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NOVEL DIMERIC PENICILLIN DERIVED INHIBITORS OF HIV-1 PROTEINASE: INTERACTION WITH THE CATALYTIC ASPARTATES

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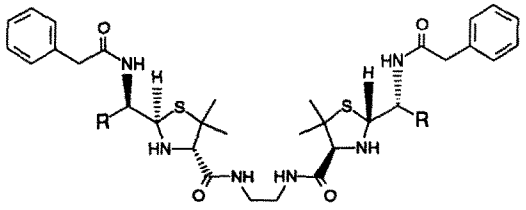
Abstract. The synthesis and enzyme activity of linker modified, dimeric, penicillin derived inhibitors is described. Variation of the linker group enabled beneficial interaction with the catalytic aspartates, however, this was offset by disruption of the original key interactions with the penicillin derived units.

Human immunodeficiency virus (HIV) is the causative agent of acquired immunodeficiency syndrome (AIDS)[1]. Inhibition of the virally encoded proteinase is recognised as an important therapeutic target for the treatment of AIDS[2]. The enzyme is a member of the aspartyl protease family[3] and has been shown to be a C_2 symmetric homodimer[4].

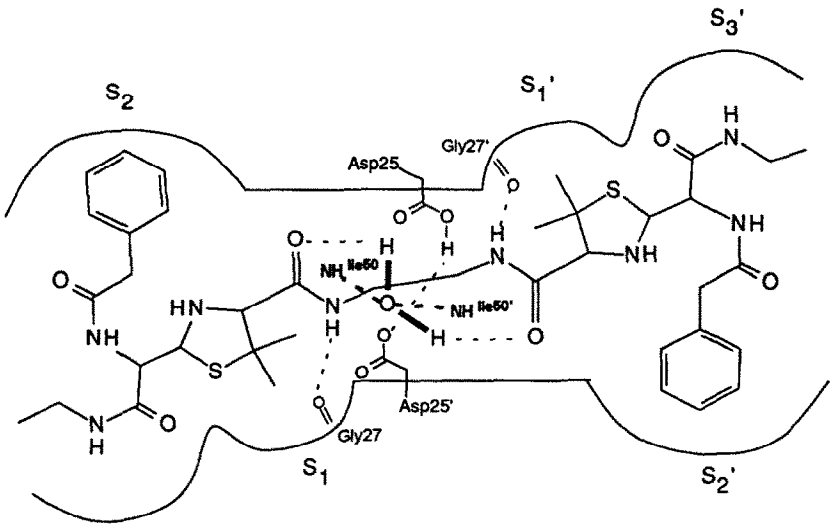
A number of groups have reported substrate based inhibitors in which the scissile bond has been replaced with a non-cleavable isostere, *e.g.*, reduced amide[5], hydroxyethylamine[6] and hydroxyethylene[7]. In an earlier communication we described an alternative approach which involved the identification, by high throughput screening, of a moderately potent inhibitor of HIV-1 proteinase[8]. From this lead, a series of potent diamides were prepared, of which compounds **1** and **2** were among the most potent, (Table I). Crystal structures of HIV-1 proteinase have been described both in the native form[4] and complexed to a number of inhibitors[9]. In view of the C_2 symmetric nature of both the enzyme and the inhibitors **1** and **2** it was postulated[8] that these compounds bound in a symmetric mode at the active site, (Figure I). Using this hypothesis, molecular modelling studies suggested that despite the potency of these inhibitors there was no favourable interaction with the catalytic aspartates, Asp25 and Asp25'. We therefore embarked on a programme to explore the potential for interaction with these key residues, by variation of the central ethylenediamide linker group in **2**.

Table I - Anti-HIV activity of Penicillin Dimers



no.	R	IC_{50}(nM)	EC_{50}(μM), (MT-4)
1	$CONHCH_2CH_3$	4.8	5.4
2	$CONHCH_2Ph$	0.9	0.29

Figure I - Schematic Representation of the Proposed Binding Mode of Compound 1 at the Active Site of HIV-1 Proteinase

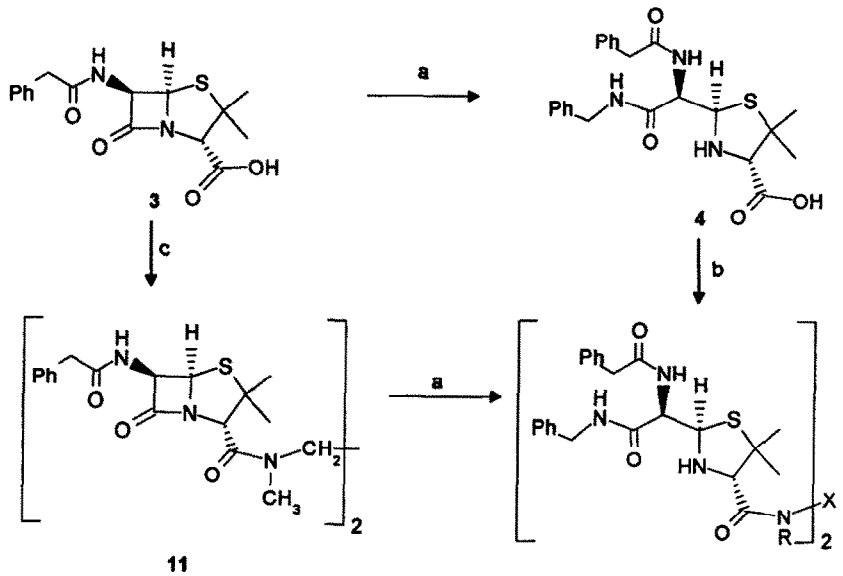


The β-lactam of penicillin G, **3**, was opened with benzylamine to afford the key intermediate **4**[10], (Scheme I). This was then coupled to a series of diamine linkers using dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole hydrate (HOBT) to afford the penicillin dimers **5-10**. Two of the required linker amines, (R,S)- and (S,S)-1,4-diaminobutane-2,3-diol were synthesised as their dihydrochloride salts according to the procedure of Kiely *et al*[11]. (R,R)-1,4-diaminobutane-2,3-diol was synthesised from commercially available dimethyl (S,S)-2,3-isopropylidene tartrate using a modification to the Kiely procedure in which an isopropylidene protecting group was employed.

Compound **12** was synthesised *via* an alternative procedure[8] in which the penicillin acid was first coupled to N,N'-dimethylethylenediamine to give the benzyl penicillin dimer **11**, (Scheme I). The β-lactams were then opened with benzylamine, in a similar manner to that described earlier, to give **12**. Finally, compound **16** was synthesised from the protected diamine **13**[13], (Scheme II). Treatment of the acid function with methyl iodide afforded the ester which was reduced using lithium aluminium hydride at 0°C to give alcohol **14**. The protecting groups were then removed under acidic conditions and the resulting diamine hydrochloride salt **15** was coupled to two equivalents of acid **4** using 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU)[14] and N,N-diisopropylethylamine (DIPEA) in DMF to afford **16**.

The features of the penicillin dimers thought to be essential for binding at the active site include the lipophilic interactions between the *gem* dimethyls of the thiazolidine and the S_1 and S_1' pockets and also between the phenylacetamido groups and the S_2 and S_2' pockets. The two carbonyls of the linker amides, (Figure I), were thought to play an important role in a tetrahedral hydrogen bonding arrangement between the NH of the Ile50 and Ile50′ residues in the flap, a water molecule and the linker carbonyls of the inhibitor.

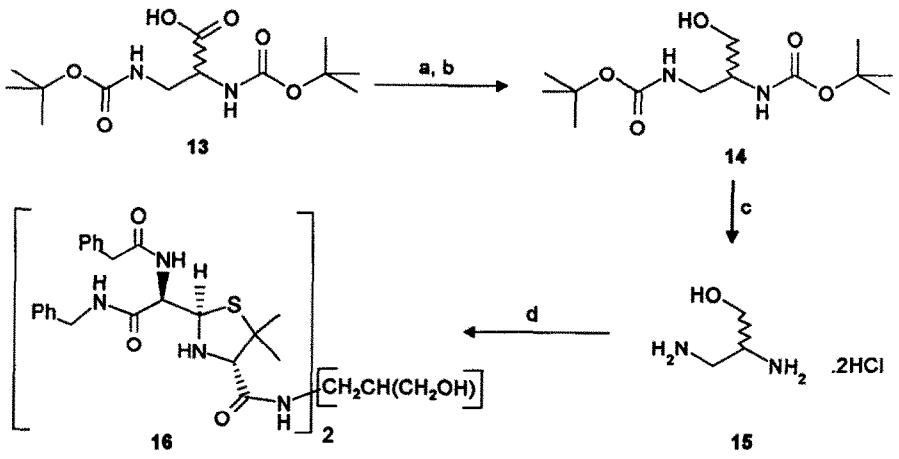
Scheme I [a]



5	X = $(CH_2)_3$	R = H
6	X = $(CH_2)_4$	R = H
7	X = $CH_2CHOHCH_2$	R = H
8	X = $CH_2CHOHCHOHCH_2$ (R,S)	R = H
9	X = $CH_2CHOHCHOHCH_2$ (S,S)	R = H
10	X = $CH_2CHOHCHOHCH_2$ (R,R)	R = H
12	X = $(CH_2)_2$	R = CH_3

[a](a) $PhCH_2NH_2$, CH_2Cl_2; (b) NH_2XNH_2, DCC, HOBT; (c) $(CH_3NHCH_2)_2$, DCC, DIPEA, CH_2Cl_2

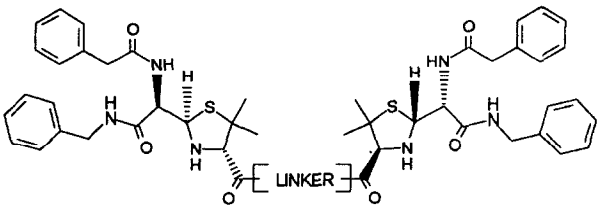
Scheme II [a]

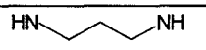
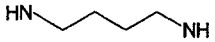
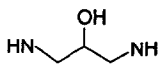
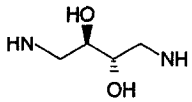
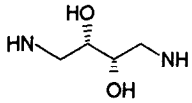
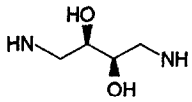
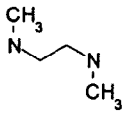
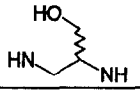


[a](a) CH_3I, K_2CO_3, DMF; (b) $LiAlH_4$, THF, 0°C; (c) HCl in MeOH; (d) **4**, TBTU, DIPEA, DMF

We speculated that the NH's of the linker amides hydrogen bonded to the backbone carbonyl of two residues Gly27 and Gly27′. Methylation of these two amides to give **12** resulted in a 10 fold loss of activity, (Table II). From this we concluded that although there was some hydrogen bonding interaction, the distance between the NH's and Gly27 and Gly27′ was likely to be greater than the optimal hydrogen bonding distance.

Table II - Activity Against HIV-1 Proteinase of Linker Modified Penicillin Dimers[8]



no.	linker	IC_{50}(nM)[15]
5		540
6		530
7		5.0
8		14
9		36
10		15
12		11
16		25

From the binding model of compound **1**, (Figure I), we surmised that in order to optimise this interaction the two NH's of the amides would need to be further from the centre of the active site. The simplest way chemically to achieve this was to increase the length of the linker. Unfortunately, extension of the linker to a propyleneamide or butyleneamide group resulted in a 500 fold decrease in activity in either case. We speculated that this decrease in activity was either as a result of the disruption of the lipophilic interaction in the S_1 and S_1' pockets, or possibly as a result of the disruption of the hydrogen bonding arrangement with the water molecule and the flaps.

In an attempt to interact with the catalytic aspartates, Asp25 and Asp25′ a hydroxymethyl group was introduced into the two carbon linker, to give dimer **16**. Unfortunately, **16** was less active than **2**, possibly because the two carbon linker was constrained at the active site, such that the hydroxymethyl group was not directed towards the aspartates. Despite the fact that the incorporation of a three carbon linker caused a decrease in activity, it did allow the introduction of a secondary alcohol to give a hydroxyethyl isostere, designed to interact with the catalytic aspartates. This was achieved with compound **7** which was 100 fold more active than **5**. Dihydroxy isosteres have also been incorporated into inhibitors of HIV proteinase, and therefore the three compounds **8** , **9** and **10** were prepared. All three analogues were at least 20 times more active than the dideoxy analogue **6**, however, their activity was not comparable with that of the hydroxyethyl isostere **7**.

We have demonstrated that with appropriate modifications to the linker it is possible to interact with the catalytic aspartates. Unfortunately, despite this extra interaction the linkers described show no improvement over the ethylenediamide linker. Compounds **5-10**, **12** and **16** all showed activity against HIV-1 *in vitro*[16], although none was as active as **2**. However, further work is in progress to optimise the interaction with the key residues at the active site without adversely affecting the lipophilic interactions in the S_1 and S_1' pockets.

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