Enantio- and Regiospecific Partial Hydrolysis of Racemic Diol Diacetates by Pig Liver Esterase

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The high enantio- and regiospecificity reported from this laboratory for the hydrolysis of the diacetate (±)-2 with pig liver esterase to yield enantiomericly pure monoacetate (+)-3a has been investigated further to define some of the structural features responsible for this unusual degree of specificity. The hydrolysis of the isomeric (±)-5 was found to proceed with identical specificity both qualitatively and quantitatively, indicating that the enzyme recognizes the overall geometry of these substrates but is unable to distinguish between the CF_2 group and the oxygen bridge. Partial hydrolysis of the monocyclic diacetate (±)-16 showed parallel enantioselectivity but proceeded with only 25% ee. These data indicate that the highly rigid 2 and 5 which present sterically well-defined targets to the enzyme result in the isolation of a single regioisomer and enantiomer as the major products of this reaction. In contrast, the conformational flexibility of 16 leads to poor differentiation between the rates of hydrolysis of the two enantiomers resulting in a low ee. It is concluded that conformationally stable molecules may offer favorable targets for regio and/or enantioselectivity in PLE reactions.

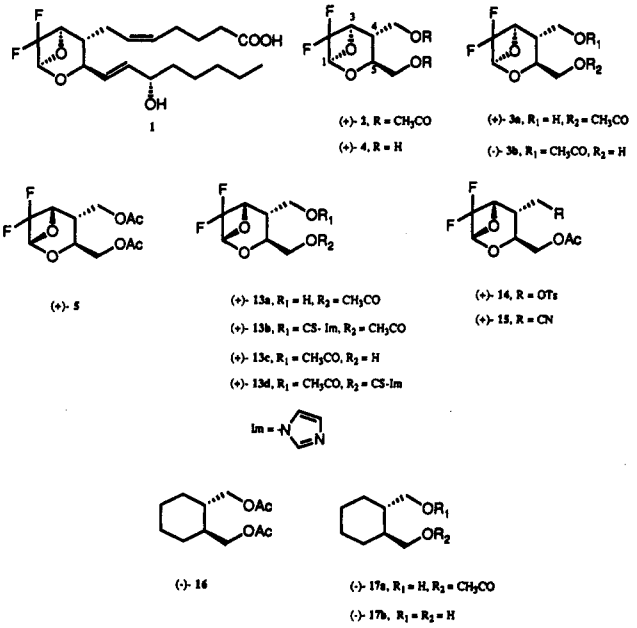
Introduction

The use of enzymes to catalyze reactions demanding high selectivity, particularly in cases of chemically sensitive substrates, has found increasing aceptance by organic chemists.[1,2] Many types of reactions involving either chemoselectivity, regioselectivity, or enantioselectivity have been described and found to be extremely useful in synthesis. The scope of the field is apparent from the excellent review by Jones,[1] himself a major contributor to the field.

In a recent paper[3] we reported the synthesis of enantiomericly pure (+)-10,10-difluorothromboxane A_2 (1) in which a key reaction was the partial hydrolysis of the racemic diol diacetate (±)-2 with pig liver esterase (PLE, E.C.3.1.1.1) to the monoacetate (+)-3a. This reaction, which proceeded in 48% yield with high regio- and enantioselectivity, not only accomplished resolution of the substrate to yield the desired enantiomer with better than 98% ee but also distinguished between the two primary acetoxy substituents, thereby permitting completion of the synthesis without the use of multiple protecting groups (Table I, entry 1). An all-chemical synthesis of 1 has also been reported.[4] It is the purpose of this paper to extend this work to related substrates and to probe the causes for the unusual enantio- and regioselectivity.

While esterases have been widely used to achieve enantiotopic selectivity with prochiral substrates,[1,5] pioneered by the Sih group,[6] and for effecting kinetic resolution of racemic substrates[7] possessing a single stereogenic center, examples of selective hydrolysis of racemates containing two or more stereogenic centers are

Chart I[a]



[a] The prefixes (+) and (-) denote the sign of the rotation of the compounds possessing the absolute configuration shown in the structural drawings. They may not coincide with those of the products used or obtained.

Table I. PLE Hydrolysis of Bis(hydroxymethyl)difluorodioxabicyclo[3.1.1]heptane Diacetates

entry	substrate[a] (mmol)	PLE u/mL	products	mmol	abs config	% ee[b]
1	(±)-2 (2.59)	360	(+)-3a	0.62	(4*R*,5*S*)	100
			(+)-3b	0.23	(4*S*,5*R*)	44[c]
2	(±)-5 (1.43)	360	(+)-13a	0.28	(4*R*,5*S*)	100
			(-)-13c	0.16	(4*S*,5*R*)	39[c]

[a] All concentrations contained 18.5 μm/mL Tris buffer at pH 8.20. Incubations for 10 min at 25 °C. [b] For details see Experimental Section. [c] The error in these values is estimated to be ± 20%. See Experimental Section for details.

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(2) Whitesides, G. M.; Wong, Ch.-H. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 617-638.
(3) Fried, J.; John, V.; Szwedo, M. J., Jr.; Chen, Ch.-K.; O'Yang, C.; Morinelli, T. A.; Okwu, A. K.; Halushka, P. V. *J. Am. Chem. Soc.* **1989**, *111*, 4510-4511.
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(5) Lam, L. K. P.; Brown, Ch. M.; De Jeso, B.; Lym, L.; Toone, E. J.; Jones, J. B. *J. Am. Chem. Soc.* **1988**, *110*, 4409-4411.
(6) Huang, F.-Ch.; Lee, L. F. H.; Mittal, R. S. D.; Ravikumar, P. R.; Chan, J. A.; Sih, Ch. J.; Caspi, E.; Eck, Ch. R. *J. Am. Chem. Soc.* **1975**, *97*, 4144-4145.
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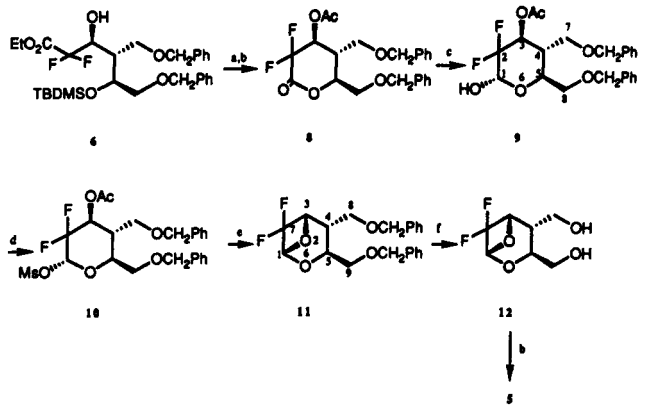
Scheme I[a]



[a] Conditions: (a) HF/CH_3CN; (b) py, Ac_2O; (c) $NaBH_4$; (d) MsCl, py; (e) KOMe; (f) Pd/C.

very rare.[8-10] Even greater is the paucity of examples testing the effectiveness of esterases to catalyze both regio- and enantiospecific hydrolysis of appropriate racemic substrates. Since the first such report describing the selective hydrolysis of diethyl (±)-α-benzylsuccinate to β-ethyl (*R*)-(+)-α-benzylsuccinate with chymotrypsin[11] only one other report has come to our attention. It describes the selective hydrolysis of α-substituted succinates with porcine pancreatic lipase (E.C.3.1.1.3) at the β-ester site with varying degrees of enantioselectivity.[12]

Results and Discussion

In probing the structural features that might be contributing to the high regio- and enantioselectivity in the hydrolysis of (±)-**2**, our first target was the diastereomeric racemate (±)-**5**, in which the two bridgehead carbons of (±)-**2** are of opposite configuration. This racemate is geometrically equivalent to (±)-**2**, but because of the exchange of the oxetane oxygen and the difluoromethylene group these compounds are not equivalent as potential hydrogen bond acceptors in their interactions with the enzyme, a fact which could change the enantiomeric and regiochemical composition of the hydrolysis products.

The synthesis of (±)-**5** is presented in Scheme I. It starts with the difluoro ester (±)-**6**,[13] which was converted to the lactone acetate **8** with HF in acetonitrile followed by acetylation (98% yield). Reduction of **8** with sodium borohydride afforded a single anomeric hemiacetal **9** in 98% yield possessing the 1α-configuration. Mesylation (88%) followed by cyclization with KOMe in MeOH yielded the oxetane **11** in 54% yield. Debenzylation with 10% Pd/C in ethyl acetate afforded the diol **12** which gave the diacetate **5** in 72% yield. It should be noted that the sensitive oxetane acetal did not suffer cleavage under any of the reaction conditions used owing to the effect of the two fluorine atoms.[13]

Table II. PLE Hydrolysis of *trans*-1,2-Bis(hydroxymethyl)cyclohexane Diacetates[a]

entry	substrate[b] (mg)	PLE u/mL	products	mol %	abs confign	% ee[c]
1	(±)-**16** (160)	32	(+)-**16**	75	(*R*,*R*)	6
			(−)-**17a**	25	(*S*,*S*)	36
2	(−)-**17a**, 25% ee (64)	64	(−)-**17a**	48	(*S*,*S*)	7
			(−)-**17b**	52	(*S*,*S*)	17
3	(+)-**16**, 12% ee (74)	64	(+)-**16**	42	(*R*,*R*)	24
			(+)-**17a**	45	(*R*,*R*)	7
			(±)-**17b**	13		0

[a] Conditions as described for (+)-**5** in the Experimental Section except for changes indicated in this table. [b] All concentrations contained 19.3 μm/mL Tris buffer at pH 8.20. Incubations for 10 min at 25 °C. [c] The error in these values is estimated to be ±20%. See Experimental Section for details.

Incubation of (±)-**5** with PLE gave as the major reaction product in 42% yield the enantiomericly pure monoacetate (+)-**13a**, together with the isomeric, partially racemic monoacetate (−)-**13c** and diol (−)-**12** (Table I, entry 2). It is interesting to note that these latter two compounds showed ee's of 39% and 56% favoring the opposite configuration. The absolute configuration of (+)-**13a**, and therefore of (−)-**13c** and (−)-**12**, was established by conversion of (+)-**13a** to the cyano acetate (+)-**15**, which was identical by ^{1}HNMR and specific rotation with the product obtained by independent synthesis.[4]

The absolute configuration of (+)-**13a** is thus identical with that of (+)-**3a** with regard to the two carbons bearing the acetoxymethyl substituents, and the same is true for (+)-**3b** and (−)-**13c** (Table I and Experimental Section). Moreover, the regiochemistry of the two reactions as well as the ratio and ee of products are likewise identical. One may conclude with confidence that it is the overall geometry of the substrate which is recognized by the enzyme with regard to both enantio- and regioselection, and that specific hydrogen bonding interactions between enzyme and substrate play no significant role in determining rates of hydrolysis. The lack of relevance of the relative stereochemistry of the bridgehead carbons suggests that the presence of the two remaining stereogenic centers is sufficient to determine the observed selectivity.[14]

An appropriate substrate to examine this question is the diacetate (±)-**16**, which lacks the oxygen bridge and contains only the two centers to which the *trans*-acetoxymethyl groups are attached. Since these two groups are of the same chirality, they are, of course, regiochemically indistinguishable. Fortunately, the absolute configuration of the diol **17b** is known,[15] which would permit correlation of the results with those obtained with the oxetanes (±)-**2** and (±)-**5**. When (±)-**16** was incubated with PLE (Table II) the *S*,*S*-enantiomer was hydrolized more rapidly yielding the *S*,*S*-enantiomer (−)-**17a** with an ee of 36% and leaving the R,R-enantiomer (+)-**16** with ee = 6% at 25% conversion (entry 1). This is precisely the enantioselectivity observed for the two oxetane substrates (±)-**2** and (±)-**5**, the important difference being the resistance of (+)-**3a** and (+)-**13a** to further hydrolysis, resulting in the isolation of these monoacetates in high

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(13) Fried, J.; Hallinan, E. A.; Szwedo, M. J., Jr. *J. Am. Chem. Soc.* **1984**, *106*, 3871–3872.
(14) It is noteworthy but not unexpected that the isomer of 10,10-difluorothromboxane A_2 possessing the 1β,3β-oxido structure proved to be a partial agonist; that is, it showed significant binding to the TXA_2 receptor but only weak agonist activity, cf. ref 4.
(15) The optically active diols **17b** were first described by: Haggis, G. H.; Owen, L. N. *J. Chem. Soc.* **1953**, 389–398. Their absolute configuration was determined by: Applequist, D. E.; Warner, N. D. *J. Org. Chem.* **1963**, 48–54.

yield and in enantiomericly pure form. The basic cyclohexane structure thus appears to account for the preference of attack of the (S,S) enantiomer. It is interesting to note that the rate of hydrolysis of (−)-**17a** is greater than that of (+)-**17a** as shown by the decrease in ee for (−)-**17a** from 25 to 7% and a corresponding increase for (−)-**17b** when partially resolved (−)-**17a** is reincubated with PLE (entry 2). As expected, reincubation of partially resolved diacetate (+)-**16** serves to increase its ee from 12 to 24% (entry 3).

It is, of course, tempting to speculate on the reasons for the exceptional success with the oxetane substrates (±)-**2** and (±)-**5**. These two substrates possess very rigid structures while the cyclohexane derivative (±)-**16** presents appreciable conformational flexibility. This latter fact permits considerable ambiguity with regard to the interaction between substrate and enzyme, whereby the transition states involving one conformer of the (+) antipode and one involving another conformer of the (−) antipode are close enough in energy to wipe out significant differences in rate between enantiomers. The conformationally rigid oxetanes present well-defined targets resulting in better differentiated rates of hydrolysis. We conclude that conformationally stable racemic molecules offer good opportunities for achieving high regio and enantioselecivity in PLE reactions.

Experimental Section: Methods and Instrumentation[4]

(3*SR*,4*RS*,5*SR*)-1-Oxo-2,2-difluoro-3β-hydroxy-4α,5β-bis[(benzyloxy)methyl]-1,5-oxidopentane (7). A solution of the difluoro ester **6** (0.90 g, 1.63 mmol) in 6 mL of 48% aqueous HF/CH_3CN (1:19 by volume) was stirred at room temperature for 2 h. It was then quenched to give 0.620 g of **7** (97%). Attempts to purify the lactone led to opening during chromatography. The purity of the sample, as shown by proton (500 MHz) NMR, was found to be >95%. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.40–7.15 (m, 10 H, 2 phenyls); 4.62 (m, 1 H, H-5), 4.60 and 4.43 (dd, 2 H, CH_2-benzyl, J_{gem} = 11.9 Hz), 4.50 (m, 2 H, CH_2-benzyl), 4.32 (dt, 1 H, H-3, $J_{H,F}$ = 12.4 Hz, $J_{H,F}$ = 9.0 Hz, $J_{3,4}$ = 4.0 Hz); 3.75 (m, 2H, H-8 and H-7); 3.62 (dd, 1 H, H-8′, J_{gem} = 11.5 Hz, $J_{5,7'}$ = 3.3 Hz), 3.50 (dd, 1 H, H-7′, J_{gem} = 9.6 Hz, $J_{4,7'}$ = 2.4 Hz), 2.58 (m, 1 H, H-4). ^{19}F-NMR ($CDCl_3$, 376.2 Hz): φ 113.9 (dd, $J_{F,F}$ = 281.4 Hz, $J_{H,F}$ = 9.4 Hz), 119.1 (dd, $J_{F,F}$ = 281.4 Hz, $J_{H,F}$ = 12.4 Hz).

(3*SR*,4*RS*,5*SR*)-1-Oxo-2,2-difluoro-3β-acetoxy-4α,5β-bis[(benzyloxy)methyl]-1,5-oxidopentane (8). To a solution of lactone **7** (0.620 g, 1.58 mmol) in pyridine (2.87 g, 36.37 mmol) was added acetic anhydride (0.843 g, 8.27 mmol) dropwise under nitrogen, and the resulting reaction mixture was allowed to stand overnight under nitrogen. It was diluted with ether (50 mL) to give 0.680 g of **8** (99%). Attempts to purify the lactone led to opening during chromatography. The purity of the sample, as shown by proton (500 MHz) NMR, was found to be >95%. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.40–7.10 (m, 10H, 2 phenyls), 5.60 (dt, $J_{H,F}$ = 10.3 Hz, $J_{3,4}$ = 8.2 Hz, 1 H, H-3); 4.70 (dt, 1 H, H-5, J = 10.6 Hz); 4.60 and 4.48 (dd, 2 H, CH_2-benzyl, J_{gem} = 11.8 Hz), 4.43 and 4.42 (dd, 2 H, CH_2-benzyl, J_{gem} = 11.9 Hz), 3.78 (dd, 1 H, H-8, J_{gem} = 11.7 Hz, $J_{5,8}$ = 2.1 Hz), 3.64 (dd, 1 H, H-8′, J_{gem} = 11.7 Hz, $J_{5,8'}$ = 3.2 Hz), 3.51 (dd, 1 H, H-7, J_{gem} = 9.9 Hz, $J_{4,7}$ = 3.4 Hz), 3.45 (dd, 1 H, H-7′, J_{gem} = 9.9 Hz, $J_{4,7'}$ = 3.1 Hz), 2.69 (m, 1 H, H-4), 1.10 (s, 3 H, OAc). ^{19}F-NMR ($CDCl_3$, 376.2 Hz): φ 112.0 (dd, $J_{F,F}$ = 282.7 Hz, $J_{H,F}$ = 10.2 Hz), 118.0 (dd, $J_{F,F}$ = 282.7 Hz, $J_{H,F}$ = 10.2 Hz).

(1*RS*,3*SR*,4*RS*,5*SR*)-1α-Hydroxy-2,2-difluoro-3β-acetoxy-4α,5β-bis[(benzyloxy)methyl]-1,5-oxidopentane (9). To a solution of acetate **9** (0.680 g, 1.56 mmol) in 15 mL of methanol was added sodium borohydride (0.238 g, 6.26 mmol) in four portions. The resulting reaction mixture was stirred at room temperature for 30 min. It was quenched with saturated NaCl solution (10 mL) to give crude hemiacetal **9** (0.668 g, 98%) which was used without further purification. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.40–7.20 (m, 10 H, 2 phenyls), 5.76 (ddd, 1 H, H-3, J_{H_3,F_a} = 21.2 Hz, J_{H_3,F_e} = 11.5 Hz, $H_{3,4}$ = 4.2 Hz), 5.24 (d, 1 H, H-1, $J_{H,F}$ = 5.7 Hz) ,4.62 and 4.47 (dd, 2 H, CH_2-benzyl, J_{gem} = 12.2 Hz), 4.45 (m, 1 H, H-5), 4.35 (s, 2 H, CH_2-benzyl), 3.69 (dd, 1 H, H-8, J_{gem} = 11.1 Hz, $J_{5,8}$ = 1.8 Hz), 3.62 (dd, 1 H, H-8′, J_{gem} = 11.1 Hz, $J_{5,8'}$ = 4.8 Hz), 3.30 (bs, 2 H, H-7, H-7′), 2.32 (m, 1 H, H-4), 2.08 (s, 3 H, OAc). ^{19}F NMR ($CDCl_3$, 376.2 Hz): φ 122.37 (dd, $J_{F,F}$ = 247.4 Hz, $J_{H,F}$ = 4.3 Hz), 124.30 and 125.0 (dd, $J_{H,F}$ = 247.6 Hz, $J_{H,F}$ = 5.7 Hz). Anal. Calcd for $C_{23}H_{26}F_2O_6$: C, 63.29; H, 6.01. Found; C, 63.09; H, 5.95.

(1*RS*,3*SR*,4*RS*,5*SR*)-1α-(Mesyloxy)-2,2-difluoro-3β-acetoxy-4α,5β-bis[(benzyloxy)methyl]-1,5-oxidopentane (10). To a solution of hemiacetal **9** (0.668 g, 1.53 mmol) in pyridine (3.145 g, 39.83 mmol) was added 0.3 g of 4-Å molecular sieve powder, and the mixture was stirred under nitrogen for 1.5 h at 22 °C. Methanesulfonyl chloride (0.995 g, 8.73 mmol) was added, and the reaction mixture was allowed to stir at 22 °C overnight. Ether was added, and the mixture was filtered through a Celite pad. The filtrate was concentrated and then passed through silica gel (pipette column) eluting with ether. Crude **10** was obtained by concentrating the ether extract (0.690 g, 88%). Purification could not be performed as the compound was found to be unstable. The purity of the sample, as shown by proton (500 MHz) NMR, was found to be >90%. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.40–7.20 (m, 10 H, 2 phenyls), 5.90 (d, 1 H, H-1, $J_{H,F}$ = 4.9 Hz), 5.68 (ddd, 1 H, H-3, J_{H_3,F_a} = 19.5 Hz, J_{H_3,F_e} = 11.6 Hz, $J_{3,4}$ = 5.7 Hz), 4.59 and 4.43 (dd, 2 H, CH_2-benzyl, J_{gem} = 11.9 Hz), 4.35 (m, 3 H, H-5 and CH_2-benzyl), 3.70 (dd, 1 H, H-8, J_{gem} = 11.3 Hz, $J_{5,8}$ = 1.6 Hz), 3.60 (dd, 1 H, H-8′, J_{gem} = 11.3 Hz, $J_{5,8'}$ = 4.2 Hz), 3.29 (bs, 2 H, H-7, H-7′) 3.13 (s, 3 H, $-SO_2CH_3$), 2.40 (m, 1 H, H-4), 2.07 (s, 3 H, OAc). A signal at δ 5.53 (ddd) indicates 5–10% of the 1β-anomer. ^{19}F NMR ($CDCl_3$, 376.2 Hz): φ 121.51 (dd, $J_{F,F}$ = 245.4 Hz, $J_{H,F}$ = 6.3 Hz), 122.2 and 122.8 (dd, $J_{F,F}$ = 254.3 Hz, $J_{H,F}$ = 5.1 Hz).

(1*SR*,3*SR*,4*RS*,5*SR*)-4,5-Bis[(benzyloxy)methyl]-7,7-difluoro-2,6-dioxabicyclo[3.1.1]heptane (11). To a solution of mesylate **10** (0.690 g, 1.34 mmol) in 10 mL of methanol was slowly added a solution of KOMe (0.375 g, 5.36 mmol) in 8 mL of methanol. The solution was then stirred under nitrogen at room temperature for 2 h. It was quenched with water and extracted with hexane. The crude material thus obtained was purified by flash column chromatography using hexane/ethyl acetate (80:20) as eluant to give 0.273 g of pure **11** (54%). The overall yield from **7** to **11** was 44.5% over the five steps. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.35–7.20 (m, 10 H, 2 phenyls), 5.61 (m, 1 H, H-1), 4.93 (m, 1 H, H-3), 4.60 and 4.61 (d's, 2 H, CH_2-benzyl, J_{gem} = 12.2 Hz), 4.47 and 4.46 (dd, 2 H, CH_2-benzyl, J_{gem} = 12.0 Hz), 4.18 (m, 1 H, H-5), 3.65 (d, 2 H, H-9, H-9′), 3.50 (m, 2 H, H-8, H-8′), 2.89 (m, 1 H, H-4). ^{19}F NMR ($CDCl_3$, 376.2 Hz): φ 103.91 (d, $J_{F,F}$ = 191.1 Hz), 132.87 (dm, $J_{F,F}$ = 190.7 Hz). Anal. Calcd for $C_{21}H_{22}F_2O_4$: C, 67.01; H, 5.89. Found: C, 67.14; H, 6.89.

(1*SR*,3*SR*,4*RS*,5*SR*)-4,5-Bis(hydroxymethyl)-7,7-difluoro-2,6-dioxabicyclo[3.1.1]heptane (12). To a suspension of 10% palladium on carbon (400 mg) in 4 mL of ethyl acetate was added the dibenzyl ether **11** (0.40 g, 1.06 mmol), and after evacuation, hydrogen was admitted. After 4 h of stirring at room temperature, 100% of the stoichiometric amount of hydrogen was consumed. Filtering through a short pipette column of Celite and elution with ethyl acetate followed by removal of the solvent gave **12** as a syrupy liquid (0.19 g, 91%). Use of 2-propanol as solvent in the hydrogenation led to partial alcoholysis of the oxetane ring. ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 5.60 (m, 1 H, H-1), 4.95 (m, 1 H, H-3), 4.17 (m, 1 H, H-5), 3.88–3.67 (m, 4 H, two CH_2OH), 2.93 (m, 1 H, H-4), 2.50 (m, 2 H, OH). ^{19}F-NMR ($CDCl_3$, 376.2 MHz): φ 114.98 (d, $J_{F,F}$ = 190.8 Hz), 143.57 (dm, $J_{F,F}$ = 191.6 Hz). Anal. Calcd for $C_7H_{10}F_2O_4$: C, 42.86; H, 5.14. Found: C, 42.64; H, 5.23.

(1*SR*,3*SR*,4*RS*,5*SR*)-4,5-Bis(acetoxymethyl)-7,7-difluoro-2,6-dioxabicyclo[3.1.1]heptane (5). To a solution of diol **12** (0.400 g, 2.04 mmol) in triethylamine (20.6 g, 204 mmol) was added acetic anhydride (2.08 g, 20.4 mmol) in 25 mL of CH_2Cl_2 dropwise over 30 min. The mixture was stirred for 1.5 h at room

temperature and then washed with water and sodium bicarbonate. The dried CH_2Cl_2 extract was passed through a short silica gel pipette column eluting with hexane/ethyl acetate (80:20) to give pure diacetate **5** as a colorless oil (0.41 g, 72%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.61 (m, 1 H, H-1), 4.90 (m, 1 H, H-3), 4.38 (dd, 1 H, H-9, J_{gem} = 11.7 Hz, $J_{5,9}$ = 2.9 Hz), 4.28 (m, 1 H, H-5), 4.17 (dd, 2 H, H-8, H,8′), 4.09 (dd, 1 H, H-9′, J_{gem} = 11.7 Hz, $J_{5,9'}$ = 5.0 Hz), 2.82 (m, 1 H, H-4), 2.14 (s, 3 H, OAc), 2.12 (s, 3 H, OAc). ^{19}F NMR ($CDCl_3$, 376.2 MHz): ϕ 102.9 (dbs, $J_{F,F}$ = 194.5 Hz), 132.41 (dt, $J_{F,F}$ = 195.6 Hz, $J_{H,F}$ = 5.9 Hz). Anal. Calcd for $C_{11}H_{14}F_2O_6$: C, 47.14; H, 5.03. Found: C, 47.14; H, 4.89.

PLE Hydrolysis of (±)-5. The diacetate (±)-**5** (0.400 g, 1.42 mmol) in 1.33 mL of absolute EtOH, gum Arabic (0.667 g), and NaCl (66.7 mg) were placed in a 250-mL round-bottom flask. To this was added 77.2 mL of 0.1 M Tris buffer (pH = 8.20, prepared from (trihydroxymethyl)methylamine in deionized water and the pH adjusted by adding concd HCl). The resulting mixture was stirred vigorously at 25 °C (water bath), and porcine liver esterase (4.7 mL, 29 916 units, Sigma) was added. The solution was stirred for precisely 10 min, and the reaction was terminated by adding ethyl acetate (100 mL). The residue was purified by flash chromatography using hexane/ethyl acetate (60:40) as eluant to separate the mixture of isomeric monoacetates (+)-**13a** and (−)-**13c** (105 mg, 31%) from the partially resolved diol (−)-**12**, which was collected by elution with ethyl acetate (175 mg, 62.5%).

Separation of the Monoacetates (+)-13a and (−)-13c as Their Thiocarbonylimidazole Derivatives (+)-13b and (−)-13d. The mixture of monoacetates (+)-**13a** and (−)-**13c** (0.105 g, 0.441 mmole) was dissolved in 1.8 mL of dry benzene, and to this solution was added 1,1′-thiocarbonyldiimidazole (0.197 g, 1.10 mmol) with stirring at room temperature under nitrogen. After 3 h the solvent was removed and the residue purified by flash column chromatography using hexane/ethyl acetate (85:15) as eluant. One-mL fractions were collected, and (−)-**13d** emerged first and was collected in fractions 20–30 (43 mg) while (+)-**13b** was collected in fractions 39–55 (65 mg). Fractions 31–38 gave a mixture of (+)-**13b** and (−)-**13d** which was purified by preparative TLC using hexane/EtOAc (9:1) as eluant. After four developments the bands were eluted with ethyl acetate and concentrated to give 19 mg of (+)-**13b** and 7 mg of (−)-**13d**. Total yield of (+)-**13b** and (−)-**13d** was 134 mg (88%).

13b: $[\alpha]^{20}_D$ = +21.87° (c = 1.71 in $CHCl_3$). Combustion analysis could not be performed as the compound was found to be unstable. The purity of the sample, as shown by proton (500 MHz) NMR, was found to be >95%. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.34 (s, 1 H, imidazole-H), 7.61 (s, 1 H, imidazole-*H*), 7.07 (s, 1 H, imidazole-*H*), 5.67 (m, 1 H, H-1), 4.98 (m, 1 H, H-3), 4.76, 4.72 (two dd, 1 H each, H-8, H-8′, J_{gem} = 11.7 Hz, $J_{4,8}$ = 5.7 Hz, $J_{4,8'}$ = 8.0 Hz), 4.40 (m, 2 H, H-5, H-9), 4.25 (dd, 1 H, H-9′, J_{gem} = 11.3 Hz, $J_{5,9'}$ = 5.6 Hz), 3.14 (m, 1 H, H-4), 2.15 (s, 3 H, OAc). ^{19}F NMR ($CDCl_3$, 376.2 MHz): ϕ 103.03 (d, $J_{F,F}$ = 192.6 Hz), 132.96 (dm, $J_{F,F}$ = 192.6 Hz).

13d: $[\alpha]^{20}_D$ = −15.12° (c = 1.10 in $CHCl_3$). Combustion analysis could not be performed as the compound was found to be unstable. The purity of the sample, as shown by proton (500 MHz) NMR, was found to be >95%. ^{1}HNMR ($CDCl_3$, 500 MHz): δ 8.40 (s, 1 H, imidazole-*H*), 7.68 (s, 1 H, imidazole-*H*), 7.04 (s, 1 H, imidazole-*H*), 5.65 (m, 1 H, H-1), 4.95 (m, 1 H, H-3), 4.90 (dd, 1 H, H-9, J_{gem} = 11.8 Hz, $J_{5,9}$ = 2.3 Hz), 4.72 (dd, 1 H, H-9′, J_{gem} = 11.8 Hz, $J_{5,9'}$ = 5.2 Hz), 4.52 (m, 1 H, H-5), 4.17 (m, 2 H, H-8, H-8′), 2.98 (m, 1 H, H-4), 2.13 (s, 3 H, OAc). ^{19}F NMR ($CDCl_3$, 376.2 MHz): ϕ 103.03 (d, $J_{F,F}$ = 192.6 Hz), 132.96 (dm, $J_{F,F}$ = 192.6 Hz).

(1*S*,3*S*,4*R*,5*S*)-4-(Hydroxymethyl)-5-(acetoxymethyl)-7,7-difluoro-2,6-dioxabicyclo[3.1.1]heptane ((+)-13a). A solution of the imidazolyl ester (+)-**13b** (68 mg, 0.195 mmol) was dissolved in 27.2 mL of 0.2 M $NaHCO_3$ (1:1 H_2O/THF by volume) and stirred for 8 h at room temperature. It was then extracted with CH_2Cl_2, and the residual material was purified by flash chromatography using hexane/ethyl acetate (70:30) as eluant to give pure alcohol (+)-**13a** as a colorless oil (46 mg, 100%), $[\alpha]^{23}_D$ = +24.51° (c = 0.45 in $CHCl_3$). Reexposure of this material to PLE for 10 min raised the $[\alpha]^{23}_D$ to +26.3°. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.61 (m, 1 H, H-1), 4.95 (m, 1 H, H-3), 4.40 (dd, 1 H, H-9, J_{gem} = 11.7 Hz, $J_{5,9}$ = 2.76 Hz), 4.28 (m, 1 H, H-5), 4.20 (dd, 1 H, H-9′, J_{gem} = 11.7 Hz, $J_{5,9'}$ = 6.6 Hz), 3.73 (m, 2 H, H-8 and H-8′), 2.74 (m, 1 H, H-4), 2.14 (s, 3 H, OAc). ^{19}F NMR ($CDCl_3$, 376.2 MHz): ϕ 103.05 (dm, $J_{F,F}$ = 190.7 Hz), 132.38 (dt, $J_{F,F}$ = 190.5 Hz, $J_{H,F}$ = 5.5 Hz). High-resolution MS: calcd for $C_7H_8O_3F_2$ (M − CH_3CO_2H, 8), 178.0441; m/z, 178.0430.

(1*S*,3*S*,4*R*,5*S*)-4-(Acetoxymethyl)-5-(hydroxymethyl)-7,7-difluoro-2,6-dioxabicyclo[3.1.1]heptane ((−)-13c). Compound (−)-**13c** (3.0 mg) was obtained as a colorless oil from **13d** (5.2 mg) as described above for the preparation of (+)-**13a**. (−)-**13c**, $[\alpha]^{20}_D$ = −11.25° (c = 0.30 in $CHCl_3$). Reincubation with PLE for 10 min leads to complete racemization. This compound is of only ancillary importance in the context of this manuscript. There was not enough material for analysis. However, the purity of the sample was found to be greater than 90% by proton (500 MHz) NMR. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.62 (m, 1 H, H-1), 4.91 (m, 1 H, H-3), 4.19 (m, 1 H, H-5), 4.12 and 4.09 (two dd, 1 H each, J_{gem} = 11.7 Hz, $J_{4,8}$ = 6.1 Hz, $J_{4,8'}$ = 7.9 Hz), 3.85 (m, 1 H, H-9), 3.68 (m, 1 H, H-9′), 3.04 (m, 1 H, H-4), 2.10 (s, 3 H, OAc). ^{19}F-NMR ($CDCl_3$, 376.2 MHz): ϕ 102.74 (dd, $J_{F,F}$ = 192.8 Hz, $J_{H,F}$ = 4.2 Hz), 132.51 (dt, $J_{F,F}$ = 189.9 Hz, $J_{H,F}$ = 6.0 Hz).

Reincubation of Partially Resolved Diol 12 with PLE. The diol **12** from PLE hydrolysis of (±)-**5** [0.117 g, $[\alpha]^{20}_D$ = −5.3° (c = 1.02 in $CHCl_3$)] was reacetylated as described earlier. The resulting (−)-**5** was resubjected to PLE hydrolysis to give 26 mg of monohydroxyacetates (+)-**13a** and (+)-**13c** and 49 mg of diol **12**. The monohydroxyacetates were separated via their thiocarbonylimidazole derivatives to give 17.2 mg of (+)-**13b**, $[\alpha]^{20}_D$ = +20.70° (c = 1.71 in $CHCl_3$), ee = 90%, and 11.1 mg of (−)-**13d**, $[\alpha]^{20}_D$ = −14.7° (c = 1.11 in $CHCl_3$).

Specific Rotation of Pure Enantiomers of Oxetane Derivatives from PLE Hydrolyses. To determine the ee of partially resolved reaction products from the PLE hydrolyses of (±)-**2** and (±)-**5** it was necessary to determine the $[\alpha]_D$ of the pure enantiomers. Since the monoacetates (+)-**3a** and (+)-**13a** are available in enantiomericly pure form of known absolute configuration it was possible to determine the $[\alpha]_D$ of the isomeric monoacetates by hydrolysis or acetylation. $[\alpha]^{23}_D$ ($CHCl_3$): **3a** (4*R*,5*S*), +49.7°; **3b** (4*S*,5*R*), +29.5°; **4** (4*R*,5*S*), +18.1°; **2** (4*R*,5*S*), +23.8°; **13a** (4*R*,5*S*), +25.4°; **13c** (4*S*,5*R*), −28.7°; **12** (4*R*,5*S*), +9.5°; **5** (4*R*,5*S*), +27.3°. The above values were used to estimate the ee values for the PLE products (+)-**3b** and (−)-**13c** shown in Table I.

(1*S*,3*S*,4*R*,5*S*)-4-[[(*p*-Toluenesulfonyl)oxy]methyl]-5-(acetoxymethyl)-7,7-difluoro-2,6-dioxabicyclo[3.1.1]heptane ((+)-14). To a solution of the alcohol (+)-**13a** (46.0 mg 0.193 mmol) in pyridine (4.91 g, 62.25 mmol) was added *p*-toluenesulfonyl chloride (285 mg, 1.5 mmol), (dimethylamino)pyridine (241 mg, 1.935 mmol), and 4-Å molecular sieve powder (150 mg) and the reaction mixture stirred at room temperature for 36 h. It was worked up with ether and water. Pure tosylate (+)-**14** was obtained as a colorless oil (65 mg, 86.6%), $[\alpha]^{20}_D$ = +21.93° (c = 0.89 in $CHCl_3$). Combustion analysis could not be performed as the compound was found to be unstable. The purity of the sample, as shown by proton (500 MHz) NMR, was found to be >95%. ^{1}H NMR (CDCl3, 500 MHz) δ 7.78 and 7.37 (AB, 4 H, phenyl, J_{AB} = 8.2 Hz), 5.58 (m, 1 H, H-1), 4.85 (m, 1 H, H-3), 4.25 (d q, 1 H, H-5, J = 6.5 Hz), 4.15–4.02 (m, 4 H, H-8, H-8′, H-9, H-9′), 2.90 (m, 1 H, H-4), 2.48 (s, 3 H, SO_2CH_3), 2.13 (s, 3 H, OAc). ^{19}F-NMR ($CDCl_3$, 376.2 MHz): ϕ, 103.38 (d, $J_{F,F}$ = 192.4 Hz), 132.73 (dt, $J_{F,F}$ = 192.6 Hz, $J_{H,F}$ = 6.1 Hz).

(1*S*,3*S*,4*R*,5*S*)-4-(Cyanomethyl)-5-(acetoxymethyl)-7,7-difluoro-2,6-dioxabicyclo[3.1.1]heptane ((+)-15). A solution of tosylate (+)-**14** (35.2 mg, 0.089 mmol) and sodium cyanide (52.0 mg, 1.06 mmol) in DMF (0.8 mL) was stirred under nitrogen at 42 °C for 36 h. The reaction was worked up by trituration with ether and then filtering, and the resulting precipitate was obtained through a layer of Celite. The filtrate was worked up and flash chromatographed over silica gel eluting with hexane/ethyl acetate (70:30) to give pure nitrile (+)-**15** (18.4 mg, 83%), $[\alpha]^{20}_D$ = +27.01° (c = 1.21 in $CHCl_3$). The proton and fluorine NMR spectra as well as the value and sign of the specific rotation are identical with those described in ref 4. This establishes the absolute configuration of **13a–d**, **14**, and **15**.

Specific Rotation of Pure Enantiomers of 17a and 17b. Since the absolute configuration of the diol **17g**, $[\alpha]_D$ = −20.2° (benzene), was known to be (*S*,*S*),[14] it was possible to determine the absolute configuration of the partially resolved products (+)-

16 and (−)-17a obtained in the PLE reaction by hydrolysis and acetylation. Enantiomericly pure (*R*,*R*)-16: $[\alpha]_D$ = +35.0° (benzene). (*S*,*S*)-17a: $[\alpha]_D$ −17.3° (benzene). These values were used to calculate the ee values shown in Table II. It is realized that the ee values derived from specific rotations are subject to considerable error. This is particularly true since three rotation values are involved in each ee calculation. We estimate the error to be in the order of ±20% of the values shown in Table II. This in no way affects the thrust of this paper, which contrasts the low ee value for (+)-16 with the high enantiomeric purity achieved for the major products derived from (+)-2 and (+)-5.

Supplementary Material Available: Proton (500-MHz) NMR spectra of compounds 7, 8, 10, (+)-13a, (+)-13b, (−)-13c, (−)-13d, and (+)-14 (8 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.