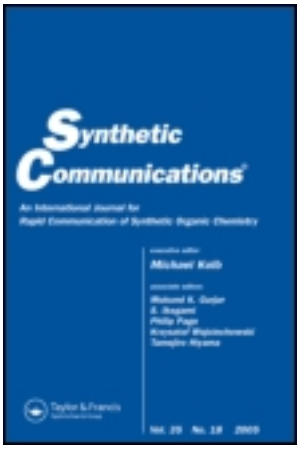

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SYNTHESIS OF VITAMIN E-CARNOSINE (VECAR): NEW ANTIOXIDANT MOLECULE WITH POTENTIAL APPLICATION IN ATHEROSCLEROSIS

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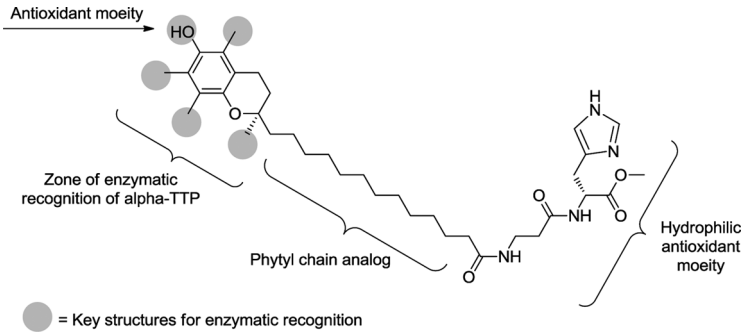
GRAPHICAL ABSTRACT



Abstract *Natural antioxidants such as carnosine and α-tocopherol (vitamin E) provide protection against several oxidative stress–related diseases such as atherosclerosis and Alzheimer's. The synthetic combination of α-tocopherol and carnosine can take advantage of the cellular transport mechanism of α-tocopherol by α-tocopherol transfer protein (α-TTP) to colocate α-tocopherol and carnosine at the interface between a lipophilic and a hydrophilic domain and protect both from oxidation. Successful synthesis of a novel heterodimer of α-tocopherol (vitamin E) and carnosine, VECAR was carried out in a total of nine steps. The VECAR design uses a 13-carbon phytyl-chain mimic to link carnosine to Trolox at the C2 carbon position. This design feature is anticipated to maintain binding to α-TTP, while maintaining the antioxidant activity of the two heterodimer components. Our results confirmed that there was no loss in antioxidant activity in VECAR using an in-vitro DPPH assay, versus α-tocopherol and Trolox.*

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Keywords Alzheimer's; antioxidant; atherosclerosis; carnosine; α-tocopherol; Trolox

INTRODUCTION

Antioxidant theory links oxidative stress with cardiovascular diseases and other major diseases such as cancer, acute inflammation, Parkinson's, and Alzheimer's.[1–7] In 2007, an estimated $430 billion was spent to treat approximately 80 million people suffering from some type of cardiovascular disease.[8,9] Antioxidant agents play vital roles in the defense system against free radicals and oxidant molecules by avoiding radical propagation (chain reaction) via radical scavenging, metal ion chelation, and coantioxidant action (antioxidant regeneration). The balance between oxidative species and antioxidant systems (natural molecules, enzymes, and proteins) defines the oxidative stress of a living system, and the oxidative stress level determines if a biological system has a high risk for diseases.[10]

Atherosclerosis in particular has been related to the oxidative process, in that high levels of oxidant molecules or low levels of antioxidants were shown to be responsible for high oxidative stress, triggering the atherosclerosis process.[11–14] The ox-LDL (i.e., the oxidized form of low-density lipoproteins, LDLs) acts at different levels, stimulating the host response and formation of foam cells. Among the lipophilic antioxidants, α-tocopherol (the most bioactive isomer of vitamin E) is one of the most potent natural antioxidants shown to protect LDLs from oxidation. To highlight the importance of α-tocopherol in humans, it has been shown that a specific enzyme α-tocopherol transfer protein (α-TTP) is responsible for α-tocopherol transport into lipoproteins. Lipoproteins then act as the main vehicle for α-tocopherol transport to different tissues.[15–17] Lipophilic antioxidants prevent oxidation of the lipid fraction of LDLs, but the more hydrophilic apolipoproteins (e.g., Apo B-100) are not protected from oxidation even in the presence of lipophilic antioxidants.[18–21]

The limitations of lipophilic natural antioxidants can be overcome with an adequate design of an alternative "natural" antioxidant formed by covalently linking the hydrophobic antioxidant with a naturally occurring hydrophilic molecule with antioxidant properties; the new structure will ensure that the component will be localized closer to apolipoproteins, have better antioxidant performance, and have potential synergetic behavior because of antioxidant–coantioxidant phenomena. Hydrophilic antioxidants have the ability to protect proteins, DNA, and carbohydrates from oxidative molecules and to regulate oxidative molecules for signaling purposes. Among hydrophilic molecules, vitamin C and carnosine play important roles in the protection of proteins and DNA from oxidative molecular attacks. Other molecules, such as flavonoids, uric acid, micronutrients, peptides, and proteins, have been researched for their potential antioxidant actions.[22–32] The dipeptide carnosine (β-alanyl-L-histidine) has been researched as a protective agent against aging because of its antioxidant properties and ability to suppress protein glycation and crosslinking. Carnosine has also been suggested as an important agent in controlling secondary problems in diabetes.[33–41] Thus, it is not surprising that analogs combining Trolox, a substructure of vitamin E, with carnosine have been reported.[42] The heterodimers used in these studies coupled Trolox directly to carnosine without inclusion of the phytyl chain component in between, which is important (vide supra) for maximizing transport to LDLs in vivo by α-TTP.

The main goal of this project was to synthesize a next-generation antioxidant molecule based on combining a more complete structure of α-tocopherol and carnosine, with potential applications in prevention of oxidative stress–related diseases, most notably atherosclerosis. The hypothesis is that conjugation of a vitamin E derivative with carnosine (referred to as VECAR) by a phytyl-type tether will result in a new molecule with antioxidant properties capable of in vivo protection of polyunsaturated fatty acids (PUFAs) and LDL from oxidation. It has been shown that fluorescent molecules tethered to α-tocopherol derivatives are successfully transported to LDL particles in vitro, and the similar design of VECAR is anticipated to also be transported actively to LDL particles via endogenous α-TTP. Studies on VECAR both in vitro and in vivo will further determine if α-tocopherol can serve as a generic vehicle for delivery of a wide array of therapeutic compounds through the lipoprotein transport mechanisms. This would be a powerful addition to methods currently used to increase bioavailability of important drug compounds.

RESULTS

Molecular Design of VECAR

The molecular design of VECAR incorporated various structural components shown to be important when modifying the structure of α-tocopherol. Several synthetic antioxidant molecules have been derived from vitamin E (e.g., Trolox, 3-oxa-chromanol derivatives, brominated α-tocopherolmethano-dimer, raxofelast, and vitamin EC) in an effort to improve bioavailability or antioxidant potential. In general, these derivatives reported in the literature use the OH moiety of α-tocopherol for adding new functionality to the molecule, which is often accomplished in one step or modify the chromanol ring, requiring more complex synthetic strategies.[43–49] An important aspect in design of derivatives is maintaining the biotransport properties that are inherent to natural α-tocopherol. There is one specific transport protein, α-TTP, that is responsible for transport of α-tocopherol to LDLs; other transport mechanisms are passive and related to the lipophilicity of the molecule. For this study, it was of interest to ensure that the α-tocopherol derivative is recognized by the α-TTP for further transport to the LDL particles. The modification of the OH or methyl groups on the chromanol ring of α-tocopherol derivatives significantly reduce or inhibit uptake of these compounds by α-TTP.[50,51] On the other hand, modification of the phytyl chain does not affect recognition of α-tocopherol derivatives; therefore, this was the site chosen for further modification with additional bioactive molecules such as the hydrophilic carnosine antioxidant.[17,51–54]

Hydrophilic molecules like carnosine are not expected to enter into lipophilic pathways in living systems, and it was of central interest to determine whether the α-tocopherol structure could shuttle bioactive molecules into LDL particles that ordinarily could not be localized there. It was envisioned that this could be accomplished by tethering the hydrophilic bioactive molecule via the phytyl tail. The phytyl tail of α-tocopherol consists of a 13-carbon chain with two chiral methyl substituents and one achiral one; however, it has been reported that substituting a straight chain for the phytyl tail does not affect the absorption and antioxidant activity in rats while maintaining the lipophilicity of the molecule.[55] Therefore,

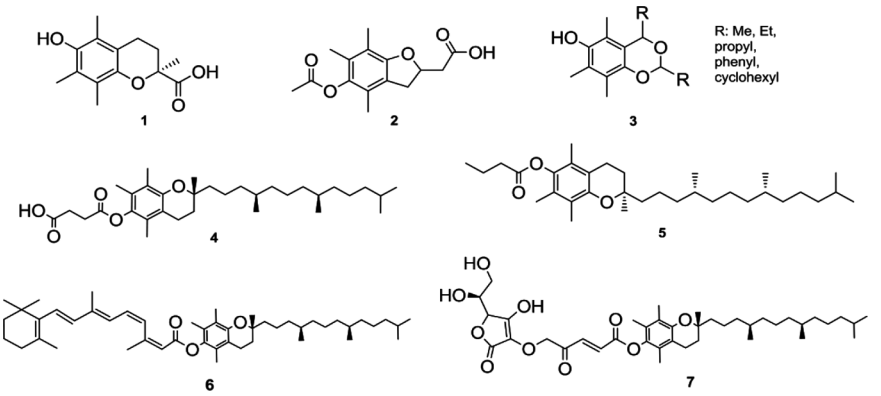


Figure 1. Vitamin E derivatives: **1**, Trolox, (S)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; **2**, Raxofelast, 2,3-dihydro-5-acetyloxy-4,6,7-trimethyl-2-benzofuranacetic acid; **3**, 3-oxa-chromanol derivatives; **4**, α-tocopherol succinate; **5**, α-tocopherol butyric acid; **6**, (E)-((R)-2,5,7,8-tetramethyl-2-((4R,8R)-4,8,12-trimethyltridecyl)chroman-6-yl) 5-(5-((S)-1,2-dihydroxyethyl)-4-hydroxy-2-oxo-2,5-dihydrofuran-3-yloxy)-4-oxopent-2-enoate; and **7**, (2Z,4Z,6E,8E)-((R)-2,5,7,8-tetramethyl-2-((4 R,8 R)-4,8,12-trimethyltridecyl) chroman-6-yl) 3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,4,6,8-tetraenoate.

the VECAR design for the synthesis in this study employed a 13-carbon tether with a carboxylate terminus for further functionalization. This has been done for similar analogs using Wittig coupling of the chromanol ring to the hydrophobic tail, and that approach was adopted for this synthesis as well. Unique to this synthesis is the conjugation of carnosine to the open end of the tail to provide a bioactive molecule with the antioxidant advantages of both lipophilic and hydrophilic components. The ability of α-tocopherol to transport a conjugated fluorescent molecule to the surface of Hep/G2 cells in culture by an α-TTP dependent pathway has been previously reported in the literature. It is anticipated that the carnosine conjugated molecule reported here will also be transported by an α-TTP dependent pathway to

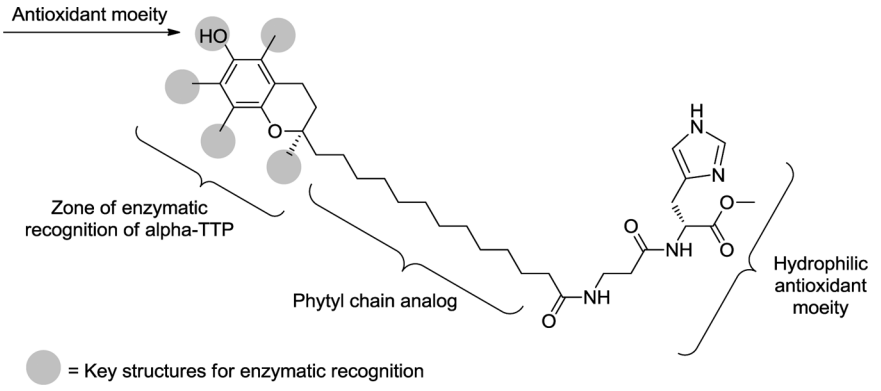
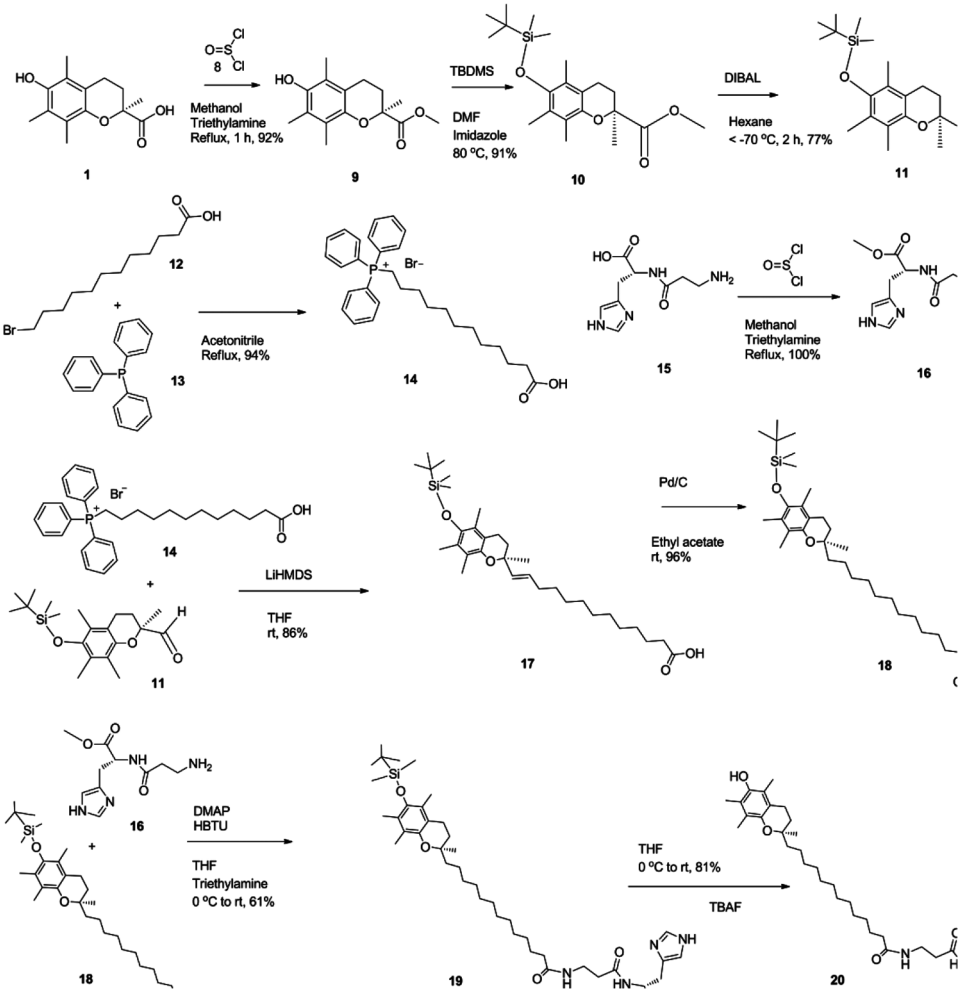


Figure 2. Schematic structure of VECAR. There is a hydrophilic section formed by the α-tocopherolchromanol ring and the carnosine dipeptide. The hydrophobic section is provided by the carbon tail that links polar heads to important sections. The circles around the chromanol ring represent the key zones for enzymatic recognition of VECAR by α-TTP enzyme.

the cells and ultimately to the LDL particles in cells, which has been shown to occur with vitamin E. Although the lipophilic vitamin E will be internalized in the LDLs, the hydrophilic carnosine may reside more toward the polar surface of the LDL particles. The complementary positioning of carnosine in polar regions of LDL particles may offer a unique and improved defense versus vitamin E alone toward oxidation of LDLs, which has been postulated as an important event in preventing atherosclerosis. Possibly more interesting is the delivery of a hydrophilic antioxidant to LDLs that could not otherwise locate there.

Synthesis of VECAR

Based on the molecular design of the VECAR molecule, the synthesis was carried outas shown in Scheme 1, involving nine steps in the overall synthesis starting



Scheme 1. Overall synthesis of VECAR.

from commercially available Trolox. Several steps follow similar procedures used by Lei and Atkinson to synthesize a vitamin E derivative with the same α-tocopherol structural component.[56] The first step was esterification of Trolox, achieved in 92% yield using thionyl chloride to form the acid chloride in the presence of methanol. This method was more dependable, reduced the reaction time from 18 h to less than 2 h, and resulted in a greater yield than the previous method reported that used p-toluenesulfonic acid to catalyze Fischer esterification using methanol. The protection of the hydroxyl group present in the chromanolring of Trolox ester **9** using *tert*-butyldimethylsilyl chloride (TBDMS) provided a white solid rather than a yellow oil as reported. It should be noted that use of excess magnesium sulfate for drying the organic extracts resulted in residual water in this product, which is difficult to remove afterward. This was significant because poor yields were observed in the subsequent DIBAL reduction when residual water was present in **10**. The third step was the reduction of the Trolox ester **10** to an aldehyde **11** using DIBAL at −78 °C over a span of 2 h. The purification of the aldehyde **11** posed some difficulty because of the small R_f in separation of the unreacted ester (which can be due to water in the reaction) with the aldehyde and required gradient elution during column chromatography. Next, the ylide (**14**) formed from 12-bromododecanoic acid (**12**), and triphenyhlphosphine was tethered to **11** through a Wittig reaction to form **17**. The double bond formed from the Wittig reaction was further reduced in the presence of hydrogen to produce **18** with yields comparable to similar steps in the literature.[56]

The coupling reaction of carnosine was complicated by the hydrophobic/hydrophilic behavior of the new molecule. Therefore, the carnosine was modified to carnosine ester (**16**) to improve its solubility in organic solvent and more importantly to avoid reactions between the carboxylic group of carnosine and its own amine terminus. Initially, esterification with diazomethane was investigated, but resulted in poor yield with several side products. Another approach was successful using thionyl chloride and methanol to form the ester without significant interference from the terminal amine. With the ester in hand, coupling of the carnosine derivative was investigated with commonly employed approaches such as using dicyclohexylcarbodiimide (DCC) with HOBt, the formation of asymmetric anhydrides, and (2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) (HBTU). The best results were achieved when HBTU was used as coupling reagent. Purification was first attempted using typical 60-Å and 300-Å pore-size silica gel; however, the product remained adsorbed under all mobile phases investigated. Thus, reverse-phase silica gel, synthesized according to the literature,[57] was employed and found to successfully separate the product from other impurities. The last step, deprotection of the silyl protecting group, was achieved using standard tetrabutylammonium fluoride (TBAF) conditions for 1.5 h; longer reactions times gave lower yields and produced more side products. Purification of the final product was again done using reverse-phase silica gel to give 26% yield overall of the isolated product.

Antioxidant Activity of VECAR by DPPH

The antioxidant behavior of VECAR was studied with the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay.[58] Measurements were performed at five concentrations [Eq. (1)] (Supplemental J) to determine IC_{50}, which is the point at which

50% of DPPH has been reduced by VECAR. The IC_{50} was found to be $24.9 \pm 1.4\,\mu M$ for VECAR; and the value for α-tocopherol alone was $24.4 \pm 1.5\,\mu M$.

These values confirm that VECAR has antioxidant activities that are similar to pure α-tocopherol, which is the main hydrophobic antioxidant used in the synthesis.

CONCLUSION

The successful synthesis of a novel heterodimer of α-tocopherol and carnosine (VECAR) was carried out in a total of nine steps. The key aspect of VECAR design was the use of a 13-hydrocarbon phytyl-chain mimic to link carnosine to Trolox at the C2 carbon position. This design feature is anticipated to not interrupt binding to α-TTP, while maintaining antioxidant activity of the two heterodimer components. Our results confirmed that there was no loss in antioxidant activity in VECAR using an in vitro DPPH assay, versus α-tocophorol and Trolox. Further studies in cell and animal matrices need to be carried out to determining the bioavailability and efficacy of the chimeric VECAR in vivo.

EXPERIMENTAL

The following chemicals were purchased from Acros Organics: Trolox 97%, *tert*-butylchlorodimethylsilane 98%, and triethylamine 99%. Methanol anhydrous 99.8%, imidazole >99.5% (GC), dimethylformamide anhydrous 99.8%, diisobutylaluminum hydride (DIBAL) 1.0 M solution in hexane, 12-bromododecanoic acid 97%, acetonitrile anhydrous 99.8%, triphenylphosphine 99%, tetrahydrofuran (THF) <99.9%, lithium bis(trimethylsilyl)-amide (LiHMDS) 1.0 M solution in THF, palladium (Pd/C) 10 wt.% (dry basis) on activated carbon, (wet, Degussa type E101 NE/W), L-carnosine 99%, and 4-(dimethylamino) pyridine (DMAP) 99% were purchased from Sigma-Aldrich. Thionyl chloride 99+% and magnesium sulfate 99.5% were purchased from Alfa Aesar. The chemicals purchased from Mallinckrodt Chemicals were methanol, ethyl acetate, toluene, ammonium chloride, chloroform, sodium hydroxide, and sodium bicarbonate. Dichloromethane and hexanes 95% n-hexane were purchased from J.T. Baker. Silica gel (porosity: 60 Å, particle size: 40–63 μm, surface area: 500–600 m^2/g, pH range: 6.5–7.5, and 300 Å particle size) was purchased from Sorbent Technologies, and *O*-benzotriazole-*N,N,N′,N′*-tetramethyl-uronium-hexafluoro-phosphate (HBTU), 99.58%, was purchased from ChemPep Inc. The following chemicals were purchased from Fisher Chemical: 1-propanol and sodium chloride. Sodium bicarbonate was purchased from EMD Chemicals.

A stock solution of DPPH in methanol at 0.4 mM was prepared and kept at −20 °C in the dark prior to use. The sample was diluted with water (pH = 3.5 prepared by addition of acetic acid) to 1 ml. The sample was added to dilute DPPH stock in methanol to obtain a final sample volume of 2 ml with a 0.1 mM DPPH concentration. The ratio between water and methanol was 1 to 1 v/v. The blank was prepared with 1 ml of water (pH 3.5) and pure methanol. The controls were prepared for each sample, with 1 ml of water (pH 3.5) and 1 ml of DPPH in methanol solution. Absorption readings were taken after 30 min at 518 nm using a Geminys 6 spectrophotometer (Thermo Scientific, Waltham, Mass.). The formula used to calculate the

rate of oxidation was

$$\% \text{ change in activity} = [(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})/\text{Abs}_{\text{control}}] \times 100 \quad (1)$$

Reverse-Phase Silica Gel

The synthesis of reverse-phase silica gel was performed for purification of VECAR after the coupling reaction. Normal-phase silica gel (100 g) was suspended in toluene (500 ml), and 21 ml (0.52 mol) of octadecyltrichlorosilane (C18, ODTS) was added dropwise. After 10 min, triethylamine (28 ml, 4 equiv.) was added to the suspension, and the final suspension was refluxed for 24 h at 125 °C. Finally, the reverse-phase silica gel was washed with toluene (1 L), DCM (1 L), ethyl acetate (1 L), and methanol (4 L). The product was dried under high vacuum.[57]

Synthesis of α-Tocopherol-Carnosine (VECAR)

The synthesis of α-tocopherol-carnosine (VECAR) was based on the protection of OH group, activation of thecarbonyl group, Wittig reaction, attachment of carnosine, and deprotection. The method is based on the work of Atkinsons and coworkers.[56,59–67]

(*S*)-Methyl 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylate (9)

Commercially available (Sigma-Aldrich) *S*-Trolox (**1**) (3.04 g, 12.1 mmol) was dissolved in 25 mL dry methanol; 2.4 mL (17.0 mmol) triethylamine was added and the mixture stirred at 0 °C for 15 min. Thionyl chloride (1.06 mL, 14.6 mmol, 1.2 equiv.) was added to the solution and stirred at 0 °C for an additional 10 min. The solution was brought to 70 °C and refluxed for 1 h, and after the reaction completed the solution was cooled to room temperature and purged with nitrogen to remove any remaining thionyl chloride. The solvent was evaporated and the brown solid was recrystallized from 75 mL methanol to give white crystals (2.95 g, 92%). ^{1}H ($CDCl_3$, 400 MHz) δ 4.31 (br, 1H, OH), 3.69 (s, 3H, OCH_3), 2.68 (m, 2H), 2.45 (m, 1 H), 2.20 (s, 3H), 2.16 (s, 3H), 2.07 (s, 3H), 1.87 (m, 1H), 1.62 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.53, 145.52, 145.31, 122.56, 121.30, 118.45, 116.87, 77.03, 52.38, 30.63, 25.45, 20.97, 12.21, 11.84, 11.26. IR ν_{max} 3527.61, 2989.30, 2926.84, 1738.34, 1454.75, 1193.27, 1113.77 cm^{-1}. MS (ESI-TOF): calcd. for $C_{15}H_{20}NaO_4$ $(M + Na)^+$: 287.1260; found: 287.1241.

(*S*)-Methyl 6-(*tert*-Butyldimethylsilyloxy)-2,5,7,8-tetramethylchroman-2-carboxylate (10)

Following previous published procedures,[56] (*S*)-methyl-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylate (**9**) (1.254 g, 4.7 mmol) was mixed with *tert*-butyldimethylsilyl chloride (1.081 g, 7.2 mmol) and imidazole (1.339 g, 19.7 mmol); and dry dimethylformamide (10 mL) was added. The mixture was stirred and heated in an oil bath under an argon atmosphere at 85 °C for 5 h, at which point none of the starting material (**9**) was detected by TLC. The reaction mixture was poured into

100 mL saturated aqueous NaCl, and then extracted with 4×50 mL ethyl acetate. The combined organic extracts were dried over magnesium sulfate and evaporated to give an oily sample with a light yellow color. The crude product was purified by column chromatography on silica gel using CH_2Cl_2–hexane = 3:1, providing a white solid after evaporation with a yield of 1.62 g (91%). ^{1}H ($CDCl_3$, 400 MHz) δ ppm 3.66 (s, 3H, OCH_3), 2.57 (m, 2H), 2.44 (m, 1H), 2.15 (s, 3H), 2.11 (s, 3H), 2.02 (s, 3H), 1.88 (m, 1H), 1.60 (s, 3H), 1.04 (s, 9H), 0.11 (s, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm 174.55, 145.90, 144.81, 126.14, 123.48, 122.70, 117.06, 77.02, 52.30, 30.58, 26.11, 25.41, 21.07, 18.62, 14.34, 13.39, 12.00, −3.28, −3.34. IR ν_{max} 2957.55, 2928.25, 2856.92, 1746.92, 1464.01, 1260.53, 1110.79 cm^{-1}. MS (ESI-TOF): calcd. for $C_{21}H_{34}NaO_4Si$ $(M + Na)^+$: 401.2124; found 401.2171.

(*S*)-6-(*tert*-Butyldimethylsilyloxy)-2,5,7,8-tetramethylchroman-2-carbaldehyde (11)

Similar to previous procedures,[56] compound **10** (3.282 g, 8.67 mmol) was added to dry hexane (35 mL) and cooled in an acetone/dry ice bath to −75 °C. Diisobutylaluminum hydride (DIBAL-H, 1.0 M in hexane, 16 mL, 16 mmol) was added using a syringe so as not to exceed −70 °C. After 2 h the reaction was quenched with dry methanol (25 mL) and stirred for 15 min. The solution was removed from the acetone/dry ice bath and was allowed to warm to −28 °C. Then water (15 mL) added to the solution by syringe. The solution was then poured into 100 mL saturated aqueous NaCl and extracted with 4×100 mL hexane / ethyl acetate (2:1). The combined organic extracts were dried over magnesium sulfate, and the solvent was evaporated. The crude product was purified by column chromatography using CH_2Cl_2–hexane (1:1 to 3:1) to give 2.33 g (77%) yield. ^{1}H ($CDCl_3$, 400 MHz) δ ppm 9.63 (s, 1H), 2.55 (m, 2H), 2.27 (m, 1H), 2.17 (s, 3H), 2.12 (s, 3H), 2.02 (s, 3H), 1.82 (m, 1H), 1.39 (s, 3H), 1.05 (s, 3H), 0.12 (s, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm 204.91, 145.56, 145.13, 126.50, 123.89, 122.84, 117.62, 80.28, 27.93, 26.08, 21.62, 20.49, 18.60, 14.35, 13.38, 12.06, −3.31, −3.34. IR ν_{max} 2928.40, 2857.11, 2799.65, 1741.47, 1463.23, 1255.44, 1101.77, 836.53 cm^{-1}. MS (ESI-TOF): calcd. for $C_{20}H_{33}O_3Si$ $(M + H)^+$: 349.2199; found 349.2023.

11-Carboxyundecyl)triphosphonium Bromide (14)

A solution of 12-bromododecanoic acid (1.66 g, 5.9 mmol) in dry acetonitrile (13 mL) was heated and stirred. Triphenylphosphine (1.637 g, 6.2 mmol) in dry acetonitrile (13 mL) was heated, stirred, and then added to the 12-bromododecanoic solution; the solution was refluxed overnight. The solvent was removed. The crystals were dissolved in DCM (14 mL). Toluene (40 mL) was added, and the solution was stirred and heated to remove the DCM. The solution was cooled, and the phosphonium salts crystallized where removed by filtration. The solution was decanted with toluene. The yield of **14** was 3.01 g (94%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 7.76–7.67 (m, 9H), 7.67–7.27 (m, 6H), 3.56–3.51 (m, 2H), 2.28 (t, 2H, $J = 7.42$ Hz), 1.53–1.45 (m, 6H), 1.17–1.11 (m, 12H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm 177.51, 135.11, 135.09, 133.61, 133.51, 130.61, 130.48, 118.60, 117.75, 34.50, 30.37, 30.21, 29.09, 29.07, 28.97, 28.94, 28.81, 24.73, 22.88, 22.52, 22.48,

22.39. IR ν_{max} 2925.16, 2848.82, 1715.11, 1436.26, 1113.18 cm^{-1}. MS (ESI-TOF): calcd. for $C_{30}H_{39}BrO_2P$ $(M + H\text{-}Br)^+$: 461.2831; found 461.2332.

(*S,E*)-13-(6-(*tert*-Butyldimethylsilyloxy)-2,5,7,8-tetramethylchroman-2-yl)tridec-12-enoic Acid (17)

The suspension of phosphonium salts (**14**) (2.50 g, 11.5 mmol) was dissolved in dry THF (40 mL) at room temperature under argon. A solution of LiHMDS in THF (1 M in THF, 16.5 mL, 11.5 mmol) was added dropwise via a syringe. The red ylide was stirred for 2 h under argon. A solution of **11** (1.45 g) in dry THF (8 mL) was added dropwise. The color changed from red to pale yellow. The suspension was stirred for an additional 3 h, until **3** could not be found by TLC. The reaction was quenched with saturated NH_4Cl (80 mL) and water (80 mL) and then extracted with ethyl acetate. The solution was dried over magnesium sulfate. After solvent removal, trituration with cold hexane removed triphenylphosphine oxide. The hexane solution was evaporated, and the crude product was purified by column chromatography on silica gel using ethyl acetate:hexane (1:1).[56] Yield of **17** was 1.90 g (86%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 5.33–5.31 (m, 2H), 2.55–2.53 (m, 2H),2.36–2.33 (m, 2H), 2.11 (s, 3H), 2.10 (s, 3H), 2.08–2.01 (m, 2H), 2.04 (s, 3H), 1.65–1.62 (m, 2H), 1.47 (s, 3H), 1.29–1.22 (m, 16H), 1.05 (s, 9 H), 0.12 (s, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm 179.99, 146.06, 144.13, 133.26, 132.49, 125.77, 123.46, 122.42, 117.83, 75.52, 34.04, 33.40, 29.97, 29.54, 29.42, 29.23, 29.05, 27.97, 27.23, 26.09, 24.67, 21.27, 18.58, 14.34, 13.40, 12.17, −3.35. IR ν_{max} 2927.02, 2855.11, 1709.96, 1462.79,1254.07, 1090.75, 837.25 cm^{-1}. MS (ESI-TOF): calcd. for $C_{32}H_{54}O_4Si$ $(M)^+$: 530.3791; found 530.3740.

(*R*)-13-(6-(*tert*-Butyldimethylsilyloxy)-2,5,7,8-tetramethylchroman-2-yl)tridecanoic Acid (18)

Pd/C (0.505 g) was added to a solution of **17** (0.95 g, 3.67 mmol) in ethyl acetate (85 mL). A hydrogen balloon was attached to the reaction mixture and it was stirred for 18 h at room temperature. The mixture was filtered and evaporated to obtain **18** as oil. The crude product was purified by column chromatography using hexane–ethyl acetate (4:1). The yield of **18** was 0.91 g (96%).[56] ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 2.60 (t, 2H, $J = 7.40$), 2.40 (t, 2H, $J = 6.28$), 2.16 (s, 3H), 2.13 (s, 3H), 2.11 (s, 3H), 1.79–1.92 (m, 2H), 1.59–1.70 (m, 4H), 1.47–1.53 (m, 2H), 1.32 (br, 16H), 1.28 (s, 3H), 1.11 (s, 9H), 0.18 (s, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm 180.52, 145.97, 144.10, 125.86, 123.52, 122.72, 117.52, 77.31, 39.66, 34.18, 31.60, 30.26, 29.71, 29.68, 29.52, 29.33, 29.14, 26.18, 24.74, 23.88, 23.70, 20.97, 18.65, 14.39, 13.47, 12.01, −3.29. IR ν_{max} 2928.58, 2853.19, 1711.92, 1252.43, 1087.10, 836.06 cm^{-1}. MS (ESI-TOF): calcd. for $C_{32}H_{56}O_4Si$ $(M + H)^+$: 533.4026; found 533.4011.

Carnosine Methyl Ester Dihydrochloride (16)

Thionyl chloride (0.25 mL, 3.3 mmol) was added dropwise to a suspension of carnosine(**15**) (602.9 mg, 2.7 mmol) in anhydrous methanol (24 mL) at 0 °C and stirred for 10 min. The solution was refluxed at 75 °C for 1 h. After cooling to room

temperature, the mixture was concentrated to give a quantitative yield. The product was used in the next step without further purification.[68] ^{1}H NMR [$(CD_3)_2SO$] δ 9.04 (s, 1H), 8.89–8.87 (d, 1 H), 8.09 (br, 3H), 7.49, (s, 1H), 4.62–4.56 (m, 1H), 3.65 (s, 3H), 3.19–3.05 (m, 2H), 2.92 (br, 2H), 2.57 (t, 2H, $J = 6.82$). ^{13}C NMR (DMSO, 100 MHz) δ ppm 171.36, 170.15, 134.06, 129.33, 117.50, 52.71, 52.01, 35.38, 32.31, 26.23. IR ν_{max} 3417.02, 3138.25, 1737.08, 1660.26, 1624.71 cm^{-1}. MS (ESI-TOF): calcd. for $C_{10}H_{17}ClN_4O_3$ $(M - H)^+$: 275.0911; found 275.0912.

Coupling Carnosine Methyl Ester with Carboxylic Acid (19)

Carboxylic acid compound **18** (840 mg, 1.59 mmol), DMAP (19 mg, 0.16 mmol), carnosine–methyl ester hydrochloride (**16**) (662 mg, 2.39 mmol), and triethylamine (1.67 mL, 5 equiv) were added to anhydrous DMF (13.4 ml). The solution was cooled to 0 °C in an ice bath. HBTU (721 mg, 1.9 mmol) in anhydrous DMF (3 mL) was added to the reaction mixture dropwise and stirred for an additional 20 min at 0 °C. The reaction was stirred at room temperature overnight. Next, the solvent was evaporated and dried under high vacuum. The washing and extraction steps were performed with chloroform and water without addition of magnesium sulfate. The organic phase was collected and evaporated under vacuum. The purification was performed with reverse-phase silica gel (C_{18}). The silica gel used was 300 Å pore size with a mixture of methanol–water (7:3) as eluent. The yield of **19** was 0.62 g (61%).[69–71] ^{1}H NMR (MeOD) δ 7.58 (s, 1H), 6.87 (s, 1H), 4.69–4.66 (m, 1H), 3.72 (s, 3H), 3.41–3.37 (m, 2H), 3.12–2.96 (m, 2H), 2.54 (t, 2H, $J = 6.62$ Hz), 2.41 (t, 2H, $J = 6.66$ Hz), 2.15 (t, 2H, $J = 7.60$ Hz), 2.07 (s, 3H), 2.03 (s, 3H; s, 3H), 1.82–1.71 (m, 2H), 1.57 (m, 3H), 1.44 (m, 3H), 1.26 (br, 16H), 1.20 (s, 3H), 1.04 (s, 9H), 0.10 (s, 6H). ^{13}C NMR (MeOD, 100 MHz) δ ppm 176.30, 173.64, 173.46, 147.22, 145.32, 136.37, 126.56, 124.42, 123.52, 118.68, 75.51, 54.10, 52.76, 40.31, 37.11, 36.82, 36.36, 32.81, 31.20, 30.74, 30.63, 30.46, 30.33, 26.94, 26.68, 24.57, 24.22, 21.84, 19.50, 14.86, 13.91, 12.26, −3.07. IR ν_{max} 3446.06, 2927.19, 2854.43, 1733.62, 1716.69, 1683.57, 1652.84, 1646.99, 1256.82, 1090.42, 837.37 cm^{-1}. MS (ESI-TOF): calcd. for $C_{42}H_{70}N_4O_6Si$ $(M + H)^+$: 755.5134; found 755.5127.

Deprotection of Hydroxyl Group of Chromanol Ring (20)

The deprotection of **19** was performed by dissolving 19 (255 mg) in dry THF (10 ml), and the addition of TBAF (2.9 mL of a 1 M THF solution) was performed dropwise via a syringe. The solution was stirred for 1.5 h at room temperature. The THF was evaporated and replaced with chloroform (200 ml). The solution was washed with water three times. The crude VECAR product was purified by flash chromatography on reverse-phase silica gel (60 Å) using gradient elution, methanol–water (30%) and methanol–water–ammonium hydroxide (8:1:1). The first two fractions collected the impurities, and pure VECAR was collected in the last fraction. The yield of **20** was 175 mg (81%). ^{1}H (MeOD, 400 MHz) δ 7.68 (s, 1H), 6.89 (s, 1H), 4.69–4.65 (m, 1H), 3.69 (s, 3H), 3.39–3.34 (m, 2H), 3.12–2.95 (m, 2H), 2.57 (t, 2H, $J = 6.9$ Hz), 2.39 (t, 2H, $J = 6.7$ Hz), 2.14 (t, 2H, $J = 3.6$ Hz), 2.11 (s, 3H), 2.07 (s, 3H), 2.03 (s, 3H), 1.79–1.72 (m, 2H), 1.59–1.53 (m, 3H), 1.43–1.39 (m, 3H), 1.27–1.26 (br, 16H), 1.19 (s, 3H). ^{13}C NMR (MeOD, 100 MHz) δ ppm

176.43, 173.70, 173.36, 146.74, 136.25, 124.42, 122.99, 122.06, 118.26, 75.42, 53.99, 52.81, 40.22, 37.11, 36.85, 36.38, 32.99, 31.21, 30.71, 30.61,30.44, 30.31, 29.87, 26.96, 24.58, 24.15, 21.77, 12.80, 12.02, 11.83. IR νmax 3445.78, 2962.90, 2928.89, 2854.65, 1733.63, 1652.73, 1646.86, 1260.60, 1083.82, 844.71 cm^{-1}. MS (ESI-TOF): calcd. for $C_{36}H_{56}N_4O_6$ $(M + H)^+$: 641.4278; found 641.4293.

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