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A Biomimetic Chromanol Cyclization Leading to α-Tocopherol**

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The diastereoselective formation of the chromanol ring is one of the most challenging reactions during the biosynthesis of tocopherols in photosynthetic organisms. We discovered the enzyme that catalyzes this transformation in the cyanobacteria *Anabaena variabilis*[1] and subsequently determined its substrate specificity[2] and recently cloned the tocopherol cyclase from the related *Anabaena* sp. into *E. coli*.[3] The enzymatic reaction mechanism was determined by isotopic labeling experiments,[4] which revealed that the reaction proceeds by *si* protonation of the double bond of **1** followed by *re* attack of the phenolic oxygen to yield γ-tocopherol (**2**; Scheme 1).

Two further observations suggest a concerted nonsynchronous cyclization, during which positive charge develops at the carbon atom that is trapped by the phenol (see **3**, Scheme 2): 1) the tetrahydroisoquinolinium terpenoid **4** as a transition-state analogue is an excellent inhibitor (IC_{50} = 1.4 nM) of tocopherol cyclase,[5] and 2) the epoxide of **1** cyclizes under acidic conditions yielding two compounds, the five-membered-ring "Baldwin" product and the six-membered ring analogue of the enzymatic product (4:6 ratio).[3,4]

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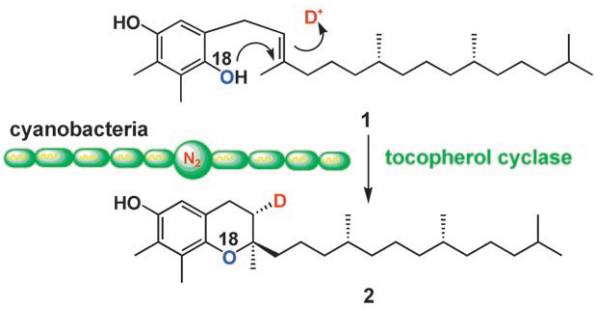
Supporting information for this article, including spectroscopic data of **5**, **6**, **13**, **18**, and **22**-*p*TsOH, is available on the WWW under http://www.angewandte.org or from the author.



Scheme 1. Chromanol ring closure catalyzed by tocopherol cyclase.

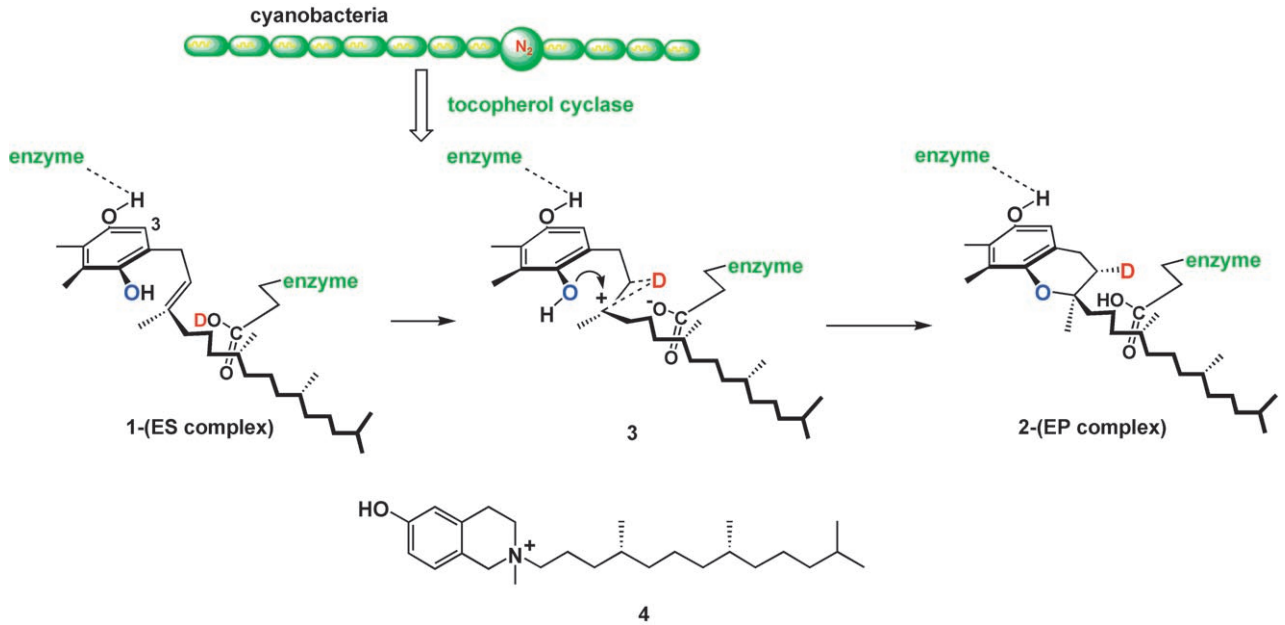
The structure of the enzyme–substrate (ES) complex of **1** is tentatively drawn in a conformation that positions the double bond in between the phenolic oxygen atom and an acid group from the protein.[3] It appears that the enzyme enforces this activated conformation in the enzyme–substrate complex; computer modeling studies show that **1**, in solution or in the gas phase, adopts a conformation in which the phytyl side chain is bent down such that the double bond is 6–7 Å apart from the phenol.

Herein, we report on a new biomimetic approach that leads to enantiomerically enriched α-tocopherol. (For earlier syntheses of tocopherols[6,7] and for two recent reports regarding access to the chromanol unit of tocopherols, see references [8,9] and references cited therein.) Our concept comprises 1) the modification of structure **1** by attaching a chiral acid at C3, b) restriction of the conformational freedom of this acid through derivatization of the phenolic OH group at C4 using a bulky substitutent, 3) the choice of a suitable chiral acid, and 4) removal/recovery of the chiral unit after cyclization and determination of the product on an α-tocopherol derivative.

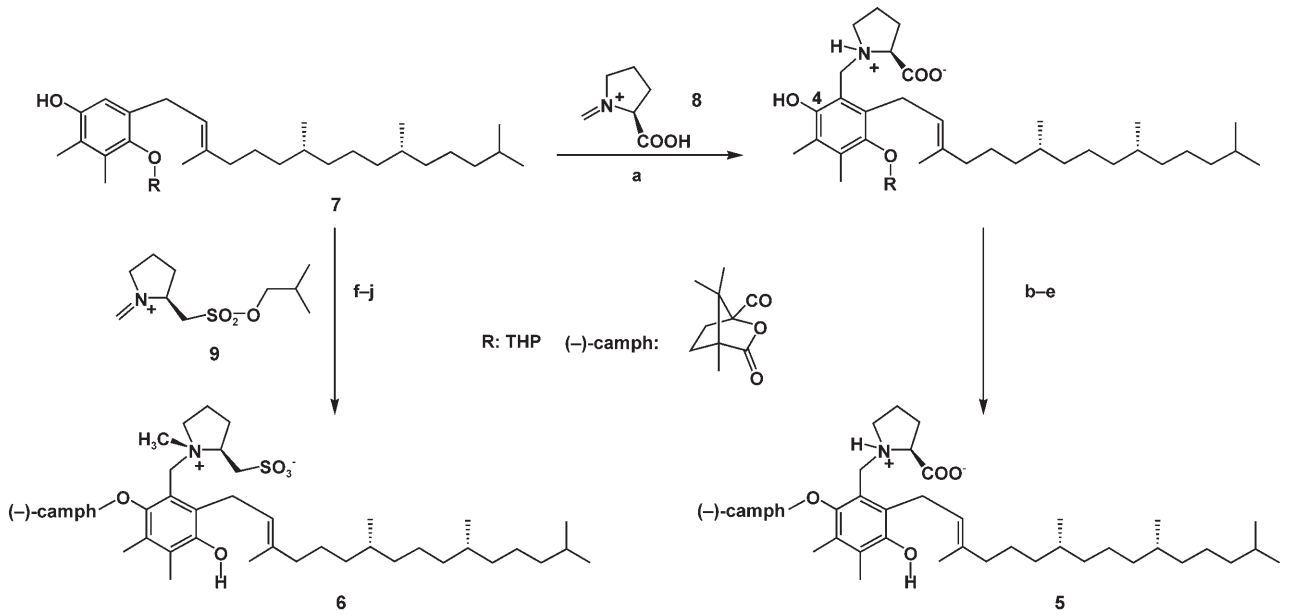
First, the target prolinyl phytylhydroquinone derivatives **5** and **6** were prepared from the monoprotected hydroquinone **7** by addition of the Mannich reagents **8** or **9** and followed by protecting group manipulations (Scheme 3). Exploratory experiments with **5** and **6** in the presence of either Lewis or Brønsted acids gave cyclized **10** and **11**, respectively, which were subsequently converted into the α-tocopheryl camphanate **12** (Scheme 4). The diastereomeric excess (*de*) was determined by HPLC, and a maximum value of 35–40% *de* was obtained in favor of the *S* configuration at C2. As the acidity of the chiral unit had no profound effect on the reaction rate, yield, and *de* value, it was concluded that systems **5** and **6** were too flexible and the distances between the acids and the double bonds were too large.

Accordingly, we thought a more-rigid acidic spacer would be advantageous to accomplish higher diasteromeric excesses and hence we prepared compound **13** (Scheme 5). Cyclization of **13** in the presence of *p*TsOH indeed confirmed the idea, as **12** was obtained with a *de* value of 80% and (2*S*)-**14** was obtained as the dominant diastereoisomer (80% overall yield). In contrast to **13**, the monoesters **15** and **16** (Figure 1) were less efficient, yielding a maximum diastereomeric excess of 47%, which indicates cooperativity of the two aspartate COOH groups in the presence of *p*TsOH. When the aspartate residue of **13** was replaced by serine or threonine to give **17** and **18**, respectively, the Lewis acid $SnCl_4$ was the additive of choice rather than *p*TsOH. For example, with (−)-(*S*,*S*)-**18** and $SnCl_4$ a *de* value of 77% for the final product **12** (80% overall yield) was observed; that is, the same value was observed, within experimental error, as that for the cyclization of **13** with *p*TsOH.

The proposed mechanism of this reaction (Figure 2) resembles the formalism described by Yamamoto and coworkers[10,11] for a binol/$SnCl_4$ system and apparently belongs to the category of "Lewis acid assisted, Brønsted acid



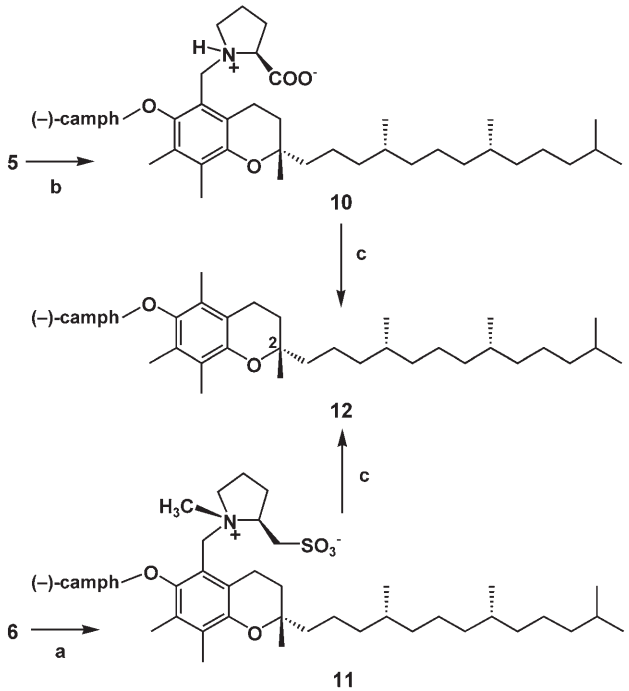
Scheme 2. The reaction mechanism of ring closure catalyzed by tocopherol cyclase. ES = enzyme–substrate; EP = enzyme–product.



Scheme 3. Synthesis of cyclization precursors **5** and **6**. Reagents and conditions: a) 1) (*S*)-proline, HCHO (35% in water), 40°C, 10 min; 2) **8** (in MeOH), **7**, 40°C, 8 h, 87%; b) $(Me)_3SiCHN_2$, MeOH, room temperature, 30 min, 98%; c) (−)-camphanoyl chloride, DMAP, CH_2Cl_2, room temperature, 2 h, 96%; d) LiI, EtOAc, 70°C, under N_2, 13 h, 70%; e) 1 N HCl, THF, room temperature, 1 h, 95%; f) **9** added to **7** in MeOH, 50°C, 2 days, 80%; g) (−)-camphanoyl chloride, DMAP, CH_2Cl_2, room temperature, 1 h, 98%; h) MeOTf, DMAP, room temperature, 4 h, 83%; i) LiI, EtOAc, 40°C, 16 h, 65%; j) 1 N HCl, THF, room temperature, 1 h, 89%. THP = tetrahydropyranyl; DMAP = (4-dimethyl)aminopyridine; Tf = trifluoromethanesulfonyl.

supported reactions" (see compound **19**; binol = 1,1′-bi-2-naphthol).

When the *S*-configured amino acids and the (−)-camphanate in **13** were replaced by their enantiomers to give

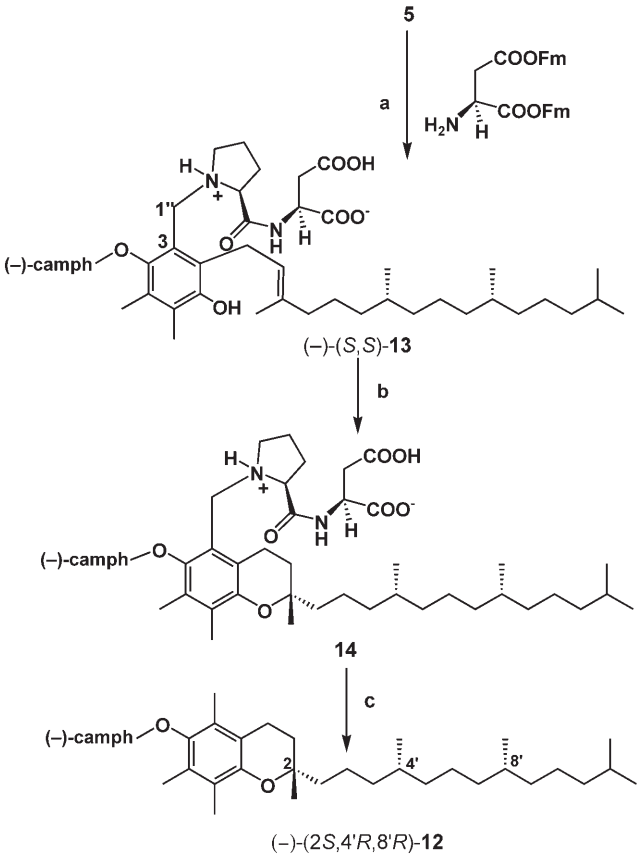


Scheme 4. Chromanol cyclization of **5** and **6**. Reagents and conditions: a) *p*TsOH (2.5 equiv), propylene carbonate, 50°C, 17 h, 70%, 38% *de*; b) $AlCl_3$ (0.7 equiv), CH_2Cl_2, room temperature, 2 days, 65%, 35% *de*; c) Pd/C (15–20%), HCOOH, MeOH, H_2 (85 bar, room temperature) 2 days, 92%. *p*TsOH = *p*-toluenesulfonic acid.

(+)-(*R*,*R*)-**20**, cyclization of the latter occurred in the presence of *p*TsOH to yield the natural (2*R*,4′*R*,8′*R*)-α-tocopherol (**21**) as the dominant diastereoisomer (70% *de*) of the reaction (Scheme 6).[12]

The superior results with the proline–aspartate phytylhydroquinone **13** relative to the monoesters **15** and **16** suggest cooperativity of the two "free" COOH units. Furthermore, as more than one equivalent of *p*TsOH is required for cyclization a Brønsted acid assisted, Brønsted acid supported reaction may be operative.[10] The structure of the reactive supramolecular assembly could be tentatively assigned from ^{1}H NMR spectroscopy of the precursor (−)-(*R*,*R*)-**22** in CH_2Cl_2 at 5°C. In the absence of *p*TsOH a slightly resolved NMR spectrum was obtained that showed the presence of two conformations in 6:4 ratio. After addition of two equivalents of *p*TsOH the system converged to one conformation that displayed a well-resolved signal pattern. From these spectra a significant NOE between a benzylic proton (at C7, in green) and the proton at the stereogenic center of the proline moiety (at C2′, in green) was observed which indicated that the proline--aspartate unit of **22** lies below the double bond (Scheme 7), ready to add the proton from the *si* face in an enzyme-like fashion.

Interestingly a further significant NOE was detected between the *meta*-positioned hydrogen atom of *p*TsOH (in red) and the allylic methyl group of the phytyl side chain. These results suggest that the second *p*TsOH molecule forms the fairly stable complex **22**-*p*TsOH through hydrogen bonding of the two S=O bonds to aspartate COOH groups to enhance the acidity of *p*TsOH. Accordingly, the proton required to initiate cyclization appears to be delivered from the second *p*TsOH moiety.



Scheme 5. Synthesis of the proline–aspartate phytylhydroquinone **13**, and its cyclization to the chromanol. Reagents and conditions: a) 1) HATU (1.4 equiv), DIPEA (6 equiv), di-Fm-aspartate (3 equiv), CH_2Cl_2, room temperature, 2 h; 2) 20% Et_2NH in CH_2Cl_2, 1 h, room temperature; iii) $KHSO_4$, 59%; b) *p*TsOH (2 equiv), CH_2Cl_2, room temperature, 2 days, 85%; c) Pd/C (15–20%), HCOOH, MeOH, H_2 (85 bar, room temperature) 2 days, 92%. HATU = *N*,*N*,*N*,*N*-tetramethyluronium hexafluorophosphate; DIPEA = diisopropylethylamine; Fm = 9-fluorenylmethyl.

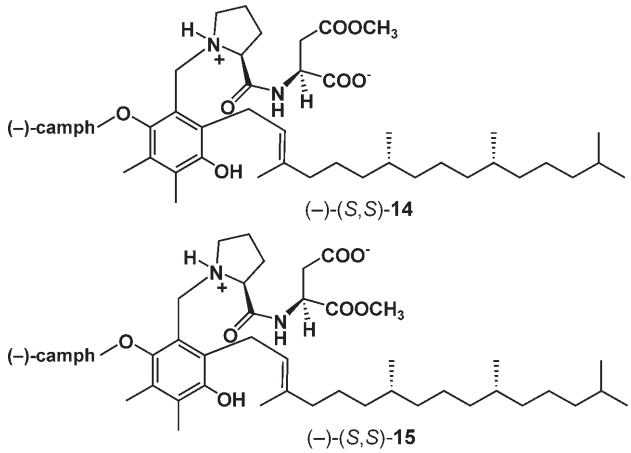


Figure 1. Aspartate monoesters for cyclization.

In conclusion, through attachment of a proline–aspartate unit to a phytylhydroquinone an enzyme-like conformation of the cyclization precursor is created that allows for diastereoface-selective preferential protonation of the double bond and concomitant attack of the phenolic oxygen atom. Similar results are obtained with a proline–threonine moiety in the presence of $SnCl_4$. To the bets of our knowledge, this is the first biomimetic cyclization that leads to the chromane structure of tocopherols with high diastereoselectivity.

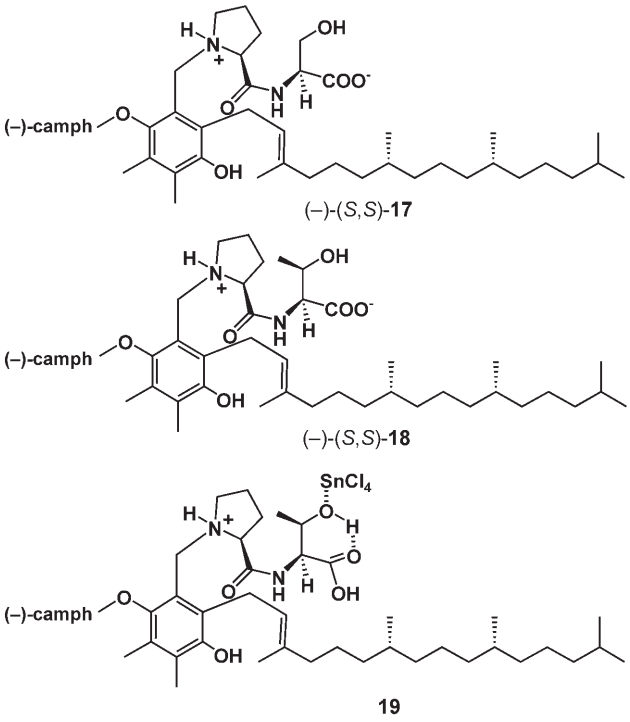
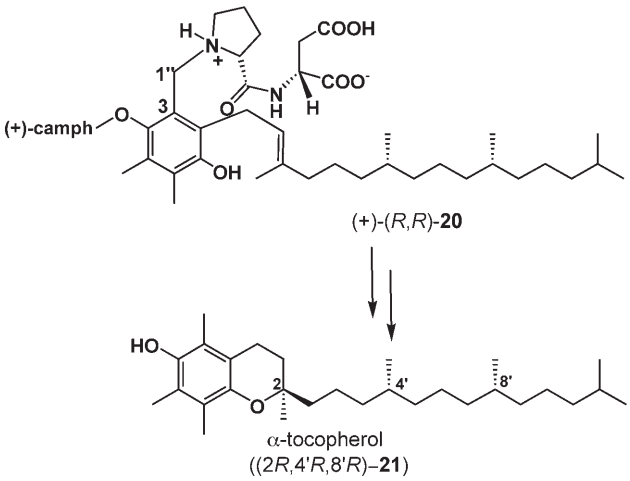


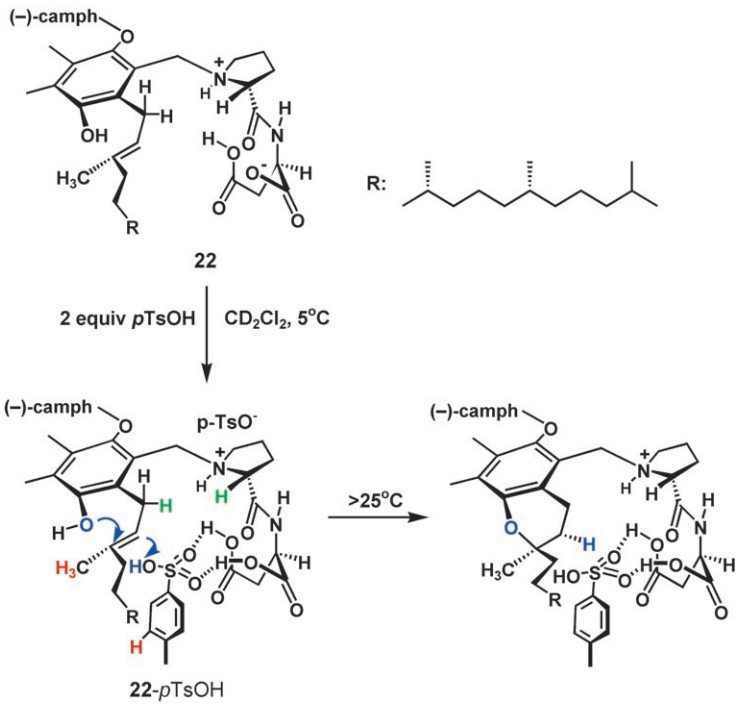
Figure 2. Proline–serine and proline–threonine cyclization precursors **16** and **17**, respectively.



Scheme 6. Cyclization of (+)-(*R*,*R*)-**20** to give natural α-tocopherol.

Experimental Section

General procedure for the acid-supported cyclization of phytylhydroquinones: Anhydrous *p*-toluenesulfonic acid (0.0715 mmol, 2 equiv; 1.23 mL of a 0.058 M solution (10 mg mL^{-1}) in CH_3CN) was added to a round-bottomed flask containing **13** (30.0 mg, 0.0358 mmol) in CH_2Cl_2 (40 mL) at room temperature under argon,



Scheme 7. Proposed mechanism for the diastereoselective protonation of a phytylhydroquinone. Colored protons show significant NOE interactions (see text for details).

and the solution was stirred for 48 h at this temperature. Saturated $NaHCO_3$ solution (10 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 × 10 mL). The organic layers were combined, dried (Na_2SO_4), and filtered. Evaporation of the solvents provided the crude product, which was taken up in CH_2Cl_2/MeOH (4:1) and filtered through celite to give **14** as a colorless solid (28 mg, 93 %). This material was used directly in the reduction step.

General procedure for reductive debenzylation: Formic acid (99 %; 15 μL) and palladium hydroxide (6 mg) on activated charcoal (15–20 % Pd, moistened with water ≈ 50 %) were added to a solution of **14** (3.0 mg, 3.58 μmol) in a mixture of MeOH (2.5 mL) and CH_2Cl_2 (0.1 mL). The reaction was stirred at room temperature under a hydrogen pressure of 85 bar for 2 days. The reaction mixture was filtered through a cotton plug, quenched with a saturated solution of $NaHCO_3$, and extracted with CH_2Cl_2 (2 × 10 mL). The combined organic layers were dried (Na_2SO_4) and filtered, and the solvents were removed under vacuum. The resulting crude product was filtered, first through celite and then through silica gel (CH_2Cl_2/MeOH 95:5) to give **12** (2.3 mg, 92 %). The diastereomeric excess (*de*) was determined by HPLC (DAICEL Chiralpac AD-H, 1 % 2-propanol in heptane; 0.5 mL min^{-1}); retention time: 28.9 min for (–)-(*S*,*R*,*R*)-**12** and 30.9 min for (–)-(*R*,*R*,*R*)-**12**, ratio 9:1). Correlations were made by admixing the camphanate of authentic all-(*R*)-α-tocopherol.

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Keywords: biomimetic synthesis · cyclization · diastereoselectivity · enzyme catalysis · peptides

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[12] The diastereo-face-selectivity depends on the configurations of the dipeptide, whereas the influence of the camphanoyl unit is negligible. The use of (–)-camphanate is purely for convenience, as tocopherols that are epimeric at C2 can be separated by HPLC and hence the *de* of the cyclization can be easily determined (C. Grütter, E. Alonso, A. Chougnet, W.-D. Woggon, unpublished results).

