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Synthesis of Peptide Analogues Containing Phosphonamidate Methyl Ester Functionality: HIV-1 Proteinase Inhibitors Possessing Unique Cell Uptake Properties

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Abstract—Stereochemically defined and epimeric phosphonamidate methyl ester-containing peptide analogues were synthesised and were found to be moderate inhibitors of the HIV-1 proteinase. All of the analogues containing the phosphonamidate ester grouping showed a marked ability to enter cells, as highlighted by the approximate equivalence of the IC_{50} values for enzyme inhibition in solution and inhibition of HIV-1 replication in virus infected cells.

Introduction

The molecular organisation of the HIV genome is known to consist of *gag*, *pol* and *env* genes which are necessary for viral replication.[1] During viral replication these genes are expressed as polyproteins which undergo enzymic cleavage to generate the functional proteins of the mature virus. Genetic and biochemical studies have demonstrated that a virally coded proteinase is responsible for the release of the proteinase, reverse transcriptase, integrase, and other proteins from the *gag–pol* fusion proteins.[2] Importantly, the HIV-1 proteinase has been proven to be essential for viral maturation since site-directed mutagenesis of the proteinase active site aspartate residues (Asp25 and Asp25') leads to the formation of non-infectious virions.[3]

Due to its critical role in viral maturation, the HIV-1 proteinase has become a prevalent target in anti-AIDS therapy and the design and synthesis of proteinase inhibitors has escalated. As a consequence, many different types of inhibitor, mainly based upon the replacement of the carboxamide moiety of the substrate by non-scissile, tetrahedral 'transition state' analogues have been tested, both in enzyme assays and in virally infected cells, and the structural requirements for effective inhibitors have begun to emerge. The results of such studies are summarised and rationalised in several useful reviews.[4–7]

While many excellent inhibitors of the proteinase have been prepared, uptake of these inhibitors into virally infected cells and inhibition of the proteinase within these cells is inefficient. Indeed, all of the inhibitors, for which data are available, are less effective in virally infected cells than in standard enzyme assays. Here we report on the synthesis and biological properties of peptidic phosphonamidate esters. These compounds are moderate to good inhibitors of the proteinase when tested in enzyme assays (IC_{50} values 1–100 μM) and, remarkably, show comparable potency in inhibiting HIV-1 replication in cells.

In a preliminary report we described the design and synthesis of a series of HIV-1 proteinase inhibitors in which the scissile amide bond within the natural substrate was replaced by a phosphonamidate or a phosphonamidate methyl ester moiety.[8]

Structurally, these moieties are close mimics of the tetrahedral transition state and/or high energy stable intermediate for peptide bond cleavage (Fig. 1). The initial synthetic targets, compounds **1** and **2**, were derived from the sequence of the proteinase-reverse transcriptase junction in the *gag–pol* polyprotein (-Leu-Asn-Phe-Pro-Ile-Ser-).[9] Because mammalian aspartic proteinases do not cleave Phe-Pro peptide bonds, the presence of the proline residue in inhibitors was expected to confer selectivity for the inhibition of the HIV-1 proteinase.[10]

The compounds were found to be moderate inhibitors of the HIV-1 proteinase. For example, phosphonamidate (**1**), when tested as a mixture of epimers at the phosphophenylalanine residue gave an IC_{50} value of 90 μM. This high value was attributed to the fact that the phosphonamidate exists in its anionic form at pH 5.5, the optimum pH for the enzyme. Studies of related phosphinic acid analogues showed that the inhibitory proficiency of the compounds increased with decreasing pH, from which it was concluded that the proteinase has a preference for the uncharged form of the phosphonic acid.[11] Similar pH-dependencies have been observed for other aspartic proteinases and it is believed that these effects result from repulsive interactions that develop between the active site Asp

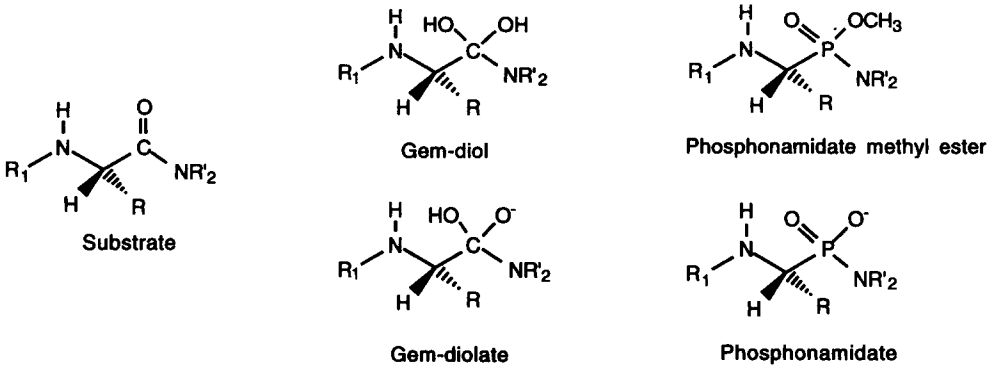
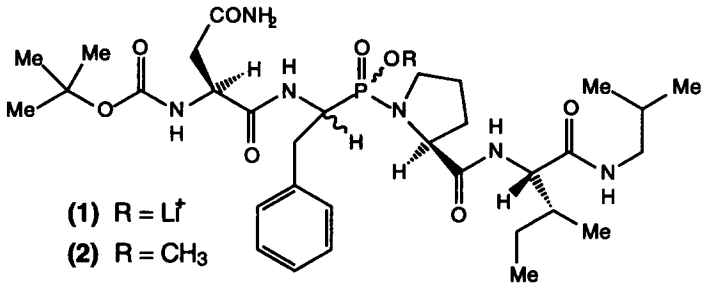


Figure 1.



(Boc-(2*S*)-Asn-Phe-ψ[P(OR)O-N]-(2*S*)-Pro-(2*S*)-Ile-NH-iBu.)

carboxylate and the negatively charged phosphonamidate as it ionises with increasing pH.[12] The most active diastereomer of the phosphonamidate methyl ester analogue (**2**) possessed (*R*)-absolute stereochemistry at the phosphophenylalanine residue [the same relative configuration as the (2*S*)-phenylalanine residue in the natural substrate], (*R*)-absolute stereochemistry at phosphorus and displayed an IC_{50} value of 30 μM as determined in enzyme assays. Remarkably, the compound showed a similar potency (IC_{50} = 45 μM) when tested as an inhibitor of viral replication in infected C8166 T-lymphoblastoid $CD4^+$ cells. Since the phosphonamidate methyl esters did inhibit the proteinase and appeared to be able to enter cells rather efficiently and would not suffer from the problem of ionisation, a systematic alteration of the binding residues flanking the central phosphonamidate methyl ester moiety was undertaken.

Synthesis

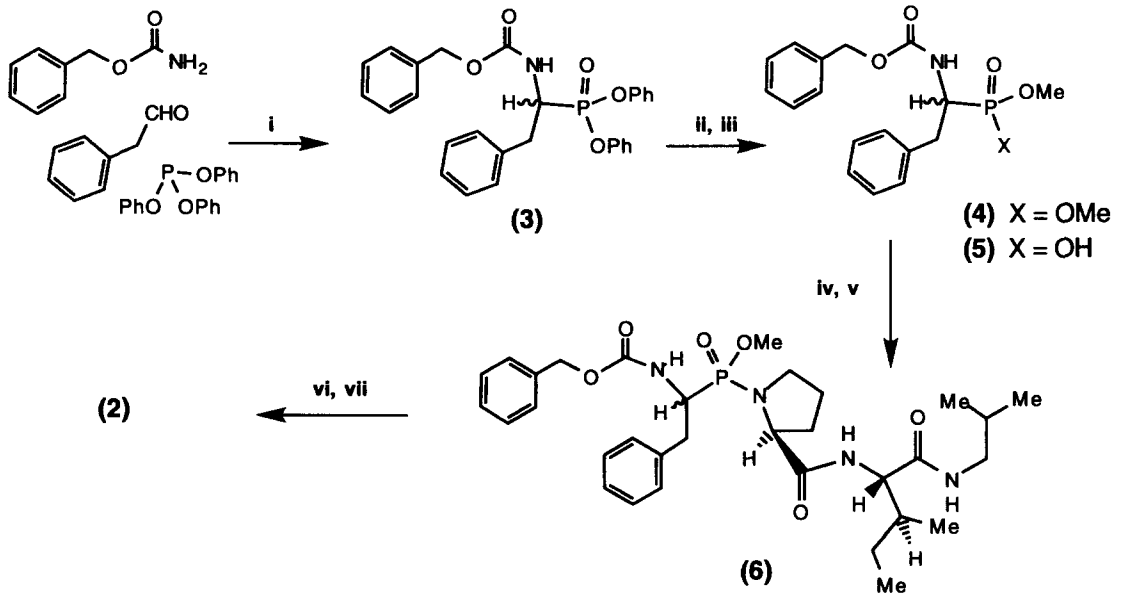
Peptidic phosphonamidate methyl esters were synthesised by reacting a range of activated aminophosphonic acid chloride half esters with various peptides, as outlined in Scheme 1.

The precursor diphenyl phosphonate (**3**) was prepared through the condensation of phenylacetaldehyde, benzyl carbamate and triphenyl phosphite in the presence of glacial acetic acid according to the method of Oleksyszyn and coworkers.[13] For the diphenylphosphonate analogue (**3**) the yield was 29%. Transesterification of the diphenyl phosphonate using sodium methoxide provided the corresponding dimethyl phosphonate (**4**) which, upon saponification, gave the methyl phosphonic acid derivative (**5**) in 63% yield.

The phosphonic acid half esters [e.g. **5**] were converted to the acid chlorides using thionyl chloride according to the method of Bartlett.[14] Treatment of the chlorophosphonate derivative with a range of N-deblocked peptides gave the phosphonamidate methyl ester analogues [e.g. **6**] in moderate to good yield (40–80%).

Replacement of phenylacetaldehyde by cyclohexylacetaldehyde in the three component coupling reaction yielded the phosphocyclohexylalanine analogue (**7**) and provided a convenient method for alteration of the binding residues at the P_1 site in the inhibitors. The dipeptide amide (**8**) was synthesised using standard peptide coupling techniques[15] and provided the $P_{1'}$ to $P_{3'}$ residues of the inhibitor. The alteration of these residues was straightforward and peptides incorporating (2*S*)-phenylalanine, (2*R*)-phenylalanine and glycine at $P_{1'}$ were synthesised. A further peptide, incorporating (2*S*)-pipecolinic acid in place of (2*S*)-proline in compound **8** was also synthesised. However, its reaction with the chlorophosphonate derived from **5** was extremely slow, presumably due to steric hindrance, and this line was not pursued further.

The effect of altering the structure of the inhibitors in the P_2 and P_3 sites was also investigated by replacing



(i) AcOH, 80 °C; (ii) 2.2 eq. NaOMe, MeOH, 20 °C; (iii) 2.5 eq. NaOH, MeOH, 20 °C; (iv) $SOCl_2$, CH_2Cl_2, 20 °C then; (v) HN-Pro-Ile-NH-*i*Bu.HCl, Net_3, CH_2Cl_2, 20 °C; (vi) Pd/C, H_2, MeOH, 20 °C; (vii) Boc-Asn, *i*BuOCOCl, NMM, THF, -15 °C.

Scheme 1.

the N-terminal protecting groups by other functionalities. All of the compounds gave the expected spectral and analytical data.

The absolute configurations of the four diastereomers of compound **2**, were assigned through an X-ray crystallographic structure determination[8] of the synthetic intermediate (**6**), by chemical degradation to known aminophosphonic acids and by a ^1H, ^{13}C and ^{31}P NMR spectroscopic comparison of the separated stereoisomers.

Biological Activity

Selected phosphonamidate methyl ester analogues were tested against the HIV-1 proteinase in enzyme assays and in cells infected with HIV-1. Enzyme activity was determined using the method of Griffiths *et al.*,[16] employing the substrate (Lys-Ala-Arg-Val-Nle-Phe(NO_2)-Glu-Ala-Nle-Gly-NH_2) and was assessed by measuring the decrease in absorbance at 300 nm caused by cleavage of the Nle-Phe(NO_2) amide bond. In control experiments, a K_m value of 33 μM[17] was obtained which compares well with the literature value of 35 μM.[16] For inhibitors, IC_{50} values were determined from duplicate Dixon plots[18] constructed from data for at least four different concentrations of the inhibitor.

In vivo inhibition of viral proliferation was measured in T-lymphoblastoid cells as detailed in the Experimental section.

Results and Discussion

All four diastereomers of the phosphonamidate methyl ester (**2**) were tested individually, both in enzyme assays and in virally infected cells (Table 1, entries 2–5). The results are striking in that they reveal a very close correlation between the inhibitory proficiency of the compounds in solution and in cells. While the differences in the potencies of the phosphonamidate (**1**) and the methyl esters appears to be small, in accord with the findings of McLeod *et al.*,[19] it is evident that there is a preference for (*R*)-absolute stereochemistry at C^α and for (*R*)-absolute stereochemistry at phosphorus in the phosphophenylalanine residue. The (*R*)-configuration at carbon is expected due to its equivalence to the (*S*)-configuration in the natural substrate. However, the preference for the (*R*)-configuration at phosphorus is difficult to interpret, particularly because the methyl group on the phosphonamidate would not be expected to interact favourably with the active site Asp residues. The relative potencies of each of the diastereomers was disappointing in the light of results obtained by Cushman *et al.*[20] which showed a strict preference for the (2*S*)-Phe epimer at P_1.

The individual diastereomers of the synthetic intermediate **6** were also tested (Table 1, entries 6–9). In this shorter compound, the P_3 *t*-butyloxycarbonyl (Boc) group of compound **2** is missing and the P_2 asparagine residue has been replaced by a benzyloxycarbonyl (Cbz) moiety. The results again highlight the preference for the (*R*)-configuration at both C^α and at phosphorus. Interestingly, these smaller inhibitors appear to be more potent than the diastereomers of compound **2**, despite lacking a P_3 residue. Most interestingly, the results for inhibition of viral replication in cells mirror, again, the results obtained for enzyme inhibition suggesting that the cells are completely accessible to the compounds.

Table 1. Summary of inhibition data

Entry number	Compound	Configuration at (C^{α},P)	IC_{50} (μM)*	
			HIV proteinase inhibition	Antiviral activity
1	Boc-N-F-PO_2-P-I-NH-iBu			
	1	(epimeric at C^{α})	90	N.D.
	Boc-N-F-[ψ]-P-I-NH-iBu			
2	2 i	(R, R)	30	45
3	2 ii	(R, S)	80	>100
4	2 iii	(S, S)	100	>100
5	2 iv	(S, R)	>100	>100
	Cbz-F-[ψ]-P-I-NH-iBu.			
6	6 i	(R, R)	5	3
7	6 ii	(R, S)	25	25
8	6 iii	(S, S)	N.D.	25
9	6 iv	(S, R)	N.D.	>100
10	Cbz-Cha-[ψ]-P-I-NH-iBu.			
	9	(mix. 2 diast.)	16	8
11	Cbz-F-[ψ]-(2S)-F-I-NH-iBu.			
	10	(mix. 4 diast.)	2	2
12	Cbz-Cha-[ψ]-(2S)-F-I-NH-iBu.			
	11	(mix. 4 diast.)	2	5
13	H_2N-F-[ψ]-(2S)-F-I-NH-iBu.			
	12	(mix. 4 diast.)	30	20
14	Boc-N-F-[ψ]-(2S)-F-I-NH-iBu.			
	13	(mix. 4 diast.)	100	>100
15	Qua-N-F-[ψ]-(2S)-F-I-NH-iBu.			
	15	(mix. 4 diast.)	45	50
16	Qua-N-Cha-[ψ]-P-I-NH-iBu.			
	17	(1 diast. unknown)	20	15
17	Cbz-F-[ψ]-(2R)-F-I-NH-iBu.			
	18	(mix. 4 diast.)	1	N.D.
18	Cbz-F-[ψ]-G-I-NH-iBu.			
	20	(mix. 4 diast.)	>30	N.D.
19	Cbz-F-[ψ]-(2S)-F-NH-iBu.			
	21	(mix. 4 diast.)	40	N.D.
20	Cbz-F-[ψ]-(2S)-F-(OMe).			
	22	(mix. 4 diast.)	100	100

*Values of IC_{50} are accurate to within ± 10%. Ro 31-8959 (**14**) gave an IC_{50} value of 0.9 nM under these conditions.
[ψ] = -$PO(OCH_3)$-.

The importance of the P_1 residue in compound **6** was investigated by replacing the phosphophenylalanine residue with a phosphocyclohexylalanine residue to give compound **9**. Compound **9** was resolved into two pairs of diastereomers. The inhibitory data for the most potent pair are given in Table 1, entry 10. These data show that Cha (cyclohexylalanine) can be used to replace the natural Phe residue in the P_1 position without a significant change in potency. Indeed, the IC_{50} value compares well with that expected for a mixture of the (*R*,*R*)- and (*R*,*S*)-diastereomers of compound **6**.

The most potent peptidic inhibitors of the HIV-1 proteinase contain either a phenylalanine residue or a phenylalanine surrogate in the $P_{1'}$ position. Thus, the (2*S*)-Pro residue at $P_{1'}$ in compound **6** was replaced with a (2*S*)-Phe residue to give phosphonamidate (**10**). Compound **10** could not be resolved into its individual diastereomers using flash silica chromatography and was, therefore, tested as a mixture (Table 1, entry 11). If the mixture contained equal amounts of all four diastereomers, in the absence of complicating effects, the most active diastereomer would be expected to possess an IC_{50} value of 500 nM. Thus, the Phe-Phe based inhibitor appears to be more potent than the most active (*R*,*R*)-diastereomer of the corresponding Phe-Pro analogue (compound **6i**). As expected from a comparison of the activities of compounds **6** and **9**, replacement of the Phe residue at P_1 in compound **10** by a Cha residue resulted in an equipotent inhibitor, compound **11**. Removal of the benzyloxycarbonyl group in compound **9** gave compound **12** which was 10-fold less potent showing that the Cbz group is compatible with the binding requirements of the S_2 pocket. Again, all of these compounds showed a remarkable correlation between enzyme inhibitory proficiency and ability to prevent HIV-1 replication in cells.

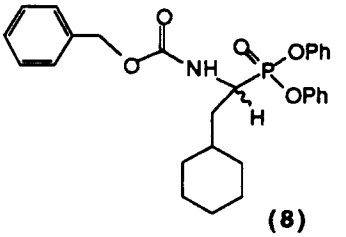
Compound **12** was extended with Boc-(2*S*)-asparagine, to give phosphonamidate methyl ester (**13**), which again showed a reduction in potency, compared to the shorter analogues **10**, **11** and **12**. Having previously observed low potency for inhibitors containing a P_3 Boc

group [e.g. compound **2**], the Boc group was replaced. Literature precedent indicated that a suitable alternative group for the P_3 position was the quinoline-2-carbonyl moiety (Qua) which had been used in the design of the highly potent Ro 31-8959 inhibitor (**14**).[21]

Accordingly, compound **15** was prepared from the aminophosphonopeptide (**12**) and Qua-(2*S*)-asparagine (**16**) using mixed anhydride chemistry. Qua-(2*S*)-asparagine (**16**) was synthesised in a simple one-pot reaction from quinaldic acid chloride, using asparagine under modified Schotten–Baumann conditions.[22]

In agreement with reported observations, introduction of the Qua residue into the P_3 position caused an increase in potency over compound **13**. However, compound **15** was 20-fold less potent than compound **10**, again highlighting the enzymes preference for the shorter analogue. Evidently, the requirements for an optimal P_3 residue in peptidic phosphonamidate methyl esters differ to those for the potent hydroxyl-containing inhibitors. One explanation could be that the differences originate from the fact that hydroxyl group-containing inhibitors can hydrogen-bond to the active site Asp residues whereas phosphonamidate methyl esters cannot. Occupation of the S_3 pocket of the enzyme can cause conformational effects that improve the key interactions with the active site Asp residues for hydroxyl group-containing inhibitors (which are more potent than those lacking such a P_3 residue) or cause adverse interactions in the case of phosphonamidates by pushing the methyl ester group closer to the catalytic Asp residues.

In a similar manner the Cbz group from compound **9** was removed and the N-terminal was extended with Qua-(2*S*)-asparagine (**16**). After chromatographic resolution a pair of diastereomers was obtained. The



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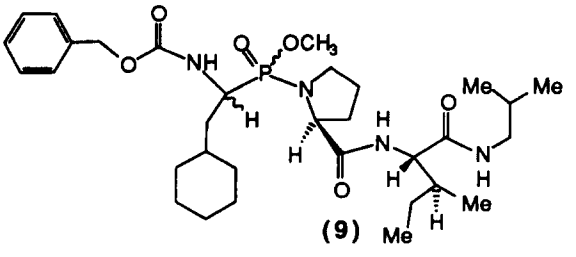

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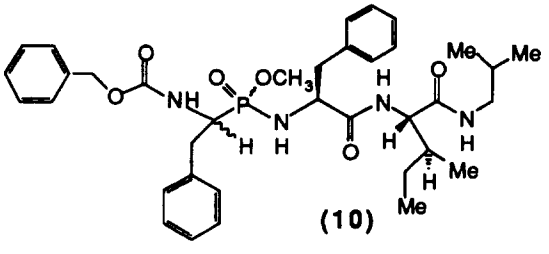

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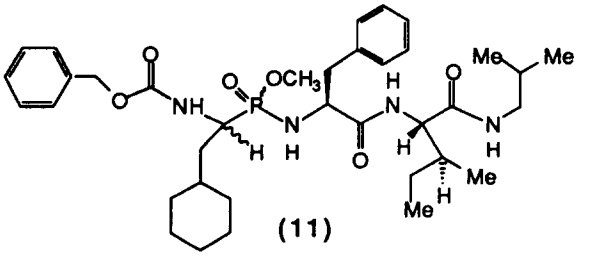

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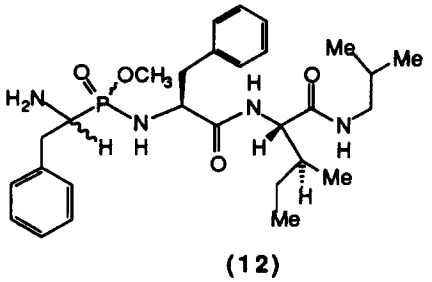

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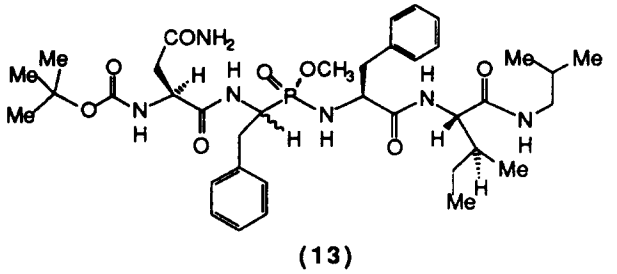

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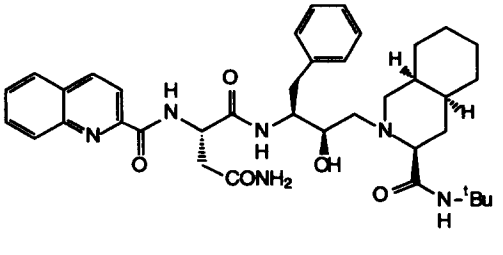

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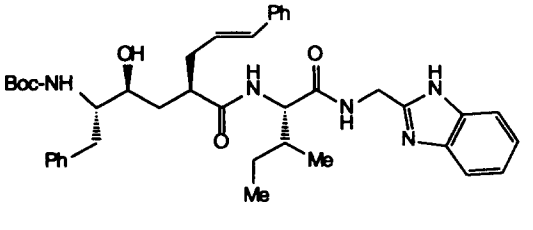

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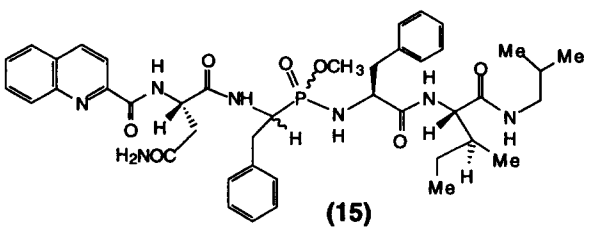

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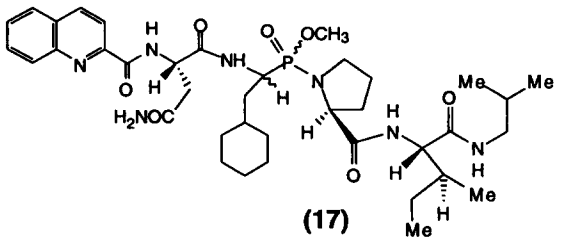

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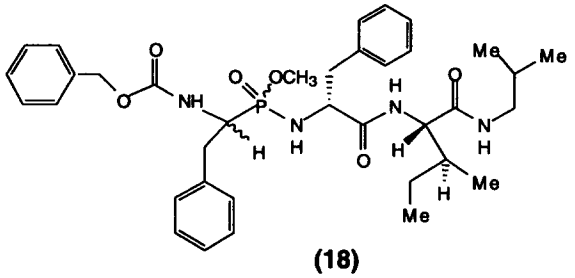

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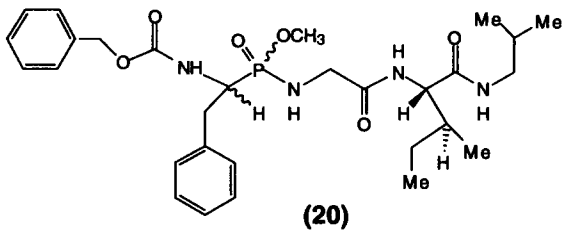

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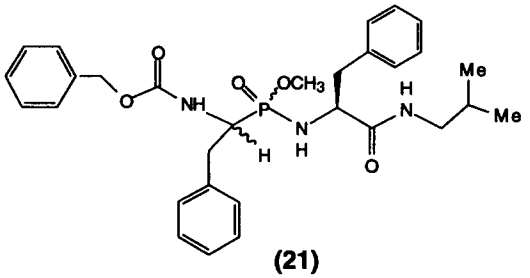

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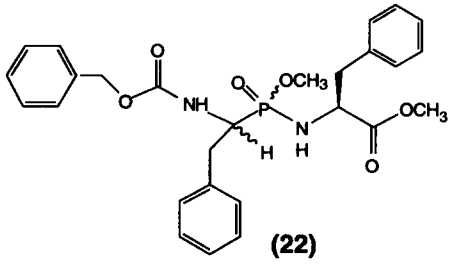

(22)

most potent diastereomer [compound **17**, absolute stereochemistry unknown] possessed an IC_{50} value of 20 μM. Again there was a close correlation with the compounds activity in virally infected cells (Table 1, entry 16).

Given that implemented alterations of the phosphonamidate methyl esters seemed to produce very small changes in potency, it was apparent that their development as useful inhibitors would be difficult. Nevertheless, the importance of the P_1· position was investigated in more depth using two approaches. In the first, the (2*S*)-Phe residue in compound **10** was replaced with the unnatural (2*R*)-Phe residue to give compound **18** which, surprisingly, was at least as potent as compound **10**. Since the S_1· pocket of the enzyme is able to accommodate groups larger than benzyl, for example, a styryl moiety in the potent hydroxyethylene inhibitor (**19**) described by Vacca *et al.*,[23] it may be that the benzyl side chain in compound **18** can achieve a conformation which allows significant hydrophobic interaction with the enzyme. In the second approach, the benzyl side chain was removed by replacing the (2*S*)-Phe residue in **10** with a Gly residue to give compound **20**. An accurate IC_{50} value for compound **20** could not be obtained due to the insolubility of the inhibitor at concentrations above 40 μM. However, the compound displayed at least a 30-fold drop in potency compared to **10**, highlighting the importance of the hydrophobic P_1· residue.

Finally, the effect of sequentially removing the P_3· and P_2· residues was examined. First, the Ile-NH-*i*Bu moiety which spans P_2· and P_3· was replaced by isobutylamine, to give compound **21**. This modification caused a 20-fold reduction in potency compared to compound **10** (Table 1, entries 11 and 19). Second, (2*S*)-Phe-Ile-NH-*i*Bu was replaced by (2*S*)-Phe-OMe to give compound **22** which displayed a 50-fold reduction in potency relative to compound **10**. Thus the P_3· and P_2· residues are important for binding in the phosphonamidate methyl ester inhibitors.

The most striking feature of the phosphonamidate inhibitors is the unique correlation of the *in vitro* IC_{50} values for the inhibition of viral replication in cells with the IC_{50} values obtained for inhibition of the enzyme in solution, see Table 1. For all of the phosphonamidate methyl esters, the ratio R (where $R = IC_{50(Enz)}/IC_{50(Virus)}$) was close to unity. Usually, peptidic inhibitors possess values of R much smaller than 1.0 due to their inability to enter cells and values can be as small as 10^{-4}. Evidently, peptidic phosphonamidate methyl esters possess a marked ability to enter cells and apparently can achieve intracellular concentrations that equal the extracellular concentration. Interestingly, the uptake properties appear to be totally independent of the size and composition of the inhibitor and the value of R is invariant for all of the compounds tested, even for those possessing high IC_{50} values. For other classes of inhibitor, R values are much smaller for large compounds than for smaller ones.

The only structural feature that differentiates the peptidic phosphonamidate methyl esters from other

reported inhibitors is the transition state isostere itself, since the residues chosen in the P_2–$P_{3'}$ positions are common to many other inhibitors.[4–7] Thus, it is tempting to suggest that the phosphonamidate methyl ester group is responsible for efficient uptake. It is interesting to note that the phosphinic acid and phosphinic acid ester inhibitors of Peyman *et al.*,[24] the closest structural analogues to the compounds described here, show low anti-HIV activity.

Summary

Although phosphonamidate-containing peptide analogues have proved to be highly potent inhibitors of the zinc proteinases, the same level of potency is not observed for the HIV-1 proteinase. This almost certainly stems from their negatively ionised nature at the pH optimum for the enzyme which results in repulsive interactions between the inhibitor and the negatively charged active site carboxylate group. Phosphonamidates, in general, are extremely acid labile and for this reason phosphonamidates offer no potential as therapeutic agents.

The problem of ionisation is not inherent in the phosphonamidate methyl ester analogues and consequently these neutral compounds have proved to be inhibitors of the HIV-1 proteinase with IC_{50} values in the μM range. However, optimisation of the methyl ester analogues through the alteration of the side chain binding residues caused only small effects in proteinase binding efficiency, in stark contrast to the effects observed for the more potent hydroxyethylene and hydroxyethylamine inhibitors. Nevertheless, the methyl phosphonamidate esters display unique cell uptake properties which may prove to be useful in the development of new therapeutic agents.

Experimental

NMR Spectra were recorded on a Bruker AM-300 FT spectrometer (^{1}H, 300 MHz; ^{13}C, 75 MHz; ^{31}P, 121.5 MHz) and a Varian Gemini FT spectrometer (^{1}H, 200 MHz; ^{13}C, 50 MHz). High field NMR spectra were obtained on a service basis at the University of Edinburgh (^{1}H, 600 MHz; ^{13}C, 150 MHz; ^{31}P, 243 MHz); ^{1}H NMR spectra were referenced on chloroform, methanol or DMSO, ^{13}C NMR spectra were referenced on chloroform, methanol or DMSO and ^{31}P spectra on external H_3PO_4. NMR Spectra are described in parts per million downfield shift from TMS and are reported consecutively as position (δ_H or δ_C), relative integral, multiplicity (*s*-singlet, *d*-doublet, *t*-triplet, *q*-quartet, *m*-multiplet, *dd*-doublet of doublets and *br*-broad), coupling constant ($J_{X,Y}$ Hz if applicable) and assignment. Infrared spectra were recorded using a Perkin Elmer 1420 ratio recording spectrometer and a Perkin Elmer 1710 FT-IR spectrometer. The samples were prepared as nujol mulls or thin films between sodium chloride discs. Absorption maxima are given in wavenumbers (cm^{-1}) relative to a polystyrene standard. Melting points were measured using electrothermal melting point apparatus and are uncorrected. Optical rotations were measured on an Optical Activity Ltd AA-100 polarimeter using 10 cm path length cells at room temperature. Mass spectra were recorded on a Kratos MS50 and obtained on a S.E.R.C. service basis at the University of Swansea using a VG ZAB E. Major fragments are given as percentages of the base peak intensity. Where appropriate, all solvents and reagents were freshly distilled prior to use. THF and ether were distilled from sodium/benzophenone under a nitrogen atmosphere; DMF and CH_2Cl_2 were distilled from CaH_2. Flash chromatography was performed according to the procedure of Still[25] using Sorbisil C60 (40–60 μm) silica gel. Analytical thin layer chromatography was carried out on 0.25 mm precoated silica gel plates (MN SIL G/UV_{254}) or on 0.1 mm precoated cellulose plates (CEL MN 300-10 /UV_{254}), and compounds were visualised by UV fluorescence, iodine vapour, ethanolic phosphomolybdic acid, aqueous potassium permanganate or ninhydrin.

Diphenyl [1-(N-benzyloxycarbonyl)-amino]-2-phenylethylphosphonate (3). Triphenyl phosphite (31.0 g, 0.1 mol), phenylacetaldehyde (18 g, 0.15 mol) and benzyl carbamate (15.1 g, 0.1 mol) were dissolved in 15 mL glacial acetic acid and stirred at room temperature until the exothermic reaction had ceased (about 1 h). The mixture was then heated to 80 °C for a further 1 h and the volatile products were then removed under reduced pressure on a boiling water bath. The yellow residue was dissolved in methanol (150 mL) and left to crystallise at −10 °C for 3 h. After this time the crystalline product was collected by filtration, dissolved in the minimum quantity of hot chloroform and a 4-fold excess of methanol added to give the product as a white solid after storage overnight at −10 °C (14.1 g, 29%) of mp 120–122 °C (lit. 119–120 °C); m/z (found: $[M + H]^+$ 487.1549. $C_{28}H_{26}NO_5P$ requires 487.1549). ν_{max} (nujol) 3287 (NH), 1720 (urethane CO), 1590 (amide II), 1262 (P=O) and 1212 cm^{-1} (P-OPh); δ_H (300 MHz, C^2HCl_3) 3.1–3.5 (2H, *m*, C<u>H</u>$_2$-CH), 4.75–4.9 (1H, *m*, C<u>H</u>), 5.12 (2H, *s*, C<u>H</u>$_2$-O), 5.34 (1H, *d*, J = 10.5 Hz, N<u>H</u>) and 7.02–7.40 (20 H, *m*, aromatic); δ_C (50.3 MHz, C^2HCl_3) 36.5 (Ph<u>C</u>H$_2$CH), 48.2 and 51.3 (*d*, PhCH$_2$<u>C</u>H, $J_{P\text{-}H}$ = 158.1 Hz), 67.7 (Ph<u>C</u>H$_2$-O), 120.8–121.2 (aromatic C-4), 125.8–130.3 (aromatic C-2,3,5,6), 136.1 and 136.7 (quaternary aromatic) and 150.4 (urethane carbonyl); δ_P (121.5 MHz, C^2HCl_3) 17.17 (88%) and 16.74 (12%); m/z (EI) 487 (M^+, 5.4%), 394 ([M − OPh]$^+$, 32), 379 ([M − PhCH$_2$OH]$^+$, 15.2), 336 ([M − H$_2$NCO–OCH$_2$Ph]$^+$, 100), 234 ([PO(OPh)$_2$]$^+$, 12.9), 145 ([PhCH$_2$CH–NHCO]$^+$, 37), 94 ([PhOH]$^+$, 65) and 77 (Ph^+, 14.9).

Dimethyl [1-(N-benzyloxycarbonyl)-amino]-2-phenylethylphosphonate (4). The diphenyl compound (**3**) (15.4 g, 31 mmol) was dissolved with warming in 200 mL of dry methanol and sodium (3.12 g, 64 mmol) in 30 mL dry methanol added dropwise with stirring. The solution

was stirred at room temperature overnight, then the volume reduced to 20 mL on a rotary evaporator and CH_2Cl_2 (200 mL) added. The solution was washed with 5% sodium hydrogen carbonate (3 × 80 mL), 0.1 M hydrochloric acid (1 × 80 mL), water (1 × 80 mL) and brine (1 × 80 mL). The solution was dried (magnesium sulphate) and the solvent removed under reduced pressure to give a yellow oil. This was purified by flash chromatography on silica gel (ethyl acetate) to give a clear oil, which gave the product as a white solid of mp 67–69 °C after trituration with petroleum ether (7.56 g, 67%). ν_{max} (nujol) 3224 (NH), 1710 (urethane CO), 1586 (amide II), 1258 (P=O) and 1057 cm^{-1} (P-OMe); δ_H (200 MHz, C^2HCl_3) 2.84–3.3 (2H, *m*, $PhC\underline{H}_2CH$), 3.62 and 3.68 (3H, *d*, J_{P-H} = 10.6 Hz, $P(O)(OCH_3)_2$), 4.40 (1H, *dt*, *J* = 7, 8.6 Hz, $PhCH_2C\underline{H}$), 4.97 (2H, *s*, $PhCH_2O$), 5.81 (1H, *d*, *J* = 7.1 Hz, -N<u>H</u>) and 7.20 (10H, *m*, Ar); δ_C (50.3 MHz, C^2HCl_3) 34.5 ($Ph\underline{C}H_2CH$), 47.4 and 50.5 (*d*, $PhCH_2\underline{C}H$, J_{P-H} = 156 Hz), 52.8 (OMe), 65.5 ($Ph\underline{C}H_2O$), 126.7–129.3 (aromatic), 137.4 and 137.7 (quaternary aromatic) and 156.1 (urethane CO); δ_P (121.5 MHz, C^2HCl_3) 26.83 (91%) and 26.21 (9%); m/z (CI) 364 ($[M + H]^+$, 100%), 259 ($[M + 2H - PhCH_2O]^+$, 12.1), 230 ($[M + H - PhCH_2OCO]^+$, 2.4), 120 ($[PhCH_2CHNH_2]^+$, 16.6), 108 ($[PhCH_2OH]^+$, 11.2) and 91 ($[PhCH_2]^+$, 13.3).

*Methyl hydrogen [1-(*N*-benzyloxycarbonyl)-amino]-2-phenylethylphosphonate (5).* The dimethyl compound (**4**) (3.63 g, 10 mmol) was dissolved in 30 mL methanol and 2 M sodium hydroxide (15 mL) added with stirring. The solution was stirred at room temperature for 12 h, after which time the solution was diluted with water (60 mL) and washed with ethyl acetate (100 mL). The aqueous layer was acidified to pH 2 with concentrated hydrochloric acid and extracted with ethyl acetate (3 × 70 mL). The organic extracts were dried (magnesium sulphate) and evaporated under reduced pressure to give the product as a white solid (2.69 g, 77%), mp 126.5–127.5 °C, m/z (found: $[M + H]^+$ 350.1152. $C_{17}H_{21}NO_5P$ requires 350.1154); ν_{max} (nujol) 3289 (OH), 2665 and 2320 (P-OH), 1687 (urethane CO), 1546 (amide II), 1219 (P=O), 1041 (P-OH) and 982 cm^{-1} (PO-C); δ_H (200 MHz, C^2HCl_3) 2.93 (2H, *m*, $PhC\underline{H}_2CH$), 3.62 (3H, *d*, J_{PH} = 10.6 Hz, $P(O)OCH_3$), 4.40 (1H, *dt*, J_{NH} = 7.1 Hz, J_{CH} = 6.3 Hz, $PhCH_2C\underline{H}$), 4.97 (2H, *s*, $PhCH_2O$), 5.64 (1H, *br*, OH), 5.81 (1H, *d*, *J* = 7.1 Hz, NH) and 7.15 (10H, *m*, Ar); δ_P (121.5 MHz, C^2HCl_3) 22.55 (9.5%) and 23.11 (90.5%); δ_C (50.31 MHz, C^2HCl_3) 38.55 ($Ph\underline{C}H_2CH$), 52.34 and 54.31 (*d*, J_{PC} = 103 Hz, $PhCH_2\underline{C}H$), 56.37 (*d*, J_{PC} = 21.1 Hz, OMe), 69.21 ($PhCH_2O$), 126.71–129.36 (aromatic), 141.34 and 142.40 (quat. aromatic) and 156.17 (urethane CO); m/z (EI) 350 ($[M + H]^+$, 5.4%), 320 ($[M - OMe + 2H]^+$, 100), 242 ($[M - Ome - PhCH_2O+2H]^+$, 18.3) and 91 ($PhCH_2^+$, 49.2).

(2S)-Prolyl-(2S)-isoleucyl-isobutylamide hydrochloride (8). HCl gas was bubbled into ethyl acetate (50 mL) at 0 °C for 1 h. Boc-(2*S*)-Pro-(2*S*)-Ile-NH-*i*Bu (1.0 g, 2.6 mmol) was added to the acidic solution and left for 1.5 h at room temperature. The solvent was removed under reduced pressure to yield a white solid, which was recrystallised from methanol/ethyl acetate (675 mg, 81%), mp 220 °C (dec.); (found: C, 56.29; H, 9.23; N, 12.93. $C_{15}H_{30}N_3O_2Cl$ requires C, 56.39; H, 9.47; N, 13.16%); m/z (found: $[M + H - HCl]^+$ 284.2338. $C_{15}H_{30}N_3O_2$ requires 284.2338); $[\alpha]_D$ –66.1° (*c* 1 in MeOH); ν_{max} (nujol) 3280 (NH str.), 1655 and 1637 (CO str. (amides)) and 1538 cm^{-1} (NH bend); δ_H (600 MHz, DMSO-d_6) 0.85 (12H, *m*, Ile and *i*Bu CH_3s), 1.10 (1H, *m*, 1 × CH_2 (Ile)), 1.45 (1H, *m*, 1 × CH_2 (Ile)), 1.67 (1H, *m*, CH (*i*Bu)), 1.74 (2H, *m*, 1 × CH_2 (Pro 3-H) and CH (Ile)), 1.84 (2H, *m*, CH_2 (Pro 4-H)), 2.29 (1H, *m*, 1 × CH_2 (Pro 3-H)), 2.80 (1H, *m*, 1 × CH_2 (*i*Bu)), 2.95 (1H, *m*, 1 × CH_2 (*i*Bu)) 3.15–3.3 (2H, *m*, CH_2 (Pro 5-H)), 4.18 (1H, *dd*, *J* = 7.80, 8.50 Hz, α CH (Ile)), 4.23 (1H, *dd*, *J* = 6.90, 8.10 Hz, α CH (Pro)), 8.15 (1H, *t*, *J* = 5.85 Hz, NH (*i*Bu)) and 8.65 (1H, *d*, *J* = 8.50 Hz, NH (Ile)); δ_C (50.3 MHz, DMSO-d_6) 11.0 ($\underline{C}H_3$ (Ile C-5)), 15.3 ($\underline{C}H_3$ (Ile C-3')), 20.0 ($\underline{C}H_3$ (*i*Bu)), 23.6 ($\underline{C}H_2$ (Pro C-4)), 24.4 ($\underline{C}H_2$ (Ile)), 28.0 ($\underline{C}H$ (*i*Bu)), 29.9 ($\underline{C}H_2$ (Pro C-3)), 36.8 ($\underline{C}H$ (Ile)), 45.4 ($\underline{C}H_2$ (Pro C-5)), 45.9 ($\underline{C}H_2$ (*i*Bu)), 57.8 (α $\underline{C}H$ (Ile)), 58.6 (α $\underline{C}H$ (Pro)), 168.2 ($\underline{C}O$ (Ile)) and 170.8 ($\underline{C}O$ (Pro)); m/z (CI) 284 ($[M + H - HCl]^+$, 100%).

Cbz-Phe-ψ[P(OMe)O-N]-(2S)-Pro-(2S)-Ile-NH-iBu (6). Using a modification of the method of Bartlett,[14] methyl hydrogen [1-(*N*-benzyloxycarbonyl)-amino]-2-phenylethylphosphonate (**5**) (1.22 g, 3.5 mmol) was dissolved in anhydrous CH_2Cl_2 (20 mL). Freshly distilled thionyl chloride (525 μL, 7 mmol) was added and the solution flushed with argon for 1 min. After 4 h, the solution was flushed again and the solvent removed under reduced pressure. The colourless oil was rediluted with anhydrous CH_2Cl_2 (10 mL) and reflushed with argon. This process was repeated and the colourless oil redissolved in CH_2Cl_2 (20 mL). To this, (2*S*)-Pro-(2*S*)-Ile-NH-*i*Bu hydrochloride (**8**) (1.12 g, 3.5 mmol) in CH_2Cl_2 (20 mL) with triethylamine (1.05 mL, 7.5 mmol) was added dropwise at 0 °C. The reaction was left at room temperature for 24 h under an atmosphere of nitrogen. The organic solution was washed with 1 M HCl (2 × 30 mL), saturated sodium bicarbonate (2 × 30 mL) and water (30 mL) and then dried over anhydrous sodium sulphate. After filtration, the solvent was removed under reduced pressure to yield an off-white solid (1.40 g, 65%). The diastereomers were resolved by silica chromatography (5% EtOH/ CH_2Cl_2);

Cbz-(R)-Phe-ψ[(S)-P(OMe)O-N]-(2S)-Pro-(2S)-Ile-NH-iBu (6i). Mp 82 °C (dec.); m/z (found: $[M + H]^+$ 615.3311. $C_{32}H_{48}N_4O_6P$ requires 615.3311); $[\alpha]_D$ –129.8° (*c* 0.5 in MeOH); ν_{max} (nujol) 3401 (NH str.), 1716 (CO str. (urethane)), 1669 and 1652 (CO str. (amide)), 1260 (P=O str.) and 1050 cm^{-1} (P-OCH_3); δ_H (600 MHz, C^2HCl_3) 0.85 (12H, *m*, Ile and *i*Bu CH_3s), 1.03 (1H, *m*, 1 × CH_2 (Ile)), 1.46 (1H, *m*, 1 × CH_2 (Ile)), 1.66–1.82 (4H, *m*, CH_2 (Pro 4-H), CH (*i*Bu) and 1 × CH_2 (Pro 3-H)), 2.00 (1H, *m*, 1 × CH_2 (Pro 3-H)), 2.15 (1H, *m*, CH (Ile)), 2.88 (1H, *m*, 1 × $ArC\underline{H}_2$), 3.00–3.20 (4H, *m*, CH_2

(*i*Bu), 1 × CH_2 (Pro 5-H) and 1 × $ArCH_2$), 3.30 (1H, *m*, 1 × CH_2 (Pro 5-H)), 3.74 (3H, *d*, $J_{P,H}$ = 10.71 Hz, $PO(OCH_3)$), 4.11 (1H, *m*, α CH (Pro)), 4.33 (1H, *dd*, *J* = 5.0, 9.3 Hz, α CH (Ile)), 4.46 (1H, *m*, CH*), 4.94 (2H, *s*, $ArCH_2O$), 5.09 (1H, *d*, *J* = 10.44 Hz, NH (urethane)), 6.93 (1H, *t*, *J* = 5.50 Hz, NH (*i*Bu)) and 7.18–7.32 (11H, *m*, aromatic and NH (Ile)); δ_C (74.76 MHz, C^2HCl_3) 11.54 (CH_3 (Ile C-5)), 15.71 (CH_3 (Ile C-3')), 20.03 (CH_3 (*i*Bu)), 24.52 (CH_2 (Ile)), 25.24 (CH_2 (Pro C-4)), 28.13 (CH (*i*Bu)), 30.54 (CH_2 (Pro C-3)), 35.69 ($ArCH_2C$), 36.32 (CH (Ile)), 46.69 and 48.60 (*d*, CH*, $J_{P,C}$ = 144.2 Hz), 46.81 (CH_2 (*i*Bu)), 46.91 (CH_2 (Pro C-5)), 50.95 and 51.05 (*d*, $PO(OCH_3)$, $J_{P,C}$ = 7.6 Hz), 57.90 (α CH (Ile)), 62.59 (α CH (Pro)), 66.84 ($ArCH_2O$), 126.61–128.96 (aromatic CHs), 135.99 and 136.25 (quat. aromatic), 155.77 (CO urethane), 170.80 (CO (Ile)) and 172.84 (CO (Pro)); δ_P (121.5 MHz, C^2HCl_3) 31.14; m/z (FAB) 637 ([M + Na]$^+$, 32%), 615 ([M + H]$^+$, 22) and 332 ([M + H – Pro-Ile-*i*Bu]$^+$, 37).

Cbz-(S)-Phe-ψ[(S)-P(OMe)O-N]-(2S)-Pro-(2S)-Ile-NH-iBu (6ii). Mp 90 °C (dec.); m/z (found: [M + H]$^+$ 615.3311. $C_{32}H_{48}N_4O_6P$ requires 615.3311); $[\alpha]_D$ –74.1° (*c* 0.5 in MeOH); ν_{max} (nujol) 3247 (NH str.), 1721 (CO str. (urethane)), 1677 and 1645 (CO str. (amide)), 1224 (P=O str.) and 1039 cm^{-1} (P-OCH_3); δ_H (600 MHz, C^2HCl_3) 0.85 (12H, *m*, Ile and *i*Bu CH_3s), 1.01 (1H, *m*, 1 × CH_2 (Ile)), 1.44 (1H, *m*, 1 × CH_2 (Ile)), 1.66 (2H, *m*, CH_2 (Pro 4-H)), 1.80 (1H, *m*, CH (*i*Bu)), 1.98 (1 × CH_2 (Pro 3-H)), 2.13 (2H, *m*, 1 × CH_2 (Pro 3-H) and CH (Ile)), 2.84 (1H, *m*, 1 × $ArCH_2$), 3.02 (1H, *m*, 1 × CH_2 (*i*Bu)), 3.10 (2H, *m*, 1 × CH_2 (Pro 5-H) and 1 × CH_2 (*i*Bu)), 3.21 (2H, *m*, 1 × CH_2 (Pro 5-H) and 1 × $ArCH_2$)), 3.69 (3H, *d*, $J_{P,H}$ = 10.62 Hz, $PO(OCH_3)$), 4.10 (1H, *m*, α CH (Pro)), 4.33 (1H, *dd*, *J* = 4.95, 9.52 Hz, α CH (Ile)), 4.49 (1H, *m*, CH*), 4.98 (2H, AB_q, *J* = 12.36 Hz, $ArCH_2O$), 5.18 (1H, *d*, *J* = 10.1 Hz, NH (urethane)) and 7.10–7.32 (12H, *m*, NH (*i*Bu), NH (Ile) and aromatic); δ_C (74.76 MHz, C^2HCl_3), 11.48 (CH_3 (Ile C-5)), 15.87 (CH_3 (Ile C-3')), 20.07 (CH_3 (*i*Bu)), 24.36 (CH_2 (Ile)), 25.08 (CH_2 (Pro C-4)), 28.08 (CH (*i*Bu)), 30.83 (CH_2 (Pro C-3)), 35.93 ($ArCH_2C$), 36.12 (CH (Ile)), 46.86 (CH_2 (*i*Bu)), 47.88 and 49.74 (*d*, CH*, $J_{P,C}$ = 139.9 Hz), 48.41 (CH_2 (Pro C-5)), 51.56 and 51.66 (*d*, $PO(OCH_3)$, $J_{P,C}$ = 7.2 Hz), 57.96 (α CH (Ile)), 61.08 (α CH (Pro)), 66.88 ($ArCH_2O$), 126.76–129.03 (aromatic CHs), 136.09 and 136.25 (quat. aromatic), 155.49 (CO urethane), 170.89 (CO (Ile)) and 172.62 (CO (Pro)); δ_P (121.5 MHz, C^2HCl_3) 30.19; m/z (FAB) 637 ([M + Na]$^+$, 10%), 615 ([M + H]$^+$, 12) and 332 ([M + H– Pro-Ile-iBu]$^+$, 21).

Cbz-(S)-Phe-ψ[(R)-P(OMe)O-N]-(2S)-Pro-(2S)-Ile-NH-iBu (6iii). Mp 186–187 °C; (found: C, 62.82; H, 7.88; N, 9.10. $C_{32}H_{47}N_4O_6P$ requires C, 62.53; H, 7.71; N, 9.11%); m/z (found: [M + H]$^+$ 615.3311. $C_{32}H_{48}N_4O_6P$ requires 615.3311); $[\alpha]_D$ –30.1° (*c* 0.5 in MeOH); ν_{max} (nujol) 3252 (NH str.), 1721 [CO str. (urethane)], 1677 and 1645 [CO str. (amide)], 1225 (P=O str.) and 1028 (P-OCH_3); δ_H (600 MHz, C^2HCl_3) 0.85 (12H, *m*, Ile and *i*Bu CH_3s), 1.06 (1H, *m*, 1 × CH_2 (Ile)), 1.42 (1H, *m*, 1 × CH_2 (Ile)), 1.74 (2H, *m*, CH_2 (Pro 4-H)), 1.80 (1H, *m*, CH (iBu)), 1.92 (1H, *m*, CH (Ile)), 1.98 and 2.12 (2H, *m*, CH_2 (Pro 3-H)), 2.89 (1H, *m*, 1 × $ArCH_2$)), 3.01 (1H, *m*, 1 × CH_2 (*i*Bu)), 3.18 (3H, *m*, 1 × CH_2 (Pro 5-H), 1 × CH_2 (*i*Bu) and 1 × $ArCH_2$), 3.29 (1H, *m*, 1 × CH_2 (Pro 5-H)), 3.66 (3H, *d*, $J_{P,H}$ = 10.68 Hz, $PO(OCH_3)$), 4.22 (1H, *dd*, *J* = 8.10, 9.30 Hz, α CH (Ile)), 4.27 (1H, *m*, α CH (Pro)), 4.46 (1H, *m*, CH*), 4.92 (2H, AB_q, *J* = 12.36 Hz, $ArCH_2O$), 6.47 (2H, *m*, NHs (urethane and *i*Bu)), 7.03 (1H, *d*, *J* = 9.30 Hz, NH (Ile)) and 7.25 (10H, *m*, aromatic); δ_C (74.76 MHz, C^2HCl_3) 10.71 (CH_3 (Ile C-5)), 15.40 (CH_3 (Ile C-3')), 20.03 (CH_3 (*i*Bu)), 24.62 (CH_2 (Ile)), 25.31 (CH_2 (Pro C-4)), 28.21 (CH (*i*Bu)), 31.48 (CH_2 (Pro C-3)), 35.71 ($ArCH_2C$), 36.25 (CH (Ile)), 46.84 (CH_2 (*i*Bu)), 47.52 (CH_2 (Pro C-5)), 48.16 and 50.12 (*d*, CH*, $J_{P,C}$ = 148.3 Hz), 51.31 and 51.41 (*d*, $PO(OCH_3)$, $J_{P,C}$ = 7.3 Hz), 57.73 (α CH (Ile)), 61.37 (α CH (Pro)), 66.65 ($ArCH_2O$), 126.49–129.13 (aromatic CHs), 136.28 and 136.70 (quat. aromatic), 156.35 (CO urethane), 171.17 (CO (Ile)) and 173.34 (CO (Pro)); δ_P (121.5 MHz, C^2HCl_3) 31.64; m/z (FAB) 637 ([M + Na]$^+$, 23%), 615 ([M + H]$^+$, 27) and 332 ([M + H - Pro-Ile-*i*Bu]$^+$, 28).

Cbz-(R)-Phe-ψ[(R)-P(OMe)O-N]-(2S)-Pro-(2S)-Ile-NH-iBu (6iv). Mp 163–164 °C; (found: C, 62.70; H, 7.92; N, 9.21. $C_{32}H_{47}N_4O_6P$ requires C, 62.53; H, 7.71; N, 9.11%); m/z (found: [M + H]$^+$ 615.3311. $C_{32}H_{48}N_4O_6P$ requires 615.3311); $[\alpha]_D$ –47.0° (*c* 0.5 in MeOH); ν_{max} (nujol) 3354 and 3209 (NH str.), 1695 (CO str. (urethane)), 1672 and 1652 (CO str. (amide)), 1252 (P=O str.) and 1036 cm^{-1} (P-OCH_3); δ_H (600 MHz, C^2HCl_3) 0.85 (12H, *m*, Ile and *i*Bu CH_3s), 1.14 (1H, *m*, 1 × CH_2 (Ile)), 1.50 (1H, *m*, 1 × CH_2 (Ile)), 1.76 (3H, *m*, CH_2 (Pro 4-H) and CH (*i*Bu)), 1.92 (1H, *m*, 1 × CH_2 (Pro 3-H)), 2.05 (2H, *m*, 1 × CH_2 (Pro 3-H)), (CH (Ile)), 2.80 (1H, *m*, 1 × $ArCH_2$)), 2.98 (1H, *m*, 1 × CH_2 (*i*Bu)), 3.07 (2H, *m*, 1 × CH_2 (*i*Bu) and 1 × CH_2 (Pro 5-H)), 3.18 (1H, *m*, 1 × CH_2 (Pro 5-H)), 3.28 (1H, *m*, 1 × $ArCH_2$), 3.60 (3H, *d*, $J_{P,H}$ = 10.53 Hz, $PO(OCH_3)$), 4.18 (1H, *dd*, *J* = 7.95, 8.85 Hz, α CH (Ile)), 4.32 (1H, *m*, α CH (Pro)), 4.45 (1H, *m*, CH*), 4.97 (2H, AB_q, *J* = 12.3 Hz, $ArCH_2O$), 5.08 (1H, *d*, *J* = 9.92 Hz, NH (urethane)), 6.61 (1H, *t*, *J* = 5.85 Hz, NH (*i*Bu)), 7.25 (10H, *m*, aromatic) and 7.45 (1H, *d*, *J* = 8.85 Hz, NH (Ile)); δ_C (74.76 MHz, C^2HCl_3) 11.02 (CH_3 (Ile C-5)), 15.51 (CH_3 (Ile C-3')), 19.98 (CH_3 (*i*Bu)), 24.67 (CH_2 (Ile)), 25.80 (CH_2 (Pro C-4)), 28.18 (CH (*i*Bu)), 31.12 (CH_2 (Pro C-3)), 35.61 ($ArCH_2C$), 35.88 (CH (Ile)), 46.73 (CH_2 (*i*Bu)), 47.55 (CH_2 (Pro C-5)), 49.46 and 50.40 (*d*, CH*, $J_{P,C}$ = 146.5 Hz), 51.51 and 51.60 (*d*, $PO(OCH_3)$, $J_{P,C}$ = 7.4 Hz), 58.22 (α CH (Ile)), 62.23 (α CH (Pro)), 66.89 ($ArCH_2O$), 126.64–128.94 (aromatic CHs), 136.04 and 136.30 (quat. aromatic), 155.77 (CO urethane), 170.91 (CO (Ile)) and 173.31 (CO (Pro)); δ_P (121.5 MHz, C^2HCl_3) 28.90; m/z (FAB) 637 ([M + Na]$^+$, 27%), 615 ([M + H]$^+$, 18) and 332 ([M + H – Pro-Ile-iBu]$^+$, 13).

Boc-(2S)-Asn-Phe-ψ[P(OMe)O-N]-(2S)-Pro-(2S)-Ile-NH-iBu (2). Cbz-(*R*)-Phe-ψ[(*S*)-P(OMe)O-N]-(2S)-Pro-(2S)-Ile-NH-*i*Bu (**6i**) (70 mg, 110 μmol) was dissolved in methanol (5 mL) and palladium/carbon catalyst (20 mg, 30% w/w) was added. The solution was stirred vigorously under a pressure of hydrogen gas (balloon) for 3 h. When the reaction had finished (by TLC), the catalyst was filtered off using Celite. The colourless solution was concentrated under reduced pressure to yield H_2N-(*R*)-Phe-ψ[(*S*)-P(OMe)O-N]-(2*S*)-Pro-(2*S*)-Ile-NH-*i*Bu as a colourless oil (47 mg, 86%).

Boc-(2*S*)-Asn (22 mg, 95 μmol) was dissolved in anhydrous THF (5 mL) with warming. *N*-Methylmorpholine (10.5 μL, 95 μmol) was added and the solution cooled to −15 °C. Isobutylchloroformate (13 μL, 95 μmol) was added and the solution left for 5 min. H_2N-(*R*)-Phe-ψ[(*S*)-P(OMe)O-N]-(2*S*)-Pro-(2*S*)-Ile-NH-*i*Bu (47 mg, 98 μmol) was dissolved in anhydrous THF (2 mL) and added dropwise to the solution at −15 °C. The reaction was left for 5 min at this temperature and a further 25 min at room temperature. The hydrochloride salts were filtered off and the THF removed under reduced pressure to yield an off-white solid which was purified by silica chromatography (10% EtOH/ CH_2Cl_2) to yield pure diastereomer (52 mg, 79%).

Boc-(2S)-Asn-(R)-Phe-ψ[(S)-P(OMe)O-N]-(2S)-Pro-(2S)-Ile-NH-iBu (2i). Mp 120 °C (dec); m/z (found: $[M + H]^+$ 695.387. $C_{33}H_{56}N_6O_8P$ requires 695.3897); $[\alpha]_D$ −127° (*c* 0.5 in MeOH); ν_{max} (nujol) 3315 (NH str.), 1683 and 1652 (CO str., br), 1251 (P=O str.) and 1030 cm^{-1} (P-OCH_3); δ_H (600 MHz, C^2HCl_3) 0.85 (12H, *m*, Ile and *i*Bu CH_3s), 1.10 (1H, *m*, 1 × CH_2 (Ile)), 1.42 (9H, *s*, Boc CH_3s), 1.50 (1H, *m*, 1 × CH_2 (Ile)), 1.78 (1H, *m*, CH (*i*Bu)), 1.88 (2H, *m*, CH_2 (Pro 4-H)), 2.05 (3H, *m*, CH_2 (Pro 3-H) and CH (Ile)), 2.46 (1H, ABX, *J* = 5.77, 15.10 Hz, 1 × CH_2 (Asn)), 2.62 (1H, *d*, *J* = 15.2 Hz, 1 × CH_2 (Asn)), 2.95 (2H, *m*, 1 × ArCH_2 and 1 × CH_2 (*i*Bu)), 3.11 (1H, *m*, 1 × CH_2 (*i*Bu)), 3.17 (2H, *m*, 1 × ArCH_2 and 1 × CH_2 (Pro 5-H)), 3.31 (1H, *m*, 1 × CH_2 (Pro 5-H)), 3.75 (3H, *d*, $J_{P,H}$ = 10.80 Hz, $PO(OCH_3)$), 4.15 (1H, *m*, α CH (Pro)), 4.28 (1H, *dd*, *J* = 6.23, 8.61 Hz, α CH (Ile)), 4.32 (1H, *m*, α CH (Asn)), 4.74 (1H, *m*, CH*), 5.68 (1H, *s*, 1 × $CONH_2$), 5.76 (1H, *d*, *J* = 6.96 Hz, NH (urethane)), 5.99 (1H, *s*, 1 × $CONH_2$), 6.95 (1H, *br*, NH (*i*Bu)), 7.18–7.28 (5H, *m*, aromatic), 7.32 (1H, *d*, *J* = 8.79 Hz, NH (Ile)) and 7.44 (1H, *d*, *J* = 8.79 Hz, NH (Asn)); δ_C (150 MHz, C^2HCl_3) 11.53 ($\underline{C}H_3$ (Ile C-5)), 15.77 ($\underline{C}H_3$ (Ile C-3')), 20.22 ($\underline{C}H_3$ (*i*Bu)), 24.89 ($\underline{C}H_2$ (Ile)), 25.53 ($\underline{C}H_2$ (Pro C-4)), 28.35 (Boc $\underline{C}H_3$s and $\underline{C}$H (*i*Bu)), 31.13 ($\underline{C}H_2$ (Pro C-3)), 35.80 (Ar$\underline{C}H_2$C), 36.70 ($\underline{C}$H (Ile)), 37.22 ($\underline{C}H_2$ (Asn)), 45.55 and 46.5 (*d*, $\underline{C}$H*, $J_{P,C}$ = 135 Hz), 47.01 ($\underline{C}H_2$ (*i*Bu)), 47.17 ($\underline{C}H_2$ (Pro C-5)), 51.45 ($PO(O\underline{C}H_3)$), 51.60 (α $\underline{C}$H (Asn)), 58.30 (α $\underline{C}$H (Ile)), 62.29 (α $\underline{C}$H (Pro)), 80.48 ($\underline{C}(CH_3)_3$), 126.65–129.26 (aromatic $\underline{C}$Hs), 136.77 (quat. aromatic), 156.6 ($\underline{C}$O urethane) and 171.13 and 173.56 ($\underline{C}$O amides); δ_P (121.5 MHz, C^2HCl_3) 30.30; m/z (FAB) 717 ($[M + Na]^+$, 100%), 695 ($[M + H]^+$, 38) and 412 ($[M + H - Pro\text{-}Ile\text{-}iBu]^+$, 17).

Boc-(2S)-Asn-(S)-Phe-ψ[(S)-P(OMe)O-N]-(2S)-Pro-(2S)-Ile-NH-iBu (2ii). This was prepared in an identical manner to the (*R,S*) diastereomer; m/z (found: $[M + H]^+$ 695.3897. $C_{33}H_{56}N_6O_8P$ requires 695.3897); $[\alpha]_D$ −54.5° (*c* 0.5 in MeOH); ν_{max} (nujol) 3287 (NH str.), 1717 (CO str. (urethane), 1657 and 1652 (CO str., br), 1253 (P=O str.) and 1043 cm^{-1} (P-OCH_3); δ_H (600 MHz, C^2HCl_3) 0.85 (12H, *m*, Ile and *i*Bu CH_3s), 1.06 (1H, *m*, 1 × CH_2 (Ile)), 1.42 (9H, *s*, Boc CH_3s), 1.49 (1H, *m*, 1 × CH_2 (Ile)), 1.82 (3H, *m*, CH (*i*Bu) and CH_2 (Pro 4-H)), 2.06–2.20 (4H, *m*, CH_2 (Pro 3-H), 1 × CH_2 (Asn) and CH (Ile)), 2.46 (1H, *d*, *J* = 14.83 Hz, 1 × CH_2 (Asn)), 2.80 (1H, *m*, 1 × ArCH_2), 3.02 (1H, *m*, 1 × CH_2 (*i*Bu)), 3.12 (1H, *m*, 1 × CH_2 (*i*Bu)), 3.27 (3H, *m*, 1 × ArCH_2 and CH_2 (Pro 5-H)), 3.76 (3H, *d*, $J_{P,H}$ = 10.62 Hz, $PO(OCH_3)$), 4.16 (1H, *m*, α CH (Pro)), 4.35 (2H, *m*, α CH (Ile) and α CH (Asn)), 4.81 (1H, *m*, CH*), 5.50 (1H, s, 1 × $CONH_2$), 5.92 (1H, *s*, 1 × $CONH_2$), 5.97 (1H, *d*, *J* = 7.87 Hz, NH (urethane)), 7.18–7.32 (7H, *m*, aromatic CHs and NHs (*i*Bu and Ile)) and 7.47 (1H, *d*, *J* = 8.06 Hz, NH (Asn)); δ_C (150 MHz, C^2HCl_3) 11.52 ($\underline{C}H_3$ (Ile C-5)), 16.0 ($\underline{C}H_3$ (Ile C-3')), 20.24 ($\underline{C}H_3$ (*i*Bu)), 24.68 ($\underline{C}H_2$ (Ile)), 25.45 ($\underline{C}H_2$ (Pro C-4)), 28.32 (Boc CH_3s and $\underline{C}$H (*i*Bu)), 31.11 ($\underline{C}H_2$ (Pro C-3)), 36.02 (Ar$\underline{C}H_2$C), 36.26 ($\underline{C}$H (Ile)), 36.99 ($\underline{C}H_2$ (Asn)), 46.75 and 47.55 (*d*, $\underline{C}$H*, $J_{P,C}$ = 120 Hz), 47.09 ($\underline{C}H_2$ (*i*Bu)), 48.52 ($\underline{C}H_2$ (Pro C-5)), 51.15 (α $\underline{C}$H (Asn)), 52.20 ($PO(O\underline{C}H_3)$), 58.32 (α $\underline{C}$H (Ile)), 61.52 (α $\underline{C}$H (Pro)), 80.3 ($\underline{C}(CH_3)_3$), 126.85–129.26 (aromatic $\underline{C}$Hs), 136.75 (quat. aromatic), 155.5 ($\underline{C}$O (urethane)) and 170.93, 171.39 and 173.19 ($\underline{C}$O amides); δ_P (121.5 MHz, C^2HCl_3) 30.43; m/z (FAB) 717 ($[M + Na]^+$, 31%), 695 ($[M + H]^+$, 22) and 412 ($[M + H - Pro\text{-}Ile\text{-}iBu]^+$, 13).

Boc-(2S)-Asn-(S)-Phe-ψ[(R)-P(OMe)O-N]-(2S)-Pro-(2S)-Ile-NH-iBu (2iii). This was prepared in an identical manner to the (*R,S*) diastereomer; m/z (found: $[M + H]^+$ 695.3897. $C_{33}H_{56}N_6O_8P$ requires 695.3897); $[\alpha]_D$ −25.6° (*c* 0.5 in MeOH); ν_{max} (nujol) 3233 (NH str.), 1700, 1684 and 1652 (CO str., br), 1224 (P=O str.) and 1045 cm^{-1} (P-OCH_3); δ_H (600 MHz, C^2HCl_3) 0.85 (12H, *m*, Ile and *i*Bu CH_3s), 1.13 (1H, *m*, 1 × CH_2 (Ile)), 1.39 (9H, *s*, Boc CH_3s), 1.51 (1H, *m*, 1 × CH_2 (Ile)), 1.82 (3H, *m*, CH (*i*Bu), 1 × CH_2 (Pro 4-H) and CH (Ile)), 1.91 (1H, *m*, 1 × CH_2 (Pro 4-H)), 2.00 (1H, *m*, 1 × CH_2 (Pro 3-H)), 2.07 (1H, *br*, 1 × CH_2 (Asn)), 2.21 (2H, *m*, 1 × CH_2 (Pro 3-H) and 1 × CH_2 (Asn)), 2.86 (1H, *m*, 1 × ArCH_2), 3.02 (1H, *m*, 1 × CH_2 (*i*Bu)), 3.16 (2H, *m*, 1 × ArCH_2 and 1 × CH_2 (Pro 5-H)), 3.26 (1H, *m*, 1 × CH_2 (*i*Bu)), 3.32 (1H, *m*, 1 × CH_2 (Pro 5-H)), 3.66 (3H, *d*, $J_{P,H}$ = 10.62 Hz, $PO(OCH_3)$), 4.25 (2H, *m*, α CH (Pro) and α CH (Ile)), 4.55 (1H, *m*, α CH (Asn)), 4.78 (1H, *m*, CH*), 5.57 (1H, *s*, 1 × $CONH_2$), 5.76 (1H, *br*, NH (urethane)), 6.01 (1H, *s*, 1 × $CONH_2$), 6.76 (1H, *br*, NH (*i*Bu)), 7.18–7.28 (5H, *m*, aromatic), 7.34 (1H, *br*, NH (Ile)) and 7.83 (1H, *d*, *J* = 8.06 Hz, NH (Asn)); δ_C (150 MHz, C^2HCl_3) 10.70 ($\underline{C}H_3$ (Ile C-5)), 15.46 ($\underline{C}H_3$ (Ile C-3')), 20.25 ($\underline{C}H_3$ (*i*Bu)), 24.87 ($\underline{C}H_2$ (Ile)), 25.97 ($\underline{C}H_2$ (Pro C-4)), 28.28 (Boc CH_3s and $\underline{C}$H (*i*Bu)), 31.69

($\underline{C}H_2$ (Pro C-3)), 35.54 (Ar$\underline{C}H_2$C), 35.86 ($\underline{C}$H (Ile)), 37.94 ($\underline{C}H_2$ (Asn)), 45.85 and 46.80 (d, $\underline{C}$H*, $J_{P,C}$ = 143 Hz), 47.09 ($\underline{C}H_2$ (Pro C-5)), 47.21 ($\underline{C}H_2$ (*i*Bu)), 51.45 (α $\underline{C}$H (Asn)), 51.85 (PO(O$\underline{C}H_3$)), 58.38 (α $\underline{C}$H (Ile)), 62.88 (α $\underline{C}$H (Pro)), 79.84 ($\underline{C}(CH_3)_3$), 126.73–129.44 (aromatic $\underline{C}$Hs), 137.14 (quat. aromatic), 155.9 ($\underline{C}$O (urethane)) and 171.60, 172.51 and 174.02 ($\underline{C}$Os); δ_P (121.5 MHz, C^2HCl_3) 30.58; m/z (FAB) 717 ([M + Na]$^+$, 57%), 695 ([M + H]$^+$, 15) and 412 ([M + H – Pro-Ile-iBu]$^+$, 10).

Boc-(2S)-Asn-(R)-Phe-ψ[(R)-P(OMe)O-N]-(2S)-Pro-(2S)-Ile-NH-iBu (2iv). This was prepared in an identical manner to the (*R,S*) diastereomer; m/z (found: [M + H]$^+$ 695.390. $C_{33}H_{56}N_6O_8P$ requires 695.3897); $[\alpha]_D$ –61° (*c* 0.5 in MeOH); ν_{max} (nujol) 3280 (NH str.), 1717 (CO str. (urethane)), 1683 and 1652 (CO str., br), 1251 (P=O str.) and 1024 cm^{-1} (P-OCH$_3$); δ_H (600 MHz, C^2HCl_3) 0.85 (12H, *m*, Ile and *i*Bu CH_3s), 1.14 (1H, *m*, 1 × CH_2 (Ile)), 1.43 (9H, *s*, Boc CH_3s), 1.53 (1H, *m*, 1 × CH_2 (Ile)), 1.77 (1H, *m*, CH (*i*Bu)), 1.82–1.92 (2H, *m*, CH_2 (Pro 4-H)), 1.95 (1H, *m*, 1 × CH_2 (Pro 3-H)), 2.05 (1H, *m*, CH (Ile)), 2.12 (1H, *m*, 1 × CH_2 (Pro 3-H)), 2.45 (1H, ABX., *J* = 6.64, 14.88 Hz, 1 × CH_2 (Asn)), 2.64 (1H, *d*, *J* = 15.10 Hz, 1 × CH_2 (Asn)), 2.84 (1H, *m*, 1 × Ar$\underline{C}H_2$), 2.99 and 3.11 (2H, *m*, CH_2 (*i*Bu)), 3.23 (1H, *m*, 1 × CH_2 (Pro 5-H)), 3.28 (2H, *m*, 1 × CH_2 (Pro 5-H) and 1 × Ar$\underline{C}H_2$), 3.62 (3H, *d*, $J_{P,H}$ = 10.62 Hz, PO(OCH$_3$)), 4.18 (1H, *t*, *J* = 8.40 Hz, α CH (Ile)), 4.38 (2H, *m*, α CH (Pro) and α CH (Asn)), 4.66 (1H, *m*, CH*), 5.56 (1H, *s*, 1 × $CONH_2$), 5.76 (1H, *d*, *J* = 7.78 Hz, NH (urethane)), 6.22 (1H, *s*, 1 × $CONH_2$), 6.81 (1H, *br*, NH (*i*Bu)), 7.05 (1H, *d*, *J* = 9.15 Hz, NH (Asn)), 7.18–7.28 (5H, *m*, aromatic) and 7.62 (1H, *d*, *J* = 8.42 Hz, NH (Ile)); δ_C (74.76 MHz, C^2HCl_3) 10.95 ($\underline{C}H_3$ (Ile C-5)), 15.58 ($\underline{C}H_3$ (Ile C-3')), 20.04 ($\underline{C}H_3$ (*i*Bu)), 24.84 ($\underline{C}H_2$ (Ile)), 25.75 ($\underline{C}H_2$ (Pro C-4)), 28.15 (Boc $\underline{C}H_3$s), 28.27 ($\underline{C}$H (*i*Bu)), 31.35 ($\underline{C}H_2$ (Pro C-3)), 35.85 (Ar$\underline{C}H_2$C), 36.58 ($\underline{C}$H (Ile)), 37.24 ($\underline{C}H_2$ (Asn)), 46.83 ($\underline{C}H_2$ (*i*Bu)), 47.83 and 49.80 (*d*, $\underline{C}$H*, $J_{P,C}$ = 148 Hz), 47.83 ($\underline{C}H_2$ (Pro C-5)), 51.32 and 51.42 (*d*, PO(O$\underline{C}H_3$), $J_{P,C}$ = 7.3 Hz), 51.52 (α $\underline{C}$H (Asn)), 58.58 (α $\underline{C}$H (Ile)), 61.66 (α $\underline{C}$H (Pro)), 80.26 ($\underline{C}(CH_3)_3$), 126.61–129.21 (aromatic $\underline{C}$Hs), 136.6 (quat. aromatic), 155.4 ($\underline{C}$O (urethane)) and 171.09, 171.42, 172.64 and 173.70 ($\underline{C}$O amides); δ_P (121.5 MHz, C^2HCl_3) 27.63; m/z (FAB) 717 ([M + Na]$^+$, 80%), 695 ([M + H]$^+$, 37) and 412 ([M + H – Pro-Ile-*i*Bu]$^+$, 19).

Boc-(2S)-Asn-Phe-ψ[P(OLi)O-N]-(2S)-Pro-(2S)-Ile-NH-iBu (1). Method 1. A solution of Boc-(*S*)-Asn-Phe-ψ[P(OMe)O-NH]-(*S*)-Pro-(*S*)-Ile-NH*i*Bu (**2i, ii**) (50 mg, 72 μmol) in dioxan (1 mL) and 1 M lithium hydroxide (288 μL, 290 μmol) was refluxed for 24 h. The white paste was filtered off and washed with acetone to remove any starting material (27 mg, 55%), m/z (derivatised as methyl ester using CH_2N_2, found: [M + H]$^+$ 695.3936. $C_{33}H_{56}N_6O_8P$ requires 695.3897) δ_H (200 MHz, MeOH-d_4) 0.90 (12H, *m*, Ile and *i*Bu CH_3s), 1.22 (1H, *m*, 1 × CH_2 (Ile)), 1.45 (9H, *s*, Boc CH_3s), 1.50 (1H, *m*, 1 × CH_2 (Ile)), 1.7–2.4 (8H, *m*, CH (*i*Bu), CH_2 (Pro 4-H), CH (Ile), CH_2 (Pro 3-H), CH_2 (Asn)), 2.75–3.5 (6H, *m*, Ar$\underline{C}H_2$, CH_2 (*i*Bu), CH_2 (Pro 5-H)), 3.85 (1H, *m*, α CH), 4.3 (2H, *m*, α CHs), 4.65 (1H, *m*, CH*) and 7.1–7.4 (5H, *m*, aromatic); δ_C (50.3 MHz, C^2HCl_3) 12.35 ($\underline{C}H_3$ (Ile C-5)), 16.64 ($\underline{C}H_3$ (Ile C-3')), 21.22 ($\underline{C}H_3$ (*i*Bu)), 26.30 ($\underline{C}H_2$ (Ile)), 28.82 ($\underline{C}H_2$ (Pro C-4)), 29.33 (Boc $\underline{C}H_3$s), 29.97 ($\underline{C}$H (*i*Bu)), 32.84 ($\underline{C}H_2$ (Pro C-3)), 38.56–41.01 (Ar$\underline{C}H_2$C), ($\underline{C}$H (Ile)), ($\underline{C}H_2$ (Asn)), 48.22–50.16 ($\underline{C}H_2$ (*i*Bu), ($\underline{C}$H*) and $\underline{C}H_2$ (Pro C-5)), 52.20 (α $\underline{C}$H (Asn)), 59.45 (α $\underline{C}$H (Ile)), 63.58 (α $\underline{C}$H (Pro)), 80.35 ($\underline{C}(CH_3)_3$), 128.17–130.89 (aromatic $\underline{C}$Hs), 138.5 (quat. aromatic), 155.8 ($\underline{C}$O (urethane)) and 174.0–176.0 ($\underline{C}$O amides); δ_P (121.5 MHz, MeOH-d_4) 18.14, 16.33;

Method 2. This was prepared using a modification of the method of McKenna.[26] To a solution of isobutylene in anhydrous CH_2Cl_2 (5 mL) (prepared by sparging with isobutylene gas for 10 min) was added freshly distilled trimethylsilylbromide (55 μL, 440 μmol) under an argon atmosphere.

H_2N-Phe-ψ[P(OMe)O-N]-(2*S*)-Pro-(2*S*)-Ile-NH-*i*Bu (2 diastereomers) (100 mg, 210 μmol) were dissolved in anhydrous CH_2Cl_2 (2 mL) and added to the TMSBr solution and the reaction left to stir at room temperature. The reaction was followed to completion by TLC (12 h) and the CH_2Cl_2 was concentrated *in vacuo* to give a yellow oil that was rediluted and evaporated twice more with CH_2Cl_2 (2 × 2 mL). The resultant oil was taken up in acetone (2 mL) and water added (0.5 mL). Removal of solvent under reduced pressure yielded a white solid (86 mg, 88%) that was used immediately in the preparation of **1**. This was prepared in an identical manner to **2** using H_2N-Phe-ψ[P(OH)O-N]-(2*S*)-Pro-(2*S*)-Ile-NH-*i*Bu (2 diastereomers) (62 mg, 130 μmol) and Boc-(2*S*)-asparagine (30 mg, 130 μmol) to produce an off-white solid (45 mg, 50%) that was dissolved in acetone and converted to **1** by the addition of 1 M lithium hydroxide (70 μL, 70 μmol).

Cbz-Cha-ψ[P(OMe)O-N]-(2S)-Pro-(2S)-Ile-NH-iBu (9). This was prepared in an identical manner to **6**, using methyl hydrogen [1-(*N*-benzyloxycarbonyl)-amino]-2-cyclohexylethylphosphonate (1.07 g, 3 mmol) to give an off-white solid (1.08 g, 58%) which was purified by silica chromatography (6% EtOH/ CH_2Cl_2), to yield two pairs of two diastereomers; diastereomers 3 and 4; m/z (found: [M + H]$^+$ 621.3781. $C_{32}H_{53}N_4O_6P$ requires 621.3781); ν_{max} (nujol) 3259 (NH str. br), 1724 and 1717 (CO str. (urethane)), 1660 and 1652 (CO str. (amide)), 1237 (P=O str.) and 1027 cm^{-1} (P-OCH$_3$); δ_H (600 MHz, C^2HCl_3) 0.90 (12H, *m*, Ile and *i*Bu CH_3s), 1.0–1.70 (17H, *m*, CH_2 (Ile), 5 × CH_2 (Cha), CH (Cha), CH_2 (Pro 4-H), *c*-HexC$\underline{H}_2$), 1.70–2.20 (4H, *m*, CH_2 (Pro 3-H), CH (*i*Bu), and CH (Ile)), 2.90–3.30 (4H, *m*, CH_2 (*i*Bu), CH_2 (Pro 5-H)), 3.61 and 3.64 (3H, *d*, $J_{P,H}$ = 10.60 Hz, PO(OCH$_3$)), 4.12–4.34 (3H, *m*, α CHs and CH*), 5.10 (2H, *m*, Ar$\underline{C}H_2$O) and 7.25–7.40 (5H, *m*, aromatic);

δ_C (150 MHz, C^2HCl_3) 10.89 and 11.40 ($\underline{C}H_3$ (Ile C-5)), 15.56 and 15.86 ($\underline{C}H_3$ (Ile C-3')), 20.17 ($\underline{C}H_3$ (*i*Bu)), 24.83 ($\underline{C}H_2$ (Ile)), 25.56 and 26.0 ($\underline{C}H_2$ (Pro C-4)), 25.94–26.46 (5 × $\underline{C}H_2$ (Cha)), 28.33 ($\underline{C}H$ (*i*Bu)), 31.63 and 31.83 ($\underline{C}H_2$ (Pro C-3)), 34.08 and 34.18 ($\underline{C}H$ (Cha)), 35.82 and 36.29 ($\underline{C}H$ (Ile)), 37.09 and 37.41 (Cha$\underline{C}H_2$), 45.00 and 45.99 (*d*, $\underline{C}H$*, $J_{P,C}$ = 148.4 Hz), 46.54 and 47.51 (*d*, $\underline{C}H$*, $J_{P,C}$ = 146.5 Hz), 46.99 ($\underline{C}H_2$ (*i*Bu)), 47.72 ($\underline{C}H_2$ (Pro C-5)), 51.8 and 52.4 (PO(O$\underline{C}H_3$)), 57.96 and 58.48 (α $\underline{C}H$ (Ile)), 61.54 and 62.14 (α $\underline{C}H$ (Pro)), 67.06 and 67.32 (Ar$\underline{C}H_2$O), 127.5–128.5 (aromatic $\underline{C}$Hs), 136.21 and 136.48 (quat. aromatic), 155.98 and 156.54 ($\underline{C}$O urethane), 170.90 and 171.22 ($\underline{C}$O (Ile)) and 173.16 and 173.45 ($\underline{C}$O (Pro)); δ_P (121.5 MHz, C^2HCl_3) 31.08 and 33.04; m/z (FAB) 643 ([M + Na]$^+$, 40%), 621 ([M + H]$^+$, 34) and 338 ([M + H – Pro-Ile-*i*Bu]$^+$, 61).

Cbz-Phe-ψ[P(OMe)O-N]-(2S)-Phe-(2S)-Ile-NH-iBu (10). This was prepared in an identical manner to **6**, using methyl hydrogen [1-(*N*-benzyloxycarbonyl)-amino]-2-phenyl-ethylphosphonate (**5**) (1.05 g, 3 mmol) and (2*S*)-Phe-(2*S*)-Ile-*i*Bu hydrochloride (1.11 g, 3 mmol) to yield an off-white solid (837 mg, 42%) which was purified by silica chromatography (10% EtOH/ CH_2Cl_2) prior to recrystallisation from methanol/ water to yield a mixture of four diastereomers (found: C, 64.96; H, 7.67; N, 8.27. $C_{36}H_{49}N_4O_6P$ requires C, 65.03; H, 7.43; N, 8.43%); m/z (found: [M + H]$^+$ 665.3468. $C_{36}H_{50}N_4O_6P$ requires 665.3468); ν_{max} (nujol) 3296 (NH str.), 1726 (CO str. (urethane)), 1694 and 1642 (CO str. (amide)), 1263 (P=O str.) and 1052 cm^{-1} (P-OCH$_3$); δ_H (500 MHz, DMSO-d_6) 0.85 (12H, *m*, Ile and *i*Bu CH_3s), 1.18 (1H, *m*, 1 × CH_2 (Ile)), 1.55 (1H, *m*, 1 x CH_2 (Ile)), 1.75 (1H, *m*, CH (*i*Bu)), 1.82 (1H, *m*, CH (Ile)), 2.28 (1H, *m*, 1 × Ar$\underline{C}H_2$CH*)), 2.45 (1H, *m*, 1 × Ar$\underline{C}H_2$CH*)), 2.80 (1H, *m*, 1 × CH_2 (Phe)), 2.90 (1H, *m*, 1 × CH_2 (Phe)), 3.02 (2H, *m*, CH_2 (*i*Bu)), 3.07, 3.24, 3.28 and 3.52 (3H, *d*, $J_{P,H}$ = 10.60 Hz, PO(OCH$_3$)), 3.90–4.30 (3H, *m*, α CHs and CH*), 4.85–5.20 (2H, *m*, Ar$\underline{C}H_2$O), 7.10–7.50 (15H, *m*, aromatic) and 7.95–8.25 (4H, *m*, NHs); δ_C (74.76 MHz, DMSO-d_6) 10.92 ($\underline{C}H_3$ (Ile C-5)), 15.27 ($\underline{C}H_3$ (Ile C-3')), 19.98 ($\underline{C}H_3$ (*i*Bu)), 24.30 ($\underline{C}H_2$ (Ile)), 27.80 ($\underline{C}H$ (*i*Bu)), 36.70 and 36.80 (Ar$\underline{C}H_2$C and $\underline{C}H$ (Ile)), 40.0 ($\underline{C}H_2$ (Phe)), 46.0 ($\underline{C}H_2$ (*i*Bu)), 49.0–52.0 ($\underline{C}H$* and PO(O$\underline{C}H_3$)), 55.51 and 57.04 (α $\underline{C}$Hs), 65.04 (Ar$\underline{C}H_2$O), 125.98-129.48 (aromatic $\underline{C}$Hs), 137.98–138.13 (quat. aromatic), 155.60 ($\underline{C}$O urethane) and 170.70 and 172.50 ($\underline{C}$O (amides)); δ_P (121.5 MHz, DMSO-d_6) 29.62, 29.78, 29.94 and 30.08; m/z (FAB) 687 ([M + Na]$^+$, 20%) and 665 ([M + H]$^+$, 79).

Cbz-Cha-ψ[P(OMe)O-N]-(2S)-Phe-(2S)-Ile-NH-iBu (11). This was prepared in an identical manner to **6**, using methyl hydrogen [1-(*N*-benzyloxycarbonyl)-amino]-2-cyclohexylethylphosphonate (1.07 g, 3 mmol) and (2*S*)-Phe-(2*S*)-Ile-*i*Bu hydrochloride (1.11 g, 3 mmol) to yield an off-white solid (1.46 g, 64%) which was purified by silica chromatography (5% EtOH/CH_2Cl_2) prior to recrystallisation from methanol/water to yield a mixture of four diastereomers (found: C, 64.41; H, 8.35; N, 8.32. $C_{36}H_{55}N_4O_6P$ requires C, 64.44; H, 8.27; N, 8.36%); m/z (found: [M + H]$^+$ 671.3937. $C_{36}H_{56}N_4O_6P$ requires 671.3937); ν_{max} (nujol) 3281 (NH str.), 1721 (CO str. (urethane)), 1665 and 1643 (CO str. (amide)) and 1263 cm^{-1} (P=O str.); δ_H (600 MHz, C^2HCl_3) 0.85 (12H, *m*, Ile and *i*Bu CH_3s), 1.0–2.20 (17H, *m*, CH_2 (Ile), 5 × CH_2 (Cha), CH (Cha), CH (Ile), CH (*i*Bu) and (*c*-Hex$\underline{C}H_2$)), 2.88–3.12 (4H, *m*, CH_2 (Phe) and CH_2 (*i*Bu)), 3.35 (3H, *d*, $J_{P,H}$ = 10.90 Hz, PO(OCH$_3$)), 3.43 (3H, *d*, $J_{P,H}$ = 10.60 Hz, PO(OCH$_3$)), 3.47 (3H, *d*, $J_{P,H}$ = 10.90 Hz, PO(OCH$_3$)), 3.59 (3H, *d*, $J_{P,H}$ = 10.90 Hz, PO(OCH$_3$)), 4.02–4.28 (3H, *m*, α CHs and CH*), 5.00–5.16 (2H, *m*, Ar$\underline{C}H_2$O) and 7.14–7.36 (10H, *m*, aromatic); δ_C (74.76 MHz, C^2HCl_3) 11.2 ($\underline{C}H_3$ (Ile C-5)), 15.5 ($\underline{C}H_3$ (Ile C-3')), 20.1 ($\underline{C}H_3$ (*i*Bu)), 24.8 ($\underline{C}H_2$ (Ile)), 25.9, 26.3, 26.5 (5 × $\underline{C}H_2$ (Cha)), 28.4 ($\underline{C}H$ (*i*Bu)), 31.7 ($\underline{C}H$ (Cha)), 34.0 ($\underline{C}H$ (Ile)), 36.5 (Cha$\underline{C}H\underline{C}H_2$), 40.0 ($\underline{C}H_2$ (Phe)), 46.0–48.0 ($\underline{C}H$*), 47.0 ($\underline{C}H_2$ (*i*Bu)), 51.6 (PO(O$\underline{C}H_3$)), 56.5 and 56.8 (α $\underline{C}H$), 58.2 and 58.4 (α $\underline{C}H$), 67.2 (Ar$\underline{C}H_2$O), 126.5–130 (aromatic $\underline{C}$Hs), 136.5–137.7 (quat. aromatic), 156.4 ($\underline{C}$O urethane) and 171.0 and 173.0 ($\underline{C}$O (amides)); δ_P (121.5 MHz, C^2HCl_3) 30.54, 31.21, 32.05 and 32.35; m/z (FAB) 693 ([M + Na]$^+$, 15%) and 671 ([M + H]$^+$, 22).

Boc-(2S)-Asn-Phe-ψ[P(OMe)O-N]-(2S)-Phe-(2S)-Ile-NH-iBu (13). This was prepared in an identical manner to **2**, using H_2N-Phe-ψ[(P(OMe)O-N]-(2*S*)-Phe-(2*S*)-Ile-NH-*i*Bu (**12**) (180 mg, 340 μmol) and (2*S*)-Boc-Asn (79 mg, 340 μmol) to yield an off-white solid (180 mg, 71%) which was purified by silica chromatography (10% EtOH/CH_2Cl_2), to yield four diastereomers; m/z (found: [M + H]$^+$ 745.4054. $C_{37}H_{57}N_6O_8P$ requires 745.4054); δ_H (200 MHz, C^2HCl_3) 0.90 (12H, *m*, Ile and *i*Bu CH_3s), 1.15 (1H, *m*, 1 × CH_2 (Ile)), 1.50 (10H, *s*, Boc CH_3s and 1 × CH_2 (Ile)), 1.80 (2H, *m*, CH (*i*Bu) and CH (Ile)), 2.20–3.30 (8H, *m*, Ar$\underline{C}H_2$CH*, CH_2 (Phe)), (CH_2 (*i*Bu) and CH_2 (Asn)), 3.40 (3H, *m*, PO(OCH$_3$)), 3.90–4.60 (4H, *m*, α CHs and CH*), 5.95 (1H, *s*, 1 × $CONH_2$), 6.65 (1H, *s*, 1 × $CONH_2$) and 7.10–7.50 (10H, *m*, aromatic); m/z (FAB) 767 ([M + Na]$^+$, 5%) and 745 ([M + H]$^+$, 25).

Qua-(2S)-asparagine (16). Quinaldic acid (2 g, 11.5 mmol) was dissolved in anhydrous CH_2Cl_2 (50 mL). Thionyl chloride (1.75 mL, 23 mmol) was added and the white precipitate redissolved with triethylamine (1.60 mL, 11.5 mmol). After 3 h the solvent was removed under reduced pressure and the acid chloride was redissolved in toluene (40 mL). To the reaction mixture was added a solution of asparagine (1.53 g, 11.5 mmol) and potassium carbonate (3.18 g, 23 mmol) in water (20 mL) and the solution stirred vigorously for 4 h. The aqueous layer was adjusted to pH 9 and separated. The aqueous layer was adjusted to pH 2 with 6 M HCl and the brown solid was filtered off, redissolved in ethanol and the solution decolourised

with charcoal. Removal of solvent under reduced pressure yielded an off-white solid which was recrystallised from ethanol (2.05 g, 62%), mp 209 °C (dec.); (found: C, 58.56; H, 4.32; N, 14.38. $C_{14}H_{13}N_3O_4$ requires C, 58.52; H, 4.56; N, 14.63%); (found: $[M + H]^+$ 288.0984. $C_{14}H_{14}N_3O_4$ requires 288.0984); $[\alpha]_D$ +23.3° (*c* 0.5 in MeOH); ν_{max} (nujol) 3384 and 3365 (NH str. (amides)), 1710 (CO str. (acid)) and 1662 (CO str. (amide)); δ_H (300 MHz, DMSO-d_6) 2.80 (2H, ABX, *J* = 15.97, 4.94 and 5.67 Hz, CH_2 (Asn)), 4.82 (1H, *m*, α CH (Asn)), 7.04 (1H, s, 1 × $CONH_2$), 7.54 (1H, *s*, 1 × $CONH_2$), 7.76 (1H, *t*, *J* = 7 Hz, CH (Qua 6-H)), 7.88 (1H, *t*, *J* = 6.9 Hz, CH (Qua 7-H)), 8.11 (2H, 2*d*, *J* = 8.23 and 7.15 Hz, 2 × CH (Qua 3-H and 5-H)), 8.18 (1H, *d*, *J* = 8.48 Hz, CH (Qua 8-H)), 8.60 (1H, *d*, *J* = 8.51 Hz, CH (Qua 4-H)) and 9.18 (1H, *d*, *J* = 8.56 Hz, NH†); δ_C (74.76 MHz, DMSO-d_6) 36.46 ($\underline{C}H_2$ (Asn)), 48.95 (α $\underline{C}$H (Asn)), 118.47 ($\underline{C}$H (Qua C-3)), 128.03 and 128.11 (2 × $\underline{C}$H Qua C-5 and C-6)), 128.88 ($\underline{C}$ (Qua C-10)), 129.14 ($\underline{C}$H (Qua C-7)), 130.53 ($\underline{C}$H (Qua C-8)), 137.94 ($\underline{C}$H (Qua C-4)), 145.90 ($\underline{C}$ (Qua C-9)), 149.47 ($\underline{C}$ (Qua C-2)), 163.37 ($\underline{C}$O amide), 171.69 ($\underline{C}ONH_2$) and 172.31 ($\underline{C}$OOH); m/z (CI) 288 ($[M + H]^+$, 12%). †Identified by D_2O shake.

Qua-(2S)-Asn-Phe-ψ[P(OMe)O-N]-(2S)-Phe-(2S)-Ile-NH-iBu (15). This was prepared in an identical manner to **2**, using H_2N-Phe-ψ[(P(OMe)O-N]-(2*S*)-Phe-(2*S*)-Ile-NH-*i*Bu (**12**) (40 mg, 76 μmol) and **16** (22 mg, 77 μmol) to yield an off-white solid (37 mg, 61%) which was purified by silica chromatography (10% EtOH/CH_2Cl_2), to yield four diastereomers; m/z (found: $[M + H]^+$ 800.3974. $C_{42}H_{55}N_7O_6P$ requires 800.3900); δ_H (200 MHz, MeOH-d_4) 0.80 (12H, *m*, Ile and *i*Bu CH_3s), 1.0–2.0 (4H, *m*, CH_2 (Ile), CH (*i*Bu) and CH (Ile)), 2.20–3.30 (8H, *m*, Ar$\underline{C}H_2$CH*, CH_2 (Phe), CH_2 (*i*Bu), CH_2 (Asn)), 3.30 (3H, *m*, PO(OC$\underline{H}_3$)), 3.50–4.60 (4H, *m*, α CHs and CH*), 7.25 (10H, *m*, aromatic) and 7.6–8.5 (6H, *m*, CHs (Qua)); m/z (FAB) 822 ($[M + Na]^+$, 100%) and 800 ($[M + H]^+$, 49).

Qua-(2S)-Asn-Cha-ψ[P(OMe)O-N]-(2S)-Pro-(2S)-Ile-NH-iBu (17). This was prepared in an identical manner to **15**, using H_2N-Cha-ψ[(P(OMe)O-N]-(2*S*)-Pro-(2*S*)-Ile-NH-*i*Bu (two diastereomers; 140 mg, 290 μmol) and **16** (83 mg, 290 μmol) to yield an off-white solid (158 mg, 73%) which was purified by silica chromatography (8% MeOH/CH_2Cl_2), to yield two diastereomers:

Diastereomer 3; ν_{max} (nujol) 3282 (NH str. br), 1700, 1696 and 1670 (CO str., br amides), 1223 (P=O str.) and 1039 cm^{-1} (P-OCH_3); δ_H (200 MHz, C^2HCl_3) 0.80 (12H, *m*, Ile and *i*Bu CH_3's), 1.0–2.20 (21H, *m*, CH_2 (Ile), 5 × CH_2 (Cha), CH (Cha), CH_2 (Pro 4-H), *c*-Hex$\underline{C}H_2$, CH_2 (Pro 3-H), CH (*i*Bu), and CH (Ile)), 2.80–3.50 (6H, *m*, CH_2 (*i*Bu), CH_2 (Pro 5-H), CH_2 (Asn), 3.65 (3H, *d*, $J_{P,H}$ = 10.60 Hz, PO(OCH_3)), 4.21 (2H, *m*, α CHs (Pro) and (Ile)), 4.60 (1H, *m*, CH*), 5.30 (1H, *m*, α CH (Asn)), 6.02 (1H, *s*, 1 × $CONH_2$), 6.85 (1H, *s*, 1 × $CONH_2$), 7.45 (1H, *br*, NH), 7.62 (1H, *t*, *J* = 7 Hz, CH (Qua 6-H)), 7.77 (1H, *t*, *J* = 6.9 Hz, CH (Qua 7-H)), 7.9 (2H, *m*, 2 × CH (Qua 3-H and 5-H)), 8.15 (1H, *d*, *J* = 8.48 Hz, CH (Qua 8-H)), 8.30 (1H, *d*, *J* = 8.51 Hz, CH (Qua 4-H)) and 9.15 (1H, *d*, *J* = 8.6 Hz, NH).

Diastereomer 4; m/z (found: $[M + H]^+$ 756.4217. $C_{38}H_{59}N_7O_7P$ requires 756.4214); δ_H (200 MHz, C^2HCl_3) 0.80 (12H, *m*, Ile and *i*Bu CH_3s), 1.0–2.20 (21H, *m*, CH_2 (Ile), 5 × CH_2 (Cha), CH (Cha), CH_2 (Pro 4-H), *c*-Hex$\underline{C}H_2$, CH_2 (Pro 3-H), CH (*i*Bu), and CH (Ile)), 2.80–3.50 (6H, *m*, CH_2 (*i*Bu), CH_2 (Pro 5-H), CH_2 (Asn)), 3.64 (3H, *d*, $J_{P,H}$ = 10.60 Hz, PO(OCH_3)), 4.21 (2H, *m*, α CHs), 4.60 (1H, *m*, CH*), 5.30 (1H, *m*, α CH (Asn)), 6.2 (1H, *s*, 1 × $CONH_2$), 6.7 (1H, *s*, 1 × $CONH_2$), 7.1 (1H, *br*, NH (*i*Bu)), 7.32 (1H, *br*, NH), 7.6–7.9 (4H, *m*, 4 × CH (Qua 3-H, 5-H, 6-H and 7-H)), 8.15 (1H, *d*, *J* = 8.48 Hz, CH (Qua 8-H)), 8.20 (1H, *d*, *J* = 8.51 Hz, CH (Qua 4-H)) and 9.28 (1H, *d*, *J* = 8.6 Hz, NH); m/z (FAB) 778 ($[M + Na]^+$, 94%) and 756 ($[M + H]^+$, 66).

(2R)-Phe-(2S)-Ile-NH-iBu hydrochloride (23). This was prepared in an identical manner to **8**, using Boc-(2*R*)-Phe (1.0 g, 3.77 mmol) and (2*S*)-Ile-NH*i*Bu.HCl (840 mg, 3.77 mmol), followed by Boc deprotection, using HCl gas to yield a white solid, which was recrystallised from methanol/ether (1.23 g, 88%), mp 252 °C (dec.); m/z (found: $[M + H - HCl]^+$ 334.2495. $C_{19}H_{32}N_3O_2$ requires 334.2494); $[\alpha]_D$ –79.6° (*c* 0.5 in MeOH); ν_{max} (nujol) 3350 and 3251 (NH str.) and 1679 and 1652 cm^{-1} (CO str. (amides)); δ_H (200 MHz, DMSO-d_6) 0.63 (3H, *d*, *J* = 7.6 Hz, CH_3 (Ile 3'-H)), 0.75 (3H, *t*, *J* = 7.5 Hz, CH_3 (Ile 5-H)), 0.87 (6H, *d*, *J* = 8 Hz, CH_3 (*i*Bu)), 1.02 (1H, *m*, 1 × CH_2 (Ile)), 1.18 (1H, *m*, 1 × CH_2 (Ile)), 1.70 (2H, *m*, CH (Ile) and CH (*i*Bu)), 2.75–3.15 (4H, *m*, CH_2 (*i*Bu) and CH_2 (Phe)), 4.13 (1H, *t*, *J* = 7.0 Hz, α CH), 4.27 (1H, *t*, *J* = 8.0 Hz, α CH), 7.30 (5H, *s*, aromatic), 8.17 (1H, *t*, *J* = 5.85 Hz, NH (*i*Bu), 8.45 (3H, *br*, NH_3^+) and 8.6 (1H, *d*, *J* = 8.76 Hz, NH (Ile)); δ_C (50.3 MHz, DMSO-d_6) 11.44 ($\underline{C}H_3$ (Ile C-5)), 15.42 ($\underline{C}H_3$ (Ile C-3')), 20.48 ($\underline{C}H_3$s (*i*Bu)), 24.42 ($\underline{C}H_2$ (Ile)), 28.12 ($\underline{C}$H (*i*Bu)), 36.89 ($\underline{C}H_2$ (Phe)), 37.35 ($\underline{C}$H (Ile)), 46.34 ($\underline{C}H_2$ (*i*Bu)), 53.34 (α $\underline{C}$H (Phe)), 57.54 (α $\underline{C}$H (Ile)), 127.25, 128.66 and 129.75 (aromatic $\underline{C}$H), 135.22 (quat. aromatic) and 168.11 and 170.32 ($\underline{C}$O amides); m/z (CI) 334 ($[M + H - HCl]^+$, 100%).

Gly-(2S)-Ile-NH-iBu hydrochloride (24). This was prepared in an identical manner to **8**, using Boc–Gly (875 mg, 5 mmol) and (2*S*)-Ile-NH*i*Bu.HCl (1.11 g, 5 mmol), followed by Boc deprotection, using HCl gas to yield a white solid, which was recrystallised from methanol/ether (1.22 g, 87%), mp 165 °C (dec.); m/z (found: $[M + H - HCl]^+$ 244.2025. $C_{12}H_{26}N_3O_2$ requires 244.2025); $[\alpha]_D$ –25.2° (*c* 0.5 in MeOH); ν_{max} (nujol) 3412 and 3280 (NH str.) and 1674 and 1654 cm^{-1} (CO str. (amides)); δ_H (200 MHz, DMSO-d_6) 0.85 (12H, *m*, Ile and *i*Bu CH_3s), 1.12 (1H, *m*, 1 × CH_2 (Ile)), 1.45 (1H, *m*, 1 × CH_2 (Ile)), 1.70 (2H, *m*, CH (Ile) and CH (*i*Bu)), 2.72–3.08 (2H, *m*, CH_2 (*i*Bu)), 3.63 (2H, *s*, CH_2 (Gly)), 4.25 (1H, *t*, *J* = 8.0 Hz, α CH (Ile)), 8.25 (4H,

br, NH_3^+ and NH (*i*Bu)) and 8.58 (1H, *d*, *J* = 8.76 Hz, NH (Ile)); δ_C (50.3 MHz, DMSO-d_6) 11.38 ($\underline{C}H_3$ (Ile C-5)), 15.64 ($\underline{C}H_3$ (Ile C-3')), 20.44 ($\underline{C}H_3$s (*i*Bu)), 24.53 ($\underline{C}H_2$ (Ile)), 28.13 ($\underline{C}H$ (*i*Bu)), 37.11 ($\underline{C}H$ (Ile)), 46.33 ($\underline{C}H_2$ (*i*Bu)), 51.0 ($\underline{C}H_2$ (Gly)), 57.50 (α $\underline{C}H$ (Ile)) and 165.88 and 170.64 ($\underline{C}O$ amides); m/z (CI) 244 ([M + H − HCl]$^+$, 100%).

Cbz-Phe-ψ[P(OMe)O-N]-(2R)-Phe-(2S)-Ile-NH-iBu (18). This was prepared in an identical manner to **6**, using methyl hydrogen [1-(*N*-benzyloxycarbonyl)-amino]-2-phenyl-ethylphosphonate (**5**) (349 mg, 1 mmol) and (2*R*)-Phe-(2*S*)-Ile-*i*Bu hydrochloride (**23**) (370 mg, 1 mmol) to yield an off-white solid (300 mg, 45%) which was purified by silica chromatography (10% EtOH/CH_2Cl_2) prior to recrystallisation from methanol/water to yield a mixture of four diastereomers, m/z (found: [M + H]$^+$, 665.3456. $C_{36}H_{50}N_4O_6P$ requires 665.3468); ν_{max} (nujol) 3269 (NH str.), 1703 (CO str. (urethane)), 1695 and 1654 (CO str. (amide)), 1261 (P=O str.) and 1049 cm^{-1} (P-OCH_3); δ_H (200 MHz, DMSO-d_6) 0.80 (12H, *m*, Ile and *i*Bu CH_3s), 1.05 (1H, *m*, 1 × CH_2 (Ile)), 1.32 (1H, *m*, 1 × CH_2 (Ile)), 1.7 (2H, *m*, CH (*i*Bu) and CH (Ile)), 2.5–3.0 (6H, *m*, Ar$\underline{C}H_2$CH*, CH_2 (Phe) and CH_2 (*i*Bu)), 3.07, 3.24, 3.28 and 3.52 (3H, *d*, $J_{P,H}$ = 10.60 Hz, PO(OCH_3)), 3.90–4.30 (3H, *m*, α CHs and CH*), 4.85–5.20 (2H, *m*, Ar$\underline{C}H_2$O), 7.10–7.50 (15H, *m*, aromatic) and 7.95–8.25 (NH's); δ_C (74.76 MHz, d_6-DMSO) 11.14 and 11.37 ($\underline{C}H_3$ (Ile C-5)), 15.59 ($\underline{C}H_3$ (Ile C-3')), 20.22 and 20.36 ($\underline{C}H_3$ (*i*Bu)), 24.36 and 24.48 ($\underline{C}H_2$ (Ile)), 28.04 and 28.15 ($\underline{C}H$ (*i*Bu)), 35.19 (Ar$\underline{C}H_2$C), 38.60 ($\underline{C}H$ (Ile)), 40.77 ($\underline{C}H_2$ (Phe)), 46.27 and 46.34 ($\underline{C}H_2$ (*i*Bu)), 49.0–52.0 ($\underline{C}H$* and PO($O\underline{C}H_3$)), 55.65, 55.81, and 57.11 (α $\underline{C}$Hs), 65.01 and 66.33 (Ar$\underline{C}H_2$O), 126.19–129.88 (aromatic $\underline{C}$Hs), 137.4–138.64 (quat. aromatic), 156.0 ($\underline{C}O$ urethane) and 171.0 ,173.2 and 173.3 ($\underline{C}O$ (amides)); δ_P (121.5 MHz, DMSO-d_6) 29.84, 30.10 and 30.24; m/z (FAB) 687 ([M + Na]$^+$, 36%) and 665 ([M + H]$^+$, 28).

Cbz-Phe-ψ[P(OMe)O-N]-Gly-(2S)-Ile-NH-iBu (20). This was prepared in an identical manner to **6**, using methyl hydrogen [1-(*N*-benzyloxycarbonyl)-amino]-2-phenyl-ethylphosphonate (**5**) (1.05 g, 3 mmol) and Gly-(2*S*)-Ile-*i*Bu hydrochloride (**24**) (840 mg, 3 mmol) to yield an off-white solid (826 mg, 48%) which was purified by silica chromatography (10% EtOH/CH_2Cl_2) prior to recrystallisation from methanol/water to yield a mixture of four diastereomers, m/z (found: [M + H]$^+$ 575.2979. $C_{29}H_{44}N_4O_6P$ requires 575.2998); ν_{max} (nujol) 3297 (NH str.), 1704 and 1696 (CO str. (urethane)), 1684 and 1657 (CO str. (amide)), 1261 (P=O str.) and 1049 cm^{-1} (P-OCH_3); δ_H (200 MHz, DMSO-d_6) 0.85 (12H, *m*, Ile and *i*Bu CH_3s), 1.1 (1H, *m*, 1 × CH_2 (Ile)), 1.45 (1H, *m*, 1 × CH_2 (Ile)), 1.7 (2H, *m*, CH (*i*Bu) and CH (Ile)), 2.6–3.2 (4H, *m*, Ar$\underline{C}H_2$CH* and CH_2 (*i*Bu)), 3.62 (5H, *m*, CH_2 (Gly) and PO(OCH_3)), 3.90–4.30 (2H, *m*, α CH (Ile) and CH*), 4.85–5.05 (2H, *m*, Ar$\underline{C}H_2$O), 7.10–7.4 (10H, *m*, aromatic) and 7.6–8.15 (NHs); δ_C (74.76 MHz, DMSO-d_6) 11.34 ($\underline{C}H_3$ (Ile C-5)), 15.61 ($\underline{C}H_3$ (Ile C-3')), 20.38 ($\underline{C}H_3$ (*i*Bu)), 24.56 ($\underline{C}H_2$ (Ile)), 28.17 ($\underline{C}H$ (*i*Bu)), 34.81 (Ar$\underline{C}H_2$C), 37.23 ($\underline{C}H$ (Ile)), 46.31 ($\underline{C}H_2$ (*i*Bu)), 49.0–52.0 ($\underline{C}H$* and PO($O\underline{C}H_3$)), 50.80 ($\underline{C}H_2$ (Gly)), 57.05 (α $\underline{C}H$ (Ile)), 65.22 and 65.33 (Ar$\underline{C}H_2$O), 126.4–129.38 (aromatic $\underline{C}$Hs), 137.4-138.64 (quat. aromatic), 156.04 ($\underline{C}O$ urethane) and 170.64 and 170.83 ($\underline{C}O$ (amides)); δ_P (121.5 MHz, DMSO-d_6) 31.01 and 31.44; m/z (FAB) 597 ([M + Na]$^+$, 100%) and 575 ([M + H]$^+$, 80).

Cbz-Phe-ψ[P(OMe)O-N]-(2S)-Phe-NH-iBu (21). This was prepared in an identical manner to **6**, using methyl hydrogen [1-(*N*-benzyloxycarbonyl)-amino]-2-phenyl-ethylphosphonate (**5**) (698 mg, 2 mmol) and (2*S*)-Phe-NH-*i*Bu (440 mg, 2 mmol) to yield, after work up, an off-white solid which was recrystallised from methanol/water to yield a mixture of four diastereomers (580 mg, 53%), m/z (found: [M + H]$^+$ 552.2627. $C_{30}H_{39}N_3O_5P$ requires 552.2627); ν_{max} (nujol) 3296 (NH str.), 1695 (CO (urethane) 1653 (CO str. (amide)), 1215 (P=O str.) and 1056 cm^{-1} (P-OCH_3); δ_H (200 MHz, DMSO-d_6) 0.85 (6H, *m*, CH_3s (*i*Bu)), 1.65 (1H, *m*, CH (*i*Bu)), 2.4–2.7 (6H, *m*, CH_2 (Phe), CH_2 (*i*Bu) and Ar$\underline{C}H_2$CH*), 3.0 and 3.40 (3H, *d*, $J_{P,H}$ = 10.60 Hz, PO(OCH_3)), 3.8–4.2 (2H, *m*, α CH (Phe) and CH*), 4.95 (2H, *m*, Ar$\underline{C}H_2$O), 5.0–5.3 (1H, *m*, NHs), 7.0–7.5 (15H, *m*, aromatic) and 8.15 (1H, *br*, NH); δ_C (74.76 MHz, DMSO-d_6) 20.33 ($\underline{C}H_3$ (*i*Bu)), 28.17 and 28.26 ($\underline{C}H$ (*i*Bu)), 33.86–34.52 (Ar $\underline{C}H_2$s), 46.31 and 46.42 ($\underline{C}H_2$ (*i*Bu)), 48.5–52.0 ($\underline{C}H$* and PO($O\underline{C}H_3$)), 55.65, 55.97, 56.09 and 56.22 (α $\underline{C}H$ (Phe)), 65.01, 65.19, 65.31 and 65.40 (Ar$\underline{C}H_2$O), 126.23–128.88 (aromatic $\underline{C}$Hs), 137.33–138.63 (quat. aromatic), 155.95 and 156.05 ($\underline{C}O$ urethane) and 172.93 and 173.04 ($\underline{C}O$ (amide)); δ_P (121.5 MHz, DMSO-d_6) 29.44, 29.89, 30.04 and 30.14; m/z (FAB) 574 ([M + Na]$^+$, 10%) and 552 ([M + H]$^+$, 35).

Cbz-Phe-ψ[P(OMe)O-N]-(2S)-Phe-OMe (22). This was prepared in an identical manner to **6**, using methyl hydrogen [1-(*N*-benzyloxycarbonyl)-amino]-2-phenyl-ethylphosphonate (**5**) (349 mg, 1 mmol) and (2*S*)-Phe-OMe hydrochloride (323 mg, 1.5 mmol) to yield, after work-up, an off-white solid which was recrystallised from acetone/petrol to yield a mixture of four diastereomers (250 mg, 49%), m/z (found: [M + H]$^+$, 511.1998. $C_{27}H_{32}N_2O_6P$ requires 511.1998); ν_{max} (nujol) 3332 and 3185 (NH str.), 1750 (CO str. (ester)), 1695 (CO (urethane)), 1653 (CO str. (amide)), 1259 (P=O str.) and 1048 cm^{-1} (P-OCH_3); δ_H (200 MHz, DMSO-d_6) 2.4–2.7 (2H, *m*, CH_2 (Phe)), 2.7–3.2 (2H, *m*, Ar$\underline{C}H_2$CH*), 3.10, 3.20, 3.35 and 3.50 (3H, *d*, $J_{P,H}$ = 10.60 Hz, PO(OCH_3)), 3.8–4.2 (2H, *m*, α CH (Phe) and CH*), 4.90 (2H, *m*, Ar$\underline{C}H_2$O), 5.2–5.7 (1H, *m*, NHs) and 7.25 (15H, *m*, aromatic CHs); δ_C (74.76 MHz, DMSO-d_6) 34–34.25 (Ar $\underline{C}H_2$s), 48.5–51.0 ($\underline{C}H$* and PO($O\underline{C}H_3$)), 51.97, 52.12 and 52.23 ($O\underline{C}H_3$ ester), 55.34, 55.58, and 55.68 (α $\underline{C}$Hs), 65.11, 65.28 and 65.39 (Ar$\underline{C}H_2$O), 126.28–130.04 (aromatic $\underline{C}$Hs), 137.56–

138.83 (quat. aromatic), 155.86 (CO urethane) and 173.86, 173.81 and 174.05 (CO (ester)); δ_P (121.5 MHz, DMSO-d_6) 28.88 and 29.64; m/z (CI) 511 ($[M + H]^+$, 40%) and 403 ($[M + H - ArCH_2OH]^+$, 58).

HIV-1 proteinase assays

The assays are based upon the hydrolytic cleavage of the decapeptide Lys-Ala-Arg-Val-Nle-(NO_2)Phe-Glu-Ala-Nle-Gly-NH_2 between the norleucine and nitrophenylalanine residues which leads to a reduction in absorbance at 300 nm. The peptide was stored as a 10 mg mL^{-1} solution in water and 2 μL of this solution was added to 1 mL aliquot of assay buffer (100 mM NaOAc, 200 mM NaCl, 1mM DTT at pH 5.6). The inhibitors to be tested were made up as 10 mM stock solutions in methanol and were used at concentrations over the range 1–100 μM. Methanol was added to a final concentration of 2% in the assay mixture and the solution was allowed to thermally equilibrate before the reaction was initiated by the addition of 15 μL of enzyme. The enzyme was stored as a 0.993 μM solution in 10 mM NaOAc containing 0.05% 2-mercaptoethanol, 1mM EDTA, 20% glycerol and 5% ethylene glycol.

Anti-viral assays: acute infection of cells (C8166 cells / HIV-1IIB)

High titre virus stocks of the human immunodeficiency virus HIV-1 (HTLV-III RF) were grown in H9 cells with RPMI 1640 (Flow Laboratories) supplemented with 10% fetal calf serum. Cell debris was removed by low speed centrifugation, and the supernatant stored at –70 °C until required. In a typical assay, C8166 T-lymphoblastoid $CD4^+$ cells were incubated with $TCID_{50}$ HIV-1 at 37 °C for 90 min and then washed three times with medium. Cell aliquots (2×10^5) were suspended in 1.5 mL growth medium in 6 mL tubes, and compounds in log dilutions [200–0.2 μM] were added immediately. The cells were then incubated at 37 °C in 5% CO_2. At 72 h post-infection 200 μl of supernatant was taken from each culture and assayed for HIV[27] using an antigen capture ELISA which recognises all the core proteins equally (Coulter Electronics, Luton, U.K.). The following controls were used: supernatants taken from uninfected and infected cells, infected cells treated with Ro-31-8959 (**14**) (Roche Products U.K. Ltd),[21] AZT (Roche Products U.K., Ltd) and ddC (Roche Products U.K. Ltd). The activities of 8959, AZT and ddC in infected cells each gave an IC_{50} of 3, 20 and 200 nM, respectively. The ELISA plates were read with a spectrophotometer. Compounds were tested in duplicate at each concentration, and the data shown are the average of two assays.

To test for compound toxicity, aliquots of 2×10^5 uninfected cells were cultured with the compounds in the same half log dilutions for 72 h. The cells were then washed with medium and resuspended in 200 μL of growth medium containing ^{14}C protein hydrolysate. After 12 h the cells were harvested and the ^{14}C incorporation measured. Uninfected, untreated cells were used as controls.

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References

1. Ratner, L.; Haseltine, W.; Pataraca, R.; Livak, K. J.; Starcich, B.; Josephs, S. F.; Doran, E. R.; Rafalski, J. A.; Whitehorn E. A.; Baumeister, K.; Ivanoff, L.; Petteway, Jr S. R.; Pearson, M. L.; Lautenberger, L. A.; Papas, T.; Ghrayeb, J.; Chang, N. T., Gallo, R. C.; Wong-staal, F. *Nature* **1985**, *313*, 277.

2. Mous, J.; Heimer, E. P.; Le Grice, S. F. J. *J. Virology* **1988**, *62*, 1433.

3. Kohl, N. E.; Emini, E. A.; Schleif, W. A.; Davis, L. J.; Heimbach, J. C.; Dixon, R. A. F.; Scolnick, E. M.; Sigal, I. S. *Proc. Natl Acad. Sci. U.S.A.* **1988**, *85*, 4686.

4. Tomasselli, A. G.; Howe, W. J.; Sawyer, T. K.; Wlodawer, A.; Heinrikson, R. L. *Chimica Oggi* **1991**, *5*, 6.

5. Huff, J. R. *J. Med. Chem.* **1991**, *34*, 2305.

6. Debouk, C. *AIDS Res. Hum. Retroviruses* **1992**, *8*, 153.

7. Martin, J. A. *Antiviral Res.* **1992**, *17*, 265.

8. Camp, N. P.; Hawkins, P. C. D.; Hitchcock, P. B.; Gani, D. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 1047.

9. Tomasselli, A. G.; Hui, J. O.; Sawyer, T. K.; Staples, D. J.; Borrow, C.; Reardon, I. M.; Have, W. J.; DeCamp, D. L.; Craik, C. S.; Heinrikson, R. L. *J. Biol. Chem.* **1990**, *265*, 14675.

10. Scharpe, S.; De Meester, I.; Hendriks, D.; Vanhoof, G.; van Sande, M.; Vriend, G. *Biochimie* **1991**, *73*, 121.

11. Grobelny, D.; Wondrak, E. M.; Galardy, R. E.; Oroszlan, S. *Biochem. Biophys. Res. Commun.* **1990**, *169*, 1111.

12. Bartlett, P. A.; Marlowe, C. K.; Giannousis, P. P.; Hansen, J. E. *Cold Spring Harbor Symp. Quant. Biol.* **1987**, *52*, 83.

13. Oleksyszyn, J.; Subotowska, L.; Mastalerz, P. *Synthesis* **1979**, 985.

14. Bartlett, P. A.; Jacobsen, N. E. *J. Am. Chem. Soc.* **1981**, *103*, 654.

15. Anderson, G. W.; Zimmerman, J. E.; Callahan, F. M.; *J. Am. Chem. Soc.* **1967**, *89*, 5012; Chen, F. M. F.; Lee, Y.; Steinauer, R.; Benoiton, N. L. *Can. J. Chem.* **1987**, *65*, 613.

16. Griffiths, J. T.; Phylip, L. H.; Konvalinka, J.; Strop, P.; Gustchina, A.; Wlodawer, A.; Davenport, R. J.; Briggs, R.; Dunn, B. M.; Kay, J. *Biochemistry* **1992**, *31*, 5193.

17. Hawkins, P. C. D.; Ph.D. Thesis, University of St. Andrews, 1993.

18. Dixon, M. *Biochem. J.* **1953**, *55*, 70.

19. McLeod, D. A.; Brinkworth, R. I.; Ashley, J. A.; Janda, K. D.; Wirsching, P. *Bioorg. Med. Chem. Lett.* **1991**, *1*, 653.

20. Cushman, M.; Oh, J.; Copeland, T. D.; Oroszlan, S.; Snyder, S. W. *J. Org. Chem.* **1991**, *56*, 4161.

21. Roberts, N. A.; Martin, J. A.; Kinchington, D.; Broadhurst, A. V.; Craig, J. C.; Duncan, I. B.; Galpin, S. A.; Handa, B. K.; Kay, J.; Krohn, A.; Lambert, R. W.; Merrett, J. H.; Mills, J. S.; Parkes, K. E. B.; Redshaw, S.; Ritchie, A. J.; Taylor, D. L.; Thomas, G. J.; Machin, P. J. *Science* **1990**, *248*, 358.

22. Schotten, C. *Chem. Ber.* **1884**, *17*, 2544; Baumann, E. *Chem. Ber.* **1886**, *19*, 3218.

23. Vacca, J. P.; Guare, J. P.; de Solms, J.; Sanders, W. M.; Giuliani, E. A.; Young, S. D.; Darke, P. L.; Zugay, J. A.; Sigal, I.; Schleif, W. A.; Quintero, J. C.; Emini, E. A.; Anderson, P. S.; Huff, J. R. *J. Med. Chem.* **1991**, *34*, 1225.

24. (a) Peyman, A.; Budt, K. H.; Spanig, J.; Stowasser, B.; Ruppert D. *Tetrahedron Lett.* **1992**, *33*, 4549; (b) Abdel-Meguid, S. S.; Zhao, B.; Murthy, K.; Winborne, E.; Choi, J. K.; DesJarlais, R.; Minnich, M.; Culp, J.; Debouck, C.; Tomaszek, T.; Meek, T. D.; Dreyer, G. B. *Biochemistry*, **1993**, *32*, 7972.

25. Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.*, **1978**, *43*, 2923.

26. McKenna, C. E.; Higa, M. T.; Cheung, N. H.; McKenna, M. E. *Tetrahedron Lett.* **1977**, 155; McKenna, C. E.; Schmidhauser, J. J. *J. Chem. Soc. Chem. Commun.* **1979**, 739.

27. Kinchington, D.; Galpin, S. A.; O'Connor, T. J.; Jeffries, D. J.; Williamson, J. D. *AIDS* **1989**, *3*, 101.

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