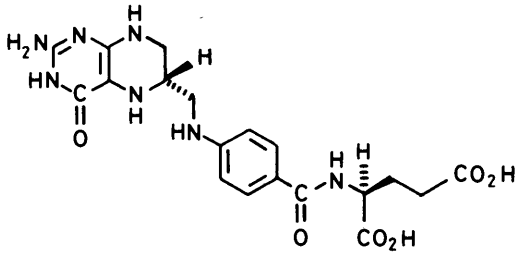
Structural Studies on Bioactive Compounds. Part 7.[1] The Design and Synthesis of α-Substituted Serines as Prospective Inhibitors of Serine Hydroxymethyltransferase

Saul J. B. Tendler,† Michael D. Threadgill,* and Michael J. Tisdale
Pharmaceutical Sciences Institute, Department of Pharmaceutical Sciences, Aston University, Birmingham B4 7ET

A short series of α-substituted analogues of serine, designed as enzyme-activated, irreversible inhibitors of serine hydroxymethyltransferase, has been prepared. (±)-α-Allyl- and (±)-α-prop-2-ynyl-serine were synthesised by appropriate alkylation of the anion derived from ethyl acetamidocyanoacetate, followed by selective reduction of the ester function and hydrolysis of protecting groups. Acetoxymethylation of the anion derived from methyl 2-(benzylideneamino)but-2-enoate gave (±)-α-vinylserine after deprotection. These novel analogues of serine were largely inactive as inhibitors of the enzyme, except that (±)-α-vinylserine showed weak competitive inhibition.

Serine hydroxymethyltransferase (SHMT, EC 2.1.2.1) catalyses the conversion of L-serine (**4**) to glycine (**5**) in the presence of tetrahydrofolic acid (FH_4) (**1**), generating a one-carbon unit in



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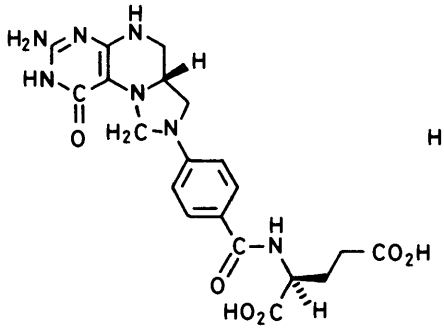

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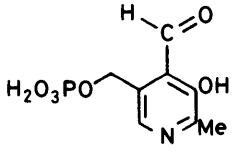
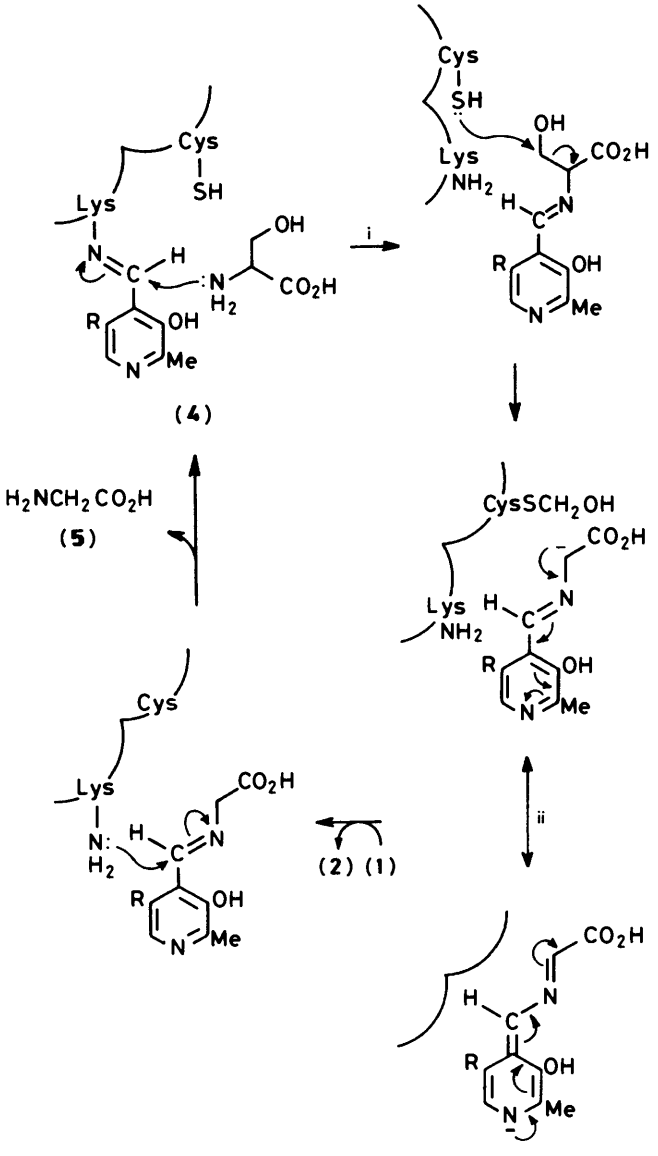


(**3**)

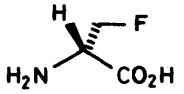
the form of N^5,N^{10}-methylenetetrahydrofolate (FH_4-CH_2) (**2**).[2] This one-carbon unit may then be used either for the biosynthesis of purines and pyrimidines or for other anabolic processes. The enzyme is a tetramer with each subunit having a coenzyme, pyridoxal phosphate (PLP) (**3**).[3] The enzyme SHMT appears to have an essential role in rapidly proliferating tumour cells which have a high requirement for L-serine.[4] In these cells, it has elevated activity in channelling one-carbon units into *de novo* nucleoside synthesis.[5] If SHMT could be inhibited, then it may be possible to inhibit the growth of these cells as a strategy for cancer chemotherapy.

† *Present address:* Division of Physical Biochemistry, National Institute for Medical Research, Mill Hill, London.



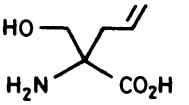
Scheme 1. Probable mechanism of serine hydroxymethyltransferase: i, Transimination; ii, delocalisation of negative charge. R = CH_2O-PO_3H^-

L-Serine (**4**) binds to the active site of the enzyme by a transimination reaction, as shown in Scheme 1, and is cleaved by a reverse-aldol reaction to a stabilised carbanion. Protonation of the carbanion followed by a reversal of the initial transimination produces glycine (**5**) and FH_4–CH_2 (**2**). D-Fluoroalanine (**6**) (a product analogue) has been shown[6] to

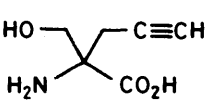


(**6**)

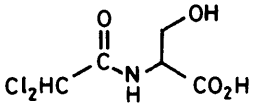
be an enzyme-activated irreversible inhibitor of this enzyme. The aim of the present work was to prepare a more effective inhibitor of this type. α-Vinylserine (**7**) was designed as an irreversible inhibitor with a proposed mechanism of action, shown in Scheme 2, analogous to the mechanism of inhibition of the PLP-dependent enzymes by α,β-unsaturated amino acids. The reverse aldol product (**8**) may be reprotonated at the terminal carbon giving an electrophilic α,β-unsaturated acid (**9**) as a soft centre to react with the thiol[7] at the active site, irreversibly inhibiting the enzyme. α-Vinyl amino acids have previously been found to be effective inhibitors of a range of PLP dependent enzymes.[8] Two other serine analogues, α-allylserine (**10**) and α-prop-2-ynylserine (**11**), with unsaturated substituents may also be proposed as irreversible inhibitors of SHMT. *N*-(Dichloroacetyl)serine (**12**) has been reported to be an active agent against experimental rodent tumours[9,10] and was also evaluated in this study.



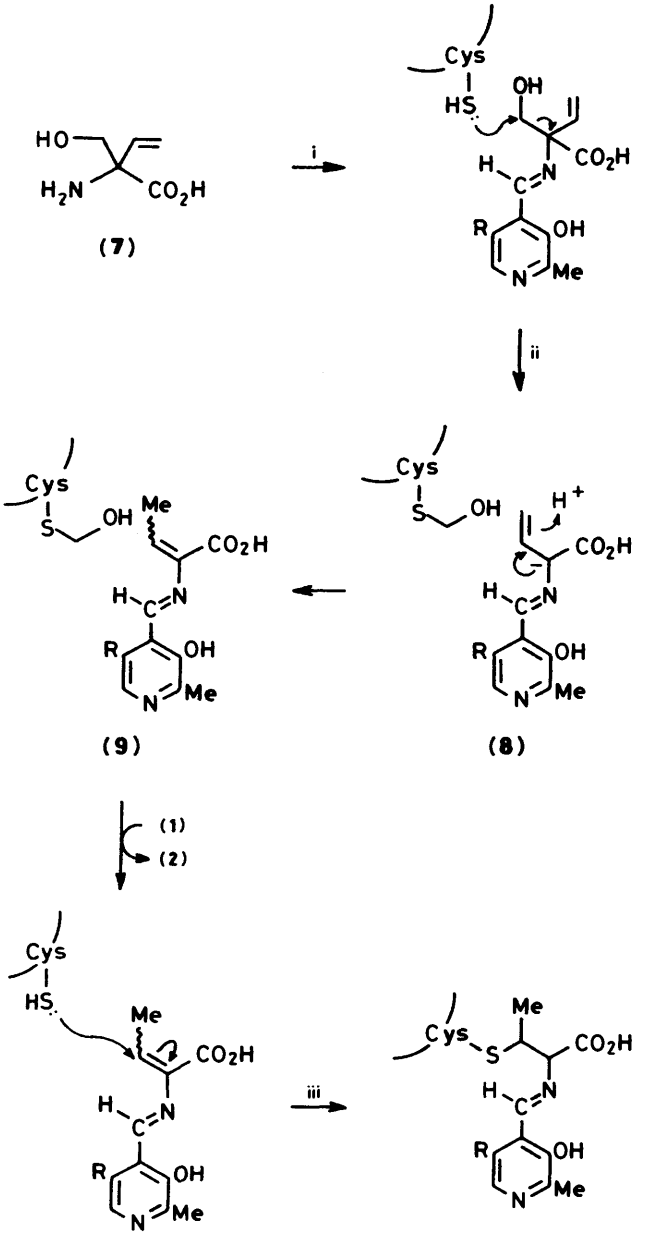
(**10**)



(**11**)



(**12**)



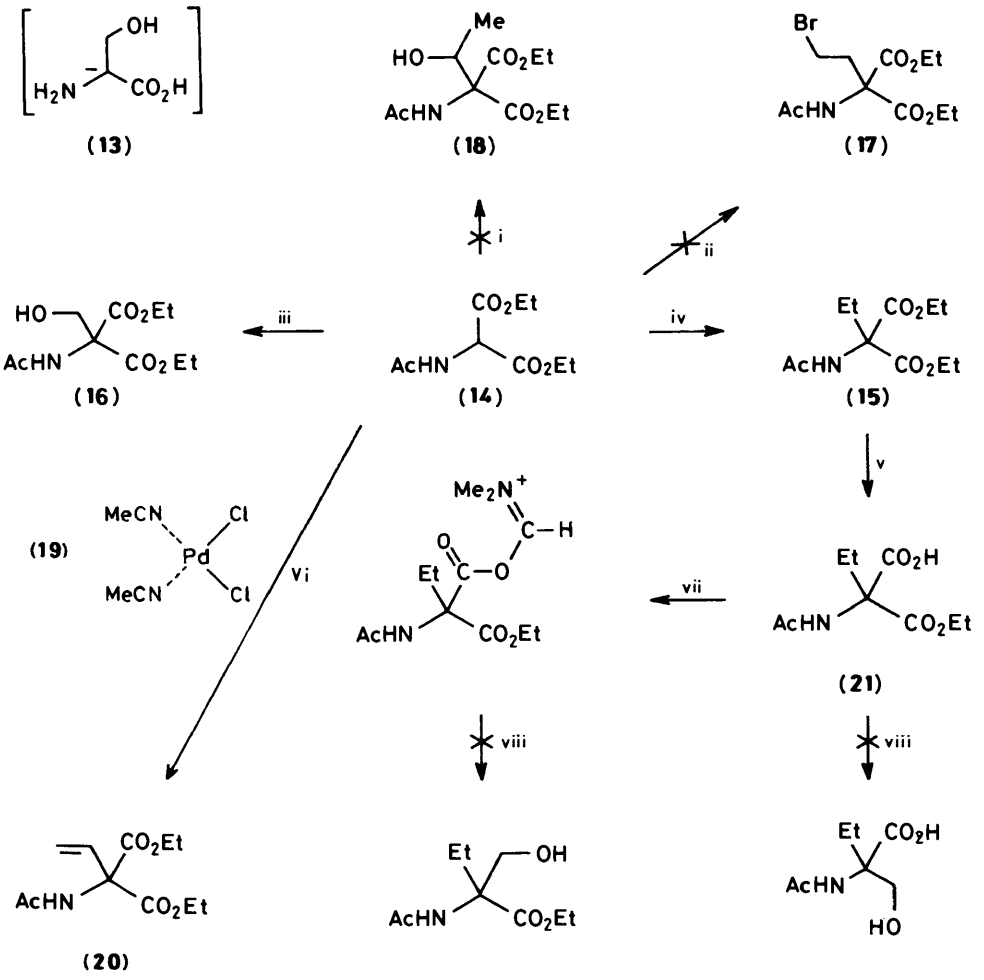
(**7**) (**8**) (**9**)

Scheme 2. Designed mechanism for the inhibition of serine hydroxymethyltransferase by (**7**): i, binding to the active site of the enzyme; ii, reverse aldol reaction; iii, conjugate addition. R = $CH_2OPO_3H^-$

Initial approaches to the introduction of substituents at the α-carbon of serine involved the use of diethyl acetamidomalonate (**14**) as a synthon for the serine α-anion (**13**) (Scheme 3). In a model sequence, the sodium derivative of (**14**) was cleanly alkylated in ethanolic solution using bromoethane to give the diester (**15**). The malonate (**14**) also reacted with aqueous formaldehyde in the presence of sodium hydroxide to afford the hydroxymethyl compound (**16**). All attempts to prepare the 2-bromoethyl compound (**17**), for possible subsequent elimination of hydrogen bromide to give a vinyl compound, were unsuccessful. Similarly, diethyl acetamidomalonate did not react with acetaldehyde to give the 1-hydroxyethyl derivative (**18**). Heyashi has reported[11] the attachment of a vinyl group to dimethyl malonate by treatment of the anion of the diester with ethylene in the presence of a stoichiometric amount of the square planar complex bis(acetonitrile)palladium(II) chloride (**19**). The synthetic scope of this potentially useful reaction is extended by our observation that ethylene reacts with diethyl acetamidosodiomalonate in the presence of (**19**) to afford the α-vinyl compound (**20**) after work-up in air. This material was, however, found to be unstable and thus to be unsuitable for the proposed synthetic route to α-vinylserine (**7**).

One ester group of the model malonate diester (**15**) was hydrolysed selectively with ethanolic potassium hydroxide, to give the monoester (**21**). Selective reduction of either the remaining ester or of the carboxylic acid should then give a protected α-ethylserine. The selective reduction of the ester group in ethyl acetamidocyanoacetate has been reported[12] to be effected by sodium borohydride but this reagent was without effect on the substrate (**21**), possibly owing to prior ionisation of the carboxylic acid. Likewise, the selective reduction of the carboxylic acid of (**21**) by sodium borohydride was unsuccessful after activation with oxalyl chloride–*N*,*N*-dimethylformamide.[13]

Scheme 4 shows the route employed for the synthesis of (**7**). (±)-Threonine (**22**), a readily available starting material with most of the required carbon skeleton, was converted to its methyl ester hydrochloride (**23**) in the usual way by treatment with boiling methanolic hydrogen chloride. Elimination of the hydroxy group could not be achieved directly, so phosphorus pentachloride was used to replace the hydroxy group, giving the



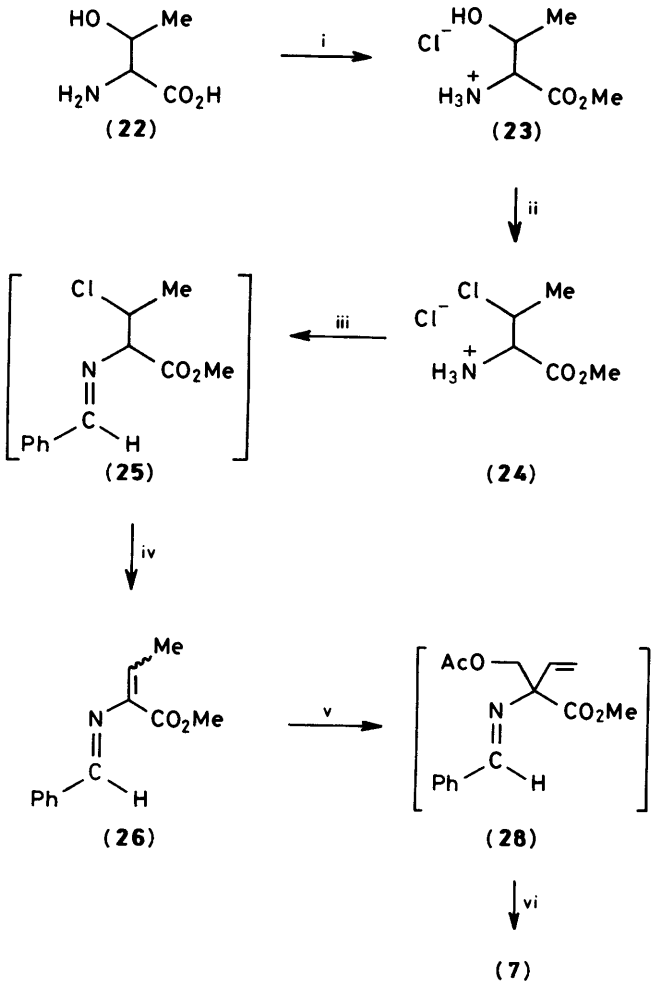
Scheme 3. Attempted syntheses of α-substituted serines using (**14**) as a synthon for (**13**): i, MeCHO–base; ii, $BrCH_2CH_2Br$–base; iii, HCHO–aq.NaOH; iv, EtBr–NaOEt–EtOH; v, KOH–EtOH; vi, NaH–THF–C_2H_4–(**19**); vii, oxalyl chloride–DMF; viii, $NaBH_4$

chloro compound (**24**) in which azirane formation is suppressed by protonation of the primary amine. Concomitant activation of the α-proton and protection was most conveniently effected by condensation of (**24**) with benzaldehyde to give the imine (**25**).

1,5-Diazabicyclo[5.4.0]undec-7-ene (DBU), a non-nucleophilic bicyclic amidine base, was used to eliminate hydrogen chloride to give methyl 2-(benzylideneamino)but-2-enoate (**26**). This dehydroamino acid represents the carbon framework for the synthesis of α-vinyl substituted amino acids by alkylation of the α-carbon.[14] The lithiated compound was prepared using lithium hexamethyldisilazide and this anion was smoothly acetoxymethylated with chloromethyl acetate (**27**) in the presence of hexamethylphosphoric triamide, providing a convenient method of introducing a protected hydroxymethyl group into the compound. Direct hydroxymethylation with formaldehyde was not possible in the non-aqueous environment required to prepare the anion; chloromethyl acetate was selected as a suitable synthon consistent with the required conditions. The chloromethyl acetate (**27**) had been previously prepared from acetyl chloride and paraformaldehyde.[15] The protecting groups were then removed from (**28**) in two stages by hydrolysis with aqueous acid and the (±)-α-vinylserine (**7**) was isolated after neutralisation with aqueous ammonia.

Selective reductions were, however, employed in the syntheses of α-allyl- and α-prop-2-ynyl-serine (**10**) and (**11**), as shown in Scheme 5. The sodium derivative of ethyl acetamidocyanoacetate (**29**) reacted smoothly with allyl bromide to give the substituted ethyl pentenoate (**30**). In this case, reduction with the more active lithium borohydride proved to be more successful than the attempts using the sodium analogue (*vide supra*). However, the immediate reduction product (**31**) could not be isolated from the resulting borate complexes, so it was hydrolysed with 6M-hydrochloric acid. Neutralisation with aqueous ammonia gave α-allylserine (**10**) in moderate yield. Ethyl acetamido(cyano)sodioacetate, prepared in absolute ethanol, failed to react with prop-2-ynyl bromide. However, the use of toluene, a less solvating solvent, facilitated the alkylation. The resulting substituted ethyl pentynoate (**32**) was then similarly reduced with lithium borohydride and the intermediate (**33**) was hydrolysed with 6M-hydrochloric acid to give α-prop-2-ynylserine (**11**) after cation exchange column chromatography using Dowex 50-X8.

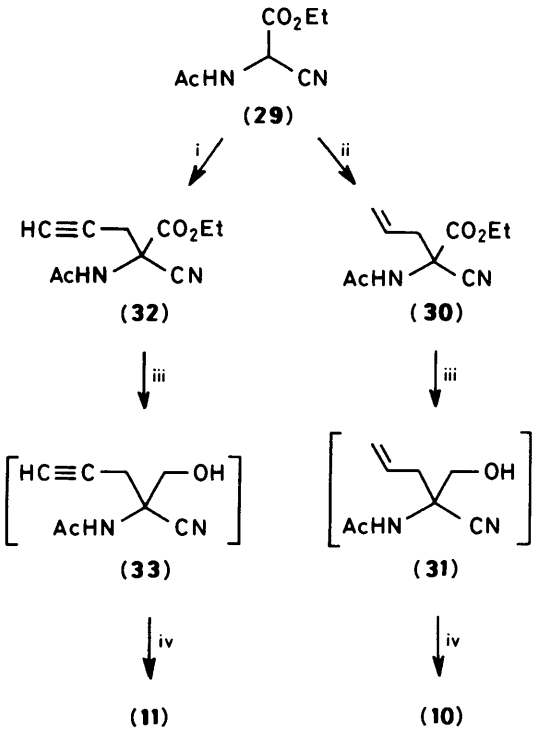
The novel α-substituted serine analogues (**7**), (**10**), and (**11**) and the *N*-dichloroacetyl compound (**12**) were initially tested against K562 human erythroid leukaemia total cell SHMT using the assay method of Taylor and Weissbach.[16] The production of [^{14}C]formaldehyde from L-[3-^{14}C]serine proceeded in a linear manner for 20 min, with total protein concentrations of up to 0.3 mg protein per 0.2 ml incubation. The activity of SHMT for the K562 total cell enzyme was determined by measuring the rate of formation of [^{14}C]formaldehyde during



Scheme 4. Synthesis of (**7**). i, MeOH–HCl; ii, PCl_5–CH_2Cl_2; iii, PhCHO–$MgSO_4$–CH_2Cl_2–Et_3N; iv, DBU–CH_2Cl_2; v, Li^+-$N(SiMe_3)_2$–$ClCH_2OAc$ (**27**)–THF–HMPA; vi, aq. HCl

20 min. Duplicate determinations were consistent within 5%. All four compounds had little inhibitory effect on the enzyme activity, at concentrations of 0.25—4 mmol l^{-1}.

To establish whether or not the substituted serines (**7**), and (**10**)—(**12**) are effective inhibitors of total cell SHMT, and to classify the type of any inhibition, the kinetics of the effects of each compound were evalulated. The rate of reaction was determined in the presence of serine (0.25—3 mM) and the test amino acid (2.5—10 mM). Only α-vinylserine deviated from the control in this assay. Lineweaver–Burk plots (Figure) show this compound to be a competitive inhibitor of total K562 cell SHMT (K_i 15.2 mmol l^{-1}). To investigate the effects of the α-substituted serine analogues on whole cells, the growth inhibitory effects of these compounds against the GM0621 and K562 leukaemia cell lines was determined. At concentrations <10 mM, the serine analogues showed no toxicity. The activation of the amino acids (**7**), (**10**), and (**11**) as inhibitors of SHMT depends on the capability of the enzyme to accept substrate analogues with substituents on the α-carbon of serine. While it is known [17] that the enzyme will accept β-substituted serines, the ability of the enzyme to catalyse the removal of the CH_2OH group from α-substituted serines has yet to be fully explored. However, it has been reported [18] that L-α-methylserine and α-hydroxymethylserine (**34**) are substrates, although the latter, (**34**), has K_m 20 mol l^{-1} (the K_m for the natural substrate, serine, is 4.3 mol l^{-1}). This suggests that the active site of the



Scheme 5. Synthesis of (**10**) and (**11**): i, $HCCCH_2Br$–NaH–PhMe; ii, H_2CCHCH_2Br–NaOEt–EtOH; iii, $LiBH_4$–THF; iv, aq. HCl

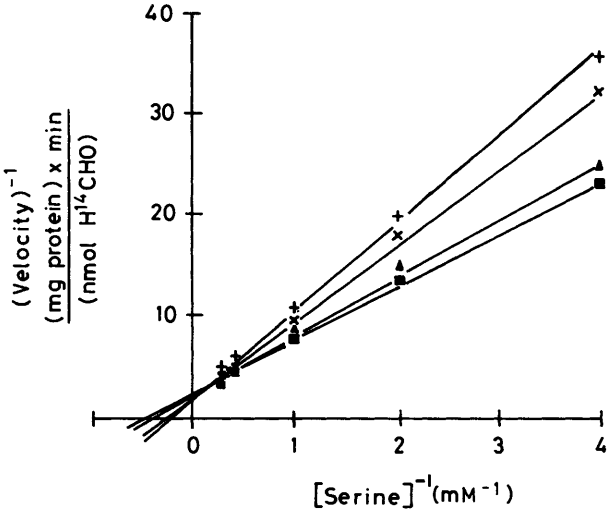
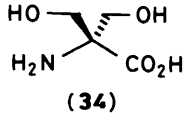


Figure. Lineweaver–Burk plots for the kinetics of inhibition of serine hydroxymethyltransferase by (**7**). Concentrations of (**7**): +10; ×5; ▲ 2.5 mol l^{-1}; ■ Control (0 mmol l^{-1})

enzyme should be able to accept α-substituted serines of similar steric bulk. The longer chain unsaturated analogues (**10**) and (**11**) caused no inhibition of the enzyme from the K562 cells.



This may suggest that either these compounds have substituents that are too bulky for the enzyme to accept or that removal of the β-proton by the enzyme, a prerequisite for the activation of

these compounds as irreversible inhibitors, does not occur. (±)-α-Vinylserine was shown to be an inhibitor (K_i 15.2 mol l^{-1}). This inhibition was, however, shown to be reversible, in contrast to the designed mechanism. The nature of the binding of (**7**) to the enzyme is not clear, although it appears to compete with serine for the binding site. Further investigations with partially purified enzymes will be conducted to reveal the true nature of this interaction.

Experimental

I.r. spectra were recorded on Pye-Unicam SP200 or Perkin-Elmer 1310 spectrometer as potassium bromide discs unless otherwise stated. ^{1}H N.m.r. spectra were recorded at 60 MHz using a Varian EM360A spectrometer and at 200 MHz with a Bruker AM200 instrument and are referenced to tetramethylsilane as the internal standard. Mass spectra were obtained using a VG Micromass 12B single focussing mass spectrometer in the electron-impact mode. M.p.s are uncorrected. Compounds are racemic unless otherwise stated.

Ethyl 2-Acetamido-2-(ethoxycarbonyl)but-3-enoate (**20**).—Dry tetrahydrofuran (38.5 ml), containing bis(acetonitrile)-palladium(II) dichloride (**19**) (500 mg, 1.9 mmol) was saturated with ethylene at −70 °C. Triethylamine (0.54 ml, 3.8 mmol) was added, followed after 15 min by diethyl acetamido(sodio)-malonate (1.9 mmol) in dry tetrahydrofuran (10 ml). The mixture was stirred at −5 °C for 1 h and warmed to ambient temperature during 70 min. After the palladium(0) had been removed by filtration, rapid chromatography of the evaporation residue (silica gel, ethyl acetate) yielded *ethyl 2-acetamido-2-(ethoxycarbonyl)but-3-enoate* (290 mg, 62%) as an unstable colourless oil, $\nu_{max.}$ 1 520, 1 640, and 1 740 cm^{-1}; δ($CDCl_3$) 1.25 (6 H, t, *J* 7 Hz, CH_2*Me*), 2.10 (3 H, s, COMe), 4.30 (4 H, q, *J* 7 Hz CH_2Me), 5.25 (1 H, d, *J* 17 Hz, 4-H), 5.30 (1 H, d, *J* 10 Hz, 4-H), 6.55 (1 H, dd, *J* 17, 10 Hz, 3-H), and 7.1 (1 H, s, NH).

Chloromethyl Acetate (**27**).—Acetyl chloride (25.9 g, 33 mmol), paraformaldehyde (9.0 g, 33 mmol HCHO) and anhydrous zinc chloride (*ca.* 100 mg) were warmed together to 50 °C. After a short induction period, the heat of reaction caused the solution to boil gently for 3 h. The mixture was filtered through basic alumina to give a yellow liquid (33.0 g). N.m.r. spectroscopy revealed this to comprise 92% chloromethyl acetate and 8% bis(chloromethyl) ether (yield of ester 85%). This material was used for later experiments without further purification and had $\nu_{max.}$ 1 760 cm^{-1}; δ($CDCl_3$) 2.10 (3 H, s, Me) and 5.60 (2 H, s, CH_2). The contaminating ether had δ($CDCl_3$) 5.55 (s, 2 × CH_2).

Methyl 2-Amino-3-chlorobutanoate Hydrochloride (**24**).—Phosphorus pentachloride (6.35 g, 30 mmol) was added to a suspension of threonine methyl ester hydrochloride (**23**) (5.0 g, 29 mmol) in dichloromethane (260 ml) during 1 h and the resulting solution was stirred at ambient temperature for 3 h then cooled to 0 °C for 16 h. The white crystals were collected by filtration, washed with diethyl ether and light petroleum (b.p. 60—80 °C), and recrystallised from acetonitrile to afford methyl 2-amino-3-chlorobutanoate hydrochloride (3.7 g, 68%) as white crystals, m.p. 169—171 °C (lit.,[19] 169—172 °C), $\nu_{max.}$ 1 500, 1 600, 1 740, and 2 500—3 000 cm^{-1}; δ(D_2O) 1.65 (3 H, d, *J* 7 Hz, 4-H_3), 3.95 (3 H, s, OMe), 4.60 (1 H, d, *J* 3 Hz, 2-H), and 4.8 (4 H, m, N^+H_3 and 3-H).

Methyl 2-(Benzylideneamino)but-2-enoate (**26**).—Benzaldehyde (4.5 g, 44 mmol) was added to a well-stirred slurry of methyl 2-amino-3-chlorobutanoate hydrochloride (**24**) (8.3 g, 44 mmol) in dichloromethane (80 ml) at 5 °C, followed by triethylamine (6.15 ml, 44 mmol) and dried magnesium sulphate (4.0 g). The resulting suspension was stirred at ambient temperature for 21 h before water (100 ml) was added. The aqueous layer was extracted with dichloromethane (3 × 50 ml) and the organic solutions were combined, washed with saturated aqueous sodium chloride, and dried ($MgSO_4$). Evaporation of the solvent under reduced pressure afforded crude methyl 2-(benzylideneamino)-3-chlorobutanoate (11.1 g), δ($CDCl_3$) 1.55 (3 H, d, *J* 7 Hz, 4-H_3), 3.75 (3 H, s, OMe), 4.10 (1 H, d, *J* 7 Hz, 2-H), 4.65 (1 H, quintet, *J* 7 Hz, 3-H), 7.3—7.9 (5 H, m, ArH), and 8.35 (1 H, s, PhC*H*). 1,5-Diazabicyclo[5.4.0]undec-5-ene (6.5 ml) was added dropwise during 5 min to the above ester in dichloromethane (80 ml) at 5 °C. After 3 h, water (100 ml) was added and the organic layer was separated and dried ($MgSO_4$). Evaporation of the solvent under reduced pressure gave an oil which, in hexane–diethyl ether (10:1), was filtered through neutral alumina (20 g) to give, after evaporation of the solvents, methyl 2-(benzylideneamino)but-2-enoate (8.4 g, 94%) as a colourless oil, $\nu_{max.}$ (liquid film) 1 580, 1 600, 1 640, and 1 715 cm^{-1}; δ($CDCl_3$) 1.90 [2.1 H, d, *J* 7 Hz, 4-H_3 (*E*)], 2.05 [0.9 H, d, *J* 7 Hz, 4-H_3 (*Z*)], 3.70 [2.1 H, s, OMe (*E*)], 3.75 [0.9 H, s, OMe (*Z*)], 5.85 [0.3 H, q, *J* 7 Hz, 3-H (*Z*)], 6.50 [0.7 H, q, *J* 7 Hz, 3-H (*E*)], 7.4 (3 H, m) and 7.8 (2 H, m) (Ar-H), 8.25 [0.3 H, s, ArC*H* (*Z*)], and 8.45 [0.7 H, s, ArC*H* (*E*)]; *m/z* 203 (M^+) and 143 (100%).

2-Amino-2-(hydroxymethyl)but-3-enoic Acid (*α-Vinylserine*) (**7**).—Lithium hexamethyldisilazide was prepared by the addition of butyl-lithium (8.0 ml, 12.3 mmol; 1.55M in hexane) to hexamethyldisilazane (2.0 g, 12.3 mmol) in tetrahydrofuran (freshly distilled from calcium hydride; 5 ml) at −70 °C under nitrogen. Hexamethylphosphoric triamide (7 ml) and methyl 2-(benzylideneamino)but-2-enoate (**26**) (2.3 g, 11.2 mmol) were then added, followed by chloromethyl acetate (**27**) (1.35 g, 12.3 mmol) in dry tetrahydrofuran (10 ml). The mixture was stirred for 1 h at −70 °C and for 2 h at ambient temperature before being diluted with saturated aqueous ammonium chloride and extracted with diethyl ether (3 × 50 ml). The combined organic extracts were washed with water (2 × 50 ml) and with saturated aqueous sodium chloride (20 ml) and dried ($MgSO_4$). Evaporation of the solvent under reduced pressure gave a yellow oil which was treated with hydrochloric acid (2M; 40 ml) for 2 min. The resulting solution was washed with diethyl ether (2 × 50 ml) and with chloroform (2 × 50 ml). Hydrochloric acid (6M; 20 ml) was added and the whole was boiled under reflux for 2 h before being washed with dichloromethane (2 × 10 ml) and treated with activated charcoal (100 mg). The filtered solution was concentrated to 10 ml under reduced pressure and neutralised by the addition of aqueous ammonia. Hot ethanol (25 ml) was added and, upon cooling, white crystals were deposited. Recrystallisation of these from aqueous ethanol yielded *2-amino-2-(hydroxymethyl)but-3-enoic acid* (170 mg, 11.6%) as fine white needles m.p. 173—175 °C (decomp.) (Found: C, 45.5; H, 7.1; N, 10.8. $C_5H_9NO_3$ requires C, 45.8; H, 6.9; N, 10.7%); $\nu_{max.}$(Nujol) 1 510, 1 590, 1 610, and 1 640 cm^{-1}; δ(D_2O) 3.80 (1 H, d, *J* 12 Hz) and 4.00 (1 H, d, *J* 12 Hz) (CH_2OH), 5.35 (1 H, d, *J* 18 Hz, 4-H), 5.44 (1 H, d, *J* 12 Hz, 4-H), and 6.02 (1 H, dd, *J* 12, 18 Hz, 3-H); *m/z* 100 ($M^+ - CH_2OH$) and 54 (100%).

Ethyl 2-Acetamido-2-cyanopent-4-enoate (**30**).—Sodium (1.15 g, 50 mmol) was dissolved in dry ethanol (30 ml) and ethyl acetamidocyanoacetate (**29**) (8.5 g, 50 mmol) and 3-bromopropene (6.5 g, 50 mmol) were added. The mixture was boiled under reflux for 16 h before the solvent was evaporated under reduced pressure. The residue was washed with water (30 ml) and recrystallised from aqueous ethanol to give ethyl 2-acetamido-2-cyanopent-4-enoate (4.1 g, 39%) as a white solid,

m.p. 83—85 °C (lit.,[20] 86—88.5 °C), $\nu_{max.}$ 1 515, 1 650, and 1 740 cm^{-1}; δ($CDCl_3$) 1.35 (3 H, t, *J* 7 Hz, CH_2Me), 2.05 (3 H, s, COMe), 2.80 (2 H, d, *J* 6 Hz, 3-H_2), 4.30 (2 H, q, *J* 7 Hz, CH_2Me), 5.2—5.9 (3 H, m, 4-H and 5-H_2), and 7.5 (1 H, s, NH); *m/z* 211 (M^+ + 1) and 95 (100%).

2-*Amino*-2-(*hydroxymethyl*)*pent*-4-*enoic Acid* (α-*allylserine*) (**10**).—Lithium borohydride (360 mg, 16 mmol) in dry tetrahydrofuran (10 ml) was added dropwise during 5 min to a suspension of ethyl 2-acetamido-2-cyanopent-4-enoate (**30**) (3.5 g, 16 mmol) in dry tetrahydrofuran (50 ml) and the mixture was boiled under reflux for 3 h before being diluted with methanol (50 ml) and acidified with hydrochloric acid (6M; 2 ml). Evaporation of the solvents under reduced pressure gave a yellow gum which, in hydrochloric acid (6M; 20 ml), was boiled under reflux for 2 h. Evaporation of the solvent and the excess of reagent under reduced pressure followed by neutralisation with aqueous ammonia gave a white powder from which 2-*amino*-2-(*hydroxymethyl*)*pent*-4-*enoic acid* (460 mg, 20%) was isolated by recrystallisation from aqueous ethanol. The white crystals had m.p. 215—220 °C (decomp.) (Found: C, 49.6; H, 7.7; N, 9.7. $C_6H_{11}NO_3$ requires C, 49.6; H, 7.6; N, 9.6%); $\nu_{max.}$ 1 510, 1 610, 1 640, 2 500—3 200, and 3 450 cm^{-1}; δ(D_2O) 2.40 (1 H, dd, *J* 14.6, 9.3 Hz) and 2.60 (1 H, dd, *J* 14.6, 6.3 Hz) (3-H_2), 3.71 (1 H, dd, *J* 11.2 Hz) and 3.95 (1 H, dd, *J* 11.2 Hz) (CH_2OH), 5.27 (1 H, d, *J* 12.2 Hz, 5-H), 5.28 (1 H, d, *J* 15.1 Hz, 5-H), and 5.7 (1 H, m, 4-H); *m/z* 126 ($M^+ - CH_2OH$) and 114 (100%).

Ethyl 2-*Acetamido*-2-*cyanopent*-4-*ynoate* (**32**).—Ethyl acetamidocyanoacetate (**29**) (17.0 g, 100 mmol) was added to a suspension of sodium hydride (50% in oil; 4.8 g, 100 mmol) in dry toluene (100 ml), followed by 3-bromopropyne (70% in toluene, 14.8 g, 180 mmol) and the mixture was boiled under reflux for 3 h. Ethyl acetate (20 ml) was added, followed by water (50 ml) and diethyl ether (50 ml). The combined organic solutions were concentrated under reduced pressure and, when allowed to stand, deposited light brown crystals. Recrystallisation of these from ethanol–light petroleum (b.p. 60—80 °C) furnished *ethyl* 2-*acetamido*-2-*cyanopent*-4-*ynoate* (5.2 g, 25%) as very pale buff crystals m.p. 87—88 °C; $\nu_{max.}$ 1 660, 1 760, and 2 500—3 500 cm^{-1}; δ($CDCl_3$) 1.40 (3 H, t, *J* 7 Hz, CH_2Me), 2.15 (3 H, s, COMe), 2.24 (1 H, t, *J* 2 Hz, 5-H), 3.10 (1 H, dd, *J* 13, 2 Hz) and 3.44 (1 H, dd, *J* 13, 2 Hz) (3-H_2), 4.41 (2 H, q, *J* 7 Hz, CH_2Me), and 7.63 (1 H, s, NH) (Found: M^+, 208.0858. Calc. for $C_{10}H_{12}N_2O_3$: *M*, 208.0848); *m/z* 179, 166, 135, and 93 (100%).

2-*Amino*-2-(*hydroxymethyl*)*pent*-4-*ynoic Acid* (α-*Propargylserine*) (**11**).—Ethyl 2-amino-2-(hydroxymethyl)pent-4-ynoate (**32**) was reduced using lithium borohydride and the intermediate was hydrolysed as described above for the synthesis of (**10**). Neutralisation with 2M-aqueous ammonia yielded a tar which, in water (5 ml), was adsorbed onto a column of Dowex 50 X8 (2 × 10 *cm*) (H^+ form). The column was washed with water until the eluates were neutral and the amino acid was eluted from the column using aqueous ammonia (2M; 100 ml). The evaporation residue was recrystallised from aqueous ethanol to give 2-*amino*-2-(*hydroxymethyl*)*pent*-4-*ynoic acid* (390 mg, 17%) as a white solid, m.p. 157—160 °C (Found: C, 50.0; H, 6.2; N, 9.7. $C_6H_9NO_3$ requires C, 50.3; H, 6.3; N, 9.8%); $\nu_{max.}$ 1 590, 1 620, and 2 500—3 400 cm^{-1}; δ(D_2O) 2.57 (1 H, dd, *J* 1.9, 2.4 Hz, 5-H), 2.75 (1 H, dd, *J* 17.6, 1.9 Hz) and 2.82 (1 H, dd, *J* 17.6, 2.4 Hz) (3-H_2), and 3.80 (1 H, d, *J* 11.2 Hz) and 3.95 (1 H, d, *J* 11.2 Hz) (CH_2OH); *m/z* 112 ($M^+ - CH_2OH$, 100%).

Assay for Activity of SHMT.—Cells were washed once with 0.9% aqueous sodium chloride and disrupted in the minium volume of 100mM Tris-HCl buffer at pH 7.1 using a sonicator probe at 10 KHz for 3 × 10 s. The suspension was then centrifuged at 3 000 × *g* for 1 h at 4 °C. Each assay contained the test compound, L-[3-^{14}C]serine (0.1 μmol), pyridoxal phosphate (0.1 μmol), tetrahydrofolic acid (0.8 μmol), 2-mercaptoethanol (2 μmol), and potassium phosphate buffer (pH 7.4; 30 μmol), and the supernatant from the disrupted cell preparation in a total volume of 0.2 ml. Reactions were initiated by the addition of the supernatant and were terminated by addition of sodium acetate buffer (1M; pH 4.5; 0.3 ml), aqueous formaldehyde (0.1M; 0.2 ml) and 5,5-dimethylcyclohexane-1,3-dione (0.4M in 50% aqueous ethanol; 0.3 ml). The mixture was heated for 5 min then cooled to 0 °C. Scintillation grade toluene (5 ml) was added and the bis(4,4-dimethyl-2,6-dioxocyclohexyl)-[^{14}C]methane was extracted into the toluene by vigorous shaking for 1 min. Following centrifugation, the radioactivity was determined by mixing a sample of the upper layer (3 ml) with Beckman E.P. scintillation fluid (10 ml) and counting by the liquid scintillation method on a Beckman LS-230 counter.

Growth Inhibition Assays.—The growth inhibitory effects of the compounds were determined against GM0621 and K562 cells, plated out into duplicate 1-ml wells of a 24-well plastics plate at a density of 5 × 10^4 cells ml^{-1}. R.P.M.I. 1 640 medium supplemented with 10% foetal calf serum was used as the culture medium with solutions of the compounds in the culture medium being added as appropriate. The cells were maintained under an atmosphere of 10% CO_2 in air at 37 °C. The cells were enumerated using a Coulter-counter during a 5 day incubation period and the percentage inhibition of growth was determined over the linear part of the growth curve.

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