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(54) PEPTIDES BIOLOGIQUEMENT ACTIFS PROVENANT DE DOMAINES FONCTIONNELS DE PROTEINES BACTERICIDES ET AUGMENTANT LA PERMEABILITE; LEURS UTILISATIONS

(54) BIOLOGICALLY ACTIVE PEPTIDES FROM FUNCTIONAL DOMAINS OF BACTERICIDAL/PERMEABILITY-INCREASING PROTEIN AND USES THEREOF

(57) Peptides ayant une séquence d'acides aminés qui est la séquence d'acides aminés d'un domaine fonctionnel de protéine humaine bactéricide/augmentant la perméabilité (BPI) ou d'une sous-séquence dudit domaine, et variantes de ladite séquence ou de ladite sous-séquence, ayant au moins une des activités biologiques BPI, telles que la liaison de l'héparine, la neutralisation de l'héparine, la liaison des lipopolysaccharides (LPS) et la neutralisation ou l'activité bactéricide desdits LPS. La présente invention présente des peptides et des compositions pharmaceutiques desdits peptides pour toute une gamme d'utilisations thérapeutiques.

(57) The present invention provides peptides having an amino acid sequence that is the amino acid sequence of a human bactericidal/permeability-increasing protein (BPI) functional domain or a subsequence thereof, and variants of the sequence or subsequence thereof, having at least one of the BPI biological activities, such as heparin binding, heparin neutralization, LPS binding, LPS neutralization or bactericidal activity. The invention provides peptides and pharmaceutical compositions of such peptides for a variety of therapeutic uses.

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(54) Title: BIOLOGICALLY ACTIVE PEPTIDES FROM FUNCTIONAL DOMAINS OF BACTERICIDAL/PERMEABILITY-INCREASING PROTEIN AND USES THEREOF

(57) Abstract

The present invention provides peptides having an amino acid sequnce that is the amino acid sequence of a human bactericidal/permeability-increasing protein (BPI) functional domain or a subsquence thereof, and variants of the sequence or subsequence thereof, having at least one of the BPI biological activities, such as heparin binding, heparin neutralization, LPS binding, LPS neutralization or bactericidal activity. The invention provides peptides and pharmaceutical compositions of such peptides for a variety of therapeutic uses.

Biologically Active Peptides from Functional Domains of Bactericidal/ Permeability-Increasing Protein and Uses Thereof

BACKGROUND OF THE INVENTION

The present invention relates to peptides derived from or based on bactericidal/permeability-increasing protein and therapeutic uses of such peptides.

Bactericidal/permeability-increasing protein (BPI) is a protein isolated from the granules of mammalian polymorphonuclear neutrophils (PMNs), which are blood cells essential in defending a mammal against invading microorganisms. Human BPI has been isolated from PMNs by acid extraction combined with either ion exchange chromatography (Elsbach, 1979, *J. Biol. Chem.* 254: 11000) or *E. coli* affinity chromatography (Weiss *et al.*, 1987, *Blood* 69: 652), and has potent bactericidal activity against a broad spectrum of Gram-negative bacteria. The molecular weight of human BPI is approximately 55,000 daltons (55kD). The complete amino acid sequence of human BPI, as well as the nucleotide sequence of DNA encoding BPI, have been elucidated by Gray *et al.*, 1989, *J. Biol. Chem.* 264: 9505, incorporated herein by reference (*see* Figure 1 in Gray *et al.*).

The bactericidal effect of BPI has been shown to be highly specific to sensitive Gram-negative species. The precise mechanism by which BPI kills Gram-negative bacteria is not yet known, but it is known that BPI must first attach to the surface of susceptible Gram-negative bacteria. This initial binding of BPI to the bacteria involves electrostatic interactions between BPI, which is a basic (i.e., positively charged) protein, and negatively charged sites on lipopolysaccharides (LPS). LPS is also known as "endotoxin" because of the potent inflammatory response that it stimulates. LPS induces the release of mediators by host inflammatory cells which may ultimately result in irreversible endotoxic shock. BPI binds to Lipid A, the most toxic and most biologically active component of LPS.

BPI is also capable of neutralizing the endotoxic properties of LPS to which it binds. Because of its Gram-negative bactericidal properties and its ability to bind to and neutralize LPS, BPI can be utilized for the treatment of mammals suffering from diseases caused by Gram-negative bacteria, including bacteremia, endotoxemia, and sepsis. These dual properties of BPI make BPI particularly useful and advantageous for such therapeutic administration.

A proteolytic fragment corresponding to the amino-terminal portion of human BPI possesses the LPS binding and neutralizing activities and antibacterial activity of the naturally-derived 55kD human holoprotein. In contrast to the amino-terminal portion, the carboxyl-terminal region of isolated human BPI displays only slightly detectable antibacterial activity (Ooi *et al.*, 1991, *J. Exp. Med.* 174: 649). One BPI amino-terminal fragment, comprising approximately the first 199 amino acid residues of the human BPI holoprotein and referred to as "$rBPI_{23}$" (*see* Gazzano-Santoro *et al.*, 1992, *Infect. Immun.* 60: 4754-4761) has been produced by recombinant means as a 23kD protein. $rBPI_{23}$ has been introduced into human clinical trials. Proinflammatory responses to endotoxin were significantly ameliorated when $rBPI_{23}$ was co-administered with LPS.

Other endotoxin binding and neutralizing peptides are known in the art. One example is *Limulus* antilipopolysaccharide factor (LALF) from horseshoe crab amebocytes (Warren *et al.*, 1992, *Infect. Immunol.* 60: 2506-2513). Another example is a cyclic, cationic lipopeptide from *Bacillus polymyxa*, termed Polymyxin B_1. Polymyxin B_1 is composed of six α,γ-diaminobutyric acid residues, one D-phenylalanine, one leucine, one threonine and a 6-methyloctanoyl moiety (Morrison and Jacobs, 1976, *Immunochem.* 13: 813-818) and is also bactericidal. Polymyxin analogues lacking the fatty acid moiety are also known, which analogues retain LPS binding capacity but are without appreciable bactericidal activity (Danner et al., 1989, Antimicrob. Agents Chemother. 33: 1428-1434). Similar properties have also been found with synthetic cyclized polymyxin analogues (Rustici et al., 1993, Science 259:361-365).

Known antibacterial peptides include cecropins and magainins. The cecropins are a family of antibacterial peptides found in the hemolymph of lepidopteran insects (Wade et al., 1990, Proc. Natl. Acad. Sci. USA 87: 4761-4765), and the magainins are a family of antibacterial peptides found in Xenopus skin and gastric mucosa (Zasloff et al., 1988, Proc. Natl. Acad. Sci. USA 85: 910-913). These peptides are linear and range from about 20 to about 40 amino acids in length. A less active mammalian cecropin has been reported from porcine intestinal mucosa, cecropin P1 (Boman et al, 1993, Infect. Immun. 61: 2978-2984). The cecropins are generally reported to be more potent than the magainins in bactericidal activity but appear to have less mammalian cell cytotoxicity. The cecropins and magainins are characterized by a continuous, amphipathic α-helical region which is necessary for bactericidal activity. The most potent of the cecropins identified to date is cecropin A. The sequence of the first ten amino acids of the cecropin A has some homology with the BPI amino acid sequence 90 - 99. However, the other 27 amino acids of cecropin A are clearly necessary for its bactericidal activity and there is little homology with BPI for those 27 amino acids. The magainins have even less homology with the BPI sequence.

Of interest to the present application are the disclosures in WO92/03535 (PCT/US91/05758) relating to compositions comprising BPI and an anionic compound, which compositions are said to exhibit (1) no bactericidal activity and (2) endotoxin neutralizing activity. Anionic compounds are preferably a protein such as serum albumin but can also be a polysaccharide such as heparin. In addition, Weiss et al. (1975, J. Clin. Invest. 55: 33-42) disclose that heparin sulfate and LPS block expression of the permeability-increasing activity of BPI. However, neither reference discloses that BPI actually neutralizes the biologic activities of heparin. Heparin binding does not necessarily imply heparin neutralization. For example, a family of heparin binding growth factors (HBGF) requires heparin as a cofactor to elicit a biological response. Examples of HBGF's include: fibroblast growth factors (FGF-1, FGF-2) and endothelial cell growth factors (ECGF-1, ECGF-2). Antithrombin III inhibition of clotting cascade proteases is another example of a heparin binding protein that requires heparin for activity and clearly does not neutralize heparin. Heparin binding proteins that do neutralize heparin (e.g., platelet factor IV, protamine, and thrombospondin are generally inhibitory of the activities induced by heparin binding proteins that use heparin as a cofactor.

BPI (including amino-terminal fragments thereof) has a number of other important biological activities. For example, BPI has been shown to have heparin binding and heparin neutralization activities in WO94/20128 (PCT/US94/02401). These heparin binding and neutralization activities of BPI are significant due to the importance of current clinical uses of heparin. Heparin is commonly administered in doses of up to 400 U/kg during surgical procedures such as cardiopulmonary bypass, cardiac catherization and hemodialysis procedures in order to prevent blood coagulation during such procedures. When heparin is administered for anticoagulant effects during surgery, it is an important aspect of post-surgical therapy that the effects of heparin are promptly neutralized so that normal coagulation function can be restored. Currently, protamine is used to neutralize heparin. Protamines are a class of simple, arginine-rich, strongly basic, low molecular weight proteins. Administered alone, protamines (usually in the form of protamine sulfate) have anti-coagulant effects. When administered in the presence of heparin, a stable complex is formed and the anti-coagulant activity of both drugs is lost. However, significant hypotensive and anaphylactoid effects of protamine have limited its clinical utility. Thus, due to its heparin binding and neutralization . . . .

activities, BPI has potential utility as a substitute for protamine in heparin neutralization in a clinical context without the deleterious side-effects which have limited the usefulness of the protamines. The additional antibacterial and anti-endotoxin effects of BPI would also be useful and advantageous in post-surgical heparin neutralization compared with protamine.

Additionally, BPI is useful in inhibiting angiogenesis due in part to its heparin binding and neutralization activities. In adults, angiogenic growth factors are released as a result of vascular trauma (wound healing), immune stimuli (autoimmune disease), inflammatory mediators (prostaglandins) or from tumor cells. These factors induce proliferation of endothelial cells (which is necessary for angiogenesis) *via* a heparin-dependent receptor binding mechanism (*see* Yayon *et al.*, 1991, *Cell* 64: 841-848). Angiogenesis is also associated with a number of other pathological conditions, including the growth, proliferation, and metastasis of various tumors; diabetic retinopathy, retrolental fibroplasia, neovascular glaucoma, psoriasis, angiofibromas, immune and non-immune inflammation including rheumatoid arthritis, capillary proliferation within atherosclerotic plaques, hemangiomas, endometriosis and Kaposi's sarcoma. Thus, it would be desirable to inhibit angiogenesis in these and other instances, and the heparin binding and neutralization activities of BPI are useful to that end.

Several other heparin neutralizing proteins are also known to inhibit angiogenesis. For example, protamine is known to inhibit tumor-associated angiogenesis and subsequent tumor growth [*see* Folkman *et al.*, 1992, *Inflammation: Basic Principles and Clinical Correlates*, 2d ed., (Galin *et al.*, eds., Review Press, N.Y.), Ch. 40, pp. 821-839] A second heparin neutralizing protein, platelet factor IV, also inhibits angiogenesis (*i.e.*, is angiostatic). Collagenase inhibitors are also known to inhibit angiogenesis (*see* Folkman *et al.*, 1992, *ibid.*) Another known angiogenesis inhibitor, thrombospondin, binds to heparin with a repeating serine/tryptophan motif instead of a basic amino acid motif (*see* Guo *et al.*, 1992, *J. Biol. Chem.* 267: 19349-19355).

Another utility of BPI involves pathological conditions associated with chronic inflammation, which is usually accompanied by angiogenesis. One example of a human disease related to chronic inflammation is arthritis, which involves inflammation of peripheral joints. In rheumatoid arthritis, the inflammation is immune-driven, while in reactive arthritis, inflammation is associated with infection of the synovial tissue with pyogenic bacteria or other infectious agents. Folkman *et al.*, 1992, *supra*, have also noted that many types of arthritis progress from a stage dominated by an inflammatory infiltrate in the joint to a later stage in which a neovascular pannus invades the joint and begins to destroy cartilage. While it is unclear whether angiogenesis in arthritis is a causative component of the disease or an epiphenomenon, there is evidence that angiogenesis is necessary for the maintenance of synovitis in rheumatoid arthritis. One known angiogenesis inhibitor, AGM1470, has been shown to prevent the onset of arthritis and to inhibit established arthritis in collagen-induced arthritis models (Peacock *et al.*, 1992, *J. Exp. Med.* 175: 1135-1138). While nonsteroidal anti-inflammatory drugs, corticosteroids and other therapies have provided treatment improvements for relief of arthritis, there remains a need in the art for more effective therapies for arthritis and other inflammatory diseases.

There continues to exist a need in the art for new products and methods for use as bactericidal agents and endotoxin neutralizing agents, and for heparin neutralization and inhibition of angiogenesis (normal or pathological). One avenue of investigation towards fulfilling this need is the determination of the functional domains of the BPI protein specifying each of these biological activities. Advantageous therapeutic embodiments would therefore comprise BPI functional domain peptides having one or more than one of the activities of BPI.

SUMMARY OF THE INVENTION

This invention provides small, readily-produced peptides having an amino acid sequence that is the amino acid sequence of a BPI functional domain or a subsequence thereof and variants of the sequence or subsequence having at least one of the biological activities of BPI, such as heparin binding, heparin neutralization, LPS binding, LPS neutralization or bactericidal activity. The functional domains of BPI discovered and described herein include: domain I, encompassing the amino acid sequence of BPI from about amino acid 17 to about amino acid 45; domain II, encompassing the amino acid sequence of BPI from about amino acid 65 to about amino acid 99; and domain III, encompassing the amino acid sequence of BPI from about amino acid 142 to about amino acid 169. Thus, the BPI functional domain peptides are based on the amino-terminal portion of human BPI.

The peptides of the invention include linear and cyclized peptides, and peptides that are linear, cyclized and branched-chain combinations of particular BPI functional domain amino acid sequences or subsequences thereof and variants of the sequence or subsequence. Combination peptides include peptides having the sequence or subsequence and variants of the sequence or subsequence of the same or different functional domains of BPI that are covalently linked together. Specifically included are combinations from two to about 10 peptides of any particular sequence or subsequence thereof and variants of that sequence or subsequence. The invention also provides peptides having additional biological activities distinct from the known biological activities of BPI, including but not limited to bactericidal activity having an altered target cell species specificity. Peptides having particular biological properties of BPI that are enhanced or decreased compared with the biological properties of BPI are also provided.

The peptides of the invention include linear and cyclized peptides, and peptides that are linear, cyclized and branched-chain amino acid substitution and additional variants of particular BPI functional domain amino acid sequences or subsequences thereof. For the substitution variants, amino acid residues at one or more positions in each of the peptides are replaced with a different amino acid residue (including atypical amino acid residues) from that found in the corresponding position of the BPI functional domain from which the specific peptide is derived. For the addition variants, peptides may include up to about a total of 10 additional amino acids, covalently linked to either the amino-terminal or carboxyl-terminal extent, or both, of the BPI functional domain peptides herein described. Such additional amino acids may duplicate amino acids in BPI contiguous to a functional domain or may be unrelated to BPI amino acid sequences and may include atypical amino acids. Linear, cyclized, and branched-chain combination embodiments of the amino acid substitution and addition variant peptides are also provided as peptides of the invention, as are cyclized embodiments of each of the aforementioned BPI functional domain peptides. In addition, peptides of the invention may be provided as fusion proteins with other functional targeting agents, such as immunoglobulin fragments. Addition variants include derivatives and modifications of amino acid side chain chemical groups such as amines, carboxylic acids, alkyl and phenyl groups.

The invention provides pharmaceutical compositions for use in treating mammals for neutralizing endotoxin, killing Gram-negative and Gram-positive bacteria and fungi, neutralizing the anti-coagulant properties of heparin, inhibiting angiogenesis, inhibiting tumor and endothelial cell proliferation, and treating chronic inflammatory disease states. The pharmaceutical compositions comprise unit dosages of the BPI peptides of this invention in solid, semi-solid and liquid dosage forms such as tablet pills, powder, liquid solution or suspensions and injectable and infusible solutions.

This invention provides peptides having an amino acid sequence which is the amino acid sequence of human BPI from about position 17 to about position 45 comprising functional domain I, having the sequence:

Domain I ASQQGTAALQKELKRIKIPDYSDSFKIKH
(SEQ ID NO:1);

and subsequences thereof which have biological activity, including but not limited to one or more of the activities of BPI, for example, bactericidal activity, LPS binding, LPS neutralization, heparin binding or heparin neutralization. Also provided in this aspect of the invention are peptides having substantially the same amino acid sequence of the functional domain I peptides having the amino acid sequence of BPI from about position 17 to about position 45 or subsequences thereof. Additionally, the invention provides peptides which contain two or more of the same or different domain I peptides or subsequence peptides covalently linked together.

This invention provides peptides having an amino acid sequence which is the amino acid sequence of human BPI from about position 65 to about position 99 comprising functional domain II, having the sequence:

Domain II SSQISMVPNVGLKFSISNANIKISGKWKAQKRFLK
(SEQ ID NO:6);

and subsequences thereof which have biological activity, including but not limited to one or more of the activities of BPI, for example, bactericidal activity, LPS binding, LPS neutralization, heparin binding or heparin neutralization. Also provided in this aspect of the invention are peptides having substantially the same amino acid sequence of the functional domain II peptides having the amino acid sequence of BPI from about position 65 to about position 99 or subsequences thereof. Additionally, the invention provides peptides which contain two or more of the same or different domain II peptides or subsequence peptides covalently linked together.

The invention also provides peptides having an amino acid sequence which is the amino acid sequence of human BPI from about position 142 to about position 169 comprising functional domain III, having the sequence:

Domain III VHVHISKSKVGWLIQLFHKKIESALRNK
(SEQ ID NO:12);

and subsequences thereof which have biological activity, including but not limited to one or more of the activities of BPI, for example, bactericidal activity, LPS binding, LPS neutralization, heparin binding or heparin neutralization. Also provided in this aspect of the invention are peptides having substantially the same amino acid sequence of the functional domain III peptides having the amino acid sequence of BPI from about position 142 to about position 169 or subsequences thereof. Additionally, the invention provides peptides which contain two or more of the same or different domain III peptides or subsequence peptides covalently linked together.

Also provided by this invention are interdomain combination peptides, wherein two or more peptides from different functional domains or subsequences and variants thereof are covalently linked together. Linear, cyclized and branched-chain embodiments of these interdomain combination peptides are provided.

The peptides of this invention have as one aspect of their utility at least one of the known activities of BPI, including LPS binding, LPS neutralization, heparin binding, heparin neutralization and bactericidal activity against Gram-negative bacteria. Additionally and surprisingly, some of the peptides of this invention have utility as bactericidal agents against Gram-positive bacteria. Another surprising and unexpected utility of some of the peptides of this invention is as fungicidal agents. Peptides of this invention provide a new class of antibiotic molecules with the dual properties of neutralizing endotoxin and killing the endotoxin-producing bacteria, useful in the treatment of mammals suffering from diseases or conditions caused by Gram-negative bacteria. Peptides of this invention that retain this dual activity and additionally have an increased antibiotic spectrum represent an additional new class of antimicrobial agents. In addition, peptides of the invention provide a class of antimicrobial agents useful in the treatment of infections by microbial strains that are resistant to traditional antibiotics but are sensitive to the permeability-increasing antimicrobial activity of peptides of the invention.

The invention also provides pharmaceutical compositions of the peptides of the invention comprising the peptides or combinations of the peptides in a pharmaceutically-acceptable carrier or diluent, both *per se* and for use in methods of treating pathological or disease states or for other appropriate therapeutic uses. Methods of using these pharmaceutical compositions for the treatment of pathological or disease states in a mammal, including humans, are also provided by the invention. Also provided by the invention are uses of BPI functional domain peptide for the manufacture of medicaments for a variety of therapeutic applications.

Specific preferred embodiments of the present invention will become evident from the following more detailed description of certain preferred embodiments and the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1a and 1b depict HPLC absorbance spectra for cyanogen bromide and proteolytic fragments of $rBPI_{23}$;

Figure 2 is a graph of LAL inhibition assay results for proteolytic fragments of $rBPI_{23}$;

Figure 3 is a graph of a heparin binding assay results using 15-mer BPI peptides;

Figure 4 is a graph of a *Limulus* Amoebocyte Lysate (LAL) inhibition assay results using 15-mer BPI peptides;

Figure 5 is a graph of a radial diffusion bactericidal assay results using 15-mer BPI peptides;

Figure 6 is a graph showing the effect of BPI functional domain peptides in a heparin binding assay;

Figures 7a and 7b are graphs showing the effects of BPI functional domain peptides on ATIII/heparin inhibition of thrombin;

Figures 8a and 8b are graphs showing the results of BPI functional domain peptides in an LAL inhibition assay;

Figures 9a, 9b, 9c, and 9d are graphs showing the results of BPI functional domain peptides in radial diffusion bactericidal assays;

Figures 9e and 9f are graphs showing the results of BPI functional domain peptides in *E. coli* broth assays;

Figures 10a, 10b, 10c, 10d and 10e are graphs showing the results of BPI functional domain combination peptides in radial diffusion bactericidal assays;

Figures 11a, 11b, 11c, 11d, 11e, 11f, 11g, 11h and 11i are graphs showing the results of BPI functional domain peptides in radial diffusion bactericidal assays;

Figures 11j and 11k are graphs showing the results of BPI functional domain peptides in bactericidal assays on bacterial cells growing in broth media;

Figure 11l is a graph showing the results of BPI functional domain peptide BPI.30 in bactericidal assays performed in human serum;

Figures 11m and 11n are graphs showing the results of BPI functional domain peptides in radial diffusion bactericidal assays using Gram-positive bacteria;

Figure 11o is a graph showing the results of BPI functional domain peptides in radial diffusion bactericidal assays in comparison with gentamicin and vancomycin using *S. aureus* cells;

Figures 11p and 11q are graphs showing the results of BPI functional domain peptides in cytotoxicity assays using *C. albicans* cells growing in broth media;

Figures 12a, 12b, 12c, 12d, 12e, 12f, and 12g are graphs showing the results of a heparin neutralization assay using BPI functional domain peptides;

Figure 13 is a schematic diagram of the structure of BPI domain II peptide BPI.2 (amino acid sequence 85-99 of the BPI sequence, SEQ ID NO:7);

Figure 14 is a schematic diagram of the structure of BPI domain III peptide BPI.11 (amino acid sequence 148-161 of the BPI sequence, SEQ ID NO:13);

Figures 15a, 15b, 15c, 15d and 15e are graphs showing the results of heparin binding assays using BPI functional domain substitution peptides;

Figure 16 is a graph showing the results of heparin binding experiments using a variety of BPI functional domain peptides;

Figures 17a and 17b are graphs of the results of Lipid A binding competition assays between synthetic BPI functional domain peptides and radiolabeled $rBPI_{23}$;

Figure 18 is a graph of the results of Lipid A binding competition assays between synthetic BPI.10 peptide and radiolabeled $rBPI_{23}$ in blood or phosphate buffered saline;

Figure 19 is a graph of the results of Lipid A binding competition assays between synthetic BPI peptides BPI.7, BPI.29 and BPI.30 *versus* radiolabeled $rBPI_{23}$;

Figures 20a and 20b are graphs of the results of Lipid A binding competition assays between BPI functional domain peptides and radiolabeled $rLBP_{25}$;

Figure 21 is a graph of the results of radiolabeled RaLPS binding experiments using BPI functional domain peptides pre-bound to HUVEC cells;

Figures 22a, 22b, 22c, 22d, 22e, 22f, 22g, and 22h are graphs showing the various parameters affecting a cellular TNF cytotoxicity assay measuring the LPS neutralization activity of BPI;

Figures 23a, 23b and 23c are graphs showing the dependence of NO production on the presence of γ-interferon and LBP in LPS-stimulated RAW 264.7 cells and inhibition of such NO production using $rBPI_{23}$;

Figures 24a, 24b, 24c, 24d, 24e, and 24f are graphs showing LPS neutralization by BPI functional domain peptides reflected in their capacity to inhibit NO production by RAW 264.7 cells stimulated by zymosan or LPS;

Figure 24g is a graph showing the IC_{50} values of synthetic BPI peptides for inhibition of LPS- or zymosan-stimulated NO production by RAW 264.7 cells;

Figure 25 is a schematic of $rBPI_{23}$ showing three functional domains;

Figure 26a is a graph showing the dependence of LPS-mediated inhibition of RAW 264.7 cell proliferation on the presence of rLBP;

Figures 26b and 26c are graphs showing patterns of BPI functional domain peptides using the assay of Example 20D;

Figure 27 is a graph showing a comparision of TNF inhibition in whole blood by various BPI functional domain peptides using the assay of Example 20E; and

Figure 28 is a graph showing the results of the thrombin clotting time assay described in Example 20G using various BPI functional domain peptides.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

This invention provides peptides having an amino acid sequence that is the amino acid sequence of at least one functional domain or subsequence thereof and variants of the sequence or subsequence of BPI. For the purposes of this invention, the term "functional domain" is intended to designate a region of the amino acid sequence of BPI that contributes to the total biological activity of the protein. These functional domains of BPI are defined by the activities of proteolytic cleavage fragments, overlapping 15-mer peptides and other synthetic peptides.

Domain I is defined as the amino acid sequence of BPI comprising from about amino acid 17 to about amino acid 45. Peptides based on this domain are moderately active in both the inhibition of LPS-induced LAL activity and in heparin binding assays, and do not exhibit significant bactericidal activity. Domain II is defined as the amino acid sequence of BPI comprising from about amino acid 65 to about amino acid 99. Peptides based on this domain exhibit high LPS and heparin binding capacity and are bactericidal. Domain III is defined as the amino acid sequence of BPI comprising from about amino acid 142 to about amino acid 169. Peptides based on this domain exhibit high LPS and heparin binding activity and are bactericidal.

The functional domains as herein defined include the continuous domains I, II and III, *i.e.*, domains comprised of a continuous portion of the BPI amino acid sequence. However, the invention also includes peptides comprising portions of BPI which are not continuous, *i.e.*, that are separated in the BPI sequence. It is recognized that some non-continuous stretches of amino acid sequence may be folded in the native protein to make such amino acid regions contiguous or in proximity, which structure can be mimicked in the peptides of the invention by covalently linking together peptides from non-continuous regions.

Peptides containing non-continuous regions of BPI amino acid sequence are one example of combination peptides provided by the invention. For the purposes of this invention, combination peptides are intended to include linear, cyclized or branched-chain peptides comprised of two or there peptides having an amino acid sequence from the same or different functional domains of BPI and subsequences thereof. Specifically disclosed are combinations containing two or more, for example, from two to about 10 functional domain peptides or subsequences thereof, and, preferably, combinations of two or three functional domain peptides (for example, homodimers, homotrimers, heterodimers and heterotrimers). Each of the component peptides comprising such combinations may have an amino acid sequence from any particular BPI functional domain amino acid sequence or subsequence thereof.

For purposes of this invention, the term "a biological activity of BPI" is intended to include, but is not limited to the biological activities of a human bactericidal/permeability-increasing protein (BPI), including, for example, a recombinant BPI holoprotein such as rBPI (SEQ ID NO:69), an amino-terminal fragment of BPI such as $rBPI_{23}$, and mutated amino-terminal fragments of BPI such as $rBPI_{21}\Delta cys$ (designated rBPI (1-193) ala^{132} in WO94/18323 (PCT/US94/01235). As disclosed in WO94/20128, rBPI has been produced having the sequence set out as SEQ ID NO:69 as shown in Gray et al. (supra) except that valine at position 151 is specified by GTG rather than GTC, and residue 185 is glutamic acid (specified by GAG) rather than lysine (specified by AAG). In addition, $rBPI_{23}$ (see also, Gazzano-Santoro et al., 1992, Infect., Immun. <u>60</u>: 4754-4761) has been produced using an expression vector containing the 31-residue signal sequence and the first 199 amino acids of the sequence of rBPI with the exceptions from the Gray et al. (supra) sequence as noted above. Such biological activities include LPS binding, LPS neutralization, heparin binding and heparin neutralization, and bactericidal activity. Specifically included is a biological activity of any peptide of this invention that is between 0.1 and 10 times the activity of BPI or of a corresponding peptide encompassing a corresponding functional domain of BPI. Also expressly included in this definition of the "biological activity . . . .

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of BPI" is a biological activity, for example bactericidal activity, that is qualitatively different than the activity of BPI or the corresponding peptide encompassing the entire corresponding domain of BPI. For example, such qualitative differences include differences in the spectrum of bacteria or other microorganisms against which the peptide is effective, relative to the amino acid sequence of the corresponding functional domain of BPI. This definition thus encompasses peptide activities, such as bactericidal activity against Gram-positive bacteria and fungicidal activity, not previously reported for BPI.

The invention provides peptides each of which has an amino acid sequence that is the amino acid sequence of one of the functional domains of human BPI or a subsequence thereof. Embodiments of such peptides include the following exemplary domain I peptides [single-letter abbreviations for amino acids can be found in G. Zubay, *Biochemistry* (2d. ed.), 1988 (MacMillen Publishing: N.Y.), p.33]:

BPI.1	QQGTAALQKELKRIK	(SEQ ID NO:4);
BPI.4	LQKELKRIKIPDYSDSFKIKHL	(SEQ ID NO:3);
BPI.14	GTAALQKELKRIKIPDYSDSFKIKHLGKGH	(SEQ ID NO:2);
and		
BPI.54	GTAALQKELKRIKIP	(SEQ ID NO:5);

the following exemplary domain II peptides:

BPI.2	IKISGKWKAQKRFLK	(SEQ ID NO:7);
BPI.3	NVGLKFSISNANIKISGKWKAQKRFLK	(SEQ ID NO:11);
and		
BPI.8	KWKAQKRFLK	(SEQ ID NO:8);

and the following exemplary domain III peptides:

BPI.5	VHVHISKSKVGWLIQLFHKKIE	(SEQ ID NO:67);
BPI.11	KSKVWLIQLFHKK	(SEQ ID NO:13);
BPI.12	SVHVHISKSKVGWLIQLFHKKIESALRNK	(SEQ ID NO:14);
BPI.13	KSKVGWLIQLFHKK	(SEQ ID NO:15);
and		
BPI.55	GWLIQLFHKKIESALRNKMNS	(SEQ ID NO:61).

It will be recognized that BPI.14, BPI.12 and BPI.55 are examples of addition variants.

The invention also provides linear and branched-chain combinations of the same or different peptides, wherein each of the peptides of the combination has an amino acid sequence that is the amino acid sequence of one of the functional domains of human BPI or a subsequence thereof. Embodiments of such peptides include the following exemplary combination domain II peptides:

BPI.9	KRFLKKWKAQKRFLK	(SEQ ID NO:51);
BPI.7	KWKAQKRFLKKWKAQKRFLK	(SEQ ID NO:54);
BPI.10.1	KRFLKKWKAQKRFLKKWKAQKRFLK	(SEQ ID NO:55);

and

BPI.10.2	QKRFLKKWKAQKRFLKKWKAQKRFLK	(SEQ ID NO:65);

and the following exemplary branched-chain domain II peptide:

MAP.1 (β-alanyl-*N*α,*N*ε-substituted-[*N*α,*N*ε(BPI.2)lysyl]lysine);

and the following exemplary combination domain III peptide:

BPI.29	KSKVGWLIQLFHKKKSKVGWLIQLFHKK	(SEQ ID NO:56);

and the following exemplary branched-chain domain III peptide:

MAP.2 (β-alanyl-*N*α,*N*ε-substituted-[*N*α,*N*ε(BPI.13)lysyl]lysine);

and the following exemplary domain II-domain III interdomain combination peptides:

BPI.30	KWKAQKRFLKKSKVGWLIQLFHKK	(SEQ ID NO:52);
BPI.63	IKISGKWKAQKRFLKKSKVGWLIQLFHKK	(SEQ ID NO:53);

and

BPI.74	KSKVGWLIQLFHKKKWKAQKRFLK	(SEQ ID No.:70).

Amino acid substitution variants are also provided, wherein the amino acid residue at one or more positions in each of the peptides is a residue different from the amino acid found in the corresponding position of the BPI functional domain from which that specific peptide is derived. For example, in one embodiment of this aspect of the invention, one position in the peptide is substituted with an alanine residue for the amino acid found at the corresponding position in the BPI amino acid sequence. In other embodiments, one position in the peptide is substituted with *e.g.*, a phenylalanine, leucine, lysine or tryptophan residue for the amino acid found at the corresponding position in the BPI amino acid sequence. Embodiments of these peptides include the following exemplary substitution domain II peptides:

BPI.15	AKISGKWKAQKRFLK	(SEQ ID NO:16);
BPI.16	IAISGKWKAQKRFLK	(SEQ ID NO:17);
BPI.17	IKASGKWKAQKRFLK	(SEQ ID NO:18);
BPI.18	IKIAGKWKAQKRFLK	(SEQ ID NO:19);
BPI.19	IKISAKWKAQKRFLK	(SEQ ID NO:20);
BPI.20	IKISGAWKAQKRFLK	(SEQ ID NO:21);
BPI.21	IKISGKAKAQKRFLK	(SEQ ID NO:22);
BPI.22	IKISGKWAAQKRFLK	(SEQ ID NO:23);
BPI.23	IKISGKWKAAKRFLK	(SEQ ID NO:24);
BPI.24	IKISGKWKAQARFLK	(SEQ ID NO:25);
BPI.25	IKISGKWKAQKAFLK	(SEQ ID NO:26);
BPI.26	IKISGKWKAQKRALK	(SEQ ID NO:27);
BPI.27	IKISGKWKAQKRFAK	(SEQ ID NO:28);
BPI.28	IKISGKWKAQKRFLA	(SEQ ID NO:29);
BPI.61	IKISGKFKAQKRFLK	(SEQ ID NO:48);
BPI.73	IKISGKWKAQFRFLK	(SEQ ID NO:62);
BPI.77	IKISGKWKAQWRFLK	(SEQ ID NO:72);
BPI.79	IKISGKWKAKKRFLK	(SEQ ID NO:73);
and		
BPI.81	IKISGKWKAFKRFLK	(SEQ ID NO:75);

and the following exemplary substitution domain III peptides:

BPI.31	ASKVGWLIQLFHKK	(SEQ ID NO:33);
BPI.32	KAKVGWLIQLFHKK	(SEQ ID NO:34);
BPI.33	KSAVGWLIQLFHKK	(SEQ ID NO:35);
BPI.34	KSKAGWLIQLFHKK	(SEQ ID NO:36);
BPI.35	KSKVAWLIQLFHKK	(SEQ ID NO:37);
BPI.36	KSKVGALIQLFHKK	(SEQ ID NO:38);
BPI.37	KSKVGWAIQLFHKK	(SEQ ID NO:39);
BPI.38	KSKVGWLAQLFHKK	(SEQ ID NO:40);
BPI.39	KSKVGWLIALFHKK	(SEQ ID NO:41);
BPI.40	KSKVGWLIQAFHKK	(SEQ ID NO:42);
BPI.41	KSKVGWLIQLAHKK	(SEQ ID NO:43);
BPI.42	KSKVGWLIQLFAKK	(SEQ ID NO:44);
BPI.43	KSKVGWLIQLFHAK	(SEQ ID NO:45);
BPI.44	KSKVGWLIQLFHKA	(SEQ ID NO:46);
BPI.82	KSKVGWLIQLWHKK	(SEQ ID NO:76);
BPI.85	KSKVLWLIQLFHKK	(SEQ ID NO:79);

BPI.86	KSKVGWLILLFHKK	(SEQ ID NO:80);
BPI.87	KSKVGWLIQLFLKK	(SEQ ID NO:81);
BPI.91	KSKVGWLIFLFHKK	(SEQ ID NO:86);
BPI.92	KSKVGWLIKLFHKK	(SEQ ID NO:87);
BPI.94	KSKVGWLIQLFFKK	(SEQ ID NO:89);
BPI.95	KSKVFWLIQLFHKK	(SEQ ID NO:90);
BPI.96	KSKVGWLIQLFHKF	(SEQ ID NO:91);
and		
BPI.97	KSKVKWLIQLFHKK	(SEQ ID NO:92).

A particular utility of such single amino acid-substituted BPI functional domain peptides provided by the invention is to identify critical residues in the peptide sequence, whereby substitution of the residue at a particular position in the amino acid sequence has a detectable effect on at least one of the biological activities of the peptide. Expressly encompassed within the scope of this invention are embodiments of the peptides of the invention having substitutions at such critical residues so identified using any amino acid, whether naturally-occurring or atypical, wherein the resulting substituted peptide has biological activity as defined herein.

Substituted peptides are also provided that are multiple substitutions, *i.e.*, where two or more different amino acid residues in the functional domain amino acid sequence are each substituted with another amino acid. For example, in embodiments of such doubly-substituted peptides, both positions in the peptide are substituted *e.g.*, with alanine, phenylalanine or lysine residues for the amino acid found at the corresponding positions in the BPI amino acid sequence. Examples of embodiments of these peptides include the multiply substituted domain II peptides:

BPI.45	IKISGKWKAAARFLK	(SEQ ID NO:31);
BPI.56	IKISGKWKAKQRFLK	(SEQ ID NO:47);
BPI.59	IKISGAWAAQKRFLK	(SEQ ID NO:30);
BPI.60	IAISGKWKAQKRFLA	(SEQ ID NO:32);
and		
BPI.88	IKISGKWKAFFRFLK	(SEQ ID NO:82);

and the exemplary multiply substituted domain III peptide:

BPI.100	KSKVKWLIKLFHKK	(SEQ ID NO:94);

and the following exemplary multiply substituted domain II substitution combination peptide:

BPI.101 KSKVKWLIKLFFKFKSKVKWLIKLFFKF (SEQ ID NO:95);

and the following exemplary multiply substituted domain II-domain III interdomain substitution combination peptide:

BPI.102 KWKAQFRFLKKSKVGWLILLFHKK (SEQ ID NO:96).

Another aspect of such amino acid substitution variants are those where the substituted amino acid residue is an atypical amino acid. Specifically encompassed in this aspect of the peptides of the invention are peptides containing D-amino acids, modified or non-naturally-occurring amino acids, and altered amino acids to provide peptides with increased stability, potency or bioavailability. Embodiments of these peptides include the following exemplary domain II peptides with atypical amino acids:

BPI.66	IKISGKW_DKAQKRFLK	(SEQ ID NO:49);
BPI.67	IKISGK$A_{\beta\text{-(1-naphthyl)}}$KAQKRFLK	(SEQ ID NO:50);
BPI.70	IKISGK$A_{\beta\text{-(3-pyridyl)}}$KAQKRFLK	(SEQ ID NO:63);
BPI.71	A_DA_DIKISGKWKAQKRFLK	(SEQ ID NO:66);
BPI.72	IKISGKWKAQKR$A_{\beta\text{-(3-pyridyl)}}$LK	(SEQ ID NO:64);
BPI.76	IKISGKWKAQF_DRFLK	(SEQ ID NO:71);
BPI.80	IKISGKWKAQ$A_{\beta\text{-(1-naphthyl)}}$RFLK	(SEQ ID NO:74);
BPI.84	IKISGK$A_{\beta\text{-(1-naphthyl)}}$KAQFRFLK	(SEQ ID NO:78);
BPI.89	IKISGK$A_{\beta\text{-(1-naphthyl)}}$KAFKRFLK	(SEQ ID NO:84);
and		
BPI.90	IKISGK$A_{\beta\text{-(1-naphthyl)}}$KAFFRFLK	(SEQ ID NO:85);

the exemplary domain III peptide with atypical amino acids:

BPI.83 KSKVG$A_{\beta\text{-(1-naphthyl)}}$LIQLFHKK (SEQ ID NO:77);

and the exemplary domain II-domain III interdomain combination peptides with atypical amino acids:

BPI.93 IKISGK$A_{\beta\text{-(1-naphthyl)}}$KAQFRFLKKSKVGWLIQLFHKK (SEQ ID NO:88);

and

BPI.98 IKISGK$A_{\beta\text{-(1-naphthyl)}}$KAQFRFLKKSKVGWLIFLFHKK (SEQ ID NO:83).

Linear and branched-chain combination embodiments of the amino acid substitution variant peptides, which create multiple substitutions in multiple domains, are also an aspect of this invention. Embodiments of these peptides include the following exemplary combination/substitution domain II peptides:

BPI.46	KWKAAARFLKKWKAQRFLK	(SEQ ID NO:57);
BPI.47	KWKAQKRFLKKWKAAARFLK	(SEQ ID NO:58);
BPI.48	KWKAAARFLKKWAAAKRFLK	(SEQ ID NO:59);
BPI.69	KWKAAARFLKKWKAAARFLKKWKAAARFLK	(SEQ ID NO:60);
and		
BPI.99	KWKAQWRFLKKWKAQWRFLKKWKAQWRFLK	(SEQ ID NO:93).

Dimerized and cyclized embodiments of each of the aforementioned BPI functional domain peptides are also provided by this invention. Embodiments of these peptides include the following exemplary cysteine-modified domain II peptides:

BPI.58	CIKISGKWKAQKRFLK	(SEQ ID NO: 9);
BPI.65(red)	CIKISGKWKAQKRFLKC	(SEQ ID NO:68);
and	S - S	
BPI.65(ox.)	CIKISGKWKAQKRFLKC	(SEQ ID NO:10).

BPI functional domain peptides described herein are useful as potent anti-bacterial agents for Gram-negative bacteria and for neutralizing the adverse effects of LPS associated with the cell membranes of Gram-negative bacteria. The peptides of the invention have, in varying amounts, additional activities of BPI, including activities not directly associated with the Gram-negative bacterial infection, such as heparin binding and neutralization. Peptides provided by this invention also may have biological activities distinct from the known biological activities of BPI. For example, some embodiments of the peptides of the invention surprisingly have been found to have a biological target range for bactericidal activity that is broader than BPI and exhibits bactericidal activity against Gram-positive as well as Gram-negative bacteria. Some embodiments of the invention have surprisingly been found to have fungicidal activity. Thus, the invention advantageously provides peptides having amino acid sequences of the biologically functional domains of BPI having distinct antimicrobial activities. Peptides of this invention that possess the dual antibacterial and anti-endotoxic properties of BPI, including those with an increased antibiotic spectrum, represent a new class of antibiotic molecules.

BPI functional domain peptides of the invention will have biological therapeutic utilities heretofor recognized for BPI protein products. For example, WO95/19784 (PCT/US95/01151), addresses use of BPI protein products in the treatment of humans exposed to Gram-negative bacterial endotoxin in circulation. WO94/20129 (PCT/US94/02463) addresses administration of BPI protein products for treatment of mycobacterial diseases. WO95/10297 (PCT/US94/11404) addresses use of BPI protein products in the treatment of conditions involving depressed reticuloendothelial system function. WO95/08344 (PCT/US94/11225), addresses synergistic combinations of BPI protein products and antibiotics. WO95/02414 (PCT/US94/07834), addresses methods of potentiating BPI protein product bactericidal activity by administration of LBP protein products. The disclosures of the above applications, thus, exemplify therapeutic uses for BPI functional domain peptides of the invention. The BPI functional domain peptides of the invention also have therapeutic utility for the treatment of pathological conditions and disease states, as disclosed in WO94/20128.

BPI functional domain peptides of the invention are thus useful in methods for: neutralizing the anti-coagulant effect of heparin; inhibiting angiogenesis (especially angiogenesis associated with ocular retinopathy); inhibiting endothelial cell proliferation (especially endometriosis and proliferation associated with implantation of fertilized ova); inhibiting malignant tumor cell proliferation (especially Kaposi's sarcoma proliferation); treating chronic inflammatory disease states (such as arthritis and especially reactive and rheumatoid arthritis); treating Gram-negative bacterial infection and the sequelae thereof; treating the adverse effects (such as increased cytokine production) of Gram-negative endotoxin in blood circulation; killing Gram-negative bacteria; treating adverse physiological effects associated with depressed reticuloendothelial system function (especially involving depressed function of Kupffer cells of the liver such as results from physical, chemical and biological insult to the liver); treating, in synergistic combination with antibiotics (such as gentamicin, polymyxin B and cefamandole nafate) Gram-negative bacterial infection and the sequelae thereof; killing Gram-negative bacteria in synergistic combination with antibiotics; treating, in combination with LBP protein products, Gram-negative bacterial infection and the sequelae thereof; killing Gram-negative bacteria in combination with LBP protein products; treating, alone or in combination with antibiotics and/or bismuth, *Mycobacteria* infection (especially infection by *M. tuberculosis*, *M. leprae* and *M. avium*); treating adverse physiological effects (such as increased cytokine production) of lipoarabinomannan in blood circulation; decontaminating fluids (such as blood, plasma, serum and bone marrow) containing lipoarabinomannan; and, treating disease states (such as gastritis and peptic, gastric and duodenal ulcers) associated with infection by bacteria of the genus *Helicobacter*. The present invention also provides pharmaceutical compositions for oral, parenteral, topical and aerosol administration comprising BPI functional domain peptides in amounts effective for the uses noted above and especially compositions additionally comprising pharmaceutically acceptable diluents, adjuvants or carriers.

With respect to uses of BPI functional domain peptides in combination with LBP protein products, as used herein, "LBP protein product" includes naturally and recombinantly product lipopolysaccharide binding protein; natural, synthetic, and recombinant biologically active polypeptide fragments and derivatives of lipopolysaccharide binding protein; and biologically active polypeptide analogs, including hybrid fusion proteins, of either LBP or biologically active fragments thereof. LBP protein products that are useful for the purposes of the present invention include LBP holoprotein which can be produced by expression of recombinant genes in transformed eucaryotic host cells such as described in WO95/00641 (PCT/US94/06931) and designated rLBP. Also described in that application are preferred LBP protein derivatives which lack CD14-mediated inflammatory properties and particularly the ability to mediate LPS activity through the CD14 receptor. Such LBP protein products are preferred for the purposes of the present invention because excessive CD14-mediated immunostimulation is generally considered undesirable, and is particularly so in subjects suffering from infection.

Preferred LBP protein derivatives are characterized as amino-terminal fragments having a molecular weight of about 25kD. Most preferred are LBP amino-terminal fragments characterized by the amino acid sequence of the first 197 amino acids of the amino-terminus of LBP, as set out in SEQ ID NOS:97 and 98, designated $rLBP_{25}$, the production of which is described in WO95/00641 (PCT/US94/06931). It is contemplated that LBP protein derivatives considerably smaller than 25 kD and comprising substantially fewer than the first 197 amino acids of the amino-terminus of the holo-LBP molecule are suitable for the purposes of the invention provided they retain the ability to bind to LPS. Moreover, it is contemplated that LBP protein derivatives comprising greater than the first 197 amino acid residues of the holo-LBP molecule including amino acids on the carboxy-terminal side of first 197 amino acids of the rLBP as disclosed in SEQ ID NOS: 97 and 98 will likewise prove useful for the purposes of the invention, provided they lack an element that promotes CD14-mediated immunostimulatory activity. It is further contemplated that those of skill in the art are capable of making additions, deletions and substitutions of the amino acid residues of SEQ ID NOS: 97 and 98 without loss of the

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desired biological activities of the molecules. Still further, LBP protein products may be obtained by deletion, substitution, addition or mutation, including mutation by site-directed mutagenesis of the DNA sequence encoding the LBP holoprotein, wherein the LBP protein product maintains LPS-binding activity and lacks CD14-mediated immunostimulatory activity. Specifically contemplated are LBP hybrid molecules and dimeric forms which may result in improved affinity of LBP for bacteria and/or increased stability *in vivo*. These include LBP/BPI hybrid proteins and LBP-Ig fusion proteins. Such hybrid proteins further include those using human gamma 1 or gamma 3 hinge regions to permit dimer formation. Other forms of dimer contemplated to have enhanced serum stability and binding affinity include fusions with Fc lacking the CH_2 domain, or hybrids using leucine or helix bundles.

BPI functional domain peptides of the invention may be generated and/or isolated by any means known in the art, including by means of recombinant production; for example U.S. Patent No. 5,028,530, and U.S. Patent No. 5,206,154 disclose novel methods for the recombinant production of polypeptides, including antimicrobial peptides. Additional procedures for recombinant production of antimicrobial peptides in bacteria have been described by Piers et al., 1993, Gene 134: 7-13. WO93/23540 (PCT/US93/04752) discloses novel methods for the purification of recombinant BPI expressed in and secreted from genetically transformed mammalian host cells in culture and discloses how one may produce large quantities of recombinant BPI suitable for incorporation into stable, homogeneous pharmaceutical preparations.

BPI functional domain peptides may also be advantageously produced using any such methods. Those of ordinary skill in the art are able to isolate or chemically synthesize a nucleic acid encoding each of the peptides of the invention. Such nucleic acids are advantageously utilized as components of recombinant expression constructs, wherein the nucleic acids are operably linked with transcriptional and/or translational control

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elements, whereby such recombinant expression constructs are capable of expressing the peptides of the invention in cultures of prokaryotic, or preferably eukaryotic cells, most preferably mammalian cells, transformed with such recombinant expression constructs.

Peptides of the invention may advantageously synthesized by any of the chemical synthesis techniques known in the art, particularly solid-phase synthesis techniques, for example, using commercially-available automated peptide synthesizers. Such peptides may also be provided in the form of combination peptides, wherein the peptides comprising the combination are linked in a linear fashion one to another and wherein a BPI sequence is present repeatedly in the peptide, with or without separation by "spacer" amino acids allowing for selected conformational presentation. Also provided are branched-chain combinations, wherein the component peptides are covalently linked via functionalities in amino acid sidechains of the amino acids comprising the peptides.

Functional domain peptides of this invention can be provided as recombinant hybrid fusion proteins comprising BPI functional domain peptides and at least a portion of at least one other polypeptide. Such proteins are described, for example, in WO93/23434 (PCT/US93/04754).

Generally, those skilled in the art will recognize that peptides as described herein may be modified by a variety of chemical techniques to produce compounds having essentially the same activity as the unmodified peptide, and optionally having other desirable properties. For example, carboxylic acid groups of the peptide, whether carboxyl-terminal or sidechain, may be provided in the form of a salt of a pharmaceutically-acceptable cation or esterified to form a C_1-C_{16} ester, or converted to an amide of formula NR_1R_2 wherein R_1 and R_2 are each independently H or C_1-C_{16} alkyl, or combined to form a heterocyclic ring, such as 5- or 6-membered. Amino groups of the peptide, whether amino-terminal or sidechain, may be in the form of a pharmaceutically-acceptable acid addition salt, such as the HCl, HBr, acetic, benzoic, toluene sulfonic, maleic, tartaric and other organic salts, or may be modified to C_1-C_{16} alkyl or dialkyl amino or further converted to an amide. Hydroxyl groups of the peptide sidechain may be converted to C_1-C_{16} alkoxy or to a C_1-C_{16} ester using well-recognized techniques. Phenyl and phenolic rings of the peptide sidechain may be substituted with one or more halogen atoms, such as fluorine, chlorine, bromine or iodine, or with C_1-C_{16} alkyl, C_1-C_{16} alkoxy, carboxylic acids and esters thereof, or amides of such carboxylic acids. Methylene groups of the peptide sidechains can be extended to homologous C_2-C_4 alkylenes. Thiols can be protected with any one of a number of well-recognized protecting groups, such as acetamide groups. Those skilled in the art will also recognize methods for introducing cyclic structures into the peptides of this invention to select and provide conformational constraints to the structure that result in enhanced binding and/or stability. For example, a carboxyl-terminal or amino-terminal cysteine residue can be added to the peptide, so that when oxidized the peptide will contain a disulfide bond, thereby generating a cyclic peptide. Other peptide cyclizing methods include the formation of thioethers and carboxyl- and amino-terminal amides and esters.

Peptidomimetic and organomimetic embodiments are also hereby explicitly declared to be within the scope of the present invention, whereby the three-dimensional arrangement of the chemical constituents of such peptido- and organomimetics mimic the three-dimensional arrangement of the peptide backbone and component amino acid sidechains in the peptide, resulting in such peptido- and organomimetics of the peptides of this invention having substantial biological activity. It is implied that a pharmacophore exists for each of the described activities of BPI. A pharmacophore is an idealized, three-dimensional definition of the structural requirements for biological activity. Peptide- and organomimetics can be designed to fit each pharmacophore with current computer modelling software (computer aided drug design). The degree of overlap between the specific activities of pharmacophores remains to be determined.

The administration of BPI functional domain peptides is preferably accomplished with a pharmaceutical composition comprising a BPI functional domain peptide and a pharmaceutically acceptable diluent, adjuvant, or carrier. The BPI functional domain peptide composition may be administered without or in conjunction with known antibiotics, surfactants, or other chemotherapeutic agents. Examples of such combinations are described in WO94/17819 (PCT/US94/01239).

Effective doses of BPI functional domain peptides for bactericidal activity, partial or complete neutralization of the anti-coagulant activity of heparin, partial or complete neutralization of LPS and other effects described herein may be readily determined by those of skill in the art according to conventional parameters, each associated with the corresponding biological activity, including, for example, the size of the subject, the extent and nature of the bacterial infection, the extent and nature of the endotoxic shock, and the quantity of heparin administered to the subject and the time since administration of the heparin. Similar determinations will be made by those of skill in this art for using the peptide embodiments of this invention for therapeutic uses envisioned and described herein.

Embodiments of the invention comprising medicaments can be prepared for oral administration, for injection, or other parenteral methods and preferably include conventional pharmaceutically acceptable carriers, adjuvents and counterions as would be known to those of skill in the art. The medicaments are preferably in the form of a unit dose in solid, semi-solid and ....

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liquid dosage forms such as tablets, pills, powders, liquid solutions or suspensions, and injectable and infusible solutions. Effective dosage ranges from about 100 μg/kg to about ~~10~~ 100 mg/kg of body weight are contemplated.

The Examples which follow are illustrative of specific embodiments of the invention, and various uses thereof. Example 1 describes the preparation of proteolytic fragments of BPI; Example 2 describes the results of bactericidal assays of the proteolytic fragments of Example 1; Example 3 describes the results of heparin binding assays using the proteolytic fragments of Example 1; Example 4 describes the results of experiments using *Limulus* amebocyte lysates to assay the LPS binding activity of the proteolytic fragments of Example 1; Example 5 describes the preparation of 15-mer peptides of BPI; Example 6 describes the results of heparin binding assays using the 15-mer peptides of Example 5; Example 7 describes the results of *Limulus* amebocyte lysates assays using the 15-mer peptides of Example 5; Example 8 describes the results of bactericidal assays of the 15-mer peptides of Example 5; Example 9 describes the preparation of BPI individual functional domain peptides; Example 10 describes the results of heparin binding assays using the BPI individual functional domain peptides of Example 9; Example 11 describes the results of heparin neutralization assays using the BPI individual functional domain peptides of Example 9; Example 12 describes the results of *Limulus* amebocyte lysates assays of LPS neutralization activity using the BPI individual functional domain peptides of Example 9; Example 13 describes the results of bactericidal assays of the BPI individual functional domain peptides of Example 9; Example 14 describes the preparation of BPI combination functional domain peptides; Example 15 describes the results of bactericidal activity assays of the BPI combination functional domain peptides of Example 14; Example 16 describes the results of additional bactericidal activity assays of the BPI combination functional domain peptides of Example 14; Example 17 describes the results of *in vivo* and *in vitro* heparin neutralization assays using the BPI combination functional domain peptides of Example 14; Example 18 describes the preparation and functional activity analysis of bactericidal activity, heparin binding activity and LPS neutralization activity assays of BPI substitution variant functional domain peptides; Example 19 provides a summary of the results of bactericidal and heparin binding assays using representative BPI functional domain peptides; Example 20 describes analysis of BPI functional domain peptides in a variety of binding and neutralization assays; Example 21 addresses a heparin neutralization assay; Example 22 describes administration of BPI functional domain peptides in model systems of collagen and bacteria-induced arthritis animal model systems exemplifying treatment of chronic inflammatory disease states; Example 23 illustrates testing of BPI functional domain peptides for angiostatic effects in a mouse malignant melanoma metastasis model system; Example 24 addresses effects of BPI functional domain peptides on endothelial cell proliferation; Example 25 describes analysis of BPI functional domain peptides in animal model systems; and Example 26 describes a protocol for testing the anti-endotoxin effects of BPI functional domain peptides of the invention *in vivo* in humans.

EXAMPLE 1

Preparation of BPI Proteolytic Fragments

Chemical cleavage and enzymatic digestion processes were applied to $rBPI_{23}$ to produce variously-sized proteolytic fragments of the recombinant BPI protein.

$rBPI_{23}$ protein was reduced and alkylated prior to proteolysis by cyanogen bromide (CNBr) or endoproteinase Asp-N. The protein was desalted by overnight precipitation upon the addition of cold (4°C) acetone (1:1 v/v) and the precipitated protein recovered by pelleting under centrifugation (5000 x *g*) for 10 minutes. The $rBPI_{23}$ protein pellet was washed twice with cold acetone and dried under a stream of nitrogen. An $rBPI_{23}$ solution was then reconstituted to a final concentration of 1 mg protein/mL in 8M urea/0.1M Tris-HCl (pH 8.1) and reduced by addition of 3.4 mM dithiothreitol (Calbiochem, San Diego, CA) for 90 minutes at 37°C. Alkylation was performed by the addition of iodoacetamide (Sigma Chemical Co., St. Louis, MO) to a final concentration of 5.3 millimolar and incubation for 30 minutes in the dark at room temperature. The reduced and alkylated protein was acetone-precipitated, centrifuged and washed as described above and the pellet was redissolved as described below for either CNBr or Asp-N digestion.

For CNBr-catalyzed protein fragmentation, the washed pellet was first dissolved in 70% trifluoroacetic acid (TFA) (Protein Sequencing Grade, Sigma Chemical Co., St. Louis, MO) to a final protein concentration of 5 mg/mL. Cyanogen bromide (Baker Analyzed Reagent, VWR Scientific, San Francisco, CA) dissolved in 70% TFA was added to give a final ratio of 2:1 CNBr to protein (w/w). This ratio resulted in an approximately 75-fold molar excess of CNBr relative to the number of methionine residues in the $rBPI_{23}$ protein. The reaction was purged with nitrogen and allowed to proceed for 24 hours in the dark at room temperature. The reaction was terminated by adding 9 volumes of distilled water, and followed by freezing (-70°C) and lyophilization.

For endoproteinase digestion, the reduced and alkylated $rBPI_{23}$ was solubilized at a concentration of 5.0 mg/mL in 8M urea/0.1M Tris-HCl (pH

8.1). An equal volume of 0.1M Tris-HCl (pH 8.1) was then added so that the final conditions were 2.5 mg/mL protein in 5M urea/0.1M Tris-HCl (pH 8.1). Endoproteinase Asp-N from *Pseudomonas fragi* (Boehringer-Mannheim, Indianapolis, IN) was added at a 1:1000 (w/w, enzyme:substrate) ratio, and digestion was allowed to proceed for 6 hours at 37°C. The reaction was terminated by addition of TFA to a final concentration of 0.1% and the samples were then fractionated by reverse phase HPLC.

The CNBr and Asp-N fragment mixtures were purified on a Zorbax™ Protein Plus C_3 column (4.6 x 250 mm, 300 Å pore size, MACMOD Analytical Inc, Chadsford, PA). A gradient ranging from 5% acetonitrile in 0.1% TFA to 80% acetonitrile in 0.1 % TFA was run over this column over a 2 hour elution period at a flow rate of 1.0 mL/min. Fragment elution was monitored at 220 nm using a Beckman™ System Gold HPLC (Beckman Scientific Instruments, San Ramon, CA). The column heating compartment was maintained at 35°C and the fractions were collected manually, frozen at -70°C and dried in a Speed Vac™ concentrator. Fragments were then solubilized in a solution of 20 mM sodium acetate (pH 4.0)/0.5 M NaCl prior to use.

Electrospray ionization mass spectrometry (ESI-MS) was performed on a VG Bio-Q™ mass spectrometer by Dr. Francis Bitsch and Mr. John Kim in the laboratory of Dr. Cedric Shackleton, Children's Hospital-Oakland Research Institute. Molecular masses were obtained by mathematical transformation of the data.

Although the DNA sequence for $rBPI_{23}$ encodes amino acid residues 1-199 of the mature protein, a significant portion of the protein that is produced is truncated at Leu-193 and Val-195, as determined by ESI-MS. The existence of these carboxyl-terminal truncations were verified by isolating the carboxyl-terminal tryptic peptides, which were sequenced and analyzed by ESI-MS.

There are six methionine residues in the $rBPI_{23}$ protein, at positions 56, 70, 100, 111, 170, and 196, and chemical cleavage by cyanogen bromide produced six major peptide fragments as predicted. The results of the CNBr cleavage experiments are summarized in Table I. The fragments were isolated by reverse phase (C_3) HPLC (Figure 1a) and their amino-terminal sequences were determined by Edman degradation. The two largest fragments (C1 and C5) were not resolved by the C_3 HPLC column and further attempts to resolve them by ion exchange chromatography were unsuccessful, presumably because they are similar in length and isoelectric point. The identities of the C1, C5 fragments within the mixture were determined by ESI-MS. The predicted mass of C1 is 6269 (Table I), taking into account the loss of 30 a.m.u. resulting from the conversion of the carboxyl-terminal methionine to homoserine during the CNBr cleavage reaction. The observed mass of 6251.51 ± 0.34 is consistent with the loss of a water molecule (18 a.m.u.) in a homoserine lactone intermediate, which may be favored over the formation of the homoserine because of the hydrophobicity of the C1 fragment C-terminal amino acids. The predicted mass of the C5 fragment is 6487 and the observed mass is 6385.84 ± 0.39 (Table I). For the C5 fragment, the C-terminal amino acids are hydrophilic, so the hydrolysis of the homoserine lactone intermediate is probably favored. From both the amino-terminal sequencing and the mass spectrum data, the C5 component represents approximately 10-25% of the material in the C1/C5 mixture.

Proteolytic cleavage with endoproteinase Asp-N was performed to provide additional fragments for the regions contained within the CNBr C1/C5 mixture. There are six aspartic acid residues within the $rBPI_{23}$ sequence at positions 15, 36, 39, 57, 105, and 116. The six major Asp-N fragments isolated by C_3 HPLC (Figure 1b) were sequenced and masses were determined by ESI-MS (Table I). A short duration digest at a 1:1000 (w/w, enzyme:substrate) ratio was used to eliminate potential non-specific cleavages, particularly at glutamic acid residues. It is evident that this digestion did not continue until completion, as one fragment (1-38) was isolated where Asp residues (amino acids 15 and 35) were not cleaved. The mass spectra of the Asp-N fragments were consistent with the predicted masses for each individual fragment. Unlike the CNBr cleavage, where the carboxyl-terminal fragment was poorly resolved, the Asp-N fragment from amino acid 116 to the carboxyl-terminus was well resolved from all of the other Asp-N fragments.

TABLE I
Summary of rBPI$_{23}$ Cleavage Fragment Analysis

CNBr Cleavage Fragments

PEAK	SEQUENCE	I.D.	MASS measured	MASS predicted
I	101-110	C4(101-111)	N.D.	1169
II	57-67	C2(57-70)	N.D.	1651
III	71-99	C3(71-100)	N.D.	3404
IV	171-194	C6(171-196)	N.D.	2929
V	1-25, 112-124	C1(1-56), C5(112-170)	6251 6486	6269 6487

Asp-N Proteolytic Fragments

PEAK	SEQUENCE	I.D.	MASS measured	MASS predicted
A	1-14	A1(1-14)	1465.5	1464
I	39-56	A3(39-56)	2145.2	2145
II	15-38	A2(15-38)	2723.6	2724
III	57-76	A4(57-104)	5442.5	5442
IV	1-38	A1 A2(1-38)	4171.4	4172
VI	116-134	A6a(116-193)	8800.3	8800
VII	116-128	A6b(116-195)	8997.1	8996

EXAMPLE 2

Bactericidal Effects of BPI Proteolytic Fragments

BPI proteolytic fragments produced according to Example 1 were screened for bactericidal effects using rough mutant *E. coli* J5 bacteria in a radial diffusion assay. Specifically, an overnight culture of *E. coli* J5 was diluted 1:50 into fresh tryptic soy broth and incubated for 3 hours at 37°C to attain log phase growth of the culture. Bacteria were then pelleted at 3,000 rpm for 5 minutes in a Sorvall™ RT6000B centrifuge (Sorvall Instruments, Newton, CT). 5 mL of 10 mM sodium phosphate buffer (pH 7.4) was added and the preparation was re-pelleted. The supernatant was decanted and 5 mL of fresh buffer was added, the bacteria were resuspended and their concentration was determined by measurement of absorbance at 590 nm (an Absorbance value of 1.00 at this wavelength equals a concentration of 1.25 x 10^9 CFU/mL in suspension). The bacteria were diluted to 4 x 10^6 CFU/mL in 10 mL of molten underlayer agarose (at approximately 45°C) and inverted repeatedly to mix in 15 mL polypropylene tubes conventionally used for this purpose.

The entire contents of such tubes were then poured into a level square petri dish and distributed evenly by rocking the dish side-to-side. The agarose hardened in less than 30 seconds and had a uniform thickness of about 1 mm. A series of wells were then punched into the hardened agarose using a sterile 3 mm punch attached to a vacuum apparatus. The punch was sterilized with 100% alcohol and allowed to air dry prior to use to avoid contaminating the bacterial culture.

5 or 10 μL of each of the BPI fragments were carefully pipetted into each well. As a negative control, dilution buffer (pH 8.3) was added to a separate well, and $rBPI_{23}$ at concentrations of 5 μg/mL and 1 μg/mL were also added as positive controls. Each plate was incubated at 37°C for 3 hours, and then 10 mL of molten overlayer agarose (at approximately 45°C) was added into the level petri dish, allowed to harden and incubated overnight at 37°C. The next day, a clear zone was seen against the lawn of bacteria in those wells having bactericidal activity. In order to visually enhance this zone, a dilute Coomassie solution (consisting of 0.002% Coomassie Brilliant Blue, 27% methanol, 15% formaldehyde (37% stock solution) and water) was poured over the agar and allowed to stain for 24 hours. The bacterial zones were measured with a micrometer.

No bactericidal activity was discerned for the $rBPI_{23}$ fragments generated by CNBr or by Asp-N digestion, when tested at amounts up to 25 pmol/well. In contrast, this assay detected measurable bactericidal activity using $rBPI_{23}$ in amounts as low as 0.75 pmol/well. Reduced and alkylated $rBPI_{23}$, on the other hand, also was not bactericidal at amounts up to 100 pmol/well, while alkylated $rBPI_{23}$ retained bactericidal activity equivalent to $rBPI_{23}$.

EXAMPLE 3

Heparin Binding by BPI Proteolytic Fragments

$rBPI_{23}$ and the BPI proteolytic fragments produced according to Example 1 were evaluated in heparin binding assays according to the methods described in Example 1 in WO94/20128. Briefly, each fragment was added to wells of a 96-well microtiter plate having a polyvinylidene difluoride membrane (Immobilon-P, Millipore, Bedford, MA) disposed at the bottom of the wells. Heparin binding of CNBr fragments was estimated using 100 picomoles of each fragment per well with a saturating concentration of ^{3}H-heparin (20 μg/mL). Positive control wells contained varying amounts of $rBPI_{23}$. The wells were dried and subsequently blocked with a 0.1% bovine serum albumin (BSA) in phosphate buffered saline, pH 7.4 (blocking buffer). Dilutions of ^{3}H-heparin (0.03-20 μCi/ml, avg. M.W. = 15,000; DuPont-NEN, Wilmington, DE) were made in the blocking buffer and incubated in the BPI peptide-containing wells for one hour at 4°C. The unbound heparin was aspirated and the wells were washed three times with blocking buffer, dried and removed for quantitation in a liquid scintillation counter (Model 1217, LKB, Gaithersburg, MD). Although BSA in the blocking buffer did show a low affinity and capacity to bind heparin, this was considered physiologically irrelevant and the background was routinely subtracted from the test compound signal. The specificity of fragment-heparin binding was established by showing that the binding of radiolabeled heparin was completely inhibited by a 100-fold excess of unlabeled heparin (data not shown).

The results, shown in Table II (as the mean values of duplicate wells ± the range between the two values), indicated that the CNBr fragments containing the amino acids 71-100 (C3) and 1-56 and 112-170 (C1,5) bound heparin to a similar extent. The CNBr fragment 171-196 also bound more heparin than the control protein (thaumatin, a protein of similar molecular weight and charge to $rBPI_{23}$).

The Asp-N fragments also demonstrated multiple heparin binding regions in $rBPI_{23}$. As seen in Table II, the 57-104 Asp-N fragment bound the highest amount of heparin, followed by the 1-38 and 116-193 fragments. These data, in combination with the CNBr fragment data, indicate that there are at least three separate heparin binding regions within $rBPI_{23}$, as demonstrated by chemically or enzymatically-generated fragments of $rBPI_{23}$, with the highest heparin binding capacity residing within residues 71-100.

TABLE II

Heparin Binding of $rBPI_{23}$ Fragments

Fragments CNBr Digest	Region	cpm ^{3}H-Heparin bound
C1,C5	1-56,112-170	82,918 ± 4,462
C2	57-70	6,262 ± 182
C3	71-100	81,655 ± 3,163
C4	101-111	4,686 ± 4
C6	171-196	26,204 ± 844
Asp-N Digest		
A1	1-38	17,002 ± 479
A2	15-38	3,042 ± 162
A3	39-56	8,664 ± 128
A4	57-104	33,159 ± 1,095
A6a	116-193	13,419 ± 309
$rBPI_{23}$	1-193	51,222 ± 1,808
Thaumatin		7,432 ± 83
Wash Buffer		6,366 ± 46

EXAMPLE 4

Effect of BPI Proteolytic Fragments on an LAL Assay

BPI proteolytic fragments produced according to Example 1 were subjected to a *Limulus* Amoebocyte Lysate (LAL) inhibition assay to determine LPS binding properties of these fragments. Specifically, each of the fragments were mixed in Eppendorf tubes with a fixed concentration of *E. coli* 0113 LPS (4 ng/mL final concentration) and incubated at 37°C for 3 hours with occasional shaking. Addition controls comprising $rBPI_{23}$ at 0.05 μg/mL were also tested. Following incubation, 360 μL of Dulbecco's phosphate buffered saline (D-PBS; Grand Island Biological Co. (GIBCO), Long Island, NY) were added per tube to obtain an LPS concentration of 200 pg/mL for the LAL assay. Each sample was then transferred into Immulon II strips (Dynatech, Chantilly, VA) in volumes of 50 μl per well.

Limulus amoebocyte Lysate (Quantitative Chromogenic LAL kit, Whitaker Bioproducts, Inc., Walkersville, MD) was added at 50 μL per well and the wells were incubated at room temperature for 25 minutes. Chromogenic substrate was then added at a volume of 100 μL per well and was well mixed. After incubation for 20 to 30 minutes at room temperature, the reaction was stopped with addition of 100 μL of 25% (v/v) acetic acid. Optical density at 405 nm was then measured in a multiplate reader (Model Vmax, Molecular Dynamics, Menlo Park, CA) with the results shown in Figure 2 in terms of percent inhibition of LPS. In this Figure, the filled circle represents $rBPI_{23}$; the open circle represents Asp-N fragment A3; the x represents Asp-N fragment A2; the filled square represents Asp-N fragment A4; the filled triangle represents Asp-N fragment A1A2; the open square represents Asp-N fragment A6a; the small open triangle represents CNBr fragment C3; and the small filled square represents CNBr fragment C1/C5.

The CNBr digest fraction containing amino acid fragments 1-56 and 112-170 inhibited the LPS-induced LAL reaction with an IC_{50} of approximately 100 nM. This IC_{50} is approximately 10-fold higher than the IC_{50} for intact $rBPI_{23}$ (9 nM) in the same assay. The other CNBr digest fragments were found to be non-inhibitory.

A slightly different result was observed with fragments generated from the Asp-N digest, where three fragments were found to be inhibitory in the LAL assay. The fragment corresponding to amino acids 116-193 exhibited LAL inhibitory activity similar to intact $rBPI_{23}$ with complete inhibition of the LPS-induced LAL reaction at 15 nM. The fragments corresponding to amino acids 57-104 and 1-38 also inhibited the LAL assay, but required 10-fold higher amounts. These results, in combination with the CNBr digest results, further supported the conclusion from previously-described experimental results that at least three regions of the rBPI$_{23}$ molecule have the ability to neturalize LBS activation of the LAL reaction, with the most potent region appearing to exist within the 116-193 aminio acid fragment.

Immunoreactivity studies of the proteolytic fragments of rBPI$_{23}$ described in Example 1 were performed using ELISA assays. In such assays, a rabbit polyclonal anti-rBPI$_{23}$ antibody, capable of blocking rBPI$_{23}$ bactericidal and LAL inhibition properties, and two different, non-blocking mouse anti-rBPI$_{23}$ monoclonal antibodies were used to probe the rBPI$_{23}$ proteolytic fragments. The polyclonal antibody was found to be immunoreactive with the 116-193 and 57-104 Asp-N fragments and with the 1-56 and 112-170 CNBr fragments, while the murine monoclonal antibodies reacted only with an Asp-N fragment representing residues 1-14 of rBPI$_{23}$.

EXAMPLE 5

Preparation of 15-mer Peptides of BPI

In order to further assess the domains of biological activity detected in the BPI fragment assays described in Examples 1-4, 15-mer synthetic peptides comprised of 15 amino acids derived from the amino acid sequence of the 23kD amino terminal fragment of BPI were prepared and evaluated for heparin-binding activity, activity in a *Limulus* Amoebocyte Lysate Inhibition (LAL) assay and bactericidal activity. Specifically, a series of 47 synthetic peptides were prepared, in duplicate, each comprising 15 amino acids and synthesized so that each peptide shared overlapping amino acid sequence with the adjacent peptides of the series by 11 amino acids, based on the sequence of rBPI$_{23}$ described in WO94/20128.

Peptides were simultaneously synthesized according to the methods of Maeji et al. (1990, *Immunol. Methods* 134: 23-33) and Gammon et al. (1991, *J. Exp. Med.* 173: 609-617), utilizing the solid-phase technology of Cambridge ....

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Research Biochemicals Ltd. under license of Coselco Mimotopes Pty. Ltd. Briefly, the sequence of $rBPI_{23}$ (1-199) was divided into 47 different 15-mer peptides that progressed along the linear sequence of $rBPI_{23}$ by initiating a subsequent peptide every fifth amino acid. This peptide synthesis technology allows for the simultaneous small scale synthesis of multiple peptides on separate pins in a 96-well plate format. Thus, 94 individual pins were utilized for this synthesis and the remaining two pins (B,B) were subjected to the same steps as the other pins without the addition of activated FMOC-amino acids. Final cleavage of the 15-mer peptides from the solid-phase pin support employed an aqueous basic buffer (sodium carbonate, pH 8.3). The unique linkage to the pin undergoes a quantitative diketopiperazine cyclization under these conditions resulting in a cleaved peptide with a cyclo(lysylprolyl) moiety on the carboxyl-terminus of each peptide. The amino-termini were not acetylated so that the free amino group could potentially contribute to anion binding reactions. An average of about 15 μg of each 15-mer peptide was recovered per well.

EXAMPLE 6

Heparin Binding by 15-mer Peptides of BPI

The BPI 15-mer peptides described in Example 5 were subjected to a heparin binding assay according to the methods described in Example 3.

The results of these experiments are shown in Figure 3, expressed as the total number of cpm bound minus the cpm bound by control wells which received blocking buffer only. These results indicated the existence of three distinct subsets of heparin-binding peptides representing separate heparin-binding functional domains in the $rBPI_{23}$ sequence. In the BPI sequence, the first domain was found to extend from about amino acid 21 to about amino acid 55; the second domain was found to extend from about amino acid 65 to about amino acid 107; and the third domain was found to extend from about amino acid 137 to about amino acid 171. Material from the blank control pins showed no heparin binding effects.

EXAMPLE 7

Effect of 15-mer Peptides of BPI on an *Limulus* Amoebocyte Lysate (LAL) Assay

The 15-mer peptides described in Example 5 were assayed for LPS binding activity using the LAL assay described in Example 4.

The results of these experiments are shown in Figure 4. The data in Figure 4 indicated at least three major subsets of peptides representing three distinct domains of the $rBPI_{23}$ protein having LPS-binding activity resulting in significant LAL inhibition. The first domain was found to extend from about amino acid 17 to about amino acid 55; the second domain was found to extend from about amino acid 73 to about amino acid 99; and the third domain was found to extend from about amino acid 137 to about amino acid 163. In addition, other individual peptides also exhibited LAL inhibition, as shown in the Figure. In contrast, material from blank control pins did not exhibit any LPS neutralizing effects as measured by the LAL assay.

EXAMPLE 8

Bactericidal Effects of 15-mer Peptides of BPI

The 15-mer peptides described in Example 5 were tested for bactericidal effects against the rough mutant strain of *E. coli* bacteria (J5) in a radial diffusion assay as described in Example 2. Products from the blank pins (B, B) were tested as negative controls.

The results of the assay are shown in Figure 5. The only 15-mer peptide found to have bactericidal activity was a peptide corresponding to amino acids 85-99 of the BPI protein. As is seen in Figure 5, the positive control wells having varying amounts of $rBPI_{23}$ also showed bactericidal activity, while the buffer and blank pin controls did not.

The results of these bactericidal assays, along with the heparin binding and LAL assays described in the above Examples, indicate that there exist discrete functional domains in the BPI protein.

The results shown in Examples 1-8 above indicate that $rBPI_{23}$ contains at least three functional domains that contribute to the total biological activity of the molecule. The first domain appears in the sequence of amino acids between about 17 and 45 and is destroyed by Asp-N cleavage at residue 38. This domain is moderately active in both the inhibition of LPS-induced LAL activity and heparin binding assays. The second functional domain appears in the region of amino acids between about 65 and 99 and its inhibition of LPS-induced LAL activity is diminished by CNBr cleavage at residue 70. This domain also exhibits the highest heparin binding capacity and contains the bactericidal peptide, 85-99. The third functional domain, between about amino acids 142 and 169, is active in the inhibition of LPS-induced LAL stimulation assay and exhibits the lowest heparin binding capacity of the three regions.

EXAMPLE 9

Preparation of BPI Individual Functional Domain Peptides

Based on the results of testing the series of overlapping peptides described in Examples 5 through 8, BPI functional domain peptides from each of the functionally-defined domains of the BPI protein were prepared by solid phase peptide synthesis according to the methods of Merrifield, 1963, *J. Am. Chem. Soc.* 85: 2149 and Merrifield *et al.*, 1966, *Anal. Chem.* 38: 1905-1914 using an Applied Biosystems, Inc. Model 432 peptide synthesizer. BPI functional domain peptides were prepared having the amino acid sequences of portions of amino acid residues 1-199 of BPI as set out in Table III below and designated BPI.2 through BPI.5 and BPI.8.

TABLE III

BPI Individual Functional Domain Peptides

Polypeptide No.	Domain	Amino Acid Region	Amino Acid Residues	MW (daltons)
BPI.2	II	85-99	15	1828.16
BPI.3	II	73-99	27	3072.77
BPI.4	I	25-46	22	2696.51
BPI.5	III	142-163	22	2621.52
BPI.8	II	90-99	10	1316.8

EXAMPLE 10

Heparin Binding Activity by BPI Individual Functional Domain Peptides

BPI individual functional domain peptides BPI.2, BPI.3, and BPI.8, along with $rBPI_{21}\Delta cys$ were assayed for heparin binding activity according to the methods described in Example 3. The results are shown in Figure 6 and indicate that BPI.3 and $rBPI_{21}\Delta cys$ had moderate heparin binding activity and BPI.2 and BPI.8 had little or no heparin binding activity.

EXAMPLE 11

Heparin Neutralization Activity of BPI Individual Functional Domain Peptides

BPI functional domain peptides BPI.2, BPI.3, BPI.4, BPI.5, BPI.6, and BPI.8, along with $rBPI_{23}$ as a positive control, were assayed for their effect on thrombin inactivation by ATIII/heparin complexes according to the method of Example 3 in WO94/20128. Specifically, a Chromostrate™ anti-thrombin assay kit (Organon Teknika Corp., Durham, NC) was used to examine the inhibition of purified thrombin by preformed ATIII/heparin complexes in plasma.

Briefly, the assay was performed in 96 well microtiter plates in triplicate with a final volume per well of 200 μL. Varying concentrations of ....

the BPI functional domain peptides ranging from 1.0 μg/mL to 100 μg/mL were assayed to determine their effect on thrombin inhibition in the presence of pre-formed ATIII/heparin complexes. The order of addition of assay components was as follows: 1) a dilution series of $rBPI_{23}$ or BPI functional domain peptides or thaumatin as a control protein, with final concentrations of 100, 50, 25, 10 and 1 μg/well, diluted in PBS in a final volume of 50 μL; 2) 50 μL plasma diluted 1:100 in a buffer supplied by the manufacturer; 3) 50 μl thrombin at 1 nKat/mL in a buffer supplied by the manufacturer; and 4) 50 μL chromogenic substrate at a concentration of 1μmol/mL in water. The reaction was allowed to proceed for 10 minutes at 37°C and stopped with the addition of 50 μL 0.1M citric acid. The colorimetric reaction was quantitated on a microplate reader as described in Example 3.

The results of these assays are shown in Figures 7a and 7b, which depict the sample concentrations as weight or molar concentrations respectively. BPI functional domain peptides BPI.3 and BPI.5 each had the most significant heparin neutralization effects. In these assays, the control protein, thaumatin, showed no neutralizing effect and was essentially equivalent to the buffer control at all protein concentrations.

EXAMPLE 12

LPS Neutralization Activity by LAL Assay of BPI Individual Functional Domain Peptides

BPI functional domain peptides BPI.2, BPI.3, and BPI.8, along with $rBPI_{23}$ as a positive control, were evaluated in the LAL assay according to the method of Example 4 herein to determine LPS binding and inhibition properties of these peptides. The experiments were performed essentially as described in Example 3 and the results are shown in Figures 8a and 8b, which depict the sample concentrations as weight or molar concentrations respectively. The results showed that BPI.3 had moderate LPS inhibition activity and that BPI.2 and BPI.8 had no significant LPS inhibition activity.

EXAMPLE 13

Bactericidal Activity Assay of BPI Individual Functional Domain Peptides

BPI functional domain peptides BPI.2, BPI.3, and BPI.8, along with $rBPI_{23}$ as a positive control, were tested for bactericidal effects against *E. coli* J5 (rough) and *E. coli* 0111:B4 (smooth) bacteria in a radial diffusion assay according to the methods of Example 2. The results of these assays are depicted in Figures 9a-9d. These results demonstrated that each of the BPI functional domain peptides BPI.2 and BPI.3 exhibited bactericidal activity while BPI.8 had little to no bactericidal activity. Each of the bactericidal peptides showing bactericidal activity tended to be more effective against the rough than the smooth *E. coli* strain.

In additional experiments, broth antibacterial assays were conducted to further determine the bactericidal activity of certain of the BPI peptides. Specifically, either *E. coli* J5 (rough) or *E. coli* 0111:B4 (smooth) bacteria were selected from single colonies on agar plates and used to inoculate 5 mL of Mueller Hinton broth and incubated overnight at 37°C with shaking. The overnight culture was diluted (~ 1:50) into 5 mL fresh broth and incubated at 37°C to log phase (~ 3 hours). Bacteria were pelleted for 5 minutes at 3000 rpm (1500 x *g*). Bacterial pellets were resuspended in 5 mL PBS and diluted to 2×10^6 cells/mL in the Mueller Hinton broth (wherein 1 OD_{570} unit equals 1.25×10^9 CFU/mL). The BPI functional domain peptides to be tested were diluted to 200 μg/mL in broth and serially diluted 2-fold in 96 well culture plates (100 μL volume). All items were at 2-fold final concentration and experiments were conducted in triplicate. Bacteria were added at 100 μL/well and the plates were incubated on a shaker at 37°C for a 20 hour period. The plates were then read on an ELISA plate multiple reader at 590 nm. One of the triplicate wells from each peptide concentration was selected for colony forming unit (CFU) determination. A 30 μL aliquot was added to 270 μL of PBS and further ten-fold serial dilutions were performed. Then a 50 μL aliquot was plated on tryptic soy agar and incubated overnight. Colonies were counted and final bacterial concentrations determined. The results of these assays are depicted in Figures 9e (for *E. coli* J5) and 9f (for *E. coli* 0111:B4). As shown in these Figures, BPI functional domain peptide BPI.3 had significant anti-bacterial activity against *E. coli* J5 bacteria and less activity against *E. coli* 0111:B4 bacteria.

EXAMPLE 14

Preparation of BPI Combination Functional Domain Peptides

Combination peptides were prepared using solid-phase chemistry as described in Example 9. The sequences of these peptides are shown in Table IV. It will be noted that the peptides designated BPI.7, BPI.9 and BPI.10 represent partial or even multiple repeats of certain BPI sequences. Specifically, BPI.7 comprises a 20-mer consisting of amino acid residues 90-99 repeated twice in a single linear peptide chain. BPI.10 comprises an approximately 50:50 admixture of a 25-mer (designated BPI.10.1; SEQ ID NO:55) and a 26-mer (designated BPI.10.2; SEQ ID NO:65) consisting of amino acid residues 94-99, 90-99, 90-99 and 93-99, 90-99, 90-99, respectively, in a single linear peptide chain. BPI.9 comprises a 16-mer comprising amino acid residues 94-99 followed by residues 90-99 in a single linear peptide chain.

These peptides were used in each of the BPI activity assays described in Examples 10-13 above. In the heparin binding assay described in Example 10 and shown in Figure 6, BPI.7 had extremely high heparin binding capacity. In the heparin neutralization assay described in Example 11 and shown in Figures 7a and 7b, BPI.7 had significant heparin neutralization effects compared with $rBPI_{23}$. In the LAL assay described in Example 12 and shown in Figures 8a and 8b, BPI.7 had significant LPS inhibition properties. In bactericidal assays using radial diffusion plates as described in Example 13 and shown in Figures 9a-9d, each of the BPI functional domain peptides BPI.7, BPI.9 and BPI.10.1 and BPI.10.2 exhibited bactericidal activity, and significant bactericidal activity was also found for BPI.7, BPI.9 and BPI.10.1 and BPI.10.2 against both rough and smooth variant strains of *E.coli* in broth assays. The BPI.10 peptides exhibited the highest bactericidal activity observed against either bacterial strain.

These bactericidal activity results obtained with peptides BPI.7 and BPI.10 showed that a linear dimer (BPI.7) and a mixture of linear multimers (BPI.10.1 and BPI.10.2) of the BPI domain II peptide KWKAQKRFLK (*i.e.*, BPI.8, SEQ ID NO:8) had bactericidal activity against *E. coli* strain J5, and that the monomer (BPI.8) showed essentially no bactericidal activity. Moreover, both the dimer and the multimer peptides had higher bactericidal activity that of BPI.9, comprising amino acids 94-99, 90-99. On the basis of these results, the additional peptides shown in Table IV were synthesized using the methods described in Example 9.

TABLE IV

BPI Combination Functional Domain Peptides

BPI peptide No.	Amino Acid Region	Amino Acid Residues	MW (daltons)
BPI.7	90-99, 90-99	20	2644.66
BPI.8	90-99	10	1316.8
BPI.9	94-99, 90-99	16	2131.34
BPI.10.1	94-99, 90-99, 90-99	25	3319.19
BPI.10.2	93-99, 90-99, 90-99	26	3447.32
BPI.13	148-161	14	1710.05
BPI.29	148-161,148-161	28	3403.1
BPI.30	90-99, 148-161	24	3023.86
BPI.63	85-99, 148-161	29	3524.4

EXAMPLE 15

Bactericidal Activity of Combination Functional Domain Peptides

The BPI combination functional domain peptides described in Example 14 were used in radial diffusion bactericidal assays essentially as described in Examples 2 and 13 above. These results are shown in Figures 10a-10e. The results shown in Figure 10a demonstrate that BPI.8, comprising one copy of a domain II peptide (amino acids 90-99), had no detectable bactericidal activity against *E. coli* J5 cells at concentrations of 1000 μg/mL. In contrast, BPI.13, comprising one copy of a domain III monomer (amino acids 148-161) showed appreciable bactericidal activity at concentrations greater than 30 μg/mL. BPI.29, comprising two copies of a domain III monomer BPI.13, had greater bactericidal activity, and BPI.30, comprising a linear combination of the domain II peptide BPI.8 and the domain III peptide BPI.13, showed the highest bactericidal activity against J5 cells, approximating that of BPI.

Figure 10b shows the results of experiments with domain II peptides comprising BPI.8, BPI.7 and BPI.10. (*See also* summary Table VIII.) Although BPI.8 showed no bactericidal activity against *E. coli* J5 cells at concentrations of 1000 μg/mL, the combination peptides BPI.7 and BPI.10 showed high levels of bactericidal activity.

Additional experiments were performed using various other bacteria as target cells to examine the range of bactericidal killing of these BPI functional domain peptides. Figure 10c shows the results of radial diffusion experiments using *E. coli* strain O7-K1. In these experiments, $rBPI_{23}$ showed no bactericidal activity at concentrations of 100 μg/mL, and low bactericidal activity even at concentrations of 1000 μg/mL. Similarly low levels of bactericidal activity were found with the peptides BPI.8 comprising the domain II (DII) monomer and BPI.13 comprising the domain III (DIII) monomer, although the amount of activity of BPI.13 was found to be higher than that of $rBPI_{23}$. Surprisingly, the domain II dimer BPI.7 and the domain II-domain III (DII-DIII) heterodimer BPI.30 showed high levels of bactericidal activity, and the domain III dimer BPI.29 showed moderate bactericidal activity. These results demonstrated that peptides of the functional BPI functional domain identified herein possess bactericidal activity qualitatively different from the bactericidal activity of the BPI molecule itself.

Figures 10d and 10e show results that further demonstrate that the homo- and heterodimers described herein have qualitatively and quantitatively different bactericidal activity spectra of susceptible bacteria. Figure 10d shows the results of radial diffusion assays using *Klebsiella pneumoniae* bacteria. The DII-DIII heterodimer BPI.30 showed the highest amount of bactericidal activity against this bacteria, the DIII homodimer BPI.29 showed moderate levels of activity, and the DII dimer (BPI.7) and DIII monomer (BPI.13) showed low levels of activity. BPI.8, comprising the DII monomer, showed no bactericidal activity at concentrations of 800 μg/mL, consistent with the lack of bactericidal activity of this peptide seen with the *E. coli* strains tested.

Figure 10e shows the levels of bactericidal activity found in radial diffusion experiments using the Gram-positive bacterium *Staphylococcus aureus*. The DII-DIII heterodimer BPI.30 showed the highest amount of bactericidal activity against this bacteria, the DIII homodimer BPI.29 showed moderate levels of activity, and DII dimer (BPI.7) and the DIII monomer (BPI.13) showed low levels of activity. BPI.8, comprising the DII monomer, showed no bactericidal activity at concentrations of 800 μg/mL, consistent with the lack of bactericidal activity of this peptide seen with the other bacteria.

These results showed that the homo- and heterodimers disclosed herein possessed varying amounts of bactericidal activity, which varied both with regard to the amount of such activity and the minimum effective concentration of the peptide necessary for bactericidal activity to be detected. These results also showed that these peptides possessed quantitatively and, more surprisingly, qualitatively different bactericidal activity than the BPI itself.

EXAMPLE 16

Additional Bactericidal Activity of BPI Combination Functional Domain Peptides

In light of the results of the experiments disclosed in Example 15, the bactericidal activity of domain II-domain III combination peptides were compared with the bactericidal activity of each of the component BPI domain II and domain III peptides, against a number of different bacteria and other microorganisms. The following BPI functional domain peptides as described above were used in radial diffusion bactericidal assays (Example 2) and broth bactericidal assays (Example 13) essentially as described in Example 15 above. These results are shown in Figures 11a-11q. These Figures show results of bactericidal assays using the following bacterial strains:

Gram-negative bacteria	BPI peptides tested
Pseudomonas aeruginosa	BPI.8, BPI.13, BPI.30
E. coli O18:K1:H7	BPI.8, BPI.13, BPI.30
Klebsiella pneumoniae	BPI.8, BPI.13, BPI.30
E. coli O75	BPI.8, BPI.13, BPI.30
Serratia marcescens	BPI.8, BPI.13, BPI.30
Proteus mirabilis	BPI.2, BPI.13, BPI.30
Salmonella typhurium	BPI.23, BPI.30
E. coli O86a:K61	BPI.23, BPI.30
E. coli O4:K12	BPI.30
Gram-positive bacteria	
Streptococcus pneumonia	BPI.29, BPI.30., BPI.48, BPI.55, BPI.13, BPI.69
Bacillus megaterium	BPI.2, BPI.7, BPI.45, BPI.46, BPI.47, BPI.48
Staphylococcus aureus	BPI.7, BPI.8, BPI.10, BPI.13, BPI.30
Fungi	
Candida albicans	BPI.30, BPI.13, BPI.29, BPI.48, BPI.2

The results of these experiments are summarized as follows. None of the BPI peptides tested showed any bactericidal activity against *S. marcescens* (Figure 11f) or *P. mirabilis* (Figure 11g). BPI.8 showed no bactericidal activity against any organism tested at concentrations up to about 2000 pmol. BPI.13 and BPI.30 showed bactericidal activity against *P. aeruginosa* (Figure 11a), *E. coli* O18:K1:H7 (Figure 11b), *K. pneumoniae* (Figure 11c), and *E. coli* O75 (Figure 11d). Additionally, BPI.30 showed bactericidal activity against *S. typhurium* (Figure 11h), and, in broth assays, *E. coli* O86a:K51 (Figure 11j) and *E. coli* O4:K12 (Figure 11k). BPI.23 showed bactericidal activity in a radial diffusion assay against *E. coli* O86a:K61 (Figure 11i).

Additionally, BPI.30 showed bactericidal activity against *E. coli* O86a:K61 in human serum (Figure 11l).

The bactericidal capacity of BPI peptides provided by the invention was also tested against Gram-positive bacteria. Surprisingly, every BPI peptide tested showed some bactericidal activity in radial diffusion assays using *S. aureus* (Figure 11e), *S. pneumoniae* (Figure 11m) and *B. megaterium* (Figure 11n) at amounts ranging between about 20 and about 2000 pmol. These results compared favorably with bactericidal activity of the antibiotics gentamicin and vancomycin (Figure 11o).

Most surprisingly, one peptide, BPI.13, was found to have fungicidal activity in a broth assay using *C. albicans* (Figures 11p and 11q). As shown in these Figures, the activity of BPI.13 is clearly distinguishable from the much lower activity levels of BPI.2, BPI.29, BPI.30, and BPI.48. These results demonstrate that the BPI functional domain peptides of the invention have antimicrobial activity qualitatively distinct from the activity previously reported for native BPI.

EXAMPLE 17

Heparin Neutralization Activity of BPI Combination Functional Domain Peptides

The *in vitro* and *in vivo* heparin neutralization capacity of the BPI combination functional domain peptides prepared in Example 14 was determined by assaying the ability of these peptides to counteract the inhibitory effect of heparin on clotting time of heparinized blood and plasma.

In vitro, the effect of BPI combination functional domain peptides was determined on heparin-mediated lengthening of activated partial thrombin time (APTT). The APTT is lengthened by the presence of endogenous or exogenous inhibitors of thrombin formation, such as therapeutically administered heparin. Thus, agents which neutralize the anti-coagulant effects of heparin will reduce the APTT measured by the test. Citrated human plasma (200 μL) was incubated for 1 minute at 37°C with either 15 μL of diluent (0.15 M

NaCl, 0.1 M Tris-HCl, pH 7.4) or 15 μL of the diluent also containing 25 μg/mL heparin (187 units/mg). Various concentrations (from 0.0 to 56 μg/mL) of $rBPI_{23}$, $rBPI_{21}\Delta cys$, or BPI combination peptides BPI.29 (the DIII homodimer) and BPI.30 (heterodimer DII + DIII) in a volume of 15 μL were added, followed immediately by 100 μL of thrombin reagent (Catalog No. 845-4, Sigma Chemical Co., St. Louis, MO). Clotting time (thrombin time) was measured using a BBL Fibrometer™ (Becton Dickenson Microbiology Systems, Cockeysville, MD). The results are shown in Figures 12a, 12b and 12e. Figure 12a shows the relative decrease caused by addition of varying amounts of $rBPI_{23}$ or $rBPI_{21}\Delta cys$ to the heparin-prolonged APTT. These results establish that each of these BPI-related proteins inhibits the heparin-mediated lengthening of APTT. Figure 12b shows that the BPI combination peptides BPI.29 and BPI.30 also inhibit the heparin-mediated lengthening of APTT. Figure 12e illustrates the results obtained with BPI.30 on a non-log scale. Figure 12g shows that BPI.29, BPI.30, and BPI.7 have the greatest effect on the clotting time of heparinized blood in the assay. BPI.3 and $rBPI_{23}$ show a smaller effect, and BPI.14, BPI.2, BPI.4, BPI.5, BPI.7, and $rLBP_{25}$, rBPI and $rBPI_{21}\Delta cys$ all show less of a decrease in clotting times of heparinized blood in this assay.

The *in vivo* effect of exemplary BPI combination peptides on APTT in heparinized rats was determined and compared with the *in vivo* effect of $rBPI_{23}$. APTT is lengthened by the presence of endogenous or exogenous inhibitors of thrombin formation, such as therapeutically administered heparin. Agents which neutralize the anti-coagulant effects of heparin will reduce the APTT as measured by this test. Sprague-Dawley rats housed under NIH guidelines were administered with 100 U/kg heparin by bolus intravenous injections *via* the animals' tail vein followed 5 minutes later by administration of varying amounts of test or control protein as compared with $rBPI_{23}$. The APTT was then determined from blood samples collected from the abdominal aorta 2 minutes after the administration of the test or control protein. The APTT of untreated animals, as well as animals treated only with a BPI peptide, was also

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determined. Figure 12c shows the dose dependence of $rBPI_{23}$ inhibition of heparin-mediated lengthening of partial thromboplastin time, and that administration of about 5mg/kg results in a APTT of the heparinized and BPI-treated animals that is almost the same as the untreated control animals. The results of similar experiments shown in Figure 12d demonstrate that the unrelated protein thaumatin has no effect on APTT times in heparinized animals. The administration of BPI.10 peptide results in a APTT in heparinized animals that is essentially the same as the APTT in control animals treated with BPI.10 alone. Similar results using BPI.30 were also obtained (Figure 12f).

These results show that BPI functional domain combination peptides (*e.g.*, BPI.10 and BPI.30) and $rBPI_{23}$ effectively neutralize heparin inhibition of coagulation proteases. Based on these characteristics, BPI combination functional domain peptides of the invention are projected to be useful in the clinical neutralization of heparin anti-coagulant effects in dosages generally corresponding functionally to those recommended for protamine sulfate, but are not expected to possess the severe hypotensive and anaphylactoid effects of that material.

EXAMPLE 18

Preparation and Functional Activity Analysis of BPI Substitution Variant Functional Domain Peptides

The results obtained above with peptides from functional domains II and III prompted a further effort to determine the functionally-important amino acid residues within these peptides. Accordingly, a series of peptides comprising the amino acid sequences of domains II and III were prepared in which one of the amino acids in the sequence was substituted with an alanine residue. Diagrams of the domain peptides used in the substitution experiments are shown in Figure 13 (domain II; IKISGKWKAQKRFLK, SEQ ID No.:7) and Figure 14 (domain III; KSKVGWLIQLFHKK, SEQ ID No.:13). These peptide series were then tested for heparin binding affinity (K_d), heparin binding capacity (Hep-CAP), LPS neutralization as determined using the *Limulus*

Ameboctye Lysate assay (LAL), and bactericidal activity against *E.coli* J5 using the radial diffusion assay (RAD), each assay as performed as described in the Examples above.

The results, shown in Table V (domain II) and Table VI (domain III), are expressed in terms of the fold difference in activity in each of these assays (except for the LAL assay where relative differences are noted) between the BPI functional domain II and domain III peptides and each alanine substituted variant peptide thereof.

For domain II peptides, most alanine-substituted peptides showed an approximately 2- to 10-fold reduction in bactericidal activity in the radial diffusion assay. Exceptions to this overall pattern include BPI.19 ($Gly_{89} \rightarrow Ala_{89}$), BPI.22 ($Lys_{92} \rightarrow Ala_{92}$), BPI.23 ($Gln_{94} \rightarrow Ala_{94}$) and BPI.24 ($Lys_{95} \rightarrow Ala_{95}$). In contrast, most alanine-substituted peptides showed no difference in the LAL assay; BPI.17 ($Ile_{87} \rightarrow Ala_{87}$) and BPI.21 ($Trp_{91} \rightarrow Ala_{91}$) showed a moderate and large decrease in activity, respectively, in this assay. For BPI.21, these results were consistent with the more than 10-fold reduction in bactericidal activity found for this peptide, indicating that amino acid 91 (a tryptophan residue in the native sequence) may be particularly important in conferring biological activity on the peptide.

The effect of alanine substitution on heparin binding and capacity was, in almost all cases, no more than 2-fold more or less than the unsubstituted peptide. One exception was the heparin binding capacity of BPI.21, which was 4-fold lower than the unsubstituted peptide. This further supports the earlier results on the particular sensitivity of the various activities of these peptides to substitution at Trp_{91}. In most cases, the effect on both the K_d of heparin binding and heparin binding capacity was consistent and of about the same magnitude. In some instances, the heparin binding capacity of the substituted peptide decreased, although the K_d increased slightly (BPI.18; $Ser_{88} \rightarrow Ala_{88}$), or decreased slightly (BPI.24). There were also instances where capacity was unchanged even though the K_d increased (BPI.20; $Lys_{90} \rightarrow Ala_{90}$) or decreased

(BPI.19). In one instance the affinity remained unaffected and the capacity decreased almost 2-fold (BPI.25; Arg_{96} → Ala_{96}).

These results indicated the existence of at least one critical residue in the domain II sequence (Trp_{91}), and that the activities of the domain II peptides were for the most part only minimally affected by alanine substitution of the other domain II amino acid residues.

For domain III peptides, most alanine-substituted peptides showed an approximately 2- to 5-fold reduction in bactericidal activity in the radial diffusion assay. Exceptions to this overall pattern include BPI.35 (Gly_{152} → Ala_{152}), BPI.39 (Gln_{156} → Ala_{156}), BPI.42 (His_{159} → Ala_{159}) and BPI.44 (Lys_{161} → Ala_{161}). Most alanine-substituted peptides showed no difference in the LAL assay; BPI.31 (Lys_{148} → Ala_{148}), BPI.32 (Ser_{149} → Ala_{149}), BPI.33 (Lys_{150} → Ala_{150}), and BPI.34 (Val_{151} → Ala_{151}) showed a moderate decrease in LPS-binding activity, and BPI.36 (Trp_{153} → Ala_{153}) and BPI.40 (Leu_{157} → Ala_{157}) showed a large decrease in LPS-binding activity in this assay. For both BPI.36 and BPI.40, these results were consistent with the approximately 5-fold reduction in bactericidal activity found for these peptides, indicating that the hydrophobic amino acids Trp_{153} and Leu_{157} in the native sequence may be particularly important in conferring biological activity on the peptide.

Effects of alanine substitution on heparin binding and capacity were of similar magnitude, being no more than about 5-fold more or less than the unsubstituted peptide. In almost every case, the type of effect of alanine substitutions on both the K_d of heparin binding and heparin binding capacity was consistent and of about the same magnitude, unlike the findings with the domain II alanine substitution peptides. In one instance (BPI.42; His_{159} → Ala_{159}), the heparin binding capacity was unaffected although the K_d declined slightly (1.2-fold). In only one instance was the K_d of heparin binding and heparin capacity increased slightly (BPI.35; Gly_{152} → Ala_{153}); an increase of only 10% was found.

Like the results found with the domain II alanine-substitution peptides, these results indicated the existence of at least one critical residue in the domain III sequence (Trp_{153}), and possibly at least one other (Leu_{157}). The results also showed that, unlike the domain II alanine-substituted peptides, almost one-half of the substitutions resulted in at least a 2-fold difference in the activities tested. In 6 cases, all four of the tested activities decreased, and in 10 instances bactericidal activity, the K_d of heparin binding and heparin capacity decreased. In only one instance (BPI.35, Gly_{152} → Ala_{152}) was the activity in the bactericidal, heparin binding K_d and heparin capacity assays found to have increased, albeit slightly.

These results indicate that alanine replacement of the hydrophobic amino acid residues Trp_{91}, Trp_{153} and Leu_{157} have the greatest effect on the activities of these BPI functional domain substitution peptides. This result is unexpected in light of the cationic nature of $rBPI_{23}$. In fact, domain II alanine substitution peptides in which lysine is replaced either by alanine or phenylalanine showed dramatic increases in activity (*e.g.*, BPI.24, BPI.73).

TABLE V

BPI DOMAIN II ALANINE SUBSTITUTION PEPTIDES

																FOLD CHANGE IN ACTIVITY			
																RAD	LAL	HEPK$_d$	HEPCAP
BPI.2	I	K	I	S	G	K	W	K	A	Q	K	R	F	L	K				
BPI.15	A															↓2.2	=	↓1.1	↓1.4
BPI.16		A														↓1.8	=	↓1.5	↓1.6
BPI.17			A													↓4.5	↓	↓1.3	↓1.8
BPI.18				A												↓1.6	=	↑1.1	↓1.3
BPI.19					A											↑1.4	=	↓1.3	=1.0
BPI.20						A										↓1.1	=	↑1.4	=1.0
BPI.21							A									↓10.4	↓↓	↓1.5	↓4.0
BPI.22								A								=1.0	=	↓1.1	↓1.5
BPI.23										A						↑2.2	=	↑2.0	↑1.4
BPI.24											A					↑3.8	=	↓2.1	↑2.1
BPI.25												A				↓3.8	=	=1.0	↓1.9
BPI.26													A			↓4.0	=	↓1.5	↓1.8
BPI.27														A		↓2.5	=	↓1.7	↓1.7
BPI.28															A	↓2.4	=	↓1.3	↓1.3

TABLE VI
BPI DOMAIN III ALANINE SUBSTITUTION PEPTIDES

	K	S	K	V	G	W	L	I	Q	L	F	H	K	K	FOLD REDUCTION IN ACTIVITY RAD	LAL	HEPK$_d$	HEPCAP
BPI.13	K	S	K	V	G	W	L	I	Q	L	F	H	K	K				
BPI.31	A														↓2.3	↓	↓3.9	↓1.8
BPI.32		A													↓1.5	↓	↓2.9	↓1.7
BPI.33			A												↓1.4	↓	↓2.0	↓1.6
BPI.34				A											↓2.2	↓	↑1.9	↓1.8
BPI.35					A										↑1.5	=	↑1.1	↑1.1
BPI.36						A									↓4.9	↓↓	↓2.6	↓4.6
BPI.37							A								↓3.8	=	↓5.2	↓2.7
BPI.38								A							↓5.0	=	↓1.7	↓1.9
BPI.39										A					↑1.1	=	↓1.3	↓1.1
BPI.40											A				↑5.2	↓↓	↓1.8	↓2.3
BPI.41												A			↓4.3	=	↓2.1	↓3.1
BPI.42													A		↑2.2	=	↓1.2	=1.0
BPI.43														A	↓1.3	=	↓2.0	↓1.3
BPI.44															↑1.2	=	↓1.7	↓1.1

EXAMPLE 19

Summary of Biological Activity of BPI Functional Domain Peptides

The distribution of the peptides into construct categories is presented in Table VII below.

The BPI functional domain peptides of this invention, or representative subsets thereof, have been assayed for the following biological activities: bactericidal activity against Gram-negative and Gram-positive bacteria, and against certain other microorganisms; LPS binding and neutralization activities; and heparin binding and heparin neutralization activities.

BPI functional domain peptides were assayed for bactericidal activity on *E. coli* J5 bacteria and for heparin binding as described in Examples 8 and 6, respectively. The assay results for exemplary peptides of the present invention are summarized in Table VIII for the Gram-negative bacteria *E. coli* J5 (rough) and *E. coli* O113 (smooth) and the Gram-positive bacteria *S. aureus*. The bactericidal activities are expressed as the amount of peptide (pmol/well and μg/well) required to generate a 30 mm^2 bactericidal zone.

TABLE VII

BPI Peptide	Seq ID No.	Peptide Sequence
I. BPI individual functional domain peptides		
Domain I Peptides		
BPI.1	4	QQGTAALQKELKRIK
BPI.4	3	LQKELKRIKIPDYSDSFKIKHL
BPI.14	2	GTAALQKELKRIKIPDYSDSFKIKHLGKGH
BPI.54	5	GTAALQKELKRIKIP
Domain II Peptides		
BPI.2	7	IKISGKWKAQKRFLK
BPI.3	11	NVGLKFSISNANIKISGKWKAQKRFLK
BPI.8	8	KWKAQKRFLK
Domain III Peptides		
BPI.5	67	VHVHISKSKVGWLIQLFHKKIE
BPI.11	13	KSKVWLIQLFHKK
BPI.12	14	SVHVHISKSKVGWLIQLFHKKIESALRNK
BPI.13	15	KSKVGWLIQLFHKK
BPI.55	61	GWLIQLFHKKIESALRNKMNS
II. Linear and branched-chain combination peptides		
Domain II Peptides		
BPI.7	54	KWKAQKRFLKKWKAQKRFLK
BPI.9	51	KRFLKKWKAQKRFLK
BPI.10.1	55	KRFLKKWKAQKRFLKKWKAQKRFLK
BPI.10.2	65	QKRFLKKWKAQKRFLKKWKAQKRFLK
MAP.1		β-Ala-$N\alpha$,$N\epsilon$-[$N\alpha$,$N\epsilon$(BPI.2)Lys]Lys

TABLE VII (cont'd.)

BPI Peptide	Seq ID No.	Peptide Sequence
II. Linear and branched-chain combination peptides (cont'd.)		
Domain III Peptides		
BPI.29	56	KSKVGWLIQLFHKKKSKVGWLIQLFHKK
MAP.2		β-Ala-$N\alpha$,$N\epsilon$-[$N\alpha$,$N\epsilon$(BPI.13)Lys]Lys
III. Single amino acid substitution peptides		
Domain II Peptides		
BPI.15	16	AKISGKWKAQKRFLK
BPI.16	17	IAISGKWKAQKRFLK
BPI.17	18	IKASGKWKAQKRFLK
BPI.18	19	IKIAGKWKAQKRFLK
BPI.19	20	IKISAKWKAQKRFLK
BPI.20	21	IKISGAWKAQKRFLK
BPI.21	22	IKISGKAKAQKRFLK
BPI.22	23	IKISGKWAAQKRFLK
BPI.23	24	IKISGKWKAAKRFLK
BPI.24	25	IKISGKWKAQARFLK
BPI.25	26	IKISGKWKAQKAFLK
BPI.26	27	IKISGKWKAQKRALK
BPI.27	28	IKISGKWKAQKRFAK
BPI.28	29	IKISGKWKAQKRFLA
BPI.61	48	IKISGKFKAQKRFLK
BPI.73	62	IKISGKWKAQFRFLK
BPI.77	72	IKISGKWKAQWRFLK
BPI.79	73	IKISGKWKAKKRFLK
BPI.81	75	IKISGKWKAFKRFLK

TABLE VII (cont'd.)

BPI Peptide	Seq ID No.	Peptide Sequence
III. Single amino acid substitution peptides (cont'd.)		
Domain III Peptides		
BPI.31	33	ASKVGWLIQLFHKK
BPI.32	34	KAKVGWLIQLFHKK
BPI.33	35	KSAVGWLIQLFHKK
BPI.34	36	KSKAGWLIQLFHKK
BPI.35	37	KSKVAWLIQLFHKK
BPI.36	38	KSKVGALIQLFHKK
BPI.37	39	KSKVGWAIQLFHKK
BPI.38	40	KSKVGWLAQLFHKK
BPI.39	41	KSKVGWLIALFHKK
BPI.40	42	KSKVGWLIQAFHKK
BPI.41	43	KSKVGWLIQLAHKK
BPI.42	44	KSKVGWLIQLFAKK
BPI.43	45	KSKVGWLIQLFHAK
BPI.44	46	KSKVGWLIQLFHKA
BPI.82	76	KSKVGWLIQLWHKK
BPI.85	79	KSKVLWLIQLFHKK
BPI.86	80	KSKVGWLILLFHKK
BPI.87	81	KSKVGWLIQLFLKK
BPI.91	86	KSKVGWLIFLFHKK
BPI.92	87	KSKVGWLIKLFHKK
BPI.94	89	KSKVGWLIQLFFKK
BPI.95	90	KSKVFWLIQLFHKK
BPI.96	91	KSKVGWLIQLFHKF
BPI.97	92	KSKVKWLIQLFHKK

TABLE VII (cont'd.)

BPI Peptide	Seq ID No.	Peptide Sequence
IV. Double amino acid substitution peptides		
Domain II Peptides		
BPI.45	31	IKISGKWKAAARFLK
BPI.56	47	IKISGKWKAKQRFLK
BPI.59	30	IKISGAWAAQKRFLK
BPI.60	32	IAISGKWKAQKRFLA
BPI.88	82	IKISGKWKAFFRFLK
Domain III Peptides		
BPI.100	94	KSKVKWLIKLFHKK
Va. Double amino acid substitution/combination peptides		
Domain II Peptides		
BPI.46	57	KWKAAARFLKKWKAQKRFLK
BPI.47	58	KWKAQKRFLKKWKAAARFLK
BPI.48	59	KWKAAARFLKKWKAAARFLK
Vb. Multiple amino acid substitution/combination peptides		
Domain II Peptides		
BPI.69	60	KWKAAARFLKKWKAAARFLKKWKAAARFLK
BPI.99	93	KWKAQWRFLKKWKAQWRFLKKWKAQWRFLK
BPI.101	95	KSKVKWLIKLFFKFKSKVKWLIKLFFKF
VIa. Atypical amino acid substitution peptides		
Domain II Peptides		
BPI.66	49	IKISGKW$_D$KAQKRFLK
BPI.67	50	IKISGKA$_{\beta\text{-(1-naphthyl)}}$KAQKRFLK
BPI.70	63	IKISGKWKA$_{\beta\text{-(3-pyridyl)}}$QKRFLK

TABLE VII (cont'd.)

BPI Peptide	Seq ID No.	Peptide Sequence
VIa. Atypical amino acid substitution peptides (cont'd.)		
Domain II Peptides		
BPI.71	66	A_DA_DIKISGKWKAQKRFLK
BPI.72	64	IKISGKWKAQKR$A_{\beta\text{-(3-pyridyl)}}$
BPI.76	71	IKISGKWKAQF_DRFLK
BPI.80	74	IKISGKWKAQ$A_{\beta\text{-(1-naphthyl)}}$RFLK
Domain III Peptides		
BPI.83	77	KSKVG$A_{\beta\text{-(1-naphthyl)}}$LIQLFHKK
VIb. Atypical amino acid double substitution peptides		
Domain II Peptides		
BPI.84	78	IKISGK$A_{\beta\text{-(1-naphthyl)}}$KAQFRFLK
BPI.89	84	IKISGK$A_{\beta\text{-(1-naphthyl)}}$KAFKRFLK
VIc. Atypical amino acid triple substitution peptides		
Domain II Peptides		
BPI.90	85	IKISGK$A_{\beta\text{-(1-naphthyl)}}$KAFFRFLK
VII. Cyclized peptides		
Domain II Peptides		
BPI.58	9	CIKISGKWKAQKRFLK
BPI.65 oxidized	10	CIKISGKWKAQKRFLKC

TABLE VII (cont'd.)

BPI Peptide	Seq ID No.	Peptide Sequence
VII. Cyclized peptides		
Domain II Peptides		
BPI.65 reduced	10	CIKISGKWKAQKRFLKC
VIIIa. Interdomain combination peptides		
Domain II-Domain III Peptides		
BPI.30	52	KWKAQKRFLKKSKVGWLIQLFHKK
BPI.63	53	IKISGKWKAQKRFLKKSKVGWLIQLFHKK
BPI.74	70	KSKVGWLIQLFHKKKWKAQKRFLK
VIIIb. Interdomain combination multiple substitution peptides		
Domain II-Domain III Peptides		
BPI.102	96	KWKAQFRFLKKSKVGWLILLFHKK
VIIIc. Atypical amino acid double substitution/interdomain combination peptides		
Domain II-Domain III Peptides		
BPI.93	88	IKISGK$A_{\beta\text{-(1-naphthyl)}}$KAQFRFLKKSKVGWLIQLFHKK
BPI.98	83	IKISGK$A_{\beta\text{-(1-naphthyl)}}$KAQFRFLKKSKVGWLIFLFHKK

TABLE VIII

Bactericidal Activity[a]

BPI Peptide	***E. coli* J5**		***E. coli* O111:B4**		***S. aureus***	
	(pmol/well)	(µg/well)	(pmol/well)	(µg/well)	(pmol/well)	µg/well)
BPI.1	-[b]	-	-	-	-	-
BPI.2	> 2733.5	> 5	-	-	-	-
BPI.3	696	2.14	-	-	N.T.[c]	N.T.
BPI.4	-	-	-	-	-	-
BPI.5	398	1.05	> 1904	> 5	N.T.	N.T.
BPI.6	-	-	-	-	-	-
BPI.7	175	0.46	> 1890.6	> 5	> 1890.6	> 5
BPI.8	> 3797.1	> 5	-	-	N.T.	N.T.
BPI.9	479	1.02	> 2345.9	> 5	N.T.	N.T.
BPI.10	102	0.41	697	2.76	N.T.	N.T.
BPI.11	638	1.06	-	-	N.T.	N.T.
BPI.12	525	1.78	-	-	N.T.	N.T.

TABLE VIII

Bactericidal Activity[a]

BPI Peptide	*E. coli* J5		*E. coli* O111:B4		*S. aureus*	
	(pmol/well)	(μg/well)	(pmol/well)	(μg/well)	(pmol/well)	μg/well)
BPI.13	441	0.75	> 2923.9	> 5	> 2923.9	>5
BPI.14	-	-	-	-	N.T.	N.T.
BPI.15	> 2797.8	> 5	-	-	N.T.	N.T.
BPI.16	> 2821.5	> 5	-	-	N.T.	N.T.
BPI.17	> 2807.2	> 5	-	-	N.T.	N.T.
BPI.18	> 2757.6	> 5	-	-	N.T.	N.T.
BPI.19	> 2712.8	> 5	-	-	N.T.	N.T.
BPI.20	> 2821.5	> 5	-	-	N.T.	N.T.
BPI.21	> 2917	> 5	-	-	N.T.	N.T.
BPI.22	> 2821.50	> 5	-	-	N.T.	N.T.
BPI.23	1330	2.36	> 2821.15	> 5	N.T.	N.T.
BPI.24	655	1.16	> 2821.50	> 5	N.T.	N.T.

TABLE VIII

Bactericidal Activity[a]

BPI Peptide	***E. coli* J5**		***E. coli* O111:B4**		***S. aureus***	
	(pmol/well)	(μg/well)	(pmol/well)	(μg/well)	(pmol/well)	μg/well)
BPI.25	> 2866.8	> 5	-	-	N.T.	N.T.
BPI.26	> 2852.1	> 5	-	-	N.T.	N.T.
BPI.27	> 2797.8	> 5	-	-	N.T.	N.T.
BPI.28	> 2821.5	> 5	-	-	N.T.	N.T.
BPI.29	442	1.5	> 1469.2	> 5	> 1469.2	> 5
BPI.30	76	0.23	608	1.84	1216	3.68
BPI.31	938	1.55	-	-	N.T.	N.T.
BPI.32	614	1.04	-	-	N.T.	N.T.
BPI.33	575	0.95	-	-	N.T.	N.T.
BPI.34	916	1.54	-	-	N.T.	N.T.
BPI.35	263	0.45	-	-	N.T.	N.T.
BPI.36	1652	2.64	-	-	N.T.	N.T.

TABLE VIII

Bactericidal Activity[a]

BPI Peptide	***E. coli* J5**		***E. coli* O111:B4**		***S. aureus***	
	(pmol/well)	(μg/well)	(pmol/well)	(μg/well)	(pmol/well)	μg/well)
BPI.37	1284	2.14	-	-	N.T.	N.T.
BPI.38	1698	2.83	-	-	N.T.	N.T.
BPI.39	316	0.52	-	-	N.T.	N.T.
BPI.40	1760	2.94	-	-	N.T.	N.T.
BPI.41	2465	4.03	-	-	N.T.	N.T.
BPI.42	265	0.44	> 3041.3	> 5	N.T.	N.T.
BPI.43	729	1.21	> 3024.8	> 5	N.T.	N.T.
BPI.44	481	0.8	2983	4.93	N.T.	N.T.
BPI.45	1302	2.23	> 1696.7	> 5	> 1696.7	> 5
BPI.46	186	0.47	> 1811.2	> 5	> 1811.2	> 5
BPI.47	98	0.25	577	1.46	> 2461.9	> 5
BPI.48	42	0.1	254	0.61	> 1390.4	> 5

TABLE VIII

Bactericidal Activity[a]

BPI Peptide	***E. coli* J5**		***E. coli* O111:B4**		***S. aureus***	
	(pmol/well)	(µg/well)	(pmol/well)	(µg/well)	(pmol/well)	µg/well)
BPI.49	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
BPI.50	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
BPI.51	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
BPI.52	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
BPI.53	-	-	-	-	N.T.	N.T.
BPI.54	-	-	-	-	N.T.	N.T.
BPI.55	299	0.75	> 1592.2	> 5	> 1592.2	> 5
BPI.56	1387	2.54	-	-	-	-
BPI.57	514	1.05	-	-	-	-
BPI.58	1050	2.03	-	-	-	-
BPI.59	> 2312.3	> 5	-	-	-	-
BPI.60	> 2136.5	> 5	-	-	-	-

TABLE VIII

Bactericidal Activity[a]

BPI Peptide	*E. coli* J5		*E. coli* O111:B4		*S. aureus*	
	(pmol/well)	(µg/well)	(pmol/well)	(µg/well)	(pmol/well)	µg/well)
BPI.61	> 2093.5	> 5	-	-	-	-
BPI.62	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
BPI.63	87	0.31	512	1.8	> 1006.3	> 5
BPI.64	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
BPI.65 oxidized	895	1.82	-	-	> 3118	> 5
BPI.65 reduced	1362	2.77	-	-	-	-
BPI.66	> 3496.7	> 5	-	-	-	-
BPI.67	> 1901.8	> 5	-	-	-	-
BPI.68	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
BPI.69	57	0.21	244	0.88	1058	3.83
BPI.70	-	-	-	-	-	-

TABLE VIII

Bactericidal Activity[a]

BPI Peptide	*E. coli* J5 (pmol/well)	*E. coli* J5 (μg/well)	*E. coli* O111:B4 (pmol/well)	*E. coli* O111:B4 (μg/well)	*S. aureus* (pmol/well)	*S. aureus* μg/well)
BPI.71	2297	4.53	-	-	-	-
BPI.72	> 1911.2	> 5	-	-	-	-
BPI.73	57	0.11	> 1810.9	> 5	> 1810.9	> 5
BPI.74	732	2.21	> 2148.2	> 5	> 2148.2	> 5
BPI.75	2030.8	4.96	-	-	> 2030.8	> 5
BPI.76	> 3906.5	> 5	-	-	-	-
BPI.77	455	0.85	-	-	1684.5	3.15
BPI.78	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
BPI.79	> 2282.9	> 5	-	-	-	-
BPI.80	655	1.24	-	-	> 1975.4	> 5
BPI.81	284	0.52	> 2344.9	> 5	> 2344.9	> 5
BPI.82	171	0.32	> 1197.8	> 5	> 1197.8	> 5

TABLE VIII

Bactericidal Activity[a]

BPI Peptide	***E. coli* J5**		***E. coli* O111:B4**		***S. aureus***	
	(pmol/well)	(μg/well)	(pmol/well)	(μg/well)	(pmol/well)	μg/well)
BPI.83	155	0.27	> 2033.5	> 5	> 2033.5	> 5
BPI.84	12	0.02	> 2016.9	> 5	> 2016.9	> 5
BPI.85	227	0.4	> 1881.2	> 5	> 1881.2	> 5
BPI.86	1520	2.58	-	-	> 2048.5	> 5
BPI.87	189	0.32	> 1535.8	> 5	> 1535.8	> 5
BPI.88	70.32	0.13	540.15	1	> 2380.0	> 5
BPI.89	229.09	0.43	> 1882.4	> 5	> 1882.4	> 5
BPI.90	83.11	0.16	1763	3.32	> 1863.3	> 5
BPI.91	> 3843.5	> 5	-	-	-	-
BPI.92	331.8	0.57	-	-	-	-
BPI.93	212.87	0.76	> 980.3	> 5	-	-
BPI.94	922.54	1.59	> 922.5	> 5	> 922.5	> 5

TABLE VIII

Bactericidal Activity[a]

BPI Peptide	*E. coli* J5		*E. coli* O111:B4		*S. aureus*	
	(pmol/well)	(µg/well)	(pmol/well)	(µg/well)	(pmol/well)	µg/well)
BPI.95	330.88	0.6	> 1397.6	> 5	-	-
BPI.96	378.33	0.65	> 2048.5	> 5	> 2048.5	> 5
BPI.97	296.58	0.53	-	-	-	-
BPI.98	> 1626.1	> 5	> 1626.1	> 5	> 1626.1	> 5
BPI.99	722.9	2.99	> 1064.1	> 5	> 1064.1	> 5
BPI.100	407.74	0.73	> 2655	> 5	-	-
BPI.101	1329.3	4.79	> 1329.3	> 5	> 1329.3	> 5
BPI.102	> 2635.6	> 5	> 2635.6	> 5	-	-
MAP.1	106	0.82	552.79	4.27	> 647.5	> 5
MAP.2	> 690.9	> 5	> 690.9	> 5	> 690.5	> 5

a Amount added to well to achieve a 30 mm^2 hold as determined by PROBIT analysis as described in Examples 15 and 16

b No detectable activity up to 5 µg/well.

c N.T. = not tested.

It will be recognized that BPI.84 peptide was found to have bactericidal activity against *E. coli* J5 bacteria that was the molar equivalent to $rBPI_{23}$.

The results of heparin binding experiments for the BPI functional domain peptides of the invention are shown in representative examples in Figures 15a-15e and Figure 16, and summarized in Table IX, wherein heparin binding data are expressed as affinity (nM) and capacity (ng). By plotting the amount of heparin bound versus increasing concentration of heparin, and fitting the data to standardized equations by non-linear least squares methods (GraFit, v.2.0, Erithacus Software, London, England), both the binding constant (K_d) and capacity are calculated.

TABLE IX

BPI Peptide	Heparin Affinity (nM)	Heparin Capacity (ng)
BPI.1	no binding	no binding
BPI.2	346.5	203.6
BPI.3	780.8	264.5
BPI.4	335.6	80.8
BPI.5	193.4	177.6
BPI.7	908.0	405.6
BPI.8	573.8	92.2
BPI.9	1141.4	212.5
BPI.10	915.7	548.9
BPI.11	743.9	290.5
BPI.12	284.6	231.5
BPI.13	984.5	369.1
BPI.14	396.4	119.3
BPI.15	315.0	145.4
BPI.16	231.0	127.25
BPI.17	266.5	113.1
BPI.18	381.2	156.6
BPI.19	266.5	203.6
BPI.20	485.1	203.6
BPI.21	231.0	50.9
BPI.22	315.0	135.7
BPI.23	693.0	285.0
BPI.24	165.0	427.6
BPI.25	346.5	107.2
BPI.26	231.0	113.1

TABLE IX (cont'd)

BPI Peptide	Heparin Affinity (nM)	Heparin Capacity (ng)
BPI.27	203.8	119.8
BPI.28	266.5	156.6
BPI.29	427.4	463.7
BPI.30	592.2	499.4
BPI.31	252.4	205.1
BPI.32	339.5	217.1
BPI.33	492.2	230.7
BPI.34	518.2	205.1
BPI.35	1083.0	406.0
BPI.36	378.7	80.2
BPI.37	189.3	136.7
BPI.38	579.1	194.3
BPI.39	757.3	335.6
BPI.40	546.9	160.5
BPI.41	468.8	119.1
BPI.42	820.4	369.1
BPI.43	492.3	283.9
BPI.44	579.1	335.6
BPI.45	152.6	160.7
BPI.46	1067.0	321.1
BPI.47	1911.0	576.4
BPI.48	1415.0	442.3
BPI.54	237.4	64.3
BPI.55	367.6	166.1
BPI.56	114.6	135.5

TABLE IX (cont'd)

BPI Peptide	Heparin Affinity (nM)	Heparin Capacity (ng)
BPI.58	194.0	231.2
BPI.59	174.9	106.7
BPI.60	64.8	120.3
BPI.61	58.3	85.2
BPI.63	599.8	305.1
BPI.65 (ox.)	159.5	190.6
BPI.65 (red.)	216.0	279.6
BPI.66	295.7	111.6
BPI.67	107.8	250.4
BPI.69	967.1	450.8
BPI.70	145.2	59.2
BPI.71	75.6	158.9
BPI.72	145.2	102.8
BPI.73	227.2	413.4
BPI.74	218.1	207.3
BPI.75	96.0	119.8
BPI.76	127.9	144.4
BPI.77	301.9	581.7
BPI.79	199.4	110.2
BPI.80	135.6	210.3
BPI.81	334.7	318.4
BPI.82	427.2	163.1
BPI.83	409.9	253.3
BPI.84	1003.2	329.2
BPI.85	682.4	233.1

TABLE IX (cont'd)

BPI Peptide	Heparin Affinity (nM)	Heparin Capacity (ng)
BPI.86	383.1	208.4
BPI.87	575.0	280.0
BPI.88	1629.0	352.8
BPI.89	1199.4	252.8
BPI.90	1231.7	274.8
BPI.91	288.1	181.2
BPI.92	667.1	227.3
BPI.93	386.7	291.5
BPI.94	406.9	216.1
BPI.95	551.2	224.5
BPI.96	468.8	203.8
BPI.97	765.4	252.2
BPI.98	683.3	1678.4
BPI.99	9097.7	971.4
BPI.100	2928.9	314.0
BPI.101	1905.0	210.9
BPI.102	4607.8	535.2
MAP.1	936.8	459.1
MAP.2	785.5	391.2
Cecropin	395.3	242.0
Magainin	3174.6	453.7
PMB Peptide	309.42	58.01
LALF	1294.1	195.3

An intriguing relationship was observed among representative BPI functional domain peptides when a multiple regression analysis was done using bactericidal activity as the predicted variable and heparin binding capacity and affinity (K_d) as the predictor variables. This analysis revealed that only heparin binding capacity was significantly related to bactericidal activity (heparin capacity, $p = 0.0001$ and heparin affinity, $p = 0.6007$). In other words, the amount of heparin that a given peptide embodiment can bind at saturation (*i.e.* capacity) has a significant relationship with bactericidal activity and not how soon a given peptide reaches 50% saturation in the heparin titration (*i.e.* affinity). From the data on LPS binding competition and neutralization, it also appears that capacity is most predictive of bactericidal activity. For examples, the results demonstrate that BPI.7, BPI.29, BPI.30, BPI.46, BPI.47, BPI.48, BPI.63, BPI.65 (reduced), BPI.69, BPI.73, BPI.58, MAP.1 and MAP.2 have extremely high heparin capacity and also are highly bactericidal. Multiple antigenic peptides (MAP peptides) are multimeric peptides on a branching lysine core as described by Posnett and Tam, 1989, *Methods in Enzymology* 178: 739-746. Conversely, BPI.2, BPI.4, BPI.8, BPI.14, BPI.53 and BPI.54 have low heparin binding capacity and accordingly have little or no bactericidal activity.

BPI interdomain combination peptides BPI.30 (comprising domain II-domain III peptides) and BPI.74 (comprising domain III-domain II peptides) were compared for bactericidal activity against Gram-negative and Gram-positive bacteria, and for heparin binding and capacity. These results surprisingly showed that inverting the order of the peptides in the combination changed the relative activity levels observed. For example, BPI.74 was found to have greatly reduced bactericidal activity compared with BPI.30. Specifically, BPI.74 had 10-fold lower bactericidal activity against *E. coli* J5 bacteria, 50-fold lower bactericidal activity against *E. coli* O111:B4 bacteria, and 3.5-fold lower bactericidal activity against *S. aureus*. A 2-fold reduction in heparin binding capacity and a 2-fold increase in heparin affinity, was also observed.

Other bactericidal and endotoxin binding proteins were examined for heparin binding activity. Cecropin A, magainin II amide, Polymyxin B peptide and

Limulus anti-LPS factor (LALF) were assayed in the direct heparin binding assay described in Example 3. The magainin II amide (Sigma, St. Louis, MO) exhibited the highest heparin binding capcity (437.7 ng heparin/2 μg peptide, K_d=3.17 μM) relative to cecropin A (Sigma, 242 ng/2 μg, K_d=395 nM), LALF (Assoc. of Cape Cod, Woods Hole, MA, 195.3 ng/2μg peptide, K_d=1.29 μM), and PMB peptide (Bachem Biosciences, Philadelphia, PA, 58.0 ng/2 μg peptide, K_d=309 mM). The magainin II amide is a substitution variant of the natural magainin sequence, where 3 alanines have been substituted at positions 8, 13, 15. The magainin II amide is reported to have less hemolytic activity than the natural magainin sequence.

The above results support the relationship between heparin binding, LPS binding and bactericidal activities demonstrated by the BPI peptide data and suggest that other LPS binding proteins will also bind to heparin. The more active bactericidal proteins, cecropin A and magainin II amide, correspondingly, have the highest heparin binding capacity of this series of other LPS binding proteins.

One type of BPI functional domain peptide addition variant incorporates the addition of D-alanine-D-alanine to either the amino- or carboxyl-terminus of a BPI functional domain peptide. The rational for this approach is to confer greater Gram-positive bactericidal activity with the addition of D-alanine. The cell wall biosynthesis in Gram-positive bacteria involves a transpeptidase reaction that specifically binds and utilizes D-alanine-D-alanine. Beta-lactam antibiotics such as the penicillins effectively inhibit this same reaction. Incorporation of D-alanine-D-alanine onto an active bactericidal peptide should target the peptide to the actively growing cell wall of Gram-positive bacteria.

In the domain II substitution series of BPI functional domain peptides, an unexpected increase was observed when Lys_{95} was substituted by alanine (BPI.24). A subsequent phenylalanine substitution at position 95 (BPI.73) resulted in improved activity compared with the alanine substitution species. Surprisingly, substitution at position 95 with D-Phe (BPI.76) resulted in dramatically reduced activity, to levels lower than the original peptide (BPI.2). This isomer effect demonstrates that the interactions of this peptide is stereospecific, and implies that BPI.73 can adopt a more active conformation compared with BPI.76. Such stereospecificity, particularly after the phenomenon has been investigated at other residues, provides an important determinant for pharmacophore development.

Peptides derived from the functional domains of BPI as defined herein have been utilized to determine that the hydrophobic amino acids (especially tryptophan) are most critical for optimal activity. This finding was unexpected due the cationic nature of BPI. In fact, for domain II, when a lysine is replaced by an alanine or phenylalanine, the activity increases dramatically (BPI.24, BPI.73). Combinations of functional domain peptides can also increase the potency of individual peptide constructs, including combinations of the most active substitution peptides from the three domains.

The purity of each newly synthesized peptide was determined by analytical reverse-phase HPLC using a VYDAC™ C-18 column (25 cm x 4.6 mm, 5μm particle size, 30 nm pore size; Separation Group, Hesperia, CA). HPLC was performed using 5% acetonitrile/0.1% trifluoroacetic acid (TFA) in water as mobile phase A, and 80% acetonitrile/0.065% TFA as mobile phase B. The eluate was monitored spectrophotometrically at 220 nm. The flow rate was 1.0 mL/min. Gradient elution conditions were selected to give optimum resolution for each peptide. Purity was expressed as the percentage that the main peak area contributed to the total peak area (see Table X). Purity and identity of the new synthesized peptides were also determined by electrospray ionization mass spectrometry using a VG Biotech Bio-Q™ mass spectrometer. Table X presents a summary of the purity analyses of exemplary peptides of the invention by mass spectroscopy and HPLC.

TABLE X

Peptide #	Protein AA Segment	MS % Purity	HPLC % Purity
BPI.1	19-33	-	-
BPI.2	85-99	57	37.2
BPI.3	73-99	-	-
BPI.4	25-46	-	-
BPI.5	42-163	-	-
BPI.6	112-127	-	-
BPI.7	(90-99) x 2	69	40.9
BPI.8	90-99	79	-
BPI.9	95-99,90-99	-	-
BPI.10	94-99, 90-99, 90-99 and 93-99, 90-99, 90-99	-	-
BPI.11	148-151,153-161	-	-
BPI.12	141-169	-	-
BPI.13	148-161	78	69
BPI.13P	148-161	100	98
BPI.14	21-50	-	13,3
BPI.15	85-99, A @ 85 (I)	66	57.6
BPI.16	85-99, A @ 86 (K)	-	84.1
BPI.17	85-99, A @ 87 (I)	86	77,67
BPI.18	85-99, A @ 88 (S)	66	70
BPI.19	85-99, A @ 88 (G)	-	69
BPI.20	85-99, A @ 90 (K)	-	66
BPI.21	85-99, A @ 91 (W)	68	65.8

TABLE X (cont'd)

Peptide #	Protein AA Segment	MS % Purity	HPLC % Purity
BPI.23	85-99, A @ 94 (Q)	-	69
BPI.24	85-99, A @ 95 (K)	-	67
BPI.25	85-99, A @ 96 (R)	-	73
BPI.26	85-99, A @ 97 (F)	-	73
BPI.27	85-99, A @ 98 (L)	-	65
BPI.28	85-99, A @ 99 (K)	-	80
BPI.29	(148-161) x 2	-	-
BPI.30	90-99,148-161	-	21
BPI.30-P	90-99,148-161	95	98
BPI.31	148-161, A @ 148 (K)	-	68
BPI.32	148-161, A @ 149 (S)	-	70
BPI.33	148-161, A @ 150 (K)	-	58
BPI.34	148-161, A @ 151 (V)	-	51
BPI.35	148-161, A @ 152 (G)	-	72
BPI.36	148-161, A @ 153 (W)	-	64
BPI.37	148-161, A @ 154 (L)	-	51
BPI.38	148-161, A @ 155 (I)	-	70
BPI.39	148-161, A @ 156 (Q)	-	53
BPI.40	148-161, A @ 157 (L)	-	53
BPI.41	148-161, A @ 158 (F)	-	63
BPI.42	148-161, A @ 159 (H)	-	59
BPI.43	148-161, A @ 160 (K)	-	53
BPI.44	148-161, A @ 161 (K)	-	70
BPI.45	85-99, A @ 94(Q)&95(K)	71	46
BPI.46	(99-90)x2, A @ 1st 94(Q)&95(K)	67	47

TABLE X (cont'd)

Peptide #	Protein AA Segment	MS % Purity	HPLC % Purity
BPI.47	(90-99)x2, A @ 2d 94(Q)&95(K)	57	34
BPI.48	(90-99)x2, A @ both 94(Q)&95(K)	68	33
BPI.54	21-35	-	-
BPI.55	152-172	-	-
BPI.56	85-99, K @ 94 (Q) & Q @ 95(K)	-	55
BPI.58	Cys-85-99	49	25.7
BPI.59	85-99, A @ 90(K)&92(K)	56	30.3
BPI.60	85-99, A @ 86(K)&99(K)	57	78.3
BPI.61	85-99, F @ 91(W)	60	59.8
BPI.63	85-99, 148-161	38	31.3
BPI.65 Rd	Cys-85-99-Cys	41	22, 34
BPI.65 Ox	Cys-85-99-Cys	-	-
BPI.66	85-99, W_D @ 91(W)	-	-
BPI.67	85-99, β-(1-naphthyl)-A @ 91	65	52
BPI.69	[90-99, A @ 94 (Q) & 95 (K)] x 3	44	54, 40
BPI.70	85-99, β-(3-pyridyl)-A @ 91	66	54
BPI.71	A_D-A_D-85-99	-	60
BPI.72	85-99, β-(3-pyridyl)-A @ 97 (F)	-	52
BPI.73	85-99, F @ 95 (K)	-	44, 39
BPI.74	148-161, 90-99	-	29
BPI.75	KKRAISFLGKKWQK	-	32
BPI.76	85-99, F_D @ 95 (K)	-	39

TABLE X (cont'd)

Peptide #	Protein AA Segment	MS % Purity	HPLC % Purity
BPI.77	85-99, W @ 95 (K)	-	38
BPI.79	85-99, K @ 94 (Q)	-	48
BPI.80	85-99, β-(1-naphthyl)-A @ 95 (K)	-	44
BPI.81	85-99, F @ 94 (Q)	-	33, 35
BPI.82	148-161, W @ 158 (F)	-	58
BPI.83	148-161, β(1-naphthyl)-A @ 153 (W)	-	63
BPI.84	85-99, β-(1-naphthyl) A @ 91 (W) & F @ 95 (K)	-	50
BPI.85	148-161, L @ 152 (G)	-	74
BPI.86	148-161, L @ 156 (Q)	-	51
BPI.87	148-161, L @ 159 (H)	-	63
BPI.88	85-99, F @ 94 (Q) & 95 (K)	-	50
BPI.89	85-99, β-(1-naphthyl) A @ 91 (W) & F @ 94 (Q)	-	50
BPI.90	85-99, β-(1-naphthyl) A @ 91 (W), F @ 94 (Q) & 95 (K)	-	63
BPI.91	148-161, F @ 156 (Q)	-	31
BPI.92	148-161, K @ 156 (Q)	-	50
BPI.93	85-99 148-161 β-(1-naphthyl) A @ 91 (W), F @ 95 (K)	-	38
BPI.94	148-161, F @ 159 (H)	-	59
BPI.95	148-161, F @ 152 (G)	-	57
BPI.96	148-161, F @ 161 (K)	-	60

TABLE X (cont'd)

Peptide #	Protein AA Segment	MS % Purity	HPLC % Purity
BPI.97	148-161, K @ 161 (G)	-	67
BPI.98	90-99, β-(1-naphthyl) A @ 91 (W), F @ 95 (K) + 91	-	31
BPI.99	[90-99, W @ 95 (K)]x3	-	-
BPI.100	148-161, K @ 152 (G) & 156 (Q)	-	-
MAP.1	βAla-Nα,Nε -[Nα,Nε(BPI.2)1Lys]Lys	54	multiple peaks
MAP.2	βAla-Nα,Nε -[Nα,Nε(BPI.13)1Lys]Lys	49	multiple peaks

BPI.13, as well as other selected peptides, were purified using a semi-preparative reverse-phase VYDAC C-18 column (25 cm x 10 mm, 10 μm particle size, 30 nm pore size). The following gradient was used to purify BPI.13: 26.7%B to 33%B/30 min. at a flow rate of 2.0 mL/min. BPI.13 was dissolved in mobile phase A at a concentration of 8.8 mg/mL and injected in a volume in 0.5 mL. Three separate injections were made and the main peak from each injection was collected. The collected material was combined and evaporated to dryness using a SpeedVac.

The purity of the recovered material (which will be referred to as BPI.13P, for purified) was determined with the analytical reverse-phase system and gradient elution conditions described above. Based on this analysis, BPI.13P was 98% pure. Purity and identity of BPI.13P was also determined by electrospray ionization mass spectometry using a VG Biotech Bio-Q mass spectrometer. The observed moleuclar mass was 1711.0 (the predicted mass was 1711.1). No impurities were detected by mass spectrometry. Recovery of BPI.13P was 55%, assuming that the desired peptide constituted 69% of the starting material.

When peptides of the invention were further purified, as described above, the magnitude of the tested biological activity of the peptides, *e.g.*, BPI.13P and BPI.30P, were found to increase when chemical purity was increased. This indicated that the observed biological activity was due to the peptide itself. In particular, the completely novel and unexpected antifungal activity of BPI.13 against *Candida albicans* (*see* Example 16), with a purity of about 69%, was further increased when the purity of the peptide preparation was increased to 98%.

EXAMPLE 20

Analysis of BPI Functional Domain Peptides using Binding and Neutralization Assays

A. LPS Binding Assays

BPI functional domain peptides were subjected to LPS binding assays.

The first of these assays was performed as described in Gazzano-Santoro *et al.*, *supra*. Briefly, a suspension of *E. coli* strain J5 Lipid A was sonicated and diluted in methanol to a concentration of 0.2μg/mL, and then 50μL aliquots were adsorbed to wells (Immulon 2 Removawell™ Strips, Dynatech). Following overnight incubation at 37°C, the wells were blocked with 215μL of a solution of D-PBS/0.1% BSA for 3 hr at 37°C. Thereafter, the blocking buffer was discarded, the wells were washed with a solution of 0.05% Tween-20 in D-PBS (D-PBS/T) and incubated overnight at 4°C with 50μL of a solution of [^{125}I]-rBPI$_{23}$ in D-PBS/T (a total of 234,000 cpm at a specific activity of 9.9 μCi/μg) and increasing concentrations of BPI functional domain peptides. After this incubation, the wells were washed three times with D-PBS/T and the bound radioactivity counted using a gamma counter. Binding to wells treated with D-PBS/BSA was considered non-specific background binding and was subtracted from the total radioactivity bound in each well to yield the amount of specifically-bound radioactivity.

The results of these experiments are shown in Figures 17a (where the concentration of each peptide is given in nM) and 17b (the identical results, with the concentration of peptide given in μg/mL). Competition experiments using unlabeled rBPI$_{23}$ are shown for comparison. These results demonstrate that all of the tested peptides have some capacity to compete with rBPI$_{23}$ for LPS binding, to differing degrees.

This experiment was repeated, comparing the LPS binding affinity of BPI.10 with rBPI$_{23}$, using twice the amount of [^{125}I]-rBPI$_{23}$ (a total of 454,000 cpm, specific activity 10 μCi/μg) and in the presence or absence of whole blood. These results are shown in Figure 18, and demonstrate that, on a molar basis, BPI.10 is within a factor of 2 as potent as rBPI$_{23}$ in competing with radiolabeled rBPI$_{23}$ in this assay.

A

The experiment was repeated using peptides BPI.7, BPI.29 and BPI.30, as in the first experiment described above except that a total of 225,000 cpm of [^{125}I]-rBPI$_{23}$ was used and Lipid A was plated at a concentration of 0.5 mg/mL. The results of this experiment are shown in Figure 19, and show that, on a molar basis, these peptides are 6- to 10-fold less potent that unlabeled rBPI$_{23}$ in binding Lipid A.

A second binding assay was developed, wherein radiolabeled recombinant LPS binding protein ([^{125}I]-rLBP) was used instead of radiolabeled rBPI$_{23}$ in competition experiments with BPI functional domain peptides BPI.2, BPI.3, BPI.4, BPI.5, BPI.7, BPI.13, BPI.14, BPI.29, BPI.30 and BPI.48. rBPI, rBPI$_{21}$Δcys, and rLBP$_{25}$ were included in these assays as controls. In these experiments, Lipid A was adsorbed to the wells at a concentration of 0.7 μg/mL in methanol. Incubation of radiolabeled rLBP (a total of 650,000 cpm and a specific activity of 3.45 μCi/μg) was performed for 2.5 hr at 37°C in the presence of BPI peptides in a series of increasing concentrations. These results are shown in Figures 20a and 20b. IC$_{50}$ values (*i.e.*, the concentration at which Lipid A binding of radiolabeled rLBP$_{25}$ is inhibited to one half the value achieved in the absence of the peptide) are shown in accompanying Table XI.

TABLE XI

Peptide	IC50: nM	μg/mL
rBPI	13	0.65
rBPI$_{21}$Δcys	30	0.69
BPI.7	100	0.26
BPI.29	130	0.44
BPI.48	200	0.48
BPI.30	250	0.75
BPI.3	250	0.75
rLBP$_{25}$	600	15
BPI.13	1000	1.7
BPI.2	1300	2.36
BPI.5	1700	4.42

In a third binding assay, a number of BPI functional domain peptides were tested for their ability to bind to radiolabeled LPS following incubation with human endothelial cells (HUVEC). This assay measures the ability to bind LPS once the BPI peptides are bound to HUVEC cells. HUVEC cells were incubated in the presence of various BPI peptides at a concentration of either 1 μg/mL or 3 μg/mL for 3 hr at 4°C in 500 μL of a solution of D-PBS/BSA. Following this incubation, the cells were washed twice with ice-cold D-PBS/BSA and then incubated for an additional 2.5 hr at 4°C in 500 μL of a solution of [^{125}I]-RaLPS (a total of 340,000 cpm at a specific activity of 4.6 x 10^6 cpm/μg) in D-PBS/BSA. The wells were washed three times with D-PBS/BSA, solubilized in 500 μL of 1M NaOH and the lysates counted using a gamma counter. These results, shown in Figure 21, indicate that BPI.29 and BPI.30 retain the capacity to bind LPS while bound to HUVEC cells.

B. LPS Neutralization Screening Assay of BPI Functional Domain Peptides using TNF Cellular Toxicity

A screening assay for LPS neutralization was developed using a tumor necrosis factor (TNF) cellular toxicity assay. A human monocytic cell line (THP-1; accession number TIB202, American Type Culture Collection, Rockville, MD) grown in media supplemented with Vitamin D produce TNF upon stimulation with LPS in a dose-dependent fashion. Mouse fibroblasts (L929 cells; ATCC No.: CCL1) are sensitive to TNF-mediated cell killing, and this cell killing is also dose-dependent. Thus, the extent of cell killing of L929 cells provides a sensitive assay for the degree of TNF induction in THP-1 cells, which in turn is a sensitive indicator of the amount of free LPS in contact with the THP-1 cells. LPS binding and neutralization by BPI functional domain peptides or rBPI$_{23}$ reduces the amount of free LPS in contact with THP-1 cells, which reduces the amount of TNF produced, which in turn reduces the amount of L929 cell killing in a standardized assay. Thus, the following assay provides a sensitive method for assessing the LPS binding and neutralization capacity of the BPI functional domain peptides of this invention.

THP-1 cells were grown in RPMI media (GIBCO™, Long Island, NY) supplemented with 10% FCS and Vitamin D in spinner culture for 3 days to a density of about 150,000 cells/mL. Cells were then plated in a round-bottomed 96-well culture plate at a density of 100,000 cells/well and incubated in RPMI media without Vitamin D or FCS in the presence of 5ng/mL *E. coli* 01113 LPS for 6 hr at 37°C. Experimental control wells also contained varying amounts of rBPI$_{23}$ or BPI functional domain peptides, in concentrations varying from about 0.1 μg/mL to about 100 μg/mL. After this incubation, the plates were centrifuged at about 600 x *g* to pellet the cells, and 50 μL of the supernatant were added to a 96-well flat bottomed culture dish prepared in parallel with 50,000 L929 cells per well in 50 μL RPMI/10% FCS.

L929 cells were prepared by monolayer growth in RPMI/10% FCS media to a density of about 1 million cells per dish, then split 1:2 on the day before the experiment and allowed to grow overnight to about 70% confluence on the day of the experiment. Actinomycin D was added to the 70% confluent culture to a final concentration of 1 μg/mL 20 min prior to plating in 96-well plates. L929 cell plates were incubated in the presence of the THP-1 supernatant for about 16 hr (overnight) at 37°C under standard conditions of mammalian cell growth. To each well was then added 20 μL of a solution prepared by diluting 100 μL of phenazine methylsulfonate in 2 mL CellTitre 96™AQ$_{\text{ueous}}$ solution (Promega, Madison, WI), containing 3-[(4,5-dimethyl)-thiozol-2-yl]-5-(3-carboxymethoxyphenyl)-2-(4-sulfonyl)-2H-tetrazolium (inner salt). The cultures were allowed to incubate for 2-4 hr at 37°C and then analyzed spectrophotometrically to determine the optical absorbance at 490nm (A490). Experimental results were evaluated relative to a semilog standard curve prepared with known amounts of TNF, varying from about 10 ng/mL to about 10 mg/mL.

The results of these experiments are shown in Figures 22a-22h. Figure 22a shows the relationship between A490 and TNF concentration in cultures of L929 cells in the presence and absence of 5 ng/mL LPS. These results show about the same linear relationship between A490 and concentration of TNF whether or not LPS was present in the assay media. Figure 22b illustrates an experiment where

TNF was incubated with L929 cells in the presence of increasing amounts of heparin. These results show a constant and characteristic A490 for TNF at concentrations of 1 ng/mL and 0.1 ng/mL, indicating that heparin does not affect L929 cell killing by TNF. Figure 22c illustrates a control experiment, showing that $rBPI_{21}\Delta cys$ decreased the amount of TNF-mediated L929 cell killing when incubated at the indicated concentrations in cultures of THP-1 cells in the presence of 5 ng/mL LPS. Figure 22d shows that heparin could compete with LPS for binding with $rBPI_{21}\Delta cys$, by inhibiting the BPI-mediated inhibition of LPS-stimulated TNF production by THP-1 cells, as measured by the L929 cell killing assay.

Figure 22e is a standard curve of A490 *versus* TNF as a measure of TNF-mediated L929 cell killing; Figure 22g shows the linearity of the standard curve in a semilog plot over a TNF concentration range of about three logs (about 1000-fold). Figure 22f shows the THP-1 cell dependence of the assay, wherein detectable amounts of TNF were most readily produced using about 100,000 THP-1 cells and LPS at a concentration of at least 5 ng/mL. Finally, Figure 22h shows that the assay was found to be dependent on THP-1 cell production of TNF in response to LPS stimulation; human histiocytic lymphoma cells (U937; ATCC No.: CRL1593) produced no detectable TNF when substituted in the assay for THP-1 cells.

This assay was used to analyze LPS binding and neutralization capacity of a number of BPI functional domain peptides of the invention. These results are shown in Table XII, and indicate that each of the peptides tested had the capacity to inhibit LPS-stimulated TNF production in THP-1 cells, as measured by TNF-mediated L929 cell killing.

TABLE XII

Peptide	IC_{50} (µg/mL)
rBPI$_{21}$Δcys	0.2
BPI.7	30
BPI.13	20
BPI.29	2-3
BPI.30	6-7
BPI.48	1

C. LPS Neutralization Screening Assay of BPI Functional Domain Peptides using a Cellular NO Production Assay

An additional LPS neutralization screening assay for BPI functional domain peptides was developed using an assay for NO production in mouse cells treated with LPS (*see* Lorsbach *et al.*, 1993, *J. Biol. Chem.* 268: 1908-1913). In this assay, mouse RAW 264.7 cells (ATCC Accession No. T1B71) were treated with bacterial LPS. The cells were incubated in 96-well plates and stimulated for 2 hours with *E. coli* O113 LPS or zymosan, in the presence or absence of γ-interferon, rLBP, fetal bovine serum (FBS) or normal human serum (NHS), or rBPI$_{21}$Δcys. After this incubation, the cells were washed with fresh media and incubated overnight in media containing 10% FCS. The NO released from the cells accumulated in the media and spontaneously converted to nitrite. This nitrite was assayed *in situ* by the Griess reaction, as follows. The nitrite was reacted with the primary amine of an added sulfanilamide and formed a diazonium salt. This salt was then reacted with added naphthylethylenediamine to form a red azo-dye. The Griess reaction was performed at room temperature in about 10 minutes. The amount of produced NO was estimated from a standard curve of Griess reaction products determined spectrophotometrically as Absorbance at a wavelength of 550nm.

The results of this assay are shown in Figures 23a to 23c. Figure 23a shows the dependence of NO production on the presence of γ-interferon. This interferon effect was found to saturate at a concentration of 100U/mL. Figure 23b shows the dependence of LPS-stimulated NO production on the presence of LBP, either added as purified recombinant protein or as a component of FBS or NHS supplements of the cell incubation media. Figure 23c shows $rBPI_{23}$-mediated inhibition of LPS-stimulated NO production, having an IC_{50} of 30-100 ng/mL. These results demonstrated that this assay is a simple, inexpensive and physiologically-relevant assay system for assessing the LPS-neutralizing activity of BPI and BPI functional domain peptides disclosed herein.

The results of such assays performed with BPI functional domain peptides are shown in Figures 24a-24g wherein the background production of NO by unstimulated cells is designated as "NO LPS". Figures 24a and 24b show inhibition of NO production stimulated by zymosan and LPS, respectively, by rBPI, $rBPI_{21}\Delta cys$ and $rLBP_{25}$. No inhibition of zymosan-stimulated NO production was seen at any concentration of BPI protein (Figure 24a). In contrast, LPS-stimulated NO production was inhibited in a concentration-dependent manner by incubation with these rBPI-related proteins (Figure 24b). Figure 24c (zymosan) and Figure 24d (LPS) shows the effects on NO production by RAW 264.7 cells of incubation with BPI.2, BPI.3, BPI.4, BPI.7 and BPI.14; $rBPI_{21}\Delta cys$ is also shown for comparison. As shown with native BPI, zymosan-stimulated NO production was not inhibited by incubation with any of the BPI functional domain peptides (with the possible exception of a small amount of inhibition by BPI.7 at high concentrations; Figure 24c). LPS-stimulated NO production, on the other hand, was inhibited efficiently by $rBPI_{21}\Delta cys$, and to a lesser degree by BPI.3 and BPI.7 (Figure 24d).

This experiment was repeated using BPI.5, BPI.13, BPI.29 and BPI.30, with $rBPI_{21}\Delta cys$ analyzed in parallel for comparison. Zymosan-stimulated NO production by RAW 264.7 cells was found to be inhibited by BPI.30 at high (~100 μg/mL) concentrations; neither any of the other BPI functional domain peptides nor $rBPI_{21}\Delta cys$ showed any inhibition of zymosan-stimulated NO production (Figure 24e). LPS-stimulated NO production was inhibited efficiently by $rBPI_{21}\Delta cys$, and to varying and lesser degrees by all of the BPI functional domain peptides tested in this experiment (Figure 24f).

The IC_{50} values (*i.e.*, the concentration of inhibitor at which zymosan or LPS-stimulated NO production by RAW 264.7 cells is reduced to one-half its value in the absence of the inhibitor) for the BPI proteins and peptides were calculated from these experiments and are showed in Figure 24g. With the exception of BPI.30, no significant inhibition of zymosan-mediated NO production was found for either the BPI functional domain peptides or $rBPI_{21}\Delta cys$, rBPI or rLBP in these experiments; the IC_{50} of BPI.30 for inhibition of zymosan-stimulated NO production was found to be between 10 and 100 μg/mL. BPI.3, BPI.5, BPI.13, BPI.29 and BPI.30 were found to have detectable levels of LPS neutralization in this assay, and the relative IC_{50} values for these peptides are shown in Figure 24g.

D. LPS Neutralization Screening Assay of BPI Functional Domain Peptides using a Cellular Proliferation Assay

An additional LPS neutralization screening assay for evaluation of BPI functional domain peptides was developed. This sensitive assay for inhibition of cellular proliferation in mouse cells treated with LPS can also be utilized for quantitation of LPS levels in human plasma upon development of a standard curve.

In this assay, mouse RAW 264.7 cells (ATCC Accession No. T1B71), maintained in RPMI 1640 media (GIBCO), supplemented with 10mM HEPES buffer (pH 7.4), 2mM L-glutamine, penicillin (100U/mL), streptomcin (100μg/mL), 0.075% sodium bicarbonate, 0.15M 2-mercaptoethanol and 10% fetal bovine serum (Hyclone, Inc., Logan, UT), were first induced by incubation in the presence of 50U/mL recombinant mouse γ-interferon (Genzyme, Cambridge, MA) for 24h prior to assay. Induced cells were then mechanically collected and centrifuged at 500 x *g* at 4°C and then resuspended in 50mL RPMI 1640 media (without supplements), re-centrifuged and again resuspended in RPMI 1640 media (without supplements). The cells were counted and their concentration adjusted to 2×10^5 cells/mL and 100μL aliquots were added to each well of a 96-well microtitre plate. The cells were then incubated for about 15 hours with *E. coli* O113 LPS (Control Standard, Assoc. of Cape Cod, Woods Hole, MA), which was added in 100μL/well aliquots at a concentration of 1ng/mL in serum-free RPMI 1640 media (this concentration being the result of titration experiments in which LPS concentration was varied between 50pg/mL and 100ng/mL). This incubation was performed in the absence or presence of BPI functional domain peptides in varying concentrations between 25ng/mL and 50μg/mL. Recombinant human BPI was used as a positive control at a concentration of 1μg/mL. Cell proliferation was quantitatively measured by the addition of 1μCi/well [^{3}H]-thymidine 5 hours after the time of initiation of the assay. After the 15-hour incubation, labeled cells were harvested onto glass fiber filters with a cell harvester (Inotech Biosystems, INB-384, Sample Processing and Filter Counting System, Lansing, MI.).

The results of this assay are shown in Figures 26a-26c. Figure 26a shows the dependence of LPS-mediated inhibition of RAW 264.7 cell proliferation of the presence of LBP, added to the reaction mixture either as a component of serum or as recombinant LBP (at a concentration of 1μg/mL). Figures 26b and 26c illustrate patterns of BPI functional domain peptide behavior found in the above assay. BPI.5 displayed an EC_{50} (*i.e.*, the peptide concentration at which the growth inhibitory effect of LPS was reversed by 50%) of 5.3 ± 0.6 μg/mL. BPI.81 was unable to reverse the growth inhibitory effect of LPS on RAW 264.7 cells, but showed additional growth inhibition with an IC_{50} (*i.e.*, the peptide concentration at which RAW cell growth was inhibited by 50% from the value without added peptide) of 14 ± 0.2 μg/mL. BPI.98 showed an EC_{50} of 0.16 ± 0.08 μg/mL and an IC_{50} of 16.5 ± 1.9 μg/mL. Finally, BPI.86 showed an EC_{50} of 0.13 ± 0.04 μg/mL and an IC_{50} of 37.5 ± 12.5 μg/mL. Results from all peptides tested with this assay are shown in Table XIII.

TABLE XIII

BPI peptide	Sequence	EC$_{50}$	IC$_{50}$
BPI.2	IKISGKWKAQKRFLK	-	-
BPI.5	HVHISKSKVGWLIQLFHKKIE	5.3±0.6	-
BPI.7	KWKAQKRFLKKWKAQKRFLK	> 50	37.5±12.5
BPI.10	QKRFLKKWKAQKRFLKKWKAQKRFLK	> 50	17.25
BPI.13	KSKVGWLIQLFHKK	1.9±0.4	37.5±12.5
BPI.13p	KSKVGWLIQLFHKK	2.0±0.3	> 50
BPI.29	KSKVGWLIQLFHKKKSKVGWLIQLFHKK	0.1±0.02	13.6±0.4
BPI.30	KWKAQKRFLKKSKVGWLIQLFHKK	1.2±1.1	10.5±1.2
BPI.46	KWKAAAKRFLKKWKAQKRFLK	1.9±1.9	18.8±0.8
BPI.47	KWKAQKRFLKKWKAAAKRFLK	0.9±0.3	9.8±0.1
BPI.48	KWKAAAKRFLKKWKAAAKRFL	1.3±0.9	5.0±0.1
BPI.63	IKISGKWKAQKRFLKKSKVGWLIQLFHKK	0.08±0.02	7.1±0.02
BPI.69	KWKAAARFLKKWKAAARFLKKWKAAARFLK	0.11±0.07	2.4±0.3
BPI.73	IKISGKWKAQFRFLK	22±10	-
BPI.74	KSKVGWLIQLFHKKKWKAQKRFLK	2.7±0.3	18.8±0.8
BPI.76	IKISGKWKAQF_DRFLK	> 50	-
BPI.77	IKISGKWKAQWRFLK	10±32	> 50
BPI.80	IKISGKWKAQ$A_{\beta\text{-(1-naphthyl)}}$RFLK	35±36	> 50
BPI.81	IKISGKWKAFKRFLK	-	14.0±0.2

TABLE XIII (cont'd)

BPI peptide	Sequence	EC_{50}	IC_{50}
BPI.82	KSKVGWLIQLWHKK	0.8±0.1	18.8±0.8
BPI.83	KSKVGWLIQL$A_{\beta\text{-(1-naphthyl)}}$HKK	1.2±0.1	37.5±12.5
BPI.84	IKISGK$A_{\beta\text{-(1-naphthyl)}}$KAQFRFLK	57±28	-
BPI.85	KSKVLWLIQLFHKK	1.3±0.1	17±15
BPI.86	KSKVGWLILLFHKK	0.13±0.04	37.5±12.5
BPI.87	KSKVGWLIQLFLKK	1.3±0.4	11.4±1.3
BPI.88	IKISGKWKAFFRFLK	> 50	6.2±7.5
BPI.89	IKISGKAβ-(1-naphthyl)KAFKRFLK	> 50	11±0.3
BPI.90	IKISGK$A_{\beta\text{-(1-naphthyl)}}$KAFFRFLK	> 50	6.3±0.7
BPI.91	KSKVGWLIFLFHKK	0.7±0.1	-
BPI.92	KSKVGWLIKLFHKK	1.9±0.1	37.5±12.5
BPI.93	IKISGK$A_{\beta\text{-(1-naphthyl)}}$KAQFRFLKKSKVGWLIQLFHKK	0.9±0.25	9.7±0.1
BPI.94	KSKVGWLIQLFFKK	1.3±0.02	23±2
BPI.95	KSKVFWLIQLFHKK	1.0±0.01	37.5±12.5
BPI.96	KSKVGWLIQLFHKF	1.6±0.2	18.8±0.8
BPI.97	KSKVKWLIQLFHKK	2.8±0.3	37.5±12.5
BPI.98	IKISGK$A_{\beta\text{-(1-naphthyl)}}$KAQFRFLKKSKVGWLIFLFHKK	0.16±0.08	16.5±1.9
MAP.1	(β-alanyl-*N*α,*N*ε-substituted-[*N*α,*N*ε(BPI.2)lysyl]lysine)	0.45±0.1	37.5±12.5
$rBPI_{21}\Delta cys$		0.08±0.05	-

-- No proliferation decrease up to 50 μg/ml.

E. LPS Neutralization Assay based on Inhibition of LPS-induced TNF Production in Whole Blood

LPS neutralization by BPI functional domain peptides of the invention was assayed in whole blood as follows. Freshly drawn blood from healthy human donors was collected into vacutainer tubes (ACD, Rutherford, N.J.) Aliquots of blood (170μL) were mixed with 10μL Ca^{++}-, Mg^{++}-free PBS containing 2.5ng/mL *E. coli* 0113 LPS, and with 20μL of varying concentrations of the BPI peptides of the invention ranging in concentration from 0.5-50μg/mL. These mixtures were then incubated for 4h at 37°C, and then the reaction stopped by the addition of 55μL ice-cold Ca^{++}-, Mg^{++}-free PBS, followed by centrifugation at 500 x *g* for 7min. Supernatants were then assayed for TNF levels using a commercial ELISA kit (Biokine™ ELISA Test, T-cell Sciences, Cambridge, MA).

The results of these experiments with representative peptides using whole blood samples from two different donors are shown in Figure 27 and Table XIV. Figure 27 shows a comparison of TNF inhibition by BPI functional domain peptides BPI.7, BPI.13 and BPI.29; results obtained using $rBPI_{21}\Delta cys$ are shown for comparison. These results are quantitated as IC_{50} values in Table XIV, and compared with LPS neutralization as assayed using NO production by RAW 264.7 cells as described in Section C above.

TABLE XIV

	IC_{50}(μg/ml)	
BPI Peptide	TNF assay	NO assay
$rBPI_{21}\Delta_{cys}$	0.65	0.4
BPI.29	5.0	2.4
BPI.13	42	16
BPI.7	Not Inhibitory	Not Inhibitory

F. LPS and Heparin Binding Assays using Tryptophan Fluorescence Quenching

The naturally-occurring amino acid tryptophan can emit light (*i.e.*, it fluoresces) having a wavelength between 300 and 400nm after excitation with light having a wavelength of between about 280nm and 290 nm, preferably 285nm. The amount of emitted light produced by such fluorescence is known to be affected by the local environment, including pH and buffer conditions, as well as binding interactions between proteins and other molecules. Some BPI functional domain peptides derived from domains II and III contain tryptophan residues, and tryptophan fluorescence was used to assay binding interactions between the BPI functional domain peptides of the invention and LPS or heparin.

Tryptophan fluorescence of the BPI functional domain peptides of the invention was determined in the presence or absence of LPS or heparin using a SPEX Fluorolog™ fluorimeter. Samples were excited with 285nm light using a 0.25nm slitwidth. Emission wavelengths were scanned between 300-400nm using a 1.25nm slitwidth. Data were accumulated as the average of three determinations performed over an approximately 5 min time span. Samples were maintained at 25°C or 37°C during the course of the experiments using a circulating water bath. Crab endotoxin binding protein (CEBP), a protein wherein the intrinsic fluorescence of tryptophan residues is affected by binding to LPS, was used as a positive control. (*See* Wainwright *et al.*, 1990, Cellular and Molecular Aspects of Endotoxin Reactions, Nowotny *et al.*, eds., Elsevier Science Publishing B.V., The Netherlands, pp. 315-325).

The results of these experiments are shown in Table XV. K_d values were determined by Scatchard-type Stern-Volmer plots of the quenching data as the negative inverse of the slope of such plots. Comparing the data for BPI.10, BPI.46 and BPI.47, it is seen that as the K_d decreased (indicating an increase in avidity for LPS), the percent fluorescence quenching increased. The differences between these peptides include replacement of basic and polar amino acid residues with non-polar residues in BPI.48 as compared with BPI.10. In contrast, as the K_d of heparin binding decreased, a corresponding increase in the percentage of fluorescence quenching was not detected. This result may indicate fundamental differences between the site or nature of heparin binding compared with LPS binding.

A

TABLE XV

BPI Peptide	# of Trp	K_d LPS (nM)	Quenching LPS (%)	K_d Heparin (μM)	Quenching Heparin (%)
BPI.10	2	124	26	1.2	67
BPI.47	2	115	41	2.2	47
BPI.48	2	83	62	0.8	41
BPI.69	3	58	72	0.4	42
BPI.73	1	66	47	0.7	19
CEBP[a]	5	19	56	0.8	54

[a] CEBP (LALF) experiments were performed at 25°C

G. Neutralization Assay Of Heparin-Mediated Lengthening of Thrombin Time

The effect of BPI functional domain peptides on heparin-mediated lengthening of thrombin time, *i.e.*, the time required for clotting of a mixture of thrombin and plasma, was examined. Thrombin time is lengthened by the presence of endogenous or exogenous inhibitors of thrombin formation, such as therapeutically administered heparin. Agents which neutralize the anti-coagulant effects of heparin will reduce the thrombin time measured by the test.

In these experiments, thrombin clotting time was determined using a MLA Electra 800 Coagulation Timer. Reconstituted plasma (200 μL, Sigma Chemical Co., No. 855-10) was incubated at 37°C for two minutes in a reaction cuvette. Thrombin Clotting Time reagent (100 μL, Baxter Diagnostics Inc., B4233-50) was added to the reaction cuvette after incubation and clotting time was then measured. Heparin sodium (13 μL, 40 μg/mL in PBS, Sigma Chemical Co., H3393) and exemplary BPI functional domain peptides (10 μL of various dilutions from about 0.05 μg/ml to about 10 μg/ml) were added to the reaction cuvette prior to plasma addition for testing of the effects of these peptides on thrombin clotting time. TCT clotting time (thrombin time) was measured using the BPI peptides indicates and the results are shown in Figure 28 and Table XVI. These results shown in Figure 28 and Table XVI below demonstrate that the tested BPI functional domain peptides neutralized heparin, as shown by inhibition of the heparin-mediated lengthening of thrombin time. The IC_{50} of this inhibition was quantitated and is shown in Table XVI.

TABLE XVI

BPI Peptide	IC_{50} (μg/ml) ± SE
BPI.10	0.115 ± 0.014
BPI.47	0.347 ± 0.041
BPI.63	0.362 ± 0.034
BPI.69	0.200 ± 0.025
BPI.73	0.910 ± 0.821
BPI.82	0.200 ± 0.073
BPI.84	0.225 ± 0.029
BPI.87	0.262 ± 0.009
BPI.88	0.691 ± 0.180
BPI.90	0.753 ± 0.210
BPI.98	0.242 ± 0.038
BPI.99	0.273 ± 0.011
BPI.100	0.353 ± 0.050
BPI.101	0.285 ± 0.088
BPI.102	0.135 ± 0.024

EXAMPLE 21

Heparin Neutralization Assay based on Inhibition of Heparin/FGF-Induced Angiogenesis into Matrigel® Basement Membrane Matrix *In Vivo*

BPI functional domain peptides of the invention are assayed for their ability to inhibit heparin-induced angiogenesis *in vivo* in mice. Liquid Matrigel®

(Collaborative Biomedical Products, Inc., Bedford, MA) is maintained at 4°C and angiogenic factors are added to the gel in the liquid state as described in Passaniti *et al.* (1992, *Lab. Invest.* 67: 519-528). Heparin (Sigma, St. Louis, MO) is dissolved in sterile PBS to various concentrations ranging from 1,250 - 10,000 U/mL. Recombinant fibroblast growth factor (bhFGF; BACHEM Bioscience Inc., Philadelphia, PA) is diluted to 200ng/mL with sterile PBS. A volume of 2.5μL dissolved heparin solution and 2.5μL recombinant bhFGF is added to 0.5mL Matrigel® per mouse injection. BPI functional domain peptides are added to this Matrigel® mixture at varying concentrations ranging from 0.5 to 50μg/mL (final concentration) in 10μL/ 0.5mL Matrigel® aliquot per experimental animal. Ten μL sterile PBS is substituted for BPI functional domain peptides in Matrigel® aliquots injected into control animals.

Male C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME) at 6-8 weeks of age are injected subcutaneously down the dorsal midline with 0.5mL aliquots of Matrigel® prepared as described above. Seven days after injection, the Matrigel® gels are excised and placed in 500μL Drabkin's reagent (Sigma). Total protein and hemoglobin content are determined for the gels stored in Drabkin's reagent after mechanical homogenization of the gels. Total protein levels are determined using a microplate assay that is commercially embodied in a kit (*DC* Protein Assay, Bio-Rad, Richmond, CA). Hemoglobin concentration is measured using Sigma Procedure #525 and reagents supplied by Sigma (St. Louis, MO) to be used with this procedure. Hemoglobin levels are expressed relative to total protein concentration.

Gels to be used for histological staining are formalin-fixed immediately after excision from the animals rather than being placed in Drabkin's reagent. Formalin-fixed gels are embedded in Tissue-Tek O.C.T. compound (Miles, Inc., Elkhart, IN) for frozen sectioning. Slides of frozen sections are stained with hematoxylin and eosin (as described by Humason, 1979, Animal Tissue Techniques, 4th Ed. W.H. feeman & Co., San Fransisco, CA, Ch.9, pp 111-131).

The effect of the BPI functional domain peptides of the invention are detected by microscopic examination of frozen stained sections for inhibition of angiogenesis relative to Matrigel® gel slices prepared without added BPI peptides. The extent of angiogenesis inhibition is quantitated using the normalized amounts of hemoglobin found in BPI peptide-containing gel slices.

EXAMPLE 22

Analysis of BPI Functional Domain Peptides in Chronic Inflammatory Disease: Collagen-Induced or Reactive Arthritis Models

BPI functional domain peptides are administered for their effects in a collagen-induced arthritis model. Specifically, arthritis is induced in mice by intradermal immunization of bovine Type II collagen at the base of the tail according to the method of Stuart *et al.* (1982, *J. Clin. Invest.* 69: 673-683). Generally, mice begin to develop arthritic symptoms at day 21 after collagen immunization. The arthritic scores of the treated mice are then evaluated in a blinded fashion over a period of 120 days for mice treated on each of days 21-25 with doses of either BPI functional domain peptides, control $rBPI_{23}$ or rBPI, or buffer which are injected intravenously *via* the tail vein.

Specifically, bovine Type II collagen (Southern Biotechnology Associates, Inc., Birmingham AL) is administered *via* intradermal injection (0.1 mg/mouse) at the base of the tail on day 0 to groups of male mice (Mouse/DBA/1J), each weighing approximately 20-25 g. BPI functional domain peptides, and $rBPI_{23}$ and rBPI are dissolved in a buffer comprised of 0.5M NaCl, 20mM sodium acetate (pH 6.0) and diluted with PBS buffer for administration at various concentrations. PBS buffer alone (0.1 mL) is administered as a control.

The collagen-induced arthritis model is also used to evaluate the performance of BPI functional domain peptides in comparison with protamine sulfate. Specifically, BPI peptides are dissolved in PBS as described above and administered at various concentrations. The other test materials are administered at the following dosages: protamine sulfate (Sigma Chemical Co., St. Louis, MO) (0.13 mg/mouse), thaumatin (0.12 mg/mouse), and PBS buffer (0.1 mL). Groups of mice receive test or control materials through intravenous injection *via* the tail vein on each of days 28 through 32 post-injection with collagen.

BPI functional domain peptides are also administered to treat reactive arthritis in a *Yersinia enterocolitica* reactive arthritis model according to the method of Yong *et al.* (1988, *Microbial Pathogenesis* 4: 305-310). Specifically, BPI peptides are administered to DBA/2J mice which have previously been injected intravenously with *Yersinia enterocolitica* cWA 0:8 T2 (*i.e.*, lacking the virulence plasmid according to Yong *et al.*, *supra*) at a dosage of 4 x 10^8 bacteria calculated to induce a non-septic arthritis in the mice. Groups of mice each receive test or control materials through intravenous injection via the tail vein.

Borrelia burgdorferi is the pathogen responsible for Lyme Disease and associated arthritis and it possesses an LPS-like complex on its cell walls which is different from but structurally related to that of *E. coli.* The effect of administration of BPI functional domain peptides on inhibition of *B. burgdorferi* LPS in a *Limulus* Amoebocyte Lysate (LAL) inhibition assay is determined. Specifically, an LAL assay according to the method of Example 4 is conducted measuring the effect of BPI peptides on *B. burgdorferi* LPS administered at 2.5μg/mL and *E. coli* 0113 LPS administered at 2ng/mL.

EXAMPLE 23

Analysis of BPI Functional Domain Peptides in Mouse Malignant Melanoma Cell Metastasis Model

BPI functional domain peptides, protamine, or buffer controls are administered to test their efficacy in a mouse malignant melanoma metastasis model. Specifically, groups of C57BL/6J mice are inoculated with 10^5 B16.F10 malignant melanoma cells *via* intravenous injection into the tail vein on day 0. BPI functional domain peptides in various concentrations are administered into the tail vein of test mice on days 1, 3, 6, 8, 10, 13, 15, 17, and 19. Protamine sulfate (0.13 mg/mouse) as a positive control, or PBS buffer (0.1 mL/mouse) as a negative control are similarly administered to additional groups of control mice. The animals are sacrificed *via* cervical dislocation on day 20 for observation of lung tissues. The lobes of each lung are perfused and inflated by injecting 3mL water into the lung *via* the trachea. Superficial tumor nodules are then counted with the aid of a dissecting microscope and the number of tumors found per group analyzed for statistically significant differences.

EXAMPLE 24

Analysis of BPI Functional Domain Peptides in a Mouse Cerebral Capillary Endothelial Cell Proliferation Assay

BPI functional domain peptides are tested for their effects in all endothelial cell proliferation assay. For these experiments, murine cerebral capillary endothelial cells (EC) as described in Bauer (1989, *Microvascular Research* 37: 148-161) are passaged in Medium 199 containing Earle's salts, L-glutamine and 2.2 g/L of sodium bicarbonate (GIBCO, Grand Island, NY), plus 10% heat inactivated fetal calf serum (FCS; Irvine Scientific, Irvine, CA) and 1% penicillin/ streptomycin (GIBCO). Harvesting of the confluent cells is performed by trypsinization with trypsin-EDTA (GIBCO) for 3 minutes. Trypsinization is stopped by adding 10 mL of the passage medium to the flask. Proliferation assays are performed on freshly harvested EC in standard flat bottom 96-well microtiter plates. A final volume of 200 μL/well is maintained for each well of the assay. A total of 4 x 10^4 EC cells is added to each well with varying concentrations of BPI peptides, or buffer control. After 48 hours of culture in a 5% CO_2 incubator, 1 μCi of [^{3}H] thymidine in 10 μL of Medium 199 is added to each well. After a 24 hour pulse, the EC cells are harvested by trypsinization onto glass microfiber filters and incorporated [^{3}H]thymidine is quantitated with a gas proportional solid phase beta counter.

Direct binding studies of BPI peptides on EC cells are performed by harvesting the 10-times passaged cells from a confluent flask and resuspending the trypsinized cells in 12.5 mL of culture medium. Then, 0.5mL of the cell suspension is added to each well of a standard 24 well tissue culture plate and incubated overnight. The plate is washed with 0.1% bovine serum albumin in phosphate buffered saline containing calcium and magnesium (GIBCO). After washing, 0.5mL BSA/PBS is added per well. Concentration dependent inhibition of EC cell proliferation is measured in terms of decreases in [^{3}H]-thymidine uptake.

EXAMPLE 25

Analysis of BPI Function Domain Peptides in Animal Models

A. Analysis in a Mouse Endotoxemia Model

BPI functional domain peptides are tested for their efficacy in a mouse experimental endotoxemia model. Groups of at least 15 mice are administered an intravenous injection of endotoxin (e.g., *E. coli* 0111:B4, Sigma Chemical Co., St. Louis, MO) at a LD_{90} dosage (e.g., 40 mg/kg). This is followed by a second intravenous injection of the test peptide in varying concentrations from about 0.1 mg/kg to about 100 mg/kg, preferably in the range of about 1 to 50 mg/kg. Injections of buffer without added peptide are used in negative control mice. The animals are observed for 7 days and mortality recorded. The efficacy of the peptides of this invention is measured by a decrease in endotoxemia-associated mortality in peptide-injected mice as compared with control mice.

B. Analysis in a Mouse Peritonitis Model

BPI functional domain peptides are tested for their efficacy in a mouse model of acute peritonitis. Groups of at least 15 mice are challenged with 10^7 live *E. coli* bacteria strain 07:K1 in 0.5 mL and then treated with 1.0 mL of a solution of BPI functional domain peptides at varying concentrations from about 0.1 mg/kg to about 100 mg/kg. Injections of buffer without added peptide are used in negative control mice. The animals are observed for 7 days and mortality recorded. Effective BPI functional domain peptides show a decrease in mortality of test group mice compared with control group mice.

EXAMPLE 26

Therapeutic Use of BPI Functional Domain Peptides in a Human *In vivo* Endotoxin Neutralization Model

A controlled, double-blind crossover study is designed and conducted as in WO95/19784 (PCT/US95/01151), to investigate the effects of BPI functional domain peptides in humans rendered endotoxemic by intravenous infusion of bacterial endotoxin.

It should be understood that the foregoing disclosure emphasizes certain specific embodiments of the invention and that all modifications or alternatives equivalent thereto are within the spirit and scope of the invention as set forth in the appended claims.

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(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "Domain I"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
Ala Ser Gln Gln Gly Thr Ala Ala Leu Gln Lys Glu Leu Lys Arg Ile
1               5                   10                  15

Lys Ile Pro Asp Tyr Ser Asp Ser Phe Lys Ile Lys His
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.14"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Gly Thr Ala Ala Leu Gln Lys Glu Leu Lys Arg Ile Lys Ile Pro Asp
1               5                   10                  15

Tyr Ser Asp Ser Phe Lys Ile Lys His Leu Gly Lys Gly His
            20                  25                  30
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.4"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Leu Gln Lys Glu Leu Lys Arg Ile Lys Ile Pro Asp Tyr Ser Asp Ser
1               5                   10                  15

Phe Lys Ile Lys His Leu
            20
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.1"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Gln Gln Gly Thr Ala Ala Leu Gln Lys Glu Leu Lys Arg Ile Lys
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.54"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Gly Thr Ala Ala Leu Gln Lys Glu Leu Lys Arg Ile Lys Ile Pro
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "Domain II"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Ser Ser Gln Ile Ser Met Val Pro Asn Val Gly Leu Lys Phe Ser Ile
1               5                   10                  15

Ser Asn Ala Asn Ile Lys Ile Ser Gly Lys Trp Lys Ala Gln Lys Arg
            20                  25                  30

Phe Leu Lys
        35
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.2"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Ile Lys Ile Ser Gly Lys Trp Lys Ala Gln Lys Arg Phe Leu Lys
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.8"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Lys Trp Lys Ala Gln Lys Arg Phe Leu Lys
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.58"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
Cys Ile Lys Ile Ser Gly Lys Trp Lys Ala Gln Lys Arg Phe Leu
1               5                   10                  15

Lys
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.65 oxidized"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Cys Ile Lys Ile Ser Gly Lys Trp Lys Ala Gln Lys Arg Phe Leu
1               5                   10                  15

Lys Cys
```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
    (A) NAME/KEY: misc_feature
    (D) OTHER INFORMATION: "BPI.3"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
Asn Val Gly Leu Lys Phe Ser Ile Ser Asn Ala Asn Ile Lys Ile Ser
1               5                   10                  15

Gly Lys Trp Lys Ala Gln Lys Arg Phe Leu Lys
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 28 amino acids
    (B) TYPE: amino acid
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
    (A) NAME/KEY: misc_feature
    (D) OTHER INFORMATION: "Domain III"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
Val His Val His Ile Ser Lys Ser Lys Val Gly Trp Leu Ile Gln Leu
1               5                   10                  15

Phe His Lys Lys Ile Glu Ser Ala Leu Arg Asn Lys
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 13 amino acids
    (B) TYPE: amino acid
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
    (A) NAME/KEY: misc_feature
    (D) OTHER INFORMATION: "BPI.11"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
Lys Ser Lys Val Trp Leu Ile Gln Leu Phe His Lys Lys
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.12"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
Ser Val His Val His Ile Ser Lys Ser Lys Val Gly Trp Leu Ile Gln
1               5                   10                  15

Leu Phe His Lys Lys Ile Glu Ser Ala Leu Arg Asn Lys
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.13"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```
Lys Ser Lys Val Gly Trp Leu Ile Gln Leu Phe His Lys Lys
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.15"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```
Ala Lys Ile Ser Gly Lys Trp Lys Ala Gln Lys Arg Phe Leu Lys
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.16"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

```
Ile Ala Ile Ser Gly Lys Trp Lys Ala Gln Lys Arg Phe Leu Lys
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.17"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

```
Ile Lys Ala Ser Gly Lys Trp Lys Ala Gln Lys Arg Phe Leu Lys
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.18"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

```
Ile Lys Ile Ala Gly Lys Trp Lys Ala Gln Lys Arg Phe Leu Lys
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.19"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

```
Ile Lys Ile Ser Ala Lys Trp Lys Ala Gln Lys Arg Phe Leu Lys
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.20"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

```
Ile Lys Ile Ser Gly Ala Trp Lys Ala Gln Lys Arg Phe Leu Lys
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids

(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.21"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

```
Ile Lys Ile Ser Gly Lys Ala Lys Ala Gln Lys Arg Phe Leu Lys
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.22"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

```
Ile Lys Ile Ser Gly Lys Trp Ala Ala Gln Lys Arg Phe Leu Lys
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.23"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

```
Ile Lys Ile Ser Gly Lys Trp Lys Ala Ala Lys Arg Phe Leu Lys
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.24"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

```
Ile Lys Ile Ser Gly Lys Trp Lys Ala Gln Ala Arg Phe Leu Lys
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.25"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

```
Ile Lys Ile Ser Gly Lys Trp Lys Ala Gln Lys Ala Phe Leu Lys
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.26"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

```
Ile Lys Ile Ser Gly Lys Trp Lys Ala Gln Lys Arg Ala Leu Lys
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.27"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

```
Ile Lys Ile Ser Gly Lys Trp Lys Ala Gln Lys Arg Phe Ala Lys
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.28"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

```
Ile Lys Ile Ser Gly Lys Trp Lys Ala Gln Lys Arg Phe Leu Ala
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.59"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Ile Lys Ile Ser Gly Ala Trp Ala Ala Gln Lys Arg Phe Leu Lys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.45"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Ile Lys Ile Ser Gly Lys Trp Lys Ala Ala Ala Arg Phe Leu Lys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.60"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Ile Ala Ile Ser Gly Lys Trp Lys Ala Gln Lys Arg Phe Leu Ala
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.31"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

```
Ala Ser Lys Val Gly Trp Leu Ile Gln Leu Phe His Lys Lys
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.32"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

```
Lys Ala Lys Val Gly Trp Leu Ile Gln Leu Phe His Lys Lys
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.33"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

```
Lys Ser Ala Val Gly Trp Leu Ile Gln Leu Phe His Lys Lys
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.34"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

```
Lys Ser Lys Ala Gly Trp Leu Ile Gln Leu Phe His Lys Lys
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.35"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

```
Lys Ser Lys Val Ala Trp Leu Ile Gln Leu Phe His Lys Lys
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.36"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

```
Lys Ser Lys Val Gly Ala Leu Ile Gln Leu Phe His Lys Lys
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.37"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

```
Lys Ser Lys Val Gly Trp Ala Ile Gln Leu Phe His Lys Lys
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.38"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

```
Lys Ser Lys Val Gly Trp Leu Ala Gln Leu Phe His Lys Lys
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.39"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

```
Lys Ser Lys Val Gly Trp Leu Ile Ala Leu Phe His Lys Lys
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids

(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.40"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

```
Lys Ser Lys Val Gly Trp Leu Ile Gln Ala Phe His Lys Lys
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.41"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

```
Lys Ser Lys Val Gly Trp Leu Ile Gln Leu Ala His Lys Lys
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.42"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

```
Lys Ser Lys Val Gly Trp Leu Ile Gln Leu Phe Ala Lys Lys
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.43"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

```
Lys Ser Lys Val Gly Trp Leu Ile Gln Leu Phe His Ala Lys
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.44"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

```
Lys Ser Lys Val Gly Trp Leu Ile Gln Leu Phe His Lys Ala
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.56"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

```
Ile Lys Ile Ser Gly Lys Trp Lys Ala Lys Gln Arg Phe Leu Lys
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.61"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

```
Ile Lys Ile Ser Gly Lys Phe Lys Ala Gln Lys Arg Phe Leu Lys
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.66"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 5..7
(D) OTHER INFORMATION: /label= D-Trp
/note= "The amino acid at position 7 is
D-tryptophan"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

```
Ile Lys Ile Ser Gly Lys Trp Lys Ala Gln Lys Arg Phe Leu Lys
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.67"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 6..8
(D) OTHER INFORMATION: /label= Substituted-Ala
/note= "The alanine at position 7 is
beta-1-naphthyl-substituted"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

```
Ile Lys Ile Ser Gly Lys Ala Lys Ala Gln Lys Arg Phe Leu Lys
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.9"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

```
Lys Arg Phe Leu Lys Lys Trp Lys Ala Gln Lys Arg Phe Leu Lys
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.30"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

```
Lys Trp Lys Ala Gln Lys Arg Phe Leu Lys Lys Ser Lys Val Gly Trp
1               5                   10                  15

Leu Ile Gln Leu Phe His Lys Lys
            20
```

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.63"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

```
Ile Lys Ile Ser Gly Lys Trp Lys Ala Gln Lys Arg Phe Leu Lys Lys
1               5                   10                  15

Ser Lys Val Gly Trp Leu Ile Gln Leu Phe His Lys Lys
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.7"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

```
Lys Trp Lys Ala Gln Lys Arg Phe Leu Lys Lys Trp Lys Ala Gln Lys
1               5                   10                  15

Arg Phe Leu Lys
            20
```

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.10.1"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

```
Lys Arg Phe Leu Lys Lys Trp Lys Ala Gln Lys Arg Phe Leu Lys Lys
1               5                   10                  15

Trp Lys Ala Gln Lys Arg Phe Leu Lys
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.29"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

```
Lys Ser Lys Val Gly Trp Leu Ile Gln Leu Phe His Lys Lys Lys Ser
1               5                   10                  15

Lys Val Gly Trp Leu Ile Gln Leu Phe His Lys Lys
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.46"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

```
Lys Trp Lys Ala Ala Ala Arg Phe Leu Lys Lys Trp Lys Ala Gln Lys
1               5                   10                  15

Arg Phe Leu Lys
            20
```

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.47"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

```
Lys Trp Lys Ala Gln Lys Arg Phe Leu Lys Lys Trp Lys Ala Ala Ala
1               5                   10                  15

Arg Phe Leu Lys
            20
```

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.48"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

```
Lys Trp Lys Ala Ala Ala Arg Phe Leu Lys Lys Trp Lys Ala Ala Ala
1               5                   10                  15

Arg Phe Leu Lys
            20
```

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.69"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

```
Lys Trp Lys Ala Ala Ala Arg Phe Leu Lys Lys Trp Lys Ala Ala Ala
1               5                   10                  15

Arg Phe Leu Lys Lys Trp Lys Ala Ala Ala Arg Phe Leu Lys
            20                  25                  30
```

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.55"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

```
Gly Trp Leu Ile Gln Leu Phe His Lys Lys Ile Glu Ser Ala Leu Arg
1               5                   10                  15

Asn Lys Met Asn Ser
            20
```

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.73"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

```
Ile Lys Ile Ser Gly Lys Trp Lys Ala Gln Phe Arg Phe Leu Lys
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.70"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 8..10
(D) OTHER INFORMATION: /label= Substituted-Ala
/note= "The alanine at position 7 is
beta-3-pyridyl-substituted"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

```
Ile Lys Ile Ser Gly Lys Ala Lys Ala Gln Lys Arg Phe Leu Lys
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.71"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 13..15
(D) OTHER INFORMATION: /label= Substituted-Ala

/note= "The alanine at position 13 is beta-3-pyridyl-substituted"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

```
Ile Lys Ile Ser Gly Lys Trp Lys Ala Gln Lys Arg Ala Leu Lys
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.10.2"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

```
Gln Lys Arg Phe Leu Lys Lys Trp Lys Ala Gln Lys Arg Phe Leu Lys
1               5                   10                  15

Lys Trp Lys Ala Gln Lys Arg Phe Leu Lys
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.72"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1..3
(D) OTHER INFORMATION: /label= D-alanine
/note= "The position 1 and position 2 alanine residues are both D-alanine"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

Ala Ala Ile Lys Ile Ser Gly Lys Trp Lys Ala Gln Lys Arg Phe Leu
1 5 10 15

Lys

(2) INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.5"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

Val His Val His Ile Ser Lys Ser Lys Val Gly Trp Leu Ile Gln Leu
1 5 10 15

Phe His Lys Lys Ile Glu
20

(2) INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.65 reduced"

(ix) FEATURE:
(A) NAME/KEY: Disulfide-bond
(B) LOCATION: 1..17

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

Cys Ile Lys Ile Ser Gly Lys Trp Lys Ala Gln Lys Arg Phe Leu Lys
1 5 10 15

Cys

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 487 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "rBPI"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

```
Met Arg Glu Asn Met Ala Arg Gly Pro Cys Asn Ala Pro Arg Trp Val
-31 -30                 -25                 -20

Ser Leu Met Val Leu Val Ala Ile Gly Thr Ala Val Thr Ala Ala Val
-15                 -10                  -5                   1

Asn Pro Gly Val Val Val Arg Ile Ser Gln Lys Gly Leu Asp Tyr Ala
              5                  10                  15

Ser Gln Gln Gly Thr Ala Ala Leu Gln Lys Glu Leu Lys Arg Ile Lys
         20                  25                  30

Ile Pro Asp Tyr Ser Asp Ser Phe Lys Ile Lys His Leu Gly Lys Gly
     35                  40                  45

His Tyr Ser Phe Tyr Ser Met Asp Ile Arg Glu Phe Gln Leu Pro Ser
 50                  55                  60                  65

Ser Gln Ile Ser Met Val Pro Asn Val Gly Leu Lys Phe Ser Ile Ser
                 70                  75                  80

Asn Ala Asn Ile Lys Ile Ser Gly Lys Trp Lys Ala Gln Lys Arg Phe
             85                  90                  95

Leu Lys Met Ser Gly Asn Phe Asp Leu Ser Ile Glu Gly Met Ser Ile
        100                 105                 110

Ser Ala Asp Leu Lys Leu Gly Ser Asn Pro Thr Ser Gly Lys Pro Thr
    115                 120                 125

Ile Thr Cys Ser Ser Cys Ser Ser His Ile Asn Ser Val His Val His
130                 135                 140                 145

Ile Ser Lys Ser Lys Val Gly Trp Leu Ile Gln Leu Phe His Lys Lys
                150                 155                 160

Ile Glu Ser Ala Leu Arg Asn Lys Met Asn Ser Gln Val Cys Glu Lys
            165                 170                 175
```

```
Val Thr Asn Ser Val Ser Ser Lys Leu Gln Pro Tyr Phe Gln Thr Leu
        180                 185                 190

Pro Val Met Thr Lys Ile Asp Ser Val Ala Gly Ile Asn Tyr Gly Leu
    195                 200                 205

Val Ala Pro Pro Ala Thr Thr Ala Glu Thr Leu Asp Val Gln Met Lys
210                 215                 220                 225

Gly Glu Phe Tyr Ser Glu Asn His His Asn Pro Pro Pro Phe Ala Pro
                230                 235                 240

Pro Val Met Glu Phe Pro Ala Ala His Asp Arg Met Val Tyr Leu Gly
            245                 250                 255

Leu Ser Asp Tyr Phe Phe Asn Thr Ala Gly Leu Val Tyr Gln Glu Ala
        260                 265                 270

Gly Val Leu Lys Met Thr Leu Arg Asp Asp Met Ile Pro Lys Glu Ser
    275                 280                 285

Lys Phe Arg Leu Thr Thr Lys Phe Phe Gly Thr Phe Leu Pro Glu Val
290                 295                 300                 305

Ala Lys Lys Phe Pro Asn Met Lys Ile Gln Ile His Val Ser Ala Ser
                310                 315                 320

Thr Pro Pro His Leu Ser Val Gln Pro Thr Gly Leu Thr Phe Tyr Pro
            325                 330                 335

Ala Val Asp Val Gln Ala Phe Ala Val Leu Pro Asn Ser Ser Leu Ala
        340                 345                 350

Ser Leu Phe Leu Ile Gly Met His Thr Thr Gly Ser Met Glu Val Ser
    355                 360                 365

Ala Glu Ser Asn Arg Leu Val Gly Glu Leu Lys Leu Asp Arg Leu Leu
370                 375                 380                 385

Leu Glu Leu Lys His Ser Asn Ile Gly Pro Phe Pro Val Glu Leu Leu
                390                 395                 400

Gln Asp Ile Met Asn Tyr Ile Val Pro Ile Leu Val Leu Pro Arg Val
            405                 410                 415

Asn Glu Lys Leu Gln Lys Gly Phe Pro Leu Pro Thr Pro Ala Arg Val
        420                 425                 430

Gln Leu Tyr Asn Val Val Leu Gln Pro His Gln Asn Phe Leu Leu Phe
    435                 440                 445

Gly Ala Asp Val Val Tyr Lys
450                 455
```

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.74"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

```
Lys Ser Lys Val Gly Trp Leu Ile Gln Leu Phe His Lys Lys Lys
1               5                   10                  15

Trp Lys Ala Gln Lys Arg Phe Leu Lys
                20
```

(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.76"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 10..12
(D) OTHER INFORMATION: /label= D-Phe
/note= "The amino acid at position 11 is D-phenylalanine"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

```
Ile Lys Ile Ser Gly Lys Trp Lys Ala Gln Phe Arg Phe Leu Lys
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.77"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

```
Ile Lys Ile Ser Gly Lys Trp Lys Ala Gln Trp Arg Phe Leu Lys
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:73:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.79"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

```
Ile Lys Ile Ser Gly Lys Trp Lys Ala Lys Lys Arg Phe Leu Lys
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:74:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.80"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 10..12

(D) OTHER INFORMATION: /label= Substituted-Ala
/note= "The alanine at position 11 is
beta-1-naphthyl-substituted"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

```
Ile Lys Ile Ser Gly Lys Trp Lys Ala Gln Ala Arg Phe Leu Lys
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:75:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.81"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

```
Ile Lys Ile Ser Gly Lys Trp Lys Ala Phe Lys Arg Phe Leu Lys
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:76:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.82"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

```
Lys Ser Lys Val Gly Trp Leu Ile Gln Leu Trp His Lys Lys
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:77:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.83"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 10..12
(D) OTHER INFORMATION: /label= Substituted-Ala
/note= "The alanine at position 6 is beta-1-naphthyl-substituted"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

```
Lys Ser Lys Val Gly Ala Lys Ile Gln Leu Phe His Lys Lys
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:78:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.84"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 6..8
(D) OTHER INFORMATION: /label= Substituted-Ala
/note= "The alanine at position 7 is beta-1-naphthyl-substituted"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

```
Ile Lys Ile Ser Gly Lys Ala Lys Ala Gln Phe Arg Phe Leu Lys
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:79:

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 14 amino acids
    (B) TYPE: amino acid
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
    (A) NAME/KEY: misc_feature
    (D) OTHER INFORMATION: "BPI.85"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

```
Lys Ser Lys Val Leu Trp Leu Ile Gln Leu Phe His Lys Lys
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:80:

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 14 amino acids
    (B) TYPE: amino acid
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
    (A) NAME/KEY: misc_feature
    (D) OTHER INFORMATION: "BPI.86"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

```
Lys Ser Lys Val Gly Trp Leu Ile Leu Leu Phe His Lys Lys
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:81:

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 14 amino acids
    (B) TYPE: amino acid
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
    (A) NAME/KEY: misc_feature
    (D) OTHER INFORMATION: "BPI.87"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

```
Lys Ser Lys Val Gly Trp Leu Ile Gln Leu Phe Leu Lys Lys
```

1 5 10

(2) INFORMATION FOR SEQ ID NO:82:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.88"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

```
Ile Lys Ile Ser Gly Lys Trp Lys Ala Phe Phe Arg Phe Leu Lys
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:83:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.98"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 6..8
(D) OTHER INFORMATION: /label= Substituted-Ala
/note= "The alanine at position 7 is
beta-1-naphthyl-substituted"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

```
Ile Lys Ile Ser Gly Lys Ala Lys Ala Gln Phe Arg Phe Leu Lys
1               5                   10                  15

Lys Ser Lys Val Gly Trp Leu Ile Phe Leu Phe His Lys Lys
                20                  25
```

(2) INFORMATION FOR SEQ ID NO:84:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.89"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 6..8
(D) OTHER INFORMATION: /label= Substituted-Ala
/note= "The alanine at position 7 is
beta-1-naphthyl-substituted"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

```
Ile Lys Ile Ser Gly Lys Ala Lys Ala Phe Lys Arg Phe Leu Lys
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:85:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.90"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 6..8
(D) OTHER INFORMATION: /label= Substituted-Ala
/note= "The alanine at position 7 is
beta-1-naphthyl-substituted"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

```
Ile Lys Ile Ser Gly Lys Ala Lys Ala Phe Phe Arg Phe Leu Lys
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:86:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.91"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

```
Lys Ser Lys Val Gly Trp Leu Ile Phe Leu Phe His Lys Lys
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:87:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.92"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

```
Lys Ser Lys Val Gly Trp Leu Ile Lys Leu Phe His Lys Lys
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:88:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.93"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 6..8
(D) OTHER INFORMATION: /label= Substituted-Ala
/note= "The alanine at position 7 is beta-1-naphthyl-substituted"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

```
Ile Lys Ile Ser Gly Lys Ala Lys Ala Gln Phe Arg Phe Leu Lys
1               5                   10                  15

Lys Ser Lys Val Gly Trp Leu Ile Gln Leu Phe His Lys Lys
                20                  25
```

(2) INFORMATION FOR SEQ ID NO:89:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.94"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

```
Lys Ser Lys Val Gly Trp Leu Ile Gln Leu Phe Phe Lys Lys
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:90:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.95"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:

```
Lys Ser Lys Val Phe Trp Leu Ile Gln Leu Phe His Lys Lys
```

1 5 10

(2) INFORMATION FOR SEQ ID NO:91:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.96"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:91:

```
Lys Ser Lys Val Gly Trp Leu Ile Gln Leu Phe His Lys Phe
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:92:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.97"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:

```
Lys Ser Lys Val Lys Trp Leu Ile Gln Leu Phe His Lys Lys
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:93:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.99"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

```
Lys Trp Lys Ala Gln Trp Arg Phe Leu Lys Lys Trp Lys Ala Gln
1               5                   10                  15

Trp Arg Phe Leu Lys Lys Trp Lys Ala Gln Trp Arg Phe Leu Lys
            20                  25                  30
```

(2) INFORMATION FOR SEQ ID NO:94:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.100"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:94:

```
Lys Ser Lys Val Lys Trp Leu Ile Lys Leu Phe His Lys Lys
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:95:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.101"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:95:

```
Lys Ser Lys Val Lys Trp Leu Ile Lys Leu Phe Phe Lys Phe Lys Ser
1               5                   10                  15

Lys Val Lys Trp Leu Ile Lys Leu Phe Phe Lys Phe
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:96:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(D) OTHER INFORMATION: "BPI.102"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:96:

```
Lys Trp Lys Ala Gln Phe Arg Phe Leu Lys Lys Ser Lys Val Gly Trp
1               5                   10                  15

Leu Ile Leu Leu Phe His Lys Lys
            20
```

(2) INFORMATION FOR SEQ ID NO:97:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1443 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1443

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 76..1443

(ix) FEATURE:
(A) NAME/KEY: misc_feature

(D) OTHER INFORMATION: "rLBP"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:97:

```
ATG GGG GCC TTG GCC AGA GCC CTG CCG TCC ATA CTG CTG GCA TTG CTG      48
Met Gly Ala Leu Ala Arg Ala Leu Pro Ser Ile Leu Leu Ala Leu Leu
-25                 -20                 -15                 -10

CTT ACG TCC ACC CCA GAG GCT CTG GGT GCC AAC CCC GGC TTG GTC GCC      96
```

```
Leu Thr Ser Thr Pro Glu Ala Leu Gly Ala Asn Pro Gly Leu Val Ala
                -5                  1               5

AGG ATC ACC GAC AAG GGA CTG CAG TAT GCG GCC CAG GAG GGG CTA TTG         144
Arg Ile Thr Asp Lys Gly Leu Gln Tyr Ala Ala Gln Glu Gly Leu Leu
        10                  15                  20

GCT CTG CAG AGT GAG CTG CTC AGG ATC ACG CTG CCT GAC TTC ACC GGG         192
Ala Leu Gln Ser Glu Leu Leu Arg Ile Thr Leu Pro Asp Phe Thr Gly
    25                  30                  35

GAC TTG AGG ATC CCC CAC GTC GGC CGT GGG CGC TAT GAG TTC CAC AGC         240
Asp Leu Arg Ile Pro His Val Gly Arg Gly Arg Tyr Glu Phe His Ser
40                  45                  50                  55

CTG AAC ATC CAC AGC TGT GAG CTG CTT CAC TCT GCG CTG AGG CCT GTC         288
Leu Asn Ile His Ser Cys Glu Leu Leu His Ser Ala Leu Arg Pro Val
                60                  65                  70

CCT GGC CAG GGC CTG AGT CTC AGC ATC TCC GAC TCC TCC ATC CGG GTC         336
Pro Gly Gln Gly Leu Ser Leu Ser Ile Ser Asp Ser Ser Ile Arg Val
            75                  80                  85

CAG GGC AGG TGG AAG GTG CGC AAG TCA TTC TTC AAA CTA CAG GGC TCC         384
Gln Gly Arg Trp Lys Val Arg Lys Ser Phe Phe Lys Leu Gln Gly Ser
        90                  95                  100

TTT GAT GTC AGT GTC AAG GGC ATC AGC ATT TCG GTC AAC CTC CTG TTG         432
Phe Asp Val Ser Val Lys Gly Ile Ser Ile Ser Val Asn Leu Leu Leu
    105                 110                 115

GGC AGC GAG TCC TCC GGG AGG CCC ACA GTT ACT GCC TCC AGC TGC AGC         480
Gly Ser Glu Ser Ser Gly Arg Pro Thr Val Thr Ala Ser Ser Cys Ser
120                 125                 130                 135

AGT GAC ATC GCT GAC GTG GAG GTG GAC ATG TCG GGA GAC TTG GGG TGG         528
Ser Asp Ile Ala Asp Val Glu Val Asp Met Ser Gly Asp Leu Gly Trp
                140                 145                 150

CTG TTG AAC CTC TTC CAC AAC CAG ATT GAG TCC AAG TTC CAG AAA GTA         576
Leu Leu Asn Leu Phe His Asn Gln Ile Glu Ser Lys Phe Gln Lys Val
            155                 160                 165

CTG GAG AGC AGG ATT TGC GAA ATG ATC CAG AAA TCG GTG TCC TCC GAT         624
Leu Glu Ser Arg Ile Cys Glu Met Ile Gln Lys Ser Val Ser Ser Asp
        170                 175                 180

CTA CAG CCT TAT CTC CAA ACT CTG CCA GTT ACA ACA GAG ATT GAC AGT         672
Leu Gln Pro Tyr Leu Gln Thr Leu Pro Val Thr Thr Glu Ile Asp Ser
    185                 190                 195

TTC GCC GAC ATT GAT TAT AGC TTA GTG GAA GCC CCT CGG GCA ACA GCC         720
Phe Ala Asp Ile Asp Tyr Ser Leu Val Glu Ala Pro Arg Ala Thr Ala
200                 205                 210                 215
```

```
CAG ATG CTG GAG GTG ATG TTT AAG GGT GAA ATC TTT CAT CGT AAC CAC        768
Gln Met Leu Glu Val Met Phe Lys Gly Glu Ile Phe His Arg Asn His
                220                 225                 230

CGT TCT CCA GTT ACC CTC CTT GCT GCA GTC ATG AGC CTT CCT GAG GAA        816
Arg Ser Pro Val Thr Leu Leu Ala Ala Val Met Ser Leu Pro Glu Glu
            235                 240                 245

CAC AAC AAA ATG GTC TAC TTT GCC ATC TCG GAT TAT GTC TTC AAC ACG        864
His Asn Lys Met Val Tyr Phe Ala Ile Ser Asp Tyr Val Phe Asn Thr
        250                 255                 260

GCC AGC CTG GTT TAT CAT GAG GAA GGA TAT CTG AAC TTC TCC ATC ACA        912
Ala Ser Leu Val Tyr His Glu Glu Gly Tyr Leu Asn Phe Ser Ile Thr
    265                 270                 275

GAT GAG ATG ATA CCG CCT GAC TCT AAT ATC CGA CTG ACC ACC AAG TCC        960
Asp Glu Met Ile Pro Pro Asp Ser Asn Ile Arg Leu Thr Thr Lys Ser
280                 285                 290                 295

TTC CGA CCC TTC GTC CCA CGG TTA GCC AGG CTC TAC CCC AAC ATG AAC       1008
Phe Arg Pro Phe Val Pro Arg Leu Ala Arg Leu Tyr Pro Asn Met Asn
                300                 305                 310

CTG GAA CTC CAG GGA TCA GTG CCC TCT GCT CCG CTC CTG AAC TTC AGC       1056
Leu Glu Leu Gln Gly Ser Val Pro Ser Ala Pro Leu Leu Asn Phe Ser
            315                 320                 325

CCT GGG AAT CTG TCT GTG GAC CCC TAT ATG GAG ATA GAT GCC TTT GTG       1104
Pro Gly Asn Leu Ser Val Asp Pro Tyr Met Glu Ile Asp Ala Phe Val
        330                 335                 340

CTC CTG CCC AGC TCC AGC AAG GAG CCT GTC TTC CGG CTC AGT GTG GCC       1152
Leu Leu Pro Ser Ser Ser Lys Glu Pro Val Phe Arg Leu Ser Val Ala
    345                 350                 355

ACT AAT GTG TCC GCC ACC TTG ACC TTC AAT ACC AGC AAG ATC ACT GGG       1200
Thr Asn Val Ser Ala Thr Leu Thr Phe Asn Thr Ser Lys Ile Thr Gly
360                 365                 370                 375

TTC CTG AAG CCA GGA AAG GTA AAA GTG GAA CTG AAA GAA TCC AAA GTT       1248
Phe Leu Lys Pro Gly Lys Val Lys Val Glu Leu Lys Glu Ser Lys Val
                380                 385                 390

GGA CTA TTC AAT GCA GAG CTG TTG GAA GCG CTC CTC AAC TAT TAC ATC       1296
Gly Leu Phe Asn Ala Glu Leu Leu Glu Ala Leu Leu Asn Tyr Tyr Ile
            395                 400                 405

CTT AAC ACC TTC TAC CCC AAG TTC AAT GAT AAG TTG GCC GAA GGC TTC       1344
Leu Asn Thr Phe Tyr Pro Lys Phe Asn Asp Lys Leu Ala Glu Gly Phe
        410                 415                 420

CCC CTT CCT CTG CTG AAG CGT GTT CAG CTC TAC GAC CTT GGG CTG CAG       1392
Pro Leu Pro Leu Leu Lys Arg Val Gln Leu Tyr Asp Leu Gly Leu Gln
```

```
    425                        430                        435

ATC CAT AAG GAC TTC CTG TTC TTG GGT GCC AAT GTC CAA TAC ATG AGA        1440
Ile His Lys Asp Phe Leu Phe Leu Gly Ala Asn Val Gln Tyr Met Arg
440                 445                 450                 455

GTT                                                                    1443
Val
```

(2) INFORMATION FOR SEQ ID NO:98:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 481 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: misc_feature

(D) OTHER INFORMATION: "rLBP"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:98:

```
Met Gly Ala Leu Ala Arg Ala Leu Pro Ser Ile Leu Leu Ala Leu Leu
-25                 -20                 -15                 -10

Leu Thr Ser Thr Pro Glu Ala Leu Gly Ala Asn Pro Gly Leu Val Ala
                 -5                   1               5

Arg Ile Thr Asp Lys Gly Leu Gln Tyr Ala Ala Gln Glu Gly Leu Leu
         10                  15                  20

Ala Leu Gln Ser Glu Leu Leu Arg Ile Thr Leu Pro Asp Phe Thr Gly
     25                  30                  35

Asp Leu Arg Ile Pro His Val Gly Arg Gly Arg Tyr Glu Phe His Ser
 40                  45                  50                  55

Leu Asn Ile His Ser Cys Glu Leu Leu His Ser Ala Leu Arg Pro Val
                 60                  65                  70

Pro Gly Gln Gly Leu Ser Leu Ser Ile Ser Asp Ser Ser Ile Arg Val
             75                  80                  85

Gln Gly Arg Trp Lys Val Arg Lys Ser Phe Phe Lys Leu Gln Gly Ser
         90                  95                 100

Phe Asp Val Ser Val Lys Gly Ile Ser Ile Ser Val Asn Leu Leu Leu
    105                 110                 115
```

```
Gly Ser Glu Ser Ser Gly Arg Pro Thr Val Thr Ala Ser Ser Cys Ser
120                 125                 130                 135

Ser Asp Ile Ala Asp Val Glu Val Asp Met Ser Gly Asp Leu Gly Trp
                140                 145                 150

Leu Leu Asn Leu Phe His Asn Gln Ile Glu Ser Lys Phe Gln Lys Val
            155                 160                 165

Leu Glu Ser Arg Ile Cys Glu Met Ile Gln Lys Ser Val Ser Ser Asp
        170                 175                 180

Leu Gln Pro Tyr Leu Gln Thr Leu Pro Val Thr Thr Glu Ile Asp Ser
    185                 190                 195

Phe Ala Asp Ile Asp Tyr Ser Leu Val Glu Ala Pro Arg Ala Thr Ala
200                 205                 210                 215

Gln Met Leu Glu Val Met Phe Lys Gly Glu Ile Phe His Arg Asn His
                220                 225                 230

Arg Ser Pro Val Thr Leu Leu Ala Ala Val Met Ser Leu Pro Glu Glu
            235                 240                 245

His Asn Lys Met Val Tyr Phe Ala Ile Ser Asp Tyr Val Phe Asn Thr
        250                 255                 260

Ala Ser Leu Val Tyr His Glu Glu Gly Tyr Leu Asn Phe Ser Ile Thr
    265                 270                 275

Asp Glu Met Ile Pro Pro Asp Ser Asn Ile Arg Leu Thr Thr Lys Ser
280                 285                 290                 295

Phe Arg Pro Phe Val Pro Arg Leu Ala Arg Leu Tyr Pro Asn Met Asn
                300                 305                 310

Leu Glu Leu Gln Gly Ser Val Pro Ser Ala Pro Leu Leu Asn Phe Ser
            315                 320                 325

Pro Gly Asn Leu Ser Val Asp Pro Tyr Met Glu Ile Asp Ala Phe Val
        330                 335                 340

Leu Leu Pro Ser Ser Ser Lys Glu Pro Val Phe Arg Leu Ser Val Ala
    345                 350                 355

Thr Asn Val Ser Ala Thr Leu Thr Phe Asn Thr Ser Lys Ile Thr Gly
360                 365                 370                 375

Phe Leu Lys Pro Gly Lys Val Lys Val Glu Leu Lys Glu Ser Lys Val
                380                 385                 390

Gly Leu Phe Asn Ala Glu Leu Leu Glu Ala Leu Leu Asn Tyr Tyr Ile
            395                 400                 405
```

```
Leu Asn Thr Phe Tyr Pro Lys Phe Asn Asp Lys Leu Ala Glu Gly Phe
        410                 415                 420

Pro Leu Pro Leu Leu Lys Arg Val Gln Leu Tyr Asp Leu Gly Leu Gln
    425                 430                 435

Ile His Lys Asp Phe Leu Phe Leu Gly Ala Asn Val Gln Tyr Met Arg
440                 445                 450                 455

Val
```

CLAIMS:

1. A peptide which is or substantially is an amino acid sequence of human bactericidal permeability increasing protein (BPI) from position 17 to position 45, a subsequence thereof or a substitution or addition or deletion variant of the sequence or the subsequence thereof, the peptide having at least one of the biological activities of BPI, namely heparin binding, heparin neutralisation, LPS binding, LPS neutralisation or bactericidal activity or having fungicidal activity.

2. A peptide according to Claim 1 having the amino acid sequence:

ASQQGTAALQKELKRIKIPDYSDSFKIKH	(SEQ ID NO: 1);
GTAALQKELKRIKIPDYSDSFKIKHLGKGH	(SEQ ID NO: 2);
LQKELKRIKIPDYSDSFKIKHL	(SEQ ID NO: 3);
QQGTAALQKELKRIK	(SEQ ID NO: 4);

or

GTAALQKELKRIKIP	(SEQ ID NO: 5).

3. A peptide which is or substantially is an amino acid sequence of human bactericidal permeability increasing protein (BPI) from position 65 to position 99, a subsequence thereof or a substitution or addition or deletion variant of the sequence or the subsequence thereof, the peptide having at least one of the biological activities of BPI, namely heparin binding, heparin neutralisation, LPS binding, LPS neutralisation or bactericidal activity or having fungicidal activity.

4. A peptide according to Claim 3 having the amino acid sequence:

SSQISMVPNVGLKFSISNANIKISGKWKAQKRFLK	(SEQ ID NO: 6);
IKISGKWKAQKRFLK	(SEQ ID NO: 7);
KWKAQKRFLK	(SEQ ID NO: 8);
CIKISGKWKAQKRFLK	(SEQ ID NO: 9);
CIKISGKWKAQKRFLKC	(SEQ ID NO:10);
NVGLKFSISNANIKISGKWKAQKRFLK	(SEQ ID NO:11);
AKISGKWKAQKRFLK	(SEQ ID NO:16);
IAISGKWKAQKRFLK	(SEQ ID NO:17);
IKASGKWKAQKRFLK	(SEQ ID NO:18);
IKIAGKWKAQKRFLK	(SEQ ID NO:19);
IKISAKWKAQKRFLK	(SEQ ID NO:20);
IKISGAWKAQKRFLK	(SEQ ID NO:21);
IKISGKAKAQKRFLK	(SEQ ID NO:22);
IKISGKWAAQKRFLK	(SEQ ID NO:23);
IKISGKWKAAKRFLK	(SEQ ID NO:24);
IKISGKWKAQARFLK	(SEQ ID NO:25);
IKISGKWKAQKAFLK	(SEQ ID NO:26);
IKISGKWKAQKRALK	(SEQ ID NO:27);
IKISGKWKAQKRFAK	(SEQ ID NO:28);
IKISGKWKAQKRFLA	(SEQ ID NO:29);
IKISGAWAAQKRFLK	(SEQ ID NO:30);
IKISGKWKAAARFLK	(SEQ ID NO:31);
IAISGKWKAQKRFLA	(SEQ ID NO:32);
IKISGKWKAKQRFLK	(SEQ ID NO:47);
IKISGKWKAQWRFLK	(SEQ ID NO:72);
IKISGKWKAKKRFLK	(SEQ ID NO:73);
IKISGKWKAFKRFLK	(SEQ ID NO:75);
IKISGKFKAQKRFLK	(SEQ ID NO:48);
IKISGKW$_{D}$KAQKRFLK	(SEQ ID NO:49);
IKISGKWKAFFRFLK	(SEQ ID NO:82);
IKISGKWKAQFRFLK	(SEQ ID NO:62);

IKISGKA$_{\beta\text{-(3-pyridyl)}}$KAQKRFLK	(SEQ ID NO:63);
IKISGKWKAQKRA$_{\beta\text{-(3-pyridyl)}}$LK	(SEQ ID NO:64);
A$_D$A$_D$IKISGKWKAQKRFLK	(SEQ ID NO:66);
IKISGKWKAQF$_D$RFLK	(SEQ ID NO:71);
IKISGKWKAQA$_{\beta\text{-(1-naphthyl)}}$RFLK	(SEQ ID NO:74);
IKISGKA$_{\beta\text{-(1-naphthyl)}}$KAQFRFLK	(SEQ ID NO:78);
IKISGKA$_{\beta\text{-(1-naphthyl)}}$KAFKRFLK	(SEQ ID NO:84);
IKISGKA$_{\beta\text{-(1-naphthyl)}}$KAFFRFLK	(SEQ ID NO:85);

or

IKISGKA$_{\beta\text{-(1-naphthyl)}}$KAQKRFLK	(SEQ ID NO:50).

5. A peptide which is or substantially is an amino acid sequence of human bactericidal permeability increasing protein (BPI) from position 142 to position 169, a subsequence thereof or a substitution or addition or deletion variant of the sequence or the subsequence thereof, the peptide having at least one of the biological activities of BPI, namely heparin binding, heparin neutralisation, LPS binding, LPS neutralisation or bactericidal activity or having fungicidal activity.

6. A peptide according to Claim 5 having the amino acid sequence:

VHVHISKSKVGWLIQLFHKKIESALRNK	(SEQ ID NO:12);
KSKVWLIQLFHKK	(SEQ ID NO:13);
VHVHISKSKVGWLIQLFHKKIE	(SEQ ID NO:67);
SVHVHISKSKVGWLIQLFHKKIESALRNK	(SEQ ID NO:14);
KSKVGWLIQLFHKK	(SEQ ID NO:15);
ASKVGWLIQLFHKK	(SEQ ID NO:33);
KAKVGWLIQLFHKK	(SEQ ID NO:34);
KSAVGWLIQLFHKK	(SEQ ID NO:35);
KSKAGWLIQLFHKK	(SEQ ID NO:36);
KSKVAWLIQLFHKK	(SEQ ID NO:37);
KSKVGALIQLFHKK	(SEQ ID NO:38);
KSKVGWAIQLFHKK	(SEQ ID NO:39);

KSKVGWLAQLFHKK	(SEQ ID NO:40);
KSKVGWLIALFHKK	(SEQ ID NO:41);
KSKVGWLIQAFHKK	(SEQ ID NO:42);
KSKVGWLIQLAHKK	(SEQ ID NO:43);
KSKVGWLIQLFAKK	(SEQ ID NO:44);
KSKVGWLIQLFHAK	(SEQ ID NO:45);
GWLIQLFHKKIESALRNKMNS	(SEQ ID NO:61);
KSKVGWLIQLWHKK	(SEQ ID NO:76);
KSKVLWLIQLFHKK	(SEQ ID NO:79);
KSKVGWLILLFHKK	(SEQ ID NO:80);
KSKVGWLIQLFLKK	(SEQ ID NO:81);
KSKVGWLIFLFHKK	(SEQ ID NO:86);
KSKVGWLIKLFHKK	(SEQ ID NO:87);
KSKVGWLIQLFFKK	(SEQ ID NO:89);
KSKVFWLIQLFHKK	(SEQ ID NO:90);
KSKVGWLIQLFHKF	(SEQ ID NO:91);
KSKVKWLIQLFHKK	(SEQ ID NO:92);
KSKVKWLIKLFHKK	(SEQ ID NO:94);
KSKVGA$_{\beta\text{-(1-naphthyl)}}$LIQLFHKK	(SEQ ID NO:77);

or

KSKVGWLIQLFHKA	(SEQ ID NO:46).

7. A peptide in which two or three of the same or different peptides according to Claims 1, 3 or 5 are directly covalently linked together.

8. A peptide according to Claim 7 having the amino acid sequence:

KRFLKKWKAQKRFLK	(SEQ ID NO:51);
KWKAQKRFLKKWKAQKRFLK	(SEQ ID NO:54);
KRFLKKWKAQKRFLKKWKAQKRFLK	(SEQ ID NO:55);
KWKAAARFLKKWKAQKRFLK	(SEQ ID NO:57);

KWKAQKRFLKKWKAAARFLK	(SEQ ID NO:58);
KWKAAARFLKKWKAAARFLK	(SEQ ID NO:59);
KWKAQWRFLKKWKAQWRFLKKWKAQWRFLK	(SEQ ID NO:93);
KWKAAARFLKKWKAAARFLKKWKAAARFLK	(SEQ ID NO:60);
QKRFLKKWKAQKRFLKKWKAQKRFLK	(SEQ ID NO:65);
KSKVKWLIKLFFKFKSKVKWLIKLFFKF	(SEQ ID NO:95);
KSKVGWLIQLFHKKKSKVGWLIQLFHKK	(SEQ ID NO:56);
KWKAQKRFLKKSKVGWLIQLFHKK	(SEQ ID NO:52);
IKISGKWKAQKRFLKKSKVGWLIQLFHKK	(SEQ ID NO:53);
KSKVGWLIQLFHKKKWKAQKRFLK	(SEQ ID NO:70);
IKISGK$A_{\beta\text{-(1-naphthyl)}}$KAQFRFLKKSKVGWLIQLFHKK	(SEQ ID NO:88);
IKISGK$A_{\beta\text{-(1-naphthyl)}}$KAQFRFLKKSKVGWLIFLFHKK	(SEQ ID NO:83);

or

KWKAQFRFLKKSKVGWLILLFHKK	(SEQ ID NO:96).

9. A peptide according to any one of Claims 1 to 8 for use as a medicament.

10. A pharmaceutical composition comprising a peptide according to any one of Claims 1 to 8 and a pharmaceutically-acceptable carrier or diluent.

11. Use of a peptide of any one of Claims 1 to 8 for the manufacture of a heparin binding medicament, a heparin binding medicament for neutralizing the anti-coagulant effect of heparin, a heparin binding medicament for inhibiting angiogenesis, a heparin binding medicament for inhibiting angiogenesis associated with ocular retinopathy, a heparin binding medicament for inhibiting endothelial cell proliferation, a heparin binding medicament for treating endometriosis by inhibiting endothelial cell proliferation, a heparin binding contraceptive, a heparin binding medicament for inhibiting malignant tumor cell proliferation, a heparin binding medicament for treatment of a chronic inflammatory disease state, a medicament for inhibiting angiogenesis, a medicament for inhibiting angiogenesis associated with ocular retinopathy, a medicament for inhibiting endothelial cell proliferation, a medicament for treating endometriosis by inhibiting endothelial cell proliferation, a contraceptive, a medicament for inhibiting malignant tumor cell proliferation, a medicament for the treatment of a chronic inflammatory disease state, a medicament for treatment of Gram-negative bacterial infection and the sequelae thereof, a medicament for treatment of the adverse effects of Gram-negative endotoxin in blood circulation, a medicament for killing Gram-negative bacteria, a medicament for treatment of adverse physiological effects associated with depressed reticuloendothelial cell or system function, a medicament for treatment of *Mycobacteria* infection, a medicament for treatment of the adverse physiological effects of the presence of lipoarabinomannan in circulation, a medicament for decontaminating a fluid containing lipoarabinomannan or a medicament for treatment of a disease state associated with infection by a species of bacteria from the genus *Helicobacter*.

12. A pharmaceutical composition according to Claim 10, further comprising an antibiotic in a synergistically effective amount.

13. A pharmaceutical composition according to Claim 10, further comprising an LBP protein product in an amount effective to potentiate the bactericidal properties of said peptide.

A

14. Use of a peptide of any one of Claims 1 to 8 for the manufacture of a medicament for treatment of Gram-negative bacterial infections and the sequelae thereof in combination with an antibiotic.

RIDOUT & MAYBEE
Toronto, Canada

Patent Agents

A

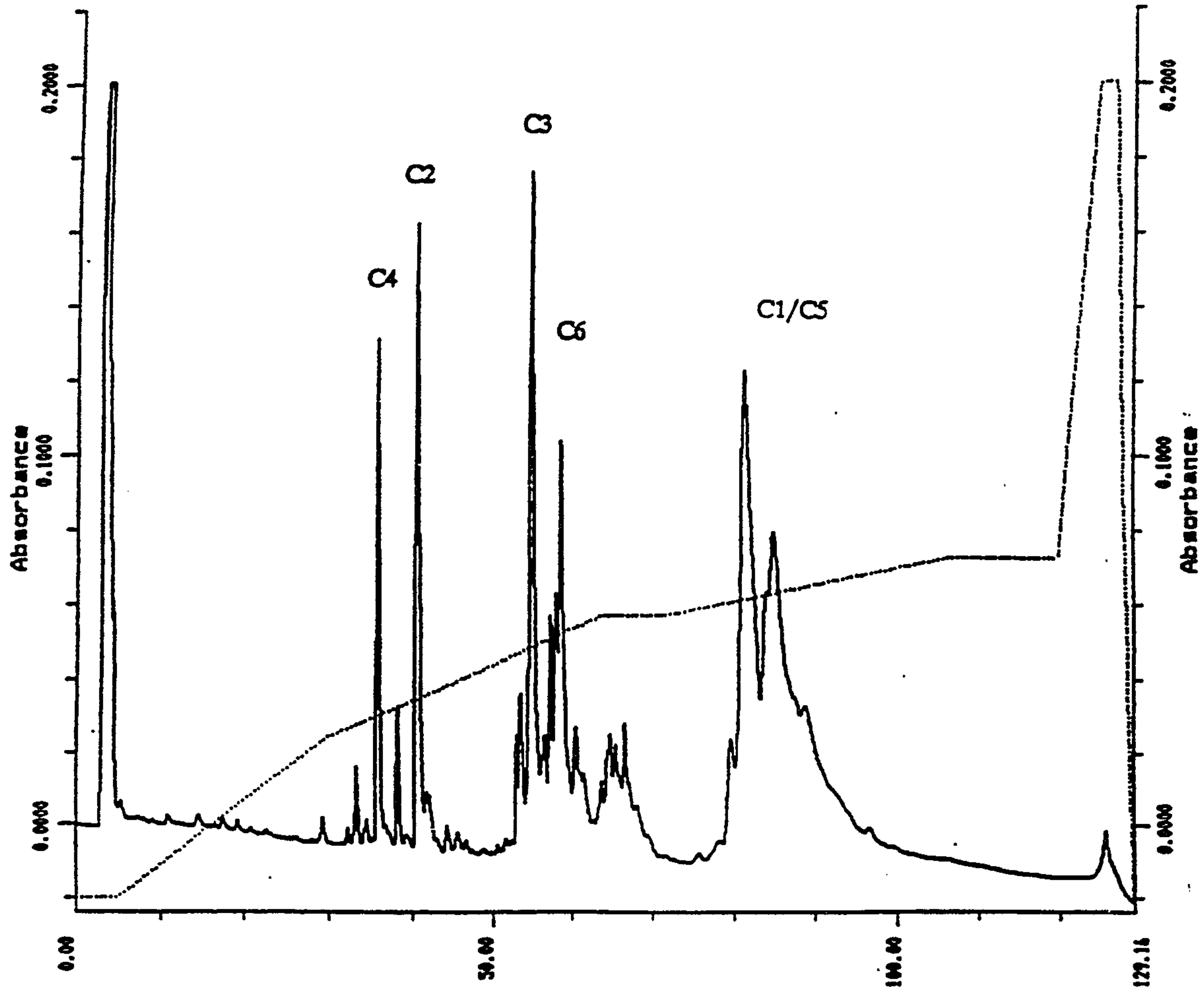
Absorbance
0.2000
0.1000
0.0000
C4
C2
C3
C6
C1/C5
0.00
50.00
100.00
129.16

FIGURE 1a

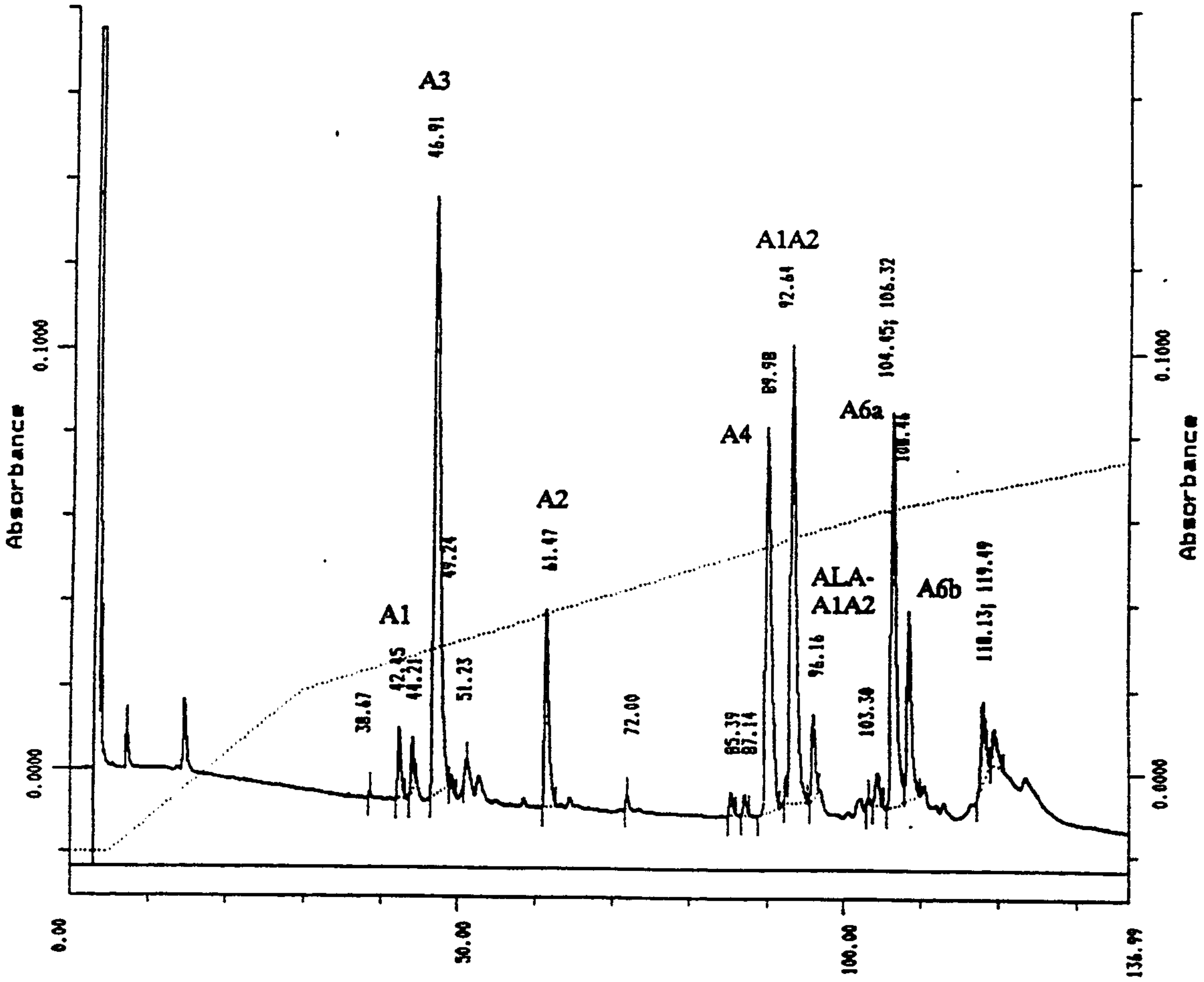
A3
46.91
A1A2
92.61
104.85; 106.32
89.98
A6a
106.46
A4
A2
61.47
49.24
ALA-
A1A2
A6b
118.13; 119.49
A1
42.85
44.21
51.23
96.16
38.47
72.00
85.39
87.14
103.30
Absorbance
0.1000
0.0000
0.00
50.00
100.00
136.99

FIGURE 1b

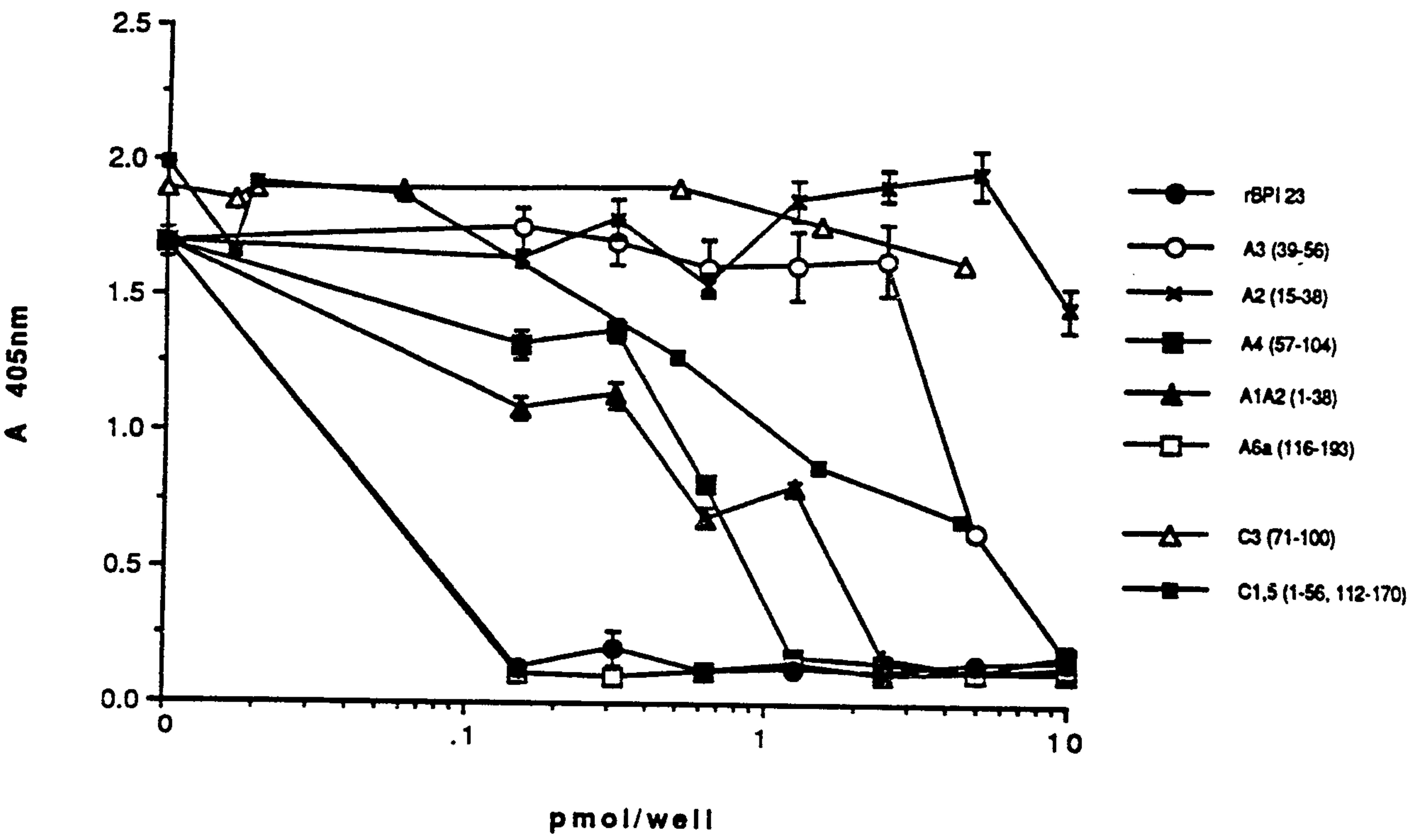
2.5
2.0
1.5
1.0
0.5
0.0
A 405nm
0
.1
1
10
pmol/well
rBPI 23
A3 (39-56)
A2 (15-38)
A4 (57-104)
A1A2 (1-38)
A6a (116-193)
C3 (71-100)
C1,5 (1-56, 112-170)

FIGURE 2

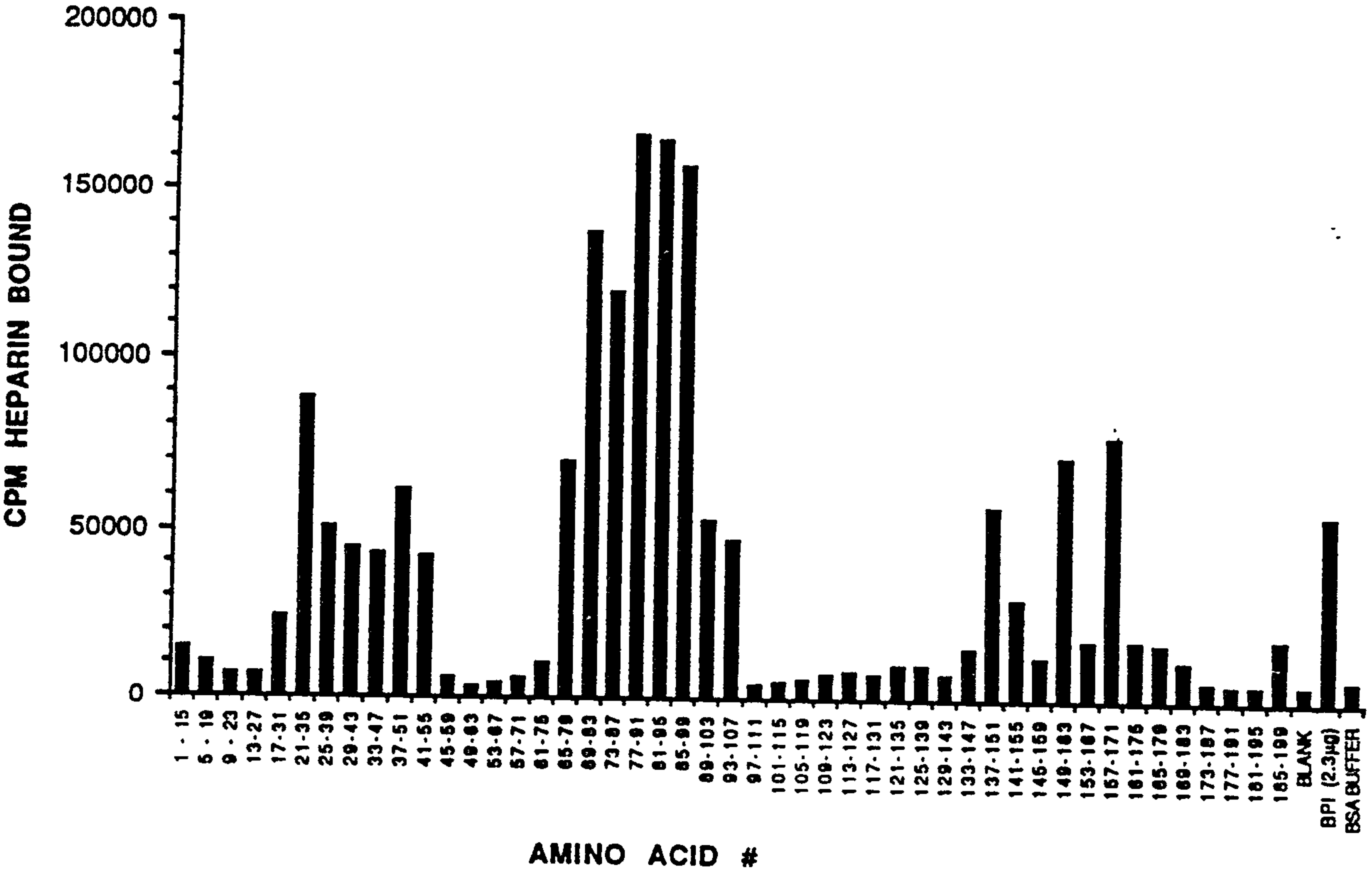
200000
150000
100000
50000
0
CPM HEPARIN BOUND
1 - 15
5 - 19
9 - 23
13-27
17-31
21-35
25-39
29-43
33-47
37-51
41-55
45-59
49-63
53-67
57-71
61-75
65-79
69-83
73-87
77-91
81-95
85-99
89-103
93-107
97-111
101-115
105-119
109-123
113-127
117-131
121-135
125-139
129-143
133-147
137-151
141-155
145-159
149-163
153-167
157-171
161-175
165-179
169-183
173-187
177-191
181-195
185-199
BLANK
BPI (2.3μg)
BSA BUFFER
AMINO ACID #

FIGURE 3

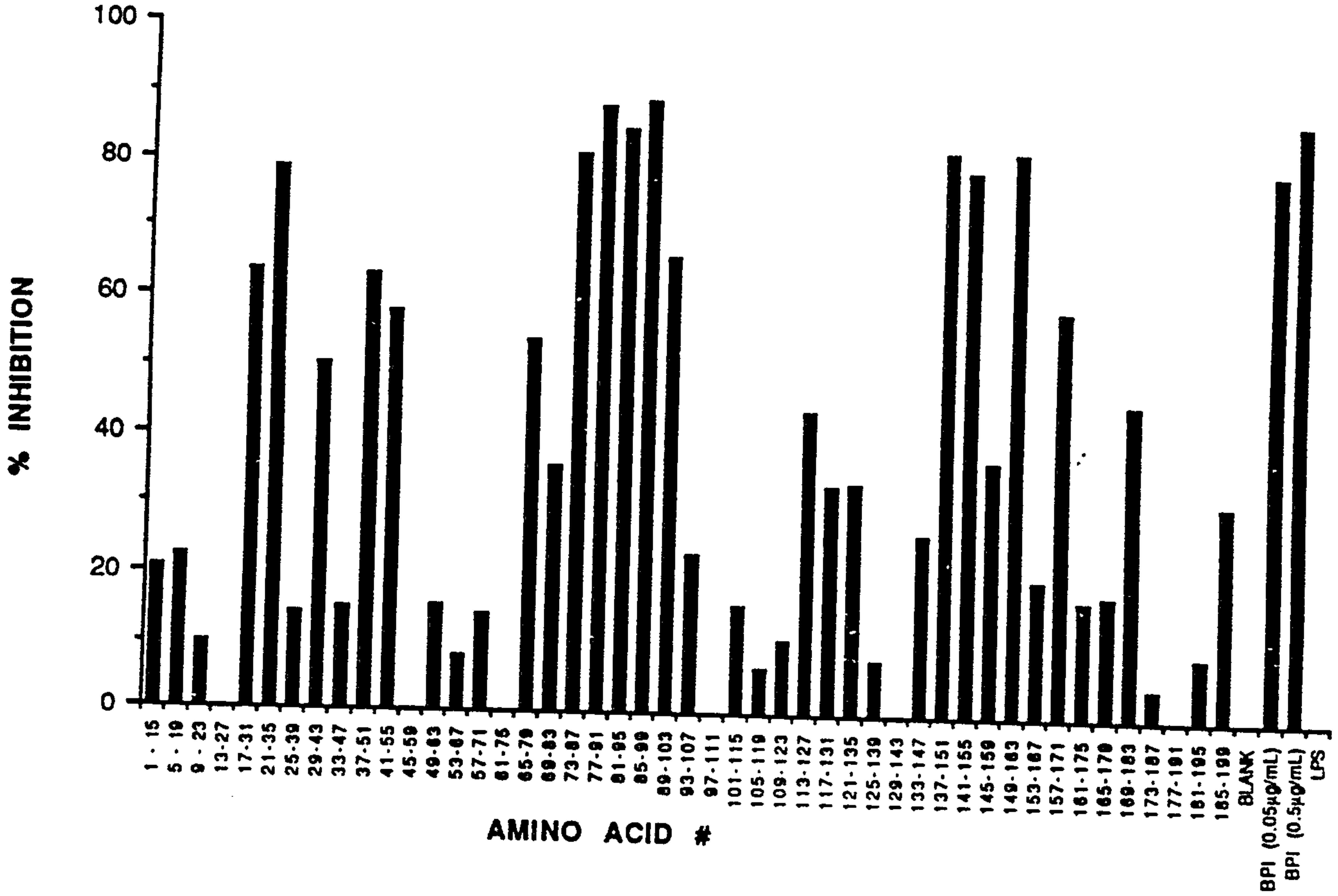
100
80
60
40
20
0
% INHIBITION
1 - 15
5 - 19
9 - 23
13-27
17-31
21-35
25-39
29-43
33-47
37-51
41-55
45-59
49-63
53-67
57-71
61-75
65-79
69-83
73-87
77-91
81-95
85-99
89-103
93-107
97-111
101-115
105-119
109-123
113-127
117-131
121-135
125-139
129-143
133-147
137-151
141-155
145-159
149-163
153-167
157-171
161-175
165-179
169-183
173-187
177-191
181-195
185-199
BLANK
BPI (0.05µg/mL)
BPI (0.5µg/mL)
LPS
AMINO ACID #

FIGURE 4

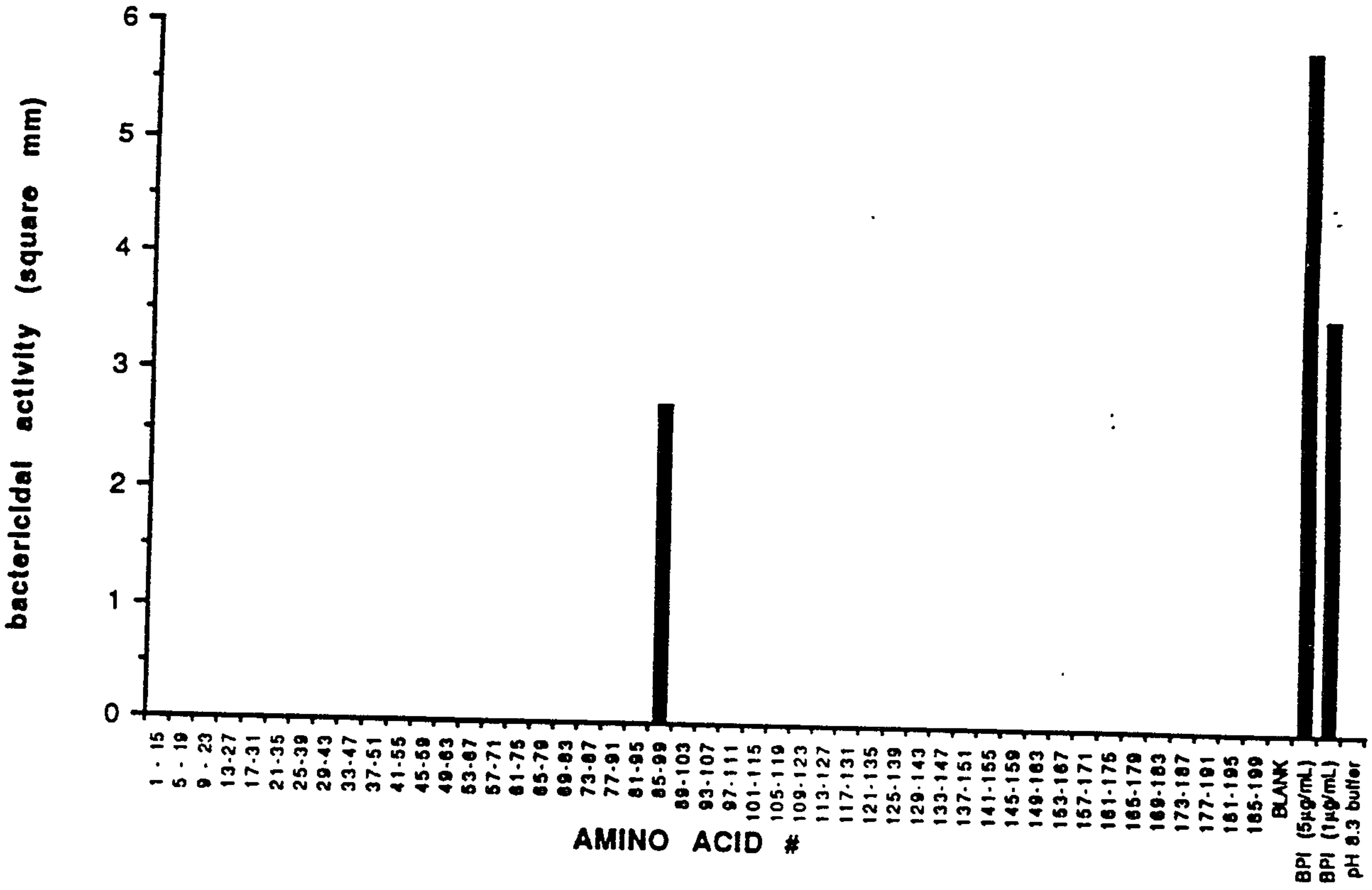
6
5
4
3
2
1
0
bactericidal activity (square mm)
1 - 15
5 - 19
9 - 23
13-27
17-31
21-35
25-39
29-43
33-47
37-51
41-55
45-59
49-63
53-67
57-71
61-75
65-79
69-83
73-87
77-91
81-95
85-99
89-103
93-107
97-111
101-115
105-119
109-123
113-127
117-131
121-135
125-139
129-143
133-147
137-151
141-155
145-159
149-163
153-167
157-171
161-175
165-179
169-183
173-187
177-191
181-195
185-199
BLANK
BPI (5µg/mL)
BPI (1µg/mL)
pH 8.3 buffer
AMINO ACID #

FIGURE 5

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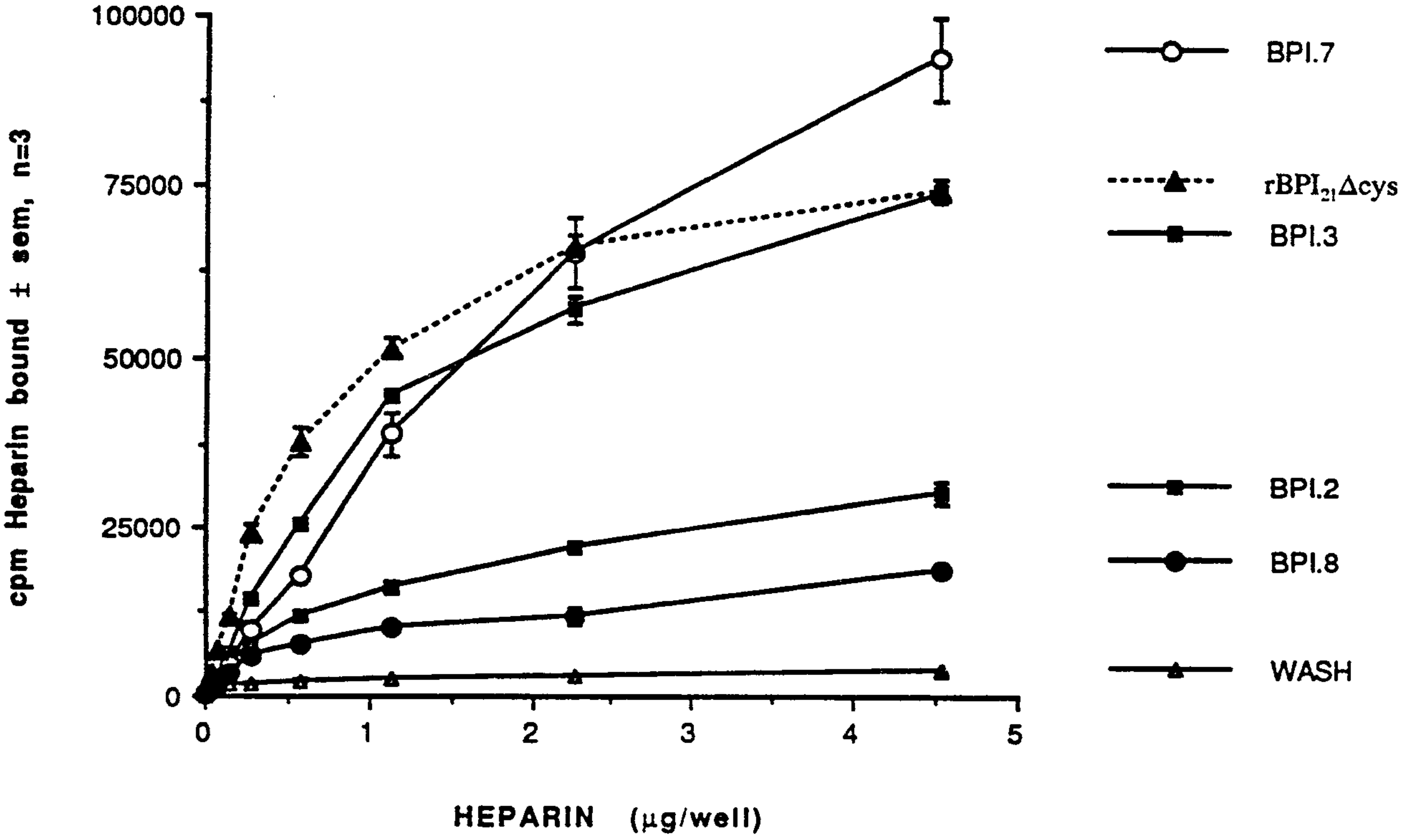
100000
75000
50000
25000
0
0
1
2
3
4
5
cpm Heparin bound ± sem, n=3
HEPARIN (µg/well)
BPI.7
rBPI$_{21}$Δcys
BPI.3
BPI.2
BPI.8
WASH

FIGURE 6

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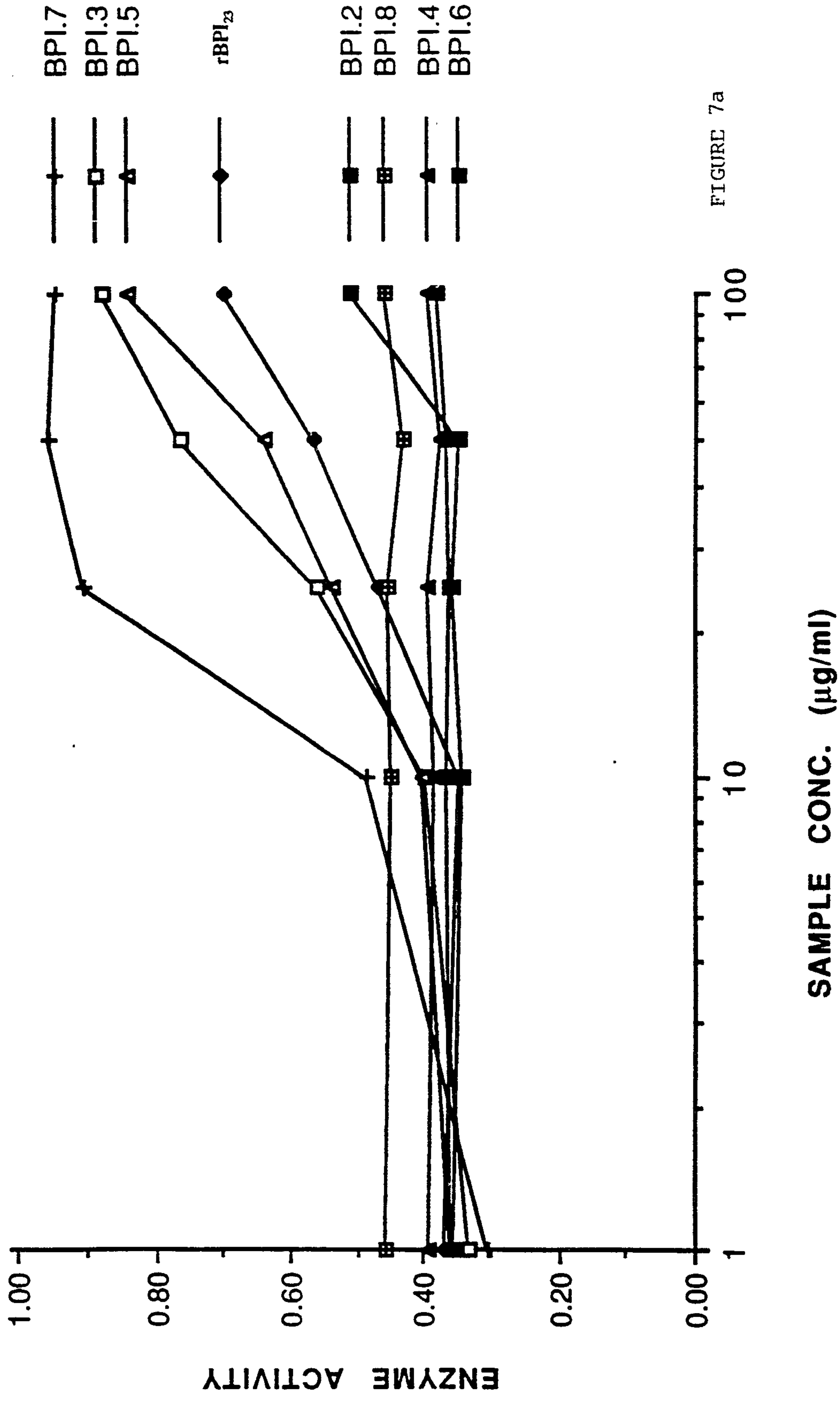

FIGURE 7a

2158058

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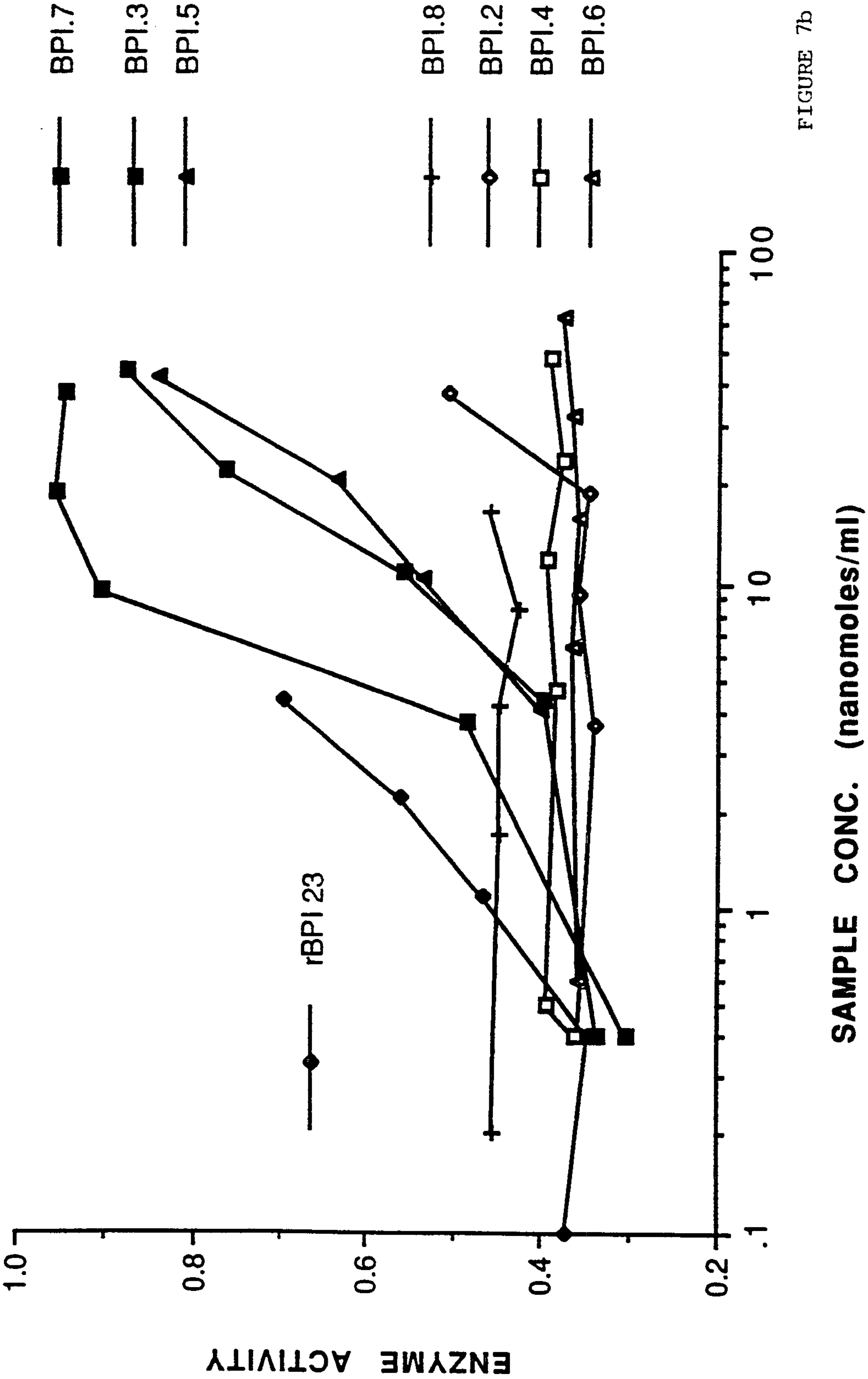

FIGURE 7b

2158058

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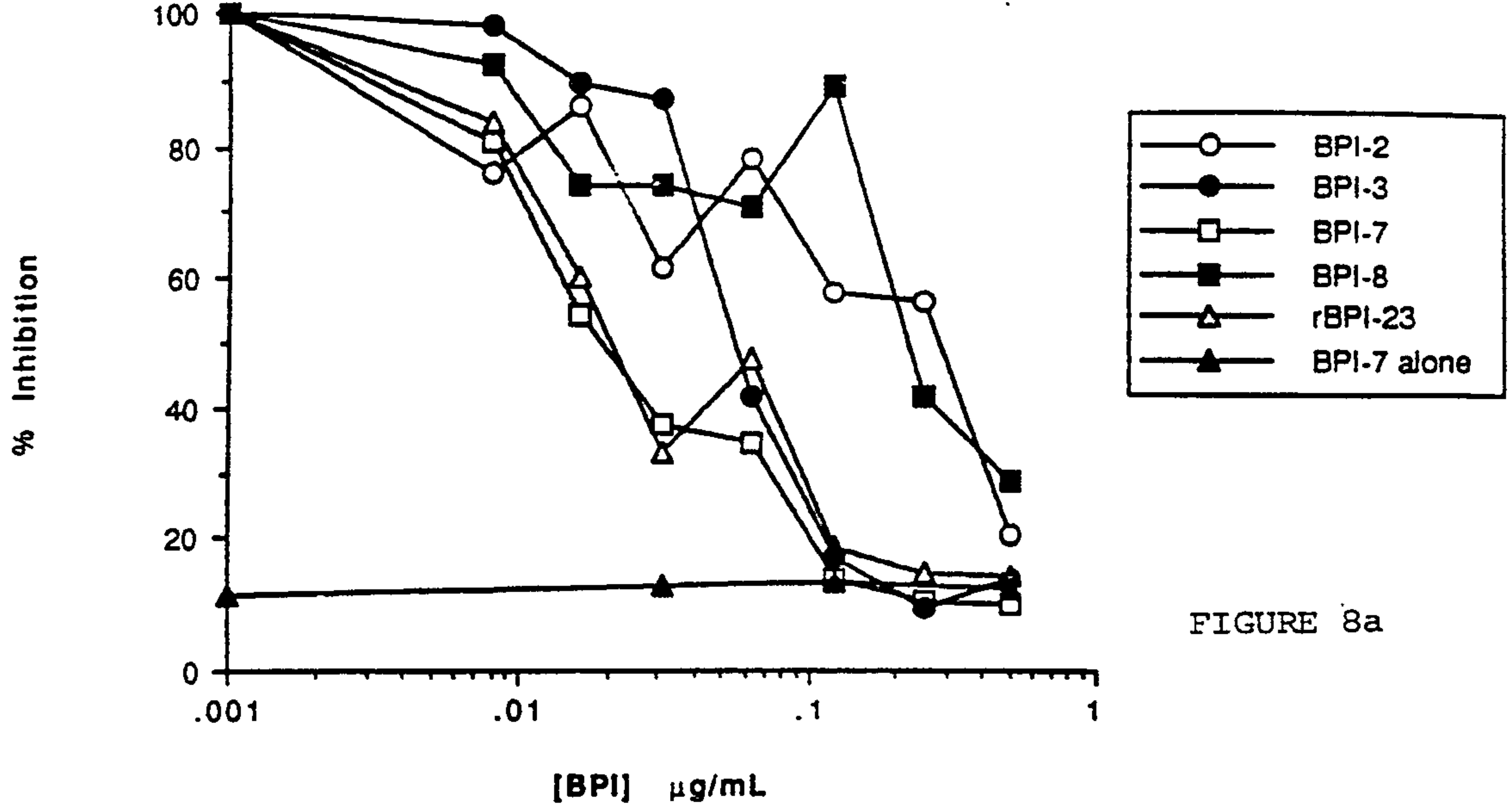

FIGURE 8a

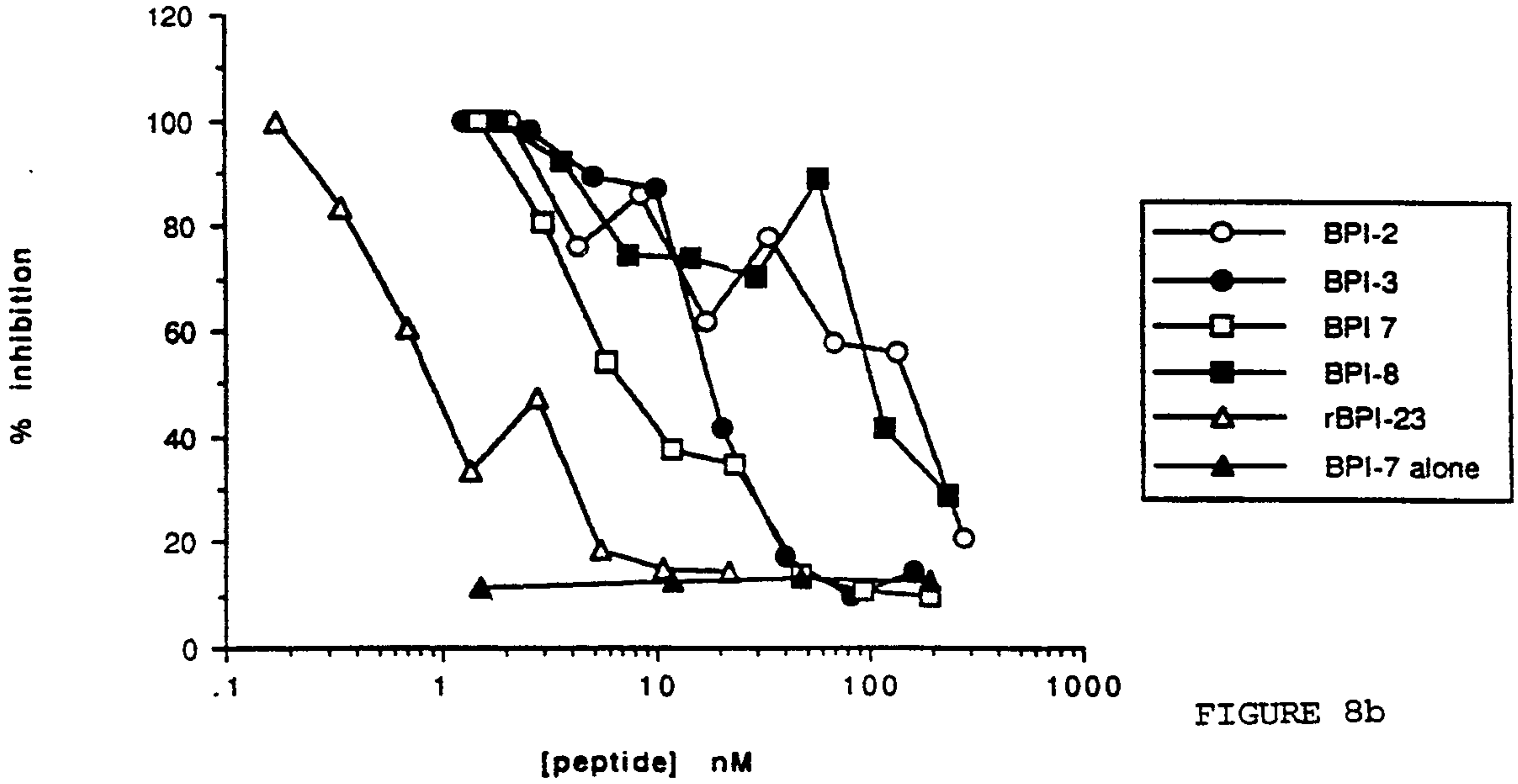

FIGURE 8b

2158058

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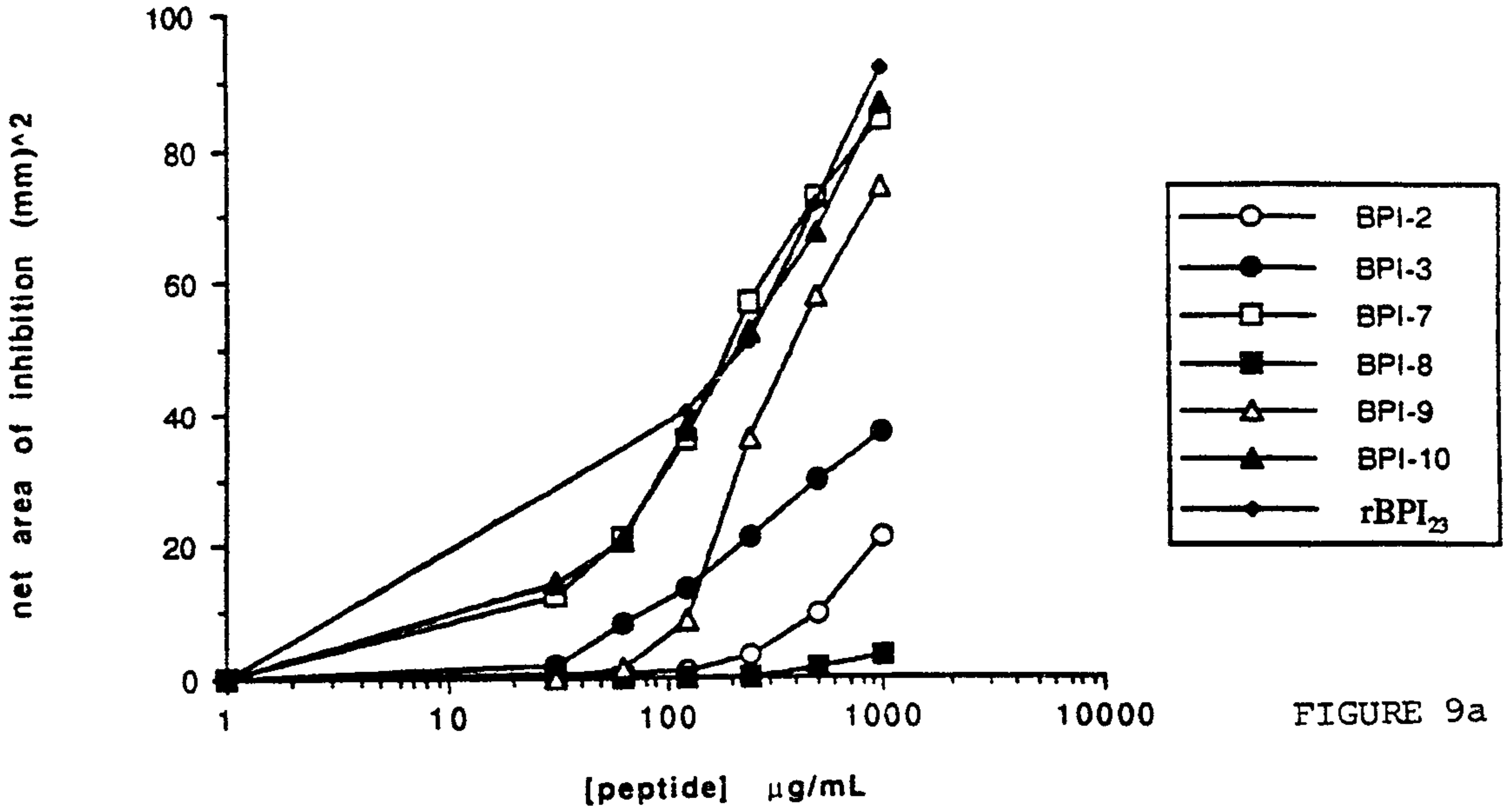

FIGURE 9a

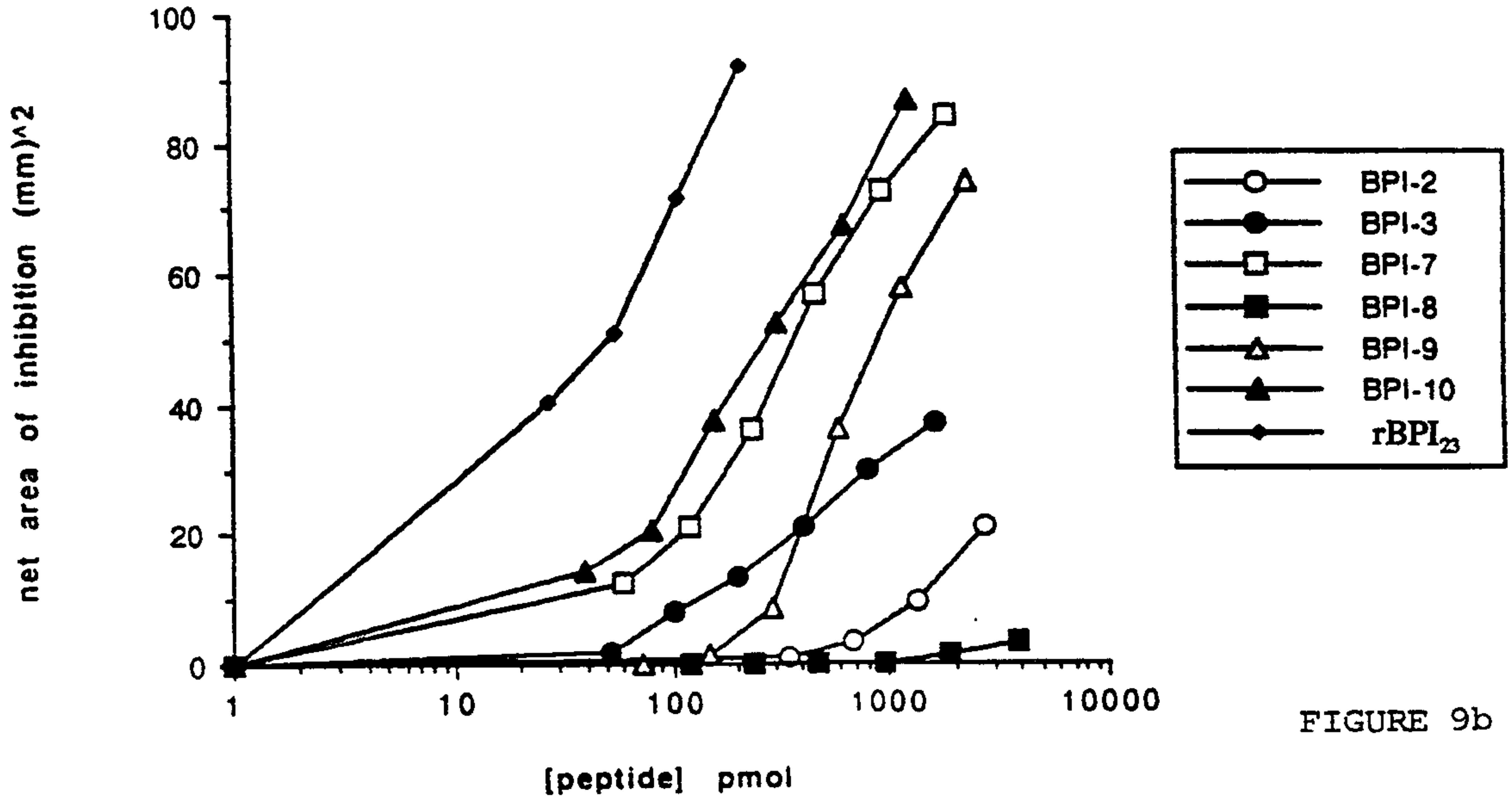

FIGURE 9b

2158058

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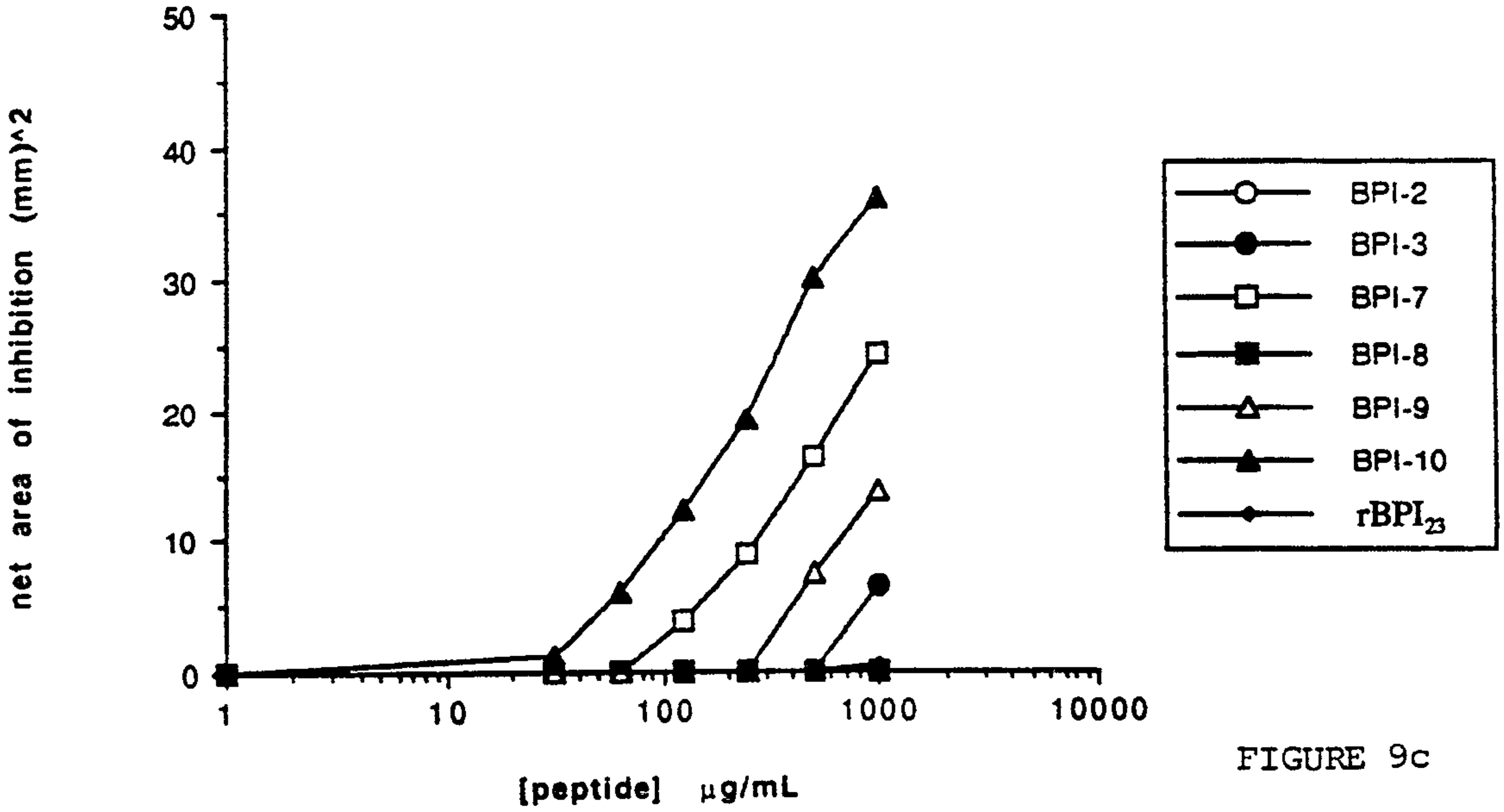

FIGURE 9c

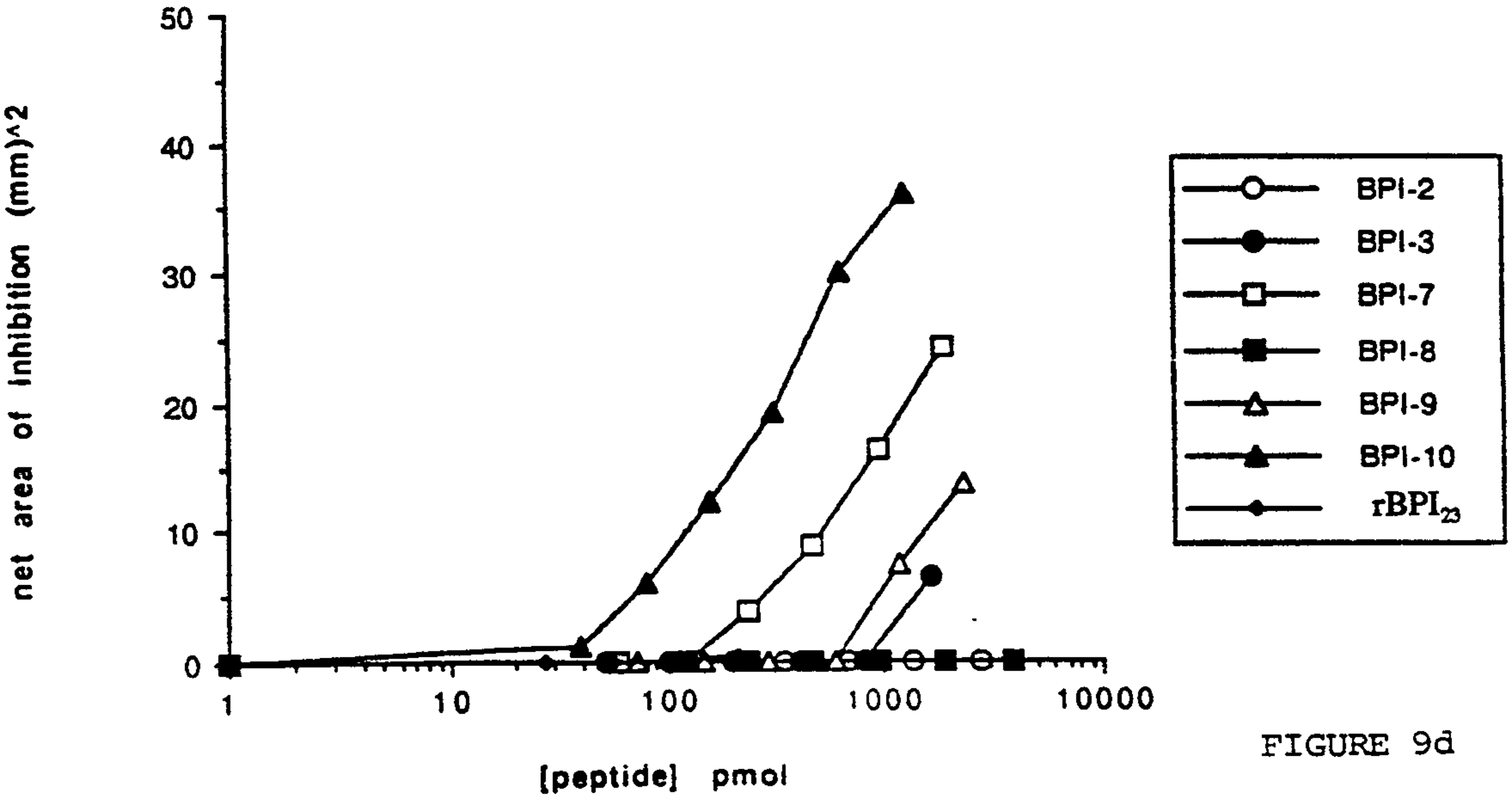

FIGURE 9d

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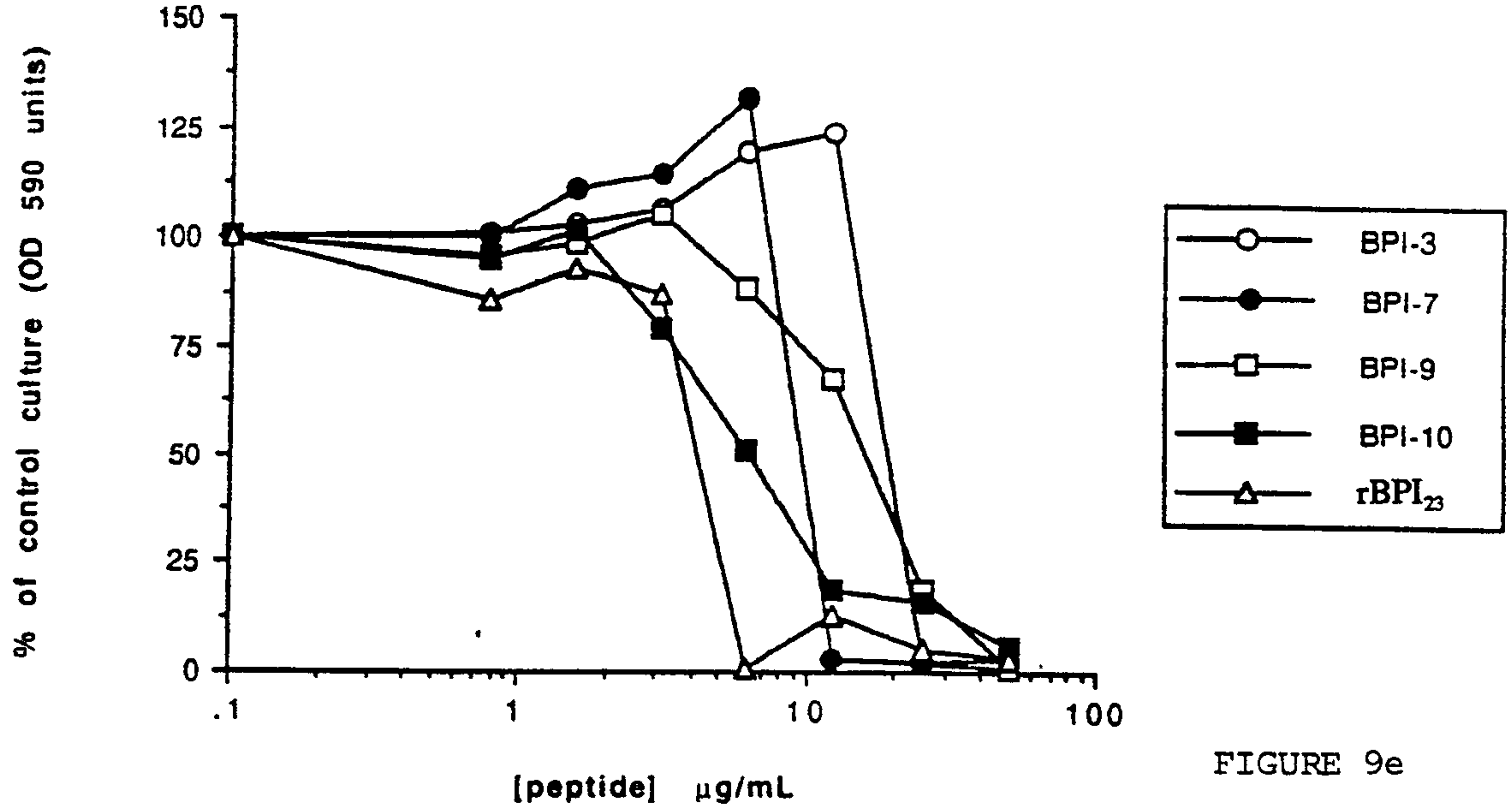

FIGURE 9e

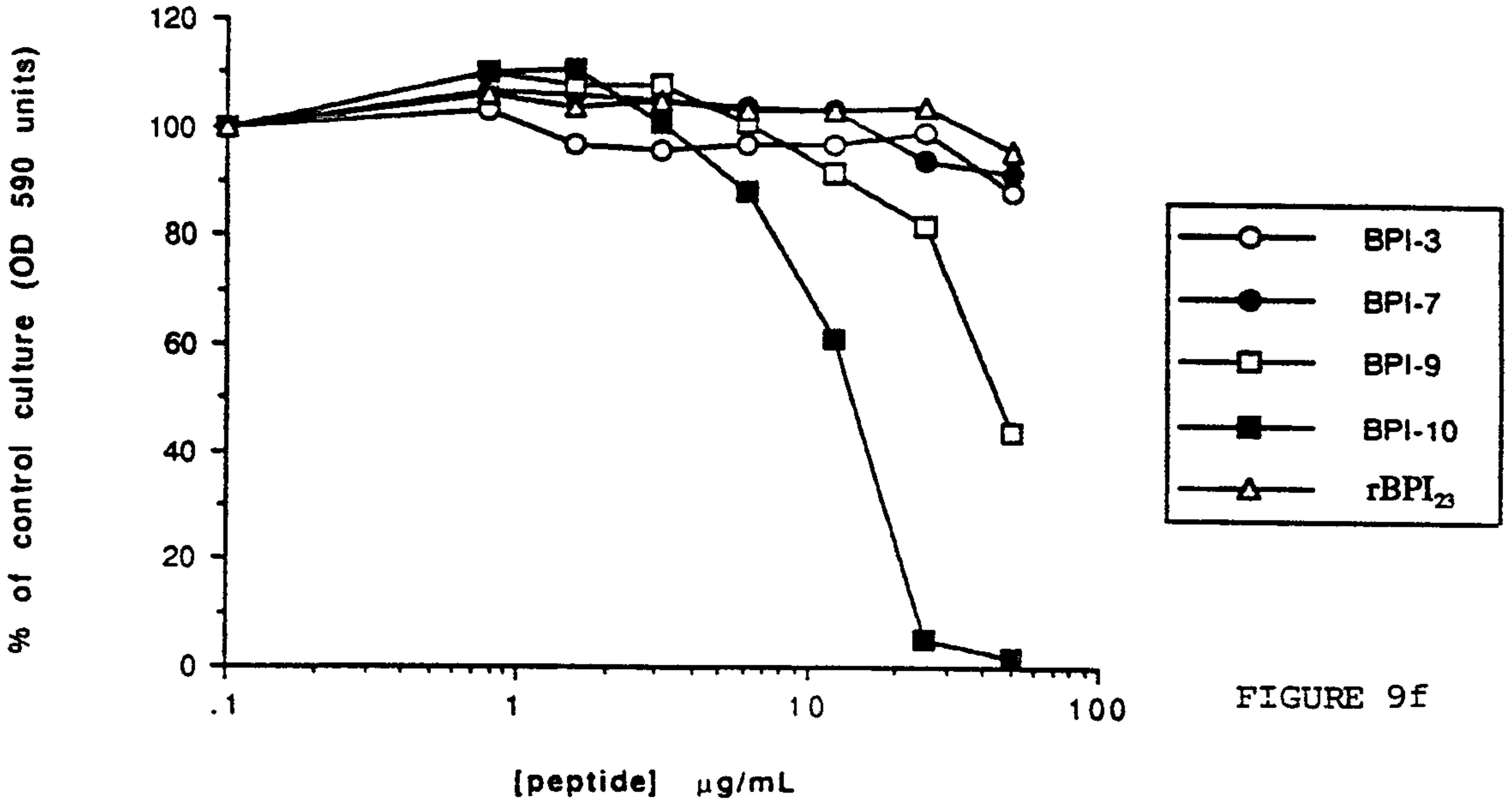

FIGURE 9f

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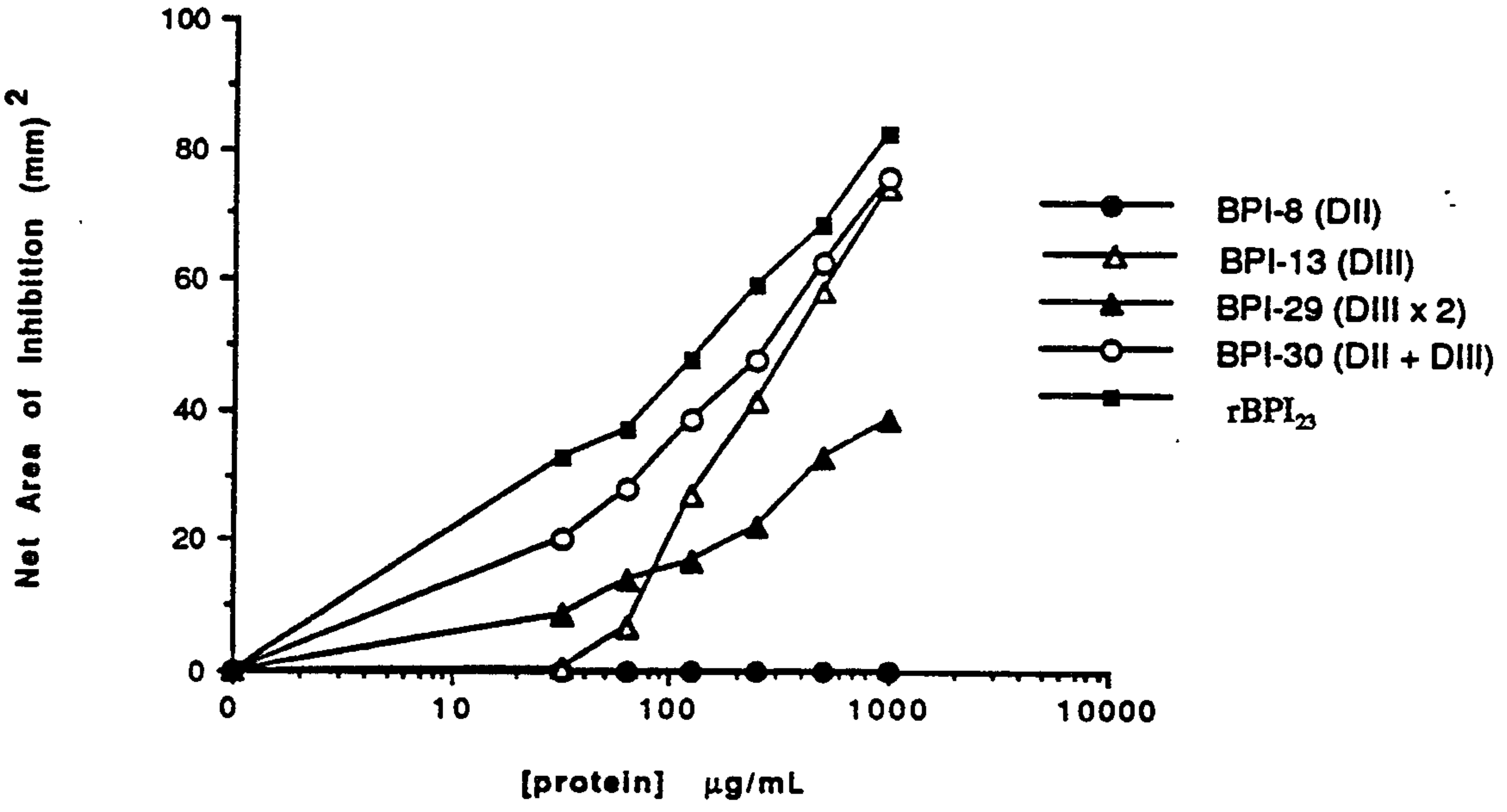

FIGURE 10a

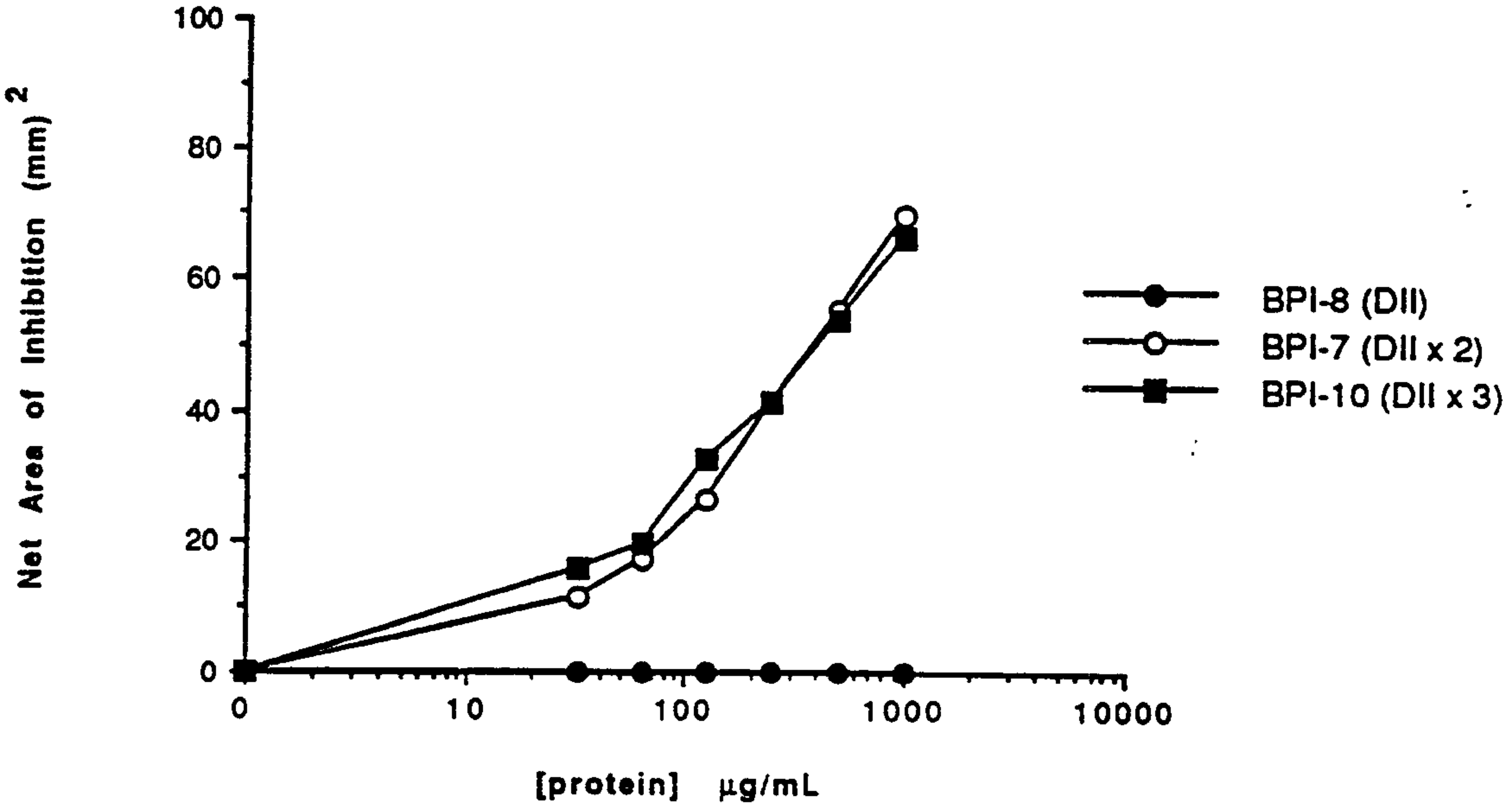
Net Area of Inhibition (mm)^2
100
80
60
40
20
0
0
10
100
1000
10000
[protein] µg/mL
BPI-8 (DII)
BPI-7 (DII x 2)
BPI-10 (DII x 3)

FIGURE 10b

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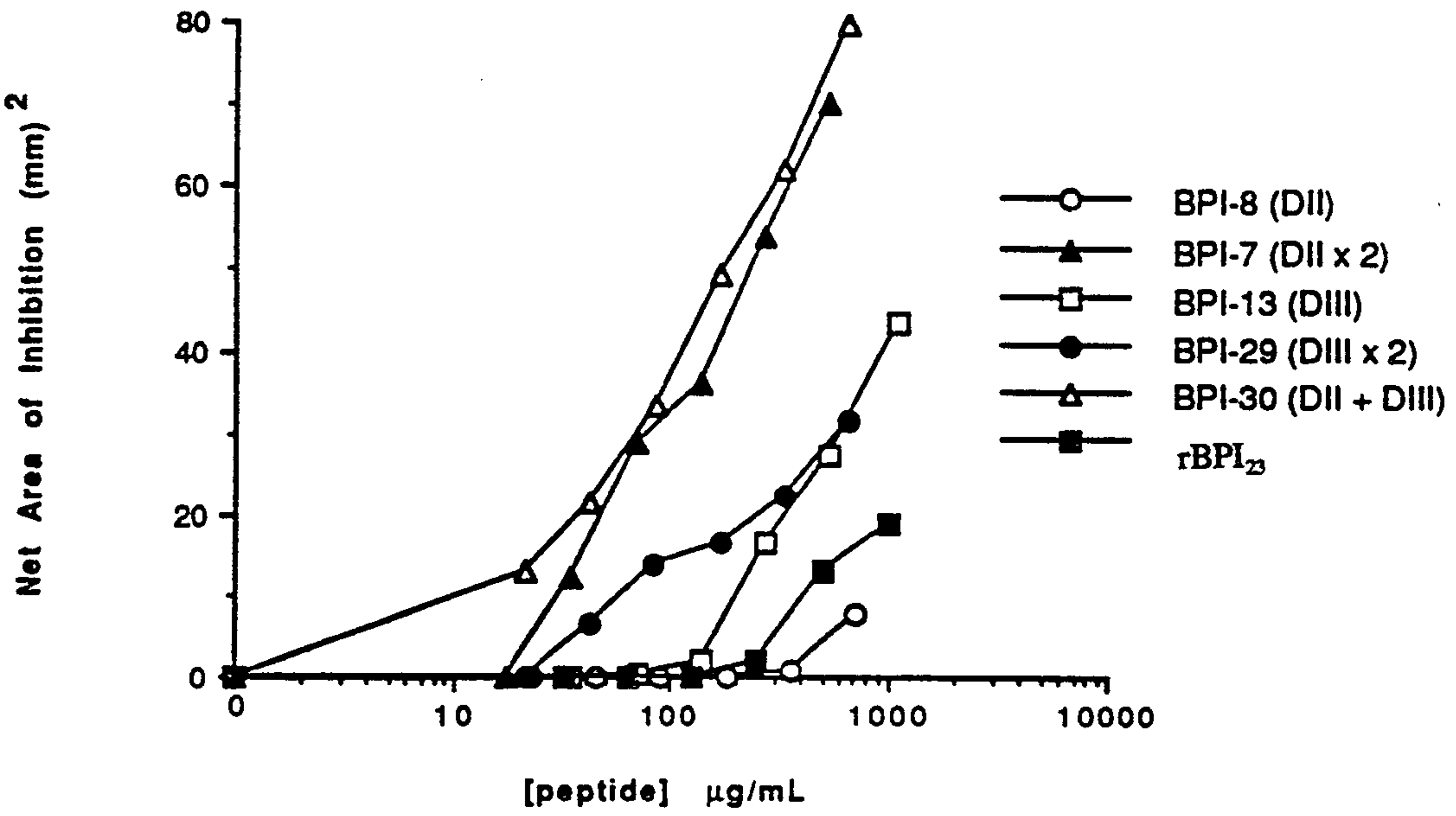

FIGURE 10c

2158058

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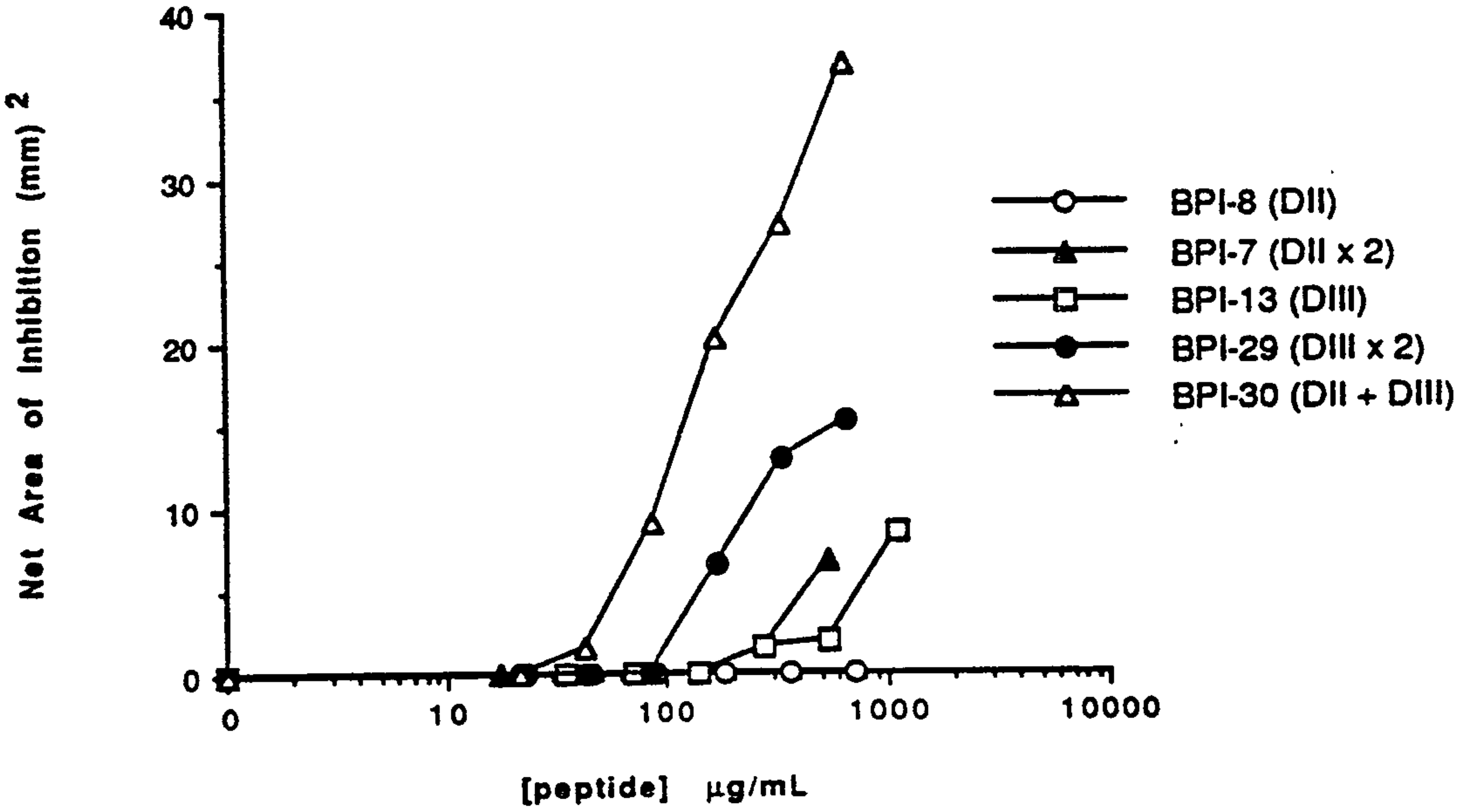

FIGURE 10d

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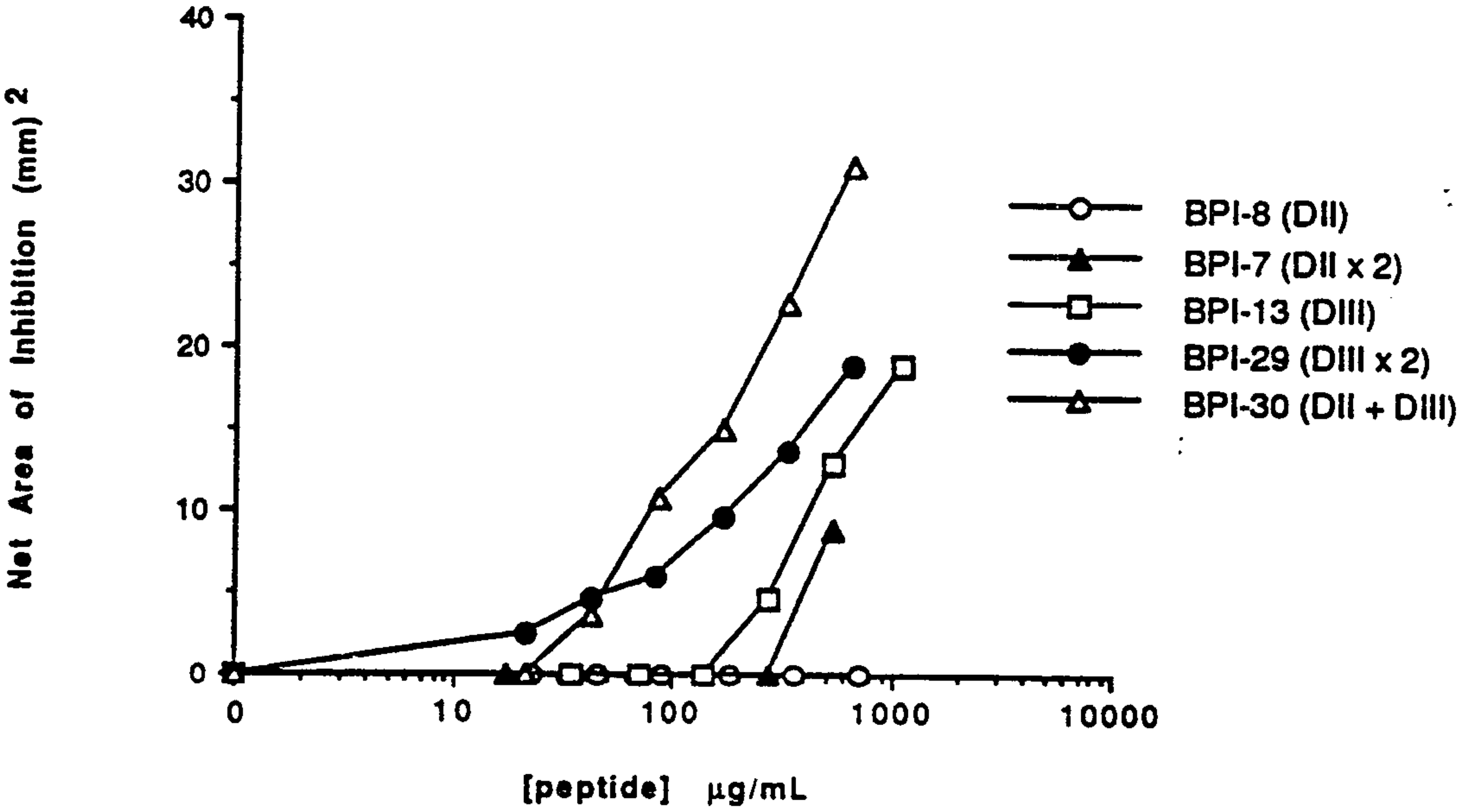

FIGURE 10e

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FIGURE 11a

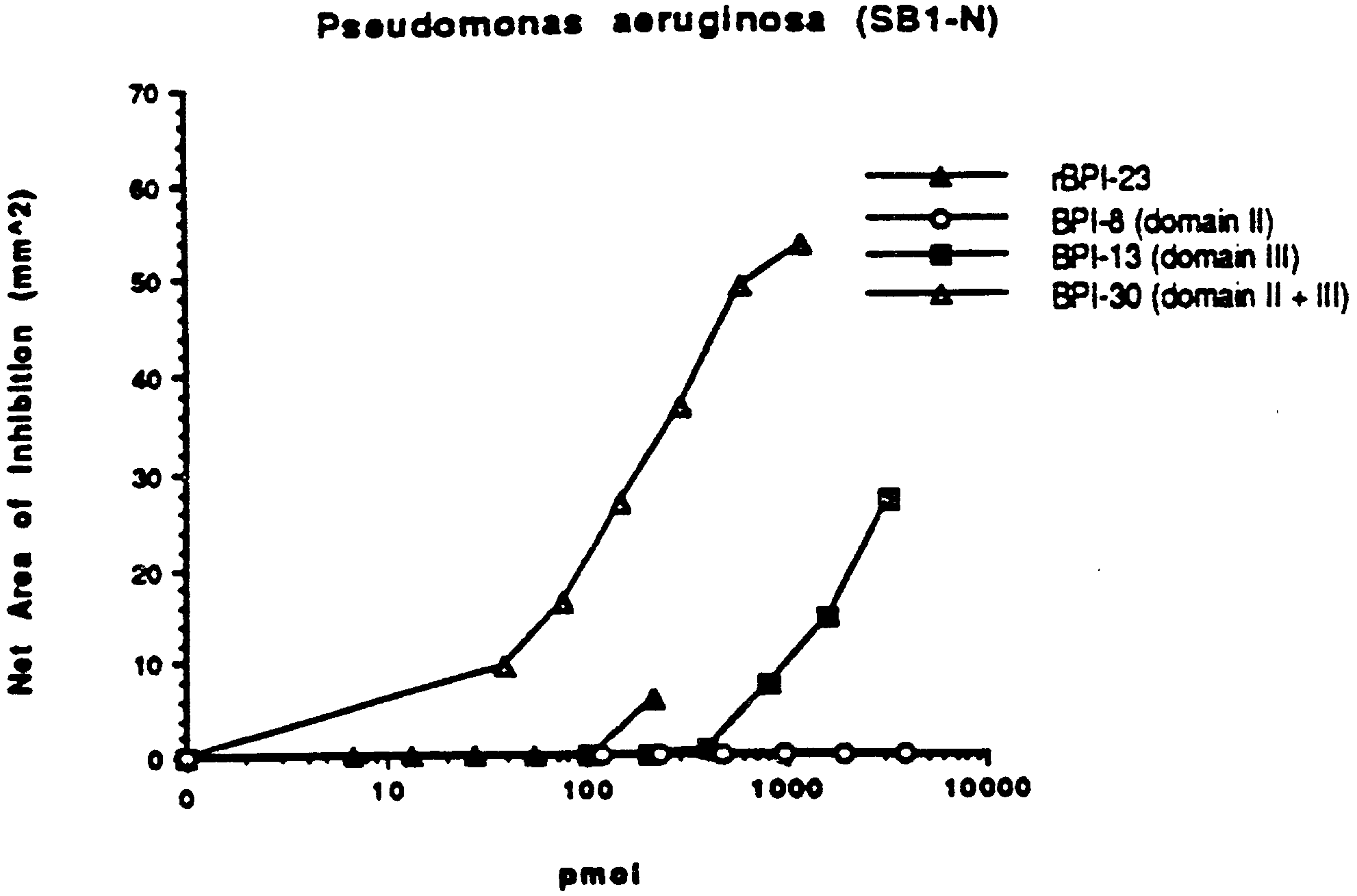

FIGURE 11b

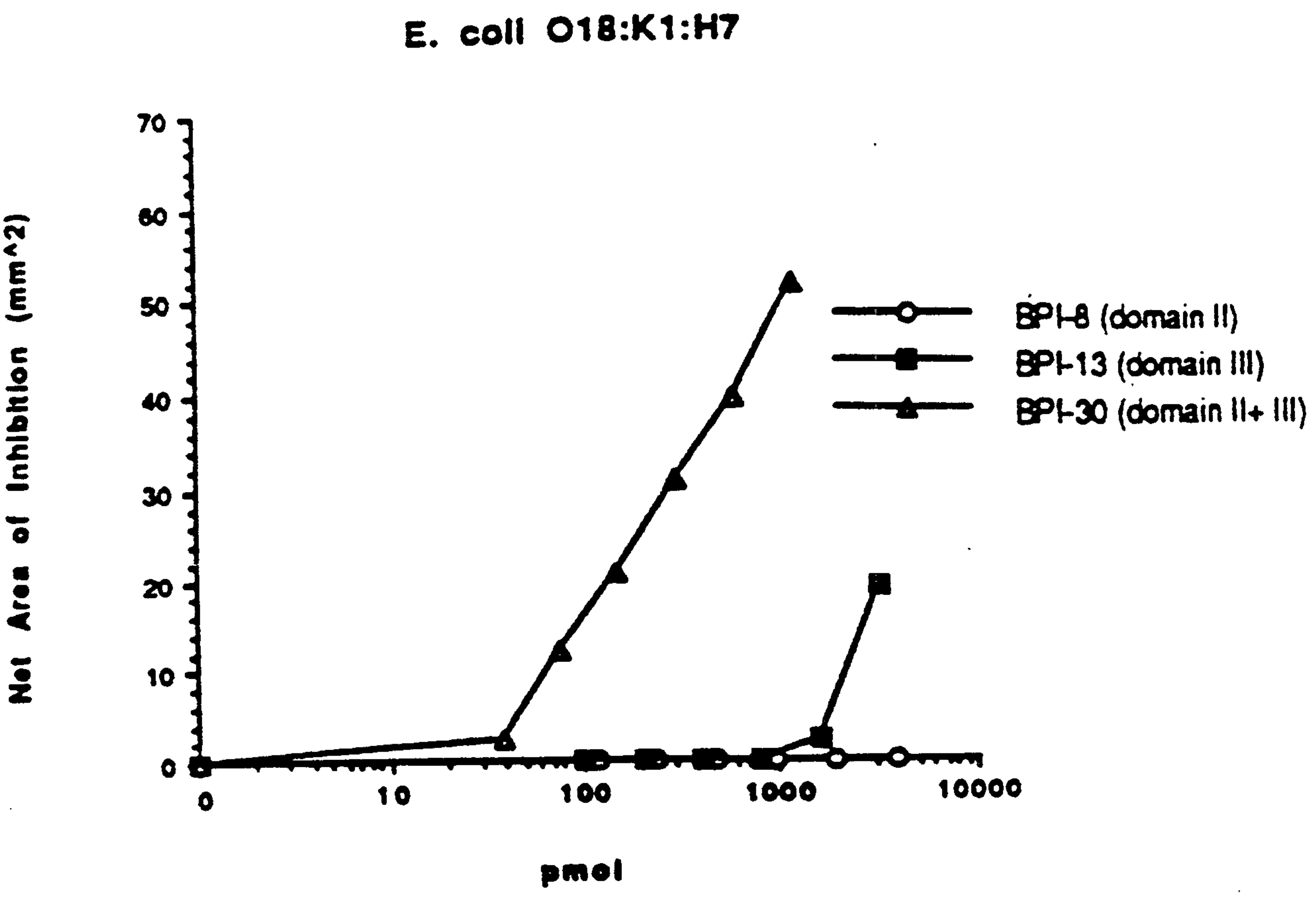
E. coli O18:K1:H7
Net Area of Inhibition (mm^2)
70
60
50
40
30
20
10
0
0
10
100
1000
10000
pmol
BPI-8 (domain II)
BPI-13 (domain III)
BPI-30 (domain II+ III)

FIGURE 11c

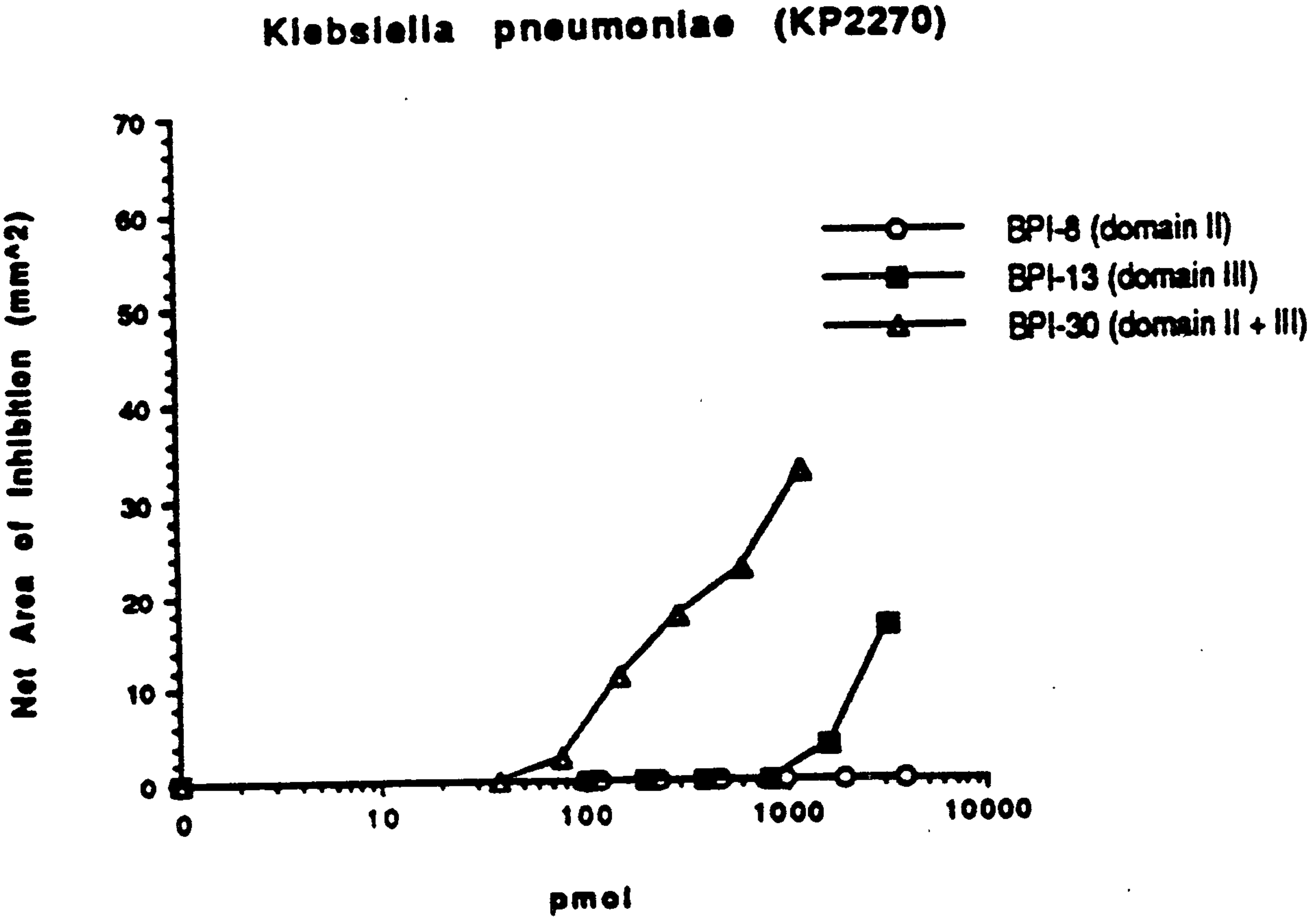
Klebsiella pneumoniae (KP2270)
Net Area of Inhibition (mm^2)
70
60
50
40
30
20
10
0
0
10
100
1000
10000
pmol
BPI-8 (domain II)
BPI-13 (domain III)
BPI-30 (domain II + III)

FIGURE 11d

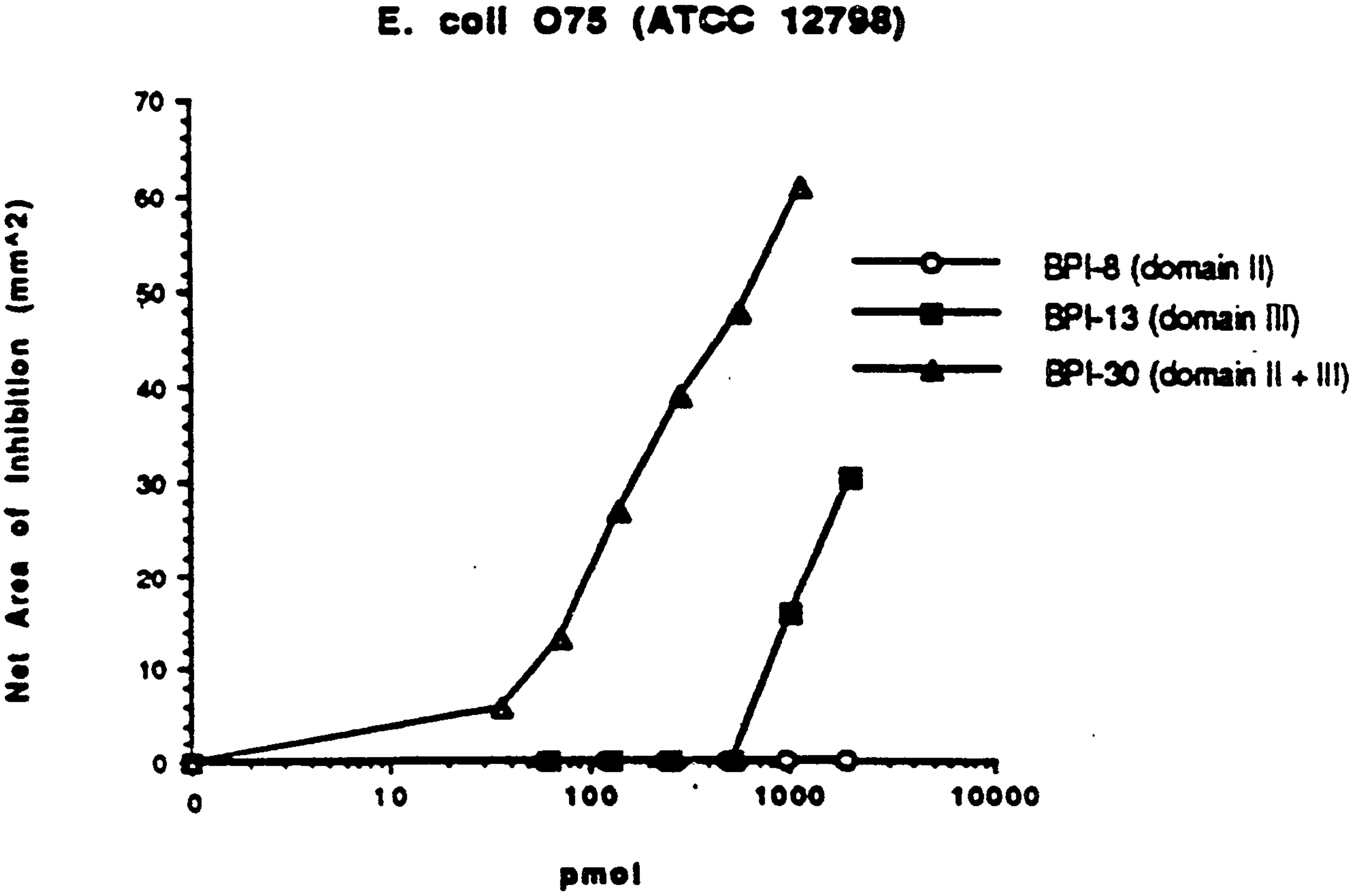
E. coli O75 (ATCC 12798)
Net Area of Inhibition (mm^2)
70
60
50
40
30
20
10
0
0
10
100
1000
10000
pmol
BPI-8 (domain II)
BPI-13 (domain III)
BPI-30 (domain II + III)

FIGURE 11e

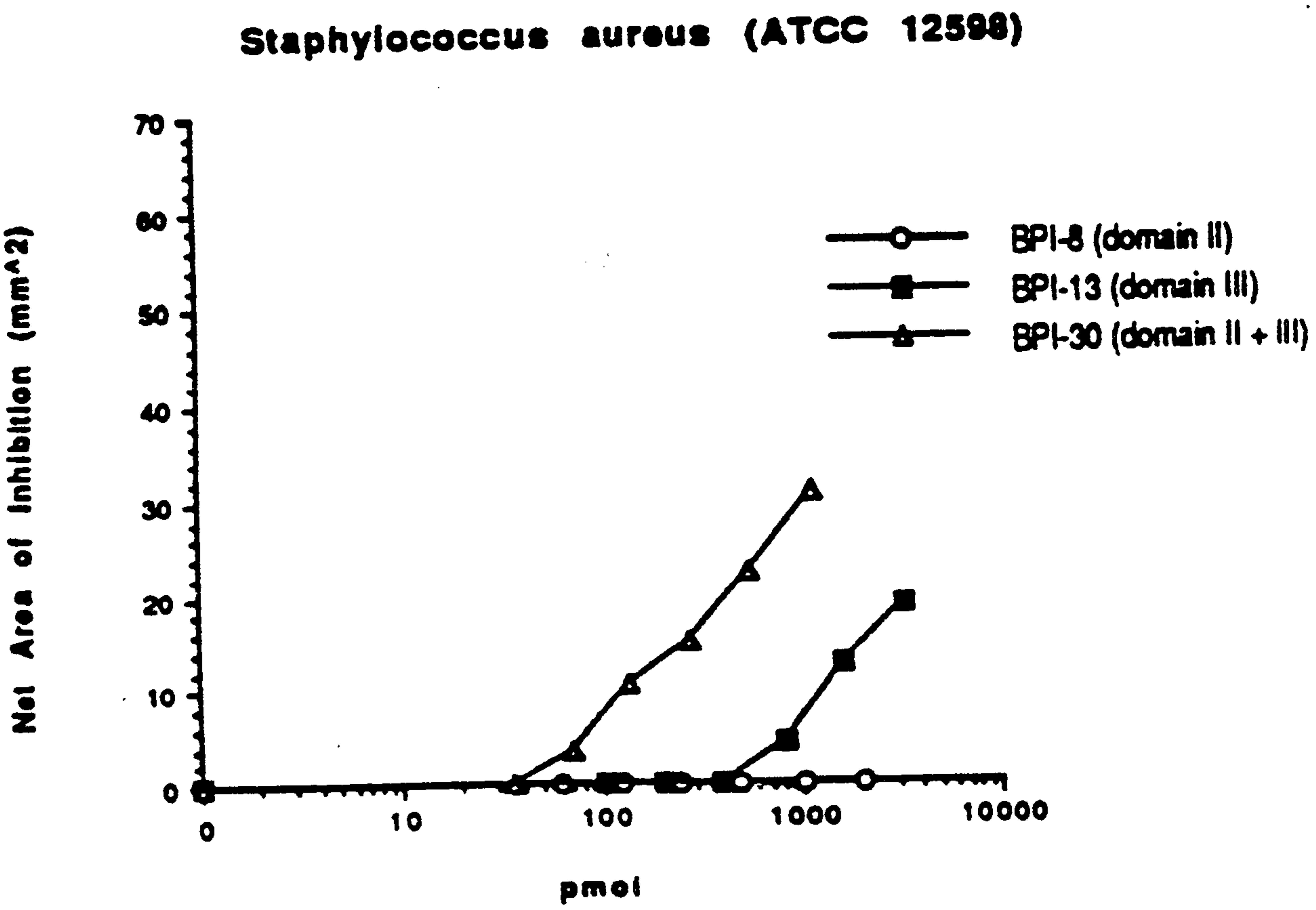
Staphylococcus aureus (ATCC 12598)
Net Area of Inhibition (mm^2)
70
60
50
40
30
20
10
0
0
10
100
1000
10000
pmol
BPI-8 (domain II)
BPI-13 (domain III)
BPI-30 (domain II + III)

FIGURE 11f

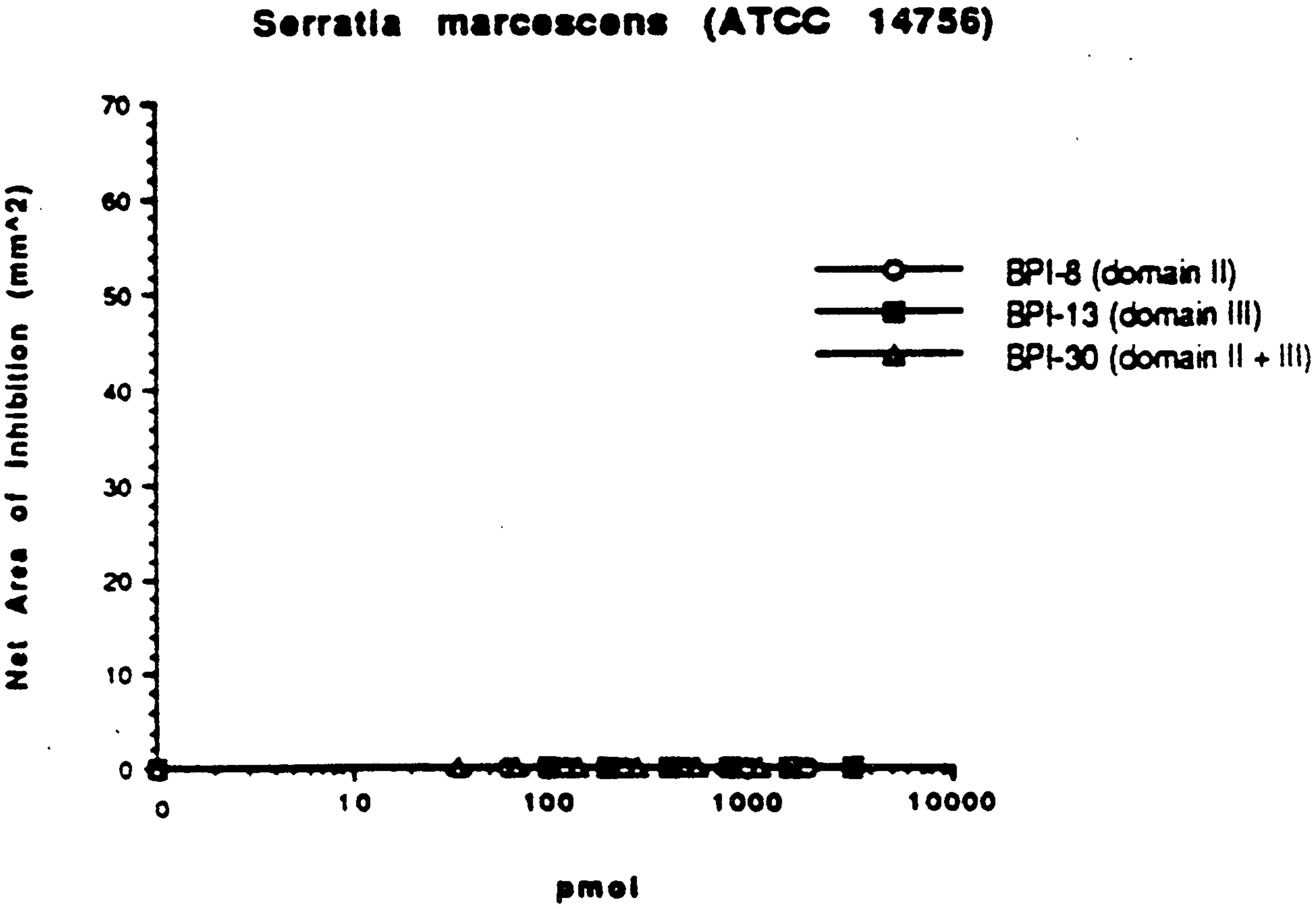
Serratia marcescens (ATCC 14756)
Net Area of Inhibition (mm^2)
70
60
50
40
30
20
10
0
0
10
100
1000
10000
pmol
BPI-8 (domain II)
BPI-13 (domain III)
BPI-30 (domain II + III)

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FIGURE 11g

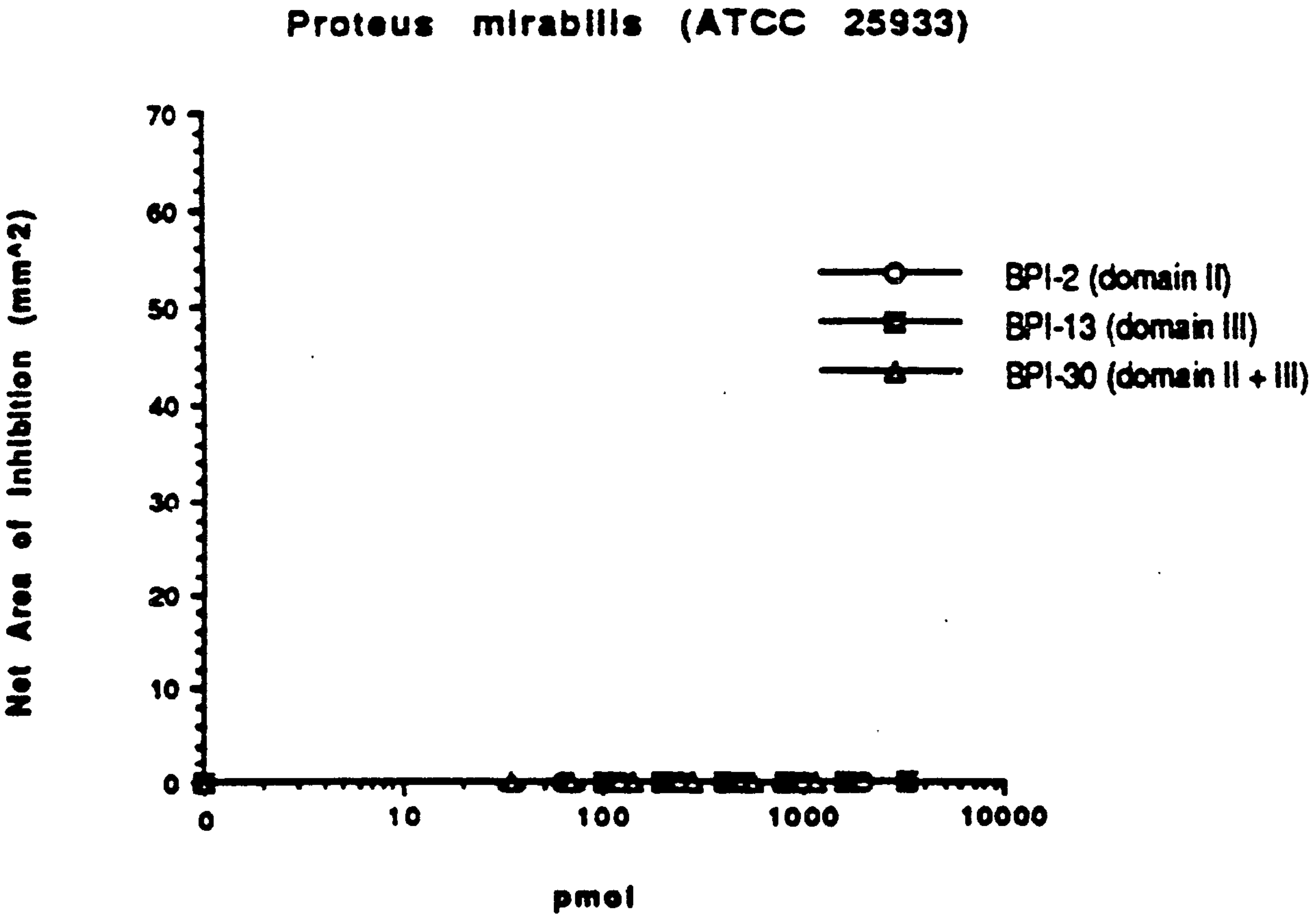

FIGURE 11h

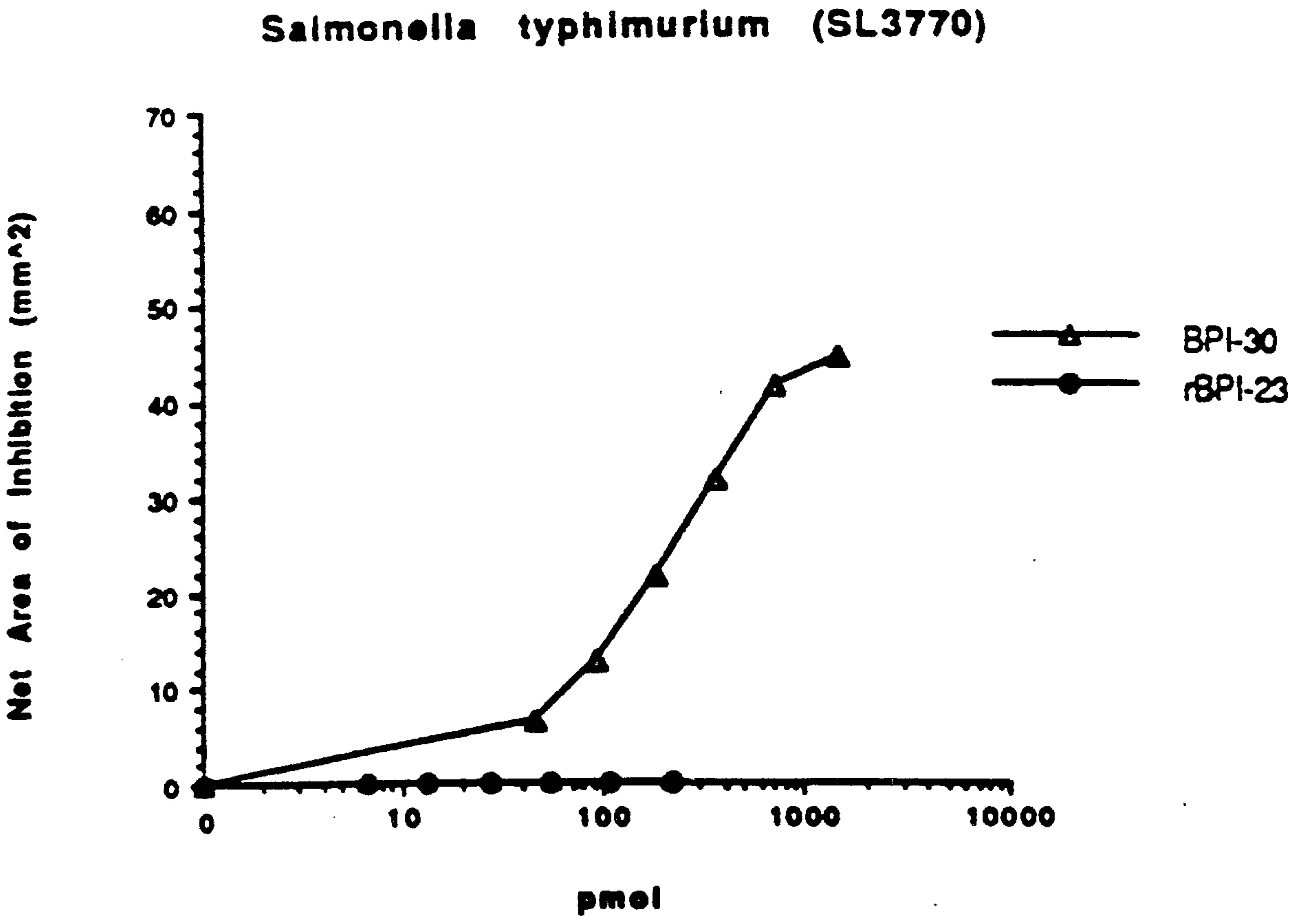
Salmonella typhimurium (SL3770)
Net Area of Inhibition (mm^2)
70
60
50
40
30
20
10
0
0
10
100
1000
10000
pmol
BPI-30
rBPI-23

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FIGURE 11i

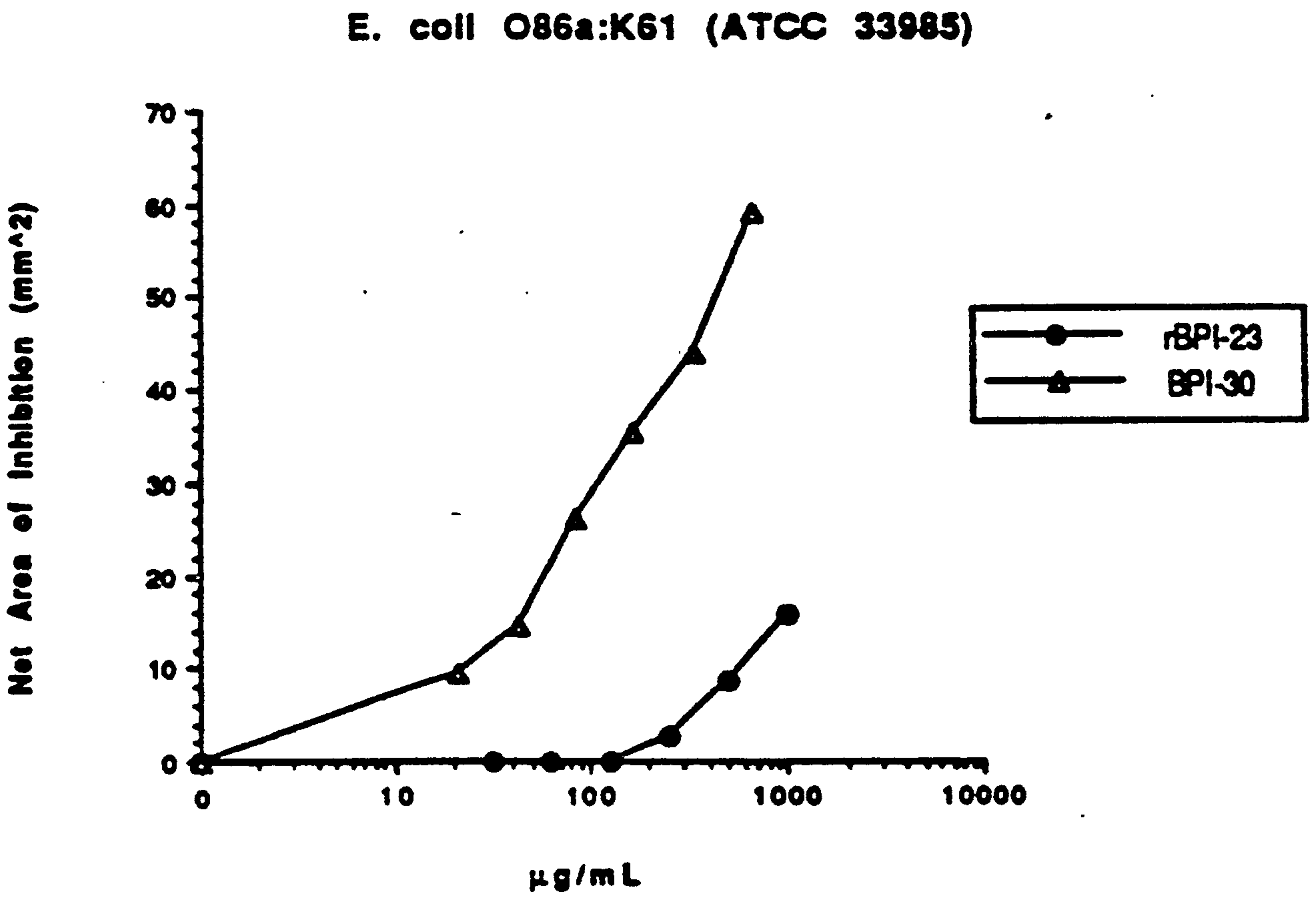

FIGURE 11j

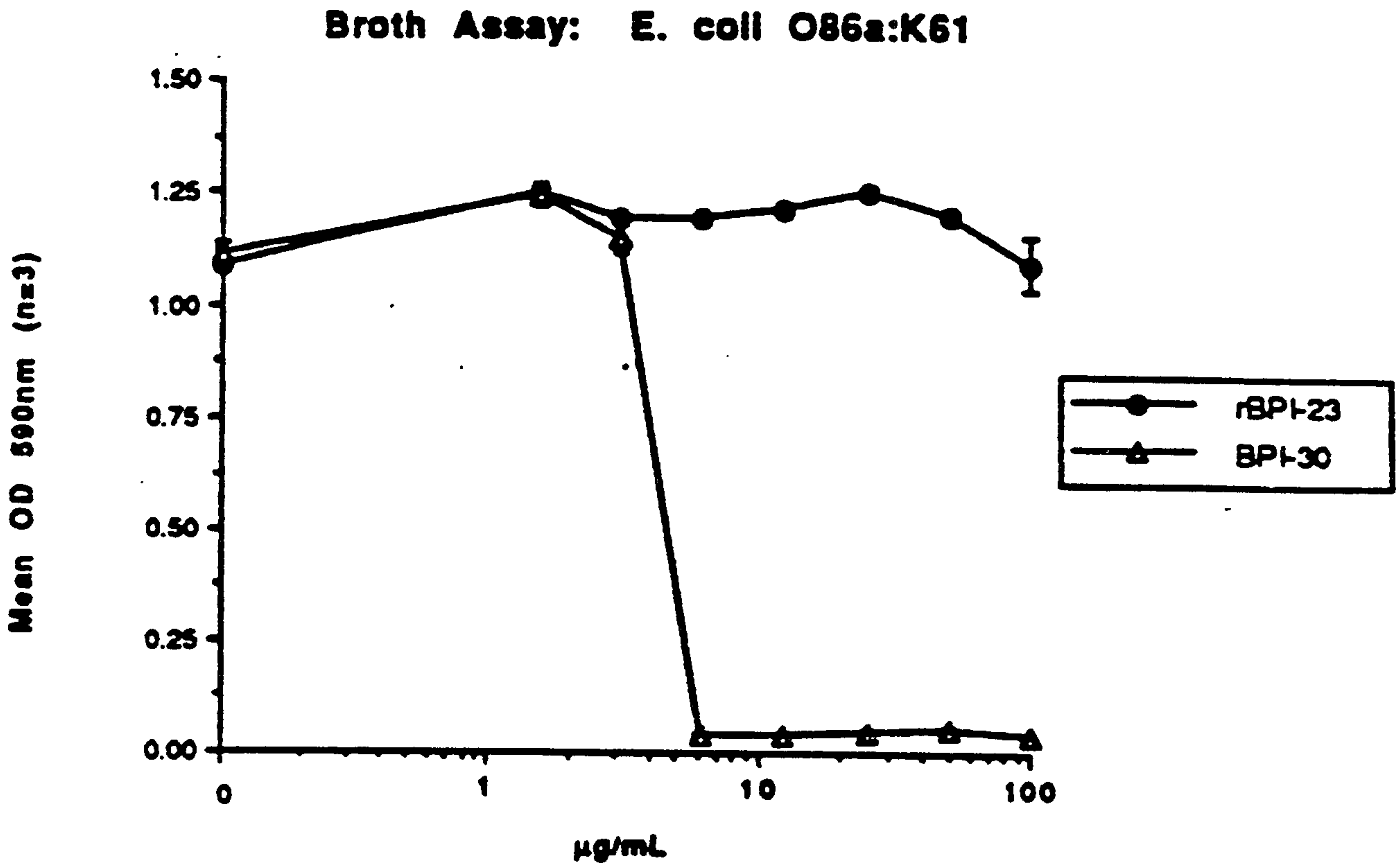
Broth Assay: E. coli O86a:K61
Mean OD 590nm (n=3)
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0
1
10
100
µg/mL
rBPI-23
BPI-30

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FIGURE 11k

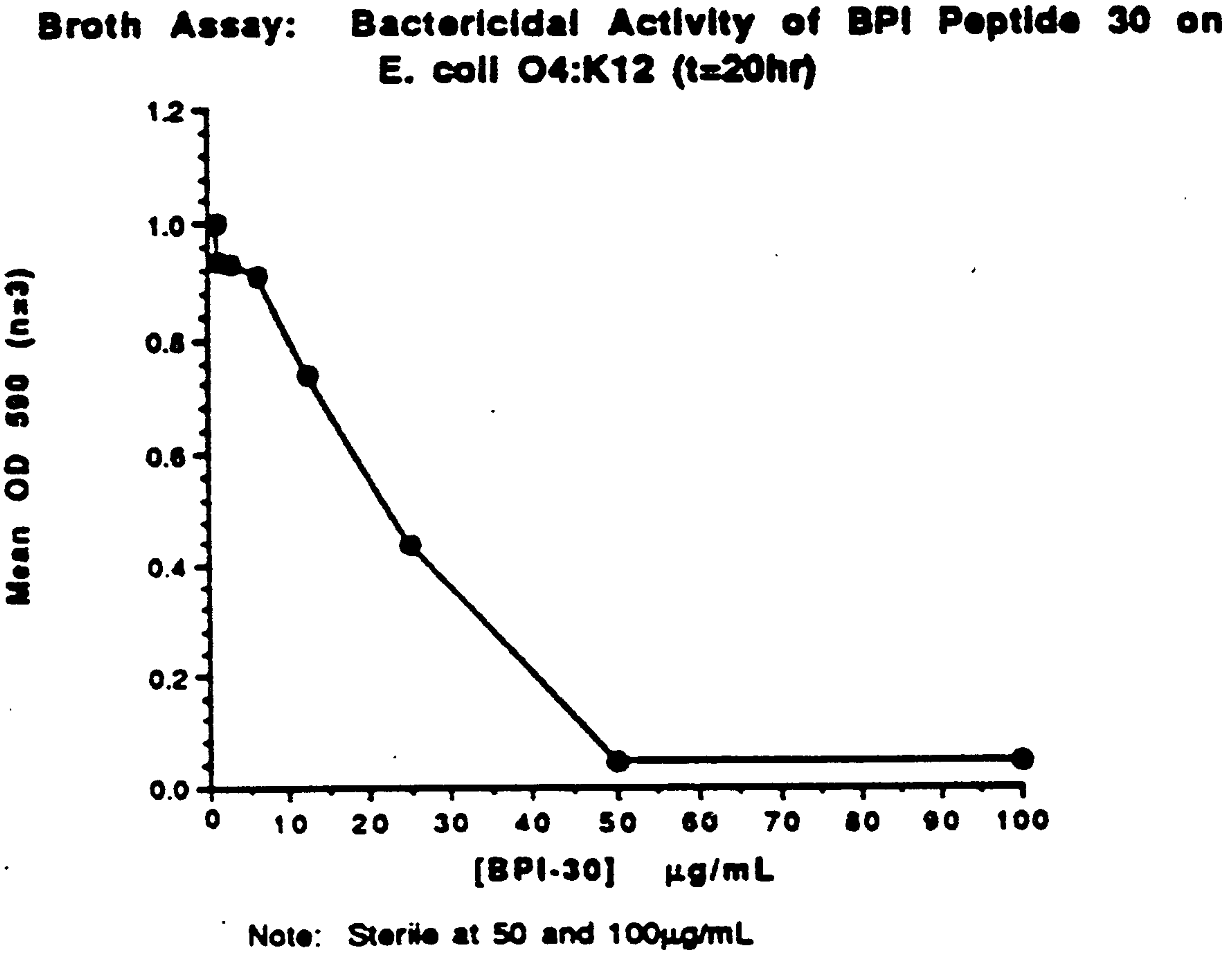

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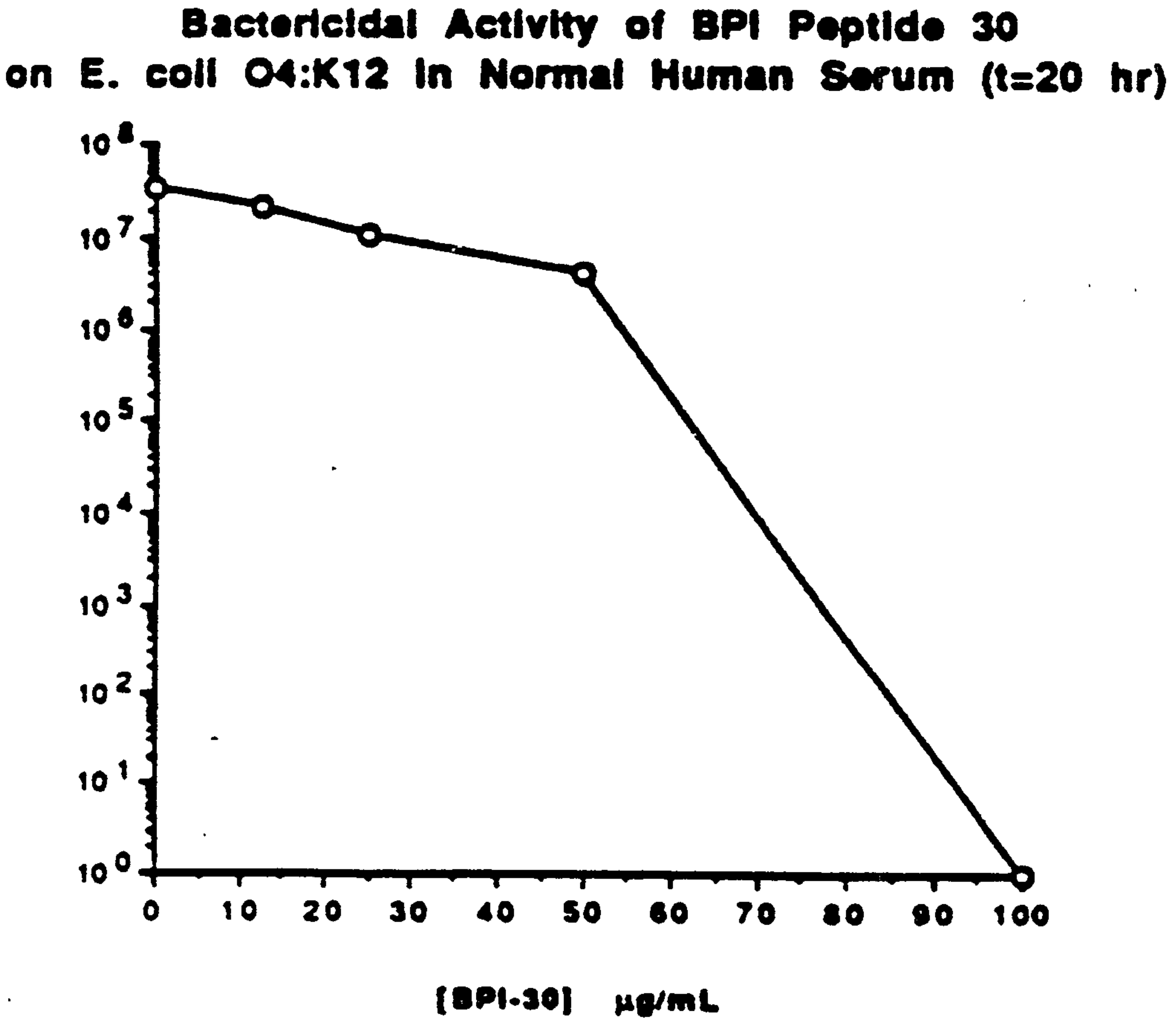
FIGURE 11 1



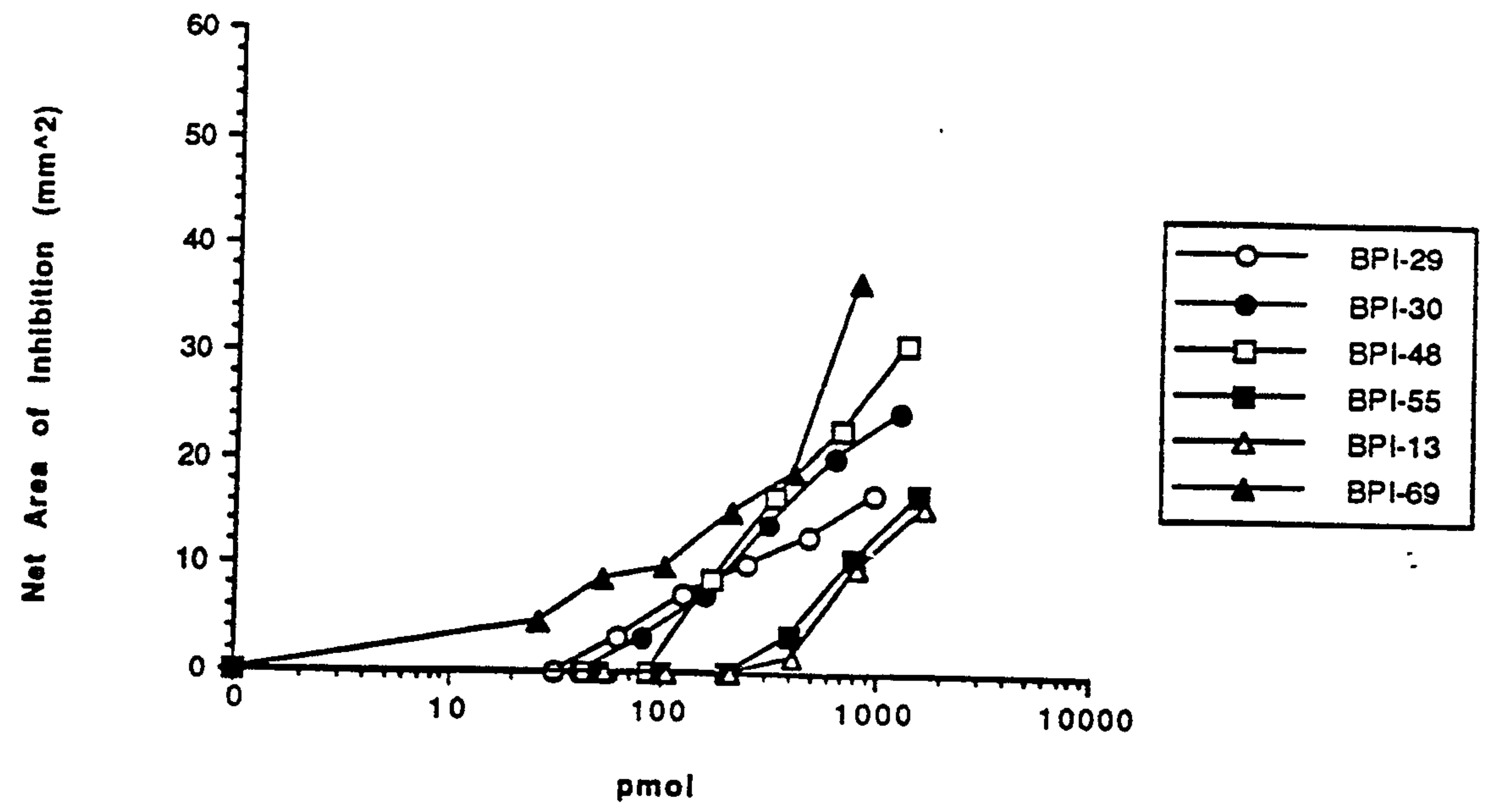


FIGURE 11m

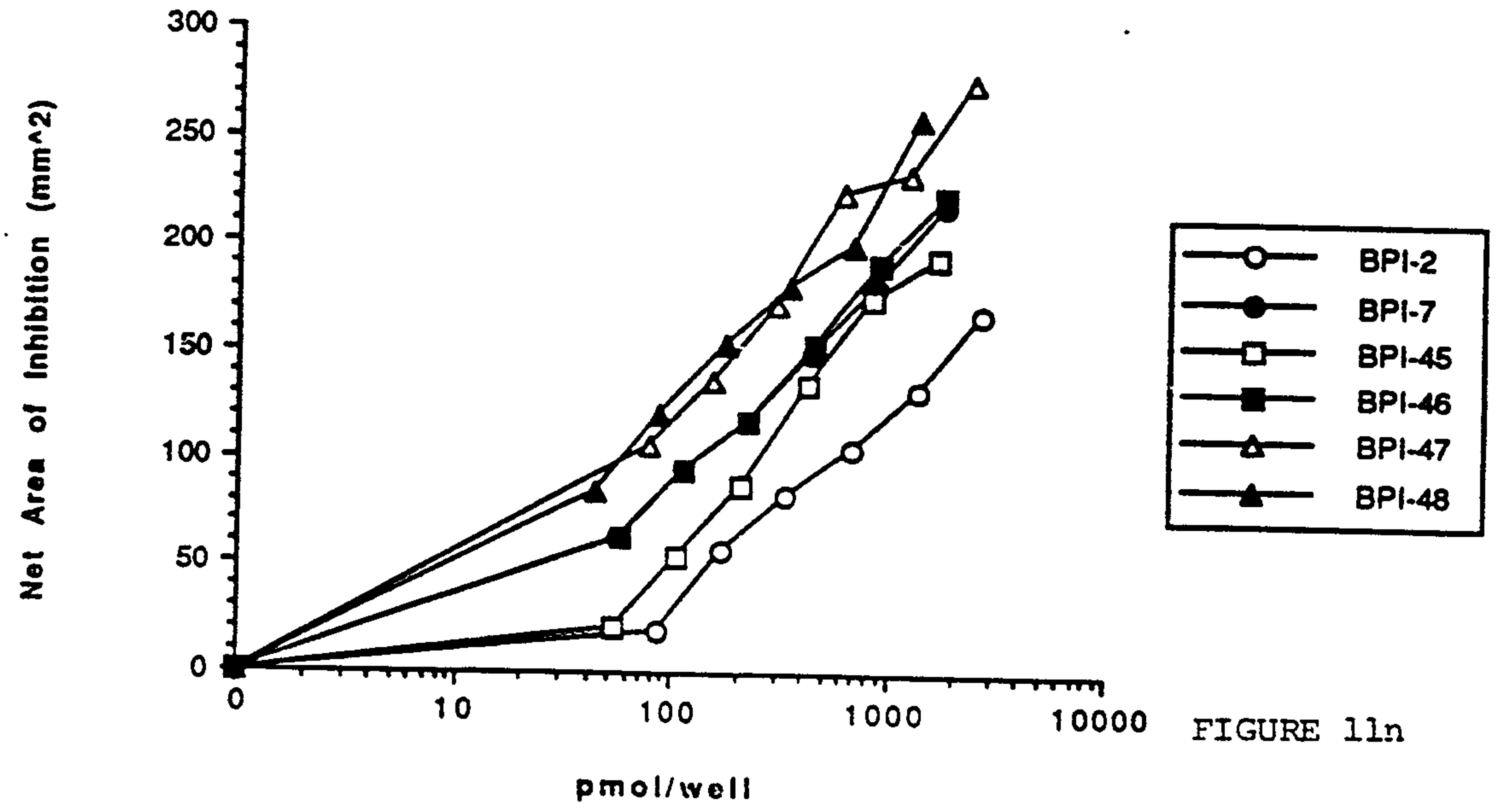


FIGURE 11n

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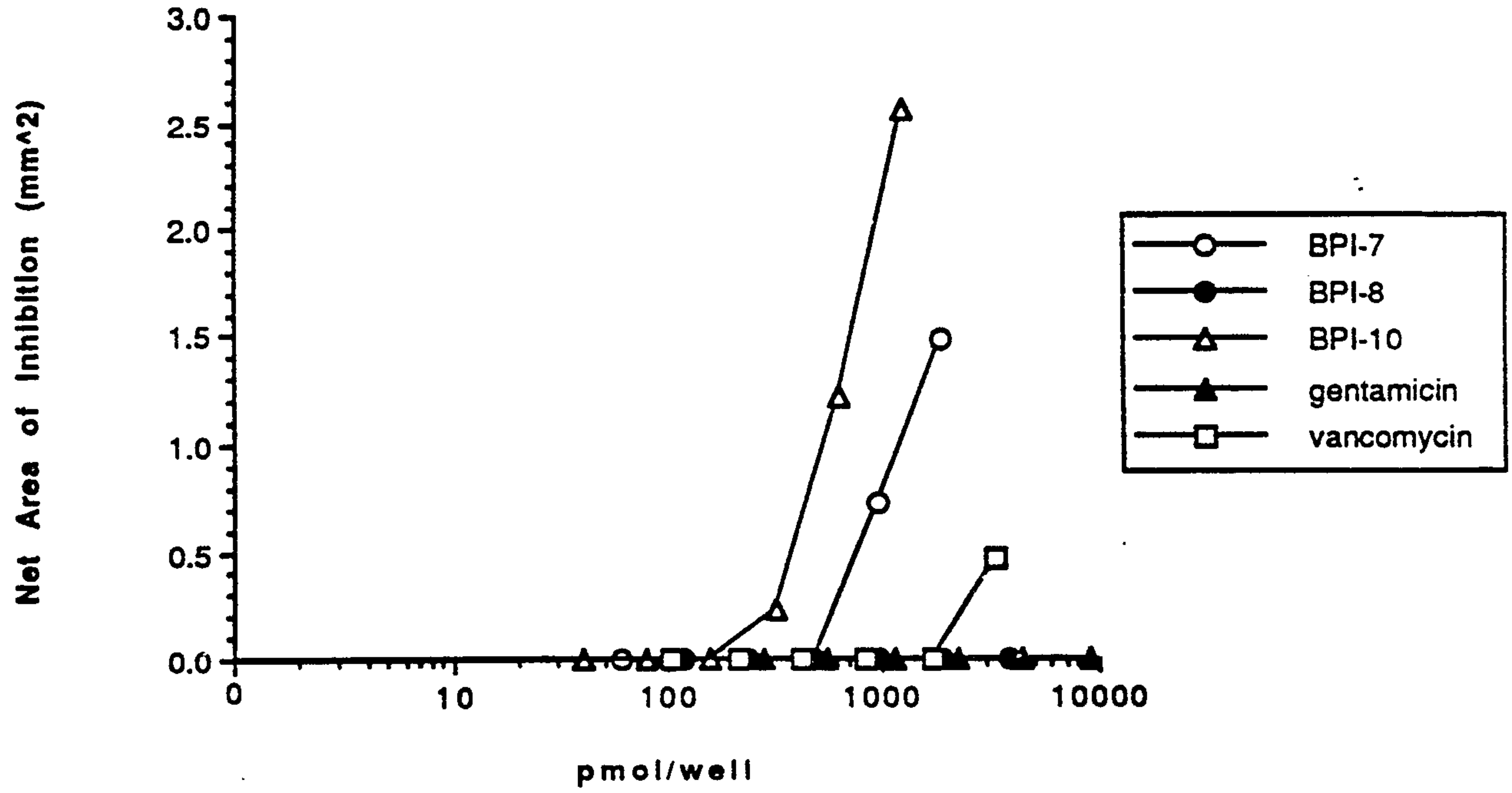

FIGURE 11o

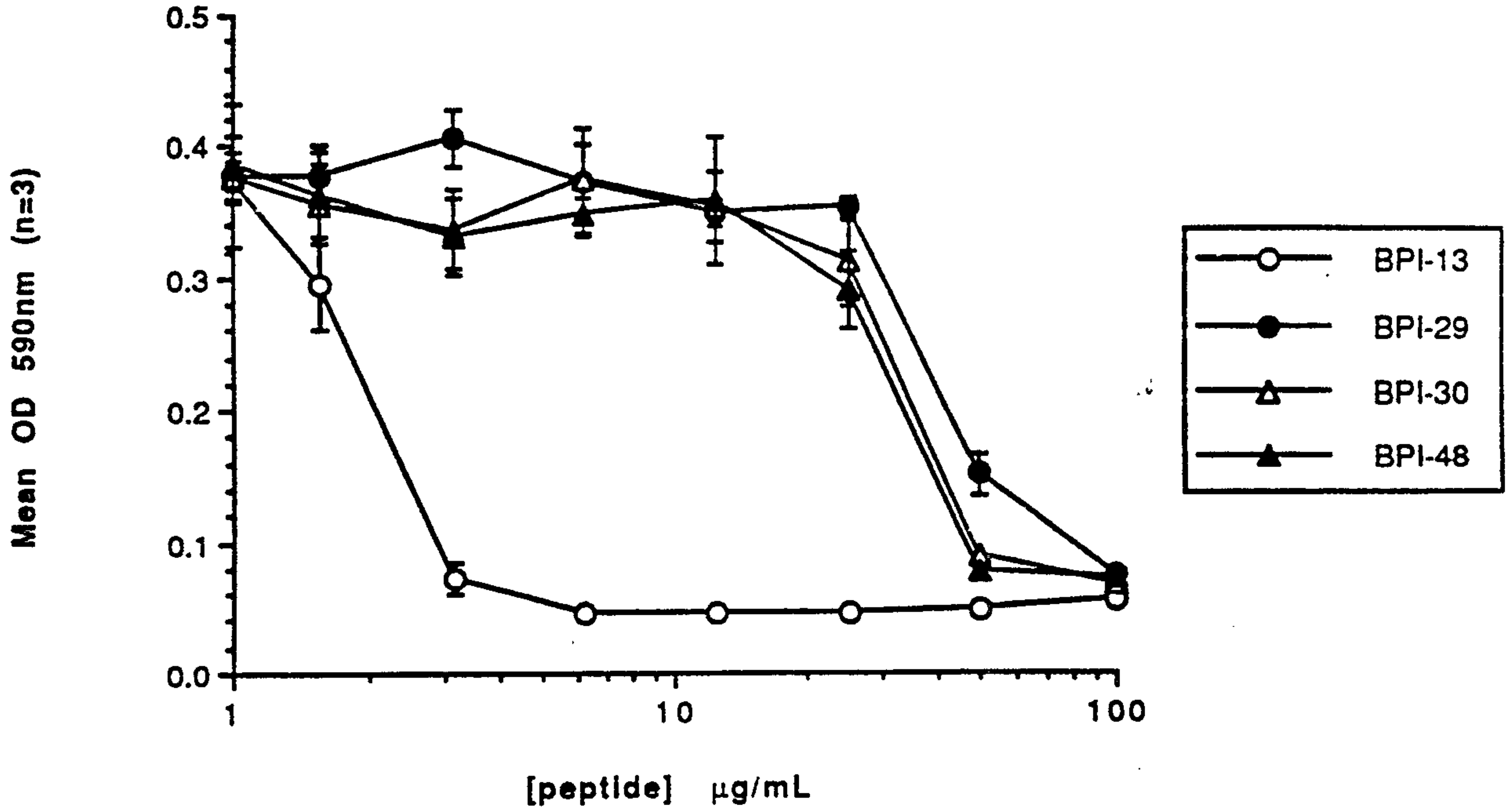
0.5
0.4
0.3
0.2
0.1
0.0
Mean OD 590nm (n=3)
1
10
100
[peptide] µg/mL
BPI-13
BPI-29
BPI-30
BPI-48

FIGURE 11p

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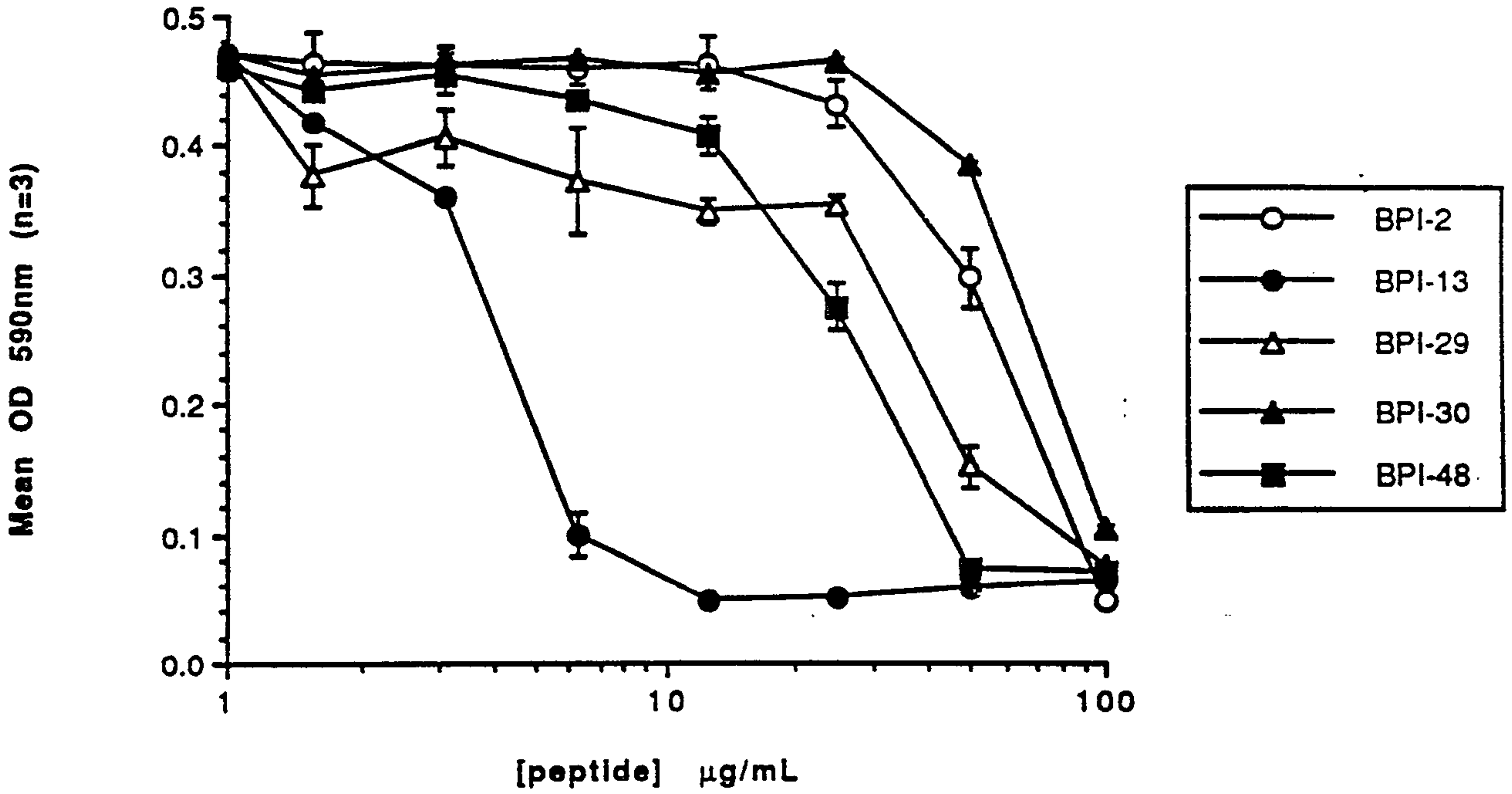

FIGURE 11q

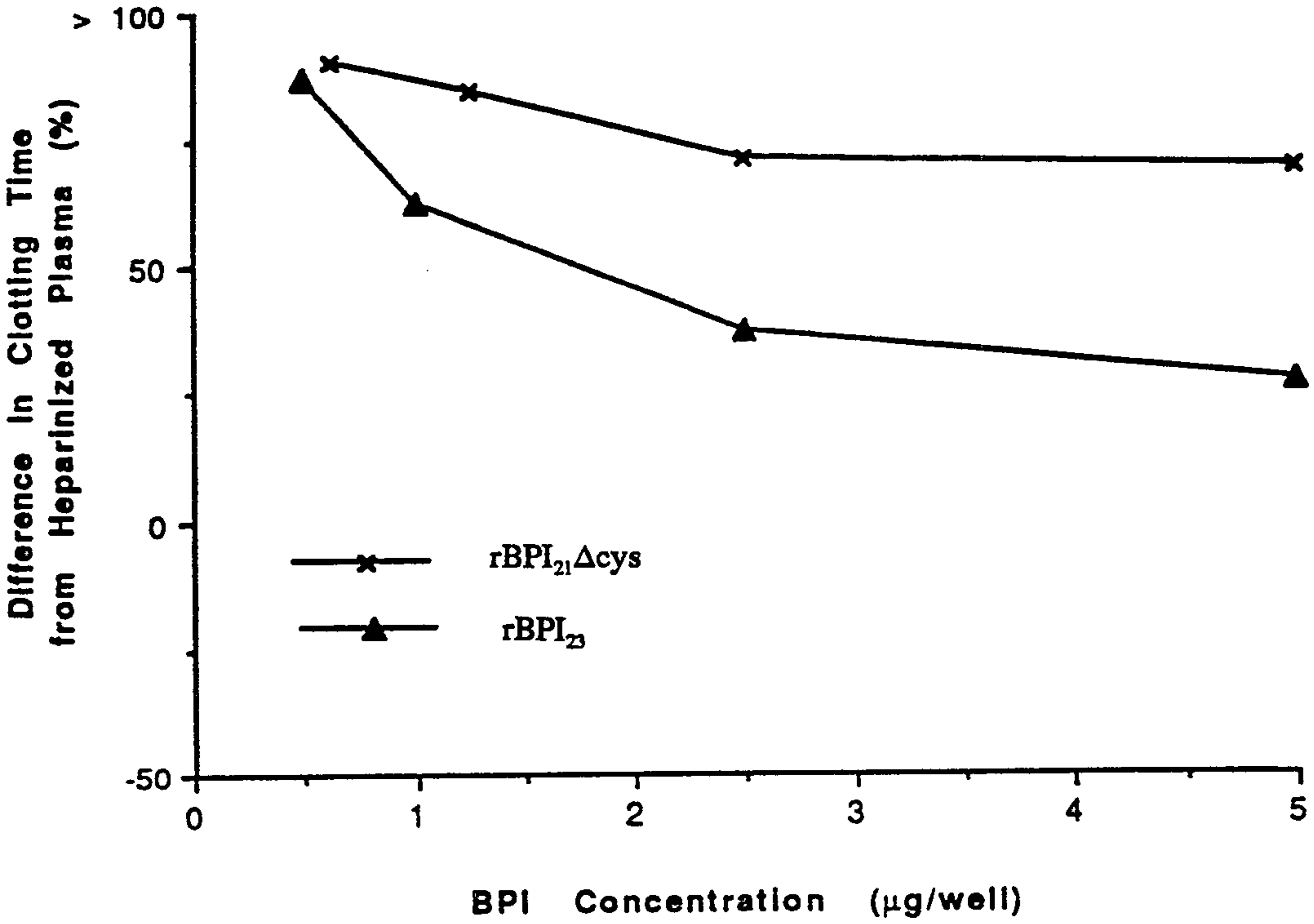
> 100
50
0
-50
0
1
2
3
4
5
Difference In Clotting Time from Heparinized Plasma (%)
BPI Concentration (µg/well)
rBPI$_{21}$Δcys
rBPI$_{23}$

FIGURE 12a

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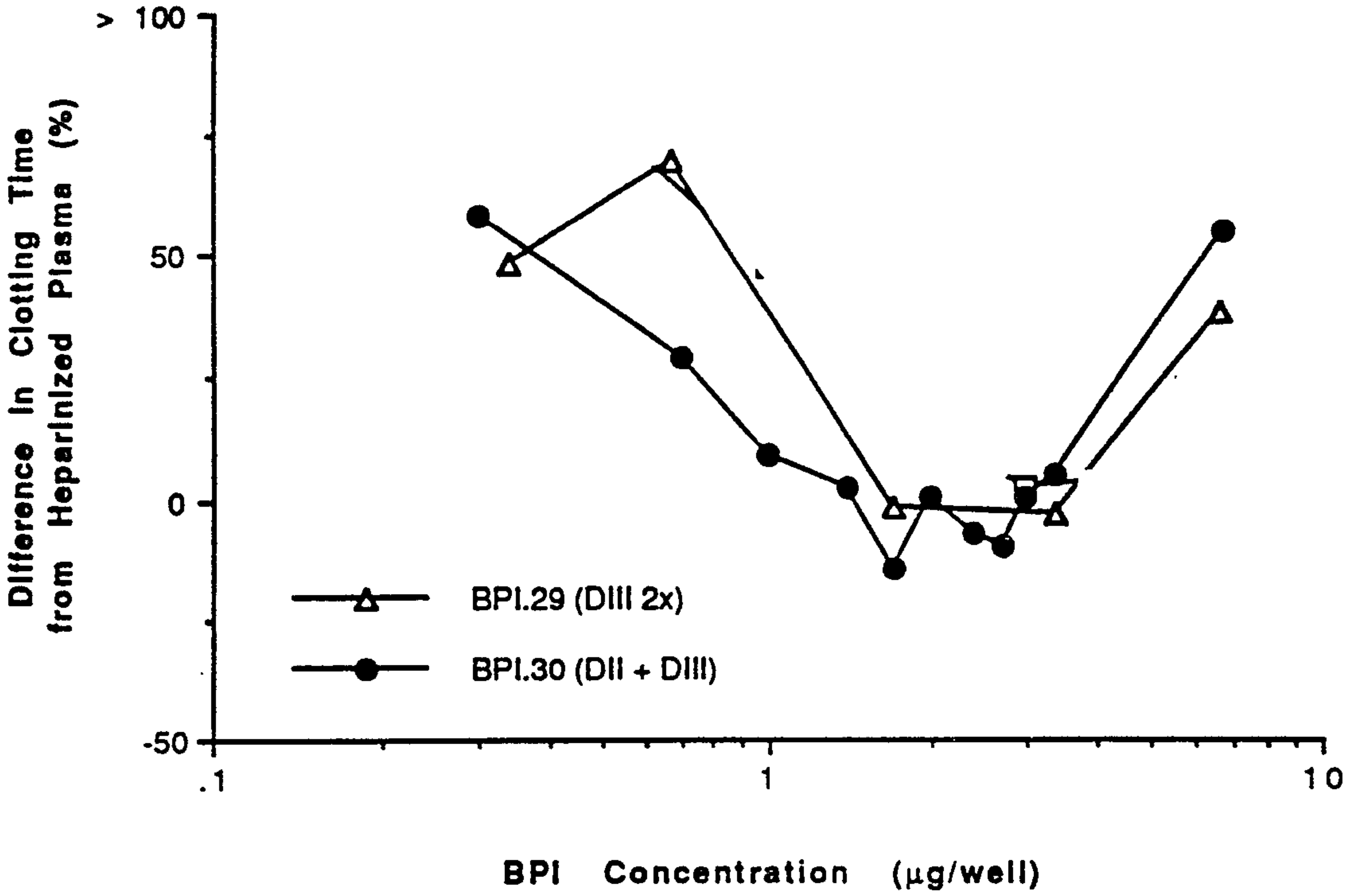

FIGURE 12b

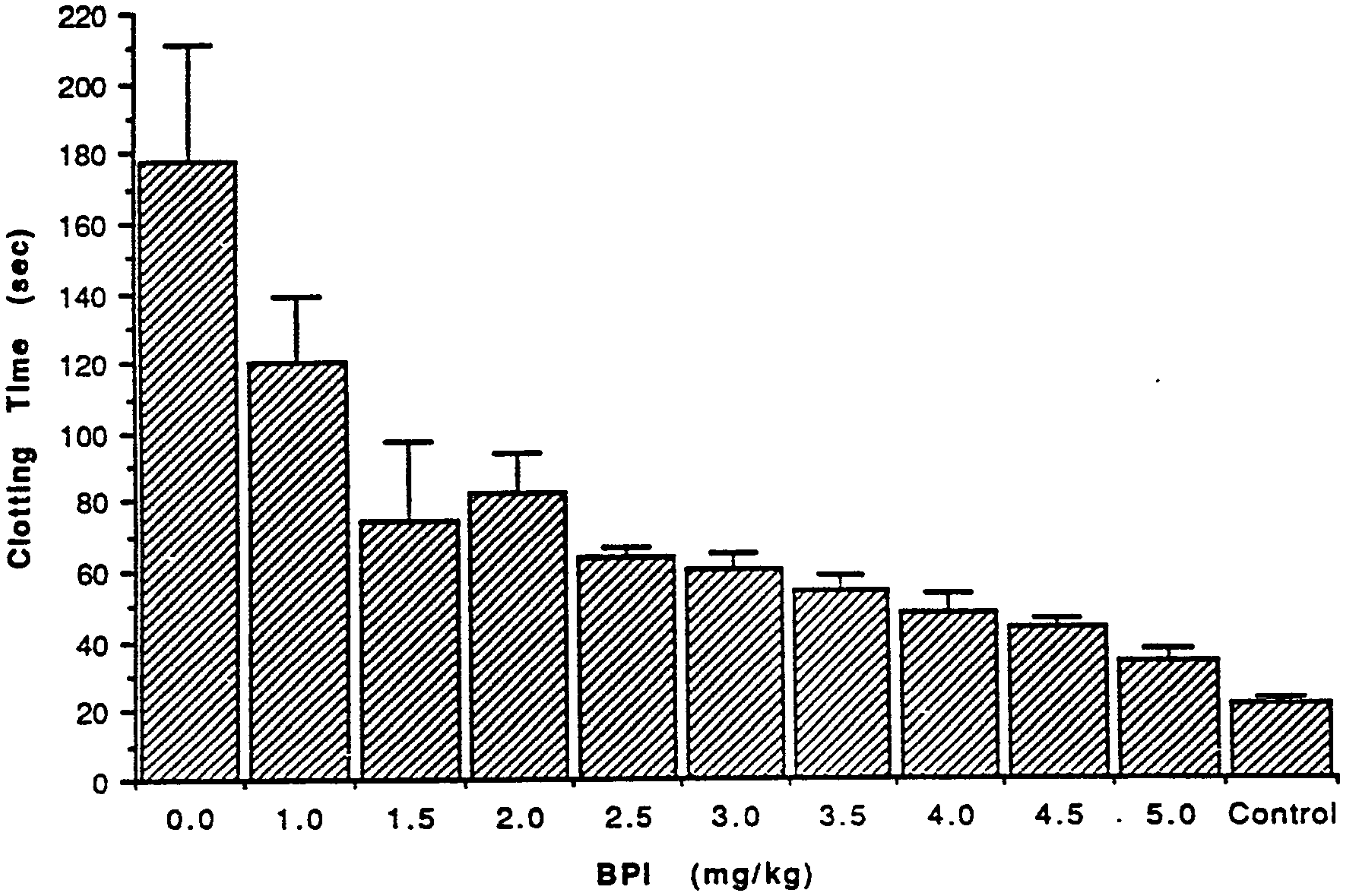
Clotting Time (sec)
220
200
180
160
140
120
100
80
60
40
20
0
0.0
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
Control
BPI (mg/kg)

FIGURE 12c

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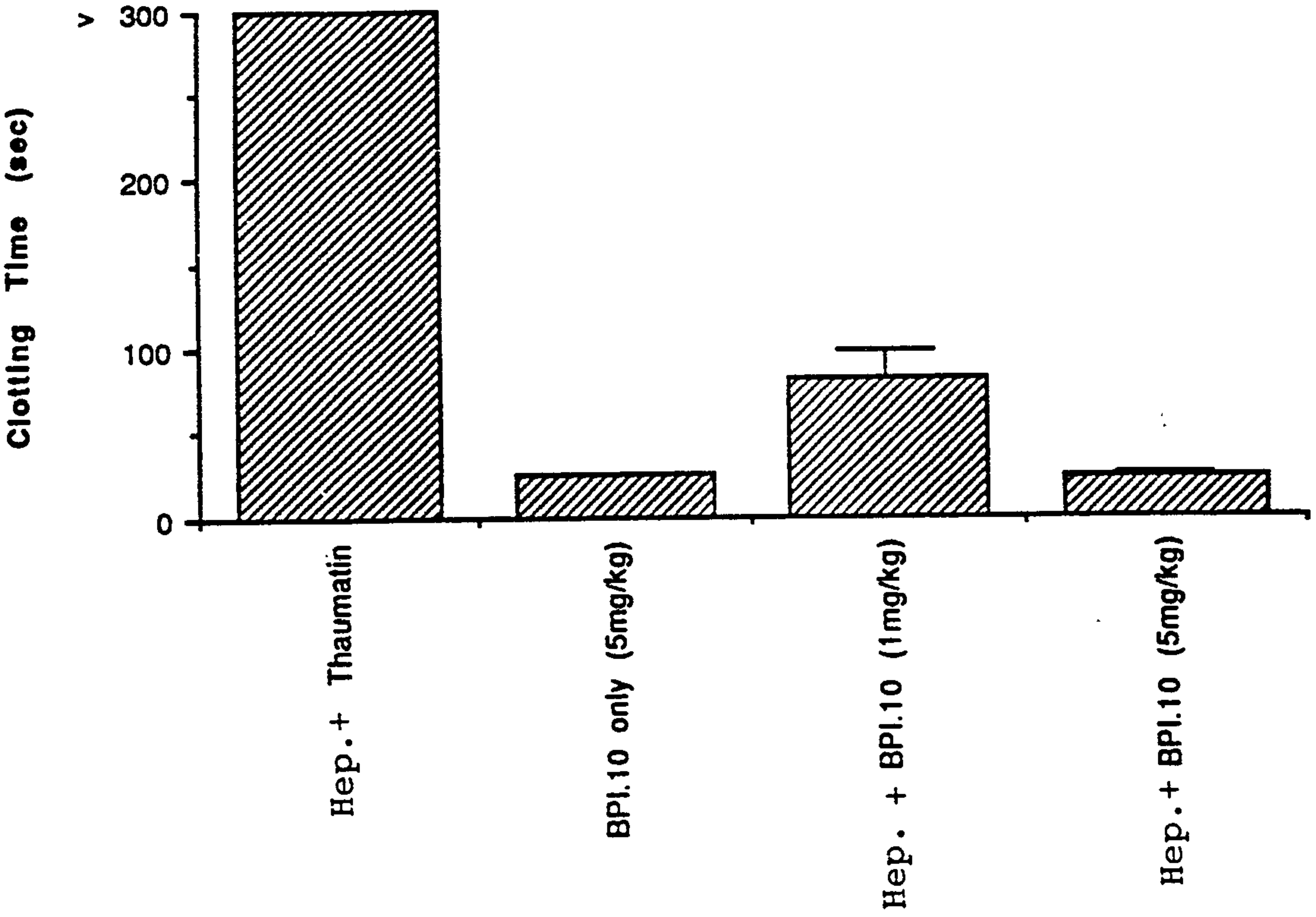

FIGURE 12d

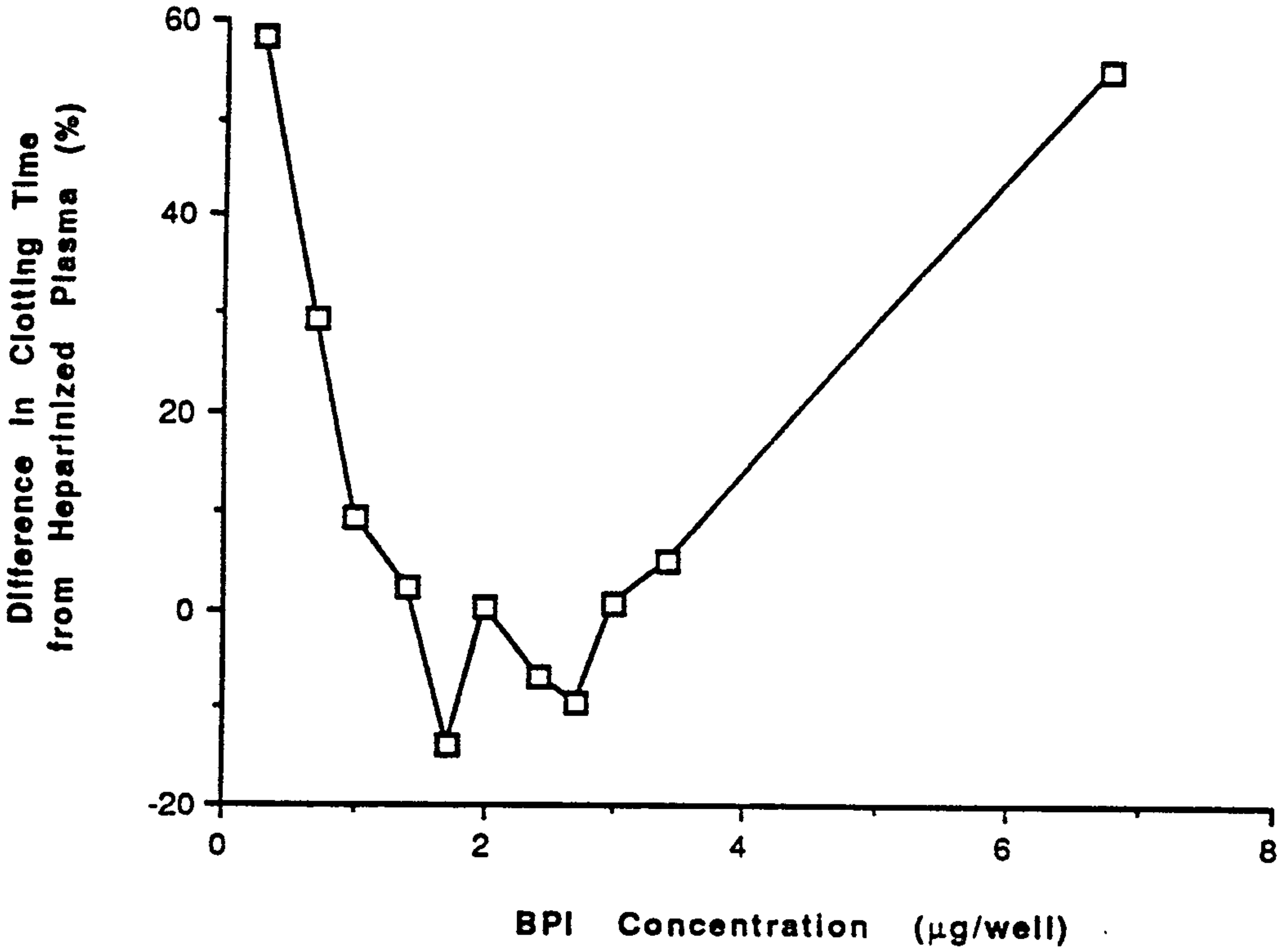
Difference In Clotting Time from Heparinized Plasma (%)
60
40
20
0
-20
0
2
4
6
8
BPI Concentration (µg/well)

FIGURE 12e

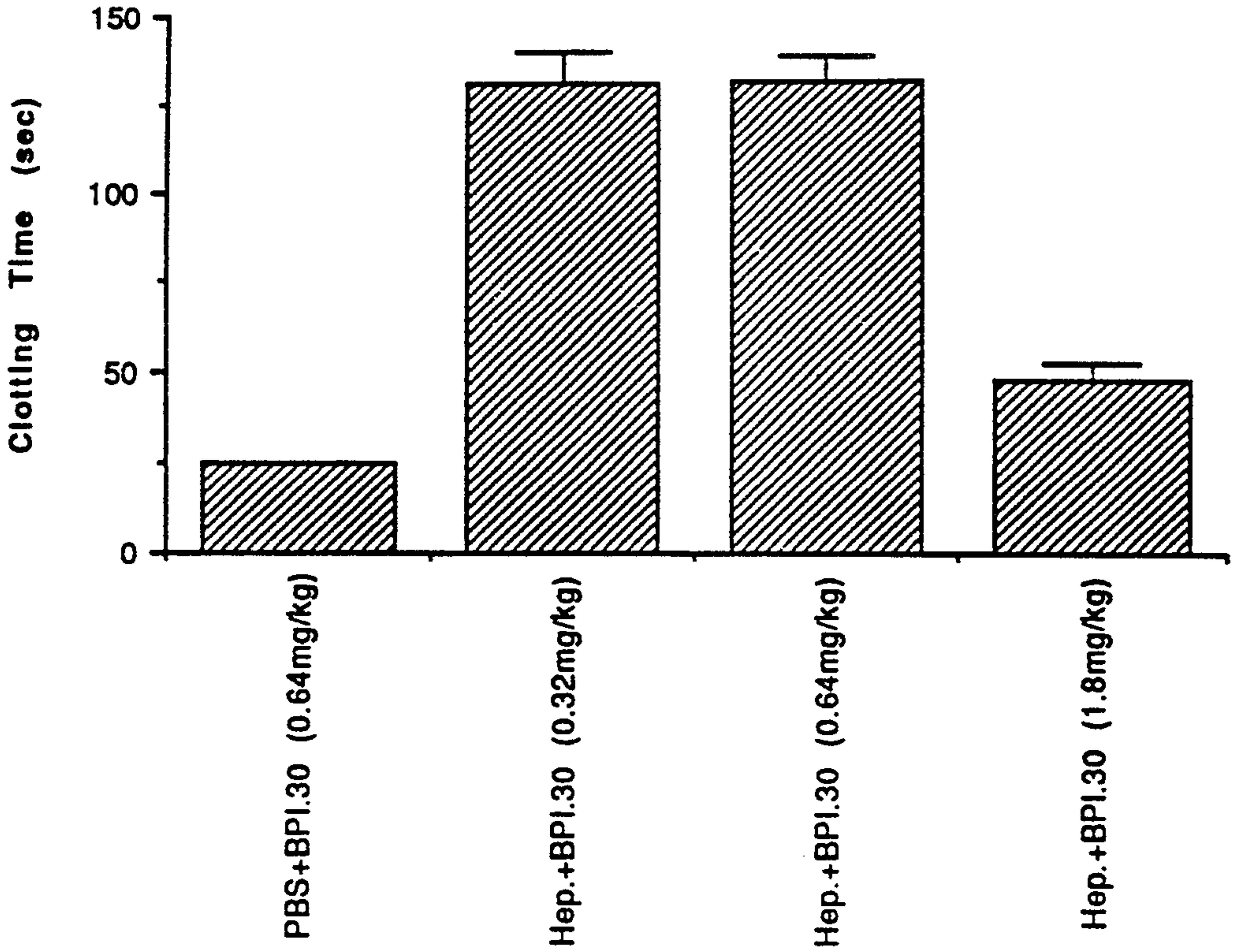
150
100
50
0
Clotting Time (sec)
PBS+BPI.30 (0.64mg/kg)
Hep.+BPI.30 (0.32mg/kg)
Hep.+BPI.30 (0.64mg/kg)
Hep.+BPI.30 (1.8mg/kg)

FIGURE 12f

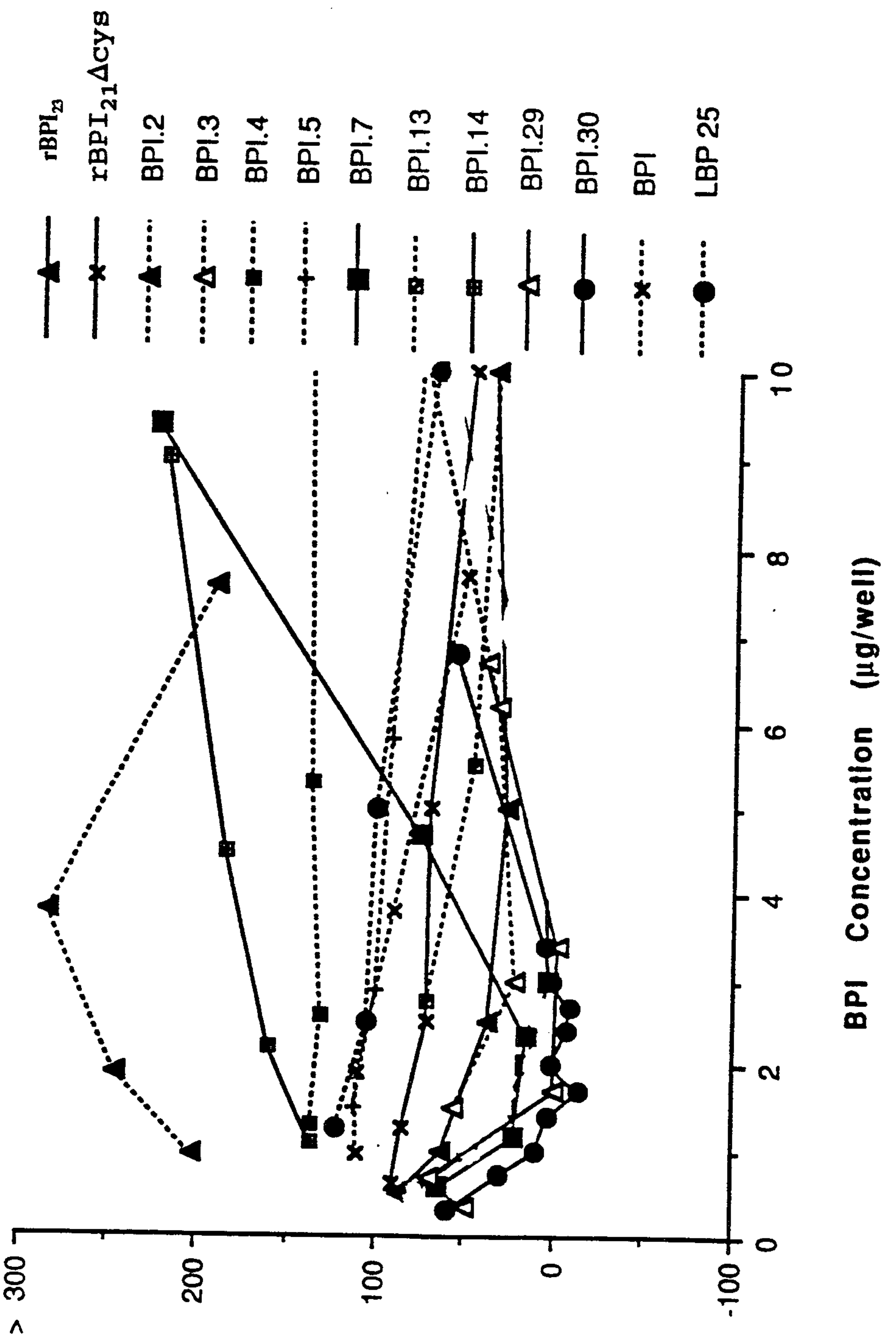
rBPI$_{23}$
rBPI$_{21}$Δcys
BPI.2
BPI.3
BPI.4
BPI.5
BPI.7
BPI.13
BPI.14
BPI.29
BPI.30
BPI
LBP 25
Difference in Clotting Time from Heparinized Plasma (%)
> 300
200
100
0
-100
0
2
4
6
8
10
BPI Concentration (µg/well)

FIGURE 12g

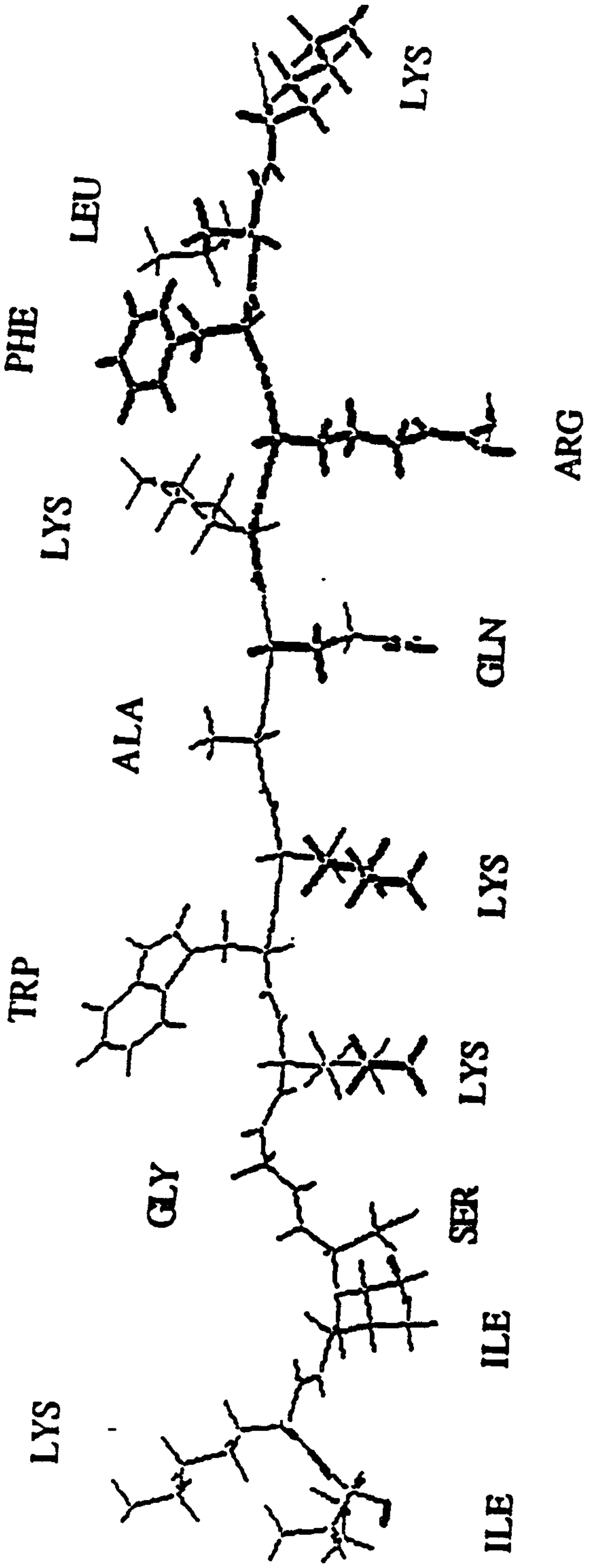

FIGURE 13

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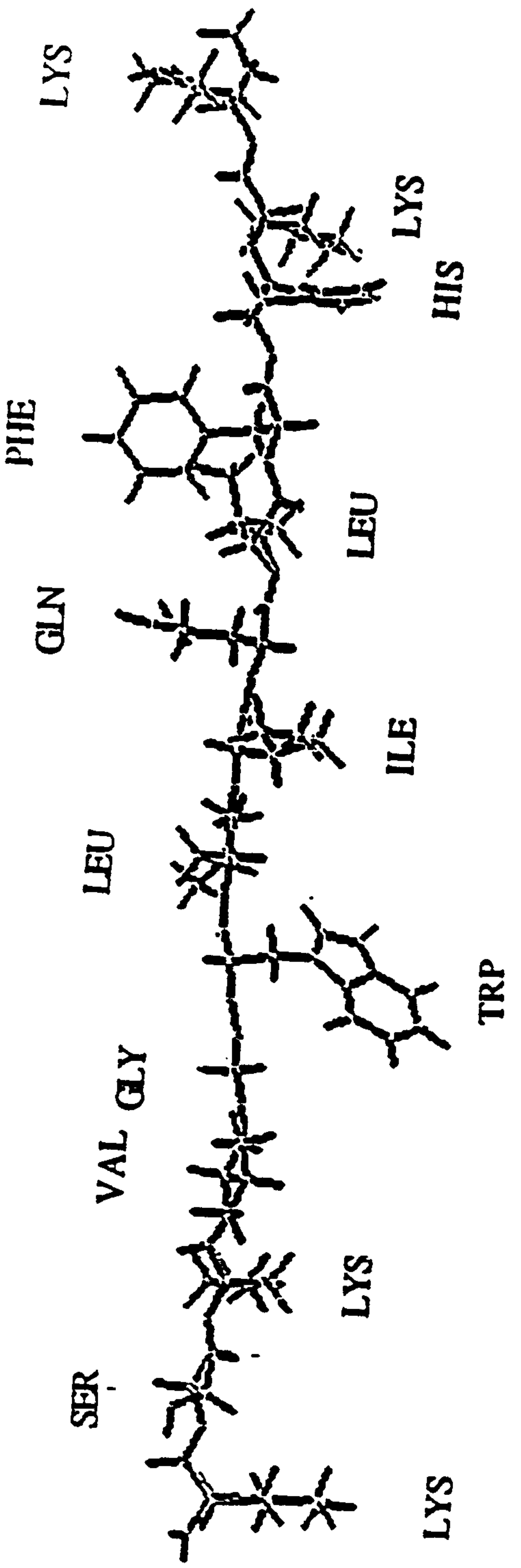

FIGURE 14

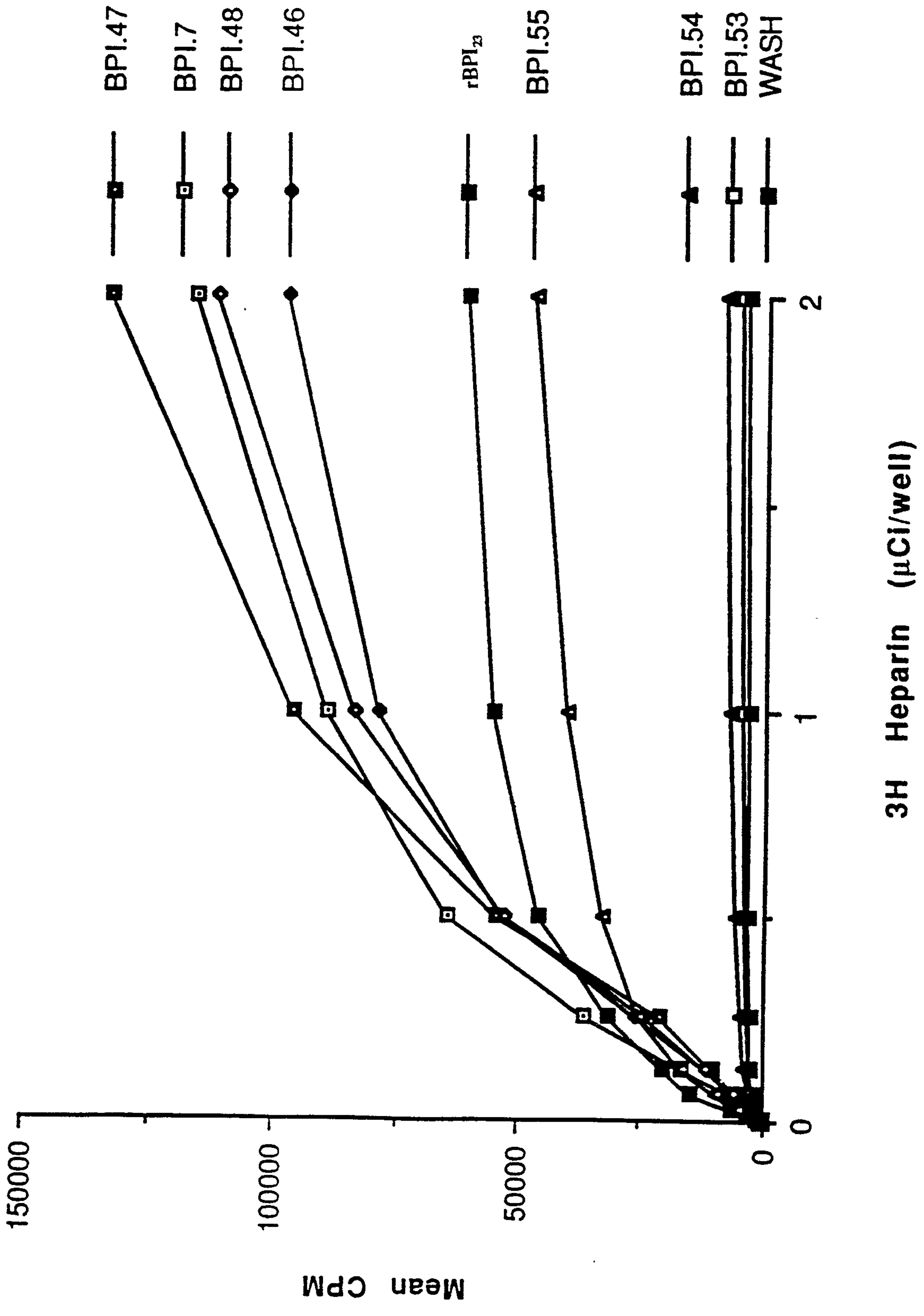
BPI.47
BPI.7
BPI.48
BPI.46
$rBPI_{23}$
BPI.55
BPI.54
BPI.53
WASH
Mean CPM
150000
100000
50000
0
0
1
2
3H Heparin (µCi/well)

FIGURE 15a

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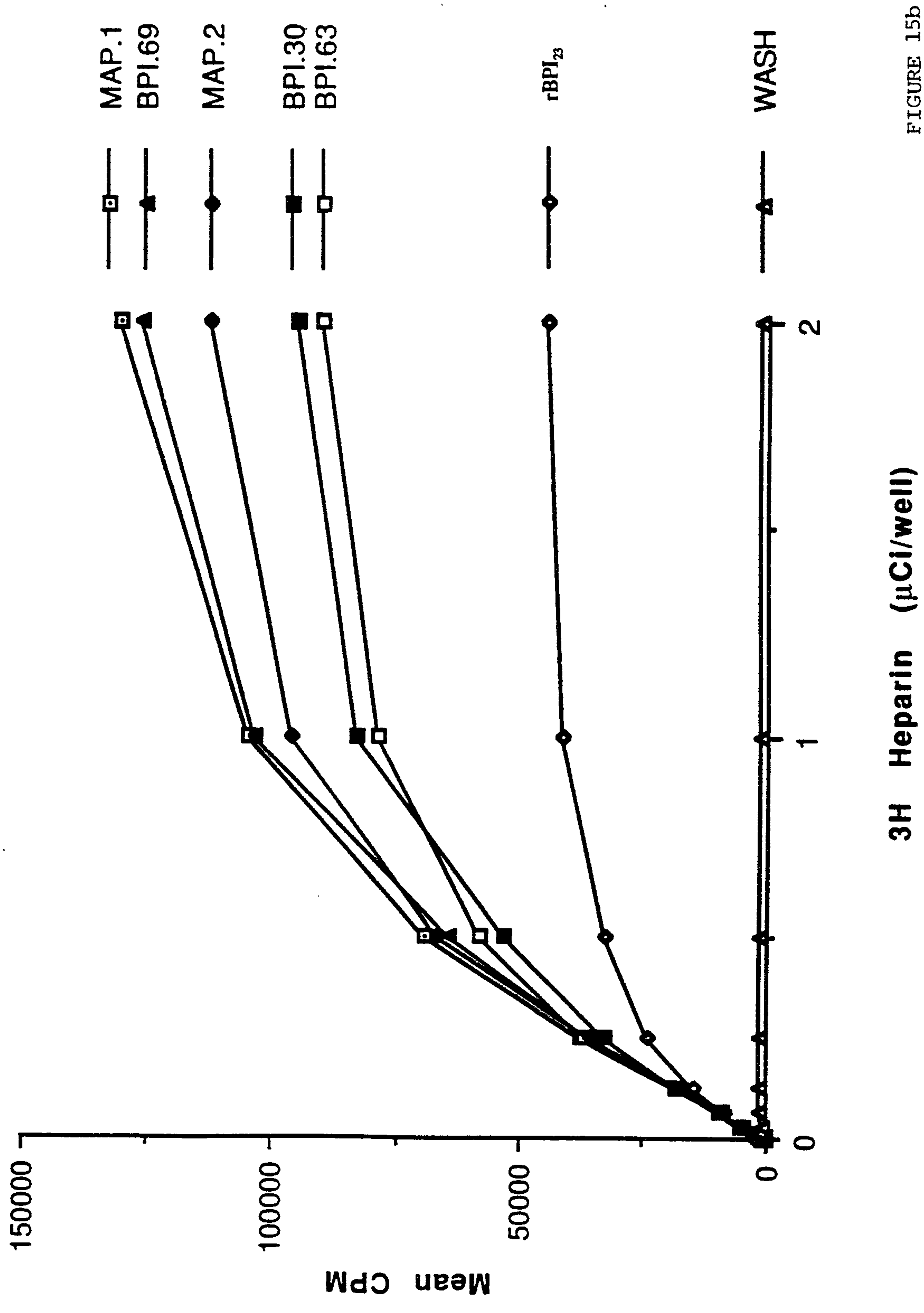

FIGURE 15b

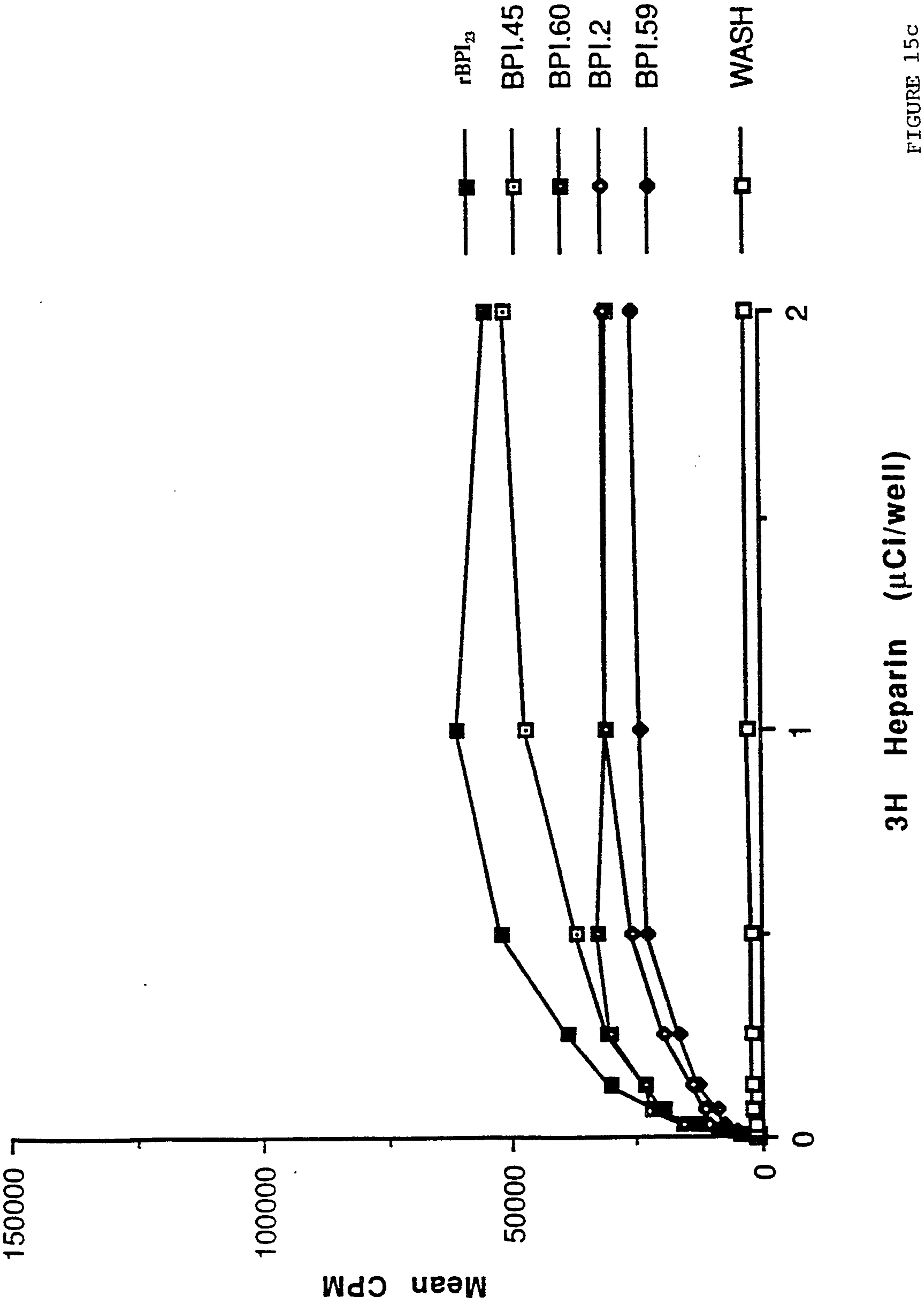
rBPI$_{23}$
BPI.45
BPI.60
BPI.2
BPI.59
WASH
Mean CPM
150000
100000
50000
0
0
1
2
3H Heparin (µCi/well)

FIGURE 15c

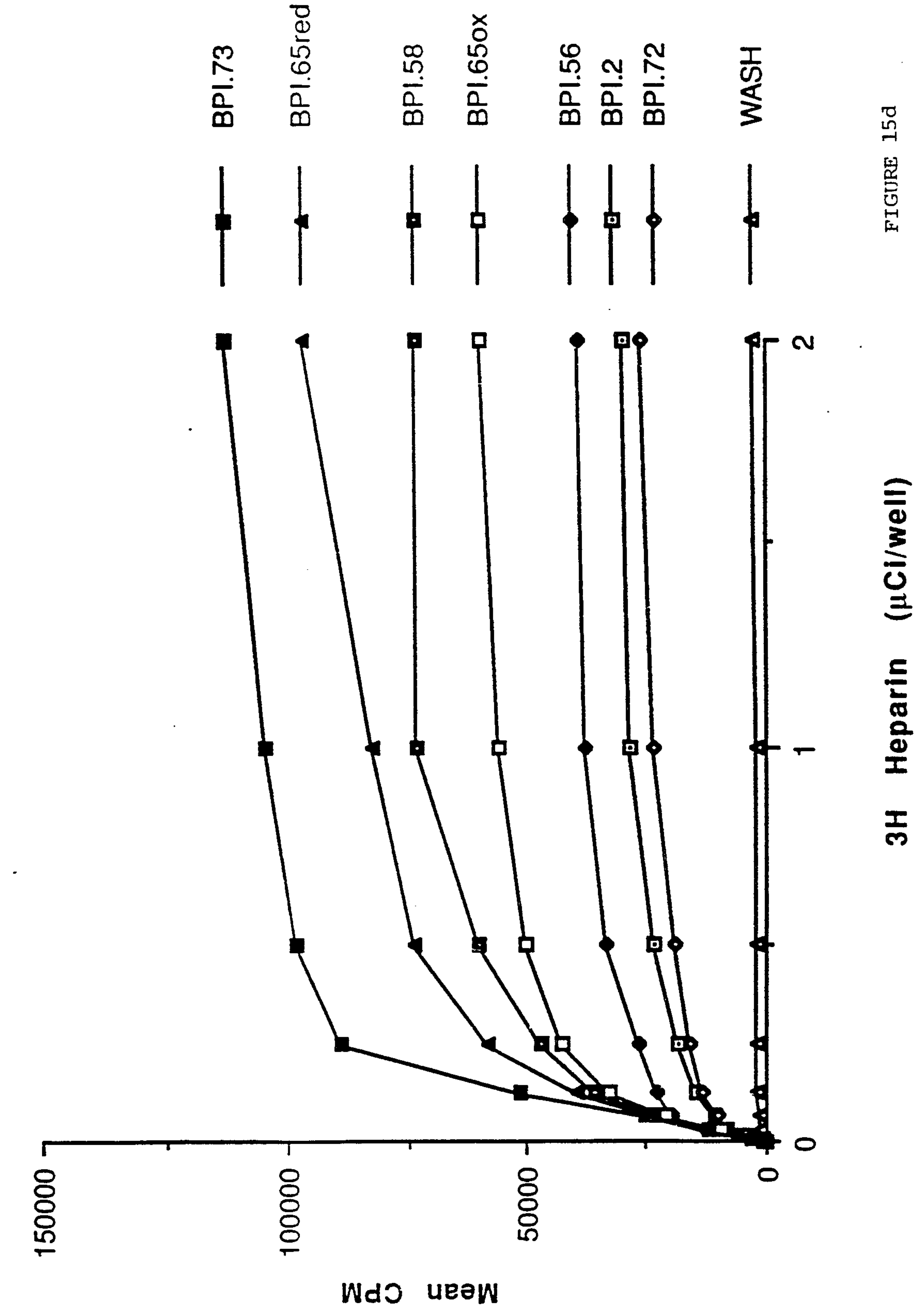
BPI.73
BPI.65red
BPI.58
BPI.65ox
BPI.56
BPI.2
BPI.72
WASH
Mean CPM
150000
100000
50000
0
0
1
2
3H Heparin (µCi/well)

FIGURE 15d

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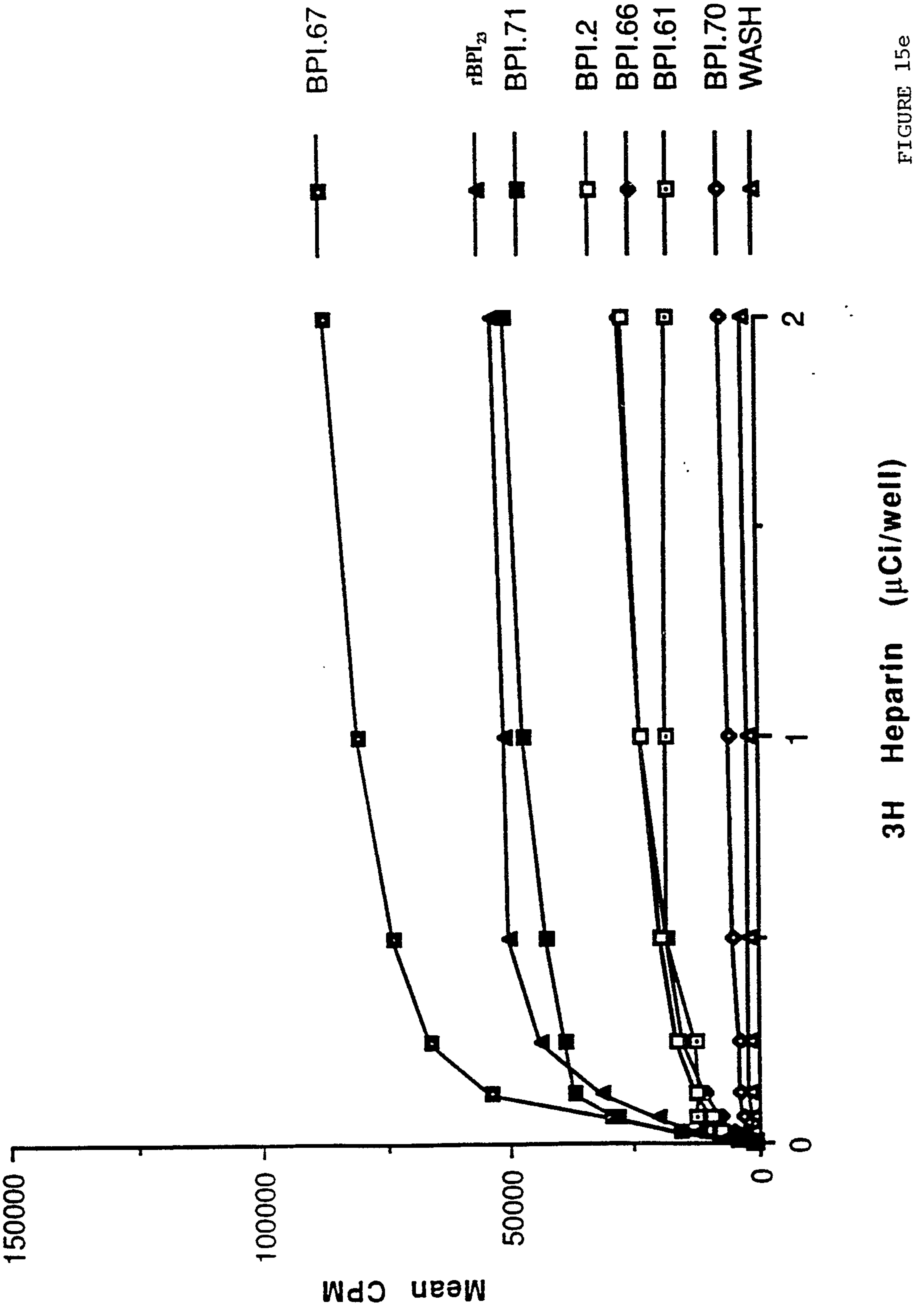

FIGURE 15e

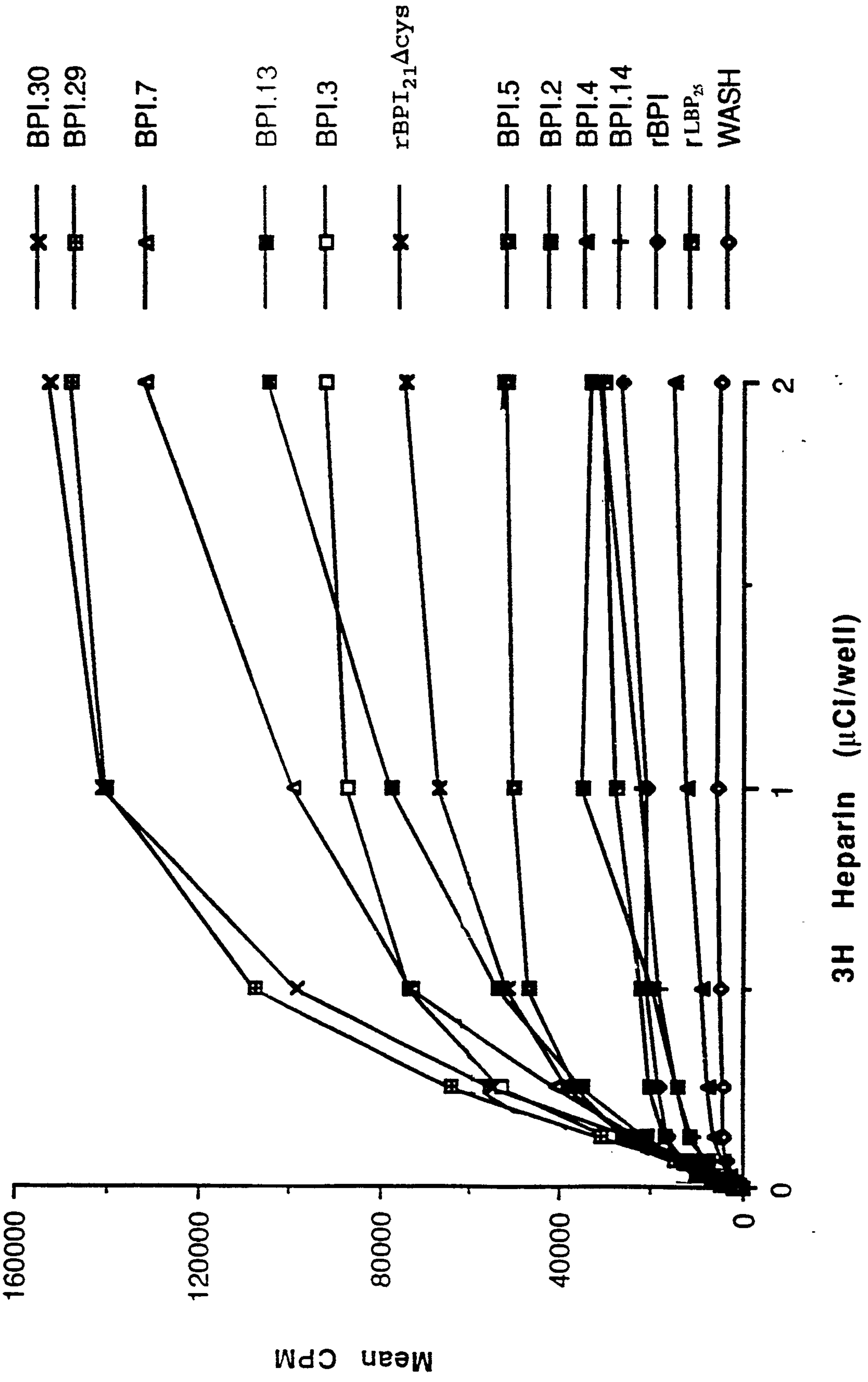
Mean CPM
160000
120000
80000
40000
0
0
1
2
3H Heparin (μCi/well)
BPI.30
BPI.29
BPI.7
BPI.13
BPI.3
$rBPI_{21}\Delta cys$
BPI.5
BPI.2
BPI.4
BPI.14
rBPI
$rLBP_{25}$
WASH

FIGURE 16

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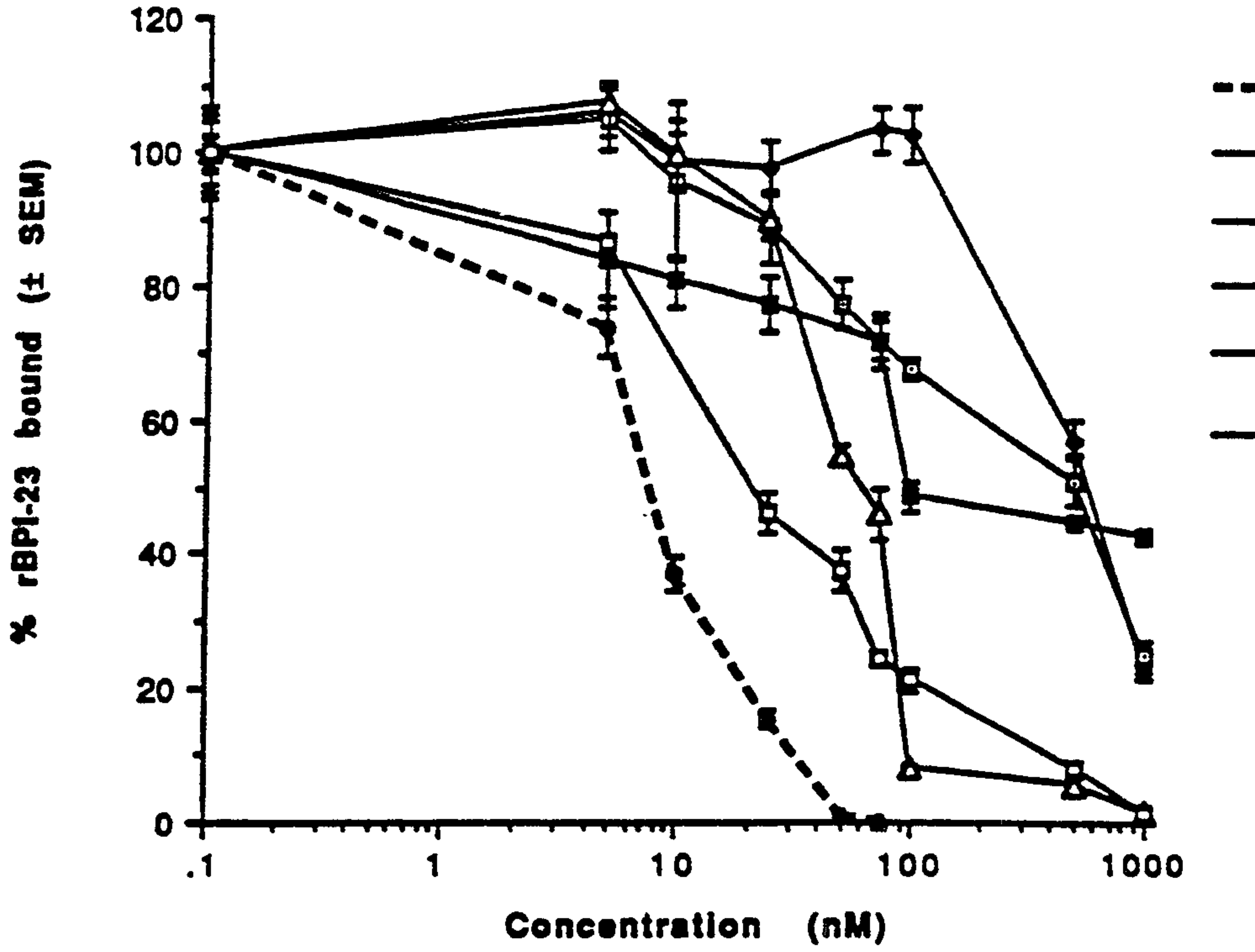

FIGURE 17a

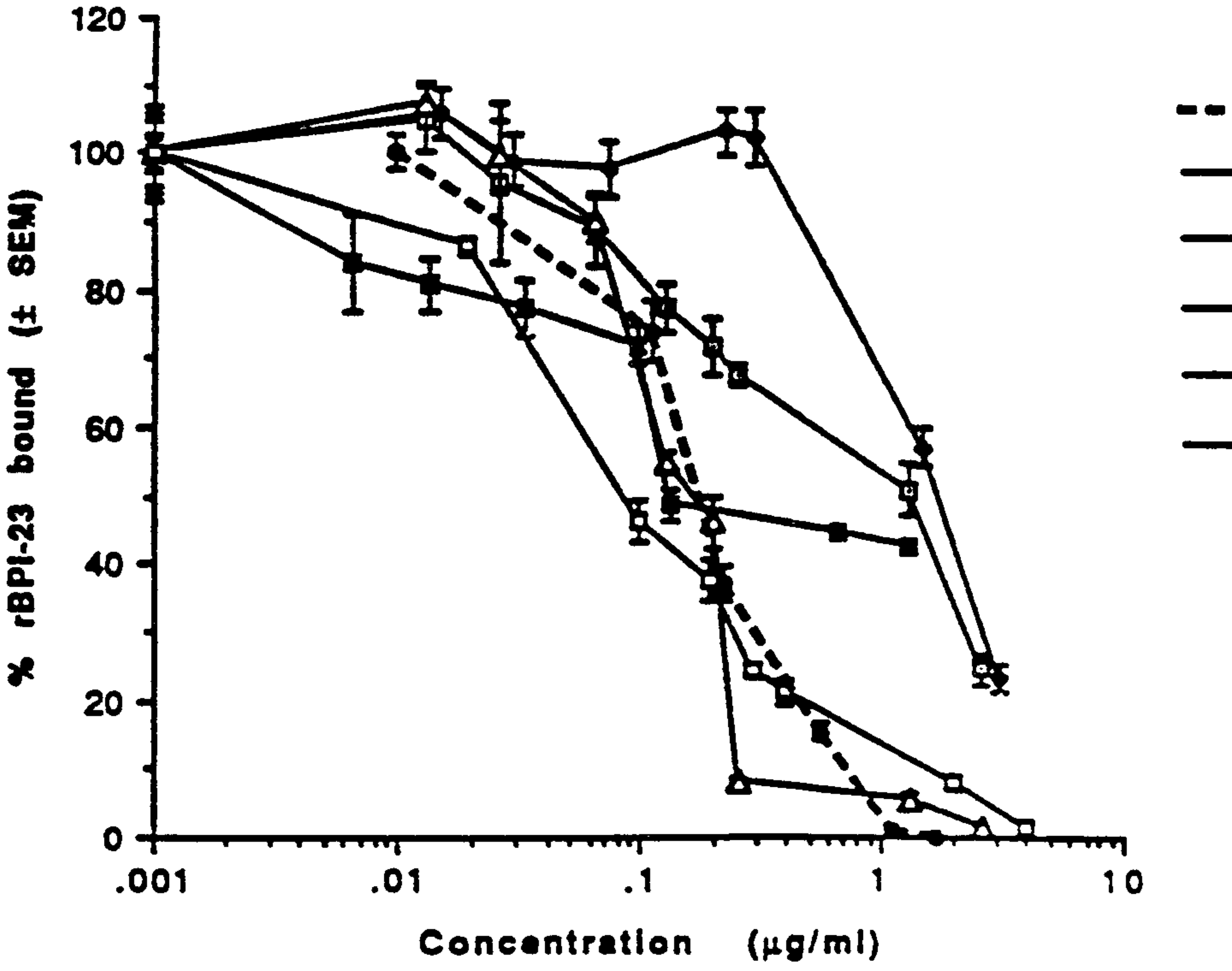

FIGURE 17b

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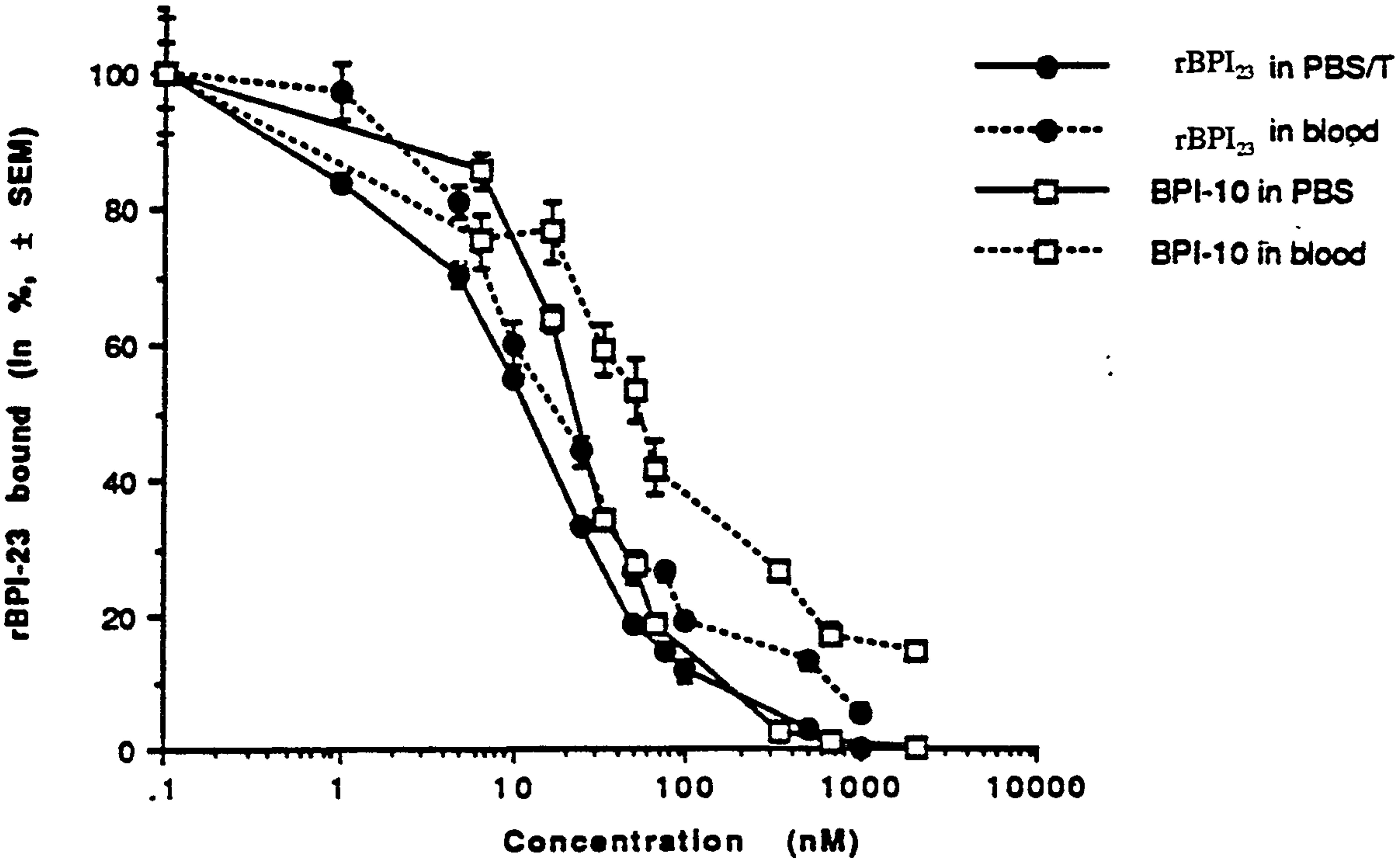

FIGURE 18

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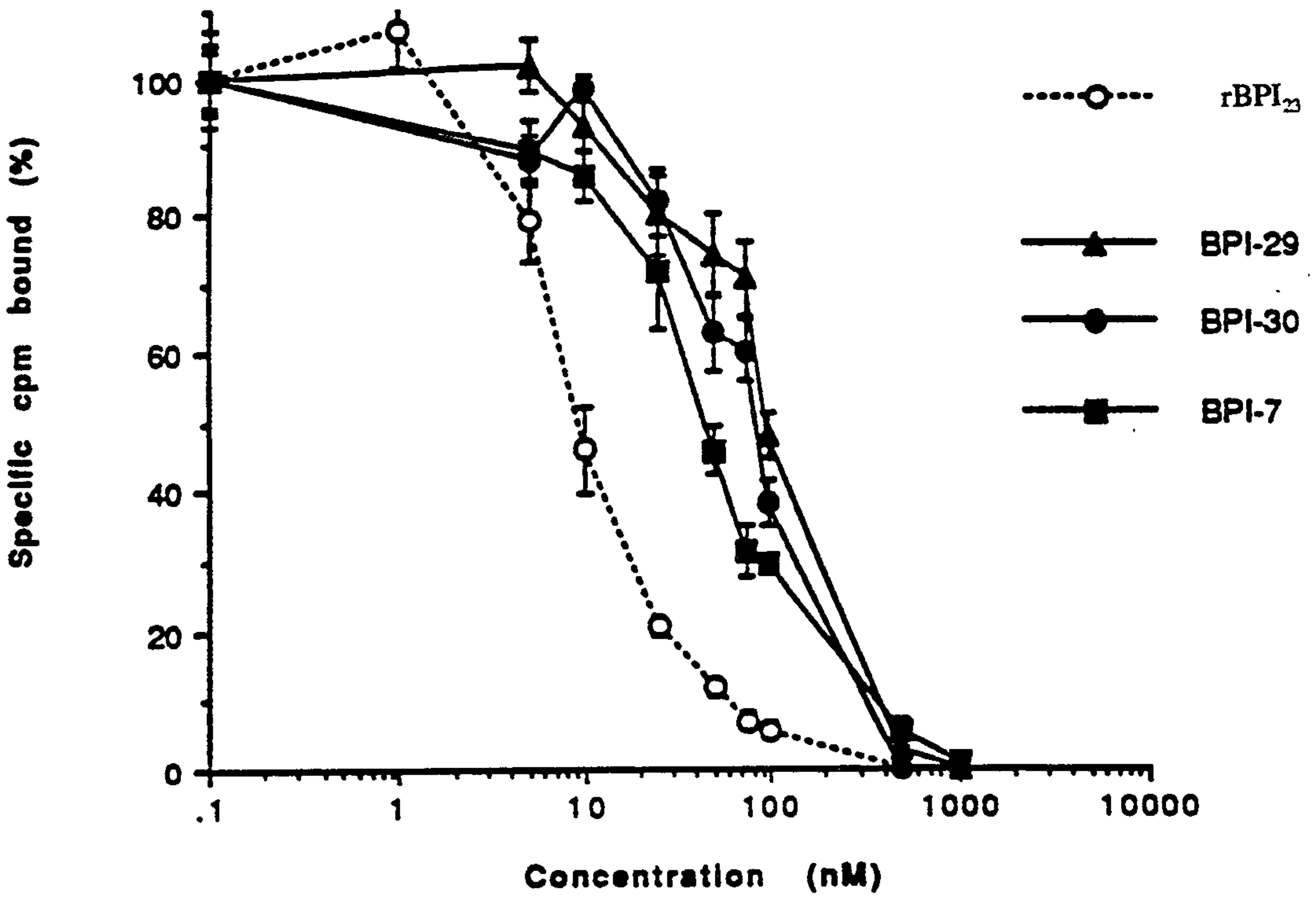

FIGURE 19

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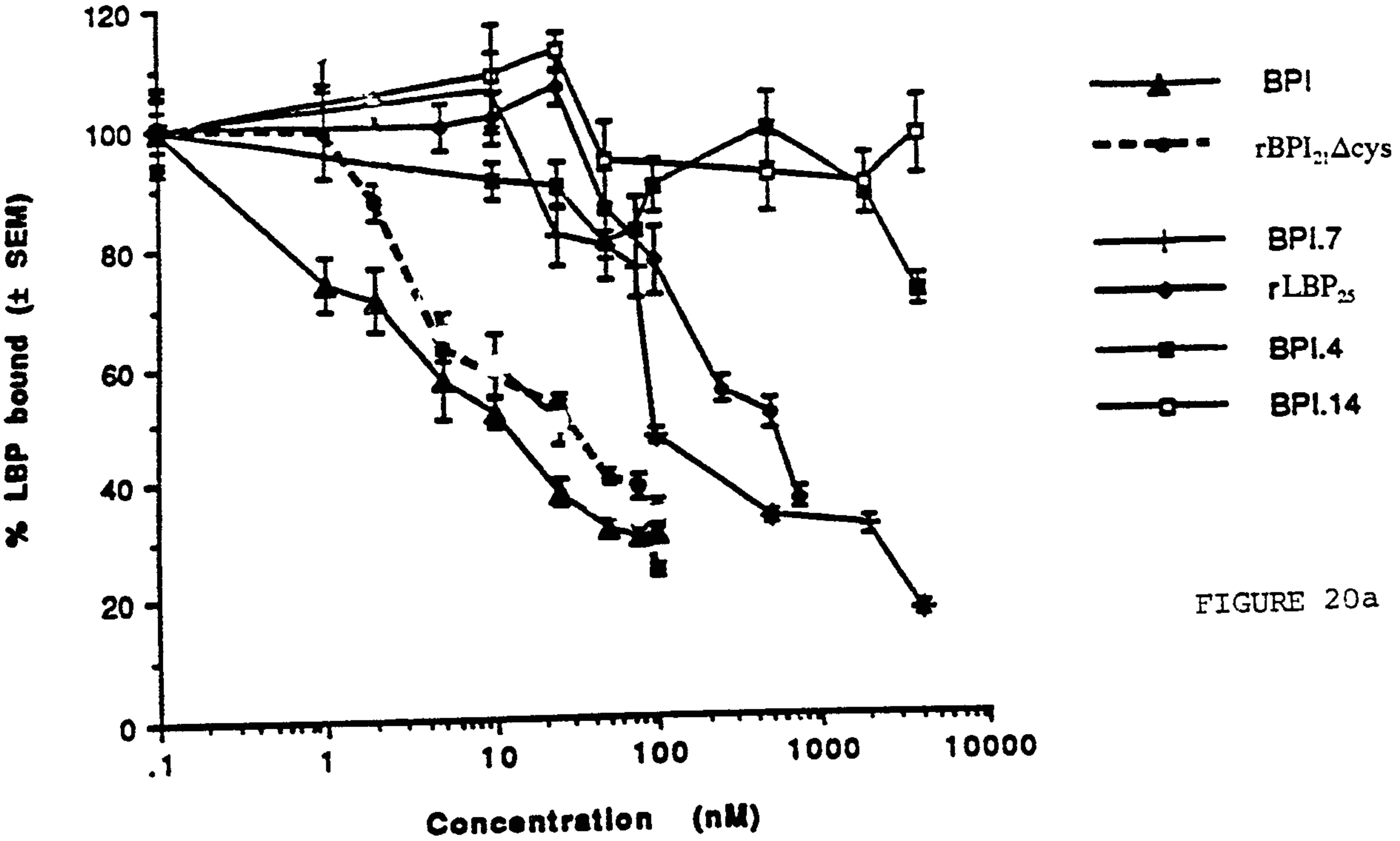


FIGURE 20a

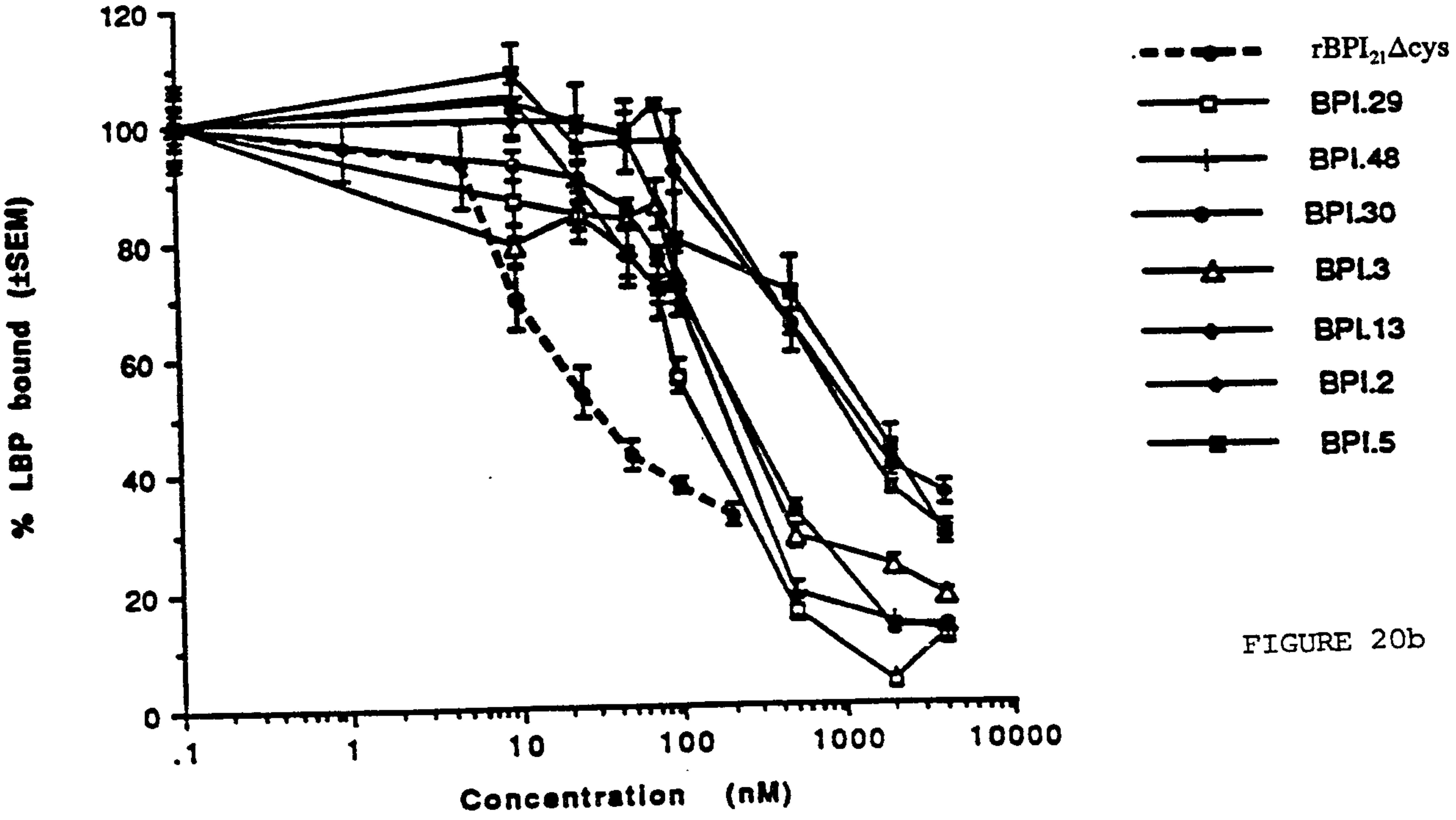


FIGURE 20b

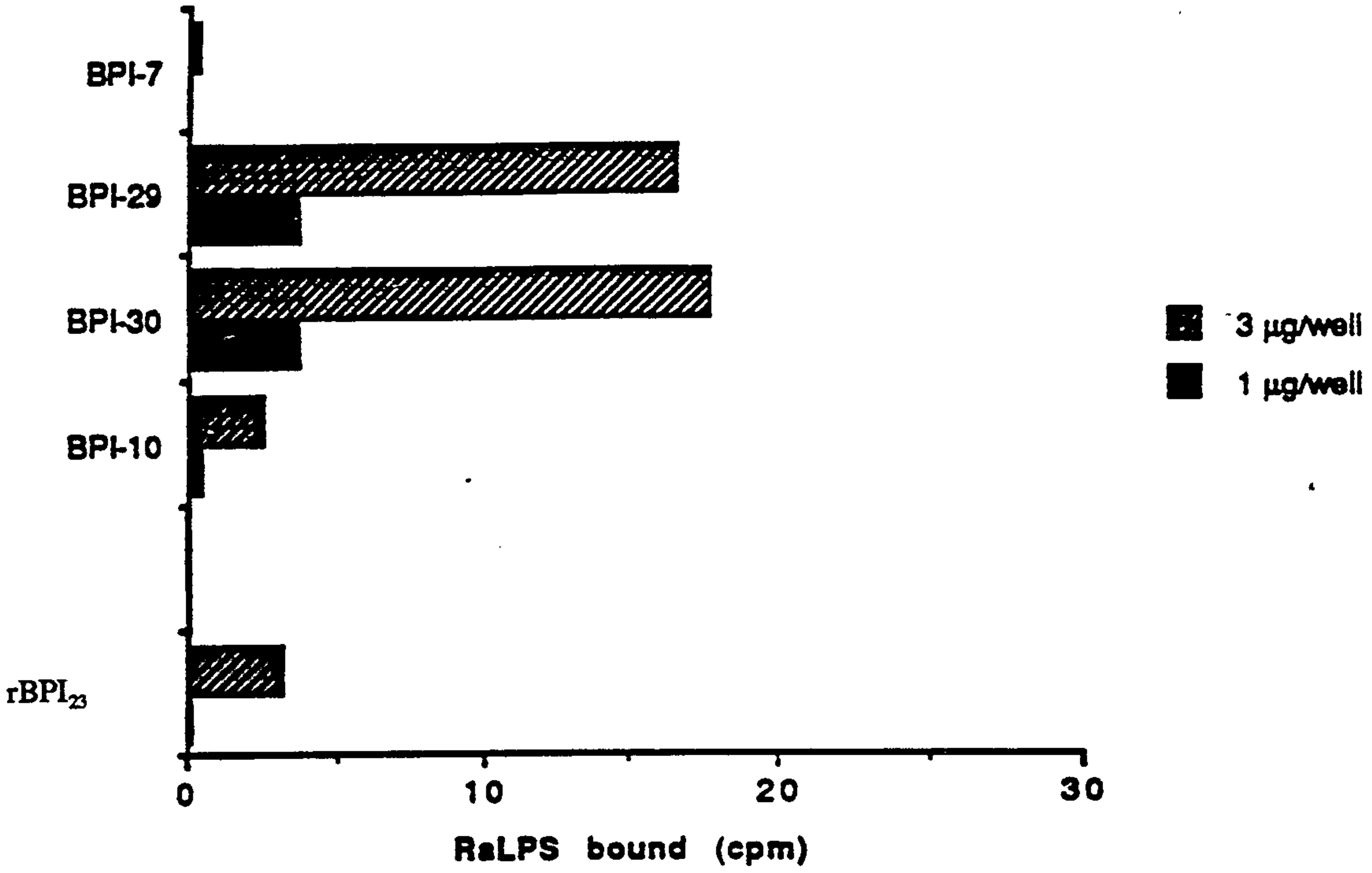
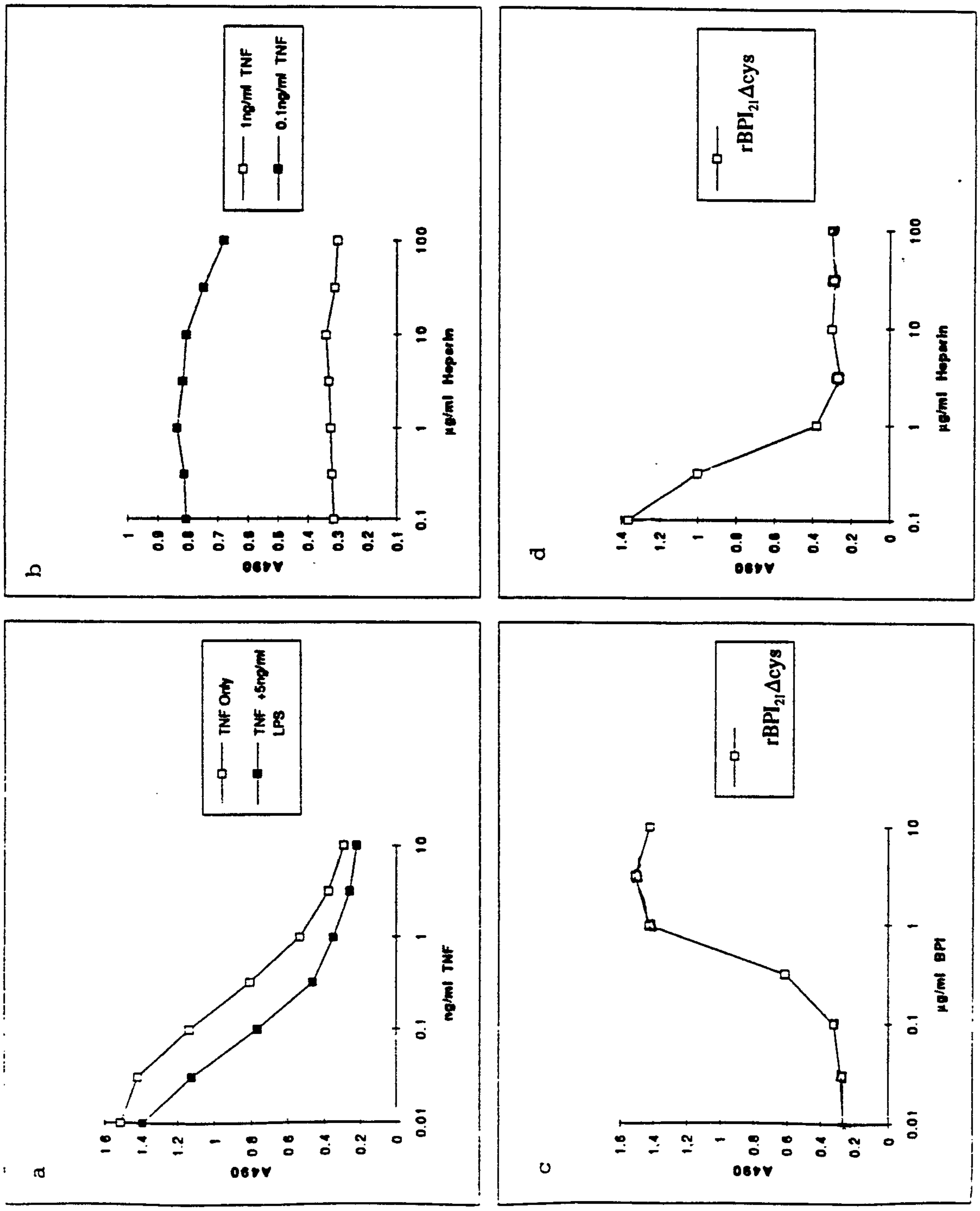
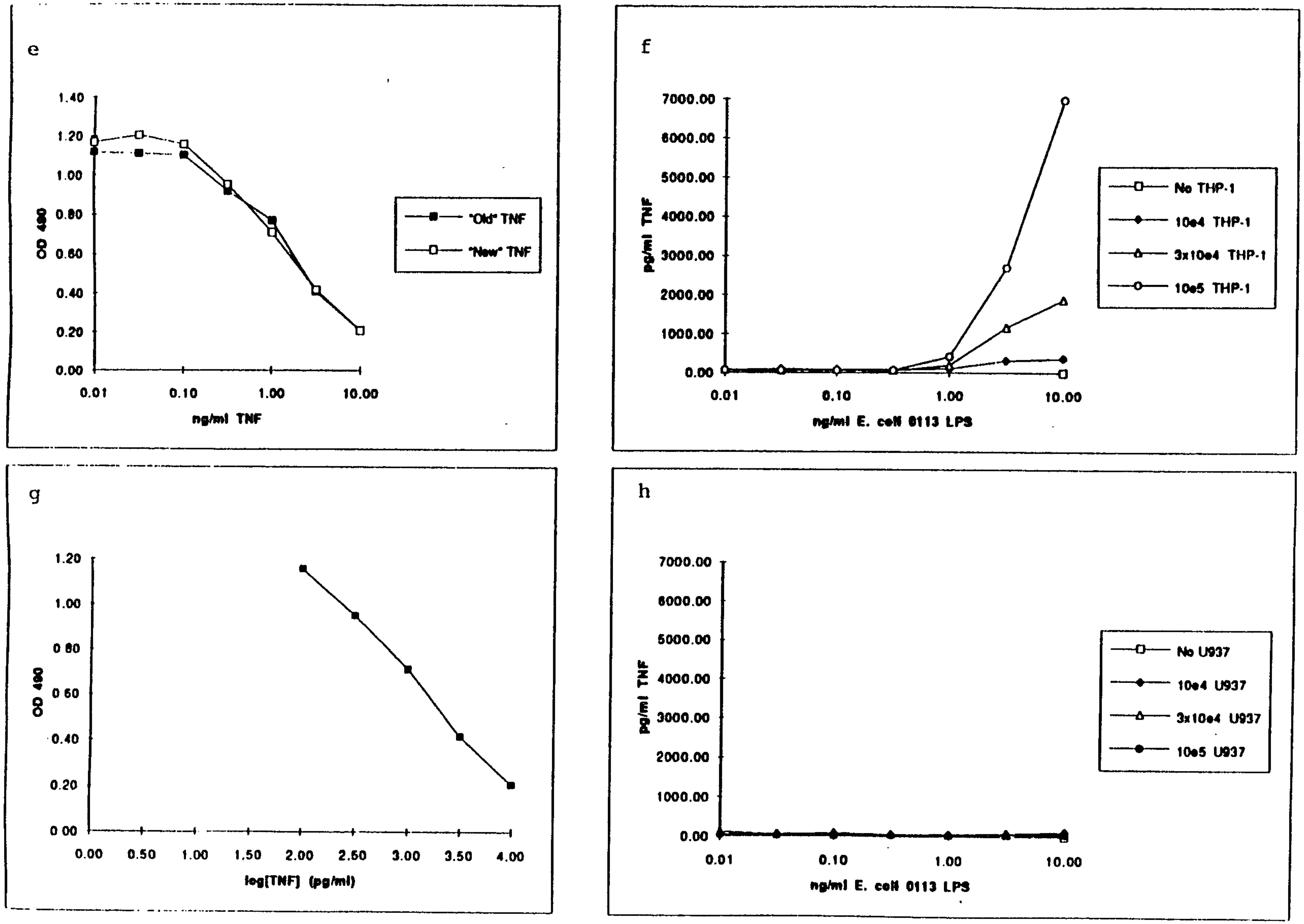

BPI-7
BPI-29
BPI-30
BPI-10
rBPI₂₃
0
10
20
30
RaLPS bound (cpm)
3 µg/well
1 µg/well

FIGURE 21



FIGURES 22a-d

FIGURES 22e - h



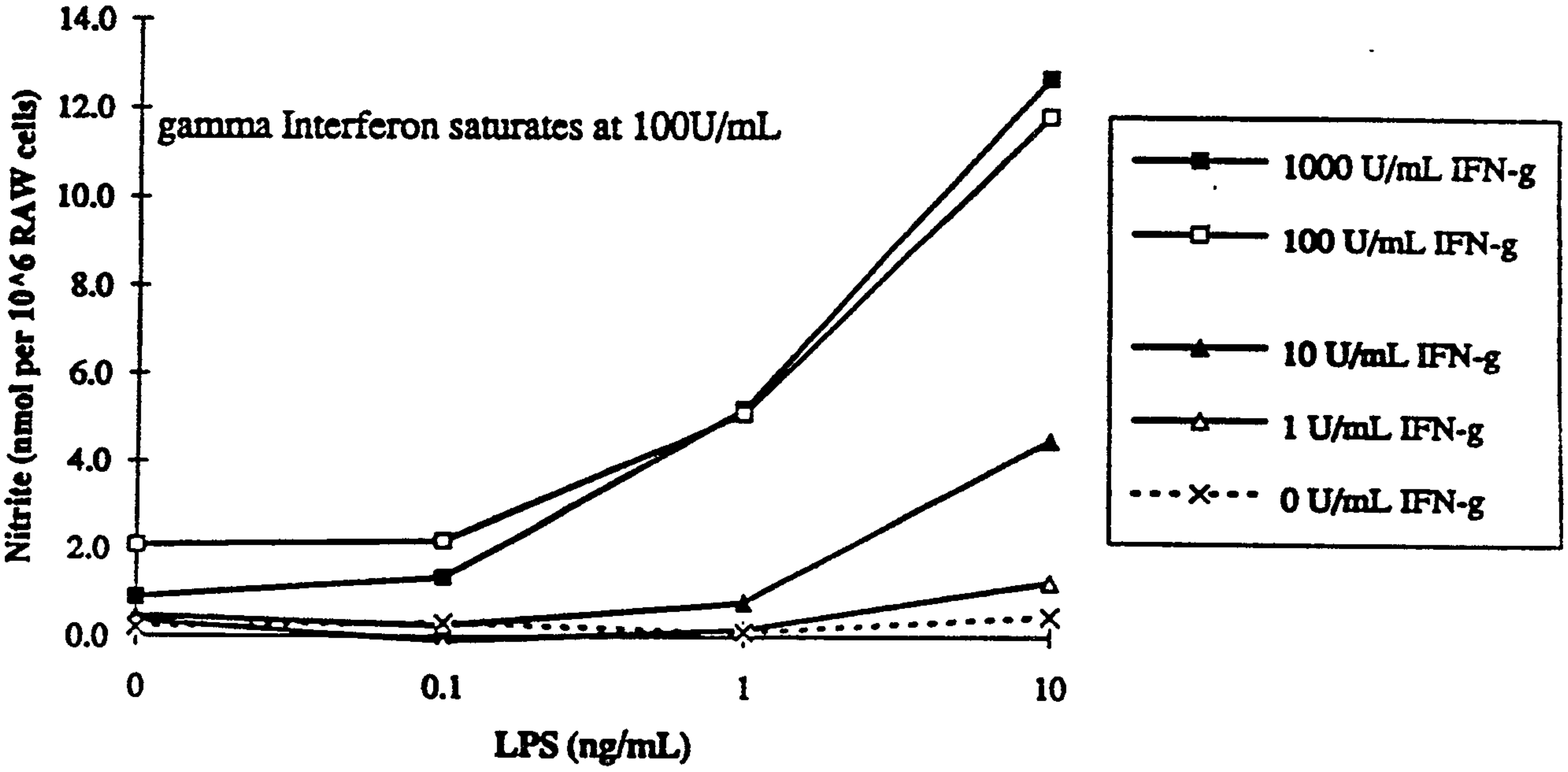

14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
Nitrite (nmol per 10^6 RAW cells)
gamma Interferon saturates at 100U/mL
0
0.1
1
10
LPS (ng/mL)
1000 U/mL IFN-g
100 U/mL IFN-g
10 U/mL IFN-g
1 U/mL IFN-g
0 U/mL IFN-g

FIGURE 23a

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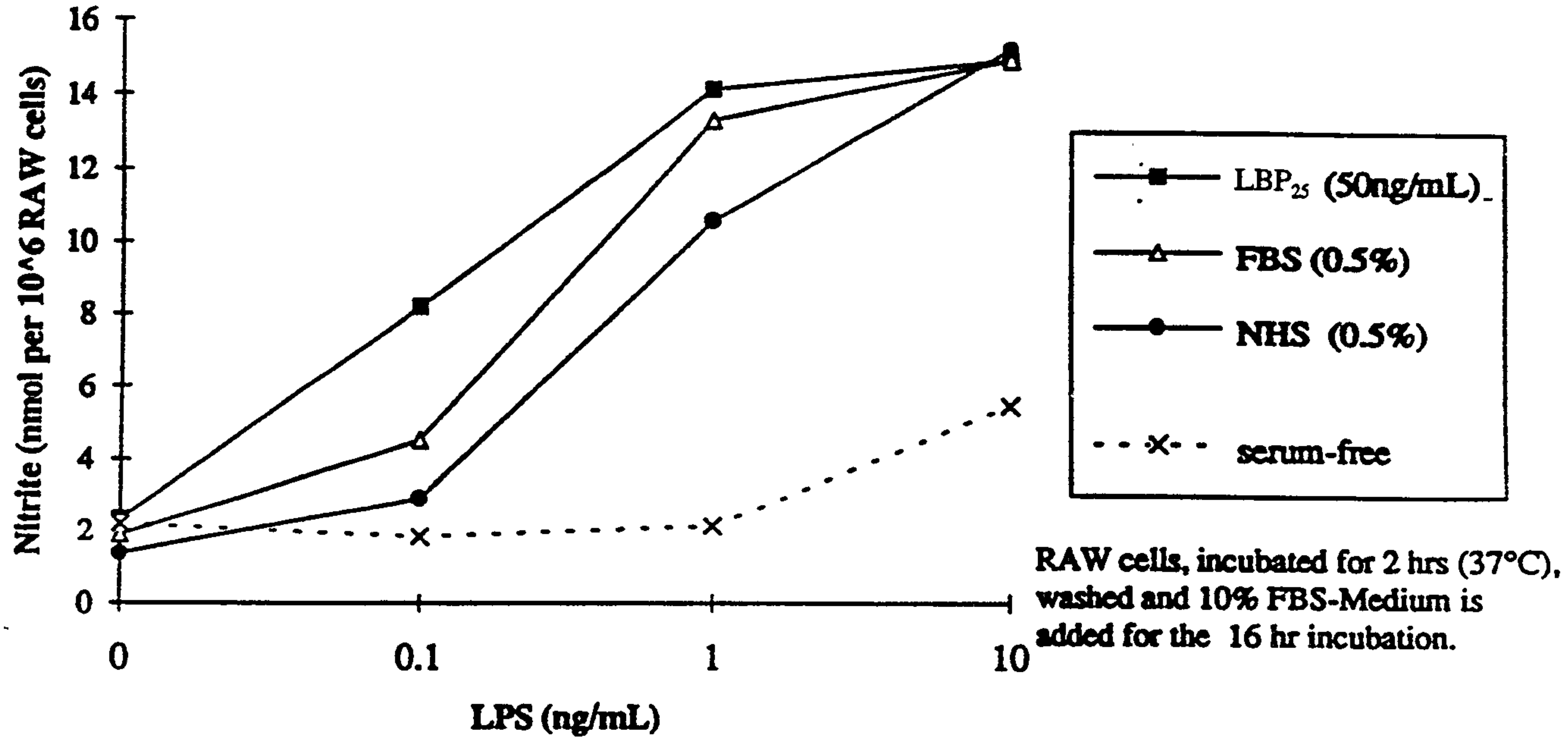

FIGURE 23b

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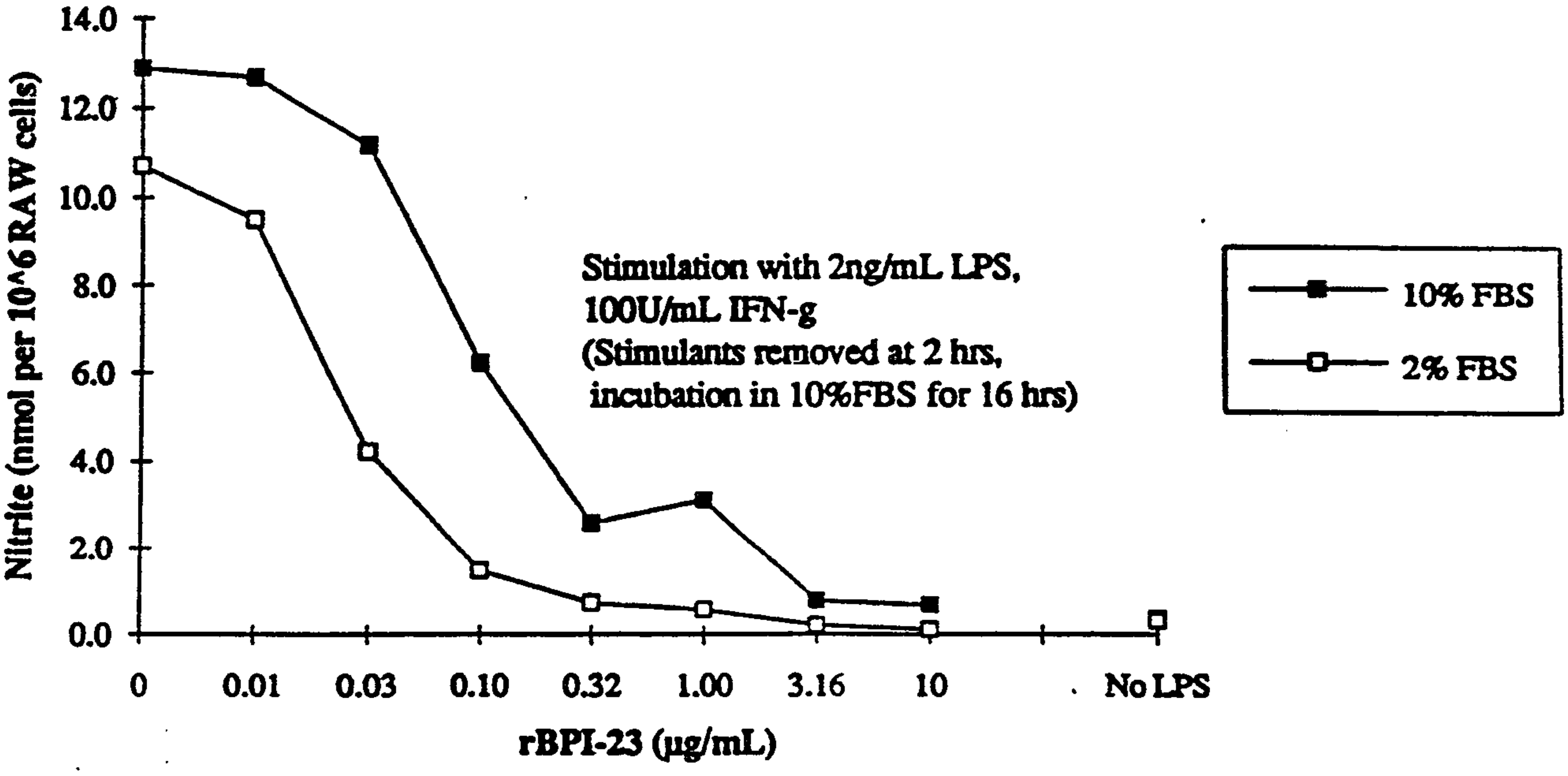

FIGURE 23c

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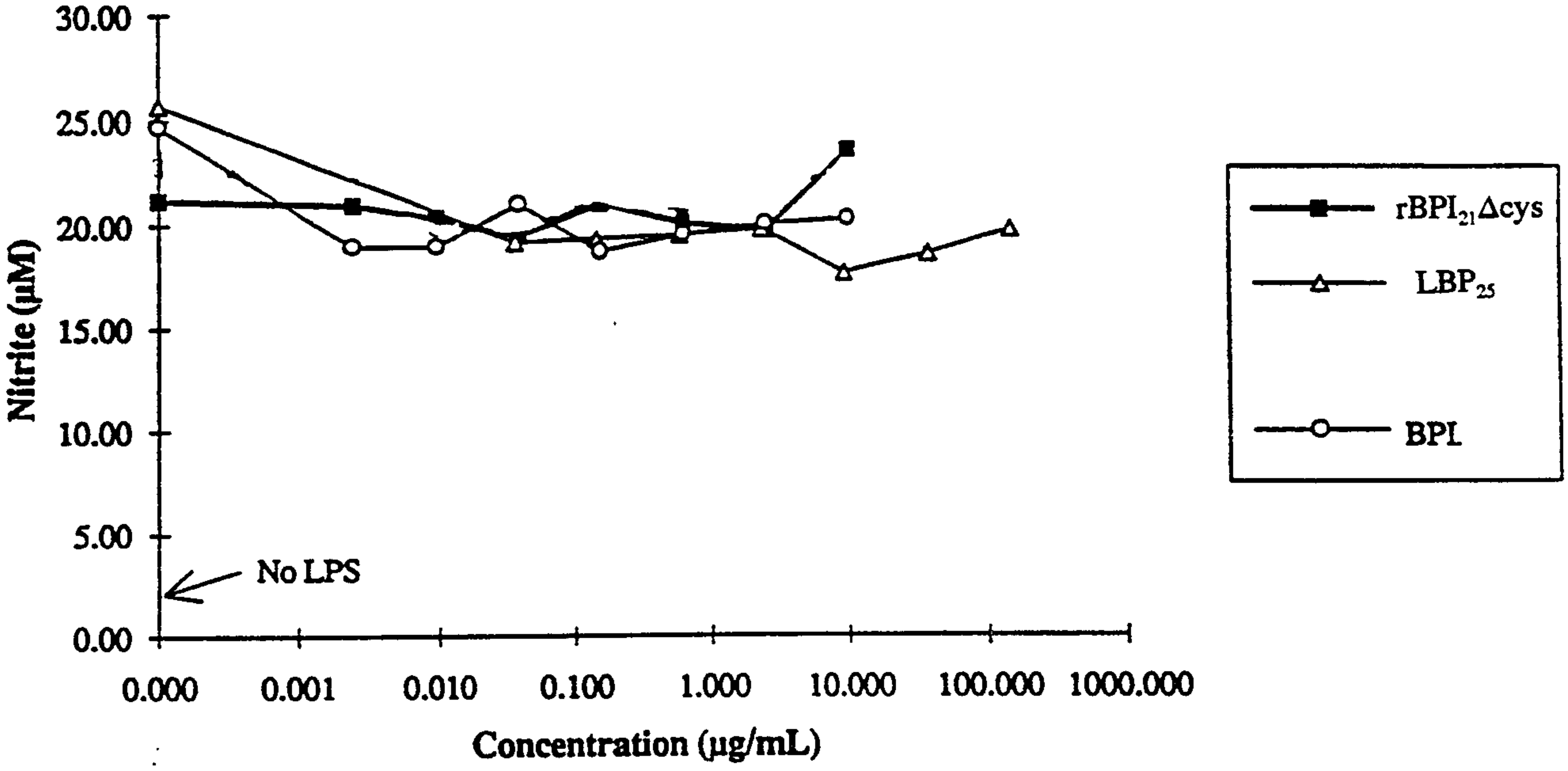

FIGURE 24a

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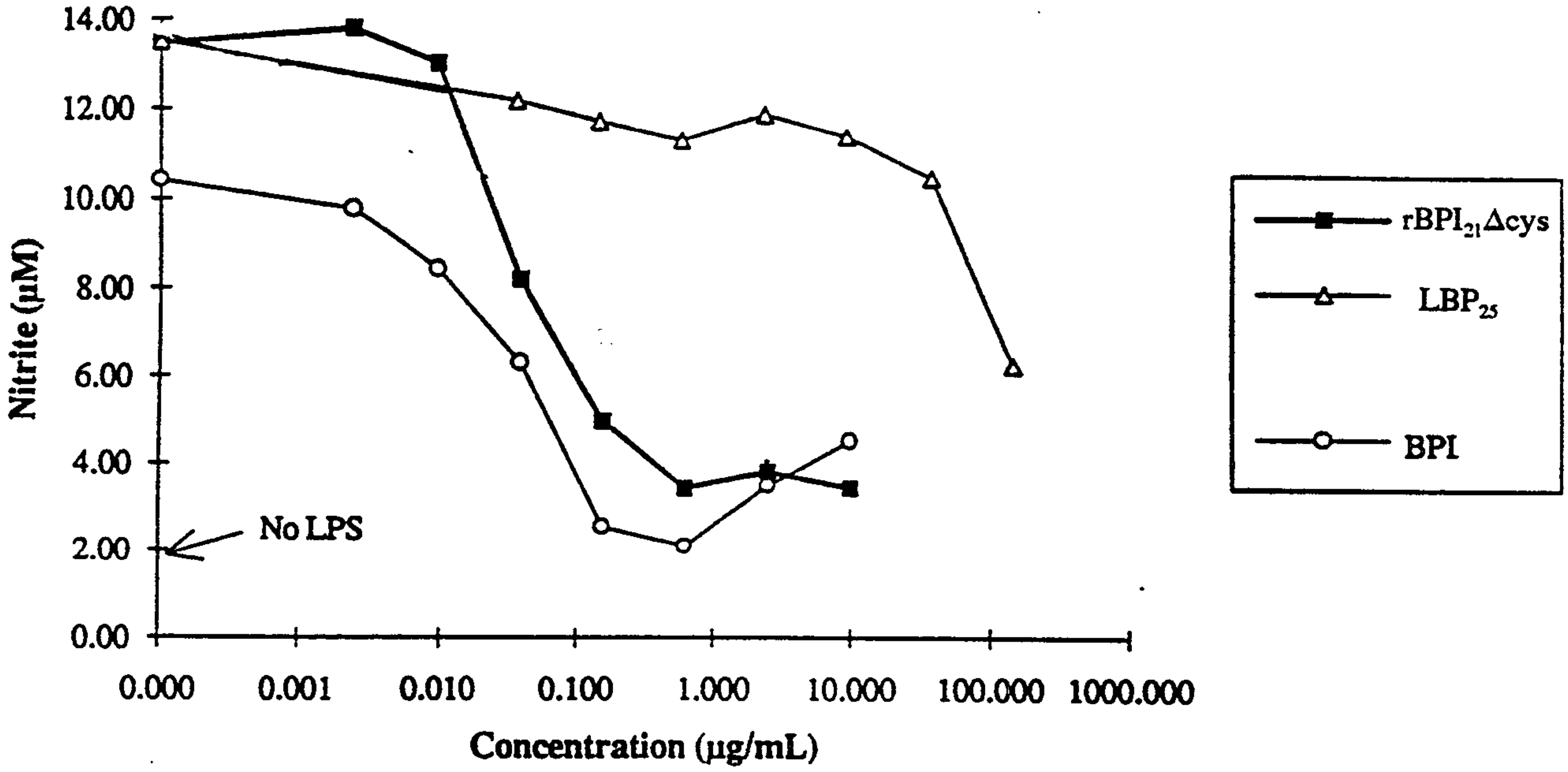

FIGURE 24b

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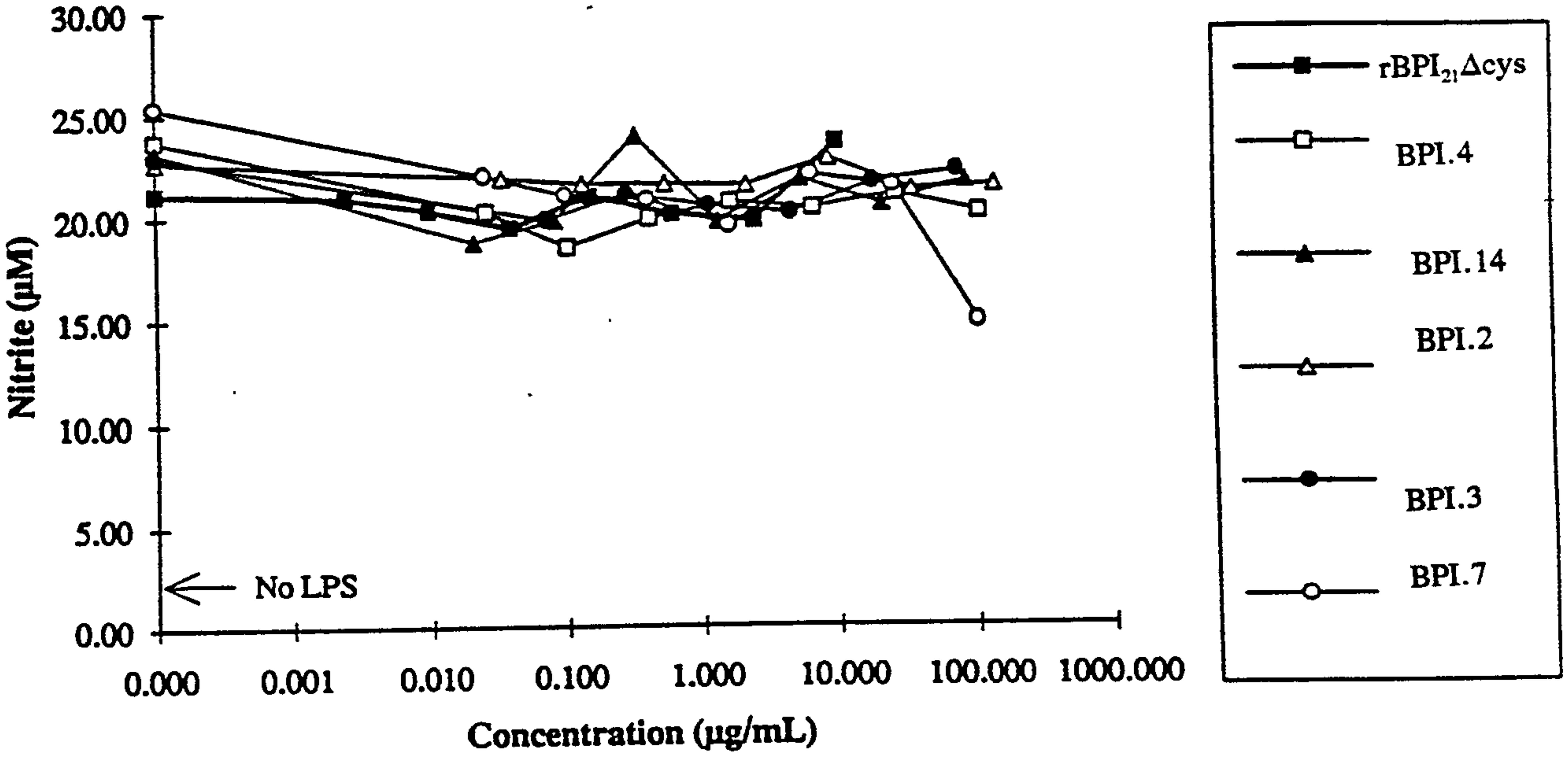

FIGURE 24c

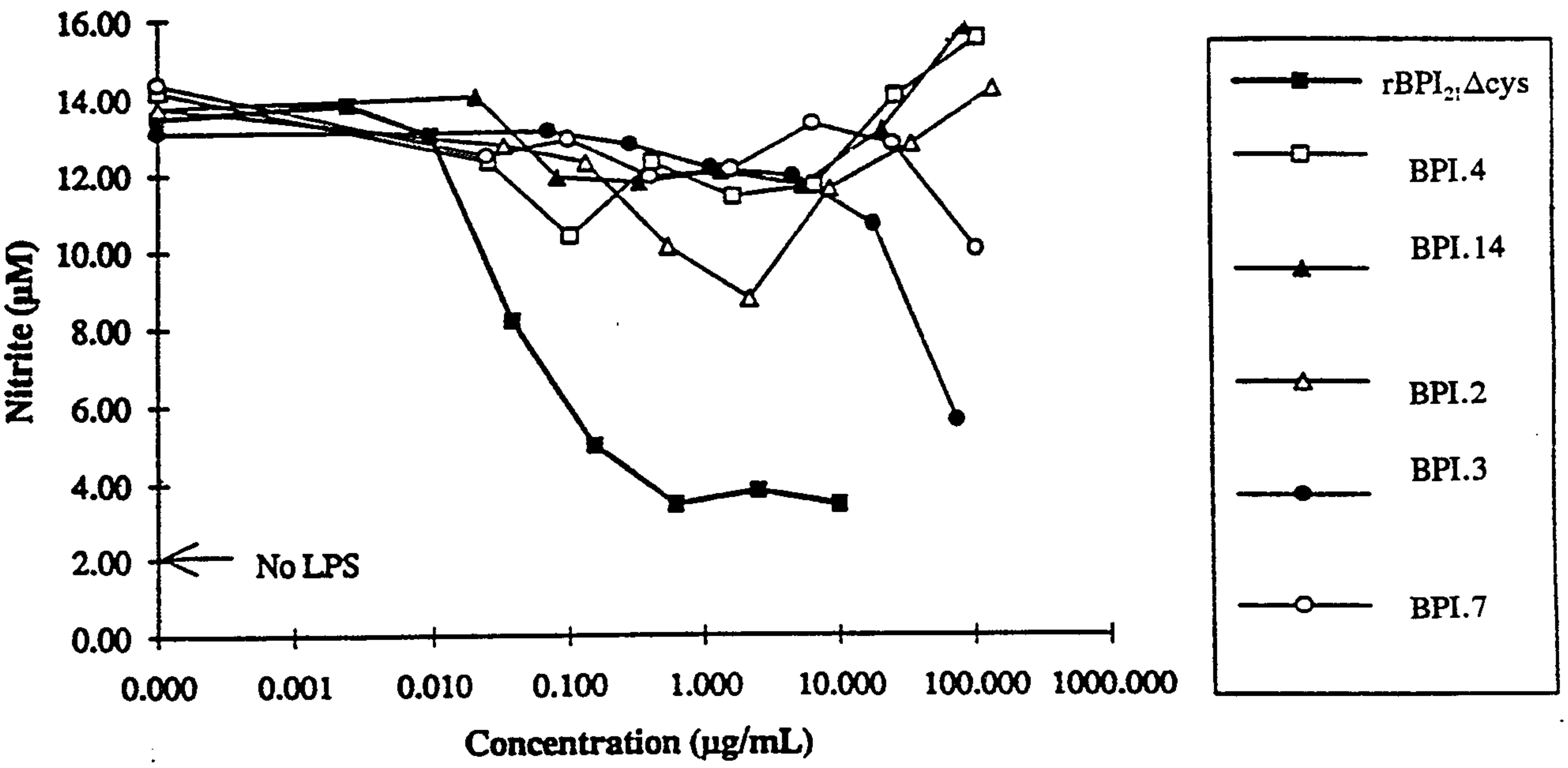
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
Nitrite (µM)
No LPS
0.000
0.001
0.010
0.100
1.000
10.000
100.000
1000.000
Concentration (µg/mL)
rBPI21Δcys
BPI.4
BPI.14
BPI.2
BPI.3
BPI.7

FIGURE 24d

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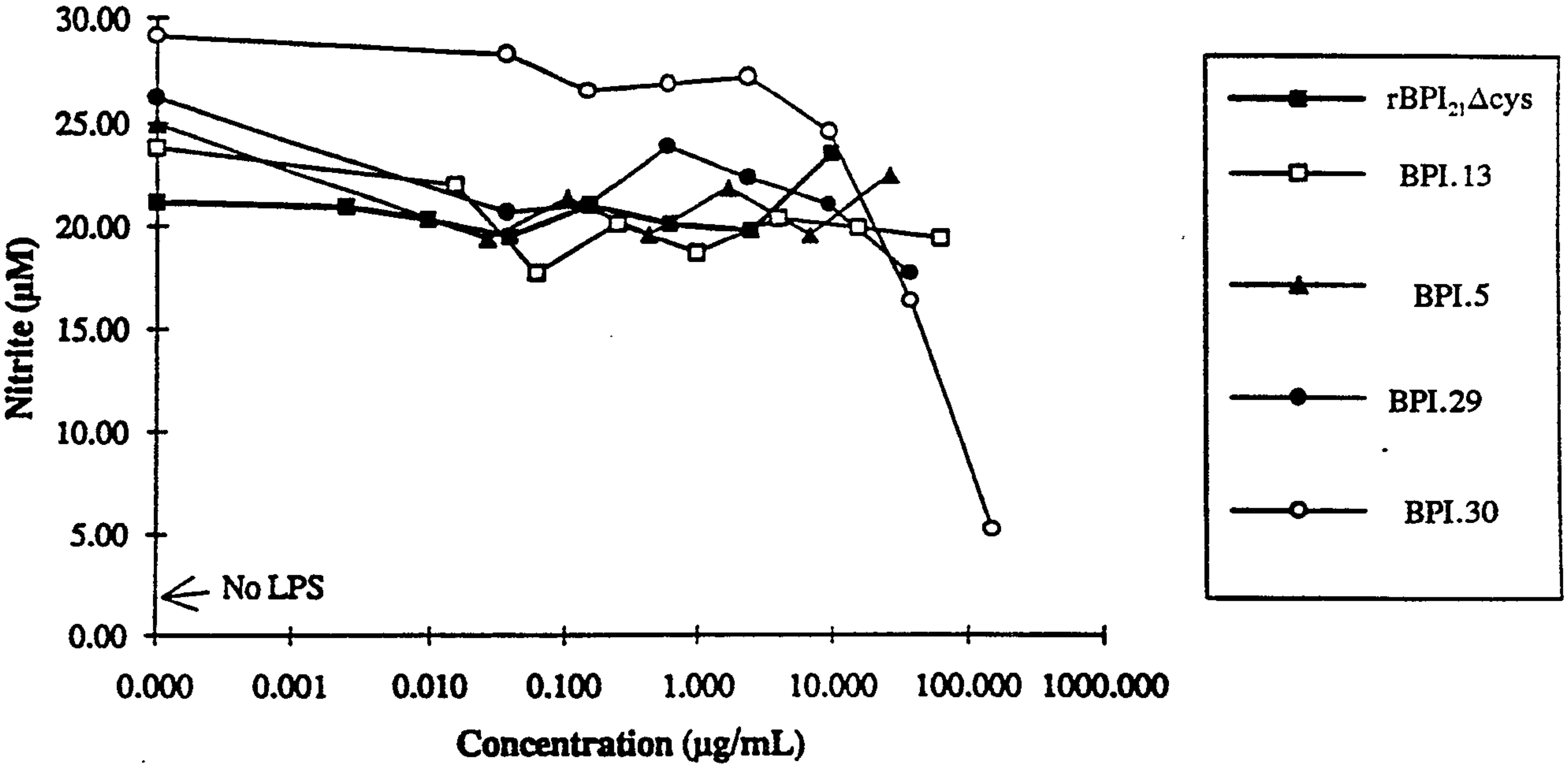

FIGURE 24e

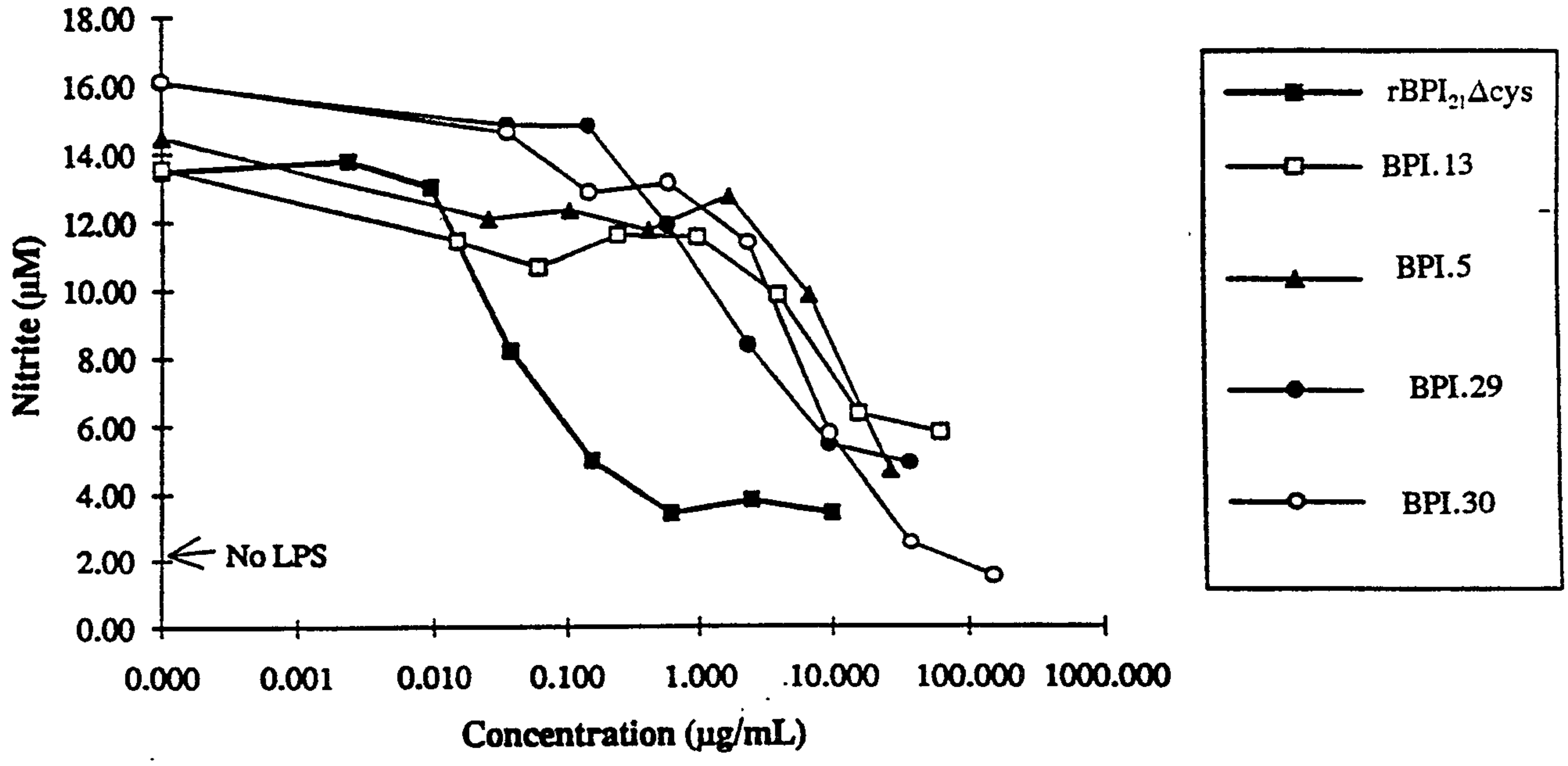
18.00
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
Nitrite (µM)
No LPS
0.000
0.001
0.010
0.100
1.000
10.000
100.000
1000.000
Concentration (µg/mL)
rBPI$_{21}$Δcys
BPI.13
BPI.5
BPI.29
BPI.30

FIGURE 24f

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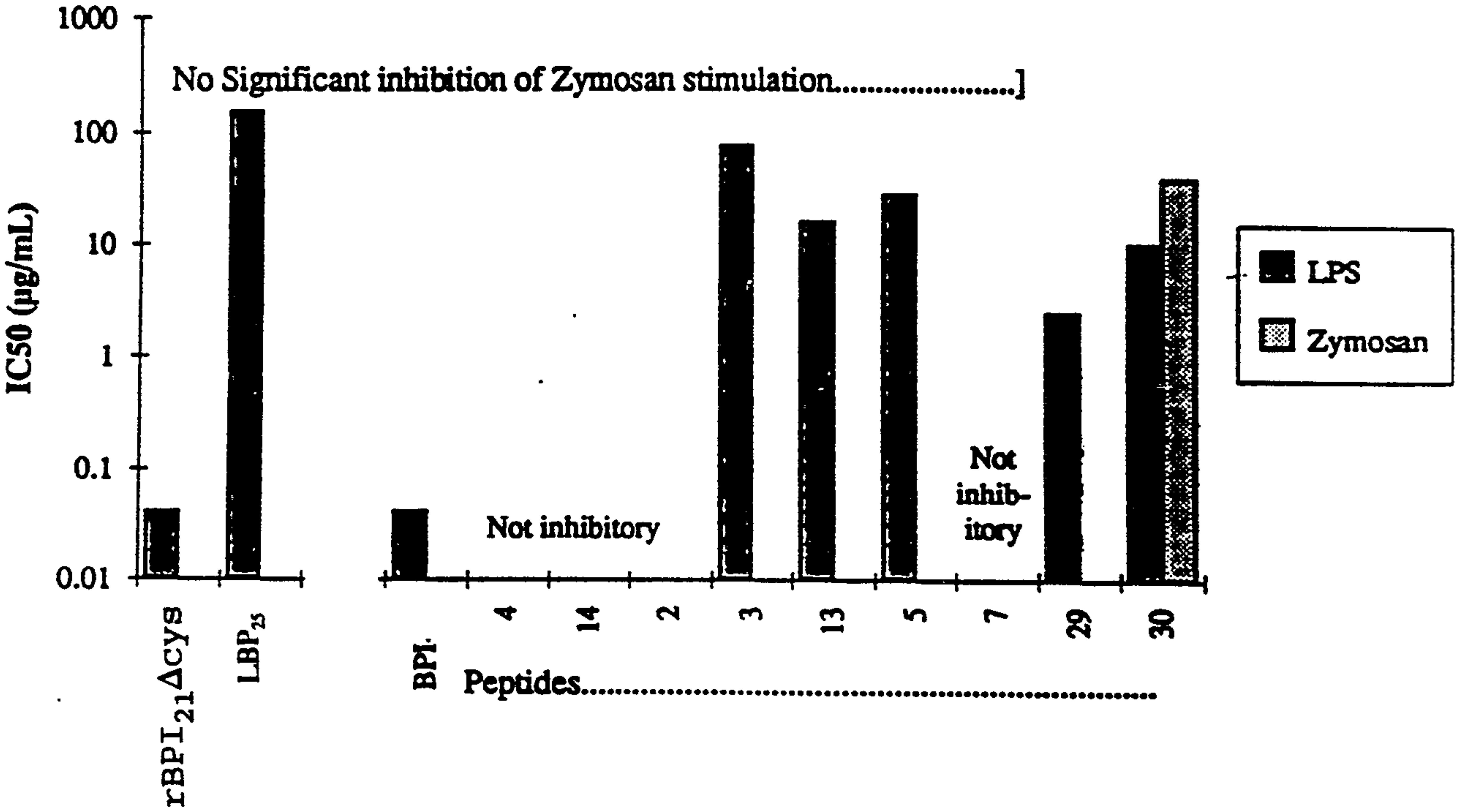

FIGURE 24g

1 VNPGVVVRISQKGLDYASQQGTAALQKELKRIKIPDYSDSFKIKHLGKGH 50

60 YSFYSMDIREFQLPSSQISMVPNVGLKFSISNANIKISGKWKAQKRFLKM 100

110 SGNFDLSIEGMSISADLKLGSNPTSGKPTITCSSCSSHINSVHVHISKSK 150

VGWLIQLFHKKIESALRNKMNSQVCEKVTNSVSSELQPYFQTLPVMTKI 199

FIGURE 25

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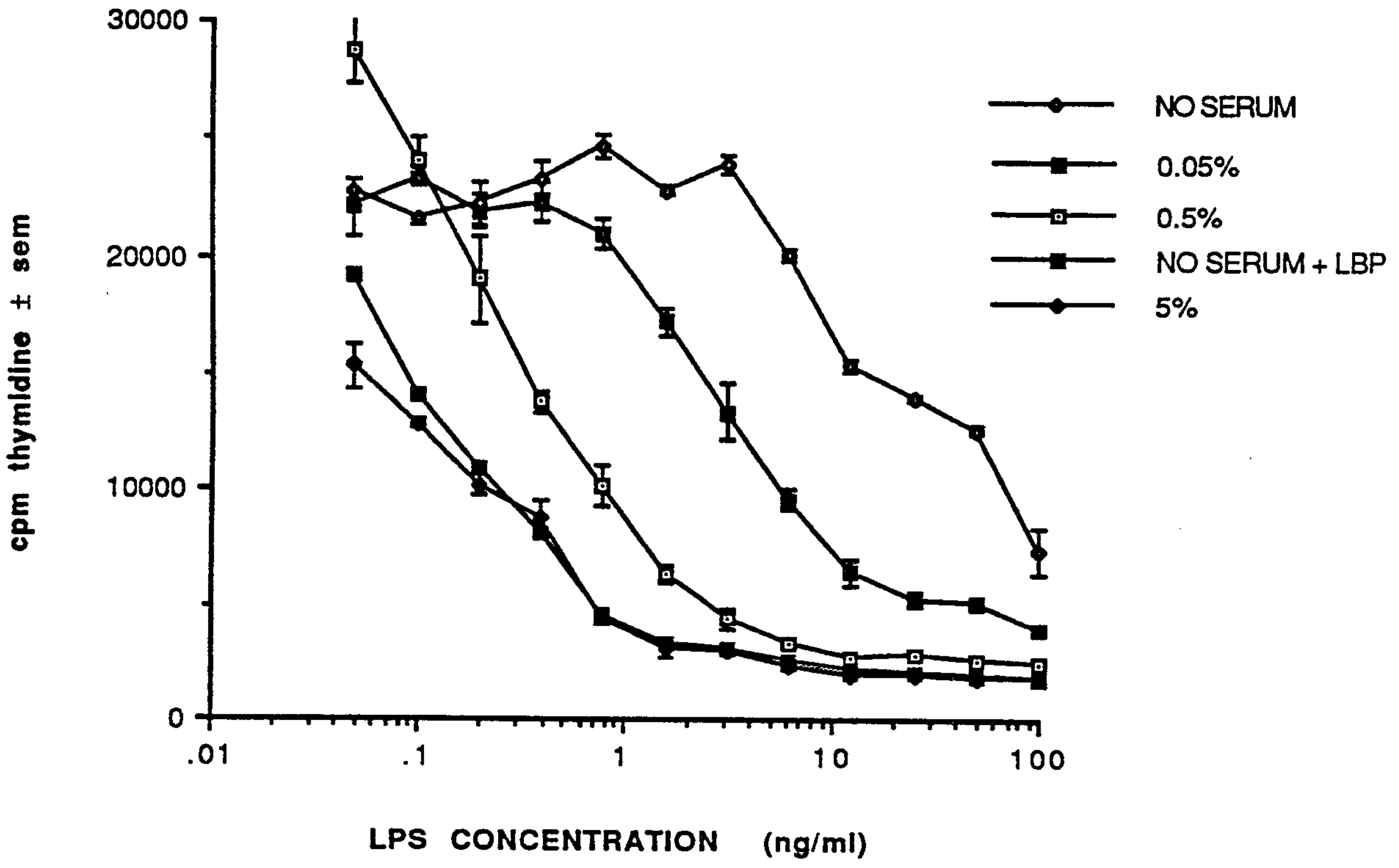
SERUM/LBP DEPENDENCE OF RAW CELL RESPONSE TO LPS

FIGURE 26a

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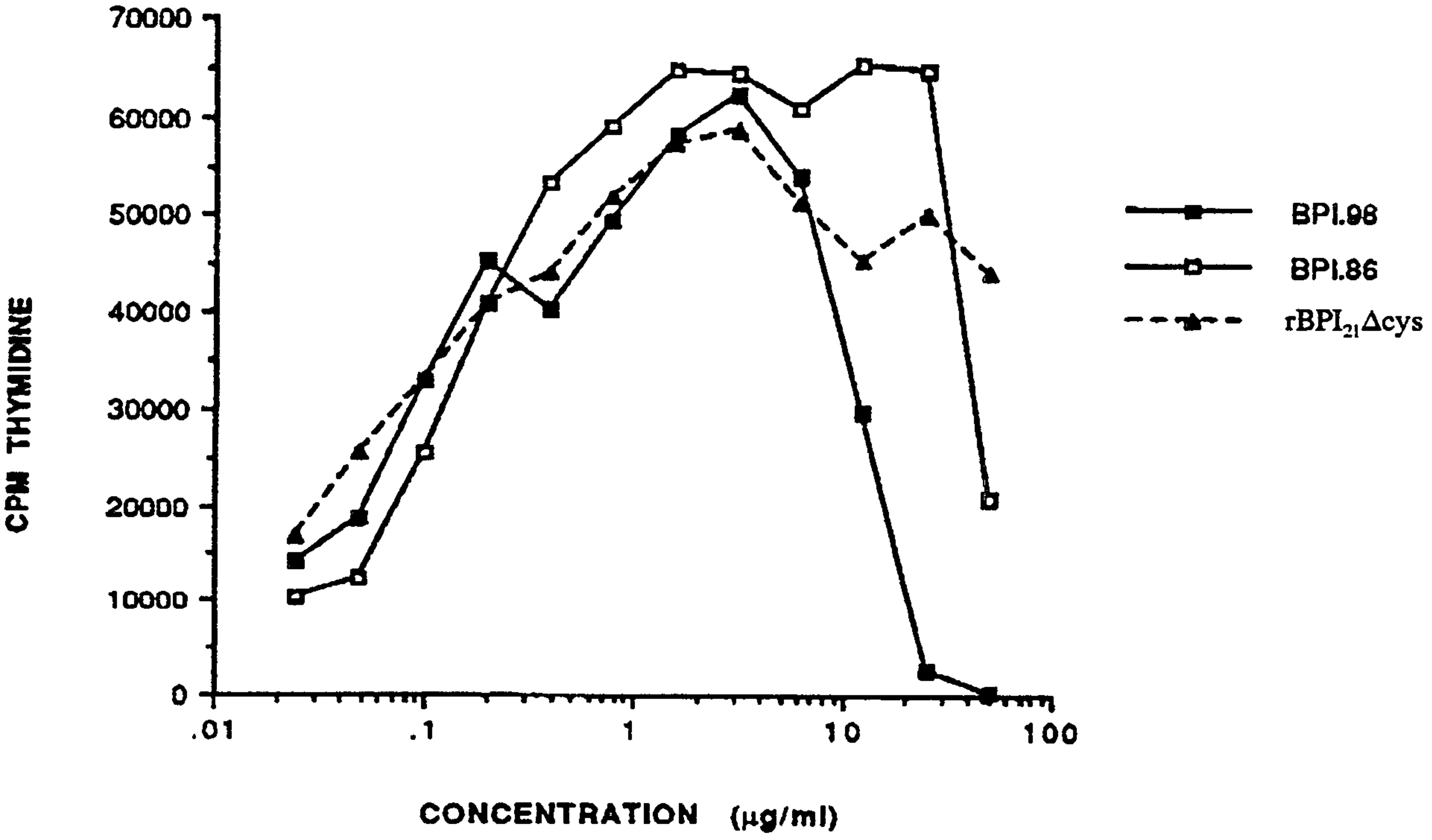

FIGURE 26b

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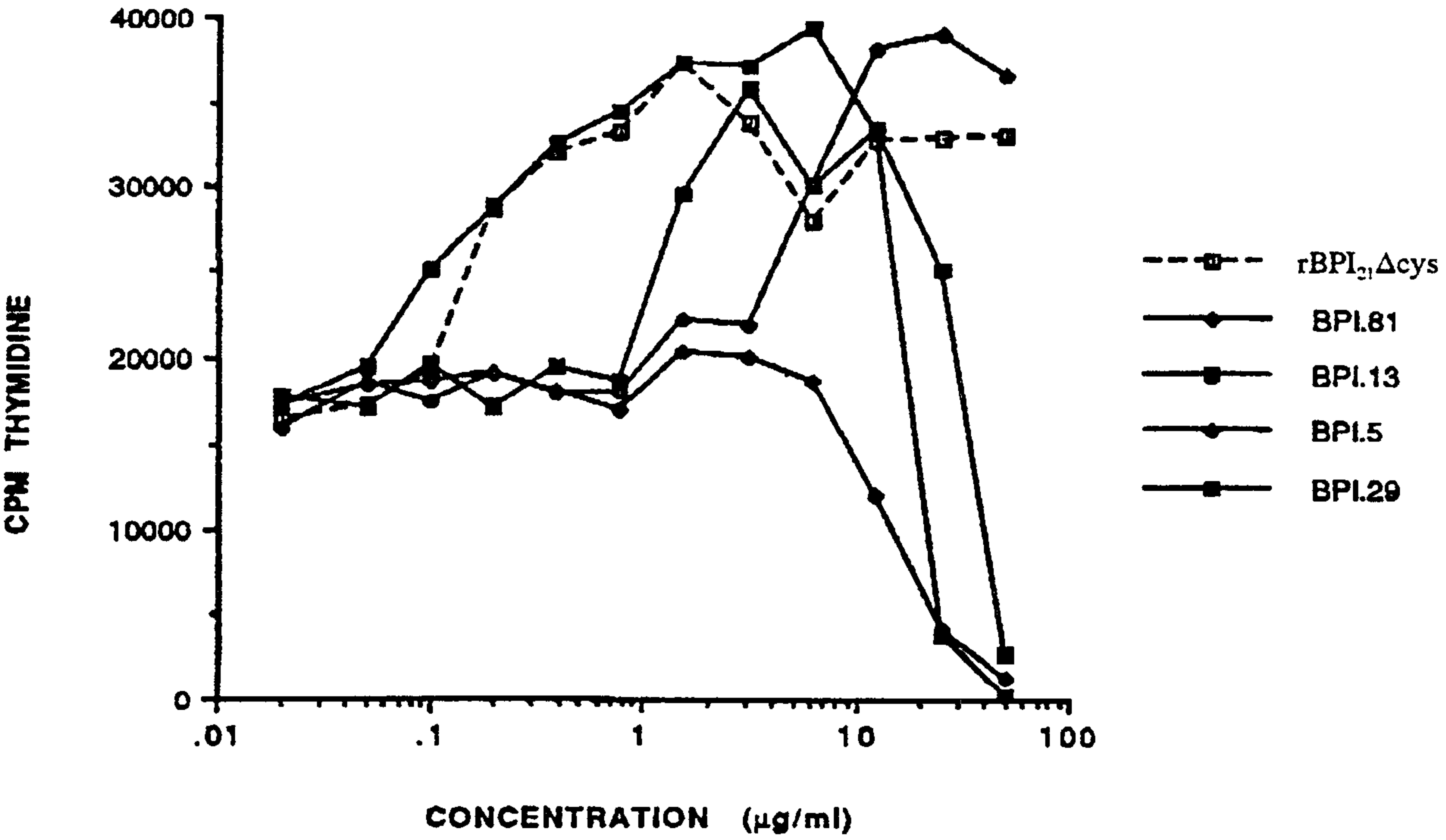

FIGURE 26c

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BPI Inhibition of TNF Production by LPS, in Whole Blood

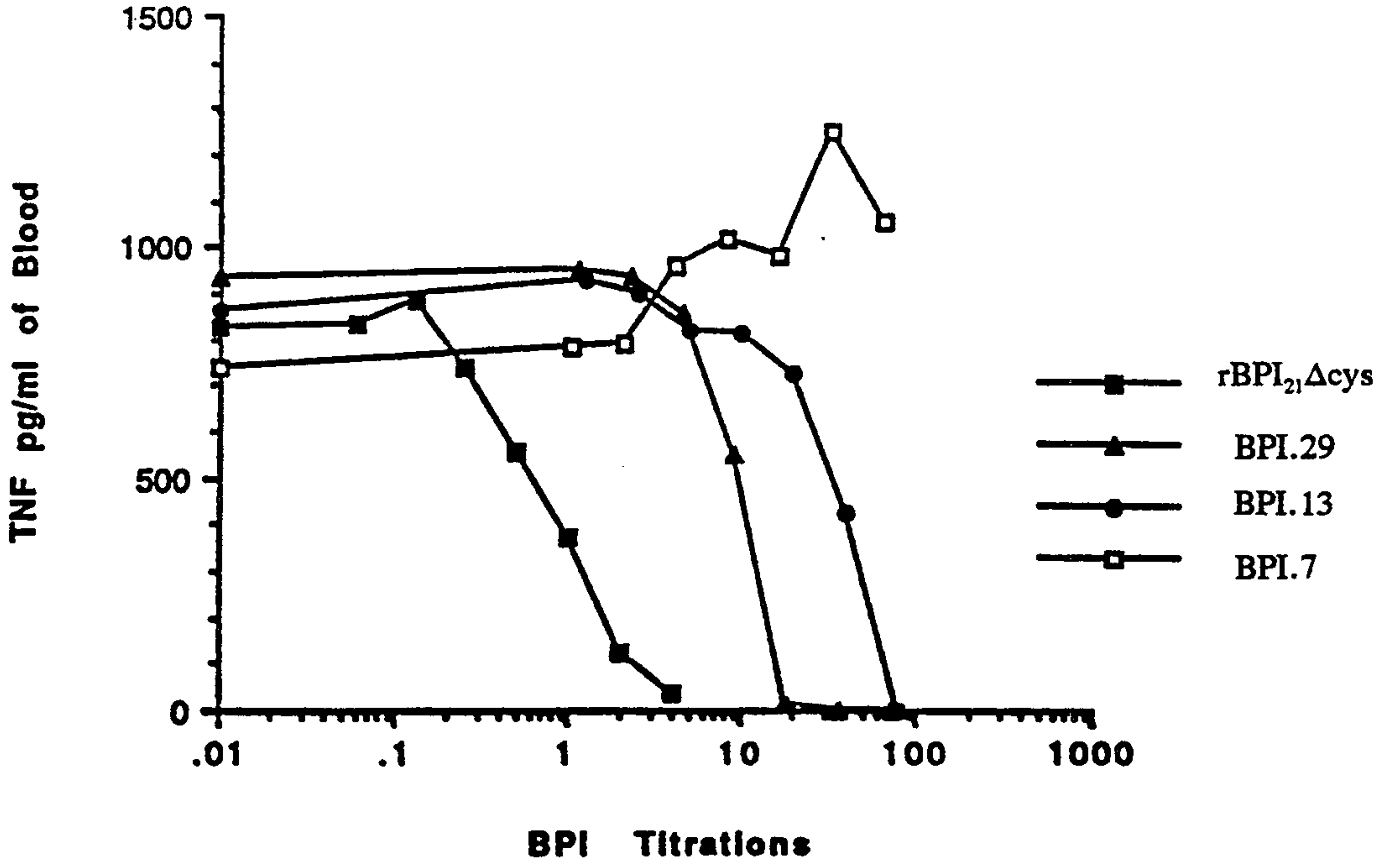

FIGURE 27a

BPI Inhibition of TNF Production by LPS, in Whole Blood

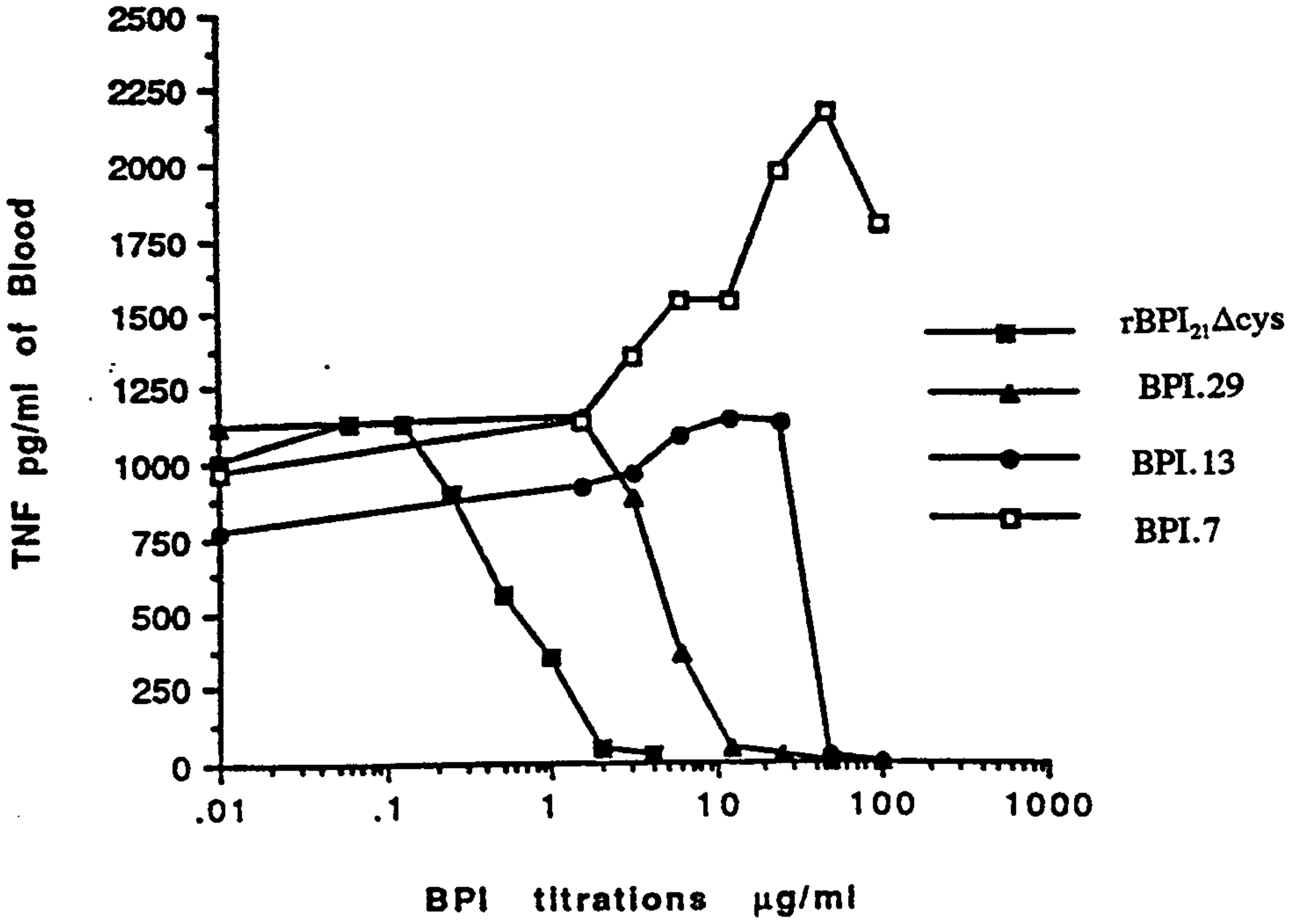

FIGURE 27b

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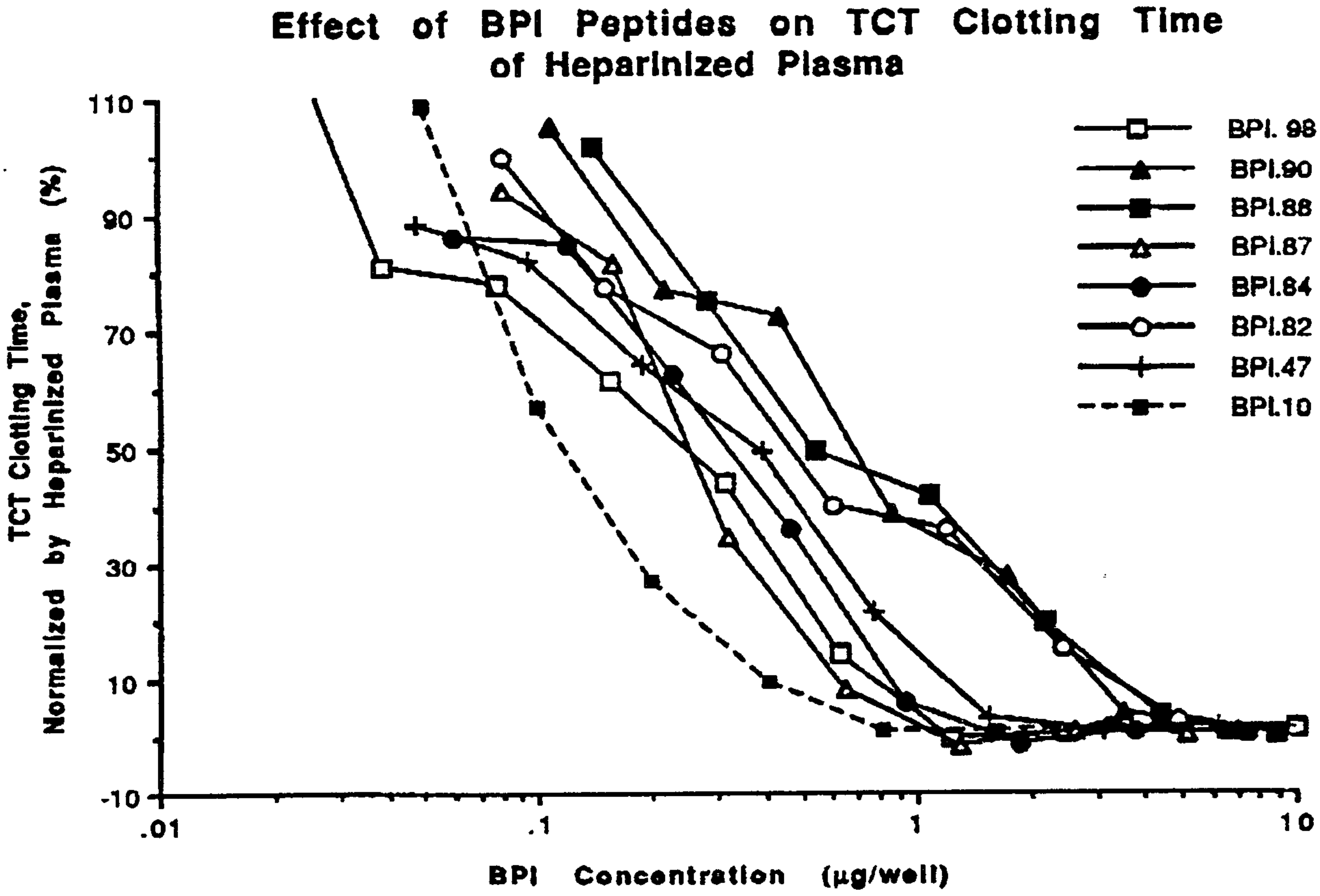

FIGURE 28