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SYNTHESES OF HOMOSERINE PHOSPHATE ANALOGS AS POTENTIAL INHIBITORS OF BACTERIAL THREONINE SYNTHASE

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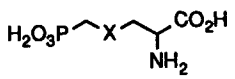
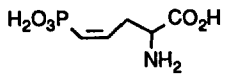
Abstract: The syntheses of five homoserine phosphate analogs (**1-4**, **Z-5**) are described. A brief summary of their potential to inhibit threonine synthase from *Escherichia coli* is given.

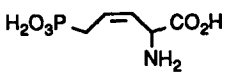
Bacterial threonine synthase (TS) [EC 4.2.99.2], a pyridoxal phosphate (PLP) dependent enzyme, catalyzes the final step in the biosynthesis of L-threonine from L-aspartic acid, i.e. the conversion of L-homoserine phosphate into L-threonine. The reaction involves the non-hydrolytic elimination of phosphate from the PLP bound substrate and subsequent addition of water to a PLP bound vinylglycine intermediate[1]. We were interested in homoserine phosphate analogs both as potential inhibitors of TS and also in order to study intermediates of this enzymic reaction by UV spectroscopy.



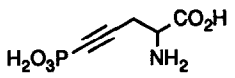
1: X = S
2: X = NH
3: X = O



Z-5



Z-6



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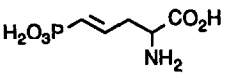
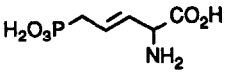


E-5



E-6

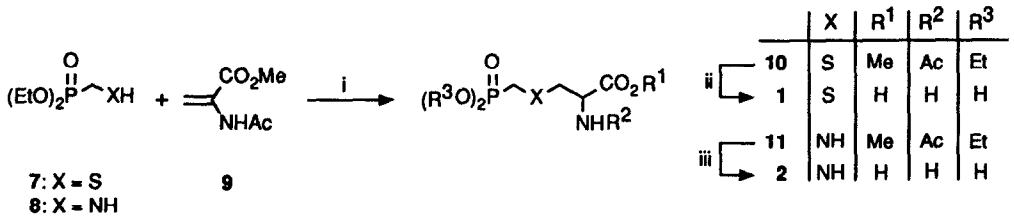
The sulfur containing phosphonic acid **1** has been reported to be a slow binding inhibitor of TS[2], and the nitrogen analog L-**2** to possess N-methyl-D-aspartic acid (NMDA) antagonistic activity[3]. Since no experimental details for the synthesis of these two compounds have been published yet, we developed syntheses for racemic and enantiomerically pure phosphonic acid **1**, as well as for the racemic analogs **2** and **3**.

The pentynoic acid **4** not only resembles homoserine phosphate, it is also a simple derivative of propargylglycine, which is a well known inhibitor of various PLP dependent enzymes[4].

Amino acid **Z-6** represents the C-terminus of two classes of naturally occuring di- and tripeptides, the rhizocticins and the plumbemycins. **Z-6**[5,6] has the L-configuration in the fungicidal rhizocticins[7], whereas the D-enantiomer is in the plumbemycins[8,9], which are active against bacteria. Moreover, Natchev has also reported that the L-enantiomer of **Z-6** displays herbicidal and fungicidal activity[6]. Inhibition studies with

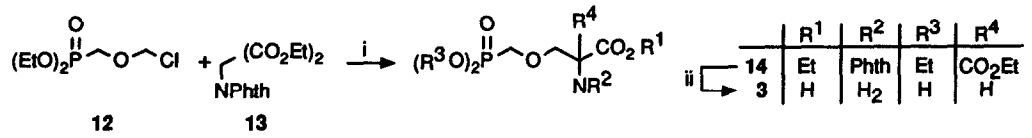
racemic Z-**6**, performed in our laboratory[10], clearly showed that this compound is an inhibitor of bacterial TS (Table 1). The amino acids E-**5**[11] and E-**6**[12] have already been described in the literature as NMDA antagonists. Therefore, we undertook the synthesis of the unsaturated amino acid Z-**5** to complete the series of pentenoic acids **5** and **6**. In this letter we describe the facile syntheses of phosphonic acids **1-4**, and Z-**5**, and give a brief summary of their action on bacterial TS.

The syntheses of compounds **1** and **2** are described in scheme 1. Michael addition of phosphonates **7**[13] and **8**[14] to methylacetamido-acrylate **9** in the presence of triethylamine in refluxing methanol[15] yielded the protected amino acids **10** and **11**, respectively. Deprotection of the sulfur compound **10** in refluxing 6N hydrochloric acid[16] and subsequent purification by ion exchange chromatography[16] afforded racemic phosphonic acid **1**[17] as an amorphous solid.



Scheme 1. *Reagents and conditions*: i. Et_3N, MeOH, reflux, for **10**: 8h :62%; for **11**: 2d :73%; ii. 1) 6N HCl, reflux, 8h; 2) Dowex 50X-200, H^+-form, eluent: H_2O :56%; iii. 1) 3.5N NaOH, reflux, 16h; 2) Dowex 50X-200, H^+-form, eluent: $H_2O \rightarrow$ 2N pyridine in H_2O; 3) TMSBr, pyridine, CH_3CN, 20°C, 2d; 4) Dowex 50X-200, H^+-form, eluent: $H_2O \rightarrow$ 2N pyridine in H_2O :85%.

The attempted deprotection of the nitrogen analog **11** under these conditions resulted mainly in a fragmentation of the molecule in a retro Michael sense, the amino acid **2** being obtained in only 29% yield. Alternatively, in a two step sequence with refluxing 3N sodium hydroxide followed by trimethylbromosilane/pyridine in acetonitrile[16] all protecting groups were removed. After purification by ion exchange chromatography the amino acid **2**[17] was obtained as a white powder in 85% yield. Since the synthesis of the oxygen analog **3** via the Michael additon route described above failed, the reaction sequence shown in scheme 2 was pursued.



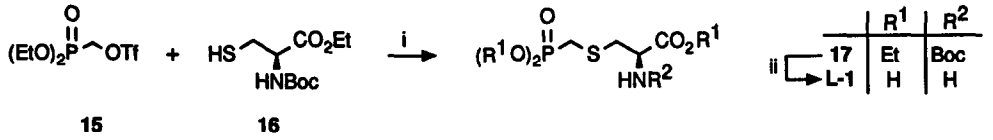
Scheme 2. *Reagents and conditions*: i. **13**, NaH, DMF; 2) **12**, 80-120°C, 5h :52%; ii. 1) 6N HCl/HOAc (5/1, v/v), reflux, 8h; 2) Dowex 50X-200, H^+-form, eluent: H_2O :56%.

Reaction of α-chloroether **12**[18] with the sodium salt of amino-malonate **13**[19] in DMF[20] afforded **14**. Deprotection and decarboxylation could be achieved by refluxing compound **14** in 6N hydrochloric acid/acetic

acid[21], and after purification by ion exchange chromatography the target compound **3**[17] was obtained as a white, amorphous solid.

A similar reaction sequence could be used for the preparation of the L-enantiomer of the sulfur containing amino acid **1** (scheme 3). Alkylation of the protected L-cysteine derivative **16**[22] with triflate **15**[23] in THF in the presence of triethylamine[23] afforded cleanly the protected phosphonic ester **17**. Deprotection and purification was carried out in the same manner as described for the racemic derivative **10**.

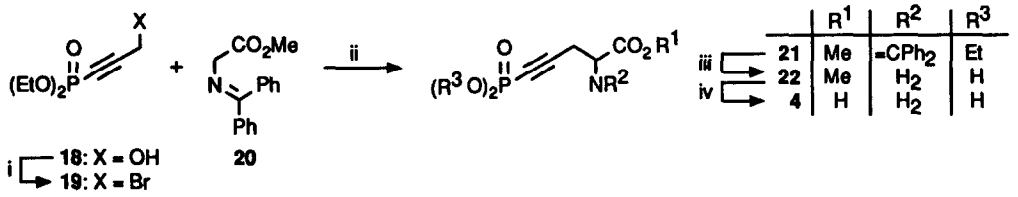
Using this reaction sequence, it should be possible to prepare the phosphonic acids **2** and **3** in enantiomerically pure form as well.



15 16

Scheme 3. *Reagents and conditions*: i. Et_3N, THF, 5°C, 2h → 20°C, 2d :75%; ii. 1) 6N HCl, reflux, 8h; 2) Dowex 50X-200, H^+-form, eluent: H_2O :70%.

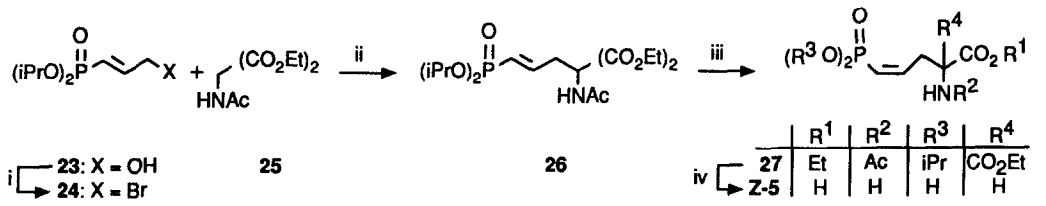
The synthesis of pentynoic acid **4** is outlined in scheme 4. Bromination of propargylalcohol **18**[24] with triphenylphosphine/ tetrabromomethane in dichloromethane[25] yielded propargylic bromide **19**. Its reaction with the anion[26] of glycine derivative **20**[27], and subsequent cleavage of the phosphonic esters and the imine with trimethylbromosilane/pyridine afforded cleanly methyl ester **22**. After saponification of the ester with 2N LiOH and purification by ion exchange chromatography, amino acid **4**[17] was obtained as a white amorphous solid.



Scheme 4. *Reagents and conditions*: i. PPh_3, CBr_4, CH_2Cl_2, 5°C, 18h :50%; ii. 1) **20**, LiHMDS, THF, -78°C; 2) **19**, THF, -78°C → 0°C, 4h; iii. Me_3SiBr, pyridine, CH_2Cl_2, 20°C, 1d :67% (based on **19**); iv. 1) 2N LiOH, 20°C, 18h; 2) Dowex 50X-200, H^+-form, eluent: H_2O :81%.

The synthesis of amino acid **Z-5** (scheme 5) was accomplished in a similar manner to the synthesis of pentynoic acid **4**. After bromination of the allylic alcohol **23**[28], the resulting bromide **24** was alkylated with the anion of aminomalonate **25**[19] to yield E-olefin **26**. Isomerization of the double bond by irradiation with a Hg medium pressure lamp (Philips HPK 125W) for 2d in a quartz vessel gave a 3:1 mixture of isomers **26** and

27, that could be separated by chromatography on SiO_2. Complete deprotection and decarboxylation of Z-isomer 27 was achieved in refluxing 6N hydochloric acid. Purification by ion exchange chromatography afforded the desired amino acid Z-5[17] as an amorphous solid.



Scheme 5. *Reagents and conditions*: i. PPh_3, CBr_4, CH_2Cl_2, 5°C, 18h :47%; ii. 1) **25**, NaH, DMF; 2) **24**, DMF, 80-100°C, 8h :27%; iii. hv, C_6H_6, 2d, **27**:16% and **26**: 43%; iv. 1) 6N HCl, reflux, 8h; 2) Dowex 50X-200, H^+-form, eluent: H_2O :52%.

The amino acids described above, as well as the already known unsaturated amino acids E-5, Z-6, and E-6 were tested for their potential to inhibit TS from *Escherichia coli* [EC 4.2.99.2] (table 1). Compounds 3, 4, and Z-5 did not inhibit the enzyme at all, although the unsaturated amino acids 4 and Z-5 did bind to the enzyme, as was shown by UV-spectroscopy[10]. The sulfur compound 1 and olefin E-5 were competitive inhibitors. Amino acids 2, Z-6, and E-6 were found to be irreversible inhibitors of the bacterial enzyme. Detailed experimental, kinetic, and spectroscopic data, as well as a discussion of these results will be published later[10].

Table 1: Kinetic constants[a] for inhibition[b] of threonine synthase from *Escherichia coli*

compound	K_i / [M]	k_{inact} / [min^{-1}]
1	$3.3 \cdot 10^{-5}$	d)
L-1	$1.1 \cdot 10^{-5}$	d)
2	$5.9 \cdot 10^{-5}$	1.44
3	c)	
4	c)	
Z-5	c)	
E-5	$5.4 \cdot 10^{-4}$	d)
Z-6	$1.0 \cdot 10^{-4}$	1.50
E-6	$4.0 \cdot 10^{-4}$	0.25

a) mean of two determinations; b) for experimental conditions see ref. 10, 29);
c) no inhibition at 10mM inhibitor concentration; d) competitive inhibitor

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17. ^{1}H NMR spectra were recorded at 300 MHz and ^{13}C NMR spectra at 75 MHz, respectively. Dioxan was used as an internal reference for spectra taken from aqueous solutions. The reported compounds possess the following physical data:

1: ^{1}H NMR (D_2O) δ 2.76 (m, 2H), 3.14 (dd, *J* = 15, 8 Hz, 1H), 3.32 (dd, *J* = 15, 5 Hz, 1H), 4.21 (dd, *J* = 8, 5 Hz, 1H); ^{13}C NMR (D_2O) δ 28.06 (d, *J* = 138.9 Hz), 34.15 (d, *J* = 5.3 Hz), 53.26, 171.71; IR (KBr) ν 1730, 1620, 1510, 1120, 1040, 915 cm^{-1}.

L-1: $[\alpha]_D^{20}$ -16.0° (*c* = 0.52, H_2O); spectroscopic data identical with racemic **1**.

2: mp 240 °C (dec.); ^{1}H NMR (D_2O + NaOD) δ 2.55 (dd, *J* = 14, 14 Hz, 1H), 2.64 (dd, *J* = 14, 14 Hz, 1H), 2.73 (dd, *J* = 12, 8.5 Hz, 1H), 2.89 (dd, *J* = 12, 5 Hz, 1H), 3.41 (dd, *J* = 8.5, 5 Hz, 1H); ^{13}C NMR (D_2O + NaOD) δ 47.61 (d, *J* = 137.4 Hz), 54.90 (d, *J* = 12.8 Hz), 55.18, 181.59; IR (KBr) ν 1642, 1180, 1118 cm^{-1}.

3: ^{1}H NMR (D_2O) δ 3.74 (m, 2H), 4.02 (dd, *J* = 11, 3.5 Hz, 1H), 4.08 (dd, *J* = 11, 5 Hz, 1H), 4.27 (dd, *J* = 5, 3.5 Hz, 1H); ^{13}C NMR (D_2O) δ 53.28, 67.06 (d, *J* = 157.8 Hz), 69.76 (d, *J* = 13.6 Hz), 170.14; IR (KBr) ν 1745, 1600, 1525, 1270, 1130, 1050, 940 cm^{-1}.

4: ^{1}H NMR (D_2O) δ 3.04 (d, *J* = 6 Hz, 1H), 3.06 (d, *J* = 6 Hz, 1H), 4.23 (dd, *J* = 6, 6 Hz, 1H); ^{13}C NMR (D_2O) δ 20.34 (d, *J* = 3.8 Hz), 50.97, 80.19 (d, *J* = 157.8 Hz), 88.89 (d, *J* = 47.6 Hz), 169.97; IR (KBr) ν 2205, 1730, 1610, 1515, 1150, 1040, 930 cm^{-1}.

Z-5: ^{1}H NMR (D_2O) δ 3.00 - 3.22 (m, 2H), 4.09 (dd, *J* = 7, 5.5 Hz, 1H), 6.04 (d, *J* = 17.5, 7.5 Hz, 1H), 6.22 (dddd, *J* = 45.5, 16, 7.5, 7 Hz, 1H); ^{13}C NMR (D_2O) δ 30.43 (d, *J* = 9.1 Hz), 51.96, 127.69 (d, *J* = 171.4 Hz), 138.52, 171.60; IR (KBr) ν 1730, 1630, 1520, 1130, 1045, 940 cm^{-1}.

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