[a]



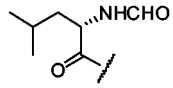
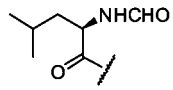
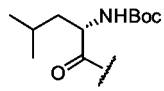
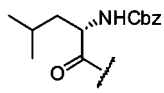
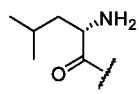
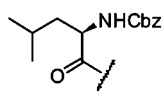
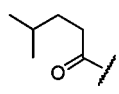
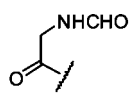
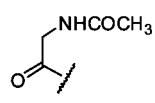
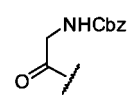
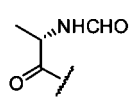
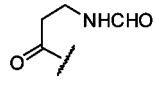
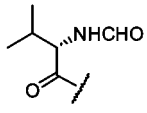
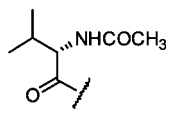
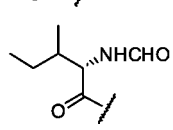
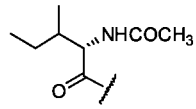
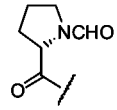
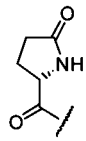
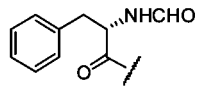
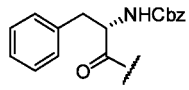
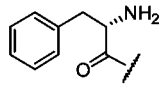
Compd	R	DAGLα (IC_{50}, μM)	MAGL (IC_{50}, μM)	CB_1 (K_i, μM)	CB_2 (K_i, μM)	FAAH (IC_{50}, μM)
THL (**1**)		1	> 10	2.5	> 10	> 50
2		5	> 10	n.t.	n.t.	> 50
3		> 25	> 10	n.t.	n.t.	> 50
4		> 25	> 10	n.t.	n.t.	> 50
5		> 25	> 10	n.t.	n.t.	> 50
6		> 25	> 10	n.t.	n.t.	> 50
7		> 25	>10	n.t.	n.t.	10.4
8		2.8	0.41	> 10	> 10	3.0
9		0.8	1.7	2.3	> 10	> 50
10		> 25	> 10	n.t.	n.t.	> 50
11		0.043	1	3.1	> 10	> 50
12		0.6	0.4	1.9	6.4	> 50
13		0.035	> 10	5.7	> 10	> 50
14		0.46	>10	8.4	> 10	> 50
15		0.016	> 10	6.0	> 10	> 50

Table 1. Continued

Compd	R	DAGLα (IC_{50}, μM)	MAGL (IC_{50}, μM)	CB_1 (K_i, μM)	CB_2 (K_i, μM)	FAAH (IC_{50}, μM)
16		0.43	> 10	8.6	> 10	6.6
17		1.1	> 10	1.7	7.4	3.4
18		0.1	3.5	2.6	> 10	> 50
19		25	> 10	n.t.	n.t.	> 50
20		> 25	> 10	n.t.	n.t.	> 50
21		~25	> 10	n.t.	n.t.	> 50
22	H	25	> 10	n.t.	n.t.	> 50

[a] Data are means of $n = 4$ separate determinations. Standard errors are not shown for the sake of clarity and were never higher than 10% of the means. Abbreviations: n.t., not tested.

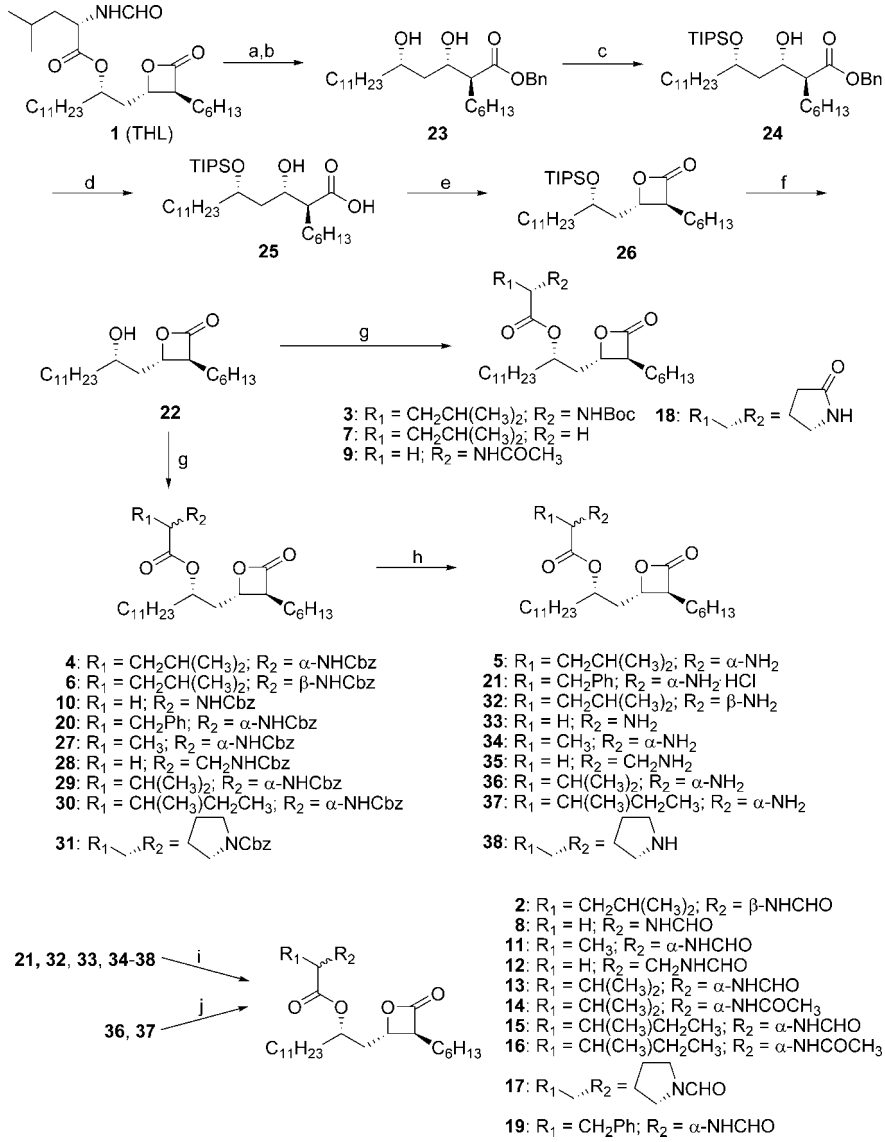
groups leads to inactivation (compare compounds **1**, **3**, **4**, **5**, and **8** with **10**). However, replacement with a $COCH_3$ group was better tolerated. Thus, even though a 13- and a 27-fold loss of potency was observed with the *N*-acetyl derivatives **14** and **16** as compared to their *N*-formyl counterparts **13** and **15**, compounds **14** and **16** are still more potent and selective DAGLα inhibitors than THL. Interestingly, substitution of CHO with $COCH_3$ in compound **8** (compound **9**) resulted in a 3.5-fold increase in the activity on DAGLα and, conversely, in a ~4-fold decrease of the inhibitory potency on MAGL. For optimal activity on DAGLα, the presence of aliphatic amino acidic residues, in particular with C-β branched chains, appears to be necessary (compare compounds **1**, **8**, **11**−**13**, and **15**). Especially noteworthy in this respect is the ~60-fold difference in the inhibitory potency between THL and compound **15** due to the different position of a methyl group. On increasing the distance between *N*-formylamino and ester groups, activity is increased ~5-fold (compare compounds **8** and **12**). Rigidification of the aliphatic side chain (compare compounds **11** and **17**) is acceptable without dramatic loss of activity. The stereochemistry at C-α has a notable effect on the activity; indeed, THL turns out to be 5-fold more active on DAGLα than its epimer **2**. All compounds exhibiting activity on DAGLα show weak or no affinity for cannabinoid receptors. Finally, none of our compounds, with the exception of **7**, **8**, **16**, and **17**, inhibits significantly FAAH up to concentrations of 50 μM.

The possibility that the newly developed compounds described here, like THL,[22] poorly penetrate into the systemic circulation when orally administered, thus limiting their use as pharmacological tools, deserves some discussion. Previously, THL has been used to inhibit DAGLα in mostly in vitro preparations,[6,10,13] and its effects on the activity of the enzyme and 2-AG tissue levels, after systemic administration, and in particular per os, have not yet been investigated. Intriguingly, however, THL was previously found to permeate the plasma membrane as it was capable of inhibiting intracellular DAGLα activity and, subsequently, the calcium-induced de novo biosynthesis of 2-AG, in intact mouse N18TG2 neuroblastoma cells.[10] We found here that, in similar experiments, compound **15**, at the concentration of 0.3 μM, behaved in exactly the same way by inhibiting the ionomycin-induced stimulation of 2-AG biosynthesis in intact N18TG2 cells. The compound exerted a 57.3 ± 5.8% inhibition of 2-AG levels, which was comparable to the 79.8 ± 6.9% inhibition observed with THL (1 μM) (means ± SEM, $N = 3$). By contrast, compound **8** (1.5 μM) enhanced 2-AG levels by 55.0 ± 4.9%, in agreement with its actions as rather selective MAGL inhibitor. Although these experiments suggest that these compounds are plasma membrane permeable, further specific studies are now required to investigate their efficacy in vivo after systemic (and particularly oral) administration and their penetration into the systemic circulation.

Conclusions

A series of THL analogues was prepared and evaluated for their inhibitory potency on metabolic enzymes of 2-AG and their affinity for cannabinoid receptors. The influence of the nature of the aminoacidic moiety and of the *N*-substitution on the activity have been explored and led to the identification of three nanomolar DAGLα inhibitors that substantially exceeded the potency and selectivity of THL (compounds **11**, **13**, and **15**) and of a potent and relatively selective inhibitor of 2-AG hydrolysis by MAGL (compound **8**). These compounds, and

Scheme 1[a]



[a] Reagents and conditions: (a) KOH, MeOH, room temp, 1 h; (b) $PhCH_2Br$, DMF, room temp, 16 h; (c) TIPSOTf, *sym*-collidine, CH_2Cl_2, −78 °C, 3 h; (d) H_2, Pd/C, AcOEt, room temp, 2.5 h; (e) $PhNTf_2$, Et_3N, CH_2Cl_2, room temp, 16 h; (f) *n*-Bu_4NF, THF, AcOH, −20 °C, 16 h; (g) $R_1R_2CHCO_2H$, DCC, CH_2Cl_2, 0 °C, 15 min, then **20**, DMAP, DMF, room temp, 16 h; (h) H_2, Pd/C, THF, room temp, 2.5 h; (i) AcOCHO, Et_3N, CH_2Cl_2, room temp, 15 min; (j) AcCl, Et_3N, CH_2Cl_2, room temp, 1 h.

particularly **8**, **13**, and **15**, qualify as useful pharmacological tools for the selective modulation of the metabolism of 2-AG with respect to that of AEA and, given the pharmaceutical success of THL, might serve as templates for the development of new therapeutic drugs. On the other hand, their pharmacokinetics still need to be assessed in order to gain a complete picture of their potential usefulness in the clinic.

Experimental Section

Chemistry. All chemical reagents were commercially available unless otherwise indicated. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Optical rotations were measured at 20 °C with a Schmidt-Haensch Polartronic D polarimeter (1 dm cell) in 1% $CHCl_3$ solutions unless otherwise indicated. IR spectra were recorded on a Perkin-Elmer 1000 FT-IR spectrophotometer as $CHCl_3$ solutions unless otherwise indicated. ^1H and ^{13}C NMR spectra were obtained on a Bruker Avance 400 or a Varian Gemini 200 spectrometer using $CDCl_3$ as solvent unless otherwise indicated and with TMS as internal standard. Satisfactory elemental analyses were obtained for the newly synthesized compounds (C, H, N ±0.4%).

Compounds **2**,[21] **3**,[18] **4**,[16h,i,n,p,20] **5**,[16i,n,p,20] **8**,[21] **12**,[21a] **13**,[21b] **18**,[19] and **22**[16i,p,q,r] have been described in the literature.

(2*S*,3*S*,5*S*)-3,5-Dihydroxy-2-hexylhexadecanoic Acid Phenylmethyl Ester (23). A solution of THL (**1**, 6.49 g, 13.1 mmol) in MeOH (175 mL) was treated with aqueous 1N KOH (28.9 mL, 28.9 mmol) and kept at room temperature for 1 h. The reaction mixture was evaporated under vacuum, and the residue redissolved in toluene and evaporated under vacuum three times. The residue (6.07 g) was dissolved in dry DMF (111 mL) and treated with benzyl bromide (3.43 mL, 28.9 mmol). After 24 h, the reaction mixture was diluted with water and extracted with AcOEt. The organic phase was washed three times with water, dried (Na_2SO_4), and evaporated under vacuum. The residue (9.02 g) was chromatographed on silica gel with hexane/AcOEt = 85/15 as eluent to give 3.03 g (50%) of **23** as an oil; $[\alpha]_D$ −1°. IR 3485, 3007, 2928, 2856, 1718, 1456, 1165 cm^{-1}. ^1H NMR (200 MHz) δ 0.87 (6H, m), 1.24−1.64 (32H, m), 2.49 (1H, dt, J = 5.4, 9.2 Hz), 3.30 (1H, br s), 3.64 (1H, m), 3.81 (1H, m), 3.97 (1H, m), 5.17 (2H, s), 7.36 (5H, m). ^{13}C NMR (50 MHz) δ 14.02, 14.10, 22.53, 22.70, 25.35, 27.26, 29.01, 29.15, 29.37, 29.64, 29.69, 31.59, 31.94, 38.00, 40.97, 51.78, 66.41, 72.52, 73.48, 126.98, 127.61, 128.35, 128.58, 135.80, 175.08. Anal. ($C_{29}H_{50}O_4$), C, H.

(2*S*,3*S*,5*S*)-2-Hexyl-5-hydroxy-3-[(triisopropylsilyl)oxy]hexadecanoic Acid Phenylmethyl Ester (24). A stirred solution of **23** (5.00 g, 10.8 mmol) in dry CH_2Cl_2 (117 mL) was treated at −78 °C and under N_2 with *sym*-collidine (3.55 mL, 27.0 mmol) and triisopropylsilyl trifluoromethanesulfonate (4.37 mL, 16.2 mmol). The mixture was stirred at a −78 °C for 3 h under N_2 and then diluted with water and extracted with CH_2Cl_2. The organic phase was washed with 2 N HCl solution and brine until neutral, dried (Na_2SO_4), and evaporated under vacuum. The residue (7.80 g) was chromatographed on silica gel with hexane/AcOEt = 95/5 as eluent to give 5.71 g (85%) of **24** as an oil; $[\alpha]_D$ +5°. IR 3494, 3025, 2928, 2858, 1727, 1465, 1265, 1164, 883 cm^{-1}. ^1H NMR (200 MHz) δ 0.88 (6H, m), 1.07 (18H, m), 1.26−1.73 (35H, m), 2.49 (1H, dt, J = 5.2, 9.4 Hz), 3.21 (1H, d, J = 5.2 Hz), 3.89 (1H, m), 4.04 (1H, m), 5.14 (1H, d, J = 12.2 Hz), 5.17 (1H, d, J = 12.2 Hz), 7.34 (5H, m). ^{13}C NMR (50 MHz) δ 12.91, 14.03, 14.10, 18.18, 18.23, 22.55, 22.70, 24.68, 27.48, 28.93, 29.19, 29.37, 29.63, 29.66, 29.92, 31.64, 31.95, 37.34, 41.33, 51.89, 66.17, 70.89, 72.02, 128.19, 128.27, 128.51, 136.04, 175.04. Anal. ($C_{38}H_{70}O_4Si$), C, H.

(3*S*,4*S*)-3-Hexyl-4-[(2*S*)-2-[(triisopropylsilyl)oxy]tridecyl]oxetan-2-one (26). A stirred solution of **24** (3.03 g, 4.84 mmol) in AcOEt (25 mL) was hydrogenated in the presence of 10% Pd/C (518 mg) at room temperature and atmospheric pressure for 2.5 h. The suspension was filtered through a short pad of celite, and the filtrate was evaporated under vacuum to give the hydroxy acid **25**, which was used in the next step without further purification. A stirred solution of **25** (2.56 g, 4.84 mmol) and TEA (1.35 mL, 9.68 mmol) in dry CH_2Cl_2 (43 mL), was treated with *N*-phenyl-bis(trifluoromethanesulfonimide) (2.59 g, 7.26 mmol) at 0 °C, and the mixture was stirred at room temperature overnight. The reaction mixture was evaporated under vacuum, and the residue (6.00 g) was chromatographed on silica gel with hexane/CH_2Cl_2 = 85/15 as eluent to give 1.91 g (77%) of **26** as an oil; $[\alpha]_D$ −19°. IR 3027, 2928, 2858, 1814, 1465, 1379, 1175, 882 cm^{-1}. ^1H NMR (200 MHz) δ 0.88 (6H, m), 1.07 (18H, m), 1.20−2.01 (35H, m), 3.28 (1H, m), 3.95 (1H, m), 4.51 (1H, dt, J = 4.6, 7.6 Hz). ^{13}C NMR (50 MHz) δ 14.01, 14.11, 18.17, 22.52, 22.70, 25.16, 26.73, 27.77, 29.04, 29.28, 29.36, 29.59, 29.65, 29.77, 31.52, 31.94, 36.30, 40.69, 56.85, 69.65, 74.72, 171.84. Anal. ($C_{31}H_{62}O_3Si$), C, H.

(3*S*,4*S*)-3-Hexyl-4-[(2*S*)-2-hydroxytridecyl]oxetan-2-one (22).[16i,p,q,r] A stirred solution of **26** (678 mg, 1.3 mmol) in THF (7.2 mL) was treated with tetrabutylammonium fluoride solution (1 M in THF) (3.9 mL, 3.9 mmol) and acetic acid (0.076 mL, 1.3 mmol) at −20 °C. The mixture was stirred at −20 °C overnight and then diluted with water and extracted with AcOEt. The organic phase was washed with brine until neutral, dried (Na_2SO_4), and evaporated under vacuum. The residue (960 mg) was chromatographed on silica gel with hexane/AcOEt = 8/2 as eluent to give 428 mg (91%) of **22**;[16i,p,q,r] mp 62−65 °C; $[\alpha]_D$ −15°. IR (KBr disks) 3553, 2956, 2924, 2850, 1813, 1470, 1136, 1091, 837 cm^{-1}. ^1H NMR (200 MHz) δ 0.89 (6H, m), 1.29−2.02 (33H, m), 3.32 (1H, ddd, J = 4.0, 6.8, 8.4 Hz), 3.76 (1H, m), 4.47 (1H, dt, J = 4.1, 6.6 Hz). ^{13}C NMR (50 MHz) δ 14.04, 14.13, 17.71, 22.55, 22.70, 25.48, 26.82, 27.84, 28.97, 29.36, 29.54, 29.59, 29.64, 29.66, 31.51, 31.93, 37.68, 41.18, 56.77, 69.27, 76.26, 171,41.

***N*-[(1,1-Dimethylethoxy)carbonyl]-L-leucine-(1*S*)-1-[[(2*S*,3*S*)-3-hexyl-4-*oxo*-2-oxetanyl]methyl]dodecyl Ester (3).**[18] To a stirred solution of Boc-Leu-OH (462 mg, 2.0 mmol) in dry CH_2Cl_2 (2 mL) was added DCC (413 mg, 2.0 mmol) at 0 °C, and the mixture was stirred at 0 °C for 15 min. The solvent was evaporated under vacuum, and the residue was dissolved in DMF (2 mL) and added to a solution of **22** (355 mg, 1.0 mmol) and DMAP (15 mg, 0.12 mmol) in DMF (1 mL). The mixture was stirred at room temperature overnight and then diluted with water and extracted with AcOEt. The organic phase was washed with brine until neutral, dried (Na_2SO_4), and evaporated under vacuum. The residue (2.031 g) was chromatographed on silica gel with hexane/AcOEt = 9/1 as eluent to give 503 mg of **3**[18] (89%) as an oil; $[\alpha]_D$ −27°. IR 3444, 3029, 2959, 2929, 2856, 1819, 1711, 1500, 1368, 1162, 1124 cm^{-1}. ^1H NMR (400 MHz) δ 0.88 (6H, m), 0.96 (6H, d, J = 6.5 Hz), 1.25−1.31 (27H, m), 1.44 (9H, s), 1.55−1.8 (6H, m), 1.96 (1H, m), 2.19 (1H, m), 3.21 (1H, dt, J = 7.6, 4.0 Hz), 4.24 (1H, m), 4.31 (1H, m), 4.84 (1H, d, J = 8.4 Hz), 5.00 (1H, m). ^{13}C NMR (100 MHz) δ 14.02, 14.12, 21.77, 22.53, 22.69, 22.93, 24.86, 25.13, 26.67, 27.68, 28.32, 28.99, 29.35, 29.47, 29.56, 29.62, 29.64, 31.50, 31.92, 34.16, 38.76, 41.54, 52.44, 57.00, 71.94, 74.50, 79.87, 155.41, 170.93, 172.95.

4-Methylpentanoic Acid (1*S*)-1-[[(2*S*,3*S*-3-Hexyl-4-*oxo*-2-oxetanyl]methyl]dodecyl Ester (7). The title compound was prepared from 4-methylpentanoic acid following the same procedure that was used for the synthesis of **3** using hexane/AcOEt = 95/5 as eluent for the chromatographic purification; yield 68%; oil; $[\alpha]_D$ −17 °. IR 3034, 2958, 2928, 2856, 1818, 1726, 1467, 1181, 1124 cm^{-1}. ^1H NMR (400 MHz) δ 0.88 (6H, m), 0.91 (6H, d, J = 6.3 Hz), 1.26−1.42 (27H, m), 1.49−1.81 (6H, m), 1.96 (1H, ddd, J = 3.6, 6.0, 12.4 Hz), 2.16 (1H, m), 2.30 (2H, t, J = 7.6 Hz), 3.21 (1H, dt, J = 7.6, 7.2 Hz), 4.32 (1H, m), 4.99 (1H, m). ^{13}C NMR (100 MHz) δ 14.02, 14.12, 22.21, 22.41, 22.53, 22.70, 25.26, 26.72, 27.68, 27.70, 29.00, 29.33, 29.36, 29.48, 29.54, 29.64, 31.51, 31.93, 32.59, 33.80, 38.82, 56.94, 70.75, 74.87, 171.10, 173.60. Anal. ($C_{28}H_{52}O_4$), C, H.

***N*-Acetyl-glycine-(1*S*)-1-[[(2*S*,3*S*)-3-hexyl-4-*oxo*-2-oxetanyl]methyl]dodecyl Ester (9).** The title compound was prepared from *N*-Acetyl-Gly-OH following the same procedure that was used for the synthesis of **3** using hexane/AcOEt = 75/25 as eluent for the chromatographic purification; yield 39%; mp 55−56°; $[\alpha]_D$ −23°. IR 3438, 3036, 2957, 2929, 2856, 1820, 1740, 1676, 1517, 1466, 1382, 1223, 1197 cm^{-1}. ^1H NMR (400 MHz) δ 0.87 (6H, m), 1.26−1.43 (26H, m), 1.59−1.80 (4H, m), 1.99 (1H, m), 2.04 (3H, s), 2.13 (1H, m), 3.21 (1H, m), 3.97 (1H, dd, J = 4.9, 18.0 Hz), 4.03 (1H, dd, J = 5.3, 15.4 Hz), 4.33 (1H, m), 5.09 (1H, m), 6.19 (1H, br s). ^{13}C NMR (100 MHz) δ 14.02, 14.12, 22.53, 22.70, 22.89, 25.20, 26.76, 27.65, 28.97, 29.32, 29.36, 29.45, 29.56, 29.63, 31.49, 31.93, 34.11, 38.87, 41.66, 57.07, 72.74, 75.06, 169.83, 170.28, 170.90. Anal. ($C_{26}H_{47}NO_5$), C, H, N.

5-*oxo*-L-Proline-(1*S*)-1-[[(2*S*,3*S*)-3-hexyl-4-*oxo*-2-oxetanyl]methyl]dodecyl Ester (18).[19] The title compound was prepared from L-pyroglutamic acid following the same procedure that was used for the synthesis of **3** using hexane/AcOEt = 6/4 as eluent for the chromatographic purification; yield 42%; mp 41−43 °C; $[\alpha]_D$ −19°. IR 3437, 3034, 2960, 2929, 2856, 1821, 1737, 1706, 1460, 1413, 1392, 1236, 1124 cm^{-1}. ^1H NMR (400 MHz) δ 0.88 (6H, m), 1.25−1.42 (26H, m), 1.59−1.80 (4H, m), 2.01 (1H, m), 2.12 (1H, m), 2.29−2.47 (4H, m), 3.20 (1H, m), 4.23 (1H, m), 4.32 (1H, m), 5.13 (1H, m), 6.48 (1H, s). ^{13}C NMR (100 MHz) δ 14.01, 14.11, 22.51, 22.69, 24.77, 25.22, 26.79, 27.60, 28.94, 29.21, 29.29, 29.34, 29.46, 29.55, 29.62, 31.48, 31.92, 34.36, 39.20, 55.61, 57.16, 73.07, 75.45, 170.68, 171.85, 177.99.

***N*-[(Phenylmethoxy)carbonyl]-L-leucine-(1*S*)-1-[[(2*S*,3*S*)-3-hexyl-4-*oxo*-2-oxetanyl]methyl]dodecyl Ester (4).**[16h,i,n,p,20] The title compound was prepared from Cbz-Leu-OH following the same procedure that was used for the synthesis of **3** using hexane/AcOEt = 9/1 as eluent for the chromatographic purification; yield 81%; mp 45−47 °C; $[\alpha]_D$ −24°. IR (KBr disks) 3335, 2959, 2918, 2850, 1842, 1731, 1693, 1536, 1285, 1193 cm^{-1}. ^1H NMR (400 MHz) δ 0.88 (6H, m), 0.93 (6H, m), 1.27−1.44 (27H, m), 1.48−1.74 (6H, m), 1.97 (1H, m), 2.16 (1H, m), 3.21 (1H, m), 4.29 (1H, m), 4.36 (1H, m), 5.01 (1H, m), 5.12 (3H, m), 7.34 (5H, m). ^{13}C NMR (100 MHz) δ 14.03, 14.13, 21.70, 22.53, 22.69, 22.94, 24.83, 25.13, 26.69, 27.65, 28.99, 29.31, 29.35, 29.45, 29.55, 29.63, 31.49, 31.92, 34.06, 38.68, 41.60, 52.81, 57.00, 67.02, 72.34, 74.57, 128.08, 128.20, 128.55, 136.26, 155.97, 170.84, 172.55.

***N*-[(Phenylmethoxy)carbonyl]-D-Leucine-(1*S*)-1-[[(2*S*,3*S*)-3-hexyl-4-*oxo*-2-oxetanyl]methyl]dodecyl Ester (6).** The title compound was prepared from Cbz-D-Leu-OH following the same procedure that was used for the synthesis of **3** using hexane/AcOEt = 8/2 as eluent for the chromatographic purification; yield 80%; mp 30−32 °C; $[\alpha]_D$ −6°. IR 3438, 3033, 2959, 2928, 2856, 1819, 1721, 1509, 1456, 1263 cm^{-1}. ^1H NMR (400 MHz) δ 0.88 (6H, m), 0.95 (6H, d, J = 6.4 Hz), 1.27−1.53 (27H, m), 1.54−1.62 (3H, m), 1.68−1.76 (3H, m), 2.01 (1H, m), 2.18 (1H, m), 3.22 (1H, m), 4.33 (2H, m), 5.00 (1H, m), 5.11 (3H, m), 7.33 (5H, m).

^{13}C NMR (100 MHz) δ 14.03, 14.12, 21.85, 22.55, 22.69, 22.86, 24.83, 25.22, 26.68, 27.66, 29.02, 29.28, 29.35, 29.47, 29.53, 29.64, 31.50, 31.92, 33.86, 38.54, 41.49, 52.82, 56.99, 67.01, 72.19, 74.44, 128.07, 128.21, 128.55, 136.24, 155.96, 171.09, 172.87. Anal. ($C_{36}H_{59}NO_6$), C, H, N.

***N*-[(Phenylmethoxy)carbonyl]-glycine-(1*S*)-1-[[(2*S*,3*S*)-3-hexyl-4-*oxo*-2-oxetanyl]methyl]dodecyl Ester (10).** The title compound was prepared from Cbz-Gly-OH following the same procedure that was used for the synthesis of **3** using hexane/AcOEt = 9/1 as eluent for the chromatographic purification; yield 65%; mp 32−35 °C; $[\alpha]_D$ −22°. IR 3448, 2957, 2928, 2856, 1820, 1723, 1517, 1466, 1223, 1197 cm^{-1}. ^{1}H NMR (400 MHz) δ 0.88 (6H, m), 1,28−1.42 (26H, m), 1.58−1.77 (4H, m), 1.99 (1H, m), 2.13 (1H, m), 3.19 (1H, m), 3.96 (2H, m), 4.32 (1H, m), 5.09 (1H, m), 5.13 (2H, s), 5.27 (1H, m), 7.32 (5H, m). ^{13}C NMR (100 MHz) δ 14.02, 14.12, 22.52, 22.69, 25.19, 26.73, 27.61, 28.95, 29.30, 29.34, 29.44, 29.54, 29.61, 31.47, 31.91, 34.08, 38.83, 42.96, 57.03, 67.11, 72.67, 74.90, 128.10, 128.22, 128.55, 136.23, 156.25, 169.76, 170.92. Anal. ($C_{32}H_{51}NO_6$), C, H, N.

***N*-[(Phenylmethoxy)carbonyl]-L-phenylalanine-(1*S*)-1-[[(2*S*,3*S*)-3-hexyl-4-*oxo*-2-oxetanyl]methyl]dodecyl Ester (20).** The title compound was prepared from Cbz-Phe-OH following the same procedure that was used for the synthesis of **3** using hexane/AcOEt = 9/1 as eluent for the chromatographic purification. Yield 80%; mp 64−66 °C; $[\alpha]_D$ −10°. IR (KBr disks) 3338, 2954, 2922, 2852, 1842, 1723, 1688, 1534, 1290, 1265, 1180 cm^{-1}. ^{1}H NMR (400 MHz) δ 0.88 (6H, m), 1.26−1.72 (30H, m), 1.85 (1H, m), 2.05 (1H, m), 3.10 (3H, m), 4.14 (1H, m), 4.60 (1H, m), 4.95 (1H, m), 5.08 (2H, s), 5.19 (1H, d, J = 8.0 Hz), 7.15 (2H, d, J = 6.8 Hz), 7.24−7.33 (8H, m). ^{13}C NMR (100 MHz) δ 14.03, 14.12, 22.53, 22.69, 25.05, 26.67, 27.61, 28.98, 29.32, 29.36, 29.43, 29.56, 29.63, 31.48, 31.92, 33.92, 38.14, 38.54, 55.09, 56.98, 72.65, 74.40, 127.24, 128.10, 128.23, 128.55, 128.69, 129.27, 130.38, 135.78, 155.65, 170.05, 171.27. Anal. ($C_{39}H_{57}NO_6$), C, H, N.

***N*-[(Phenylmethoxy)carbonyl]-L-alanine-(1*S*)-1-[[(2*S*,3*S*)-3-hexyl-4-*oxo*-2-oxetanyl]methyl]dodecyl Ester (27).** The title compound was prepared from Cbz-Ala-OH following the same procedure that was used for the synthesis of **3** using CH_2Cl_2/AcOEt = 95/5 as eluent for the chromatographic purification; yield 78%; mp 36−38 °C; $[\alpha]_D$ −23°. IR 3436, 2957, 2929, 2856, 1820, 1720, 1508, 1455, 1337, 1240, 1197, 1062 cm^{-1}. ^{1}H NMR (400 MHz) δ 0.87 (6H, m), 1.25 (26H, m), 1.42 (3H, d, J = 6.8 Hz), 1.58−1.71 (4H, m), 1.97 (1H, m), 2.13 (1H, m), 3.19 (1H, m), 4.29 (1H, m), 4.35 (1H, m), 5.03 (1H, m), 5.11 (2H, s), 5.30 (1H, m), 7.34 (5H, m). ^{13}C NMR (100 MHz) δ 14.00, 14.11, 18.49, 22.51, 22.69, 25.13, 26.70, 27.64, 28.95, 29.31, 29.35, 29.44, 29.55, 29.62, 31.48, 31.92, 34.19, 38.83, 49.88, 57.03, 66.93, 72.59, 74.78, 128.09, 128.19, 128.55, 136.30, 155.61, 170.81, 172.55. Anal. ($C_{33}H_{53}NO_6$), C, H, N.

***N*-[(Phenylmethoxy)carbonyl]-β-alanine-(1*S*)-1-[[(2*S*,3*S*)-3-hexyl-4-*oxo*-2-oxetanyl]methyl]dodecyl Ester (28).** The title compound was prepared from Cbz-β-Ala-OH following the same procedure that was used for the synthesis of **3** using hexane/AcOEt = 85/15 as eluent for the chromatographic purification; yield 81%; oil; $[\alpha]_D$ −22°. IR 3452, 3029, 2929, 2856, 1820, 1723, 1513, 1466, 1280, 1236, 1189, 1131 cm^{-1}. ^{1}H NMR (400 MHz) δ 0.88 (6H, m), 1.25−1.41 (26H, m), 1.57−1.77 (4H, m), 1.96 (1H, m), 2.10 (1H, m), 2.54 (2H, m), 3.18 (1H, m), 3.47 (2H, m), 4.30 (1H, m), 5.05 (1H, m), 5.09 (2H, s), 5.29 (1H, br s), 7.34 (5H, m). ^{13}C NMR (100 MHz) δ 14.01, 14.11, 22.51, 22.69, 25.26, 26.74, 27.62, 28.93, 29.34, 29.46, 29.55, 29.62, 31.47, 31.91, 34.22, 34.71, 36.63, 38.95, 57.04, 66.72, 71.70, 75.17, 128.07, 128.11, 128.51, 136.53, 156.30, 170.83, 171.97. Anal. ($C_{33}H_{53}NO_6$), C, H, N.

***N*-[(Phenylmethoxy)carbonyl]-L-valine-(1*S*)-1-[[(2*S*,3*S*)-3-hexyl-4-*oxo*-2-oxetanyl]methyl]dodecyl Ester (29).** The title compound was prepared from Cbz-Val-OH following the same procedure that was used for the synthesis of **3** using hexane/AcOEt = 9/1 as eluent for the chromatographic purification; yield 65%; mp 58−61 °C; $[\alpha]_D$ −18°. IR 3437, 2959, 2929, 2856, 1820, 1723, 1509, 1466, 1346, 1236, 1125 cm^{-1}. ^{1}H NMR (400 MHz) δ 0.88 (9H, m), 0.99 (3H, d, J = 6.7 Hz), 1.26−1.42 (26H, m), 1.59−1.75 (4H, m), 1.97 (1H, m), 2.17 (2H, m), 3.20 (1H, m), 4.28 (2H, m), 5.00 (1H, m), 5.11 (2H, s), 5.32 (1H, d, J = 9.4 Hz), 7.31 (5H, m). ^{13}C NMR (100 MHz) δ 14.02, 14.13, 17.33, 19.28, 22.52, 22.70, 25.16, 26.69, 27.66, 28.97, 29.32, 29.36, 29.45, 29.55, 29.63, 30.95, 31.49, 31.92, 33.95, 38.58, 57.03, 59.32, 67.04, 72.44, 74.46, 128.11, 128.19, 128.55, 136.30, 156.30, 170.89, 171.53. Anal. ($C_{35}H_{57}NO_6$), C, H, N.

***N*-[(Phenylmethoxy)carbonyl]-L-isoleucine-(1*S*)-1-[[(2*S*,3*S*)-3-hexyl-4-*oxo*-2-oxetanyl]methyl]dodecyl Ester (30).** The title compound was prepared from Cbz-Ile-OH following the same procedure that was used for the synthesis of **3** using hexane/AcOEt = 9/1 as eluent for the chromatographic purification; yield 68%; mp 42−44 °C; $[\alpha]_D$ −16°; IR 3437, 3038, 2960, 2929, 2856, 1820, 1722, 1509, 1465, 1335, 1226, 1213, 1197 cm^{-1}. ^{1}H NMR (200 MHz) δ 0.85−0.97 (12H, m), 1.26 (28H, m), 1.61−1.78 (4H, m), 1.98 (2H, m), 2.15 (1H, m), 3.21 (1H, dt, J = 7.6, 4.0 Hz), 4.29 (2H, m), 5.00 (1H, m), 5.11 (2H, s), 5.30 (1H, d, J = 9.0 Hz), 7.32 (5H, m). ^{13}C NMR (50 MHz) δ 11.56, 13.99, 14.09, 15.67, 22.52, 22.69, 24.90, 25.16, 26.71, 27.72, 28.97, 29.34, 29.45, 29.55, 29.63, 31.49, 31.92, 34.00, 37.71, 38.66, 57.13, 58.88, 67.06, 72.46, 74.47, 128.08, 128.19, 128.55, 136.36, 156.14, 170.67, 171.41. Anal. ($C_{36}H_{59}NO_6$), C, H, N.

***N*-[(Phenylmethoxy)carbonyl]-L-proline-(1*S*)-1-[[(2*S*,3*S*)-3-hexyl-4-*oxo*-2-oxetanyl]methyl]dodecyl Ester (31).** The title compound was prepared from Cbz-Pro-OH following the same procedure that was used for the synthesis of **3** using hexane/AcOEt = 85/15 as eluent for the chromatographic purification; yield 96%; oil; $[\alpha]_D$ −48°. IR 2957, 2928, 2856, 1819, 1740, 1698, 1467, 1455, 1420, 1356, 1229, 1123 cm^{-1}. ^{1}H NMR (400 MHz) δ 0.87 (6H, m), 1.19−1.57 (26H, m), 1.67−1.75 (4H, m), 1.83−2.23 (6H, m), 3.13 and 3.18 (1H, 2m), 3.48−3.60 (2H, m), 4.19 and 4.36 (1H, 2m), 4.36 (1H, m), 4.95 and 5.02 (1H, 2m), 5.06 and 5.15 (2H, 2m), 7.30−7.36 (5H, m). ^{13}C NMR (100 MHz) δ 14.02, 14.11, 22.53, 22.69, 24.38, 25.17, 26.66, 27.58, 27.64, 29.00, 29.35, 29.47, 29.56, 29.63, 29.88, 30.84, 31.49, 31.92, 34.19, 34.34, 38.86, 46.49, 47.01, 56.96, 58.97, 59.58, 66.97, 72.00, 74.84, 127.70, 127.79, 127.98, 128.44, 128.50, 136.59, 136.76, 154.85, 170.83, 171.18, 172.23, 172.29. Anal. ($C_{35}H_{55}NO_6$), C, H, N.

L-Leucine-(1*S*)-1-[[(2*S*,3*S*)-3-hexyl-4-*oxo*-2-oxetanyl]methyl]dodecyl Ester (5).[16i,n,p,20] A stirred solution of **4** (105 mg, 0.25 mmol) in THF (6 mL) was hydrogenated in the presence of 10% Pd/C (47 mg) at room temperature and atmospheric pressure for 2.5 h. The suspension was filtered through a short pad of celite and the filtrate was evaporated under vacuum. The residue was chromatographed on silica gel with hexane/AcOEt = 7/3 as eluent to give 56 mg of **5**[16i,n,p,20] (48%); oil; $[\alpha]_D$ −16° ($CHCl_3$, c = 0.5). IR 3503, 3433, 2958, 2928, 2856, 1820, 1728, 1602, 1467, 1126 cm^{-1}. ^{1}H NMR (400 MHz) δ 0.88 (6H, m), 0.95 (6H, t, J = 7.6 Hz), 1.26−1.78 (35H, m), 1.98 (1H, m), 2.17 (1H, m), 3.21 (1H, m), 3.46 (1H, m), 4.30 (1H, m), 5.02 (1H, m). ^{13}C NMR (100 MHz) δ 14.02, 14.12, 21.74, 22.53, 22.70, 23.06, 24.82, 25.25, 26.72, 27.57, 27.69, 29.00, 29.35, 29.48, 29.55, 29.63, 31.50, 31.93, 34.14, 38.86, 44.01, 53.11, 57.01, 71.62, 74.81, 170.88, 175.34. Anal. ($C_{28}H_{53}NO_4$), C, H, N.

L-Phenylalanine-(1*S*)-1-[[(2*S*,3*S*)-3-hexyl-4-*oxo*-2-oxetanyl]methyl]dodecyl Ester Hydrochloride (21). The title compound was prepared from **20** following the same procedure that was used for the synthesis of **5** using hexane/AcOEt = 8/2 as eluent for the chromatographic purification. The amine was dissolved in anhydrous Et_2O and was treated with gaseous HCl to give a precipitate of the corresponding hydrochloride; yield 80%; mp 87−91 °C; $[\alpha]_D$ −18° (MeOH, c = 1). IR (KBr disks) 3314, 3161, 2956, 2922, 2853, 2622, 1819, 1727, 1597, 1523, 1469, 1376, 1278, 1125 cm^{-1}. ^{1}H NMR (400 MHz, CD_3OD) δ 0.96 (6H, m), 1.26−1.41 (26H, m), 1.67 (2H, m) 1.77 (2H, m), 2.08 (2H, m), 3.19 (1H, dd, J = 7.9, 14.2 Hz), 3.34 (2H, m), 4.37 (2H, m), 5.15 (1H, m), 7.34−7.41 (5H, m). ^{13}C NMR (100 MHz, CD_3OD) δ 14.41, 14.46, 23.63, 23.74, 26.03, 27.83, 28.66, 30.14, 30.47, 30.57, 30.75, 32.75, 33.08, 34.99, 37.46, 39.48, 55.18, 57.92, 75.98, 76.63, 128.93, 130.18, 130.48, 135.70, 169.87, 172.91. Anal. ($C_{31}H_{52}ClNO_4$), C, H, N.

D-Leucine-(1*S*)-1-[[(2*S*,3*S*)-3-hexyl-4-*oxo*-2-oxetanyl]methyl]dodecyl Ester (32). Prepared as above from **6** and used in the next step without further purification.

Glycine-(1*S*)-1-[[(2*S*,3*S*)-3-hexyl-4-*oxo*-2-oxetanyl]methyl]dodecyl Ester (33). Prepared as above from **10** and used in the next step without further purification.

L-Alanine-(1*S*)-1-[[(2*S*,3*S*)-3-hexyl-4-*oxo*-2-oxetanyl]methyl]dodecyl Ester (34). Prepared as above from **27** and used in the next step without further purification.

***β*-Alanine-(1*S*)-1-[[(2*S*,3*S*)-3-hexyl-4-*oxo*-2-oxetanyl]methyl]dodecyl Ester (35).** Prepared as above from **28** and used in the next step without further purification.

L-Valine-(1*S*)-1-[[(2*S*,3*S*)-3-hexyl-4-*oxo*-2-oxetanyl]methyl]dodecyl Ester (36). Prepared as above from **29** and used in the next step without further purification.

L-Isoleucine-(1*S*)-1-[[(2*S*,3*S*)-3-hexyl-4-*oxo*-2-oxetanyl]methyl]dodecyl Ester (37). Prepared as above from **30** and used in the next step without further purification.

L-Proline-(1*S*)-1-[[(2*S*,3*S*)-3-hexyl-4-*oxo*-2-oxetanyl]methyl]dodecyl Ester (38). Prepared as above from **31** and used in the next step without further purification.

***N*-Formyl-D-leucine-(1*S*)-1-[[(2*S*,3*S*)-3-hexyl-4-*oxo*-2-oxetanyl]methyl]dodecyl Ester (2).**[21] A solution of crude **32** (117 mg, 0.25 mmol) was stirred with formic acetic anhydride (0.24 mL, 3.2 mmol) in dry CH_2Cl_2 (0.74 mL) at room temperature for 15 min and then diluted with water and extracted with AcOEt. The organic phase was washed with saturated $NaHCO_3$ and brine until neutral, dried (Na_2SO_4), and evaporated under vacuum. The residue (115 mg) was chromatographed on silica gel with hexane/AcOEt = 7/3 as eluent to give 82 mg (66%) of **2**;[21] mp 40−43 °C; $[\alpha]_D$ −4°. IR (KBr disks) 3385, 2958, 2921, 2853, 1842, 1707, 1685, 1671, 1468, 1384, 1291, 1258 cm^{-1}. ^1H NMR (400 MHz) δ 0.89 (6H, m), 0.96 (6H, d, J = 6.0), 1.54−1.78 (33H, m), 2.00 (1H, m), 2.18 (1H, m), 3.23 (1H, m), 4.37 (1H, m), 4.65 (1H, m), 5.03 (1H, m), 6.19 (1H, d, J = 8.0), 8.20 (1H, s). ^{13}C NMR (100 MHz) δ 14.03, 14.12, 21.86, 22.53, 22.69, 22.82, 24.87, 25.21, 26.68, 27.64, 28.98, 29.26, 29.34, 29.46, 29.52, 29.63, 31.48, 31.91, 33.83, 38.53, 41.36, 49.69, 56.99, 72.50, 74.60, 160.77, 171.07, 172.27.

***N*-Formyl-glycine-(1*S*)-1-[[(2*S*,3*S*)-3-hexyl-4-*oxo*-2-oxetanyl]methyl]dodecyl Ester (8).**[21] The title compound was prepared from **33** following the same procedure that was used for the synthesis of **2** using hexane/AcOEt = 6/4 as eluent for the chromatographic purification; yield 21%; oil; $[\alpha]_D$ −24° ($CHCl_3$, c = 0.5). IR 3428, 2957, 2928, 2856, 1820, 1742, 1690, 1508, 1467, 1379, 1197 cm^{-1}. ^1H NMR (400 MHz) δ 0.88 (6H, m), 1.26−1.43 (26H, m), 1.60−1.82 (4H, m), 2.02 (1H, m), 2.13 (1H, m), 3.21 (1H, m), 4.03 (1H, dd, J = 5.5, 18.4 Hz), 4.13 (1H, dd, J = 5.8, 18.2 Hz), 4.34 (1H, m), 5.12 (1H, m), 6.24 (1H, br s), 8.26 (1H, s). ^{13}C NMR (100 MHz) δ 14.00, 14.11, 22.50, 22.67, 25.16, 26.73, 27.60, 28.93, 29.28, 29.33, 29.41, 29.52, 29.60, 31.45, 31.89, 34.07, 38.87, 40.11, 57.04, 73.03, 75.08, 161.05, 169.24, 170.86.

N-Formyl-L-alanine-(1*S*)-1-[[(2*S*,3*S*)-3-hexyl-4-*oxo*-2-oxetanyl]methyl]dodecyl Ester (11). The title compound was prepared from **34** following the same procedure that was used for the synthesis of **2** using CH_2Cl_2/AcOEt = 9/1 as eluent for the chromatographic purification; yield 21%; mp 31−32 °C; $[\alpha]_D$ −29°. IR 3423, 2957, 2929, 2856, 1821, 1735, 1686, 1505, 1456, 1380, 1197, 1123 cm^{-1}. ^1H NMR (400 MHz) δ 0.88 (6H, m), 1.25−1.35 (26H, m), 1.45 (3H, d, J = 7.2 Hz), 1.60−1.81 (4H, m), 2.00 (1H, m), 2.14 (1H, m), 3.22 (1H, m), 4.30 (1H, m), 4.65 (1H, m), 5.07 (1H, m), 6.22 (1H, br s), 8.19 (1H, s). ^{13}C NMR (100 MHz) δ 14.01, 14.11, 18.37, 22.52, 22.70, 25.13, 26.75, 27.65, 28.96, 29.31, 29.35, 29.44, 29.55, 29.63, 31.49, 31.93, 34.22, 38.93, 47.13, 57.12, 73.03, 74.98, 160.39, 170.72, 172.13. Anal. ($C_{26}H_{47}NO_5$), C, H, N.

***N*-Formyl-*β*-alanine-(1*S*)-1-[[(2*S*,3*S*)-3-hexyl-4-*oxo*-2-oxetanyl]methyl]dodecyl Ester (12).**[21a] The title compound was prepared from **35** following the same procedure that was used for the synthesis of **2** using hexane/AcOEt = 6/4 as eluent for the chromatographic purification; yield 73%; oil, $[\alpha]_D$ −26°. IR 3442, 2957, 2929, 2856, 1821, 1727, 1686, 1508, 1466, 1390, 1231, 1126 cm^{-1}. ^1H NMR (400 MHz) δ 0.87 (6H, m), 1.26−1.42 (26H, m), 1.59−1.81 (4H, m), 2.00 (1H, m), 2.11 (1H, m), 2.56 (2H, m), 3.21 (1H, m), 3.58 (2H, m), 4.36 (1H, m), 5.10 (1H, m), 6.31 (1H, br s), 8.15 (1H, s). ^{13}C NMR (100 MHz) δ 14.02, 14.13, 22.52, 22.69, 25.24, 26.78, 27.60, 28.95, 29.32, 29.35, 29.47, 29.55, 29.63, 31.48, 31.92, 33.50, 34.33, 39.08, 57.09, 72.02, 75.68, 161.31, 170.82, 172.01.

***N*-Formyl-L-valine-(1*S*)-1-[[(2*S*,3*S*)-3-hexyl-4-*oxo*-2-oxetanyl]methyl]dodecyl Ester (13).**[21b] The title compound was prepared from **36** following the same procedure that was used for the synthesis of **2** using CH_2Cl_2/AcOEt = 95/5 as eluent for the chromatographic purification; yield 57%; mp 43−45 °C; $[\alpha]_D$ −24°. IR 3425, 2959, 2929, 2856, 1821, 1733, 1690, 1505, 1466, 1309, 1195, 1127 cm^{-1}. ^1H NMR (400 MHz) δ 0.87 (6H, m), 0.92 (3H, d, J = 6.8 Hz), 1.00 (3H, d, J = 6.8 Hz), 1.25−1.40 (26H, m), 1.62−1.79 (4H, m), 2.01 (1H, dt, J = 4.7, 15.1 Hz), 2.21 (2H, m), 3.22 (1H, m), 4.29 (1H, m), 4.63 (1H, dd, J = 4.8, 8.8 Hz), 5.03 (1H, m), 6.18 (1H, d, J = 8.8 Hz), 8.27 (1H, s). ^{13}C NMR (100 MHz) δ 14.02, 14.13, 17.45, 19.28, 22.52, 22.70, 25.14, 26.73, 27.68, 28.96, 29.33, 29.35, 29.44, 29.55, 29.62, 31.04, 31.49, 31.92, 33.97, 38.65, 55.90, 57.12, 72.87, 74.66, 160.94, 170.75, 171.02.

***N*-Acetyl-L-valine-(1*S*)-1-[[(2*S*,3*S*)-3-hexyl-4-*oxo*-2-oxetanyl]methyl]dodecyl Ester (14).** To a stirred solution of **36** (162 mg, 0.36 mmol) and TEA (0.20 mL, 1.44 mmol) in dry CH_2Cl_2 (1 mL) was added acetyl chloride (0.086 mL, 1.21 mmol). The mixture was stirred at room temperature for 1 h and then diluted with water and extracted with AcOEt. The organic phase was washed with brine, dried (Na_2SO_4) and evaporated under vacuum. The residue (170 mg) was chromatographed on silica gel with hexane/AcOEt = 75/25 as eluent to give 112 mg of **14** (68%); mp 62−63 °C; $[\alpha]_D$ −26°. IR 3435, 2959, 2929, 2856, 1821, 1731, 1676, 1509, 1466, 1373, 1309, 1124 cm^{-1}. ^1H NMR (400 MHz) δ 0.88−0.93 (9H, m), 0.97 (3H, d, J = 6.8 Hz), 1.26−1.29 (26H, m), 1.41−1.79 (4H, m), 1.99 (1H, m), 2.05 (3H, s), 2.16 (2H, m), 3.23 (1H, m), 4.30 (1H, m), 4.53 (1H, dd, J = 5.1, 8.5 Hz), 5.01 (1H, m), 6.10 (1H, d, J = 8.6 Hz). ^{13}C NMR (100 MHz) δ 14.01, 14.12, 17.70, 19.19, 22.51, 22.69, 23.19, 25.12, 26.71, 27.67, 28.95, 29.34, 29.44, 29.55, 29.61, 31.03, 31.48, 31.91, 33.98, 38.64, 57.10, 57.44, 72.62, 74.78, 170.11, 170.79, 171.49. Anal. ($C_{29}H_{53}NO_5$), C, H, N.

***N*-Formyl-L-isoleucine-(1*S*)-1-[[(2*S*,3*S*)-3-hexyl-4-*oxo*-2-oxetanyl]methyl]dodecyl Ester (15).** The title compound was prepared from **37** following the same procedure that was used for the synthesis of **2** using CH_2Cl_2/AcOEt = 95/5 as eluent for the chromatographic purification; yield 56%; mp 57−59 °C; $[\alpha]_D$= −24°. IR 3425, 3024, 2960, 2929, 2857, 1821, 1732, 1689, 1502, 1465, 1197 cm^{-1}. ^1H NMR (400 MHz) δ 0.88−0.97 (12H, m), 1.26−1.29 (28H, m), 1.62−1.78 (4H, m), 1.94−2.03 (2H, m), 2.17 (1H, m), 3.23 (1H, m), 4.29 (1H, m), 4.65 (1H, m), 5.02 (1H, m), 6.29 (1H, d, J = 8.5 Hz), 8.24 (1H, s). ^{13}C NMR (100 MHz) δ 11.32, 11.53, 14.01, 14.12, 15.59, 22.53, 22.70, 24.94, 25.13, 26.74, 27.69, 28.97, 29.45, 29.55, 29.62, 31.49, 31.92, 33.95, 37.64, 38.64, 55.49, 57.14, 72.84, 74.67, 160.85, 170.74, 170.94. $C_{29}H_{53}NO_5$, C, H, N.

***N*-Acetyl-L-isoleucine-(1*S*)-1-[[(2*S*,3*S*)-3-hexyl-4-*oxo*-2-oxetanyl]methyl]dodecyl Ester (16).** The title compound was prepared from **37** following the same procedure that was used for the synthesis of **14** using hexane/AcOEt = 75/25 as eluent for the chromatographic purification; yield 60%; mp 89−91 °C; $[\alpha]_D$ −22°. IR 3435, 2960, 2929, 2856, 1821, 1731, 1675, 1509, 1465, 1379, 1236, 1197, 1123 cm^{-1}. ^1H NMR (200 MHz) δ 0.85−0.97 (12H, m), 1.26−1.29 (28H, m), 1.60−1.99 (6H, m), 2.04 (3H, s), 2.18 (1H, m), 3.23 (1H, dt, J = 4.2, 7.5 Hz), 4.29 (1H, m), 4.56 (1H, dd, J = 5.0, 8.6 Hz), 5.02 (1H, m), 6.02 (1H, d, J = 8.6 Hz). ^{13}C NMR (50 MHz) δ 11.55, 13.99, 14.09, 15.55, 22.52, 22.69, 23.21, 25.14, 26.74, 27.72, 28.96, 29.35, 29.45, 29.55, 29.62, 31.49, 31.93, 34.04, 37.72, 38.71, 56.98, 57.20, 72.68, 74.79, 169.85, 170.66, 171.40. Anal. ($C_{30}H_{55}NO_5$), C, H, N.

***N*-Formyl-L-proline-(1*S*)-1-[[(2*S*,3*S*)-3-hexyl-4-*oxo*-2-oxetanyl]methyl]dodecyl Ester (17).** The title compound was prepared from **38** following the same procedure that was used for the synthesis of **2** using hexane/AcOEt = 6/4 as eluent for the

chromatographic purification; yield 47%; mp 49−50 °C; $[\alpha]_D$ −66°. IR 3523, 2957, 2929, 2856, 1819, 1739, 1667, 1466, 1419, 1379, 1276, 1127, 1090 cm^{-1}. ^1H NMR (400 MHz) δ 0.87 (6H, m), 1.26−1.28 (26H, m), 1.59−1.82 (4H, m), 1.92−2.11 (5H, m), 2.25 (1H, m), 3.19 (1H, m), 3.54 and 3.61 (1H, 2m), 3.64 (1H, m), 4.33 (1H, m), 4.40 (1H, dd, J = 3.6, 8.4 Hz), 5.03 and 5.11 (1H, 2m), 8.26 and 8.29 (1H, 2s). ^{13}C NMR (100 MHz) δ 14.03, 14.13, 22.53, 22.70, 22.82, 24.09, 25.15, 25.22, 26.68, 26.78, 27.59, 28.98, 29.35, 29.46, 29.58, 29.63, 31.50, 31.92, 34.35, 38.84, 46.33, 56.86, 56.99, 57.14, 58.95, 72.34, 73.21, 75.01, 160.71, 161.63, 171.15, 171.25. Anal. ($C_{28}H_{49}NO_5$), C, H, N.

***N*-Formyl-L-phenylalanine-(1*S*)-1-[[(2*S*,3*S*)-3-hexyl-4-*oxo*-2-oxetanyl]methyl]dodecyl Ester (19).** The title compound was prepared from **21** following the same procedure that was used for the synthesis of **2** using hexane/AcOEt = 7/3 as eluent for the chromatographic purification; yield 100%; mp 59−63 °C; $[\alpha]_D$ −20°. IR (KBr disks) 3346, 2955, 2918, 2849, 1844, 1719, 1654, 1522, 1464, 1381, 1280, 1176 cm^{-1}. ^1HNMR (400 MHz) δ 0.88 (6H, m), 1.25−1.43 (26H, m), 1.59 (2H, m), 1.71 (2H, m), 1.89 (1H, dt, J = 4.4, 14.8 Hz), 2.07 (1H, m), 3.13 (3H, m), 4.17 (1H, dt, J = 5.1, 6.9 Hz), 4.90 (1H, q, J = 7.0 Hz), 4.98 (1H, m), 6.07 (1H, d, J = 7.5 Hz), 7.17 (2H, d, J = 6.7 Hz), 7.28 (3H, m), 8.16 (1H, s). ^{13}C NMR (100 MHz) δ 14.03, 14.12, 22.53, 22.69, 25.05, 26.69, 27.61, 28.97, 29.32, 29.35, 29.43, 29.56, 29.62, 31.48, 31.92, 33.93, 37.81, 38.60, 52.11, 57.04, 73.03, 74.56, 127.34, 128.71, 129.26, 135.62, 160.51, 170.77, 170.81. Anal. ($C_{32}H_{51}NO_5$), C, H, N.

CB_1R and CB_2R Binding Assays. The affinity of compounds for either CB_1 or CB_2 receptors was evaluated using membranes from HEK cells transfected with either the human recombinant CB_1 or CB_2 receptor cDNA and [^{3}H]-(−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol ([^{3}H]-CP-55,940; K_d = 0.18 nM for CB_1 and K_d = 0.31 nM for CB_2) as the high affinity ligand, as described by the manufacturer (Perkin-Elmer, Italy). Displacement curves were generated by incubating drugs with a fixed concentration of [^{3}H]CP-55,940 (0.14 nM for CB_1 and 0.084 nM for CB_2 binding assay). In all cases, K_i values were calculated by applying the Cheng−Prusoff equation to the IC_{50} values (obtained by GraphPad) for the displacement of the bound radioligand by increasing concentrations of the test compounds.

Assay of Fatty Acid Amide Hydrolase (FAAH). The effect on the enzymatic hydrolysis of AEA was measured by using membranes prepared from rat brain, incubated with increasing concentrations of the test compounds and [^{14}C]AEA (2.4 μM) in 50 mM Tris-HCl, pH 9, for 30 min at 37 °C. [^{14}C]Ethanolamine produced from [^{14}C]AEA hydrolysis was measured by scintillation counting of the aqueous phase after extraction of the incubation mixture with 2 volumes of $CHCl_3/CH_3OH$ = 1/1 (by volume). Data are expressed as the concentration exerting 50% inhibition of AEA hydrolysis (IC_{50}), calculated by GraphPad.

Assay of DAGL Activity. Confluent COS-7 cells, overexpressing the human recombinant DAGLα, were harvested in tris−HCl buffer, pH 7 and homogenized in a homogenizer (Dounce). The homogenates were centrifuged at 4 °C at 800*g* (5 min) and then at 10000*g* (25 min). The 10000*g* membrane fraction was incubated in incubation buffer (Tris−HCl 50 mM) at pH 7.0 at 37 °C for 20 min, with synthetic *sn*-1-[^{14}C]oleoyl-2-arachidonoylglycerol (1.0 mCi/mmol, 25 μM). After the incubation, lipids were extracted with 2 volumes of $CHCl_3$/MeOH = 2/1 (by volume) and the extracts were lyophilized under vacuum. Extracts were fractionated by TLC on silica on plastic plates using $CHCl_3$/MeOH/NH_4OH (85/15/1 by volume) as the eluting system. Bands corresponding to [^{14}C]oleic acid were cut, and their radioactivity was counted with a β-counter.

Assay of MAGL Activity. The 10000*g* cytosolic fraction from COS-7 cells, which contains high levels of MAGL, was incubated in Tris−HCl 50 mM, at pH 7.0 at 37 °C for 20 min, with synthetic 2-[^{3}H]arachidonoylglycerol (1.0 mCi/mmol, 25 μM). After the incubation, lipids were extracted with 2 volumes of $CHCl_3$/MeOH = 2/1 (by volume) and the extracts were lyophilized under vacuum. Extracts were fractionated by TLC on silica on plastic plates using $CHCl_3$/MeOH/NH_4OH (85/15/1 by volume) as the eluting system. Bands corresponding to [^{3}H]arachidonic acid were cut, and their radioactivity was counted with a β-counter.

Assay of DAGLα Inhibitory Activity in Intact Cells. Confluent mouse N18TG2 neuroblastoma cells, cultured as described[10] and attached to 10 cm Petri dishes, were incubated for 20 min at 37 °C in 5 mL of medium containing 3 μM ionomycin in the presence of vehicle or THL (1 μM) or compound **15** (0.3 μM) or compound **8** (1.5 μM). After terminating the incubation by addition of 5 mL of ice-cold CH_3OH, cells were scraped off of the Petri dishes, transferred to 50 mL Falcon tubes, and extracted three times with 5 mL of $CHCl_3$ for lipid extraction. Lyophilized lipids were then analyzed for the presence of 2-AG by isotope-dilution liquid chromatography mass spectrometry as described previously.[10] Ionomycin-stimulated levels of 2-AG in N18TG2 cells were 57.6 ± 2.7 pmol/mg of extracted lipid. The effects of the inhibitors were compared by using ANOVA, followed by the Bonferroni's test.

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Supporting Information Available: Elemental analysis data for compounds **5**−**7**, **9**−**11**, **14**−**17**, **19**−**21**, **23**, **24**, **26**−**31**. This material is available free of charge via the Internet at http://pubs.acs.org.

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