Inhibition of HIV-1 Protease by a Subunit of Didemnaketal A

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Inhibition of HIV-1 protease is one of the most effective ways to treat AIDS,[1] but the emergence of drug resistant viral strains[2] mandates new approaches. Natural product screening has uncovered novel peptidomimetic protease inhibitors,[3] some of which have been used to develop potent and specific inhibitors of medicinally relevant enzymes.[4,5] Didemnaketals A **(1)** and B **(2)** (Figure 1) were isolated from the *Ascidian didemnum* sp. at Auluptagel Island, Palau, and found to inhibit HIV-1 protease with an IC_{50} of 2 μM and 10 μM, respectively.[6] The structures of the didemnaketals were characterized by Faulkner and colleagues through extensive NMR studies, but the absolute configurations of eight stereogenic centers in the pyran rings and the pentaester side chain were not determined due to limited supply of **1**. Because the didemnaketals are potent, non-nitrogen containing HIV-1 protease inhibitors with novel structures and good initial activity, we decided to synthesize analogues of **1** to determine the minimum structure needed to inhibit HIV-1 protease. We report here that simplified analogues of **1** inhibit HIV-1 protease by an unusual mechanism.

Since most reported HIV-1 protease inhibitors contain a free hydroxyl group that interacts with the catalytic aspartic acid carboxyl groups in the active site, we synthesized analogues of the C8 to C21 portion of didemnaketal A that contains the α-hydroxyl ketone moiety. Three stereochemically ambiguous methyl groups were deleted to generate compound **3** (Figure 1). Four pairs of diastereomers of compound **3** and all intermediates were synthesized as mixtures of pairs of diastereomers from the known diol **4**[7] by means of a 14-step transformation outlined in Scheme 1.[8] However, none of the spiroketal diastereomers **3** synthesized by this route inhibited HIV-1 protease.

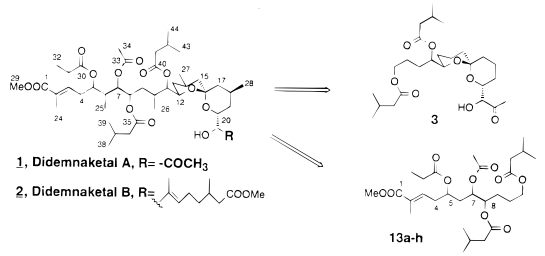
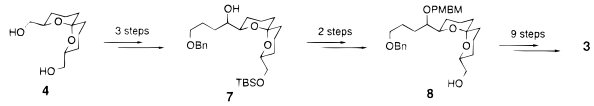
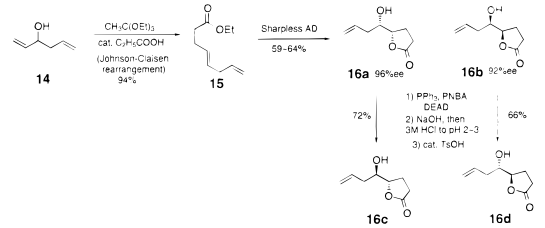


Figure 1. Didemnaketal A and B and simplified analogs.

Scheme 1



Scheme 2



We then turned our attention to the pentaester side-chain portion of **1**. Removal of the C6 and C10 methyl groups from the C1−C11 fragment produced compound **13** (Figure 1) for which eight diastereomers are possible. These were synthesized from 1,5-diene-3-ol (**14**) (Scheme 2) via Johnson−Claisen rearrangement[9] to form the achiral diene **15** (94% yield). Sharpless asymmetric dihydroxylation (AD)[10] was used to convert **15** to the hydroxy lactone **16a** (*S*,*S*) and its enantiomer **16b** (*R*,*R*) in 59−64% yield and 92% and 96% ee, respectively. Mitsunobu inversion[11] of the alcohols gave the two other diastereomers **16c** (*R*,*S*) and **16d** (*S*,*R*) in 66−72% yield.

Hydroxy lactones **16a**−**d** (Scheme 3) were converted to their THP ethers[12] and then reduced to the corresponding diols **17a**−**d** by $LiAlH_4$ in THF. Esterification of the diols **17a**−**d**, followed by removal of the THP group with CAN in MeOH,[13] gave alcohols **18a**−**d** in 59−71% yield over four steps. Acylation of alcohols **18a**−**d** afforded triesters **19a**−**d**. Ozonolysis of the triesters, followed by treatment with the allylborane derived from (+) and (−) *B*-methoxydiisopinylcamphenylborane,[14] afforded eight hydroxytriesters **20a**−**h** in 71−79% yield (these were obtained in ratios of 5:1 to 8:1, depending on the diastereomer). Esterification of hydroxytriesters **20a**−**h**, followed by ozonolysis of tetraesters **21a**−**h** and Wittig homologation, produced the desired eight pentaesters **13a**−**h** in 70−77% yield over 2 steps. Pentaesters **13a**−**h** were assayed for inhibition of HIV-1 protease

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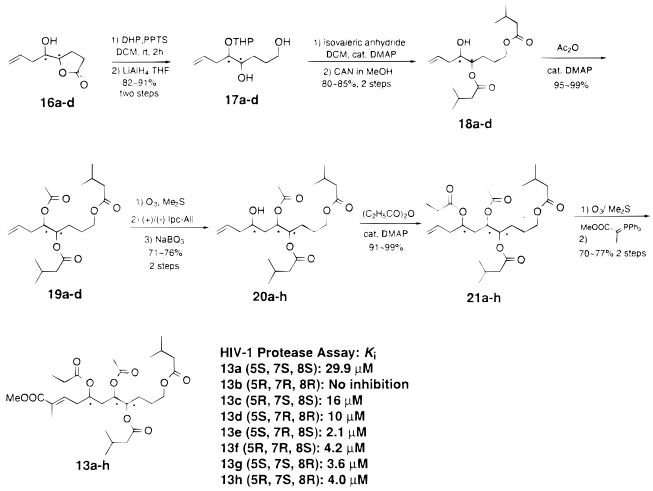
Scheme 3



(Scheme 3).[15] Compound **13e** (5*S*,7*R*,8*S*) was the best inhibitor with a K_i value of 2.1 μM, which is comparable in potency to that reported for **1** (because pentaester **13e** is an analogue of **1** and pentaesters **13e**−**h** have very similar K_i values, it is not possible to assign the stereocenters in the parent didemnaketals A and B from these inhibition data). Additional analogues (not shown) that contained hydroxyl groups in place of ester groups were not active, suggesting that full activity does not require a free hydroxyl group in this class of HIV-1 protease inhibitors.

The novel structures of pentaesters **13e**−**h**, which lack the free hydroxyl group found in conventional HIV-1 protease inhibitors, prompted us to examine the mechanism of inhibition carefully. HIV-1 protease is composed of two identical subunits which spontaneously dimerize to form active enzyme.[16] Since blocking of the active site or dissociation of the dimer will inhibit the enzyme, we chose to analyze this system by using the kinetic method of Zhang et al.,[17] which was developed to differentiate between pure competitive inhibition and noncompetitive or dissociative inhibition for inhibitors of HIV-1 protease. Kinetic data obtained and plotted according to the method of Zhang gave parallel lines for inhibition of HIV-1 protease by analogue **13e** (Figure 2). Additional experiments established that the inhibition was reversible and no evidence for enzyme precipitation was detected. These results are consistent with the pentaester inhibiting dimerization of HIV-1 protease monomers (competitive inhibitors give intersecting lines[17]).

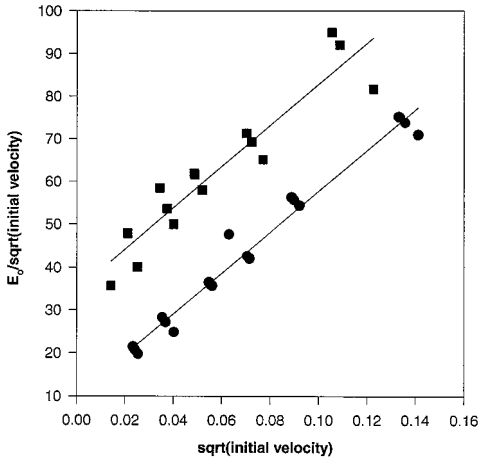


Figure 2. Dissociation kinetics of HIV-1 protease with **13e**.

By systematically modifying the structure of didemnaketal A, we have discovered a novel class of HIV-1 protease inhibitors that acts by an unusual mechanism. Compound **13e** is only the fourth non-nitrogen containing HIV-1 protease inhibitor reported to date,[18] and two previous examples[19,20] have been used to develop HIV-1 protease inhibitors in clinical trials. All clinically established HIV-1 protease inhibitors have been designed to bind in the active site of the enzyme and to block the binding of the substrate; only a few dimerization inhibitors of HIV-1 PR are known,[21] but these offer a new approach to inhibiting mutant strains of HIV-1 protease. Efforts to develop more potent and stable analogues of pentaester **13e** are in progress.

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Supporting Information Available: Expanded Scheme 1 for preparation of compounds **3**. Full experimental details for the synthesis of inhibitor **13e** and intermediates **15, 16a, 16c, 17c, 18c, 19c, 20e, 21e** (6 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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(15) (a) For fluorimetric assay conditions, see: Peranteau, A. G.; Kuzmic, P.; Angell, Y.; Garcia-Echeverria, C.; Rich, D. H. *Anal. Biochem.* **1995**, *227*, 242. Kinetic constants for all compounds were estimated from a series of inhibitor concentrations at constant enzyme levels. All assays were done in triplicate at each inhibitor concentration. The dose response data were then nonlinearly fit using KineTic (BioKin, Ltd). Normal fits were obtained and inhibition was reversible upon dilution. (b) The plasmid Pmon5888 was a generous gift of M. Gustafason. The HIV-1 protease gene was subcloned into Pet-17b (Novagen) and expressed in *Escherichia coli* by IPTG induction. The isolated inclusion bodies were purified by the method of Gustafason *et al.* with a modification of an additional DEAE-Sepharose column as the final purification step. See: Gustafason, M. E.; Junger, K. D., Foy, B. A.; Baez, J. A.; Bishop, B. F.; Rangwala, S. H.; Michner, M. L.; Leimgruber, R. M.; Houseman, K. A.; Mueller, R. A.; Matthews, B. K.; Olins, P. O.; Grabner, R. W.; Hershman, A. *Protein Expression Purif.* **1995**, *6*, 512−518.

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