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Article

Synthesis and Cytotoxicity Studies of Stilbene Long-Chain Fatty Acid Conjugates

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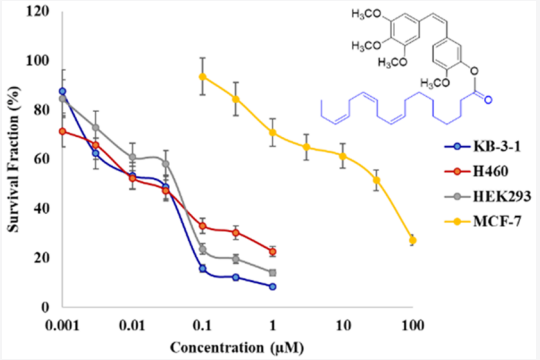
ABSTRACT: A series of 16 conjugates of the tubulin polymerization inhibitor combretastatin A4 (CA-4) and other functionally related stilbene with four 18-carbon fatty acids, namely, stearic, oleic, linoleic, and linolenic acids, have been synthesized in good yields. These new derivatives have been evaluated against the KB-3-1 (human epidermoid carcinoma), NCI-H460 (human lung cancer), HEK293 (human embryonic kidney), and MCF-7 (human breast adenocarcinoma) cell lines for antiproliferative activity, with the exhibited cytotoxic activities comparable with those of CA-4 and colchicine. Compounds **22** and **23**, CA-4 conjugates of linoleic and linolenic acids, respectively, were determined to have exhibited the most active in vitro assays, with compound **23** exhibiting very similar activity to the parent compound against the NCI-H460 cell line. Our studies further delineated the structurally required *Z*-geometry of the stilbene moiety and that conjugation of the less active *E*-stilbenes with the most active fatty acid had minimal or no improvement in their respective activities.



Considering the persistent global challenge of achieving long-term and permanent treatment solutions for various forms of cancer, researchers have intensified efforts based on contemporary approaches such as gene therapy alongside traditional chemotherapeutic approaches.[1−3] Among the small molecular chemical entities investigated is the stilbene combretastatin-A4 (CA-4) isolated by the Pettit group from the South African willow plant *Combretum caffrum* Kuntze. Based on chemotherapeutic assays, CA-4 has been demonstrated to be among the most potent of the isolated classes of stilbenes. Indeed, the phosphate prodrug **1**, having increased aqueous solubility relative to the parent phenol, has advanced through phase II clinical trials in treating patients with advanced recurrent or metastatic anaplastic thyroid cancer (Figure 1).[4−8] Also shown in Figure 1 is the structure of the tubulin inhibitor colchicine.

One of the major drawbacks to the use of chemotherapeutic agents like CA-4 is the systemic toxicity largely resulting from the lack of target specificity, and this has led to increased efforts toward refining the strategy of targeted drug delivery. In one promising approach, various anticancer agents have been conjugated with fatty acids as carriers with the aim of achieving improved target specificity through increased uptake and accumulation of the drug in the tumor environment.[9]

In a previous study, we reported our findings on the cytotoxicity of substituted benzophenones conjugated with various fatty acids differing in the degree of unsaturation and validated increased bioactivity paralleling the degree of unsaturation.[10] The benzophenone scaffolds were examined considering earlier reports that they interact with microtubules in a manner similar to CA-4, binding to the colchicine site of β-tubulin (Figure 2).

With the benefits of fatty acid conjugation established[11] and being cognizant of the dose-limiting cardiotoxic side effects associated with CA-4 administration, we embarked on the study of the current series of CA-4-inspired long-chain fatty acid conjugates anticipating that the incorporated natural fatty acid carriers might further increase the bioactivity profile of both stereoisomeric forms of the functionalized stilbenes, allowing for probable drug administration at reduced concentrations. A persistent and unavoidable caveat to the (*Z*)-stilbenes including CA-4 and its derivatives is the observed isomerization to the less bioactive (*E*)-diastereomer. This has

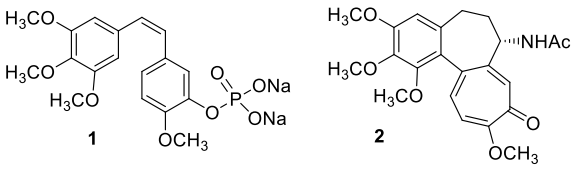


Figure 1. Structures of CA-4 phosphate and colchicine.

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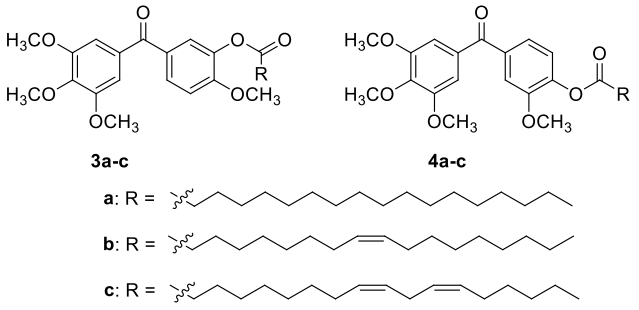


Figure 2. Structures of phenstatin and isophenstatin conjugates.

led us to additionally synthesize and probe the preliminary effects of fatty acid conjugation on these systems. Indeed, the potential to modify the biological properties of chemotherapeutic agents through conjugation with fatty acids has been harnessed by other investigators. Most recently, Ojike et al. reported their findings on the antiproliferative activities of four polyunsaturated fatty acid–CA4 (PUFA–CA4) conjugates, attributing the observed cytotoxicity to the inhibition of microtubule assembly.[12] Concurrently and independently, our team synthesized and characterized the targeted series of 16 fatty acid–stilbene conjugates, which were tested against four tumor cell lines. Varying degrees of antiproliferative activities were observed, consistent with previously reported findings and with the outcomes from the studies performed by the Lavignac group.[12]

In another recent study, Callmann et al. reported the antitumor activity of an 18-carbon α,ω-dicarboxylic acid conjugate of paclitaxel, which readily forms a noncovalent complex with human serum albumin (HSA), a well-studied drug carrier.[13] In this alternate pro-drug strategy, the dicarboxylic acid functions as the binding motif for the drug carrier, and this mimics the interactions between HSA and natural long-chain fatty acids. As previously alluded to, we synthesized strategically and examined both stereoisomeric forms of the stilbene–fatty acid conjugates to assess the extent to which the incorporated fatty acid would alter the cytotoxicity of the stilbenes, and more significantly the impact on the bioactivity of the otherwise low-activity (*E*)-stilbenes. Also surveyed were the incorporation of the more saturated and more economical fatty acids that are anticipated to be more amenable to large-scale applications.

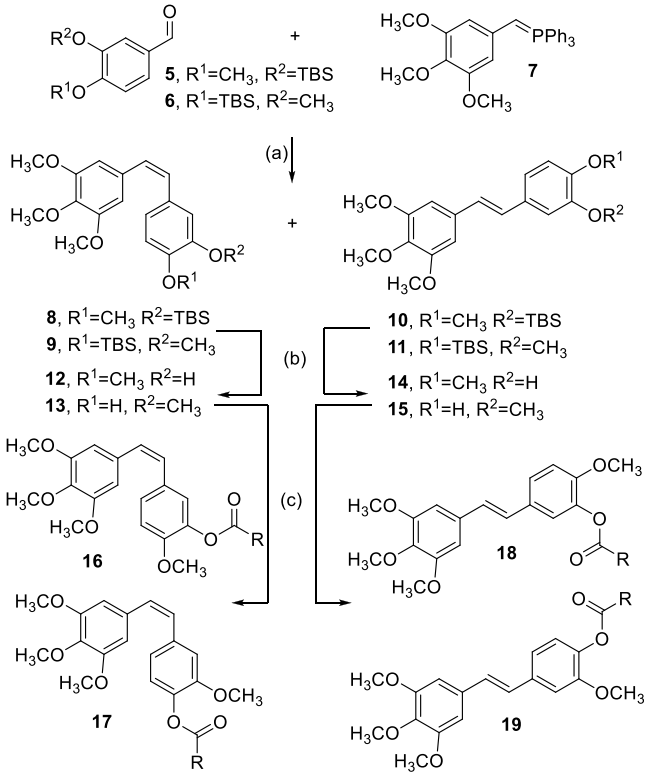
RESULTS AND DISCUSSION

Syntheses of the (*Z*)- and (*E*)-stilbenes were accomplished in a straightforward manner in modest yields by the Wittig olefination approach as reported in the literature, and as summarized in Scheme 1, with the photosensitive *Z*/*E* product mixtures being separated by centrifugal chromatography.[14]

All preliminary steps prior to the olefination reaction were optimized through the incorporation of microwave irradiation with yields in excess of 95%. In our hands, formation of the alkene functionality under microwave irradiation proved elusive, with the starting materials being largely recovered.[15] The silyl deprotection step prior to esterification was also smoothly accomplished in the microwave synthesizer.

Conjugation of the stilbenes with the respective fatty acids was mediated by the coupling agent dicyclohexylcarbodiimide, DCC, and with 2,6-dimethylaminopyridine, DMAP, as additive and is also illustrated in Scheme 1.[16] The 16 new conjugates targeted were successfully synthesized by this approach and

Scheme 1. Synthesis of (*Z*)- and (*E*)-Stilbene–Fatty Acid Conjugates[a]



[a]Reagents and conditions: (a) *n*-BuLi, THF, 0 °C, 65% combined yield of **8** and **10**, 3.6:1 ratio; 44% combined yield of **9** and **11**, 1.6:1 ratio; (b) TBAF, THF, 0 °C, 4 h, 37–91%; (c) RCO_2H, DCC, DMAP,CH_2Cl_2, MW, rt, 12 min.

were fully characterized with analytical data to completely authenticate their chemical structures. Product yields varied, ranging between 25% and 80%. Further attempts at improving the output of the lower yielding reactions such as extended reaction times, modifications of the reaction stoichiometry and physical parameters, and by changing the reaction additives failed to increase product yields. Depicted in Figures 3 and 4 are the 16 conjugates with respective isolated yields given in parentheses. Compound **13** was observed to gradually isomerize into **15**; thus mixtures of these stilbenes were esterified, and the respective conjugates subsequently separated.

In vitro antiproliferative studies of all 16 conjugates were performed by a modified MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric assay.[17] Cytotoxicity studies were performed against the KB-3-1 (human epidermoid carcinoma), NCI-H460 (human lung cancer), HEK293 (human embryonic kidney), and the MCF-7 (human breast adenocarcinoma) cell lines. Also included in Table 1 are the testing data for the parent stilbenes and the natural product colchicine as controls. CA-4 and other tubulin-active agents have been shown to bind to the colchicine site of endothelial tubulin.[18]

Consistent with previous findings from a group of phenstatin conjugates, antiproliferative activity was observed to increase slightly as the tethered fatty acid becomes less saturated.[4] As expected, the derivatized stilbenes generally exhibited reduced cytotoxicity relative to the parent phenols in these in vitro assays. These findings also revealed that both the *trans* stilbene

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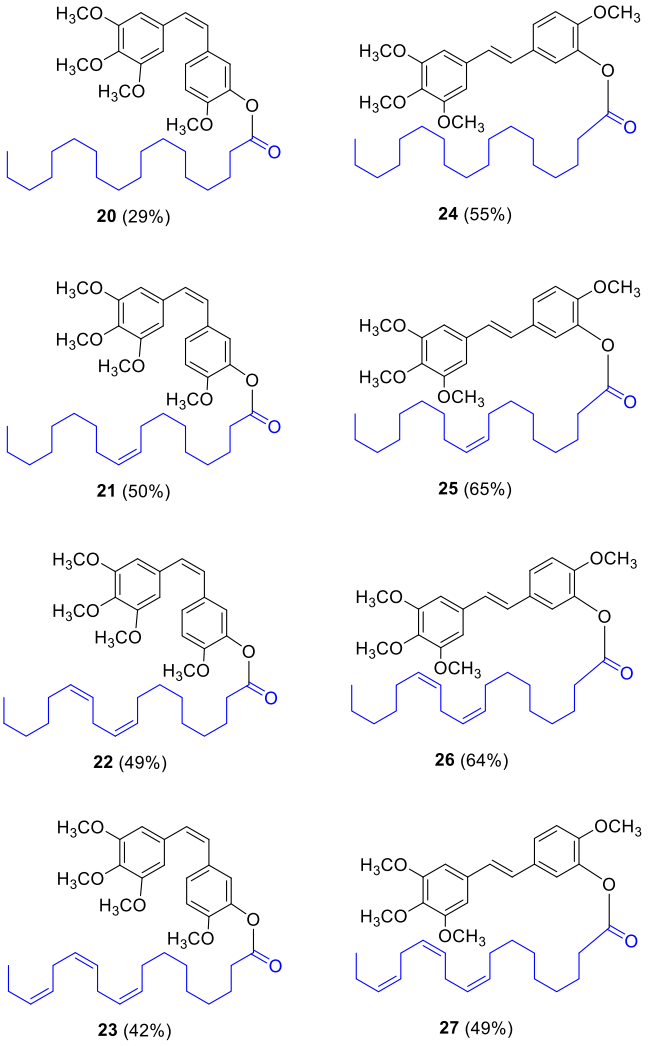

Figure 3. Structures and % yields of the stilbene−fatty acid conjugates **20**−**27**.

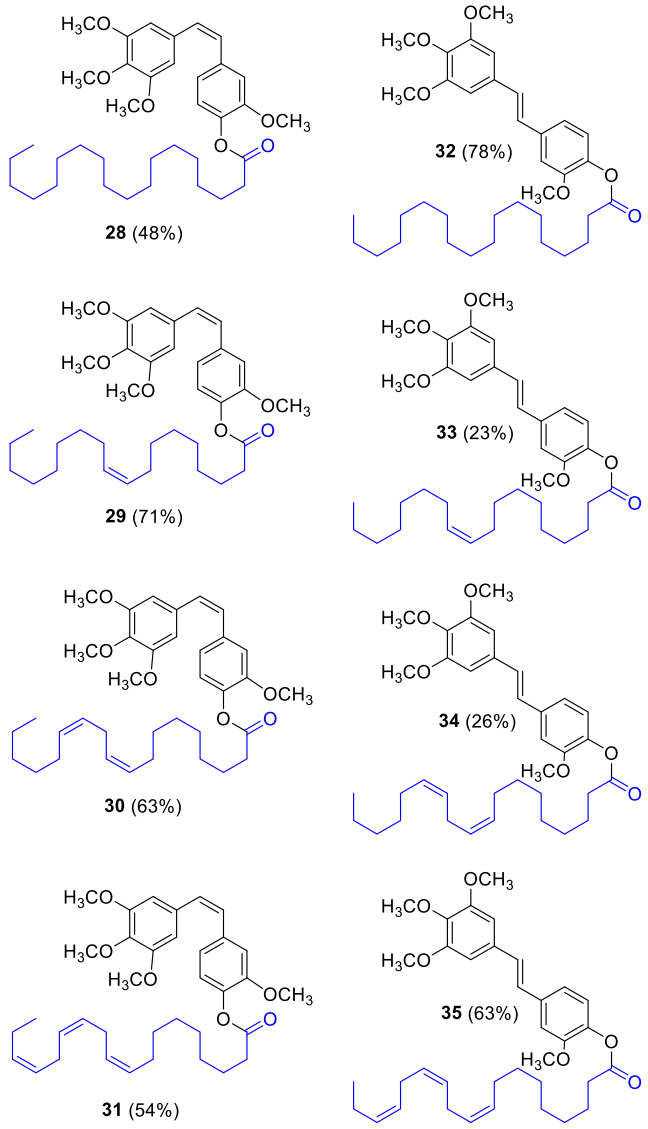

Figure 4. Structures and % yields of the stilbene−fatty acid conjugates **28**−**35**.

parents and their fatty acid conjugates were generally less bioactive than their *cis* counterparts. Indeed, the most active of the (*E*) derivatives were compounds **26** and **27**. Compound **23** has been shown to be most active overall, and particularly against the NCI-H460 cell line, with an IC_{50} value of 0.01 μM.

Against the HEK293 cell line, compound **22** showed the best activity, with an average IC_{50} value of 0.02 μM. The IC_{50} values of compounds **20**−**23** against the KB-3-1 cells were very similar, ranging between 0.02 and 0.03 μM. Compound **22** was determined to be the most active against the HEK293 cell line, with an IC_{50} value of 0.02 μM, while compound **20** gave the best results against the MCF-7 cells (IC_{50} value of 0.24 μM). Comparing IC_{50} values for compounds **26** and **27** against the KB-3-1 and MCF-7 lines, 4.9 and 2.4 μM respectively, reveals an approximate 2-fold increase in bioactivity of compound **27** over compound **26**. It is also noteworthy that the isomerized diastereomeric stilbene **35** exhibited significantly reduced cytotoxicity; at best 200 times weaker than compound **27** against the HEK293 cell line.

The associated conjugates **28** through **31** showed activity that seemed to vary with the cell line investigated. Conjugates **32** through **35** were the least active, generally giving IC_{50} values greater than 100 μM.

In conclusion, we have demonstrated the effects of incorporating long-chain fatty acids in the structure of a bioactive natural product and the extent to which biological properties have been altered. Furthermore, the synthetic utility of incorporating microwave technology in the seamless generation of these conjugates under relatively mild conditions has been established.

EXPERIMENTAL SECTION

General Experimental Procedures. All synthetic transformations were performed in oven-dried glassware. Solutions of air-sensitive reagents were transferred through a syringe under N_2 gas. All reagents were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Dichloromethane was distilled over calcium hydride, while tetrahydrofuran (THF) was distilled over benzophenone and sodium metal under N_2 gas. Microwave-assisted syntheses were accomplished using a CEM Discover Synthesizer (CEM Corporation, Matthews, NC, USA.) All TLC plates were visualized under a UV lamp (254 or 365 nm) or with iodine vapor. Column chromatography was performed using silica gel as a stationary phase and eluting with mixtures of hexane and ethyl acetate. Centrifugal chromatography was performed using a Chromatotron (T-Squared Technology, Inc., San

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Table 1. Summary of Cytotoxicity Data of Synthesized Compounds

	$IC_{50} \pm SD$ (μM)[a]			
compound	KB-3-1[b]	NCI-H460[c]	HEK293[d]	MCF-7[e]
2	0.02 ± 0.002	0.06 ± 0.002	0.02 ± 0.002	0.06 ± 0.006
12	0.01 ± 0.001	0.01 ± 0.001	0.01 ± 0.001	0.09 ± 0.019
14	0.10 ± 0.015	0.25 ± 0.032	0.20 ± 0.021	0.11 ± 0.008
20	0.03 ± 0.004	0.03 ± 0.002	0.03 ± 0.004	0.24 ± 0.027
21	0.03 ± 0.003	0.31 ± 0.045	0.03 ± 0.002	>10
22	0.02 ± 0.002	0.02 ± 0.002	0.02 ± 0.002	>10
23	0.03 ± 0.003	0.01 ± 0.002	0.023 ± 0.003	>10
24	>10	>10	2.90 ± 0.170	>10
26	4.90 ± 0.361	2.40 ± 0.261	2.60 ± 0.296	>10
27	2.40 ± 0.367	>10	2.40 ± 0.212	>10
28	3.10 ± 0.297	>10	3.90 ± 0.593	7.70 ± 0.304

[a]Concentration inhibiting 50% of cell growth for a 72 h exposure period of test samples. Data represent mean values ± standard deviation for three independent experiments. [b]KB-3-1, human epidermoid carcinoma cell line. [c]NCI-H460, human lung cancer cell line. [d]HEK293, human embryonic kidney cell line. [e]MCF-7, human breast adenocarcinoma cell line.

Bruno, CA, USA). Melting points were determined using a Mel-Temp 200W melting point apparatus (Laboratory Devices, Inc.). Infrared (IR) spectra were obtained on a PerkinElmer Spectrum 2 FT-IR spectrometer using a PIKE MIRacle ATR (Attenuated Total Reflectance) accessory. ^{1}H NMR spectra and ^{13}C NMR spectra were obtained on a Bruker Advance DPX spectrometer at 400 and 100 MHz, respectively. NMR samples were prepared by using ~0.75 mL of $CDCl_3$ (Cambridge Isotope Laboratories Inc.), and all spectra were obtained with reference to the solvent peak of $CDCl_3$ at 7.27 ppm for ^{1}H NMR spectra and 77.23 ppm for ^{13}C NMR spectra. ^{1}H NMR spectra were reported as follows: chemical shift δ (ppm); singlet (s), doublet (d), triplet (t), quartet/quintet (q), multiplet (m), coupling constants J = Hz, and integration reported as the number of protons present. Elemental analyses were performed by Atlantic Microlab Inc. (Norcross, GA, USA).

Synthesis of Compounds 8−11. A 10 mL reaction vessel was charged with 3,4,5-trimethoxybenzaldehyde (235 mg, 1.20 mmol) and sodium borohydride (46.9 mg, 1.24 mmol) in 6.0 mL of absolute ethanol. The mixture was irradiated at 150 W, 100 psi, at 25 °C with moderate stirring for 12 min. The reaction mixture was transferred to a separatory funnel, to which cold brine (6 mL) and ethyl acetate (6 mL) were added, and the organic layer was separated. The aqueous layer was further extracted with eight additional 6 mL portions of ethyl acetate, and the organic layers were combined and dried over anhydrous magnesium sulfate. Subsequent gravity filtration and evaporation under reduced pressure afforded pure 3,4,5-trimethoxybenzyl alcohol (217 mg, 95%).

Three 10 mL reaction vessels were charged with 721 mg (3.64 mmol), 586 mg (2.96 mmol), and 451 mg (2.28 mmol) of 3,4,5-trimethoxybenzyl alcohol, respectively. To each vessel were added dry THF (6 mL) and 1 equiv of PBr_3. The reaction mixtures were irradiated at 100 W, 100 psi at 50 °C for 6 min. To the combined reaction mixtures was added 18 mL of cold saturated sodium bicarbonate as an extraction solvent. The aqueous layer was further extracted with six 20 mL portions of diethyl ether. Drying of the organic layer over magnesium sulfate, followed by filtration and evaporation under reduced pressure, yielded the pure 3,4,5-trimethoxybenzyl bromide (2.27 g, 98%).

A mixture of 3,4,5-trimethoxybenzyl bromide (258 mg, 0.988 mmol) and triphenylphosphine (284 mg, 1.08 mmol) in THF (4.0 mL) was microwaved at 150 W, 100 psi at 50 °C for 30 min. After cooling to room temperature, the mixture was transferred to a round-bottomed flask, and the solvent evaporated under reduced pressure. Diethyl ether was added to the residue, which was stored at −20 °C for 48 h. The ethereal supernatant was subsequently decanted leaving behind the pure phosphonium bromide salt quantitatively as an off-white solid.

A mixture of vanillin (or isovanillin, 338.9 mg, 2.23 mmol), DMAP (278.7 mg, 2.28 mmol), TBSCl (542.1 mg, 3.60 mmol), and triethylamine (0.6 mL, 826 mg, 8.17 mmol) in 6.0 mL of CH_2Cl_2 was irradiated at 150 W, 100 psi, at 25 °C for 1 h. The reaction mixture was subsequently combined with 6 mL of brine and the organic layer separated. The aqueous layer was further extracted with eight additional 6 mL portions of CH_2Cl_2. Drying of the combined organic layers with anhydrous $MgSO_4$, gravity filtration, and flash chromatographic purification afforded the pure TBS ether (95%).

Formation of stilbenes was accomplished by the established Wittig protocol. Thus, a 200 mL round-bottomed flask equipped for magnetic stirring and fitted with a pressure-equalizing dropping funnel was charged with the phosphonium bromide salt (3.99 g, 7.62 mmol) in 70 mL of THF. The reaction mixture was cooled to −10 °C, and a solution of *n*-BuLi in hexanes (7.2 mL, 1.6 M, 11.4 mmol) added dropwise via syringe. Following the addition of the base, the mixture was cooled to −78 °C for 5 min, after which a solution of the silylated vanillin (1.83 g, 6.86 mmol) in 30 mL of THF was added dropwise with stirring. After 5 min, the mixture was allowed to warm to 0 °C and then quenched by pouring into 100 mL of cold brine. The mixture was next extracted with ethyl acetate (50 mL × 4), and the combined organic layers were dried over anhydrous $MgSO_4$. Subsequent filtration and chromatographic purification afforded the diastereomeric mixture of **9** and **11** (1.31 g, 44%) in a ratio of 1.6:1. Compound **9**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.13 (6H, s), 0.98 (9H, s), 3.61 (3H, s), 3.70 (6H, s), 3.83 (3H, s), 6.43 (1H, d, J = 12.1 Hz), 6.49 (1H, d, J = 12.1 Hz), 6.54 (2H, s), 6.76 (3H, m). Compound **11**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.17 (6H, s), 1.01 (9H, s), 3.87 (6H, s), 3.91 (6H, s), 6.72 (2H, s), 6.84 (1H, d, J = 8.1 Hz), 6.89 (1H, d, J = 16.2 Hz), 6.96 (1H, d, J = 16.1 Hz), 6.98 (1H, dd, J = 2.0, 8.2 Hz), 7.02 (1H, d, J = 1.9 Hz). Physical data and spectroscopic information for compounds **8** and **10** were consistent with such data that have been reported in the literature.[12]

(*Z*)-2-Methoxy-5-(3,4,5-trimethoxystyryl)phenol (12). In a round-bottomed flask, compound **8** (2.34 g, 5.43 mmol) was dissolved in dry THF (25 mL), and TBAF (2.16 g, 8.26 mmol) was added. Under a positive atmosphere of N_2 gas, the mixture was cooled in an ice−brine bath and stirred for four hours. In a separatory funnel, cold saturated ammonium chloride (25 mL) was added, and the reaction mixture was poured in. The aqueous layer was extracted four times with ethyl acetate. The combined organic layer obtained was dried with magnesium sulfate and filtered. Flash chromatographic purification on silica gel (hexane−ethyl acetate, 1:1) afforded compound **12** (1.24 g, 72%) as a white solid (lit. mp 117−118 °C): ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.70 (6H, s), 3.84 (3H, s), 3.86 (3H, s), 5.54 (1H, s), 6.41 (1H, d, J = 12.2 Hz), 6.47 (1H, d, J = 12.2 Hz), 6.53 (2H, s), 6.73 (1H, d, J = 8.33 Hz), 6.79 (1H, dd, J = 2.0, 8.3 Hz), 6.92 (1H, d, J = 2.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.9, 56.0, 60.9, 106.0, 110.3, 115.0, 121.1, 129.0, 129.5, 130.6, 132.7, 137.1, 145.2, 145.8, 152.9; anal. calcd for $C_{18}H_{20}O_5$: C 68.35, H 6.37, found: C 68.10, H 6.83.

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(*E*)-2-Methoxy-5-(3,4,5-trimethoxystyryl)phenol (14). Compound **14** was prepared according to the method adopted for compound **12**. Thus, ether **10** (2.07 g, 4.81 mmol) reacted with TBAF (1.92 g, 7.34 mmol) to afford the *trans* isomer **14** (1.39 g, 91%), isolated as a white solid (lit. mp 103−104 °C): ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.82 (3H, s), 3.86 (3H, s), 3.87 (6H, s), 5.96 (1H, s), 6.69 (2H, s), 6.79 (1H, d, J = 8.4 Hz), 6.85 (1H, d, J = 16.2 Hz), 6.88 (1H, d, J = 16.2 Hz), 6.95 (1H, dd, J = 1.9, 8.3 Hz), 7.14 (1H, d, J = 2.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.9, 56.1, 60.9, 103.4, 110.8, 111.9, 119.3, 127.0, 127.9, 130.9, 133.4, 137.7, 145.9, 146.6, 153.4; anal. calcd for $C_{18}H_{20}O_5$: C 68.35, H 6.37, found: C 68.53, H 6.47.

(*E*)-2-Methoxy-4-(3,4,5-trimethoxystyryl)phenol (15). Compound **15** was prepared according to the method adopted for compound **12**. Thus, ether **9** (1.96 g, 4.55 mmol) reacted with TBAF (1.83 g, 7.00 mmol) to afford the *trans* isomer **15** (0.56 g, 37%), isolated as a white solid, mp 139−140 °C. The *cis* isomer **13** completely isomerized to **15**, hence was not isolated. IR ν_{max} 3395, 2937, 2833, 1579, 1514, 1466, 1448, 1427, 1338, 1225, 1210, 1167, 1119, 1031, 988, 958, 848, 818, 792 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.87 (3H, s), 3.92 (6H, s), 3.95 (3H, s), 5.69 (1H, s), 6.72 (2H, s), 6.88 (1H, d, J = 16.4 Hz), 6.90 (1H, d, J = 8.5 Hz), 6.95 (1H, d, J = 16.2 Hz), 7.01 (1H, d, J = 1.9 Hz), 7.03 (1H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.9, 56.1, 61.0, 103.3, 108.2, 114.6, 120.4, 126.5, 128.2, 133.4, 137.6, 145.6, 146.7, 153.4; anal. calcd for $C_{18}H_{20}O_5$: C 68.34, H 6.37, found: C 68.17, H 6.42.

(*Z*)-2-Methoxy-5-(3,4,5-trimethoxystyryl)phenyl stearate (20). In a reaction tube, a mixture of compound **12** (273.3 mg, 0.864 mmol), stearic acid (247.7 mg, 0.871 mmol), DCC (196.0 mg, 0.950 mmol), and DMAP (106.2 mg, 0.869 mmol) was dissolved in CH_2Cl_2 (4 mL). The mixture was irradiated under the following conditions: 100 W, 25 °C, 100 psi, at 2 min intervals (4 times) for a total time of 8 min. The reaction mixture was prepurified by flash chromatography (hexanes−ethyl acetate, 1:1) and then subjected to centrifugal chromatography (hexane−ethyl acetate, 3:1), which yielded compound **20** (0.15 g, 29%) as a white solid (mp 61−62 °C): IR ν_{max} 2916, 2852, 1759, 1578, 1506, 1462, 1412, 1130, 1240, 1128, 1028, 851, 797, 713 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (3H, t, J = 6.6 Hz,), 1.35 (28 H, m), 1.72 (2H, q, J = 7.6 Hz), 2.52 (2H, t, J = 7.5), 3.70 (6H, s), 3.78 (3H, s), 3.83 (3H, s), 6.43 (1H, d, J = 12.2 Hz), 6.46 (1H, d, J = 12.3 Hz), 6.51 (2H, s), 6.84 (1H, d, J = 8.5 Hz), 7.00 (1H, d, J = 2.1 Hz), 7.11 (1H, dd, J = 2.1, 8.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 25.0, 29.0, 29.3, 29.4, 29.5, 29.6, 29.7, 29.8, 32.0, 34.0, 55.9, 56.0, 60.9, 105.8, 111.9, 123.2, 127.6, 128.6, 128.7, 129.5, 132.5, 137.2, 139.6, 150.3, 153.0, 171.7; anal. calcd for $C_{36}H_{54}O_6$: C 74.19, H 9.34, found: C, 74.29, H 9.34.

2-Methoxy-5-((*Z*)-3,4,5-trimethoxystyryl)phenyl oleate (21). Compound **21** was prepared according to the procedure described for **20**: from compound **12** (273.7 mg, 0.865 mmol), oleic acid (246.0 mg, 0.871 mmol), DCC (197.5 mg, 0.957 mmol), and DMAP (106.5 mg, 0.872 mmol), which yielded **21** as a clear oil (0.25 g, 50%): IR ν_{max} 3003, 2924, 2854, 1763, 1613, 1578.7, 1508, 1455, 1426, 1238, 1127, 1009, 852, 772 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.89 (3H, t, J = 6.3 Hz), 1.36 (20H, m), 1.72 (2H, q, J = 7.5 Hz), 2.01 (4H, m), 2.52 (2H, t, J = 7.4 Hz), 3.70 (6H, s), 3.79 (3H, s), 3.84 (3H, s), 5.35 (2H, m), 6.43 (1H, d, J = 12.2 Hz), 6.47 (1H, d, J = 12.3 Hz), 6.51 (2H, s), 6.84 (1H, d, J = 8.5 Hz), 7.00 (1H, d, J = 2.1 Hz), 7.11 (1H, dd, J = 2.1, 8.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 25.0, 27.2, 27.3, 29.0, 29.2, 29.3, 29.5, 29.7, 29.8, 31.9, 34.0, 55.9, 60.9, 105.8, 112.0, 123.2, 128.6, 129.5, 129.8, 130.0, 130.1, 132.5, 137.2, 139.6, 150.3, 153.0, 171.7; anal. calcd for $C_{36}H_{52}O_6$: C 74.45, H 9.02, found: C 74.17, H 9.23.

2-Methoxy-5-((*Z*)-3,4,5-trimethoxystyryl)phenyl (9*Z*,12*Z*)-octadeca-9,12-dienoate (22). Compound **22** was prepared according to the procedure described for **20**: from compound **12** (284.0 mg, 0.898 mmol), linoleic acid (250.2 mg, 0.892 mmol), DCC (203.1 mg, 0.984 mmol), and DMAP (110.9 mg, 0.908 mmol), which yielded compound **22** as a clear oil (0.25 g, 49%): IR ν_{max} 2932, 2698, 1635, 1576, 1514, 1449, 1406, 1369, 1154, 1023, 877, 780 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.89 (3H, t, J = 6.9 Hz), 1.30 (14H, m), 1.72 (2H, q, J = 7.3 Hz), 2.05 (4H, m), 2.52 (2H, t, J = 7.5 Hz), 2.78 (2H, t, J = 6.4 Hz), 3.69 (6H, s), 3.77 (3H, s), 3.83 (3H, s), 5.35 (4H, m), 6.42 (1H, d, J = 12.2 Hz), 6.46 (1H, d, J = 12.2 Hz), 6.50 (2H, s), 6.82 (1H, d, J = 8.5 Hz), 7.00 (1H, d, J = 2.1 Hz), 7.10 (1H, dd, J = 2.1, 8.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.6, 24.7, 25.0, 25.6, 26.4, 27.2, 29.0, 29.1, 29.2, 29.4, 29.6, 30.9, 31.5, 32.7, 33.9, 55.8, 55.9, 60.8, 105.9, 112.0, 123.1, 127.6, 127.9, 128.1, 128.6, 129.5, 130.0, 130.1, 130.2, 132.4, 137.2, 139.6, 150.3, 153.0, 171.6; anal. calcd for $C_{36}H_{50}O_6$: C 74.71, H 8.71, found: C 73.92, H 8.79.

2-Methoxy-5-((*Z*)-3,4,5-trimethoxystyryl) phenyl-(9*Z*,12*Z*,15*Z*)-octadeca-9,12,15-trienoate (23). Compound **23** was prepared according to the procedure described for **20**: from compound **12** (288.7 mg, 0.913 mmol), linolenic acid (251.8 mg, 0.904 mmol), DCC (207.9 mg, 1.01 mmol), and DMAP (114.2 mg, 0.935 mmol), which yielded compound **23** as a clear oil (0.22 g, 42%): IR ν_{max} 2934, 2701, 1635, 1576, 1515, 1450, 1369, 1281, 1154, 1023, 877, 780 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_{3,}$) δ 0.97 (3H, t, J = 7.5 Hz), 1.36 (8H, m), 1.72 (2H, m), 2.08 (4H, m), 2.52 (2H, t, J = 7.48 Hz), 2.81 (4H, t, J = 5.99 Hz), 3.70 (6H, s), 3.79 (3H, s), 3.83 (3H, s), 5.36 (6H, m), 6.43 (1H, d, J = 12.2 Hz), 6.47 (1H, d, J = 12.4 Hz), 6.51 (2H, s), 6.84 (1H, d, J = 8.5 Hz), 7.00 (1H, d, J = 2.0 Hz), 7.11 (1H, dd, J = 2.1, 8.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3, 20.6, 25.0, 25.5, 25.6, 27.2, 29.0, 29.2, 29.6, 34.0, 55.9, 56.0, 60.9, 105.9, 112.0, 123.2, 127.1, 127.6, 127.8, 128.3, 128.6, 129.5, 130.1, 130.3, 132.0, 132.5, 137.2, 139.6, 150.3, 153.0, 171.7; anal. calcd for $C_{36}H_{48}O_6$: C 74.97, H 8.39, found: C 73.98, H 8.38.

(*E*)-2-Methoxy-5-(3,4,5-trimethoxystyryl)phenyl stearate (24). Compound **24** was prepared according to the procedure described for **20**: from compound **14** (288.7 mg, 0.913 mmol), stearic acid (177.0 mg, 0.622 mmol), DCC (140.8 mg, 0.683 mmol), and DMAP (79.5 mg, 0.651 mmol), from which **24** was isolated (0.21 g, 55%) as a white solid (mp 76−77 °C): IR ν_{max} 3021, 2957, 2918, 2851, 1752, 1580, 1513, 1464, 1424, 1253, 1127, 955, 799, 773, 714 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (3H, t, J = 7.0 Hz), 1.41 (28H, m), 1.78 (2H, q, J = 7.6 Hz), 2.59 (2H, t, J = 7.5 Hz), 3.83 (3H, s), 3.86 (3H, s), 3.90 (6H, s), 6.69 (2H, s), 6.87 (1H, d, J = 16.2 Hz), 6.91 (1H, d, J = 7.1 Hz), 6.92 (1H, d, J = 15.7 Hz), 7.22 (1H, d, J = 2.1 Hz), 7.29 (1H, dd, J = 2.0, 8.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 22.7, 25.1, 29.1, 29.3, 29.4, 29.6, 29.7, 31.9, 34.1, 55.9, 56.1, 61.0, 103.4, 112.4, 120.4, 125.2, 127.0, 127.5, 130.6, 133.2, 137.8, 140.1, 150.7, 153.4, 171.9; anal. calcd for $C_{36}H_{54}O_6$, C 74.19, H 9.34;, found: C 74.11, H 9.39.

2-Methoxy-5-((*E*)-3,4,5-trimethoxystyryl)phenyl oleate (25). Compound **25** was similarly prepared according to the procedure described for **20**: from compound **14** (215.8 mg, 0.682 mmol), oleic acid (192.7 mg, 0.682 mmol), DCC (159.6 mg, 0.774 mmol), and DMAP (87.6 mg, 0.717 mmol), which yielded **25** (0.26 g, 65%) also isolated as a white solid (mp 38−39 °C): IR ν_{max} 3003, 2921, 2850, 1756, 1584, 1516, 1466, 1428, 1324, 1282, 1247, 1124, 1009, 970, 822, 774 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (3H, t, J = 7.1 Hz), 1.36 (20H, m), 1.78 (2H, m), 2.02 (4H, m), 2.59 (2H, t, J = 7.5 Hz), 3.81 (3H, s), 3.86 (3H, s), 3.88 (6H, s), 5.36 (2H, m), 6.69 (2H, s), 6.86 (1H, d, J = 16.2 Hz), 6.92 (1H, d, J = 16.1 Hz), 6.92 (1H, d, J = 8.6 Hz), 7.22 (1H, d, J = 2.1 Hz), 7.26 (1H, dd, J = 2.2, 8.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 22.7, 25.1, 27.2, 27.3, 29.1, 29.2, 29.3, 29.6, 29.7, 29.8, 31.9, 34.1, 55.9, 56.1, 60.9, 103.4, 112.4, 120.3, 125.2, 127.0, 127.5, 129.7, 130.0, 130.6, 133.2, 137.8, 140.1, 150.7, 153.4, 171.8; anal. calcd for $C_{36}H_{52}O_6$: C 74.45, H 9.02, found: C 74.62, H 9.15.

2-Methoxy-5-((*E*)-3,4,5-trimethoxystyryl)phenyl-(9*Z*,12*Z*)-octadeca-9,12-dienoate (26). Compound **26** was similarly prepared according to the procedure described for **20**: from compound **14** (207.9 mg, 0.657 mmol), linoleic acid (199.3 mg, 0.711 mmol), DCC (152.3 mg, 0.738 mmol), and DMAP (81.4 mg, 0.666 mmol), which yielded **26** (0.24 g, 64%) isolated as a white solid (mp 36−37 °C): IR ν_{max} 3006, 2921, 2851, 1757, 1583, 1509, 1452, 1428, 1322, 1246, 1125, 1005, 975, 825, 772, 716 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.89 (3H, t, J = 7.0 Hz), 1.31 (14H, m), 1.78 (2H, q, J = 7.6 Hz), 2.05 (4H, m), 2.60 (2H, t, J = 7.5 Hz), 2.78 (2H, t, J = 6.4 Hz), 3.84 (3H, s), 3.86 (3H, s), 3.91 (6H, s), 5.35 (4H, m),

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6.70 (2H, s), 6.87 (1H, d, J = 16.2 Hz), 6.93 (1H, d, J = 17.2 Hz), 6.94 (1H, d, J = 8.4 Hz), 7.23 (1H, d, J = 2.1 Hz), 7.30 (1H, dd, J = 2.1, 8.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.6, 25.1, 25.7, 29.1, 29.2, 29.4, 29.6, 31.5, 34.1, 56.0, 56.1, 61.0, 103.4, 112.4, 120.4, 125.2, 127.0, 127.5, 127.9, 128.1, 130.0, 130.25 130.56, 133.12, 137.8, 140.1, 150.7, 153.4, 171.6; anal. calcd for $C_{36}H_{50}O_6$: C 74.71, H 8.71, found: C 74.93, H 8.86.

2-Methoxy-5-((*E*)-3,4,5-trimethoxystyryl)phenyl-(9*Z*,12*Z*,15*Z*)-octadeca-9,12,15-trienoate (27). Compound **27** was also prepared according to the procedure described for **20**: from a mixture of compound **14** (251.6 mg, 0.795 mmol), linolenic acid (237.9 mg, 0.854 mmol), DCC (193.7 mg, 0.939 mmol), and DMAP (98.2 mg, 0.804 mmol), which yielded **27** (0.23 g, 49%) isolated as a white solid (mp 41−42 °C): IR ν_{max} 3009, 2929, 2849, 1756, 1583, 1509, 1451, 1428, 1322, 1246, 1124, 1025, 1004, 972, 824, 773, 724 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.98 (3H, t, J = 7.5 Hz), 1.41 (8H, m), 1.78 (2H, m), 2.08 (4H, m), 2.60(2H, t, J = 7.5 Hz), 2.81 (4H, t, J = 5.8 Hz), 3.84 (3H, s), 3.86 (3H, s), 3.90 (6H, s), 5.36 (6H, m), 6.70 (2H, s), 6.87 (1H, d, J = 16.2 Hz), 6.93 (1H, d, J = 16.6 Hz), 6.94 (1H, d, J = 8.5 Hz), 7.23 (1H, d, J = 2.1 Hz), 7.30 (1H, dd, J = 2.1, 8.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3, 20.6, 25.0, 25.6, 27.2, 29.1, 29.2, 29.6, 34.1, 56.0, 56.1, 61.0, 103.4, 112.4, 120.4, 125.2, 127.0, 127.1, 127.5, 127.8, 128.3, 130.3, 130.6, 132.0, 133.2, 137.8, 140.1, 150.7, 153.4, 171.8; anal. calcd for $C_{36}H_{48}O_6$: C 74.97, H 8.39, found: C 74.92, H 8.44.

(*Z*)-2-Methoxy-4-(3,4,5-trimethoxystyryl)phenyl stearate (28). In a reaction tube, a mixture of compound **13** and compound **15** (249.5 mg, 0.789 mmol), stearic acid (225.3 mg, 0.792 mmol), DCC (179.4 mg, 0.870 mmol), and DMAP (96.4 mg, 0.789 mmol) were dissolved in CH_2Cl_2 (4 mL). The mixture was irradiated under the following conditions: 100 W, 25 °C, 100 psi, for 6 min. The reaction mixture was prepurified by column chromatography (hexanes−ethyl acetate, 1:1) to isolate the isomeric mixture. The mixture containing the *cis* and *trans* isomers was separated on the chromatotron (hexane−ethyl acetate, 3:1), which yielded **28** (0.22 g, 48%) as a white solid (mp 63−64 °C) and **32** (0.12 g, 26%) also isolated as a white solid. Compound **32** was subsequently prepared in a higher yield as described separately. Compound **28**: IR ν_{max} 2916, 2850, 1766, 1656, 1582, 1504, 1463, 1416, 1334, 1271, 1233, 1133, 1034, 1001, 874, 854, 715 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (3H, t, J = 7.0 Hz), 1.38 (28H, m), 1.74 (2H, q, J = 7.6 Hz), 2.56 (2H, t, J = 7.5), 3.64 (3H, s), 3.69 (6H, s), 3.82 (3H, s), 6.48 (2H, s), 6.52 (1H, d, J = 12.2 Hz), 6.56 (1H, d, J = 12.1 Hz), 6.87 (2H, m), 6.92 (1H, d, J = 8.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 22.7, 25.1, 26.4, 29.1, 29.3, 29.4, 29.5, 29.6, 29.7, 30.9, 31.9, 32.8, 34.1, 55.7, 56.0, 60.9, 106.0, 112.8, 121.5, 122.7, 129.4, 130.4, 132.4, 136.0, 137.2, 138.9, 150.8, 152.9, 171.9; anal. calcd for $C_{36}H_{54}O_6$: C 74.19, H 9.34, found: C 74.70, H 9.62.

2-Methoxy-4-((*Z*)-3,4,5-trimethoxystyryl)phenyl oleate (29). Compound **29** was prepared according to the procedure described for compound **28** from a mixture of compounds **13** and **15** (249.5 mg, 0.789 mmol), oleic acid (225.3 mg, 0.798 mmol), DCC (176.3 mg, 0.855 mmol), and DMAP (95.6 mg, 0.782 mmol). This combination yielded **29** (0.31 g, 71%) as a pale yellow oil and **33** (0.10 g, 23%) isolated as a white wax-like solid. Compound **29**: IR ν_{max} 2916, 2851, 1766, 1656, 1582, 1504, 1463, 1416, 1335, 1271, 1246, 1233, 1133, 1034, 1001, 874, 854.3, 715 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (3H, t, J = 7.0 Hz), 1.34 (20H, m), 1.74 (2H, m), 2.01 (4H, m), 2.56 (2H, t, J = 7.5 Hz), 3.64 (3H, s), 3.69 (6H, s), 3.82 (3H, s), 5.35 (2H, m), 6.48 (2H, s), 6.51 (1H, d, J = 12.2 Hz), 6.56 (1H, d, J = 12.1 Hz), 6.87 (2H, m), 6.92 (1H, d, J = 8.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 25.0, 27.2, 25.5, 26.4, 27.2, 29.1, 29.2, 29.3, 29.5, 30.9, 31.9, 32.8, 34.0, 55.7, 55.9, 60.9, 106.0, 112.8, 121.5, 122.6, 129.3, 129.7, 130.0, 130.4, 132.3, 136.1, 137.2, 138.9, 150.8, 152.9, 171.8; anal. calcd for $C_{36}H_{52}O_6$: C 74.45, H 9.02, found: C 74.71, H 9.37.

2-Methoxy-4-((*E*)-3,4,5-trimethoxystyryl)phenyl oleate (33). IR ν_{max} 3003, 2919, 2852, 1767, 1580, 1509, 1464, 1422, 1337, 1246, 1202, 1123, 1035, 1004, 980, 914, 853, 715 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (3H, t, J = 7.1 Hz), 1.36 (20H, m), 1.77 (2H, q, J = 7.5 Hz), 2.02 (4H, m), 2.58 (2H, t, J = 7.5 Hz), 3.87 (3H, s), 3.88 (3H, s), 3.92 (6H, s), 5.36 (2H, m), 6.73 (2H, s), 6.97 (2H, s), 7.01 (1H, d, J = 7.9 Hz), 7.07 (2H, d, J = 9.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 22.7, 25.1, 27.2, 27.3, 29.1, 29.2, 29.6, 29.7, 29.8, 31.9, 34.1, 55.8, 56.1, 61.0, 103.5, 110.0, 119.1, 123.0, 127.6, 128.8, 129.8, 130.0, 132.9, 136.2, 138.0, 139.4, 151.3, 153.4, 171.9; anal. calcd for $C_{36}H_{52}O_6$: C 74.45, H 9.02, found: C 74.54, H 9.06.

2-Methoxy-4-((*Z*)-3,4,5-trimethoxystyryl)phenyl-(9*Z*,12*Z*)-octadeca-9,12-dienoate (30). Compound **30** was prepared according to the procedure described for compound **28** from a mixture of compounds **13** and **15** (241.1 mg, 0.762 mmol), linoleic acid (261.8 mg, 0.933 mmol), DCC (177.7 mg, 0.861 mmol), and DMAP (95.0 mg, 0.777 mmol). This combination yielded **30** (0.28 g, 63%) and **34** (0.11 g, 26%), both isolated as clear, pale yellow oils. Compound **30**: IR ν_{max} 2992, 2916, 2852, 1766, 1582, 1504, 1463, 1416, 1334, 1271, 1246, 1233, 1133, 1034, 1001, 874, 854, 789, 715 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.89 (3H, t, J = 7.0 Hz), 1.30 (14H, m), 1.75 (2H, q, J = 7.6 Hz), 2.05 (4H, m), 2.55 (2H, t, J = 7.5 Hz), 2.78 (2H, t, J = 6.2 Hz), 3.63 (3H, s), 3.68 (6H, s), 3.82 (3H, s), 5.36 (4H, m), 6.48 (2H, s), 6.51 (1H, d, J = 12.2 Hz), 6.55 (1H, d, J = 12.1 Hz), 6.86 (2H, m), 6.92 (1H, d, J = 8.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.6, 25.0, 25.6, 26.4, 27.2, 29.0, 29.2, 29.4, 29.6, 30.9, 31.5, 32.7, 34.0, 55.7, 55.9, 60.8, 106.0, 112.8, 121.5, 122.6, 127.9, 128.1, 129.3, 130.0, 130.2, 130.4, 132.3, 136.1, 137.2, 138.9, 150.8, 152.9, 171.8; anal. calcd for $C_{36}H_{50}O_6$: C 74.71, H 8.71, found: C 74.56, H 8.97.

2-Methoxy-4-((*E*)-3,4,5-trimethoxystyryl)phenyl-(9*Z*,12*Z*)-octadeca-9,12-dienoate (34): IR ν_{max} 2921, 2851, 1767, 1657, 1580, 1507, 1453, 1421, 1127, 1033, 853 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.89 (3H, t, J = 7.0 Hz), 1.30 (14H, m), 1.77 (2H, q, J = 7.6 Hz), 2.05 (4H, m), 2.59 (2H, t, J = 6.9 Hz), 2.78 (2H, t, J = 6.4 Hz), 3.87 (3H, s), 3.88 (3H, s), 3.92 (6H, s), 5.35 (4H, m), 6.73 (2H, s), 6.97 (2H, s), 7.01 (1H, d, J = 8.0 Hz), 7.07 (2H, d, J = 9.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.6, 25.1, 25.7, 27.2, 29.1, 29.2, 29.3, 29.6, 31.5, 34.1, 55.9, 56.1, 61.0, 103.5, 110.0, 119.2, 122.0, 127.6, 127.9, 128.1, 128.8, 130.1, 130.3 132.9, 136.1, 138.0, 139.4, 151.3, 153.4, 171.9; anal. calcd for $C_{36}H_{50}O_6$: C 74.71, H 8.71, found: C 74.00, H 8.67.

2-Methoxy-4-((*Z*)-3,4,5-trimethoxystyryl)phenyl-(9*Z*,12*Z*,15*Z*)-octadeca-9,12,15-trienoate (31): Compound **31** was prepared according to the procedure described for compound **28** from a mixture of compounds **13** and **15** (176.4 mg, 0.558 mmol), linolenic acid (159.6 mg, 0.573 mmol), DCC (126.6 mg, 0.614 mmol), and DMAP (68.3 mg, 0.559 mmol). This combination yielded **31** (0.17 g, 54%) isolated as a clear oil, and **35** (0.11 g, 35%) was isolated as a white solid (mp 51−52 °C). Compound **31**: IR ν_{max} 2995, 2917, 2852, 1766, 1657, 1583, 1504, 1463, 1417, 1335, 1272, 1233, 1133, 1034, 1001, 875, 855, 715 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.97 (3H, t, J = 7.5 Hz), 1.37 (8H, m), 1.75 (2H, q, J = 7.5 Hz), 2.08 (4H, m), 2.55 (2H, t, J = 7.5 Hz), 2.81 (4H, t, J = 6.0 Hz), 3.64 (3H, s), 3.69 (6H, s), 3.82 (3H, s), 5.35 (6H, m), 6.48 (2H, s), 6.51 (1H, d, J = 12.2 Hz), 6.56 (1H, d, J = 12.1 Hz), 6.87 (2H, m), 6.92 (1H, d, J = 8.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3, 20.6, 25.0, 25.5, 25.6, 27.2, 29.0, 29.2, 29.2, 29.6, 34.0, 55.7, 55.9, 60.9, 106.0, 112.8, 121.5, 122.7, 127.1, 127.7, 127.8, 128.3, 129.33, 130.3, 130.4, 132.0, 132.3, 137.2, 139.0, 150.8, 152.9, 171.8; anal. calcd for $C_{36}H_{48}O_6$: C 74.97, H 8.39, found: C 74.05, H 8.35.

(*E*)-2-Methoxy-4-(3,4,5-trimethoxystyryl)phenyl stearate (32). In a reaction tube, compound **15** (122.3 mg, 0.387 mmol), stearic acid (110.0 mg, 0.387 mmol), DCC (91.1 mg, 0.442 mmol), and DMAP (48.8 mg, 0.399 mmol) were dissolved in CH_2Cl_2 (4 mL). The mixture was irradiated under the following conditions: 100 W, 25 °C, 100 psi, for 6 min. The reaction mixture was prepurified by flash chromatography (hexanes-ethyl acetate, 1:1), and subsequently by centrifugal chromatography (hexanes−ethyl acetate, 1:1). The pure product **32** (0.18 g, 78%) was isolated as a white solid (mp 95−96 °C): IR ν_{max} 3001, 2920, 2850, 1770, 1579, 1509, 1464, 1421, 1338, 1243, 1204, 1120, 1035, 1005, 981, 912, 853, 721 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (3H, t, J = 7.0 Hz), 1.39 (28H, m), 1.76 (2H, q, J = 7.5 Hz), 2.58 (2H, t, J = 7.5 Hz), 3.86 (3H, s), 3.87 (3H,

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s), 3.90 (6H, s), 6.72 (2H, s), 6.96 (2H, s), 7.00 (1H, d, J = 8.0 Hz), 7.07 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 22.7, 25.1, 29.1, 29.3, 29.4, 29.6, 29.7, 32.0, 34.1, 55.8, 56.1, 61.0, 103.5, 110.0, 119.1, 123.0, 127.5, 128.8, 132.9, 136.2, 138.0, 139.4, 151.3, 153.4, 171.9; anal. calcd for $C_{36}H_{54}O_6$: C 74.19, H 9.34, found: C 73.94, H 9.34.

2-Methoxy-4-((*E*)-3,4,5-trimethoxystyryl)phenyl-(9*Z*,12*Z*,15*Z*)-octadeca-9,12,15-trienoate (35). Compound **35** was prepared according to the procedure described for **32** from compound **15** (241.6 mg, 0.764 mmol), linolenic acid (212.6 mg, 0.763 mmol), DCC (173.3 mg, 0.840 mmol), and DMAP (93.3 mg, 0.764 mmol), from which **35** (0.28 g, 63%) was isolated as a white solid (mp 51−52 °C). Compound **35**: IR ν_{max} 3006, 2926, 2851, 1764, 1579, 1509, 1450, 1422, 1328, 1246, 1203, 1117, 1035, 1004, 980, 911, 852, 830, 717 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.98 (3H, t, J = 7.5 Hz), 1.40 (8H, m), 1.77 (2H, q, J = 7.6 Hz), 2.08 (4H, m), 2.58 (2H, t, J = 7.5 Hz), 2.82 (4H, t, J = 5.8 Hz), 3.87 (6H, s), 3.91 (6H, s), 5.36 (6H, m), 6.73 (2H, s), 6.96 (2H, s), 7.00 (1H, d, J = 8.0 Hz), 7.06 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3, 20.6, 25.1, 25.6, 25.7, 27.2, 29.1, 29.2, 29.6, 34.1, 55.8, 56.1, 61.0, 103.6, 110.0, 119.1, 123.0, 127.1, 127.6, 127.8, 128.3, 128.8, 130.3, 132.0, 132.9, 136.2, 138.0, 139.4, 151.3, 153.4, 171.9; anal. calcd for $C_{36}H_{48}O_6$: C 74.97, H 8.39, found: C 74.87, H 8.42.

Biological Testing. Human epidermoid carcinoma (KB-3-1), NCI-H460 (human lung cancer cell line), HEK293 (human embryonic kidney cells), and the MCF-7 (human breast adenocarcinoma) cell lines were cultured in DMEM medium supplemented with 10% FBS and 1% P/S and maintained in 5% CO_2 at 37 °C. The cells were trypsinized, resuspended, and seeded into a 96-well plate at a density of ∼5 × 10^4 cells per well. The cell viability was determined by MTT assay. Once the cells were attached, different concentrations of the test drugs were added into each well ranging from 0.1 to 100 μM. Cell viability was measured after 68 h by adding MTT (4 mg/mL) to the plate. After 4 h of incubation, discarding the supernatant, 100 μL of DMSO was added to all wells. The plates were analyzed spectrophotometrically at 570 nm. The concentration at which around 50% of cells survived (IC_{50}) was calculated. IC_{50} values were determined using the software GraphPad Prism (version 8).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.0c00027.

Copies of IR, ^{1}H NMR, ^{13}C NMR spectra, and cytotoxicity data (PDF)

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Notes

The authors declare no competing financial interest.

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