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C_2-Symmetric Phosphinic Acid Inhibitors of HIV Protease

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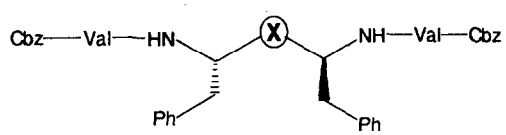
Abstract: The synthesis of the previously not accessible symmetrical bis(α-aminoalkyl)phosphinic acids is demonstrated. These compounds are valuable central building blocks in the synthesis of HIV protease inhibitors.

Among the numerous intervention points that can be employed in the development of drugs for AIDS therapy, the virally encoded human immunodeficiency virus (HIV) protease has emerged as one of the most popular targets[1,2]. HIV protease, an aspartic proteinase, is responsible for the posttranslational processing of HIV polyprotein products, especially the gag and gag-pol polyproteins[2]. Blockade of these processing steps leads to viral particles which are morphologically immature and noninfectious[3]. HIV protease functions as a C_2-symmetric homodimer[4]. Recently this led to the synthesis of C_2-symmetric HIV protease inhibitors like 1a and 1b that match the symmetry of the enzyme[5-7]. The basic structure of these inhibitors is shown in Figure 1.



1a X= CH(OH)
1b X= CH(OH)-CH(OH)
2a X= P(O)OH

Figure 1: C_2-symmetric inhibitors of HIV protease

X stands for a symmetric mimetic of the carbonyl group in the transition state of hydrolysis, while the hydrophobic periphery reflects the primary specificity of HIV protease for hydrophobic side chains. Because of the similarity of the tetrahedral phosphorous moiety and the tetrahedral intermediate formed on the path to peptide hydrolysis, phosphorous analogs of peptides have been shown to be potent transition state analogs of peptidases[8]. Grobelny et al.[9] were able to show that phosphinic acid isosteres of hexapeptides are powerful inhibitors of HIV protease. However, the application of the C_2-symmetry principle using phosphinic acid analogs like 2a, has not been possible yet due to the lack of the central symmetric building blocks, bis(α-aminoalkyl)phosphinic acids. In this work we present a synthetic route to

this type of compounds, their incorporation into peptidic inhibitors and the substantial activity against HIV protease of the latter.

While bis(aminomethyl)phosphinic acid 3 has already been obtained by Maier in 1979[10], the synthesis of bis(α-amino-arylmethyl))phosphinic acids has only recently been published by Tyka et al.[11]. They reacted α-amino-arylmethylphosphinic acid with with arylidenebisamides (for a reaction scheme see ref. 11). However, only moderate yields were obtained and, moreover, alkylidenebisamides do not react which is a serious limitation of the method. From the various routes that have been used to obtain α-aminophosphonic acids[12], the alkylation of nucleophilic precursors such as Schiff bases[12,13] appeared to be the method of choice in the synthesis of symmetrically substituted bis(α-aminoalkyl)phosphinic acids. As an example, the synthesis of bis(α-amino-2-phenylethyl)phosphinic acid is outlined in Figure 2. It starts from the parent compound, bis(aminomethyl)phosphinic acid 3.

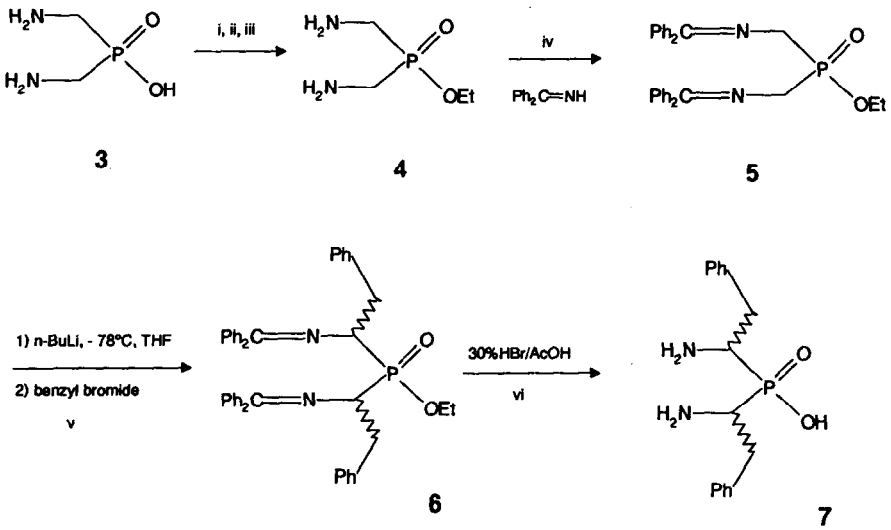
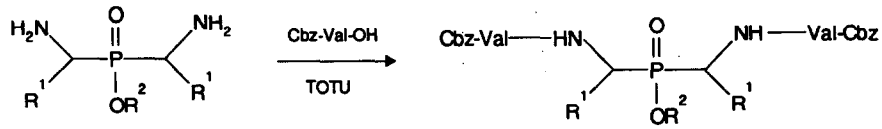


Figure 2: Synthesis of bis(α-amino-2-phenylethyl)phosphinic acid. i) Boc_2O, NaOH; ii) EtOH,DCC; iii) HCl,MeOH; iv) CH_2Cl_2, 24h, 53%; v) 24h at -78°C, 95%; vi) 7: R=H, 30% HBr/AcOH, 95%.- 8: R=Et, 10% aq. HCl,

3 is esterified in a three step procedure by protection of the amino groups with the t-butoxycarbonyl group, esterification with DCC/ethanol in THF, and subsequent deblocking of the amino groups in HCl/ methanol. The hydrochloride of the resulting ester 4 is treated with the imine of benzophenone in CH_2Cl_2 for 24 hours and the crystalline bis(diphenylmethylene-α-aminomethyl)phosphinic acid ethylester 5 was obtained in 53% yield after purification on silica. Alkylation of 5 was achieved by treatment with 2.2 equivalents of n-BuLi at -78°C followed by addition of an excess of benzyl bromide resulting in a mixture of three stereoisomers 6. The reaction should be held at -78 °C for at least 24 hours. If the temperature is raised too

early, the monoalkylated product predominates. Bis(α-amino-2-phenylethyl)phosphinic acid 7 is liberated from 6 by treatment with 30% HBr in AcOH. Treatment of 6 with 10% aq. HCl selectively cleaves the imino groups and yields the corresponding ester 8.



7,8,9 2,10,11

Figure 3: Synthesis of C_2-symmetric phosphinic acid HIV protease inhibitors. 7, 2: R^1=CH_2Ph, R^2=H; 8, 10: R^1=$PhCH_2$, R^2=Et; 9, 11: R^1=H, R^2=H;

Coupling of the central building blocks with Cbz-valine (Figure 3) is carried out by standard peptide chemistry using TOTU[14] as coupling reagent. TOTU offers the advantage that coupling can be carried out even in the presence of water, since 9 dissolves only in aqueous DMF. Coupling yields are about 75%.

Inhibition of HIV protease was determined at pH 5.5 using a synthetic substrate[15]. Preliminary results show that the mixture of stereoisomers 2 has an IC_{50}[16] value of 36 nM. We expect that the isomer 2a with the correct stereochemistry at the α-carbon atoms (R,R) will be even more powerful. On the other hand the ester 10 and the derivative 11 which lacks the benzyl groups exhibit IC_{50} values > 10 μM, showing that the free phosphonic acid as well as the hydrophobic side chains seem to be requirements for optimal inhibition.

In this paper we demonstrated the synthesis of the previously not accessible bis(α-aminoalkyl)phosphinic acids and their use as central building blocks in HIV protease inhibitors with substantial activity. The stereocontrolled synthesis of these compounds, as well as the determination of the pH dependence of their inhibitory activity are under way.

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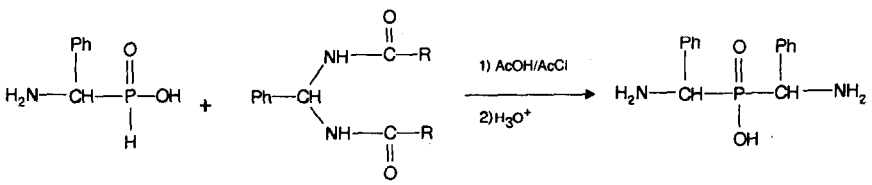

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16) IC_{50}: inhibitor concentration reducing the enzyme activity to 50%.

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