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# DESCRIPTION

## CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority of U.S. Application Serial No. 14/284,609, filed on May 22, 2014, which is non-provisional application filed under 37 CFR §1.53(b), claims the benefit under 35 USC §119(e) of U.S. Provisional Application Serial No. 61/829,461 filed on 31 May 2013,

## SUBMISSION OF SEQUENCE LISTING ON ASCII TEXT FILE

[0002] The content of the following submission on ASCII text file is incorporated herein by reference in its entirety: a computer readable form (CRF) of the Sequence Listing (file name: P4960R2WO\_PCTSequenceListing.txt, date recorded: May 30, 2014, size: 195,240 bytes).

## FIELD OF THE INVENTION

[0003] The invention relates to anti-wall teichoic acid ("anti-WTA") antibodies conjugated to rifamycin-type antibiotics and to use of the resultant antibody-antibiotic conjugates in the treatment of infectious diseases.

## BACKGROUND OF THE INVENTION

[0004] Pathogenic bacteria are a substantial cause of sickness and death in both humans and animals. Prominent among these is *Staphylococcus aureus* (*S. aureus*; SA) which is the leading cause of bacterial infections in humans worldwide. *S. aureus* can cause a range of illnesses, from minor skin infections to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), bacteremia, and sepsis. Its incidence ranges from skin, soft tissue, respiratory, bone, joint, endovascular to wound infections. It is still one of the five most common causes of nosocomial infections and is often the cause of postsurgical wound infections. Each year, some 500,000 patients in American hospitals contract a staphylococcal infection.

[0005] Over the last several decades infection with *S. aureus* is becoming increasingly difficult to treat largely due to the emergence of methicillin-resistant *S. aureus* (MRSA) that is resistant to all known beta-lactam antibiotics (Boucher, H.W. et al. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 48, 1-12 (2009)). The circumstances

are so acute, that by 2005, infection with MRSA was reported to be the leading cause of death due to a single infectious agent - responsible for over 15,000 deaths in the United States (DeLeo, F.R. & Chambers, H.F. Reemergence of antibiotic-resistant *Staphylococcus aureus* in the genomics era. *The Journal of Clinical Investigation* 119:2464-2474 (2009)). Vancomycin, linezolid and daptomycin have become the antibiotics of choice for treating invasive MRSA infections (Boucher, H., Miller, L.G. & Razonable, R.R. Serious infections caused by methicillin-resistant *Staphylococcus aureus*. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 51 Suppl 2, S183-197 (2010)). However, reduced susceptibility to vancomycin and cross-resistance to linezolid and daptomycin have also been reported in MRSA clinical strains (Nannini, E., Murray, B.E. & Arias, C.A. Resistance or decreased susceptibility to glycopeptides, daptomycin, and linezolid in methicillin-resistant *Staphylococcus aureus*. *Current opinion in pharmacology* 10, 516-521 (2010)). Over time, the vancomycin dose necessary to overcome resistance has crept upward to levels where nephrotoxicity occurs. Thus, mortality and morbidity from invasive MRSA infections remains high despite these antibiotics.

**[0006]** Although SA is generally thought to be an extracellular pathogen, investigations going back at least 50 years have revealed its ability to infect and survive in various types of host cells, both professional phagocytes and non-phagocytic cells (Gresham, H.D. et al. Survival of *Staphylococcus aureus* inside neutrophils contributes to infection. *J Immunol* 164, 3713-3722 (2000); Anwar, S., Prince, L.R., Foster, S.J., Whyte, M.K. & Sabroe, I. The rise and rise of *Staphylococcus aureus*: laughing in the face of granulocytes. *Clinical and Experimental Immunology* 157, 216-224 (2009); Fraunholz, M. & Sinha, B. Intracellular *staphylococcus aureus*: Live-in and let die. *Frontiers in cellular and infection microbiology* 2, 43 (2012); Garzoni, C. & Kelley, W.L. Return of the Trojan horse: intracellular phenotype switching and immune evasion by *Staphylococcus aureus*. *EMBO molecular medicine* 3:115-117 (2011)). This facultative intracellular persistence enables host immune evasion, long-term colonization of the host, maintenance of a chronically infected state, and is likely a cause for clinical failures of, and relapses after, conventional antibiotic therapy. Furthermore, exposure of intracellular bacteria to suboptimal antibiotic concentrations may encourage the emergence of antibiotic resistant strains, thus making this clinical problem more acute. Consistent with these observations, treatment of patients with invasive MRSA infections such as bacteremia or endocarditis with vancomycin or daptomycin was associated with failure rates greater than 50% (Kullar, R., Davis, S.L., Levine, D.P. & Rybak, M.J. Impact of vancomycin exposure on outcomes in patients with methicillin-resistant *Staphylococcus aureus* bacteremia: support for consensus guidelines suggested targets. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 52, 975-981 (2011); Fowler, V.G., Jr. et al. Daptomycin versus standard therapy for bacteremia and endocarditis caused by *Staphylococcus aureus*. *The New England journal of medicine* 355, 653-665 (2006); Yoon, Y.K., Kim, J.Y., Park, D.W., Sohn, J.W. & Kim, M.J. Predictors of persistent methicillin-resistant *Staphylococcus aureus* bacteraemia in patients treated with vancomycin. *The Journal of antimicrobial chemotherapy* 65:1015-1018 (2010)). Therefore, a more successful anti-staphylococcal therapy should include the elimination of intracellular bacteria.



**[0007]** Most of today's antibacterials chemically are semisynthetic modifications of various natural compounds. These include, for example, the beta-lactam antibacterials, which include the penicillins (produced by fungi in the genus *Penicillium*), the cephalosporins, and the carbapenems. Antimicrobial compounds that are still isolated from living organisms include the aminoglycosides, whereas other antibacterials - for example, the sulfonamides, the quinolones, and the oxazolidinones, are produced solely by chemical synthesis. In accordance with this, many antibacterial compounds are classified on the basis of chemical/biosynthetic origin into natural, semisynthetic, and synthetic. Another classification system is based on biological activity; in this classification, antibacterials are divided into two broad groups according to their biological effect on microorganisms: bactericidal agents kill bacteria, and bacteriostatic agents slow down or stall bacterial growth.

**[0008]** Ansamycins are a class of antibiotics, including rifamycin, rifampin, rifampicin, rifabutin, rifapentine, rifalazil, ABL-1657, and analogs thereof, that inhibit bacterial RNA polymerase and have exceptional potency against gram-positive and selective gram-negative bacteria (Rothstein, D.M., et al (2003) Expert Opin. Invest. Drugs 12(2):255-271; US 7342011; US 7271165).

**[0009]** Immunotherapies have been reported for preventing and treating *S. aureus* (including MRSA) infections. US2011/0262477 concerns uses of bacterial adhesion proteins Eap, Emp and AdsA as vaccines to stimulate immune response against MRSA. WO2000071585 describes isolated monoclonal antibodies reactive to specific *S. aureus* strain isolates. US20110059085A1 suggests an Ab-based strategy utilizing IgM Abs specific for one or more SA capsular antigens, although no actual antibodies were described.

**[0010]** Teichoic acids (TA) are bacterial polysaccharides found within the cell wall of Gram-positive bacteria including SA. Wall teichoic acids (WTA) are those covalently linked to the peptidoglycan (PDG) layer of the cell wall; whereas lipoteichoic acids (LTA) are those covalently linked to the lipids of the cytoplasmic membrane. Xia et al. (2010) Intl. J. Med. Microbiol. 300:148-54. These glycopolymers play crucial roles in bacterial survival under disadvantageous conditions and in other basic cellular processes. The known WTA structures vary widely between bacterial species. *S. aureus* TAs are composed of repetitive polyol phosphate subunits such as ribitol phosphate or glycerol phosphate. Given their structural diversity and variability, WTAs are considered attractive targets for antibodies and as vaccines, *ibid*.

**[0011]** Antibody-drug conjugates (ADC), also known as immunoconjugates, are targeted chemotherapeutic molecules which combine ideal properties of both antibodies and cytotoxic drugs by targeting potent cytotoxic drugs to antigen-expressing tumor cells (Teicher, B.A. (2009) Curr. Cancer Drug Targets 9:982-1004), thereby enhancing the therapeutic index by maximizing efficacy and minimizing off-target toxicity (Carter, P.J. and Senter P.D. (2008) The Cancer J. 14(3):154-169; Chari, R.V. (2008) Acc. Chem. Res. 41:98-107. ADC comprise a targeting antibody covalently attached through a linker unit to a cytotoxic drug moiety. Immunoconjugates allow for the targeted delivery of a drug moiety to a tumor, and intracellular

accumulation therein, where systemic administration of unconjugated drugs may result in unacceptable levels of toxicity to normal cells as well as the tumor cells sought to be eliminated (Polakis P. (2005) Curr. Opin. Pharmacol. 5:382-387). Effective ADC development for a given target antigen depends on optimization of parameters such as target antigen expression levels, tumor accessibility (Kovtun, Y.V. and Goldmacher V.S. (2007) Cancer Lett. 255:232-240), antibody selection (US 7964566), linker stability (Erickson et al (2006) Cancer Res. 66(8):4426-4433; Doronina et al (2006) Bioconjugate Chem. 17:114-124; Alley et al (2008) Bioconjugate Chem. 19:759-765), cytotoxic drug mechanism of action and potency, drug loading (Hamblett et al (2004) Clin. Cancer Res. 10:7063-7070) and mode of linker-drug conjugation to the antibody (Lyon, R. et al (2012) Methods in Enzym. 502:123-138; Xie et al (2006) Expert. Opin. Biol. Ther. 6(3):281-291; Kovtun et al (2006) Cancer Res. 66(6):3214-3121; Law et al (2006) Cancer Res. 66(4):2328-2337; Wu et al (2005) Nature Biotech. 23(9):1137-1145; Lambert J. (2005) Current Opin. in Pharmacol. 5:543-549; Hamann P. (2005) Expert Opin. Ther. Patents 15(9):1087-1103; Payne, G. (2003) Cancer Cell 3:207-212; Trail et al (2003) Cancer Immunol. Immunother. 52:328-337; Syrigos and Epenetos (1999) Anticancer Res. 19:605-614).

**[0012]** The concept of ADC in cancer therapy has also been expanded into antibacterial therapy, in this case the drug portion is an antibiotic, resulting in antibody-antibiotic conjugate (AAC). US 5545721 and US 6660267 describe synthesis of a non-specific immunoglobulin-antibiotic conjugate that binds to the surface of target bacteria via the antibiotic, and uses thereof for treating sepsis. US 7569677 and related patents suggest prophetically antibiotic-conjugated antibodies that have an antigen-binding portion specific for a bacterial antigen (such as SA capsular polysaccharide), but lack a constant region that reacts with a bacterial Fc-binding protein (e.g., staphylococcal protein A).

**[0013]** WO 2004/050846 describes the use of compositions comprising anti-WTA antibodies for use as vaccines for treating staphylococcal infections by binding to *S. aureus* to block infection.

**[0014]** US 6,322,788 describes antibodies capable of binding to a bacterial antigen that lack the ability to be bound by bacterial Fc-binding proteins.

**[0015]** WO 2006/081711 describes antibody-drug conjugates using auristatin peptides, including MeVal-Val-Dil-Dap-Norephedrine (MMAE) and MeVal-Val-Dil-Dap-Phe (MMAF).

## SUMMARY OF THE INVENTION

**[0016]** The invention is defined in the appended claims.

**[0017]** Any example in agreement with the invention as defined in the claims forms an embodiment of the invention.

**[0018]** The invention provides compositions referred to as "antibody-antibiotic conjugates," or "AAC") comprising an antibody conjugated by a covalent attachment to one or more rifamycin-type antibiotic moieties as set out in the claims.

**[0019]** The antibody-antibiotic conjugates of the invention may employ an anti-WTA monoclonal antibody, comprising a light chain and a H chain, the L chain comprising CDR L1, CDR L2, and CDR L3 and the H chain comprising CDR H1, CDR H2 and CDR H3, wherein the CDR L1, CDR L2, and CDR L3 and CDR H1, CDR H2 and CDR H3 comprise the amino acid sequences of the CDRs of each of Abs 4461 (SEQ ID NO. 1-6), 4624 (SEQ ID NO. 7-12), 4399 (SEQ ID NO. 13-18), and 6267 (SEQ ID NO. 19-24) respectively, as shown in Tables 6A and 6B.

**[0020]** In one example, the isolated anti-WTA monoclonal antibody comprises a heavy chain variable region comprising a heavy chain variable region (VH), wherein the VH comprises at least 95% sequence identity over the length of the VH region selected from the VH sequence of SEQ ID NO.26, SEQ ID NO.28, SEQ ID NO.30, SEQ ID NO.32 of antibodies 4461, 4624, 4399, and 6267, respectively. In one example, this antibody further comprises a L chain variable region (VL) wherein the VL comprises at least 95% sequence identity over the length of the VL region selected from the VL sequence of SEQ ID NO.25, SEQ ID NO.27, SEQ ID NO.29, SEQ ID NO.31 of antibodies 4461, 4624, 4399, and 6267, respectively. In another example, the isolated anti-WTA monoclonal antibody comprises a L chain variable region (VL) wherein the VL comprises at least 95% sequence identity over the length of the VL region selected from the VL sequence of SEQ ID NO.25, SEQ ID NO.27, SEQ ID NO.29, SEQ ID NO.31 of antibodies 4461, 4624, 4399, and 6267, respectively. In any of the preceding examples, the sequence identity may be 96%, 97%, 98%, 99% or 100%.

**[0021]** In more specific examples, the antibody comprises:

1. (i) VL of SEQ ID NO. 25 and VH of SEQ ID NO. 26;
2. (ii) VL of SEQ ID NO. 27 and VH of SEQ ID NO. 28;
3. (iii) VL of SEQ ID NO. 29 and VH of SEQ ID NO. 30; or
4. (iv) VL of SEQ ID NO. 31 and VH of SEQ ID NO. 32.

**[0022]** In some examples, the isolated anti-WTA (wall teichoic acid) monoclonal antibody comprising a light (L) chain and a heavy (H) chain, wherein:

1. (a) the L chain comprising CDR L1 comprising the sequence of KSSQSVLSRANNYYVA (SEQ ID NO:1), CDR L2 comprising the sequence of WASTREF (SEQ ID NO:2), and CDR L3 comprising the sequence of QQYYTSRRT (SEQ ID NO:3); and the H chain comprising CDR H1 comprising the sequence of DYYMH (SEQ ID NO:4), CDR H2 comprising the sequence of WINPKSGGTNYAQRFG (SEQ ID NO:5), and CDR H3 comprising the sequence of DCGSGGLRDF (SEQ ID NO:6);
2. (b) the L chain comprising CDR L1 comprising the sequence of RSNQNLLSSNNNYLA

- (SEQ ID NO:7), CDR L2 comprising the sequence of WASTRES (SEQ ID NO:8), and CDR L3 comprising the sequence of QQYYANPRT (SEQ ID NO:9); and the H chain comprising CDR H1 comprising the sequence of DYYIH (SEQ ID NO:10), CDR H2 comprising the sequence of WINPNTGGTYAQQKFRD (SEQ ID NO:11), and CDR H3 comprising the sequence of DCGRGGLRDI (SEQ ID NO:12);
3. (c) the L chain comprising CDR L1 comprising the sequence of KSNQNVCLASSNDKNYLA (SEQ ID NO:13), CDR L2 comprising the sequence of WASIRES (SEQ ID NO:14), and CDR L3 comprising the sequence of QQYYTNPRT (SEQ ID NO:15); and the H chain comprising CDR H1 comprising the sequence of DYYIH (SEQ ID NO:16), CDR H2 comprising the sequence of WINPNTGGTNYAQQKFQG (SEQ ID NO:17), and CDR H3 comprising the sequence of DCGNAGLRDI (SEQ ID NO:18); or
4. (d) the L chain comprising CDR L1 comprising the sequence of KSSQNVLYSSNNKNYLA (SEQ ID NO:19), CDR L2 comprising the sequence of WASTRES (SEQ ID NO:20), and CDR L3 comprising the sequence of QQYYTSPPYT (SEQ ID NO:21); and the H chain comprising CDR H1 comprising the sequence of SYWIG (SEQ ID NO:22), CDR H2 comprising the sequence of IIHPGDSKTRYSPSFQG (SEQ ID NO:23), and CDR H3 comprising the sequence of LYCSGGSCYSDRAFSSLGAGGYYYGMGV (SEQ ID NO:24).

**[0023]** In any one of the preceding embodiments, the antibody may be an antigen-binding fragment lacking a Fc region. In some embodiments, the antibody is a F(ab) or F(ab')<sub>2</sub>. In some embodiments, the antibody further comprises a heavy chain constant region and/or a light chain constant region, wherein the heavy chain constant region and/or the light chain constant region comprise one or more amino acids that are substituted with cysteine residues. In some embodiments, the heavy chain constant region comprises amino acid substitution A118C and/or S400C, and/or the light chain constant region comprises amino acid substitution V205C, wherein the numbering is according to the EU numbering. In some examples, the heavy chain constant region comprises the amino acid sequence of SEQ ID NO:149 and/or the light chain constant region comprises the amino acid sequence of SEQ ID NO:151. In some examples, the heavy chain constant region comprises the amino acid sequence of SEQ ID NO:149 and the light chain constant region comprises the amino acid sequence of SEQ ID NO:150. In some examples, the heavy chain constant region comprises the amino acid sequence of SEQ ID NO:148 and the light chain constant region comprises the amino acid sequence of SEQ ID NO:151.

**[0024]** In one aspect, the Ab of any one of the preceding embodiments binds WTA alpha.

**[0025]** In another aspect, the invention employs an isolated anti-WTA monoclonal antibody comprising a light chain and a H chain, the L chain comprising CDR L1, CDR L2, and CDR L3 and/or the H chain comprising CDR H1, CDR H2 and CDR H3, wherein the CDR L1, CDR L2, and CDR L3 and CDR H1, CDR H2 and CDR H3 comprise the amino acid sequences of the corresponding CDRs of each of Abs shown in Figure 14 (SEQ ID NO. 33-110). In another

aspect, the invention provides an isolated anti-WTA monoclonal antibody comprising a light chain and a H chain, the L chain comprising CDR L1, CDR L2, and CDR L3 and/or the H chain comprising CDR H1, CDR H2 and CDR H3, wherein the CDR L1, CDR L2, and CDR L3 and CDR H1, CDR H2 and CDR H3 comprise the amino acid sequences of the corresponding CDRs of each of Abs shown in Figure 15A, 15B, 16A and 16B (antibodies 6078, 6078.v2HC-Cys, 6078.v2LC-Cys, 6078.v3HC-Cys, 6078.v3LC-Cys, 6078.v4HC-Cys, 6078.v4LC-Cys, 6078.v4HCLC-Cys, 4497, 4497.v8HC-Cys, 4497.v8LC-Cys, and 4497.v8HCLC-Cys). In a specific embodiment these Abs bind WTA beta.

**[0026]** In another aspect, the invention employs an isolated anti-WTA monoclonal antibody, specifically anti-WTA beta monoclonal antibody which comprises a L chain variable region (VL) wherein the VL comprises at least 95% sequence identity over the length of the VL region selected from the VL sequence corresponding to each of the antibodies 6078, 6263, 4450, 6297, 6239, 6232, 6259, 6292, 4462, 6265, 6253, 4497, and 4487 respectively, as shown in Figures 17A-1 to 17A-2 at Kabat positions 1-107. In further examples, the antibody further comprises a heavy chain variable region comprising a heavy chain variable region (VH), wherein the VH comprises at least 95% sequence identity over the length of the VH region selected from the VH sequences corresponding to each of the antibodies 6078, 6263, 4450, 6297, 6239, 6232, 6259, 6292, 4462, 6265, 6253, 4497, and 4487 respectively, as shown in Figures 17B-1 to 17B-2 at Kabat positions 1-113. In any of the preceding embodiments, the sequence identity may be 96%, 97%, 98%, 99% or 100%. In a more specific example of the antibody, the VH comprises the sequence of SEQ ID NO. 112 and the VL comprises the SEQ ID NO. 111.

**[0027]** In another aspect, the invention employs an isolated anti-WTA monoclonal antibody, specifically anti-WTA beta monoclonal antibody which comprises a L chain variable region (VL) wherein the VL comprises at least 95% sequence identity over the length of the VL region selected from the VL sequence corresponding to each of the antibodies as shown in Figures 15A or 16A (antibodies 6078, 6078.v2HC-Cys, 6078.v2LC-Cys, 6078.v3HC-Cys, 6078.v3LC-Cys, 6078.v4HC-Cys, 6078.v4LC-Cys, 6078.v4HCLC-Cys, 4497, 4497.v8HC-Cys, 4497.v8LC-Cys, and 4497.v8HCLC-Cys) at Kabat positions 1-107. In further examples, the antibody further comprises a heavy chain variable region comprising a heavy chain variable region (VH), wherein the VH comprises at least 95% sequence identity over the length of the VH region selected from the VH sequences corresponding to each of the antibodies as shown in Figures 15B and 16B (antibodies 6078, 6078.v2HC-Cys, 6078.v2LC-Cys, 6078.v3HC-Cys, 6078.v3LC-Cys, 6078.v4HC-Cys, 6078.v4LC-Cys, 6078.v4HCLC-Cys, 4497, 4497.v8HC-Cys, 4497.v8LC-Cys, and 4497.v8HCLC-Cys) at Kabat positions 1-113. In any of the preceding embodiments, the sequence identity may be 96%, 97%, 98%, 99% or 100%. In an additional embodiment, this antibody further comprises a VL having at least 95% sequence identity to SEQ ID NO. 119.

**[0028]** The anti-WTA beta Cys-engineered H and L chain variants can be paired in any of the following combinations to form full Abs for conjugating to linker-Abx intermediates to generate anti-WTA AACs of the invention. In one embodiment, the L chain comprises the sequence of SEQ ID NO.121 and the H chain comprises the sequence of SEQ ID NO. 124. In another

embodiment, the isolated antibody comprises a L chain of SEQ ID NO. 123 and a H chain comprising a sequence of SEQ ID NO.124 or SEQ ID NO.157. In a particular embodiment, the anti- WTA beta antibody as well as the anti-WTA beta AAC of the invention comprises a L chain of SEQ ID NO. 123.

**[0029]** Yet another example is an antibody that binds to the same epitope as each of the anti-WTA alpha Abs of Figure 13A and Figure 13B. Also provided is an antibody that binds to the same epitope as each of the anti-WTA beta Abs of Figure 14, Figures 15A and 15B, Figures 16A and 16B, and Figures 17A and 17B.

**[0030]** In a further embodiment, the anti-WTA beta and anti-WTA alpha antibodies of the present invention are antigen-binding fragments lacking the Fc region, preferably F(ab')<sub>2</sub> or F(ab). Thus, the present invention provides antibody-antibiotic conjugates wherein the WTA antibody is a F(ab')<sub>2</sub> or F(ab).

**[0031]** In another aspect, the invention employs an anti-WTA monoclonal antibody that binds to WTA beta.

**[0032]** In some examples, the isolated anti-WTA (wall teichoic acid) monoclonal antibody comprising a light (L) chain and a heavy (H) chain, wherein:

1. (a) the L chain comprising CDR L1 comprising the sequence of KSSQSIFRTSRNKNLLN (SEQ ID NO:99), CDR L2 comprising the sequence of WASTRKS (SEQ ID NO: 100), and CDR L3 comprising the sequence of QQYFSPPYT (SEQ ID NO:101); and the H chain comprising CDR H1 comprising the sequence of SFWMH (SEQ ID NO:102), CDR H2 comprising the sequence of FTNNEGTTTAYADSVRG (SEQ ID NO: 103), and CDR H3 comprising the sequence of GDGGLDD (SEQ ID NO:104); or
2. (b) the L chain comprising CDR L1 comprising the sequence of KSSQSIFRTSRNKNLLN (SEQ ID NO:99), CDR L2 comprising the sequence of WASTRKS (SEQ ID NO:100), and CDR L3 comprising the sequence of QQYFSPPYT (SEQ ID NO:101); and the H chain comprising CDR H1 comprising the sequence of SFWMH (SEQ ID NO:102), CDR H2 comprising the sequence of FTNNEGTTTAYADSVRG (SEQ ID NO:103), and CDR H3 comprising the sequence of GEGGLDD (SEQ ID NO:118).

**[0033]** In some embodiments, the isolated anti-WTA monoclonal antibody comprises a heavy chain variable region (VH), wherein the VH comprises an amino acid sequence having at least 95% sequence identity over the length of the VH sequence SEQ ID NO.120 or SEQ ID NO.156. In some embodiments, the antibody further comprises a light chain variable region (VL), wherein the VL comprises an amino acid sequence having at least 95% sequence identity over the length of the VL sequence SEQ ID NO.119. In some embodiments, the isolated anti-WTA monoclonal antibody comprising a light chain variable region (VL), wherein the VL comprises an amino acid sequence having at least 95% sequence identity over the length of the VL

sequence SEQ ID NO.119. In any of the preceding embodiments, the sequence identity may be 96%, 97%, 98%, 99%, or 100%. In some embodiments, the isolated anti-WTA monoclonal antibody comprising a light chain variable region (VL) and a heavy chain variable region (VH), wherein the VL comprises the sequence of SEQ ID NO.119 and the VH comprises the sequence SEQ ID NO.120 or SEQ ID NO.156.

**[0034]** In some embodiments described above, the antibody is an antigen-binding fragment lacking a Fc region. In some embodiments, the antibody is a F(ab) or F(ab')<sub>2</sub>. In some embodiments, the antibody further comprises a heavy chain constant region and/or a light chain constant region, wherein the heavy chain constant region and/or the light chain constant region comprise one or more amino acids that are substituted with cysteine residues. In some embodiments, the heavy chain constant region comprises amino acid substitution A118C or S400C, and/or the light chain constant region comprises amino acid substitution V205C, wherein the numbering is according to the EU numbering. In some embodiments, the heavy chain constant region comprises the amino acid sequence of SEQ ID NO:149 and/or the light chain constant region comprises the amino acid sequence of SEQ ID NO:151. In some embodiments, the heavy chain constant region comprises the amino acid sequence of SEQ ID NO:149 and the light chain constant region comprises the amino acid sequence of SEQ ID NO:150. In some embodiments, the heavy chain constant region comprises the amino acid sequence of SEQ ID NO:148 and the light chain constant region comprises the amino acid sequence of SEQ ID NO:151.

**[0035]** In some embodiments, the isolated anti-WTA monoclonal antibody comprising a heavy chain and a light chain, wherein the heavy chain comprises the sequence of SEQ ID NO:146, SEQ ID NO.147, SEQ ID NO.157 or SEQ ID NO.124, and the light chain comprises the sequence of SEQ ID NO.121. In some embodiments, the isolated anti-WTA monoclonal antibody comprising a heavy chain and a light chain, wherein the heavy chain comprises the sequence of SEQ ID NO:146, SEQ ID NO.147, SEQ ID NO.157 or SEQ ID NO.124, and the light chain comprises the sequence of SEQ ID NO.123. In some embodiments, the isolated anti-WTA monoclonal antibody comprising a heavy chain and a light chain, wherein the heavy chain comprises the sequence of SEQ ID NO:146, SEQ ID NO.147, SEQ ID NO.157 or SEQ ID NO.124, and the light chain comprises the sequence of SEQ ID NO.145.

**[0036]** In some embodiments of any of the antibodies described above, the antibody is not an IgM isotype. In some embodiments of any of the antibodies described above, the antibody is an IgG (e.g., IgG1, IgG2, IgG3, IgG4), IgE, IgD, or IgA (e.g., IgA1 or IgA2) isotype. In some embodiments of any of the antibodies described above, the antibody is produced by a host cell in cell culture. In some embodiments, the host cell is a non-human cell. In some embodiments, the host cell is prokaryotic or eukaryotic. In some embodiments, the host cell is a mammalian cell (e.g., a human or non-human mammalian cell). In some embodiments, the host cell is a CHO cell.

**[0037]** Also described is an isolated nucleic acid encoding any of the antibodies disclosed herein. Also described is a vector comprising a nucleic acid encoding any of the antibodies

disclosed herein. The expression vector may be an expression vector.

**[0038]** Also described is a host cell comprising a nucleic acid encoding any of the antibodies disclosed herein. The host cell may be prokaryotic or eukaryotic. The host cell may be a mammalian cell (e.g., a human or non-human mammalian cell). In some embodiments, the host cell is a CHO cell.

**[0039]** Also described is a method of producing an antibody comprising culturing a host cell comprising a nucleic acid encoding any of the antibodies disclosed herein under conditions suitable for expression of the nucleic acid; and recovering the antibody produced by the cell. The method may further comprise purifying the antibody.

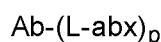
**[0040]** Another aspect of the invention is an antibody-antibiotic conjugate (AAC) compound comprising an anti-wall teichoic acid (WTA) antibody of the invention, covalently attached by a peptide linker to a rifamycin-type antibiotic as set out in the claims. In some embodiments the anti-wall teichoic acid (WTA) antibody binds to *Staphylococcus aureus*. In some embodiments, the anti-wall teichoic acid antibody binds to methicillin-resistant *Staphylococcus aureus* (MRSA).

**[0041]** In some examples of the antibody-antibiotic conjugates disclosed herein, the antibody comprises: i) L chain and H chain CDRs of SEQ ID NOs 99-104 or ii) the VL of SEQ ID NO.119 or SEQ ID NO. 123 paired with the VH of SEQ ID NO.120 or SEQ ID NO. 156; or iii) the VL of SEQ ID NO.111 paired with the VH of SEQ ID NO.112.

**[0042]** In some examples, the rifamycin-type antibiotic is a rifalazil-type antibiotic. In some embodiments, the rifamycin-type antibiotic comprises a quaternary amine attached to the peptide linker. In some embodiments, the antibiotic is attached to the antibody via a peptide linker which is attached to an engineered cysteine of the anti-WTA antibody. The engineered cysteine may be in the L or H chain of the modified antibody. In some embodiments, the cysteine residue is in the L chain. In some embodiments, the cysteine residue is in the H chain.

**[0043]** The antibody-antibiotic conjugate compounds of the invention can comprise a peptide linker which is a *S. aureus* cysteine protease cleavable linker; such linkers include a staphopain B or a staphopain A cleavable linker. In one embodiment, the *S. aureus* protease is an endopeptidase. In another embodiment the linker is a host protease cleavable linker preferably a human protease cathepsin B cleavable linker (e.g., a val-cit dipeptide linker).

**[0044]** An exemplary embodiment of an antibody-antibiotic conjugate compound has the formula:



wherein:



Ab is the anti-wall teichoic acid antibody;

L is the peptide linker having the formula:



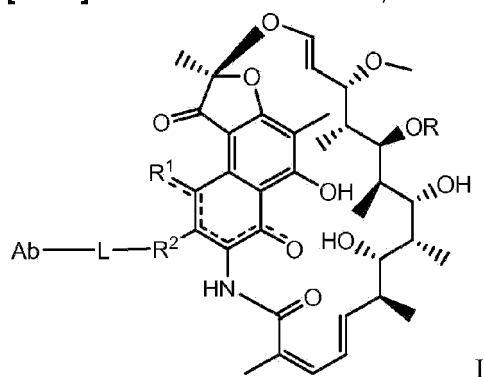
where Str is a stretcher unit; Pep is a peptide of two to twelve amino acid residues, and Y is a spacer unit;

abx is the rifamycin-type antibiotic; and

p is an integer from 1 to 8.

**[0045]** In one embodiment, the antibody-antibiotic conjugate compounds of any of the preceding comprise a antibiotic antibody ratio (AAR) of 2 or 4.

**[0046]** In some embodiments, the antibody-antibiotic conjugate compound is of the formula I:



wherein:

the dashed lines indicate an optional bond;

R is H, C<sub>1</sub>-C<sub>12</sub> alkyl, or C(O)CH<sub>3</sub>;

R<sup>1</sup> is OH;

R<sup>2</sup> is CH=N-(heterocyclyl), wherein the heterocyclyl is optionally substituted with one or more groups independently selected from C(O)CH<sub>3</sub>, C<sub>1</sub>-C<sub>12</sub> alkyl, C<sub>1</sub>-C<sub>12</sub> heteroaryl, C<sub>2</sub>-C<sub>20</sub> heterocyclyl, C<sub>6</sub>-C<sub>20</sub> aryl, and C<sub>3</sub>-C<sub>12</sub> carbocyclyl;

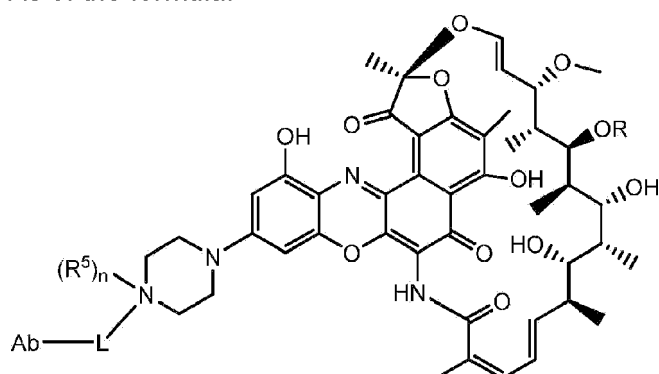
or R<sup>1</sup> and R<sup>2</sup> form a five- or six-membered fused heteroaryl or heterocyclyl, and optionally forming a spiro or fused six-membered heteroaryl, heterocyclyl, aryl, or carbocyclyl ring, wherein the spiro or fused six-membered heteroaryl, heterocyclyl, aryl, or carbocyclyl ring is optionally substituted H, F, Cl, Br, I, C<sub>1</sub>-C<sub>12</sub> alkyl, or OH;

L is the peptide linker attached to  $R^2$  or the fused heteroaryl or heterocyclyl formed by  $R^1$  and  $R^2$ ; and

Ab is the anti-wall teichoic acid (WTA) antibody.

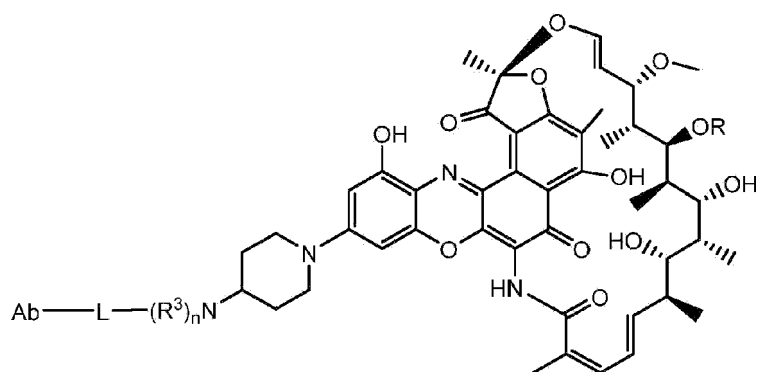
**[0047]** In some of these embodiments, the antibody-antibiotic conjugate compound of formula I is of the formula:

**[0048]** In some of these embodiments, the antibody-antibiotic conjugate compound of formula I is of the formula:



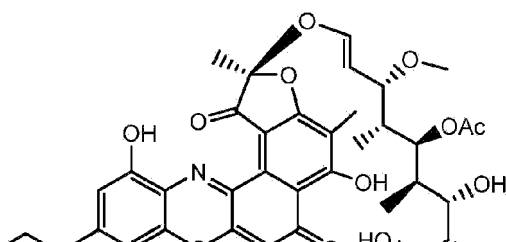
wherein  $R^5$  is independently selected from H and  $C_1$ - $C_{12}$  alkyl; and n is 0 or 1.

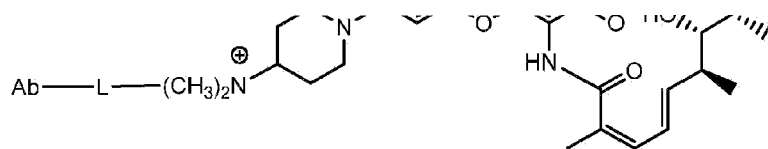
**[0049]** In some of these embodiments, the antibody-antibiotic conjugate compound of formula I is of the formula:



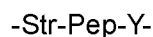
wherein  $R^3$  is independently selected from H and  $C_1$ - $C_{12}$  alkyl; and n is 1 or 2.

**[0050]** In some particular embodiments, the antibody-antibiotic conjugate compound of formula I is of the formula:

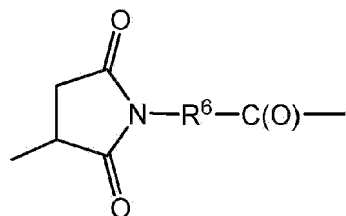




**[0051]** In some embodiments, provided is an antibody-antibiotic conjugate (AAC) compound comprising an anti-wall teichoic acid (WTA) antibody of the invention, covalently attached by a peptide linker to a rifamycin-type antibiotic, where the peptide linker has the formula:

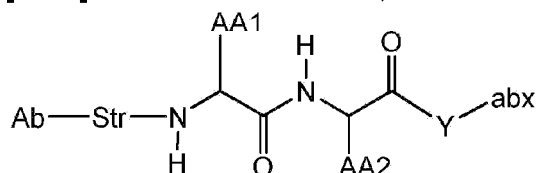


where Str is a stretcher unit covalently attached to the anti-wall teichoic acid (WTA) antibody; Pep is a peptide of two to twelve amino acid residues, and Y is a spacer unit covalently attached to the rifamycin-type antibiotic. In some of these embodiments, Str has the formula:



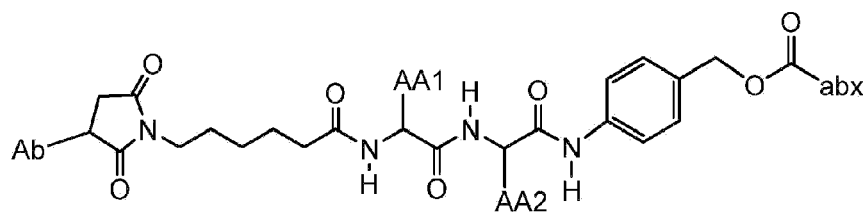
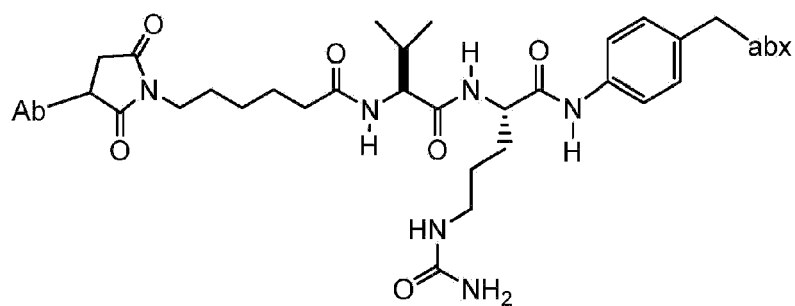
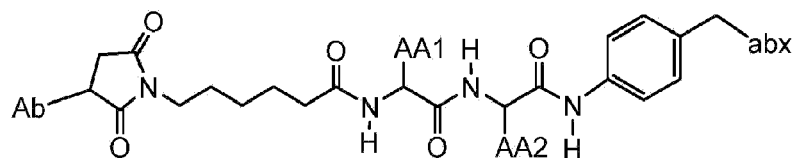
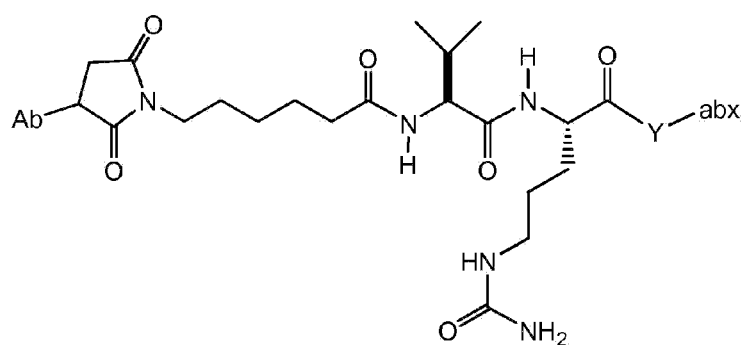
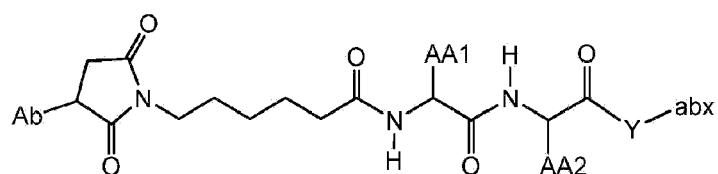
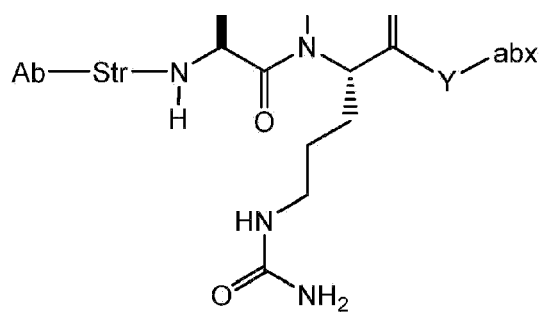
wherein  $R^6$  is selected from the group consisting of  $C_1$ - $C_{10}$  alkylene-,  $-C_3$ - $C_8$  carbocyclo-,  $-O$ -( $C_1$ - $C_8$  alkyl)-,  $-$ arylene-,  $-C_1$ - $C_{10}$  alkylene-arylene-,  $-$ arylene- $C_1$ - $C_{10}$  alkylene-,  $-C_1$ - $C_{10}$  alkylene-( $C_3$ - $C_8$  carbocyclo)-,  $-(C_3$ - $C_8$  carbocyclo)- $C_1$ - $C_{10}$  alkylene-,  $-C_3$ - $C_8$  heterocyclo-,  $-C_1$ - $C_{10}$  alkylene-( $C_3$ - $C_8$  heterocyclo)-,  $-(C_3$ - $C_8$  heterocyclo)- $C_1$ - $C_{10}$  alkylene-,  $-(CH_2CH_2O)_r$ , and  $-(CH_2CH_2O)_r-CH_2$ ; and  $r$  is an integer ranging from 1 to 10. In one variation,  $R^6$  is  $-(CH_2)_5$ . In some of these embodiments, Pep comprises two to twelve amino acid residues independently selected from glycine, alanine, phenylalanine, lysine, arginine, valine, and citrulline. In one variation, Pep is selected from valine-citrulline (val-cit, vc); phenylalanine-lysine (fk); GGAFAGGG (SEQ ID NO: 126); tpm-cit; GPImeLFF (SEQ ID NO: 129); valine-citrulline-phenylalanine (val-cit-phe); GGAF (SEQ ID NO: 131); and LAFG (SEQ ID NO: 128). In some of these embodiments, Y comprises para-aminobenzyl or para-aminobenzyloxycarbonyl.

**[0052]** In some embodiments, the antibody-antibiotic conjugate compound is of the formula:

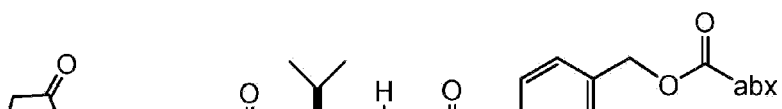


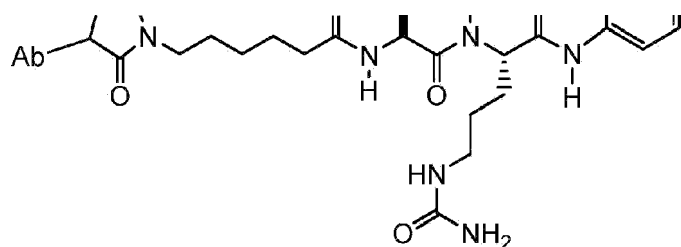
where Ab, Str, Y and abx are as defined herein, and AA1 and AA2 are independently selected from an amino acid side chain. In some of these embodiments, the amino acid side chain is independently selected from H,  $-CH_3$ ,  $-CH_2(C_6H_5)$ ,  $-CH_2CH_2CH_2CH_2NH_2$ ,  $-CH_2CH_2CH_2NHC(NH)NH_2$ ,  $-CHCH(CH_3)CH_3$ , and  $-CH_2CH_2CH_2NHC(O)NH_2$ . In some of these embodiments, the antibody-antibiotic conjugate compound is of the formula:



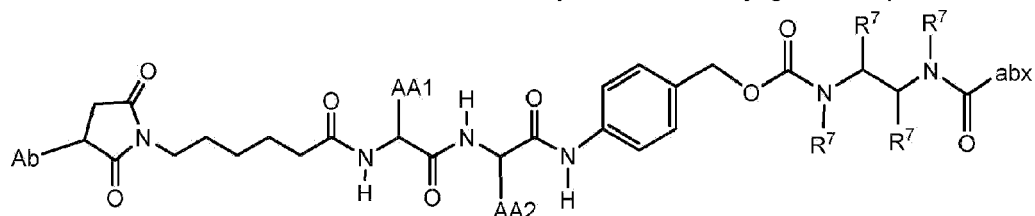


or

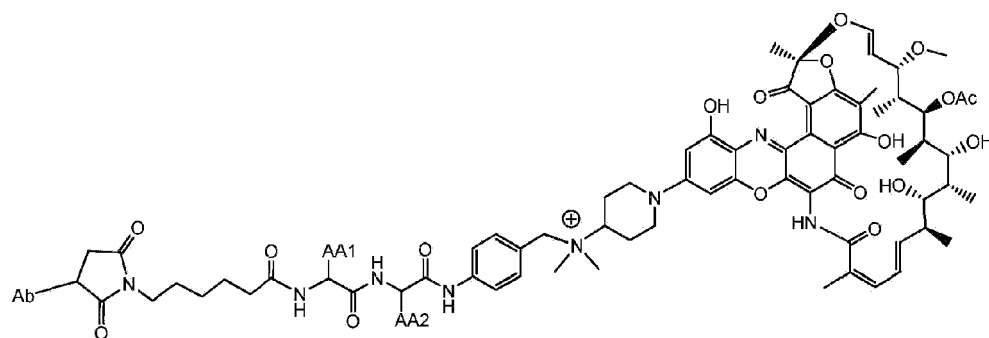
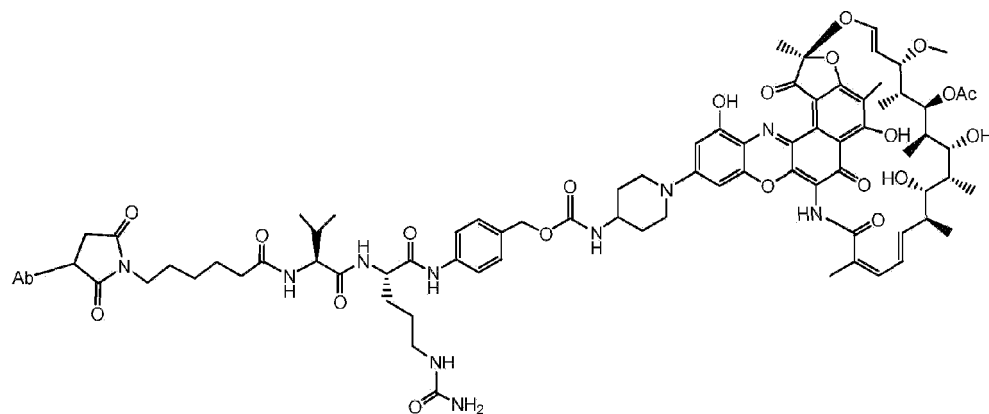
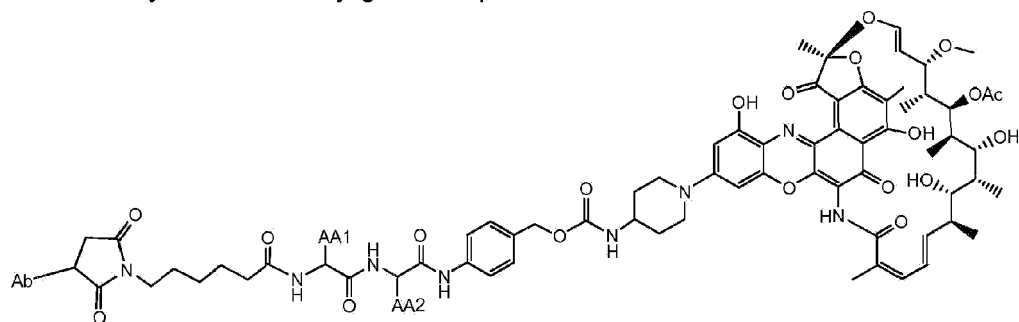




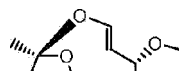
In some of these embodiments, the antibody-antibiotic conjugate compound is of the formula:

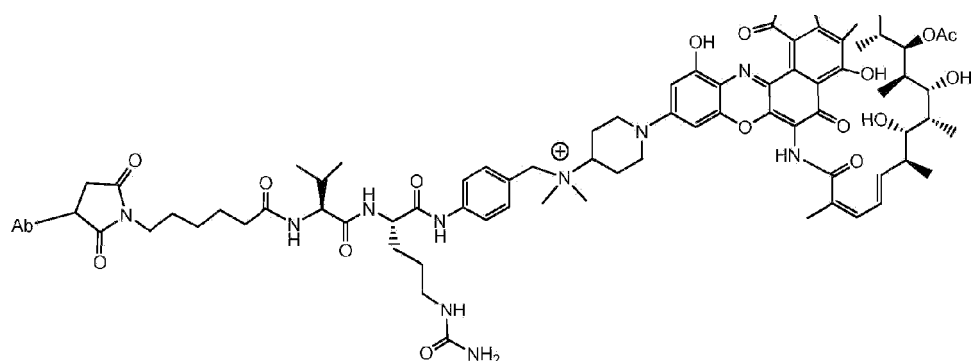


where  $R^7$  is independently selected from H and  $C_1$ - $C_{12}$  alkyl. In some of these embodiments, the antibody-antibiotic conjugate compound is of the formula:



or





**[0053]** Another aspect of the invention is a pharmaceutical composition comprising an antibody-antibiotic conjugate compound of the invention.

**[0054]** In one aspect, the present invention provides an antibody-antibiotic conjugate as set out in the claims for use in a method of treating a bacterial infection in a patient, wherein the bacterial infection is a *Staphylococcus aureus* infection. In some embodiments, the patient has been diagnosed with a Staph aureus infection. In some embodiments, treating the bacterial infection comprises reducing bacterial load.

**[0055]** In one aspect, the present invention provides an antibody-antibiotic conjugate as set out in the claims for use in a method of reducing the number of intracellular *Staphylococcus aureus* bacterial cells in the host cells of a *Staphylococcus aureus* infected patient without reducing the number of the host cells. This use may comprise killing persister bacterial cells (e.g, staph A) in vivo by contacting the persister bacteria with an AAC of any of the preceding embodiments.

**[0056]** In another embodiment, the medical use further comprises administering a second therapeutic agent. In a further embodiment, the second therapeutic agent is an antibiotic including an antibiotic against Staph aureus in general or MRSA in particular.

**[0057]** In one embodiment, the second antibiotic administered in combination with the antibody-antibiotic conjugate compound of the invention is selected from the structural classes: (i) aminoglycosides; (ii) beta-lactams; (iii) macrolides/cyclic peptides; (iv) tetracyclines; (v) fluoroquinolones/fluoroquinolones; (vi) and oxazolidinones.

**[0058]** In one embodiment, the second antibiotic administered in combination with the antibody-antibiotic conjugate compound of the invention is selected from clindamycin, novobiocin, retapamulin, daptomycin, GSK-2140944, CG-400549, sitafloxacin, teicoplanin, triclosan, naphthiridone, radezolid, doxorubicin, ampicillin, vancomycin, imipenem, doripenem, gemcitabine, dalbavancin, and azithromycin.

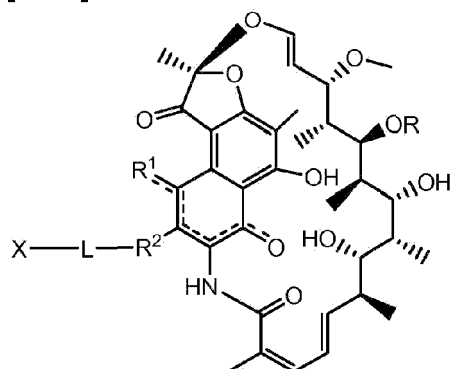
**[0059]** In some embodiments herein, the bacterial load in the subject has been reduced to an undetectable level after the treatment. In one embodiment, the patient's blood culture is negative after treatment as compared to a positive blood culture before treatment. In some

embodiments herein, the bacterial resistance in the subject is undetectable or low. In some embodiments herein, the subject is not responsive to treatment with methicillin or vancomycin.

**[0060]** Another aspect of the invention is a process for making an antibody or an antibody-antibiotic conjugate compound of the invention comprising conjugating a rifamycin-type antibiotic to an anti-wall teichoic acid (WTA) antibody as set out in the claims.

**[0061]** Another aspect of the invention is a kit for treating a bacterial infection comprising a pharmaceutical composition of the invention and instructions for use as set out in the claims.

**[0062]** Also described is a linker-antibiotic intermediate having Formula II:



II

wherein:

the dashed lines indicate an optional bond;

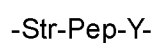
R is H, C<sub>1</sub>-C<sub>12</sub> alkyl, or C(O)CH<sub>3</sub>;

R<sup>1</sup> is OH;

R<sup>2</sup> is CH=N-(heterocyclyl), wherein the heterocyclyl is optionally substituted with one or more groups independently selected from C(O)CH<sub>3</sub>, C<sub>1</sub>-C<sub>12</sub> alkyl, C<sub>1</sub>-C<sub>12</sub> heteroaryl, C<sub>2</sub>-C<sub>20</sub> heterocyclyl, C<sub>6</sub>-C<sub>20</sub> aryl, and C<sub>3</sub>-C<sub>12</sub> carbocyclyl;

or R<sup>1</sup> and R<sup>2</sup> form a five- or six-membered fused heteroaryl or heterocyclyl, and optionally forming a spiro or fused six-membered heteroaryl, heterocyclyl, aryl, or carbocyclyl ring, wherein the spiro or fused six-membered heteroaryl, heterocyclyl, aryl, or carbocyclyl ring is optionally substituted H, F, Cl, Br, I, C<sub>1</sub>-C<sub>12</sub> alkyl, or OH;

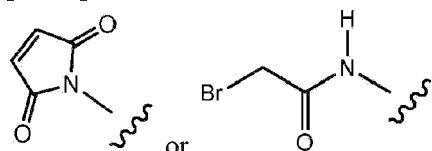
L is a peptide linker attached to R<sup>2</sup> or the fused heteroaryl or heterocyclyl formed by R<sup>1</sup> and R<sup>2</sup>; and having the formula:



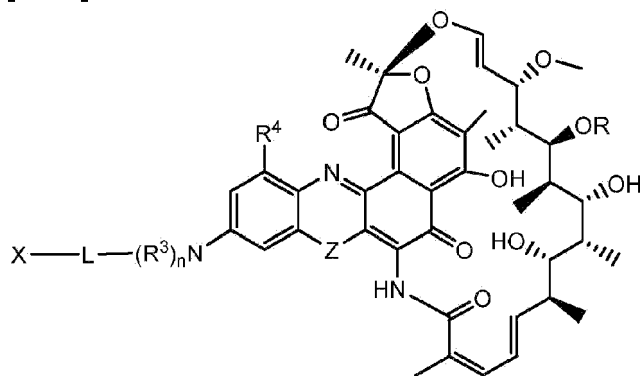
where Str is a stretcher unit; Pep is a peptide of two to twelve amino acid residues, and Y is a spacer unit; and

X is a reactive functional group selected from maleimide, thiol, amino, bromide, bromoacetamido, iodoacetamido, p-toluenesulfonate, iodide, hydroxyl, carboxyl, pyridyl disulfide, and N-hydroxysuccinimide.

[0063] In some of the linker-antibiotic intermediate of Formula II, X is



[0064] The the linker-antibiotic intermediate of Formula II may have the formula:



wherein

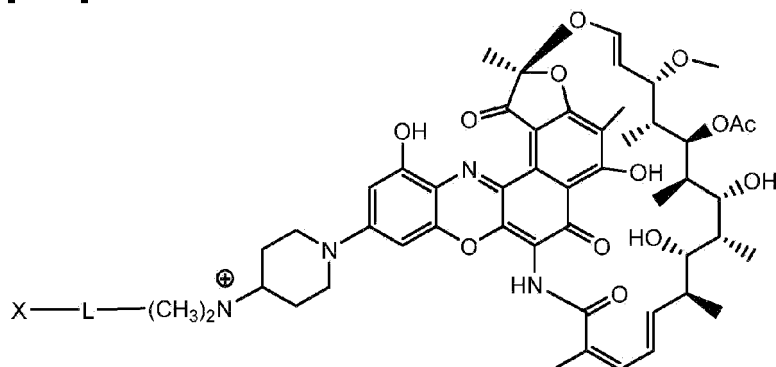
$R^3$  is independently selected from H and  $C_1$ - $C_{12}$  alkyl;

n is 1 or 2;

$R^4$  is selected from H, F, Cl, Br, I,  $C_1$ - $C_{12}$  alkyl, and OH; and

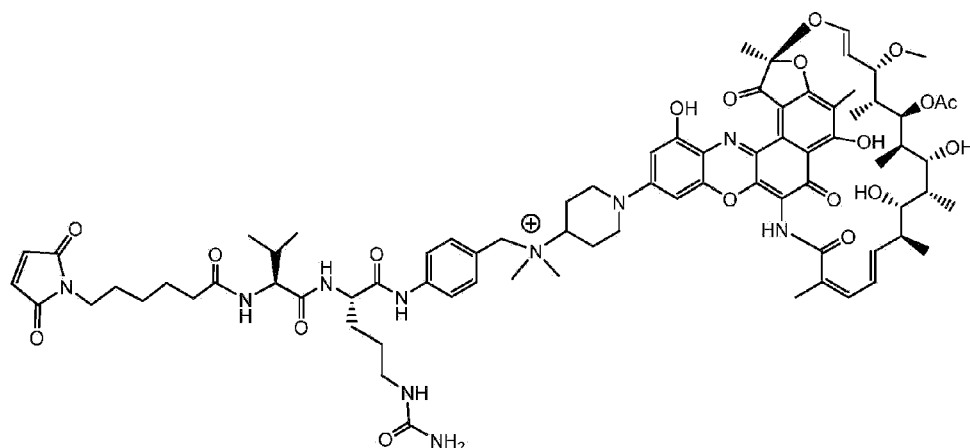
Z is selected from NH,  $N(C_1$ - $C_{12}$  alkyl), O and S.

[0065] The the linker-antibiotic intermediate of Formula II may have the formula:





or



[0066] Also provided is a method of killing intracellular *Staph aureus* in the host cells of a *Staph aureus* infected patient without killing the host cells comprising administering an anti-WTA-antibiotic conjugate detailed herein.

[0067] It is to be understood that one, some, or all of the properties of the various embodiments described herein may be combined to form other embodiments of the present invention. These and other aspects of the invention will become apparent to one of skill in the art.

## BRIEF DESCRIPTION OF THE DRAWINGS

[0068]

Figure 1 shows that exposure to vancomycin or rifampicin kills MRSA gradually. Vancomycin was tested at 2  $\mu\text{g/mL}$  (open square) and 20  $\mu\text{g/mL}$  (closed square). Rifampicin was tested at 0.02  $\mu\text{g/mL}$  (open triangle) and 0.2  $\mu\text{g/mL}$  (closed triangle).

Figure 2 shows infected peritoneal cells were able to transfer infection to osteoblasts in the presence of vancomycin.

Figure 3 shows the cell wall of Gram-positive bacteria, such as *S. aureus* with a cartoon representation of wall teichoic acids (WTA), Lipo teichoic acid (LTA) and the Peptidoglycan (PGN) sheaths that stabilize the cell membrane and provide attachment sites.

Figure 4 shows the chemical structure and glycosyl modifications of Wall Teichoic Acid (WTA), described in detail under Definitions.

Figure 5 shows a possible mechanism of drug activation for antibody-antibiotic conjugates (AAC). Active antibiotic (Abx) is released after internalization of the AAC inside mammalian cells.

Figures 6A and 6B summarize the characteristics of the Abs from the primary screening of a library of mAbs showing positive ELISA binding to cell wall preparations from USA300 or Wood46 strain *S. aureus* strains, as described in Example 21. Of the Abs that bind to WTA, 4 are specific to WTA alpha and 13 bind specifically to WTA beta.

Figure 7A shows an *in vitro* macrophage assay demonstrating that AAC kill intracellular MRSA.

Figure 7B shows intracellular killing of MRSA (USA300 strain) with 50 µg/mL of the thio-S4497-HC-A118C-pipBOR **102** in macrophages, osteoblasts (MG63), Airway epithelial cells (A549), and human umbilical vein endothelial cells (HUVEC) compared to naked, unconjugated anti-WTA antibody S4497. The dashed line indicates the limit of detection for the assay.

Figure 7C shows comparison of AAC made with linker-antibiotic intermediates LA-**51** and LA-**54** (Table 2). MRSA was opsonized with S4497 antibody alone or with AAC: AAC-**102** or AAC-**105** (Table 3) at various concentrations ranging from 10 µg/mL to .003 µg/mL.

Figure 7D shows AAC kills intracellular bacteria without harming the macrophages.

Figure 7E shows recovery of live USA300 from inside macrophages from the macrophage cell lysis above. Few (10,000 fold fewer) live *S. aureus* were recovered from macrophages infected with S-4497-AAC opsonized bacteria compared to naked antibody treated controls.

Figure 8A shows *in vivo* efficacy of thio-S4497-HC-A118C-MC-vc-PAB-pipBOR **102** AAC in an intraperitoneal infection model in A/J mice. Mice were infected with MRSA by intraperitoneal injection and treated with 50 mg/Kg of S4497 antibody alone or with 50 mg/Kg of **102** AAC (HC-A114C Kabat = HC-A118C EU) by intraperitoneal injection. Mice were sacrificed 2 days post infection and the total bacterial load was assessed in the peritoneal supernatant (Extracellular bacteria), peritoneal cells (Intracellular bacteria) or in the kidney.

Figure 8B shows intravenous, *in vivo*, infection model in A/J mice. Mice were infected with MRSA by intravenous injection and treated with 50 mg/Kg of S4497 antibody, 50 mg/Kg of thio-S4497-HC-A118C-MC-vc-PAB-pipBOR **102** AAC or a simple mixture of 50 mg/Kg of S4497 antibody + .5 mg/Kg of free rifamycin. The grey dashed line indicates the limit of detection for each organ.

Figure 9A shows efficacy of thio-S4497-HC-A118C-MC-vc-PAB-pipBOR **102** AAC in an intravenous infection model by titration of the S4497-pipBOR AAC.

Figure 9B shows thio-S4497-HC-A118C-MC-vc-PAB-dimethylpipBOR **105** AAC is more efficacious than thio-S4497-HC-A118C-MC-vc-PAB-pipBOR **102** AAC in an intravenous infection model by titration. Treatments with S4497 Antibody, **102** AAC or thio-S4497-HC-A118C-MC-vc-PAB-dimethylpipBOR **112** AAC were administered at the indicated doses 30 minutes after infection. Mice were sacrificed 4 days after infection and the total number of surviving bacteria per mouse (2 kidneys pooled) was determined by plating.

Figure 9C shows that thio-S4497-HC-A118C-MC-vc-PAB-dimethylpipBOR **105** AAC is more efficacious than S4497 Antibody or dimethylpipBOR 7 antibiotic alone in an intravenous

infection model. CB17.SCID mice infected with  $2 \times 10^7$  CFU of MRSA by intravenous injection. One day after infection, the mice were treated with 50 mg/Kg of S4497 antibody, 50 mg/Kg of AAC **105** or with 0.5 mg/Kg of dimethyl-pipBOR **7**, the equivalent dose of antibiotic that is contained in 50 mg/Kg of AAC). Mice were sacrificed 4 days after infection and the total number of surviving bacteria per mouse (2 kidneys pooled) was determined by plating.

Figure 10A shows the prevalence of anti-*S. aureus* antibodies in human serum. *S. aureus* infected patients or normal controls contain high amounts of WTA specific serum antibody with same specificity as anti-WTA S4497. Binding of various wild-type (WT) serum samples to MRSA that expressed the S4497 antigen was examined versus binding to a MRSA strain TarM/TarS DKO (double knockout) mutant which lacks the sugar modifications that are recognized by the S4497 antibody.

Figure 10B shows an AAC is efficacious in the presence of physiological levels of human IgG (10 mg/mL) in an *in vitro* macrophage assay with the USA300 strain of MRSA. The thio-S4497-HC-A118C-MC-vc-PAB-dimethylpipBOR **105** is efficacious in the presence of 10 mg/mL of human IgG. The USA300 strain of MRSA was opsonized with AAC alone, or with AAC diluted in 10 mg/mL of human IgG. The total number of surviving intracellular bacteria was assessed 2 days post infection.

Figure 10C shows an *in vivo* infection model demonstrating that AAC is efficacious in the presence of physiological levels of human IgG. The combined data are from 3 independent experiments using two separate preparations of thio-S4497-HC-A118C-MC-vc-PAB-dimethylpipBOR **105** or **112** AAC. Mice treated with the AAC had a greater than 4-log reduction in bacterial loads (Students t-test  $p = .0005$ ).

Figure 11A shows *in vivo* infection model demonstrating that AAC are more efficacious than the current standard of care (SOC) antibiotic vancomycin in mice that are reconstituted with normal levels of human IgG. Mice were treated with S4497 antibody (50 mg/Kg), vancomycin (100 mg/Kg), thio-S4497-HC-A118C-MC-vc-PAB-dimethylpipBOR **105** AAC (50 mg/Kg), or an AAC made with an isotype control antibody that does not recognize MRSA, thio-hu-anti gD 5B5-HC-A118C-MC-vc-PAB-dimethylpipBOR **110** AAC (50 mg/Kg).

Figure 11B shows the relative binding of anti-*Staph. aureus* antibodies to USA300 strain isolated from kidneys in an *in vivo* infection model, as determined by FACS. The S4497 antibody recognizes an N-acetylglucosamine modification that is linked to wall teichoic acid (WTA) via a beta-anomeric bond on the cell wall of *S. aureus*. The S7578 antibody binds to a similar N-acetylglucosamine modification that is joined to WTA via an alpha-anomeric bond. The rF1 antibody is a positive control anti-MRSA antibody that recognizes sugar modifications found on a family of SDR-repeat containing cell wall anchored proteins. The gD antibody is a negative control human IgG<sub>1</sub> that does not recognize *S. aureus*.

Figure 11C shows *in vivo* infection model demonstrating that AAC, thio-S6078-HC A1 14C-LCWT-MC-vc-PAB-dimethylpipBOR **129** is more efficacious than naked anti-WTA antibody S4497, according to the same regimen as Figure 11A, in mice that are reconstituted with normal levels of human IgG. Mice were treated with S4497 antibody (50 mg/Kg), or thio-

S6078-HC A114C-LCWT-MC-vc-PAB-dimethylpipBOR **129** AAC (50 mg/Kg).

Figure 12 shows a growth inhibition assay demonstrating that AAC are not toxic to *S. aureus* unless the linker is cleaved by cathepsin B. A schematic cathepsin release assay (Example 20) is shown on the left. AAC is treated with cathepsin B to release free antibiotic. The total amount of antibiotic activity in the intact vs. the cathepsin B treated AAC is determined by preparing serial dilutions of the resulting reaction and determining the minimum dose of AAC that is able to inhibit the growth of *S. aureus*. The upper right plot shows the cathepsin release assay for thio-S4497-HC-A118C-MC-vc-PAB-pipBOR **102** and the lower right plot shows the cathepsin release assay for thio-S4497-HC-A118C-MC-vc-PAB-dimethylpipBOR **105**.

Figure 13A shows an amino acid sequence alignment of the light chain variable regions (VL) of four human anti-WTA alpha antibodies (SEQ ID NOS 25, 27, 29 and 31, respectively, in order of appearance). The CDR sequences CDRL1, L2 and L3 according to Kabat numbering are underlined.

Figure 13B shows an amino acid sequence alignment of the heavy chain variable regions (VH) of the four human anti-WTA alpha antibodies of Figure 13A. The CDR sequences CDR H1, H2 and H3 according to Kabat numbering are underlined (SEQ ID NOS 26, 28, 30 and 32, respectively, in order of appearance).

Figure 14 shows the CDR sequences of the L and H chains of 13 human anti-WTA beta antibodies (SEQ ID NOS 33-110).

Figures 15A-1 and 15A-2 show an alignment of the full length L chain (light chain) of anti-WTA beta Ab 6078 (unmodified) and its variants, v2, v3, v4 (SEQ ID NOS 113, 113, 115, 113, 115, 113, 115 and 115, respectively, in order of appearance). The CDR sequences CDRL1, L2 and L3 according to Kabat numbering are underlined. Boxes show the contact residues and CDR residues according to Kabat and Chothia. L chain variants that contain an engineered Cys are indicated by the C in the black box the end of the constant region (at EU residue no. 205 in this case). The variant designation, e.g., v2LC-Cys means variant 2 containing a Cys engineered into the L chain. HCLC-Cys means each of the H and L chains contain an engineered Cys. Variants 2, 3 and 4 have changes in the beginning of the H chain as shown in Figures 15B.

Figures 15B-1, 15B-2, 15B-3, 15B-4 show an alignment of the full length H chain (heavy chain) of anti-WTA beta Ab 6078 (unmodified) and its variants, v2, v3, v4 (SEQ ID NOS 114, 139-144 and 143, respectively, in order of appearance) which have changes in the beginning of the H chain. H chain variants that contain an engineered Cys are indicated by the C in the black box the end of the constant region (at EU residue no. 118 in this case).

Figures 16A-1 and 16A-2 show an alignment of the full length L chain of anti-WTA beta Ab 4497 (unmodified) and Cys engineered L chains (SEQ ID NOS 121, 123, 145 and 145, respectively, in order of appearance). The CDR sequences CDRL1, L2 and L3 according to Kabat numbering are underlined. Boxes show the contact residues and CDR residues according to Kabat and Chothia. L chain variants that contain an engineered Cys are indicated by the C in the dotted box near the end of the constant region (at EU residue no. 205 in this

case).

Figures 16B-1, 16B-2, 16B-3 show an alignment of the full length H chain of anti-WTA beta Ab 4497 (unmodified) and its v8 variant with D altered to E in CDR H3 position 96, with or without the engineered Cys (SEQ ID NOS 146-147, 157 and 147, respectively, in order of appearance). H chain variants that contain an engineered Cys are indicated by the C in the black box the end of the constant region (at EU residue no. 118 in this case).

Figures 17A-1, 17A-2, 17A-3 show an amino acid sequence alignment of the full length light chain of the thirteen human anti-WTA beta antibodies (SEQ ID NOS 113, 158-167, 121 and 168, respectively, in order of appearance). The variable region (VL) spans Kabat amino acid positions 1 to 107. The CDR sequences CDRL1, L2 and L3 according to Kabat numbering are underlined.

Figures 17B-1 to 17B-6 show an amino acid sequence alignment of the full length heavy chain of the thirteen human anti-WTA beta antibodies of Figures 17A-1, 17A-2, 17A-3 (SEQ ID NOS 114, 169-176, 133-134, 138 and 127, respectively, in order of appearance). The variable region (VH) spans Kabat amino acid positions 1-113. The CDR sequences CDR H1, H2 and H3 according to Kabat numbering are underlined. H chain Eu position 118 marked by an asterisk can be changed to Cys for drug conjugation. Residues highlighted in black can be replaced with other residues that do not affect antigen binding to avoid deamidation, aspartic acid isomerization, oxidation or N-linked glycosylation.

Figure 18A shows binding of Ab 4497 mutants to *S. aureus* cell wall as analyzed by ELISA.

Figure 18B shows a comparison of Ab 4497 and its mutants (SEQ ID NOS 177, 177, 177-178, 178-179, 179-180, 180 and 180, respectively, in order of appearance) in the highlighted amino acid positions and their relative antigen binding strength as tested by ELISA.

Figure 19 shows the results of FACS analysis of Ab 6078 WT and mutants binding to protein A deficient strain of USA300 (USA300-SPA), as described in Example 23. The mutants showed unimpaired binding to *S. aureus*.

Figure 20 shows that pre-treatment with 50 mg/kg of free antibodies is not efficacious in an intravenous infection model. Balb/c mice were given a single dose of vehicle control (PBS) or 50 mg/Kg of antibodies by intravenous injection 30 minutes prior to infection with  $2 \times 10^7$  CFU of USA300. Treatment groups included an isotype control antibody that does not bind to *S. aureus* (gD), an antibody directed against the beta modification of wall teichoic acid (4497) or an antibody directed against the alpha modification of wall teichoic acid (7578). Control mice were given twice daily treatments with 110 mg/Kg of vancomycin by intraperitoneal injection (Vanco).

Figure 21 and Figure 22 show that AACs directed against either the beta modification of wall teichoic acid or the alpha modification of wall teichoic acid are efficacious in an intravenous infection model using mice that are reconstituted with normal levels of human IgG. CB17.SCID mice were reconstituted with human IgG using a dosing regimen optimized to yield constant

levels of at least 10 mg/mL of human IgG in serum and infected with  $2 \times 10^7$  CFU of USA300 by intravenous injection. Treatment was initiated 1 day after infection with buffer only control (PBS), 60 mg/Kg of beta-WTA AAC (**136** AAC) or 60 mg/Kg of alpha-WTA AAC (**155** AAC).

Figure 23A and Figure 23B show the synthesis of linker-antibiotic intermediate **51** from 2-nitrobenzene-1,3-diol **1**.

Figure 24 shows the synthesis of linker-antibiotic intermediate, MC-vc-PAB-dimethylpipBOR **54** from TBS-protected benzoxazino rifamycin **4**.

Figure 25A and Figure 25B show the synthesis of dimethyl pipBOR **7** from (5-fluoro-2-nitro-1,3-phenylene)bis(oxy)bis(methylene)dibenzene **9**.

Figure 26 shows the structure of a FRET peptide substrate for cleavage validation, mal-K(TAMRA)GGAFAGGGK(fluorescein) (SEQ ID NO: 125) containing the most abundant residues in P1, P2, and P3 from the REPLi protease activity screen. Flanking Gly residues from the REPLi FRET peptide structure were conserved. A thiol-reactive maleimide group on the N-terminus allowed for conjugation to antibodies with reactive cysteines. Upon cleavage of the FRET peptide, the quenching effect is lost and an increase in fluorescence is observed.

Figure 27 shows the structure of thioFAB S4497-MC-GGAFAGGG-(pipBOR) ("core peptide" disclosed as SEQ ID NO: 126), a tool compound used for identifying active fractions containing the protease of interest. Mal-GGAFAGGG-DNA31 ("core peptide" disclosed as SEQ ID NO: 126) was conjugated to THIOFAB 4497. THIOFABs contain one reactive cysteine. The *S. aureus* protease cleaved the linker C-terminal to Ala, releasing Gly-Gly-Gly-(pipBOR).

Figure 28 and Figure 29 show Mal-K(tamra)GGAFAGGGK(fluorescein) (SEQ ID NO: 125) AAC are cleaved in both Wood46 (Figure 28) and USA300 (Figure 29) when conjugated to an antibody that binds *S. aureus* (thio-S4497) and not when conjugated to an antibody that does not bind to *S. aureus* (thio-trastuzumab). Fluorescence intensity measured over time of thioMAB 4497 mal-K(tamra)GGAFAGGGK(fluorescein) (SEQ ID NO: 125) incubated with log phase cultures of Wood46 and USA300 MRSA. The thioMAB 4497 FRET peptide conjugates made from mal-K(TAMRA)GGAFAGGGK(fluorescein) (SEQ ID NO: 125) of Figure 26 show an increase in fluorescence in both strains, indicating that the experimental linker is cleaved by a *S. aureus* protease and that the protease is present in the clinically relevant strain of MRSA, USA300 (Figure 29). Cell density affects the rate of cleavage, with cleavage occurring earlier in cultures of the higher cell density ( $10^8$  cells/ml). The isotope control conjugate (thio-trastuzumab) did not show an increase in fluorescence in any condition.

Figure 30 show two optimized linkers for staphopain B cleavage in AAC. Linkers optimized for cleavage by staphopain B, including residue preferences for P4 and P1'. Linkers were designed using data from the REPLi screen. QSY7 was added to the C-terminus of each linker to act as an antibiotic surrogate.

Figure 31 shows results from the Macrophage Assay, demonstrating that staphopain cleavable AAC is able to kill intracellular bacteria. The USA300 strain of *S. aureus* was incubated with

various doses (100 µg/mL, 10 µg/mL, 1 µg/mL or 0.1 µg/mL) of S4497 antibody alone, thio-S4497 HC WT (v8), LC V205C-MC-vc-PAB-(dimethylpipBOR) AAC-192 or thio-S4497 HC v1-MP-LAFG-PABC-(piperazinoBOR) ("core peptide" disclosed as SEQ ID NO: 128) AAC-193 to permit binding of the AACs to the bacteria (Figure 31). After 1 hour incubation, the opsonized bacteria were fed to murine macrophages and incubated at 37 °C for 2 hours to permit phagocytosis. After phagocytosis was complete, the infection mix was replaced with normal growth media supplemented with 50 µg/mL of gentamycin to kill any remaining extracellular bacteria and the total number of surviving intracellular bacteria was determined 2 days later by plating serial dilutions of the macrophage lysates on Tryptic Soy Agar plates. The staphopain cleavable AAC was able to kill intracellular USA300 with similar potency compared to the cathepsin B cleavable AAC. Gray dashed line indicates the limit of detection for the assay (10 CFU/well).

Figure 32 shows results from the Macrophage Assay, demonstrating that staphopain cleavable AAC is able to kill intracellular bacteria. AACs target antibiotic killing to *S. aureus* via antigen specific binding of the antibody. The Wood46 strain of *S. aureus* was chosen for this experiment because it does not express protein A, a molecule that binds to the Fc region of IgG antibodies. The Wood46 strain of *S. aureus* was incubated with 10 µg/mL or 0.5 µg/mL of S4497 antibody, Isotype control-AAC containing a cathepsin B cleavable linker thio-trastuzumab HC A118C-MC-vc-PAB-(dimethyl-pipBOR) AAC-101, thio-S4497 HC WT (v8), LC V205C-MC-vc-PAB-(dimethylpipBOR) AAC-192, Isotype control-AAC containing a staphopain cleavable linker thio-trastuzumab HC A118C-MP-LAFG-PABC-(piperazinoBOR) ("core peptide" disclosed as SEQ ID NO: 128), or thio-S4497 HC v1-MP-LAFG-PABC-(piperazinoBOR) ("core peptide" disclosed as SEQ ID NO: 128) AAC-193 for 1 hour to permit binding of the AACs to bacteria. To limit non-specific binding of the AACs, the opsonized bacteria were centrifuged, washed once and resuspended in buffer before being fed to murine macrophages. After phagocytosis was complete, the infection mix was replaced with normal growth media supplemented with 50 µg/mL of gentamycin to kill any remaining extracellular bacteria and the total number of surviving intracellular bacteria was determined 2 days later by plating serial dilutions of the macrophage lysates on Tryptic Soy Agar plates. The 4497-AAC containing a staphopain cleavable linker was able to kill all detectable intracellular bacteria, whereas the isotype control AAC showed no activity.

Figures 33 and 34 show Staphopain AAC is active in vivo in a murine intravenous infection model. CB17.SCID mice were reconstituted with human IgG using a dosing regimen optimized to yield constant levels of at least 10 mg/mL of human IgG in serum. Mice were treated with 4497 antibody (50 mg/kg), AAC-215 with staphopain cleavable linker (50 mg/kg,) or an isotype control, anti-gD AAC containing staphopain cleavable linker (50 mg/kg). Mice were given a single dose of AAC-215 on day 1 post infection by intravenous injection. Total number of surviving bacteria in 2 kidneys (Figure 33) or in heart (Figure 34) was determined by plating.

Figures 35 and 36 show results from the in vitro Macrophage Assay for thio-S6078 AAC. In Figure 35, thio-S6078.v4.HC-WT, LC-Cys-MC-vc-PAB-(dimethylpipBOR) AAC was effective at killing intracellular bacteria at doses at or above 0.5 µg/mL with an antibiotic loading of 2.0 (AAC-173) or 3.9 (AAC-171) dimethylpipBOR antibiotics (LA-54) per thio-S6078 antibody. In

Figure 36, thio-S6078.v4.HC-WT, LC-Cys-MC-vc-PAB-(piperazBOR) was effective at killing intracellular bacteria at doses at or above 0.5 µg/mL with an antibiotic loading of 1.8 (AAC-174) or 3.9 (AAC-172) piperazBOR antibiotics (LA-65) per thio-S6078 antibody.

Figures 37 and 38 show results from *in vivo* efficacy of thio-S6078 AAC in a murine intravenous infection model. CB17.SCID mice were reconstituted with human IgG using a dosing regimen optimized to yield constant levels of at least 10 mg/mL of human IgG in serum. Mice were infected with USA300 and treated with vehicle control (PBS), thio-S6078.v4.HC-WT, LC-Cys-MC-vc-PAB-(dimethylpipBOR) AAC with an antibiotic loading of 2.0 (AAC-173) or 3.9 (AAC-171) dimethylpipBOR antibiotics (LA-54) per thio-S6078 antibody (Figure 37) and thio-S6078.v4.HC-WT, LC-Cys-MC-vc-PAB-(piperazBOR) with an antibiotic loading of 1.8 (AAC-174) or 3.9 (AAC-172) piperazBOR antibiotics (LA-65) per thio-S6078 antibody (Figure 38). Mice were given a single dose of AAC on day 1 post infection by intravenous injection and sacrificed on day 4 post infection. The total number of surviving bacteria in 2 kidneys was determined by plating. Treatment with AAC containing lower antibiotic loading reduced bacterial burdens by approximately 1,000-fold and treatment with the AAC containing higher antibiotic loading reduced bacterial burdens by more than 10,000-fold.

## DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

**[0069]** Reference will now be made in detail to certain embodiments of the invention, examples of which are illustrated in the accompanying structures and formulas. While the invention will be described in conjunction with the enumerated embodiments, including methods, materials and examples, such description is non-limiting and the invention is intended to cover all alternatives, modifications, and equivalents, whether they are generally known, or incorporated herein. In the event that one or more of the incorporated literature, patents, and similar materials differs from or contradicts this application, including but not limited to defined terms, term usage, described techniques, or the like, this application controls. Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. One skilled in the art will recognize many methods and materials similar or equivalent to those described herein, which could be used in the practice of the present invention. The present invention is in no way limited to the methods and materials described.

### I. GENERAL TECHNIQUES

**[0070]** The techniques and procedures described or referenced herein are generally well understood and commonly employed using conventional methodology by those skilled in the art, such as, for example, the widely utilized methodologies described in Sambrook et al., *Molecular Cloning: A Laboratory Manual* 3d edition (2001) Cold Spring Harbor Laboratory



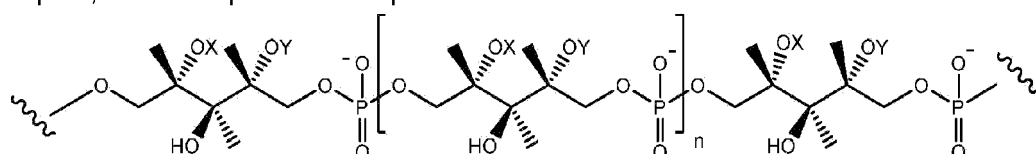
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**[0071]** The nomenclature used in this Application is based on IUPAC systematic nomenclature, unless indicated otherwise. Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs, and are consistent with: Singleton et al (1994) Dictionary of Microbiology and Molecular Biology, 2nd Ed., J. Wiley & Sons, New York, NY; and Janeway, C., Travers, P., Walport, M., Shlomchik (2001) Immunobiology, 5th Ed., Garland Publishing, New York.

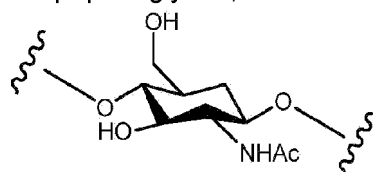
## II. DEFINITIONS

**[0072]** When indicating the number of substituents, the term "*one or more*" refers to the range from one substituent to the highest possible number of substitution, i.e. replacement of one hydrogen up to replacement of all hydrogens by substituents. The term "*substituent*" denotes an atom or a group of atoms replacing a hydrogen atom on the parent molecule. The term "*substituted*" denotes that a specified group bears one or more substituents. Where any group may carry multiple substituents and a variety of possible substituents is provided, the substituents are independently selected and need not to be the same. The term "*unsubstituted*" means that the specified group bears no substituents. The term "*optionally substituted*" means that the specified group is unsubstituted or substituted by one or more substituents, independently chosen from the group of possible substituents. When indicating the number of substituents, the term "*one or more*" means from one substituent to the highest possible number of substitution, i.e. replacement of one hydrogen up to replacement of all hydrogens by substituents.

**[0073]** The term "wall teichoic acid" (WTA) means anionic glycopolymers that are covalently attached to peptidoglycan via phosphodiester linkage to the C6 hydroxyl of the N-acetyl muramic acid sugars. While the precise chemical structure can vary among organisms, in one embodiment, WTA is a ribitol teichoic acid with repeating units of 1,5-phosphodiester linkages of D-ribitol and D-alanyl ester on position 2 and glycosyl substituents on position 4. The glycosyl groups may be N-acetylglucosaminyl  $\alpha$  (alpha) or  $\beta$  (beta) as present in *S. Aureus*. The hydroxyls on the alditol/sugar alcohol phosphate repeats are substituted with cationic D-alanine esters and monosaccharides, such as N-acetylglucosamine. In one aspect, the hydroxyl substituents include D-alanyl and alpha ( $\alpha$ ) or beta ( $\beta$ ) GlcNHAc. In one specific aspect, WTA comprises a compound of the formula:



where the wavy lines indicate repeating linkage units or the attachment sites of Polyalditol-P or the peptidoglycan, where X is D-alanyl or -H; and Y is  $\alpha$  (alpha)-GlcNHAc or  $\beta$  (beta)-GlcNHAc.



GlcNHAc

**[0074]** In *S. aureus*, WTA is covalently linked to the 6-OH of N-acetyl muramic acid (MurNAc) via a disaccharide composed of N-acetylglucosamine (GlcNAc)-1-P and N-acetylmannoseamine (ManNAc), which is followed by two or three units of glycerol-phosphates. The actual WTA polymer is then composed of 11-40 ribitol-phosphate (Rbo-P) repeating units. The step-wise synthesis of WTA is first initiated by the enzyme called TagO, and *S. aureus* strains lacking the TagO gene (by artificial deletion of the gene) do not make any WTA. The repeating units can be further tailored with D-alanine (D-Ala) at C2-OH and/or with N-acetylglucosamine (GlcNAc) at the C4-OH position via  $\alpha$ - (alpha) or  $\beta$ - (beta) glycosidic linkages. Depending of the *S. aureus* strain, or the growth phase of the bacteria the glycosidic linkages could be  $\alpha$  -,  $\beta$  -, or a mixture of the two anomers.

**[0075]** The term "antibiotic" (abx or Abx) includes any molecule that specifically inhibits the growth of or kill micro-organisms, such as bacteria, but is non-lethal to the host at the concentration and dosing interval administered. In a specific aspect, an antibiotic is nontoxic to the host at the administered concentration and dosing intervals. Antibiotics effective against bacteria can be broadly classified as either bactericidal (*i.e.*, directly kills) or bacteriostatic (*i.e.*, prevents division). Anti-bactericidal antibiotics can be further subclassified as narrow-spectrum or broad-spectrum. A broad-spectrum antibiotic is one effective against a broad range of bacteria including both Gram-positive and Gram-negative bacteria, in contrast to a narrow-spectrum antibiotic, which is effective against a smaller range or specific families of bacteria. Examples of antibiotics include: (i) aminoglycosides, *e.g.*, amikacin, gentamicin, kanamycin,

neomycin, netilmicin, streptomycin, tobramycin, paromycin, (ii) ansamycins, e.g., geldanamycin, herbimycin, (iii) carbacephems, e.g., loracarbef, (iv), carbapenems, e.g., ertapenem, doripenem, imipenem/cilastatin, meropenem, (v) cephalosporins (first generation), e.g., cefadroxil, cefazolin, cefalotin, cefalexin, (vi) cephalosporins (second generation), e.g., cefaclor, cefamandole, cefoxitin, cefprozil, cefuroxime, (vi) cephalosporins (third generation), e.g., cefixime, cefdinir, cefditoren, cefoperazone, cefotaxime, cefpodoxime, ceftazidime, ceftibuten, ceftizoxime, ceftriaxone, (vii) cephalosporins (fourth generation), e.g., cefepime, (viii), cephalosporins (fifth generation), e.g., ceftobiprole, (ix) glycopeptides, e.g., teicoplanin, vancomycin, (x) macrolides, e.g., axithromycin, clarithromycin, dirithromycin, erythromycin, roxithromycin, troleandomycin, telithromycin, spectinomycin, (xi) monobactams, e.g., axtreonam, (xii) penicilins, e.g., amoxicillin, ampicillin, axlocillin, carbenicillin, cloxacillin, dicloxacillin, flucloxacillin, mezlocillin, meticillin, nafcillin, oxacillin, penicillin, peperacillin, ticarcillin, (xiii) antibiotic polypeptides, e.g., bacitracin, colistin, polymyxin B, (xiv) quinolones, e.g., ciprofloxacin, enoxacin, gatifloxacin, levofloxacin, lemevofloxacin, moxifloxacin, norfloxacin, ofloxacin, trovafloxacin, (xv) sulfonamides, e.g., mafenide, prontosil, sulfacetamide, sulfamethizole, sulfanilamide, sulfasalazine, sulfisoxazole, trimethoprim, trimethoprim-sulfamethoxazole (TMP-SMX), (xvi) tetracyclines, e.g., demeclocycline, doxycycline, minocycline, oxytetracycline, tetracycline and (xvii) others such as arspenamine, chloramphenicol, clindamycin, lincomycin, ethambutol, fosfomycin, fusidic acid, furazolidone, isoniazid, linezolid, metronidazole, mupirocin, nitrofurantoin, platensimycin, pyrazinamide, quinupristin/dalfopristin, rifampin/rifampicin or tinidazole.

**[0076]** As used herein, the term "WTA antibody" refers to any antibody that binds WTA whether WTA alpha or WTA beta. The terms "anti-wall teichoic acid alpha antibody" or "anti-WTA alpha antibody" or "anti-aWTA" or "anti-aGlcNac WTA antibody" are used interchangeably to refer to an antibody that specifically binds wall teichoic acid (WTA) alpha. Similarly, the terms "anti-wall teichoic acid beta antibody" or "anti-WTA beta antibody" or "anti-βWTA" or "anti-βGlcNac WTA antibody" are used interchangeably to refer to an antibody that specifically binds wall teichoic acid (WTA) beta. The terms "anti-Staph antibody" and "an antibody that binds to Staph" refer to an antibody that is capable of binding an antigen on *Staphylococcus aureus* ("Staph" or "S. aureus") with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting Staph. In one embodiment, the extent of binding of an anti-Staph antibody to an unrelated, non-Staph protein is less than about 10% of the binding of the antibody to MRSA as measured, e.g., by a radioimmunoassay (RIA). In certain embodiments, an antibody that binds to Staph has a dissociation constant (Kd) of  $\leq 1\mu\text{M}$ ,  $\leq 100\text{ nM}$ ,  $\leq 10\text{ nM}$ ,  $\leq 5\text{ nM}$ ,  $\leq 4\text{ nM}$ ,  $\leq 3\text{ nM}$ ,  $\leq 2\text{ nM}$ ,  $\leq 1\text{ nM}$ ,  $\leq 0.1\text{ nM}$ ,  $\leq 0.01\text{ nM}$ , or  $\leq 0.001\text{ nM}$  (e.g.,  $10^{-8}\text{ M}$  or less, e.g. from  $10^{-8}\text{ M}$  to  $10^{-13}\text{ M}$ , e.g., from  $10^{-9}\text{ M}$  to  $10^{-13}\text{ M}$ ). In certain embodiments, an anti-Staph antibody binds to an epitope of Staph that is conserved among Staph from different species.

**[0077]** The term "methicillin-resistant *Staphylococcus aureus* " (MRSA), alternatively known as multidrug resistant *Staphylococcus aureus* or oxacillin-resistant *Staphylococcus aureus* (ORSA), refers to any strain of *Staphylococcus aureus* that is resistant to beta-lactam antibiotics, which include the penicillins (e.g., methicillin, dicloxacillin, nafcillin, oxacillin, etc.)

and the cephalosporins. "Methicillin-sensitive *Staphylococcus aureus*" (MSSA) refers to any strain of *Staphylococcus aureus* that is sensitive to beta-lactam antibiotics.

**[0078]** The term "minimum inhibitory concentration" ("MIC") refers to the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. Assays for determining MIC are known. One method is as described in Example 18 below.

**[0079]** The term "antibody" herein is used in the broadest sense and specifically covers monoclonal antibodies, polyclonal antibodies, dimers, multimers, multispecific antibodies (e.g., bispecific antibodies), and antigen binding antibody fragments thereof, (Miller et al (2003) J. of Immunology 170:4854-4861). Antibodies may be murine, human, humanized, chimeric, or derived from other species. An antibody is a protein generated by the immune system that is capable of recognizing and binding to a specific antigen (Janeway, C., Travers, P., Walport, M., Shlomchik (2001) Immunology, 5th Ed., Garland Publishing, New York). A target antigen generally has numerous binding sites, also called epitopes, recognized by CDRs on multiple antibodies. Each antibody that specifically binds to a different epitope has a different structure. Thus, one antigen may be recognized and bound by more than one corresponding antibody. An antibody includes a full-length immunoglobulin molecule or an immunologically active portion of a full-length immunoglobulin molecule, i.e., a molecule that contains an antigen binding site that immunospecifically binds an antigen of a target of interest or part thereof, such targets including but not limited to, cancer cell or cells that produce autoimmune antibodies associated with an autoimmune disease, an infected cell or a microorganism such as a bacterium. The immunoglobulin (Ig) disclosed herein can be of any isotype except IgM (e.g., IgG, IgE, IgD, and IgA) and subclass (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2). The immunoglobulins can be derived from any species. In one aspect, the Ig is of human, murine, or rabbit origin. In a specific embodiment, the Ig is of human origin.

**[0080]** The "class" of an antibody refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called  $\alpha$ ,  $\delta$ ,  $\epsilon$ ,  $\gamma$ , and  $\mu$ , respectively.

**[0081]** "Native antibodies" refer to naturally occurring immunoglobulin molecules with varying structures. For example, native IgG antibodies are heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light chains and two identical heavy chains that are disulfide-bonded. From N- to C-terminus, each heavy chain has a variable region (VH), also called a variable heavy domain or a heavy chain variable domain, followed by three constant domains (CH1, CH2, and CH3). Similarly, from N- to C-terminus, each light chain has a variable region (VL), also called a variable light domain or a light chain variable domain, followed by a constant light (CL) domain. The light chain of an antibody may be assigned to one of two types, called kappa ( $\kappa$ ) and lambda ( $\lambda$ ), based on the amino acid sequence of its constant domain.

**[0082]** The terms "full length antibody," "intact antibody," and "whole antibody" are used herein interchangeably to refer to an antibody having a structure substantially similar to a native antibody structure or having heavy chains that contain an Fc region as defined herein.

**[0083]** An "antigen-binding fragment" of an antibody refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, F(ab')<sub>2</sub>; diabodies; linear antibodies; single-chain antibody molecules (e.g. scFv); and multispecific antibodies formed from antibody fragments.

**[0084]** The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variant antibodies, e.g., containing naturally occurring mutations or arising during production of a monoclonal antibody preparation (e.g., natural variation in glycosylation), such variants generally being present in minor amounts. One such possible variant for IgG1 antibodies is the cleavage of the C-terminal lysine (K) of the heavy chain constant region. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. Thus, the modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by a variety of techniques, including but not limited to the hybridoma method, recombinant DNA methods, phage-display methods, and methods utilizing transgenic animals containing all or part of the human immunoglobulin loci, such methods and other exemplary methods for making monoclonal antibodies being described herein. In addition to their specificity, the monoclonal antibodies are advantageous in that they may be synthesized uncontaminated by other antibodies.

**[0085]** The term "chimeric antibody" refers to an antibody in which a portion of the heavy and/or light chain is derived from a particular source or species, while the remainder of the heavy and/or light chain is derived from a different source or species.

**[0086]** A "human antibody" is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human or a human cell or derived from a non-human source that utilizes human antibody repertoires or other human antibody-encoding sequences. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues.

**[0087]** A "humanized antibody" refers to a chimeric antibody comprising amino acid residues from non-human HVRs and amino acid residues from human FRs. In certain embodiments, a humanized antibody will comprise substantially all of at least one, and typically two, variable

domains, in which all or substantially all of the HVRs (e.g., CDRs) correspond to those of a non-human antibody, and all or substantially all of the FRs correspond to those of a human antibody. A humanized antibody optionally may comprise at least a portion of an antibody constant region derived from a human antibody. A "humanized form" of an antibody, e.g., a non-human antibody, refers to an antibody that has undergone humanization.

**[0088]** The term "variable region" or "variable domain" refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three hypervariable regions (HVRs). (See, e.g., Kindt et al. *Kuby Immunology*, 6th ed., W.H. Freeman and Co., page 91 (2007).) A single VH or VL domain may be sufficient to confer antigen-binding specificity. Furthermore, antibodies that bind a particular antigen may be isolated using a VH or VL domain from an antibody that binds the antigen to screen a library of complementary VL or VH domains, respectively. See, e.g., Portolano et al., *J. Immunol.* 150:880-887 (1993); Clarkson et al., *Nature* 352:624-628 (1991).

**[0089]** The term "hypervariable region," "HVR," or "HV," when used herein refers to the regions of an antibody variable domain which are hypervariable in sequence ("complementarity determining regions" or "CDRs") and/or form structurally defined loops and/or contain the antigen-contacting residues ("antigen contacts"). Generally, antibodies comprise six HVRs; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). In native antibodies, H3 and L3 display the most diversity of the six HVRs, and H3 in particular is believed to play a unique role in conferring fine specificity to antibodies. See, e.g., Xu et al., *Immunity* 13:37-45 (2000); Johnson and Wu, in *Methods in Molecular Biology* 248:1-25 (Lo, ed., Human Press, Totowa, NJ, 2003). Indeed, naturally occurring camelid antibodies consisting of a heavy chain only are functional and stable in the absence of light chain (Hamers-Casterman et al., (1993) *Nature* 363:446-448; Sheriff et al., (1996) *Nature Struct. Biol.* 3:733-736).

**[0090]** A number of HVR delineations are in use and are encompassed herein. The Kabat Complementarity Determining Regions (CDRs) are based on sequence variability and are the most commonly used (Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)). Chothia refers instead to the location of the structural loops (Chothia and Lesk, (1987) *J. Mol. Biol.* 196:901-917). For antigen contacts, refer to MacCallum et al. *J. Mol. Biol.* 262: 732-745 (1996). The AbM HVRs represent a compromise between the Kabat HVRs and Chothia structural loops, and are used by Oxford Molecular's AbM antibody modeling software. The "contact" HVRs are based on an analysis of the available complex crystal structures. The residues from each of these HVRs are noted below.

Loop	Kabat	AbM	Chothia	Contact
L1	L24-L34	L24-L34	L26-L32	L30-L36
L2	L50-L56	L50-L56	L50-L52	L46-L55
L3	L89-L97	L89-L97	L91-L96	L89-L96

Loop	Kabat	AbM	Chothia	Contact
H1	H31-H35B	H26-H35B	H26-H32	H30-H35B (Kabat numbering)
H1	H31-H35	H26-H35	H26-H32	H30-H35 (Chothia numbering)
H2	H50-H65	H50-H58	H53-H55	H47-H58
H3	H95-H102	H95-H102	H96-H101	H93-H101

**[0091]** HVRs may comprise "extended HVRs" as follows: 24-36 or 24-34 (L1), 46-56 or 50-56 (L2) and 89-97 or 89-96 (L3) in the VL and 26-35 (H1), 50-65 or 49-65 (H2) and 93-102, 94-102, or 95-102 (H3) in the VH. Unless otherwise indicated, HVR residues, CDR residues and other residues in the variable domain (e.g., FR residues) are numbered herein according to Kabat et al., *supra*.

**[0092]** The expression "variable-domain residue-numbering as in Kabat" or "amino-acid-position numbering as in Kabat," and variations thereof, refers to the numbering system used for heavy-chain variable domains or light-chain variable domains of the compilation of antibodies in Kabat et al., *supra*. Using this numbering system, the actual linear amino acid sequence may contain fewer or additional amino acids corresponding to a shortening of, or insertion into, a FR or HVR of the variable domain. For example, a heavy-chain variable domain may include a single amino acid insert (residue 52a according to Kabat) after residue 52 of H2 and inserted residues (e.g. residues 82a, 82b, and 82c, etc. according to Kabat) after heavy-chain FR residue 82. The Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence.

**[0093]** "Framework" or "FR" refers to variable domain residues other than hypervariable region (HVR) residues. The FR of a variable domain generally consists of four FR domains: FR1, FR2, FR3, and FR4. Accordingly, the HVR and FR sequences generally appear in the following sequence in VH (or VL): FR1-H1(L1)-FR2-H2(L2)-FR3-H3(L3)-FR4.

**[0094]** An "acceptor human framework" for the purposes herein is a framework comprising the amino acid sequence of a light chain variable domain (VL) framework or a heavy chain variable domain (VH) framework derived from a human immunoglobulin framework or a human consensus framework, as defined below. An acceptor human framework "derived from" a human immunoglobulin framework or a human consensus framework may comprise the same amino acid sequence thereof, or it may contain amino acid sequence changes. In some embodiments, the number of amino acid changes are 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less. In some embodiments, the VL acceptor human framework is identical in sequence to the VL human immunoglobulin framework sequence or human consensus framework sequence.

**[0095]** A "human consensus framework" is a framework which represents the most commonly occurring amino acid residues in a selection of human immunoglobulin VL or VH framework

sequences. Generally, the selection of human immunoglobulin VL or VH sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a subgroup as in Kabat et al., Sequences of Proteins of Immunological Interest, Fifth Edition, NIH Publication 91-3242, Bethesda MD (1991), vols. 1-3. In one embodiment, for the VL, the subgroup is subgroup kappa I as in Kabat et al., supra. In one embodiment, for the VH, the subgroup is subgroup III as in Kabat et al., supra.

**[0096]** "Affinity" refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (e.g., an antibody) and its binding partner (e.g., an antigen). Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g., antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant ( $K_d$ ). Affinity can be measured by common methods known in the art, including those described herein.

**[0097]** An "affinity matured" antibody refers to an antibody with one or more alterations in one or more hypervariable regions (HVRs), compared to a parent antibody which does not possess such alterations, such alterations resulting in an improvement in the affinity of the antibody for antigen.

**[0098]** The term "epitope" refers to the particular site on an antigen molecule to which an antibody binds.

**[0099]** An "antibody that binds to the same epitope" as a reference antibody refers to an antibody that blocks binding of the reference antibody to its antigen in a competition assay by 50% or more, and conversely, the reference antibody blocks binding of the antibody to its antigen in a competition assay by 50% or more. An exemplary competition assay is provided herein.

**[0100]** A "naked antibody" refers to an antibody that is not conjugated to a heterologous moiety (e.g., a cytotoxic moiety) or radiolabel. The naked antibody may be present in a pharmaceutical formulation.

**[0101]** "Effector functions" refer to those biological activities attributable to the Fc region of an antibody, which vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g. B cell receptor); and B cell activation.

**[0102]** "Antibody-dependent cell-mediated cytotoxicity" or ADCC refers to a form of cytotoxicity in which secreted Ig bound onto Fc receptors (FcRs) present on certain cytotoxic cells (e.g., natural killer (NK) cells, neutrophils and macrophages) enable these cytotoxic effector cells to bind specifically to an antigen-bearing target cell and subsequently kill the target cell with cytotoxins. The antibodies "arm" the cytotoxic cells and are required for killing of the target cell



by this mechanism. The primary cells for mediating ADCC, NK cells, express Fc $\gamma$ (gamma)RIII only, whereas monocytes express Fc $\gamma$ (gamma)RI, Fc $\gamma$ (gamma)RII and Fc $\gamma$ (gamma)RIII. Fc expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.* 9: 457-92 (1991). To assess ADCC activity of a molecule of interest, an in vitro ADCC assay, such as that described in US 5,500,362 or US 5,821,337 may be performed. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and natural killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed in vivo, e.g., in an animal model such as that disclosed in Clynes et al., *PNAS USA* 95:652-656 (1998).

**[0103]** "Phagocytosis" refers to a process by which a pathogen is engulfed or internalized by a host cell (e.g., macrophage or neutrophil). Phagocytes mediate phagocytosis by three pathways: (i) direct cell surface receptors (for example, lectins, integrins and scavenger receptors) (ii) complement enhanced - using complement receptors (including CRI, receptor for C3b, CR3 and CR4) to bind and ingest complement opsonized pathogens, and (iii) antibody enhanced - using Fc Receptors (including Fc $\gamma$ gammaRI, Fc $\gamma$ gammaRIIA and Fc $\gamma$ gammaRIIIA) to bind antibody opsonized particles which then become internalized and fuse with lysosomes to become phagolysosomes. In the present invention, it is believed that pathway (iii) plays a significant role in the delivery of the anti-MRSA AAC therapeutics to infected leukocytes, e.g., neutrophils and macrophages (Phagocytosis of Microbes: complexity in Action by D. Underhill and A Ozinsky. (2002) *Annual Review of Immunology*, Vol 20:825).

**[0104]** "Complement dependent cytotoxicity" or "CDC" refers to the lysis of a target cell in the presence of complement. Activation of the classical complement pathway is initiated by the binding of the first component of the complement system (C1q) to antibodies (of the appropriate subclass) which are bound to their cognate antigen. To assess complement activation, a CDC assay, e.g., as described in Gazzano-Santoro et al., *J. Immunol. Methods* 202: 163 (1996), may be performed.

**[0105]** The term "Fc region" herein is used to define a C-terminal region of an immunoglobulin heavy chain. The term includes native-sequence Fc regions and variant Fc regions. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy-chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. The C-terminal lysine (residue 447 according to the EU numbering system - also called the EU index, as described in Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991) of the Fc region may be removed, for example, during production or purification of the antibody, or by recombinantly engineering the nucleic acid encoding a heavy chain of the antibody. Accordingly, a composition of intact antibodies may comprise antibody populations with all K447 residues removed, antibody populations with no K447 residues removed, and antibody populations having a mixture of antibodies with and without the K447 residue. The term "Fc receptor" or "FcR" also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus. Guyer et al., *J. Immunol.* 117: 587 (1976) and Kim et al., *J. Immunol.* 24: 249 (1994). Methods of measuring

binding to FcRn are known (see, e.g., Ghetie and Ward, *Immunol. Today* 18: (12): 592-8 (1997); Ghetie et al., *Nature Biotechnology* 15 (7): 637-40 (1997); Hinton et al., *J. Biol. Chem.* 279(8): 6213-6 (2004); WO 2004/92219 (Hinton et al.). Binding to FcRn in vivo and serum half-life of human FcRn high-affinity binding polypeptides can be assayed, e.g., in transgenic mice or transfected human cell lines expressing human FcRn, or in primates to which the polypeptides having a variant Fc region are administered. WO 2004/42072 (Presta) describes antibody variants which improved or diminished binding to FcRs. See also, e.g., Shields et al., *J. Biol. Chem.* 9(2):6591-6604 (2001).

**[0106]** The carbohydrate attached to the Fc region may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH2 domain of the Fc region. See, e.g., Wright et al. (1997) *TIBTECH* 15:26-32. The oligosaccharide may include various carbohydrates, e.g., mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the "stem" of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in an IgG may be made in order to create IgGs with certain additionally improved properties. For example, antibody modifications are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. Such modifications may have improved ADCC function. See, e.g. US 2003/0157108 (Presta, L.); US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Examples of publications related to "defucosylated" or "fucose-deficient" antibody modifications include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; WO2002/031140; Okazaki et al., *J. Mol. Biol.* 336: 1239-1249 (2004); Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004). Examples of cell lines capable of producing defucosylated antibodies include Lee 13 CHO cells deficient in protein fucosylation (Ripka et al. *Arch. Biochem. Biophys.* 249:533-545 (1986); US Pat. Appl. Pub. No. 2003/0157108 A1, Presta, L; and WO 2004/056312 A1, Adams et al., especially at Example 11), and knockout cell lines, such as alpha- 1,6-fucosyltransferase gene, FUT8, knockout CHO cells (see, e.g., Yamane-Ohnuki et al., *Biotech. Bioeng.* 87: 614 (2004); Kanda, Y. et al, *Biotechnol. Bioeng.*, 94(4):680-688 (2006); and WO2003/085107).

**[0107]** An "isolated antibody" is one which has been separated from a component of its natural environment. In some embodiments, an antibody is purified to greater than 95% or 99% purity as determined by, for example, electrophoretic (e.g., SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (e.g., ion exchange or reverse phase HPLC). For review of methods for assessment of antibody purity, see, e.g., Flatman et al., *J. Chromatogr. B* 848:79-87 (2007).

**[0108]** An "isolated nucleic acid" refers to a nucleic acid molecule that has been separated from a component of its natural environment. An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different

from its natural chromosomal location.

**[0109]** "Isolated nucleic acid encoding an anti-WTA beta antibody" refers to one or more nucleic acid molecules encoding antibody heavy and light chains, including such nucleic acid molecule(s) in a single vector or separate vectors, and such nucleic acid molecule(s) present at one or more locations in a host cell.

**[0110]** As use herein, the term "specifically binds to" or is "specific for" refers to measurable and reproducible interactions such as binding between a target and an antibody, which is determinative of the presence of the target in the presence of a heterogeneous population of molecules including biological molecules. For example, an antibody that specifically binds to a target (which can be an epitope) is an antibody that binds this target with greater affinity, avidity, more readily, and/or with greater duration than it binds to other targets. In one embodiment, the extent of binding of an antibody to a target unrelated to WTA-beta is less than about 10% of the binding of the antibody to the target as measured, e.g., by a radioimmunoassay (RIA). In certain embodiments, an antibody that specifically binds to WTA beta has a dissociation constant ( $K_d$ ) of  $\leq 1\mu\text{M}$ ,  $\leq 100\text{ nM}$ ,  $\leq 10\text{ nM}$ ,  $\leq 1\text{ nM}$ , or  $\leq 0.1\text{ nM}$ . In certain embodiments, an antibody specifically binds to an epitope on that is conserved from different species. In another embodiment, specific binding can include, but does not require exclusive binding.

**[0111]** "Binding affinity" generally refers to the strength of the sum total of non-covalent interactions between a single binding site of a molecule (e.g., an antibody) and its binding partner (e.g., an antigen). Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity that reflects a 1:1 interaction between members of a binding pair (e.g., antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant ( $K_d$ ). Affinity can be measured by common methods known in the art, including those described herein. Low-affinity antibodies generally bind antigen slowly and tend to dissociate readily, whereas high-affinity antibodies generally bind antigen faster and tend to remain bound longer. A variety of methods of measuring binding affinity are known in the art, any of which can be used for purposes of the present invention. Specific illustrative and exemplary embodiments for measuring binding affinity are described in the following.

**[0112]** In one embodiment, the " $K_d$ " or " $K_d$  value" according to this invention is measured by a radiolabeled antigen-binding assay (RIA) performed with the Fab version of an antibody of interest and its antigen as described by the following assay. Solution-binding affinity of Fabs for antigen is measured by equilibrating Fab with a minimal concentration of ( $^{125}\text{I}$ )-labeled antigen in the presence of a titration series of unlabeled antigen, then capturing bound antigen with an anti-Fab antibody-coated plate (see, e.g., Chen et al., (1999) J. Mol. Biol. 293:865-881). To establish conditions for the assay, microtiter plates (DYNEX Technologies, Inc.) are coated overnight with 5  $\mu\text{g/ml}$  of a capturing anti-Fab antibody (Cappel Labs) in 50 mM sodium carbonate (pH 9.6), and subsequently blocked with 2% (w/v) bovine serum albumin in PBS for two to five hours at room temperature (approximately 23°C). In a non-adsorbent plate (Nunc

#269620), 100 pM or 26 pM [ $^{125}$ I]-antigen are mixed with serial dilutions of a Fab of interest (e.g., consistent with assessment of the anti-VEGF antibody, Fab-12, in Presta et al., *Cancer Res.* 57:4593-4599 (1997)). The Fab of interest is then incubated overnight; however, the incubation may continue for a longer period (e.g., about 65 hours) to ensure that equilibrium is reached. Thereafter, the mixtures are transferred to the capture plate for incubation at room temperature (e.g., for one hour). The solution is then removed and the plate washed eight times with 0.1% TWEEN-20<sup>TM</sup> surfactant in PBS. When the plates have dried, 150  $\mu$ l/well of scintillant (MICROSCINT-20<sup>TM</sup>; Packard) is added, and the plates are counted on a TOPCOUNT<sup>TM</sup> gamma counter (Packard) for ten minutes. Concentrations of each Fab that give less than or equal to 20% of maximal binding are chosen for use in competitive binding assays.

**[0113]** According to another embodiment, the  $K_d$  is measured by using surface-plasmon resonance assays using a BIACORE<sup>®</sup>-2000 or a BIACORE<sup>®</sup>-3000 instrument (BIAcore, Inc., Piscataway, NJ) at 25°C with immobilized antigen CM5 chips at ~10 response units (RU). Briefly, carboxymethylated dextran biosensor chips (CM5, BIAcore Inc.) are activated with N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) according to the supplier's instructions. Antigen is diluted with 10 mM sodium acetate, pH 4.8, to 5  $\mu$ g/ml (~0.2  $\mu$ M) before injection at a flow rate of 5  $\mu$ l/minute to achieve approximately 10 response units (RU) of coupled protein. Following the injection of antigen, 1 M ethanolamine is injected to block unreacted groups. For kinetics measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in PBS with 0.05% TWEEN 20<sup>TM</sup> surfactant (PBST) at 25°C at a flow rate of approximately 25  $\mu$ l/min. Association rates ( $k_{on}$ ) and dissociation rates ( $k_{off}$ ) are calculated using a simple one-to-one Langmuir binding model (BIAcore<sup>®</sup> Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant ( $K_d$ ) is calculated as the ratio  $k_{off}/k_{on}$ . See, e.g., Chen et al., *J. Mol. Biol.* 293:865-881 (1999). If the on-rate exceeds  $10^6 \text{ M}^{-1} \text{ s}^{-1}$  by the surface-plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence-emission intensity (excitation = 295 nm; emission = 340 nm, 16 nm band-pass) at 25 °C of a 20 nM anti-antigen antibody (Fab form) in PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow-equipped spectrophotometer (Aviv Instruments) or a 8000-series SLM-AMINCO<sup>TM</sup> spectrophotometer (ThermoSpectronic) with a stirred cuvette.

**[0114]** An "on-rate," "rate of association," "association rate," or " $k_{on}$ " according to this invention can also be determined as described above using a BIACORE<sup>®</sup>-2000 or a BIACORE<sup>®</sup>-3000 system (BIAcore, Inc., Piscataway, NJ).

**[0115]** The terms "host cell," "host cell line," and "host cell culture" are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants" and "transformed cells," which include the primary transformed cell and progeny derived therefrom without regard to the number of

passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein.

**[0116]** The term "vector," as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as "expression vectors".

**[0117]** "Percent (%) amino acid sequence identity" with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available from Genentech, Inc., South San Francisco, California, or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, including digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

**[0118]** In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows: 100 times the fraction  $X/Y$ , where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described.

**[0119]** The term "rifamycin-type antibiotic" means the class or group of antibiotics having the

structure of, or similar structure to, rifamycin.

**[0120]** The term "rifalazil-type antibiotic" means the class or group of antibiotics having the structure of, or similar structure to, rifalazil.

**[0121]** When indicating the number of substituents, the term "one or more" refers to the range from one substituent to the highest possible number of substitution, i.e. replacement of one hydrogen up to replacement of all hydrogens by substituents. The term "substituent" denotes an atom or a group of atoms replacing a hydrogen atom on the parent molecule. The term "substituted" denotes that a specified group bears one or more substituents. Where any group may carry multiple substituents and a variety of possible substituents is provided, the substituents are independently selected and need not to be the same. The term "unsubstituted" means that the specified group bears no substituents. The term "optionally substituted" means that the specified group is unsubstituted or substituted by one or more substituents, independently chosen from the group of possible substituents. When indicating the number of substituents, the term "one or more" means from one substituent to the highest possible number of substitution, i.e. replacement of one hydrogen up to replacement of all hydrogens by substituents.

**[0122]** The term "alkyl" as used herein refers to a saturated linear or branched-chain monovalent hydrocarbon radical of one to twelve carbon atoms ( $C_1$ - $C_{12}$ ), wherein the alkyl radical may be optionally substituted independently with one or more substituents described below. In another embodiment, an alkyl radical is one to eight carbon atoms ( $C_1$ - $C_8$ ), or one to six carbon atoms ( $C_1$ - $C_6$ ). Examples of alkyl groups include, but are not limited to, methyl (Me,  $-CH_3$ ), ethyl (Et,  $-CH_2CH_3$ ), 1-propyl (n-Pr, n-propyl,  $-CH_2CH_2CH_3$ ), 2-propyl (i-Pr, i-propyl,  $-CH(CH_3)_2$ ), 1-butyl (n-Bu, n-butyl,  $-CH_2CH_2CH_2CH_3$ ), 2-methyl-1-propyl (i-Bu, i-butyl,  $-CH_2CH(CH_3)_2$ ), 2-butyl (s-Bu, s-butyl,  $-CH(CH_3)CH_2CH_3$ ), 2-methyl-2-propyl (t-Bu, t-butyl,  $-C(CH_3)_3$ ), 1-pentyl (n-pentyl,  $-CH_2CH_2CH_2CH_2CH_3$ ), 2-pentyl ( $-CH(CH_3)CH_2CH_2CH_3$ ), 3-pentyl ( $-CH(CH_2CH_3)_2$ ), 2-methyl-2-butyl ( $-C(CH_3)_2CH_2CH_3$ ), 3-methyl-2-butyl ( $-CH(CH_3)CH(CH_3)_2$ ), 3-methyl-1-butyl ( $-CH_2CH_2CH(CH_3)_2$ ), 2-methyl-1-butyl ( $-CH_2CH(CH_3)CH_2CH_3$ ), 1-hexyl ( $-CH_2CH_2CH_2CH_2CH_2CH_3$ ), 2-hexyl ( $-CH(CH_3)CH_2CH_2CH_2CH_3$ ), 3-hexyl ( $-CH(CH_2CH_3)(CH_2CH_2CH_3)$ ), 2-methyl-2-pentyl ( $-C(CH_3)_2CH_2CH_2CH_3$ ), 3-methyl-2-pentyl ( $-CH(CH_3)CH(CH_3)CH_2CH_3$ ), 4-methyl-2-pentyl ( $-CH(CH_3)CH_2CH(CH_3)_2$ ), 3-methyl-3-pentyl ( $-C(CH_3)(CH_2CH_3)_2$ ), 2-methyl-3-pentyl ( $-CH(CH_2CH_3)CH(CH_3)_2$ ), 2,3-dimethyl-2-butyl ( $-C(CH_3)_2CH(CH_3)_2$ ), 3,3-dimethyl-2-butyl ( $-CH(CH_3)C(CH_3)_3$ ), 1-heptyl, 1-octyl, and the like.

**[0123]** The term "alkylene" as used herein refers to a saturated linear or branched-chain divalent hydrocarbon radical of one to twelve carbon atoms ( $C_1$ - $C_{12}$ ), wherein the alkylene radical may be optionally substituted independently with one or more substituents described below. In another embodiment, an alkylene radical is one to eight carbon atoms ( $C_1$ - $C_8$ ), or one to six carbon atoms ( $C_1$ - $C_6$ ). Examples of alkylene groups include, but are not limited to, methylene ( $-CH_2-$ ), ethylene ( $-CH_2CH_2-$ ), propylene ( $-CH_2CH_2CH_2-$ ), and the like.

**[0124]** The term "alkenyl" refers to linear or branched-chain monovalent hydrocarbon radical of two to eight carbon atoms ( $C_2-C_8$ ) with at least one site of unsaturation, i.e., a carbon-carbon,  $sp^2$  double bond, wherein the alkenyl radical may be optionally substituted independently with one or more substituents described herein, and includes radicals having "cis" and "trans" orientations, or alternatively, "E" and "Z" orientations. Examples include, but are not limited to, ethylenyl or vinyl ( $-CH=CH_2$ ), allyl ( $-CH_2CH=CH_2$ ), and the like.

**[0125]** The term "alkenylene" refers to linear or branched-chain divalent hydrocarbon radical of two to eight carbon atoms ( $C_2-C_8$ ) with at least one site of unsaturation, i.e., a carbon-carbon,  $sp^2$  double bond, wherein the alkenylene radical may be optionally substituted independently with one or more substituents described herein, and includes radicals having "cis" and "trans" orientations, or alternatively, "E" and "Z" orientations. Examples include, but are not limited to, ethylenylene or vinylenylene ( $-CH=CH-$ ), allyl ( $-CH_2CH=CH-$ ), and the like.

**[0126]** The term "alkynyl" refers to a linear or branched monovalent hydrocarbon radical of two to eight carbon atoms ( $C_2-C_8$ ) with at least one site of unsaturation, i.e., a carbon-carbon,  $sp$  triple bond, wherein the alkynyl radical may be optionally substituted independently with one or more substituents described herein. Examples include, but are not limited to, ethynyl ( $-C\equiv CH$ ), propynyl (propargyl,  $-CH_2C\equiv CH$ ), and the like.

**[0127]** The term "alkynylene" refers to a linear or branched divalent hydrocarbon radical of two to eight carbon atoms ( $C_2-C_8$ ) with at least one site of unsaturation, i.e., a carbon-carbon,  $sp$  triple bond, wherein the alkynylene radical may be optionally substituted independently with one or more substituents described herein. Examples include, but are not limited to, ethynylene ( $-C\equiv C-$ ), propynylene (propargylene,  $-CH_2C\equiv C-$ ), and the like.

**[0128]** The terms "carbocycle", "carbocyclyl", "carbocyclic ring" and "cycloalkyl" refer to a monovalent non-aromatic, saturated or partially unsaturated ring having 3 to 12 carbon atoms ( $C_3-C_{12}$ ) as a monocyclic ring or 7 to 12 carbon atoms as a bicyclic ring. Bicyclic carbocycles having 7 to 12 atoms can be arranged, for example, as a bicyclo [4,5], [5,5], [5,6] or [6,6] system, and bicyclic carbocycles having 9 or 10 ring atoms can be arranged as a bicyclo [5,6] or [6,6] system, or as bridged systems such as bicyclo[2.2.1]heptane, bicyclo[2.2.2]octane and bicyclo[3.2.2]nonane. Spiro moieties are also included within the scope of this definition. Examples of monocyclic carbocycles include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, 1-cyclopent-1-enyl, 1-cyclopent-2-enyl, 1-cyclopent-3-enyl, cyclohexyl, 1-cyclohex-1-enyl, 1-cyclohex-2-enyl, 1-cyclohex-3-enyl, cyclohexadienyl, cycloheptyl, cyclooctyl, cyclononyl, cyclodecyl, cycloundecyl, cyclododecyl, and the like. Carbocyclyl groups are optionally substituted independently with one or more substituents described herein.

**[0129]** "Aryl" means a monovalent aromatic hydrocarbon radical of 6-20 carbon atoms ( $C_6-C_{20}$ ) derived by the removal of one hydrogen atom from a single carbon atom of a parent

aromatic ring system. Some aryl groups are represented in the exemplary structures as "Ar". Aryl includes bicyclic radicals comprising an aromatic ring fused to a saturated, partially unsaturated ring, or aromatic carbocyclic ring. Typical aryl groups include, but are not limited to, radicals derived from benzene (phenyl), substituted benzenes, naphthalene, anthracene, biphenyl, indenyl, indanyl, 1,2-dihydronaphthalene, 1,2,3,4-tetrahydronaphthyl, and the like. Aryl groups are optionally substituted independently with one or more substituents described herein.

**[0130]** "Arylene" means a divalent aromatic hydrocarbon radical of 6-20 carbon atoms ( $C_6$ - $C_{20}$ ) derived by the removal of two hydrogen atom from a two carbon atoms of a parent aromatic ring system. Some arylene groups are represented in the exemplary structures as "Ar". Arylene includes bicyclic radicals comprising an aromatic ring fused to a saturated, partially unsaturated ring, or aromatic carbocyclic ring. Typical arylene groups include, but are not limited to, radicals derived from benzene (phenylene), substituted benzenes, naphthalene, anthracene, biphenylene, indenylene, indanylene, 1,2-dihydronaphthalene, 1,2,3,4-tetrahydronaphthyl, and the like. Arylene groups are optionally substituted with one or more substituents described herein.

**[0131]** The terms "heterocycle," "heterocyclyl" and "heterocyclic ring" are used interchangeably herein and refer to a saturated or a partially unsaturated (i.e., having one or more double and/or triple bonds within the ring) carbocyclic radical of 3 to about 20 ring atoms in which at least one ring atom is a heteroatom selected from nitrogen, oxygen, phosphorus and sulfur, the remaining ring atoms being C, where one or more ring atoms is optionally substituted independently with one or more substituents described below. A heterocycle may be a monocycle having 3 to 7 ring members (2 to 6 carbon atoms and 1 to 4 heteroatoms selected from N, O, P, and S) or a bicycle having 7 to 10 ring members (4 to 9 carbon atoms and 1 to 6 heteroatoms selected from N, O, P, and S), for example: a bicyclo [4,5], [5,5], [5,6], or [6,6] system. Heterocycles are described in Paquette, Leo A.; "Principles of Modern Heterocyclic Chemistry" (W.A. Benjamin, New York, 1968), particularly Chapters 1, 3, 4, 6, 7, and 9; "The Chemistry of Heterocyclic Compounds, A series of Monographs" (John Wiley & Sons, New York, 1950 to present), in particular Volumes 13, 14, 16, 19, and 28; and J. Am. Chem. Soc. (1960) 82:5566. "Heterocyclyl" also includes radicals where heterocycle radicals are fused with a saturated, partially unsaturated ring, or aromatic carbocyclic or heterocyclic ring. Examples of heterocyclic rings include, but are not limited to, morpholin-4-yl, piperidin-1-yl, piperazinyl, piperazin-4-yl-2-one, piperazin-4-yl-3-one, pyrrolidin-1-yl, thiomorpholin-4-yl, S-dioxothiomorpholin-4-yl, azocan-1-yl, azetidin-1-yl, octahydropyrido[1,2-a]pyrazin-2-yl, [1,4]diazepan-1-yl, pyrrolidinyl, tetrahydrofuranyl, dihydrofuranyl, tetrahydrothienyl, tetrahydropyranyl, dihydropyranyl, tetrahydrothiopyranyl, piperidino, morpholino, thiomorpholino, thioxanyl, piperazinyl, homopiperazinyl, azetidiny, oxetanyl, thietanyl, homopiperidinyl, oxepanyl, thiepanyl, oxazepinyl, diazepinyl, thiazepinyl, 2-pyrrolinyl, 3-pyrrolinyl, indolinyl, 2H-pyranyl, 4H-pyranyl, dioxanyl, 1,3-dioxolanyl, pyrazolinyl, dithianyl, dithiolanyl, dihydropyranyl, dihydrothienyl, dihydrofuranyl, pyrazolidinylimidazolinyl, imidazolidinyl, 3-azabicyclo[3.1.0]hexanyl, 3-azabicyclo[4.1.0]heptanyl, azabicyclo[2.2.2]hexanyl, 3H-indolyl quinoliziny and N-pyridyl ureas. Spiro moieties are also



included within the scope of this definition. Examples of a heterocyclic group wherein 2 ring atoms are substituted with oxo (=O) moieties are pyrimidinonyl and 1,1-dioxo-thiomorpholinyl. The heterocycle groups herein are optionally substituted independently with one or more substituents described herein.

**[0132]** The term "heteroaryl" refers to a monovalent aromatic radical of 5-, 6-, or 7-membered rings, and includes fused ring systems (at least one of which is aromatic) of 5-20 atoms, containing one or more heteroatoms independently selected from nitrogen, oxygen, and sulfur. Examples of heteroaryl groups are pyridinyl (including, for example, 2-hydroxypyridinyl), imidazolyl, imidazopyridinyl, pyrimidinyl (including, for example, 4-hydroxypyrimidinyl), pyrazolyl, triazolyl, pyrazinyl, tetrazolyl, furyl, thienyl, isoxazolyl, thiazolyl, oxadiazolyl, oxazolyl, isothiazolyl, pyrrolyl, quinolinyl, isoquinolinyl, tetrahydroisoquinolinyl, indolyl, benzimidazolyl, benzofuranyl, cinnolinyl, indazolyl, indolizinyl, phthalazinyl, pyridazinyl, triazinyl, isoindolyl, pteridinyl, purinyl, oxadiazolyl, triazolyl, thiadiazolyl, thiadiazolyl, furazanyl, benzofurazanyl, benzothiophenyl, benzothiazolyl, benzoxazolyl, quinazolinyl, quinoxalinyl, naphthyridinyl, and furopyridinyl. Heteroaryl groups are optionally substituted independently with one or more substituents described herein.

**[0133]** The heterocycle or heteroaryl groups may be carbon (carbon-linked), or nitrogen (nitrogen-linked) bonded where such is possible. By way of example and not limitation, carbon bonded heterocycles or heteroaryls are bonded at position 2, 3, 4, 5, or 6 of a pyridine, position 3, 4, 5, or 6 of a pyridazine, position 2, 4, 5, or 6 of a pyrimidine, position 2, 3, 5, or 6 of a pyrazine, position 2, 3, 4, or 5 of a furan, tetrahydrofuran, thiofuran, thiophene, pyrrole or tetrahydropyrrole, position 2, 4, or 5 of an oxazole, imidazole or thiazole, position 3, 4, or 5 of an isoxazole, pyrazole, or isothiazole, position 2 or 3 of an aziridine, position 2, 3, or 4 of an azetidine, position 2, 3, 4, 5, 6, 7, or 8 of a quinoline or position 1, 3, 4, 5, 6, 7, or 8 of an isoquinoline.

**[0134]** By way of example and not limitation, nitrogen bonded heterocycles or heteroaryls are bonded at position 1 of an aziridine, azetidine, pyrrole, pyrrolidine, 2-pyrroline, 3-pyrroline, imidazole, imidazolidine, 2-imidazoline, 3-imidazoline, pyrazole, pyrazoline, 2-pyrazoline, 3-pyrazoline, piperidine, piperazine, indole, indoline, 1H-indazole, position 2 of a isoindole, or isoindoline, position 4 of a morpholine, and position 9 of a carbazole, or  $\beta$ -carboline.

**[0135]** A "metabolite" is a product produced through metabolism in the body of a specified compound or salt thereof. Metabolites of a compound may be identified using routine techniques known in the art and their activities determined using tests such as those described herein. Such products may result for example from the oxidation, reduction, hydrolysis, amidation, deamidation, esterification, deesterification, enzymatic cleavage, and the like, of the administered compound. Accordingly, the invention includes metabolites of compounds of the invention, including compounds produced by a process comprising contacting a Formula I compound of this invention with a mammal for a period of time sufficient to yield a metabolic product thereof.

**[0136]** The term "pharmaceutical formulation" refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

**[0137]** A "sterile" formulation is aseptic or free from all living microorganisms and their spores.

**[0138]** A "stable" formulation is one in which the protein therein essentially retains its physical and chemical stability and integrity upon storage. Various analytical techniques for measuring protein stability are available in the art and are reviewed in *Peptide and Protein Drug Delivery*, 247-301, Vincent Lee Ed., Marcel Dekker, Inc., New York, New York, Pubs. (1991) and Jones, A. *Adv. Drug Delivery Rev.* 10: 29-90 (1993). Stability can be measured at a selected temperature for a selected time period. For rapid screening, the formulation may be kept at 40 °C for 2 weeks to 1 month, at which time stability is measured. Where the formulation is to be stored at 2-8 °C, generally the formulation should be stable at 30 °C or 40 °C for at least 1 month and/or stable at 2-8°C for at least 2 years. Where the formulation is to be stored at 30 °C, generally the formulation should be stable for at least 2 years at 30 °C and/or stable at 40 °C for at least 6 months. For example, the extent of aggregation during storage can be used as an indicator of protein stability. Thus, a "stable" formulation may be one wherein less than about 10% and preferably less than about 5% of the protein are present as an aggregate in the formulation. In other embodiments, any increase in aggregate formation during storage of the formulation can be determined.

**[0139]** An "isotonic" formulation is one which has essentially the same osmotic pressure as human blood. Isotonic formulations will generally have an osmotic pressure from about 250 to 350 mOsm. The term "hypotonic" describes a formulation with an osmotic pressure below that of human blood. Correspondingly, the term "hypertonic" is used to describe a formulation with an osmotic pressure above that of human blood. Isotonicity can be measured using a vapor pressure or ice-freezing type osmometer, for example. The formulations of the present invention are hypertonic as a result of the addition of salt and/or buffer.

**[0140]** "Carriers" as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers that are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN®, polyethylene glycol (PEG), and PLURONICS™.

**[0141]** A "pharmaceutically acceptable carrier" refers to an ingredient in a pharmaceutical

formulation, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative. A "pharmaceutically acceptable acid" includes inorganic and organic acids which are nontoxic at the concentration and manner in which they are formulated. For example, suitable inorganic acids include hydrochloric, perchloric, hydrobromic, hydroiodic, nitric, sulfuric, sulfonic, sulfinic, sulfanilic, phosphoric, carbonic, etc. Suitable organic acids include straight and branched-chain alkyl, aromatic, cyclic, cycloaliphatic, arylaliphatic, heterocyclic, saturated, unsaturated, mono, di- and tricarboxylic, including for example, formic, acetic, 2-hydroxyacetic, trifluoroacetic, phenylacetic, trimethylacetic, t-butyl acetic, anthranilic, propanoic, 2-hydroxypropanoic, 2-oxopropanoic, propandioic, cyclopentanepropionic, cyclopentane propionic, 3-phenylpropionic, butanoic, butandioic, benzoic, 3-(4-hydroxybenzoyl)benzoic, 2-acetoxybenzoic, ascorbic, cinnamic, lauryl sulfuric, stearic, muconic, mandelic, succinic, embonic, fumaric, malic, maleic, hydroxymaleic, malonic, lactic, citric, tartaric, glycolic, glyconic, gluconic, pyruvic, glyoxalic, oxalic, mesylic, succinic, salicylic, phthalic, palmoic, palmeic, thiocyanic, methanesulphonic, ethanesulphonic, 1,2-ethanedisulfonic, 2-hydroxyethanesulfonic, benzenesulphonic, 4-chlorobenzenesulfonic, naphthalene-2-sulphonic, p-toluenesulphonic, camphorsulphonic, 4-methylbicyclo[2.2.2]-oct-2-ene-1-carboxylic, glucoheptonic, 4,4'-methylenebis-3-(hydroxy-2-ene-1-carboxylic acid), hydroxynapthoic.

**[0142]** "Pharmaceutically-acceptable bases" include inorganic and organic bases which are non-toxic at the concentration and manner in which they are formulated. For example, suitable bases include those formed from inorganic base forming metals such as lithium, sodium, potassium, magnesium, calcium, ammonium, iron, zinc, copper, manganese, aluminum, N-methylglucamine, morpholine, piperidine and organic nontoxic bases including, primary, secondary and tertiary amines, substituted amines, cyclic amines and basic ion exchange resins, [e.g.,  $N(R')_4^+$  (where  $R'$  is independently H or  $C_{1-4}$  alkyl, e.g., ammonium, Tris)], for example, isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, ethanolamine, 2-diethylaminoethanol, trimethamine, dicyclohexylamine, lysine, arginine, histidine, caffeine, procaine, hydrabamine, choline, betaine, ethylenediamine, glucosamine, methylglucamine, theobromine, purines, piperazine, piperidine, N-ethylpiperidine, polyamine resins and the like. Particularly preferred organic non-toxic bases are isopropylamine, diethylamine, ethanolamine, trimethamine, dicyclohexylamine, choline, and caffeine.

**[0143]** Additional pharmaceutically acceptable acids and bases useable with the present invention include those which are derived from the amino acids, for example, histidine, glycine, phenylalanine, aspartic acid, glutamic acid, lysine and asparagine.

**[0144]** "Pharmaceutically acceptable" buffers and salts include those derived from both acid and base addition salts of the above indicated acids and bases. Specific buffers and/ or salts include histidine, succinate and acetate.

**[0145]** A "pharmaceutically acceptable sugar" is a molecule which, when combined with a protein of interest, significantly prevents or reduces chemical and/or physical instability of the protein upon storage. When the formulation is intended to be lyophilized and then

reconstituted, "pharmaceutically acceptable sugars" may also be known as a "lyoprotectant". Exemplary sugars and their corresponding sugar alcohols include: an amino acid such as monosodium glutamate or histidine; a methylamine such as betaine; a lyotropic salt such as magnesium sulfate; a polyol such as trihydric or higher molecular weight sugar alcohols, e.g. glycerin, dextran, erythritol, glycerol, arabitol, xylitol, sorbitol, and mannitol; propylene glycol; polyethylene glycol; PLURONICS®; and combinations thereof. Additional exemplary lyoprotectants include glycerin and gelatin, and the sugars mellibiose, melezitose, raffinose, mannotriose and stachyose. Examples of reducing sugars include glucose, maltose, lactose, maltulose, iso-maltulose and lactulose. Examples of non-reducing sugars include non-reducing glycosides of polyhydroxy compounds selected from sugar alcohols and other straight chain polyalcohols. Preferred sugar alcohols are monoglycosides, especially those compounds obtained by reduction of disaccharides such as lactose, maltose, lactulose and maltulose. The glycosidic side group can be either glucosidic or galactosidic. Additional examples of sugar alcohols are glucitol, maltitol, lactitol and iso-maltulose. The preferred pharmaceutically-acceptable sugars are the non-reducing sugars trehalose or sucrose. Pharmaceutically acceptable sugars are added to the formulation in a "protecting amount" (e.g. pre-lyophilization) which means that the protein essentially retains its physical and chemical stability and integrity during storage (e.g., after reconstitution and storage).

**[0146]** The "diluent" of interest herein is one which is pharmaceutically acceptable (safe and non-toxic for administration to a human) and is useful for the preparation of a liquid formulation, such as a formulation reconstituted after lyophilization. Exemplary diluents include sterile water, bacteriostatic water for injection (BWFI), a pH buffered solution (e.g. phosphate-buffered saline), sterile saline solution, Ringer's solution or dextrose solution. In an alternative embodiment, diluents can include aqueous solutions of salts and/or buffers.

**[0147]** A "preservative" is a compound which can be added to the formulations herein to reduce bacterial activity. The addition of a preservative may, for example, facilitate the production of a multi-use (multiple-dose) formulation. Examples of potential preservatives include octadecyldimethylbenzyl ammonium chloride, hexamethonium chloride, benzalkonium chloride (a mixture of alkylbenzyldimethylammonium chlorides in which the alkyl groups are long-chain compounds), and benzethonium chloride. Other types of preservatives include aromatic alcohols such as phenol, butyl and benzyl alcohol, alkyl parabens such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol, 3-pentanol, and m-cresol. The most preferred preservative herein is benzyl alcohol.

**[0148]** An "individual" or "subject" or "patient" is a mammal. Mammals include, but are not limited to, domesticated animals (e.g., cows, sheep, cats, dogs, and horses), primates (e.g., humans and non-human primates such as monkeys), rabbits, and rodents (e.g., mice and rats). In certain embodiments, the individual or subject is a human.

**[0149]** As used herein, "treatment" (and grammatical variations thereof such as "treat" or "treating") refers to clinical intervention designed to alter the natural course of the individual, tissue or cell being treated during the course of clinical pathology. Desirable effects of

treatment include, but are not limited to, decreasing the rate of disease progression, ameliorating or palliating the disease state, and remission or improved prognosis, all measurable by one of skill in the art such as a physician. In one embodiment, treatment can mean alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, decreasing the rate of infectious disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some embodiments, antibodies of the invention are used to delay development of a disease or to slow the progression of an infectious disease.

**[0150]** As used herein, "in conjunction with" refers to administration of one treatment modality in addition to another treatment modality. As such, "in conjunction with" refers to administration of one treatment modality before, during or after administration of the other treatment modality to the individual.

**[0151]** The term "phagosome" refers to an internalized membrane-enclosed endocytic vessel of a phagocytic cell. It can be initiated by direct-, antibody- or complement- enhanced phagocytosis. The term "phagolysosome" refers to an internalized cellular vessel that has fused with one or more lysosomes.

**[0152]** Bacteria are traditionally divided into two main groups, Gram-positive (Gm+) and Gram-negative (Gm-), based upon their Gram-stain retention. Gram-positive bacteria are bounded by a single unit lipid membrane, and they generally contain a thick layer (20-80 nm) of peptidoglycan responsible for retaining the Gram-stain. Gram-positive bacteria are those that are stained dark blue or violet by Gram staining. In contrast, Gram-negative bacteria cannot retain the crystal violet stain, instead taking up the counterstain (safranin or fuchsine) and appearing red or pink. Gram-positive cell walls typically lack the outer membrane found in Gram-negative bacteria.

**[0153]** The term "bacteremia" refers to the presence of bacteria in the bloodstream which is most commonly detected through a blood culture. Bacteria can enter the bloodstream as a severe complication of infections (like pneumonia or meningitis), during surgery (especially when involving mucous membranes such as the gastrointestinal tract), or due to catheters and other foreign bodies entering the arteries or veins. Bacteremia can have several consequences. The immune response to the bacteria can cause sepsis and septic shock, which has a relatively high mortality rate. Bacteria can also use the blood to spread to other parts of the body, causing infections away from the original site of infection. Examples include endocarditis or osteomyelitis.

**[0154]** A "therapeutically effective amount" is the minimum concentration required to effect a measurable improvement of a particular disorder. A therapeutically effective amount herein may vary according to factors such as the disease state, age, sex, and weight of the patient, and the ability of the antibody to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the antibody are outweighed by the therapeutically beneficial effects. In one embodiment, a therapeutically

effective amount is an amount effective to reduce bacteremia in an *in vivo* infection. In one aspect, a "therapeutically effective amount" is at least the amount effective to reduce the bacterial load or colony forming units (CFU) isolated from a patient sample such as blood by at least one log relative to prior to drug administration. In a more specific aspect, the reduction is at least 2 logs. In another aspect, the reduction is 3, 4, 5 logs. In yet another aspect, the reduction is to below detectable levels. In another embodiment, a therapeutically effective amount is the amount of an AAC in one or more doses given over the course of the treatment period, that achieves a negative blood culture (i.e., does not grow out the bacteria that is the target of the AAC) as compared to the positive blood culture before or at the start of treatment of the infected patient.

**[0155]** A "prophylactically effective amount" refers to an amount effective, at the dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically but not necessarily, since a prophylactic dose is used in subjects prior to, at the earlier stage of disease, or even prior to exposure to conditions where the risk of infection is elevated, the prophylactically effective amount can be less than the therapeutically effective amount. In one embodiment, a prophylactically effective amount is at least an amount effective to reduce, prevent the occurrence of or spread of infection from one cell to another.

**[0156]** "Chronic" administration refers to administration of the medicament(s) in a continuous as opposed to acute mode, so as to maintain the initial therapeutic effect (activity) for an extended period of time. "Intermittent" administration is treatment that is not consecutively done without interruption, but rather is cyclic in nature.

**[0157]** The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, combination therapy, contraindications and/or warnings concerning the use of such therapeutic products.

**[0158]** The term "chiral" refers to molecules which have the property of non-superimposability of the mirror image partner, while the term "achiral" refers to molecules which are superimposable on their mirror image partner.

**[0159]** The term "stereoisomers" refers to compounds which have identical chemical constitution, but differ with regard to the arrangement of the atoms or groups in space.

**[0160]** "Diastereomer" refers to a stereoisomer with two or more centers of chirality and whose molecules are not mirror images of one another. Diastereomers have different physical properties, e.g. melting points, boiling points, spectral properties, and reactivities. Mixtures of diastereomers may separate under high resolution analytical procedures such as electrophoresis and chromatography.

**[0161]** "Enantiomers" refer to two stereoisomers of a compound which are non-superimposable mirror images of one another.

**[0162]** Stereochemical definitions and conventions used herein generally follow S. P. Parker, Ed., McGraw-Hill Dictionary of Chemical Terms (1984) McGraw-Hill Book Company, New York; and Eliel, E. and Wilen, S., Stereochemistry of Organic Compounds (1994) John Wiley & Sons, Inc., New York. Many organic compounds exist in optically active forms, i.e., they have the ability to rotate the plane of plane-polarized light. In describing an optically active compound, the prefixes D and L, or R and S, are used to denote the absolute configuration of the molecule about its chiral center(s). The prefixes d and l or (+) and (-) are employed to designate the sign of rotation of plane-polarized light by the compound, with (-) or l meaning that the compound is levorotatory. A compound prefixed with (+) or d is dextrorotatory. For a given chemical structure, these stereoisomers are identical except that they are mirror images of one another. A specific stereoisomer may also be referred to as an enantiomer, and a mixture of such isomers is often called an enantiomeric mixture. A 50:50 mixture of enantiomers is referred to as a racemic mixture or a racemate, which may occur where there has been no stereoselection or stereospecificity in a chemical reaction or process. The terms "racemic mixture" and "racemate" refer to an equimolar mixture of two enantiomeric species, devoid of optical activity.

**[0163]** The term "protecting group" refers to a substituent that is commonly employed to block or protect a particular functionality while other functional groups react on the compound. For example, an "amino-protecting group" is a substituent attached to an amino group that blocks or protects the amino functionality in the compound. Suitable amino-protecting groups include, but are not limited to, acetyl, trifluoroacetyl, t-butoxycarbonyl (BOC), benzyloxycarbonyl (CBZ) and 9-fluorenylmethylenoxycarbonyl (Fmoc). For a general description of protecting groups and their use, see T. W. Greene, Protective Groups in Organic Synthesis, John Wiley & Sons, New York, 1991, or a later edition.

**[0164]** The term "about" as used herein refers to the usual error range for the respective value readily known to the skilled person in this technical field. Reference to "about" a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter per se.

**[0165]** As used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly indicates otherwise. For example, reference to an "antibody" is a reference to from one to many antibodies, such as molar amounts, and includes equivalents thereof known to those skilled in the art, and so forth.

### III. COMPOSITIONS AND METHODS

#### ANTIBODY-ANTIBIOTIC CONJUGATES (AAC)

**[0166]** The AAC compounds of the invention include those with antibacterial activity, effective

against a number of human and veterinary Gram positive, Gram negative pathogens, including the Staphylococci. In an exemplary embodiment, the AAC compounds include a cysteine-engineered antibody conjugated, i.e. covalently attached by a linker, to a rifamycin-type antibiotic moiety. The biological activity of the rifamycin-type antibiotic moiety is modulated by conjugation to an antibody. The antibody-antibiotic conjugates (AAC) of the invention selectively deliver an effective dose of an antibacterial to an infection site whereby greater selectivity, i.e. a lower efficacious dose, may be achieved while increasing the therapeutic index ("therapeutic window").

**[0167]** The invention provides novel antibacterial therapy that aims to prevent antibiotic escape by targeting populations of bacteria that evade conventional antibiotic therapy. The novel antibacterial therapy is achieved with an Antibody Antibiotic Conjugate (AAC) in which an antibody specific for cell wall components found on *S. aureus* (including MRSA) is chemically linked to a potent antibiotic (a derivative of rifamycin). The antibiotic is joined to the antibody via a protease cleavable, peptide linker that is designed to be cleaved by cathepsin B, a lysosomal protease found in most mammalian cell types (Dubowchik et al (2002) Bioconj. Chem. 13:855-869). The AAC acts as a pro-drug in that the antibiotic is inactive (due to the large size of the antibody) until the linker is cleaved. Since a significant proportion of *S. aureus* found in a natural infection is taken up by host cells, primarily neutrophils and macrophages, at some point during the course of infection in the host, and that the time spent inside host cells provides a significant opportunity for the bacterium to evade antibiotic activity. The AACs of the invention are designed to bind to *S. aureus* and release the antibiotic inside the phagolysosome after bacteria are taken up by host cells. By this mechanism, AAC are able to concentrate the active antibiotic specifically in a location where *S. aureus* is poorly treated by conventional antibiotics. While the invention is not limited or defined by an particular mechanism of action, the AAC improve antibiotic activity via three potential mechanisms: (1) The AAC delivers antibiotic inside mammalian cells that take up the bacteria, thereby increasing the potency of antibiotics that diffuse poorly into the phagolysosomes where bacteria are sequestered. (2) AAC opsonize bacteria - thereby increasing uptake of free bacteria by phagocytic cells - and release the antibiotic locally to kill the bacteria while they are sequestered in the phagolysosome. (3) AAC improve the half-life of antibiotics *in vivo* (improved pharmacokinetics) by linking the antibiotic to an antibody. Improved pharmacokinetics of AAC enable delivery of sufficient antibiotic in regions where *S. aureus* is concentrated while limiting the overall dose of antibiotic that needs to be administered systemically. This property should permit long-term therapy with AAC to target persistent infection with minimal antibiotic side effects.

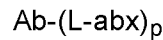
**[0168]** The present application describes the generation of novel conjugated anti-WTA antibody therapeutic agents and their use in the treatment of infections with Gram positive (Gm+) bacteria including *S. aureus* infections. These antibodies are capable of targeting populations of Gm+ bacteria that evade convention antibiotic therapy.

**[0169]** An antibody-antibiotic conjugate compound of the invention comprises an anti-wall teichoic acid beta (WTA beta) antibody covalently attached by a peptide linker to a rifamycin-



type antibiotic.

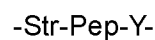
**[0170]** In one example, the antibody-antibiotic conjugate has the formula:



wherein:

Ab is the anti-wall teichoic acid antibody;

L is the peptide linker having the formula:



where Str is a stretcher unit; Pep is a peptide of two to twelve amino acid residues, and Y is a spacer unit;

abx is the rifamycin-type antibiotic; and

p is an integer from 1 to 8.

**[0171]** The number of antibiotic moieties which may be conjugated via a reactive linker moiety to an antibody molecule may be limited by the number of free cysteine residues, which are introduced by the methods described herein. Exemplary AAC of Formula I therefore comprise antibodies which have 1, 2, 3, or 4 engineered cysteine amino acids (Lyon, R. et al (2012) Methods in Enzym. 502:123-138).

## ANTI-WALL TEICHOIC (WTA) ANTIBODIES

**[0172]** Disclosed herein are certain anti-WTA Abs and conjugated anti-WTA antibodies that bind to WTA expressed on a number of Gm+ bacteria including *Staphylococcus aureus*. Anti-WTA antibodies may be selected and produced by the methods taught in US 8283294; Meijer PJ et al (2006) J Mol Biol. 358(3):764-72; Lantto J, et al (2011) J Virol. 85(4):1820-33, and in Example 21 below. The invention provides compositions of these anti-WTA Abs.

**[0173]** The cell wall of Gram-positive bacteria is comprised of thick layer of multiple peptidoglycan (PGN) sheaths that not only stabilize the cell membrane but also provide many sites to which other molecules could be attached (Figure 3). A major class of these cell surface glycoproteins are teichoic acids ("TA"), which are phosphate-rich molecules found on many glycan-binding proteins (GPB). TA come in two types: (1) lipo teichoic acid ("LTA"), which are

anchored to the plasma membrane and extend from the cell surface into the peptidoglycan layer; and (2) wall TA (WTA), which are covalently attached to peptidoglycan and extend through and beyond the cell wall (Figure 3). WTA can account for as much as 60% of the total cell wall mass in GPB. As a result, it presents a highly expressed cell surface antigen.

**[0174]** The chemical structures of WTAs vary among organisms. In *S. aureus*, WTA is covalently linked to the 6-OH of N-acetyl muramic acid (MurNAc) via a disaccharide composed of N-acetylglycosamine (GlcNAc)-1-P and N-acetylmannoseamine (ManNAc), which is followed by about two or three units of glycerol-phosphates (Figure 4) The actual WTA polymer is then composed of about 11-40 ribitol-phosphate (Rbo-P) repeating units. The step-wise synthesis of WTA is first initiated by the enzyme called TagO, and *S. aureus* strains lacking the TagO gene (by deletion of the gene) do not make any WTA. The repeating units can be further tailored with D-alanine (D-Ala) at C2-OH and/or with N-acetylglucosamine (GlcNAc) at the C4-OH position via  $\alpha$ - (alpha) or  $\beta$ - (beta) glycosidic linkages. Depending of the *S. aureus* strain, or the growth phase of the bacteria the glycosidic linkages could be  $\alpha$  -,  $\beta$  -, or a mixture of the two anomers. These GlcNAc sugar modifications are tailored by two specific *S. aureus*-derived glycosyltransferases (Gtfs): TarM Gtf mediates  $\alpha$  -glycosidic linkages, whereas TarS Gtfs mediates  $\beta$ - (beta) glycosidic linkages.

**[0175]** Given significant evidence that intracellular stores of MRSA are protected from antibiotics, the novel therapeutic compositions of the invention were developed to prevent this method of antibiotic evasion by using a *S. aureus* specific antibody to tether an antibiotic onto the bacteria such that when the bacteria is engulfed or otherwise internalized by a host cell in vivo, it brings the antibiotic along into the host cell.

**[0176]** In one aspect, the invention provides anti-WTA antibodies which are anti-WTA $\alpha$  or anti-WTA $\beta$ . In another aspect, the invention provides anti-Staph aureus Abs. The exemplary Abs were cloned from B cells from *S. aureus* infected patients (as taught in Example 21). In one embodiment the anti-WTA and anti-Staph aureus Abs are human monoclonal antibodies. The invention encompasses chimeric Abs and humanized Abs comprising the CDRs of the present WTA Abs.

**[0177]** For therapeutic use, the WTA Abs of the invention for conjugation to antibiotics to generate AACs, can be of any isotype except IgM. In one embodiment, the WTA Abs are of the human IgG isotype. In more specific embodiments, the WTA Abs are human IgG1.

**[0178]** Figures 6A and 6B lists the Abs that are anti-WTA $\alpha$  or anti-WTA  $\beta$ . Throughout the specification and figures, the Abs designated by a 4-digit number (e.g., 4497) may also be referred to with a preceding "S", e.g., S4497; both names refer to the same antibody which is the wild type (WT) unmodified sequence of the antibody. Variants of the antibody are indicated by a "v" following the antibody no., e.g., 4497.v8. Unless specified (e.g. as by a variant number), the amino acid sequences shown are the original, unmodified/unaltered sequences. These Abs can be altered at one or more residues, for example to improve the pK, stability, expression, manufacturability (e.g., as described in the Examples below), while maintaining

substantially about the same or improved binding affinity to the antigen as compared to the wild type, unmodified antibody. Variants of the present WTA antibodies having conservative amino acid substitutions are encompassed by the invention. Below, unless specified otherwise, the CDR numbering is according to Kabat and the Constant domain numbering is according to EU numbering.

**[0179]** Figure 13A and Figure 13B provide the amino acid sequence alignment of the Light chain Variable regions (VL) and the Heavy chain Variable region (VH), respectively of four human anti-WTA alpha antibodies. The CDR sequences CDR L1, L2, L3 and CDR H1, H2, H3 according to Kabat numbering are underlined.

**Tables 6A:** Light chain CDR sequences of the anti-WTA $\alpha$ .

Antibody	CDR L1	CDR L2	CDR L3
4461	KSSQSVLSRANNYYVA (SEQ ID NO.1)	WASTREF (SEQ ID NO.2)	QQYYTSRRT (SEQ ID NO.3)
4624	RSNQNLSSSNYYLA (SEQ ID NO.7)	WASTRES (SEQ ID NO.8)	QQYYANPRT (SEQ ID NO.9)
4399	KSNQNVLASSNDKNYLA (SEQ ID NO.13)	WASIRES (SEQ ID NO.14)	QQYYTNPRT (SEQ ID NO.15)
6267	KSSQNVLYSSNNKNYLA (SEQ ID NO.19)	WASTRES (SEQ ID NO.20)	QQYYTSPPYT (SEQ ID NO.21)

**Tables 6B:** Heavy chain CDR sequences of the anti-WTA $\alpha$ .

Antibody	CDR H1	CDR H2	CDR H3
4461	DYYMH (SEQ ID NO.4)	WINPKSGGTNYAQRFGG (SEQ ID NO.5)	DCGSGGLRDF (SEQ ID NO.6)
4624	DYYIH (SEQ ID NO.10)	WINPNTGGTYAQRFRD (SEQ ID NO.11)	DCGRGGLRDI (SEQ ID NO.12)
4399	DYYIH (SEQ ID NO.16)	WINPNTGGTNYAQRFGG (SEQ ID NO.17)	DCGNAGLRDI (SEQ ID NO.18)
6267	SYWIG (SEQ ID NO.22)	IIHPGDSKTRYSPSFQG (SEQ ID NO.23)	LYCSGGSCYSDR AFSSLGAGGYYY YGMGV (SEQ ID NO.24)

**[0180]** The sequences of the each pair of VL and VH are as follows:

**4461 Light Chain Variable Region**

DIQMTQSPDLSAVSLGERATINCKSSQSVLSRANNYYVAWYQHKGQPPKLLIYWASTREFGVPDRFSGSGSGT  
DFTLTINSQAEDVAVYYCQQYYTSRRTFGQGTKVEIK (SEQ ID NO. 25)

**4461 Heavy Chain Variable Region**

QVQLVQSGAEVRKPGASVKVSKASGYSFTDYYMHVVRQAPGQGLEWMGWWINPKSGGTNYAQRFGQGRVTMT

GDTISAAAYMDLASLTSDDTAVYYCVKDCGSGGLRDFWGQGTTTVTVSS (SEQ ID NO. 26)

#### 4624 Light Chain Variable Region

DIQMTQSPDSLVSLSGERATINCRSNQNLSSNNNYLAWYQQKPGQPLKLLIYWASTRESGVPDRFSGSGSGTDF  
TLTISSLQAEDVAVYYCQYYANPRTFGQGTKVEIK (SEQ ID NO. 27)

#### 4624 Heavy Chain Variable Region

QVQLQQSRVEVKRPGTSVKVSCKTSYTFSDYYIHVVRLAPGQGLELMGWINPNTGGTYAQKFRDRVMTTRDT  
SIATAYLEMSSLTSDDTAVYYCAKDCGRGGLRDIWGPGTMTVTVSS (SEQ ID NO. 28)

#### 4399 Light Chain Variable Region

EIVLTQSPDSLAVSLGERATINCKSNQNVLASSNDKNYLAWFQHKPGQPLKLLIYWASIRESGVPDRFSGSGSGTDF  
TLTISSLRAEDVAVYYCQYYTNPRTFGQGTKVEFN (SEQ ID NO. 29)

#### 4399 Heavy Chain Variable Region

EVQLVQSGAEVKKPGTSVKVSCKASGYTFTDYYIHVVRLAPGQGLELMGWINPNTGGTNYAQKFQGRVTMTRD  
TSIATAYMELSSLTSDDTAVYYCAKDCGNAGLRDIWGQGTTTVTVSS (SEQ ID NO. 30)

#### 6267 Light Chain Variable Region

DIQLTQSPDSLAVSLGERATINCKSSQNVLYSSNNKNYLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGSGTD  
FTLTISLQAEDVAVYYCQYYTSPPYTFGQGTKLEIE (SEQ ID NO. 31)

#### 6267 Heavy Chain Variable Region

EVQLVQSGAEVKKPGESLKISCKGSGSYFTSYWIGWVRQMPGKGLEWMGIIHPGDSKTRYSPSFQGGQVTISADKSI  
STAYLQWNSLKASDTAMYYCARLYCSGGSCYSDFSSLGAGGYYYYGMGVWGQGTTTVTVSS (SEQ ID NO.  
32).

**[0181]** Examples are provided of an isolated monoclonal antibody that binds wall teichoic acid (WTA) comprising a light chain and a H chain, the L chain comprising CDR L1, L2, L3 and the H chain comprising CDR H1, H2, H3 wherein the CDR L1, L2, L3 and H1, H2, H3 comprise the amino acid sequences of the CDRs of each of Abs 4461 (SEQ ID NO. 1-6), 4624 (SEQ ID NO. 7-12), 4399 (SEQ ID NO. 13-18), and 6267 (SEQ ID NO. 19-24) respectively, as shown in Table 6A and 6B.

**[0182]** In other examples, the isolated monoclonal Ab that binds WTA comprises a H chain variable region (VH) and a L chain variable region (VL), wherein the VH comprises at least 95% sequence identity over the length of the VH region sequence of the each of antibodies 4461, 4624, 4399, and 6267, respectively. In yet another specific aspect, the sequence identity is 96%, 97%, 98%, 99% or 100%.

**[0183]** Also described are anti-WTA beta Abs comprising the L and H chain CDR sequences as shown in Figure 14. In one embodiment, the isolated anti-WTA beta monoclonal Abs comprise the CDR L1, L2, L3 and H1, H2, H3 selected from the group consisting of the CDRs of each of the 13 Abs in Figure 14. In another embodiment, the invention provides an isolated anti-WTA beta Abs comprising at least 95% sequence identity over the length of the V region domains of each of 13 antibodies. In yet another specific aspect, the sequence identity is 96%, 97%, 98%, 99% or 100%.

**[0184]** Of the 13 anti-WTA beta Abs, 6078 and 4497 were modified to create variants i) having an engineered Cys in one or both L and H chains for conjugation to linker-antibiotic intermediates; and ii) wherein the first residue in the H chain Q is altered to E (v2) or the first two residues QM were changed to EI or EV (v3 and v4).

**[0185]** Figures 15A-1 and 15A-2 provide the amino acid sequence of the full length L chain of anti-WTA beta Ab 6078 (unmodified) and its variants, v2, v3, v4. L chain variants that contain an engineered Cys are indicated by the C in the black box the end of the constant region (at EU residue no. 205 in this case). The variant designation, e.g., v2LC-Cys means variant 2 containing a Cys engineered into the L chain. HCLC-Cys means both the H and L chains of the antibody contain an engineered Cys. Figures 15B-1 to 15B-4 show an alignment of the full length H chain of anti-WTA beta Ab 6078 (unmodified) and its variants, v2, v3, v4 which have changes in the first or first 2 residues of the H chain. H chain variants that contain an engineered Cys are indicated by the C in the black box the end of the constant region (at EU residue no. 118).

#### 6078 Light Chain Variable Region (VL)

DIVMTQSPSILSASVGDRVTITCRASQTISGWLAWYQQKPAEAPKLLIYKASTLESGVPSRFSGSGSGTEFTLTISLQ  
PDDFGIYYCQQYKSYSFNFGQGTKVEIK (SEQ ID NO.111)

#### 6078 Heavy Chain Variable Region (VH)

XX<sub>1</sub>QLVQSGAEVKKPGASVKVSCEASGYLTSDINWVRQATGQGPEWMGWMNANSNGNTGYAQKFQGRVTLT  
GDTISSTAYMELSSLRSEDTAVYYCARSSILVRGALGRYFDLWGRGTLVTVSS (SEQ ID NO.112)  
wherein X is Q or E; and X<sub>1</sub> is M, I or V.

#### 6078 Light Chain

DIVMTQSPSILSASVGDRVTITCRASQTISGWLAWYQQKPAEAPKLLIYKASTLESGVPSRFSGSGSGTEFTLTISLQ  
PDDFGIYYCQQYKSYSFNFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQS  
GNSQESVTEQDSKSDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO.113)

#### 6078 Cysteine-engineered Light Chain

DIVMTQSPSILSASVGDRVTITCRASQTISGWLAWYQQKPAEAPKLLIYKASTLESGVPSRFSGSGSGTEFTLTISLQ  
PDDFGIYYCQQYKSYSFNFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQS  
GNSQESVTEQDSKSDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPCTKSFNRGEC (SEQ ID NO.115)

#### 6078 WT full length Heavy Chain

QMQLVQSGAEVKKPGASVKVSCEASGYLTSDINWVRQATGQGPEWMGWMNANSNGNTGYAQKFQGRVTLT  
GDTISSTAYMELSSLRSEDTAVYYCARSSILVRGALGRYFDLWGRGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAAL  
GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKS  
CDKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ  
YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGF  
YPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPG  
(SEQ ID NO.114)

#### 6078 variant (v2, v3, or v4) full length Heavy Chain

EXQLVQSGAEVKKPGASVKVSCEASGYLTSDINWVRQATGQGPEWMGWMNANSNGNTGYAQKFQGRVTLT  
GDTISSTAYMELSSLRSEDTAVYYCARSSILVRGALGRYFDLWGRGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAAL  
GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKS  
CDKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ  
YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGF

YPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG  
(SEQ ID NO.116) wherein X can be M, I or V.

#### 6078 variant (v2, v3 or v4), Cys-engineered Heavy Chain

EXQLVQSGAEVKKPGASVKVSCEASGYTLTSYDINWVRQATGQGPEWMGWMNANSNGNTGYAQKFQGRVTLT  
GDTISSTAYMELSSLRSEDTAVYYCARSSILVRGALGRYFDLWGRGTLVTVSSCSTKGPSVFPLAPSSKSTSGGTAALG  
CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC  
DKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY  
NSTYRVVSVLTVLHQDWLNGKEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY

PSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG  
(SEQ ID NO.117)

wherein X is M, I or V.

**[0186]** Also described is an isolated anti-WTA beta antibody comprising a heavy chain and a light, wherein the heavy chain comprises a VH having at least 95% sequence identity to SEQ ID NO. 112. In an additional embodiment, this antibody further comprises a VL having at least 95% sequence identity to SEQ ID NO. 111. In a example, the anti-WTA beta antibody comprises a light chain and a heavy chain, wherein the L chain comprises a VL of SEQ ID NO. 111 and the H chain comprises a VH of SEQ ID NO. 112. In a yet more specific example, the isolated anti-WTA beta antibody comprises a L chain of SEQ ID NO. 113 and a H chain of SEQ ID NO. 114.

**[0187]** The 6078 Cys-engineered H and L chain variants can be paired in any of the following combinations to form full Abs for conjugating to linker-Abx intermediates to generate anti-WTA AACs of the invention. The unmodified L chain (SEQ ID NO.113) can be paired with a Cys-engineered H chain variant of SEQ ID NO. 117; the variant can be one wherein X is M, I or V. The Cys-engineered L chain of SEQ ID NO. 115 can be paired with: the H chain of SEQ ID NO.114; a H chain variant of SEQ ID NO.116; or a Cys-engineered H chain variant of SEQ ID NO.117 (in this version, both H and L chains are Cys engineered). In a particular example, the anti-WTA beta antibody and the anti-WTA beta AAC of the invention comprises a L chain of SEQ ID NO. 115 and H chain of SEQ ID NO.116.

**[0188]** Figures 16A-1 and 16A-2 provide the full length L chain of anti-WTA beta Ab 4497 (unmodified) and its v8 variants. L chain variants that contain an engineered Cys are indicated by the C in the black box the end of the constant region (at EU residue no. 205). Figures 16B-1, 16B-2, 16B-3 show an alignment of the full length H chain of anti-WTA beta Ab 4497 (unmodified) and its v8 variant with D altered to E in CDR H3 position 96, with or without the engineered Cys. H chain variants that contain an engineered Cys are indicated by the C in the black box the end of the constant region (at EU residue no. 118 in this case). Unmodified CDR H3 is GDGGLDD (SEQ ID NO.104); 4497v8 CDR H3 is GEGGLDD (SEQ ID NO.118).

#### 4497 Light Chain Variable Region

DIQLTQSPDLSAVSLGERATINCKSSQIFRTSRNKNLLNWWYQQRPGQPPRLLIHWASTRKSGVPDRFSGSGFGTDFTLTITSLQAEDVAIYYCQYFSPPYTFGQGTKLEIK (SEQ ID NO. 119)

**4497 Heavy Chain Variable Region**

EVQLVESGGGLVQPGGSLRLSCSASGFSFNSFWMHWRVQVPGKGLVWISFTNNEGTTTAYADSVRGRFIISRDNA  
KNTLYLEMNNLRGEDITAVYYCARGDGGLDDWGQGLTVTVSS (SEQ ID NO. 120)

**4497.v8 Heavy Chain Variable Region**

EVQLVESGGGLVQPGGSLRLSCSASGFSFNSFWMHWRVQVPGKGLVWISFTNNEGTTTAYADSVRGRFIISRDNA  
KNTLYLEMNNLRGEDITAVYYCARGEGGLDDWGQGLTVTVSS (SEQ ID NO. 156)

**4497 Light Chain**

DIQLTQSPDSLAVSLGERATINCKSSQSIFRTSRNKNLLNWWYQQRPGQPPRLIIHWASTRKSGVPDRFSGSGFGTDF  
TLTITSLQAEDVAIYYCQYFSPPYTFGQGTLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV  
DNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO.  
121)

**4497 v.8 Heavy Chain**

EVQLVESGGGLVQPGGSLRLSCSASGFSFNSFWMHWRVQVPGKGLVWISFTNNEGTTTAYADSVRGRFIISRDNA  
KNTLYLEMNNLRGEDITAVYYCARGEGGLDDWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE  
PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC  
PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV  
LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWE  
SNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO.  
122 )

**4497 -Cys Light Chain**

DIQLTQSPDSLAVSLGERATINCKSSQSIFRTSRNKNLLNWWYQQRPGQPPRLIIHWASTRKSGVPDRFSGSGFGTDF  
TLTITSLQAEDVAIYYCQYFSPPYTFGQGTLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV  
DNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO. 123)

**4497.v8- Heavy Chain**

EVQLVESGGGLVQPGGSLRLSCSASGFSFNSFWMHWRVQVPGKGLVWISFTNNEGTTTAYADSVRGRFIISRDNA  
KNTLYLEMNNLRGEDITAVYYCARGEGGLDDWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE  
PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC  
PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV  
LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWE  
SNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO.  
157; the same as SEQ ID NO.122).

**4497.v8 -Cys Heavy Chain**

EVQLVESGGGLVQPGGSLRLSCSASGFSFNSFWMHWRVQVPGKGLVWISFTNNEGTTTAYADSVRGRFIISRDNA  
KNTLYLEMNNLRGEDITAVYYCARGEGGLDDWGQGLTVTVSSCSTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE  
PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC  
PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV  
LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWES  
NGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO.  
124)

**[0189]** Another isolated anti-WTA beta antibody provided by the invention comprises a heavy chain and a light, wherein the heavy chain comprises a VH having at least 95% sequence identity to SEQ ID NO. 120. In an additional embodiment, this antibody further comprises a VL

having at least 95% sequence identity to SEQ ID NO. 119. In a specific embodiment, the anti-WTA beta antibody comprises a light chain and a heavy chain, wherein the L chain comprises a VL of SEQ ID NO. 119 and the H chain comprises a VH of SEQ ID NO. 120. In a yet more specific example, the isolated anti-WTA beta antibody comprises a L chain of SEQ ID NO. 121 and a H chain of SEQ ID NO. 122.

**[0190]** The 4497 Cys-engineered H and L chain variants can be paired in any of the following combinations to form full Abs for conjugating to linker-Abx intermediates to generate anti-WTA AACs of the invention. The unmodified L chain (SEQ ID NO.121) can be paired with a Cys-engineered H chain variant of SEQ ID NO. 124. The Cys-engineered L chain of SEQ ID NO. 123 can be paired with: the H chain variant of SEQ ID NO.157; or a Cys-engineered H chain variant of SEQ ID NO.124 (in this version, both H and L chains are Cys engineered). In a particular embodiment, the anti-WTA beta antibody and the anti-WTA beta AAC of the invention comprises a L chain of SEQ ID NO. 123.

**[0191]** Yet another example is an antibody that binds to the same epitope as each of the anti-WTA alpha Abs of Figure 13A and Figure 13B. Also provided is an antibody that binds to the same epitope as each of the anti-WTA beta Abs of Figure 14, Figures 15A and 15B, and Figures 16A and 16B.

**[0192]** Binding of anti-WTA antibodies to WTA is influenced by the anomeric orientation of GlcNAc-sugar modifications on WTA. WTA are modified by N-acetylglucosamine (GlcNAc) sugar modifications at the C4-OH position via  $\alpha$ - or  $\beta$ -glycosidic linkages, by TarM glycosyltransferase or TarS glycosyltransferase, respectively. Accordingly, cell wall preparations from glycosyltransferase mutant strains lacking TarM ( $\Delta$ TarM), TarS ( $\Delta$ TarS), or both TarM and TarS ( $\Delta$ TarM/ $\Delta$ TarS) were subjected to immunoblotting analysis with antibodies against WTA. WTA antibody (S7574) specific to  $\alpha$ -GlcNAc modifications on WTA does not bind to cell wall preparation from  $\Delta$ TarM strain. Vice versa, a WTA antibody (S4462) specific to  $\beta$ -GlcNAc modifications on WTA does not bind to cell wall preparation from  $\Delta$ TarS strain. As expected, both these antibodies do not bind to cell wall preparations from a deletion strain lacking both glycosyltransferases ( $\Delta$ TarM/ $\Delta$ TarS) and also the strain lacking any WTA ( $\Delta$ TagO). According to such analysis, antibodies have been characterized as anti-  $\alpha$ -GlcNAc WTA mAbs, or as anti-  $\beta$ -GlcNAc WTA mAbs as listed in the Table in Figures 6A and 6B.

**[0193]** Cysteine amino acids may be engineered at reactive sites in an antibody and which do not form intrachain or intermolecular disulfide linkages (Junutula, et al., 2008b Nature Biotech., 26(8):925-932; Dornan et al (2009) Blood 114(13):2721-2729; US 7521541; US 7723485; WO2009/052249, Shen et al (2012) Nature Biotech., 30(2):184-191; Junutula et al (2008) Jour of Immun. Methods 332:41-52). The engineered cysteine thiols may react with linker reagents or the linker-antibiotic intermediates of the present invention which have thiol-reactive, electrophilic groups such as maleimide or alpha-halo amides to form AAC with cysteine engineered antibodies (thioMabs) and the antibiotic (abx) moieties. The location of the antibiotic moiety can thus be designed, controlled, and known. The antibiotic loading can be controlled since the engineered cysteine thiol groups typically react with thiol-reactive linker



reagents or linker-antibiotic intermediates in high yield. Engineering an anti-WTA antibody to introduce a cysteine amino acid by substitution at a single site on the heavy or light chain gives two new cysteines on the symmetrical tetramer antibody. An antibiotic loading near 2 can be achieved and near homogeneity of the conjugation product AAC.

**[0194]** In certain embodiments, it may be desirable to create cysteine engineered anti-WTA antibodies, e.g., "thioMAbs," in which one or more residues of an antibody are substituted with cysteine residues. In particular embodiments, the substituted residues occur at accessible sites of the antibody. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible sites of the antibody and may be used to conjugate the antibody to other moieties, such as antibiotic moieties or linker-antibiotic moieties, to create an immunoconjugate, as described further herein. In certain embodiments, any one or more of the following residues may be substituted with cysteine, including V205 (Kabat numbering) of the light chain; A118 (EU numbering) of the heavy chain; and S400 (EU numbering) of the heavy chain Fc region. Nonlimiting exemplary cysteine engineered heavy chain A118C (SEQ ID NO: 149) and light chain V205C (SEQ ID NO:151) mutants of an anti-WTA antibody are shown. Cysteine engineered anti-WTA antibodies may be generated as described (Junutula, et al., 2008b Nature Biotech., 26(8):925-932; US 7521541; US-2011/0301334).

**[0195]** In another example, the invention relates to an isolated anti-WTA antibody comprising a heavy chain and a light, wherein the heavy chain comprises a wild-type heavy chain constant region sequence or cysteine-engineered mutant (ThioMab) and the light chain comprises a wild-type light chain constant region sequence or cysteine-engineered mutant (ThioMab). In one aspect, the heavy chain has at least 95% sequence identity to:

Heavy chain (IgG1) constant region, wild-type

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTQSWNSGALTSGVHTF  
PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKT  
HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFN  
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK  
ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE  
WESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA  
LHNHYTQKSLSLSPGK

(SEQ ID NO:148)

Heavy chain (IgG1) constant region, A118C "ThioMab"

CSTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTQSWNSGALTSGVHTF  
PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKT  
HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFN  
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK  
ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE  
WESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA  
LHNHYTQKSLSLSPGK

(SEQ ID NO:149)

and the light chain has at least 95% sequence identity to:

Light chain (kappa) constant region, wild-type

RTVAAPSVFIFPPSDEQLKSGTASVVCLTNNFYPREAKVQWKVDNALOSGNS

QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN  
GEC

(SEQ ID NO:150)

Light chain (kappa) constant region, V205C "ThioMab"

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS  
QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPCTKSFN  
EC

(SEQ ID NO:151)

**[0196]** The AAC of the invention include cysteine engineered anti-WTA antibodies where one or more amino acids of a wild-type or parent anti-WTA antibody are replaced with a cysteine amino acid. Any form of antibody may be so engineered, i.e. mutated. For example, a parent Fab antibody fragment may be engineered to form a cysteine engineered Fab, referred to herein as "ThioFab." Similarly, a parent monoclonal antibody may be engineered to form a "ThioMab." It should be noted that a single site mutation yields a single engineered cysteine residue in a ThioFab, while a single site mutation yields two engineered cysteine residues in a thioMab, due to the dimeric nature of the IgG antibody. Mutants with replaced ("engineered") cysteine (Cys) residues are evaluated for the reactivity of the newly introduced, engineered cysteine thiol groups.

**[0197]** The antibodies described herein may be produced using host cells in culture. Host cells may be transformed with vectors (expression or cloning vectors) comprising one or more nucleic acids encoding the antibodies described herein. The cells may be cultured under conditions suitable for producing the antibodies, and antibodies produced by the cell may be further purified. Suitable cells for producing antibodies may include prokaryotic, yeast, or higher eukaryotic (e.g., mammalian) cells. In some embodiments, a mammalian cell (a human or a non-human mammalian cell) is used. In some embodiments, a Chinese Hamster Ovary (CHO) cell is used.

**[0198]** Mammalian cells may be cultured, and propagation of mammalian cells in culture (tissue culture) has become a routine procedure. Examples of mammalian host cell lines may include, without limitation, monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham et al., J. Gen Virol. 36:59 (1977)); baby hamster kidney cells (BHK, ATCC CCL 10); mouse sertoli cells (TM4, Mather, Biol. Reprod. 23:243-251 (1980)); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather et al., Annals N.Y. Acad. Sci. 383:44-68 (1982)); MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2). Other useful mammalian host cell lines include myeloma cell lines such as NS0 and Sp2/0. For a review of certain mammalian host cell lines suitable for

antibody production, see, e.g., Yazaki and Wu, *Methods in Molecular Biology*, Vol. 248 (B. K. C. Lo, ed., Humana Press, Totowa, N.J., 2003), pp. 255-268.

**[0199]** Plant cell cultures of cotton, corn, potato, soybean, petunia, tomato, duckweed (Leninaceae), alfalfa (*M. truncatula*), and tobacco can also be utilized as hosts.

**[0200]** Suitable prokaryotic cells for this purpose include eubacteria, such as Gram-negative or Gram-positive organisms, for example, Enterobacteriaceae such as *Escherichia*, e.g., *E. coli*, *Enterobacter*, *Erwinia*, *Klebsiella*, *Proteus*, *Salmonella*, e.g., *Salmonella typhimurium*, *Serratia*, e.g., *Serratia marcescens*, and *Shigella*, as well as *Bacilli* such as *B. subtilis* and *B. licheniformis* (e.g., *B. licheniformis* 41P disclosed in DD 266,710 published 12 Apr. 1989), *Pseudomonas* such as *P. aeruginosa*, and *Streptomyces*. One preferred *E. coli* cloning host is *E. coli* 294 (ATCC 31,446), although other strains such as *E. coli* B, *E. coli* X1776 (ATCC 31,537), and *E. coli* W3110 (ATCC 27,325) are suitable. These examples are illustrative rather than limiting.

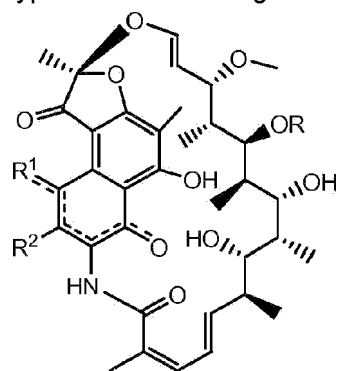
**[0201]** In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for antibody-encoding vectors. *Saccharomyces cerevisiae*, or common baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as *Schizosaccharomyces pombe*; *Kluyveromyces* hosts such as, e.g., *K. lactis*, *K. fragilis* (ATCC 12,424), *K. bulgaricus* (ATCC 16,045), *K. wickerhamii* (ATCC 24,178), *K. waltii* (ATCC 56,500), *K. drosophilum* (ATCC 36,906), *K. thermotolerans*, and *K. marxianus*; *yarrowia* (EP 402,226); *Pichia pastoris* (EP 183,070); *Candida*; *Trichoderma reesia* (EP 244,234); *Neurospora crassa*; *Schwanniomyces* such as *Schwanniomyces occidentalis*; and filamentous fungi such as, e.g., *Neurospora*, *Penicillium*, *Tolypocladium*, and *Aspergillus* hosts such as *A. nidulans* and *A. niger*.

## RIFAMYCIN-TYPE ANTIBIOTIC MOIETIES

**[0202]** The antibiotic moiety (abx) of the antibody-antibiotic conjugates (AAC) of the invention is a rifamycin-type antibiotic or group that has a cytotoxic or cytostatic effect. The rifamycins are a group of antibiotics that are obtained either naturally by the bacterium, *Nocardia mediterranei*, *Amycolatopsis mediterranei* or artificially. They are a subclass of the larger Ansamycin family which inhibit bacterial RNA polymerase (Fujii et al (1995) *Antimicrob. Agents Chemother.* 39:1489-1492; Feklistov, et al (2008) *Proc Natl Acad Sci USA*, 105(39): 14820-5) and have potency against gram-positive and selective gram-negative bacteria. Rifamycins are particularly effective against mycobacteria, and are therefore used to treat tuberculosis, leprosy, and mycobacterium avium complex (MAC) infections. The rifamycin-type group includes the "classic" rifamycin drugs as well as the rifamycin derivatives rifampicin (rifampin, CA Reg. No. 13292-46-1), rifabutin (CA Reg. No. 72559-06-9; US 2011/0178001), rifapentine and rifalazil (CA Reg. No. 129791-92-0, Rothstein et al (2003) *Expert Opin. Investig. Drugs* 12(2):255-271; Fujii et al (1994) *Antimicrob. Agents Chemother.* 38:1118-1122. Many

rifamycin-type antibiotics share the detrimental property of resistance development (Wichelhaus et al (2001) J. Antimicrob. Chemother. 47:153-156). Rifamycins were first isolated in 1957 from a fermentation culture of *Streptomyces mediterranei*. About seven rifamycins were discovered, named Rifamycin A, B, C, D, E, S, and SV (US 3150046). Rifamycin B was the first introduced commercially and was useful in treating drug-resistant tuberculosis in the 1960s. Rifamycins have been used for the treatment of many diseases, the most important one being HIV-related Tuberculosis. Due to the large number of available analogues and derivatives, rifamycins have been widely utilized in the elimination of pathogenic bacteria that have become resistant to commonly used antibiotics. For instance, Rifampicin is known for its potent effect and ability to prevent drug resistance. It rapidly kills fast-dividing bacilli strains as well as "persisters" cells, which remain biologically inactive for long periods of time that allow them to evade antibiotic activity. In addition, rifabutin and rifapentine have both been used against tuberculosis acquired in HIV-positive patients.

**[0203]** Antibiotic moieties (abx) of the Formula I antibody-antibiotic conjugates are rifamycin-type moieties having the structure:



wherein:

the dashed lines indicate an optional bond;

R is H, C<sub>1</sub>-C<sub>12</sub> alkyl, or C(O)CH<sub>3</sub>;

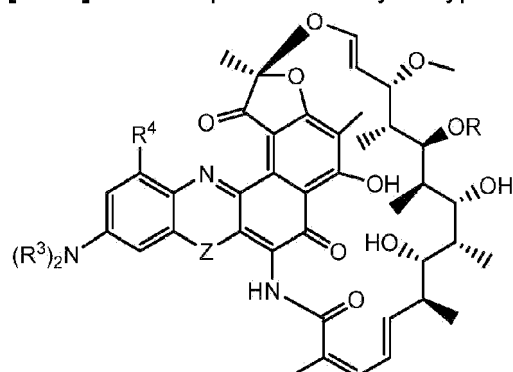
R<sup>1</sup> is OH;

R<sup>2</sup> is CH=N-(heterocyclyl), wherein the heterocyclyl is optionally substituted with one or more groups independently selected from C(O)CH<sub>3</sub>, C<sub>1</sub>-C<sub>12</sub> alkyl, C<sub>1</sub>-C<sub>12</sub> heteroaryl, C<sub>2</sub>-C<sub>20</sub> heterocyclyl, C<sub>6</sub>-C<sub>20</sub> aryl, and C<sub>3</sub>-C<sub>12</sub> carbocyclyl;

or R<sup>1</sup> and R<sup>2</sup> form a five- or six-membered fused heteroaryl or heterocyclyl, and optionally forming a spiro or fused six-membered heteroaryl, heterocyclyl, aryl, or carbocyclyl ring, wherein the spiro or fused six-membered heteroaryl, heterocyclyl, aryl, or carbocyclyl ring is optionally substituted H, F, Cl, Br, I, C<sub>1</sub>-C<sub>12</sub> alkyl, or OH; and

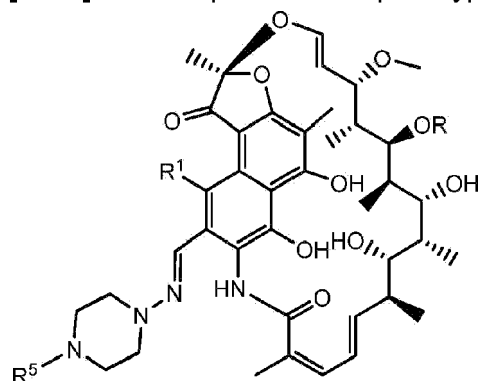
where the peptide linker L is covalently attached to R<sup>2</sup>.

[0204] An example of a rifamycin-type moiety is:



wherein  $R^3$  is independently selected from H and  $C_1$ - $C_{12}$  alkyl;  $R^4$  is selected from H, F, Cl, Br, I,  $C_1$ - $C_{12}$  alkyl, and OH; and Z is selected from NH,  $N(C_1$ - $C_{12}$  alkyl), O and S; and where the peptide linker L is covalently attached to the nitrogen atom of  $N(R^3)_2$ .

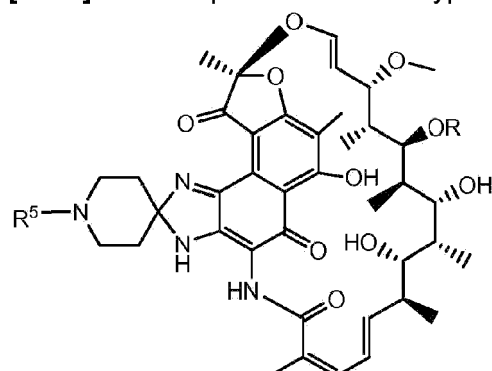
[0205] An example of a rifampicin-type moiety is:



wherein

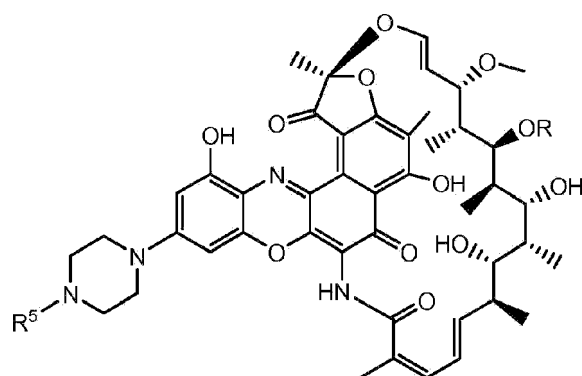
$R^5$  is selected from H and  $C_1$ - $C_{12}$  alkyl; and where the peptide linker L is covalently attached to the nitrogen atom of  $NR^5$ .

[0206] An example of a rifabutin-type moiety is:



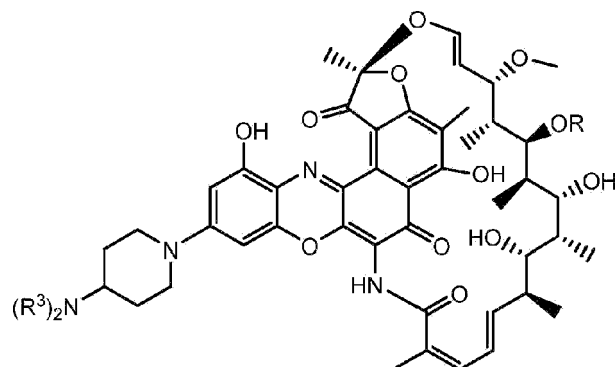
wherein  $R^5$  is selected from H and  $C_1$ - $C_{12}$  alkyl; and where the peptide linker L is covalently attached to the nitrogen atom of  $NR^5$ .

[0207] An embodiment of a benzoxazinorifamycin-type moiety is:



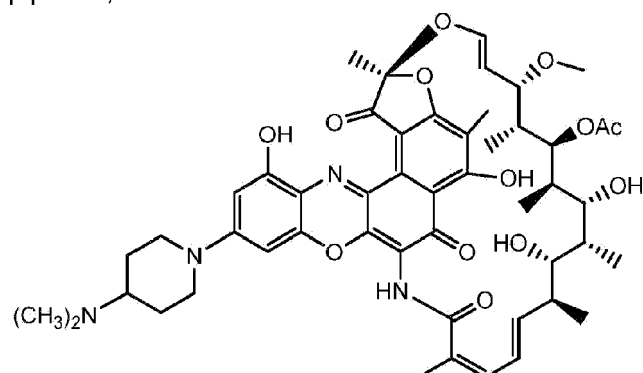
wherein  $R^5$  is selected from H and  $C_1$ - $C_{12}$  alkyl; and where the peptide linker L is covalently attached to the nitrogen atom of  $NR^5$ .

**[0208]** An embodiment of a benzoxazinorifamycin-type moiety, referred to herein as pipBOR, is:



wherein  $R^3$  is independently selected from H and  $C_1$ - $C_{12}$  alkyl; and where the peptide linker L is covalently attached to the nitrogen atom of  $N(R^3)_2$ .

**[0209]** An embodiment of a benzoxazinorifamycin-type moiety, referred to herein as dimethyl pipBOR, is:

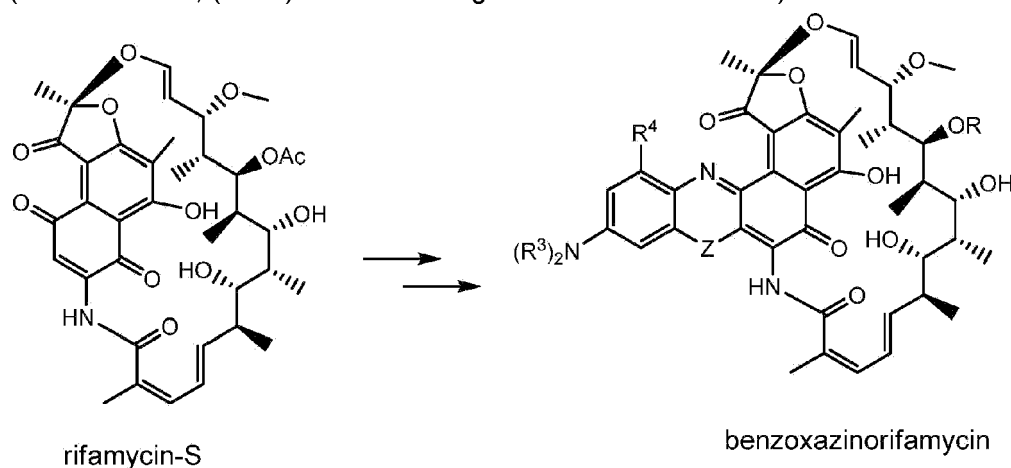


where the peptide linker L is covalently attached to the nitrogen atom of  $N(CH_3)_2$ .

**[0210]** The semi-synthetic derivative rifamycin S, or the reduced, sodium salt form rifamycin SV, can be converted to Rifalazil-type antibiotics in several steps, where R is H, or Ac,  $R^3$  is independently selected from H and  $C_1$ - $C_{12}$  alkyl;  $R^4$  is selected from H, F, Cl, Br, I,  $C_1$ - $C_{12}$  alkyl,

and OH; and Z is selected from NH, N(C<sub>1</sub>-C<sub>12</sub> alkyl), O and S, as exemplified in Figures 9-11. Benzoxazino (Z = O), benzthiazino (Z = S), benzdiazino (Z = NH, N(C<sub>1</sub>-C<sub>12</sub> alkyl)) rifamycins may be prepared (US 7271165). Benzoxazinorifamycin (BOR), benzthiazinorifamycin (BTR), and benzdiazinorifamycin (BDR) analogs that contain substituents are numbered according to the numbering scheme provided in formula A at column 28 in US 7271165. By "25-O-deacetyl" rifamycin is meant a rifamycin analog in which the acetyl group at the 25-position has been removed. Analogs in which this position is further derivatized are referred to as a "25-O-deacetyl-25-(substituent)rifamycin", in which the nomenclature for the derivatizing group replaces "substituent" in the complete compound name.

**[0211]** Rifamycin-type antibiotic moieties can be synthesized by methods analogous to those disclosed in US 4610919; US 4983602; US 5786349; US5981522; US 4859661; US 7271165; US 2011/0178001; Seligson, et al., (2001) *Anti-Cancer Drugs* 12:305-13; *Chem. Pharm. Bull.*, (1993) 41:148). Rifamycin-type antibiotic moieties can be screened for antimicrobial activity by measuring their minimum inhibitory concentration (MIC), using standard MIC in vitro assays (Tomioka et al., (1993) *Antimicrob. Agents Chemother.* 37:67).



## PEPTIDE LINKERS

**[0212]** A "peptide linker" (L) is a bifunctional or multifunctional moiety which is covalently attached to one or more antibiotic moieties (abx) and an antibody unit (Ab) to form antibody-antibiotic conjugates (AAC) of Formula I. Peptide linkers in AAC are substrates for cleavage by intracellular proteases, including lysosomal conditions. Proteases includes various cathepsins and caspases. Cleavage of the peptide linker of an AAC inside a cell may release the rifamycin-type antibiotic with anti-bacterial effects.

**[0213]** The amount of active antibiotic released from cleavage of AAC can be measured by the Caspase release assay of Example 20.

**[0214]** Antibody-antibiotic conjugates (AAC) can be conveniently prepared using a linker

reagent or linker-antibiotic intermediate having reactive functionality for binding to the antibiotic (abx) and to the antibody (Ab). In one exemplary embodiment, a cysteine thiol of a cysteine engineered antibody (Ab) can form a bond with a functional group of a linker reagent, an antibiotic moiety or antibiotic-linker intermediate.

**[0215]** The peptide linker moiety of an AAC In one aspect, a linker reagent or linker-antibiotic intermediate has a reactive site which has an electrophilic group that is reactive to a nucleophilic cysteine present on an antibody. The cysteine thiol of the antibody is reactive with an electrophilic group on a linker reagent or linker-antibiotic, forming a covalent bond. Useful electrophilic groups include, but are not limited to, maleimide and haloacetamide groups.

**[0216]** Cysteine engineered antibodies react with linker reagents or linker-antibiotic intermediates, with electrophilic functional groups such as maleimide or  $\alpha$ -halo carbonyl, according to the conjugation method at page 766 of Klussman, et al (2004), *Bioconjugate Chemistry* 15(4):765-773, and according to the protocol of Example 19.

**[0217]** In another embodiment, the reactive group of a linker reagent or linker-antibiotic intermediate contains a thiol-reactive functional group that can form a bond with a free cysteine thiol of an antibody. Examples of thiol-reaction functional groups include, but are not limited to, maleimide,  $\alpha$ -haloacetyl, activated esters such as succinimide esters, 4-nitrophenyl esters, pentafluorophenyl esters, tetrafluorophenyl esters, anhydrides, acid chlorides, sulfonyl chlorides, isocyanates and isothiocyanates.

**[0218]** In another embodiment, a linker reagent or antibiotic-linker intermediate has a reactive functional group which has a nucleophilic group that is reactive to an electrophilic group present on an antibody. Useful electrophilic groups on an antibody include, but are not limited to, pyridyl disulfide, aldehyde and ketone carbonyl groups. The heteroatom of a nucleophilic group of a linker reagent or antibiotic-linker intermediate can react with an electrophilic group on an antibody and form a covalent bond to an antibody unit. Useful nucleophilic groups on a linker reagent or antibiotic-linker intermediate include, but are not limited to, hydrazide, oxime, amino, thiol, hydrazine, thiosemicarbazone, hydrazine carboxylate, and arylhydrazide. The electrophilic group on an antibody provides a convenient site for attachment to a linker reagent or antibiotic-linker intermediate.

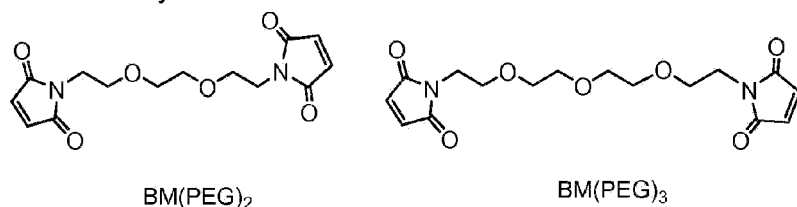
**[0219]** A peptide linker may comprise one or more linker components. Exemplary linker components include a peptide unit, 6-maleimidocaproyl ("MC"), maleimidopropanoyl ("MP"), valine-citrulline ("val-cit" or "vc"), alanine-phenylalanine ("ala-phe"), and p-aminobenzyloxycarbonyl ("PAB"), N-succinimidyl 4-(2-pyridylthio) pentanoate ("SPP"), and 4-(N-maleimidomethyl) cyclohexane-1 carboxylate ("MCC"). Various linker components are known in the art, some of which are described below.

**[0220]** In another embodiment, the linker may be substituted with groups that modulate solubility or reactivity. For example, a charged substituent such as sulfonate ( $-\text{SO}_3^-$ ) or ammonium, may increase water solubility of the reagent and facilitate the coupling reaction of



the linker reagent with the antibody or the antibiotic moiety, or facilitate the coupling reaction of Ab-L (antibody-linker intermediate) with abx, or abx-L (antibiotic-linker intermediate) with Ab, depending on the synthetic route employed to prepare the AAC.

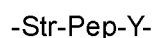
**[0221]** The AAC of the invention expressly contemplate, but are not limited to, those prepared with linker reagents: BMPEO, BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, sulfo-SMPB, SVSB (succinimidyl-(4-vinylsulfone)benzoate), and bis-maleimide reagents such as DTME, BMB, BMDB, BMH, BMOE, BM(PEG)<sub>2</sub>, and BM(PEG)<sub>3</sub>. Bis-maleimide reagents allow the attachment of the thiol group of a cysteine engineered antibody to a thiol-containing antibiotic moiety, label, or linker intermediate, in a sequential or convergent fashion. Other functional groups besides maleimide, which are reactive with a thiol group of a cysteine engineered antibody, antibiotic moiety, or linker-antibiotic intermediate include iodoacetamide, bromoacetamide, vinyl pyridine, disulfide, pyridyl disulfide, isocyanate, and isothiocyanate.



**[0222]** Useful linker reagents can also be obtained via other commercial sources, such as Molecular Biosciences Inc.(Boulder, CO), or synthesized in accordance with procedures described in Toki et al (2002) J. Org. Chem. 67:1866-1872; Dubowchik, et al. (1997) Tetrahedron Letters, 38:5257-60; Walker, M.A. (1995) J. Org. Chem. 60:5352-5355; Frisch et al (1996) Bioconjugate Chem. 7:180-186; US 6214345; WO 02/088172; US 2003130189; US2003096743; WO 03/026577; WO 03/043583; and WO 04/032828.

**[0223]** In another embodiment, the peptide linker moiety of an AAC comprises a dendritic type linker for covalent attachment of more than one antibiotic moiety through a branching, multifunctional linker moiety to an antibody (Sun et al (2002) Bioorganic & Medicinal Chemistry Letters 12:2213-2215; Sun et al (2003) Bioorganic & Medicinal Chemistry 11:1761-1768). Dendritic linkers can increase the molar ratio of antibiotic to antibody, i.e. loading, which is related to the potency of the AAC. Thus, where a cysteine engineered antibody bears only one reactive cysteine thiol group, a multitude of antibiotic moieties may be attached through a dendritic linker.

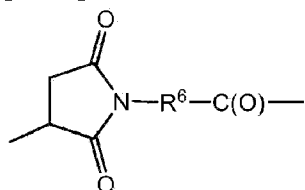
**[0224]** In certain embodiments of Formula I AAC, the peptide linker has the formula:



where Str is a stretcher unit covalently attached to the anti-wall teichoic acid (WTA) antibody; Pep is a peptide of two to twelve amino acid residues, and Y is a spacer unit covalently

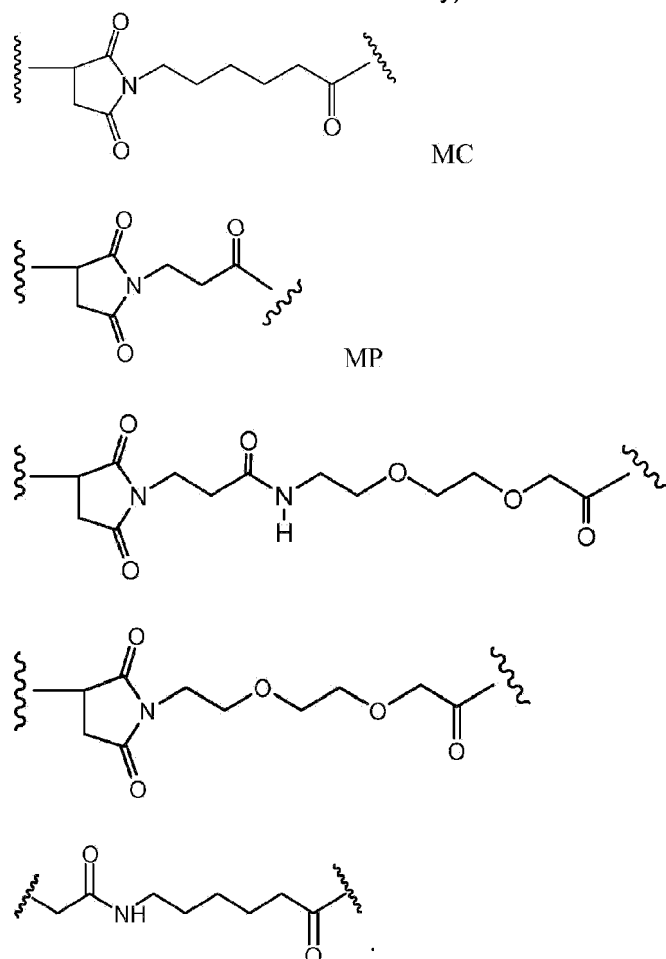
attached to the rifamycin-type antibiotic. Exemplary embodiments of such linkers are described in US 7498298.

**[0225]** In one embodiment, a stretcher unit "Str" has the formula:



wherein  $R^6$  is selected from the group consisting of  $C_1-C_{10}$  alkylene-,  $-C_3-C_8$  carbocyclo-,  $-O-(C_1-C_8 \text{ alkyl})-$ ,  $-$ arylene-,  $-C_1-C_{10}$  alkylene-arylene-,  $-$ arylene- $C_1-C_{10}$  alkylene-,  $-C_1-C_{10}$  alkylene- $(C_3-C_8 \text{ carbocyclo})-$ ,  $-(C_3-C_8 \text{ carbocyclo})-C_1-C_{10}$  alkylene-,  $-C_3-C_8$  heterocyclo-,  $-C_1-C_{10}$  alkylene- $(C_3-C_8 \text{ heterocyclo})-$ ,  $-(C_3-C_8 \text{ heterocyclo})-C_1-C_{10}$  alkylene-,  $-(CH_2CH_2O)_r-$ , and  $-(CH_2CH_2O)_r-CH_2-$ ; and  $r$  is an integer ranging from 1 to 10.

**[0226]** Exemplary stretcher units are shown below (wherein the wavy line indicates sites of covalent attachment to an antibody):



**[0227]** A peptide unit "Pep" comprises two or more amino acid residues that occur naturally, including the twenty major amino acids as well as minor amino acids such as citrulline, which

are well known in the field of biochemistry. Amino acids are distinguished by their side chain. The peptide unit thus comprises two or more amino acid side chains, including but not limited to,  $-\text{CH}_3$  (alanine),  $-\text{CH}_2\text{CH}_2\text{CH}_2\text{NHC}(\text{NH})\text{NH}_2$  (arginine),  $-\text{CH}_2\text{C}(\text{O})\text{NH}_2$  (asparagine),  $-\text{CH}_2\text{CO}_2\text{H}$  (aspartic acid),  $-\text{CH}_2\text{CH}_2\text{CH}_2\text{NHC}(\text{O})\text{NH}_2$  (citrulline),  $-\text{CH}_2\text{SH}$  (cysteine),  $-\text{CH}_2\text{CH}_2\text{CO}_2\text{H}$  (glutamic acid),  $-\text{CH}_2\text{CH}_2\text{C}(\text{O})\text{NH}_2$  (glutamine),  $-\text{H}$  (glycine),  $-\text{CH}_2(\text{imidazolyl})$  (histidine),  $-\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_3$  (isoleucine),  $-\text{CH}_2\text{CH}(\text{CH}_3)\text{CH}_3$  (leucine),  $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2$  (lysine),  $-\text{CH}_2\text{CH}_2\text{SCH}_3$  (methionine),  $-\text{CH}_2(\text{C}_6\text{H}_5)$  (phenylalanine),  $-\text{CH}_2\text{CH}_2\text{CH}_2-$  (proline),  $-\text{CH}_2\text{OH}$  (serine),  $-\text{CH}(\text{OH})\text{CH}_3$  (threonine),  $-\text{CH}_2(\text{indole})$  (tryptophan),  $-\text{CH}_2(\text{p-C}_6\text{H}_4\text{OH})$  (tyrosine),  $-\text{CHCH}(\text{CH}_3)\text{CH}_3$  (valine). See page 1076-1077, "Organic Chemistry" 5th Ed. John McMurry, Brooks/Cole pub. (2000). The amino acid residues of the peptide unit include all stereoisomers, and may be in the D or L configurations. In one embodiment, Pep comprises two to twelve amino acid residues independently selected from glycine, alanine, phenylalanine, lysine, arginine, valine, and citrulline. In one such embodiment, the amino acid unit allows for cleavage of the linker by a protease, thereby facilitating release of the antibiotic from the AAC upon exposure to intracellular proteases, such as lysosomal enzymes (Doronina et al. (2003) Nat. Biotechnol. 21:778-784). Exemplary amino acid units include, but are not limited to, a dipeptide, a tripeptide, a tetrapeptide, a pentapeptide, and a hexapeptide. Exemplary dipeptides include: valine-citrulline (vc or val-cit), alanine-phenylalanine (af or ala-phe); phenylalanine-lysine (fk or phe-lys); or N-methyl-valine-citrulline (Me-val-cit). Exemplary tripeptides include: glycine-valine-citrulline (gly-val-cit), valine-citrulline-phenylalanine (val-cit-phe), and glycine-glycine-glycine (gly-gly-gly). Additional peptide linkers include GGAFFAGGG (SEQ ID NO: 126); tpm-cit; GPImeLFF (SEQ ID NO: 129); and LAFG (SEQ ID NO: 128). Peptide linkers can be prepared by forming a peptide bond between two or more amino acids and/or peptide fragments. Such peptide bonds can be prepared, for example, according to the liquid phase synthesis method (E. Schroder and K. Lübke (1965) "The Peptides", volume 1, pp 76-136, Academic Press) which is well known in the field of peptide chemistry. Amino acid units can be designed and optimized in their selectivity for enzymatic cleavage by a particular enzyme, for example, a tumor-associated protease, cathepsin B, C and D, or a plasmin protease.

**[0228]** In one embodiment, spacer unit Y comprises para-aminobenzyl or para-aminobenzylloxycarbonyl. A "non-self-immolative" spacer unit is one in which part or all of the spacer unit remains bound to the antibiotic moiety upon enzymatic (e.g., proteolytic) cleavage of the AAC. Examples of non-self-immolative spacer units include, but are not limited to, a glycine spacer unit and a glycine-glycine spacer unit. Other combinations of peptidic spacers susceptible to sequence-specific enzymatic cleavage are also contemplated. For example, enzymatic cleavage of an AAC containing a glycine-glycine spacer unit by a tumor-cell associated protease would result in release of a glycine-glycine-antibiotic moiety from the remainder of the AAC. In one such embodiment, the glycine-glycine-antibiotic moiety is then subjected to a separate hydrolysis step in the tumor cell, thus cleaving the glycine-glycine spacer unit from the antibiotic moiety.

**[0229]** A spacer unit allows for release of the antibiotic moiety without a separate hydrolysis

step. A spacer unit may be "self-immolative" or a "non-self-immolative." In certain embodiments, a spacer unit of a linker comprises a p-aminobenzyl unit (PAB). In one such embodiment, a p-aminobenzyl alcohol is attached to an amino acid unit via an amide bond, a carbamate, methylcarbamate, or carbonate between the p-aminobenzyl group and the antibiotic moiety (Hamann et al. (2005) Expert Opin. Ther. Patents (2005) 15:1087-1103). In one embodiment, the spacer unit is p-aminobenzylloxycarbonyl (PAB).

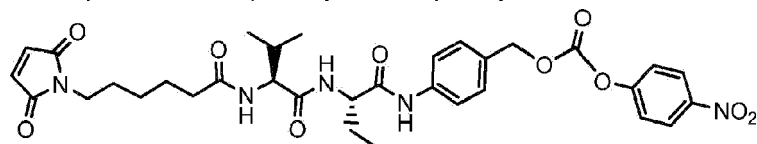
**[0230]** In one embodiment, the antibiotic forms a quaternary amine, such as the dimethylaminopiperidyl group, when attached to the PAB spacer unit of the peptide linker. Examples of such quaternary amines are linker-antibiotic intermediates (LA) are **54, 61, 66, 67, 73, 74, 76, 78, 79, 83, 84** from Table 2. The quaternary amine group may modulate cleavage of the antibiotic moiety to optimize the antibacterial effects of the AAC. In another embodiment, the antibiotic is linked to the PABC spacer unit of the peptide linker, forming a carbamate functional group in the AAC. Such carbamate functional group may also optimize the antibacterial effects of the AAC. Examples of PABC carbamate linker-antibiotic intermediates (LA) are **51, 52, 53, 55, 56, 57, 58, 62, 63, 64, 65, 72, 75, 80, 81, 87** from Table 2. Other linker-antibiotic intermediates (LA) employ amide (**59, 69, 70, 71, 77, 82, 85**) or phenolic (**60, 68, 86**) groups.

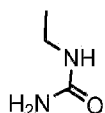
**[0231]** Other examples of self-immolative spacers include, but are not limited to, aromatic compounds that are electronically similar to the PAB group such as 2-aminoimidazol-5-methanol derivatives (US 7375078; Hay et al. (1999) Bioorg. Med. Chem. Lett. 9:2237) and ortho- or para-aminobenzylacetals. Spacers can be used that undergo cyclization upon amide bond hydrolysis, such as substituted and unsubstituted 4-aminobutyric acid amides (Rodrigues et al (1995) Chemistry Biology 2:223), appropriately substituted bicyclo[2.2.1] and bicyclo[2.2.2] ring systems (Storm et al (1972) J. Amer. Chem. Soc. 94:5815) and 2-aminophenylpropionic acid amides (Amsberry, et al (1990) J. Org. Chem. 55:5867). Elimination of amine-containing drugs that are substituted at glycine (Kingsbury et al (1984) J. Med. Chem. 27:1447) is also exemplary of self-immolative spacers useful in AAC.

#### LINKER-ANTIBIOTIC INTERMEDIATES USEFUL FOR AAC

**[0232]** Linker-antibiotic intermediates (LA) of Formula II and Table 2 were prepared by coupling a rifamycin-type antibiotic moiety with a peptide-linker reagent, as exemplified in Figures 23-25 and Examples 1-17. Linker reagents were prepared by methods described in WO 2012/113847; US 7659241; US 7498298; US 20090111756; US 2009/0018086; US 6214345; Dubowchik et al (2002) Bioconjugate Chem. 13(4):855-869, including:

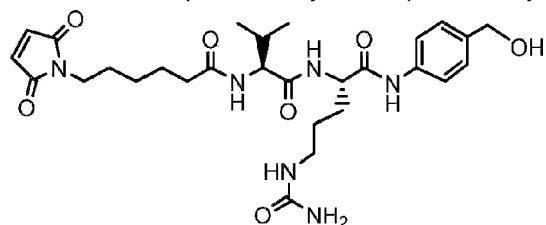
4-((S)-2-((S)-2-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl 4-nitrophenyl carbonate **6**





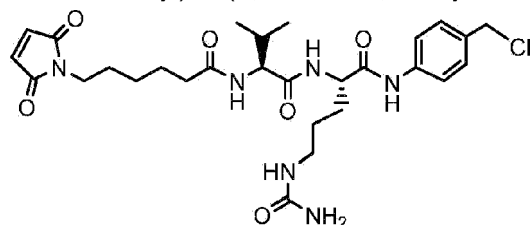
6

6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N-((S)-1-((S)-1-(4-(hydroxymethyl)phenylamino)-1-oxo-5-ureidopentan-2-ylamino)-3-methyl-1-oxobutan-2-yl)hexanamide **8**



8

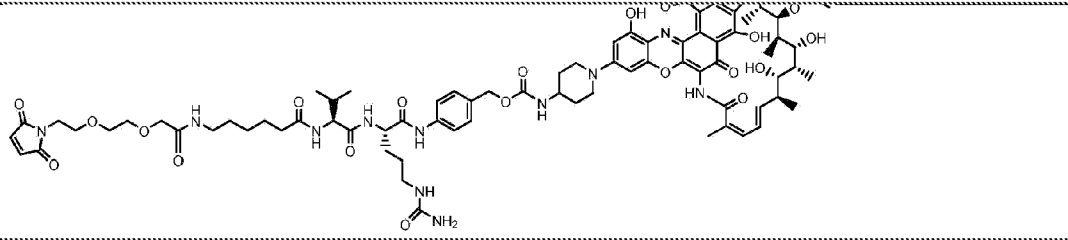
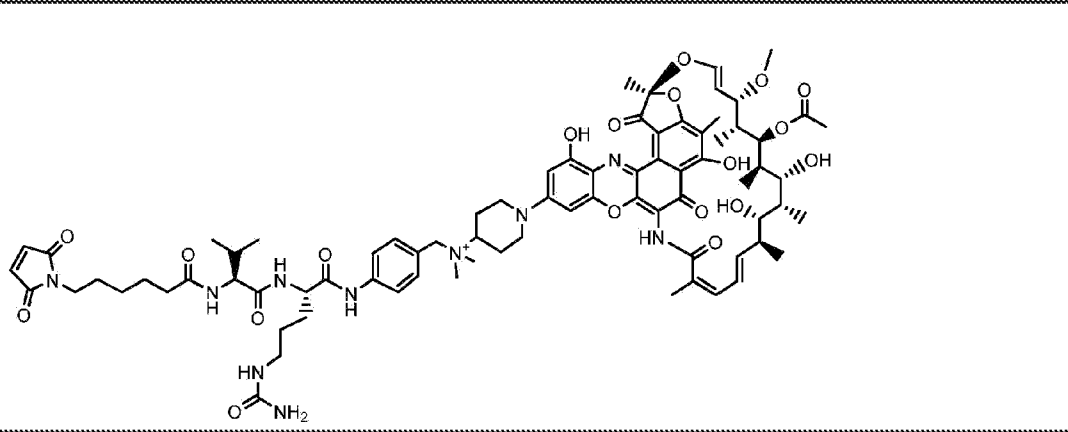
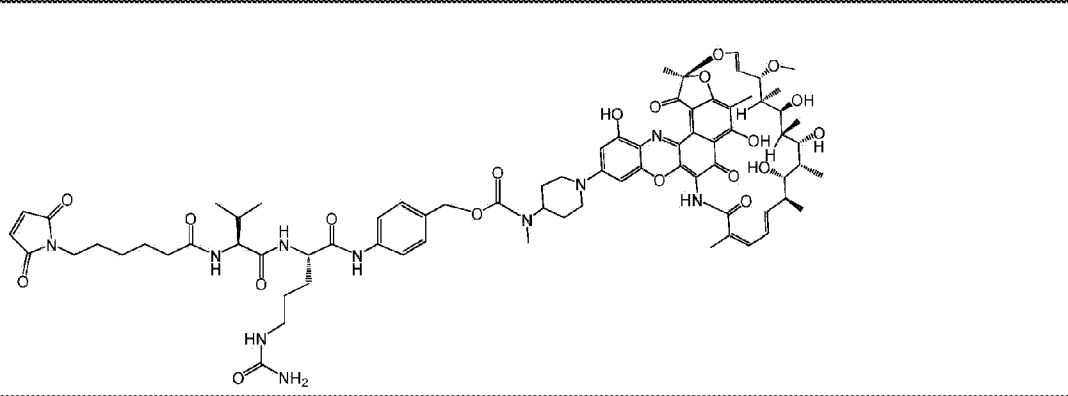
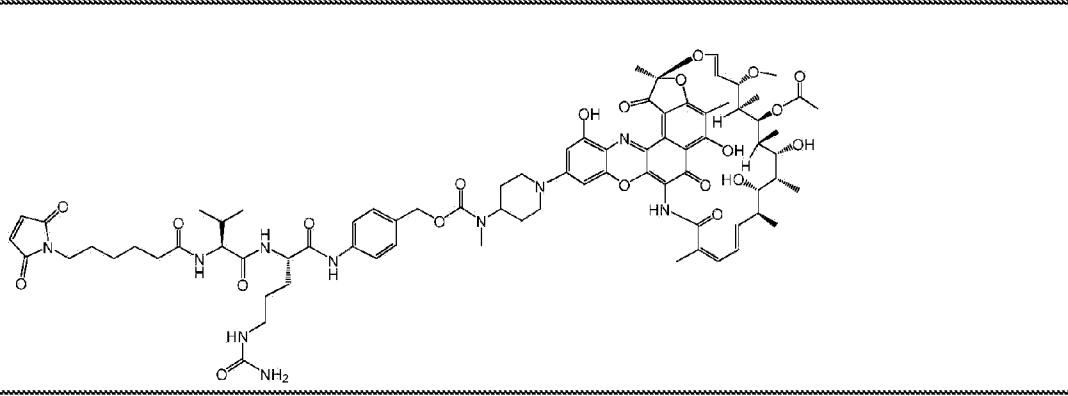
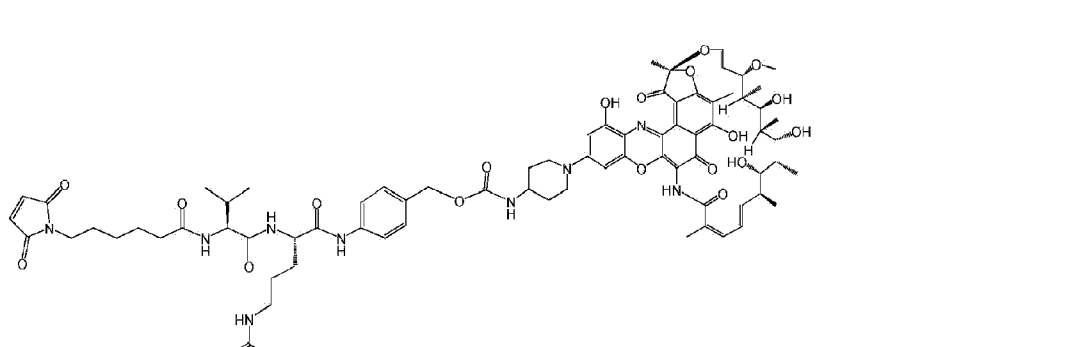
N-((S)-1-((S)-1-(4-(chloromethyl)phenylamino)-1-oxo-5-ureidopentan-2-ylamino)-3-methyl-1-oxobutan-2-yl)-6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamide **9**

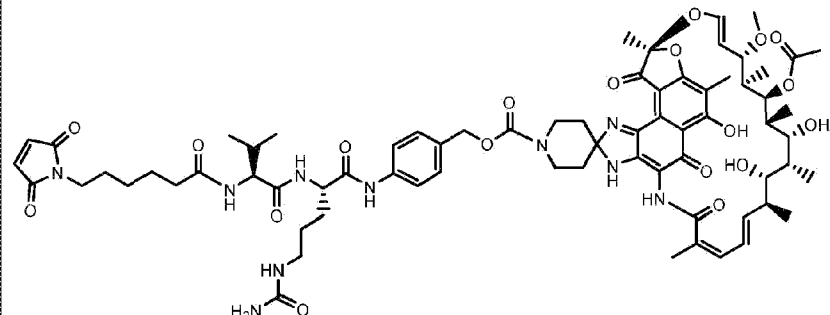
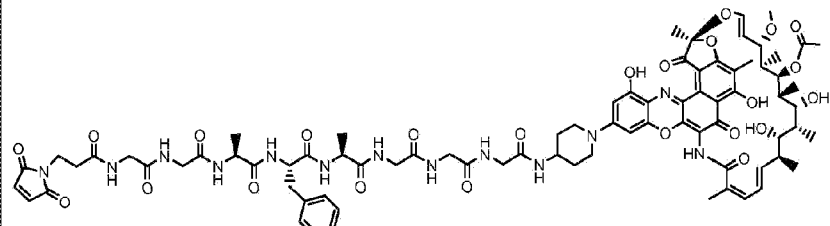
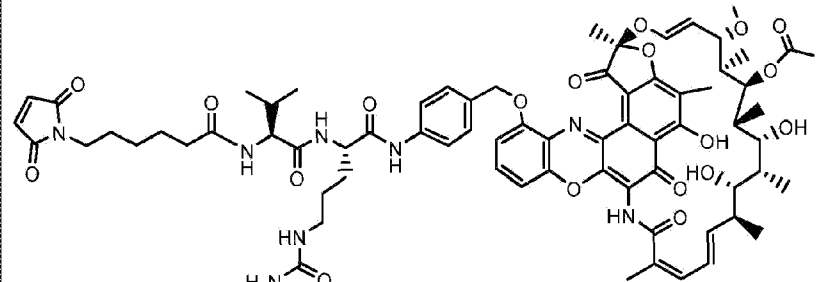
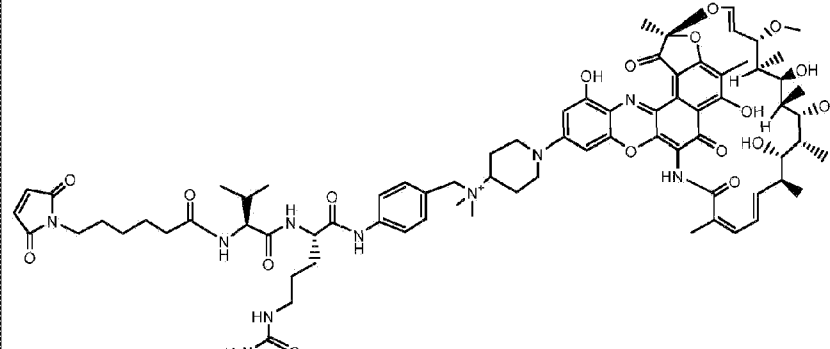
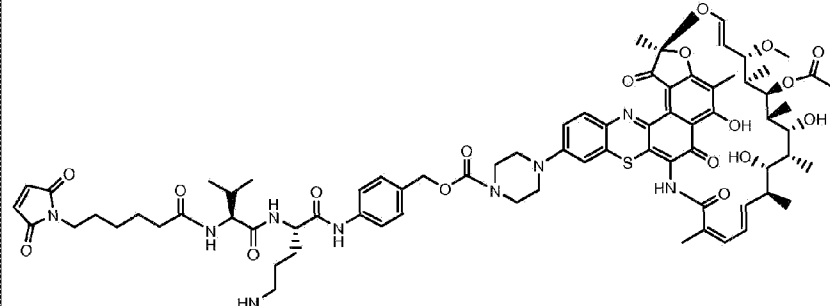



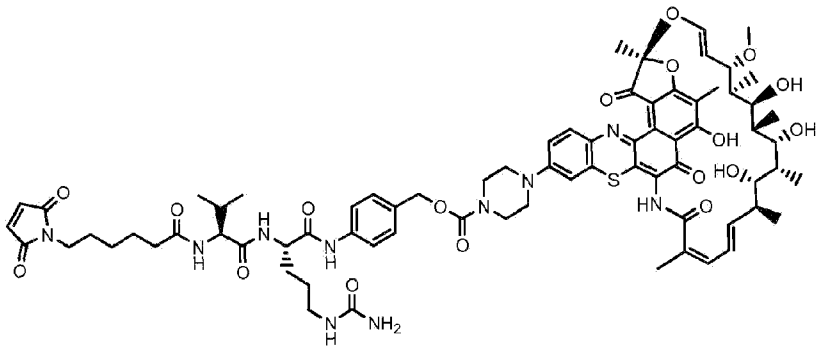
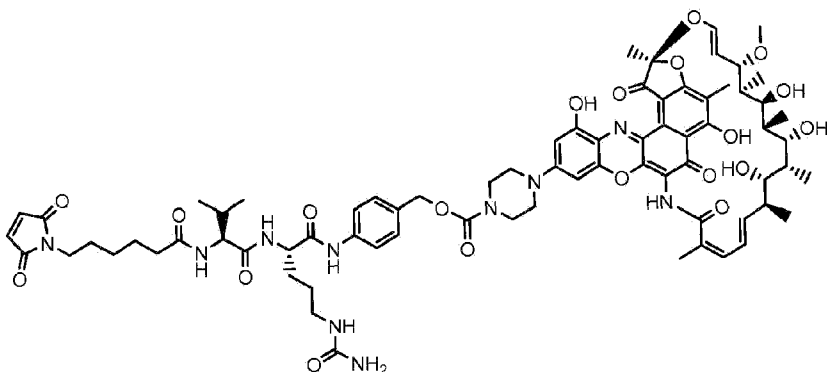
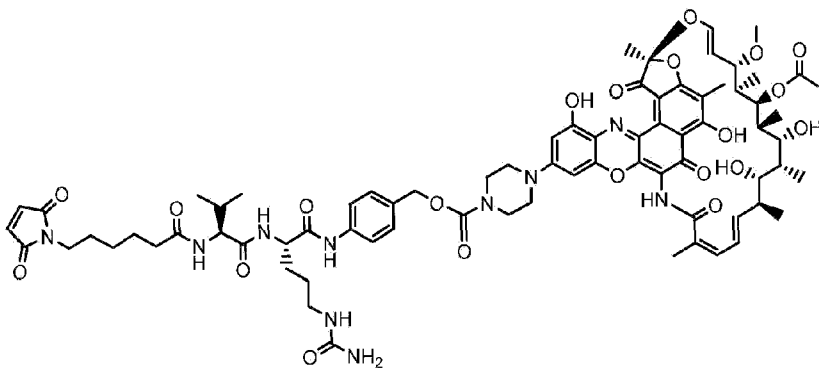
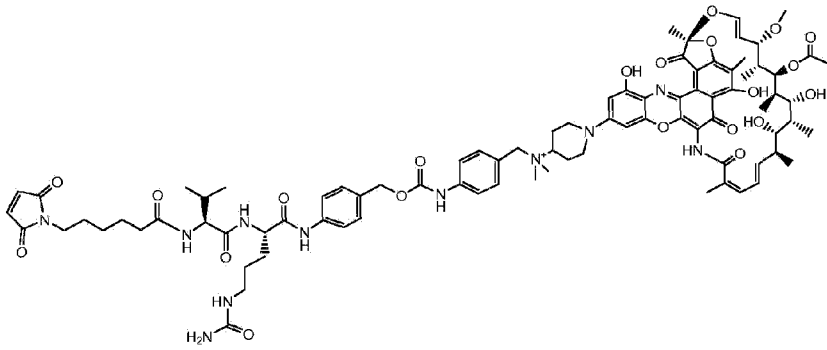
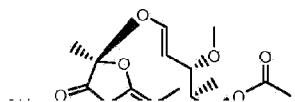
9

Table 2 Linker-antibiotic intermediates (LA)

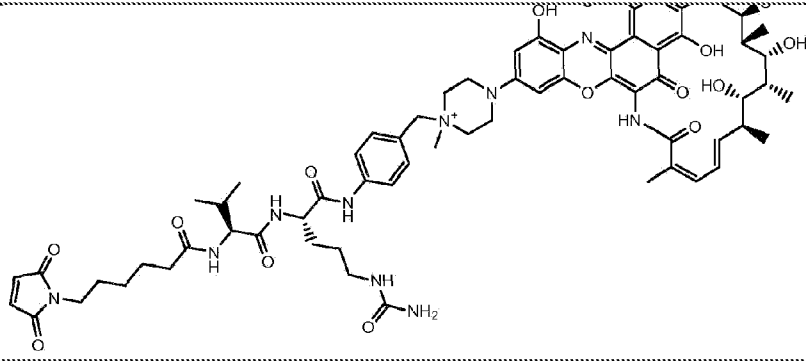
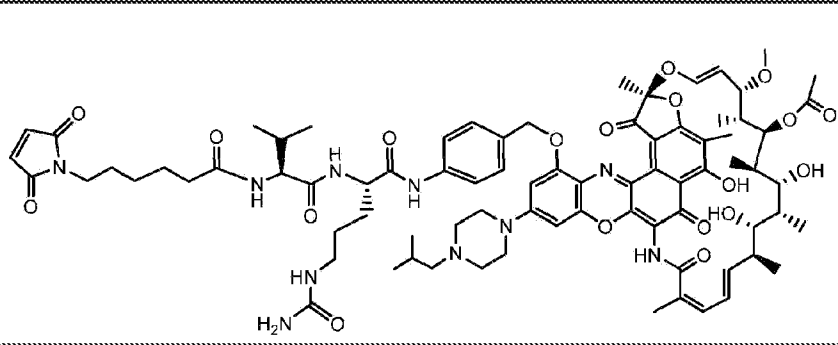
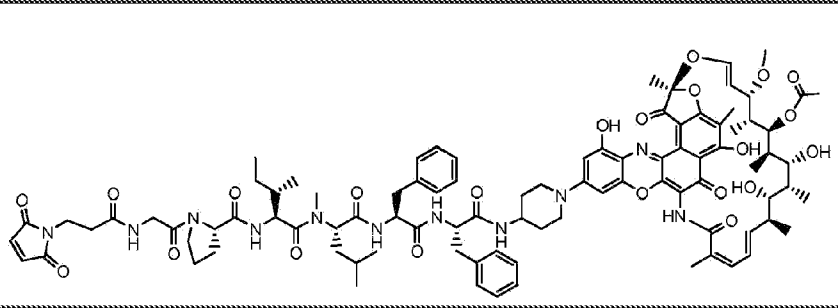
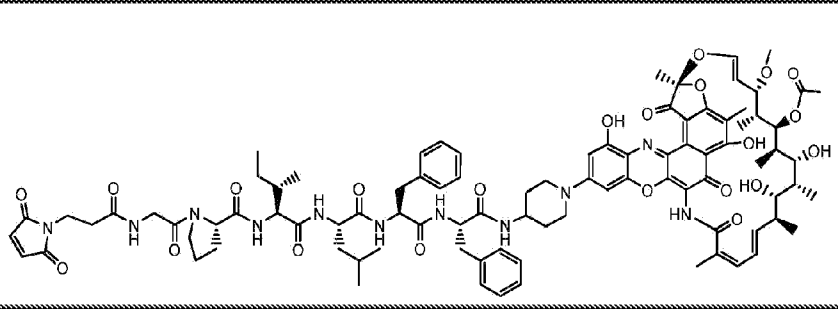
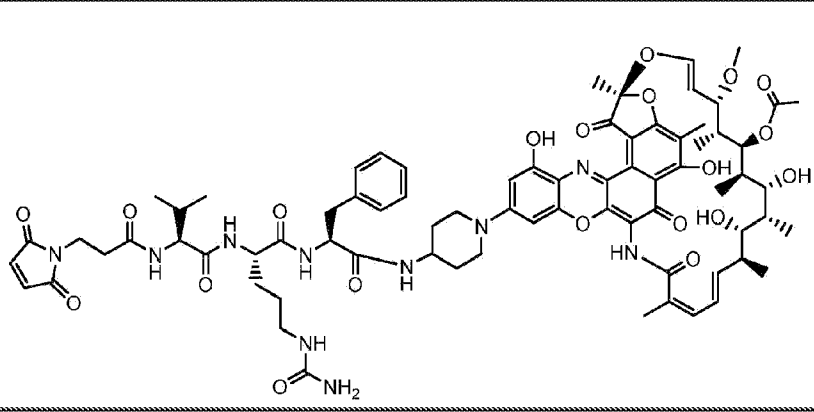

LA No.	Structure
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LA-52	
LA-53	

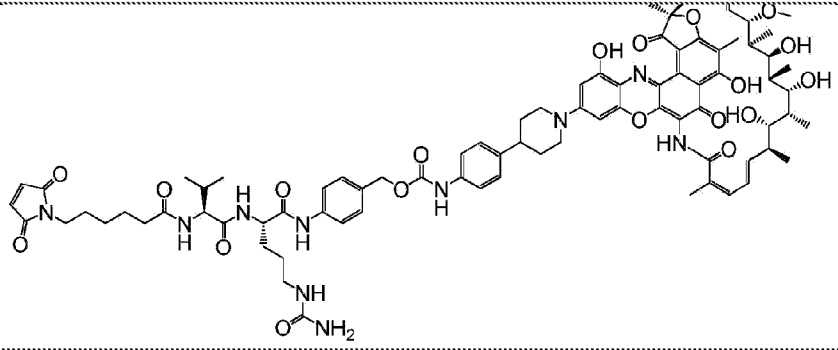
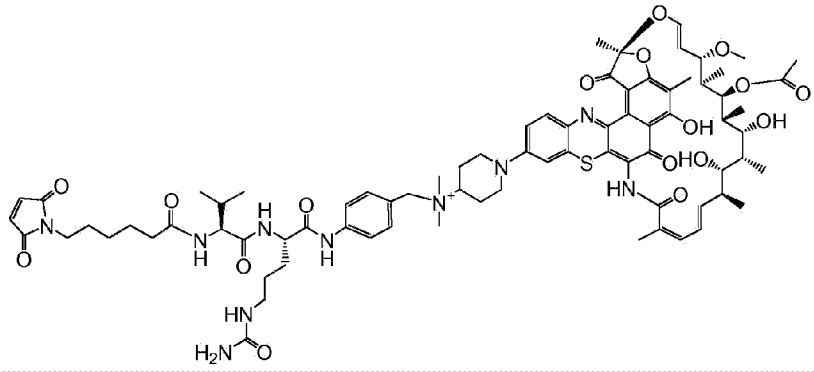
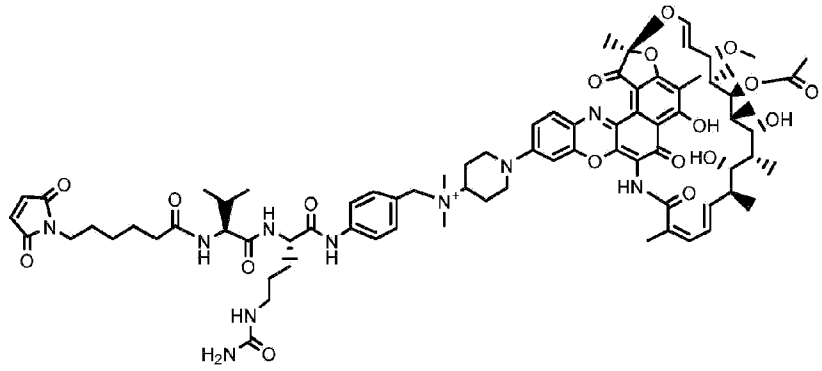
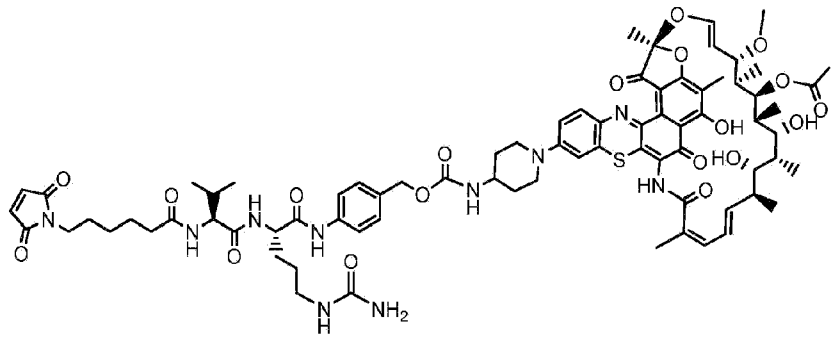
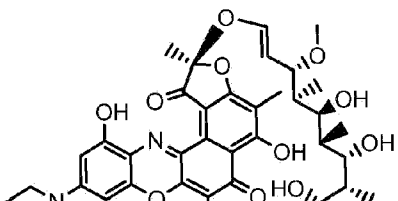
LA No.	Structure
	
LA-54	
LA-55	
LA-56	
LA-57	

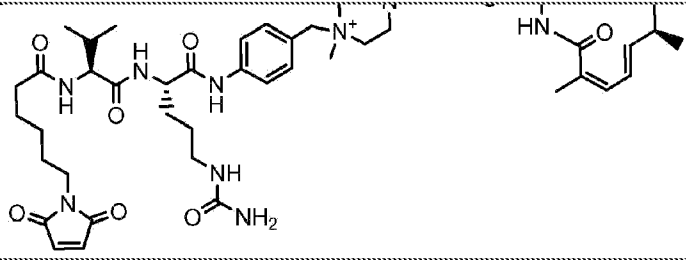
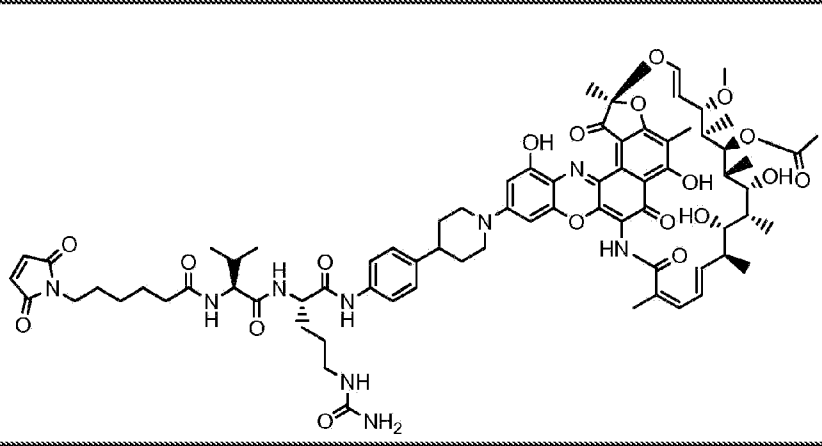
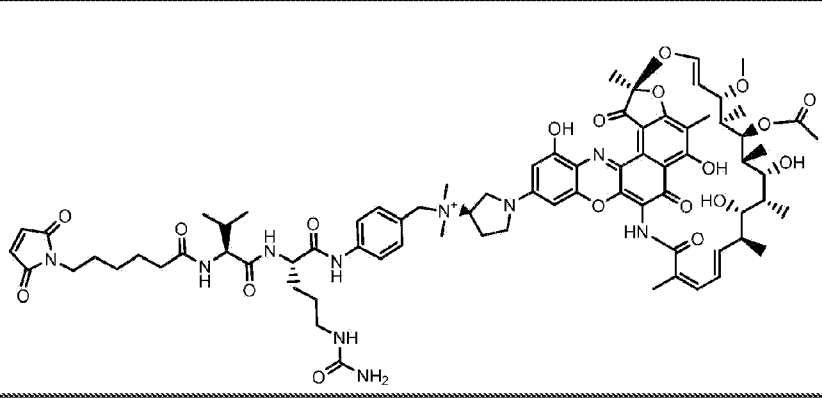
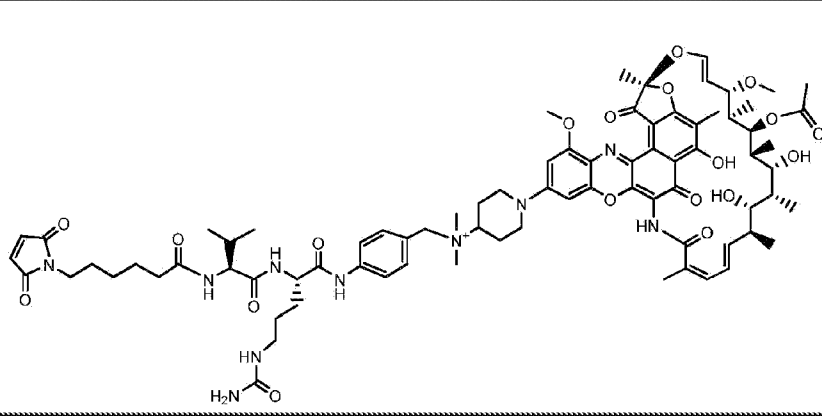
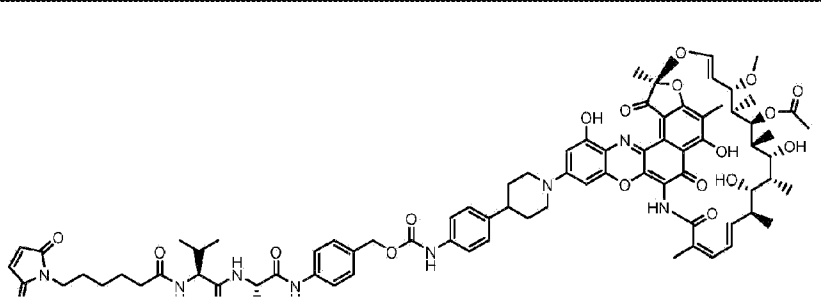
LA No.	Structure
LA-58	 <p>Chemical structure of LA-58, a complex molecule featuring a large polycyclic core with multiple hydroxyl groups and a side chain containing a piperidine ring, an amide, and a terminal amino group.</p>
LA-59	 <p>Chemical structure of LA-59, a complex molecule featuring a large polycyclic core with multiple hydroxyl groups and a side chain containing a piperidine ring, an amide, and a terminal amino group.</p>
LA-60	 <p>Chemical structure of LA-60, a complex molecule featuring a large polycyclic core with multiple hydroxyl groups and a side chain containing a piperidine ring, an amide, and a terminal amino group.</p>
LA-61	 <p>Chemical structure of LA-61, a complex molecule featuring a large polycyclic core with multiple hydroxyl groups and a side chain containing a piperidine ring, an amide, and a terminal amino group.</p>
LA-62	 <p>Chemical structure of LA-62, a complex molecule featuring a large polycyclic core with multiple hydroxyl groups and a side chain containing a piperidine ring, an amide, and a terminal amino group.</p>

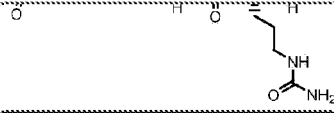
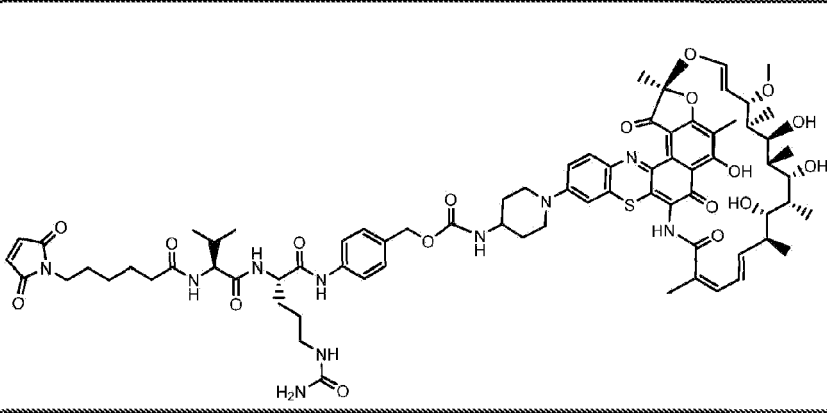
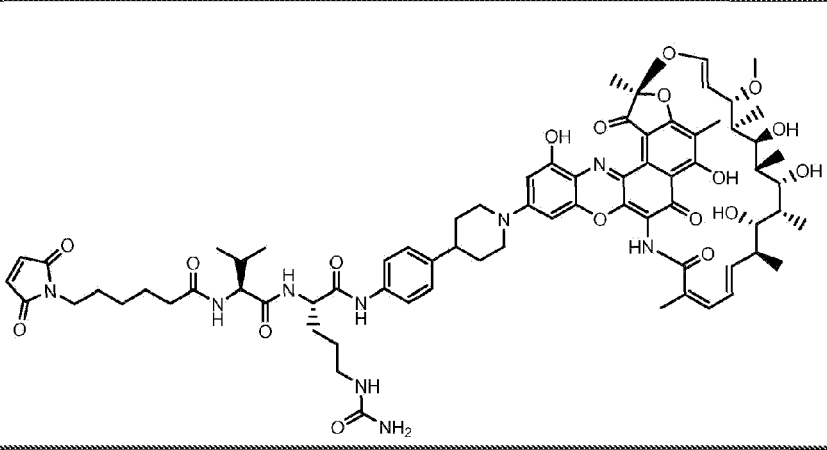
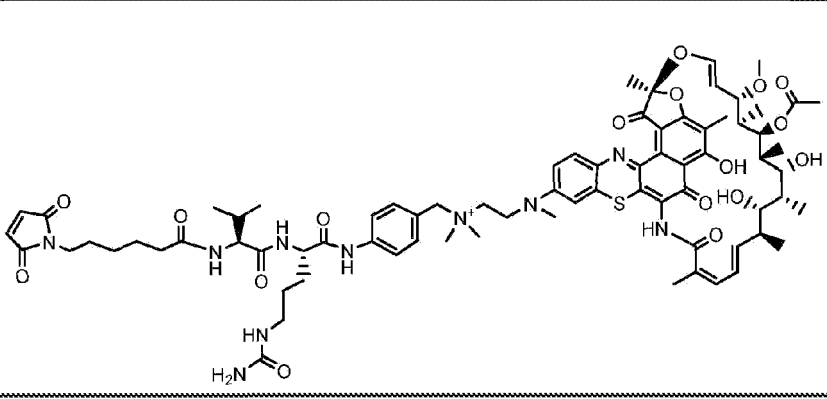
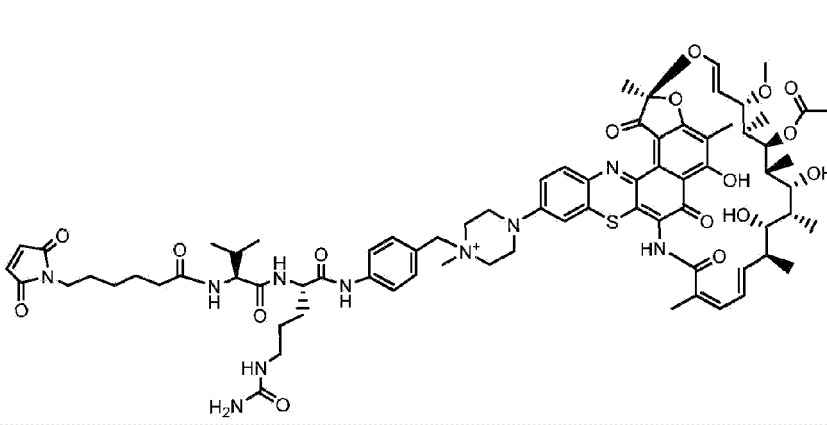
LA No.	Structure
	
LA-63	
LA-64	
LA-65	
LA-66	
LA-67	

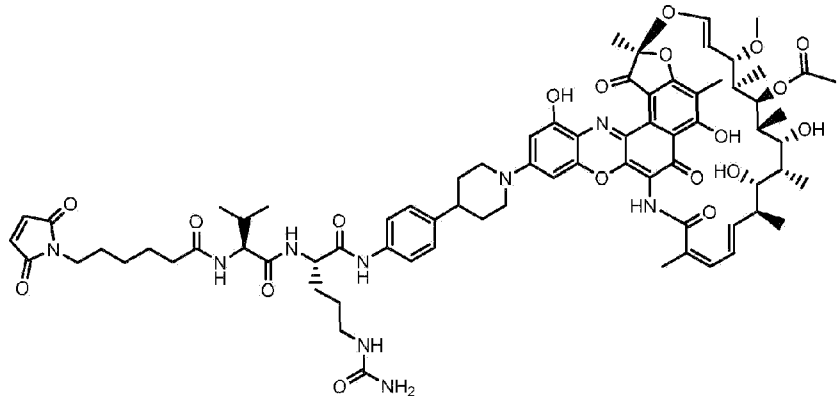
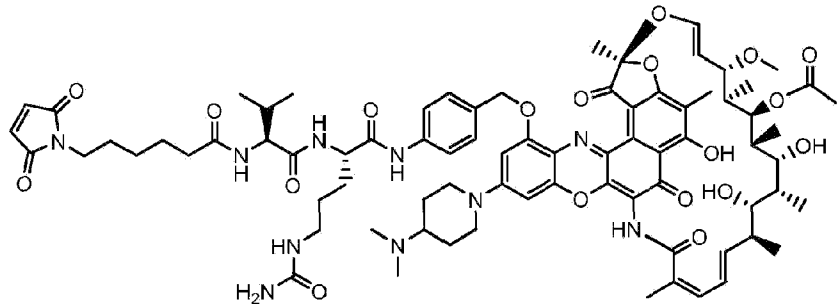
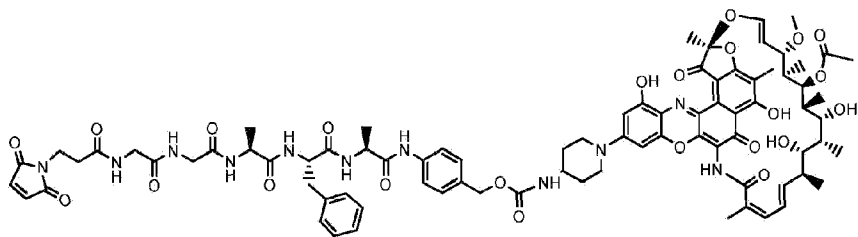
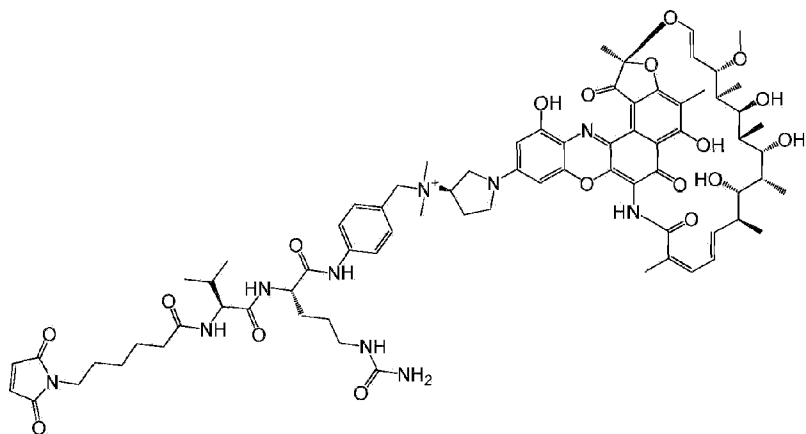
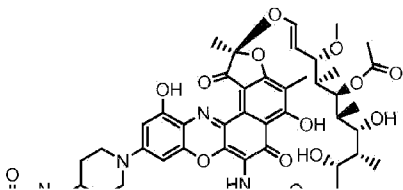


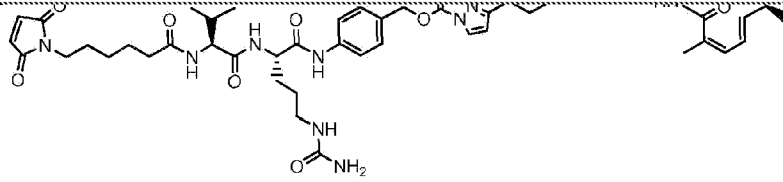
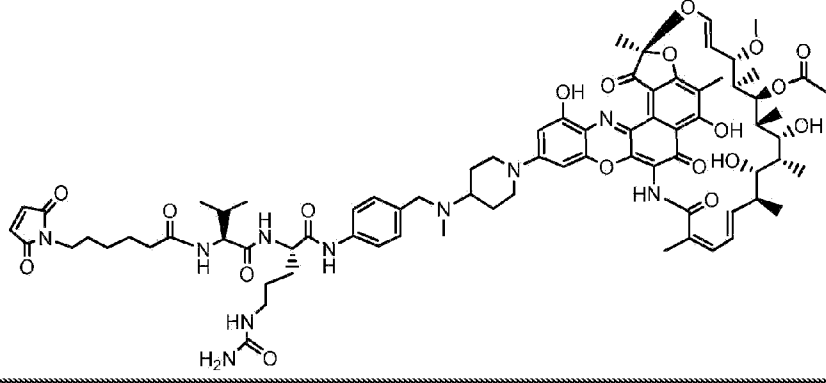
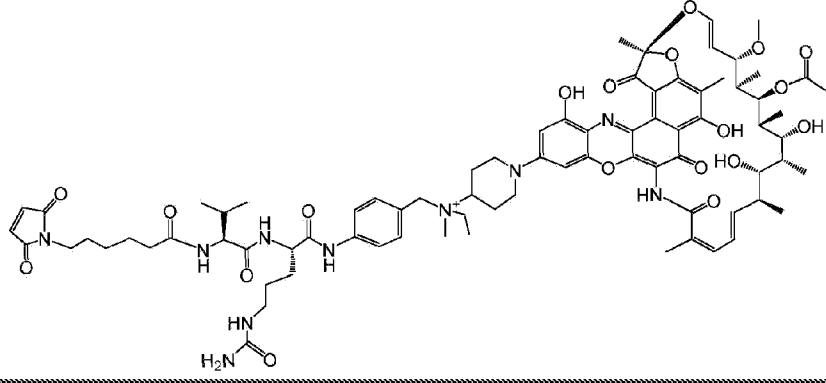
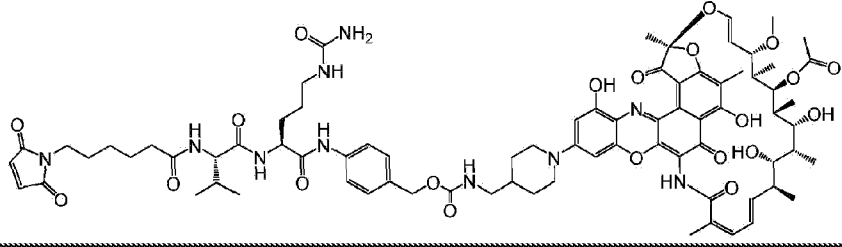
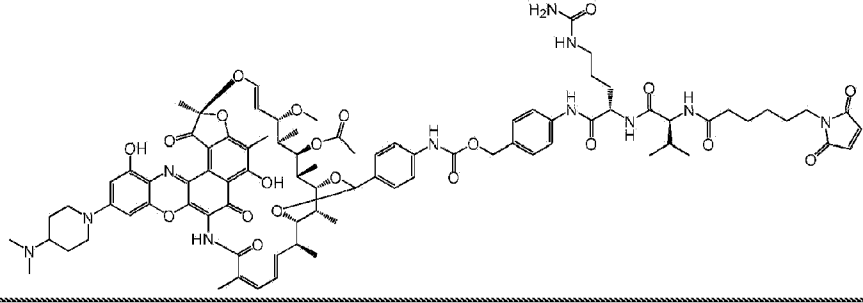

LA No.	Structure
	
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LA-69	
LA-70	
LA-71	
LA-72	

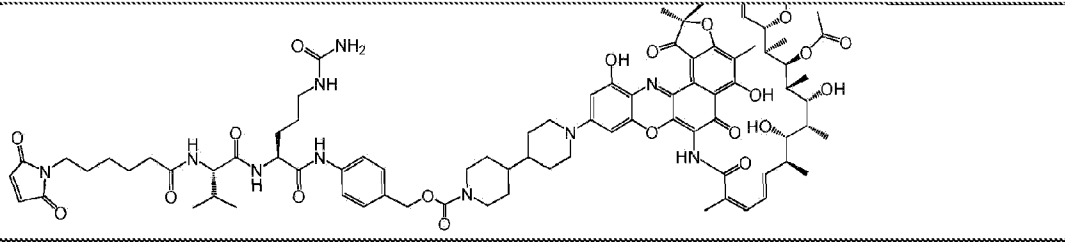
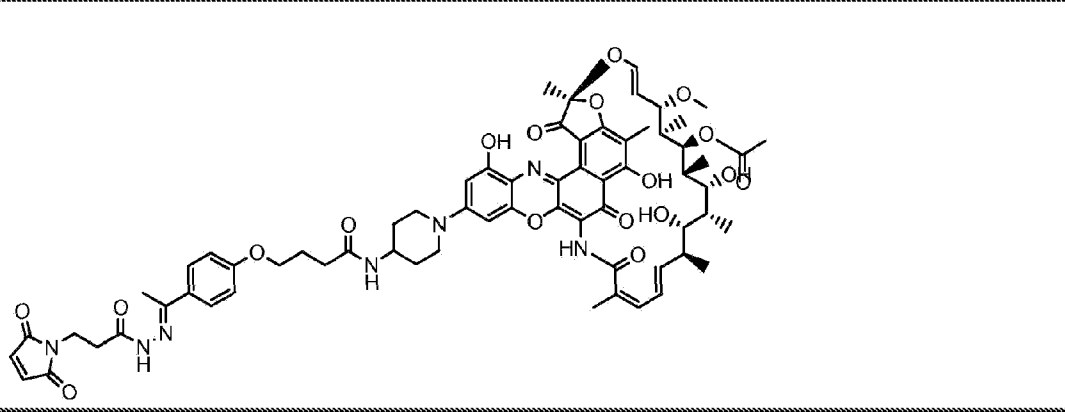
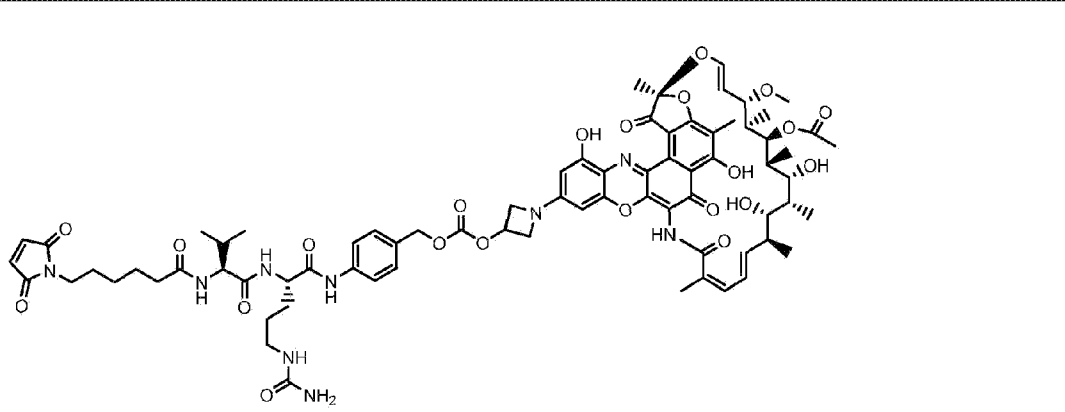
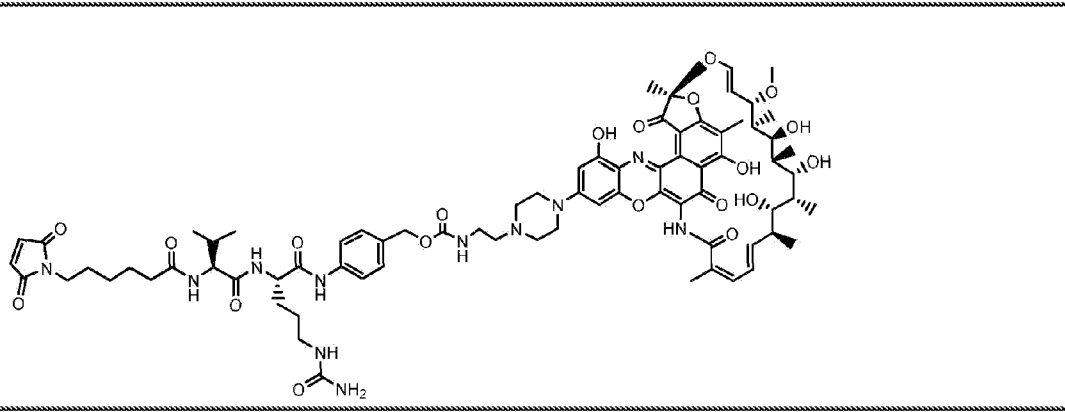
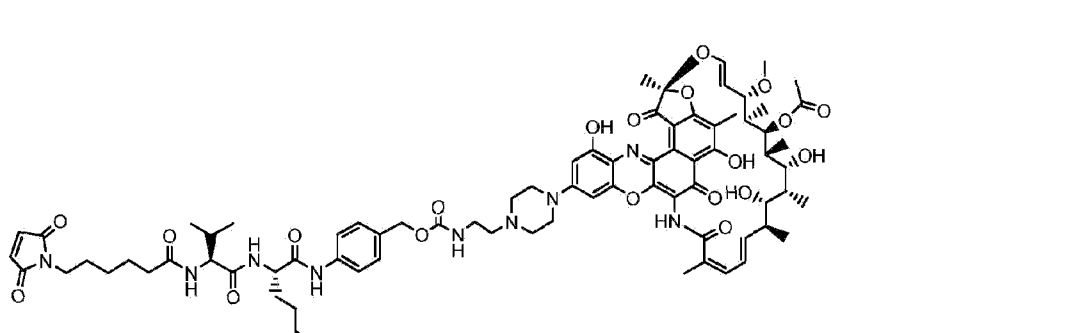
LA No.	Structure
	
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LA-74	
LA-75	
LA-76	


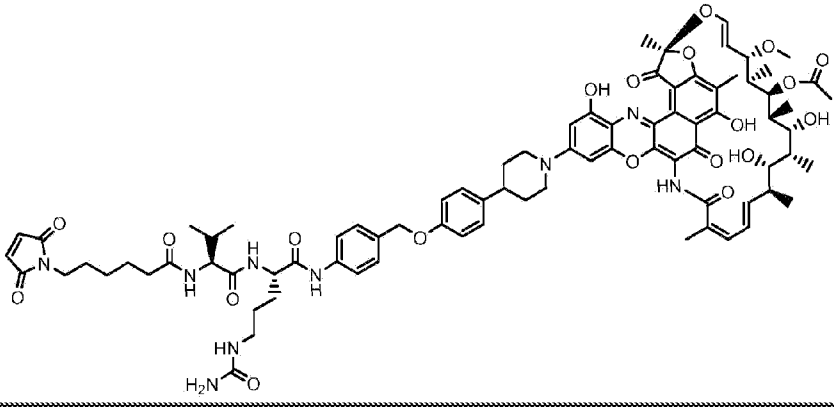
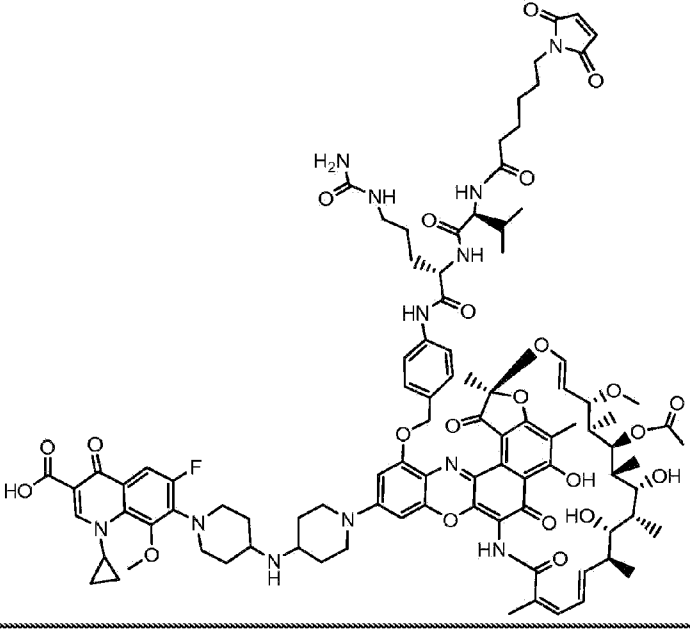
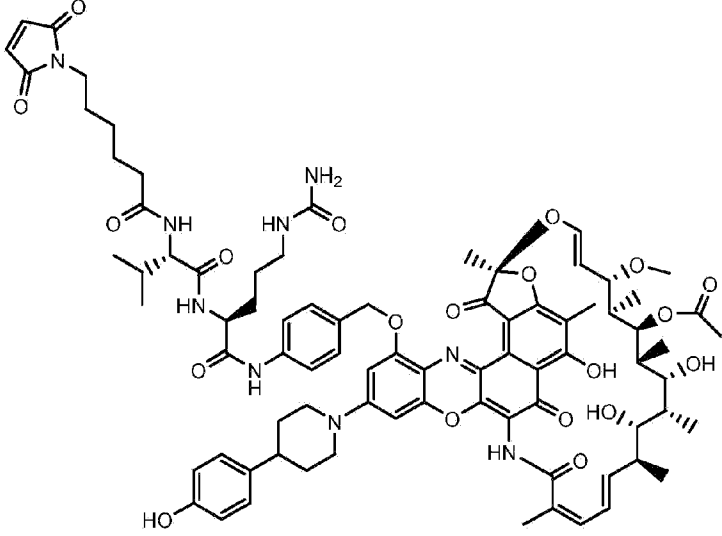
LA No.	Structure
	
LA-77	
LA-78	
LA-79	
LA-80	

LA No.	Structure
	
LA-81	
LA-82	
LA-83	
LA-84	

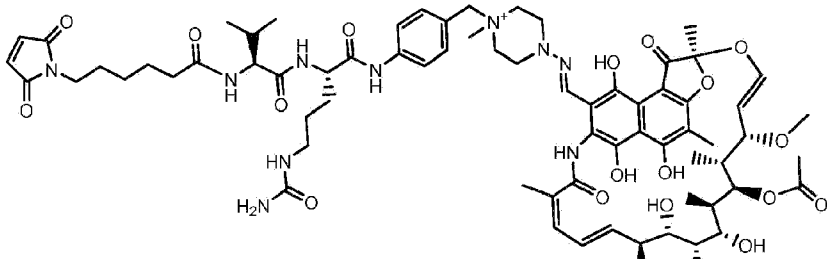
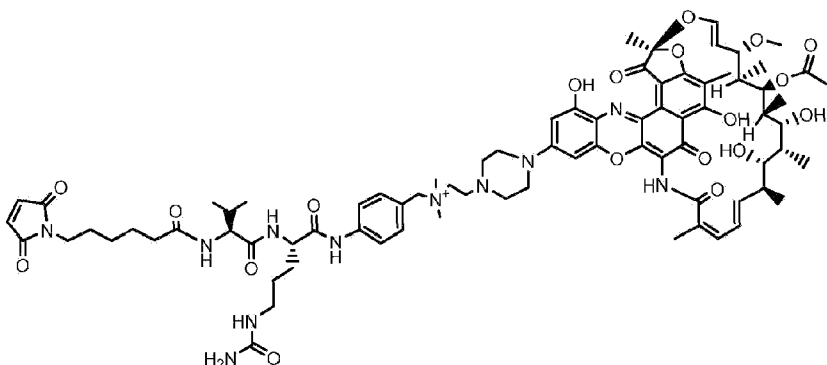
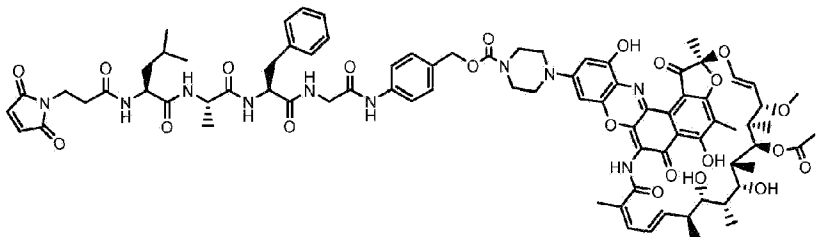
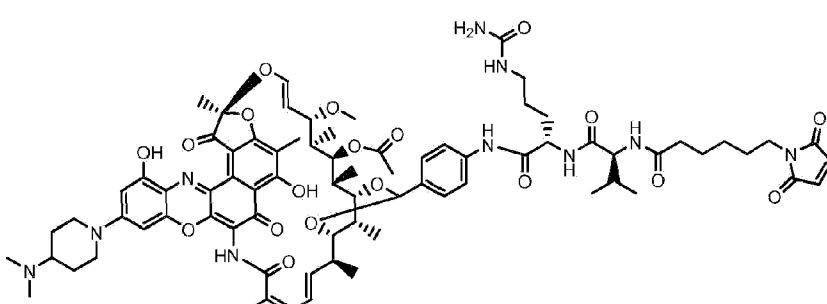
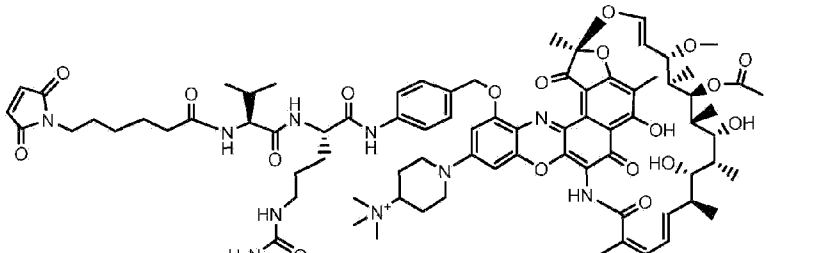

LA No.	Structure
LA-85	 <p>Chemical structure of LA-85, a complex molecule featuring a large polycyclic core with multiple hydroxyl groups and a fused ring system. The structure is substituted with a long chain containing a pyrrolidine ring, a carbonyl group, and a terminal amide group. The chain also includes a piperidine ring and a phenyl group.</p>
LA-86	 <p>Chemical structure of LA-86, a complex molecule featuring a large polycyclic core with multiple hydroxyl groups and a fused ring system. The structure is substituted with a long chain containing a pyrrolidine ring, a carbonyl group, and a terminal amide group. The chain also includes a piperidine ring and a phenyl group.</p>
LA-87	 <p>Chemical structure of LA-87, a complex molecule featuring a large polycyclic core with multiple hydroxyl groups and a fused ring system. The structure is substituted with a long chain containing a pyrrolidine ring, a carbonyl group, and a terminal amide group. The chain also includes a piperidine ring and a phenyl group.</p>
LA-88	 <p>Chemical structure of LA-88, a complex molecule featuring a large polycyclic core with multiple hydroxyl groups and a fused ring system. The structure is substituted with a long chain containing a pyrrolidine ring, a carbonyl group, and a terminal amide group. The chain also includes a piperidine ring and a phenyl group.</p>
LA-89	 <p>Chemical structure of LA-89, a complex molecule featuring a large polycyclic core with multiple hydroxyl groups and a fused ring system. The structure is substituted with a long chain containing a pyrrolidine ring, a carbonyl group, and a terminal amide group. The chain also includes a piperidine ring and a phenyl group.</p>

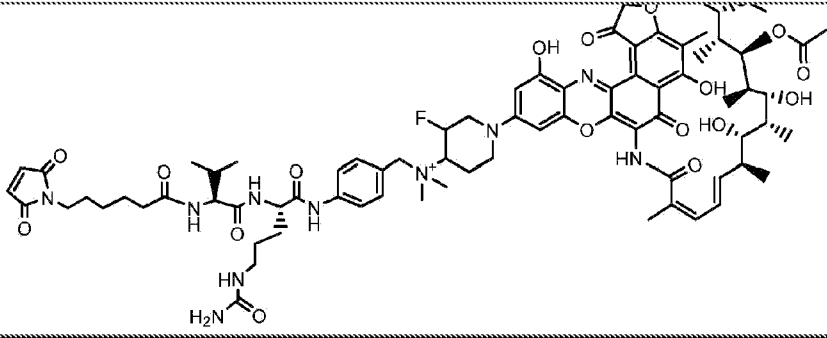
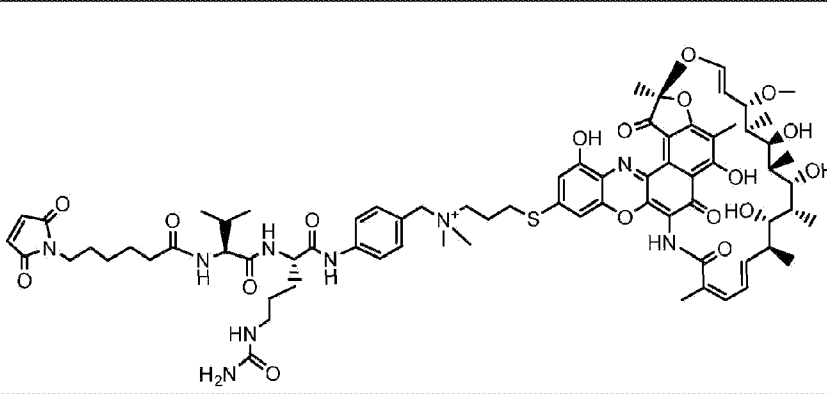
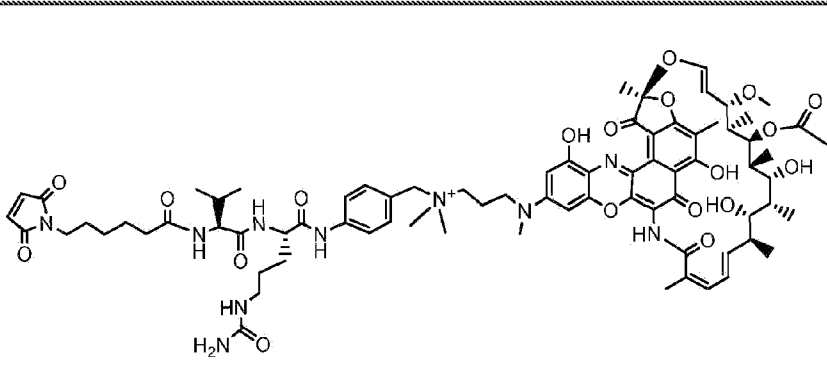
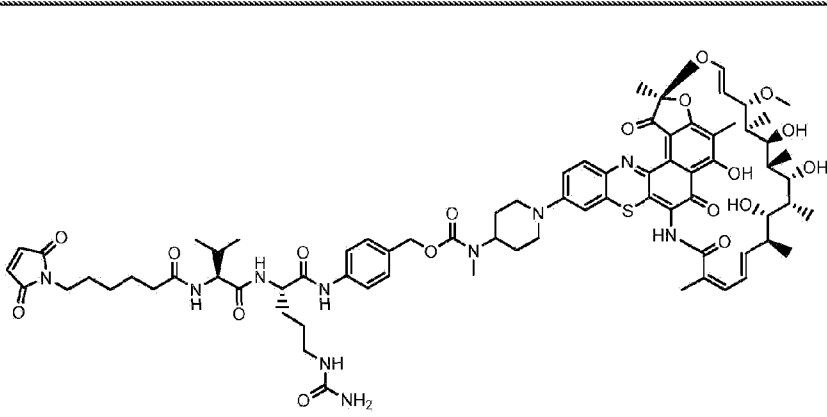
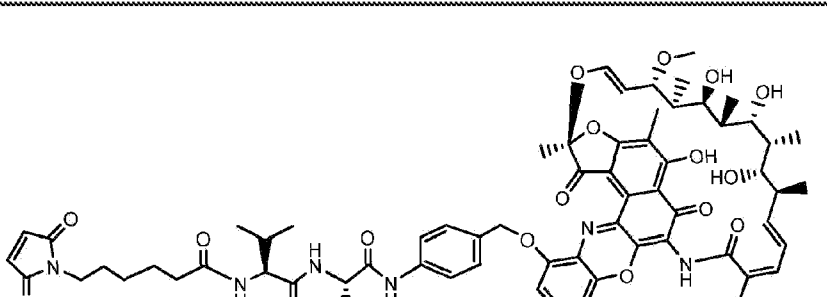
LA No.	Structure
	
LA-90	
LA-91	
LA-92	
LA-93	
LA-94	


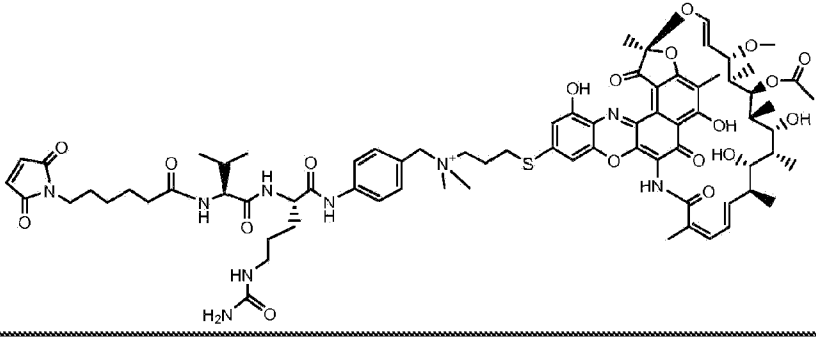
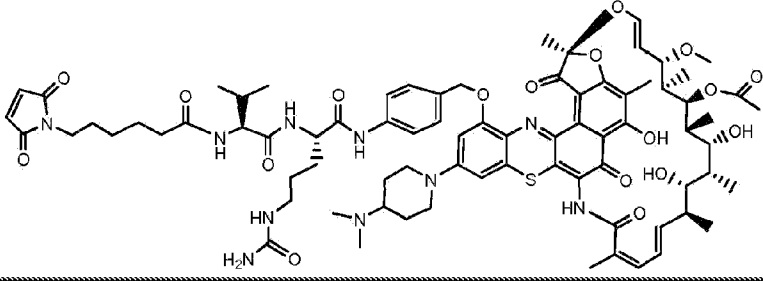
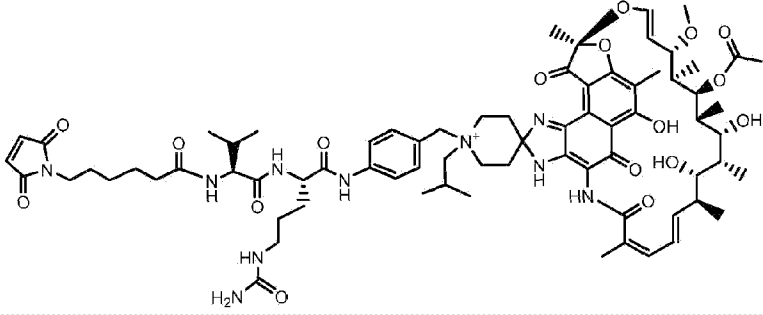
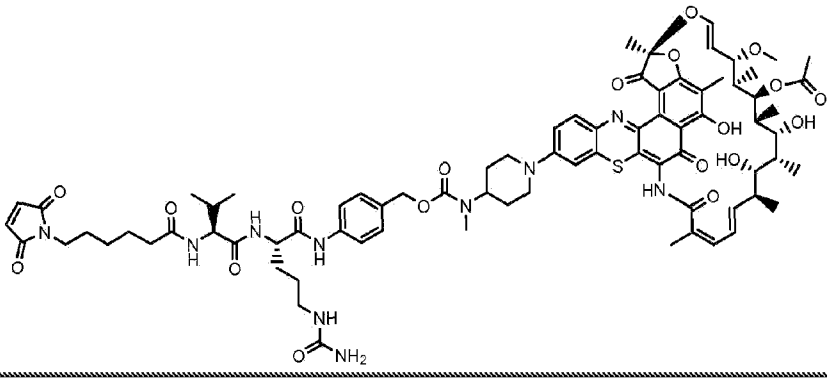
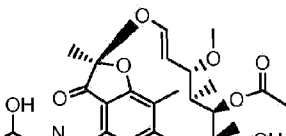
LA No.	Structure
	
LA-95	
LA-96	
LA-97	
LA-98	

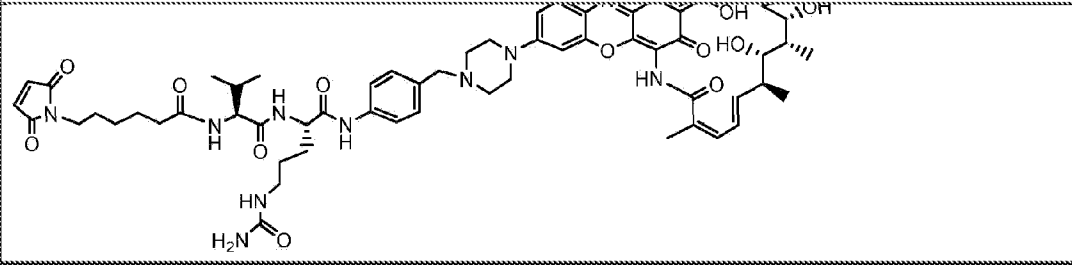
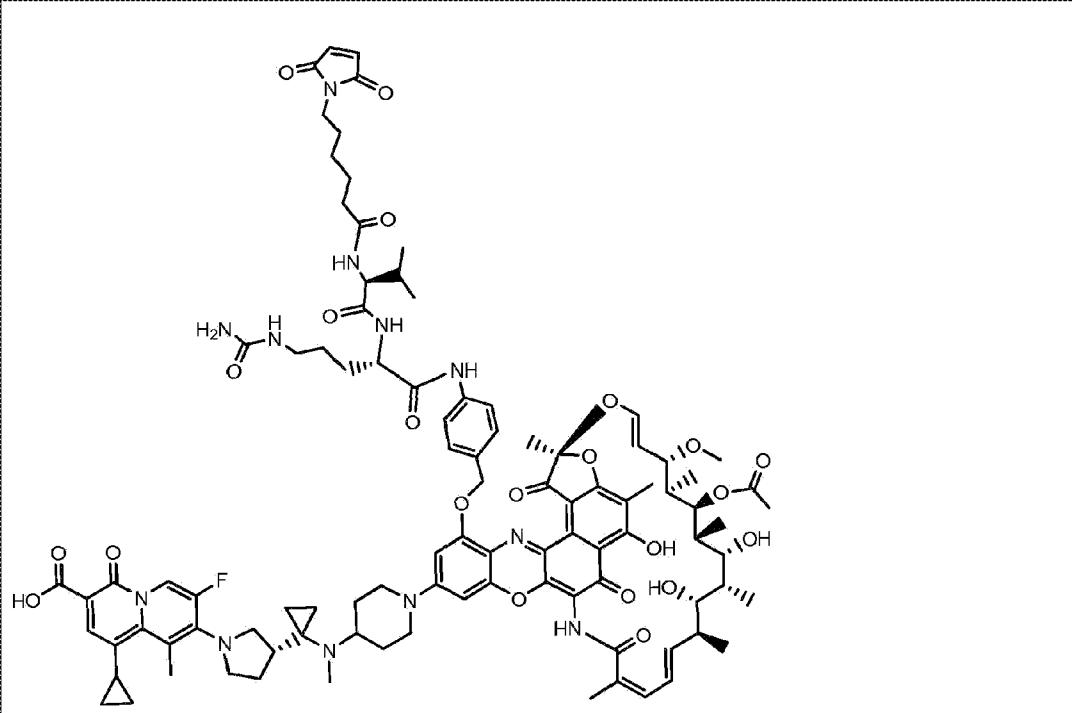
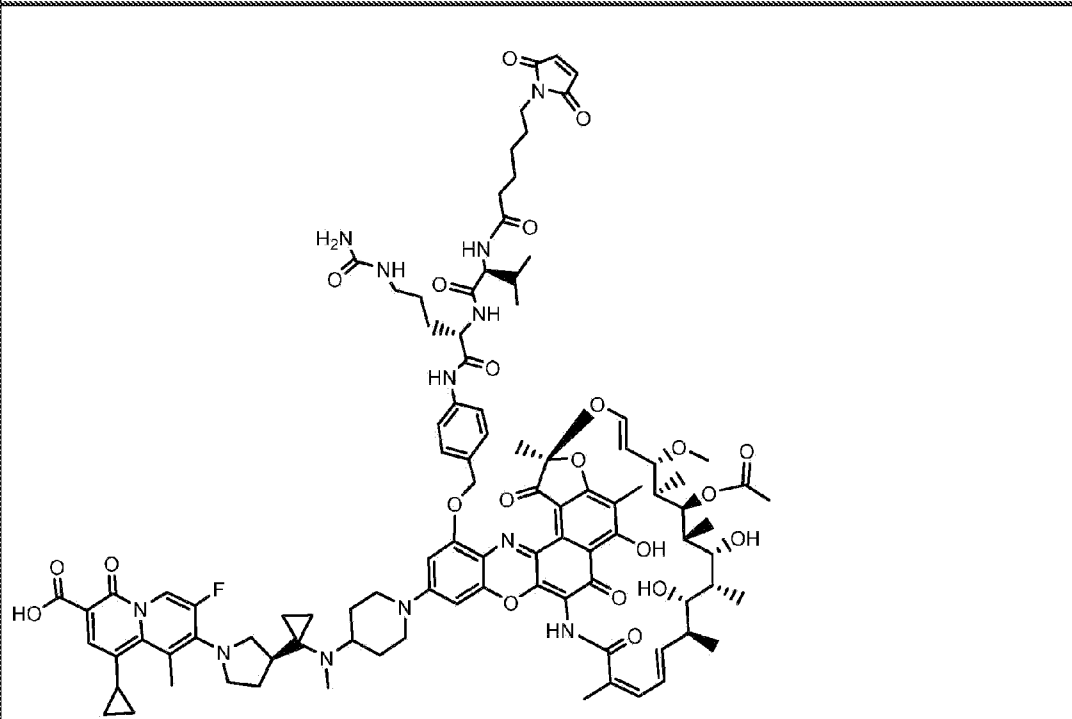
LA No.	Structure
	
LA-99	
LA-100	
LA-101	



LA No.	Structure
LA-102	 <p>Chemical structure of LA-102, a complex molecule featuring a pyridine ring, a long aliphatic chain, a piperazine ring, and a complex polycyclic system with multiple hydroxyl groups and a carboxylic acid derivative.</p>
LA-103	 <p>Chemical structure of LA-103, a complex molecule featuring a pyridine ring, a long aliphatic chain, a piperazine ring, and a complex polycyclic system with multiple hydroxyl groups and a carboxylic acid derivative.</p>
LA-104	 <p>Chemical structure of LA-104, a complex molecule featuring a pyridine ring, a long aliphatic chain, a piperazine ring, and a complex polycyclic system with multiple hydroxyl groups and a carboxylic acid derivative.</p>
LA-105	 <p>Chemical structure of LA-105, a complex molecule featuring a pyridine ring, a long aliphatic chain, a piperazine ring, and a complex polycyclic system with multiple hydroxyl groups and a carboxylic acid derivative.</p>
LA-106	 <p>Chemical structure of LA-106, a complex molecule featuring a pyridine ring, a long aliphatic chain, a piperazine ring, and a complex polycyclic system with multiple hydroxyl groups and a carboxylic acid derivative.</p>
LA-107	 <p>Chemical structure of LA-107, a complex molecule featuring a pyridine ring, a long aliphatic chain, a piperazine ring, and a complex polycyclic system with multiple hydroxyl groups and a carboxylic acid derivative.</p>

LA No.	Structure
	
LA-108	
LA-109	
LA-110	
LA-111	

LA No.	Structure
	
LA-112	
LA-113	
LA-114	
LA-115	
LA-116	

LA No.	Structure
	 <p>Chemical structure of LA-116, showing a complex molecule with a central core containing a quinoline and a pyridine ring, substituted with a fluorine atom and a cyclopropyl group. The molecule is linked via a piperidine ring to a phenyl group, which is further connected to a chain containing a pyrrolidine ring, a carboxylic acid group, and a terminal amide group.</p>
LA-117	 <p>Chemical structure of LA-117, showing a complex molecule with a central core containing a quinoline and a pyridine ring, substituted with a fluorine atom and a cyclopropyl group. The molecule is linked via a piperidine ring to a phenyl group, which is further connected to a chain containing a pyrrolidine ring, a carboxylic acid group, and a terminal amide group.</p>
LA-118	 <p>Chemical structure of LA-118, showing a complex molecule with a central core containing a quinoline and a pyridine ring, substituted with a fluorine atom and a cyclopropyl group. The molecule is linked via a piperidine ring to a phenyl group, which is further connected to a chain containing a pyrrolidine ring, a carboxylic acid group, and a terminal amide group.</p>

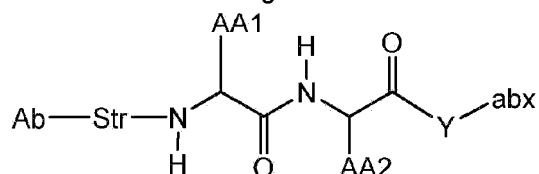
## EMBODIMENTS OF ANTIBODY-ANTIBIOTIC CONJUGATES

**[0233]** The S4497 antibody was linked to derivatives of rifamycin in Table 3 termed pipBOR and others via a protease cleavable, peptide linker. The linker is designed to be cleaved by lysosomal proteases including cathepsins B, D and others, which recognize peptide units, including the Valine-Citrulline (val-cit, vc) dipeptide (Dubowchik et al (2002) Bioconj. Chem. 13:855-869). Generation of the linker-antibiotic intermediate consisting of the antibiotic and the MC-vc-PAB linker and others, is described in detail in Examples 1-17. The linker is designed such that cleavage of the amide bond at the PAB moiety separates the antibody from the antibiotic in an active state.

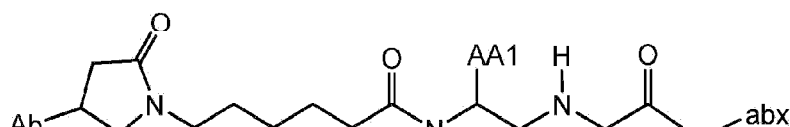
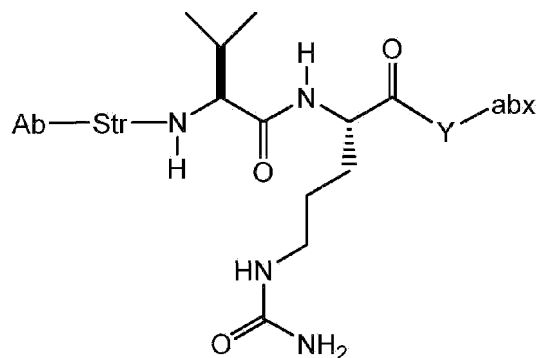
**[0234]** The AAC termed S4497-dimethyl-pipBOR is identical to the S4497-pipBOR AAC except for the dimethylated amino on the antibiotic and the oxycarbonyl group on the linker.

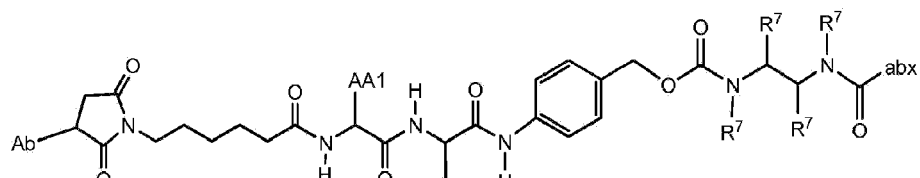
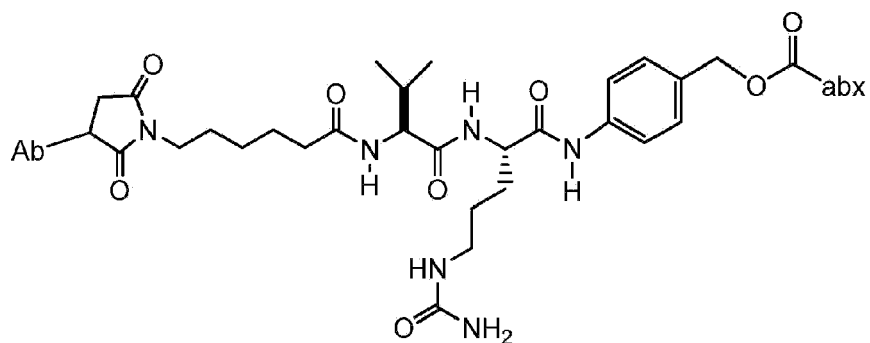
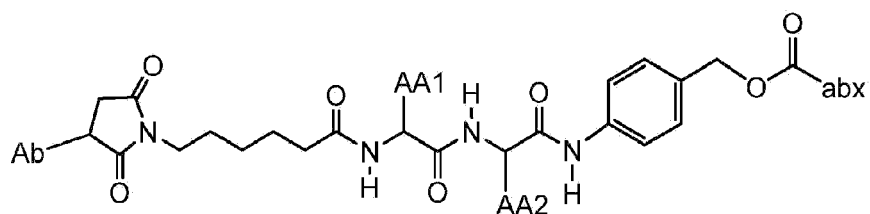
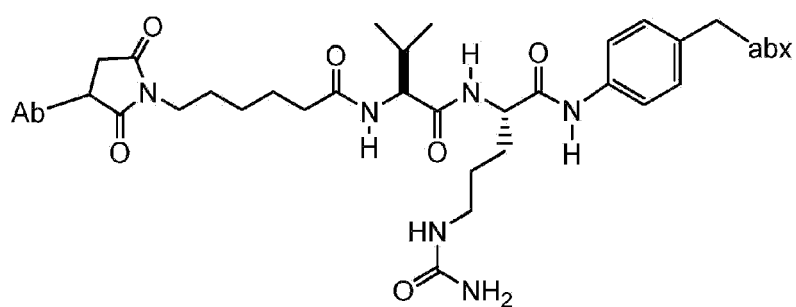
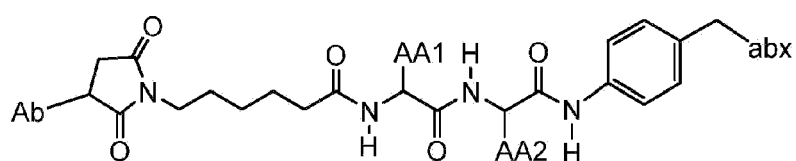
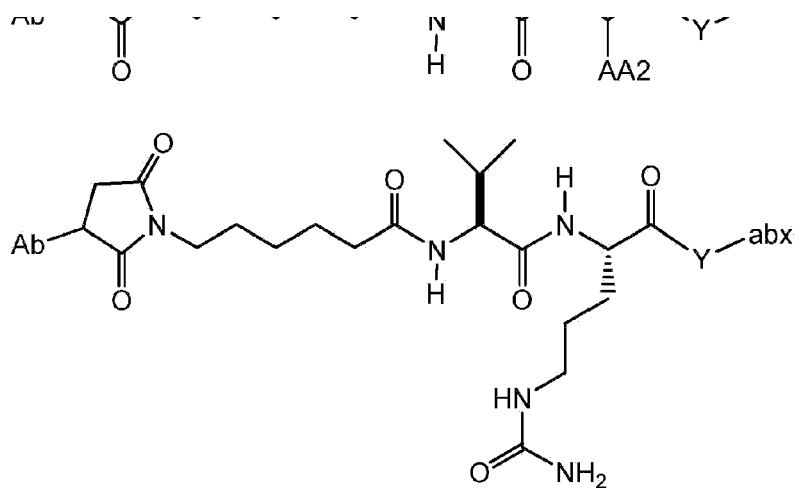
**[0235]** Figure 5 shows a possible mechanism of drug activation for antibody-antibiotic conjugates (AAC). Active antibiotic (Ab) is only released after internalization of the AAC inside mammalian cells. The Fab portion of the antibody in AAC binds *S. aureus* whereas the Fc portion of the AAC enhances uptake of the bacteria by Fc-receptor mediated binding to phagocytic cells including neutrophils and macrophages. After internalization into the phagolysosome, the Val-Cit linker is cleaved by lysosomal proteases releasing the active antibiotic inside the phagolysosome.

**[0236]** An embodiment of the antibody-antibiotic conjugate (AAC) compounds of the invention includes the following:

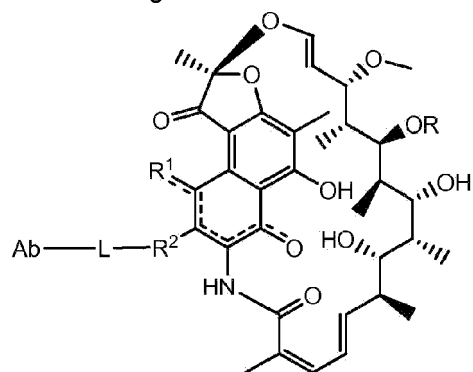


where AA1 and AA2 are independently selected from an amino acid side chain, including H, -CH<sub>3</sub>, -CH<sub>2</sub>(C<sub>6</sub>H<sub>5</sub>), -CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NH<sub>2</sub>, -CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NHC(NH)NH<sub>2</sub>, -CHCH(CH<sub>3</sub>)CH<sub>3</sub>, and -CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NHC(O)NH<sub>2</sub>; and including the formulas:.





[0237] An example of the antibody-antibiotic conjugate compounds of the invention includes the following:



wherein:

the dashed lines indicate an optional bond;

R is H, C<sub>1</sub>-C<sub>12</sub> alkyl, or C(O)CH<sub>3</sub>;

R<sup>1</sup> is OH;

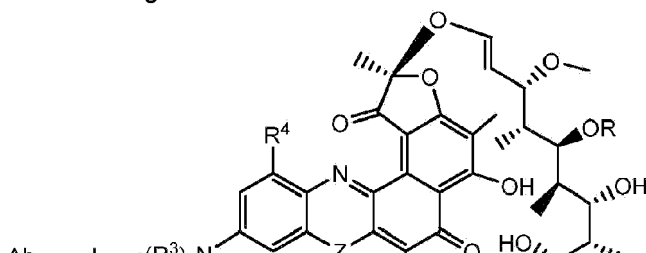
R<sup>2</sup> is CH=N-(heterocyclyl), wherein the heterocyclyl is optionally substituted with one or more groups independently selected from C(O)CH<sub>3</sub>, C<sub>1</sub>-C<sub>12</sub> alkyl, C<sub>1</sub>-C<sub>12</sub> heteroaryl, C<sub>2</sub>-C<sub>20</sub> heterocyclyl, C<sub>6</sub>-C<sub>20</sub> aryl, and C<sub>3</sub>-C<sub>12</sub> carbocyclyl;

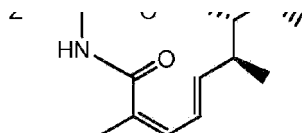
or R<sup>1</sup> and R<sup>2</sup> form a five- or six-membered fused heteroaryl or heterocyclyl, and optionally forming a spiro or fused six-membered heteroaryl, heterocyclyl, aryl, or carbocyclyl ring, wherein the spiro or fused six-membered heteroaryl, heterocyclyl, aryl, or carbocyclyl ring is optionally substituted H, F, Cl, Br, I, C<sub>1</sub>-C<sub>12</sub> alkyl, or OH;

L is the peptide linker attached to R<sup>2</sup> or the fused heteroaryl or heterocyclyl formed by R<sup>1</sup> and R<sup>2</sup>; and

Ab is the anti-wall teichoic acid (WTA) antibody.

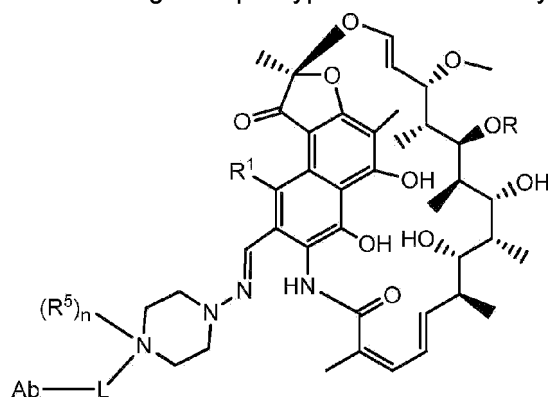
[0238] An example of the antibody-antibiotic conjugate compounds of the invention includes the following:





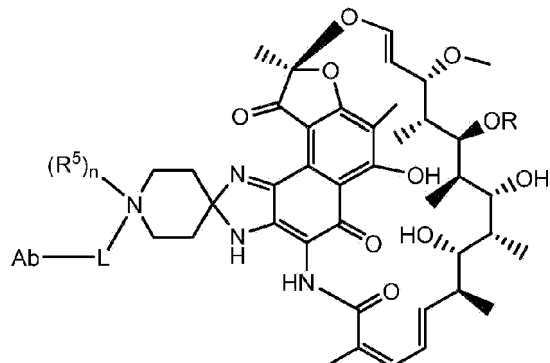
wherein  $R^3$  is independently selected from H and  $C_1$ - $C_{12}$  alkyl;  $n$  is 1 or 2;  $R^4$  is selected from H, F, Cl, Br, I,  $C_1$ - $C_{12}$  alkyl, and OH; and  $Z$  is selected from NH,  $N(C_1$ - $C_{12}$  alkyl), O and S.

**[0239]** An example of the antibody-antibiotic conjugate compounds of the invention includes the following rifampin-type antibiotic moiety:



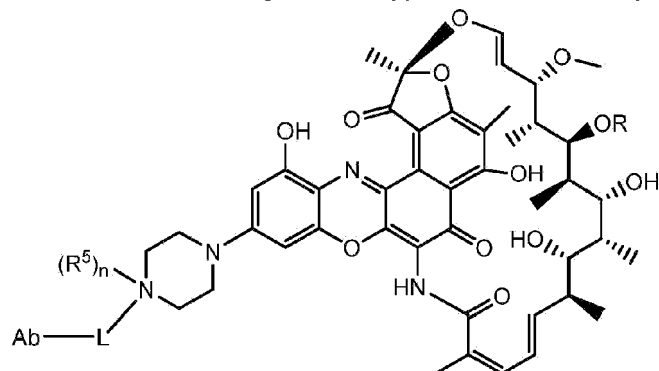
wherein  $R^5$  is selected from H and  $C_1$ - $C_{12}$  alkyl; and  $n$  is 0 or 1.

**[0240]** An example of the antibody-antibiotic conjugate compounds of the invention includes the following rifabutin-type antibiotic moiety:



wherein  $R^5$  is selected from H and  $C_1$ - $C_{12}$  alkyl; and  $n$  is 0 or 1.

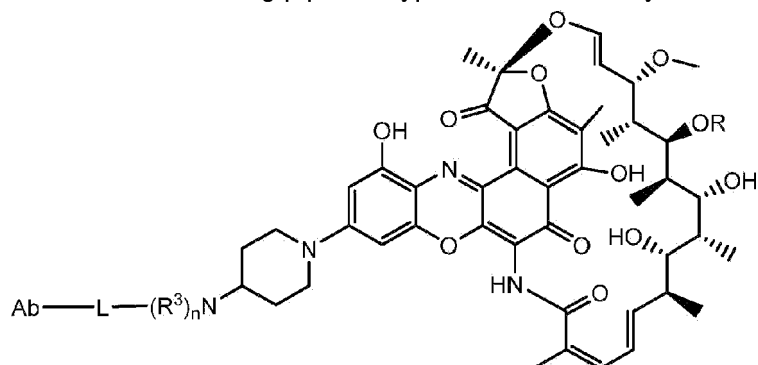
**[0241]** An embodiment of the antibody-antibiotic conjugate compounds of the invention includes the following rifalazil-type antibiotic moiety:





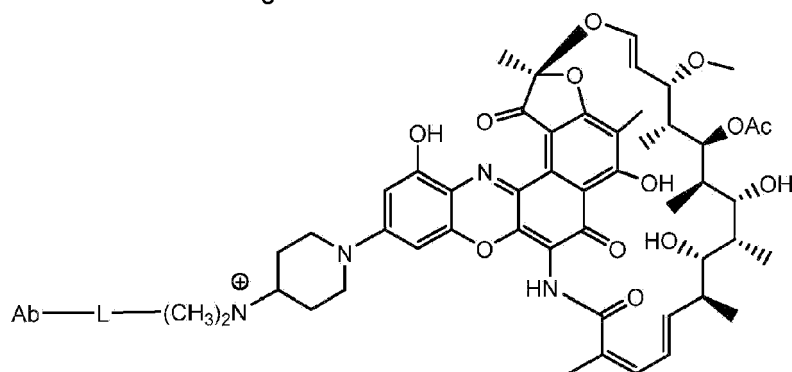
wherein  $R^5$  is independently selected from H and  $C_1$ - $C_{12}$  alkyl; and n is 0 or 1.

**[0242]** An embodiment of the antibody-antibiotic conjugate compounds of the invention includes the following pipBOR-type antibiotic moiety:

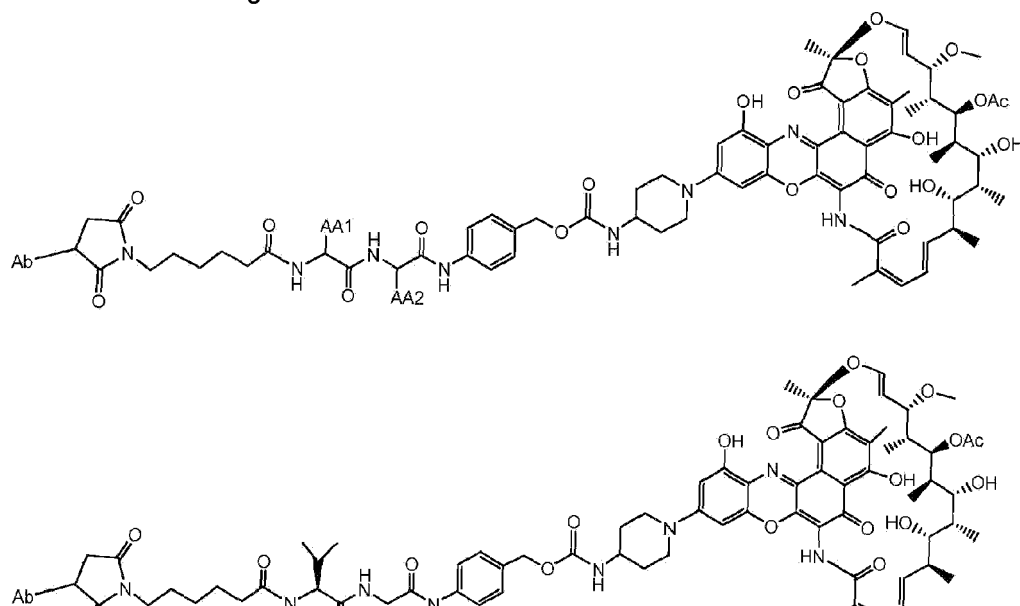


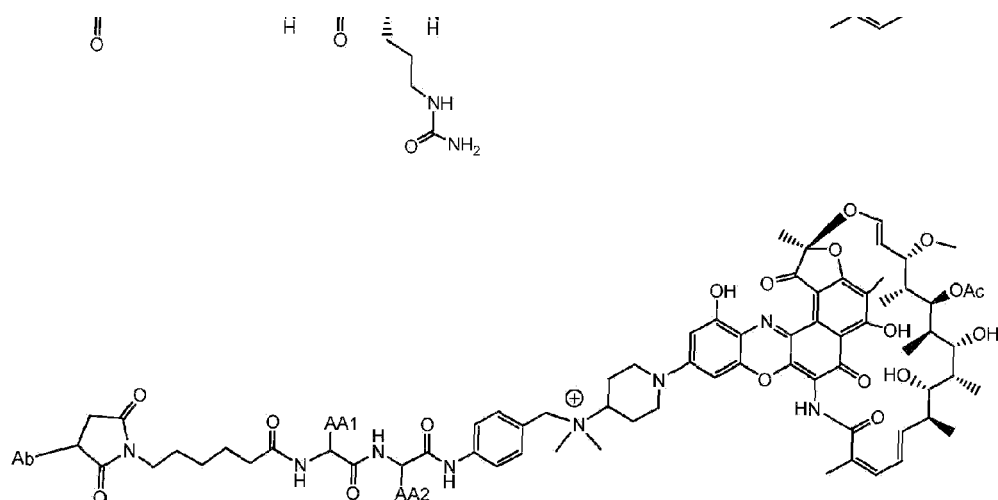
wherein  $R^3$  is independently selected from H and  $C_1$ - $C_{12}$  alkyl; and n is 1 or 2.

**[0243]** An embodiment of the antibody-antibiotic conjugate compounds of the invention includes the following:

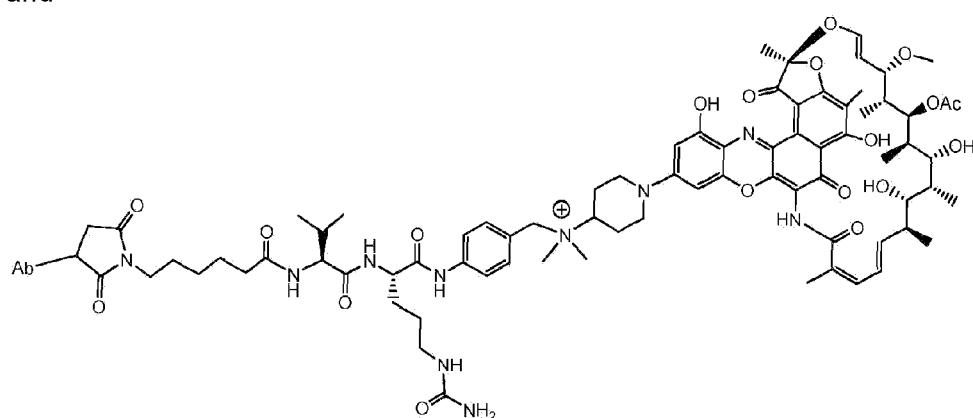


**[0244]** Further embodiments of the antibody-antibiotic conjugate compounds of the invention include the following:





and



## ANTIBIOTIC LOADING OF AAC

**[0245]** Antibiotic loading is represented by  $p$ , the average number of antibiotic (abx) moieties per antibody in a molecule of Formula I. Antibiotic loading may range from 1 to 20 antibiotic moieties ( $D$ ) per antibody. The AAC of Formula I include collections or a pool of antibodies conjugated with a range of antibiotic moieties, from 1 to 20. The average number of antibiotic moieties per antibody in preparations of AAC from conjugation reactions may be characterized by conventional means such as mass spectroscopy, ELISA assay, and HPLC. The quantitative distribution of AAC in terms of  $p$  may also be determined. In some instances, separation, purification, and characterization of homogeneous AAC where  $p$  is a certain value from AAC with other antibiotic loadings may be achieved by means such as reverse phase HPLC or electrophoresis.

**[0246]** For some antibody-antibiotic conjugates,  $p$  may be limited by the number of attachment sites on the antibody. For example, where the attachment is a cysteine thiol, as in the exemplary embodiments above, an antibody may have only one or several cysteine thiol groups, or may have only one or several sufficiently reactive thiol groups through which a linker

may be attached. In certain embodiments, higher antibiotic loading, e.g.  $p > 5$ , may cause aggregation, insolubility, toxicity, or loss of cellular permeability of certain antibody-antibiotic conjugates. In certain embodiments, the antibiotic loading for an AAC of the invention ranges from 1 to about 8; from about 2 to about 6; from about 2 to about 4; or from about 3 to about 5; about 4; or about 2.

**[0247]** In certain embodiments, fewer than the theoretical maximum of antibiotic moieties are conjugated to an antibody during a conjugation reaction. An antibody may contain, for example, lysine residues that do not react with the antibiotic-linker intermediate or linker reagent, as discussed below. Generally, antibodies do not contain many free and reactive cysteine thiol groups which may be linked to an antibiotic moiety; indeed most cysteine thiol residues in antibodies exist as disulfide bridges. In certain embodiments, an antibody may be reduced with a reducing agent such as dithiothreitol (DTT) or tricarboylethylphosphine (TCEP), under partial or total reducing conditions, to generate reactive cysteine thiol groups. In certain embodiments, an antibody is subjected to denaturing conditions to reveal reactive nucleophilic groups such as lysine or cysteine.

**[0248]** The loading (antibiotic/antibody ratio, "AAR") of an AAC may be controlled in different ways, e.g., by: (i) limiting the molar excess of antibiotic-linker intermediate or linker reagent relative to antibody, (ii) limiting the conjugation reaction time or temperature, and (iii) partial or limiting reductive conditions for cysteine thiol modification.

**[0249]** It is to be understood that where more than one nucleophilic group reacts with an antibiotic-linker intermediate or linker reagent followed by antibiotic moiety reagent, then the resulting product is a mixture of AAC compounds with a distribution of one or more antibiotic moieties attached to an antibody. The average number of antibiotics per antibody may be calculated from the mixture by a dual ELISA antibody assay, which is specific for antibody and specific for the antibiotic. Individual AAC molecules may be identified in the mixture by mass spectroscopy and separated by HPLC, e.g. hydrophobic interaction chromatography (see, e.g., McDonagh et al (2006) Prot. Engr. Design & Selection 19(7):299-307; Hamblett et al (2004) Clin. Cancer Res. 10:7063-7070; Hamblett, K.J., et al. "Effect of drug loading on the pharmacology, pharmacokinetics, and toxicity of an anti-CD30 antibody-drug conjugate," Abstract No. 624, American Association for Cancer Research, 2004 Annual Meeting, March 27-31, 2004, Proceedings of the AACR, Volume 45, March 2004; Alley, S.C., et al. "Controlling the location of drug attachment in antibody-drug conjugates," Abstract No. 627, American Association for Cancer Research, 2004 Annual Meeting, March 27-31, 2004, Proceedings of the AACR, Volume 45, March 2004). In certain embodiments, a homogeneous AAC with a single loading value may be isolated from the conjugation mixture by electrophoresis or chromatography. Cysteine-engineered antibodies of the invention enable more homogeneous preparations since the reactive site on the antibody is primarily limited to the engineered cysteine thiol. In one embodiment, the average number of antibiotic moieties per antibody is in the range of about 1 to about 20. In some embodiments the range is selected and controlled from about 1 to 4.

## METHODS OF PREPARING ANTIBODY-ANTIBIOTIC CONJUGATES

**[0250]** An AAC of Formula I may be prepared by several routes employing organic chemistry reactions, conditions, and reagents known to those skilled in the art, including: (1) reaction of a nucleophilic group of an antibody with a bivalent linker reagent to form Ab-L via a covalent bond, followed by reaction with an antibiotic moiety (abx); and (2) reaction of a nucleophilic group of an antibiotic moiety with a bivalent linker reagent, to form L-abx, via a covalent bond, followed by reaction with a nucleophilic group of an antibody. Exemplary methods for preparing an AAC of Formula I via the latter route are described in US 7498298 .

**[0251]** Nucleophilic groups on antibodies include, but are not limited to: (i) N-terminal amine groups, (ii) side chain amine groups, e.g. lysine, (iii) side chain thiol groups, e.g. cysteine, and (iv) sugar hydroxyl or amino groups where the antibody is glycosylated. Amine, thiol, and hydroxyl groups are nucleophilic and capable of reacting to form covalent bonds with electrophilic groups on linker moieties and linker reagents including: (i) active esters such as NHS esters, HOBt esters, haloformates, and acid halides; (ii) alkyl and benzyl halides such as haloacetamides; (iii) aldehydes, ketones, carboxyl, and maleimide groups. Certain antibodies have reducible interchain disulfides, i.e. cysteine bridges. Antibodies may be made reactive for conjugation with linker reagents by treatment with a reducing agent such as DTT (dithiothreitol) or tricarboylethylphosphine (TCEP), such that the antibody is fully or partially reduced. Each cysteine bridge will thus form, theoretically, two reactive thiol nucleophiles. Additional nucleophilic groups can be introduced into antibodies through modification of lysine residues, e.g., by reacting lysine residues with 2-iminothiolane (Traut's reagent), resulting in conversion of an amine into a thiol. Reactive thiol groups may be introduced into an antibody by introducing one, two, three, four, or more cysteine residues (e.g., by preparing variant antibodies comprising one or more non-native cysteine amino acid residues).

**[0252]** Antibody-antibiotic conjugates of the invention may also be produced by reaction between an electrophilic group on an antibody, such as an aldehyde or ketone carbonyl group, with a nucleophilic group on a linker reagent or antibiotic. Useful nucleophilic groups on a linker reagent include, but are not limited to, hydrazide, oxime, amino, hydrazine, thiosemicarbazone, hydrazine carboxylate, and arylhydrazide. In one embodiment, an antibody is modified to introduce electrophilic moieties that are capable of reacting with nucleophilic substituents on the linker reagent or antibiotic. In another embodiment, the sugars of glycosylated antibodies may be oxidized, e.g. with periodate oxidizing reagents, to form aldehyde or ketone groups which may react with the amine group of linker reagents or antibiotic moieties. The resulting imine Schiff base groups may form a stable linkage, or may be reduced, e.g. by borohydride reagents to form stable amine linkages. In one embodiment, reaction of the carbohydrate portion of a glycosylated antibody with either galactose oxidase or sodium meta-periodate may yield carbonyl (aldehyde and ketone) groups in the antibody that can react with appropriate groups on the antibiotic (Hermanson, Bioconjugate Techniques). In another embodiment, antibodies containing N-terminal serine or threonine residues can react with sodium meta-

periodate, resulting in production of an aldehyde in place of the first amino acid (Geoghegan & Stroh, (1992) Bioconjugate Chem. 3:138-146; US 5362852). Such an aldehyde can be reacted with an antibiotic moiety or linker nucleophile.

**[0253]** Nucleophilic groups on an antibiotic moiety include, but are not limited to: amine, thiol, hydroxyl, hydrazide, oxime, hydrazine, thiosemicarbazone, hydrazine carboxylate, and arylhydrazide groups capable of reacting to form covalent bonds with electrophilic groups on linker moieties and linker reagents including: (i) active esters such as NHS esters, HOBt esters, haloformates, and acid halides; (ii) alkyl and benzyl halides such as haloacetamides; (iii) aldehydes, ketones, carboxyl, and maleimide groups.

**[0254]** The antibody-antibiotic conjugates (AAC) in Table 3 were prepared by conjugation of the described anti-WTA antibodies and linker-antibiotic intermediates of Table 2, and according to the described methods in Example 24. AAC were tested for efficacy by *in vitro* macrophage assay (Example 18) and *in vivo* mouse kidney model (Example 19).

**Table 3** Antibody-antibiotic conjugates (AAC)

AAC No.	AAC formula	linker-abx LA No. (Table 2)	AAR *
AAC-101	thio-trastuzumab HC A118C-MC-vc-PAB-(dimethyl-pipBOR)	LA-54	1.8
AAC-102	thio-S4497-HC-A118C-MC-vc-PABC-(pipBOR)	LA-51	1.9
AAC-103	thio-S4497-HC-A114C-MC-fk-PABC-(pipBOR)	LA-52	1.0
AAC-104	thio-S4497-HC-A114C-MP-vc-PABC-(pipBOR)	LA-53	1.8
AAC-105	thio-S4497-HC-A118C-MC-vc-PAB-(dimethylpipBOR)	LA-54	1.73
			1.9
AAC-106	thio-S4462-HC-A118C-MC-vc-PAB-(dimethylpipBOR)	LA-54	
AAC-107	thio-S4497-HC-A118C-MC-vc-PABC-(monomethylpip, desacetylBOR)	LA-55	1.75
AAC-108	thio-S4497-HC-A118C-MC-vc-PABC-(monomethylpipBOR)	LA-56	1.5
AAC-109	thio-S4497-HC-A118C-MC-vc-PABC-(pip, desacetylBOR)	LA-57	1.9
AAC-110	thio-hu-anti gD 5B5-HC-A118C-MC-vc-PAB-(dimethylpipBOR)	LA-54	1.94
AAC-111	thio-S4497-HC-A118C-MC-vc-PABC-(rifabutin)	LA-58	1.6
AAC-112	thio-S4497-HC-A118C-MC-vc-PAB-(dimethyl-pipBOR)	LA-54	1.65
AAC-113	thio-S4497 HC-MC-GGAFAGGG-(pipBOR) ("core peptide" disclosed as SEQ ID NO: 126)	LA-59	1.6
AAC-114	thio-S4462 HC-MC-GGAFAGGG-(pipBOR)	LA-59	1.8

AAC No.	AAC formula	linker-abx LA No. (Table 2)	AAR *
	("core peptide" disclosed as SEQ ID NO: 126)		
AAC-115	thio-Tmab LC-MC-GGAFAGGG-(pipBOR) ("core peptide" disclosed as SEQ ID NO: 126)	LA-59	1.7
AAC-116	thio-S7578-MC-vc-PAB-(dimethyl-pipBOR)	LA-54	tbd
AAC-117	thio-S4497-HC-A118C-MC-vc-PABC-(pipBOR)	LA-51	tbd
AAC-118	thio-S4497-HC-A118C-MC-vc-PAB-(oxyBOR)	LA-60	1.8
AAC-119	thio-S4497-HC-A118C-MC-vc-PAB-(dimethylpip, desacetylBOR)	LA-61	1.7
AAC-120	thio-S4497-HC-A118C-MC-vc-PABC- (piperazBTR)	LA-62	1.8
AAC-121	thio-S4497-HC-A118C-MC-vc-PABC-(piperaz, desacetylBTR)	LA-63	1.8
AAC-122	thio-S4497-HC-A118C-MC-vc-PAB- (dimethylpipBOR)	LA-54	1.9
AAC-123	thio-S4497-HC-A118C-MC-vc-PAB- (dimethylpipBOR)	LA-54	1.9
AAC-124	thio-S4497-HC-A118C-MC-vc-PABC- (piperazBTR)	LA-62	1.8
AAC-125	thio-S4497-HC-A118C-MC-vc-PABC-(piperaz, desacetylBOR)	LA-64	0.9
AAC-126	thio-S4497-HC-A118C-MC-vc-PABC- (piperazBOR)	LA-65	1.7
AAC-127	thio-S4497-HC-A118C-MC-vc-PABC-PAB- (dimethylpipBOR)	LA-66	1.7
AAC-128	thio-S4497-HC-A118C-MC-vc-PAB- (methylpiperaz BOR)	LA-67	1.9
AAC-129	thio-S6078-HC A114C-LCWT-MC-vc-PAB- (dimethylpipBOR)	LA-54	1.6
AAC-130	thio-S4497-HC-A118C-MC-vc-PAB-(oxy, isopropylpipBOR)	LA-68	1.8
AAC-131	thio-S4497-HC-A118C-MC-tpm-cit-PAB- (dimethylpipBOR)	LA-69	1.8
AAC-132	thio-S4497 HC MC-GPIImLFF-(pipBOR) ("core peptide" disclosed as SEQ ID NO: 129)	LA-69	1.3
AAC-133	thio-S4497 HC MC-GPILFF-(pipBOR) ("core peptide" disclosed as SEQ ID NO: 130)	LA-70	1.2
AAC-134	thio-S4497 HC MC-val-cit-phe-(pipBOR)	LA-71	1.7
AAC-135	thio-S4497-HC-A118C-MC-vc-PAB-	LA-54	1.9

AAC No.	AAC formula	linker-abx LA No. (Table 2)	AAR *
	(dimethylpipBOR)		
AAC-136	thio-S4497.v1 HC WT, LC V205C- MC-vc-PAB- (dimethylpipBOR)	LA-54	2
AAC-137	thio-S4497.v1 HC WT, LC V205C-MC-vc-PAB- (piperazBOR)	LA-65	tbd
AAC-138	thio-S4497 HC WT v8, LC V205C- MC-vc-PAB- (dimethylpipBOR)	LA-54	1.9
AAC-139	thio-S4497-HC-A118C-MC-vc-PAB- (dimethylpipBOR)	LA-54	1.8
AAC-140	thio-S4497 HC WT (v8), LC V205C-MC-vc-PAB- (piperazBOR)	LA-65	1.6
AAC-141	thio-S6078-HCA114C-LCWT-MC-vc-PAB- (dimethylpipBOR)	LA-54	1.6
AAC-142	thio-S4497 HC WT (v8), LC V205C-MC-vc-PAB- (dimethylpipBOR)	LA-54	1.7
AAC-143	thio-S4497 HC-MP-GGAFA-PAB-(pipBOR) ("core peptide" disclosed as SEQ ID NO: 131)	LA-87	1.55
AAC-144	thio-S4497 v1HC-MC-vc-PAB-(phenylpipBOR)	LA-72	1.7
AAC-145	thio-S4497 v1HC-MC-vc-PAB-(dimethylBTR)	LA-73	1.7
AAC-146	thio-Tmab HC A118C-MP-GGAFA-PABC- (pipBOR) ("core peptide" disclosed as SEQ ID NO: 131)	LA-87	1.3
AAC-147	thio-S4497 v1HC-MC-vc-PAB-(dimethylpipBOR)	LA-74	1.9
AAC-148	thio-S4497v1 HC-MC-vc-PABC-(pipBTR)	LA-75	1.9
AAC-149	thio-S4497v1 HC-MC-vc-PAB-(methylpiperaz, desacetylBOR)	LA-76	2
AAC-150	thio-S4497v1 HC-MC-vc-(phenylpipBOR)	LA-77	1.8
AAC-151	thio-S4497-HC-A118C-MC-vc-PAB-(3- dimethylaminopyrrolBOR)	LA-78	tbd
AAC-152	thio-S4497-HC-A118C-MC-vc-PAB-(O-methyl, dimethylpipBOR)	LA-79	1.7
AAC-153	thio-S4497-HC-A118C-MC-vc-PABC- (phenylpipBOR)	LA-80	tbd
AAC-154	thio-S4497v1 HC WT, LC V205C-MC-vc-PAB- (dimethylpipBOR)	LA-54	1.8
AAC-155	thio-S7578-HC WT-LC V205C-MC-vc-PAB- (dimethylpipBOR)	LA-54	1.9
AAC-156	thio-S4497v8-LC-MC-vc-PAB-(3-	LA-78	tbd

AAC No.	AAC formula	linker-abx LA No. (Table 2)	AAR *
	dimethylaminopyrrolBOR)		
AAC-157	thio-S4497v8-LC-MC-vc-PABC-(desacetyl, pipBTR)	LA-81	2.2
AAC-158	thio-S4497v8-LC-MC-vc-(desacetyl, phenylpipBOR)	LA-82	-
AAC-159	thio-S4497v8-LC-MC-vc-PAB- (dimethylamino,methylaminoethylBTR)	LA-83	2.2
AAC-160	thio-S4497v8-LC-MC-vc-PAB- (methylpiperazBTR)	LA-84	tbd
AAC-161	thio-S4497v8-LC-MC-vc-(phenylpipBOR)	LA-85	tbd
AAC-162	thio-S4497v8-LC-MC-vc-PAB-(oxy, dimethylaminopipBOR)	LA-86	tbd
AAC-163	thio-S4497-v8-LCV205C-MC-LAFG-PAB- (dimethylamino-3-pyrroloBOR) ("core peptide" disclosed as SEQ ID NO: 128)	LA-88	2.2
AAC-164	thio-S4497-v8-LCV205C-MC-vc- PABpyrazolo(pipBOR)	LA-89	tbd
AAC-165	thio-S4497-v8-LCV205C-MC-vc-PAB- (monomethylaminopipBOR)	LA-90	1.3
AAC-166	thio-S4497 HC WT (v8), LC V205C-MC-vc-PAB- (dimethylpipBOR)	LA-54	1.8
AAC-167	thio-S4497 HC WT (v8), LC V205C-MC-vc-PAB- (methyl,ethylaminopipBOR)	LA-91	2.0
AAC-168	thio-S4497 HC WT (v8), LC V205C-MC-vc- PABC-(aminomethylpipBOR)	LA-92	1.6
AAC-169	thio-S4497 WT (V8), LC V205C-MC-vc-PABC- C21,C23-phenylacetal-(dimethylaminopipBOR)	LA-93	1.6
AAC-170	thio-S4497 WT (V8), LC V205C-MC-vc- PABCpip-(pipBOR)	LA-94	1.4
AAC-171	thio-S6078 v4 HC-Cys LC-Cys-MC-vc-PAB- (dimethylpipBOR)	LA-54	3.9
AAC-172	thio-S6078 v4 HC-CYS, LC-CYS-MC-vc-PAB- (piperazBOR)	LA-65	3.9
AAC-173	thio-S6078 v4 HC-WT, LC-Cys-MC-vc-PAB- (dimethylpipBOR)	LA-54	2.0
AAC-174	thio-S6078.v4.HC-WT, LC-CYS-MC-vc-PAB- (piperazBOR)	LA-65	1.8
AAC-175	thio-S4497 HC WT (v8), LC V205C-MP- hydrazidePP-(pipBor)	LA-95	2.0



AAC No.	AAC formula	linker-abx LA No. (Table 2)	AAR *
AAC-176	thio-S4497 HC WT (v8), LC V205C-MC-vc-PABC-(azetidinyIBOR)	LA-96	tbd
AAC-177	thio-S4497-v8-LCV205C-MC-vc-PABC-(ethylpiperazino, desacetylBOR)	LA-97	1.6
AAC-178	thio-S4497-v8-LCV205C-MC-vc-PABC-(ethylaminopiperazinoBOR)	LA-98	1.7
AAC-179	thio-S4497-v8-LCV205C-MC-vc-PABphenyl-(pipBOR)	LA-99	1.9
AAC-180	thio-S4497-v8-LCV205C-MC-vc-PAB-(fluoroquinolone, oxyBOR)	LA-100	1.9
AAC-181	thio-S4497-v8-LCV205C-MC-vc-PAB-(phenoxypip, oxyBOR)	LA-101	1.6
AAC-182	thio-S6078 v4 HC-CYS, LC-CYS-MC-vc-PAB-(oxy, dimethylaminopipBOR)	LA-86	4.0
AAC-183	thio-S6078 v4 HC-CYS, LC-CYS-MC-vc-PABC-(desacetyl, pipBTR)	LA-81	3.8
AAC-184	thio-S6078 v4 HC-CYS, LC-CYS-MC-vc-PAB-(oxy, isopropylpipBOR)	LA-68	tbd
AAC-185	thio-S6078 v4 HC-CYS, LC-CYS-MC-vc-PAB-(methylrifampicin)	LA-102	tbd
AAC-186	thio-S4497 HC WT (v8), LC V205C-MC-vc-PAB-(dimethylpipBOR)	LA-54	1.9
AAC-187	thio-S6078 v4 HC-CYS, LC-CYS-MC-vc-PAB-(dimethylpipBOR)	LA-54	3.8
AAC-188	thio-S6078 v4 HC-WT, LCCYS-MC-vc-PAB-(oxy, isopropylpipBOR)	LA-68	1.6
AAC-189	thio-S6078 v4 HC-WT, LC-CYS-MC-vc-PAB-(methylrifampicin)	LA-102	1.8
AAC-190	thio-S7578-HC-WT-LC-Cys-MC-vc-PAB-(dimethylpipBOR)	LA-54	1.9
AAC-191	thio-S4497-v8-LC-V205C-MC-vc-PAB-(dimethylaminoethylpiperazinoBOR)	LA-103	1.7
AAC-192	thio-S4497 HC WT (v8), LC V205C-MC-vc-PAB-(dimethylpipBOR)	LA-54	1.9
AAC-193	thio-S4497 HC v1-MP-LAFG-PABC-(piperazinoBOR) ("core peptide" disclosed as SEQ ID NO: 128)	LA-104	1.8
AAC-194	thio-S6078 v4 HC-WT, LC-CYS-MC-vc-PAB-(piperazBOR)	LA-65	1.8

AAC No.	AAC formula	linker-abx LA No. (Table 2)	AAR *
AAC-195	thio-S4497-v8-LC V205C-MC-vc-C21,C23-anilinoacetal-(dimethylaminopipBOR)	LA-105	2.0
AAC-196	thio-S4497-v8-LC V205C-MC-vc-anilino-(trimethylammonium-pip, oxyBOR)	LA-106	2.1
AAC-197	thio-S4497-v8-LC V205C-MC-vc-PAB-(dimethylammonium, fluoropipBOR)	LA-107	2.0
AAC-198	thio-S4497-v8-LC V205C-MC-vc-PAB-(dimethylammonium, thiopropyl, desacetyl BOR)	LA-108	1.9
AAC-199	thio-S4497-v8-LC V205C-MC-vc-PAB-(dimethylammonium, methylaminopropylBOR)	LA-109	1.8
AAC-200	thio-S4497-v8-LC V205C-MC-vc-PAB-(dimethylpipBOR)	LA-54	1.0
AAC-201	thio-S4497-v8-LC V205C-MC-vc-PABC-(methylaminopip, desacetyl BOR)	LA-110	1.9
AAC-202	thio-S4497-v8-LC V205C-MC-vc-PAB-(oxy, pip, desacetyl BOR)	LA-111	1.9
AAC-203	thio-S4497-v8-LC V205C-MC-vc-PAB-(methylrifampicin)	LA-102	1.8
AAC-204	thio-S4497-v8-LC-cys-MC-vc-PAB-(dimethylammonium, thiopropyl BOR)	LA-112	2.0
AAC-205	thio-S4497-v8-LC V205C-MC-vc-PAB-(oxy, dimethylaminopipBOR)	LA-113	1.8
AAC-206	thio-S4497-v8-LC V205C-MC-vc-PAB-(N-isobutylrifabutin)	LA-114	1.9
AAC-207	thio-S4497-v8-LC V205C-MC-vc-PAB-(methylrifampicin)	LA-102	1.8
AAC-208	thio-S4497-v8-LC V205C-MC-vc-PAB-(dimethylammonium, fluoropipBOR)	LA-107	1.8
AAC-209	thio-S4497-v8-LC cys-D10-MC-vc-PAB-(dimethylpipBOR)	LA-54	1.9
AAC-210	thio-S4497-v8-LC cys-MC-vc-PABC-(monomethylpipBTR)	LA-115	1.5
AAC-211	thio-S4497-v8-LC cys-MC-vc-PAB-(piperazinoBOR)	LA-116	tbd
AAC-212	thio-S4497-v8-LC cys-MC-vc-PAB-(R-fluoroquinolone, oxyBOR)	LA-117	tbd
AAC-213	thio-S4497-v8-LC cys-MC-vc-PAB-(S-fluoroquinolone, oxyBOR)	LA-118	tbd

AAC No.	AAC formula	linker-abx LA No. (Table 2)	AAR *
AAC-214	thio-S4497-v8-LC-cys-MC-vc-PAB-(dimethylpip, desacetylBOR)	LA-61	2.0
AAC-215	thio-S4497 LC v8-MP-LAFG-PABC-(piperazinoBOR) ("core peptide" disclosed as SEQ ID NO: 128)	LA-104	1.6
AAC-216	thio-S4497 HC WT (v8), LC V205C-MC-vc-PAB-(piperazBOR)	LA-65	1.9
AAC-217	thio-S4497-v8-LCV205C-MC-vc-PAB-(monomethylaminopipBOR)	LA-90	1.8
AAC-218	thio-S4497 HC WT (v8), LC V205C-MC-vc-PAB-(dimethylpipBOR)	LA-54	1.9
AAC-219	thio-S4497 HC WT (v8), LC V205C-MC-vc-PABC-(piperazBOR)	LA-65	1.9
AAC-220	thio-S4497-v8-LCV205C-MC-vc-PAB-(piperazinoBOR)	LA-116	tbd
AAC-221	thio-S4497-v8-LCV205C-MC-vc-PAB-(methylpiperaz BOR)	LA-67	tbd
AAC-222	thio-S4497 LC v8 -MP-LAFG-PABC-(piperazinoBOR) ("core peptide" disclosed as SEQ ID NO: 128)	LA-104	tbd
<p>* AAR = antibiotic/antibody ratio average  Wild-type ("WT"), cysteine engineered mutant antibody ("thio"), light chain ("LC"), heavy chain ("HC"), 6-maleimidocaproyl ("MC"), maleimidopropanoyl ("MP"), valine-citrulline ("val-cit" or "vc"), alanine-phenylalanine ("ala-phe"), p-aminobenzyl ("PAB"), and p-aminobenzyloxycarbonyl ("PABC")  tbd = to be determined  HC-A114C (Kabat) = HC-A118C (EU)</p>			

#### IN VITRO ANALYSIS DEMONSTRATING THAT AAC KILL INTRACELLULAR MRSA

**[0255]** *In vitro* experiments confirm that the AAC release active antibiotic only after the linker between the antibody and the antibiotic is cleaved by an appropriate enzyme such as cathepsin B. MRSA was cultured overnight in normal bacterial growth media and up to 10 µg/mL of AAC. Incubation of MRSA with the S4497-pipBOR or S4497-dimethyl-pipBOR AACs did not result in inhibition of bacterial growth unless the AACs were pre-treated with cathepsin B to release the active antibiotic. An *in vitro* assay utilizing murine peritoneal macrophages confirmed that AAC release active antibiotic and kill MRSA inside phagocytic cells (Example 18). An AAC comprising antibody rF1, which binds to a family of cell wall associated proteins was conjugated to a rifamycin derivative. *S. aureus* (Newman strain) was treated with various

doses of the rF1-AAC or with equivalent doses of either antibody alone, rifampicin alone or a mixture of antibody and free rifampicin to permit antibody binding to the bacteria (opsonization) and after 1 hour incubation the opsonized bacteria were fed to macrophages (Figure 7A).

**[0256]** Figure 7A shows an *in vitro* macrophage assay demonstrating that AAC kill intracellular MRSA. *S. aureus* (Newman) was incubated with rF1 antibody alone, free rifampicin alone, a simple mixture of the rF1 antibody plus free rifampicin combined at the same ratio of antibody to antibiotic found in the AAC, or the rF1-AAC for 1 hour and added to murine macrophages. Macrophages were incubated at 37 °C for 2 hours to permit phagocytosis. After phagocytosis was complete, the infection mix was replaced with normal growth media supplemented with 50 µg/mL of gentamycin to inhibit the growth of extracellular bacteria and the total number of surviving intracellular bacteria was determined 2 days after infection by plating.

**[0257]** The macrophages were infected for 2 hours and the infection was removed and replaced with media containing gentamycin to kill any remaining extracellular bacteria that were not taken up by the macrophages. After 2 days, macrophages were lysed and the total number of surviving intracellular bacteria was determined by plating on agar plates. Analysis revealed that treatment with the AAC resulted in more than 100 fold reduction in the number of intracellular bacteria compared to treatment with a simple mixture of the rF1 antibody plus free rifampicin combined at the same antibody to antibiotic ratio found in the AAC (Figure 7A).

**[0258]** MRSA is able to invade a number of non-phagocytic cell types including osteoblasts and various epithelial and endothelial cell types (Garzoni and Kelly, (2008) Trends in Microbiology). MRSA is able to infect an osteoblast cell line (MG63), an airway epithelial cell line (A549) and primary cultures of human umbilical vein endothelial cells (HUVEC). Figure 7B shows intracellular killing of MRSA (USA300 strain) with 50 µg/mL of S4497-pipBOR AAC **102** in macrophages, osteoblasts (MG63), Airway epithelial cells (A549), and human umbilical vein endothelial cells (HUVEC) where naked, unconjugated antibody S4497 does not. These cell types likely express lower overall levels of cathepsin B than professional phagocytic cells such as macrophages, however MRSA treated with 50 µg/mL the was effectively killed after internalization into all three of these cell lines. The dashed line indicates the limit of detection for the assay.

**[0259]** *In vitro* analysis was performed to compare the activity of AAC made with variations in the linker that joins the antibody to the antibiotic. The S4497-dimethyl-pipBOR AAC is more potent than the S4497-pipBOR AAC in the macrophage intracellular killing assay. The S4497-pipBOR AAC and the S4497-dimethyl-pipBOR AAC were titrated to determine the minimum effective dose in our macrophage intracellular killing assay (Figure 7C). Treatment with at least 2 µg/mL of AAC may be necessary to achieve optimal clearance of intracellular bacteria.

**[0260]** Figure 7C shows comparison of AAC made with pipBOR **51** vs. dimethyl-pipBOR (diMe-pipBOR) **54**. MRSA was opsonized with S4497 antibody alone or with AACs: S4497-pipBOR **102** or S4497-diMethyl-pipBOR **105** at various concentrations ranging from 10 µg/mL to 0.003 µg/mL. These data revealed that for both AAC, optimal killing occurred when AAC were tested

at more than 2 µg/mL, with a dose dependent loss in activity that became evident at 0.4 µg/mL. The overall level of killing was significantly superior with the S4497 dimethyl-pipBOR AAC **105**. Treatment with higher doses of the S4497-dimethyl-pipBOR AAC **105** eliminated the intracellular bacteria to below the limit of detection and over 300 fold killing using a suboptimal dose of .4 µg/mL of AAC was observed.

**[0261]** Figure 7D shows AAC kills intracellular bacteria without harming the macrophages. The USA300 strain of *S. aureus* was pre-incubated with 50 µg/mL of the S4497 anti-*S. aureus* antibody (antibody) or with 50 µg/mL of thio-S4497-HC-A118C-MC-vc-PAB-dimethylpipBOR **105** AAC, for 1 hour to permit binding of antibody to the bacteria. Opsonized bacteria were added to murine peritoneal macrophages at a multiplicity of infection of 10-20 bacteria per macrophage and incubated at 37 °C for 2 hours to permit phagocytosis. After phagocytosis was complete, free bacteria were removed and the macrophages were cultured for 2 days in normal growth media supplemented with 50 µg/mL of gentamycin to kill non-internalized bacteria. At the end of the culture period, survival of macrophages was assessed by detecting release of cytoplasmic lactate dehydrogenase (LDH) into the culture supernatant. The total amount of LDH released from each well was compared to control wells containing macrophages that were lysed by addition of detergent to the wells. The extent of macrophage cell lysis in wells treated with detergent, uninfected macrophages, macrophages infected with USA300 pre-opsonized with S4497 antibody or macrophages infected with USA300 pre-opsonized with thio-S4497-HC-A118C-MC-vc-PAB-dimethylpipBOR **105** AAC was measured.

**[0262]** Figure 7E shows recovery of live USA300 from inside macrophages from the macrophage cell lysis above. Macrophages were lysed and serial dilutions of the cell lysate were plated to enumerate the number of surviving intracellular bacteria.

**[0263]** Figure 9 shows a growth inhibition assay demonstrating that AAC are not toxic to *S. aureus* unless the linker is cleaved by cathepsin B. A schematic cathepsin release assay (Example 20) is shown on the left. AAC is treated with cathepsin B to release free antibiotic. The total amount of antibiotic activity in the intact vs. the cathepsin B treated AAC is determined by preparing serial dilutions of the resulting reaction and determining the minimum dose of AAC that is able to inhibit the growth of *S. aureus*. The upper right plot shows the cathepsin release assay for thio-S4497-HC-A118C-MC-vc-PAB-pipBOR **102** and the lower right plot shows the cathepsin release assay for thio-S4497-HC-A118C-MC-vc-PAB-dimethylpipBOR **105**.

#### IN VIVO EFFICACY OF ANTIBODY ANTIBIOTIC CONJUGATES:

**[0264]** An *in vivo* peritonitis model in mice was established to test the efficacy of AAC. In this model, mice are infected by intraperitoneal injection (I.P.) of MRSA and the bacterial load is monitored 2 days after infection in the peritoneal fluid and kidney. Bacteria harvested from the peritoneum could be found either as free floating extracellular bacteria or internalized inside peritoneal cells - primarily neutrophils and macrophages - that are recruited to the site of the

infection. Although extracellular bacteria identified in this model appeared to be sensitive to antibiotic treatment, the intracellular bacteria were shown to be unresponsive to treatment with a number of clinically relevant antibiotics including rifampin (Sandberg et al (2009) Antimicrobial Agents Chemother) and therefore appeared to be an excellent target to test efficacy of our AAC.

**[0265]** Figure 8A shows *in vivo* efficacy of the S4497-pipBOR AAC **102**. Intraperitoneal infection model in A/J mice. Mice were infected with  $5 \times 10^7$  CFU of MRSA by intraperitoneal injection and treated with 50 mg/Kg of S4497 antibody alone or with 50 mg/Kg of the S4497-pipBOR AAC **102** by intraperitoneal injection (protocol 11-2032A). Mice were sacrificed 2 days post infection and the total bacterial load was assessed in the peritoneal supernatant (Extracellular bacteria), peritoneal cells (Intracellular bacteria) or in the kidney.

**[0266]** A/J mice were infected with USA300 and administered 50 mg/Kg of either S4497 antibody or S4497-pipBOR AAC **102** thirty minutes after infection. After 2 days, the mice were sacrificed and bacterial loads were monitored in the peritoneal wash and the kidney. To distinguish between extracellular and intracellular bacteria, the peritoneal wash was centrifuged gently to separate the supernatant, containing extracellular bacteria, and the peritoneal cells. Peritoneal cells were treated with lysostaphin to kill any contaminating extracellular bacteria and lysed to enumerate the total number of intracellular bacteria at the time of harvest. Although mice treated with antibody alone harbored between  $10^5$  and  $10^6$  CFU of both intracellular and extracellular bacteria in the peritoneal wash and between  $10^4$  and  $10^6$  bacteria in the kidney, the mice treated with the S4497-pipBOR AAC cleared the infection to below the limit of detection. These data revealed that although the AAC is designed to release active antibiotic inside the phagolysosome, excellent clearance of both the intracellular and extracellular pools of MRSA was observed. Since extracellular bacteria are not killed directly by the AAC, the fact that these bacteria were also cleared by AAC treatment suggests that either a significant fraction of the extracellular bacteria is taken up by cells at some time during the infection, or that the AAC is able to enhance uptake of extracellular bacteria thereby increasing the relative proportion of bacteria that are intracellular where they are effectively killed by the AAC.

**[0267]** Efficacy of the AAC in an intravenous infection model was also examined. In this model, *S. aureus* is taken up by circulating neutrophils shortly after infection such that the majority of bacteria found in blood are associated with host cells within minutes after infection (Rogers, et al (1956) J. Exp. Med. 103:713-742). A/J Mice were infected with  $2 \times 10^6$  CFU of MRSA by intravenous injection, and then treated with 50 mg/Kg of AACs by intravenous injection 30 minutes post infection. In this model, the primary site of infection is the kidney, and mice develop large abscesses that are detectable by two days post infection and fail to be cleared for up to 30 days in the absence of treatment. Treatment with 50 mg/Kg of the S4497-pipBOR AAC **102** cleared the infection in all of the mice tested (Figure 8B).

**[0268]** Figure 8B shows intravenous infection model in A/J mice. Mice were infected with  $2 \times 10^6$

CFU by intravenous injection and treated with 50 mg/Kg of S4497 antibody, 50 mg/Kg of S4497-pipBOR AAC **102** or a simple mixture of 50 mg/Kg of S4497 antibody + .5 mg/Kg of free rifamycin. Treatments were delivered by IV injection 30 minutes post infection and kidneys were harvested 4 days post infection. The grey dashed line indicates the limit of detection for each organ. Control groups treated with 50 mg/Kg of S4497 antibody alone, or with a simple mixture of 50 mg/Kg of S4497 antibody plus 0.5 mg/kg free rifamycin (the equivalent dose of antibiotic present in 50 mg/Kg of AAC) were not efficacious.

**[0269]** Efficacy of AAC made with pipBOR and dimethyl-pipBOR antibiotic moieties was compared *in vivo* in the intravenous infection model in A/J mice. The S4497-pipBOR AAC **102** (Figure 9A) or the S4497-dimethyl-pipBOR AAC **105** (Figure 9B) were administered at various doses ranging from 50 mg/Kg to 2 mg/Kg 30 minutes after infection and kidneys were examined 4 days after infection to determine the total bacterial load. Figure 9A shows efficacy of pipBOR AAC **102** in an intravenous infection model by titration of the S4497-pipBOR AAC **102**. Seven week old female A/J Mice were infected with  $2 \times 10^6$  CFU of MRSA (USA300 strain) by intravenous injection into the tail vein. Figure 9B shows efficacy of diMethyl-pipBOR AAC **105** in the intravenous infection model by titration of the S4497-dimethyl-pipBOR AAC **105**. Treatments with S4497 antibody, AAC **102** or AAC **105** were administered at the indicated doses 30 minutes after infection. Mice were sacrificed 4 days after infection and the total number of surviving bacteria per mouse (2 kidneys pooled) was determined by plating.

**[0270]** Both AAC were effective at the highest dose of 50 mg/Kg, however the S4497-pipBOR AAC **102** was only partially efficacious at lower doses. The S4497-dimethyl-pipBOR AAC **105** yielded complete bacterial clearance at doses above 10 mg/Kg. Subsequent experiments indicated that doses above 15 mg/Kg were required for consistent bacterial clearance. Figures 9A and 9B show thio-S4497-HC-A118C-MC-vc-PAB-dimethylpipBOR **105** AAC is more efficacious than thio-S4497-HC-A118C-MC-vc-PAB-pipBOR **102** AAC in an intravenous infection model indicating an effect of the carbamate (**51**) and dimethylpiperidyl (**54**) structural distinction between **102** and **105**, respectively.

**[0271]** Mice were treated with the AAC 30 minutes after infection. To better replicate conditions likely to occur in MRSA patients seeking treatment, it was determined whether the AAC is effective at clearing an established infection and that linking of the antibiotic to an anti-S. aureus antibody provides a definite advantage over treatment with antibiotic alone. To this end, the efficacy of AAC with an equivalent dose of the antibiotic dimethyl-pipBOR was compared.

**[0272]** Figure 9C shows CB17.SCID mice infected with  $2 \times 10^7$  CFU of MRSA by intravenous injection (protocol 12-2418). One day after infection, the mice were treated with 50 mg/Kg of S4497 antibody, 50 mg/Kg of S4497 dimethyl-pipBOR AAC **105** or with 0.5 mg/Kg of dimethyl-pipBOR antibiotic **7**, the equivalent dose of antibiotic that is contained in 50 mg/Kg of AAC). Mice were sacrificed 4 days after infection and the total number of surviving bacteria per mouse (2 kidneys pooled) was determined by plating. Treatment with 50 mg/Kg of S4497-dimethyl-pipBOR AAC was clearly efficacious when given 1 day post infection, whereas treatment with the equivalent dose of dimethyl-pipBOR alone failed to clear the infection.

## TREATMENT WITH AN AAC IS EFFICACIOUS IN THE PRESENCE OF HUMAN ANTIBODIES AND SUPERIOR TO TREATMENT WITH THE CURRENT STANDARD OF CARE (SOC) VANCOMYCIN

**[0273]** The S4497 antibody was cloned from B cells derived from *S. aureus* infected patients. This raised the concern that normal human serum, or serum present in MRSA infected patients may contain anti-MRSA antibodies that would compete for binding with our AAC. To address this, human serum derived from normal healthy donors and a panel of MRSA patients was tested to estimate the overall level of anti-MRSA antibodies that recognize the same antigen as the AAC. An ELISA based assay using cell wall preparations from MRSA was developed. To limit non-antigen specific binding to the cell wall preparations in these assays, a strain of MRSA that is deficient in the gene for protein A was utilized. Protein A binds to the Fc region of IgG antibodies. Binding of various wild-type (WT) serum samples to MRSA that expressed the S4497 antigen (Figure 10A, WT) was examined versus binding to a MRSA strain TarM/TarS DKO (double knockout) mutant which lacks the sugar modifications that are recognized by the S4497 antibody. Figure 10A shows prevalence of anti-*S. aureus* antibodies in human serum. *S. aureus* infected patients or normal controls contain high amounts of WTA specific serum antibody with same specificity as anti-WTA S4497.

**[0274]** A standard curve was generated using a monoclonal antibody that binds well to the same antigen that is recognized by S4497. By comparing the level of binding in serum samples to the signal obtained from the antibody used to generate the standard curve, the level of anti-MRSA antibodies present in serum samples derived from normal healthy donors or MRSA patients, or in total IgG preparations isolated from normal serum was estimated (Figure 10A). Normal human serum contains 10-15 mg/mL of total IgG (Manz et al. (2005) Annu Rev. Immunol. 23:367). Analysis of anti-MRSA reactivity in the different serum samples revealed that up to 300 µg/mL of these antibodies are potentially reactive with the same antigen recognized by S4497 and are therefore likely to compete for binding with the AAC.

**[0275]** The S4497 antibody was used to generate AAC for properties including very high binding on MRSA (estimated 50,000 binding sites per bacterium). Sufficient numbers of AAC may be able to bind to MRSA even in the presence of the competing antibodies found in human serum. To test this directly, the S4497-dimethyl-pipBOR AAC in buffer supplemented with 10 mg/mL of human IgG (Figure 10B, +IGIV) was titrated and the level of intracellular killing was measured in the macrophage intracellular killing assay.

**[0276]** Figure 10B shows an *in vivo* infection model demonstrating that AAC is efficacious in the presence of physiological levels of human IgG. *In vitro* macrophage assay with the USA300 strain of MRSA shows that S4497-dimethyl-pipBOR AAC **105** is efficacious in the presence of 10 mg/mL of human IgG. The USA300 strain of MRSA was opsonized with AAC alone, or with AAC diluted in 10 mg/mL of human IgG for 1 hour at 37 °C with shaking. The opsonized bacteria were added directly to murine peritoneal macrophages and incubated for 2 hours to



permit phagocytosis. After infection, the macrophage cultures were maintained in complete media supplemented with gentamycin and the total number of surviving intracellular bacteria was assessed 2 days post infection. These data revealed that although the human IgG did inhibit AAC killing at the lower doses, excellent killing was achieved using doses above 10 µg/mL, an antibody concentration that is readily achievable *in vivo*. Normal serum IgG can diminish the functional effect of **105** AAC. Since maximal macrophage intracellular killing activity of an AAC may require both high antigen binding and efficient interaction with FcRs (for opsonophagocytosis), preexisting serum antibodies may both compete for binding to WTA and the corresponding formed immune complexes compete for binding to FcRs on macrophages.

**[0277]** To confirm that the AAC would be effective in the presence of competing human antibodies *in vivo*, the *in vivo* infection model was modified to generate mice that express normal levels of human IgG in the serum. CB17:SCID mice, that lack both T cells and B cells and therefore do not have antibodies in the serum (Bosna & Carroll, (1991) Ann Rev Immunol. 9:323, were reconstituted with 10 mg/mL of human IgG by daily dosing of highly concentrated human IgG (IGIV). Preliminary studies confirmed that these mice, termed SCID:hulgG, indeed had sustained levels of at least 10 mg/mL of human IgG in the serum and that these mice were equally susceptible to infection with MRSA compared to untreated controls. SCID:hulgG mice were infected with MRSA and treated with either S4497 antibody or with the S4497-dimethyl-pipBOR AAC (50 mg/Kg) 1 day after infection. Four days after infection the bacterial load in the kidneys (Fig 10C) was assessed.

**[0278]** Figure 10C shows the combined data from 3 independent experiments using 2 separate preparations of the thio-S4497-HC-A118C-MC-vc-PAB-dimethyl-pipBOR AAC **105** or **112**. CB17.SCID mice were reconstituted with human IgG using a dosing regimen optimized to yield constant levels of at least 10 mg/mL of human IgG in serum. Mice were treated with S4497 antibody (50 mg/Kg), or S4497-dimethyl-pipBOR AAC (50 mg/Kg). Mice treated with the AAC had a greater than 4-log reduction in bacterial loads (Students t-test  $p=0.0005$ ). Bacterial loads were on average over 10,000 fold lower in the mice treated with the S4497-dimethyl-pipBOR AAC compared to mice treated with S4497 antibody control, indicating that the AAC was clearly effective even in the presence of high levels of competing human anti-MRSA antibodies.

**[0279]** Efficacy of the AAC was compared with that of treatment with vancomycin, the current standard of care treatment for MRSA infections. Figure 11A shows *in vivo* infection model demonstrating that AAC is more efficacious than the current standard of care (SOC) antibiotic vancomycin in mice that are reconstituted with normal levels of human IgG. CB17.SCID mice were reconstituted with human IgG using a dosing regimen optimized to yield constant levels of at least 10 mg/mL of human IgG in serum. Mice were treated with S4497 antibody (50 mg/Kg), vancomycin (100 mg/Kg), S4497-dimethyl-pipBOR AAC (50 mg/Kg, **112** or an AAC made with an isotype control antibody that does not recognize MRSA, thio-hu-anti gD 5B5-HC-A118C-MC-vc-PAB-dimethylpipBOR AAC **110** (50 mg/Kg). Mice receiving AACs were given a single dose of AAC on day 1 post infection by intravenous injection. Mice receiving vancomycin treatments were given twice daily injections of the antibiotic by intraperitoneal injection. All mice

were sacrificed on day 4 post infection, and the total number of surviving bacteria per mouse (2 kidneys pooled) was determined by plating.

**[0280]** Treatment with vancomycin is effective at treating MRSA infection in our murine intravenous infection model if the treatment is initiated 30 minutes after infection. Twice-daily dosing with 100 mg/Kg of vancomycin failed to clear the infection, and was only able to reduce bacterial loads by about 50 fold, when treatment was initiated more than 1 day post infection (Figure 11A). Strikingly, treatment with a single dose of the S4497-dimethyl-pipBOR AAC 1 day after infection was able to clear the infection in the majority of mice. Surprisingly, treatment with control AAC made with a human IgG antibody that does not recognize *S. aureus* (gD-AAC) had some efficacy in this model. The gD antibody does not recognize *S. aureus* through its antigen binding site, however the antibody is able to bind to protein A found on *S. aureus*.

**[0281]** Figure 11C shows *in vivo* infection model demonstrating that AAC, thio-S6078-HC A114C-LCWT-MC-vc-PAB-dimethylpipBOR **129** is more efficacious than naked anti-WTA antibody S4497, according to the same regimen as Figure 11A, in mice that are reconstituted with normal levels of human IgG. CB17.SCID mice were reconstituted with human IgG using a dosing regimen optimized to yield constant levels of at least 10 mg/mL of human IgG in serum. Mice were treated with S4497 antibody (50 mg/Kg), or thio-S6078-HC A114C-LCWT-MC-vc-PAB-dimethylpipBOR **129** AAC (50 mg/Kg).

**[0282]** FACS analysis showed that staining with high concentrations of the gD antibody on bacteria isolated from an *in vivo* infection yields low level binding to *S. aureus* relative to binding of anti-MRSA antibodies to MRSA isolated from infected kidneys (Figure 11B). Mice were infected with MRSA by intravenous injection and infected kidneys were removed 3 days post infection and homogenized. Anti-MRSA or control antibodies were labeled with Alexa-488 and tested at a range of concentrations between 0.08 µg/mL and 50 µg/mL. The S4497 antibody recognizes an N-acetylglucosamine modification that is linked to wall teichoic acid (WTA) via a beta-anomeric bond on the cell wall of *S. aureus*. The S7578 antibody binds to a similar N-acetylglucosamine modification that is joined to WTA via an alpha-anomeric bond. The rF1 antibody is a positive control anti-MRSA antibody that recognizes sugar modifications found on a family of SDR-repeat containing cell wall anchored proteins. The gD antibody is a negative control human IgG<sub>1</sub> that does not recognize *S. aureus*. Although the overall level of binding with the gD antibody is significantly lower than that obtained with the S4497 antibody (estimated to be at least 30 fold lower by FACS analysis, Figure 11B), the limited efficacy seen with the gD-AAC indicates that even low level binding of an AAC on MRSA *in vivo* is sufficient to yield efficacy that appeared equivalent to the reduction in CFUs obtained with vancomycin.

**[0283]** The above data clearly demonstrate that AAC are able to kill intracellular MRSA and that the S4497-pipBOR, and S4497 dimethyl-pipBOR AAC are effective at limiting infection with MRSA both *in vitro* and *in vivo*. AAC of the invention act by killing bacteria inside mammalian cells and thereby provide a unique therapeutic that is more effective at killing populations of bacteria that are resistant to treatment with vancomycin.

**[0284]** Figure 20 shows that pre-treatment with 50 mg/kg of free antibodies is not efficacious in an intravenous infection model. Balb/c mice were given a single dose of vehicle control (PBS) or 50 mg/Kg of antibodies by intravenous injection 30 minutes prior to infection with  $2 \times 10^7$  CFU of USA300. Treatment groups included an isotype control antibody that does not bind to *S. aureus* (gD), an antibody directed against the beta modification of wall teichoic acid (4497) or an antibody directed against the alpha modification of wall teichoic acid (7578). Control mice were given twice daily treatments with 110 mg/Kg of vancomycin by intraperitoneal injection (Vanco). All mice were sacrificed on day 4 post-infection, and the total number of surviving bacteria in kidneys (2 kidneys pooled) was determined by plating. Although pre-treatment with vancomycin cleared the infection in all of the mice tested, pre-treatment with antibodies directed against the cell wall of *S. aureus* had no effect on bacterial loads.

**[0285]** Figures 21 and 22 show that AACs directed against either the beta modification of wall teichoic acid or the alpha modification of wall teichoic acid are efficacious in an intravenous infection model using mice that are reconstituted with normal levels of human IgG. CB17.SCID mice were reconstituted with human IgG using a dosing regimen optimized to yield constant levels of at least 10 mg/mL of human IgG in serum and infected with  $2 \times 10^7$  CFU of USA300 by intravenous injection. Treatment was initiated 1 day after infection with buffer only control (PBS), 60 mg/Kg of beta-WTA AAC ( **136** AAC) or 60 mg/Kg of alpha-WTA AAC ( **155** AAC). The mice were sacrificed on day 4 post infection, and the total number of surviving bacteria in kidneys (2 kidneys pooled, Figure 21) and heart (Figure 22) was determined by plating. Treatment with the beta-WTA AAC resulted in a 100,000 fold reduction in bacterial load in the kidney compared to mice treated with the vehicle control. Treatment with the alpha-WTA AAC resulted in an average 9,000 fold reduction in bacterial load in the kidney.

**[0286]** To date, it remains uncertain why the currently available antibiotics are often ineffective at killing intracellular stores of bacteria. Antibiotics could fail because they do not reach sufficient concentrations inside cells, either because they do not enter the phagolysosomal compartment where intracellular stores of bacteria reside, or because they may be subject to the activity of efflux pumps that remove the antibiotic from mammalian cells. Antibiotics may be damaged by harsh conditions found inside the phagolysosome including low pH, reducing agents and oxidizing agents that are released specifically to kill the phagocytosed bacterium. Alternatively, antibiotics may fail because the bacteria up regulate defense mechanisms or fail to divide inside the phagolysosome and are therefore rendered transiently insensitive to antibiotics. The relative importance of these mechanisms of antibiotic resistance will differ for different pathogens and for each antibiotic. The antibiotic component of our AAC, pipBOR and dimethyl-pipBOR are indeed more potent than rifampicin at killing intracellular MRSA when tested as free antibiotics. The linkage of these antibiotics to an antibody provides a real dose-dependent increase in efficacy that is apparent *in vivo* (Figure 9C). In this case, improved efficacy of the AAC over antibiotic alone is likely due to a combination of its ability to opsonize bacteria and to improved pharmacokinetics of AAC. Most free antibiotics are rapidly cleared *in vivo* and require repeated dosing with high concentrations of antibiotic to maintain sufficient antibiotic concentrations in serum. In contrast, AAC have long half-lives in serum due to the antibody portion of the molecule. Since AAC release the antibiotic only after binding to *S.*

aureus and being transported along with the bacterium into the confined space of the phagolysosome, they concentrate small doses of antibiotic specifically in a niche where most antibiotics fail. Therefore, in addition to targeting protected reservoirs of intracellular bacteria, AAC may facilitate the use of more potent antibiotics that may prove too toxic for use as a single agent by limiting the release of the antibiotic to where it is most needed.

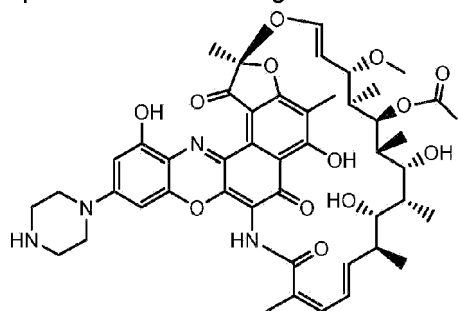
**[0287]** Figures 35 and 36 show results from the *in vitro* Macrophage Assay for thio-S6078 AAC. *S. aureus* (USA300 NRS384) was incubated with unconjugated S6078 antibody at 50 u/mL and AAC at 50 µg/mL, 5 µg/mL, .5 µg/mL or 0.05 µg/mL for 1 hour to permit binding of the antibody to the bacteria. The resulting opsonized bacteria were fed to murine macrophages and incubated at 37 °C to permit phagocytosis. After 2 hours, the infection mix was removed and replaced with normal growth media supplemented with 50 µg/mL of gentamycin to kill any remaining extracellular bacteria. The total number of surviving intracellular bacteria was determined 2 days later by plating serial dilutions of the macrophage lysates on Tryptic Soy Agar plates. In Figure 35, thio-S6078.v4.HC-WT, LC-Cys-MC-vc-PAB-(dimethylpipBOR) AAC was effective at killing intracellular bacteria at doses at or above 0.5 µg/mL with an antibiotic loading of 2.0 (AAC-173) or 3.9 (AAC-171) dimethylpipBOR antibiotics (LA-54) per thio-S6078 antibody. In Figure 36, thio-S6078.v4.HC-WT, LC-Cys-MC-vc-PAB-(piperazBOR) was effective at killing intracellular bacteria at doses at or above 0.5 µg/mL with an antibiotic loading of 1.8 (AAC-174) or 3.9 (AAC-172) piperazBOR antibiotics (LA-65) per thio-S6078 antibody.

**[0288]** Figures 37 and 38 show results from *in vivo* efficacy of thio-S6078 AAC in a murine intravenous infection model. CB17.SCID mice were reconstituted with human IgG using a dosing regimen optimized to yield constant levels of at least 10 mg/mL of human IgG in serum. Mice were infected with USA300 and treated with vehicle control (PBS), thio-S6078.v4.HC-WT, LC-Cys-MC-vc-PAB-(dimethylpipBOR) AAC with an antibiotic loading of 2.0 (AAC-173) or 3.9 (AAC-171) dimethylpipBOR antibiotics (LA-54) per thio-S6078 antibody (Figure 37) and thio-S6078.v4.HC-WT, LC-Cys-MC-vc-PAB-(piperazBOR) with an antibiotic loading of 1.8 (AAC-174) or 3.9 (AAC-172) piperazBOR antibiotics (LA-65) per thio-S6078 antibody (Figure 38). Mice were given a single dose of AAC on day 1 post infection by intravenous injection and sacrificed on day 4 post infection. The total number of surviving bacteria in 2 kidneys was determined by plating. Treatment with AAC containing lower antibiotic loading reduced bacterial burdens by approximately 1,000-fold and treatment with the AAC containing higher antibiotic loading reduced bacterial burdens by more than 10,000-fold.

## STAPHOPAIN B CLEAVABLE LINKERS FOR ANTIBODY-ANTIBIOTIC CONJUGATES

**[0289]** A protease cleavable linker is described herein to be cleaved by staphopain B, a secreted *S. aureus* endopeptidase. To design a linker cleaved specifically by the *Staphylococcus aureus* bacterium, the protease activity of *S. aureus* culture supernatant was characterized using a FRET peptide library. From this screen, a unique substrate specificity was identified. Using this substrate, the enzyme responsible for activity was purified from

culture supernatant and identified as staphopain B. Based on this identification, a staphopain B optimized linker was generated and linked to piperazino-rifamycin:



**[0290]** Piperazino-rifamycin is a potent rifalazil-like antibiotic. The resultant AAC has demonstrated efficacy in *in vitro* and *in vivo* models of MRSA infection, providing a novel mechanism by which to target MRSA infections. Staphopain B is a secreted cysteine protease from the papain family of endopeptidases (CAS Reg. No. 347841-89-8, Sigma-Aldrich #S3951, Filipek et al (2005) J. Biol. Chem. 280 (15): 14669-74) and has evolved to have a unique substrate specificity, preferring bulky aromatic side chains in the P2 position. Expression of staphopain B is controlled by the *agr* (or accessory gene regulator) quorum sensing system (Janzon, L. and S. Arvidson (1990) The EMBO journal 9(5): 1391-1399) as part of the staphylococcal proteolytic cascade (SCP). Agr modulates the expression of secreted proteases and other virulence factors of *S. aureus* including aureolysin, V8, and staphopain A (Shaw, L., E. Golonka, et al. (2004) Microbiology 150(Pt 1): 217-228). Staphopain B has been implicated as a potent virulence factor due to its ability to degrade host connective tissue as well as augment several immune system proteins (Imamura, T., S. Tanase, et al. (2005) Journal of experimental medicine 201(10): 1669-1676; Potempa, J., A. Dubin, et al. (1988) Journal of biological chemistry 263(6): 2664-2667; Ohbayashi, T., A. Irie, et al. (2011) Microbiology 157(Pt 3): 786-792; Smagur, J., K. Guzik, et al. (2009); Biological chemistry 390(4): 361-371; Smagur, J., K. Guzik, et al. (2009); Journal of innate immunity 1(2): 98-108; Kulig, P., B. A. Zabel, et al. (2007); Journal of immunology 178(6): 3713-3720). Staphopain B's role as an important virulence factor makes it an attractive target for protease mediated antibiotic release.

**[0291]** Identifying substrates cleaved by *Staphylococcus aureus* proteases:

To identify substrates readily cleaved by *S. aureus* endopeptidases, supernatant from an overnight culture of Wood46 strain *S. aureus* was incubated with a commercially available FRET peptide library. The Wood46 strain has a constitutively active *agr* locus, thus the Wood46 strain exhibits increased protease expression compared to wild type. The FRET peptide library, Rapid Endopeptidase Profiling Library or PepSets™ REPLi (Mimotopes, Victoria, Australia), consists of 512 wells with 8 internally quenched fluorogenic peptides per well in a 96-well plate format. The peptides fluoresce upon cleavage allowing for proteolytic activity to be monitored in real-time. Each peptide has a tripeptide variable core flanked by a series of glycine residues on either side and an additional two lysine residues at the C-terminus for solubility. Supernatant from the Wood46 culture was added to the library and plates were incubated overnight at 37 °C. Wells showing greater than a 15-fold increase in fluorescence (12 out of 512 wells total) were analyzed by LC-MS (Agilent Q-TOF) to determine the cleavage

products. Cleavage sites were ranked based on frequency (Table 4). Among the top hits, a pattern in substrate specificity was observed, specifically a preference for bulky hydrophobic side chains of Phe and Tyr in the P2 position.

**Table 4.** Amino acid preferences of REPLi sequences cleaved by Wood46 secreted proteases. Abundance at each position (%)

Residues	P4	P3	P2	P1	P1'	P2'
G	71.4	50.0	0.0	28.6	35.7	100.0
I/L	21.4	7.1	0.0	0.0	7.1	0.0
A/V	0.0	14.3	0.0	50.0	28.6	0.0
N/Q	7.1	7.1	0.0	0.0	7.1	0.0
S/T	0.0	14.3	0.0	14.3	0.0	0.0
F/Y	0.0	0.0	100.0	0.0	21.4	0.0
K/R	0.0	7.1	0.0	7.1	0.0	0.0

**[0292]** Abundance at each position of amino acid residues present in the FRET peptides of wells that showed the greatest increase in fluorescence. REPLi peptides contain the sequence MCA-Gly-Gly-Gly-Xaa-Yaa-Zaa-Gly-Gly-DPA-Lys-Lys (SEQ ID NO: 132), where Xaa, Yaa, and Zaa vary. Glycine residues present in the table represent the Gly residues that flank the variable core. While Gly residues are the most abundant in several positions, they give little insight to substrate specificity. When designing linkers, preference was given to amino acids from the variable core. Amino acids that were not in any of the top hits were omitted from the table.

**[0293]** Design and conjugation of a FRET substrate cleaved by a MRSA protease *in vitro*:

A peptide was designed and synthesized using the most frequent residues in the cleaved peptides from the REPLi screen using specificity information for P1, P2, and P3. The peptide had the sequence GGAFAGGG (SEQ ID NO: 126), with cleavage expected between GGAF (SEQ ID NO: 131) and GGG. The peptide was synthesized using solid phase synthesis incorporating fluorescent dyes, tetramethylrhodamine (TAMRA) and fluorescein as a FRET pair (Figure 26) with maleimido-propionic-acid added to the N-terminus to allow for conjugation to antibody cysteine residues. The resultant mal-FRET-peptide, maleimido-propionic (MP)-Lys(TAMRA)-Gly-Gly-Ala-Phe-Ala-Gly-Gly-Gly-Lys(fluorescein) ("core peptide" disclosed as SEQ ID NO: 125), was conjugated to the cysteine-engineered thioMab antibody, thio-S4497. The mal-FRET-peptide was also conjugated to cysteine-engineered anti-Her2 thioMab trastuzumab, a nonbinding control.

**[0294]** The thio-S4497-MP-K(Tamra)GGAFAGGGK(Fluorescein) ("core peptide" disclosed as SEQ ID NO: 125) FRET conjugate and non-binding control FRET conjugate, thio-trastuzumab-MP-K(Tamra)GGAFAGGGK(Fluorescein) ("core peptide" disclosed as SEQ ID NO: 125), were incubated with log phase cultures of Wood46 (Figure 28) and the wild type, USA300 (Figure 29), at cell densities of  $10^8$  cells/ml and  $10^7$  cells/ml in tryptic soy broth (TSB). The final

concentration of MP-Lys(TAMRA)-Gly-Gly-Ala-Phe-Ala-Gly-Gly-Gly-Lys(fluorescein) ("core peptide" disclosed as SEQ ID NO: 125) for all wells was 2 $\mu$ M. Fluorescence was monitored over time at 37 °C, excitation  $\lambda$ 495nm/ emission  $\lambda$ 518nm. An increase in fluorescence was observed with the 4497-mal-FRET-peptide conjugate in both Wood46 and USA300, indicating that the FRET peptide is cleaved by a *S. aureus* protease and that the protease is present in both strains. The linker unit MP-K(Tamra)GGAFAGGGK(Fluorescein) ("core peptide" disclosed as SEQ ID NO: 125) is cleaved in both Wood46 and USA300 when conjugated to an antibody that binds *S. aureus*. Validating the cleavage of this model linker in USA300 was critical due to its relevance to the clinical strain of MRSA that a potential therapeutic antibody-antibiotic conjugate (AAC) would target. The thio-S4497-MP-K(Tamra)GGAFAGGGK(Fluorescein) ("core peptide" disclosed as SEQ ID NO: 125) FRET conjugate shows an increase in fluorescence in both strains, indicating that the linker is cleaved by a *S. aureus* protease and that the protease is present in the clinically relevant strain of MRSA, USA300. Cell density affects the rate of cleavage, with cleavage occurring earlier in cultures of the higher cell density. The non-binding control thio-trastuzumab-MP-K(Tamra)GGAFAGGGK(Fluorescein) ("core peptide" disclosed as SEQ ID NO: 125) conjugate did not show an increase in fluorescence in any condition tested.

**[0295]** Based on the cleaved substrate from the cell based assays, linker-antibiotic intermediate LA-59 (Table 2) was prepared and conjugated to antibodies to form anti-MRSA heavy chain, cysteine engineered thio-S4497 (AAC-113) and thio-S4462 (AAC-114), and anti-HER2 light chain thio-trastuzumab (AAC-115) of Table 3. The GGAFAGGG (SEQ ID NO: 126) linked AAC demonstrated better rates of cleavage than the FRET-peptide when incubated with concentrated supernatant from a Wood46 overnight culture, indicating that the linker-antibiotic is a better substrate for the unknown protease of interest. Cleavage occurred at the expected site between alanine and glycine in the GGAFAGGG-linked ("core peptide" disclosed as SEQ ID NO: 126) AAC (AAC-113, AAC-114, AAC-115). This linker-antibiotic (LA-59) is not an optimized delivery system for the antibiotic because upon cleavage, GGG-rifamycin as opposed to free rifamycin is released. While the therapeutic potential of this linker-antibiotic may be uncertain, its ability to be efficiently cleaved by protease make it a useful tool compound to identify fractions containing the active protease of interest. Linker-antibiotic intermediate LA-59 was conjugated to the Fab portion of the thio-S4497 antibody (Scheer, J. M., W. Sandoval, et al. (2012). PloS one 7(12): e51817). Cysteine-engineered Fab antibodies, "thioFABs", have one reactive cysteine that enables the site-specific conjugation of one thiol reactive compound. thioFAB S-4497 was reacted with a 3 fold molar excess of LA-59 over thioFAB for 1hr in 50mM TRIS pH 7.5, 150mM NaCl at room temperature. Excess LA-59 was separated from AAC by diafiltration in PBS. The resultant conjugate, thioFAB S4497-MC-GGAFAGGG-(pipBOR) ("core peptide" disclosed as SEQ ID NO: 126) (Figure 27), was used as a tool compound to identify active fractions, with cleavage of the linker detected by LC-MS analysis.

**[0296]** Optimizing linkers for efficient cleavage by staphopain B:

The linker-antibiotic intermediate LA-59, MC-GGAFAGGG-(pipBOR) ("core peptide" disclosed as SEQ ID NO: 126), has substrate residues optimized for the P1, P2, and P3 positions. Using the results from the REPLi screen, two new linkers were designed and synthesized

incorporating a residue preference for P4 (Figure 30). Maleimido-propionic-Leu-Ala-Phe-Ala-Ala ("core peptide" disclosed as SEQ ID NO: 136) and maleimido-propionic-Leu-Ala-Phe-Gly-Ala ("core peptide" disclosed as SEQ ID NO: 135) were synthesized using solid phase synthesis. Isoleucine and Leucine were the most frequent residues in P4 in the REPLi screen (disregarding Glycine). Only one residue, Leucine, was chosen to limit the number of linkers synthesized. Ala and Gly were alternated in the P1 position to examine the effect on cleavability. A residue in P1', Ala, was also included. QSY®7 (xanthylum, 9-[2-[[4-[[[2,5-dioxo-1-pyrrolidinyl]oxy]carbonyl]-1-piperidinyl]sulfonyl]phenyl]-3,6-bis(methylphenylamino)-NHS ester, chloride, CAS Reg. No. 304014-12-8, Life Technologies) was added to the C-terminus of both linkers to act as an antibiotic surrogate (Figure 30). Incorporating QSY®7 allowed for the cleavability of these linkers to be evaluated without consuming costly and labor-intensive antibiotics.

**[0297]** The experimental mal-peptide-QSY7 linkers were conjugated to THIOFAB S4497 to evaluate the cleavability of these linkers. Conjugations were performed as previously described. The resultant THIOFAB S4497 linker-QSY7 conjugates were assessed for cleavability by staphopain B, staphopain A, and human cathepsin B at pH 7.2 (Table 5). Like staphopain B, staphopain A is a secreted cysteine protease of *S. aureus* from the papain family of proteases. It is structurally similar to staphopain B, but has a broader substrate specificity (Filipek, R., M. Rzychon, et al. (2003). The Journal of biological chemistry 278(42): 40959-40966). Cathepsin B, a mammalian cysteine lysosomal protease, is also a member of the papain family of endopeptidases. It is thought to cleave the valine-citruline linkers used in other AAC linkers described in this patent.

**Table 5.** Cleavage of optimized linkers by staphopains and human cathepsin B

		Staphopain A	Staphopain B	Cathepsin B
MP-LAFGA-QSY7 ("core peptide" disclosed as SEQ ID NO: 135) (Figure 30)	Percent cleavage pH 7.2	38	100	100
	Cleavage product	A-QSY7	A-QSY7	A-QSY7
MP-LAFAA-QSY7 ("core peptide" disclosed as SEQ ID NO: 136) (Figure 30)	Percent cleavage pH 7.2	23	100	100
	Cleavage product	A-QSY7	A-QSY7	A-QSY7, QSY7
MP-LAFG-PABC-(piperazinoBOR) ("core peptide" disclosed as SEQ ID NO: 128) LA-104	Percent cleavage pH 5	100	100	100
	Percent cleavage pH 7.2	46	100	64
	Cleavage product	piperazino-rifamycin	piperazino-rifamycin	



**[0298]** Table 5 shows data from cleavage of optimized linkers conjugated to thioFAB by staphopain A, staphopain B, and Cathepsin B. The final linker-antibiotic, MP-LAFG-piperazino-rifamycin ("core peptide" disclosed as SEQ ID NO: 128) is efficiently cleaved by all three proteases. Cleavage by the staphopains results in the release free rifamycin. Cleavage by cathepsin B releases Phe-Gly-piperazino-rifamycin (Example 26).

**[0299]** Design and conjugation of a staphopain B cleavable linker that releases free antibiotic: From the cleavage assays of the experimental linkers tested, mal-LAFGA ("core peptide" disclosed as SEQ ID NO: 135) was selected for antibiotic attachment. Further optimization of this linker was required to achieve free antibiotic release upon proteolytic cleavage. To accomplish this, Ala in P1' was replaced by p-aminobenzyl (PAB) or p-aminobenzyloxycarbonyl (PABC). Piperazino-rifamycin was added to the C-terminus of this linker to complete the linker-antibiotic intermediates LA-88 MC-LAFG-PAB-(dimethylamino-3-pyrroloBOR) ("core peptide" disclosed as SEQ ID NO: 128) and LA-104, MP-LAFG-PABC-(piperazinoBOR) ("core peptide" disclosed as SEQ ID NO: 128). Upon cleavage after Gly in the P1 position, the PAB and PABC groups are designed to self-immolate, releasing free antibiotic. LA-88 was conjugated to form thio-S4497-v8-LCV205C-MC-LAFG-PAB-(dimethylamino-3-pyrroloBOR) AAC-163 ("core peptide" disclosed as SEQ ID NO: 128) (Table 3). LA-104 was conjugated to form AAC-193, AAC-215, and AAC-222. Cleavage assays of AAC 193 with staphopain A, staphopain B, and cathepsin B were performed at pH 7.2 and pH 5. These pH values were selected to either mimic plasma (pH 7.2) or the environment of the phagolysosome (pH 5). Staphopain B achieved 100% cleavage at both pH 5 and 7.2. Staphopain A showed 100% cleavage at pH 5 and 64% cleavage at pH 7.2.

**[0300]** The substitution of PABC for Ala in the P1' group changed the location at which cathepsin B cleaves the linker. Upon cleavage by cathepsin B, Phe-Gly-PABC-(piperazinoBOR) was released. As a therapeutic, potential cleavage of this linker by cathepsin B would most likely occur in the lysosomal compartment of macrophages. Under these circumstances, other proteases, including staphopain B, may further process FG-PABC-piperazino-rifamycin to liberate free antibiotic.

**[0301]** Linker-antibiotic intermediate, MP-LAFG-PABC-(piperazinoBOR) ("core peptide" disclosed as SEQ ID NO: 128) LA-104 was conjugated to thioMAB S4497 to evaluate the *in vitro* and *in vivo* efficacy of the resultant AACs AAC-193, AAC-215, AAC-222. Two control conjugates were also made by conjugating LA-104 to thioMAB anti-Her2 and thioMAB anti-gD, both isotype controls. The light chain linked, thioMAB 4497 MP-LAFG-PABC-piperazino-rifamycin conjugate ("core peptide" disclosed as SEQ ID NO: 128) AAC-215 had a drug to antibody ratio (DAR) of 1.6, as did the thioMAB anti-gD control conjugate. The thioMAB anti-Her2 mal-LAFG-PABC-piperazino-rifamycin ("core peptide" disclosed as SEQ ID NO: 128) conjugate had a DAR of 1.5.

**[0302]** *S. aureus* culture supernatant was screened using a FRET peptide library to identify

substrates readily cleaved by secreted proteases. The results of this screen showed the overwhelming majority of measured protease activity may be attributed to one secreted cysteine protease of *S. aureus*, staphopain B. Peptide linkers designed for cleavage by staphopain B were optimized and an efficiently cleaved substrate was identified that released free antibiotic. The resultant linker is also cleaved by *S. aureus* protease staphopain A and human protease cathepsin B, both also cysteine proteases.

**[0303]** When conjugated to an antibody that binds *S. aureus*, the resultant AAC show efficacy both *in vitro* and *in vivo*. The endogenous endopeptidases of MRSA provide a mechanism to target MRSA infections and release payload in a disease specific manner. The ability of this linker to be cleaved by secreted proteases of *S. aureus* allows for targeting of MRSA present in both human neutrophils as well as extracellularly in host plasma/tissue. This dual targeting may enable the release of a high concentration of antibiotic at both intra- and extra-cellular sites of infection.

**[0304]** Staphopain A and B expression are up-regulated in human neutrophils and are thought to be important virulence factors (Burlak, C., et al. (2007) Cellular microbiology 9(5):1172-1190), making them attractive targets for protease mediated release of antibiotic. Human cathepsin B also cleaves the linker, presenting an alternate pathway of release. The observed efficacy of the AAC is likely to be the result of multiple proteases, both from *S. aureus* and the host, involved in the release of antibiotic or antibiotic moieties. A serum-stable linker that is cleaved by an assortment of proteases provides a release mechanism that may outmaneuver resistance mutations of the bacterium.

## METHODS OF TREATING AND PREVENTING INFECTIONS WITH ANTIBODY-ANTIBIOTIC CONJUGATES

**[0305]** The AAC of the invention are useful as antimicrobial agents effective against a number of human and veterinary Gram positive bacteria, including the Staphylococci, for example *S. aureus*, *S. saprophyticus* and *S. simulans*; Listeria, for example *Listeria monocytogenes*; Enterococci, for example *E. faecalis*; Streptococci, for example *S. pneumoniae*; Clostridium, for example *C. difficile*.

**[0306]** Persistent bacteremia can result from internalization into host cells. While not entirely understood, internalized pathogens are able to escape immune recognition by surviving inside host cells. Such organisms include *S. aureus*, *Salmonella* (e.g., *S. typhi*, *S. choleraesuis* and *S. enteritidis*), *Legionella* (e.g., *L. pneumophila*), *Listeria* (e.g., *L. monocytogenes*), *Brucella* (e.g., *B. abortus*, *B. melitensis*, *B. suis*), *Chlamydia* (*C. pneumoniae*, *C. trachomatis*), *Rickettsia* spp., *Shigella* (e.g., *S. flexneri*), and *Mycobacteria*.

**[0307]** Following entry into the bloodstream, *S. aureus* can cause metastatic infection in almost any organ. Secondary infections occur in about one-third of cases before the start of therapy (Fowler et al., (2003) Arch. Intern. Med. 163:2066-2072), and even in 10% of patients after the

start of therapy (Khatib et al., (2006) Scand. J. Infect. Dis., 38:7-14). Hallmarks of infections are large reservoirs of pus, tissue destruction, and the formation of abscesses (all of which contain large quantities of neutrophils). While only about 5% of patients develop complications if the bacteremia is extinguished within 48 hours, the levels rises to 40%, if bacteraemia persists beyond three days.

**[0308]** While *S. aureus* is generally considered to be an extracellular pathogen that secretes toxins, evidence exists that it can survive inside endothelial cells, keratinocytes, fibroblasts, and osteoclasts (Alexander et al, (2001) Appl. Microbiol. Biotechnol. 56:361-366; Garzoni et al, (2009) Trends Microbiol. 17:59-65). Neutrophils and macrophages are key components of the host innate immune response to bacterial infection. These cells internalize *S. aureus* by phagocytosis, which may be enhanced by antibody, complement, or host lectins such as mannose binding protein, which can bind simultaneously to pathogen and neutrophils, macrophages, and other professional phagocytes. As previously mentioned, *S. aureus* not only survives in the lysosomal environment, but may actually exploit it as a mechanism for developing persistence in the host.

**[0309]** The antibody-antibiotic conjugates (AAC) of the invention have significant therapeutic advantages for treating intracellular pathogens, including those residing in phagolysosomes. In one embodiment, the pathogen is internalized into leukocyte cells, and the intracellular component is a phagolysosome. In an intact AAC, the antibody variable region binds to a cell surface antigen on the bacteria (opsonization). Not to be limited by any one theory, in one mechanism, by the antibody component of the AAC binding to the bacterial cell surface, phagocytes are attracted to the bacterium. The Fc portion of the antibody binds to an Fc receptor on the phagocyte, facilitating phagocytosis. After the AAC-bacteria complex is phagocytosed, upon fusing to lysosome, the AAC linker is cleaved by exposure to phagolysosomal enzymes, releasing an active antibiotic. Due to the confined space and relatively high local Abx concentration (about  $10^4$  per bacterium), the result is that the phagolysosome no longer supports the survival of the intracellular pathogen (Figure 5). Because the AAC is essentially an inactive prodrug, the therapeutic index of the antibiotic can be extended relative to the free (unconjugated) form. The antibody provides pathogen specific targeting, while the cleavable linker is cleaved under conditions specific to the intracellular location of the pathogen. The effect can be both directly on the opsonized pathogen as well as other pathogens that are co-localized in the phagolysosome. In a specific aspect, the pathogen is *S. aureus*.

**[0310]** Antibiotic tolerance is the ability of a disease-causing pathogen to resist killing by antibiotics and other antimicrobials and is mechanistically distinct from multidrug resistance (Lewis K (2007). "Persister cells, dormancy and infectious disease". Nature Reviews Microbiology 5 (1): 48-56. doi:10.1038/nrmicro1557). Rather, this form of tolerance is caused by a small sub-population of microbial cells called persisters (Bigger JW (14 October 1944). "Treatment of staphylococcal infections with penicillin by intermittent sterilization". Lancet 244 (6320): 497-500). These cells are not multidrug resistant in the classical sense, but rather are dormant cells that are tolerant to antibiotic treatment that can kill their genetically identical

siblings. This antibiotic tolerance is induced by a non-or extremely slow dividing physiological state. When antimicrobial treatment fails to eradicate these persister cells, they become a reservoir for recurring chronic infections. The antibody-antibiotic conjugates of the invention possess a unique property to kill these persister cells and suppress the emergence of multidrug tolerant bacterial populations.

**[0311]** In another embodiment, the AAC of the invention may be used to treat infection regardless of the intracellular compartment in which the pathogen survives.

**[0312]** In another embodiment, AACs could also be used to target bacteria in planktonic or biofilm form (example: *S. aureus*, *K. pneumoniae*, *E. coli*, *A. baumannii*, *P. aeruginosa* and Enterobacteriaceae) by antibody-mediated opsonization, leading to internalization by professional phagocytes. When the phagosome fuses with a lysosome, sufficiently high concentrations of free antibiotic will be released from the AAC in the acidic or proteolytic environment of the phagolysosome to kill the phagocytosed pathogen.

**[0313]** Methods of treating infection with antibody-antibiotic conjugates (AAC) of the invention include treating bacterial lung infections, such as *S. aureus* pneumonia or tuberculosis infections, bacterial ocular infections, such as trachoma and conjunctivitis, heart, brain or skin infections, infections of the gastrointestinal tract, such as travellers' diarrhea, osteomyelitis, ulcerative colitis, irritable bowel syndrome (IBS), Crohn's disease, and IBD (inflammatory bowel disease) in general, bacterial meningitis, and abscesses in any organ, such as muscle, liver, meninges, or lung. The bacterial infections can be in other parts of the body like the urinary tract, the bloodstream, a wound or a catheter insertion site. The AACs of the invention are useful for difficult-to-treat infections that involve biofilms, implants or sanctuary sites (e.g., osteomyelitis and prosthetic joint infections), and high mortality infections such as hospital acquired pneumonia and bacteremia. Vulnerable patient groups that can be treated to prevent *Staphylococcal aureus* infection include hemodialysis patients, immune-compromised patients, patients in intensive care units, and certain surgical patients. In another aspect, the invention provides a method of killing, treating, or preventing a microbial infection in an animal, preferably a mammal, and most preferably a human, that includes administering to the animal an AAC or pharmaceutical formulation of an AAC of the invention. The invention further features treating or preventing diseases associated with or which opportunistically result from such microbial infections. Such methods of treatment or prevention may include the oral, topical, intravenous, intramuscular, or subcutaneous administration of a composition of the invention. For example, prior to surgery or insertion of an IV catheter, in ICU care, in transplant medicine, with or post cancer chemotherapy, or other activities that bear a high risk of infection, the AAC of the invention may be administered to prevent the onset or spread of infection.

**[0314]** The bacterial infection may be caused by a bacteria with an active and inactive form, and the AAC is administered in an amount and for a duration sufficient to treat both the active and the inactive, latent form of the bacterial infection, which duration is longer than is needed to treat the active form of the bacterial infection.

**[0315]** Analysis of various Gram+ bacteria found WTA beta expressed on all *S. aureus*, including MRSA and MSSA strains, as well as non-aureus Staph strains such as *S. saprophyticus* and *S. simulans*. WTA alpha (Alpha-GLcNAc ribitol WTA) is present on most, but not all *S. aureus*, and also present on *Listeria monocytogenes*. WTA is not present on Gram-negative bacteria. Therefore one aspect of the invention is a method of treating a patient infected with *S. aureus* or *S. saprophyticus* or *S. simulans* by administering a therapeutically effective amount of an anti-WTA beta-AAC of the invention. Another aspect of the invention is a method of treating a patient infected with *S. aureus* or *Listeria monocytogenes* by administering a therapeutically effective amount of an anti-WTA alpha-AAC of the invention. The invention also contemplates a method of preventing infections by *S. aureus* or *S. saprophyticus* or *S. simulans* by administering a therapeutically effective amount of an anti-WTA beta-AAC of the invention in hospital settings such as surgery, burn patient, and organ transplantation.

**[0316]** The patient needing treatment for a bacterial infection as determined by a physician of skill in the art may have already been, but does not need to be diagnosed with the kind of bacteria that he/she is infected with. Since a patient with a bacterial infection can take a turn for the worse very quickly, in a matter of hours, the patient upon admission into the hospital can be administered the anti-WTA-AACs of the invention along with one or more standard of care Abx such as vancomycin. When the diagnostic results become available and indicate the presence of, e.g., *S. aureus* in the infection, the patient can continue with treatment with the anti-WTA AAC. Therefore, in one embodiment of the method of treating a bacterial infection or specifically a *S. aureus* infection, the patient is administered a therapeutically effective amount of an anti-WTA beta AAC. In the methods of treatment or prevention of the present invention, an AAC of the invention can be administered as the sole therapeutic agent or in conjunction with other agents such as those described below. The AACs of the invention show superiority to vancomycin in the treatment of MRSA in pre-clinical models. Comparison of AACs to SOC can be measured, e.g., by a reduction in mortality rate. The patient being treated would be assessed for responsiveness to the AAC treatment by a variety of measurable factors. Examples of signs and symptoms that clinicians might use to assess improvement in their patients includes the following: normalization of the white blood cell count if elevated at diagnosis, normalization of body temperature if elevated (fever) at the time of diagnosis, clearance of blood cultures, visual improvement in wound including less erythema and drainage of pus, reduction in ventilator requirements such as requiring less oxygen or reduced rate of ventilation in a patient who is ventilated, coming off of the ventilator entirely if the patient is ventilated at the time of diagnosis, use of less medications to support a stable blood pressure if these medications were required at the time of diagnosis, normalization of lab abnormalities that suggest end-organ failure such as elevated creatinine or liver function tests if they were abnormal at the time of diagnosis, and improvement in radiologic imaging (e.g. chest x-ray that previously suggested pneumonia showing resolution). In a patient in the ICU, these factors might be measured at least daily. Fever is monitored closely as is white blood cell count including absolute neutrophil counts as well as evidence that a "left shift" (appearance of blasts indicating increased neutrophil production in response to an active infection) has resolved.

**[0317]** In the context of the present methods of treatment of the invention, a patient with a bacterial infection is considered to be treated if there is significant measurable improvement as assessed by the physician of skill in the art, in at least two or more of the preceding factors compared to the values, signs or symptoms before or at the start of treatment or at the time of diagnosis. In some embodiments, there is measurable improvement in 3, 4, 5, 6 or more of the aforementioned factors. In some embodiments, the improvement in the measured factors is by at least 50%, 60%, 70%, 80%, 90%, 95% or 100% compared to the values before treatment. Typically, a patient can be considered completely treated of the bacterial infection (e.g., *S. aureus* infection) if the patient's measurable improvements include the following: i) repeat blood or tissue cultures (typically several) that do not grow out the bacteria that was originally identified ; ii) fever is normalized; iii) WBC is normalized; and iv) evidence that end-organ failure (lungs, liver, kidneys, vascular collapse) has resolved either fully or partially given the pre-existent co-morbidities that the patient had.

### ***Dosing***

**[0318]** In any of the foregoing aspects, in treating an infected patient, the dosage of an AAC is normally about 0.001 to 1000 mg/kg/day. In one embodiment the patient with a bacterial infection is treated at an AAC dose in the range of about 1 mg/kg to about 100mg/kg, typically about 5mg/kg to about 90mg/kg, more specifically 10mg/kg to 50 mg/kg. The AAC may be given daily (e.g., a single dose of 5 to 50 mg/kg/day) or less frequently (e.g., a single dose of 5, 12.5, or 25 mg/kg/week). One dose may be split over 2 days, for example, 25mg/kg on one day and 25mg/kg the next day. The patient can be administered a dose once every 3 days (q3D), once a week to every other week (qOW), for a duration of 1-8 weeks. In one embodiment, the patient is administered an AAC of the invention via IV once a week for 2-6 weeks with standard of care (SOC) to treat the bacterial infection such as a staph A infection. Treatment length would be dictated by the condition of the patient or the extent of the infection, e.g. a duration of 2 weeks for uncomplicated bacteremia, or 6 weeks for bacteremia with endocarditis.

**[0319]** In one embodiment, an AAC administered at an initial dose of 2.5 to 100 mg/kg for one to seven consecutive days, followed by a maintenance dose of 0.005 to 10 mg/kg once every one to seven days for one month.

### ***Route of administration***

**[0320]** For treating the bacterial infections, the AACs of the invention can be administered at any of the preceding dosages intravenously (i.v.) or subcutaneously. In one embodiment, the WTA-AAC is administered intravenously. In a specific embodiment, the WTA-AAC administered via i.v. is a WTA-beta AAC, more specifically, wherein the WTA-beta antibody is one selected from the group of Abs with amino acid sequences as disclosed in Figure 14, Figure 15A,

Figure 15B, Figure 16A, and Figure 16B.

### ***Combination therapy***

**[0321]** An AAC may be administered in conjunction with one or more additional, e.g. second, therapeutic or prophylactic agents as appropriate as determined by the physician treating the patient.

**[0322]** In one embodiment, the second antibiotic administered in combination with the antibody-antibiotic conjugate compound of the invention is selected from the structural classes: (i) aminoglycosides; (ii) beta-lactams; (iii) macrolides/cyclic peptides; (iv) tetracyclines; (v) fluoroquinolones/fluoroquinolones; (vi) and oxazolidinones. See: Shaw, K. and Barbachyn, M. (2011) Ann. N.Y. Acad. Sci. 1241:48-70; Sutcliffe, J. (2011) Ann. N.Y. Acad. Sci. 1241:122-152.

**[0323]** In one embodiment, the second antibiotic administered in combination with the antibody-antibiotic conjugate compound of the invention is selected from clindamycin, novobiocin, retapamulin, daptomycin, GSK-2140944, CG-400549, sitafloxacin, teicoplanin, triclosan, naphthyridone, radezolid, doxorubicin, ampicillin, vancomycin, imipenem, doripenem, gemcitabine, dalbavancin, and azithromycin.

**[0324]** Additional examples of these additional therapeutic or prophylactic agents are anti-inflammatory agents (e.g., non-steroidal anti-inflammatory drugs (NSAIDs; e.g., detoprofen, diclofenac, diflunisal, etodolac, fenoprofen, flurbiprofen, ibuprofen, indomethacin, ketoprofen, meclofenamate, mefenamic acid, meloxicam, nabumetone, naproxen sodium, oxaprozin, piroxicam, sulindac, tolmetin, celecoxib, rofecoxib, aspirin, choline salicylate, salsalate, and sodium and magnesium salicylate) and steroids (e.g., cortisone, dexamethasone, hydrocortisone, methylprednisolone, prednisolone, prednisone, triamcinolone)), antibacterial agents (e.g., azithromycin, clarithromycin, erythromycin, gatifloxacin, levofloxacin, amoxicillin, metronidazole, penicillin G, penicillin V, methicillin, oxacillin, cloxacillin, dicloxacillin, nafcillin, ampicillin, carbenicillin, ticarcillin, mezlocillin, piperacillin, azlocillin, temocillin, cephalothin, cephapirin, cephadrine, cephaloridine, cefazolin, cefamandole, cefuroxime, cephalexin, cefprozil, cefaclor, loracarbef, cefoxitin, cefmoxazole, cefotaxime, ceftizoxime, ceftriaxone, cefoperazone, ceftazidime, cefixime, cefpodoxime, ceftibuten, cefdinir, cefpirome, cefepime, BAL5788, BAL9141, imipenem, ertapenem, meropenem, astreonom, clavulanate, sulbactam, tazobactam, streptomycin, neomycin, kanamycin, paromycin, gentamicin, tobramycin, amikacin, netilmicin, spectinomycin, sisomicin, dibekalin, isepamicin, tetracycline, chlortetracycline, demeclocycline, minocycline, oxytetracycline, methacycline, doxycycline, telithromycin, ABT-773, lincomycin, clindamycin, vancomycin, oritavancin, dalbavancin, teicoplanin, quinupristin and dalopristin, sulphamylamide, para-aminobenzoic acid, sulfadiazine, sulfisoxazole, sulfamethoxazole, sulfathalidine, linezolid, nalidixic acid, oxolinic acid, norfloxacin, perfloxacin, enoxacin, ofloxacin, ciprofloxacin, temafloxacin, lomefloxacin, fleroxacin, grepafloxacin, sparfloxacin, trovafloxacin, clinafloxacin, moxifloxacin, gemifloxacin, sitafloxacin, daptomycin, garenoxacin, ramoplanin, faropenem, polymyxin, tigecycline,

AZD2563, or trimethoprim), antibacterial antibodies including antibodies to the same or different antigen from the AAC targeted Ag, platelet aggregation inhibitors (e.g., abciximab, aspirin, cilostazol, clopidogrel, dipyridamole, eptifibatide, ticlopidine, or tirofiban), anticoagulants (e.g., dalteparin, danaparoid, enoxaparin, heparin, tinzaparin, or warfarin), antipyretics (e.g., acetaminophen), or lipid lowering agents (e.g., cholestyramine, colestipol, nicotinic acid, gemfibrozil, probucol, ezetimibe, or statins such as atorvastatin, rosuvastatin, lovastatin simvastatin, pravastatin, cerivastatin, and fluvastatin). In one embodiment the AAC of the invention is administered in combination with standard of care (SOC) for *S. aureus* (including methicillin-resistant and methicillin-sensitive strains). MSSA is usually typically treated with nafcillin or oxacillin and MRSA is typically treated with vancomycin or cefazolin.

**[0325]** These additional agents may be administered within 14 days, 7 days, 1 day, 12 hours, or 1 hour of administration of an AAC, or simultaneously therewith. The additional therapeutic agents may be present in the same or different pharmaceutical compositions as an AAC. When present in different pharmaceutical compositions, different routes of administration may be used. For example, an AAC may be administered intravenous or subcutaneously, while a second agent may be administered orally.

## PHARMACEUTICAL FORMULATIONS

**[0326]** The present invention also provides pharmaceutical compositions containing the AAC, and to methods of treating a bacterial infection using the pharmaceutical compositions containing AAC. Such compositions may further comprise suitable excipients, such as pharmaceutically acceptable excipients (carriers) including buffers, acids, bases, sugars, diluents, glidants, preservatives and the like, which are well known in the art and are described herein. The present methods and compositions may be used alone or in combinations with other conventional methods and/or agents for treating infectious diseases. In one aspect, the invention further provides pharmaceutical formulations comprising at least one antibody of the invention and/or at least one antibody-antibiotic conjugate (AAC) thereof. In some embodiments, a pharmaceutical formulation comprises 1) an antibody of the invention and/or an AAC thereof, and 2) a pharmaceutically acceptable carrier. In some embodiments, a pharmaceutical formulation comprises 1) an antibody of the invention and/or an AAC thereof, and optionally, 2) at least one additional therapeutic agent.

**[0327]** Pharmaceutical formulations comprising an antibody or AAC of the invention are prepared for storage by mixing the antibody or AAC having the desired degree of purity with optional physiologically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)) in the form of aqueous solutions or lyophilized or other dried formulations. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, histidine and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride); phenol, butyl or



benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g., Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG). Pharmaceutical formulations to be used for *in vivo* administration are generally sterile, readily accomplished by filtration through sterile filtration membranes.

**[0328]** Active ingredients may also be entrapped in microcapsule prepared, for example, by co-acervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsule and poly-(methylmethacrylate) microcapsule, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nanoparticles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980).

**[0329]** Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody or AAC of the invention, which matrices are in the form of shaped articles, e.g., films, or microcapsule. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and  $\gamma$  ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated antibodies or AAC remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37 °C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

**[0330]** An antibody may be formulated in any suitable form for delivery to a target cell/tissue. For example, antibodies may be formulated as liposomes, a small vesicle composed of various types of lipids, phospholipids and/or surfactant which is useful for delivery of a drug to a mammal. The components of the liposome are commonly arranged in a bilayer formation, similar to the lipid arrangement of biological membranes. Liposomes containing the antibody

are prepared by methods known in the art, such as described in Epstein et al., (1985) Proc. Natl. Acad. Sci. USA 82:3688; Hwang et al., (1980) Proc. Natl Acad. Sci. USA 77:4030; US 4485045; US 4544545; WO 97/38731; US 5013556.

**[0331]** Particularly useful liposomes can be generated by the reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of the antibody of the present invention can be conjugated to the liposomes as described in Martin et al., (1982) J. Biol. Chem. 257:286-288 via a disulfide interchange reaction. A chemotherapeutic agent is optionally contained within the liposome (Gabizon et al., (1989) J. National Cancer Inst. 81(19):1484).

## METHODS AND COMPOSITIONS FOR DIAGNOSTICS AND DETECTION

**[0332]** In certain embodiments, any of the antibodies provided herein is useful for detecting the presence of MRSA in a biological sample. The term "detecting" as used herein encompasses quantitative or qualitative detection. A "biological sample" comprises, e.g., blood, serum, plasma, tissue, sputum, aspirate, swab, etc.

**[0333]** In one embodiment, an anti-WTA antibody for use in a method of diagnosis or detection is provided. In a further aspect, a method of detecting the presence of WTA in a biological sample is provided. In certain embodiments, the method comprises contacting the biological sample with an anti-WTA antibody as described herein under conditions permissive for binding of the anti-WTA antibody to WTA, and detecting whether a complex is formed between the anti-WTA antibody and WTA in the biological sample. Such method may be an *in vitro* or *in vivo* method. In one embodiment, an anti-MRSA antibody is used to select subjects eligible for therapy with an anti-MRSA antibody, e.g. where MRSA is a biomarker for selection of patients.

**[0334]** In one exemplary embodiment, an anti-WTA antibody is used *in vivo* to detect, e.g., by *in vivo* imaging, an MRSA-positive infection in a subject, e.g., for the purposes of diagnosing, prognosing, or staging treatment of an infection, determining the appropriate course of therapy, or monitoring response of the infection to therapy. One method known in the art for *in vivo* detection is immuno-positron emission tomography (immuno-PET), as described, e.g., in van Dongen et al., (2007) The Oncologist 12:1379-1389 and Verel et al., (2003) J. Nucl. Med. 44:1271-1281. In such embodiments, a method is provided for detecting an Staph-positive infection in a subject, the method comprising administering a labeled anti-Staph antibody to a subject having or suspected of having an Staph-positive infection, and detecting the labeled anti-Staph antibody in the subject, wherein detection of the labeled anti-Staph antibody indicates a Staph-positive infection in the subject. In certain of such embodiments, the labeled anti-Staph antibody comprises an anti-Staph antibody conjugated to a positron emitter, such as  $^{68}\text{Ga}$ ,  $^{18}\text{F}$ ,  $^{64}\text{Cu}$ ,  $^{86}\text{Y}$ ,  $^{76}\text{Br}$ ,  $^{89}\text{Zr}$ , and  $^{124}\text{I}$ . In a particular embodiment, the positron emitter is

<sup>89</sup>Zr.

**[0335]** In further embodiments, a method of diagnosis or detection comprises contacting a first anti-Staph antibody immobilized to a substrate with a biological sample to be tested for the presence of Staph, exposing the substrate to a second anti-Staph antibody, and detecting whether the second anti-Staph antibody is bound to a complex between the first anti-Staph antibody and Staph in the biological sample. A substrate may be any supportive medium, e.g., glass, metal, ceramic, polymeric beads, slides, chips, and other substrates. In certain embodiments, a biological sample comprises a cell or tissue (e.g., biopsy material, including cancerous or potentially cancerous colorectal, endometrial, pancreatic or ovarian tissue). In certain embodiments, the first or second anti-Staph antibody is any of the antibodies described herein. In such embodiments, the second anti-WTA antibody may be anti-WTA antibodies S4497, S4462, S6978, S4487, or antibodies derived from them as described herein.

**[0336]** Exemplary disorders that may be diagnosed or detected according to any of the above embodiments include MRSA-positive infection, using test such as immunohistochemistry (IHC) or in situ hybridization (ISH). In some embodiments, a Staph-positive infection is an infection that expresses Staph according to a reverse-transcriptase PCR (RT-PCR) assay that detects Staph mRNA. In some embodiments, the RT-PCR is quantitative RT-PCR (Francois P and Schrenzel J (2008). "Rapid Diagnosis and Typing of Staphylococcus aureus". Staphylococcus: Molecular Genetics. Caister Academic Press; Mackay IM, ed. (2007)), and real time PCR ("Real-Time PCR in Microbiology: From Diagnosis to Characterization. Caister Academic Press).

**[0337]** In certain embodiments, labeled anti-wall teichoic acid (WTA) antibodies are provided. Labels include, but are not limited to, labels or moieties that are detected directly (such as fluorescent, chromophoric, electron-dense, chemiluminescent, and radioactive labels), as well as moieties, such as enzymes or ligands, that are detected indirectly, e.g., through an enzymatic reaction or molecular interaction. Exemplary labels include, but are not limited to, the radioisotopes <sup>32</sup>P, <sup>14</sup>C, <sup>125</sup>I, <sup>3</sup>H, and <sup>131</sup>I, fluorophores such as rare earth chelates or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luciferases, e.g., firefly luciferase and bacterial luciferase (US 4737456), luciferin, 2,3-dihydrophthalazinediones, horseradish peroxidase (HRP), alkaline phosphatase,  $\beta$ -galactosidase, glucoamylase, lysozyme, saccharide oxidases, e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase, heterocyclic oxidases such as uricase and xanthine oxidase, coupled with an enzyme that employs hydrogen peroxide to oxidize a dye precursor such as HRP, lactoperoxidase, or microperoxidase, biotin/avidin, spin labels, bacteriophage labels, stable free radicals, and the like. In another embodiment, a label is a positron emitter. Positron emitters include but are not limited to <sup>68</sup>Ga, <sup>18</sup>F, <sup>64</sup>Cu, <sup>86</sup>Y, <sup>76</sup>Br, <sup>89</sup>Zr, and <sup>124</sup>I. In a particular embodiment, a positron emitter is <sup>89</sup>Zr.

**[0338]** Clinically, the symptoms of infections with MRSA are similar to those of methicillin-sensitive Staphylococcus aureus (MSSA), and include abscesses and cellulitis. Often, the

abscesses are accompanied by an areas of central necrosis. Furuncles (boils) are also common, particularly in the context of a MRSA outbreak. Lesions may also be misreported as a spider bite due the general redness which progresses to a necrotic center. Additionally, infections can appear as impetigo, folliculitis, deep-seated abscesses, pyomyositis, osteomyelitis, necrotizing fasciitis, staphylococcal toxic-shock syndrome, pneumonia and sepsis. Serious systemic infections are more common among persons with a history of injection drug use, diabetes or other immunocompromising conditions.

**[0339]** Standard treatment options for MRSA infections include conservative, mechanical options such as: (i) warm soaks and compresses, (ii) incision and drainage, and (iii) remove of foreign device (e.g., catheter) causing the infection. For more serious infections, especially those displaying cellulitis, antibiotics (Abx) are prescribed. For mild to moderate infections, antibiotics include trimethoprim-sulfamethoxazole (TMP-SMX), clindamycin, doxycycline, minocycline, tetracycline, rifampin, vancomycin, linezolid. Typically, a treatment regimen occurs for 5-10 with periotic reexamination and evaluation both during and after the treatment period.

**[0340]** Additional treatment options include decolonization, especially in the setting where a patient experiences recurring infection or where they are in an environment where a MRSA outbreak is ongoing. Decolonization is a procedure where the flora inhibiting the nares of the patient is removed. This is done through topical application of 2% mupirocin ointment applied generously within both nostrils for 5-10 days and topical cleansing with chlorhexidine gluconate 4% for 5 days.

## ARTICLES OF MANUFACTURE

**[0341]** In another aspect of the invention, an article of manufacture containing materials useful for the treatment, prevention and/or diagnosis of the disorders described above is provided. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the disorder and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an antibody or immunoconjugate of the invention. The label or package insert indicates that the composition is used for treating the condition of choice. Moreover, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises an antibody or immunoconjugate of the invention; and (b) a second container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent. The article of manufacture in this embodiment of the invention may further comprise a package insert indicating that the compositions can be used to treat a particular condition. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable

buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution or dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

## EXAMPLES

[0342] The following are examples of methods and compositions of the invention. It is understood that various other embodiments may be practiced, given the general description provided above.

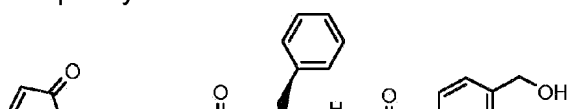
### Example 1 MC-vc-PAB-pipBOR 51

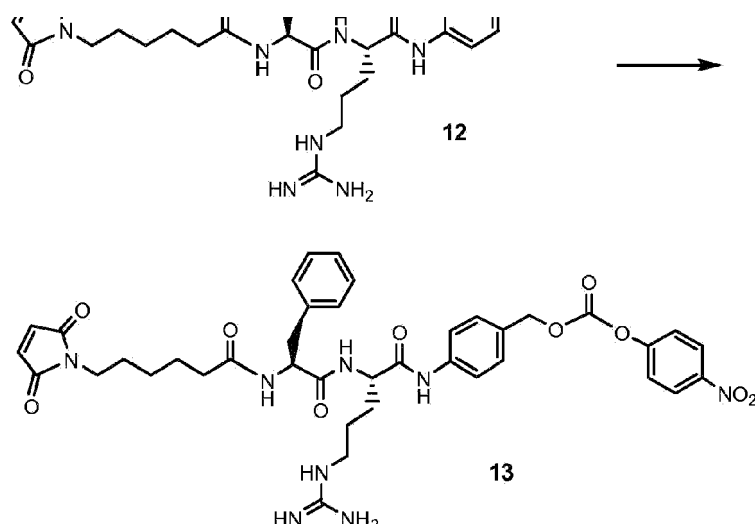
[0343] 2-Nitrobenzene-1,3-diol **1** was hydrogenated under hydrogen gas with palladium/carbon catalyst in ethanol solvent to give 2-aminobenzene-1,3-diol **2**, isolated as the hydrochloride salt (Figures 23A and 23B). Mono-protection of **2** with tertbutyldimethylsilyl chloride and triethylamine in dichloromethane/tetrahydrofuran gave 2-amino-3-(tert-butyldimethylsilyloxy)phenol **3**. Rifamycin S (ChemShuttle Inc., Fremont, CA, US 7342011; US 7271165; US 7547692) was reacted with **3** by oxidative condensation with manganese oxide or oxygen gas in toluene at room temperature to give TBS-protected benzoxazino rifamycin **4**. Reaction of **4** with piperidin-4-amine and manganese oxide gave piperidyl benzoxazino rifamycin (pipBOR) **5**.

[0344] Piperidyl benzoxazino rifamycin (pipBOR) **5** (0.02 mmol) and 4-((S)-2-((S)-2-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl 4-nitrophenyl carbonate **6** (0.02 mmol) were mixed in DMF (0.4 ml) at room temperature (RT). To this was added 2.5 equivalents of N,N'-diisopropylethylamine. The solution was stirred from one to about 12 hours and was monitored by LC/MS. Upon completion, the solution was diluted with DMF and injected onto HPLC and purified under acidic conditions to give MC-vc-PAB-pipBOR **51**. M/Z = 1498.9. Yield 40%

### Example 2 MC-fk-PAB-pipBOR 52

[0345] Following the procedure of Example 1, 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N-((S)-1-((S)-5-guanidino-1-(4-(hydroxymethyl)phenylamino)-1-oxopentan-2-ylamino)-1-oxo-3-phenylpropan-2-yl)hexanamide **12** was converted to 4-((S)-2-((S)-2-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamido)-3-phenylpropanamido)-5-guanidinopentanamido)benzyl 4-nitrophenyl carbonate **13**.

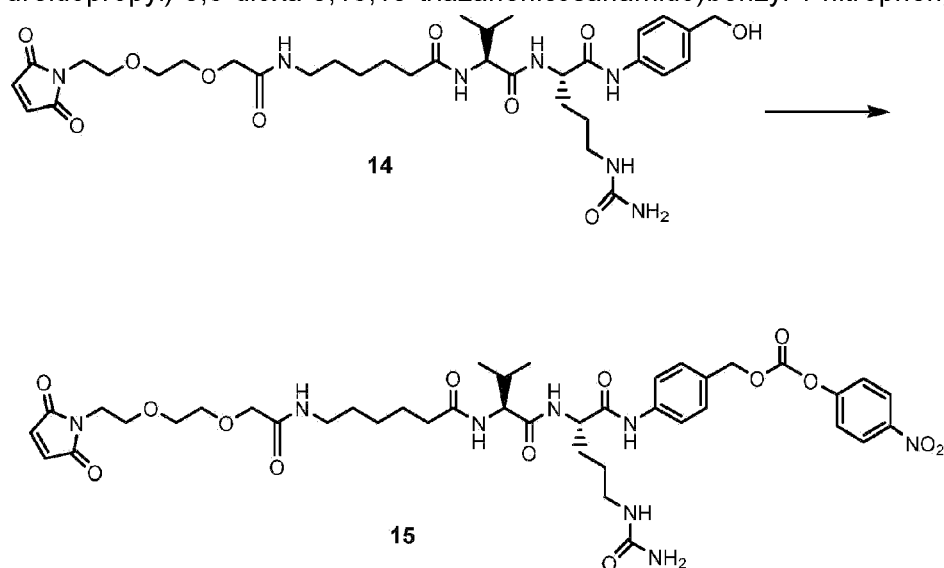




**[0346]** Piperidyl benzoxazino rifamycin (pipBOR) **5** (0.02 mmol) and **13** (0.02 mmol) were mixed in DMF (0.4 ml) at room temperature (RT). To this was added 2.5 equivalents of N,N'-diisopropylethylamine. The solution was stirred from one to about 12 hours and was monitored by LC/MS. Upon completion, the solution was diluted with DMF and injected onto HPLC and purified under acidic conditions to give MC-fk-PAB-pipBOR **52**. M/Z = 1545.8. Yield 32%

### Example 3 MP-vc-PAB-pipBOR **53**

**[0347]** Following the procedure of Example 1, 6-(2-(2-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)ethoxy)acetamido)-N-((S)-1-((S)-1-(4-(hydroxymethyl)phenylamino)-1-oxo-5-ureidopentan-2-ylamino)-3-methyl-1-oxobutan-2-yl)hexanamide **14** was converted to 4-((17S,20S)-1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-17-isopropyl-8,15,18-trioxo-20-(3-ureidopropyl)-3,6-dioxo-9,16,19-triazahenicosanamido)benzyl 4-nitrophenyl carbonate **15**.



**[0348]** Piperidyl benzoxazino rifamycin (pipBOR) **5** (0.02 mmol) and **15** (0.02 mmol) were mixed in DMF (0.4 ml) at room temperature (RT). To this was added 2.5 equivalents of N,N'-diisopropylethylamine. The solution was stirred from one to about 12 hours and was monitored by LC/MS. Upon completion, the solution was diluted with DMF and injected onto HPLC and purified under acidic conditions to give MP-vc-PAB-pipBOR **53**. M/Z = 1644.8. Yield 57%

**Example 4 MC-vc-PAB-dimethylpipBOR 54**

**[0349]** Reaction of N,N-dimethylpiperidin-4-amine with TBS-protected benzoxazino rifamycin **4** gave dimethylpiperidyl benzoxazino rifamycin (dimethyl pipBOR) **7** (Figure 24).

**[0350]** Alternatively, (5-fluoro-2-nitro-1,3-phenylene)bis(oxy)bis(methylene)dibenzene **9** was hydrogenated under hydrogen gas with palladium/carbon catalyst in tetrahydrofuran/methanol solvent to remove the benzyl groups to give 2-amino-5-fluorobenzene-1,3-diol **10**. Commercially available Rifamycin S or Rifamycin SV sodium salt (ChemShuttle Inc., Fremont, CA) was reacted with 2-amino-5-fluorobenzene-1,3-diol **10** by oxidative condensation in air or potassium ferric cyanide in ethylacetate at 60 °C to give fluoro benzoxazino rifamycin **11**. Displacement of fluoro with N,N-dimethylpiperidin-4-amine gave dimethyl pipBOR **7** (Figures 25A and 25B).

**[0351]** 6-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N-((S)-1-((S)-1-(4-(hydroxymethyl)phenylamino)-1-oxo-5-ureidopentan-2-ylamino)-3-methyl-1-oxobutan-2-yl)hexanamide **8**, prepared according to procedures in WO 2012113847; US 7659241; US 7498298; US 20090111756; US 20090018086; US 6214345; Dubowchik et al (2002) Bioconjugate Chem. 13(4):855-869 (1.009 g, 1.762 mmol, 1.000, 1009 mg) was taken up in N,N-dimethylformamide (6 mL, 77 mmol, 44, 5700 mg). To this was added a solution of thionyl chloride (1.1 equiv., 1.938 mmol, 1.100, 231 mg) in dichloromethane (DCM) (1 mL, 15.44 mmol, 8.765, 1325 mg) in portions dropwise (1/2 was added over 1 hour, stirred one hour at room temperature (RT) then added the other half over another hour). The solution remained a yellow color. Another 0.6 eq of thionyl chloride was added as a solution in 0.5 mL DCM dropwise, carefully. The reaction remained yellow and was stirred sealed overnight at RT. The reaction was monitored by LC/MS to 88% product benzyl chloride **9**. Another 0.22 eq of thionyl chloride was added dropwise as a solution in 0.3 mL DCM. When the reaction approached 92% benzyl chloride **9**, the reaction was bubbled with N<sub>2</sub>. The concentration was reduced from 0.3 M to 0.6 M. The product N-((S)-1-((S)-1-(4-(chloromethyl)phenylamino)-1-oxo-5-ureidopentan-2-ylamino)-3-methyl-1-oxobutan-2-yl)-6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamide **9** was stored in the refrigerator as a 0.6 M solution and used as is. M/Z 591.3, 92% yield.

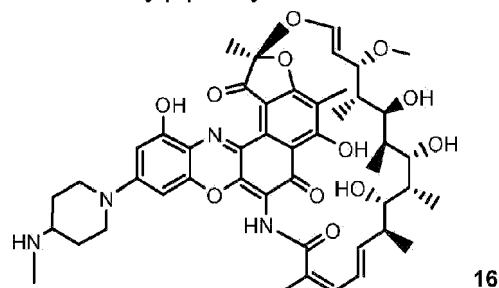
**[0352]** In a flask, N-((S)-1-((S)-1-(4-(chloromethyl)phenylamino)-1-oxo-5-ureidopentan-2-ylamino)-3-methyl-1-oxobutan-2-yl)-6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamide **9**, (0.9 mmol) was cooled to 0 °C and dimethylpiperidyl benzoxazino rifamycin (dimethyl pipBOR) **7** (0.75 g, 0.81 mmol, 0.46, 750 mg) was added. The mixture was diluted with another 1.5 mL

of DMF to reach 0.3 M. Stirred open to air for 30 minutes. N,N-diisopropylethylamine (3.5 mmol, 3.5 mmol, 2.0, 460 mg) was added and the reaction stirred overnight open to air. Over the course of 4 days, 4 additions of 0.2eq N,N-diisopropylethylamine base was added while the reaction stirred open to air, until the reaction appeared to stop progressing. The reaction was diluted with DMF and purified on HPLC (20-60% ACN/FA·H<sub>2</sub>O) in several batches to give MC-vc-PAB-dimethylpipBOR **54**.

M/Z=1482.8 yield: 32%

#### **Example 5 MC-vc-PAB-monomethylpip, desacetylBOR 55**

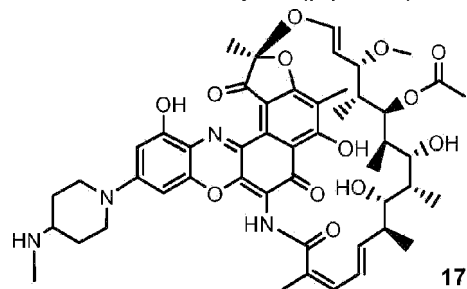
**[0353]** Following the procedures of Example 1, N-methylpiperidin-4-amine and TBS-protected desacetyl, benzoxazino rifamycin were reacted with manganese oxide to give monomethylpiperidyl benzoxazino rifamycin (pipBOR) **16**.



**[0354]** 4-((S)-2-((S)-2-(6-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl 4-nitrophenyl carbonate **6** and **16** were reacted to give MC-vc-PAB-monomethylpip, desacetylBOR **55** in 26% yield (M/Z = 1456.5).

#### **Example 6 MC-vc-PAB-monomethylpipBOR 56**

**[0355]** Following the procedures of Example 1, N-methylpiperidin-4-amine and TBS-protected, benzoxazino rifamycin **4** were reacted with manganese oxide to give monomethylpiperidyl benzoxazino rifamycin (pipBOR) **17**.



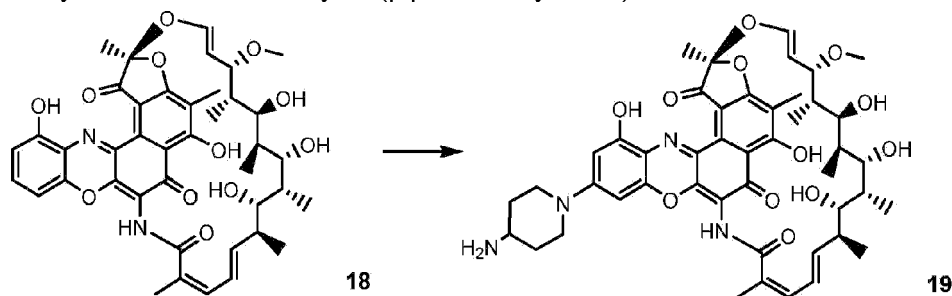
**[0356]** 4-((S)-2-((S)-2-(6-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl 4-nitrophenyl carbonate **6** and **17** were



reacted to give MC-vc-PAB-monomethylpipBOR **56** in 25% yield ( $M/Z = 1471.0$ ).

**Example 7** MC-vc-PAB- pip, desacetylBOR 57

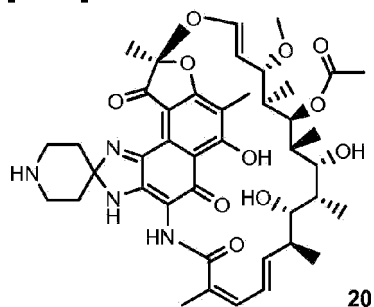
**[0357]** Following the procedures of Example 1, piperidin-4-amine and TBS-protected desacetyl, benzoxazino rifamycin **18** were reacted with manganese oxide to give piperidyl, des-acetyl benzoxazino rifamycin (pip desacetyl BOR) **19**.



**[0358]** Piperidyl, des-acetyl benzoxazino rifamycin **19** (0.02 mmol) and 4-((S)-2-((S)-2-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl 4-nitrophenyl carbonate **6** (0.02 mmol) were reacted to give MC-vc-PAB-pip,desacetylBOR **57**. M/Z = 1456.6. Yield 13%

### Example 8 MC-vc-PAB-rifabutin 58

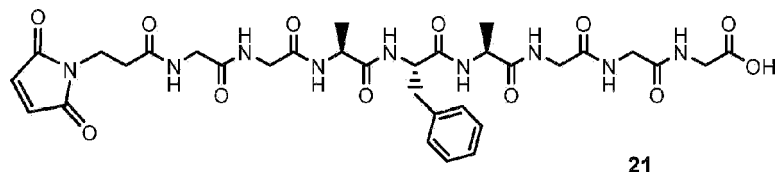
**[0359]**



**[0360]** Following the procedures of Example 1, des-isobutyl rifabutin **20** (0.02 mmol) and 4-((S)-2-((S)-2-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl 4-nitrophenyl carbonate **6** (0.02 mmol) were reacted to give MC-vc-PAB-rifabutin **58**. M/Z=1389.6. Yield 21%

**Example 9** MC-GGAFAGGG-pipBOR ("core peptide" disclosed as SEQ ID NO: 126)59

[0361]

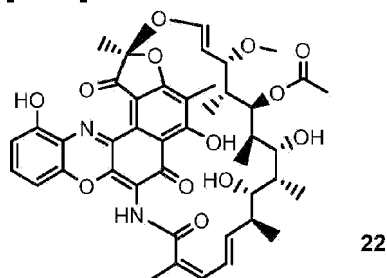


21

[0362] Following the procedures of Example 1, maleimide peptide **21** was coupled with piperidyl benzoxazino rifamycin (pipBOR) **5** under standard amide bond forming conditions to give MC-GGAFAGGG-pipBOR ("core peptide" disclosed as SEQ ID NO: 126) **59**.  $M/Z=1626.0$ . Yield 13%

#### Example 10 MC-vc-PAB-rif **60**

[0363]

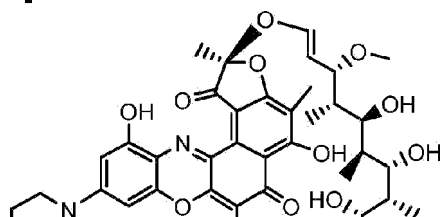


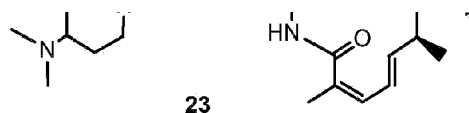
22

[0364] In a small vial, 0.05 mL of a 0.6 M solution of N-((S)-1-((S)-1-(4-(chloromethyl)phenylamino)-1-oxo-5-ureidopentan-2-ylamino)-3-methyl-1-oxobutan-2-yl)-6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamide **9**, prepared by the procedure of Example 4 was cooled to 0 °C and 1 equiv. of benzoxazino rifamycin **22** was added and the mixture was stirred for 5 minutes. To this 0 °C solution was added  $K_2CO_3$  (15 eq) and the sides of the vial were washed with 0.05 mL of DMF. The reaction was stirred open to air to room temperature for 1-4 hours. When all **9** was consumed, the solids were filtered off, and the collected filtrate was diluted with DMF. Purification by HPLC gave MC-vc-PAB-rif **60** in 11% yield ( $M/Z = 1356.9$ ).

#### Example 11 MC-vc-PAB-dimethylpip, desacetylBOR **61**

[0365]

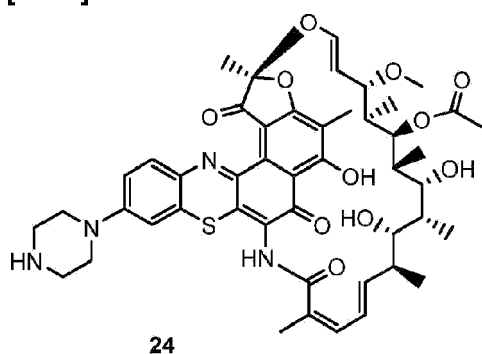




**[0366]** Following the procedures of Example 4, N-((S)-1-((S)-1-(4-(chloromethyl)phenylamino)-1-oxo-5-ureidopentan-2-ylamino)-3-methyl-1-oxobutan-2-yl)-6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamide **9** was reacted with dimethylpiperidyl, desacetyl benzoxazino rifamycin (dimethyl, desacetyl pipBOR) **23** to give MC-vc-PAB-dimethylpip, desacetylBOR **61**. M/Z = 1440.66

**Example 12 MC-vc-PAB-piperazBTR 62**

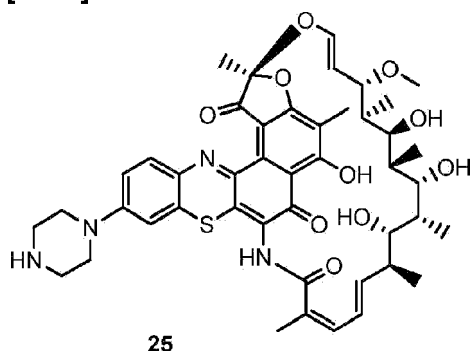
**[0367]**



**[0368]** Following the procedures of Example 1, 4-((S)-2-((S)-2-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl 4-nitrophenyl carbonate **6** was reacted with piperazino benzthiazino rifamycin (piperazBTR) **24** to give MC-vc-PAB-piperazBTR **62**. M/Z = 1483.7

**Example 13 MC-vc-PAB- piperaz, desacetylBTR 63**

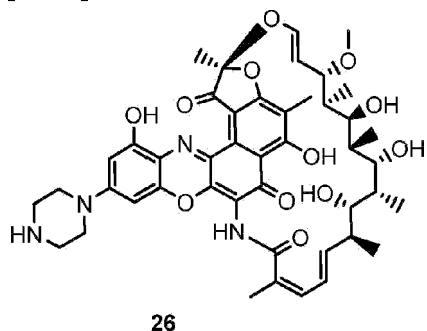
**[0369]**



[0370] Following the procedures of Example 1, 4-((S)-2-((S)-2-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl 4-nitrophenyl carbonate **6** was reacted with piperazino, desacetyl benzthiazino rifamycin (pipBTR) **25** to give MC-vc-PAB- piperaz, desacetylBTR **63**. M/Z = 1441.6

**Example 14 MC-vc-PAB-piperaz, desacetylBOR 64**

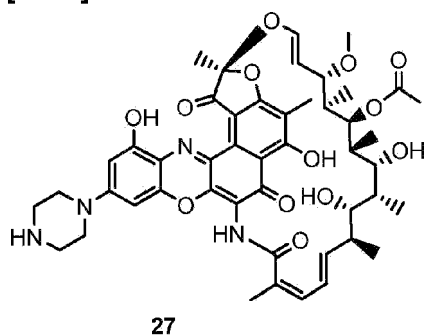
[0371]



[0372] Following the procedures of Example 1, 4-((S)-2-((S)-2-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl 4-nitrophenyl carbonate **6** was reacted with piperazyl, desacetyl benzoxazino rifamycin (desacetyl pipBOR) **26** to give MC-vc-PAB-piperaz, desacetylBOR **64**. M/Z = 1441.6

**Example 15 MC-vc-PAB-piperazBOR 65**

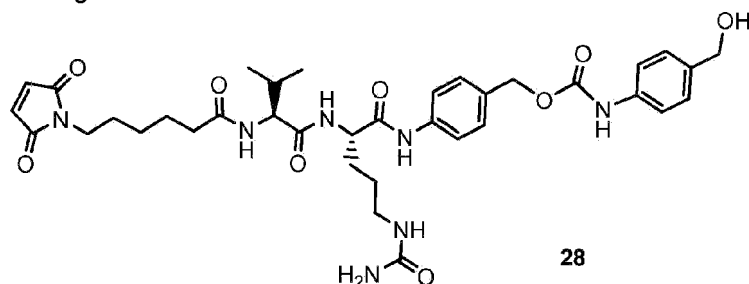
[0373]



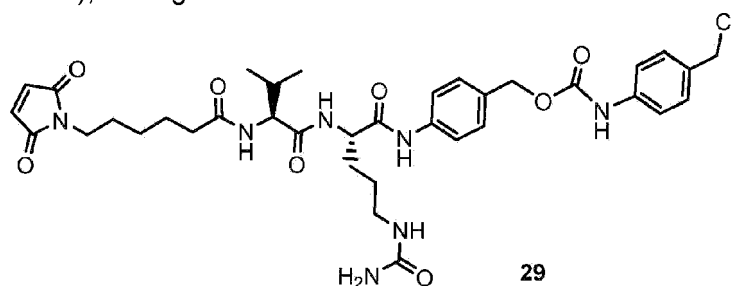
[0374] Following the procedures of Example 1, 4-((S)-2-((S)-2-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl 4-nitrophenyl carbonate **6** was reacted with piperazyl benzoxazino rifamycin (piperazylBOR) **27** to give MC-vc-PAB-piperazBOR **65**. M/Z = 1482.7

**Example 16 MC-vc-bisPAB-dimethylpipBOR 66**

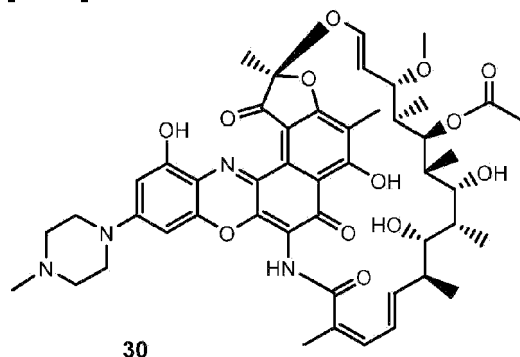
**[0375]** In a vial, 4-((S)-2-((S)-2-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl 4-nitrophenyl carbonate **6** (1.56 g, 2.11 mmol, 100 mass%) was taken up in DMF (55 equiv., 116 mmol, 55.0, 8.5 g) and stirred at RT. To this cloudy yellow mixture was added (4-aminophenyl)methanol (PAB, 1.1 equiv., 2.33 mmol, 1.10, 286 mg) and 1-hydroxybenzotriazole (0.37 equiv., 0.782 mmol, 0.370, 106 mg) followed by N,N'-diisopropylethylamine (1 equiv., 2.11 mmol, 1.00, 276 mg). The reaction was stirred for 2 hours and monitored by LC/MS. An additional 1 equivalent of N,N'-diisopropylethylamine (Hunigs Base) and 100 mg of (4-aminophenyl)methanol were added. The reaction was stirred overnight at RT sealed. About 0.5L of diethyl ether was added dropwise to precipitate out product. The ether was decanted, the solid was redissolved in DMF, and purified directly onto HPLC in several batches to give 4-((S)-2-((S)-2-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl 4-(hydroxymethyl)phenylcarbamate **28** (0.435g) in 28% overall isolated yield (M/Z: 722.5), having the structure:



**[0376]** Following the procedure of Example 4, 4-((S)-2-((S)-2-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl 4-(hydroxymethyl)phenylcarbamate **28** was reacted with thionyl chloride to give 4-((S)-2-((S)-2-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl 4-(chloromethyl)phenylcarbamate **29** in 47% isolated yield (M/Z: 740.4), having the structure:



**[0377]** Following the procedure of Example 4, 4-((S)-2-((S)-2-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl 4-(chloromethyl)phenylcarbamate **29** was reacted with dimethylpiperidyl benzoxazino rifamycin (dimethyl pipBOR) **7** to give MC-vc-bisPAB-dimethylpipBOR **66** in 5% yield (M/Z: 1632.1)

**Example 17 MC-vc-PAB-methylpiperaz BOR 67****[0378]**

**[0379]** Following the procedure of Example 4, N-((S)-1-((S)-1-(4-(chloromethyl)phenylamino)-1-oxo-5-ureidopentan-2-ylamino)-3-methyl-1-oxobutan-2-yl)-6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamide **9** was reacted with methylpiperazino benzoxazino rifamycin (methyl piperazBOR) **30** to give MC-vc-PAB-methylpiperaz BOR **67**. M/Z = 1454.68

**Example 18 Intracellular MRSA are protected from antibiotics**

**[0380]** This example provides evidence that MRSA can survive intracellularly *in vivo*. In an infection, intracellular MRSA are protected from and able to survive antibiotic treatment (such as SOC Vancomycin), enabling transfer of infection from one cell to another.

**[0381]** *MIC determinations for extracellular bacteria:* The MIC for extracellular bacteria was determined by preparing serial 2-fold dilutions of the antibiotic in Tryptic Soy Broth. Dilutions of the antibiotic were made in quadruplicate in 96 well culture dishes. MRSA (NRS384 strain of USA300) was taken from an exponentially growing culture and diluted to  $1 \times 10^4$  CFU/mL. Bacteria was cultured in the presence of antibiotic for 18-24 hours with shaking at 37 °C and bacterial growth was determined by reading the Optical Density (OD) at 630 nM. The MIC was determined to be the dose of antibiotic that inhibited bacterial growth by >90%.

**[0382]** *MIC determinations for intracellular bacteria:* Intracellular MIC was determined on bacteria that were sequestered inside mouse peritoneal macrophages. Macrophages were plated in 24 well culture dishes at a density of  $4 \times 10^5$  cells/mL and infected with MRSA (NRS384 strain of USA300) at a ratio of 10-20 bacteria per macrophage. Macrophage cultures were maintained in growth media supplemented with 50 µg/mL of gentamycin to inhibit the growth of extracellular bacteria and test antibiotics were added to the growth media 1 day after infection. The survival of intracellular bacteria was assessed 24 hours after addition of the antibiotics.

Macrophages were lysed with Hanks Buffered Saline Solution supplemented with .1% Bovine Serum Albumin and .1% Triton-X, and serial dilutions of the lysate were made in Phosphate Buffered Saline solution containing 0.05% Tween-20. The number of surviving intracellular bacteria was determined by plating on Tryptic Soy Agar plates with 5% defibrinated sheep blood.

**[0383]** *Isolation of peritoneal macrophages:* Peritoneal macrophages were isolated from the peritoneum of 6-8 week old Balb/c mice (Charles River Laboratories, Hollister, CA). To increase the yield of macrophages, mice were pre-treated by intraperitoneal injection with 1 mL of thioglycolate media (Becton Dickinson). The thioglycolate media was prepared at a concentration of 4% in water, sterilized by autoclaving, and aged for 20 days to 6 months prior to use. Peritoneal macrophages were harvested 4 days post treatment with thioglycolate by washing the peritoneal cavity with cold phosphate buffered saline. Macrophages were plated in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Fetal Calf Serum, and 10 mM HEPES, without antibiotics, at a density of  $4 \times 10^5$  cells/well in 24 well culture dishes. Macrophages were cultured over night to permit adherence to the plate. This assay was also utilized to test intracellular killing in non-phagocytic cell types. MG63 (CRL-1427) and A549 (CCL185) cell lines were obtained from ATCC and maintained in RPMI 1640 tissue culture media supplemented with 10 mM Hepes and 10 % Fetal Calf Serum (RPMI-10). HUVEC cells were obtained from Lonza and maintained in EGM Endothelial Cell Complete Media (Lonza, Walkersville, MD).

**[0384]** *Infection of macrophages with opsonized MRSA:* The USA300 strain of MRSA (NRS384) was obtained from the NARSA repository (Chantilly, Virginia). Some experiments utilized the Newman strain of *S. aureus* (ATCC25904). In all experiments bacteria were cultured in Tryptic Soy Broth. To assess intracellular killing with AAC, USA300 was taken from an exponentially growing culture and washed in HB (Hanks Balanced Salt Solution supplemented with 10 mM HEPES and 0.1% Bovine Serum Albumin). AAC or antibodies were diluted in HB and incubated with the bacteria for 1 hour to permit antibody binding to the bacteria (opsonization), and the opsonized bacteria were used to infect macrophages at a ratio of 10-20 bacteria per macrophage ( $4 \times 10^6$  bacteria in 250  $\mu$ L of HB per well. Macrophages were pre-washed with serum free DMEM media immediately before infection, and infected by incubation at 37 °C in a humidified tissue culture incubator with 5% CO<sub>2</sub> to permit phagocytosis of the bacteria. After 2 hours, the infection mix was removed and replaced with normal growth media (DMEM supplemented with 10% Fetal Calf Serum, 10 mM HEPES and gentamycin was added at 50  $\mu$ g/ml to prevent growth of extracellular bacteria. At the end of the incubation period, the macrophages were washed with serum free media, and the cells were lysed in HB supplemented with 0.1% triton-X (lyses the macrophages without damaging the intracellular bacteria). In some experiments viability of the macrophages was assessed at the end of the culture period by detecting release of cytoplasmic lactate dehydrogenase (LDH) into the culture supernatant using an LDH Cytotoxicity Detection Kit (Product 11644793001, Roche Diagnostics Corp, Indianapolis, IN). Supernatants were collected and analyzed immediately according to the manufacturer's instructions. Serial dilutions of the lysate were made in

phosphate buffered saline solution supplemented with 0.05% Tween-20 (to disrupt aggregates of bacteria) and the total number of surviving intracellular bacteria was determined by plating on Tryptic Soy Agar with 5% defibrinated sheep blood.

**[0385]** *Generation of MRSA infected peritoneal cells.* 6-8 week old female A/J mice (JAX™ Mice, Jackson Laboratories) were infected with  $1 \times 10^8$  CFU of the NRS384 strain of USA300 by peritoneal injection. The peritoneal wash was harvested 1 day post infection, and the infected peritoneal cells were treated with 50 µg/mL of lysostaphin diluted in Hepes Buffer supplemented with 0.1% BSA (HB buffer) for 30 minutes at 37 °C. Peritoneal cells were then washed 2x in ice cold HB buffer. The peritoneal cells were diluted to  $1 \times 10^6$  cells/mL in RPMI 1640 tissue culture media supplemented with 10 mM Hepes and 10 % Fetal Calf Serum, and 5 µg/mL vancomycin. Free MRSA from the primary infection was stored overnight at 4 °C in Phosphate Buffered Saline Solution as a control for extracellular bacteria that were not subject to neutrophil killing.

**[0386]** *Transfer of infection from peritoneal cells to osteoblasts:* MG63 osteoblast cell line was obtained from ATCC (CRL-1427) and maintained in RPMI 1640 tissue culture media supplemented with 10 mM Hepes and 10 % Fetal Calf Serum (RPMI-10). Osteoblasts were plated in 24 well tissue culture plates and cultured to obtain a confluent layer. On the day of the experiment, the osteoblasts were washed once in RPMI (without supplements). MRSA or infected peritoneal cells were diluted in complete RPMI-10 and vancomycin was added at 5 µg/mL immediately prior to infection. Peritoneal cells were added to the osteoblasts at  $1 \times 10^6$  peritoneal cells/mL. A sample of the cells was lysed with 0.1% triton-x to determine the actual concentration of live intracellular bacteria at the time of infection. The actual titer for all infections was determined by plating serial dilutions of the bacteria on Tryptic Soy Agar with 5% defibrinated sheep blood.

**[0387]** MG63 osteoblasts were plated in 4 well glass chamber slides and cultured in RPMI 1640 tissue culture media supplemented with 10 mM Hepes and 10 % Fetal Calf Serum (RPMI-10) until they formed confluent layers. On the day of infection, the wells were washed with serum free media and infected with a suspension of infected peritoneal cells, or with the USA300 strain of MRSA diluted in complete RPMI-10 supplemented with 5 µg/mL of vancomycin. One day after infection, the cells were washed with phosphate buffered saline (PBS) and fixed for 30 minutes at room temperature in PBS with 2% paraformaldehyde. Wells were washed 3X in PBS and permeabilized with PBS with 0.1% saponin for 30 minutes at room temperature.

**[0388]** *Immunofluorescence:* MRSA was identified by staining with 20 µg/mL of rabbit anti-Staph 20920, (abcam, Cambridge, MA) followed by anti-rabbit Rhodamine (Jackson ImmunoResearch, 711-026-152). The cell membranes of peritoneal cells were stained with Cholera-Toxin-Beta subunit-biotin (Invitrogen, Carlsbad, CA) followed by streptavidin Cy5 (BD Biosciences San Jose, CA). Binding of the cholera-toxin to peritoneal cells was confirmed by co-staining with anti-CD 11b Alexa 488 clone M1/70 (BD biosciences). Slides were mounted



with Prolong Gold with DAPI (Invitrogen, Carlsbad CA). Slides were viewed using a Leica SPE confocal microscope. Images were collected as a series of Z-stacks and compiled to generate the maximum projection images shown.

**[0389]** Survival of *S. aureus* inside mammalian cells provides a viable niche that permits persistent infection in the presence of antibiotic therapy. *S. aureus* is able to infect and survive inside a number of mammalian cell types including neutrophils, macrophages, osteoblasts and epithelial cells (Garzoni, C. and W. L. Kelley (2009) Trends Microbiol 17(2): 59-65). To test directly whether intracellular MRSA is protected from antibiotics, a number of clinically approved antibiotics were compared for their ability to kill extracellular MRSA cultured in standard bacterial growth media, with their ability to kill intracellular MRSA that is sequestered inside murine macrophages (Table 1). Murine peritoneal macrophages were selected for this analysis because these cells represent a genetically normal primary cell type that is a natural component of the innate immune response to *S. aureus*. Analysis confirmed that these cells are easily infected and cultured *in vitro*. MRSA is able to survive intracellularly for up to six days after infection of the macrophages (Kubica, M., K. Guzik, et al. (2008) PLoS One 3(1): e1409). To test the intracellular effect of antibiotics, macrophages were infected with MRSA, and cultured in the presence of gentamycin, an antibiotic that is known to be inactive inside the phagolysosome due to poor cellular uptake of the antibiotic (Vaudaux, P. and F. A. Waldvogel (1979) Antimicrob Agents Chemother 16(6): 743-749). Test antibiotics were added to the culture media (in addition to gentamycin) one day after infection at a range of doses chosen to include the clinically achievable serum levels (shown as serum C<sub>max</sub> in Table 1). This analysis revealed that although extracellular MRSA is highly susceptible to growth inhibition by low doses of vancomycin, daptomycin, linezolid or rifampicin in liquid culture, all four antibiotics failed to kill the same strain of intracellular MRSA that was sequestered inside macrophages. Remarkably, even rifampicin, which is reported to be one of the best antibiotics for treating intracellular infections such as tuberculosis yielded minimal killing of intracellular MRSA over the time and dose range of the experiment.

**Table 1** Minimum inhibitory concentrations (MIC) of several antibiotics

Antibiotics (Abx)	Extracellular MRSA MIC (µg/mL)	Intracellular MRSA MIC (µg/mL)	Serum C <sub>max</sub> (µg/mL)
Vancomycin	1	>100	10-40
Daptomycin	4	>100	80
Linezolid	0.3	>20	10
Rifampicin	0.004	>20	20

**[0390]** The above data confirmed that intracellular bacteria are protected from antibiotics during the time that they are sequestered inside cells. However, MRSA is not thought to be a true intracellular pathogen in that it is not able to infect neighboring cells by direct cell to cell transfer, and the majority of infected cells will eventually lyse releasing the intracellular bacteria. Therefore, it remained possible that the intracellular pool, once released, would inevitably be exposed to extracellular antibiotics at least transiently, even if the bacteria were

immediately taken up by neighboring cells. Uptake of free MRSA by macrophages requires between 15 and 90 minutes (data not shown), suggesting that if the bacteria were able to resist a brief exposure to antibiotic, it could remain protected in the intracellular niche by moving sequentially from a dying cell to a new host. To determine whether a brief exposure to antibiotics was sufficient to kill MRSA, vancomycin, the current standard of care treatment for MRSA infections, and rifampin were tested. MRSA was taken from an actively growing culture and diluted to  $1 \times 10^6$  bacteria/mL in normal growth media. Antibiotics were added at two doses representing between 2x and 10x the expected minimum inhibitory concentration (MIC). Samples were removed at various times between 30 minutes and 5 hours, and the antibiotic was removed by centrifugation and dilution. The total number of surviving bacteria in the culture was determined by plating on agar plates.

**[0391]** Figure 1 shows comparison of the time of kill for vancomycin (vanco) and rifampicin (Rifa) on actively dividing MRSA. MRSA was cultured for 5 hours in TSB media in the presence of antibiotics. At the indicated times, a sample of the culture was taken and the antibiotic was removed by centrifugation. The total number of surviving bacteria was determined at each time point by plating. Vancomycin was tested at 2  $\mu\text{g/mL}$  (open square) and 20  $\mu\text{g/mL}$  (closed square). Rifampin was tested at 0.02  $\mu\text{g/mL}$  (open triangle) and 0.2  $\mu\text{g/mL}$  (closed triangle). These data (Figure 1) revealed that although both antibiotics were able to inhibit bacterial growth effectively, and by 5 hours a 100 fold loss in viable bacteria was observed, the bacteria were killed gradually over the 5 hour observation period and 90% of the bacteria remained viable during the first two hours of antibiotic treatment permitting ample time for potential uptake by host cells.

**[0392]** Intracellular stores of MRSA were assayed for transfer of infection to a permissive intracellular niche in the presence of vancomycin. *S. aureus* can survive inside osteoblasts, and intracellular stores of *S. aureus* have been observed in patients with osteomyelitis, a condition where chronic infection with *S. aureus* is known to be recalcitrant to antibiotic treatment (Thwaites and Gant, (2011) Nature Reviews Microbiology 9:215-222; Ellington et al., (2006) J. Orthopedic Research 24(1): 87-93; Bosse et al., (2005) J. Bone and Joint Surgery, 87(6): 1343-1347). An *in vitro* assay was developed using an osteoblast cell line MG63 since this cell line was reported to be capable of harboring intracellular *S. aureus* (Garzoni and Kelly, (2008) Trends in Microbiology). This assay confirmed that MRSA is able to infect MG63 cells, and viable intracellular bacteria can be recovered from infected MG63 cells for up to 6 days *in vitro*. To generate a pool of intracellular *S. aureus*, peritoneal cells were harvested from mice that were infected by peritoneal injection of MRSA (Figure 2).

**[0393]** Figure 2 shows transfer of infection from infected peritoneal cells to osteoblasts in the presence of vancomycin. To generate a pool of intracellular *S. aureus*, A/J mice were infected with MRSA and infected peritoneal cells were taken 1 day post infection. Similarly generated cells have been reported to harbor viable intracellular bacteria that are capable of transferring infection in an *in vivo* infection model (Gresham et al J Immunol 2000; 164:3713-3722). The infected peritoneal cells consisted of a mixture of primarily neutrophils and macrophages and approximately 10% of the cells harbored intracellular bacteria. The cells were treated with

lysostaphin to remove extracellular bacteria and suspended in growth media supplemented with 5 µg/mL of vancomycin. A sample of the peritoneal cells used for infection was lysed to determine the precise dose of viable intracellular MRSA at the time infection was initiated, and various doses of free extracellular MRSA were also diluted into media with vancomycin for comparison. The peritoneal cells (intracellular MRSA), or free bacteria (extracellular MRSA) were then added to monolayers of MG63 osteoblasts and cultured for 4 hours (open bars) or 1 day (closed bars). The total number of surviving intracellular bacteria in each well was determined by plating cell lysates on agar plates. Intracellular MRSA were protected from vancomycin compared to the extracellular MRSA controls. Wells infected with  $3 \times 10^4$  intracellular bacteria yielded 8,750 intracellular bacteria (about 1 third of the infection dose) 1 day after infection, whereas the extracellular bacteria were efficiently killed as infection with a similar dose of free MRSA yielded only 375 intracellular bacteria 1 day post infection

**[0394]** Immunofluorescence microscopy also demonstrated transfer of infection from peritoneal cells to MG63 osteoblasts. Peritoneal cells were collected from mice 1 day after infection with MRSA and treated with lysostaphin to kill any contaminating extracellular bacteria (Intracellular Infection). Free MRSA was taken from an actively growing culture and washed in PBS (Extracellular Infection). The total number of viable bacteria in the Intracellular and Extracellular infection samples was confirmed by plating on agar plates and both samples were suspended in media supplemented with 5 µg/mL of vancomycin immediately before addition to confluent layers of MG63 osteoblasts cultured in chamber slides. One day after infection, the MG63 cells were washed to remove extracellular bacteria, permeabilized and stained with an anti-*S. aureus* antibody to identify intracellular MRSA and cholera toxin which bound preferentially to the peritoneal cell membranes. All of the cell nuclei were co-stained with DAPI to confirm that the MG63 monolayer was intact. Slides were examined by confocal microscopy.

**[0395]** Wells infected with peritoneal cells contained a confluent monolayer of MG63 cells and peritoneal macrophages were clearly visible on top of the MG63 layer. Many of the macrophages were clearly infected with MRSA which is visible as clusters of red bacteria in the single color image or white particles in the overlay image. In addition to the infected macrophages, clear examples were observed of bacteria that were associated only with the MG63 cells. These infected MG63 cells were also visible in wells that were infected with the free MRSA. Infection with free MRSA required a much higher inoculum to achieve a similar level of infection in the MG63 cells.

**[0396]** The above results established that both free MRSA and intracellular MRSA are able to survive and infect MG63 cells in the presence of vancomycin. Bacteria from the intracellular infection were significantly better able to survive vancomycin treatment than the free bacteria under these conditions. Infection with  $3 \times 10^4$  CFU of intracellular bacteria yielded  $8.7 \times 10^3$  CFUs of intracellular bacteria 1 day post infection. Infection with a similar dose of free bacteria yielded only 375 intracellular bacteria 1 day post infection, indicating that the intracellular bacteria were up to 20 times better able to survive than the free bacteria. All infection doses recovered more intracellular bacteria (between 1.5 to 6 times) when wells were harvested 1 day vs. 4 hours after infection. Since vancomycin completely inhibits growth when added to

free MRSA (Figure 1), these data suggest that the MRSA must have replicated at some time despite constant exposure to vancomycin in the culture media. Although MRSA does not replicate significantly inside murine macrophages (our unpublished observations), there is considerable evidence that *S. aureus* is able to escape the phagolysosome and replicate within the cytoplasm of non-phagocytic cell types (Jarry, T. M., G. Memmi, et al. (2008) Cell Microbiol 10(9): 1801-1814). Together the above observations suggest that even under constant exposure to vancomycin, free MRSA can infect cells and intracellular MRSA can transfer from one cell to another cell. These observations reveal a potential mechanism for maintenance and even spread of infection that could occur in the presence of constant antibiotic therapy.

#### **Example 19 In vivo infection models.**

**[0397] Peritonitis Model.** 7 week old female A/J mice (Jackson Laboratories) were infected by peritoneal injection with  $5 \times 10^7$  CFU of USA300. Mice were sacrificed 2 days post infection and the peritoneum was flushed with 5 mL of cold phosphate buffered saline solution (PBS). Kidneys were homogenized in 5 mL of PBS as described below for the intravenous infection model. Peritoneal washes were centrifuged for 5 minutes at 1,000 rpm at 4°C in a table top centrifuge. The supernatant was collected as the extracellular bacteria and the cell pellet containing peritoneal cells was collected as the intracellular fraction. The cells were treated with 50 µg/mL of lysostaphin for 20 minutes at 37°C to kill contaminating extracellular bacteria. Peritoneal cells were washed 3x in ice cold PBS to remove the lysostaphin prior to analysis. To count the number of intracellular CFUs, peritoneal cells were lysed in HB (Hanks Balanced Salt Solution supplemented with 10 mM HEPES and .1% Bovine Serum Albumin) with 0.1% Triton-X, and serial dilutions of the lysate were made in PBS with 0.05% tween-20.

**[0398] Intravenous infection Model:** 7 week old female mice were used for all in vivo experiments and infections were carried out by intravenous injection into the tail vein. A/J mice (Jackson Lab) were infected with a dose of  $2 \times 10^6$  CFU. Balb/c mice (Charles River Laboratories, Hollister, CA) were infected with a dose of  $2 \times 10^7$  CFU. For studies examining the role of competing human IgG (SCID IVIG model), CB17.SCID mice (Charles River Laboratories, Hollister, CA) were reconstituted with GammaGard S/D IGIV Immune Globulin (ASD Healthcare, Brooks KY) using a dosing regimen optimized to achieve constant serum levels of >10 mg/mL of human IgG. IGIV was administered with an initial intravenous dose of 30 mg per mouse followed by a second dose of 15 mg/mouse by intraperitoneal injection after 6 hours, and subsequent daily dosings of 15 mg per mouse by intraperitoneal injection for 3 consecutive days. Mice were infected 4 hours after the first dose of IGIV with  $2 \times 10^7$  CFU of MRSA diluted in phosphate buffered saline by intravenous injection. Mice that received vancomycin were treated with twice daily intraperitoneal injections of 100 mg/Kg of vancomycin starting between 6 and 24 hours post infection for the duration of the study. Experimental therapeutics (AAC, anti-MRSA antibodies or free dimethyl-pipBOR antibiotic) were diluted in phosphate buffered saline and administered with a single intravenous injection 30 minutes to 24 hours after infection. All mice were sacrificed on day 4 after infection, and kidneys were

harvested in 5 mL of phosphate buffered saline. The tissue samples were homogenized using a GentleMACS Dissociator™ (Miltenyi Biotec, Auburn, CA). The total number of bacteria recovered per mouse (2 kidneys) was determined by plating serial dilutions of the tissue homogenate in PBS .05% Tween on Tryptic Soy Agar with 5% defibrinated sheep blood.

#### **Example 20 Cathepsin / Caspase release assay**

**[0399]** To quantify the amount of active antibiotic released from AAC following treatment with cathepsin B, AAC were diluted to 200 µg/mL in cathepsin buffer (20 mM Sodium Acetate, 1 mM EDTA, 5 mM L-Cysteine). See: page 863 of Dubowchik et al (2002) Bioconj. Chem. 13:855-869. Cathepsin B (from bovine spleen, SIGMA C7800) was added at 10 µg/mL and the samples were incubated for 1 hour at 37 °C. As a control, AAC were incubated in buffer alone. The reaction was stopped by addition of 10 volumes of bacterial growth media, Tryptic Soy Broth pH 7.4 (TSB). To estimate the total release of active antibiotic, serial dilutions of the reaction mixture were made in quadruplicate in TSB in 96 well plates and the USA300 strain of *S. aureus* was added to each well at a final density of  $2 \times 10^3$  CFU/mL. The cultures were incubated over night at 3 °C with shaking and bacterial growth was measured by reading absorbance at 630 nM using a plate reader.

#### **Example 21 Production of anti-WTA Antibodies**

##### ***Antibody generation, screening and selection***

**[0400]** Abbreviations: MRSA (methicillin-resistant *S. aureus*); MSSA (methicillin-sensitive *S. aureus*); VISA (vancomycin intermediate-resistant *S. aureus*); LTA (lipoteichoic acid); TSB (tryptic soy broth); CWP (cell wall preparation).

**[0401]** Human IgG antibodies were cloned from peripheral B cells from patients post *S. aureus* infection using the Symplex™ technology (Symphogen, Lyngby, Denmark) which conserves the cognate pairing of antibody heavy and light chains, as described in US 8,283,294: "Method for cloning cognate antibodies"; Meijer PJ et al. Journal of Molecular Biology 358:764-772 (2006); and Lantto J et al. J Virol. 85(4):1820-33 (Feb 2011); Plasma and memory cells were used as genetic source for the recombinant full-length IgG repertoires. Individual antibody clones were expressed by transfection of mammalian cells as described in Meijer PJ, et al. Methods in Molecular Biology 525: 261-277, xiv. (2009). Supernatants containing full length IgG1 antibodies were harvested after seven days and used to screen for antigen binding by indirect ELISA in the primary screening. A library of mAbs showing positive ELISA binding to cell wall preparations from USA300 or Wood46 strain *S. aureus* strains was generated. Antibodies were subsequently produced in 200-ml transient transfections and purified with Protein A chromatography (MabSelect SuRe, GE Life Sciences, Piscataway, NJ) for further

testing. For larger scale antibody production, antibodies were produced in CHO cells. Vectors coding for VL and VH were transfected into CHO cells and IgG was purified from cell culture media by protein A affinity chromatography.

Table 7 : List of antigens used to isolate the Abs

Ag	Description	Vendor/source	Coating
WTA	Wall Teichoic acid (WTA) from Staph A. Cat.No. R84500 (2 mg/vial), lot no. 5E14909.	Meridian Life Sciences	2 µg/ml
PGN	Peptidoglycan from Staphylococcus aureus; Cat no. 77140, lot no. 1396845	Sigma	2 µg/ml
CW #1	CW USA300, RPMI, iron deplet. Stationary Phase	Genentech, 100x	
CW #3	CW USA300, TSB. Stationary Phase	Genentech, 500X	
CW #4	CW Wood46, TSB. Stationary Phase	Genentech, 500X	
CW#1 and CW#3 were always mixed together in making the ELISA coating:			

**[0402]** Figure 6 summarizes the primary screening of the antibodies by the ELISA. All (except 4569) were isolated when screened with the USA300 Cell wall prep mixture (iron depleted:TSB in a 96:4 ratio). All GlcNAc beta (except 6259), SDR, and PGN (4479) mAbs were also positive for PGN and WTA in primary screening. All GlcNAc alpha were found exclusively by screening for binding with the USA300 CW mix. The 4569 (LTA specific) was found by screening on Wood46 CWP.

***Selection of anti-WTA mAb from the library using ex vivo flow cytometry***

**[0403]** Each mAb within this library was queried for three selection criteria: (1) relative intensity of mAb binding to the MRSA surface, as an indication of high expression of the corresponding cognate antigen which would favor high antibiotic delivery; (2) consistency of mAb binding to MRSA isolated from a diverse variety of infected tissues, as an indication of the stable expression of the cognate antigen at the MRSA surface in vivo during infections; and (3) mAb binding capacity to a panel of clinical *S. aureus* strains, as an indication of conservation of expression of the cognate surface antigen. To this end, flow cytometry was used to test all of these pre-selected culture supernatants of mAbs in the library for reactivity with *S. aureus* from a variety of infected tissues and from different *S. aureus* strains.

**[0404]** All mAbs in the library were analyzed for their capacity to bind MRSA from infected kidneys, spleens, livers, and lungs from mice which were infected with MRSA USA300; and within hearts or kidneys from rabbits which were infected with USA300 COL in a rabbit endocarditis model. The capacity of an antibody to recognize *S. aureus* from a variety of

infected tissues raises the probability of the therapeutic antibody being active in a wide variety of different clinical infections with *S. aureus*. Bacteria were analyzed immediately upon harvest of the organs, i.e. without subculture, to prevent phenotypic changes caused by *in vitro* culture conditions. We had previously observed that several *S. aureus* surface antigens, while being expressed during *in vitro* culture, lost expression in infected tissues. Antibodies directed against such antigens would be unlikely to be useful to treat infections. During the analysis of this mAb library on a variety of infected tissues, this observation was confirmed for a significant number of antibodies, which showed significant binding to *S. aureus* bacteria from culture, but absence of binding to bacteria from all of the tested infected tissues. Some antibodies bound to bacteria from some but not all tested infected tissues. Therefore, in the present invention, we selected for antibodies that were able to recognize bacteria from all infection conditions tested. Parameters that were assessed were (1) relative fluorescence intensity, as a measure for antigen abundance; (2) number of organs that stained positive, as a measure for stability of antigen expression; and (3) mAb binding capacity to a panel of clinical *S. aureus* strains as an indication of conservation of expression of the cognate surface antigen. Fluorescence intensity of the test antibodies was determined as relative to an isotype control antibody that was directed against a non-relevant antigen, for example, IgG1 mAb anti-herpes virus gD:5237 (referenced below). mAbs against WTA-beta not only showed the highest antigen abundance, but also showed very consistent binding to MRSA from all infected tissues tested and specified above.

**[0405]** Additionally, we tested the capacity of these mAbs to bind to the following *S. aureus* strains, which were cultured *in vitro* in TSB: USA300 (MRSA), USA400 (MRSA), COL (MRSA), MRSA252 (MRSA), Wood46 (MSSA), Rosenbach (MSSA), Newman (MSSA), and Mu50 (VISA). Anti-WTA beta mAbs but not anti-WTA alpha mAbs were found to be reactive with all of these strains. The analysis of binding to different strains indicated that WTA beta is more conserved than WTA alpha and therefore more suitable for AAC.

**[0406]** Example 22 Characterization of antibodies with specificity against wall teichoic acids on *S. aureus*.

***i) Confirming WTA specificity of Abs***

**[0407]** Cell wall preparations (CWP) from a *S. aureus* wild-type (WT) strain and a *S. aureus* mutant strain lacking WTA ( $\Delta$ TagO; WTA-null strain) were generated by incubating 40 mg of pelleted *S. aureus* strains with 1 mL of 10 mM Tris-HCl (pH 7.4) supplemented with 30% raffinose, 100  $\mu$ g/ml of lysostaphin (Cell Sciences, Canton, MA), and EDTA-free protease inhibitor cocktail (Roche, Pleasanton, CA), for 30 min at 37 °C. The lysates were centrifuged at 11,600 x g for 5 min, and the supernatants containing cell wall components were collected. For immunoblot analysis, proteins were separated on a 4-12% Tris-glycine gel, and transferred to a nitrocellulose membrane (Invitrogen, Carlsbad, CA), followed by blotting with indicated test antibodies against WTA, or with control antibodies against PGN and LTA.

**[0408]** Immunoblotting shows that the antibodies against WTA bind to WT cell wall preparations from WT *S. aureus* but not to cell wall preparations from the  $\Delta$ TagO strain lacking WTA. The control antibodies against peptidoglycan (anti-PGN) and lipoteichoic acid (anti-LTA) bind well to both cell wall preparations. These data indicate the specificity of the test antibodies against WTA.

***ii) Flow cytometry to determine extent of mAb binding to MRSA surface***

**[0409]** Surface antigen expression on whole bacteria from infected tissues was analyzed by flow cytometry using the following protocol. For antibody staining of bacteria from infected mouse tissues, 6-8 weeks old female C57B1/6 mice (Charles River, Wilmington, MA) were injected intravenously with  $10^8$  CFU of log phase-grown USA300 in PBS. Mouse organs were harvested two days after infection. Rabbit infective endocarditis (IE) was established as previously described in Tattavin P. et al. *Antimicrobial agents and chemotherapy* 54: 610-613 (2010). Rabbits were injected intravenously with  $5 \times 10^7$  CFU of stationary-phase grown MRSA strain COL, and heart vegetations were harvested eighteen hours later. Treatment with 30 mg/kg of vancomycin was given intravenously b.i.d. 18 h after infection with  $7 \times 10^7$  CFU stationary-phase

**[0410]** To lyse mouse or rabbit cells, tissues were homogenized in M tubes (Miltenyi, Auburn, CA) using a gentleMACS cell dissociator (Miltenyi), followed by incubation for 10 min at RT in PBS containing 0.1% Triton-X100 (Thermo), 10  $\mu$ g/mL of DNaseI (Roche) and Complete Mini protease inhibitor cocktail (Roche). The suspensions were passed through a 40 micron filter (BD), and washed with HBSS without phenol red supplemented with 0.1% IgG free BSA (Sigma) and 10 mM Hepes, pH 7.4 (HB buffer). The bacterial suspensions were next incubated with 300  $\mu$ g/mL of rabbit IgG (Sigma) in HB buffer for 1 h at room temperature (RT) to block nonspecific IgG binding. Bacteria were stained with 2  $\mu$ g/mL of primary antibodies, including rF1 or isotype control IgG1 mAb anti-herpes virus gD:5237 (Nakamura GR et al., *J Virol* 67: 6179-6191 (1993)), and next with fluorescent anti-human IgG secondary antibodies (Jackson ImmunoResearch, West Grove, PA). In order to enable differentiation of bacteria from mouse or rabbit organ debris, a double staining was performed using 20  $\mu$ g/mL mouse mAb 702 anti-*S. aureus* peptidoglycan (Abcam, Cambridge, MA) and a fluorochrome-labeled anti-mouse IgG secondary antibody (Jackson ImmunoResearch). The bacteria were washed and analyzed by FACSCalibur (BD). During flow cytometry analysis, bacteria were gated for positive staining with mAb 702 from double fluorescence plots.

***iii) Measuring binding affinity to S. aureus and antigen density on MRSA***

**[0411]** Table 8 shows equilibrium binding analysis of MRSA antibodies binding to Newman- $\Delta$ SPA strain, and the antigen density on the bacterium.

Table 8



MRSA Antibody	Specificity	aveK <sub>D</sub> , nM (n=2)	Antigen Density, aveSites/Bacterium
4497	b-WTA	2.5	50,000
4462	b-WTA	3.1	43,000
6263	b-WTA	1.4	22,000
6297	b-WTA	1.1	21,000
7578	a-WTA	0.4	16,000
rF1	SDR-glyco	0.3	1600

[0412] The K<sub>D</sub> and antigen density were derived using a radioligand cell binding assay under the following assay conditions: DMEM + 2.5% mouse serum binding buffer; solution binding for 2hrs at room temperature (RT); and using 400,000 bacteria/well.

[0413] Ab 6263 is 6078-like in that the sequences are very similar. Except for the second residue (R versus G) in CDR H3, all the other L and H chain CDR sequences are identical.

### **Example 23 Engineering WTA antibody mutants**

[0414] In summary, the VH region of each of the anti-WTA beta Abs were cloned out and linked to human H chain gamma1 constant region and the VL linked to kappa constant region to express the Abs as IgG1. In some cases the wild type sequences were altered at certain positions to improve the antibody stability as described below. Cysteine engineered Abs (ThioMabs) were then generated.

#### ***i. Linking Variable regions to Constant regions***

[0415] The VH regions of the WTA beta Abs identified from the human antibody library above were linked to human  $\gamma$ 1 constant regions to make full length IgG1 Abs. The L chains were kappa L chains.

#### ***ii. Generating stability variants***

[0416] The WTA Abs in Figure 14, (see in particular, Figures 15A, 15B, 16A, 16B) were engineered to improve certain properties (such as to avoid deamidation, aspartic acid isomerization, oxidation or N-linked glycosylation) and tested for retention of antigen binding as well as chemical stability after amino acid replacements. Single stranded DNA of clones encoding the heavy or light chains was purified from M13KO7 phage particles grown in *E. coli*

CJ236 cells using a QIAprep Spin M13 kit (Qiagen). 5' phosphorylated synthetic oligonucleotides with the sequences:

5'- CCCAGACTGCACCAGCTGGATCTCTGAATGTACTCCAGTTGC- 3' (SEQ ID NO. 152)

5'- CCAGACTGCACCAGCTGCACCTCTGAATGTACTCCAGTTGC- 3' (SEQ ID NO. 153)

5'CCAGGGTTCCCTGGCCCCAWTMGTCAAGTCCASCWKACCTCTTGACAGTAA

TAGACAGC- 3' (SEQ ID NO. 154);

and

5'- CCTGGCCCCAGTCGTCAAGTCCTCCTTCACCTCTTGACAGTAATAGACAGC-3' (SEQ ID NO. 155) (IUPAC codes)

were used to mutate the clones encoding the antibodies by oligonucleotide-directed site mutagenesis as described by site-specific mutagenesis following the methodology as described in Kunkel, T.A. (1985). Rapid and efficient site-specific mutagenesis without phenotypic selection. *Proceedings of the National Academy of Sciences USA* 82(2): 488-492. Mutagenized DNA was used to transform *E. coli* XL1-Blue cells (Agilent Technologies) and plated on Luria Broth plates containing 50 µg/ml Carbenicillin. Colonies were individually picked and grown in liquid Luria Broth media containing 50 µg/ml Carbenicillin. Miniprep DNA was sequenced to confirm the presence of mutations.

**[0417]** For Ab 6078, the second amino acid in the VH, met (met-2), is prone to oxidation. Therefore met-2 was mutated to Ile or Val, to avoid oxidation of the residue. Since the alteration of met-2 may affect binding affinity, the mutants were tested for binding to Staph CWP by ELISA.

**[0418]** CDR H3 "DG" or "DD" motifs were found to be prone to transform to iso-aspartic acid. Ab 4497 contains DG in CDR H3 positions 96 and 97 (see Figure 18B) and was altered for stability. CDR H3 is generally critical for antigen binding so several mutants were tested for antigen binding and chemical stability (see Figure 18A). Mutant D96E (v8) retains binding to antigen, similar to wild-type Ab 4497 (Figure 18A; Figure 18B), and is stable and does not form iso-aspartic acid.

### ***Staph CWP ELISA***

**[0419]** For analysis of 6078 antibody mutants, a lysostaphin-treated USA300 ΔSPA *S. aureus* cell well preparation (WT) consisting of  $1 \times 10^9$  bugs/ml was diluted 1/100 in 0.05 Sodium Carbonate pH 9.6 and coated onto 384-well ELISA plates (Nunc; Neptune, NJ) during an overnight incubation at 4°C. Plates were washed with PBS plus 0.05% Tween-20 and blocked during a 2-hour incubation with PBS plus 0.5% bovine serum albumin (BSA). This and all subsequent incubations were performed at room temperature with gentle agitation. Antibody samples were diluted in sample/standard dilution buffer (PBS, 0.5% BSA, 0.05% Tween 20, 0.25% CHAPS, 5 mM EDTA, 0.35M NaCl, 15 ppm Proclin , (pH 7.4)), added to washed plates,

and incubated for 1.5 - 2 hours. Plate-bound anti-*S. aureus* antibodies were detected during a 1-hour incubation with a peroxidase-conjugated goat anti-human IgG(Fc) F(ab')<sub>2</sub> fragment (Jackson ImmunoResearch; West Grove, PA) diluted to 40 ng/mL in assay buffer (PBS, 0.5% BSA, 15 ppm Proclin, 0.05% Tween 20). After a final wash, tetramethyl benzidine (KPL, Gaithersburg, MD) was added, color was developed for 5-10 minutes, and the reaction was stopped with 1 M phosphoric acid. The plates were read at 450 nm with a 620 nm reference using a microplate reader.

### ***iii. Generating Cys engineered mutants (ThioMabs)***

**[0420]** Full length ThioMabs were produced by introducing a Cysteine into the H chain (in CH1) or the L chain (C<sub>K</sub>) at a predetermined position as previously taught and described below to allow conjugation of the antibody to a linker-antibiotic intermediate. H and L chains are then cloned into separate plasmids and the H and L encoding plasmids co-transfected into 293 cells where they are expressed and assembled into intact Abs. Both H and L chains can also be cloned into the same expression plasmid. IgG1 are made having 2 engineered Cys, one in each of H chains, or 2 engineered Cys, one in each of the L chains, or a combination of 2 H and 2L chains each with engineered Cys (HCLCCys) were generated by expressing the desired combination of cys mutant chains and wild type chains.

**[0421]** Figures 15A and 15B shows the 6078 WT and mutant Abs with the combination of HC Cys and LC Cys. The 6078 mutants were also tested for their ability to bind protein A deficient USA300 Staph A from overnight culture. From the results from the FACS analysis as shown in Figure 19, the mutant Abs bound USA300 similarly to the 6078 WT (unaltered) antibody; the amino acid alterations in the mutants did not impair binding to Staph A. gD is a non-specific negative control antibody.

### **Example 24 Preparation of Anti-WTA Antibody-Antibiotic Conjugates**

**[0422]** Anti-wall teichoic acid antibody-antibiotic conjugates (AAC) Table 3 were prepared by conjugating an anti-WTA antibody to a linker-antibiotic intermediate, including those from Table 2. Prior to conjugation, the anti-WTA antibodies were partially reduced with TCEP using standard methods in accordance with the methodology described in WO 2004/010957. The partially reduced antibodies were conjugated to the linker-antibiotic intermediate using standard methods in accordance with the methodology described, e.g., in Doronina et al. (2003) Nat. Biotechnol. 21:778-784 and US 2005/0238649 A1. Briefly, the partially reduced antibodies were combined with the linker-antibiotic intermediate to allow conjugation of the linker-antibiotic intermediate to reduced cysteine residues of the antibody. The conjugation reactions were quenched, and the AAC were purified. The antibiotic load (average number of antibiotic moieties per antibody) for each AAC was determined and was between about 1 to about 2 for the anti-wall teichoic acid antibodies engineered with a single cysteine mutant site.

**[0423]** *Reduction/Oxidation of ThioMabs for Conjugation:* Full length, cysteine engineered monoclonal antibodies (ThioMabs - Junutula, et al., 2008b Nature Biotech., 26(8):925-932; Dornan et al (2009) Blood 114(13):2721-2729; US 7521541; US 7723485; WO2009/052249, Shen et al (2012) Nature Biotech., 30(2):184-191; Junutula et al (2008) Jour of Immun. Methods 332:41-52) expressed in CHO cells were reduced with about a 20-40 fold excess of TCEP (tris(2-carboxyethyl)phosphine hydrochloride or DTT (dithiothreitol) in 50 mM Tris pH 7.5 with 2 mM EDTA for 3 hrs at 37 °C or overnight at room temperature.(Getz et al (1999) Anal. Biochem. Vol 273:73-80; Soltec Ventures, Beverly, MA). The reduced ThioMab was diluted and loaded onto a HiTrap S column in 10 mM sodium acetate, pH 5, and eluted with PBS containing 0.3M sodium chloride. Alternatively, the antibody was acidified by addition of 1/20<sup>th</sup> volume of 10 % acetic acid, diluted with 10 mM succinate pH 5, loaded onto the column and then washed with 10 column volumes of succinate buffer. The column was eluted with 50 mM Tris pH7.5, 2 mM EDTA.

**[0424]** The eluted reduced ThioMab was treated with 15 fold molar excess of DHAA (dehydroascorbic acid) or 200 nM aqueous copper sulfate (CuSO<sub>4</sub>). Oxidation of the interchain disulfide bonds was complete in about three hours or more. Ambient air oxidation was also effective. The re-oxidized antibody was dialyzed into 20 mM sodium succinate pH 5, 150 mM NaCl, 2 mM EDTA and stored frozen at -20 °C.

**[0425]** *Conjugation of Thio-Mabs with linker-antibiotic intermediates:* The deblocked, reoxidized, thio-antibodies (ThioMab) were reacted with 6-8 fold molar excess of the linker-antibiotic intermediate of Table 2 (from a DMSO stock at a concentration of 20 mM) in 50 mM Tris, pH 8, until the reaction was complete (16-24 hours) as determined by LC-MS analysis of the reaction mixture.

**[0426]** The crude antibody-antibiotic conjugates (AAC) were then applied to a cation exchange column after dilution with 20 mM sodium succinate, pH 5. The column was washed with at least 10 column volumes of 20 mM sodium succinate, pH 5, and the antibody was eluted with PBS. The AAC were formulated into 20 mM His/acetate, pH 5, with 240 mM sucrose using gel filtration columns. AAC were characterized by UV spectroscopy to determine protein concentration, analytical SEC (size-exclusion chromatography) for aggregation analysis and LC-MS before and after treatment with Lysine C endopeptidase.

**[0427]** Size exclusion chromatography was performed using a Shodex KW802.5 column in 0.2M potassium phosphate pH 6.2 with 0.25 mM potassium chloride and 15% IPA at a flow rate of 0.75 ml/min. Aggregation state of AAC was determined by integration of eluted peak area absorbance at 280 nm.

**[0428]** LC-MS analysis was performed using an Agilent QTOF 6520 ESI instrument. As an example, an AAC generated using this chemistry was treated with 1:500 w/w Endoproteinase Lys C (Promega) in Tris, pH 7.5, for 30 min at 37 °C. The resulting cleavage fragments were loaded onto a 1000A, 8 um PLRP-S column heated to 80°C and eluted with a gradient of 30%

B to 40% B in 5 minutes. Mobile phase A: H<sub>2</sub>O with 0.05% TFA. Mobile phase B: acetonitrile with 0.04% TFA. Flow rate: 0.5ml/min. Protein elution was monitored by UV absorbance detection at 280 nm prior to electrospray ionization and MS analysis. Chromatographic resolution of the unconjugated Fc fragment, residual unconjugated Fab and antibiotic-Fab was usually achieved. The obtained m/z spectra were deconvoluted using Mass Hunter™ software (Agilent Technologies) to calculate the mass of the antibody fragments.

**Example 25 Identification and purification of staphopain B as the protease responsible for cleavage**

**[0429]** Supernatant from a 3 liter overnight culture of Wood46 was concentrated and buffer exchanged using TFF (10kDa) into 50 mM sodium phosphate pH 7. The sample was loaded on Q Sepharose FF and proteins were separated chromatographically using a gradient of 0-300mM NaCl in 50mM sodium phosphate pH 7. Active fractions were identified by incubating with thioFAB S4497-MC-GGAFAGGG-(pipBOR) ("core peptide" disclosed as SEQ ID NO: 126) of Figure 27 and assessing for cleavage of the linker at the expected site by LC-MS analysis. Active fractions were pooled and supplemented with ammonium sulphate to a concentration of 2M. Proteins were further purified by hydrophobic interaction chromatography on Phenyl Sepharose using a gradient of 2-0M ammonium sulfate in 50mM TRIS pH 7.5. Again, active fractions were identified using the tool compound. These fractions were pooled and further purified on Mono Q in 50mM Sodium Acetate pH 5.5 using a salt gradient of 0-1M NaCl. Active fractions from this chromatography step were identified as before, pooled and applied to size exclusion chromatography in PBS. Active fractions were identified and determined to contain a single protein of interest by SDS-PAGE.

**[0430]** Enriched active fractions from the Q Sepharose purification were characterized to identify the class of protease responsible for activity. The protease was found to be inhibited by N-ethylmaleimide, indicating that the enzyme is likely a cysteine protease. After reviewing the known secreted cysteine proteases of *Staphylococcus aureus*, staphopain B was identified to have similar substrate specificity to the specificity observed in the REPLi screen (Kalinska, M., T. Kantyka, et al. (2012). Biochimie 94(2): 318). Purified staphopain B was purchased (Sigma-Aldrich) and incubated with thioFAB S4497-MC-GGAFAGGG-(pipBOR) ("core peptide" disclosed as SEQ ID NO: 126) from Figure 27. Staphopain B was found to cleave the linker at the same site as the active protease purified from Wood46 supernatant. Staphopain B and the active protease purified from culture supernatant were incubated with Alexa Fluor® 488 C<sub>5</sub> maleimide (Invitrogen, Life Technologies, Thermo Fisher Scientific Inc.) to label the active-site cysteines. Samples were run on SDS-PAGE to identify the protease as staphopain B. SDS-PAGE gels of active fractions from SEC purification were run alongside purified staphopain B, with and without Alexa Fluor 488.

**[0431]** Enriched active fractions for the Q sepharose purification were also analyzed by proteomic mass spectrometry. Ten micrograms of active fractions B11, B12, and C02 and ten

micrograms of an active fractions, 1, 2, 3, and ten micrograms of an inactive fraction were run on SDS-PAGE. Bands were excised and subjected to overnight digestions by trypsin. The digested samples were analyzed by LC-MS/MS and tandem mass spectral results were submitted for database searching using the Mascot search algorithm. Staphopain B was the top hit for cysteine proteases present in the active fractions. Autolysin, which is also a cysteine protease, also appears as a top hit, with the highest abundance of unique peptides occurring in the inactive fraction, the negative control, thus autolysin was omitted from consideration. Mass spectral proteomic analysis of active fractions from the Q Sepharose purifications show a high abundance of staphopain B. T restle data was filtered for *S. aureus* proteins and ranked by number of peptides in Active fraction 1.

**[0432]** The active protease was purified from Wood46 *S. aureus* culture supernatant. The cells were grown overnight at 37°C in 3 liters of TSB. Cells were removed by centrifugation at 10,000 x g for 10 min. The supernatant was collected and passed through two 0.22um filters. Next, it was concentrated and buffer exchanged into 50mM Sodium Phosphate pH 7 using TFF with a 10 kD membrane. The sample was concentrated ten-fold to a volume of 300ml. The sample was loaded on Q Sepahrose FF (GE Healthcare Biosciences AB) and proteins were separated chromatographically using a gradient of 0-300mM NaCl in 50mM sodium phosphate pH 7. Active fractions were pooled and supplemented with ammonium sulfate to a concentration of 2M. Proteins were further purified by hydrophobic interaction chromatography on Phenyl Sepharose (GE Healthcare Biosciences AB) using a gradient of 2-0M ammonium sulfate in 50mM TRIS pH 7.5. Active fractions were pooled and further purified on Mono Q (GE Healthcare Biosciences AB) in 50mM Sodium Acetate pH 5.5 using a salt gradient of 0-1M NaCl. Active fractions from this chromatography step were identified as before, pooled and applied to size exclusion chromatography (Zenix-150, Sepax Technologies) in PBS.

**[0433]** To identify fractions containing our active protease of interest, 100ul from each fraction was transferred to a 96-well plate where it was incubated with 25ug of THIOFAB 4497 mal-GGAFAGGG-DNA31 ("core peptide" disclosed as SEQ ID NO: 126). Fractions from each chromatography step were analyzed for activity, and 100ul was used regardless of protein concentration. Samples were incubated overnight at 37°C and subsequently analyzed by LC-MS to identify fractions where protease cleavage of the linker-antibiotic had occurred. Pooled active fractions from the Q Sepharose FF chromatography were measured to have a total protein concentration of 14 mg/ml. 200 µg of the pool was diluted to 2mg/ml in PBS and was incubated with and without N-ethylmaleimide (NEM, Sigma) at a concentration of 0.1mM final. Samples were incubated at room temperature for 1 hour. 25ug of THIOFAB 4497 mal-GGAFAGGG-DNA31 ("core peptide" disclosed as SEQ ID NO: 126) was added to both samples and incubated at 37°C for 2 hours. At 2 hours, samples were analyzed by LC-MS to identify whether protease cleavage of the linker-antibiotic had occurred. About 50ul of the active fractions from SEC were incubated with 0.1mM Alexa Fluor 488 C<sub>5</sub> maleimide (Invitrogen) for 1 hour regardless of protein concentration for labeling. Cleavage assays with purified proteases were performed by incubating 5uM of the thioFAB conjugate with 50nM protease in a final volume of 100ul. Assays were either performed in PBS pH 7.2, 4mM L-Cys, 2.5 mM EDTA or 100mM Sodium Citrate pH 5, 100mM NaCl, 4mM L-Cys, 2.5mM EDTA.

Samples were incubated for 2 hours at 37°C. At 2 hours, cleavage reactions were quenched by diluting 1:1 with 1% TFA. Samples were subsequently run on LC-MS to determine percent linker cleavage. Percent cleavage was determined by integrating the A<sub>280</sub> chromatograms of the cleaved and intact species. The antibiotic or chromophore moieties added on the C-terminus of the linkers add significant hydrophobicity, such that thioFAB with intact linkers and thioFAB with cleaved linkers are baseline resolved.

**[0434]** For proteomic analysis of enriched fractions, ten micrograms of enriched active fractions from the Q Sepahrose® (GE Healthcare Life Sciences) purification were loaded onto a 4-12% Bis-Tris gel (Life Technologies). Entire gel lanes were excised and divided from top to bottom into 11 bands. The gel bands were destained with 50% acetonitrile/50 mM ammonium bicarbonate, reduced with dithiothreitol (50mM final concentration) for 30 minutes at 50 °C, and alkylated with iodoacetamide (50 mM final concentration) at room temperature in the dark for 30 minutes. The samples were then digested at 37 °C overnight with 0.02 µg/µl trypsin (Promega) in 50 mM ammonium bicarbonate. The digested samples were injected onto a 100 µm inner diameter capillary column (NanoAcquity UPLC column, 100µm x 100mm, 1.7µm, BEH130 C18, Waters Corp) and separated by capillary reverse phase chromatography on a NanoAcquity UPLC system (Waters Corp). Samples were loaded in 0.1% trifluoroacetic acid in water and eluted with a gradient of 2-90% Buffer B (where Buffer A is 0.1% formic acid/2%acetonitrile/98%water and Buffer B is 0.1% formic acid/ 2% water/98%acetonitrile) at 1.00 µl/min with a total analysis time of 45 minutes. Peptides were eluted directly into an LTQ-Orbitrap XL (ThermoFisher) mass spectrometer and ionized using an ADVANCE source (Michrom-Bruker) with a spray voltage of 1.4 kV. Mass spectral data were acquired using a method comprising of one full MS scan (375-1600 m/z) in the Orbitrap at resolution of 60,000 M/ΔM at m/z 400 followed by collision-induced dissociation (CID) of the top 8 most abundant ions detected in the full MS scan in a cycle repeated throughout the LC gradient in the linear ion trap. Tandem mass spectral results were submitted for database searching using the Mascot® search algorithm version 2.3.02 (Matrix Sciences) against a concatenated target-decoy database, Uniprot ver 2010\_12, comprising of *S. aureus* proteins and common laboratory contaminants. The data was searched with tryptic specificity, variable modifications of cysteine carbamidomethylation (+57.0215 Da) and methionine oxidation (+15.995 Da), allowing 2 miscleavages, 20 ppm precursor ion mass, and 0.5 Da fragment ion mass tolerance specified. Peptide spectral matches were filtered using a linear discriminant algorithm (LDA) to a false discovery rate (FDR) of 1%.

#### **Example 26 Staphopain cleavage of thioFab FRET peptides and AAC**

**[0435]** 5µM of thioFAB 4497 MP-LAFGA-QSY7("core peptide" disclosed as SEQ ID NO: 135) and thioFAB 4497 MP-LAFAA-QSY7 ("core peptide" disclosed as SEQ ID NO: 136) (Figure 30) were incubated with 50nM of protease in PBS pH 7.2, 4mM L-Cys, 2.5 mM EDTA for 2 hours at 37 °C. At 2 hours, cleavage reactions were quenched by diluting 1:1 with 1% TFA. Samples were subsequently run on LC-MS to determine percent linker cleavage. All the proteases tested cleaved the two linkers, although at varying degrees and locations (Table 4). Staphopain

A and staphopain B cleave the linkers at the same sites: MP-LAFG↓A-QSY7 ("core peptide" disclosed as SEQ ID NO: 135) and MP-LAFA↓A-QSY7 ("core peptide" disclosed as SEQ ID NO: 136). Staphopain B achieves 100% cleavage of both linkers at the concentration tested. Cathepsin B also achieved 100% cleavage for both linkers under these conditions, although the sites of cleavage were mixed. Cathepsin B cleaved mal-LAFGA-QSY7 ("core peptide" disclosed as SEQ ID NO: 135) exclusively at MP-LAFG↓A-QSY7 ("core peptide" disclosed as SEQ ID NO: 135), while it cleaved mal-LAFAA-QSY7 ("core peptide" disclosed as SEQ ID NO: 136) at both MP-LAFA↓A-QSY7 ("core peptide" disclosed as SEQ ID NO: 136) and MP-LAFAA↓-QSY7 ("core peptide" disclosed as SEQ ID NO: 136). MP-LAFAA-QSY7 ("core peptide" disclosed as SEQ ID NO: 136) cleavage by staphopain A was 23%, while cleavage of m MP-LAFGA-QSY7 ("core peptide" disclosed as SEQ ID NO: 135) was 38%.

**[0436]** 5µM of AAC-193 was incubated with 50 nM of protease in either PBS pH 7.2, 4 mM L-Cys, 2.5 mM EDTA or 100 mM Sodium Citrate pH 5, 100 mM NaCl, 4 mM L-Cys, 2.5 mM EDTA for 2 hours at 37 °C. At 2 hours, cleavage reactions were quenched by diluting 1:1 with 1% TFA. Samples were subsequently run on LC-MS to determine percent linker cleavage. The optimized linker-antibiotic was efficiently cleaved by all proteases tested. Upon cleavage by staphopain A and staphopain B, free piperazino-rifamycin was released. Staphopain B achieved 100% cleavage at both pH 5 and 7.2. Staphopain A showed 100% cleavage at pH 5 and 64% cleavage at pH 7.2.

**Example 27 Antibody-antibiotic conjugate, thio-S4497 LC v8-MP-LAFG-PABC-(piperazinoBOR) ("core peptide" disclosed as SEQ ID NO: 128) AAC-215, inhibits *S. aureus* *in vitro*:**

**[0437]** 1x10<sup>8</sup> stationary phase Wood46 bacteria were suspended in 10 µL of HB buffer (Hanks Balanced Salt Solution supplemented with 0.1% Bovine Serum Albumin) containing 100 µg/mL of thio-S4497 LC v8-MP-LAFG-PABC-(piperazinoBOR) ("core peptide" disclosed as SEQ ID NO: 128) AAC-215 or thio-S4497-HC-A118C-MC-vc-PABC-(piperazBOR) AAC-126. The latter uses a valine-citrulline (vc) cathepsin B cleavable linker to deliver the same antibiotic.

**[0438]** After 1 hour, samples were diluted 10-fold by addition of 90 µL of HB, or 90 uL of cathepsin B (10 µg/mL of Cathepsin B in 20 mM Sodium Acetate, 1 mM EDTA, 5 mM L-Cysteine pH 5) and incubated at 37 °C for an additional 3 hours. Release of active antibiotic was inferred by determining whether incubation of AACs with the bacteria was able to inhibit subsequent bacterial growth. Bacteria/AAC suspensions were spotted directly onto Tryptic Soy Agar plates and bacterial growth was visualized after overnight incubation at 37 °C. Active drug is released from AAC conjugated with MC-LAFG-PAB-(piperazinoBOR) ("core peptide" disclosed as SEQ ID NO: 128) linker-antibiotic intermediate.

**[0439]** Bacteria that were incubated without AAC grew well and were not affected by cathepsin B treatment. Bacteria treated with AAC-126 containing the cathepsin B cleavable linker, valine-



citrulline (vc) grew well after incubation in HB buffer alone, but failed to grow after treatment with AAC-126 + Cathepsin B, indicating that the enzyme treatment was required to release active antibiotic. In contrast, bacteria incubated with AAC-215 containing the staphopain cleavable linker LAFG (SEQ ID NO: 128) failed to grow after incubation with HB buffer alone, suggesting that the bacterial suspension contained enzymatic activity that was sufficient to release active antibiotic from the staphopain cleavable linker AAC.

**Example 28 Antibody-antibiotic conjugate, thio-S4497 HC v1-MP-LAFG-PABC-(piperazinoBOR) ("core peptide" disclosed as SEQ ID NO: 128) AAC-193 kills intracellular MRSA in a macrophage assay:**

**[0440]** The USA300 strain of *S. aureus* was incubated with various doses (100 µg/mL, 10 µg/mL, 1 µg/mL or 0.1 µg/mL) of S4497 antibody alone, thio-S4497 HC WT (v8) (SEQ ID NO: 137), LC V205C-MC-vc-PAB-(dimethylpipBOR) ("core peptide" disclosed as SEQ ID NO: 128) AAC-192, or thio-S4497 HC v1-MP-LAFG-PABC-(piperazinoBOR) AAC-193 to permit binding of the AAC to the bacteria (Figure 31).

**S4497 HC WT (v8) 446aa**

EVQ LVE SGG GLV QPG GSL RLS CSA SGF SFN SFW MHW VRQ VPG KGL VWI SFT NNE GTT TAY  
 ADS VRG RFI ISR DNA KNT LYL EMN NLR GED TAV YYC ARG DGG LDD WGQ GTL VTV SSA STK  
 GPS VFP LAP SSK STS GGT AAL GCL VKD YFP EPV TVS WNS GAL TSG VHT FPA VLQ SSG LYS  
 LSS VVT VPS SSL GTQ TYI CNV NHK PSN TKV DKK VEP KSC DKT HTC PPC PAP ELL GGP SVF  
 LFP PKP KDT LMI SRT PEV TCV VVD VSH EDP EVK FNW YVD GVE VHN AKT KPR EEQ YNS TYR  
 VVS VLT VLH QDW LNG KEY KCK VSN KAL PAP IEK TIS KAK GQP REP QVY TLP PSR EEM TKN  
 QVS LTC LVK GFY PSD IAV EWE SNG QPE NNY KTT PPV LDS DGS FFL YSK LTV DKS RWQ QGN  
 VFS CSV MHE ALH NHY TQK SLS LSP GK

(SEQ ID NO: 137)

**[0441]** After 1 hour incubation, the opsonized bacteria were fed to murine macrophages and incubated at 37 C for 2 hours to permit phagocytosis. After phagocytosis was complete, the infection mix was replaced with normal growth media supplemented with 50 µg/mL of gentamycin to kill any remaining extracellular bacteria and the total number of surviving intracellular bacteria was determined 2 days later by plating serial dilutions of the macrophage lysates on Tryptic Soy Agar plates (Figure 31). The staphopain cleavable AAC was able to kill intracellular USA300 with similar potency compared to the cathepsin B cleavable AAC. Gray dashed line indicates the limit of detection for the assay (10 CFU/well).

**Example 29 AAC target antibiotic killing to *S. aureus* via antigen specific binding of the antibody:**

**[0442]** The Wood46 strain of *S. aureus* was chosen because it does not express protein A, a molecule that binds to the Fc region of IgG antibodies. The Wood46 strain of *S. aureus* was incubated with 10 µg/mL or 0.5 µg/mL of S4497 antibody, Isotype control-AAC containing a cathepsin B cleavable linker, thio-trastuzumab HC A118C-MC-vc-PAB-(dimethyl-pipBOR) AAC-

101, thio-S4497 HC WT (v8), LC V205C-MC-vc-PAB-(dimethylpipBOR) AAC-192, Isotype control-AAC containing a staphopain cleavable linker, thio-trastuzumab HC A118C-MP-LAFG-PABC-(piperazinoBOR) ("core peptide" disclosed as SEQ ID NO: 128) or thio-S4497 HC v1-MP-LAFG-PABC-(piperazinoBOR) ("core peptide" disclosed as SEQ ID NO: 128) AAC-193 for 1 hour to permit binding of the AAC to bacteria (Figure 32). To limit non-specific binding of the AAC, the opsonized bacteria were centrifuged, washed once and resuspended in buffer before being fed to murine macrophages. After phagocytosis was complete, the infection mix was replaced with normal growth media supplemented with 50 µg/mL of gentamycin to kill any remaining extracellular bacteria and the total number of surviving intracellular bacteria was determined 2 days later by plating serial dilutions of the macrophage lysates on Tryptic Soy Agar plates (Figure 32). The AAC containing a staphopain cleavable linker, AAC-193, was able to kill all detectable intracellular bacteria, whereas the isotype control AAC showed no activity. The Macrophage Assay demonstrates that staphopain cleavable AAC are able to kill intracellular bacteria. Antibody-antibiotic conjugate, thio-S4497 LC v8-MP-LAFG-PABC-(piperazinoBOR) ("core peptide" disclosed as SEQ ID NO: 128) AAC-215 is efficacious *in vivo* in a MRSA infection model:

**[0443]** CB17.SCID mice were reconstituted with human IgG using a dosing regimen optimized to yield constant levels of at least 10 mg/mL of human IgG in serum. Mice were treated with 4497 antibody (50 mg/kg), AAC-215 with staphopain cleavable linker (50 mg/kg,) or an isotype control, anti-gD AAC containing staphopain cleavable linker (50 mg/kg). Mice were given a single dose of AAC-215 on day 1 post infection by intravenous injection. All mice were sacrificed on day 4 post infection, and the total number of surviving bacteria in 2 kidneys (Figure 33) or in heart (Figure 34) was determined by plating. Treatment with AAC-215 containing a staphopain cleavable linker reduced bacterial loads to below the limit of detection in 6 out of the 8 mice tested, whereas the isotype control AAC showed limited activity. The dashed line indicates the limit of detection for the assay (333 CFU/mouse).

### **Example 30 Growth of *S. aureus* and protease activity profiling:**

**[0444]** *Staphylococcus aureus* strain Wood46 (ATCC10832) was cultured overnight at 37 °C in tryptic soy broth (TSB) with shaking. Cultures were centrifuged at 10,000 x g for 10 min. Supernatant was collected and passed through two 0.22µm filters.

**[0445]** *S. aureus* protease activity profiling: 150 ml of culture supernatant was concentrated and buffer exchanged into phosphate buffered saline (PBS) using TFF (Millipore Pellicon XL Cassette Biomax 10kDa) to a final volume of 38 ml with a final total protein concentration of 1 mg/ml. Protease activity assay of Wood46 supernatant was performed using the Rapid Endopeptidase Profiling Library or REPLi (Mimotopes, Victoria, Australia). The library consists of 3375 internally quenched fluorogenic peptides in a 96-well format arranged in 512 groups. Library peptides contain the sequence MCA-Gly-Gly-Gly-Xaa-Yaa-Zaa-Gly-Gly-DPA-Lys-Lys (SEQ ID NO: 132) where MCA corresponds to 7-methoxycoumarin-4-acetic acid (fluorescent

donor) and DPA corresponds to N<sup>b</sup>-(2,4-dinitrophenyl)-L-2,3-diaminopropionic acid (fluorescence acceptor). Wells containing 5nmol of the FRET peptides were solubilized in 5ul of 50% acetonitrile (Sigma). 50 µl (microliters) of the concentrated Wood46 supernatant and 50ul of PBS were added to each well. Plates were incubated at 37 °C and fluorescence measurements were taken at 0, 30, 60, 140, and 170 minutes. Fluorescence data were obtained on a Tecan Sapphire<sup>2</sup>, excitation λ320nm/ emission λ400nm. Endpoint fluorescence intensity fold change was calculated as  $F_{\text{final}}/F_{\text{initial}}$ .

**[0446]** Substrate cleavage sites were determined by LC-MS performed with an Agilent Q-TOF using an ESI source. 10ul from each well were injected and separated by reversed phase chromatography on a Waters Xbridge OST C18 2.5um column (4.6 x 50mm) using an Agilent 1260 HPLC system. Samples were eluted with a gradient of 2-90% Buffer B (where Buffer A is 0.05% trifluoroacetic acid/99.95% water and Buffer B is 0.04% % trifluoroacetic acid/99.96 % acetonitrile) at 500 µl/min with a total analysis time of 20 minutes. Peptides were eluted directly into a Q-TOF mass spectrometer. Cleavage products were assigned based on comparing observed molecular weights with calculated masses corresponding to cleavage at each possible site.

**Example 31 Synthesis of maleimido FRET peptide linker:**

**[0447]** The maleimido FRET peptide, (MP-Lys(TAMRA)-Gly-Gly-Ala-Phe-Ala-Gly-Gly-Gly-Lys(fluorescein) ("core peptide" disclosed as SEQ ID NO: 125) of Figure 26 was synthesized by standard Fmoc solid-phase chemistry using a PS3 peptide synthesizer (Protein Technologies, Inc.). 0.1 mmol of Fmoc-Lys(Boc)-Rink amide resin (Novabiochem) was used to generate a C-terminal carboxamide. Fmoc-Lys(Mtt)-OH (Novabiochem) was added at the first N-terminal residue to allow for additional side-chain chemistry after removal of the Mtt group. A fluorescence acceptor, 5(6)-carboxy tetramethylrhodamine, or TAMRA (Novabiochem), was attached to this side-chain amine on the resin after the Mtt group was removed by three consecutive 30 min washes of 1% TFA in dichloromethane with 3% triisopropylsilane (TIS). The reaction was allowed to proceed for 20 hours. After this step, the terminal Fmoc group is removed by 20% piperidine in DMF and coupled with maleimido-propionic acid (Bachem AG) by HBTU. The TAMRA-labeled intermediate peptides were cleaved off from the resin with 95:2.5:2.5 trifluoroacetic acid (TFA)/TIS/water (v/v/v) for 2 hours at room temperature with gentle shaking. The cleavage solution was filtered and evaporated under a stream of nitrogen to remove the TFA. The crude intermediates dissolved in a mixture of water and acetonitrile were subjected to further purification by reverse-phase HPLC with a Jupiter 5µl C4 column (5 µm, 10 mm x 250 mm) from Phenomenex. After lyophilization, the purified intermediates were then reacted with 10 eq. NHS-fluorescein (Thermo Scientific) in 50/50 phosphate buffered saline (PBS)/dimethylformamide (DMF) (v/v) for 20 hours to label the free amine at the C-terminal lysine. The FRET peptide was then purified and lyophilized as described above. All reaction mixtures and final products were analyzed and confirmed by LC-MS.

**[0448]** Solid phase synthesis of linkers: All linkers described were synthesized using standard Fmoc solid-phase chemistry on a PS3 peptide synthesizer (Protein Technologies, Inc.). 0.1mM of Fmoc-amino acid-Wang resin (Novabiochem) was used for all linkers to generate a C-terminal carboxyl. Linkers were purified as described above. QSY7 amine (Invitrogen) attachment was accomplished by reacting purified linkers with a 1.1 fold molar excess of QSY7 amine, a 1.1 fold molar excess of HATU, and a 2.2 fold molar excess of DIEA in DMF overnight at room temperature. Linker-QSY7 species were then purified as described above. All reaction mixtures and final products were analyzed and confirmed by LC-MS.

**Example 32 Cell based FRET cleavage assays:**

**[0449]** Cultures of Wood46 and USA300 were inoculated with a 1:200 dilution of overnight cultures (0.1ml in 20 ml) in TSB and incubated at 37°C with shaking. Strains were cultured to exponential phase of growth and plated at cell densities of  $10^8$  cells/ml and  $10^7$  cells/ml in tryptic soy broth (TSB). thioMAB FRET peptide conjugates were added to wells at a final FRET peptide concentration of 2µM. Plates were incubated at 37 °C and fluorescence was monitored over time, excitation  $\lambda$ 495nm/ emission  $\lambda$ 518nm, for 210 minutes.

**[0450]** Cleavage of thioFAB FRET peptide and thioFAB mal-GGAFAGGG-DNA31 ("core peptide" disclosed as SEQ ID NO: 126) by concentrated active supernatant: thioFAB S4497 conjugated with either MP-Lys(TAMRA)-Gly-Gly-Ala-Phe-Ala-Gly-Gly-Gly-Lys(fluorescein) ("core peptide" disclosed as SEQ ID NO: 125) or MP-Gly-Gly-Ala-Phe-Ala-Gly-Gly-Gly-(pipBOR) LA-59 ("core peptide" disclosed as SEQ ID NO: 126) were incubated with concentrated Wood46 supernatant that had been processed as described above. thioFAB S4497 and supernatant were mixed 1:1 on a milligram basis (25 µg of thioFAB S4497 with 25 µg of proteinaceous supernatant) in PBS. Samples were incubated for 2 hours at 37 °C. At 2 hours, the reaction was quenched by diluting 1:1 with 0.1% TFA. Samples were analyzed by LC-MS to determine amount of cleavage and cleavage products.

**[0451]** Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the invention.

**SEQUENCE LISTING**

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Ala

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1				5				

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&lt;400&gt; 4

Asp	Tyr	Tyr	Met	His
1				5

&lt;210&gt; 5

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&lt;400&gt; 5

Trp	Ile	Asn	Pro	Lys	Ser	Gly	Gly	Thr	Asn	Tyr	Ala	Gln	Arg	Phe	Gln
1				5				10						15	

Gly

&lt;210&gt; 6

&lt;211&gt; 10

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&lt;400&gt; 6

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&lt;210&gt; 7

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<400> 7

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1				5		

<210> 9

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Gln	Gln	Tyr	Tyr	Ala	Asn	Pro	Arg	Thr
1				5				

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&lt;400&gt; 11

Trp	Ile	Asn	Pro	Asn	Thr	Gly	Gly	Thr	Tyr	Tyr	Ala	Gln	Lys	Phe	Arg
1				5					10					15	

Asp

&lt;210&gt; 12

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&lt;400&gt; 12

Asp	Cys	Gly	Arg	Gly	Gly	Leu	Arg	Asp	Ile
1				5					10

&lt;210&gt; 13

&lt;211&gt; 17

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&lt;400&gt; 13

Lys	Ser	Asn	Gln	Asn	Val	Leu	Ala	Ser	Ser	Asn	Asp	Lys	Asn	Tyr	Leu
1				5					10					15	

Ala

&lt;210&gt; 14

&lt;211&gt; 7

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Trp	Ala	Ser	Ile	Arg	Glu	Ser
1				5		



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Trp	Ile	Asn	Pro	Asn	Thr	Gly	Gly	Thr	Asn	Tyr	Ala	Gln	Lys	Phe	Gln
1				5					10					15	

Gly

<210> 18

<211> 10

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&lt;400&gt; 18

Asp	Cys	Gly	Asn	Ala	Gly	Leu	Arg	Asp	Ile
1				5					10

&lt;210&gt; 19

&lt;211&gt; 17

&lt;212&gt; PRT

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&lt;221&gt; source

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&lt;400&gt; 19

Lys	Ser	Ser	Gln	Asn	Val	Leu	Tyr	Ser	Ser	Asn	Asn	Lys	Asn	Tyr	Leu
1				5					10					15	

Ala

&lt;210&gt; 20

&lt;211&gt; 7

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&lt;220&gt;

&lt;221&gt; source

&lt;223&gt; /note="Description of Artificial Sequence: Synthetic peptide"

&lt;400&gt; 20

Trp	Ala	Ser	Thr	Arg	Glu	Ser
1				5		

&lt;210&gt; 21

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&lt;400&gt; 21

Gln	Gln	Tyr	Tyr	Thr	Ser	Pro	Pro	Tyr	Thr
1				5					10

&lt;210&gt; 22

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<400> 22

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<210> 23

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Ile Ile His Pro Gly Asp Ser Lys Thr Arg Tyr Ser Pro Ser Phe Gln  
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Gly

<210> 24

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Leu Gly Ala Gly Gly Tyr Tyr Tyr Tyr Gly Met Gly Val  
20 25

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1 5 10 15

Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Val Leu Ser Arg

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      20              25              30
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Ala Asn Asn Asn Tyr Tyr Val Ala Trp Tyr Gln His Lys Pro Gly Gln
    35              40              45

Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Phe Gly Val
    50              55              60

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65              70              75              80

Ile Asn Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln
      85              90              95

Tyr Tyr Thr Ser Arg Arg Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
    100              105              110

Lys

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1              5              10              15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Asp Tyr
      20              25              30

Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
    35              40              45

Gly Trp Ile Asn Pro Lys Ser Gly Gly Thr Asn Tyr Ala Gln Arg Phe
    50              55              60

Gln Gly Arg Val Thr Met Thr Gly Asp Thr Ser Ile Ser Ala Ala Tyr
65              70              75              80

Met Asp Leu Ala Ser Leu Thr Ser Asp Asp Thr Ala Val Tyr Tyr Cys
      85              90              95

Val Lys Asp Cys Gly Ser Gly Gly Leu Arg Asp Phe Trp Gly Gln Gly
    100              105              110

Thr Thr Val Thr Val Ser Ser
    115

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&lt;210&gt; 27

&lt;211&gt; 112

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&lt;400&gt; 27

Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro	Asp	Ser	Leu	Ser	Val	Ser	Leu	Gly
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Glu	Arg	Ala	Thr	Ile	Asn	Cys	Arg	Ser	Asn	Gln	Asn	Leu	Leu	Ser	Ser
		20					25					30			

Ser	Asn	Asn	Asn	Tyr	Leu	Ala	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Gln	Pro
	35						40					45			

Leu	Lys	Leu	Leu	Ile	Tyr	Trp	Ala	Ser	Thr	Arg	Glu	Ser	Gly	Val	Pro
50						55				60					

Asp	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile
65					70					75					80

Ser	Ser	Leu	Gln	Ala	Glu	Asp	Val	Ala	Val	Tyr	Tyr	Cys	Gln	Gln	Tyr
				85					90					95	

Tyr	Ala	Asn	Pro	Arg	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Val	Glu	Ile	Lys
			100					105					110		

&lt;210&gt; 28

&lt;211&gt; 119

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&lt;221&gt; source

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&lt;400&gt; 28

Gln	Val	Gln	Leu	Gln	Gln	Ser	Arg	Val	Glu	Val	Lys	Arg	Pro	Gly	Thr
1				5				10						15	

Ser	Val	Lys	Val	Ser	Cys	Lys	Thr	Ser	Gly	Tyr	Thr	Phe	Ser	Asp	Tyr
			20					25					30		

Tyr	Ile	His	Trp	Val	Arg	Leu	Ala	Pro	Gly	Gln	Gly	Leu	Glu	Leu	Met
		35					40					45			

Gly	Trp	Ile	Asn	Pro	Asn	Thr	Gly	Gly	Thr	Tyr	Tyr	Ala	Gln	Lys	Phe
50						55						60			

Arg Asp Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ala Thr Ala Tyr  
65 70 75 80

Leu Glu Met Ser Ser Leu Thr Ser Asp Asp Thr Ala Val Tyr Tyr Cys  
85 90 95

Ala Lys Asp Cys Gly Arg Gly Gly Leu Arg Asp Ile Trp Gly Pro Gly  
100 105 110

Thr Met Val Thr Val Ser Ser  
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Glu Ile Val Leu Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly  
1 5 10 15

Glu Arg Ala Thr Ile Asn Cys Lys Ser Asn Gln Asn Val Leu Ala Ser  
20 25 30

Ser Asn Asp Lys Asn Tyr Leu Ala Trp Phe Gln His Lys Pro Gly Gln  
35 40 45

Pro Leu Lys Leu Leu Ile Tyr Trp Ala Ser Ile Arg Glu Ser Gly Val  
50 55 60

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr  
65 70 75 80

Ile Ser Ser Leu Arg Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln  
85 90 95

Tyr Tyr Thr Asn Pro Arg Thr Phe Gly Gln Gly Thr Lys Val Glu Phe  
100 105 110

Asn

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 1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr  
 20 25 30

Tyr Ile His Trp Val Arg Leu Ala Pro Gly Gln Gly Leu Glu Leu Met  
 35 40 45

Gly Trp Ile Asn Pro Asn Thr Gly Gly Thr Asn Tyr Ala Gln Lys Phe  
 50 55 60

Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ala Thr Ala Tyr  
 65 70 75 80

Met Glu Leu Ser Ser Leu Thr Ser Asp Asp Thr Ala Val Tyr Tyr Cys  
 85 90 95

Ala Lys Asp Cys Gly Asn Ala Gly Leu Arg Asp Ile Trp Gly Gln Gly  
 100 105 110

Thr Thr Val Thr Val Ser Ser  
 115

&lt;210&gt; 31

&lt;211&gt; 114

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&lt;221&gt; source

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&lt;400&gt; 31

Asp Ile Gln Leu Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly  
 1 5 10 15

Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Asn Val Leu Tyr Ser  
 20 25 30

Ser Asn Asn Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln  
 35 40 45

Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val  
 50 55 60

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr  
 65 70 75 80

Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln  
 85 90 95

Tvr Tvr Thr Ser Pro Pro Tvr Thr Phe Glv Gln Glv Thr Lvs Leu Glu

100 105 110

Ile Glu

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 1 5 10 15

Ser Leu Lys Ile Ser Cys Lys Gly Ser Gly Tyr Ser Phe Thr Ser Tyr  
 20 25 30

Trp Ile Gly Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met  
 35 40 45

Gly Ile Ile His Pro Gly Asp Ser Lys Thr Arg Tyr Ser Pro Ser Phe  
 50 55 60

Gln Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr  
 65 70 75 80

Leu Gln Trp Asn Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys  
 85 90 95

Ala Arg Leu Tyr Cys Ser Gly Gly Ser Cys Tyr Ser Asp Arg Ala Phe  
 100 105 110

Ser Ser Leu Gly Ala Gly Gly Tyr Tyr Tyr Tyr Gly Met Gly Val Trp  
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Gly Gln Gly Thr Thr Val Thr Val Ser Ser  
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<400> 33

Arg Ala Ser Gln Thr Ile Ser Gly Trp Leu Ala  
 1 5 10



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<400> 34  
Lys Ala Ser Thr Leu Glu Ser  
1 5

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Gln Gln Tyr Lys Ser Tyr Ser Phe Asn  
1 5

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1 5

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1 5 10 15

Gly

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<400> 38

Ser	Ser	Ile	Leu	Val	Arg	Gly	Ala	Leu	Gly	Arg	Tyr	Phe	Asp	Leu
1				5					10					15

<210> 39

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<400> 39

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1				5					10	

<210> 40

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1				5		

<210> 41

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Gln Gln Tyr Lys Ser Tyr Ser Phe Asn  
 1 5

&lt;210&gt; 42

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&lt;400&gt; 42

Ser Tyr Asp Ile Asn  
 1 5

&lt;210&gt; 43

&lt;211&gt; 17

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Trp Met Asn Ala Asn Ser Gly Asn Thr Gly Tyr Ala Gln Lys Phe Gln  
 1 5 10 15

Gly

&lt;210&gt; 44

&lt;211&gt; 15

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; source

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Ser Ser Ile Leu Val Arg Gly Ala Leu Gly Arg Tyr Phe Asp Leu  
 1 5 10 15

&lt;210&gt; 45

&lt;211&gt; 12

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<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 46

Glu	Thr	Ser	Ser	Arg	Ala	Thr
1			5			

<210> 47

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 47

His	Lys	Tyr	Gly	Ser	Gly	Pro	Arg	Thr
1			5					

<210> 48

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 48

Asn	Tyr	Asp	Phe	Ile
1			5	

<210> 49

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 49

Trp	Met	Asn	Pro	Asn	Ser	Tyr	Asn	Thr	Gly	Tyr	Gly	Gln	Lys	Phe	Gln
1				5					10					15	

Gly

<210> 50

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 50

Ala	Val	Arg	Gly	Gln	Leu	Leu	Ser	Glu	Tyr
1				5					10

<210> 51

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 51

Arg	Ala	Ser	Gln	Ser	Val	Ser	Ser	Ser	Tyr	Leu	Ala
1				5					10		

<210> 52

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 52

Asp	Ala	Ser	Ser	Arg	Ala	Thr
1				5		

<210> 53

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 53

Gln	Lys	Tyr	Gly	Ser	Thr	Pro	Arg	Pro
1				5				

<210> 54

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 54

Ser	Tyr	Asp	Ile	Asn
1				5

<210> 55

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 55

Trp	Met	Asn	Pro	Asn	Ser	Gly	Asn	Thr	Asn	Tyr	Ala	Gln	Arg	Phe	Gln
1				5					10					15	

Gly

<210> 56

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 56

Glu	Arg	Trp	Ser	Lys	Asp	Thr	Gly	His	Tyr	Tyr	Tyr	Tyr	Gly	Met	Asp
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

1 - - 5 - - 10 - - - 15 -

Val

<210> 57

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 57

Arg	Ala	Ser	Leu	Asp	Ile	Thr	Asn	His	Leu	Ala
1				5					10	

<210> 58

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 58

Glu	Ala	Ser	Ile	Leu	Gln	Ser
1				5		

<210> 59

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 59

Glu	Lys	Cys	Asn	Ser	Thr	Pro	Arg	Thr
1				5				

<210> 60

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 60

Asn Tyr Asp Ile Asn  
1 5

<210> 61

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 61

Trp Met Asn Pro Ser Ser Gly Arg Thr Gly Tyr Ala Pro Lys Phe Arg  
1 5 10 15

Gly

<210> 62

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 62

Gly Gly Gly Tyr Tyr Asp Ser Ser Gly Asn Tyr His Ile Ser Gly Leu  
1 5 10 15

Asp Val

<210> 63

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 63

Arg Ala Ser Gln Ser Val Gly Ala Ile Tyr Leu Ala  
1 5 10

<210> 64

<211> 7



<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 64

Gly	Val	Ser	Asn	Arg	Ala	Thr
1			5			

<210> 65

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 65

Gln	Leu	Tyr	Thr	Ser	Ser	Arg	Ala	Leu	Thr
1				5					10

<210> 66

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 66

Ala	Tyr	Ala	Met	Asn
1			5	

<210> 67

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 67

Ser	Ile	Thr	Lys	Asn	Ser	Asp	Ser	Leu	Tyr	Tyr	Ala	Asp	Ser	Val	Lys
1				5					10					15	

Gly

<210> 68  
 <211> 10  
 <212> PRT  
 <213> Artificial Sequence  
  
 <220>  
 <221> source  
 <223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 68  
 Leu Ala Ala Arg Ile Met Ala Thr Asp Tyr  
 1                      5                      10

<210> 69  
 <211> 11  
 <212> PRT  
 <213> Artificial Sequence  
  
 <220>  
 <221> source  
 <223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 69  
 Arg Ala Ser Gln Gly Ile Arg Asn Gly Leu Gly  
 1                      5                      10

<210> 70  
 <211> 7  
 <212> PRT  
 <213> Artificial Sequence  
  
 <220>  
 <221> source  
 <223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 70  
 Pro Ala Ser Thr Leu Glu Ser  
 1                      5

<210> 71  
 <211> 9  
 <212> PRT  
 <213> Artificial Sequence  
  
 <220>  
 <221> source  
 <223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 71  
 Leu Gln Asp His Asn Tyr Pro Pro Thr  
 -                      -

1 5

<210> 72

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 72

Tyr Tyr Ser Met Ile

1 5

<210> 73

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 73

Ser Ile Asp Ser Ser Ser Arg Tyr Leu Tyr Tyr Ala Asp Ser Val Lys  
1 5 10 15

Gly

<210> 74

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 74

Asp Gly Asp Asp Ile Leu Ser Val Tyr Arg Gly Ser Gly Arg Pro Phe  
1 5 10 15

Asp Tyr

<210> 75

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 75

Arg	Ala	Ser	Gln	Gly	Ile	Arg	Asn	Gly	Leu	Gly
1				5					10	

<210> 76

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 76

Pro	Ala	Ser	Thr	Leu	Glu	Ser
1				5		

<210> 77

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 77

Leu	Gln	Asp	His	Asn	Tyr	Pro	Pro	Ser
1				5				

<210> 78

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 78

Tyr	Tyr	Ser	Met	Ile
1				5

<210> 79

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 79

Ser	Ile	Asp	Ser	Ser	Ser	Arg	Tyr	Arg	Tyr	Tyr	Thr	Asp	Ser	Val	Lys
1				5					10					15	

Gly

<210> 80

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 80

Asp	Gly	Asp	Asp	Ile	Leu	Ser	Val	Tyr	Gln	Gly	Ser	Gly	Arg	Pro	Phe
1				5					10					15	

Asp Tyr

<210> 81

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 81

Arg	Ala	Ser	Gln	Ser	Val	Arg	Thr	Asn	Val	Ala
1				5					10	

<210> 82

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 82

Gly	Ala	Ser	Thr	Arg	Ala	Ser
1				5		

<210> 83  
<211> 9  
<212> PRT  
<213> Artificial Sequence  
  
<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 83  
Leu Gln Tyr Asn Thr Trp Pro Arg Thr  
1                    5

<210> 84  
<211> 5  
<212> PRT  
<213> Artificial Sequence  
  
<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 84  
Thr Asn Asp Met Ser  
1                    5

<210> 85  
<211> 17  
<212> PRT  
<213> Artificial Sequence  
  
<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 85  
Thr Ile Ile Gly Ile Asp Asp Thr Thr His Tyr Ala Asp Ser Val Arg  
1                    5                    10                    15

Gly  
  
<210> 86  
<211> 7  
<212> PRT  
<213> Artificial Sequence  
  
<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 86

Asn Ser Gly Ile Tyr Ser Phe  
1 5

<210> 87

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 87

Arg Ala Ser Gln Asp Ile Gly Ser Ser Leu Ala  
1 5 10

<210> 88

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 88

Ala Thr Ser Thr Leu Gln Ser  
1 5

<210> 89

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 89

Gln Gln Leu Asn Asn Tyr Val His Ser  
1 5

<210> 90

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 90  
Asp Tyr Ala Met Gly  
1 5

<210> 91  
<211> 17  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 91  
Val Val Thr Gly His Ser Tyr Arg Thr His Tyr Ala Asp Ser Val Lys  
1 5 10 15

Gly

<210> 92  
<211> 12  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 92  
Arg Ile Trp Ser Tyr Gly Asp Asp Ser Phe Asp Val  
1 5 10

<210> 93  
<211> 11  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 93  
Arg Ala Ser Gln Ser Ile Gly Asp Arg Leu Ala  
1 5 10

<210> 94  
<211> 7  
<212> PRT  
<213> Artificial Sequence



<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 94

Trp Ala Ser Asn Leu Glu Gly  
1 5

<210> 95

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 95

Gln Gln Tyr Lys Ser Gln Trp Ser  
1 5

<210> 96

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 96

Ser Tyr Ala Met Asn  
1 5

<210> 97

<211> 16

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 97

Tyr Ile Ser Ser Ile Glu Thr Ile Tyr Tyr Ala Asp Ser Val Lys Gly  
1 5 10 15

<210> 98

<211> 13

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 98

Asp	Arg	Leu	Val	Asp	Val	Pro	Leu	Ser	Ser	Pro	Asn	Ser
1				5					10			

<210> 99

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 99

Lys	Ser	Ser	Gln	Ser	Ile	Phe	Arg	Thr	Ser	Arg	Asn	Lys	Asn	Leu	Leu
1				5					10					15	

Asn

<210> 100

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 100

Trp	Ala	Ser	Thr	Arg	Lys	Ser
1				5		

<210> 101

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 101

Gln	Gln	Tyr	Phe	Ser	Pro	Pro	Tyr	Thr
1				5				

<210> 102

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 102

Ser	Phe	Trp	Met	His
1				5

<210> 103

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 103

Phe	Thr	Asn	Asn	Glu	Gly	Thr	Thr	Thr	Ala	Tyr	Ala	Asp	Ser	Val	Arg
1				5					10					15	

Gly

<210> 104

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 104

Gly	Asp	Gly	Gly	Leu	Asp	Asp
1				5		

<210> 105

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 105

Arg	Ala	Ser	Gln	Phe	Thr	Asn	His	Tyr	Leu	Asn
1				5					10	

<210> 106

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 106

Val	Ala	Ser	Asn	Leu	Gln	Ser
1			5			

<210> 107

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 107

Gln	Gln	Ser	Tyr	Arg	Thr	Pro	Tyr	Thr
1			5					

<210> 108

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 108

Ser	Gly	Tyr	Tyr	Asn
1			5	

<210> 109

<211> 16

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 109

Tyr	Ile	Leu	Ser	Gly	Ala	His	Thr	Asp	Ile	Lys	Ala	Ser	Leu	Gly	Ser
1			5				10						15		

1 5 10 15

<210> 110  
 <211> 11  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <221> source  
 <223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 110  
 Ser Gly Val Tyr Ser Lys Tyr Ser Leu Asp Val  
 1 5 10

<210> 111  
 <211> 107  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <221> source  
 <223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 111  
 Asp Ile Val Met Thr Gln Ser Pro Ser Ile Leu Ser Ala Ser Val Gly  
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Thr Ile Ser Gly Trp  
 20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Ala Glu Ala Pro Lys Leu Leu Ile  
 35 40 45

Tyr Lys Ala Ser Thr Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly  
 50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro  
 65 70 75 80

Asp Asp Phe Gly Ile Tyr Tyr Cys Gln Gln Tyr Lys Ser Tyr Ser Phe  
 85 90 95

Asn Phe Gly Gln Gly Thr Lys Val Glu Ile Lys  
 100 105

<210> 112  
 <211> 124  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<220>

<221> VARIANT

<222> 1

<223> Xaa = Gln or Glu

<220>

<221> VARIANT

<222> 2

<223> Xaa = Met or Ile or Val

<400> 112

Xaa	Xaa	Gln	Leu	Val	Gln	Ser	Gly	Ala	Glu	Val	Lys	Lys	Pro	Gly	Ala
1				5					10					15	
Ser	Val	Lys	Val	Ser	Cys	Glu	Ala	Ser	Gly	Tyr	Thr	Leu	Thr	Ser	Tyr
		20					25					30			
Asp	Ile	Asn	Trp	Val	Arg	Gln	Ala	Thr	Gly	Gln	Gly	Pro	Glu	Trp	Met
		35				40					45				
Gly	Trp	Met	Asn	Ala	Asn	Ser	Gly	Asn	Thr	Gly	Tyr	Ala	Gln	Lys	Phe
	50				55					60					
Gln	Gly	Arg	Val	Thr	Leu	Thr	Gly	Asp	Thr	Ser	Ile	Ser	Thr	Ala	Tyr
65				70				75					80		
Met	Glu	Leu	Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys
			85					90					95		
Ala	Arg	Ser	Ser	Ile	Leu	Val	Arg	Gly	Ala	Leu	Gly	Arg	Tyr	Phe	Asp
			100				105						110		
Leu	Trp	Gly	Arg	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser				
		115					120								

<210> 113

<211> 214

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 113

Asp	Ile	Val	Met	Thr	Gln	Ser	Pro	Ser	Ile	Leu	Ser	Ala	Ser	Val	Gly
1				5					10					15	
Asp	Arg	Val	Thr	Ile	Thr	Cys	Arg	Ala	Ser	Gln	Thr	Ile	Ser	Gly	Trp
		20					25						30		
Leu	Ala	Trp	Tyr	Gln	Gln	Lys	Pro	Ala	Glu	Ala	Pro	Lys	Leu	Leu	Ile
		35				40					45				
Tyr	Lys	Ala	Ser	Thr	Leu	Glu	Ser	Gly	Val	Pro	Ser	Arg	Phe	Ser	Gly
	50				55					60					
Ser	Gly	Ser	Gly	Thr	Glu	Phe	Thr	Leu	Thr	Ile	Ser	Ser	Leu	Gln	Pro
65				70					75					80	
Asp	Asp	Phe	Gly	Ile	Tyr	Tyr	Cys	Gln	Gln	Tyr	Lys	Ser	Tyr	Ser	Phe

85 90 95  
 Asn Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala  
 100 105 110  
 Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly  
 115 120 125  
 Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala  
 130 135 140  
 Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln  
 145 150 155 160  
 Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser  
 165 170 175  
 Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr  
 180 185 190  
 Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser  
 195 200 205  
 Phe Asn Arg Gly Glu Cys  
 210

&lt;210&gt; 114

&lt;211&gt; 453

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; source

&lt;223&gt; /note="Description of Artificial Sequence: Synthetic polypeptide"

&lt;400&gt; 114

Gln Met Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala  
 1 5 10 15  
 Ser Val Lys Val Ser Cys Glu Ala Ser Gly Tyr Thr Leu Thr Ser Tyr  
 20 25 30  
 Asp Ile Asn Trp Val Arg Gln Ala Thr Gly Gln Gly Pro Glu Trp Met  
 35 40 45  
 Gly Trp Met Asn Ala Asn Ser Gly Asn Thr Gly Tyr Ala Gln Lys Phe  
 50 55 60  
 Gln Gly Arg Val Thr Leu Thr Gly Asp Thr Ser Ile Ser Thr Ala Tyr  
 65 70 75 80  
 Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys  
 85 90 95

110 115 120 125 130 135 140 145 150 155 160 165 170 175 180 185 190 195 200 205 210

Ala Arg Ser Ser Ile Leu Val Arg Gly Ala Leu Gly Arg Tyr Phe Asp  
 100 105 110  
 Leu Trp Gly Arg Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys  
 115 120 125  
 Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly  
 130 135 140  
 Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro  
 145 150 155 160  
 Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr  
 165 170 175  
 Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val  
 180 185 190  
 Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn  
 195 200 205  
 Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro  
 210 215 220  
 Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu  
 225 230 235 240  
 Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp  
 245 250 255  
 Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp  
 260 265 270  
 Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly  
 275 280 285  
 Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn  
 290 295 300  
 Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp  
 305 310 315 320  
 Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro  
 325 330 335  
 Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu  
 340 345 350  
 Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn  
 355 360 365  
 Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile  
 370 375 380



Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr  
385 390 395 400

Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys  
405 410 415

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys  
420 425 430

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu  
435 440 445

Ser Leu Ser Pro Gly  
450

<210> 115

<211> 214

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 115

Asp Ile Val Met Thr Gln Ser Pro Ser Ile Leu Ser Ala Ser Val Gly  
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Thr Ile Ser Gly Trp  
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Ala Glu Ala Pro Lys Leu Leu Ile  
35 40 45

Tyr Lys Ala Ser Thr Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly  
50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro  
65 70 75 80

Asp Asp Phe Gly Ile Tyr Tyr Cys Gln Gln Tyr Lys Ser Tyr Ser Phe  
85 90 95

Asn Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala  
100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly  
115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala  
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln  
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser  
165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr  
180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Cys Thr Lys Ser  
195 200 205

Phe Asn Arg Gly Glu Cys  
210

<210> 116

<211> 453

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<220>

<221> VARIANT

<222> 2

<223> Xaa = Met or Ile or Val

<400> 116

Glu Xaa Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala  
1 5 10 15  
Ser Val Lys Val Ser Cys Glu Ala Ser Gly Tyr Thr Leu Thr Ser Tyr  
20 25 30  
Asp Ile Asn Trp Val Arg Gln Ala Thr Gly Gln Gly Pro Glu Trp Met  
35 40 45  
Gly Trp Met Asn Ala Asn Ser Gly Asn Thr Gly Tyr Ala Gln Lys Phe  
50 55 60  
Gln Gly Arg Val Thr Leu Thr Gly Asp Thr Ser Ile Ser Thr Ala Tyr  
65 70 75 80  
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys  
85 90 95  
Ala Arg Ser Ser Ile Leu Val Arg Gly Ala Leu Gly Arg Tyr Phe Asp  
100 105 110  
Leu Trp Gly Arg Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys  
115 120 125  
Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly  
130 135 140  
Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro  
145 150 155 160  
Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr  
165 170 175  
Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val  
180 185 190  
Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn  
195 200 205  
Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro  
210 215 220  
Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu  
225 230 235 240  
Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp  
245 250 255  
Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp  
260 265 270  
Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly

```

      275      -      280      -      285      -      -
Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
290      295      300
Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
305      310      315      320
Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro
325      330      335
Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu
340      345      350
Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn
355      360      365

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
370      375      380
Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
385      390      395      400
Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
405      410      415
Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
420      425      430
Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
435      440      445
Ser Leu Ser Pro Gly
450

```

&lt;210&gt; 117

&lt;211&gt; 453

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; source

&lt;223&gt; /note="Description of Artificial Sequence: Synthetic polypeptide"

&lt;220&gt;

&lt;221&gt; VARIANT

&lt;222&gt; 2

&lt;223&gt; Xaa = Met or Ile or Val

&lt;400&gt; 117

```

Glu Xaa Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1      5      10      15
Ser Val Lys Val Ser Cys Glu Ala Ser Gly Tyr Thr Leu Thr Ser Tyr
20      25      30
Asp Ile Asn Trp Val Arg Gln Ala Thr Gly Gln Gly Pro Glu Trp Met
35      40      45
Gly Trp Met Asn Ala Asn Ser Gly Asn Thr Gly Tyr Ala Gln Lys Phe
50      55      60
Gln Gly Arg Val Thr Leu Thr Gly Asp Thr Ser Ile Ser Thr Ala Tyr
65      70      75      80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85      90      95
Ala Arg Ser Ser Ile Leu Val Arg Gly Ala Leu Gly Arg Tyr Phe Asp
100      105      110
Leu Trp Gly Arg Gly Thr Leu Val Thr Val Ser Ser Cys Ser Thr Lys
115      120      125
Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly
130      135      140
Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro
145      150      155      160
Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
165      170      175
Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
180      185      190
Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn

```

```

195      200      205
Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro
210      215      220
Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu
225      230      235      240
Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
245      250      255

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
260      265      270
Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
275      280      285
Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
290      295      300
Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
305      310      315      320
Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro
325      330      335
Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu
340      345      350
Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn
355      360      365
Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
370      375      380
Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
385      390      395      400
Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
405      410      415
Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
420      425      430
Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
435      440      445
Ser Leu Ser Pro Gly
450

```

&lt;210&gt; 118

&lt;211&gt; 7

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; source

&lt;223&gt; /note="Description of Artificial Sequence: Synthetic peptide"

&lt;400&gt; 118

Gly Glu Gly Gly Leu Asp Asp

1 5

&lt;210&gt; 119

&lt;211&gt; 113

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; source

&lt;223&gt; /note="Description of Artificial Sequence: Synthetic polypeptide"

&lt;400&gt; 119

```

Asp Ile Gln Leu Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
1           5           10           15

```

Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Ile Phe Arg Thr  
20 25 30

Ser Arg Asn Lys Asn Leu Leu Asn Trp Tyr Gln Gln Arg Pro Gly Gln  
35 40 45

Pro Pro Arg Leu Leu Ile His Trp Ala Ser Thr Arg Lys Ser Gly Val  
50 55 60

Pro Asp Arg Phe Ser Gly Ser Gly Phe Gly Thr Asp Phe Thr Leu Thr  
65 70 75 80

Ile Thr Ser Leu Gln Ala Glu Asp Val Ala Ile Tyr Tyr Cys Gln Gln  
85 90 95

Tyr Phe Ser Pro Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile  
100 105 110

Lys

<210> 120

<211> 116

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 120

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
1 5 10 15

Ser Leu Arg Leu Ser Cys Ser Ala Ser Gly Phe Ser Phe Asn Ser Phe  
20 25 30

Trp Met His Trp Val Arg Gln Val Pro Gly Lys Gly Leu Val Trp Ile  
35 40 45

Ser Phe Thr Asn Asn Glu Gly Thr Thr Thr Ala Tyr Ala Asp Ser Val  
50 55 60

Arg Gly Arg Phe Ile Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr  
65 70 75 80

Leu Glu Met Asn Asn Leu Arg Gly Glu Asp Thr Ala Val Tyr Tyr Cys  
85 90 95

Ala Arg Gly Asp Gly Gly Leu Asp Asp Trp Gly Gln Gly Thr Leu Val  
100 105 110

Met Val Ser Ser

III Val Ser Ser

115

<210> 121

<211> 220

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 121

Asp Ile Gln Leu Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly  
1 5 10 15

Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Ile Phe Arg Thr  
20 25 30

Ser Arg Asn Lys Asn Leu Leu Asn Trp Tyr Gln Gln Arg Pro Gly Gln  
35 40 45

Pro Pro Arg Leu Leu Ile His Trp Ala Ser Thr Arg Lys Ser Gly Val  
50 55 60

Pro Asp Arg Phe Ser Gly Ser Gly Phe Gly Thr Asp Phe Thr Leu Thr  
65 70 75 80

Ile Thr Ser Leu Gln Ala Glu Asp Val Ala Ile Tyr Tyr Cys Gln Gln  
85 90 95

Tyr Phe Ser Pro Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile  
100 105 110

Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp  
115 120 125

Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn  
130 135 140

Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu  
145 150 155 160

Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp  
165 170 175

Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr  
180 185 190

Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser

195

200

205

Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys  
 210 215 220

<210> 122

<211> 445

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 122

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ser Ala Ser Gly Phe Ser Phe Asn Ser Phe  
 20 25 30

Trp Met His Trp Val Arg Gln Val Pro Gly Lys Gly Leu Val Trp Ile  
 35 40 45

Ser Phe Thr Asn Asn Glu Gly Thr Thr Thr Ala Tyr Ala Asp Ser Val  
 50 55 60

Arg Gly Arg Phe Ile Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr  
 65 70 75 80

Leu Glu Met Asn Asn Leu Arg Gly Glu Asp Thr Ala Val Tyr Tyr Cys  
 85 90 95

Ala Arg Gly Glu Gly Gly Leu Asp Asp Trp Gly Gln Gly Thr Leu Val  
 100 105 110

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala  
 115 120 125

Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu  
 130 135 140

Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly  
 145 150 155 160

Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser  
 165 170 175

Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu

180

185

190

Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr

195		200		205
Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr				
210		215		220
Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe				
225		230		235
Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro				
	245		250	255
Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val				
	260		265	270
Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr				
	275		280	285
Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val				
	290		295	300
Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys				
305		310		315
Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser				
	325		330	335
Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro				
	340		345	350
Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val				
	355		360	365
Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly				
	370		375	380
Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp				
385		390		395
Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp				
	405		410	415
Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His				
	420		425	430
Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly				
	435		440	445

&lt;210&gt; 123

&lt;211&gt; 220

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; source

&lt;223&gt; /note="Description of Artificial Sequence: Synthetic polypeptide"



&lt;400&gt; 123

Asp Ile Gln Leu Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly  
 1 5 10 15

Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Ile Phe Arg Thr  
 20 25 30

Ser Arg Asn Lys Asn Leu Leu Asn Trp Tyr Gln Gln Arg Pro Gly Gln  
 35 40 45

Pro Pro Arg Leu Leu Ile His Trp Ala Ser Thr Arg Lys Ser Gly Val  
 50 55 60

Pro Asp Arg Phe Ser Gly Ser Gly Phe Gly Thr Asp Phe Thr Leu Thr  
 65 70 75 80

Ile Thr Ser Leu Gln Ala Glu Asp Val Ala Ile Tyr Tyr Cys Gln Gln  
 85 90 95

Tyr Phe Ser Pro Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile  
 100 105 110

Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp  
 115 120 125

Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn  
 130 135 140

Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu  
 145 150 155 160

Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp  
 165 170 175

Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr  
 180 185 190

Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser  
 195 200 205

Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys  
 210 215 220

&lt;210&gt; 124

&lt;211&gt; 444

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; source

&lt;223&gt; /note="Description of Artificial Sequence: Synthetic polypeptide"

&lt;400&gt; 124

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly

```

1           5           10           15

Ser Leu Arg Leu Ser Cys Ser Ala Ser Gly Phe Ser Phe Asn Ser Phe
      20                25                30

Trp Met His Trp Val Arg Gln Val Pro Gly Lys Gly Leu Val Trp Ile
      35                40                45

Ser Phe Thr Asn Asn Glu Gly Thr Thr Thr Ala Tyr Ala Asp Ser Val
      50                55                60

Arg Gly Arg Phe Ile Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
      65                70                75                80

Leu Glu Met Asn Asn Leu Arg Gly Glu Asp Thr Ala Val Tyr Tyr Cys
      85                90                95

Ala Arg Gly Glu Gly Gly Leu Asp Asp Trp Gly Gln Gly Thr Leu Val
      100                105                110

Thr Val Ser Ser Cys Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala
      115                120                125

Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu
      130                135                140

Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
      145                150                155                160

Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
      165                170                175

Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu
      180                185                190

Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr
      195                200                205

Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr
      210                215                220

Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe
      225                230                235                240

Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
      245                250                255

Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val
      260                265                270

Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
      275                280                285

Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val
      290                295                300

```

Leu Thr Val Leu His Gln Asp Trp Leu Gly Lys Glu Tyr Lys Cys Lys  
305 310 315 320

Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys  
325 330 335

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser  
340 345 350

Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys  
355 360 365

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln  
370 375 380

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly  
385 390 395 400

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln  
405 410 415

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn  
420 425 430

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly  
435 440

<210> 125

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<220>

<221> MOD\_RES

<222> (1)..(1)

<223> Lys(TAMRA)

<220>

<221> MOD\_RES

<222> (10)..(10)

<223> Lys(fluorescein)

<400> 125

Lys Gly Gly Ala Phe Ala Gly Gly Gly Lys  
1 5 10

<210> 126

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 126

Gly Gly Ala Phe Ala Gly Gly Gly  
1 5

<210> 127

<211> 448

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 127

Gln Met Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu  
1 5 10 15

Thr Leu Ser Leu Ser Cys Ser Val Ser Gly Ala Ser Ala Ser Ser Gly  
20 25 30

Tyr Tyr Asn Trp Val Arg Gln Thr Pro Gly Gly Gly Leu Glu Trp Ile  
35 40 45

Ala Tyr Ile Leu Ser Gly Ala His Thr Asp Ile Lys Ala Ser Leu Gly  
50 55 60

Ser Arg Val Ala Val Ser Val Asp Thr Ser Lys Asn Gln Val Thr Leu  
65 70 75 80

Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Thr Tyr Tyr Cys Ala  
85 90 95

Arg Ser Gly Val Tyr Ser Lys Tyr Ser Leu Asp Val Trp Gly Gln Gly  
100 105 110

Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe  
115 120 125

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu  
130 135 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp  
145 150 155 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu  
165 170 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser  
180 185 190

```

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
195                               200                205

Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
210                               215                220

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
225                               230                235                240

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
245                               250                255

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
260                               265                270

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
275                               280                285

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
290                               295                300

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305                               310                315                320

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
325                               330                335

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
340                               345                350

Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr
355                               360                365

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
370                               375                380

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
385                               390                395                400

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
405                               410                415

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
420                               425                430

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
435                               440                445

```

&lt;210&gt; 128

&lt;211&gt; 4

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic peptide"  
  
<400> 128  
Leu Ala Phe Gly  
1  
  
<210> 129  
<211> 6  
<212> PRT  
<213> Artificial Sequence  
  
<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic peptide"  
  
<220>  
<221> MOD\_RES  
<222> (3)..(3)  
<223> Ile(me)  
  
<400> 129  
Gly Pro Ile Leu Phe Phe  
1                      5  
  
<210> 130  
<211> 6  
<212> PRT  
<213> Artificial Sequence  
  
<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic peptide"  
  
<400> 130  
Gly Pro Ile Leu Phe Phe  
1                      5  
  
<210> 131  
<211> 5  
<212> PRT  
<213> Artificial Sequence  
  
<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic peptide"  
  
<400> 131

Gly Gly Ala Phe Ala  
1 5

<210> 132

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<220>

<221> MOD\_RES

<222> (4)..(6)

<223> Any amino acid

<220>

<221> MOD\_RES

<222> (9)..(9)

<223> DPA

<400> 132

Gly Gly Gly Xaa Xaa Xaa Gly Gly Xaa Lys Lys  
1 5 10

<210> 133

<211> 450

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 133

Glu Val Gln Leu Val Gln Ser Gly Gly Asp Leu Val Gln Pro Gly Gly  
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr  
20 25 30

Ala Met Gly Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Leu  
35 40 45

Ser Val Val Thr Gly His Ser Tyr Arg Thr His Tyr Ala Asp Ser Val  
50 55 60

Lys Gly Arg Phe Ile Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Phe  
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Trp Trp Cys

----- 85 ----- 90 ----- 95 -----  
 Ala Lys Arg Ile Trp Ser Tyr Gly Asp Asp Ser Phe Asp Val Trp Gly  
 100 105 110  
 Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser  
 115 120 125  
 Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala  
 130 135 140  
 Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val  
 145 150 155 160  
 Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala  
 165 170 175  
 Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val  
 180 185 190  
 Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His  
 195 200 205  
 Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys  
 210 215 220  
 Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly  
 225 230 235 240  
 Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met  
 245 250 255  
 Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His  
 260 265 270  
 Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val  
 275 280 285  
 His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr  
 290 295 300  
 Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly  
 305 310 315 320  
 Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile  
 325 330 335  
 Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val  
 340 345 350  
 Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser  
 355 360 365  
 Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu  
 370 375 380



Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro  
385 390 395 400

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val  
405 410 415

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met  
420 425 430

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser  
435 440 445

Pro Gly  
450

<210> 134

<211> 450

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 134

Glu Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Gly  
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr  
20 25 30

Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
35 40 45

Ser Tyr Ile Ser Ser Ile Glu Thr Ile Tyr Tyr Ala Asp Ser Val Lys  
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr Leu  
65 70 75 80

Gln Met Asn Ser Leu Arg Asp Glu Asp Thr Ala Val Tyr Tyr Cys Ala  
85 90 95

Arg Asp Arg Leu Val Asp Val Pro Leu Ser Ser Pro Asn Ser Trp Gly  
100 105 110

Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser  
115 120 125

Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala  
130 135 140

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val  
145 150 155 160

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala  
 165 170 175

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val  
 180 185 190

Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His  
 195 200 205

Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys  
 210 215 220

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly  
 225 230 235 240

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met  
 245 250 255

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His  
 260 265 270

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val  
 275 280 285

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr  
 290 295 300

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly  
 305 310 315 320

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile  
 325 330 335

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val  
 340 345 350

Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser  
 355 360 365

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu  
 370 375 380

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro  
 385 390 395 400

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val  
 405 410 415

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met  
 420 425 430

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser  
 435 440 445

Pro Gly  
 450

430

&lt;210&gt; 135

&lt;211&gt; 5

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; source

&lt;223&gt; /note="Description of Artificial Sequence: Synthetic peptide"

&lt;400&gt; 135

Leu	Ala	Phe	Gly	Ala
1				5

&lt;210&gt; 136

&lt;211&gt; 5

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; source

&lt;223&gt; /note="Description of Artificial Sequence: Synthetic peptide"

&lt;400&gt; 136

Leu	Ala	Phe	Ala	Ala
1				5

&lt;210&gt; 137

&lt;211&gt; 446

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; source

&lt;223&gt; /note="Description of Artificial Sequence: Synthetic polypeptide"

&lt;400&gt; 137

Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	Gly
1			5						10					15	

Ser	Leu	Arg	Leu	Ser	Cys	Ser	Ala	Ser	Gly	Phe	Ser	Phe	Asn	Ser	Phe
			20					25					30		

Trp	Met	His	Trp	Val	Arg	Gln	Val	Pro	Gly	Lys	Gly	Leu	Val	Trp	Ile
		35					40					45			

Ser	Phe	Thr	Asn	Asn	Glu	Gly	Thr	Thr	Thr	Ala	Tyr	Ala	Asp	Ser	Val
	50					55					60				

Arg	Gly	Arg	Phe	Ile	Ile	Ser	Arg	Asp	Asn	Ala	Lys	Asn	Thr	Leu	Tyr
65					70					75				80	

Leu Glu Met Asn Asn Leu Arg Gly Glu Asp Thr Ala Val Tyr Tyr Cys  
 85 90 95

Ala Arg Gly Asp Gly Gly Leu Asp Asp Trp Gly Gln Gly Thr Leu Val  
 100 105 110

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala  
 115 120 125

Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu  
 130 135 140

Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly  
 145 150 155 160

Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser  
 165 170 175

Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu  
 180 185 190

Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr  
 195 200 205

Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr  
 210 215 220

Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe  
 225 230 235 240

Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro  
 245 250 255

Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val  
 260 265 270

Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr  
 275 280 285

Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val  
 290 295 300

Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys  
 305 310 315 320

Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser  
 325 330 335

Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro  
 340 345 350

Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val  
 355 360 365

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly  
 370 375 380

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp  
385 390 395 400

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp  
405 410 415

Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His  
420 425 430

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys  
435 440 445

<210> 138

<211> 445

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 138

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
1 5 10 15

Ser Leu Arg Leu Ser Cys Ser Ala Ser Gly Phe Ser Phe Asn Ser Phe  
20 25 30

Trp Met His Trp Val Arg Gln Val Pro Gly Lys Gly Leu Val Trp Ile  
35 40 45

Ser Phe Thr Asn Asn Glu Gly Thr Thr Thr Ala Tyr Ala Asp Ser Val  
50 55 60

Arg Gly Arg Phe Ile Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr  
65 70 75 80

Leu Glu Met Asn Asn Leu Arg Gly Glu Asp Thr Ala Val Tyr Tyr Cys  
85 90 95

Ala Arg Gly Asp Gly Gly Leu Asp Asp Trp Gly Gln Gly Thr Leu Val  
100 105 110

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala  
115 120 125

Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu  
130 135 140

Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly  
145 150 155 160

Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser  
165 170 175

Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu  
 180 185 190  
 Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr  
 195 200 205  
 Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr  
 210 215 220  
 Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe  
 225 230 235 240  
 Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro  
 245 250 255  
 Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val  
 260 265 270  
 Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr  
 275 280 285  
 Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val  
 290 295 300  
 Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys  
 305 310 315 320  
 Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser  
 325 330 335  
 Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro  
 340 345 350  
 Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val  
 355 360 365  
 Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly  
 370 375 380  
 Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp  
 385 390 395 400  
 Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp  
 405 410 415  
 Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His  
 420 425 430  
 Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly  
 435 440 445

&lt;210&gt; 139

&lt;211&gt; 453

&lt;212&gt; PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 139

Glu Met Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala  
1 5 10 15

Ser Val Lys Val Ser Cys Glu Ala Ser Gly Tyr Thr Leu Thr Ser Tyr  
20 25 30

Asp Ile Asn Trp Val Arg Gln Ala Thr Gly Gln Gly Pro Glu Trp Met  
35 40 45

Gly Trp Met Asn Ala Asn Ser Gly Asn Thr Gly Tyr Ala Gln Lys Phe  
50 55 60

Gln Gly Arg Val Thr Leu Thr Gly Asp Thr Ser Ile Ser Thr Ala Tyr  
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys  
85 90 95

Ala Arg Ser Ser Ile Leu Val Arg Gly Ala Leu Gly Arg Tyr Phe Asp  
100 105 110

Leu Trp Gly Arg Gly Thr Leu Val Thr Val Ser Ser Cys Ser Thr Lys  
115 120 125

Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly  
130 135 140

Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro  
145 150 155 160

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr  
165 170 175

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val  
180 185 190

Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn  
195 200 205

Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro  
210 215 220

Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu  
225 230 235 240

Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp  
245 250 255

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp  
260 265 270

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly  
275 280 285

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn  
290 295 300

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp  
305 310 315 320

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro  
325 330 335

Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu  
340 345 350

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn  
355 360 365

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile  
370 375 380

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr  
385 390 395 400

Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys  
405 410 415

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys  
420 425 430

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu  
435 440 445

Ser Leu Ser Pro Gly  
450

<210> 140

<211> 453

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 140

Glu Met Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala  
1 5 10 15

Ser Val Lys Val Ser Cys Glu Ala Ser Gly Tyr Thr Leu Thr Ser Tyr  
20 25 30



Asp Ile Asn Trp Val Arg Gln Ala Thr Gly Gln Gly Pro Glu Trp Met  
 35 40 45

Gly Trp Met Asn Ala Asn Ser Gly Asn Thr Gly Tyr Ala Gln Lys Phe  
 50 55 60

Gln Gly Arg Val Thr Leu Thr Gly Asp Thr Ser Ile Ser Thr Ala Tyr  
 65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys  
 85 90 95

Ala Arg Ser Ser Ile Leu Val Arg Gly Ala Leu Gly Arg Tyr Phe Asp  
 100 105 110

Leu Trp Gly Arg Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys  
 115 120 125

Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly  
 130 135 140

Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro  
 145 150 155 160

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr  
 165 170 175

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val  
 180 185 190

Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn  
 195 200 205

Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro  
 210 215 220

Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu  
 225 230 235 240

Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp  
 245 250 255

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp  
 260 265 270

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly  
 275 280 285

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn  
 290 295 300

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp  
 305 310 315 320

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro  
 325 330 335

Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu  
340 345 350

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn  
355 360 365

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile  
370 375 380

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr  
385 390 395 400

Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys  
405 410 415

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys  
420 425 430

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu  
435 440 445

Ser Leu Ser Pro Gly  
450

<210> 141

<211> 453

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 141

Glu Ile Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala  
1 5 10 15

Ser Val Lys Val Ser Cys Glu Ala Ser Gly Tyr Thr Leu Thr Ser Tyr  
20 25 30

Asp Ile Asn Trp Val Arg Gln Ala Thr Gly Gln Gly Pro Glu Trp Met  
35 40 45

Gly Trp Met Asn Ala Asn Ser Gly Asn Thr Gly Tyr Ala Gln Lys Phe  
50 55 60

Gln Gly Arg Val Thr Leu Thr Gly Asp Thr Ser Ile Ser Thr Ala Tyr  
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys  
85 90 95

Ala Arg Ser Ser Ile Leu Val Arg Gly Ala Leu Gly Arg Tyr Phe Asp  
100 105 110

Leu Trp Gly Arg Gly Thr Leu Val Thr Val Ser Ser Cys Ser Thr Lys  
 115 120 125

Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly  
 130 135 140

Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro  
 145 150 155 160

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr  
 165 170 175

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val  
 180 185 190

Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn  
 195 200 205

Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro  
 210 215 220

Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu  
 225 230 235 240

Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp  
 245 250 255

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp  
 260 265 270

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly  
 275 280 285

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn  
 290 295 300

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp  
 305 310 315 320

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro  
 325 330 335

Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu  
 340 345 350

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn  
 355 360 365

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile  
 370 375 380

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr  
 385 390 395 400

Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys  
 405 410 415

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys  
 420 425 430

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu  
 435 440 445

Ser Leu Ser Pro Gly  
 450

<210> 142

<211> 453

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 142

Glu Ile Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala  
 1 5 10 15

Ser Val Lys Val Ser Cys Glu Ala Ser Gly Tyr Thr Leu Thr Ser Tyr  
 20 25 30

Asp Ile Asn Trp Val Arg Gln Ala Thr Gly Gln Gly Pro Glu Trp Met  
 35 40 45

Gly Trp Met Asn Ala Asn Ser Gly Asn Thr Gly Tyr Ala Gln Lys Phe  
 50 55 60

Gln Gly Arg Val Thr Leu Thr Gly Asp Thr Ser Ile Ser Thr Ala Tyr  
 65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys  
 85 90 95

Ala Arg Ser Ser Ile Leu Val Arg Gly Ala Leu Gly Arg Tyr Phe Asp  
 100 105 110

Leu Trp Gly Arg Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys  
 115 120 125

Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly  
 130 135 140

Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro  
 145 150 155 160

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr  
 165 170 175

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val  
 180 185 190

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Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn
  195                      200                      205

Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro
  210                      215                      220

Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu
  225                      230                      235                      240

Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
                245                      250                      255

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
                260                      265                      270

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
                275                      280                      285

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
  290                      295                      300

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
305                      310                      315                      320

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro
                325                      330                      335

Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu
                340                      345                      350

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn
  355                      360                      365

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
  370                      375                      380

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
  385                      390                      395                      400

Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
                405                      410                      415

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
                420                      425                      430

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
  435                      440                      445

Ser Leu Ser Pro Gly
  450

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&lt;210&gt; 143

&lt;211&gt; 453

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; source

&lt;223&gt; /note="Description of Artificial Sequence: Synthetic polypeptide"

&lt;400&gt; 143

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala  
 1 5 10 15

Ser Val Lys Val Ser Cys Glu Ala Ser Gly Tyr Thr Leu Thr Ser Tyr  
 20 25 30

Asp Ile Asn Trp Val Arg Gln Ala Thr Gly Gln Gly Pro Glu Trp Met  
 35 40 45

Gly Trp Met Asn Ala Asn Ser Gly Asn Thr Gly Tyr Ala Gln Lys Phe  
 50 55 60

Gln Gly Arg Val Thr Leu Thr Gly Asp Thr Ser Ile Ser Thr Ala Tyr  
 65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys  
 85 90 95

Ala Arg Ser Ser Ile Leu Val Arg Gly Ala Leu Gly Arg Tyr Phe Asp  
 100 105 110

Leu Trp Gly Arg Gly Thr Leu Val Thr Val Ser Ser Cys Ser Thr Lys  
 115 120 125

Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly  
 130 135 140

Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro  
 145 150 155 160

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr  
 165 170 175

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val  
 180 185 190

Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn  
 195 200 205

Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro  
 210 215 220

Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu  
 225 230 235 240

Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp  
245 250 255

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp  
260 265 270

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly  
275 280 285

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn  
290 295 300

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp  
305 310 315 320

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro  
325 330 335

Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu  
340 345 350

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn  
355 360 365

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile  
370 375 380

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr  
385 390 395 400

Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys  
405 410 415

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys  
420 425 430

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu  
435 440 445

Ser Leu Ser Pro Gly  
450

<210> 144

<211> 453

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 144

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala  
1 5 10 15

Ser Val Lys Val Ser Cys Glu Ala Ser Gly Tyr Thr Leu Thr Ser Tyr  
20 25 30

Asp Ile Asn Trp Val Arg Gln Ala Thr Gly Gln Gly Pro Glu Trp Met  
35 40 45

Gly Trp Met Asn Ala Asn Ser Gly Asn Thr Gly Tyr Ala Gln Lys Phe  
50 55 60

Gln Gly Arg Val Thr Leu Thr Gly Asp Thr Ser Ile Ser Thr Ala Tyr  
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys  
85 90 95

Ala Arg Ser Ser Ile Leu Val Arg Gly Ala Leu Gly Arg Tyr Phe Asp  
100 105 110

Leu Trp Gly Arg Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys  
115 120 125

Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly  
130 135 140

Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro  
145 150 155 160

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr  
165 170 175

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val  
180 185 190

Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn  
195 200 205

Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro  
210 215 220

Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu  
225 230 235 240

Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp  
245 250 255

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp  
260 265 270

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly  
275 280 285

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn  
290 295 300

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp  
305 310 315 320

Leu Asn Gly Lys Glu Thr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro



Asn Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Asn Glu  
325 330 335

Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu  
340 345 350

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn  
355 360 365

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile  
370 375 380

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr  
385 390 395 400

Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys  
405 410 415

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys  
420 425 430

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu  
435 440 445

Ser Leu Ser Pro Gly  
450

<210> 145

<211> 220

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 145

Asp Ile Gln Leu Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly  
1 5 10 15

Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Ile Phe Arg Thr  
20 25 30

Ser Arg Asn Lys Asn Leu Leu Asn Trp Tyr Gln Gln Arg Pro Gly Gln  
35 40 45

Pro Pro Arg Leu Leu Ile His Trp Ala Ser Thr Arg Lys Ser Gly Val  
50 55 60

Pro Asp Arg Phe Ser Gly Ser Gly Phe Gly Thr Asp Phe Thr Leu Thr  
65 70 75 80

Ile Thr Ser Leu Gln Ala Glu Asp Val Ala Ile Tyr Tyr Cys Gln Gln  
85 90 95

Tyr Phe Ser Pro Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile  
 100 105 110

Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp  
 115 120 125

Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn  
 130 135 140

Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu  
 145 150 155 160

Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp  
 165 170 175

Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr  
 180 185 190

Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser  
 195 200 205

Ser Pro Cys Thr Lys Ser Phe Asn Arg Gly Glu Cys  
 210 215 220

<210> 146

<211> 445

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 146

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ser Ala Ser Gly Phe Ser Phe Asn Ser Phe  
 20 25 30

Trp Met His Trp Val Arg Gln Val Pro Gly Lys Gly Leu Val Trp Ile  
 35 40 45

Ser Phe Thr Asn Asn Glu Gly Thr Thr Thr Ala Tyr Ala Asp Ser Val  
 50 55 60

Arg Gly Arg Phe Ile Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr  
 65 70 75 80

Leu Glu Met Asn Asn Leu Arg Gly Glu Asp Thr Ala Val Tyr Tyr Cys  
 85 90 95

Ala Arg Gly Asp Gly Gly Leu Asp Asp Trp Gly Gln Gly Thr Leu Val  
 100 105 110

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala  
 115 120 125  
 Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu  
 130 135 140  
 Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly  
 145 150 155 160  
 Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser  
 165 170 175  
 Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu  
 180 185 190  
 Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr  
 195 200 205  
 Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr  
 210 215 220  
 Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe  
 225 230 235 240  
 Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro  
 245 250 255  
 Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val  
 260 265 270  
 Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr  
 275 280 285  
 Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val  
 290 295 300  
 Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys  
 305 310 315 320  
 Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser  
 325 330 335  
 Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro  
 340 345 350  
 Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val  
 355 360 365  
 Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly  
 370 375 380  
 Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp  
 385 390 395 400  
 Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp  
 405 410 415

Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His  
 420 425 430

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly  
 435 440 445

<210> 147

<211> 445

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 147

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ser Ala Ser Gly Phe Ser Phe Asn Ser Phe  
 20 25 30

Trp Met His Trp Val Arg Gln Val Pro Gly Lys Gly Leu Val Trp Ile  
 35 40 45

Ser Phe Thr Asn Asn Glu Gly Thr Thr Thr Ala Tyr Ala Asp Ser Val  
 50 55 60

Arg Gly Arg Phe Ile Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr  
 65 70 75 80

Leu Glu Met Asn Asn Leu Arg Gly Glu Asp Thr Ala Val Tyr Tyr Cys  
 85 90 95

Ala Arg Gly Glu Gly Gly Leu Asp Asp Trp Gly Gln Gly Thr Leu Val  
 100 105 110

Thr Val Ser Ser Cys Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala  
 115 120 125

Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu  
 130 135 140

Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly  
 145 150 155 160

Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser  
 165 170 175

Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu  
 180 185 190

Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr  
 195 200 205

Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr  
 210 215 220  
 Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe  
 225 230 235 240  
 Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro  
 245 250 255  
 Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val  
 260 265 270  
 Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr  
 275 280 285  
 Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val  
 290 295 300  
 Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys  
 305 310 315 320  
 Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser  
 325 330 335  
 Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro  
 340 345 350  
 Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val  
 355 360 365  
 Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly  
 370 375 380  
 Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp  
 385 390 395 400  
 Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp  
 405 410 415  
 Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His  
 420 425 430  
 Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly  
 435 440 445  
 <210> 148  
 <211> 330  
 <212> PRT  
 <213> Homo sapiens  
 <400> 148  
 Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys  
 1 5 10 15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr  
 20 25 30  
 Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser  
 35 40 45  
 Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser  
 50 55 60  
 Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr  
 65 70 75 80  
 Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys  
 85 90 95  
 Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys  
 100 105 110  
 Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro  
 115 120 125  
 Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys  
 130 135 140  
 Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp  
 145 150 155 160  
 Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu  
 165 170 175  
 Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu  
 180 185 190  
 His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn  
 195 200 205  
 Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly  
 210 215 220  
 Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu  
 225 230 235 240  
 Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr  
 245 250 255  
 Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn  
 260 265 270  
 Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe  
 275 280 285  
 Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn  
 290 295 300  
 Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr  
 305 310 315 320

Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys

325

330

<210> 149

<211> 330

<212> PRT

<213> Homo sapiens

<400> 149

Cys Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys  
1 5 10 15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr  
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser  
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser  
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr  
65 70 75 80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys  
85 90 95

Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys  
100 105 110

Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro  
115 120 125

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys  
130 135 140

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp  
145 150 155 160

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu  
165 170 175

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu  
180 185 190

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn  
195 200 205

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly  
210 215 220

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu  
225 230 235 240

Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr  
245 250 255

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn  
260 265 270

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe  
275 280 285

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn  
290 295 300

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr  
305 310 315 320

Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys  
325 330

<210> 150

<211> 107

<212> PRT

<213> Homo sapiens

<400> 150

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu  
1 5 10 15

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe  
20 25 30

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln  
35 40 45

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser  
50 55 60

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu  
65 70 75 80

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser  
85 90 95

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys  
100 105

<210> 151

<211> 107

<212> PRT

<213> Homo sapiens

<400> 151

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu  
1 5 10 15



Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe  
                   20                                  25                                  30

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln  
                   35                                  40                                  45

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser  
           50                                  55                                  60

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu  
   65                                  70                                  75                                  80

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser  
                   85                                  90                                  95

Pro Cys Thr Lys Ser Phe Asn Arg Gly Glu Cys  
                   100                                  105

<210> 152

<211> 42

<212> DNA

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 152

cccagactgc accagctgga tctctgaatg tactccagtt gc           42

<210> 153

<211> 41

<212> DNA

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 153

ccagactgca ccagctgcac ctctgaatgt actccagttg c           41

<210> 154

<211> 61

<212> DNA

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

&lt;400&gt; 154

ccagggttcc ctggccccaw tmgtcaagtc cascwkcacc tcttgacacag taatagacag 60  
c 61

&lt;210&gt; 155

&lt;211&gt; 52

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; source

&lt;223&gt; /note="Description of Artificial Sequence: Synthetic oligonucleotide"

&lt;400&gt; 155

cctggcccca gtcgtcaagt cctccttcac ctctgcaca gtaatagaca gc 52

&lt;210&gt; 156

&lt;211&gt; 116

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; source

&lt;223&gt; /note="Description of Artificial Sequence: Synthetic polypeptide"

&lt;400&gt; 156

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly 1  
1 5 10 15

Ser Leu Arg Leu Ser Cys Ser Ala Ser Gly Phe Ser Phe Asn Ser Phe 20  
20 25 30

Trp Met His Trp Val Arg Gln Val Pro Gly Lys Gly Leu Val Trp Ile 35  
35 40 45

Ser Phe Thr Asn Asn Glu Gly Thr Thr Thr Ala Tyr Ala Asp Ser Val 50  
50 55 60

Arg Gly Arg Phe Ile Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr 65  
65 70 75 80

Leu Glu Met Asn Asn Leu Arg Gly Glu Asp Thr Ala Val Tyr Tyr Cys 85  
85 90 95

Ala Arg Gly Glu Gly Gly Leu Asp Asp Trp Gly Gln Gly Thr Leu Val 100  
100 105 110

Thr Val Ser Ser 115  
115

&lt;210&gt; 157

&lt;211&gt; 445

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; source

&lt;223&gt; /note="Description of Artificial Sequence: Synthetic polypeptide"

&lt;400&gt; 157

Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	Gly
1			5					10					15		

Ser	Leu	Arg	Leu	Ser	Cys	Ser	Ala	Ser	Gly	Phe	Ser	Phe	Asn	Ser	Phe
		20						25					30		

Trp	Met	His	Trp	Val	Arg	Gln	Val	Pro	Gly	Lys	Gly	Leu	Val	Trp	Ile
		35					40					45			

Ser	Phe	Thr	Asn	Asn	Glu	Gly	Thr	Thr	Thr	Ala	Tyr	Ala	Asp	Ser	Val
	50					55					60				

Arg	Gly	Arg	Phe	Ile	Ile	Ser	Arg	Asp	Asn	Ala	Lys	Asn	Thr	Leu	Tyr
65					70					75					80

Leu	Glu	Met	Asn	Asn	Leu	Arg	Gly	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys
			85						90					95	

Ala	Arg	Gly	Glu	Gly	Gly	Leu	Asp	Asp	Trp	Gly	Gln	Gly	Thr	Leu	Val
		100						105					110		

Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala
	115						120					125			

Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu	Gly	Cys	Leu
	130						135				140				

Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn	Ser	Gly
145					150					155					160

Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser
				165					170					175	

Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	Ser	Leu
		180						185					190		

Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser	Asn	Thr
		195					200					205			

Lys	Val	Asp	Lys	Lys	Val	Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr
	210					215					220				

Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe
225					230					235					240

Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

245 250 255

Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val  
260 265 270

Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr  
275 280 285

Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val  
290 295 300

Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys  
305 310 315 320

Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser  
325 330 335

Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro  
340 345 350

Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val  
355 360 365

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly  
370 375 380

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp  
385 390 395 400

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp  
405 410 415

Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His

420

425

430

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly  
435 440 445

<210> 158

<211> 214

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 158

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ile Leu Ser Ala Ser Val Gly  
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Thr Ile Ser Gly Trp  
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Ala Glu Ala Pro Lys Leu Leu Ile  
 35 40 45

Tyr Lys Ala Ser Thr Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly  
 50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro  
 65 70 75 80

Asp Asp Phe Gly Ile Tyr Tyr Cys Gln Gln Tyr Lys Ser Tyr Ser Phe  
 85 90 95

Asn Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala  
 100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly  
 115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala  
 130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln  
 145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser  
 165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr  
 180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser  
 195 200 205

Phe Asn Arg Gly Glu Cys  
 210

<210> 159

<211> 215

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 159

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly  
 1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Phe Val Ser Arg Thr  
 20 25 30

Ser Leu Ala Trp Phe Gln Gln Lys Pro Gly Gln Pro Pro Arg Leu Leu  
 35 40 45

Ile Tyr Glu Thr Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser  
 50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu  
 65 70 75 80

Pro Glu Asp Phe Ala Met Tyr Tyr Cys His Lys Tyr Gly Ser Gly Pro  
 85 90 95

Arg Thr Phe Gly Gln Gly Thr Lys Val Glu Val Lys Arg Thr Val Ala  
 100 105 110

Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser  
 115 120 125

Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu  
 130 135 140

Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser  
 145 150 155 160

Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu  
 165 170 175

Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val  
 180 185 190

Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys  
 195 200 205

Ser Phe Asn Arg Gly Glu Cys  
 210 215

<210> 160

<211> 215

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 160

Glu Thr Thr Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly  
 1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser  
 20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Lys Val Leu

35                                      40                                      45  
 Ile Tyr Asp Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser  
     50                                      55                                      60  
 Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu  
     65                                      70                                      75                                      80  
 Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Lys Tyr Gly Ser Thr Pro  
                                     85                                      90                                      95  
 Arg Pro Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala  
                                     100                                      105                                      110  
 Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser  
                                     115                                      120                                      125  
 Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu  
                                     130                                      135                                      140  
 Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser  
     145                                      150                                      155                                      160  
 Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu  
                                     165                                      170                                      175  
 Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val  
                                     180                                      185                                      190  
 Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys  
                                     195                                      200                                      205  
 Ser Phe Asn Arg Gly Glu Cys  
     210                                      215

&lt;210&gt; 161

&lt;211&gt; 214

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; source

&lt;223&gt; /note="Description of Artificial Sequence: Synthetic polypeptide"

&lt;400&gt; 161

Asp Val Val Met Thr Gln Ser Ser Ser Ser Leu Ser Ala Ser Val Gly  
     1                                      5                                      10                                      15  
 Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Leu Asp Ile Thr Asn His  
                                     20                                      25                                      30  
 Leu Ala Trp Tyr Gln Gln Lys Pro Gly Glu Leu Pro Lys Leu Leu Ile  
                                     35                                      40                                      45

Tyr Glu Ala Ser Ile Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly  
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro  
65 70 75 80

Glu Asp Val Ala Thr Tyr Tyr Cys Glu Lys Cys Asn Ser Thr Pro Arg  
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala  
100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly  
115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala  
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln  
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser  
165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr  
180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser  
195 200 205

Phe Asn Arg Gly Glu Cys  
210

<210> 162

<211> 216

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 162

Glu Ile Val Met Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly  
1 5 10 15

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

Tyr Leu Ala Trp Tyr Gln Gln Glu Pro Gly Arg Ala Pro Thr Leu Leu  
35 40 45



Phe Tyr Gly Val Ser Asn Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser  
50 55 60

Cys Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu  
65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Leu Tyr Thr Ser Ser Arg  
85 90 95

Ala Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val  
100 105 110

Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys

115

120

125

Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg  
130 135 140

Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn  
145 150 155 160

Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser  
165 170 175

Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys  
180 185 190

Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr  
195 200 205

Lys Ser Phe Asn Arg Gly Glu Cys  
210 215

&lt;210&gt; 163

&lt;211&gt; 214

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; source

&lt;223&gt; /note="Description of Artificial Sequence: Synthetic polypeptide"

&lt;400&gt; 163

Glu Ile Val Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly  
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Gly  
20 25 30

Leu Gly Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro Lys Leu Leu Ile  
35 40 45

Tyr Pro Ala Ser Thr Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly

50                                      55                                      60  
 Ser Gly Ser Asp Arg Asp Phe Thr Leu Thr Ile Thr Ser Leu Gln Pro  
 65                                      70                                      75                                      80  
 Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Asp His Asn Tyr Pro Pro  
                                     85                                      90                                      95  
 Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala  
                                     100                                      105                                      110  
 Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly  
                                     115                                      120                                      125  
 Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala  
                                     130                                      135                                      140  
 Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln  
 145                                      150                                      155                                      160  
 Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser  
                                     165                                      170                                      175  
 Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr  
                                     180                                      185                                      190  
 Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser  
                                     195                                      200                                      205  
 Phe Asn Arg Gly Glu Cys  
 210

<210> 164

<211> 214

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 164

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly  
 1                                      5                                      10                                      15  
 Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Gly  
                                     20                                      25                                      30  
 Leu Gly Trp Tyr Gln Gln Ile Pro Gly Lys Ala Pro Lys Leu Leu Ile  
                                     35                                      40                                      45  
 Tyr Pro Ala Ser Thr Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly  
 50                                      55                                      60

Ser Gly Ser Asp Arg Asp Phe Thr Leu Thr Ile Thr Ser Leu Gln Pro  
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Asp His Asn Tyr Pro Pro  
85 90 95

Ser Phe Ser Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala  
100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly  
115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala  
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln  
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser  
165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr  
180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser  
195 200 205

Phe Asn Arg Gly Glu Cys  
210

<210> 165

<211> 214

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 165

Asp Ile Gln Met Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly  
1 5 10 15

Glu Thr Val Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Arg Thr Asn  
20 25 30

Val Ala Trp Tyr Arg His Lys Ala Gly Gln Ala Pro Met Ile Leu Val  
35 40 45

Ser Gly Ala Ser Thr Arg Ala Ser Gly Ala Pro Ala Arg Phe Ser Gly  
50 55 60

Ser Gly Tyr Gly Thr Glu Phe Thr Leu Thr Ile Thr Ser Leu Gln Ser

65                      70                      75                      80

Glu Asp Phe Ala Val Tyr Tyr Cys Leu Gln Tyr Asn Thr Trp Pro Arg  
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Val Lys Arg Thr Val Ala Ala  
100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly  
115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala  
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln  
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser  
165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr  
180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser  
195 200 205

Phe Asn Arg Gly Glu Cys  
210

<210> 166

<211> 214

<212> PRT

### <213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 166

Asp Val Val Met Thr Gln Ser Pro Ser Phe Leu Ser Ala Ser Val Gly  
1 5 10 15

Asp Arg Val Thr Leu Thr Cys Arg Ala Ser Gln Asp Ile Gly Ser Ser  
20 25 30

Leu Ala Trp Tyr Gln Gln Arg Pro Gly Lys Ala Pro Asn Leu Leu Ile  
35 40 45

Tyr Ala Thr Ser Thr Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly

50

55

60

Ser Gly Phe Gly Thr Glu Phe Thr Leu Thr Ile Ser Thr Leu Gln Pro  
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Leu Asn Asn Tyr Val His  
85 90 95

Ser Phe Gly Pro Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala  
100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly  
115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala  
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln  
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser  
165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr  
180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser  
195 200 205

Phe Asn Arg Gly Glu Cys  
210

<210> 167

<211> 213

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 167

Glu Thr Thr Leu Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Gly Asp Arg  
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Val Leu Ile

35

40

45

Tyr Trp Ala Ser Asn Leu Glu Gly Gly Val Pro Ser Arg Phe Ser Gly  
50 55 60

Thr Gly Ser Gly Thr Gly Phe Ala Leu Thr Ile Ser Gly Leu Gln Pro

Thr Gly Ser Gly Thr Glu Phe Ala Leu Thr Ile Ser Gly Leu Gln Phe  
 65 70 75 80

Asp Asp Leu Ala Thr Tyr Tyr Cys Gln Gln Tyr Lys Ser Gln Trp Ser  
 85 90 95

Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro  
 100 105 110

Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr  
 115 120 125

Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys  
 130 135 140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu  
 145 150 155 160

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser  
 165 170 175

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala  
 180 185 190

Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe  
 195 200 205

Asn Arg Gly Glu Cys  
 210

<210> 168

<211> 214

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 168

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly  
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Phe Thr Asn His Tyr  
 20 25 30

Leu Asn Trp Tyr Gln His Lys Pro Gly Arg Ala Pro Lys Leu Met Ile  
 35 40 45

Ser Val Ala Ser Asn Leu Gln Ser Gly Val Pro Ser Arg Phe Thr Gly  
 50 55 60

Ser Glu Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Gly Leu Gln Pro  
 65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Arg Thr Pro Tyr  
85 90 95

Thr Phe Gly Gln Gly Ser Arg Leu Glu Met Lys Arg Thr Val Ala Ala  
100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly  
115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala  
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln  
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser  
165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr  
180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser  
195 200 205

Phe Asn Arg Gly Glu Cys  
210

<210> 169

<211> 453

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 169

Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala

1 5 10 15

Ser Val Lys Val Ser Cys Glu Ala Ser Gly Tyr Thr Leu Thr Ser Tyr  
20 25 30

Asp Ile Asn Trp Val Arg Gln Ala Thr Gly Gln Gly Pro Glu Trp Met  
35 40 45

Gly Trp Met Asn Ala Asn Ser Gly Asn Thr Gly Tyr Ala Gln Lys Phe  
50 55 60

Gln Gly Arg Val Thr Leu Thr Gly Asp Thr Ser Ile Ser Thr Ala Tyr  
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys  
 85 90 95

Ala Gly Ser Ser Ile Leu Val Arg Gly Ala Leu Gly Arg Tyr Phe Asp  
 100 105 110

Leu Trp Gly Arg Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys  
 115 120 125

Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly  
 130 135 140

Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro  
 145 150 155 160

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr  
 165 170 175

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val  
 180 185 190

Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn  
 195 200 205

Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro  
 210 215 220

Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu  
 225 230 235 240

Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp  
 245 250 255

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp  
 260 265 270

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly  
 275 280 285

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn  
 290 295 300

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp  
 305 310 315 320

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro  
 325 330 335

Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu  
 340 345 350

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn  
 355 360 365

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile  
 370 375 380



Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr  
385 390 395 400

Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys  
405 410 415

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys  
420 425 430

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu  
435 440 445

Ser Leu Ser Pro Gly  
450

<210> 170

<211> 448

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 170

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Val Lys Pro Gly Ala  
1 5 10 15

Ser Leu Lys Val Ser Cys Lys Ala Ser Gly Tyr Ile Ile Ile Asn Tyr  
20 25 30

Asp Phe Ile Trp Val Arg Gln Ala Thr Gly Gln Gly Pro Glu Trp Met  
35 40 45

Gly Trp Met Asn Pro Asn Ser Tyr Asn Thr Gly Tyr Gly Gln Lys Phe  
50 55 60

Gln Gly Arg Val Thr Met Thr Trp Asp Ser Ser Met Ser Thr Ala Tyr  
65 70 75 80

Met Glu Leu Ser Ser Leu Thr Ser Ala Asp Thr Ala Val Tyr Tyr Cys  
85 90 95

Ala Arg Ala Val Arg Gly Gln Leu Leu Ser Glu Tyr Trp Gly Gln Gly  
100 105 110

Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe  
115 120 125

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu  
130 135 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp  
145 150 155 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu  
 165 170 175  
 Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser  
 180 185 190  
 Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro  
 195 200 205  
 Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys  
 210 215 220  
 Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro  
 225 230 235 240  
 Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser  
 245 250 255  
 Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp  
 260 265 270  
 Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn  
 275 280 285  
 Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val  
 290 295 300  
 Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu  
 305 310 315 320  
 Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys  
 325 330 335  
 Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr  
 340 345 350  
 Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr  
 355 360 365  
 Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu  
 370 375 380  
 Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu  
 385 390 395 400  
 Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys  
 405 410 415  
 Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu  
 420 425 430  
 Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly  
 435 440 445

&lt;210&gt; 171

&lt;211&gt; 455

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; source

&lt;223&gt; /note="Description of Artificial Sequence: Synthetic polypeptide"

&lt;400&gt; 171

Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala  
 1 5 10 15

Ser Val Lys Val Ser Cys Arg Ala Ser Gly Tyr Thr Phe Thr Ser Tyr  
 20 25 30

Asp Ile Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met  
 35 40 45

Gly Trp Met Asn Pro Asn Ser Gly Asn Thr Asn Tyr Ala Gln Arg Phe  
 50 55 60

Gln Gly Arg Leu Thr Met Thr Lys Asn Thr Ser Ile Asn Thr Ala Tyr  
 65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys  
 85 90 95

Ala Thr Glu Arg Trp Ser Lys Asp Thr Gly His Tyr Tyr Tyr Tyr Gly  
 100 105 110

Met Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser  
 115 120 125

Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr  
 130 135 140

Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro  
 145 150 155 160

Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val  
 165 170 175

His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser  
 180 185 190

Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile  
 195 200 205

Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val  
 210 215 220

Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala  
 225 230 235 240

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro  
 245 250 255

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val  
 260 265 270  
 Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val  
 275 280 285  
 Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln  
 290 295 300  
 Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln  
 305 310 315 320  
 Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala  
 325 330 335  
 Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro  
 340 345 350  
 Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr  
 355 360 365  
 Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser  
 370 375 380  
 Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr  
 385 390 395 400  
 Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr  
 405 410 415  
 Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe  
 420 425 430  
 Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys  
 435 440 445  
 Ser Leu Ser Leu Ser Pro Gly  
 450 455

<210> 172

<211> 456

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 172

Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Val Lys Arg Pro Gly Ala  
 1 5 10 15  
 Ser Val Lys Val Ser Cys Glu Ala Ser Gly Tyr Thr Val Ser Asn Tyr  
 20 25 30

Asp Ile Asn Trp Val Arg Gln Ala Thr Gly Gln Gly Leu Glu Trp Met  
 35 40 45

Gly Trp Met Asn Pro Ser Ser Gly Arg Thr Gly Tyr Ala Pro Lys Phe  
 50 55 60

Arg Gly Arg Val Thr Met Thr Arg Ser Thr Ser Ile Ser Thr Ala Tyr  
 65 70 75 80

Met Glu Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Val Tyr Tyr Cys  
 85 90 95

Ala Arg Gly Gly Gly Tyr Tyr Asp Ser Ser Gly Asn Tyr His Ile Ser  
 100 105 110

Gly Leu Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala  
 115 120 125

Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser  
 130 135 140

Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe  
 145 150 155 160

Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly  
 165 170 175

Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu  
 180 185 190

Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr  
 195 200 205

Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys  
 210 215 220

Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro  
 225 230 235 240

Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys  
 245 250 255

Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val  
 260 265 270

Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr  
 275 280 285

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu  
 290 295 300

Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His  
 305 310 315 320

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys  
                           325                          330                          335

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln  
                           340                          345                          350

Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met  
                           355                          360                          365

Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro  
                           370                          375                          380

Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn  
                           385                          390                          395                          400

Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu  
                           405                          410                          415

Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val  
                           420                          425                          430

Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln  
                           435                          440                          445

Lys Ser Leu Ser Leu Ser Pro Gly  
                           450                          455

<210> 173

<211> 448

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 173

Gln Ile Thr Leu Lys Glu Ser Gly Gly Gly Leu Ile Lys Pro Gly Gly

1                          5                          10                          15

Ser Leu Arg Leu Ser Cys Ala Thr Ser Gly Phe Pro Phe Ser Ala Tyr  
                           20                          25                          30

Ala Met Asn Trp Val Arg Gln Ala Pro Gly Arg Gly Leu Glu Trp Val  
                           35                          40                          45

Ser Ser Ile Thr Lys Asn Ser Asp Ser Leu Tyr Tyr Ala Asp Ser Val  
                           50                          55                          60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Gly Asn Ser Leu Tyr  
                           65                          70                          75                          80

Leu Gln Met Asn Ser Leu Arg Val Glu Asp Thr Ala Val Tyr Tyr Cys

														85															90															95														
Ala	Thr	Leu	Ala 100		Ala	Arg	Ile	Met	Ala 105		Thr	Asp	Tyr	Trp	Gly 110	Gln	Gly																																									
Thr	Leu	Val 115	Thr	Val	Ser	Ser	Ala 120		Ser	Thr	Lys	Gly	Pro 125		Ser	Val	Phe																																									
Pro	Leu 130	Ala	Pro	Ser	Ser	Lys 135		Ser	Thr	Ser	Gly	Gly 140	Thr	Ala	Ala	Leu																																										
Gly 145	Cys	Leu	Val	Lys	Asp 150	Tyr	Phe	Pro	Glu	Pro 155		Val	Thr	Val	Ser	Trp																																										
Asn	Ser	Gly	Ala	Leu 165	Thr	Ser	Gly	Val	His 170	Thr	Phe	Pro	Ala	Val	Leu																																											
Gln	Ser	Ser	Gly 180	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser																																											
Ser	Ser	Leu 195	Gly	Thr	Gln	Thr	Tyr 200	Ile	Cys	Asn	Val	Asn 205	His	Lys	Pro																																											
Ser	Asn 210	Thr	Lys	Val	Asp	Lys 215	Lys	Val	Glu	Pro	Lys 220	Ser	Cys	Asp	Lys																																											
Thr 225	His	Thr	Cys	Pro	Pro 230	Cys	Pro	Ala	Pro	Glu 235	Leu	Leu	Gly	Gly	Pro 240																																											
Ser	Val	Phe	Leu	Phe 245	Pro	Pro	Lys	Pro	Lys 250	Asp	Thr	Leu	Met	Ile 255	Ser																																											
Arg	Thr	Pro	Glu 260	Val	Thr	Cys	Val	Val 265	Val	Asp	Val	Ser	His	Glu	Asp																																											
Pro	Glu 275	Val	Lys	Phe	Asn	Trp	Tyr 280	Val	Asp	Gly	Val	Glu 285	Val	His	Asn																																											
Ala	Lys 290	Thr	Lys	Pro	Arg	Glu 295	Glu	Gln	Tyr	Asn	Ser 300	Thr	Tyr	Arg	Val																																											
Val 305	Ser	Val	Leu	Thr	Val 310	Leu	His	Gln	Asp	Trp 315	Leu	Asn	Gly	Lys	Glu 320																																											
Tyr	Lys	Cys	Lys	Val 325	Ser	Asn	Lys	Ala	Leu	Pro 330	Ala	Pro	Ile	Glu 335	Lys																																											
Thr	Ile	Ser	Lys 340	Ala	Lys	Gly	Gln	Pro 345	Arg	Glu	Pro	Gln	Val	Tyr	Thr																																											
Leu	Pro	Pro 355	Ser	Arg	Glu	Glu	Met 360	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr																																											
Cys	Leu 370	Val	Lys	Gly	Phe	Tyr 375	Pro	Ser	Asp	Ile	Ala 380	Val	Glu	Trp	Glu																																											

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu  
385 390 395 400

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys  
405 410 415

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu  
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Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly  
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<211> 456

<212> PRT

<213> Artificial Sequence

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<221> source

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Ser Met Ile Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
35 40 45

Ser Ser Ile Asp Ser Ser Ser Arg Tyr Leu Tyr Tyr Ala Asp Ser Val  
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Gln Asn Ser Leu Tyr  
65 70 75 80

Leu Gln Met Ser Gly Leu Arg Val Glu Asp Thr Ala Val Tyr Tyr Cys  
85 90 95

Ala Arg Asp Gly Asp Asp Ile Leu Ser Val Tyr Arg Gly Ser Gly Arg  
100 105 110

Pro Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala  
115 120 125

Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser  
130 135 140

Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe  
145 150 155 160

Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly  
165 170 175



Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu  
 180 185 190  
 Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr  
 195 200 205  
 Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys  
 210 215 220  
 Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro  
 225 230 235 240  
 Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys  
 245 250 255  
 Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val  
 260 265 270  
 Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr  
 275 280 285  
 Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu  
 290 295 300  
 Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His  
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 Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys  
 325 330 335  
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 Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro  
 370 375 380  
 Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn  
 385 390 395 400  
 Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu  
 405 410 415  
 Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val  
 420 425 430  
 Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln  
 435 440 445  
 Lys Ser Leu Ser Leu Ser Pro Gly  
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&lt;210&gt; 175

&lt;211&gt; 456

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; source

&lt;223&gt; /note="Description of Artificial Sequence: Synthetic polypeptide"

&lt;400&gt; 175

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Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Phe Asn Tyr Tyr  
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Ser Met Ile Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
 35 40 45

Ser Ser Ile Asp Ser Ser Ser Arg Tyr Arg Tyr Tyr Thr Asp Ser Val  
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Gln Asn Ser Leu Tyr  
 65 70 75 80

Leu Gln Met Ser Ala Leu Arg Val Glu Asp Thr Ala Val Tyr Tyr Cys  
 85 90 95

Ala Arg Asp Gly Asp Asp Ile Leu Ser Val Tyr Gln Gly Ser Gly Arg  
 100 105 110

Pro Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala  
 115 120 125

Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser  
 130 135 140

Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe  
 145 150 155 160

Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly  
 165 170 175

Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu  
 180 185 190

Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr  
 195 200 205

Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys  
 210 215 220

Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro  
 225 230 235 240

Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys  
 245 250 255

Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val  
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 Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr  
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 Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu  
 290 295 300  
 Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His  
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 340 345 350  
 Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met  
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 Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro  
 370 375 380  
 Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn  
 385 390 395 400  
 Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu  
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 Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val  
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<210> 176

<211> 445

<212> PRT

<213> Artificial Sequence

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<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

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Asp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Gln Trp Val  
 35 40 45  
 Ser Thr Ile Ile Gly Ile Asp Asp Thr Thr His Tyr Ala Asp Ser Val  
 50 55 60  
 Arg Gly Arg Phe Thr Val Ser Arg Asp Thr Ser Lys Asn Met Val Tyr  
 65 70 75 80  
 Leu Gln Met Asn Ser Leu Arg Val Glu Asp Thr Ala Leu Tyr Tyr Cys  
 85 90 95  
 Val Lys Asn Ser Gly Ile Tyr Ser Phe Trp Gly Gln Gly Thr Leu Val  
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 Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala  
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 Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu  
 130 135 140  
 Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly  
 145 150 155 160  
 Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser  
 165 170 175  
 Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu  
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 195 200 205  
 Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr  
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 Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe  
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 275 280 285  
 Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val  
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325 330 335  
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&lt;211&gt; 7

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&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; source

&lt;223&gt; /note="Description of Artificial Sequence: Synthetic peptide"

&lt;400&gt; 177

Ala Arg Gly Asp Gly Gly Leu  
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&lt;210&gt; 178

&lt;211&gt; 7

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; source

&lt;223&gt; /note="Description of Artificial Sequence: Synthetic peptide"

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Ala Arg Gly Asp Ala Gly Leu  
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&lt;210&gt; 179

&lt;211&gt; 7

&lt;212&gt; PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 179

Ala Arg Gly Glu Gly Gly Leu  
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<212> PRT

<213> Artificial Sequence

<220>

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<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 180

Ala Arg Gly Ala Gly Gly Leu  
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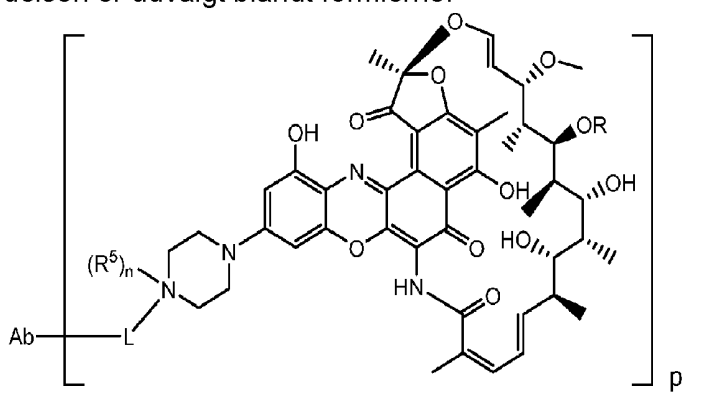
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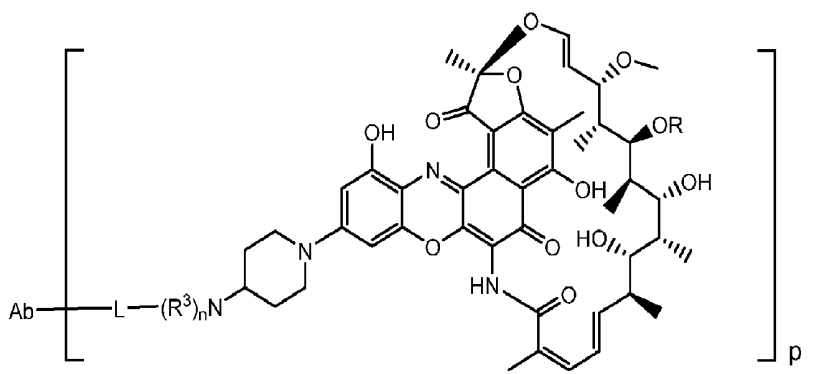
**Patentkrav**

1. Antistof-antibiotikum-konjugatforbindelse omfattende et monoklonalt anti-væg-teichoinsyre-(WTA)-antistof, hvor det monoklonale anti-væg-teichoinsyre-antistof binder til *Staphylococcus aureus*, og er kovalent bundet til et antibiotikum af en rifamycin-type ved en *S. aureus* endopeptidase- eller cystein-protease-spaltbar peptid-linker (L), hvor antistof-antibiotikum-konjugatforbindelsen er udvalgt blandt formlerne:



hvor

$R^5$  er uafhængigt udvalgt blandt H og  $C_1$ - $C_{12}$  alkyl; og  
 $n$  er 0 eller 1; og



hvor

$R^3$  er uafhængigt udvalgt blandt H og  $C_1$ - $C_{12}$  alkyl; og  
 $n$  er 1 eller 2;

hvor R er H,  $C_1$ - $C_{12}$  alkyl eller  $C(O)CH_3$ ;

L er peptid-linkeren med formelen:

## -Str-Pep-Y-

5 hvor Str er en stretcher-enhed; Pep er et peptid af to til tolv aminosyrerester, og Y er en spacer-enhed;  
 Ab er det monoklonale anti-væg-teichoinsyre-antistof; og  
 p er et heltal fra 1 til 8;  
 hvor VL'en af det monoklonale anti-WTA-antistof omfatter -CDR L1, som omfatter sekvensen af KSSQSIFRTSRNKNLLN (SEQ ID NO:99), CDR L2, som  
 10 omfatter sekvensen af WASTRKS (SEQ ID NO:100), og CDR L3, der omfatter sekvensen af QQYFSPPYT (SEQ ID NO:101); og VH'en af det monoklonale anti-WTA-antistof omfatter CDR H1, som omfatter sekvensen af SFWMH (SEQ ID NO:102), CDR H2, der omfatter sekvensen af FTNNEGTTTAY-ADSVRG (SEQ ID NO: 103), og CDR H3, som omfatter sekvensen af GEG-  
 15 GLDD (SEQ ID NO:118) eller GDGGLDD (SEQ ID NO:104).

20 **2.** Antistof-antibiotikum-konjugatforbindelse ifølge krav 1, hvor antistoffet omfatter en VL omfattende aminosyresekvensen SEQ ID NO:119.

**3.** Antistof-antibiotikum-konjugatforbindelse ifølge krav 1 eller krav 2, hvor antistoffet omfatter en VH omfattende aminosyresekvensen af SEQ ID NO:156.

25 **4.** Antistof-antibiotikum-konjugatforbindelse ifølge et af kravene 1 til 3, hvor antistoffet omfatter en VL og en VH, hvor VL'en omfatter sekvensen af SEQ ID NO:119, og VH'en omfatter sekvensen SEQ ID NO:156.

30 **5.** Antistof-antibiotikum-konjugatforbindelse ifølge krav 1, hvor antistoffet omfatter en letkæde (LC), hvor LC'en omfatter aminosyresekvensen af SEQ ID NO:121, og en tungkæde (HC), hvor HC'en omfatter aminosyresekvensen af SEQ ID NO:146, SEQ ID NO:147, SEQ ID NO:157 eller SEQ ID NO:124.

**6.** Antistof-antibiotikum-konjugatforbindelse ifølge krav 1, hvor antistoffet omfatter en letkæde (LC), hvor LC'en omfatter aminosyresekvensen af SEQ ID



NO:123, og en tungkæde (HC), hvor HC'en omfatter aminosyresekvensen af SEQ ID NO:146, SEQ ID NO:147, SEQ ID NO:157 eller SEQ ID NO:124.

5       **7.** Antistof-antibiotikum-konjugatforbindelse ifølge krav 1, hvor antistoffet omfatter en letkæde (LC), hvor LC'en omfatter aminosyresekvensen af SEQ ID NO:145, og en tungkæde (HC), hvor HC'en omfatter aminosyresekvensen af SEQ ID NO:146, SEQ ID NO:147, SEQ ID NO:157 eller SEQ ID NO:124.

10       **8.** Antistof-antibiotikum-konjugatforbindelse ifølge krav 1 eller krav 2, hvor antistoffet omfatter en VH omfattende aminosyresekvensen af SEQ ID NO:120.

15       **9.** Antistof-antibiotikum-konjugatforbindelse ifølge et af kravene 1, 2 eller krav 8, hvor antistoffet omfatter en VL og en VH, hvor VL'en omfatter sekvensen af SEQ ID NO:119, og VH'en omfatter sekvensen SEQ ID NO:120.

20       **10.** Antistof-antibiotikum-konjugatforbindelse ifølge krav 1, hvor antistoffet omfatter en letkæde (LC), hvor LC'en omfatter aminosyresekvensen af SEQ ID NO:121, og en tungkæde (HC), hvor HC'en omfatter aminosyresekvensen af SEQ ID NO:146.

25       **11.** Antistof-antibiotikum-konjugatforbindelse ifølge krav 1, hvor antistoffet omfatter en letkæde (LC), hvor LC'en omfatter aminosyresekvensen af SEQ ID NO:123, og en tungkæde (HC), hvor HC'en omfatter aminosyresekvensen af SEQ ID NO:147.

30       **12.** Antistof-antibiotikum-konjugatforbindelse ifølge krav 1, hvor antistoffet omfatter en letkæde (LC), hvor LC'en omfatter aminosyresekvensen af SEQ ID NO:145, og en tungkæde (HC), hvor HC'en omfatter aminosyresekvensen af SEQ ID NO:157.

**13.** Antistof-antibiotikum-konjugatforbindelse ifølge krav 1, hvor antistoffet omfatter en letkæde (LC), hvor LC'en omfatter aminosyresekvensen af SEQ ID NO:145, og en tungkæde (HC), hvor HC'en omfatter aminosyresekvensen af

SEQ ID NO:147.

**14.** Antistof-antibiotikum-konjugatforbindelse ifølge et af de foregående krav, hvor antibiotikummet af rifamycin-typen omfatter en kvaternær amin, som er bundet til peptid-linkeren.

**15.** Antistof-antibiotikum-konjugatforbindelse ifølge krav 14, hvor peptid-linkeren er bundet til en cystein eller en manipuleret cystein af anti-WTA-antistoffet.

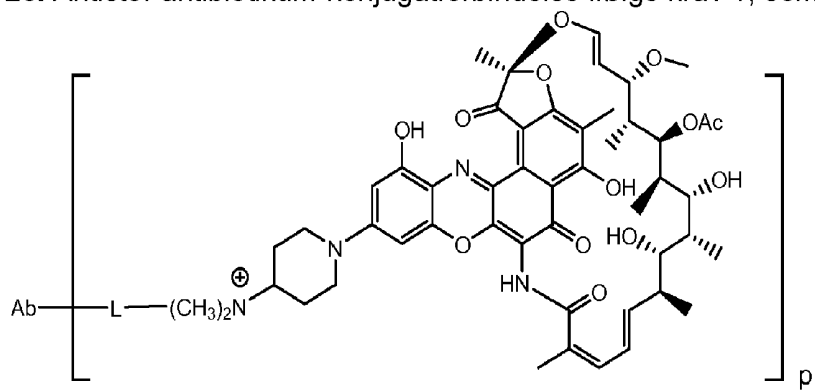
**16.** Antistof-antibiotikum-konjugatforbindelse ifølge krav 1, hvor peptid-linkeren er en staphopain B- eller en staphopain A-spaltbar linker.

**17.** Antistof-antibiotikum-konjugatforbindelse ifølge krav 16, hvor peptid-linkeren er en spaltbar linker af humant protease-cathepsin B.

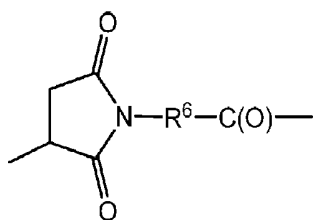
**18.** Antistof-antibiotikum-konjugatforbindelse ifølge krav 17, hvor peptid-linkeren er en val-cit-dipeptid-linker.

**19.** Antistof-antibiotikum-konjugatforbindelse ifølge et af de foregående krav, hvor p er 2 til 4.

**20.** Antistof-antibiotikum-konjugatforbindelse ifølge krav 1, som har formelen:



**21.** Antistof-antibiotikum-konjugatforbindelse ifølge et af de foregående krav, hvor Str har formelen:



hvor  $R^6$  er udvalgt fra gruppen bestående af  $C_1$ - $C_{10}$  alkylen-,  $-C_3$ - $C_8$  carbocyclo-,  $-O$ -( $C_1$ - $C_8$  alkyl)-, -arylen-,  $-C_1$ - $C_{10}$  alkylen-arylen-, -arylen- $C_1$ - $C_{10}$  alkylen-,  $-C_1$ - $C_{10}$  alkylen-( $C_3$ - $C_8$  carbocyclo)-, -( $C_3$ - $C_8$  carbocyclo)- $C_1$ - $C_{10}$  alkylen-,  $-C_3$ - $C_8$  heterocyclo-,  $-C_1$ - $C_{10}$  alkylen-( $C_3$ - $C_8$  heterocyclo)-, -( $C_3$ - $C_8$  heterocyclo)- $C_1$ - $C_{10}$  alkylen-,  $-(CH_2CH_2O)_r$  og  $-(CH_2CH_2O)_r-CH_2-$ ; og  $r$  er et heltal i området fra 1 til 10.

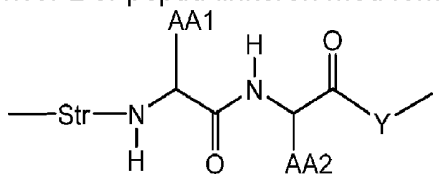
**22.** Antistof-antibiotikum-konjugatforbindelse ifølge krav 21, hvor  $R^6$  er  $-(CH_2)_5-$ .

**23.** Antistof-antibiotikum-konjugatforbindelse ifølge et af de foregående krav, hvor Pep omfatter to til tolv aminosyrerester uafhængigt udvalgt blandt glycin, alanin, phenylalanin, lysin, arginin, valin og citrullin.

**24.** Antistof-antibiotikum-konjugatforbindelse ifølge krav 23, hvor Pep er udvalgt blandt valin-citrullin (val-cit, vc); phenylalanin-lysin (fk); og valin-citrullin-phenylalanin (val-cit-phe).

**25.** Antistof-antibiotikum-konjugatforbindelse ifølge et af de foregående krav, hvor Y omfatter para-aminobenzyl eller para-aminobenzyloxycarbonyl.

**26.** Antistof-antibiotikum-konjugatforbindelse ifølge et af de foregående krav, hvor L er peptid-linkeren med formen:

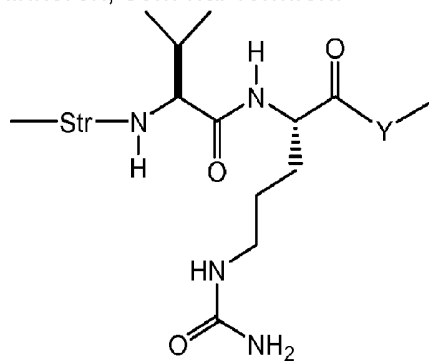


hvor AA1 og AA2 er uafhængigt udvalgt fra en aminosyresidekæde.

**27.** Antistof-antibiotikum-konjugatforbindelse ifølge krav 26, hvor aminosyre-sidekæden er uafhængigt udvalgt blandt H,  $-\text{CH}_3$ ,  $-\text{CH}_2(\text{C}_6\text{H}_5)$ ,  $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2$ ,  $-\text{CH}_2\text{CH}_2\text{CH}_2\text{NHC}(\text{NH})\text{NH}_2$ ,  $-\text{CHCH}(\text{CH}_3)\text{CH}_3$  og  $-\text{CH}_2\text{CH}_2\text{CH}_2\text{NHC}(\text{O})\text{NH}_2$ .

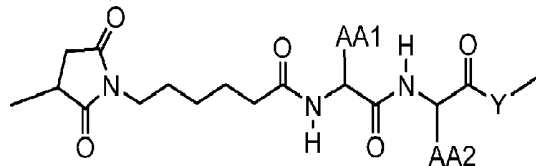
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**28.** Antistof-antibiotikum-konjugatforbindelse ifølge krav 26, hvor L er peptid-linkeren, som har formelen:



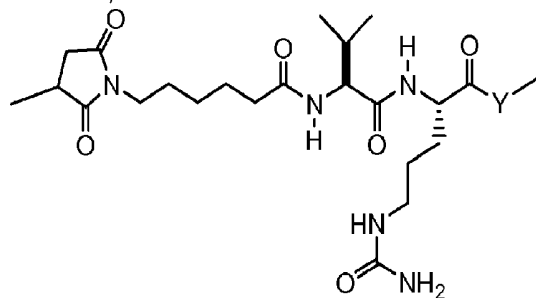
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**29.** Antistof-antibiotikum-konjugatforbindelse ifølge krav 26, hvor L er peptid-linkeren, som har formelen:



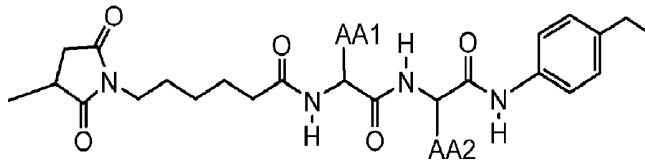
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**30.** Antistof-antibiotikum-konjugatforbindelse ifølge krav 29, hvor L er peptid-linkeren, som har formelen:



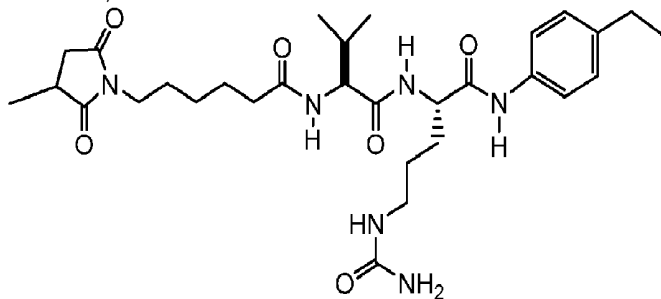
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**31.** Antistof-antibiotikum-konjugatforbindelse ifølge krav 26, hvor L er peptid-linkeren, som har formelen:



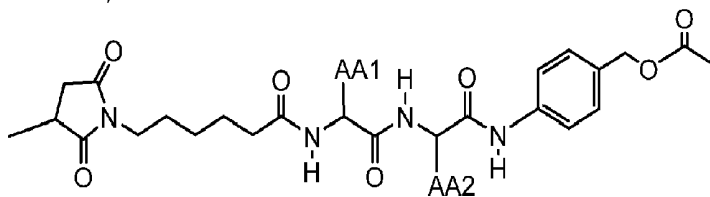
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**32.** Antistof-antibiotikum-konjugatforbindelse ifølge krav 31, hvor L er peptid-linkeren, som har formelen:



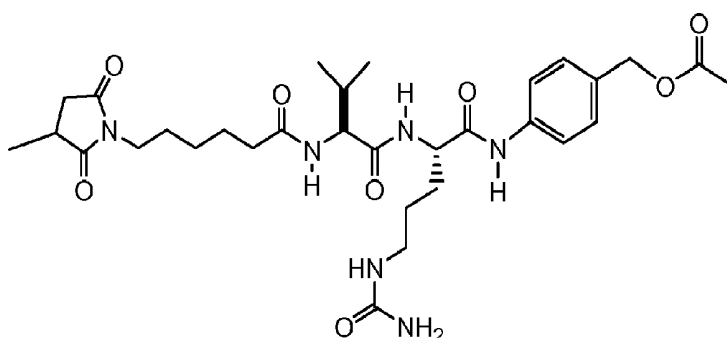
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**33.** Antistof-antibiotikum-konjugatforbindelse ifølge krav 26, hvor L er peptid-linkeren, som har formelen:

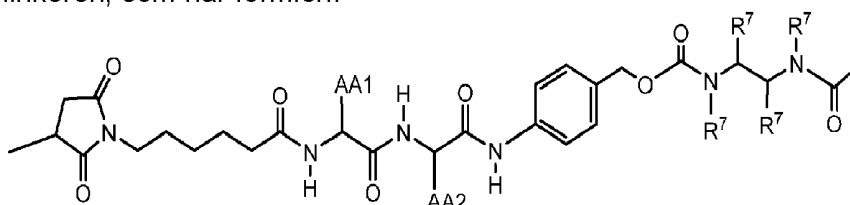


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**34.** Antistof-antibiotikum-konjugatforbindelse ifølge krav 33, hvor L er peptid-linkeren, som har formelen:

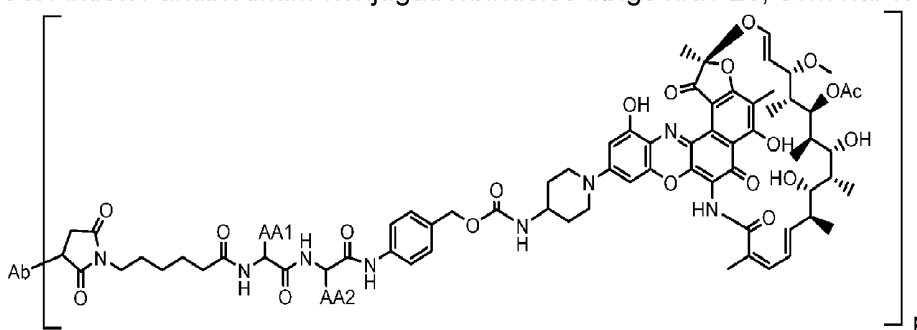


**35.** Antistof-antibiotikum-konjugatforbindelse ifølge krav 26, hvor L er peptid-linkeren, som har formlen:

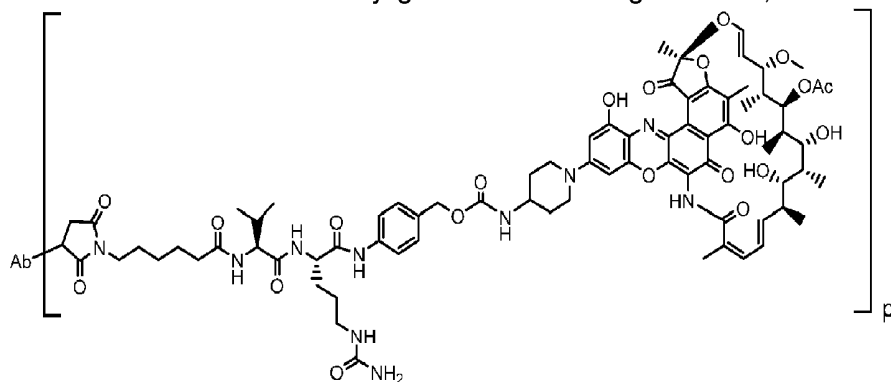


hvor R<sup>7</sup> er uafhængigt udvalgt blandt H og C<sub>1</sub>-C<sub>12</sub> alkyl.

**36.** Antistof-antibiotikum-konjugatforbindelse ifølge krav 26, som har formelen:

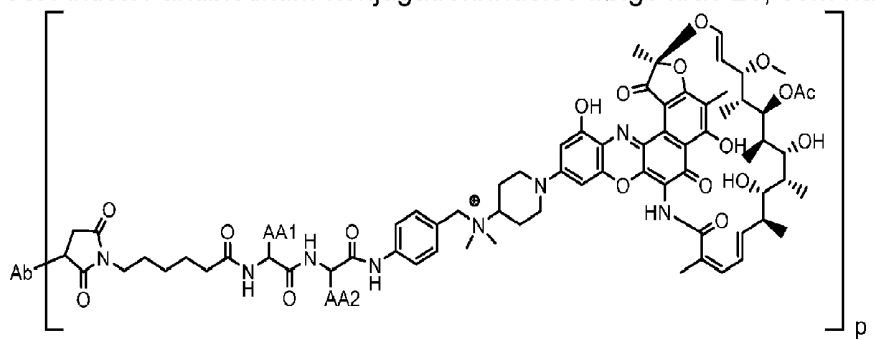


**37.** Antistof-antibiotikum-konjugatforbindelse ifølge krav 36, som har formelen:

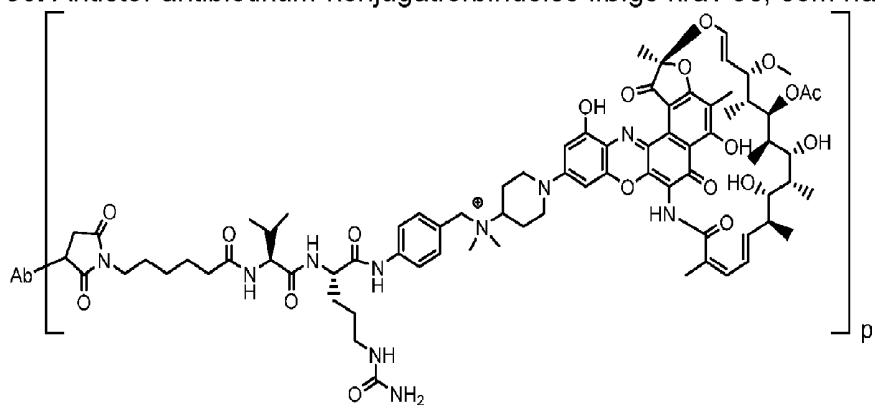


5

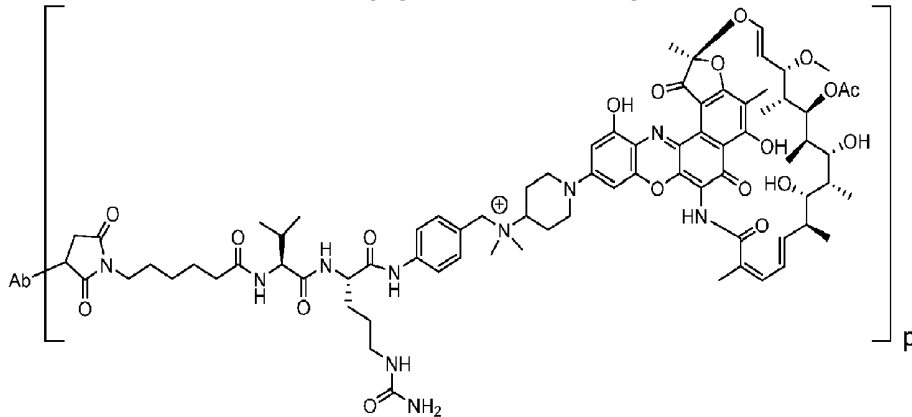
**38.** Antistof-antibiotikum-konjugatforbindelse ifølge krav 26, som har formelen:



**39.** Antistof-antibiotikum-konjugatforbindelse ifølge krav 38, som har formelen:



40. Antistof-antibiotikum-konjugatforbindelse ifølge krav 1, som har formelen:



hvor Ab er et monoklonalt anti-væg-teichoinsyre- (WTA)-antistof, som binder  
 5 til *Staphylococcus aureus*, hvor VL'en af det monoklonale anti-væg-teichon-  
 syre-antistof omfatter CDR L1, der omfatter sekvensen af KSSQSIFRTSRN-  
 KNLLN (SEQ ID NO:99), CDR L2, som omfatter sekvensen af WASTRKS  
 (SEQ ID NO:100), og CDR L3, der omfatter sekvensen af QQYFSPPYT (SEQ  
 ID NO:101); og VH'en af det monoklonale anti-WTA-antistof omfatter CDR H1,  
 10 som omfatter sekvensen af SFWMH (SEQ ID NO:102), CDR H2, som omfatter  
 sekvensen af FTNNEGTTTAYADSVRG (SEQ ID NO:103), og CDR H3, som  
 omfatter sekvensen af GEGGLDD (SEQ ID NO:118); og  
 p er et heltal fra 1 til 8.

15 **41.** Farmaceutisk sammensætning, som omfatter antistof-antibiotikum-konju-  
 gatforbindelsen ifølge et af de foregående krav og et farmaceutisk acceptabelt  
 bærestof, glidemiddel, fortyndingsmiddel eller hjælpestof.

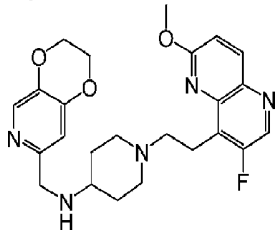
20 **42.** Fremgangsmåde til fremstilling af antistof-antibiotikum-konjugatforbindel-  
 sen ifølge et af kravene 1 til 40, omfattende konjugering af antibiotikummet af  
 rifamycin-typen med det monoklonale WTA-antistof.

25 **43.** Antistof-antibiotikum-konjugatforbindelse ifølge et af kravene 1 til 40 til an-  
 vendelse inden for terapi.



- 44.** Antistof-antibiotikum-konjugatforbindelse ifølge et af kravene 1 til 40 til anvendelse i en fremgangsmåde til behandling af en bakterieinfektion i en patient, hvor bakterieinfektionen er en *Staphylococcus aureus*-infektion.
- 5      **45.** Antistof-antibiotikum-konjugatforbindelse til anvendelse i fremgangsmåden ifølge krav 44, hvor bakteriebelastning i patienten er reduceret ved hjælp af behandlingen.
- 10      **46.** Antistof-antibiotikum-konjugatforbindelse ifølge et af kravene 1 til 40 til anvendelse i en fremgangsmåde til reduktion af antallet af intracellulære *Staphylococcus aureus*-bakterieceller i værtscellerne af en *Staphylococcus aureus*-inficeret patient uden reduktion af antallet af værtscellerne.
- 15      **47.** Antistof-antibiotikum-konjugatforbindelse til anvendelse i fremgangsmåden ifølge krav 46, hvor antallet af persistente bakterieceller er reduceret.
- 20      **48.** Antistof-antibiotikum-konjugatforbindelse til anvendelse i fremgangsmåden ifølge et af kravene 44 til 47, hvor patienten er et menneske.
- 25      **49.** Antistof-antibiotikum-konjugatforbindelse til anvendelse i fremgangsmåden ifølge et af kravene 44 til 48, endvidere omfattende indgivelse af et andet terapeutisk middel.
- 30      **50.** Antistof-antibiotikum-konjugatforbindelse til anvendelse i fremgangsmåden ifølge krav 49, hvor det andet terapeutiske middel er et antibiotikum.
- 30      **51.** Antistof-antibiotikum-konjugatforbindelse til anvendelse i fremgangsmåden ifølge krav 50, hvor det andet terapeutiske middel er et antibiotikum udvalgt fra strukturklassen bestående af (i) aminoglycosider; (ii) beta-lactamer; (iii) macrolider/cykliske peptider; (iv) tetracycliner; (v) fluorquinoliner/fluorquinoloner; og (vi) oxazolidinoner.

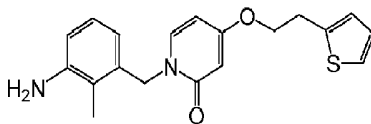
**52.** Antistof-antibiotikum-konjugatforbindelse til anvendelse i fremgangsmåden ifølge krav 50, hvor det andet terapeutiske middel er et antibiotikum udvalgt fra gruppen bestående af clindamycin, novobiocin, retapamulin, daptomycin, GSK-2140944 (gepotidacin) med strukturen:



5

GSK-2140944 ,

CG-400549 med strukturen:

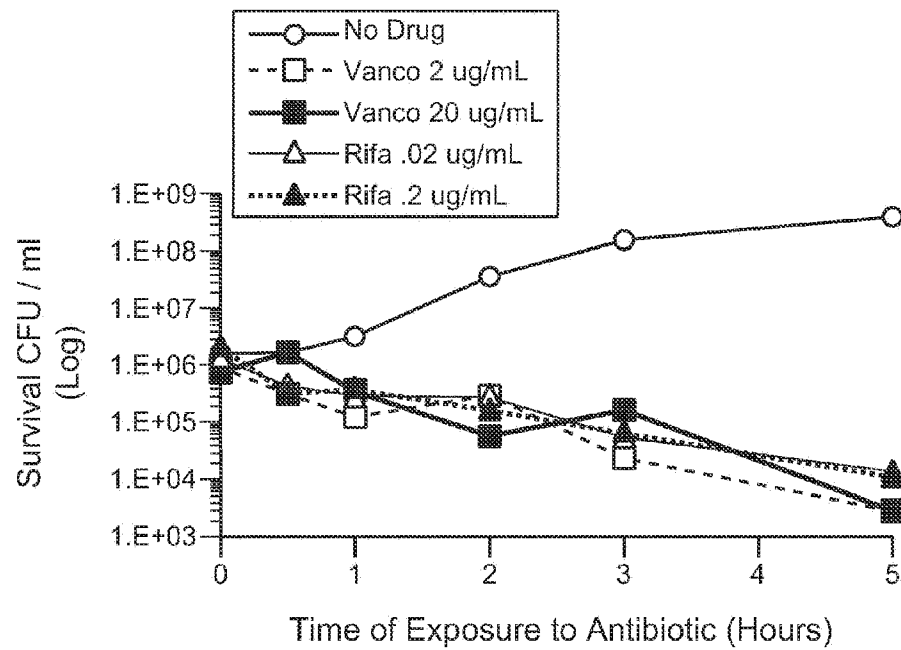


CG-400549 ,

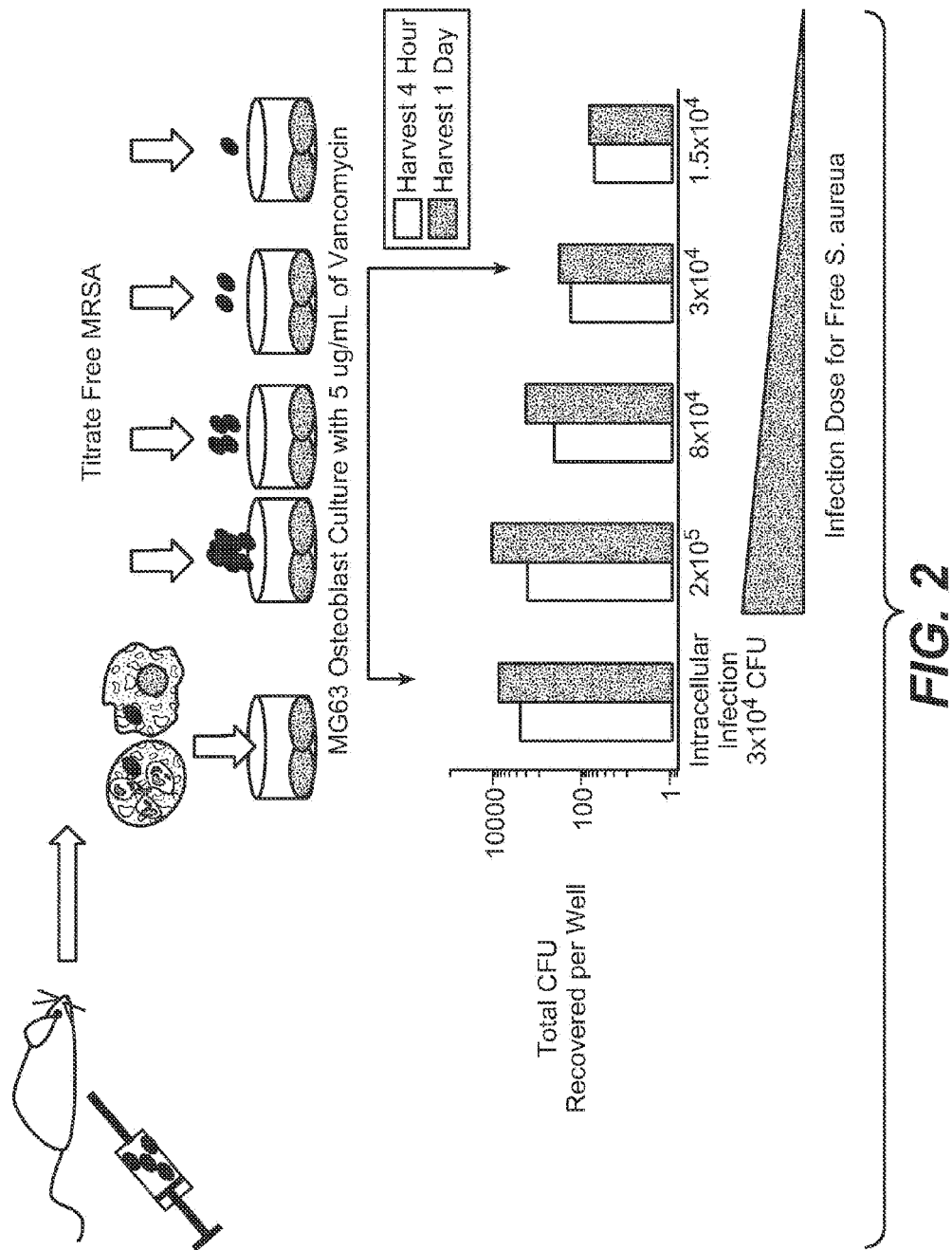
sitafloxacin, teicoplanin, triclosan, naphthyridon, radezolid, doxorubicin, ampicillin, vancomycin, imipenem, doripenem, gemcitabin, dalbavancin og azithromycin.

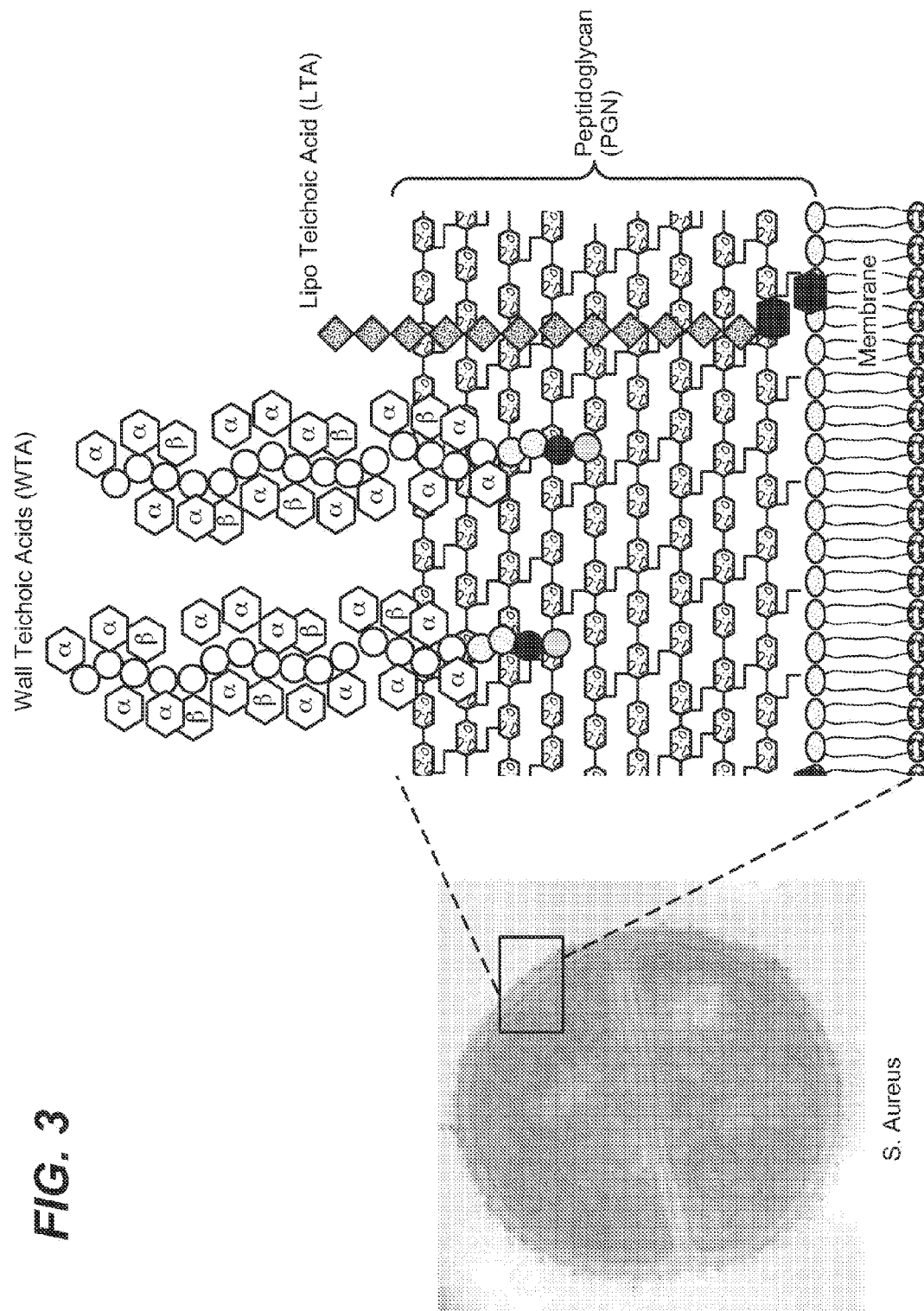
10

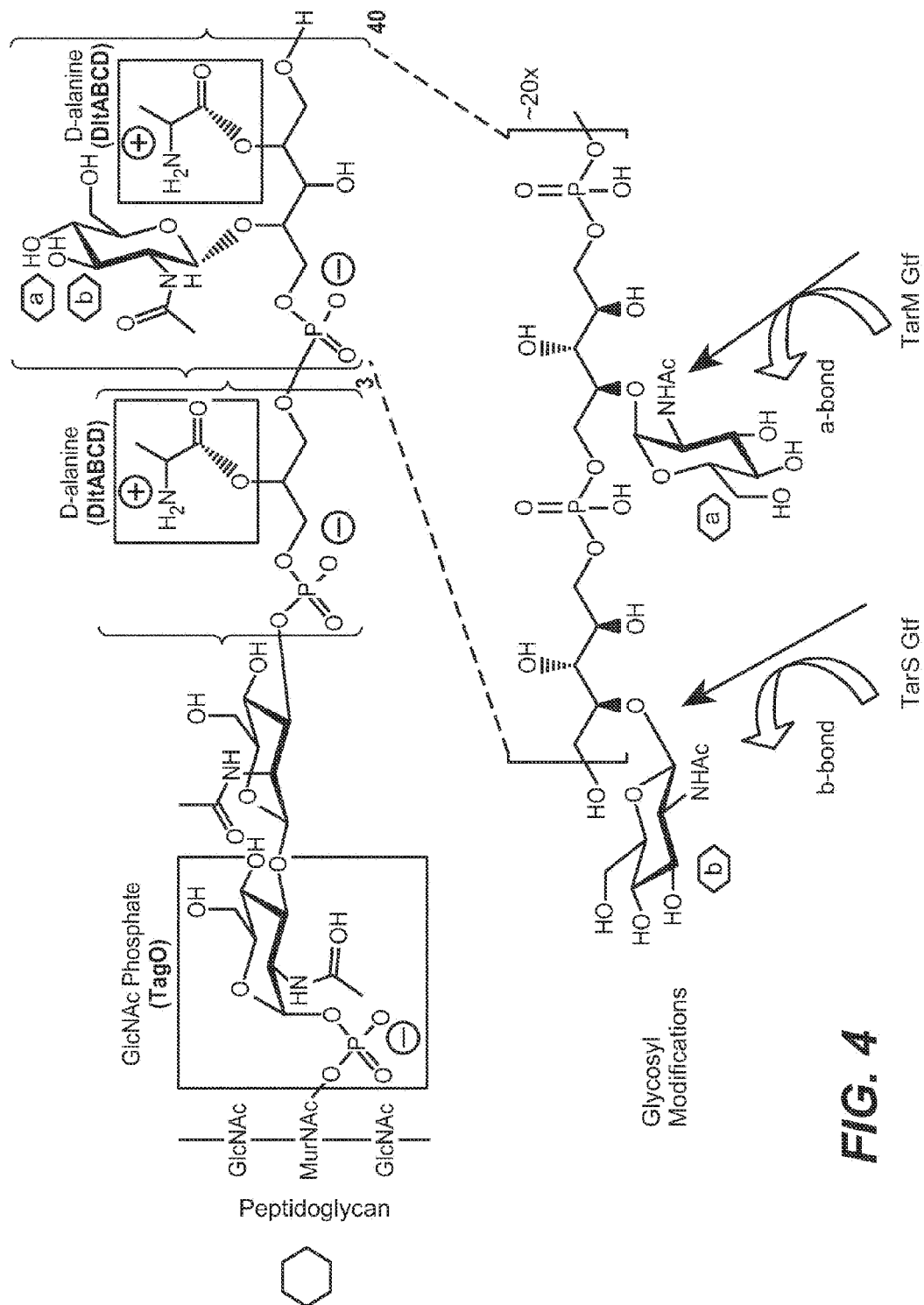
# DRAWINGS



**FIG. 1**





**FIG. 4**

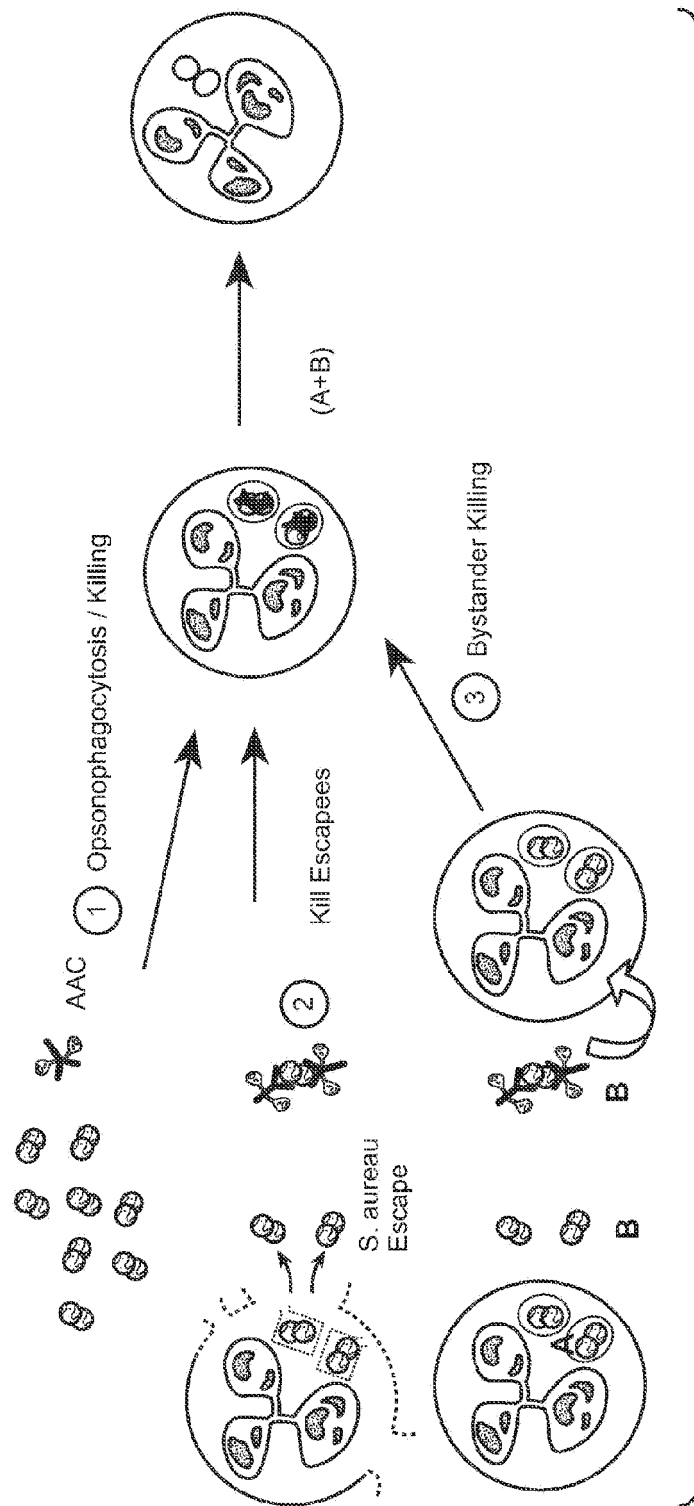


FIG. 5

**FIG. 6**

FIG. 6A

FIG. 6B

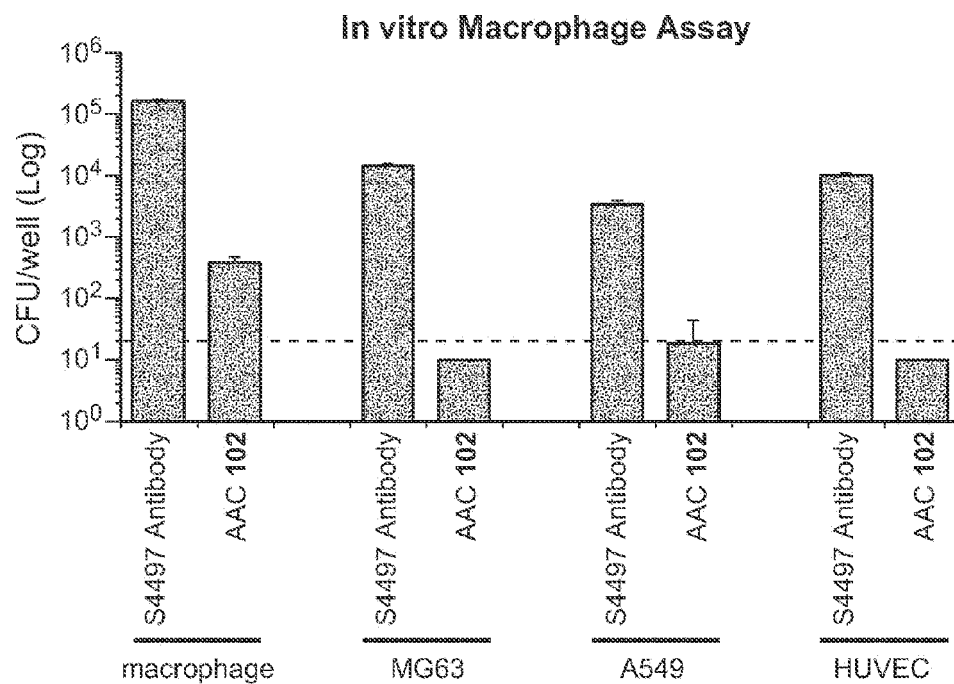
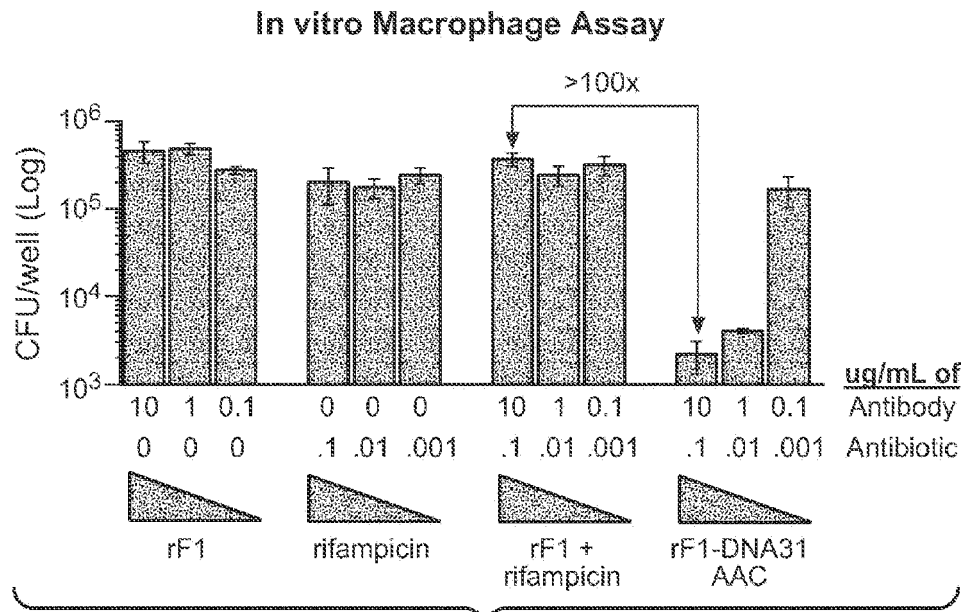
**FIG. 6A**

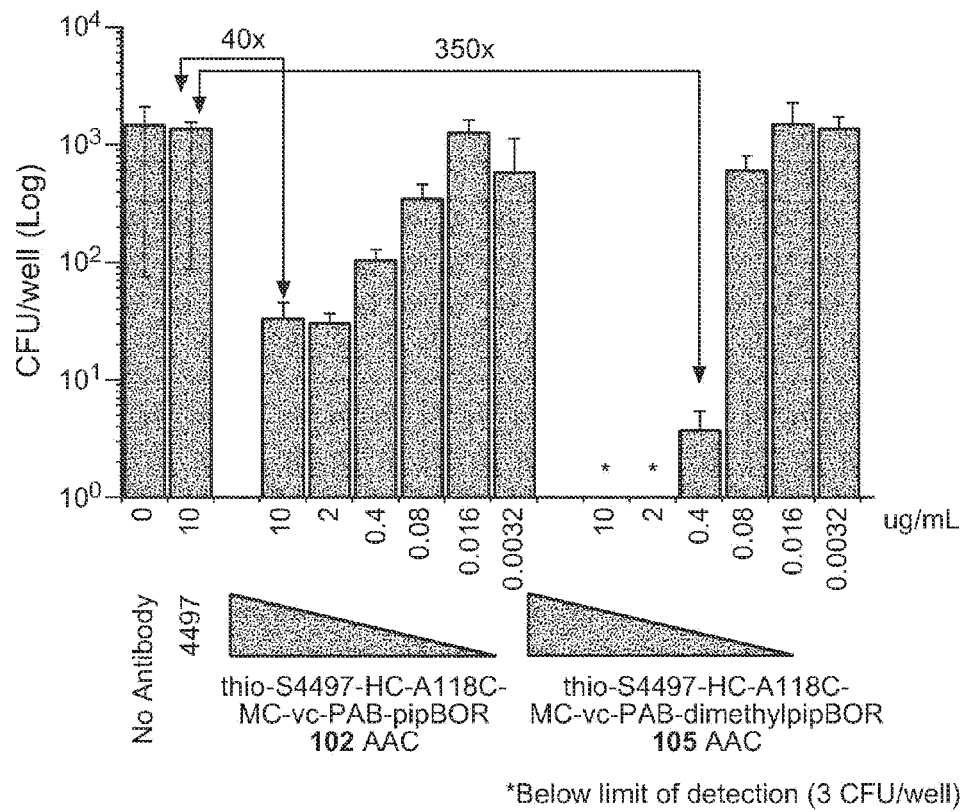
Antibody	Antigen	GlcNAc	KD(nM)	Antigen Density Ave. Sites Bacterium	Donor	Source Cells	Binding to Coating(s) in Primary Screening Ag (ELISA)
4497	WTA	beta	2.5	50	327	PB/PC	WTA; PGN; CW USA300 stat (iron depl: TSB in 96:4 ratio)
4462	WTA	beta	3.1	43	326	sMBC	WTA; PGN; CW USA300 stat (iron depl: TSB in 96:4 ratio)
4450	WTA	beta			326	sMBC	WTA; PGN; CW USA300 stat (iron depl: TSB in 96:4 ratio)
4487	WTA	beta			327	PB/PC	WTA; PGN; CW USA300 stat (iron depl: TSB in 96:4 ratio)
6078	WTA	beta			349	sMBC	PGN+WTA (1:1); CW USA300 stat (iron depl: TSB in 96:4 ratio)
6263	WTA	beta	1.4	22,000	350	PB/PC	PGN+WTA (1:1); CW USA300 stat (iron depl: TSB in 96:4 ratio)
6297	WTA	beta	1.1	21,000	350	PB/PC	PGN+WTA (1:1); CW USA300 stat (iron depl: TSB in 96:4 ratio)
6239	WTA	beta			350	PB/PC	PGN+WTA (1:1); CW USA300 stat (iron depl: TSB in 96:4 ratio)
6292	WTA	beta			350	PB/PC	PGN+WTA (1:1); CW USA300 stat (iron depl: TSB in 96:4 ratio)
6232	WTA	beta			350	PB/PC	PGN+WTA (1:1); CW USA300 stat (iron depl: TSB in 96:4 ratio)
6259	WTA	beta			350	PB/PC	CW USA300 stat (iron depl: TSB in 96:4 ratio)
6253	WTA	beta			350	PB/PC	PGN+WTA (1:1); CW USA300 stat (iron depl: TSB in 96:4 ratio)
6265	WTA	beta			350	PB/PC	PGN+WTA (1:1); CW USA300 stat (iron depl: TSB in 96:4 ratio)

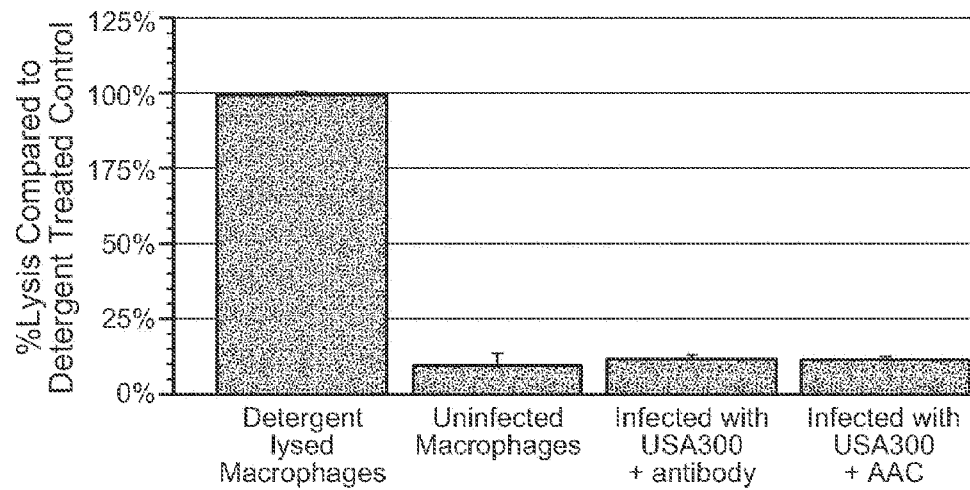
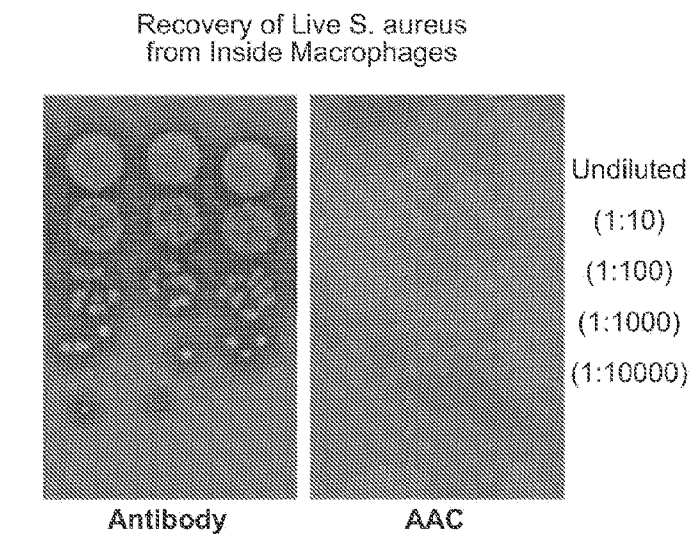


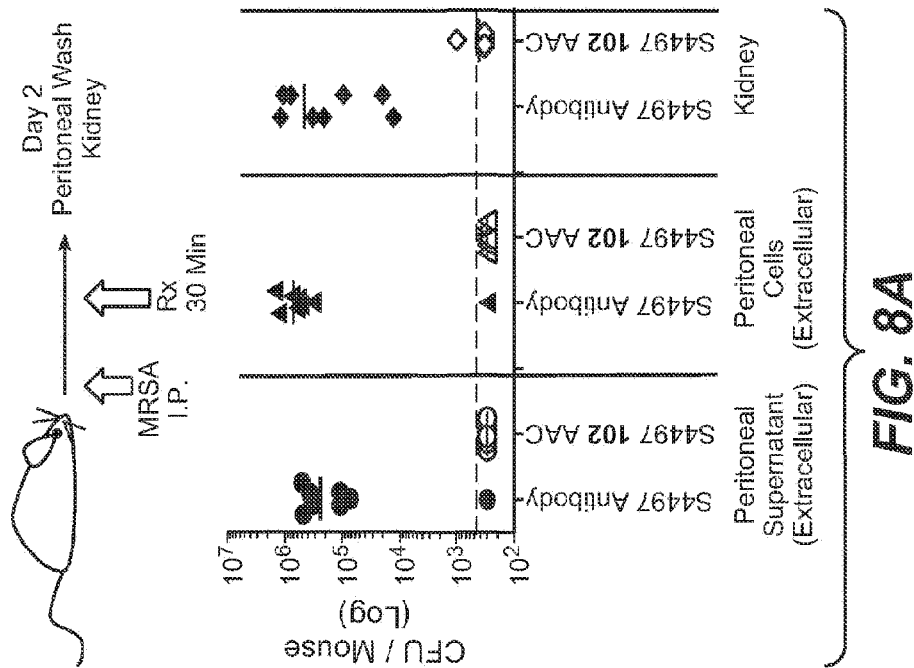
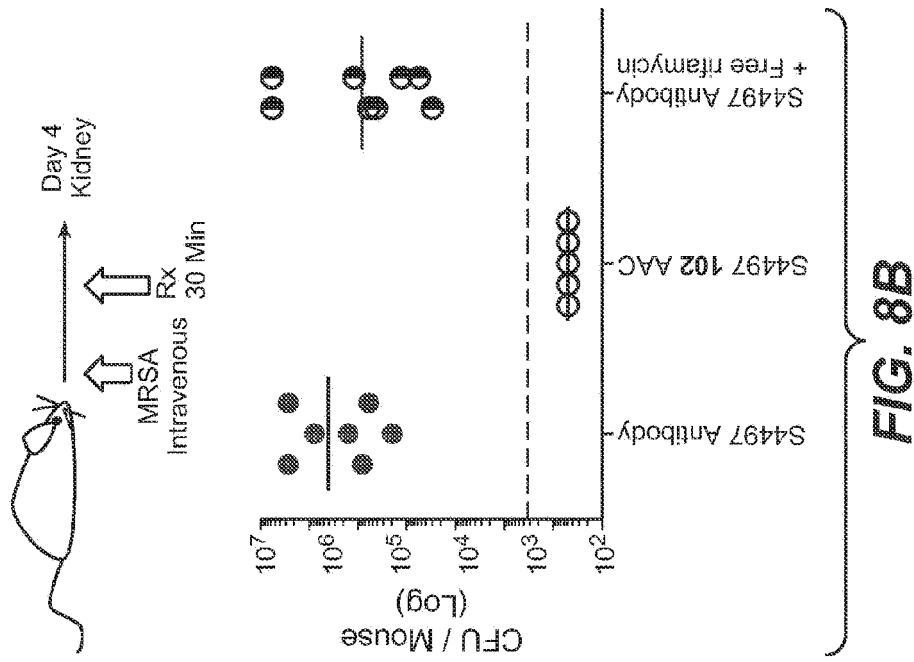
4461(7574)	WTA	alpha			326	sMBC	CW USA300 stat (iron depl: TSB in 96:4 ratio)
4624(7578)	WTA	alpha	0.4	16	326	sMBC	CW USA300 stat (iron depl: TSB in 96:4 ratio)
4399	WTA	alpha			326	sMBC	CW USA300 stat (iron depl: TSB in 96:4 ratio)
6267	WTA	alpha			350	PB/PC	CW USA300 stat (iron depl: TSB in 96:4 ratio)
rF1	SDR-proteins	?	0.3	1600			
4516(7577)	SDR-proteins	?			327	PB/PC	WTA; PGN; CW USA300 stat (iron depl: TSB in 96:4 ratio)
6234	SDR-proteins	?			350	PB/PC	PGN+WTA (1:1); CW USA300 stat (iron depl: TSB in 96:4 ratio)
6060	SDR-proteins	?			349	sMBC	PGN+WTA (1:1); CW USA300 stat (iron depl: TSB in 96:4 ratio)
4569	LTA	?			327	PB/PC	CW Wood46 stat TSB
4479	PGN				327	PB/PC	WTA; PGN; CW USA300 stat (iron depl: TSB in 96:4 ratio)

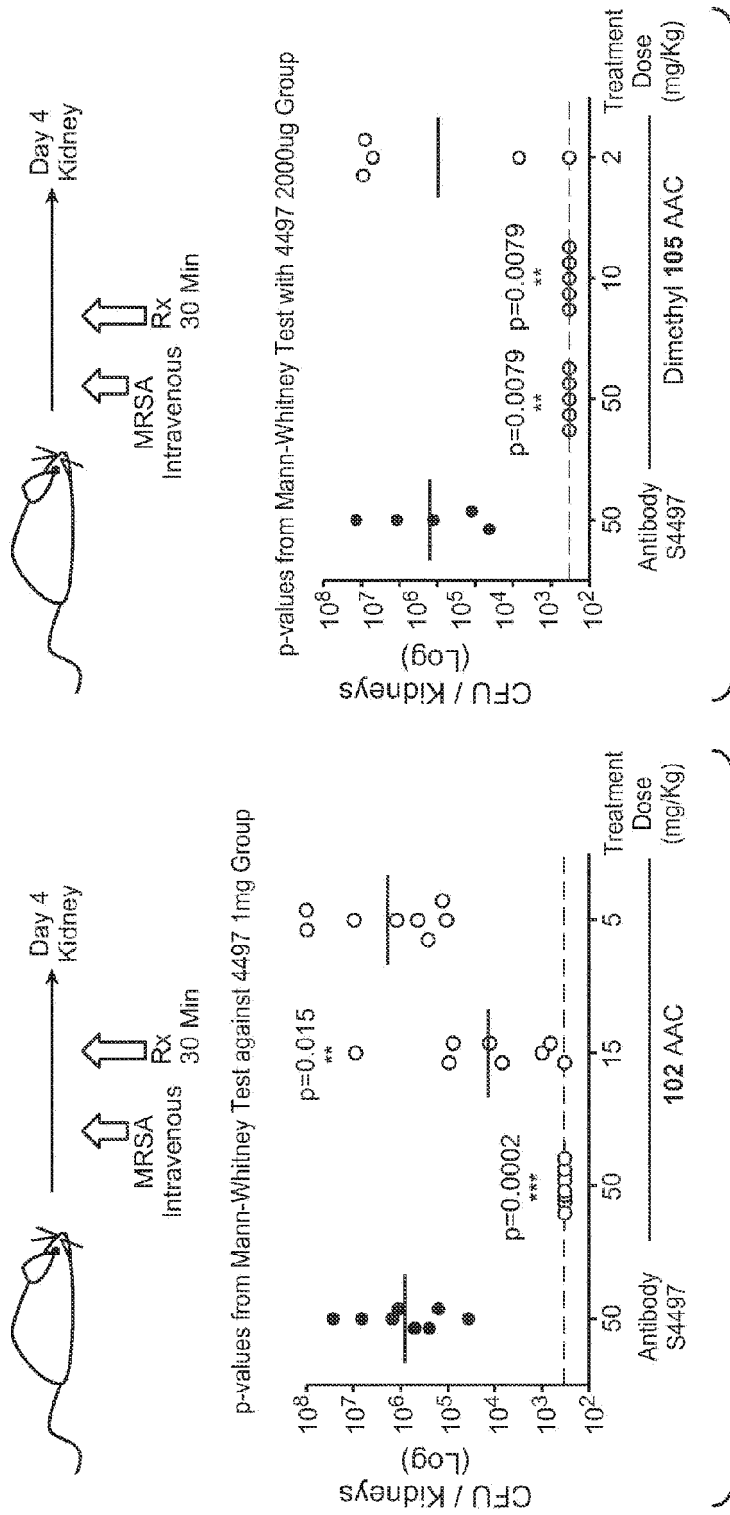
**FIG. 6B**

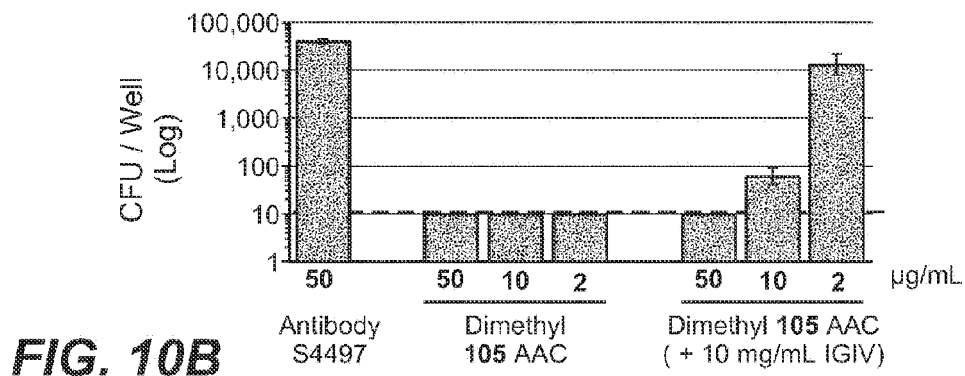
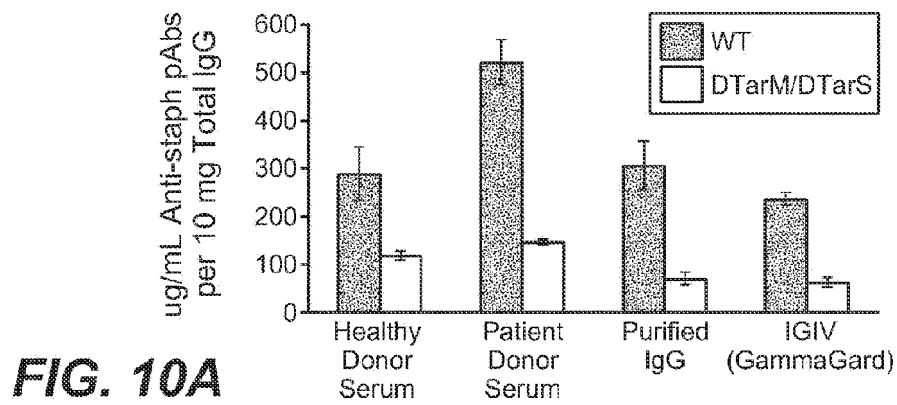
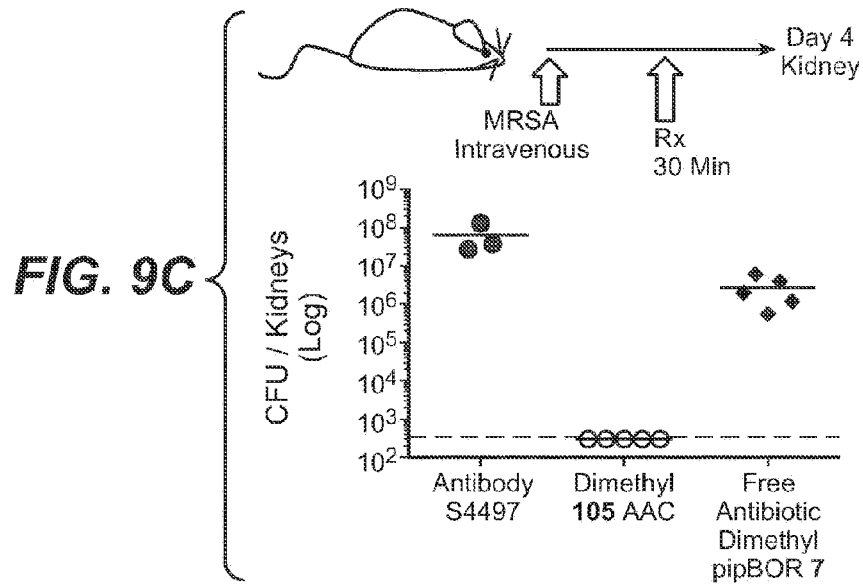


**FIG. 7C**

**FIG. 7D****FIG. 7E**







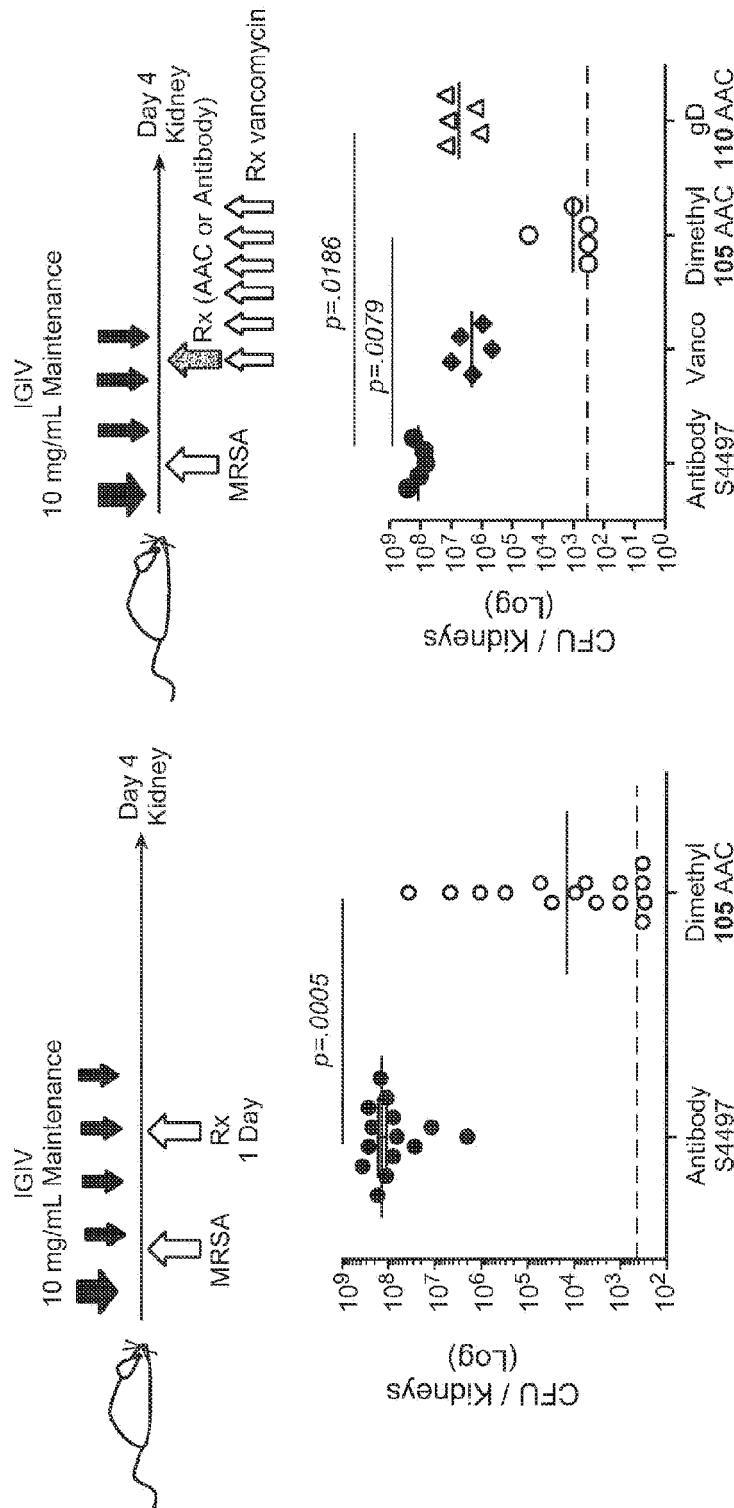
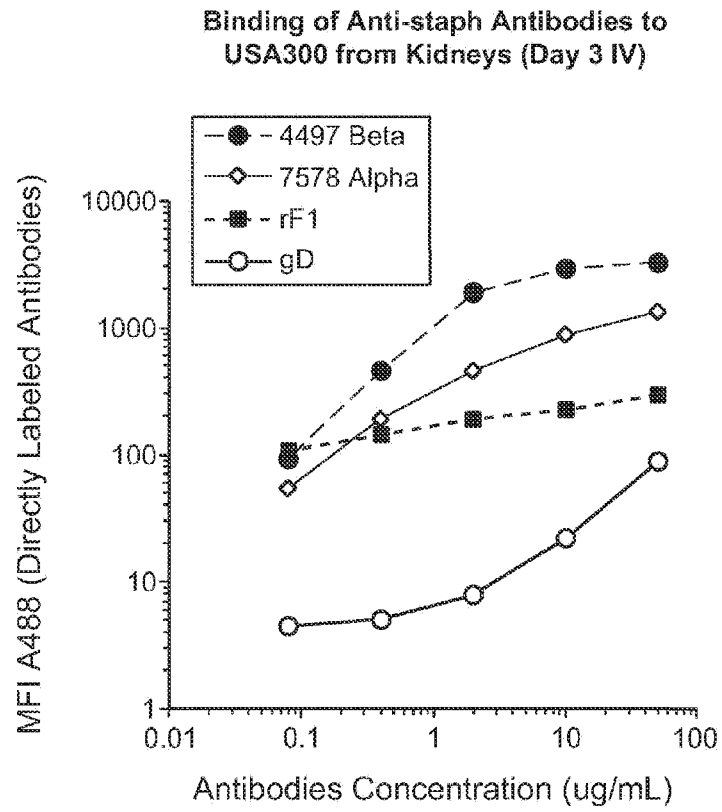


FIG. 10C

FIG. 11A



**FIG. 11B**

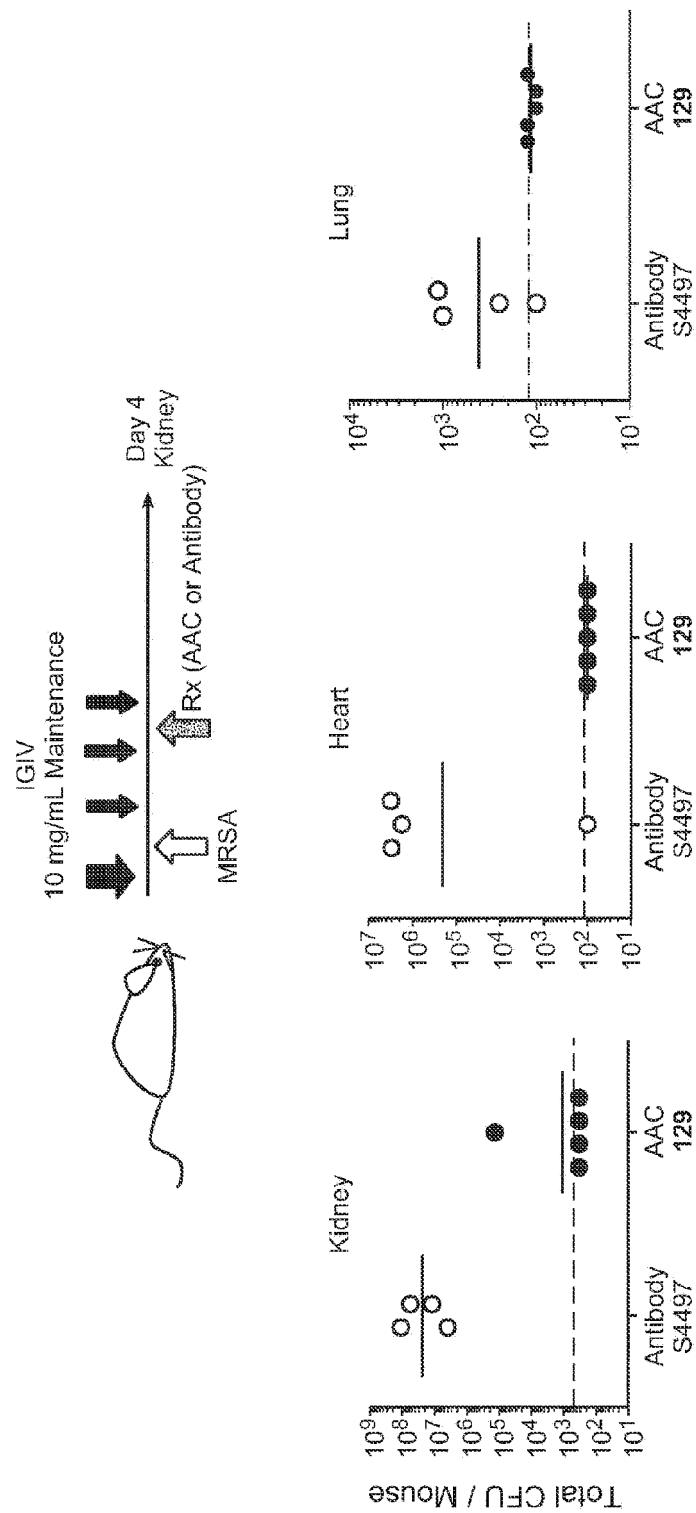
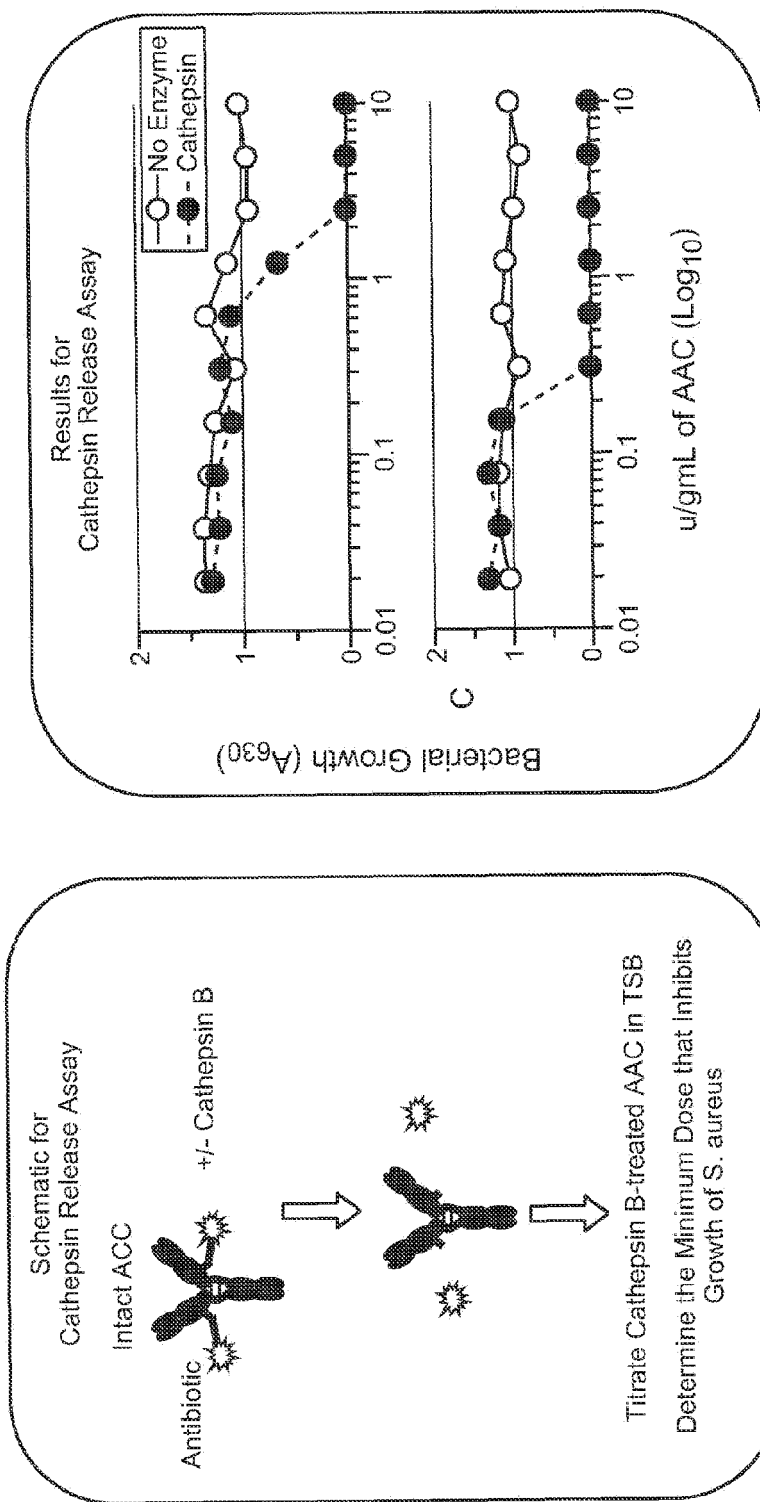


FIG. 11C

**FIG. 12**

CDR Sequences According to Kabat Definition are Underlined  
Light Chain Variable Region

			CDR L1 - Chothia																				CDR L1 - Kabat																				CDR L1 - Contact																			
			CDR L2 - Contact																				CDR L2 - Chothia																				CDR L2 - Kabat																			
			CDR L3 - Contact																				CDR L3 - Chothia																				CDR L3 - Kabat																			
Kabat Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	27a	27b	27c	27d	27e	27f	28	29	30	31	32	33	34	35	36																				
4461	D	I	Q	M	T	Q	S	P	D	S	L	A	V	S	L	G	E	R	A	T	I	N	C	K	S	S	Q	S	V	L	S	R	A	N	N	N	N	Y	Y	V	A	W	Y																			
4624	D	I	Q	M	T	Q	S	P	D	S	L	S	V	S	L	G	E	R	A	T	I	N	C	R	S	N	Q	N	L	L	-	S	S	S	N	N	N	Y	L	A	W	Y																				
4399	E	I	V	L	T	Q	S	P	D	S	L	A	V	S	L	G	E	R	A	T	I	N	C	K	S	N	Q	N	V	L	A	S	S	N	D	K	N	Y	L	A	W	F																				
6267	D	I	Q	L	T	Q	S	P	D	S	L	A	V	S	L	G	E	R	A	T	I	N	C	K	S	S	Q	N	V	L	Y	S	S	N	N	K	N	Y	L	A	W	Y																				
			CDR L2 - Contact																				CDR L2 - Chothia																				CDR L2 - Kabat																			
Kabat Number	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78																				
4461	Q	H	K	P	G	Q	P	P	K	L	L	I	Y	W	A	S	T	R	E	F	G	V	P	D	R	F	S	G	S	G	S	G	T	D	F	T	L	T	I	N	S	L																				
4624	Q	Q	K	P	G	Q	P	L	K	L	L	I	Y	W	A	S	T	R	E	S	G	V	P	D	R	F	S	G	S	G	S	G	T	D	F	T	L	T	I	S	S	L																				
4399	Q	H	K	P	G	Q	P	L	K	L	L	I	Y	W	A	S	T	R	E	S	G	V	P	D	R	F	S	G	S	G	S	G	T	D	F	T	L	T	I	S	S	L																				
6267	Q	Q	K	P	G	Q	P	L	K	L	L	I	Y	W	A	S	T	R	E	S	G	V	P	D	R	F	S	G	S	G	S	G	T	D	F	T	L	T	I	S	S	L																				
			CDR L3 - Contact																				CDR L3 - Chothia																				CDR L3 - Kabat																			
Kabat Number	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	95a	96	97	98	99	100	101	102	103	104	105	106	107																																
4461	Q	A	E	D	V	A	V	Y	Y	C	Q	Q	Y	Y	T	S	R	.	R	T	F	G	Q	G	T	K	V	E	I	K																																
4624	Q	A	E	D	V	A	V	Y	Y	C	Q	Q	Y	Y	A	N	P	.	R	T	F	G	Q	G	T	K	V	E	I	K																																
4399	R	A	E	D	V	A	V	Y	Y	C	Q	Q	Y	Y	T	N	P	.	R	T	F	G	Q	G	T	K	V	E	F	N																																
6267	Q	A	E	D	V	A	V	Y	Y	C	Q	Q	Y	Y	T	S	P	P	Y	T	F	G	Q	G	T	K	L	E	I	E																																

FIG. 13A

CDR H2 - Contact

CDR H2 - Chotria

CDR H2 - Kabat

CDR H3 - Contact

CDR H3 - Chothia

CDR H3 - Kabat

KabatNumber 102 103 104 105 106 107 108 109 110 111 112 113

4461	F	W	G	G	G	T	T	V	V
4624	I	W	G	E	G	T	K	V	V
4399	I	W	G	G	G	T	T	V	V
6267	V	W	G	G	G	T	T	V	V

**FIG. 13B**

Antibody	CDR L1	CDR L2	CDR L3	CDR H1	CDR H2	CDR H3
6078	RASQTISGWLA (SEQ ID NO:33)	KASTLES (SEQ ID NO:34)	QQYKSYSFN (SEQ ID NO:35)	SYDIN (SEQ ID NO:36)	WMNANSNGNTGYAQKFQG (SEQ ID NO:37)	SSLVRGALGRYFDL (SEQ ID NO:38)
6263	RASQTISGWLA (SEQ ID NO:39)	KASTLES (SEQ ID NO:40)	QQYKSYSFN (SEQ ID NO:41)	SYDIN (SEQ ID NO:42)	WMNANSNGNTGYAQKFQG (SEQ ID NO:43)	SSLVRGALGRYFDL (SEQ ID NO:44)
4450	RASQFVSRITSLA (SEQ ID NO:45)	ETSSRAT (SEQ ID NO:46)	HKYGSGPRT (SEQ ID NO:47)	NYDFI (SEQ ID NO:48)	WMNPNSYNTGYGQKFQG (SEQ ID NO:49)	AVRGQLLSEY (SEQ ID NO:50)
6297	RASQSVSSSYLA (SEQ ID NO:51)	DASSRAT (SEQ ID NO:52)	QKYGSTPRP (SEQ ID NO:53)	SYDIN (SEQ ID NO:54)	WMNPNSNGNTGYAQKFQG (SEQ ID NO:55)	ERWSKDTGHYYYGMDV (SEQ ID NO:56)
6239	RASLDITNHLA (SEQ ID NO:57)	EASILQS (SEQ ID NO:58)	EKCNSPTPT (SEQ ID NO:59)	NYDIN (SEQ ID NO:60)	WMNPSSSGRTGYAPKFRG (SEQ ID NO:61)	GGGYDSSGNYHISGLDV (SEQ ID NO:62)
6232	RASQSVGAIVLA (SEQ ID NO:63)	GVSNRAT (SEQ ID NO:64)	QLYTSSRALT (SEQ ID NO:65)	AYAMN (SEQ ID NO:66)	SITKNDSLSYYADSVKG (SEQ ID NO:67)	LAARIMATDY (SEQ ID NO:68)
6259	RASQGIKRLG (SEQ ID NO:69)	PASTLES (SEQ ID NO:70)	LQDHNYPPT (SEQ ID NO:71)	YYSMI (SEQ ID NO:72)	SIDSSSRVLYYADSVKG (SEQ ID NO:73)	DGDDILSVYRGSGRPFDY (SEQ ID NO:74)
6292	RASQGIKRLG (SEQ ID NO:75)	PASTLES (SEQ ID NO:76)	LQDHNYPPT (SEQ ID NO:77)	YYSMI (SEQ ID NO:78)	SIDSSSRVRYTDSVKG (SEQ ID NO:79)	DGDDILSVYQSGSRPFDY (SEQ ID NO:80)
4462	RASQSVRTNVA (SEQ ID NO:81)	GASTRAS (SEQ ID NO:82)	LQYNTWPRT (SEQ ID NO:83)	TNDMS (SEQ ID NO:84)	TIIGDDTTTHYADSVRG (SEQ ID NO:85)	NSGIYSF (SEQ ID NO:86)
6265	RASQDIGSSLA (SEQ ID NO:87)	ATSTLOS (SEQ ID NO:88)	QQLNNYVHS (SEQ ID NO:89)	DYAMG (SEQ ID NO:90)	VVTGHSYRTHYADSVKG (SEQ ID NO:91)	RIWSYGDDSEFV (SEQ ID NO:92)
6253	RASQSIGDRLA (SEQ ID NO:93)	WASNLEG (SEQ ID NO:94)	QQYKSQWS (SEQ ID NO:95)	SYAMN (SEQ ID NO:96)	YISSIETIYADSVKG (SEQ ID NO:97)	DRLVDVPLSPNS (SEQ ID NO:98)
4497	KSSQSIFRTSRNKNLLN (SEQ ID NO:99)	WASTRKS (SEQ ID NO:100)	QQYFSPPYT (SEQ ID NO:101)	SFWMH (SEQ ID NO:102)	FTNNEGTTTAYADSVRG (SEQ ID NO:103)	GDGGLDD (SEQ ID NO:104)
4487	RASQFTNHLYN (SEQ ID NO:105)	VASNLOS (SEQ ID NO:106)	QQSYRTPT (SEQ ID NO:107)	SGYYN (SEQ ID NO:108)	YILSCAHTDIKASLGS (SEQ ID NO:109)	SGVYSKYSLDV (SEQ ID NO:110)

6263 has same CDR sequences as 6078.

**FIG. 14**

## CDR Sequences According to Kabat Definition are Underlined

## Light Chain

Kabat Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
6078	D	I	V	M	T	Q	S	P	S	I	L	S	A	S	V	G	D	R	V	T	I	T	C	R	A	S	Q	T	I	S	G	W	L	A	W	Y	Q	Q	K	P	A	E
6078.v2HC-Cys	D	I	V	M	T	Q	S	P	S	I	L	S	A	S	V	G	D	R	V	T	I	T	C	R	A	S	Q	T	I	S	G	W	L	A	W	Y	Q	Q	K	P	A	E
6078.v2LC-Cys	D	I	V	M	T	Q	S	P	S	I	L	S	A	S	V	G	D	R	V	T	I	T	C	R	A	S	Q	T	I	S	G	W	L	A	W	Y	Q	Q	K	P	A	E
6078.v3HC-Cys	D	I	V	M	T	Q	S	P	S	I	L	S	A	S	V	G	D	R	V	T	I	T	C	R	A	S	Q	T	I	S	G	W	L	A	W	Y	Q	Q	K	P	A	E
6078.v3LC-Cys	D	I	V	M	T	Q	S	P	S	I	L	S	A	S	V	G	D	R	V	T	I	T	C	R	A	S	Q	T	I	S	G	W	L	A	W	Y	Q	Q	K	P	A	E
6078.v4HC-Cys	D	I	V	M	T	Q	S	P	S	I	L	S	A	S	V	G	D	R	V	T	I	T	C	R	A	S	Q	T	I	S	G	W	L	A	W	Y	Q	Q	K	P	A	E
6078.v4LC-Cys	D	I	V	M	T	Q	S	P	S	I	L	S	A	S	V	G	D	R	V	T	I	T	C	R	A	S	Q	T	I	S	G	W	L	A	W	Y	Q	Q	K	P	A	E
6078.v4HCLC-Cys	D	I	V	M	T	Q	S	P	S	I	L	S	A	S	V	G	D	R	V	T	I	T	C	R	A	S	Q	T	I	S	G	W	L	A	W	Y	Q	Q	K	P	A	E

Kabat Number	CDRL2 - Contact																																											
	CDRL2 - Chothia																CDRL2 - Kabat																											
	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84		
6078	A	P	K	L	L	I	Y	X	A	S	T	L	E	S	G	V	P	S	R	F	S	G	S	G	S	G	S	G	T	E	F	T	L	T	I	S	S	L	Q	P	D	D	F	G
6078.v2HC-Cys	A	P	K	L	L	I	Y	X	A	S	T	L	E	S	G	V	P	S	R	F	S	G	S	G	S	G	S	G	T	E	F	T	L	T	I	S	S	L	Q	P	D	D	F	G
6078.v2LC-Cys	A	P	K	L	L	I	Y	X	A	S	T	L	E	S	G	V	P	S	R	F	S	G	S	G	S	G	S	G	T	E	F	T	L	T	I	S	S	L	Q	P	D	D	F	G
6078.v3HC-Cys	A	P	K	L	L	I	Y	X	A	S	T	L	E	S	G	V	P	S	R	F	S	G	S	G	S	G	S	G	T	E	F	T	L	T	I	S	S	L	Q	P	D	D	F	G
6078.v3LC-Cys	A	P	K	L	L	I	Y	X	A	S	T	L	E	S	G	V	P	S	R	F	S	G	S	G	S	G	S	G	T	E	F	T	L	T	I	S	S	L	Q	P	D	D	F	G
6078.v4HC-Cys	A	P	K	L	L	I	Y	X	A	S	T	L	E	S	G	V	P	S	R	F	S	G	S	G	S	G	S	G	T	E	F	T	L	T	I	S	S	L	Q	P	D	D	F	G
6078.v4LC-Cys	A	P	K	L	L	I	Y	X	A	S	T	L	E	S	G	V	P	S	R	F	S	G	S	G	S	G	S	G	T	E	F	T	L	T	I	S	S	L	Q	P	D	D	F	G
6078.v4HCLC-Cys	A	P	K	L	L	I	Y	X	A	S	T	L	E	S	G	V	P	S	R	F	S	G	S	G	S	G	S	G	T	E	F	T	L	T	I	S	S	L	Q	P	D	D	F	G

Kabat Number	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126
6078	I	Y	Y	C	Q	Q	Y	K	S	Y	S	P	N	P	C	Q	G	T	K	V	E	I	K	R	T	V	A	A	P	S	V	F	-	F	P	P	S	D	E	Q	L	K
6078.v2HC-Cys	I	Y	Y	C	Q	Q	Y	K	S	Y	S	P	N	P	C	Q	G	T	K	V	E	I	K	R	T	V	A	A	P	S	V	F	-	F	P	P	S	D	E	Q	L	K
6078.v2LC-Cys	I	Y	Y	C	Q	Q	Y	K	S	Y	S	P	N	P	C	Q	G	T	K	V	E	I	K	R	T	V	A	A	P	S	V	F	-	F	P	P	S	D	E	Q	L	K

## Constant Region, Eu Number

FIG. 15A-1

Kabat Number	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	
6078.v3HC-Cys	I	Y	Y	C	Q	Y	K	S	Y	S	Y	S	F	N	F	G	Q	G	T	K	V	E	I	K	R	T	V	A	A	P	S	V	F	I	T	P	P	S	D	E	Q	L	K
6078.v3LC-Cys	I	Y	Y	C	Q	Y	K	S	Y	S	Y	S	F	N	F	G	Q	G	T	K	V	E	I	K	R	T	V	A	A	P	S	V	F	I	T	P	P	S	D	E	Q	L	K
6078.v4HC-Cys	I	Y	Y	C	Q	Y	K	S	Y	S	Y	S	F	N	F	G	Q	G	T	K	V	E	I	K	R	T	V	A	A	P	S	V	F	I	T	P	P	S	D	E	Q	L	K
6078.v4LC-Cys	I	Y	Y	C	Q	Y	K	S	Y	S	Y	S	F	N	F	G	Q	G	T	K	V	E	I	K	R	T	V	A	A	P	S	V	F	I	T	P	P	S	D	E	Q	L	K
6078.v4HCLC-Cys	I	Y	Y	C	Q	Y	K	S	Y	S	Y	S	F	N	F	G	Q	G	T	K	V	E	I	K	R	T	V	A	A	P	S	V	F	I	T	P	P	S	D	E	Q	L	K
Eu Number	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	
6078	S	G	T	A	S	V	V	C	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	
6078.v2HC-Cys	S	G	T	A	S	V	V	C	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	
6078.v2LC-Cys	S	G	T	A	S	V	V	C	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	
6078.v3HC-Cys	S	G	T	A	S	V	V	C	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	
6078.v3LC-Cys	S	G	T	A	S	V	V	C	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	
6078.v4HC-Cys	S	G	T	A	S	V	V	C	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	
6078.v4LC-Cys	S	G	T	A	S	V	V	C	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	
6078.v4HCLC-Cys	S	G	T	A	S	V	V	C	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	
Eu Number	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	
6078	X	D	S	T	Y	S	L	S	S	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L
6078.v2HC-Cys	X	D	S	T	Y	S	L	S	S	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L
6078.v2LC-Cys	X	D	S	T	Y	S	L	S	S	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L
6078.v3HC-Cys	X	D	S	T	Y	S	L	S	S	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L
6078.v3LC-Cys	X	D	S	T	Y	S	L	S	S	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L
6078.v4HC-Cys	X	D	S	T	Y	S	L	S	S	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L
6078.v4LC-Cys	X	D	S	T	Y	S	L	S	S	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L
6078.v4HCLC-Cys	X	D	S	T	Y	S	L	S	S	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L
Eu Number	211	212	213	214																																							
6078	R	G	E	C																																							
6078.v2HC-Cys	R	G	E	C																																							
6078.v2LC-Cys	R	G	E	C																																							
6078.v3HC-Cys	R	G	E	C																																							
6078.v3LC-Cys	R	G	E	C																																							
6078.v4HC-Cys	R	G	E	C																																							
6078.v4LC-Cys	R	G	E	C																																							

FIG. 15A-2



## Heavy Chain

Kabat Number	CDR H1 - Contact										CDR H1 - Chothia										CDR H1 - Kabat																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
6078	Q	M	Q	I	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	E	A	S	G	Y	T	L	T	S	Y	D	I	N	W	V	R	Q	A	T	G
6078.v2HC-Cys	E	M	Q	I	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	E	A	S	G	Y	T	L	T	S	Y	D	I	N	W	V	R	Q	A	T	G
6078.v2LC-Cys	E	M	Q	I	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	E	A	S	G	Y	T	L	T	S	Y	D	I	N	W	V	R	Q	A	T	G
6078.v3HC-Cys	E	I	Q	I	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	E	A	S	G	Y	T	L	T	S	Y	D	I	N	W	V	R	Q	A	T	G
6078.v3LC-Cys	E	I	Q	I	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	E	A	S	G	Y	T	L	T	S	Y	D	I	N	W	V	R	Q	A	T	G
6078.v4HC-Cys	E	V	Q	I	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	E	A	S	G	Y	T	L	T	S	Y	D	I	N	W	V	R	Q	A	T	G
6078.v4LC-Cys	E	V	Q	I	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	E	A	S	G	Y	T	L	T	S	Y	D	I	N	W	V	R	Q	A	T	G
6078.v4HCLC-Cys	E	V	Q	I	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	E	A	S	G	Y	T	L	T	S	Y	D	I	N	W	V	R	Q	A	T	G

Kabat Number	CDR H2 - Contact										CDR H2 - Chothia										CDR H2 - Kabat																					
	43	44	45	46	47	48	49	50	51	52	52a	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	82a
6078	Q	G	P	E	W	M	G	W	M	N	A	N	S	G	N	T	G	Y	A	Q	K	F	Q	G	R	V	T	L	T	G	D	T	S	I	S	T	A	Y	M	E	L	S
6078.v2HC-Cys	Q	G	P	E	W	M	G	W	M	N	A	N	S	G	N	T	G	Y	A	Q	K	F	Q	G	R	V	T	L	T	G	D	T	S	I	S	T	A	Y	M	E	L	S
6078.v2LC-Cys	Q	G	P	E	W	M	G	W	M	N	A	N	S	G	N	T	G	Y	A	Q	K	F	Q	G	R	V	T	L	T	G	D	T	S	I	S	T	A	Y	M	E	L	S
6078.v3HC-Cys	Q	G	P	E	W	M	G	W	M	N	A	N	S	G	N	T	G	Y	A	Q	K	F	Q	G	R	V	T	L	T	G	D	T	S	I	S	T	A	Y	M	E	L	S
6078.v3LC-Cys	Q	G	P	E	W	M	G	W	M	N	A	N	S	G	N	T	G	Y	A	Q	K	F	Q	G	R	V	T	L	T	G	D	T	S	I	S	T	A	Y	M	E	L	S
6078.v4HC-Cys	Q	G	P	E	W	M	G	W	M	N	A	N	S	G	N	T	G	Y	A	Q	K	F	Q	G	R	V	T	L	T	G	D	T	S	I	S	T	A	Y	M	E	L	S
6078.v4LC-Cys	Q	G	P	E	W	M	G	W	M	N	A	N	S	G	N	T	G	Y	A	Q	K	F	Q	G	R	V	T	L	T	G	D	T	S	I	S	T	A	Y	M	E	L	S
6078.v4HCLC-Cys	Q	G	P	E	W	M	G	W	M	N	A	N	S	G	N	T	G	Y	A	Q	K	F	Q	G	R	V	T	L	T	G	D	T	S	I	S	T	A	Y	M	E	L	S

FIG. 15B-1

CDR H3 - Contact		CDR H3 - Chothia		CDR H3 - Kabat		Cons.	
Kabat Number	6078	Eu Number	6078	6078.v2HC-Cys	6078.v2LC-Cys	6078.v3HC-Cys	6078.v3LC-Cys
82b	S	120	T	K	G	P	S
82c	S	121	T	K	G	P	S
83	L	122	R	S	E	E	E
84	S	123	R	S	E	E	E
85	E	124	S	E	E	E	E
86	C	125	C	T	A	V	Y
87	T	126	T	A	V	Y	Y
88	A	127	A	V	Y	Y	Y
89	V	128	V	Y	Y	Y	Y
90	Y	129	Y	Y	Y	Y	Y
91	Y	130	Y	Y	Y	Y	Y
92	C	131	C	A	R	S	S
93	A	132	A	R	S	S	S
94	R	133	R	S	S	S	S
95	S	134	S	S	S	S	S
96	S	135	S	S	S	S	S
97	I	136	I	L	V	R	G
98	L	137	L	V	R	G	A
99	V	138	V	R	G	A	L
100	V	139	V	R	G	A	L
101	V	140	V	R	G	A	L
102	V	141	V	R	G	A	L
103	V	142	V	R	G	A	L
104	V	143	V	R	G	A	L
105	V	144	V	R	G	A	L
106	V	145	V	R	G	A	L
107	V	146	V	R	G	A	L
108	V	147	V	R	G	A	L
109	V	148	V	R	G	A	L
110	V	149	V	R	G	A	L
111	V	150	V	R	G	A	L
112	V	151	V	R	G	A	L
113	V	152	V	R	G	A	L
114	V	153	V	R	G	A	L
115	V	154	V	R	G	A	L
116	V	155	V	R	G	A	L
117	V	156	V	R	G	A	L
118	V	157	V	R	G	A	L
119	V	158	V	R	G	A	L
120	V	159	V	R	G	A	L
121	V	160	V	R	G	A	L
122	V	161	V	R	G	A	L
123	V	162	V	R	G	A	L
124	V	163	V	R	G	A	L
125	V	164	V	R	G	A	L
126	V	165	V	R	G	A	L
127	V	166	V	R	G	A	L
128	V	167	V	R	G	A	L
129	V	168	V	R	G	A	L
130	V	169	V	R	G	A	L
131	V	170	V	R	G	A	L
132	V	171	V	R	G	A	L
133	V	172	V	R	G	A	L
134	V	173	V	R	G	A	L
135	V	174	V	R	G	A	L
136	V	175	V	R	G	A	L
137	V	176	V	R	G	A	L
138	V	177	V	R	G	A	L
139	V	178	V	R	G	A	L
140	V	179	V	R	G	A	L
141	V	180	V	R	G	A	L
142	V	181	V	R	G	A	L
143	V	182	V	R	G	A	L
144	V	183	V	R	G	A	L
145	V	184	V	R	G	A	L
146	V	185	V	R	G	A	L
147	V	186	V	R	G	A	L
148	V	187	V	R	G	A	L
149	V	188	V	R	G	A	L
150	V	189	V	R	G	A	L
151	V	190	V	R	G	A	L
152	V	191	V	R	G	A	L
153	V	192	V	R	G	A	L
154	V	193	V	R	G	A	L
155	V	194	V	R	G	A	L
156	V	195	V	R	G	A	L
157	V	196	V	R	G	A	L
158	V	197	V	R	G	A	L
159	V	198	V	R	G	A	L
160	V	199	V	R	G	A	L
161	V	200	V	R	G	A	L
162	V	201	V	R	G	A	L
163	V	202	V	R	G	A	L
164	V	203	V	R	G	A	L
165	V	204	V	R	G	A	L
166	V	205	V	R	G	A	L
167	V	206	V	R	G	A	L
168	V	207	V	R	G	A	L
169	V	208	V	R	G	A	L
170	V	209	V	R	G	A	L
171	V	210	V	R	G	A	L
172	V	211	V	R	G	A	L
173	V	212	V	R	G	A	L
174	V	213	V	R	G	A	L
175	V	214	V	R	G	A	L
176	V	215	V	R	G	A	L
177	V	216	V	R	G	A	L
178	V	217	V	R	G	A	L
179	V	218	V	R	G	A	L
180	V	219	V	R	G	A	L
181	V	220	V	R	G	A	L
182	V	221	V	R	G	A	L
183	V	222	V	R	G	A	L
184	V	223	V	R	G	A	L
185	V	224	V	R	G	A	L
186	V	225	V	R	G	A	L
187	V	226	V	R	G	A	L
188	V	227	V	R	G	A	L
189	V	228	V	R	G	A	L
190	V	229	V	R	G	A	L
191	V	230	V	R	G	A	L
192	V	231	V	R	G	A	L
193	V	232	V	R	G	A	L
194	V	233	V	R	G	A	L
195	V	234	V	R	G	A	L
196	V	235	V	R	G	A	L
197	V	236	V	R	G	A	L
198	V	237	V	R	G	A	L
199	V	238	V	R	G	A	L
200	V	239	V	R	G	A	L
201	V	240	V	R	G	A	L
202	V	241	V	R	G	A	L
203	V	242	V	R	G	A	L
204	V	243	V	R	G	A	L
205	V	244	V	R	G	A	L
206	V	245	V	R	G	A	L
207	V	246	V	R	G	A	L
208	V	247	V	R	G	A	L
209	V	248	V	R	G	A	L
210	V	249	V	R	G	A	L
211	V	250	V	R	G	A	L
212	V	251	V	R	G	A	L
213	V	252	V	R	G	A	L
214	V	253	V	R	G	A	L
215	V	254	V	R	G	A	L
216	V	255	V	R	G	A	L
217	V	256	V	R	G	A	L
218	V	257	V	R	G	A	L
219	V	258	V	R	G	A	L
220	V	259	V	R	G	A	L
221	V	260	V	R	G	A	L
222	V	261	V	R	G	A	L
223	V	262	V	R	G	A	L
224	V	263	V	R	G	A	L
225	V	264	V	R	G	A	L
226	V	265	V	R	G	A	L
227	V	266	V	R	G	A	L
228	V	267	V	R	G	A	L
229	V	268	V	R	G	A	L
230	V	269	V	R	G	A	L
231	V	270	V	R	G	A	L
232	V	271	V	R	G	A	L
233	V	272	V	R	G	A	L
234	V	273	V	R	G	A	L
235	V	274	V	R	G	A	L
236	V	275	V	R	G	A	L
237	V	276	V	R	G	A	L
238	V	277	V	R	G	A	L
239	V	278	V	R	G	A	L
240	V	279	V	R	G	A	L
241	V	280	V	R	G	A	L
242	V	281	V	R	G	A	L
243	V	282	V	R	G	A	L
244	V	283	V	R	G	A	L
245	V	284	V	R	G	A	L
246	V	285	V	R	G	A	L
247	V	286	V	R	G	A	L
248	V	287	V	R	G	A	L
249	V	288	V	R	G	A	L
250	V	289	V	R	G	A	L
251	V	290	V	R	G	A	L
252	V	291	V	R	G	A	L
253	V	292	V	R	G	A	L
254	V	293	V	R	G	A	L
255	V	294	V	R	G	A	L
256	V	295	V	R	G	A	L
257	V	296	V	R	G	A	L
258	V	297	V	R	G	A	L
259	V	298	V	R	G	A	L
260	V	299	V	R	G	A	L
261	V	300	V	R	G	A	L
262	V	301	V	R	G	A	L
263	V	302	V	R	G	A	L
264	V	303	V	R	G	A	L
265	V	304	V	R	G	A	L
266	V	305	V	R	G	A	L
267	V	306	V	R	G	A	L
268	V	307	V	R	G	A	L
269	V	308	V	R	G	A	L
270	V	309	V	R	G	A	L
271	V	310	V	R	G	A	L
272	V	311	V	R	G	A	L
273	V	312	V	R	G	A	L
274	V	313	V	R	G	A	L
275	V	314	V	R	G	A	L
276	V	315	V	R	G	A	L
277	V	316	V	R	G	A	L
278	V	317	V	R	G	A	L
279	V	318	V	R	G	A	L
280	V	319	V	R	G	A	L
281	V	320	V	R	G	A	L
282	V	321	V	R	G	A	L
283	V	322	V	R	G	A	L
284	V	323	V	R	G	A	L
285	V	324	V	R	G	A	L
286	V	325	V	R	G	A	L
287	V	326	V	R	G	A	L
288	V	327	V	R	G	A	L
289	V	328	V	R	G	A	L
290	V	329	V	R	G	A	L
291	V	330	V	R	G	A	L
292	V	331	V	R	G	A	L
293	V	332	V	R	G	A	L
294	V	333	V	R	G	A	L
295	V	334	V	R	G	A	L
296	V	335	V	R	G	A	L
297	V	336	V	R	G	A	L
298	V	337	V	R	G	A	L
299	V	338	V	R	G	A	L
300	V	339	V	R	G	A	L

FIG. 15B-2

Eu Number	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	241	242	243	244	245
6078	H	K	P	S	N	T	K	V	I	K	K	V	E	P	K	S	C	D	K	T	H	I	C	E	P	C	P	A	E	I	L	L	G	G	P	S	V	P	L	P	P	P
6078.v2HC-Cys	H	K	P	S	N	T	K	V	I	K	K	V	E	P	K	S	C	D	K	T	H	I	C	E	P	C	P	A	E	I	L	L	G	G	P	S	V	P	L	P	P	P
6078.v2LC-Cys	H	K	P	S	N	T	K	V	I	K	K	V	E	P	K	S	C	D	K	T	H	I	C	E	P	C	P	A	E	I	L	L	G	G	P	S	V	P	L	P	P	P
6078.v3HC-Cys	H	K	P	S	N	T	K	V	I	K	K	V	E	P	K	S	C	D	K	T	H	I	C	E	P	C	P	A	E	I	L	L	G	G	P	S	V	P	L	P	P	P
6078.v3LC-Cys	H	K	P	S	N	T	K	V	I	K	K	V	E	P	K	S	C	D	K	T	H	I	C	E	P	C	P	A	E	I	L	L	G	G	P	S	V	P	L	P	P	P
6078.v4HC-Cys	H	K	P	S	N	T	K	V	I	K	K	V	E	P	K	S	C	D	K	T	H	I	C	E	P	C	P	A	E	I	L	L	G	G	P	S	V	P	L	P	P	P
6078.v4LC-Cys	H	K	P	S	N	T	K	V	I	K	K	V	E	P	K	S	C	D	K	T	H	I	C	E	P	C	P	A	E	I	L	L	G	G	P	S	V	P	L	P	P	P
6078.v4HCLC-Cys	H	K	P	S	N	T	K	V	I	K	K	V	E	P	K	S	C	D	K	T	H	I	C	E	P	C	P	A	E	I	L	L	G	G	P	S	V	P	L	P	P	P
Eu Number	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287
6078	K	P	K	D	T	L	M	I	S	R	T	P	E	V	I	C	V	V	V	D	V	S	H	E	J	P	E	V	K	F	H	N	V	V	D	G	V	E	V	H	N	A
6078.v2HC-Cys	K	P	K	D	T	L	M	I	S	R	T	P	E	V	I	C	V	V	V	D	V	S	H	E	J	P	E	V	K	F	H	N	V	V	D	G	V	E	V	H	N	A
6078.v2LC-Cys	K	P	K	D	T	L	M	I	S	R	T	P	E	V	I	C	V	V	V	D	V	S	H	E	J	P	E	V	K	F	H	N	V	V	D	G	V	E	V	H	N	A
6078.v3HC-Cys	K	P	K	D	T	L	M	I	S	R	T	P	E	V	I	C	V	V	V	D	V	S	H	E	J	P	E	V	K	F	H	N	V	V	D	G	V	E	V	H	N	A
6078.v3LC-Cys	K	P	K	D	T	L	M	I	S	R	T	P	E	V	I	C	V	V	V	D	V	S	H	E	J	P	E	V	K	F	H	N	V	V	D	G	V	E	V	H	N	A
6078.v4HC-Cys	K	P	K	D	T	L	M	I	S	R	T	P	E	V	I	C	V	V	V	D	V	S	H	E	J	P	E	V	K	F	H	N	V	V	D	G	V	E	V	H	N	A
6078.v4LC-Cys	K	P	K	D	T	L	M	I	S	R	T	P	E	V	I	C	V	V	V	D	V	S	H	E	J	P	E	V	K	F	H	N	V	V	D	G	V	E	V	H	N	A
6078.v4HCLC-Cys	K	P	K	D	T	L	M	I	S	R	T	P	E	V	I	C	V	V	V	D	V	S	H	E	J	P	E	V	K	F	H	N	V	V	D	G	V	E	V	H	N	A
Eu Number	288	289	290	291	292	293	294	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329
6078	K	T	K	P	R	E	E	Q	Y	N	S	T	Y	R	V	V	S	V	E	T	V	I	H	Q	J	W	L	N	G	K	E	Y	X	C	K	V	S	N	K	A	L	P
6078.v2HC-Cys	K	T	K	P	R	E	E	Q	Y	N	S	T	Y	R	V	V	S	V	E	T	V	I	H	Q	J	W	L	N	G	K	E	Y	X	C	K	V	S	N	K	A	L	P
6078.v2LC-Cys	K	T	K	P	R	E	E	Q	Y	N	S	T	Y	R	V	V	S	V	E	T	V	I	H	Q	J	W	L	N	G	K	E	Y	X	C	K	V	S	N	K	A	L	P
6078.v3HC-Cys	K	T	K	P	R	E	E	Q	Y	N	S	T	Y	R	V	V	S	V	E	T	V	I	H	Q	J	W	L	N	G	K	E	Y	X	C	K	V	S	N	K	A	L	P
6078.v3LC-Cys	K	T	K	P	R	E	E	Q	Y	N	S	T	Y	R	V	V	S	V	E	T	V	I	H	Q	J	W	L	N	G	K	E	Y	X	C	K	V	S	N	K	A	L	P
6078.v4HC-Cys	K	T	K	P	R	E	E	Q	Y	N	S	T	Y	R	V	V	S	V	E	T	V	I	H	Q	J	W	L	N	G	K	E	Y	X	C	K	V	S	N	K	A	L	P
6078.v4LC-Cys	K	T	K	P	R	E	E	Q	Y	N	S	T	Y	R	V	V	S	V	E	T	V	I	H	Q	J	W	L	N	G	K	E	Y	X	C	K	V	S	N	K	A	L	P
6078.v4HCLC-Cys	K	T	K	P	R	E	E	Q	Y	N	S	T	Y	R	V	V	S	V	E	T	V	I	H	Q	J	W	L	N	G	K	E	Y	X	C	K	V	S	N	K	A	L	P

FIG. 15B-3

Eu Number	330	331	332	333	334	335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365	366	367	368	369	370	371		
6078	A	P	I	E	K	T	I	S	K	A	K	G	Q	P	R	E	P	Q	V	Y	E	L	P	P	S	R	S	R	E	B	M	T	K	N	Q	V	S	L	T	C	L	V	K	G
6078.v2HC-Cys	A	P	I	E	K	T	I	S	K	A	K	G	Q	P	R	E	P	Q	V	Y	E	L	P	P	S	R	S	R	E	B	M	T	K	N	Q	V	S	L	T	C	L	V	K	G
6078.v2LC-Cys	A	P	I	E	K	T	I	S	K	A	K	G	Q	P	R	E	P	Q	V	Y	E	L	P	P	S	R	S	R	E	B	M	T	K	N	Q	V	S	L	T	C	L	V	K	G
6078.v3HC-Cys	A	P	I	E	K	T	I	S	K	A	K	G	Q	P	R	E	P	Q	V	Y	E	L	P	P	S	R	S	R	E	B	M	T	K	N	Q	V	S	L	T	C	L	V	K	G
6078.v3LC-Cys	A	P	I	E	K	T	I	S	K	A	K	G	Q	P	R	E	P	Q	V	Y	E	L	P	P	S	R	S	R	E	B	M	T	K	N	Q	V	S	L	T	C	L	V	K	G
6078.v4HC-Cys	A	P	I	E	K	T	I	S	K	A	K	G	Q	P	R	E	P	Q	V	Y	E	L	P	P	S	R	S	R	E	B	M	T	K	N	Q	V	S	L	T	C	L	V	K	G
6078.v4LC-Cys	A	P	I	E	K	T	I	S	K	A	K	G	Q	P	R	E	P	Q	V	Y	E	L	P	P	S	R	S	R	E	B	M	T	K	N	Q	V	S	L	T	C	L	V	K	G
6078.v4HCLC-Cys	A	P	I	E	K	T	I	S	K	A	K	G	Q	P	R	E	P	Q	V	Y	E	L	P	P	S	R	S	R	E	B	M	T	K	N	Q	V	S	L	T	C	L	V	K	G
Eu Number	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394	395	396	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413		
6078	F	Y	P	S	D	I	A	V	E	W	E	S	N	G	Q	F	E	N	H	Y	K	T	T	P	P	F	V	L	D	S	D	G	S	F	F	L	V	S	K	L	T	V	D	
6078.v2HC-Cys	F	Y	P	S	D	I	A	V	E	W	E	S	N	G	Q	F	E	N	H	Y	K	T	T	P	P	F	V	L	D	S	D	G	S	F	F	L	V	S	K	L	T	V	D	
6078.v2LC-Cys	F	Y	P	S	D	I	A	V	E	W	E	S	N	G	Q	F	E	N	H	Y	K	T	T	P	P	F	V	L	D	S	D	G	S	F	F	L	V	S	K	L	T	V	D	
6078.v3HC-Cys	F	Y	P	S	D	I	A	V	E	W	E	S	N	G	Q	F	E	N	H	Y	K	T	T	P	P	F	V	L	D	S	D	G	S	F	F	L	V	S	K	L	T	V	D	
6078.v3LC-Cys	F	Y	P	S	D	I	A	V	E	W	E	S	N	G	Q	F	E	N	H	Y	K	T	T	P	P	F	V	L	D	S	D	G	S	F	F	L	V	S	K	L	T	V	D	
6078.v4HC-Cys	F	Y	P	S	D	I	A	V	E	W	E	S	N	G	Q	F	E	N	H	Y	K	T	T	P	P	F	V	L	D	S	D	G	S	F	F	L	V	S	K	L	T	V	D	
6078.v4LC-Cys	F	Y	P	S	D	I	A	V	E	W	E	S	N	G	Q	F	E	N	H	Y	K	T	T	P	P	F	V	L	D	S	D	G	S	F	F	L	V	S	K	L	T	V	D	
6078.v4HCLC-Cys	F	Y	P	S	D	I	A	V	E	W	E	S	N	G	Q	F	E	N	H	Y	K	T	T	P	P	F	V	L	D	S	D	G	S	F	F	L	V	S	K	L	T	V	D	
Eu Number	414	415	416	417	418	419	420	421	422	423	424	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445	446											
6078	K	S	R	W	Q	Q	G	N	V	F	S	C	S	V	M	H	E	A	L	H	N	H	Y	T	Q	X	S	L	S	L	S	P	G											
6078.v2HC-Cys	K	S	R	W	Q	Q	G	N	V	F	S	C	S	V	M	H	E	A	L	H	N	H	Y	T	Q	X	S	L	S	L	S	P	G											
6078.v2LC-Cys	K	S	R	W	Q	Q	G	N	V	F	S	C	S	V	M	H	E	A	L	H	N	H	Y	T	Q	X	S	L	S	L	S	P	G											
6078.v3HC-Cys	K	S	R	W	Q	Q	G	N	V	F	S	C	S	V	M	H	E	A	L	H	N	H	Y	T	Q	X	S	L	S	L	S	P	G											
6078.v3LC-Cys	K	S	R	W	Q	Q	G	N	V	F	S	C	S	V	M	H	E	A	L	H	N	H	Y	T	Q	X	S	L	S	L	S	P	G											
6078.v4HC-Cys	K	S	R	W	Q	Q	G	N	V	F	S	C	S	V	M	H	E	A	L	H	N	H	Y	T	Q	X	S	L	S	L	S	P	G											
6078.v4LC-Cys	K	S	R	W	Q	Q	G	N	V	F	S	C	S	V	M	H	E	A	L	H	N	H	Y	T	Q	X	S	L	S	L	S	P	G											
6078.v4HCLC-Cys	K	S	R	W	Q	Q	G	N	V	F	S	C	S	V	M	H	E	A	L	H	N	H	Y	T	Q	X	S	L	S	L	S	P	G											

FIG. 15B-4

CDR Sequences According to Kabat Definition are Underlined  
Light Chain

		CDR L1 - Chothia		CDR H1 - Contact	
		CDR L1 - Kabat			
Kabat Number	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 27a 27b 27c 27d 27e 27f 28 29 30 31 32 33 34 35 36				
4497	D I Q L T Q S P D S L A V S I G E R A T I N C K S S Q S I F R T S R N K N L L N W Y				
4497.v6-HC-Cys	D I Q L T Q S P D S L A V S L G E R A T I N C K S S Q S I F R T S R N K N L L N W Y				
4497.v6-LC-Cys	D I Q L T Q S P D S L A V S L G E R A T I N C K S S Q S I F R T S R N K N L L N W Y				
4497.v6-HCLC-Cys	D I Q L T Q S P D S L A V S L G E R A T I N C K S S Q S I F R T S R N K N L L N W Y				
		CDR L2 - Contact			
		CDR L2 - Chothia			
		CDR L2 - Kabat			
Kabat Number	37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78				
4497	Q Q R P G Q P P R L L I H W A S T R R K S G V P D R F S G S G F G T D F T L I I T S L				
4497.v6-HC-Cys	Q Q R P G Q P P R L L I H W A S T R R K S G V P D R F S G S G F G T D F T L I I T S L				
4497.v6-LC-Cys	Q Q R P G Q P P R L L I H W A S T R R K S G V P D R F S G S G F G T D F T L I I T S L				
4497.v6-HCLC-Cys	Q Q R P G Q P P R L L I H W A S T R R K S G V P D R F S G S G F G T D F T L I I T S L				
		CDR L3 - Contact		Constant Region, Eu Number	
		CDR L3 - Chothia			
		CDR L3 - Kabat			
Kabat Number	79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120				
4497	Q A E D V A I Y Y C Q Q Y F S P P V T F G Q G T K L E I K R T V A A P S V F I F P P				
4497.v6-HC-Cys	Q A E D V A I Y Y C Q Q Y F S P P V T F G Q G T K L E I K R T V A A P S V F I F P P				
4497.v6-LC-Cys	Q A E D V A I Y Y C Q Q Y F S P P V T F G Q G T K L E I K R T V A A P S V F I F P P				
4497.v6-HCLC-Cys	Q A E D V A I Y Y C Q Q Y F S P P V T F G Q G T K L E I K R T V A A P S V F I F P P				

FIG. 16A-1

Eu Number	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162
4497	S	D	E	Q	I	K	S	G	T	A	S	V	V	C	L	L	N	N	F	Y	P	R	E	A	K	V	Q	W	X	V	D	N	A	L	Q	S	G	N	S	Q	E	S
4497.v6-HC-Cys	S	D	E	Q	I	K	S	G	T	A	S	V	V	C	L	L	N	N	F	Y	P	R	E	A	K	V	Q	W	X	V	D	N	A	L	Q	S	G	N	S	Q	E	S
4497.v8-LC-Cys	S	D	E	Q	I	K	S	G	T	A	S	V	V	C	L	L	N	N	F	Y	P	R	E	A	K	V	Q	W	X	V	D	N	A	L	Q	S	G	N	S	Q	E	S
4497.v8-HCLC-Cys	S	D	E	Q	I	K	S	G	T	A	S	V	V	C	L	L	N	N	F	Y	P	R	E	A	K	V	Q	W	X	V	D	N	A	L	Q	S	G	N	S	Q	E	S
Eu Number	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204
4497	V	T	E	Q	E	S	K	D	S	T	Y	S	L	S	S	T	L	T	L	S	K	A	D	Y	E	K	E	K	V	Y	A	C	E	V	T	H	Q	G	L	S	S	P
4497.v6-HC-Cys	V	T	E	Q	E	S	K	D	S	T	Y	S	L	S	S	T	L	T	L	S	K	A	D	Y	E	K	E	K	V	Y	A	C	E	V	T	H	Q	G	L	S	S	P
4497.v8-LC-Cys	V	T	E	Q	E	S	K	D	S	T	Y	S	L	S	S	T	L	T	L	S	K	A	D	Y	E	K	E	K	V	Y	A	C	E	V	T	H	Q	G	L	S	S	P
4497.v8-HCLC-Cys	V	T	E	Q	E	S	K	D	S	T	Y	S	L	S	S	T	L	T	L	S	K	A	D	Y	E	K	E	K	V	Y	A	C	E	V	T	H	Q	G	L	S	S	P
Eu Number	205	206	207	208	209	210	211	212	213	214																																
4497	V	T	K	S	F	H	R	G	E	C																																
4497.v6-HC-Cys	V	T	K	S	F	H	R	G	E	C																																
4497.v8-LC-Cys	V	T	K	S	F	H	R	G	E	C																																
4497.v8-HCLC-Cys	V	T	K	S	F	H	R	G	E	C																																

FIG. 16A-2

FIG. 16B-1

449736-HCLG-C/S  
K A K G Q P R E D Q V Y F L P P S R E E E N T K N Q V S I T C I V K G F Y P S D I A V



Eu Number	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394	395	396	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421
4497	E	W	E	S	N	G	Q	P	E	N	N	Y	K	T	T	P	P	V	L	D	S	D	G	S	F	F	L	Y	S	X	I	T	V	D	K	S	R	W	Q	Q	G	N
4497.v8-HC-Cys	E	W	E	S	N	G	Q	P	E	N	N	Y	K	T	T	P	P	V	L	D	S	D	G	S	F	F	L	Y	S	X	I	T	V	D	K	S	R	W	Q	Q	G	N
4497.v8-LC-Cys	E	W	E	S	N	G	Q	P	E	N	N	Y	K	T	T	P	P	V	L	D	S	D	G	S	F	F	L	Y	S	X	I	T	V	D	K	S	R	W	Q	Q	G	N
4497.v8-HCLC-Cys	E	W	E	S	N	G	Q	P	E	N	N	Y	K	T	T	P	P	V	L	D	S	D	G	S	F	F	L	Y	S	X	I	T	V	D	K	S	R	W	Q	Q	G	N
Eu Number	422	423	424	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445	446																	
4497	V	F	S	C	S	V	M	H	E	A	L	H	N	H	Y	T	Q	K	S	I	S	L	S	P	G																	
4497.v8-HC-Cys	V	F	S	C	S	V	M	H	E	A	L	H	N	H	Y	T	Q	K	S	I	S	L	S	P	G																	
4497.v8-LC-Cys	V	F	S	C	S	V	M	H	E	A	L	H	N	H	Y	T	Q	K	S	I	S	L	S	P	G																	
4497.v8-HCLC-Cys	V	F	S	C	S	V	M	H	E	A	L	H	N	H	Y	T	Q	K	S	I	S	L	S	P	G																	

FIG. 16B-3

## CDR Sequences According to Kabat Definition are Underlined

## Light Chain

CDR Sequences According to Kabat Definition are Undefined																																													
Kabat Number	Light Chain																																												
	CDRL1 - Chothia																																												
	CDRL1 - Kabat																																												
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	27a	27b	27c	27d	27e	27f	28	29	30	31	32	33	34	35	36				
6078	Q	I	V	M	T	Q	S	P	S	I	L	S	A	S	V	G	D	R	V	T	I	T	C	R	A	S	Q	.	.	.	.	.	.	.	T	I	S	G	G	L	A	W	Y		
6263	Q	I	Q	L	T	Q	S	P	S	I	L	S	A	S	V	G	D	R	V	T	I	T	C	R	A	S	Q	.	F	.	.	.	.	.	.	T	I	S	G	G	L	A	W	Y	
4450	E	I	V	L	T	Q	S	P	S	I	L	S	A	S	V	G	D	R	V	T	I	T	C	R	A	S	Q	.	.	.	.	.	.	.	V	S	R	I	S	L	A	W	F		
6297	E	T	L	T	Q	S	P	S	P	G	T	L	S	L	S	P	G	E	R	A	T	L	S	C	R	A	S	Q	S	.	.	.	.	.	.	V	S	S	S	Y	L	A	W	Y	
6239	Q	V	V	M	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T	I	T	C	R	A	S	Q	S	.	.	.	.	.	.	.	D	I	T	N	H	L	A	W	Y	
6232	E	I	V	L	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T	I	T	C	R	A	S	Q	S	.	.	.	.	.	.	.	V	G	A	I	Y	L	A	W	Y	
6259	E	I	V	L	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T	I	T	C	R	A	S	Q	.	.	.	.	.	.	.	.	G	I	R	N	G	L	G	W	Y	
6292	Q	I	Q	M	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T	I	T	C	R	A	S	Q	.	.	.	.	.	.	.	.	.	G	I	R	N	G	L	G	W	Y
4462	Q	I	Q	M	T	Q	S	P	A	T	L	S	V	S	P	G	E	T	V	T	L	T	S	C	R	A	S	Q	.	.	.	.	.	.	.	.	S	V	R	I	N	V	A	W	Y
6265	Q	V	V	M	T	Q	S	P	S	T	L	S	A	S	V	G	D	R	V	T	I	T	C	R	A	S	Q	.	.	.	.	.	.	.	.	.	D	I	G	S	S	L	A	W	Y
6253	E	T	T	L	T	Q	S	P	S	T	L	S	A	S	V	G	D	R	V	T	I	T	C	R	A	S	Q	.	.	.	.	.	.	.	.	.	S	I	G	D	R	L	A	W	Y
4497	Q	I	Q	L	T	Q	S	P	D	S	L	A	V	S	L	G	E	R	A	T	I	N	C	K	S	S	Q	S	I	F	R	T	S	R	N	K	N	L	L	N	W	Y			
4487	Q	I	Q	L	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T	I	T	C	R	A	S	Q	.	.	.	.	.	.	.	.	.	F	T	N	H	Y	L	N	W	Y

## CDR L2 - Contact

CDR L2 - Chothia
CDR L2 - Kabat

Kabat Number	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78		
6078	Q	Q	K	P	A	E	A	E	A	P	K	L	L	I	Y	K	A	S	T	L	E	S	G	V	P	S	R	F	S	G	S	G	T	E	F	T	L	T	I	S	S	L		
6263	Q	Q	K	P	G	Q	P	P	K	L	L	L	I	Y	K	A	S	T	L	E	S	G	V	P	S	R	F	S	G	S	G	S	G	T	E	F	T	L	T	I	S	S	L	
4450	Q	Q	K	P	G	Q	P	P	K	L	L	L	I	Y	E	T	S	S	R	A	T	G	I	P	D	R	F	S	G	S	G	S	G	T	D	F	T	L	T	I	S	R	L	
6297	Q	Q	K	P	G	Q	P	P	K	L	L	L	I	Y	E	T	S	S	R	A	T	G	I	P	D	R	F	S	G	S	G	S	G	T	D	F	T	L	T	I	S	R	L	
6239	Q	Q	K	P	G	E	L	P	K	L	L	L	I	Y	E	A	S	I	L	Q	S	G	V	P	S	R	F	S	G	S	G	S	G	T	D	F	T	L	T	I	S	S	L	
6232	Q	Q	E	P	G	R	A	P	T	L	L	L	I	Y	P	A	S	T	L	E	S	G	V	P	S	R	F	S	G	S	G	S	G	D	R	D	F	T	L	T	I	T	S	L
6259	Q	Q	T	P	G	K	A	P	K	L	L	L	I	Y	P	A	S	T	L	E	S	G	V	P	S	R	F	S	G	S	G	S	G	D	R	D	F	T	L	T	I	T	S	L
6292	Q	Q	I	P	G	K	A	P	K	L	L	L	I	Y	P	A	S	T	L	E	S	G	V	P	S	R	F	S	G	S	G	S	G	T	E	F	T	L	T	I	T	S	L	
4462	R	H	K	A	G	Q	A	P	M	I	L	L	V	S	G	A	S	T	R	A	S	G	V	P	S	R	F	S	G	S	G	S	G	T	E	F	T	L	T	I	T	S	L	
6265	Q	Q	R	P	G	K	A	P	K	V	L	L	I	Y	A	T	S	T	L	Q	S	G	V	P	S	R	F	S	G	S	G	S	G	T	E	F	T	L	T	I	S	T	L	
6253	Q	Q	K	P	G	K	A	P	K	V	L	L	I	Y	H	A	S	T	R	K	S	G	V	P	S	R	F	S	G	S	G	S	G	T	E	F	F	A	L	T	I	S	G	L
4497	Q	Q	R	P	G	Q	P	P	R	L	L	L	I	H	A	S	T	R	K	S	G	V	P	S	R	F	S	G	S	G	S	G	T	E	F	T	L	T	I	T	S	C	L	
4487	Q	H	K	P	G	R	A	P	K	L	L	L	I	S	V	A	S	N	L	Q	S	G	V	P	S	R	F	T	G	S	E	S	G	T	D	F	T	L	T	I	S	C	L	

FIG. 17A-1

CDR L3 - Contact			CDR L3 - Chothia			CDR L3 - Kabat			Constant Region, Eu Number																																			
Kabat Number	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	95a	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119		
6078	Q	P	D	D	F	G	I	Y	Y	C	Q	Q	Q	Y	K	S	V	S	.	F	H	F	G	Q	G	T	K	V	E	I	K	R	E	V	A	A	P	S	V	F	I	F	P	
6263	Q	P	D	D	F	G	I	Y	Y	C	Q	Q	Y	K	S	V	S	.	F	H	F	G	Q	G	T	K	V	E	I	K	R	E	V	A	A	P	S	V	F	I	F	P		
4450	E	P	D	D	F	A	M	Y	Y	C	H	K	Y	K	S	G	P	.	R	T	P	F	G	Q	G	T	K	V	E	I	K	R	E	V	A	A	P	S	V	F	I	F	P	
6297	E	P	D	D	F	A	V	Y	Y	C	Q	K	Y	G	S	G	P	.	R	T	P	F	G	Q	G	T	K	V	E	I	K	R	E	V	A	A	P	S	V	F	I	F	P	
6239	Q	P	D	D	F	A	T	Y	Y	C	E	K	Y	G	S	G	P	.	R	T	P	F	G	Q	G	T	K	V	E	I	K	R	E	V	A	A	P	S	V	F	I	F	P	
6232	E	P	D	D	F	A	V	Y	Y	C	Q	L	Y	T	S	S	R	A	L	T	P	F	G	Q	G	T	K	V	E	I	K	R	E	V	A	A	P	S	V	F	I	F	P	
6259	Q	P	D	D	F	A	T	Y	Y	C	L	Q	D	H	N	Y	P	.	P	T	P	F	G	Q	G	T	K	V	E	I	K	R	E	V	A	A	P	S	V	F	I	F	P	
6292	Q	P	D	D	F	A	T	Y	Y	C	L	Q	D	H	N	Y	P	.	P	T	P	F	G	Q	G	T	K	V	E	I	K	R	E	V	A	A	P	S	V	F	I	F	P	
4462	Q	S	E	D	F	A	V	Y	Y	C	L	Q	Y	N	T	N	P	.	R	T	P	F	G	Q	G	T	K	V	E	I	K	R	E	V	A	A	P	S	V	F	I	F	P	
6265	Q	P	D	D	F	A	T	Y	Y	C	Q	Q	L	N	N	Y	V	.	E	S	P	F	G	Q	G	T	K	V	E	I	K	R	E	V	A	A	P	S	V	F	I	F	P	
6253	Q	P	D	D	L	A	T	Y	Y	C	Q	Q	Q	Y	K	S	Q	.	V	S	P	F	G	Q	G	T	K	V	E	I	K	R	E	V	A	A	P	S	V	F	I	F	P	
4497	Q	A	E	D	V	A	I	Y	Y	C	Q	Q	Q	Y	F	S	P	P	.	Y	T	P	F	G	Q	G	T	K	V	E	I	K	R	E	V	A	A	P	S	V	F	I	F	P
4487	Q	P	E	D	F	A	T	Y	Y	C	Q	Q	S	Y	R	T	P	.	Y	T	P	F	G	Q	G	T	K	V	E	I	K	R	E	V	A	A	P	S	V	F	I	F	P	

Eu Number	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161
6078	P	S	D	E	Q	L	X	S	G	T	A	S	V	V	C	L	L	N	H	F	Y	P	R	E	A	K	V	Q	W	K	V	D	N	A	L	Q	S	G	N	S	Q	E
6263	P	S	D	E	Q	L	X	S	G	T	A	S	V	V	C	L	L	N	H	F	Y	P	R	E	A	K	V	Q	W	K	V	D	N	A	L	Q	S	G	N	S	Q	E
4450	P	S	D	E	Q	L	X	S	G	T	A	S	V	V	C	L	L	N	H	F	Y	P	R	E	A	K	V	Q	W	K	V	D	N	A	L	Q	S	G	N	S	Q	E
6297	P	S	D	E	Q	L	X	S	G	T	A	S	V	V	C	L	L	N	H	F	Y	P	R	E	A	K	V	Q	W	K	V	D	N	A	L	Q	S	G	N	S	Q	E
6239	P	S	D	E	Q	L	X	S	G	T	A	S	V	V	C	L	L	N	H	F	Y	P	R	E	A	K	V	Q	W	K	V	D	N	A	L	Q	S	G	N	S	Q	E
6232	P	S	D	E	Q	L	X	S	G	T	A	S	V	V	C	L	L	N	H	F	Y	P	R	E	A	K	V	Q	W	K	V	D	N	A	L	Q	S	G	N	S	Q	E
6259	P	S	D	E	Q	L	X	S	G	T	A	S	V	V	C	L	L	N	H	F	Y	P	R	E	A	K	V	Q	W	K	V	D	N	A	L	Q	S	G	N	S	Q	E
6292	P	S	D	E	Q	L	X	S	G	T	A	S	V	V	C	L	L	N	H	F	Y	P	R	E	A	K	V	Q	W	K	V	D	N	A	L	Q	S	G	N	S	Q	E
4462	P	S	D	E	Q	L	X	S	G	T	A	S	V	V	C	L	L	N	H	F	Y	P	R	E	A	K	V	Q	W	K	V	D	N	A	L	Q	S	G	N	S	Q	E
6265	P	S	D	E	Q	L	X	S	G	T	A	S	V	V	C	L	L	N	H	F	Y	P	R	E	A	K	V	Q	W	K	V	D	N	A	L	Q	S	G	N	S	Q	E
6253	P	S	D	E	Q	L	X	S	G	T	A	S	V	V	C	L	L	N	H	F	Y	P	R	E	A	K	V	Q	W	K	V	D	N	A	L	Q	S	G	N	S	Q	E
4497	P	S	D	E	Q	L	X	S	G	T	A	S	V	V	C	L	L	N	H	F	Y	P	R	E	A	K	V	Q	W	K	V	D	N	A	L	Q	S	G	N	S	Q	E
4487	P	S	D	E	Q	L	X	S	G	T	A	S	V	V	C	L	L	N	H	F	Y	P	R	E	A	K	V	Q	W	K	V	D	N	A	L	Q	S	G	N	S	Q	E

FIG. 17A-2

Eu Number 162 163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198 199 200 201 202 203

6078 S V T E Q D S K D S T Y S L S S E L T L S K A D Y E K H K V Y A C E V T H Q G L S S

6263 S V T E Q D S K D S T Y S L S S E L T L S K A D Y E K H K V Y A C E V T H Q G L S S

4450 S V T E Q D S K D S T Y S L S S E L T L S K A D Y E K H K V Y A C E V T H Q G L S S

6297 S V T E Q D S K D S T Y S L S S E L T L S K A D Y E K H K V Y A C E V T H Q G L S S

6239 S V T E Q D S K D S T Y S L S S E L T L S K A D Y E K H K V Y A C E V T H Q G L S S

6232 S V T E Q D S K D S T Y S L S S E L T L S K A D Y E K H K V Y A C E V T H Q G L S S

6259 S V T E Q D S K D S T Y S L S S E L T L S K A D Y E K H K V Y A C E V T H Q G L S S

4462 S V T E Q D S K D S T Y S L S S E L T L S K A D Y E K H K V Y A C E V T H Q G L S S

6265 S V T E Q D S K D S T Y S L S S E L T L S K A D Y E K H K V Y A C E V T H Q G L S S

6253 S V T E Q D S K D S T Y S L S S E L T L S K A D Y E K H K V Y A C E V T H Q G L S S

4497 S V T E Q D S K D S T Y S L S S E L T L S K A D Y E K H K V Y A C E V T H Q G L S S

4487 S V T E Q D S K D S T Y S L S S E L T L S K A D Y E K H K V Y A C E V T H Q G L S S

Eu Number 204 205 206 207 208 209 210 211 212 213 214

6078 P V T X S F N R G E C

6263 P V T X S F N R G E C

4450 P V T X S F N R G E C

6297 P V T X S F N R G E C

6239 P V T X S F N R G E C

6232 P V T X S F N R G E C

6259 P V T X S F N R G E C

6292 P V T X S F N R G E C

4462 P V T X S F N R G E C

6265 P V T X S F N R G E C

6253 P V T X S F N R G E C

4497 P V T X S F N R G E C

4487 P V T X S F N R G E C \*

\* Light chain Eu position 205 marked by asterisk can be changed to Cys for drug conjugation.

**FIG. 17A-3**

## Heavy Chain

Heavy Chain	CDR H1 - Contact										CDR H1 - Chothia										CDR H1 - Kabat																									
Kabat Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42				
6078	Q	M	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	E	A	S	G	Y	T	L	T	S	Y	D	I	N	W	V	R	Q	A	T	G				
6263	Q	V	Q	L	Q	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	E	A	S	G	Y	T	L	T	S	Y	D	I	N	W	V	R	Q	A	T	G				
4450	E	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	K	A	S	G	Y	T	L	T	S	Y	D	I	N	W	V	R	Q	A	T	G				
6297	Q	V	Q	L	Q	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	E	A	S	G	Y	T	L	T	S	Y	D	I	N	W	V	R	Q	A	P	G				
6239	Q	V	Q	L	Q	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	E	A	S	G	Y	T	L	T	S	N	Y	D	I	N	W	V	R	Q	A	T	G			
6232	Q	I	T	L	K	E	S	G	G	G	L	I	K	P	G	G	S	L	R	L	S	C	A	T	S	G	E	F	P	F	S	A	Y	A	M	I	N	W	V	R	Q	A	P	G		
6259	E	V	Q	L	V	Q	S	G	G	G	L	V	K	P	G	E	S	L	R	L	S	C	A	A	S	G	E	F	S	F	D	Y	S	M	I	N	W	V	R	Q	A	P	G			
6292	Q	V	Q	L	Q	Q	S	G	G	G	L	V	N	P	G	E	S	L	R	L	S	C	A	A	S	G	E	F	S	F	N	Y	S	M	I	N	W	V	R	Q	A	P	G			
4462	Q	V	Q	L	Q	Q	S	G	G	G	L	E	Q	P	G	G	S	L	R	L	S	C	A	A	S	G	E	F	T	F	N	T	N	D	M	S	W	V	R	Q	A	P	G			
6265	E	V	Q	L	V	Q	S	G	G	G	L	V	Q	P	G	G	S	L	R	L	S	C	A	A	S	G	E	F	T	F	S	S	D	Y	A	M	N	W	V	R	Q	A	P	G		
6253	E	V	Q	L	V	Q	S	G	G	G	L	V	Q	P	G	G	S	L	R	L	S	C	A	A	S	G	E	F	T	F	S	S	F	R	M	E	N	W	V	R	Q	A	P	G		
4497	E	V	Q	L	V	E	S	G	G	G	L	V	Q	V	Q	P	G	G	S	L	R	L	S	C	A	A	S	G	E	F	T	F	N	S	F	R	M	E	N	W	V	R	Q	A	P	G
4487	Q	M	Q	L	Q	E	S	G	P	G	L	V	K	P	S	E	T	L	S	L	S	C	S	V	S	G	A	S	S	A	S	S	C	G	Y	N	W	V	R	Q	T	P	G			

## CDR H2 - Contact

## CDR H2 - Chothia

## CDR H2 - Kabat

Kabat Number	43	44	45	46	47	48	49	50	51	52	52a	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	82a	
6078	Q	G	P	E	E	W	W	G	W	M	N	A	N	S	G	N	T	G	Y	A	Q	X	F	Q	G	R	V	T	L	T	G	D	T	S	I	S	T	A	Y	M	E	L	S
6263	Q	G	P	E	E	W	W	G	W	M	N	A	N	S	G	N	T	G	Y	A	Q	X	F	Q	G	R	V	T	L	T	G	D	T	S	I	S	T	A	Y	M	E	L	S
4450	Q	G	P	E	E	W	W	G	W	M	N	A	N	S	G	N	T	G	Y	A	Q	X	F	Q	G	R	V	T	L	T	G	D	T	S	I	S	T	A	Y	M	E	L	S
6297	Q	G	L	E	W	W	G	W	M	N	A	N	S	G	N	T	G	Y	A	Q	X	F	Q	G	R	V	T	L	T	G	D	T	S	I	S	T	A	Y	M	E	L	S	
6239	Q	G	L	E	W	W	G	W	M	N	A	N	S	G	N	T	G	Y	A	Q	X	F	Q	G	R	V	T	L	T	G	D	T	S	I	S	T	A	Y	M	E	L	S	
6232	K	G	L	E	W	V	S	S	I	T	K	N	S	D	S	L	L	Y	Y	A	D	S	V	K	G	R	F	T	I	S	R	D	N	A	Q	N	S	L	Y	L	Q	M	S
6259	K	G	L	E	W	V	S	S	I	T	K	N	S	D	S	L	L	Y	Y	A	D	S	V	K	G	R	F	T	I	S	R	D	N	A	Q	N	S	L	Y	L	Q	M	S
6292	K	G	L	E	W	V	S	S	I	T	K	N	S	D	S	L	L	Y	Y	A	D	S	V	K	G	R	F	T	I	S	R	D	N	A	Q	N	S	L	Y	L	Q	M	S
4462	K	G	L	E	W	V	S	S	I	T	K	N	S	D	S	L	L	Y	Y	A	D	S	V	K	G	R	F	T	I	S	R	D	N	A	Q	N	S	L	Y	L	Q	M	S
6265	K	G	L	E	W	V	S	S	I	T	K	N	S	D	S	L	L	Y	Y	A	D	S	V	K	G	R	F	T	I	S	R	D	N	A	Q	N	S	L	Y	L	Q	M	S
6253	K	G	L	E	W	V	S	S	I	T	K	N	S	D	S	L	L	Y	Y	A	D	S	V	K	G	R	F	T	I	S	R	D	N	A	Q	N	S	L	Y	L	Q	M	S
4497	K	G	L	V	W	I	S	F	T	N	N	E	G	T	T	A	Y	A	D	S	V	K	G	R	F	T	I	S	R	D	N	A	Q	N	S	L	Y	L	Q	M	S		
4487	G	G	L	E	W	I	A	Y	I	L	.	S	G	A	H	T	D	I	K	A	S	L	G	S	R	R	V	A	V	S	V	D	T	S	K	N	Q	V	T	L	R	L	S

FIG. 17B-1

CDR H3 - ConIact	
CDR H3 - Chothia	
CDR H3 - Kabat	
Kabat Number	82b 82c 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100 100a 100b 100c 100d 100e 100f 100g 100h 100i 100j 101 102 103 104 105 106 107 108 109 110 111 112
6078	S L R S E D T A V Y Y C A R S S I L V R G A L G R Y F . . . D E W G R G T E V T V S
6263	S L R S E D T A V Y Y C A G S S I L V R G A L G R Y F . . . D L W G R G T E V T V S
4450	S L T S E D T A V Y Y C A R A R E R G Q L L S . . . E Y W G Q Q G T E V T V S
6297	S L T S E D T A V Y Y C A T A R E R G Q L L S . . . E Y W G Q Q G T E V T V S
6239	S L T S E D T A V Y Y C A R C G G Y Y D S S G N Y H I S G L D V W G Q Q G T E V T V S
6232	S L R V E D T A V Y Y C A T L A A R I M A T . . . D Y W G Q Q G T E V T V S
6259	G L R V E D T A V Y Y C A R D G D D I L S V V Y P G S S G R R P F D Y W G Q Q G T E V T V S
6292	A L R V E D T A V Y Y C A R D G D D I L S V V Y P G S S G R R P F D Y W G Q Q G T E V T V S
4462	S L R V E D T A V Y Y C V K E S G I Y . . . S F W G Q Q G T E V T V S
6265	S L R A E D T A V Y Y C A K R I W S Y G D D S F . . . D V W G Q Q G T E V T V S
6253	S L R D E D T A V Y Y C A R D R L V D V P L S S P . . . N S W G Q Q G T E V T V S
4497	N L R G E D T A V Y Y C A R C D G L . . . D D W G Q Q G T E V T V S
4487	S V F A A D T A F Y Y C A R S S G V Y S E Y S L . . . D V W G Q Q G T E V T V S

## Constant Region, Eu Numbering System Used

Kabat Number	113 118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150 151 152 153 154 155 156 157 158
6078	S A S T K G P S V F P L A P S S K S T S G G T A A L G C L V K D Y F P E P V T V S
6263	S A S T K G P S V F P L A P S S K S T S G G T A A L G C L V K D Y F P E P V T V S
4450	S A S T K G P S V F P L A P S S K S T S G G T A A L G C L V K D Y F P E P V T V S
6297	S A S T K G P S V F P L A P S S K S T S G G T A A L G C L V K D Y F P E P V T V S
6239	S A S T K G P S V F P L A P S S K S T S G G T A A L G C L V K D Y F P E P V T V S
6232	S A S T K G P S V F P L A P S S K S T S G G T A A L G C L V K D Y F P E P V T V S
6259	S A S T K G P S V F P L A P S S K S T S G G T A A L G C L V K D Y F P E P V T V S
4462	S A S T K G P S V F P L A P S S K S T S G G T A A L G C L V K D Y F P E P V T V S
6265	S A S T K G P S V F P L A P S S K S T S G G T A A L G C L V K D Y F P E P V T V S
6253	S A S T K G P S V F P L A P S S K S T S G G T A A L G C L V K D Y F P E P V T V S
4497	S A S T K G P S V F P L A P S S K S T S G G T A A L G C L V K D Y F P E P V T V S
4487	S A S T K G P S V F P L A P S S K S T S G G T A A L G C L V K D Y F P E P V T V S

FIG. 17B-2

Eu Number 159 160 161 162 163 164 155 156 157 158 169 170 171 172 173 174 175 176 177 178 179 180 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198 199 200  
6078 N S G A L T S G V H T F P A V L Q S S G L Y S L S S V V T V P S S S L G T Q T Y I C  
6263 N S G A L T S G V H T F P A V L Q S S G L Y S L S S V V T V P S S S L G T Q T Y I C  
4450 N S G A L T S G V H T F P A V L Q S S G L Y S L S S V V T V P S S S L G T Q T Y I C  
6297 N S G A L T S G V H T F P A V L Q S S G L Y S L S S V V T V P S S S L G T Q T Y I C  
6239 N S G A L T S G V H T F P A V L Q S S G L Y S L S S V V T V P S S S L G T Q T Y I C  
6232 N S G A L T S G V H T F P A V L Q S S G L Y S L S S V V T V P S S S L G T Q T Y I C  
6259 N S G A L T S G V H T F P A V L Q S S G L Y S L S S V V T V P S S S L G T Q T Y I C  
6292 N S G A L T S G V H T F P A V L Q S S G L Y S L S S V V T V P S S S L G T Q T Y I C  
4462 N S G A L T S G V H T F P A V L Q S S G L Y S L S S V V T V P S S S L G T Q T Y I C  
6265 N S G A L T S G V H T F P A V L Q S S G L Y S L S S V V T V P S S S L G T Q T Y I C  
6253 N S G A L T S G V H T F P A V L Q S S G L Y S L S S V V T V P S S S L G T Q T Y I C  
4497 N S G A L T S G V H T F P A V L Q S S G L Y S L S S V V T V P S S S L G T Q T Y I C  
4487 N S G A L T S G V H T F P A V L Q S S G L Y S L S S V V T V P S S S L G T Q T Y I C

Eu Number 201 202 203 204 205 206 207 208 209 210 211 212 213 214 215 216 217 218 219 220 221 222 223 224 225 226 227 228 229 230 231 232 233 234 235 236 237 238 239 240 241 242  
6078 N V N E K P S N T K V D K K V E P K S C D K T H T C P P C P A P E L L G G P S V P L  
6263 N V N E K P S N T K V D K K V E P K S C D K T H T C P P C P A P E L L G G P S V P L  
4450 N V N E K P S N T K V D K K V E P K S C D K T H T C P P C P A P E L L G G P S V P L  
6297 N V N E K P S N T K V D K K V E P K S C D K T H T C P P C P A P E L L G G P S V P L  
6239 N V N E K P S N T K V D K K V E P K S C D K T H T C P P C P A P E L L G G P S V P L  
6232 N V N E K P S N T K V D K K V E P K S C D K T H T C P P C P A P E L L G G P S V P L  
6259 N V N E K P S N T K V D K K V E P K S C D K T H T C P P C P A P E L L G G P S V P L  
4462 N V N E K P S N T K V D K K V E P K S C D K T H T C P P C P A P E L L G G P S V P L  
6265 N V N E K P S N T K V D K K V E P K S C D K T H T C P P C P A P E L L G G P S V P L  
6253 N V N E K P S N T K V D K K V E P K S C D K T H T C P P C P A P E L L G G P S V P L  
4497 N V N E K P S N T K V D K K V E P K S C D K T H T C P P C P A P E L L G G P S V P L  
4487 N V N E K P S N T K V D K K V E P K S C D K T H T C P P C P A P E L L G G P S V P L

FIG. 17B-3

Eu Number 243 244 245 246 247 248 249 250 251 252 253 254 255 256 257 258 259 260 261 262 263 264 265 266 267 268 269 270 271 272 273 274 275 276 277 278 279 280 281 282 283 284  
6078 F P P K P K D T L M I S R T P E V T C V V V D V S H E E D P E V K F M W Y V D G V E V  
6263 F P P K P K D T L M I S R T P E V T C V V V D V S H E E D P E V K F M W Y V D G V E V  
4450 F P P K P K D T L M I S R T P E V T C V V V D V S H E E D P E V K F M W Y V D G V E V  
6297 F P P K P K D T L M I S R T P E V T C V V V D V S H E E D P E V K F M W Y V D G V E V  
6239 F P P K P K D T L M I S R T P E V T C V V V D V S H E E D P E V K F M W Y V D G V E V  
6232 F P P K P K D T L M I S R T P E V T C V V V D V S H E E D P E V K F M W Y V D G V E V  
6259 F P P K P K D T L M I S R T P E V T C V V V D V S H E E D P E V K F M W Y V D G V E V  
6292 F P P K P K D T L M I S R T P E V T C V V V D V S H E E D P E V K F M W Y V D G V E V  
4462 F P P K P K D T L M I S R T P E V T C V V V D V S H E E D P E V K F M W Y V D G V E V  
6265 F P P K P K D T L M I S R T P E V T C V V V D V S H E E D P E V K F M W Y V D G V E V  
6253 F P P K P K D T L M I S R T P E V T C V V V D V S H E E D P E V K F M W Y V D G V E V  
4497 F P P K P K D T L M I S R T P E V T C V V V D V S H E E D P E V K F M W Y V D G V E V  
4487 F P P K P K D T L M I S R T P E V T C V V V D V S H E E D P E V K F M W Y V D G V E V

Eu Number 285 286 287 288 289 290 291 292 293 294 295 296 297 298 299 300 301 302 303 304 305 306 307 308 309 310 311 312 313 314 315 316 317 318 319 320 321 322 323 324 325 326  
6078 H N A K T K P R E E Q Y N S T Y R V Y S V L T V L H Q Q D W L N G K E Y X C K V S N X  
6263 H N A K T K P R E E Q Y N S T Y R V Y S V L T V L H Q Q D W L N G K E Y X C K V S N X  
4450 H N A K T K P R E E Q Y N S T Y R V Y S V L T V L H Q Q D W L N G K E Y X C K V S N X  
6297 H N A K T K P R E E Q Y N S T Y R V Y S V L T V L H Q Q D W L N G K E Y X C K V S N X  
6239 H N A K T K P R E E Q Y N S T Y R V Y S V L T V L H Q Q D W L N G K E Y X C K V S N X  
6232 H N A K T K P R E E Q Y N S T Y R V Y S V L T V L H Q Q D W L N G K E Y X C K V S N X  
6259 H N A K T K P R E E Q Y N S T Y R V Y S V L T V L H Q Q D W L N G K E Y X C K V S N X  
6292 H N A K T K P R E E Q Y N S T Y R V Y S V L T V L H Q Q D W L N G K E Y X C K V S N X  
4462 H N A K T K P R E E Q Y N S T Y R V Y S V L T V L H Q Q D W L N G K E Y X C K V S N X  
6265 H N A K T K P R E E Q Y N S T Y R V Y S V L T V L H Q Q D W L N G K E Y X C K V S N X  
6253 H N A K T K P R E E Q Y N S T Y R V Y S V L T V L H Q Q D W L N G K E Y X C K V S N X  
4497 H N A K T K P R E E Q Y N S T Y R V Y S V L T V L H Q Q D W L N G K E Y X C K V S N X  
4487 H N A K T K P R E E Q Y N S T Y R V Y S V L T V L H Q Q D W L N G K E Y X C K V S N X

FIG. 17B-4



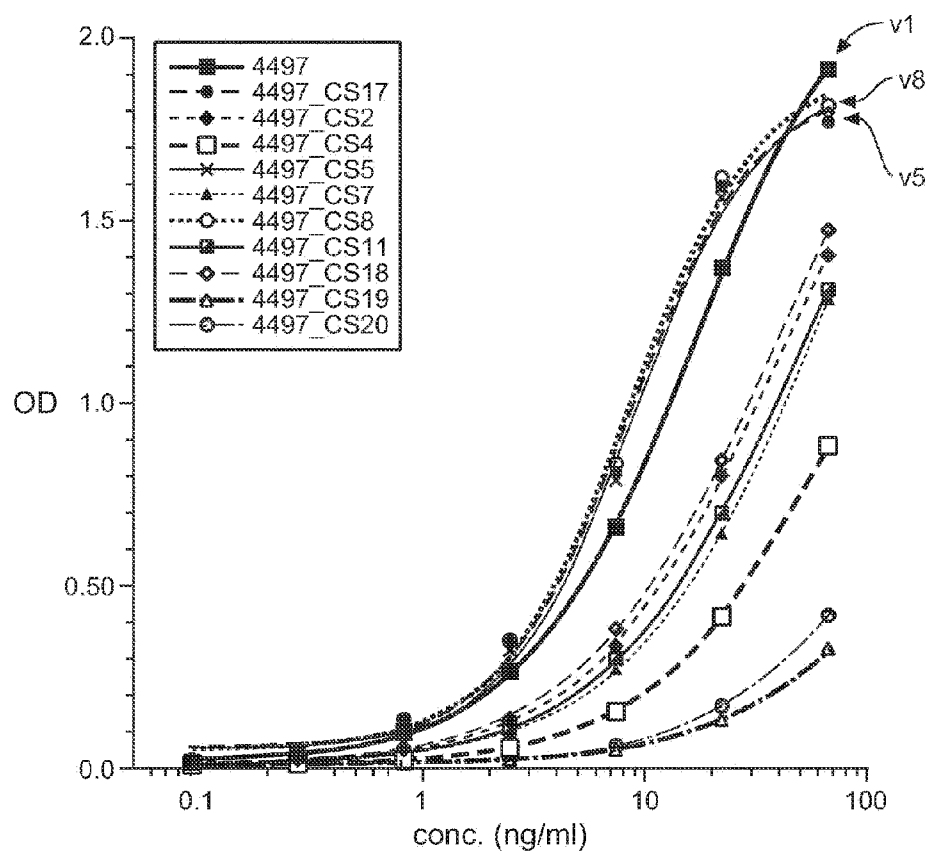
EU Number 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341 342 343 344 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360 361 362 363 364 365 366 367 368  
6078 A L P A P I I X T I S X A K G Q P R E P Q V Y C L P P P S E E E M T K N Q V S L T C L  
6263 A L P A P I I X K T I S K A K G Q P R E P Q V Y C L P P P S E E E M T K N Q V S L T C L  
4450 A L P A P I I X K T I S K A K G Q P R E P Q V Y C L P P P S E E E M T K N Q V S L T C L  
6297 A L P A P I I X K T I S K A K G Q P R E P Q V Y C L P P P S E E E M T K N Q V S L T C L  
6239 A L P A P I I X K T I S K A K G Q P R E P Q V Y C L P P P S E E E M T K N Q V S L T C L  
6232 A L P A P I I X K T I S K A K G Q P R E P Q V Y C L P P P S E E E M T K N Q V S L T C L  
6259 A L P A P I I X K T I S K A K G Q P R E P Q V Y C L P P P S E E E M T K N Q V S L T C L  
6292 A L P A P I I X K T I S K A K G Q P R E P Q V Y C L P P P S E E E M T K N Q V S L T C L  
4462 A L P A P I I X K T I S K A K G Q P R E P Q V Y C L P P P S E E E M T K N Q V S L T C L  
6265 A L P A P I I X K T I S K A K G Q P R E P Q V Y C L P P P S E E E M T K N Q V S L T C L  
6253 A L P A P I I X K T I S K A K G Q P R E P Q V Y C L P P P S E E E M T K N Q V S L T C L  
4497 A L P A P I I X K T I S K A K G Q P R E P Q V Y C L P P P S E E E M T K N Q V S L T C L  
4487 A L P A P I I X K T I S K A K G Q P R E P Q V Y C L P P P S E E E M T K N Q V S L T C L

EU Number 369 370 371 372 373 374 375 376 377 378 379 380 381 382 383 384 385 386 387 388 389 390 391 392 393 394 395 396 397 398 399 400 401 402 403 404 405 406 407 408 409 410  
6078 V K G F Y P S D I A V E W E S N G Q P E N N Y X T T P P P V L D S D G S Z F L Y S K L  
6263 V K G F Y P S D I A V E W E S N G Q P E N N Y X T T P P P V L D S D G S Z F L Y S K L  
4450 V K G F Y P S D I A V E W E S N G Q P E N N Y X T T P P P V L D S D G S Z F L Y S K L  
6297 V K G F Y P S D I A V E W E S N G Q P E N N Y X T T P P P V L D S D G S Z F L Y S K L  
6239 V K G F Y P S D I A V E W E S N G Q P E N N Y X T T P P P V L D S D G S Z F L Y S K L  
6232 V K G F Y P S D I A V E W E S N G Q P E N N Y X T T P P P V L D S D G S Z F L Y S K L  
6259 V K G F Y P S D I A V E W E S N G Q P E N N Y X T T P P P V L D S D G S Z F L Y S K L  
4462 V K G F Y P S D I A V E W E S N G Q P E N N Y X T T P P P V L D S D G S Z F L Y S K L  
6265 V K G F Y P S D I A V E W E S N G Q P E N N Y X T T P P P V L D S D G S Z F L Y S K L  
6253 V K G F Y P S D I A V E W E S N G Q P E N N Y X T T P P P V L D S D G S Z F L Y S K L  
4497 V K G F Y P S D I A V E W E S N G Q P E N N Y X T T P P P V L D S D G S Z F L Y S K L  
4487 V K G F Y P S D I A V E W E S N G Q P E N N Y X T T P P P V L D S D G S Z F L Y S K L

FIG. 17B-5

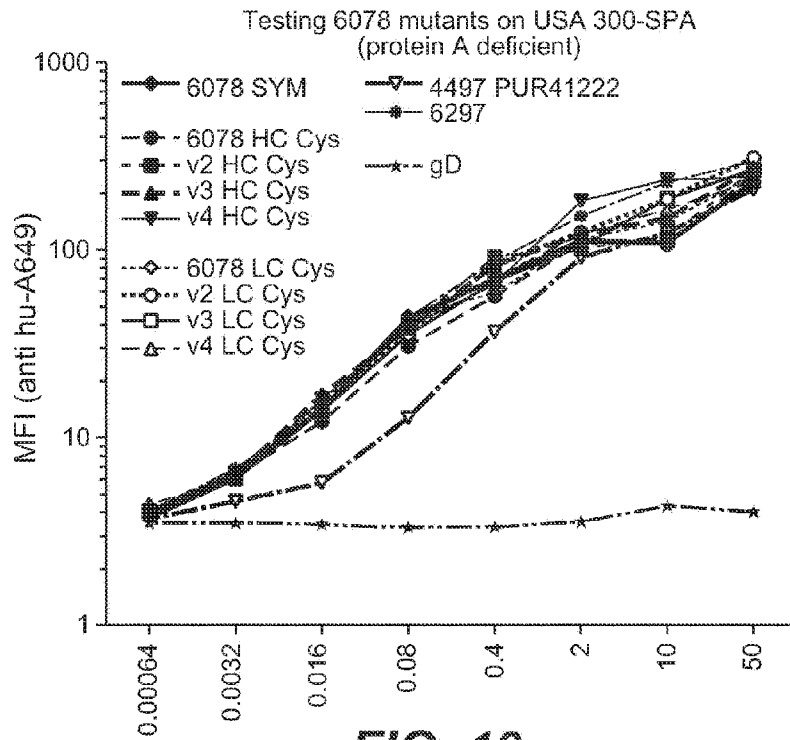
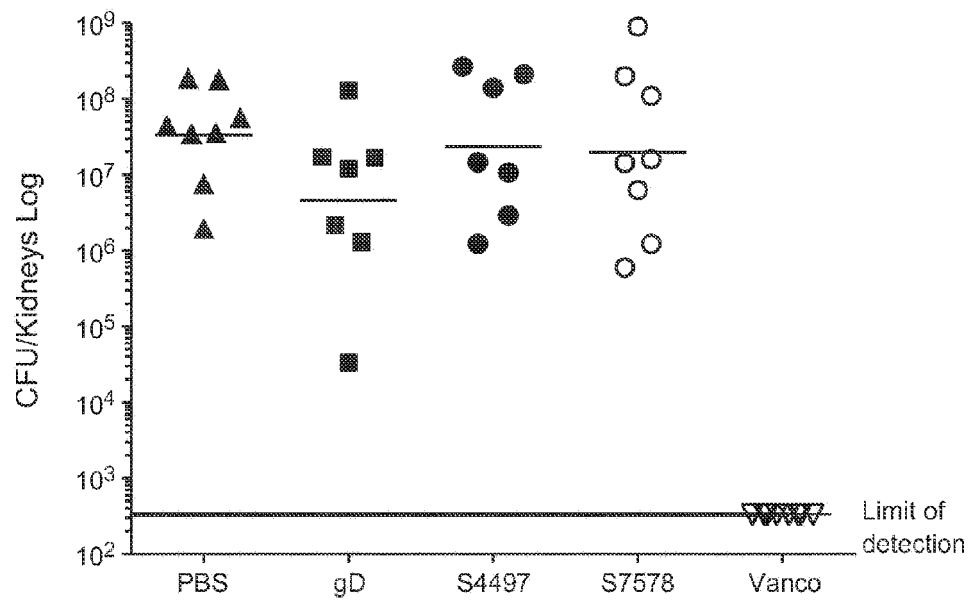
EU Number	411	412	413	414	415	416	417	418	419	420	421	422	423	424	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445	446
6078	T	V	D	K	S	R	W	Q	Q	G	N	V	F	S	C	S	V	M	H	E	A	L	H	N	H	Y	T	Q	K	S	L	S	L	S	P	G
6263	T	V	D	K	S	R	W	Q	Q	G	N	V	F	S	C	S	V	M	H	E	A	L	H	N	H	Y	T	Q	K	S	L	S	L	S	P	G
4450	T	V	D	K	S	R	W	Q	Q	G	N	V	F	S	C	S	V	M	H	E	A	L	H	N	H	Y	T	Q	K	S	L	S	L	S	P	G
6297	T	V	D	K	S	R	W	Q	Q	G	N	V	F	S	C	S	V	M	H	E	A	L	H	N	H	Y	T	Q	K	S	L	S	L	S	P	G
6239	T	V	D	K	S	R	W	Q	Q	G	N	V	F	S	C	S	V	M	H	E	A	L	H	N	H	Y	T	Q	K	S	L	S	L	S	P	G
6232	T	V	D	K	S	R	W	Q	Q	G	N	V	F	S	C	S	V	M	H	E	A	L	H	N	H	Y	T	Q	K	S	L	S	L	S	P	G
6259	T	V	D	K	S	R	W	Q	Q	G	N	V	F	S	C	S	V	M	H	E	A	L	H	N	H	Y	T	Q	K	S	L	S	L	S	P	G
6292	T	V	D	K	S	R	W	Q	Q	G	N	V	F	S	C	S	V	M	H	E	A	L	H	N	H	Y	T	Q	K	S	L	S	L	S	P	G
4462	T	V	D	K	S	R	W	Q	Q	G	N	V	F	S	C	S	V	M	H	E	A	L	H	N	H	Y	T	Q	K	S	L	S	L	S	P	G
6265	T	V	D	K	S	R	W	Q	Q	G	N	V	F	S	C	S	V	M	H	E	A	L	H	N	H	Y	T	Q	K	S	L	S	L	S	P	G
6253	T	V	D	K	S	R	W	Q	Q	G	N	V	F	S	C	S	V	M	H	E	A	L	H	N	H	Y	T	Q	K	S	L	S	L	S	P	G
4497	T	V	D	K	S	R	W	Q	Q	G	N	V	F	S	C	S	V	M	H	E	A	L	H	N	H	Y	T	Q	K	S	L	S	L	S	P	G
4487	T	V	D	K	S	R	W	Q	Q	G	N	V	F	S	C	S	V	M	H	E	A	L	H	N	H	Y	T	Q	K	S	L	S	L	S	P	G

FIG. 17B-6

**FIG. 18A**

	93	94	95	96	97	98	99	101	102	ELISA
WT (v1)	A	R	G	D	G	G	L	D	D	+++
v7				D	G				E	+
v2				D	G				Y	+
v4				D	A				D	+
v19				D	A				E	+/-
v8				E	G				D	+++
v20				E	G				E	+/-
v5				A	G				D	++
v11				A	G				E	+
v18				A	G				Y	+

**FIG. 18B**

**FIG. 19****FIG. 20**

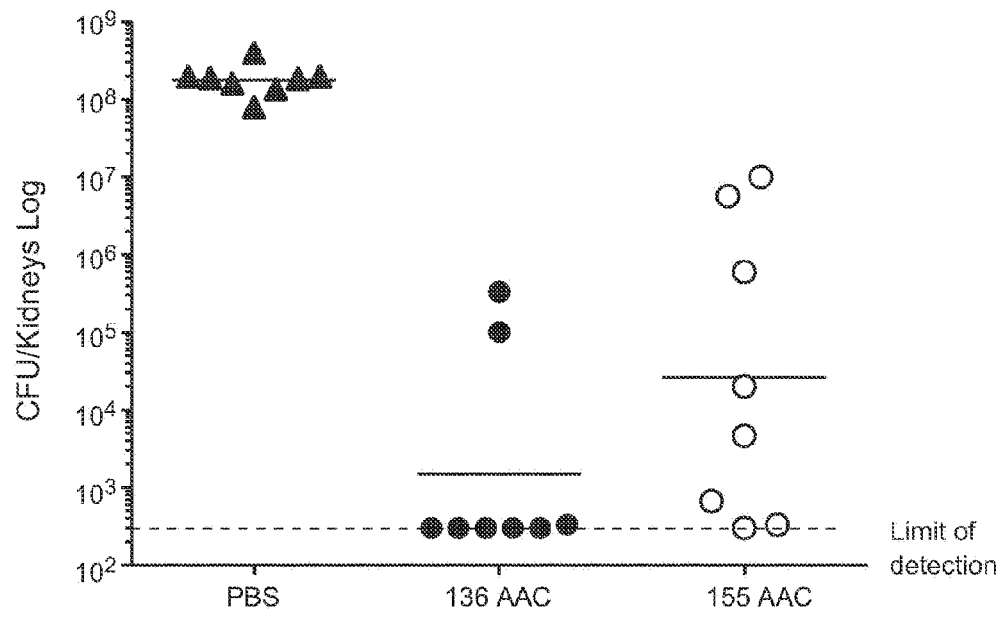
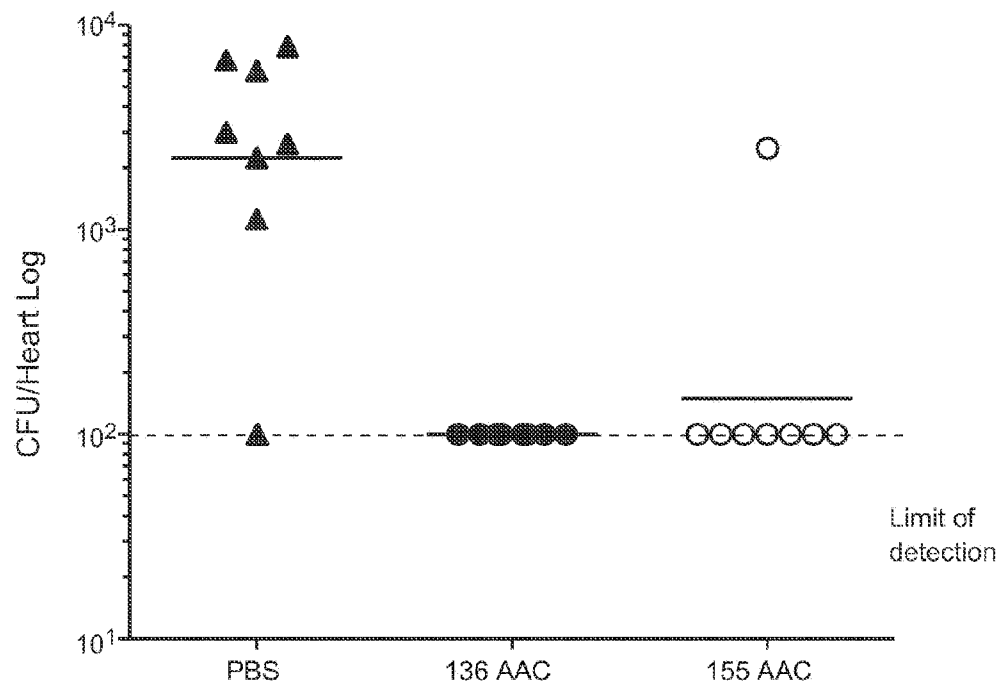
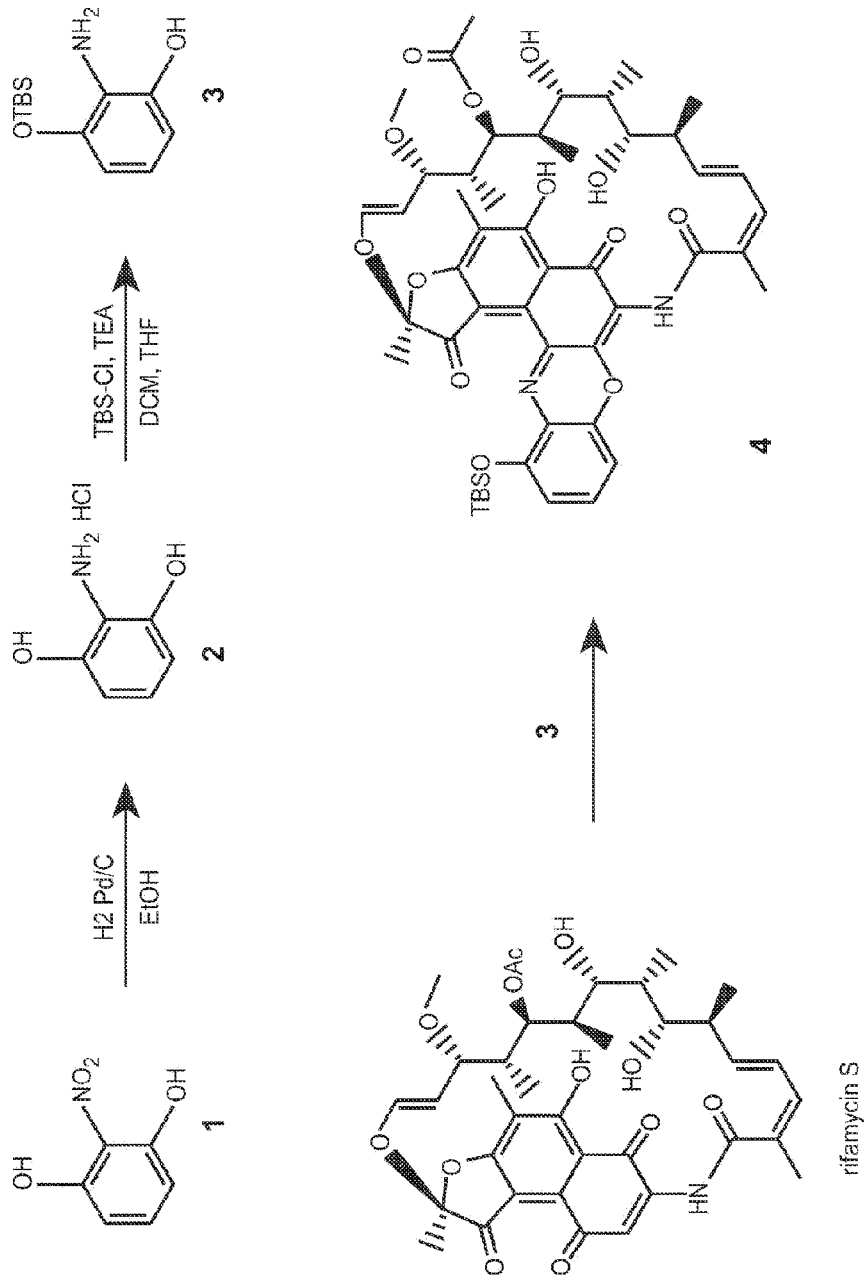
**FIG. 21****FIG. 22**

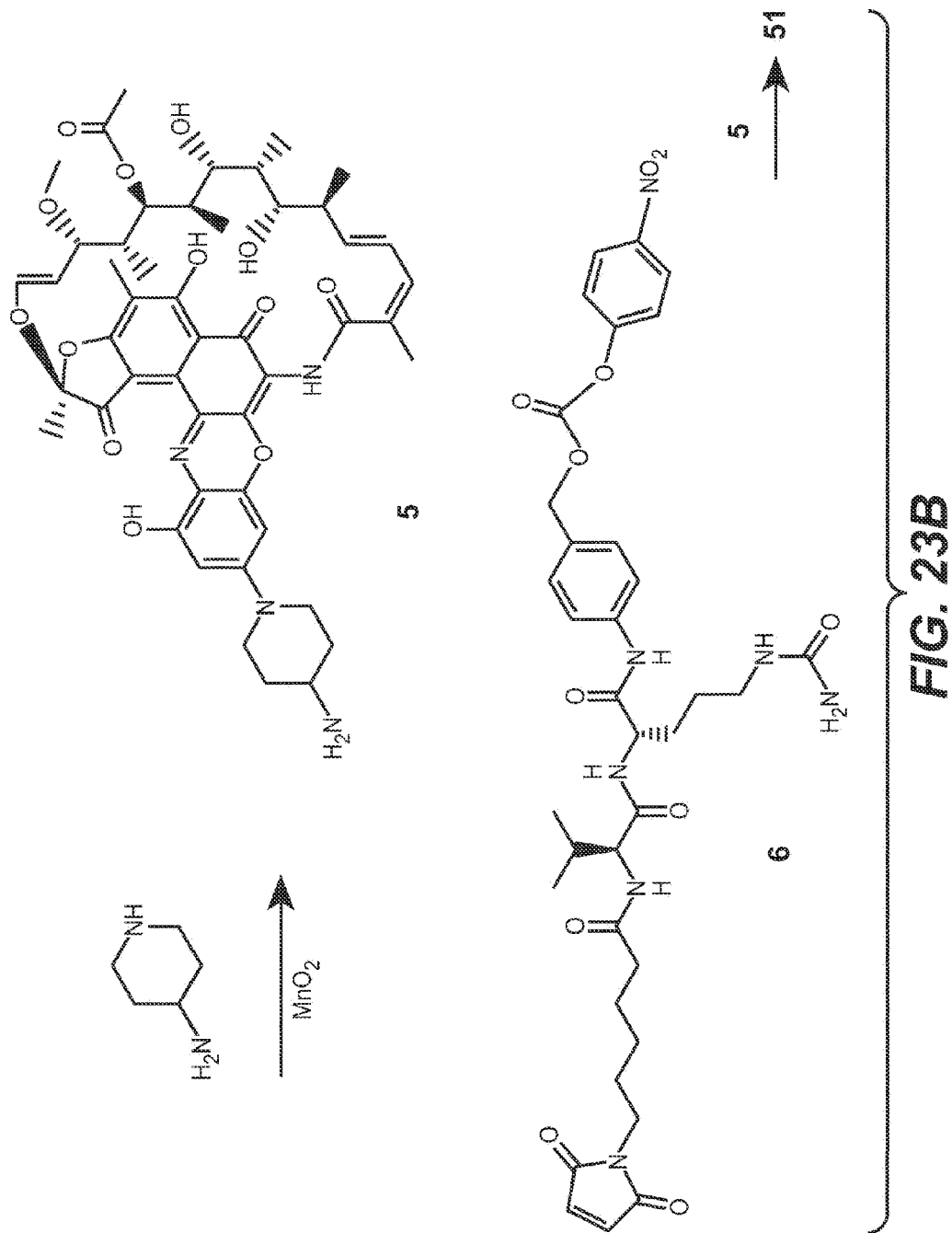
FIG. 23A

FIG. 23B

**FIG. 23**



**FIG. 23A**



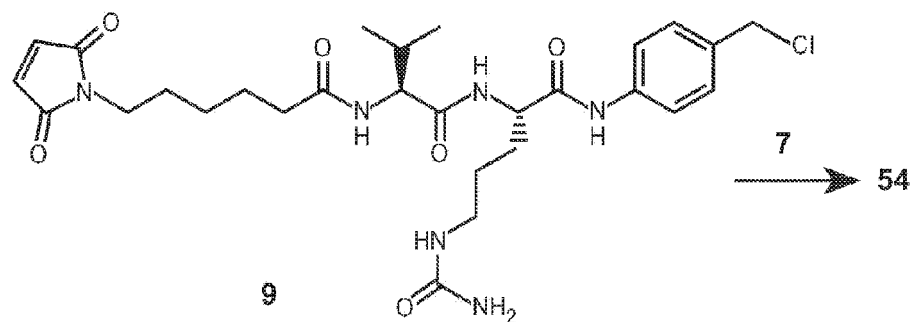
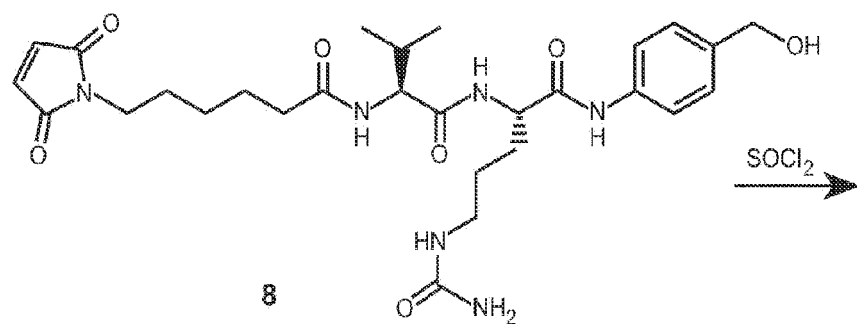
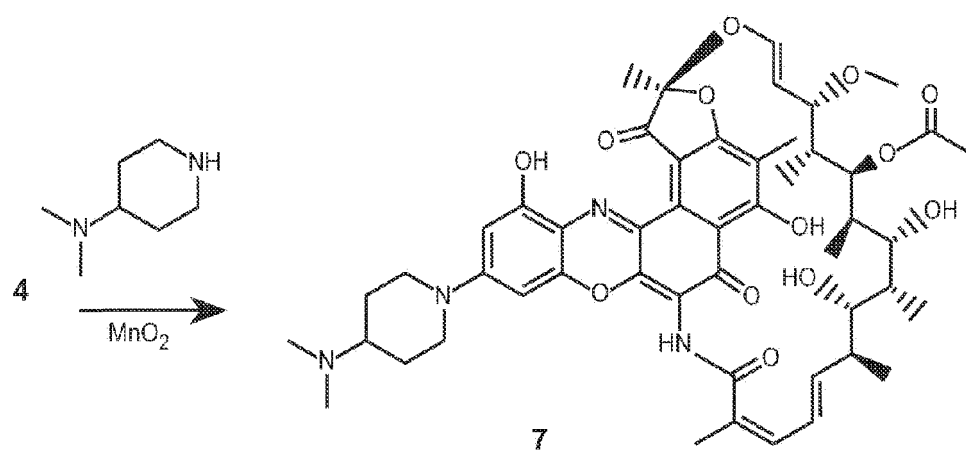
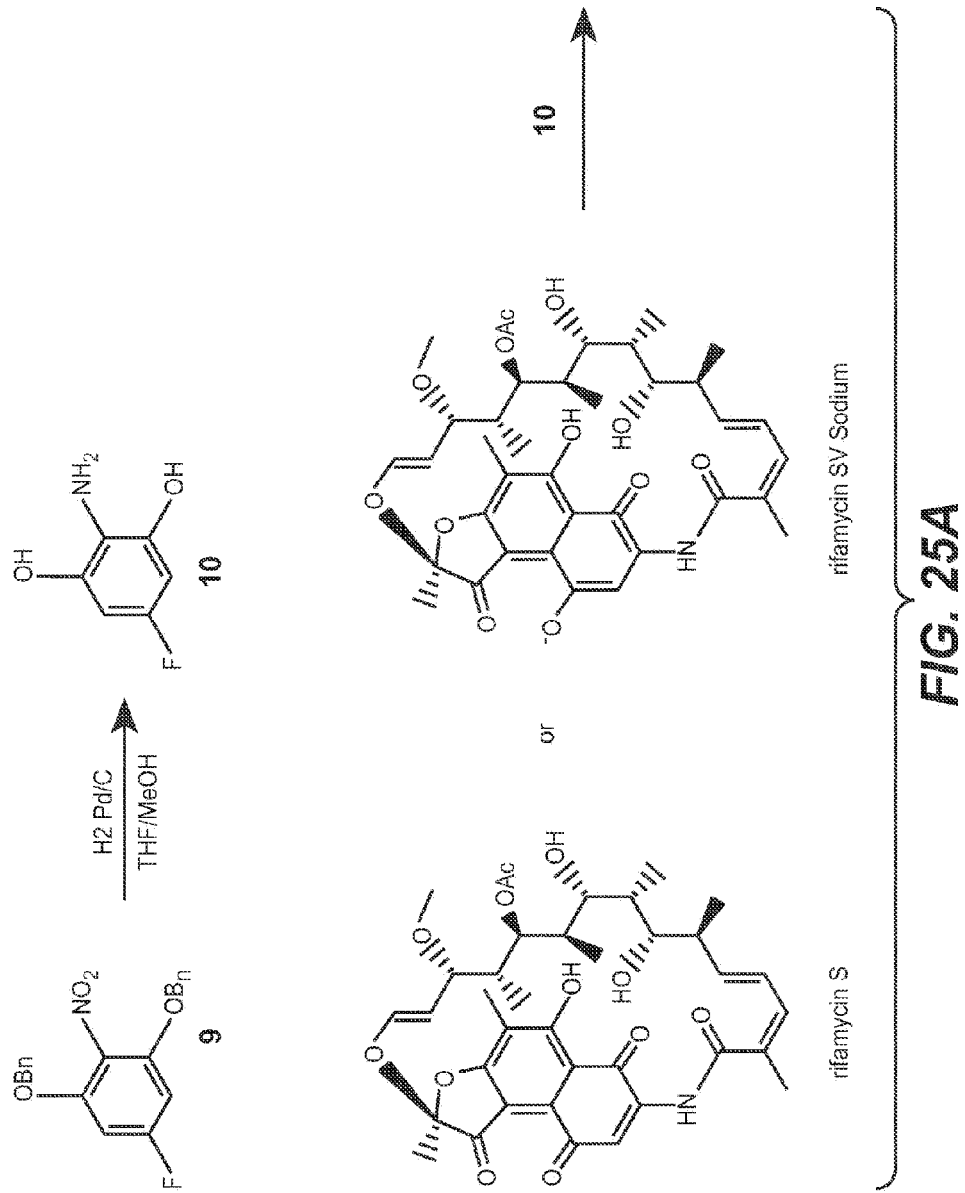
**FIG. 24**



FIG. 25A

FIG. 25B

**FIG. 25**



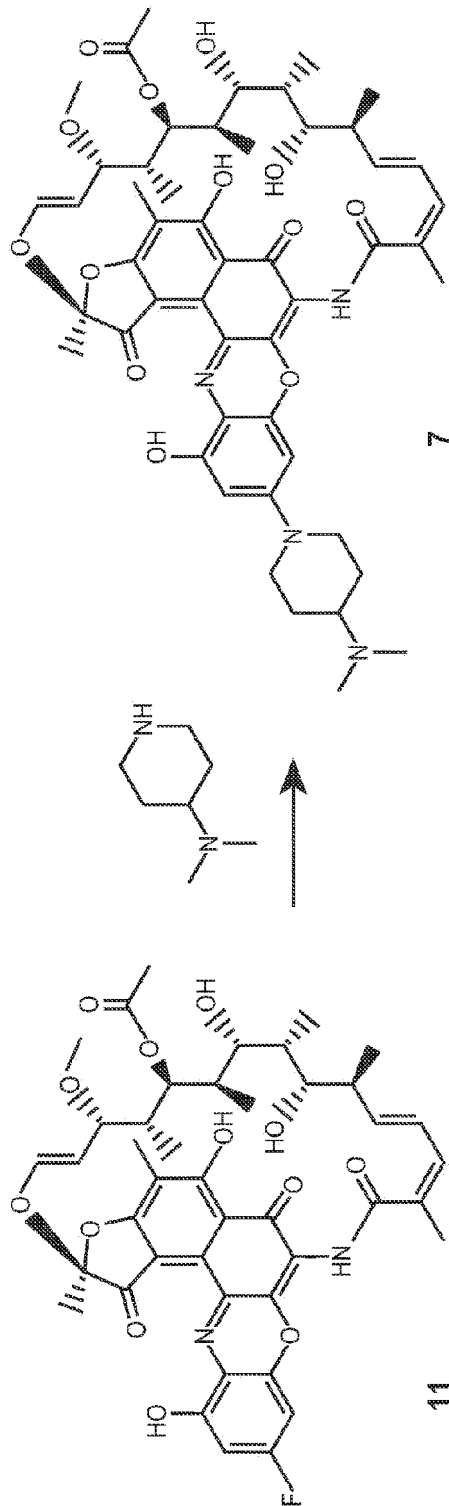
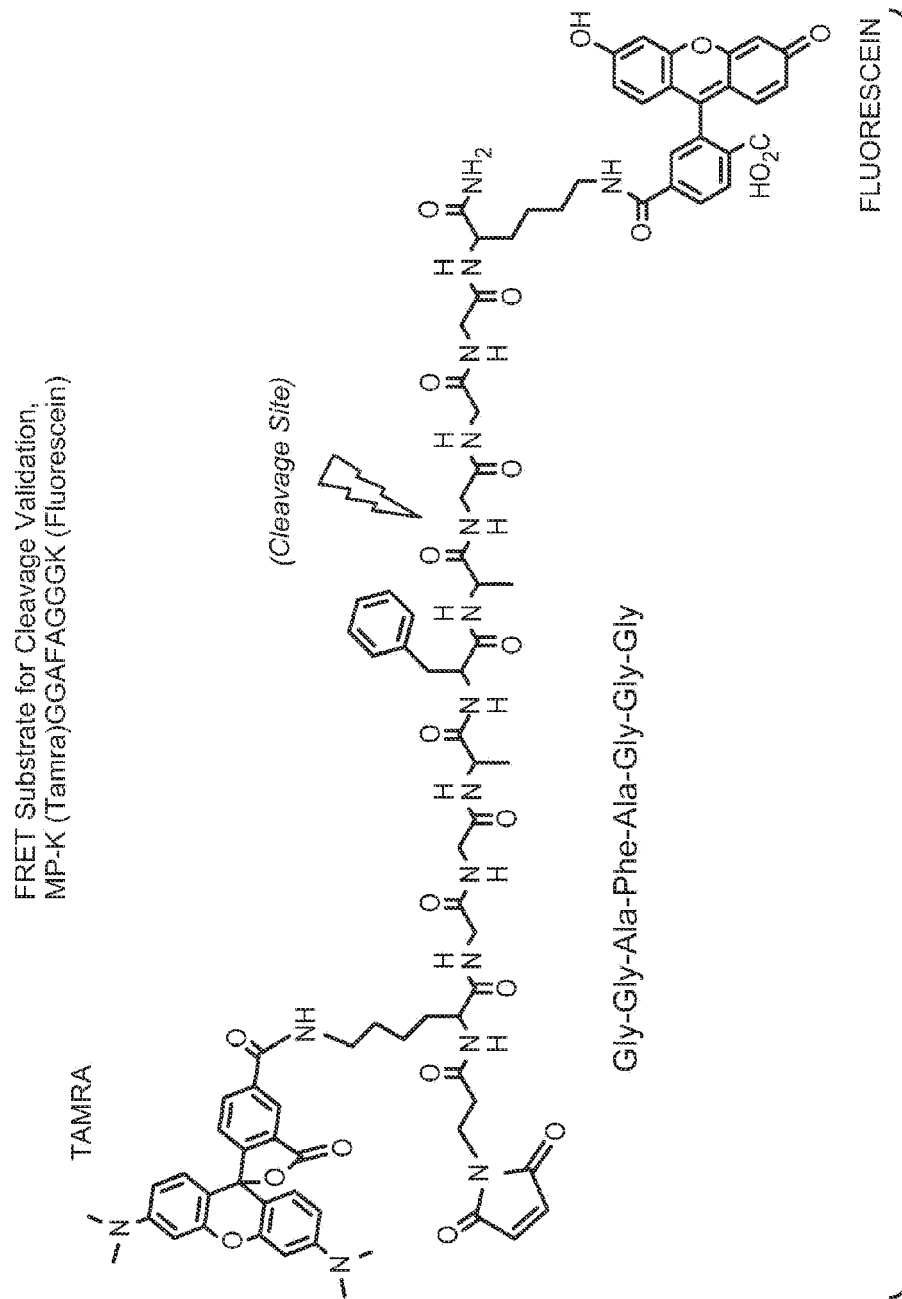


FIG. 25B

**FIG. 26**

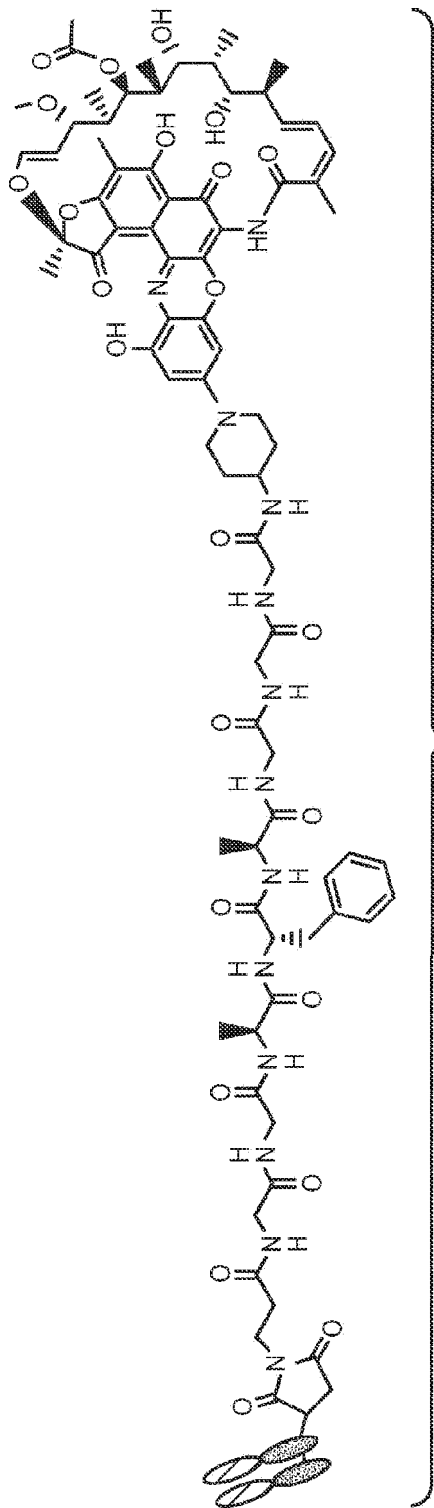
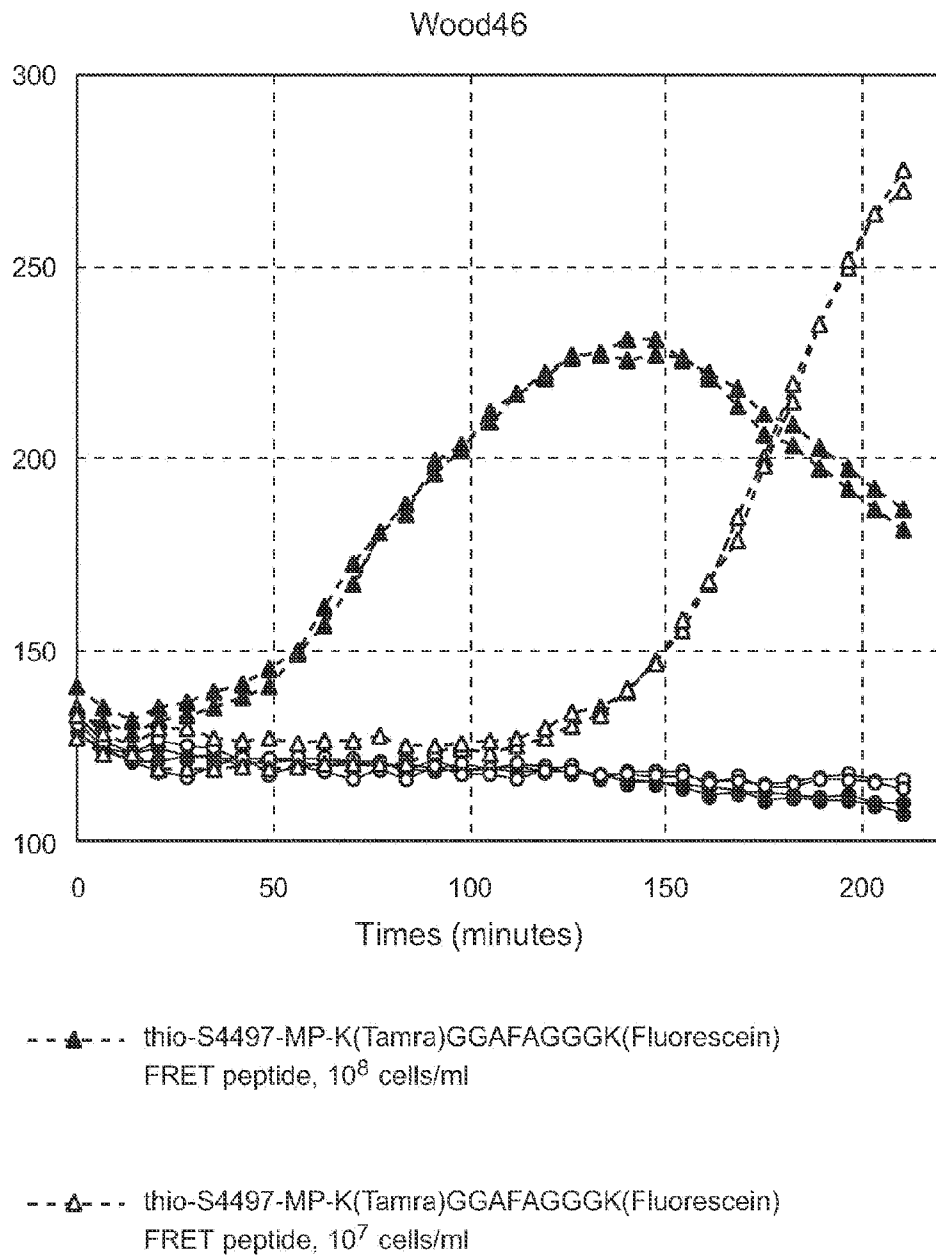
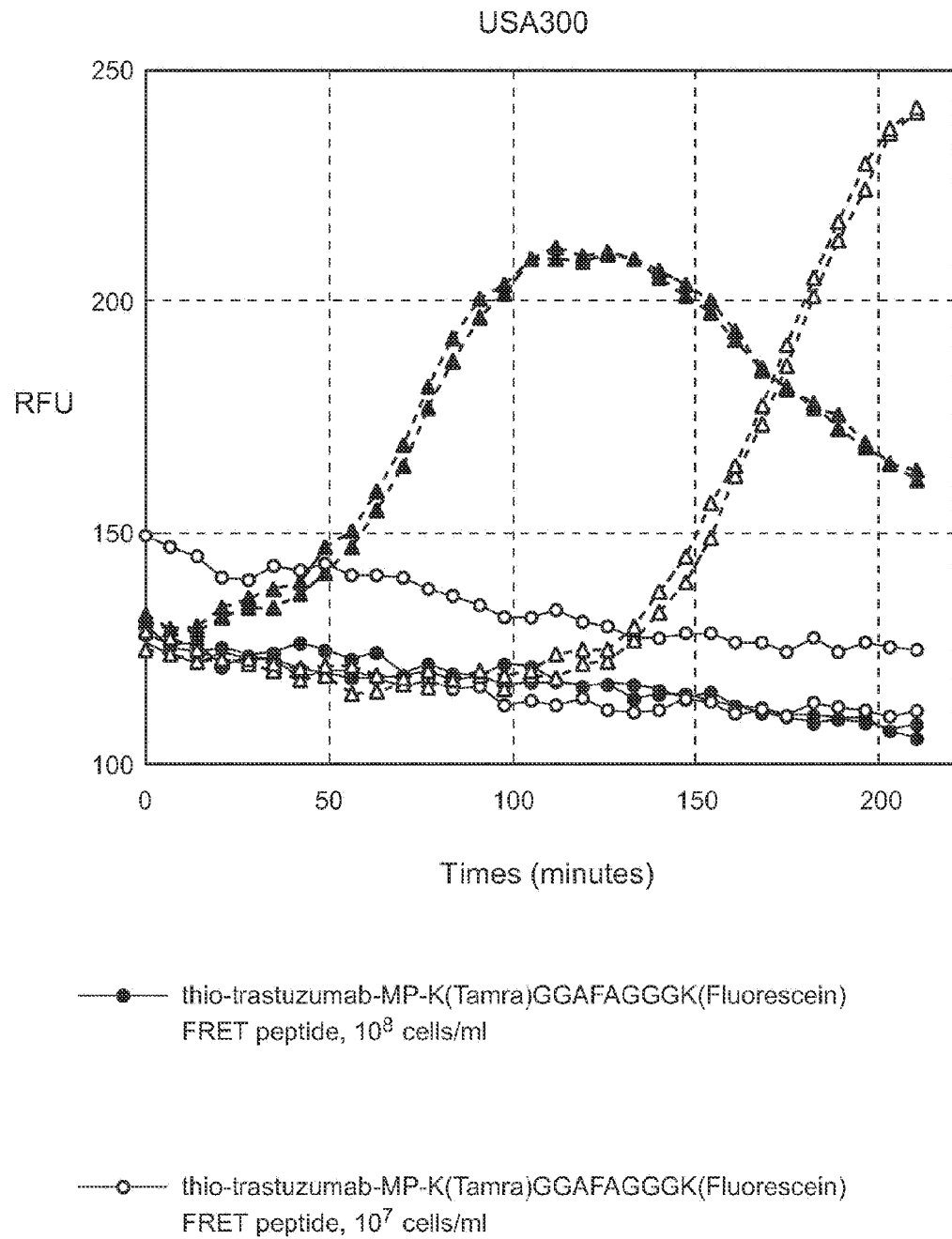
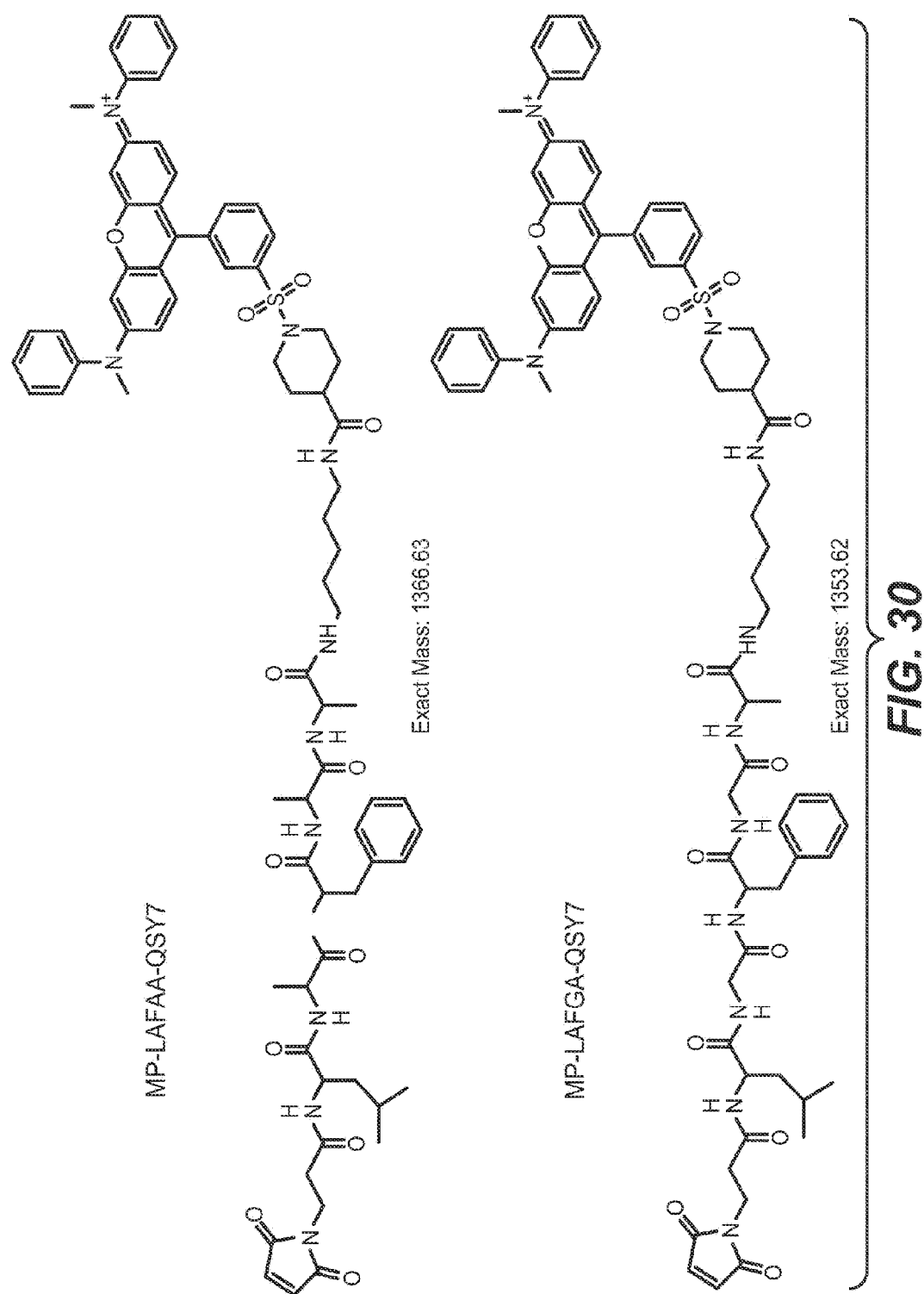
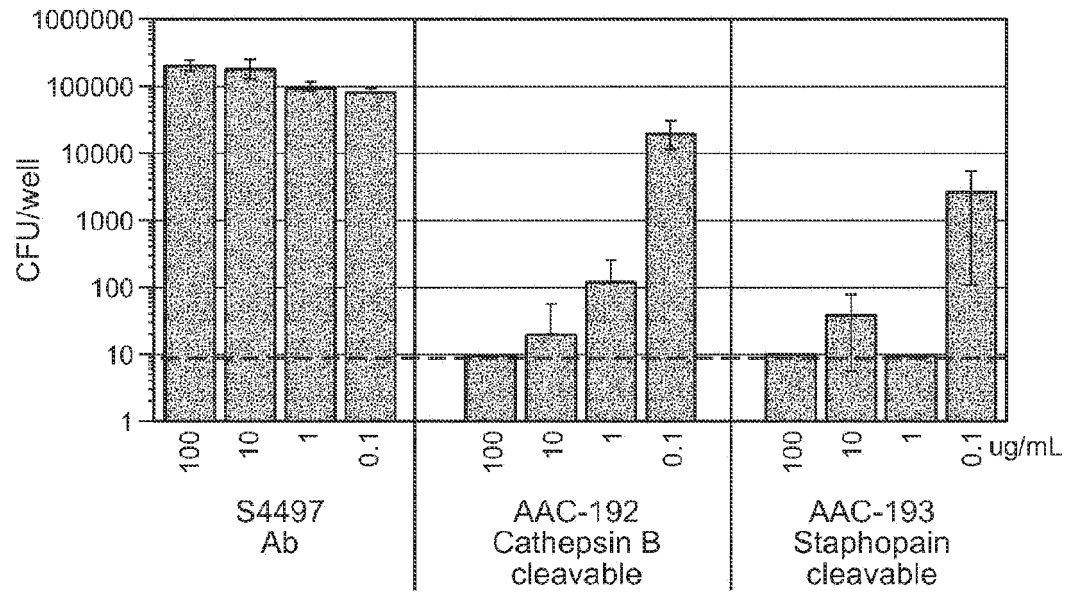
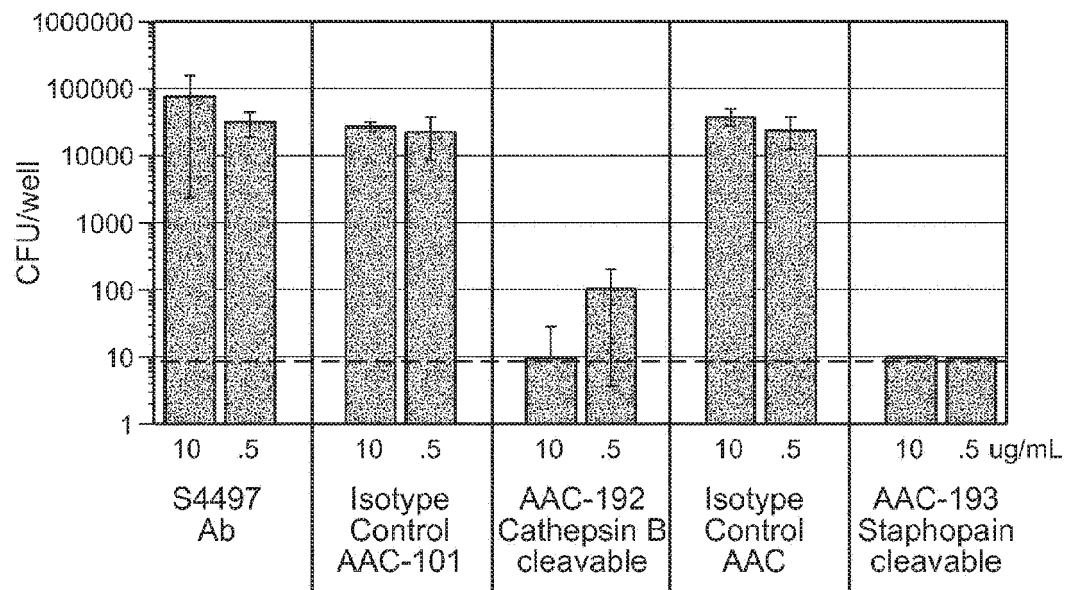


FIG. 27

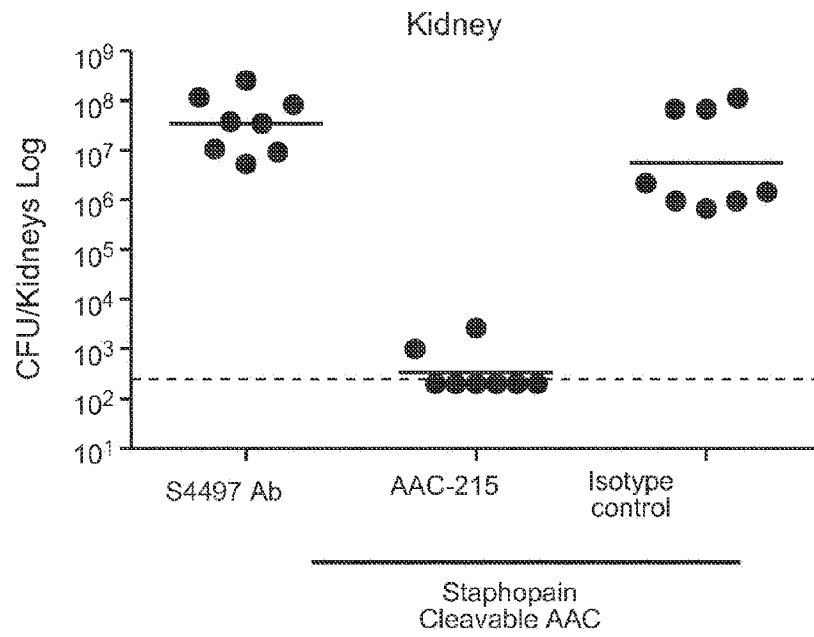
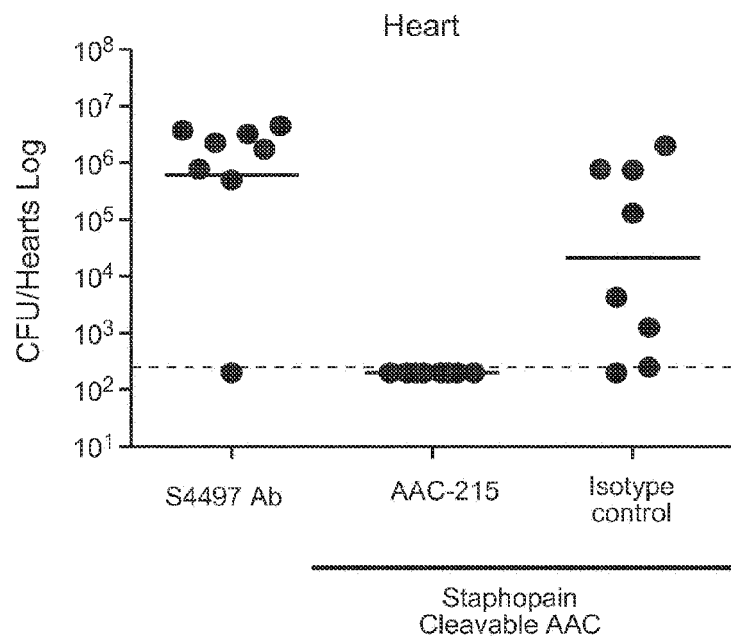
**FIG. 28**

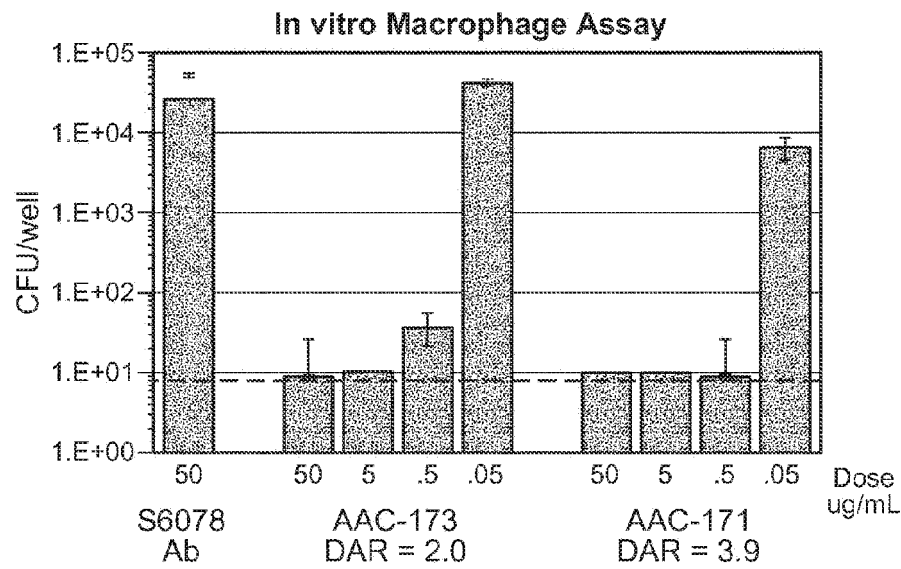
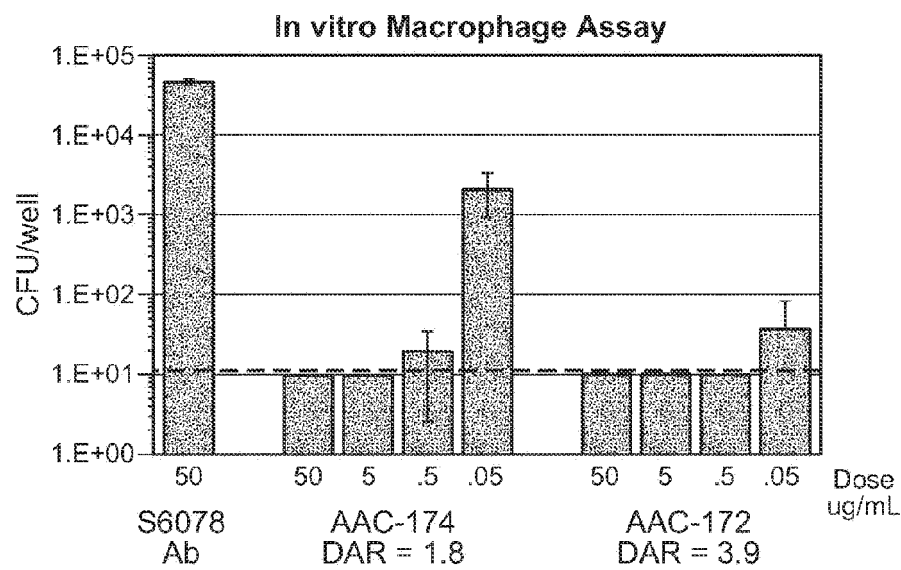
**FIG. 29**

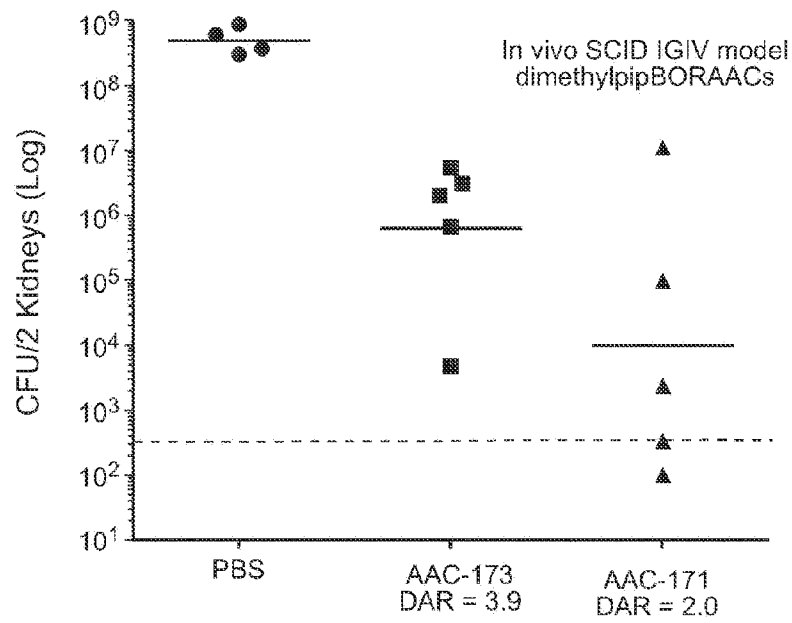
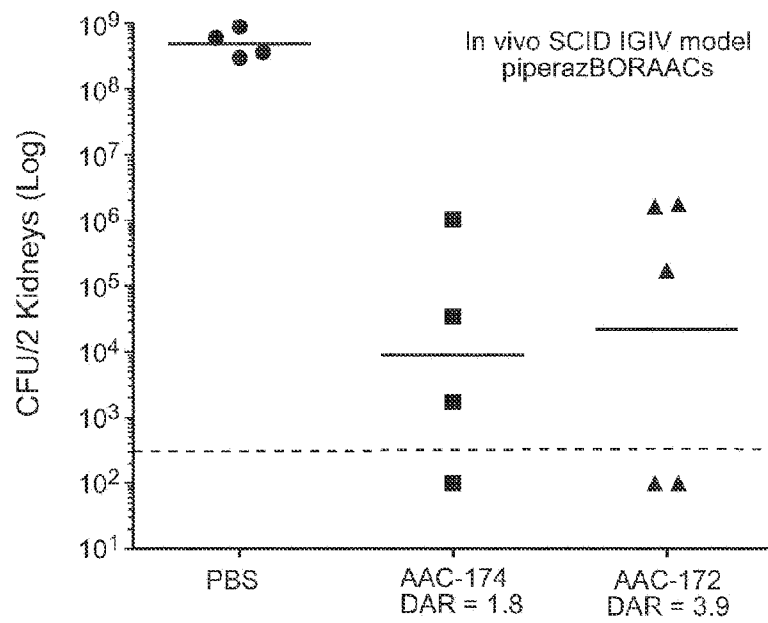


**FIG. 31****FIG. 32**



**FIG. 33****FIG. 34**

**FIG. 35****FIG. 36**

**FIG. 37****FIG. 38**