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SYNTHESIS OF POTENTIAL INHIBITORS OF THE ENZYME ASPARTATE TRANSCARBAMOYLASE [1]

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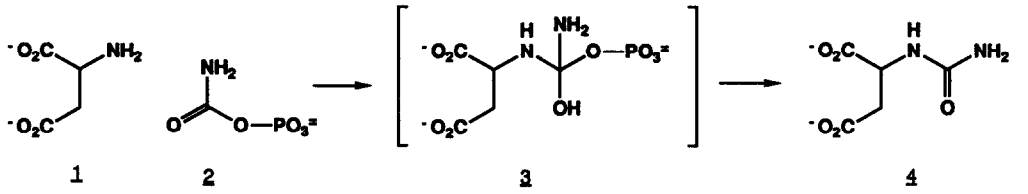
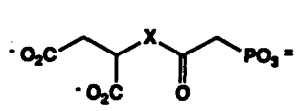
***ABSTRACT**: Two difluoromethylenephosphonates, 7 and 8, and the phosphinylmethylenephosphonate 9 were prepared as potential inhibitors of aspartate transcarbamoylase (ATCase). The synthesis of compound 8 was facilitated by development of a new direct method for converting nitroalkanes to alkanoic acids. Of the three phosphonates, the amide 7 proved to be a good inhibitor of mung bean ATCase (I_{50} ~ 5μM).*

Aspartate transcarbamoylase (EC 2.1.3.2., ATCase) catalyses the condensation of L-aspartate (1) with carbamoyl phosphate (2) to give N-carbamoyl-L-aspartate (4). This is the first committed step in de novo pyrimidine biosynthesis, and inhibitors of this enzyme are of potential interest both as agrochemicals[2] and pharmaceuticals.[3]



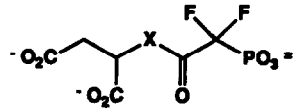
1 2 3 4

N-(Phosphonoacetyl)-L-aspartate, PALA (5)[4] and (4,5-dicarboxy-2-ketopentyl)phosphonate, DIKEP (6)[5] are both potent inhibitors of ATCase (K_i values about 10^{-8} M and 10^{-7} M, respectively). These compounds are multi-substrate analogues,[6] which may also exhibit some transition state characteristics.[3] In both PALA (5) and DIKEP (6) a methylenephosphonate group has been used as a stable replacement for a phosphate moiety. Although this is a good isosteric replacement, the difference in electronegativity between oxygen and methylene carbon means it is less good electronically. Work by Blackburn et al[7] led us to postulate that this electronic disparity would be overcome in the difluoromethylenephosphonate analogues 7 and 8, thereby leading to tighter enzyme binding. In addition, the highly electrophilic nature of the difluoroketone 8 means that it should readily react with a water molecule (possibly one of those present in the active site[8]) to give the hydrate 10. Both 10 and the phosphinate 9 can be regarded as mimics of the high energy tetrahedral reaction intermediate 3. "Transition state" inhibitors based on α-fluoroketones or tetrahedral phosphorus are well documented, and have the potential for very tight binding.[9] This communication describes the synthesis and ATCase-inhibitory properties of the three phosphonates 7, 8 and 9.



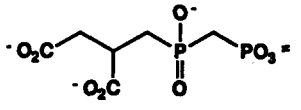
5 X = NH

6 X = CH_2



7 X = NH

8 X = CH_2



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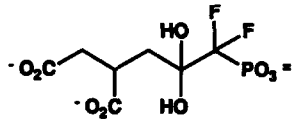

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The amide 11[10] ($[\alpha]_D^{25}$ 39°(c 2, $CHCl_3$)) was synthesised by coupling L-aspartic acid dimethyl ester hydrochloride with diethyl difluorophosphonoacetic acid[11] (Scheme I). De-esterification with trimethylsilyl bromide[12] followed by hydroxide yielded the required amide 7, isolated as the tetra-lithium salt monohydrate ($[\alpha]_D^{25}$ 3.6° (c 2, H_2O)).[10]

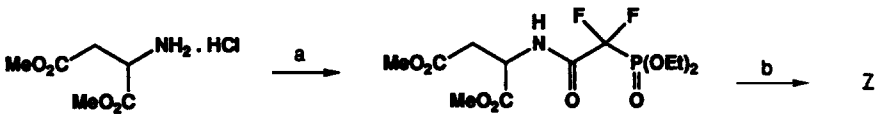
The ketone 8 was synthesised as outlined in Scheme II. Michael addition of nitromethane to diethyl itaconate yielded the nitro compound 12.[10] This material was cleanly converted by basic buffered $KMnO_4$ into the carboxylic acid 13.[10,13] This reaction is noteworthy in that although $KMnO_4$ has been used to convert nitroalkanes to aldehydes,[14] and aldehydes to acids,[15] it has not been previously reported as a reagent for direct conversion. In fact we have been able to find very few alternative mild methods for achieving this transformation.[16] Our method tolerates a variety of functional groups (ester, amide, primary alcohol, benzylic methylene) and generally gives analytically pure products without chromatography. The acid 13 was converted with oxalyl chloride[17] into the acid chloride 14, which was directly coupled with [(diethylphosphinyl)difluoromethyl]zinc bromide[18] to yield 15.[10] De-esterification as before yielded the ketone 8 (again isolated as the tetralithium salt monohydrate)[10] in 42% overall yield.

The synthesis of the final target, the phosphinate 9, was achieved as shown in Scheme III. Michael addition of ethyl hypophosphite[19] to diethyl itaconate yielded the phosphinate 16.[10] The sodium salt of 16 was condensed with diethyl phosphonomethyltriflate[20,21] to yield 17.[10] This reaction is of interest in that, coupled with recent advances in the synthesis of P-H phosphinates,[22] it provides a new method for preparing the biologically interesting phosphinylmethylenephosphonates.[23] Finally, acid catalysed hydrolysis yielded the phosphinate 9, isolated as the pentasodium salt dihydrate.[10]

Compounds 7, 8 and 9 were tested for activity against mung bean ATCase.[24] Unfortunately, both the difluoroketone 8 and the phosphinate 9 were inactive at the highest concentrations measured (100 μM). This may be because ATCase catalyses via a "closed transition state",[25] so that it can only bind inhibitors with a ground state trigonal carbonyl. The difluoroketone 8 probably exists as the hydrate 10 under the assay conditions,[26] and is thus unable to bind. Gratifyingly, the amide 7 was a good inhibitor of ATCase (I_{50} ~ 5μM), though it was less tightly bound than PALA 5 (I_{50} ~ 1μM). It has been suggested that the carbonyl group of PALA is protonated at the active site of ATCase.[27] This may, at least in part, explain the difference in inhibition levels of 5 and 7. None of the compounds exhibited any significant agrochemical activity.

ACKNOWLEDGEMENTS: We wish to thank Drs A.C. Baillie, C.G. Earnshaw, K. Wright and the late Mr B.J. Wright of Schering for helpful discussions during the course of this work. We also wish to thank Mr. J. Cant for performing the enzyme assays.

SCHEME I



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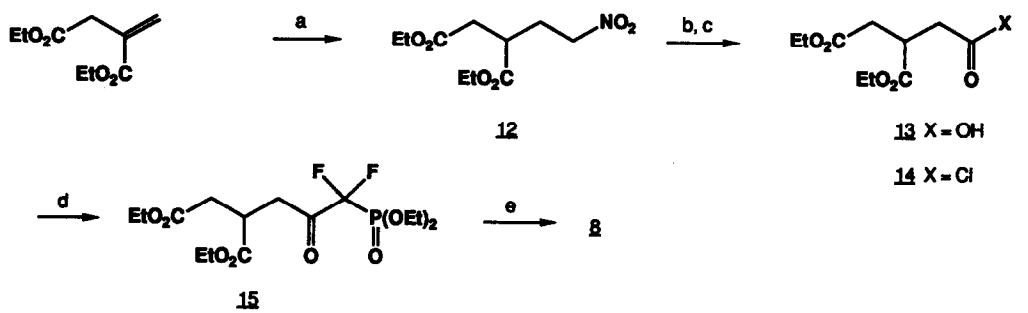
(a) 1eq $(EtO)_2P(O)CF_2CO_2H$, 1eq 1-hydroxybenzotriazole, 1.1eq DCC, CH_2Cl_2, r.t., 2 days; 46%. (b) (i) 5eq TMSBr, r.t., 5h; (ii) 1M NaOH, r.t., 5h; (iii) 'Amberlite' IR-120, H^+ form; (iv) 4eq LiOH; 98%.

SCHEME II



12

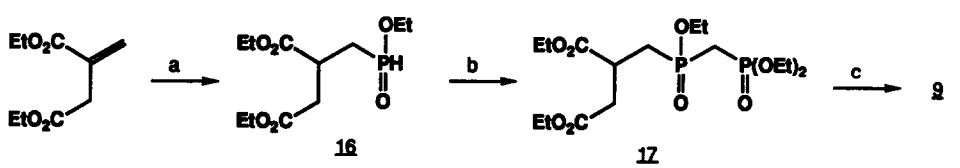
13 X = OH

14 X = Cl

15

(a) 40eq CH_3NO_2, 1eq NaOEt, EtOH, Δ, 5h; 73%. (b), to give 13: (i) 2eq KOH, 5eq K_2HPO_4, tBuOH, H_2O then 4eq $KMnO_4$, 25°C, 30 min; (ii) sat'd Na_2SO_3; (iii) 2M HCl; 96%. (c) to give 14: 1.05 eq $(COCl)_2$, PhMe, 40°C, 3 h; quant. (d) 1.05 eq $(EtO)_2P(O)CF_2ZnBr$, 0.08 eq CuBr, DME, 0°C→rt, 20h; 60%. (e) (i) 5 eq TMSBr, r.t., 3 days; (ii) 1M NaOH, r.t., 5h; (iii) 'Amberlite' IR-120, H^+ form; (iv) 4eq LiOH; 98%.

SCHEME III



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17

(a) 1eq $H_2P(O)OEt$, EtOH, 80°C, 24 h; 41%. (b) 1eq $(EtO)_2P(O)CH_2OSO_2CF_3$, 1eq NaH, DME, r.t., 80 min; 74%. (c) (i) 6M HCl, Δ, 16 h; (ii) 5eq NaOH; 73%.

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