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Preparation of Novel Hydroxyethyl Amine Isosteres as Potential Cathepsin D Inhibitors

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Abstract

Cathepsin D is a lysosomal aspartic protease found in all mammalian cells and is considered to be one of the main catabolic proteinases. Cathepsin D has been suggested to play a role in the metastatic potential of several types of cancer. A high activated cathepsin D level in breast tumor tissue has been associated with an increased incidence of relapse and metastasis. High levels of active cathepsin D have also been found in colon cancer, prostate cancer, uterine cancer, and ovarian cancer. Hydroxyethyl isosteres with cyclic tertiary amine have proven to be clinically useful as inhibitors of aspartyl proteases similar to cathepsin D in activity, such as the HIV-1 aspartyl protease. We have undertaken the design, via computer molecular modeling, and the synthesis of (hydroxyethyl)amine isostere inhibitors, which are similar to potent inhibitors of the aspartyl HIV-1 protease. We now report the preparation of six compounds that contain novel hydroxyethyl isosteres with cyclic tertiary amines.

Introduction

Aspartyl proteases are among the most biologically important proteolytic enzymes. Some of the more serious medical problems, such as cardiac disease, AIDS, Alzheimer's disease, as well as colorectal and breast cancer, and other cancers, either result directly from, or are characterized by, uncontrolled aspartyl protease activity (Mimoto et al., 2000; Moore et al., 2000; Nakaya et al., 2001; Messmer et al., 2001). The HIV-1 aspartyl proteinase, which is responsible for the maturation of HIV into the infectious viral particles (Darke and Heff, 1994), has become an important therapeutic target for treatment of acquired immunodeficiency syndrome (AIDS) (Debnath, 1999; Alterman et al., 1998; Backbro et al., 1997; Hulten et al., 1997).

Cathepsin D is an aspartyl lysosomal protease similar to the HIV-1 aspartyl protease in substrate specificity. Cathepsin D is clearly involved in the process of tumor invasion and metastasis (Thorpe et al., 1998; Vetvicka et al., 1997; Laury-Kleintop et al., 1995; Losch et al., 1996; Mordente et al., 1998; Reig et al., 1996). In fact, cathepsin D has recently emerged as a prognostic indicator in several cancers, including breast cancer (Thorpe et al., 1998), prostrate (Mordente et al., 1998), and colon (Reig et al., 1996). Also cathepsin D has recently been associated with the development of Alzheimer's disease (Moore, 2000). Specific proteinase inhibitors, useful in investigations of the mechanisms and pathways of intracellular protein degradation, could lead to the development of therapeutic agents for treatment of many types of carcinomas, as well as Alzheimer's disease.

Pepsin, renin, cathepsin D, and recently the HIV-1 aspartyl protease are among the best characterized aspartyl proteases. All of these proteases are inhibited by pepstatin A (Kratzel et al., 1998; Kratzel et al., 1999; Scholtz et al., 1994), a pentapeptide like compound which contains two unusual -amino acid statines [(3S,4S)-4-amino-3-hydroxy-6-methylheptanoic acid]. Pepstatin, which contains a reactive hydroxyl group, forms the tetrahedral intermediate by reacting with the essential carboxyl group in the active site of the proteinase (Rich and Bernatowicz, 1985; Payne et al., 1991). Although pepstatin A was found to be a potent inhibitor of the HIV-1 aspartyl protease, the peptidic nature of the inhibitor resulted in poor bioavailability. In order to improve bioavailability and improve in vivo half-life, recent work has focused on smaller inhibitors that contain a non-peptide functionality in place of the peptide bond cleavage site of the substrate (Skulnick et al., 1997; Reich et al., 1996). The use of hydroxyethyl isosteres with cyclic tertiary amines has lead to compounds with enhanced oral absorption (Smith et al., 1997). In recent years the (R)-hydroxyethylamine insert was incorporated as a key component of many clinically used, highly potent, HIV-1 protease inhibitors. Initially several compounds that contain hydroxyethyl amine isosteres with flexible alkyl amines were developed (Beaulieu et al., 1997), but they suffered limited in vivo half-lives and were not therapeutically useful.

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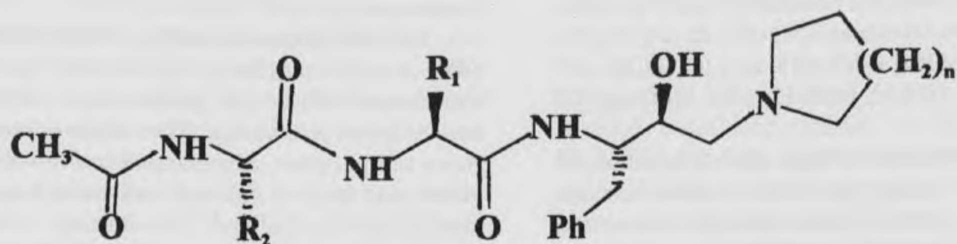


Fig. 1. Target molecules with peptide and non-peptide portions.

Molecular modeling (HYPERCHEM) has shown that a six member ring forming the tertiary amine may be able to orient the backbone of the inhibitor toward a bioactive conformation while providing more of a non-peptide functionality which greatly improves the half-life of the inhibitor in vivo. A comparison of the structural and stereochemical requirements of the S_3', S_2', and S_1' as well as the S_1, S_2, and the S_3, subsites of cathepsin D along with projected interactions with various inhibitor side chains suggests modifications which may be introduced to improve potency. Several investigators have shown, through crystallographic studies or molecular modeling, that either a cyclohexyl group of a reduced phenylalanine or the phenyl group of a phenyalanine-type statine (hydroxyethylene) or hydroxyethyl amine is easily positioned in the S_1 site of the HIV-1 aspartyl protease (Payne et al., 1991; McConnell et al., 1991). Other studies show that a bulky amine or amide might fit reasonably well into the S_2', and S_3', sites (Paul et al., 1995). Therefore, we set out to synthesize compounds that contain a peptide portion to accommodate the S_1, S_2, and S_3 subsites and a non-peptide hydroxyethyl isostere portion with a cyclic tertiary amine to accommodate the S_1', S_2', and S_3', enzyme subsites. The general structure of our synthetic target is shown in Figure 1.

Materials and Methods

Anhydrous solvents were "anhydrous grade" from Aldrich Chemical Company. Dry solvents were distilled from sodium just prior to use. All other solvents were HPLC grade. Reagents were purchased from Sigma-Aldrich Chemical Company. Thin layer chromatography (TLC) was run on Whatman PE SIL G/UV 250 µm silica gel plates. Column chromatography was run on either Aldrich TLC grade silica gel 2-25µm particle size BET surface area ~500 m^2/g, average pore diameter 60Å, or Sigma Sephadex LH-20, lipophilic, bead size 20-100 µm. The ^1H NMR spectra were collected either on a Bruker 200 MHz AC 200 superconducting spectrometer or on a Hitachi 60 MHz R1200 RS NMR spectrometer. ^1H NMR of final compounds and major intermediates were collected on the 200 MHz spectrometer, while the spectra of minor intermediates were collected on the 60 MHz NMR spectrometer. The spectral data were processed NTNMR software produced by TeleMag.

BOC-L-phenylalaninal (1).--Commercially available BOC-L-phenylalanine methyl ester (10.2 g, 36.5 mmol) in 200 mL dry tetrahydrofuran (THF) was chilled to -80°C under a N_2 atmosphere. The cold solution was slowly treated with 92 mL (91.25 mmol, 2.5 equivalents) of 1 M diisobutyl aluminum hydride (DIBAL) in hexanes over 20 minutes. The mixture was stirred at -80°C under N_2 for four hours. The reaction was quenched by the addition of 100 mL 0.01 M aqueous HCl. The mixture was then partitioned between the layers of dichloromethane (400 mL) and 5% aqueous sodium potassium tartarate (400 mL). The aqueous layer was extracted with another 400 mL of dichloromethane. The organic layers were combined and washed three times with 250 mL portions of distilled water. The organic layer was dried over anhydrous magnesium sulfate and concentrated under reduced pressure to produce a colorless viscous oil. The crude aldehyde was purified by silica gel flash chromatography using dichloromethane as the mobile phase, (final mass 5.80 g). TLC (CH_2Cl_2) R_f = 0.39. ^1H NMR ($CDCl_3$/TMS, 60MHz) δ 1.0 (9H, s), 2.3 (2H, d), 4.1 (1H, m), 4.9 (1H, m), 6.9 (5H,s), 9.9 (1H,s).

3(S)-BOCamino-4-phenyl-1-butene (2).--A precooled (0 °C) solution of triphenylmethyl phosphonium bromide (12.8 g, 0.035 mol) in 50 mL dry THF was slowly treated over 10 minutes with 20 mL of 1.6 M n-butyl lithium (0.032 mol) under N_2 atmosphere. The solution was stirred for four hours at 0°C. A solution of BOC-L-phenylalaninal (5.0 g, 0.020 mol) in 25 mL dry THF was added. The mixture was allowed to warm to room temperature and was refluxed for 24 hours under N_2 atmosphere. The reaction was cooled and then quenched by the addition of 100 mL 0.01 M aqueous HCl saturated with NaCl. The mixture was extracted three times with 200 mL portions of ether. The

organic layers were pooled, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure to give a white powder (3.5 g). TLC (ether/hexanes, 1/1, v/v) $R_f = 0.36$. ^1H NMR ($CDCl_3$/TMS, 60MHz) δ 1.0 (9H, s), 2.2 (2H, d), 4.2 (1H, m), 4.9 (1H, m), 5.1 (2H, d, J=10 Hz), 5.3 (1H, m), 6.9 (5H,s).

3(S)-BOCamino-4-phenyl-2-oxirane (3).--A solution of 5.5 g (4 equivalents, 32 mmol) meta-chloroperoxybenzoic acid (MCPBA) in 200 mL methylene chloride was mixed with a solution of 2.0 g (8 mmol) 3(S)-BOCamino-4-phenyl-1-butene (2) in 100 mL methylene chloride. The mixture was stirred at room temperature for 24 hours. The mixture was then filtered and concentrated under reduced pressure. The oily residue was partitioned between the layers of ether (400 mL) and distilled water (400 mL). The organic layer was removed and saved. The aqueous layer was extracted with another 400 mL ether. The ether layers were combined, washed with distilled water (400 mL), 5% aqueous $NaHCO_3$ (400 mL), and distilled water again (400 mL). The organic layer was dried over anhydrous magnesium sulfate and concentrated under reduced pressure to produce a mixture of epoxide diastereomers (1.53 g). The diastereomers were separated by silica gel (grade 710, 4-20 μm, surface area 480 m^2/g) column chromatography (column size 4" dia. x 5' length) using 10% ethyl acetate/hexanes as the mobile phase.

3(S)-BOCamino-4-phenyl-2(R)-oxirane (3a).--0.597 g. TLC (35% ethyl acetate/hexanes) $R_f = 0.21$. ^1H NMR ($CDCl_3$/TMS,200MHz) δ 0.9572 (9H, s), 2.451 (2H, d), 3.525 (2H, d), 3.705 (1H, d of t), 4.531 (1H, m), 4.715 (1H, m, exchangable), 7.105 (5H,s).

3(S)-BOCamino-4-phenyl-2(S)-oxirane (3b).--0.535 g. TLC (35% ethyl acetate/hexanes) $R_f = 0.18$. ^1H NMR ($CDCl_3$/TMS,200MHz) δ 0.9805 (9H, s), 2.401 (2H, d), 3.529 (2H, d), 4.203 (1H, d of t), 4.502 (1H, m), 4.675 (1H, m, exchangable), 7.059 (5H,s).

3(S)-BOCamino-4-phenyl-1-N-piperdine-2(S)-butanol (4a).--A solution of **3a** (1.0 g, 3.1 mmol) in 100 mL dry THF was treated with 1.0 mL (10 mmol) piperdine. The solution was refluxed for 48 hrs. The mixture was then cooled to room temperature, concentrated under reduced pressure to about one half its volume, and partitioned between ethyl acetate (200 mL) and 5% aqueous sodium potassium tartarate (200 mL) containing 1.0 g NaCl. The organic layer was washed with distilled water (100 mL) and dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure to give a white solid (0.867 g). The crude product was purified by silica gel column chromatography (2.5 cm x 60 cm length) using 50% ethyl acetate/hexanes as the mobile phase to give 0.673 g. TLC (50% ethyl acetate/hexanes) $R_f = 0.54$. ^1H NMR ($CDCl_3$/TMS,200MHz) δ 0.9892 (9H, s), 1.654 (2H, t), 1.768 (4H, m), 2.130 (4H, t), 2.443 (2H, d), 2.905 (2H, d), 3.855 (1H, d of t), 4.4921 (1H, d of t), 4.925 (1H, m, exchangable), 7.085 (5H,s).

3(S)-BOCamino-4-phenyl-1-N-pyrrolidine-2(S)-butanol (4b).--A solution of **3a** (1.0 g, 3.1 mmol) in 100 mL dry THF was treated with 1.7 mL (20 mmol) pyrrolidine. The solution was refluxed for 48 hrs. The mixture was then cooled to room temperature, concentrated under reduced pressure to about one half its volume, and partitioned between ethyl acetate (200 mL) and 5% aqueous sodium potassium tartarate (200 mL) containing 1.0 g NaCl. The organic layer was washed with distilled water (100 mL) and dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure to give a white solid (0.927 g). The crude product was purified by silica gel column chromatography (2.5 cm x 60 cm length) using 50% ethyl acetate/hexanes as the mobile phase to give 0.673 g. TLC (50% ethyl acetate/hexanes) $R_f = 0.34$. ^1H NMR ($CDCl_3$/TMS,200MHz) δ 0.9990 (9H, s), 1.859 (4H, t), 2.330 (4H, t), 2.456 (2H, d), 2.927 (2H, d), 3.892 (1H, d of t), 4.501 (1H, d of t), 4.935 (1H, m, exchangable), 7.125 (5H,s).

3(S)-amino-4-phenyl-1-N-piperdine-2(S)-butanol dihydrochloride (5a).--0.50 g of 4a was dissolved in 50 mL cold (0°C) 2 M HCl in chloroform. The mixture was stirred at 0°C for 1 hour. Cold diethyl ether (250 mL) was added to induce precipitation of the product. The liquid was decanted, and the precipitant was washed twice with cold ether (100 mL). The crude solid was dissolved in 20 mL methanol and then recrystallized by the addition of 250 mL cold ether. The white solid was again washed twice with cold ether (100 mL) and dried under reduced pressure (0.46 g). TLC (10% ethanol/ethyl acetate) $R_f = 0.35$ ^1H NMR (D_2O, 60MHz) δ 1.7 (2H, t), 1.9 (4H, m), 2.2 (4H, t), 2.5 (2H, d), 2.9 (2H, d), 3.7 (1H, m), 4.5 (1H, m), 7.1 (5H,s).

3(S)-amino-4-phenyl-1-N-pyrrolidine-2(S)-butanol dihydrochloride (5b).--0.55 g of 4b was dissolved in 50 mL cold (0°C) 2 M HCl in chloroform. The mixture was stirred at 0°C for 1 hour. Cold diethyl ether (250 mL) was added to induce precipitation of the product. The liquid was decanted, and the precipitant was washed twice with cold ether (100 mL). The crude solid was dissolved in 20 mL methanol and then recrystallized by the addition of 250 mL cold ether. The white solid was again washed twice with cold ether (100 mL) and dried under reduced pressure (0.47 g). TLC (10% ethanol/ethyl acetate) $R_f = 0.23$ ^1H NMR (D_2O, 60MHz) δ 2.0 (4H, t), 2.3 (4H, m), 2.6 (2H, d), 3.0 (2H, d), 3.8 (1H, m), 4.4 (1H, m), 7.0 (5H,s).

General Procedure for Preparation of Carbobenzoxy-amino acids (6a-c).--A solution of the appropriate amino acid (0.050 moles) in 250 mL 1 M aqueous NaOH was chilled to 0°C and slowly treated (over 1 hour) with (0.055 moles) benzyl chloroformate. The mixture was stirred for three hours at 0°C, allowed to slowly warm, and stirred

overnight at room temperature. The basic solution was then washed twice with dichloromethane (150 mL) and then acidified with 6 M aqueous HCl. The acidic mixture was then extracted twice with dichloromethane (150 mL). The organic layers were pooled, washed with distilled water (100 mL), dried over anhydrous magnesium sulfate, and concentrated under reduced pressure.

Carbobenzoxy-L-alanine (6a).--TLC (ethyl acetate/hexanes, 1/1, v/v) $R_f = 0.45$. ^1H NMR ($CDCl_3$/TMS, 60MHz) δ 2.4 (3H, d), 4.4 (1H, t), 4.7 (1H, m), 5.3 (2H, s), 7.3 (5H,s), 11.2 (1H, m).

Carbobenzoxy-L-valine (6b).--TLC (ethyl acetate/hexanes, 1/1, v/v) $R_f = 0.80$. ^1H NMR ($CDCl_3$/TMS, 60MHz) δ 1.5 (6H, d), 2.3 (1H, m), 4.3 (1H, t), 4.7 (1H, m), 5.2 (2H, s), 7.2 (5H,s), 11.3 (1H, m).

Carbobenzoxy-L-leucine (6c).--TLC (ethyl acetate/hexanes, 1/2, v/v) $R_f = 0.35$. ^1H NMR ($CDCl_3$/TMS, 60MHz) δ 1.3 (6H, d), 2.2 (3H, m), 4.5 (1H, t), 4.9 (1H, m), 5.3 (2H, s), 7.3 (5H,s), 11.4 (1H, m).

General Procedure for Preparation of Carbobenzoxy-dipeptide methyl esters (7a-c).--A cold (-10°C) solution of 0.015 mole of the appropriate carbobenzoxy-amino acid (6a-c) in 20 mL anhydrous dimethyl formamide (DMF) was treated with 2.0 mL (0.015 mole) anhydrous triethylamine. After 10 minutes the cold solution was treated with 1.2 mL (0.014 mole) ethyl chloroformate. The mixture was stirred for 1 hour at -10°C and then combined with a precooled (0°C) solution containing 2.60 g (0.015 mole) L-phenylalanine methyl ester hydrochloride and 2.0 mL (0.015 mole) triethyl amine in 20 mL anhydrous DMF. The mixture was stirred at 0°C for 3 hours, allowed to warm to room temperature, and stirred overnight. The mixture was then partitioned between the layers of ethyl acetate (200 mL) and aqueous 1 M NaOH (150 mL). The organic layer was washed with distilled water (150 mL), aqueous 1 M HCl (150 mL), and distilled water (150 mL) again. The organic layer was dried over anhydrous magnesium sulfate and evaporated under reduced pressure.

Carbobenzoxy-L-alanyl-L-phenylalanine methyl ester (7a).--TLC (chloroform) $R_f = 0.18$ ^1H NMR ($CDCl_3$/TMS, 60MHz) δ 2.3 (3H, d), 2.7 (2H, d), 3.8 (3H, s), 4.5 (2H, m), 4.7 (1H, m), 5.0 (1H, m), 5.4 (2H, s), 6.9 (5H, s), 7.3 (5H,s).

Carbobenzoxy-L-valyl-L-phenylalanine methyl ester (7b).--TLC (chloroform) $R_f = 0.27$. ^1H NMR ($CDCl_3$/TMS, 60MHz) δ 1.5 (6H, d), 2.3 (1H, m), 2.6 (2H, d), 3.7 (3H, s), 4.6 (2H, m), 4.7 (1H, m), 5.0 (1H, m), 5.3 (2H, s), 7.0 (5H, s), 7.3 (5H,s).

Carbobenzoxy-L-leucyl-L-phenylalanine methyl ester (7c).--TLC (chloroform) $R_f = 0.38$. ^1H NMR ($CDCl_3$/TMS, 60MHz) δ 1.2 (6H, d), 2.2 (3H, m), 2.7 (2H, d), 3.6 (3H, s), 4.6 (2H, m), 4.7 (1H, m), 5.0 (1H, m), 5.5 (2H, s), 7.0 (5H, s), 7.2 (5H,s).

General Procedure for Preparation of Carbobenzoxy-dipeptide acids (8a-c).--A solution of the appropriate carbobenzoxy-dipeptide methyl ester (0.010 to 0.015 mole) in 100 mL methanol was mixed with 50 mL aqueous 1 M NaOH. The mixture was stirred overnight at room temperature. The resulting mixture was diluted with 200 mL distilled water and acidified to pH 3.0 with aqueous 6 M HCl. The mixture was then concentrated to about one half volume under reduced pressure. The cloudy mixture was extracted twice with ethyl acetate (150 mL). The organic layers were combined, washed with distilled water (150 mL), dried over anhydrous magnesium sulfate, and then evaporated under reduced pressure.

Carbobenzoxy-L-alanyl-L-phenylalanine (8a).--TLC (chloroform) $R_f = 0.18$ ^1H NMR ($CDCl_3$/TMS, 60MHz) δ 2.3 (3H, d), 2.7 (2H, d), 4.6 (2H, m), 4.9 (1H, m), 5.0 (1H, m), 5.4 (2H, s), 6.9 (5H, s), 7.5 (5H,s), 10.8 (1H, m).

Carbobenzoxy-L-valyl-L-phenylalanine (8b).--TLC (chloroform) $R_f = 0.27$. ^1H NMR ($CDCl_3$/TMS, 60MHz) 1.5 (6H, d), δ 2.3 (1H, m), 2.6 (2H, d), 4.5 (2H, m), 4.8 (1H, m), 5.0 (1H, m), 5.3 (2H, s), 7.0 (5H, s), 7.4 (5H,s), 11.0 (1H, m).

Carbobenzoxy-L-leucyl-L-phenylalanine (8c).--TLC (chloroform) $R_f = 0.38$. ^1H NMR ($CDCl_3$/TMS, 60MHz) δ 1.2 (6H, d), 2.2 (3H, m), 2.7 (2H, d), 4.5 (2H, m), 4.7 (1H, m), 5.0 (1H, m), 5.5 (2H, s), 7.0 (5H, s), 7.4 (5H,s), 11.2 (1H, m).

General Procedure for Coupling Cbz-dipeptide to 3(S)-amino-4-phenyl-1-N-piperdine (or pyrrolidine)-2(S)-butanol (9a-f).--A precooled solution (-15°C) of the appropriate carbobenzoxy-dipeptide (8a-c) (0.35 mmol) in 10 mL anhydrous DMF was treated with 56 µL (0.40 mmol) triethyl amine. The mixture was allowed to react at -15°C for 30 minutes and was then treated with 34 µL (0.35 mmol) ethyl chloroformate. The mixture was stirred under N_2 atmosphere for 1 hour at -15°C. A precooled (0°C) solution containing 0.32 mmole of either 5a or 5b in 25 mL anhydrous DMF and 125 µL (1.0 mmole) triethylamine was then added to the mixed anhydride of the Cbz-dipeptide. The combined mixture was stirred under N_2 at 0°C for 4 hours, allowed to warm to room temperature, and stirred overnight at room temperature. The mixture was partitioned between the layers of ethyl acetate (250 mL) and 0.01 M aqueous NaOH. The organic layer was removed and saved. The aqueous layer was extracted again with another 250 mL ethyl acetate. The organic layers were pooled, washed with distilled water (100 mL), dried over anhydrous magnesium sulfate, and evaporated under reduced pressure.

3(S)-[Cbz-L-alanyl-L-phenylalanylamino]-4-phenyl-1-N-piperdine-2(S)-butanol (9a).--0.157 g. TLC (10% ethanol/ethyl acetate) $R_f = 0.17$, ^1H NMR (DMSO-d_6 60 MHz) δ 1.7 (2H, d), 1.9 (4H, m), 2.0 (4H, t), 2.4 (5H, m), 2.7 (2H, d), 3.1 (2H, d), 3.7 (1H, m), 4.6 (3H, m), 5.0 (1H, m), 5.4 (2H, s), 6.9 (5H, s), 7.1 (5H,s), 7.3 (5H, s).

3(S)-[Cbz-L-valyl-L-phenylalanylamino]-4-phenyl-1-N-piperdine-2(S)-butanol (9b).--0.135 g. TLC (10% ethanol/ethyl acetate) $R_f = 0.23$, ^1H NMR (DMSO-d_6 60 MHz) δ 1.3 (6H, d), 1.5 (2H, t), 1.8 (4H, m), 1.9 (4H, t), 2.5 (3, m), 2.7 (2H, d), 3.1 (2H, d), 3.7 (1H, m), 4.6 (3H, m), 5.0 (1H, m), 5.4 (2H, s), 6.8 (5H, s), 7.1 (5H,s), 7.4 (5H, s).

3(S)-[Cbz-L-leucyl-L-phenylalanylamino]-4-phenyl-1-N-piperdine-2(S)-butanol (9c).--0.128 g. TLC (10% ethanol/ethyl acetate) $R_f = 0.26$, ^1H NMR (DMSO-d_6 60 MHz) δ 1.3 (6H, d), 1.5 (2H, t), 1.8 (4H, m), 1.9 (4H, t), 2.5 (7, m), 2.7 (2H, d), 3.1 (2H, d), 3.7 (1H, m), 4.6 (3H, m), 5.0 (1H, m), 5.4 (2H, s), 6.9 (5H, s), 7.3 (5H,s), 7.5 (5H, s).

3(S)-[Cbz-L-alanyl-L-phenylalanylamino]-4-phenyl-1-N-pyrrolidine-2(S)-butanol (9d).--0.167 g. TLC (15% ethanol/ethyl acetate) $R_f = 0.23$, ^1H NMR (DMSO-d_6 60 MHz) δ 1.8 (4H, t), 2.0 (4H, m), 2.4 (5H, m), 2.9 (2H, d), 3.3 (2H, d), 3.8 (1H, m), 4.6 (3H, m), 5.0 (1H, m), 5.3 (2H, s), 6.8 (5H, s), 7.1 (5H,s), 7.5 (5H, s).

3(S)-[Cbz-L-valyl-L-phenylalanylamino]-4-phenyl-1-N-pyrrolidine-2(S)-butanol (9e).--0.117 g. TLC (15% ethanol/ethyl acetate) $R_f = 0.35$, ^1H NMR (DMSO-d_6 60 MHz) δ 1.3 (6H, d), 1.7 (4H, m), 209 (4H, t), 2.4 (3, m), 2.8 (2H, d), 3.2 (2H, d), 3.8 (1H, m), 4.7 (3H, m), 5.0 (1H, m), 5.3 (2H, s), 6.8 (5H, s), 7.2 (5H,s), 7.5 (5H, s).

3(S)-[Cbz-L-leucyl-L-phenylalanylamino]-4-phenyl-1-N-pyrrolidine-2(S)-butanol (9f).--0.142 g. TLC (15% ethanol/ethyl acetate) $R_f = 0.41$, ^1H NMR (DMSO-d_6 60 MHz) δ 1.3 (6H, d), 1.8 (6H, m), 2.0 (4H, t), 2.4 (3, m), 2.9 (2H, d), 3.3 (2H, d), 3.8 (1H, m), 4.7 (3H, m), 5.0 (1H, m), 5.3 (2H, s), 6.8 (5H, s), 7.2 (5H,s), 7.5 (5H, s).

General Procedure for Preparation of 3(S)-[Acetyl-dipeptide-amino]-4-phenyl-1-N- piperdine (or pyrrolidine)-2(S)-butanol hydrochloride (10a-f).--A solution of the carbobenzy protected compound (9a-f) (0.20 mmol) in 250 mL methanol and 1 mL 0.01 M aqueous HCl was treated with 0.050 g pre-moistened 10% Pd-C to form a slurry in a 3 neck flask. H_2 gas was bubbled (1 atm) through the rapidly stirring mixture at room temperature for three hours. The mixture was then filtered to remove the catalyst, and the solvent was evaporated under reduced pressure. The crude amine hydrochloride was dissolved in 10 mL dimethyl sulfoxide (DMSO) and treated with 125 µL (0.10 mole) triethyl amine. The mixture was stirred at room temperature for 30 minutes. Acetic anhydride (95 µL, 1.0 mmol) was added, and the mixture was stirred overnight at room temperature. Cold diethyl ether (200 mL) was added to precipitate the product. The liquid was decanted, and the white solid was washed three times with cold ether (100 mL). The crude product was purified by Sephadex LH-20 column chromatography (column size 5 cm dia. x 80 cm) using methanol as the mobile phase.

3(S)-[Acetyl-L-alanyl-L-phenylalanylamino]-4-phenyl-1-N-piperdine-2(S)-butanol (10a).--0.52 g. TLC (1-butanol/H_2O/acetic acid, 15/2/1) $R_f = 0.67$, ^1H NMR (methanol-d_4 200 MHz) δ 1.689 (2H, d), 1.987 (4H, m), 2.010 (4H, t), 2.305 (3H, d), 2.210 (2H, s) 2.462 (4H, m), 2.597 (2H, d), 3.715 (1H, m), 4.610 (3H, m), 4.984 (3H, m), 6.949 (5H, s), 7.212 (5H,s).

3(S)-[Acetyl-L-valyl-L-phenylalanylamino]-4-phenyl-1-N-piperdine-2(S)-butanol (10b).--0.63 g. TLC (1-butanol/H_2O/acetic acid, 15/2/1) $R_f = 0.72$, ^1H NMR (methanol-d_4 200 MHz) δ 1.452 (6H, d), 1.689 (2H, d), 1.989 (4H, m), 2.016 (4H, t), 2.291 (1H, m), 2.219 (2H, s) 2.458 (4H, m), 2.599 (2H, d), 3.720 (1H, m), 4.613 (3H, m), 4.989 (3H, m), 6.959 (5H, s), 7.203 (5H,s).

3(S)-[Acetyl-L-leucyl-L-phenylalanylamino]-4-phenyl-1-N-piperdine-2(S)-butanol (10c).--0.41 g. TLC (1-butanol/H_2O/acetic acid, 15/2/1) $R_f = 0.79$, ^1H NMR (methanol-d_4 200 MHz) δ 1.298 (6H, d), 1.689 (2H, d), 1.990 (4H, m), 2.017 (4H, t), 2.199 (3H, m), 2.220 (2H, s) 2.455 (4H, m), 2.601 (2H, d), 3.721 (1H, m), 4.614 (3H, m), 4.990 (3H, m), 6.960 (5H, s), 7.213 (5H,s).

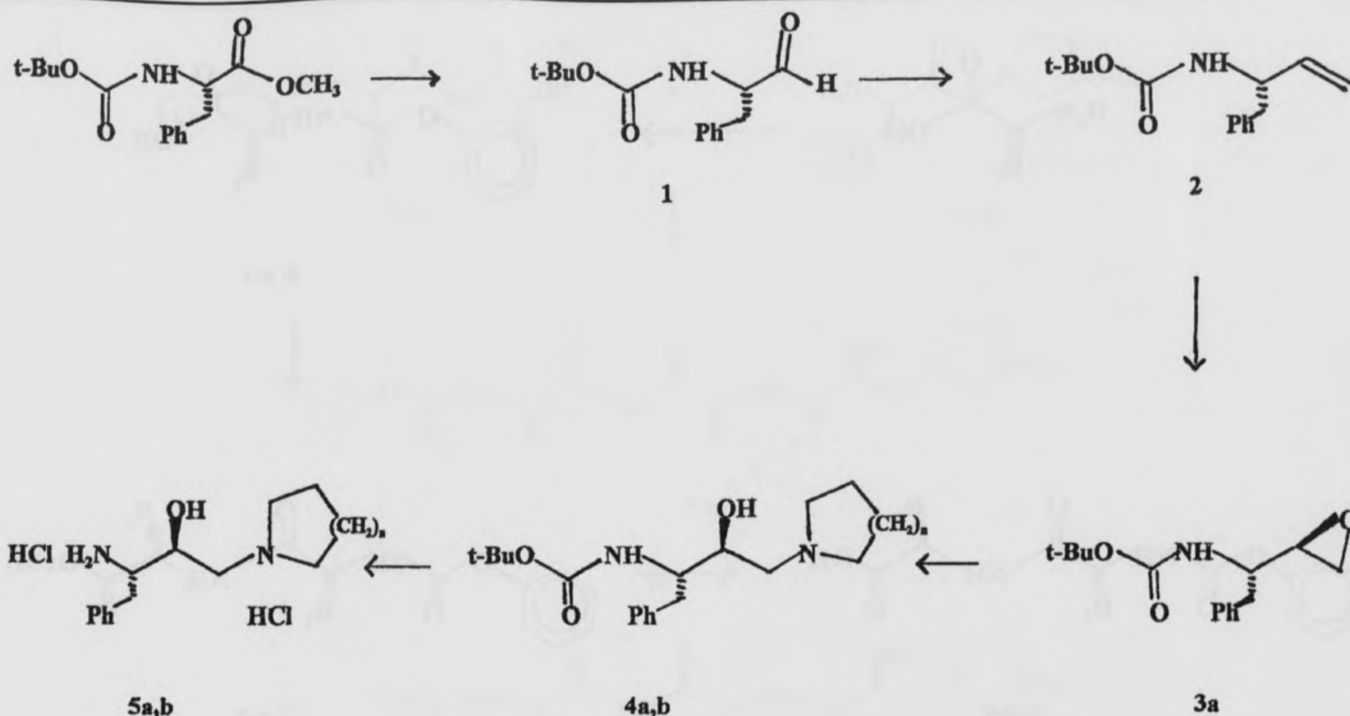
3(S)-[Acetyl-L-alanyl-L-phenylalanylamino]-4-phenyl-1-N-pyrrolidine-2(S)-butanol (10d).--0.71 g. TLC (1-butanol/H_2O/acetic acid, 15/2/1) $R_f = 0.55$, ^1H NMR (methanol-d_4 200 MHz) δ 1.999 (4H, t), 2.135 (4H, t), 2.315 (3H, d), 2.211 (2H, s) 2.457 (4H, m), 2.631 (2H, d), 3.715 (1H, m), 4.615 (3H, m), 4.991 (3H, m), 6.999 (5H, s), 7.242 (5H,s).

3(S)-[Acetyl-L-valyl-L-phenylalanylamino]-4-phenyl-1-N-pyrrolidine-2(S)-butanol (10e).--0.75 g. TLC (1-butanol/H_2O/acetic acid, 15/2/1) $R_f = 0.68$, ^1H NMR (methanol-d_4 200 MHz) δ 1.452 (6H, d), 2.009 (4H, t), 2.160 (4H, t), 2.396 (1H, m), 2.220 (2H, s) 2.488 (4H, m), 2.601 (2H, d), 3.722 (1H, m), 4.655 (3H, m), 4.993 (3H, m), 6.979 (5H, s), 7.301 (5H,s).

3(S)-[Acetyl-L-leucyl-L-phenylalanylamino]-4-phenyl-1-N-pyrrolidine-2(S)-butanol (10f).--0.81 g. TLC (1-butanol/H_2O/acetic acid, 15/2/1) $R_f = 0.81$, ^1H NMR (methanol-d_4 200 MHz) δ 1.299 (6H, d), 1.990 (4H, t), 2.020 (4H, t), 2.197 (3H, m), 2.221 (2H, s) 2.462 (4H, m), 2.611 (2H, d), 3.725 (1H, m), 4.615 (3H, m), 4.997 (3H, m), 6.976 (5H, s), 7.200 (5H,s).

Results and Discussion

Our synthetic plan of the potential cathepsin D inhibitors involved three phases: (a) preparation of the protected hydroxyethyl amine isostere portion (b) preparation of the carbobenzoxy protected dipeptide portion and (c) condensation and deblocking of the peptide and non-peptide portions. The hydroxyethyl amine isosteres were prepared from a tert- butoxycarbonyl- (BOC) chiral amino aminoalkyl epoxide (Scheme 1). The novel chiral aminoalkyl epoxides were first reported by Evans et al. (1985) and have been subsequently used successfully in the preparation of



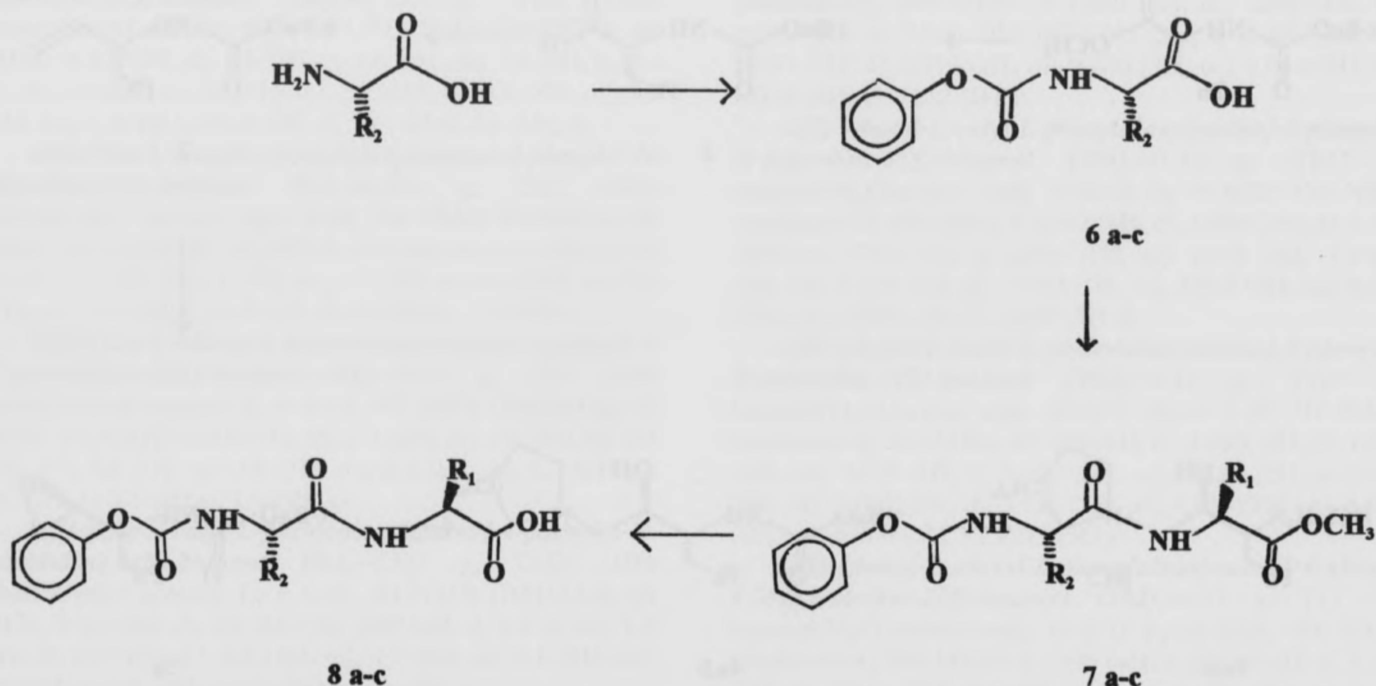
Scheme 1

several HIV-1 aspartyl protease inhibitors with hydroxyethyl amine isosteres (Fassler et al., 1996; Barrish et al., 1994). The alkene was prepared from a protected chiral aminoaldehyde by reaction with a ylide (triphenyl phosphonium methylide). The olefin was converted to the chiral epoxide with meta-chloroperoxybenzoic acid (MCPBA) in methylene chloride. This reaction takes place with retention of stereochemical configuration at the α-carbon of the protected aminoaldehyde. The ylide attack on the diastereotopic faces of the aldehyde is nonspecific, and the epoxide obtained is a separable mixture of isomers easily distinguished by NMR adsorption on the C_2 proton (δ 3.7 and 4.1) (Evans et al., 1985). Separation of the diastereomers was accomplished by silica gel column chromatography using ethyl acetate/hexanes (25% v/v) as the mobile phase. Normally the 2S,3S epoxide is utilized to prepare HIV-1 protease inhibitors with the desired R-hydroxyethyl amine isostere (Fassler et al., 1996; Barrish et al., 1994). However, since the S-hydroxyethyl amine isostere is reported to be the more active isomer for cathepsin D inhibition (Kick, 1997), we utilized the 2R,3S protected amino epoxide in our synthesis (Scheme 1). Piperdine or pyrrolidine was used as a nucleophile in the preparation of the cyclized tertiary amines. The BOC protecting group was removed from the primary amine with non-aqueous acid (2 M HCl in chloroform).

Preparation of the protected peptide portion of the target molecules was accomplished in the following manner. A basic solution of the chosen amino acid was condensed with benzyl chloroformate to provide, after acidification and purification, the carbobenzoxy (Cbz) protected amino acid. The Cbz-amino acid was then coupled to a separate amino acid methyl ester by a mixed anhydride coupling technique utilizing ethyl chloroformate. The C-terminus of the Cbz protected dipeptide methyl ester was then deblocked in aqueous sodium hydroxide, followed by acidification (Scheme 2). In the third phase of our synthesis, the Cbz-protected dipeptide was condensed with ethyl chloroformate and then reacted with the basified primary amine of the hydroxyethyl amine isostere portion (Scheme 3). The Cbz protecting group of the resulting compound was then removed and replaced with an acetyl group. The final product was purified by sephadex HP chromatography and characterized by TLC and ^{1}H NMR.

Conclusions

Our synthetic route shows a great deal of promise for the future synthesis of similar hydroxyethyl amine isosteres. The synthetic inhibitors will be screened for their inhibition of cathepsin D first by spectrophotometric assay techniques, and then detailed kinetic data will be determined by more



Scheme 2

sensitive fluorometric techniques (Kick et al., 1997). The inhibition constants for these synthetic compounds will be reported in the near future.

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Literature Cited

Alterman, M., M. Bjorsne, A. Muhlman, B. Classon, I. Kvarnstrom, H. Danielson, P. O. Markgren, U. Nillroth, T. Unge, A. Hallberg, and **B. Samuelsson.** 1998. Design and Synthesis of New Potent C_2-Symmetric HIV-1 Protease Inhibitors. J. Med. Chem. 41:3782-3792.

Backbro, K., S. Lowgren, K. Osterland, J. Atepo, and **T. Unge.** 1997. Unexpected Binding Mode of a Cyclic Sulfamide HIV-1 Protease Inhibitor. J. Med. Chem. 40:898-902.

Barrish, J. C., E. Gordon, M. Alam, P. F. Lin, G. S. Bisacchi, P. Chen, P. T. W. Cheng, P. T. W., and **A.W. Fritz.** 1994. Aminodiol HIV Protease Inhibitors 1. Design, Synthesis, and Preliminary SAR. J. Med. Chem. 37:1758-1768.

Beaulieu, P. L., D. Wernic, A. Abraham, P. C. Anderson, T. Bogri, Y. Bosquet, G. Groteau, I. Guse, D. Lamarre, F. Liard, W. Paris, D. Thibeault, S. Pav, and **L. Tong.** 1997. Potent HIV Protease Inhibitors Containing a Novel (Hydroxyethyl)amide Isostere. J. Med. Chem. 40:2164-2176.

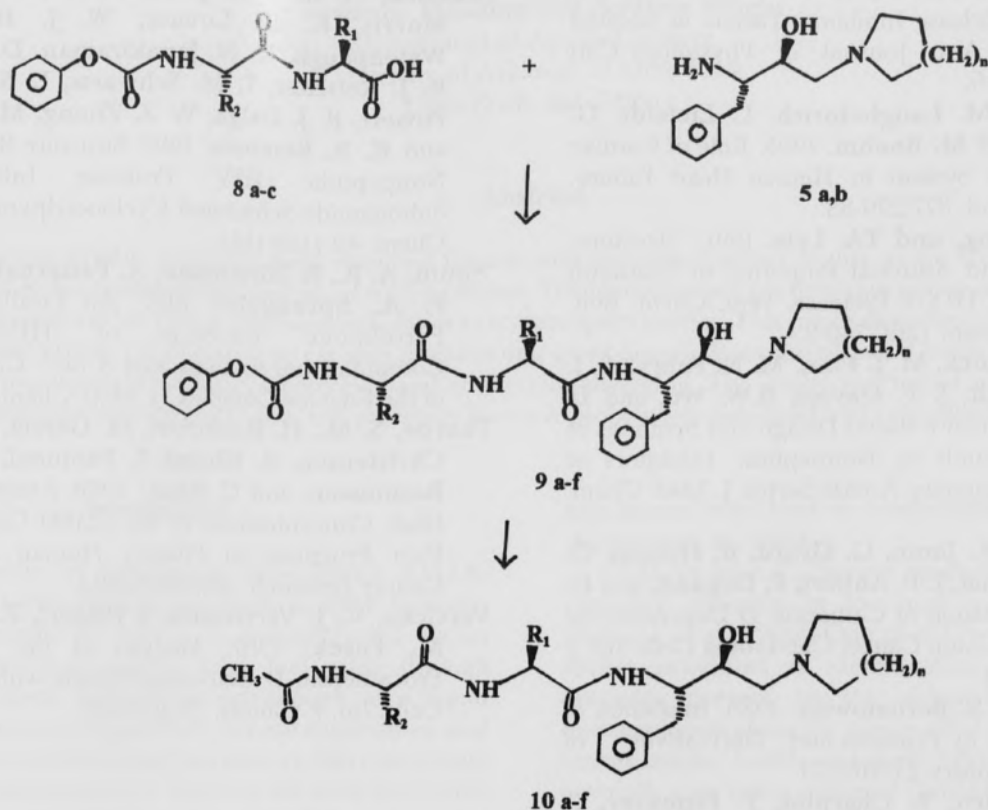
Darke, P. L and **J. R. Huff.** 1994. HIV-1 Protease as an Inhibitor Target for the Treatment of AIDS. Advances in Pharmacology 25:399-454.

Debnath, A. K. 1999. Three-Dimensional Quantitative Structure-Activity Relationship Study on Cyclic Urea Derivatives as HIV-1 Protease Inhibitors. J. Med. Chem. 42:249-259.

Evans, B. E., K. E. Rittle, C. F. Homnick, J. P. Springer, J. Hirshfield, and **D. F. Veber.** 1985 A Stereocontrolled Synthesis of Hydroxyethylene Dipeptide Isosteres Using Novel, Chiral Aminoalkyl Epoxides and -(aminoalkyl)–Lactones. J. Org. Chem. 50:4615-4625.

Fassler, A., G. Bold, H. G. Capraro, R. Cozens, J. Mestan, B. Poncioni, J. Rosel, M. Tintelnot-Blomleg, and **M. Lang.** 1996. Aza-Peptide Analogs as Potent HIV-1 Protease Inhibitors with Oral Bioavailability. J. Med. Chem. 39:3203-3216.

Hulten, J., NM. Bonham, U. Nillroth, T. Hansson, G. Zuccarello, A. Bouzide, J. Agnist, B. Classon, H. Danielson, A. Karlen, I. Kvarnstrom, B. Samuelson, and **A. Hallberg.** 1997. Cyclic HIV-1 Protease Inhibitors Derived from Mannitol: Synthesis,



Scheme 3

Inhibitory Potencies, and Computational Predictions of Binding Affinities. J. Med. Chem. 40:885-897.

Kick, E. K., D. C. Roe, A. G. Skillman, G. Liu, T. J. Ewing, Y. Sun, I. D. Kuntz, and **J. A. Ellman J.A.** 1997. Structure-Based Design and Combinatorial Chemistry Yield Low Nanomolar Inhibitors of Cathepsin D. Chemistry and Biology 4:297-307.

Kratzel, M., R. Hiessbock, and **A. Bernkop-Schnurch.** 1998. Auxiliary Agents for Peroral Administration of Peptide and Protein Drugs: Synthesis and Evaluation of Novel Pepstatin Analogs. J. Med. Chem. 41:2339-2344.

Kratzel, M., B. Schlichtner, R. Kirchmayer, and **A. Bernkop-Schnurch.** 1999. Simplified Pepstatins: Synthesis and Evaluation of N-Terminally Modified Analogs. J. Med. Chem. 42:2041-2045.

Laury-Kleintop, L. D., E. C. Coronel, M. K. Lange, T. Tachovsky, S. Longo, S. Tucker, and **J. A. Alhadeff.** 1995. Western Blotting and Isoform Analysis of Cathepsin D from Normal and Malignant Human Breast Cell Lines. Breast Cancer Research and Treatment 35: 211-220.

Losch, A., P. Kohlberger, G. Gitsch, A. Kaider, G. Breitenecker, and C. Kainz. 1996. Lysosomal Protease Cathepsin D is a Prognostic Marker in Endometrial Cancer. British J. Cancer 73:1525-1533.

McConnell, R. M., D. Frizzell, A. Camp, A. Evans, W. Jones, and **C. Cagle.** 1991. New Pepstatin Analogues: Synthesis and Pepsin Inhibition. J. Med. Chem. 34: 2298-2300.

Messmer, U. K., C. Pereda-Fernandez, and J. Manderscheid. 2001. Dexamethazone Inhibits TNA–Induced Apoptosis and IAP Protein Downregulation in MCF-7 Cells. British Journal of Pharmacology, 133:467-476.

Mimoto, T., N. Hattori, H. Takaku, S. Kisanuki, and **J. Imai.** 2000. Structure-Activity Relationships of Orally Potent Tripeptide-Based HIV Protease Inhibitors Containing Hydroxymethylcarbonyl Isostere. Chem. Pharm. Bull. 48:1310-1326.

Mordente, J. A., J. P. Cherry, J. Chapman, M. S. Choudhury, H. Tazaki, C. Mallouh, and **S. Konno.** 1998. Maturation of Cathepsin D as a Possible Predictor of Prostate Cancer Progression. Molecular Urology 2:1-5.

Moore, C. L., D. D. Leatherwood, T. S. Diehl, D. J. Selkoe, and **M. S. Wolfe.** 2000. Difluoro Ketone Peptidomimetics Suggest a Large S_1 Pocket for Alzheimer's -Secretase: Implications for Inhibitor Design. J. Med. Chem. 43:3434-3442.

Nakaya, K., R. Mizuno, and **T. Ohhashi.** 2001. B16-BL6 Melanoma Cells Release Inhibitory Factors in Isolated Lymph Vessels. Am. Journal of Physiology-Cell Physiology. 281: 0-6.

Paul, M., P. Stock, M. Langheinrich, L. Liefeldt, G. Schoenfelder, and **M. Boehm.** 1995. Role of Cardiac Renin-Angiotensin System in Human Heart Failure. Adv. Exp. Med. Biol. 377:279-83.

Payne, LS., SD. Young, and TA. Lyle. 1991. Structure-Activity Studies and Antiviral Properties of Transition State Inhibitors of HIV-1 Protease. Pept. Chem. Biol. Proc. Am. Pept. Symp. 12th. 740-2.

Reich, S. H., M. Melnick, M. J. Pino, M. N. Fuhry, A. J. Trippe, K. Appelt, J. F. Davies, B.W. Wu, and **L. Musick.** 1996. Structure-Based Design and Synthesis of Substituted 2-Butanols as Nonpeptidic Inhibitors of HIV Protease: Secondary Amide Series. J. Med. Chem. 39:2781-2794.

Reig, S. H., I. Kim, A. Janin, G. Grard, B. Hemon, O. Moreau, N. Porchet, J. P. Aubert, P. Degand, and **G. Huet.** 1996. Regulation of Cathepsin D Dependent on the Phenotype of Colon Cancer Carcinoma Cells. Int. J. Cancer 72:479-484.

Rich, D. H., and M. S. Bernatowicz. 1985. Inhibition of Aspartyl Proteases by Pepstatin and Derivatives of Pepstatin. Biochemistry 24:3165-73.

Scholtz, D., A. Billich, B. Charpiot, P. Ettmayer, P. Lehr, B. Rosenwirth, E. Schreiner, and **H. Gstach.** 1994. Inhibitors of HIV-1 Proteinase Containing 2-Heterosubstituted 4-Amino-3-Hydroxy-5-Phenyl-pentanoic Acid: Synthesis, Enzyme Inhibition, and Antiviral Activity. J. Med. Chem. 37:3079-3089.

Skulnick, H. I., P. D. Johnson, P. A. Aristoff, J. K. Morris, K. D. Lovasz, W. J. Howe, K. D. Watenpaugh, M. N. Janakiraman, D. J. Anderson, R. J. Reischer, T. M. Schwartz, L. S. Banitt, P. L. Possert, R. J. Dalga, W. Z. Zhong, M. G. Williams, and **K. R. Romines.** 1997. Structure-Based Design of Nonpeptidic HIV Protease Inhibitors: The Sulfonamide-Substituted Cyclooctylpyranones. J. Med. Chem. 40:1149-1164.

Smith, A. B., R. Hirschman, A. Pasternak, W. Yao, and P. A. Sprengeler. 1997. An Orally Bioavailable Pyrrolinone Inhibitor of HIV-1 Protease: Computational Analysis, and X-ray Crystal Structure of the Enzyme Complex. J. Med. Chem. 40:2440-2444.

Thorpe, S. M., H. Rochefort, M. Garcia, G. Freiss, I. J. Christensen, S. Khalaf, F. Paolueel, B. Pau, B. B. Rasmussen, and **C. Rose.** 1998. Association Between High Concentrations of M. 52,000 Cathepsin D and Poor Prognosis in Primary Human Breast Cancer. Cancer Research. 49:6008-60014.

Vetvicka, V., J. Vetvickova, I. Hilgert, Z. Voburka, and **M. Fusek.** 1997. Analysis of the Interaction of Procathepsin D Activation Peptide with Breast Cancer Cells. Int. J. Cancer. 73:403-409.