Bioorganic & Medicinal Chemistry Letters, Vol.2, No.9, pp. 1047-1052, 1992
Printed in Great Britain

0960-894X/92 $5.00 + .00

SYNTHESIS OF STEREOCHEMICALLY DEFINED PHOSPHONAMIDATE-CONTAINING PEPTIDES: INHIBITORS FOR THE HIV-1 PROTEINASE

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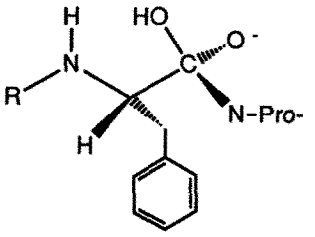
(*Received* 25 *May* 1992)

Abstract -Phosphonamidate-containing peptidic substrate analogues of the HIV-1 *gag-pol* proteinase-reverse transcriptase junction {-Phe-ψ[PO_2-N]-(*S*)-Pro- and -Phe-ψ[P(OMe)O-N]-(*S*)-Pro-}, mimicks for the transition states for proteolysis, have been synthesised. The absolute stereochemistry at C-1 of the phosphonophenylalanine residue was determined by X-ray crystallography. Boc-(S)-Asn-Phe-ψ[PO_2-N]-(*S*)-Pro-(S)-Ile-NH-*i*-Bu and Boc-(S)-Asn-(R)-Phe-ψ[P(OMe)O-N]-(*S*)-Pro-(S)-Ile-NH-*i*-Bu inhibit the HIV-1 proteinase.

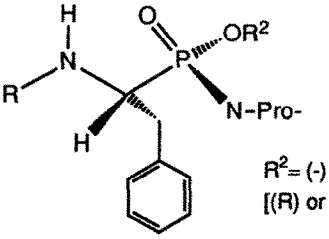
In a continuing search for therapies for AIDS the HIV-1 proteinase, a member of the aspartic proteinase family, has been identified as a promising target. Proteinase activity is vital for maturation of the virus and several approaches have been taken to producing inhibitors, based upon substituting the scissile bond with non-cleavable analogues.[1] The recent report of Janda and coworkers[2] on epimeric phosphonamidate containing dipeptides as inhibitors for the HIV-1 proteinase has prompted us to disclose our progress in this area.

The phosphonamidate moiety was employed as the non-cleavable part of the inhibitor due to its very close electronic and geometric resemblance to the tetrahedral transition state/high energy stable intermediate for peptide bond cleavage, Figure 1.

It possesses all the hydrogen bonding capacity of the transition state, the correct overall charge and the correct geometry, shape and size. Therefore, in addition to potential as inhibitors, phosphonoamidate analogues of natural substrates, for example compound (**1**), are suited to probing the mechanism of the proteinase. Such compounds also uniquely provide the opportunity to examine protein-substrate interactions in detail.



Tetrahedral intermediate

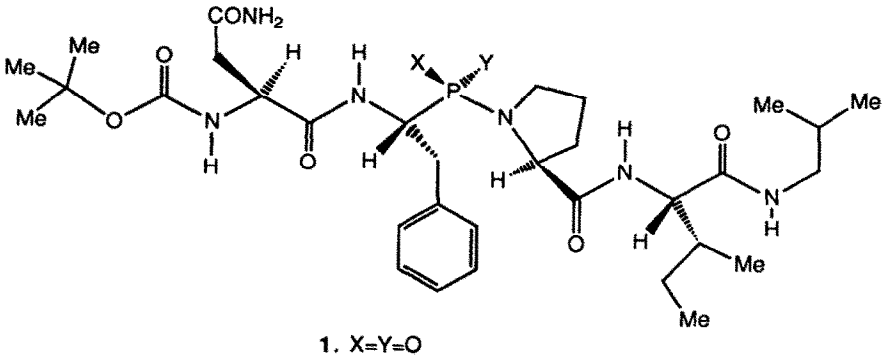


R^2= (-) or, Me
[(R) or (S) at P]

Phosphonamidate

Figure 1

The structures of the first targets, compounds {**1**, R = (-) and **2**, R = Me, (R) or (S) at phosphorus} were derived from the sequence of the proteinase-reverse transcriptase junction in the *gag-pol* polyprotein (Leu-Asn-Phe-Pro-Ile-Ser).[3] This sequence has been used in the design of several successful inhibitors.[4] The incorporation of the unique Phe-Pro scissile bond site into the design was expected to confer selectivity for the inhibition of the HIV-1 proteinase over the mammalian aspartic proteinases.[5]

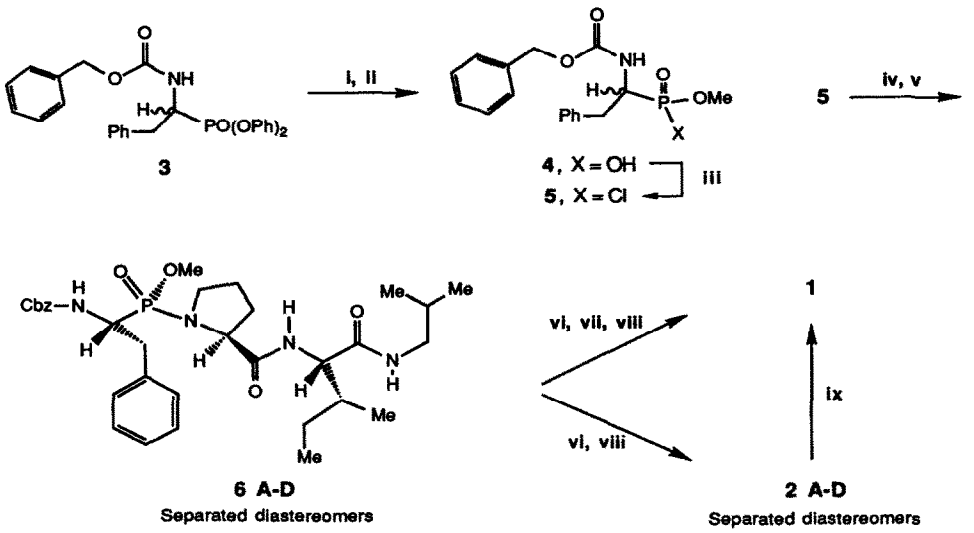


1. X=Y=O

2. (**R**) is X=OMe, Y=O; (**S**) is X=O, Y= OMe

Compounds (**1**) and (**2**) were synthesized from the diphenyl phosphonate (**3**) as outlined in Scheme 1. The diphenyl phosphonate was prepared in 31% yield through the condensation of phenylacetaldehyde, benzyl carbamate and triphenyl phosphite according to the method of Olekszyn and coworkers.[6]

Transesterification of (**3**) in methanol gave the dimethyl phosphonate which yielded the methyl phosphonic acid (**4**) upon treatment with sodium hydroxide in methanol. Conversion to the acid chloride was achieved using thionyl chloride and treatment of this chlorophosphonate (**5**) with a range of amines gave the corresponding phosphonamidates in moderate to good yield.



Scheme 1

i) NaOMe, MeOH, RT, 12 h., 72%; ii) NaOH, MeOH, RT, o/n, H^+ work-up, 63%; iii)$SOCl_2$, CH_2Cl_2, RT, 4 h., then; iv) H_2N-Pro-Ile-NH-*i*-Bu. HCl, NEt_3, CH_2Cl_2, RT, 24 h., 52% over both steps; v) Chromatographic resolution on silica, EtOH:CH_2Cl_2 5:95; vi) Pd/C, H_2, MeOH, RT, 3h., 75 %; vii) TMSBr, CH_2Cl_2, RT, 24 h., then H_2O, 88%; viii) Boc-Asn, iBuOCOCl, NMM, THF, -15°C, 15 min., 84%; ix) 4 eq. LiOH, dioxane, RT, 24 h., 54%.

Reaction of the chlorophosphonate (**5**) with pre-formed H_2N-(S)-Pro-(S)-Ile-NH-*i*-Bu in dichloromethane in the presence of triethylamine gave all four stereoisomers of the phophonamidate (**6**) in 52% yield. Each stereoisomer was separated by column chromatography on silica and the isomers were designated isomers A-D in order of the elution. Each diastereomer displayed a well resolved ^{31}P-coupled doublet due to the methyl phosphonate ester group in ^1H-NMR spectra and the C-2 proline proton resonated at δ 4.11, 4.10, 4.27 and 4.32 ppm for diastereomers A-D respectively. The absolute stereochemistry at C-1 and at phosphorus in the phosphonophenylalanine residue of isomer D (Figure 2)[7] was determined by X-ray crystallography and the absolute configurations of the other isomers were assigned on the basis of chemical degradations and ^1H-, ^{13}C- and ^{31}P-NMR spectroscopy.[8]

Compound (**6**) either in resolved form, or as a mixture of isomers was hydrogenolysed to deblock the amino terminal. Reaction with N-Boc-(2S)-asparagine using standard peptide coupling techniques gave the methyl protected phosphonamidates (**2, A-D**) in 84% yield. Each diastereomer of compound (**2**) was tested as an inhibitor of the HIV-1 proteinase, Table 1.

The deprotected phosphonamidate (**1**) was prepared in moderate yield upon the treatment of diastereomers of compound (**2**) with lithium hydroxide or lithium propylthiolate. The reactions did not proceed smoothly and the lithium salts were difficult to purify. Thus compound (**1**) was prepared *via* an alternative route in which the benzyloxycarbonyl and phosphonamidate methyl ester protecting groups were sequentially removed through catalytic hydrogenolysis followed by treatment of the resulting free amine with trimethylsilyl bromide, prior to reaction with Boc-(S)-asparagine, Scheme 1. When compounds (**6 A** and **B**) were treated in this manner a mixture of two diastereomers (**1 A** and **B**) differing in configuration at C-1 of the phosphonophenylalanine residue were obtained. These were also tested as inhibitors for the proteinase, see Table 1 below. All spectroscopic and analytical data for the compounds and for all of the synthetic intermediates were consistent with the assigned structures. Full details will be reported elsewhere.[10]

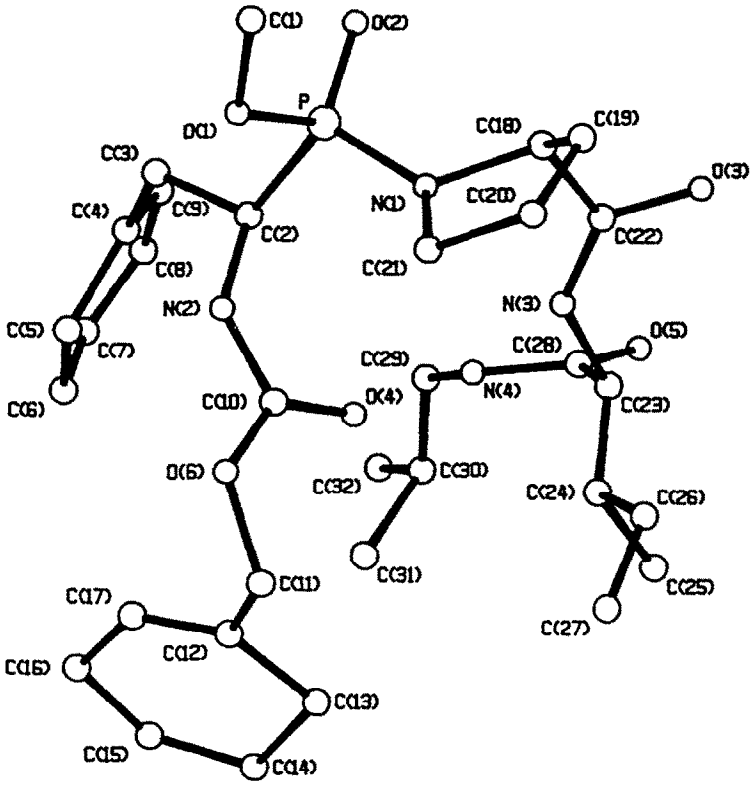


Figure 2: Crystal structure of stereoisomer 6D.

The inhibitory properties of the transition state analogues {**1**, (RS) at C-1, and **2A-D**} against the HIV-1 proteinase were tested at pH 5.6 and at 37°C using the substrate Lys-Ala-Arg-Val-Nle-Nph-Glu-Ala-Nle-Gly-NH_2, Table 1. In each case the potential inhibitor was preincubated with the enzyme for a period of 15 minutes prior to the addition of substrate and the assessment of enzyme activity. Note that assays in which the inhibitor was added last showed higher enzyme activities and, therefore, higher IC_{50} values to those shown in Table 1.

Table 1

	Compound	IC_{50} (μM)
2A	Boc-(S)-Asn-(**R**)-Phe-ψ[(**S**)-P(OMe)O-N]-(*S*)-Pro-(S)-Ile-NH-*i*-Bu	80
2B	Boc-(S)-Asn-(**S**)-Phe-ψ[(**S**)-P(OMe)O-N]-(*S*)-Pro-(S)-Ile-NH-*i*-Bu	98
2C	Boc-(S)-Asn-(**S**)-Phe-ψ[(**R**)-P(OMe)O-N]-(*S*)-Pro-(S)-Ile-NH-*i*-Bu	400
2D	Boc-(S)-Asn-(**R**)-Phe-ψ[(**R**)-P(OMe)O-N]-(*S*)-Pro-(S)-Ile-NH-*i*-Bu	30
1	Boc-(S)-Asn-(**RS**)-Phe-ψ[PO_2-N]-(*S*)-Pro-(S)-Ile-NH-*i*-Bu	93

The results indicate that phosphonamidate **2D** binds to the proteinase most effectively. This isomer possesses (R)-absolute stereochemistry at C^{α} of the phosphonophenylalanine residue, the same *relative* configuration as the (2S)-phenylalanine residue in the natural substrate. The fact that isomer **2D** inhibits more efficiently that any of the other isomers, in particular, isomer **2A** indicates that the preferred configuration at phosphorus is (R). At pH 5.6 the IC_{50} for compound **1** was much higher than expected, possibly due to repulsive interactions between the negatively charged phosphonamidate and the active site carboxylate.[11] Structural studies to define the mode of inhibitor binding and kinetic work on the cause of time dependent inhibition is in progress.

Acknowledgements: We thank the SERC for a studentship to P.C.D.H., the University of St. Andrews for a studentship to N.P.C., Dr. I. Sadler, University of Edinburgh, for 600 MHz ^1H- and 150 MHz ^{13}C- NMR spectra, the MRC AIDS Directed Programme for samples of the HIV-1 proteinase and substrate and the British Technology Group.

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7. Crystallographic data have been deposited at the Cambridge Data Centre. The crystals of 6D are orthorhombic, $P2_12_12_1$ (No. 19) a = 9.265 (5), b = 15.654 (10), c = 23.929 (11) Å; V =3470 Å^3 ; Z = 4; d_c = 1.18 g.cm^{-3}. Crystal size was 0.25 x 0.15 x 0.02 mm. Total unique reflections = 3462, R = 0.118 for 738 reflections with $|F^2| > 2\sigma(F^2)$.

8. Data for stereoisomer 6D: δ_H (600 MHz, $CDCl_3$) 0.9 (12H, m, CH_3's 31,32,27,25), 1.14 & 1.50 (2H, m, CH_2 (26)), 1.76 (3H, m, CH_2 (20) and CH (30)), 1.92 (1H, m, 1 of CH_2 (21)), 2.05 (2H, m, 1 of CH_2 (21) and CH (24)), 2.80 (1H, m, 1 of CH_2 (3)), 2.98 (1H, m, 1 of CH_2 (29)), 3.07 (2H, m, 1 of CH_2 (29) and 1 of CH_2 (19)), 3.18 (1H, m, 1 of CH_2 (19)), 3.28 (1H, m, 1 of CH_2 (3)), 3.60 (3H, d, $J_{P,H}$ 10.7 Hz, $POOCH_3$), 4.18 (1H, AB q, J 7.66 Hz, CH (23)), 4.32 (1H, m, CH (18)), 4.45 (1H, m, CH (2)), 4.97 (2H, AB q, J 12.3 Hz, CH_2 (1)), 5.08 (1H, d, J 9.88 Hz, NH (2)), 6.61 (1H, t, J 5.85 Hz, NH (4)), 7.25 (10H, m, aromatic CH), 7.45 (1H, d, J 8.77 Hz, NH (3)); δ_C (149.52 MHz, $CDCl_3$) 11.02 ($\underline{C}H_3$ (27)), 15.51 ($\underline{C}H_3$ (25), 19.98 ($\underline{C}H_3$ (31,32), 24.67 ($\underline{C}H_2$(26)), 25.80 & 25.87 ($\underline{C}H_2$ (20)), 28.18 ($\underline{C}H$ (30)), 31.12 & 31.20 (CH_2 (21)), 35.61 ($\underline{C}H_2$ (3)), 35.88 ($\underline{C}H$ (24)), 46.73 ($\underline{C}H_2$ (29)), 47.55 ($\underline{C}H_2$ (19)), 49.46 & 50.40 ($J_{P,C}$ 146.5 Hz, $\underline{C}H$ (2)), 51.51 & 51.60 ($J_{P,C}$ 7.4 Hz, $\underline{C}H_3$ (1)), 58.22 (α $\underline{C}H$ (23)), 62.23 (α $\underline{C}H$ (18)), 66.89 ($\underline{C}H_2$ (3)), 126.64-128.94 (aromatic $\underline{C}H$'s), 136.04 & 136.30 (quaternary aromatic), 155.77 ($\underline{C}O$ (10)), 170.91 ($\underline{C}O$ (28)), 173.31 ($\underline{C}O$ (22)); δ_P (121.5 MHz, $CDCl_3$) 28.90; Acc. m/z (FAB) 615.3311; $C_{32}H_{47}N_4O_6P$ requires 615.33115.

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