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Asymmetric Synthesis and Biological Activity of nor-α-Tocopherol, a New Vitamin E Analogue

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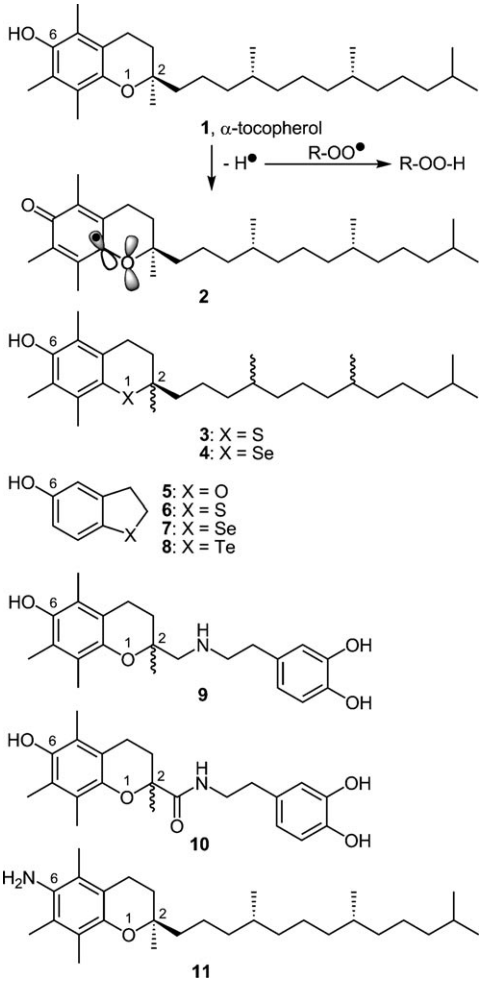
The vitamin E analogues (2*R*,4′*R*,8′*R*)-nor-α-tocopherol (94% *de*) and (2*RS*,4′*R*,8′*R*)-nor-α-tocopherol have been synthesized from (all *R*)-hexahydrofarnesol and phytol, respectively. According to in vitro experiments with murine macrophages nor-α-tocopherol is an anti-inflammatory compound more potent than α-tocopherol.

Introduction

α-Tocopherol (**1**) is the biologically most significant member of the vitamin E family and is known to act as a very efficient radical-chain-breaking antioxidant in tissues.[1] Ingold's investigations suggested[1] that the antioxidant reactivity of the chromanol system in vitamin E compounds is attributable to stereoelectronic factors: that is, the lone pair of O1 is favorably oriented to stabilize the tocopheroxyl radical **2** produced by homolysis of the phenolic OH group.[2] Hence, in attempts to improve the antioxidant properties of **1**, the racemic vitamin E analogues **3** and **4** in which the oxygen is replaced by sulfur and selenium were synthesized.[3–5] It was expected that the larger heteroatoms should stabilize the adjacent radical better than oxygen. These compounds, however, were less efficient antioxidants than **1**.[6,7] In contrast, replacement of the six-membered heterocycle with a five-membered ring (see **5**–**8**) led to greater radical chain-breaking capacities in a two-phase lipid peroxidation model system.[7,8]



More recent studies have reported on the replacement of the chiral side chain in **1** by shorter units with polar functional groups (see, for example, **9** and **10**).[9] These compounds show remarkably increased antioxidant potencies in relation to **1**. Changing of the phenol group of **1** for an amino group yields tocopheryl amines such as **11**, which shows antiproliferative effects on the neuroblastoma glioma C6 cancer cell line 100 times more efficient than **1**.[10] The fact that tocopherols as well as tocopherol analogues display various biological activities[11–12] beyond their well established antioxidant properties led us to synthesize the unexplored chromanol **12** (Scheme 1). Like **1**, this tocopherol analogue shows the same *R* chirality at the stereogenic centers C2/C4′/C8′; however, the methyl group at C2 is replaced by a hydrogen and **12** is hence named nor-α-tocopherol.

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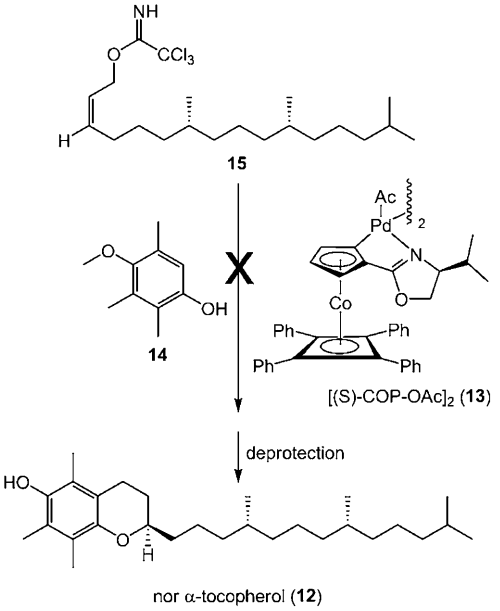


Scheme 1. Attempted synthesis of **12**.

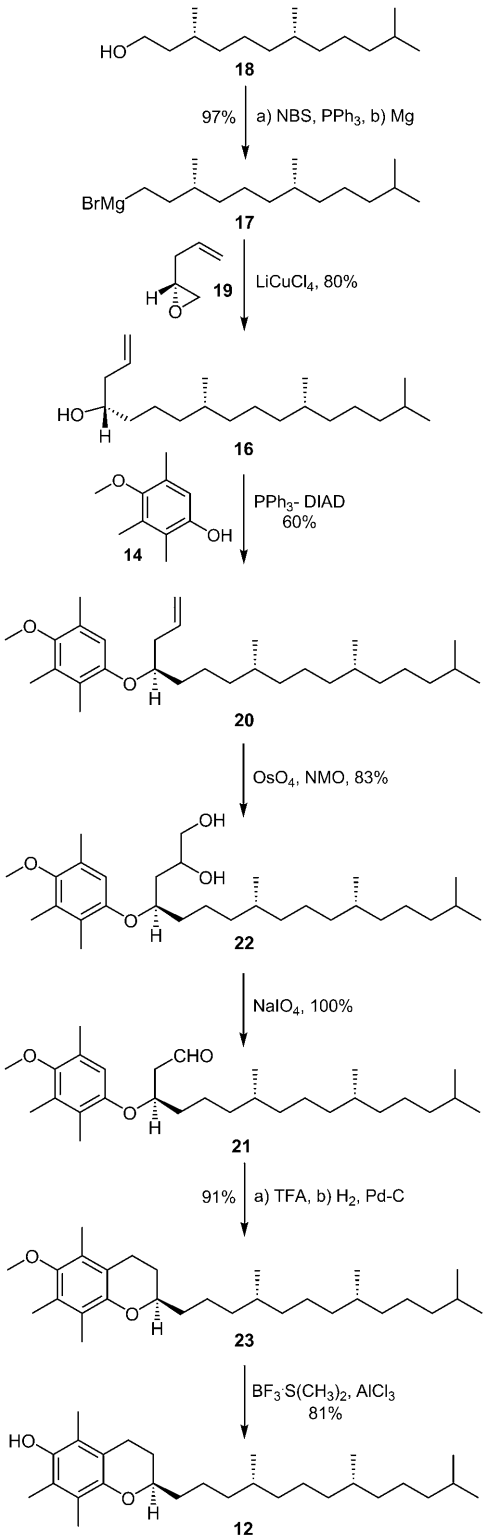
Results and Discussion

Synthesis of nor-α-tocopherol

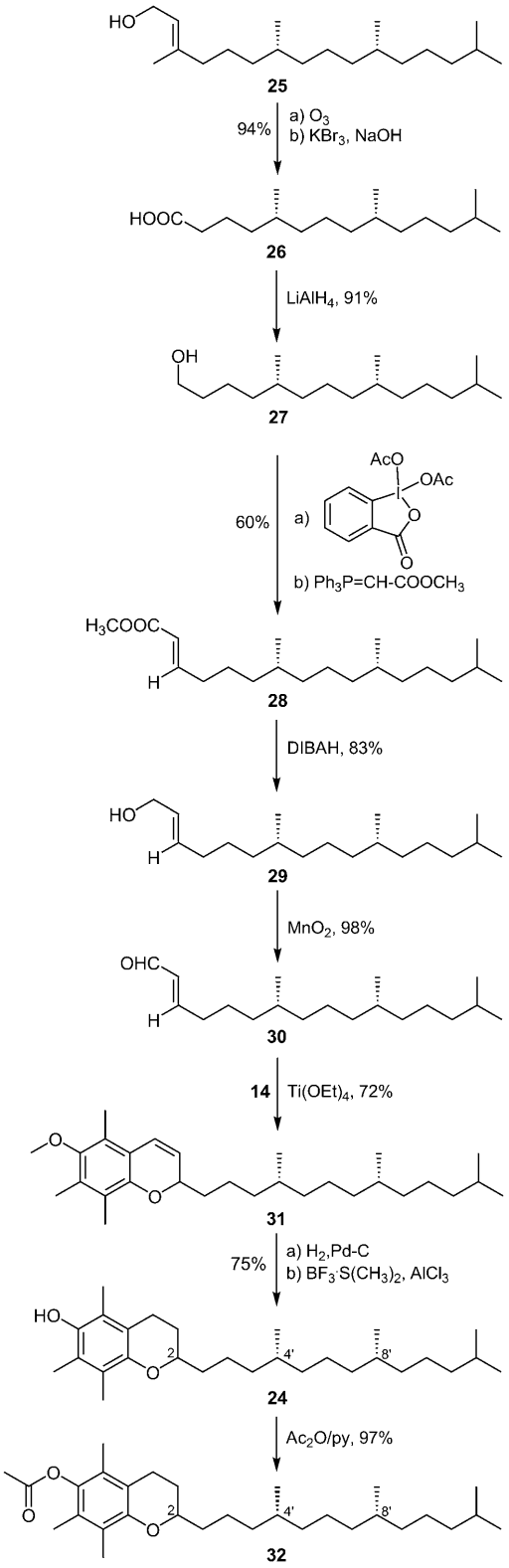
Our first approach directed towards enantiomerically enriched nor-α-tocopherol (**12**) involved the use of a procedure published by Overman et al.[13] in which couplings of *Z*-configured allyl trichloroacetimidates with various phenols in the presence of catalytic amounts of the Pd complex **13** were performed (Scheme 1). Unfortunately, with the phenol **14** and the activated olefin **15** this reaction did not work at all and it seems that the scope of this procedure is limited to monosubstituted phenols and short olefins.

Accordingly we developed a more elaborate, but nevertheless efficient, route to **12** (Scheme 2). For the synthesis of the key intermediate **16** two building blocks were required: 1) the Grignard reagent **17**,[14] available from (3*R*,7*R*)-hexahydrofarnesol **18**,[15] and 2) the allyl epoxide **19**, which was accessible from commercially available (*R*)-epichlorohydrin.[16] The reaction between **17** and **19**, catalyzed by lithium cuprate,[17–18] gave the alcohol **16** in 80% yield and 97% *de* (diastereomeric excess), determined on the corresponding *p*-nitrobenzoate. Unlike in cases of tertiary alcohols[14] the Mitsunobu reaction between the non-activated **16** and the phenol **14** proceeded readily and gave the phenol ether **20** in 60% yield with complete inversion of configuration. Ozonolysis of the double bond of **20** was unsatisfactory, so cleavage of the olefin to the aldehyde **21** was pursued in two steps via the diol **22** in excellent yield. Acid-catalyzed cyclization to the chromane system and subsequent hydrogenation was accomplished in 91% yield and the resulting chromanol ether **23** was finally deprotected to give nor-α-tocopherol (**12**) in 94% *de*.

For the preliminary biological experiments reported here larger amounts of nor-α-tocopherol were required, and so we developed a route to (2*RS*,4′*R*,8′*R*)-nor-α-tocopherol (**24**), racemic at C2 (Scheme 3). For this purpose phytol (**25**) was converted into the known acid **26**[19] in analogy with a published procedure.[20] Reduction to the alcohol **27** followed by Dess–Martin oxidation to the corresponding aldehyde and subsequent Wittig reaction furnished the unsaturated ester **28**. Re-



Scheme 2. Synthesis of nor-α-tocopherol (**12**).



Scheme 3. Synthesis of (2*R*/*S*,4′*R*,8′*R*)-nor-α-tocopherol (**24**).

duction to the alcohol **29** and subsequent oxidation yielded the desired aldehyde **30** ready for coupling with the phenol **14**. Lewis-acid-catalyzed ring closure gave the chromene **31**, which was conveniently hydrogenated and deprotected to afford pure **24**. In order to prepare stable stock solutions for biological experiments, **24** was quantitatively acetylated to **32**.

Evaluation of the biological activity of (2*RS*,4′*R*,8′*R*)-nor-α-tocopherol (24)

As mentioned above, the antioxidant activities of tocopherols and analogues are to a large extent related to stabilization of the radical center adjacent to O1. In order to compare this property for tocopherol and nor-tocopherol, the electron distributions in the SOMOs of the chromanyl radicals were calculated. As shown in Figure 1 for the two units **33** and **34**, corresponding to α-tocopherol and nor-α-tocopherol, respectively, the electron distributions are superimposable, and hence no significant difference in the in vitro antioxidant activities of **12** and **24** versus α-tocopherol (**1**) would be expected. Accordingly we focused our investigations on tocopherol features[11–12] unrelated to its antioxidant activity.

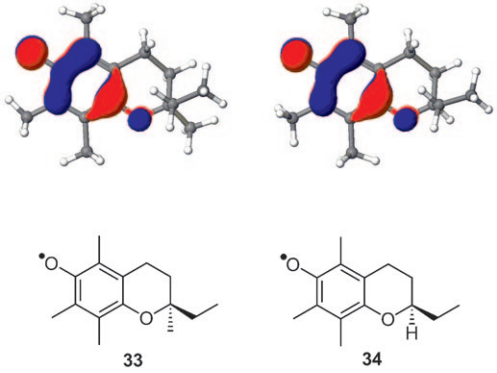


Figure 1. SOMOs of the chromanyl radicals **33** and **34**.

An increasing volume of evidence suggests a role for α-tocopherol in modulating redox-regulated signal transduction pathways both in cultured cells and in vivo.[21–24] Although no common transcription factor for α-tocopherol-sensitive molecular targets has yet been identified, different genes have been reported to be affected by α-tocopherol, including those involved in the regulation of inflammation and atherogenesis.[25] Monocytes, when stimulated with the endotoxin lipopolysaccharide (LPS), differentiate into macrophages and produce large amounts of pro-inflammatory mediators, including tumor necrosis factor α (TNFα), interleukin 1β (IL-1β), and interleukin 6 (IL-6). Furthermore, release of pro-inflammatory molecules triggers nitric oxide production by activation of the macrophage-inducible NO synthase (iNOS). For our cell culture studies we used LPS-stimulated murine RAW264.7 monocytes to investigate the potential anti-inflammatory activity of (2*RS*,4′*R*,8′*R*)-nor-α-tocopherol (**24**) relative to **12** (94% *de*) and to α-tocopherol (**1**).

It is important to note that for initial biological experiments a stable stock solution of the phenol ester **32** was used. However, **32** is hydrolyzed to **24** under conditions of incubation

with cell cultures. Accordingly the results reported in Figures 2–4 refer to the biological activity of (2*RS*,4′*R*,8′*R*)-nor-α-tocopherol (**24**).

We determined the expression of pro-inflammatory molecules after LPS stimulation (100 ng mL^{-1}) in RAW264.7 cells preincubated with 50 μmol L^{-1} nor-α-tocopheryl acetate **32**, which is a noncytotoxic concentration. Relative mRNA levels of TNFα, IL-1β, IL-6, and iNOS were significantly decreased in cells incubated with the nor-α-tocopherol relative to controls (Figure 2).

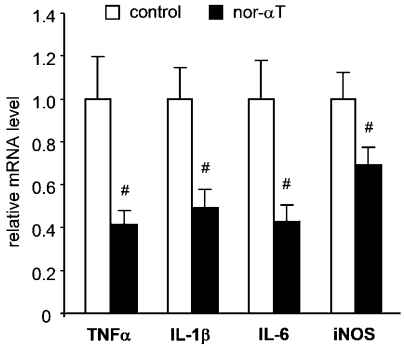


Figure 2. Effect of **24** (nor-αT) on mRNA levels of pro-inflammatory genes in murine macrophages. RAW264.7 cells were preincubated with **32** (50 μmol L^{-1}) for 24 h and subsequently stimulated with LPS. Total RNA was isolated after 1 h (for TNFα) and 6 h (for IL-1β, IL-6, and iNOS). mRNA levels were measured by real-time RT-PCR. Values are means ± SD ($n = 6$). # indicates significant differences ($p < 0.5$) between nor-αT-treated and untreated control cells.

Similar results for (2*RS*,4′*R*,8′*R*)-nor-α-tocopherol (**24**) were obtained with regard to proinflammatory cytokine levels in the cell culture supernatant (Figure 3) and iNOS protein levels in cell lysates of our RAW264.7 cells (Figure 4). In fact, (2*RS*,4′*R*,8′*R*)-nor-α-tocopherol (**24**) significantly decreased LPS-induced secretion of TNF-α, IL-1β, and IL6 relative to untreated controls, as well as cellular iNOS protein levels.

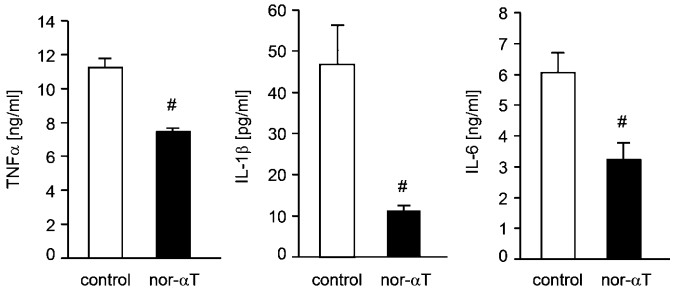


Figure 3. Effect of **24** (nor-αT) on secretion of the proiinflammatory cytokines TNF-α, IL-1β, and IL6. Cells were preincubated with **32** (50 μmol L^{-1}) for 24 h and subsequently stimulated with LPS. Cell culture supernatants were collected after 24 h and cytokine production was measured by ELISA. Values are means ± SD ($n = 3$). # indicates significant differences ($p < 0.5$) between nor-αT and untreated controls.

As far as the inhibition of LPS-induced TNF-α, IL-1β, and IL-6 gene expression is concerned, (2*RS*,4′*R*,8′*R*)-nor-α-tocopherol

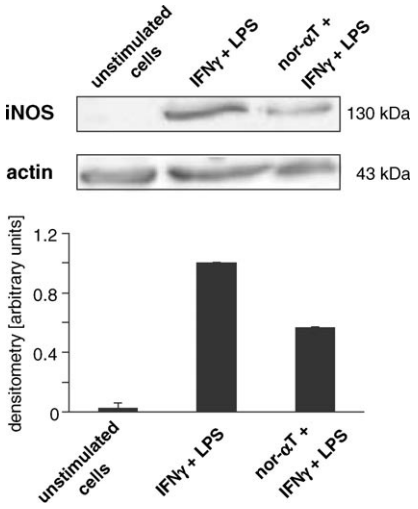


Figure 4. Effect of **24** (nor-αT) on iNOS protein level in murine macrophages. Cells were preincubated with **32** (50 μmol L^{-1}) for 24 h and stimulated with interferon γ (IFNγ) and LPS. Whole cells were lyzed after 24 h and protein levels were assessed by Western Blotting. Relative protein intensities were determined by densitometry.

proved to be (1.8 ± 0.3)-, (3.0 ± 0.3)-, and (1.8 ± 0.1) times more potent than α-tocopherol (**1**).

No significant difference between (2*RS*,4′*R*,8′*R*)-nor-α-tocopherol (racemic at C2, **24**) and compound **12** [(2*R*,4′*R*,8′*R*), 94% *de*] with regard to inhibition of inflammatory gene expression in our RAW264.7 cells was observed.

Conclusions

Nor-α-tocopherol is a potent anti-inflammatory molecule in murine macrophages in vitro. These properties are unrelated to the absolute configuration at C2, because **12** and **24** displayed the same biological activities. However, nor-α-tocopherol is a significantly better inflammatory agent than α-tocopherol (**1**).

Experimental Section

General: Chemicals and solvents were purchased from commercial suppliers or purified by standard techniques. For TLC, silica gel plates (Merck AG, 60 F254) were used and compounds were visualized by irradiation with UV light and/or by treatment with a solution of phosphomolybdic acid, cerium(IV) sulfate, and concd. H_2SO_4 in water (2:4:40:160 by weight) followed by heating. Flash chromatography was performed with Fluka silica gel 60 (particle size 0.040–0.063 mm). ^{1}H and ^{13}C NMR spectra were recorded with a Bruker DPX NMR (400 MHz and 500 MHz) spectrometer at ambient temperature. Chemical shifts are given in δ units relative to tetramethylsilane (TMS, $\delta = 0$ ppm), and the coupling constants (J) are given in Hz. If not otherwise stated, spectra were recorded in $CDCl_3$ at ambient temperature; for ^{1}H NMR spectra tetramethylsilane was used as internal standard and for ^{13}C NMR spectra $CDCl_3$ was used as internal standard ($\delta = 77.2$ ppm). Melting points are uncorrected. IR spectroscopy was performed with a PerkinElmer

1600 FTIR apparatus. Electron-spray ionization mass spectra (ESI-MS) were recorded with a Bruker Esquire 3000 plus spectrometer. HPLC was carried out with an intelligent pump, detector, and integrator and a Hewlett Packard S1100 or a Shimadzu LC-20AB/SPD-M20A instrument. Microanalyses were performed with a PerkinElmer 240 Analyzer by Werner Kirsch at the Department of Chemistry, University of Basel. Optical rotations were measured with a PerkinElmer Polarimeter 341 at $\lambda = 589$ nm.

Preparation of 16: *N*-Bromosuccinimide (4.63 g, 26 mmol) was added portionwise over 30 min at 4–8 °C to a solution of (3*R*,7*R*)-hexahydrofarnesol (**18**, 5.25 g, 23 mmol) and triphenylphosphine (7.23 g, 27.6 mmol) in dichloromethane (35 mL). The reaction mixture was stirred for 20 min and then concentrated on a rotary evaporator. The two-phase residue was triturated with hexane and the solid material was filtered off and washed with hexane. The concentrated filtrate was purified by chromatography on SiO_2 (hexane) to afford the corresponding bromide (6.55 g, 98%) as a colorless oil. This (874 mg, 3.0 mmol) was dissolved in THF (4 mL) and added over 15 min to a suspension of magnesium turnings (85 mg, 3.5 mmol) in THF (1 mL)/dibromoethane (15 μL, 0.17 mmol). The mixture was stirred at RT for 30 min and at 50 °C for 4 h.

In a second flask, a solution of (*R*)-2-allyloxirane (**19**, 190 mg, 2.26 mmol) in THF (4 mL) was cooled to −70 °C, the Grignard reagent **17** was added by syringe, and the mixture was stirred at −70 °C for 5 min. The mixture was stirred at −70 °C for 5 min. A catalytic amount of dilithium tetrachlorocuprate(II) (0.1 M solution in THF, 40 μmol) was then added. After stirring at −70 °C for 15 min the reaction mixture was allowed to warm slowly to room temperature while additional dilithium tetrachlorocuprate solution (80 μmol) was added portionwise. The mixture was stirred for 2 h, quenched with half-saturated aq. NH_4Cl, and extracted with *tert*-butyl methyl ether (TBME). The organic layer was washed with brine, dried ($MgSO_4$), and concentrated on a rotary evaporator. The colorless oil was purified by column chromatography on SiO_2 (hexane/EtOAc 10:1) to afford **16** (491 mg, 73%) as a colorless, viscous oil. $[\alpha]_D^{20} = -4.5°$ ($c = 1.47$ in $CHCl_3$); ^{1}H NMR (400 MHz,$CDCl_3$): $\delta = 5.78–5.88$ (m, 1H), 5.11–5.16 (m, 2H), 3.6–3.7 (m, 1H), 2.25–2.35 (m, 1H), 2.10–2.18 (m, 1H), 1.0–1.6 (m, 22H), 0.84–0.88 ppm (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 135.33$, 118.50, 71.10, 42.40, 39.77, 37.84, 37.78, 37.69, 37.56, 37.40, 33.20, 33.16, 28.39, 25.21, 24.88, 23.54, 23.13, 23.04, 20.16, 20.10 ppm; IR (neat): $\tilde{\nu} = 3348$, 1640 cm^{-1}; EI-MS: *m/z* (%): 255.3 (55) [$M-C_3H_5$]; elemental analysis calcd (%) for $C_{20}H_{40}O$ (296.54): C 81.01, H 13.60; found: C 80.78, H 13.37. Determination of diastereomeric excess $de = 97$%: HPLC on Chiralpak AD-H column (heptane/propan-2-ol 99.8:0.2), UV 254 nm; 0.5 mL min^{-1}; major diastereoisomer $t_R = 18.7$ min, minor diastereoisomer $t_R = 23.2$ min.

Preparation of 20: A solution of the alcohol **16** (490 mg, 1.65 mmol), 4-methoxy-2,3,5-trimethylphenol (**14**, 399 mg, 2.4 mmol), and triphenylphosphine (629 mg, 2.4 mmol) in toluene (5 mL) was cooled in an ice bath. Diisopropyl azodicarboxylate (525 mg, 2.6 mmol) in toluene (2.5 mL) was added dropwise over 15 min and the mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated and the residual viscous oil was partitioned between hexane and aqueous methanol (85%, *v/v*). The aq. methanol phase was reextracted with hexane and the combined hexane phases were dried ($MgSO_4$) and concentrated in vacuo. The residual oil was purified by chromatography on SiO_2 (hexane/EtOAc 25:1) to yield **20** (438 mg, 60%) as a colorless, viscous oil. $[\alpha]_D^{20} = +3.9$ ($c = 1.08$ in $CHCl_3$); ^{1}H NMR (400 MHz,$CDCl_3$): $\delta = 6.52$ (s, 1H), 5.86 (ddt, $J = 17$, 10, 7 Hz, 1H), 5.04–5.14 (m, 2H), 4.18 (q, $J = 6$ Hz, 1H), 3.66 (s, 3H), 2.34–2.46 (m, 2H), 2.26 (s, 3H), 2.20 (s, 3H), 2.12 (s, 3H), 1.0–1.65 (m, 21H), 0.84–0.88 ppm (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 152.43$, 150.87, 135.07, 130.98, 128.03, 125.50, 117.49, 113.75, 78.37, 60.52, 39.79, 38.71, 37.86, 37.75, 37.71, 37.47, 34.44, 33.21, 33.12, 28.40, 25.22, 24.88, 23.23, 23.14, 23.05, 20.17, 20.11, 16.76, 13.15, 12.65 ppm; IR (neat): $\tilde{\nu} =$ 1641, 1479, 1229, 1090 cm^{-1}; EI-MS: *m/z* (%): 444.4 (10) [*M*], 166.1 (100); elemental analysis calcd (%) for $C_{30}H_{52}O_2$ (444.74): C 81.02, H 11.78; found: C 80.86, H 11.65.

Preparation of 22: A solution of 4-methylmorpholine *N*-oxide monohydrate (270 mg, 2.0 mmol) in water (4 mL) and an aqueous solution of osmium tetroxide (4%, 480 μL, 75 μmol) were added to a solution of the olefin **20** (427 mg, 0.96 mmol) in acetone (16 mL). The reaction mixture was stirred at room temperature for 20 h. It was partitioned between diethyl ether and water, and the organic phase was washed with sat. aq. Na_2SO_3 and brine, dried, and concentrated. The residual oil was purified by chromatography on SiO_2 (hexane/EtOAc 5:4) to afford **7** (382 mg, 83%) as a colorless, viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 6.60$ (s, 1H), 4.39–4.50 (m, 1H), 3.96–4.04 (m, 1H), 3.64–3.72 (m, 1H), 3.66 (s, 1.5H), 3.65 (s, 1.5H), 3.46–3.55 (m, 1H), 3.14 (d, $J = 2$ Hz, 0.5H), 2.59 (d, $J = 4$ Hz, 0.5H), 2.25 (s, 3H), 2.19 (s, 3H), 2.10 (s, 3H), 1.0–2.05 (m, 24H), 0.80–0.88 ppm (m, 12H); IR (neat): $\tilde{\nu} = 3387$, 1479, 1229, 1090 cm^{-1}; EI-MS: *m/z* (%): 478.4 (4) [*M*], 166.1 (100); elemental analysis calcd (%) for $C_{30}H_{54}O_4$ (478.75): C 75.26, H 11.37; found: C 75.01, H 11.23.

Preparation of 21: Sodium periodate (300 mg, 1.3 mmol) was added to a solution of the diol **20** (123.6 mg, 0.26 mmol) in acetone/water (4:1, 5 mL). The heterogeneous mixture was stirred at room temperature for 2 h. It was partitioned between TBME and water, and the organic phase was washed with sat. brine, dried, and concentrated in vacuo to afford the aldehyde **21** (109.5 mg, 95%) as a colorless oil. $[\alpha]_D^{20} = -11.1$ ($c = 1$, in hexane); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 9.82$ (t, $J = 2$ Hz, 1H), 6.56 (s, 1H), 4.66 (q, $J =$ 6 Hz, 1H), 3.65 (s, 3H), 2.78 (ddd, $J = 16$, 6, 3 Hz, 1H), 2.69 (ddd, $J =$ 16, 6, 3 Hz, 1H), 2.25 (s, 3H), 2.18 (s, 3H), 2.08 (s, 3H), 1.0–1.8 (m, 21H), 0.82–0.88 ppm (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta =$ 201.60, 151.61, 151.44, 131.29, 128.37, 125.70, 114.02, 74.38, 60.51, 48.44, 39.77, 37.84, 37.73, 37.69, 37.28, 35.18, 33.20, 33.10, 28.39, 25.21, 24.87, 23.14, 23.04, 20.15, 20.03, 16.73, 13.15, 12.65; IR (neat): $\tilde{\nu} = 1723$, 1476, 1455, 1227, 1088 cm^{-1}; EI-MS: *m/z* (%): 446.4 (21) [*M*], 166.1 (100); elemental analysis calcd (%) for $C_{29}H_{50}O_3$ (446.71): C 77.97, H 11.28; found: C 78.17, H 11.21.

Preparation of 23: A solution of the aldehyde **21** (109.5 mg, 0.26 mmol) in dichloromethane (5 mL) was cooled to 0 °C. Trifluoroacetic acid (50 μL) was added and the solution was stirred at 0 °C for 25 min. Palladium on carbon (10%, 40 mg) was then added, the argon was replaced with hydrogen (1 atm), and the chromane was hydrogenated at room temperature for 1 h. The filtered reaction mixture was concentrated in vacuo. The residual oil was purified by chromatography on SiO_2 (hexane/EtOAc 80:1) to afford **23** (96.1 mg, 91%) as a white solid; m.p. 39–41 °C; $[\alpha]_D^{20} =$ +49.0 ($c = 1$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 3.86$ (m, 1H), 3.64 (s, 3H), 2.63 (m, 2H), 2.20 (s, 3H), 2.14 (s, 3H), 2.12 (s, 3H), 2.00 (m, 1H), 1.0–1.75 (m, 22H), 0.84–0.89 ppm (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 150.08$, 149.63, 128.13, 126.47, 123.10, 119.00, 75.31, 60.80, 39.79, 37.88, 37.77, 37.72, 37.32, 36.16, 33.22, 33.19, 28.40, 28.31, 25.23, 24.89, 23.66, 23.35, 23.15, 23.05, 20.17, 20.16, 12.92, 12.13, 12.08 ppm; IR (neat): $\tilde{\nu} = 1456$, 1251, 1091 cm^{-1}; EI-MS: *m/z* (%): 430.4 (100) [*M*]; elemental analysis calcd (%) for $C_{29}H_{50}O_2$ (430.71): C 80.87, H 11.70; found: C 80.77, H 11.59.

Preparation of 12: A solution of the chromane **23** (94.6 mg, 0.22 mmol) in dichloromethane (3 mL) was cooled to 0 °C. Boron trifluoride methyl sulfide complex (0.5 mL), anhydrous aluminum chloride (360 mg), and acetonitrile (2 mL) were added, and the mixture was stirred at room temperature for 4 h. The mixture was partitioned between ice-cold aqueous $NaHCO_3$ and TBME, and the organic phase was washed with sat. brine, dried, and concentrated on a rotary evaporator. The crude product was purified by chromatography on SiO_2 (hexane/EtOAc 30:1 to 12:1) to afford nor-α-tocopherol (**12**, 73.8 mg, 81%) as a colorless, wax-like solid. $[\alpha]_D^{20} = +42.5$ ($c = 1$ in $CHCl_3$); ^{1}H NMR (400 MHz,$CDCl_3$): $\delta = 4.19$ (s, 1H), 3.82 (m, 1H), 2.65 (m, 2H), 2.16 (s, 3H), 2.13 (s, 3H), 2.10 (s, 3H), 2.00 (m, 1H), 1.0–1.75 (m, 19H), 0.83–0.88 ppm (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 147.44$, 145.25, 122.75, 121.26, 119.13, 118.73, 75.21, 39.79, 37.88, 37.77, 37.72, 37.32, 36.09, 33.22, 33.19, 28.47, 28.40, 25.23, 24.89, 23.70, 23.39, 23.15, 23.05, 20.18, 20.16, 12.56, 12.15, 11.67 ppm; IR (neat): $\tilde{\nu} = 3350$, 1460, 1254, 1084 cm^{-1}; EI-MS: *m/z* (%): 416.4 (100) [*M*]; ESI-MS (MeOH, neg): *m/z* (%): 415 [*M*−H], 831 [2*M*−H]; elemental analysis calcd (%) for $C_{28}H_{48}O_2$ (416.69): C 80.71, H 11.61; found: C 80.77, H 11.53. Determination of diastereomeric excess *de* = 94%: HPLC on Chiralpak AD-H column (heptane/propan-2-ol 99:1), UV 295 nm; 0.8 mL min^{-1}; major diastereomer $t_R = 12.1$ min, minor diastereomer $t_R = 12.9$ min.

Preparation of 27: The alcohol **27** was prepared from phytol **25**;[19,20] a different synthesis of racemic **27** has been reported previously.[26] Characteristic data for **27**: $[\alpha]_D^{20} = +1.1$ ($c = 1.05$ in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 3.64$ (t, $J = 6.40$ Hz, 2H), 1.05–1.56 (m, 21H), 0.85 ppm (m, 12H); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 63.32$, 39.56, 37.62, 37.54, 37.47, 37.00, 33.35, 32.98, 32.96, 28.17, 24.98, 24.66, 23.41, 22.91, 22.81, 19.94, 19.86 ppm; ESI-MS: *m/z* (%): 279.2 $[M+Na]^+$; elemental analysis calcd (%) for $C_{17}H_{36}O$: C 79.61, H 14.15; found: C 79.57, H 13.98.

Preparation of 28: A solution of Dess–Martin periodinane (2.34 g, 5.4 mmol) in dichloromethane (5 mL) was added under Ar to a solution of the alcohol **27** (1 g, 3.90 mmol) in dichloromethane (12 mL). The reaction mixture was stirred at RT for 2 h and quenched with a solution of $Na_2S_2O_3$ (7.2 g) in saturated aq. NH_4Cl (32 mL). The mixture was extracted twice with ether. The extract was washed with brine, dried over Na_2SO_4, and concentrated under vacuum to afford the crude aldehyde as colorless oil used immediately for the next step.

The freshly prepared aldehyde (382 mg, 1.50 mmol) in dimethyl sulfoxide (1 mL was added) under Ar to a solution of $Ph_3P=CHCOOMe$ (1.0 g, 3 mmol) in dimethyl sulfoxide (4 mL). The reaction mixture was stirred overnight, quenched with methanol/water (85:15, *v/v*), and extracted with hexane. The organic phases were washed with brine and dried over Na_2SO_4. After concentration, a residue was purified by flash chromatography on SiO_2 with dichloromethane/hexane (1:1, *v/v*) to afford the ester **28** (300 mg, yield 63%) as a colorless oil. $[\alpha]_D^{20} = -1.9$ ($c = 1.22$ in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 6.97$ (dt, $J = 15.60$, 7.00 Hz, 1H), 5.82 (dt, $J = 15.60$, 1.40 Hz, 1H), 3.72 (s, 3H), 2.19 (m, 2H), 1.04–1.52 (m, 19H), 0.85 ppm (m, 12H); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 167.40$, 150.04, 120.99, 51.55, 39.55, 37.59, 37.47, 36.69, 32.97, 32.82, 32.74, 28.17, 25.75, 24.98, 24.62, 22.90, 22.81, 19.92, 19.81 ppm; ESI-MS: *m/z* (%): 333.3 $[M+Na]^+$, 643.3 $[2M+Na]^+$; elemental analysis calcd (%) for $C_{20}H_{38}O_2$: C 77.36, H 12.33; found: C 77.57, H 12.09.

Preparation of 29: A solution of DIBAL-H in hexane (1 M, 2 mL) was added at 0 °C under Ar to a solution of the ester **28** (300 mg, 0.96 mmol) in dry ether (4 mL). The reaction mixture was allowed to warm to RT and stirred for another 2 h. The reaction was quenched with $NaSO_4 \cdot 10H_2O$ and the mixture was stirred at RT for 1 h. The solid was filtered and washed twice with ether. The combined organic solvents were concentrated under vacuum to afford the allyl alcohol **29** (230 mg, yield 83%) as a colorless oil. $[\alpha]_D^{20} = -0.8$ ($c = 1.10$ in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 5.66$ (m, 2H), 4.08 (t, $J = 5.2$ Hz, 2H), 2.03 (m, 2H), 1.50 (m, 1H), 1.02–1.45 (m, 19H), 0.85 ppm (m, 12H); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 133.85$, 128.99, 64.07, 39.56, 37.62, 37.54, 37.48, 36.76, 32.98, 32.87, 32.74, 28.17, 26.82, 24.98, 24.65, 22.91, 22.81, 19.94, 19.89 ppm; ESI-MS: *m/z* (%): 305.3 $[M+Na]^+$; elemental analysis calcd (%) for $C_{20}H_{38}O_2$: C 80.78, H 13.56; found: C 80.90, H 13.30.

Preparation of 30: Activated MnO_2 (869 mg, 10 mmol) was added at RT to a solution of the allylic alcohol **29** (225 mg, 0.80 mmol) in dichloromethane (5 mL) and the reaction mixture was stirred for 4 h. The solid was then filtered through a celite pad and washed three times with dichloromethane. The combined organic layers were concentrated under vacuum to afford the unsaturated aldehyde **30** (220 mg, yield 98%) as a colorless oil. $[\alpha]_D^{20} = -2.2$ ($c = 1.10$ in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 9.50$ (d, $J = 8.00$ Hz, 1H), 6.85 (dt, $J = 15.60$, 6.80 Hz, 1H), 6.11 (ddt, $J = 15.60$, 8.00, 1.40 Hz, 1H), 2.32 (m, 2H), 1.02–1.49 (m, 19H), 0.85 ppm (m, 12H); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 194.32$, 159.21, 133.17, 39.54, 37.56, 37.45, 37.44, 36.67, 33.24, 32.96, 32.80, 28.15, 25.58, 24.97, 24.60, 22.89, 22.80, 19.92, 19.78 ppm; ESI-MS: *m/z* (%): 303.3 $[M+Na]^+$.

Preparation of 24: A freshly prepared solution of $Ti(OEt)_4$ in toluene (0.9 mL) was added to a solution of the hydroquinone **14** (83.1 mg, 0.5 mmol) in toluene (1 mL). The reaction mixture was heated at reflux for 30 min. The aldehyde **30** (225 mg, 0.8 mmol) was then added to the mixture, which was heated at reflux for another 3.5 h. The reaction mixture was quenched with sat. aq. NH_4Cl and extracted with ether. The combined organic layers were washed with brine and dried over Na_2SO_4. After concentration under vacuum the residue was purified by flash chromatography on SiO_2 with hexane/ethyl acetate (49:1, *v/v*) as a eluent to afford the chromene **31** (150 mg, yield 72%) as a yellowish oil. The air- and light-sensitive **31** was immediately hydrogenated and deprotected as described for the transformation of **23** to **12**. Finally, (2*RS*,4′*R*,8′*R*)-nor-α-tocopherol (**24**, 117 mg, 80%) was obtained as a colorless oil, spectroscopically identical with **12**.

Preparation of 32: (2*RS*,4′*R*,8′*R*)-nor-α-Tocopherol (**24**, 25.1 mg, 0.06 mmol) was dissolved in pyridine (0.5 mL), and acetic anhydride (0.1 mL,109 mg, 1.06 mmol) was added by syringe. The mixture was stirred for 3 h at RT and was then poured into HCl (10%, 5 mL) and extracted three times with dichloromethane (30 mL). The combined organic phases were washed twice with water (20 mL) and finally dried over Na_2SO_4. The solution was concentrated under vacuum and the pure product **32** was obtained as slightly yellow oil (26.9 mg, 0.59 mmol, 97%). ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 3.85$ (m, 1H), 2.65–2.62 (m, 2H), 2.33 (s, 3H), 2.11 (s, 3H), 2.02 (s, 3H), 1.96 (s, 3H), 1.8–1.6 (m, 2H), 1.6–1.05 (m, 21H), 0.9–0.8 ppm (m, 12H).

Cell culture experiments: RAW264.7 cells were cultured in Dulbecco's modified Eagle's medium supplemented with fetal bovine serum (10%), together with penicillin (100 U mL^{-1}) and streptomycin (100 μg mL^{-1}, all reagents from PAA). Cells were grown in a humidified atmosphere at 37 °C and 5% CO_2. For induction of cytokine production, cells were stimulated with LPS from *Salmonella enteriditis* (Sigma, 100 ng mL^{-1}). For measurement of iNOS levels, cells were stimulated with IFNγ (Invitrogen, 5 ng mL^{-1}) and LPS (100 ng mL^{-1}). Total RNA was isolated by chloroform/water separation and isopropyl alcohol precipitation. RNA concentration was

measured by reading the absorbance at 260 nm on a spectrophotometer (DU800, Beckmann Coulter) and RNA quality was checked by calculating the 260/280 nm ratio. Primer sequences for real-time RT-PCR experiments were designed with primer3 software and were obtained from MWG/Eurofins. One-step quantitative reverse transcriptase PCR was carried out with the QuantiTec®SYBR®Green RT-PCR kit (Qiagen) on a Rotorgene cycler 6000 (Corbett Life Science). Relative mRNA levels of genes were quantified as the ratios between expression level of the target and housekeeping gene (GAPDH). Cytokine concentrations in cell culture supernatants were measured with the aid of commercial ELISA kits (R&D Systems). Protein levels of iNOS were determined by Western Blotting in whole cell extracts. Protein (60 μg) was loaded on a SDS-PAGE gel (10%) and separated by electrophoresis. Protein bands were transferred to a nitrocellulose membrane (BioRad). The membrane was incubated with respective antibodies for iNOS (Stressgen) and actin (Santa Cruz Biotechnology). Subsequent to incubation with the secondary antibody (BioRad) protein bands were visualized with the aid of a Pierce® ECL Western Blotting Substrate Kit (Thermo Scientific). Relative intensities of the bands were quantified by densitometry and expressed as the ratios between target protein (iNOS) and loading control (actin).

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