130 °C, retention time 3 min) to give **17**: ^{1}H NMR ($CDCl_3$) δ 2.42 (s, 3, CH_3), 7.30 (s, 4, Ar); IR (CCl_4) 1823 cm^{-1} (C=O); mass spectrum *m/e* (rel intensity) 354 (98, M^+), 285 (100, $M^+ - CF_3$) 257 (74, $M^+ - CF_3CO$), 241 (46, $M^+ - CF_3CO_2$). The unidentified material showed singlets at δ 5.38 and 7.42 ($CDCl_3$, rel intensity 1:2) but was not examined further.

Similar treatment of **11** gave **18**: ^{1}H NMR (CCl_4) δ 7.0–8.0 (m, Ar); IR (CCl_4) 1830 cm^{-1} (C=O). And **12** gave **19**: ^{1}H NMR (CCl_4) δ 7.46 (s, Ar); IR (CCl_4) 1820 cm^{-1} (C=O).

The analogous reaction of **14** gave **21**: ^{1}H NMR (CCl_4) δ 7.60 (s, Ar); IR (CCl_4) 1825 cm^{-1} (C=O). An authentic sample of **21** was prepared by the reaction of $PhC(CF_3)(CN)OH$[4b] (1 mmol) with 0.75 mL of $(CF_3CO)_2O$ and a drop of pyridine for 2 days at 0 °C followed by evaporation of the solvent.

Trifluoroethanolysis of 13. A solution of **13** (80 mg, 0.22 mmol) and 20 mg (0.32 mmol) of urea in 10 mL of TFE was kept 5 days at 25 °C, poured into ice water–ether, and extracted with ether; the extract was then dried and evaporated and purified by VPC (OV 17 column, 170 °C, retention time 15 min) to give *p*-tolyl-1-cyano-2,2,2-trifluoroethyl 2,2,2-trifluoroethyl ether (**20**) as the only observed product: ^{1}H NMR ($CDCl_3$) δ 2.41 (s, 3, CH_3), 3.7–4.0 (m, 2, CH_2CF_3), 7.2–7.6 (q, 4, Ar); mass spectrum *m/e* (rel intensity) 298 (82, $M^+ + 1$), 229 (86, $M^+ - CF_3$), 199 (43, $M^+ - CF_3CH_2O$), 119 (100, $TolCO^+$). Anal. Calcd for $C_{12}H_9F_6NO$ (297.21): C, 48.50; H, 3.05; N, 4.71. Found: C, 48.47; H, 3.25; N, 4.56.

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Appendix: Calculation of *k*(OTs)/*k*(OPNB) Ratios

The rate constant for solvolysis of *p*-AnisCH(OPNB)CH_3 (**36**) at 25 °C in 70% acetone is 4.53×10^{-7} s^{-1} (extrapolated from data[7a] at higher temperature) and a rate constant for *p*-AnisCH(OTs)CH_3 (**37**) under the same conditions of 2.6×10^2 s^{-1} is obtained from the *mY* correlation $\log K = 1.23 Y_{OTs} + 1.95$ for **37** derived from calculated log *k* values of **37** in 97% HFIP and 97% TFE of 6.39 and 4.20, respectively. These log *k* values are obtained from the relations $\log k = -5.94\sigma^+ + 1.76$ (97% HFIP) and $\log k = -5.05\sigma^+ + 0.26$ (97% TFE) for ArCH(OTs)CH_3[7b] and the Y_{OTs} value of 0.38 for 70% acetone extrapolated from published data for 10 to 60% acetone.[8] These values give *k*(OTs)/*k*(OPNB) = 5.7×10^8.

Combination of the rate ratios *k*(*t*-BuCl)/*k*(*t*-BuOPNB) = 2.6×10^4 (80% acetone)[10a,b] and *k*(*exo*-2-NbOTs)/*k*(*exo*-2-NbCl) = 1.7×10^3 (60% acetone, Nb = norbornyl)[10c,d] gives *k*(OTs)/*k*(OPNB) = 4.4×10^7. Combination of the rate ratio *k*(*t*-BuCl)/*k*(*t*-BuOPNB) = 2.6×10^4 (80% acetone)[10a,b] and *k*(*endo*-2-NbOTs)/*k*(*endo*-2-NbCl) = 36 (60% acetone)[10c,d] gives *k*(OTs)/*k*(OPNB) = 9.4×10^6. A rate ratio *k*(OTs)/*k*(OPNB) = 2×10^9 may be derived from other reported comparisons.[11]

Organic Synthesis with Enzymes. 3.[1] TBADH-Catalyzed Reduction of Chloro Ketones. Total Synthesis of (+)-(*S*,*S*)-(*cis*-6-Methyltetrahydropyran-2-yl)acetic Acid: A Civet Constituent

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Abstract: Highly enantioselective reduction of aliphatic chloro ketones catalyzed by *Thermoanaerobium brockii* alcohol dehydrogenase (TBADH) afforded the corresponding *S* chloro alcohols, which are new and useful chiral bifunctional building blocks. The synthetic potential of these compounds was illustrated by syntheses of several optically pure cyclic ethers. In particular, (*S*)-(+)-5-chloropentan-2-ol was used for the total synthesis of (+)-(*S*,*S*)-(*cis*-6-methyltetrahydropyran-2-yl) acetic acid, a natural constituent of the perfume material civet. Two of the key steps in the synthesis involve organopalladium chemistry: a Pd(0)-catalyzed intramolecular allylic etherification followed by a Pd(II)-catalyzed Wacker oxidation of the disubstituted olefin.

A most general and reliable approach to the total synthesis of optically active compounds takes advantage of chiral starting materials that are readily available from natural sources. Carbohydrates, tartaric acid, malic acid, lactic acid, amino acids, etc., have been extensively used as a "chiral pool"[2] for preparing a variety of highly functionalized chiral building blocks (chirons[3]). Interestingly, simple aliphatic chirons are less readily available from the above-mentioned chiral pool compounds, as their preparation usually requires multistep removal of functional groups. Enzymes present attractive alternatives for the preparation of new chiral compounds that are not easily derived from natural products.

In the preceding paper we introduced a wide variety of chiral aliphatic secondary alcohols that were efficiently produced by *Thermoanaerobium brockii* alcohol dehydrogenase (TBADH)[4] catalyzed reduction. However, since most of these alcohols possess

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(1) Part 2: see ref 4.
(2) Seebach, D.; Kalinowski, H. O. *Nachr. Chem. Tech.* **1976**, *24*, 415.
(3) For the employment of chiral building blocks from natural sources, see: (a) Hanessian, S. *Total Synthesis of Natural Products: The "Chiron" Approach*; Pergamon: Oxford, 1983. (b) Seebach, D.; Hungerbuhler, E. In *R. Scheffold: Modern Synthetic Methods 1980*; Sauerlaender: Aarau, 1980; p 91.
(4) Keinan, E.; Hafeli, E. K.; Seth, K. K.; Lamed, R. *J. Am. Chem. Soc.* **1986**, *108*, 162–169.

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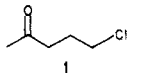
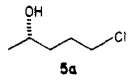
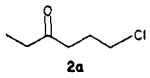
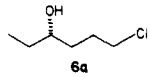
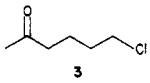
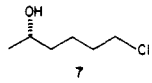
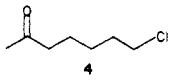
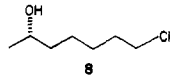
Table I. Reduction of ω-Chloro Ketones with TBADH[a]

starting matl	product	rl rate[b]	$[\alpha]_D$,[c] deg	ee,[d] %	abs confign
 1	 5a	1.5	15.03	98	*S*
 2a	 6a	0.15	8.74	>99	*S*
 3	 7	1.5	10.97	>99	*S*
 4	 8	1.5	8.26	>99	*S*

[a] The reaction was carried out in a column of TBADH–Eupergrit-C[4] operated at 37 °C. For details, see the Experimental Section. [b] Relative rates of reduction were determined by GC analysis by comparing the residence time in the column required to achieve 50% conversion for different substrates. The reduction rate of 2-hexanone was arbitrarily assigned a unit value. [c] Optical rotations were measured in $CHCl_3$ in a 10-cm cell. [d] Enantiomeric excess was determined by HPLC by using (*R*)-(−)-1-(1-naphthyl)ethyl isocyanate as described in ref 4.

only the single hydroxyl function, they are not very useful synthetic building blocks. In order to provide greater synthetic flexibility, the chiron should carry more than one functional group. We, therefore, turned our attention to aliphatic ketones bearing a second functionality that are still compatible with the enzymatic system. In this paper we introduce new chiral bifunctional building blocks—aliphatic chloro alcohols—that are produced from the corresponding chloro ketones by reduction with TBADH. The synthetic potential of these compounds is illustrated by short syntheses of several optically pure cyclic ethers.

Results and Discussion

The rationale for choosing the primary chloride as a second functional group was based on the atomic volume of the chloride substituent, which is similar to that of a methyl group.[5] A chloro alkanone might well be accepted by the enzyme in place of the higher homologous alkanone. For example, 5-chloropentan-2-one (**1**) and 2-hexanone may fit equally well into the active site of the enzyme.

Indeed, enzymatic reduction of these two ketones with TBADH proceeded at a comparable rate. Unfortunately, large-scale reduction of chloro ketones to chloro alcohols could not be carried out by batch processing, as the product and, even more so, the starting material are quite unstable in the aqueous reaction medium. Their decomposition liberates HCl, which lowers the pH and inactivates the enzyme. We solved this problem rather easily by using immobilized enzyme[4] packed in a column kept at 37 °C. The reduction was carried out in a flow system in which reservoirs of both the starting material and product were kept at 3 °C. Since the unstable compounds were exposed to 37 °C only briefly (their passage time through the column), no decomposition of either chloro ketone or chloro alcohol could be observed.

Table I lists four chloro ketones that were reduced to the corresponding *S* secondary alcohols by this method. The flow rate in all cases was adjusted to achieve about 50–70% conversion, and product was separated from the starting material by fractional distillation.

An obvious synthetic use of the chiral chloro alcohols is for the production of cyclic ethers via cyclization under basic conditions. This simple transformation is best achieved by mere distillation of the chloroalcohol over sodium hydride. The chiral THF and THP derivatives thus obtained are listed in Table II. Analysis by complexation gas chromatograpy using chiral nickel(II) β-diketonates[6] (Figure 1) demonstrated the remarkable optical purity

Table II. Synthesis of Chiral Cyclic Ethers

starting matl	product	$[\alpha]_D$,[a] deg	ee,[b] %	abs confign
5a	 9	19.36	98	*S*
6a	 10	16.85	>99	*S*
7	 11	11.81	>99	*S*

[a] Optical rotations were measured in $CHCl_3$ in a 10-cm cell. [b] Enantiomeric excess was determined by complexation GC; see Figure 1 and ref 6.

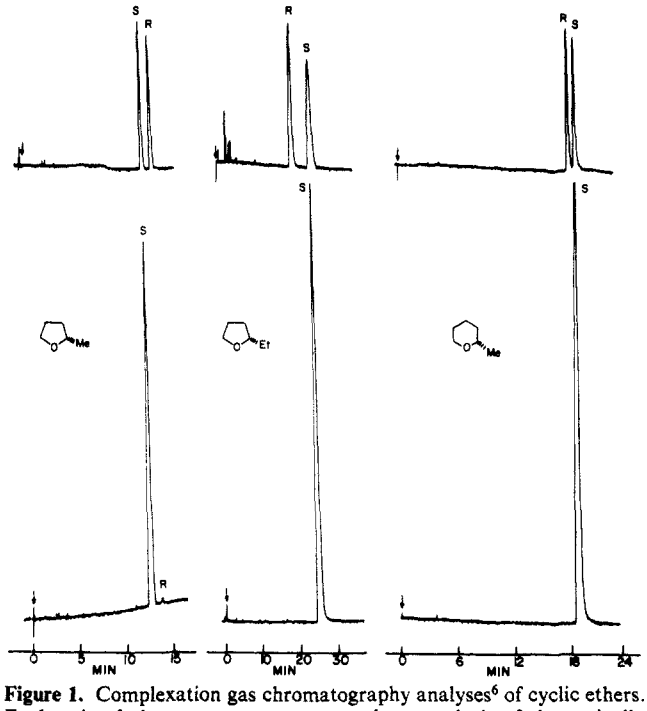


Figure 1. Complexation gas chromatography analyses[6] of cyclic ethers. Each pair of chromatograms corresponds to analysis of the optically active ether shown (lower chromatogram) and of an authentic racemic compound (upper chromatogram).

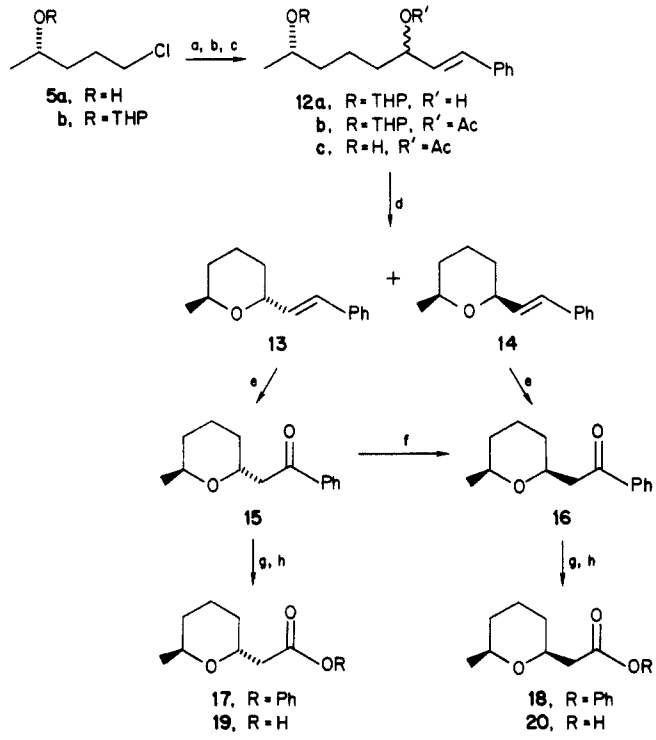
of these cyclic ethers **9–11**, in excellent agreement with the optical purity of their precursors **5a**, **6a**, and **7**, as was determined by HPLC (Table II).

Using continuously operating reactors with immobilized TBADH is particularly advantageous for large-scale production

(5) Pauling, L. *The Nature of the Chemical Bond*; Cornell University Press: Ithaca, NY, 1960; pp 257–264.

(6) Determination of the enantiomeric excess of the cyclic ethers **9–11** was carried out by Prof. V. M. Schurig and D. Wistuba at the Institut für Organische Chemie, Tübingen. The analysis was performed by complexation gas chromatography using a glass capillary column with nickel bis[3-(heptafluorobutyryl)-1(*R*)-camphorate] in a silicon oil phase. For details, see: (a) Schurig, F. V.; Burkle, W. *J. Am. Chem. Soc.* **1982**, *104*, 7573. (b) Schurig, F. V.; Weber, R. *J. Chromatogr.* **1984**, *289*, 321.

Scheme I[a]



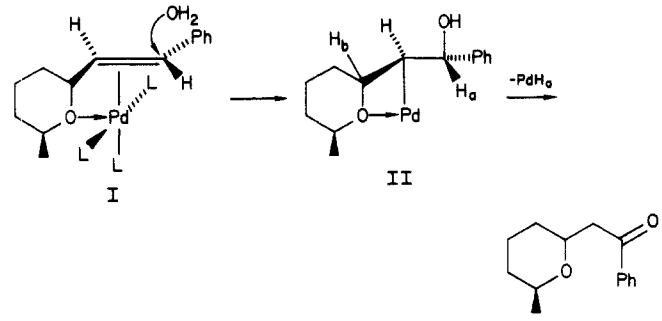
[a] Key: (a) dihydropyran, TsOH, 15 h, room temperature, 98%. (b) Mg; cinnamaldehyde; NH_4Cl, H_2O, 67%. (c) (1) Ac_2O, pyridine. (2) $HClO_4$, CH_3OH, H_2O 81%. (d) $Pd(PPh_3)_4$ (0.03 equiv), THF, 20 h, room temperature, 94%. (e) $PdCl_2$, CuCl, O_2, H_2O, DMF, 50 °C, 72 h, 35–44%. (f) $PhCO_2H$, $CHCl_3$ reflux 16 h, 100%. (g) CF_3CO_3H, CH_2Cl_2, Na_2HPO_4, 0 °C, 3 h, 94–98%. (h) KOH, H_2O, CH_3OH reflux, 3 h, 82–99%.

of optically pure cyclic ethers[7] that are not readily available by other methods. Easy access to large quantities of such chiral solvents may open interesting possibilities for asymmetric synthesis in chiral media as has already been demonstrated with respect to 2-methyltetrahydrofuran.[7b]

The chiral aliphatic chloro alcohols, described above, are excellent bifunctional building blocks that may be conveniently employed for syntheses of natural products containing chiral carbinol centers.[8] This advantage is demonstrated by the total synthesis of (+)-(*S*,*S*)-(*cis*-6-methyltetrahydropyran-2-yl)acetic acid (**20**), a naturally occurring heterocycle that was isolated from the perfume material civet,[9] a glandular secretion of the civet cat (*Viverra civetta*). Its total synthesis has attracted much attention in recent years.[10]

The synthesis of **20** is outlined in Scheme I. The chiral alcohol (*S*)-(+)-5-chloropentan-2-ol (**5a**) was protected as a tetrahydropyranyl (THP) derivative, **5b**, prior to conversion to the corresponding Grignard reagent, which was reacted with cinnamaldehyde. Subsequent acetylation of the resulting alcohol **12a** gave the allylic acetate **12b** as a mixture of two diastereomers in

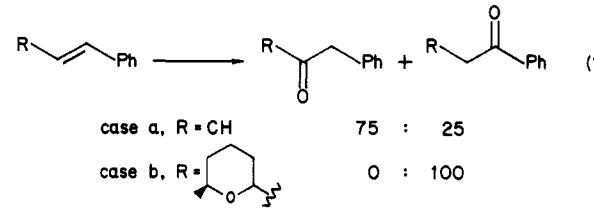
Scheme II



equal quantities (evident from 270-MHz ^1H NMR). Acidic removal of the THP protecting group afforded **12c**, an excellent substrate for Pd(0)-catalyzed intramolecular allylic etherification.[11] Indeed, exposure of a THF solution of **12c** to 3 mol % of $Pd(PPh_3)_4$ at room temperature resulted in efficient cyclization (91%), without need to further activate the nucleophilic hydroxyl group.[11] The resulting isomeric tetrahydropyrans **13** and **14** are easy to separate by chromatography. However, to obtain the target molecule **20**, such a separation is unnecessary as the cis and trans isomeric ketones **15** and **16**, obtained from a Wacker-type oxidation of the mixture of two olefins **13** and **14**, converged cleanly under acid catalysis to the cis isomer **16**.[12]

Interestingly, very little has been reported on the Wacker oxidation of disubstituted olefins.[13] Its use in organic synthesis has been mainly limited to conversion of monosubstituted olefins into methyl ketones.[14] The regioselectivity observed in our case, namely exclusive oxidation of the benzylic rather than the homobenzylic carbon, is fortunately the desired one. Nevertheless, this situation is quite surprising on grounds of the known stereochemical course[15] and expected regioselectivity of the Wacker process. It has been demonstrated both theoretically[16a] and experimentally[16b] that a nucleophile approaching a coordinated olefin from the face opposite to the metal is expected to attack the carbon that is substituted by an electron-donating group (or the position remote from an electron-withdrawing substituent). This tendency has been demonstrated in the Wacker oxidation of indene to β-indanone[17a] and of para-substituted styrenes to acetophenone and phenylacetaldehydes.[17b]

Indeed, we also found this prediction to be valid in the case of a Wacker oxidation of a model substrate, β-methylstyrene, which yielded phenylacetone and propiophenone in a 3:1 ratio (case a in eq 1). However, when this oxidation was repeated with β-



(7) (a) Novak, E. R.; Tarbell, D. S. *J. Am. Chem. Soc.* **1967**, *89*, 73. (b) Iffland, D. C.; Davis, J. E. *J. Org. Chem.* **1977**, *42*, 4150. (c) Robinson, P. L.; Barry, C. N.; Bass, S. W.; Jarvis, S. E.; Evans, S. A., Jr. *J. Org. Chem.* **1983**, *48*, 5398. (b) Arad-Yellin, R.; Green, B. S.; Knossow, M.; Tsoucaris, G. *J. Am. Chem. Soc.* **1983**, *105*, 4561.

(8) Keinan, E.; Seth, K. K.; Ghirlando, R.; Lamed, R., submitted for publication.

(9) (a) Maurer, B.; Grieder, A.; Thommen, W. *Helv. Chim. Acta* **1979**, *62*, 44. (b) Maurer, B.; Thommen, W. *Ibid.* **1979**, *62*, 1096. (c) van Dorp, D. A.; Ward, J. P. *Experientia* **1981**, *37*, 917.

(10) For previous synthesis of the optically active natural product, see: (a) Seebach, D.; Pohmakotr, M. *Helv. Chim. Acta* **1979**, *62*, 843. Seebach, D.; Pohmakotr, M.; Schregenberger, C.; Weidmann, B.; Mali, R. S.; Pohmakotr, S. *Ibid.* **1982**, *65*, 419. (b) Lichtenthaler, F. W.; Klingler, F. D.; Jarglis, P. *Carbohydr. Res.* **1984**, *132*, C1. For synthesis of the racemic compound, see: (c) Semmelhack, M. F.; Bodurow, C. *J. Am. Chem. Soc.* **1984**, *106*, 1496. (d) Bates, H. A.; Deng, P. N. *J. Org. Chem.* **1983**, *48*, 4479. (e) Kim, Y.; Mundy, B. P. *Ibid.* **1982**, *47*, 3556. (f) Ley, S. V.; Lygo, B.; Molines, H.; Morton, J. A. *J. Chem. Soc., Chem. Commun.* **1982**, 1251.

(11) (a) Keinan, E.; Sahai, M.; Roth, Z.; Nudelman, A.; Herzig, J. *J. Org. Chem.* **1985**, *50*, 3558–3566. (b) Stanton, S. A.; Felman, S. W.; Parkhurst, C. S.; Godleski, S. A. *J. Am. Chem. Soc.* **1983**, *105*, 1964. (c) Stork, G.; Poirer, J. M. *J. Am. Chem. Soc.* **1983**, *105*, 1073.

(12) This equilibration, which gives the thermodynamically more stable cis isomer, could also be carrier out under basic catalysis, as was reported[10a] for the *tert*-butyl ketone **21**.

(13) Tsuji, J. *Organic Synthesis with Palladium Compounds*; Springer: New York, 1980; pp 6–12.

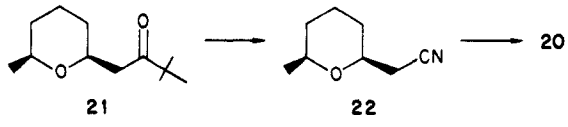
(14) (a) Tsuji, J. *Top. Curr. Chem.* **1980**, *91*, 29. (b) Tsuji, J.; Shimizu, I.; Kobayashi, Y. *Isr. J. Chem.* **1984**, *24*, 153.

(15) (a) Hegedus, L. S. *Tetrahedron* **1984**, *40*, 2415. (b) Backvall, J. E. *Acc. Chem. Res.* **1983**, *16*, 335.

(16) (a) Eisenstein, O.; Hoffmann, R. *J. Am. Chem. Soc.* **1981**, *103*, 4308. (b) Chang, T. C. T.; Foxman, B. M.; Rosenblum, M.; Stockman, C. *J. Am. Chem. Soc.* **1981**, *103*, 7361.

(17) (a) Zakharova, L. M.; Vrgaftik, M. N.; Moiseev, I. I.; Katsman, L. A. *Izv. Akad. Nauk SSSR* **1970**, 700. (b) Okada, H.; Noma, T.; Katsuyama, Y.; Hashimoto, H. *Bull. Chem. Soc. Jpn.* **1968**, *41*, 1395. (c) Jira, R. *Tetrahedron Lett.* **1971**, 1225. (d) Zaw, K.; Lautens, M.; Henry, P. M. *Organometallics* **1985**, *4*, 1286.

Scheme III



tetrahydropyranyl styrenes, **13** and **14** (case b in eq 1), we observed the opposite regioselectivity, leading to the phenyl ketones **15** or **16** exclusively. The substituent electronic effect is sufficiently large to explain the observed regioselectivity in case a but is too small to account for that of case b (the reported values for σ_p of CH_3, Ph, and CH_2OR are –0.17,[18a] –0.01,[18a] and +0.03,[18b] respectively).

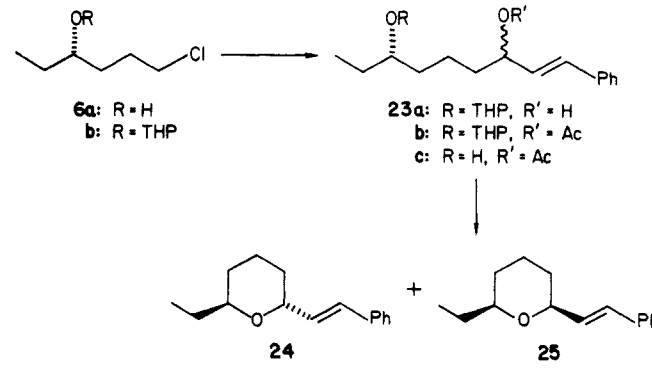
The regioselectivity observed in case b may be alternatively rationalized by a nonsymmetrical bonding of palladium to the olefin, caused by intramolecular coordination to the tetrahydropyranyl oxygen (structure I in Scheme II). This slippage of the metal away from the phenyl substituent would encourage nucleophilic attack of a water molecule at the benzylic carbon, leading to intermediate II. Decomposition of this η^1-Pd complex via β-hydride elimintion of Pd–H_a would yield the observed product **15** or **16**. However, complex II may also decompose via competing pathways such as elimination of Pd–H_b or deoxypalladium[19] to give polar, yet unidentified, byproducts. This may accout for the low yields of ketones **15** and **16**. Although no attempts were made to optimize this reaction, it seems likely that yields may be improved by appropriate modifications of reaction conditions.

Interestingly, analogous regioselectivity has been observed in the Wacker oxidation of allylic alcohols, which yielded products having two oxygen functions at the 1- and 3-positions.[17c,d]

Baeyer–Villiger oxidation of the phenyl ketones **15** and **16** was difficult to carry out. These ketones were completely inert to *m*-chloroperbenzoic acid (mCPBA) in CH_2Cl_2 at room temperature, even over several days. When more vigorous conditions were applied (mCPBA, 48 h in refluxing $CHCl_3$), slow decomposition of the ketone to a complex mixture of polar products was observed. Similar resistance to direct oxidation was previously encountered[10a] in a number of analogous ketones, including **16** and **21**, and the problem has been circumvented in that case by a three-step route (Scheme III)[10a] involving formation of the corresponding oxime, followed by Beckmann fragmentation to the nitrile **22**, which was then hydrolyzed to give **20** in moderate yield. We found, however, that the desired direct oxidation could be efficiently achieved with a more powerful oxidant. Both ketones **15** or **16** are smoothly and quantitatively oxidized to the corresponding phenyl esters **17** or **18** by treatment with anhydrous trifluoroperacetic acid[20] in CH_2Cl_2 for less than 3 h at 0 °C.

Finally, basic hydrolysis of the esters **18** or **17** afforded the naturally occurring civet constituent **20**[21,22] or its trans isomer, **19**, respectively. Interestingly, the chemical shifts and coupling constants measured in the ^1H NMR spectra of both **19** and **20** were found to be highly dependent on concentration and temperature, probably due to a reversible dimerization process of the carboxylic acid present in both compounds.[22] Thus, the NMR spectrum of **20** was identical with that of an authentic sample[21] only when measured under the same set of conditions.

Scheme IV



Starting with (*S*)-(+)-6-chlorohexan-3-ol (**6a**) and carrying out a set of transformations similar to those described in Scheme I, two new 2,6-disubstituted tetrahydropyran derivatives **24** and **25** (Scheme IV) were obtained.

Conclusion

The thermostable alcohol dehydrogenase extracted from *Thermoanaerobium brockii* (TBADH), previously found to catalyze asymmetric reduction of simple aliphatic ketones, was shown to be an extremely useful catalyst, particularly in its immobilized form, for enantioselective reduction of aliphatic chloro ketones, providing a new family of chiral bifunctional building blocks. Some of these were successfully used for production of optically pure cyclic ethers, as well as for total synthesis of enantiomerically pure (+)-(*S*,*S*)-(*cis*-6-methyltetrahydropyran-2-yl)acetic acid. Our synthetic approach toward this natural component of the perfume civet represents application of both applied enzymology and organometallic chemistry. Other natural products containing asymmetric carbinol centers, such as lasiodiplodin, zearalenone, ferrulactone II, and other macrolides, are currently being synthesized in our laboratories from enzymatically produced chiral building blocks.[8]

Experimental Section

General Methods. Elemental analyses were performed by the Microanalytical Laboratory of the Weizmann Institute of Science. Infrared spectra were recorded on a Perkin-Elmer 467 grating spectrometer and are given in units of inverse centimeters. Proton NMR spectra were measured in deuterated chloroform on a Varian FT-80A or Bruker WH-270 NMR spectrometer. All chemical shifts are reported in units downfield from Me_4Si, and *J* values are given in Hertz. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broadened. Mass spectra were recorded on a Varian Mat 731 spectrometer or on a Finnigan 4500 GC–MS. Measurements of pH were carried out on a Radiometer-Copenhagen PHM-62. Thin-layer chromatography (TLC) was performed on aluminum sheets precoated with silica gel (Merck, Kieselgel 60, F-254, Art. 5549). Column chromatographic separations were performed on silica gel (Merck, Kieselgel 60, 230–400 mesh, Art. 9385) under a pressure of 1 atm (flash chromatography). Preparative TLC was performed on glass plates precoated with silica gel (Merck, Kieselgel 60 F-254, Art. 5717). Distillations of products were performed in a Kugelrohr apparatus; the temperature given are pot temperatures. GLC analyses were performed on a Spectra-Physics SP-7100 (FI detector) equipped with a 0.125-in. × 6-ft column packed with 10% Carbowax 20 on Chromosorb W. Preparative GLC separations were performed on a Varian Aerograph 90P (TC detector) equipped with a $^1/_2$-in. × 12-ft column packed with 15% Carbowax 20M on Chromosorb W. Reactions that required anhydrous conditions were carried out in flame-dried flasks under a nitrogen or argon atmosphere in dry, freshly distilled solvents.

Reduction of Chloro Ketones with Immobilized TBADH. A powder of TBADH on Eupergit-C[4] (prepared from 10 g of Eupergit-C[4] and 45 mL of crude TBADH extract) was packed in a double-walled glass column that was kept at 37 °C by circulating water. The reaction mixture (aqueous solution containing 0.3–1.0% substrate, 10–30% 2-propanol, 50 mM Tris-HCl buffer (pH 8), 0.025 mM NADP, and 3 mM mercaptoethanol) was passed through the column by using a peristaltic pump at a flow rate (usually between 0.2 and 1.0 mL/min) adjusted to

(18) (a) McDaniel, D. H.; Brown, H. C. *J. Org. Chem.* **1958**, *23*, 420. (b) Exner, O. *Collect. Czech. Chem. Commun.* **1966**, *31*, 65.

(19) Keinan, E.; Sahai, M.; Kirson, I. *J. Org. Chem.* **1983**, *48*, 2550 and references cited therein.

(20) (a) A solution of anhydrous CF_3CO_3H was prepared from H_2O_2 (100%) (provided by Dr. Z. Ludmer of the Israel Institute for Biological Research) and trifluoroacetic anhydride in CH_2Cl_2. See: Noyori, R.; Sato, T.; Kobayashi, H. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 2661.

(21) Compound **20** was identical (NMR, IR, MS) with an authentic sample provided by Dr. B. Maurer of Firmenich, Geneva (synthesized by Prof. D. Seebach[10a]). The optical rotation of our compound, $[\alpha]^{22}_D$ +43.85° (C_6H_6, *c* 2.52), was higher than that reported in ref 10a, $[\alpha]^{22}_D$ +31.97° (C_6H_6 *c* 1.2), and in ref 10b, $[\alpha]^{22}_D$ +35° (chloroform,[22] *c* 0.5).

(22) Our compound whose optical rotation in benzene was +43.85° showed rotation of +18.60° in chloroform. Therefore, we assume that the reported rotation[10b] of 35° refers to benzene and not to chloroform as was reported (probably by mistake).

(23) For detailed NMR study of compounds **19** and **20** see: Keinan, E.; Sahai, M.; Seth, K. K.; Berman, E., submitted for publication.

achieve 50% conversion of the ketone to the alcohol (as was indicated by GC analysis). The whole system was placed in a cold room to assure that all components except the column itself were kept at 4 °C. The mixture was collected and worked up with a large excess of ammonium sulfate, followed by extraction with diethyl ether. The extract was dried over sodium sulfate or by a short silica gel column, followed by removal of the solvent under reduced pressure. Pure samples of chloro alcohols were obtained by either fractional distillation or preparative GC. The results are given in Table I.

(*S*)-(+)-2-Methyltetrahydrofuran (9). (*S*)-(+)-5-Chloropentan-2-ol (**5a**) (1.0 g, 8.1 mmol) was cooled to 0 °C without solvent, sodium hydride (350 mg, 14.5 mmol) was slowly added, and the mixture was stirred at room temperature for 3 h. The resulting thick slurry was then subjected to Kugelrohr distillation [100 °C (60 mm)] to give **9**:0.70 g, 99.7%; $[\alpha]_D$ +19.36° ($CHCl_3$, *c* 28.5); NMR (80 MHz, $CDCl_3$) 4.08–3.47 (m, 3 H), 1.92–1.36 (m, 4 H), 1.23 (d, *J* = 6.3 Hz, 3 H).

(*S*)-(+)-2-Ethyltetrahydrofuran (10). Cyclization of (*S*)-(+)-6-chlorohexan-3-ol (**6a**) (500 mg, 3.6 mmol) was carried out as described above to give **10**:300 mg, 82%; $[\alpha]_D$ +16.85° ($CHCl_3$, *c* 4.8); NMR (80 MHz, $CDCl_3$) 3.75 (m, 3 H), 2.1–1.3 (m, 6 H), 0.93 (t, *J* = 7.6 Hz, 3 H); IR (neat) 2965, 2940, 2850, 1465, 1455, 1380, 1080, 1045, 925; mass spectrum, *m/e* (rel intens) 100 (6, M^+), 99 (3), 83 (6), 72 (11), 71 (100), 70 (24), 59 (11), 57 (21), 55 (24).

(*S*)-(+)-2-Methyltetrahydropyran (11). Cyclization of (*S*)-(+)-6-chlorohexan-3-ol (**7**) (1.0 g, 7.3 mmol) was carried out as described above to give **11**: 0.695 g, 95%; $[\alpha]_D$ +11.81° ($CHCl_3$, *c* 8.7); NMR (80 MHz, $CDCl_3$) 3.88 (br d, *J* = 10.0 Hz, 1 H), 3.68 (m, 2 H), 1.8–1.3 (m, 6 H), 1.15 (d, *J* = 6.2 Hz, 3 H); IR (neat) 2980, 2925, 2860, 1460, 1447, 1375, 1310, 1275, 1215, 1125, 1085, 1045, 930, 740, 720, 650; mass spectrum, *m/e* (rel intens) 100 (39, M^+), 99 (9), 86 (19), 85 (100), 81 (11), 70 (8), 67 (34), 59 (11), 57 (51), 56 (81), 55 (73).

(2*S*)-(+)-5-Chloro-2-(tetrahydropyranyl-2-oxy)pentane (5b). (2*S*)-(+)-5-Chloropentan-2-ol (**5a**) (6.0 g, 49 mmol) was dissolved in 25 mL of CH_2Cl_2, together with dihydropyran (5.04 g, 60 mmol). The solution was cooled to 0 °C, and then *p*-toluenesulfonic acid (0.3 g, 1.7 mmol) was added. The mixture was stirred at 0 °C for 15 h and at room temperature for 1 h, then washed with aqueous $NaHCO_3$, and dried over $MgSO_4$, and the solvent was removed under reduced pressure. The residue was Kugelrohr distilled [100–120 °C (0.2 mm)], affording **5b**: 9.9 g, 98%; $[\alpha]_D$ +25.22° ($CHCl_3$, *c* 8.6); R_f 0.51 (SiO_2, hexane/ethyl acetate, 8:1); NMR (270 MHz, $CDCl_3$) 4.68 (br t, *J* = 2.9 Hz) and 4.63 (br t, *J* = 2.9 Hz) (at a 1:1 ratio, together 1 H), 3.84 (m, 2 H), 3.55 (m, 3 H), 2.0–1.5 (m, 10 H), 1.24 (d, *J* = 6.4 Hz) and 1.13 (d, *J* = 6.1 Hz) (at a 1:1 ratio, together 3 H); IR (neat) 2930, 2860, 2840, 1460, 1450, 1440, 1370, 1350, 1330, 1200, 1180, 1170, 1150, 1130, 1070, 1030, 1020, 995, 870, 720, 650; mass spectrum, *m/e* (rel intens) 207 (0.2, M^+), 205 (0.2, M^+), 129 (10), 107 (14), 105 (50), 101 (34), 86 (12), 85 (100), 69 (75), 68 (10), 67 (33), 63 (5), 57 (50), 56 (91), 55 (55).

(7*S*)-(+)-3-Hydroxy-1-phenyl-7-(tetrahydropyranyl-2-oxy)oct-1-ene (12a). A solution of 1,2-dibromomethane (10 mL) in ether (100 mL) was added, under argon atmosphere, to a flame-dried flash containing magnesium turnings (10 g, 411 mmol), resulting in reflux and a vigorous gas evolution. The chloride **5b** (9.48 g, 45.9 mmol) was then added, and the mixture was stirred for 4 h at room temperature and then cooled to −20 °C. Freshly distilled cinnamaldehyde (9.96 g, 75.4 mmol) was added, and the mixture was stirred at −20 °C for 20 min, allowed to warm to room temperture, and then quenched with an aqueous slurry of NH_4Cl. The mixture was dried over Na_2SO_4, the solvent was removed under reduced pressure, and the residue was subjected to flash chromatography (hexane/ethyl acetate, 3:2), affording **12a**: 9.35 g, 67%; $[\alpha]_D$ +8.83° ($CHCl_3$, *c* 3.0); R_f 0.64 (SiO_2, hexane/ethyl acetate, 1:1); NMR (270 MHz, $CDCl_3$) 7.42–7.16 (m, 5 H), 6.56 (d, *J* = 16.0 Hz, 1 H), 6.21 (dd, *J* = 16.0, 6.7 Hz) and 6.20 (dd, *J* = 16.0, 6.7 Hz) (at a 1:1 ratio, together 1 H), 4.67 (br t, *J* = 3.1 Hz) and 4.62 (br t, *J* = 3.1 Hz) (at a 1:1 ratio, together 1 H), 4.27 (br q, *J* = 5.9 Hz, 1 H), 3.96–3.65 (m, 2 H), 3.46 (m, 1 H), 1.85–1.38 (m, 12 H), 1.22 (d, *J* = 6.2 Hz) and 1.10 (d, *J* = 6.1 Hz) (at a 1:1 ratio, together 3 H); IR (neat) 3600–3100, 2940, 2870, 1490, 1450, 1370, 1200, 1140, 1120, 1075, 1040, 1020, 995, 970, 750, 695; mass spectrum, *m/e* (rel intens) 221 (2), 220 (6), 203 (5), 186 (3), 160 (3), 156 (2), 147 (3), 146 (3), 145 (2), 144 (10), 134 (26), 132 (16), 130 (15), 116 (20), 106 (15), 105 (14), 104 (16), 92 (24), 86 (100), 78 (12), 68 (13), 58 (11), 56 (15).

(7*S*)-(+)-3-Acetoxy-1-phenyl-7-(tetrahydropyranyl-2-oxy)oct-1-ene (12b). The alcohol **12a** (1.4 g, 4.6 mmol) was dissolved in dry pyridine (7 mL) to which acetic anhydride (3 mL) was added, and the mixture was stirred overnight at room temperture. The reaction mixture was worked up with 10% aqueous $CuSO_4$ to remove traces of pyridine and then dried over Na_2SO_4. Removal of the solvent under reduced pressure afforded the acetate **12b**: 1.55 g, 97%; $[\alpha]_D$ +7.85° ($CHCl_3$, *c* 1.3); R_f 0.34 (SiO_2, hexane/ethyl acetate, 8:1); NMR (80 MHz, $CDCl_3$) 7.33 (m, 5 H), 6.60 (d, *J* = 10.4 Hz, 1 H), 6.11 (br dd, *J* = 10.4, 7.2 Hz, 1 H), 5.50 (br q, *J* = 6.1 Hz, 1 H), 4.68 (m, 1 H), 3.8 (m, 2 H), 3.5 (m, 1 H), 2.06 (s, 3 H), 1.6 (m, 12 H), 1.21 (d, *J* = 6.2 Hz) and 1.09 (d, *J* = 6.2 Hz) (at a 1:1 ratio, together 3 H); IR (neat) 2955, 2920, 2860, 1730, 1490, 1445, 1370, 1350, 1235, 1028, 1020, 965, 745, 690; mass spectrum, *m/e* (rel intens) 215 (3), 203 (12), 202 (13), 185 (7), 184 (4), 169 (4), 143 (13), 133 (26), 129 (28), 117 (23), 115 (37), 105 (17), 104 (11), 103 (15), 91 (38), 86 (100), 79 (9), 78 (9), 77 (20), 68 (32), 58 (25), 56 (28).

(7*S*)-(+)-3-Acetoxy-7-hydroxy-1-phenyloct-1-ene (12c). Compound **12b** (5.56 g, 16.1 mmol) was dissolved in a mixture of methanol (80 mL) and water (20 mL) containing concentrated $HClO_4$ (0.2 mL). The mixture was stirred overnight at room temperature, and then brought to neutral pH by treatment with K_2CO_3, and the solvent was removed under reduced pressure. The residue was worked up with ether and water, and the ether solution was concentrated and subjected to flash chromatography (hexane/ethyl acetate, 1:1), affording the deprotected acetate **12c**: 3.48 g, 83%; $[\alpha]_D$ +3.85° ($CHCl_3$, *c* 1.3); R_f 0.57 (SiO_2, hexane/ethyl acetate, 1:1); NMR (80 MHz, $CDCl_3$) 7.3 (m, 5 H), 6.62 (d, *J* = 16 Hz, 1 H), 6.10 (dd, *J* = 16, 7.0 Hz, 1 H), 5.34 (q, *J* = 6.8 Hz, 1 H), 3.79 (br sextet, *J* = 6 Hz, 1 H), 2.07 (s, 3 H), 1.8–1.3 (m, 6 H), 1.12 (d, *J* = 6.2 Hz, 3 H); IR (neat) 3600–3100, 2960, 2920, 2850, 1723, 1490, 1460, 1445, 1370, 1240, 1100, 1085, 1025, 965, 665, 630; mass spectrum, *m/e* (rel intens) 220 (1, M^+ − 42), 219 (7), 203 (5), 202 (31, M^+ − AcOH), 201 (12), 169 (19), 158 (15), 155 (15), 147 (22), 146 (26), 145 (16), 144 (14), 143 (24), 142 (15), 141 (20), 134 (12), 133 (88), 131 (42), 130 (33), 129 (64), 128 (53), 126 (13), 117 (30), 116 (15), 115 (76), 111 (25), 105 (31), 104 (47), 103 (22), 97 (11), 92 (10).

(2*S*,6*S*)-(*E*)-2-(*cis*-6-Methyltetrahydropyran-2-yl)-1-phenylethene (14) and (2*R*,6*S*)-(*E*)-2-(*trans*-6-Methyltetrahydropyran-2-yl)-1-phenylethene (13). 3-Acetoxy-7-hydroxy-1-phenyloct-1-ene (**12c**) (2.266 g, 8.6 mmol) was dissolved in dry THF (10 mL) to which $Pd(PPh_3)_4$ (329 mg, 0.28 mmol, 3.2 mol %) was added, and the solution was stirred at room temperature until no starting material could be detected by TLC (20 h). The mixture was concentrated and subjected to flash chromatography (hexane/ethyl acetate, 95:5), followed by Kugelrohr distillation [150–170 °C (0.2 mm)] to give 1.03 g (59%) of **14** and 0.61 g (35%) of **13**.

Physical Properties of the Cis Isomer 14: $[\alpha]_D$ −33.13° ($CHCl_3$, *c* 1.6); R_f 0.84 (SiO_2, hexane/ethyl acetate, 14:1); ^1H NMR (270 MHz, $CDCl_3$) 7.3 (m, 5 H), 6.58 (d, *J* = 16.0 Hz, 1 H), 6.22 (dd, *J* = 16.0, 6.1 Hz, 1 H), 4.00 (ddd, *J* = 10.3, 7.1, 2.4 Hz, 1 H), 3.55 (d, q, d, *J* = 10.8, 6.2, 1.7 Hz, 1 H), 1.88 (m, 1 H), 1.75–1.3 (m, 5 H), 1.23 (d, *J* = 6.2 Hz, 3 H); ^{13}C NMR (22.63 MHz, $CDCl_3$) 137.16, 131.12, 129.93, 128.42 (2 C), 127.40, 126.48 (2 C), 77.07, 73.94, 33.16, 31.70, 23.72, 22.26; IR (neat) 2967, 2925, 2915, 1485, 1435, 1380, 1360, 1305, 1195, 1180, 1145, 1080, 1067, 1030, 980, 960, 740, 690; mass spectrum, *m/e* (rel intens) 202 (42, M^+), 187 (1), 148 (2), 147 (2), 146 (5), 145 (4), 133 (18), 132 (9), 131 (36), 115 (18), 111 (6), 105 (38), 104 (100), 103 (16), 97 (11), 91 (34), 84 (7), 78 (15), 77 (21), 70 (27), 69 (27), 65 (7), 55 (56). Anal. Calcd: C, 83.17; H, 8.91. Found: C, 84.84; H, 8.70.

Physical Properties of the Trans Isomer 13: $[\alpha]_D$ −17.63° ($CHCl_3$, *c* 1.6); R_f 0.72 (SiO_2 hexane/ethyl acetate, 14:1); ^1H NMR (270 MHz, $CDCl_3$) 7.3 (m, 5 H), 6.56 (dd, *J* = 16.4, 1.3 Hz, 1 H), 6.34 (dd, *J* = 16.4, 5.1 Hz, 1 H), 4.5 (m, 1 H), 4.0 (m, 1 H), 1.83 (m, 1 H), 1.68 (m, 4 H), 1.35 (m, 1 H), 1.21 (d, *J* = 6.4 Hz, 3 H); ^{13}C NMR (22.63 MHz, $CDCl_3$) 138.08, 130.96, 130.58, 128.53 (2 C), 127.40, 126.37 (2 C), 72.05, 67.36, 32.35, 29.71, 20.65, 18.87; IR (neat) 2980, 2920, 1490, 1445, 1380, 1340, 1200, 1130, 1080, 1040, 920, 750, 645; mass spectrum, *m/e* (rel intens) 202 (32, M^+), 187 (1), 159 (3), 146 (5), 145 (5), 131 (34), 115 (23), 105 (33), 104 (100), 103 (19), 97 (9), 91 (36), 84 (6), 78 (17), 70 (25), 69 (27), 65 (10), 55 (61). Anal. Calcd: C, 83.17; H, 8.91. Found: C, 82.14; H, 8.96.

Wacker Oxidation of β-Methylstyrene. β-Methylstyrene (107 mg, 0.9 mmol), cuprous chloride (82 mg), and $PdCl_2$ (15 mg, 0.08 mmol) were mixed with DMF (1 mL) and water (1 mL). The mixture was stirred under oxygen atmosphere for 16 h and then extracted with pentane. The solvent was removed under reduced pressure to give phenylacetone and propiophenone (together 115 mg, 95%) at a 3:1 ratio, as determined by ^1H NMR.

(2*R*,6*S*)-(*trans*-6-Methyltetrahydropyran-2-yl)acetophenone (15). Compound **13** (100 mg, 0.49 mmol) was added to a suspension of $PdCl_2$ (45 mg, 0.25 mmol) and CuCl (150 mg, 1.5 mmol) in H_2O/DMF (1:1, 1.5 mL). The mixture was stirred at 50 °C under oxygen atmosphere for 72 h and then diluted with water and extracted with ether. The ether extract was dried over Na_2SO_4 and the solvent removed under reduced pressure. The residue was subjected to preparative TLC, giving 35 mg of unreacted starting material **13** and 31 mg (44%) of compound **15**: $[\alpha]_D$ −26.67° ($CHCl_3$, *c* 1.5); R_f 0.43 (SiO_2, hexane/ethyl acetate, 14:1); NMR (80 MHz, $CDCl_3$) 8.00 (m, 2 H), 7.55 (m, 3 H), 4.43 (m, 1 H), 3.95 (m, 1 H), 3.37 (dd, *J* = 16, 7.6 Hz, 1 H), 3.01 (dd, *J* = 16, 6.4 Hz,

1 H), 1.67 (m, 6 H), 1.18 (d, J = 6.4 Hz, 3 H); IR (neat) 3000, 2960, 2890, 1700, 1610, 1590, 1460, 1390, 1300, 1280, 1230, 1210, 1190, 1060, 1040, 770, 610; mass spectrum, m/e (rel intens) 218 (1, M^+), 185 (1), 176 (2), 175 (2), 162 (14), 147 (9), 133 (3), 120 (18), 106 (8), 105 (100), 99 (12), 98 (6), 91 (3), 81 (28), 78 (11), 69 (7), 65 (2), 55 (36).

(*S*,*S*)-(*cis*-6-Methyltetrahydropyran-2-yl)acetophenone (16). Oxidation of **14** was carried out as described for the trans isomer **13**, yielding 35% of **16**: $[\alpha]_D$ −15.0° ($CHCl_3$, c 1.0); R_f 0.68 (SiO_2, hexane/ethyl acetate, 14:1); NMR (270 MHz, $CDCl_3$) 7.96 (br d, J = 8.4 Hz, 2 H), 7.54 (t, J = 7.3 Hz, 1 H), 7.45 (dd, J = 8.4, 7.3 Hz, 2 H), 3.98 [m (5 lines), 1 H], 3.49 (d, q, d, J = 10.8, 6.1, 1.6 Hz, 1 H), 3.31 (dd, J = 16.1, 5.9 Hz, 1 H), 2.97 (dd, J = 16.1, 6.6 Hz, 1 H), 1.79 (m, 2 H), 1.59 (m, 2 H), 1.24 (m, 2 H), 1.14 (d, J = 6.2 Hz, 3 H); IR (neat) 2980, 2960, 2930, 1680, 1590, 1575, 1444, 1368, 1340, 1282, 1215, 1177, 1065, 986, 887, 750, 690; mass spectrum, m/e (rel intens) 218 (1, M^+), 176 (2), 175 (2), 162 (15), 161 (3), 147 (8), 133 (1), 120 (17), 106 (8), 105 (100), 99 (4), 81 (7), 78 (10), 77 (60), 69 (5), 55 (15).

Isomerization of 15 to 16. Compound **15** (22 mg, 0.10 mmol) was dissolved in $CHCl_3$ (1 mL) containing benzoic acid (49 mg, 0.4 mmol). The mixture was refluxed overnight and washed with aqueous bicarbonate. The solvent was removed under reduced pressure to give 22 mg (100%) of **16**, which was found to be pure by TLC and ^1H NMR.

Phenyl (2*R*,6*S*)-(*trans*-6-Methyltetrahydropyran-2-yl)acetate (17). A freshly prepared solution of trifluoroperacetic acid[20] (0.25 mmol) in CH_2Cl_2 was added to a cold (0 °C) solution of **15** (30 mg, 0.14 mmol) in 1 mL of CH_2Cl_2 containing anhydrous Na_2HPO_4 (80 mg, 0.56 mmol). The mixture was stirred at 0 °C for 3 h and filtered, and the solvent was removed under reduced pressure, affording the phenyl ester **17**: 31 mg, 98%; $[\alpha]_D$ −36.43° ($CHCl_3$, c 1.4); R_f 0.50 (SiO_2, hexane/ethyl acetate, 14:1; NMR (270 MHz, $CDCl_3$) 7.34 (m, 2 H), 7.20 (m, 1 H), 7.07 (m, 2 H), 4.42 (dddd, J = 8.7, 6.5, 5.6, 3.8 Hz, 1 H), 4.30 (quintet of d, J = 6.5, 3.2 Hz, 1 H), 2.93 (dd, J = 14.5, 8.7 Hz, 1 H), 2.66 (dd, J = 14.5, 5.6 Hz, 1 H), 1.72 (m, 4 H), 1.39 (m, 2 H), 1.22 (d, J = 6.5 Hz, 3 H); IR (neat) 2967, 2917, 2870, 1747, 1588, 1490, 1455, 1412, 1370, 1335, 1197, 1187, 1160, 1050, 1010, 947, 930, 757, 690; mass spectrum, m/e (rel intens) 234 (1, M^+), 192 (1), 141 (28), 100 (6), 99 (100), 98 (17), 94 (40), 81 (88), 77 (11), 65 (20), 57 (14), 55 (53).

Phenyl (2*S*,6*S*)-(*cis*-6-Methyltetrahydropyran-2-yl)acetate (18). Baeyer–Villiger oxidation of **16** was carried out as described for the oxidation of **15**. Starting from **16** (74 mg, 0.34 mmol), Na_2HPO_4 (200 mg, 1.4 mmol) and CF_3CO_3H (0.64 mmol) in 2 mL of CH_2Cl_2 afforded **18**: 74 mg, 94%); $[\alpha]_D$ +14.8° ($CHCl_3$, c 1.0); R_f 0.59 (SiO_2, hexane-/ethyl acetate, 14:1); NMR (270 MHz, $CDCl_3$) 7.36 (t, J = 7.5 Hz, 2 H), 7.22 (t, J = 7.5 Hz, H), 7.08 (d, J = 7.7 Hz, 2 H), 3.90 (m, 1 H), 3.51 (d, q, d, J = 10.8, 6.0, 1.6 Hz, 1 H), 2.77 (dd, J = 14.9, 7.7 Hz, 1 H), 2.63 (dd, J = 14.9, 5.6 Hz, 1 H), 1.62 (m, 4 H), 1.33 (m, 2 H), 1.19 (d, J = 6.2 Hz, 3 H); IR (neat) 2975, 2960, 2840, 1750, 1585, 1480, 1370, 1335, 1280, 1245, 1190, 1160, 1065, 760, 685; mass spectrum, m/e (rel intens) 234 (1, M^+), 141 (31), 123 (2), 99 (100), 95 (13), 94 (35), 83 (2), 82 (5), 81 (84), 77 (9), 71 (6), 69 (10), 68 (11), 65 (15), 57 (12), 56 (3), 55 (54).

(2*R*,6*S*)-(−)-(*trans*-6-Methyltetrahydropyran-2-yl)acetic Acid (19). The phenyl ester **17** (22.5 mg, 0.096 mmol) was dissolved in 1 mL of a 1:1 mixture of methanol and 10% aqueous KOH. The mixture was refluxed for 3 h and then diluted with water and washed with ether. The aqueous solution was acidified with 10% aqeuous HCl to pH 3, mixed with excess NaCl, and extracted with ether. The ether solution was washed with brine and dried over Na_2SO_4, and the solvent was removed under reduced pressure. The residue was subjected to Kugelrohr distillation [150–170 °C (0.2 mm)] to give **19**: 15 mg, 99%; $[\alpha]_D$ −39.52° ($CHCl_3$, c 1.45); NMR (270 MHz, $CDCl_3$) 6.8 (br s, 1 H), 4.22 (dddd, J = 8.7, 7.3, 4.7, 3.9 Hz, 1 H), 4.07 (m, 1 H), 2.63 (dd, J = 15.5, 8.7 Hz, 1 H), 2.41 (dd, J = 15.5, 4.7 Hz, 1 H), 1.71 (m, 4 H), 1.38 (m, 2 H), 1.17 (d, J = 6.6 Hz, 3 H); IR (neat) 2970, 1700, 1400, 1250, 1120–1000, 860, 790; mass spectrum, m/e (rel intens) 158 (1, M^+), 157 (1), 147 (3), 140 (6), 125 (13), 116 (11), 115 (14), 102 (27), 99 (23), 98 (16), 89 (13), 86 (22), 81 (41), 71 (28), 69 (22), 68 (23), 60 (22), 55 (100).

(2*S*,6*S*)-(+)-(*cis*-6-Methyltetrahydropyran-2-yl)acetic Acid (20). Treatment of **18** (72 mg, 0.3 mmol) with KOH solution in methanol/water was carried out as described for compound **17**, affording, after distillation, **20**: 40 mg, 82%; $[\alpha]^{22}_D$ +18.60° ($CHCl_3$, c 2.77); $[\alpha]^{22}_D$ +43.85° (C_6H_6, c 2.52) [lit.[10a] $[\alpha]^{22}_D$ +31.97° (C_6H_6, c 1.2)]; ^1H NMR (270 MHz, $CDCl_3$) 6.6 (br s, 1 H), 3.76 (m, 1 H), 3.55 (d, q, d, J = 10.8, 6.2, 2.0 Hz, 1 H), 2.58 (dd, J = 16.2, 7.3 Hz, 1 H), 2.51 (dd, J = 16.2, 5.2 Hz, 1 H), 1.83 (m, 1 H), 1.6 (m, 3 H), 1.26 (m, 2 H), 1.21 (d, J = 6.2 Hz, 3 H); ^{13}C NMR (67.89 MHz, $CDCl_3$) 174.45, 74.61, 73.98, 41.19, 32.77, 30.78, 23.17, 22.00; IR (neat) 3600–3000, 2980, 2920, 2845, 1700, 1430–1410, 1360, 1290, 1200, 1170, 1060, 1130, 990, 927, 865, 840; mass spectrum, m/e (rel intens) 158 (3, M^+), 143 (2), 140 (2), 125 (9), 116 (16), 115 (19), 112 (7), 102 (38), 99 (18), 98 (19), 97 (14), 89 (21), 86 (29), 85 (3), 84 (8), 81 (25), 73 (11), 72 (3), 70 (26), 69 (22), 68 (34), 67 (12), 60 (42), 58 (23), 57 (14), 56 (10), 55 (100), 54 (23), 53 (15).

(*S*)-(+)-5-Chloro-3-(tetrahydropyranyl-2-oxy)hexane (6b). (*S*)-(+)-5-Chlorohexan-3-ol (**6a**) (2.127 g, 15.58 mmol) was treated with dihydropyran (1.68 g, 20.0 mmol) as described in the preparation of **5b** to give **6b**: 3.15 g, 92%; R_f 0.51 (SiO_2, hexane/ethyl acetate, 8:1). NMR (80 MHz, $CDCl_3$) 4.58 (m, 1 H), 3.86 (m, 1 H), 3.52 (m, 4 H), 2.2–1.1 (m, 12 H), 0.92 (t, J = 5.1 Hz) and 0.86 (t, J = 5.1 Hz) (at a 1:1 ratio, together 3 H); IR (neat) 2957, 2932, 2877, 2852, 1465, 1455, 1440, 1380, 1353, 1340, 1210, 1200, 1188, 1175, 1155, 1130, 1115, 1080, 1030, 1000, 990, 900, 865, 800, 710, 650; mass spectrum, m/e (rel intens) 143 (2), 119 (9), 101 (11), 85 (100), 83 (17), 67 (14), 57 (27), 56 (75), 55 (50).

(7*S*)-3-Hydroxy-1-phenyl-7-(tetrahydropyranyl-2-oxy)non-1-ene (23a). The protected chlorohexanol **6b** (2.0 g, 9.0 mmol) was reacted with magnesium (2.0 g) and then with cinnamaldehyde (2.1 g, 15.9 mmol) as described for compound **5b** to give **23a**: 1.87 g, 65%; $[\alpha]_D$ +2.92° ($CHCl_3$, c 2.5); R_f 0.69 (SiO_2, hexane/ethyl acetate, 8:1) NMR (270 MHz, $CDCl_3$) 7.4 (m, 5 H), 6.57 (d, J = 15.9 Hz, 1 H), 6.22 (dd, J = 15.9, 6.6 Hz) and 6.21 (dd, J = 15.9, 6.6 Hz) (at a 1:1 ratio, together 1 H), 4.62 (m, 1 H), 4.30 (m, 1 H), 3.90 (m, 1 H), 3.57 (m, 1 H), 3.46 (m, 1 H), 1.7–1.5 (m, 14 H), 0.93 (t, J = 7.8 Hz) an 0.87 (t, J = 7.5 Hz) (at a 1:1 ratio, together 3 H); IR (neat) 3600–3100, 2940, 2870, 1470, 1455, 1385, 1355, 1265, 1205, 1135, 1120, 1075, 1025, 995, 750, 695.

(7*S*)-3-Acetoxy-1-phenyl-7-(tetrahydropyranyl-2-oxy)non-1-ene (23b). Acylation of **23a** (0.65 g, 2.0 mmol) was carried out as described for alcohol **11a** to give **23b**: 0.752 g, 99.6%; R_f 0.30 (SiO_2, hexane/ethyl acetate, 8:1); NMR (80 MHz, $CDCl_3$) 7.3 (m, 5 H), 6.62 (d, J = 15.2 Hz, 1 H), 6.10 (br dd, J = 15.2, 7.2 Hz, 1 H), 5.36 (br q, J = 7.2 Hz, 1 H), 4.58 (m, 1 H), 3.88 (m, 1 H), 3.72 (m, 2 H), 2.06 (s, 3 H), 1.8–1.3 (m, 14 H), 0.92 (t, J = 5.3 Hz) and 0.85 (t, J = 5.3 Hz) (at a 1:1 ratio, together 3 H); IR (neat) 2980, 2880, 1735, 1480, 1450, 1440, 1375, 1255, 1250, 1245, 1200, 1135, 1080, 1035, 1005, 970, 760, 700.

(7*S*)-3-Acetoxy-7-hydroxy-1-phenylnon-1-ene (23c). Deprotection of **23b** (0.88 g, 2.4 mmol) was carried out as described for compound **11b** to give **23c**: 0.555 g, 82%; R_f 0.67 (SiO_2, hexane/ethyl acetate, 1:1); NMR (80 MHz, $CDCl_3$) 7.3 (m, 5 H), 6.62 (d, J = 15.6 Hz, 1 H), 6.11 (dd, J = 15.6, 7.0 Hz, 1 H), 5.52 (br q, J = 7.1 Hz, 1 H), 3.48 (m, 1 H), 2.07 (s, 3 H), 1.6–1.2 (m, 8 H), 0.93 (t, J = 6.7 Hz, 3 H); IR (neat) 3600–3100, 2920, 2860, 1730, 1460, 1450, 1370, 1235, 1010, 965, 695.

(2*S*,6*S*)-(*E*)-2-(*cis*-6-Ethyltetrahydropyran-2-yl)-1-phenylethene (25) and (2*R*,6*S*)-(*E*)-2-(*trans*-6-Ethyltetrahydropyran-2-yl)-1-phenylethene (24). Cyclization of the hydroxy acetate **23c** (270 mg, 0.98 mmol) was carried out as described for compound **11c** by using $Pd(PPh_3)_4$ (58 mg, 0.05 mmol) to give **25** (116 mg, 55%) and **24** (92 mg, 44%).

Physical Properties of the Cis Isomer 25: $[\alpha]_D$ −34.83° ($CHCl_3$, c 1.2); R_f 0.62 (SiO_2, hexane/ethyl acetate, 8:1); NMR (270 MHz, $CDCl_3$) 7.39 (d, J = 6.7 Hz, 2 H), 7.29 (br t, J = 6.7 Hz, 2 H), 7.19 (br t, J = 6.7 Hz, 1 H), 6.58 (dd, J = 16.1, 0.8 Hz, 1 H), 6.24 (dd, J = 16.1, 5.8 Hz, 1 H), 3.99 (dddd, J = 11.0, 5.8, 2.1, 0.8 Hz, 1 H), 3.30 (d, t, d, J = 11.1, 6.2, 1.6 Hz, 1 H), 1.9 (m, 1 H), 1.7–1.2 (m, 7 H), 0.96 (t, J = 7.5 Hz, 3 H); IR (neat) 2930, 2870, 1490, 1465, 1445, 1440, 1375, 1355, 1345, 1335, 1200, 1120, 1100, 1085, 1040, 1000, 965, 885, 755, 745, 700, 695; mass spectrum, m/e (rel intens) 218 (1, M^+), 201 (1), 187 (3), 169 (3), 159 (5), 148 (19), 143 (6), 133 (39), 131 (48), 130 (54), 129 (21), 128 (19), 115 (30), 111 (9), 105 (59), 104 (100), 103 (21), 91 (78), 84 (37), 83 (20), 78 (21), 77 (30), 69 (25), 68 (48), 65 (12), 57 (16), 56 (36), 55 (90); ^{13}C (22.63 MHz, $CDCl_3$) 131.31, 129.55, 128.42 (2 C), 128.09, 127.29, 126.45 (2 C), 79.32, 78.14, 32.07, 30.80, 29.44, 23.72, 9.98. Anal. Calcd: C, 83.33; H, 9.26. Found: C, 83.85; H, 9.21.

Physical Properties of the Trans Isomer 24: $[\alpha]_D$ −25.5° ($CHCl_3$, c 0.2); R_f 0.54 (SiO_2, hexane/ethyl acetate, 8:1); ^1H NMR (270 MHz, $CDCl_3$) 7.41 (br d, J = 6.7 Hz, 2 H), 7.32 (t, J = 6.7 Hz, 2 H), 7.23 (t, J = 6.7 Hz, 1 H), 6.47 (dd, J = 16.2, 1.5 Hz, 1 H), 6.31 (dd, J = 16.2, 4.9 Hz, 1 H), 4.51 (m, 1 H), 3.68 (q, d, J = 6.5, 3.3 Hz, 1 H), 1.81 (m, 1 H), 1.73–1.57 (m, 5 H), 1.47–1.26 (m, 2 H), 0.94 (t, J = 7.9 Hz, 3 H); IR (neat) 2930, 2840, 1490, 1460, 1445, 1435, 1370, 1322, 1307, 1195, 1085, 1070, 1040, 1000, 960, 905, 740, 690; mass spectrum, m/e (rel intens), 216 (36, M^+), 201 (1), 187 (4), 169 (4), 159 (5), 148 (16), 143 (9), 133 (37), 131 (38), 130 (54), 129 (26), 128 (22), 117 (12), 115 (28), 111 (9), 105 (56), 104 (100), 98 (3), 91 (85), 84 (35), 83 (20), 78 (19), 77 (26), 69 (25), 68 (46), 65 (14), 57 (19), 56 (34), 55 (79); ^{13}C (22.63 MHz, $CDCl_3$) 136.68, 130.85, 130.76, 128.54 (2 C), 127.41, 126.37 (2 C), 72.82, 71.88, 30.16, 29.94, 27.35, 19.00, 10.11. Anal. Calcd: C, 83.33; H, 9.21. Found: C, 83.75; H, 9.20.

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Catalytic Oxidation of Dithiols by a Semisynthetic Enzyme

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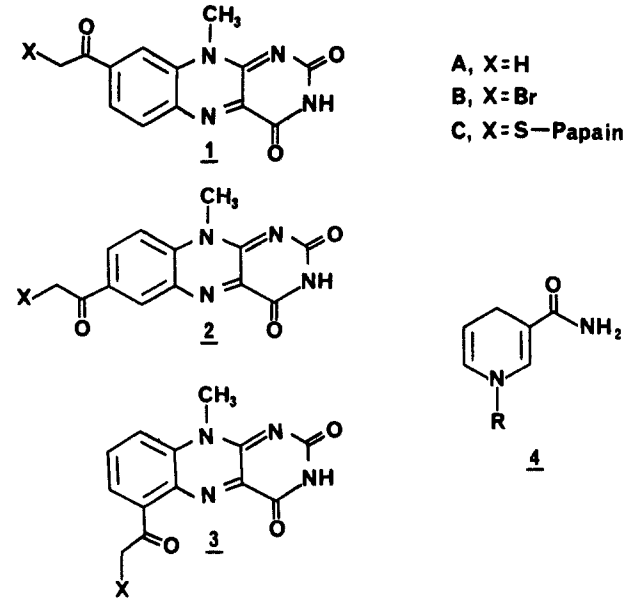
Abstract: The semisynthetic enzyme flavopapain (**1C**), obtained from the alkylation of Cys-25 of papain with 8α-(bromoacetyl)-10-methylisoalloxazine (**1B**), was found to be an effective catalyst for the oxidation of dithiols to disulfides. The k_2/K_s values for the oxidation of *d,l*-dihydrolipoamide and *d,l*-dihydrolipoic acid determined from anaerobic single-reaction stopped-flow kinetics were 4400 and 3400 M^{-1} s^{-1}, respectively. These values were, respectively, 126 and 200 times larger than the second-order rate constants for oxidation of *d,l*-dihydrolipoamide and *d,l*-dihydrolipoic acid by the model flavin 8-acetyl-10-methylisoalloxazine (**1A**). Under aerobic turnover conditions using the synthetic dye MTT as an electron acceptor, the k_{cat} and K_m values for the oxidation of *d,l*-dihydrolipoamide by **1C** were in approximate agreement with the k_2 and K_s values, indicating that the rate-limiting step of the catalytic cycle is substrate oxidation rather than oxidation of dihydroflavopapain. When compared with flavopapains **2C** and **3C** [obtained as above but by modification with 7α- and 6α-(bromoacetyl)-10-methylisoalloxazine (**2B** and **3B**, respectively)], flavopapain **1C** is the most efficient catalyst. The circular dichroic spectra of flavopapains **1C**, **2C**, and **3C** were recorded, and the dissociation constants of the sulfite addition complexes of **1C** and **2C** were determined. From these kinetic and physical studies, the differences in catalytic activity of **1C**, **2C**, and **3C** were judged to be due to changes in the flavin orientation within the active site and the ability to fit the substrate into a productive reaction conformation.

The chemical modification of existing protein molecules to produce new catalysts represents an important approach to enzyme engineering.[1] In our work, we have covalently linked isoalloxazine derivatives to proteolytic enzymes such as papain to create new redox enzymes.[2] Specifically, the Cys-25 thiol of papain was treated with α-(bromoacetyl)isoalloxazines **1B**, **2B**, and **3B** to produce "flavopapain" semisynthetic enzymes **1C**, **2C**, and **3C**, respectively[3] (Chart I). These flavopapains have been shown to be catalysts for the oxidation of *N*-alkyl-1,4-dihydronicotinamides **4**. When coupled with artificial electron acceptors, flavopapain **1C** has been shown to be a particularly efficient oxidation catalyst.[4]

In view of papain's broad specificity as a hydrolytic enzyme, it appeared likely that the flavopapain enzymes might bind other classes of substrates and catalyze different kinds of redox reactions. Among the many oxidation reactions catalyzed by flavins is the oxidation of thiols to disulfides. Indeed, flavopapain **2C** was shown to mediate the oxidation of dithiols, albeit with only modest rate acceleration relative to the model flavin compound **2A**.[5] We now report the first demonstration of efficient turnover catalysis of thiol oxidation by a synthetic catalyst, flavopapain **1C**. We also report on the results of several studies designed to aid in understanding the molecular basis of the different catalytic efficiencies of flavopapains **1C**, **2C**, and **3C**.

Chart I



Experimental Section

Instrumentation. Scanning UV-visible spectrophotometry was carried out on either a Perkin-Elmer λ-5 or a Varian Cary 219 spectrophotometer equipped with thermostated cell compartments. Stopped-flow measurements were made on a Durrum-Gibson stopped-flow spectrophotometer with a thermostated syringe compartment. All solutions were preequilibrated in the thermostated compartments for 5 min prior to recording data. Circular dichroism spectra were recorded on an AVIV 60DS CD spectrometer. High-pressure liquid chromatography analyses were performed on a Waters Associates liquid chromatograph equipped with a Altex reversed-phase G-18 analytical column.

Materials. *d,l*-Lipoamide and *d,l*-dihydrolipoic acid were purchased from Sigma. *d,l*-Dihydrolipoamide was prepared according to literature

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