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Novel analogs of D-*ef*-MAPP and B13. Part 1: Synthesis and evaluation as potential anticancer agents

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Abstract—A series of novel isosteric analogs of the ceramidase inhibitors, (1*S*,2*R*)-*N*-myristoylamino-phenylpropanol-1 (D-*e*-MAPP) and (1*R*,2*R*)-*N*-myristoylamino-4′-nitro-phenylpropandiol-1,3 (B13), with modified targeting and physicochemical properties were designed, synthesized, and evaluated as potential anticancer agents. When MCF7 cells were treated with the analogs, results indicated that the new analogs were of equal or greater potency compared to the parent compounds. Their activity was predominantly defined by the nature of the modification of the *N*-acyl hydrophobic interfaces: *N*-acyl analogs (class A), urea analogs (class B), *N*-alkyl analogs (class C, lysosomotropic agents), and ω-cationic-*N*-acyl analogs (class D, mitochondriotropic agents). The most potent compounds belonged to either class D, the aromatic ceramidoids, or to class C, the aromatic *N*-alkylaminoalcohols. Representative analogs selected from this study were also evaluated by the National Cancer Institute In Vitro Anticancer Drug Discovery Screen. Again, results showed a similar class-dependent activity. In general, the active analogs were non-selectively broad spectrum and had promising activity against all cancer cell lines. However, some active analogs of the D-*e*-MAPP family were selective against different types of cancer. Compounds LCL85, LCL120, LCL385, LCL284, and LCL204 were identified to be promising lead compounds for therapeutic development.

1. Introduction

Cancer has proven to be a difficult disease to treat, and few effective drugs are available. Identification of novel, efficient, selective, and less toxic anticancer agents remains an important and challenging goal in medicinal chemistry.[1–3]

Sphingolipid (SPL) metabolism provides a rich network of bioactive molecules that play key roles in the regulation of diverse cell functions. Ceramide (Cer) has emerged as a key modulator of cancer cell growth and apoptosis.[4] Sphingosine 1-phosphate (S1P), generated from Cer by the action of ceramidases (CDases) to yield sphingosine (Sph) which is phosphorylated by sphingosine kinase (SK), promotes growth and opposes Cer-mediated apoptosis.[5,6] Because of the roles of Cer and S1P in regulating cell growth and cell death, their metabolic and signaling pathways have emerged as potential targets for anticancer therapy.[7] Many approaches have been explored to increase endogenous Cer, and a few appear promising.[7,8] These include the application of cell permeable short-chain Cers,[9–11] liposomal formulations,[12,13] site-specific cationization,[14–17] and induction of endogenous Cer by modulation of SPL metabolizing enzymes.[18–34]

In the course of our investigation of SPLs' chemistry and the search for new molecules that mimic the action of SPLs and/or regulate their metabolism, we have focused on analogs that affect Cer metabolism, especially the Cer-Sph-S1P balance, and display particular desired properties, and we have also attempted to target them to specific sub-cellular compartments.[14–17,27,28,31]

Previously, we synthesized a set of lipophilic phenyl-*N*-acyl-amino-alcohols (aromatic analogs of Cer, Scheme 1) and evaluated their structure-dependent inhibitory effects in HL-60 cells.[20,21] We found that analog activity

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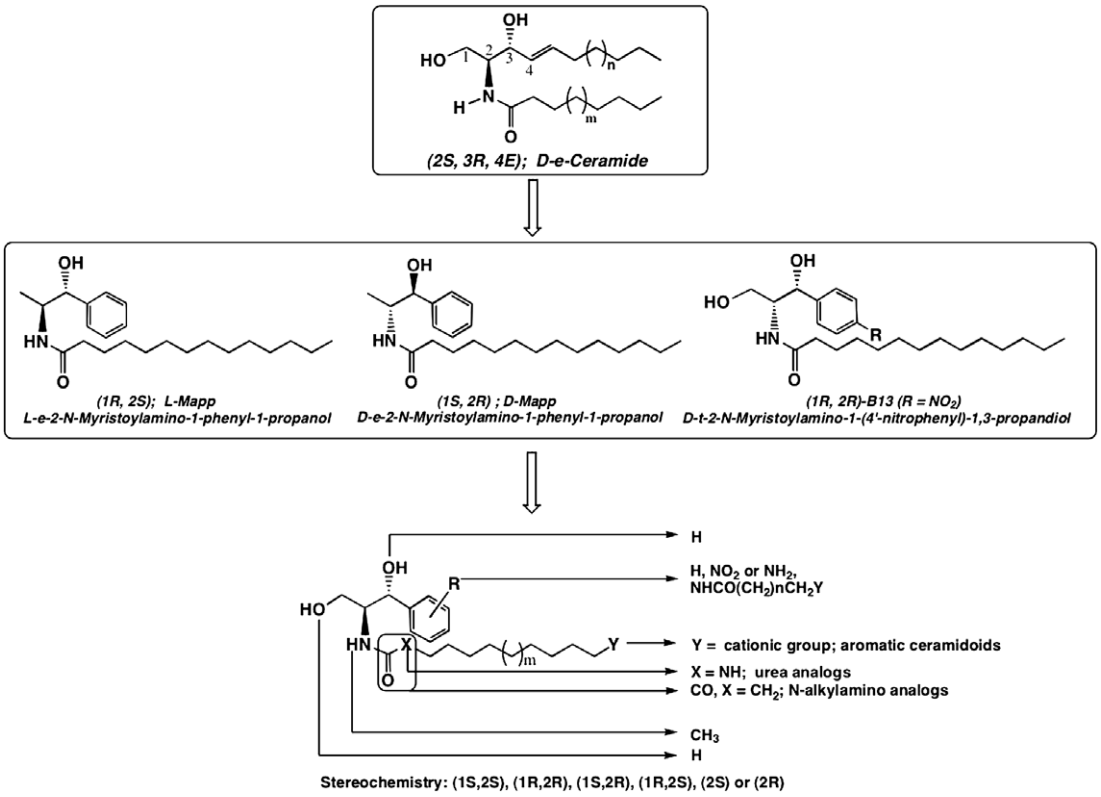


Scheme 1. Ceramide, D-*e*-MAPP and B13 structures and design for aromatic analogs.

depended on stereochemistry and the chain length of the *N*-acyl moiety. Analogs with the natural Cer configuration were not active compounds, whereas their stereoisomers inhibited cell growth. The active isomers were *N*-dodecanoyl (C_{12}), *N*-myristoyl (C_{14}), and *N*-palmitoyl (C_{16}) homologs, with C_{14}-homologs being the most potent. Shorter (C_2–C_{10}) and longer (C_{18}–C_{24}) homologs were considerably less active. Pilot modification of some *N*-myristoyl analogs (amides) to their *N*-myristyl-counterpart (*N*-alkyl-amines) increased their inhibitory activity in HL-60 cells (A. Bielawska, data not published).

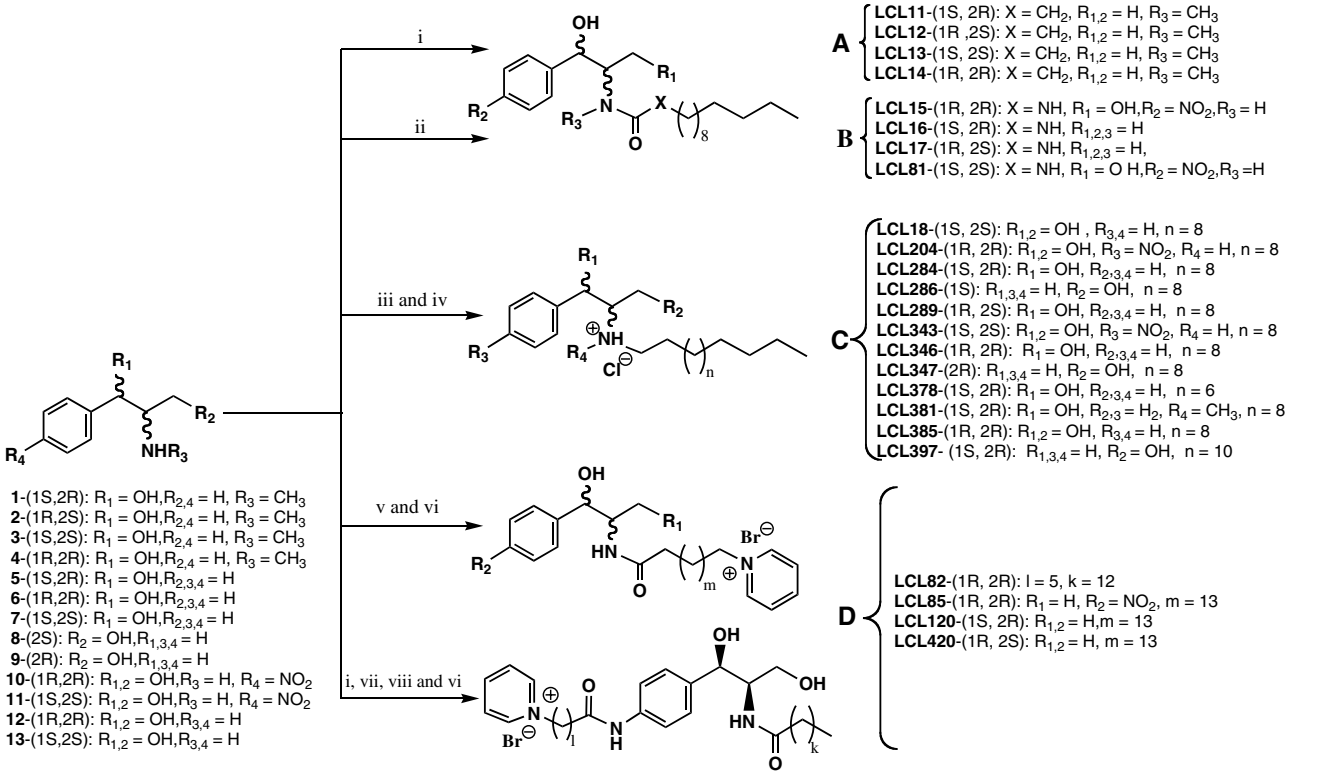
Further investigations on the active analogs confirmed their inhibitory effects on ceramidases (CDases). HL-60 cell experiments and in vitro studies showed that the most potent analogs from this group, D-*e*-MAPP, stereospecifically inhibited alkaline CDase, whereas its enantiomer, L-*e*-MAPP, served as a substrate for this enzyme.[20] Biological activity of D-*e*-MAPP was later confirmed by several investigators.[35–40] Another active analog: B13 (Scheme 1), which differs from D-*e*-MAPP in stereochemistry and functional groups, inhibited acid CDase.[32,34] Treatment with B13 caused the release of cytochrome C and induced apoptosis.[34] Biological activity of B13 was also demonstrated in malignant melanoma, colon, and prostate cancer cells, and in animal experiments of in vivo cancer growth.[32–34]

D-*e*-MAPP, B13, and Cers are *N*-acylamino alcohols with two distinct elements of rigidity: a phenyl ring or long chain alkenyl unit and the amido group joined to the chiral backbone of the propane chain (Scheme 1). These structural features determine their conformation(s) and ability to interact effectively with putative targets, via hydrogen bonding and van der Waals hydrophobic interactions. Therefore, the aromatic analogs of Cer constitute attractive models for the development of new bioactive lipophilic molecules. The effects of additional aromatic analogs on SPL metabolism and SPL intracellular trafficking have been reported, demonstrating that the phenyl-amino-alcohol foundation is recognized by many enzymes of SPL metabolism and serves as a useful structural benchmark for development of bioactive compounds.[18,19,22–26,29,41]

In a previous study, we showed that isosteric replacement of the amide group of Cer by urea or *N*-alkylamine units generated inhibitors of neutral CDase, thus illustrating the usefulness of this approach.[42] Moreover, in another recent study, we developed the concept of the fixed positive charge-dependent cellular targeting Cer, and demonstrated that fixed cationic Cer analogs target preferentially to the mitochondria.[14,16,17]

Extending these findings to the aromatic analogs of Cer, we suspected that introduction of specific structural features and other modifications of −CO, X, Y, R, and R_1-functionalities as well as altering the stereochemistry of D-*e*-MAPP and B13 structures (Scheme 1) will improve and modify their physicochemical and targeting properties to specific compartments.

These new compounds are shown in Scheme 2. Based on targeting behavior of alkylamines, we expected that



Scheme 2. Synthetic outline for the new analogs of D-*e*-MAPP and B13. Reagents and conditions: (i) Myristoyl chloride, 50% CH_3COONa, THF, rt; (ii) dodecyl isocyanate, C_2H_5OH, THF, rt; (iii) $CH_3(CH_2)_pCHO$ (p = 10, 12, 14), $NaBH_3CN$, CH_3OH, CH_3COOH, rt; (iv) 1 M HCl in diethyl ether, ethyl acetate, rt; (v) 16-bromohexadecanoyl chloride, 50% CH_3COONa, THF, rt; (vi) pyridine, toluene, 75–85 °C, 4 h; (vii) Zn, CH_3COOH, rt; (viii) 6-bromohexanoyl chloride, 50% CH_3COONa, THF, rt.

some analogs will locate to lysosomes (e.g., *N*-alkylamino analogs, class C).[43–46] In contrast, fixed cations are expected to act as a mitochondriotropic agents (aromatic ceramidoids, class D).[17] Finally, neutral analogs (parent amides, *N*-methyl amides, class A, and urea analogs, class B) may show no compartmental preferences as was shown for exogenous Cers.[47,48]

Here we describe the synthesis, full NMR characterization, basic physicochemical properties, and growth suppression of breast carcinoma MCF7 cells of new analogs of D-*e*-MAPP and B13. Additionally, we present results from the National Cancer Institute for a full screening against a 60-human-tumor-cell assay (the NCI's 60-cell line assay) performed for the selected analogs from classes A–D.

2. Results and discussion

2.1. Chemistry

The compounds designed and synthesized in this study represent the second generation of analogs that are based on structural and stereochemical modifications of D-*e*-MAPP and B13 (Scheme 1). The selected model compounds generally contain C_{14}-hydrocarbon chains in their *N*-acyl parts. Structural analogs contain a modified proton donor–acceptor ability of the functional groups at C1-, C2-, and C3-positions and substituents of the phenyl ring. The stereochemical isomers are: (1*R*,2*S*), (1*R*,2*R*), (1*S*,2*R*) (1*S*,2*S*), (2*R*), and (2*S*).

We introduce the following groups of compounds: class A, *N*-methyl, *N*-myristoyl analogs of D-*e*-MAPP (and the parent compounds); class B, urea analogs, for which the *N*-acyl group is replaced by a nonhydrolyzable urea group; class C, *N*-alkylamino analogs for which the *N*-acyl moiety was reduced to an *N*-alkylamino group; and class D, analogs containing an ω-pyridinium salt unit in the *N*-acyl group (shown in Scheme 2).

2.2. Synthesis

The synthesis of the target compounds was carried out efficiently according to the procedures presented in Scheme 2. Four different approaches, based on the previously reported procedures for the preparation of Cer,[42] their mimics[17,19,42] and varied D-*e*-MAPP, and B13 analogs,[21,22,49] were utilized to synthesize the amphiphilic compounds LCL11–LCL420. Specifically, *N*-methyl amides LCL11–LCL14 were prepared by N-acylation of the corresponding stereoisomers of amines[1–4] with myristoyl chloride under a basic, two-phase system. The urea analogs of D-*e*-MAPP and B13, LCL15, LCL16, LCL17 and LCL81, which have the C_{12}-alkyl chains mimicking the C_{13}-alkyl chains of the parent amides, were prepared by condensation of the corresponding amines 5, 6, 10, and 11 with dodecyl isocyanate. Lipophilic amine analogs of D-*e*-Mapp, L-*e*-Mapp, and B13

were prepared by the reductive amination of dodecyl, myristyl, and hexadecyl aldehydes with 1, 5–11, using $NaCNBH_3$ in a glacial acetic acid-methanol solution, subsequent to the transformation of the formed di- or trialkyl amine free bases to their corresponding hydrochloride salts LCL18–LCL397. Cationic analogs LCL85, LCL120, and LCL420, which have a fixed positive charge located in their *N*-acyl chains, were prepared by N-acylation of 5, 6, and 10 with 16-bromo-hexadecanoyl chloride after the process of conjugation of the formed ω-bromo-amides,[14,15] and [16] with pyridine. Analog LCL82, which has a pyridinium salt moiety attached at the *para*-position of the phenyl ring, was prepared in a three-step synthetic process starting from B13. Thus, the nitro group of B13 was reduced with Zn in CH_3COOH to afford a 4-amino-derivative.[17] This compound was N-acylated with 6-bromo-hexanoyl chloride to form the intermediate ω-bromo-di-amide,[18] which was conjugated in the final step with pyridine to give LCL82.

All synthesized model compounds were characterized by NMR, MS, and polarimetry, confirming their structural homogeneity and high optical purity (listed in Section 4).

2.2.1. Cellular levels. The cellular levels of representative analogs from class A–D in MCF7 cells were established by high-performance liquid chromatography-mass spectrometry (HPLC–MS) analysis. Experimental data from treating cells with the representative analogs (10 μM) over the indicated time showed a very fast cellular uptake for all tested analogs with the highest extent for analogs from class C (Fig. 1). Concentrations of LCL284 and LCL204 after 0.5 h of treatment were established as ~3600 pmols/1×10^6 cells whereas for the other analogs, the cellular levels were in the range of 800–1700 pmols/1×10^6 cells with the lowest levels being the class D analogs. Progressive increases up to 5 h were observed for all analogs. At this time point, LCL204 and LCL284 were present at concentrations two times higher than the other analogs. No significant differences were noted for the representative analogs from classes B and D compared to D-*e*-MAPP and B13.

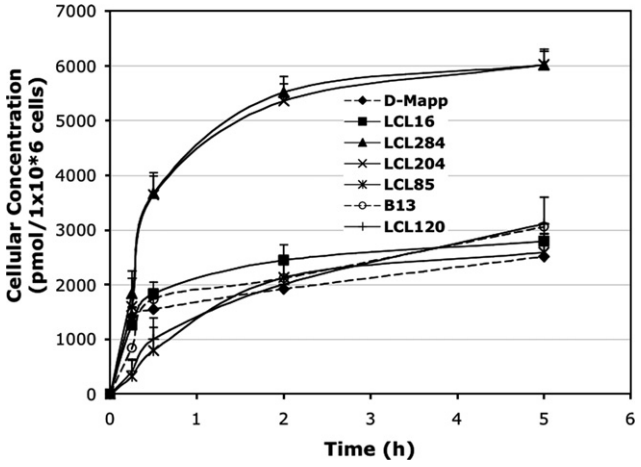


Figure 1. Cellular concentration of selected new analogs in MCF7 cells (10 μM treatment over time).

2.2.2. Inhibitory effects on MCF7 cell growth. The antiproliferative effects of the newly obtained compounds were examined in MCF7 breast carcinoma cells and compared to the activity of D-*e*-MAPP, B13, and their enantiomers (L-*e*-MAPP and LCL5). Data are shown in Figures 2 and 3. IC_{50} values represent the drug concentration that reduces the cell number by 50% during the drug incubation for 48 h, signifying the growth inhibitory power of the agents.

D-*e*-MAPP and B13 were less potent in MCF7 cells compared to HL-60 cells.[20,21] D-*e*-MAPP and B13 inhibited cell growth in a dose-dependent manner (IC_{50} at 48 h, 30 μM and 14 μM, respectively), whereas L-*e*-MAPP was inactive up to 50 μM (the highest concentration tested). This compound was also inactive in HL-60 cells.[21]

The inhibitory profiles for the representative D-*e*-MAPP and B13 analogs from classes A–D are shown in Figure 2a and b, respectively. IC_{50} values for all synthesized analogs were calculated from studies similar to those shown in Figure 2 as established for the 48-h time point, and the IC_{50} for the compounds ranged from 2.3 μM to ~35.0 μM (Fig. 3). The potency of these analogs depended on the modifications made in the *N*-acyl- part of D-*e*-MAPP or B13. The *N*-methyl analogs and urea analogs (classes A and B) were active with $IC_{50} > 10$ μM, whereas the *N*-alkyl-amino analogs and cationic analogs

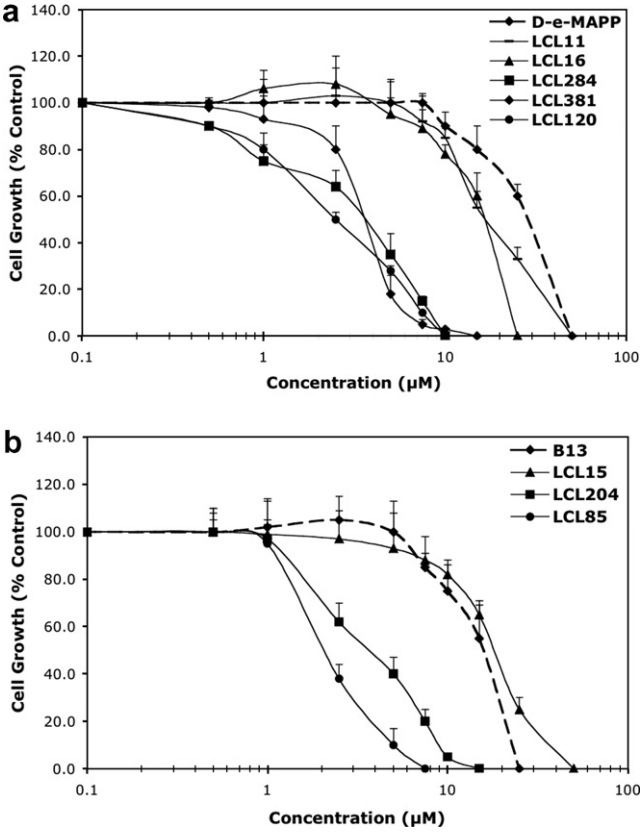


Figure 2. Inhibitory effect on MCF7 cell growth (48 h). (a) Concentration-dependent effect for the representative D-*e*-MAPP analogs. (b) Concentration-dependent effect for the representative B13 analogs.

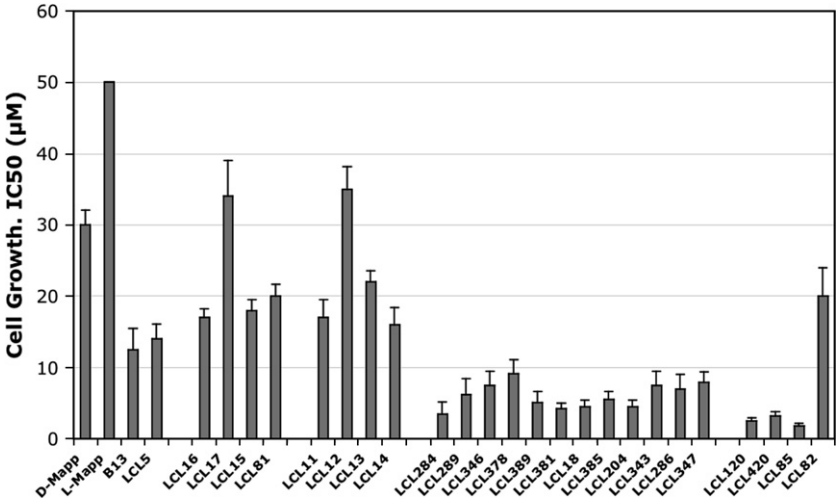


Figure 3. IC_{50} profile for all synthesized compounds in MCF7 cells at 48 h.

(class C, D) were active with $IC_{50} < 10$ μM. Interestingly, all new analogs of inactive L-*e*-MAPP were also potent (LCL12, LCL17, LCL289, and LCL420).

Additionally, activities of representative analogs from classes C and D were compared to the activity of the corresponding analogs of Cer: *N*-myristyl-sphingosine (C_{14}-ceramine, LCL342) and CCPS analogs (LCL30).[17,42] The results showed that C_{14}-ceramine was inactive up to 25 μM (the highest concentration tested) and activity of LCL30 was comparable to the activity of analogs from class D (not shown). The potency of the analogs from class B was compared to D-*e*-C6-urea-Cer (LCL25), which represents a cell-permeable, short-chain analog of C16-urea-Cer.[42] The results showed that activity of LCL25 (IC_{50} ~15 μM, data not shown) was similar to class B analogs.

As shown in Figure 2a, *N*-methyl-D-*e*-MAPP (LCL11) and D-*e*-urea-MAPP (LCL16) were twice as effective as D-*e*-MAPP (similar IC_{50} values; however, at a higher concentration, LCL16 was more effective than LCL11 resulting in 80% and 40% inhibition of growth, respectively). The cationic analog LCL120 and the *N*-myristyl analogs (LCL284) were ~10× more potent than D-*e*-MAPP. B13 analogs followed the pattern observed for D-*e*-MAPP analogs with the exception of urea-B13 (LCL15). The low activity of this compound was also reported for several different cell lines.[49]

Analogs from class A, with LCLs[11–14] representing the full set of stereoisomers, were more potent than D-*e*-MAPP itself (Fig. 3). The least potent was the (1*R*,2*S*) isomer bearing the same stereochemistry of L-*e*-MAPP and its (1*S*,2*S*) diastereoisomer. The (1*S*,2*R*) isomer bearing stereochemistry of D-*e*-MAPP and its (1*R*,2*R*) diastereoisomer were more active. In general, isomers with the threo-configuration were more potent than their erythro diastereoisomers and the 2R isomers were more active than 2S counterparts.

As shown in Figure 3, urea analogs (class B) of D- and L-*e*-MAPP (LCL16 and LCL17) had increased activity, whereas the urea analog of B13 (LCL15) and its enantiomer (LCL81) were slightly less potent compared to the parent compounds (D-*e*-MAPP, B13 and their enantiomers, respectively). D-*e*-urea-MAPP, LCL16, was more potent than its enantiomer, LCL17; however, no enantiospecificity was found for urea B13 (LCL15 and LCL81 were equally potent).

All *N*-myristyl-phenylamino-alcohols (class C) ably inhibited MCF7 cell growth (IC_{50} 3.5–8.0 μM, Fig. 3). IC_{50} values for LCL284 and LCL204, analogs of the parent compounds, were established at 3.5 μM and 4.5 μM, respectively. From available *N*-myristyl isomers of D-*e*-MAPP, the most active was LCL284 bearing the stereochemistry of D-*e*-MAPP, followed by its enantiomer LCL289 (IC_{50}: 3.5 μM and 5.5 μM, respectively). LCL346, the threo-isomer, bearing (1*R*,2*R*) stereochemistry, was less potent than its erythro-diastereoisomer, LCL284. LCL286 and LCL347, the isomers of LCL284, which contain the primary instead of the secondary OH group, were half as potent as LCL284. LCL381, the *N*-methyl analog of LCL284, representing a tertiary lipophilic amine, was almost equally potent as LCL284. Shorter (C_{12}–, LCL378) and longer (C_{16}–, LCL379) homologs of LCL284 were only a little less potent than LCL284 (LCL378 $IC_{50} = 9.0$ μM; LCL397 IC_{50}: 5.0 μM). Similar chain-length dependency was observed previously for the *N*-acyl analogs.[20,21]

The stereochemical effect of B13 analogs on cell growth was studied for LCL204/LCL343 and LCL385/LCL18 pairs. These compounds represent (1*R*,2*R*) and (1*S*,2*S*) enantiomeric pairs of the threo-isomers only. As shown in Figure 3, the (1*R*,2*R*) isomers were more potent than the (1*S*,2*S*) isomers.

The effects of phenyl ring substituents on cell growth were studied using LCL204 and LCL385 as model compounds. These two analogs have the same stereochemistry (1*R*,2*R*), contain two hydroxyl groups, and differ only in the presence or absence of the *p*-NO_2 group in the phenyl ring. LCL385 was almost equally potent as LCL204 (Fig. 3).

In conclusion, all compounds representing class C were much more potent than the parent compounds, and only a small influence of the structural changes (hydroxyl group location and number, chiral center stereochemistry, and phenyl ring substituents types) was noticed for their activity.

The independently synthesized lipophilic amine, AD2646, which shares the structure of LCL204 (hydrochloride of AD2646)—a compound we previously synthesized and studied—was shown to act as a bioactive molecule in HL-60 cells.[22]

Analogs from class D, LCL120, LCL85, and LCL420, were as potent as the analogs from class C (Figs. 2a, b, and 3). LCL120, LCL85, and LCL420 (analogs of D-*e*-MAPP, B13 and L-*e*-MAPP) had the following IC_{50} values: 2.5 μM, 2.3 μM, and 2.4 μM, respectively. Somewhat surprisingly, again the analog of inactive L-*e*-MAPP became active. Additionally, we found that the location of the pyridinium disk in the molecule played a significant role on the activity of this class of compounds. Comparison of LCL85 and LCL82 (contains an ω-pyridinium disk in *N*-acyl part of 4′-substituent of the phenyl ring, Scheme 2) showed that LCL82 had low activity (IC_{50} = 20 μM, Fig. 3), whereas LCL85 was one of the most active compounds (IC_{50} = 2.3 μM). A similar observation (activity/phenyl substitution dependency) was noticed when the para-NO_2 group from B13 was replaced by the amino group. LCL83 was inactive up to 25 μM (the highest concentration tested, data not shown).

These results indicate that all modifications introduced into *N*-acyl part of D-*e*-MAPP, L-*e*-MAPP, and B13 structures generated analogs with higher activity than the parent compounds. The most significant improvements were observed for L-*e*-MAPP analogs.

From our previous work,[21] we have learned that stereochemistry of phenyl-*N*-acyl-amino alcohols plays an important role on their activity. Analogs with the configuration of natural Cer were not active as was shown for L-*e*-MAPP, whereas their stereoisomers inhibited cell growth with D-MAPP, the enantiomer of L-*e*-MAPP, being the most potent. This effect may be related to the fact that L-*e*-MAPP was metabolized on its *N*-amido group, whereas D-*e*-MAPP inhibited this process suggesting involvement of amidase/ceramidase enzymes.[20] However, this enantiospecific effect was not observed for the new analogs. Forced localization of LCL120 and LCL420 (enantiomeric pair of aromatic ceramidoids) to mitochondria demonstrated almost equal potency suggesting an effect on some non-enantiospecific enzymes. Enantiomeric pairs of class B and C analogs, where the *N*-amido group was replaced by urea or *N*-alkyl groups also showed a similar activity suggesting that these analogs can be recognized by some enzymes (ceramidases) but do not serve as their substrates.

Considering cellular levels of the analogs from classes C and D (Fig. 1), the most active analogs were the cationic analogs: LCL85, LCL120, and LCL420.

2.2.3. Evaluation of the selected analogs for potential anticancer activity in the NCI's in vitro human disease-oriented tumor cell line screening panel (the NCI's 60-cell line assay). Twenty-three compounds selected from the previous study were evaluated in the in vitro human disease-oriented tumor cell line screening panel developed by the NCI in accordance with the protocol of the Drug Evaluation Branch, National Cancer Institute, Bethesda, MD.[50–54]

The panel cell lines used in this test were: leukemia, non-small cell lung cancer, colon cancer, central nervous system cancer, melanoma, ovarian cancer, renal cancer, prostate cancer, and breast cancer. The details of the cell lines used are shown in Tables 2–4. The cytotoxic and/or growth inhibitory effects were tested in vitro against the panel of 60 human tumor cell lines derived from nine neoplastic diseases at 10-fold dilution of five concentrations ranging from 10^{-4} to 10^{-8} M. The growth percentage was evaluated spectrophotometrically. For each tested compound, three parameters were measured for each cell line: GI_{50} (drug concentration that reduces net protein by 50% during the incubation), TGI (total growth inhibition, signifying toxicity), and LC_{50} (drug concentration resulting in 50% reduction in measured protein at the end of the drug treatment, compared to the beginning, signifies net cell loss). The $\log_{10}GI_{50}$, and $\log_{10}LC_{50}$ and $\log_{10}TGI$ were determined and defined as the mean of the $\log_{10}$ of the individual GI_{50}, TGI, and LC_{50} values. The average GI_{50} values collected from the 60-cell line assay for the 23 tested compounds are shown in Table 1. Compounds with $\log_{10}GI_{50}$ values = −4 and <−4 were considered active.

Full profiles (60 cell lines) of the GI_{50}, TGI, and LC_{50} values for the selected analogs LCL284, LCL289, LCL286, LCL204, LCL385, LCL120, LCL420, and LCL85 are shown in Tables 2–4, respectively.

From Table 1, we can conclude that all tested compounds were active in this test showing MGMID Log_{10}-GI_{50} values from −4.02 to −5.77 (1.71–95.0 μM); δ, 0.11–3.03; range, 0.3–3.30.

D-*e*-MAPP and B13 had a broad spectrum of activity with the GI_{50} values ∼−4.0 (∼100 μM); δ < 1; and range <1, indicating no cell specificity. Other analogs from class A were more potent (GI_{50} ∼−4.9, ∼11 μM) but the δ values were also <1. Interestingly, LCL12, the analog of L-*e*-MAPP, had a high GI_{50} (−4.96) with a δ and range >3. This compound was specifically active in SNB-57 and MDA-MS-435 cancer cell lines (not shown).

Class B analogs had GI_{50} values ∼−4.8 (∼15 μM). LCL16 and LCL15, urea analogs of the parent compounds, had δ values <1 and range values <1. However, LCL17 and LCL81, the enantiomers of LCL16 and LCL15 had δ and range values >1, showing cancer cell specificity to HOP-92 and BT-549 by LCL17 and BT-549 by LCL81 (data not shown).

Table 1. Average GI_{50} values collected from the NCI's 60-cell line assay

	MG MID $Log_{10}GI_{50}$	Delta	Range
Class A			
D-Mapp	−4.03	0.32	0.35
B13	−4.02	0.47	0.49
LCL5	−4.90	0.75	1.48
LCL11	−4.84	0.37	0.60
LCLI2	−4.96	3.03	3.30
Class B			
LCL16	−4.76	0.15	0.30
LCLI7	−4.84	2.33	2.57
LCL15	−4.84	0.54	0.72
LCL81	−4.83	1.20	1.36
Class C			
LCL18	−5.73	0.15	1.01
LCL204	−5.58	0.32	1.06
LCL385	−5.70	0.24	1.20
LCL343	−5.67	0.38	1.17
LCL284	−5.75	1.31	2.09
LCL289	−5.71	0.11	0.94
LCL346	−5.77	1.46	2.42
LCL286	−5.74	2.26	3.16
LCL347	−5.77	2.23	3.27
LCL381	−5.68	0.15	1.01
Class D			
LCL85	−5.30	1.13	1.73
LCL120	−5.67	2.33	3.29
LCL420	−5.42	2.58	4.00
LCL82	−5.03	0.85	1.25

The most active were analogs from classes C and D, with GI_{50} values from −5.03 to −5.77 (1.71–9.2 μM).

Class C analogs had GI_{50} values −5.58 to −5. 77 (2.61–1.71 μM) with a broad spectrum of δ values (0.15–2.26), ranging from 0.94 to 3.27. From this group, LCL284, LCL346, LCL286, and LCL347 had $\delta > 1$ indicating cancer cell specificity. Remaining analogs showed a broad spectrum of antitumor activity against the nine tumor subpanels tested with LCL385 being the most potent.

Class D, ω-cationic ceramidoids: LCL82, LCL85, LCL120, and LCL420 had GI_{50} values from −5.03 to −5.67, $\delta \sim 1$ (0.85, 1.13, 2.33, and 2.58, respectively) and range values >1 (1.25, 1.73, 3.29, and 4.0, respectively) indicating cancer cell specificity. Again, the least potent was LCL82 (∼9.2 μM).

A full screen on the in vitro tumor 50% growth inhibition (GI_{50}, M) for the selected analogs from classes C and D, LCL284, LCL289, LCL246, LCL204, LCL385, and LCL120, LCL420, LCL85 is shown in Table 2. We can conclude that all presented analogs were potent at the level of ∼1 μM with broad-spectrum antitumor activity. LCL284 was specifically active in HL-60 (TB) leukemia cells (−7.06, 86 nM) but showed low activity in SK-MEL-5 cells (−4.97, 11 μM). Low activity in SK-MEL-5 cells (−4.88, 12 μM) was also detected for LCL289, the enantiomer of LCL284. LCL286, an isomer of LCL284 containing the primary OH group instead of the secondary OH group present in LCL284, was very potent in BT-549 breast cancer cells (−8.00, ∼10 nM). LCL120 was specifically very potent in CCRF-CEM leukemia cells (<−8.00, <10 nM) and was selective for breast cancer cells NCI-ADR-RES versus BT-549 (v4.71, 19.5 μM and −7.16, 68 nM, respectively). Similar selectivity was detected for LCL420, the enantiomer of LCL120 (>4.00 vs −8.00). B13 analogs LCL385, LCL204, and LCL85 were also very active (GI_{50} ∼−5.5, 2.8 μM) with broad-spectrum activity and low specificity, except for LCL85.

A full screen on the in vitro total growth inhibition (TGI, M) by these selected analogs is shown in Table 3. Data indicate that all tested analogs were cytostatic with TGI values between −4.84 and −5.5 (14.3–2.8 μM). LCL120 and LCL420 showed some safety specificity to NCI-ADR-RES breast cancer cells and HCT-15 colon cancer cells (∼−4, ∼100 μM).

A full screen on the in vitro tumor 50% lethal concentration (LC_{50}, M) of these analogs is shown in Table 4. Data indicate that all tested analogs were cytotoxic with LC_{50} values between >−4.00 and −5.00 (10–100 μM). In most cases, cytotoxic effects of the class D compounds were lower than class C compounds.

3. Summary and conclusions

Based on the chemical structure differences and cell activity presented here, compounds were grouped into 4 classes (A, B, C, and D). Class A: *N*-acyl analogs (neutral); class B: urea analogs (neutral); class C: *N*-alkyl analogs (lysosomotropic analogs); class D: ω-cationic analogs (mitochondriotropic analogs).

All new analogs showed an increased inhibitory effect on MCF7 cell growth, compared to their parent compounds. Class A and class B were active with $IC_{50} > 10$ μM, whereas analogs from classes C and D were active with $IC_{50} < 10$ μM. New analogs of inactive L-*e*-MAPP were also potent.

An SAR study from the NCI's 60 cell-based assay showed that all tested compounds were active, with GI_{50} values from −4.02 to −5.77; δ values from 0.11 to 3.03, and a range values of 0.3–3.30. These results indicate promising activity against all cancer cell lines and specificity of particular analogs to certain cancers. Again, the most active analogs were from classes C and D, with GI_{50}values from −5.03 to −5.77 (∼1–10 μM). Cationic analogs were more specific for certain cell lines. Analogs from the B13 family had a non-selective spectrum of activity, whereas analogs of the D-*e*-MAPP family were selective for particular cancer cell lines. Compounds LCL85, LCL420, LCL120, LCL385, LCL204, LCL286, LCL289, and LCL284 proved to be the most promising derivatives identified from this series. LCL85, LCL120, LCL420, and LCL385 had potent anticancer activity and lower toxicity, and LCL284, LCL289, and LCL286 had potent anticancer activity and cancer specificity. These attributes, combined with

Table 2. Full screen, in vitro tumor 50% growth inhibition ($\log_{10}GI_{50}$)

Cell line	Panel							
	LCL284	LCL289	LCL286	LCL204	LCL385	LCL120	LCL420	LCL85
Leukemia								
CCRF-CEM	−5.84	−5.62	−5.76	−5.70	−5.80	<−8.00	ND	5.00
HL-60(TB)	−7.06	−5.70	−5.75	−5.69	−5.48	−5.95	−5.83	−4.84
K-562	−5.85	−5.78	−5.74	−5.73	−5.65	−6.08	−6.04	−5.43
MOLT-4	−6.06	−5.64	−5.69	−5.79	−5.68	−5.60	−5.31	−4.97
RPMI-8226	−5.74	−5.70	−5.81	−5.66	−5.78	−6.54	ND	−4.70
Non-small cell lung cancer								
A549/ATCC	−5.76	−5.72	−5.69	−5.45	−5.77	−5.36	−5.29	−5.40
EKVS	−5.79	−5.70	−5.33	−5.70	−5.50	−5.59	−5.44	−4.89
HOP-62	−5.73	−5.71	−5.91	−5.72	−5.83	−5.57	−5.15	−5.86
HOP-92	−5.81	−5.82	−5.91	−5.90	−5.85	−7.39	ND	−5.68
NCI-H226	−5.73	−5.71	−5.72	−4.98	−5.49	−5.38	−5.19	−4.95
NCI-H23	−5.67	−5.74	−5.68	−4.84	−5.45	−5.53	−5.53	−4.74
NCI-H322M	−5.72	−5.69	−5.70	−5.32	−5.67	−4.96	−4.84	−5.41
NCI-H460	−5.75	−5.72	−5.74.	−5.72	−5.73	−5.53	−5.55	−6.43
NCI-H522	−5.72	−5.69	−5.68	−5.70	−5.70	−5.83	−5.69	−5.53
Colon cancer								
COLO 205	−5.72	−5.76	−5.78	−5.75	−5.73	−5.86	−5.98	−5.64
HCC-2998	−5.73	−5.74	−5.65	−5.62	−5.78	−5.82	−5.81	−5.87
HCT-116	−5.73	−5.73	−5.69	−5.71	−5.76	−5.47	−5.46	−5.78
HCT-15	−5.74	−5.72	−5.77	−5.73	−5.72	−4.75	−4.24	−5.14
HT29	−5.75	−5.73	−5.69	−5.73	−5.77	−5.80	−5.88	−5.84
KM12	−5.74	−5.73	−5.73	−5.73	−5.73	−5.76	−5.56	−5.93
SW-620	−5.67	−5.69	−5.70	−5.70	−5.62	−5.37	−5.16	−4.94
CNS cancer								
SF-268	−5.75	−5.75	−5.75	−5.68	−5.78	−5.78	−5.49	−4.89
SF-295	−5.74	−5.75	−5.75	−5.65	−5.73	−5.08	−4.84	−5.57
SF-539	−5.87	−5.75	−5.77	−5.78	−5.81	−5.69	−5.47	−5.07
SNB-19	−5.76	−5.77	−5.77	−5.76	−5.75	−5.80	−5.51	−4.90
SNB-75	−5.68	−5.77	−5.66	−5.40	−5.64	−5.53	−5.77	−4.80
U251	−5.73	−5.74	−5.76	−5.73	−5.75	−5.85	−5.53	−5.25
Melanoma								
LOX IMVI	−5.74	−5.75	−5.80	−5.76	−5.75	−5.45	−5.39	−5.66
MALME-3M	−5.71	−5.69	−5.70	−5.61	−5.68	−5.78	−5.61	−4.82
M14	−5.73	−5.71	−5.65	−5.69	−5.73	−5.74	−5.20	−5.32
SK-MEL-2	−5.70	−5.75	−5.57	−5.48	−5.67	−5.66	−5.54	−5.40
SK-MEL-28	−5.73	−5.72	−5.72	−5.72	−5.74	−5.77	−5.63	−5.47
SK-MEL-5	−4.97	−4.88	−5.75	−5.61	−5.67	−5.80	−5.79	−4.92
UACC-257	−5.78	−5.73	−5.68	−5.37	−5.77	−5.80	−5.65	−4.79
UACC-62	−5.74	−5.74	−5.70	−5.19	−5.74	−5.83	−5.73	−4.87
Ovarian cancer								
IGROV1	−5.79	−5.74	−5.72	−5.72	−5.81	−5.79	−5.50	−5.60
OVCAR-3	−5.76	−5.78	−5.78	−5.09	−5.72	−5.96	−5.88	−5.38
OVCAR-4	−5.67	−5.70	−5.72	−5.72	−5.72	−5.77	−5.78	−4.95
OVCAR-5	−5.70	−5.77	−5.62	−5.43	−5.68	−5.28	−4.96	−5.50
OVCAR-8	−5.74	−5.72	−5.72	−5.64	−5.73	−5.56	−5.41	−5.42
SK-OV-3	−5.63	−5.63	−5.66	−5.05	−5.17	−5.51	−5.48	−4.70
Renal cancer								
786-0	−5.76	−5.80	−5.74	−5.79	−5.79	−5.58	−4.99	−5.77
A498	−5.50	−5.55	−4.84	−5.17	−4.74	−4.89	−4.75	−5.05
ACHN	−5.73	−5.75	−5.77	−5.73	−5.76	−4.77	−4.66	−5.41
CAKI-1	−5.71	−5.71	−5.78	−5.12	−5.78	−5.86	−4.85	−5.85
RXF 393	−5.86	−5.78	−5.77	−5.74	−5.94	−5.34	−4.92	−5.30
SN12C	−5.75	−5.74	−5.73	−5.66	−5.76	−5.76	−5.46	−5.49
TK-10	−5.73	−5.70	−5.69	−4.98	−5.70	−5.31	−4.84	−5.60
UO-31	−5.76	−5.76	−5.36	−5.37	−5.42	−4.72	−4.54	−4.79
Prostate cancer								
PC3	−5.73	−5.69	−5.79	−5.76	−5.80	−5.86	ND	−5.69
DU-145	−5.83	−5.73	−5.84	−5.81	−5.78	−5.46	−4.90	−5.63

Table 2 (*continued*)

Cell line	Panel							
	LCL284	LCL289	LCL286	LCL204	LCL385	LCL120	LCL420	LCL85
Breast cancer								
MCF7	−5.74	−5.73	−5.76	−5.72	−5.74	−5.80	−5.83	−5.39
NCI-ADR-RES	−5.74	−5.77	−5.73	−5.69	−5.68	−4.71	>−4.00	−4.79
MDA-MB-231/ATCC	−5.76	−5.72	−5.70	−5.70	−5.76	−5.76	−5.49	−5.62
HS 578T	−5.60	−5.66	−5.62	−5.68	−5.55	−5.75	−5.62	−4.93
MDA-MB-435	−5.75	−5.75	−5.76	−5.72	−5.75	−5.80	−5.58	−5.63
BT-549	−5.95	−5.82	−8.00	−5.82	−5.89	−7.16	−8.00	−5.48
T-47D	−5.64	−5.68	−5.78	−5.42	−5.72	−5.81	−6.03	−4.79
MG-MID	−5.75	−5.71	−5.74	−5.58	−5.70	−5.67	−5.42	−5.30
Delta	1.31	0.11	2.26	0.32	0.24	2.33	2.58	1.13
Range	2.09	0.94	3.16	1.06	1.20	3.29	4.00	1.73

the synthetic ease of their preparation, suggest that these analogs are promising lead structures for cancer chemotherapy development.

4. Experimental

4.1. Chemistry

All solvents, general reagents, and starting amino alcohols 1–13 (Scheme 2) were purchased from Aldrich-Sigma & Fluka. D-*e*-MAPP, L-*e*-MAPP, (1*R*,2*R*)-B13, and (1*S*,2*S*)-B13 were prepared from the corresponding amino alcohols 5, 6, 10 and 11 as described previously.[20,21] Purity of these compounds was confirmed by ^{1}H NMR and the optical rotation values: D-*e*-MAPP: $[\alpha]_D^{23}$ +14.1 (*c* 1, MeOH); $[\alpha]_{365}^{23}$ +43.9 (*c* 1, MeOH); L-*e*-MAPP: $[\alpha]_D^{23}$ −14.9 (*c* 1, MeOH); $[\alpha]_{365}^{23}$ −45.6 (*c* 1, MeOH). (1*R*,2*R*)-B13: $[\alpha]_D^{24}$ −29.3 (*c* 1, $CHCl_3$); (1*S*,2*S*)-B13: $[\alpha]_D^{24}$ +29.6 (*c* 1, $CHCl_3$). Reaction progress was monitored with analytical normal and reverse-phase thin layer chromatography (NP TLC or RP TLC) using aluminum sheets with 0.25 mm silica gel 60-F_{254}(Merck) or 0.150 mm C18-silica gel (Sorbent Technologies). Detection was performed with PMA reagent (ammonium heptamolybdate tetrahydrate cerium sulfate, 5:2, g/g) in 125 mL of 10% H_2SO_4 and the Dragendorff reagent (Fluka) following heating of the TLC plates at 170 °C or with UV (254 nm). Flash chromatography was performed using EM Silica Gel 60 (230–400 mesh) with the indicated eluent systems. Melting points were determined in open capillaries on an Electrothermal IA 9200 melting point apparatus and are reported uncorrected. Optical rotation data were acquired using a Jasco P-1010 polarimeter. ^{1}H NMR spectra were recorded on Bruker AVANCE 500 MHz spectrometer equipped with Oxford Narrow Bore Magnet. Chemical shifts are reported in ppm on the δ scale from the internal standard of residual chloroform (7.26 ppm). Mass spectral data were recorded in a positive ion electrospray ionization (ESI) mode on Thermo Finnigan TSQ 7000 triple quadrupole mass spectrometer. Samples were infused in a methanol solution with an ESI voltage of 4.5 kV and capillary temperature of 200 °C.[55] Specific structural features were established by fragmentation pattern upon electrospray (ESI/MS/MS) conditions, specific for each of the compound studied.

4.1.1. General procedure for the preparation of LCL11–14 (class A). To a well-stirred solution of an appropriate 2-*N*-methylamino-1-phenyl-1-propanol (1–4, 0.83 g, 5.0 mmol) or their hydrochloride salts (1.0 g, 5.0 mmol) in a mixture of 50% aqueous solution of sodium acetate (24 mL) and THF (50 mL), myristoyl chloride (1.23 g, 5.0 mmol) was added dropwise at room temperature (rt). The reaction mixture was stirred for 4 h. The organic phase was separated and the aqueous layer was extracted twice with ethyl acetate (2 × 10 mL). The combined organic phases were dried over anhydrous magnesium sulfate and evaporated under reduced pressure to dryness to give a crude product. This material was purified by flash column chromatography (elution with $CHCl_3$/MeOH/concd NH_4OH, 10:2:0.01, v/v/v) following crystallization from suitable solvents.

4.1.1.1. (1*S*,2*R*)-2-(*N*-Methyl,*N*-tetradecanoylamino)-1-phenyl-1-propanol (LCL11). Prepared from **1** in 78% yield after crystallization from hexane–ethyl acetate (1:1, v/v) as a white microcrystalline powder, mp 34–36 °C; TLC ($CHCl_3$/MeOH, 5:1, v/v), R_f 0.74; $[\alpha]_D^{25}$ +10.2 (*c* 1, MeOH); $[\alpha]_{365}^{25}$ +29.8 (*c* 1, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$)δ 7.34 (m, 4H, 2,3,5,6-ArH), 7.27 (m, 1H, 4-ArH), 4.84 (m, 0.8H, 1-H), 4.67 (m, 0.2H, 1-H), 4.47 (m, 0.8H, 2-H), 4.0 (m, 0.2H, 2-H), 2.83 (s, 0.6H, NCH_3), 2.67 (s, 2.4H, NCH_3), 2.25 (m, 2H, $COCH_2$), 1.58 (m, 2H, $COCH_2CH_2$), 1.23 (m, 20H, CH_2), 1.19 (d, 3H, J = 6.8, $CHCH_3$) 0.87 (t, 3H, J = 7.0, CH_3); ESI-MS (CH_3OH, relative intensity, %) *m*/*z* 376.6 (MH^+, 100). Calcd for $[C_{24}H_{42}NO_2]^+$ *m*/*z* 376.31.

4.1.1.2. (1*R*,2*S*)-2-(*N*-Methyl,*N*-tetradecanoylamino)-1-phenyl-1-propanol (LCL12). Prepared from **2** in 76% yield after crystallization from *n*-hexane–ethyl acetate (1:1, v/v) as a white powder; $[\alpha]_D^{25}$ −10.9 (*c* 1, MeOH); $[\alpha]_{365}^{25}$ −31.2 (*c* 1, MeOH). Remaining data are identical as reported for LCL11.

4.1.1.3. (1*S*,2*S*)-2-(*N*-Methyl,*N*-tetradecanoylamino)-1-phenyl-1-propanol (LCL13). Prepared from **3** in 78% yield after crystallization from hexane–ethyl acetate (1:1, v/v) as a white microcrystalline powder, mp 30–32 °C; TLC ($CHCl_3$/MeOH, 5:1, v/v), R_f 0.66; $[\alpha]_D^{25}$ +69.2 (*c* 1, MeOH); $[\alpha]_{365}^{25}$ +264.0 (*c* 1, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33 (m, 4H, 2,3,5,6-

Table 3. Full screen, in vitro total growth inhibition ($Log_{10}TGI$)

Cell line	Panel							
	LCL284	LCL289	LCL286	LCL204	LCL385	LCL120	LCL420	LCL85
Leukemia								
CCRF-CEM	−5.48	−5.29	−5.43	−5.40	−5.45	ND	ND	−4.62
HL-60(TB)	−5.60	−5.40	−5.39	−5.32	−5.43	−5.50	−5.36	−4.52
K-562	−5.55	−5.45	−5.39	−5.42	−5.31	−5.40	−4.80	−4.90
MOLT-4	−5.66	−5.33	−5.36	−5.45	ND	−5.19	−4.73	−4.62
RPMI-8226	−5.44	−5.36	−5.47	−5.33	−5.44	−5.72	ND	−4.41
Non-small cell lung cancer								
A549/ATCC	−5.48	−5.44	−5.33	−4.71	−5.49	−4.36	−4.66	−4.77
EKVS	−5.51	−5.42	−4.77	−5.37	−4.76	−5.06	−4.83	−4.55
HOP-62	−5.48	−5.46	−5.59	−5.46	−5.55	−5.05	−4.69	−5.55
HOP-92	−5.52	−5.51	−5.55	−5.56	−5.54	−6.04	ND	−5.25
NCI-H226	−5.44	−5.42	−5.42	−4.46	−4.93	−4.64	−4.57	−4.56
NCI-H23	−5.39	−5.45	−5.35	−4.46	−5.01	−4.93	−4.81	−4.46
NCI-H322M	−5.48	−5.43	−5.41	−4.83	−5.41	−4.64	−4.50	−4.88
NCI-H460	−5.48	−5.46	−5.47	−5.45	−5.46	−4.94	−4.88	−5.78
NCI-H522	−5.45	−5.41	−5.40	−5.40	−5.42	−5.44	−4.88	−5.13
Colon cancer								
COLO 205	−5.47	−5.48	−5.50	−5.45	−5.47	−5.52	−5.31	−5.30
HCC-2998	−5.46	−5.49	−5.43	−5.25	−5.51	−5.52	−5.13	−5.54
HCT-116	−5.49	−5.46	−5.43	−5.46	−5.50	−4.88	−4.83	−5.52
HCT-15	−5.49	−5.46	−5.50	−5.46	−5.47	−4.49	>−4.00	−4.70
HT29	−5.49	−5.45	−5.40	−5.46	−5.51	−5.46	−4.93	−5.48
KM12	−5.50	−5.47	−5.47	−5.47	−5.48	−5.50	−4.94	−5.61
SW-620	−5.38		−5.39	−5.33		−4.76	−4.70	−4.62
CNS cancer								
SF-268	−5.46	−5.47	−5.45	−5.31	−5.49	−5.50	−4.88	−4.55
SF-295	−5.47	−5.47	−5.48	−5.33	−5.45	−4.62	−4.56	−4.99
SF-539	−5.57	−5.47	−5.47	−5.50	−5.51	−5.26	−4.85	−4.68
SNB-19	−5.51	−5.50	−5.50	−5.45	−5.49	−5.51	−4.88	−4.59
SNB-75	−5.41	−5.47	−5.32	−4.75	−5.25	−5.27	−4.95	−4.40
U251	−5.48	−5.49	−5.49	−5.46	−5.50	−5.56	−4.86	−4.70
Melanoma								
LOX IMVI	−5.48	−5.49	−5.52	−5.49	−5.50	−4.87	−4.83	−5.31
MALME-3M	−5.47	−5.44	−5.45	−5.31	−5.42	−5.51	−5.23	−4.54
MI4	−5.47	−5.45	−5.41	−5.43	−5.48	−5.41	−4.71	−4.75
SK-MEL-2	−5.40	−5.43	−5.26	−5.09	−5.32	−5.36	−5.13	−4.86
SK-MEL-28	−5.49	−5.46	−5.46	−5.47	−5.49	−5.50	−5.22	−4.88
SK-MEL-5	−5.46	−4.32	−5.49	−5.15	−5.39	−5.53	−5.41	−4.61
UACC-257	−5.48	−5.45	−5.32	−4.68	−5.46	−5.47	−5.08	−4.42
UACC-62	−5.46	−5.47	−5.40	−4.62	−5.43	−5.54	−5.24	−4.57
Ovarian cancer								
IGROV1	−5.51	−5.45	−5.43	−5.44	−5.49	−5.48	−4.80	−5.19
OVCAR-3	−5.49	−5.50	−5.52	−4.51	−5.39	−5.62	−5.19	−4.78
OVCAR-4	−5.42	−5.43	−5.46	−5.45	−5.46	−5.27	−4.91	−4.56
OVCAR-5	−5.46	−5.50	−5.26	−4.85	−5.42	−4.74	−4.62	−5.13
OVCAR-8	−5.42	−5.39	−5.42	−5.25	−5.42	−4.87	−4.64	−4.77
SK-OV-3	−5.32	−5.32	−5.34	−4.56	−4.61	−4.87	−4.92	−4.33
Renal cancer								
786-0	−5.50	−5.51	−5.49	−5.51	−5.52	−5.07	−4.65	−5.50
A498	−5.19	−5.00	−4.56	−4.57	−4.37	−4.55	−4.49	−4.68
ACHN	−5.48	−5.48	−5.50	−5.47	−5.50	−4.48	−4.35	−4.87
CAKI-1	−5.47	−5.47	−5.51	−4.62	−5.45	−4.52	−4.56	−5.25
RXF 393	−5.56	−5.50	−5.46	−5.41	−5.61	−4.62	−4.53	−4.67
SN12C	−5.46	−5.46	−5.43	−5.32	−5.48	−5.44	−4.83	−4.87
TK-10	−5.48	−5.46	−5.43	−4.58	−5.33	−4.80	−4.55	−5.19
UO-31	−5.49	−5.47	−4.67	−4.73	−4.75	−4.38	−4.02	−4.51
Prostate caner								
PC-3	−5.48	−5.43	−5.55	−5.50	−5.53	−5.40	ND	−5.30
DU-145	−5.55	−5.46	−5.51	−5.54	−5.50	−4.82	−4.60	−4.96

Table 3 (*continued*)

Cell line	Panel							
	LCL284	LCL289	LCL286	LCL204	LCL385	LCL120	LCL420	LCL85
Breast cancer								
MCF7	−5.47	−5.47	−5.49	−5.46	−5.48	−5.43	−5.32	ND
NCI-ADR-RES	−5.45	−5.47	−5.46	−5.37	−5.42	−4.39	>−4.00	−4.49
MDA-MB-231/ATCC	−5.47	−5.45	−5.44	−5.41	−5.47	−5.44	−4.84	−5.21
HS 578T	−5.26	−5.26	−5.30	−5.28	−5.19	−5.39	−4.87	−4.51
MDA-MB-435	−5.50	−5.49	−5.50	−5.48	−5.50	−5.53	−5.14	−5.17
BT-549	−5.62	−5.53	−5.75	−5.54	−5.58	−5.97	−6.07	−4.80
T-47D	−5.38	−5.40	−5.48	−4.85	−5.44	−5.19	−4.97	−4.47
MG-MID	−5.45	−5.42	−5.40	−5.21	−5.38	−4.96	−4.84	−4.87
Delta	0.21	0.11	0.35	0.35	0.23	1.79	1.23	0.91
Range	1.20	1.21	1.19	1.10	1.24	2.64	2.07	1.45

ArH), 7.24 (m, 1H, 4-ArH), 4.58 (m, 0.8H, 1-H), 4.40 (m, 1.2H, 1-H and 2-H), 4.0 (m, 0.2H, 2-H), 2.90 (s, 0.6H, NCH_3), 2.79 (s, 2.4H, NCH_3), 2.30 (m, 2H, $COCH_2$), 1.60 (m, 2H, $COCH_2CH_2$), 1.25 (m, 20H, CH_2), 1.11 (d, 2.4H, $J = 7.0$, $CHCH_3$), 0.98 (d, 0.6H, $J = 6.7$, $CHCH_3$), 0.87 (t, 3H, $J = 7.0$, CH_3); ESI-MS (CH_3OH, relative intensity, %) *m/z* 376.5 (MH^+, 100). Calcd for $[C_{24}H_{42}NO_2]^+$ *m/z* 376.31.

4.1.1.4. (1*R*,2*R*)-2-(*N*-Methyl,*N*-tetradecanoylamino)-1-phenyl-1-propanol (LCL14). Prepared from **4** in 72% yield after crystallization from *n*-hexane–ethyl acetate (1:1, v/v) as a white powder; $[\alpha]_D^{25}$ −68.7 (*c* 1, MeOH); $[\alpha]_{365}^{25}$ −263.0 (*c* 1, MeOH). Remaining data are identical as reported for LCL13.

4.1.2. General procedure for the preparation of LCL15-17 and LCL81 (class B). To a well-stirred solution of the appropriate 2-amino-1-phenyl-1-propanol or 2-amino-1-phenyl-1,3-propandiol (5, 6, 10, or 11, 3.0 mmol) in anhydrous tetrahydrofuran (12 mL) and anhydrous ethanol (1.0 mL), dodecyl isocyanate (98%, 0.90 mL, 3.75 mmol) was added dropwise over 1 min. Reaction mixture was stirred under dry nitrogen at rt for 4 h and evaporated under reduced pressure to dryness, and the residue was dried in a high vacuum at rt for 2 h. The crude product was purified by gradient flash column chromatography (ethyl acetate–*n*-hexane, 6:1, v/v, following pure ethyl acetate) and recrystallized from a suitable solvent.

4.1.2.1. (1*R*,2*R*)-3-Dodecyl-1-[1′-hydroxy-2′-hydroxymethyl-1′-(4″-nitrophenyl)ethyl]-urea (LCL15). Prepared from **10** in 69% yield after recrystallization from *n*-hexane–diethyl ether (1:3, v/v) as a pale yellow microcrystalline powder, mp 82–84 °C; TLC (ethyl acetate) R_f 0.17 (lit. R_f 0.14)[49]; $[\alpha]_D^{20}$ −53.6 (*c* 1, MeOH) (lit. $[\alpha]_D^{20}$ −50.98, *c* 0.4, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 8.17 (d, 2H, $J = 8.9$, 3,5-ArH), 7.63 (d, 2H, $J = 8.3$, 2,6-ArH), 5.11 (d, 1H, $J = 2.4$, 1-H), 3.90 (m, 1H, 2-H), 3.69 (dd, 1H, $J = 10.6$ and 7.9, 3-Ha), 3.56 (dd, 1H, $J = 10.6$ and 5.5, 3-Hb), 2.90 (m, 2H, $NHCH_2$), 1.28 (m, 20H, CH_2), 0.89 (t, 3H, $J = 7.0$, CH_3); ESI-MS (CH_3OH, relative intensity, %) *m/z* 424.2 (MH^+, 15), 267.1 (100). Calcd for $[C_{22}H_{38}N_3O_5]^+$ *m/z* 424.3.

4.1.2.2. (1*S*,2*R*)-3-Dodecyl-1-(1′-hydroxy-2′-methyl-1′-phenylethyl)-urea (LCL16). Prepared from **5** in 73% yield after recrystallization from *n*-hexane–diethyl ether (1:2, v/v) as white microcrystalline needles, mp 68.5–70 °C; TLC ($CHCl_3$–MeOH, 5:1, v/v) R_f 0.36; TLC (*n*-hexane–ethyl acetate, 2:1, v/v) R_f 0.48 (lit. R_f 0.46)[49]; $[\alpha]_D^{20}$ +12.0 (*c* 1, MeOH), (lit. $[\alpha]_D^{20}$ +15.81, *c* 0.2, MeOH)[49]; $[\alpha]_{365}^{20}$ +25.4 (*c* 1, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30 (m, 4H, 2,3,5,6-ArH), 7.25 (m, 1H, 4-ArH), 4.77 (d, 1H, $J = 1.3$, 1-H), 4.55 (br s, 2H, NH or OH), 4.35 (1H, NH or OH), 4.17 (m, 1H, 2-H), 3.12 (m, 2H, $NHCH_2$), 1.47 (m, 2H, $NHCH_2CH_2$), 1.23(m, 18H, CH_2), 0.98 (d, 3H, $J = 6.8$, $CHCH_3$) 0.87 (t, 3H, $J = 7.0$, CH_3); ESI-MS (CH_3OH, relative intensity, %) *m/z* 363.2 (MH^+, 20), 345.2 ($[MH-H_2O]^+$, 100). Calcd for $[C_{22}H_{39}N_2O_2]^+$ *m/z* 363.29.

4.1.2.3. (1*R*,2*S*)-3-Dodecyl-1-(1′-hydroxy-2′-methyl-1′-phenylethyl)urea (LCL17). Prepared from **6** in 75% yield, $[\alpha]_D^{21}$ −11.5 (*c* 1, MeOH); $[\alpha]_{365}^{21}$ −27.5 (*c* 1, MeOH), (lit. $[\alpha]_D^{20}$ −27.27, *c* 0.1, MeOH).[49] The remaining analytical data are identical as reported for LCL16.

4.1.2.4. (1*S*,2*S*)-3-Dodecyl-1-[1′-hydroxy-2′-hydroxymethyl-1′-(4″-nitrophenyl)ethyl]-urea (LCL81). Prepared from **11** in 70% yield. $[\alpha]_D^{21}$ +46.4 (*c* 1, MeOH), (lit. $[\alpha]_D^{21}$ +52.15, *c* 0.3, MeOH).[49] The remaining analytical data are identical as reported for LCL15.

4.1.3. General procedure for the preparation of class C analogs: LCL18, 204, 284, 286, 289, 343, 346, 347, 378, 381, 385, and 397. To a well-stirred mixture of the appropriate 2-amino-1-phenyl-propanols (4.4 mmol) and aldehydes (3.5 mmol) in methanol (15 mL) and glacial acetic acid (1.0 mL), sodium cyanoborohydride (300 mg, 4.77 mmol) was added portion-wise over 1 min at room temperature and reaction mixture was stirred for an additional 30 min. The reaction mixture was evaporated under a reduced pressure to dryness and the obtained residue was dried under high vacuum (~1 Torr at room temperature over 6 h). The crude product was purified by flash column chromatography using chloroform–methanol–concentrated ammonium hydroxide (5:1:0.1; v/v/v) to give a pure product as a

Table 4. Full screen, in vitro tumor 50% lethal concentration ($\log_{10}LC_{50}$)

Cell line	Panel							
	LCL284	LCL289	LCL286	LCL204	LCL385	LCL120	LCL420	LCL85
Leukemia								
CCRF-CEM	−5.12	>−4.00	−5.11	ND	−5.10	ND	ND	−4.24
HL-60(TB)	−5.18	−5.09	−5.03	−4.82	−5.07	ND	−4.50	−4.20
K-562	−5.25	−5.13	−5.03	−5.11	>−4.00	>−4.00	−4.11	−4.41
MOLT-4	−5.31	ND	−5.03	−5.12	ND	>4.00	−4.20	−4.27
RPM1-8226	−5.14	ND	−5.13	>−4.00	−5.11	−5.29	ND	−4.12
Non-small cell lung cancer								
A549/ATCC	−5.20	−5.15	−4.85	>−4.00	−5.22	>−4.00	−4.17	−4.17
EKVS	−5.22	ND	−4.34	−5.05	−4.04	>−4.00	−4.37	−4.22
HOP-62	−5.23	−5.21	−5.27	−5.20	−5.27	−4.42	−4.35	−5.24
HOP-92	−5.23	ND	−5.19	−5.21	−5.23	−5.24		−4.68
NCI-H226	−5.15	ND	ND	−4.00	>−4.00	>−4.00	−4.05	−4.17
NCI-H23	−5.10	−5.17	−5.01	−4.08	−4.19	−4.25	−4.31	−4.19
MCI-H322M	−5.23	−5.18	−5.13	−4.37	−5.14	−4.31	−4.28	−4.44
MCI-H460	−5.21	−5.20	−5.21	ND	ND	>−4.00	−4.38	−4.96
MCI-H522	−5.18	−5.13	ND	ND	ND	ND	−4.30	−4.51
Colon cancer								
COLO 205	−5.21	−5.21	−5.22	ND	−5.21	ND	−4.50	−4.87
HCC-2998	−5.19	−5.24	−5.21	−4.53	−5.25	−5.22	−4.51	−5.21
HCT-116	−5.24	−5.19	−5.17	−5.21	−5.25	−4.23	−4.41	−5.26
HCT-15	−5.23	−5.20	−5.23	ND	−5.22	−4.23	>−4.00	−4.33
HT29	−5.24	−5.17	−5.11	ND	−5.25	−5.12	−4.46	−5.12
KM12	−5.25	−5.20	−5.21	−5.21	−5.23	−5.24	−4.44	−5.30
SW-620	−5.10	>−4.00	−5.09	−4.00	>−4.00	−4.18	−4.34	−4.31
CNS cancer								
SF-268	−5.17	−5.18	−5.15	>−4.00	ND	−5.22	−4.36	−4.21
SF-295	−5.21	−5.19	−5.21	−5.01	−5.17	−4.20	−4.27	−4.42
SF-539	−5.26	ND	−5.17	−5.22	−5.22	>−4.00	−4.40	−4.34
SNB-19	ND	−5.23	−5.23	ND	ND	ND	−4.42	−4.28
SNB-75	−5.15	−5.18	−4.85	−4.22	−4.56	−5.01	−4.42	>−4.00
U251	−5.23	−5.23	ND	ND	−5.25	−5.28	−4.41	−4.23
Melanoma								
LOX IMVI	−5.23	−5.23	−5.24	−5.22	−5.24	−4.29	−4.35	−4.91
MALME-3M	−5.22	−5.19	−5.20	−5.02	−5.17	−5.24	−4.70	−4.27
M14	−5.21	−5.20	−5.17	−5.16	−5.23	−5.08	−4.33	−4.31
SK-MEL-2	−5.09	ND	>−4.00	>−4.00	>−4.00	ND	−4.22	−4.03
SK-MEL-28	−5.24	−5.20	−5.20	−5.22	−5.25	−5.24	−4.69	−4.44
SK-MEL-5	−4.00	>−4.00	−5.22	>−4.00	ND	−5.26	−5.02	−4.31
UACC-257	−5.19	−5.17	−4.75	−4.03	−5.14	ND	−4.41	−4.05
UACC-62	−5.18	−5.20	−5.10	−4.12	−5.12	−5.24	−4.60	−4.26
Ounrijifj cancer								
1GROV1	−5.24	ND	ND	ND	ND	ND	−4.24	−4.65
OVCAR-3	−5.22	ND	−5.26	−4.00	ND	ND	−4.58	−4.26
OVCAR-4	−5.17	−5.16	−5.20	−5.17	ND	>−4.00	−4.37	−4.18
OVCAR-5	−5.21	−5.24	−4.80	−4.14	−5.15	−4.30	−4.29	−4.61
OVCAR-8	ND	ND	ND	>−4.00	ND	>−4.00	>−4.00	−4.09
SK-OV-3	−5.01	−5.01	−5.03	−4.10	−4.10	−4.22	−4.45	>−4.00
Renal cancer								
786-0	−5.24	−5.23	−5.23	−5.24	−5.25	−4.46	−4.31	−5.24
A498	−4.13	>−4.00	−4.28	−4.03	>−4.00	−4.21	−4.24	−4.33
ACHN	−5.23	−5.22	−5.23	−5.21	−5.25	−4.19	−4.04	−4.44
CAK1-1	−5.24	−5.23	−5.25	−4.17	−5.11	−4.18	−4.27	−4.59
RXF393	−5.26	−5.22	−5.16	−5.08	−5.29	−4.01	−4.13	−4.20
SN12C	−5.17	−5.17	−5.13	>−4.00	−5.19	−5.11	−4.32	−4.38
TK-10	−5.24	−5.21	−5.17	−4.18	−4.86	−4.39	−4.25	−4.65
UO-31	−5.23	−5.18	>−4.00	−4.06	−4.10	−4.03	>−4.00	−4.22
Prostate cancer								
PC-3	−5.23	ND	−5.25	ND	−5.26	−4.82	ND	−4.80
DU-145	−5.28	−5.19	−5.22	−5.27	−5.21	−4.31	−4.30	−4.48

Table 4 (*continued*)

Cell line	Panel							
	LCL284	LCL289	LCL286	LCL204	LCL385	LCL120	LCL420	LCL85
Breast cancer								
MCF7	−5.20	−5.21	−5.22	ND	ND	−5.07	−4.68	−4.37
NCI-ADR-RES	ND	ND	−5.20	−5.05	−5.16	−4.07	>−4.00	−4.20
MDA-MB-231/ATCC	−5.19	−5.19	−5.18	−5.11	−5.18	−5.12	−4.37	−4.56
HS 578T	−4.33	>−4.00	−4.96	−4.00	>−4.00	−5.04	>−4.00	−4.09
MDA-MB-435	−5.25	−5.24	−5.25	−5.24	−5.25	−5.26	−4.59	−4.61
BT-549	−5.30	−5.24	−5.34	−5.25	−5.26	−5.47	−5.06	−4.39
T-47D	−5.11	−5.12	−5.16	−4.06	ND	>−4.00	−4.23	−4.16
MG-MID	−5.15	−5.06	−5.07	−4.61	−4.93	−4.23	−4.35	−4.44
Delta	0.16	0.18	0.27	0.66	0.36	1.78	0.71	0.86
Range	1.31	1.24	1.34	1.27	1.29	2.41	1.06	1.30

white microcrystalline powder or a waxy solid. These products were dissolved in anhydrous ethyl acetate (15 mL) and 1 M HCl solution in a dry diethyl ether (7.0 mL) was added dropwise at +4 °C. The reaction mixture was stirred at rt for an additional 10 min. The mixture was evaporated under reduced pressure to dryness and the afforded residue was dried in a high vacuum for 2 h at room temperature. The crude hydrochlorides were recrystallized from a suitable solvent as listed below.

4.1.3.1. (1*R*,2*R*)-2-(*N*-Tetradecylamino)-1-(4′-nitrophenyl)-1,3-propandiol hydrochloride (LCL204). Prepared from **10** and tetradecyl aldehyde in 57% yield. Analytical sample of LCL204 was obtained by crystallization from ethyl acetate–*n*-hexane (1:1, v/v) as a white powder, mp 101–103 °C. TLC ($CHCl_3$/MeOH/concd NH_4OH, 5:1:0.1, v/v/v) $R_f = 0.35$; $[\alpha]_D^{20}$ − 30.5 (*c* 1, MeOH); ^1H NMR (500 MHz, CD_3OD) δ 8.28 (d, 2H, *J* = 8.8, ArH), 7.72 (d, 2H, *J* = 8.8, ArH), 5.02 (d, 1H, *J* = 9.2, 1-H), 3.67 (dd, 1H, *J* = 12.5, 3.4, 1-Ha), 3.38 (dd, 1H, *J* = 12.5, 4.1, 1-Hb), 3.34 (m, 1H, 2-H), 3.15 (m, 2H, $NHCH_2$), 1.73 (m, 2H, $NHCH_2CH_2$), 1.30 (m, 22H, CH_2), 0.89 (t, 3H, *J* = 7.1, CH_3); ESI-MS (CH_3OH, relative intensity, %) *m*/*z* 409.5 (M^+, 100), 391 ($[M-H_2O]^+$, 10). Calcd for $[C_{23}H_{41}N_2O_4]^+$ *m*/*z* 409.31. Anal. Calcd for $C_{23}H_{41}ClN_2O_4$(445.0): C, 62.07; H, 9.29; N, 6.29; Cl, 7.97. Found: C, 61.29; H, 9.22; N, 6.21; Cl, 7.86.

4.1.3.2. (1*S*,2*S*)-2-(*N*-Tetradecylamino)-1-(4′-nitrophenyl)-1,3-propandiol hydrochloride (LCL343). Prepared from **11** and tetradecyl aldehyde in 61% yield, $[\alpha]_D^{20}$ + 31.5 (*c* 1, MeOH). The remaining analytical data are identical as reported for LCL204.

4.1.3.3. (1*R*,2*R*)-2-*N*-(Tetradecylamino)-1-phenyl-1,3-propandiol hydrochloride (LCL385). Prepared from **12** and tetradecyl aldehyde in 59% yield. Analytical sample was obtained by crystallization from *n*-hexane–ethyl acetate (4:2, v/v) as a white powder, mp > 170 (with decomp.); $[\alpha]_D^{21}$ − 40.0 (*c* 1, MeOH). ^1H NMR (500 MHz, $CDCl_3$) δ 7.38 (d, 1H, *J* = 6.8, NH), 7.30 (m, 5H, ArH), 5.13 (d, 1H, *J* = 9.4, 1-H), 3.73 (dd, 1H, *J* = 13.4, 2.3, 1-Ha), 3.53 (dd, 1H, *J* = 13.4, 4.7, 1-Hb), 3.16 (m, 1H, 2-H), 3.08 (m, 2H, $NHCH_2$), 1.85 (m, 2H, $NHCH_2CH_2$), 1.30 (m, 2H, $NHCH_2CH_2CH_2$), 1.20 (m, 20H, CH_2), 0.82 (t, 3H, *J* = 7.1, CH_3); ESI-MS (CH_3OH, relative intensity, %) *m*/*z* 364.4 (M^+, 100). Calcd for $[C_{23}H_{42}NO_2]^+$ *m*/*z* 364.32.

4.1.3.4. (1*S*,2*S*)2-(*N*-Tetradecylamino)-1-phenyl-1,3-propandiol hydrochloride (LCL18). Prepared from **13** and tetradecyl aldehyde in 60% yield, $[\alpha]_D^{21}$ + 38.5 (*c* 1, MeOH). The remaining analytical data are identical as reported for LCL385.

4.1.3.5. (1*S*,2*R*)-2-*N*-(Tetradecylamino)-1-phenyl-1-propanol hydrochloride (LCL284). Prepared from **5** and tetradecyl aldehyde in 64% yield. Analytical sample was obtained by crystallization from anhydrous ethyl acetate as a white powder, mp > 177 °C (with decomp.); TLC ($CHCl_3$/MeOH/concd NH_4OH, 8:1:0.1, v/v/v) $R_f = 0.65$; $[\alpha]_D^{20}$ + 14.5 (*c* 1, MeOH) and −16.7 (*c* 1, $CHCl_3$); $[\alpha]_{365}^{20}$ + 41.2 (*c* 1, MeOH) and −58.0 (*c* 1, $CHCl_3$); ^1H NMR (500 MHz, CD_3OD) δ 7.40 (m, 4H, 2,3,5,6-ArH), 7.38 (m, 1H, 4-ArH), 5.13 (d, 1H, *J* = 3.0, 1-H), 3.46 (dd, 1H, *J* = 3.0, 6.7, 2-H), 3.09 (t, 2H, *J* = 4.3, $NHCH_2$), 1.74 (m, 2H, $NHCH_2CH_2$), 1.30 (m, 22H, CH_2), 1.05 (d, 3H, *J* = 6.8, $CHCH_3$) 0.89 (t, 3H, *J* = 7.1, CH_3); ESI-MS (CH_3OH, relative intensity, %) *m*/*z* 348.3 (M^+, 100). Calcd for $[C_{23}H_{42}NO]^+$ *m*/*z* 348.33. Anal. Calcd for $C_{23}H_{42}ClNO$ (384.04): C, 71.93; H, 11.02; N, 3.65; Cl, 9.23. Found: C, 71.77; H, 11.08; N, 3.69; Cl, 9.53.

4.1.3.6. (1*R*,2*S*)-2-(*N*-Tetradecylamino)-1-phenyl-1-propanol hydrochloride (LCL289). Prepared from **6** and tetradecyl aldehyde in 62% yield, $[\alpha]_D^{20}$ − 13.3 (*c* 1, MeOH) and +16.0 (*c* 1, $CHCl_3$); $[\alpha]_{365}^{20}$ − 40.5 (*c* 1, MeOH) and +59.0 (*c* 1, $CHCl_3$). Remaining analytical data are identical as reported for LCL284.

4.1.3.7. (1*R*,2*R*)-2-(*N*-Tetradecylamino)-1-phenyl-1-propanol hydrochloride (LCL346). Prepared from **7** and tetradecyl aldehyde in 60% yield. Analytical sample was obtained by crystallization from anhydrous ethyl acetate as a white powder, mp > 155°C; $[\alpha]_D^{23}$ − 33.2 (*c* 1, $MeOH_)$ and $[\alpha]_{365}^{23}$ − 107.3 (*c* 1, MeOH); ^1H NMR (500 MHz, MeOD) δ 9.84 (br s, 1H, NH), 8.33 (br s, 1H, NH), 7.35 (m, 5H, 2,6-ArH), 4.83 (d, 1H, *J* = 7.6, 1-H), 3.40 (m, 1H, $NHCH_2$), 3.16 (m, 1H, $NHCH_2$), 2.90 (m, 1H, 2-H), 1.94 (m, 2H, $NHCH_2CH_2$), 1.61 (m, 2H, CH_2), 1.25 (m, 24H, CH_3 and CH_2), 0.87 (t, 3H, *J* = 7.1, CH_3); ESI-MS (CH_3OH, relative intensity,

%) *m/z* 348.3 (M^+, 100). Calcd for $[C_{23}H_{42}NO]^+$ *m/z* 348.33.

4.1.3.8. (2*S*)-2-*N*-(Tetradecylamino)-1-phenyl-3-propanol hydrochloride (LCL286). Prepared from **8** and tetradecyl aldehyde in 65% yield. Analytical sample was obtained by crystallization from anhydrous ethyl acetate as a white powder, mp >165 °C (with decomp.); $[\alpha]_D^{24}$ −7.70 (*c* 1, MeOH) and $[\alpha]_{365}^{24}$ −25.6 (*c* 1, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.25 (br s, 1H, NH), 8.89 (br s, 1H, NH), 7.27 (m, 5H, 2-6-ArH), 3.81 (dd, 1H, *J* = 2.4 and 13.0, 1-Ha), 3.72 (dd, 1H, *J* = 6.6 and 13.0, 1-Hb), 3.26 (m, 1H, 2-H), 3.19 (dd, 1H, *J* = 5.4 and 13.6, 3-Ha), 3.01 (dd, 1H, *J* = 9.4 and 13.6, 3-Hb), 2.93 (m, 1H, NHCH_2), 2.83 (m, 1H, NHCH_2) 1.82 (m, 2H, NHCH$_2$CH_2), 1.18 (m, 23H, CH_2), 0.81 (t, 3H, *J* = 7.1, CH_3); ESI-MS (CH_3OH, relative intensity, %) *m/z* 348.4 (M^+, 100). Calcd for $[C_{23}H_{42}NO]^+$ *m/z* 348.33.

4.1.3.9. (2*R*)-2-(*N*-Tetradecylamino)-1-phenyl-3-propanol hydrochloride (LCL347). Prepared from **9** and tetradecyl aldehyde in 61% yield, $[\alpha]_D^{20}$ +5.22 (*c* 1, MeOH) and $[\alpha]_{365}^{20}$ +19.4 (*c* 1, MeOH). Remaining analytical data are identical as reported for LCL286.

4.1.3.10. (1*S*,2*R*)-2-(*N*-Methyl,*N*-tetradecylamino)-1-phenyl-1-propanol hydrochloride (LCL381). Prepared from **1** and tetradecyl aldehyde in 59% yield. An analytical sample was obtained by crystallization from anhydrous ethyl acetate as a white powder, mp 107–109 °C; $[\alpha]_D^{24}$ +5.54 (*c* 1, MeOH) and $[\alpha]_{365}^{20}$ +18.2 (*c* 1, MeOH). ^{1}H NMR (500 MHz, CD_3OD) δ.43 (m, 4H, 2,3,5 and 6-ArH), 7.35 (m, 1H, 4-ArH), 5.21 (d, 1H, *J* = 2.1, 1-H), 3.53 (m, 1H, 2-H), 2.96 (s, 3H, NHCH_3), 1.78 (m, 2H, NHCH_2), 1.42 (m, 4H, CH_2 and NHCH$_2$CH_2), 1.28 (m, 23H, CH_2), 1.14 (d, 3H, *J* = 6.8, CHCH_3) 0.89 (t, 3H, *J* = 7.1, CH_3); ESI-MS (CH_3OH, relative intensity, %) *m/z* 362.3 (M^+, 100). Calcd for $[C_{24}H_{44}NO]^+$ *m/z* 362.34.

4.1.3.11. (1*S*,2*R*)-2-(*N*-Dodecylamino)-1-phenyl-1-propanol hydrochloride (LCL378). Prepared from **5** and dodecyl aldehyde in 58% yield. Analytical sample was obtained by crystallization from anhydrous ethyl acetate as a white powder, mp >198 °C (with decomp.); TLC ($CHCl_3$/MeOH/concd NH_4OH, 8:1:0.1, v/v/v) R_f = 0.60; $[\alpha]_D^{23}$ +8.3 (*c* 0.5, MeOH) and $[\alpha]_{365}^{23}$ +32.0 (*c* 0.5, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 7.39 (m, 4H, 2,3,5,6-ArH), 7.36 (m, 1H, 4-ArH), 5.11(d, 1H, *J* = 3.1, 1-H), 3.43 (dd, 1H, *J* = 3.1, 6.8, 2-H), 3.10 (t, 2H, *J* = 4.5, NHCH_2), 1.72 (m, 2H, NHCH$_2$CH_2), 1.30 (m, 20H, CH_2), 1.03 (d, 3H, *J* = 6.8, CHCH_3) 0.88 (t, 3H, *J* = 7.1, CH_3); ESI-MS (CH_3OH, relative intensity, %) *m/z* 320.4 (M^+, 100). Calcd for $[C_{21}H_{38}NO]^+$ *m/z* 320.29.

4.1.3.12. (1*S*,2*R*)-2-(*N*-Hexadecylamino)-1-phenyl-1-propanol hydrochloride (LCL397). Prepared from 5 and hexadecyl aldehyde in 62% yield. Analytical sample was obtained by crystallization from anhydrous ethyl acetate as a white powder, mp >195 °C (with decomp.); TLC ($CHCl_3$/MeOH/concd NH_4OH, 8:1:0.1, v/v/v) R_f = 0.67; $[\alpha]_D^{23}$ +14.8 (*c* 0.5, MeOH) and $[\alpha]_{365}^{20}$ +38.6 (*c* 0.5, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 7.41 (m, 4H, 2,3,5,6-ArH), 7.37 (m, 1H, 4-ArH), 5.12(d, 1H, *J* = 3.0, 1-H), 3.44 (dd, 1H, *J* = 3.1, 6.7, 2-H), 3.08 (t, 2H, *J* = 4.2, NHCH_2), 1.73 (m, 2H, NHCH$_2$CH_2), 1.30 (m, 24H, CH_2), 1.04 (d, 3H, *J* = 6.8, CHCH_3) 0.89 (t, 3H, *J* = 7.1, CH_3); ESI-MS (CH_3OH, relative intensity, %) *m/z* 376.5 (M^+, 100). Calcd for $[C_{25}H_{46}NO]^+$ *m/z* 376.36.

4.1.4. General procedure for the preparation of LCL82, LCL85, LCL120 and LCL420 (class D)

- Preparation of ω-Br analogs **14**–**17**.

4.1.4.1. (1*S*,2*R*)-2-*N*-(16′-Bromohexadecanoylamino)-1-phenyl-1-propanol (14). Prepared from **1** and 16-bromohexadecanoyl chloride[17] according to the general procedure for class A. The crude product was purified by flash column chromatography ($CHCl_3$/MeOH/concd NH_4OH, 10:1:0.05, v/v/v) to give a pure bromo-analog 14 in 79% yield. Analytical sample of 14 was obtained by recrystallization from *n*-hexane–ethyl acetate (3:1, v/v) to give a white microcrystalline powder, mp 77–78,5°C; TLC R_f ($CHCl_3$/MeOH, 8:1, v/v) R_f 0.68. $[\alpha]_D^{22}$ +13.4 (*c* 1, MeOH); $[\alpha]_{365}^{22}$ +37.8 (*c* 1, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32 (m, 4H, 2,3,5,6-ArH), 7.27 (m, 1H, 4-ArH), 5.46 (d, 1H, *J* = 7.4, NH), 4.83 (d, 1H, *J* = 2.8, 1-H), 4.34 (m, 1H, 2-H), 3.39 (t, 2H, *J* = 6.9, CH_2Br), 2.17 (t, 2H, *J* = 7.1, COCH_2), 1.84 (p, 2H, *J* = 7.2, CH_2CH$_2$Br), 1.61 (m, 2H, COCH$_2$CH_2), 1.40 (m, 2-H, CH_2CH$_2$CH$_2$Br), 1.25(m, 20H, CH_2), 1.01 (d, 3H, *J* = 6.9, CHCH_3); ESI-MS (CH_3OH, relative intensity, %) *m/z* 468.3 (MH^+, 100). Calcd for $[C_{25}H_{43}BrNO_2]^+$ *m/z* 468.24.

4.1.4.2. (1*R*,2*S*)-2-*N*-(16′-Bromohexadecanoylamino)-1-phenyl-1-propanol (15). Prepared from **2** and 16-bromohexadecanoyl chloride[17] in 75% yield according to the general procedure for class A. $[\alpha]_D^{20}$ −13.2 (*c* 1, MeOH); $[\alpha]_{365}^{20}$ −38.5 (*c* 1, MeOH). Remaining analytical data are identical as reported for 14.

4.1.4.3. (1*R*,2*R*)-2-*N*-(16′-Bromohexadecanoylamino)-1-(4′-nitrophenyl)-1,3-propandiol (16). Prepared from **10** and 16-bromohexadecanoyl chloride[17] in 72% yield according to the general procedure for class A. The crude product was purified by flash column chromatography ($CHCl_3$/MeOH/concd NH_4OH, 10:1:0.05, v/v/v) to give a pure corresponding bromo-analog 16 (457 mg, 75% yield) as a pale yellow powder. Analytical sample of 16 was obtained by recrystallization from *n*-hexane–ethyl acetate (2:1, v/v) to give a white microcrystalline powder, mp 79-81°C; TLC R_f ($CHCl_3$/MeOH, 8:1, v/v) R_f 0.38. $[\alpha]_D^{20}$ −1.90 (*c* 1, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.19 (d, 2H, *J* = 8.8, 3,5-ArH), 7.55 (d, 2H, *J* = 8.4, 2,6-ArH), 6.08 (d, 1H, *J* = 7.9, NH), 5.20 (d, 1H, *J* = 4.7, 1-H), 4.13 (m, 1H, 2-H), 3.88 (d, 2H, *J* = 4.3, 3-Hab), 3.40 (t, 2H, *J* = 6.8, CH_2Br), 2.11 (m, 2H, COCH_2), 1.83 (p, 2H, *J* = 7.2, CH_2CH$_2$Br), 1.47(m, 2H, COCH$_2$CH_2), 1.43 (m, 2-H, CH_2CH$_2$CH$_2$Br), 1.23 (m, 20H, CH_2); ESI-MS (CH_3OH, relative intensity, %) *m/z* 529.3 (MH^+, 100). Calcd for $[C_{25}H_{42}BrN_2O_5]^+$ *m/z* 529.22.

4.1.4.4. (1*R*,2*R*)-2-*N*-(Tetradecanoylamino)-1-(4′-aminophenyl)-1,3-propandiol (17). To a well-stirred and cooled mixture of Zn dust (1.1 g) and 80% acetic acid (20 mL), B13 (1.05 g, 2.5 mmol) was added portion-wise over 5 min. The mixture was stirred for an additional 10 min and then gently heated to 50 °C to dissolve the formed white solid. The hot reaction mixture was filtered off and the filtrate was cooled down to rt and refrigerated for 30 min. The formed precipitate was separated by filtration and the filtrate was kept in the freezer (−10 °C) overnight. The formed crude product was separated by filtration, washed with 0.5 N NH_4OH solution, water and dried at rt for 24 h. This material was recrystallized twice from ethanol–water (3:4, v/v) to give pure 17 (0.340 g, 29%) as a pale yellow microcrystalline powder, mp 124–126°C. TLC ($CHCl_3$/MeOH/concd NH_4OH, 4:1:0.1, v/v/v) $R_f = 0.41$; $[\alpha]_D^{24}$ − 16.5 (*c* 0.5, MeOH); ^1H NMR (500 MHz, CD_3OD) δ 7.55 (d, 2H, *J* = 8.1, ArH), 7. 31 (d, 2H, *J* = 8.5, ArH), 5.02 (d, 1H, *J* = 3.2, 1-H), 4.00 (m, 1H, 2-H), 3.71 (dd, 1H, *J* = 7.1 and 10.8, 3-Ha), 3.51(dd, 1H, *J* = 5.7 and 10.8, 3-Hb), 2.09 (m, 2H, $COCH_2$), 1.43 (m, 2H, $COCH_2CH_2$), 1.28 (m, 23H, CH_2), 0.89 (t, 3H, *J* = 7.1, CH_3); ESI-MS (CH_3OH, relative intensity, %) *m*/*z* 393.4. (MH^+, 100). Calcd for $[C_{23}H_{41}N_2O_3]^+$ *m*/*z* 393.3.

4.1.4.5. (1*R*,2*R*)-2-*N*-(Tetradecanoylamino)-1-[4′-*N*-(6″-bromohexanoyl)-aminophenyl]-1,3-propandiol (18). Prepared from **17** and 6-bromohexanoyl chloride in 63% yield according to the general procedure for class A. Analytical sample of 18 was obtained by recrystallization from ethyl acetate to give a white microcrystalline powder, mp 131-133°C. TLC ($CHCl_3$/MeOH, 9:1, v/v) $R_f = 0.37$; ^1H NMR (500 MHz, CD_3OD) δ 7.55 (d, 2H, *J* = 8.1, ArH), 7. 31 (d, 2H, *J* = 8.5, ArH), 5.02 (d, 1H, *J* = 3.2, 1-H), 4.00 (m, 1H, 2-H), 3.71 (dd, 1H, *J* = 7.1 and 10.8, 3-Ha), 3.51(dd, 1H, *J* = 5.7 and 10.8, 3-Hb), 2.09 (m, 2H, $COCH_2$), 1.43 (m, 2H, $COCH_2CH_2$), 1.28 (m, 23H, CH_2), 0.89 (t, 3H, *J* = 7.1, CH_3); ESI-MS (CH_3OH, relative intensity, %) *m*/*z* 569.4 (MH^+, 100). Calcd for $[C_{29}H_{49}BrN_2O_4]^+$*m*/*z* 569.29.

- Preparation of aromatic ceramidoids (ω-pyridinium analogs).

4.1.5. General procedure for cationization of 14–18. Mixtures of 14–16 and 18 (0.50 mmol), anhydrous pyridine (2 mL), and anhydrous toluene (2 mL) were heated in a sealed glass test-tube in an oil bath at 75–85 °C over 6 h. After completion, the reaction mixture was cooled and evaporated to dryness. The residues were dried under the high vacuum (~1 Torr at rt over 6 h), washed twice with ethyl acetate–*n*-hexane (4:1, v/v/, 2 × 10 mL), and the afforded crude products were purified as described below.

4.1.5.1. (1*S*,2*R*)2-*N*-[16-(1′-Pyridinium)-hexadecanoylamino]-1-phenyl-1-propanol bromide (LCL120). Prepared from **14**. Crude product was dissolved in distilled water (10 mL) and extracted with dichloromethane (2 × 4.0 mL). The organic fractions were extracted back with water (2 × 4 mL) and all the aqueous fractions were combined and evaporated under reduced pressure at room temperature. The obtained residue was lyophilized in a high vacuum to give a pure LCL120 in 81% as a colorless oil. Analytical sample of LCL120 was prepared by recrystallization from anhydrous acetone–ethanol (8:1, v/v) to give a colorless semi-solid. TLC ($CHCl_3$/$(CH_3)_2CO$/MeOH/CH_3COOH/H_2O, 20:8:8:2:1, v/v) R_f 0.49; RP TLC (C18 Silica, CH_3CN/MeOH/1 M NH_4Cl (aq), 4:1:1.5, v/v) R_f 0.46; $[\alpha]_D^{21}$ + 9.1 (*c* 1, MeOH); $[\alpha]_{365}^{21}$ + 30.4 (*c* 1, MeOH); ^1H NMR (500 MHz, CD_3OD) δ 9.00 (d, 2H, *J* = 5.6, 2,5-H_{Py}) 8.59 (t, 1H, *J* = 7.9, 4-H_{Py}), 8.11 (t, 2H, *J* = 7.0, 3,5-H_{Py}), 7.36 (m, 2H, ArH), 7.28 (m, 2H, ArH), 7.21 (m, 1H, ArH), 4.62 (m, 3H, 1-H and C(16)H_2-pyridinium ring), 4.12 (m, 1H, 2-H), 2.07 (m, 2H, $COCH_2$), 2.01 (m, 2H, C(15)H_2C(16)H_2-pyridinium ring), 1.45 (m, 2H, $COCH_2CH_2$), 1.38 (m, 2H, C(14)H_2C(15)H_2C(16)H_2-pyridinium ring), 1.25 (m, 20H, CH_2), 1.08 (d, 3H, *J* = 6.8, CH_3); ESI-MS (CH_3OH, relative intensity, %) *m*/*z* 467.3 (M^+, 100). Calcd for $[C_{30}H_{47}N_2O_2]^+$ *m*/*z* 467.36.

4.1.5.2. (1*R*,2*S*)-2-*N*-[16-(1′-Pyridinium)-hexadecanoylamino]-1-phenyl-1-propanol bromide (LCL420). Prepared from **15** as described for LCL120 in 76% yield. $[\alpha]_D^{21}$ − 8.54 (*c* 1, MeOH); $[\alpha]_{365}^{21}$ − 29.6 (*c* 1, MeOH). Remaining analytical data are identical as reported for LCL120.

4.1.5.3. (1*R*,2*R*)-2-*N*-[16-(1′-Pyridinium)-hexadecanoylamino]-1-(4″-nitrophenyl)-1,3-propandil bromide (LCL85). Obtained from **16**. Crude product was dissolved in distilled water (10 mL) and extracted with dichloromethane (2 × 4.0 mL). The organic fractions were extracted back with water (2 × 4 mL) and all the aqueous fractions were combined and evaporated under reduced pressure. The obtained residue was lyophilized in a high vacuum to give a pure LCL85 as a pale yellow semi-solid (220 mg, 75% yield). Analytical sample of LCL85 was prepared by recrystallization from anhydrous acetone–ethyl acetate (8:1, v/v) to give a pale yellow microcrystalline powder, mp 52–53.5 °C. TLC ($CHCl_3$/$(CH_3)_2CO$/MeOH–CH_3COOH/H_2O, 20:8: 8:2:1, v/v) R_f 0.45; RP TLC (C18 Silica, CH_3CN/MeOH/1 M NH_4Cl (aq), 4:1:1.5, v/v) R_f 0.51 $[\alpha]_D^{25}$ − 1.55 (*c* 1, MeOH); ^1H NMR (500 MHz, CD_3OD) δ 9.00 (dd, 2H, *J* = 6.6, 1.2, 2,5-H_{Py}) 8.59 (t, 1H, *J* = 7.8, 4-H_{Py}), 8.16 (d, 2H, *J* = 8.9, ArH), 8.11 (t, 2H, *J* = 7.2, 3,5-H_{Py}), 7.63 (d, 2H, *J* = 8.9, ArH), 5.13 (m, 1H, 1-H), 4.63 (t, 2H, *J* = 7.7, C(16)H_2-pyridinium ring), 4.18 (m, 1H, 2-H), 3.76 (dd, 1H, *J* = 10.7, 7.6, 3-Ha), 3.56 (dd, 1H, *J* = 10.7, 6.0, 3-Hb), 2.07 (m, 2H, $COCH_2$), 2.01 (m, 2H, C(15)H_2C(16)H_2-pyridinium ring), 1.30 (m, 24H, $COCH_2CH_2$, C(14)H_2C(15)H_2C(16)H_2-pyridinium ring and CH_2); ESI-MS (CH_3OH, relative intensity, %) *m*/*z* 528.5 (M^+, 100). Calcd for $[C_{30}H_{46}N_3O_5]^+$ *m*/*z* 528.34.

4.1.5.4. (1*R*,2*R*)-2-*N*-(Tetradecanoylamino)-1-[4′-*N*-[(6″-pyridinium)-hexanoyl]aminophenyl]-1,3-propandiol bromide (LCL82). Obtained from **18**. Crude product was dissolved in a warm mixture of acetone–THF (1:1, v/v) and kept in refrigerator overnight. The obtained solid was separated by filtration, washed with ethyl acetate–acetone (1:1, v/v) and dried in a high vacuum to give a pure

LCL82 in 84% yield as a white solid. Analytical sample of LCL82 was prepared by recrystallization from anhydrous acetone–THF (1:2, v/v) to give a white microcrystalline powder, mp >122 °C. TLC ($CHCl_3$/$(CH_3)_2CO$/MeOH–CH_3COOH/H_2O, 20:8:8:2:1, v/v) R_f 0.31; RP TLC (C18 Silica, CH_3CN/MeOH/1 M NH_4Cl (aq), 4:1:1.5, v/v) R_f 0.38; $[\alpha]_D^{25}$ −7.65 (*c* 0.5, MeOH) and $[\alpha]_{365}^{25}$ −11.7 (*c* 0.5, MeOH); ^1H NMR (500 MHz, CD_3OD) δ 8.99 (dd, 2H, J = 6.5, 1.2, 2,5-H_{Py}) 8.58 (t, 1H, J = 7.9, 4-H_{Py}), 8.08 (t, 2H, J = 7.2, 3,5-H_{Py}), 7.49 (d, 2H, J = 8.9, ArH), 7.32 (d, 2H, J = 8.6, ArH), 4.92 (d, 1H, J = 3.8, 1-H), 4.65 (t, 2H, J = 7.5, C(6) H_2-pyridinium ring), 4.08 (m, 1H, 2-H), 3.68 (dd, 1H, J = 10.9, 6.7, 3-Ha), 3.48 (dd, 1H, J = 10.7, 5.7, 3-Hb), 2.39 (t, 2H, J = 7.2, 4-NHCOCH_2), 2.13 (t, 2H, J = 7.6, 2-NHCOCH_2), 2.07 (m, 2H, C(5) H_2C(6)H_2-pyridinium ring), 1.77 (m, 2H, 4-NHCOCH$_2$CH_2), 1.46 (m, 4H, 4-NHCOCH$_2$CH$_2$CH_2 and C(4) H_2C(5)H_2C(6)H_2-pyridinium ring), 1.27(m, 22H, CH_2), 0.88 (t, 3H, J = 7.1, CH_3); ESI-MS (CH_3OH, relative intensity, %) *m/z* 568.6 (M^+, 100). Calcd for $[C_{34}H_{54}N_3O_4]^+$ *m/z* 568.41.

4.2. Biology

4.2.1. Cell culture. MCF7 cells (breast adenocarcinoma, pleural effusion) were purchased from American type Culture Collection (ATCC, Rockville, MD, USA) and grown in RPMI 1640 media (Life Technologies, Inc) supplemented with 10% fetal calf serum (FCS, Summit Biotechnology, CO, USA) and maintained under standard incubator conditions (humidified atmosphere 95% air, 5% CO_2 37 °C). A parallel set of cells was used to determine cell proliferation and to prepare lipid extracts for MS analysis.

4.2.2. Cell proliferation. MCF7 cells were seeded at a density of ~50% (corresponding to 1×10^6 cells) in 10 ml of 10% fetal calf serum (FCS) and, after 24 h incubation, were treated with LCL compounds (0–50 μM) dissolved in ethanol, keeping ethanol level at 0.1% and the changes in cell numbers after 24 h or 48 h were determined and expressed as a percentage of the untreated controls. Control cells were prepared under identical condition and treated with the same amount of ethanol. Briefly, media were removed, cells were washed twice with PBS, detached using 1% trypsin and centrifuged at 800 rpm. Cell pellets were resuspended in PBS and Trypan blue (Sigma Chemicals, St. Louis, MO, USA) was added (1:1 dilution). Under a light microscope, the percentage of unstained and stained cells was assessed. The IC_{50} represents the drug concentration resulting in 50% growth inhibition compared to control cells.

4.2.3. The NCI's 60-cell assay. The human tumor cell lines of the cancer screening panel were grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. Cells were inoculated into 96-well microtiter plates in 100 μL at plating densities ranging from 5000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37 °C, 5% CO_2, 95% air, and 100% relative humidity for 24 h prior to addition of experimental drugs. Experimental drugs were solubilized in dimethyl sulfoxide at 400-fold the desired final maximum concentration. Following drug addition, the plates were incubated for an additional 48 h at 37 °C, 5% CO_2, 95% air, and 100% relative humidity.[50–54]

4.2.4. Cellular levels of exogenously added new analogs by HPLC–MS analysis. Cellular levels of the new analogs used for MCF7 cell treatment were established by HPLC–MS performed by the Lipidomics Core at MUSC on Thermo Finnigan TSQ 7000, triple-stage quadrupole mass spectrometer operating in a Multiple Reaction Monitoring (MRM) positive-ionization mode.[55] Quantitative analysis of the cellular level of D-*e*-MAPP, B13, and their analogs was based on the calibration curves generated by spiking an artificial matrix with known amounts of target standards and an equal amount of the internal standard (IS) as described.[56] The target analyte to IS peak area ratios from the samples were similarly normalized to their respective IS and compared to the calibration curves using a linear regression model.

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