Total Synthesis of (-)-Chorismic Acid and (-)-Shikimic Acid

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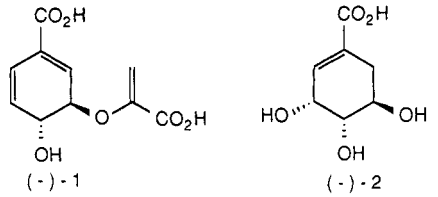
A survey of the enantioselective hydrolyses of **5a–h** and **6a–c** with commercially available lipases and cholesterol esterases is reported. A procedure for the preparative-scale synthesis of enantiomerically pure (+)-4 and (-)-4 by the enantioselective hydrolysis of **6a** or **6b** with cholesterol esterase from bovine pancreas is described. Enantiomerically pure (-)-3 is prepared from either (+)-4 or (-)-4. A short total synthesis of (-)-chorismic acid (22%) from (-)-3 and of (-)-shikimic acid (94%) from (-)-4 is reported.

(-)-Chorismic acid [(-)-1] is the branch-point intermediate in the biosynthesis of aromatic substances from (-)-shikimic acid [(-)-2] in bacteria, fungi, and higher plants.[1] The synthesis of (±)-1 has been accomplished



in our laboratory[2] and in Ganem's laboratory.[3] Numerous syntheses of (±)-2[4] have been reported. The resolution of (±)-2 has been accomplished.[4a,b] Syntheses of (-)-2 from (-)-quinic acid,[5] D-mannose,[6] and D-arabinose[7] have been described. Of these, the synthesis by Fleet and co-workers is the most notable.[6b] A total synthesis of (-)-2 utilizing an asymmetric Diels–Alder reaction has been accomplished by Masamune and co-workers.[8] Thus far, the only preparation of an enantiomerically pure intermediate for the synthesis of (-)-1, and analogues of (-)-1 for enzymatic investigations, is that which utilizes (-)-quinic acid as starting material.[9]

We have found racemic epoxy alcohols 3 and 4, readily prepared from methyl cyclohex-3-enecarboxylate, to be versatile intermediates for the synthesis of chorismate-type structures.[2b] Consequently, a convenient procedure for the resolution of 3 and 4 was of considerable interest. Described below are (1) enzyme-catalyzed kinetic resolutions that provide the pure enantiomers of 3 and 4, (2) the synthesis of (-)-1 from (-)-3, and (3) the synthesis of (-)-2 from (-)-4.

The enantioselective, enzyme-catalyzed hydrolysis of glycidol esters reported by Ladner and Whitesides[10] appeared to be an attractive procedure for the kinetic resolution of ester derivatives of (±)-3 and (±)-4. Sharpless and co-workers have described the dependence of enantiomeric excess on the relative rate of reaction of each enantiomer in chemical kinetic resolutions.[11] The relative reactivity of enantiomers (which can be described in terms of the enantiomeric ratio (*E*); see Table I, footnote *d*), the extent of conversion, and the enantiomeric excess of substrate and product in enzyme-catalyzed kinetic resolutions have been correlated by Sih and co-workers.[12]

A survey of the enantioselective hydrolyses of (±)-**5a–h**, derivatives of (±)-3, with commercially available lipases and cholesterol esterases is provided in Table I. In every case, with the exception of entry 12, the major enantiomer obtained was (+)-3. No hydrolysis of the methyl ester was observed except when using the enzyme pig liver esterase (EC 3.1.1.1, Sigma, 1600 units/mL). In this case the methyl ester was cleanly removed in preference to the side-chain ester, with no kinetic resolution observed. The enantiomeric excess (ee) of the hydrolysis product was generally only a few percent higher for reactions carried out at 0–5 °C rather than at 24 °C (entries 1 and 2). In the hydrolysis of *n*-butyrate ester **5a**, cholesterol esterase from bovine pancreas (entry 5) gave greater ee than the lipases (entries 1–4) or cholesterol esterase from porcine pancreas (entry 6); although cholesterol esterase from *Pseudomonas fluorescens* gave even greater ee (entry 7), the slow reaction rate and high cost of the enzyme eliminated consideration for use on a synthetic scale. Increasing the chain length of the acyl moiety (entry 8) beyond the *n*-butyrate did not improve greatly the ee of (+)-3

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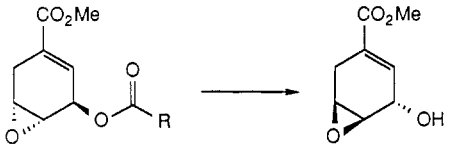
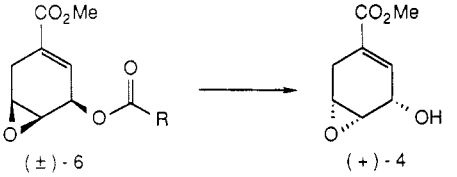
Table I. Enantioselective Hydrolysis of (±)-5 to (+)-3



entry	compd	R substr[a]	% ee (+)-3	enzyme[b] (units/mmol substr)	% conversn[c]	E[d]	temp, °C	reactn time, h
1	**5a**	$(CH_2)_2CH_3$	62	A (6500)	47	7	24	7
2	**5a**	$(CH_2)_2CH_3$	70	A (31 000)	39	9	0–5	7.5
3	**5a**	$(CH_2)_2CH_3$	61	A[e] (20 000)	32	5	24	10.5
4	**5a**	$(CH_2)_2CH_3$	48	B (20 000)	40	4	0–5	6
5	**5a**	$(CH_2)_2CH_3$	78	C (53)	40	14	0–5	4.5
6	**5a**	$(CH_2)_2CH_3$	45	D (50)	39	3	0–5	3
7	**5a**	$(CH_2)_2CH_3$	88	E (105)	37	26	0–5	50
8	**5b**	$(CH_2)_4CH_3$	80	C (50)	47	19	0–5	5
9	**5c**	$(CH_2)_8CH_3$	70	C (155)	38	9	24	31
10	**5d**	$CH(CH_3)_2$	83	A (41 000)	30	15	0–5	4.5
11	**5d**	$CH(CH_3)_2$	80	A (40 000)	40	15	0–5	5
12	**5d**	$CH(CH_3)_2$	18[f]	B (40 000)	32	2	0–5	31
13	**5d**	$CH(CH_3)_2$	60	C (77)	38	6	0→24	21
14	**5e**	$CH_2CH(CH_3)_2$	*g*	A (40 000)	14		0→24	48
15	**5e**	$CH_2CH(CH_3)_2$	*g*	C (98)	16		0→24	96
16	**5f**	$C(CH_3)_3$	*g*	A (40 000)	16		24	96
17	**5g**	CH_2Cl	8	A (40 000)	46	1	0–5	7
18	**5g**	CH_2Cl	3	C (50)	55	1	0–5	2.5
19	**5h**	CH_2OMe	11	C (52)	51	1	0–5	1
20	**5i**	MTPA						

[a] Esters were prepared following the general procedure described in the Experimental Section, except for entries 17 and 18 in which case (dimethylamino)propylamine was omitted and an aqueous workup was used. In general, 0.3–0.4 mmol of substrate was used in a reaction. [b] Key: A, lipase (EC 3.1.1.3, Sigma type VII, from *Candida cylindracea*, 500 units/mg); B, lipase (EC 3.1.1.3, Sigma type II, from porcine pancreas, 13 units/mg with triacetin); C, cholesterol esterase (EC 3.1.1.13, Sigma, from bovine pancreas, 1.5 or 1.22 units/mg); D, cholesterol esterase (EC 3.1.1.13, Sigma, from porcine pancreas, 0.58 units/mg); E, cholesterol esterase (EC 3.1.1.13, Sigma, from *P. fluorescens*, 6.3 units/mg). [c] Measured by integration of the ^1H NMR spectrum. [d] E is equal to the ratio of the specificity constants for the two enantiomers and is a constant independent of the extent of conversion for a reaction. It is therefore somewhat more general than ee and can be calculated by the expression formulated by Sih and co-workers[12] $E = \ln [1 - c(1 + ee(P))]/\ln [1 - c(1 - ee(P))]$, where c is the extent of conversion and ee(P) is the ee of the product. [e] Type VII lipase was immobilized by the procedure of Cambou and Klibanov.[13] [f] (−)-**3** was obtained under these conditions. [g] The percent ee was not determined due to the low conversion.

Table II. Enantioselective Hydrolysis of (±)-6 to (+)-4



entry	compd	R substr[a]	% ee (+)-4	enzyme[b] (units/mmol substr)	% conversn[c]	E[d]	temp, °C	reactn time, h
21	**6a**	$(CH_2)_2CH_3$	91	C (50)	40	39	0–5	3
22	**6b**	$(CH_2)_4CH_3$	93	C (7)	41	54	0–5	47
23	**6b**	$(CH_2)_4CH_3$	89	C (9)	40	31	24	4
24	**6c**	$CH(CH_3)_2$	55	A (40 000)	39	5	0–5	7
25	**6d**	MTPA						

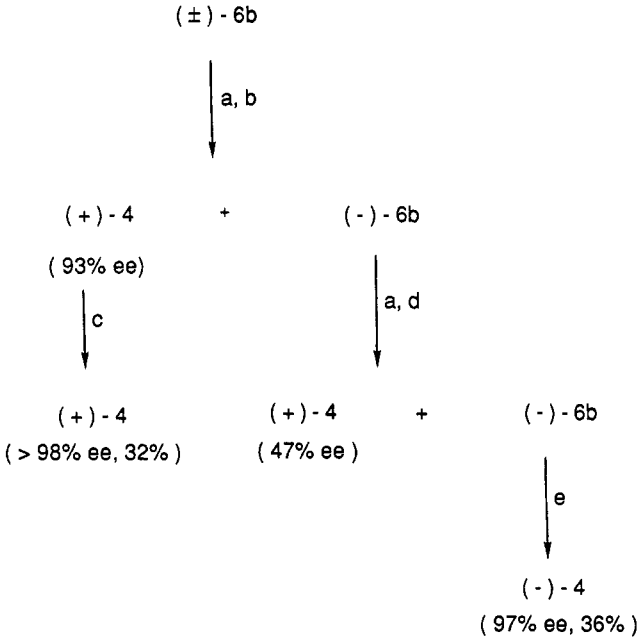
[a–d] See corresponding footnotes for Table I.

obtained. In fact, increasing chain length led to decreased water solubility and tended to give a substrate that would become gummy under the reaction conditions, and the result was lower ee (entry 9). With the exception of isobutyrate ester **5d** with type VII lipase (entries 10 and 11), chain branching resulted in decreased ee (entries 12 and 13) or a drastic decrease in reaction rate (entries 14–16). Although the hydrolyses of **5g** and **5h** proceeded at reasonable rates (entries 17–19), very little ee of (+)-**3** was observed.

The enantioselective hydrolyses of (±)-**6a,b**, the *n*-butyrate and *n*-hexanoate esters of (±)-**4** (Table II, entries 21–23), with cholesterol esterase from bovine pancreas provided (+)-**4** (~90% ee). In contrast to the reasonable ee obtained from hydrolysis of **5d** with type VII lipase (Table I, entries 10 and 11), isobutyrate ester **6c** (entry 24) gave (+)-**4** with only 55% ee.

For preparative-scale synthesis of the pure enantiomers of **3** and **4**, enantioselective hydrolyses of (±)-**6a,b** with cholesterol esterase from bovine pancreas were the most attractive kinetic resolution procedures (Scheme I). When the hydrolysis of (±)-**6b** (~4 g) was allowed to proceed to 41% conversion at 0–5 °C, (+)-**4** (93% ee) was obtained; and one recrystallization provided enantiomerically pure (+)-**4** in 32% yield (theoretical yield 50%). Further enzyme-catalyzed hydrolysis of recovered hexanoate ester (−)-**6b** for an additional 13% conversion (54% total) gave (+)-**4** (47% ee) and unhydrolyzed (−)-**6b** which was sub-

Scheme I[a]



[a] Key: (a) cholesterol esterase (bovine pancreas), H_2O, pH 7.8, 0–5 °C; (b) 41% conversion; (c) recrystallization from ethyl ether/petroleum ether; the other diastereomer not detected in the ^1H NMR spectrum of the Mosher ester; (d) 13% conversion; (e) NaOMe, MeOH.

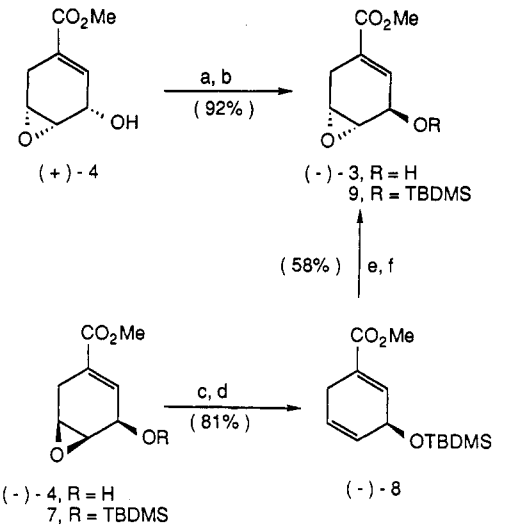
jected to methoxide-catalyzed ester interchange to provide (−)-4 (97% ee) in 36% yield (theoretical yield 50%). The absolute stereochemistry of (+)-4 and (−)-4 was established by comparison with (−)-4 prepared from (−)-methyl shikimate.[14]

It is interesting to point out an important consequence of the theory behind enzyme catalyzed kinetic resolutions; namely, the ee of the faster reacting enantiomer will always be lower than the ee of the slower reacting enantiomer at a comparable extent of conversion. For example, the above data suggest the enantiomeric ratio (E) to have a value of ~50 for the hydrolysis of (±)-**6b** with cholesterol esterase from bovine pancreas. It can be calculated that with E = 50, the ee of the product at 40% conversion should equal 92.8%, while the ee of remaining substrate at 60% conversion should equal 99.996%.[12]

Larger scale kinetic resolutions (20–30 g) were carried out at 24 °C rather than 0–5 °C in order to minimize the quantity of enzyme necessary to maintain a reasonable rate of hydrolysis. Also, the hydrolysis of (±)-**6a** was found to be more reproducible on a large scale than (±)-**6b**. Both were crystalline solids, but **6a** would remain a finely dispersed solid throughout the reaction, whereas **6b** would tend to clump up and become gummy, which greatly reduced the efficiency of the resolution. The ee of (+)-**4** obtained upon hydrolysis of (±)-**6a** at 24 °C was essentially the same as obtained at 0–5 °C with (±)-**6b**.

Inversion of configuration at the carbinol carbon of (+)-**4** was readily accomplished by the Mitsunobu reaction[15] followed by methoxide-catalyzed ester interchange to afford pure (−)-**3** in 92% yield (Scheme II). In addition, (−)-**4** could also be converted to (−)-**3** by inversion of configuration at the oxirane carbons. Protection of the hydroxyl group of (−)-**4** by reaction with *tert*-butyldimethylsilyl chloride (TBDMS-Cl) followed by deoxygenation[2b,16] gave (−)-**8** (81%). Epoxidation of (−)-**8** and subsequent desilylation gave (−)-**3** in 47% yield from (−)-**4**. The overall yield of (−)-**3** from (±)-**4** was 46%.

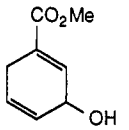
Scheme II[a]



[a] Key: (a) *i*-$PrO_2CN{=}NCO_2$-*i*-Pr, Ph_3P, AcOH, THF; (b) NaOMe, MeOH; (c) TBDMS-Cl, Et_3N, DMAP, CH_2Cl_2; (d) $MeO_2CC(N_2)CO_2Me$, rhodium octanoate, C_6H_6; (e) mCPBA, Na_2HPO_4, CH_2Cl_2; (f) *n*-Bu_4NF, THF.

Alternatively, both of the enantiomers of **3** could be produced by cholesterol esterase (bovine pancreas) mediated hydrolysis of (±)-**5b**, although in about one-third the yield that was realized when starting with (±)-**6b**. Following the procedure outlined for (±)-**6b**, (+)-**3** was obtained in 12% yield (theoretical yield 50%) and (−)-**3** was obtained in 15% yield (theoretical yield 50%).

In addition to the esterase-catalyzed kinetic resolution, we were obligated to consider the Sharpless asymmetric epoxidation of (±)-**10** as a kinetic resolution procedure to



(±)-10

obtain the pure enantiomers of **4**. Although epoxidation of 3-hydroxycyclohexene with 0.6 equiv of *tert*-butyl hydroperoxide under conditions of the Sharpless asymmetric epoxidation gave epoxide with only 30% ee,[11] asymmetric epoxidation studies of cyclohexadienols have not been reported. Racemic **10**[17] was prepared in 57% yield by desilylation of (±)-**8**, prepared from (±)-**4** by the procedure indicated in Scheme II for enantiomerically pure material. Unfortunately, (±)-**10** did not undergo reaction at a reasonable rate in the Sharpless asymmetric epoxidation reaction.[18]

The synthesis of (−)-**1** from (−)-**3** in overall yield of 22% is outlined in Scheme III. The reaction of (−)-**3** and trimethyl diazophosphonoacetate with $Rh_2(n\text{-}C_7H_{15}CO_2)_4$ catalysis provided **11**, which was converted to the monoanion and quenched with gaseous formaldehyde to give (−)-**12**.[19] Transformation of (−)-**12** to (−)-**1** was accomplished as indicated in Scheme III and described previously for the synthesis of (±)-**1**.[2b]

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(15) (a) Mitsunobu, O. *Synthesis* **1981**, 1–28. (b) Volante, R. P. *Tetrahedron Lett.* **1981**, *22*, 3119–3122.
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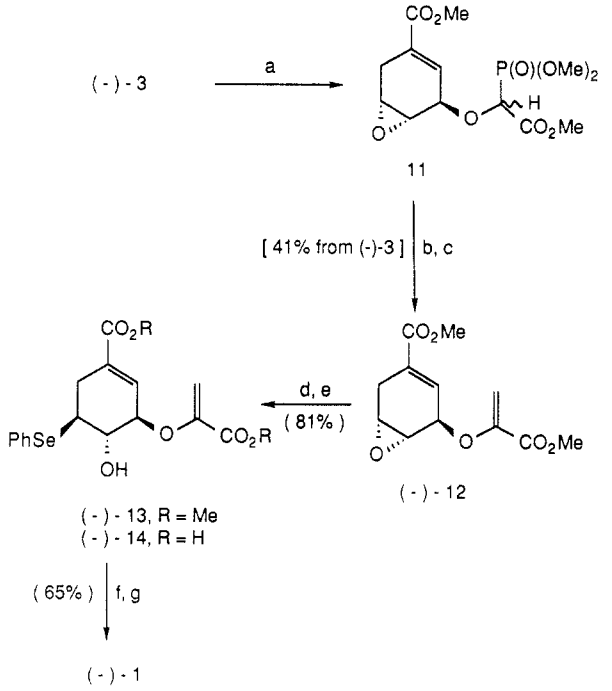
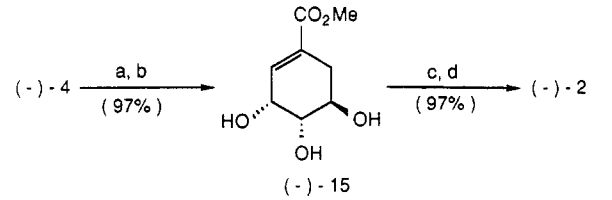
(19) Lesuisse, D.; Berchtold, G. A., manuscript in preparation.

Scheme III[a]



[a] Key: (a) $MeO_2CC(N_2)P(O)(OMe)_2$, rhodium octanoate, C_6H_6, reflux; (b) $LiN(SiMe_3)_2$, THF, −78 °C; (c) H_2CO, −78 °C; (d) $(PhSe)_2$, $NaBH_4$, MeOH; (e) NaOH, THF/H_2O, 0–5 °C; (f) H_2O_2, acetone, −30 °C; (g) 3,5-dimethoxyaniline (DMA), room temperature.

Scheme IV[a]



[a] Key: (a) 80% AcOH, heat; (b) NaOMe, MeOH; (c) NaOH, THF/H_2O; (d) H_3O^+.

The total synthesis of (−)-methyl shikimate [(−)-**15**] and (−)-**2** from (−)-**4** was readily accomplished in overall yield of 94% (Scheme IV). As previously described, (−)-**4** was converted to (−)-**15**,[14] which was saponified to give (−)-shikimic acid [(−)-**2**].

In summary, the enantioselective cholesterol esterase catalyzed hydrolysis procedure has provided a convenient synthesis of the pure enantiomers of **3** and **4**. The synthetic utility of these materials has been demonstrated by the first total synthesis of (−)-**1** from (−)-**3** and a convenient total synthesis of (−)-**2** from (−)-**4**.

Experimental Section[20]

Esters of Methyl (1β,2β,6β)-2-Hydroxy-7-oxabicyclo[4.1.0]hept-3-ene-4-carboxylate (5a–h) and Methyl (1α,2β,6α)-2-Hydroxy-7-oxabicyclo[4.1.0]hept-3-ene-4-carboxylate (6a–c). General Procedure. To epoxy alcohol **3** or **4** (170 mg, 1.0 mmol) dissolved in CH_2Cl_2 (10 mL) were added Et_3N (0.18 mL, 1.3 mmol, 1.3 equiv) and 4-(dimethylamino)-pyridine (DMAP, spatula tip full), and the system was flushed with N_2. The acid chloride (1.3 mmol, 1.3 equiv) was added via syringe over several minutes to the stirred solution at room temperature. When TLC analysis indicated complete reaction (usually within several hours), (dimethylamino)propylamine (0.038 mL, 0.3 mmol, 0.3 equiv) was added to quench any excess acid chloride, and the solution was concentrated. The residual oil was flash chromatographed on silica gel [ethyl acetate/petroleum ether (1:5)] to give between 90 and 100% yields of the esters **5a–h** and **6a–c**.

(+)- and (−)-Methyl (1β,2α,6β)-2-Hydroxy-7-oxabicyclo-[4.1.0]hept-3-ene-4-carboxylate [(+)- and (−)-4]. To epoxy alcohol (±)-**4** (2.10 g, 0.012 mol) dissolved in CH_2Cl_2 (20 mL) were added Et_3N (2.25 mL, 0.016 mol, 1.3 equiv) and DMAP (76 mg, 0.0006 mol, 0.05 equiv); the system was flushed with N_2 and cooled to ~10 °C. Hexanoyl chloride (2.25 mL, 0.016 mol, 1.3 equiv) was added via syringe over 5 min giving a copious precipitate. The mixture was allowed to warm to room temperature and was stirred for 8 h. The reaction was quenched by addition of (dimethylamino)propylamine (0.47 mL, 0.0037 mol, 0.3 equiv) followed by suction filtration. The filtrate was concentrated, and the residual oil was flash chromatographed on silica gel [ethyl acetate/petroleum ether (1:5)] to give 3.30 g of hexanoate (±)-**6b** (100%) as a colorless oil: ^1H NMR δ 6.60 (1 H, m), 5.81 (1 H, m), 3.76 (3 H, s), 3.53 (2 H, br m), 3.10 (1 H, d, J = 19 Hz), 2.53 (1 H, dq, J = 19 Hz, J = 2.6 Hz), 2.42 (2 H, t, J = 7.5 Hz), 1.69 (2 H, m), 1.34 (4 H, m), 0.91 (3 H, m). In subsequent experiments **6b** was obtained as a low-melting solid, mp 37–38 °C.

In a similar fashion (±)-**4** was converted to crystalline (±)-**6a**: ^1H NMR δ 6.60 (1 H, m), 5.81 (1 H, m), 3.76 (3 H, s) 3.53 (2 H, br m), 3.09 (1 H, d, J = 20 Hz), 2.54 (1 H, dm, J = 20 Hz), 2.41 (2 H, t, J = 7.3 Hz), 1.70 (2 H, septet, J = 7.3 Hz), 0.99 (3 H, t, J = 7.3 Hz); mp 46–47 °C.

Method A. Hexanoate (±)-**6b** (4.16 g, 0.016 mol) was suspended in H_2O (100 mL) and cooled to 0–5 °C while the mixture was stirred vigorously.[22] Cholesterol esterase from bovine pancreas (52 mg, 5.0 units/mmol substrate)[23] was added, and the pH was adjusted to 7.8 by addition of 0.2 M NaOH and thereafter maintained at this level by continued addition of 0.2 M NaOH with a syringe pump–pH controller unit.[24] After 30 h (10.5 mg, 1.0 unit/mmol) and 43 h (5.6 mg, 0.5 unit/mmol) additional enzyme was added. After 47 h a total of 31.0 mL of 0.2 M NaOH (0.0062 mol, 0.4 equiv)[25] had been added. The solution was saturated with NaCl and extracted with ethyl acetate (6 × 100 mL). The extracts were dried over $MgSO_4$, filtered, and concentrated to give 3.62 g of oil. ^1H NMR indicated 41% conversion. The mixture was flash chromatographed on silica gel [ethyl acetate/petroleum ether (1:10) 500 mL, (1:5) 500 mL, (1:1) 500 mL], giving 2.35 g of hexanoate (−)-**6b** (96% yield at 41% conversion) and 1.00 g of epoxy alcohol (+)-**4** (93% yield at 41% conversion, 93% ee). The epoxy alcohol was recrystallized from ethyl ether/petroleum ether to give 0.83 g (77%, >98% ee)[26] of fluffy white crystals. This represents a yield of 32% (theoretical yield 50%) of (+)-**4**: mp 80–81 °C; $[\alpha]^{25}_D$ +57.0° (4.06, $CHCl_3$).

Method B. Crystalline butyrate (±)-**6a** (27.43 g, 0.11 mol), ground to a fine powder by a mortal and pestle, was suspended in H_2O (600 mL) by vigorous mechanical stirring. The pH was adjusted to ~7 by addition of 1.0 M NaOH, cholesterol esterase from bovine pancreas (280 mg, 3.0 units/mmol substrate)[23] was added, and the pH was maintained at 7.7 by continued addition of 1.0 M NaOH with a syringe pump–pH controller unit.[24] After 14.5 h more cholesterol esterase (16 mg, 0.2 unit/mmol) was added.

(20) ^1H NMR were obtained in $CDCl_3$ at 250 or 270 MHz, and ^{13}C NMR were obtained in $CDCl_3$ at 67.9 MHz. Mosher esters were made from (+)-α-methoxy-α-[(trifluoromethyl)phenyl]acetyl chloride [(+)-MTPA-Cl] and ~5 mg of substrate following the general procedure for esters of **3** and **4** as outlined in the Experimental Section: Hill, J. G.; Sharpless, K. B.; Exon, C. M.; Regenye, R. *Org. Synth.* **1985**, *63*, 66–78. (+)-MTPA-Cl was made from (+)-MTPA according to the procedure of Mosher and co-workers: Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543–2549. Flash chromatography refers to the procedure developed by Still and co-workers.[21]

(21) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923–2925.

(22) If solidification should occur at this point, care must be taken to fragment the material into fine pieces.

(23) EC 3.1.1.13, Sigma, 1.5 or 1.22 units/mg of solid.

(24) Syringe pump: Sage 341A, VWR Scientific. pH controller: Accumet 805MP, Fisher Scientific.

(25) This does not include the amount of base needed initially to adjust the pH.

(26) The other diastereomer was not detected in the ^1H NMR spectrum of the Mosher ester.

After 19 h a total of 46.0 mL of 1.0 M NaOH (0.046 mol, 0.4 equiv)[25] had been added. The majority of the remaining solid butyrate was collected by suction filtration, washing the solid with H_2O (2 × 20 mL). The aqueous filtrate was extracted with hexanes (250 mL, 5 × 150 mL); the combined extracts were dried over $MgSO_4$, filtered, and concentrated to give 1.61 g of white solid. The solid collected previously was dissolved in CH_2Cl_2 (100 mL), dried over $MgSO_4$, filtered, and concentrated to give 14.24 g of white solid. The combined total of 15.85 g (96% yield at 40% conversion) of solid butyrate (−)-**6a** was obtained, which was pure by ^1H NMR. The aqueous layer was saturated with NaCl and extracted with ethyl acetate (6 × 150 mL). The extracts were dried over $MgSO_4$, filtered, and concentrated to give 7.43 g of solid epoxy alcohol (+)-**4** (96% yield at 40% conversion, 91% ee). ^1H NMR indicated ~1% butyrate contamination of the alcohol. The epoxy alcohol was recrystallized as before to give enantiomerically pure (+)-**4**.[26]

Enantiomerically enriched (−)-**6b** (2.35 g, 8.75 mmol) obtained above was suspended in H_2O (50 mL), and the resultant mixture was cooled to 0–5 °C while being stirred vigorously. Cholesterol esterase from bovine pancreas (29 mg, 5.0 units/mmol of substrate)[23] was added, and the pH was adjusted to 7.8 by addition of 0.2 M NaOH and thereafter maintained at this level by continued addition of 0.2 M NaOH with a syringe pump–pH controller unit.[24] After 27.5 h (14 mg, 2.4 units/mmol), 44 h (7 mg, 1.2 units/mmol), and 68 h (4 mg, 0.7 unit/mmol), additional enzyme was added. After 89 h a total of 11.2 mL of 0.2 M NaOH (2.24 mmol, 0.26 equiv)[25,27] had been added. The solution was saturated with NaCl and extracted with ethyl acetate (6 × 100 mL). The extracts were dried over $MgSO_4$, filtered, and concentrated to give 2.09 g of oil. ^1H NMR indicated 23% conversion.[28] The mixture was flash chromatographed on silica gel [ethyl acetate/petroleum ether (1:5) 200 mL, (1:1) 200 mL) to give 1.74 g of (−)-**6b** (96% yield at 23% conversion) and 0.30 g of (+)-**4** (88% yield at 23% conversion, 47% ee).

To a freshly prepared solution of NaOMe (150 mg of Na, 6.5 mmol, 1.0 equiv in 10 mL of MeOH) cooled to 0–5 °C under a N_2 atmosphere was added (−)-**6b** (1.74 g, 6.5 mmol) in 10 mL of MeOH via cannula, with additional MeOH (2 × 2.5 mL) to complete the transfer. The mixture was stirred at 0–5 °C for 1 h, acetic acid (0.38 mL, 6.5 mmol, 1.0 equiv) was added, and the solution was concentrated to ~2-mL volume. This was taken up in ethyl acetate (100 mL) and washed with H_2O (10 mL) saturated with NaCl. The aqueous portion was extracted with ethyl acetate (4 × 20 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated to give 1.03 g of oil. The oil was flash chromatographed on silica gel [ethyl acetate/petroleum ether (1:5) 200 mL, (1:1) 300 mL) to give 0.94 g of epoxy alcohol (−)-**4** (85%, 97% ee)[29] as a crystalline solid. This represents a yield of 36% (theoretical yield 50%) of (−)-**4**: mp 78–79.5 °C (ethyl ether/petroleum ether) (lit.[14] mp 81–82 °C); $[\alpha]^{25}_D$ −58.5° (4.13, $CHCl_3$) [lit.[14] $[\alpha]^{25}_D$ −54.4° (4.04, $CHCl_3$)].

(−)-Methyl (1β,2β,6β)-2-Hydroxy-7-oxabicyclo[4.1.0]hept-3-ene-4-carboxylate [(−)-3 from (+)-4]. To (+)-**4** (1.34 g, 7.90 mmol, >98% ee) dissolved in dry THF (50 mL, from Na) under N_2 were added triphenylphosphine (3.33 g, 11.9 mmol, 1.5 equiv) and acetic acid (0.73 mL, 11.9 mmol, 1.5 equiv). Diisopropyl azodicarboxylate (2.50 mL, 11.9 mmol, 1.5 equiv) was added dropwise over 10 min, giving a clear, colorless solution that was stirred at room temperature. After 36 h the mixture was concentrated; the crude acetate was dissolved in dry MeOH (10 mL) and cooled to 0–5 °C. To this solution was added 0.5 M NaOMe (117 mg Na, 5.1 mmol, 0.5 equiv in 10 mL of MeOH) over 20 min via syringe pump.[24] After 1 h, acetic acid (0.30 mL, 5.2 mmol, 0.6 equiv) was added, and the solution was concentrated to ~ 15-mL volume. This was taken up in ethyl acetate (50 mL) and washed with H_2O (10 mL) saturated with NaCl. The aqueous portion was extracted with ethyl acetate (4 × 20 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated to give 9.51 g of crude solid. Flash chromatography on silica gel [ethyl acetate/petroleum ether (1:5) 1700 mL, (1:2) 1200 mL] gave 5.19 g of (−)-**3** contaminated with triphenylphosphine oxide. The solid was triturated with 15 mL of ethyl acetate/petroleum ether (1:1) and suction filtered, and the filtrate was concentrated to give 3.99 g of solid. Flash chromatography on silica gel [ethyl acetate/petroleum ether (1:5) 3500 mL, (1:3) 1600 mL, (1:2) 4300 mL] gave 1.24 g (92%, >98% ee)[26] of crystalline solid. This represents a yield of 29% from (±)-**4** to (−)-**3**: mp 65–66.5 °C; $[\alpha]^{25}_D$ −53.9° (4.20, $CHCl_3$).

(−)-Methyl 3-[[(Dimethylethyl)dimethylsilyl]oxy]cyclohexa-1,4-diene-1-carboxylate [(−)-8]. To (−)-**4** (0.869 g, 5.11 mmol) dissolved in CH_2Cl_2 (15 mL) were added Et_3N (0.890 mL, 6.38 mmol, 1.25 equiv), DMAP (0.312 g, 2.55 mmol, 0.5 equiv), and *tert*-butyldimethylsilyl chloride (0.961 g, 6.38 mmol, 1.25 equiv) as a CH_2Cl_2 solution (5 mL). The system was flushed with N_2 and, after stirring for 24 h at room temperature, the mixture was washed with 5% HCl (10 mL, 3 × 10 mL of CH_2Cl_2 back-extractions) and 5% $NaHCO_3$ (10 mL, 3 × 10 mL of CH_2Cl_2 back-extractions). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated to give 1.518 g of crude oil. The oil was flash chromatographed on silica gel [ethyl acetate/petroleum ether (1:10)] to give 1.451 g (100%) of **7** as a colorless oil: ^1H NMR δ 6.62 (1 H, m), 4.71 (1 H, m), 3.75 (3 H, s), 3.43 (2 H, br m), 3.05 (1 H, d, J = 19.5 Hz), 2.42 (1 H, dq, J = 19.5 Hz, J = 2.6 Hz), 0.95 (9 H, s), 0.17 (6 H, s).

To the *tert*-butyldimethylsilyl-protected epoxy alcohol **7** (1.450 g, 5.10 mmol) dissolved in dry benzene (40 mL, from Na) was added rhodium octanoate dimer (82 mg, 0.11 mmol, 0.02 equiv) with the system under N_2. To this bright green solution was added dimethyl diazomalonate (2.422 g, 15.3 mmol, 3.0 equiv)[30] as a benzene solution (40 mL) via cannula, and the mixture was stirred at room temperature. Vigorous evolution of N_2 was evident, and the solution turned brown. After 30 min the entire mixture was flash chromatographed on silica gel [ethyl acetate/petroleum ether (1:10)] to give 0.328 g (22%, corrected for aromatic) of (−)-**8** contaminated with 10% methyl *m*-(*tert*-butyldimethylsiloxy)-benzoate. An additional 0.964 g of oil was further purified by flash chromatography on silica gel [ethyl acetate/petroleum ether (1:10)] to give 0.847 g (59%, corrected for aromatic) of (−)-**8** contaminated with 5% methyl *m*-(*tert*-butyldimethylsiloxy)-benzoate. This gave a combined yield of 81% of (−)-**8**: IR (neat) 1725, 1680, 1645 cm^{-1}; ^1H NMR δ 6.86 (1 H, m), 5.93 (1 H, dm, J = 9.5 Hz), 5.76 (1 H, dm, J = 9.5 Hz), 4.85 (1 H, m), 3.78 (3 H, s), 2.98 (1 H, dm, J = 21 Hz), 2.80 (1 H, dm, J = 21 Hz), 0.93 (9 H, s), 0.14 (6 H, s); ^{13}C NMR δ 166.6 (s), 137.3 (d), 128.4 (s), 126.6 (d), 125.1 (d), 63.5 (d), 51.4 (q), 25.5 (q and t), 17.9 (s), −4.8 (q); mass spectrum, m/e (relative intensity) 268 (M^+, 2.3), 253 (1.3), 237 (1.6), 211 (8.2), 209 (5.1), 136 (1.3), 105 (100); high-resolution mass spectrum for $C_{14}H_{24}O_3Si$, calcd 268.1495, found 268.1494; $[\alpha]^{20}_D$ −7.4° (3.11, $CHCl_3$, corrected for aromatic).

(−)-Methyl (1β,2β,6β)-2-Hydroxy-7-oxabicyclo[4.1.0]hept-3-ene-4-carboxylate [(−)-3 from (−)-4]. To diene (−)-**8** (1.174 g, 4.10 mmol, corrected for aromatic) dissolved in CH_2Cl_2 (20 mL) were added Na_2HPO_4 (0.657 g, 4.63 mmol, 1.1 equiv) and mCPBA (1.005 g, 80%, 4.66 mmol, 1.1 equiv), and the mixture was stirred at room temperature. After 58 h the mixture was washed with saturated aqueous Na_2SO_3 (1 × 10 mL, 3 × 10 mL of CH_2Cl_2 back-extractions) and 5% $NaHCO_3$ (1 × 20 mL, 3 × 10 mL of CH_2Cl_2 back-extractions). The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated to give 1.290 g of crude oil. ^1H NMR indicated a 3:1 ratio of trans to cis epoxides.[31] The oil was flash chromatographed on silica gel [ethyl acetate/petroleum ether (1:15) 500 mL, (1:10) 200 mL, (1:2) 100 mL] to give 0.253 g (22%) of pure cis epoxide **7** and 0.707 g (61%) of pure trans epoxide **9**: ^1H NMR δ 6.67 (1 H, m), 4.63 (1 H, br s), 3.76 (3 H, s), 3.43 (1 H, m), 3.18 (1 H, br s), 2.90 (1 H, dm, J = 20 Hz), 2.68 (1 H, dq, J = 20 Hz, J = 2.5 Hz), 0.93 (9 H, s), 0.17 (3 H, s), 0.14 (3 H, s). Additionally, 0.061 g (5%) of a bisepoxide

(27) This amount of base was calculated to give ~15% conversion based on the original amount of (±)-**6b**, or a total of 56% conversion for the two experiments.

(28) This would give ~13% conversion, or a total of 54% conversion for the two experiments.

(29) Some of the aromatization that occurred can be avoided by adding the NaOMe solution to the epoxy alcohol (see next experiment).

(30) Ando, W.; Yagihara, T.; Tozune, S.; Imai, I.; Suzuki, J.; Toyama, T.; Nakaido, S.; Migita, T. *J. Org. Chem.* **1972**, *37*, 1721–1727.

(31) Replacement of the *tert*-butyldimethylsilyl protecting group with a triisopropylsilyl protecting group gave no improvement in stereoselectivity.

was obtained: ^{1}H NMR δ 4.57 (1 H, br s), 3.77 (3 H, s), 3.29 (1 H, br s), 3.24 (1 H, m), 3.04 (1 H, m), 2.94 (1 H, dd, J = 17 Hz, J = 3.1 Hz), 2.73 (1 H, d, J = 17 Hz), 0.93 (9 H, s), 0.17 (6 H, s).

To trans epoxide **9** (0.707 g, 2.49 mmol) dissolved in dry THF (15 mL, from Na) under a N_2 atmosphere and cooled to 0–5 °C was added 1.0 M n-Bu_4NF (2.6 mL, 2.6 mmol, 1.05 equiv, in THF) over 15 min via syringe. After 1.5 h the reaction was quenched by addition of H_2O (20 mL) followed by extraction with ethyl acetate (6 × 25 mL), with addition of NaCl to break an emulsion. The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated to give 1.49 g of crude (−)-**3** as an oil. Flash chromatography on silica gel [ethyl acetate/petroleum ether (1:1)] gave 0.401 g (95%) of crystalline (−)-**3**, which was identical with (−)-**3** obtained before. This represents a yield of 17% from racemic **4**. Thus, a total yield of 46% of (−)-**3** was obtained from (±)-**4**.

(−)-Methyl (1β,2β,6β)-2-Hydroxy-7-oxabicyclo[4.1.0]hept-3-ene-4-carboxylate [(−)-3 from (±)-3]. Following the general procedure, (±)-**3** was converted in 95% yield to hexanoate (±)-**5b**: ^{1}H NMR δ 6.73 (1 H, m), 5.68 (1 H, m), 3.76 (3 H, s), 3.45 (1 H, m), 3.25 (1 H, m), 2.99 (1 H, dm, J = 20 Hz), 2.70 (1 H, dq, J = 20 Hz, J = 2.5 Hz), 2.35 (2 H, t, J = 7.5 Hz), 1.65 (2 H, m), 1.31 (4 H, m), 0.90 (3 H, t, J = 6.7 Hz).

Following the procedure outlined for (±)-**6b**, (±)-**5b** (0.658 g, 2.45 mmol) was hydrolyzed under the action of cholesterol esterase from bovine pancreas,[23] by a total of 20.3 mg (30.5 units/mmol substrate) over 47 h. After 9.8 mL of 0.1 M NaOH (0.98 mmol, 0.4 equiv) had been added, the reaction was worked up as before to give 0.584 g of oil. ^{1}H NMR indicated 45% conversion. The oil was flash chromatographed on silica gel [ethyl acetate/petroleum ether (1:5) 150 mL, (1:1) 150 mL] to give 0.333 g of **5b** (92% yield at 45% conversion) and 0.159 g of (+)-**3** (84% yield at 45% conversion, 74% ee). The epoxy alcohol was twice recrystallized from ethyl ether/petroleum ether to give 47 mg (25%, >98% ee)[26] of fluffy white crystals. This represented a yield of 12% (theoretical yield 50%) of (+)-**3**: mp 65–67 °C; $[\alpha]^{25}_D$ +55.3° (4.06, $CHCl_3$).

Following the procedure for (−)-**6b**, enantiomerically enriched **5b** was hydrolyzed under the action of cholesterol esterase from bovine pancreas,[23] with a total of 7.4 mg (9.0 units/mmol substrate) over 47 h. After 6.0 mL of 0.05 M NaOH (0.3 mmol, 0.24 equiv)[32] had been added, the reaction was worked up as before to give 0.296 g of oil. ^{1}H NMR indicated 22% conversion.[33] The mixture was flash chromatographed on silica gel [ethyl acetate/petroleum ether (1:5) 120 mL, (1:1) 120 mL] to give 0.240 g of hexanoate **5b** (92% yield at 22% conversion) and 47 mg of **3** (100% yield at 22% conversion).

To hexanoate **5b** (0.240 g, 0.89 mmol) dissolved in MeOH (5 mL) and cooled to 0–5 °C was added 0.5 M NaOMe (1.79 mL, 0.89 mmol, 1.0 equiv) over 15 min via syringe pump.[24] After 1.5 h acetic acid (0.051 mL, 0.89 mmol, 1.0 equiv) was added, and the solution was concentrated to ~2-mL volume. The mixture was taken up in ethyl acetate (25 mL) and washed with saturated aqueous NaCl (5 mL). The aqueous portion was extracted with ethyl acetate (3 × 10 mL), and the combined organic extracts were dried over $MgSO_4$, filtered, and concentrated to give 0.147 g of pale yellow solid. Flash chromatography on silica gel [ethyl acetate/petroleum ether (1:5) 100 mL, (1:1) 150 mL] gave 0.137 g of (−)-**3** (90%, 83% ee) as a crystalline solid. Recrystallization from ethyl ether/petroleum ether gave 62 mg (41%, >98% ee)[26] of fluffy white needles. This represented a yield of 15% (theoretical yield 50%) of (−)-**3** which was identical with (−)-**3** obtained before.

Methyl 3-Hydroxycyclohexa-1,4-diene-1-carboxylate [(±)-10]. Racemic **8** was prepared in the same manner as described for enantiomerically pure (−)-**8**, starting from a 4:1 mixture of trans to cis epoxy alcohols.

To (±)-**8** (0.457 g, 1.70 mmol) dissolved in dry THF (25 mL, from Na) under a N_2 atmosphere and cooled to ~5 °C was added 1.0 M n-Bu_4NF (1.80 mL, 1.80 mmol, 1.06 equiv, in THF) over ~30 s. After stirring for 10 min, the reaction was quenched by pouring the mixture into ethyl acetate (50 mL) and washing with H_2O (50 mL), with addition of saturated aqueous NaCl (3 mL) to break an emulsion. The aqueous layer was extracted with ethyl acetate (5 × 25 mL), and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated to give 0.297 g of crude oil. Flash chromatography on silica gel [ethyl acetate/petroleum ether (1:5) 400 mL, (1:1) 200 mL] gave 0.150 g (57%) of (±)-**10** as an oil that solidified upon cooling in the freezer: mp 40–41 °C; ^{1}H NMR, identical with that for (±)-**10** previously reported.[17]

(−)-Methyl (1β,2β,6β)-[[1-(Methoxycarbonyl)ethenyl]-oxy]-7-oxabicyclo[4.1.0]hept-3-ene-4-carboxylate [(−)-12]. To (−)-**3** (0.552 g, 3.24 mmol) dissolved in dry benzene (25 mL, from Na) under a N_2 atmosphere were added trimethyl diazophosphonoacetate (1.89 g, 4.55 mmol, 1.4 equiv)[34] and rhodium octanoate dimer (51 mg, 0.065 mmol, 0.02 equiv). The mixture was heated to a gentle reflux. After 2 h the solution was cooled and concentrated to a green oil that was flash chromatographed on silica gel [ethyl acetate/petroleum ether (2:1) 1300 mL, (3:1) 750 mL, (5:1) 600 mL, (10:1) 1000 mL] to give 0.685 g (~60%) of a colorless oil. ^{1}H NMR indicated ~96% purity of **11**: ^{1}H NMR δ 6.78 (1 H, m), 4.59 (1 H, m), 4.58 and 4.55 (1 H, d, J = 19.6 Hz), 3.89–3.77 (12 H, br), 3.48–3.37 (2 H, m), 2.96 (1 H, dm, J = 19.5 Hz), 2.69 (1 H, d, J = 19.5 Hz). The remaining ~4% was identified by ^{1}H NMR to be a diene that was the product of deoxygenation of the epoxide:[2b,16] ^{1}H NMR δ 6.93 (1 H, m), 6.21 (1 H, m), 5.83 (1 H, m), 5.04 (1 H, m), 4.37 (1 H, m), 3.91–3.80 (12 H, br), 2.96 (2 H, m).

To phosphonate **11** (0.685 g, 1.96 mmol) dissolved in dry THF (50 mL, from Na) under a N_2 atmosphere and cooled to −78 °C (dry ice/acetone) was added 1.0 M lithium bis(trimethylsilyl)amide (2.15 mL, 2.15 mmol, 1.1 equiv, in hexanes) over 10 min. After the mixture was stirred an additional 5 min, gaseous formaldehyde (generated from paraformaldehyde, 0.280 g, 20 mmol, 10 equiv)[35] was bubbled into the cold solution over 30 min, and stirring was continued for another 15 min. The reaction was quenched at −78 °C by addition of saturated aqueous NH_4Cl (20 mL) followed by extraction with ethyl acetate (50 mL, 5 × 20 mL). The combined extracts were dried over $MgSO_4$, filtered, and concentrated to give 0.427 g of solid. Recrystallization from methanol gave 0.281 g [34% from (−)-**3**] of (−)-**12**. The mother liquor was concentrated and flash chromatographed on silica gel [ethyl acetate/petroleum ether (1:5)] to give an additional 57 mg [7% from (−)-**3**] of crystalline solid for a combined yield of 41% for the two steps to (−)-**12**: mp 114–116 °C (MeOH); $[\alpha]^{25}_D$ −120° (1.12, $CHCl_3$).

(−)-Chorismic Acid [(−)-1]. The preparation of (−)-**13** was carried out as previously described[2b] for (±)-**13**; $[\alpha]^{25}_D$ −14.1° (1.76, $CHCl_3$).

The conversion of (−)-**13** to (−)-**14** was carried out as previously described[2b] for (±)-**14**, to give a foam; $[\alpha]^{25}_D$ −6.6° (1.59, ethanol).

The conversion of (−)-**14** to (−)-**1** was carried out as previously described[2b] for (±)-**1**, to give a white solid: mp 105–108 °C dec (solid triturated with ethyl acetate) [lit.[36] mp 148–149 °C dec (ethyl acetate/petroleum ether); lit.[36] mp 112 °C dec (ethyl ether/petroleum ether); lit.[36] mp 115 °C dec (ethyl acetate/carbon tetrachloride)]; $[\alpha]^{21}_D$ −274° (0.16, H_2O) [lit.[36] $[\alpha]^{25}_D$ −295.5 ± 3° (0.2, H_2O)].

(−)-Shikimic Acid [(−)-2]. Following the previously published procedure,[14] (−)-**4** was converted to (−)-methyl shikimate [(−)-**15**]: mp 113–114 °C (ethyl acetate) (lit.[37] mp 113–114 °C); $[\alpha]^{25}_D$ −128° (1.79, ethanol) [lit.[6b] $[\alpha]^{20}_D$ −125° (1.88, ethanol)].

To (−)-methyl shikimate (108 mg, 0.57 mmol) dissolved in THF/H_2O (1:1, 8 mL) was added 1.0 M NaOH (0.63 mL, 0.63 mmol, 1.1 equiv). After the mixture was stirred for 5.5 h at room temperature, Amberlite IR-120 (plus) ion-exchange resin was added and then removed by suction filtration after 10 min, with

(32) This amount of base was calculated to give ~13% conversion based on the original amount of (±)-**5b**, or a total of 58% for the two experiments.

(33) This would give ~12% conversion, or a total of 57% conversion for the two experiments.

(34) This compound was contaminated with p-toluenesulfonamide and was of ~50% purity by weight. It was prepared according to the procedure used to make the corresponding triethyl ester: Regitz, M.; Anschütz, W.; Liedhegener, A. *Chem. Ber.* **1968**, *101*, 3734.

(35) Paraformaldehyde was heated at an oil bath temperature of ~180 °C with a constant stream of N_2 passing over it and through $^1/_4$-in. Teflon tubing into the reaction mixture.

(36) Edwards, J. M.; Jackman, L. M. *Aust. J. Chem.* **1965**, *18*, 1227–1239.

(37) Fischer, H. O. L.; Dangschat, G. *Helv. Chim. Acta* **1934**, *17*, 1200–1207.

washing of the resin with H_2O (2 × 10 mL). The filtrate was concentrated to give 100 mg (100%) of an oil that crystallized under vacuum. ^1H NMR indicated ~97% stereochemical purity of (-)-**2**:[38] mp 183-184.5 °C; $[\alpha]^{21}_D$ -170° (1.18, H_2O). There was no depression of melting point with authentic (-)-**2**:[39] mp 185-186 °C [lit.[40] mp 183-184 °C (corrected)]; $[\alpha]^{21}_D$ -184° (1.17, H_2O) [lit.[41] $[\alpha]^{18}_D$ -184° (4.03, H_2O)].

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Registry No. (-)-**1**, 617-12-9; (-)-**2**, 138-59-0; (+)-**3**, 106861-59-0; (-)-**3**, 106861-60-3; (±)-**3**, 76947-23-4; (+)-**4**, 106861-61-4; (-)-**4**, 76985-84-7; (±)-**4**, 76985-85-8; (±)-**5a**, 106780-56-7; (±)-**5b**, 106780-57-8; (±)-**5c**, 106780-58-9; (±)-**5d**, 106780-59-0; (±)-**5e**, 106780-60-3; (±)-**5f**, 106780-61-4; (±)-**5g**, 106780-62-5; (±)-**5h**, 106780-63-6; (±)-**6a**, 106861-62-5; (-)-**6a**, 106861-65-8; (±)-**6b**, 106861-63-6; (-)-**6b**, 106861-66-9; (±)-**6c**, 106861-64-7; **7**, 106780-64-7; (-)-**8**, 106780-65-8; (±)-**8**, 106861-67-0; **9**, 106861-68-1; (±)-**10**, 106780-66-9; **11**, 106780-67-0; (-)-**12**, 106861-69-2; (-)-**13**, 106861-70-5; (-)-**14**, 106861-71-6; (-)-**15**, 40983-58-2; E.C.3.1.1.3, 9001-62-1; E.C.3.1.1.13, 9026-00-0.

Supplementary Material Available: ^1H NMR spectral data for **5a,c-i** and **6c-d** (2 pages). Ordering information is given on any current masthead page.

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Synthesis of Oligophosphopeptides and Related ATP γ-Peptide Esters as Probes for cAMP-Dependent Protein Kinase

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The synthesis of N- and C-terminally blocked heptapeptide substrates for cAMP-dependent protein kinase and the corresponding phosphoheptapeptide products has been accomplished by solution-phase techniques. The chemically synthesized heptapeptides were evaluated as substrates for the enzyme. ATP γ-peptide esters incorporating portions of the peptide substrate sequence also have been synthesized. The synthesis of Ac-Leu-Arg(NO_2)-Arg(NO_2)-Ala-(*O*-phospho)Ser-Leu-Gly-OMe, a potential precursor to the corresponding Arg-containing ATP γ-peptide ester, has been investigated by attempted displacement of peptide mesylates with tetra-*n*-butylammonium di-*tert*-butyl phosphate and by 85% phosphoric acid ring-opening of aziridine peptides.

Introduction

The heptapeptide Leu-Arg-Arg-Ala-Ser-Leu-Gly, also known as the "kemptide", is an excellent substrate for cAMP-dependent protein kinase,[1] being phosphorylated with kinetic parameters comparable to those of natural protein substrates.[2] It has thus received much attention in the last decade and has greatly facilitated the study of this enzyme. The corresponding phosphopeptide, Leu-Arg-Arg-Ala-(*O*-phospho)Ser-Leu-Gly, is important for product identification in the biochemical phosphorylation of the kemptide as well as for product inhibition studies of protein kinase and has thus far been obtained only through enzymatic means.[3,4] The enzymatic syntheses, however, are limited in scale and require additional purification steps. Solid-phase techniques of peptide synthesis, which have been successfully employed in the preparation of Leu-Arg-Arg-Ala-Ser-Leu-Gly,[2] have not been widely used in the synthesis of phosphopeptides.[5] Furthermore, certain commercial solid-phase preparations of the kemptide have been shown to contain significant impurities.[6] We report here the synthesis of the related peptides **1a** and **1b**, in which both the N- and C-termini are blocked, and the corresponding phosphopeptides **2a** and **2b**. The solution-phase methods employed in this work allow for the complete characterization of every intermediate in the synthetic scheme and make large-scale preparations feasible. The availability of blocked peptide kinase substrates and the putative phosphopeptide products should facilitate a variety of kinetic and spectroscopic studies of the protein kinase-catalyzed reaction and allow for a more extensive evaluation of blocked vs. free peptides as kinase substrates.[7,8] We also have investigated the synthesis of the phosphopeptide Ac-Leu-Arg(NO_2)-Arg(NO_2)-Ala-(*O*-phospho)Ser-Leu-Gly-OMe (**3**) as a potential intermediate in the synthesis of ATP γ-peptide esters, which have been proposed as multisubstrate adduct inhibitors of this enzyme.[9]

Ac-Leu-Arg-Arg-Ala-Ser-Leu-Gly-R

1a, R = OMe
b, R = NHMe

Ac-Leu-Arg-Arg-Ala-Ser(OPO$_3$H$_2$)-Leu-Gly-R

2a, R = OMe
b, R = NHMe

Results and Discussion

Synthesis of Peptides. The peptides **1a** and **1b** were synthesized by using a 3 + 4 coupling of their smaller

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