Pergamon

Bioorganic & Medicinal Chemistry, Vol. 2, No. 9, pp. 919-925, 1994

0968-0896/94 $7.00 + .00

0968-0896(94)00111-1

HIV-1 Protease Inhibitors: Ketomethylene Isosteres with Unusually High Affinity Compared with Hydroxyethylene Isostere Analogs

Anne Marinier,[a,†] Mihaly V. Toth,[a,‡] Kathryn Houseman,[b] Richard Mueller,[b] and Garland R. Marshall[c,*]

[a]*Department of Molecular Biology and Pharmacology, Washington University, St. Louis, MO 63130, U.S.A.*
[b]*Searle Research and Development, Skokie, IL 60077, U.S.A.*
[c]*Center for Molecular Design and Department of Molecular Biology and Pharmacology, Washington University, St. Louis, MO 63130, U.S.A.*

Abstract—HIV protease is a member of the aspartic proteinase family of proteolytic enzymes which include pepsin and renin. In contrast to the enhanced affinity seen with renin and pepsin upon conversion of the transition-state isostere, ketomethylene, to the hydroxyethylene, a set of HIV protease inhibitors showed a reduction in affinity. This implies that interactions with the active site of other segments of the inhibitor than those of the transition-state analog must predominate in the case of HIV protease, and that observations made on mammalian aspartic proteinases do not necessarily apply to viral aspartic proteinases.

Introduction

The human immunodeficiency virus (HIV), the pathogen responsible for acquired immunodeficiency syndrome (AIDS), is a retrovirus which expresses the genetic content of its RNA by directing the synthesis of polyproteins by the host. These precursors are then processed by proteolysis to give essential viral enzymes and structural proteins. A virally encoded enzyme, HIV protease, is responsible for the specific cleavages of the polyproteins associated with the *gag/pol* gene.[1] HIV protease has been shown to be essential in the replication of the virus by mutation. Inhibition of HIV protease as a therapeutic intervention for AIDS treatment appears, therefore, to be a logical strategy. Inhibition of virally encoded proteases as an approach to antiviral therapy has already been demonstrated[2] and other recent observations can rationalize this approach. Isolation and sequence analysis of HIV-1[3] and HIV-2[4] proteases have determined the analogy with aspartic proteinases.[5] Modification by site-directed mutagenesis of Asp-25 prevents processing of the polyprotein[6,7] and inhibits cleavage of gag p55.[8] Asp-25 corresponds to the active site residue of other aspartic proteinases by sequence homology. Also, mutation of amino acids adjacent to this presumed active site has shown inhibition of gag processing.[9]

HIV protease cleaves the virally encoded polyproteins at different sites to liberate the gag proteins (p17, p24 and p15) as well as the protease itself and reverse transcriptase. These cleavage sites contain Met-Met, Tyr-Pro, Phe-Pro and Leu-Pro[10] as the cleaved amide bond. Thus, small peptides overlapping each of these cleavage sites become candidates for chemical modification as potential inhibitors. This approach involving modification of known substrate sequences is well accepted for the design of enzyme inhibitors. The cleaved amide bond is substituted by another functionality resistant to hydrolysis. Hence, most of the recognition sites are still present, but the compound is not hydrolyzed by the enzyme. Particular attention has been directed toward these isosteric peptide bond replacements with tetrahedral geometry replacing the carbonyl in efforts to synthesize transition-state analogs with improved properties as drugs.[11] The link to aspartic proteases has allowed rapid development of HIV protease inhibitors based on the experience in development of renin inhibitors. The publication of the crystal structure of HIV-1 protease[12] and complexes with inhibitors[13] provided the three-dimensional information necessary for optimization and *de novo* design through molecular modeling and computational chemistry.[14]

Szelke *et al.*[15] have prepared substrate analogs having a reduced peptide bond, $\Psi[CH_2NH]$, as inhibitors of renin. In an analogous approach, we have synthesized peptide substrates containing dipeptide analogs of Met-Met, Leu-Pro, Tyr-Pro and Phe-Pro, in which the amide bond has been reduced to give the $\Psi[CH_2NH]$ linkage.[16] These peptides, having fewer than seven residues, have shown good affinity for HIV-1 protease with K_i's in the micromolar to submicromolar range.

We wish to report the synthesis of a new series of peptide inhibitors containing a ketomethylene linkage, $\Psi[COCH_2]$, as the amide isostere together with their activities. The most active inhibitor of the previous series having a reduced linkage, MVT-101 (Ac-Thr-Ile-Nle$\Psi[CH_2NH]$Nle-Gln-Arg-NH_2, K_i=789 nM) was chosen

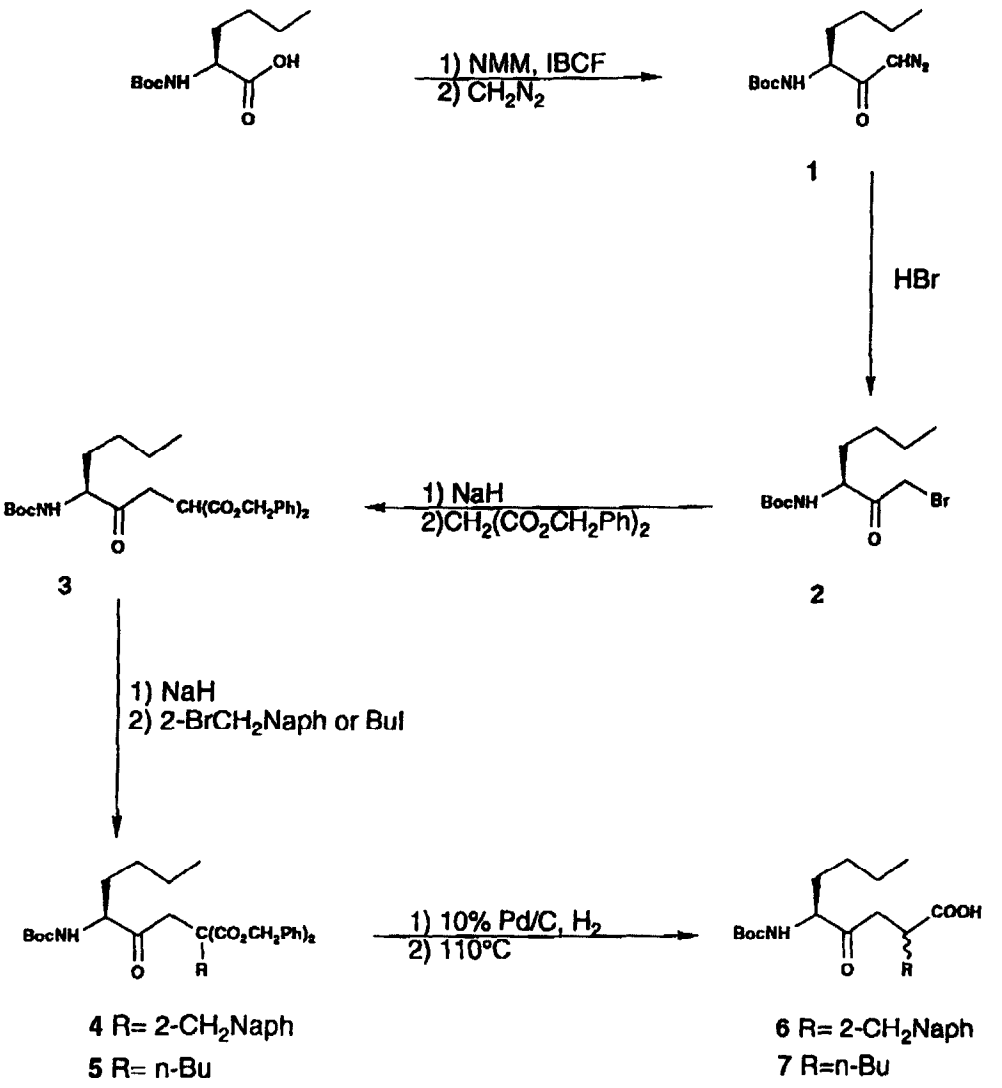
†Current Address: Bristol-Myers Squibb Pharmaceutical Research Institute, 100, blvd de l'Industrie, Candiac Quebec J5R 1J1, Canada.
‡Current Address: Monsanto Company, Mail Zone AA2E, 700 Chesterfield Village Pkwy, Chesterfield, MO 63198, U.S.A.

as the parent compound for this study. We have also prepared and tested the several corresponding hydroxyethylene analogs in which the residues' sequence was determined by the best ketomethylene compounds. By analogy with other mammalian aspartic proteinase inhibitors, the peptides having a hydroxyethylene isostere bond should demonstrate a better affinity since it was shown, for example with pepsin,[17] that an enhancement of 10–40 fold is usually noticed when the ketone is transformed into the hydroxyl functionality. We were interested to see if a similar rationale would apply to HIV-1 protease.

Results and Discussion

Synthesis

The dipeptides containing the keto-methylene isostere bond were synthesized according to the sequence of reactions shown in Scheme I based on the procedure of Kaltenbronn *et al.*[18] Boc-norleucine was first converted into the diazoketone derivative **1** by treatment with diazomethane at 0 °C. The crude compound was then reacted with hydrogen bromide to give the bromide **2** in 83 % yield after crystallization. Condensation with the anion derived from dibenzylmalonate gave the malonate **3** which was then treated with sodium hydride to yield the corresponding anion. Alkylation with 2-bromomethylnaphthalene or *n*-butyliodide afforded the intermediate **4** which was finally decarboxylated by hydrogenation and heating to give the desired dipeptide **5** (42 %). This compound was obtained as a mixture of diastereomers containing the ketomethylene isostere. This dipeptide isostere was then incorporated as a unit by the solid-phase synthesis protocol using the *p*-methylbenzhydrylamine polymer and 4 equivalents of Boc-amino acids. Three of the most potent inhibitors were then reduced using sodium borohydride to give the corresponding hydroxyethylene analogs. Only a single peak was detected for each hydroxyethylene analog by HPLC even though two diastereoisomers were expected. We consider it unlikely that a single diastereoisomer resulted from the reduction even though asymmetric induction of the wrong stereoisomer might account for the anomalous loss of activity upon reduction.



Scheme I.

Experimental

^{1}H NMR spectra were recorded on a Varian Gemini-300 NMR spectrometer at 300 MHz with tetramethylsilane (TMS) used as the internal standard. Mass spectra were obtained with a Finnigan 3300 spectrometer, or with a VG ZAB-SE with a 11-250J data system in the FAB mode. Infrared spectra were done with a Perkin–Elmer 1710 FT spectrometer and optical rotations were performed on a Perkin–Elmer 241 polarimeter. Column chromatography was carried out on Universal scientific silica gel 60. TLC were performed on silica gel (precoated Merck silica gel GF plates, 250 microns, Analtech). Preparative HPLC was carried out using a Beckman 110A pump and a Beckman 420 gradient liquid chromatograph. Analytical HPLC was performed on a Spectra-Physics apparatus (SP4290 integrator, SP8800 ternary HPLC pump and SP8450 UV/VIS detector). Two solvent systems were used for HPLC: A = 100 % water and 0.05 % trifluoroacetic acid, B = 90 % acetonitrile, 10 % water and 0.038 % trifluoroacetic acid.

Solution-phase synthesis of the dipeptides

(S)-(1-Butyl-3-diazo-2-oxopropyl)carbamic acid, 1,1-dimethylethyl ester 1. A solution of Boc-norleucine (6.9 g, 30 mmol) in ethyl acetate (80 mL) was cooled to –25 °C and *N*-methylmorpholine (3.3 mL, 30 mmol) and isobutylchloroformate (3.9 mL, 30 mmol) was added. The mixture was stirred for 15 min at –25 °C and ethyl ether (50 mL) was then added. After cooling the mixture to –50 °C, the white precipitate was filtered under nitrogen and the cold filtrate was treated with a solution of diazomethane in ethyl ether (130 mL, 0.4 M, 52 mmol). Mixing was allowed to proceed overnight, gradually warming to room temperature. Excess of diazomethane was removed by bubbling a nitrogen stream through the solution and the solvent was then evaporated. The residual oil was dissolved in ethyl acetate (100 mL) and was washed with a saturated solution of sodium bicarbonate (1 × 100 mL) and brine (1 × 100 mL). The organic layer was dried over sodium sulfate, filtered and concentrated to yield 7.5 g (98 %) of crude product **1**.

(S)-(3-Bromo-1-butyl-2-oxopropyl)carbamic acid, 1,1-dimethylethyl ester 2. A solution of diazoketone **1** (7.5 g crude, 29.4 mmol) in ethyl acetate (100 mL) was cooled to –20 °C and hydrogen bromide gas was introduced until no more nitrogen gas evolved (about 20 min). The mixture was poured on saturated sodium bicarbonate solution and extracted with EtOAc. The combined organic layers were washed with a saturated solution of sodium bicarbonate (2 × 100 mL) and brine (1 × 100 mL), dried over sodium sulfate, filtered and concentrated. The residue was crystallized from hexane to afford 7.5 g (83 %) of bromide **2**.

2-[3-[[(1,1-Dimethylethoxy) carbonyl] -amino] -2- oxoheptyl]propanedioic acid, bis(phenylmethyl) ester 3. A suspension of sodium hydride (1.1 g, 28 mmol, 60 % in mineral oil) in hexane was washed free of mineral oil and then suspended in tetrahydrofuran (40 mL). A solution of dibenzylmalonate (6.1 mL, 24.3 mmol) in tetrahydrofuran (50 mL) was added slowly and the solution was stirred for 1 h and then cooled to 0 °C. A solution of bromide **2** (7.5 g, 24.3 mmol) in THF (20 mL) was added and the mixture was stirred at 0 °C for 30 min and then at room temperature for 1 h. The residue was concentrated and dissolved in ethyl acetate (100 mL), washed with 1 M citric acid (2 × 100 mL), a saturated solution of sodium bicarbonate (2 × 100 mL) and brine (1 × 100 mL). The organic layer was dried over sodium sulfate, filtered and concentrated to give 13 g (99 %) of crude product **3**.

2-[3-[[(1,1-Dimethylethoxy) carbonyl] -amino] -2- oxoheptyl]-2-(2-methylnaphthyl)propanedioic acid, bis(phenylmethyl) ester 4. Procedure A: a suspension of sodium hydride (0.55 g, 14.0 mmol, 60 % in mineral oil) in hexane was washed free of mineral oil and then suspended in dimethylformamide (25 mL). To this suspension was added slowly a solution of malonate **3** (5.1 g, 10 mmol) in dimethylformamide (25 mL) and the mixture was then treated with a solution of 2-bromomethylnaphthalene (3.3 g, 15 mmol) in dimethylformamide (25 mL) and stirred for 12 h. The solvent was evaporated and the residue was dissoved in ethyl acetate (100 mL) and washed with a solution of 10 % potassium hydrogen sulfate (2 × 100 mL), a saturated solution of sodium bicarbonate (2 × 100 mL) and brine (1 × 100 mL). The organic layer was dried over sodium sulfate, filtered and concentrated to yield 8.6 g of crude product **4**.

Boc-NleΨ[COCH$_2$]2-naphthalalanine 6. Procedure B: A solution of malonate **4** (8.6 g crude) in methanol (100 mL) was treated with 10 % Pd/C and was stirred in a hydrogen atmosphere (40 psi) for 12 h. The mixture was filtered and the solvent was removed under reduced pressure. The residue was taken up in toluene (100 mL) and heated at reflux for 3 h. The solvent was removed under reduced pressure and the residue was dissolved in ethyl acetate (100 mL), washed with a solution of 10 % potassium hydrogen sulfate (2 × 100 mL) and brine (1 × 100 mL). The organic layer was dried over sodium sulfate, filtered and concentrated. The residue was recrystallized from EtOAc and hexane (1:3) to afford 1.8 g (42 % for 2 steps) of dipeptide **6**. NMR δ, $CDCl_3$ (ppm): 7.26 (7H, m, -$C_{10}H_7$), 5.06 (1H, d, -N*H*-CO-), 4.20 (1H, m, -C*H*((CH_2)$_3$$CH_3$)-), 3.46–2.40 (5H, m, -CO-C*H*$_2$-C*H*(CO_2H)-C*H*$_2$-$C_{10}H_7$), 1.39 (9H, s, -OC(*CH*$_3$)$_3$), 1.95–0.70 (9H, m, -(*CH*$_2$)$_3$-*CH*$_3$). IR, $CHCl_3$ (cm^{-1}): 3391, 2959, 2930, 2870, 1712, 1682, 1505, 1455, 1248, 1164. FABMS: 428 (MH^+).

2-[3-[[(1,1-Dimethylethoxy) carbonyl] -amino] -2- oxoheptyl]-2-butylpropanedioic acid, bis(phenylmethyl) ester 5. Procedure A was applied using the following quantities: malonate **3** (6.5 g crude, 12.8 mmol), sodium hydride (0.55 g, 60 % in mineral oil, 14 mmol), *n*-butyliodide (2.7 mL, 24 mmol), dimethylformamide (70 mL) and afforded 7.2 g of crude compound **5**.

Boc-NleΨ[COCH$_2$]Nle 7. Procedure B was applied using the following quantities of reagents: malonate **5** (3.6 g, 6.4 mmol), methanol (60 mL), 10 % palladium in charcoal (1

g), toluene (50 mL) and afforded 1.2 g (55 % for two steps) of compound **7**. NMR δ, $CDCl_3$ (ppm): 11.20 (1H, s, $-CO_2H$), 5.20 (1H, m, -NH-CO-), 4.23 (1H, m, $-CH((CH_2)_3CH_3)-$), 2.99–2.42 (3H, m, $-CO-CH_2-CH(CO_2H)-(CH_2)_3-CH_3$), 1.40 (9H, s, $-OC(CH_3)_3$), 1.93–0.72 (18H, m, $2 \times -(CH_2)_3-CH_3$).

Solid-phase synthesis

The peptides were prepared by solid-phase synthesis using the *p*-methylbenzhydrylamine polymer (0.33 g, 0.33 mmol). The following synthetic protocol was used for the incorporation of the Boc-amino acids:

Deprotection: -TFA (10 mL), 1 min.
-TFA (10 mL), 5 min.
-DMF (10 mL), 2 × 2 min.

Neutralization: -DIPEA (10 mL, 10 % in DMF), 2 min.
-DMF (10 mL) , 2 × 2 min.

Coupling: -4 equivalents of Boc-amino acid, 4 equivalents of benzotriazol-1-yloxy-tris-(dimethylamino)phosphonium hexafluorophosphate (BOP reagent) and 12 equivalents of DIPEA in DMF (10 mL) for 1 h.
-The coupling in DMF was repeated if the Kaiser test was positive.
-The dipeptide analog containing the ketomethylene isostere was incorporated in this protocol as a unit and its synthesis is described below.

Cleavage: -HF:anisole (9:1).[19]
-Solution of crude peptide in 30 % acetic acid and lyophilization.

Purification: -Reverse-phase HPLC on a C-18 semipreparative column using water and CH_3CN as solvents.

*Abz-Thr-Ile-Nle*Ψ*[COCH$_2$]Nle-Gln-Arg-NH$_2$*. The peptide was prepared by solid phase synthesis using 0.125 g of resin (0.125 mmol), Boc-Arg(Tos), Boc-Gln, Boc-NleΨ[$COCH_2$]Nle-OH, Boc-Ile, Boc-Thr(Bzl) and 2-aminobenzoic acid. The peptide was cleaved with HF, yielding 60 mg of crude peptide as a mixture of diastereoisomers. By reverse-phase HPLC, 2 mg of each diastereoisomer were purified, FABMS: 860.4 (MH^+). Amino acid analysis: Arg, Gln, Ile, Thr.

*Abz-Thr-Ile-Nle*Ψ*[COCH$_2$]2-Nal-Gln-Arg-NH$_2$*. The peptide was prepared by solid phase synthesis using 0.125 g of resin (0.125 mmol), Boc-Arg(Tos), Boc-Gln, Boc-NleΨ[$COCH_2$]Nle-OH, Boc-Ile, Boc-Thr(Bzl) and 2-aminobenzoic acid. The peptide was cleaved with HF, yielding 58 mg of crude peptide as a mixture of diastereoisomers. By reverse-phase HPLC, 2 mg of each diastereoisomer were purified, FABMS: 944.6 (MH^+). Amino acid analysis: Arg, Gln, Ile, Thr.

*Abz-Thr-Ile-Phe*Ψ*[COCH$_2$]Ala-Gln-Arg-NH$_2$*. The peptide was prepared by solid phase synthesis using 0.125 g of resin (0.125 mmol), Boc-Arg(Tos), Boc-Gln, Boc-PheΨ[$COCH_2$]Ala-OH, Boc-Ile, Boc-Thr(Bzl) and 2-aminobenzoic acid. The peptide was cleaved with HF, yielding 60 mg of crude peptide. By reverse-phase HPLC, 2 mg were purified, FABMS: 852.4 (MH^+). Amino acid analysis: Arg, Gln, Ile, Thr.

*Qua-Thr-Ile-Nle*Ψ*[COCH$_2$]2-Nal-Gln-Arg-NH$_2$*. The peptide was prepared by solid phase synthesis using 0.25 g of resin (0.14 mmol), Boc-Arg(Tos), Boc-Gln, Boc-NleΨ[$COCH_2$]2-Nal-OH, Boc-Ile, Boc-Thr(Bzl) and quinaldic acid. The peptide was cleaved with HF, yielding 67 mg of crude peptide as a mixture of diastereoisomers. By reverse-phase HPLC, 2 mg of each diastereoisomer were purified, FABMS: 980.4 (MH^+). Amino acid analysis: Arg, Gln, Ile, Thr.

*Qua-Ile-Nle*Ψ*[COCH$_2$]2-Nal-Gln-Arg-NH$_2$*. The peptide was prepared by solid phase synthesis using 0.175 g of resin (0.10 mmol), Boc-Arg(Tos), Boc-Gln, Boc-NleΨ[$COCH_2$]2-Nal-OH, Boc-Ile and quinaldic acid. The peptide was cleaved with HF, yielding 43 mg of crude peptide as a mixture of diastereoisomers. By reverse-phase HPLC, 2 mg of each diastereoisomer were purified, FABMS: 879.3 (MH^+). Amino acid analysis: Arg, Gln, Ile.

*Qua-Nle*Ψ*[COCH$_2$]2-Nal-Gln-Arg-NH$_2$*. The peptide was prepared by solid phase synthesis using 0.175 g of resin (0.10 mmol), Boc-Arg(Tos), Boc-Gln, Boc-NleΨ[$COCH_2$]2-Nal-OH and quinaldic acid. The peptide was cleaved with HF, yielding 175 mg of crude peptide as a mixture of diastereoisomers. By reverse-phase HPLC, 2 mg of each diastereoisomer were purified, FABMS: 766.5 (MH^+). Amino acid analysis: Arg, Gln.

*Qui-Thr-Ile-Nle*Ψ*[COCH$_2$]Nle-Gln-Arg-NH$_2$*. The peptide was prepared by solid phase synthesis using Boc-Arg(Tos), Boc-Gln, Boc-NleΨ[$COCH_2$]Nle-OH, Boc-Ile, Boc-Thr(Bzl) and 2-quinoxaloyl chloride. The peptide was cleaved with HF, yielding a mixture of diastereoisomers. By reverse-phase HPLC, 2 mg of each diastereoisomer were purified, FABMS: 897.3 (MH^+). Amino acid analysis: Arg, Gln, Ile, Thr.

*Qui-Ile-Nle*Ψ*[COCH$_2$]Nle-Gln-Arg-NH$_2$*. The peptide was prepared by solid phase synthesis using Boc-Arg(Tos), Boc-Gln, Boc-NleΨ[$COCH_2$]Nle-OH, Boc-Ile and 2-quinoxaloyl chloride. The peptide was cleaved with HF, yielding a mixture of diastereoisomers. By reverse-phase HPLC, 2 mg of each diastereoisomer were purified, FABMS: 796.6 (MH^+). Amino acid analysis: Arg, Gln, Ile.

*Qui-Nle*Ψ*[COCH$_2$]Nle-Gln-Arg-NH$_2$*. The peptide was prepared by solid phase synthesis using Boc-Arg(Tos), Boc-Gln, Boc-NleΨ[$COCH_2$]Nle-OH and 2-quinoxaloyl chloride. The peptide was cleaved with HF, yielding 85 mg of crude peptide as a mixture of diastereoisomers. By reverse-phase HPLC, 2 mg of each diastereoisomer were purified, FABMS: 683.3 (MH^+). Amino acid analysis: Arg, Gln.

Qui-Thr-Ile-NleΨ[$COCH_2$]2-Nal-Gln-Arg-NH_2. The peptide was prepared by solid phase synthesis using Boc-Arg(Tos), Boc-Gln, Boc-NleΨ[$COCH_2$]2-Nal-OH, Boc-Ile, Boc-Thr(Bzl) and 2-quinoxaloyl chloride. The peptide was cleaved with HF, yielding 88 mg of crude peptide as a mixture of diastereoisomers. By reverse-phase HPLC, 2 mg of each diastereoisomer were purified, FABMS: 981 (MH^+). Amino acid analysis: Arg, Gln, Ile, Thr.

Qui-Ile-NleΨ[$COCH_2$]2-Nal-Gln-Arg-NH_2. The peptide was prepared by solid phase synthesis using Boc-Arg(Tos), Boc-Gln, Boc-NleΨ[$COCH_2$]2-Nal-OH, Boc-Ile and 2-quinoxaloyl chloride. The peptide was cleaved with HF, yielding 80 mg of crude peptide as a mixture of diastereoisomers. By reverse-phase HPLC, 2 mg of each diastereoisomer were purified, FABMS: 880.5 (MH^+). Amino acid analysis: Arg, Gln, Ile.

Qui-NleΨ[$COCH_2$]2-Nal-Gln-Arg-NH_2. The peptide was prepared by solid phase synthesis using Boc-Arg(Tos), Boc-Gln, Boc-NleΨ[$COCH_2$]2-Nal-OH and 2-quinoxaloyl chloride. The peptide was cleaved with HF, yielding 90 mg of crude peptide as a mixture of diastereoisomers. By reverse-phase HPLC, 2 mg of each diastereoisomer were purified. The compounds being very hygroscopic and not very stable, FABMS: 766 (MH^+).

Qui-Thr-Ile-NleΨ[$COCH_2$]2-Nal-Gln-NH_2. The peptide was prepared by solid phase synthesis using Boc-Gln, Boc-NleΨ[$COCH_2$]2-Nal-OH, Boc-Ile, Boc-Thr(Bzl) and 2-quinoxaloyl chloride. The peptide was cleaved with HF, yielding 57 mg of crude peptide as a mixture of diastereoisomers. By reverse-phase HPLC, 2 mg of each diastereoisomer were purified, FABMS: 825 (MH^+). Amino acid analysis: Gln, Ile, Thr.

Qui-Ile-NleΨ[$COCH_2$]2-Nal-Gln-NH_2. The peptide was prepared by solid phase synthesis using Boc-Gln, Boc-NleΨ[$COCH_2$]2-Nal-OH, Boc-Ile and 2-quinoxaloyl chloride. The peptide was cleaved with HF, yielding 81 mg of crude peptide as a mixture of diastereoisomers. By reverse-phase HPLC, 2 mg of each diastereoisomer were purified, FABMS: 724.3 (MH^+). Amino acid analysis: Gln, Ile.

Qui-NleΨ[$COCH_2$]2-Nal-Gln-NH_2. The peptide was prepared by solid phase synthesis using Boc-Gln, Boc-NleΨ[$COCH_2$]2-Nal-OH and 2-quinoxaloyl chloride. The peptide was cleaved with HF, yielding an oil as crude peptide, as a mixture of diastereoisomers. By reverse-phase HPLC, 2 mg of each diastereoisomer were purified. The compounds being very hygroscopic and not very stable, FABMS: 610 (MH^+).

Qui-Thr-Ile-NleΨ(CHOHCH_2)2-Nal-Gln-Arg-NH_2. To a stirred solution of Qui-Thr-Ile-NleΨ[$COCH_2$]2-Nal-Gln-Arg-NH_2 (2.0 mg) in MeOH (0.5 mL) was added sodium borohydride (2 mg) at room temperature. The mixture was stirred at room temperature for 3 h. The solvent was evaporated and the residual mixture was purified by HPLC to give the hydroxyethylene compound (1 mg), FABMS: 983 (MH^+). Amino acid analysis: Thr, Ile, Gln, Arg.

Qua-Thr-Ile-NleΨ(CHOHCH_2)Nle-Gln-Arg-NH_2. To a stirred solution of Qua-Thr-Ile-NleΨ[$COCH_2$]2-Nal-Gln-Arg-NH_2 (2.0 mg) in MeOH (0.5 mL) was added sodium borohydride (2 mg) at room temperature. The mixture was stirred at room temperature for 3 h. The solvent was evaporated and the residual mixture was purified by HPLC to give the hydroxyethylene compound (1 mg), FABMS: 982.3 (MH^+). Amino acid analysis: Thr, Ile, Gln, Arg.

Abz-Thr-Ile-NleΨ(CHOHCH_2)Nle-Gln-Arg-NH_2. To a stirred solution of Qui-Thr-Ile-NleΨ[$COCH_2$]2-Nal-Gln-Arg-NH_2 (2.0 mg) in MeOH (0.5 mL) was added sodium borohydride (2 mg) at room temperature. The mixture was stirred at room temperature for 3 h. The solvent was evaporated and the residual mixture was purified by HPLC to give the hydroxyethylene compound (1 mg), FABMS: 946.6 (MH^+). Amino acid analysis: Thr, Ile, Gln, Arg.

Enzyme assays

The HIV-1 protease assays were conducted according to the procedure of Toth and Marshall.[20] Inhibition of enhanced fluorescence due to reduced cleavage of the substrate hexapeptide 2-Abz-Thr-Ile-Nle-Phe(*p*-NO_2)-Gln-Arg-NH_2, derived from the p24/p15 cleavage site in the presence of different concentrations of inhibitor allow the determination of inhibitor efficacy.

Results

The results of the HIV-1 protease assays of the ketomethylene isosteric analogs are shown in Table 1. Most of the inhibitors yield IC_{50}'s in the nanomolar range. In comparison with the reduced-peptide linked inhibitors, the ketomethylene linkage seems to be more efficient in binding to HIV-1 protease. This behaviour can be rationalized by the presence of two hydrogen bonds with the carbonyl of the ketomethylene isostere and one of the aspartic acids of the active site as easily demonstrated by molecular modeling. In the case of the reduced-peptide bond isosteres, these interactions are absent although a potential electrostatic interaction between the protonated nitrogen and an ionized Asp residue of the active site would be expected to compensate.

This study demonstrates also that arginine in P_3' position is essential for good binding (see entries 13, 14 and 15) since the inhibition fell down in the micromolar range when the latter is missing in the peptides. Also, the number of residues required for optimal binding is at least six (see entries 1, 2, 4, 7, and 10), even if some peptides having only five residues gave good IC_{50}'s (see entries 5, 8 and 11). It can be seen finally that the 2-naphthylalanine sidechain in the P_1' position is more effective than norleucine.

Surprisingly, the reduction of these peptides with borohydride did not enhance the affinity (Table 2) for HIV-1

protease as was expected based on the results with renin. The peptides having an hydroxyethyl functionality as the isosteric bond showed a 10-fold reduced inhibition as compared with the ketomethylene parent. Assuming that a 1:1 mixture of the two isomers for the carbon bearing the hydroxyl group was obtained and that one diastereoisomer is active,[21] it seemed logical to expect, at least, the same inhibition as the ketomethylene analogs. It appears that in these particular peptides, an analogy with the mammalian aspartic proteases is obviously not appropriate. Modeling studies[22] to understand this unexpected behaviour are in progress.

Acknowledgements

The authors acknowledge partial support of this research by the National Institutes of Health (Research Cooperative Agreement grant AI27302 and program project grant GM24483).

Table 1. Ketomethylene isosteres as inhibitors of HIV-1 protease

ENTRY	PEPTIDE	IC_{50} (μM)[a]	
1	Abz-Thr-Ile-NleΨ[$COCH_2$]Nle-Gln-Arg-NH_2	0.423	0.0063
2	Abz-Thr-Ile-NleΨ[$COCH_2$]2-Nal-Gln-Arg-NH_2		0.0048
3	Abz-Thr-Ile-PheΨ[$COCH_2$]Ala-Gln-Arg-NH_2		0.033
4	Qua-Thr-Ile-NleΨ[$COCH_2$]2-Nal-Gln-Arg-NH_2	1.540	.0046
5	Qua-Ile-NleΨ[$COCH_2$]2-Nal-Gln-Arg-NH_2		0.0085
6	Qua-NleΨ[$COCH_2$]2-Nal-Gln-Arg-NH_2		1.44
7	Qui-Thr-Ile-NleΨ[$COCH_2$]Nle-Gln-Arg-NH_2	1.47	0.029
8	Qui-Ile-NleΨ[$COCH_2$]Nle-Gln-Arg-NH_2	1.37	0.087
9	Qui-NleΨ[$COCH_2$]Nle-Gln-Arg-NH_2	26 % at 10 μM	2 % at 10 μM
10	Qui-Thr-Ile-NleΨ[$COCH_2$]2-Nal-Gln-Arg-NH_2	1.24	0.0073
11	Qui-Ile-NleΨ[$COCH_2$]2-Nal-Gln-Arg-NH_2	1.60	0.0102
12	Qui-NleΨ[$COCH_2$]2-Nal-Gln-Arg-NH_2		not determined
13	Qui-Thr-Ile-NleΨ[$COCH_2$]2-Nal-Gln-NH_2	8.08	1.11
14	Qui-Ile-NleΨ[$COCH_2$]2-Nal-Gln-NH_2		7.98
15	Qui-NleΨ[$COCH_2$]2-Nal-Gln-NH_2		7 % at 10 μM

[a] A single value is for the most active diastereomer. Two values are for the two isolated diastereomers.

[b] The abbreviations used are Abz = *o*-aminobenzoic acid, 2-Nal = β-naphthalylalanine, Qua = quinaldic acid, and Qui = quinolic acid.

Table 2. Comparison table of the IC_{50}'s of the corresponding ketone and alcohol isosteres

PEPTIDE	IC_{50} (nM)	
	ψ[$COCH_2$]	Ψ[$CHOHCH_2$]
Abz-Thr-Ile-NleΨ[isostere]Nle-Gln-Arg-NH_2	6.3	38
Qua-Thr-Ile-NleΨ[isostere]2-Nal-Gln-Arg-NH_2	4.6	37
Qui-Thr-Ile-NleΨ[isostere]2-Nal-Gln-Arg-NH_2	7.3	85

References

1. Krausslich, H. G.; Wimmer, E. *Ann. Rev. Biochem.* **1988**, *57*, 701.

2. Korant, B. D.; Towatori, T.; Ivanoff, L.; Petteway, Jr S.; Brzin, J.; Lenarcic, B.; Turk, V. *J. Cell Biochem.* **1986**, *32*, 91.

3. Ratner, L.; Haseltine, W.; Patarca, R.; Livak, K. J.; Starcich, B.; Josephs, S. F.; Doran, E. R.; Rafalski, J. A.; Whitehorn, E. A .; Baumeister, K.; Ivanoff, L.; Petteway, S. R.; Pearson, M. L.; Lautenberger, J. A.; Papas, T. S.; Ghrayeb, J.; Chang, N. T.; Gallo, R. C.; Wong-Staal, F. *Nature* **1985**, *313*, 277.

4. Guyader, M.; Emerman, M.; Sonigo, P.; Clavel, F.; Montagnier, L.; Alizon, M. *Nature* **1987**, *326*, 662.

5. Rich, D. H. *Proteinase Inhibitors*, pp. 179–217, Barrett, A. J.; Salvesen, G., Eds; Elsevier Science Publ.; Amsterdam, 1986.

6. Seelmeier, S.; Schmidt, S.; Turk, V.; von der Kolm, K. *Proc. Natl Acad. Sci. USA* **1988**, *85*, 6612.

7. Mous, J.; Heimer, E. P.; Le Grice, S. F. J. *J. Virology* **1988**, *62*, 1433.

8. Kohl, N. E.; Emini, E. A.; Schleif, W. A.; Davis, L. J.; Heimbach, J. C.; Dixon, A. F.; Scolnick, E. M.; Sigal, I. S. *Proc. Nat. Acad. Sci. USA* **1988**, *85*, 4686.

9. Loeb, D. D.; Hutchison, C. A.; Edgell, M. E.; Farmerie, W. G.; Swanstrom, R. J. *J. Virol.* **1989**, *63*, 111.

10. (a) Schneider, J.; Kent, S. B. H. *Cell* **1988**, *54*, 363; (b) Bock, M. G.; Dipardo, R. M.; Evans, B. E.; Freidinger, R. M.; Rittle, K. E.; Payne, L. S.; Boger, J.; Whitter, W. L.; LaMont, B. L.; Ulm, E. H.; Blaine, E. H.; Schorn, T. W.; Veber, D. F. *J. Med. Chem.***1988**, *31*, 1918; (c) Kotler, M.; Katz, R. A.; Leis, J.; Skalka, A. M. *J. Biol. Chem.* **1988**, *264*, 3428.

11. Spatola, A. F. *Chemistry and Biochemistry of Amino Acids, Peptides and Proteins*, Vol. 7, pp. 267–358, Weinstein, B., Ed.; Marcel Dekker; New York, 1983.

12. (a) Miller, M.; Jaskolski, M.; Rao, J. K. M.; Leis, J.; Wlodawer, A. *Nature* **1989**, *337*, 576; (b) Navia, M. A.; Fitzgerald, P. M. D.; McKeevers, B. M.; Leu, C. T.; Heimbach, J. C.; Herber, W. K.; Sigal, I. S.; Darke, P. L.; Springer, J. P. *Nature* **1989**, *337*, 615.

13. Miller, M.; Schneider, J.; Sathyanarayana, B. K.; Toth, M. V.; Marshall, G. R.; Clawson, L.; Selk, L.; Kent, S. B. H.; Wlodawer, A. *Science* **1989**, *246*, 1149.

14. (a) Appelt, K. *Perspect. Drug Discov. Des.* **1993**, *1*, 23; (b) Moore, M. L.; Dreyer, G. B. *Perspect. Drug Discov. Des.* **1993**, *1*, 85.

15. Szelke, M.; Leckie, B.; Hallett, A.; Jones, D. M.; Sueiros, J.; Atrash, B.; Lever, A. F. *Nature* **1982**, *299*, 555.

16. Toth, M. V.; Chiu, F.; Glover, G.; Kent, S. B. H.; Ratner, L.; Vander Heyden, N.; Green, J.; Rich, D. H.; Marshall, G. R. *Peptides: Chemistry, Structure and Biology*, pp. 835–838, Rivier, J. E.; Marshall, G. R., Eds; ESCOM; Leiden, 1990.

17. (a) Holladay, M. W.; Salituro, F. G.; Rich, D. H. *J. Med. Chem.* **1987**, *30*, 374; (b) Rich, D. H.; Bernatowicz, M. S.; Schmidt, P. G. *J. Am. Chem. Soc.* **1982**, *104*, 3535.

18. Kaltenbronn, J. S.; Hudsepth, E. A.; Lunney, B. M.; Michniewicz, B.; Woo, P. K. W.; Essenburg, A. D. *J. Med. Chem.* **1990**, *33*, 838.

19. Tam, J. P. *Macromolecular Sequencing and Synthesis: Selected Methods and Applications*, Chapter 13, pp. 153–184; Alan R. Liss, Inc.; New York, 1988.

20. Toth, M. V.; Marshall, G. R. *Intl J. Pept. Prot. Res.* **1990**, *36*, 544.

21. (a) Bott, R.; Subramanian, E.; Davies, D. R. *Biochemistry* **1982**, *21*, 6956; (b) James, M. N. G.; Sielecki, A.; Salituro, F.; Rich, D. H.; Hofmann, T. *Proc. Natl Acad. Sci. USA* **1982**, *79*, 6137; (c) Foundling, S. I.; Cooper, J.; Watson, F. E.; Cleasby, A.; Pearl, L. H.; Sibanda, B. L.; Hemmings, A.; Wood, S. P.; Blundell, T. L.; Vallner, J. M.; Norey, C. G.; Kay, J.; Boger, J.; Dumm, B. M.; Leckie, B. J.; Jones, D. M.; Atrash, B.; Hallet A.; Szelke, M. *Nature* **1987**, *327*, 349.

22. Waller, C. L.; Oprea, T. I.; Giolitti, A.; Marshall, G. R. *J. Med. Chem.* **1993**, *36*, 4152.

(*Received* 2 *June* 1994)