Synthesis of D,L-γ-Hydroxyphosphinothricin, a Potent New Inhibitor of Glutamine Synthetase

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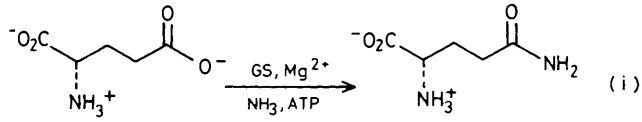
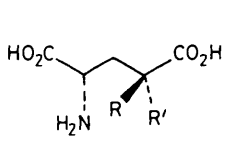
A flexible synthesis of D,L-γ-hydroxyphosphinothricin (GHPPT), a potent inhibitor of the enzyme glutamine synthetase, is described which features silicon-mediated addition of ethyl methylphosphinate to benzyl 2-benzyloxycarbonylamino-4-oxobutyrate; selective deprotections yield the title compound and various derivatives.

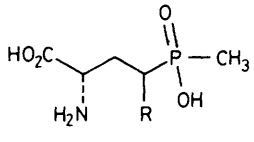
The enzyme glutamine synthetase (GS; E.C. 6.3.1.2) catalyses an important reaction in nitrogen metabolism, the conversion of L-glutamate into L-glutamine [reaction (i)].[1] While L-glutamic acid serves as the natural substrate for the enzyme, it was reported some time ago that the γ-hydroxy analogues (**1a** and **b**) also function as remarkably efficient substrates, the *threo*-isomer (**1a**) possessing a lower K_m value than that of L-glutamic acid itself.[2,3] We have been engaged in the design of analogues of phosphinothricin (PPT) (**2a**), a naturally occurring phosphinic acid mimic of L-glutamic acid[4,5]



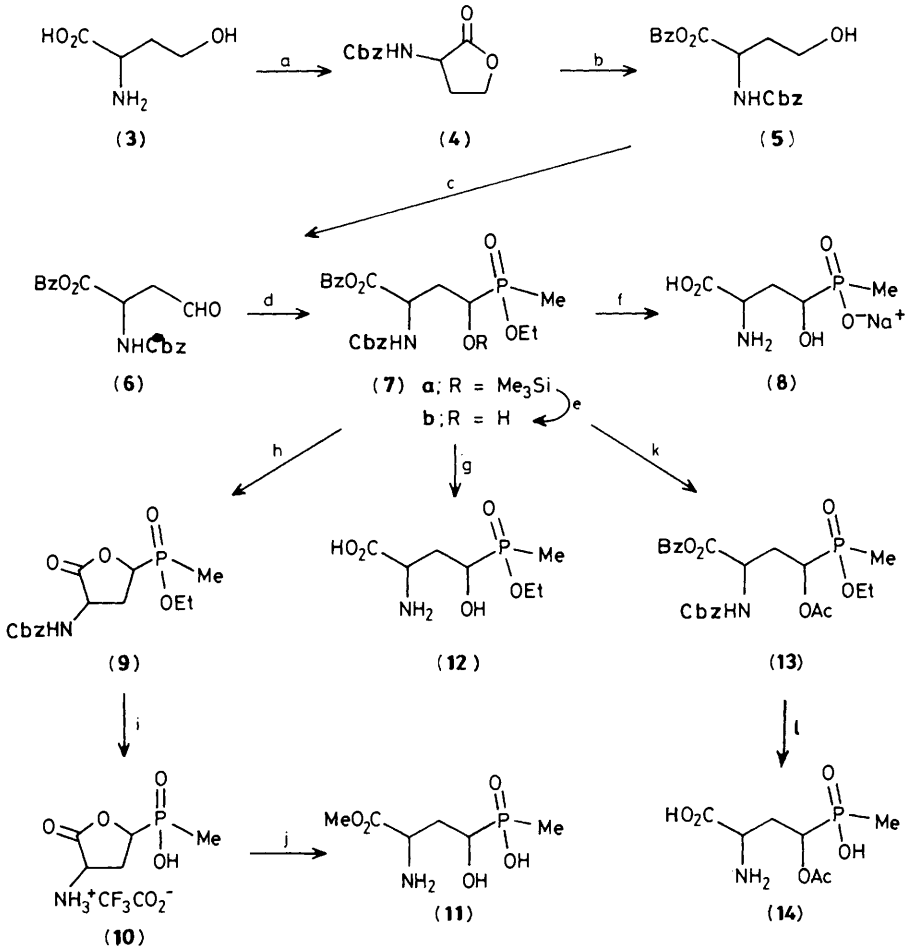
(i)



(**1**) **a**; R = OH, R′ = H
b; R = H, R′ = OH



(**2**) **a**; R = H, PPT
b; R = OH, GHPPT



Scheme 1. *Reagents:* a, CbzCl, H_2O, $NaHCO_3$; 100 °C (0.1 mm Hg), 83%; b, NaOH, MeOH; $PhCH_2Br$, Me_2NCHO, 23 °C, 24 h, 80%; c, Me_2SO, CH_2Cl_2, $(COCl)_2$, −78 °C; Et_3N, 100%; d, MeP(O)HOEt, BSA, CH_2Cl_2, 23 °C, 90%; e, HF, H_2O, MeCN, 95%; f, Me_3SiBr, CH_2Cl_2, 23 °C; H_2, 10% Pd/C, H_2O, MeOH, 1 equiv. NaOH, 55%; g, H_2, 10% Pd/C, H_2O, EtOH, 55%; h, CF_3CO_2H, CH_2Cl_2, 23 °C, 71% from (**7a**) or 100% from (**7b**); i, Me_3SiBr, CH_2Cl_2, 23 °C; H_2, 10% Pd/C, H_2O, MeOH; CF_3CO_2H, 55 °C, 98%; j, MeOH, reflux, 50%; k, Ac_2O, 4-*N*,*N*-dimethylaminopyridine, pyridine, 95%; l, Me_3SiBr, CH_2Cl_2, 23 °C; H_2, 10% Pd/C, H_2O, EtOH, 47%.

and an effective herbicide.[6,7] It was of interest to investigate the effect of introducing substituents found in alternative GS substrates into the structure of transition state analogue inhibitors such as (**2a**).[8] We report here an efficient synthesis of D,L-γ-hydroxyphosphinothricin (GHPPT), a novel phosphinothricin analogue which functions as a potent competitive inhibitor of glutamine synthetase.

A flexible route was developed which also afforded selectively functionalized derivatives of interest in probing structure–activity relationships in GS inhibitors. D,L-homoserine (**3**) was converted *via* the lactone (**4**)[9] into the *N*-Cbz benzyl ester (**5**) in 66% yield. Previously described methods for oxidizing (**5**) to benzyl 2-benzyloxycarbonylamino-4-oxobutyrate (**6**) proved unsatisfactory;[10] however, Swern conditions (oxalyl chloride, Me_2SO in CH_2Cl_2 at −78 °C followed by triethylamine) were found to yield (**6**) almost quantitatively, regardless of scale. Addition of ethyl methylphosphinate to (**6**) in the presence of bis(trimethylsilyl)acetamide(BSA)[11] smoothly gave the silyl ether (**7a**) and the alcohol (**7b**) in 63 and 27% yields, respectively, after silica gel chromatography (EtOAc/hexane, followed by EtOAc/Pr^iOH). The ether (**7a**) and the alcohol (**7b**) were both obtained as mixtures of four chromatographically inseparable diastereoisomers. Treatment of (**7a**) with bromotrimethylsilane in methylene chloride, followed by hydrogenation in aqueous methanol containing an equivalent of sodium hydroxide, filtration through Celite, and precipitation with acetone afforded the sodium salt of D,L-γ-hydroxyphosphinothricin (GHPPT) (**8**) in 55% yield. It was obtained as a hygroscopic solid, m.p. 100—120 °C, consisting of a 56:44 mixture of diastereoisomers.†

The alcohol (**7b**) served as a key intermediate for the derivatives (**14**) (GAPPT), (**10**), (**11**), and (**12**) (see Scheme 1).

Under standard assay conditions‡ with bacterial (*E. coli*) glutamine synthetase,§ in which the observed substrate K_m value for glutamate was 3.3 mM, a value of 1.6 μM was obtained for K_i of the inhibitor (**8**) (GHPPT), one of the most potent binding constants yet seen for inhibition of GS under reversible conditions.[4,5] A K_i value of 33 μM was determined for (**14**) (GAPPT). Both compounds were comparably effective inhibitors of GS from sheep brain and sorghum leaf. The compounds (**8**) (GHPPT), (**14**) (GAPPT), (**10**), and (**11**) all showed strong herbicidal activity against a broad range of plant species. Interestingly, the phosphinate ester (**12**) was completely inactive, supporting the hypothesis that enzyme mediated phosphorylation of the phosphinic acid moiety by ATP is required for inhibition.[5] Biochemical studies in this series will be described in greater detail elsewhere.

Received, 8th June 1987; Com. 777

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† All compounds isolated gave satisfactory spectroscopic (^1H and ^{31}P n.m.r.) and analytical data. Selected physical data include (spectral resonances corresponding to the major diastereoisomer in isomeric mixtures are denoted with an asterisk): (**8**), m.p. 100—120 °C (gradual decomp.); ^1H n.m.r. (300 MHz; D_2O) δ 1.25 (d, 3H, $^2J_{CP}$ 14.2 Hz), 1.90—2.35 (series of m, 2H), and 3.69—3.93 (m, 2H); ^{13}C n.m.r.(90.6 MHz; D_2O; 56:44 diastereomeric mixture) δ 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), and 175.86, 176.10*; ^{31}P n.m.r. (40.3 MHz; D_2O) δ 40.47.

(**10**), m.p. 110—115 °C (decomp.); ^1H n.m.r. [400 MHz; $(CD_3)_2SO$; 60:40 diastereoisomeric mixture] δ 1.31* (d, 3H, $^2J_{CP}$ 14.0 Hz), 1.32 (d, $^2J_{CP}$ 14.9 Hz), 2.30—2.46* (m, 1H), 2.66* (m, 0.6 H), 2.79 (m, 0.4 H), 4.37* (m, 1H), 4.61* (t, 0.6 H, *J* 7.6 Hz), and 4.76 (dd, 0.4 H, *J* 4.0 and 9.3 Hz); ^{13}C n.m.r. [100.6 MHz; $(CD_3)_2SO$] δ 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; ^{31}P n.m.r. [40.3 MHz, $(CD_3)_2SO$] δ 35.47* and 37.01.

(**14**) m.p. 124—126 °C (decomp.) ^1H n.m.r. (400 MHz; D_2O; 90:10 diastereoisomeric mixture) δ 1.27 (d, $^2J_{CP}$ 14.5 Hz), 1.28* (d, 3H, $^2J_{CP}$ 14.4), 2.15 (s), 2.17* (s, 3H), 2.28—2.50* (m, 2H), 4.02* (dd, 1H, *J* 4.9 and 9.1 Hz), 4.12 (t, *J* 5.9 Hz), 5.05—5.13* (m, 1H), and 5.48 (m); ^{13}C n.m.r. (75.4 MHz; D_2O; major isomer only) δ 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); ^{31}P n.m.r.(40.3 MHz, D_2O) δ 37.62.

‡ Values of K_i were determined by the Lineweaver-Burke method, utilizing data obtained from assays measuring either phosphate or ADP release under conditions in which irreversible inhibition was not significant.

§ Generously provided by Professor Joseph J. Villafranca.