

Biotransformations in the Synthesis of Enantiopure Bioactive Molecules

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Introduction

Recognition that chirality plays a crucial role in nature has fostered much effort in enantioselective synthesis. Although the concept of enzyme applications^{1–4} to asymmetric synthesis has been long recognized, it is only recently that these catalysts are attracting the attention of the nonspecialist. The ability to discriminate between enantiomers of a racemic substrate is of well-recognized preparative value.⁵ An enzymatic resolution gives access to both enantiomers, which is desirable if both are required. However, if only one enantiomer is needed, a 50% conversion constitutes the maximum yield. Enzymes are also capable of differentiating enantiotopic groups of prochiral and meso compounds. In contrast to the kinetic resolution of a racemic material, the theoretical yield of these conversions is 100%. The enzymatic desymmetrization of meso compounds has gained popularity in recent years and constitutes a highly effective approach for the synthesis of enantiomerically pure compounds.⁶ I will present here an overview of work from our laboratory involving the use of enzymes and fermentations in desymmetrizations and in resolutions for the production of enantiopure intermediates and the use of the latter in the synthesis of a variety of bioactive target molecules.

The molecular machinery of lipases is much like that of the serine proteases and consists of a catalytic triad of amino acids—serine, histidine, and aspartic (or glutamic) acid. This machinery first transfers the acyl group of an ester (or other acyl derivative) to the hydroxyl group of the serine residue to form the acylated enzyme. The acyl group is subsequently transferred to an external nucleophile

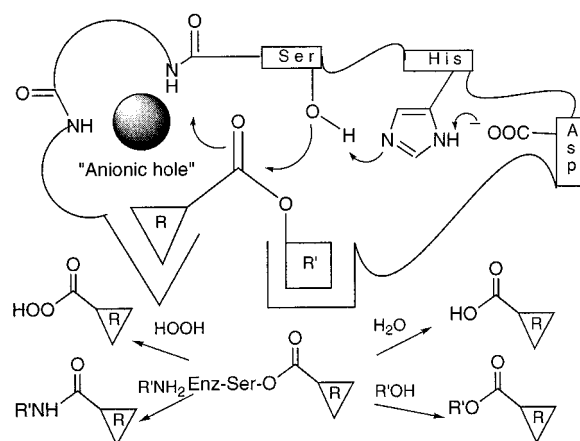


FIGURE 1. Mechanism of action of esterases and capture of the acyl/enzyme complex with various nucleophiles.

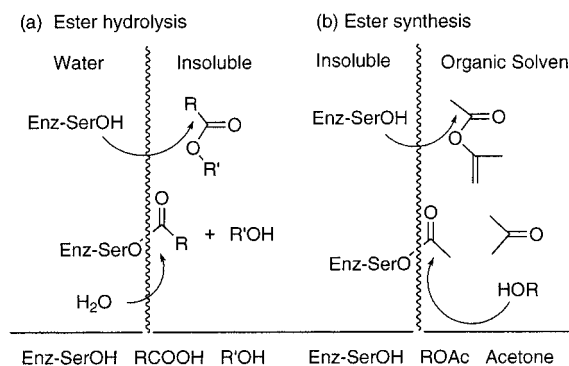
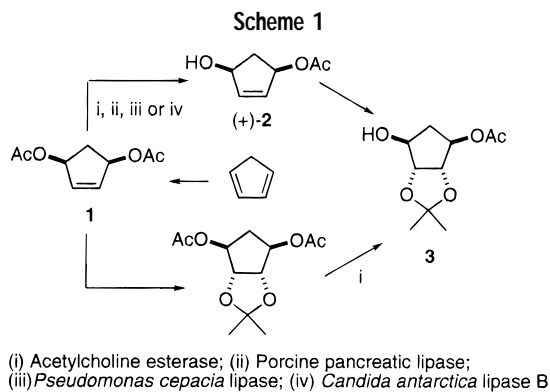


FIGURE 2. Lipases at interfaces. (a) Hydrolysis of an insoluble ester. (b) Transesterification involving an insoluble biocatalyst and the organic soluble acylating agent and substrate.

phile with return of the enzyme to its pre-acylated state to start the process over again. A variety of nucleophiles can participate in this process: water results in hydrolysis, an alcohol results in esterification or transesterification, an amine results in amidation, hydrogen peroxide results in the formation of a peracid (which can epoxidize an available olefin). The shape nature of the protein creates hydrophilic or lipophilic pockets⁷ within the enzyme active site which result in enantio-, diastereo-, or chemoselectivity. This selectivity can be manifested during formation of the acyl-enzyme intermediate or in the subsequent nucleophilic attack or both (Figure 1).

In general, cells produce lipases to hydrolyze extracellular fats. Because the natural substrates are water insoluble, lipases are especially structured to act at a water/organic interface. For this reason lipases appear to be optimum among the enzymes to operate in organic solvents;⁸ in this case the interface is between the insoluble enzyme with its essential water of hydration and the organic solvent containing an acylating agent, *e.g.*, isopropenyl acetate, and a substrate to be acylated (Figure 2). As a consequence of shape selective binding, enzymatic ester hydrolysis can be enantiocomplementary to the corresponding enzymatic ester formation, *e.g.*, hydrolysis of an (*RS*)-acetate may result in the formation of the (*R*)-acetate and (*S*)-alcohol whereas exposure of the

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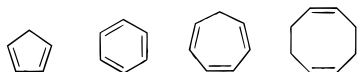


corresponding (*RS*)-alcohol to the same enzyme under acetylating conditions may result in the (*S*)-acetate and the (*R*)-alcohol.⁹ Note that in each case the enzyme possesses the same enantioselectivity, in the above examples preferentially hydrolyzing the (*S*)-acetate and acetylating the (*S*)-alcohol.

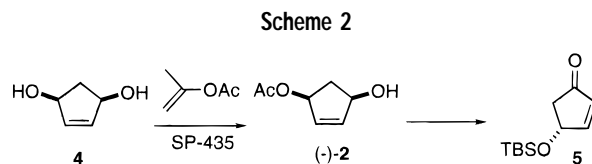
In contrast to hydrolase enzymes such as the lipases, oxidoreductase systems are generally much more complex and require recycling of cofactors. For this reason it is often more expedient, or even necessary, to carry out transformations involving these systems with growing cells, *i.e.*, by fermentation.¹⁰ For example, the arene dioxygenase system chemistry discussed in this Account requires NADH and consists of a flavoprotein reductase, a ferredoxin, and a tetrameric iron-sulfur protein.

Enzymatic Desymmetrizations

Our laboratory is engaged in a program of synthesis of bioactive molecules by routes involving the following features: Readily available cyclic starting materials are subjected to stereospecific oxidation reactions which serve to set relative ring stereochemistry and produce compounds of the stereochemical class meso. These meso compounds are desymmetrized by use of enzymes to set absolute stereochemistry about the ring. Further manipulation then provides useful new enantiopure intermediates which are carried on to target molecules. In many instances, regioselective ring cleavage reactions are used to unveil acyclic compounds with fixed absolute stereochemistry. Our approaches to such enantiopure intermediates have utilized the following as starting materials:



Chemistry Based on Cyclopentadiene. In the five-membered ring series our chemistry has begun with the addition of singlet oxygen to cyclopentadiene. This cycloaddition reaction, which is recurrent in our program, has the advantage of cleanly setting oxygen functionality *cis* at the termini of cyclic dienes. A variety of enzymes have been found useful in the production of enantiopure cyclopentenol (+)-**2** from **1** (Scheme 1).¹¹ The enantiomeric (-)-**2** has been prepared from the diol **4** by



enzymatic acetylation in organic media with isopropenyl acetate in the presence of the biocatalyst SP-435 (immobilized, recombinant *Candida antarctica* lipase B from Novo Nordisk) (Scheme 2).¹²

The key chiral intermediate, enone **6**, can be prepared in either enantiomeric series from **3**. Direct oxidation of **3** leads to (+)-**6** whereas protective group manipulations and oxidation at the former acetoxy-bearing carbon of **3** leads to the enantiomer (-)-**6**.^{13–15} This ability to enter into either enantiomeric series in a straightforward fashion regardless of the enantiomeric outcome of the enzymatic desymmetrization is a consistent advantage of working with cyclic meso diol derivatives as substrates. Enone **8** has been prepared using related chemistry with an equilibration of *cis*-protected diol compound **7** to the *trans* isomer at the last stage.¹⁶

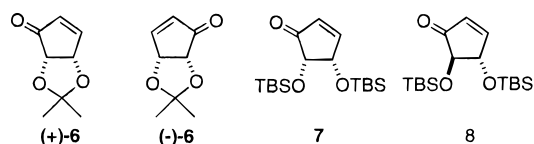


Figure 3 illustrates examples of target molecules that we have prepared from the various cyclopentenones. As noted earlier, ring cleavage reactions are used to unveil stereochemically secure acyclic materials; it is frequently the case that subsequently these ring-opened materials are transformed to heterocyclic targets as exemplified in Scheme 3.

Chemistry Based on Benzene. *cis*-Dihydroxylation appears to be the first step in the degradation of aromatic compounds by lower organisms; degradation is continued by subsequent action of a dehydrogenase. About 1970 Gibson²³ discovered that the mutant *Pseudomonas putida* 39/D, a source of the enzyme “toluene dioxygenase”, lacked the dehydrogenase and allowed for the accumulation of the diols from arenes. It appeared to us that meso-3,5-cyclohexadiene-1,2-diol (**10**), available by oxidation of benzene by *P. putida* 39/D (or by strains of *Escherichia coli* (*E. coli* JM109 (pDTG601)) containing the genetic code for toluene dioxygenase)²⁴ would be an ideal starting material for the development of enantioselective syntheses of various cyclitols and related materials which are currently of interest as glycosidase inhibitors. The diol **10** was protected as its acetone **11** and the latter converted to the meso-2,3-diprotected conduritol A (**12**) by addition of singlet oxygen and thiourea reduction. The meso diol **12** was treated with crude lipase from *Pseudomonas cepacia* (Amano PS-30 lipase) (*ca.* 1:1 substrate to crude lipase) in isopropenyl acetate at 55 °C for 2 days to yield monoacetate **13** (90% yield, >98% ee). Variants on Mitsunobu chemistry were very successful for the inver-

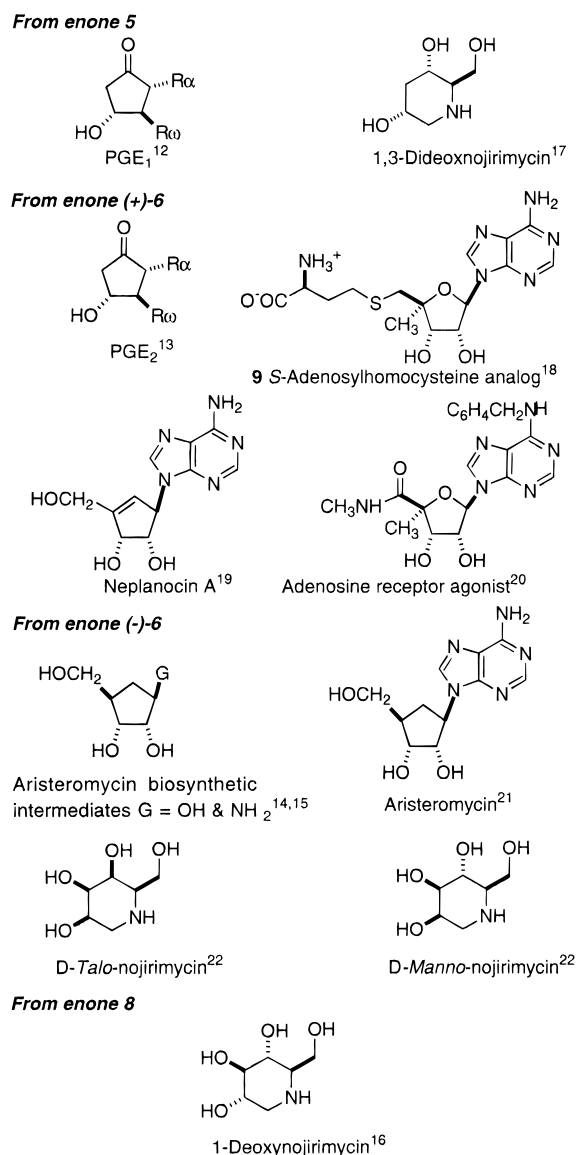
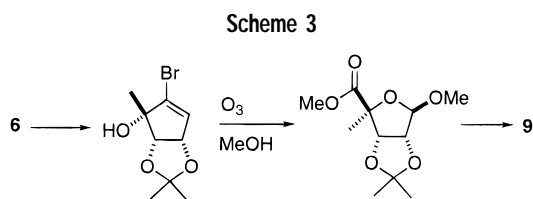
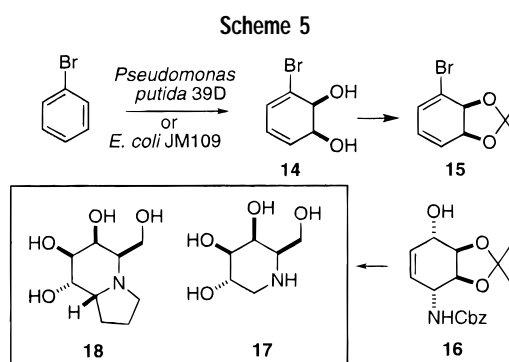
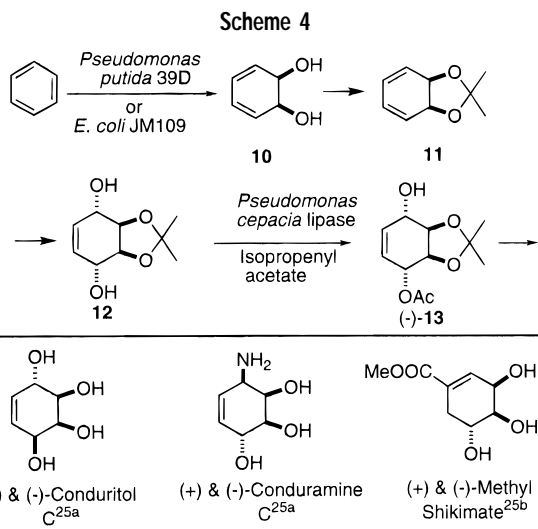


FIGURE 3. Targets prepared from cyclopentenones.



sion of oxygenated centers and the introduction of amino substituents (Scheme 4).²⁵

The biooxidation of numerous mono-substituted benzenes by *P. putida* 39/D or *E. coli* JM109 leads directly to the enantiopure diols which are versatile intermediates for exploitation in target molecule synthesis.^{24,26} Biooxidation of bromobenzene leads to diol **14** (Scheme 5); protection of the diol and addition of an acylnitroso dienophile followed by reduction steps led to **16**. The latter served as a key intermediate in the preparation of (+)-1-deoxy-galacto-nojirimycin (**17**) and the related indolizidine **18** (Scheme 5).²⁷ In these and subsequent examples, the carbocyclic ring is converted to a nitrogen



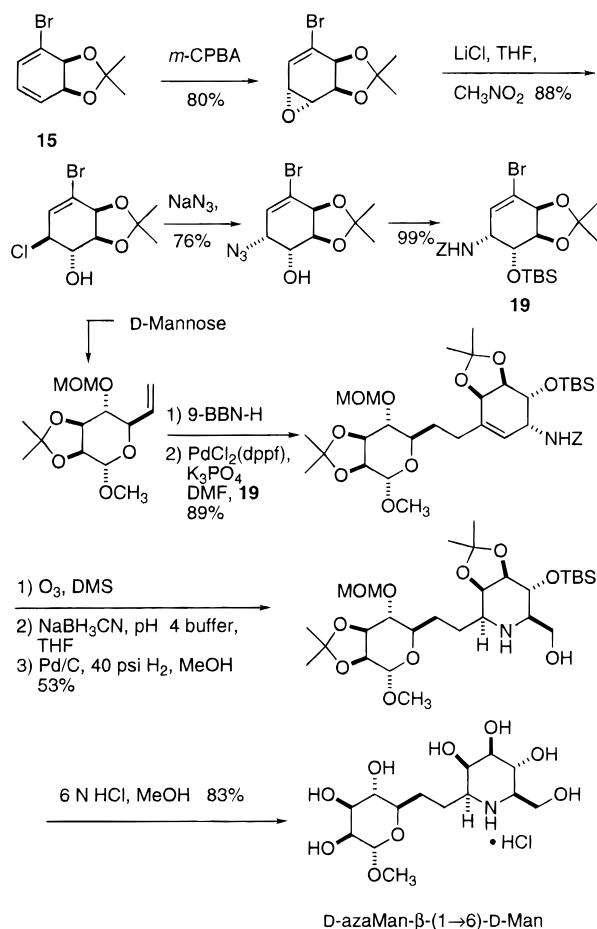
heterocycle by ozonolysis followed by internal reductive amination.²⁸

An efficient method for the production of variously linked "aza-C-disaccharides" has been developed utilizing the enantiopure diol **14** as a source of the protected aminotriol **19**. Details for the production of the model system D-azaMan- β -(1,6)-D-Man(OMe) are illustrated in Scheme 6.^{29,30} Reduction of the intermediate imine gives rise to ss-linked aza-C-glycosides,^{31,32} whereas various α -linked saccharide mimics are available by variants of the route illustrated in Scheme 7.³³ This technology has been extended to provide a variety of saccharide wherein internal units are replaced with simplified scaffolds (Scheme 8).^{33,34}

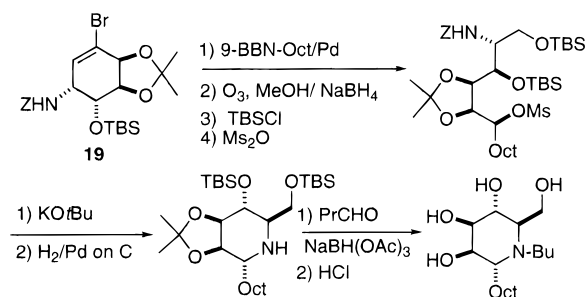
Chemistry Based on Cycloheptatriene. Cycloheptatriene has been transformed to tropone by a number of processes. We favor a variant based on the procedure of Reingold wherein tropylium ion is treated with carbonate to initiate a disproportionation which provides tropone and cycloheptatriene. Tropone undergoes a reduction to 3,5-cycloheptadienol (**20**) with sodium borohydride in methanol (Scheme 9). The meso diol **21** and its diastereomer **23** were prepared utilizing singlet oxygen chemistry and Bäckvall Pd(II) chemistry, respectively. These diols were cleanly desymmetrized using *P. cepacia* lipase in isopropenyl acetate to provide optically pure **22** and **24**, respectively (Scheme 10).³⁵

The preparation of **22** nicely illustrates that utilization of enzymes in organic media often provides significant

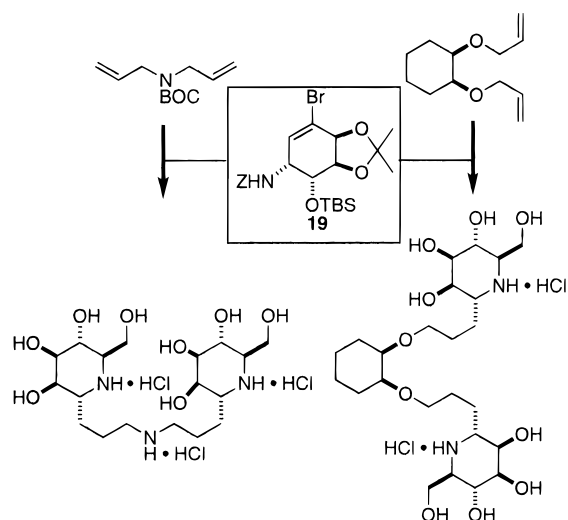
Scheme 6



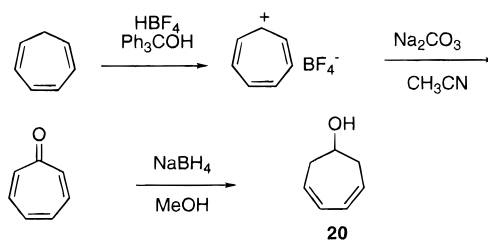
Scheme 7



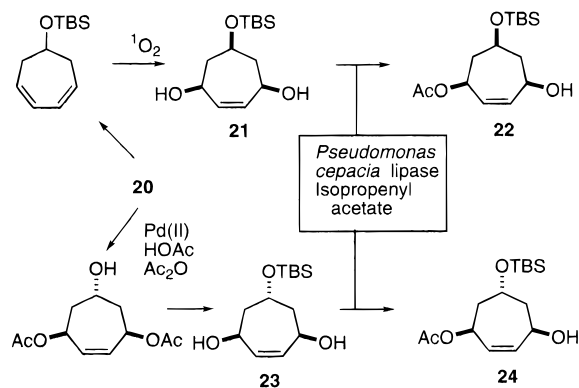
Scheme 8



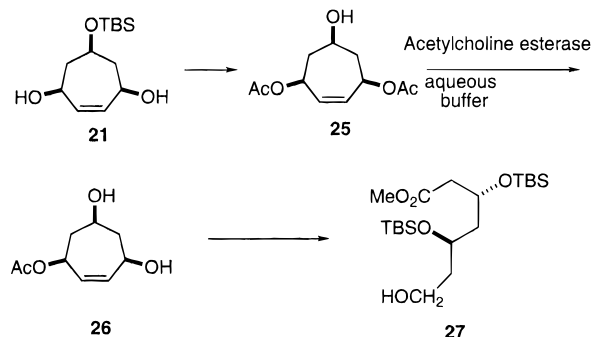
Scheme 9



Scheme 10



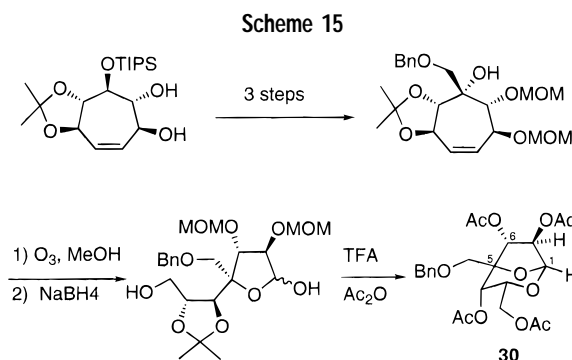
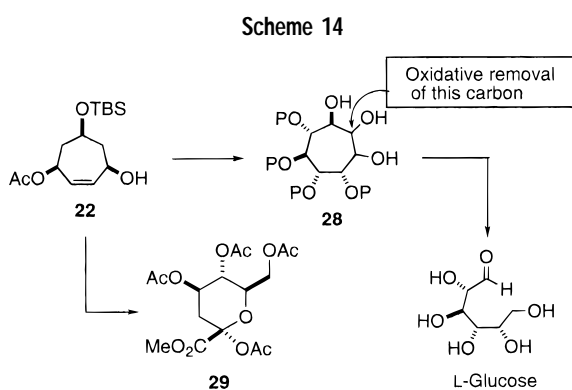
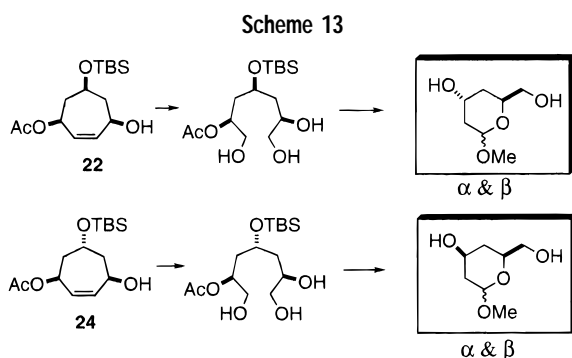
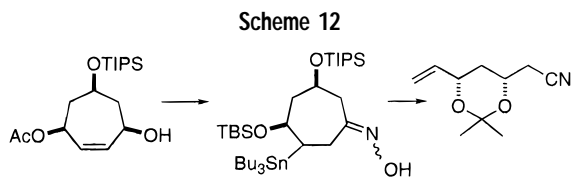
Scheme 11



advantages in a synthetic sequence. In the aqueous-based sequence the diacetate **25** was formed from compound **21**, the *tert*-butyldimethylsilyl protecting group was removed to increase water solubility, and the meso diacetate **25** was desymmetrized using acetylcholine esterase in aqueous phosphate buffer to provide diol (+)-**26**. The manipulation of (+)-**26** to the important compactin analogue intermediate **27** required the differentiation of the allylic and non-allylic free hydroxyl groups as well as the reinstallation of the silyl protecting group (Scheme 11).³⁶ By switching to enzyme chemistry in organic media three steps (the conversion to the diacetate and the removal and replacement of the *tert*-butyldimethylsilyl) were eliminated in the overall conversion of **21** to **27**.

Building blocks for skipped polyols have been prepared from **24** and its TIPS analog utilizing tin-directed frag-

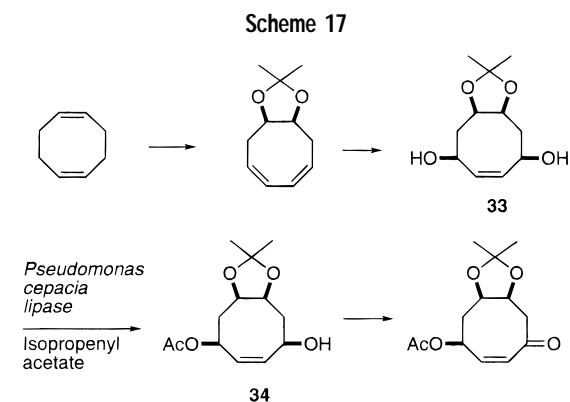
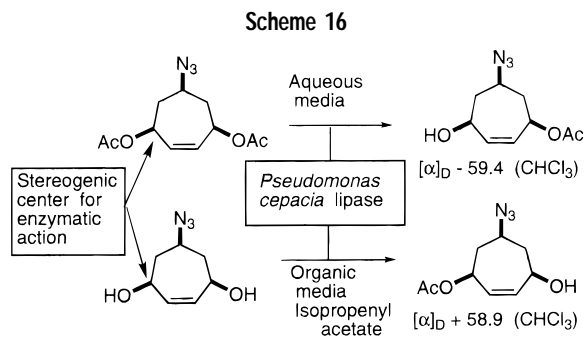
mentations (Scheme 12).³⁷ We have found these cycloheptatriene-derived enantiopure intermediates to be useful in the synthesis of a variety of carbohydrate derivatives. All eight enantiopure methyl glycosides of the 2,4-



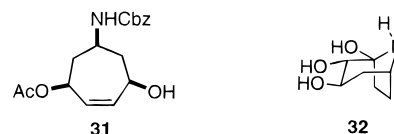
dideoxyhexoses have been prepared from **22** and **24**, four of which are illustrated in Scheme 13.³⁸

Stereoselective oxidation of **21** to **28** allowed us to complete the transformation of cycloheptatriene to L-glucose (Scheme 14).³⁹ Compound **22** has also served as a precursor for the synthesis of the 3-deoxy-D-arabino-heptulosonic acid derivative **29**.⁴⁰ The related triisopropylsilyl derivative has been transformed to the zaragozic acid core structure **30** utilizing tandem Rubottom reactions and $\text{BnOCH}_2\text{SnBu}_3$ to introduce the additional oxygenated centers prior to ring cleavage by ozonolysis (Scheme 15).⁴¹

Scheme 16 illustrate the enantiocomplementary outcome of enzymatic reactions in aqueous and organic media. In chemistry related to that shown in Scheme 16,



intermediate **31** has been processed to both enantiomers of the tropane alkaloid calystegine A₃ (**32**).⁴²



Chemistry Based on Cyclooctadiene. The enantiopure tetraol derivative **34** has been prepared from 1,5-cyclooctadiene. The key step in the sequence was the enzymatic desymmetrization of meso diol **33** using *P. cepacia* lipase and isopropenyl acetate (Scheme 17).⁴³

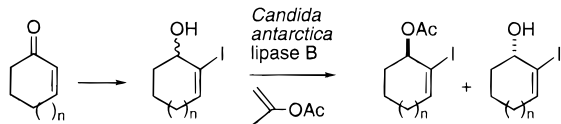
Resolutions

Our program has emphasized enzymatic desymmetrizations but has not ignored enzymatic resolutions. The latter can be applied in cases where the former is not suitable or is less direct. Select examples of enzymatic resolutions carried out in our laboratory in pursuit of bioactive molecules are outlined below.

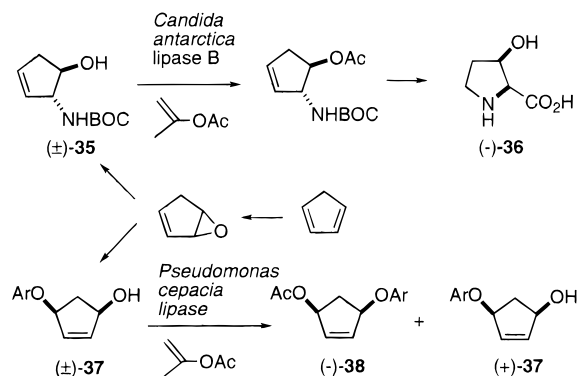
Simple cycloalkenols (or esters thereof) do not respond well to enzymatic resolutions; this is perhaps a reflection of the small steric difference in the CH_2 and CH groups flanking the carbinol center. We have recently developed a method for the α -iodination of cycloalkenones to provide 2-iodo-2-cycloalken-1-ones.⁴⁴ Reductions of the latter with $\text{NaBH}_4/\text{CeCl}_3$ gave 2-iodo-2-cycloalken-1-ols which were nicely resolved using *Candida antarctica* lipase B (SP-435) and isopropenyl acetate (Scheme 18).⁴⁵

Cyclopentadiene was readily monoepoxidized with peracetic acid. Direct ring opening of the epoxide with ammonia led to a racemic amino alcohol, shown as its BOC derivative **35**. The latter was cleanly resolved using

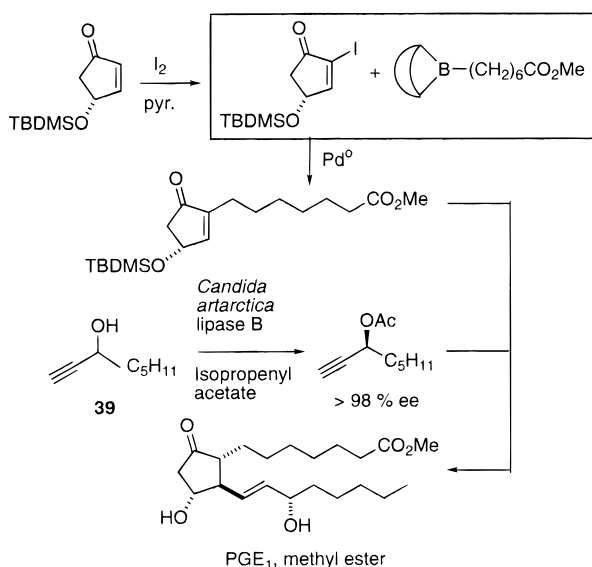
Scheme 18



Scheme 19



Scheme 20

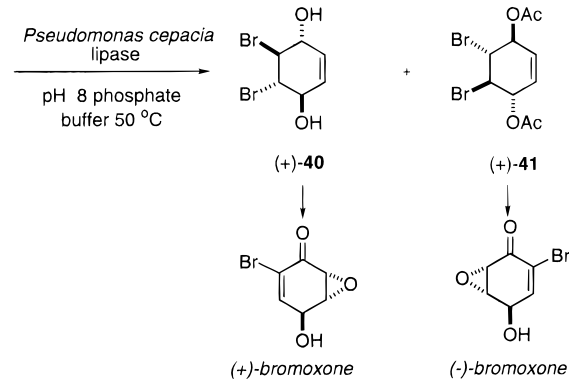
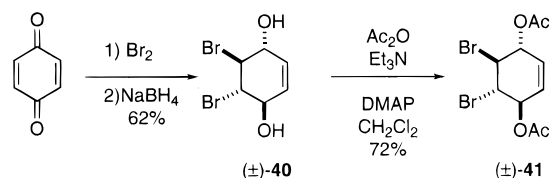


C. antarctica lipase B (SP-435) and isopropenyl acetate to provide a basis for the synthesis of (2*S*,3*R*)-3-hydroxyproline (**36**) (Scheme 19).⁴⁶

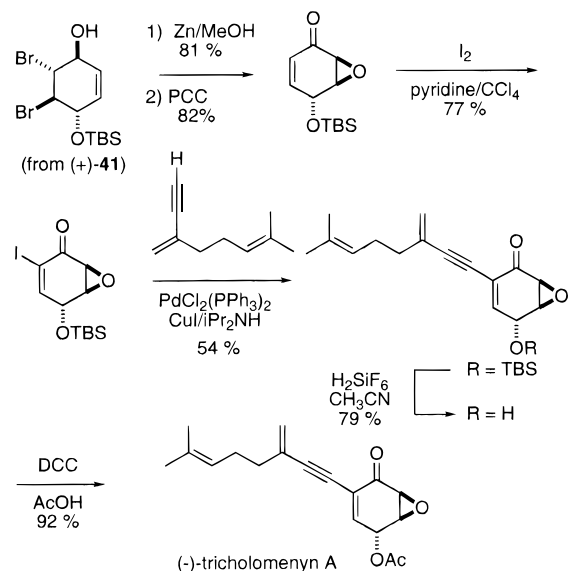
Cyclopentadiene monoepoxide in the presence of a phenol and a Pd catalyst provided racemic **37**. Kinetic resolution of (±)-**37** using *P. cepacia* lipase (Amano PS-30) and isopropenyl acetate provided (–)-**38** (45% yield, >98% ee) and (+)-**37** (49% yield, >95% ee) (Scheme 19).¹⁶ The latter compounds are readily transformed to enones (+)- and (–)-**6**. A similar resolution has been achieved in the hydrolysis direction by Siddiqui and Schneller.⁴⁷

As noted previously the biocatalyst SP-435 has been used in the synthesis of enone **6** (Scheme 2). The same biocatalyst has been found effective for the resolution of **39**, a lower side-chain intermediate for prostaglandins. Our very short synthesis of PGE₁ illustrated below relies on this biocatalyst for introduction of absolute stereochemistry in both the ring and side chain (Scheme 20).¹² The key coupling was achieved by the Suzuki process

Scheme 21

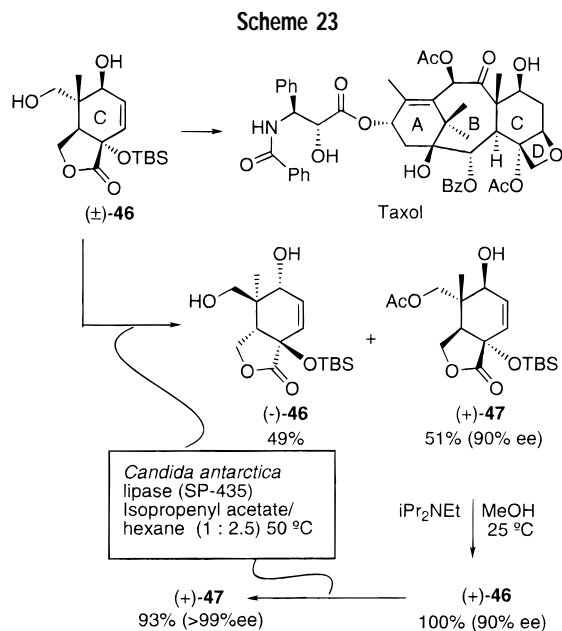


Scheme 22



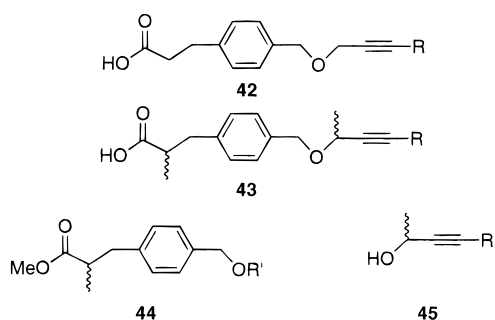
involving an α -iodoenone. α -Iodoenones which are necessary for the success of this procedure are prepared by the general method noted above involving elemental iodine and pyridine.⁴⁴ This coupling methodology provides a general solution to many problems encountered in alternative procedures for α -substitution of ketones.

The C_2 -symmetric diol (±)-**40**, prepared via bromination/reduction of *p*-benzoquinone, was converted into the known diacetate (±)-**41**. The hydrolysis of (±)-**41** with crude *P. cepacia* lipase (Amano PS-30) in pH 8 phosphate buffer at 50 °C was effective; the diacetate (+)-**41** (26%, >98% ee) and the diol (+)-**40** (47%, 90% ee) were obtained (Scheme 21).⁴⁸ Note that in this case each enantiomer of the diacetate contains two carbinol centers of the same absolute configuration and that double hydrolysis occurs in the case of the *R,R*-enantiomer. These enantiopure intermediates (after recrystallization) have been used in the preparation of the natural (+) and unnatural enanti-



mers of the epoxyquinol natural product, bromoxone.⁴⁸ α -Iodocycloalkenones readily participate in Sonagashira coupling as illustrated in the benzoquinone-based synthesis of tricholomenyn A in Scheme 22.⁴⁹

We have been interested in compounds that exhibit 12-lipoxygenase inhibitory activity.⁵⁰ One group of compounds that has been a focus of our studies are arylpropanoic acids of the general structure **42**. In connection with structure–activity studies we have prepared a series of methylated analogues including all possible absolute stereochemical isomers of **43**. The intermediates for the preparation of the various **43** isomers were prepared by enzymatic kinetic resolutions of **44** (aqueous media) and **45** (organic media) using *P. cepacia* lipase (Amano PS-30).⁵¹



In the Nicolaou total synthesis of taxol,⁵² relative stereochemistry of the core tetracycle was relayed from ring C intermediate **46**. This intermediate was expeditiously constructed starting with a Diels–Alder reaction facilitated by a boronic ester tether (Scheme 23).⁵² Treatment of (±)-**46** with isopropenyl acetate/hexane (1:2.5) in the presence of SP-435 at 50 °C for 24 h gave the results outlined in Scheme 23. Monoacetate **47** (ee 90%), readily separated from unreacted diol **46** by flash chromatography, was quantitatively deacetylated with Hunig's base in methanol. The resulting alcohol, (+)-**46**, was resubjected

to enzymatic transesterification to provide (+)-**47** in 93% yield and >99% ee. The overall production of enantiopure (+)-**47** from (±)-**46** was 47% (out of a theoretical 50%).⁵³ These results nicely illustrate the opportunity of enantiopurity enhancement which can be achieved by appropriate sequential kinetic resolutions or by coupling any enantioselective reaction with a subsequent kinetic resolution.⁵⁴

Concluding Remarks

It is hoped that this Account will persuade the reader of the remarkable advantages that can accrue by the incorporation of enzymatic steps into a synthetic design. The advantages of enantioselectivity of enzymatic reactions can often be enhanced by use of meso compounds, and the range of useful substrates can be extended by use of enzymes in organic media.

Nature's catalysts—the enzymes—should be considered by synthetic chemists in the same context as man-made catalysts; their use in a synthetic sequence may provide unique advantages of efficiency, stereoselectivity, and environmental friendliness. Although enzymes are designed by nature to carry out a specific molecular transformation on a specific substrate, when encouraged to do so, lipases in particular can be remarkably promiscuous in regard to substrates and conditions and, yet, remain exquisitely chemo-, regio-, and enantioselective.

I express my gratitude to my students and collaborators who have participated in the work herein reviewed and whose names appear in the references. Our work cited in this review has been largely supported by grants from the National Science Foundation. In addition, the support of various projects by the NIH, 3M Company, S. C. Johnson Wax, Ono Pharmaceutical, Mitsubishi Paper, and Merck is gratefully acknowledged. We thank Dr. Gregg Whited of Genencor International for carrying out the biooxidations of arenes described in this Account and Novo Nordisk for gifts of SP-435.

References

- (1) For recent specialized books see: (a) Faber, K. *Biotransformations in Organic Chemistry*; Springer-Verlag: Berlin, Heidelberg, 1997. (b) Wong, C.-H.; Whitesides, G. M. *Enzymes in Synthetic Organic Chemistry*; Elsevier Science Ltd.: Oxford, 1994. (c) Faber, K.; Griengl, H. In *Chirality - From Weak Bosons to the α -Helix*; Janoschek, R., Ed.; Springer-Verlag: Berlin Heidelberg, 1991; pp 103-140. (d) Servi, S. *Microbial Reagents in Organic Synthesis*; NATO ASI Series C; Kluwer: Dordrecht, 1992. (e) Abramowicz, D. A., Ed. *Biocatalysis*; Van Nostrand Reinhold: New York, 1990. (f) Drauz, K., Waldmann, H., Eds. *Enzyme Catalysis in Organic Synthesis Vol. 1 & 2*; VCH: Weinheim, 1995. (g) Gerhartz, W., Ed. *Enzymes in Industry*; VCH: Weinheim, 1990. (h) Sheldon, R. A. *Chirotechnology: Industrial Syntheses of Optically Active Compounds*; Marcel Dekker, Inc.: New York, 1993.
- (2) For recent special issues of periodicals see: (a) *Proceedings of the European Symposium on Biocatalysis, Graz 1993*; Crout, D. H. G., Griengl, H., Faber, K., Roberts, S. M., Eds. *Biocatalysis* **1994**, 9 (1-4). (b) *Proceedings of the International Biorganic*

- Symposium "Biotransformations in Organic Chemistry: Principles and Applications", Interlaken 1993*; Kieslich, K., Leuenberger, H. G. W., Seebach, D., Eds. *Chimia* **1993**, 47 (4). (c) *Enzymes in Organic Synthesis*; Crout, D. H. G., Roberts, S. M., Jones, J. B., Eds. *Tetrahedron: Asymmetry* **1993**, 4 (5–6). (d) *Biocatalysis in Organic Chemistry*; Meijer, E. M., Ed. *Recl. Trav. Chim. Pays-Bas* **1991**, 110 (5). (e) *Enantioselective Synthesis using Biological Systems*; Azerad, R., Sih, C. J., Eds. *Biocatalysis* **1990**, 3 (1–2).
- (3) For recent general reviews see: (a) Azerad, R. *Bull. Soc. Chim. Fr.* **1995**, 132, 17. (b) Wong, C.-H.; Halcomb, R. L.; Ichikawa, Y.; Kajimoto, T. *Angew. Chem., Int. Ed. Engl.* **1995**, 34, 412, 521. (c) Santaniello, E.; Ferraboschi, P.; Grisenti, P.; Manzocchi, A. *Chem. Rev.* **1992**, 92, 1071. (d) Drueckhammer, D. G.; Hennen, W. J.; Pederson R. L.; Barbas, C. F., III; Gautheron, C. M.; Krach, T.; Wong, C.-H. *Synthesis* **1991**, 499. (e) Leuenberger, H. G. W. *Pure Appl. Chem.* **1990**, 62, 753.
 - (4) Selected specialized reviews. Bakers yeast: (a) Csuk, R. Glänzer, B. I. *Chem. Rev.* **1991**, 49–97. Hydrolytic enzymes: (b) Boland, W.; Fröbl, C.; Lorenz, M. *Synthesis* **1991**, 1049–1072. (c) Faber, K.; Riva, S. *Synthesis* **1992**, 895. *Pseudomonas fluorescens* lipase: (d) Xie, Z.-F. *Tetrahedron: Asymmetry* **1991**, 2, 773. Pig liver esterase: (e) Ohno, M.; Otsuka, M. *Org. React.* **1989**, 37, 1. (f) Zhu, L.-M.; Tedford, M. C. *Tetrahedron* **1990**, 46, 6587. Enzymes in the synthesis of chiral drugs: (g) Margolin, A. L. *Enzyme Microb. Technol.* **1993**, 15, 266. Enzymatic protecting group techniques: (h) Waldmann, H.; Sebastian, D. *Chem. Rev.* **1994**, 94, 911–937. Enzymatic C–C bond formation: (i) Fessner, W.-D.; Walter, C. *Topics in Current Chemistry*; Springer-Verlag: Berlin, 1996; Vol. 184.
 - (5) For a discussion of the quantitation of this discrimination see: Chen, C. S.; Wu, S.H.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1987**, 104, 2812.
 - (6) For recent reviews of enzymatic desymmetrizations see: (a) Schoffers, E.; Golebiowski, A.; Johnson, C. R. *Tetrahedron (Report)* **1996**, 52, 3769. (b) Danielli, B.; Lesma, G.; Passarella, D.; Riva, S. In *Chiral Synthons via Enzyme-Mediated Asymmetrization of Meso Compounds in Advances in the Use of Synthons in Organic Chemistry*; Dondoni, A., Padwa, A., Eds.; JAI Press Ltd.: London, 1993; p 143.
 - (7) Jones has pioneered the mapping of active sites by evaluation of substrates. For a model of the active site of pig liver esterase based on a study of more than 100 substrates see: Provencher, L.; Wynn, H.; Jones, J. B.; Krawczyk, A. R. *Tetrahedron: Asymmetry* **1993**, 4, 2025. For a simple model for prediction of enantioselectivity of *Candida rugosa* lipase see: Kazlauskas, R. J.; Weissfloch, A. N. E.; Rappaport, A. T.; Cuccia, L. A. *J. Org. Chem.* **1991**, 56, 2656.
 - (8) (a) Chen, C.-S.; Sih, C. J. *Angew. Chem., Int. Ed. Engl.* **1989**, 28, 695. (b) Klibanov, A. M. *Trends Biochem. Sci.* **1989**, 14, 141. (c) Klibanov, A. M. *Acc. Chem. Res.* **1990**, 23, 114. (d) Zaks, A.; Klibanov, A. M. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, 82, 3192. (e) Santaniello, E.; Ferraboschi, P.; Grisenti, P. *Enzyme Microb. Technol.* **1993**, 15, 367.
 - (9) Wang, Y.-F.; Wong, C.-H. *J. Org. Chem.* **1988**, 53, 3127. Faber, K.; Ottolina, G.; Riva, S. *Biocatalysis* **1993**, 8, 9.
 - (10) Roberts, S. M., Wiggins, K., Casy, G., Eds. *Preparative Biotransformations: Whole Cells and Isolated Enzymes*; Wiley: Chichester, 1993.
 - (11) Johnson, C. R.; Bis, S. J. *Tetrahedron Lett.* **1992**, 33, 7287.
 - (12) Johnson, C. R.; Braun, M. P. *J. Am. Chem. Soc.* **1993**, 115, 11014; U.S. Patent 5,359,110, 1994.
 - (13) Johnson, C. R.; Penning, T. D. *J. Am. Chem. Soc.* **1988**, 110, 4726; U.S. Patent 4,873,360, 1989.
 - (14) Parry, R. J.; Haridas, K.; De Jong, R. L.; Johnson, C. R. *Tetrahedron Lett.* **1990**, 31, 7549.
 - (15) Parry, R. J.; Haridas, K.; De Jong, R. L.; Johnson, C. R. *Chem. Commun.* **1991**, 10, 740.
 - (16) Johnson, C. R.; Nerurkar, B. M.; Golebiowski, A.; Sundram, H.; Esker, J. L. *Chem. Commun.* **1995**, 1139.
 - (17) Johnson, C. R.; Golebiowski, A.; Braun, M. P.; Sundram, H. *Tetrahedron Lett.* **1994**, 35, 1833.
 - (18) Johnson, C. R.; Esker, J. L.; Van Zandt, M. C. *J. Org. Chem.* **1994**, 59, 5854.
 - (19) Medich, J.; Kunen, K.; Johnson, C. R. *Tetrahedron Lett.* **1987**, 28, 4131.
 - (20) Siddiqi, S. M.; Jacobson, K. A.; Esker, J. L.; Olah, M. E.; Ji, X.-d.; Melman, N.; Tiwari, K. N.; Secrist, J. A., III; Schneller, S. W.; Cristalli, G.; Stiles, G. L.; Johnson, C. R.; Ijzerman, A. P. *J. Med. Chem.* **1995**, 38, 1174.
 - (21) Johnson, C. R.; De Jong, R. L. Unpublished results.
 - (22) Johnson, C. R.; Golebiowski, A.; Schoffers, E.; Sundram, H.; Braun, M. P. *Synlett* **1995**, 313.
 - (23) Gibson, D. T.; Hensley, M.; Yoshioka, H. Mabry, T. J. *Biochemistry* **1970**, 9, 1626.
 - (24) For recent reviews of the use of diols obtained by microbiological oxidations of arenes see: Hudlicky, T.; Thorpe, A. J. *Chem. Commun.* **1996**, 1993–2000. Carless, H. A. J. *Tetrahedron: Asymmetry* **1992**, 3, 795. Hudlicky, T.; Entwistle, D. A.; Pitzer, K. K.; Thorpe, A. J. *Chem. Rev.* **1996**, 96, 1195. Hudlicky, T. In *Green Chemistry: Designing Chemistry for the Environment*; Anastas, P. T., Williamson, T., Eds.; ACS Symposium Series; American Chemical Society: Washington, DC, **1996**, 626, 180. Widdowson, D. A.; Ribbons, D. W.; Thomas, S. D. *Janssen Chim. Acta* **1990**, 8, 3.
 - (25) (a) Johnson, C. R.; Plé, P. A.; Su, L.; Heeg, M. J.; Adams, J. P. *Synlett* **1992**, 388–390. (b) Johnson, C. R.; Adams, J. P.; Collins, M. A. *J. Chem. Soc., Perkin Trans. 1* **1993**, 1.
 - (26) For example, the diols derived from toluene and chlorobenzene have been transformed to enone (+)-**6** (Hudlicky, T.; Luna, H.; Barbieri, G.; Kwart, L.D. *J. Am. Chem. Soc.* **1988**, 110, 4735. Hudlicky, T.; Natchus, M. G.; Nugent, T. *Synth. Commun.* **1992**, 22, 151).
 - (27) Johnson, C. R.; Golebiowski, A.; Sundram, H.; Miller, M. W.; Dwaihy, R. L. *Tetrahedron Lett.* **1995**, 36, 653.
 - (28) For a detailed discussion of the oxidative cleavage/recyclization strategy with regard to the synthesis of carbohydrates and related substances see: Hudlicky, T. *Chem. Rev.* **1996**, 96, 3. Hudlicky, T. Reed, J. W. In *Advances in Asymmetric Synthesis*; Hassner, A., Ed.; JAI Press: London, 1995; Vol. 1, p 271.
 - (29) Johnson, C. R.; Miller, M. W.; Golebiowski, A.; Sundram, H.; Ksebati, M. B. *Tetrahedron Lett.* **1994**, 35, 8991.
 - (30) Johns, B. A.; Elbein, A. D.; Pan, Y. T.; Johnson, C. R. *J. Am. Chem. Soc.* **1997**, 119, 4856.
 - (31) Johnson, C. R.; Johns, B. A. *J. Org. Chem.* **1997**, 62, 6046.
 - (32) Johnson, C. R.; Johns, B. A. *Synlett* **1997**, 1406.
 - (33) Johnson, C. R.; Johns, B. A. *Tetrahedron Lett.* **1997**, 38, 7977.
 - (34) Johnson, C. R.; Johns, B. A., *Tetrahedron Lett.* **1998**, 39, 749.

- (35) Johnson, C. R.; Golebiowski, A.; McGill, T. K. Steensma, D. H. *Tetrahedron Lett.* **1991**, *32*, 2597.
- (36) Johnson, C. R.; Senanayake, C. H. *J. Org. Chem.* **1989**, *54*, 735.
- (37) Scialdone, M. A.; Johnson, C. R. *Tetrahedron Lett.* **1995**, *36*, 43.
- (38) Johnson, C. R.; Golebiowski, A.; Steensma, D. H. *Tetrahedron Lett.* **1991**, *32*, 3931.
- (39) Johnson, C. R.; Golebiowski, A.; Steensma, D. H. *J. Am. Chem. Soc.* **1992**, *114*, 9414.
- (40) Johnson, C. R.; Kozak, J. *J. Org. Chem.* **1994**, *59*, 2910.
- (41) Xu, Y.; Johnson, C. R. *Tetrahedron Lett.* **1997**, *38*, 1117.
- (42) Johnson, C. R.; Bis, S. J. *J. Org. Chem.* **1995**, *60*, 615.
- (43) Johnson, C. R.; Harikrishnan, L. S.; Golebiowski, A. *Tetrahedron Lett.* **1994**, *35*, 7735.
- (44) Johnson, C. R.; Adams, J. P.; Braun, M. P.; Senanayake, C. B.W.; Uskoković, M. R.; Wovkulich, P. M. *Tetrahedron Lett.* **1992**, *33*, 917.
- (45) Johnson, C. R.; Sakaguchi, H. *Synlett* **1992**, 813.
- (46) Sundram, H.; Golebiowski, A.; Johnson, C. R. *Tetrahedron Lett.* **1995**, *35*, 6975.
- (47) Siddiqi, S. M.; Schneller, S. W.; Ikeda, S. ; Snoeck, R.; Andrei, G.; Balzarini, J.; DeClercq, E. *Nucleosides Nucleotides* **1993**, *12*, 185.
- (48) Johnson, C. R.; Miller, M. W. *J. Org. Chem.* **1995**, *60*, 6674.
- (49) Miller, M. W.; Johnson, C. R. *J. Org. Chem.* **1997**, *62*, 1582.
- (50) Gorins, G.; Kuhnert, L.; Johnson, C. R.; Marnett, L. J. *J. Med. Chem.* **1996**, *39*, 4871.
- (51) Johnson, C. R.; Gorins, G.; Marnett, L. J.; Honn, K. V. U.S. Patent 5,238,832, 1993.
- (52) Nicolaou, K. C.; Ueno, H.; Liu, J.-J.; Nantermet, P. G.; Yang, Z.; Renaud, J.; Paulvannan, K.; Chaddha, R. *J. Am. Chem. Soc.* **1995**, *117*, 813 and preceding papers.
- (53) Johnson, C. R.; Xu, Y.; Nicolaou, K. C.; Yang, Z.; Guy, R. K.; Dong, J. G.; Berova, N. *Tetrahedron Lett.* **1995**, *36*, 3291.
- (54) For selected additional examples of sequential kinetic resolutions see Guo, Z.-W.; Wu, S.-H.; Chen, C.S.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1990**, *112*, 4943. Macfarland, E. L. A.; Roberts, S. M., Turner, N. L. *Chem. Commun.* **1990**, 569. Chen, C.-S.; Liu, Y.-C. *J. Org. Chem.* **1991**, *56*, 1966.

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