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Solid-phase synthesis of a combinatorial library of dihydroceramide analogues and its activity in human alveolar epithelial cells

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Abstract—Solid-phase synthesis of a small combinatorial library of dihydroceramide analogues as mixtures of *erythro* and *threo* diastereomers is described. Some dihydroceramide analogues cause growth arrest and apoptosis in a dose-dependent manner in human alveolar epithelial cells. This activity is likely due to the *threo* isomers, as evidenced by cellular studies with a pair of diastereomerically pure *N*-acyldihydrosphingosines. The apoptotic activity reported in this work provides information for the design of new compounds that may provide the basis for the generation of biochemical tools for the study of different pathologies where ceramide and/or dihydroceramide are involved.

1. Introduction

Ceramide (Cer) is a member of an important group of fatty acid metabolites that play important roles in the regulation of many cell functions. From a structural standpoint, Cer derives from a 2-*N*-(acylamino)-1,3-diol core with an unsaturated hydrocarbon chain, which is biosynthetically produced by (*E*)-4 desaturation of dihydroceramide (Fig. 1). Cer plays a number of important physiological functions in cell regulation and homeostasis.[1–3] Thus, the addition of exogenous Cer or the enhancement of its intracellular levels induces cell differentiation, cell cycle arrest, apoptosis, or cell senescence in various cell types.[4,5] As a result, much effort has been put in the design of Cer analogues as potential inhibitors of enzymes involved in Cer biosynthesis and/or catabolism, with the aim of altering the intracellular levels of this lipid, which should allow a thorough study of its participation in cell regulatory processes.

Chronic obstructive pulmonary disease (COPD) is characterized by chronic inflammation of the airways and progressive destruction of lung parenchyma, a process that in most cases is initiated by cigarette smoking. Among the several mechanisms postulated to be involved in the pathogenesis of COPD, disruption of the balance between apoptosis and replenishment of structural cells in the lung has been recently reviewed.[6] An increase in apoptotic alveolar epithelial and endothelial cells in the lungs of COPD patients has been shown. Moreover, Petrache et al.[7] reported increased lung Cer levels in emphysema patients, suggesting that Cer upregulation might be an important pathogenetic element in the development of emphysema.

It is well accepted that exogenously added short-chain D-*erythro*-dihydroceramides (DHCs) do not have apoptotic activity in different cell lines,[8–10] while L-*threo N*-acetylsphinganine (L-*threo*-C2-DHC) caused cell death in HL-60 cells.[8] Nevertheless, it is not clear whether long-chain DHCs induce apoptosis, since accumulation

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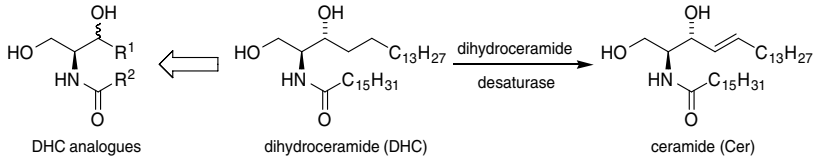


Figure 1. General structure of the DHC analogues synthesized in this work and biosynthetic transformation of DHC into Cer.

of DHC, but not Cer, along with cell death, has been reported in both human leukaemia cells treated with gamma-tocopherol and prostate cancer cells.[11,12]

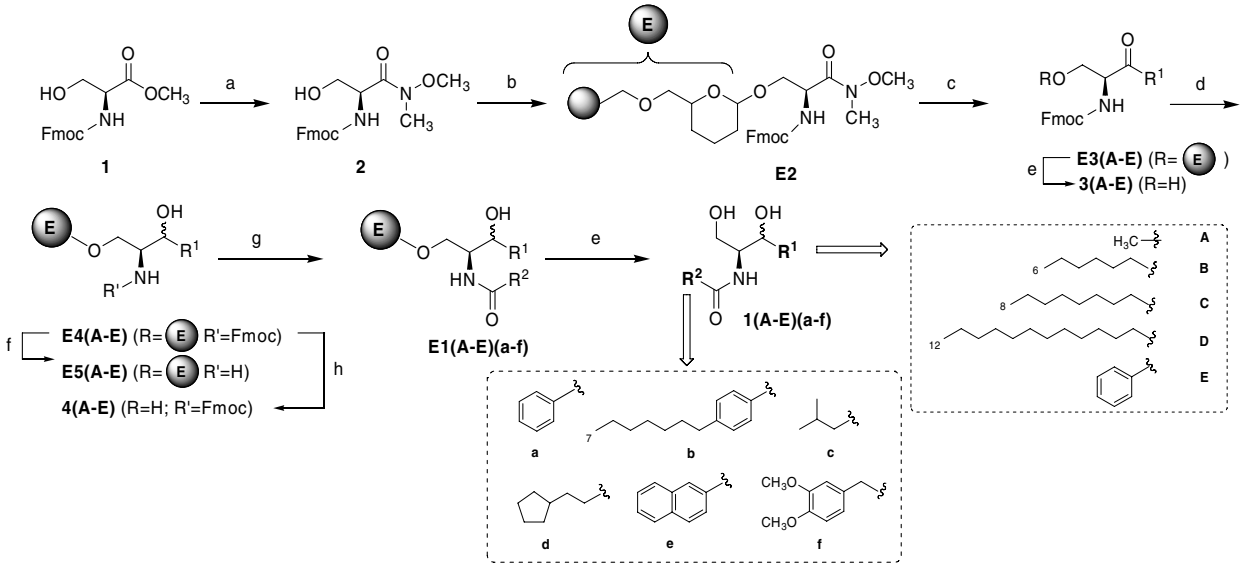
In this context, we herein describe the solid-phase synthesis of a small combinatorial library of DHC analogues **1**(**A–E**)(**a–f**) obtained by systematic variation of the sphingoid aliphatic chain and the *N*-acyl moiety (Scheme 1).[13,14] We also present preliminary data on the activity of the library members in A549 cells, a human alveolar epithelial cell type used in COPD studies.[15–17]

2. Library design

Library design was based on the reactivity of a suitably *N*,*O*-diprotected supported Weinreb amide **E2** (Scheme 1). This was obtained by acid catalyzed condensation[18] of Ellman resin (**E**)[19] with Weinreb amide **2**, obtained from commercially available Fmoc L-serine methyl ester (**1**). The acid-sensitive tetrahydropyranyl linker present in the resin was considered suitable for anchoring the primary hydroxyl group of the Weinreb amide **2**, whereas orthogonal amino protection was granted by means of the base-sensitive *N*-Fmoc protecting group.

We reasoned that reaction of resin **E2** with a set of Grignard reagents should furnish supported α-aminoketones **E3**. Subsequent stereoselective carbonyl reduction to **E4**, removal of the Fmoc protecting group and further *N*-acylation would be required to give resins **E1** as precursors of the desired DHC analogues **1** after cleavage from the resin.[20]

The design of a chemical library implies the proper choice of building blocks in order to guarantee the maximum diversity of the library members. This is especially important when applied to small libraries for preliminary exploratory studies. In our case, a series of 13 commercially available Grignard reagents (R^1MgBr) and 22 acylating agents (R^2COCl) were combined to afford a virtual library of 286 members. After enumeration with MOE program,[21] energy minimization was carried out with force field MMFF94 with 0.01 kcal/mol gradient for convergence. A set of 60 standard 2D and 3D descriptors was calculated, containing physical-chemistry, spatial, topological indices and information content indices. Finally, dimensionality of the data was reduced by principal component analysis (PCA) down to seven components to account for 90% of the variance. The chemical space was clustered (partitioned) using the complete-linkage hierarchical clustering method (HRC-complete). PRALINS (Program for Rational Analysis of Libraries in silico) was employed for the diversity evaluation.[22] A full-array (6×6 combinatorial) sub-library of 36 compounds was selected in order to optimize population coverage (i.e., total number of compounds included in represented clusters/total number of molecules in library). Simulated annealing was employed as



Scheme 1. Reagents: (a) $HN(OCH_3)CH_3{\cdot}HCl$, CH_2Cl_2, Me_3Al; (b) Ellman resin, PPTS, 1,2-DCE; (c) R^1MgX, THF; (d) $Zn(BH_4)_2$, THF, see Table 1; (e) TFA, CH_2Cl_2/EtOH; (f) piperidine, DMF; (g) from **E5**(**A–E**): R^2COCl, 50% NaOAc–H_2O; (h).

optimization algorithm. Besides population coverage, the representativity of the selection was measured in terms of space coverage (i.e., number of clusters represented by the subset divided by the number of 36 total hierarchical clusters that match the selection size). The resulting combinatorial subset consisting of 6 Grignard reagents (**A–E**, see Scheme 1, and allylmagnesium bromide) and 6 acylating agents (**b–f**, see Scheme 1, and trifluoroacetyl chloride) achieved 84% of population coverage (61% space coverage). However, unexpected reactivity problems found in the course of the library development prompted us to reconsider the initial design. Thus, allylmagnesium bromide was disregarded, since no allylation adduct was ever detected from reaction of resin **E2** with this Grignard reagent. On the other side, acylations of **E5(A–E)** with trifluoroacetyl chloride proved problematic due to the adventitious trifluoroacetamide hydrolysis observed in the course of the reaction. For this reason, this acylating reagent was replaced by the more robust benzoyl chloride (**a**, Scheme 1).

Therefore, the final synthesized combinatorial library was composed of 30 compounds, arising from 5 Grignard reagents (**A–E**) and 6 acylating agents (**a–f**) (Scheme 1). This modified subset represents a 60% population coverage (44% space coverage) compared to the maximum theoretical optimal value of 71% population coverage (67% space) as a result of the above modifications.[23]

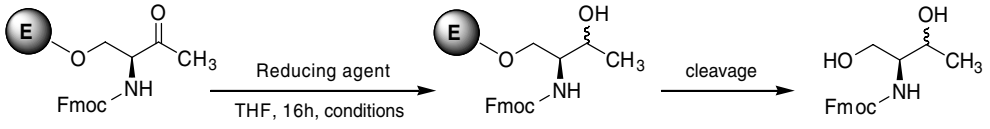
3. Chemistry

Functionalized Ellman resin **E2** (0.65 mmol/g) was obtained as described in Scheme 1. The synthetic sequence was first optimized with MeMgBr. Thus, reaction of **E2** with 2.2 mmol of a 3 M MeMgBr solution in THF under N_2 afforded ketone **E3A**, as evidenced by a carbonyl signal at 208.2 ppm in gel-phase ^{13}C NMR and the disappearance of the characteristic singlets at 3.33 and 3.86 ppm of the N–CH_3 and N–OCH_3 groups, respectively, of the Weinreb amide moiety in the MAS (magic angle spinning) ^1H NMR. The reaction outcome was also confirmed by spectroscopic analysis of the cleaved adduct **3A**.[20] Reduction of ketone **E3A** to **E4A** was tested under a variety of reducing agents and reaction conditions (Table 1).[24] The diastereoselectivity of the process was determined from HPLC analysis of a sample obtained by cleavage of a small resin aliquot.[20] Isomers were identified by co-injection of the resulting **4A** (mixture of *erythro* and *threo* isomers) with a standard of *N*-Fmoc L-threoninol obtained independently.[25]

The use of $Zn(BH_4)_2$ afforded a modest diastereoselectivity (entry 1), which could not be improved with the use of a chelating Lewis acid such as $ZnCl_2$ (entry 2)[26] or a lower reaction temperature (entry 3). On the other side, reduction of **E3A** with L-selectride[24,27] afforded a complex mixture (entry 4), whereas aluminium hydride reagents showed dissimilar results. Thus, lithium tri-*tert*-butoxyaluminium hydride[24] afforded a 1:1 mixture of *erythro* and *threo* isomers (entry 5), whereas DIBAH[28] afforded **E4A** in excellent diastereoselectivity (entry 6). Unfortunately, this result could not be reproduced from resins **E3(B–E)**, since complex reaction mixtures were obtained in all cases. For this reason, $Zn(BH_4)_2$ at rt was the method of choice for reduction of ketone resins **E3(A–E)** in our library of DHC analogues.

The remaining synthetic sequence was optimized from **E4A** (7:3 mixture of diastereomers). Thus, cleavage of the *N*-Fmoc protecting group under basic conditions (20% piperidine in DMF and CH_2Cl_2, two cycles)[29] afforded **E5A** in excellent yield.[30] Acylation of **E5A** was first optimized with benzoyl chloride under Schotten-Baumann conditions (50% aq NaOAc/THF). The reaction was monitored by the Kaiser test,[31] which revealed total consumption of the starting supported amine after 16 h at rt. Interestingly, clean mixtures of *erythro*- and *threo*-*N*-acylated **1Aa**[30] were obtained with

Table 1. Reduction of resin **E3A**



E3A **E4A** **4A**

Entry	Reducing agent	Conditions	**E4A** (*erythro:threo*)[a]
1	$Zn(BH_4)_2$	rt	7:3
2	$Zn(BH_4)_2$	rt ($ZnCl_2$)[b]	7:3
3	$Zn(BH_4)_2$	−78 °C	7:3
4	L-Selectride[c]	−50 °C	Complex mixture
5	$LiAlH[OC(CH_3)_3]_3$	rt	1:1
6	DIBAH[d]	rt	9:1[e]

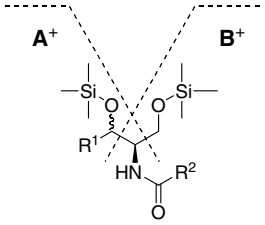
[a] Determined by HPLC analysis of **4A** after cleavage from the resin (see Ref 20).
[b] $ZnCl_2$ (3 equiv/mol, based on the loading of **E3A**).
[c] L-Selectride: $Li[CH(CH_3)CH_2CH_3]_3BH$.
[d] DIBAH: $(i\text{-}Bu)_2AlH$.
[e] Complex mixtures for **E4(B–E)**, see text.

no trace of the corresponding *N*,*O*-diacylated adducts. These adducts were observed, however, in acylation experiments carried out with *p*-nitrophenyl benzoate in an attempt to accelerate the acylation step by using an activated ester.[14,30,32]

This methodology was applied to the selected building blocks (Grignard reagents **B–E** and acylating agents **b–f**) shown in Scheme 1 to give the 5×6 combinatorial library of DHC analogues. Thus, addition of Grignard reagents **B–E** to the supported Weinreb amide **E2** afforded the expected resins **E3(B–E)**, which were submitted to $Zn(BH_4)_2$ reduction,[30] Fmoc removal and final acylation-cleavage to give compounds **1(A–E)(a–f)**. Library members were analyzed by ^1H NMR and HRMS for identity verification after cleavage from the resin. Diastereomeric composition (*erythro*/*threo*) and purities were confirmed by GC-MS of the corresponding bis-*O*-trimethylsilyl adducts obtained by derivatization with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BTMSTFA)[33] (Table 2). The diastereomeric ratio (dr) was calculated by integration of peaks corresponding to the two adducts, which were identified by the diagnostic fragments in their corresponding mass spectra[33] (see Table 2). In addition, stereochemical assignment was inferred from comparison of retention times with those of related compounds of known stereochemistry, such as the diastereomeric pairs of *N*-hexanoyl and *N*-octanoyl D-*erythro* and L-*threo* sphinganines, for which *threo* isomers showed lower retention times than the corresponding *erythro* counterparts.[34] As expected, the major formation of *erythro* isomers is in agreement with the observed reaction outcome for **1Aa**.

Each building block behaved similarly in terms of purities of the final library members, with no significant differences resulting from variation of either the Grignard reagent or the acylating agent, as shown in Table 3.

Table 2. GC-MS Analysis of DHC library as *bis*-(OTMS) derivatives



Compound	Peaks				Retention time (min)			Purity (%)	dr[a]
	$[M]^+$	$[M-15]^+$	A^+	B^+	*erythro*	*threo*	Δ rt		
1Aa	353	338	250	236	27.07	26.29	0.78	70	7:3
1Ab	451	436	348	334	36.37	35.95	0.42	75	6:4
1Ac	333	318	230	216	18.63	17.65	0.98	80	6:4
1Ad	373	358	270	256	26.25	25.41	0.84	76	7:3
1Ae	403	388	300	286	33.45	32.76	0.69	77	6:4
1Af	427	412	324	310	32.58	31.72	0.86	71	6:4
1Ba	423	408	320	236	31.24	30.43	0.81	85	6:4
1Bb	521	506	418	334	40.65	40.14	0.51	67	6:4
1Bc	403	388	300	216	25.69	24.54	1.15	83	6:4
1Bd	443	428	340	256	32.08	31.08	1.00	74	7:3
1Be	473	458	370	286	38.38	37.65	0.73	73	6:4
1Bf	497	482	394	310	37.24	36.30	0.94	63	6:4
1Ca	451	436	348	236	33.85	33.12	0.73	82	6:4
1Cb	549	534	446	334	42.84	42.31	0.53	70	6:4
1Cc	431	416	328	216	28.77	27.67	1.10	77	6:4
1Cd	471	456	368	256	34.87	33.87	1.00	68	7:3
1Ce	501	486	398	286	40.60	39.96	0.64	76	1:1
1Cf	525	510	422	310	39.52	38.65	0.87	70	6:4
1Da	507	492	404	236	39.34	38.64	0.70	89	7:3
1Db	605	590	502	334	47.26	46.75	0.51	69	6:4
1Dc	487	472	384	216	34.72	33.78	0.94	84	6:4
1Dd	527	512	424	256	40.14	39.25	0.89	73	6:4
1De	557	542	454	286	45.34	44.70	0.64	65	6:4
1Df	581	566	478	310	44.25	43.45	0.80	59	7:3
1Ea	415	400	312	236	31.32		[b]	88	[b]
1Eb	513	498	410	334	41.19		[b]	81	[b]
1Ec	395	380	292	216	25.89	25.42	0.47	83	[b]
1Ed	435	420	332	256	32.68		[b]	83	[b]
1Ee	465	450	362	286	39.03		[b]	78	[b]
1Ef	489	474	386	310	37.83	37.41	0.42	85	[b]

[a] dr: diastereomeric ratio (*erythro*/*threo*).
[b] Unresolved peaks prevented dr ratio analysis.

Table 3. Purities of subsets of compounds within the combinatorial library[a]

R1	Mean % ± SD (n = 6)	R2	Mean % ± SD (n = 5)
A	75 ± 3.8	**a**	83 ± 6.9
B	74 ± 8.6	**b**	72 ± 5.0
C	74 ± 5.4	**c**	81 ± 2.6
D	73 ± 11.4	**d**	75 ± 4.9
E	83 ± 3.4	**e**	74 ± 4.7
		f	70 ± 8.9

[a] Purities of individual compounds are given in Table 2.

4. Biological activity

Cytotoxicity of the individual library members was tested in A549 human alveolar cells using the MTT test (see Experimental). Since the MTT test is a measure of living cells, a decrease in MTT-positive cells can be due either to decreased growth, apoptosis or necrosis. Previous observations that apoptotic activity of different Cer analogues in HL60 human myeloid leukaemic cells was dependent on foetal bovine serum (FBS) concentration in the medium[35] prompted us to test our library with A549 cells in the presence or absence of FBS using *N*-hexanoylsphingosine (C6-Cer) and *N*-hexanoyldihydrosphingosine (C6-DHC) as positive and negative controls, respectively.[36] In our hands, A549 survival in the presence of C6-Cer was dependent on FBS concentration in the medium. C6-Cer was not cytotoxic at concentrations up to 1 mM when FBS was present at 5% or lower concentrations, whereas a dose–response curve was observed at higher FBS concentrations (see Fig. 3 for 0% and 10% FBS). On the other hand, in agreement with previously published data,[36] C6-DHC was inactive at concentrations up to 1 mM, at the different FBS concentrations tested (0–10%) (Fig. 2).

Survival of A549 cells in the presence of the DHC analogues **1(A–E)(a–f)** for three days with or without 10% FBS in the incubation medium was evaluated using the MTT test. Active compounds showed the IC_{50} values depicted in Figure 2 and caused a time-dependent and dose-dependent inhibition of cell viability (Fig. 3). For the sake of simplicity, compounds with IC_{50} higher than 250 μM are not shown. Compounds **1Aa**, **1Ac**, **1Ad**, **1Ae** and **1Ec** were not toxic at 1 mM, whereas compounds **1Af**, **1Ba**, **1Bc**, **1Ea**, **1Ed** and **1Ee** inhibited cell viability with IC_{50} of 410, 290, 302, 689, 597 and 580 μM, respectively.

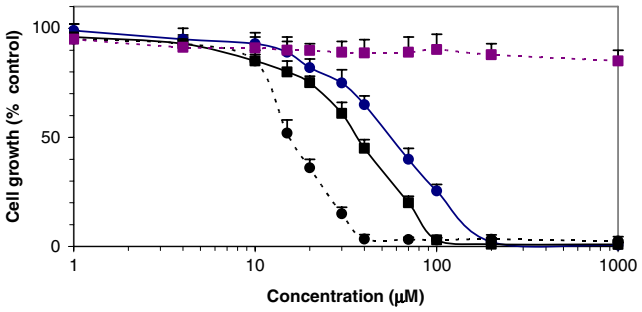


Figure 3. Cytotoxicity of **1Dc** (circle) and C6-Cer (square) in A549 cells incubated with 10% FBS (continuous line) or without FBS (dotted line) for 72 h. Error bars represent standard deviations of nine experiments.

On the other hand, compounds **1Bb**, **1Bd**, **1Cc**, **1Cf**, **1Dc** and **1Eb** showed a twofold increase in cytotoxicity (IC_{50}) in the absence of FBS (Fig. 2). Among the different acyl groups tested (R^2, see Scheme 1), *N*-benzoyl derivatives **b** and **f** were the most cytotoxic ones, showing IC_{50}'s close to that of C6-Cer (IC_{50} for **1Bb**, **1Cb**, **1Df**, and **1Eb** were in the range of 20–40 μM; C6-Cer: IC_{50} = 35 μM). Regarding the contribution of the R^1 groups on cell toxicity, an increase in the hydrocarbon chain length led to more toxic compounds (compare compounds **1D** with compounds **1A**), while aromatic **1E** were inactive, with the exception of the above-mentioned **1Eb**. However, it is worth mentioning that compound **1Db**, representing one of the best theoretical combinations of R^1 and R^2 groups, showed a somewhat lower cytotoxicity in A549 cells than the remaining **D** members (Fig. 2). This could be explained by the high lipophilicity (estimated log *P*)[37] of **1Db**, which could well be above the optimum for maximal toxicity.

Since the library was composed of a mixture of D-*erythro* and L-*threo* adducts (see Table 2), D-*erythro*-dihydrosp-

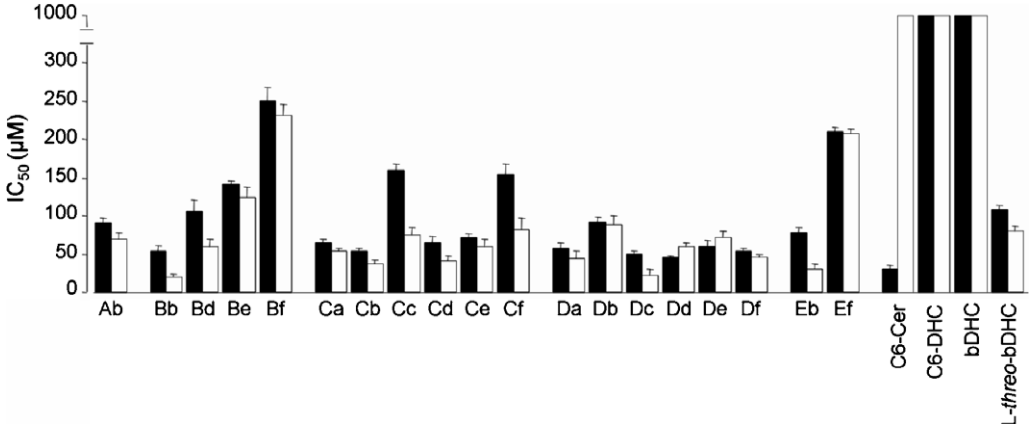


Figure 2. Cytotoxicity (IC_{50} values) of selected DHC analogues of genaral structure **1** in A549 cells incubated with 10% FBS (full bars) or without FBS (empty bars) for 72 h. Values for C6-Cer, C6-DHC, **b-DHC** and L-*threo*-b-DHC are also shown. Error bars represent standard deviations of nine experiments.

hingosine and its diastereomeric L-*threo* counterpart (safingol) were independently *N*-acylated with the 4-heptylbenzoyl moiety (**b**, Scheme 1)[38] to give b-DHC and L-*threo*-b-DHC, respectively (Fig. 4). As shown in Figure 2, b-DHC was non-toxic, while the diastereomeric L-*threo*-b-DHC caused cell death with an IC_{50} value of 108 μM, indicating that the *threo* isomer is likely responsible for the cytotoxicity produced by the mixture.

As stated above, a decrease in MTT-positive cells can be due either to decreased growth, apoptosis or necrosis. In this context, it has been reported that short-chain Cer (C6-Cer) induces the generation of endogenous long-chain Cer in A549 cells, thus producing cell cycle arrest and growth inhibition at 20 μM,[10,36] while A549 cells treated with 50 μM C6-Cer exhibited apoptotic morphology and cell death.[39,40] Therefore, the effect of active compounds at different concentrations and incubation times was investigated by flow cytometry. As shown in Figure 5B, about 92% of the cell population was arrested in the G_0/G_1 phase of the cell cycle upon treatment of A549 cells with the highly cytotoxic **1Cb** (6:4 *erythro*/*threo*, see Table 2), a higher percentage than that obtained in control cells (Fig. 5A). Similar results were achieved with library members listed in Table 4 and also with L-*threo*-b-DHC. A good correlation between the percentage of cell population arrested in the G_0/G_1 phase (Table 4) and IC_{50} values for the active compounds (Fig. 2) can be inferred from the above data. Conversely, C6-DHC or b-DHC did not lead to cell cycle arrest.

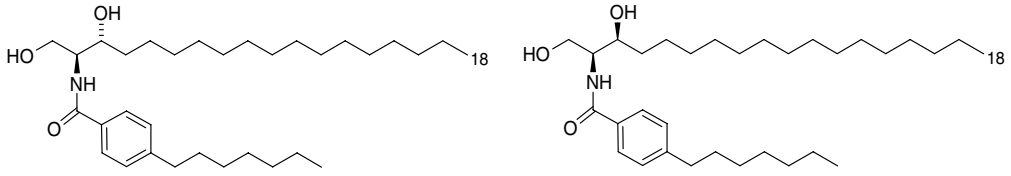
In order to assert that apoptosis is responsible for cell death observed in Figure 4, A549 cells were incubated with active DHC analogues and stained with Annexin V and propidium iodide (PI). As shown in Figure 6, an increase in the number of apoptotic cells was observed after treatment with compound **1Cb** (Fig. 6B), L-*threo*-b-DHC (Fig. 6D), C6-Cer and other active DHC analogues described in this paper. On the contrary, b-DHC (Fig. 6C) and C6-DHC were inactive. These results are in agreement with those reported for C6-Cer, a compound that causes cell cycle arrest or apoptosis in A549 cells, depending on the used dose.[10,36,39,40]

As mentioned above, the apoptogenic activity of the *erythro*/*threo* DHCs reported here is probably caused by the L-*threo* isomers. In agreement with the results found in this work, the apoptotic activity of L-*threo*-C2-DHC and its homo analogue in HL-60 cells has been

Table 4. Effect of active DHC analogues on A549 cell cycle population[a]

Compound	Cell cycle phase		
	G_0/G_1	S	G_2
Control	70.3	27.0	2.7
1Bb	86.5	8.1	5.4
1Ca	89.6	8.4	2.0
1Cb	92.6	3.4	4.0
1Cc	84.5	10.5	5.0
1Cd	94.4	3.4	2.2
1Ce	91.2	5.4	3.4
1Cf	84.0	10.5	5.5
1Da	83.0	12.8	4.2
1Db	88.2	7.4	4.4
1Dc	89.8	5.2	4.4
1De	90.4	7.4	2.2
1Df	86.5	5.4	7.0
L-*threo*-b-DHC	83.1	11.5	5.4

[a] Data are expressed as percentage of each cycle phase, and calculated as described under Experimental.



(**b-DHC**)
D-*erythro*-*N*-(4-heptylbenzoyl)dihydrosphingosine

(**L-*threo*-b-DHC**)
L-*threo*-*N*-(4-heptylbenzoyl)dihydrosphingosine

Figure 4. *N*-acylated dihydrosphingosine (b-DHC) and *N*-acylated safingol (L-*threo*-b-DHC) synthesized for comparative cytotoxicity studies.

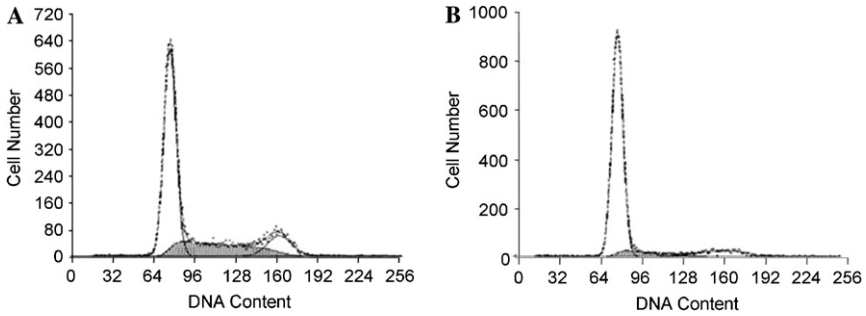


Figure 5. Cell cycle profiles of A549 cells grown in the absence (A) or presence (B) of **1Cb** for 72 h.

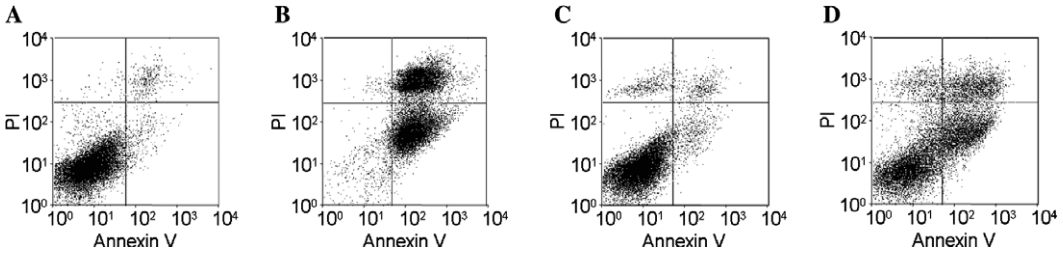


Figure 6. Flow cytometry analyses using Annexin V and propidium iodide as dyes of A549 cells incubated without (A) or with **1Cb** (B), **b-DHC** (C) and L-*threo*-b-DHC (D).

reported, whereas their corresponding *erythro* diastereomers were inactive.[8,41] In addition, cytotoxicity of DHC analogues (racemic *erythro* and *threo*) containing bulky acyl groups has been reported in U937 cells.[14]

The above data are in apparent conflict with a report showing that a phenethylisothiocyanate derivative of D-*erythro*-DHC was cytotoxic in HL-60 cells.[42] However, the particular nature of the *N*-acyl group in this latter case may be responsible for the different behaviour of this compound. On the other hand, the apoptogenic role of long-chain D-*erythro*-DHCs is not clear in the light of the results in human leukaemia cells treated with gamma-tocopherol and prostate cancer cells, where accumulation of DHC, but not of Cer, along with cell death has been reported.[11,12] Furthermore, a recent report[43] describes that both long- and short-chain *erythro*-DHCs do not simply lack apoptogenic activity,[8,10] but counteract the apoptotic effect of ceramide by inhibiting Cer channel formation in mitochondria in early apoptosis.[43,44] Therefore, in the light of this last report,[43] the *erythro*-DHC isomers here described might be anti-apoptogenic. The cytotoxicity of the pure *threo* isomers would thus probably be higher than that of the corresponding *erythro*/*threo* mixtures, in which their effect could be masked by the cytoprotective *erythro* counterparts. Even in that case, IC_{50} values for the most active library members (mixtures *erythro*/*threo*) were in the range of those obtained for Cer (see Fig. 2).

Reports on the metabolism of L-*threo*-DHC indicate that this compound is metabolised to dihydrosphingomyelin, but not to dihydroglucosylceramide by rat liver Golgi fractions,[45] whereas L-*threo*-DHC generated after cell treatment with safingol (L-*threo*-dihydrosphingosine) was converted into the corresponding glycoconjugates, as well as into dihydrosphingomyelin, in both fibroblasts and two types of neuronal cells.[46] Our data from the library of DHC analogues pointed out that active compounds most probably have a L-*threo* configuration, show cytotoxicity by an apoptogenic mechanism and fit into a narrow range of hydrophobicity. The metabolic fate of the L-*threo* isomers reported here is currently under investigation. Likewise, the mechanism/s underlying the apoptogenic effect of these compounds and the putative activity of the D-*erythro* isomers as Cer channel formation inhibitors are also being studied in our laboratories.

5. Conclusions

Solid-phase synthesis of a small combinatorial library of DHC analogues as mixtures of *erythro* and *threo* diastereomers is described. Building blocks have been selected by means of the PRALINS program in order to explore the maximum diversity among the library members. Cellular studies show that some DHC analogues cause growth arrest and apoptosis in a dose-dependent manner in human alveolar epithelial cells. The activity may be attributed to the corresponding *threo* isomers, as evidenced by cellular experiments performed with a selected pair of diastereomerically pure *N*-acyldihydrosphingosine analogues. Maximum apoptotic activity is exhibited by compounds with estimated log *P* values of 2.5–4.5. The overall results reported here provide information for the design of new compounds that may be potentially useful as biochemical tools for the study of different pathologies where Cer and/or DHC are involved, such as COPD. The intracellular signal transduction mechanisms for these new DHC analogues are under investigation in our laboratory.

6. Experimental

6.1. Biological protocols

6.1.1. Cytotoxicity assay in A549 cells. Human alveolar epithelial A549 cells were obtained from the American Type Culture Collection (ATCC) and grown in HAM F12 with glutamine medium supplemented with 10% foetal bovine serum (FBS). Cells were kept at 37 °C in 5% CO_2/95% air. At the time of the experiments, cells were seeded in medium with 10% FBS at a density of 10^5 cells per well in 96-well plates. Twenty-four hours later, media were replaced with fresh medium containing different percentages of FBS, and compounds were added to give final concentrations of 10–1000 μM. Cells were incubated at 37 °C in 5% CO_2/95% air for 3 days. The number of viable cells was quantified by the estimation of its dehydrogenase activity, which reduces 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to water-insoluble formazan, which was dissolved in DMSO and measured at 570 nm with a Multiskan plate reader (Labsystems). D-*erythro*-C6-Cer and D-*erythro*-C6-DHC were used as reference compounds. All compounds were dissolved in ethanol and control experiments were performed with ethanol (0.1%).

6.1.2. Evolution of cell cycle profiles by flow cytometry. DHC analogues were incubated with A549 cells in HAM F12 medium supplemented with 10% FBS, for 3 days. Next, cells were collected by brief trypsinization, washed with PBS, 10 mM EDTA at 4 °C, washed with PBS, 10 mM EDTA, 1% BSA at 4 °C, resuspended in PBS, 70% ethanol at 4 °C, 10^6 cell/ml and stored at −20 °C. On the day of the analysis, cells were washed with PBS and treated with DNAse-free RNAse (0.05 mg/ml) at 37 °C for 1 h. Cells were then stained with propidium iodide for 15 min and analyzed with a FACS flow cytometer (Coulter). Cells treated with 0.1% ethanol were used as controls.

6.1.3. Evaluation of apoptosis by annexin V and propidium iodide staining. Cells, collected by brief trypsinization, were stained with an Alexa Fluor 488 Annexin V/propidium iodide staining kit (Molecular Probes, Inc. Oregon), and apoptosis was evaluated by FACS (Coulter XL) with Coulter EPICS. The externalization of phosphatidylserine of the plasma membrane, a marker of apoptosis, was recognized by annexin V conjugated with fluorescein; propidium iodide penetrates into the plasma membrane of cells that have lost membrane integrity.

6.2. Chemistry

6.2.1. General. Solvents were distilled prior to use and dried by standard methods.[47] Grignard reagents were used from commercial sources. Chemical shifts are reported in delta units (δ), parts per million (ppm) relative to the singlet at 7.24 ppm of $CDCl_3$ for ^1H and in ppm relative to the centre line of a triplet at 77.0 ppm of $CDCl_3$ for ^{13}C. Magic angle spinning ^1H NMR (MAS-^1H NMR) were carried out in a Brucker DMX500 at 500 MHz and at a constant temperature of 27 °C. Gel-phase NMR experiments were conducted by placing a previously dried small amount of resin in a NMR tube and dropwise addition of the minimum volume of solvent required for swelling the resin under continuous gentle stirring to remove air bubbles. HPLC analyses were performed in a Merck D-6000 chromatography system (L-6000 photodiode array detector) using a LiChroCART® RP-18 (Merck) column (5 μm and 3.9× 150 mm), eluted with mixtures of MilliQ deionized water and HPLC-grade acetonitrile containing 0.1% TFA, at 1 ml/min. HPLC-MS analyses were obtained on a Hewlett Packard MSD system. GC-MS were performed in a Fisons (8000 series) equipped with a DB-5 column (J&W) (25 m × 0.25 μm × 0.22 mm) coupled to a MD-800 detector and He as carrier gas.

6.2.2. (*S*)-2-[(9H-Fluoren-9-yl)methoxycarbonylamino]-3-hydroxy-*N*-methoxy-*N*- methylpropionamide (2). A solution of *N*,*O*-dimethylhydroxylamine (HCl) (9.2 g, 93.8 mmol) in CH_2Cl_2 (120 mL) under Ar at 0 °C was treated with 43.4 mL Me_3Al (2 M in CH_2Cl_2). The reaction mixture was stirred at rt for 30 min, treated with a solution of commercially available **1** (8 g, 23.5 mmol) in CH_2Cl_2 (80 mL) and heated to reflux for 16 h. The reaction was next quenched by pouring it over a mixture of 1 N HCl (240 mL) and crushed ice, and extracted with CH_2Cl_2 (3× 100 mL). Usual workup of the organic extracts afforded a crude which was crystallized from MeOH to afford 9.5 g (78%) of amide **2**. ^1H NMR (300 MHz, $CDCl_3$): 3.21 (s, 3H, N–Me); 3.75 (s, 3H, N–O*Me*); 3.85 (t, J = 5.0 Hz, 2H, CH_2–OH); 4.19 (t, J = 7.0 Hz, 1H, C*H*(Fmoc)); 4.36 (d, J = 7.0 Hz, 2H, CH_2(Fmoc)); 4.86 (sa, 1H, C*H*–NH); 6.05 (d, J = 8.0 Hz, 1H, N*H*); 7.28 (tt, J_1 = 7.0 Hz, J_2 = 2.0 Hz, 1H, CH_{ar}(Fmoc)); 7.37 (t, J = 7.5 Hz, 2H, CH_{ar}(Fmoc)); 7.58 (dd, J_1 = 7.0 Hz, J_2 = 3.5 Hz, 2H, CH_{ar}(Fmoc)); 7.73 (d, J = 7.5 Hz, 2H, CH_{ar}(Fmoc)); ^{13}C NMR (75 MHz, $CDCl_3$): 170.5 (*C*=O(amide)); 156.4 (C=O(Fmoc)); 143.7 (C_8); 141.2 (*C*13), 127.6; 127.0; 125.1; 119.9; 67.1 (*C*H2(Fmoc)); 63.3 (*C*H$_2$–OH); 61.6 (*C*H–NH); 52.8 (N–OMe); 47.0 (C_7); 32.1 (N–Me); IR (film): 3415, 3321, 3065, 2943, 2893, 1715, 1654, 1526, 1450, 1250, 1057, 739 cm^{-1}; $[\alpha]_D^{20}$ −5.1 (c = 1, MeOH).

6.2.3. Resin E2 by anchorage of amide 2 to Ellman resin. In a 100 mL flask provided with mechanical stirrer are placed, under Ar, 2 g (loading: 0.94 mmol/g) of Ellman resin,[19] 4.2 g (11.3 mmol) of amide **2** and 1 g (5.6 mmol) of PPTS. 1,2-DCE (25 mL) was next added and the reaction mixture was gently stirred for 16 h at 80 °C. The resin was next filtered and successively washed with CH_2Cl_2 (3× 6 mL), DMF/H_2O (1:1, 3× 6 mL), DMF (3× 6 mL), THF (3× 6 mL), CH_2Cl_2 (3× 6 mL) and dried to give 2.5 g of resin **E2** (loading 0.65 mmol/g); MAS-^1H NMR (500 MHz): 3.33 (s, N–Me); 3.86 (s, N–OMe); 4.10 (s, CH_2–OH); 4.37 (s, C*H*(Fmoc)); 4.52 (s, CH_2(Fmoc)); 5.07 (s, C*H*–NH); 7.19 (sa, CH_{ar}(Fmoc) and polystyrene); 7.42 (sa, CH_{ar}(Fmoc)); 7.75 (s, CH_{ar}(Fmoc)); 7.86 (s, CH_{ar}(Fmoc)); Gel phase ^{13}C NMR (75 MHz, $CDCl_3$, diagnostic signals): 170.2 (*C*=O(amide)); 155.9 (C=O(Fmoc)); 67.8 (*C*H$_2$(Fmoc)); 66.9 (*C*H$_2$–OH); 61.3 (*C*H–NH); 53,3 (N–OMe); 46.9 (*C*H(Fmoc)); 32.1 (N–Me); IR (KBr): 3655, 3433, 3320, 3083, 3016, 2923, 2851, 1944, 1877, 1805, 1712, 1681, 1604, 1485, 1449, 1250, 903, 764 cm^{-1}.

6.2.4. Reaction of resin E2 with Grignard reagents. synthesis of resins E3(A–E). General method. A suspension of resin **E2** (1 g, 0.65 mmol) in THF (8 mL) was placed in a Pyrex® tube under Ar and treated at rt with 2.2 mmol of a THF solution of the required Grignard reagent **A–E** (**A**: 3 M MeMgBr; **B**: 2 M hexylMgBr; C: 2 M octylMgBr; **D**: 2 M dodecylMgBr; **E**: 2 M phenylMgBr, see Scheme 1). After gentle agitation for 16 h at rt, the resin was filtered and washed successively with CH_2Cl_2 (3× 6 mL), DMF/H_2O (1:1) (3× 6 mL), DMF (3× 6 mL), THF (3× 6 mL), CH_2Cl_2 (3× 6 mL) and dried. Resin **E3A**: 734.6 mg (loading: 0.51 mmol/g); MAS-^1H NMR (500 MHz): 2.44 (s, Me); 4.23 (s, CH_2–OH); 4.46 (s, C*H*(Fmoc)); 4.71 (s, CH_2(Fmoc)); 5.08 (s, C*H*–NH); 7.32 (sa, CH_{ar}(Fmoc) and polystyrene); 7.49 (sa, CH_{ar}(Fmoc)); 7.84 (s, CH_{ar}(Fmoc)); 7.95 (s, CH_{ar}(Fmoc)); Gel phase ^{13}C NMR (75 MHz, $CDCl_3$, diagnostic signals): 208.2 (*C*=O(ketone)); 170.2 (*C*=O(amide)); 154.4 (C=O(Fmoc)); 67.9 (*C*H$_2$(Fmoc)); 66.9 (*C*H$_2$–OH); 60.7 (*C*H–NH); 47.2 (*C*H(Fmoc)); Resin **E3B**: 768 mg (loading: 0.53 mmol/g); Resin **E3C**: 746 mg (loading: 0.54 mmol/g); Resin **E3D**: 705 mg (loading: 0.58 mmol/g); Resin **E3E**: 779 mg (loading: 0.52 mmol/g).

6.2.5. Resins E4(A–E) from reaction of resins E3(A–E) with $Zn(BH_4)_2$. General method. A suspension of the corresponding resin **E3(A–E)** in THF (3 mL) was placed in a Pyrex® tube and treated with a 0.4 M solution of $Zn(BH_4)_2$ in THF (3 equiv/mol, based on the loading of the starting resin). The reaction mixture was agitated at rt for 16 h and filtered. The resin was washed successively with CH_2Cl_2 (3× 6 mL), DMF/H_2O (1:1) (3× 6 mL), DMF (3× 6 mL), THF (3× 6 mL), CH_2Cl_2 (3× 6 mL) and dried. Following this procedure, resin **E3A** (800 mg) afforded 732 mg of resin **E4A** (*erythro*/*threo*: 7/3, loading 0.47 mmol/g); MAS-^{1}H NMR (500 MHz): 1.42 (s, Me); 4.12 (s, CH_2–OH); 4.40 (s, C*H*(Fmoc)); 4.70 (s, CH_2(Fmoc)); 5.04 (s, C*H*–NH); 7.25 (broad, CH_{ar}(Fmoc) and polystyrene); 7.45 (sa, CH_{ar}(Fmoc)); 7.78 (s, CH_{ar}(Fmoc)); 7.90 (s, CH_{ar}(Fmoc)). Gel phase ^{13}C NMR (75 MHz, $CDCl_3$, diagnostic signals): 157.7 (C═O(Fmoc)); 73.2 (CH_2(Fmoc)); 68.7, 66.5 (*C*H_2 and *C*H–OH); 55.4 (*C*HNH and polystyrene); 40.4 (*C*H(Fmoc) and polystyrene); 17.8 (Me). Similarly, from 800 mg of resins **E3(B–E)**, resins **E4B** (716 mg, loading: 0.47 mmol/g), **E4C** (716 mg, loading: 0.48 mmol/g), and **E4D** (758 mg, loading: 0.55 mmol/g), and **E4E** (674 mg, loading: 0.44 mmol/g) were obtained.

6.2.6. Resins E5(A–E) from deprotection of resins E4(A–E). General method. The starting resin **E4** (600 mg) was placed in a Pyrex® tube and treated in successive cycles with 10 mL of a 20% solution of piperidine in DMF (2 cycles) and 10 mL of a 20% solution of piperidine in CH_2Cl_2 (2 additional cycles). The reaction mixture was agitated at rt for 1 h in each cycle and filtered before the following cycle. Final washings with CH_2Cl_2 (3 × 6 mL) afforded the required resins. Resin **E5A**: 514 mg. Resin **E5B**: 505 mg. Resin **E5C**: 532 mg. Resin **E5D**: 514 mg. Resin **E5E**: 551 mg.

6.2.7. Acylation of resins E5(A–E) followed by cleavage of functionalized Ellman resins. General acylation method. A suspension of the starting resin in THF (2 mL) in a Pyrex® tube provided with a septum was treated with 2.5 mL of an aqueous 50% NaOAc solution. After gentle agitation for 10 min at rt, a solution of the acylating agent **a**–**f** (1 mol/equiv, based on the resin loading, in 2 mL THF) was added dropwise. The reaction mixture was next agitated at rt for 16 h or until negative Kaiser test.[31] The reaction mixture was filtered and the resin is successively washed with 10% aqueous NaOH (6× 3 mL), DMF (10× 3 mL), EtOH (4× 6 mL), THF (6× 6 mL), CH_2Cl_2 (6× 6 mL) and dried. Resin **E1Aa**: Gel phase ^{13}C NMR (75 MHz, $CDCl_3$, diagnostic signals): 167.7 (*amide*); 68.5, 68.3 (*C*H_2 O and *C*H–OH); 53.3 (*C*H–NH); 17.5 (Me). Resins **E1(A–E)(a–f)** were cleaved following the general method (Section 6.2.9).

6.2.8. Cleavage of functionalized Ellman resins. General method. A suspension of the resin in 3.5 mL of a 6:4 mixture of CH_2Cl_2/EtOH was treated with neat TFA (2 mL) and gently agitated at rt for 1 h. The reaction mixture was next filtered and the resin washed with CH_2Cl_2 (3× 6 mL), EtOH (3× 6 mL) and again CH_2Cl_2 (3× 6 mL). The combined washings were evaporated to dryness to obtain the corresponding cleavage products. For GC-MS of (bis-(OTMS)) derivatives, see Table 2.

Compound **1Aa** (31 mg from 190 mg of resin **E1Aa**): ^{1}H NMR (500 MHz, $CDCl_3$): 1.33 (d, J = 6.5 Hz, 3H, Me); 3.71 (m, 3H, CH_2–OH and C*H*–NH); 3.78 (m, 1H, C*H*–OH); 7.53 (m, 3H, CH_{ar}); 7.95 (m, 2H, CH_{ar}); HRMS: calcd for $C_{11}H_{15}NO_3$ $[M+Na]^+$: 232.0950; found: 232.0945.

Compound **1Ba** (41 mg from 190 mg of resin **E1Ba**): ^{1}H NMR (500 MHz, $CDCl_3$): 0.88 (t, J = 6.0 Hz, 3H, CH_3); 1.30 (broad, 6H, CH_2); 1.51 (m, 2H, CH_2); 3.83 (m, 3H, CH_2–OH and C*H*–NH); 4.12 (m, 1H, C*H*–OH); 7.46 (m, 2H, CH_{ar}); 7.54 (m, 1H, CH_{ar}); 7.81 (m, 2H, CH_{ar}); HRMS: calcd for $C_{16}H_{25}NO_3$ $[M+Na]^+$: 302.1732; found: 302.1738.

Compound **1Ca** (53 mg from 200 mg of resin **E1Ca**): ^{1}H NMR (500 MHz, $CDCl_3$): 0.87 (t, J = 7.0 Hz, 3H, Me); 1.29 (broad, 12H, CH_2); 1.50 (m, 2H CH_2); 3.76 (m, 2H, CH_2–OH); 3.85 (m, 1H, C*H*–NH); 4.12 (m, 1H, C*H*–OH); 7.46 (m, 2H, CH_{ar}); 7.53 (m, 1H, CH_{ar}); 7.81 (m, 2H, CH_{ar}); HRMS: calcd for $C_{18}H_{29}NO_3$ $[M+Na]^+$: 330.2045; found: 330.2052.

Compound **1Da** (35 mg from 160 mg of resin **E1Da**): ^{1}H NMR (500 MHz, $CDCl_3$): 0.89 (t, J = 7.0 Hz, 3H, Me); 1.29 (broad, 12H, CH_2); 1.50 (m, 2H CH_2); 3.75 (m, 2H, CH_2–OH); 3.84 (m, 1H, C*H*–NH); 4.12 (m, 1H, C*H*–OH); 7.46 (m, 2H, CH_{ar}); 7.53 (m, 1H, CH_{ar}); 7.81 (m, 2H, CH_{ar}); HRMS: calcd for $C_{22}H_{37}NO_3$ $[M+Na]^+$: 386.2671; found: 386.2664.

Compound **1Ea** (30 mg from 160 mg of resin **E1Da**): ^{1}H NMR (500 MHz, $CDCl_3$): 3.86 (broad, 2H, CH_2–OH); 4.37 (broad, 1H, C*H*–NH); 4.90 (m, 1H, C*H*–OH); 7.25 (m, 5H, CH_{ar}); 7.45 (m, 4H, CH_{ar}); 7.55 (m, 3H, CH_{ar}); 7.82 (m, 2H, CH_{ar}); HRMS: calcd for $C_{16}H_{17}NO_3$ $[M+Na]^+$: 294.1106; found: 294.1100.

Compound **1Ab** (50 mg from 220 mg of resin **E1Ab**): ^{1}H NMR (500 MHz, $CDCl_3$): 0.89 (t, J = 7.0 Hz, 3H, CH_3); 1.24 (d, J = 6.5 Hz, 3H, Me); 1.32 (m, 8H, CH_2); 1.63 (m, 2H, CH_2(β phenyl)); 2.66 (m, 2H, CH_2 (α phenyl)); 3.71 (m, 3H, CH_2–OH and C*H*–NH); 3.78 (m, 1H, C*H*–OH); 7.27 (m, 2H, CH_{ar}); 7.76 (m, 2H, CH_{ar}); HRMS: calcd for $C_{18}H_{29}NO_3$ $[M+Na]^+$: 330.2045; found: 330.2050.

Compound **1Bb** (56 mg from 198 mg of resin **E1Bb**): ^{1}H NMR (500 MHz, $CDCl_3$): 0.88 (t, J = 6.5 Hz, 6H, CH_3); 1.31 (broad, 16H, CH_2); 1.53 (m, 2H CH_2); 1.63 (m, 2H, CH_2 (β phenyl)); 2.66 (t, J = 7.5 Hz, 2H, CH_2(α phenyl)); 3.73 (m, 3H, CH_2–OH and C*H*–NH); 4.10 (m, 1H, C*H*–OH); 7.27 (m, 2H, CH_{ar}); 7.76 (m, 2H, CH_{ar}); HRMS: calcd for $C_{23}H_{39}NO_3$ $[M+Na]^+$: 400.2828; found: 400.2819.

Compound **1Cb** (109 mg from 393 mg of resin **E1Cb**): ^{1}H NMR (500 MHz, $CDCl_3$): 0.88 (t, J = 6.5 Hz, 6H, CH_3); 1.29 (broad, 20H, CH_2); 1.53 (m, 2H CH_2); 1.63

(m, 2H, CH_2(β phenyl)); 2.65 (t, J = 7.0 Hz, 2H, CH_2 (α phenyl)); 3.78 (m, 3H, CH_2–OH and CH–NH); 4.11 (m, 1H, CH–OH); 7.27 (m, 2H, CH_{ar}); 7.76 (m, 2H, CH_{ar}); HRMS: calcd for $C_{25}H_{43}NO_3$ $[M+Na]^+$: 428.3141; found: 428.3157.

Compound **1Db** (86 mg from 313 mg of resin **E1Db**): ^{1}H NMR (500 MHz, $CDCl_3$): 0.88 (t, J = 7.0 Hz, 6H, CH_3); 1.26 (broad, 28H, CH_2); 1.49 (m, 2H CH_2); 1.63 (m, 2H, CH_2 (β phenyl)); 2.65 (t, J = 7.5 Hz, 2H, CH_2(α phenyl)); 3.73 (m, 3H, CH_2–OH and CH–NH); 4.11 (m, 1H, CH–OH); 7.26 (m, 2H, CH_{ar}); 7.76 (m, 2H, CH_{ar}); HRMS: calcd for $C_{29}H_{51}NO_3$ $[M+Na]^+$: 484.3767; found: 484.3754.

Compound **1Eb** (83 mg from 220 mg of resin **E1Eb**): ^{1}H NMR (500 MHz, $CDCl_3$): 0.88 (t, J = 6.5 Hz, 3H, CH_3); 1.31 (broad, 8H, CH_2); 1.59 (m, 2H CH_2(β phenyl)); 2.62 (t, J = 7.5 Hz, 2H, CH_2(α phenyl)); 3.86 (m, 2H, CH_2–OH); 4.37 (m, 1H, CH–NH); 4.90 (m, 1H, CH–OH); 7.25 (m, 5H, CH_{ar}); 7.45 (m, 2H, CH_{ar}); 7.57 (m, 2H, CH_{ar}); HRMS: calcd for $C_{23}H_{31}NO_3$ $[M+Na]^+$: 392.2202; found: 392.2217.

Compound **1Ac** (34 mg from 190 mg of resin **E1Ac**): ^{1}H NMR (500 MHz, $CDCl_3$): 0.96 (d, 6H, CH_3 (isopropyl)); 1.16 (m, 3H, Me); 2.10 (m, 3H, CH_2 and CH (isopropyl)); 3.62 (m, 3H, CH_2–OH and CH–NH); 3.80 (m, 1H, CH–OH); HRMS: calcd for $C_9H_{19}NO_3$ $[M+Na]^+$: 212.1263; found: 212.1269.

Compound **1Bc** (39 mg from 190 mg of resin **E1Bc**): ^{1}H NMR (500 MHz, $CDCl_3$): 0.89 (t, J = 7.0 Hz, 3H, CH_3); 0.96 (d, 6H, CH_3 (isopropyl)); 1.31 (broad, 8H, CH_2); 1.54 (m, 2H, CH_2); 2.10 (m, 3H, CH_2 and CH (isopropyl)); 3.64 (m, 3H, CH_2–OH and CH–NH); 3.82 (m, 1H, CH–OH); HRMS: calcd for $C_{14}H_{29}NO_3$ $[M+Na]^+$: 282.2045; found: 282.2039.

Compound **1Cc** (52 mg from 200 mg of resin **E1Cc**): ^{1}H NMR (500 MHz, $CDCl_3$): 0.89 (t, J = 7.5 Hz, 3H, CH_3); 0.95 (d, 3H, CH_3 (isopropyl)); 1.30 (broad, 10H, CH_2); 1.44 (m, 2H CH_2); 2.10 (m, 3H, CH_2 and CH (isopropyl)); 3.64 (m, 3H, CH_2–OH and CH–NH); 3.81 (m, 1H, CH–OH); HRMS: calcd for $C_{16}H_{33}NO_3$ $[M+Na]^+$: 310.2358; found: 310.2348.

Compound **1Dc** (37 mg from 160 mg of resin **E1Dc**): ^{1}H NMR (500 MHz, $CDCl_3$): 0.88 (t, J = 7.5 Hz, 3H, CH_3); 0.94 (d, 3H, CH_3 (isopropyl)); 1.29 (broad, 10H, CH_2); 1.44 (m, 2H CH_2); 2.10 (m, 3H, CH_2 and CH (isopropyl)); 3.66 (m, 3H, CH_2–OH and CH–NH); 3.81 (m, 1H, CH–OH); HRMS: calcd for $C_{20}H_{41}NO_3$ $[M+Na]^+$: 366.2984; found: 366.2995.

Compound **1Ec** (25 mg from 160 mg of resin **E1Ec**): ^{1}H NMR (500 MHz, $CDCl_3$): 0.96 (d, 3H, CH_3 (isopropyl)); 2.12 (m, 3H, CH_2 and CH (isopropyl)); 3.86 (m, 2H, CH_2–OH); 4.37 (m, 1H, CH–NH); 4.90 (m, 1H, CH–OH); 7.46 (m, 2H, CH_{ar}); 7.53 (m, 1H, CH_{ar}); 7.81 (m, 2H, CH_{ar}); HRMS: calcd for $C_{14}H_{21}NO_3$ $[M+Na]^+$: 274.1419; found: 274.1424.

Compound **1Ad** (26 mg from 190 mg of resin **E1Ad**): ^{1}H NMR (500 MHz, $CDCl_3$): 1.12 (m, 2H, CH_2); 1.21 (d, J = 6 Hz, 3H, CH_3); 1.63 (m, 5H, 2 CH_2 and CH); 1.79 (m, 4H, 2 CH_2); 2.25 (m, 2H, CH_2); 3.63 (m, 3H, CH_2–OH and CH–NH); 3.79 (m, 1H, CH–OH); HRMS: calcd for $C_{12}H_{23}NO_3$ $[M+Na]^+$: 252.1576; found: 252.1570.

Compound **1Bd** (38 mg from 190 mg of resin **E1Bd**): ^{1}H NMR (500 MHz, $CDCl_3$): 0.90 (t, J = 6.5 Hz, 3H, CH_3);.1.12 (m, 2H, CH_2); 1.32 (broad, 8H, CH_2); 1.43 (m, 2H CH_2); 1.63 (m, 5H, 2 CH_2 and CH); 1.79 (m, 4H, 2 × CH_2); 2.25 (m, 2H, CH_2); 3.64 (m, 3H, CH_2–OH and CH–NH); 3.81 (m, 1H, CH–OH); HRMS: calcd for $C_{17}H_{33}NO_3$ $[M+Na]^+$: 322.2358; found: 322.2371.

Compound **1Cd** (42 mg from 200 mg of resin **E1Cd**): ^{1}H NMR (500 MHz, $CDCl_3$): 0.89 (t, J = 6.5 Hz, 3H, CH_3); 1.12 (m, 2H, CH_2); 1.29 (broad, 12H, CH_2); 1.54 (m, 2H CH_2); 1.63 (m, 5H, 2 CH_2 and CH); 1.79 (m, 4H, 2 CH_2); 2.25 (m, 2H, CH_2); 3.66 (m, 3H, CH_2–OH and CH–NH); 3.81 (m, 1H, CH–OH); HRMS: calcd for $C_{19}H_{37}NO_3$ $[M+Na]^+$: 350.2671; found: 350.2666.

Compound **1Dd** (30 mg from 160 mg of resin **E1Dd**): ^{1}H NMR (500 MHz, $CDCl_3$): 0.90 (t, J = 6.5 Hz, 3H, CH_3); 1.13 (m, 2H, CH_2); 1.29 (broad, 20H, CH_2); 1.54 (m, 2H CH_2); 1.64 (m, 5H, 2 CH_2 and CH); 1.79 (m, 4H, 2 CH_2); 2.30 (m, 2H, CH_2); 3.65 (m, 3H, CH_2–OH and CH–NH); 3.80 (m, 1H, CH–OH); HRMS: calcd for $C_{23}H_{45}NO_3$ $[M+Na]^+$: 406.3297; found: 406.3309.

Compound **1Ed** (25 mg from 160 mg of resin **E1Ed**): ^{1}H NMR (500 MHz, $CDCl_3$): 1.01 (m, 2H, CH_2); 1.57 (m, 2H CH_2); 1.67 (m, 5H, 2 CH_2 and CH); 1.79 (m, 4H, 2 CH_2); 2.07 (m, 2H, CH_2); 3.69 (m, 2H, CH_2–OH); 4.17 (m, 1H, CH–NH); 5.02 (m, 1H, CH–OH); 7.24 (m, 5H, CH_{ar}); 7.31 (m, 2H, CH_{ar}); 7.40 (m, 2H, CH_{ar}); HRMS: calcd for $C_{17}H_{25}NO_3$ $[M+Na]^+$: 314.1732; found: 314.1726.

Compound **1Ae** (35 mg from 190 mg of resin **E1Ae**): ^{1}H NMR (500 MHz, $CDCl_3$): 1.21 (d, J = 6 Hz, 3H, CH_3); 3.65 (m, 3H, CH_2–OH and CH–NH); 3.82 (m, 1H, CH–OH); 7.52 (m, 3H, CH_{ar}); 7.95 (m, 3H, CH_{ar}); 8.20 (m, 1H, CH_{ar}); HRMS: calcd for $C_{15}H_{17}NO_3$ $[M+Na]^+$: 282.1106; found: 282.1100.

Compound **1Be** (38 mg from 190 mg of resin **E1Be**): ^{1}H NMR (500 MHz, $CDCl_3$): 0.91 (t, J = 7.0 Hz, 3H, CH_3); 1.34 (broad, 8H, CH_2); 1.62 (m, 2H, CH_2); 3.75 (m, 3H, CH_2–OH and CH–NH); 3.92 (m, 1H, CH–OH); 7.53 (m, 3H, CH_{ar}); 7.95 (m, 3H, CH_{ar}); 8.21 (m, 1H, CH_{ar}); HRMS: calcd for $C_{20}H_{27}NO_3$ $[M+Na]^+$: 352.1889; found: 352.1897.

Compound **1Ce** (53 mg from 200 mg of resin **E1Ce**): ^{1}H NMR (500 MHz, $CDCl_3$): 0.89 (t, J = 7.0 Hz, 3H, CH_3); 1.29 (broad, 12H, CH_2); 1.61 (m, 2H, CH_2); 3.79 (m, 3H, CH_2–OH and CH–NH); 4.23 (m, 1H, CH–OH); 7.54 (m, 3H, CH_{ar}); 7.94 (m, 3H, CH_{ar}); 8.21 (m, 1H, CH_{ar}); HRMS: calcd for $C_{22}H_{31}NO_3$ $[M+Na]^+$: 380.2202; found: 380.2195.

Compound **1De** (31 mg from 160 mg of resin **E1De**): ^{1}H NMR (500 MHz, $CDCl_3$): 0.89 (t, J = 6.5 Hz, 3H, CH_3); 1.28 (broad, 20H, CH_2); 1.61 (m, 2H, CH_2); 3.78 (m, 3H, CH_2–OH and CH–NH); 3.92 (m, 1H, CH–OH); 7.55 (m, 3H, CH_{ar}); 7.94 (m, 3H, CH_{ar}); 8.20 (m, 1H, CH_{ar}); HRMS: calcd for $C_{26}H_{39}NO_3$ $[M+Na]^+$: 436.2828; found: 436.2845.

Compound **1Ee** (25 mg from 160 mg of resin **E1Ee**): ^{1}H NMR (500 MHz, $CDCl_3$): 3.69 (m, 2H, CH_2–OH); 4.17 (m, 1H, CH–NH); 5.02 (m, 1H, CH–OH); 7.24 (m, 5H, CH_{ar}); 7.31 (m, 2H, CH_{ar}); 7.40 (m, 2H, CH_{ar}); 7.56 (m, 3H, CH_{ar}); 7.93 (m, 3H, CH_{ar}); 8.22 (m, 1H, CH_{ar}); HRMS: calcd for $C_{20}H_{19}NO_3$ $[M+Na]^+$: 344.1263; found: 344.1270.

Compound **1Af** (44 mg from 190 mg of resin **E1Af**): ^{1}H NMR (500 MHz, $CDCl_3$): 1.13 (d, J = 6 Hz, 3H, CH_3); 3.49 (s, 2H, CH_2); 3.67 (m, 3H, CH_2–OH and CH–NH); 3.81 (m, 7H, CH–OH and 2× O–CH_3); 6.88 (m, 3H, CH_{ar}); HRMS: calcd for $C_{13}H_{19}NO_5$ $[M+Na]^+$: 292.1161; found: 292.1155.

Compound **1Bf** (41 mg from 190 mg of resin **E1Bf**): ^{1}H NMR (500 MHz, $CDCl_3$): 0.88 (t, J = 7.0 Hz, 3H, CH_3); 1.29 (broad, 8H, CH_2); 1.44 (m, 2H, CH_2); 3.48 (s, 2H, CH_2); 3.58 (m, 3H, CH_2–OH and CH–NH); 3.81 (m, 7H, CH–OH and 2× O–CH_3); 6.87 (m, 3H, CH_{ar}); HRMS: calcd for $C_{18}H_{29}NO_5$ $[M+Na]^+$: 362.1944; found: 362.1952.

Compound **1Cf** (47 mg from 200 mg of resin **E1Cf**): ^{1}H NMR (500 MHz, $CDCl_3$): 0.89 (t, J = 7.0 Hz, 3H, CH_3); 1.29 (broad, 12H, CH_2); 1.46 (m, 2H CH_2); 3.45 (s, 2H, CH_2); 3.65 (m, 3H, CH_2–OH and CH–NH); 3.81 (m, 7H, CH–OH and 2× O–CH_3); 6.88 (m, 3H, CH_{ar}); HRMS: calcd for $C_{20}H_{33}NO_5$ $[M+Na]^+$: 390.2257; found: 390.2263.

Compound **1Df** (34 mg from 160 mg of resin **E1Df**): ^{1}H NMR (500 MHz, $CDCl_3$): 0.89 (t, J = 6.5 Hz, 3H, CH_3); 1.28 (broad, 20H, CH_2); 1.56 (m, 2H, CH_2); 3.48 (s, 2H, CH_2); 3.78 (m, 3H, CH_2–OH and CH–NH); 3.92 (m, 1H, CH–OH); 3.65 (m, 3H, CH_2–OH and CH–NH); 3.81 (m, 7H, CH–OH and 2× O–CH_3); 6.88 (m, 3H, CH_{ar}); HRMS: calcd for $C_{24}H_{41}NO_5$ $[M+Na]^+$: 446.2883; found: 446.2875.

Compound **1Ef** (33 mg from 160 mg of resin **E1Ef**): ^{1}H NMR (500 MHz, $CDCl_3$): 3.35 (s, 2H, CH_2); 3.68 (m, 2H, CH_2–OH); 3.77 (s, 3H, O–CH_3); 3.80 (s, 3H, O–CH_3); 4.16 (m, 1H, CH–NH); 4.78 (m, 1H, CH–OH); 6.62 (dd, J_1 = 8.5 Hz, J_2 = 2.0 Hz, 1H, CH_{ar}); 6.88 (m, 2H, CH_{ar}); 7.24 (m, 5H, CH_{ar}); 7.31 (m, 2H, CH_{ar}); 7.40 (m, 2H, CH_{ar}); HRMS: calcd for $C_{18}H_{21}NO_5$ $[M+Na]^+$: 354.1318; found: 354.1307.

Compound **3A** (16.6 mg from 100 mg of resin **E3A**): ^{1}H NMR (500 MHz, CD_3OD): 2.17 (s, 3H, CH_3); 3.76 (dd, J_1 = 11.0 Hz, J_2 = 4.5 Hz, 1H,CHH–OH); 3.87 (dd, J_1 = 11.0 Hz, J_2 = 6.1 Hz, 1H,CHH–OH); 4.22 (dd, J_1 = 13.0 Hz, J_2 = 4.5 Hz, 1H, CH(Fmoc)); 4.23 (d, J = 4.5 Hz, 1H, CH–NH); 4.38 (m, 2H, CH_2(Fmoc)); 7.30 (t, J = 7.5 Hz, 1H, CH_{ar}(Fmoc)); 7.38 (t, J = 7.5 Hz, 2H, CH_{ar}(Fmoc)); 7.66 (dd, J_1 = 7.0 Hz, J_2 = 3.0 Hz, 2H, CH_{ar}(Fmoc)); 7.78 (d, J = 7.5 Hz, 2H, CH_{ar}(Fmoc)); IR (film) 3389, 2943, 2863, 1694, 1466, 1208, 1167, 810 cm^{-1}.

Compound **3B** (20.9 mg from 100 mg of resin **E3B**): ^{1}H NMR (500 MHz, CD_3OD): 0.88 (t, J = 6.5 Hz, 3H, CH_3); 1.28 (broad, 6H, CH_2); 1.56 (sc, 2H, CH_2); 2.53 (t, J = 7.0 Hz, 2H, CH_2); 3.73 (dd, J_1 = 11.5 Hz, J_2 = 5.0 Hz, 1H, CHH–OH); 3.81 (dd, J_1 = 11.5 Hz, J_2 = 4.5 Hz, 1H, CHH–OH); 4.23 (m, 2H, CH(Fmoc) and CH–NH); 4.39 (m, 2H, CH_2(Fmoc)); 7.31 (t, J = 7.5 Hz, 1H, CH_{ar}(Fmoc)); 7.39 (t, J = 7.5 Hz, 2H, CH_{ar}(Fmoc)); 7.67 (dd, J_1 = 7.0 Hz, J_2 = 3.0 Hz, 2H, CH_{ar}(Fmoc)); 7.79 (d, J = 7.5 Hz, 2H, CH_{ar}(Fmoc)) ppm; IR (film): 3392, 2930, 2858, 1683, 1452, 1206, 1144, 802 cm^{-1}.

Compound **3C** (22.8 mg from 100 mg of resin **E3B**): ^{1}H NMR (500 MHz, CD_3OD): 0.87 (t, J = 6.5 Hz, 3H, CH_3); 1.27 (broad, 10H, CH_2); 1.55 (m, 2H, CH_2); 2.53 (t, J = 7.0 Hz, 2H, CH_2); 3.72 (dd, J_1 = 11.0 Hz, J_2 = 5.0 Hz, 1H, CHH–OH); 3.80 (dd, J_1 = 11.0 Hz, J_2 = 4.5 Hz, 1H,CHH–OH); 4.23 (m, 2H, CH(Fmoc) and CH–NH); 4.38 (m, 2H, CH_2(Fmoc)); 7.31 (t, J = 7.5 Hz, 1H, CH_{ar}(Fmoc)); 7.38 (t, J = 7.5 Hz, 2H, CH_{ar}(Fmoc)); 7.67 (dd, J_1 = 7.0 Hz, J_2 = 3.0 Hz, 2H, CH_{ar}(Fmoc)); 7.79 (d, J = 7.5 Hz, 2H, CH_{ar}(Fmoc)); IR (film): 3393, 2926, 2855, 1682, 1204, 1142, 802 cm^{-1}.

Compound **3D** (27.8 mg from 100 mg of resin **E3D**): ^{1}H NMR (500 MHz, CD_3OD): 0.88 (t, J = 6.5 Hz, 3H, CH_3), 1.24 (broad, 18 H, CH_2), 1.54 (m, 2H, C$H_{2(\beta)}$ (C=O)), 2.53 (t, J = 7.0 Hz, 2H, C$H_{2(\alpha)}$ (C=O)), 3.74 (dd, J_1 = 11.5 Hz, J_2 = 5.0 Hz, 1H, CHH(OH)); 3.80 (dd, J_1 = 11.5 Hz, J_2 = 4.5 Hz, 1H, CHH(OH)); 4.23 (m, 2H, CH(Fmoc) and CH(NH)); 4.31 (dd, J_1 = 10.5 Hz, J_2 = 7.0 Hz, 1H, CHH(Fmoc)); 4.45 (dd, J_1 = 10.5 Hz, J_2 = 4.5 Hz, 1H, CHH(Fmoc)); 7.30 (t, J = 7.5 Hz, 2H, CH_{ar}(Fmoc)); 7.38 (t, J = 8 H, 2H, CH_{ar}(Fmoc)); 7.66 (sc, 2H, CH_{ar}(Fmoc)); 7.78 (d, J = 7.5 Hz, 2H, CH_{ar}(Fmoc)); IR (film): 3391, 2919, 2850, 1679, 1449, 1203, 1142, 801, 724 cm^{-1}.

Compound **3E** (20.1 mg from 100 mg of resin **E3E**): ^{1}H NMR (500 MHz, CD_3OD): 3.79 (dd, J_1 = 11.5 Hz, J_2 = 6.5 Hz, 1H, CHH(OH)); 3.85 (dd, J_1 = 11.5 Hz, J_2 = 4.5 Hz, 1H, CHH(OH)); 4.20 (t, J = 7.0 Hz, 2H, CH(Fmoc)); 4.36 (m, 2H, CH_2(Fmoc)); 5.32 (t, J = 4.5 Hz, 1H, CH(NH)); 7.28 (t, J = 7.5 Hz, 2H, CH_{ar}(Fmoc)); 7.36 (t, J = 8H, 2H, CH_{ar}(Fmoc)); 7.49 (t, J = 7.5 Hz, 2H, CH_{ar}(m–C = O)); 7.62 (sc, 3H, CH_{ar}(Fmoc) iCH_{ar}(p-C = O)); 7.75 (d, J = 7.5 Hz, 2H, CH_{ar}(Fmoc)); 8.00 (d, J = 8.0 Hz, 2H, CH_{ar}(o-C=O)) ppm; IR (film): 3392, 1681, 1449, 1204, 1142, 802, 723 cm^{-1}.

Compound **4A** (15.4 mg from 100 mg of resin **E4A**): ^{1}H NMR (500 MHz, CD_3OD) (major isomer): 1.30 (d, J = 6.5 Hz, 3H, CH_3); 3.71 (m, 3H, CH_2–OH and CH–NH); 3.78 (m, 1H, CH–OH); 4.21 (m, 1H, CH(Fmoc)); 4.37 (m, 2H, CH_2(Fmoc)); 7.29 (t, J = 7.5 Hz, 1H,

CH_{ar}(Fmoc)); 7.37 (t, J = 7.0 Hz, 2H, CH_{ar}(Fmoc)); 7.65 (t, J = 6.5 Hz, 2H, CH_{ar}(Fmoc)); 7.78 (d, J = 7.0 Hz, 2H, CH_{ar}(Fmoc)); IR (film): 3399, 2497, 1680, 1450, 1250, 1157, 800, 739 cm^{-1}.

Compound **4B** (18.8 mg from 100 mg of resin **E4B**): ^{1}H NMR (500 MHz, $CDCl_3$) (major isomer): 0.89 (t, J = 6.5 Hz, 3H, CH_3); 1.29 (broad, 8H, CH_2); 1.53 (m, 2H CH_2); 3.56 (m, 3H, CH_2–OH and CH–NH); 3.78 (m, 1H, CH–OH); 4.22 (m, 1H, CH(Fmoc)); 4.38 (m, 2H, CH_2(Fmoc)); 7.30 (t, J = 7.0 Hz, 1H, CH_{ar}(Fmoc)); 7.38 (t, J = 7.0 Hz, 2H, CH_{ar}(Fmoc)); 7.67 (d, J = 6.5 Hz, 2H, CH_{ar}(Fmoc)); 7.79 (d, J = 7.5 Hz, 2H, CH_{ar}(Fmoc)); IR (film): 3392, 2493, 1678, 1453, 1209, 1151, 802, 728 cm^{-1}.

Compound **4C** (20.4 mg from 100 mg of resin **E4B**): ^{1}H NMR (500 MHz, $CDCl_3$) (major isomer): 0.88 (t, J = 7.5 Hz, 3H, CH_3); 1.29 (broad, 12H, CH_2); 1.53 (sc, 2H CH_2); 3.55 (broad, 3H, CH_2–OH and CH–NH); 3.78 (broad, 1H, CH–OH); 4.22 (m, 1H, CH(Fmoc)); 4.38 (m, 2H, CH_2(Fmoc)); 7.30 (t, J = 7.0 Hz, 1H, CH_{ar}(Fmoc)); 7.38 (t, J = 7.5 Hz, 2H, CH_{ar}(Fmoc)); 7.67 (d, J = 7.0 Hz, 2H, CH_{ar}(Fmoc)); 7.79 (d, J = 7.5 Hz, 2H, CH_{ar}(Fmoc)); IR (film): 3401, 2504, 1674, 1451, 1209, 1149, 802, 727 cm^{-1}.

Compound **4D** (26.5 mg from 100 mg of resin **E4D**): ^{1}H NMR (500 MHz, $CDCl_3$) (major isomer): 0.88 (t, J = 7.5 Hz, 3H, CH_3); 1.28 (broad, 20H, CH_2); 1.54 (m, 2H CH_2); 3.55 (m, 3H, CH_2–OH and CH–NH); 3.78 (m, 1H, CH–OH); 4.22 (m, 1H, CH(Fmoc)); 4.38 (m, 2H, CH_2(Fmoc)); 7.30 (t, J = 7.5 Hz, 1H, CH_{ar}(Fmoc)); 7.38 (t, J = 7.5 Hz, 2H, CH_{ar}(Fmoc)); 7.66 (d, J = 7.0 Hz, 2H, CH_{ar}(Fmoc)); 7.79 (d, J = 7.5 Hz, 2H, CH_{ar}(Fmoc)); IR (film): 3368, 2916, 1670, 1150, 1205, 1147, 800, 727 cm^{-1}.

Compound **4E** (26.5 mg from 100 mg of resin **E4D**): ^{1}H NMR (500 MHz, $CDCl_3$) (major isomer): 3.89 (m, 2H, CH_2(OH)), 4.20 (m, 2H, CH(Fmoc)); 4.36 (m, 2H, CH_2(Fmoc)); 5.15 (m, 1H, CH–OH); 5.31 (t, J = 4.5 Hz, 1H, CH(NH)); 7.28 (t, J = 7.5 Hz, 2H, CH_{ar}(Fmoc)); 7.36 (t, J = 8 H, 2H, CH_{ar}(Fmoc)); 7.49 (t, J = 7.5 Hz, 2H, CH_{ar}(m-C=O)); 7.62 (sc, 3H, CH_{ar}(Fmoc) and CH_{ar}(p-C=O)); 7.76 (d, J = 7.5 Hz, 2H, CH_{ar}(Fmoc)); 8.01 (d, J = 8.0 Hz, 2H, CH_{ar}(o-C=O)); IR (film): 3392, 2505, 1672, 1450, 1205, 1147, 973, 802, 726 cm^{-1}.

6.2.9. D-*erythro*-*N*-(4-heptylbenzoyl)dihydrosphingosine (b-DHC). A solution of D-*erythro*-dihydrosphingosine (14 mg, 0.046 mmol) in THF (2 mL) was treated with a solution of NaOAc (50% in H_2O) and the resulting biphasic mixture was vigorously stirred for 5 min. 4-Heptylbenzoyl chloride (13 μL, 0.055 mmol) was next added dropwise at rt and the resulting reaction mixture was stirred overnight at rt. Solvent was removed in vacuo, the residue was taken up in CH_2Cl_2 and the organic phase was washed with H_2O (3 × 10 mL), dried and evaporated to dryness to give crude amide. Flash chromatography (CH_2Cl_2/MeOH 50:1) afforded pure **b-DHC** (22 mg, 95% yield); ^{1}H NMR (500 MHz, $CDCl_3$): 7.73 (d, J = 8.22 Hz, 2H), 7.24 (d, J = 8.22 Hz, 2H), 7.14 (broad, 1H), 4.10 (dd, J_1 = 11.5 Hz; J_2 = 3.3 Hz, 1H), 4.03 (m, 1H), 3.89 (m, 1H), 3.85 (m, 1H), 2.65 (t, J = 7.58 Hz, 2H), 1.62 (m, 4H), 1.30 (m, 34H), 0.89 (t, J = 6.80 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): 167.9, 147.3, 131.6, 128.8, 127.3, 74.4, 62.6, 54.3, 36.0, 34.7, 32.1, 32.0, 31.4, 29.9, 29.8, 29.8, 29.6, 29.4, 29.3, 26.2, 22.9, 22.8, 14.3, 14.3; IR (film) 3284, 2918, 2850, 1636, 1541, 1468, 1073, 1042 cm^{-1}; $[\alpha]_D$ +12.1 (c = 1.0, $CHCl_3$, 20 °C).

6.2.10. L-*threo*-*N*-(4-heptylbenzoyl)dihydrosphingosine (L-*threo*-b-DHC). Following the above procedure, L-*threo*-dihydrosphingosine (safingol) (5 mg, 0.017 mmol) afforded 8.3 mg (89%) of the acylated compound; ^{1}H NMR (500 MHz, $CDCl_3$): 7.74 (d, J = 8.15 Hz, 2H), 7.25 (d, J = 8.15 Hz, 2H), 6.91 (broad, 1H), 4.12 (m, 1H), 4.07 (m, 1H), 3.94 (m, 2H), 2.87 (broad, 1H), 2.65 (t, J = 7.64 Hz, 2H), 1.62 (m, 2H), 1.54 (m, 2H), 1.27 (m, 36H), 0.89 (t, J = 6.64 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): 168.2, 147.3, 131.7, 128.8, 127.2, 73.4, 65.8, 36.0, 34.7, 32.1, 32.0, 31.4, 29.9, 29.9, 29.8, 29.8, 29.7, 29.7, 29.5, 29.4, 29.3, 25.8, 22.9, 22.8, 14.3, 14.3; IR (film): 3284, 2918, 2850, 1636, 1541, 1468, 1073, 1042 cm^{-1}; $[\alpha]_D$ +7.81 (c = 0.41, $CHCl_3$, 20 °C).

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