Preparation of novel HIV-protease inhibitors

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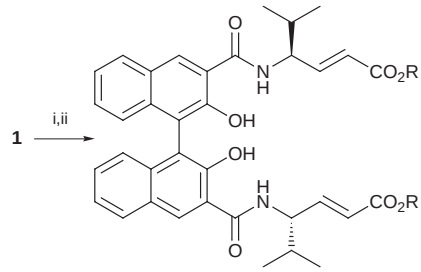
The synthesis and biological properties of new HIV-1-protease inhibitors involving amino acids or dipeptides attached to binaphthol, biphenol or embonic acid are described.

Inspite of recent progress in the development of therapeutics for the treatment of AIDS, a number of problems persist.[1] A well-known approach concerns the design and synthesis of HIV-protease inhibitors. The HIV-protease is a well characterized viral enzyme consisting of two units, each composed of 99 amino acids, which join together to form the C_2-symmetric active homo-dimer. Consequently, one strategy has been to prepare C_2-symmetric inhibitors in the form of peptide mimetics, one family of active compounds being C_2-symmetric 1,2-diols flanked by short peptide units.[2] The hydroxy moities have been shown to participate in the interaction with the HIV-protease *via* H-bonding, the configuration at the two stereogenic centers of the diol playing an important role with respect to the degree of binding. We speculated that similar compounds based on chiral binaphthol or biphenol units in place of the traditional diols could constitute a new class of HIV-1-protease inhibitors, specifically because the enhanced acidity of such moieties would be expected to lead to stronger H-bonding. Here we present the initial results of this strategy.

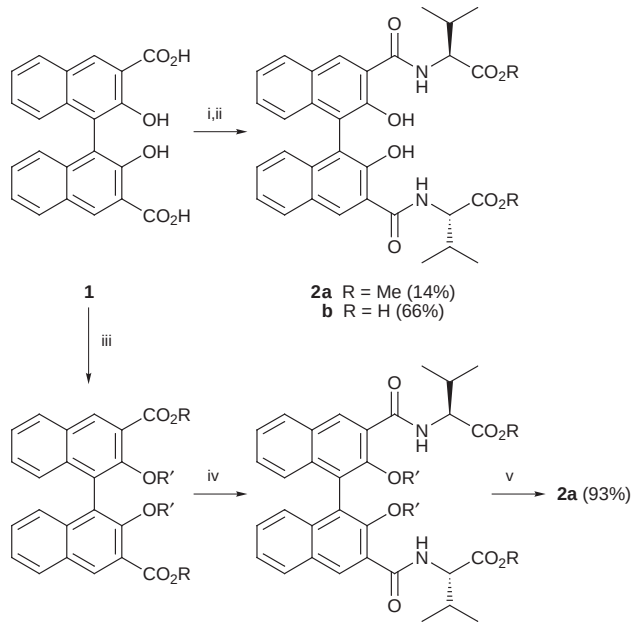
We first envisioned peptides of the type **2b** and **5b** based on racemic 2,2′-dihydroxy-1,1′-binaphthyl-3,3′-dicarboxylic acid **1**. Direct coupling with valine methyl ester resulted in a 14% yield of the desired diastereomeric dipeptide esters **2a**, which were hydrolyzed to the acids **2b**. In order to improve the synthesis, the hydroxy functions of **1** were first protected by benzyl groups (Scheme 1). The diastereomeric mixture of **2b** was separated by HPLC to provide analytically pure (*R*,*S*,*S*)-**2b** and (*S*,*S*,*S*)-**2b**. In the case of the dipeptide **5b** with two vinylogous amino acid residues, only direct coupling was carried out (Scheme 2).



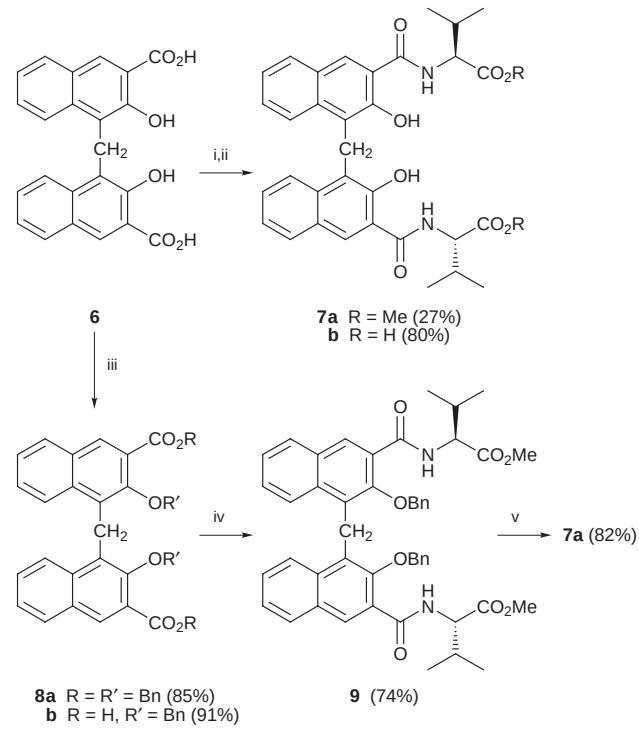
5a R = Et (17%)
b R = H (65%)

Scheme 2 *Reagents*: i, NaOH, NHS, DCC; NEt_3, L-$HCl \cdot H_2NCHPr^iCH{=}CHCO_2Et$; ii, LiOH, H_2O

Although there is a limited degree of rotational freedom with respect to the axis going through the two naphthyl units, the C_2-symmetric dipeptides **2b** and **5b** are in fact fairly rigid. In order to introduce more conformational flexibility, we prepared dipeptide **7b** based on embonic acid **6** (Scheme 3). Although the



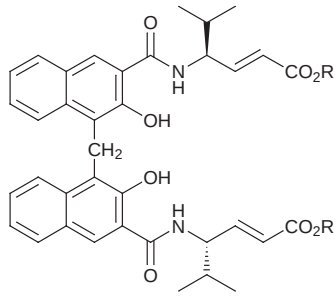
Scheme 1 *Reagents*: i, NaOH, *N*-hydroxysuccinimide (NHS), DCC, L-$H_2NCHPr^iCO_2Me$; ii, LiOH, H_2O; iii, BnBr, K_2CO_3; iv, NHS, DCC, L-$H_2NCHPr^iCO_2Me$; v, $Pd(OH)_2$, cyclohexene



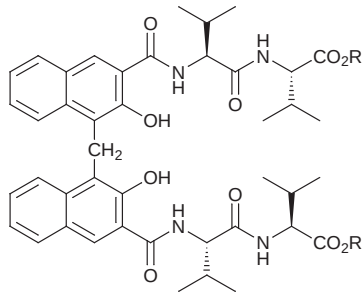
Scheme 3 *Reagents*: i, NaOH, NHS, DCC, L-$H_2NCHPr^iCO_2Me$; ii, LiOH, H_2O; iii, BnBr, K_2CO_3, KOH, H_2O; iv, NHS, DCC, L-$H_2NCHPr^iCO_2Me$; v, $Pd(OH)_2$, cyclohexene

latter is achiral, conformational enantiomers (or diastereomers in the case of **7b**) are likely, especially upon binding to the C_2-symmetric HIV-protease. The dipeptide **10b** incorporating vinylogous valine was synthesized analogously.

In order to test the biological effect of extending the peptidic side-arms, the tetrapeptide **11b** was prepared in a direct manner by NHS–DCC-mediated coupling of **6** with L-valinyl-L-valine ethyl ester. Here, as in all previous cases, the compounds were purified by HPLC and characterized by standard spectroscopic and analytical means.

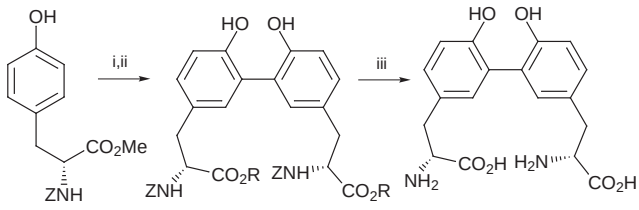


10a R = Me (32%)
b R = H (80%)



11a R = Et (46%)
b R = H (16%)

Finally, the biphenol derivative **13** was prepared by oxidative coupling[3] of *N*-benzyloxycarbonyl-L-tyrosine methyl ester **12**. Deprotection delivered the dipeptide **14** (Scheme 4).



12 (Z = benzyloxycarbonyl) **13a** R = Me (52%) **b** R = H (94%) **14** (90%)

Scheme 4 *Reagents*: i, VOF_3; ii, LiOH, H_2O; iii, H_2, Pd-C

In order to screen the ability of the compounds to inhibit the HIV-1-protease, the IC_{50} values were measured using standard procedures.[4] Table 1 shows that several compounds have activities similar to a number of other HIV-protease inhibitors which have been reported in recent years.[2] It is interesting to note that in the case of **5b** (but not **2b**) the absolute configuration of the binaphthol backbone plays a significant role in the degree of HIV-protease inhibition. Specifically, the (*R*,*S*,*S*)-compound is considerably more active than the (*S*,*S*,*S*)-diastereomer. However, the conformationally more flexible compounds based on embonic acid **6** are more active, especially the tetrapeptide **11b**.

Theoretically, the mode of action of the above compounds can either be due to active-site inhibition of the HIV-protease or to a possible inhibition of dimerization of the two 99-amino acid

Table 1 Properties of synthesized compounds

Compound	Solubility/μM	IC_{50}/μM
(*R*,*S*,*S*)-**2b**	> 65	47
(*S*,*S*,*S*)-**2b**	> 65	48
(*R*,*S*,*S*)-**5b**	> 50	46
(*S*,*S*,*S*)-**5b**	> 50	13
7b	> 100	40
10b	~ 30	8
11b	> 10	2.8
13b	> 100	24
14	> 100	240

units (*i.e*. prevention of homo-dimer formation). In order to shed some light on these aspects, kinetic studies using the model of Zhang were carried out on select compounds, *i.e*. dissociative inhibition constants (K_i) and competitive inhibition constants (K_c) were measured.[5] Accordingly, in the case of the most active compound **11b**, the K_i and K_c values turned out to be 6.9 and 2.0 μM, respectively. This means that active site (competitive) inhibition dominates, although dissociative inhibition plays some role. Mixed inhibition also pertains to the related dipeptide **7b** (K_i = 15.8 μM; K_c = 6.2 μM). In contrast, the mechanism of action of the tyrosin derivative **13b** appears to be based primarily on the inhibition of dimerization of the monomeric HIV-protease units (K_i = 6.8 μM; K_c = 229 μM). This still needs to be studied more closely, *e.g*. using light scattering. However, preliminary molecular modelling is in line with these conclusions.

In summary, we have designed and prepared new HIV-1-protease inhibitors based on naphtholic and phenolic units to which amino acids or dipeptides are attached. Although the respective activities are lower than those of the most potent drugs currently known,[1,2] the discovery of these new lead structures allows for the (combinatorial) synthesis of analogs which may show improved performance.

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Notes and References

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