Design and Fast Synthesis of C-Terminal Duplicated Potent C_2-Symmetric P1/P1′-Modified HIV-1 Protease Inhibitors

Mathias Alterman,[†] Hans O. Andersson,[||] Neeraj Garg,[†] Göran Ahlsén,[‡] Seved Lövgren,[||] Björn Classon,[⊥] U. Helena Danielson,[‡] Ingmar Kvarnström,[▽] Lotta Vrang,[§] Torsten Unge,[||] Bertil Samuelsson,[⊥] and Anders Hallberg*[,†]

Department of Organic Pharmaceutical Chemistry, Uppsala University, BMC, Box 574, SE-751 23 Uppsala, Sweden, Department of Biochemistry, Uppsala University, BMC, Box 576, SE-751 23 Uppsala, Sweden, Medivir AB, Lunastigen 7, SE-141 44 Huddinge, Sweden, Department of Chemistry, Linköping University, SE-581 83 Linköping, Sweden, Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, SE-106 91 Stockholm, Sweden, and Department of Molecular Biology, Uppsala University, BMC, Box 590, SE-751 24 Uppsala, Sweden

Received March 16, 1999

An analysis of the X-ray structure of a complex of HIV-1 protease with a linear C_2-symmetric C-terminal duplicated inhibitor guided the selection of a series of diverse target compounds. These were synthesized with the objective to identify suitable P1/P1′ substituents to provide inhibitors with improved antiviral activity. Groups with various physical properties were attached to the *para*-positions of the P1/P1′ benzyloxy groups in the parent inhibitor. A *p*-bromobenzyloxy compound, prepared in only three steps from commercially available starting materials, was utilized as a common precursor in all reactions. The subsequent coupling reactions were completed within a few minutes and relied on palladium catalysis and flash heating with microwave irradiation. All of the compounds synthesized exhibited good inhibitory potency in the protease assay, with K_i values ranging from 0.09 to 3.8 nM. A 30-fold improvement of the antiviral effect in cell culture, compared to the parent compound, was achieved with four of the inhibitors. The differences in K_i values were not correlated to the differences in antiviral effect, efficiency against mutant virus, or reduced potency in the presence of human serum. The poorest enzyme inhibitors in fact belong to the group with the best antiviral effect. The binding features of two structurally related inhibitors, cocrystallized with HIV-1 protease, are discussed with special emphasis on the interaction at the enzyme/water phase.

Introduction

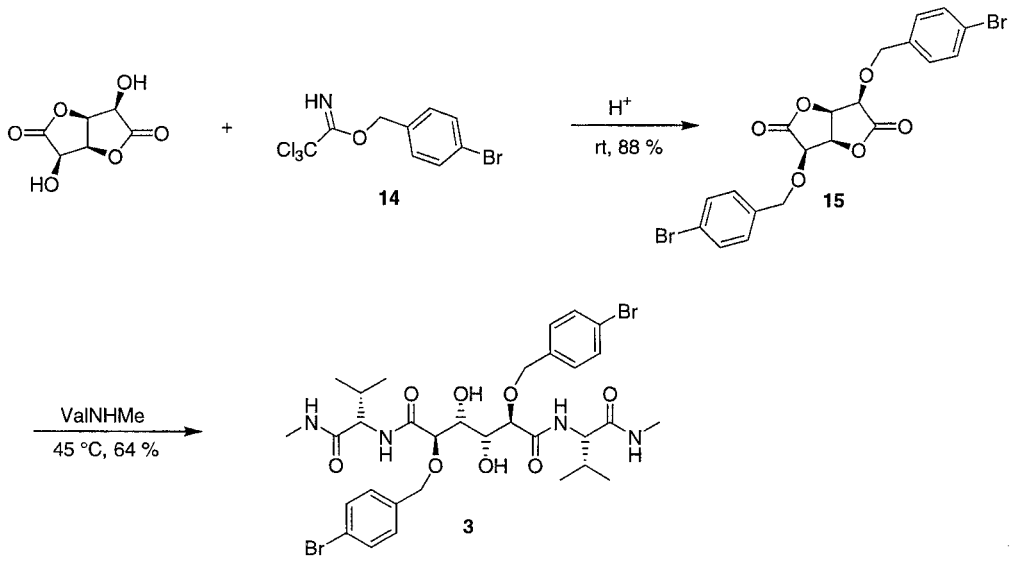
The etiological agent of acquired immune deficiency syndrome (AIDS), the human immunodeficiency virus (HIV), is a retrovirus of the lentivirus subfamily.[1] The RNA genome of HIV encodes an essential aspartic protease, which has become one of the prime targets for chemotherapeutic intervention in the treatment of AIDS.[2] The C_2-symmetric dimeric protease processes the viral precursor polyproteins *gag* and *gag/pol* into structural proteins and enzymes. Four HIV protease inhibitors, saquinavir, ritonavir, indinavir, and nelfinavir, have been approved to date for AIDS therapy, mainly in combination with reverse transcriptase inhibitors. This therapy has led to reduction of the viral load, and an increase in the number of $CD4^+$ lymphocytes has been demonstrated in HIV-infected individuals.[3] However, side effects[4] and the clinical emergence of resistant mutants[5] suggest that there will be a need for an ever-increasing armament of new protease inhibitors, preferably with unique resistance profiles. Such inhibitors shall exhibit good bioavailability and be easy to synthesize at low cost.

We are engaged in a program where derivatized carbohydrates are employed as C_2-symmetric HIV-1 protease inhibitors.[6] Application of the concept of C-terminal duplication combined with suitable selection of the P2/P2′ substituents provided previously a series of potent protease inhibitors with P1/P1′ benzyloxy groups, e.g. **1**, which could be prepared in three steps from commercially available starting materials.[6d] With the objective of identifying inhibitors with improved antiviral activity, the X-ray crystal structure of a complex of HIV-1 protease with the inhibitor **1** was examined. Molecular modeling suggested that larger groups in the *para*-positions of the P1/P1′ benzyloxy side chains, located near the exterior surface of the active site and close to the solvent, could be accommodated readily. The *para*-position was anticipated to serve as a handle for modification aimed at improved antiviral activity. So far, the effect of modifications to the P1 (or P1′) side chain of linear nonsymmetrical inhibitors on enzyme inhibitory potency, antiviral activity, and oral bioavailability has been the subject of only a relatively limited number of studies.[7] The P1/P1′ structure−activity relationships were found to differ among the structural classes of inhibitors investigated, and unfortunately the predictability of the pharmacokinetic behavior is often poor.[7a,b,i]

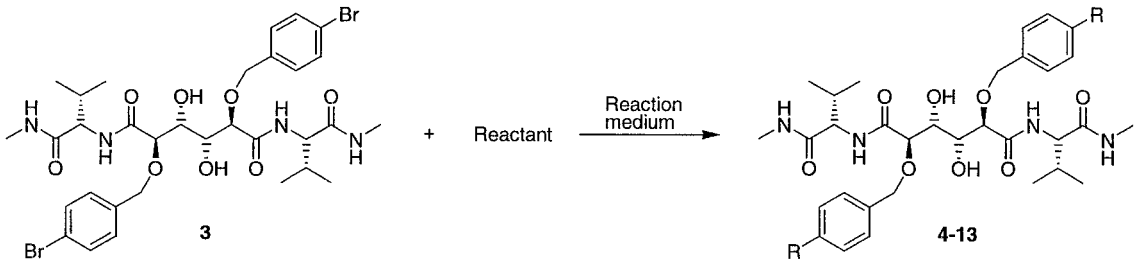
* Corresponding author.
[†] Department of Organic Pharmaceutical Chemistry, Uppsala University.
[‡] Department of Biochemistry, Uppsala University.
[§] Medivir AB.
[▽] Linköping University.
[⊥] Stockholm University.
[||] Department of Molecular Biology, Uppsala University.

10.1021/jm9910371 CCC: $18.00
Published on Web 09/03/1999

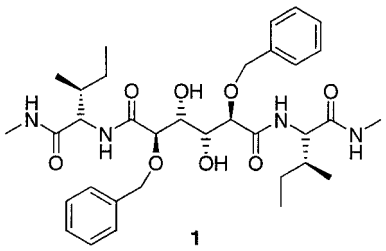
Scheme 1

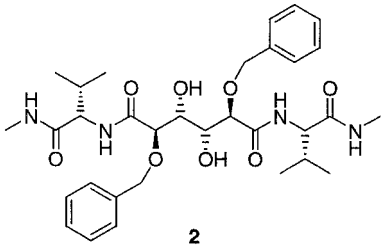


Scheme 2



We aimed at exploring the P1/P1′ sites of the carbohydrate-derived inhibitor **2**, an equipotent valine ana-





logue of **1**, and selected a variety of diverse functional groups to be coupled to the *para*-position of the P1/P1′ benzyloxy substituents. Herein we report rapid microwave-promoted palladium-catalyzed syntheses of a series of new P1/P1′-modified derivatives, **4**−**13**, all prepared from one single precursor. In addition to inhibition analysis on purified enzyme, the antiviral effect in cell culture using wild type and mutant virus and also the effect with 50% human serum are presented. Finally, the characteristic binding features of two of the inhibitors are discussed.

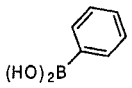
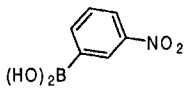
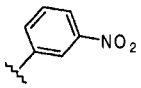
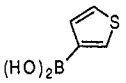
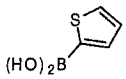
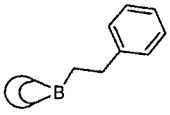
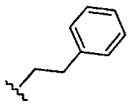
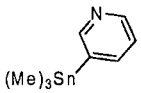
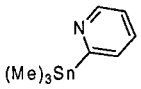
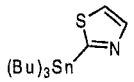
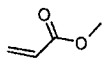
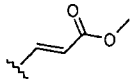
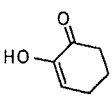
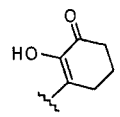
Result and Discussion

Chemistry. The synthesis of the bromo precursor **3** was accomplished, as depicted in Scheme 1, following a similar procedure to that previously reported for the synthesis of **1** and **2**.[6d] Thus, the L-1,4:3,6-mannarodilactone, prepared in one step from commercially available L-mannonic-γ-lactone, was alkylated in dioxane with 4-bromobenzyl imidate **14** to deliver **15**, which precipitated from the reaction medium and was eventually isolated in 88% yield. Subsequent treatment of **15** with excess valine methylamide in 1,2-dichloroethane afforded the bromo derivative **3**, which precipitated as a white powder and was isolated in 64% yield.

The target compounds **4**−**13** (Scheme 2) were prepared by palladium-catalyzed coupling reactions promoted by microwave irradiation[8] as shown in Table 1. We have recently studied flash heating with model compounds and devised a protocol that allowed the Suzuki, Stille, and Heck couplings to be completed in minutes[9] instead of hours or days[10] which is frequently required with traditional heating techniques. Here we demonstrate that additionally the more complex organopalladium precursor **3**, with potential for undesired water elimination, constitutes a suitable substrate in all of the reaction types.

The aryl- and heteroarylboronic acids were reacted with **3** in a sealed vessel in the presence of a catalytic amount of palladium tetrakis(triphenylphosphine) with sodium carbonate as base.[10b] Microwave irradiation at 45 W for 4 min in a mixture of dimethoxyethane, ethanol, and water allowed isolation of the compounds **4**−**7** in high yields. A microwave induced 9-BBN cou-

Table 1. Synthetic Methods for Preparation of C_2-Symmetric Carbohydrate-Derived Inhibitors Modified in P1/P1′, Enzyme Inhibition, and Antiviral Activity in Cell Culture

Cmpd. no.	Reactant	Reaction medium	Time (Min)	Effect (W)	R-group	Yield (%)	K_i[a] (nM)	ED_{50}[b] (μM)	ED_{50}mutants[c] (μM)	ED_{50}hs[d] (μM)
2							0.4	1.5	nd[e], 20[f]	20
3							0.3	0.8	1.2[e], 1.0[f]	2.1
4		$Pd(PPh_3)_4$, Na_2CO_3, DME, EtOH, H_2O	4	45		93	0.7	0.04	0.4[e], 0.7[f]	0.8
5		$Pd(PPh_3)_4$, Na_2CO_3, DME, EtOH, H_2O	4	45		85	1.4	0.05	1.1[e], 0.04[f]	1.1
6		$Pd(PPh_3)_4$, Na_2CO_3, DME, EtOH, H_2O	4	45		96	1.2	0.04	0.3[e], 0.2[f]	0.8
7		$Pd(PPh_3)_4$, Na_2CO_3, DME, EtOH, H_2O	4	45		86	1.2	0.04	0.3[e], 0.2[f]	1.0
8		$Pd(PPh_3)_4$, Na_2CO_3, DMF, THF	2	60		38	3.8	2.1	nd	nd
9		$Pd(PPh_3)_4$, DIEA, DMF, CuO	2	60		50	0.3	2.5	>3[e], >3[f]	20
10		$Pd(PPh_3)_4$, DIEA, DMF, CuO	2	60		54	0.6	0.8	nd	3.6
11		$Pd(PPh_3)_4$, DIEA, DMF, Ag_2O	2	60		53	0.6	0.3	>3[e], >3[f]	0.4
12		$Pd(OAc)_2$, $P(o\text{-tol})_3$, DMF, H_2O	2	60		76	0.09	0.8	nd	5.7
13		$Pd(OAc)_2$, $P(o\text{-tol})_3$, DMF, H_2O	48 h	100 °C[g]		52	0.3	3.7	nd	20

[a] Standard error 20%. [b] ED_{50} for reference substances tested in the same assay: ritonavir (ED_{50} 0.06 μM), indinavir (ED_{50} 0.06 μM), saqinavir (ED_{50} 0.01 μM), nelfinavir (ED_{50} 0.04 μM). [c] nd, not determined. [d] Compounds tested with 50% human AB+ serum. [e] Mutations in MT4/HIV-1: V32I, M46I, V82A. [f] Mutations in MT4/HIV-1: M46I, V82F, V84I. [g] Executed with traditional thermal heating.

pling, which has not been reported previously, was used for the preparation of **8**. Thus, phenylethyl 9-BBN was synthesized by hydroboration of styrene,[11] whereafter the bromo precursor **3**, palladium tetrakis(triphenylphosphine), and sodium carbonate were added to the reaction vessel. Exposure to microwave irradiation (60 W) for 2 min delivered **8,** although in moderate yield (38%).

Trimethylheteroaryl tin or tributylheteroaryl tin compounds were employed as coupling partners in the Stille reactions.[10c] The reactions were conducted at 60 W for

2 min in the presence of palladium tetrakis(triphenylphosphine) and with dimethylformamide as solvent to furnish **9**−**11**. In the coupling reactions with the pyridyl tin compounds, cupric oxide was employed as additive, but in the corresponding coupling with the thiazolyl tin derivative, silver(I) oxide was found to be more suitable.[12]

Heck reactions with methyl acrylate and with 1,2-cyclohexanedione[13] were conducted with microwave irradiation for 2 min (60 W) in aqueous dimethylformamide.[10a] The isolation of the dione **13** was not successful by standard chromatography. Starting material contaminated the product, and all efforts to achieve full conversion of **3**, e.g. increased power, prolonged reaction time, or addition of a second portion of catalyst, failed and led to formation of degradation products. Therefore the reaction was executed with traditional heating at 100 °C for 48 h to afford pure **13** in 53% yield.

It should be emphasized that the reaction protocols described here permit all reaction types to be performed with an aryl bromide. Thus, it is not necessary to employ the more reactive aryl iodides, as arylpalladium precursors, although aryl iodides should be useful also in flash heated reactions.[9]

HIV Protease Inhibition. The HIV-1 protease was cloned and heterologously expressed in *Escherichia coli* and purified as described elsewhere.[14] The K_i values for the synthesized compounds were determined by a fluorometric assay[15] (Table 1).

In Vitro Anti-HIV Activity. The anti-HIV activity was assayed in MT4 cells according to a previously published procedure[15] using the colorimetric XTT assay to monitor the cytopathogenic effects. Anti-HIV activity measured in the presence of human serum (hs) was performed in MT4 cells with human AB+ serum. Mutants were obtained by passaging cell-free virus in the presence of stepwise-increased concentrations of ritonavir[15] (Table 1).

Structure−Activity Relationships and X-ray Crystallographic Data. As suggested from modeling, all target compounds examined (**3**−**13**) exhibited good inhibitory potencies in the protease enzyme assay with K_i's in the nanomolar range (Table 1). We first replaced the P1 and P1′ phenyl groups with biphenyl moieties and thereby obtained an inhibitor (**4**) with a slightly higher K_i than that of **2**. More importantly, a 40-fold increase in antiviral activity was observed. The diphenylethane derivative **8** was prepared to map the region closer to the exterior of the enzymatic cleft. This modification resulted in an increased K_i value, and the antiviral activity dropped 50-fold as compared to **4**. This is contrary to expectations based on previous studies of related P1/ P1′ benzyloxyphenyl analogues, where the antiviral activity was often found to be enhanced strongly by this carbon chain extension.[7l] We decided to replace the *p*-hydrogen atom with various lipophilic, electron-rich, and electron-deficient heterocycles, with hydrogen bond-accepting capacities, since Thompson et al. have demonstrated with several examples that *p*-oxygen substituents in P1 or P1′ enhance cell penetration relative to the related parent compounds.[7m] Both the 2- and 3-substituted electron-rich thienyl compounds **6** and **7** were less potent than the parent compound **2**. However, both of these compounds demonstrated a pronounced antiviral activity (ED_{50} 0.04 μM) and were similar in activity to the phenyl analogue **4** (ED_{50} 0.04 μM), ritonavir (ED_{50} 0.06 μM), and indinavir (ED_{50} 0.06 μM), evaluated in the same assay system. Derivatives **9** and **10**, with electron-deficient weakly basic pyridine rings, which were expected to increase water solubility, were approximately equipotent to **2** in the protease assay but showed no obvious advantages in the cell system. An increase in antiviral activity was encountered with the thiazole **11**, as compared to the parent compound **2** and the pyridyl compounds **9** and **10**, corroborating with recent findings by Bold et al.[7a] Compound **5**, with electron-withdrawing 3-nitrophenyl groups in the *para*-positions but lacking basic nitrogens, was the weakest inhibitor in the series with respect to the enzyme assay. Despite this, the nitro compound, unlike the pyridyl compounds **9** and **10**, exhibited good antiviral activity in the cell system. The linear unsaturated ester **12**, on the other hand, which was the most potent enzyme inhibitor in the series is 20-fold less efficient than the nitro compound **5** in exerting an antiviral effect in the cell assay.

It was previously reported that a tethered polar carboxyl group attached to the *para*-position of a P1′ substituent gives high affinity to the protease but results in a low antiviral effect. This was anticipated to be attributed to poor cell penetration.[7b,e] A cyclic unsaturated hydroxy ketone, which combined hydrogen bond-donating and hydrogen bond-accepting capacity and was designed to be reminiscent of the classical hydroxyketocoumairn, a bioiostere of a carboxyl group,[16] was attached to the P1/ P1′ aryl groups. Although binding to the protease with good affinity was achieved with **13**, the antiviral effect (ED_{50} 3.7 μM) was still poor.

The most active compounds in the cell assay were evaluated further in cell culture with mutant virus, selected by increased concentrations of ritonavir. Three amino acids were substituted in the protease of the mutant virus used. All of the compounds **4**−**7** suffered a 10-fold decrease in antiviral activity with the mutant virus, except for compound **5**, which retained activity against one of the mutants. Despite this loss the inhibitors were in fact 20-fold more active than the lead compound **2** against one mutant virus. For comparison, compounds **3**, **9**, and **11** were also included in this study. The antiviral activity of compounds **9** and **11** was low against mutant virus, but the bromo compound **3** exhibited similar antiviral activity in all of the assays used.

Nonspecific protein binding of the inhibitors was assessed in a modified cell assay with 50% of human serum. A dramatic decrease of the antiviral activity was encountered for most of the tested inhibitors, e.g. more than a 20-fold drop in antiviral activity for the four most active compounds (**4**−**7**). Surprisingly, the antiviral activity of the thiazole derivative **11** remained at the same level in the presence of serum.

Compounds **4**, **6**, **7**, and **9** were chosen for evaluation of oral absorption in rats. At 30−40 mg/kg all of the compounds failed to attain measurable blood levels (<0.02 μg/mL). Nelfinavir tested in the same assay gave 1.9 μM at 26 mg/kg.

The 3-thienyl and 3-pyridyl compounds **6** and **9** were selected for cocrystallization with the HIV-1 protease

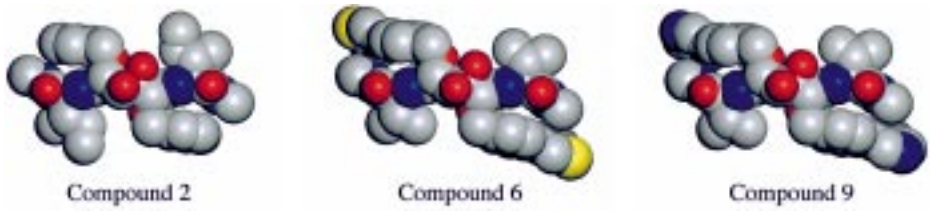


Figure 1. Compounds **1**, **6**, and **9** adopt the same overall conformation. The P1 and P2 arms are in contact with each other through close-packing interactions. In this figure the inhibitor compounds are rotated such that the central hydroxyl is pointing toward the reader. This figure was drawn with the program Molscript2.02.[18]

and crystallographic structure determination. The structures were determined with 2.1 and 2.0 Å resolutions for the complexes with the HIV-1 protease and compounds **6** and **9**, respectively. As expected, the previously determined compound **1** and compounds **6** and **9** have the same overall conformation (Figure 1). The aromatic side groups of the P1/P1′ arms pack closely to the backbone and side groups of the P2/P2′ arms. The inhibitors are chemically C_2-symmetrical with two central hydroxyl groups, which both could be used alternatively for the binding to the catalytic aspartate residues. Due to the 3D conformation of the inhibitor, only one of these hydroxyls can be utilized for binding to the aspartates. This arrangement leads to an asymmetric binding of the inhibitor to the protease, which in turn induces deviations from exact symmetrical arrangement of the protein monomers. Interestingly, only one arrangement of the hydroxyls is found in the crystal structure. The deviation from exact symmetry is also revealed by the slightly different conformations of the P1/P1′ arms and the waters and residues surrounding them.

Comparison of the three protease inhibitor structures shows how the water molecules at the entrances to the S1/S1′ sites, which are also at the entrance to the S3/S3′ sites, are displaced by the penetrating thienyl and pyridyl groups (Figure 2). In the complex of compound **1** with the protease, four water molecules are firmly bound at the entrance at each side of the protease dimer. These water molecules are linked to each other in a hydrogen bond network, which also involves Arg8/108. The thienyl and pyridyl rings of the extended P1/P1′ arms displace three of these water molecules on both sides. In the compound **6** structure (Figure 2c,d), two new water molecules are bound to the thienyl ring and Arg8/108. Since the K_i values of compounds **2**, **6**, and **9** do not differ significantly, it could be concluded that the net entropy, from release of the waters and binding the larger compounds **6** and **9**, seems to balance the net enthalpy from loss of the water hydrogen bonds and creations of the thienyl and pyridyl interactions.[17] The structures also demonstrate that these elongated P1/P1′ arms fill out the entrances completely (Figure 2). The electron density for the thiophene ring does not uniquely define the position for the sulfur atom. This observation indicates that the thiophene ring may be bound in two orientations, with approximately equal occupancy in both orientations. Whether this is also the situation for the pyridine cannot be excluded. However, the positions of coordinated water molecules support the orientation represented in Figures 1 and 2.

The protein backbone and side chains contract slightly at the entrances to the S1/S1′−S3/S3′ sites as the result

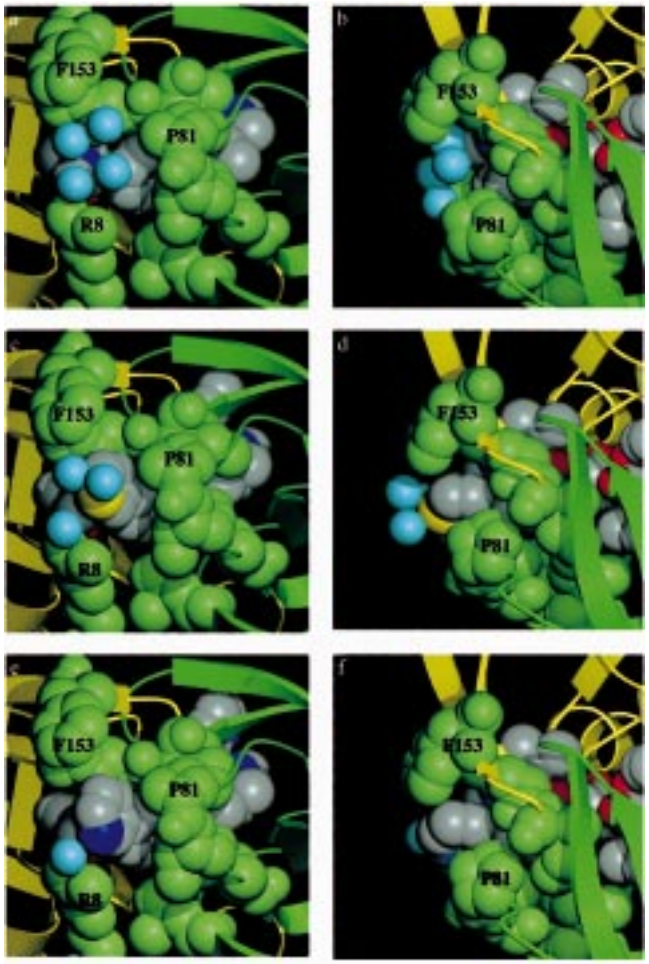


Figure 2. Displacement of water molecules to fill the entrance to the S1/S1′−S3/S3′ sites. In green are marked amino acids, which make up the entrance and are in contact with the inhibitors. In the structure of compound **1** in complex with the protease, four water molecules (light blue) are bound through a hydrogen bond network covering the entrance (a,b). Filling out the entrance by extension of the P1/P1′ inhibitor arms with thienyl (compound **6**, c,d) and pyridyl (compound **9**, e,f) results in displacement of water molecules. The view in panels b, d, and f is 90° away from the view in panels a, c, and d and from the top of the molecule. This figure was drawn with the program Molscript2.02.[18]

of interactions with the elongated P1/P1′ arms. Due to close-packing interactions between Pro81/181 and the thienyl and pyridyl groups, the loop aa78−83/178−183 has moved 0.3−0.4 Å toward the P1/P1′ arms. Interactions with backbone atoms of Gly47/147 and the side chain of Phe53/153 have caused the flaps (aa47−53/147−153) to move 0.2−0.5 Å. In the compound **6** complex, the side chain of Arg8/108 has moved toward the thienyl groups and packs at van der Waals distances

to the aromatic ring. In the compound **9** complex, Arg8/108 has moved away from the inhibitor. The structure studies of these protease inhibitor complexes demonstrate the flexibility in the protease molecule, which confirms a previous observation by Nugiel et al. who studied the same region of the protease.[7d]

Conclusion

In summary, application of the concept of C-terminal duplication and concomitant modifications of the P1/P1′ positions has led to the potent protease inhibitors **4**−**7** with a 30-fold improvement of the antiviral effect as compared to the parent compound **2** in vitro. These compounds were also consistently more potent than **2** in the presence of human serum. With regard to HIV protease inhibition, it has been demonstrated that a wide variety of groups are tolerated in P1/P1′ and that the inhibitory potency in the enzyme assay is largely retained in all of the compounds synthesized. We observed no significant alteration in the K_i values as a result of a possible water displacement from the S1/S1′ sites by thienyl and pyridyl groups. This is consistent with the view of the *para*-positions of P1/P1′ being essentially "neutral" to the thermodynamics of enzyme binding.[7m] We conclude that the K_i values did not always translate to antiviral activity in the cell-based assay and that the poorest enzyme inhibitors **5** and **6**, although still inhibiting in the nanomolar range, belong to the group with the best antiviral effect. Furthermore, we have developed fast and flexible protocols for the synthesis of the inhibitors, starting from a commercially available carbohydrate and relying on palladium-catalyzed coupling reactions promoted by microwave irradiation as key reactions. These reactions constitute the first example of the application of palladium-catalyzed reactions conducted with flash heating to the area of medicinal chemistry of which we are aware. We foresee that this heating technique will find many applications in particular in the context of combinatorial chemistry.

Experimental Section

Chemistry. General Information. Melting points were recorded on an electrothermal melting point apparatus and are uncorrected. Optical rotations were obtained on a Perkin-Elmer 241 polarimeter. Specific rotations ($[\alpha]_D$) are reported in deg/dm, and the concentration (*c*) is given in g/100 mL in the specified solvent. ^{1}H and ^{13}C NMR spectra were recorded on a Jeol JNM-EX 270 spectrometer at 270.2 and 67.8 MHz, respectively. Chemical shifts are given as δ values (ppm) downfield from tetramethylsilane. Infrared spectra were recorded on a Perkin-Elmer 1600 series FTIR instrument. Elemental analyses were performed by Mikro Kemi AB, Uppsala, Sweden, or Analytische Laboratorien, Lindlar, Germany, and were within ±0.4% of calculated values. Column chromatography was performed on silica gel 60 (0.04−0.063 mm; E. Merck). Circular chromatography was performed on Chromatotron 7924T (TC Research) with 1-mm thick silica gel 60 (0.04−0.063 mm; E. Merck) plates and gradient elution. Thin-layer chromatography was performed on precoated silica gel F-254 plates (0.25 mm; E. Merck) and visualized with UV light and H_2SO_4 in ethanol or ninhydrin. The palladium tetrakis(triphenylphosphine) was freshly made according to procedures described by Heck.[19] All microwave reactions were carried out in heavy-walled Pyrex tubes, sealed with screw-cap-fitted Teflon septa. The microwave treatment was performed with a MicroWell 10 single-mode cavity (Labwell AB, Uppsala, Sweden). It is not recommended to repeat these reactions in a multimode domestic microwave oven producing nonuniform irradiation. **Caution! It is important to note that when carrying out microwave-heated reactions in closed vessels, quite large pressures may build up, and therefore it is imperative that an appropriate septum is utilized as a pressure relief device.** Standard workup: organic layers were dried with $MgSO_4$ and concentrated in vacuo.

(4-Bromobenzyl)-2,2,2-trichloroacetimidate (14). To a stirred solution of 4-bromobenzyl alcohol (50.0 g, 0.267 mmol) in toluene (250 mL) under a nitrogen atmosphere was slowly added sodium (1.23 g, 0.0535 mmol). The solution was left for 20 min. To this was added 2,2,2-trichloroacetonitrile (26.7 mL, 0.267 mmol). The slight exothermic reaction was stirred for 1 h at room temperature. The orange-colored mixture was filtered through a glass funnel and concentrated. Drying in vacuo gave 84.3 g (95%) of (4-bromobenzyl)-2,2,2-trichloroacetimidate as a slightly grayish solid. The product is 95% pure (^{1}H NMR) and can be used without further purification: IR (KBr) ν 3332, 1668 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.41 (br s, 1H), 7.51 (m, 2H), 7.31 (m, 2H), 5.29 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 162.3, 138.4, 131.7, 129.4, 122.3, 69.9. A small portion was recrystallized from toluene and submitted for elemental analysis. Anal. ($C_9H_7Cl_3NO\cdot 1/8H_2O$) C, H, N.

2,5-*O*-Bis(4-bromobenzyl)-L-mannaro-1,4:3,6-dilactone (15). A dried 1-L round flask was charged with L-1,4:3,6-mannarodilactone (5.00 g, 28.7 mol) and dry dioxane (400 mL) under nitrogen a atmosphere. The mixture was heated until the dilactone was completely dissolved, and then the oil bath was removed. To the stirred solution were added a small portion of triflic acid (0.1 mL) and (4-bromobenzyl)-2,2,2-trichloroacetimidate (28.6 g, 86.2 mmol). The rest of the triflic acid (0.6 mL) was added dropwise under vigorous stirring. The exothermic reaction was stirred for 2 h. The product precipitated from the solution and was collected by filtration. The white crystals were washed with dioxane and dried in vacuo. Additional product was collected by filtering the filtrate through a funnel with 2 cm of $NaHCO_3$ and 2 cm of silica. The filtrate was concentrated and dried in vacuo overnight. The remaining solid was washed with diethyl ether (100 mL). The ether was decanted off, and the washing procedure was repeated three times. The product was filtered and dried in vacuo to give totally 12.9 g (88%) of compound **15** as white crystals: IR (KBr) ν 3050, 2882, 1797 cm^{-1}; $[\alpha]_D = -110°$ (c = 1.02, DMSO, 22 °C); ^{1}H NMR (DMSO-d_6) δ 7.60 (d, J = 8.25 Hz, 4H), 7.36 (d, J = 8.25 Hz, 4H), 5.25 (d, J = 3.6 Hz, 2H), 4.89 (d, J = 3.6 Hz, 2H) 4.75 (m, 4H); ^{13}C NMR (DMSO-d_6) δ 171.7, 136.4, 131.6, 130.1, 121.2, 74.9, 74.4, 71.3. Anal. ($C_{20}H_{16}Br_2O_6$) C, H.

***N*1,*N*6-Bis[(1*S*)-2-methyl-1-(methylcarbamoyl)propyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-bis(4-bromobenzyloxy)-3,4-dihydroxyhexanediamide (3).** Compound **15** (6.00 g, 11.7 mmol) was dissolved in 1,2-dichloroethane (100 mL). To the solution was added 6 equiv of valine methylamide (9.15 g, 70.3 mmol). The flask was sealed with a silicon septum and heated to 50 °C for 48 h. The white product precipitated from the solution and was collected by filtration. The product was washed with $CHCl_3$ (30 mL) and dried in vacuo to give 5.79 g (64%) of compound **3** as off-white crystals: IR (KBr) ν 3290, 3099, 2962, 1642 cm^{-1}; $[\alpha]_D = +3.9°$ (c = 1.03, DMSO, 22 °C); ^{1}H NMR (DMSO-d_6) δ 7.88 (q, J = 4.6 Hz, 2H), 7.72 (d, J = 8.9 Hz, 2H), 7.52 (d, J = 8.6 Hz, 4H), 7.28 (d, J = 8.6 Hz, 4H), 4.81 (d, J = 7.6, 2H), 4.43 (s, 4H), 4.16 (dd, J = 8.9, 6.6 Hz, 2H), 3.99 (d, J = 7.9 Hz, 2H), 3.83 (app d, 2H), 2.59 (d, J = 4.6 Hz, 6H), 1.97 (m, 2H), 0.85 (d, J = 6.6 Hz, 6H), 0.82 (d, J = 6.9 Hz, 6H); ^{13}C NMR (DMSO-d_6) δ 171.1, 170.4, 137.5, 131.1, 129.7, 120.6, 79.3, 70.3, 69.7, 57.7, 30.6, 25.5, 19.3, 18.2. A small portion was recrystallized from $CHCl_3$ to give **3** as off-white crystals, which were subjected to elemental analysis. Anal. ($C_{32}H_{44}Br_2N_4O_8$) C, H, N.

General Method for the Preparation of Compounds 4−7. Method I. A mixture of **3** (30 mg, 0.039 mmol), arylboronic acid (0.19 mmol), 2 M aqueous $NaCO_3$ (39 μL, 0.078 mmol), tetrakis(triphenylphosphine)palladium (2.2 mg, 0.0019

mmol), EtOH (60 μL), H_2O (80 μL), and 1,2-dimethoxyethane (240 μL) was placed in a Pyrex tube and degassed under a nitrogen flow for 5 min. The tube was sealed with a Teflon septum and irradiated in a microwave reactor for 4 min at 45 W. The reaction mixture was allowed to cool and then diluted with $CHCl_3$ (50 mL). The organic layer was washed with saturated aqueous $NaHCO_3$ (3 × 20 mL), dried, filtered, and concentrated. The crude product was purified by circular chromatography (CH_2Cl_2 → CH_2Cl_2/MeOH (20:1)) to give pure **4**−**7**.

***N*1,*N*6-Bis[(1*S*)-2-methyl-1-(methylcarbamoyl)propyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-bis(4-phenylbenzyloxy)-3,4-dihydroxyhexanediamide (4).** The title compound was prepared in 93% yield (28 mg, 0.036 mmol) according to method I, using phenylboronic acid (24 mg): IR (KBr) ν 3305, 3080, 2957, 1649 cm^{-1}; $[\alpha]_D = +1.9°$ ($c = 0.99$, DMSO, 21 °C); ^1H NMR ($CDCl_3$) δ 7.58 (m, 8H), 7.44 (m, 10H), 7.25 (d, $J = 9.2$ Hz, 2H), 7.03 (q, $J = 4.3$ Hz, 2H), 5.27 (br s, 2H), 4.71 (m, 4H), 4.35 (dd, $J = 9.2$, 4.3 Hz, 2H), 4.21 (br s, 2H), 4.15 (br s, 2H), 2.68 (d, $J = 4.6$ Hz, 6H), 2.49 (m, 2H), 0.94 (d, $J = 6.6$ Hz, 6H), 0.83 (d, $J = 6.6$ Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 172.5, 170.6, 141.7, 140.5, 135.3, 129.0, 128.7, 127.7, 127.7, 127.2, 82.0, 73.6, 73.3, 58.4, 29.0, 26.2, 19.8, 17.1. Anal. ($C_{44}H_{54}N_4O_8$) C, H, N.

***N*1,*N*6-Bis[(1*S*)-2-methyl-1-(methylcarbamoyl)propyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-bis[4-(3-nitrophenyl)benzyloxy]-3,4-dihydroxyhexanediamide (5).** The title compound was prepared in 85% yield (28 mg, 0.033 mmol) according to method I, using 3-nitrophenylboronic acid (32 mg): IR (KBr) ν 3286, 3083, 2962, 1647 cm^{-1}; $[\alpha]_D = -6.5°$ ($c = 1.00$, DMSO, 22 °C); ^1H NMR (DMSO-d_6) δ 8.41 (m, 2H), 8.21 (dd, $J = 7.9$, 1.5 Hz, 2H), 8.13 (d, $J = 8.4$ Hz, 2H), 7.91 (q, $J = 4.7$ Hz, 2H), 7.8−7.7 (m, 8H), 7.48 (d, $J = 8.3$ Hz, 4H), 4.87 (d, $J = 7.4$ Hz, 2H), 4.54 (s, 4H), 4.19 (dd, $J = 6.6$, 8.8 Hz, 2H), 4.07 (d, $J = 7.8$ Hz, 2H), 3.91 (app t, 2H), 2.60 (d, $J = 4.6$ Hz, 6H), 1.92 (m, 2H), 0.86 (d, $J = 6.6$ Hz, 6H), 0.84 (d, $J = 6.6$ Hz, 6H); ^{13}C NMR (DMSO-d_6) δ 171.1, 170.4, 148.4, 141.5, 138.5, 136.9, 133.2, 130.4, 128.4, 126.8, 122.1, 120.9, 79.6, 70.7, 69.8, 57.6, 30.6, 25.4, 19.2, 18.1. Anal. ($C_{44}H_{52}N_6O_{12}\cdot H_2O$) C, H, N.

***N*1,*N*6-Bis[(1*S*)-2-methyl-1-(methylcarbamoyl)propyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-bis[4-(3-thienyl)benzyloxy]-3,4-dihydroxyhexanediamide (6).** The title compound was prepared in 96% yield (29 mg, 0.037 mmol) according to method I, using 3-thiopheneboronic acid (25 mg): IR (KBr) ν 3291, 3100, 2962, 1645 cm^{-1}; $[\alpha]_D = -5.2°$ ($c = 1.00$, DMSO, 22 °C); ^1H NMR (DMSO-d_6) δ 7.89 (q, $J = 4.3$ Hz, 2H), 7.84 (dd, $J = 3.0$, 1.5 Hz, 2H), 7.72 (d, $J = 9.1$ Hz, 2H), 7.68 (d, $J = 7.9$ Hz, 4H), 7.62 (dd, $J = 5.0$, 3.0 Hz, 2H), 7.54 (dd, $J = 5.1$, 1.5 Hz, 2H), 7.36 (d, $J = 7.9$ Hz, 4H), 4.86 (d, $J = 7.3$ Hz, 2H), 4.48 (s, 4H), 4.19 (dd, $J = 6.4$, 9.0 Hz, 2H), 4.04 (d, $J = 7.4$ Hz, 2H), 3.90 (app t, 2H), 2.60 (d, $J = 4.3$ Hz, 6H), 1.91 (m, 2H), 0.86 (d, $J = 6.3$ Hz, 6H), 0.84 (d, $J = 6.3$ Hz, 6H); ^{13}C NMR (DMSO-d_6) δ 171.1, 170.4, 141.4, 137.0, 134.4, 128.1, 127.0, 126.1, 125.9, 120.8, 79.6, 71.7, 69.8, 57.3, 30.3, 24.7, 19.2, 18.1. Anal. ($C_{40}H_{50}N_4O_8S_2\cdot 1/2H_2O$) C, H, N.

***N*1,*N*6-Bis[(1*S*)-2-methyl-1-(methylcarbamoyl)propyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-bis[4-(2-thienyl)benzyloxy]-3,4-dihydroxyhexanediamide (7).** The title compound was prepared in 86% yield (26 mg, 0.034 mmol) according to method I, using 2-thiopheneboronic acid (25 mg): IR (KBr) ν 3304, 2960, 1647 cm^{-1}; $[\alpha]_D = -0.1°$ ($c = 0.89$, DMSO, 21 °C); ^1H NMR (DMSO-d_6) δ 7.89 (q, $J = 4.5$ Hz, 2H), 7.72 (d, $J = 9.0$ Hz, 2H), 7.62 (d, $J = 8.3$ Hz, 4H), 7.53 (dd, $J = 5.1$, 1.2 Hz, 2H), 7.49 (dd, $J = 3.4$, 1.2 Hz, 2H), 7.37 (d, $J = 8.3$ Hz, 4H), 7.12 (dd, $J = 3.6$, 5.1 Hz, 2H), 4.83 (d, $J = 7.1$ Hz, 2H), 4.48 (s, 4H), 4.16 (dd, $J = 8.6$, 6.6 Hz, 2H), 4.03 (d, $J = 7.6$ Hz, 2H), 3.88 (app t, 2H), 2.59 (d, $J = 4.6$ Hz, 6H), 1.97 (m, 2H), 0.85 (d, $J = 6.6$ Hz, 6H), 0.83 (d, $J = 6.8$ Hz, 6H); ^{13}C NMR (DMSO-d_6) δ 171.1, 170.4, 143.1, 137.3, 133.0, 131.1, 129.7, 128.3, 125.2, 123.7, 79.3, 70.7, 69.5, 57.6, 30.6, 25.4, 19.3, 18.1. Anal. ($C_{40}H_{50}N_4O_8S_2$) C, H, N.

***N*1,*N*6-Bis[(1*S*)-2-methyl-1-(methylcarbamoyl)propyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-bis[4-(2-phenylethyl)benzyloxy]-3,4-dihydroxyhexanediamide (8).** A dried Pyrex tube was charged with styrene (22 μL, 0.19 mmol) and THF (20 μL) under a nitrogen atmosphere and then cooled on an ice bath. 9-BBN (0.5 M) in THF (390 μL, 0.19 mmol) was added via a syringe through a silicon septum. The reaction mixture was allowed to reach room temperature and stirred for 4 h. To the reaction mixture were added **3** (30 mg, 0.039 mmol), K_2CO_3 (21 mg, 0.16 mmol), tetrakis(triphenylphosphine)palladium (2.7 mg, 0.0023 mmol), and DMF (1 mL) under nitrogen atmosphere. The stir bar was removed from the mixture, and the tube was sealed with a Teflon septum and irradiated in the microwave reactor for 2 min at 60 W. After cooling, $CHCl_3$ (50 mL) was added to the reaction mixture. The organic layer was separated and subsequently washed with saturated aqueous $NaHCO_3$ (3 × 20 mL), dried, filtered, and concentrated. The crude product was purified by circular chromatography (CH_2Cl_2 → CH_2Cl_2/MeOH (20:1)) to give pure **8** (12 mg, 0.015 mmol) in 38% yield: IR (KBr) ν 3306, 3087, 2926, 1650 cm^{-1}; $[\alpha]_D = -9.1°$ ($c = 1.21$, $CHCl_3$, 21 °C); ^1H NMR (DMSO-d_6) δ 7.88 (q, $J = 4.6$ Hz, 2H), 7.68 (d, $J = 9.0$ Hz, 2H), 7.29−7.13 (m, 18H), 4.82 (d, $J = 7.2$ Hz, 2H), 4.42 (s, 4H), 4.18 (d, $J = 9.0$, 6.4 Hz, 2H), 3.99 (d, $J = 7.4$ Hz, 2H), 3.85 (app t, 2H), 2.85 (s, 8H), 2.59 (d, $J = 4.6$ Hz, 6H), 1.98 (m, 2H), 0.85 (d, $J = 6,5$ Hz, 6H), 0.83 (d, $J = 6.5$ Hz, 6H); ^{13}C NMR (DMSO-d_6) δ 171.0, 170.5, 141.4, 140.8, 135.4, 128.3, 128.2, 127.6, 125.7, 79.4, 71.1, 69.9, 57.5, 37.0, 36.7, 30.5, 25.4, 19.2, 18.1. Anal. ($C_{48}H_{62}N_4O_8$) C, H, N.

General Method for the Preparation of Compounds 9−11. Method II. A mixture of **3** (30 mg, 0.039 mmol), aryltrialkyltin (0.19 mmol), tetrakis(triphenylphosphine)-palladium (2.2 mg, 0.0019 mmol), metal oxide (0.039 mmol), and DMF (1 mL) was placed in a Pyrex tube and degassed under a nitrogen flow for 5 min. The tube was sealed with a Teflon septum and irradiated in a microwave reactor for 2 min at 60 W. After cooling, $CHCl_3$ (50 mL) was added to the reaction mixture. The organic layer was separated and subsequently washed with saturated aqueous $NaHCO_3$ (3 × 20 mL), dried, filtered, and concentrated. The crude product was dissolved in acetonitrile (100 mL), washed with isohexane (3 × 20 mL), concentrated, and then purified by circular chromatography (CH_2Cl_2 → CH_2Cl_2/MeOH (9:1)) to give pure **9**−**11**.

***N*1,*N*6-Bis[(1*S*)-2-methyl-1-(methylcarbamoyl)propyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-bis[4-(3-pyridyl)benzyloxy]-3,4-dihydroxyhexanediamide (9).** The title compound was prepared in 50% yield (15 mg, 0.020 mmol) according to method II, using trimethyl-3-pyridyltin[20] (47 mg) and CuO (3.1 mg): IR (KBr) ν 3432, 3080, 2921, 1650 cm^{-1}; $[\alpha]_D = -5.0°$ ($c = 1.4$, DMSO, 21 °C); ^1H NMR (DMSO-d_6) δ 8.87 (dd, $J = 0.7$, 2.3 Hz, 2H), 8.56 (dd, $J = 1.7$, 4.6 Hz, 2H), 8.05 (ddd, $J = 7.9$, 2.5, 1.7 Hz, 2H), 7.90 (q, $J = 4.7$ Hz, 2H), 7.75 (d, $J = 8.9$ Hz, 2H), 7.70 (d, $J = 8.3$ Hz, 3H), 7.48 (m, 2H), 7.46 (d, $J = 8.3$ Hz, 2H), 4.85 (d, $J = 7.4$ Hz, 2H), 4.52 (s, 4H), 4.18 (dd, $J = 8.9$, 6.5 Hz, 2H), 4.06 (d, $J = 7.9$ Hz, 2H), 3.89 (app t, 2H), 2.59 (d, $J = 4.5$ Hz, 6H), 1.98 (m, 2H), 0.86 (d, $J = 6.7$ Hz, 6H), 0.83 (d, $J = 6.7$ Hz, 6H); ^{13}C NMR (DMSO-d_6) δ 171.1, 170.4, 148.4, 147.6, 138.0, 136.2, 135.3, 134.0, 128.3, 126.7, 123.8, 79.4, 70.7, 69.8, 57.6, 30.5, 25.4, 19.2, 18.1. Anal. ($C_{42}H_{52}N_6O_8\cdot H_2O$) C, H, N.

***N*1,*N*6-Bis[(1*S*)-2-methyl-1-(methylcarbamoyl)propyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-bis[4-(2-pyridyl)benzyloxy]-3,4-dihydroxyhexanediamide (10).** The title compound was prepared in 54% yield (16 mg, 0.021 mmol) according to method II, using trimethyl-2-pyridyltin[20] (47 mg) and CuO (3.1 mg): IR (KBr) ν 3304, 3085, 2959, 1650 cm^{-1}; $[\alpha]_D = -4.3°$ ($c = 0.47$, MeOH, 21 °C); ^1H NMR (DMSO-d_6) δ 8.65 (ddd, $J = 4.8$, 1.2, 0.8 Hz, 2H), 8.05 (d, $J = 8.3$ Hz, 4H), 7.96−7.82 (m, 6H), 7.76 (d, $J = 8.9$ Hz, 2H), 7.45 (d, $J = 8.3$ Hz, 4H), 7.34 (ddd, $J = 7.2$, 4.8, 1.3 Hz, 2H), 4.87 (d, $J = 7.3$ Hz, 2H), 4.53 (s, 4H), 4.19 (dd, $J = 8.8$, 6.8 Hz, 2H), 4.06 (d, $J = 7.6$ Hz, 2H), 3.93 (app t, 2H), 2.60 (d, $J = 4.5$ Hz, 6H), 1.98 (m, 2H), 0.86 (d, $J = 6.7$ Hz, 6H), 0.83 (d, $J = 6.6$ Hz, 6H); ^{13}C NMR (DMSO-d_6) δ 171.1, 170.4, 155.7, 149.5, 138.9, 137.9, 137.2, 127.8, 126.3, 122.5, 120.1, 79.5, 70.8, 69.7, 57.6, 30.5, 25.4, 19.2, 18.1. Anal. ($C_{42}H_{52}N_6O_8\cdot 1/2H_2O$) C, H, N.

***N*1,*N*6-Bis[(1*S*)-2-methyl-1-(methylcarbamoyl)propyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-bis[4-(2-thiazolyl)benzyloxy]-3,4-dihydroxyhexanediamide (11).** The title compound was prepared

in 53% yield (16 mg, 0.021 mmol) according to method II, using tributyl-2-thiazolyltin (73 mg) and Ag_2O (9.0 mg): IR (KBr) ν 3363, 3081, 2960, 1650 cm^{-1}; $[\alpha]_D = -2.3°$ ($c = 0.47$, DMSO, 21 °C); ^1H NMR ($CD_3OD-CDCl_3$, 3:1) δ 7.90 (d, $J = 8.3$ Hz, 4H), 7.82 (d, $J = 3.3$ Hz, 2H), 7.49 (d, $J = 3.3$ Hz, 2H), 7.48 (d, $J = 8.3$ Hz, 4H), 4.68 (s, 4H), 4.24 (q, $J = 5.9$ Hz, 2H), 4.18 (m, 2H), 4.10 (m, 2H), 2.72 (d, $J = 4.6$ Hz, 6H), 2.19 (m, 2H), 0.92 (d, $J = 6.9$ Hz, 6H), 0.88 (d, $J = 6.6$ Hz, 6H); ^{13}C NMR ($CD_3OD-CDCl_3$, 3:1) δ 173.3, 172.7, 170.0, 144.0, 140.4, 133.8, 129.4, 127.5, 120.4, 81.1, 72.8, 72.2, 59.3, 31.1, 26.5, 19.9, 18.1. Anal. ($C_{38}H_{48}N_6O_8S_2$) C, H, N.

***N*1,*N*6-Bis[(1*S*)-2-methyl-1-(methylcarbamoyl)propyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-bis{4-[(*E*)-2-methoxycarbonylethenyl]-benzyloxy}-3,4-dihydroxyhexanediamide (12).** A mixture of **3** (30 mg, 0.039 mmol), methyl acrylate (35 μg, 0.39 mmol), diisopropylethylamine (27 μL, 0.16 mmol), $Pd(OAc)_2$ (0.87 mg, 0.0039 mmol), PPh_3 (2.6 mg, 0.093 mmol), H_2O (0.15 mL), and DMF (0.85 mL) was added to a Pyrex tube and degassed under a nitrogen flow for 5 min. The tube was sealed with a Teflon septum and irradiated in a microwave reactor for 2 min at 60 W. After cooling, $CHCl_3$ (50 mL) was added to the reaction mixture. The organic layer was separated and subsequently washed with saturated aqueous $NaHCO_3$ (3 × 20 mL), dried, filtered, and concentrated. The crude product was purified by circular chromatography ($CH_2Cl_2 \rightarrow CH_2Cl_2$/MeOH (20:1)) to give pure **12** (23 mg, 0.029 mmol) in 76% yield: IR (KBr) ν 3307, 3098, 2960, 1650 cm^{-1}; $[\alpha]_D = -6.7°$ ($c = 1.00$, DMSO, 21 °C); ^1H NMR (DMSO-d_6) δ 7.88 (q, $J = 4.7$ Hz, 2H), 7.74 (d, $J = 8.9$ Hz, 2H), 7.68 (d, $J = 8.1$ Hz, 4H), 7.65 (d, $J = 15.7$ Hz, 2H), 7.37 (d, $J = 8.1$ Hz, 4H), 6.62 (d, $J = 16.2$ Hz, 2H), 4.84 (d, $J = 7.4$ Hz, 2H), 4.49 (s, 4H), 4.17 (dd, $J = 6.4$, 9.0 Hz, 2H), 4.02 (d, $J = 7.6$ Hz, 2H), 3.86 (app t, 2H), 2.59 (d, $J = 4.6$ Hz, 6H), 1.97 (m, 2H), 0.84 (d, $J = 6.4$ Hz, 6H), 0.82 (d, $J = 6.6$ Hz, 6H); ^{13}C NMR (DMSO-d_6) δ 171.1, 170.3, 166.6, 144.2, 140.6, 133.2, 128.2, 127.9, 117.6, 79.4, 70.6, 69.7, 57.6, 51.4, 30.5, 25.4, 19.2, 18.1. Anal. ($C_{40}H_{54}N_4O_{12}\cdot H_2O$) C, H, N.

***N*1,*N*6-Bis[(1*S*)-2-methyl-1-(methylcarbamoyl)propyl](2*R*,3*R*,4*R*,5*R*)-2,5-bis[4-(3-cyclohexane-1,2-dionyl)-benzyloxy]-3,4-dihydroxyhexanediamide (13).** A mixture of **3** (30 mg, 0.039 mmol), 1,2-cyclohexanedione (87 mg, 0.78 mmol), diisopropylethylamine (27 μL, 0.16 mmol), $Pd(OAc)_2$ (0.87 mg, 0.0039 mmol), P(o-tol)$_3$ (2.8 mg, 0.093 mmol), H_2O (0.15 mL), and DMF (0.85 mL) was added to a Pyrex tube with a magnetic stir bar and degassed under a nitrogen flow for 5 min and sealed. The reaction was heated to 100 °C and stirred for 48 h. After cooling, $CHCl_3$ (50 mL) was added to the reaction mixture. The organic phase was separated and subsequently washed with saturated aqueous $NaHCO_3$ (3 × 20 mL), dried, filtered, and concentrated. The crude product was purified on column chromatography ($CHCl_3$/MeOH (20:1)) to give pure **13** (17 mg, 0.020 mmol) in 52% yield: IR (KBr) ν 3314, 3106, 2960, 1652 cm^{-1}; $[\alpha]_D = -7.3°$ ($c = 1.01$, $CHCl_3$, 19 °C); ^1H NMR (DMSO-d_6) δ 8.39 (s, 2H), 7.89 (q, $J = 4.6$ Hz, 2H), 7.72 (d, $J = 9.0$ Hz, 2H), 7.67 (d, $J = 8.3$ Hz, 4H), 7.34 (d, $J = 8.3$ Hz, 4H), 4.83 (d, $J = 7.3$ Hz, 2H), 4.48 (s, 4H), 4.18 (dd, $J = 8.9$, 6.4 Hz, 2H), 3.88 (app t, 2H), 2.70 (m, 2H), 2.60 (d, $J = 4.5$ Hz, 6H), 2.52 (m, 2H), 1.98 (m, 4H), 0.85 (d, $J = 6.3$ Hz, 6H), 0.83 (d, $J = 5.6$ Hz, 6H); ^{13}C NMR (DMSO-d_6) δ 195.0, 171.1, 170.4,144.1, 137.4, 137.1, 128.1, 127.5, 127.0, 79.5, 70.9, 69.9, 57.6, 36.4, 30.5, 28.4, 25.4, 22.3, 19.3, 18.1. Anal. ($C_{44}H_{58}N_4O_{12}\cdot 2H_2O$) C, H, N.

In Vitro Anti-HIV Activity. The anti-HIV activity was assayed in MT4 cells according to a previously reported procedure[15] using the colorimetric XTT assay to monitor the cytopathogenic effects. Briefly, MT4 cells were seeded into 96-well plates (2×10^4 cells/well) and infected with HIV-1, IIIb (10−50 $TCID_{50}$/well). Test compounds were dissolved in DMSO to 10 mg/mL stock solutions, further diluted in medium, and added in the desired concentration to the cells. The cell viability was measured after 6 days by the XTT method, and the 50% dose (ED_{50}) was determined.

The anti-HIV activity measurements in the presence of human serum were performed with MT4 cells that were infected with HIV, IIIb as described above. Test compounds were diluted in human AB+ serum (Sigma) and added to the cells after 1 h of virus adsorption. The final medium contained 50% human serum and 10% fetal calf serum. The viral replication was analyzed after 6 days by determining the amount of HIV p24 antigen.

Mutant HIV was achieved by passaging cell-free virus in the presence of stepwise increased concentrations of ritonavir. When virus was grown in the highest possible nontoxic concentration, it was passaged once in medium without compound and the supernatant was frozen in aliquots at −70 °C. The cellular DNA was analyzed with respect to the nucleotide sequence of the HIV protease gene.

Oral Absorption. Male Sprague−Dawley rats (weight: approximately 200 g) from B&K Universal AB, Sollentuna, Sweden, were used for this study. The rats were fasted 18 h before administration of the compound. The test compounds were dissolved in propylene glycol in an administration volume of 4 mL/kg. Two rats were used for each compound in the screening of plasma levels after oral administration of **4** (40 mg/kg), **6** (40 mg/kg), **7** (40 mg/kg), **9** (30 mg/kg), and nelfinavir (26 mg/kg). The rats were dosed by gavage at time 0, and blood samples were collected in heparin (20 IE/mL) from the tail vein at 0.5, 1, 2, and 4 h. Blood samples were immediately centrifuged at 3000−3500*g* for 10 min, and the plasma was stored at −18 °C until assayed. After protein precipitation, the plasma samples were directly injected onto the HPLC system. Drug concentrations in plasma were determined by reversed-phase liquid chromatography and UV detection.

Crystallography. The details of the crystallization and the structure determination will be published elsewhere. Briefly, complexes of HIV-1 PR with the compounds **1**, **6**, and **9** were crystallized in the space group *P*21212. The cell parameters were (compound **1**) $a = 59.0$, $b = 86.8$, $c = 47.0$ Å, (compound **6**) $a = 58.1$, $b = 86.1$, $c = 46.1$ Å, and (compound **9**) $a = 58.5$, $b = 86.7$, $c = 46.5$ Å. X-ray diffraction data to 1.8, 2.1, and 2.0 Å resolution, respectively, were collected at the synchrotron stations D41 at LURE, Paris, France, and I711 at MAX-lab, Lund, Sweden. Data were processed with DENZO[21] and scaled with SCALEPACK.[22] The completeness of data was 82.6% with an R_{sym}[23] of 6.4% for the PR/**1** complex, for the PR/**6** complex the corresponding values were 93.1% and 7.2%, and for the PR/**9** complex the corresponding values were 93.2% and 12.8%. The protein model coordinates from 1AJV were used for molecular replacement calculations. Refinements were done using the program package XPLOR.[24] The structures were refined to an $R_{crystal}$ (R_{free}) factor[25] of 19.1% (22.8%) using 1.8 Å data for the PR/**1** complex, 19.3% (26.2%) using 2.1 Å data for the PR/**6** complex, and 18.1% (24.1%) using 2.0 Å data for the PR/**9** complex. Model building were made using the program O.[26]

Acknowledgment. We gratefully acknowledge support from the Swedish Foundation for Strategic Research (SSF), the Swedish Research Council for Engineering Sciences (TFR), Medivir AB, Huddinge, Sweden, and the Swedish Medical Research Council (MFR).

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JM9910371