Inhibitors of Pyrimidine Biosynthesis. Part 1. Synthesis of Potential Transition-state Analogues of Aspartate Transcarbamylase

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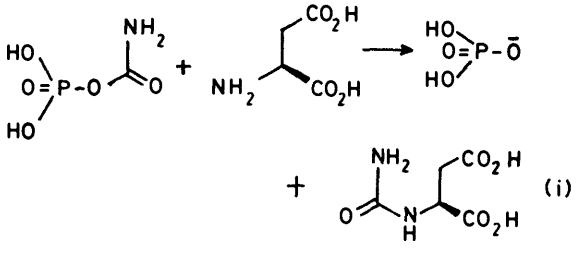
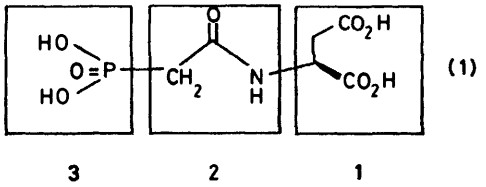
A systematic variation of the structure of a transition-state analogue of aspartate transcarbamylase has been carried out. A new, and general, synthesis of these analogues, starting from the appropriate amino-acid is described.

THE *de novo* biosynthesis of pyrimidines from non-pyrimidine precursors comprises six enzymically catalysed steps converting hydrogencarbonate and ammonia into uridylic acid (UMP). Inhibition of this pathway has been established as a good therapeutic target.[1,2] In particular, blockage of the pathway should be lethal to organisms, such as the malarial parasite,[3] which rely exclusively on biosynthesis for their pyrimidine requirements for nucleic acid production.

Chemical and biochemical efforts to exploit this target have concentrated on the later stages of the pathway involving transformations of the fully formed pyrimidine ring (orotate to UMP) and it seemed attractive to us, for a variety of reasons, to attempt to inhibit the earlier steps of the pathway. The justifications for this approach were that the area had been little explored; that the allosteric control point would be early on the pathway (and once defined would be a good target), and that enzymological differences between species had been described.[4] The subject of this paper is the design and synthesis of a series of potential inhibitors of the second enzyme in the pyrimidine biosynthetic pathway, aspartate transcarbamylase.



(i)



(1)

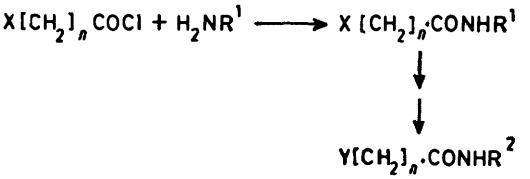
3 2 1

The enzyme catalyses the nucleophilic substitution reaction between L-aspartic acid and carbamoyl phosphate giving carbamoyl-L-aspartate and phosphate [eqn. (i)].

Phosphonoacetyl-L-aspartic acid (PALA) (1), a putative transition-state analogue, is a potent inhibitor of the enzyme [5] and has anti-proliferative [6,7] and antitumour activity [8,9] in culture. We have used this structure as a template for a systematic investigation of the requirements for binding to the enzyme, and have developed a new and quite general synthesis of PALA and analogues.

For the purposes of structural variation it is convenient to consider the analogue (1) as a combination of three units: 1, the amino-acid portion, 2, the ' reacting centre,' and 3, the phosphonate portion, which are illustrated schematically on (1).

The synthesis of all the structural variations considered here, can be described by the generalised equation (ii) where X and Y represent variations at site 3; n, variations at site 2; and R^1 and R^2 variations at site 1.



(ii).

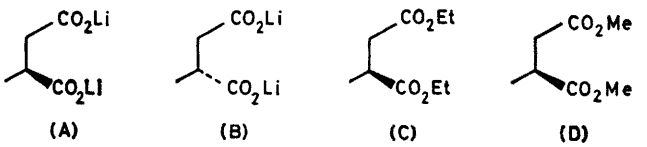
Variations at the amino-acid portion (site 1) have been made by an elaboration of equation (ii) shown in Scheme 1. These analogues can be further classified into two main types: (a) PALA esters and (b) compounds with substitution of different amino-acids for the L-Asp residue in PALA.

The chloroacylamino-acid esters (4) were made by refluxing the appropriate amino-acid esters with the chloroacyl chlorides in ethyl acetate. Purification was achieved by distillation giving the products as oils or low melting solids. The Michaelis–Arbuzov reactions of (4) with triethyl phosphite (TEP) proceeded smoothly at reflux temperature to give the diethoxyphosphinylalkyl amino-acid esters (5) as distillable oils in good yields.

In order to circumvent the problem of selective hydrolysis of the phosphonate ester moieties of (5) in the presence of the amide group, an alternative strategy was adopted in order to make the phosphonic acids (7).

Tris(trimethylsilyl)phosphite (TMSP) [10,11] underwent the analogous Michaelis–Arbuzov reaction to TEP, when excess of the reagent was used as solvent and the reaction mixture was refluxed for long periods under dry conditions. Fractional distillation of the reaction

mixture produced the bis(trimethylsilyl)phosphinoylalkyl amino-acid esters (6) as high-boiling viscous oils. These moisture-sensitive compounds were characterised by n.m.r. before being hydrolysed to the phosphonic acids

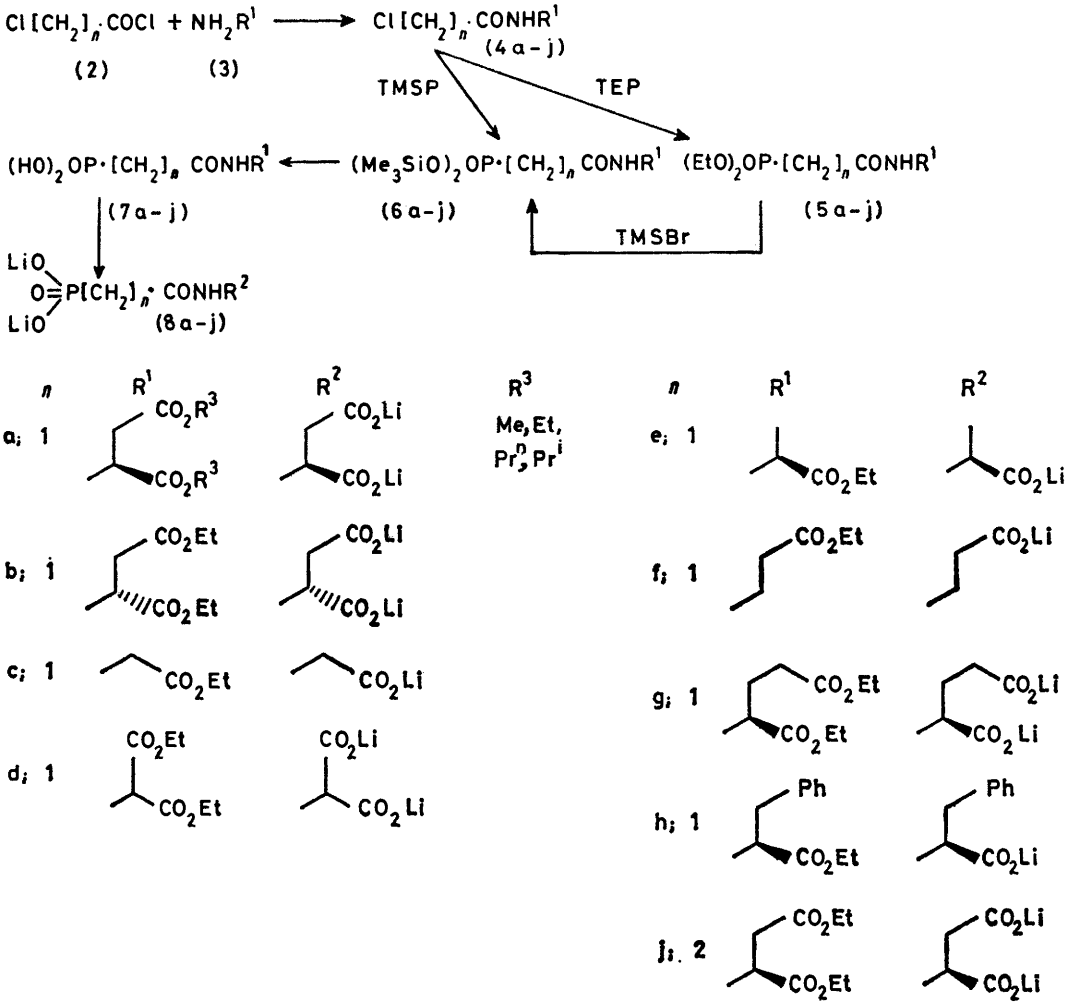


(7) with water at room temperature. The acids were obtained as stable crystalline solids.

The amino-acid ester groups of (7) were subsequently removed by titration with the theoretical amount of 1M-aqueous lithium hydroxide using a pH-stat with the end-point set to pH 11.5. The solutions were evaporated under high vacuum and subsequently dried *in vacuo* at 50 °C to give the hygroscopic solids (8) which were characterised by n.m.r. and microanalysis.

Measurement of the optical rotation of the tetralithium salt of PALA [8; $n = 1$, $R^2 = (A)$] gave a value $\{[\alpha]_D^{23} +13.2°\ (H_2O)\}$ very similar to the $[\alpha]_D$ reported in the literature,[5] thus providing evidence for the absence of racemisation throughout the sequence of Scheme 1. This is further supported by the optical rotation $\{[\alpha]_D^{22} = -16.9°\ (H_2O)\}$ of the enantiomeric compound [8; $n = 1$, $R^2 = (B)$], which was also made using the transformations of Scheme 1.

Although the TMSP route obviated the problem of selective hydrolysis of the phosphonate esters mentioned above, the transformation of [5; $n = 1$, $R^1 = (C)$] to [6; $n = 1$, $R^1 = (C)$] was achieved on an analytical scale using trimethylsilyl bromide (TMSBr).[12] This reaction was conveniently monitored by n.m.r., by correlating the appearance of ethyl bromide with the concomitant disappearance of the phosphonate ester signals. In contrast the related reagent trimethylsilyl iodide [13] (TMSI) was less selective than TMSBr in that both phosphonate and carboxylate esters were cleaved under the same reaction conditions. This was established by the appearance in the n.m.r. spectrum of the reaction mixture of ethyl and methyl iodide signals and by the



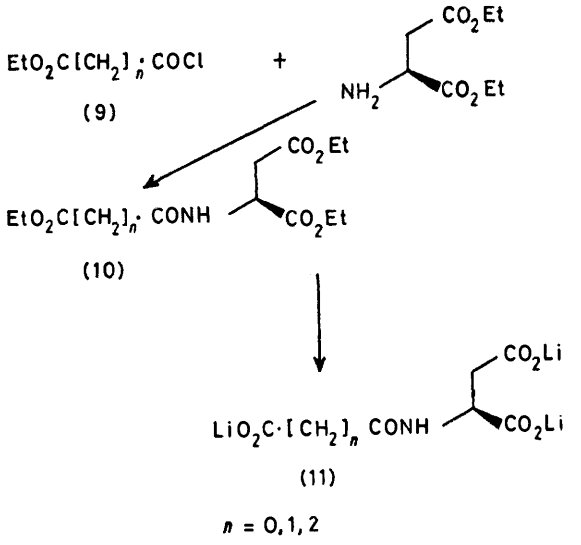
SCHEME 1

corresponding loss of ethyl phosphonate and methyl carboxylate signals from [5; $n = 1$, $R^1 = (D)$] on treatment with TMSI. This reactivity difference between TMSBr and TMSI is in agreement with the results of McKenna and Schmidhauser [14] and contrasts with the claim of Blackburn and Ingleson [15] for the selectivity of TMSI.

The overall yield of PALA tetralithium salt [8; $n = 1$, $R^2 = (A)$] from diethyl L-aspartate hydrochloride was 29% with the isolation and characterisation of all the intermediates illustrated in Scheme 1. This compares favourably with the existing small-scale literature procedures [5,6,16] and combined with the simpler manipulative procedures presented here supports our contention that this is an improved method for the synthesis of PALA and its analogues.

Variations of the central position (site 2) of the parent structure (1) have been limited to increasing the length of this unit by inserting an extra methylene (*i.e.* $n = 2$ in Scheme 1), based on the fact that the phosphate group must move away from the site of nucleophilic attack as the reaction proceeds. These compounds 4i—8i were synthesised using the reactions of Scheme 1.

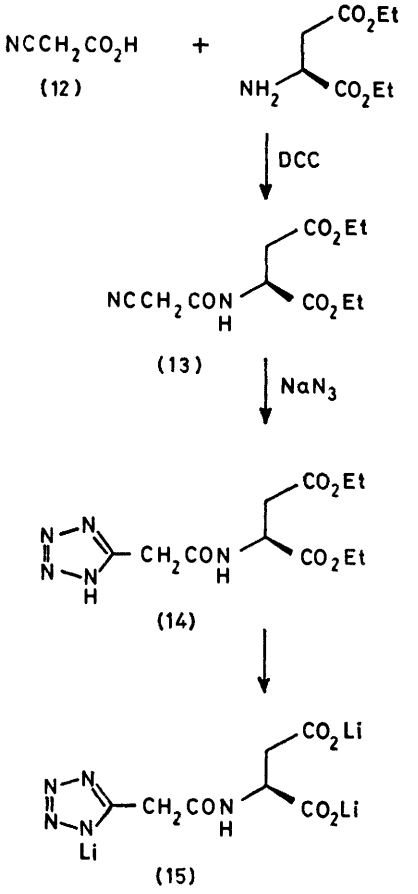
Replacement of the phosphonate group at site 3 by other anionic groups has been carried out in order to investigate the enzyme-binding requirement at this position. Replacement by carboxy was achieved using the method exemplified in Scheme 2.



SCHEME 2

The ester-acid chlorides (9) were condensed with diethyl-L-aspartate by refluxing overnight in dry ethyl acetate. The tri-ester products (10) were isolated as distillable oils and characterised before saponification to the trilithium salts (11) at pH $\gtrsim$ 11.5. Under these conditions the product (11; $n = 0$) was optically active $\{[\alpha]_D^{22}$ +26° $(H_2O)\}$; however when a similar hydrolysis was carried out without pH control, the optical rotation was much lower $\{[\alpha]_D^{22}$ +4.09° $(H_2O)\}$ thus suggesting that partial racemisation does occur at high pH values.

Condensation of cyanoacetic acid (12) with diethyl-L-aspartate in the presence of dicyclohexylcarbodi-imide (DCC) produced the cyanoamide (13) as a distillable oil which subsequently crystallised to a low-melting solid. Conversion into the tetrazole (14) using sodium azide, followed by controlled pH hydrolysis produced the trilithium salt (15) (Scheme 3). Although this compound exhibited the expected i.r. and n.m.r. characteristics all attempts to obtain a satisfactory microanalysis have failed.



SCHEME 3

Testing of this series of compounds against aspartate transcarbamylase *in vitro* and careful kinetic analysis of the inhibition data should delineate the requirements for tight binding at, and close to, the active site of the enzyme. With the first results of this ' receptor-mapping exercise ' then being available it should be possible to continuously refine the structural parameters for binding using extensions of the synthetic methods described in order to produce compounds of higher potency.

EXPERIMENTAL

I.r. spectra were determined with a Perkin-Elmer 157G or 297 instrument for KBr discs, Nujol mulls, or liquid films. ^{1}H N.m.r. spectra were recorded using a Perkin-Elmer R12B (60 MHz) or a JEOL JNM-PMX60 (60 MHz) spectrometer operating in the continuous-wave mode and a Bruker WP-

80 (80 MHz) or a Bruker HFX-90 (90 MHz) instrument operating in the Fourier-transform mode [tetramethylsilane or 2,2,3,3-tetradeuterio-3-(trimethylsilyl)propionic acid, sodium salt as internal standard]. Resonances are reported as p.p.m. downfield from tetramethylsilane position on the δ scale.

All products were routinely checked for homogeneity by t.l.c. on silica-gel plates (E. Merck, 60F254, 0.25 mm) using the solvents indicated in the text. The spots were located either by filtered u.v. light ($\nu_{max.}$ 254 and 365 nm), iodine, 1% t-butyl hypochlorite in cyclohexane followed by 1% starch/1% potassium iodide in water, Hanes–Isherwood reagent,[17a] or Dittmer–Lester reagent.[17b] Optical rotations were determined on a Bendix N.P.L. automatic polarimeter. Water of hydration reported in the microanalytical figures

The reaction mixture was then fractionally distilled *in vacuo* which first removed the excess of triethyl phosphite and then gave the *product* (22.49 g, 81%) [t.l.c. in chloroform–methanol (8 : 1 v/v) R_F 0.55 with trace impurity at R_F 0.7], $\nu_{max.}$ (liquid film) 3 280, 1 740, and 1 680 cm^{-1}, δ($CDCl_3$), 1.05—1.37 (2 t, 12 H, CH_3CH_2), 2.87 (d, 2 H, *J* 23 Hz, CH_2P), 2.78—2.95 (m, 2 H, CH_2CH), 3.85—4.45 (q + quint, 8 H, CH_2CH_3), 4.64—4.98 (m, 1 H, $CHCH_2$), and 7.28—7.67br (d, 1 H, NH). The *N*-(diethoxyphosphinoyl)acetyl compounds shown in Table 2 were prepared in an analogous manner.

N-(*Dihydroxyphosphinyl*)*acetoyl*-L-*aspartic Acid Diethyl Ester* (7a; R^3 = Et).—*N*-Chloroacetyl-L-aspartic acid diethyl ester (4a; R^3 = Et) (5.32 g, 20 mmol) and tris(trimethylsilyl) phosphite [11] (12 g, 40 mmol) were refluxed under nitrogen for 7 h. The reaction mixture was then fractionally distilled *in vacuo* which removed the excess of tris(trimethylsilyl) phosphite, b.p. 36 °C at 0.05 Torr. After changing the receiver the residue was distilled to give the bis(trimethylsilyl) phosphonate (6a; R^3 = Et) as a moisture-sensitive viscous oil (5.35 g, 59%), b.p. 146—158 °C at 0.07 Torr, R_F 0—0.15 in chloroform–methanol (1/1 v/v), $\nu_{max.}$ 3 260—3 180, 1 740, and 1 650 cm^{-1}, δ($CDCl_3$*) 0.19 (s, 18 H), (Me_3SiO_2), 1.13 (t, 6 H, *J* 8 Hz, CH_3CH_2), 2.68—2.86 (m, 2 H, CH_2CH), 2.70 (d, 2 H, *J* 21 Hz, CH_2P), 4.02 (q, 2 H, *J* 8 Hz), 4.09 (q, 2 H, *J* 8 Hz, CH_2CH_3), 4.50—4.90 (m, 1 H, $CHCH_2$), and 7.25—7.64br (d, 1 H, NH).

The bis(trimethylsilyl) phosphonate (6a; R^3 = Et) (5.0 g, 11 mmol) and water (20 ml) were stirred at room temperature for 1 h, by which time two immiscible layers were present. The total reaction mixture was evaporated at 40 °C to give a colourless oil which crystallised on trituration with ethanol. Recrystallisation from ethanol gave the *phosphonic acid* (7a; R^3 = Et) (2.74 g, 80%), $[\alpha]_D^{22}$ +1.35° (H_2O), $\nu_{max.}$ (KBr) 3 350, 1 745, 1 720, and 1 645 cm^{-1}, δ(D_2O) 1.25 (t, 6 H, *J* 8 Hz, CH_3CH_2), 2.94 (d, 2 H, *J* 21 Hz, CH_2P), 2.95 (d, 2 H, *J* 6 Hz, CH_2CH), 4.18 (q, 2 H, *J* = 8 Hz) and 4.23 (q, 2 H, *J* 8 Hz) (CH_2CH_3) and *ca.* 4.9

* N.m.r. solvent without tetramethysilane as standard.

TABLE 1

N-Chloroacetylamino-acid esters $ClCH_2CONHR$ (4a—h)

Compound	Reflux time/h	Yield (%)	M.p. or B.p. (°C) (Torr)	Formula	Analysis Found (%) C	H	N	Required (%) C	H	N
(4a; R^3 = Me)	13½	66	118—119 (0.07)	$C_8H_{12}ClNO_5$	40.4	5.4	6.2	40.4	5.0	5.9
(4a; R^3 = Et) [a]	8—9	62	126—129 (0.04)	$C_{10}H_{16}ClNO_5$	44.8	6.1	5.0	45.1	6.0	5.2
(4a; R^3 = Pr^n)	9	48	156—164 (0.04)	$C_{12}H_{20}ClNO_5 \cdot \frac{1}{4}H_2O$	48.6	7.1	5.2	48.3	6.9	4.7
(4a; R^3 = Pr^i)	9	37	120—122 (0.01)	$C_{12}H_{20}ClNO_5$	48.6	6.5	4.6	49.0	6.8	4.8
(4c) [b]	7½	72	62.5	$C_6H_{10}ClNO_3 \cdot \frac{1}{4}H_2O$	39.1	5.5	7.5	39.1	5.7	7.6
(4e) [c]	9	50	90—94 (0.05)	$C_7H_{12}ClNO_3 \cdot \frac{1}{4}H_2O$	42.4	6.0	7.0	42.4	6.3	7.0
(4f) [d]	7	82	110—127 (0.05)	$C_7H_{12}ClNO_3 \cdot \frac{1}{4}H_2O$	42.7	6.5	7.0	42.4	6.3	7.0
(4g)	7	69	140 (0.02)	$C_{11}H_{18}ClNO_5$	47.3	6.5	5.1	47.2	6.4	5.0
(4h) [e]	5½	82	67	$C_{13}H_{16}ClNO_2$	58.0	6.0	5.0	58.0	6.0	5.2
(4d) [f]	12	80.5	101—102	$C_5H_{14}ClNO_5$	42.9	5.5	5.4	42.9	5.6	5.6
(4b)	12	68	120—124 (0.075)	$C_{10}H_{16}ClNO_5$	45.1	6.0	5.2	45.2	6.0	5.3

[a] Ref. 18. [b] O. Diels and H. Heintzel, *Ber.*, 1905, **38**, 304. [c] E. Fischer and A. Schulze, *Ber.*, 1907, **40**, 950. [d] T. A. Mastryukova, A. E. Shipov, G. V. Zhdanova, Yu. S. Kagan, E. A. Ershova, N. A. Guserva, and M. I. Kalbachnik, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1978, 503 (*Chem. Abs.*, 1978, **88**, 190,979p). [e] W. S. Fones, *J. Org. Chem.*, 1952, **17**, 1661. [f] J. C. Sheehan and A. K. Bose, *J. Amer. Chem. Soc.*, 1951, **73**, 1761.

was confirmed either by n.m.r. or by loss on drying and re-analysis. Reaction times were not optimised.

N-*Chloroacetyl*-L-*aspartic Acid Diethyl Ester* (4a; R^3 = Et).[18]—Diethylamine (7.15 ml, 5.0 g, 68 mmol) was added to L-aspartic acid diethyl ester hydrochloride (11.3 g, 50 mmol) in benzene (150 ml) with stirring. Dry ether (350 ml) was then added and the precipitate of diethylamine hydrochloride was filtered off. The filtrate was evaporated at 30 °C to a pale yellow oil which was dissolved in dry ethyl acetate (250 ml). This solution was mixed with chloroacetyl chloride (4.2 ml, 6.0 g, 53 mmol) and refluxed with exclusion of moisture for 8 h. After standing overnight the solvent was removed at 40 °C and the residual oil was distilled *in vacuo* to give the product (8.24 g, 62%), pure by t.l.c. [R_F 0.8 in chloroform–methanol (1 : 1 v/v)], $\nu_{max.}$ (film) 3 320, 1 735, and 1 675 cm^{-1}, δ($CDCl_3$) 1.19 (t, 6 H, *J* 8 Hz, CH_3CH_2), 2.76—2.97 (m, 2 H, CH_2CH), 4.04 (s, 2 H, CH_2Cl), 3.90—4.38 (2q, 4 H, *J* 8 Hz), and 4.62—4.96 (m, 1 H, $CHCH_2$). The other *N*-chloroacetyl compounds shown in Table 1 were prepared similarly.

N-(*Diethoxyphosphinoyl*)*acetyl*-L-*aspartic Acid Diethyl Ester* 5a; R^3 = Et).—*N*-Chloroacetyl-L-aspartic acid diethyl ester (4a; R^3 = Et) (20 g, 75 mmol) and triethyl phosphite (120 ml) were refluxed for 24 h with exclusion of moisture.

(m ?, 1 H, C*H*CH_2, obscured by HOD). The *N*-phosphonoacetyl amino-acid esters shown in Table 3 were prepared similarly.

N-(*Dihydroxyphosphinoyl*)*acetyl-*L*-aspartic acid Tetralithium Salt* (8a).[5,6,16]—*N*-(Dihydroxyphosphinoyl)acetyl-L-aspartic acid diethyl ester (7a; R^3 = Et) (0.933 g, 3 mmol) in water (10 ml) was titrated with 1.0M-aqueous lithium hydroxide (12 ml, 12 mmol) on a pH-stat set to an end point of pH 11.5.

After the addition of base the mixture was stirred for a further 24 h then evaporated at 40 °C and 0.5 Torr. The evaporation process was repeated three times after trituration with ethanol. The hygroscopic *solid* obtained was dried over phosphorus pentaoxide at room temperature at 0.5 Torr for 6 h then at 80 °C at 0.5 Torr for a further 12 h, R_F (PEI cellulose plates) 0.4 in 1.2M-aqueous lithium chloride [Found (after drying at 150 °C): C, 25.7; H, 2.45; N, 4.85; P, 11.1. $C_6H_6NO_8PLi_4$ requires C, 25.8; H, 2.15; N, 5.0; P, 11.1%], $[\alpha]_D^{23}$ +13.2° (H_2O), $\nu_{max.}$ (KBr) 3 400br and 1 600br, δ(D_2O) 2.52 (d, 2 H, *J* 17 Hz, CH_2P), 2.44—2.74 (m, 2 H, C*H*$_2$CH), and 4.18—4.48 (m, 1 H, C*H*CH_2). The lithium salts shown in Table 4 were prepared in a similar manner; however the drying procedure was modified to and interval of 24 h at 50 °C and 250 Torr. Several of the products were exceedingly hygroscopic and weighings and transfer operations were carried out in a dry-box under nitrogen.

N-(3-*Chloropropionyl*)-L-*aspartic Acid Diethyl Ester* (4j).—This was prepared in an identical manner to the chloroacetyl compound (4a; R^3 = Et) by refluxing L-aspartic acid diethyl ester with 3-chloropropionyl chloride for 11 h. Distillation *in vacuo* gave the *product* (4j) (29%), b.p. 160 °C at 0.3 Torr which crystallised on cooling. Recrystallisation from ethyl acetate–light petroleum (b.p. 60—80 °C) gave m.p. 41—42 °C, R_F 0.5 in chloroform–methanol (1 : 1 v/v) (Found: C, 47.5; H, 6.4; N, 5.2. $C_{11}H_{18}ClNO_5$ requires C, 47.3; H, 6.5; N, 5.0%), $\nu_{max.}$ (KBr) 3 320, 1 730, and 1 640 cm^{-1}, δ($CDCl_3$) 1.27 (t, 6 H, *J* 8 Hz, C*H*$_3$CH$_2$), 2.70 (t, 2 H, *J* 6 Hz, C*H*$_2$CONH), 2.82—3.04 (m, 2 H, C*H*$_2$CH), 3.80 (t, 2 H, *J* 6 Hz, ClC*H*$_2$CH$_2$), 4.17 (q, 2 H, *J* 8 Hz) and 4.23 (q 2 H, *J* 8 Hz) (2 C*H*$_2$CH$_3$), 4.68—5.04 (m, 1 H, C*H*CH_2), and 6.4—6.9br (d, 1 H, NH).

N-(3-*Diethoxyphosphinoyl*)*propionyl-*L*-aspartic Acid Diethyl Ester* (5j).—This ester was prepared by refluxing *N*-3-chloropropionyl-L-aspartic acid diethyl ester (4j) with triethyl phosphite for 10 h in a similar manner to the preparation of the acetyl compound (5a; R^3 = Et). Distillation *in vacuo* removed the excess of triethyl phosphite and left a residual viscous yellow *oil* (65%), R_F 0.55 in chloroform–methanol (8 : 1 v/v) (Found: C, 45.9; H, 7.4; N, 3.3. $C_{15}H_{28}NO_8P{\cdot}\frac{1}{2}H_2O$ requires C, 46.2; H, 7.4; N, 3.6), $\nu_{max.}$ (liquid film) 3 280, 1 740, and 1 685 cm^{-1} δ($CDCl_3$) 1.14—1.60 (m, 12 H, 4 C*H*$_3$CH$_2$), 1.78—2.80 (m, 4 H, PCH$_2$C*H*$_2$CO), 2.85—3.05 (m, 2 H, C*H*$_2$CH), 3.88—4.52 (m, 8 H, 4 C*H*$_2$CH$_3$), 4.68—5.07 (m, 1 H, C*H*CH_2), and 6.8—7.2br (d, 1 H, NH).

TABLE 2

N-(Diethoxyphosphinyl)acetylamino-acid esters $(EtO)_2P(O)CH_2CONHR^1$ (5a—h)

Compound	Reflux time/h	Yield (%)	M.p. or B.p. (°C) (Torr)	Formula	Analysis Found (%) C	H	N	Required (%) C	H	N
(5a; R^3 = Me)	8	33	187 (0.008)	$C_{12}H_{22}NO_8P$	42.2	6.4	4.1	42.5	6.5	4.1
(5a; R^3 = Et)	24	81	184—209 (0.04)	$C_{14}H_{26}NO_8P{\cdot}\frac{1}{2}H_2O$	44.9	7.4	3.6	44.7	7.2	3.7
(5a; R^3 = Pr^n)	*ca.* 19	61	180—184 (0.04)	$C_{16}H_{30}NO_8P$	48.4	7.7	3.6	48.4	7.5	3.8
(5c)	6	73	143—145 (0.005)	$C_{10}H_{20}NO_6P{\cdot}\frac{1}{2}H_2O$	41.6	7.6	4.9	41.4	7.2	4.8
(5e)	5	42	150 (0.005)	$C_{11}H_{22}NO_6P{\cdot}\frac{1}{4}H_2O$	44.3	8.0	4.7	44.1	7.5	4.7
(5f)	6	83	160—164 (0.001)	$C_{11}H_{22}NO_6P$	44.8	7.3	4.7	44.7	7.5	4.7
(5g)	8	40	178—180 (0.005)	$C_{15}H_{28}NO_8P{\cdot}\frac{1}{4}H_2O$	46.6	7.6	3.7	46.7	7.4	3.6
(5h)	*ca.* 24	74	177—181 (0.02)	$C_{17}H_{26}NO_6P$	55.0	7.0	3.8	55.1	7.0	3.9
(5b)	*ca.* 24	57	158—170 (0.025)	$C_{14}H_{26}NO_8P$	45.5	7.1	4.0	45.8	7.1	3.8

TABLE 3

N-Phosphonoacetylamino-acid esters $(HO)_2P(O)CH_2CONHR^1$ (7a—h)

Compound	Reflux time/h	Yield (%)	M.p. (°C)	Formula	Analysis Found (%) C	H	N	Required (%) C	H	N
(7a; R^3 = Me)	7	11	124—125	$C_8H_{14}NO_8P$	33.5	5.0	4.7	33.9	5.0	5.0
(7a; R^3 = Et)	7	47	118—119	$C_{10}H_{18}NO_8P$	38.2	5.9	4.6	38.6	5.8	4.5
(7a; R^3 = Pr^n)	7	34	63	$C_{12}H_{22}NO_8P{\cdot}1\frac{1}{2}H_2O$	39.2	6.5	4.0	39.3	6.8	3.8
(7a; R^3 = Pr^i)	28	36	75—77	$C_{12}H_{22}NO_8P{\cdot}H_2O$	40.1	6.6	4.0	40.3	6.8	3.9
(7c)	17	24	142—143	$C_6H_{12}NO_6P$	31.6	5.5	6.3	32.0	5.3	6.2
(7e)	10	38	124—125	$C_7H_{14}NO_6P$	34.6	6.1	5.6	35.1	5.9	5.9
(7f)	6	45	116—117	$C_7H_{14}NO_6P$	34.8	6.0	6.0	35.1	5.9	5.9
(7g)	$7\frac{1}{2}$	7	108—110 (decomp.)	$C_{11}H_{20}NO_8P{\cdot}\frac{1}{2}H_2O$	39.2	6.1	4.1	39.5	6.3	4.2
(7h)	6	38	136—137	$C_{13}H_{18}NO_6P$	49.1	5.9	4.4	49.5	5.7	4.4
(7b)	64	15	117—118	$C_{10}H_{18}NO_8P$	38.3	5.8	4.5	38.6	5.8	4.5
(7d)	19	17	145—146	$C_9H_{16}NO_6P{\cdot}H_2O$	34.5	5.4	4.5	34.3	5.7	4.4

N-(3-*Dihydroxyphosphinoylpropionyl*)-L-*aspartic Acid Diethyl Ester* (7j).—This ester was prepared in a similar manner to the acetyl compound (7a; R^3 = Et) by refluxing *N*-(3-chloropropionyl)-L-aspartic acid diethyl ester (4j), with an excess of tris(trimethylsilyl) phosphite (TMSP) for 12 h. Distillation *in vacuo* first removed excess TMSP (b.p. 28 °C at 0.04 Torr) and then gave the bis(trimethylsilylphosphonate) (6j), b.p. 176—180 °C at 0.1 Torr, as a yellow oil. Hydrolysis with water and evaporation gave the *phosphonic acid* (7j) as a yellow gum (73%) (Found: C, 36.4; H, 6.3; N, 4.1. $C_{11}H_{20}NO_8P\cdot 2H_2O$ requires C, 36.6; H, 6.7; N, 3.9%), δ([$^2H_6$]DMSO) 1.18 (t, 6 H, *J* 7 Hz, 2 CH_3CH_2), 1.40—2.84 (m, *ca.* 6 H, CH_2CH and CH_2CH_2P), 4.06 (q, 4 H, *J* 7 Hz, 2 CH_2CH_3), 4.32—4.80 (m, 1 H, $CHCH_2$), 5.75br [s, *ca.* 7 H*, $(HO)_2OP$ and *ca.* $2H_2O$], and 8.2—8.5br (d, 1 H, NH).

N-(3-*Dihydroxyphosphinoylpropionyl*)-L-*aspartic Acid Tetralithium Salt* (8j).—This salt was prepared in an analogous manner to the tetralithium salt (8a) described above, except that the reaction mixture was heated on a steam-bath for 1 h to complete the hydrolysis (monitored by n.m.r.).

* Corrected value by subtraction of solvent water.

TABLE 4

N-Phosphonoacetylamino-acid lithium salts $(LiO)_2P(O)CH_2CONHR^2$ (8a—h)

Compound	Formula	Analysis Found (%) C	H	N	Required (%) C	H	N
(8a) [a]	$C_6H_6NO_8PLi_4\cdot 2H_2O$	23.2	3.3	4.4	22.9	3.2	4.4
(8c)	$C_4H_5NO_6PLi_3\cdot H_2O$	20.4	3.2	5.6	20.6	3.0	6.0
(8e)	$C_5H_7NO_6PLi_3\cdot 1\frac{1}{2}H_2O$	23.4	4.1	5.2	23.4	3.9	5.5
(8f)	$C_5H_7NO_6PLi_3\cdot H_2O$	24.4	3.2	5.6	24.3	3.6	5.7
(8g)	$C_7H_8NO_8PLi_4\cdot 2\frac{1}{2}H_2O$	24.8	3.5	4.0	24.85	3.8	4.1
(8h)	$C_{11}H_{11}NO_6PLi_3\cdot 3H_2O$	36.5	4.5	3.9	36.8	4.7	3.9
(8b)	$C_6H_6NO_8PLi_4\cdot 3H_2O$	21.7	3.2	4.1	21.6	3.6	4.2
(8d)	$C_5H_4NO_8PLi_4\cdot 2\frac{1}{2}H_2O$	19.7	2.6	4.1	19.4	2.9	4.5

[a] Refs. 5 and 6.

The *product* was dried as described above and had R_F 0.75 (PEI–cellulose) in 1.2M-aqueous lithium chloride (Found: C, 27.0; H, 3.25; N, 4.4. $C_7H_8NO_8PLi_4\cdot 1H_2O$ requires C, 27.0; H, 3.2; N, 4.5%).

N-*Ethoxalyl-L-aspartic Acid Diethyl Ester* (10; n = 0).—Diethyl-L-aspartate, (5.0 g, 26.45 mmol), generated from the hydrochloride as described above, and ethoxalyl chloride (3.5 g, 26.45 mmol) in dry ethyl acetate (30 ml) were refluxed for 12 h with exclusion of moisture. The solvent was removed at 40 °C and the residual oil was distilled *in vacuo* to give the *product* (10; *n* = 0) (4.21 g, 55%), $\nu_{max.}$ (liquid film) 3 330, 1 770, and 1 710 cm^{-1}, δ($CDCl_3$) 1.1—1.6 (m, 9 H, CH_3CH_2), 2.8—3.1 (m, 2 H, CH_2CH), 3.9—4.6 (m, 6 H, CH_2CH_3), 4.6—5.0 (m, 1 H, $CHCH_2$), 7.9br (d, 1 H, NH). *N*-Ethoxycarbonylacetyl-L-aspartic acid diethyl ester (10; *n* = 1) and *N*-(3-ethoxycarbonylpropionyl)-L-aspartic acid diethyl ester (10; *n* = 2) were made in an analogous manner and have the properties described in Table 5.

N-*Oxalyl-L-aspartic Acid Trilithium salt* (11; n = 0).—*N*-Ethoxalyl-L-aspartic acid triethyl ester (10; *n* = 0) (0.862 g, 2.98 mmol) in water (30 ml) was titrated with 1.0M-aqueous lithium hydroxide (8.95 ml, 8.95 mmol) in a pH-stat set to an end-point of pH 11.5. After the addition of base the solution was stirred for 5 days, and then evaporated at 40 °C and 0.5 Torr. The residual solid was dried at 60 °C at 250 Torr for 3 days to give the *product* (0.239 g), $[\alpha]_D^{22}$ +26° (H_2O), $\nu_{max.}$ (KBr) 3 400, 1 580, and 1 420 cm^{-1}, δ(D_2O) 2.58—3.00 (m, 2 H, CH_2CH), and 4.35—4.53 (m, 1 H, $CHCH_2$). An identical experiment carried out without pH control gave a product with $[\alpha]_D^{22}$ +4.09° (H_2O). The trilithium salts of *N*-carboxyacetyl- and *N*-(3-carboxypropionyl)-L-aspartic acids (11; *n* = 1,2) were made in an identical manner and have the properties shown in Table 6.

N-*Cyanoacetyl-L-aspartic Acid Diethyl Ester* (13).—L-Aspartic acid diethyl ester hydrochloride (30.0 g, 0.13 mol) was converted into the free base as described above. Cyanoacetic acid (11.06 g, 0.13 mol) was added to a solution of the free base in dichloromethane (50 ml) at 0—5 °C and dicyclohexylcarbodi-imide (26.78 g, 0.13 mol) in dichloromethane (60 ml) was added slowly to the mixture with stirring. Dicyclohexylurea precipitated after *ca.* 2 min and dichloromethane (100 ml) was added to facilitate stirring. After 2 h the reaction mixture was filtered and the solvent removed from the filtrate at 40 °C to give an orange

TABLE 5

N-Ethoxyacyl-L-aspartic acid diethyl esters (10)

Compound	Yield (%)	B.p. (°C)/Torr	Formula	Analysis Found (%) C	H	N	Required (%) C	H	N
(10; *n* = 0)	55	158—164/0.15	$C_{12}H_{19}NO_7$	49.9	6.75	4.95	49.85	6.55	4.85
(10; *n* = 1)	37	107/0.02—180/0.06 [a]	$C_{13}H_{21}NO_7$	51.8	7.1	4.6	51.5	6.95	4.6
(10; *n* = 2)	29	169—175/0.01	$C_{14}H_{23}NO_7$	53.4	7.45	4.6	53.0	7.25	4.4

[a] Cold finger distillation. Oil bath temperatures quoted.

TABLE 6

N-Carboxyacyl-L-aspartic acid tri-lithium salts (11)

Compound	Formula	Analysis Found (%) C	H	N	Required (%) C	H	N
(11; *n* = 0)	$C_6H_4Li_3NO_7\cdot H_2O$	30.2	2.9	5.55	29.9	2.5	5.8
(11; *n* = 1)	$C_7H_6Li_3NO_7\cdot 2H_2O$	30.35	3.3	4.9	30.75	3.65	5.15
(11; *n* = 2)	$C_8H_8Li_3NO_7\cdot 2H_2O$	33.95	4.1	4.4	33.45	4.2	4.9

oil which was distilled *in vacuo*. The product distilled as an oil (23.4 g, 70%), b.p. 163—171 °C at 0.02 Torr which crystallised on storing at 4 °C (Found: C, 51.6; H, 6.3; N, 11.0. $C_{11}H_{16}N_2O_5$ requires C, 51.6; H, 6.25; N, 10.9%) $\nu_{max.}$(Nujol) 3 350, 2 260, 1 730, and 1 655 cm^{-1}, $\delta(CDCl_3)$ 1.25 (t, 6 H, *J* 7 Hz, CH_3CH_2), 2.72—3.00 (m, 2 H, CH_2CH), 3.50 (s, 2 H, CH_2CN), 3.90—4.50 (m, 4 H, CH_2CH_3), 4.60—4.93 (m, 1 H, $CHCH_2$), and 2.77 (d, 1 H, NH).

N-*Tetrazolylacetyl*-L-*aspartic Acid Diethyl Ester* (14).—*N*-Cyanoacetyl-L-aspartic acid diethyl ester (13) (6.16 g, 24 mmol) was dissolved in redistilled dimethylformamide (50 ml) and ammonium chloride (1.29 g, 24 mmol) and sodium azide (1.56 g, 24 mmol) were added. The resulting suspension was stirred for 2 h at 125 °C, then cooled to room temperature, and filtered. The filtrate was evaporated to a brown oil at 40 °C. Saturated aqueous sodium hydrogencarbonate (5 ml) was added, the solution filtered, and the aqueous filtrate extracted with chloroform (3 × 20 ml) to remove unchanged starting material. The aqueous layer was acidifed to pH 3 by careful addition of concentrated hydrochloric acid and cooled in ice. The white solid which precipitated was filtered off and recrystallised from water, then dried over P_2O_5 *in vacuo* at room temperature to afford the *ester* (1.43 g, 20%), m.p. 135.5—138.5 °C (Found: C, 44.0; H, 5.6; N, 23.2. $C_{11}H_{17}N_5O_5$ requires C, 44.15; H, 5.7; N, 23.4%), $\nu_{max.}$ (KBr) 3 355, 2 980—2 320, 1 760, 1 650, and 1 570 cm^{-1}. $\delta([^2H_6]DMSO)$ 1.17 (t, 6 H, *J* 6 Hz, 2 CH_3CH_2), 2.85 (d, 2 H, *J* 6 Hz, CH_2CH), 4.0—4.50 (m, 6 H, 2 CH_2CH_3 and CH_2CONH), 3.93—4.37 (m, 1 H, $CHCH_2$), and 8.7br (d, 1 H, NH).

N-*Tetrazolylacetyl*-L-*aspartic Acid Trilithium Salt* (15).—Hydrolysis of *N*-tetrazolylacetyl-L-aspartic acid diethyl ester (14) was carried out in an identical manner to the hydrolysis of (7a; R^3 = Et). Evaporation of the reaction mixture and drying of the residue at 60 °C and 0.1 Torr overnight gave the product, m.p. 285 °C (decomp.), $\nu_{max.}$ (KBr) 3 400br, 1 600, and 1 410 cm^{-1}, $\delta(D_2O)$ 2.51—2.83 (m, 2 H, CH_2CH), 3.95 (s, 2 H, CH_2CO), and 4.29—4.65 (m, 1 H, $CHCH_2$). No satisfactory microanalysis could be obtained for this compound.

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