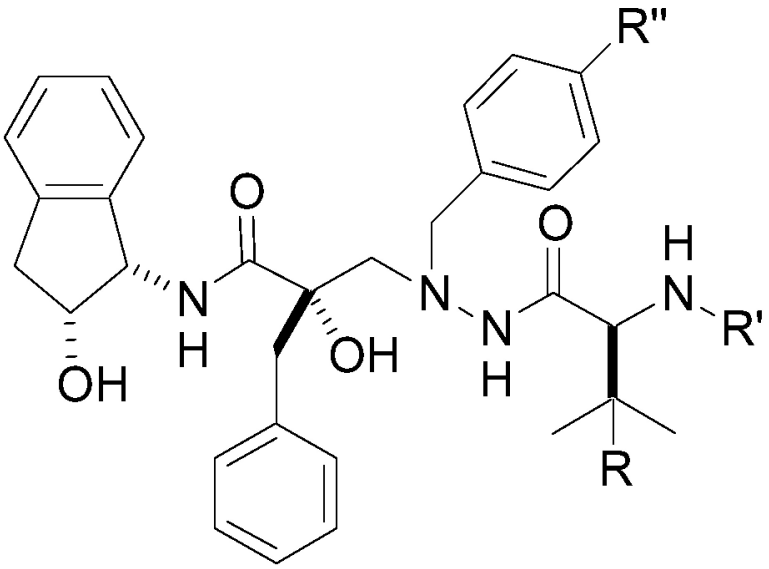
Journal of Medicinal Chemistry

Brief Article

A New Class of HIV-1 Protease Inhibitors Containing a Tertiary Alcohol in the Transition-State Mimicking Scaffold

Jenny K. Ekegren, Torsten Unge, Mayada Zreik Safa, Hans Wallberg, Bertil Samuelsson, and Anders Hallberg

J. Med. Chem., **2005**, 48 (25), 8098-8102• DOI: 10.1021/jm050790t • Publication Date (Web): 16 November 2005



R = H = Me

R' = C(O)OBn = C(O)OMe = SO_2Me = C(O)NHBn

R'' = H = Br

More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 1 articles that cite this article, as of the time of this article download

Journal of Medicinal Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Brief Articles

A New Class of HIV-1 Protease Inhibitors Containing a Tertiary Alcohol in the Transition-State Mimicking Scaffold

Jenny K. Ekegren,[†] Torsten Unge,[‡] Mayada Zreik Safa,[†] Hans Wallberg,[§] Bertil Samuelsson,[§] and Anders Hallberg*,[†]

Department of Medicinal Chemistry, Organic Pharmaceutical Chemistry, BMC, Uppsala University, Box 574, SE-751 23 Uppsala, Sweden, Department of Cell and Molecular Biology, Structural Biology, BMC, Uppsala University, Box 596, SE-751 24 Uppsala, Sweden, and Medivir AB, Lunastigen 7, SE-141 44 Huddinge, Sweden

Received August 10, 2005

Novel HIV-1 protease inhibitors encompassing a tertiary alcohol as part of the transition-state mimicking unit have been synthesized. Variation of the $P_1'-P_3'$ residues and alteration of the tertiary alcohol absolute stereochemistry afforded 10 inhibitors. High potencies for the compounds with (*S*)-configuration at the carbon carrying the tertiary hydroxyl group were achieved with K_i values down to 2.4 nM. X-ray crystallographic data for a representative compound in complex with HIV-1 protease are presented.

Introduction

Since the first cases of AIDS were identified in 1981, followed by isolation of the HIV virus a few years later, the epidemic has expanded worldwide, leaving almost no region unaffected by the disease.[1,2] Recent figures from UNAIDS reveal that almost 5 million people were newly infected with HIV in 2004 and a total of 39 million people were living with HIV/AIDS.[3] For patients with access to therapy, most of whom live in the developed world, drugs and accurate medical care have turned the former fatal infection into a treatable, chronic infectious disease.[4−6] With the use of antiretroviral therapy, a new threat in the battle against HIV has appeared: drug resistance. In the U.S., about 50% of the patients receiving therapy are infected with viruses resistant to at least one of the available anti-HIV drugs on the market.[7−10] Taking these different aspects into account, the need for new drugs is obvious.[11]

HIV protease inhibitors used in combination therapy with reverse transcriptase inhibitors are important as significant suppression of viral loads in HIV patients has been accomplished.[12−15] Seven HIV protease inhibitors have reached the market so far. However, the therapy is far from being free of complications; severe side effects such as dyslipidaemia, hypersensitivity, and lipodystrophy are limiting adherence and therefore clinical efficacy.[16] The oral bioavailability of the HIV protease inhibitors is generally low. However, the development of atazanavir led to the first drug in which high oral bioavailability was achieved (Figure 1). Furthermore, this drug exhibits excellent antiviral activity against wild-type and saquinavir- and indinavir-resistant strains of HIV.[17−20]

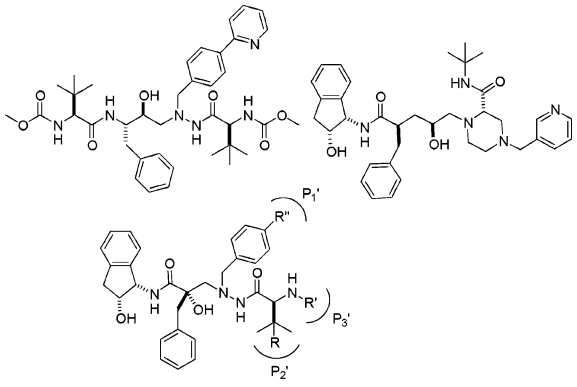


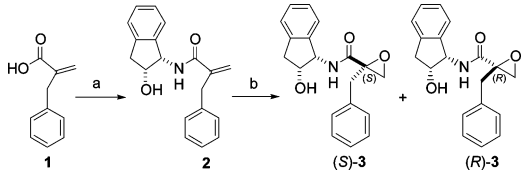
Figure 1. Atazanavir (top left), indinavir (top right), and the generic structure of the new class of inhibitors (bottom).

All inhibitors in clinic encompass a secondary hydroxyl group as part of the transition-state mimicking unit. There are only a few examples reported where tertiary alcohols have been used as parts of transition-state mimics in protease inhibitors.[21−25] In those cases, introduction of the tertiary alcohol generally provided less potent inhibitors compared to the corresponding compounds with a secondary alcohol. In one study of aspartyl protease inhibitors (not including HIV protease inhibitors) by Rich et al., the most potent compound with a tertiary hydroxyl group had the opposite stereochemistry compared to the most potent compound comprising a secondary hydroxyl group.[21−23] We were encouraged to explore the transition-state mimic present in the generic structure shown in Figure 1, as initial modeling suggested that a tertiary alcohol built into this core structure could favorably interact with Asp25 and Asp125 of the HIV protease. Furthermore, we speculate that a hydroxyl group in α-position to an amide could enable partial masking of the amide functionality and the hydroxyl group via internal hydrogen bonding that in principle should improve transcellular transport. This

* To whom correspondence should be addressed. Phone: +46-18-4714284. Fax: +46-18-4714474. E-mail: Anders.Hallberg@orgfarm.uu.se.
[†] Department of Medicinal Chemistry, Uppsala University.
[‡] Department of Cell and Molecular Biology, Uppsala University.
[§] Medivir AB.

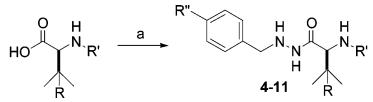
10.1021/jm050790t CCC: $30.25
Published on Web 11/16/2005

Scheme 1[a]



[a] Reagents: (a) (1*S*,2*R*)-1-amino-2-indanol, EDC, NMM, HOBT, EtOAc, room temp, 68%; (b) mCPBA, CH_2Cl_2, reflux, 53%.

Scheme 2[a]



4 R=H R'=CO_2Bn R'' H **8** R=Me R'=SO_2Me R''=H
5 R=Me R'=CO_2Bn R''=H **9** R=Me R'=C(O)NHBn R''=H
6 R=H R'=CO_2Me R''=H **10** R=Me R'=CO_2Me R''=Br
7 R=Me R'=CO_2Me R''=H **11** R=H R'=CO_2Bn R''=Br

[a] Reagents: (a) benzylhydrazine·2HCl/Et_3N or 4-bromobenzylhydrazine and EDC, HOBT, NMM, EtOAc, room temp, 50−79%.

concept, which takes advantage of intramolecular hydrogen bonds to increase apparent lipophilicity and to increase membrane penetration, was successfully applied by Ashwood et al. in NK1 receptor antagonists.[26] In the generic structure in Figure 1 the tertiary alcohol unit on the prime side is linked to the ethyl(hydrazine) moiety found in atazanavir and on the nonprime side to the (1*S*)-amino-(2*R*)-hydroxyindane structure originating from indinavir.[27−29] We herein report the synthetic protocols, HIV-1 protease inhibition data, and anti-HIV activities for these new HIV protease inhibitors in cell assays. Furthermore, we present X-ray crystallographic data for a representative compound of this class.

Results

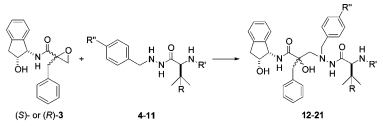
Chemistry. The transition-state mimicking tertiary alcohol utilized in the new HIV protease inhibitors originated from the optically active 2,2-disubstituted epoxide **3**, which was readily available in both diastereomeric forms (Scheme 1). The synthesis of **3** started from 2-benzylacrylic acid **1**[30] and (1*S*,2*R*)-1-amino-2-indanol, which were coupled using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), *N*-methylmorpholine (NMM), and 1-hydroxybenzotriazole (HOBT). The resulting α,*β*-unsaturated amide **2** was epoxidized by 3-chloroperoxybenzoic acid (mCPBA), yielding the two diastereomers of epoxide **3** in a 50:50 mixture, here denoted (*S*)-**3** and (*R*)-**3** where *S* and *R* refer to the absolute configuration of the asymmetric carbon in the epoxide ring. The two diastereomers readily separated upon column chromatography, yielding the pure isomers.

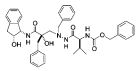
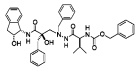
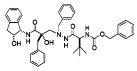
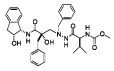
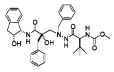
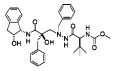
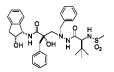
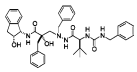
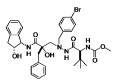
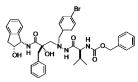
The synthetic strategy was to ring-open **3** with different hydrazides to obtain a series of inhibitors.[17,18,31] The hydrazides were synthesized from L-valine or L-*tert*-leucine to assess the impact of an isopropyl versus a *tert*-butyl group as P_2' side chains (Scheme 2). Different P_3' substituents were incorporated into the scaffold by starting from commercially available Cbz-protected L-valine/L-*tert*-leucine or from the corresponding unprotected amino acids, which were first N-derivatized. Functionalization with an *N*-methoxycarbonyl group was described previously.[17,18] The functionalized amino acids were coupled with benzylhydrazine using EDC, NMM, and HOBT, affording the desired hydrazides in 50−67% yield after column chromatography (**4**−**9**, Scheme 2). However, the urea derivative **9** turned out to be unstable, and after filtration through a short silica column the crude product was used without further purification in the inhibitor synthesis. To investigate the effect of a larger substituent in the inhibitor P_1' side chain, two brominated hydrazides were synthesized (**10** and **11**, Scheme 2). Starting from 4-bromobenzylhydrazine,[32] prepared according to a published procedure from 4-bromobenzyl bromide and hydrazine monohydrate,[33] the two bromohydrazides were produced in 54% and 79% yields, respectively.

Two different strategies were used for the synthesis of the HIV protease inhibitors from epoxide **3** and the hydrazides **4**−**11** (Table 1). In the first approach the two reagents were stirred in *i*PrOH at 80 °C for 3−7 days, and the reactions were monitored by RPLC−MS (method A). Later, an improved procedure was used where $Ti(OiPr)_4$[34,35] catalyzed the ring opening of **3** in THF and a reaction time of only 3 h was now required (method B). Purification by preparative RPLC−MS resulted in highly pure products; however, this also seemed to have a negative effect on the isolated yields compared to purification by column chromatography only. To establish which stereochemistry of the transition-state mimicking tertiary alcohol generated the most potent inhibitors, two pairs of inhibitors were synthesized having an opposite absolute configuration at the carbon bearing the tertiary hydroxyl group. Hydrazide **4** was used to ring-open epoxides (*S*)-**3** and (*R*)-**3**, affording the diastereomeric inhibitors **12** and **13**. Likewise, hydrazide **7** was utilized to afford inhibitors **16** and **17** (Table 1).

HIV Protease Inhibition, MT4 Cell-Based Anti-HIV Activity, Permeability, and Metabolic Stability in Vitro. The K_i and EC_{50} values for **12**−**21** are in Table 1. The K_i values for the diastereomer pairs **12/13** and **16/17** revealed that the compounds with (*S*)-configuration were at least 40 times more potent than the compounds with (*R*)-configuration. The *N*-methoxycarbonyl group in the P_3' position clearly gave more potent inhibitors than the *N*-benzyloxycarbonyl group and the sulfonamide and urea derivatives (cf. **16** with **14**, **18**, and **19**; Table 1). Incorporation of a bromo atom in the para position of the P_1' benzyl group provided the most potent inhibitor with $K_i = 2.4$ nM (**20**, Table 1). EC_{50} values below 10 μM were achieved for all compounds with an *N*-methoxycarbonyl group in the P_3' position and (*S*)-configuration at the carbon bearing the tertiary alcohol, while the *N*-benzyloxycarbonyl compounds exhibited values above that limit (cf. **16/14** and **15/12**, Table 1). A *tert*-butyl group in the P_2' position combined with the favorable *N*-methoxycarbonyl at the P_3' position rendered a compound twice as active as the corresponding isopropyl derivative in the cell assay (cf. **16** and **15**, Table 1). The most potent compound within this series was **20** as it also had the best cell activity data with an EC_{50} of 1.1 μM. **16** and **20** were evaluated in selected in vitro resistant HIV-1 isolates from serial passages of a symmetric diol-based inhibitor[36] and ritonavir, respectively (Table 1). The two compounds exhibited equipotent activity in these mutant MT4/HIV-1 protease assays (V32I, M46I, V82A and V32I,

Table 1. Synthesis of Inhibitors **12**−**21** and Biological Data



Compound	Structure	From epoxide/hydrazide[a]	Purification[b]	Yield (%)	K_i (nM)[c]	EC_{90} (μM)[c]
12		(*S*)-**3/4**	1	55	23	> 10
13		(*R*)-**3/4**	2	39	870	> 10
14		(*S*)-**3/5**	1 + 2	40	18	> 10
15		(*S*)-**3/6**	1 + 2	19	9	4.8
16[f,g]		(*S*)-**3/7**	1 + 2	46	6	1.9 2.0[d] 1.6[e]
17		(*R*)-**3/7**	2	53	2200	> 10
18		(*S*)-**3/8**	1 + 2	43	63	> 10
19		(*S*)-**3/9**	2	25	22	> 10
20[f,g]		(*S*)-**3/10**	1	55	2.4	1.1 0.9[d] 0.8[e]
21		(*S*)-**3/11**	2	13	17	> 10

[a] All compounds were synthesized according to method A except **20**, which was prepared according to method B (see Supporting Information). [b] Purification methods: (1) column chromatography, silica; (2) RPLC−MS. [c] Indinavir: $K_i = 0.31$ nM[14] and $EC_{50} = 0.041$ μM.[13,38] [d] Mutations in MT4/HIV-1 protease: V32I, M46I, V82A. [e] Mutations in MT4/HIV-1 protease: V32I, M46I, A71V, V82A.[f] **16** Papp(Caco-2) = 35×10^{-6} cm/s; **20** Papp(Caco-2) = 42×10^{-6} cm/s. [g] **16** $Cl_{int} = 527$ μL min^{-1} mg^{-1}; **20** $Cl_{int} = 266$ μL min^{-1} mg^{-1}.

M46I, A71V, V82A, respectively) as in the wild-type MT4/HIV-1 protease assay. The in vitro DMPK profile for **16** and **20** was also evaluated. As we hoped, **16** (Papp = 35×10^{-6} cm/s) and **20** (Papp = 42×10^{-6} cm/s) were found to exhibit excellent permeability in the Caco-2 cell assay, more than 6 times better than atazanavir (Papp = 5.3×10^{-6} cm/s) in the same assay. We propose that the high permeability is attributed to the masking of the amide and tertiary hydroxyl functionalities by an intramolecular hydrogen bond network. The fast intrinsic clearance of **16** ($Cl_{int} = 527$ μL min^{-1} mg^{-1}) and **20** ($Cl_{int} = 266$ μL min^{-1} mg^{-1}) is believed to be due to metabolic hydroxylation of the benzylic position of the aminoindanol.[37]

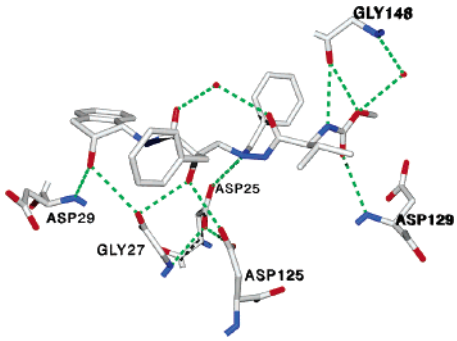


Figure 2. X-ray crystal structure of **15** cocrystallized with HIV-1 protease. Hydrogen-bonding interactions between the inhibitor and key residues of the enzyme are represented by green dashed lines.

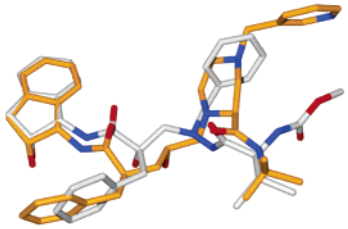


Figure 3. Superimposition of the X-ray structures of inhibitor **15** (gray) and indinavir (orange) in the active site of HIV-1 protease.

X-ray Crystallographic Data. Cocrystallization of inhibitor **15** with HIV-1 protease followed by X-ray diffraction data provided a 3D structure with 1.8 Å resolution (PDB code 2bqv). The hydrogen-bonding interactions between the inhibitor and the enzyme are shown in Figure 2. The tertiary alcohol forms a hydrogen bond to one of the active site aspartates, Asp125. Furthermore, the β-nitrogen of the hydrazido group was found to form a weak hydrogen bond to the other active site aspartate residue, Asp25 (3.2 Å). A distinct hydrogen bond pattern was also observed between the P_3' carbamate group and Asp129 and Gly148 as well as Gly149.

Superimposition of the X-ray structures of **15** and indinavir (PDB code 1hsg) reveals significant differences between the two inhibitors (Figure 3). Whereas the positions of the P_2 aminoindanol and the P_2' isopropyl groups overlap well with the corresponding residues in indinavir, the positions of the P_1/P_1' groups differ considerably in **15**. Both groups penetrate less deeply into the enzyme subsites, and the discrepancies are about 1.4 Å on the P_1 side and 4.5 Å on the P_1' side compared to indinavir. In addition, the angle at which the P_1' benzyl group penetrates into the S_1' pocket is different from that of indinavir. The most striking differences appear when the hydrogen bond pattern between the enzyme catalytic aspartates and the transition-state mimicking scaffolds of **15** and indinavir is analyzed (Figure 4). Whereas indinavir positions the central hydroxyl group in close contact (within 2.6−3.0 Å) with all four oxygen atoms of the active site aspartate residues, **15** positions the tertiary alcohol and the β-nitrogen of the hydrazido group symmetrically over the active site. This arrangement leads to the tertiary alcohol forming a less tight hydrogen bond to Asp125 (3.1 Å) compared to indinavir. In addition, the tertiary

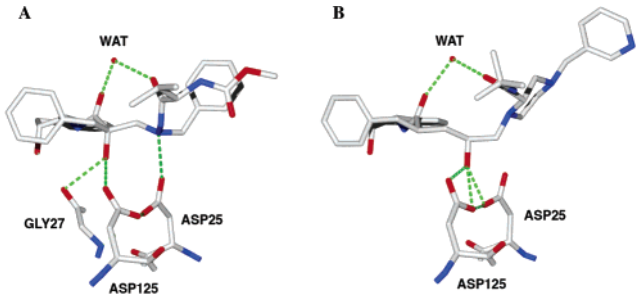


Figure 4. Comparison of hydrogen bond pattern in complexes of HIV-1 protease and inhibitor **15** (A) and indinavir (B). Hydrogen bonds are represented by green dashed lines.

hydroxyl group is hydrogen-bonded to the main chain oxygen of Gly27 (3.0 Å), a feature not observed with indinavir.

Discussion

The observed lowering of K_i when the benzyl group of the P_1' residue was elongated with a *p*-bromo substituent (cf. **20** and **16**, Table 1) might be explained by favorable interactions at the enzyme surface. The extension of this residue approaches the enzyme/solvent interface, and a more efficient displacement of solvent molecules at the enzyme surface, mediated by the polar bromine compared to the hydrophobic phenyl lacking a para substituent, can occur. Large P_3' residues will occupy parts of the $S_3'-S_1'$ spanning pocket and could interfere negatively with the phenyl P_1' group, as deduced from modeling. It was therefore less surprising that a P_3' benzyl group resulted in less efficient inhibitors, as was also observed in the atazanavir series.[17,18] In contrast, the relatively small (and more easily solvated) methyl carbamate group that gave lower K_i and EC_{50} values (below 10 μM) can be well accommodated in the $S_3'-S_1'$ pocket (cf. **16/14** and **15/12**, Table 1). The 3D structure revealed that the carbamate group in **15** is in a hydrogen bond network where the three heteroatoms of the carbamate participate (Figure 2). We replaced the carbamate with the powerful hydrogen bond accepting/donating urea fragment and hoped that lower K_i values could be achieved in the enzyme assay by this manipulation. However, no improvement was encountered (cf. **14** and **19**, Table 1). An unfavorable alignment of the urea imposed by the large benzyl group present in **19** when binding to the enzyme could account for this observation. Modeling suggested that a methyl sulfonamide could provide an alternative and proper substitute for the methyl carbamate in **15**. This alteration was also largely unsuccessful. Hence, **18** was 10 times less potent than **16** (Table 1), but more importantly it did not exhibit any inhibitory effect in the cell assay. The fact that only the methyl carbamates and not the methylsulfonamide compound are efficient in the cell assays is difficult to rationalize. The increased cellular potency achieved when switching from valine to *tert*-leucine for the P_3' methyl carbamate compounds (cf. **16** and **15**, Table 1) was in agreement with previously reported observations from the atazanavir series.[18] Even though the *tert*-leucine compounds **16** and **20**, with K_i of 6 and 2.4 nM, exert antiviral activities in the cell assay, it is notable that the EC_{50} values are not impressive (1.9 and 1.1 μM). The same types of assay afforded indinavir a K_i of 0.31 nM in the HIV-1 protease assay and an EC_{50} of 41 nM in the MT4 cell assay.[13,14,38] Thus, indinavir is 10 times better than **20** in the enzyme assay and ~25 times more effective in the cell assay. The data from the Caco-2 cell assay (**16** Papp = 35×10^{-6} cm/s; **20** Papp = 42×10^{-6} cm/s) predict excellent cell membrane penetration for these compounds. As a comparison, atazanavir exhibited a Papp of 5.3×10^{-6} cm/s and indinavir a Papp of 1.7×10^{-6} cm/s in the same assay. With the exception of the reports from Rich et al.,[21-23] there seems to be few studies addressing the potential of tertiary alcohols as part of transition-state substitutes. The data presented herein suggest that further efforts could be devoted to more extensive elaboration of this structural element as a central core in inhibitors of aspartyl proteases. Carboxylic acid derivatives with an α-hydroxyl group are prone to form intramolecular hydrogen bonds in solution.[39] The present investigation, as deduced from the Papp values from the Caco-2 assay, supports the hypothesis that compounds with an inherent ability to form intramolecular hydrogen bonds are more likely to exhibit high permeability. However, the compounds herein do not seem to be efficiently transported into the intracellular compartments of HIV-1 infected MT4 cells and the moderate antiviral activities observed are not easy to rationalize.

Conclusion

In summary, new HIV-1 protease inhibitors encompassing a rarely used tertiary alcohol based transition-state mimicking scaffold have been synthesized. Variations at the $P_1'-P_3'$ side chains of the inhibitors resulted in compounds with K_i values in the low nanomolar range (down to 2.4 nM). Results from the enzyme assay established that the compounds comprising the tertiary alcohol with (*S*)-configuration were more potent enzyme inhibitors than those with (*R*)-configuration. The absolute stereochemistry was confirmed by X-ray crystallography data obtained from ligand **15** cocrystallized with the HIV-1 protease at a resolution of 1.8 Å. The results confirm that a tertiary alcohol can serve as part of a transition-state mimicking scaffold in HIV-protease inhibitors. The moderate antiviral activities encountered in HIV-1 infected MT4 cells are difficult to explain, in particular since excellent Papp values from Caco-2 assays support the assumption that masking of the hydroxyl group and the adjacent amide bond by intramolecular hydrogen bonds should render compounds with high cell membrane permeability. It is notable that the best compound (**20**) in cell assays was equally potent toward two common mutated HIV proteases as toward the wild-type protease. An optimization of the compounds within this series aiming at inhibitors with improved cellular potency is in progress.

Experimental Section

General Procedures for Synthesis of Inhibitors. Method A. Epoxide **3** and hydrazide were dissolved in *i*PrOH, and the mixture was stirred at 80 °C for 72−168 h. Evaporation of the solvent followed by purification (column chromatography and/or RPLC−MS) afforded the products in 13−55% yield.

Method B. Epoxide **3** and hydrazide were dissolved in dry THF, and $Ti(OiPr)_4$ was added under N_2 atmosphere. Stirring at room temperature for 2.5 h and then at 40 °C for 30 min

was followed by the addition of saturated $NaHCO_3$ (aq) and Et_2O. The resulting mixture was stirred at room temperature for 10 min. Filtration and separation of the two phases, drying of the organic phase (Na_2SO_4), and evaporation followed by purification by column chromatography afforded the product in 55% yield.

Acknowledgment. We gratefully acknowledge the Swedish Foundation for Strategic Research (SSF) and the Swedish Research Council (VR) for financial support and Johan Gising for preparative work.

Supporting Information Available: Experimental details and spectroscopic data for compounds **2**−**21** (^{1}H NMR, ^{13}C NMR, MS, optical rotation), procedures for enzyme and Caco-2 assays and liver microsome stability evaluation, and table of elemental analysis data and X-ray structure determination details. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Barre-Sinoussi, F.; Chermann, J. C.; Rey, F.; Nugeyre, M. T.; Chamaret, S.; Gruest, J.; Dauguet, C.; Axler-Blin, C.; Vezinet-Brun, F.; Rouzioux, C.; Rozenbaum, W.; Montagnier, L. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science* **1983**, *220*, 868−871.

(2) Gallo, R. C.; Sarin, P. S.; Gelmann, E. P.; Robert-Guroff, M.; Richardson, E.; Kalyanaraman, V. S.; Mann, D.; Sidhu, G. D.; Stahl, R. E.; Zolla-Pazner, S.; Leibowitch, J.; Popovic, M. Isolation of human T-cell leukemia virus in acquired immune deficiency syndrome (AIDS). *Science* **1983**, *220*, 865−867.

(3) *AIDS Epidemic Update: December 2004*; Joint United Nations Programme on HIV/AIDS (UNAIDS)/WHO, 2004.

(4) Pomerantz, R. J.; Horn, D. L. Twenty years of therapy for HIV-1 infection. *Nat. Med.* **2003**, *9*, 867−873.

(5) Peterlin, B. M.; Trono, D. Hide, shield and strike back: how HIV-infected cells avoid immune eradication. *Nat. Rev. Immunol.* **2003**, *3*, 97−107.

(6) *2004 Report on the Global AIDS Epidemic*; Joint United Nations Programme on HIV/AIDS (UNAIDS), 2004.

(7) Clavel, F.; Hance, A. J. HIV drug resistance. *N. Engl. J. Med.* **2004**, *350*, 1023−1035.

(8) Werber, Y. HIV drug market. *Nat. Rev. Drug Discovery* **2003**, *2*, 513−514.

(9) Menéndez-Arias, L. Targeting HIV: antiretroviral therapy and development of drug resistance. *Trends Pharmacol. Sci.* **2002**, *23*, 381−388.

(10) de Mendoza, C.; Soriano, V. Resistance to HIV protease inhibitors: mechanisms and clinical consequences. *Curr. Drug Metab.* **2004**, *5*, 321−328.

(11) De Clercq, E. New approaches toward anti-HIV chemotherapy. *J. Med. Chem.* **2005**, *48*, 1297−1313.

(12) Abdel-Rahman, H. M.; Al-Karamany, G. S.; El-Koussi, N. A.; Youssef, A. F.; Kiso, Y. HIV protease inhibitors: peptidomimetic drugs and future perspectives. *Curr. Med. Chem.* **2002**, *9*, 1905−1922.

(13) Randolph, J. T.; DeGoey, D. A. Peptidomimetic inhibitors of HIV protease. *Curr. Top. Med. Chem.* **2004**, *4*, 1079−1095.

(14) Rodríguez-Barrios, F.; Gago, F. HIV protease inhibition: limited recent progress and advances in understanding current pitfalls. *Curr. Top. Med. Chem.* **2004**, *4*, 991−1007.

(15) Bursavich, M. G.; Rich, D. H. Designing non-peptide peptidomimetics in the 21st century: inhibitors targeting conformational ensembles. *J. Med. Chem.* **2002**, *45*, 541−558.

(16) Carr, A. Toxicity of antiretroviral therapy and implications for drug development. *Nat. Rev. Drug Discovery* **2003**, *2*, 624−634.

(17) Fässler, A.; Bold, G.; Capraro, H.-G.; Cozens, R.; Mestan, J.; Poncioni, B.; Rösel, J.; Tintelnot-Blomley, M.; Lang, M. Aza-peptide analogs as potent human immunodeficiency virus type-1 protease inhibitors with oral bioavailability. *J. Med. Chem.* **1996**, *39*, 3203−3216.

(18) Bold, G.; Fässler, A.; Capraro, H.-G.; Cozens, R.; Klimkait, T.; Lazdins, J.; Mestan, J.; Poncioni, B.; Rösel, J.; Stover, D.; Tintelnot-Blomley, M.; Acemoglu, F.; Beck, W.; Boss, E.; Eschbach, M.; Hurlimann, T.; Masso, E.; Roussel, S.; Ucci-Stoll, K.; Wyss, D.; Lang, M. New aza-dipeptide analogues as potent and orally absorbed HIV-1 protease inhibitors: candidates for clinical development. *J. Med. Chem.* **1998**, *41*, 3387−3401.

(19) Wang, F.; Ross, J. Atazanavir: a novel azapeptide inhibitor of HIV-1 protease. *Formulary* **2003**, *38*, 691−702.

(20) Raja, A.; Lebbos, J.; Kirkpatrick, P. Fresh from the pipeline: Atazanavir sulphate. *Nat. Rev. Drug Discovery* **2003**, *2*, 857−858.

(21) Rich, D. H.; Bernatowicz, M. S.; Agarwal, N. S.; Kawai, M.; Salituro, F. G.; Schmidt, P. G. Inhibition of aspartic proteases by pepstatin and 3-methylstatine derivatives of pepstatin. Evidence for collected-substrate enzyme inhibition. *Biochemistry* **1985**, *24*, 3165−3173.

(22) Rich, D. H. Pepstatin-derived inhibitors of aspartic proteinases. A close look at an apparent transition-state analog inhibitor. *J. Med. Chem.* **1985**, *28*, 263−273.

(23) Agarwal, N. S.; Rich, D. H. Inhibition of cathepsin D by substrate analogs containing statine and by analogs of pepstatin. *J. Med. Chem.* **1986**, *29*, 2519−2524.

(24) Godfrey, J. D., Jr.; Gordon, E. M.; Von Langen, D. J. Synthesis of peptide-derived amino alcohols II. Synthetic methodology for the preparation of tertiary alcohols. *Tetrahedron Lett.* **1987**, *28*, 1603−1606.

(25) Kim, B. M.; Guare, J. P.; Hanifin, C. M.; Arford-Bickerstaff, D. J.; Vacca, J. P.; Ball, R. G. A convergent synthesis of novel conformationally restricted HIV-1 protease inhibitors. *Tetrahedron Lett.* **1994**, *35*, 5153−5156.

(26) Ashwood, V. A.; Field, M. J.; Horwell, D. C.; Julien-Larose, C.; Lewthwaite, R. A.; McCleary, S.; Pritchard, M. C.; Raphy, J.; Singh, L. Utilization of an intramolecular hydrogen bond to increase the CNS penetration of an NK1 receptor antagonist. *J. Med. Chem.* **2001**, *44*, 2276−2285.

(27) Lyle, T. A.; Wiscount, C. M.; Guare, J. P.; Thompson, W. J.; Anderson, P. S.; Darke, P. L.; Zugay, J. A.; Emini, E. A.; Schleif, W. A.; Quintero, J. C.; Dixon, R. A. F.; Sigal, I. S.; Huff, J. R. Benzocycloalkyl amines as novel C-termini for HIV protease inhibitors. *J. Med. Chem.* **1991**, *34*, 1228−1230.

(28) Askin, D.; Eng, K. K.; Rossen, K.; Purick, R. M.; Wells, K. M.; Volante, R. P.; Reider, P. J. Highly diastereoselective reaction of a chiral, non-racemic amide enolate with (*S*)-glycidyl tosylate. Synthesis of the orally active HIV-1 protease inhibitor L-735,-524. *Tetrahedron Lett.* **1994**, *35*, 673−676.

(29) Dorsey, B. D.; Levin, R. B.; McDaniel, S. L.; Vacca, J. P.; Guare, J. P.; Darke, P. L.; Zugay, J. A.; Emini, E. A.; Schleif, W. A.; Quintero, J. C.; Lin, J. H.; Chen, I.-W.; Holloway, M. K.; Fitzgerald, P. M. D.; Axel, M. G.; Ostovic, D.; Anderson, P. S.; Huff, J. R. L-735,524: The design of a potent and orally bioavailable HIV protease inhibitor. *J. Med. Chem.* **1994**, *37*, 3443−3451.

(30) Liu, X.; Hu, E.; Tian, X.; Mazur, A.; Ebetino, F. H. Enantioselective synthesis of phosphinyl peptidomimetics via an asymmetric Michael reaction of phosphinic acids with acrylate derivatives. *J. Organomet. Chem.* **2002**, *646*, 212−222.

(31) Sham, H. L.; Zhao, C.; Stewart, K. D.; Betebenner, D. A.; Lin, S.; Park, C. H.; Kong, X.-P.; Rosenbrook, W., Jr.; Herrin, T.; Madigan, D.; Vasavanonda, S.; Lyons, N.; Molla, A.; Saldivar, A.; Marsh, K. C.; McDonald, E.; Wideburg, N. E.; Denissen, J. F.; Robins, T.; Kempf, D. J.; Plattner, J. J.; Norbeck, D. W. A novel, picomolar inhibitor of human immunodeficiency virus type 1 protease. *J. Med. Chem.* **1996**, *39*, 392−397.

(32) Savin, V. I. *N*-Nitrenes. IV. Synthesis of unsymmetrical bibenzyls. *Zh. Org. Khim.* **1992**, *28*, 43−50.

(33) Otteneder, M.; Plastaras, J. P.; Marnett, L. J. Reaction of malondialdehyde−DNA adducts with hydrazines—development of a facile assay for quantification of malondialdehyde equivalents in DNA. *Chem. Res. Toxicol.* **2002**, *15*, 312−318.

(34) Chong, J. M.; Sharpless, K. B. Nucleophilic openings of 2,3-epoxy acids and amides mediated by Ti(O-*i*-Pr)$_4$. Reliable C-3 selectivity. *J. Org. Chem.* **1985**, *50*, 1560−1563.

(35) Caron, M.; Sharpless, K. B. Ti(O-*i*-Pr)$_4$-mediated nucleophilic openings of 2,3-epoxy alcohols. A mild procedure for regioselective ring-opening. *J. Org. Chem.* **1985**, *50*, 1557−1560.

(36) Alterman, M.; Bjoersne, M.; Muehlman, A.; Classon, B.; Kvarnstroem, I.; Danielson, H.; Markgren, P.-O.; Nillroth, U.; Unge, T.; Hallberg, A.; Samuelsson, B. Design and synthesis of new potent C2-symmetric HIV-1 protease inhibitors. Use of L-mannaric acid as a peptidomimetic scaffold. *J. Med. Chem.* **1998**, *41*, 3782−3792.

(37) Balani, S. K.; Arison, B. H.; Mathai, L.; Kauffman, L. R.; Miller, R. R.; Stearns, R. A.; Chen, I. W.; Lin, J. H. Metabolites of L-735,524, a potent HIV-1 protease inhibitor, in human urine. *Drug Metab. Dispos.* **1995**, *23*, 266−270.

(38) Molla, A.; Vasavanonda, S.; Kumar, G.; Sham, H. L.; Johnson, M.; Grabowski, B.; Denissen, J. F.; Kohlbrenner, W.; Plattner, J. J.; Leonard, J. M.; Norbeck, D. W.; Kempf, D. J. Human serum attenuates the activity of protease inhibitors toward wild-type and mutant human immunodeficiency virus. *Virology* **1998**, *250*, 255−262.

(39) Carrell, H. L.; Glusker, J. P.; Piercy, E. A.; Stallings, W. C.; Zacharias, D. E.; Davis, R. L.; Astbury, C.; Kennard, C. H. L. Metal chelation versus internal hydrogen bonding of the α-hydroxy carboxylate group. *J. Am. Chem. Soc.* **1987**, *109*, 8067−8071.

JM050790T