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Evaluation of "Norpeptides" as Potential Inhibitors of HIV-Proteases†

Kevin Burgess* and Biman Pal
Department of Chemistry, Texas A&M University, College Station, TX 77843, U.S.A.

Abstract—Peptide mimics of substrates for HIV-proteases were prepared. These "norpeptides" are identical to a fragment of the HIV-polyprotein except that a crucial scissile bond was deleted, and an α,β-disubstituted amino acid spans the P_1 and P_1' site. Thus all four stereoisomers of **LeuΨ[]Ala** (i.e. $H_2NCH(CH_2{}^iPr)CH(Me)CO_2H$, **1**) were incorporated into Ac-Ala-Arg-Val-**LeuΨ[]Ala**-Glu-Ala-NH_2 (all other residues being L-amino acids), and tested with respect to inhibition of HIV-1 and HIV-2 proteases.

Transition-state analogs are compounds designed to resemble intermediates in enzyme-mediated reactions and bind tightly to the enzyme in question, thus inhibiting it. This mode of thinking has dominated the search for HIV-protease inhibitors as potential pharmaceuticals for anti-AIDS chemotherapy,[1] and other branches of medicinal chemistry for which beneficial effects might be obtained by impairing the action of a protease.[2-16] Consequently, transition state analogs have been formed from phosphinates,[8] sulfoxides,[17] fluoroketones,[8] hydroxyethylene-based compounds,[6,7,12,18,19] and a variety of other structures.

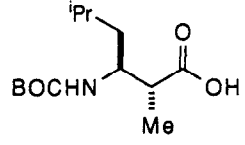
It occurred to us that for peptide-based substrates encapsulated in an enzyme, contacts at the active site may account for a relatively small fraction of the free energy of binding since the total number of interactions peripheral to the active site is relatively high. This might be particularly true for a heptapeptide bound in the "cylindrical pocket" formed by HIV-1 protease.[20-22] Transition-state mimics should only enhance binding to the enzyme functionalities directed towards the scissile bond, while the peripheral amino acids form a variety of hydrogen bonds, hydrophobic interfaces, and electrostatic complements along the enzyme cavity.

This paper describes "norpeptides" designed to test for inhibition via substrate/enzyme interactions which exclude the active site. These peptidomimetics contain α,β-disubstituted β-amino acids spanning the P_1 and P_1' positions, hence there is no transition-state mimicry, and not even a carbonyl group to interact at this position; however, all the peripheral amino acids could be retained. As a first test of this approach norpeptide mimics of Ac-Ala-Arg-Val-Leu-Ala-Glu-Ala-NH_2 were prepared (Scheme I). This peptide is a direct analog of one of the sites in the HIV-polyprotein which is cleaved by HIV-proteases.[23] The peptidomimetics **1** incorporate the α,β-disubstituted β-amino acids **2** at the Leu-Ala cleavage point in the parent peptide, but do not have an amide bond to be cleaved at this position. Consequently, inhibition of the parent enzyme is a very crude measure of how well the peripheral amino acids hold the peptide substrate in place.

The required α,β-disubstituted β-amino acids **2** were prepared via the hydroboration methodology previously developed in our laboratories, as summarized in Scheme II.[24] The sequence beginning with a D-amino acid is shown, but in fact both D- and L-starting materials were used. Control of relative stereochemistry was achieved using 9-BBN to give the *syn* diastereomer, and BH_3 to give the *anti*.

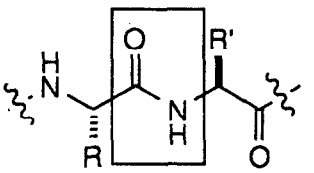
The four stereoisomeric α,β-disubstituted β-amino acids **2** were incorporated into Ac-Ala-Arg-Val-**LeuΨ[]Ala**-Glu-Ala-NH_2 using a solid phase approach with MBHA resin,[25] the (benzotriazolyloxy)tris(dimethylamino)-phosphonium hexafluorophosphate (BOP)/1-hydroxybenzotriazole (HOBt) coupling system,[26] tosyl side chain protection for arginine and benzyl protection for the glutamic acid side chain.

The four diastereomeric "norpeptides" were originally tested for inhibition of HIV-1 protease using the published fluorogenic assay.[27] Only one peptidomimetic, the one incorporating (2*R*,3*S*)-**2**, showed any activity, and that was very low (IC_{50} = 11 μM). Unfortunately this same assay was unavailable to us for screening for inhibition of HIV-2 protease. Consequently, a second assay was used with both enzymes. This assay apparently does not register low levels of activity because none of the peptidomimetics showed any inhibition of HIV-1 or HIV-2 protease.

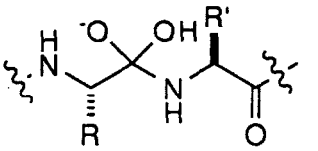


(2*R*,3*S*)-**2**

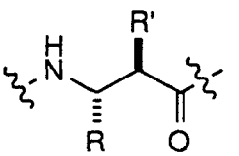
†This paper is dedicated to Professor C. J. Sih.



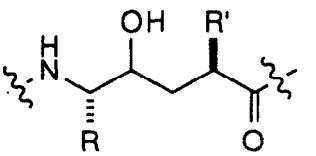
scissile bond in parent peptide



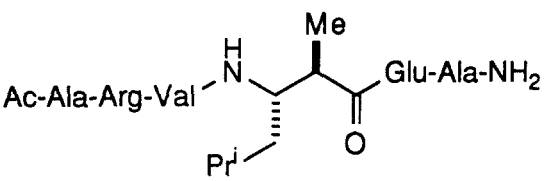
intermediate in hydrolysis



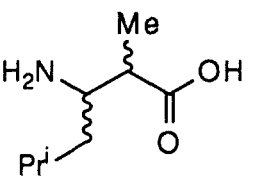
"norpeptide" analog



typical transition state analog (hydroxyethylene)

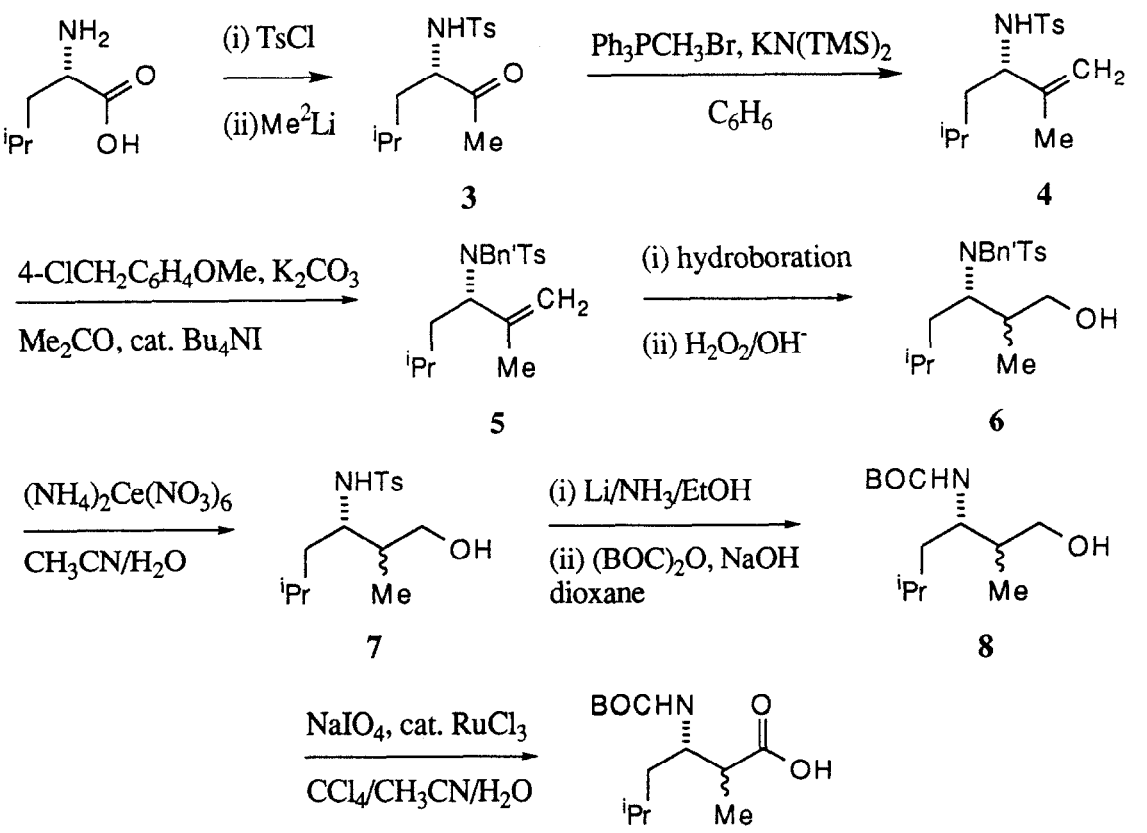


1



2

Scheme I. Transition state analogs, and "norpeptides" from α,β-disubtituted β-amino acids (e.g. **2**).



Scheme II.

It is curious, though possibly fortuitous, that the only "norpeptide" stereoisomer in this series to show any hint of activity in this series was that wherein the absolute configuration most closely corresponds to the natural -Leu-Ala- junction (although one center has *R*-configuration because the priorities of the groups effectively change in the β-amino acid). Peptides containing the Leu-Ala scissile bond are more effectively processed by HIV-2 than by HIV-1,[28] so lack of activity with the former enzyme is perhaps significant. However, the largely negative results obtained in this study do not prove that the concept of using β-amino acids at the cleavage point is generally inapplicable. The simplistic discussion of these peptidomimetics given in this paper ignores subtle conformational effects that are sequence dependent, and the fact that deletion of two atoms from the linear chain may significantly perturb the peripheral interactions in this case, though perhaps not in others. On this basis we present "norpeptides" as a possibility for further investigations.

Experimental

Published procedures were followed to obtain the α,β-disubstituted β-amino acids[24] and for the tests for inhibition of HIV proteases.[27,29]

Peptide synthesis

The peptide AcARVL*AENH_2 was prepared using *N-tert*-butyloxycarbonyl (Boc) amino acid derivatives on MBHA resin. The following side chain protecting groups were used: Glu(OBzl), Arg(Ts). Manual peptide synthesis was carried out in a 30 mL vessel fitted with a coarse glass frit by using a manual wrist action shaker (Burrel, Model 75). The assembly of the protected peptide was carried out at room temperature using the following reaction step cycles. First the Boc protecting group was removed from the α-amino group of the resin-bound amino acid with TFA (50% TFA in CH_2Cl_2 for 1 and 20 min). The deprotected peptide–resin was then neutralized with 10% DIEA in CH_2Cl_2 (2 x 5 min). Amino acids were coupled to the free α-amino group by the addition of 3 equiv. of Boc-amino acid, 3 equiv. of BOP and of HOBt, and 5.3 equiv. of DIEA in 10 mL of DMF. The reaction was allowed to proceed for a total of 2 h. The efficiency of the coupling was monitored at each step by a ninhydrin assay.[30] If there was an incomplete coupling, the resin was first neutralized with 10% DIEA and the coupling steps were repeated.

Cleavage of the peptide from the resin was done at Multiple Peptide Systems, Inc. (San Diego, CA). Dried resin was placed in the HF apparatus. Side chain protecting groups as well as the peptide–resin bond were cleaved under "high HF" conditions (90% HF, 10% *p*-cresol) at 0 °C for 2 h. After removal of HF under vacuum, the peptide–resin residue was placed on a fritted funnel and washed with anhydrous ether. The peptide was then dissolved in 10% aqueous acetic acid and filtered through, leaving the resin on the frit. The crude peptide solution was lyophilized and the peptide purified by semi-preparative reversed-phase HPLC on a Vydac C18 column using a 30 min linear gradient of 5–65% acetonitrile/water/0.05% TFA with a flow rate of 2 mL/min and detector set at 230 nm. The desired peptide fraction was collected, lyophilized and characterized by amino acid analysis, and FAB mass spectroscopy (m/z = 727).

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