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TOWARDS NEW INHIBITORS OF D-ALANINE:D-ALANINE LIGASE : THE SYNTHESIS OF 3-AMINO BUTENYLPHOSPHONIC AND AMINOPHOSPHONAMIDIC ACIDS.

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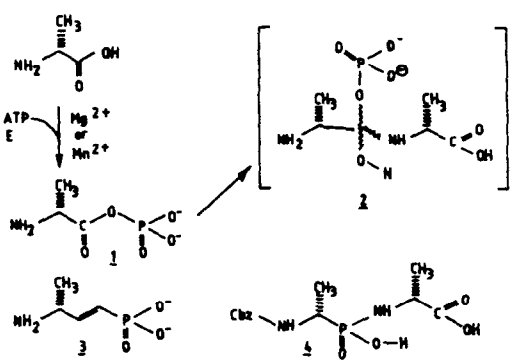
SUMMARY : The condensation of sodium salt of tetraethyl methylenediphosphonate with N-Cbz alaninal followed by the standard acidolytic removal of protecting groups provides an efficient method for the synthesis of 3-aminobutenylphosphonic acid E 3 ; N-Cbz-aminophosphonamidic acid 4 was prepared from diphenyl 1-amino ethylphosphonic acid through methyl phosphochloridate alanine methyl ester condensation in the presence of triethylamine and alkaline partial deprotection.

Many clinically useful antimicrobial agents display their selective toxicity by interfering with the biosynthesis of peptidoglycan, a structure only present in bacteria (1). The dipeptide D-alanyl-D-alanine is known to be an essential precursor of this macromolecule. Its biosynthesis involves the initial conversion of the L-alanine of the host to D-alanine by alanine racemase, followed by the coupling of two molecules of D-alanine by a D-alanine: D-alanine ligase (2). In recent years, inhibitors of alanine racemase have been extensively studied (2,3) whereas little information on the ligase has been reported. Among the described substrate or product analogues (4-7), no suicide or transition state inhibitors have been designed so far for the D-alanine:D-alanine ligase.

An efficient approach to the synthesis of potent selective enzyme inhibitors has been the design of molecules which mimic the presumed transition state of the enzyme catalytic reaction (8-10). As the ligase is known to require ATP for its activity (activation of the carboxylic acid function as a mixed anhydride), and to possess two binding sites for D-alanine, one can imagine the reaction to proceed in two steps as shown in scheme 1 (2,4).

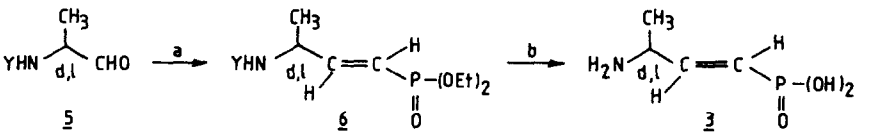


SCHEME 1 : Postulated mechanism of D-Alanine : D-Alanine ligase

Therefore, it may be expected that 3-aminobutenylphosphonic acid 3 could be a stable analogue of the hypothetical transient D-alanyl acylphosphate 1. Likewise, α-aminophosphonamidic acid 4 may be stable mimic of the tetrahedral carbon intermediate 2 possibly involved in the second step of D-alanyl-D-alanine biosynthesis. This strategy has been successfully investigated for the discovery of new potent peptidase inhibitors (11-15).

We want to report here the first results obtained in the synthesis of these new rationally designed inhibitors 3 and 4.

The well-documented Wittig-Horner condensation of the tetraalkyl methylenediphosphonate with a suitable aldehyde (16-18), was selected to synthesize protected amino-3-butenylphosphonic esters 6. Accordingly, the reaction of N-Cbz-alaninal 5 (19) with the sodium salt of tetraethyl methylenediphosphonate (20) proceeded in good yield to give 6 (scheme 2).

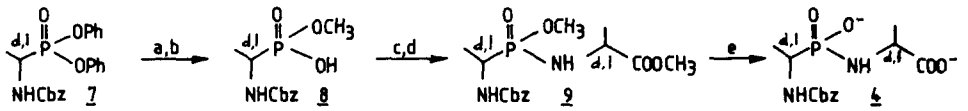


SCHEME 2 . Y = BOC, Cbz ; a : $NaCH\left[P(OEt)_2\right]_2$; b : HCl - AcOH

Like the related reactions of stabilized ylides with aldehydes, this condensation shows a preference for the formation of the more stable E-vinylphosphonate 6. Furthermore, we have found that the standard acidolytic removal of protective groups afforded the desired unsaturated phosphonic acid 3 in good yield.

In a typical procedure, to a solution of 5 (Y=Cbz : 3 g, 0.015 mole) in dry THF was added dropwise at 0° the sodium salt of tetraethyl methylenediphosphonate (3.2 g, 0.11 mole) in THF under nitrogen. After 3 h stirring, the reaction mixture was poured into water. The usual extractive work-up and purification on silicagel chromatography gave pure 6 (Y=Cbz) as a colourless oil (2.1 g, 62 % yield) (21). For deprotection, 6 (Y=Cbz : 1.7 g, 0.005 mole) was dissolved in glacial acetic acid (5 ml) and hydrochloric acid (50 ml), heated under reflux for 7-8 h (7). After removal of solvents, dissolution of the residue in ethanol, and precipitation with propylene oxyde, the pure 3 is obtained as a white solid (0.53 g, 70 % yield, M.P. 220°C) (21).

The fully protected α-aminophosphonamidic acid 9 was synthesized from the readily available diphenyl 1-aminoethylphosphonic acid 7 (22) as starting material, as depicted in scheme 3 :



SCHEME 3 - a : CH_3OH KF, 18CROWN-6 ; b:NaOH/dioxane ; c : $SOCl_2$ / $CHCl_3$; d : Ala - OMe , NEt_3 ; e : NaOH

The coupling of phosphochloridate with a protected amino-acid has been proved useful to prepare phosphinic acid dipeptide analogues (11-15,23). Thus, after methanol ester interchange, partial alkaline hydrolysis and $SOCl_2$ chlorination, the resulting phosphochloridate reacted with alanine methyl ester in the presence of triethylamine affording N-benzyloxycarbonyldimethyl ester phosphonamidate 9. Alkaline cleavage of carboxyl and phosphonamidic methyl esters was achieved finally to give 4 in satisfactory yield.

Typically, the diphenyl phosphonate 7 (20.6 g, 0.05 mole) and potassium fluoride dihydrate (46.9 g, 0.5 mole) dissolved in 300 ml methanol and 18-crown-6 (0.5 g) were heated to boiling 15 mn and left at room temperature overnight (24). Usual extractive work-up and purification gave the oily dimethyl phosphonate (12.56 g, 88 % yield) which was dissolved (4 g, 0.014 mole) in 60 ml dioxane and stirred overnight with a solution of sodium hydroxyde N in 60 ml water. The solvent was removed, the residue dissolved in 90 ml water and acidified to pH=1. The methylphosphonate 8 precipitated as a white solid (M.P. 108°C, 44 % yield) (2). To a solution of 8 (1.47 g, 5.4 mmoles) in $CHCl_3$ (10.5 ml), $SOCl_2$ (0.39 ml, 5.4 mmoles) was added. After 4 h stirring the mixture was evaporated under vacuo to provide the crude phosphochloridate which was dissolved in $CHCl_3$ (10 ml) and cooled. A solution of methylalaninate (0.9 g, 6.48 mmoles), trimethylamine (1.2 g, 11.9 mmoles) and $CHCl_3$ (10 ml) was then added dropwise and left under stirring for 20 mn. After work-up, the crude residue was purified on silicagel column chromatography and the pure oily phosphonamide 9 was obtained (M.P. 4°C, 36 % yield) (21). The methyl ester protecting groups was conveniently removed by basic hydrolysis of 9 (0.63 g, 1.76 mmole) with a solution of NaOH, 2N (2.63 ml) on stirring 24 h at room temperature. Then lyophilisation afforded 4 as a carboxylic and phosphinic sodium salt (0.850 g).

The phosphonic 3 and phosphonamidic 4 compounds are indeed good competitive inhibitors of D-alanine:D-alanine ligase. They have Ki's close to 10^{-6}M a value which is two degrees of magnitude smaller than the Km.

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21. Isolated yield. Selected analytical data. Compounds :
    6 : ^{1}H-NMR ($CDCl_3$/TMS int.) δ ppm : 5.76, 6.70 (ABXY system, 2H, $J_{H_1H_2}$=17.1 Hz ; $^{2}J_{H_1P}$=18.9 Hz ; $^{3}J_{H_2P}$=21.8 Hz ; $^{3}J_{H_2H_3}$=4.6 Hz ; $J_{H_1H_3}$=1.7 Hz) ; 4.46 (m, 1H).
    3 : ^{13}C-NMR (D_2O, 250 MHz) δ ppm : 140.92 ; 127.92 (d, $^{1}J_{CP}$=175 Hz) ; 49.75 (d, $^{3}J_{CP}$=22) ; 18.38.
    8 : ^{1}H-NMR ($CDCl_3$/TMS int.) δ ppm : 1.35 (dd, 3H, $^{3}J_{HH}$=7 Hz, $^{3}J_{HP}$=17 Hz) ; 3.55 (d, 3H, $^{3}J_{HP}$=10 Hz).
    9 : ^{1}H-NMR ($CDCl_3$/TMS int.) δ ppm : 1.1-1.8 (m, 6H) ; 3.5-4.4 (m, 9H) ; M.S. (NH_3) : 376 $(M+NH_4)^+$, 359 $(M+H)^+$, base peak, 108 $(PhCH_2OH)^+$.
    4 : ^{1}H-NMR (D_2O) : δ ppm : 1.02 (m, 6H) ; 3.39 (m, 2H).
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