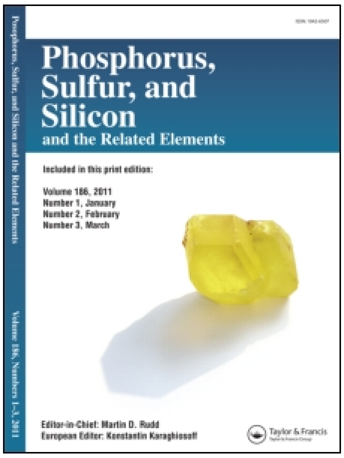

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SUBSTRATE RELATED O,O-DIALKYLDIPEPTIDYLY ψ CARBOXYBENZYLPHOSPHONATES, A NEW TYPE OF THROMBIN INHIBITOR

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SUBSTRATE RELATED *O,O*-DIALKYLDIPEPTIDYLΨ CARBOXYBENZYLPHOSPHONATES, A NEW TYPE OF THROMBIN INHIBITOR

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O,O-Dialkyl α-hydroxybenzylphosphonates (**1**) were coupled to substrate derived dipeptides (**2**), using an active ester methodology approach to generate a series of phosphonotripeptides (**3**) with a carboxy ester linkage between the "P1" and "P2" positions of the molecule. These compounds inhibit thrombin in the micromolar range.

Keywords: carboxyesters; pseudopeptides; thrombin; thrombin inhibitors

INTRODUCTION

Peptides possessing surrogate amide bond replacements have stimulated considerable interest in recent years; as they have been used to investigate a wide variety of biological systems.[1] Many of the isosteric modifications developed offer greater resilience toward proteolytic degradation; and elicit a higher degree of conformational freedom. A series of phosphorus derived carboxyester pseudopeptide analogues based upon the "fibrinogen-like" recognition sequence, "Phe-Pro-Arg" have been prepared,

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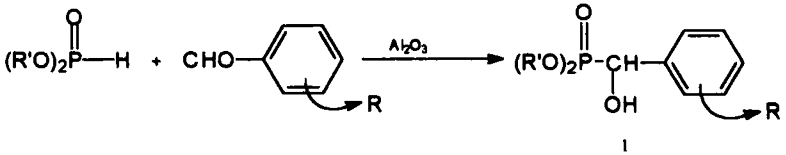
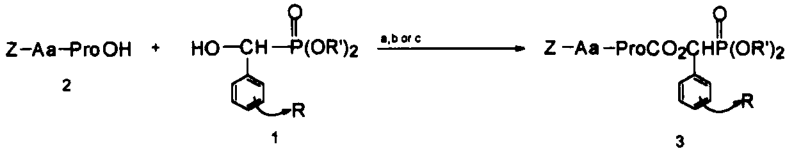
where there is an increase in the flexibility of the "P1-P2" bond, as compared to the corresponding position in the aminophosphonate analogues.[2] This has farreaching implications for the design of anti-thrombotic agents with a greater specificity toward thrombin.[3]

The *O,O*-dialkyldipeptidylycarboxybenzylphosphonates (**3**) were synthesised by coupling biologically active *O,O*-dialkyl α-hydroxybenzylphosphonates (**1**, prepared by the Pudovik reaction on Al_2O_3 at R.T., Scheme 1[4]), to substrate derived dipeptides (**2**, Scheme 2).[5] The coupling reagents used were: (a) N,N′-carbonyldiimidazole (CDI) in the presence of allyl bromide or methyl iodide;[6] (b) 1-hydroxybenzotriazole (HOBt) in the presence of dimethylaminopyridine (DMAP) and dicyclohexylcarbodiimide (DCC);[7] and (c) 1-hydroxy 7-azabenzotriazole (HOAt) in the presence of DMAP and DCC.[8] The phosphonotripeptides (**3**) were purified by gel filtration through Sephadex LH20, using methanol as the eluting solvent, and were generally isolated in good yield (see Table) as white, fluffy, crystalline powders. Method (a) was found to be the easiest to manipulate, due to the formation of a negligible amount of by-product.



SCHEME 1



SCHEME 2

TABLE *O,O*-Dialkyldipeptidylψcarboxybenzylphosphonates (**3**)

Entry	Aa		*R′*	*d* ^{31}*P(CDCl$_3$)* *(ppm)*	*K$_i$* *(μm)*	*Method*	*Yield* *(%)*
1	D-Dpa	3-CF_3O	iPr	15.16, 15.18	20.3	a	62
2	D-Phe	4-CH_3CH_2O	iPr	16.49, 16.58	13.62	a	83
3	D-Dpa	2,3,4-$(CH_3O)_3$	iPr	17.05, 17.11	19.4	a	76
4	DL-Dpa	3-CH_3O	iPr	16.02–16.28	206.0	a	85
5	DL-Dpa	3-NO_2	Et	16.33–16.96	107.0	a	52
6	D-Phe	4-CH_3O_2C	iPr	15.13, 15.21	77.3	b	87
7	D-Dpa	4-CN	Et	16.54, 16.71	55.5	c	41
8	D-Dpa	3,4,5-$(CH_3O)_3$	Et	17.70, 18.26	28.5	b	46
9	DL-Dpa	4-CH_3O_2C	Et	17.01–17.53	260.0	b	72
10	DL-Dpa	H	Et	17.86–18.31	16.0	b	89
11	DL-Dpa	4-NO_2	Et	16.20–16.81	6.2	b	58

NB (a) CDI in the presence of allyl bromide or methyl iodide; (b) HOBt/DCC in the presence of DMAP; (c) HOAt/DCC in the presence of DMAP.

RESULTS AND DISCUSSION

The compounds were fully characterised by elemental analysis; ^{1}H, ^{13}C, ^{31}P and, where appropriate, ^{19}F N.M.R. spectroscopy; FABMS (3-NOBA) or electrospray MS (methanol). Authentication by ^{31}P N.M.R. spectroscopy showed that the products (**3**) exhibited typically a higher field signal for the phosphorus centre (doublet or doublet of doublets, ca 15 – 18 ppm); as compared to the α-hydroxyphosphonate precursors (**1**) which resonated as lower field singlets (ca 20 – 22 ppm; cf also peptidylaminophosphonates ca 16 – 28 ppm).[2] This feature is synonymous with an electron-rich phosphorus species. The absence of the latter signal showed that the reaction had been completed (any unreacted α-hydroxyphosphonate [**1**] was found to have a much longer retention time on the Sephadex LH20 column, and could therefore be conveniently separated). The compounds (**3**), afforded in comparable yield to the peptidylaminophosphonates,[2] do not appear to be affected by racemisation of the L- proline centre following coupling; as evidenced by observation of the expected number of signals in the ^{31}P N.M.R. spectra. These new antithrombotic agents competitively inhibit thrombin in the micromolar range (i.e. similar potency to the peptidylaminophosphonates), and due to their relatively simple preparation provide enormous scope for structural modification. It is envisaged that chiral α-hydroxyphosphonates[9] may be similarly coupled to peptides based upon the "Phe-Pro-Arg" sequence and provide the enantioselectivity[10] that is required for clinically viable and potent antithrombotic drugs.

Acknowledgements

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This oily residue was gel-filtered through Sephadex LH20 using MeOH as the eluting solvent. The eluant was concentrated under reduced pressure to afford the compound (**3**) as a white fluffy solid.

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