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New Hybrid Transition State Analog Inhibitors of HIV Protease with Peripheric C_2-Symmetry

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Abstract: The synthesis of novel hybrid transition state analogs, a combination of phosphinic acid- and hydroxymethylene-type inhibitors of HIV protease, is demonstrated. These nonsymmetrical structures, placed in a peripheric C_2-symmetrical environment, inhibit HIV protease in the lower nanomolar range.

In view of developing drugs for Aids-therapy the virally-encoded HIV protease has emerged as one of the most attacked targets for interfering with the viral life cycle [1]. The key function of this aspartic protease for viral replication is posttranslational processing of HIV polyprotein products, especially Pr 55 gag and Pr 160 gag/pol [2,3]. The proteinase may also be essential at an earlier step in the viral life cycle by hydrolysis of the NC proteins surrounding the viral RNA following expulsion of the viral capsid into a newly infected cell [4].

HIV protease functions as a C_2-symmetric homodimer [5,6]. Primarily hydrophobic side chains are required for peptidomimetic inhibitors of HIV protease. Various structures resembling the transition state of enzymatic peptide bond cleavage inserted in the inhibitor center allow tight binding to the catalytic site of HIV protease (Asp 25, 225) [1]. Recently the synthesis of C_2-symmetric HIV protease inhibitors that match the symmetry of the enzyme was reported [7-10]. Such compounds employing a P_1-P_1'-dihydroxyethylene (A) [7,9,10] or a -phosphinate (B) [8,11] modification have proven to be excellent inhibitors of HIV protease.

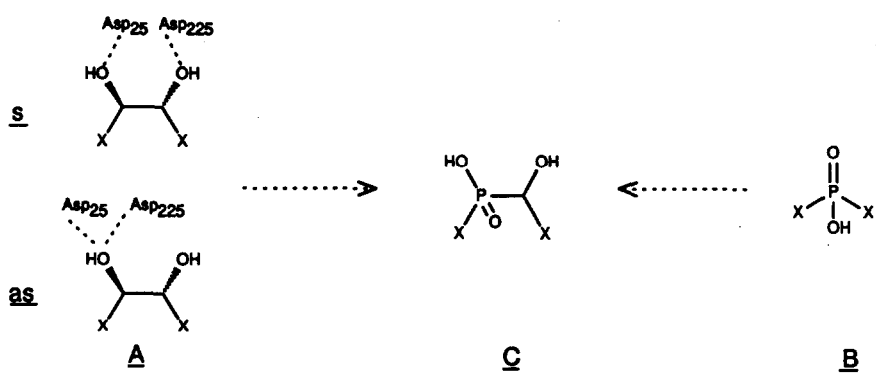
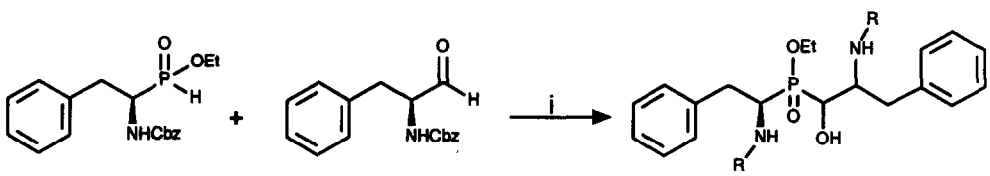


Figure 1: Transition state analog inhibitors of HIV protease with (peripheric) C_2-symmetry

It is an open question, whether the catalytic carboxyl groups of the enzyme (Asp 25, 225) bind in a symmetrical (s) or an asymmetrical (as) way to the dihydroxyethylene moiety A (Figure 1). In case of an asymmetrical preference a slight distortion of inhibitor symmetry in the centerpart combined with peripheric C_2-symmetry might increase binding affinity and maintain specificity. Therefore we decided to synthesize the asymmetrical structure C which connects a phosphinic acid moiety with a hydroxymethylene group. This represents a hybrid between two excellent transition state analogs A and B placed in a peripheric C_2-symmetric environment.

In this work we present a synthetic route to this type of central building blocks and their conversion into powerful inhibitors of HIV protease. Since the formation of phosphorus-carbon bonds via addition of phosphinic acid derivatives to aldehydes is well known [12], this strategy was applied for the synthesis of the central building block (Figure 2). As lipophilic side chains with L-amino acid configuration in the P_1-P_1'-position are important for enzyme specificity [1], we used Cbz-protected (R)-α-amino-phosphinic acid ethylester 1 [13] and Cbz-protected (S)-phenyl-alaninal 2 [14] as starting materials. Base catalysis with tert. amine gave the desired coupling product 3a as a mixture of 3 major diastereomers (ratio $3a_1$: $3a_2$: $3a_3$ = 3.4 : 1.7 : 1, 47% overall yield) [15]. Removal of the Cbz-protecting groups was carried out by hydrogenation using Pd/C as a catalyst and provides 3b in 87% yield.



1 2 3

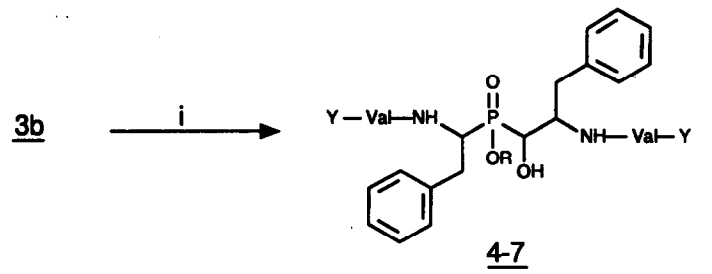
i(3a): $CHCl_3$, NEt_3 (47% yield)
i(3b): a) i(3a) ; b) H_2, Pd/C (87% yield)

3a:R=Cbz
3b:R=H

Figure 2 : Synthesis of the central building block (3)

Coupling of the central building block 3b using the mixture of the three diastereomers with L-Cbz-valine is carried out by standard peptide chemistry with TOTU [16] as coupling reagent and provides 4 in 70 % yield. Trimethylsilyl bromide was used for cleavage of the phosphinic ester 4 to give inhibitor 5 (22 % yield) (Figure 3).

To introduce L-naphthylalanine into the P_3-P_3'-position 4 was hydrogenated, coupled with Boc-protected L-naphthylalanine applying standard peptide chemistry and treated with trifluoroacetic acid providing ethylester 6. Conversion of 6 into the free phosphinic acid 7 was carried out by treatment with trimethylsilyl bromide followed by hydrolysis.



	R	Y	IC_{50} (HIV Protease) [nM]	IC_{50} (Renin) [nM]
4	Et	Cbz	70.0	$> 10^5$
5	H	Cbz	1.3	$> 10^5$
6	Et	Nal	15.0	$> 10^5$
7	H	Nal	0.5	$> 10^5$

i (4) : Cbz-Val-OH, TOTU (70 % yield).

i (5) : a) i (4) ; b) TMS-Br, H_2O (22 % yield).

i (6) : a) i (4) ; b) H_2, Pd/C (90 % yield) ; c) Boc-Nal-OH, HOBT, DCC (63 % yield) ; d) TFA (80 % yield).

i (7) : a) i (6) ; b) TMS-Br, H_2O (50 % yield).

Figure 3 : Synthesis and enzyme-inhibiting activity of peripheric C_2-symmetric HIV protease inhibitors

Inhibition of HIV protease was determined at pH 5.5 using a synthetic substrate [17]. IC_{50}-values of the free phosphinic acids 5 and 7 are in the lower nanomolar range (Figure 3). Using L-naphthylalanine as terminus (P_3, P_3') the IC_{50} value drops by a factor of 3 compared to Cbz. Further side chain modifications in positions P_1-P_3 are under way. The decrease of binding affinity of the esters 4 and 6 compared to 5 and 7 respectively might be due to the lack of additional hydrogen bond formation and/or steric hindrance between the ethyl group and one of the catalytic aspartates. Human renin, another important aspartic protease, is not inhibited by compounds 4-7 underlining the specificity of these inhibitors. For a better understanding of the binding situation we are working on the preparation of crystals of protease-inhibitor-complexes for X-ray studies as well as on the stereocontrolled synthesis of these compounds.

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15) The ratio of the isolated diastereomeres is $3a_1$: $3a_2$: $3a_3$=3.4 : 1.7 : 1. Incubation of 1 or 2 under reaction conditions i (3a) shows, that racemisation occurs only on 2 and not on 1. As expected, reaction of the S-enantiomer of 1 with 2 gives the enantiomers of the three major diastereomers of the 3a-mixture as main products (identical NMR-spectra, opposite optical rotations). This is in agreement with fast racemisation of 2 under coupling conditions.

$3a_1$: 270 MHz-^{1}H-NMR (d^6-DMSO): d=7.63 (d, J=9Hz,1H,NH), 7.12-7.38 (m, 21H,Ph-H,NH), 5.93 (dd, J_1=8.3Hz, J_2=8Hz,1H,OH), 4.92 (m, 4H,Ph-CH_2-O), 3.92-4.36 (m, 5H,N-CH,O-CH,CH_3-CH_2), 3.26 (m, 2H,Ph-CH_2), 2.76 (m, 2H,Ph-CH_2), 1.19 (t, J=7Hz,3H,CH_3).

$3a_2$: 270 MHz-^{1}H-NMR (d^6-DMSO): δ=7.60 (d, J=9Hz,1H,NH), 7.12-7.38 (m, 20H,Ph-H), 6.92 (d, J=9Hz,1H,NH), 5.84 (dd, J_1=J_2=8Hz,1H,OH), 4.97 (s, 2H,Ph-CH_2-O), 4.95 (d, J=13Hz,1H,Ph-CH_2-O), 4.87 (d, J=13Hz,1H, Ph-CH_2-O), 4.40 (m, 1H,N-CH), 4.25 (m, 1H,N-CH), 3.96 (dq, J1=J2=7Hz,2H,CH_3-CH_2), 3.84 (m, 1H,O-CH), 2.63-3.10 (m, 4H,Ph-CH_2), 1.10 (t, J=7Hz,3H,CH_3).

$3a_3$: 270 MHz-^{1}H-NMR (d^6-DMSO): δ=7.53 (d, J=9Hz,1H,NH), 7.12-7.38 (m, 20H,Ph-H), 6.90 (d, J=9Hz,1H,NH), 5.68 (dd, J_1=8.3Hz, J_2=8Hz,1H,OH), 4.98 (s, 2H,Ph-CH_2-O), 4.95 (d, J=13Hz,1H,Ph-CH_2-O), 4.85 (d, J=13Hz,1H, Ph-CH_2-O), 3.84-4.27 (m, 5H,N-CH,O-CH,CH_3-CH_2), 3.00 (m, 2H,Ph-CH_2), 2.75 (m, 2H,Ph-CH_2), 1.18 (t, J=7Hz, 3H,CH_3).
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Dedicated to Professor Klaus Hafner on the occasion of his sixty/fifth birthday

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