Synthesis of diphenyl phosphonate analogues of tyrosine and tryptophan and derived peptides as chymotrypsin inhibitors

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The synthesis of α-aminophosphonate analogues of tyrosine and tryptophan, *e.g.* 4 and 5 respectively, and their incorporation into proline-containing dipeptides is reported; of the sequences synthesised, the dipeptide Z-Pro-Trp^{P}(OPh)$_2$ is the only derivative that functions as an irreversible inactivator of the serine proteinase chymotrypsin, in contrast, the tyrosine analogue 4 behaves as a competitive reversible inhibitor of the enzyme and the tryptophan analogue 5 behaves as a slow-binding inhibitor.

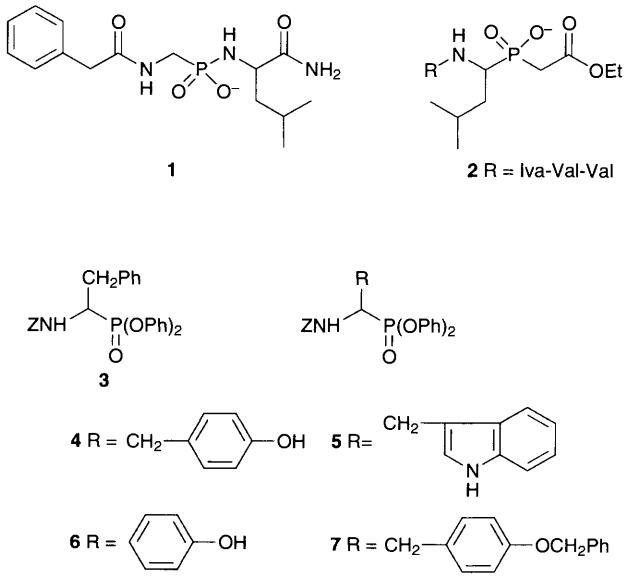
α-Aminophosphonates, when incorporated into appropriate recognition sequences, function as potent inhibitors of a number of peptidases and proteinases. For example, potent inhibitors of the zinc-dependent metallopeptidase thermolysin and the aspartyl proteinase penicillopepsin are obtained when the scissile amide bonds in appropriate substrate molecules are replaced by a phosphonic acid moiety to give analogues such as **1**[1] and **2**,[2] respectively.

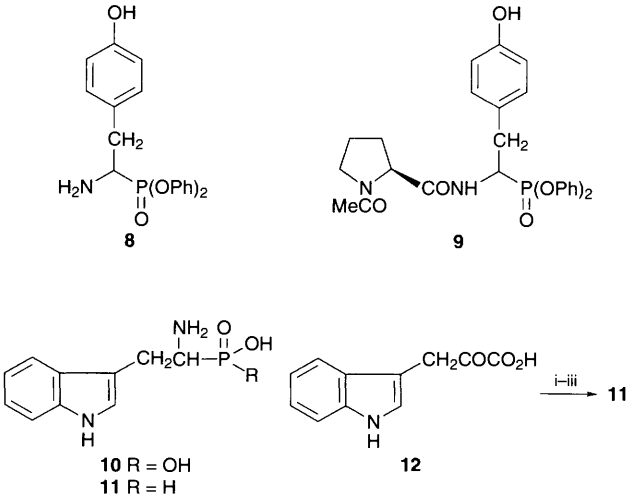
The high potency of these compounds is thought to be attributable to their ability to function as transition state analogues of the tetrahedral intermediate formed during the hydrolysis of the scissile amide bond of substrate molecules, catalysed by this class of enzymes.[3]

We[4] and others[5] have shown that phenyl esters of α-aminophosphonates act as highly efficient, irreversible inhibitors of serine proteinases, a large group of enzymes with a wide range of important biological functions, and yet show no activity towards the closely related cysteine proteinases. In addition, excellent levels of inhibitory discrimination between individual enzymes within the serine proteinase class can also be achieved using these reagents. For example, the diphenyl phosphonate **3**, an analogue of phenylalanine, irreversibly inhibits chymotrypsin with a second order rate constant of 4.5×10^4 dm^3 mol^{-1} min^{-1} † and yet shows no appreciable activity towards elastase or trypsin.

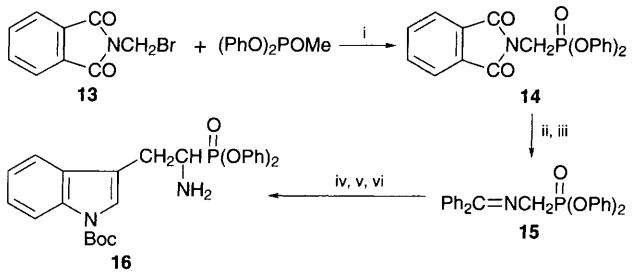
Although the phosphonic acid analogue of tyrosine has been reported[6] the methods of synthesis are not easily applicable to the corresponding and unknown diphenyl ester **4**. Oleksyszyn[7] has developed a convenient route which directly provides *N*-protected diphenyl esters of α-aminophosphonates through the reaction of triphenyl phosphite, benzyl carbamate, and the appropriate aldehyde. Reaction of 4-hydroxybenzaldehyde under these conditions provided the (4-hydroxyphenyl)glycine analogue **6** (76%).‡ In view of the difficulties associated with the synthesis of (4-hydroxyphenyl) acetaldehyde, required for the direct preparation of the tyrosine analogue **4** by this method, our preferred approach to **4** involved a similar reaction of the corresponding *O*-benzyl protected aldehyde to give **7** (74%). Deprotection could be achieved in poor yield (33%) by hydrogenolysis at 70 psi using 20% Pd(OH)$_2$ on carbon in acetic acid.§ However, reaction of **7** with 1% HBr in acetic acid at 20 °C for 72 h gave **8** as its hydrobromide salt in virtually quantitative yield. Treatment of **8** with *N*-(benzyloxycarbonyloxy)succinimide¶ and triethylamine in dry THF gave diphenyl 1-(*N*-benzyloxycarbonylamino)-2-(4-hydroxyphenyl)ethanephosphonate **4**, while coupling of **8** with *N*-acetyl-L-proline, using the mixed anhydride method, provided *N*-acetyl-L-Pro-Tyr^{P}(OPh)$_2$ **9** (73%).

The synthesis of the tryptophan phosphonic **10**[8] and phosphonous **11**[9] acid analogues, in low yields, has been reported. The former route is long and inefficient and in both approaches conversion to the unknown diphenyl ester **5** would not be trivial. An approach *via* the phosphonous acid **11**[9] (Scheme 1)‖ is attractive since we have prepared a range of such compounds





Scheme 1 *Reagents and conditions*: i, $Ph_2CHN^+H_3$ $H_2PO_2^-$; ii, H_3O^+; iii, propylene oxide



Scheme 2 *Reagents and conditions*: i, xylene, reflux; ii, NH_2NH_2. H_2O, AcOH, THF; iii, Ph_2C = NH; iv, $KN(SiMe)_3$, THF, −78 °C; v, (*N*-Boc-indol-3-yl)methyl bromide; vi, TFA, CH_2Cl_2

with high optical purity.[10] However, in our hands, this method (Scheme **1**) gave, at best, trace amounts of **11**.

The tryptophan analogue was ultimately prepared by the route shown in Scheme **2**. Although requiring a long reaction time, the Arbusov reaction of **13** with diphenylmethyl phosphite provides **14** in excellent yield. Deprotection and imine formation provided **15** which was alkylated, *via* its anion,[11] with indol-3-ylmethyl bromide. Finally, treatment with TFA in CH_2Cl_2 at room temperature achieved differential deprotection of the α-nitrogen to give **16** (45% overall from **13**) as a colourless oil. Treatment of **16** with benzyl chloroformate and triethylamine in dry CH_2Cl_2, followed by removal of the Boc protecting group with TFA, gave the diphenyl 1-(*N*-benzyloxycarbonylamino)phosphonate **5**, while a similar sequence of reactions involving coupling of **16** with *N*-Z-L-proline, using the mixed anhydride method, provided *N*- Z-L-Pro-Trp^P(OPh)$_2$ **17**.

Preliminary results show that each of the newly synthesised phenyl phosphonates, with the exception of **9**, exhibited inhibitory activity when tested against chymotrypsin. Of the sequences synthesised, the dipeptide Z-Pro-Trp^P(OPh)$_2$ is the only derivative that functions as an irreversible inhibitor of this serine proteinase, exhibiting a second-order rate constant for inactivation of $8.6 \pm 0.7 \times 10^4$ dm^3 mol^{-1} min^{-1}. In contrast, the simple tyrosine and tryptophan analogues **4** and **5**, respectively, interact with this proteinase in a reversible manner. The former acts purely as a competitive reversible inhibitor with $K_i = 0.86 \pm 0.09$ μmol dm^{-3}, whereas the latter behaves as a slow-binding reversible inhibitor with $K_{i(Final)} = 0.042 \pm 0.005$ μmol dm^{-3}. The basis for these differing inhibitory modalities is currently being investigated.

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Footnotes

† Incorporation of this analogue into the extended peptide sequence Boc-Val-Pro-Phe^P(OPh)$_2$ results in a 1000 fold increase in activity.

‡ All new compounds provided satisfactory analytical data and were characterised by mass spectrometry and ^{1}H, ^{13}C and ^{31}P NMR in $CDCl_3$ solution unless specified. *Selected data* for **4**: MS(ES) *m/z* = 503 (M)$^+$; δ_H (300 MHz) 2.95 (m, 1 H), 3.32 (m, 1 H), 4.76 (m, 1 H), 5.02 (s, 2 H), 5.19 (d, 1 H, ^3J 10 Hz), 6.66 (d, 2 H, ^3J 8.2 Hz), 7.0 (d, 2 H, ^3J 8.2 Hz), 7.1–7.4 (m, 15 H); $\delta_P\{^1H\}$ (202 MHz) 18.65. For **8**. HBr, oil, δ_H(300 MHz, $CDCl_3$/[$^2H_6$]Me_2SO) 3.45 (m, 2 H), 4.1 (m, 1 H), 6.8 (d, 2 H, ^3J = 7.5 Hz), 7.0–7.4 (m, 12 H); $\delta_P\{^1H\}$ (202 MHz, $CDCl_3$/[$^2H_6$]Me_2SO) 13.86. For **16**, MS(EI) *m/z* = 626 (M)$^+$, 292, 130 and 91; δ_H(300 MHz) 1.66 (s, 9 H), 3.02–3.10 (m, 1 H), 3.48–3.58 (m, 1 H), 3.71–3.79 (m, 1 H), 7.06–7.36 (m, 14 H), 7.52–7.56 (m, 2 H), 8.14–8.16 (m, 1 H); $\delta_P\{^1H\}$ (202 MHz) 21.46.

§ We have experienced substantial difficulty in carrying out hydrogenations in the presence of phosphonate and similar functions and these difficulties are compounded in the present case by competitive loss of phenyl ester groups under vigorous hydrogenation conditions.

¶ Attempts to prepare **4** by treatment of **8** with benzyl chloroformate and triethylamine lead instead to the *O*-benzyloxycarbonyl derivative.

‖ Indol-3-ylpyruvic acid **12** is used in place of indol-3-ylacetaldehyde due to the instability of the latter compound. Attempts to synthesise **5** directly from **12** by reaction with triphenyl phosphite and benzyl carbamate were unsuccessful.

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