Pergamon

Tetrahedron 58 (2002) 6305–6310

TETRAHEDRON

Efficient method to prepare hydroxyethylamine-based aspartyl protease inhibitors with diverse P_1 side chains

Masao Chino, Masahiro Wakao and Jonathan A. Ellman*

Department of Chemistry, Center for New Directions in Organic Synthesis, University of California, Berkeley, CA 94720, USA

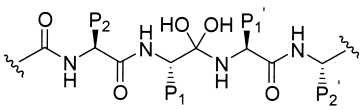
Received 20 February 2002; revised 4 April 2002; accepted 22 April 2002

Abstract—An efficient procedure for the solid-phase synthesis of hydroxyethylamine-based aspartyl protease inhibitors is described. The 1,2-bromo alcohol **4** is the key intermediate and is prepared in four-steps in good overall yield from commercial available amino acids. © 2002 Published by Elsevier Science Ltd.

1. Introduction

Combinatorial synthesis sequences designed to target protein families are particularly important because a sufficiently versatile and robust library synthesis approach can potentially be used to identify ligands to *any* member of that protein family.[1,2] We have demonstrated the power of this approach by developing a strategy for the solid-phase synthesis of libraries of compounds[3,4] to the aspartyl protease family,[5] which includes the extremely important drug targets, HIV protease[6] and β secretase implicated in Alzheimer's disease.[7]

We designed our library synthesis sequence to provide inhibitors based upon the hydroxyethylamine isostere (Fig. 1), which is a key pharmacophore for targeting aspartyl proteases and is present in several of the approved drugs for the treatment of AIDs.[5,6] This library synthesis approach has resulted in the rapid identification of single digit nanomolar and high picomolar small molecule inhibitors to cathepsin D[3,8] implicated in neurodegenerative disease[9] and cancer metastasis[10] and to the plasmepsins I and II,[11] which are key proteases in the life cycle of the malaria parasite.



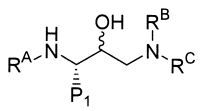
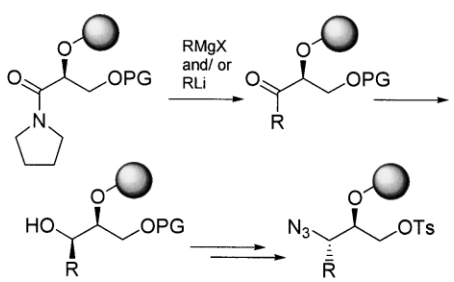


Figure 1. Hydroxyethylamine inhibitors.

To develop potent and selective aspartyl protease inhibitors it is essential that an appropriate P_1 side chain be identified. In our previously reported method,[3,4] the P_1 side chain is introduced by addition of Grignard reagents to a support-bound amide followed by functional group manipulations (Scheme 1). While this method is effective for introducing hydrophobic side chains, Grignard reagents are not compatible with a variety of functionality. For example, yapsin, a recently identified aspartyl protease that is implicated in peptide hormone processing, prefers basic lysine and arginine side chains at the P_1 position in peptide substrates.[12] Our previously reported method cannot be used to incorporate these side chains. Here we describe a practical and efficient sequence for the preparation of hydroxyethylamine-based inhibitors that is amenable to the incorporation of a wide range of functionality at the P_1 position.

2. Results and discussion

The key intermediate, 1,2-bromo alcohol **4**, is prepared in a straightforward four-step sequence from side-chain

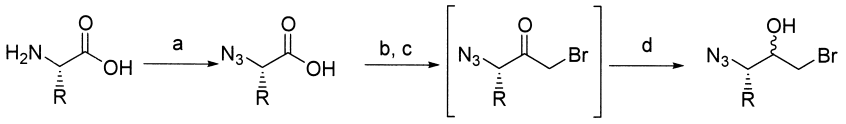


Scheme 1.

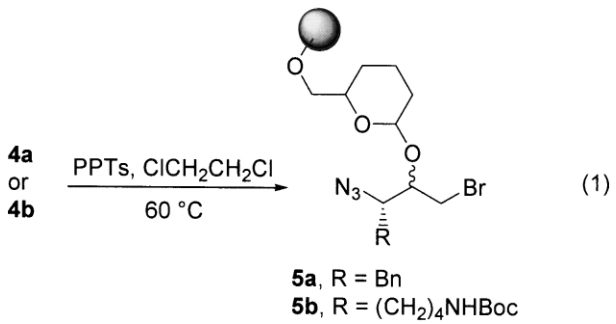
Keywords: combinatorial chemistry; hydroxyethylamine isosteres; aspartyl protease inhibitors.

* Corresponding author. Tel.: +1-510-642-4488; fax: +1-510-642-8369; e-mail: jellman@uclink.berkeley.edu

0040–4020/02/$ - see front matter © 2002 Published by Elsevier Science Ltd.
PII: S0040-4020(02)00629-4



Scheme 2. *Reagents and conditions*: (a) TfN_3, K_2CO_3, cat $CuSO_4$, aqueous MeOH, CH_2Cl_2, rt, 92% yield for **2a**, 68% yield for **2b**; (b) isobutyl chloroformate, *N*-methylmorpholine, THF, −40°C, then CH_2N_2, 0°C; (c) aqueous HBr, CH_2Cl_2, 0°C; (d) $NaBH_4$, THF, H_2O, 65% yield over 3 steps for **4a**, 60% yield over 3 steps for **4b**.

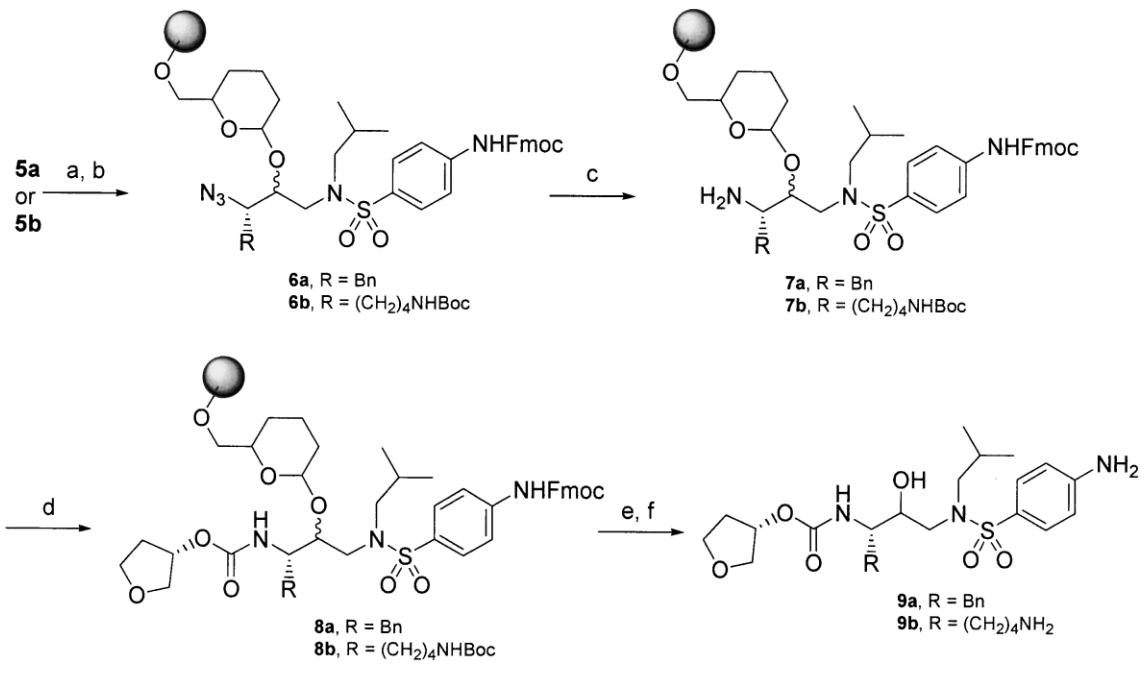


Scheme 3.

protected amino acids (Scheme 2). Because a very large number of natural and unnatural amino acids are commercially available, this approach provides access to bromo alcohols **4** incorporating a large number of diverse P_1 side chains. First, the amino acid is stereospecifically converted to α-azido carboxylic acid **2** by using trifluoromethansulfonyl azide according to the procedures of Wong[13] and Pelletier.[14] The α-bromo ketone **3** is then prepared in a one-pot sequence by activation of carboxylic acid **2** with isobutyl chloroformate and *N*-methylmorpholine followed by addition of diazomethane and then aqueous HBr. Reduction with $NaBH_4$ provides the desired alcohol **4** as a 1:1 ratio of stereoisomers. The 1:1 mixture of alcohol stereoisomers is ideal for the discovery of inhibitors since it is well documented that for aspartyl protease inhibitors either alcohol stereoisomer can provide the greatest inhibitory activity depending upon both the protease that is targeted and the functionality displayed on the inhibitor.[5]

The efficiency of the sequence was demonstrated by the preparation of **4a** and **4b**, which incorporate the phenylalanine and protected lysine side chains, in 58 and 41% overall yields, respectively. In addition, minimal epimerization (<2%) occurs during the halomethylation and reduction steps as determined by chiral HPLC analysis of **4a** and **4b**. The enantiomers of **4a** and **4b** were used as HPLC standards and were prepared from the corresponding D-amino acids.

For the solid-phase synthesis of aspartyl protease inhibitors, the secondary alcohol intermediate **4** is coupled to DHP resin (Scheme 3).[15] While neither camphorsulfonic acid nor toluenesulfonic acid were effective catalysts for loading alcohol **4** to support, pyridinium toluenesulfonate (PPTs) resulted in good loading efficiencies providing **5a** and **5b** in 0.57 and 0.58 mmol/g loading levels, respectively. Because the conditions to load alcohol **4** are acidic and may result in cleavage of the Boc group, prior to further synthetic transformations **5b** was treated with *tert*-butyl carbonic anhydride to ensure that the amine side chain was completely protected.



Scheme 4. *Reagents and conditions*: (a) *i*$BuNH_2$, NMP, 80°C; (b) *N*-Fmoc-4-aminobenzenesulfonyl choride, *i*Pr_2NEt, THF, rt; (c) $SnCl_2$, PhSH, Et_3N, THF, rt; (d) *N*-succinimidyl carbonate of 3-(*S*)-hydroxytetrahydrofuran, *i*Pr_2NEt, THF, rt; (e) 20% piperidine in NMP; (f) 95:5 THF/H_2O, 15 min.

To demonstrate that support-bound intermediates **5** can effectively be converted to aspartyl protease inhibitors, the FDA approved HIV protease inhibitor amprenavir,[16] **9a**, was synthesized (Scheme 4). A derivative of amprenavir, which incorporates the lysine side chain at the P_1 position, **9b**, was also prepared to demonstrate the compatibility of the solid-phase sequence with reactive side chain functionality. The syntheses were initiated by adding isobutylamine to **5a** and **5b** followed by reaction of the resulting secondary amines with *N*-Fmoc-4-aminobenzenesulfonyl chloride to provide the tertiary sulfonamide intermediates **6a** and **6b**. Reduction of the azide with thiophenol/Et_3N/$SnCl_2$ (4:5:1) as described by Bartra and co-workers provided the amines **7a** and **7b**,[17] which were acylated with the *N*-succinimidyl carbonate of 3-(*S*)-hydroxytetrahydrofuran to provide carbamates **8a** and **8b**. Removal of the Fmoc protecting groups with 20% piperidine in NMP followed by treatment with 95:5 TFA/water for 15 min provided inhibitors **9a** (amprenavir) and **9b** in 66 and 70% overall yields after reverse-phase HPLC purification, respectively.

3. Conclusion

In summary, we report an efficient procedure for the solid-phase synthesis of hydroxyethylamine-based aspartyl protease inhibitors with 1,2-bromoalcohol **4** serving as the key intermediate. Because **4** is prepared in four straightforward steps from readily available amino acids in good overall yields and without racemization, this method should provide rapid access to inhibitors incorporating a large number of different side chains at the P_1 position.

4. Experimental

4.1. General methods

Materials were obtained from commercial suppliers and employed without further purification unless otherwise stated. DHP resin was purchased from NovaBiochem (San Diego, CA). The following solvents were distilled under N_2 from the specified drying agents: tetrahydrofuran (THF) and diethyl ether (Et_2O) from sodium/benzophenone ketyl, and methylene chloride (CH_2Cl_2), dichloroethane and pyridine from calcium hydride. Diazomethane was generated in situ using the following procedure.[18] To a 0.7 M suspension of *p*-toluenesulfonylmethylnitrosamide (Diazold) in absolute ethanol was added small aliquots of 40 wt% KOH aqueous solution until the Diazold suspension became white. Diazomethane gas was produced and transferred by cannula (two fine-polished pipets connected by rubber tubing) under positive N_2 flow into the stirring THF solution of the second reagent. Infrared spectra (IR) were recorded with a Perkin–Elmer 1600 Series Fourier transform spectrometer using NaCl plates, and only partial spectral data is listed. All ^1H and ^{13}C NMR spectra were obtained in $CDCl_3$ unless otherwise stated, and chemical shifts for ^1H and ^{13}C NMR are recorded in parts per million relative to the internal solvent peak at 7.26 and 77.0 ppm, respectively. Coupling constants are reported in hertz. LC–MS analyses were conducted using a Hewlett Packard 1100 MSD with an Agilent Zorbax SB-C18 column.

4.2. Trifluoromethanesulfonyl azide (triflyl azide)[13]

A solution of NaN_3 (595 mg, 9.15 mmol) in 1.5 mL of H_2O was cooled in an ice bath and treated with 2.5 mL of CH_2Cl_2. The resulting biphasic mixture was stirred vigorously and treated with Tf_2O (523 mg, 1.85 mmol) over a period of 5 min. The reaction mixture was stirred at 0°C for 2 h. The organic phase was then separated and the aqueous phase was extracted twice with CH_2Cl_2. The total volume of the reagent solution was 5 mL. The combined organic layers were washed once with saturated Na_2CO_3 solution and the resulting triflyl azide solution used without further purification. WARNING: According to the literature report the triflyl azide solution should not be heated or concentrated.

4.2.1. Synthesis of α-azido acid 2a (R = Bn). The diazo transfer reaction was performed according to the procedure of Pelletier for converting α-amino acids to α-azido acids.[14] L-Phe (460 mg, 2.79 mmol) was combined with K_2CO_3 (577.5 mg, 4.19 mmol) and $CuSO_4$ pentahydrate (6.98 mg, 27.9 μmol), distilled H_2O (9 mL), and CH_3OH (18 mL). Triflyl azide in CH_2Cl_2 (15 mL, ~5.55 mmol, 2 equiv.) prepared according to the procedure above was added with stirring, and the resulting mixture was stirred at ambient temperature overnight. Subsequently, the organic solvents were removed under reduced pressure and the aqueous slurry was diluted with H_2O (50 mL). This slurry was then acidified to pH 6 with 2N HCl aqueous solution and diluted with 0.25 M, pH 6.2 phosphate buffer (50 mL) and extracted with EtOAc (4×) to remove the sulfonamide byproduct. The aqueous phase was then acidified to pH 2 with 2N HCl aqueous solution. The product was obtained from another round of EtOAc extractions (3×). The EtOAc extracts were combined, dried (Na_2SO_4) and evaporated to dryness giving 474 mg (89% yield) of the α-azido acid **2a** (R = Bn) as a pale yellow oil. The spectral data correlated exactly with the literature values.[14]

4.2.2. Synthesis of α-azido acid 2b (R = $(CH_2)_4$NHBoc). Compound **2b** was prepared according to the procedure used to prepare **2a**. From 686 mg (2.79 mmol) of *N*-ε-Boc-Lys was obtained 516 mg (68% yield) of **2b** as a pale yellow oil. The spectral data correlated exactly with the literature values.[14]

4.2.3. Bromomethyl alcohol 4a (R = Bn). Isobutyl chloroformate (287 μL, 2.2 mmol) was added to a solution of azido acid **2a** (382 mg, 2.0 mmol) and *N*-methylmorpholine (242 μL, 2.2 mmol) in THF (20 mL) at −40°C, and the reaction mixture was stirred for 15 min. The reaction mixture was then filtered and diazomethane (from 1.33 g, 6.2 mmol of 1-methyl-3-nitro1-nitrosoguanidine and 1.8 mL of 40 wt% aqueous KOH in 10 mL of EtOH) was added slowly. The reaction flask was stoppered and was maintained at 0°C in a refrigerator overnight. The reaction mixture was then treated with 48% HBr aq (410 μL) and was stirred for 15 min. The reaction mixture was diluted with EtOAc (20 mL) and then was extracted with 15 wt% aqueous citric acid, then washed with saturated sodium bicarbonate (with CO_2 evolution), and then washed with brine. The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure.

The residue was dissolved in 20 mL of 95:5 THF/H_2O. To the resulting solution was added sodium borohydride (0.1 g, 2.6 mmol) gradually at rt. The reaction mixture was stirred for 1 h and then was neutralized with aqueous 1N HCl. After extraction with EtOAc (3×20 mL), the organic extracts were washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure. Purification by silica-gel chromatography with 85:15 hexanes/EtOAc afforded 350 mg (65% yield over 3 steps) of **4a** (R=Bn) as a white solid: IR (NaCl): 3418, 2107, 1262, 1050 cm^{-1}. ^1H NMR (500 MHz): δ 2.33 (brs, 1H), 2.40 (brs, 1H), 2.84 (dd, J=14.0, 8.6 Hz, 1H), 3.03 (dd, J=13.7, 7.9 Hz, 1H), 3.08 (dd, J=13.7, 6.7 Hz, 1H), 3.15 (dd, J=14.0, 3.6 Hz, 1H), 3.48 (dd, J=10.3, 5.4 Hz, 1H), 3.53 (dd, J=10.4, 6.7 Hz, 1H), 3.61–3.79 (m, 3H×2), 7.26–7.30 (m, 3H×2), 7.33–7.39 (m, 2H×2). ^{13}C NMR (125 MHz): δ 35.2, 36.9, 37.0, 37.4, 64.8, 66.0, 71.9, 72.2, 127.0, 127.1, 128.7, 128.8, 129.2, 129.4, 136.6, 136.9. Anal. Calcd for $C_{10}H_{12}BrN_3O$: C, 44.46; H, 4.48; N, 15.56. Found: C, 44.73; H, 4.64; N, 15.28.

Determination of enantiopurity of bromomethyl alcohol **4a**. Both enantiomers of **4a** were synthesized from L- and D-Phe. The HPLC analysis of a 1:1 mixture of these compounds (5 μL, concentration=2.5 mg/mL) using a DAICEL Chiralcel column with *i*PrOH/hexane (2:98) as an eluent at a flow rate of 1.0 mL/min at wavelength of 210 nm afforded four peaks of equal area corresponding to the four possible diastereomers. The retention time for **4a** prepared from L- and D-Phe was 20.8 and 22.2 min and 16.5 and 31.0 min, respectively. HPLC analysis of **4a** derived from L-Phe established that racemization was less than 2.5% [rt (peak area), 16.5 min (<1%), 20.8 min (46%), 22.2 min (52%), 31.0 min (<1%)].

4.2.4. Bromomethyl alcohol 4b (R=$(CH_2)_4$NHBoc). Compound **4b** was prepared according to the procedure used to prepare **4a** starting with 545 mg (2.0 mmol) of **2b**. Purification by silica-gel chromatography with 70:30 hexanes/EtOAc afforded 420 mg (60% yield for 3 steps) of **4b** (R=$(CH_2)_4$NHBoc) as a white solid: IR (NaCl): 3433, 2925, 2359, 2099, 1688, 1524 cm^{-1}. ^1H NMR (500 MHz, in acetone-d_6): δ 1.38 (s, 9H×2), 1.38–1.79 (m, 6H×2), 3.03–3.13 (m, 2H×2), 3.37–3.44 (m, 1H), 3.48–3.66 (m, 5H), 3.82–3.86 (m, 1H), 3.87–3.91 (m, 1H), 5.98 (brs, 1H×2). ^{13}C NMR (125 MHz): δ 22.9, 23.1, 28.4, 29.6, 29.9, 35.1, 37.1, 44.8, 54.3, 63.5, 64.7, 72.3, 72.6, 156.1, 156.2. HRMS (FABMS) calcd for $[M]^+$ ($C_{12}H_{23}BrLiN_4O_3$) 357.13135, found 357.111960.

Determination of enantiopurity of bromomethyl alcohol **4b**. Both enantiomers of **4b** were synthesized from L- and D-*N*-ε-Boc-Lys and were then benzoylated to introduce a chromophore for HPLC analysis. To a solution of **4b** (64.0 mg, 0.182 mmol) prepared from L-*N*-ε-Boc-Lys in 2 mL of CH_2Cl_2 were added pyridine (64.4 μL, 0.797 mmol), 4-(dimethylamino)pyridine (DMAP, 2.4 mg, 0.020 mmol), and benzoyl chloride (46.3 μL, 0.399 mmol). The mixture was stirred at ambient temperature overnight. The reaction was quenched by addition of sat. $NaHCO_3$ aq. (3 mL). The aqueous layer was extracted with CH_2Cl_2 (3×5 mL). The organic layers were combined, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica-gel chromatography with 80:20 hexane/EtOAc to afford 80.3 mg of benzoylated compound **4b** (97%). In the same manner, the **4b** prepared from D-*N*-ε-Boc-Lys was converted to the corresponding benzoylated compound. ^1H NMR (400 MHz, $CDCl_3$, for 1:1 mixture): δ ppm 1.42 (s, 9H×2), 1.40–1.74 (m, 6H×2), 3.03–3.20 (br, 2H×2), 3.56–3.88 (m, 3H×2), 4.55 (brs, 1H×2), 5.23 (m, 1H), 5.31 (m, 1H), 7.45 (m, 2H×2), 7.60 (m, 1H×2), 8.06 (m, 2H×2). HRMS calcd for $C_{19}H_{27}BrN_4O_4$+H 455.12939, found 455.12966. The HPLC analysis of a 1:1 mixture of these compounds (5 μL, concentration=2.5 mg/mL) using a DAICEL Chiralcel column with EtOH/hexane (1.5:98.5) as an eluent at a flow rate of 1.0 mL/min at wavelength of 230 nm afforded four peaks of equal area corresponding to the four possible diastereomers. The retention time (RT) for L- and D-form was 23.3 and 26.3 min and 19.7 and 21.9 min, respectively. HPLC analysis of **4b** derived from L-*N*-ε-Boc-Lys established that no racemization had occurred during the synthesis sequence [rt (peak area), 23.3 min (50%), 26.3 min (50%)].

4.2.5. *N*-Fmoc-4-aminobenzenesulfonic acid.[19] Sulphanilic acid (6.80 g, 35.6 mmol) was dissolved in a solution of saturated aqueous $NaHCO_3$ (17 mL) and reacted with FmocCl (0.92 g, 4.82 mmol) at pH 8 with mechanical stirring. The reaction mixture was stirred for 12 h, and then the precipitated solid was filtered off and washed with Et_2O. The filtrate was dried under reduced pressure. The dry residue was dissolved in a mixture of dry toluene (25 mL) and anhydrous DMF (2.5 mL) and thionyl chloride (1.00 mL, 13.7 mmol) was then added at 0°C. The reaction mixture was stirred for 12 h at room temperature, then poured into an ice-water mixture and then neutralized with $NaHCO_3$. The aqueous phase was extracted with EtOAc (2×25 mL). The organic layer was washed with brine, dried ($NaSO_4$), and concentrated. Recrystallization of the crude product from benzene and hexane afforded 811 mg (54% yield) of desired product as a white crystalline solid: IR (NaCl): 2360, 1716, 1589, 1524, 1373, 1320, 1220, 1170 cm^{-1}. ^1H NMR (500 MHz): δ 4.28 (t, J=6.0 Hz, 1H), 4.66 (d, J=6.0 Hz, 2H), 7.35 (td, J=7.5, 1.0 Hz, 2H), 7.44 (t, J=7.5 Hz, 2H), 7.53–7.60 (m, 2H), 7.61 (d, J=7.5 Hz, 2H), 7.80 (d, J=7.5 Hz, 2H), 7.96 (d, J=7.5 Hz). ^{13}C NMR (125 MHz): δ 46.9, 67.3, 118.1, 120.1, 124.7, 127.2, 128.0, 128.7, 138.1, 141.4, 143.3, 144.1, 152.5. Anal. Calcd for $C_{21}H_{16}ClNO_4S$: C, 60.94; H, 3.90; N, 3.38. Found: C, 61.14; H, 4.05; N, 3.30.

4.3. General solid-phase synthesis methods

All solid-phase reaction mixtures were stirred at the slowest rate. For the general solid-phase workup procedure, the reaction solution was filtered away from the support-bound material using polypropylene cartridges with 70 μm PE frits (Speed Accessories) attached to Teflon stopcocks. Cartridges and stopcocks were purchased from Applied Separations (Allentown, PA). The support-bound material was thoroughly washed with various solvents as described in the specific experimental sections.

4.3.1. Support-bound bromide 5a (R=Bn). To a mixture of 144 mg (0.141 mmol) of DHP resin in 0.5 mL of

dichloroethane at room temperature was added a solution of 93.5 mg (0.212 mmol) of alcohol **4a** (R = Bn) in 0.5 mL of dichloroethane. The reaction mixture was stirred for 10–12 h at 60°C followed by the addition of 88.5 mg (0.353 mmol) of PPTs. The resin was then washed with NMP (3×), THF (3×), CH_2Cl_2 (3×), and Et_2O (3×), flushed with N_2, and then dried under reduced pressure, and stored at −20°C.

The loading level of the resin was determined according to the following procedure. Resin **5a** (30.4 mg) was treated with 95:5 TFA/H_2O (1.0 mL). Stirring was carried out by rotating at room temperature on a wrist action shaker for 15 min. After filtration, the resin was washed with 95:5 TFA/H_2O (1×) and CH_2Cl_2 (2×), and the combined filtrates were concentrated under reduced pressure to provide alcohol **4a** as colorless oil. The loading level was then determined by integration of the NMR signals of **4a** against a known amount of *p*-xylene as an internal standard (500 MHz, MeOH-d_4). The loading level was 0.576 mequiv./g, which corresponds to a 59% loading efficiency based on the 0.98 mequiv./g loading level reported for the purchased DHP resin.

4.3.2. Support-bound bromide 5b (R = $(CH_2)_4$NHBoc). To a mixture of 144 mg (0.141 mmol) of DHP resin in 0.5 mL of dichloroethane at room temperature was added a solution of 74.4 mg (0.212 mmol) of alcohol **4b** (R = $(CH_2)_4$NHBoc) in 0.5 mL of dichloroethane. The reaction mixture was stirred for 10–12 h at 60°C followed by the addition of 88.5 mg (0.353 mmol) of PPTs. The reaction mixture was transferred to a polypropylene cartridge with a 70 μm PE frit attached to a Teflon stopcock for the work up process. The resin was immediately washed with NMP (3×), THF (3×), CH_2Cl_2 (3×), and Et_2O (3×), flushed with N_2, and then dried under reduced pressure.

Because some of the *N*-Boc group might be cleaved under the acidic conditions of the resin-loading step, the resin was subjected to Boc_2O to ensure complete protection. The reaction vessel was charged with 0.353 M Boc_2O (77.0 mg, 0.353 mmol) in THF (1.0 mL) at room temperature followed by the addition of 61 μL (0.35 mmol) of $i\,Pr_2NEt$. Mixing was accomplished by rotating on a wrist-action shaker at room temperature for 10–12 h. The reaction solution was then drained and the resin was immediately washed with NMP (3×), THF (3×), CH_2Cl_2 (3×), and Et_2O (3×), flushed with N_2, dried under reduced pressure, and stored at −20°C.

The loading level of the resin was determined according to the following procedure. Resin **5b** (23.9 mg) was treated with 95:5 TFA/H_2O (1.0 mL). Stirring was carried out by rotating at room temperature on a wrist action shaker for 15 min. After filtration, the resin was washed with 95:5 TFA/H_2O (1×) and CH_2Cl_2 (2×), and the combined filtrates were concentrated under reduced pressure to provide alcohol **4b** (lacking the Boc group) as colorless oil. The loading level was then determined by integration of the NMR signals of **4b** (lacking the Boc group) against a known amount of *p*-xylene as an internal standard (500 MHz, MeOH-d_4. The loading level was 0.570 mequiv./g, 58% loading efficiency based on the 0.98 mequiv./g loading level reported for the purchased DHP resin.

4.3.3. Synthesis of aspartyl protease inhibitor 9. Support-bound bromide **5** (~10 μmol) was added to a vial. A 1.0 M solution of *n*-butylamine in NMP (1 mL) was then added to the vial. The vial was sealed and then the reaction mixture was heated at 80°C for 36 h. The reaction mixture was then transferred to a polypropylene cartridge with a 70 mm PE frit attached to a Teflon stopcock for the work up process. The resin was washed with NMP (3×), THF (3×), CH_2Cl_2 (3×), and ether (3×), and then dried under vacuum. Acylation with 0.3 M sulfonyl chloride[7] and 0.6 M $i\,Pr_2EtN$ in THF (1.0 mL) was carried out overnight. The resin was washed with NMP (3×), THF (3×), CH_2Cl_2 (3×), and ether (3×), and then dried under vacuum. Reduction of the azide was accomplished using 0.2 M $SnCl_2$, 0.8 M PhSH, and 1.0 M Et_3N in THF (1 mL) for 4 h[8]. The resin was washed with 50 vol% aqueous THF solution (3×), THF (3×), CH_2Cl_2 (3×) and ether (3×), and then dried under vacuum. The resulting support-bound amine was then acylated using 1 mL of a THF stock solution that was 0.3 M in the *N*-succinimidyl carbonate of 3-(*S*)-hydroxytetrahydrofuran[9] and 0.6 M in $i\,Pr_2EtN$. After allowing the acylation reaction to proceed overnight, the resin was washed with NMP (4×), THF (2×), CH_2Cl_2 (3×) and ether (3×). Resin **5** was then treated with 20 vol% piperidine in NMP (1 mL) for 30 min. The resin was washed with NMP (3×), THF (3×), CH_2Cl_2 (3×), and ether (3×) and dried under vacuum. The resulting resin was treated with 95:5 TFA/H_2O for 15 min followed by filtration. The resin was then rinsed with 95:5 TFA/H_2O (1×) and CH_2Cl_2 (2×). The combined filtrates were concentrated to dryness. Toluene was added to azeotrope the TFA during the concentration step. The mixture was then purified by reverse-phase HPLC (Varian Microsorb C18 (Si) column R 0089100C5) using a linear gradient starting with 5:95, 0.1% TFA/acetonitrile: 0.1% TFA/H_2O and ending with 0.1% TFA/acetonitrile over 120 min. Purified product **9** was stored at −20°C.

4.3.4. Inhibitor 9a (R = Bn, amprenavir (vx 478)). The solid-phase synthesis procedure was followed starting with 19.1 mg (11.0 μmol) of support-bound bromide **5a**. Reverse-phase HPLC purification gave 3.7 mg (66% yield) of inhibitor **13a** as a white powder: IR (NaCl): 2921, 1708, 1689, 1595, 1314, 1148 cm^{-1}. ^1H NMR (500 MHz, in MeOH-d_4): δ 0.86–0.91 (m, 6H), 1.28–2.19 (m, 9H), 2.75–3.17 (m, 6H), 3.51–3.91 (m, 7H), 6.70 (d, *J* = 8.7 Hz, 2H), 7.47 (d, *J* = 8.7 Hz, 2H). HRMS (FABMS) calcd for $[M+H]^+$ ($C_{25}H_{36}N_3O_6S$) 506.2325, found 506.2330.

4.3.5. Inhibitor 9b (R = $(CH_2)_4$NHBoc). The solid-phase synthesis procedure was followed starting with 15.5 mg (8.8 μmol) of support-bound bromide **5b**. Reverse-phase HPLC purification provided 3.0 mg (70% yield) of inhibitor **13b** as a colorless oil: IR (NaCl): 3399, 1678, 1203, 1139 cm^{-1}. ^1H NMR (500 MHz, in MeOH-d_4): δ 0.72–0.98 (m, 6H), 1.53–2.33 (m, 5H), 2.53–3.20 (m, 5H), 3.42–3.87 (m, 5H), 4.93–5.09 (m, 1H), 6.67–6.70 (m, 1H), 7.16–7.79 (m, 8H). HRMS (FABMS) calcd for $[M+Na]^+$ ($C_{22}H_{38}N_4ONaO_6S$) 509.2410, found 509.2397.

Acknowledgments

This research was supported by NIH grant GM50451. Support for M. C. from Mitsubishi Pharma Corporation is also gratefully acknowledged. M. W. thanks the Japan Society for the Promotion of Science (JSPS Fellows No. 01798).

References

1. Macdonald, S. J. F.; Smith, P. W. *Drug Discov. Today* **2001**, *6*, 947–953.
2. Maly, D. J.; Huang, L.; Ellman, J. A. *ChemBiolChem.* **2002**, *3*, 16–37.
3. Lee, C. E.; Kick, E. K.; Ellman, J. A. *J. Am. Chem. Soc.* **1998**, *20*, 9735–9748.
4. Kick, E. K.; Ellman, J. A. *J. Med. Chem.* **1995**, *38*, 1427–1430.
5. For an excellent summary of aspartyl protease targets and inhibitors see: Leung, D.; Abbenante, G.; Fairlie, D. P. *J. Med. Chem.* **2000**, *43*, 305–341.
6. Thompson, L. A.; Tebben, A. J. *Annu. Rep. Med. Chem.* **2001**, *36*, 247–256.
7. Wolfe, M. S. *J. Med. Chem.* **2001**, *44*, 2039–2060.
8. Kick, E. K.; Roe, D. C.; Skillman, A. G.; Liu, G.; Ewing, T. J. A.; Sun, Y.; Kuntz, I. D.; Ellman, J. A. *Chem. Biol.* **1997**, *4*, 297–309.
9. Bi, X.; Lin, B.; Haque, T.; Lee, C. E.; Skillman, A. G.; Kuntz, I. D.; Ellman, J. A.; Lynch, G. *J. Neurochem.* **2000**, *74*, 1469–1477.
10. Garcia, M.; Platet, N.; Liaudet, E.; Laurent, V.; Derocq, D.; Brouillet, J. P.; Rochefort, H. *Stem Cells* **1996**, *14*, 642–650.
11. Haque, T. S.; Skillman, A. G.; Lee, C. E.; Habashita, H.; Gluzman, I. Y.; Ewing, T. J. A.; Goldberg, D. E.; Kuntz, I. D.; Ellman, J. A. *J. Med. Chem.* **1999**, *42*, 1428–1440.
12. Olsen, V.; Cawley, N. X.; Brandt, J.; Egel-Mitani, M.; Loh, Y. P. *Biochem. J.* **1999**, *339*, 407–411.
13. Alper, P. B.; Hung, S.-C.; Wong, C.-H. *Tetrahedron Lett.* **1996**, *34*, 6029–6032.
14. Lundquist, IV J. T.; Pelletier, J. C. *Org. Lett.* **2001**, *3*, 781–783.
15. Thompson, L. A.; Ellman, J. A. *Tetrahedron Lett.* **1994**, *35*, 9333–9336.
16. Kim, E. E.; Baker, C. T.; Dwyer, M. D.; Murcko, M. A.; Rao, B. G.; Tung, R. D.; Navia, M. A. *J. Am. Chem. Soc.* **1995**, *117*, 1181–1182.
17. Bartra, M.; Romea, P.; Urpi, G.; Vilarrasa, J. *Tetrahedron* **1990**, *46*, 587–594.
18. Lombardi, P. *Chem. Ind.* **1990**, 708.
19. *N*-Fmoc-4-aminobenzenesulfonyl chloride was prepared according to the following literature procedure: Jameson, G. W.; Elmore, D. T. *Biochem. J.* **1974**, *141*, 555–565.