Enzymatic Cyclization of Hydroxylated Surrogate Squalenoids with Bakers' Yeast†

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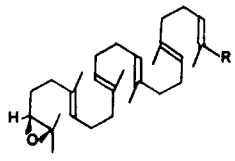
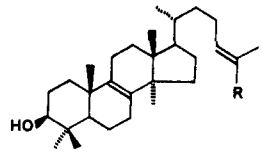
Abstract: An approach to the asymmetric synthesis of remote-functionalized sterols based on the enantioselective enzymatic cyclization of the C-1 hydroxylated surrogate substrate **3a** with bakers' yeast is described. Thus, incubation of the functionalized squalene analogue (-)-**3a** with bakers' yeast in phosphate buffer leads to the stereospecific formation of the C-28 hydroxylated sterol (+)-**4a** in 40.5% yield. The enzymatic cyclization of the isomeric surrogate substrate **3b** does not afford the sterol **4b** but gives exclusively the partially cyclized product **4c** in 53% yield. The dramatically different cyclization paths found for **3a** and **3b** suggest that the key interactions that determine the fate of a structurally modified substrate are those between the enzyme and the β-face of the chair-boat-chair folded substrate conformation.

We recently demonstrated the use of bakers' yeast as an effective reagent for the preparative-scale enantioselective enzymatic cyclization of (±)-squalene oxide (**1a**) to (+)-lanosterol (**2a**) and further demonstrated that this method could be applied to the asymmetric synthesis of the naturally occurring lanostane sterol (+)-ganoderic acid Z via its ester **2b**.[1] The successful cyclization





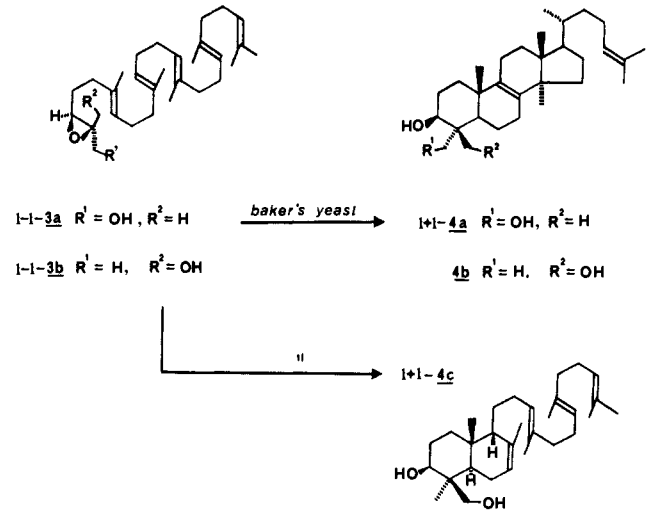
(±)-**1a** R = CH_3
(±)-**1b** R = CO_2CH_3

(+)-**2a** R = CH_3
(+)-**2b** R = CO_2CH_3

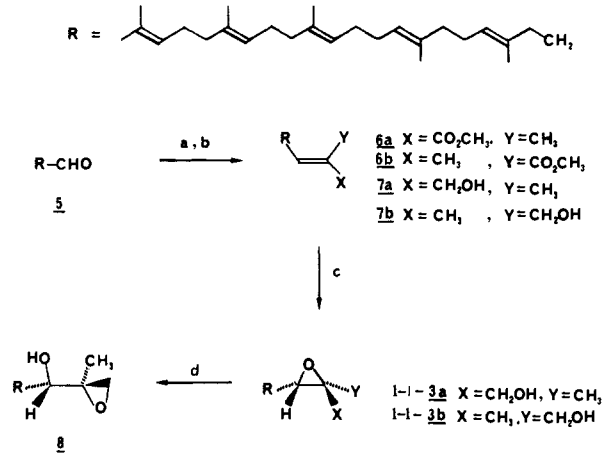
of the functionalized substrate **1b** led us to consider the application of this method to the cyclization of other types of heteroatom modified substrates, namely hydroxylated substrates. Such an approach would be potentially useful for the synthesis of remote-functionalized sterols, such as **4a** or **4b** (cf. Scheme I), that would be difficult to prepare by conventional synthetic methods.[2] However, the enzymatic tolerance of a nucleophilic heterogroup, with respect to both substrate recognition and cyclization, was uncertain, particularly if the group is positioned near the "ring-forming sites" of the substrate backbone. Consequently, we decided to investigate the enzymatic cyclization of the surrogate substrates **3a** and **3b** in order to determine whether the rigidly enzyme controlled cyclization process would allow these surrogates to be converted to the sterols **4a** and **4b**, respectively, despite the juxtaposition of the internal nucleophilic hydroxy group at C-1.[3,4] Also, in keeping with the proposed chair-boat-chair conformation required for cyclization of the natural substrate,[5] surrogates **3a** and **3b** would serve as unique probes for assessing the heteroatom's effect on opposing topological regions in the active site. Furthermore, a study of these substrates would finally clarify the long-standing concerns about the effects of substrate lipophilicity on enzymatic recognition.

We now report our findings for the unprecedented *enzymatic cyclization* of the *hydroxylated substrates* **3a** and **3b**, where the cyclization of **3a** proceeds to the expected sterol **4a** without nucleophilic intervention of the heterogroup. The enantioselectivity of the enzyme is also complemented by the stereospecific formation of **4a** as the sole sterol product. An entirely different cyclization pathway was found for substrate **3b**. The enzyme does induce an efficient cyclization of the substrate, but only as far as the bicyclic structure **4c**. Hence, although the enzyme easily tolerates heteroatom modifications, steric and/or electronic effects operating at the β-face[6] of the conformationally folded substrate dramatically

Scheme I



Scheme II[a]



[a] (a) for **6a**, $Ph_3P{=}C(CH_3)CO_2CH_3$, benzene; for **6b**, $(CF_3CH_2O)_2P(=O)CH(CH_3)CO_2CH_3$, $KN(TMS)_2$, 18-crown-6, THF; (b) $LiAlH_4$; (c) for **7a**, $TiCl_4$/*t*-BuOOH/(+)-DET, CH_2Cl_2; for **7b**, same as for **3a** except (-)-DET; (d) 1:1 *t*-BuOH/0.5 N NaOH.

appear to alter the course of the cyclization pathway. Notwithstanding, the unprecedented cyclization of substrate **3a** to furnish

† Dedicated to Professor E. J. Corey on the occasion of his 60th birthday.

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sterol (+)-**4a** broadens the scope of the enzymatic method and contributes to its merit for the asymmetric synthesis of sterol intermediates that may be transformed to naturally occurring C-28-oxidized lanosterols.[7]

Results and Discussion

Preparation of Surrogate Substrates. The hydroxylated substrates (−)-**3a** and (−)-**3b** were synthesized in optically active form as outlined in Scheme II. Condensation of the readily accessible aldehyde **5**[8] with 1.5 equiv of $Ph_3P{=}C(CH_3)CO_2CH_3$[9] in benzene at 60 °C afforded a 66% yield of the *E* unsaturated ester **6a** after chromatographic separation of *E*/*Z* isomers. The *Z* unsaturated ester **6b** was prepared independently, but in a similar fashion, employing Still's modification[10] of the Horner–Emmons reaction, which involved the addition of aldehyde **5** to the anion derived from methyl 2-[bis(trifluoroethyl)phosphono]propionate and $KN(TMS)_2$/18-crown-6 in THF at −78 °C to afford an 85% yield of a 10:1 mixture of **6b**/**6a**. The small amount of undesired *E* isomer was easily removed by silica gel chromatography. Each of the esters, **6a** and **6b**, was reduced separately with $LiAlH_4$ to give the *E* and *Z* allylic alcohols **7a** and **7b** in 75% and 68% yields, respectively. Lastly, asymmetric epoxidation of the *E* alcohol **7a** with $Ti(O\text{-}i\text{-}Pr)_4$/(+)-diethyl tartrate/*t*-BuOOH under the catalytic conditions described by Sharpless[11] gave an 88% yield of (−)-**3a**. The optical purity of **3a** ($[\alpha]^{23}_D$ −3.31°, *c* 1, EtOH) was determined to be 87% ee by HPLC and ^1H NMR analysis of the corresponding MTPA ester[12] and represents a slight improvement over the 80% ee previously reported for the stoichiometric Sharpless epoxidation of alcohol **7a**.[13] The *Z* allylic alcohol **7b** was similarly converted to the epoxide **3b** (93% yield, 78% ee) with the exception that (−)-diethyl tartrate was used for the epoxidation. The absolute configuration at C-3 in both **3a** and **3b** corresponds to the correct configuration recognized by 2,3-oxidosqualene-lanosterol cyclase;[14] however, the use of enantiomerically enriched substrates, while beneficial from a synthetic point of view, is not mandatory.

Enzymatic Cyclization with Bakers' Yeast. The ultrasonically disrupted yeast homogenates used for the preparative-scale enzymatic reactions were prepared from bakers' yeast (*Saccharomyces cereviciae*) as described previously.[1] Active homogenates may also be prepared from Fleischmann's brand yeast or *Saccharomyces ellipsoideus* (wine yeast, Red Star brand). As was shown in our previous study, ultrasonic irradiation of the yeast for at least 1.5 h at 0 °C is essential for producing homogenates with good conversion efficiency. In the present study, all incubations were conducted anaerobically at 23 °C with yeast:substrate ratios of 100:1 (w/w) in phosphate buffer. Thus, incubation of 1.00 g of surrogate substrate (−)-**3a** and 14 g of Triton X-100[15] with 1 L of sonicated bakers' yeast homogenate afforded a new sterol corresponding to a 40.5% isolated yield of **4a** (based on 87% ee for **3a**). An overall conversion of 66.2% could be achieved with two cycles of incubation. The synthetic sterol **4a** (R_f 0.24, SiO_2, 1:2 ethyl acetate/hexane) was easily separated from the yeast's native sterols,[16] ergosterol (R_f 0.59) and lanosterol (trace amounts, R_f 0.79), by column chromatography after extractive workup with ether. A control incubation using a boiled yeast homogenate failed to produce sterol **4a**; substrate **3a** was recovered unchanged. All analytical and spectral data for the synthetic sterol were consistent with the expected structure **4a**. The 4α stereochemistry of the hydroxymethyl appendage was evident from the ^1H NMR spectrum, which displayed chemical shifts (3.41 and 3.80 ppm) and a coupling constant (J_{AB} = 12 Hz) for the CH_2OH methylene protons consonant with the values associated with an equatorial hydroxymethyl substituent in naturally occurring terpenoids but not with an axial hydroxymethyl substituent.[17] The absolute stereochemistry of the enzymatic product **4a** was assigned on the basis of the enantioselectivity of the cyclase, which accepts only substrates bearing the same absolute configuration at C-3 as 3(*S*)-squalene oxide.[14] The decrease in the enantiomeric excess (87% ee → 79% ee) of the unreacted starting material **3a** recovered from the incubation mixture correlated well with both the expected enantioselectivity of the enzymatic reaction and the yield of the sterol product **4a**. In addition, that only the 3*S*-(−)-**3a** enantiomer was a suitable substrate for the enzyme was confirmed by the failure of the 3*R*-(+) enantiomer of **3a** to be converted to a sterol under identical incubation conditions; this enantiomer was recovered unchanged. Furthermore, repeated recrystallization of **4a** led to no change in the specific rotation ($[\alpha]^{23}_D$ +56.6°, *c* 1, $CHCl_3$) after three recrystallizations from acetone.

Apart from sterol **4a** and the aforementioned native sterols, none of the 4β isomeric sterol **4b** was detected in the reaction mixture as determined by thin-layer chromatography on 10% $AgNO_3$ impregnated silica gel. Thus, the stereospecific cyclization of **3a** supported our initial hypothesis that the rigid conformational control elements near the epoxide terminus can be used to advantage for preventing the heterogroup from intervening in the cyclization process. We also recovered all material from the incubation corresponding to the chromatographic region of substrate **3a** and confirmed the identity of this material (HPLC and NMR) to be only unreacted **3a**, thereby excluding the remote possibility that the substrate was enzymatically transformed to other acyclic products such as the isomeric epoxide 1,2-epoxysqualen-3-ol (**8**) (see Scheme II) via an isomerization of **3a**; a reaction that readily occurs under nonenzymatic, basic-catalyzed conditions.[18] Also, we did not detect products resulting from hydrolysis of the terminal epoxide moiety as has been observed previously for the enzymatic cyclization of C-1 noroxidosqualene analogues.[3]

In view of the successful enzymatic conversion of surrogate **3a** to **4a**, we anticipated substrate **3b** would be an equally suitable substrate for the sterol cyclase. As expected, the incubation of 0.500 g of **3b** with 500 mL of sonicated yeast homogenate produced a single, new product (53% isolated yield, based on 78% ee for **3b**) appearing in the chromatographic region similar to, but clearly distinguishable from, sterol **4a**. However, ^1H and ^{13}C NMR spectra showed this product to be the bicyclic compound **4c** and not the lanosterol analogue **4b**. Since the NMR spectrum of **4c** confirmed the Δ^7 double bond and the hydroxymethyl 4β

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(6) Here, "β-face" follows the conventional use of the term as applied to steroid chemistry and refers to the topological region above the presumed chair–boat–chair folded conformation of the substrate.

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(10) Still, W. C.; Gennari, C. *Tetrahedron Lett.* **1983**, *24*, 4405–8.

(11) Hanson, R. M.; Sharpless, K. B. *J. Org. Chem.* **1986**, *51*, 1922–25.

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(13) Yamada, Y.; Seo, C. H.; Okada, H. *Agric. Biol. Chem.* **1981**, *45*, 1741–2.

(14) Barton, D. H. R.; Jarman, T. R.; Watson, K. D.; Widdowson, D. A.; Boar, R.; Damp, S. K. *J. Chem. Soc., Chem. Commun.* **1974**, 861.

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stereochemistry,[17] we inferred that **4c** also possessed the 5α and 9β stereochemistry. Also, **4c** was obtained in optically active form ($[\alpha]^{23}_D$ +9.4°, $CHCl_3$) in keeping with the enantioselective cyclization of substrates **1a,b** and **3a**.

We did not observe an appreciable difference in the incubation times required to convert either **3a** or **3b** to cyclic products, but both surrogates required longer incubation times (48 h) than did the natural substrate that cyclizes to lanosterol in 84% yield within 12 h.[1] We tentatively attributed these slower conversion rates to a decrease in the substrate's affinity for the enzyme due to the decrease in the substrate's lipophilicity. Although steric factors should not be discounted, their contribution to the enzymic binding of **3a** and **3b** seems less significant since substrates of similar steric demand, such as homosqualene oxide (i.e. **3**, $R^1 = CH_3$, $R^2 = H$), are known to be efficiently converted to homolanosterol[3,4] by using the same incubation conditions and times typically employed for the natural substrate. Study of these and related substrate analogues, designed to probe the effects of steric and polarity factors on substrate recognition and cyclization, is planned.

Conclusion

A broader scope for the synthesis of sterols by the enzymatic cyclization of squalene analogues with bakers' yeast is demonstrated herein by the conversion of the functionalized substrate **3a** to the remote-functionalized sterol **4a**. Yet, the hydroxylated substrate **3b** is only partially cyclized to the bicyclic terpenoid **4c**.[19] This contrast presently led us to presume that the enzyme's normal cyclizing function may be more sensitive to the topological orientation of structural changes made along the polyene backbone than the nucleophilic nature of the modified group or its influence on the substrate's lipophilicity. Experiments designed to further test this hypothesis and expand the synthetic scope of the enzymatic reaction by investigating modified substrates bearing strategically positioned heteroatoms is in progress.

Experimental Section

HPLC analyses were performed with use of a 15 cm SiO_2 (5 μm) column. Elemental analyses were performed by Atlantic Microlabs, Atlanta, GA.

Bakers' yeast (*S. cereviciae*) grade type II was purchased from Sigma and stored at 4 °C prior to use. Yeast stored for more than 3 months showed appreciable loss in enzymatic efficiency. Ultrasonic irradiation of yeast suspensions was conducted with a Branson Model 450 Sonifier, Cell Disruptor, equipped with a 0.5-in. diameter titanium immersion tip.

Preparation of Methyl (*E*,*E*,*E*,*E*,*E*)-2,6,10,15,19,23-Hexamethyltetracosa-2,6,10,14,18,22-hexaenoate (6a). To a suspension of 6.60 g (18.9 mmol) of $Ph_3P{=}C(CH_3)CO_2CH_3$[9] in 24 mL of benzene under a nitrogen atmosphere was added a solution of 4.0 g (12.7 mmol) of aldehyde **5**[8] in 24 mL of benzene. The mixture was heated at 60 °C for 3 h, cooled to room temperature, diluted with 200 mL of ether, washed successively with 100 mL of water and 2 × 100 mL of brine, and dried over anhydrous magnesium sulfate. Evaporation of the solvent gave a viscous yellow oil, which was chromatographed on flash silica gel in 1:4 benzene/hexane to afford 3.81 g (66%) of **6a** as a colorless oil: IR (TF) 3010, 1745, 1720 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.60 (br s, 18 H), 1.85 (s, 3 H), 2.00 (br s, 20 H), 3.70 (s, 3 H), 5.12 (m, 5 H), 6.75 (t, J = 7 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 12.6, 16.3, 16.3, 16.3, 17.5, 25.9, 26.7, 26.7, 27.4, 27.4, 28.3, 28.3, 28.7, 38.2, 39.6, 39.6, 51.7, 124.3, 124.3, 124.4, 124.4, 125.2, 127.4, 131.2, 133.8, 133.8, 134.9, 135.1, 142.3, 168.7. Anal. Calcd for $C_{31}H_{50}O_2$: C, 81.88; H, 11.08. Found: C, 81.93; H, 11.07.

Preparation of Methyl (*Z*,*E*,*E*,*E*,*E*,*E*)-2,6,10,15,19,23-Hexamethyltetracosa-2,6,10,14,18,22-hexaenoate (6b). To a solution of 1.17 g (3.52 mmol) of methyl 2-[bis(trifluoroethyl)phosphono]propionate[10] and 4.0 g of 18-crown-6 in 60 mL of THF at −78 °C under a nitrogen atmosphere was added dropwise over a 5-min period 2.52 mL of a 1.4 M solution of potassium hexamethyldisilazide in THF. The resulting pale brown solution was stirred at −78 °C for 5 min followed by the addition of a solution of 1.20 g (3.12 mmol) of aldehyde **5** in 3 mL of THF. After being stirred for 20 min at −78 °C, the reaction mixtue was quenched by the addition of 50 mL of 2 M aqueous ammonium chloride solution and diluted with 200 mL of ether. The organic phase was washed with 2 × 100 mL of water and 2 × 100 mL of brine and dried over anhydrous magnesium sulfate. Evaporation of solvent gave a pale yellow oil, which was chromatographed on a silica gel column in 1:20 ethyl acetate/hexane to afford 1.19 g (85%) of a mixture of **6b**/**6a** (10:1 as determined by HPLC eluting with 86:14 hexane/CH_2Cl_2). Rechromatography of the mixture on a flash/silica gel column in 1:4 benzene/hexane afforded pure **6b** as a colorless oil: IR (TF) 3090, 2950, 1760, 1745 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.60 (br s, 21 H), 2.00 (br s, 20 H), 3.70 (s, 3 H), 5.10 (m, 5 H), 5.90 (t, J = 6 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 16.4, 16.4, 16.4, 16.4, 26.5, 26.5, 26.5, 26.5, 27.9, 27.9, 27.9, 27.9, 39.0, 39.0, 39.6, 39.6, 39.6, 60.2, 124.2, 124.2, 124.2, 124.3, 124.3, 126.6, 130.9, 133.8, 134.8, 134.8, 134.8, 143.1, 168.0. Anal. Calcd for $C_{31}H_{50}O_2$: C, 81.88; H, 11.08. Found: C, 81.95; H, 11.08.

Preparation of (*E*,*E*,*E*,*E*,*E*,*E*)-2,6,10,15,19,23-Hexamethyltetracosa-2,6,10,14,18,22-hexaen-1-ol (7a). To a suspension of 100 mg of $LiAlH_4$ in 50 mL of ether at 0 °C under a nitrogen atmosphere was added dropwise over a 10-min period a solution of 3.63 g (8.0 mmol) of ester **6a**, and the mixture was stirred for 30 min at 0 °C. The reaction mixture was quenched by careful dropwise addition 50 mL of a saturated aqueous solution of potassium sodium tartrate and diluted with 200 mL of ether. The organic phase was separated and washed successively with 2 × 100 mL of water and 100 mL of brine and dried over anhydrous magnesium sulfate. Evaporation of solvent followed by chromatography of the crude product on a flash silica gel column in 1:3 ethyl acetate/hexane afforded 2.56 g (75%) of the *E* allylic alcohol **7a**: IR (TF) 3350, 3090, 2920, 1450 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.40 (s, 3 H), 1.55 (br s, 18 H), 2.00 (br s, 20 H), 3.90 (s, 2 H), 5.15 (m, 6 H). These spectral data for **7a** were consistent with those previously reported for this compound synthesized by an alternate route.[13]

Preparation of (*Z*,*E*,*E*,*E*,*E*,*E*)-2,6,10,15,19,23-Hexamethyltetracosa-2,6,10,14,18,22-hexaen-1-ol (7b). The procedure describe for the preparation of **7a** was repeated with 1.10 g (2.4 mmol) of the *Z* ester **6b** and 50 mg of $LiAlH_4$ in 20 mL of ether at 0 °C. Chromatography of the crude product on a flash silica gel column in 1:10 ethyl acetate/hexane gave 0.695 g (68%) of **7b** as a colorless oil: IR (TF) 3360, 3015, 1010 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.62 (br s, 18 H), 1.82 (s, 3 H), 2.04 (br s, 20 H), 4.13 (s, 2 H), 5.15 (m, 6 H); ^{13}C NMR δ 15.8, 15.8, 15.8, 15.8, 15.8, 17.4, 21.0, 25.4, 26.1, 26.5, 26.5, 28.1, 28.1, 39.6, 39.6, 39.6, 39.6, 61.1, 123.0, 123.0, 123.0, 123.0, 124.7, 127.6, 130.7, 134.1, 134.4, 134.4, 134.6, 134.7. Anal. Calcd for $C_{30}H_{50}O$: C, 84.44; H, 11.81. Found: C, 84.39; H, 11.80.

Preparation of (2*R*,3*S*)-(*E*,*E*,*E*,*E*)-2,6,10,15,19,23-Hexamethyl-2,3-epoxytetracosa-6,10,14,18,22-pentaen-1-ol (3a). To a solution of 173 mg (0.80 mmol) of (+)-diethyl tartrate and 0.4 g of powdered molecular sieves (4A) in 80 mL of anhydrous CH_2Cl_2 at −20 °C under a nitrogen atmosphere was added 228 mg (0.80 mmol) of Ti(*O*-*i*-Pr)$_4$ followed by 1.20 mL (6.4 mmol) of a 5.3 M solution of *t*-BuOOH in CH_2Cl_2. After the mixture was stirred for 20 min at −20 °C, a solution of 1.60 g (4.0 mmol) of allylic alcohol **7a** in 40 mL of CH_2Cl_2 was added dropwise over a 10-min period. The mixture was stirred for 1 h at −20 °C and diluted with 200 mL of CH_2Cl_2 followed by the addition of 100 mL of a solution of saturated aqueous sodium bicarbonate. The organic phase was washed with 2 × 100 mL of water and 100 mL of brine, and dried over anhydrous sodium carbonate. Evaporation of the solvent followed by column chromatography on flash silica gel in 1:10 ethyl acetate/hexane afforded 1.56 g (88%) of substrate **3a**: $[\alpha]^{23}_D$ −3.31° (*c* 1 in EtOH); IR (TF) 3450, 3020, 1040 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.30 (s, 3 H), 1.65 (br s, 18 H), 2.05 (br s, 20 H), 3.00 (t, J = 6 Hz, 1 H), 3.62 (br s, 2 H), 5.15 (m, 5 H). The optical purity of **3a** was determined by conversion of the epoxy alcohol **3a** to its corresponding (−)-MTPA ester as previously described.[13] The enantiomeric excess was determined to be 87% ee (lit.[13] 80% ee) from the relative integral of the methyl group on the oxide ring in the NMR spectrum of the MTPA ester and was confirmed by HPLC analysis on a silica column in 14:86 CH_2Cl_2/hexane (flow 1 mL/min), displaying retention times of 16.49 and 17.23 min for (−)-**3a** MTPA ester and the (+)-**3a** MTPA ester, respectively.

The (+)-**3a** enantiomer was also prepared in 83% yield ($[\alpha]^{23}_D$ +3.33°) from 0.40 g (1 mmol) of **7a** as described above for (−)-**3a** except (−)-diethyl tartrate was used. The optical purity of (+)-**3a** was determined to be 88% via the MTPA ester as described above. The ^1H and ^{13}C NMR spectra for (+)-**3a** were identical with those for (−)-**3a**.

Preparation of (2*S*,3*R*)-(*E*,*E*,*E*,*E*)-2,6,10,15,19,23-Hexamethyl-2,3-epoxytetracosa-6,10,14,18,22-pentaen-1-ol (3b). To a solution of 56 mg (0.27 mmol) of (−)-diethyl tartrate and 0.2 g of powdered molecular sieves (4A) in 25 mL of anhydrous CH_2Cl_2 at −20 °C under a nitrogen

(19) A striking example of another structurally modified substrate that does not follow the normal cyclization pathway has been recently reported by Krief et al.[20a] It was elegantly demonstrated that changing the stereochemistry of the Δ^{18}-bond leads only to a tricyclic product. We also note that the formation of a bicyclic product from the enzymatic cyclization of a partially cyclic substrate has also been reported.[20b]

(20) (a) Krief, A.; Schauder, J.-R.; Guittet, E.; Herve du Penhoat, C.; Lallemand, J.-Y. *J. Am. Chem. Soc.* **1987**, *109*, 7910–11. (b) Van Tamelen, E. E.; Hopla, R. E. *Ibid.* **1979**, *101*, 6112–14.

atmosphere was added 78 mg (0.27 mmol) of Ti(*O*-*i*-Pr)$_4$ followed by 0.423 mL (2.24 mmol) of a 5.3 M solution of *t*-BuOOH in CH_2Cl_2. After the mixture was stirred for 20 min at −20 °C, a solution of 581 mg (1.4 mmol) of allylic alcohol **7b** in 5 mL of CH_2Cl_2 was added dropwise over a 10-min period. The mixture was stirred for 1 h at −20 °C and diluted with 200 mL of CH_2Cl_2 followed by the addition of 100 mL of a solution of saturated aqueous sodium bicarbonate. The organic phase was washed with 2 × 100 mL of water and 100 mL of brine and dried over anhydrous sodium carbonate. Evaporation of the solvent followed by column chromatography on flash silica gel in 1:10 ethyl acetate/hexane afforded 565 mg (93%) of substrate **3b**: $[\alpha]^{23}_D$ −5.80° (*c* 1, EtOH); IR (TF) 3480, 1040 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.39 (s, 3 H), 1.62 (br s, 18 H), 2.20 (br s, 20 H), 2.85 (t, J = 6 Hz, 1 H), 3.68 (br s, 2 H), 5.17 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 15.9, 15.9, 15.9, 15.9, 26.6, 26.6, 26.6, 26.6, 26.6, 28.2, 28.2, 36.4, 39.7, 39.7, 39.7, 39.7, 39.7, 61.1, 63.9, 64.6, 124.2, 124.2, 124.2, 124.2, 125.2, 131.0, 133.6, 134.8, 134.8, 135.0. Anal. Calcd for $C_{30}H_{50}O_2$: C, 81.39; H, 11.38. Found: C, 81.42; H, 11.38. The optical purity of **3b** was determined to be 78% ee via the corresponding (−)-MTPA ester as described for **3a**. HPLC retention times for (−)-**3b** MTPA ester and (+)-**3b** MTPA ester were 22.18 and 24.51 min, respectively, in 35:65 CH_2Cl_2/hexane (flow 0.5 mL/min).

General Procedure for the Generation of Cyclase Active Yeast Homogenates. Bakers' yeast (50 g) was evenly dispersed with the aid of a glass rod in 250 mL of 0.10 M phosphate buffer, pH 7.4, and the suspension was cooled to 0 °C. An ultrasonic probe was supported above the yeast suspension so that the tip extends approximately 2 cm below the level of the liquid. The suspension was irradiated for 1.5 h with a power output of approximately 40 W/cm. At this power level, cavitation causes slow warming of the suspension; therefore it is essential to apply sufficient external cooling (ice/acetone bath) to maintain the internal temperature below 20 °C. After irradiation, the suspension is diluted with 250 mL of buffer to give 500 mL total volume of homogenate. For incubations requiring larger amounts of homogenate, we have found the best results are obtained by combining the appropriate volumes from 500-mL batches of homogenate instead of sonicating larger amounts of the yeast and buffer. The homogenate may be stored at 0 °C for at least 12 h prior to use without appreciable loss of activity.

Enzymatic Cyclization of Substrate 3a. To a mixture of 1.00 g (2.35 mmol) of substrate **3a** and 14 g of Triton X-100 was added 1 L of the sonicated yeast homogenate prepared as described above, and the mixture was stirred at 23 °C under a nitrogen atmosphere. After 48 h, the mixture was diluted with 250 mL of 15% KOH/MeOH followed by an additional 500 mL of MeOH and extracted with ether. The organic phase was washed successively with 2 × 200 mL of 20% aqueous KOH, 2 × 100 mL of water, and 200 mL of brine and dried over anhydrous magnesium sulfate. Evaporation of the solvent gave a yellow syrup, which was chromatographed on a flash silica gel column eluting successively with 1:10, 1:5, and 1:3 ethyl acetate/hexane to afford 0.379 g (40.5% yield based on 87% ee for **3a**) of sterol **4a**: $[\alpha]^{23}_D$ +56.6° (*c* 1 in $CHCl_3$, this value was unchanged after three recrystallizations of **4a** from acetone); mp 174–174.5 °C; IR (KBr) 3340, 3005, 1370 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.70 (s, 3 H), 0.89 (s, 3 H), 0.92 (s, 3 H), 1.02 (s, 3 H), 1.60 (s, 3 H), 1.67 (s, 3 H), 2.68 (br s, 2 H, OH), 3.41 and 3.80 (AB q, J_{AB} = 12 Hz, 2 H, CH_2O), 3.77 (m, 1 H), 5.08 (t, J = 6 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 17.9, 18.4, 20.9, 23.0, 24.8, 26.1, 27.2, 27.2, 29.9, 30.9, 30.9, 36.8, 36.8, 38.2, 41.9, 43.5, 44.4, 44.4, 44.5, 44.5, 49.0, 49.8, 49.8, 63.5, 78.4, 128.3, 130.8, 134.3, 134.3; HRMS calcd for $C_{30}H_{50}O_2$ 442.3811, obsd m/e 442.3806 (M^+), 427, 409, 351, 69.

The optical activity of the recovered unreacted starting material **3a** (0.552 g) was determined to be 79% ee after conversion to the corresponding MTPA ester as described in the preparation of **3a**.

Enzymatic Cyclization of 3b. To a mixture of 0.500 g of substrate **3b** (1.17 mmol) and 7 g of Triton X-100 was added 500 mL of the bakers' yeast homogenate, prepared as described above, and the mixture was stirred at 23 °C under a nitrogen atmosphere. After 48 h, the mixture was diluted with 125 mL of 15% KOH/MeOH followed by 250 mL of MeOH and extracted with ether. The organic phase was washed successively with 20% aqueous KOH, water, and brine and dried over anhydrous magnesium sulfate. Evaporation of the solvent, followed by column chromatography on flash silica gel, eluting with 1:5 ethyl acetate/hexane, gave 0.220 g (52.8%) of **4c** as a colorless oil: IR (TF) 3450, 3030, 2930, 1460 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.18 (s, 3 H), 1.25 (s, 3 H), 1.61 (br s, 18 H), 2.00 (br s, 15 H), 2.68 (br s, 2 H), 3.35 and 3.90 (AB q, J_{AB} = 10 Hz, 2 H, CH_2O), 3.62 (m, 1 H), 5.13 (m, 3 H), 5.30 (m, 1 H); HRMS calcd for $C_{30}H_{50}O_2$ 422.3811, obsd m/e 442.3801 (M^+), 379, 223, 69.

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