135974-45-7; **6f**, 136031-64-6; **6g**, 135974-46-8; **6h**, 135974-47-9; **6i**, 135974-48-0; **6j**, 135974-49-1; **6k**, 135974-50-4; **6l**, 135974-51-5; **6m**, 135974-52-6; **6n**, 135974-53-7; **6o**, 135974-54-8; **6p**, 136031-65-7; **6q**, 136031-66-8; samarium iodide, 13813-25-7.

Supplementary Material Available: Details of the X-ray crystallographic structure determinations described within the paper, including structure data, atomic coordinates, bond lengths and angles, and isotropic and anisotropic thermal parameters (108 pages). Ordering information is given on any current masthead page.

Synthesis and Analysis of 506BD, a High-Affinity Ligand for the Immunophilin FKBP

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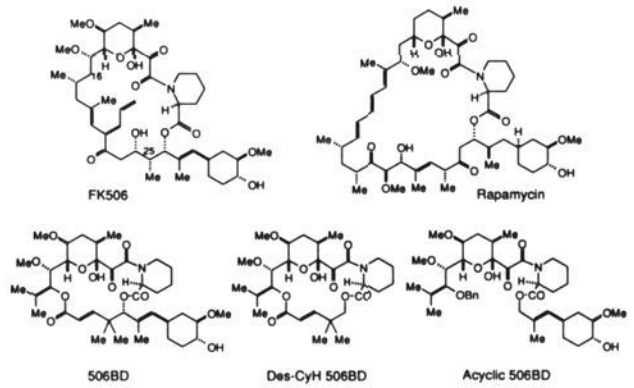
Abstract: The design, synthesis, and analysis of a high-affinity ligand, 506BD, for the human immunophilin FKBP is described. The synthesis illustrates a novel hydroboration reaction that proceeds with unusual regio- and stereochemical control. In accord with expectations regarding the structural requirements for FKBP binding, 506BD potently inhibits the rotamase activity of FKBP (inhibitory constant K_i = 5 nM). The significance of these and other findings with regard to a biological model for immunophilin-ligand actions is described.

Investigations of FK506, rapamycin, and cyclosporin A (CsA) are providing insights into the mechanisms of signal transduction in the cytoplasm of cells, the "black box" of the signal transduction pathway (Figure 1).[1] FK506 and CsA inhibit the exocytosis of secretory vesicles in mast cells following stimulation of the IgE receptor[2] as well as the transcription of the interleukin-2 (IL-2) gene in T cells following stimulation of the T-cell receptor.[3,4] The ability of rapamycin to inhibit the actions of FK506 but not CsA in both cell lines implies that these signaling pathways share common features.[2] We have suggested that complexes of these three molecules with cytosolic receptors termed immunophilins (immunosuppressant binding proteins) function as the inhibitory agents.[1,4,5] Whereas complexes of CsA with a cyclophilin (cyclosporin A binding protein) and FK506 with an FKBP (FK506 and rapamycin binding protein) inhibit the aforementioned signaling pathways in mast cells and T cells, a complex of rapamycin with an FKBP (possibly the same FKBP associated with FK506) inhibits lymphokine receptor mediated pathway in the T cell that results in cell proliferation.[4,6] Recently, the "immunophilin complex" hypothesis received strong support from studies on *Saccharomyces cerevisiae*, an organism that is highly sensitive to the antiproliferative actions of rapamycin. Deletion of the yeast FKBP gene resulted in a mutant strain that is resistant to rapamycin; transfection of either yeast or human FKBP into the mutant strain returned rapamycin sensitivity.[7] Related findings on *S. cerevisiae* have been reported with FK506[8] (see Note Added in Proof).

Following the discovery of cyclophilin[9] and FKBP,[10,11] the use of the expression cassette polymerase chain reaction (ECPCR) method[12,13] led to the overexpression of the human forms of both proteins in *Escherichia coli*.[14,15] The availability of these recombinant proteins has facilitated investigations of binding interactions with ligands,[16] enzyme properties,[17,18] and structure determinations.[19-22] Cyclophilin and FKBP are rotamase enzymes: they catalyze the interconversion of the cis and trans rotamers of peptidyl-prolyl amide bonds of peptide and protein substrates in vitro.[10,11,23,24] This functional property encouraged an alternative biological proposal. It had been suggested that immunophilins constitute a component of signaling pathways and that the T-cell-inhibitory properties of immunophilin ligands may be associated with their ability to inhibit the actions of immunophilins.[25,26] The studies reported in this article resulted in the

(1) Schreiber, S. L. *Science* **1991**, *251*, 283-287.
(2) Hultsch, T.; Albers, M. W.; Schreiber, S. L.; Hohman, R. J. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 6229-6233.
(3) Dumont, F. J.; Staruch, M. J.; Koprak, S. L.; Melino, M. R.; Sigal, N. H. *J. Immunol.* **1990**, *144*, 251-258.
(4) Bierer, B. E.; Mattila, P. S.; Standaert, R. F.; Herzenberg, L. A.; Burakoff, S. J.; Crabtree, G.; Schreiber, S. L. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 9231-9235.
(5) Bierer, B. E.; Somers, P. K.; Wandless, T. J.; Burakoff, S. J.; Schreiber, S. L. *Science* **1990**, *250*, 556-559.
(6) Dumont, F. J.; Melino, M. R.; Staruch, M. J.; Koprak, S. L.; Fischer, P. A.; Sigal, N. H. *J. Immunol.* **1990**, *144*, 1418-1424.
(7) Koltin, Y.; Faucette, L.; Bergsma, D. J.; Levy, M. A.; Cafferkey, R.; Koser, P. L.; Johnson, R. K.; Livi, G. P. *Mol. Cell. Biol.* **1991**, *11*, 1718-1723.
(8) Heitman, J.; Movva, N. R.; Hiestand, P. C.; Hall, M. N. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 1948-1952.
(9) Handschumacher, R. E ; Harding, M. W.; Rice, J.; Drugge, R. J.; Speicher, D. W. *Science* **1984**, *226*, 544-547.
(10) Harding, M. W.; Galat, A.; Uehling, D. E.; Schreiber, S. L. *Nature* **1989**, *341*, 758-760.
(11) Siekierka, J. J.; Hung, S. H. Y.; Poe, M.; Lin, C. S.; Sigal, N. H. *Nature* **1989**, *341*, 755-758.
(12) MacFerrin, K. D.; Terranova, M. P.; Schreiber, S. L.; Verdine, G. L. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 1937-1941.
(13) Schreiber, S. L.; Verdine, G. L. *Tetrahedron* **1991**, *47*, 2543-2562.
(14) Human FKBP: Standaert, R. F.; Galat, A.; Verdine, G. L.; Schreiber, S. L. *Nature* **1990**, *346*, 671-674.
(15) Human cyclophilin: Liu, J.; Albers, M. W.; Chen, C.-m.; Schreiber, S. L.; Walsh, C. T. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 2304-2308.
(16) Rosen, M. K.; Standaert, R. F.; Galat, A.; Nakatsuka, M.; Schreiber, S. L. *Science* **1990**, *248*, 863-866.
(17) Albers, M. W.; Walsh, C. T.; Schreiber, S. L. *J. Org. Chem.* **1990**, *55*, 4984-4986.
(18) Harrison, R. K.; Stein, R. L. *Biochemistry* **1990**, *29*, 1684-1689.
(19) Rosen, M. K.; Michnick, S. W.; Karplus, M.; Schreiber, S. L. *Biochemistry* **1991**, *30*, 4774-4789.
(20) Wandless, T. J.; Michnick, S. W.; Rosen, M. K.; Karplus, M.; Schreiber, S. L. *J. Am. Chem. Soc.* **1991**, *113*, 2339-2341.
(21) Michnick, S. W.; Rosen, M. K.; Wandless, T. J.; Karplus, M.; Schreiber, S. L. *Science* **1991**, *251*, 836-839. See also: Moore, J. A.; Peattie, D. A.; Fitzgibbon, M. J.; Thomson, J. A. *Nature* **1991**, *351*, 248-250.
(22) Van Duyne, G. D.; Standaert, R. F.; Karplus, P. A.; Schreiber, S. L.; Clardy, J. *Science* **1991**, *251*, 839-842.
(23) Fischer, G.; Wittmann-Liebold, B.; Lang, K.; Kiefhaber, T.; Schmid, F. X. *Nature* **1989**, *337*, 476-478.
(24) Takahashi, N.; Hayano, T.; Suzuki, M. *Nature* **1989**, *337*, 473-476.
(25) Wicker, L. S.; Boltz, R. C.; Matt, V.; Nichols, E. A.; Peterson, L. B.; Sigal, N. H. *Eur. J. Immunol.* **1990**, *20*, 2277-2283.

0002-7863/91/1513-8045$02.50/0



Structures of FKBP Ligands.

Figure 1. Structures of FKBP ligands.

preparation of agents that distinguished these biological models.

Important problems in this field include the determination of the structural basis for high-affinity interactions of immunophilin ligands with immunophilins and the biological consequences of complexation. We have attempted to design and synthesize novel cyclophilin ligands on the basis of the structure of CsA;[27,28] a preliminary account of our early efforts has been reported.[29] Described herein are the results of a related approach to the study of the interactions of FKBP and two natural product ligands, FK506 and rapamycin.

When structural information concerning a receptor or its bound ligands is lacking, the solid-state and solution conformations of unbound ligands are often utilized as models for the design of nonnatural ligands. A potential problem arises from the possibility that the bound (biologically relevant) conformation of the ligand is not the same as that observed in the unbound ligand. In fact, recent evidence from NMR studies indicates a markedly different conformation of CsA bound to cyclophilin versus the uncomplexed ligand, vividly demonstrating the limitations of this approach.[30] Yet, at the outset of these studies, the X-ray structures of FK506[31] and rapamycin[32] represented the only structural data available. This information was used to facilitate the design of a new FKBP ligand, termed 506BD (FK506 Binding Domain). In this article, we present the results of these initial investigations. With the three-dimensional solution structure of FKBP[21] and the X-ray structure of the FKBP–FK506 complex now determined[22] and insights into the geometry of the FKBP–rapamycin complex now available,[20] a second series of investigations has recently been initiated that take into account this structural information. Thus, a comparison of the receptor-structure-based approach to the generally more available ligand-structure-based approach utilized in this study should soon be possible.

Design Considerations

The ligand-structure-based approach requires the identification of residues necessary for binding and the design of elements that will ensure that the conformation necessary for binding is heavily populated. Regarding the first point, little data on structure–binding relationships existed to guide the choice of a binding domains, but considerations of possible mechanisms of rotamase

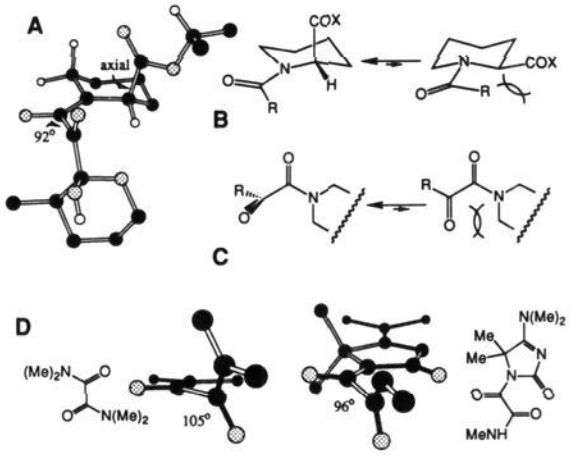


Figure 2. A: FK506 substructure from X-ray. B: $A^{1,3}$ strain favors axial acyl group. C: $A^{1,3}$ strain favors perpendicular dicarbonyl. D: Two illustrations from CSD of twisted dicarbonyl functionality.

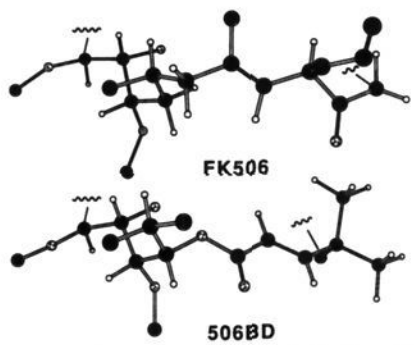


Figure 3. Schematic illustrating structural similarities of the effector domain of FK506 and the scaffolding domain of 506BD.

inhibition led us to focus on the pipecolinyl and tricarbonyl moieties. It was reasoned that the pipecolinyl ring of FK506 and rapamycin mimics a proline and the tricarbonyl acts as an electrophile or twisted amide surrogate. Comparison of the FK506 and rapamycin structures suggested that the pyranose and cyclohexyl rings might play a role in recognition as well. During the course of these investigations, we gathered indirect evidence for the importance of the pyranose moiety of these molecules in binding to FKBP by examining peptide substrate specificity in the FKBP rotamase assay.[17,18] In addition, we reasoned that the connecting chains of the macrocyclic loops of FK506 and rapamycin function as the biological effector elements that determine the distinct biological properties of these molecules. To ascertain the extent to which the cyclohexyl ring contributes to binding, a descyclohexyl variant of 506BD (des-CyH 506BD) was prepared; an acyclic variant of 506BD was also designed to determine the importance of the macrocyclic ring (Figure 1).

Local conformational preferences within FK506 were identified that account for the observed conformation of the macrolactam in the crystal structure. Within the putative binding domain, $A^{1,3}$ strain introduces conformational biases at the pipecolinyl ring and adjacent α-keto amide. To avoid $A^{1,3}$ repulsions, the keto carbonyl must be approximately perpendicular to the adjacent amide carbonyl and the acyl substituent must be in an axial orientation on the pipecolinyl ring (Figure 2). Although most unconstrained 1,2-dicarbonyl systems are found in the anti conformation (180° torsion angle), a search of the Cambridge Structural Database for N,N-disubstituted amides with an adjacent carbonyl function revealed that the torsion angle between the carbonyl substituents ranges from 92 to 105° in the 14 examples of this substructure. Two of these are shown in Figure 2.[33]

(26) Siekierka, J. J.; Wiederrecht, G. W.; Greulich, H.; Boulton, D.; Hung, S. H. Y.; Cryan, J.; Hodges, P. J.; Sigal, N. H. *J. Biol. Chem.* **1990**, *265*, 21011–21015.
(27) Dorsey, B. D.; Anthony, N. J.; Schreiber, S. L. Unpublished results.
(28) Hawley, R. C. Ph.D. Thesis, Yale University, New Haven, CT, 1989.
(29) Schreiber, S. L.; Anthony, N. J.; Borsey, B. D.; Hawley, R. C. *Tetrahedron Lett.* **1988**, *29*, 6577–6580.
(30) Fesik, S. W.; Gampe, R. T.; Holzman, T. F.; Egan, D. A.; Edalji, R.; Luly, J. R.; Simmer, R.; Helfrich, R.; Kishore, V.; Rich, D. H. *Science* **1990**, *250*, 1406–1409.
(31) Tanaka, H.; Kuroda, A.; Marusawa, H.; Hatanaka, H.; Kino, T.; Goto, T.; Hashimoto, M.; Taga, T. *J. Am. Chem. Soc.* **1987**, *109*, 5031–5033.
(32) Neil Swindells, D. C.; White, P. S.; Findlay, J. A. *Can. J. Chem.* **1978**, *56*, 2491–2492.
(33) Cambridge Structural Database Refcodes: TMOXAM and BUSSAJ.

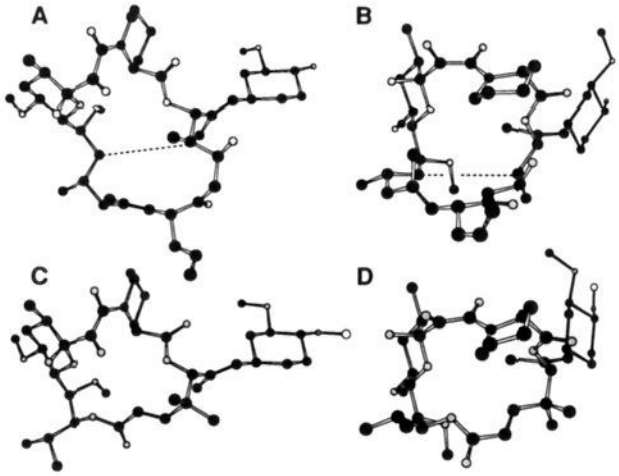


Figure 4. Three-dimensional representations of A, FK506 crystal structure (cis rotamer, dashed line illustrates attachment sites for enoate spacer); B, FK506 (possible trans rotamer, dashed line illustrates attachment sites for enoate spacer); C, 506BD (cis rotamer); and D, 506BD (trans rotamer).

The conformation of a flexible molecule is largely governed by forces that minimize the torsional strain of σ bonds and the steric strain of nonbonded atoms. Staggering sp^3–sp^3 bonds while eclipsing sp^2–sp^3 bonds and minimizing gauche butane, –gauche/+gauche (*syn*-pentane), and allylic $A^{1,3}$ strain along the C14–C26 chain (FK506 numbering) results in the local conformation of FK506 observed in the solid state (Figure 3). The trans enoate scaffolding element of 506BD was chosen to constrain the distance between C16 and C25 in order to accommodate the conformational preferences of the binding domain. From the perspective of the excised C17–C25 element, the enoate linker is positioned so as to satisfy the aforementioned conformational biases of this fragment as well. Thus, the bridging of these two atoms, as represented by the dashed line in Figure 4A, was expected to allow either domain to maintain its preferred conformation.

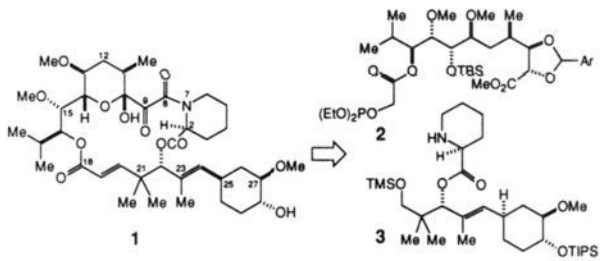
At the intersection of the scaffolding and binding domains of 506BD, an isopropyl substituent was introduced to restrict rotation about the C15–C16 bond by presenting a *syn*-pentate interaction in the undesired conformer; this constraint is present in FK506. Although the conformation of FK506 bound to FKBP was unknown at the time of design, we thought it prudent for 506BD to be capable of sampling both the cis and trans amide rotamer conformations of FK506. Consequently, the trans enoate scaffolding element was designed to bridge FK506 from the *pro-S* hydrogen of C16 to the methyl substituent of C25. This scaffolding plan is accommodated by both the observed cis and a possible trans conformation (Figure 4).

Results and Discussion

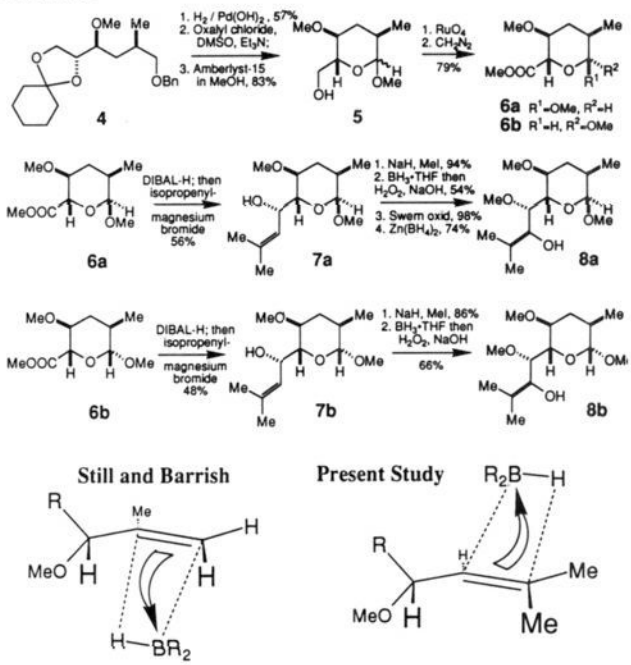
The synthetic design of 506BD was guided to some extent by the experience gained in this laboratory from the total synthesis of FK506,[34] yet the syntheses diverge where the greater simplicity of the 506BD structure could be used to an advantage. Accordingly, retrosynthetic disconnections at the carbon–nitrogen bond of the amide and at the double bond of the enoate provide two fragments, the C8–C19 fragment **2** and the pipecolinyl ester of the cyclohexyl fragment **3** (Scheme I). Analogous to the total synthesis of FK506, the C9 and C10 carbonyls were masked as a protected diol. The greater simplicity within the scaffolding domain of 506BD provided flexibility in the coupling and cyclization sequence.

The synthesis of the C10–C16 fragment of 506BD is outlined in Scheme II. The oxygenated six-carbon fragment **4** was prepared by the method of Williams and Benbow; a cyclohexylideneglyceraldehyde was coupled with a Grignard reagent

Scheme I



Scheme II



derived from (*S*)-(+)-methyl 3-hydroxy-2-methylpropionate.[35] Debenzylation and oxidation provided an aldehyde that was internally protected as a mixture of O-methyl acetals **5** with concomitant deprotection of the cyclohexylidene acetal by exposure to acidic resin in refluxing methanol. The initial synthetic plan called for the oxidation of alcohol **5** to an aldehyde and subsequent addition of a Grignard reagent, which was expected to provide the correct stereochemistry of the methoxy substituent adjacent to the pyranose ring at C15 via a metal chelate with the ring and carbonyl oxygens. As the aldehyde proved to be unstable, isolation was obviated by a one-pot reduction–Grignard addition sequence[36] with the coresponding methyl esters **6**. Oxidation of alcohols **5** to the corresponding acids[37] followed by esterification with diazomethane gave the methyl esters **6**. At this point, the two anomers were separated (**6a**/**6b** = 1.7:1) and carried through the next sequence of reactions separately. One-pot DIBAL-H reduction of the ester **6a** followed by the addition of isopropenylmagnesium bromide gave alcohol **7a** as a 6:1 mixture of diastereomers. The same reaction sequence with **6b** provided **7b** as a 4:1 mixture of diastereomers. In each instance, the major isomer (depicted) results from an apparent chelation-controlled addition. After methylation of **7a** and **7b**, the major isomers were separated and subjected to a hydroboration–oxidation sequence. A curious disparity exists in the stereoselectivity of the hydroboration reaction between the methyl ethers of **7a** and **7b**, which differ only in the stereochemistry at the anomeric center. The methyl ether of **7b**, with the equatorial OMe substituent, provided only the desired

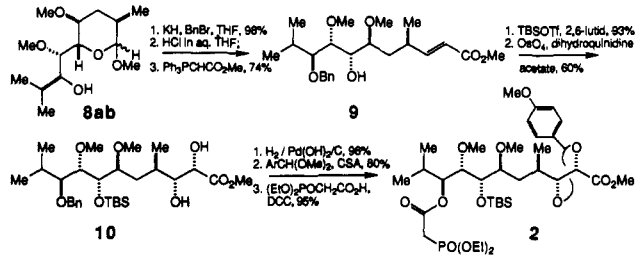
(34) Nakatsuka, M.; Ragan, J. A.; Sammakia, T.; Smith, D. B.; Uehling, D. E.; Schreiber, S. L. *J. Am. Chem. Soc.* **1990**, *112*, 5583–5601.

(35) Williams, D. R.; Benbow, J. W. *J. Org. Chem.* **1988**, *53*, 4643–4644.

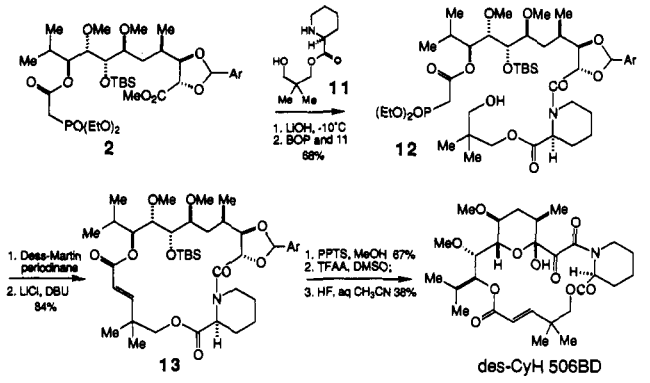
(36) Burke, S. D.; Deaton, D. N.; Olsen, R. J.; Armistead, D. M.; Blough, B. E. *Tetrahedron Lett.* **1987**, *28*, 3905–3906.

(37) Carlsen, H. J.; Katsuki, T.; Martin, V. S.; Sharpless, K. B. *J. Org. Chem.* **1981**, *46*, 3936–3938.

Scheme III



Scheme IV

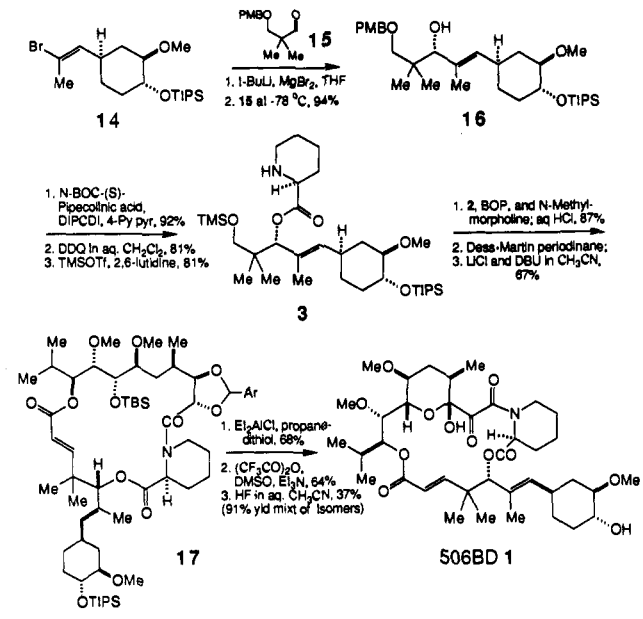


alcohol **8b**, whereas the methyl ether of **7a**, with the axial OMe substituent, produced a 2:1 mixture of alcohols, with the desired isomer **8a** predominating. We note that both the regio- and diastereofacial selectivity in these reactions are opposite to that normally observed in hydroboration reactions of allylic ethers that are disubstituted at the olefinic carbon adjacent to the carbon bearing the allylic ether (Scheme II).[38] The change in regiochemistry was anticipated on the basis of the olefin substitution pattern. The facial selectivity is consistent with the addition of the boron atom anti to the neighboring methoxyl in a transition-state geometry that minimizes $A^{1,3}$ strain about the trisubstituted olefin (Scheme II). Thus, the outcome of this reaction is analogous to hydroborations of cyclic allylic ethers, such as a 3-methoxycyclohexene derivative.[39] However, the significant difference in selectivity seen with the methyl ethers of **7a** and **7b** was not anticipated and is not easily understood. This mixture of alcohols **8a** was oxidized to the ketone and reduced with $Zn(BH_4)_2$.[40] Reduction with chelation control at the α-alkoxy group provided only the desired alcohol (depicted). X-ray diffraction analysis of the crystalline alcohol **8a** allowed an unequivocal assignment of stereochemistry at all centers in question (see supplementary material).

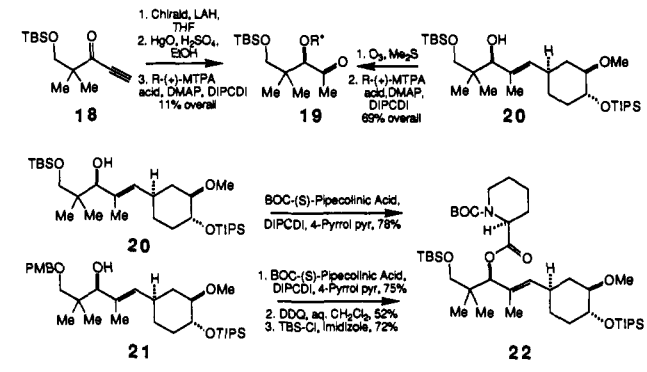
To ensure that the series of reactions on the minor anomer resulted in the same stereochemical relationship of alkoxy groups, both compounds, **8a** and **8b**, were benzylated, hydrolyzed to the lactol, and treated with methyl phosphoranylideneacetate to yield the α,β-unsaturated ester **9**, at which point a comparison by ^1H NMR was possible (Scheme III). As these products were identical, they were then combined for further processing. The unsaturated carbons were dihydroxylated with osmium tetraoxide and dihydroquinidine acetate,[41] which was used to avoid the formation of a mixture of diastereomers. The stereochemistry shown (**10**) is that predicted by the empirical rule suggested by Sharpless. In preparation for coupling, the benzyl ether was removed by hydrogenolysis, the diol was protected as the *p*-methoxybenzylidene acetal, and the remaining free hydroxyl was acylated to provide the ester phosphonate **2**.

A variant of 506BD that lacked both the extraannular trisubstituted olefin and cyclohexyl moiety was formulated in order

Scheme V



Scheme VI



to determine the importance of this substituent for binding to FKBP. The synthesis also served as a model system to explore the best coupling–cyclization sequence and to aid in the optimization of the vicinal diol oxidation (Scheme IV). Initially, formation of the enoate by a Horner–Emmons coupling preceded closure of the macrocycle through macrolactamization. Subsequently, it was found that a modified sequence of reactions that reversed the order of coupling and cyclization improved efficiency. Thus, selective hydrolysis of the methyl ester within **2** was accomplished by exposure to LiOH at −10 °C. The resulting acid was reacted with BOP (benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate) to form the activated ester and then coupled to the amine **11**, which resulted in the formation of amide **12**. Oxidation of **12** with the Dess–Martin periodinane and cyclization with DBU and dry LiCl[42] gave the macrocycle **13** in 60% overall yield from **2**. Deprotection of the PMB acetal was accomplished with pyridinium *p*-toluenesulfonate (PPTS) in methanol. The vicinal diol was then oxidized under modified Swern conditions,[43] and the resultant diketone was subjected to aqueous hydrogen fluoride in acetonitrile to yield des-CyH 506BD.

The synthesis of the cyclohexyl-equipped pipecolinyl ester fragment **3** is outlined in Scheme V. Metalation and hydroxyalkylation of vinyl bromide **14**, which was prepared in the course of our synthesis of FK506, led to a 1:1 mixture of alcohol **16** and its carbinol epimer. The diastereomers were separated by flash chromatography, and the undesired diastereomer was recycled

(38) Still, W. C.; Barrish, J. *J. Am. Chem. Soc.* **1983**, *105*, 2487–2489.
(39) Schreiber, S. L.; Smith, D. B. *J. Org. Chem.* **1989**, *54*, 9–10.
(40) Nakata, T.; Tanaka, T.; Oishi, T. *Tetrahedron Lett.* **1983**, *24*, 2653–2656.
(41) Hentges, S. G.; Sharpless, K. B. *J. Am. Chem. Soc.* **1980**, *102*, 4263–4265.
(42) Blanchette, M. A.; Choy, W.; Davis, J. T.; Essenfeld, A. P.; Masamune, S.; Roush, W. R.; Sakai, T. *Tetrahedron Lett.* **1984**, *25*, 2183–2186.
(43) Amon, C. M.; Banwell, M. G.; Gravatt, G. L. *J. Org. Chem.* **1987**, *52*, 4851–4855.

by an oxidation–reduction sequence. The stereochemistry of **16** and its alcohol epimer was assigned following the correlative studies shown in Scheme VI. Acetylide addition to the *tert*-butyldimethylsilyl (TBS) ether of 2,2-dimethyl-3-hydroxypropionaldehyde and subsequent oxidation gave ketone **18**. Reduction with LAH in the presence of Darvon alcohol[44] followed by conversion of the acetylene to the methyl ketone and esterification with (*R*)-(+)-methoxy(trifluoromethyl)phenylacetic acid provided the methyl ketone **19**. This compound proved to be identical with the product of ozonolysis and Mosher esterification of **20**, which was prepared in analogy to **16**. Finally, a stereochemical correlation of **20** and **21** followed the preparation of **22**. On the basis of the literature precedent for the facial selectivity in the ketone reduction,[44] the stereochemistry of **21** is as shown in Scheme VI. Accordingly, the diastereomeric alcohol **16**, which contains the proper stereochemistry for 506BD synthesis, was used in subsequent synthetic transformations.

As in our FK506 synthesis, alcohol **16** was esterified with *N*-BOC-(*S*)-(−)-pipecolinic acid without complications due to epimerization. In final preparation for fragment coupling, the PMB group was removed with DDQ and the urethane was deprotected with trimethylsilyl triflate to yield **3**. The coupling of fragments **2** and **3** gave macrocycle **17**. With the exception of a mild acid treatment subsequent to coupling and the deprotection of the *p*-methoxybenzylidene acetal with diethylaluminum chloride and propanedithiol, the sequence of reactions that produced 506BD paralleled the sequence described for des-CyH 506BD (Scheme V). The final deprotection step gives a 2:1 mixture of 506BD and its seven-membered hemiacetal isomer (assigned by ^{13}C NMR analysis in analogy to the work of T. K. Jones et al.).[45] As the two isomers were slow to interconvert in nonpolar solvent systems, they were separated by high-pressure liquid chromatography (HPLC). The individual isomers were found to undergo negligible interconversion in chloroform solution, but in more polar solvents, such as DMSO, interconversion was significantly accelerated. After 36 h, an apparent equilibrium ratio of 1:2, favoring the seven-membered hemiacetal, was reached.

The analysis of the solution conformation of 506BD (**1**) was undertaken by ^1H NMR methods. Due to carbonyl deshielding, there is a pronounced difference in chemical shift between the equatorial (*pro-R*) and axial (*pro-S*) protons on the carbon adjacent to the nitrogen for the cis amide rotamer of FK506. In the trans amide rotamer, the carbonyl has rotated away from these protons and the difference in chemical shift decreases (FK506 cis *pro-R* H = δ 4.43, cis *pro-S* H = δ 3.01; FK506 trans *pro-R* H = δ 3.74, trans *pro-S* H = δ 3.40; 506BD cis *pro-R* H = δ 4.41, cis *pro-S* H = δ 2.45). Thus, it is clear from a comparison of the chemical shifts of these protons in 506BD with the corresponding signals for the cis and trans rotamers of FK506 that the cis amide rotamer of 506BD predominates (8:1). The similarity in conformation of the pyranose rings and neighboring appendages in FK506 and 506BD was evident upon comparison of the vicinal coupling constants.[5] These data suggest that the solution conformation of the major cis rotamer of 506BD is indeed similar to that anticipated by application of the previously described design principles.

To test the affinity of 506BD for FKBP, two assays were performed: one measured rotamase inhibition and the other measured binding to the protein. Equilibrium-binding and rotamase-inhibition studies with FKBP, which were carried out as previously described,[4] were complicated by the equilibrium between 506BD and its seven-membered hemiacetal isomer that occurs in the aqueous solutions necessary for these assays. The results of experiments that differed in the ratios of the two isomers indicate that the seven-membered hemiacetal has a lower affinity toward FKBP. Although we were unable to suppress the isomerization completely, when minimized, a K_d of 20 nM was determined for 506BD. Thus, 506BD is only 20-fold less efficient in displacing [^3H]dihydro FK506 from FKBP than is FK506 itself. Furthermore, 506BD was found to inhibit the rotamase activity of FKBP, with K_i = 5 nM. For comparison, the binding and inhibition data for both des-CyH 506BD (K_d = 1.8 μM, K_i = 0.3 μM) and the acyclic analogue of 506BD (K_d = 300 nM, K_i = 20 nM) were determined. It can be seen from these data that both substrates have weaker affinities for FKBP, with the cyclohexyl appendage of 506BD and thus presumably of FK506, contributing a factor of approximately 60 toward binding and the macrocyclic constraint a factor of 2–4. We assume the difference between the measured K_i and K_d values reflects limitations in these measurements.

During the course of the synthesis of 506BD, it came to light that FK506 and rapamycin interrupt distinct T-cell and mast-cell signaling pathways and that they inhibit each others actions in a variety of functional assays.[3,4] Our interpretation of these results is to view FK506 and rapamycin as dual-domain agents, consisting of a binding domain responsible for binding to immunophilin and an effector domain that determines the signaling pathway with which the drug will interfere. According to this view, the immunophilin serves to present the differing effector elements of the two signaling inhibitors by forming receptor–ligand complexes with the common binding domains. The specificity for the different signaling pathways is then determined by the geometry of the immunophilin–ligand complex, which determines the specificity of further protein interactions. The mutual inhibition of the two drugs results from their competitive binding to the common receptor site.

The results of biological assays with 506BD support this hypothesis. Equipped with the common binding domains of FK506 and rapamycin, 506BD blocks the specific actions of both of these drugs at concentrations consistent with their relative affinities to FKBP.[5] The "immunophilin-complex" hypothesis imparts a specific role for the effector elements of FK506 and rapamycin. As 506BD lacks an effector element, it was not anticipated to inhibit either the T-cell-receptor or lymphokine-receptor signaling pathways. These expectations were confirmed experimentally in studies that have been detailed elsewhere.[5] 506BD has also been found to distinguish the actions of FK506 and CsA on mast-cell exocytosis.[2] The movement of secretory vesicles to the cell membrane and subsequent release of inflammatory substances (histamine, serotonin) follows the activation of a signal transduction pathway that originates at the IgE receptor. This IgE receptor-mediated exocytosis, or degranulation, is inhibited by concentrations of CsA and FK506 that are comparable to those required to inhibit the T cell receptor mediated transcription of the IL-2 gene. 506BD potently inhibits the action of FK506 but not CsA in this system. These results illustrate that the inhibition of FKBP rotamase activity is not a sufficient requirement for inhibiting exocytosis (506BD does not inhibit exocytosis) and that the actions of CsA and FK506 are mediated by distinct receptors. These studies also illustrate that common mechanisms for signal transmission through the cytoplasm are operative in pathways leading to exocytosis and transcription. As mentioned in the introduction, the methods of yeast genetics have recently provided a confirmation of the "immunophilin-complex" hypothesis.[7,8]

Retrospective Analysis

Recently, the solution structure of FKBP has been determined by NOE-restrained molecular dynamics,[21] and the crystal structure of the FKBP–FK506 complex has been solved to 1.7 Å resolution by X-ray diffractometry.[22] The conformation of bound FK506 is depicted in Figure 5 (A). The solid-state bound conformation of FK506 differs from the solid-state unbound conformer at several key torsion angles that include C14–C15, C15–C16, C16–C17, and N7–C8. The difference of the amide bond is most obvious: the trans rotamer binds the protein, whereas the cis rotamer crystallizes in the unbound state. This 180° rotation about N7–C8 is accommodated by the effector domain with little change in local conformation other than −120°/+120°/−120° rotations in the three torsion angles about the carbon chain between C14 and C17.

(44) Brinkmeyer, R. S.; Kapoor, V. M. *J. Am. Chem. Soc.* **1977**, *99*, 8339–8341.

(45) Jones, T. K.; Reamer, R. A.; Desmond, R.; Mills, S. G. *J. Am. Chem. Soc.* **1990**, *112*, 2998–3017.

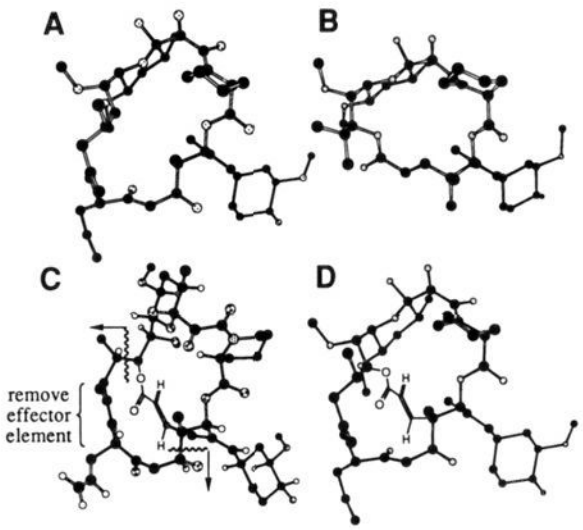


Figure 5. A: Conformation of FK506 bound to FKBP (X-ray). B: Predicted conformation of 506BD bound to FKBP. C: Conformation of FK506 in the solid state (X-ray). The line drawing of an enoate spacer illustrates the sites of bridging in the design of 506BD. D: Illustration of A (above), but with the enoate added in order to illustrate the bridging sites in 506BD and to facilitate a model of "bound 506BD" (see B above).

The conformation of the effector loop (C18–C26) in the unbound conformation of FK506 is similar to that observed in the bound state (Figure 5, compare C vs D). The scaffolding domain for FK506 was devised to bridge the binding and effector domains (by building a connector between C16 and C25 of FK506), and the principles of conformational analysis, especially as applied to the effector element, suggested the *pro-S* C16 hydrogen and the C25 methine were suitable candidates for attachment sites. Indeed, the distance between C16 and C25 in the unbound (C) and bound (D) states of FK506 is essentially unchanged. Thus, the enoate scaffolding element facilitates access to the bound conformation of 506BD by fixing the distance between C16 and C25 to that observed in the bound conformation of FK506. However, many other conformational features of FK506, particularly in the regions that make contact with the protein, were not anticipated. On the basis of the conformation of FK506 bound to FKBP (see Figure 5, A and D), a likely conformation of 506BD bound to FKBP is shown in Figure 5 (B). This hypothetical conformation is quite different from those that guided our thoughts as we formulated the 506BD structure (compare to C and D, Figure 4). Indeed, ongoing efforts to experimentally determine the structure of 506BD bound to FKBP may provide even more surprises.

Conclusion

A nonnatural product, 506BD, was designed on the basis of structural information available for FK506, synthesized, and found to be a high-affinity ligand for the immunophilin FKBP. Conformational analysis demonstrated that the solution conformations of the common structural elements of FK506 and 506BD are similar, in accord with expectations based on conformational analysis. As anticipated from our biological model, 506BD does not inhibit TCR-mediated events, such as IL-2 transcription in analogy to FK506, or lymphokine-receptor-associated events, such as T-cell proliferation in analogy to rapamycin. In accord with its high affinity toward FKBP, 506BD is observed to inhibit the specific actions of both immunosuppressants. FK506 and rapamycin appear to be comprised of a common FKBP-binding element (found in 506BD) and distinct effector elements. It would appear that complexes of these drugs with either the same FKBP or FKBPs with similar drug binding sites are able to interfere with distinct signal transmission processes, which have been localized to the cytoplasm of the cell. Future efforts will focus on the use of structural information for FKBP–ligand complexes in order to prepare higher affinity ligands for FKBP receptors. The ability to equip ultra-high-affinity ligands with a family of "effector elements", such as those found on FK506 and rapamycin, may yield powerful new probe reagents for further investigations of the black box of cellular signal transduction.

Experimental Procedures

A. General Procedures. All reactions were performed in oven-dried glassware under a positive pressure of nitrogen or argon. Air- and moisture-sensitive compounds were introduced via syringe or cannula through a rubber septum.

B. Physical Data. Melting points are uncorrected. Optical rotations were recorded on a JASCO DIP-0181 digital polarimeter with a sodium lamp (589 nm, D line) at the temperature indicated. Infrared spectra were recorded on a Nicolet 5PC FT-IR spectrometer. Proton magnetic resonance spectra (^{1}H NMR) were recorded on Bruker AM-500 (500 MHz), AM-400 (400 MHz), and AM-300 (300 MHz) spectrometers. Chemical shifts are reported in ppm from tetramethylsilane with the solvent resonance as an internal standard (chloroform, 7.27 ppm). For compounds that have more than one rotamer, the chemical shift of the corresponding signal for the minor rotamer is given in brackets. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broadened, m = multiplet), coupling constants (hertz), integration. ^{13}C NMR spectra were recorded with complete proton decoupling on a Bruker AM-500 (125.5 MHz) or AM-400 (100 MHz) spectrometer. Low- and high-resolution mass spectra were obtained by Dr. Andrew Tyler of the Harvard Chemistry Department Mass Spectrometry Facility. Combustion analyses were performed by Spang Microanalytical (Eagle Harbor, MI) or Atlantic Microlabs, Inc. (Norcross, GA).

C. Chromatography. Reactions were monitored by thin-layer chromatography (TLC) using E. Merck silica gel 60F glass plates (0.25 mm). Components were visualized by illumination with long-wave ultraviolet light, exposure to iodine vapor, and/or by dipping in a aqueous ceric ammonium molybdate or ethanolic *p*-anisaldehyde solution followed by heating. Solvents for chromatography were HPLC grade. Liquid chromatography was performed by forced flow (flash chromatography) of the indicated solvent system on E. Merck silica gel 60 (230–400 mesh).

D. Solvents and Reagents. All reagents and solvents were analytical grade and were used as received with the following exceptions. Tetrahydrofuran (THF), benzene, toluene, and diethyl ether were distilled from sodium metal benzophenone ketyl. Dichloromethane, triethylamine, acetonitrile, pyridine, and diisopropylamine were distilled from calcium hydride. Dimethyl sulfoxide (DMSO), and dimethylformamide (DMF) were distilled from calcium hydride at reduced pressure and stored over 4-Å molecular sieves.

(2*S*)-Methyl 3-(Benzyloxy)-2-methylpropionate. (*S*)-(+)-Methyl 3-hydroxy-2-methylpropionate (24.43 g, 207 mmol) was dissolved in 500 mL of dichloromethane and 1000 mL of cyclohexane. Benzyl 2,2,2-trichloroacetimidate (42.3 mL, 228 mmol) was added via syringe, and the solution was allowed to cool to 0 °C over a period of 60 min. Dropwise addition of triflic acid (1.25 mL, 14 mmol) initiated the reaction and caused the precipitation of a fine solid. The mixture was allowed to warm to ambient temperature after 3 h, and stirring was continued for 2 h at room temperature. The mixture was filtered through a short pad of Celite, and pyridine (5 mL) was added to the filtrate. Rotary evaporation gave an oil that was purified by silica gel chromatography (20:1 to 10:1 hexane/ethyl acetate), and the protected ester was isolated as a clear oil (33.10 g, 159 mmol, 77%): $[\alpha]^{23}_D$ +10.9° (*c* 0.1.71, $CHCl_3$); IR (film) 3032 m, 2980 m, 2951 m, 2865 m, 1771 sh, 1740 (C=O), 1497 w, 1455 m, 1364 m, 1312 w, 1250 m, 1202 s, 1179 s; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38–7.28 (mult, 5 H), 4.54 (s, 2 H), 3.71 (s, 3 H), 3.67 (dd, J = 9.2, 7.4, 1 H), 3.51 (dd, J = 9.3, 5.9, 1 H), 2.80 (sextet, J = 7.1, 6.0, 1 H), 1.19 (d, J = 7.1, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.2, 138.1, 128.3, 127.5, 127.4, 73.0, 71.9, 51.6, 40.1, 13.9; MS (FAB) *m/e* 209 (M + H).

(2*S*)-3-(Benzyloxy)-2-methyl-1-propanol. The methyl ester prepared above (33.10 g, 159 mmol) was dried by rotary evaporation of 50 mL of benzene and then taken up in 1000 mL of dichloromethane and cooled to −78 °C. A 1.0 M solution of DIBAH (325 mL, 325 mmol) was added carefully to the stirred solution over 20 min, with care taken to prevent vigorous gas evolution. The solution was kept at −78 °C for 30 min and then allowed to warm to ambient temperature. The reaction was quenched carefully with 50 mL of saturated Na^+/K^+ tartrate (Rochelle's salt) and then poured into a solution of 1000 mL of saturated Rochelle's salt and stirred for 2 h. The layers were partitioned, the aqueous layer was extracted with ethyl acetate (4 × 250 mL), and the combined organic extracts were dried over $MgSO_4$. Rotary evaporation of the organic phase provided the alcohol (28.0 g, 155 mmol, 98%): $[\alpha]^{23}_D$ +3.7° (*c* 1.03, $CHCl_3$); IR (film) 3550–3250 m (OH), 2960 m, 2926 m, 2874 s, 1497 w, 1455 m, 1364 m, 1208 w, 1098 s, 1042 m, 990 m, 737 s; ^{1}H

NMR (400 MHz, $CDCl_3$) δ 7.39–7.29 (mult, 5 H), 4.53 (s, 2 H), 3.62–3.59 (mult, 2 H), 3.54 (dd, J = 9.2, 4.8, 1 H), 3.44 (dd, J = 8.9, 7.8, 1 H), 2.84 (s, 1 H), 2.10–2.05 (mult, 1 H), 0.90 (d, J = 6.9, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.0, 128.3, 127.6, 127.5, 75.0, 73.2, 67.3, 35.5, 13.4; MS (FAB) m/e 181 (M + H).

(2*S*)-3-(Benzyloxy)-2-methylpropyl Bromide. The benzyl-protected alcohol prepared above (28.0 g, 155 mmol) was placed in a 1000-mL pear-shaped flask and dried by rotary evaporation of 60 mL of benzene. The alcohol was dissolved in 400 mL of dichloromethane and cooled to 0 °C over 45 min. Triphenylphosphine (52.9 g, 202 mmol) was added in one portion, followed after 5 min by *N*-bromosuccinamide (35.9 g, 202 mmol); the solution was stirred for 6 h at ambient temperature. Concentration of the amber reaction mixture gave a very viscous oil, which after elution with hexanes and filtration (to remove triphenylphosphine oxide) gave a clear amber oil after a second concentration. Purification of the oil by silica gel chromatography (10:1 hexane/ethyl acetate) provided the desired bromide as a clear, colorless oil (31.0 g, 127 mmol, 82%): $[\alpha]^{23}_D$ +11.7° (c 3.68, $CHCl_3$); IR (film) 2965 m, 2932 m, 2859 s, 1653 m, 1497 w, 1474 m, 1362 m, 1233 w, 1100 s, 1028 w, 737 m, 698 m; ^1H NMR (400 MHz, $CDCl_3$) δ 7.40–7.29 (mult, 5 H), 4.54 (s, 2 H), 3.54–3.50 (mult, 2 H), 3.45–3.42 (mult, 2 H), 2.15 (mult, 1 H), 1.05 (d, J = 6.6, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.3, 128.3, 127.6, 127.5, 73.1, 72.7, 38.2, 35.6, 15.8; MS (FAB) m/e 243 (M + H). Anal. Calcd for $C_{11}H_{15}OBr$: C, 54.34; H, 6.22; Br, 32.86. Found: C, 54.47; H, 6.13; Br, 32.90.

(1*R*,2*R*,*S*,4*R*)-5-(Benzyloxy)-1-(2,2-pentamethylene-1,3-dioxolan-4-yl)-4-methylpentan-2-ol. A 500-mL three-neck flask was fitted with a septum, a glass stopper, and a reflux condenser and charged with magnesium (3.00 g, 123 mmol, activated by treatment with 1 N HCl followed by water, ethanol, and acetone) and 20 mL of THF. Four drops of the alkyl bromide from above were added via syringe followed by dibromoethane (0.35 mL, 4.1 mmol). The remainder of the alkyl bromide (10.0 g in 10 mL of THF, 41.1 mmol) was added to the stirred mixture via a syringe pump over 4 h, carefully adjusting the rate of addition so the temperature remained between 30 and 35 °C. After complete addition of the bromide, the mixture was stirred for 4 h at room temperature. Cyclohexylidene ketal protected glyceraldehyde (5.8 g, 34 mmol, freshly prepared) was dissolved in 300 mL of THF and cooled to −78 °C. (Note: Preparation of the glyceraldehyde was according to literature precedent; however, solvent polymerization after addition of the Grignard reagent occurred unless care was taken to wash the aldehyde with aqueous sodium bicarbonate before concentration.) Transfer of the Grignard solution to the aldehyde was performed dropwise via cannula, followed by stirring at −78 °C for 90 min and then warming to room temperature. The reaction was quenched by dilution with ether followed by aqueous NH_4Cl. The organic phase was separated and the aqueous layer was extracted with ether (2 × 100 mL). The combined organic solutions were dried ($MgSO_4$), concentrated, and purified by silica gel chromatography (20:1 to 10:1 to 5:1 to 3:1 to 2:1 hexane/ethyl acetate). The desired alcohol (a 4:1 mixture of diastereomers) was isolated as a clear, colorless oil (7.61 g, 22.8 mmol, 55%): $[\alpha]^{23}_D$ +12.6° (c 0.23, $CHCl_3$); IR (film) 3550–3300 m (OH), 2934 m, 2863 s, 1451 m, 1437 w, 1366 m, 1281 m, 1252 m, 1163 w, 1144 w, 1100 m, 929 m, 847 m, 737 w, 698 w; ^1H NMR (400 MHz, $CDCl_3$) δ 7.38–7.28 (mult, 5 H), 4.52 (s, 2 H), 4.03–3.91 (mult, 3 H), 3.90–3.79 (mult, 1 H), 3.43–3.31 (mult, 2 H), 2.97 (s, 1 H), 2.78 (s, 1 H), 2.12–2.06 (mult, 1 H), 1.65–1.59 (mult, 10 H), 1.48–1.40 (mult, 2 H), 1.01–0.96 (mult, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.0, 128.4, 128.3, 127.7, 109.5, 79.0, 78.3, 76.3, 75.5, 73.3, 73.0, 70.4, 69.3, 65.7, 65.4, 38.1, 38.0, 36.3, 34.9, 34.8, 30.5, 30.2, 25.2, 24.0, 23.8, 18.0, 17.1; MS (FAB) m/e 335 (M + H). Anal. Calcd for $C_{20}H_{30}O_4$: C, 71.82; H, 9.04. Found: C, 71.86; H, 9.01.

(1*R*,2*R*,*S*,4*R*)-5-(Benzyloxy)-1-(2,2-pentamethylene-1,3-dioxolan-4-yl)-2-methoxy-4-methylpentane (4). A 500-mL flask was charged with sodium hydride (7.3 g, 180 mmol, 60% dispersion in mineral oil), dimethyl sulfoxide (200 mL), and THF (50 mL). The gray slurry was cooled to 0 °C in an ice water bath. The carbinol described above (20.15 g, 60 mmol) was added to the slurry via cannula in THF (5 × 10 mL). The mixture was allowed to warm to room temperature and stir for 90 min, at which time iodomethane (18.8 mL, 300 mmol) was added to the mixture via syringe followed by stirring at room temperature overnight. The slurry was diluted with ether and quenched with aqueous NH_4Cl. The organic layer was washed with distilled water and then saturated $NaHCO_3$; the aqueous solutions were combined and extracted with one portion of ether. The organics were combined, dried over $MgSO_4$, concentrated, and purified by silica gel chromatography (10:1 to 5:1 hexane/ether) to provide methyl ether **4** as a clear, colorless oil (19.8 g, 56.8 mmol, 94%): $[\alpha]^{23}_D$ +2.4° (c 3.55, $CHCl_3$); IR (film) 2934 s, 2861 m, 1451 m, 1366 w, 1163 m, 1143 w, 1102 s, 1042 m, 930 m, 698 m; ^1H NMR (400 MHz, $CDCl_3$) δ 7.37 (mult, 5 H), 4.50 (s, 2 H), 4.05–3.95 (mult, 3 H), 3.86–3.83 (mult, 1 H), 3.51–3.28 (mult, 5 H), 2.07–2.00 (mult, 1 H), 1.64–1.56 (mult, 10 H), 1.40–1.29 (mult, 2 H), 1.03–0.98 (mult, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.6, 128.2, 127.5, 127.4, 127.3, 109.8, 109.5, 79.5, 78.3, 77.9, 76.1, 75.4, 72.9, 72.8, 65.6, 65.5, 58.9, 58.7, 36.0, 35.7, 34.9, 34.8, 30.2, 29.8, 25.1, 24.0, 23.8, 18.3, 16.8; MS (FAB) m/e 349 (M + H); HRMS calcd for $C_{21}H_{32}O_4$ (M + H) 335.2216, found (FAB) 335.2213. Anal. Calcd for $C_{21}H_{32}O_4$: C, 72.38; H, 9.26. Found: C, 72.20; H, 9.09.

(1*R*,2*S*,4*R*)-1-(2,2-Pentamethylene-1,3-dioxolan-4-yl)-2-methoxy-4-methylpentan-5-ol. Benzyl ether **4** (33.2 g, 95.5 mmol) was dissolved in 500 mL of ethyl acetate and 250 mL of methanol. The solution was degased by aspirator/argon purge (six cycles). The catalyst, $Pd(OH)_2/C$ (2.0 g, 10% palladium content), was added in 4 equal portions followed by aspirator degassing. The catalyst/solvent mixture was placed under an atmosphere of hydrogen and allowed to stir for 16 h at room temperature. The mixture was thoroughly degassed and then filtered through a short plug of Celite 545; care was taken to flush the pad thoroughly with ethyl acetate, never allowing air to pass freely through the plug. The clear filtrate was concentrated and purified by silica gel chromatography (10:1 to 5:1 to 4:1 to 3:1 to 2:1 hexane/ethyl acetate) giving the desired diastereomer in 44 fractions followed by 23 mixed fractions. Mixed fractions were repurified and combined to give the desired alcohol as a clear, colorless oil (14.11 g, 54.6 mmol, 57%): $[\alpha]^{23}_D$ +1.8° (c 0.72, $CHCl_3$); IR (film) 3500–3250 m (OH), 2936 s, 2865 m, 1462 w, 1449 m, 1366 m, 1333 w, 1281 m, 1070 m, 1042 m, 986 w, 936 m, 909 m, 847 m; ^1H NMR (400 MHz, $CDCl_3$) δ 3.97–3.87 (mult, 3 H), 3.70 (dd, J = 8.0, 6.5, 1 H), 3.35–3.19 (mult, 5 H), 1.82–1.74 (mult, 1 H), 1.52–1.40 (mult, 10 H), 1.35–1.25 (mult, 2 H), 0.82 (d, J = 7.0, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 109.3, 79.5, 77.2, 67.3, 65.5, 58.1, 35.8, 34.8, 34.5, 32.1, 24.9, 23.7, 23.5, 17.5; MS (FAB) m/e 259 (M + H). Anal. Calcd for $C_{14}H_{26}O_4$: C, 65.09; H, 10.14. Found: C, 64.95; H, 10.27.

(2*R*,*S*,3*R*,5*S*,6*R*)-2,5-Dimethoxy-6-(hydroxymethyl)-3-methyltetrahydropyran (5). A 1000-mL flask was charged with oxalyl chloride (5.75 mL, 66 mmol) and 500 mL of dichloromethane and cooled to −78 °C. Dimethyl sulfoxide (9.0 mL, 127 mmol) was added dropwise and the solution was stirred for 25 min. The alcohol described above (13.10 g, 50.7 mmol) was then added dropwise to the cooled solution via cannula as a 2.0 M solution in dichloromethane; the solution stirred for 45 min, at which point triethylamine (35.4 mL, 254 mmol) was added and the solution was allowed to warm to room temperature over 60 min. The clear solution was diluted with ether and washed with water. The layers were separated and the aqueous layer was back-extracted with 1 portion of ether. The organic layers were combined, dried over $MgSO_4$, and concentrated, leaving an oil that was azeotroped with 30 mL of benzene. The oil was then diluted with 500 mL of methanol and allowed to stir at room temperature as 5.5 g of Amberlyst-15 was added. The slurry was stirred for 24 h, and was then filtered through a short bed (approx 1 cm) of Celite 545, concentrated, and purified by silica gel chromatography (2:1 to 1:1 hexane/ether), providing **5** as a colorless oil (8.0 g, 42 mmol, 83% for two steps): $[\alpha]^{23}_D$ +!58° (c 2.52, $CHCl_3$); IR (film) 3575–3250 m (OH), 2934 m, 2897 m, 2832 w, 1464 m, 1456 m, 1383 w, 1361 w, 1188 m, 1167 w, 1134 s, 1062 m, 955 w, 939 m, 929 m; ^1H NMR (400 MHz, $CDCl_3$) δ 4.43 (d, J = 3.2, 1 H), 3.99 (d, J = 8.4, 1 H), 3.87–3.70 (mult), 3.54–3.49 (mult), 3.35–3.34 (mult), 3.24–3.17 (mult), 2.25–2.18 (mult), 1.98–1.93 (mult), 1.82–1.75 (mult), 1.46–1.38 (q, J = 11.9, 1 H), 1.12–1.08 (mult, 1 H), 0.96–0.92 (mult); ^{13}C NMR (100 MHz, $CDCl_3$) δ 107.7, 100.7, 78.2, 75.7, 75.4, 70.7, 63.0, 62.9, 56.8, 56.6, 56.0, 54.7, 35.6, 35.0, 34.0, 31.0, 16.3, 16.2; MS (FAB) m/e 191 (M + H). Anal. Calcd for $C_9H_{18}O_4$: C, 56.82; H, 9.54. Found: C, 57.03; H, 9.44.

(2*R*,*S*,3*R*,5*S*,6*S*)-6-Carbomethoxy-2,5-dimethoxy-3-methyltetrahydropyran (6a and 6b). Primary alcohol **5** (8.0 g, 42.0 mmol) was dissolved in 150 mL of carbon tetrachloride, 150 mL of acetonitrile, and 225 mL of distilled water and cooled to 0 °C. Ruthenium(III) chloride trihydrate (330 mg, 1.26 mmol) was added in 1 portion, followed by sodium metaperiodate (36.0 g, 168 mmol) added over 20 min via a solid addition funnel. The ice bath was then removed, and the dark solution was allowed to stir for 2 h at room temperature. The reaction was closely monitored by TLC and was quenched when done by addition of 300 mL of dichloromethane. The aqueous layer was separated and extracted further with dichloromethane (3 × 100 mL). The organic extracts were dried over $MgSO_4$ and filtered through a 1-cm plug of silica over a 1-cm plug of Celite 545. The dark solution was concentrated and then diluted with 600 mL of ether in a 1000-mL Erlenmeyer flask. Esterification of the acid was accomplished by treatment with diazomethane (generated in a separate flask from 18 g of diazald and 4.5 g of potassium hydroxide in 250 mL of 10:1 ethanol/ether and bubbled through the reaction vessel in a stream of dry nitrogen) until TLC indicated complete consumption of the starting material. Nitrogen was bubbled through the yellow reaction mixture until it became clear. Concentration and silica gel chromatography (10:1 to 5:1 hexane/ethyl acetate) gave 4.73 g (21.7

mmol) of the major anomer **6a** and 2.50 g (11.5 mmol) of the minor anomer **6b** (79% overall for two steps). Characterization of the major anomer **6a**: $[\alpha]^{23}_D$ +154° (*c* 1.42, $CHCl_3$); IR (film) 2936 m, 2903 sh, 1754 s (C=O), 1464 w, 1456 m, 1439 w, 1284 w, 1221 m, 1190 m, 1134 m, 1074 w, 1026 m, 964 w, 952 m, 936 w, 922 m; ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.48 (d, *J* = 3.3, 1 H), 4.01 (d, *J* = 9.7, 1 H), 3.78 (s, 3 H), 3.46–3.42 (ddd, *J* = 14.3, 9.6, 4.5, 1 H), 3.36 (s, 3 H), 3.31 (s, 3 H), 1.98–1.94 (mult, 1 H), 1.89–1.87 (mult, 1 H), 1.45 (q, *J* = 12.1, 1 H), 0.92 (d, *J* = 6.9, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.2, 101.0, 76.4, 70.9, 56.4, 55.1, 52.3, 33.4, 31.1, 16.1; MS (FAB) *m/e* 241 (M + Na). Anal. Calcd for $C_{10}H_{18}O_5$: C, 55.03; H, 8.31. Found: C, 55.05; H, 8.27. Characterization of the minor anomer **6b**: $[\alpha]^{23}_D$ −54.0° (*c* 1.22, $CHCl_3$); IR (film) 2955 m, 2936 m, 2832 sh, 1754 s (C=O), 1462 m, 1439 m, 1389 w, 1287 m, 1215 w, 1200 m, 1130 m, 1103 w, 1034 m, 1007 m, 974 m, 885 w; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.98 (d, *J* = 8.4, 1 H), 3.80 (d, *J* = 10.6, 1 H), 3.79 (s, 3 H), 3.50–3.44 (mult, 4 H), 3.32 (s, 3 H), 2.26–2.20 (ddd, *J* = 12.9, 8.8, 4.4, 1 H), 1.75–1.65 (mult, 1 H), 1.10–1.09 (mult, 1 H), 0.94 (d, *J* = 6.7, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.8, 107.9, 78.1, 76.0, 56.9, 56.8, 52.2, 35.4, 34.4, 16.4; MS (FAB) *m/e* 241 (M + Na). Anal. Calcd for $C_{10}H_{18}O_5$: C, 55.03; H, 8.31. Found: C, 55.13; H, 8.26.

[2*S*,3*R*,5*S*,6*R*,6(1*S*)]-2,5-Dimethoxy-6-(1-hydroxy-3-methyl-2-butenyl)-3-methyltetrahydropyran (7a). A dry 100-mL three-necked round-bottom flask (fitted with a reflux condenser, stopper, and a septum) was charged with magnesium metal (2.37 g, 98 mmol) and 60 mL of THF. A portion of 1-bromo-2-methylpropene (1.1 mL, 1.1 mmol) was added to the suspension followed by dibromoethane (0.28 mL, 3.25 mmol), and then after 5 min, the remainder of the vinyl bromide (5.5 mL, 5.5 mmol) was added dropwise. The reaction was heated to reflux for 60 min and allowed to stir at room temperature for 60 min. In the meantime, pyranose **6a** (4.73 g, 21.7 mmol) was dried by evaporation of 25 mL of benzene, dissolved in 290 mL of ethyl ether, cooled to −78 °C. After 10 min, DIBAH (24.0 mL, 24 mmol of a 1.0 M solution in hexane) was added to the ester and the clear solution was stirred at −78 °C for 45 min. The cloudy Grignard suspension was then added to the reaction via cannula, and the solution was stirred for 60 min at 0 °C, followed by cooling to −78 °C and quenching with 5 mL of saturated aqueous NH_4Cl. The mixture was allowed to warm to room temperature and poured into 75 mL of Rochelle's salt and 75 mL of ethyl ether. Separation of the organic layer was followed by back-extraction of the aqueous layer (2 × 100 mL ether). The organic phases were combined, dried over $MgSO_4$, and concentrated, and the resulting oil was purified by silica gel chromatography (40% ether in hexane), providing the desired carbinol as a clear, colorless oil (2.961 g, 12.1 mmol, 56%): $[\alpha]^{23}_D$ +171° (*c* 0.81, $CHCl_3$); IR (film) 3550–3300 m (OH), 2975 sh, 2934 m, 2899 m, 2832 w, 1453 m, 1383 m, 1360 w, 1210 m, 1190 m, 1167 m, 1138 m, 1103 m, 1059 w, 1030 m, 974 w, 953 m; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.44 (d, *J* = 8.7, 1 H), 4.59 (t, *J* = 7.1, 1 H), 4.44 (d, *J* = 3.2, 1 H), 3.38 (s, 3 H), 3.37–3.33 (mult, 2 H), 3.30 (s, 3 H), 2.10 (d, *J* = 7.3, 1 H), 1.98–1.95 (mult, 1 H), 1.82–1.77 (mult, 1 H), 1.73 (d, *J* = 7.9, 6 H), 1.42–1.39 (dd, *J* = 8.0, 4.9, 1 H), 0.92 (d, *J* = 6.9, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 135.4, 124.9, 100.9, 74.5, 73.3, 66.4, 56.2, 54.6, 33.9, 31.2, 25.8, 18.2, 16.2; MS (FAB) *m/e* 267 (M + Na). Anal. Calcd for $C_{13}H_{24}O_4$: C, 63.91; H, 9.90. Found: C, 64.05; H, 9.64.

[2*S*,3*R*,5*S*,6*S*,6(1*S*)]-2,5-Dimethoxy-6-(1-methoxy-3-methyl-2-butenyl)-3-methyltetrahydropyran. Carbinol **7a** (2.961 g, 12.1 mmol) was placed in a 250-mL flask, dissolved in 140 mL of THF, and cooled to 0 °C. Sodium hydride (1.45 g, 36 mmol, 60% dispersion in mineral oil) was added and stirring was continued for 60 min at room temperature. The slurry was cooled to 0 °C and iodomethane (3.8 mL, 61 mmol) was added via syringe. The slurry was allowed to warm to room temperature and stir for 90 min. The reaction was quenched with 50 mL of aqueous NH_4Cl, and the slurry was extracted with ether (3 × 30 mL). The organic phases were combined, dried over $MgSO_4$, concentrated, and purified by silica gel chromatography (5:1 hexane/ether), producing the desired methyl ether as a clear, colorless oil (2.953 g, 11.4 mmol, 94%): $[\alpha]^{23}_D$ +185° (*c* 1.24, $CHCl_3$); IR (film) 2975 m, 2934 m, 2897 m, 2822 w, 1183 w, 1140 m, 1113 m, 1099 m, 1082 m, 1059 m, 1030 m, 1005 w, 970 m, 953 w, 938 w; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.39 (d, *J* = 9.3, 1 H), 4.44 (d, *J* = 3.3, 1 H), 4.24 (dd, *J* = 9.4, 1.5, 1 H), 3.45–3.40 (ddd, *J* = 14.2, 9.6, 4.6, 1 H), 3.39–3.28 (mult, 1 H), 3.38 (s, 3 H), 3.27 (s, 3 H), 1.96–1.92 (ddd, *J* = 11.9, 8.7, 4.3, 1 H), 1.89–1.83 (mult, 1 H), 1.76 (d, *J* = 1.1, 3 H), 1.70 (d, *J* = 1.3, 3 H), 1.39–1.32 (q, *J* = 12.1, 1 H), 0.89 (d, *J* = 6.9, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 135.9, 122.8, 100.8, 74.2, 73.9, 73.5, 56.6, 56.2, 54.3, 33.8, 31.8, 25.9, 18.2, 16.2; MS (FAB) *m/e* 281 (M + Na). Anal. Calcd for $C_{14}H_{26}O_4$: C, 65.09; H, 10.14. Found: C, 65.18; H, 9.97.

[2*S*,3*R*,5*S*,6*S*,6(1*S*,2*R*,*S*)]-2,5-Dimethoxy-6-(2-hydroxy-1-methoxy-3-methylbutyl)-3-methyltetrahydropyran. The pyranose described above (2.953 g, 11.4 mmol) was dried by evaporation of 30 mL of benzene and was diluted with 100 mL of THF and cooled to 0 °C. Borane (17.1 mL, 17.1 mmol, 1.0 M solution in THF) was added dropwise, and the clear solution was stirred at 0 °C for 2.5 h. Sodium hydroxide (57 mL, 57 mmol, 1.0 N aqueous solution) was then carefully added dropwise, followed by hydrogen peroxide (6.5 g, 57 mmol, 30% aqueous solution). The solution was warmed to room temperature and allowed to stir for 90 min. The reaction was diluted with ether and washed with water (2 × 75 mL). The combined aqueous phases were back-extracted with ether, and the organic phases were combined, dried over $MgSO_4$, concentrated, and purified by silica gel chromatography (2:1 to 1:1 hexane/ether), leaving the desired carbinol, a crystalline white solid, as a 2:1 mixture of diastereomers (1.709 g, 54%).

[2*S*,3*R*,5*S*,6*S*,6(1*R*)]-2,5-Dimethoxy-6-(1-methoxy-3-methyl-2-oxobutyl)-3-methyltetrahydropyran. The mixture of carbinol diastereomers (1.709 g, 6.18 mmol) was placed in a 15-mL flask and dried by azeotrope with benzene. Oxalyl chloride (3.7 mL, 7.4 mmol, 2.0 M solution in dichloromethane) was placed in a 100-mL flask with 60 mL of dichloromethane and cooled to −78 °C. Dimethyl sulfoxide (1.10 mL, 15.5 mmol) was added dropwise and the solution stirred for 28 min at −78 °C. The alcohol was then added as a solution in dichloromethane via cannula (3 × 2 mL washes), and this cloudy suspension was stirred at −78 °C for 35 min. Triethylamine (4.31 mL, 31 mmol) was added via syringe, and the solution was warmed to room temperature and stirred for 30 min. The solution was diluted with 100 mL of ether and washed with 100 mL of water. The organic phase was separated, dried ($MgSO_4$), concentrated, and purified by silica gel chromatography (3:1 hexane/ether), providing the desired ketone as a clear, colorless oil (1.668 g, 6.08 mmol, 98%): IR (film) 2975 m, 2934 m, 2880 m, 2830 m, 1711 s (C=O), 1466 m, 1458 m, 1381 m, 1346 w, 1210 m, 1186 m, 1138 m, 1100 s, 1076 m, 1049 s, 1024 m, 972 m, 955 m; ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.35 (d, *J* = 3.2, 1 H), 4.09 (d, *J* = 2.1, 1 H), 3.87–3.85 (dd, *J* = 9.6, 2.1, 1 H), 3.48 (s, 3 H), 3.43–3.38 (ddd, *J* = 14.1, 9.5, 4.5, 1 H), 3.35 (s, 3 H), 3.19 (s, 3 H), 3.10–3.05 (qn, *J* = 6.8, 1 H), 2.00–1.96 (ddd, *J* = 12.0, 8.5, 4.2, 1 H), 1.86–1.80 (mult, 1 H), 1.45–1.38 (q, *J* = 11.9, 1 H), 1.09 (d, *J* = 6.9, 3 H), 1.08 (d, *J* = 6.7, 3 H), 0.89 (d, *J* = 6.9, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 185.6, 101.2, 84.0, 73.7, 72.3, 59.9, 55.7, 55.1, 35.7, 33.6, 30.9, 18.6, 17.8, 16.3; MS (FAB) *m/e* 297 (M + Na). Anal. Calcd for $C_{14}H_{26}O_5$: C, 61.29; H, 9.55. Found: C, 61.67; H, 9.86.

[2*S*,3*R*,5*S*,6*S*,6(1*S*,2*S*)]-2,5-Dimethoxy-6-(2-hydroxy-1-methoxy-3-methylbutyl)-3-methyltetrahydropyran (8a). The ketone described above (1.640 g, 5.98 mmol) was placed in a 250-mL flask, dissolved in 80 mL of ethyl ether, and cooled to 0 °C. After cooling for 30 min, $Zn(BH_4)_2$ (100 mL, 15 mmol, 0.15 M solution in ethyl ether) was added via syringe. The reaction was quenched carefully at 0 °C after 30 min with 3 mL of water followed by 10 mL of aqueous NH_4Cl. The layers were separated, and the aqueous phase was back-extracted with 50 mL of ether. The organics were combined, dried over $MgSO_4$, concentrated, and purified by silica gel chromatography (2:1 to 1:1 hexane/ether). Carbinol **8a** was isolated as a crystalline white solid (1.218 g, 4.41 mmol, 74%): mp 82 °C; $[\alpha]^{23}_D$ +146° (*c* 0.25, $CHCl_3$); IR (film) 3540–3400 m (OH), 2975 m, 2957 m, 2938 m, 2870 m, 2834 w, 1462 m, 1450 m, 1381 m, 1188 m, 1169 w, 1136 m, 1111 s, 1092 m, 1053 m, 1009 w, 968 m, 955 w; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.46 (d, *J* = 3.3, 1 H), 3.86 (d, *J* = 9.5, 1 H), 3.60 (d, *J* = 4.8, 1 H), 3.58–3.50 (mult, 2 H), 3.48 (s, 3 H), 3.40 (s, 3 H), 3.36 (s, 3 H), 2.91 (d, *J* = 6.7, 1 H), 2.02–1.97 (ddd, *J* = 11.9, 8.5, 4.2, 1 H), 1.90–1.78 (mult, 2 H), 1.44–1.35 (q, *J* = 9.1, 1 H), 1.07 (d, *J* = 6.6, 3 H), 0.92 (d, *J* = 6.8, 3 H), 0.90 (d, *J* = 6.7, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 101.5, 76.6, 75.6, 73.7, 70.8, 58.2, 55.9, 33.4, 31.2, 30.7, 29.7, 19.3, 18.9, 16.4; MS (FAB) *m/e* 299 (M + Na). Anal. Calcd for $C_{14}H_{28}O_5$: C, 60.84; H, 10.21. Found: C, 61.05; H, 9.85.

[2*S*,3*R*,5*S*,6*S*,6(1*S*,2*S*)]-2,5-Dimethoxy-6-[2-(benzyloxy)-1-methoxy-3-methylbutyl]-3-methyltetrahydropyran. A 100-mL flask was charged with potassium hydride (1.0 g, 8.7 mmol, 35% dispersion in mineral oil) and 50 mL of THF and cooled to 0 °C. Cooling for 10 min was followed by addition of **8a** (1.200 g, 4.34 mmol) in 3 mL of THF via cannula. The slurry was stirred for 95 min while warming to room temperature. After cooling to 0 °C, the alkoxide was then quenched with benzyl bromide (1.55 mL, 13 mmol), and the slurry was stirred for 30 min at room temperature. The excess potassium hydride was quenched carefully at 0 °C with aqueous NH_4Cl, followed by dilution with ether, separation of layers, and back-extraction of the aqueous phase with ether. The combined organic phases were dried ($MgSO_4$), concentrated, and purified by silica gel chromatography (3:1 hexane/ether), leaving the desired benzyl ether as a clear, colorless oil (1.565 g, 4.27 mmol, 98%): $[\alpha]^{23}_D$ +93.7° (*c* 0.38, $CHCl_3$); IR (film) 2959 m, 2932 m, 2901 m, 2876 m, 2832 w, 1454 m, 1188 m, 1134 m, 1115 s, 1107 s, 1053 s, 1028 m, 968 w, 735 w, 698 w; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36–7.23 (mult, 5 H), 4.74 (d, *J* = 11.4, 1 H), 4.62 (d, *J* = 11.4, 1 H), 4.50 (d, *J* = 3.3, 1 H), 3.80 (d, *J* = 9.6, 1 H), 3.66 (d, *J* = 7.2, 1 H), 3.58–3.54 (mult, 1 H), 3.53 (s, 3 H), 3.37–3.32 (mult, 1 H), 3.36 (s, 3 H), 3.31 (s, 3 H),

2.14–2.08 (mult, 1 H), 2.04–2.00 (ddd, J = 11.9, 8.3, 4.1, 1 H), 1.90–1.85 (mult, 1 H), 1.47–1.40 (q, J = 11.8, 1 H), 1.07 (d, J = 7.0, 3 H), 1.04 (d, J = 6.8, 3 H), 0.93 (d, J = 6.9, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 139.4, 128.1, 127.1, 101.9, 82.6, 78.9, 74.5, 73.6, 70.5, 60.6, 56.2, 55.1, 33.8, 30.6, 29.3, 21.2, 17.0, 16.4; MS (FAB) m/e 389 (M + Na). Anal. Calcd for $C_{21}H_{34}O_5$: C, 68.82; H, 9.35. Found: C, 69.08; H, 9.24.

[2*R*,3*R*,5*S*,6*R*,6(1*S*)]-2,5-Dimethoxy-6-(1-hydroxy-3-methyl-2-butenyl)-3-methyltetrahydropyran (7b). Methyl ester **6b** (1.829 g, 8.38 mmol) was converted to carbinol **7b** following the same procedure used to transform the major anomer **6a**. The desired carbinol was isolated (819.3 mg, 3.35 mmol, 40% for two steps) as a clear, colorless oil after purification by silica gel chromatography (40% ether in hexane): $[\alpha]^{23}_D$ +4.5° (c 0.40, $CHCl_3$); IR (film) 3450–3300 m (OH), 2957 sh, 2932 m, 2876 m, 2857 m, 2828 m, 1456 w, 1389 w, 1377 w, 1184 m, 1130 m; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.42 (d, J = 9.0, 1 H), 4.58–4.53 (ddd, J = 11.0, 9.2, 1.8, 1 H), 3.98 (d, J = 8.4, 1 H), 3.50 (s, 3 H), 3.41–3.34 (mult, 1 H), 3.39 (s, 3 H), 3.14–3.12 (dd, J = 9.2, 2.0, 1 H), 2.26–2.19 (mult, 2 H), 1.75 (d, J = 7.8, 3 H), 1.73 (d, J = 7.8, 3 H), 1.68–1.58 (mult, 1 H), 1.13–1.04 (q, J = 11.1, 1 H), 0.94 (d, J = 6.6, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.8, 124.5, 107.8, 80.7, 74.3, 66.6, 56.8, 56.6, 35.7, 35.0, 25.9, 18.2, 16.3; MS (FAB) m/e 267 (M + Na). Anal. Calcd for $C_{13}H_{24}O_4$: C, 63.91; H, 9.90. Found: C, 63.82; H, 9.76.

[2*R*,3*R*,5*S*,6*S*,6(1*S*)]-2,5-Dimethoxy-6-(1-methoxy-3-methyl-2-butenyl)-3-methyltetrahydropyran. Carbinol **7b** (819.3 mg, 3.35 mmol) was methylated following the same protocol as the conversion described above. The methyl ether (744.8 mg, 2.88 mmol, 86%) was isolated as a clear, colorless oil: $[\alpha]^{23}_D$ +22.4° (c 0.49, $CHCl_3$); IR (film) 2978 sh, 2955 sh, 2932 m, 2856 m, 1464 m, 1449 w, 1387 m, 1190 m, 1179 w, 1130 m, 1100 m, 1074 m, 1046 m, 989 w; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.40 (d, J = 9.6, 1 H), 4.21 (dd, J = 9.6, 2.0, 1 H), 3.86 (d, J = 8.4, 1 H), 3.51–3.43 (mult, 1 H), 3.47 (s, 3 H), 3.40 (s, 3 H), 3.27 (s, 3 H), 3.07–3.05 (dd, J = 9.2, 2.0, 1 H), 2.22–2.18 (ddd, J = 12.7, 9.0, 4.5, 1 H), 1.79 (d, J = 1.0, 3 H), 1.73 (d, J = 1.1, 3 H), 1.69–1.64 (mult, 1 H), 1.08–1.01 (q, J = 11.4, 1 H), 0.93 (d, J = 6.6, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 136.2, 122.3, 108.1, 81.1, 74.2, 74.1, 57.0, 56.3, 56.1, 36.2, 35.0, 26.1, 18.3, 16.4; MS (FAB) m/e 281 (M + Na). Anal. Calcd for $C_{14}H_{26}O_4$: C, 65.09; H, 10.14. Found: C, 64.97; H, 10.22.

[2*R*,3*R*,5*S*,6*S*,6(1*S*,2*S*)]-2,5-Dimethoxy-6-(2-hydroxy-1-methoxy-3-methylbutyl)-3-methyltetrahydropyran (8b). The pyranose described above (260 mg, 1.00 mmol) was dried by evaporation of 5 mL of benzene and then dissolved in 15 mL of THF at 0 °C. A 1.0 M solution of borane in THF (1.5 mL, 1.5 mmol) was added dropwise. The procedure was identical with the hydroboration–oxidation protocol described previously from this point forward. The desired crystalline, white solid **8b**, was isolated as a single isomer (184.0 mg, 0.66 mmol, 66%): mp 58 °C; $[\alpha]^{23}_D$ −12.6° (c 1.88, $CHCl_3$); IR (film) 3550–3450 m (OH), 2959 m, 2934 m, 2876 sh, 1464 m, 1389 w, 1223 m, 1196 w, 1182 m, 1130 m, 1099 m, 1072 m, 1039 m, 1010 m, 988 m, 849 w; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.89 (d, J = 8.4, 1 H), 3.59–3.56 (mult, 1 H), 3.53–3.44 (mult, 3 H), 3.42 (s, 3 H), 3.39 (s, 3 H), 3.32 (s, 3 H), 2.67 (d, J = 7.2, 1 H), 2.24–2.20 (ddd, J = 12.8, 8.7, 4.3, 1 H), 1.75–1.68 (sx, J = 6.8, 1 H), 1.65–1.59 (mult, 1 H), 1.04–1.01 (mult, 1 H), 1.00 (d, J = 6.6, 3 H), 0.89 (d, J = 6.6, 3 H), 0.88 (d, J = 6.6, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 108.2, 78.3, 76.7, 75.1, 73.2, 58.0, 56.6, 56.2, 35.5, 34.5, 31.0, 19.4, 18.4, 16.3; MS (FAB) m/e 299 (M + Na). Anal. Calcd for $C_{14}H_{28}O_5$: C, 60.84; H, 10.21. Found: C, 60.81; H, 10.25.

[2*R*,3*R*,5*S*,6*S*,6(1*S*,2*S*)]-2,5-Dimethoxy-6-[2-(benzyloxy)-1-methoxy-3-methylbutyl]-3-methyltetrahydropyran. Carbinol **8b** (486.2 mg, 1.76 mmol) was benzylated following the same protocol as the transformation of **8a** to its benzyl ether. The desired benzyl ether (621.0 mg, 1.69 mmol, 96%) was isolated as a clear, colorless oil after silica gel chromatography: $[\alpha]^{23}_D$ −16.1° (c 1.11, $CHCl_3$); IR (film) 2959 m, 2932 m, 2876 w, 2828 w, 1464 m, 1454 m, 1389 m, 1341 m, 1196 w, 1182 m, 1154 m, 1116 w, 1069 m, 963 m; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38–7.27 (mult, 5 H), 4.63 (d, J = 2.2, 1 H), 3.90 (d, J = 8.4, 1 H), 3.60 (d, J = 2.6, 1 H), 3.58–3.52 (mult, 2 H), 3.51 (s, 3 H), 3.50 (s, 3 H), 3.43–3.39 (mult, 1 H), 3.37 (s, 3 H), 2.33–2.29 (ddd, J = 12.6, 8.6, 4.3, 1 H), 2.13–2.08 (mult, 1 H), 1.71–1.65 (mult, 1 H), 1.11–1.04 (mult, 2 H), 1.09 (d, J = 7.0, 3 H), 1.02 (d, J = 6.9, 3 H), 0.95 (d, J = 6.6, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 139.2, 128.2, 127.2, 126.9, 107.6, 81.8, 78.2, 77.3, 74.5, 73.7, 60.4, 56.7, 55.3, 35.4, 34.9, 28.9, 21.1, 16.5, 15.8; MS (FAB) m/e 389 (M + Na). Anal. Calcd for $C_{21}H_{34}O_5$: C, 68.82; H, 9.35. Found: C, 68.90; H, 9.33.

[2*R*,*S*,3*R*,5*S*,6*S*,6(1*S*,2*S*)]-2-Hydroxy-6-[2-(benzyloxy)-1-methoxy-3-methylbutyl]-5-methoxy-3-methyltetrahydropyran. To a solution of methyl acetal (585 mg, 1.60 mmol) in 16 mL of THF was added 6.5 mL of a 4.5 N aqueous solution of HCl. After heating for 2 h at 60 °C, the reaction mixture was cooled to room temperature and carefully transferred to a separatory funnel containing 100 mL of EtOAc and 30 mL of saturated aqueous $NaHCO_3$. Gentle swirling of the separatory funnel was followed by more vigorous agitation. After removal of the aqueous layer, the organic layer was washed with additional saturated aqueous $NaHCO_3$ until the organic solution had a pH of 7. The aqueous phase was back-extracted with EtOAc, and the combined organic solutions were dried over $MgSO_4$ and concentrated. The resulting yellow oil was purified by flash chromatography (20% to 30% to 50% EtOAc in hexanes) to give 542 mg of a mixture of the two lactols: $[\alpha]^{23}_D$ +42.9° (c 0.375, $CHCl_3$); IR (film) 3430 (br), 2959, 2934, 2876, 1454, 1095, 1068, 734; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36–7.24 (mult, 10 H), 5.01 (d, J = 2.8, 21 H), 4.71 (d, J = 11.4, 1 H), 4.64–4.57 (q, J = 12.6, 4 H), 4.11 (d, J = 7.3, 1 H), 4.00 (d, J = 9.7, 1 H), 3.66–3.45 (mult, 6 H), 3.49 (s, 3 H), 3.48 (s, 3 H), 3.36 (s, 3 H), 3.35 (s, 3 H), 3.36–3.33 (mult, 2 H), 2.29 (t, J = 4.3, 1 H), 2.27 (t, J = 4.3, 1 H), 2.06–2.00 (mult, 4 H), 1.88–1.84 (mult, 4 H), 1.72–1.68 (mult, 2 H), 1.52–1.43 (mult, 4 H), 1.08–1.05 (mult, 6 H), 1.01–0.99 (mult, 9 H), 0.93 (d, J = 6.9, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 139.3, 128.3, 128.2, 127.3, 127.25, 127.2, 100.9, 94.4, 81.9, 81.3, 78.6, 78.2, 77.8, 74.5, 74.4, 74.2, 73.6, 69.9, 60.5, 55.4, 54.9, 37.2, 35.2, 33.8, 29.9, 29.3, 29.0, 21.1, 21.0, 16.6, 16.6, 16.3, 15.9; HRMS (calcd for $C_{20}H_{32}O_5Na$ (M + Na) 375.216, found (FAB) 375.216.

(2*E*,4*R*,6*S*,7*S*,8*R*,9*S*)-7-Hydroxy-6,8-dimethoxy-4,10-dimethyl-9-(phenylmethoxy)-2-undecenoic Acid, Methyl Ester (9). A mixture of freshly purified lactol (900 mg, 2.56 mmol) and recrystallized (hexane/benzene) methyl (triphenylphosphoranylidene)acetate (850 mg, 2.56 mmol) was refluxed in 25 mL of acetonitrile. Additional portions (425 mg, 1.28 mmol) of the phosphoranylidene were added at 24-h intervals. After a total reaction time of 72 h, the reaction mixture was cooled to room temperature, concentrated, and purified by flash chromatography (10% to 20% to 30% EtOAc in hexane) to give 673 mg (73.5%) of the desired α,β-unsaturated ester, which was contaminated by 5% (approximated by ^{1}H NMR integration) of an undesired diastereomer tentatively assigned as the γ-methyl epimer: $[\alpha]^{23}_D$ −12.7° (c 0.355, $CHCl_3$); IR (film) 3478 (br), 2965, 2933, 1719, 1656, 1110, 913, 739; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28–7.17 (mult, 5 H), 6.86–6.81 (dd, J = 15.7, 8.6, 1 H), 4.75 (d, J = 10.9, 1 H), 4.51 (d, J = 10.9, 1 H), 3.64 (s, 3 H), 3.63–3.60 (mult, 1 H), 3.50 (d, J = 3.3, 1 H), 3.40 (s, 3 H), 3.46–3.34 (mult, 2 H), 3.29 (s, 3 H), 3.23–3.19 (ddd, J = 8.1, 8.1, 3.2, 1 H), 2.56–2.52 (mult, 1 H), 1.86–1.79 (mult, 1 H), 1.77–1.71 (mult, 1 H), 1.58–1.49 (mult, 1 H), 1.00 (d, J = 6.8, 3 H), 0.98 (d, J = 6.7, 3 H), 0.86 (d, J = 6.9, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.4, 154.9, 138.2, 128.3, 127.8, 127.7, 119.6, 84.9, 78.9, 78.0, 75.5, 72.5, 57.9, 57.5, 51.3, 37.7, 33.6, 30.2, 20.8, 19.8, 19.1; HRMS calcd for $C_{23}H_{36}O_6Na$ (M + Na) 431.241, found (FAB) 431.240.

(2*E*,4$_R$,6*S*,7*S*,8*S*,9*S*)-7-[(*tert*-Butyldimethylsilyl)oxy]-6,8-dimethoxy-4,10-dimethyl-9-(phenylmethoxy)-2-undecenoic Acid, Methyl Ester. To a −78 °C solution of the hydroxy enoate (313 mg, 0.76 mmol) in 7.6 mL of methylene chloride was added 2,6-lutidine (0.142 mL, 1.21 mmol), followed by *tert*-butyldimethylsilyl triflate (0.209 mL, 0.912 mmol). The reaction was warmed to room temperature for 15 min, quenched with MeOH (0.5 mL), diluted with ether (40 mL), and then washed sequentially with aqueous $NaHCO_3$ (10 mL), H_2O (10 mL), and brine (10 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated. Flash chromatography with 5% EtOAc in hexanes gave 370 mg (93%) of the desired silyl ether: $[\alpha]^{23}_D$ −42.1° (c 0.76, $CHCl_3$); IR (film) 2953, 2927, 1723, 1654, 1101, 835, 735; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35–7.26 (mult, 5 H), 6.75–6.70 (dd, J = 15.6, 7.2, 1 H), 5.78 (d, J = 15.6, 1 H), 4.67 (d, J = 11.5, 1 H), 4.53 (d, J = 11.5, 1 H), 3.99–3.97 (dd, J = 7.7, 1.4, 1 H), 3.70 (s, 3 H), 3.4 (s, 3 H), 3.12 (s, 3 H), 3.12–3.06 (mult, 3 H), 2.57–2.52 (mult, 1 H), 1.82–1.75 (mult, 2 H), 1.24–1.19 (ddd, J = 1.5, 10.5, 14.6, 1 H), 1.05 (d, J = 6.7, 3 H), 1.03–1.01 (mult, 1 H), 1.00 (d, J = 6.7, 3 H), 0.95 (d, J = 6.8, 3 H) 0.91 (s, 9 H), 0.07 (s, 6 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.9, 154.5, 138.7, 128.2, 127.6, 127.4, 120.2, 85.2, 83.1, 79.8, 73.7, 72.9, 59.6, 56.6, 51.3, 36.3, 33.4, 30.0, 26.1, 20.8, 20.7, 18.5, 18.4, −4.3, −4.6; HRMS calcd for $C_{29}H_{50}O_6SiNa$ (M + Na) 545.328, found (FAB) 545.329.

(2*S*,3*R*,4*R*,6*S*,7*S*,8*S*,9*S*)-7-[(*tert*-Butyldimethylsilyl)oxy]-2,3-dihydroxy-6,8-dimethoxy-4,10-dimethyl-9-(phenylmethoxy)undecanoic Acid, Methyl Ester (10). To a mixture of the α,β-unsaturated ester (875 mg, 1.67 mmol) and dihydroquinidine acetate (737 mg, 2.0 mmol) in 16.7 mL of toluene was added dropwise 5.14 mL of a 0.39 M toluene solution of OsO_4. Upon addition of a drop of the osmium tetraoxide, the solution turned brown locally then faded, and eventually the entire solution became dark green. After 5 h the reaction was diluted with additional toluene, and hydrogen sulfide was bubbled through until the dark green solution turned brown and a black precipitate formed (approx 3 min). The precipitate was removed by filtration through a pad of Celite. The brown filtrate was concentrated and subjected to flash chromatography (10% then 15% EtOAc in hexanes) to yield 540 mg (60.4% of a single diastereomer) of the diol **10**: $[\alpha]^{23}_D$ −10.5° (c 0.665,

$CHCl_3$); IR (film) 3499 (br), 2958, 2931, 1742, 1249, 1112, 833, 738; ^1H NMR (500 MHz, $CDCl_3$) δ 7.34–7.26 (mult, 5 H), 4.68 (d, J = 11.5, 1 H), 4.56 (d, J = 11.5, 1 H), 4.21 (d, J = 8.2, 1 H), 4.03–4.01 (dd, J = 7.6, 1.5, 1 H), 3.80 (s, 3 H), 3.65 (d, J = 5.0, 1 H), 3.54–3.50 (mult, 1 H), 3.42 (s, 3 H), 3.37 (d, J = 9.7 Hz, 1 H), 3.23–3.21 (mult, 1 H), 3.14 (s, 3 H), 3.14–3.12 (mult, 1 H), 2.88 (d, J = 7.7, 1 H), 2.19–2.14 (mult, 1 H), 2.03–1.97 (ddd, J = 4.9, 9.8, 10.3, 1 H), 1.94–1.87 (mult, 1 H), 1.51–1.47 (mult, 1 H), 1.04 (d, J = 6.7, 3 H), 1.0 (d, J = 6.9, 3 H), 0.95 (d, J = 6.9, 3 H), 0.89 (s, 9 H), 0.08 (s, 3 H), 0.06 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.4, 138.6, 128.3, 127.6, 127.5, 85.3, 82.3, 79.8, 75.9, 73.8, 73.2, 71.7, 59.6, 55.9, 52.5, 32.4, 31.4, 29.9, 26.1, 20.8, 18.5, 18.3, 16.9, −4.6; HRMS calcd for $C_{29}H_{52}O_8SiNa$ (M + Na) 579.333, found (FAB) 579.334.

(2*S*,3*R*,4*R*,6*S*,7*S*,8*S*,9*S*)-7-[(*tert*-Butyldimethylsilyl)oxy]-2,3,9-trihydroxy-6,8-dimethoxy-4,10-dimethylundecanoic Acid, Methyl Ester. A mixture of 505 mg (0.95 mmol) of **10** and 10% $Pd(OH)_2/C$ (100 mg) in 9 mL of EtOAc was stirred under an atmosphere of H_2 for 2 h. After argon purging, the reaction was diluted with EtOAc (35 mL), and the catalyst was removed by filtration through Celite. Concentration gave 426 mg (96%) of the triol: $[\alpha]^{23}_D$ −23.5° (*c* 0.26, $CHCl_3$); IR (film) 3488, 2957, 2931, 1742, 1254, 1121, 1095, 837, 774; ^1H NMR (500 MHz, $CDCl_3$) δ 4.25 (d, J = 7.7, 1 H), 4.19 (dd, J = 4.4, 1.1, 1 H), 3.63–3.53 (mult, 4 H), 3.44 (s, 3 H), 3.39 (d, J = 1.9, 1 H), 3.35 (s, 3 H), 3.09–3.07 (dd, J = 9.2, 4.5, 1 H), 2.98 (d, J = 7.8, 1 H), 2.15 (mult, 1 H), 1.86–1.83 (mult, 3 H), 1.02 (d, J = 6.9, 3 H), 0.98 (d, J = 7.0, 3 H), 0.91 (s, 9 H), 0.89 (d, J = 6.8, 3 H), 0.13 (s, 3 H), 0.11 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.5, 81.3, 79.9, 76.2, 75.4, 73.3, 71.7, 59.1, 56.9, 52.7, 32.9, 32.7, 29.2, 25.9, 20.0, 18.2, 16.8, 14.9, −4.5, −4.9; HRMS calcd for $C_{22}H_{46}O_8SiNa$ (M + Na) 489.286, found (FAB) 489.288.

(2*S*,3*R*,4*R*,6*S*,7*S*,8*S*,9*S*)-7-[(*tert*-Butyldimethylsilyl)oxy]-9-hydroxy-6,8-dimethoxy-2,3-[[(4-methoxyphenyl)methylene]dioxy]-4,10-dimethylundecanoic Acid, Methyl Ester. The triol described above (426 mg, 0.91 mmol) and the dimethyl acetal of anisaldehyde (0.820 mL, 4.55 mmol) were stirred together in 9 mL of a 2:1 mixture of benzene/THF in the presence of 4-Å molecular sieves (200 mg, crushed and flame dried) for 5 min. The reaction mixture was treated with camphorsulfonic acid (105 mg, 0.455 mmol) and stirred at ambient temperature for 2 h. The sieves were removed by filtration through glass wool. The filtrate was diluted with EtOAc and neutralized by successive washings with aqueous saturated $NaHCO_3$ (3 × 20 mL), H_2O (1 × 20 mL), and brine (1 × 20 mL). The organic layer was dried over $MgSO_4$, filtered, concentrated, and subjected to flash chromatography (5%, 10%, 15%, 20%, then 30% EtOAc in hexanes) to give 466 mg (80%) of the *p*-methoxybenzylidene acetal: $[\alpha]^{23}_D$ −19.0° (*c* 0.31, $CHCl_3$); IR (film) 3499 (br), 2958, 2931, 1753, 1616, 1248, 1089, 831; ^1H NMR (500 MHz, $CDCl_3$) δ 7.44 (d, J = 8.8, 2 H), 6.87 (d, J = 8.8, 2 H), 5.93 (s, 1 H), 4.43 (d, J = 5.8, 1 H), 4.13–4.09 (mult, 2 H), 3.80 (s, 6 H), 3.55–3.50 (mult, 2 H), 3.40 (s, 3 H), 3.33 (s, 3 H), 3.28 (d, J = 2.7, 1 H), 3.08–3.05 (dd, J = 4.5, 8.9, 1 H), 1.89–1.83 (mult, 2 H) 1.66–1.57 (mult, 1 H), 1.09 (d, J = 6.9, 3 H), 0.92 (d, J = 7.3, 3 H), 0.91 (s, 9 H), 0.86 (d, J = 6.8, 3 H), 0.12 (s, 3 H), 0.10 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.4, 160.6, 128.4, 113.6, 104.6, 85.1, 81.9, 81.6, 76.6, 75.3, 73.2, 58.8, 57.7, 55.3, 52.3, 34.2, 33.9, 28.9, 25.8, 19.9, 18.1, 17.1, 14.9, −4.7, −5.1; HRMS calcd for $C_{30}H_{52}O_9SiNa$ (M + Na) 607.328, found (FAB) 607.327.

(2*S*,3*R*,4*R*,6*S*,7*S*,8*S*,9*S*)-7-[(*tert*-Butyldimethylsilyl)oxy]-9-[2-(diethylphosphono)acetoxy]-6,8-dimethoxy-2,3-[[(4-methoxyphenyl)methylene]dioxy]-4,10-dimethylundecanoic Acid, Methyl Ester (2). A solution of (diethylphosphono)acetic acid (41.6 mg, 0.212 mmol) in 0.5 mL of dichloromethane was added to a solution of the hydroxy ester described above (103 mg, 0.177 mmol) in 1 mL of dichloromethane. Upon addition of dicyclohexylcarbodiimide (43.6 mg, 0.212 mmol) to the reaction, a white precipitate immediately began to form. The heterogeneous mixture was stirred for 20 min, quenched with MeOH (0.1 mL), concentrated, and purified by flash chromatography (30%, 40%, then 50% EtOAc, in hexanes). The resulting colorless oil, contaminated by a white solid, was taken up in 50% ether in hexanes (10 mL), filtered, and concentrated to give 128 mg (95%) of the desired phosphonate **2**: $[\alpha]^{23}_D$ −9.4° (*c* 0.18, $CHCl_3$); IR (film) 2958, 2931, 1732, 1616, 1252, 1094, 1026, 831; ^1H NMR (500 MHz, $CDCl_3$) δ 7.41 (d, J = 8.7, 2 H), 6.86 (d, J = 8.7, 2 H), 5.89 (s, 1 H), 4.81 (dd, J = 3.3, 5.0, 1 H), 4.4 (d, J = 5.9, 1 H), 4.19–4.10 (mult, 5 H), 3.81 (mult, 1 H), 3.79 (s, 6 H), 3.41 (s, 3 H), 3.45 (mult, 1 H), 3.24 (s, 3 H), 31.5 (dd, J = 3.2, 7.4, 1 H), 2.95–2.90 (dd, J = 2.7, 21.7, 2 H), 2.25 (mult, 1 H), 1.95 (mult, 1 H), 1.71–1.61 (mult, 2 H), 1.33 (t, J = 7.1, 3 H), 1.05 (d, J = 6.9, 3 H), 0.92 (d, J = 6.7, 3 H), 0.88 (s, 9 H), 0.76 (d, J = 6.7, 3 H), 0.05 (s, 3 H), 0.04 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.3, 165.2, 165.1, 160.7, 128.6, 128.5, 113.6, 113.5, 104.7, 85.8, 84.1, 80.9, 79.5, 72.9, 62.6, 62.5, 60.6, 56.7, 55.2, 34.9, 33.9, 33.8, 33.7, 28.4, 25.9, 20.0, 18.4, 17.7, 17.4, 16.3; HRMS calcd for $C_{36}H_{63}O_{13}PSiNa$ (M + Na) 785.367, found (FAB) 785.369.

[1(1*R*,3*R*,4*R*),1*E*,3*S*]-3-Hydroxy-5-[(4-methoxybenzyl)oxy]-1-[3-methoxy-4-[(triisopropylsilyl)oxy]cyclohex-1-yl]-2,4,4-trimethyl-1-pentene (16). Vinyl bromide **14** (377.7 mg, 0.93 mmol) was placed in a 75-mL flask and dried by evaporation of xylenes (2 × 10 mL). The flask was thoroughly purged with argon and the oil was dissolved in 10 mL of THF and cooled to −78 °C. After 30 min a solution of *tert*-butyllithium (1.38 mL, 2.1 mmol, 1.55 M in pentane) was added and the yellow solution stirred for 60 min at −78 °C. A solution of $MgBr_2$ (1.11 mL, 1.11 mmol, 1.0 M in 3:1 ether/benzene) was added dropwise, and the cloudy solution was stirred for 20 min at −78 °C. The aldehyde **15** (270 mg, 1.18 mmol) was precooled to −78 °C and added to the anion via cannula (3 × 2 mL of THF). The clear solution was stirred for 30 min at −78 °C and was then allowed to warm to ambient temperature over 60 min. The solution was diluted with dichloromethane (30 mL) and quenched with saturated NH_4Cl (30 mL). The aqueous phase was back-extracted (2 × 20 mL CH_2Cl_2); the combined organic phase was dried ($MgSO_4$), concentrated, and purified by flash chromatography (10:4:1 hexanes/CH_2Cl_2/ether), yielding a 1:1 mixture of carbinol diastereomers (126 mg of **16**, 355 mg mixture). Data for **16**: $[\alpha]^{23}_D$ −10.4° (*c* 1.01, $CHCl_3$); IR (film) 3550–3300 w (OH), 2938 s, 2894 sh, 2867 mult, 1594 mult, 1464 w, 1248 mult, 1175 w, 1138 mult, 1111 mult, 1084 mult, 1038 mult, 1015 w, 884 mult, 735 w; ^1H NMR (500 MHz, $CDCl_3$) δ 7.25 (d, J = 8.6, 1 H), 6.88 (d, J = 8.7, 1 H), 5.14 (d, J = 9.0, 1 H), 4.40 (s, 2 H), 3.88 (d, J = 3.3, 1 H), 3.82 (s, 3 H), 3.60 (d, J = 3.8, 1 H), 3.56 (ddd, J = 13.2, 8.5, 4.9, 1 H), 3.39 (s, 3 H), 3.33 (d, J = 8.7, 1 H), 3.25 (d, J = 8.8, 1 H), 2.98 (ddd, J = 12.8, 8.4, 4.5, 1 H), 2.29–2.25 (mult, 1 H), 2.06–2.01 (mult, 1 H), 1.96–1.92 (mult, 1 H), 1.68 (d, J = 1.1, 3 H), 1.63–1.56 (mult, 1 H), 1.42–1.38 (mult, 1 H), 1.37–1.33 (mult, 1 H), 1.31–1.27 (mult, 1 H), 1.08 (s, 21 H), 1.05–0.96 (mult, 2 H), 0.90 (s, 3 H), 0.88 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.3, 134.1, 133.2, 129.9, 129.2, 113.9, 84.5, 84.2, 80.3, 75.0, 73.3, 57.4, 55.2, 38.9, 36.1, 35.0, 34.7, 34.2, 31.6, 30.8, 26.9, 23.6, 22.6, 20.8, 18.1, 13.9, 12.6; HRMS calcd for $C_{32}H_{56}O_5Si$ (M + Na) 571.380, found (FAB) 571.379.

[1(1*R*,3*R*,4*R*),1*E*,3(2*S*),3*S*]-3-[[[*N*-(*tert*-Butoxycarbonyl)piperidin-2-yl]carbonyl]oxy]-5-[(4-methoxybenzyl)oxy]-1-[3-methoxy-4-[(triisopropylsilyl)oxy]cyclohex-1-yl]-2,4,4-trimethyl-1-pentene. Alcohol **16** (395 mg, 0.72 mmol) was combined with *N*-BOC-(*S*)-pipecolinic acid (660 mg, 2.88 mmol) and 4-pyrrolidinopyridine (320 mg, 2.16 mmol) and azeotropically dried with benzene (3 × 2 mL). The mixture was evacuated under high vacuum, purged with argon, dissolved in dichloromethane (7 mL), and cooled to −30 °C before addition of diisopropylcarbodiimide (0.56 mL, 3.6 mmol). The solution was stirred for 1 h at −20 °C and then stored in the freezer overnight. After 20 h total, the reaction was quenched with H_2O (0.10 mL) and warmed to room temperature. After 30 min of vigorous stirring, the reaction mixture was extracted with EtOAc (2 × 25 mL). The organic extracts were combined, dried over $MgSO_4$, filtered, concentrated, and purified by flash chromatography (10:4:1 hexane/CH_2Cl_2/Et_2O) to yield 504 mg (91.6%) of the pipecolinate ester as a 1.1:1 mixture of rotamers. Data for major rotamer: $[\alpha]^{23}_D$ −39.1° (*c* 2.71, $CHCl_3$); IR (film) 2936, 2867, 1737, 1695, 1612, 1247, 1152, 1110, 730; ^1H NMR (500 MHz, $CDCl_3$) δ 7.23 (d, J = 8.7 Hz, 2 H), 6.86 (d, J = 8.7 Hz, 2 H), 5.19 [5.10] (d, J = 9.0 Hz, 1 H), 5.09 (s, 1 H), 4.89 [4.73] (mult, 1 H), 4.36 (s, 2 H), 4.04 [3.90] (d, J = 12.7 Hz, 1 H), 3.79 (s, 3 H), 3.56–3.53 (ddd, J = 4.8, 8.4, 13.1 Hz, 1 H), 3.37 (s, 3 H), 3.18 (d, J = 7.2 Hz, 1 H), 3.11 (d, J = 7.2 Hz, 1 H), 3.01–2.8 (mult, 3 H), 2.3–2.1 (mult, 3 H), 2.05–1.88 (3 H), 1.68 (s, 3 H), 1.65–1.5 (mult, 3 H), 1.47 (s, 9 H), 1.23–1.1 (mult, 2 H), 1.03 (s, 9 H), 0.9 (s, 3 H), 0.82 (s, 6 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 133.8, 129.0, 128.9, 113.6, 74.9, 72.8, 57.4, 55.2, 36.0, 35.0, 28.4, 18.1, 18.0, 12.6; HRMS calcd for $C_{43}H_{73}NO_8SiNa$ (M + Na) 782.500, found (FAB) 782.500.

[1(1*R*,3*R*,4*R*),1*E*,3(2*S*),3*S*]-3-[[[*N*-(*tert*-Butoxycarbonyl)piperidin-2-yl]carbonyl]oxy]-1-[3-methoxy-4-[(triisopropylsilyl)oxy]cyclohex-1-yl]-2,4,4-trimethyl-1-penten-5-ol. A mixture of the *p*-methoxybenzyl ether described above (300 mg, 0.39 mmol) and DDQ (109 mg, 0.47 mmol) were stirred in 4 mL of wet dichloromethane. (Wet dichloromethane was prepared by vigorous shaking with 1 mL of H_2O.) As the reaction progressed, the solution turned from green to orange with formation of a dark orange precipitate. The entire reaction mixture was loaded directly on to a silica gel column and purified by flash chromatography (10:1 CH_2Cl_2/Et_2O) to yield 201 mg (80.5%) of the primary alcohol as a 1.2:1 mixture of rotamers. Data for major rotamer: $[\alpha]^{23}_D$ −51.4° (*c* 0.810, $CHCl_3$); IR (film) 3467 (br), 2936, 2867, 1737, 1689, 1159, 1117, 730; ^1H NMR (500 MHz, $CDCl_3$) δ 5.22 [5.20] (d, J = 7.6 Hz, 1 H), 5.07 [5.11] (s, 1 H), 4.83 [4.73] (d, J = 3.8, 1 H), 3.90 [4.05] (d, J = 11.4 Hz, 1 H), 3.57–3.52 (mult, 1 H), 3.37 (s, 3 H), 3.20 (d, J = 11.3, 1 H) 3.01–2.91 (mult, 3 H), 2.21–2.16 (mult, 4 H), 2.0–1.9 (mult, 3 H), 1.69 (s, 3 H), 1.66–1.49 (mult, 4 H), 1.45 [1.42] (s, 9 H), 1.38–1.09

(mult, 2 H), 1.06 (s, 18 H), 0.90 (s, 3 H), 0.85 (s, 6 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.0, 171.7, 155.9, 155.2, 134.7, 134.4, 130.6, 130.5, 90.2, 84.3, 82.2, 82.0, 80.0, 74.8, 69.4, 69.3, 57.5, 57.4, 54.8, 53.8, 42.3, 41.1, 40.0, 39.8, 35.8, 34.9, 33.9, 30.4, 28.4, 28.3, 26.9, 26.6, 24.8, 24.6, 22.0, 21.8, 20.7, 20.4, 20.3, 18.1, 15.1, 12.8, 12.6, 12.4; HRMS calcd for $C_{35}H_{65}NO_7SiNa$ (M + Na) 662.443, found (FAB) 662.442.

[1(1*R*,3*R*,4*R*),1*E*,3(2*S*),3*S*]-3-[(Piperidin-2-ylcarbonyl)oxy]-5-[(trimethylsilyl)oxy]-1-[3-methoxy-4-[(triisopropylsilyl)oxy]cyclohex-1-yl]-2,4,4-trimethyl-1-pentene (3). To a cooled (0 °C) solution of the primary alcohol described above (121 mg, 0.19 mmol) and 2,6-lutidine (0.088 mL, 0.76 mmol) in 3 mL of dichloromethane was added trimethylsilyl triflate (0.090 mL, 0.48 mmol). The reaction was stirred at 0 °C for 30 min and then quenched with MeOH (0.25 mL). After concentration, the reaction mixture was loaded with 0.5 mL of dichloromethane on to a silica gel column and aged for 5 min before elution with 4% MeOH in dichloromethane to yield 94 mg (80.8%) of **3**: $[\alpha]^{23}_D$ −27.0° (*c* 0.420, $CHCl_3$); IR (film) 2937, 2866, 1740, 1251, 1107, 879; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.17 (d, *J* = 9.1 Hz, 1 H), 4.99 (s, 1 H), 3.56–3.50 (ddd, *J* = 4.8, 8.4, 13.1 Hz, 1 H), 3.35–3.33 (mult, 1 H), 3.37 (s, 3 H), 3.47 (d, *J* = 9.7 Hz, 1 H), 3.19 (d, *J* = 9.7 Hz, 1 H), 3.08–3.05 (mult, 1 H), 2.98–2.92 (mult, 1 H), 2.69–2.63 (mult, 1 H), 2.24–2.21 (mult, 1 H), 2.01–1.88 (mult, 5 H), 1.77–1.75 (mult, 1 H), 1.65 (s, 3 H), 1.58–1.34 (mult, 6 H), 1.06 (s, 18 H), 1.04–0.94 (mult, 3 H), 0.87 (s, 3 H), 0.81 (s, 3 H), 0.06 (s, 9 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.5, 133.5, 130.9, 120.0, 84.3, 81.1, 74.8, 68.7, 58.4, 57.3, 45.5, 39.6, 35.6, 34.9, 34.0, 30.5, 29.2, 25.8, 24.1, 21.1, 20.7, 18.1, 18.0, 15.1, 12.6; HRMS calcd for $C_{33}H_{65}NO_5Si_2Na$ (M + Na) 634.430, found (FAB) 634.432.

Phosphonoacetate Precursor to 17. To a cooled (−20 °C) solution of **2** (216 mg, 0.28 mmol) in a 4:1:1 mixture of THF/MeOH/H_2O was added 35.7 mg (0.85 mmol) of LiOH. The resulting heterogeneous solution was maintained in a temperature range of −15 °C to −10 °C for 2 h and then acidified with 0.1 N HCl to a pH of 6. During acidification, the reaction temperature was kept as low as possible without allowing the solution to freeze. The resulting mixture was extracted with EtOAc and the organic solution dried over $MgSO_4$, filtered, and concentrated to give 197 mg (90.8%) of the free acid. Without further purification, the acid was azeotroped with benzene (2 × 5 mL), dissolved in 2 mL of dichloromethane, and treated with *N*-methylmorpholine (0.184 mL, 1.68 mmol) and (benzotriazolyl-1-oxy)tris(dimethylamino)phosphonium hexafluorophosphate (371 mg, 0.84 mmol). A solution of **3** (171 mg, 0.279 mmol) in 0.5 mL of dichloromethane was added, and the resulting solution was stirred at ambient temperature for 3 h. The reaction mixture was diluted with EtOAc (40 mL), washed with 5% HCl (1 × 5 mL), saturated aqueous $NaHCO_3$ (2 × 10 mL), H_2O (1 × 10 mL), and brine (1 × 10 mL). The organic solution was concentrated, taken up in 5 mL of THF, and treated with 10% HCl (1.0 mL) for 5 min to remove the trimethylsilyl ether. The reaction mixture was quenched with saturated $NaHCO_3$ (5 mL) and extracted with EtOAc (2 × 30 mL). The organic extracts were dried ($MgSO_4$), concentrated, and purified by flash chromatography (50% EtOAc in hexanes) to yield 285 mg (86.7%) of coupled phosphonoacetate–alcohol, which exists as a 2:1 mixture of rotamers. Data for the major rotamer: $[\alpha]^{23}_D$ −32.8° (*c* 0.305, $CHCl_3$); IR (film) 3441, 2937, 2868, 1733, 1648, 1617, 1248, 1111, 911, 726; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40–7.37 (d, *J* = 8.7, 2 H), 6.87–6.84 (d, *J* = 8.7, 2 H), 5.77 [5.49] (s, 1 H), 5.28 [4.95] (d, *J* = 4.4, 1 H), 5.21 [5.11] (d, *J* = 8.6, 1 H), 5.07 [4.68] (s, 1 H), 4.80 [4.71] (dd, *J* = 5.0, 3.6, 1 H), 4.67 (t, *J* = 6.6, 1 H), 4.48 [4.51] (d, *J* = 6.4, 1 H), 4.18–4.11 (mult, 4 H), 3.80–3.84 (mult, 1 H), 3.78 (s, 3 H), 3.54–3.44 (mult, 2 H), 3.37 (s, 3 H), 3.36 (s, 3 H), 3.26 (s, 3 H), 3.25–3.11 (mult, 3 H), 2.97–2.85 (mult, 4 H), 2.24–2.18 (mult, 2 H), 2.04 (mult, 1 H), 1.99–1.89 (mult, 3 H), 1.68 (s, 3 H), 1.68–1.35 (mult, 4 H), 1.35–1.29 (mult, 6 H), 1.2–1.0 (mult, 18 H), 0.95–0.85 (mult, 3 H), 0.85–0.75 (mult, 12 H), 0.74 (d, *J* = 6.9, 3 H), 0.62 (d, *J* = 14.3, 3 H), 0.0 (s, 6 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.6, 170.5, 169.6, 165.1, 160.7, 160.5, 134.3, 131.0, 130.7, 129.1, 128.3, 1130.5, 103.7, 84.2, 84.1, 83.6, 82.4, 82.02, 81.7, 81.1, 80.0, 79.5, 75.8, 75.3, 74.1, 73.0, 72.8, 69.2, 62.5, 60.7, 57.4, 56.8, 55.2, 52.6, 43.3, 39.9, 39.4, 35.9, 34.9, 34.8, 34.2, 33.9, 33.4, 32.9, 30.5, 28.5, 27.9, 26.5, 25.9, 25.2, 24.7, 21.9, 21.6, 20.8, 20.2, 20.0, 19.7, 18.4, 18.1, 18.0, 17.9, 17.7, 16.3, 16.3, 15.1, 12.5; HRMS calcd for $C_{65}H_{116}NO_{17}PSi_2Na$ (M + Na) 1292.742, found (FAB) 1292.749.

(9*S*,10*R*)-14-[(*tert*-Butyldimethylsilyl)oxy]-9,10-[[(4-methoxyphenyl)methylene]dioxy]-9,10-tetrahydro-27-[(triisopropylsilyl)oxy]-506BD (17). A mixture of the alcohol described above (285 mg, 0.223 mmol) and Dess–Martin periodinane (190 mg, 0.466 mmol) was stirred in 3 mL of dichloromethane for 1.5 h. The excess periodinane was quenched by addition of 1 mL of a 5% $Na_2S_2O_3$ solution, followed by vigorous stirring of the biphasic mixture for 10 min. The reaction mixture was diluted with EtOAc (30 mL) and washed successively with 5% $Na_2S_2O_3$ (5 mL), saturated $NaHCO_3$ (2 × 5 mL), H_2O (1 × 5 mL), and brine (1 × 5 mL). The organic solution was dried over $MgSO_4$, concentrated, and taken up in 200 mL of acetonitrile. To this dilute solution of aldehyde was added 94 mg (2.2 mmol) of dry LiCl and 0.33 mL (2.2 mmol) of diazobicycloundecene. The resulting white cloudy solution was allowed to react for 2.5 h, before being diluted with EtOAc (350 mL) and washed with 5% HCl (2 × 50 mL), saturated $NaHCO_3$ (2 × 50 mL), and brine (1 × 50 mL). The organic layer was dried over $MgSO_4$, concentrated, and purified by flash chromatography (10%, 15%, then 20% EtOAc in hexanes) to give 168 mg (67%) of **17** as a mixture of acetal diastereomers: $[\alpha]^{23}_D$ −58.3° (*c* 0.40, $CHCl_3$); IR (thin film) 2936, 2867, 1737, 1710, 1647, 1616, 1255, 1107, 739; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42 (d, *J* = 8.7, 2 H), 7.09 (d, *J* = 16.0, 1 H), 6.89 (d, *J* = 8.7, 2 H), 5.74–5.71 (d, *J* = 16.0, 1 H), 5.69 (s, 1 H), 5.29 (d, *J* = 5.2, 1 H), 5.25 (d, *J* = 8.9, 1 H), 4.99 (s, 1 H), 4.91 (d, *J* = 5.3, 1 H), 4.81 (s, 1 H), 4.48 (d, *J* = 5.9, 1 H), 4.36 (d, *J* = 2.8, 1 H), 3.90 (mult, 1 H), 3.80 (s, 3 H), 3.57–3.53 (mult, 1 H), 3.45 (s, 3 H), 3.37 (s, 3 H), 3.29 (s, 3 H), 3.11 (mult, 1 H), 3.05 (t, *J* = 11.9, 1 H), 2.98–2.93 (mult, 1 H), 2.28–2.21 (mult, 3 H), 2.02 (mult, 2 H), 1.94–1.91 (mult, 1 H), 1.73–1.53 (mult, 6 H), 1.57 (s, 3 H), 1.47–1.37 (mult, 4 H), 1.21 (d, *J* = 6.9, 3 H), 1.06 (s, 18 H), 0.97 (d, *J* = 6.9, 3 H), 0.94 (s, 9 H), 0.92 (mult, 15 H), 0.11 (s, 3 H), 0.08 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.0, 169.2, 166.1, 160.6, 154.9, 129.4, 128.8, 128.4, 113.7, 103.3, 84.2, 74.7, 57.3, 55.2, 52.9, 43.8, 40.9, 35.6, 34.9, 33.9, 30.5, 28.5, 26.3, 26.1, 26.0, 25.3, 21.1, 20.4, 18.4, 18.1, 18.0, 12.3; HRMS calcd for $C_{61}H_{103}NO_{13}Si_2Na$ (M + Na) 1136.687, found (FAB) 1136.684.

(9*S*,10*R*)-14-[(*tert*-Butyldimethylsilyl)oxy]-9,10-tetrahydro-27-[(triisopropylsilyl)oxy]-506BD. To a cooled (−78 °C) solution of **17** (85 mg, 0.083 mmol) in 2 mL of dichloromethane was added diethylaluminum chloride (0.25 mL of a 1.0 M solution in dichloromethane) followed by propanedithiol (0.025 mL, 0.25 mmol). The cold bath was removed and after the reaction proceeded at ambient temperature for 4 h, additional portions of Et_2AlCl (0.05 mL of 1.0 M solution) and propanedithiol (0.005 mL) were added. After a total reaction time of 5 h, the reaction was quenched with H_2O (0.5 mL), diluted with EtOAc (4 mL), and treated with saturated Rochelle's salt (2 mL). The resulting biphasic mixture was separated, dried ($MgSO_4$), and concentrated. The excess propanedithiol was removed in vacuo, and the resulting oil was subjected to flash chromatography (2% EtOAc in hexanes then 1% MeOH and 30% EtOAc in hexane) to give 51 mg (67.5%) of the resultant diol as a mixture of rotamers: $[\alpha]^{23}_D$ −69.5° (*c* 0.325, $CHCl_3$); IR (film) 3467 (br), 29.5, 2867, 1721, 1647, 1462, 1115, 730; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.16–7.13 (d, *J* = 15.8 Hz, 1 H), 5.77–5.74 (d, *J* = 15.8 Hz, 1 H), 5.46 (d, *J* = 4.7 Hz, 1 H), 5.25 (d, *J* = 8.7 Hz, 1 H), 4.99 (s, 1 H), 4.8 (mult, 1 H), 4.56 (d, *J* = 7.4 lHz, 1 H), 3.88 (mult, 1 H), 3.81 (d, *J* = 7.9 Hz, 1 H), 3.70 (d, *J* = 2.8, 1 H), 3.57–3.52 (mult, 2 H), 3.45 (s, 3 H), 3.36 (s, 3 H), 3.17 (mult, 1 H), 3.11 (mult, 1 H), 2.24–1.90 (mult, 6 H), 1.74–1.63 (mult, 3 H), 1.60 (s, 3 H), 1.54–1.33 (mult, 4 H), 1.06 (s, 18 H), 0.97 (d, *J* = 6.8, 3 H), 0.92–0.89 (mult, 6 H), 0.90 (s, 6 H), 0.07 (s, 3 H), 0.06 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.3, 136.4, 129.6, 118.1, 85.7, 84.2, 74.7, 68.2, 57.4, 52.6, 43.4, 40.5, 35.6, 34.9, 33.9, 30.5, 29.6, 28.6, 26.3, 25.9, 25.4, 25.3, 22.6, 20.6, 20.1, 18.4, 18.1, 18.0, 14.3, 12.6, −4.5, −4.8; HRMS calcd for $C_{53}H_{97}NO_2Si_2Na$ (M + Na) 1018.645, found (FAB) 1018.650.

14-[(*tert*-Butyldimethylsilyl)oxy]-27-[(triisopropylsilyl)oxy]-506BD. To a −78 °C solution of DMSO (0.046 mL, 0.65 mmol) in 1.5 mL of dichloromethane was added trifluoroacetic anhydride (0.046 mL, 0.33 mmol). After 15 min, the diol derived from **17** (described above) (66 mg, 0.065 mmol) was added in 0.5 mL of dichloromethane via syringe with two 0.1-mL rinses. The resulting solution was stirred for 1 h and then treated with triethylamine (0.23 mL, 1.63 mmol). The resulting yellow solution was warmed to room temperature and stirring continued for 10 min. The reaction was quenched by the addition of 1 mL of H_2O. EtOAc was added (20 mL) and the organic layer was separated and dried over $MgSO_4$. After filtration and concentration, the crude product was purified by flash chromatography (10% EtOAc in hexanes) to yield 41.8 mg (64%) of a bright yellow oil, which was a mixture of the desired tricarbonyl and the hydrate as well as 10.3 mg of monooxidized product. Data listed for major rotamer (3:1 mixture) of the tricarbonyl product: $[\alpha]^{23}_D$ −85.0° (*c* 0.060, $CHCl_3$); IR ($CHCl_3$) 2934, 2866, 1720, 1649, 1462, 1109; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.97 [6.91] (d, *J* = 15.9, 1 H), 5.63 [5.80] (d, *J* = 15.9, 1 H), 5.17 (d, *J* = 8.9, 1 H), 5.13 (d, *J* = 5.0, 1 H), 5.03 (dd, *J* = 7.1, 7.1, 1 H), 4.98 [4.85] (s, 1 H), 4.40 (d, *J* = 13.2, 1 H), 3.85 (d, *J* = 9.0, 1 H), 3.45–3.43 (mult, 1 H), 3.36 (s, 3 H), 3.33–3.26 (mult, 1 H), 3.26 (s, 3 H), 3.14–3.08 (mult, 1 H), 2.96 (s, 3 H), 2.85–2.81 (mult, 1 H), 2.28 (d, *J* = 9.1, 1 H), 2.11–2.07 (mult, 3 H), 2.04–1.91 (mult, 2 H), 1.83–1.80 (mult, 1 H), 1.73 (d, *J* = 13.5, 1 H), 1.65–1.60 (mult, 2 H), 1.52 (s, 3 H), 1.44–1.40 (mult, 2 H), 1.23–0.71 (mult, 54 H), 0.12 (s, 3 H), 0.10 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 200.0, 184.8, 169.3, 166.0, 153.1, 136.7, 129.4, 128.3, 120.2, 119.6, 86.9, 84.2, 74.7, 60.7, 57.2, 56.2, 51.9, 44.1, 40.8, 35.5, 34.9, 33.9, 30.4, 28.6, 26.3, 26.1, 26.0, 25.5, 25.1, 24.8, 21.5, 20.2, 18.6, 18.2, 18.0,

17.2, 14.3, 12.6; HRMS calcd for $C_{53}H_{93}NO_{12}Si_2Na$ (M + Na) 1014.613, found (FAB) 1014.610.

506BD (1). To a solution of the tricarbonyl described above (18.3 mg, 18.3 mmol) in 1 mL of acetonitrile was added 1 mL of a 48% solution of HF in acetonitrile. After 1 h the reaction was quenched by adding the reaction mixture to a stirring, biphasic mixture of ether (10 mL) and saturated aqueous $NaHCO_3$ (10 mL). The ether layer was washed with three additional portions of $NaHCO_3$, dried over $MgSO_4$, filtered, and concentrated. Flash chromatography (70% EtOAc in hexane) gave 8 mg of a mixture of **1** and the seven-membered ring hemiacetal isomer (resulting from cyclization of the secondary alcohol onto the C9 ketone, hereafter referred to as **iso-1**) enriched in **1** as well as 4 mg of a mixture enriched in **iso-1**. The hemiacetal isomers were separated by HPLC on a 10 mm × 25 cm silica gel column (2-mg portions, 5.0 mL/min, 254-nm wavelength detector, 40% EtOAc in hexane) to give 5 mg of pure **1** (retention time = 7.2 min) and 2.5 mg of pure **iso-1** (retention time = 9.6 min). Data for **1**: $[\alpha]^{23}_D$ -45.8° (*c* 0.085, $CHCl_3$); IR (film) 3480 (br), 2930, 2856, 1734, 1716, 1651, 1633, 1456, 1269, 1230, 1095; ^1H NMR (500 MHz, $CDCl_3$) δ 6.84-6.81 [6.96-6.91] (d, *J* = 16.1, 1 H, C20), 5.61-5.58 [5.78-5.75] (d, *J* = 16.1, 1 H, C19), 5.39 (s, 1 H, OH of hemiacetal), 5.15-5.12 [5.19] (dd, *J* = 10.1, 4.1, 1 H, C16), 5.13 [5.21] (s, 1 H, C2), 5.04 (d, *J* = 9.2, 1 H, C24), 4.82 (s, 1 H, C22), 4.52-4.49 [3.65] (d, *J* = 13.2, 1 H, C6), 4.09-4.07 (dd, *J* = 9.5, 1.3, 1 H, C14), 3.69 (dd, *J* = 3.0, 1.4, 1 H, C15), 3.45-3.30 (mult, 2 H, C13, C27), 3.37 (s, 3 H), 3.36 (s, 3 H), 3.35 (s, 3 H), 2.97-2.92 (mult, 1 H, C28), 2.86-2.81 (t, *J* = 13.6, 1 H, C6), 2.39-2.16 (mult, 2 H), 2.15-2.06 (mult, 2 H), 2.05-1.99 (mult, 1 H), 1.98-1.88 (mult, 1 H), 1.82-1.65 (mult, 2 H), 1.61 (s, 3 H), 1.58-1.53 (mult, 1 H), 1.42-1.52 (mult, 2 H), 1.41-1.26 (mult, 2 H), 1.25-1.02 (mult, 4 H), 1.1 (s, 3 H), 1.0 (s, 3 H), 0.97 (d, *J* = 6.7, 3 H), 0.93-0.91 (mult, 6 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.8, 169.2, 166.5, 150.9, 135.7, 130.1, 129.9, 121.4, 98.6, 86.1, 84.3, 84.1, 75.9, 75.1, 75.0, 74.0, 73.5, 70.9, 58.1, 56.6, 56.4, 56.2, 41.1, 40.7, 40.2, 35.2, 34.3, 34.0, 33.5, 32.4, 31.2, 31.1, 30.2, 30.0, 29.7, 28.8, 26.8, 26.7, 24.4, 23.3, 20.6, 19.8, 18.9, 16.4, 15.5; HRMS calcd for $C_{38}H_{59}NO_{12}Na$ (M + Na) 744.393, found (FAB) 744.395. Data for **iso-1**: $[\alpha]^{23}_D$ -45.8° (*c* 0.085, $CHCl_3$); IR (film) 2930, 2856, 1728, 1709, 1633, 1456, 1269, 1230, 1095; ^1H NMR (500 MHz, $CDCl_3$) δ 7.12-7.08 (d, *J* = 16.1, 1 H, C20), 5.67-5.63 (d, *J* = 16.1, 1 H, C19), 5.22 (s, 1 H, OH of hemiacetal), 5.24-5.21 (dd, *J* = 2.2, 10.0, 1 H, C16), 5.10-5.07 (d, *J* = 9.9 Hz, 1 H, C24), 5.03 (s, 1 H, C22), 4.65-4.62 (d, *J* = 12.9, 1 H, C6), 3.49-3.47 (d, *J* = 9.9, 1 H, C14), 3.46-3.43 (d, *J* = 9.9, 1 H, C15), 3.36 (s, 3 H), 3.29 (s, 3 H), 3.28 (s, 3 H), 3.26-3.20 (mult, 1 H, C13), 3.19-3.09 (mult, 1 H, C6), 2.99-2.94 (mult, 1 H, C11), 2.88-2.83 (mult, 1 H, C27), 2.59 (s, 1 H), 2.3-1.89 (mult, 10 H), 1.73-1.61 (mult, 2 H), 1.52-0.72 (mult, 16 H), 1.38 (s, 3 H), 1.05 (s, 3 H), 0.94 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 211.2, 165.6, 152.9, 135.9, 129.9, 120.2, 98.7, 86.8, 84.0, 83.2, 78.1, 76.4, 74.8, 73.5, 59.1, 56.8, 56.5, 52.5, 44.1, 40.4, 40.2, 38.1, 35.1, 34.3, 31.1, 30.2, 29.7, 29.5, 27.2, 25.3, 22.9, 21.5, 19.7, 16.6, 15.6; HRMS calcd for $C_{38}H_{59}NO_{12}Na$ (M + Na) 744.393, found (FAB) 744.393.

Acknowledgment. We thank the NIGMS (GM-38627) for support of this research. A National Science Foundation Predoctoral Fellowship to T.J.W. and an American Cancer Society Fellowship to P.K.S. are gratefully acknowledged. We especially thank Professor Jon Clardy and Dr. G. D. Van Duyne for their important contributions to the FKBP ligand studies and for the X-ray structure determination of compound **8a**.

Note Added in Proof. The Ca^{2+}, calmodulin-dependent protein phosphatase calcineurin (PP2B) has recently been shown to be a common target of cyclophilin-CSA and FKBP-FK506 complexes in mammalian cells: Liu, J.; Farmer, J. D.; Lane, W. S.; Friedman, J.; Weissman, I.; Schreiber, S. L. *Cell* **1991**, *66*, 807-815.

Supplementary Material Available: Tables of crystal data, atomic coordinates, bond lengths and angles, and isotropic and anisotropic displacement coefficients (7 pages); tables of observed and calculated structure factors (5 pages). Ordering information is given on any current masthead page.

Secondary H/D Isotope Effects in Methyl-Transfer Reactions Decrease with Increasing Looseness of the Transition Structure

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Abstract: The barriers of identity gas-phase methyl-transfer reactions $X^- + CH_3X \rightarrow XCH_3 + X^-$ are directly related to the looseness of their transition structures, and inversely related to the secondary kinetic isotope effects in the methyl group ($k_H/k_D < 1$). This means that the isotope effects decrease with increasing looseness of the transition structure, in contrast to current belief. Similar trends are observed at the 4-31G, 6-31+G*, and MP2/6-31+G* computational levels, and at each level, the C-H bond lengths of the transition structures do not depend upon X. The isotope effects are inverse primarily as a result of the increase in C-H (C-D) stretching frequencies (C-H (C-D) bond shortening) that accompanies the tetrahedral to trigonal change in geometry. Because of the constant C-H bond lengths in the transition structures, the largest isotope effect (smallest k_H/k_D) is seen with the substrate having the longest C-H bond. The contributions to the isotope effects from the complementary changes in the C-H (C-D) bending vibrations along the reaction coordinates are normal.

Introduction

There are interesting correlations between the energy changes along the reaction coordinates of S_N2 reactions, eq 1, and the changes in geometry that attend these reactions.[1] In the gas phase, the reaction coordinate is double-welled,[2] because of the presence of stable reactant and product ion-molecule complexes ($X^-\cdots CH_3Y$ and $XCH_3\cdots Y^-$).

$$X^- + CH_3Y \rightarrow XCH_3 + Y^- \quad (1)$$

For identity reactions (X = Y), ΔE^*, the central barrier computed at the 4-31G level[3] is linearly related to ΔE_{def}, the energy required

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‡ Holder of a Canada Council Killam Research Fellowship, 1990-1992.

(1) (a) Mitchell, D. J.; Schlegel, H. B.; Shaik, S. S.; Wolfe, S. *Can. J. Chem.* **1985**, *63*, 1642. (b) Shaik, S. S.; Schlegel, H. B.; Wolfe, S. *J. Chem. Soc., Chem. Commun.* **1988**, 1322.

(2) Brauman, J. I.; Olmstead, W. N.; Lieder, C. A. *J. Am. Chem. Soc.* **1974**, *96*, 4030. Farneth, W. E.; Brauman, J. I. *Ibid.* **1976**, *98*, 7891. Olmstead, W. N.; Brauman, J. I. *Ibid.* **1977**, *99*, 4219.

(3) Mitchell, D. J. Ph.D. Thesis, Queen's University, Kingston, Ontario, 1981.

0002-7863/91/1513-8056$02.50/0