

Portage Transport of Toxophoric Agent, *N*-hydroxyalanine, through Oligopeptide Permease in *Escherichia coli*

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Di- and tri-peptides containing DL-*N*-hydroxyalanine were prepared. DL-*N*-Hydroxyalanine was linked, *via* its primary amino group, to the α -carbon of glycine residues in dipeptide synthon (L-alanyl(α -DL-*N*-hydroxyalanyl)DL-glycine) **5**, and tripeptide synthon (L-alanyl-L-alanyl(α -DL-*N*-hydroxyalanyl) DL-glycine) **12**. **5** proved to be 19 times more potent than DL-*N*-hydroxyalanine when tested *in vitro* for the ability to inhibit the growth of *E. coli*. However, **12** gave comparable potency to DL-*N*-hydroxyalanine itself.

Introduction

In recent years, a variety of naturally occurring antibiotics have been recognized. They are analogues or derivatives of small peptides and function by entering susceptible microorganisms via peptide permease and attacking intracellular targets; the cell growth inhibitory agent may be the intact peptide or a moiety released from it by intracellular hydrolysis.^{1,2,14,15} In *Escherichia coli* (*E. coli*), it has been found that there are separate peptide transport systems for dipeptides and for oligopeptides.¹ *Candida albicans* has also been shown to have peptide transport systems although the diversity of this organism has yet to be conclusively defined.^{3,4,14}

The factors that determine the recognition of peptides by microbial peptide transport systems have been the subject of numerous investigations.¹⁵ A significant observation that has emerged from this studies, primarily in bacteria, is that peptide transport systems generally possess little demonstrable side chain specificity.¹⁴ The possibility that the peptide transport system could be exploited therapeutically were demonstrated in *E. coli* where normally impermeant amino acids were shown to enter these cells by a peptide carrier mechanism when incorporated into the backbone of peptide by α -linkage on glycine.⁵ The method consists of constructing a peptide in which an internal or C-terminal glycine residue bears an α -substituent that is a good leaving group. Intracellular hydrolytic release of the glycine amino group allows the leaving group to be expelled and express its antibacterial properties. Many naturally and synthetic peptide examples that contain growth inhibitory amino acids have been reported and considerable interest has been expressed in utilizing this approach as a means of developing novel chemotherapeutic agents.^{7,10-14} The development of the wide-spectrum antimicrobial agent, alaphosphin [L-alanyl-L-(1-aminoethyl) phosphonic acid] is a powerful example of the peptide transport.⁸ It is obvious that amino acid antimetabolites, normally impermeable to the cell of *E. coli*, should be transported into the cell when incorporated into a peptide chain. Bacilysin, dipeptide antibiotic isolated from a culture of *Bacillus Subtilis*, provide another example for this approach to an antifungal drug. Anticapsin,⁹ a "warhead" amino acid in bacilysin and a potent inhibitor of glucosamine synthetase, is

poorly active against whole cell, but is effectively transported into cells as peptide.¹⁰ To date the peptide transport system has been successfully applied for the delivery of different kinds of toxophoric agents such as thiophenol, phenol, aniline,¹¹ fluorouracil,¹² sulfanilic acid, sulfanilamide¹³ and 2-aminopimelic acid.¹⁵ The aims of the investigation were to demonstrate that the bacterial peptide permease could recognize and transport peptide mimetics containing a glycine residue with α -substituted compounds linked through a sulfur, oxygen, or nitrogen atom and that these compounds would serve as substrates for cytoplasmic peptidases.

N-Hydroxyamino acids are components of ferrichrome and ferrichrome A,¹⁶ mycobactin,¹⁷ and a number of other naturally occurring antibiotics,^{18,19} while *N*-hydroxyamino acids have not been found as free form in nature. The known reactivity of *N*-substituted hydroxyamines with carbonyl groups,^{20,21} including pyridoxal-5'-phosphate,²² suggested that *N*-hydroxyamino acids would be potent irreversible inhibitors of pyridoxal-9'-dependent enzyme.²³ This study suggests that *N*-hydroxyalanine is as useful potent amino acid antagonist as a irreversible inhibitor of transaminase. In the biosynthesis of the peptidoglycan layer of the bacterial cell wall, three enzymes, alanine racemase, D-Ala-D-Ala ligase, and D-Ala-D-Ala adding enzyme were involved.²⁴ The racemase and ligase are targets of the antibacterial agents such as *O*-carbonyl-D-serine²⁵ and D-cycloserine,²⁶ respectively.

In this paper, we describe the use of *N*-hydroxyalanine as a "warhead" component in peptides and the *in vitro* activity of these peptides against *E. coli* on the basis of the two inhibition mechanisms of DL-*N*-OH-Ala mentioned above. Evidence is presented that supports peptide transport mediated entry of these substances to gain access to intracellular targets.

Experimental

All melting points (mp) and boiling points (bp) are uncorrected. ¹H-NMR spectra were recorded on a General Electric GN-260 spectrometer using tetramethylsilane as internal standard (δ scale). Flash chromatography was performed on Merk Silica gel 60 (230-400 mesh ASTM) using nitrogen pressure. Analytical thin-layer chromatography (TLC) was carried out on precoated (0.25 mm) Merk silica gel F-254 plates. R_f values of TLC and purity were determined

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in the following solvent systems: A, chloroform-methanol (9/1, v/v); B, chloroform-methanol (9/2, v/v); C, chloroform-methanol-acetic acid (7/2/1, v/v/v); D, *n*-butanol-acetic acid-water (4/1/1, v/v/v). Compounds were visualized by uv, ninhydrin, and KI/starch. Reversed phase HPLC was performed on a LiChrograph system utilizing a Merck column (25×0.4 cm) packed with LiChrospher 100 RP-18 (10 μm) and methanol-water solvent (10-50% gradient) system.

DL-N-Hydroxyalanine, DL-N-OH-Ala.²⁷ A mixture of pyruvic acid 470 mg, 4 mmol, and HCl·NH₂OH (300 mg, 4 mmol) was taken in water (40 ml). 1N KOH was added to raise the pH to approximately 5. A large excess of NaBH₃CN (720 mg, 12 mmol) was added and the solution was stirred at room temperature for 48h. The pH of the reaction mixture was adjusted to 1 with conc. HCl. Filtration and evaporation under vacuum gave crude white solid. Further purification was performed by Dowex 50W-X8 column chromatography. The column was equilibrated and eluted with 0.3 M pyridine, and the pH was adjusted to 2.5 with formic acid. The fraction containing DL-*N*-OH-Ala were pooled together, and evaporated to give white pure product; yield 344 mg (82%), mp. 145-148°C, *R_f* (D) 0.59. NMR (D₂O) δ_H 1.4 (d, 3H, CH₃), 4.0 (q, 1H, alanyl-CH-). Anal. Found: C, 34.11; H, 6.82; N, 13.51. Calcd. for C₃H₇NO₃: C, 34.29; H, 6.67; N, 13.33%.

Benzyloxycarbonyl-L-alanylamine, Z-L-Ala-NH₂ (1).

Dry ammonia gas was led in a gentle stream to the stirred solution of benzyloxycarbonyl-L-alanyl-*p*-nitrophenylester (3.44 g, 10 mmol) in tetrahydrofuran (100 ml). One hour after dry ammonia gas was passed, the flask was stoppered and kept at room temperature overnight. The solvent was concentrated to give crude product. Purification by flash chromatography, eluted with 5% methanol in dichloromethane gave the compound as a white crystalline solid (2.15 g, 96%), mp. 218-220°C, *R_f* (C) 0.47. NMR (DMSO-*d*₆) δ_H 1.2 (d, 6H, βCH₃), 4.1 (q, 1H, -CH-), 5.0 (s, 2H, benzylic CH₂), 7.1 (d, 1H, NH), 7.4 (s, 5H, aromatic), 7.5 (d, 1H, NH).

Benzyloxycarbonyl-L-alanyl-α-DL-hydroxyglycine, Z-L-Ala-DL-Gly(α-OH) (2). A mixture of glyoxylic acid monohydrate (2.3 g, 25 mmol) and Benzyloxycarbonyl-L-alanine amide, Z-L-Ala-NH₂ (5.0 g, 22.5 mmol) in 50 ml of acetone was refluxed at 65°C for 12h. Methylene chloride (100 ml) was added and the reaction mixture was stored in refrigerator overnight. The resulting solid was filtered to give 2.0 g of white solid, mp. 124-127°C. The filtrate was allowed to stand for 1hr at room temperature to give an additional 3.15 g of solid product; yield 5.15 g (77%), *R_f* (C) 0.36 NMR (DMSO-*d*₆) δ_H 1.2 (d, 3H, -CH₃), 4.1 (m, 1H, alanyl CH), 5.0 (s, 2H, benzylic -CH₂-), 5.4 (d, 1H, gly -CH), 6.3 (b, 1H, -OH), 7.3 (s, 5H, aromatic).

Benzyloxycarbonyl-L-alanyl-α-DL-acetoxylglycine, Z-L-Ala-DL-Gly(α-OAc) (3). Z-L-Ala-DL-Gly(α-OH) (300 mg, 1 mmol) was suspended to 20 ml of acetic anhydride and cooled to 0°C. Then 50 ml of pyridine was added and the reaction mixture was stored at 5°C for 1h. Concentration of the reaction mixture produced a residue that was triturated with a mixture of petroleum ether/Et₂O (1:1 v/v) to afford a white solid; yield 350 mg (92%), mp. 115-118°C, *R_f* (C) 0.44. NMR (CDCl₃) δ_H 1.2 (d, 3H, alanyl CH₃), 2.0 (s, 3H, acetyl CH₃), 4.3 (q, 1H, alanyl CH), 5.08 (s, 2H, benzylic CH₂), 6.2 (d, 1H, glycylic CH), 7.25 (s, 5H, aromatics).

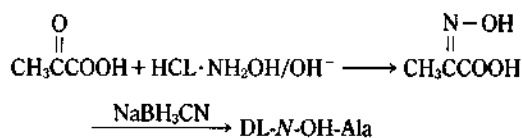
Benzyloxycarbonyl-L-alanyl-α-DL-N-hydroxyalanyl-glycine, Z-L-Ala-DL-Gly(α-DL-N-OH-Ala) (4). 3 (4.16 mg, 0.1 mmol), DL-*N*-OH-Ala (11 mg, 0.12 mmol), and triethylamine (3 ml, 0.2 mmol) were combined in 5 ml of dimethylformamide (DMF) and stirred for 20h. The solvent was removed by evaporation *in vacuo*, and the resulting pale yellow syrup was dissolved in 14 ml of H₂O and purified by prep-HPLC eluting with MeOH/H₂O(1:1 v/v). Fractions containing the product were combined, evaporated, and lyophilized to give 37 mg (72%) of pure product; mp. 121-125°C, *R_f* (C) 0.34. NMR (acetone-*d*₆) δ_H 1.25 (overlap doublets, two alanyl CH₃), 3.6-3.7 (m, 1H, *N*-hydroxyalanyl CH), 4.40 (m, 1H, alanyl CH), 5.1 (s, 2H, benzylic CH₂), 5.7 (m, 1H, glycylic CH), 7.3 (s, 5H, aromatics).

L-Alanyl-α-DL-N-hydroxyalanyl-glycine, L-Ala-DL-Gly(α-DL-N-OH-Ala) (5). The protected peptide 4 (45 mg, 0.1 mmol), 10% Pd-C (10 mg), 1,4-cyclohexadiene (0.3 ml), and methanol (3 ml) were stirred for 30 min at room temperature. The reaction mixture was filtered with the aid of celite and the solvent was removed by evaporation *in vacuo* to give the desired product (37 mg, 91%), mp. 178-181°C, *R_f* (D) 0.52. NMR (acetone-*d*₆) δ_H 1.45 (overlapping doublets, 3H, alanyl CH₃), 4.15 (m, 1H, alanyl CH), 4.0-4.1 (m, 1H *N*-hydroxyalanyl-CH), 5.9 (s, 1H, glycylic -CH-), 7.2 (s, 5H, aromatic). Anal. Found: C, 36.09; H, 5.35; N, 15.79. Calcd. for C₈H₁₅N₃O₇: C, 36.23; H, 5.66; N, 15.85%.

Benzyloxycarbonyl-L-alanyl-L-alanylamine, Z-L-Ala-L-Ala-NH₂ (8)²⁸. To a mixture of Z-L-Ala (265 mg) and Ala-NH₂ (164 mg, 1 mmol) in DMF were added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) (200 mg, 1.1 mmol) and 1-hydroxybenzotriazole (HOBT) (160 mg, 1.1 mmol). The pH of the reaction mixture was adjusted to neutral with Et₃N at 0°C and stirred for 12h. Concentration of the reaction mixture gave yellow oil. The residue was diluted with 100 ml of ethyl acetate (EtOAc) and washed with water (2×25 ml), NaHCO₃ (2×25 ml), 0.5N citric acid (3×25 ml), brine water (3×25 ml), dried over anhydrous MgSO₄, and evaporated *in vacuo* to afford crude oily product. Purification by flash chromatography, eluting with 3% (v/v) methanol in dichloromethane gave the title compound as a white solid (357 mg, 87%), mp. 233-240°C, *R_f* (B) 0.73. NMR (DMSO-*d*₆) δ_H 1.2 (d, 6H, alanyl CH₃), 4.0 (q, 1H, alanyl CH), 4.4 (q, 1H, alanyl CH), 5.0 (s, 2H, benzylic CH₂) 7.0 (s, 1H, amide NH), 7.3 (s, 5H, aromatic).

Benzyloxycarbonyl-L-alanyl-L-alanyl-α-DL-hydroxyglycine, Z-L-Ala-L-Ala-DL-Gly(α-OH) (9). This compound was synthesized by the same method of 2 from Z-L-Ala-L-Ala-NH₂ (2.93 g, 10 mmole) and glyoxylic acid monohydrate (920 mg, 10 mmole). Yield 2.8 g (76%), mp. 205-207°C, *R_f* (C) 0.34. NMR (DMSO-*d*₆) δ_H 1.2 (d, 6H, -CH₃), 4.0 (m, 1H, alanyl -CH), 4.3 (m, 1H, alanyl-CH), 5.0 (s, 2H, benzylic -CH₂-), 5.4(d, 1H, glycylic -CH), 6.4(b, 1H, -OH), 7.3(s, 5H, aromatic).

Benzyloxycarbonyl-L-alanyl-L-alanyl-α-DL-acetoxylglycine pyridinium salt, Z-L-Ala-L-Ala-DL-Gly(α-OAc)-OH·Py (10). A mixture of Z-L-Ala-L-Ala-DL-Gly(α-OH)-OH (400 mg, 1.1 mmol), 50 ml of pyridine and 20 ml of acetic anhydride was stirred at 5°C for 1.5h. The reaction mixture was treated as described for the preparation of dipeptide 3; yield 420 mg (91%), mp. 135-137°C, *R_f* (C) 0.40. NMR (DMSO-*d*₆) δ_H 1.1 (d, 6H, alanyl-CH₃), 1.9 (s, 1H, -CH₃), 2.0



Scheme 1.

(s, 2H, -CH₃), 4.0 (m, 1H, alanyl -CH-), 4.3 (m, 1H, alanyl -CH-), 5.0 (s, 2H, -CH₂), 6.2 (d, 1H, glycyl -CH-), 7.3 (s, 5H, aromatic), 8.6 (s, 1H, pyridine)

Benzoyloxycarbonyl-L-alanyl-L-alanyl-α-DL-N-hydroxyalanylglycine, Z-L-Ala-L-Ala-DL-Gly(α-DL-N-OH-Ala) (11). This compound was synthesized from Z-L-Ala-L-Ala-DL-Gly(α-OAc)-OH·Py (51 mg, 0.1 mmol) **10** and DL-N-OH-Ala (12 mg, 0.1 mmol) in a similar manner as described for the preparation of dipeptide **4**; yield 39 mg (68%), mp. 139-145°C, R_f (C) 0.33. NMR (acetone-d₆) δ_H 1.25 (overlap d, two alanyl -CH₃), 3.6-3.7 (m, 1H, N-hydroxyalanyl -CH-), 4.0 (m, 1H, alanyl -CH-), 4.3 (m, 1H, alanyl -CH-), 5.1 (s, 2H, benzylic -CH₂), 6.0 (m, 1H, glycyl -CH-), 7.3 (s, 5H, aromatic).

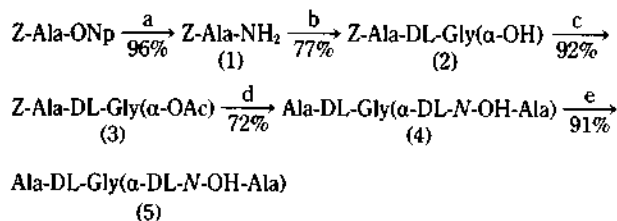
L-Alanyl-L-alanyl-DL-α-DL-N-hydroxyalanylglycine, L-Ala-L-Ala-DL-Gly(α-DL-N-OH-Ala) (12). This compound was synthesized from Z-L-Ala-L-Ala-DL-(α-DL-N-OH-Ala) (28 mg, 0.05 mmol) **11**, by the same method of dipeptide **5**; yield 21 mg (93%), mp. 192-198°C, R_f (D) 0.55. NMR (DMSO-d₆) δ_H 1.45 (overlap d, 6H, alanyl -CH₃), 3.8 (m, 1H, alanyl -CH-), 4.05 (m, 1H, alanyl -CH-), 4.0-4.1 (m, 1H N-hydroxyalanyl -CH-), 5.9 (s, 1H, glycyl -CH-), 7.2 (s, 5H, aromatic). Anal. Found: C, 39.05; H, 5.98; N, 16.72. Calcd. for C₁₁H₂₀N₄O₅: C, 39.29; H, 5.95; N, 16.67%.

Biological activity. For disk diffusion assay, seeded plated of *E. coli* were prepared as follows; cells were grown in liquid medium (10X A-C medium) for periods of 24-48h at 37°C²⁹ and agar was inoculated with 1 ml of the above inoculum. The seeded agar (15 ml) was poured into 150-mm petri-dish. The peptides, dissolved in water, were absorbed onto 6.35-mm paper disks (Schleicher and Schull Co.), which were then placed onto the seeded plates. The plates were incubated for 24h at 37°C, after which time zones of growth inhibition were observed.

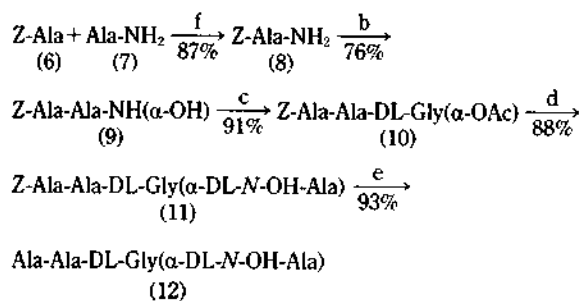
Results and Discussion

Synthesis. Synthesis of N-hydroxyalanyl peptides was accomplished as outlined in Scheme 1. Initially, DL-N-hydroxyalanine was synthesized from the oxime intermediate followed by reduction with NaBH₃CN in water.

Reaction of Z-L-Ala-NH₂ **1** with glyoxylic acid produced dipeptide **2** in 77% yield. Subsequent treatment of **2** with catalytic amount of N,N-dimethylaminopyridine (DMAP) in acetic anhydride produced the peptide acetate **3** in 92% yield. Reaction of **3** with DL-N-OH-Ala gave Z-L-Ala-DL-Gly(α-DL-N-OH-Ala) **4** in 72% yield. Removal of the N-protecting group by phase transfer hydrolysis afforded the required dipeptide as a mixture of diastereoisomers in 91% yield (Scheme 2). For the synthesis of tripeptide, firstly, Z-L-Ala-L-Ala-NH₂ **8** was synthesized by the coupling L-Ala-NH₂ to Z-L-Ala-OH with water soluble carbodiimide, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), and 1-hydroxybenzotriazole (HOBT) in 87% yield.²¹ Then the rest of the reaction steps were followed as the synthetic scheme of dipeptide



Scheme 2. Synthesis of dipeptide synthon of α-DL-N-OH-Ala: (a) NH₃ in THF; (b) glyoxylic acid hydrate, 65°C, 12hr in methylene chloride; (c) Ac₂O, DMAP; (d) DL-N-OH-Ala, NEt₃ in DMF; (e) 10% Pd-C, 1,4-cyclohexadiene in methanol. HONP: *p*-nitrophenol. DMA: dimethyl amino pyridine. Amino acids devoid of configurational description are L-isomers.



Scheme 3. Synthesis of tripeptide synthon of α-DL-N-OH-Ala. (b) Glyoxylic acid hydrate, 65°C, 12hr in methylene chloride; (c) Ac₂O, DMAP; (d) DL-N-OH-Ala, DMF; (e) 10% Pd-C, 1,4-cyclohexadiene in methanol; (f) EDC/HOBT in DMF. EDC: 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, HOBT: 1-hydroxybenzotriazole. Amino acids devoid of configurational description are L-isomers.

to produce L-Ala-L-Ala-DL-Gly(α-DL-N-OH-Ala) **12** in 43% overall yield (Scheme 3). It is generally recognized that peptides containing D-amino acids are poor substrate for peptide permeases.^{30,31} It was therefore important to isolate the all L-stereoisomer or to assess its content in a mixture of peptide synthones. Unfortunately, we were not able to separate the diastereomers of N-hydroxyalanyl di- and tripeptides by HPLC. Therefore, we tried the other method to measure the amount of all L-isomer in the product. Integration of glycine singlets in the ¹H-NMR spectra of stereoisomeric mixture of both acetoxy- and DL-N-hydroxyalanyl peptides provided the relative amounts (Figure 1),^{12,32} but give the stereochemical assignments of each isomers. In an attempt to prove the relative ratio between LL and LD-isomers, N-hydroxyalanyl di- and tripeptides were hydrolyzed with aminopeptidase, leading to the formation of glyoxylate from the peptides containing N-hydroxyalanine.⁶ The amount of glyoxylate released was measured with lactic acid dehydrogenase and NADH.³³ The reaction velocity was determined by a decrease in absorbance at 340 nm resulting from the oxidation of NADH at pH 7.3. The result showed that the dipeptide contained 43% of LL isomer and tripeptide contained 29% of LLL isomer.

Biological activity. Transport systems for peptides have been found in a wide variety of microorganisms.⁵ *E. coli* has been studied most extensively in this regard and

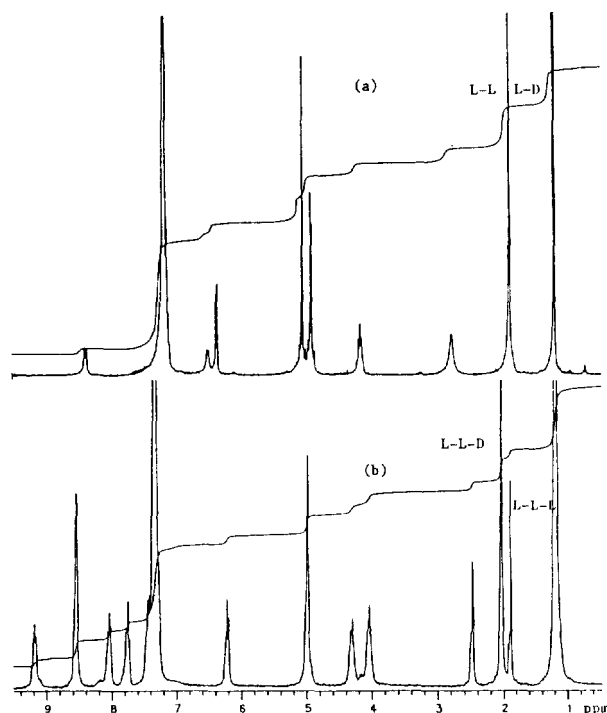


Figure 1. 260 MHz $^1\text{H-NMR}$ spectra of the two α -acetoxy peptides. (a) Z-L-Ala-DL-Gly (α -OAc) in acetone- d_6 , (b) Z-L-Ala-DL-Gly (α -OAc) in DMSO- d_6 .

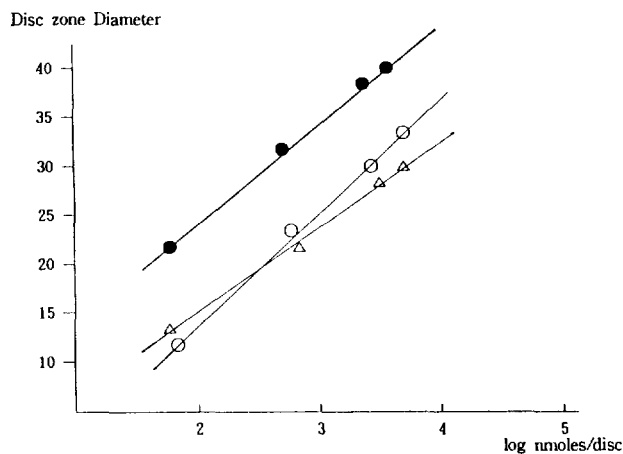


Figure 2. Activity of dipeptide and tripeptide derivatives. (○) DL-*N*-OH-Ala, (●) L-Ala-DL-Gly (α -DL-*N*-OH-Ala), (△) L-Ala-L-Ala-DL-Gly (α -DL-*N*-OH-Ala). Zone of inhibition were caused on *E. coli* seeded agar plates, medium (10 \times A-C)²⁷ solutions of the compounds were added to filter paper disks (6 mm diameter) and the disks were transferred to the plates. After incubation at 37°C for 8hr the diameters of the zones of inhibition were measured.

has been shown to possess three distinct system, one for peptides, another for oligopeptides, and a third for tripeptides composed of amino acids with hydrophobic side chains.^{34,35,37} Peptide required for the evaluation of this concept were prepared as illustrated in Scheme 2 and 3. In order to demonstrate the feasibility of such peptide carriers, DL-*N*-OH-Ala was selected under the assumption that it would

elicit a strong inhibitory response once inside the cell. Since DL-*N*-OH-Ala itself is slightly active as shown in Figure 2, due to its poor transport ability, our attention turned to the preparation of dipeptide derivative of DL-*N*-OH-Ala in order to improve the uptake of DL-*N*-OH-Ala, which is linked through its primary amino group to the α -carbon of a glycine residue at the carboxyl terminal of the dipeptide. Thus, L-Ala-DL-Gly(α -DL-*N*-OH-Ala) proved to be eight times more active than the corresponding underivatized moiety, *N*-hydroxyalanine (Figure 2), despite of the previous result³⁶ that dipeptide synthon containing the unnatural amino acids bind less effectively to its transport system than the oligopeptide transport system does. Precisely, dipeptides bind to its transport system so poorly (116000 times) that the entry rate of the compound fell below that of the corresponding underivatized moiety. It is generally accepted that the peptide permeases are stereospecific so that only the LL isomer of the dipeptide is allowed to be penetrated through dipeptide permease, while the LD isomer is not.^{30,31} LL-Dipeptide constitutes 43% of the mixture, therefore, the enhancement of biological activity was actually 19-fold. This represents another example where a compound with limited ability to penetrate the bacterial cell can be transported through portage transport *via* dipeptide transport system.

As an attempt to improve the antibacterial activity, the tripeptide (L-Ala-L-Ala-DL-Gly(α -DL-*N*-OH-Ala)) containing *N*-hydroxyalanine at the α -position of glycine was prepared as shown in Scheme 3. The resulted tripeptide proved to be less active than the corresponding dipeptide. From this result, it is conceivable that both enzyme, alanine racemase and transaminase, are inhibited by *N*-hydroxyalanine which is transported through membrane permease, but the difference of cellular concentration of alanine substrate plays another important role in causing a different inhibition rate of the target enzyme. Although high concentration of DL-*N*-hydroxyalanine would be accumulated in the bacterial cell through tripeptide carrier system, penetration of twice the amount of L-Ala will be simultaneously occurred through oligopeptide transport system. Therefore, the low inhibitory activity of tripeptide could be considered as a result of increased cellular concentration of L-Ala that could compete with DL-*N*-OH-Ala, Presumably at the two cytoplasmic enzyme sites (racemase and transaminase). However, it is important to establish whether the lack of activity of this compound was due to the failure at the transport level or the absence of inhibitory action against pyridoxal-5'-phosphate dependent enzyme, alanine transaminase or rapid oxidation of DL-*N*-OH-Ala by amino acid oxidase in the cytosole. The experiments are ongoing to interpret the results, which will be published in the following paper.

However, our results demonstrate that DL-*N*-OH-Ala can be transported into *E. coli* by peptide carrier. Being transported into the cell, the peptides are presumably hydrolyzed by intracellular peptidases, resulting the release of the toxic compound DL-*N*-OH-Ala. Furthermore, this study supports the hypothesis that the attachment of an antimetabolite to carrier peptides could provide a viable mechanism for the introduction of the impermeable molecule into *E. coli*.

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Synthesis and Crystal Structure of UP_2S_6

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The new ternary phase UP_2S_6 has been prepared and structurally characterized. The compound is isostructural with ZrP_2S_6 and ThP_2S_6 but is different from TiP_2S_6 . The structure has been determined by a single crystal X-ray diffraction technique. UP_2S_6 crystallizes in the tetragonal system ($C_{4h}^2-P4_2/m$, $a=6.797(7)$ Å, $c=9.738(12)$ Å) with two formula units in the unit cell. The structure can be described in terms of U^{4+} and $P_2S_6^{4-}$ ions. This hexathiohypodiphosphate anion ($P_2S_6^{4-}$) has ideally staggered conformation. The U^{4+} cation is coordinated by 8 sulfur atoms in a slightly distorted dodecahedral geometry (42m). The distribution of sulfur atoms is very well optimized for this geometry.

Introduction

For metals with oxidation number 4, the compounds $[M^{4+}][P_2S_6^{4-}]$ ($M=Ti, Zr$, and Th) represent new structural ty-

pes unrelated to the known hexathiohypodiphosphates of divalent metals ($[M^{2+}]_2[P_2S_6^{4-}]$). Attempts to prepare 8-12 μm IR-transmitting new phases in the metal/P/S system were made to investigate the effect of a highly oxidized metal