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Synthesis and Characterization of Lipoamino Acids Prepared from Aspartic Acid

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Abstract
We synthesized the derivatives of Aspartic acid ranging from alkyl chain length of C_{10} to C_{14} in excellent yields by a facile route. The method involves preparation of copper (II) complexes of Aspartic acid by regioselective coordination of amine and carboxylic groups which are in 1, 2 relationships. The acid functional group in diacidic amino acid is free which underwent further transformations such as salt formation by the use of *N, N, N′, N′*-Tetramethylguanidine and esterification by the treatment with suitable alkyl halide. The free *β*-esters of Aspartic Acid (A10, A12, A14) were isolated by precipitation and washing with acetone of the respective copper complexes and subsequent treatment with an excess of ethylene diamine tetra acetic acid acid (EDTA) disodium salt in water. Their structures have been elucidated by UV, IR, 1H NMR and mass spectra.

Keywords
Lipoamino acid, Aspartic acid, Liposomal drug delivery

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INTRODUCTION
Amino acids are naturally hydrophilic molecules, potentially anionic or cationic depending on the pH. Grafting one or more hydrocarbon chains gives them an amphiphilic character, which amplifies their biological effects or surface properties. This is the essence of lipoamino acid technology. On the one hand, grafting hydrocarbon chains (acylation) improves their solubilization in hydrophobic media (lipophilization). This facilitates their vectorization in living media and increases their bioavailability to create active ingredients with multiple biological targets. On the other hand, depending on the hydrocarbon chain selected, the amphiphilic character gives the possibility of creating bio sourced surfactants.

Lipoaminoacids are complex lipids with amino acid moiety which is linked with a long chain alcohol or fatty acid or glycerolipd. These lipoaminoacid are present in bacteria, lower plants, and even human beings with complex structures. Generally, two types of complex lipoamino acids are present namely a. Lipoaminoacid in which the amino acid is linked via ester bond. e.g., Siolipin b. Lipoamino acid in which the amino acid is linked via ether bond. e.g., Betain lipids. Lipoamino acid combines structural features of lipids with those of amino acids [1-2]. They are highly lipophilic due to the long alkyl side chains yet show polar and conformational behavior characteristic of amino acids. Due to their natural and simple structure they show low toxicity and quick biodegradation. The combination of polar amino acids (hydrophilic moiety) and non-polar long chain compounds (hydrophobic moiety) for building up the amphiphilic structure has produced molecules with high surface

activity. They constitute an interesting alternative to conventional surfactants because of their low toxicity, biodegradability, and renewable sources of raw materials. They have been reported recently to act as permeability enhancers across GI tract and as nontoxic and biodegradable emulsifiers and surfactants. Due to the existence of specific amino acid binding sites at the membrane barriers and the amphiphilic nature of lipoamino acids, they have a potential to act as targeting ligands. Thus, it was thought useful to explore the possibility of using these amphiphiles for drug delivery applications.

Lipophilicity is a key factor for diffusion of drugs across various biological barriers [3]. Lipoamino acids are introduced to increase the lipophilicity along with amphiphilicity exclusively to interact with cell membranes [4-5]. The side chain modification of LAA allows refining the physicochemical properties to achieve the highest cellular uptake of drugs. LAA is used to design peptide prodrugs which can cross the BBB; however, LAA connected with N-terminals results stability of amides against enzymatic degradation in brain [6].

The purpose of the investigation is to synthesize lipoamino acids of Aspartic acid by introducing various alkyl chain lengths ranging from C_{10} to C_{14} resulting β-Esters of Aspartic Acid so that they have α amino and carboxylic acid groups free and long alkyl side chain in their structure. We selected Aspartic acid as the starting material to synthesize above said compounds as they are dicarboxylic acids and provide a site to carry out esterification at β-carboxylic group so that long alkyl chain could be coupled. We selected Aspartic acid and Glutamic acid as the starting material to synthesize above said compounds as they are dicarboxylic acids and provide a site to carry out esterification at β-carboxylic group and γ carboxylic group, respectively, so that long alkyl chain could be coupled. A number of methods are available to carry out this synthesis [7-11]. However, these methods require lengthy multistep procedures, not sufficiently site-specific or give rather low yields. Hence above methods were advanced and optimized by Heeswijk and Eenink et al1982 to obtain excellent yield [12].

L-amino acid transporter (LAT-1) acts as major route for transportation of aromatic amino acid into the living cells. This transport system is very vital for the penetration of amino acids through blood brain barrier and placenta barrier [13]. The transport ability of the transporter depends upon the size. e.g. big sized transporter transport large neutral amino acid [14]. The characteristic feature of LAT-1 is that it possesses broad substrate selectivity that facilitates to accept amino acid related compound. The lipid alpha- amino acids, a class of compounds synthesized by combining amino acids with suitable fatty acids. Lipoaminoacids (LAA) are selective substrates for LAT-1 since they have amino as well as carboxylic acid groups attached to the same carbon along with a hydrophobic chain [15]. LAA plays very vital role to facilitate the drug to interact with cell membrane. They also enhance the penetrability across various biological barriers [16]. The interesting feature of LAA is that it will increase the biological uptake and intracellular concentration of drug [17-18]. When it is conjugated with suitable drug candidate. Hence LAA is very much useful in novel drug delivery system particularly to deliver the drugs, vaccines, and genes [19]. Many novel drug delivery systems e.g., Liposomal drug delivery, lipid emulsions and nanoparticles were successfully adopted LAA to deliver, the therapeutic drug candidates to the target site [20]. They targeted the drug delivery using LAA will not only improve the efficacy of the drug but also reduces the toxicity there by improve therapeutic index [21].

MATERIALS AND METHODS

All the chemicals were of synthetic grade and commercially procured from Qualigen, Mumbai and CDH, New Delhi. Melting points were determined in open capillary method and were uncorrected. Purity of the compound was checked on Silica Gel TLC plates. IR spectra were recorded on FT-IR8400S, Fourier Transform (SHIMADZU) Infrared spectrophotometer using Br disc method. The proton magnetic resonance spectra (H-NMR) were recorded on Perkin Elmer Spectrophotometer- 300MHz.in DMSO *d6*using TMS as an internal standard. Chemical shifts are reported in δ values downfield from internal Trimethyl Silane (TMS). Coupling constants (J) are given in Hz. Multiplicity of signals are expressed as an s (singlet), d (doublet), t (triplet), m (multiplet) and brs (broad singlet). Mass Spectra were recorded on Waters Quattro Micro Mass Spectrophotometer by using Electron spray ionization (ESI). Synthesis of the title compounds was carried out as depicted in Scheme-I. (Figure 1)

Synthesis of β-esters of Aspartic acid (A10, A12, A14):

General procedure for synthesis of L-Aspartic acid Copper (II) complex Copper (II) Salt Octahydrate

A solution of copper (II) acetate monohydrate (41.2 g, 206 mmol) in water (750 ml) was added dropwise (1 h) to a stirred solution of L-aspartic acid (26.6 g, 200 mmol) in water (1100 ml) at 70 °C. The mixture was kept at room temperature for 2 days to complete precipitation and the product was isolated by

suction, washed with water, ethanol, and ether, and dried at 50 °C; yield: 98%.

General procedure for synthesis of L-Aspartic Acid Copper (II) Complex N, N, N', N'-Tetramethyl guanidinium Salt

L-Aspartic Acid Complex Salt 2 in Solution: N, N, N', N'-Tetramethylguanidine (1.4 ml, 11.2 mmol) was added slowly (30 min) to a stirred mixture of both finely powdered aspartic acid (2, 0.75 g, 5.6 mmol), in dimethylformamide (5 ml) and water (0.6 ml). After dissolution of all solids (2h), dimethylformamide (4 ml) was added. The deep blue solution was used directly for the preparation of the 5-esters 5.

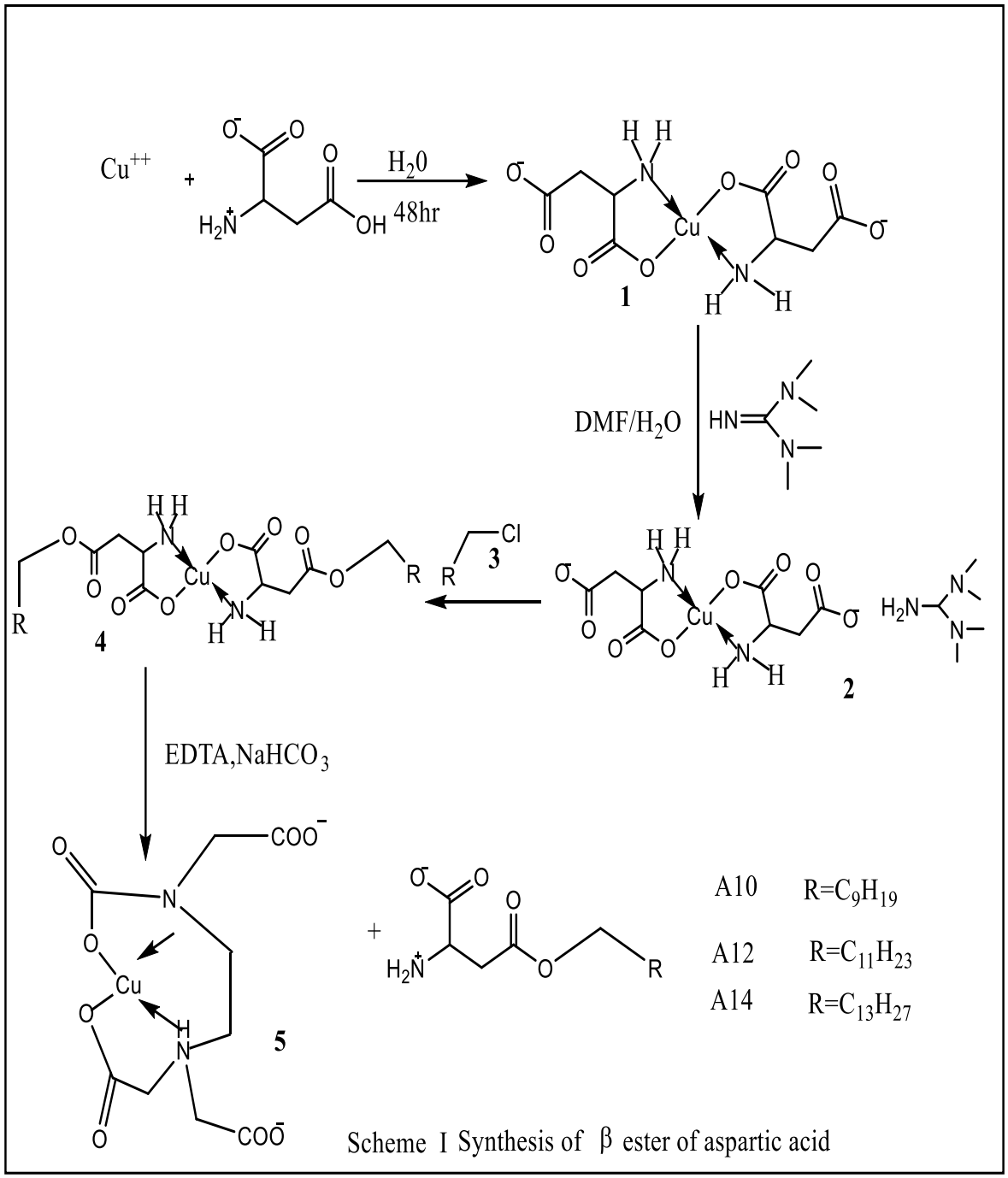

Figure 1: Synthesis of β ester of aspartic acid

General procedure for synthesis of Aspartic Acid β-Alkyl Ester Copper (II) Complexes

To the stirred solution of complex salt 2 prepared as above, the alkyl halide 3(11.8 mmol) was added at once, the resultant mixture was stirred for 24 h at 40 °C. The slurry or cake obtained was stirred with acetone until a fine precipitate was obtained (0.25-16 h), filtered, and washed with acetone. The solids

are suspended in water (25-50 ml) and stirred until all lumps have been converted into a fine precipitate which was isolated by suction, and washed with water, and dried.

General procedure for synthesis of Aspartic Acid β-Alkyl Esters

The wet cake of 4 (or dried and finely powdered 4,) was transferred to a supersaturated aqueous solution of EDTA disodium salt, freshly prepared by the portion wise addition of ethylene diamine tetra acetic acid (EDTA; 1.6 g) to a stirred solution of sodium hydrogen carbonate (0.9 g) in water (11 ml). The resultant mixture was vigorously shaken until compound 4 has been completely decomposed (0.5-1 h). Slight warming, and/ or dilution with water (15 ml), and acetone (5 ml) will be necessary to obtain favorable results. The mixture was cooled (10 °C), filtered and the crystalline 5 was washed with cold water and dried in vacuum. All esters are homogeneous on T.L.C (silica gel, butanol/acetic acid/water, 18/2/5, ninhydrine-and us. visualization).

Compound code: A10.

MF $C_{14}H_{27}NO_4$; MWt 273; yield 80% ; IR (KBr, ν max, cm-)2921, 1730, 1585, 1520, 1476, 1415, 1365, 1311, 1243, 1186, 973, 861, 669, 520. ; **^{1}H NMR (δ ppm, 200MHz)** 0.90(t,J=7.10) 3 -COO-$(CH_2)_9$-CH_3, 1.22-1.54 (m) 14 -COO-CH_2-CH_2-$(CH_2)_7$-CH_3, 1.78(m) 2 -COO-CH_2-CH_2-$(CH_2)_7$-CH_3 3.48 (d, J=5.3) 2 -NH_2-CH-CH_2-COO-, 4.34 (t, J=7.1)2 -COO-CH_2- $(CH_2)_8CH_3$ 4.77 (t, J=5.3) 1 -NH_2-CH-COO- ; ESI+MS, 30 (eV) m/z, (%): 274 (M^+, 100%), 275 (M^{+2} 20%)

Compound code: A12

MF: $C_{16}H_{31}NO_4$, M Wt: 301.;Yield: 80 %.;IR (KBr, ν max, cm^{-1}):2920, 2852, 1732, 1584, 1521, 1415, 1365, 1242, 1208, 1022, 861, 717, 669, 519 cm^{-1} ^{1}H NMR (δ ppm, 200MHz) 0.79 (t, J=6.20) 3 -COO-$(CH_2)_{11}$-CH_3 1.10-1.38 (m) 18 -COO-CH_2-CH_2-$(CH_2)_9$-CH_3 1.57-1.75 (m) 2 -COO-CH_2-CH_2-$(CH_2)_9$-CH_3 3.37(d, J=5.3) 2 -NH_2-CH-CH_2-COO- 4.65 (t, J=5.3) 1 -NH_2-CH-COO-; ESI+MS, 30 (eV) m/z, (%):302 (M^+, 100%), 303 (M^{+2} 20%)

Compound code: A14.

MF: $C_{18}H_{35}NO_4$ MWt: 329;Yield: 80 % IR (KBr, ν max, cm^{-1}): 2919, 2851, 1730, 1518, 1410, 1329, 1226, 1186, 1083, 997, 857, 825, 179, 673, 538 cm^{-1}; ^{1}H NMR (TFA-d, δ ppm, 200MHz) 0.79 (t, J=6.20) -COO-$(CH_2)_{13}$-CH_3 ,1.55-2.00 (m) 22 -COO-CH_2-CH_2-$(CH_2)_{11}$-CH_3 2.11-2.35 (m) 2 -COO-CH_2-CH_2-$(CH_2)_{11}$-CH_3 3.02(d, J=7.0) 2 -NH_2-CH-CH_2-COO- 3.42 (t, J=7.1) 2 COO-CH_2- $(CH_2)_{12}$-CH_3 4.76 (t, J=6.2) 1 -NH_2-CH-COO- Mass Spectrum: Waters Quattro Micro (ESI). ESI+MS, (30 eV) m/z, (%): 330(M^+, 100%), 331(M^{+2} 20%), 284 (33%).

RESULTS AND DISCUSSION

As shown in Scheme-I, three *β*-esters of Aspartic Acid have been synthesized in excellent yields by copper complex method. The method involves preparation of copper (II) complexes of aspartic acid by regioselective coordination of amine and carboxylic groups which are in 1,2 relationship, remaining acid functional group in diacidic amino acid is free which underwent further transformations such as salt formation by the use of *N, N,N′,N′*-Tetramethyl guanidine and esterification by the treatment with suitable alkyl halide. The free *β*-esters of Aspartic Acid (A10, A12, A14) were isolated by precipitation and washing with acetone of the respective copper complexes and subsequent treatment with an excess of ethylenediaminetetraacetic acid (EDTA) disodium salt in water. All compounds prepared by this method were obtained in fair to good yields and were homogeneous on T.L.C. (no α-ester) in the crude state.

The compounds thus obtained have been characterized by their physical, analytical, and spectral data. The IR spectrum of the compound A10 showed characteristic peaks at 2921, 1730 cm^{-1} for CH, str and, Ester, C=O str. 1585, 1520 cm^{-1} for C-O str, 1476, 1415, 1365 cm^{-1}(strong) for Aliphatic chain, 1686 cm^{-1}(strong) for C=O stretch of Ester, 1585 cm^{-1}(strong) for N-H bend of 1° NH_2 , 1243cm^{-1} (strong) for C-N stretch aliphatic amino acids. The presence of peak at 1730 cm^{-1} confirmed the ester functionality in the product **(Fig 1.1a).**

The ^{1}H NMR spectrum of the compound A10 showed the characteristic signals at δ =4.77(t, 1H) for amine attached NH_2-C-H. The signal at δ =4.34(t, 2H) is indicating the two protons -COO-CH_2- $(CH_2)_8CH_3$ and signal at δ =3.48(d, 2H) shown to indicate the presence of -NH_2-CH-CH_2-COO-. The prominent signals at δ =1.78(m, 2H) shown the presence of -COO-CH_2-CH_2-$(CH_2)_7$-CH_3. The signal at δ =1.22-1.54(m, 14H) indicates the presence of aliphatic alkyl chain -COO-CH_2-CH_2-(CH_2)$_7$-CH_3, Triplet signal at δ =0.90(t, 3H) shown the presence of terminal methyl group -COO-$(CH_2)_9$-CH_3.

Further the mass spectrum of the compound A10 showed a prominent molecular ion at M^+ at m/z, (%): 274 and M^{+2} at m/z, (%)275 with an intensity of 100 % and 20 % respectively confirmed the assigned structure.

In a similar way remaining *β*-esters of Aspartic Acid have been characterized and confirmed the molecular formulae of the assigned structures by their spectral data. A detailed account of physical and spectral data has been presented in the experimental section.

CONCLUSION

Lipoamino acids have been reported to possess several interesting properties. Their amphiphilic nature, biodegradability, and the reports that they can act as absorption enhancers were the major factors that provided impetus to the present work. Apart from this, Lipoamino acids with a specific structure can possibly act as recognition ligands for amino acid transporters available at the membrane barriers. Lipoamino acids of Aspartic acid with varying carbon chain length could be prepared in good yields by a facile method. Thus, Lipoamino acids of Aspartic acid were found to possess interesting properties. Their ability to act as targeting ligand along with ability to act as penetration enhancers make them unique agents for drug delivery. Particularly their ability to target the drug to the brain is noteworthy. Thus, these ligands show promise as penetration enhancers and targeting ligands and are worth exploring further for drug delivery applications.

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REFERENCES

[1] Gorshein A., **Biochim Biophys Acta, 152- 358, (1968)
[2] Brown AE et al., Isolation and characterization of a novel lipid, 1(3), 2-diacylglyceryl-(3)-o-4'- (N, N, N-trimethyl) homoserine from Ochromonas danica. Biochemistry,13: 3476, (1974)
[3] Pajouhesh, H. Lenz, G.R., Medicinal chemical properties of successful central nervous system drugs. NeuroRx, 2: 541–553, (2005)
[4] Tóth, I.; Malkinson, J.P.; Flinn, N.S.; Drouillat, B.; Horváth, A.; Erchegyi, J.; Idei, M.; Venetianer, A.; Artursson, P.; Lazorova, L.; et al., Novel lipoamino acid- and liposaccharide-based system for peptide delivery: Application for oral administration of tumor-selective somatostatin analogues. J. Med. Chem, 42:4010–4013, (1999)
[5] Blanchfield, J.T.; Toth, I. Lipids., sugars and liposaccharides in drug delivery 2: An update. Curr. Med. Chem, 11: 2375–2382 (2004)
[6] Polgar, L.; Szeltner, Z. Structure, function and biological relevance of prolyl oligopeptidase. Curr. Prot. Pept. Sci, 9: 96–107, (2008)
[7] Feijen, J et.al., Synthesis and viscosity of poly (y-p-biphenyl methyl-L-glutamate) in benzene/ dichloroacetic acid mixtures, a comparison with poly (y-benzyl-L-glutamate) *Makromol.Chem*. 175; 3193, (1974)
[8] Guttman, S, Boissonas, R.A., Synthese du N-acetyl-L-Seryl-l-tyrosyl-L-seryl L-methionyl-gamma-L-glutamate etc. benzyle et de peptide apparentes *Helv.Chem. Acta*, 41:1852, (1958)
[9] King, F. E., Clark-Lewis, J.W., Smith, G.R., *J. Chem. Soc, 1046, (1954)*
[10] Ledger, R Stewart, F.H.C., Aust. J.Chem. *Chem. Soc, 1046, (1965)*
[11] Loucheux, M.H., Parrod, M.J., *Acad.Sci.Ser*, *C*: 267-614,(1968)
[12] Van Heeswijk,W. A. R, Fenik, M J D Feijen J. Synthesis, 744,(1984)
[13] Christensen HN. Role of amino acid transport and counter transport in nutrition and metabolism. Physiol Rev, 70:43–77, (1990)
[14] Pardridge WM. Brain metabolism: a perspective from the blood– brain barrier. Physiol Rev, 63:1481–1535, (1983)
[15] Hiroshi U, Kanai Y, Kim DK, Wempe MF, Chairroungdua A, Morimoto E, et al. Transport of amino acid-related compounds mediated by L-type amino acid transporter (LAT1): insights into the mechanisms of substrate recognition. Mol Pharmacol, 61:729–737, (2002)
[16] Toth I. A novel chemical approach to drug delivery: lipidic amino acid conjugates. J Drug Target, 2:217–239, (1994)
[17] Pignatello R, Jansen G, Kathmann I, Puglisi G, Toth I. Lipoamino acid conjugates of methotrexate with antitumor activity. J Pharm Sci, 87:367–371, (1998)
[18] Wong A, Toth I. Lipid, sugar and liposaccharide-based delivery systems. Curr Med Chem, 8:1123–36, (2001)
[19] Zhong W, Skwarczynski M, Toth I. Lipid core peptide system for gene, drug, and vaccine delivery. Aust J Chem, 62:956–67, (2009)

[20] Bumol TF. 4th Inter. Conf. Monoclonal antibody immunoconjugates for cancer. San Diego; 1989; 50.
[21] Youssef M, Fattal E, Alonso MJ, Robot TL. Effectiveness of nanoparticle bound ampicillin in the treatment of Listeria monocytogenes infection in athymic nude mice. Antimicrob Agents Chemother. 1988; 31:104.