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Synthesis of New Thienyl Ring Containing HIV-1 Protease Inhibitors: Promising Preliminary Pharmacological Evaluation against Recombinant HIV-1 Proteases[§]

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A series of new thienyl ring containing analogues of nelfinavir and saquinavir with different substitution patterns were synthesized from suitable enantiopure diols. Their inhibitory activity against wild type recombinant HIV-1 protease was evaluated. In general thienyl groups spaced from the core by a methylene group gave products showing IC_{50} in the nanomolar range, irrespective of the type and the substitution pattern of the heterocycle. The range of activity of the two most active compounds is substantially maintained or even increased against two commonly selected mutants, under drug pressure, such as V32I and V82A.

Introduction

The global AIDS epidemic is one of the greatest medical challenges of our time. Drug discovery for anti HIV-1[a] agents has greatly accelerated in recent decades.[1] Combination therapy or highly active antiretroviral therapy (HAART) has dramatically changed the history of the infection as well as its morbidity and mortality, becoming the major current treatment for AIDS.[2] HIV/AIDS has become a manageable disease in developed countries, where novel approaches such as structured treatment interruptions (STIs) may be employed. On the basis of the evidence that viral protease is essential to the life cycle of HIV-1, protease inhibitors have been introduced into combination-therapy regimens, resulting in a marked improvement in clinical outcomes.[3] Nevertheless, strains of HIV-1 become resistant to the currently available drugs at an alarming rate, which accounts for the urgent need for new, broad-spectrum protease inhibitors, which are effective against both new mutants and the wild-type virus.[4]

In recent years, several pharmaceutical companies have developed, or are in the process of developing, an increasing number of second-generation protease inhibitors, either with or without a flexible structure.[5] To date, nine inhibitors against the HIV protease have been approved by the FDA. However, the virus has shown a very high degree of adaptability. Its high mutation rate is caused by the error-prone viral reverse transcriptase and its fast replication rate. This leads, especially under the additional selection pressure of HAART, to the development of resistant strains of the virus. The pronounced cross-resistance of all approved inhibitors explains the ongoing need to seek new inhibitors.

HIV protease has a C_2-symmetric homodimeric structure. As such, it selectively cleaves the Phe-Pro (Tyr-Pro) moiety of the virus homoprotein[6] and the rational design of inhibitors based on substrate models is possible. Increasing the structural diversity of inhibitor scaffolds may at least diminish the accelerated development of multidrug-resistant variants.

Most of commercially available anti HIV protease drugs have a peptidomimetic structure based on the tetrahedral transition state mimetic concept, in which a nonhydrolyzable hydroxyethylene or dihydroxyethylene or hydroxyethylamine moiety is used as the central core of the molecule.

With structural diversity in mind, we explored the possibility of introducing a thienyl ring in the core of several commercial anti HIV protease drugs. It is well-known that a thienyl ring mimics the phenyl group of phenylalanine in peptidomimetics[7] and in many drugs.[8] Furthermore, a recent study on C_2-symmetric diol-based HIV protease inhibitors has shown that the introduction of a thienyl ring instead of a phenyl group greatly enhances the antiviral activity of the compound.[9]

Thus, nelfinavir and saquinavir were chosen as model structures into which a thienyl fragment was introduced. In particular, the two cores were modified in the residues containing the phenyl moiety, as shown in structures **A** and **B** in Figure 1. We replaced them with a series of homo- or polycyclic thienyl ring-containing fragments and kept the same (*R*) absolute configuration of the carbon bearing the central hydroxyl group and the same relative configuration of the two vicinal stereocenters. To investigate the effect of chain length on enzyme inhibition, we also introduced a series of CH_2 spaced thienyl fragments.

Chemistry

Usually, the synthetic route to the commercial inhibitors employs a convergent synthesis in which an electrophilic chiral amino alcohol moiety is the key building block for the central core.[10] Once formed, it is subsequently bonded to suitable side chains. This general approach was also followed in our case,

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[§]Dedicated to the memory of Prof. A. M. Tamburro.

[a]Abbreviations: HIV-1, human immunodeficiency virus type 1; HAART, highly active antiretroviral therapy; STI, structured treatment interruption; FDA, Food and Drug Administration; WT, wild type; PHIQ, perhydroisoquinoline; HOBt, 1-hydroxybenzotriazole; NMM, 4-methylmorpholine; EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide.

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but we developed original synthetic routes for the preparation of the fragment bearing heterocyclic P_1 substituent (Scheme 1). Accordingly, the retrosynthesis required the regio- and stereoselective introduction of the nitrogen group from the diol **D** and the Sharpless asymmetric dihydroxylation of trans α,β-unsaturated ester **E**, which introduced stereogenic centers with the right relative and absolute configuration.

Recently we successfully exploited such an approach to the synthesis of two novel thienyl derivatives of nelfinavir and saquinavir **1** and **2** (Figure 2).[11] This prompted us to widen its scope.

Because of the shortage of reports on asymmetric oxidation of heteroaromatic acrylates and crotonates, we developed an efficient Sharpless asymmetric dihydroxylation of thienyl and benzothienyl derivatives, which afforded chiral 1,2-diols chemoselectively in high ee and good chemical yield[12] (Figure 3).

An azido group was introduced using two different approaches. According to our recently described procedure,[11] **3** was transformed into the corresponding cyclic sulfite. Then it was submitted to regioselective and stereospecific nucleophilic ring-opening reaction with sodium azide, affording the azido hydroxy ester **7**. Final reduction with BH_3SMe_2 and catalytic $NaBH_4$ gave the key intermediate azidodiol **8**[13] (Scheme 2).

A different strategy was chosen for analogues containing an additional methylene group. Thus, starting from the dihydroxybutyl esters **4a**−**c**, reduction by BH_3SMe_2 gave triols **10a**−**c** in almost quantitative yield (Scheme 3). Various reagents for the formation of cyclic acetals were tested to perform selective protection of hydroxyl groups on carbons 1 and 2. Among them, freshly prepared 3,3-dimethoxypentane[14] was the most efficient. The free hydroxyl was then transformed into a mesylate[15] and substituted with the azido group. Final hydrolysis gave the azidodiols **13** in modest to good overall yield.

The common perhydroisoquinolinic residue was linked by selective activation of primary hydroxyl group as a mesitylene sulfonyl derivative and subsequent reaction with the commercially available substituted perhydroisoquinoline (Scheme 4). Finally, the azidoalcohols were transformed into amino alcohols **18** and **19** by Pd catalyzed hydrogenation in excellent yield.

Such amino alcohols are common intermediates for both nelfinavir and saquinavir analogues, which are different only in the P_2 ligands. Thus, for the synthesis of nelfinavir analogues, amino alcohols were condensed with 3-acetoxy-2-methylbenzoic acid. Final deacetylation of the phenolic group afforded the target compounds **22** and **23** (Scheme 5).

The characteristic dipeptide unit of saquinavir derivatives **26** was prepared by coupling of asparagine *tert*-butyl ester **25** and quinaldic acid **24** (Scheme 6). Hydrolysis with TFA afforded the acid **27**,[11] which was coupled with amino alcohols **18** and **19**, affording the desired saquinavir derivatives **28** and **29**.

A noteworthy fact was that each synthetic sequence for the most active compounds **23a** and **29c**, once optimized, has been easily repeated in just 2 working weeks by a single operator.

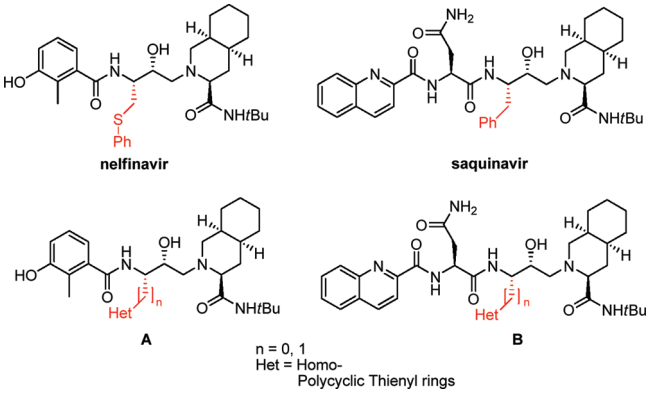


Figure 1. Structures of nelfinavir and saquinavir and their thienyl derivatives **A** and **B**.

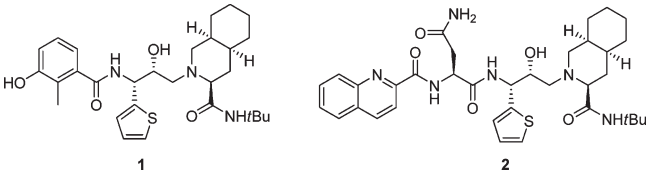


Figure 2. Structures of thienyl derivatives of nelfinavir (**1**) and saquinavir (**2**).

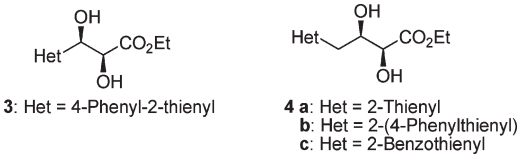


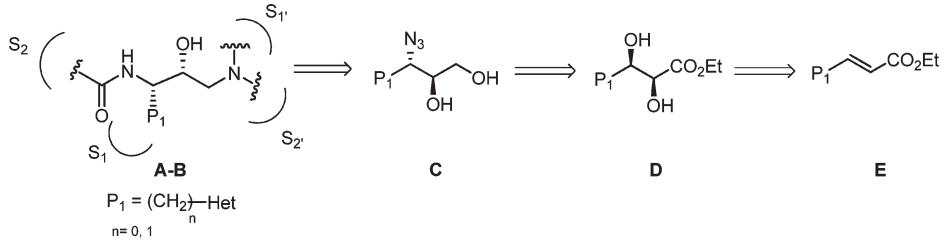
Figure 3. Starting thienyl and benzothienyl dihydroxy esters.

Biological Evaluation

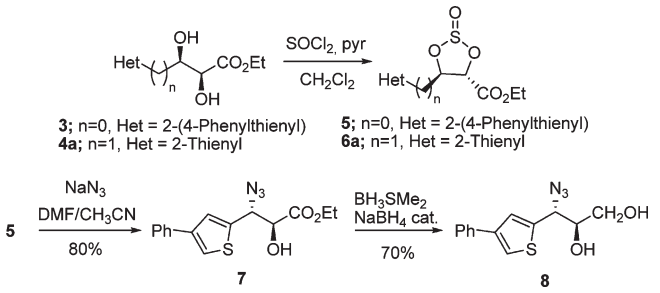
The inhibitory activities of these new thienyl derivatives of nelfinavir and saquinavir on a recombinant wild-type HIV protease were evaluated, and the results are given in Tables 1 and 2.

From the reported data it is apparent that the presence of a thienyl ring directly linked to the core generally causes a drop in the activity, when compared with that of the original commercial drugs. Indeed, it falls into the micromolar range for compounds **1**, **22** (Table 1) and **2**, **28** (Table 2). The need of a methylene spacer between the core and the heterocyclic groups is proved by the general high activity of the corresponding derivatives **23a**−**c** and **29a**−**c**, which falls in the nanomolar range. In these cases the longer and more flexible chain at P_1 likely enters the S_1 protease subsite more deeply and the derivatives are better mimics of the model nelfinavir and saquinavir. Small differences in the activity can also be observed upon changing the type of heterocyclic moiety. Among the nelfinavir analogues (Table 1), unsubstituted

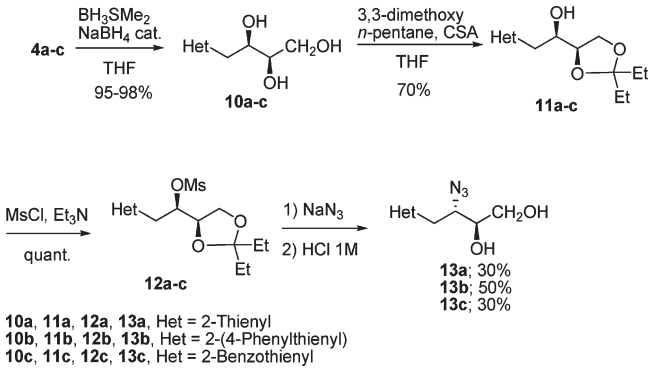
Scheme 1. Retrosynthetic Approach to the Thienyl Cores



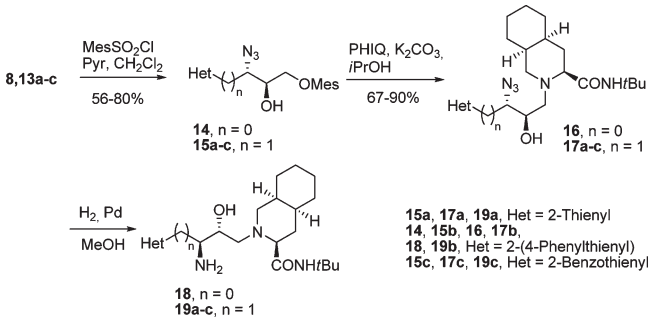
Scheme 2. Synthesis of Azidodiol **8**



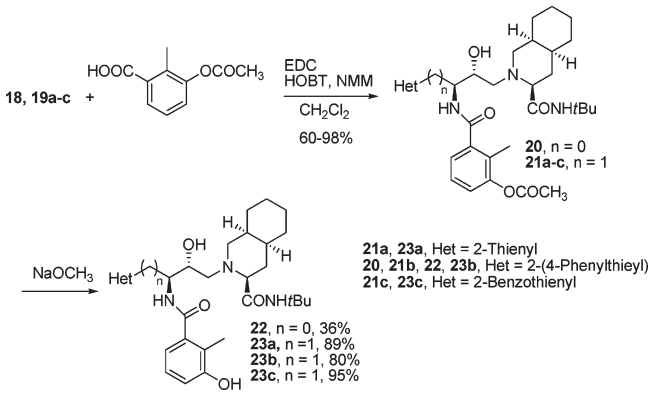
Scheme 3. Syntheses of Azidodiols **13a−c**



Scheme 4. Syntheses of Amino Alcohols **18** and **19a−c**



Scheme 5. Syntheses of Compounds **22** and **23a−c**



2-thienyl derivative **23a** reaches a promising IC_{50} = 2.9 nM, while the more sterically demanding 4-phenyl-2-thienyl and 2-benzothienyl derivatives **23b** and **23c** show slightly higher IC_{50} values.

Saquinavir analogues take advantage of a more extended heterocyclic moiety. Both 4-phenyl-2-thienyl **29b** and 2-benzothienyl derivative **29c** show low IC_{50} values in the nanomolar range, with the latter compound having a remarkable

Scheme 6. Syntheses of Compounds **28** and **29a−c**

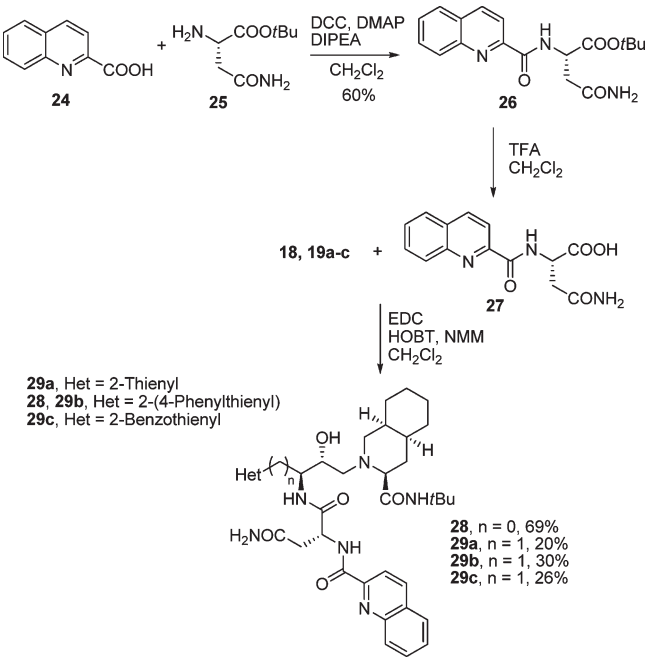
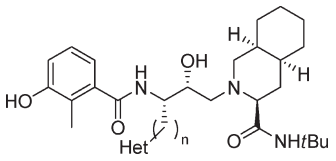
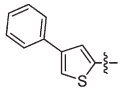
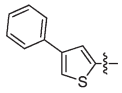
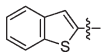


Table 1. Inhibitory Activity of Nelfinavir and Its Thienyl Derivatives **22** and **23a−c**



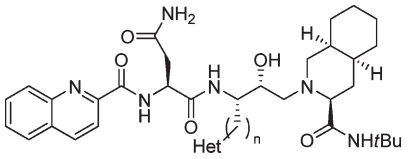
Entry	n	Het	Compound	IC_{50}[a] (μM)
1	1	Ph-S		0.0019
2	0		**1**	30.0
3	0		**22**	4.0
4	1		**23a**	0.0029
5	1		**23b**	0.006
6	1		**23c**	0.015

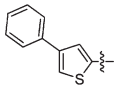
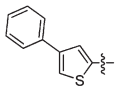
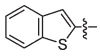
[a] IC_{50} values were obtained by measuring the initial rates of hydrolysis of the fluorogenic substrate Abz-Thr-Ile-Nle-Phe(NO_2)-Gln-Arg. Results are the mean of at least three independent experiments.

IC_{50} = 0.6 nM. The data in Table 2 also show that an extended lipophilic interaction between the side chain and the enzyme subsite S_1 is mandatory for the proper binding of these inhibitors.

We have also evaluated, in a preliminary test, the activity of the most promising compounds **23a** and **29c** against two HIV protease mutants that are commonly selected under drug pressure, namely, V32I and V82A.[16] The residual enzyme activities in

Table 2. Inhibitory Activity of Saquinavir and Its Derivatives **28** and **29a−c**



Entry	n	Het	Compound	IC_{50}[a](μM)
1	1	Ph		0.0004
2	0		**2**	5.0
3	0		**28**	1.5
4	1		**29a**	0.021
5	1		**29b**	0.0012
6	1		**29c**	0.0006

[a] IC_{50} values were obtained by measuring the initial rates of hydrolysis of the fluorogenic substrate Abz-Thr-Ile-Nle-Phe(NO_2)-Gln-Arg. Results are the mean of at least three independent experiments.

Table 3. Inhibitory Activity on HIV Protease Mutants

	residual enzyme activity[a] (%)		
	WT	V32I	V82A
23a, 1 nM	34	97	60
23a, 10 nM	5	32	
29c, 1 nM	53	54	37

[a] Residual enzyme activity values were obtained by measuring the initial rates of hydrolysis of the fluorogenic substrate Abz-Thr-Ile-Nle-Phe(NO_2)-Gln-Arg. Results are the mean of at least three independent experiments.

the presence of either **23a** or **29c** are reported in Table 3 and compared with the residual activities of the WT enzyme measured during the same kinetic run.[17] The activity of 1 nM **23a** is entirely lost on V32I, while it is 2-fold reduced on V82A. When the concentration of **23a** is increased to 10 nM, its activity is restored against V32I. Thus, **23a** appears more sensitive to the V32I mutation and its activity is seen to decrease by 1 order of magnitude while the activity is largely maintained against V82A. A similar behavior has also been shown by nelfinavir against the V82A mutant.[18]

In contrast, 1 nM **29c** fully maintains its activity against V32I and is even somewhat more active against V82A than against the WT enzyme. These are promising results, since saquinavir is conversely about 20-fold less active against V32I than against the WT protease.[19]

Conclusion

We have synthesized, in a simple and easily reproducible sequence, a series of thienyl containing analogues of HIV protease reference inhibitors nelfinavir and saquinavir in order to test the usefulness of such heteroaromatic residues as mimics of P_1 groups. Various derivatives possessing a flexible side chain gave promising results against the wild type HIV-1 protease, and two of them, **23a** and **29c**, showed activity in the same nanomolar range as the marketed drugs. It is noteworthy that such a little change in the core structure of marketed HIV protease inhibitors nelfinavir and saquinavir does not affect their activity against the wild type HIV protease. Even more important and somewhat surprising is the discovery that when **23a** and **29c** were preliminarily tested against two commonly selected mutants under drug pressure, such as V32I and V82A, the minor structural changes did not substantially lead to cross-resistance to these two new compounds. In particular, the activity of **29c** is completely retained or even enhanced with respect to that of saquinavir. Ongoing work is in progress with more specific inhibition activity test of the most promising compounds and with further research for new and simplified potential HIV inhibitors.

Experimental Section

General. Unless otherwise specified, materials were purchased from commercial suppliers and used without further purification. THF, toluene, and diethyl ether were distilled from sodium/benzophenone immediately before use. Dichloromethane was distilled from P_2O_5. DMF was freshly distilled and stored over 4 Å sieves. Moisture-sensitive reactions were conducted in oven- or flame-dried glassware under argon atmosphere. All reactions were magnetically stirred and monitored by thin-layer chromatography using precoated silica gel (60 F_{254}) plates. Column chromatography was carried out on Merck silica gel (63−200 μm particle size) by progressive elution with different solvents mixtures. Mass spectra were obtained by GC/MS with electron impact ionization. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ solutions at 500 and 125 MHz, respectively. Chemical shifts (δ) were expressed in ppm and coupling constants (J) in hertz. Optical rotations were determined at the sodium D line at 25 °C. HPLC analyses were performed using Chiralcel OJ-H column with UV detection at 235 nm. Estimated purity of all compounds by combustional analysis was always at least 95%.

(+)-(2*S*,3*R*)-3-Azido-2-hydroxy-3-(4-phenylthiophen-2-yl)propionic acid ethyl ester (7) was obtained in 80% yield as a brown oil. R_f = 0.5 (*n*-hexane/EtOAc 8:2). $[\alpha]_D$ +47.7 (*c* 1.24, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.25 (t, J = 8.0 Hz, 3H), 3.32 (s, 1H), 4.25 (q, J = 8.0 Hz, 2H), 4.66 (d, J = 3.0 Hz, 1H), 5.12 (d, J = 3.0 Hz, 1H), 7.58 (d, J = 7.0 Hz, 2H), 7.45 (m, 5H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 14.4, 62.8, 62.9, 73.7, 122.0, 126.5, 127.7, 129.2, 135.6 136.3, 142.1, 171.2. Anal. Calcd for $C_{15}H_{15}N_3O_3S$: C, 56.77; H, 4.76; N, 13.24; S, 10.10. Found: C, 56.71; H, 4.86; N, 13.15; S, 10.10.

(+)-(2*S*,3*R*)-3-Azido-3-(4-phenylthiophen-2-yl)propane-1,2-diol (8) was isolated in 70% yield as a white solid. Mp = 96−98 °C. R_f = 0.4 (petroleum ether/AcOEt 6:4). $[\alpha]_D$ +154.0 (*c* 1.20, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 2.24 (s, 1H), 2.70 (s, 1H), 3.77 (m, 2H), 3.94 (dd, J_1 = 6.0 Hz, J_2 = 4.0 Hz, 1H), 4.93 (d, J = 6.0 Hz, 1H), 7.34 (t, J = 7.0 Hz, 1H), 7.43 (m, 3H), 7.49 (s, 1H), 7.60 (d, J = 7.0 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 62.9, 63.1, 74.2, 121.6, 121.8, 126.6, 127.1, 129.1, 135.5, 142.4, 148.2. Anal. Calcd for $C_{13}H_{13}N_3O_2S$: C, 56.71; H, 4.76; N, 15.26; S, 11.65. Found: C, 56.65; H, 4.81; N, 15.20; S, 11.75.

(+)-(2*R*,3*S*)-4-(Thiophen-2-yl)butane-1,2,3-triol (10a) was isolated in 98% yield as a colorless oil. R_f = 0.2 ($CHCl_3$/MeOH 95:5). $[\alpha]_D$ +14.7 (*c* 0.55, $CHCl_3$). ^1H NMR (500 MHz, CD_3OD) δ (ppm): 3.00 (A part of an ABX system, J_{AB} = 15 Hz, J_{AX} = 8.0 Hz, 1H), 3.15 (B part of an ABX system, J_{AB} = 15 Hz, J_{BX} = 6.0 Hz, 1H), 3.55 (m, 1H), 3.60 (A part of an ABX system, J_{AB} = 11 Hz, J_{AX} = 6.5 Hz, 1H), 3.65 (B part of an ABX system, J_{AB} = 11 Hz, J_{BX} = 5.0 Hz, 1H), 3.84 (m, 1H), 6.92 (m, 2H), 7.20 (m, 1H). ^{13}C NMR (125 MHz, CD_3OD) δ (ppm): 33.7, 63.4, 72.5, 73.0, 123.5, 125.6, 126.5, 141.3. Anal. Calcd for $C_8H_{12}O_3S$: C, 51.04; H, 6.43; S, 17.03. Found: C, 51.10; H, 6.50; S, 17.05.

General Procedure for the Synthesis of Cyclic Acetals 11. A solution of the triol (**10**, 0.371 mmol) and 3,3-dimethoxypentane (0.927 mmol, 122 mg) in dry THF (4 mL) under Ar atmosphere was stirred for 15 min at room temperature. Then camphorsulfonic acid (0.148 mmol, 35 mg) was added at 0 °C, and the mixture was allowed to warm to room temperature and was stirred for 4 h. The reaction was quenched by adding Et_3N (0.15 mmol, 15 mg), and after solvent removal the crude material was purified by chromatography on silica gel (petroleum ether/AcOEt 8:2), affording the desired product.

(+)-(*R*)-1-((*R*)-2,2-Diethyl-1,3-dioxolan-4-yl)-2-(thiophen-2-yl)-ethanol (11a) was isolated in 70% yield as a colorless oil. R_f = 0.6 (petroleum ether/AcOEt 8:2). $[\alpha]_D$ +1.66 (*c* 1.75, $CHCl_3$). ^{1}H NMR (500 MHz, CD_3OD) δ (ppm): 0.91 (t, J = 8.0 Hz, 3H), 0.93 (t, J = 8.0 Hz, 3H), 1.64 (q, J = 8.0 Hz, 2H), 1.71 (dq, J_1 = 8.0 Hz, J_2 = 3.0 Hz, 2H), 3.02 (A part of an ABX system, J_{AB} = 15.5 Hz, J_{AX} = 5.5 Hz, 1H), 3.07 (B part of an ABX system, J_{AB} = 15.5 Hz, J_{BX} = 7.5 Hz, 1H), 3.72 (A part of an ABX system, J_{AB} = 8.0 Hz, J_{AX} = 8.0 Hz, 1H), 3.79 (m, 1H), 3.99 (B part of an ABX system J_{AB} = 8.0 Hz, J_{BX} = 7.0 Hz, 1H), 4.06 (m, 1H), 6.90 (d, J = 3.0 Hz, 1H), 6.97 (m, 1H), 7.20 (d, J = 5.0 Hz, 1H). ^{13}C NMR (125 MHz, CD_3OD) δ (ppm): 8.3, 8.5, 29.3, 29.8, 34.7, 66.5, 73.1, 78.8, 113.5, 124.6, 126.4, 127.2, 139.8. EI-MS *m*/*z*:, 256 (1) [M^+], 227 (100). Anal. Calcd for $C_{13}H_{20}O_3S$: C, 60.91; H, 7.86; S, 12.51. Found: C, 60.80; H, 7.91; S, 12.45.

Synthesis of Mesylates 12. To a solution of the alcohol (**11**, 0.25 mmol) and Et_3N (0.38 mmol, 0.05 mL) in dry CH_2Cl_2 (2 mL) under argon atmosphere, methanesulfonyl chloride (0.38 mmol, 0.03 mL) was added at 0 °C. The solution was stirred for 1 h and then warmed to room temperature, dissolved in $CHCl_3$ (10 mL), and washed with saturated aqueous NH_4Cl (10 mL) and with saturated aqueous NaCl (10 mL). The organic phase was dried on anhydrous Na_2SO_4, the solvent was evaporated under reduced pressure, and the crude was purified on silica gel, except for **19** which was used in the subsequent reaction as crude product.

Synthesis of the Azidodiols 13. To a solution of the starting mesylate (**12**, 0.12 mmol) in DMF (2 mL), solid NaN_3 (0.24 mmol, 16 mg) was added at 0 °C, and the solution was kept stirring at 100 °C. After 15 h the solution was cooled to room temperature, EtOAc (10 mL) was added, and the mixture was washed with H_2O (10 mL × 2). The organic phase was dried on anhydrous Na_2SO_4, and the solvent was evaporated under reduced pressure. To the solution of the crude in THF (3 mL), 1 M aqueous HCl (3 mL) was added, and the reaction mixture was warmed to 60 °C for 2 h. Then EtOAc (10 mL) and saturated aqueous $NaHCO_3$ (10 mL) were added until pH 7 was obtained. The organic phase was separated, washed with saturated aqueous NaCl, and dried on anhydrous Na_2SO_4. The solvent was distilled under reduced pressure, and the crude was purified by chromatography on silica gel.

(+)-(2*S*,3*S*)-3-Azido-4-(thiophen-2-yl)butane-1,2-diol (13a) was isolated in 30% yield as a colorless oil. R_f = 0.25 (petroleum ether/AcOEt 6/4). $[\alpha]_D$ +32.0 (*c* 0.5, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.93 (br s, 2H), 3.05 (A part of an ABX system, J_{AB} = 15.0 Hz, J_{AX} = 8.0 Hz, 1H), 3.28 (B part of an ABX system, J_{AB} = 15.0 Hz, J_{BX} = 3.0 Hz, 1H), 3.74 (m, 4H), 6.97 (m, 2H), 7.23 (d, J = 5.0 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 31.6, 63.4, 65.5, 72.9, 125.1, 126.9, 127.3, 139.3. Anal. Calcd for $C_8H_{11}N_3O_2S$: C, 45.06; H, 5.20; N, 15.70; S, 15.04. Found: C, 45.10; H, 5.15; N, 15.72; S, 15.10.

Synthesis of Mesitylenes 14 and 15. To a cold (0 °C) stirred solution of azidodiol (**8** or **13**, 1.43 mmol) in CH_2Cl_2 (3.7 mL), pyridine (236 mg, 2.86 mmol) and mesitylenesulfonyl chloride (344 mg, 1.57 mmol) were added. The reaction mixture was stirred at 0 °C for 4 h and then at 20 °C for 20 h. The solvent was evaporated under reduced pressure, and the crude mixture was dissolved in EtOAc and washed with cold 2 M aqueous HCl (5 mL), water, saturated aqueous $NaHCO_3$, and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated, and the crude product was purified by chromatography on silica gel (petroleum ether/EtOAc 8:2).

(2*S*,3*S*)-3-Azido-2-hydroxy-4-(thiophen-2-yl)butyl 2,4,6-trimethylbenzensulfonate (15a) was prepared running the reaction at 50 °C for 24 h and was isolated in 64% yield as a colorless oil. R_f = 0.8 (petroleum ether/AcOEt 6:4). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.34 (s, 3H), 2.65 (s, 6H), 3.07 (A part of an ABX system, J_{AB} = 15.0 Hz, J_{AX} = 8.0 Hz, 1H), 3.30 (B part of an ABX system, J_{AB} = 15.0 Hz, J_{BX} = 3.5 Hz, 1H), 3.74 (m, 4H), 4.07 (A part of an ABX system, J_{AB} = 11.0 Hz, J_{AX} = 6.0 Hz, 1H), 4.17 (B part of an ABX system, J_{AB} 11.0 Hz, J_{BX} = 3.0 Hz, 1H), 6.97 (m, 2H), 7.23 (d, J = 5.0 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 21.0, 22.6, 31.0, 64.2, 70.0, 70.6, 124.9, 126.9, 127.0, 129.7, 131.9, 140.0, 143.8. Anal. Calcd for $C_{17}H_{21}N_3O_4S_2$: C, 51.63; H, 5.35; N, 10.62; S, 16.22. Found: C, 51.55; H, 5.40; N, 10.56; S, 16.30.

Synthesis of Aminoazido Alcohols 16 and 17. To a solution of mesitylen azidoalcohol (**14** or **15**, 0.32 mmol) in *i*-PrOH (10.8 mL), PHIQ (132 mg, 0.55 mmol) and K_2CO_3 (90 mg, 0.65 mmol) were added. The mixture was stirred at 50 °C for 16−26 h, then *i*-PrOH was evaporated under reduced pressure and the residue dissolved in EtOAc (20 mL). The organic layer was washed with saturated aqueous NH_4Cl and brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The resulting residue was purified by chromatography on silica gel (petroleum ether/EtOAc 7:3).

(−)-(3*S*,4a*S*,8a*S*)-2-((2*R*,3*S*)-3-Azido-2-hydroxy-4-(thiophen-2-yl)-butyl)-*N*-*tert*-butyldecahydroisoquinoline-3-carboxamide (17a) was isolated in 89% yield as white solid. Mp = 119−121 °C. R_f = 0.2 (petroleum ether/AcOEt 8:2). $[\alpha]_D$ −71.15 (*c* 1.3, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.35 (s, 9H), 1.53 (m, 14 H), 2.44 (m, 2H), 2.72 (m, 2H), 2.89 (m, 1H), 3.04 (A part of an ABX system, J_{AB} = 15.5 Hz, J_{AX} = 9.5 Hz, 1H), 3.31 (B part of an ABX system, J_{AB} = 15.5 Hz, J_{BX} = 3.0 Hz, 1H), 3.63 (m, 2H), 5.86 (br s, 1H), 6.96 (m, 2H), 7.22 (d, J = 5.0 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 21.0, 26.0, 26.3, 28.9, 30.5, 30.8, 31.3, 33.5, 36.3, 51.4, 58.5, 61.1, 66.8, 70.8, 71.0, 124.9, 126.7, 127.3, 140.0, 173.8. Anal. Calcd for $C_{22}H_{35}N_5O_2S$: C, 60.94; H, 8.14; N, 16.15; S, 7.39. Found: C, 60.90; H, 8.18; N, 16.20; S, 7.35.

General Procedure for the Preparation of Amino Alcohols 18 and 19. A mixture of azido alcohol (**16** or **17**, 1 mmol) and Pd/C 10% (60 mg) in 20 mL of CH_3OH was kept stirring at room temperature under H_2 (1 atm). After 2 h, the catalyst was filtered off and the mixture was evaporated under reduced pressure. The products were isolated by chromatographic purification on silica gel of the crude product.

(−)-(3*S*,4a*S*,8a*S*)-2-((2*R*,3*S*)-3-Amino-2-hydroxy-4-(thiophen-2-yl)-butyl)-*N*-*tert*-butyldecahydroisoquinoline-3-carboxamide (19a) was isolated in 82% yield as a brown oil. R_f = 0.4 ($CHCl_3$/CH_3OH 9/1). $[\alpha]_D$ −113.9 (*c* 0.8 $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.35 (s, 9H), 1.60 (m, 12H), 2.32 (d, J = 11.0 Hz, 1H), 2.56 (A part of an ABX system, J_{AB} = 13.0 Hz, J_{AX} = 8.0 Hz, 1H), 2.79 (B part of an ABX system, J_{AB} = 13.0 Hz, J_{BX} = 3.0 Hz, 1H), 2.80 (dd, J_1 = 3.0 Hz, J_2 = 11.0 Hz, 1H), 3.15 (m, 3H), 3.32 (m, 1H), 3.98 (m, 1H), 4.73 (br s, 3H), 6.43 (br s, 1H), 6.93 (m, 1H), 6.97 (s, 1H), 7.16 (d, J = 5.0 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 20.7, 21.0, 25.9, 26.5, 29.0, 30.1, 30.5, 31.0, 32.8, 33.2, 51.5, 58.6, 60.3, 70.1, 124.9, 127.1, 127.5, 140.0, 173.8. Anal. Calcd for $C_{22}H_{37}N_3O_2S$: C, 64.83; H, 9.15; N, 10.31; S, 7.87. Found: C, 64.85; H, 9.10; N, 10.35; S, 7.90.

Synthesis of Nelfinavir and Saquinavir Analogues: Preparation of Amides 22, 23, 28, and 29. To a solution of the amino alcohol (**18** or **19**, 1.15 mmol) in anhydrous CH_2Cl_2 (10 mL), 2-methyl-3-oxyacetylbenzoic acid (223 mg, 1.15 mmol, for nelfinavir analogues) or (*S*)-4-amino-4-oxo-2-(quinoline-2-carboxamido)-butanoic acid **27** (330 mg, 1.15 mmol, for saquinavir analogues), HOBt (155 mg, 1.15 mmol), and NMM (0.25 mL, 2.3 mmol) were added under argon atmosphere. The mixture was cooled to

0 °C. Then EDC (264 mg, 1.38 mmol) was slowly added. After 1 h at 0 °C, the mixture was allowed to reach room temperature. After reaction completion, AcOEt (20 mL) was added and the mixture was washed with H_2O, saturated aqueous $NaHCO_3$, 10% aqueous citric acid, saturated aqueous $NaHCO_3$, and finally brine. The organic phase was dried over anhydrous Na_2SO_4 and filtered and the solvent evaporated under reduced pressure. The products were isolated by chromatographic purification on silica gel of the crude product.

(−)-3-((2*S*,3*R*)-4-((3*S*,4a*S*,8a*S*)-3-(*tert*-Butylcarbamoyl)octahydroisoquinolin-2(1*H*)-yl)-3-hydroxy-1-(thiophen-2-yl)butan-2-ylcarbamoyl)-2-methylphenyl acetate (21a) was isolated, after 4 h of reaction, in 81% yield as a brown oil. R_f = 0.4 ($CHCl_3/CH_3OH$ 95:5). $[\alpha]_D$ −48.7 (*c* 1.14 $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.19 (s, 9H), 1.20−2.15 (12H), 2.17 (s, 3H), 2.37 (s, 3H), 2.39 (m, 2H), 3.00 (m, 1H), 3.18 (m, 1H), 3.74 (m, 1H), 4.02 (m, 1H), 4.59 (m, 1H), 5.74 (br s, 1H), 6.92 (m, 1H), 6.97 (m, 2H), 7.04 (d, *J* = 8.0 Hz, 1H), 7.12 (d, *J* = 8.0 Hz, 1H), 7.18 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 12.8, 20.8, 25.9, 26.2, 28.4, 28.4, 30.4, 30.8, 30.8, 33.5, 36.0, 51.1, 56.2, 58.8, 59.5, 70.5, 71.0, 123.7, 123.9, 124.8, 125.8, 126.4, 126.9, 128.8, 137.8, 141.2, 149.6, 169.0, 170.4, 173.8. Anal. Calcd for $C_{32}H_{45}N_3O_5S$: C, 65.84; H, 7.77; N, 7.20; S, 5.49. Found: C, 65.75; H, 7.85; N, 7.22; S, 5.53.

Synthesis of Nelfinavir Analogues: Preparation of Final Products 22 and 23. To a stirring solution of amide (**20** or **21**, 1.0 mmol) in CH_3OH (15 mL), Na (5 mg, 0.15 mmol) was carefully added at room temperature. After 1 h the solvent was evaporated under reduced pressure and the residue dissolved in AcOEt (20 mL). The mixture was washed with H_2O (2 × 20 mL), dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. The products were isolated by chromatography on silica gel.

(−)-(3*S*,4a*S*,8a*S*)-*N*-*tert*-Butyl-2-((2*R*,3*S*)-2-hydroxy-3-(3-hydroxy-2-methylbenzamido)-4-(4-phenylthiophen-2-yl)butyl)decahydroisoquinoline-3-carboxamide (23b) was isolated in 70% yield as a colorless oil. R_f = 0.5 (petroleum ether/AcOEt 4:6). $[\alpha]_D$ −43.1 (*c* 1.5, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.5 (m, 12H), 1.17 (s, 9H), 1.85 (s, 3H), 2.26 (m, 2H), 2.52 (m, 1H), 2.61 (m, 1H), 2.96 (m, 1H), 3.15 (m, 1H), 3.62 (m, 1H), 4.02 (m, 1H), 4.56 (bs, 1H), 5.90 (bs, 1H), 6.70 (d, *J* = 7.0 Hz, 1H), 6.77 (d, *J* = 7.0 Hz, 1H), 6.84 (m, 2H), 7.28 (m, 2H), 7.35 (t, *J* = 8.0 Hz, 2H), 7.54 (d, *J* = 7.0 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 12.3, 20.5, 25.7, 26.2, 28.4, 30.3, 30.7, 33.4, 35.9, 51.2, 55.7, 58.8, 60.4, 70.3, 71.2, 116.8, 118.8, 122.5, 125.4, 126.2, 126.3, 127.0, 128.7, 135.9, 137.5, 141.9, 154.8, 171.7, 174.25. Anal. Calcd for $C_{36}H_{47}N_3O_4S$: C, 69.98; H, 7.67; N, 6.80; S, 5.19. Found: C, 70.05; 7.70; N, 6.70; S, 5.20.

(−)-(*R*)-*N*^{1}-((2*S*,3*R*)-1-(Benzo[*b*]thiophen-2-yl)-4-((3*S*,4a*S*,8a*S*)-3-(*tert*-butylcarbamoyl)octahydroisoquinolin-2(1*H*)-yl)-3-hydroxybutan-2-yl)-2-(quinoline-2-carboxamido)succinamide (29c) was obtained after 24 h of reaction and isolated in 26% yield as a brown oil. R_f = 0.3 ($CHCl_3/CH_3OH$ 9:1). $[\alpha]_D$ −1.02 (*c* 0.5, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.27 (s, 9H), 1.51 (13 H), 3.15 (m, 7H), 4.01 (m, 1H), 4.47 (m, 1H), 5.78 (bs, 1H), 6.15 (bs, 1H), 6.25 (bs, 1H), 6.97 (bs, 2H), 7.04 (bs, 1H), 7.41 (m, 3H), 7.77 (m, 3H), 8.11 (m, 2H), 9.12 (d, *J* = 7.0 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 20.6, 25.7, 26.0, 28.6, 28.6, 29.7, 30.6, 31.8, 35.8, 45.7, 50.1, 50.2, 50.9, 58.8, 70.5, 118.5, 121.8, 122.7, 123.3, 123.7, 127.6, 128.2, 129.4, 130.1, 130.1, 137.4, 139.4, 139.8, 142.0, 146.5, 148.6, 164.8, 170.5, 172.7. Anal. Calcd for $C_{40}H_{50}N_6O_5S$: C, 66.09; H, 6.93; N, 11.56; S, 4.41. Found: C, 66.15; H, 6.85; N, 11.58; S, 4.42.

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Supporting Information Available: General experimental procedures, spectroscopic data for compounds **5**, **6a**, **9**, **10b**,**c**, **11b**,**c**, **12b**,**c**, **13b**,**c**, **14**, **15b**,**c**, **16**, **17b**,**c**, **18**, **19b**,**c**, **20**, **21b**,**c**, 22, **23a**−**c**, **28**, **29a**,**b**, and enzyme assays procedure. This material is available free of charge via the Internet at http://pubs.acs.org.

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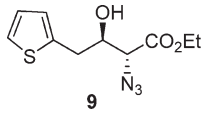

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