Design and Synthesis of γ-Oxygenated Phosphinothricins as Inhibitors of Glutamine Synthetase

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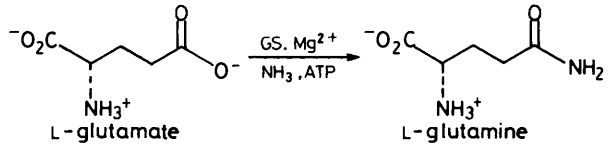
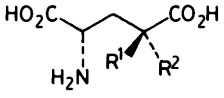
The ability of L-γ-hydroxyglutamic acids to act as substrates of the enzyme glutamine synthetase (GS) was exploited as a rationale for the synthesis of γ-oxygenated analogues of the naturally occurring GS inhibitor phosphinothricin (PPT). The potent new inhibitor DL-γ-hydroxyphosphinothricin (GHPPT) was prepared *via* a key reaction involving the silicon-mediated addition of ethyl methylphosphinate to benzyl 2-benzyloxycarbonylamino-4-oxobutyrate. The resulting intermediate was also converted to various derivatives useful in probing structure–activity relationships in the GHPPT series. The γ-oxygenated phosphinothricins display enzyme inhibitory activity as well as *in vivo* phytotoxicity.

Enzymes utilizing a variety of substrates for reaction catalysis are often subject to inhibition by variously substituted inhibitors, with particularly potent inhibition occurring when the latter function as transition-state analogues.[1] The extent of correlation between substrate efficacy of a congeneric series of substrates and the potency of analogously substituted inhibitors has been suggested as a criterion for transition-state analogue inhibition.[2] This paper describes an approach to the synthesis of glutamine synthetase inhibitors which employs the kinetic interrelationship of substrate and inhibitor effectiveness as a rationale for inhibitor design.

The enzyme glutamine synthetase (GS, E.C. 6.3.1.2) catalyses a reaction of central importance in plant metabolism, the conversion of L-glutamate to L-glutamine (Scheme 1).[3] This reaction is a key step for the incorporation of ammonia into the carbon products of photosynthesis. The amide functionality of glutamine provides the nitrogen required in the formation of α-amino acids *via* transamination and, as the precursor to carbamoyl phosphate, it also furnishes nitrogen for the urea cycle and pyrimidine biosynthesis.[4]

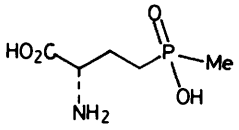


Scheme 1.

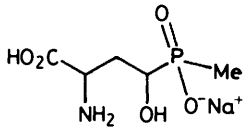


(1)

a; $R^1 = R^2 = H$
b; $R^1 = H, R^2 = OH$
c; $R^1 = OH, R^2 = H$



(2), PPT



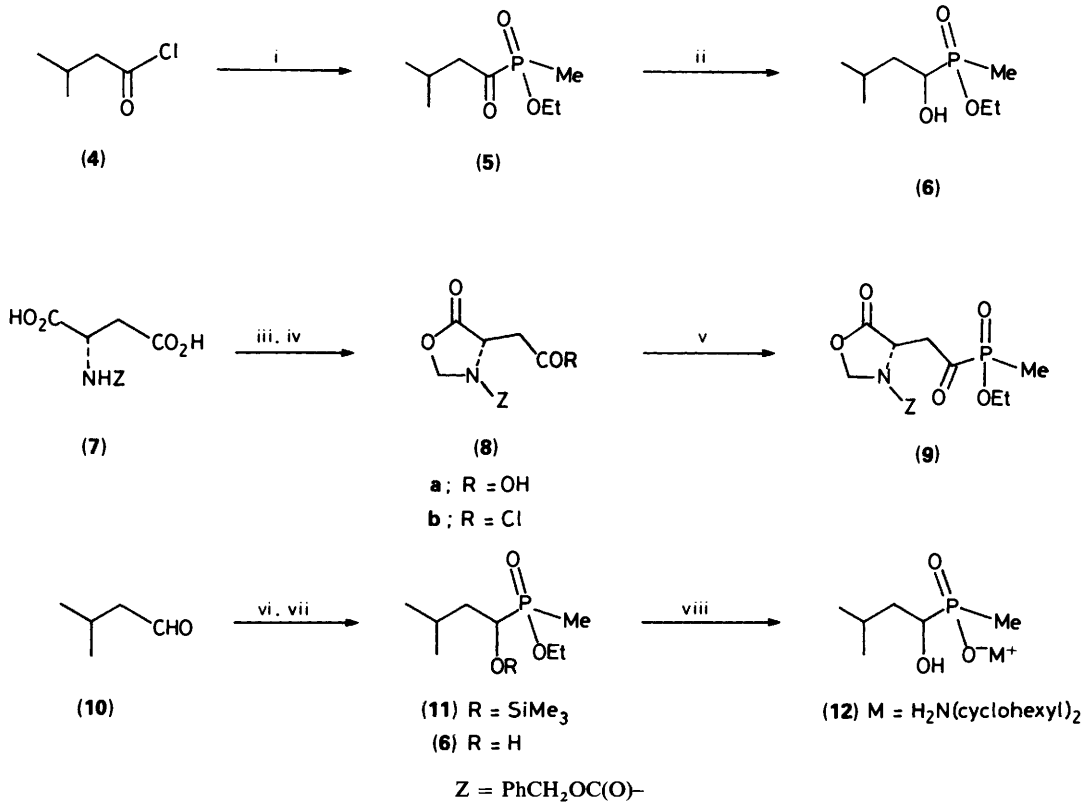
(3), GHPPT

Whilst L-glutamic acid (**1a**) is the natural substrate for glutamine synthetase, the classic investigations of Meister document the ability of γ-hydroxylated glutamic acids (**1b**) and (**1c**) also to function as substrates, the *threo*-isomer (**1c**) possessing a lower K_m value for ovine brain glutamine synthetase than that of L-glutamic acid itself.[5–7] We have been engaged in the design of analogues of phosphinothricin (PPT) (**2**), a phosphinic acid mimic of L-glutamic acid which is produced by various streptomycete species.[8] Phosphinothricin is a potent transition-state analogue inhibitor of glutamine synthetase[9] and is also an effective herbicide against a broad range of plant species.[10] We have recently described an approach to the design of α- and γ-alkyl-substituted phosphinothricins which takes advantage of structural variability in the substrates of glutamine synthetase.[11] We report herein the synthesis and evaluation of DL-γ-hydroxyphosphinothricin (**3**, GHPPT), which is a potent new inhibitor of glutamine synthetase, as well as related γ-oxygenated phosphinothricins. These phosphinothricin analogues display enzyme-inhibitory properties as well as *in vivo* phytotoxicity, making them useful both as probes for defining the requirements of phosphinothricin binding at the enzyme active site, and as agents for the control of plant growth.[12]

Results and Discussion

Synthesis of γ-Oxygenated Analogues of Phosphinothricin.—Hydrogenation of oxophosphinates to give hydroxyphosphinates (Scheme 2) was effective for (**5**), the product of Michaelis–Arbuzov reaction of isovaleryl chloride (**4**) with diethyl methylphosphonite. However, this approach failed in the case of a protected amino acid. Esterification of (**7**) as the oxazolinone (**8a**),[13] followed by acyl chloride formation and Michaelis–Arbuzov reaction, afforded the labile oxophosphinate (**9**). Attempted reduction of (**9**) yielded only complex mixtures, apparently resulting from decomposition of the product under the acidic conditions.

An alternative construction of hydroxyphosphinates involves phosphonite addition to aldehydes. The usually sluggish addition of trialkyl phosphite esters to aldehydes[14] undergoes



Scheme 2. *Reagents:* i, $MeP(OEt)_2$, PhMe, reflux, 54%; ii, HOAc, EtOAc, PtO_2,H_2 (1 atm), 81%; iii, paraformaldehyde, *p*-$MeC_6H_4SO_3H$, PhMe, reflux, remove H_2O, 95%; iv, $SOCl_2$, 100%; v, $MeP(OEt)_2$, PhMe, 50 °C, 60%; vi, MeP(O)H(OEt), $MeCON(SiMe_3)_2$, CH_2Cl_2, 23 °C, 73%; vii, H_2O, EtOH, 23%; viii, Me_3SiBr, CH_2Cl_2; H_2O, THF; EtOAc, H_2N(cyclohexyl)$_2$, 82%.

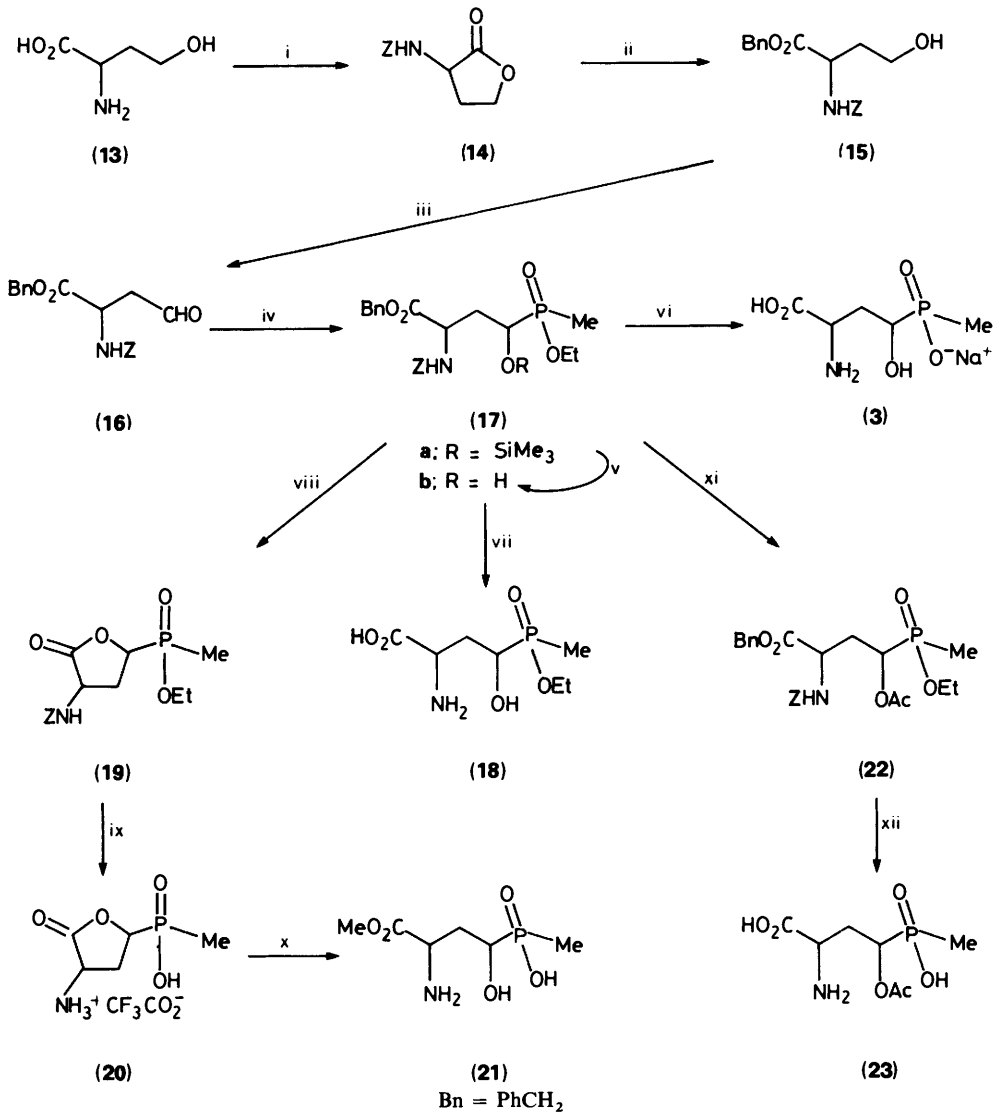
dramatic acceleration with use of silylated phosphites,[15] and silylated phosphonites have also proved advantageous for phosphinate synthesis *via* conjugate addition and Michaelis–Arbuzov reactions.[16] We have found that use of a silylated phosphonite ester for addition to aldehydes provides a simple and direct route to the desired γ-hydroxyphosphinates (Scheme 2).[17] Thus, reaction of isovaleraldehyde (**10**) with ethyl methylphosphinate in the presence of bis(trimethylsilyl)-acetamide provided the silyl ether (**11**) which was cleaved with aqueous ethanol to give the carbinol (**6**). Dealkylation of (**6**) with bromotrimethylsilane[18] afforded the salt (**12**) after work-up with dicyclohexylamine.

This methodology was useful for the development of a flexible route to DL-γ-hydroxyphosphinothricin (**3**). D,L-Homoserine (**13**) (Scheme 3) was converted *via* the lactone (**14**)[19] to the hydroxy ester (**15**), which was oxidized quantitatively under Swern conditions to give the aldehyde (**16**).[20] Addition of ethyl methylphosphinate to (**16**) in the presence of bis(trimethylsilyl)-acetamide smoothly gave the silyl ether (**17a**) and the alcohol (**17b**) in 63 and 27% yields, respectively, after silica gel chromatography. The ether (**17a**) and the alcohol (**17b**) were each obtained as a mixture of four chromatographically inseparable diastereoisomers. Treatment of (**17a**) with bromotrimethylsilane, followed by hydrogenation in methanol containing sodium hydroxide (1 equiv.), afforded in 55% yield the sodium salt of DL-γ-hydroxyphosphinothricin (GHPPT) (**3**), as a 56:44 mixture of carbinol diastereoisomers.[21]

We were interested in observing the effect on biological activity of blocking various positions of the GHPPT parent structure (**3**). The alcohol (**17b**), which could be obtained quantitatively by desilylation of (**17a**) with aqueous HF, served as a useful intermediate for the preparation of various derivatives of GHPPT (Scheme 3). Hydrogenation of (**17b**) gave the ethyl phosphinate ester (**18**), whilst lactonisation of (**17a**) to give (**19**), followed by deprotection and methanolysis, furnished the methyl carboxylate ester (**21**). Acetylation of (**17b**), followed by phosphinate ester cleavage and hydrogenation, afforded DL-γ-acetoxyphosphinothricin (GAPPT) (**23**). GAPPT prepared in this way was free of any GHPPT as determined by ^{31}P NMR spectroscopy.

γ-Oxygenated Analogues of Phosphinothricin as Inhibitors of Glutamine Synthetase.—A competitive inhibitor's potency under steady-state conditions is characterised by the inhibition constant K_i, which can be determined from graphical treatment of enzyme activity in the presence of varying concentrations of both substrate and inhibitor.[22] The phosphinothricin analogues prepared in this study were evaluated as inhibitors of plant glutamine synthetase isolated from sorghum seedlings.[23] The experimentally determined K_i values are listed in the Table, together with Meister's reported substrate K_m values for the corresponding glutamic acid analogues.[5] For purposes of comparison, both DL-phosphinothricin and the γ-methyl analogue (GMPPT) (**24**)[11] were also evaluated as inhibitors of this enzyme.

Although DL-phosphinothricin possessed the lowest inhibition constant of the series (Table, entry 1), GHPPT (**3**) (entry 2) was almost as potent a competitive inhibitor. The γ-methylated analogue (**24**) (entry 3) was a somewhat weaker inhibitor. Despite the presence of a large functional group at the γ-position, the γ-acetoxy analogue GAPPT (**23**) (entry 4) exhibited significant GS inhibition. The γ-lactone (**20**), whilst showing potent inhibition of GS, was found to be in equilibrium with GHPPT (**3**), and kinetic evaluation was not further pursued. Finally, the esters (**18**) and (**21**) were inactive as inhibitors of the sorghum enzyme, supporting the hypothesis



Scheme 3. *Reagents:* i, $PhCH_2OCOCl$, H_2O, $NaHCO_3$; 100 °C (0.1 mmHg), 83%; ii, NaOH, MeOH; $PhCH_2Br$, Me_2NCHO, 23 °C, 24 h, 80%; iii, Me_2SO, CH_2Cl_2, $(COCl)_2$, −78 °C; Et_3N, 100%; iv, MeP(O)H(OEt), $MeCON(SiMe_3)_2$, CH_2Cl_2, 23 °C, 90%; v, HF, H_2O, MeCN, 95%; vi, Me_3SiBr, CH_2Cl_2, 23 °C; H_2, 10% Pd/C, H_2O, MeOH, NaOH (1 equiv.), 55%; vii, H_2, 10% Pd/C, H_2O, EtOH, 55%; viii, CF_3CO_2H, CH_2Cl_2, 23 °C, 71% from (**17a**) or 100% from (**17b**); ix, Me_3SiBr, CH_2Cl_2, 23 °C; H_2, 10% Pd/C, H_2O, MeOH; CF_3CO_2H, 55 °C, 98%; x, MeOH, reflux, 50%; xi, Ac_2O, 4-*N*,*N*-dimethylaminopyridine, pyridine, 95%; xii, Me_3SiBr, CH_2Cl_2, 23 °C; H_2, 10% Pd/C, H_2O, EtOH, 47%.

Table. Substituted phosphinothricin K_i values and corresponding glutamate K_m values.

Entry	Compound	K_i/μM[a]	K_m/mM[b]
1	(2) PPT	8	3.9
2	(3) GHPPT	14	2.4[c]
3	(24) GMPPT[e]	67	3.6[c]
4	(23) GAPPT	178	*d*

[a] Relative standard deviations are ≤ 15%. [b] As reported in ref 5. [c] The *threo*-isomer. [d] Not available. [e] GMPPT (**24**) was prepared as described in ref. 11.

that charged acidic groups at both positions are required for GS binding. A free methylphosphinic acid moiety is required for the phosphorylation event thought to accompany enzyme inactivation.[9]

Time-dependent kinetic methods were used to characterise further the interaction of these compounds with plant GS.[11] The figure illustrates time-course experiments in which sorghum GS was incubated for 15 min with ATP, $MgCl_2$, and 5 × K_i concentrations of inhibitor, followed by 50-fold dilution and continuous assay of enzyme activity. Under these conditions the greatest recovery of enzyme activity was observed for GMPPT (**24**), whilst somewhat less enzyme reactivation was seen after incubation with the γ-hydroxy analogue GHPPT (**3**). Despite its larger size, the γ-acetoxy group of GAPPT (**23**) was best accommodated at the active site of GS, based on the extent of enzyme recovery observed after incubation with (**23**). Inhibition with DL-phosphinothricin resulted in slight but measurable recovery of activity. Both $MgCl_2$ and ATP were required for enzyme inhibition and recovery, supporting the hypothesis that the phosphinothricins undergo enzyme-mediated phosphorylation in the same manner as do the glutamic acids.[9] If this is the case, then recovery of GS activity is likely to reflect dissociation of the phosphorylated inhibitors from the enzyme active site.

As discussed elsewhere,[11] the phosphinothricins are assumed to bind to the enzyme active site in a fully staggered conformation. The *erythro*-isomer (**25**) of the γ-methyl analogue

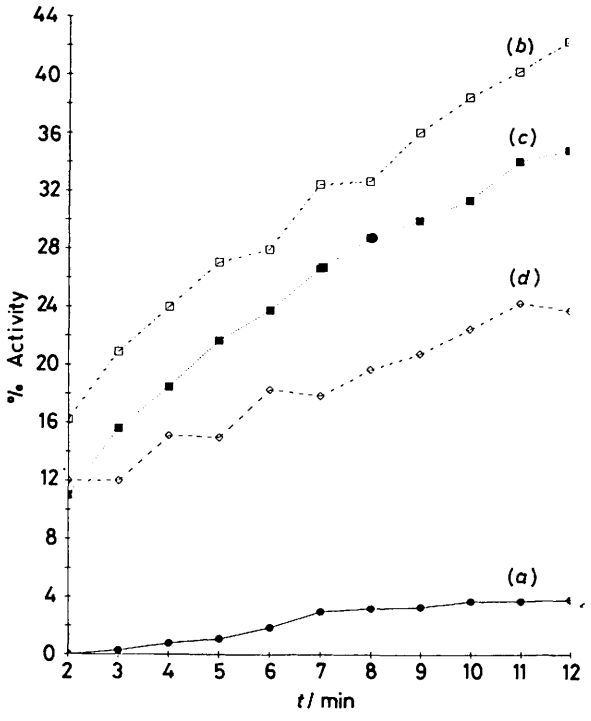
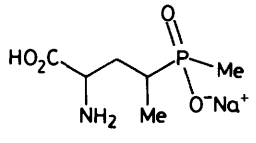
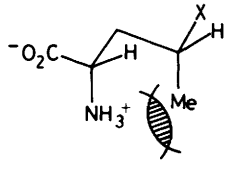


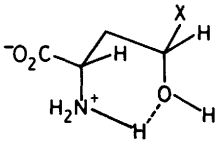
Figure. Recovery curves of (*a*) PPT (**2**), (*b*) GMPPT (**24**), (*c*) GHPPT (**3**), and (*d*) GAPPT (**23**) with sorghum GS.



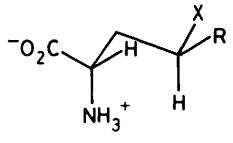
(**24**), GMPPT



(**25**) X = $P(Me)O_2^-$



(**26**) X = $P(Me)O_2^-$



(**27**) X = $P(Me)O_2^-$
R = Me, OH

GMPPT cannot adopt such a conformation, whilst the *erythro*-isomer (**26**) of the γ-hydroxy analogue GHPPT can do so readily as a result of hydrogen bonding. The *threo*-isomer (**27**) of both analogues can also bind to the enzyme in this conformation. The ability of both diastereoisomers of GHPPT (**3**) to interact with GS partially accounts for the favorable K_i value observed for this inhibitor as compared with GMPPT.

The novel inhibitor GHPPT (**3**) displayed potent *in vivo* herbicidal activity against a broad range of plant species, as did the lactone (**20**). Both the acetate GAPPT (**23**) and the methyl carboxylate ester (**21**) were almost as active, suggesting that plants possess metabolic esterase activity capable of generating GHPPT from these derivatives. On the other hand, the ethyl phosphinate ester (**18**) was herbicidally inactive, indicating that plants are incapable of dealkylating the phosphinate ester.

In conclusion, we have demonstrated that considerations of substrate variability for the enzyme glutamine synthetase can be used to design novel, biologically active analogues of the natural product phosphinothricin. Specifically, the effectiveness of L-γ-hydroxyglutamic acids as GS substrates provided a rationale for the design of γ-oxygenated analogues of phosphinothricin. Besides their ability to function as inhibitors of glutamine synthetase, such analogues also possess desirable phytotoxic properties. In addition, these amino acids can be used to study subtle interspecies variations in the enzyme active site. Thus, we have found that kinetic evaluation of DL-γ-hydroxyphosphinothricin GHPPT (**3**) as an inhibitor of *Escherichia coli* glutamine synthetase provides an inhibition of K_i value of 1.6 μM,* one of the most potent binding constants determined for inhibition of GS under reversible conditions by the phosphinothricins.[9] The *X*-ray structure determination of bacterial (*Salmonella typhimurium*) glutamine synthetase offers the further possibility of direct observation of the dynamic effects of bound phosphinothricin analogues on the enzyme conformation.[24]

Experimental

General Methods.—Solvents were dried as necessary prior to use: acetonitrile, dichloromethane, dimethylformamide, toluene, and triethylamine were stored over 3 Å molecular sieves; ethanol was distilled from CaH_2; tetrahydrofuran, dimethyl sulphoxide, and diethyl ether were anhydrous grade. All reactions were run under a nitrogen atmosphere. Preparative TLC was performed on 12 cm diameter circular silica gel plates (Merck PF-254/$CaSO_4$) using a Harrison Research Model 7924 Chromatotron. Flash chromatography was generally performed in a sintered-glass funnel[25] using 40–63 μm Merck silica gel with gradient elution. M.p.s were determined on a Mel-Temp apparatus and are uncorrected. Elemental analyses were performed by Atlantic Microlabs, Inc. Some phosphinic acids tended to retain water and/or solvent, and underwent decomposition upon attempted desiccation *in vacuo*. ^1H NMR spectra were recorded on Varian EM-360L (60 MHz), Bruker WM-360 (360 MHz), Varian XL300 (300 MHz), or Varian XL400 (400 MHz) spectrometers. Chemical shifts are reported as δ_H values relative to internal tetramethylsilane or sodium [2,2,3,3-$^2H_4$]propionate. ^{13}C NMR spectra were recorded on Bruker WM-360 (90.5 MHz), Varian XL300 (75.4 MHz), or Varian XL400 (100.6 MHz) spectrometers. Chemical shifts are reported as δ_C values relative to tetramethylsilane using the deuteriated solvent as the standard or relative to the internal instrument lock on D_2O. In diastereoisomeric mixtures, the major diastereoisomer is denoted with an asterisk. ^{19}F NMR spectra were recorded on a Varian EM-360L (56.4 MHz) spectrometer and chemical shifts are reported as δ_F values relative to trichlorofluoromethane (0 ppm) using an external standard of α,α,α-trifluorotoluene (−63.9 ppm). ^{31}P NMR spectra were recorded on a JEOL FX-100 (40.3 MHz) or Varian XL300 (120.9 MHz) spectrometer and chemical shifts are reported as δ_P values in ppm relative to external H_3PO_4. Mass spectra were recorded on Finnegan 4500 (EI, CI) or VG 70E (FAB, glycerol matrix) instruments.

Ethyl DL-*Methyl*(3-*methyl*-1-*oxobutyl*)*phosphinate* (**5**).—Diethyl methylphosphonite (17.5 ml, 0.117 mol) was added *via* a syringe pump over 1 h under a continuous N_2 stream to a refluxing solution of isovaleryl chloride (**4**) (14.09 g, 0.117 mol) in dry toluene (125 ml). The product was distilled through a

* Determined under standard assay conditions[26] in which the observed substrate K_m for glutamate was 3.3mM.

short Vigreux column to afford 12.19 g (54%) of the *ester* (**5**) as a colourless oil, b.p. 61–64 °C at 0.3 mmHg (Found: C, 49.8; H, 9.0. $C_8H_{17}O_3P$ requires C, 50.0; H, 8.9%); δ_H(360 MHz; $CDCl_3$) 0.96 (d, 3 H, *J* 6.7 Hz), 1.36 (t, 3 H, *J* 6.9 Hz), 1.58 (d, 3 H, *J* 14.2 Hz), 2.27 (m, 1 H), 2.70 (dd, 1 H, *J* 6.7, 6.8 Hz), 2.84 (dd, 1 H, *J* 6.7, 6.8 Hz), and 4.15 (m, 2 H); δ_C(90.5 MHz; $CDCl_3$) 10.5 (d, $^1J_{CP}$ 91.1 Hz), 16.3 (d, $^3J_{CP}$ 5.4 Hz), 22.1, 23.3 (d, $^3J_{CP}$ 2.5 Hz), 50.3 (d, $^2J_{CP}$ 43.5 Hz), 61.5 (d, $^2J_{CP}$ 7.0 Hz), and 215.0 (d, $^1J_{CP}$ 105.3 Hz); δ_P (40.3 MHz; $CDCl_3$) 32.8.

Ethyl DL-(1-*Hydroxy*-3-*methylbutyl*)*methylphosphinate* (**6**).—(*A*) *From ketone* (**5**). A mixture of ketone (**5**) (3.60 g, 18.8 mmol) and PtO_2 (0.36 g) in acetic acid (10 ml) was hydrogenated at atmospheric pressure for 15 h. Since the reaction was incomplete, EtOAc (10 ml) and additional PtO_2 (0.18 g) were added and hydrogenation was continued for 7 h. The product was filtered through Celite, concentrated, taken up in dichloromethane, washed with saturated $NaHCO_3$ and brine, dried ($MgSO_4$), and concentrated. The crude product was filtered through silica gel (10% MeOH–$CHCl_3$) to afford 2.95 g (81%) of the *hydroxy ester* (**6**) as a colourless oil (Found: C, 46.3; H, 9.7. $C_8H_{19}O_3P$·0.75 H_2O requires C, 46.3; H, 9.95%); δ_H(60 MHz; $CDCl_3$) 0.92, 0.98 (2 × d, 3 H, *J* 6 Hz), 1.30 (t, 3 H, *J* 7 Hz), 1.45 (d, 3 H, *J* 14 Hz), 1.15–2.50 (m, 3 H), 3.55–4.30 (m, 1 H), 4.07 (dq, 2 H, *J* 7, 7 Hz), and 4.97 (ddd, 1 H, *J* 4, 6, 14 Hz); δ_P(40.3 MHz; $CDCl_3$) 55.2 (0.86P) and 56.2 (0.14P).

(*B*) *From isovaleraldehyde* (**10**). A mixture of ethyl methylphosphinate (1.5 ml, 10.0 mmol), bis(trimethylsilyl)-acetamide (2.70 ml, 11.0 mmol), and isovaleraldehyde (**10**) (1.10 ml, 10.0 mmol) in CH_2Cl_2 (10 ml) was stirred for 4 h at room temperature. The mixture was poured into water (30 ml) and was extracted with CH_2Cl_2. The organic layers were washed with water and brine, dried ($MgSO_4$), and concentrated. The crude product (**11**) was distilled to afford a colourless oil (1.95 g, 73%), b.p. 59–65 °C at 0.2 mmHg.

The silyl ether (**11**) was heated to reflux in 20% aqueous ethanol (12 ml) for 6 h. ^{31}P NMR spectroscopy indicated complete conversion to products and an isomer ratio of 58:42. The mixture was concentrated, taken up in CH_2Cl_2, washed with water and brine, dried ($MgSO_4$), and concentrated to give 0.45 g of (**6**) (23%, a low yield because of the solubility of the product in water) as a colourless oil; δ_H(60 MHz; $CDCl_3$) 0.92, 0.98 (2 × d, 3 H, *J* 6 Hz), 1.30 (t, 3 H, *J* 7 Hz), 1.42, 1.45 (2 × d, 3 H, *J* 14 Hz), 1.15–2.50 (m, 3 H), 3.60–4.20 (m, 1 H), 4.10, 4.13 (2 × dq, 2 H, *J* 7, 7 Hz), and 4.40–5.30 (m, 1 H); δ_P(40.3 MHz; $CDCl_3$) 54.8 (0.33P) and 55.1 (0.67P).

DL-(1-*Hydroxy*-3-*methylbutyl*)*methylphosphinic Acid, Dicyclohexylamine Salt* (**12**).—The phosphinate (**6**) (1.51 g, 7.78 mmol), prepared from (**5**), was dissolved in dry dichloromethane (10 ml) and treated with bromotrimethylsilane (3.1 ml, 23.5 mmol). The mixture was stirred for 6 h at room temperature then concentrated under high vacuum, and the residue was dissolved in 10% aqueous tetrahydrofuran (THF) (20 ml). The mixture was stirred at room temperature overnight and concentrated to afford 1.42 g (110%) of the phosphinic acid as an orange, viscous oil.

The crude phosphinic acid was dissolved in ethyl acetate (20 ml) and treated with dicyclohexylamine (1.73 ml, 8.7 mmol). The resulting white precipitate was filtered off, washed with EtOAc and light petroleum, and dried under high vacuum to afford 2.22 g (82 %) of the *salt* (**12**) as a white solid, m.p. 150–152 °C (Found: C, 60.6; H, 10.8; N, 4.1. $C_{18}H_{38}NO_3P$·0.5 H_2O requires C, 60.65; H, 11.0; N, 3.9%); δ_H(60 MHz; $CDCl_3$) 0.87, 0.94 (2 × d, 3 H, *J* 6 Hz), 1.00–2.32 (m, 27 H), 1.18 (d, 3 H, *J* 14 Hz), 2.60–3.20 (m, 2 H), 3.20–3.70 (m, 1 H), and 6.20–8.40 (br s, 2 H); δ_P(40.3 MHz; $CDCl_3$) 36.5.

Benzyl DL-(2-*Oxotetrahydrofuran*-3-*yl*)*carbamate* (**14**).—Benzyl chloroformate (126 ml, 0.84 mol) was added dropwise to a stirred mixture of DL-homoserine (**13**) (100.0 g, 0.84 mol) and sodium hydrogen carbonate (157.5 g, 1.88 mol) in water (1 l) at room temperature over 1 h. The mixture was stirred overnight, acidified to pH 3 with concentrated HCl, and extracted with EtOAc. The organic layers were washed with brine, dried ($MgSO_4$), and concentrated to a viscous oil. The product was heated overnight under high vacuum (35 °C) followed by 1 h on a steam bath to afford 164.67 g (83%) of the lactone (**14**) as a white solid. A portion (11.82 g) of the product was recrystallised ($CHCl_3$–light petroleum) to give white crystals (9.16 g), m.p. 107.5–108 °C (lit.,[19] 93–97 °C), R_F 0.35 (50% EtOAc–hexane) (Found: C, 61.2; H, 5.6; N, 5.9. Calc. for $C_{12}H_{13}NO_4$: C, 61.3; H, 5.6; N, 5.95%); δ_H(60 MHz; $CDCl_3$) 1.75–2.90 (series of m, 2 H), 3.90–4.60 (m, 3 H), 5.07 (s, 2 H), 5.65 (br d, 1 H, *J* 8 Hz), and 7.33 (s, 5 H).

Benzyl DL-N-(*Benzyloxycarbonyl*)*homoserinate* (**15**).—A solution of sodium hydroxide (18.24 g, 0.455 mol) in anhydrous methanol (200 ml) was added to a stirred suspension of the lactone (**14**) (107.0 g, 0.455 mol) in methanol (250 ml). The resulting solution was stirred for 1.5 h at room temperature and concentrated to afford a white solid. The carboxylate salt was dissolved in dry dimethylformamide (DMF) (450 ml) and benzyl bromide (54 ml, 0.454 mol) was added. The mixture was stirred at room temperature for 1 day, poured into water (1.5 l), and extracted with ether. The ether layers were washed with water and brine, dried ($MgSO_4$), and concentrated to give the alcohol (**15**) as a white solid (147.00 g, 94%). The product was recrystallised from ether to afford 99.09 g. A second crop gave an additional 25.14 g for a total yield of 124.23 g (80%), m.p. 63.5–65 °C (lit.,[20a] 60–61 °C) (Found: C, 66.5; H, 6.2; N, 4.1. Calc. for $C_{19}H_{21}NO_5$: C, 66.5; H, 6.2; N, 4.1%); δ_H(60 MHz; $CDCl_3$) 1.40–2.35 (m, 2 H), 2.90 (m, 1 H), 3.57 (m, 2 H), 4.00–4.68 (m, 1 H), 5.07, 5.13 (2 × s, 4 H), 5.82 (br d, 1 H, *J* 7.2 Hz), and 7.33 (s, 10 H).

Benzyl DL-2-(*Benzyloxycarbonylamino*)-4-*oxobutanoate*[20a] (**16**).—Anhydrous dimethyl sulphoxide (DMSO) (17.0 ml, 0.22 mol) in dichloromethane (30 ml) was added dropwise to a stirred solution of oxalyl chloride (10.0 ml, 0.11 mol) in dry dichloromethane (250 ml) at −78 °C. The solution was stirred for 2 min and the alcohol (**15**) (34.3 g, 0.1 mol) in dichloromethane (100 ml) was added over 3 min. The resulting slurry was stirred for 15 min and triethylamine (70 ml, 0.5 mol, stored over 3 Å molecular sieves) was added. The mixture was stirred for an additional 5 min at −78 °C, then allowed to warm at room temperature for 10 min and poured into water (0.5 l). The layers were separated, the aqueous layer was extracted with dichloromethane, and the combined organic layers were washed with 0.6M HCl, saturated $NaHCO_3$, and brine, dried ($MgSO_4$), and concentrated to afford 34.48 g (101%) of the *aldehyde* (**16**) as a pale yellow oil (Found: C, 66.7; H, 5.65; N, 4.0. $C_{19}H_{19}NO_5$ requires C, 66.85; H, 5.6; N, 4.1%); δ_H(60 MHz; $CDCl_3$) 3.00 (d, 2 H, *J* 5 Hz), 4.35–4.80 (m, 1 H), 5.03, 5.18 (2 × s, 4), 5.80 (br d, 1 H, *J* 6 Hz), 7.05 (s, 10 H), and 9.70 (s, 1 H).

Benzyl DL-N-*Benzyloxycarbonyl*-4-[*ethoxy*(*methyl*)*phosphinyl*]-O-*trimethylsilylhomoserinate* (**17a**).—The aldehyde (**16**) (3.36 g, 9.85 mmol) in dry dichloromethane (100 ml) was treated with ethyl methylphosphinate (1.02 ml, 9.85 mmol) and bis(trimethylsilyl)acetamide (2.70 ml, 10.8 mmol), and was stirred at room temperature overnight. The solution was washed with 2M HCl and saturated $NaHCO_3$, dried ($MgSO_4$), and concentrated to an oil which was purified by flash chromatography (EtOAc–hexane) to give 3.25 g (63%) of the

trimethylsilyl ether (**17a**) as a colourless oil (Found: C, 57.3; H, 7.0; N, 2.7. $C_{25}H_{36}NO_7PSi$ requires C, 57.6; H, 7.0; N, 2.7%); δ_H(60 MHz; $CDCl_3$) 0.09. 0.12. 0.14, 0.16 (4 × s, 9 H), 1.19, 1.29, 1.38 (3 × d, 3 H, *J* 14.3 Hz), 1.25, 1.28, 1.30 (3 × t, 3 H, *J* 7.0 Hz), 1.95–2.40 (m, 2 H), 3.90–4.08 (m, 3 H), 4.50 (m, 1 H), 5.03–5.24 (m, 4 H), 5.67, 5.98, 6.16, 6.33 (4 × br d, 1 H, *J* 8.6 Hz), and 7.32 (s, 10 H); δ_P(40.3 MHz; $CDCl_3$) 50.9 (0.39P), 51.3 (0.18P), 55.2 (0.30P), and 55.6 (0.15P).

Further elution of the silica gel with 40% Pr^iOH–EtOAc gave the alcohol (**17b**) (1.18 g, 27%).

Benzyl DL-N-*Benzyloxycarbonyl*-4-[*ethoxy*(*methyl*)*phosphinyl*]*homoserinate* (**17b**).—The aldehyde (**16**) (34.08 g, 0.10 mol) in dry dichloromethane (500 ml) cooled in an ice-bath was treated with ethyl methylphosphinate (10.4 ml, 0.10 mol) and bis(trimethylsilyl)acetamide (27.2 ml, 0.11 mol). The mixture was stirred at room temperature overnight, washed with 2M HCl and saturated $NaHCO_3$, dried ($MgSO_4$), and concentrated to an oil.

The crude trimethylsilyl ether (**17a**) was dissolved in acetonitrile (250 ml) and treated with aqueous HF (49%; 12.2 ml, 0.3 mol) for 0.5 h at room temperature. Saturated aqueous $NaHCO_3$ (200 ml) was added, the layers were separated, and the aqueous layer was extracted with dichloromethane. The combined organic layers were washed with water, dried ($MgSO_4$), concentrated, and flash chromatographed (CH_2Cl_2→EtOAc→50% Pr^iOH–EtOAc) to afford 38.11 g (85%) of the *alcohol* (**17b**) as a colourless oil (Found: C, 58.7; H, 6.3; N, 3.0. $C_{22}H_{28}NO_7P$ requires C, 58.8; H, 6.3; N, 3.1%); δ_H(400 MHz; $CDCl_3$) 1.19–1.38 (4 × t, 3 H, *J* 7.0 Hz), 1.40, 1.45, 1.49 (3 × d, 3 H, *J* 14.4 Hz), 1.94–2.38 (m, 2 H), 3.74–4.69 (series of m, 5 H), 5.08–5.24 (m, 4 H), 5.83, 5.85 (2 × d, 0.6 H, *J* 3.6 Hz), 6.05, 6.08 (2 × d, 0.4 H, *J* 7.6 Hz), and 7.34 (br s, 10H); δ_P(40.3 MHz; $CDCl_3$) 53.1 (0.30P), 53.6 (0.51P), and 53.9 (0.19P); *m*/*z* (FAB MS) 450 (M^+ + H).

Sodium DL-(3-*Amino*-3-*carboxy*-1-*hydroxypropyl*)*methylphosphinate* (*GHPPT*) (**3**).—A solution of the phosphinate (**17a**) (10.58 g, 20.3 mmol) in dry dichloromethane (35 ml) was treated with bromotrimethylsilane (5.40 ml, 40.6 mmol) and stirred for 2 h at room temperature. The reaction mixture was concentrated under high vacuum and the residue was dissolved in methanol (80 ml). Palladium/carbon (10%; 0.44 g) was added and the mixture was hydrogenated at atmospheric pressure overnight. Since the reaction was not complete, the suspension was filtered through Celite and concentrated, and was taken up in 25% aqueous methanol. Additional 10% Pd/C (0.50 g) and 2.5M NaOH (8.12 ml, 20.3 mmol) were added and the mixture was hydrogenated for an additional 3 h. The reaction mixture was filtered through Celite and concentrated, and the product was precipitated with acetone. The precipitate was dried, dissolved in water, and lyophilised to afford 2.45 g (55%) of GHPPT (**3**) as an off-white hygroscopic solid, m.p. 100–120 °C (gradual decomp.) (Found: C, 22.75; H, 6.0; N, 5.2. $C_5H_{11}NaNO_5P$·2.5H_2O requires C, 22.7; H, 6.1; N, 5.3%); δ_H(300 MHz; D_2O) 1.25 (d, 3 H, $^2J_{CP}$ 14.2 Hz), 1.90–2.35 (series of m, 2 H), and 3.69–3.93 (m, 2 H); δ_C(90.6 MHz; D_2O; 56:44 diastereoisomeric mixture) 12.78* (d, $^1J_{CP}$ 91.9 Hz), 32.09*, 32.35, 53.59* (d, $^3J_{CP}$ 11.8 Hz), 54.30 (d, $^3J_{CP}$ 11.6 Hz), 68.62* (d, $^1J_{CP}$ 110.2 Hz), 69.65 (d, $^1J_{CP}$ 109.7 Hz), 175.86, and 176.10*; δ_P(40.3 MHz; D_2O) 40.47.

DL-4-[*Ethoxy*(*methyl*)*phosphinyl*]*homoserine* (**18**).—A mixture of the alcohol (**17b**) (2.45 g, 5.46 mmol) and 10% Pd/C (0.50 g) in absolute ethanol (30 ml) was hydrogenated at atmospheric pressure overnight. The mixture was filtered through Celite and concentrated. Since the reaction was incomplete, the residue was dissolved in 10% aqueous ethanol (50 ml), 10% Pd/C (1.00 g) was added, and hydrogenation was continued overnight. The suspension was filtered through Celite and the filtrate was treated with ether. The resulting precipitate was filtered off, washed with ether and pentane, and dried under high vacuum to afford 0.68 g (55%) of the *hydroxy ester* (**18**) as a white solid, m.p. 167–169 °C (decomp.) (Found: C, 36.3; H, 7.1; N, 5.9. $C_7H_{16}NO_5P$·0.4 H_2O requires C, 36.2; H, 7.3; N, 6.0%); δ_H(300 MHz; D_2O) 1.32, 1.33 (2 × t, 3 H, *J* 7.2 Hz), 1.62, 1.63 (2 × d, 3 H, *J* 13.5 Hz), 2.00–2.40 (m, 2 H), and 3.85–4.35 (m, 4 H); δ_P(40.3 MHz; D_2O) 57.1 (0.55P) and 58.0 (0.45P).

Benzyl DL-5-[*Ethoxy*(*methyl*)*phosphinyl*]-2-*oxotetrahydrofuran*-3-*ylcarbamate* (**19**).—The trimethylsilyl ether (**17a**) (2.01 g, 3.86 mmol) in dry dichloromethane (20 ml) was treated with trifluoroacetic acid (4 ml) and stirred at room temperature overnight. The mixture was concentrated, azeotroped with toluene, and chromatographed on a 4 mm Chromatotron plate (20% Pr^iOH–EtOAc) to afford 0.91 g (71%) of the *lactone* (**19**) as a colourless oil (Found: C, 52.0; H, 6.0; N, 3.9. $C_{15}H_{20}NO_6P$·0.3H_2O requires C, 52.0; H, 6.0; N, 4.0%); ν_{max}($CHCl_3$) 1 790 (γ-lactone) and 1 715 cm^{-1} (carbamate); δ_H(60 MHz; $CDCl_3$) 1.32 (t, 3 H, *J* 7.0 Hz), 1.56, 1.58, 1.61 (3 × d, 3 H, *J* 14.0 Hz), 2.20–3.00 (m, 2 H), 4.02–4.22 (m, 2 H), 4.38–4.72 (m, 2 H), 5.11 (m, 2 H), 5.91, 5.99, 6.05, 6.30 (4 × br d, 1 H, *J* 7.2 Hz), and 7.34 (m, 5 H); δ_P(40.3 MHz; $CDCl_3$) 45.5 (0.33P), 47.6 (0.33P), and 48.0 (0.34P); *m*/*z* (FAB MS) 342 (M^+ + H).

DL-5-[*Hydroxy*(*methyl*)*phosphinyl*]-2-*oxotetrahydrofuran*-3-*ylammonium Trifluoroacetate* (**20**).—The lactone (**19**) (6.56 g, 18.8 mmol) was dissolved in dry dichloromethane (150 ml) and bromotrimethylsilane (4.9 ml, 38 mmol) was added. The mixture was stirred at room temperature overnight, concentrated, taken up in toluene–propylene oxide, and reconcentrated. The residue was taken up in 10% aqueous methanol (100 ml), 10% Pd/C (1.10 g) was added, and the solution was hydrogenated at atmospheric pressure overnight. The suspension was filtered through Celite, concentrated and lyophilised. ^1H and ^{31}P NMR spectroscopy indicated a mixture of 40–50% of the methyl ester and desired lactone. The crude product was dissolved in trifluoracetic acid (20 ml) and heated at 55 °C overnight. The mixture was concentrated and addition of anhydrous ether precipitated the product. The product was washed with ether and pentane, and dried under high vacuum to afford the lactone trifluoroacetate salt (**20**) (5.42 g, 98%), m.p. 110–115 °C (decomp.) (Found: C, 30.3; H, 4.4; N, 4.2. $C_7H_{11}F_3NO_6P$·0.12Et_2O·0.12H_2O requires C, 30.3; H, 4.4; N, 4.4%); δ_H(400 MHz; [$^2H_6$]DMSO; 60:40 diastereoisomeric mixture) 1.31* (d, 3 H, $^2J_{CP}$ 14.0 Hz), 1.32 (d, $^2J_{CP}$ 14.9 Hz), 2.30–2.46* (m, 1 H), 2.66* (m, 0.6H), 2.79 (m, 0.4H), 4.37* (m, 1 H), 4.61* (t, 0.6H, *J* 7.6 Hz), and 4.76 (dd, 0.4H, *J* 4.0, 9.3 Hz); δ_C(100.6 MHz; [$^2H_6$]DMSO) 14.84* (d, $^1J_{CP}$ 96.5 Hz), 28.58, 29.33*, 50.46, 51.37* (d, $^3J_{CP}$ 4.7 Hz), 77.62* (d, $^1J_{CP}$ 109.8 Hz), 78.34 (d, $^1J_{CP}$ 106.6 Hz), 118.80* (q, $^1J_{CF}$ 291.6 Hz), 165.28* (q, $^2J_{CF}$ 35.4 Hz), 175.91*, and 175.98; δ_P(40.3 MHz; [$^2H_6$]DMSO) 35.47*, and 37.01.

On standing in D_2O, (**20**) gave 19% of the ring-opened compound after 1.5 h and 77% after 19 h.

Methyl DL-4-[*Hydroxy*(*methyl*)*phosphinyl*]*homoserinate* (**21**).—The lactone salt (**20**) (1.00 g, 3.41 mmol) was dissolved in methanol (10 ml) and heated to reflux for 6 h. The product was precipitated with anhydrous ether and two crops were obtained. The combined crops were dissolved in water, filtered, and lyophilized to give the ester (**21**) as a glass (0.56 g, 50%), m.p. 90–92 °C (decomp.) (Found: C, 30.1; H, 6.0; N, 5.3. $C_6H_{14}NO_5P$·0.4CF_3CO_2H·0.8H_2O requires C, 30.1; H, 5.95;

N, 5.2%); ^{1}H and ^{13}C NMR spectra (400 MHz) showed that partial relactonization had occurred (23%): δ_H(400 MHz; D_2O) 1.61, 1.62, 1.68 (minor) (3 × d, 3 H, *J* 13.5 Hz), 2.40–2.77 (m, 2 H), 4.09–4.24 (m, 1 H), 4.16, 4.17 (2 × s, 3 H), and 4.69 (m, 1 H); δ_P(40.3 MHz; D_2O) 33.9 (0.23P) and 41.7 (0.77P).

Benzyl DL-O-*Acetyl*-N-*benzyloxycarbonyl*-4-[*ethoxy*(*methyl*)*phosphinyl*]*homoserinate* (**22**).—The alcohol (**17b**) (1.17 g, 2.61 mmol) in dry dichloromethane (20 ml) was treated with acetic anhydride (0.40 ml, 4.24 mmol), pyridine (0.34 ml, 4.24 mmol), and 4-dimethylaminopyridine (5 mg). The mixture was stirred at room temperature overnight and was poured into 2M HCl (50 ml). The layers were separated and the aqueous layer was extracted with dichloromethane. The organic layers were washed with saturated $NaHCO_3$, dried ($MgSO_4$), and concentrated to afford an oil. The oil was taken up in ether and precipitated with pentane to give 1.22 g (95%) of the *ester* (**22**) as a gummy solid (Found: C, 57.9; H, 6.2; N, 2.8. $C_{24}H_{30}NO_8P$·0.35H_2O requires C, 57.9; H, 6.2; N, 2.8%); δ_H(300 MHz; $CDCl_3$) 1.25, 1.26, 1.28, 1.29 (4 × t, 3 H, *J* 7.0 Hz), 1.416, 1.422, 1.446 (3 × d, 3 H, *J* 13.9 Hz), 1.92, 1.94, 2.11, 2.12 (4 × s, 3 H), 2.15–2.60 (m, 2 H), 3.92–4.15 (m, 2 H), 4.47–4.64 (m, 1 H), 5.06–5.18 (m, 4 H), 5.22–5.43 (m, 1 H), 5.54, 5.57 (2 × m, 1 H), and 7.34 (m, 10 H); δ_P(40.3 MHz; $CDCl_3$) 47.6 (0.25P), 48.5 (0.24P), 49.0 (0.23P), and 49.6 (0.28P); *m/z* (FAB MS) 492 (M^+ + H).

DL-O-*Acetyl*-4-[*hydroxy*(*methyl*)*phosphinyl*]*homoserine*, 90:10 *Diastereoisomeric Mixture* (*GAPPT*) (**23**).—Bromotrimethylsilane (2.90 ml, 22.0 mmol) was added to a solution of the acetate (**22**) (6.75 g, 13.7 mmol) in dry dichloromethane (100 ml). The mixture was stirred at room temperature overnight, concentrated, and treated with propylene oxide (5 ml) in toluene (200 ml). The solution was set aside at room temperature for 2 h and concentrated. The residue was taken up in 10% aqueous ethanol (100 ml), 10% Pd/C (1.00 g) was added, and the stirred suspension was hydrogenated at atmospheric pressure overnight. Additional 10% Pd/C (1.00 g) was added and hydrogenation was continued for 6 h. The suspension was filtered through Celite, concentrated to an aqueous solution, and precipitated with acetone–ether. The mother liquors were concentrated, lyophilised, taken up in methanol–ethanol (1:1), and precipitated with acetone. The filtered precipitates were combined, taken up in water and lyophilised to afford 1.54 g (47%) of the ester (**23**) as a white powder, m.p. 124–126 °C (decomp.) (Found: C, 32.2; H, 6.25; N, 5.35. $C_7H_{14}NO_6P$·1.2H_2O requires C, 32.2; H, 6.3; N, 5.4%); δ_H(400 MHz; D_2O; 90:10 diastereoisomeric mixture) 1.27 (d, $^2J_{CP}$ 14.5 Hz), 1.28* (d, 3 H, $^2J_{CP}$ 14.4), 2.15 (s), 2.17* (s, 3 H), 2.28–2.50* (m, 2 H), 4.02* (dd, 1 H, *J* 4.9, 9.1 Hz), 4.12 (t, *J* 5.9 Hz), 5.05–5.13* (m, 1 H), and 5.48 (m); δ_C(75.4 MHz; D_2O; major isomer only) 15.39 (d, $^1J_{CP}$ 95.6 Hz), 23.04, 32.96, 53.64 (d, $^3J_{CP}$ 9.1 Hz), 71.96 (d, $^1J_{CP}$ 107.1 Hz), 174.42, and 176.16 (d, $^3J_{CP}$ 4.3 Hz); δ_P(40.3 MHz; D_2O) 37.62.

Evaluation of Phosphinothricin Analogues as Inhibitors of Glutamine Synthetase.—Cytosolic glutamine synthetase was isolated from aetiolated sorghum seedlings according to an established procedure.[23] *E. coli* glutamine synthetase was a gift from Professor Villafranca.[9a] Steady-state glutamine synthetase activity was determined by endpoint measurement of the phosphate released upon enzymatic hydrolysis of ATP.[26] Time-dependent GS activity was determined by continuous measurement of ADP released.[27] Procedures for graphical determination of inhibition included the Lineweaver–Burk and Dixon methods.[28] Reported constants represent average values taken from several plots and showed relative standard deviations of less than 15%.

Acknowledgements

We thank Professor Joseph J. Villafranca (Pennsylvania State University) for many helpful discussions and for a generous gift of *E. coli* glutamine synthetase.

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Paper 9/03025E
Received 17*th July* 1989
Accepted 23*rd September* 1989