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Syntheses of new modified Phe-Pro peptides. Use of proline replacements in potential HIV inhibitors.

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Abstract

The syntheses consisting of replacement of proline amino acid by a 3-pyrrolidinone ring in Phe-Pro analogues are described. Preliminary anti-HIV studies demonstrated the potential activity of this new class of compounds.

Introduction

Many potent and selective inhibitors of HIV-1 protease, an essential enzyme in the retroviral life cycle, are based on a transition-state mimic concept which incorporates non hydrolysable hydroxyethylene and hydroxyethylamine isosteres.[1] C-2 symmetric inhibitors based on the symmetric disposition of the HIV-1 protease structure have also been described.[2] While retroviral proteases are highly specific with respect to the *in vivo* cleavage of their polyprotein substrates, there is no absolute sequence homology at the cleavage sites, as shown by the sequences of these cleavage sites or by peptide analogues containing those cleavage sites.[3,4,5] However, one class of cleavage sites is highly conserved in retroviral polyproteins and displays an unusual sequence specificity.[6] It consists of a pentapeptide (Ser/Thr)-X-Y-(Tyr/Phe)-Pro. The cleavage occurs between the aromatic residues Tyr or Phe and the Pro residue. Since endopeptidases which cleave an X-Pro bond are rare, we anticipated that it might be possible to design protease inhibitors based on modifications mimicking the five membered ring of the proline residue.

We described herein the syntheses of new Phe-Pro dipeptide analogues in which the proline residue was replaced by a 3-pyrrolidinone. These new analogues were tested for their activity on HIV replication in MT-4 infected cells.

Chemistry

Two routes were investigated for the synthesis of the new analogues (Scheme 1). The first route (a→f) was rather long : it gave the secondary alcohol after 5 steps consisting essentially of manipulation of protecting groups.

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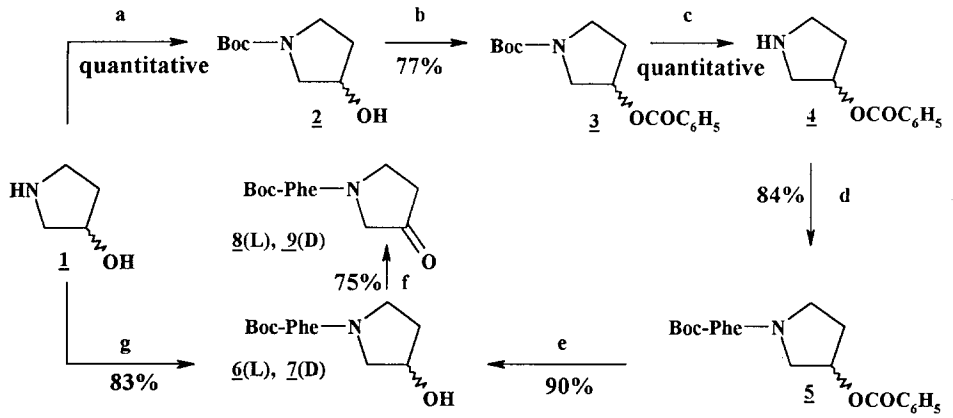
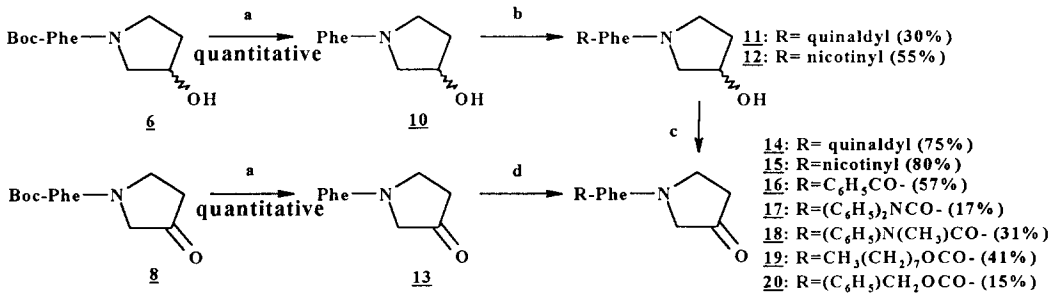


Scheme 1: (a) $(Boc)_2O/CH_2Cl_2$ (b) benzoyl choride, TEA $/CH_2Cl_2$ (c) TFA/CH_2Cl_2 (d) N-(*tert*-butoxycarbonyl)-L-phenylalanine, BOP, TEA/CH_2Cl_2 (e) $NaOH/THF,H_2O$ (f) DMSO, trifluoro acetic anhydride, TEA/CH_2Cl_2. (g) N-(*tert*-butoxycarbonyl)-L-(or D)-phenylalanine, BOP, TEA/CH_2Cl_2

Compound **6** was directly oxidized using either 2,2,6,6-tetramethylpiperidinyl-1-oxy (TEMPO)[7] or trifluoro acetic anhydride in Swern conditions, to give the target compound **8**. The Swern oxidation was quantitative. More recently we were able to shorten considerably this sequence. Augustyns et al.[8] have shown recently that coupling of a N-Boc protected peptide to 3-hydroxypyrrolidine was possible in the presence of BOP reagent without hydroxyl protection. In our experiments, it appeared that the use of DCC/HOBT instead of BOP or PyBOP gave the best yields. The resulting derivative **6** was then oxidized as described above to give the target compound **8**. Both pure L- and D-Phe were used in the syntheses.

Compounds **8** and **9** were then equipped with the substituents required for the biological studies. The two synthetic routes investigated are summarized in scheme 2.



Scheme 2: (a) TFA/CH_2Cl_2; (b) DCC/HOBT in TEA/CH_2Cl_2, quinaldic or nicotinic acid; (c) DMSO, trifluoro acetic anhydride, TEA/CH_2Cl_2; (d) RCl, TEA/CH_2Cl_2

The structures of all these new analogues were determined by ^{1}H NMR, mass spectra and elemental analyses. Examination of the ^{1}H NMR spectra indicated that most compounds existed in solution as a mixture of two rotamers around the amide bond.[8,9]

Antiviral Activity

On table 1, the inhibition of viral spread T-lymphoid infected cells by the newly synthesized L-Phe-Pro analogues in HIV-BRU MT-4 human is indicated. The results concerning the D-Phe-Pro derivatives are in progress. The partition coefficients of the compounds were also calculated.

Table 1: Anti HIV Activity.

Compound	Log P[a]	IC_{50} µM[b]	CC_{50} µM[c]
6	3.15 ± 0.51	50	100
8	3.97 ± 0.58	10	100
14	3.44 ± 0.63	10	>100
15	2.31 ± 0.62	0.1-1	>100
16	3.17 ± 0.61	inactive	50
17	4.86 ± 0.67	inactive	>100
18	3.33 ± 0.61	inactive	50
19	5.38 ± 0.57	inactive	50
20	3.97 ± 0.58	1	50
Boc-Phe-Pro	3.96 ± 0.53	inactive	>100

[a] Log P determination were performed using ACD (Advanced Chemistry Development, Inc.)/LogP 1.0 base calculations.
[b] IC_{50}= concentration required to inhibit *syncitia* formation by 50% on MT-4 cells.
[c] CC_{50}= concentration required to cause 50% death of uninfected MT-4 cells.

Some of these analogues, in particular compound **15** showed a good anti-HIV activity (IC_{50} 0.1-1µM). It is obvious that the substitution of the pyrrolidine ring of proline by a 3-pyrrolidinone in the Phe-Pro sequence, is largely responsible for the observed inhibition effect. The hydroxy analogue **6** of the ketone **8** showed a five-fold decrease of anti-HIV activity. Concurrently, the substitution of the amino Phe terminal group was studied. Biological activity data revealed that anti-HIV activity depends on the chemical structure of Phe N-substituents. Indeed, nicotinyl group (Log P 2.31) gave an increased anti-HIV activity while more lipophilic substituent like octylcarbamate (Log P 5.38) led to an inactive compound. At this stage, the study demonstrated that moderately potent inhibitors of HIV-1 protease incorporating pyrrolidinone moiety could be obtained. These preliminary results were encouraging since some of these inhibitors displayed an anti-HIV activity *on in vitro* infected

cells. Their inhibitory effect on HIV purified recombinant protease is currently under investigation. However, they are examples of compounds being poorly effective in purified enzyme but which elicited high antiviral activity in cell culture experiments.[10] Consequently, these new types of Phe-Pro analogues are new lead compounds which could be optimized *via* systematic variations of the N-terminal Phe substituent. These derivatives, which bear a ketone group, are chemically different from pyrrolidinone inhibitors of HIV protease recently described by Smith et al.[11]. We hope to optimize these new anti-HIV inhibitors by structural modifications of Phe-pyrrolidinone.

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References and Notes

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