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Stereoselective synthesis of C_{18}-guggultetrol and C_{18}-phytosphingosine analogues from D-fructose

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ABSTRACT

A series of C_{18}-guggultetrol stereo isomers and C_{18}-phytosphingosine regio/stereo isomers were synthesised in a stereoselective fashion involving metal mediated fragmentation, stereoselective reduction, 1,4 *O*→*O* silyl migration, and Grubbs' cross metathesis as key steps. D-Fructose was used as a raw material for the preparation of all the analogues. The isophytosphingosine derivatives were evaluated against their 5-LOX (5-lipoxigenase) inhibitory activity.

1. Introduction

Guggultetrols[1] are aliphatic long chain 1,2,3,4-tetrols isolated from the gum resin of *Commiphora mukul,* a small tree of the family Burseraceae, endemic to the Hindustan peninsula. Two major compounds in guggultetrols series identified are D-*xylo*-C_{18}-guggultetrol (~50%) and D-*xylo*-C_{20}-guggultetrol (40%) (Fig. 1). The crude resin, called *guggulu* in Sanskrit, has been used especially for the treatment of rheumatoid arthritis and lipid disorders in Ayurveda, the traditional system of ancient Indian medicine.[2] Along with guggultetrols two other steroids namely *Z* and *E*-pregna-4,17(20)-diene-3,16-diones (*Z*- and *E*-guggulsterones) were also isolated. However the recent studies of randomized, placebo-controlled clinical trials revealed that guggulsterones do not improve the serum cholesterol levels in adults with hypercholesterolemia.[3] On the other hand, very few reports are available in the literature for the synthesis of guggultetrols[4] and their isomers[5] and no biological studies were reported on these fatty alcohols. Since guggultetrols are structurally similar to the naturally occurring phytosphingolipids (Fig. 1), which are known to play multiple biological activities,[6] these are also expected to exhibit interesting biological properties.

Recent work on the biological importance of D-*ribo*-C_{18}-phytosphingosine[7] made it an attractive target to the synthetic community. As a result a number of methods were published to prepare phytosphingosines as well as their stereoisomers.[8] Interestingly, the regioisomers of these amino-triols, where the amine is positioned on the C-1, C-3, or C-4 carbon are very less. To the best of our knowledge, there is only one recent report in which the amine was placed on the C-4 carbon and it was called isophytosphingosine.[9] The stereoisomers of this isophytosphingosine showed excellent cytotoxic activity in B16 murine melanoma cells, in some cases better activity than the D-*ribo*-C_{18}-phytosphingosine itself. As part of our research program aimed at developing enantioselective methods for the synthesis of bioactive molecules starting from commercially inexpensive carbohydrate raw materials,[10] herein we report the synthesis of guggultetrol epimers as well as the stereo and regioisomers of phytosphingolipids from the commercially inexpensive ketose, D-fructose. Further, the synthetic analogues of phytosphingolipids were evaluated against their 5-LOX (5-lipoxigenase) inhibitory activity.

2. Results and discussion

The key intermediates **2** and **3**, for the preparation of guggultetrol epimers as well as phytosphingosine analogues, were synthesized on a large scale from commercially available inexpensive raw material D-fructose **1** in a series of eight steps (**2** in 27% and **3** in 22% overall yield) involving only three column chromatographic purifications.[10] Cross metathesis of alcohols **2** and **3** with tetradec-1-ene using Grubbs II generation catalyst provided the alkenols **4**[10a] and **5**[10b] which were further hydrogenated with 10%

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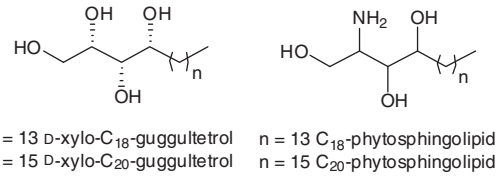


Figure 1. General structure of guggultetrols and phytosphingolipids.

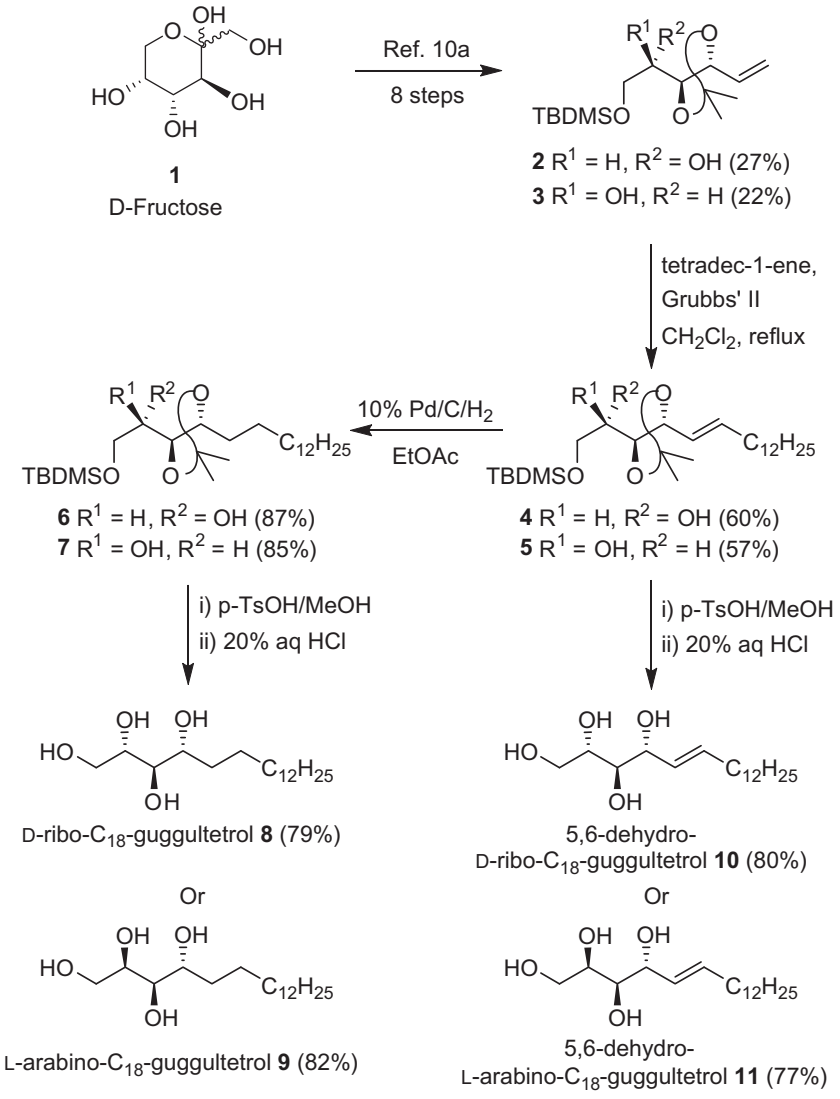
Pd/C under hydrogen atmosphere to give alcohols **6**[10a] and **7**[10a] respectively in good yield. A one-pot deprotection of TBDMS and acetonide protective groups using *p*-TsOH/MeOH followed by 20% aqueous HCl provided D-*ribo*-C_{18}-guggultetrol **8** and L-*arabino*-C_{18}-guggultetrol **9** respectively, in excellent yield as single enantiomers. On the other hand, global deprotection of TBDMS and acetonide protecting groups of alkenols **4** and **5** under similar reaction conditions mentioned above furnished unsaturated guggultetrol analogues **10** and **11** (Scheme 1).

The synthesis of phytosphingosine analogues was envisaged again starting from the key intermediate enols **2** and **3**. Thus, the reaction of **2** and **3** with Ph_3P/DIAD/phthalimide in dry THF under Mitsunobu reaction conditions provided enols **12**[11] and **13**[11] respectively, via a one-pot 1,4 *O*→*O* silyl migration followed by nucleophilic substitution with phthalimide at the primary carbon center.[11] Cross-metathesis of olefins **12** and **13** with tetradec-1-ene provided the protected 5,6-unsaturated phytosphingosine analogues **14** and **15**. Pd/C/H_2 mediated hydrogenation of the double bond provided compounds **16** and **17** in good yield. A one-pot deprotection of acid-sensitive TBDMS and acetonide protective groups, by using 80% aqueous CH_3COOH at 50 °C provided the phthalimide protected regioisomeric phytosphingosine analogues **18** and **19** in excellent yield. Finally deprotection of phthalimide achieved by stirring the reaction mixture in $H_2NNH_2{\cdot}H_2O$/MeOH gave phytosphingosine analogues **20** and **21** as white solids (Scheme 2).

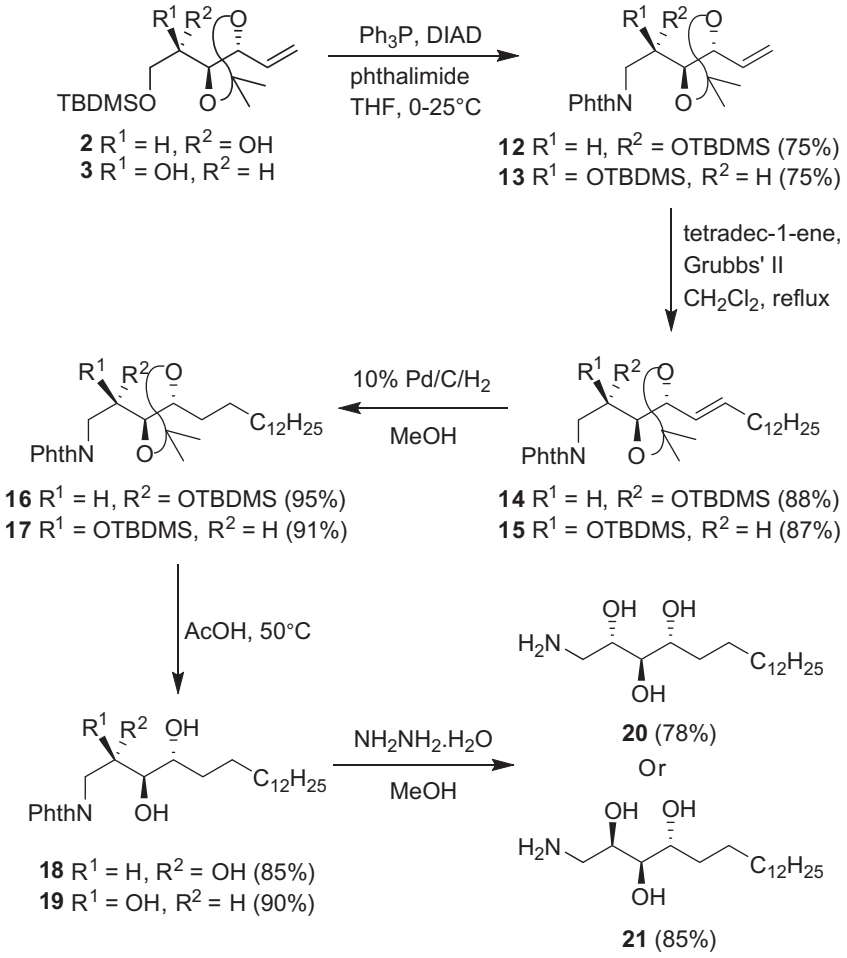
Further we also planned to synthesize 5,6-unsaturated isomeric phytosphingosine derivatives. Thus, the deprotection of alcohol protective groups in olefins **14** and **15** with AcOH at 50 °C furnished 5,6-unsaturated triols **22** and **24**. Final the removal of phthalimide protective group using $NH_2NH_2{\cdot}H_2O$/MeOH provided the 5,6-unsaturated isomeric phytosphingosine derivatives **23** and **25** respectively in good yield (Scheme 3).

3. In vitro 5-lipoxigenase (5-LOX) enzyme assay

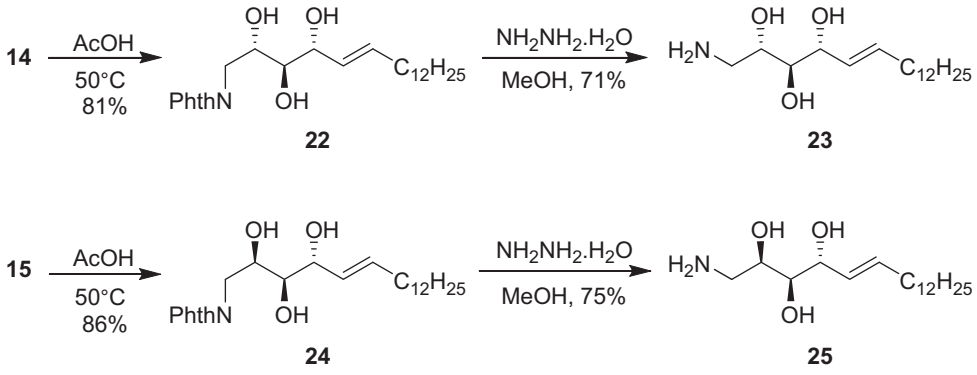
It has been shown that 5-LOX and its metabolites, leukotrienes (LTs) and 5-hydroxyeicosatetraenoic acid (5-HETE) are important



Scheme 1. Stereoselective synthesis of guggultetrol analogues.



Scheme 2. Stereoselective synthesis of isomeric phytosphingosine analogues.



Scheme 3. Synthesis of 5,6-unsaturated isomeric phytosphingosine analogues.

pro-inflammatory mediators participating in various forms of acute and sub acute inflammation as well as potential survival factors for prostate cancer cells and the inhibition of 5-LOX triggered massive apoptosis. Thus, the development of 5-LOX inhibitors gains much importance in order to reduce the side effects caused by the present drugs in the market. For example, COX-2 inhibition by celecoxib (a selective COX-2 inhibitor, drug present in the market) in cancer cell lines was shown to increase the formation of 5-HETE, which is having tumor cell proliferative property.[12] In our studies the isophytosphingosine analogues **20**, **21**, **23,** and **25** were tested in vitro for their inhibitory properties against 5-LOX enzyme

Table 1
IC_{50} values of the isophytosphingosine derivatives against 5-LOX enzyme inhibition

Compound	5-LOX IC_{50} (μM)
20	>100
23	46
21	50
25	0.96
NDGA	1.8

NDGA: nordihydroguaiaretic acid.

using nordihydroguaiaretic acid (NDGA) as a standard 5-LOX inhibitor (IC_{50} = 1.8 μM).[13] Out of the 4 molecules tested compound **21** and **23** showed moderate inhibition and the compound **25** exhibited excellent inhibitory activity with IC_{50} = 0.96 μM (Table 1). Comparing the activities of compounds **20** and **23**, possessing D-*ribo* configuration and compounds **21** and **25**, possessing L-*arabino* configuration, indicates that incorporation of unsaturation at 5 position increases the 5-LOX inhibitory activity.

4. Conclusion

In conclusion, an efficient method for the stereoselective synthesis of D-*ribo*-C_{18}-guggultetrol, L-*arabino*-C_{18}-guggultetrol, and their 5,6-unsaturated counterparts as well as regioisomers of phytosphingosines were developed starting from D-fructose. This is the first report on the synthesis of the isophytosphingosine analogue in which the amine was placed on an achiral carbon. Further all the phytosphingosine regioisomers were evaluated for their in vitro 5-LOX inhibitory activity. Synthesis of other regio-isomers of phytosphingosine family and the evaluation of their bioactivities were under progress.z

5. Experimental

5.1. General

All the reactions were carried out under inert atmosphere with dry solvents under anhydrous conditions unless otherwise mentioned. TLC was run on Silica Gel 60 F254 (Merck) and the spots were detected by staining with H_2SO_4 in methanol (5%, V/V) or phosphomolybdic acid in ethanol (5%, W/V) and heat. Silica-gel (100–200 mesh) was used as a stationary phase for column chromatography. NMR spectra were recorded at 25 °C on a Bruker AvanceIII 400 (400 MHz for ^1H and 100 MHz for ^{13}C) or 500 (500 MHz for ^1H and 125 MHz for ^{13}C) instrument with $CDCl_3$ or CD_3OD as solvent and residual $CHCl_3$ (δ_H 7.26 ppm) or CH_3OH (δ_H 3.31 ppm) as internal standard for ^1H and $CDCl_3$ (δ_C 77.0 ppm) or CD_3OD (δ_C 49.0 ppm) as internal standard for ^{13}C. Chemical shifts are given in δ (ppm) and coupling constants (J) in Hz. IR spectra were recorded on JASCO FT/IR-5300. Elemental analyses were recorded on a Thermo Finnigan Flash EA 1112 analyzer. Mass spectra were recorded on Shimadzu-LCMS-2010A mass spectrometer.

5.2. (2*S*,3*R*,4*R*)-Octadecane-1,2,3,4-tetraol (8)

To a stirred solution of **6** (60 mg, 0.12 mmol) in methanol (2 mL) was added *p*-toluenesulfonic acid (36.4 mg, 0.19 mmol). The resultant mixture was stirred at room temperature for 2 h. Then 20% aq HCl (1.5 mL) was added to the mixture and stirred for another 2 h. Removal of the solvents under reduced pressure provided a viscous residue. To the residue was added EtOAc (20 mL) and water (10 mL). The organic layer was separated and the aqueous layer was extracted with EtOAc (3 × 20 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated. Column chromatography of the crude product using CH_2Cl_2/MeOH (95:5) provided compound **8** (31 mg, 79%) as a colorless solid. Mp: 97–99 °C. R_f (5% MeOH/CH_2Cl_2): 0.47; $[\alpha]_D^{25}$ +9 (*c* 0.9 in MeOH). IR (KBr, cm^{-1}): ν_{max} 3418, 3292, 2918, 2851, 1471, 1419, 1331, 1269, 1101; ^1H NMR (400 MHz, CD_3OD): δ 3.77 (dd, 1H, J = 2.4, 10.8 Hz), 3.69–3.59 (m, 3H), 3.46 (t, 1H, J = 6.4 Hz), 1.71–1.65 (m, 1H), 1.57 (br s, 1H), 1.32–1.29 (br m, 24H), 0.90 (t, 3H, J = 6.0 Hz); ^{13}C NMR (100 MHz, CD_3OD): δ 76.0, 74.4, 74.0, 64.6, 33.4, 33.1, 30.9, 30.8, 30.5, 23.7, 14.4; Low-resolution MS (ESI): m/z: 319 $(M+1)^+$; Anal. Calcd for $C_{18}H_{38}O_4$: C, 67.88; H, 12.03. Found: C, 67.71; H, 12.11.

5.3. (2*R*,3*R*,4*R*)-Octadecane-1,2,3,4-tetraol (9)

Compound **9** was synthesized following the procedure described for compound **8**: mp: 87–90 °C. Yield 82%. R_f (5% MeOH/CH_2Cl_2) 0.41; $[\alpha]_D^{25}$ +5 (*c* 1 in MeOH). IR (KBr, cm^{-1}): ν_{max} 3391, 3292, 2920, 2849, 1469, 1323, 1298, 1076; ^1H NMR (400 MHz, CD_3OD): δ 3.89 (td, 1H, J = 2.0, 6.4 Hz), 3.62–3.60 (m, 3H), 3.33 (d, 1H, J = 2.0 Hz), 1.78–1.73 (m, 1H), 1.57–1.55 (m, 1H), 1.38–1.29 (br m, 24H), 0.90 (t, 3H, J = 6.4 Hz). ^{13}C NMR (100 MHz, CD_3OD): δ 74.6, 74.1, 72.2, 64.7, 33.5, 33.0, 30.8, 30.7, 30.6, 30.5, 30.4, 30.3, 23.7, 14.4. Low-resolution MS (ESI): m/z: 319 $(M+1)^+$. Anal. Calcd for $C_{18}H_{38}O_4$: C, 67.88; H, 12.03. Found: C, 67.75; H, 12.09.

5.4. (2*S*,3*R*,4*R*,*E*)-Octadec-5-ene-1,2,3,4-tetraol (10)

To a stirred solution of **4** (60 mg, 0.12 mmol) in methanol (2 mL) was added *p*-toluenesulfonic acid (36.6 mg, 0.19 mmol). The resultant mixture was stirred at room temperature for 2 h. Then 20% aq HCl (2 mL) was added to the mixture and stirring continued for another 2 h. Removal of the solvents under reduced pressure provided a viscous residue. To the residue was added EtOAc (10 mL) and water (5 mL). The organic layer was separated and the aqueous layer was extracted with EtOAc (3 × 20 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated. Column chromatography of the crude product using CH_2Cl_2/MeOH (95:5) provided compound **10** (32.0 mg, 80%) as a colorless solid. Mp: 69–72 °C. R_f (5% MeOH/CH_2Cl_2) 0.46; $[\alpha]_D^{25}$ +1 (*c* 0.8 in MeOH). IR (KBr, cm^{-1}): ν_{max} 3339, 2920, 2849, 1649, 1518, 1466, 1325, 1228, 1051; ^1H NMR (400 MHz, CD_3OD): δ 5.74 (dt, 1H, J = 6.8, 15.2 Hz), 5.58 (dd, 1H, J = 7.6, 15.6 Hz), 4.19 (dd, 1H, J = 4.0, 7.6 Hz), 3.76 (dd, 1H, J = 2.4, 10.8 Hz), 3.61 (dd, 1H, J = 5.6, 11.2 Hz), 3.56–3.53 (m, 2H), 2.10–2.03 (m, 2H), 1.43–1.38 (m, 2H), 1.33–1.29 (br m, 18H), 0.90 (t, 3H, J = 6.4 Hz); ^{13}C NMR (100 MHz, CD_3OD): δ 135.1, 129.9, 75.9, 75.1, 74.0, 64.7, 33.6, 33.0, 30.8, 30.7, 30.6, 30.5, 30.4, 30.3, 23.7, 14.4; Low-resolution MS (ESI): m/z: 317 $(M+1)^+$; Anal. Calcd for $C_{18}H_{36}O_4$: C, 68.31; H, 11.47. Found: C, 68.21; H, 11.36.

5.5. (2*R*,3*R*,4*R*,*E*)-Octadec-5-ene-1,2,3,4-tetraol (11)

Compound **11** was synthesized following the procedure described for compound **10**: Yield 77%. R_f (5% MeOH/CH_2Cl_2): 0.39; $[\alpha]_D^{25}$ +4 (*c* 0.6 in MeOH). IR (KBr, cm^{-1}): ν_{max} 3447, 2918, 2836, 1685, 1523, 1466, 1325, 1024; ^1H NMR (400 MHz, CD_3OD): δ 5.74 (dt, 1H, J = 6.4, 15.6 Hz), 5.59 (dd, 1H, J = 6.8, 15.6 Hz), 4.09 (t, 1H, J = 7.2 Hz), 3.85 (td, 1H, J = 2.8, 6.8 Hz), 3.61 (dd, 2H, J = 3.2, 5.6 Hz), 3.41 (dd, 1H, J = 2.4, 5.6 Hz), 2.10–2.05 (m, 2H), 1.43–1.38 (m, 2H), 1.33–1.29 (br m, 18H), 0.90 (t, 3H, J = 6.4 Hz); ^{13}C NMR (100 MHz, CD_3OD): δ 134.3, 131.4, 74.6, 74.1, 72.2, 64.7, 33.5, 33.0, 30.8, 30.7, 30.6, 30.5, 30.4, 30.3, 23.7, 14.4; Low-resolution MS (ESI): m/z: 317 $(M+1)^+$; Anal. Calcd for $C_{18}H_{36}O_4$: C, 68.31; H, 11.47. Found: C, 68.21; H, 11.55.

5.6. 2-((*S*)-2-(*tert*-Butyldimethylsilyloxy)-2-((4*R*,5*S*)-2,2-dimethyl-5-((*E*)-tetradec-1-enyl)-1,3-dioxolan-4-yl)ethyl)isoindoline-1,3-dione (14)

Compound **12** (275 mg, 0.63 mmol) and 1-tetradecene (0.50 g, 2.5 mmol) were dissolved in CH_2Cl_2 (25 mL) at room temperature. Grubbs II generation catalyst (4 mol %) was added to the solution and then the reaction mixture was refluxed under nitrogen for 24 h. After cooling the reaction mixture was concentrated and purified by column chromatography with EtOAc:hexane (6:94) to afford compound **14** (305 mg, 88%) as a colorless liquid. R_f (10% EtOAc/hexane): 0.65. $[\alpha]_D^{25}$ −30 (*c* 2 in $CHCl_3$). IR (neat, cm^{-1}): ν_{max}

3474, 2928, 2854, 1774, 1718, 1614, 1467, 1396; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.82 (d, 2H, J = 3.2 Hz), 7.70 (d, 2H, J = 2.0 Hz), 5.81–5.79 (br m, 2H), 4.64 (t, 1H, J = 12.8 Hz), 4.20 (t, 1H, J = 6.0 Hz), 4.10 (dd, 1H, J = 4.0, 6.8 Hz), 3.89 (dd, 1H, J = 6.8, 13.6 Hz), 3.74 (dd, 1H, J = 6.4, 14.0 Hz), 2.13–2.10 (m, 2H), 1.42–1.38 (m, 2H), 1.33 (s, 3H), 1.26–1.23 (br m, 21H), 0.86 (t, 3H, J = 5.6 Hz), 0.83 (s, 9H), 0.01 (s, 3H), −0.06 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.3, 136.7, 133.8, 132.2, 126.2, 123.1, 108.0, 80.3, 79.1, 68.6, 41.3, 32.4, 31.9, 29.7, 29.6, 29.5, 29.3, 29.0, 27.0, 25.8, 22.7, 17.8, 14.1, −4.5, −4.6. Low-resolution MS (ESI): m/z: 599 $(M)^+$; Anal. Calcd for $C_{35}H_{57}NO_5Si$: C, 70.07; H, 9.58; N, 2.33. Found: C, 70.21; H, 9.51; N, 2.45.

5.7. 2-((*R*)-2-(*tert*-Butyldimethylsilyloxy)-2-((4*R*,5*S*)-2,2-dimethyl-5-((*E*)-tetradec-1-enyl)-1,3-dioxolan-4-yl)ethyl)isoindoline-1,3-dione (15)

Compound **15** was synthesized following the procedure described for compound **14**: Yield 87%. R_f (10% EtOAc/hexane): 0.60. $[\alpha]_D^{25}$ +10 (c 1 in $CHCl_3$). IR (neat, cm^{-1}): ν_{max} 3477, 2926, 2854, 1774, 1720, 1618, 1467, 1394; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.83 (dd, 2H, J = 3.2, 5.6 Hz), 7.71 (dd, 2H, J = 3.2, 5.6 Hz), 5.73 (dt, 1H, J = 6.4, 15.2 Hz), 5.62 (dd, 1H, J = 8.8, 15.6 Hz), 4.56 (dd, 1H, J = 6.0, 8.8 Hz), 4.15–4.10 (m, 1H), 4.03 (dd, 1H, J = 6.0, 8.0 Hz), 3.78–3.68 (m, 2H), 2.12–2.07 (m, 2H), 1.49 (s, 3H), 1.40–1.38 (m, 2H), 1.33 (s, 3H), 1.25–1.23 (br m, 18H), 0.87 (t, 3H, J = 6.0 Hz), 0.75 (s, 9H), 0.06 (s, 3H), −0.12 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.3, 137.0, 133.9, 132.1, 125.8, 123.1, 108.1, 80.1, 78.6, 69.1, 40.1, 32.3, 31.9, 29.7, 29.6, 29.5, 29.3, 28.8, 28.0, 25.6, 25.3, 22.7, 18.0, 14.1, −4.4, −4.9; Low-resolution MS (ESI): m/z: 600 $(M)^+$; Anal. Calcd for $C_{35}H_{57}NO_5Si$: C, 70.07; H, 9.58; N, 2.33. Found: C, 70.16; H, 9.49; N, 2.41.

5.8. 2-((*S*)-2-(*tert*-Butyldimethylsilyloxy)-2-((4*R*,5*S*)-2,2-dimethyl-5-tetradecyl-1,3-dioxolan-4-yl)ethyl)iso-indoline-1,3-dione (16)

To a stirred solution of compound **14** (110 mg, 0.18 mmol) in MeOH (6 mL) was added 10% palladium on charcoal (30 mg). Then the mixture was degassed and stirred under hydrogen atmosphere at room temperature overnight. After completion of the reaction (by TLC) the suspension was filtered through a pad of celite and concentrated. The crude product was purified by column chromatography using hexane:EtOAc (19:1) to afford compound **16** (104 mg, 95%) as a colorless liquid. R_f (10% EtOAc/hexane): 0.68; $[\alpha]_D^{25}$ −10 (c 1 in $CHCl_3$). IR (KBr, cm^{-1}): ν_{max} 2922, 2849, 1776, 1720, 1604, 1468, 1398; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.82 (dd, 2H, J = 3.2, 5.6 Hz), 7.69 (dd, 2H, J = 3.2, 5.6 Hz), 4.21 (dd, 1H, J = 6.8, 12.8 Hz), 4.14–4.09 (m, 1H), 4.03 (t, 1H, J = 6.0 Hz), 3.91 (dd, 1H, J = 6.8, 13.6 Hz), 3.74 (dd, 1H, J = 6.4, 13.6 Hz), 1.61–1.53 (m, 2H), 1.29–1.23 (br m, 24H), 1.18 (s, 3H), 1.15 (s, 3H), 0.86 (t, 3H, J = 6.4 Hz), 0.82 (s, 9H), 0.06 (s, 3H), −0.01 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.5, 133.8, 132.3, 123.0, 107.9, 80.5, 78.0, 67.8, 42.3, 31.9, 29.8, 29.7, 29.6, 29.5, 29.4, 27.5, 26.2, 25.7, 25.2, 22.7, 17.9, 14.1, −3.9. −4.6; Low-resolution MS (ESI): m/z: 603 $(M+1)^+$; Anal. Calcd for $C_{35}H_{59}NO_5Si$: C, 69.84; H, 9.88; N, 2.33. Found: C, 69.71; H, 9.81; N, 2.43.

5.9. 2-((*R*)-2-(*tert*-Butyldimethylsilyloxy)-2-((4*R*,5*S*)-2,2-dimethyl-5-tetradecyl-1,3-dioxolan-4-yl)-ethyl)iso-indoline-1,3-dione (17)

Compound **17** was synthesized following the procedure described for compound **16**: Yield 91%. R_f (5% EtOAc/hexane): 0.64. $[\alpha]_D^{25}$ +13 (c 0.7 in $CHCl_3$). IR (neat, cm^{-1}): ν_{max} 2926, 2854, 1776, 1720, 1614, 1467, 1392; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.85 (dd, 2H, J = 3.2, 5.6 Hz), 7.72 (dd, 2H, J = 3.2, 5.6 Hz), 4.17 (td, 1H, J = 3.6, 8.4 Hz), 4.13–4.07 (m, 1H), 3.96 (dd, 1H, J = 5.2, 7.6 Hz), 3.84 (dd, 1H, J = 8.4, 13.6 Hz), 3.59 (dd, 1H, J = 3.6, 14.0 Hz), 1.64–1.61 (m, 2H), 1.46 (s, 3H), 1.33 (s, 3H), 1.25 (br s, 24H), 0.87 (t, 3H, J = 6.4 Hz), 0.74 (s, 9H), 0.04 (s, 3H), −0.15 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.2, 133.9, 132.0, 123.1, 107.8, 79.5, 77.1, 69.0, 40.9, 31.9, 29.8, 29.6, 29.5, 29.3, 28.2, 25.9, 25.8, 25.5, 22.6, 18.0, 14.1, −4.4. −4.9; Low-resolution MS (ESI): m/z: 603 $(M+1)^+$; Anal. Calcd for $C_{35}H_{59}NO_5Si$: C, 69.84; H, 9.88; N, 2.33. Found: C, 69.92; H, 9.81; N, 2.29.

5.10. 2-((2*S*,3*R*,4*R*)-2,3,4-Trihydroxyoctadecyl)isoindoline-1,3-dione (18)

A solution of compound **16** (90 mg, 0.14 mmol) in 80% aqueous acetic acid (5 mL) was stirred at 50 °C for 15 h. After completion of the reaction (by TLC) the reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The residual acetic acid was co-evaporated with toluene to give a white solid. Flash column chromatography of this residue with EtOAc:hexane (1:1) provided compound **18** (56.6 mg, 85%) as a white foam. R_f (50% EtOAc/hexane): 0.61. $[\alpha]_D^{25}$ −1.2 (c 0.6 in $CHCl_3$). IR (KBr, cm^{-1}): ν_{max} 3485, 3312, 3302, 2916, 2849, 1780, 1707, 1467, 1392; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.85 (dd, 2H, J = 3.2, 5.6 Hz), 7.72 (dd, 2H, J = 2.8, 5.6 Hz), 4.11–3.98 (m, 3H), 3.82–3.77 (m, 1H), 3.39 (br s, 1H), 3.27 (br s, 2H), 2.52 (br s, 1H), 1.77–1.68 (m, 1H), 1.53–1.45 (m, 2H), 1.30–1.26 (br m 23H), 0.88 (t, 3H, J = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.5, 134.2, 131.9, 123.5, 73.9, 73.8, 72.9, 41.0, 32.8, 31.8, 29.6, 29.5, 29.3, 25.4, 22.6, 14.0; Low-resolution MS (ESI): m/z: 448 $(M+1)^+$; Anal. Calcd for $C_{26}H_{41}NO_5$: C, 69.77; H, 9.23; N, 3.13. Found: C, 69.71; H, 9.15; N, 3.18.

5.11. 2-((2*R*,3*R*,4*R*)-2,3,4-Trihydroxyoctadecyl)isoindoline-1,3-dione (19)

Compound **19** was synthesized following the procedure described for compound **18**: Yield 90%. R_f (50% EtOAc/hexane): 0.56. $[\alpha]_D^{25}$ +1 (c 0.4 in $CHCl_3$). IR (KBr, cm^{-1}): ν_{max} 3435, 3306, 2918, 2849, 1778, 1714, 1467, 1361; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.86 (dd, 2H, J = 3.2, 5.6 Hz), 7.73 (dd, 2H, J = 2.8, 5.6 Hz), 4.19–4.17 (m, 1H), 4.00 (dd, 1H, J = 7.6, 14.4), 3.87 (dd, 1H, J = 4.8, 14.0), 3.78–3.77 (m, 1H), 3.39 (t, 1H, J = 5.2 Hz), 3.28 (d, 1H, J = 4.8 Hz), 3.01 (d, 1H, J = 7.2 Hz), 2.36 (d, 1H, J = 5.6 Hz), 1.62–1.45 (m, 3H), 1.31–1.26 (br m, 23H), 0.88 (t, 3H, J = 6.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.0, 134.2, 132.0, 123.5, 74.2, 72.9, 69.8, 41.6, 33.6, 31.9, 29.7, 29.6, 29.5, 29.3, 25.9, 22.6, 14.0; Low-resolution MS (ESI): m/z: 448 $(M+1)^+$; Anal. Calcd for $C_{26}H_{41}NO_5$: C, 69.77; H, 9.23; N, 3.13. Found: C, 69.85; H, 9.16; N, 3.21.

5.12. (2*S*,3*R*,4*R*)-1-Aminooctadecane-2,3,4-triol (20)

To a stirred solution of compound **18** (50 mg, 0.11 mmol) in dry CH_3OH (3 mL) was added hydrazine hydrate (11.2 mg, 0.22 mmol) at room temperature. The resulting mixture was stirred overnight. After completion of the reaction the solvent was evaporated under reduced pressure. The obtained residue was purified by flash column chromatography using $CHCl_3$:MeOH:NH_4OH (80:20:0.5) to give compound **20** (27.5 mg, 78%) as a colorless solid. Mp: 90–93 °C. R_f ($CHCl_3$/MeOH/NH_4OH 80:20:0.5): 0.24. $[\alpha]_D^{25}$ +13 (c 0.5 in $CHCl_3$). IR (KBr, cm^{-1}): ν_{max} 3425, 2918, 2851, 1469, 1396; ^{1}H NMR (400 MHz, CD_3OD): δ 3.64–3.58 (m, 2H), 3.38 (t, 1H, J = 6.4 Hz), 2.90 (dd, 1H, J = 3.6, 13.2 Hz), 2.72 (dd, 1H, J = 7.2, 13.2 Hz), 1.72–1.66 (m, 1H), 1.57–1.54 (m, 1H), 1.29 (br s, 24H), 0.90 (t, 3H, J = 6.8 Hz); ^{13}C NMR (100 MHz, CD_3OD): δ 76.7, 74.5, 74.0, 44.8, 33.6, 33.08 30.9, 30.8, 30.7, 30.5, 26.7, 23.7, 14.5; Low-resolution MS (ESI): m/z: 318 $(M+1)^+$; Anal. Calcd for

$C_{18}H_{39}NO_3$: C, 68.09; H, 12.38; N, 4.41. Found: C, 68.19; H, 12.28; N, 4.51.

5.13. (2*R*,3*R*,4*R*)-1-Aminooctadecane-2,3,4-triol (21)

Compound **21** was synthesized by following the procedure described for compound **20**: mp: 86–89 °C. Yield 85%. R_f ($CHCl_3$/MeOH/NH_4OH, 80:20:0.5): 0.26. $[\alpha]_D^{25}$ +6 (*c* 0.9 in $CHCl_3$). IR (KBr, cm^{-1}): ν_{max} 3356, 2918, 2851, 1467, 1342; ^1H NMR (400 MHz, CD_3OD): δ 3.87 (ddd, 1H, *J* = 2.0, 4.4, 8.0 Hz), 3.58 (td, 1H, *J* = 2.8, 8.0 Hz), 3.22 (dd, 1H, *J* = 2.0, 8.0 Hz), 2.85 (dd, 1H, *J* = 8.0, 12.8 Hz), 2.77 (dd, 1H, *J* = 4.4, 13.2 Hz), 1.79–1.73 (m, 1H), 1.59–1.55 (m, 1H), 1.32–1.26 (br m, 24H), 0.90 (t, 3H, *J* = 6.8 Hz); ^{13}C NMR (100 MHz, CD_3OD): δ 76.0, 72.6, 71.7, 45.5, 34.7, 33.0, 30.9, 30.8, 30.7, 30.5, 26.6, 23.7, 14.4; Low-resolution MS (ESI): *m*/*z*: 318 $(M+1)^+$; Anal. Calcd for $C_{18}H_{39}NO_3$: C, 68.09; H, 12.38; N, 4.41. Found: C, 68.21; H, 12.29; N, 4.38.

5.14. 2-((2*S*,3*R*,4*R*,*E*)-2,3,4-Trihydroxyoctadec-5-enyl)isoindoline-1,3-dione (22)

A solution of compound **14** (100 mg, 0.16 mmol) in 80% aqueous acetic acid (8 mL) was stirred at 50 °C for 15 h. After completion of the reaction (by TLC) the reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The residual acetic acid was co-evaporated with toluene to give a white solid. Flash column chromatography of this residue with EtOAc:hexane (1:1) provided compound **22** (63.7 mg, 86%) as a white foam. R_f (40% EtOAc/hexane): 0.58. $[\alpha]_D^{25}$ +2 (*c* 1 in $CHCl_3$). IR (KBr, cm^{-1}): ν_{max} 3479, 2918, 2852, 1776, 1714, 1618, 1464, 1388; ^1H NMR (400 MHz, $CDCl_3$,): δ 7.85 (dd, 2H, *J* = 3.2, 5.6 Hz), 7.73 (dd, 2H, *J* = 3.2, 5.6 Hz), 5.81 (dt, 1H, *J* = 6.4, 13.6 Hz), 5.57 (dd, 1H, *J* = 7.2, 15.8 Hz), 4.26 (t, 1H, *J* = 6.0), 4.13–4.06 (m, 1H), 3.99 (dd, 1H, *J* = 5.2, 14.8 Hz), 3.93–3.89 (m, 1H), 3.45–3.41 (m, 1H), 3.27 (d, 1H, *J* = 4.0 Hz), 3.16 (d, 1H, *J* = 4.0 Hz), 2.08–2.03 (m, 2H), 1.38–1.33 (m, 2H), 1.26–1.24 (br m, 18H), 0.88 (t, 3H, *J* = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.5, 135.8, 134.3, 131.8, 127.9, 123.6, 74.7, 73.4, 72.6, 41.0, 32.3, 31.9, 29.6, 29.5, 29.4, 29.3, 29.2, 29.0, 22.6, 14.0; Low-resolution MS (ESI): *m*/*z*: 446 $(M+1)^+$; Anal. Calcd for $C_{26}H_{39}NO_5$: C, 70.08; H, 8.82; N, 3.14. Found: C, 70.15; H, 8.79; N, 3.22.

5.15. (2*S*,3*R*,4*R*,*E*)-1-Aminooctadec-5-ene-2,3,4-triol (23)

To a stirred solution of compound **22** (100 mg, 0.22 mmol) in dry CH_3OH (3 mL) was added hydrazine hydrate (22.4 mg, 0.45 mmol) at 25 °C. The resulting mixture was stirred overnight. After completion of the reaction the solvent was evaporated under reduced pressure. The obtained residue was purified by flash column chromatography using $CHCl_3$:MeOH:NH_4OH (80:20:0.5) to give compound **23** (50 mg, 71%) as a colorless solid. Mp: 76–79 °C. R_f ($CHCl_3$/MeOH/NH_4OH 80:20:0.5): 0.18. IR (KBr, cm^{-1}): ν_{max} 3418, 2918, 2851, 1614, 1467; ^1H NMR (400 MHz, CD_3OD): δ 5.75 (dt, 1H, *J* = 6.4, 15.6 Hz), 5.58 (dd, 1H, *J* = 7.2, 15.2 Hz), 4.14 (dd, 1H, *J* = 5.2, 7.2 Hz), 3.60–3.55 (m, 1H), 3.47 (dd, 1H, *J* = 5.2, 7.2 Hz), 2.95 (dd, 1H, *J* = 3.6, 13.2 Hz), 2.77 (dd, 1H, *J* = 7.2, 13.2 Hz), 2.10–2.05 (m, 2H), 1.42–1.38 (m, 2H), 1.29 (br s 18H), 0.90 (t, 3H, *J* = 6.8 Hz); ^{13}C NMR (100 MHz, CD_3OD): δ 135.1, 130.0, 76.9, 75.0, 73.0, 44.6, 33.6, 33.1, 30.8, 30.7, 30.6, 30.5, 30.4, 23.7, 14.4; Low-resolution MS (ESI): *m*/*z*: 316 $(M+1)^+$; Anal. Calcd for $C_{18}H_{37}NO_3$: C, 68.53; H, 11.82; N, 4.44. Found: C, 68.45; H, 11.75; N, 4.51.

5.16. 2-((2*R*,3*R*,4*R*,*E*)-2,3,4-Trihydroxyoctadec-5-enyl)isoindoline-1,3-dione (24)

Compound **24** was synthesized following the procedure described for compound **22**: mp: 122–125 °C. Yield 81%. R_f (40% EtOAc/hexane): 0.53. $[\alpha]_D^{25}$ +2 (*c* 1.7 in $CHCl_3$). IR (KBr, cm^{-1}): ν_{max} 3489, 2922, 2856, 1776, 1714, 1618, 1466; ^1H NMR (400 MHz, $CDCl_3$): δ 7.85 (dd, 2H, *J* = 3.2, 5.2 Hz), 7.73 (dd, 2H, *J* = 3.2, 5.6 Hz), 5.79 (dt, 1H, *J* = 6.8, 15.2 Hz), 5.52 (dd, 1H, *J* = 6.8, 15.6 Hz), 4.29 (t, 1H, *J* = 6.0), 4.18–4.15 (m, 1H), 4.00 (dd, 1H, *J* = 8.0, 14.4 Hz), 3.83 (dd, 1H, *J* = 4.4, 14.0 Hz), 3.46 (dd, 1H, *J* = 1.6, 5.2 Hz), 2.07–2.02 (m, 2H), 1.37–1.30 (m, 2H), 1.23 (br s, 18H), 0.87 (t, 3H, *J* = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.0, 134.9, 134.2, 131.9, 128.2, 123.5, 74.8, 72.9, 69.5, 41.5, 32.3, 31.9, 29.6, 29.5, 29.3, 29.2, 29.0, 22.8, 14.1; Low-resolution MS (ESI): *m*/*z*: 445 $(M)^+$; Anal. Calcd for $C_{26}H_{39}NO_5$: C, 70.08; H, 8.82; N, 3.14. Found: C, 70.21; H, 8.75; N, 3.21.

5.17. (2*R*,3*R*,4*R*,*E*)-1-Aminooctadec-5-ene-2,3,4-triol (25)

Compound **25** was synthesized following the procedure described for compound **23**: mp: 73–76 °C. Yield 75%. R_f ($CHCl_3$/MeOH/NH_4OH 80:20:0.5): 0.25. $[\alpha]_D^{25}$ +8 (*c* 0.4 in MeOH). IR (KBr, cm^{-1}): ν_{max} 3385, 2920, 2851, 1626, 1467, 1340; ^1H NMR (400 MHz, CD_3OD): δ 5.74 (dt, 1H, *J* = 6.4, 15.6 Hz), 5.58 (dd, 1H, *J* = 6.8, 15.2 Hz), 4.06 (t, 1H, *J* = 6.8 Hz), 3.82–3.78 (m, 1H), 3.28 (d, 1H, *J* = 2.4), 2.82–2.73 (m, 1H), 2.10–2.05 (m, 2H), 1.42–1.38 (m, 2H), 1.29 (br s, 18H), 0.90 (t, 3H, *J* = 6.4 Hz); ^{13}C NMR (100 MHz, CD_3OD): δ 134.4, 131.5, 75.8, 74.1, 72.3, 45.5, 33.5, 33.0, 30.8, 30.7, 30.6, 30.5, 30.4, 30.3, 23.7, 14.4; Low-resolution MS (ESI): *m*/*z*: 316 $(M+1)^+$; Anal. Calcd for $C_{18}H_{37}NO_3$: C, 68.53; H, 11.82; N, 4.44. Found: C, 68.45; H, 11.75; N, 4.38.

5.18. In vitro 5-LOX inhibitory assay

5-LOX from potato tubers was purified and assayed as per the reported protocol.[13] Enzyme activity was measured using polar graphic method with a Clark's oxygen electrode on Strathkelvin Instruments, model 782, RC-300. Typical reaction mixture contained 50–100 μL of enzyme and 10 μL of substrate [AA-133 μM (final concentration)] 2 mL 100 mM phosphate buffer pH 6.3 and the final volume made upto 3 mL with double distilled water. Since LOXs are oxygen-consuming enzymes, the concentration of oxygen decrease in the reaction mixture was taken as a measure of enzyme activity. Reaction was allowed to proceed at 25 °C and the maximum slope generated was taken for calculating enzyme activity. The activity was expressed as units/mg protein, where one unit is defined as one μmole of oxygen consumed per minute.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carres.2012.07.016.

References

1. (a) Patil, V. D.; Nayak, U. R.; Dev, S. *Tetrahedron* **1972**, *28*, 2341–2352; (b) Patil, V. D.; Nayak, U. R.; Dev, S. *Tetrahedron* **1973**, *29*, 1595–1598; (c) Dev, S. *Pure Appl. Chem.* **1989**, *61*, 353–356.
2. (a) Satyavati, G. V. *Indian J. Med. Res.* **1988**, *87*, 327–335; (b) Sastry, V. V. S. *Bull. Indian Inst. Hist. Med.* **1976**, *6*, 102–116.
3. Szapary, P. O.; Wolfe, M. L.; Bloedon, L. T.; Cucchiara, A. J.; DerMarderosian, A. H.; Cirigliano, M. D.; Rader, D. J. *JAMA* **2003**, *290*, 765–772.
4. (a) Prasad, K. R.; Chandrakumar, A. *Tetrahedron* **2007**, *63*, 1798–1805; (b) George, S.; Suryavanshi, G.; Sudalai, A. *Tetrahedron: Asymmetry* **2010**, *21*, 558–561.
5. (a) Palameta, B.; Zambeli, N. J. *J. Org. Chem.* **1964**, *29*, 1031–1035; (b) Kjer, A.; Kjer, D.; Skrydstrup, T. *Tetrahedron* **1986**, *42*, 1439–1448; (c) Kumar, V.; Dev, S. *Tetrahedron* **1987**, *43*, 5933–5948.

6. (a) Riethmuller, J.; Riehle, A.; Grassme', H.; Gulbins, E. *Biochim. Biophys. Acta, Biomembr.* **2006**, *1758*, 2139–2147; (b) Snook, C. F.; Jones, J. A.; Hannun, Y. A. *Biochim. Biophys. Acta, Mol. Cell Biol. Lipids* **2006**, *1761*, 927–946; (c) Liao, J.; Tao, J.; Lin, G.; Liu, D. *Tetrahedron* **2005**, *61*, 4715–4733.
7. (a) Lee, J. S.; Min, D. S.; Park, C.; Park, C. S.; Cho, N. J. *FEBS Lett.* **2001**, *499*, 82–86; (b) Park, M. T.; Kang, J. A.; Choi, J. A.; Kang, C. M.; Kim, T. H.; Bae, S.; Kang, S.; Kim, S.; Choi, W. I.; Cho, C. K.; Chung, H. Y.; Lee, Y. S.; Lee, S. J. *Clin. Cancer Res.* **2003**, *9*, 878–885; (c) Park, M. T.; Choi, J. A.; Kim, M. J.; Um, H. D.; Bae, S.; Kang, C. M.; Cho, C. K.; Kang, S. M.; Chung, H. Y.; Lee, Y. S.; Lee, S. J. *J. Biol. Chem.* **2003**, *278*, 50624–50634; (d) Nagahara, Y.; Shinomiya, T.; Kuroda, S.; Kaneko, N.; Nishio, R.; Ikekita, M. *Cancer Sci.* **2005**, *96*, 83–92.
8. For reviews on synthesis: (a) Howell, A. R.; Ndakala, A. J. *Curr. Org. Chem.* **2002**, *6*, 365–391; (b) Morales-Serna, J. A.; Llaveria, J.; Diaz, Y.; Isabel, M. M.; Castillón, S. *Curr. Org. Chem.* **2010**, *14*, 2483–2521.
9. Rives, A.; Baudoin-Dehoux, C.; Saffon, N.; Andrieu-Abadie, N.; Génisson, Y. *Org. Biomol. Chem* **2011**, 9, 8163–8170.
10. (a) Perali, R. S.; Mandava, S.; Chalapala, S. *Tetrahedron* **2011**, *67*, 9283–9290; (b) Perali, R. S.; Mandava, S.; Bandi, R. *Tetrahedron* **2011**, *67*, 4031–4035.
11. Perali, R. S.; Mandava, S.; Chunduri, V. R. *Tetrahedron Lett.* **2011**, *52*, 3045–3047.
12. Ye, Y. N.; Wu, W. K.; Shin, V. Y.; Bruce, I. C.; Wong, B. C.; Cho, C. H. *Carcinogenesis* **2005**, *26*, 827–834.
13. Reddanna, P.; Whelan, J.; Maddipati, K. R.; Reddy, C. C. *Methods Enzymol.* **1990**, *187*, 268–277.