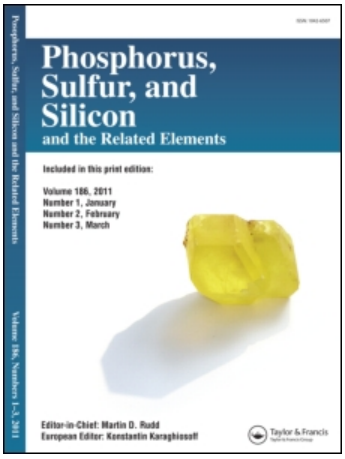

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THE SYNTHESIS OF PHOSPHONATE ANALOGUES OF AMINO ACIDS AND PEPTIDES

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Abstract Diphenyl phosphonate ester analogues of phenylalanine and peptides derived from these have been prepared and are shown to specifically inhibit chymotrypsin. Phosphonate isosters of serine and tyrosine phosphates appropriately protected for use in peptide synthesis have been prepared.

Phosphorus-containing amino acid analogues show a range of interesting biological properties[1]. Serine proteinases are known to be involved in a wide range of normal and pathological processes including emphysema, cystic fibrosis, cancer and myocardial infarction.[2]

We have synthesised a variety of targeted phosphonate analogues (e.g. Z.Phe.$^{P}(OR)_2$) and derived peptides as specific serine proteinase (e.g. chymotrypsin and trypsin) inhibitors. Although the alkyl phosphonate esters were inactive, the diphenyl esters had substantial and specific activity. The kinetic plots for the inactivation of chymotrypsin with Z.Phe.$^{P}(OPh)_2$ and derived peptides show that these phosphonates behave as potent competitive irreversible inactivators of this proteinase since (a) inhibition is time dependent and increases with t and (b) increasing inhibitor concentration leads to increasing rate of inhibition. Inhibitor constants of the simple Cbz.Phe.$^{P}(OPh)_2$ and other known inhibitors of chymotrypsin are shown in the Table.

Cbz.Phe.$^{P}(OPh)_2$ is substantially more active than the corresponding α–chloroketone which is an archetypal inhibitor of chymotrypsin. Extension of the chain length appropriately to a

tripeptide provides superb inactivators of chymotrypsin which are 10^3 to 10^4x more active than Cbz.Phe.$^P(OPh)_2$. Recently published work[3] on the activation of chymotrypsin, cathepsin G and elastase by similar peptide sequences confirms many of our results and indicates the stability of these compounds to a variety of degradation processes *in vitro*.

Inhibitor Sequence	K_i (μM)	k_3 (min^{-1})	k_3/K_i (M^{-1},min^{-1})
Cbz.Phe.$^P(OPh)_2$	56±11	1.3±0.3	2.3±0.1 x 10^4
Cbz.Phe.CH_2Cl	-----	-------	4.1 x 10^3 *
CBz.Phe.H	138	N.A.	N.A.
Boc.Ala.Phe. $^P(OPh)_2$	---	-------	1.1 x 10^2
Boc.Ala.Phe.H	1140 **	N.A.	N.A.

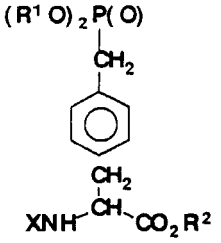
Taken from *Shaw and Ruscica (1971) and **Sharma *et al* (!979)

The mechanism of inhibition almost certainly involves phosphorylation of serine OH with consequent loss of OPh from phosphorus. We have synthesised biotin derivatives of Phe.$^P(OPh)_2$ and Val.$^P(OPh)_2$ and the fact that these compounds can be used as reagents to specifically detect the presence of chymotrypsin/cathepsin G and elatase, respectively, in SDS-PAGE is proof that covalent bonding occurs between the enzyme and the inhibitor.

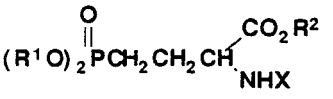
PHOSPHONATE ISOSTERS OF SERINE AND TYROSINE PHOSPHATES

Our interest in phosphonate analogues has recently encompassed phosphonate isosters (**1**) and (**2**) of phosphorylated serine and tyrosine and, ultimately, their incorporation into peptides. Protein phosphorylation/dephosphorylation *via* kinases and phosphatases, respectively, is an important control mechanism in cellular processes and it is likely that these enzymes play an important role in tumourgenesis.[4]

The selective modulation of the activity of such enzymes should allow a more precise determination of the contribution of an individual kinase or phosphatase to a metabolic process and may



(1)

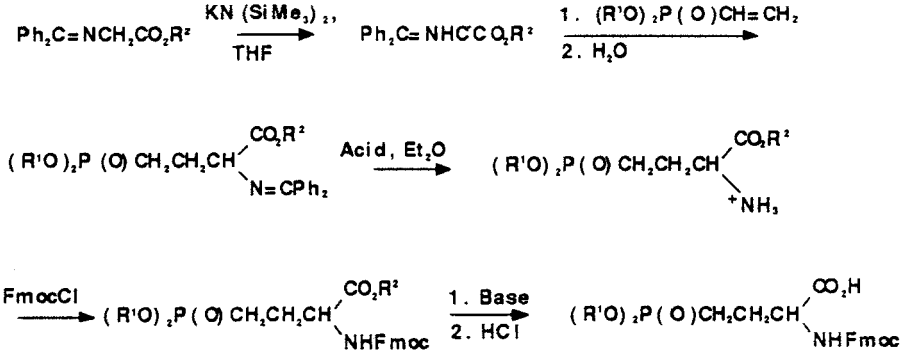


(2)

offer a rational and novel route to antitumour agents.

Available synthetic routes to (1)[5] and (2)[6] are generally long and do not provide convenient routes to derivatives appropriately protected at phosphorus and nitrogen but with the free carboxylic acid function required for peptide synthesis. We have now prepared such derivatives in good yield by short convenient routes, based on a method[7] previously used to prepare *fully unprotected* phosphinathricin derivatives.

We have obtained the fully protected isoster (Scheme 1).



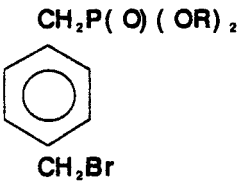
Scheme 1

Depending on the conditions used, we were able to selectively remove the imine protecting group or this group *and* the carboxylic ester function by acid treatment. This latter reaction is clearly very useful since, followed by N-protection, it provides, in three-steps, the phosphonate isoster in a form suitable for direct use in peptide synthesis. In no case did competitive phosphonate ester hydrolysis present a problem although small amounts of ester exchange occurred in mixed ester cases.

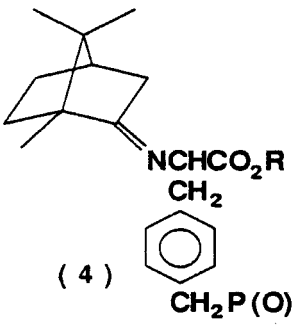
The alternative selective removal of the imine group alone, followed by Fmoc protection and mild alkaline hydrolysis also gave the free carboxylic acid derivative in excellent yield.

We have also prepared the tyrosine phosphonate isoster (**1**, R^2= **H**, R^1 = **Alkyl, Ar**) by a similar route from α,α'-dibromo-*p*-xylene using an Arbusov reaction with trialkyl phosphites or diphenylethyl phosphite to generate the phosphonate (**3**).

We are currently investigating routes, based on Scheme 1, to single enantiomers of (**1**) and (**2**). The tyrosine imine (**4**) has been obtained by this method as a 70:30 mixture of diastereomers which can be separated by HPLC.



(3)



(4)

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