Communications to the Editor

Expedient Method for the Solid-Phase Synthesis of Aspartic Acid Protease Inhibitors Directed toward the Generation of Libraries

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Introduction. Aspartic acid proteases are a widely distributed family of enzymes that play important roles in fungi, plants, vertebrates, and retroviruses.[1] The aspartic acid proteases (characterized by having two aspartic acid residues in the active site) catalyze the hydrolysis of amide bonds with specificity for peptide bonds located between large hydrophobic residues. A number of aspartic acid proteases are important pharmaceutical targets, including renin,[2] cathepsin D,[3] the human immunodeficiency virus (HIV) protease,[4,5] human t-cell leukemia virus type 1 (HTLV-1) protease,[6] and *candida albicans* aspartic acid protease.[7]

Potent inhibitors of these enzymes can be readily accessed by the incorporation of an isostere that mimics the geometry of the tetrahedral intermediate in place of the scissile bond of the peptide substrate.[2b,8] Unfortunately, these inhibitors have limited therapeutic utility, due to the poor oral availability and/or short-circulating half-lives that result from their peptidic nature. For this reason, there has been a great deal of work toward the development of aspartic acid protease inhibitors that display *nonpeptide* functionality about the isostere of the tetrahedral intermediate.[2,4] In order to generate a therapeutically useful inhibitor the compound must have both high affinity and favorable pharmokinetic properties. The combination of these two requirements render an *a priori* design of inhibitor structure based on the peptide substrate very challenging. Therefore, the identification of potent and bioavailable inhibitors has required the time-consuming synthesis and evaluation of a large number of different nonpeptidic compounds.

Herein we report a general and high-yielding solid-phase method for the rapid display of *nonpeptide* functionality about molecules incorporating the (hydroxyethyl)amine and (hydroxyethyl)urea isosteres.[9] Of the possible isosteres upon which to construct a library of potential nonpeptide inhibitors, the (hydroxyethyl)-amine and (hydroxyethyl)urea isosteres were selected for two reasons. First, several orally available HIV-1 protease inhibitors that incorporate these isosteres have been identified (Figure 1),[4] including compounds that are currently in clinical trials for the treatment of HIV infection. Second, we believed that solid-phase methods could be developed to display a wide range of diverse functionality about these isosteres.

Initially we chose to display functionality from scaffold **1**,[10] which provides access to known HIV-1 protease inhibitors. The scaffold was first coupled to dihydropyran functionalized polystyrene support by employing

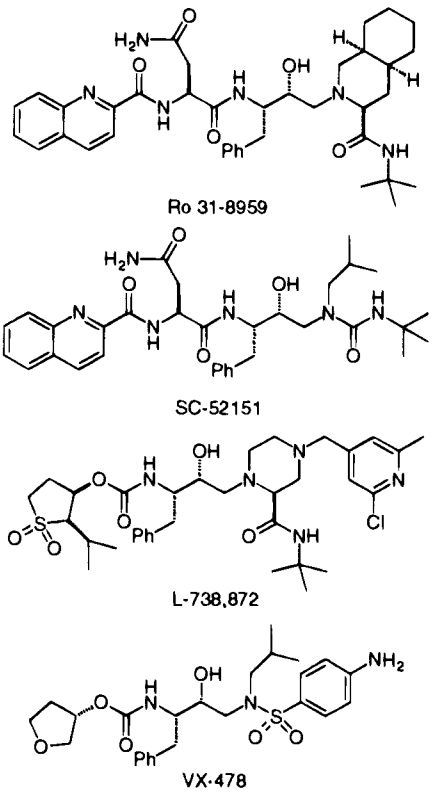
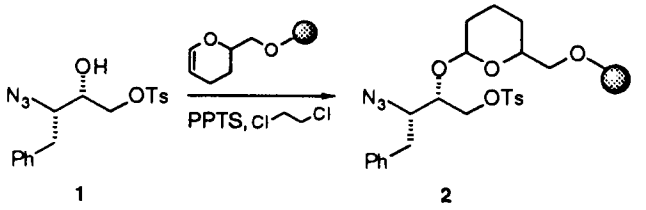


Figure 1.

Scheme 1



pyridinium *p*-toluenesulfonate in 1,2-dichloroethane (Scheme 1).[11] The reaction progress was qualitatively monitored by IR by following the appearance of the azide stretch. The exact loading level of the resin was based on the mass balance of recovered alcohol **1**, which was obtained by subjecting a portion of the resin **2** to cleavage by 95:5 trifluoroacetic acid (TFA)/water.[12]

The synthesis was initiated by displacement of the primary tosyl alcohol with either functionalized or unfunctionalized primary or secondary amines, including amines found in known HIV-1 protease inhibitors (Scheme 2, **3a–h** and **4i**). After coupling of the primary amines, the resulting secondary amine products **3a–g** can be converted to ureas by reaction with isocyanates (**4a–f**) or by stepwise treatment with triphosgene followed by amine addition (**4g**).[13,14] The ability to employ either a preformed isocyanate or the stepwise procedure to synthesize the ureas provides ready incorporation of functionality from both commercially available isocyanates and the even larger pool of commercially available amines. Notably, substituted ureas have been employed successfully at this site in a number of

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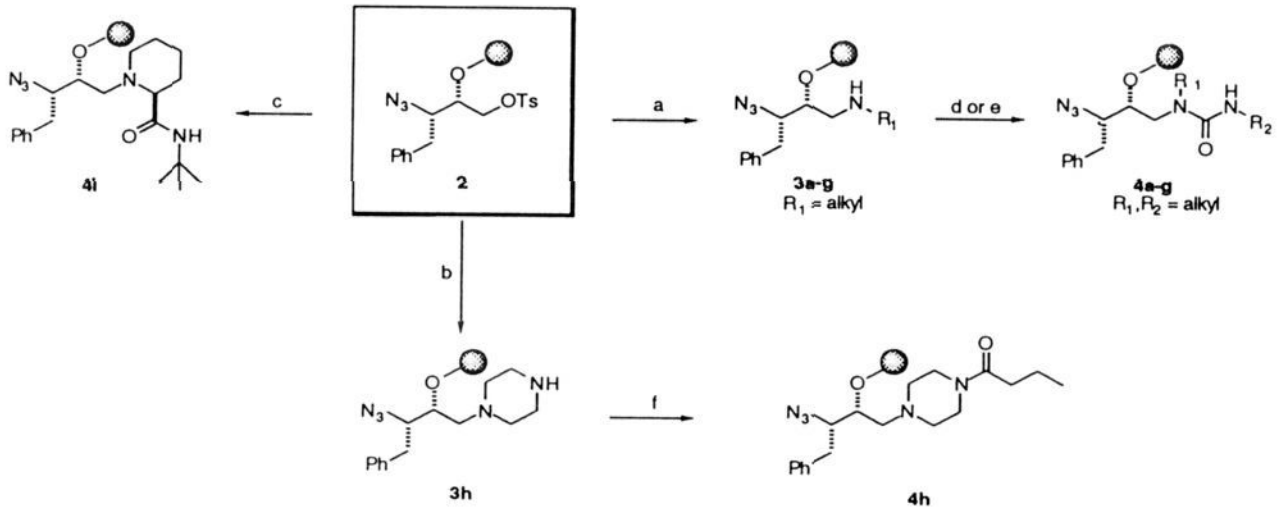
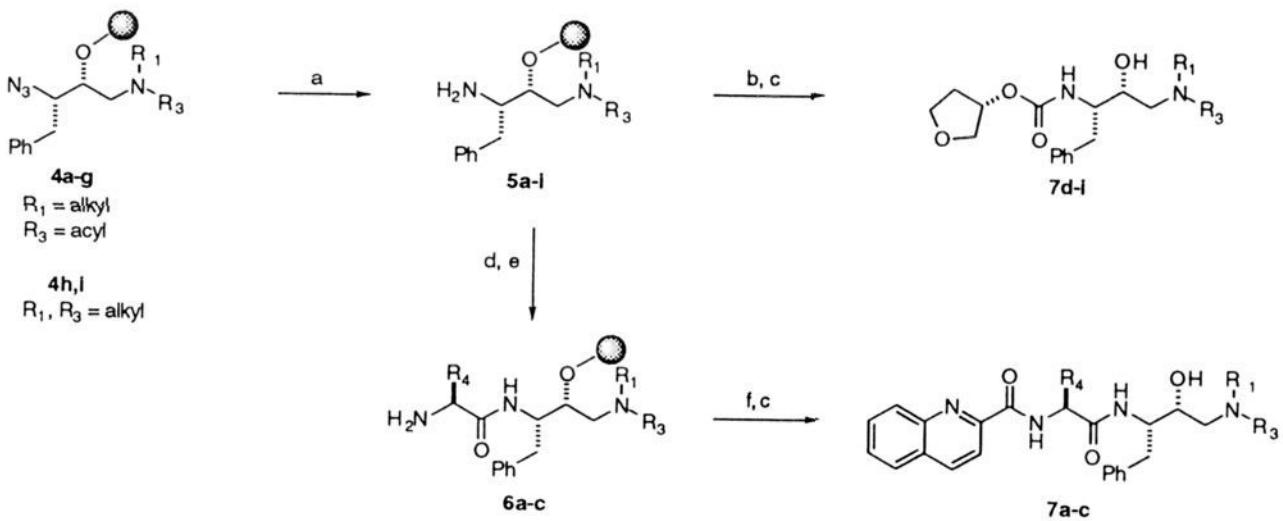
Scheme 2[a]



[a] (a) R_1NH_2, NMP, 80 °C; (b) piperazine, NMP, 80 °C; (c) *N-tert*-butyl-L-pipecolinamide, NMP, 95 °C; (d) R_2NCO, $ClCH_2CH_2Cl$; (e) (i) $OC(OCCl_3)_2$, Et_3N, cat. DMAP, THF, (ii) 4-(3-aminopropyl)morpholine, THF; (f) butyryl chloride, *i*-Pr_2EtN, CH_2Cl_2.

Scheme 3[a]



[a] (a) $SnCl_2$:HSPh:Et_3N (1:4:5), THF, (b) 3(*S*)-tetrahydrofuranylsuccinimidyl, *i*-Pr_2EtN, CH_2Cl_2; (c) 95:5 TFA/H_2O; (d) Fmoc amino acid, PyBOP, HOBt, *i*-Pr_2EtN (3 equiv), DMF; (e) 20% piperidine in DMF; (f) pentafluorophenyl ester of quinaldic acid, HOBt, Et_3N, DMF.

aspartic acid protease inhibitors.[4b,15] In addition, acyl chlorides can be employed to provide amides; for example, the piperazine derivative **3h** was acylated with butyryl chloride to provide **4h** (Scheme 2).

The synthesis about the P_1 site of the inhibitor was initiated by reduction of the azide using thiophenol/Et_3N/$SnCl_2$ (4:5:1) as described by Bartra and coworkers (Scheme 3).[16] The reduction was relatively rapid (<4 h at room temperature), and the reaction progress was easily monitored by IR by following the disappearance of the azide stretch. Although a number of alternative methods are available for reducing azides to amines, most of these methods are heterogeneous in nature, are slow, and/or require protic solvents that do not effectively solvate the polystyrene resin.

The resulting primary amine (**5**) can then be acylated to provide carbamate or amide products that can be further derivatized. For example, the coupling of **5a** with *N*-Fmoc-Asn(Trt)-OH under PyBOP/HOBt coupling conditions[17] was followed by removal of the Fmoc protecting group with 20% piperidine in DMF to provide **6a** (Scheme 3). Subsequently, the free amine was coupled with the pentafluorophenyl ester of quinaldic acid.[18,19] The concomitant removal of the trityl protecting group and the cleavage of the material from the solid support with 95:5 TFA/water for 20 min[12] provided the HIV-1 protease inhibitor **7a** (Figure 2), developed by Monsanto,[4b] in 85% overall yield for the six-step process. To demonstrate the versatility of the method, compounds **7b** and **7c** were prepared by incorporating the sterically hindered amino acid, valine, and the functionalized amino acid, tyrosine, in place of asparagine in 83% and 74% overall yields, respectively. Alternatively, reaction of amines **5d**−**i** with the activated *N*-succinimidyl carbonate of 3(*S*)-hydroxytetrahydrofuran provides carbamates **7d**−**i**.[20] The tetrahydrofuran urethane has been shown to bind tightly to the S_2 region of HIV-1 protease.[4c,21] Cleavage of the material from the solid support with 95:5 TFA/water for 20 min[12] provided analytically pure derivatives **7d**−**i** after chromatography in 47−86% overall yield based on the initial loading of alcohol **1**.

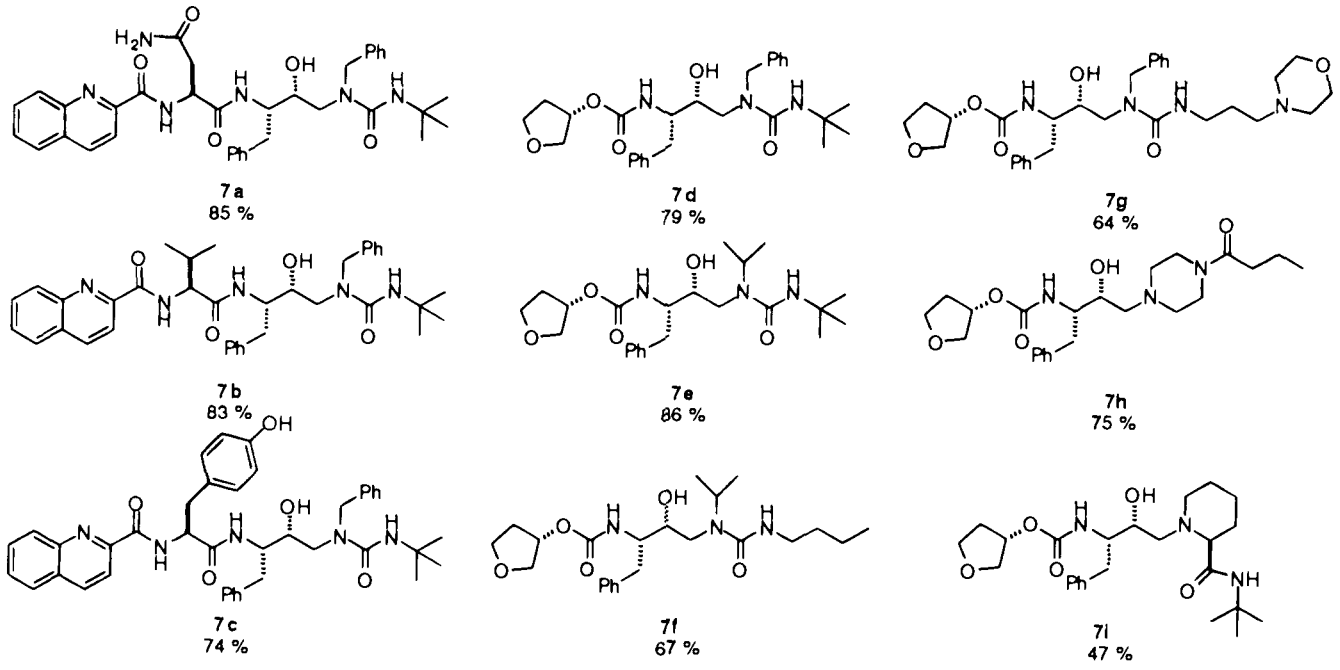


Figure 2. (Hydroxyethyl)amine and (hydroxyethyl)urea derivatives synthesized on solid support. Yields of analytically pure material after chromatography were determined from mass balance and were based upon the initial loading of alcohol **1**. Elemental analyses were all within ±0.4% of theoretical value.

In summary, we have been able to obtain good yields (47–86%) of molecules incorporating the (hydroxyethyl)-amine and (hydroxyethyl)urea isosteres after four to six chemical transformations on solid support. In addition, we have been able to incorporate many of the functional groups and structures that are present in known inhibitors of HIV-1 protease and renin, thereby demonstrating the generality of the synthesis sequence. Employing the described synthesis method, the simultaneous synthesis of a library of potential aspartic acid protease inhibitors is in progress, as is the evaluation of the library against a number of aspartic acid protease targets.[22]

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Supplementary Material Available: Experimental details for the synthesis and characterization of compounds **7a–i** (5 pages). Ordering information is given on any current masthead page.

References

(1) Dunn, B. M., Ed. *Structure and Function of the Aspartic Proteinases*; Plenum Press: New York, 1991.
(2) (a) Greenlee, W. J. Renin Inhibitors. *Med. Res. Rev.* **1990**, *10*, 173–236. (b) Wiley, R. A.; Rich, D. H. Peptidomimetics Derived from Natural Products. *Med. Res. Rev.* **1993**, *13*, 327–384.
(3) Baldwin, E. T.; Bhat, T. N.; Gulnik, S.; Hosur, M. V.; Sowder, R. C.; Cachau, R. E.; Collins, J.; Silva, A. M.; Erickson, J. W. Crystal Structures of Native and Inhibited Forms of Human Cathepsin D: Implication for Lysosomal Targeting and Drug Design. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 6796–6800.
(4) HIV protease inhibitors based on the (hydroxyethyl)amine or -urea template: (a) Roberts, N. A.; Martin, J. A.; Kinchington, D.; Broadhurst, A. V.; Craig, J. C.; Duncan, I. B.; Galpin, S. A.; Handa, B. K.; Kay, J.; Kröhn, A.; Lambert, R. W.; Merrett, J. H.; Mills, J. S.; Parkes, K. E. B.; Redshaw, S.; Ritchie, A. J.; Taylor, D. L.; Thomas, G. J.; Machin, P. J. Rational Design of Peptide-Based HIV Proteinase Inhibitors. *Science* **1990**, *248*, 358–361 (Ro 31-8959). (b) Getman, D. P.; DeCrescenzo, G. A.; Heintz, R. M.; Reed, K. L.; Talley, J. J.; Bryant, M. L.; Clare, M.; Houseman, K. A.; Marr, J. J.; Mueller, R. A.; Vazquez, M. L.; Shieh, H-S.; Stallings, W. C.; Stegeman, R. A. Discovery of a Novel Class of Potent HIV-1 Protease Inhibitors Containing the (*R*)-(Hydroxyethyl)urea Isostere. *J. Med. Chem.* **1993**, *36*, 288–291 (SC-52151). (c) Kim, B. M.; Vacca, J. P.; Guare, J. P.; Hanifin, C. M.; Michelson, S. R.; Darke, P. L.; Zugay, J. A.; Emini, E. A.; Schleif, W.; Lin, J. H.; Chen, I.-W.; Vastag, K.; Ostovic, D.; Anderson, P. S.; Huff, J. R. A New Hydroxyethylamine Class of HIV-1 Protease Inhibitors with High Antiviral Potency and Oral Bioavailability. *BioMed. Chem. Lett.* **1994**, *4*, 2273–2278 (L-738,872). (d) Kim, E. E.; Baker, C. T.; Dwyer, M. D.; Murcko, M. A.; Rao, B. G.; Tung, R. D.; Navia, M. A. Crystal Structure of HIV-1 Protease in Complex with VX-478, a Potent and Orally Bioavailable Inhibitor of the Enzyme. *J. Am. Chem. Soc.* **1995**, *117*, 1181–1182 (VX-478).
(5) HIV protease inhibitors based on other templates: (a) Smith, A. B.; Hirschmann, R.; Pasternak, A.; Akaishi, R.; Guzman, M. C.; Jones, D. R.; Keenan, T. P.; Sprengeler, P. A.; Darke, P. L.; Emini, E. A.; Holloway, M. K.; Schleif, W. A. Design and Synthesis of Peptidomimetic Inhibitors of HIV-1 Protease and Renin. Evidence for Improved Transport. *J. Med. Chem.* **1994**, *37*, 215–218. (b) Lam, P. Y. S.; Jadhav, P. K.; Eyermann, C. J.; Hodge, C. N.; Ru, Y.; Bacheler, L. T.; Meek, J. L.; Otto, M. J.; Rayner, M. M.; Wong, Y. N.; Chang, C.-H.; Weber, P. C.; Jackson, D. A.; Sharpe, T. R.; Erickson-Viitanen, S. Rational Design of Potent, Bioavailable, Nonpeptide Cyclic Ureas as HIV Protease Inhibitors. *Science* **1994**, *263*, 380–384. (c) Norbeck, D.; Kempf, D.; Marsh, K.; Paul, D. Knigge, M.; Vasavanonda, S.; Clement, J.; Kohlbrenner, W.; Sham, H.; Wideburg, N.; Plattner, J. Discovery of Potent, Orally Bioavailable Inhibitors of HIV Protease. In *Discovery of Potent, Orally Bioavailable Inhibitors of HIV Protease*; VII International Conference on AIDS; Amsterdam, 1992. (d) Varney, M. D.; Appelt, K.; Kalish, V.; Reddy, M. R.; Tatlock, J.; Palmer, C. L.; Romines, W. H.; Wu, B.-W.; Musick, L. Crystal-Structure-Based Design and Synthesis of Novel C-Terminal Inhibitors of HIV Protease. *J. Med. Chem.* **1994**, *37*, 2274–2284. For reviews, see: (e) Huff, J. R. HIV Protease: A Novel Chemotherapeutic Target for AIDS. *J. Med. Chem.* **1991**, *34*, 2305–2314. (f) Wlodawer, A.; Erickson, J. W. Structure-Based Inhibitors of HIV-1 Protease. *Annu. Rev. Biochem.* **1993**, *62*, 543–585. (g) See reference 2b.

(6) Saiga, A.; Tanaka, T.; Orita, S.; Sato, A.; Sato, S.; Hachisu, T.; Abe, K.; Kimura, Y.; Kondo, Y.; Fujiwara, T.; Igarashi, H. Human T-cell Leukemia Virus Type 1 Protease Protein Expressed in *Escherichia coli* Possesses Aspartic Proteinase Activity. *Arch. Virol.* **1993**, *128*, 195–210.

(7) Fusek, M.; Smith, E. A.; Monod, M.; Dunn, B.; Foundling, S. I. Extracellular Aspartic Proteinases from *Candida albicans*, *Candida tropicalis*, and *Candida parapsilosis* Yeasts Differ Substantially in Their Specificities. *Biochemistry* **1994**, *33*, 9791–9799.

(8) Owens, R. A.; Gesellchen, P. D.; Houchins, B. J.; DiMarchi, R. D. The Rapid Identification of HIV Protease Inhibitors Through the Synthesis and Screening of Defined Peptide Mixtures. *Biochem. Biophys. Res. Commun.* **1991**, *181*, 402–408.

(9) In seminal work in this area, Owens and co-workers have previously incorporated statine isosteres into a peptide library to identify HIV protease inhibitors (see ref 7); however, in order to display nonpeptide functionality about the isostere, new solid-phase synthesis methods are required.

(10) (a) Ghosh, A. K.; McKee, S. P.; Lee, H. Y.; Thompson, W. J. A Facile and Enantiospecific Synthesis of 2(*S*)- and 2(*R*)-[1′(*S*)-Azido-2-phenylethyl] oxirane. *J. Chem. Soc., Chem. Commun.* **1992**, 273–274. (b) Caron, M.; Carlier, P. R.; Sharpless, K. B. Regioselective Azide Opening of 2,3-Epoxy Alcohols by $[Ti(O\text{-}i\text{-}Pr)_2(N_3)_2]$: Synthesis of α-Amino Acids. *J. Org. Chem.* **1988**, *53*, 5185–5187.

(11) Thompson, L. A.; Ellman, J. A. Straightforward and General Method for Coupling Alcohols to Solid Supports. *Tetrahedron Lett.* **1994**, *35*, 9333–9336.

(12) The product is not stable to TFA for long periods of time. The cleavage was determined to be complete in 20 min, because significant amounts of product were not obtained upon resubjection of the resin to the cleavage cocktail.

(13) Majer, P.; Randad, R. S. A Safe and Efficient Method for Preparation of *N*-*N*′-Unsymmetrically Disubstituted Ureas Utilizing Triphosgene. *J. Org. Chem.* **1994**, *59*, 1937–1938.

(14) The acylation reactions were followed with a bromophenyl blue indicator for the presence of the secondary amine. Krchnák, V.; Vágner, J.; Safár, P.; Lebl, M. Noninvasive Continuous Monitoring of Solid-phase peptide Synthesis by Acid-base Indicator. *Czech. Chem. Commun.* **1988**, *53*, 2542–2548.

(15) Raddatz, P.; Schmitges, C. J.; Minck, K. O. Amino Acid Derivatives. Eur. Pat. Appl. 264, 795, 1987.

(16) Bartra, M.; Romea, P.; Urpí, F.; Vilarrasa, J. A Fast Procedure for the Reduction of Azides and Nitro Compounds Based on the Reducing Ability of $Sn(SR)_3^-$ Species. *Tetrahedron* **1990**, *46*, 587–594.

(17) Disappearance of the primary amine was monitored by either a bromophenyl blue[14] or ninhydrin indicator. Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. Color Test for Detection of Free Terminal Amino Groups in the Solid-Phase Synthesis of Peptides. *Anal. Biochem.* **1970**, *34*, 595–598.

(18) Cho, S. Y. S.; Jungheim, L. N.; Baxter, A. J. Novel HIV-1 Protease Inhibitors Containing a β-Hydroxy Sulfide Isostere. *Biomed. Chem. Lett.* **1994**, *4*, 715–720.

(19) The coupling of quinaldic acid with amine **6a** was also performed with PyBOP/HOBt in similar yield (unpublished results).

(20) Ghosh, A. K.; Duong, T. T.; McKee, S. P.; Thompson, W. J. *N*,*N*′-Disuccinimidyl Carbonate: A Useful Reagent for Alkoxycarbonylation of Amines. *Tetrahedron Lett.* **1992**, *33*, 2781–2784.

(21) Ghosh, A. K.; Thompson, W. J.; McKee, S. P.; Duong, T. T.; Lyle, T. A.; Chen, J. C.; Darke, P. L.; Zugay, J. A.; Emini, E. A.; Schleif, W. A.; Huff, J. R.; Anderson, P. S. 3-Tetrahydrofuran and Pyran Urethanes as High-Affinity P_2-Ligands for HIV-1 Protease Inhibitors. *J. Med. Chem.* **1993**, *36*, 292–294.

(22) The introduction of various side chains in the P_1 site of the inhibitors will diversify the library to include potential inhibitors for a variety of aspartic acid proteases.

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