



Expedient solid-phase synthesis of both symmetric and asymmetric diol libraries targeting aspartic proteases

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ABSTRACT

C₂-symmetric diols have been shown to be highly potent against HIV-1 protease (PR). However, gaining access to these compounds has been hampered by the need of multistep solution-phase reactions which are often tedious and inefficient. In this Letter, we have disclosed a solid-phase strategy for rapid preparation of small molecule-based, symmetric and asymmetric diols as potential HIV-1 protease inhibitors. Upon biological screening, we found one of them, SYM-5, to be a potent and selective inhibitor (K_i = 400 nM) against HIV-1 protease.

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A key challenge in current drug discovery is the development of high-throughput (HT) amenable chemical reactions that allow rapid synthesis of diverse chemical libraries of enzyme inhibitors. In recent years, one reaction that has received much attention is the amide-bond formation between an amine and a carboxylic acid using suitable activating/coupling reagents.¹ The reaction is highly efficient in that it often generates desired products in nearly quantitative yields, thus allowing direct in situ biological screening to be carried out. This strategy has been successfully adopted, in both solution- and solid-phase, by several groups for the rapid discovery of small molecule inhibitors.^{2,3} The solution phase amide-forming strategy is comparatively easier to implement but has the following problems:² (1) undesired chemicals (starting materials, reagents, by-products, etc) are present during the biological screening and therefore often cause false results; (2) multi-step reactions cannot be carried out without proper purification of the intermediates; and (3) excessive starting materials/reagents normally cannot be used to drive an otherwise-difficult coupling to completion. Solid-phase synthesis could potentially solve many of these problems, and has been widely adopted in the synthesis of biopolymers (e.g., oligonucleotides and peptides) as well as small molecules (e.g., in combinatorial chemistry). In most cases, however, low-quality products are generated and need to be laboriously purified before screening. We have recently become interested in developing highly efficient solid-phase strategies that enable large-scale synthesis of high-quality compound collections

which are suitable for direct in situ biological screening.^{3,4} With these new chemical tools, we have successfully developed small molecule inhibitors targeting different classes of proteases, including cysteine and metallo-proteases. Herein, by making use of the amide-forming reaction, we report the solid-phase synthesis of both symmetric and asymmetric diols as putative inhibitors of aspartic proteases. Many aspartic proteases are known therapeutic targets.^{5,6} For example, HIV-1 protease is one of the main targets of AIDS.⁵ Renin is currently being used to treat hypertension.^{6a} Cathepsin D is involved in breast cancer metastasis.^{6b} β -Secretase is related to Alzheimer disease.^{6c} Plasmepsins, key aspartic proteases from malaria involved in the hemoglobin degrading pathway in parasite-infected macrophages, are being pursued to treat malaria.^{6d}

HIV-1 protease (PR) is one of the best known aspartic proteases, and an attractive target for the treatment of AIDS.⁵ The enzyme consists of two identical, non-covalently associated subunits of 99 amino acid residues formed in an C₂-symmetric fashion. The highly symmetrical active site is formed at the dimer interface. Over the years, many HIV-1 protease inhibitors (PI) have been developed.^{5a} Two of the most effective PI are shown in Figure 1 (top two): they contain a C₂-symmetric, diol-containing moiety which mimics the transition state of amide hydrolysis. Wong's peptidic inhibitor developed in 1998,^{5b} for example, has a K_i of 1.5 nM against HIV-1 PR. Its poor bioavailability (e.g., peptide-based, large MW) however makes it ineffective in cell-based assay. Abbott's Ritonavir,^{5c} an FDA-approved drug and improved version of Wong's inhibitor, is nearly C₂-symmetric, and much more effective against AIDS due to its smaller size (MW = 820) and fewer

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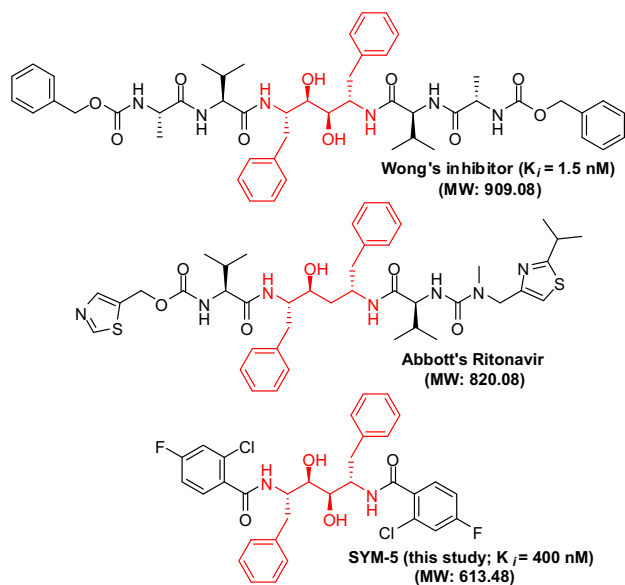
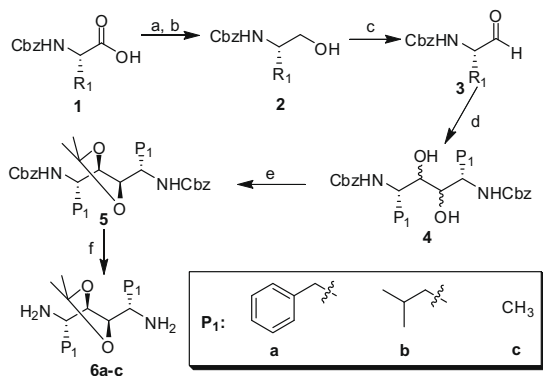


Figure 1. Structures of two known diol-containing HIV-1 protease inhibitors (top two) and the inhibitor identified from this study (bottom). The core diol structures are highlighted (Red). Top two are C_2 -symmetric or near symmetric. The newly identified one was shown to have good inhibition against HIV-1 PR ($K_i = 400$ nM).

amide bonds. The rapid emergence of drug-resistant HIV-1 PR, however, has rendered many of these PI ineffective. Thus, there is an urgent need to develop chemistry that permits rapid and efficient synthesis of new PI. Another paradigm shift in HIV PI research is the introduction of asymmetric PI which could be more effective against some of the drug-resistant HIV strains.^{5d} In the current study, we have developed a solid-phase strategy for rapid synthesis of potential symmetric and asymmetric PI (Schemes 1 and 2). As a proof of concept, the strategy was successfully used to synthesize 75 diol-containing compounds, which, upon direct in situ screening, revealed a small (MW = 613) and potent HIV-1 PI ($K_i = 400$ nM).

In our strategy, we adopted a solution-cum-solid phase strategy, in which the most important component of the inhibitors—the diaminodiol core group, was synthesized in solution (Scheme 1) and purified to homogeneity before being installed onto the commercially available 4-formyl-3-methoxyphenoxy (FMP) resin,⁷ followed by diversification with a variety of acids (Scheme 2). As shown in Scheme 1, following previously published procedures,^{5c} three different C_2 -symmetric diaminodiol, **6a–c**, were made from commercially available Cbz-protected amino acids **1**. Upon reduction, the

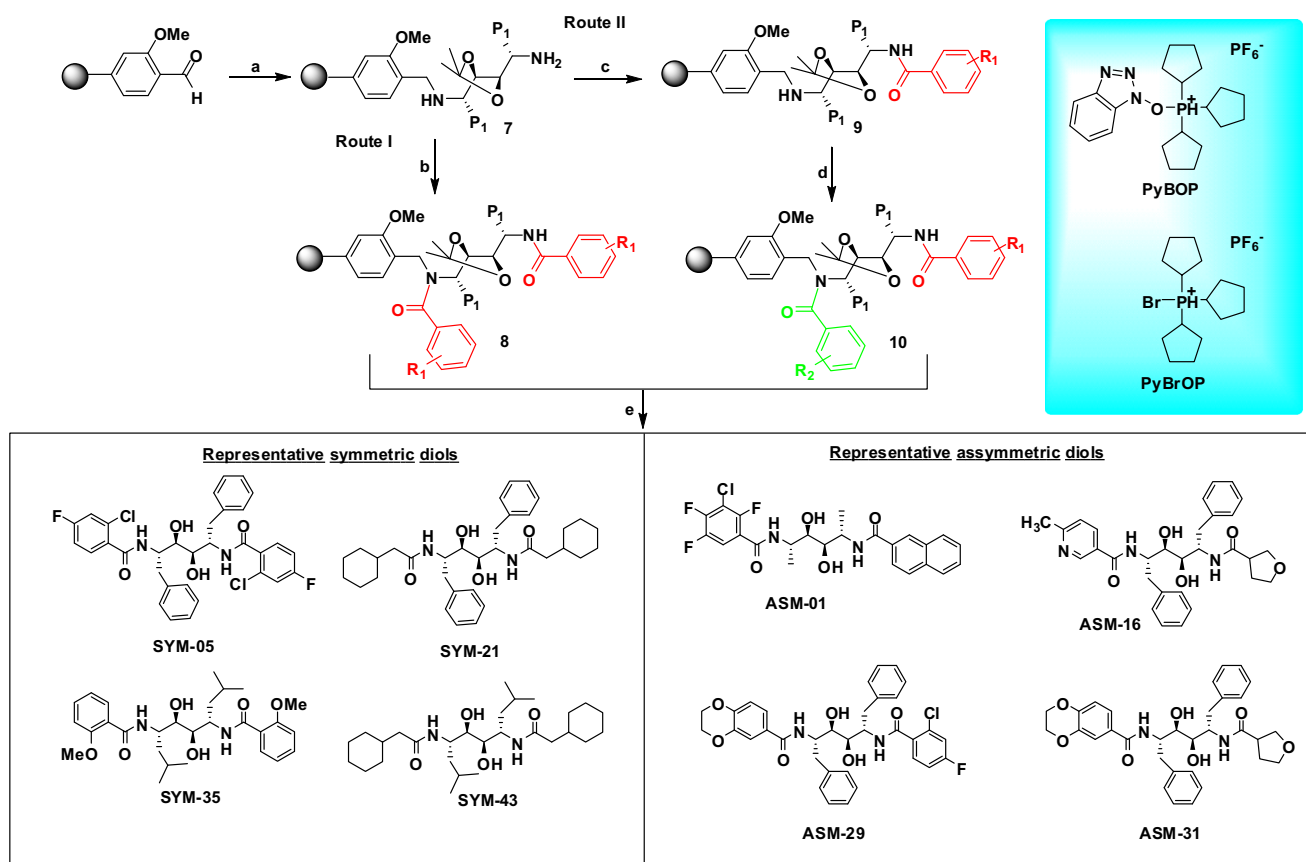


Scheme 1. Solution-phase synthesis of the diol warheads. (a) Isochloroformate, NMM, DCE, -15°C . (b) NaBH_4 , THF, 0°C , 70–77%. (c) Dess–Martin, DCM, rt, 60–70%. (d) VCl_3 , 1,3-dimethylimidazole, Zn, dry THF, reflux, 10–20%. (e) 2,2-dimethoxypropane, p -TsOH, acetone, rt, 60–70%. (f) H_2 , Pd/C, MeOH/EA, rt, 60–70%.

corresponding amino alcohols **2** was oxidized to the aldehydes **3** using Dess–Martin reagent. Subsequently, pinacol homocoupling reaction between two equivalents of **3** in the presence of VCl_3 afforded diastereomeric diols **4** in a single-step transformation ($\sim 20\%$ yield). Subsequent protection of the dihydroxyl group with 2,2-dimethoxypropane, flash chromatography to remove minor diastereomeric impurities, and deprotection of the Cbz groups using H_2 (in Pd/C) gave the resulting enantiomerically pure diaminodiol, **6a–c**, in moderate yield (50% in 2 steps).

In the subsequent solid-phase reactions (Scheme 2), the aldehyde-containing FMP resin was used for capturing of **6** by reductive amination, followed by amide bond-forming reaction with acids. As previously shown,³ key advantages of this method include (i) chemical modification of the library building blocks (i.e., diol core & acids) is unnecessary; (ii) it's solid-phase, enabling a large library to be constructed efficiently; (iii) it's robust, giving high-quality products. The IRORI™ directed sorting technology was used in the synthesis of the 75-member library.⁴ First, the three diaminodiol, **6a–c**, were treated with FMP resin in the presence of $\text{Na}(\text{OAc})_3\text{BH}/2\%$ glacial acetic acid in DCE to give the resin-bound amines, **7**, which contain an 1° and 2° amine each. Initially, simultaneous acylation of both the 1° and 2° amines with the same acid building blocks was met with some difficulties under most coupling conditions (e.g., DIC/HBTU/TBTU/HATU); in most cases, only the monoacylated product (at 1° amine position) was generated. This was clearly due to the chemical and steric difference of the two amines in **7**. We therefore took advantage of this to make both symmetric and asymmetric diols (Scheme 2). An exhaustive testing of (**7** + acid) coupling under a variety of different coupling conditions finally gave rise to the following two sets of optimized conditions: (I) to make symmetric diols, Route I was used in which PyBOP/DIEA coupling with the acid (10 equiv) was carried out to give **8**; (II) to make asymmetric diols, Route II was used in which PyBOP/HOAt coupling with the first acid (5 equiv) was performed, giving **9**, followed by PyBOP/DIEA coupling with the second acid (5 equiv) to give **10**. Finally, cleavage of the products from the resin using an optimized TFA cocktail (5:4:1 TFA/DCM/water) gave a total of 44 C_2 -symmetric and 31 asymmetric diol inhibitors (representative compounds are shown in Scheme 2; see Supplementary data for complete list). To ensure the crude products generated from our strategy were sufficiently pure for direct in situ screening, the compounds were further characterized by LCMS and NMR; in most cases the desired products were obtained with good purity ($>90\%$ and 60–95% for symmetric and asymmetric diols, respectively).⁸

Next, the inhibitory activity of these 75 diol-based inhibitors was determined against HIV-1 PR, plasmepsin I (PM I) and plasmepsin II (PM II) using a standard fluorescence microplate assay method. First, an inhibitor fingerprint of the library against the aspartic proteases was obtained, from which six potential hits (**SYM-5**, **-21**, **-35**, **-43** and **ASM-16**, **-29**) were identified. Detailed inhibition studies were then carried out to obtain the corresponding IC_{50}/K_i of these compounds, and the results are summarized in Table 1. The best inhibitor against HIV-1 PR was found to be **SYM-5**, with IC_{50} and K_i values of 395.4 and 400 nM, respectively (Fig. 2).⁹ Significantly, it showed a very poor inhibition against PM I and PM II ($>25\ \mu\text{M}$), indicating that this inhibitor is highly specific only towards HIV-1 PR. None of the asymmetric compounds was able to inhibit 50% of the HIV-1 PR activity at a concentration of $25\ \mu\text{M}$, which is expected since the assay was done with the C_2 -symmetric wildtype HIV-1 PR. However, two of these compounds exhibited moderate activity against PM II ($\text{IC}_{50} = 12.6$ and $11.7\ \mu\text{M}$ for **ASM-16** and **-29**, respectively), which may be developed into potent inhibitors in future. **SYM-21** and **SYM-43** were identified to be moderate and selective inhibitors of PM II and PM I, respectively. These results thus validate our strategy as a feasible method for future discovery of other aspartic protease inhibitors.



Scheme 2. Solid-phase synthesis of both symmetric and asymmetric diols. (a) Na(OAc)₃BH, 2% AcOH in DCE, rt. (b) acid (10 equiv), PyBrOP/DIEA in DMF, rt. (c) acid 1 (5 equiv), PyBOP/HOAt/DIEA in DMF, rt. (d) acid 2 (5 equiv), PyBrOP/DIEA in DMF, rt. (e) TFA/DCM/H₂O (5/4/1), rt.

Table 1
Inhibition of the six selected inhibitors

| Enzyme | IC ₅₀ (K _i) in nM | | | | | |
|----------|--|---------|---------|---------|---------|---------|
| | SYM-5 | SYM-21 | SYM-35 | SYM-43 | ASM-16 | ASM-29 |
| HIV-1 PR | 395.4 (400) | >25,000 | 1313 | >25,000 | >25,000 | >25,000 |
| PM I | >25,000 | 4391 | >25,000 | 2642 | >25,000 | >25,000 |
| PM II | >25,000 | 3908 | >25,000 | >25,000 | 12,657 | 11,773 |

In summary, we have developed a solid-phase amide-forming approach for rapid assembly of both symmetric and asymmetric diol-containing small molecules as potential aspartic protease inhibitors. One potent and selective inhibitor of HIV-1 PR was discovered which should possess better bioavailability properties (fewer amide bonds, lower MW) than its parental compounds. The clear advantage in our strategy lies in its feasibility for solid-phase synthesis of asymmetric diols. When compared with other

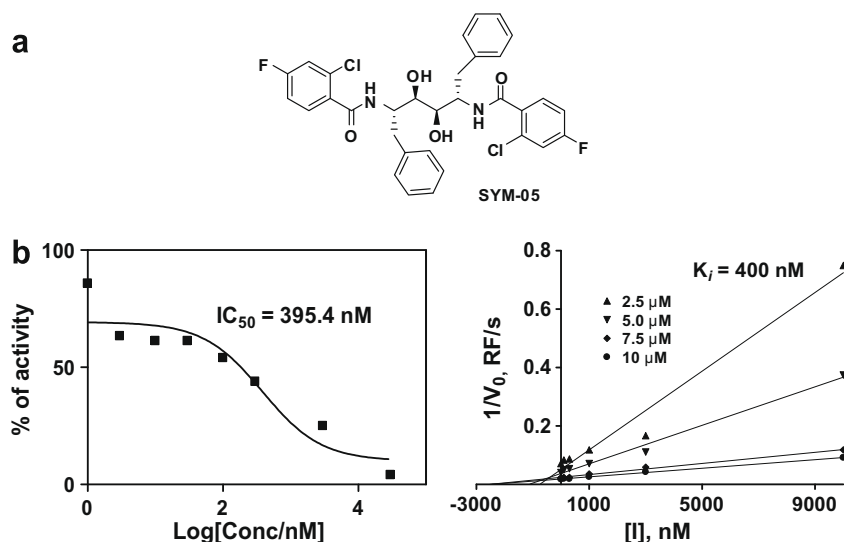


Figure 2. (a) Structure of SYM-5. (b) IC₅₀ (left) and K_i (right) of SYM-5 against HIV-1 PR.

potent HIV-1 protease inhibitors, our compound pales in terms of its inhibitory activity. Nevertheless, we believe the chemical strategy disclosed herein may be readily expanded in future for the construction of much bigger compound libraries which may lead to candidate compounds with improved biological activities.

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Supplementary data

Supplementary data (detailed experimental procedures and results) associated with this article can be found, in the online version, at [doi:10.1016/j.bmcl.2009.03.041](https://doi.org/10.1016/j.bmcl.2009.03.041).

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8. The purity of the compounds were estimated based on the LC profile of each compound (see Supplementary data) obtained under the UV channel of 254 nm (all 75 compounds in this study contain aromatic moieties).
9. The inhibition experiments were carried out using commercially available HIV-1 protease kit (see Supplementary data for details), and the screening conditions were adjusted to ensure IC₅₀ and K_i values obtained can be directly compared with Wong's and Abbott's compounds.