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NOVEL, EXTENDED TRANSITION STATE MIMIC IN HIV-1 PROTEASE INHIBITORS WITH PERIPHERAL C_2-SYMMETRY

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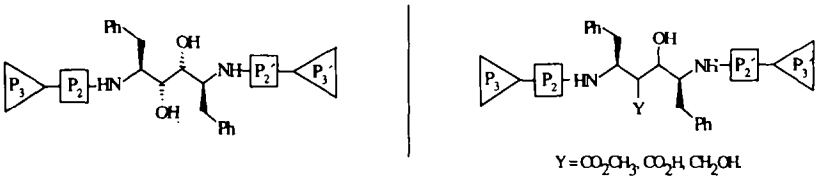
Abstract: Extension of hydroxyethylene containing transition state mimics in HIV-protease inhibitors by carboxy or hydroxymethyl substituents led to potent competitive enzyme inactivation as well as inhibition of HIV-1 replication in MT4-cells. Two diastereomeric series are discussed.

The human immunodeficiency virus type 1 (HIV-1) encodes an aspartic proteinase essential for viral replication. It has been identified as a promising target for the chemotherapy of HIV-infections[1], because effective inhibition of the viral enzyme leads to the formation of immature, non-infectious virions[2]. Numerous inhibitors, with structures resembling the transition state of the enzymatic cleavage-process of the peptide bond, have been described. Especially HIV-protease inhibitors employing dihydroxyethylene replacement of the scissile amide bond of the natural substrate, and reflecting the C_2-symmetry of the apo-enzyme, had been demonstrated to be excellent inhibitors of HIV protease (Figure 1[3])[4].

Figure 1[3]:



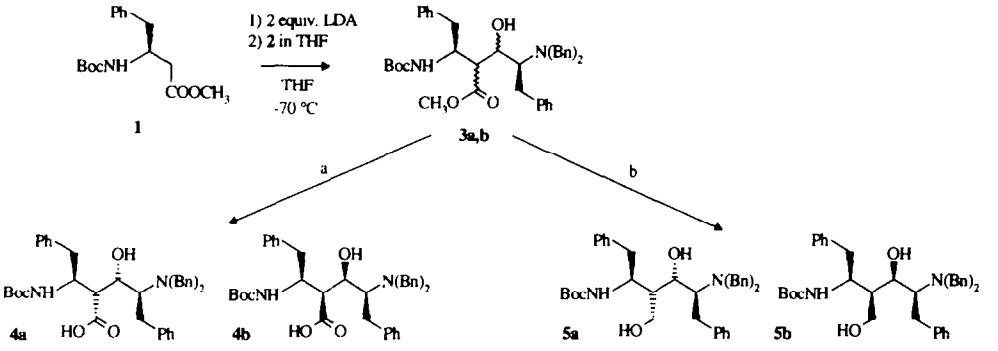
Dihydroxyethylene containing inhibitors

modified hydroxyethylene containing inhibitors

By CAMD-studies we became interrested to study the influence on inhibitory potential when one of the hydroxyl group is replaced by the more bulky carboxy or hydroxymethyl substituents. These modifications in the central part of the inhibitor, while preserving the peripheral C_2-symmetry of the molecules, might give new insights into the steric requirements around the catalytic aspartates[5]. We have recently reported on the efficient synthesis of two diastereoisomeres of these central building blocks[6]. The results of their incorporation into HIV-protease inhibitors are described here.

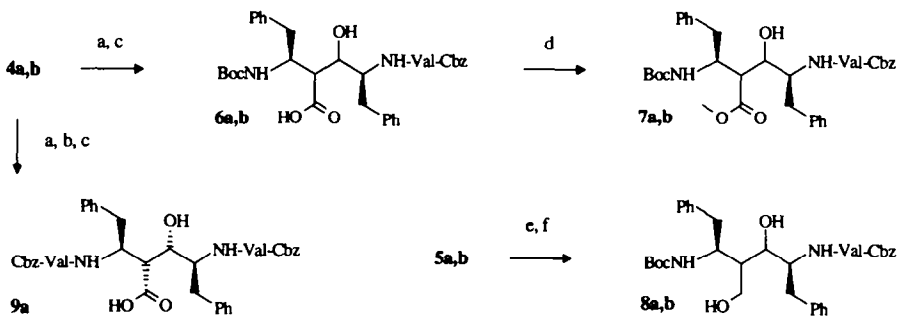
Since lipophilic side chains of L-configurated amino acids in the P_1-P_1'-position are important for enzyme

specificity, the central building block (**3a,b**) was synthesized via aldol addition of N,N-dibenzyl protected L-phenylalaninal **2** to enantiomerically pure, dilithiated 3*(S)*-amino-methyl-butanoate **1**. Diastereoselectivity varied from 1:1.5 to 1:20 for **3a**: **3b**, depending on experimental conditions and protecting groups used in **1** (abs. configuration of separated **3a** and **3b** has been determined relative to the known *(S)*-configuration of the phenylalanine fragments)[6]. The two diastereomers (**3a,b**) were more easily separable by column chromatography after saponification or reduction of the ester-functionality (**4a,b**; **5a,b**; *scheme* 1). Compounds **4** and **5** represent the central building blocks for the synthesis of protease inhibitors with carboxy-hydroxy- or hydroxymethyl-hydroxyethylene substructure as transition state mimic.



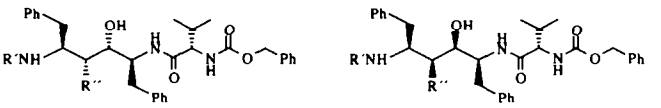
Scheme 1. (a) i) DMF, 5N aqueous KOH; r.t., 16h; ii) isolated yield after separation: 28% **4a**, 42% **4b**
(b) i) LAH, THF, -30°C→r.t., 12h; ii) isolated yield after separation: 22% **5a**, 35% **5b**.

Reductive deprotection of carboxylic acid derivatives **4**, and subsequent acylation with Cbz-valine provided **6**, which were converted to the corresponding ester derivatives **7**[7]. Analogously, after deprotection, alcohol derivatives **5** were transformed to **8** by coupling with the Cbz-valine fragment (*scheme* 2)[3]. The molecules **6**, **7**, and **8** exhibited weak inhibition of HIV-1 protease in a peptide cleavage assay (table 1)[8]. In terms of inhibitory potential a clear preference of stereochemistry and as well as functional group, present in the core of these truncated inhibitors, became visible: diastereomers B (**6b**, **7b**, **8b**) turned out to be less tolerated by the enzyme, when compared to diastereomers A (**6a**, **7a**, **8a**). In both series of diastereomers a superiority of the hydroxymethyl substituent (**8a**, **8b**) to carboxylic acid or ester group was demonstrated. This result could be further confirmed by transformation of **4a** to **9a** (*scheme* 2; table 1). Carboxy group bearing inhibitor **9a**, spanning the S3 to S3´subsites[3], was less potent (K_i = 0.43 μM)[8,9] than its truncated, hydroxymethyl substituted congener **8a** (K_i = 0.29 μM). Therefore, we decided to perform further structure-activity studies in the "hydroxymethyl" series.



Scheme 2. (a) Pd_{black}, H_2, MeOH, 50°C, 12h; (b) CF_3SO_3H, CH_2Cl_2, r.t., 1h; (c) Cbz-Val-OSu, DMF, r.t., 48h; (d) diazomethane in Et_2O, THF, r.t., 3h; (e) Pd_{black}, H_2, MeOH+4%HCOOH, r.t., 12h; (f) Cbz-aa-OH, EDC.HCl, DHOBT, DMF, r.t., 48h.

Table 1. Comparison of the Novel Transition State Mimics

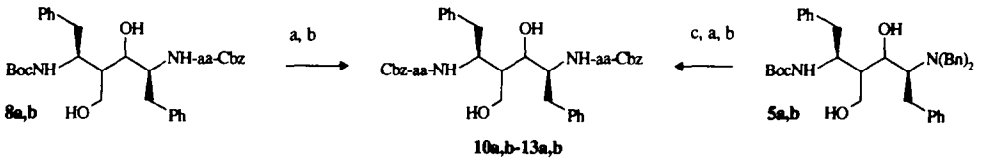


		diastereomer A		diastereomer B	
R′	R″	#	K_i or % inhibition[a]	#	K_i
Boc	COOH	**6a**	18%	**6b**	n.a.[b]
Boc	$COOCH_3$	**7a**	20%	**7b**	n.a.
Boc	CH_2-OH	**8a**	0.29 µM	**8b**	3.7 µM
Cbz-Val	COOH	**9a**	0.43 µM		

[a] % inhibition at 12.5 µM of compound tested; [b] not active at 12.5 µM

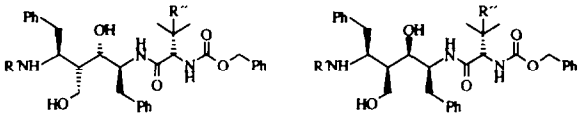
To study the influence of P_2-modifications, derivatives **8** were deprotected, and subsequently coupled with Cbz-protected amino acids (aa) to give inhibitors **10a,b** - **12a,b** (*scheme* 3). As summarized in table 2, extension of the inhibitory module (**8**) by Cbz-Asn resulted in decreased activity (**10**). This was in agreement with earlier observation in other PheΨPhe-transition state mimics, namely that Asn is not well tolerated in P_2[10]. A significant enhancement of inhibition of HIV-1 proteinase could be achieved by introduction of more hydrophobic P_2-residues, like Cbz-valine (**11**) or Cbz-*tert.* leucine (**12**). Again the more potent compounds belonged to the series of diastereomer A (**10a** -**12a**). Substitution of valine (**11a**) by the more space filling *tert.* leucine (**12a**) led only in the case of diastereomer A to increased enzyme inhibition, while the same modification performed on diastereomer B (**11b**→**12b**) nearly abolished activity. Surprisingly the inhibitory potency of **12b** could be rescued by exchange of valine in P_2' against *tert.* leucine to give **13b**. The same modification applied to diastereomer

A had no effect on improving enzyme inhibition (**12a→13a**).



Scheme 3. (a) 6N HCl in dioxane, r.t., 15 min.; (b) Cbz-aa-OH, EDC.HCl, DHOBT, DMF, r.t., 48h; (c) Pd_{black}, H_2, MeOH+4%HCOOH, r.t., 12h.

Table 2. Hydroxymethyl-hydroxyethylene Containing Inhibitors Modified in P_2 and P_2'



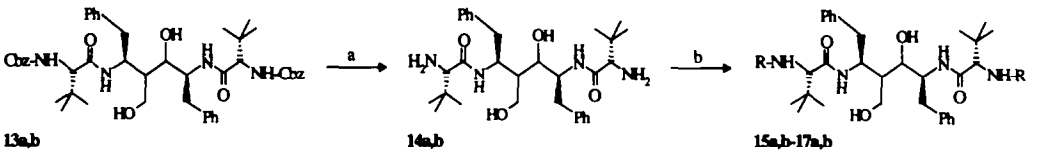
		diastereomer A			diastereomer B	
R′	R″		K_i[μM]	ED_{50}[μM][11]	K_i[μM] or % inhibition[a)]	ED_{50}[μM][11]
Boc	H	**8a**	0.290	>10	**8b** 3.7	>10
Cbz-Asn	H	**10a**	0.340	>10	**10b** 32%	>10
Cbz-Val	H	**11a**	0.027	3.7	**11b** 0.510	>10
Cbz-tert.Leu	H	**12a**	0.016	2.5	**12b** 39%	>10
Cbz-tert.Leu	CH_3	**13a**	0.013	1.6	**13b** 0.255	>10

[a)] % inhibition at 12.5 μM of compound tested

The first compounds out of this series which exhibited antiviral activity *in vitro*[11] were **11a - 13a**. However, although **11a - 13a** were inhibitors of HIV-proteinase in the lower nanomolar range, there was a remarkable discrepancy between enzyme inhibition and antiviral activity. We assumed that this phenomenon may be caused by insufficient cellular uptake, due to the highly hydrophobic character of the compounds. Therefore we substituted the terminal phenyl groups by more polar heterocyclic residues, and anticipated that these modifications should not lead to decrease or even loss of enzyme inhibition, because these groups are located close to the boundary of the active site and the surrounding water.

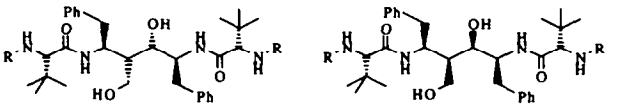
For synthesis of the desired molecules, derivatives **5a,b** were fully deprotected (*scheme* 3) and the intermediate diamines were then converted to the double Cbz-protected inhibitors **13** by coupling with Cbz-*tert.* leucine. Again reductive deprotection of **13** afforded **14**, which was subsequently coupled with benzimidazole

containing residues to give inhibitors **15** - **17** (*scheme* 4).



Scheme 4: (a) 10%Pd/C, MeOH+10%TEA, r.t., 1h; (b) **15,17**: R-COOH, EDC.HCl, DHOBT, DMF, r.t., 48h; **16**: R-CH_2-OH, bis(hydroxybenztriazolyl)carbonate, pyridine, CH_2Cl_2, r.t., 12h.

Table 3. Hydroxymethyl-hydroxyethylene Containing Inhibitors Modified in P_3 and P_3'



		diastereomer A			diastereomer B	
R		K_i [μM] or % inhibition [a]	ED_{50}[μM]		K_i[μM] or % inhibition [a]	ED_{50}[μM]
Cbz	**13a**	0.013	1.65	**13b**	0.255	>10
H	**14a**	22%	>10	**14b**	8%	>10
(Benzimidazole-2-yl)- CO	**15a**	31%	>10	**15b**	42%	>10
(Benzimidazole-2-yl)-CH_2OCO	**16a**	0.013	0.50	**16b**	0.029	4.7
(Benzimidazole-2-yl)-$(CH_2)_2CO$	**17a**	0.009	0.52	**17b**	0.160	>10

[a] % inhibition at 12.5μM of compound tested

As summarized in table 3, removal of the Cbz-protecting groups of **13** to give **14** resulted in a dramatic loss of activity, indicating unfavorable interactions of the terminal amino functions within the active site. Acylation of **14** with benzimidazolecarboxylic acid did not restore activity. Only when the proper distance (derived from analogy to Cbz-protecting group) between heterocyclic moiety and amino groups was reintroduced, potent inhibitors (**16**, **17**) were obtained. The interplay of the outer subsite contacts (S_3,S_3') with the core binding (S_1,S_1') is illustrated by the fact that replacement of the P_3/P_3' Cbz moiety (**13a/13b**) by benzimidazole carbamate groups (**16a/16b**) resulted in a flattening out of the stereochemical dependence on enzyme inhibition potency, while the difference in K_i in **17a** and **17b** is almost the same as in **13a** and **13b**.

In summary, introduction of the more polar benzimidazole heterocycle in **16a** or **17a** led to the desired reduction of the gap between enzyme inhibition and antiviral activity compared to **13**. **16a** and **17a** are potent HIV protease inhibitors with submicromolarer activity *in vitro*.

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