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A Convenient Synthesis of N-Protected Diphenyl Phosphonate Ester Analogues of Ornithine, Lysine and Homolysine.

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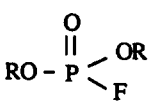
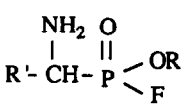
Abstract: *A 3-step synthesis to the title compounds has been developed which provides them differentially protected at nitrogen. These could then be selectively deprotected using hydrazine hydrate or hydrogenolysis over Pd/C.*

Phosphorus based reagents[1] have played an important and diverse role in the investigation of many physiological processes. Of particular interest over recent years has been their effective use as transition state analogue inhibitors[2] of the serine protease family of enzymes [trypsin, chymotrypsin, elastase, etc.]. These enzymes are known to be involved in many normal and pathological states, and their inopportune activation can result in the exacerbation if not the triggering of a range of diseases such as emphysema, cystic fibrosis, cancer, etc. In view of this the development of specific inactivators of this class of enzyme offers a basis for the rational design of therapeutic agents and the unequivocal assessment of the contribution of individual enzymes to homeostasis and pathophysiology.



(1)



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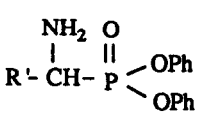


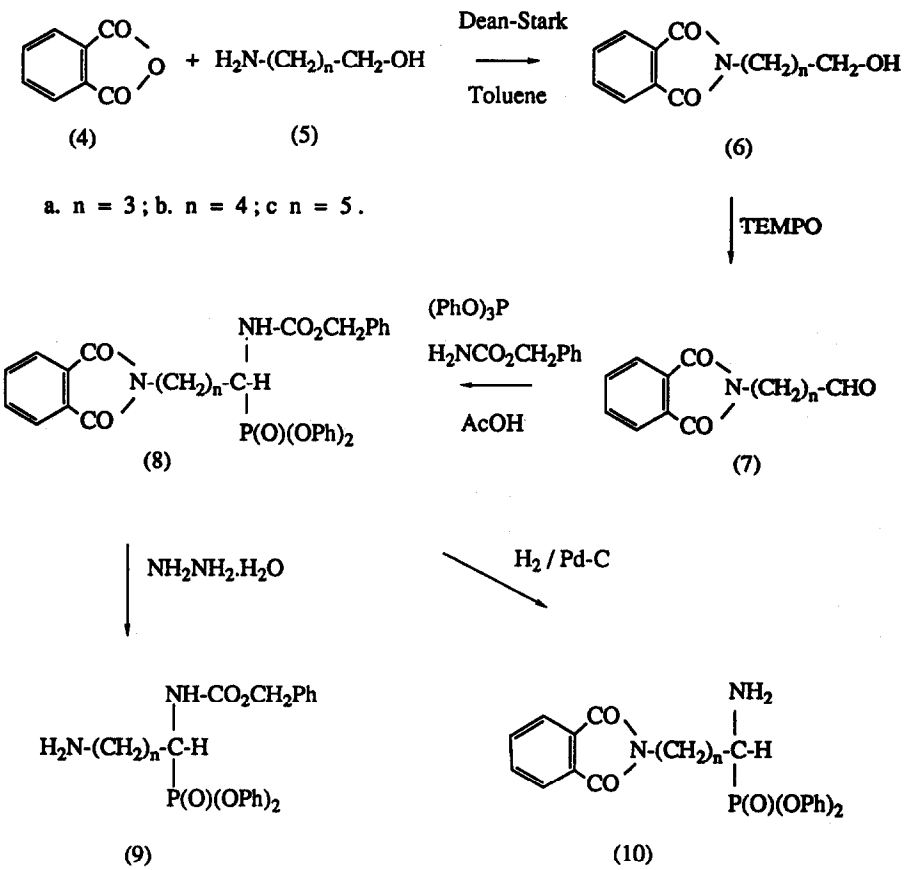
(3)

The classical inhibitors of these enzymes, fluorophosphates such as diisopropyl fluorophosphate [D.F.P.] [(1), R = -$CH(CH_3)_2$] are known[3] to react stoichiometrically with the active site serine via a phosphorylation reaction forming a covalently bound adduct. However such inhibitors are nondiscriminatory in action since they lack the structural features essential to normal substrate-enzyme selectivity.

α–Aminoalkyl phosphonic acid derivatives (2)[4] on the other hand, which are analogues of the natural

amino acids involved in normal peptidyl recognition sequences should have a much greater potential for providing more selectivity in inhibition. To react effectively with the active site the phosphonic acid residue in these required activation by the presence of a good leaving group at phosphorus. However, this had the disadvantage of greatly reducing inhibitor stability at biological pH thereby limiting considerably their usefulness.

More recently α-aminoalkyl diphenyl phosphonate ester analogues of peptidyl amino acids [(3),R' = $CH(CH_3)_2$, $-CH_2Ph$] have also been examined[5] as possible inhibitors. The results show that these are able more effectively to combine stability at biological pH with reactivity at the active site, and are highly specific irreversible inhibitors of serine protease enzymes.



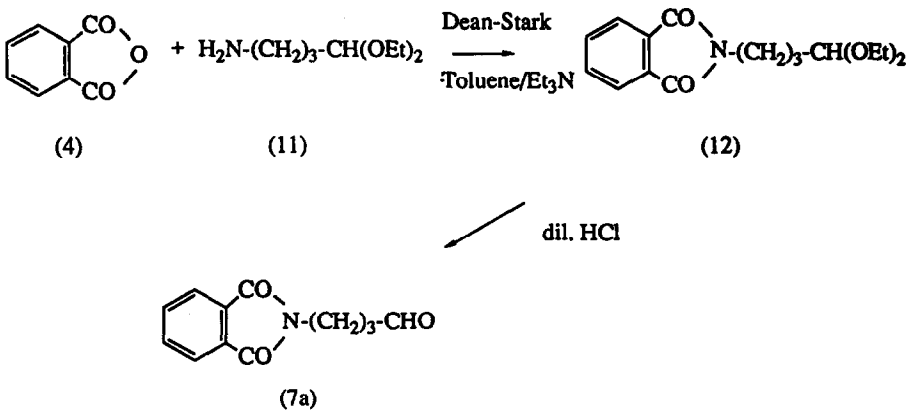
Scheme 1.

As an extension of our work in this area we required to synthesise the analogous diphenyl phosphonate esters of ornithine, lysine, and homolysine. A previous synthesis of the ornithine and lysine analogues has been reported[6] but this provided them in their unprotected form by a method not easily adaptable to our needs. A

lengthy preparation of the m-chlorophenyl phosphonate analogue of lysine has also been published[7] but this has an acetate group attached at the α-nitrogen. It would be expected that the conditions required for its removal would also result in deprotection at phosphorus. Since neither route was appropriate to our needs we have developed a short convenient synthesis of these compounds which we now wish to report[8]. Our approach is outlined in Scheme 1.

The main requirement for our route was to provide the diphenyl phosphonate ester analogues differentially protected at nitrogen to allow for their selective deprotection during peptide synthesis. Of the available procedures capable of being adapted to our needs the amidoalkylation of triphenyl phosphite first reported by Oleksyszyn et.al[9]. seemed most applicable. This required preparation of suitably protected amino-aldehydes. The phthalimido protection group was chosen for this as it is convenient to incorporate and is also able to resist the conditions used for assembly of the N-C-P skeleton, and the deprotection of the α-amino group. A drawback to its use is the inconsistency sometimes experienced during removal with hydrazine hydrate. However, in our case no such problems were experienced.

The procedure involves initial preparation of phthalimido protected amino - alcohols (6) by heating equivalent amounts of phthalic anhydride (4) and amino-alcohol (5) in toluene under Dean-Stark conditions. This gives the Pth-alcohols (6) in high yield (up to 98%). Subsequent TEMPO-catalysed hypochlorite oxidation[10] gave the Pth-aldehydes (7) (90-95%). Treatment of the Pth-aldehydes (7) with benzyl carbamate (1 Equiv.) and triphenyl phosphite (1 Equiv.) in glacial acetic acid at 80-85 °C for 1hr. provided the crude products. Evaporation under vacuum on a rotavapor gave a reddish-brown oil which was then dissolved in methanol and left at -20 °C for 2-3 hrs. The white solid which precipitates could be recrystallised from methanol to give the N-protected diphenyl phosphonate esters (8) in 50-55% yield[11].



(4) (11) (12)

(7a)

Scheme 2.

Selective deprotection of these compounds could be achieved at the α–nitrogen by catalytic hydrogenolysis over Pd/C in ethanol at atmospheric pressure[12], and at the terminal nitrogen by stirring at r.t. over 3 days with 100% hydrazine hydrate[13] (1.2 equiv.) in T.H.F.

The aldehyde used to prepare the ornithine analogue can also be obtained from 4-aminobutyraldehyde diethyl acetal (11) Scheme 2. by first forming its Pth-protected derivative (12) under Dean-Stark conditions in the presence of triethylamine(10% in toluene). Hydrolysis of the Pth-protected acetal (12) using dil.HCl at room temperature provided the Pth-protected aldehyde (7a),(95-98%).

We are currently examining these compounds and their derivatives as possible inhibitors of trypsin-type enzymes.

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11 The spectral data for all compounds are in accord with their structures. Data for compounds (8) are as follows: (**8a**): n=3, M.P.72-74 °C; $C_{32}H_{29}N_2O_7P$ Requires: C 65.75, H 4.97, N 4.79, Found: C 65.35, H 4.99 N 4.73; ^{1}H NMR($CDCl_3$, 300M Hz) δ 1.7-2.2(m, 4H, -CH_2CH_2-), 3.75(t,2H, N-CH_2-), 4.5(m, 1H, C-H), 5.12 (q, 2H, OCH_2Ph), 5.25(d, 1H, -NH), 7.02-7.40(m, 15H, Ar- H), 7.7(m), 7.83(m), (4H,-Pth): (**8b**): n=4, M.P.112-114 °C; $C_{33}H_{31}N_2O_7P$ Requires: C 66.22, H 5.18, N 4.68, Found: C 65.90, H 5.16, N 4.65; ^{1}H NMR($CDCl_3$, 300M Hz) δ 1.5 - 2.2(m, 6H, -$(CH_2)_3$-), 3.70(t, 2H, N-CH_2-), 4.5(m, 1H, -CH), 5.12(q, 2H, OCH_2Ph), 5.25(d, 1H, NH), 7.02-7.40(m, 15H, Ar-H), 7.7(m), 7.83(m), (4H,-Pth); (**8c**): n=5, M.P. 105-107 °C; $C_{34}H_{33}N_2O_7P$ Requires C 66.67, H 5.39, N 4.58, Found: C 66.36, H 5.35, N 4.56; ^{1}NMR($CDCl_3$, 300M Hz) δ 1.2-1.8(m, 6H, -(CH_3)-), 1.90-2.10(m, 2H, -CH_2-CH), 3.70(t, 2H, N-CH_2-), 4.5(CH_2),5.12(m,3H, NH and OCH_2Ph), 7.02-7.40(m, 15H, Ar-H), 7.7(m), 7.9(m), (4H,-Pth).

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