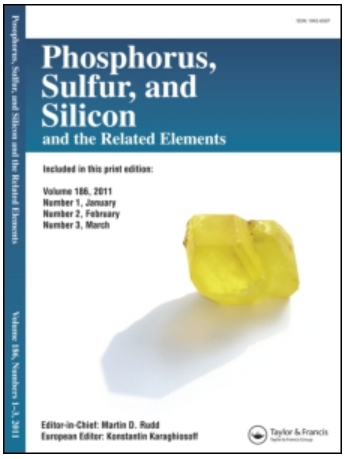

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The Synthesis of Phosphinic Acid Based Proteinase Inhibitors

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The phenyl phosphinate analogues of valine and phenylalanine with extended chains at phosphorus have been prepared as racemic mixtures and also in high optical purity. Kinetic data clearly indicate specific inhibitory activity for analogues having (R) configuration at α-carbon. A similar synthetic approach provides a simplified route to reported C_2 symmetrical HIV inhibitors.

Keywords: Serine proteinase; phosphinate analogues; C_2 symmetrical; HIV inhibitors

α-AMINOALKYLPHOSPHINATE SERINE PROTEINASE INHIBITORS

Diphenyl α- aminoalkylphosphonate ester analogues of α-amino acids can act as highly potent and selective inhibitors of serine proteinases[1], a large group of enzymes with a wide range of biological functions[2]. The inhibition mechanism is now generally understood to involve displacement of a phenoxy group at phosphorus by the active site serine of the enzyme resulting in a covalently bound, phosphorylated enzyme.

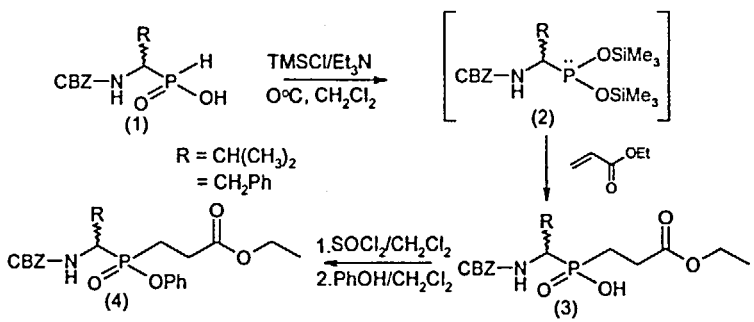
Diphenyl α- aminoalkyl phosphonates exhibit single point recognition for the enzyme by binding at its S_1 primary binding site, such binding also determines specificity for a particular serine proteinase. Extension of the analogue using appropriate amino acid residues increases recognition between enzyme and inhibitor and leads to enhanced inhibition values (Table 1).

Table 1. Second order rate constants $k_{ob}/[I](M^{-1}s^{-1})$[1]
(porcine pancreatic elastase and human leukacyte elastase)

Inhibitor	PPE	HLE
Z-Val^P(OPh)$_2$	9.00	2.80×10^2
Z-Ala-Ala-Val^P(OPh)$_2$	3.40×10^2	1.30×10^3
Boc-Val-Pro-Val^P(OPh)$_2$	1.10×10^4	2.70×10^4

Analogues extended at nitrogen increase recognition in the S binding region of the enzyme and are readily available using normal amino acid coupling techniques. However, extension at phosphorus enabling recognition in the S' binding region is synthetically more demanding and less well known. One recent study by Bartlett [3] detailed evidence of binding at S'_1 and S'_2 sites. However, the examples reported which contained P-O bond coupling to the extended chain, were subject to enzymatic degredation during assays making inhibition values obtained unreliable.

We have now synthesised a number of these extended chain analogues containing the more stable P-C bond coupling at phosphorus, and have examined their inhibitory properties against human neutrophile elastase (HNE) (valine analogue) (Table 2), and chymotrypsin (phenylalanine analogue) (Table 3).



Scheme 1

The analogues were synthesised from the corresponding phosphonous acids (1)[4] using a previously reported procedure[5] involving in situ generation of an intermediate silyl phosphonite (2) by addition of trimethylsilyl chloride/triethylamine mixture to the phosphonous acid (1) at low temperature under nitrogen followed by reaction with an activated alkene (Scheme 1). Treatment of the resulting phosphinic acid (3) with thionyl chloride/phenol yielded the required phenyl ester (4). Since the phosphonous acid analogues could also be prepared in optically pure form at the α-carbon[4][6] these were used to generate the extended chain analogues and the resulting diasteriomeric mixtures examined.

Table 2 Second order rate constants[A=k/K$_i$] (human neutrophile elastase) ($M^{-1}s^{-1}$)

Inhibitor	Diast. Ratio	α-Carbon Conf.	$[\alpha]_D^{c=1}$ EtOH	HNE
Z-Val^P(OPh)($CH_2CH_2CO_2Et$)	89:11	R	-2.2	$1.08x10^2$
	95:5	S	+5.6	inactive
	racemic	-	-	$0.47x10^2$

The kinetic data (Tables 2&3) shows that the diasteriomeric mixtures generated from phosphonous acid having (S) configuration at the α-carbon are inactive while those generated from phosphonous acid with (R) configuration at the α-carbon are active inhibitors, and as expected show higher values than their respective racemic mixtures.

Table 3 Second order rate constants[A=k/K$_i$] (chymotrypsin) ($M^{-1}s^{-1}$)

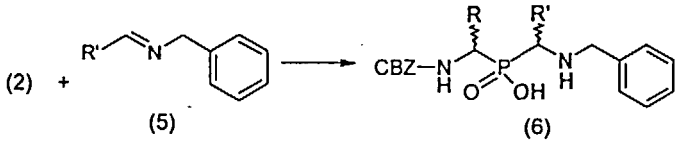
Inhibitor	Diast. Ratio	α-Carbon Conf.	$[\alpha]_D^{c=1}$ EtOH	Chym.
Z-Phe^P(OPh)($CH_2CH_2CO_2Et$)	91:9	R	-10.2	$2.0x10^4$
	94:6	S	+12.6	inactive
	racemic	-	-	$2.12x10^3$

Further elaboration of these extended chain analogues through addition of appropriate amino acid residues at this new carboxylic acid site should enable increased recognition within the S' binding region to be attained.

C_2 SYMMETRICAL HIV PROTEINASE INHIBITORS

The virally encoded HIV-1 proteinase essential for retroviral replication has emerged as a key target for development of AIDS therapy[7]. The active site of this proteinase exhibits twofold symmetry[8] with the S_1 and S'_1 subsites indistinguishable in its native state. One approach to inhibition of this proteinase is the design of C_2 symmetrical substrates. Among the structures examined those based on phosphinic acid derivatives have been a focus of considerable attention. Several syntheses of these C_2 symmetrical phosphinic acid inhibitors have been reported[9], however the methods employed have been cumbersome and inefficient. We have now developed a significantly simplified route to these compounds by making use of the methodology already described for preparing the extended chain phosphinate analogues. This new approach involves addition of the silyl phosphonite (2) to an imine (5) generated from a

suitable aldehyde and benzylamine (Scheme 2).



Scheme 2

Table 4 C_2 Symmetrical Analogues (6)

	a	b	c	d	e
R	$CH(CH_3)_2$	$CHCH_2(CH_3)_2$	Ph	CH_2Ph	$CH_2C_6H_{11}$
R'	$CH(CH_3)_2$	$CHCH_2(CH_3)_2$	Ph	$CH_2C_6H_{11}$	$CH_2C_6H_{11}$

The C_2 symmetrical compounds (6)(Table 4) prepared in this way have the added advantage of possessing differing protecting groups at the two nitrogens enabling selective deprotection and extension. Due to the instability[10] of the required imine we have so far been unable to apply this approach to synthesis of the dibenzyl analogue, which is known to be the more biologically active of the reported derivatives. However, the benzyl-cyclohexylmethyl and di(cyclohexylmethyl) analogues have both been obtained.

Using the enantiomerically pure phosphonous acids we have also been able to prepare these compounds with a degree of optical purity. Work is now in progress to develop and evaluate this route to provide individual enantiomers.

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