hexadiene[13]), was ineffective at dimer splitting in preliminary experiments (CH_3CN; λ_{irrad} = 436 nm), possibly because of a need for the deprotonated form of the reduced flavin.[14]

Dimer photosplitting by **1b** and **1c** in low-polarity environments demonstrates the feasibility of pyrimidine dimer recognition for the purpose of dimer repair. Dimers apparently engage in Watson–Crick hydrogen bonding to their complementary bases when formed in double-stranded DNA,[15] but there is currently no evidence regarding hydrogen bonding of photolyases to dimers in single- or double-stranded DNA. Whether photolyases utilize hydrogen bonding in the recognition, orienting, and splitting of thymine dimers in DNA awaits further studies of the natural system.

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X-ray Crystal Structure of the HIV Protease Complex with L-700,417, an Inhibitor with Pseudo C_2 Symmetry

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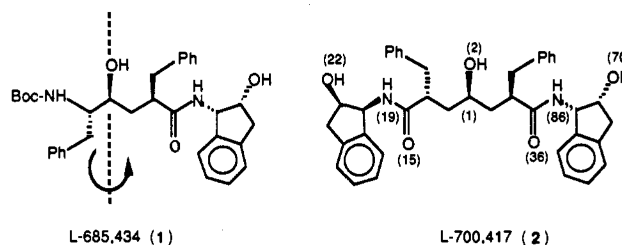
The human immunodeficiency virus type 1 protease (HIVP) is responsible for the processing of the polyprotein products of the *gag* and *pol* genes into their mature forms.[1,2] The enzyme is an aspartyl protease and is active as a dimer with the active site shared between subunits.[3–5] If the action of the protease is inhibited or blocked genetically, virus infection can be arrested.[6–8] Because of the essential role that the protease plays in virus maturation, it appears to be an excellent target for the development of an antiviral agent.

Among the most potent inhibitors of aspartyl proteases are compounds containing a central secondary alcohol which interacts with two catalytic Asp residues,[9] and inclusion of such a hydroxyl group into any inhibitor design would be desirable. Other desirable features in an HIVP inhibitor would include hydrophobic substituents to project into the specificity pockets of the enzyme[10] and hydrogen-bond donors to interact with the carbonyl of Gly 27 which projects up from the floor of the active site from each subunit.[11,12] Because the native enzyme can adopt a conformation in which a 2-fold symmetry axis extends through the active site,[3–5] it might also be desirable to include approximate C_2 symmetry through the central alcohol of inhibitors. Compounds which take advantage of this C_2 symmetry have recently been reported by Erickson et al.[13] Using a similar strategy, we have designed a new set of pseudosymmetric inhibitors based upon L-685,434,[14] (**1**), a hydroxyethylene-containing HIVP inhibitor which utilizes the novel 2-hydroxy-1-aminoindan as a P_2' amino acid[15] replacement. Rotation of the C-terminal half of **1** around the



central hydroxyl-bearing carbon led to the design of L-700,417[16] (**2**). Compound **2** inhibits HIVP activity[17] with an IC_{50} of 0.67 nM and was active in halting the spread of HIV-1 (IIIB) infection in human T-lymphoid cell culture[18] with a minimal 100% inhibitory concentration of 100 nM. To further evaluate and improve on the inhibitor design, we have determined the high-resolution crystal structure of the complex of **2** with HIVP.

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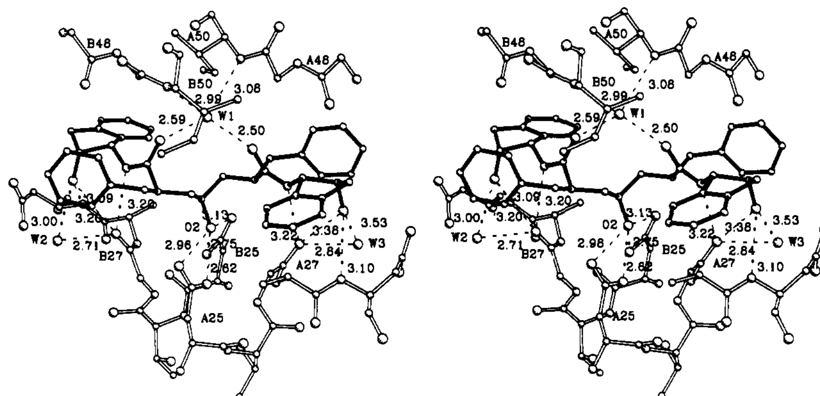

Figure 1. Stereodrawing of a ball and stick figure of selected active-site residues of the final refined structure of the complex of HIVP with **2**. The enzyme is shown in open bonds, the inhibitor in closed bonds, and dotted lines indicate possible intermolecular hydrogen bonds. Interatomic distances are shown for possible hydrogen bonds.

Cocrystals of HIVP and **2**[19] were isomorphous with crystals of the HIVP–acetylpepstatin (HIVP–APS) complex,[11] indicating that the assumption of asymmetry by the protease in enzyme–inhibitor complexes is not a consequence of the binding of an asymmetric inhibitor. Subunit asymmetry appears to arise from inherent asymmetric modes of flexibility which are driven by the clash of residues A50 and B50 in conformations with exact subunit symmetry.[3-5,11] Difference electron density maps[20] clearly indicated that **2** was bound by the protease at the active site with high occupancy. However, because of the symmetric nature of the inhibitor, it could be fit[21] into the difference electron density in either of two orientations. The orientations differ by rotation of the inhibitor in the active site by 180° about an approximate 2-fold axis which relates the two HIVP subunits; after refinement, atomic positions in the orientations differ significantly only at O2 and C1 of the central alcohol. Because each refined orientation fits the electron density map equally well and because difference maps do not distinguish between the possibilities, it is likely that both orientations are present in the crystal structure.[22] After refinement,[19] the crystallographic R factor for the comparison of observed and calculated structure factors was 0.18 with root-mean-square deviations from ideal bond and angle distances of 0.015 Å and 0.037 Å.[23]

Several intermolecular hydrogen bonds are formed between **2** and HIVP and contribute to the stability of the complex. The central hydroxyl group (O2) is within hydrogen-bonding distance of each of the carboxylate oxygens of both Asp A25[24] and Asp B25 (Figure 1). Hydrogen bonds are also donated from the amide (N19 and N86) and hydroxyl (O22 and O70) groups of the indanol amide residues to the carbonyl oxygens of Gly A27 and Gly B27, though these appear to be poor hydrogen bonds. The indanol hydroxyl groups also accept hydrogen bonds from the amide groups of Asp A29 and Asp B29. Additional hydrogen bonds which may contribute to complex stability are formed to three trapped moelcules of solvent that form hydrogen bonds to the protein as well as the inhibitor. Hydrogen bonds are formed between the carbonyl oxygens of the inhibitor (O15 and O36) and water W1 and then from the water molecule to the amide nitrogens of residues A50 and B50 with nearly ideal tetrahedral geometry (Figure 1). Water molecules W2 and W3, which donate hydrogen bonds to the hydroxyl groups of the indanols (O22 and O70), also donate hydrogen bonds to the carbonyl groups of Gly B27 and Gly A27. Distances and angles are not optimal for all five of the hydrogen bonds to each indanol of the inhibitor, suggesting that the energetic contributions of the interactions are individually of very low energy or that not all of the interactions occur simultaneously. Inhibitors might be improved if these solvent sites could be replaced by inhibitor substituents.

In contrast to the relatively poor hydrogen-bonding interactions, hydrophobic interactions are extensive. The amount of hydrophobic surface removed from solvent upon complex formation[25,26] is 792 $Å^2$ and could contribute as much as 19 kcal/mol toward complex stability.[27] The phenyl rings of the inhibitor project into the P_1/P_1' binding sites on the protease, where they pack against Leu A23, Val A80, Pro A81, Val A82, and Ile A84 in one subsite and residues B23 and B80–B84 in the other subsite. Residues B80–B84 adjust by up to 1 Å relative to their positions in the HIVP–APS complex,[11] apparently to accommodate the separation between phenyl rings in the inhibitor. The indan rings project into the P_2/P_2' binding sites and pack against the side chains of Ala B28, Val B32, Ile B47, Ile B50, Leu B76, and Ile B84 and the main-chain portion of residues B28–B32 in one subsite and against the same residues from the A chain in the other subsite. Efficient packing of the inhibitor into the active site succeeds in removing 94% of the hydrophobic surface of the inhibitor from bulk solvent.

The structures of protease complexes with APS[11] and other ligands[12,13,28,29] represent HIVP complexes with inhibitors that are primarily peptidyl, while **2** retains very little peptide character. One disadvantage of the nonpeptidyl design of **2** is that the carbonyl oxygen of Gly 48 on each HIVP subunit is buried upon inhibitor binding and is not stabilized by hydrogen bonding to either the inhibitor or solvent, though the dipole is aligned directly above the dipole of the indanol hydroxyl group. To alleviate the

(19) The HIVP complex with **2** was cocrystallized (enzyme and inhibitor in a 1:2 ratio) from NaCl by vapor diffusion as described previously for the HIVP–APS complex, and the crystals were isomorphous with the orthorhombic HIVP–APS crystals ($P2_12_12$, a = 58.88 Å, b = 86.80 Å, c = 46.79 Å).[11] Data were collected from a single crystal of enzyme–inhibitor complex using Cu Kα X-rays (λ = 1.5418) and a Siemens multiwire X-ray area detector equipped with a graphite monochromator and processed using the Xengen software (Howard, A. J.; Gilliland, G. L.; Finzel, B. C.; Poulos, T. L.; Ohlendorf, D. H.; Selemme, F. R. *J. Appl. Crystallogr.* **1987**, *20*, 383–387). The data extended to 2.1-Å resolution, was 97.2% complete, had 70% of the intensities observed greater than $2\sigma(I)$, and had an overall R_{merge} equal to 0.07. All measured reflections were used in refinement (Hendrickson, W. A. *Methods Enzymol.* **1985**, *115*, 252–270. Smith, J. L.; Corfield, P. W. R.; Hendrickson, W. A.; Low, B. W. *Acta Crystallogr.* **1988**, *A44*, 357–358).

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unfavorable interaction, conformational adjustments occur in the "flap" region where the main-chain torsional angles ψ_{48} and φ_{49} rotate by as much as 40° relative to their angles in the HIVP-APS complex.[11] This contact with the indan ring is replaced by a hydrogen bond to an amide group in more peptidyl inhibitors,[11-13,28,29] and affinity might be improved by incorporating a substituent capable of donating a hydrogen bond to the carbonyl of Gly 48. In another adjustment, which is only partially successful in optimizing interactions, ψ_{A27} rotates so as to move the carbonyl oxygen of Gly A27 away from the catalytic Asp residues and toward the indanol hydrogen-bond donors. Because the inhibitor appears unable to form good hydrogen bonds with both ends of the active site simultaneously (Figure 1), decreasing the number of bonds separating inhibitor amides might improve inhibitory potency.

The design of **2** was successful in that it produced an inhibitor with high affinity (0.7 nM) which interacts with the protease in a nearly symmetric manner. The indanol ring system is a novel substituent that simultaneously provides good hydrophobic interactions and is involved in as many as three hydrogen bonds with the protein. However, the presence of unsolvated carbonyl groups, unoptimized hydrogen bonding, and several water-mediated interactions between the protein and inhibitor indicate that inhibitors can be improved.

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Stereocontrolled Total Synthesis of (±)-Pentalenolactone P Methyl Ester

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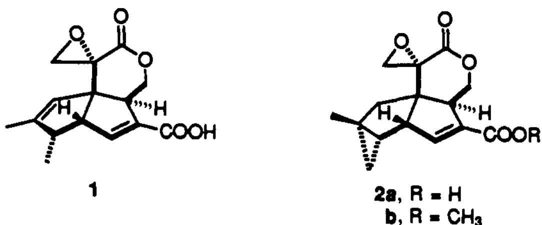
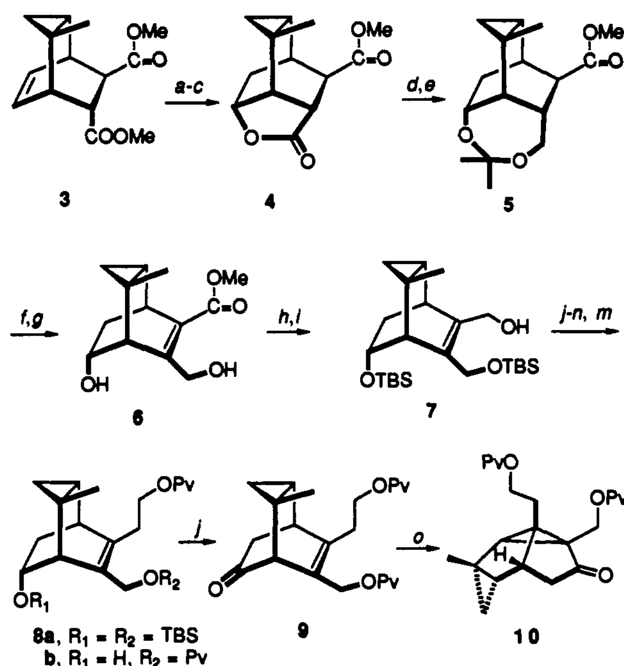
The isolation in 1957[1,2] of pentalenolactone (**1**) has proven to be a harbinger of many exciting discoveries involving *Streptomyces*-derived triquinane sesquiterpenes.[3] Of these, the identification and characterization by Seto et al.[4] of pentalenolactone P (**2a**) could provide the key to our detailed understanding of the biosynthetic origin of this class of antibiotics. Cane et al. have



suggested two possible pathways for the formation of **2a**.[3] From the catabolic perspective, its protonation with rupture of the cyclopropane ring could be the source of a centrally important tertiary carbocation intermediate. Strikingly, pentalenolactone P is the first member of this class to contain a fused three-membered ring, *which is seen to reside on the highly congested concave surface of the molecule.* As a consequence of the latent reactivity of **2a** and its unusual structural features, we have undertaken a stereocontrolled synthesis of this highly functionalized pentacyclic lactone as its stable methyl ester **2b**.

Scheme I



^{a}NaOH, CH_3OH, H_2O, room temperature. ^{b}$Hg(OAc)_2$, MeOH, room temperature, $NaBH_4$, -78 °C. ^{c}CH_2N_2. ^{d}$NaBH_4$, MeOH, room temperature. ^{e}$CH_3C(OMe)_2CH_3$, (TsOH), THF. ^{f}LDA, THF, -78 °C; PhSeCl. ^{g}MCPBA, $NaHCO_3$, CH_2Cl_2, room temperature. ^{h}TBSCl, imid, DMF, room temperature. ^{i}Dibal-H, CH_2Cl_2, -78 °C. ^{j}$(n\text{-}Pr)_4NRuO_4$ (TPAP), 4-methylmorpholine *N*-oxide (NMO), 4-Å sieves, CH_2Cl_2, room temperature. ^{k}$Ph_3P{=}CH_2$, THF, 0 °C → room temperature. ^{l}9-BBN, THF, room temperature; $NaBO_3$. ^{m}PvCl, Et_3N, DMAP, CH_2Cl_2, room temperature. ^{n}HF (48%), CH_3CN, 0 °C. ^{o}$h\nu$, 3000 Å, acetone, room temperature.

The requisite trans relationship of the cyclopropane and lactone components was immediately secured by Diels-Alder addition of fumaroyl chloride to 1-methylcycloheptatriene[5] followed directly by methanolysis to give **3** (80%, Scheme I).[6] Once lactone **4** had been produced (75%),[7] chemoselective distinction between the pair of carbonyl groups was made feasible (87%), leading ultimately to **5** (76%). Despite considerable steric shielding about the carbomethoxy group in **5**, phenylselenenylation of the enolate could be satisfactorily accomplished. Direct oxidation of this intermediate with buffered MCPBA gave dihydroxy ester **6** in an overall yield of 72%. Evidently, the substantial strain introduced upon installation of the conjugated double bond accelerates acetal cleavage and hydrolysis. Silylation of the hydroxyl groups in **6** and ester reduction provided **7** (80%) and set the stage for regioselective chain extension.

Toward this end, perruthenate oxidation[8] of **7** delivered the aldehyde, which was directly subjected to Wittig olefination. This two-step sequence gave rise efficiently (93%) to the conjugated diene. As anticipated, its hydroboration-oxidation[9] afforded **8a** after pivaloylation (87%). Desilylation with HF in acetonitrile at 0 °C[10] and subsequent selective acylation with pivaloyl chloride

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