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(54) Title: ANTI-STAPHYLOCOCCUS AUREUS ANTIBODY RIFAMYCIN CONJUGATES AND USES THEREOF

• **Concept of TAC:**
antibiotic is released from TAC by phagolysosomal proteases

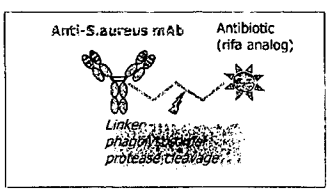

FIG. 2

(57) Abstract: The invention provides anti-Staphylococcus aureus antibody rifamycin antibiotic conjugates and methods of using same.

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ANTI-STAPHYLOCOCCUS AUREUS ANTIBODY RIFAMYCIN CONJUGATES AND USES THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 62/087,184, filed December 3, 2014, which is incorporated herein by reference in its entirety for all purposes.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on November 20, 2015, is named P32433-WO_SL.txt and is 190541 bytes in size.

TECHNICAL FIELD

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 Staphylococcus infections.

BACKGROUND OF THE INVENTION

Staphylococcus aureus (*S. aureus*; SA) is the leading cause of bacterial infections in humans worldwide and represents a major health problem in both hospital and community settings. However, *S. aureus* is not exclusively a pathogen and commonly colonizes the anterior nares and skin of healthy individuals. When infection does occur, the most serious infections such as endocarditis, osteomyelitis, necrotizing pneumonia and sepsis occur following dissemination of the bacteria into the bloodstream (Lowy, F.D. (1998) "Staphylococcus aureus infections" *N Engl J Med* **339**, 520-532). Over the last several decades, infection with *S. aureus* has become increasingly difficult to treat due to the emergence and rapid spread of methicillin-resistant *S. aureus* (MRSA) that is resistant to all known beta-lactam antibiotics (Boucher, H.W., *et al.* (2009) "Bad bugs, no drugs: no

ESKAPE! An update from the Infectious Diseases Society of America" *Clin Infect Dis* 48, 1-12). Invasive MRSA infections are hard to treat, with a mortality rate of ~20% and are the leading cause of death by an infectious agent in the USA. Vancomycin, linezolid and daptomycin have thus become the few antibiotics of choice for treating invasive MRSA infections (Boucher, H., Miller, L.G. & Razonable, R.R. (2010) "Serious infections caused by methicillin-resistant Staphylococcus aureus" *Clin Infect Dis* 51 Suppl 2, S183-197). However, reduced susceptibility to vancomycin and cross-resistance to linezolid and daptomycin have already been reported in MRSA clinical strains (Nannini, E., Murray, B.E. & Arias, C.A. (2010) "Resistance or decreased susceptibility to glycopeptides, daptomycin, and linezolid in methicillin-resistant Staphylococcus aureus" *Curr Opin Pharmacol* **10**, 516-521). 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.

Investigations have revealed that *S. aureus* is able to invade and survive inside mammalian cells including the phagocytic cells that are responsible for bacterial clearance (Thwaites, G.E. & Gant, V. (2011) Are bloodstream leukocytes Trojan Horses for the metastasis of Staphylococcus aureus? *Nat Rev Microbiol* **9**, 215-222); Rogers, D.E., Tompsett, R. (1952) "The survival of staphylococci within human leukocytes" *J. Exp. Med* **95**, 209-230); Gresham, H.D., *et al.* (2000) "Survival of Staphylococcus aureus inside neutrophils contributes to infection" *J Immunol* **164**, 3713-3722); Kapral, F.A. & Shayegani, M.G. (1959) "Intracellular survival of staphylococci" *J Exp Med* **110**, 123-138; Anwar, S., et al. (2009) "The rise and rise of Staphylococcus aureus: laughing in the face of granulocytes" *Clin Exp Immunol* **157**, 216-224); Fraunholz, M. & Sinha, B. (2012) "Intracellular Staphylococcus aureus: live-in and let die" *Front Cell Infect Microbiol* **2**, 43); Garzoni, C. & Kelley, W.L. (2011) "Return of the Trojan horse: intracellular phenotype switching and immune evasion by Staphylococcus aureus" *EMBO Mol Med* **3**, 115-117). *S. aureus* is taken up by host phagocytic cells, primarily neutrophils and macrophages, within minutes following intravenous infection (Rogers, D.E. (1956) "Studies on Bacterimia: Mechanisms Relating to the Persistence of Bacteremia in Rabbits Following the Intravanous Injection of Staphylococci" *JEM* **103**, 713). While the majority of the bacteria are effectively killed by these cells, incomplete clearance of *S. aureus* inside blood borne phagocytes can allow these infected cells to act as "Trojan horses" for dissemination of the bacteria away from the initial site of infection. Indeed, patients with normal neutrophil counts may be more prone to

disseminated disease than those with reduced neutrophil counts (Thwaites, G.E. & Gant, V. (2011) supra). Once delivered to the tissues, *S. aureus* can invade various non-phagocytic cell types, and intracellular *S. aureus* in tissues is associated with chronic or recurrent infections. 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.

Ansamycins are a class of antibiotics, including rifamycin, rifampin, rifampicin, rifabutin, rifapentine, rifalazil, ABI-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).

Immunotherapies have been reported for preventing and treating *S. aureus* (including MRSA) infections. US 2011/0262477 concerns uses of bacterial adhesion proteins Eap, Emp and AdsA as vaccines to stimulate immune response against MRSA. WO 2000/071585 describes isolated monoclonal antibodies reactive to specific *S. aureus* strain isolates. US 2011/0059085A1 suggests an Ab-based strategy utilizing IgM Abs specific for one or more SA capsular antigens, although no actual antibodies were described.

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).

Non-specific immunoglobulin-antibiotic conjugates are described that bind to the surface of target bacteria via the antibiotic for treating sepsis (US 5,545,721; US 6,660,267). Antibiotic-conjugated antibodies are described 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 (US 7569677).

In view of the alarming rate of resistance of MRSA to conventional antibiotics and the resultant mortality and morbidity from invasive MRSA infections, there is a high unmet need for new therapeutics to treat S. aureus infections. The present invention satisfies this need, and provides compositions and methods that overcome the limitations of current therapeutic compositions as well as offer additional advantages that will be apparent from the detailed description below.

SUMMARY OF THE INVENTION

The present invention provides a unique therapeutic that includes the elimination of intracellular bacteria. The present invention demonstrates that such a therapeutic is efficacious in-vivo where conventional antibiotics like vancomycin fail.

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.

An aspect of the invention is an antibody-antibiotic conjugate compound comprising an anti-wall teichoic acid (WTA) antibody, covalently attached by a protease-cleavable, non-peptide linker to a rifamycin-type antibiotic.

An exemplary embodiment of the invention is an antibody-antibiotic conjugate of claim 1 having the formula:

$$Ab–(PML–abx)_p$$

wherein:

Ab is the anti-wall teichoic acid antibody;

PML is a protease-cleavable, non-peptide linker having the formula:

$$-Str-PM-Y-$$

where Str is a stretcher unit; PM is a peptidomimetic unit, and Y is a spacer unit;

abx is the rifamycin-type antibiotic; and

p is an integer from 1 to 8.

The antibody-antibiotic conjugate compounds of any of the preceding embodiments can comprise any one of the anti-wall teichoic acid (WTA) Abs described herein. These anti-WTA antibodies bind to Staphylococcus aureus. In one embodiment, the antibody is an anti-WTAα monoclonal antibody. In exemplary anti-WTAα antibodies, the Ab is a monoclonal antibody comprising a light (L) chain and a heavy (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 1A and 1B.

In some embodiments, the anti-WTA antibody comprises 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. The antibodies may further comprise 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 embodiment, the antibody-antibiotic conjugate compound of the invention comprises an anti-WTAβ monoclonal antibody. An exemplary anti-WTAβ antibody comprises 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 corresponding CDRs of each of Abs shown in Figure 12 (SEQ ID NO. 33-110).

Another anti-WTAβ antibody useful to generate the AACs of the invention 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 Figure 15A-1, 15A-2, 15A-3 at Kabat positions 1-107. This antibody may further comprise 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 Figure 15B-1 to 15B-6 at Kabat positions 1-113.

In another anti-WTAβ antibody, the VL comprises the sequence of SEQ ID NO. 111 and the VH comprises the sequence of SEQ ID NO. 112 wherein X is Q or E and X_1 is M, I or V.

The invention provides an anti-WTAβ useful to generate an AAC of the invention wherein the antibody light chain contains an engineered cysteine and comprises the sequence of SEQ ID NO. 115 and the H chain comprises the SEQ ID NO. 116 wherein X is M, I or V. In an alternative pairing L and H chains, the antibody light chain comprises the sequence of SEQ ID NO. 113 and the H chain contains an engineered cysteine and comprises the SEQ ID NO. 117 wherein X is M, I or V. A Cys may be engineered into each of the L and H chains; in one example of such a WTAβ antibody, light chain contains an engineered cysteine and comprises the sequence of SEQ ID NO. 115, and the H chain contains an engineered cysteine and comprises the SEQ ID NO. 117 wherein X is M, I or V.

Another anti-WTAβ antibody useful for conjugation comprises a VH and a VL, wherein the VH comprises at least 95% sequence identity over the length of the VH of SEQ ID NO. 156 and the VL comprises at least 95% sequence identity over the length of the VL of sequence SEQ ID NO. 119. In a specific embodiment, the anti-WTAβ antibody comprises a VH comprising the sequence of SEQ ID NO. 156 and a VL comprising the sequence of the SEQ ID NO. 119.

The anti-WTAβ antibody of the invention may comprise a L chain comprising the sequence of SEQ ID NO.121 and a H chain comprising the sequence of SEQ ID NO. 124. In another example, the anti-WTAβ antibody comprises a L chain comprising the sequence of SEQ ID NO. 123 and a H chain comprising the sequence of SEQ ID NO. 157 or SEQ ID NO. 124.

In other embodiments, the antibody comprises: i) L chain and H chain CDRs of SEQ ID NOs 99-104 or the L chain and H chain CDRs of SEQ ID NOs. 33-38; 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.

In some embodiments of the AACs of the invention, the antibody binds to the same epitope as the antibody of any one of the preceding embodiments.

The antibody of any one of the preceding embodiments may be an antigen-binding fragment lacking a Fc region. In some embodiments, the antibody is a F(ab) or $F(ab')_2$. 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 system is according to EU numbering.

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.

An exemplary embodiment of the invention is pharmaceutical composition comprising the antibody-antibiotic conjugate compound, and a pharmaceutically acceptable carrier, glidant, diluent, or excipient.

The anti-WTA-AACs of the invention are useful as antimicrobial agents effective to treat human and veterinary Staphylococci, for example S. aureus, S. saprophyticus and S. simulans as well as Listeria, for example Listeria monocytogenes. In a specific aspect, the AACs of the invention are useful to treat *S. aureus* infections. Thus, the invention also provides a method of treating a Staphylococcal infection in a human or veterinary patient comprising administering to the patient a therapeutically-effective amount of an antibody-

antibiotic conjugate of any one of the preceding embodiments. In one embodiment the bacterial infection is a *Staphylococcus aureus* infection. In some embodiments, the patient has been diagnosed with a S. aureus infection. In some embodiments, treating the bacterial infection comprises reducing the bacterial load or counts. In one embodiment, the method of treatment is administered to patients where the bacterial infection including S. aureus has led to bacteremia. In specific embodiments the method is used to treat Staphylococcal endocarditis or osteomyelitis. In one embodiment, the antibody-antibiotic conjugate compound is administered to the infected patient at a dose in the range of about 50mg/kg to 100mg/kg.

Also provided is method of killing intracellular *S. aureus* in the cells of a *S. aureus* infected patient without killing the host cells by administering an anti-WTA-antibiotic conjugate compound of any of the above embodiments. Another method is provided for killing persister Staphylococcal bacterial cells (e.g, *S. aureus*) in vivo by contacting the persister bacteria with an AAC of any of the preceding embodiments.

In another embodiment, the method of treatment 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.

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) fluoroquinolines/fluoroquinolones; (vi) and oxazolidinones.

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, napthyridone, radezolid, doxorubicin, ampicillin, vancomycin, imipenem, doripenem, gemcitabine, dalbavancin, and azithromycin.

In some embodiments herein, the bacterial load in the infected patient 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.

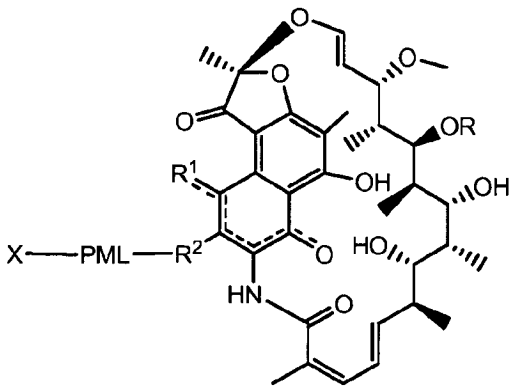
An exemplary embodiment of the invention is a process for making the antibody-antibiotic conjugate comprising conjugating a rifamycin-type antibiotic to an anti-wall teichoic acid (WTA) antibody.

An exemplary embodiment of the invention is a kit for treating a bacterial infection, comprising:

a) the pharmaceutical composition comprising the antibody-antibiotic conjugate compound, and a pharmaceutically acceptable carrier, glidant, diluent, or excipient; and

b) instructions for use.

An aspect of the invention is an antibiotic-linker intermediate having Formula II:



II

wherein:

the dashed lines indicate an optional bond;

R is H, C_1–C_{12} alkyl, or $C(O)CH_3$;

R^1 is OH;

R^2 is CH=N–(heterocyclyl), wherein the heterocyclyl is optionally substituted with one or more groups independently selected from $C(O)CH_3$, C_1–C_{12} alkyl, C_1–C_{12} heteroaryl, C_2–C_{20} heterocyclyl, C_6–C_{20} aryl, and C_3–C_{12} carbocyclyl;

or R^1 and R^2 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_1–C_{12} alkyl, or OH;

PML is a protease-cleavable, non-peptide linker attached to R^2 or the fused heteroaryl or heterocyclyl formed by R^1 and R^2; and having the formula:

–Str–PM–Y–

where Str is a stretcher unit; PM is a peptidomimetic unit, 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.

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

Figure 1: Intracellular stores of MRSA are protected from vancomycin in vivo and in vitro. Figure 1A shows a schematic of the experimental design for generating free bacteria (planktonic) vs. intracellular bacteria. Four cohorts of mice were infected by intravenous injection with roughly equivalent doses of viable free bacteria or intracellular bacteria and selected groups were treated with vancomycin immediately after infection and then once per day (see Example 2). Figure 1B and Figure 1C show bacterial loads in kidney and brain, respectively of infected mice 4 days post infection. The dashed line indicates the limit of detection for the assay. Figure 1D shows that MRSA is protected from vancomycin when cultured on a monolayer of infectable cells. (ND = none detected). Figure 1E and Figure 1F show that MRSA is able to grow in the presence of vancomycin when cultured on a monolayer of infectable cells. MRSA (free bacteria) was seeded in media, media + vancomycin, or media + vancomycin and plated on a monolayer of MG63 osteoblasts (Figure 1E) or Human Brain Microvascular Endothelial Cells (HBMEC, Figure 1F). Extracellular bacteria (free bacteria) grew well in media alone, but were killed by vancomycin. In wells containing a monolayer of mammalian cells (Intracellular + vanco) a fraction of the bacteria were protected from vancomycin during the first 8 hours after infection and were able to expand within the intracellular compartment over 24 hours. Error bars show standard deviation for triplicate wells.

Figure 2: shows the concept of an Antibody Antibiotic Conjugate (AAC). In one example, the AAC consists of an antibody directed against an epitope on the surface of S.

aureus linked to a potent rifamycin-type antibiotic (e.g. Rifalog) via a linker that is cleaved by lysosomal proteases.

Figure 3 shows a possible mechanism of drug activation for antibody-antibiotic conjugates (AAC). AACs bind to extracellular bacteria via the antigen binding domain (Fab) of the antibody and promote uptake of the opsonized bacteria via Fc-mediated phagocytosis. The linker is cleaved by lysosomal proteases such as cathepsin B. Following cleavage of the linker, the linker is hydrolyzed releasing free antibiotic inside the phagolysosome. The free antibiotic kills the opsonized and phagocytosed bacteria along with any previously internalized bacteria residing in the same compartment.

Figure 4 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 5 shows the chemical structure and glycosyl modifications of Wall Teichoic Acid (WTA), described in detail under Definitions.

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 3. Of the Abs that bind to WTA, 4 are specific to WTA alpha and 13 bind specifically to WTA beta.

Figure 7A shows titration of Alexa-488 labeled anti-β-GlcNAC WTA or anti-α-GlcNAC WTA antibody on MRSA isolated directly from infected mouse kidneys. The anti-CMV-gD antibody served as an antibody isotype control. Figure 7B shows that the antibody used to generate the AAC recognizes an epitope on Wall Teichoic Acid that is mediated by the glycosyltransferase TarS. FACS analysis with the anti-β-GlcNAC WTA antibody or an isotype control on Wt USA300, USA300-TarM or USA300-TarS.

Figure 8 shows selection of a potent rifamycin-type antibiotic (rifalog) dimethylpipBOR for its ability to kill non-replicating MRSA.

Figure 9: Growth inhibition assay demonstrating that intact TAC (a form of AAC) does not kill planktonic bacteria unless the antibiotic is released by treatment with cathepsin

B. TAC was incubated in buffer alone (open circles) or treated with cathepsin B (closed circles). The intact TAC was not able to prevent bacterial growth after overnight incubation. Pretreatment of the TAC with cathepsin B released sufficient antibiotic activity to prevent bacterial growth at .6 μg/mL of TAC, which is predicted to contain .006 μg/mL of antibiotic.

Figure 10 shows treatment of S. aureus infected mice with anti-WTA-PML AAC greatly reduced or eradicated bacterial counts in infected organs as compared to naked antibody, as described in Example 10. Figure 10A is a schematic showing the timeline of the experiment and injection time points as described in Example 10. Figure 10B shows treatment with AAC (DAR2) from Table 3 reduced bacterial load in the kidneys by approximately 7,000-fold. Figure 10C shows that treatment with AAC (DAR2) reduced bacterial burdens in the heart by approximately 500-fold.

Figure 11A provides an amino acid sequence alignment of the light chain variable regions (VL) of four human anti-WTA alpha antibodies, 4461. 4624, 4399, 6267 (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 11B shows an amino acid sequence alignment of the heavy chain variable regions (VH) of the four human anti-WTA alpha antibodies of Figure 11A. 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 12 shows the CDR sequences of the L and H chains of 13 human anti-WTA beta antibodies (SEQ ID NOS 33-110).

Figures 13A-1 and 13A-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 near 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 13B.

Figures 13B-1, 13B-2, 13B-3, 13B-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 at the start of the constant region (at EU residue no. 118 in this case).

Figures 14A-1 and 14A-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 14B-1, 14B-2, 14B-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 at the start of the constant region (at EU residue no. 118 in this case).

Figures 15A-1, 15A-2, 15A-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 15B-1 to 15B-6 show an amino acid sequence alignment of the full length heavy chain of the thirteen human anti-WTA beta antibodies of Figures 15A-1, 15A-2, 15A-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 16 shows a comparison of Ab 4497 and its mutants in the highlighted amino acid positions and their relative antigen binding strength as tested by ELISA. Figure 16 discloses SEQ ID NOS 177, 177, 177, 178, 178, 179, 179, 180, 180 and 180, respectively, in order of appearance.

Figure 17 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 $2x10^7$ 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).

DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

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.

All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety.

I. GENERAL TECHNIQUES

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 Press, Cold Spring Harbor, N.Y.; *Current Protocols in Molecular Biology* (F.M. Ausubel, et al. eds., (2003)); the series *Methods in Enzymology* (Academic Press, Inc.): *PCR 2: A Practical Approach* (M.J. MacPherson, B.D. Hames and G.R. Taylor eds. (1995)), Harlow and Lane, eds. (1988) *Antibodies, A Laboratory Manual*, and *Animal Cell Culture* (R.I. Freshney, ed. (1987)); *Oligonucleotide Synthesis* (M.J. Gait, ed., 1984); *Methods in Molecular Biology*, Humana Press; *Cell Biology: A Laboratory Notebook* (J.E. Cellis, ed., 1998) Academic Press; *Animal Cell Culture* (R.I. Freshney), ed., 1987); *Introduction to Cell and Tissue Culture* (J.P. Mather and P.E. Roberts, 1998) Plenum Press; *Cell and Tissue Culture: Laboratory Procedures* (A. Doyle, J.B. Griffiths, and D.G. Newell, eds., 1993-8) J. Wiley and Sons; *Handbook of Experimental Immunology* (D.M. Weir and C.C. Blackwell, eds.); *Gene Transfer Vectors for Mammalian Cells* (J.M. Miller and M.P. Calos, eds., 1987); *PCR: The Polymerase Chain Reaction*, (Mullis et al., eds., 1994); *Current Protocols in Immunology* (J.E. Coligan et al., eds., 1991); *Short Protocols in Molecular Biology* (Wiley and Sons, 1999); *Immunobiology* (C.A. Janeway and P. Travers, 1997); *Antibodies* (P. Finch, 1997); *Antibodies: A Practical Approach* (D. Catty., ed., IRL Press, 1988-1989); *Monoclonal Antibodies: A Practical Approach* (P. Shepherd and C. Dean, eds., Oxford University Press, 2000); *Using Antibodies: A Laboratory Manual* (E. Harlow and D. Lane (Cold Spring Harbor Laboratory Press, 1999); *The Antibodies* (M. Zanetti and J. D. Capra, eds., Harwood Academic Publishers, 1995); and *Cancer: Principles and Practice of Oncology* (V.T. DeVita et al., eds., J.B. Lippincott Company, 1993).

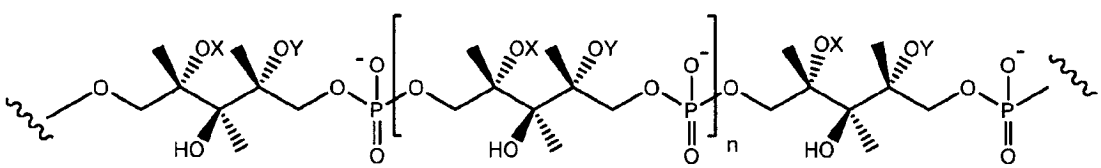
The nomenclature used in this Application is based on IUPAC systematic nomenclature, unless indicated otherwise. Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs, and are 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.

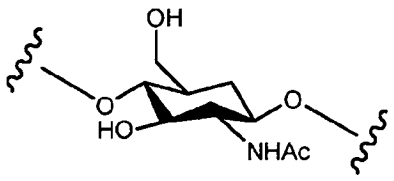
II. DEFINITIONS

"Antibody Antibiotic Conjugate" or AAC is a compound composed of an antibody that is chemically linked to an antibiotic by a linker. The antibody binds an antigen or epitope on a bacterial surface, for example, a bacterial cell wall component. As used in this invention, the linker is a protease-cleavable, non-peptide linker that is designed to be cleaved by proteases, including cathepsin B, a lysosomal protease found in most mammalian cell types (Dubowchik et al (2002) Bioconj. Chem. 13:855-869). A diagram of the AAC with its 3 components is depicted in Figure 2. "THIOMAB™ Antibiotic Conjugate" or "TAC" is a form of AAC in which the antibody is chemically conjugated to a linker-antibiotic unit via one or more cysteines, generally a cysteine that is recombinantly engineered into the antibody at specific site(s) on the antibody to not interfere with the antigen binding function.

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 α (alpha) or β (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 (α) or beta (β) 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 α (alpha)-GlcNHAc or β (beta)-GlcNHAc.



GlcNHAc

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 α- (alpha) or β-(beta) glycosidic linkages. Depending of the S. aureus strain, or the growth phase of the bacteria the glycosidic linkages could be α -, β -, or a mixture of the two anomers.

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-αWTA" or "anti-αGlcNac 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 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 non-toxic 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.,* ertapenum, doripenem, imipenem/cilastatin, meropenem, (v) cephalosporins (first generation), *e.g.,* cefadroxil, cefazolin, cefalotin, cefalexin, (vi) cephalosporins (second generation), e.g., ceflaclor, 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, dirithromycine, erythromycin, roxithromycin, troleandomycin, telithromycin, spectinomycin, (xi) monobactams, *e.g.*, axtreonam, (xii) penicilins, *e.g.*, amoxicillin, ampicillin, axlocillin, carbenicillin, cloxacillin, dicloxacillin, flucloxacillin, mezlocillin, meticillin, nafcilin, oxacillin, penicillin, peperacillin, ticarcillin, (xiii) antibiotic polypeptides, *e.g.*, bacitracin, colistin, polymyxin B, (xiv) quinolones, e.g., ciprofloxacin, enoxacin, gatifloxacin, levofloxacin, lemefloxacin, moxifloxacin, norfloxacin, orfloxacin, 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.

Staphylococcus aureus is also referred to herein as Staph A or *S. aureus* in short. 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 in 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.

The terms "anti-Staph a antibody" and "an antibody that binds to Staph a" refer to an antibody that is capable of binding an antigen on *Staphylococcus aureus* ("S. aureus") with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting S. aureus. In one embodiment, the extent of binding of an anti-Staph a antibody to an unrelated, non-Staph a 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 a has a dissociation constant (Kd) of $\leq 1\mu M$, ≤ 100 nM, ≤ 10 nM, , ≤ 5 Nm, , ≤ 4 nM, , ≤ 3 nM, , ≤ 2 nM, ≤ 1 nM, ≤ 0.1 nM, ≤ 0.01 nM, or ≤ 0.001 nM (e.g., 10^{-8} M or less, e.g. from 10^{-8} M to 10^{-13} M, e.g., from 10^{-9} M to 10^{-13} M). In certain embodiments, an anti-Staph a antibody binds to an epitope of Staph a that is conserved among Staph from different species.

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. Assay for determining MIC are known. One method is as described in the Example section below.

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) Immuno Biology, 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.

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., IgG_1,

IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α, δ, ε, γ, and μ, respectively.

"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 (κ) and lambda (λ), based on the amino acid sequence of its constant domain.

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.

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, $F(ab')_2$; diabodies; linear antibodies; single-chain antibody molecules (e.g. scFv); and multispecific antibodies formed from antibody fragments.

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.

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.

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.

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.

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).

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).

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
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

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*.

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.

"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.

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.

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.

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).

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.

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

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.

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.

"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.

"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 Fcγ(gamma)RIII only, whereas monocytes express Fcγ(gamma)RI, Fcγ(gamma)RII and Fcγ(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).

"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 FcγgammaRI, FcγgammaRIIA and FcγgammaRIIIA) 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).

"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.

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 13 CHO cells deficient in protein fucosylation (Ripka et al. Arch. Biochem. Biophys. 249:533-545 (1986); US Pat. Appl. Pub. No. 2003/0157108 Al, Presta, L; and WO 2004/056312 Al, 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).

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).

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.

"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.

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 (Kd) of $\leq 1\mu M$, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM, or ≤ 0.1

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.

"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 (Kd). 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.

In one embodiment, the "Kd" or "Kd 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}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 μg/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™ surfactant in PBS. When the plates have dried, 150 μl/well of scintillant (MICROSCINT-20™; Packard) is

added, and the plates are counted on a TOPCOUNT™ 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.

According to another embodiment, the Kd is measured by using surface-plasmon resonance assays using a BIACORE®-2000 or a BIACORE®-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 μg/ml (~0.2 μM) before injection at a flow rate of 5 μl/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™ surfactant (PBST) at 25°C at a flow rate of approximately 25 μl/min. Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using a simple one-to-one Langmuir binding model (BIAcore® Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (Kd) 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 M^{-1} 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™ spectrophotometer (ThermoSpectronic) with a stirred cuvette.

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®-2000 or a BIACORE®-3000 system (BIAcore, Inc., Piscataway, NJ).

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.

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".

"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.

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.

The term "rifamycin-type antibiotic" means the class or group of antibiotics having the structure of, or similar structure to, rifamycin.

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

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.

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.

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.

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.

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 vinylene (–CH=CH–), allyl (–CH_2CH=CH–), and the like.

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≡CH), propynyl (propargyl, -CH_2C≡CH), and the like.

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≡C-), propynylene (propargylene, -CH_2C≡C-), and the like.

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.

"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.

"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.

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, azetidinyl, 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-azabicyco[3.1.0]hexanyl, 3-azabicyclo[4.1.0]heptanyl, azabicyclo[2.2.2]hexanyl, 3H-indolyl quinolizinyl 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.

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.

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.

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 β-carboline.

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.

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.

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

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.

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.

"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 dextrins; 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™.

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 tri-carboxylic, 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-acetoxy-benzoic, 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-chorobenzenesulfonic, napthalene-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.

"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.

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.

"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.

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).

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.

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.

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.

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, the AACs, TACs of the invention are used to delay development of a disease or to slow the progression of an infectious disease or reduce the bacterial load in the blood stream and/or in infected tissues and organs.

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.

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.

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 at least 3, 4, 5 logs. In yet another aspect, the reduction is to below detectable levels using assays known in the art

including assays exemplified herein. 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.

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.

"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.

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.

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.

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.

"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.

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

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.

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.

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 (ad describes) embodiments that are directed to that value or parameter per se.

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)

The experimental results herein are a strong indication that therapies aimed at eliminating intracellular bacteria will improve clinical success. Towards this aim, the present invention provides a unique therapeutic that selectively kills *S. aureus* organisms that have invaded intracellular compartments of host cells. The present invention demonstrates that such a therapeutic is efficacious in in-vivo models where conventional antibiotics like vancomycin fail.

The invention provides an 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, non-peptide linker that is designed to be cleaved by proteases, including cathepsin B, a lysosomal protease found in most mammalian cell types (Dubowchik et al (2002) Bioconj. Chem. 13:855-869). A diagram of the AAC with its 3 components is depicted in Figure 2. Not to be limited by any one theory, one mechanism of action of the AAC is schematized in Figure 3. 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, 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 AACs 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. Since thousands of AACs can

bind to a single bacterium, this platform releases sufficient antibiotics in these intracellular niches to sustain maximal antimicrobial killing. Furthermore, as more bacteria are released from pre-existing intracellular reservoirs, the fast on-rate of this antibody-based therapy ensures immediate "tagging" of these bacteria before they can escape to neighboring or distant cells, thus mitigating further spread of the infection. (3) AAC improve the half-life of antibiotics *in vivo* (improved pharmacokinetics) by linking the antibiotic to an antibody, as compared to antibiotics which are cleared rapidly from serum. 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.

An antibody-antibiotic conjugate compound comprising an anti-wall teichoic acid (WTA) antibody, covalently attached by a protease-cleavable, non-peptide linker to a rifamycin-type antibiotic.

An exemplary embodiment is the antibody-antibiotic conjugate having the formula:

$$Ab–(PML–abx)_p$$

wherein:

Ab is the anti-wall teichoic acid antibody;

PML is the protease-cleavable, non-peptide linker having the formula:

$$–Str–PM–Y–$$

where Str is a stretcher unit; PM is a peptidomimetic unit, and Y is a spacer unit;

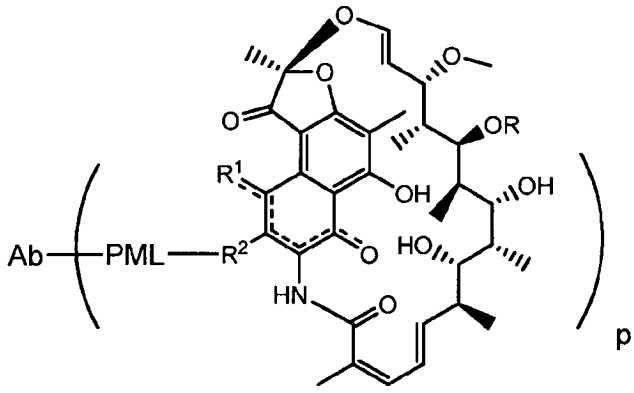
abx is the rifamycin-type antibiotic; and

p is an integer from 1 to 8.

The rifamycin-type antibiotic may be a rifalazil-type antibiotic.

The rifamycin-type antibiotic may comprise a quaternary amine attached to the protease-cleavable, non-peptide linker.

An exemplary embodiment of the antibody-antibiotic conjugate has Formula I:



I

wherein:

the dashed lines indicate an optional bond;

R is H, C_1–C_{12} alkyl, or $C(O)CH_3$;

R^1 is OH;

R^2 is CH=N–(heterocyclyl), wherein the heterocyclyl is optionally substituted with one or more groups independently selected from $C(O)CH_3$, C_1–C_{12} alkyl, C_1–C_{12} heteroaryl, C_2–C_{20} heterocyclyl, C_6–C_{20} aryl, and C_3–C_{12} carbocyclyl;

or R^1 and R^2 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_1–C_{12} alkyl, or OH;

PML is the protease-cleavable, non-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.

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 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

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 Examples 3-4 below.

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 4). 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 4). 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.

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 5) 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 α- (alpha) or β-(beta) glycosidic linkages. Depending of the S. aureus strain, or the growth phase of the bacteria the glycosidic linkages could be α -, β -, or a mixture of the two anomers. These GlcNAc sugar modifications are tailored by two specific S. aureus-derived glycosyltransferases (Gtfs): TarM Gtf mediates α -glycosidic linkages, whereas TarS Gtfs mediates β-(beta)glycosidic linkages.

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.

The anti-WTA antibody of an AAC of the present invention can be an anti-WTAα or anti-WTAβ antibody. The exemplary anti-WTA Abs provided throughout the specification were cloned from B cells from *S. aureus* infected patients (as taught in the Examples below). In one embodiment the anti-WTA and anti-Staph aureus Abs are human monoclonal antibodies. The AAC or TAC of the invention encompass chimeric Abs and humanized Abs comprising the CDRs of the present WTA Abs.

For the therapeutic uses of this invention, the WTA Abs conjugated 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.

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., 4497v8. 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.

For conjugation to produce a TAC compound, the anti-WTA antibodies of the invention may comprise engineered Cys in one or both L and H chains for conjugation to linker-antibiotic intermediate, as taught below.

Figure 11A and Figure 11B 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.

Table 1A: Light chain CDR sequences of the anti-WTAα.

Antibody	CDR L1	CDR L2	CDR L3
4461	KSSQSVLSRANNNYYVA (SEQ ID NO.1)	WASTREF (SEQ ID NO.2)	QQYYTSRRT (SEQ ID NO.3)
4624	RSNQNLLSSSNNNYLA (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)

Table 1B: Heavy chain CDR sequences of the anti-WTAα.

Antibody	CDR H1	CDR H2	CDR H3
4461	DYYMH (SEQ ID NO.4)	WINPKSGGTNYAQRFQG (SEQ ID NO.5)	DCGSGGLRDF (SEQ ID NO.6)
4624	DYYIH (SEQ ID NO.10)	WINPNTGGTYYAQKFRD (SEQ ID NO.11)	DCGRGGLRDI (SEQ ID NO.12)
4399	DYYIH (SEQ ID NO.16)	WINPNTGGTNYAQKFQG (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)

The sequences of the each pair of VL and VH are as follows:

4461 Light Chain Variable Region

DIQMTQSPDSLAVSLGERATINCKSSQSVLSRANNNYYVAWYQHKPGQPPKLLIYW ASTREFGVPDRFSGSGSGTDFTLTINSLQAEDVAVYYCQQYYTSRRTFGQGTKVEIK (SEQ ID NO. 25)

4461 Heavy Chain Variable Region

QVQLVQSGAEVRKPGASVKVSCKASGYSFTDYYMHWVRQAPGQGLEWMGWINPK SGGTNYAQRFQGRVTMTGDTSISAAYMDLASLTSDDTAVYYCVKDCGSGGLRDFW GQGTTVTVSS (SEQ ID NO. 26)

4624 Light Chain Variable Region

DIQMTQSPDSLSVSLGERATINCRSNQNLLSSSNNNYLAWYQQKPGQPLKLLIYWAS TRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYANPRTFGQGTKVEIK (SEQ ID NO. 27)

4624 Heavy Chain Variable Region

QVQLQQSRVEVKRPGTSVKVSCKTSGYTFSDYYIHWVRLAPGQGLELMGWINPNTG GTYYAQKFRDRVTMTRDTSIATAYLEMSSLTSDDTAVYYCAKDCGRGGLRDIWGPG TMVTVSS (SEQ ID NO. 28)

4399 Light Chain Variable Region

EIVLTQSPDSLAVSLGERATINCKSNQNVLASSNDKNYLAWFQHKPGQPLKLLIYWA SIRESGVPDRFSGSGSGTDFTLTISSLRAEDVAVYYCQQYYTNPRTFGQGTKVEFN (SEQ ID NO. 29)

4399 Heavy Chain Variable Region

EVQLVQSGAEVKKPGTSVKVSCKASGYTFTDYYIHWVRLAPGQGLELMGWINPNTG GTNYAQKFQGRVTMTRDTSIATAYMELSSLTSDDTAVYYCAKDCGNAGLRDIWGQ GTTVTVSS (SEQ ID NO. 30)

6267 Light Chain Variable Region

DIQLTQSPDSLAVSLGERATINCKSSQNVLYSSNNKNYLAWYQQKPGQPPKLLIYWA STRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYTSPPYTFGQGTKLEIE (SEQ ID NO. 31)

6267 Heavy Chain Variable Region

EVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIGWVRQMPGKGLEWMGIIHPGDS KTRYSPSFQGQVTISADKSISTAYLQWNSLKASDTAMYYCARLYCSGGSCYSDRAFS SLGAGGYYYYGMGVWGQGTTVTVSS (SEQ ID NO. 32).

For production of an AAC compound, the invention provides an isolated monoclonal antibody that binds wall teichoic acid alpha (WTAα) 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 1A and Table 1B above.

In another embodiment, the isolated monoclonal Ab that binds WTAα 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%.

The present invention also provides an AAC comprising an anti-WTA beta antibody from the list of Abs exemplified in Figure 12. In one embodiment, the isolated anti-WTA beta monoclonal Ab comprises 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 12. 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%.

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).

Figures 13A-1 and 13A-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 13B-1 to 13B-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)

DIVMTQSPSILSASVGDRVTITCRASQTISGWLAWYQQKPAEAPKLLIYKASTLESGV PSRFSGSGSGTEFTLTISSLQPDDFGIYYCQQYKSYSFNFGQGTKVEIK (SEQ ID NO.111)

6078 Heavy Chain Variable Region (VH)

XX_1QLVQSGAEVKKPGASVKVSCEASGYTLTSYDINWVRQATGQGPEWMGWMNA NSGNTGYAQKFQGRVTLTGDTSISTAYMELSSLRSEDTAVYYCARSSILVRGALGRY FDLWGRGTLVTVSS (SEQ ID NO.112) wherein X is Q or E; and X_1 is M, I or V.

6078 Light Chain

DIVMTQSPSILSASVGDRVTITCRASQTISGWLAWYQQKPAEAPKLLIYKASTLESGV PSRFSGSGSGTEFTLTISSLQPDDFGIYYCQQYKSYSFNFGQGTKVEIKRTVAAPSVFIF PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO.113)

6078 Cysteine-engineered Light Chain

DIVMTQSPSILSASVGDRVTITCRASQTISGWLAWYQQKPAEAPKLLIYKASTLESGV PSRFSGSGSGTEFTLTISSLQPDDFGIYYCQQYKSYSFNFGQGTKVEIKRTVAAPSVFIF PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS LSSTLTLSKADYEKHKVYACEVTHQGLSSPCTKSFNRGEC (SEQ ID NO.115)

6078 WT full length Heavy Chain

QMQLVQSGAEVKKPGASVKVSCEASGYTLTSYDINWVRQATGQGPEWMGWMNAN
SGNTGYAQKFQGRVTLTGDTSISTAYMELSSLRSEDTAVYYCARSSILVRGALGRYF
DLWGRGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG
ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKS
CDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
EKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP
G (SEQ ID NO.114)

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

EXQLVQSGAEVKKPGASVKVSCEASGYTLTSYDINWVRQATGQGPEWMGWMNAN
SGNTGYAQKFQGRVTLTGDTSISTAYMELSSLRSEDTAVYYCARSSILVRGALGRYF
DLWGRGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG
ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKS
CDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
EKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP
G (SEQ ID NO.116) wherein X can be M, I or V.

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

EXQLVQSGAEVKKPGASVKVSCEASGYTLTSYDINWVRQATGQGPEWMGWMNAN
SGNTGYAQKFQGRVTLTGDTSISTAYMELSSLRSEDTAVYYCARSSILVRGALGRYF
DLWGRGTLVTVSSCSTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG
ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKS
CDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
EKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP
G (SEQ ID NO.117) wherein X is M, I or V.

In one embodiment, the invention provides 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 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. 111 and the H chain comprises a VH of SEQ ID NO. 112. In a yet more specific embodiment, the isolated anti-WTA beta antibody comprises a L chain of SEQ ID NO. 113 and a H chain of SEQ ID NO. 114.

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 embodiment, 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.

Figures 14A-1 and 14A-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 near the end of the constant region (at EU residue no. 205). Figures 14B-1, 14B-2, 14B-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 at the beginning of the constant region C_H1 (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

DIQLTQSPDSLAVSLGERATINCKSSQSIFRTSRNKNLLNWYQQRPGQPPRLLIHWAS TRKSGVPDRFSGSGFGTDFTLTITSLQAEDVAIYYCQQYFSPPYTFGQGTKLEIK (SEQ ID NO. 119)

4497 Heavy Chain Variable Region

EVQLVESGGGLVQPGGSLRLSCSASGFSFNSFWMHWVRQVPGKGLVWISFTNNEGT TTAYADSVRGRFIISRDNAKNTLYLEMNNLRGEDTAVYYCARGDGGLDDWGQGTL VTVSS (SEQ ID NO. 120)

4497.v8 Heavy Chain Variable Region

EVQLVESGGGLVQPGGSLRLSCSASGFSFNSFWMHWVRQVPGKGLVWISFTNNEGT TTAYADSVRGRFIISRDNAKNTLYLEMNNLRGEDTAVYYCARGEGGLDDWGQGTL VTVSS (SEQ ID NO. 156)

4497 Light Chain

DIQLTQSPDSLAVSLGERATINCKSSQSIFRTSRNKNLLNWYQQRPGQPPRLLIHWAS TRKSGVPDRFSGSGFGTDFTLTITSLQAEDVAIYYCQQYFSPPYTFGQGTKLEIKRTV AAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO. 121)

4497 v.8 Heavy Chain

EVQLVESGGGLVQPGGSLRLSCSASGFSFNSFWMHWVRQVPGKGLVWISFTNNEGT TTAYADSVRGRFIISRDNAKNTLYLEMNNLRGEDTAVYYCARGEGGLDDWGQGTL VTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO. 122)

4497 -Cys Light Chain

DIQLTQSPDSLAVSLGERATINCKSSQSIFRTSRNKNLLNWYQQRPGQPPRLLIHWAS TRKSGVPDRFSGSGFGTDFTLTITSLQAEDVAIYYCQQYFSPPYTFGQGTKLEIKRTV AAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD

SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO. 123)

4497.v8- Heavy Chain

EVQLVESGGGLVQPGGSLRLSCSASGFSFNSFWMHWVRQVPGKGLVWISFTNNEGT
TTAYADSVRGRFIISRDNAKNTLYLEMNNLRGEDTAVYYCARGEGGLDDWGQGTL
VTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF
PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP
CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH
NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG
QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL
DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO. 157; the same as SEQ ID NO.122).

4497.v8 -Cys Heavy Chain

EVQLVESGGGLVQPGGSLRLSCSASGFSFNSFWMHWVRQVPGKGLVWISFTNNEGT
TTAYADSVRGRFIISRDNAKNTLYLEMNNLRGEDTAVYYCARGEGGLDDWGQGTL
VTVSSCSTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF
PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP
CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH
NAKTKPREEQYNSTYRVVSVLTVLHQDWLGKEYKCKVSNKALPAPIEKTISKAKGQ
PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD
SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO. 124)

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 embodiment, the isolated anti-WTA beta antibody comprises a L chain of SEQ ID NO. 121 and a H chain of SEQ ID NO. 122.

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.

Yet another embodiment is an antibody that binds to the same epitope as each of the anti-WTA alpha Abs of Figure 11A and Figure 11B. Also provided is an antibody that binds to the same epitope as each of the anti-WTA beta Abs of Figure 12, Figures 13A and 13B, and Figures 14A and 14B.

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 α- or β-glycosidic linkages, by TarM glycosyltransferase or TarS glycosyltransferase, respectively. Accordingly, cell wall preparations from glycosyltransferase mutant strains lacking TarM (ΔTarM), TarS (ΔTarS), or both TarM and TarS (ΔTarM/ΔTarS) were subjected to immunoblotting analysis with antibodies against WTA. WTA antibody (S7574) specific to α-GlcNAc modifications on WTA does not bind to cell wall preparation from ΔTarM strain (Meijer, P.J., *et al.* (2006) "Isolation of human antibody repertoires with preservation of the natural heavy and light chain pairing." *Journal of molecular biology* **358**, 764-772). Vice versa, a WTA antibody (S4462) specific to β-GlcNAc modifications on WTA does not bind to cell wall preparation from ΔTarS strain. As expected, both these antibodies do not bind to cell wall preparations from a deletion strain lacking both glycosyltransferases (ΔTarM/ΔTarS) and also the strain lacking any WTA (ΔTagO). According to such analysis, antibodies have been characterized as anti- α-GlcNAc WTA mAbs, or as anti- β-GlcNAc WTA mAbs as listed in the Table in Figures 6A and 6B.

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 (THIOMAB™ or 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.

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.

In another embodiment, the invention provides an isolated anti-WTA antibody for conjugation to produce an AAC, the 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

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF
PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKT
HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA
LHNHYTQKSLSLSPGK

(SEQ ID NO:148)

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

CSTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF
PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKT
HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA
LHNHYTQKSLSLSPGK

(SEQ ID NO:149)

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

Light chain (kappa) constant region, wild-type

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS
QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR
GEC

(SEQ ID NO:150)

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

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS
QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPCTKSFNRG
EC

(SEQ ID NO:151)

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.

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.

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.

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

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 marcescans*, 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.

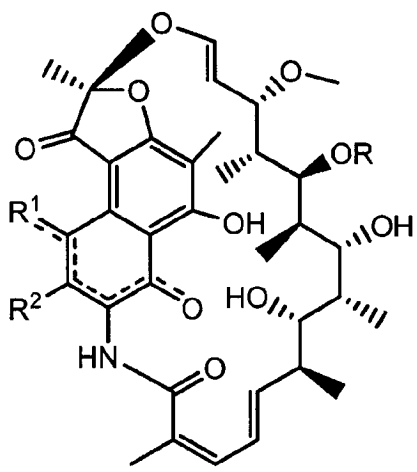
In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for antibody-encoding vectors. *Saccharomyces cerevisiae*, or common baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other 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. wickeramii* (ATCC 24,178), *K. waltii* (ATCC 56,500), *K. drosophilarum* (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

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.

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_1–C_{12} alkyl, or $C(O)CH_3$;

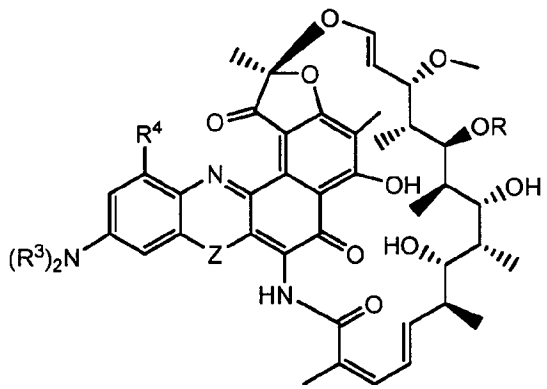
R^1 is OH;

R^2 is CH=N–(heterocyclyl), wherein the heterocyclyl is optionally substituted with one or more groups independently selected from $C(O)CH_3$, C_1–C_{12} alkyl, C_1–C_{12} heteroaryl, C_2–C_{20} heterocyclyl, C_6–C_{20} aryl, and C_3–C_{12} carbocyclyl;

or R^1 and R^2 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_1–C_{12} alkyl, or OH; and

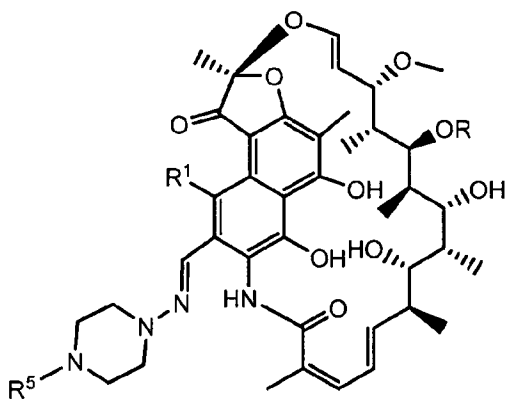
where the non-peptide linker PML is covalently attached to R^2.

An embodiment 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 non-peptide linker PML is covalently attached to the nitrogen atom of $N(R^3)_2$.

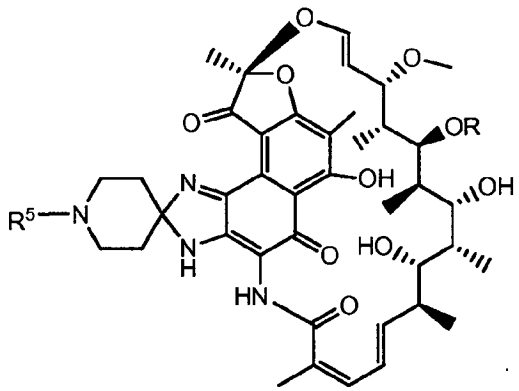
An embodiment of a rifampicin-type moiety is:



wherein

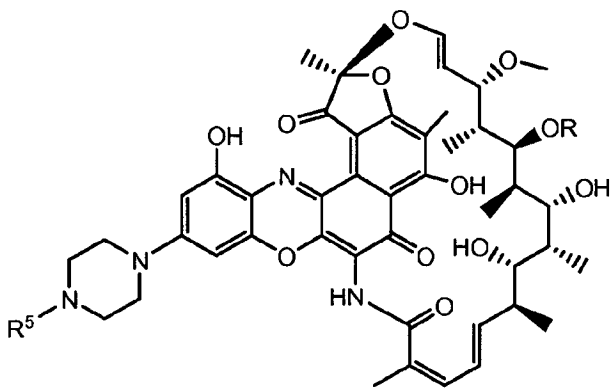
R^5 is selected from H and C_1–C_{12} alkyl; and where the non-peptide linker PML is covalently attached to the nitrogen atom of NR^5.

An embodiment of a rifabutin-type moiety is:



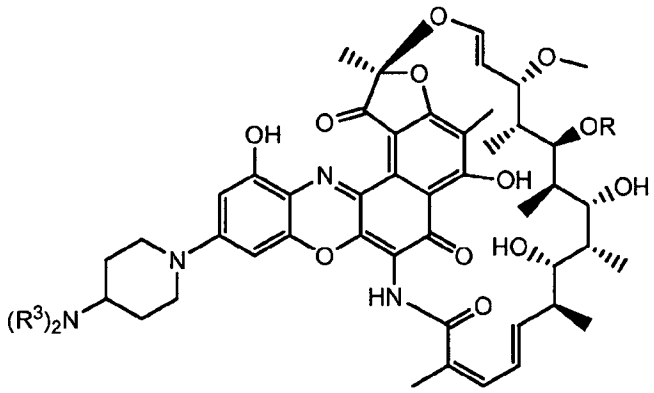
wherein R^5 is selected from H and C_1–C_{12} alkyl; and where the non-peptide linker PML is covalently attached to the nitrogen atom of NR^5.

An embodiment of a benzoxazinorifamycin-type moiety is:



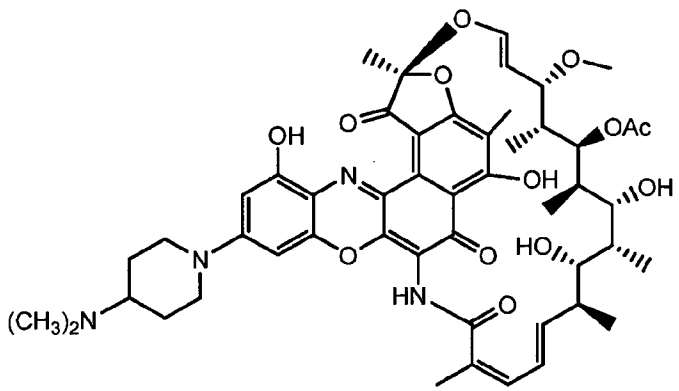
wherein R^5 is selected from H and C_1–C_{12} alkyl; and where the non-peptide linker PML is covalently attached to the nitrogen atom of NR^5.

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 non-peptide linker PML is covalently attached to the nitrogen atom of $N(R^3)_2$.

An embodiment of a benzoxazinorifamycin-type moiety, referred to herein as dimethylpipBOR, is:

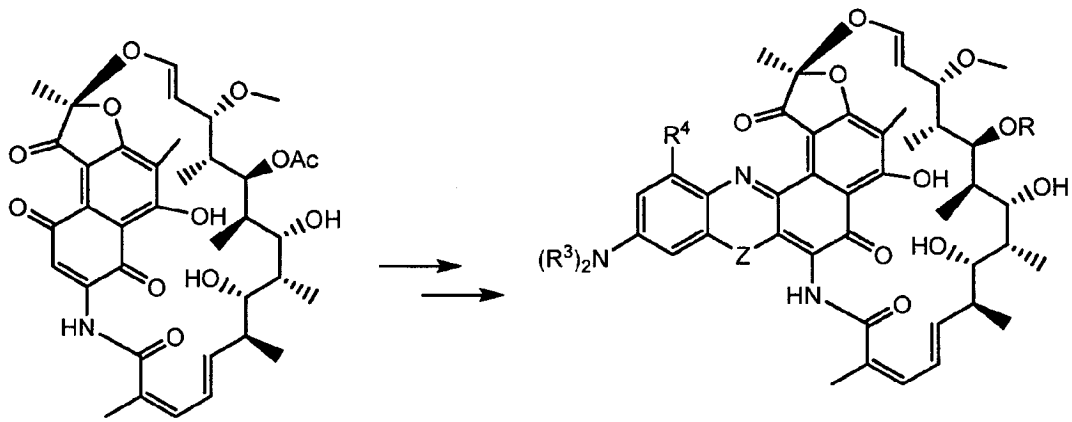


where the non-peptide linker PML is covalently attached to the dimethylamino nitrogen atom.

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, $N(C_1$–C_{12} alkyl), O and S (see, e.g., Fig. 23A and B, and Fig. 25A and B in WO 2014/194247) . Benzoxazino (Z = O), benzthiazino (Z = S), benzdiazino (Z = NH, $N(C_1$–C_{12} 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, which is incorporated by reference for this purpose. 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.

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, and WO 2014/194247, each of which is hereby incorporated by reference). 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).



rifamycin-S benzoxazinorifamycin

PROTEASE-CLEAVABLE NON-PEPTIDE LINKERS

A "protease-cleavable, non-peptide linker" (PML) 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. Protease-cleavable, non-peptide linkers in AAC are substrates for cleavage by intracellular proteases, including under lysosomal conditions. Proteases includes various cathepsins and caspases. Cleavage of the non-peptide linker of an AAC inside a cell may release the rifamycin-type antibiotic with anti-bacterial effects.

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.

The PML moiety of an AAC may comprise one amino acid residue.

The PML moiety of an AAC comprises a peptidomimetic unit.

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.

Cysteine engineered antibodies react with linker reagents or linker-antibiotic intermediates, with electrophilic functional groups such as maleimide or α-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.

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, α-haloacetyl, activated esters such as succinimide esters, 4-nitrophenyl esters, pentafluorophenyl esters, tetrafluorophenyl esters, anhydrides, acid chlorides, sulfonyl chlorides, isocyanates and isothiocyanates.

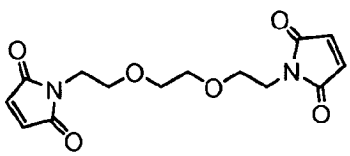
In another embodiment, a linker reagent or antibiotic-linker intermediate has a reactive functional group which has a nucleophilic group that is reactive to an electrophilic group present on an antibody. Useful electrophilic groups on an antibody include, but are not limited to, pyridyl disulfide, aldehyde 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.

A PML moiety may comprise one or more linker components. Exemplary linker components include a single amino acid such as citrulline ("cit"), 6-maleimidocaproyl ("MC"), maleimidopropanoyl ("MP"), and p-aminobenzyloxycarbonyl ("PAB"), N-succinimidyl 4-(2-pyridylthio) pentanoate ("SPP"), and 4-(N-maleimidomethyl)

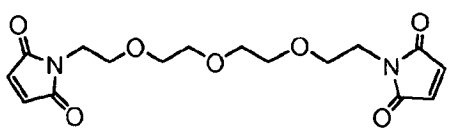
cyclohexane-1 carboxylate ("MCC"). Various linker components are known in the art, some of which are described below.

In another embodiment, the linker may be substituted with groups that modulate solubility or reactivity. For example, a charged substituent such as sulfonate ($-SO_3^-$) 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.

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, $BM(PEG)_2$, and $BM(PEG)_3$. 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.



$BM(PEG)_2$



$BM(PEG)_3$

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.

In another embodiment, the PML 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.

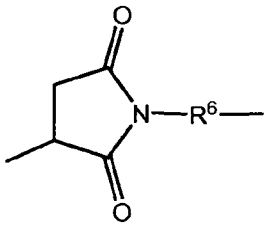
In certain embodiments of Formula I AAC, the protease-cleavable, non-peptide linker PML has the formula:

$$-Str-PM-Y-$$

where Str is a stretcher unit; PM is a peptidomimetic unit, and Y is a spacer unit; abx is the rifamycin-type antibiotic; and

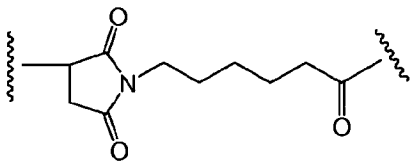
p is an integer from 1 to 8.

In one embodiment, a stretcher unit "Str" has the formula:

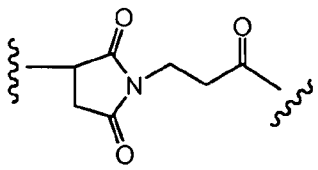


wherein R^6 is selected from the group consisting of C_1-C_{12} alkylene, C_1-C_{12} alkylene-C(=O), C_1-C_{12} alkylene-NH, $(CH_2CH_2O)_r$, $(CH_2CH_2O)_r$-C(=O), $(CH_2CH_2O)_r$-CH_2, and C_1-C_{12} alkylene-NHC(=O)CH_2CH(thiophen-3-yl), where r is an integer ranging from 1 to 10.

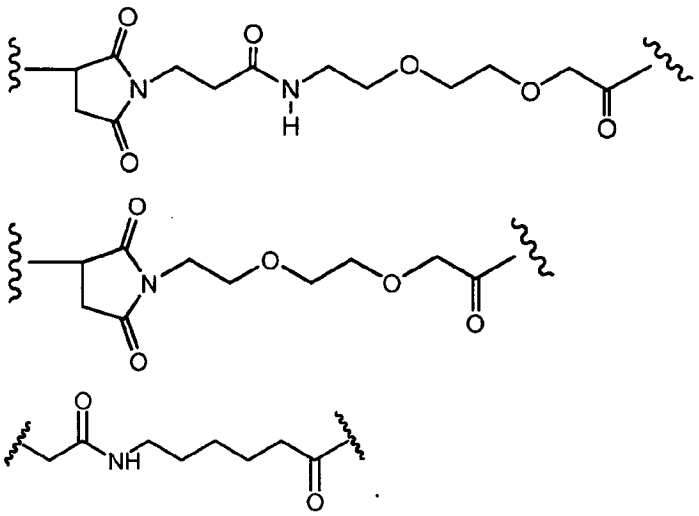
Exemplary stretcher units are shown below (wherein the wavy line indicates sites of covalent attachment to an antibody):



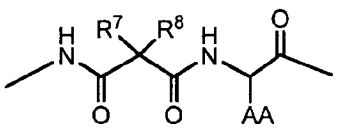
MC



MP



In one embodiment, PM has the formula:



where R^7 and R^8 together form a C_3-C_7 cycloalkyl ring, and

AA is an amino acid side chain 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 one embodiment, spacer unit Y comprises para-aminobenzyl (PAB) or para-aminobenzyloxycarbonyl (PABC).

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-aminobenzyloxycarbonyl (PAB).

In one embodiment, the antibiotic forms a quaternary amine, such as the dimethylaminopiperidyl group, when attached to the PAB spacer unit of the non-peptide linker PML. Examples of such quaternary amines are linker-antibiotic intermediates (PLA) are PLA-1 to 4 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 non-peptide linker PML, 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 (PLA) are PLA-5 and PLA-6 from Table 2.

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.

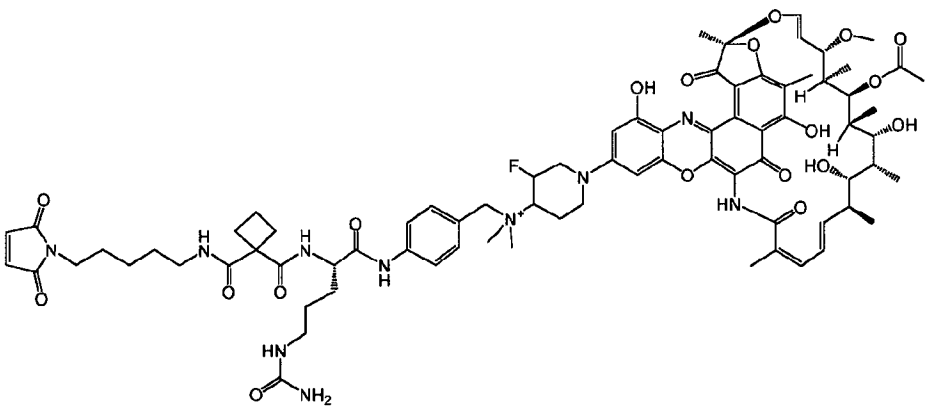
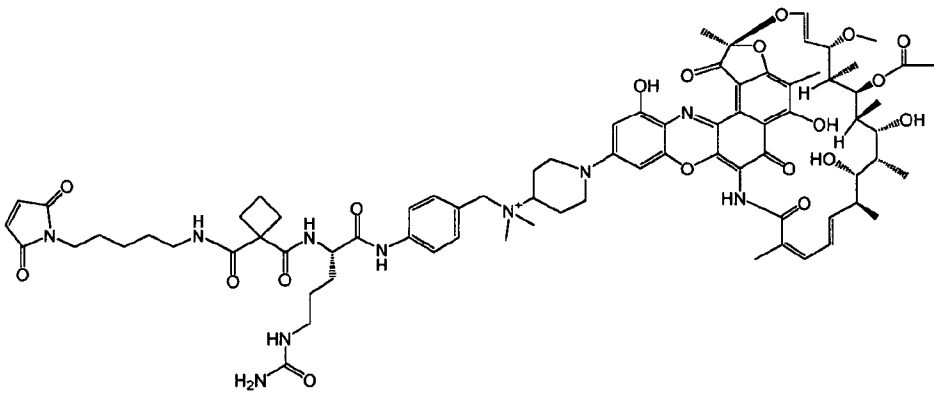
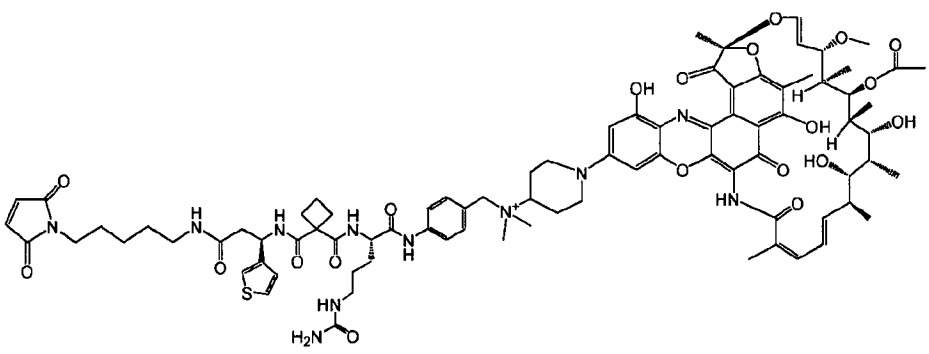
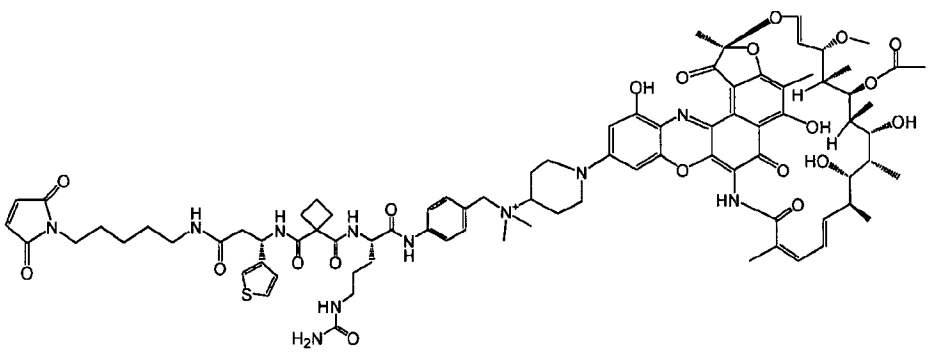
The amount of active antibiotic released from cleavage of AAC can be measured by the Caspase release assay of Example 8.

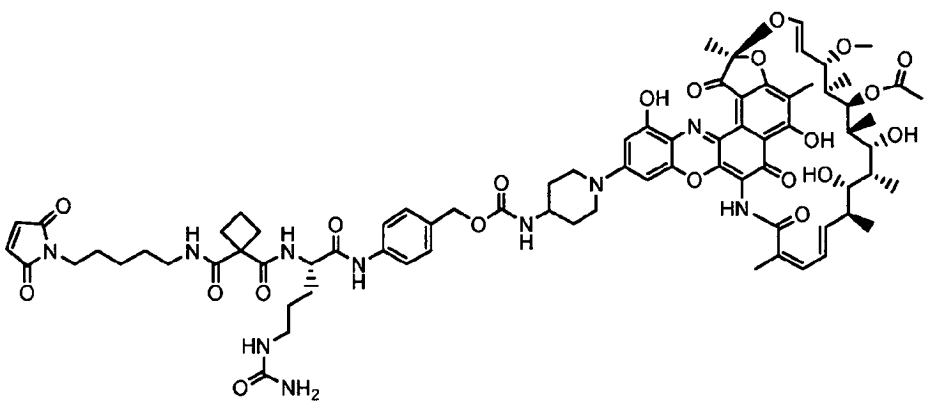
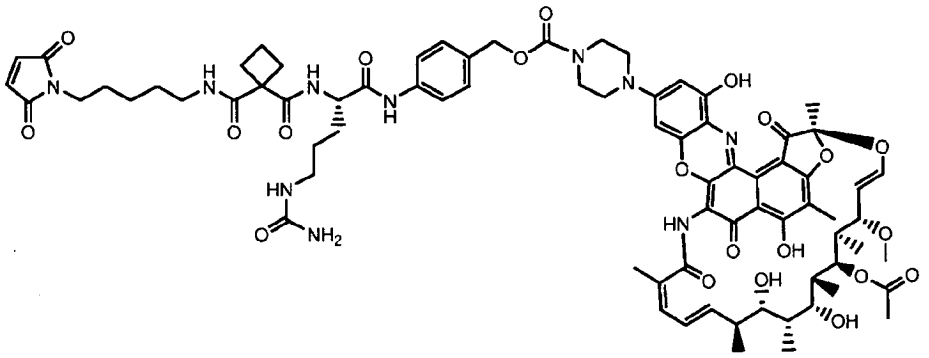
LINKER-ANTIBIOTIC INTERMEDIATES USEFUL FOR AAC

PML Linker-antibiotic intermediates (PLA) of Formula II and Table 2 were prepared by coupling a rifamycin-type antibiotic moiety with a linker reagent, Examples 11-21. 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

Table 2 PML Linker-antibiotic intermediates

LA No.	Structure

PLA-1	
PLA-2	
PLA-3	
PLA-4	

PLA-5	
PLA-6	

EMBODIMENTS OF ANTIBODY-ANTIBIOTIC CONJUGATES

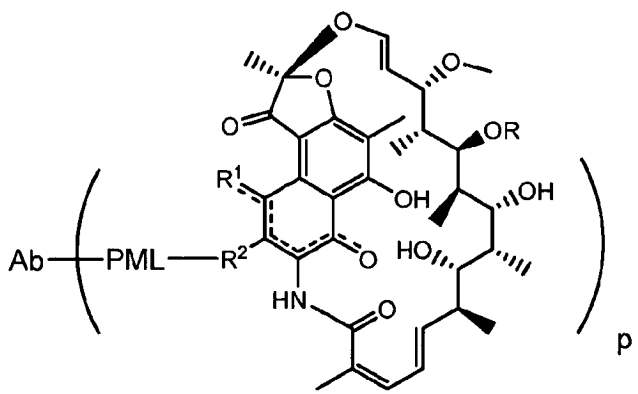
Cysteine engineered, anti-WTA antibodies were linked via the free cysteine thiol group to derivatives of rifamycin, termed pipBOR and others, via a protease cleavable, non-peptide linker to form the antibody-antibiotic conjugate compounds (AAC) in Table 3. The linker is designed to be cleaved by lysosomal proteases including cathepsins B, D and others, Generation of the linker-antibiotic intermediate consisting of the antibiotic and the PML linker and others, is described in detail in Examples 11-21. 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.

The AAC named "dimethylpipBOR" is identical to the "pipBOR" AAC except for the dimethylated amino on the antibiotic and the oxycarbonyl group on the linker.

Figure 3 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 linker may be cleaved by lysosomal proteases releasing the active antibiotic inside the phagolysosome.

An embodiment of the antibody-antibiotic conjugate (AAC) compounds of the invention includes Formula I:



I

wherein:

the dashed lines indicate an optional bond;

R is H, C_1–C_{12} alkyl, or $C(O)CH_3$;

R^1 is OH;

R^2 is CH=N–(heterocyclyl), wherein the heterocyclyl is optionally substituted with one or more groups independently selected from $C(O)CH_3$, C_1–C_{12} alkyl, C_1–C_{12} heteroaryl, C_2–C_{20} heterocyclyl, C_6–C_{20} aryl, and C_3–C_{12} carbocyclyl;

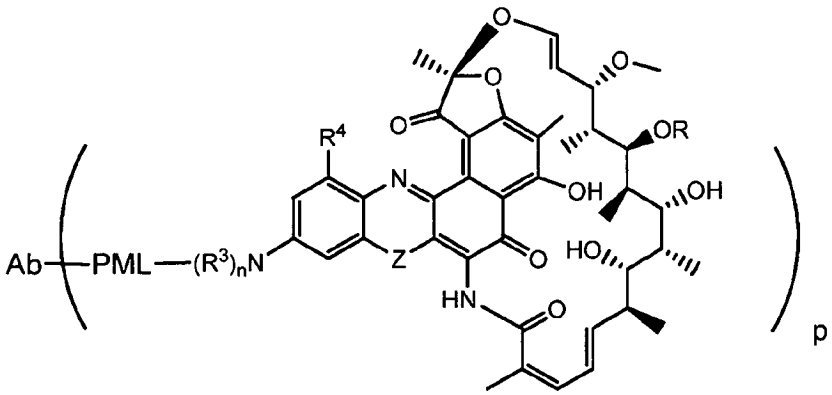
or R^1 and R^2 form a five- or six-membered fused heteroaryl or heterocyclyl, and optionally forming a spiro or fused six-membered heteroaryl, heterocyclyl, aryl, or carbocyclyl ring, wherein the spiro or fused six-membered heteroaryl, heterocyclyl, aryl, or carbocyclyl ring is optionally substituted H, F, Cl, Br, I, C_1–C_{12} alkyl, or OH;

PML is the protease-cleavable, non-peptide linker attached to R^2 or the fused heteroaryl or heterocyclyl formed by R^1 and R^2;

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

p is an integer from 1 to 8.

Another embodiment of the antibody-antibiotic conjugate (AAC) compounds of the invention includes the formula:



wherein

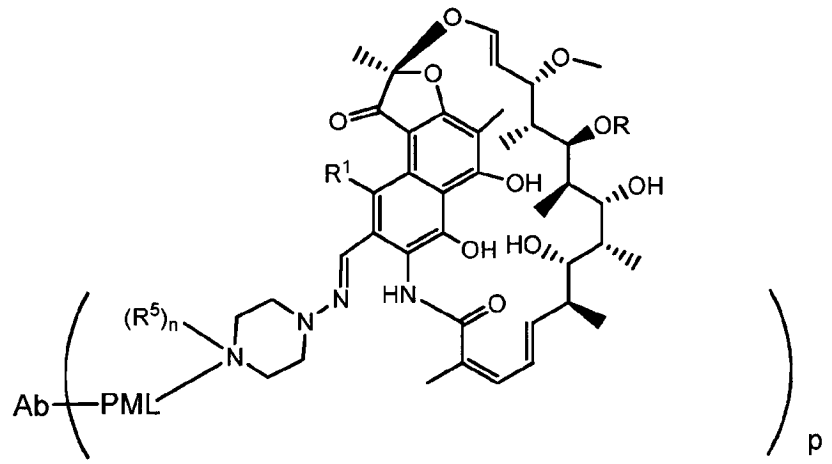
R^3 is independently selected from H and C_1–C_{12} alkyl;

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.

Another embodiment of the antibody-antibiotic conjugate (AAC) compounds of the invention includes the formula:

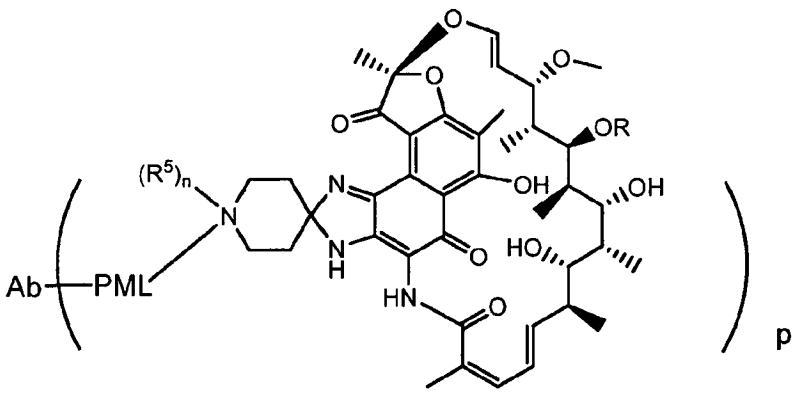


wherein

R^5 is selected from H and C_1–C_{12} alkyl; and

n is 0 or 1.

Another embodiment of the antibody-antibiotic conjugate (AAC) compounds of the invention includes the formula:

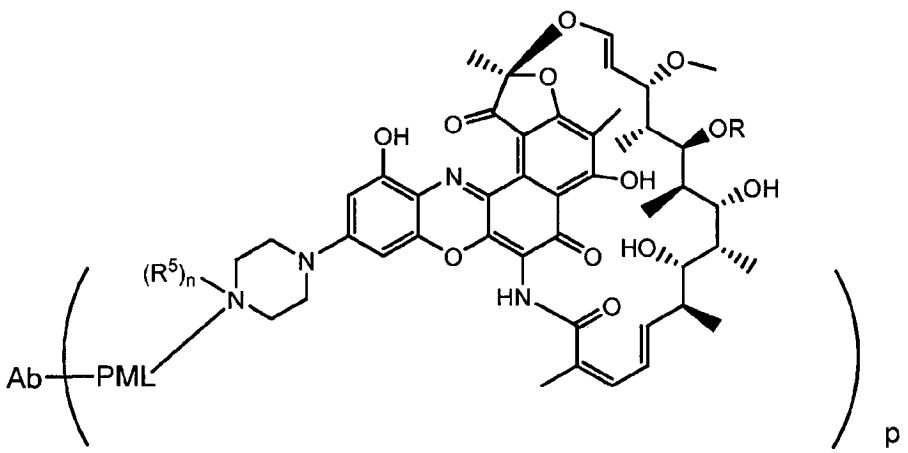


wherein

R^5 is selected from H and C_1–C_{12} alkyl; and

n is 0 or 1.

Another embodiment of the antibody-antibiotic conjugate (AAC) compounds of the invention includes the formula:

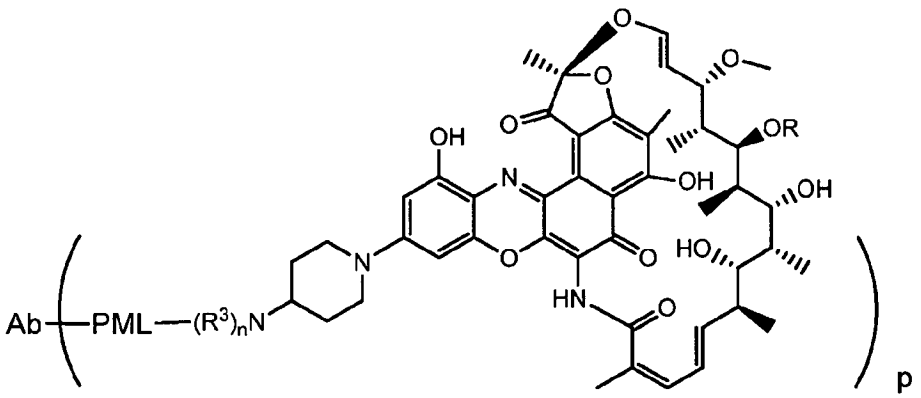


wherein

R^5 is independently selected from H and C_1–C_{12} alkyl; and

n is 0 or 1.

Another embodiment of the antibody-antibiotic conjugate (AAC) compounds of the invention includes the formula:

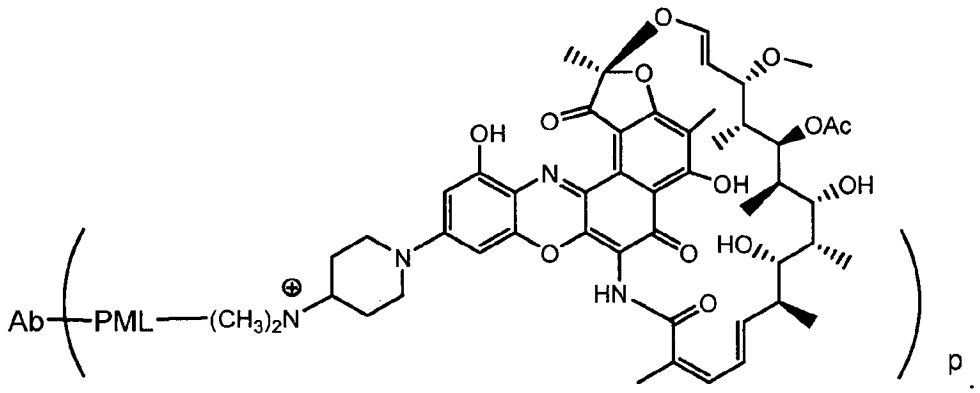


wherein

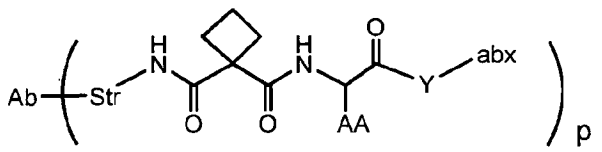
R^3 is independently selected from H and C_1–C_{12} alkyl; and

n is 1 or 2.

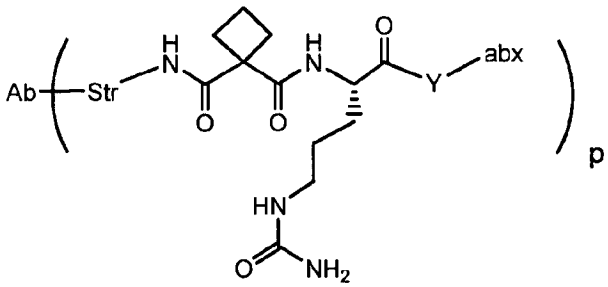
Another embodiment of the antibody-antibiotic conjugate (AAC) compounds of the invention includes the formula:



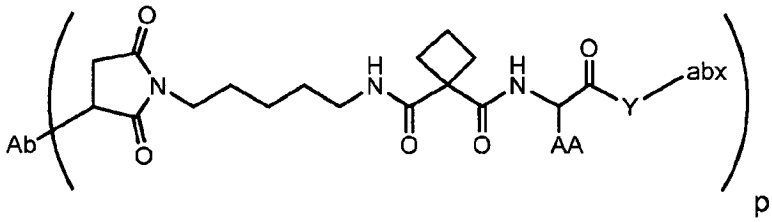
Another embodiment of the antibody-antibiotic conjugate (AAC) compounds of the invention includes the formula:



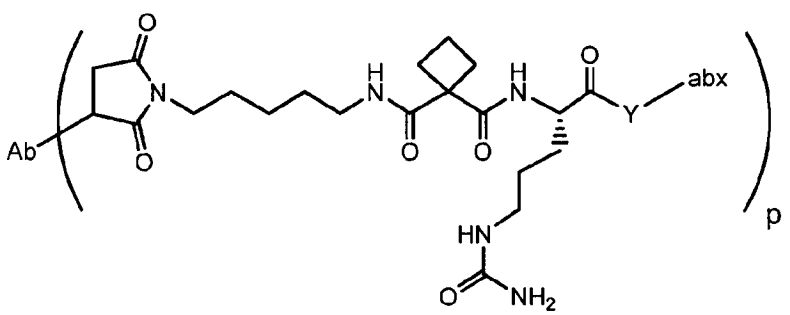
Another embodiment of the antibody-antibiotic conjugate (AAC) compounds of the invention includes the formula:



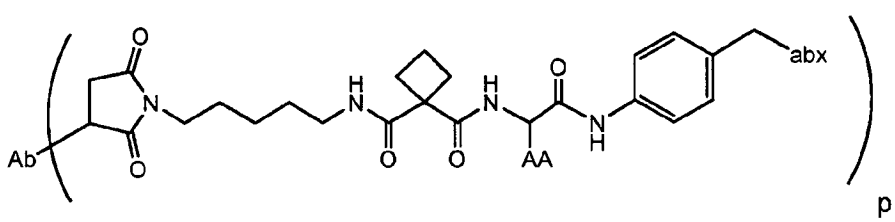
Another embodiment of the antibody-antibiotic conjugate (AAC) compounds of the invention includes the formula:



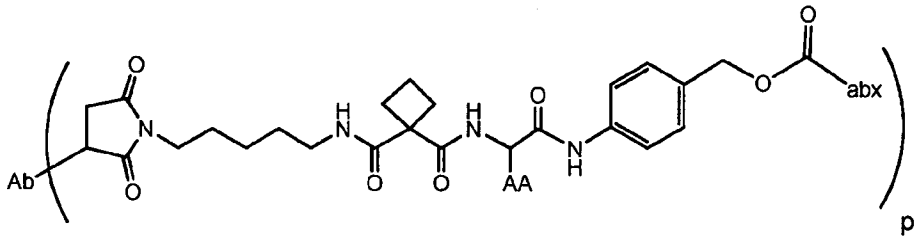
Another embodiment of the antibody-antibiotic conjugate (AAC) compounds of the invention includes the formula:



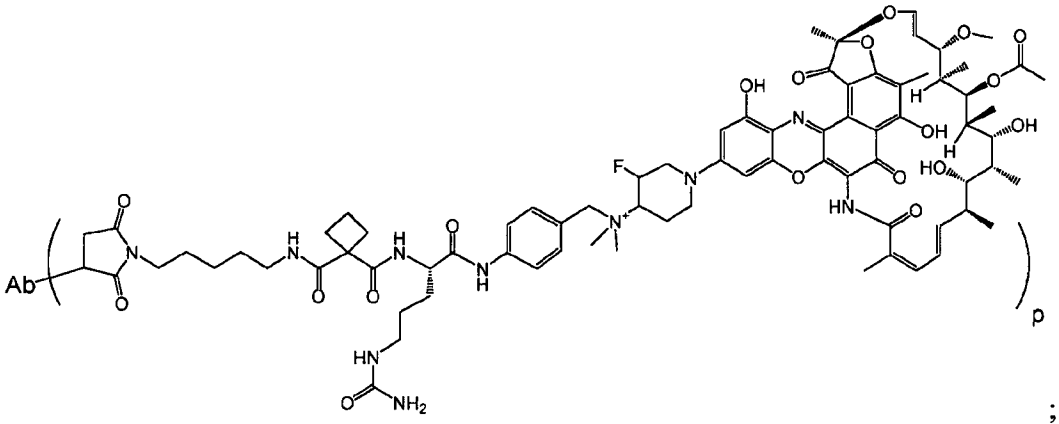
Another embodiment of the antibody-antibiotic conjugate (AAC) compounds of the invention includes the formulas:



and



Another embodiment of the antibody-antibiotic conjugate (AAC) compounds of the invention includes the formulas:



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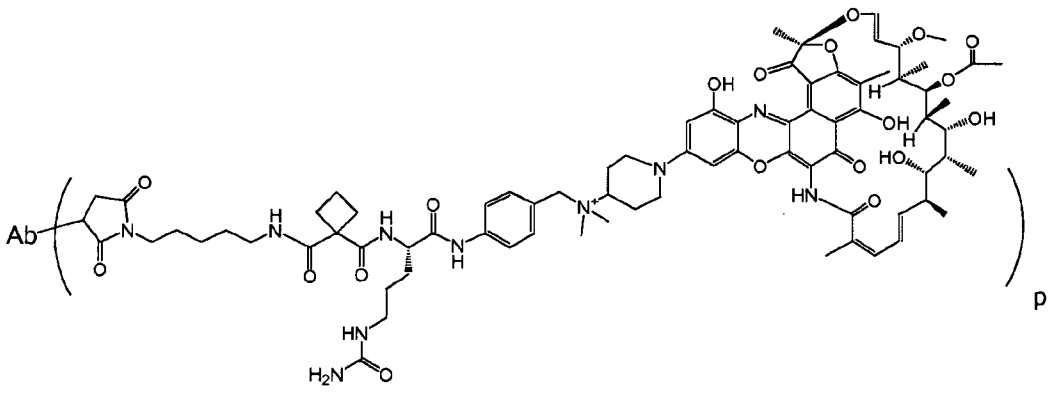
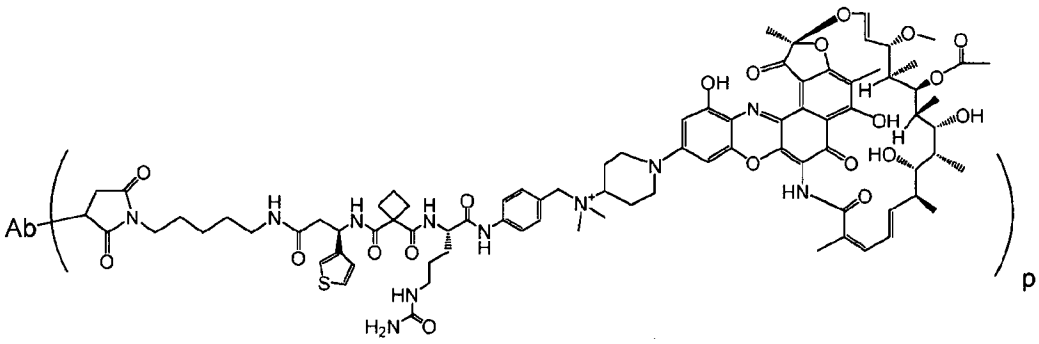


;



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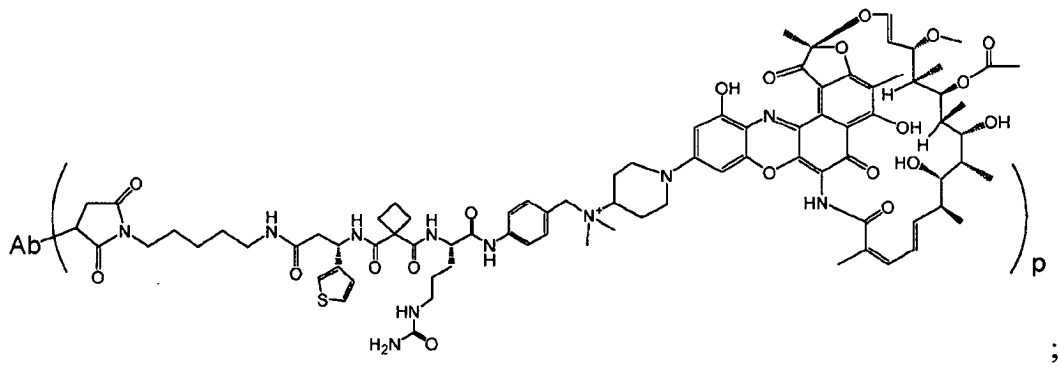

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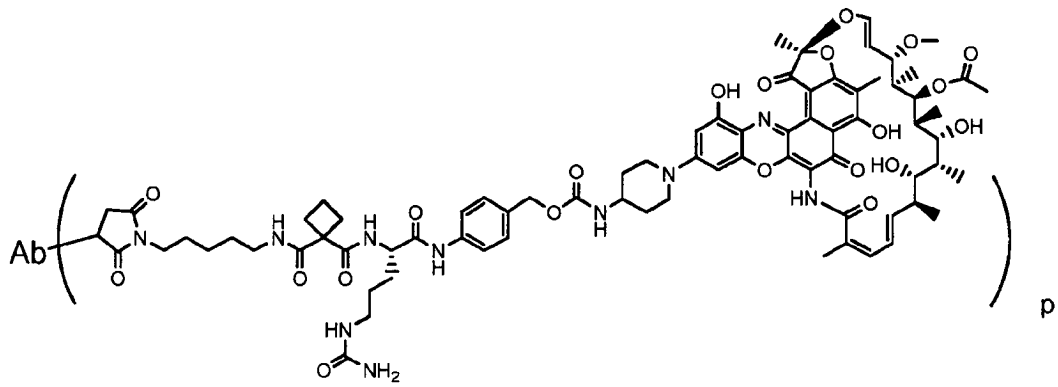

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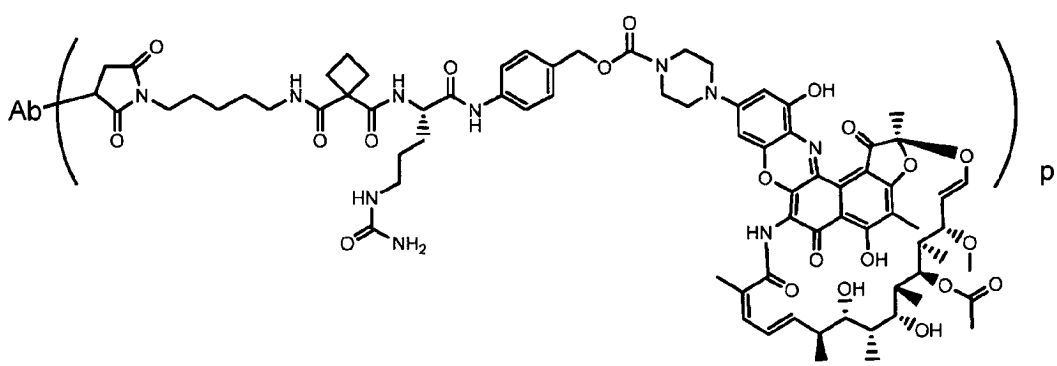
and



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ANTIBIOTIC LOADING OF AAC

Antibiotic loading is represented by p, the 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.

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.

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 tricarbonylethylphosphine (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.

The loading (antibiotic/antibody ratio, "AAR") of an AAC, also may be referred to herein as drug to antibody ratio (DAR), 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.

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

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, which is expressly incorporated herein by reference.

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 tricarbonylethylphosphine (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).

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.

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.

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 7. AAC were tested for efficacy by *in vitro* macrophage assay (Example 9) and *in vivo* mouse kidney model (Example 10).

Table 3 WTA Antibody-PML-antibiotic conjugates (AAC)

AAC No.	AAC formula	linker-abx PLA No.	AAR *
101	thio-S6060-HC-WT/LC-cys-MC-(CBDK-cit)-PAB-(dimethylpipBOR)	PLA-2	1.9
102	thio-S4497-LC-cys-MC-(CBDK-cit)-PAB-(dimethylpipBOR)	PLA-2	1.8
103	thio-S4497-LC-V205C-MC-((*R*)-thiophen-3-yl-CBDK-cit)-PAB-(dimethylpipBOR)	PLA-3	1.8
104	thio-S4497-LC-V205C-MC-((*S*)-thiophen-3-yl-CBDK-cit)-PAB-(dimethylpipBOR)	PLA-4	1.5
105	thio-S4497-LC-MC-(CBDK-cit)-PABC-(piperazBTR)	PLA-6	-
106	thio-S4497-HC-A118C-MC-(CBDK-cit)-PAB-(dimethylpipBOR)	PLA-2	1.8
107	thio-S4497-HC-A118C-MC-(CBDK-cit)-PABC-(pipBOR)	PLA-5	2.0

* AAR = antibiotic/antibody ratio average

Wild-type ("WT"), cysteine engineered mutant antibody ("thio"), light chain ("LC"), heavy chain ("HC"), 6-maleimidocaproyl ("MC"), maleimidopropanoyl ("MP"), cyclobutyldiketo ("CBDK"), citrulline ("cit"), cysteine ("cys"), p-aminobenzyl ("PAB"), and p-aminobenzyloxycarbonyl ("PABC")

METHODS OF TREATING AND PREVENTING INFECTIONS WITH ANTIBODY-ANTIBIOTIC CONJUGATES

The anti-WTA-AACs of the invention are useful as antimicrobial agents effective against human and veterinary Staphylococci, for example S. aureus, S. saprophyticus and S. simulans. In a specific aspect, the AACs of the invention are useful to treat *S. aureus* infections.

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 abcesses (all of which contain large quantities of neutrophils). About 40% of patients develop complications if the bacteremia persists beyond three days.

The proposed mechanism of action of an AAC has been described above (under subheading Antibody-Antibiotic Conjugates). The anti-WTA antibody-antibiotic conjugates (AAC) of the invention have significant therapeutic advantages for treating intracellular pathogens. The AAC linker is cleaved by exposure to phagolysosomal enzymes, releasing an active antibiotic. Due to the confined space and relatively high local antibiotic concentration (about 10^4 per bacterium), the result is that the phagolysosome no longer supports the survival of the intracellular pathogen. 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. 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.

In another embodiment, the anti-WTA-AAC of the invention may be used to treat infection regardless of the intracellular compartment in which the pathogen survives.

In another embodiment, anti-WTA-AACs of the invention could also be used to target Staphylococci bacteria in planktonic or biofilm form. Bacterial infections treatable with antibody-antibiotic conjugates (AAC) of the invention include treating bacterial pulmonary infections, such as S. aureus pneumonia, osteomyelitis, recurrent rhinosinusitis, bacterial endocarditis, bacterial ocular infections, such as trachoma and conjunctivitis, heart, brain or skin infections, infections of the gastrointestinal tract, such as travellers' diarrhea, 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 anti-WTA 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.

The bacterial infection may be caused by 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.

Analysis of various Gram+ bacteria found WTA beta expressed on all S. aureus, including MRSA and MSSA strains, as well as 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– bacteria. Therefore one aspect of the invention is a method of treating a patient infected with one or more of S. aureus, 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 and/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 one or more of 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.

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 or ciprofloxacin. 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.

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. If 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 (heart, lungs, liver, kidneys, vascular collapse) has resolved either fully or partially given the pre-existent co-morbidities that the patient had.

Dosing

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 150mg/kg, typically about 5mg/kg to about 150mg/kg, more specifically about 25mg/kg to 125 mg/kg, 50mg/kg to 125mg/kg , even more specifically at about 50mg/kg to 100mg/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, 10, 25 or 50 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.

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

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 12, Figure 13A1 and A2 & Figure 13B1-B4, Figure 14A1-A2 & Figure 14B1-B3, and Figure 15A1-A3 and Figure 15B1-B6.

Combination therapy

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.

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) fluoroquinolines/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.

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, napthyridone, radezolid, doxorubicin, ampicillin, vancomycin, imipenem, doripenem, gemcitabine, dalbavancin, and azithromycin.

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, meclofenameate, mefenamic acid, meloxicam, nabumeone, naproxen sodium, oxaprozin, piroxicam, sulindac, tolmetin, celecoxib, rofecoxib, aspirin, choline salicylate, salsalte, 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, cepalothin, cephapirin, cephradine, cephaloridine, cefazolin, cefamandole, cefuroxime, cephalexin, cefprozil, cefaclor, loracarbef, cefoxitin, cefmatozole, cefotaxime, ceftizoxime, ceftriaxone, cefoperazone, ceftazidime, cefixime, cefpodoxime, ceftibuten, cefdinir, cefpirome, cefepime, BAL5788, BAL9141, imipenem, ertapenem, meropenem, astreonam, 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 dalfopristin, sulphanilamide, 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.

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

The present invention also provides pharmaceutical compositions containing the WTA-AACs, 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 conventions methods and/or agents for treating infectious diseases. In some embodiments, a pharmaceutical formulation comprises 1) a anti-WTAβ-AAC of the invention, and 2) a pharmaceutically acceptable carrier. In some embodiments, a pharmaceutical formulation comprises 1) an AAC of the invention and optionally, 2) at least one additional therapeutic agent.

Pharmaceutical formulations comprising an AAC of the invention are prepared for storage by mixing the 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 dextrins; 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.

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, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980).

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 γ 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.

An AAC may be formulated in any suitable form for delivery to a target cell/tissue. For example, AACs 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.

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.

MATERIALS AND METHODS

Bacterial strains:

All experiments were done with MRSA-USA300 NRS384 obtained from NARSA (http://www.narsa.net/control/member/repositories) unless noted otherwise.

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 $1x10^4$ CFU/mL. The 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%.

MIC determinations for intracellular bacteria

Intracellular MIC was determined on bacteria that were sequestered inside mouse peritoneal macrophages (see below for generation of murine peritoneal macrophages). Macrophages were plated in 24 well culture dishes at a density of $4x10^5$ cells/mL and

infected with MRSA at a ratio of 10-20 bacteria per macrophage. Macrophage cultures were maintained in growth media supplemented with 50 ug/mL of gentamycin (an antibiotic that is active only on extracellular bacteria) 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 .05% Tween-20. The number of surviving intracellular bacteria was determined by plating on Tryptic Soy Agar plates with 5% defibrinated sheep blood.

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 $4x10^5$ 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).

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 ($4x10^6$ bacteria in 250 µL 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_2 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 µg/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.

Generation of MRSA infected peritoneal cells.

6-8 week old female A/J mice (JAX™ Mice, Jackson Laboratories) were infected with $1x10^8$ 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 $1x10^6$ 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.

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 $1x10^6$ 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.

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.

In vivo transfer of infection model:

USA300 stocks were prepared for infection from actively growing cultures in tryptic soy broth. Bacteria were washed 3X in phosphate buffered saline (PBS) and aliquots were frozen at -80° C in PBS 25% glycerol. Intracellular Bacteria Infections: A/J mice were chosen for these experiments because they are easily infected with relatively low doses of MRSA ($2x10^6$ CFU/mouse). 7 week old female A/J mice were obtained from Jackson Lab and infected by peritoneal injection with $5x10^7$ CFU of USA300. Mice were sacrificed 1 day post infection and the peritoneum was flushed with 5 mL of cold PBS. Peritoneal washes were centrifuged for 5 minutes at 1,000 rpm at 4° C in a table top centrifuge. The cell pellet containing peritoneal cells was collected and cells were treated with 50 ug/mL of lysostaphin (Cell Sciences Inc. Canton MA, CRL 309C) for 20 minutes at 37° C to kill contaminating extracellular bacteria. Peritoneal cells were washed 3x in ice cold PBS to remove the lysostaphin. Peritoneal cells from donor mice were pooled, and recipient mice were injected

with cells derived from 5 donors per each recipient by intravenous injection into the tail vein. To determine the number of live intracellular CFUs, a sample of the peritoneal cells were lysed in HB (Hanks Balanced Salt Solution supplemented with 10 mM HEPES and .1% Bovine Serum Albumin) with .1% Triton-X, and serial dilutions of the lysate were made in PBS with .05% tween-20. Free Bacteria Infections: A/J mice were infected with various doses of free bacteria using a fresh aliquot of the glycerol stocks utilized for the peritoneal injections. Actual infection doses were confirmed by CFU plating. For the data shown in Figure 1A the actual infection dose for Intracellular Bacteria was $1.8x10^6$ CFU/mouse, and the actual infection dose for Free Bacteria was $2.9x10^6$ CFU/mouse. Selected mice were treated with a single dose of 100 mg/Kg of vancomycin by intravenous injection immediately after infection.

In vitro transfer of infection to non-phagocytic cells.

Generation of MRSA infected peritoneal cells: 6-8 week old female A/J mice (Jackson Lab) were infected with $1x10^8$ 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 ug/mL of lysostaphin diluted in Hepes Buffer supplemented with .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 $1x10^6$ cells/mL in RPMI 1640 tissue culture media supplemented with 10 mM Hepes and 10 % Fetal Calf Serum, and 5 ug/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.

Infection of osteoblasts or HBMEC. MG63 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). HBMEC cells (Catalog #1000) and ECM media (catalog# 1001) were obtained from SciencCell Research Labs (Carlsbad, CA). Cells were plated in 24 well tissue culture plates and cultured to obtain a confluent layer. On the day of the experiment, the cells were washed once in RPMI (without supplements). MRSA or infected peritoneal cells were diluted in complete RPMI-10 and vancomycin was added at 5 ug/mL immediately prior to infection. Peritoneal cells were added to the osteoblasts at $1x10^6$ peritoneal cells/mL. A sample of the cells was lysed with .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.

Generation of the anti-S. aureus antibodies.

The human IgG antibodies against anti-beta-GlcNAc WTA mAb were cloned from peripheral B cells from patients post *S. aureus* infection using a monoclonal antibody discovery technology which conserves the cognate pairing of antibody heavy and light chains [38]. Individual antibody clones were expressed by transfection of mammalian cells (Meijer, P.J., Nielsen, L.S., Lantto, J. & Jensen, A.(2009) Human antibody repertoires. *Methods Mol Biol* **525**, 261-277, xiv; Meijer, P.J., *et al.* (2006) "Isolation of human antibody repertoires with preservation of the natural heavy and light chain pairing." *Journal of molecular biology* **358**, 764-772). Supernatants containing full-length IgG1 antibodies were harvested after seven days and used to screen for antigen binding by ELISA. These antibodies were positive for binding to cell wall preparations from USA300. 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. Isolation and usage of these antibodies were approved by the regional ethical review board.

Conjugation of the linker drug to antibody.

Construction and production of the THIOMAB variant of Anti-WTA antibody (Ab) was done as follows. A cysteine residue was engineered at the Val 205 position of Anti-WTA Ab light chain to produce its THIOMAB™ cysteine-engineered antibody variant. This thio Anti-WTA was conjugated to PML Linker-antibiotic intermediates from Table 2. The antibody was reduced in the presence of fifty-fold molar excess DTT overnight. The reducing agent and the cysteine and glutathione blocks were purified away using HiTrap SP-HP column (GE Healthcare). The antibody was reoxidized in the presence of fifteen-fold molar excess dehydroascorbic acid (MP Biomedical) for 2.5 hours. The formation of interchain disulfide bonds was monitored by LC/MS. A three-fold molar excess of the PML linker antibiotic intermediate over protein was incubated with the THIOMAB for one hour. The antibody drug conjugate was purified by filtration through a 0.2 um SFCA filter (Millipore). Excess free linker drug was removed by filtration. The conjugate was buffer exchanged into 20 mM histidine acetate pH 5.5 / 240 mM sucrose by dialysis. The number of conjugated

rifamycin-type antibiotics per mAb was quantified by LC/MS analysis as the antibiotic/antibody ratio (AAR). Purity was also assessed by size exclusion chromatography.

Mass Spectrometric Analysis

LC/MS analysis was performed on a 6530 Accurate-Mass Quadrupole Time-of-Flight (Q-TOF) LC/MS (Agilent Technologies). Samples were chromatographed on a PRLP-S column, 1000 Å, 8 μm (50 mm ×2.1 mm, Agilent Technologies) heated to 80 °C. A linear gradient from 30–60% B in 4.3 minutes (solvent A, 0.05% TFA in water; solvent B, 0.04% TFA in acetonitrile) was used and the eluent was directly ionized using the electrospray source. Data was collected and deconvoluted using the Agilent Mass Hunter qualitative analysis software. Before LC/MS analysis, antibody drug conjugate was treated with lysyl endopeptidase (Wako) for 30 minutes at 1:100 w/w enzyme to antibody ratio, pH 8.0, and 37 °C to produce the Fab and the Fc portion for ease of analysis. The antibiotic to antibody ration (AAR) (used interchangeably herein with drug to antibody ratio (DAR)) was calculated using the abundance of Fab and Fab+1 calculated by the MassHunter software.

Analysis of bacteria isolated from infected mice: Balb/c mice were infected with $1x10^7$ CFU of MRSA (USA300) by intravenous injection and kidneys were harvested on day 3 post infection. Kidneys were homogenized using a GentleMACS dissociator in 5 mL volume per 2 kidneys using M-Tubes and the program RNA01.01 (Miltenyi Biotec, Auburn, CA). Homogenization buffer was: PBS + 0.1% Triton-X100, 10 ug/mL DNAase (Bovine pancreas grade II, Roche) and protease inhibitors (Complete protease inhibitor cocktail, Roche 11-836-153001). After homogenization, the samples were incubated at room temperature for 10 minutes and then diluted with ice cold PBS and filtered through a 40 uM cell strainer. Tissue homogenates were washed 2x in ice cold PBS and then suspended in a volume of 0.5 mL per 2 kidneys in HB buffer (Hanks Balanced Salt Solution supplemented with 10 mM HEPES and .1% Bovine Serum Albumin). The cell suspension was filtered again and 25 uL of the bacterial suspension was taken for each staining reaction.

Flow Cytometry to compare expression of anti-MRSA antibodies: Antibody staining for flow cytometry Bacteria ($1x10^7$ of in vitro grown bacteria, or 25 uL of tissue homogenate described above) were suspended in HB (above) and blocked by incubation with 400 μg/mL (microgram per milliliter) of mouse IgG (SIGMA, I5381) for 1 hour. Fluorescently labeled antibodies were added directly to the blocking reaction and incubated at room temperature for an additional 10-20 minutes. Bacteria were washed 3X in HB buffer and then fixed in PBS

2% paraformaldehyde prior to FACS analysis. Test antibodies (anti-βWTA:4497, anti-αWTA:7578 or isotype control:gD) were conjugated with Alexa-488 using amine reactive reagents (Invitrogen, succinimidyl-ester of Alexa Fluor 488, NHS-A488). Antibodies in 50 mM sodium phosphate were reacted with a 5-10 fold molar excess of NHS-A488 in the dark for 2-3 hrs at room temperature. The labeling mixture was applied to a GE Sepharose S200 column equilibrated in PBS to remove excess reactants from the conjugated antibody. The number of A488 molecules/antibody was determined using the UV method as described by the manufacturer.

For analysis of bacteria in tissue homogenates a non-competing anti-*S. aureus* antibody (rF1- Hazenbos, W.L., *et al.* (2013) Novel staphylococcal glycosyltransferases SdgA and SdgB mediate immunogenicity and protection of virulence-associated cell wall proteins. *PLoS Pathog* 9, e1003653was conjugated to Alexa-647 to distinguish *S. aureus* from similar sized particles. Test antibodies were examined at a range of doses from 80 ng/mL to 50 ug/mL. Flow cytometry was performed using a Beckton Dickson FACS ARIA (BD Biosciences, San Jose CA) and analysis was performed using FlowJo analysis software (Flow Jo LLC, Ashland OR).

Time of kill for free antibiotics on non-replicating bacteria: *S. aureus* (USA300) was taken from an overnight stationary phase culture, washed once in Phosphate Buffered Saline (PBS) and suspended at $1x10^7$ CFU/mL in PBS with no antibiotic or with $1x10^{-6}$M antibiotic in a 10 mL volume in 50 mL polypropylene centrifuge tubes. The bacteria were incubated at 37°C overnight with shaking. At each time point, three 1 mL samples were removed from each culture and centrifuged to collect the bacteria. Bacteria were washed once with PBS to remove the antibiotic and the total number of surviving bacteria was determined by plating serial dilutions of the bacteria on agar plates.

Killing of persisters cells by free antibiotics: *S. aureus* (USA300) was taken from an overnight stationary phase culture, washed once in Tryptic Soy Broth (TSB) and then adjusted to a final concentration of $1x10^7$ CFU/ml in a total volume of 10 mL of either TSB or TSB with ciprofloxicin (0.05 mM). Cultures were incubated with shaking at 37°C for 6 hours and then the second antibiotic, either rifampicin (1 ug/ml) or another rifamycin-type antibiotic (1 ug/ml) was added. At the indicated times, samples were removed from each culture, washed once with PBS to remove the antibiotic and re-suspended in PBS. The total

number of surviving bacteria was determined by plating serial dilutions of the bacteria on agar plates. At the final time point the remainder of each culture was collected and plated.

Cathepsin release assay for AAC: To quantify the amount of active antibiotic released from AACs following treatment with cathepsin B, AACs were diluted to 200 ug/mL in cathepsin buffer (20 mM Sodium Acetate, 1 mM EDTA, 5 mM L-Cysteine pH 5). Cathepsin B (from bovine spleen, SIGMA C7800) was added at 10 ug/mL and the samples were incubated for 1 hour at 37° C. As a control, AACs were incubated in buffer alone. The reaction was stopped by addition of 9 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 MRSA (USA300) was added to each well at a final density of $2x10^3$ CFU/mL. The cultures were incubated over night at 37° C with shaking and bacterial growth was measured by reading absorbance at 630 nM using a plate reader.

Synthesis of S4497 antibody FRET conjugate for the phagolysosomal processing.

A maleimide FRET peptide was synthesized and conjugated to the S4497 cysteine-engineered, THIOMAB™ antibody. The FRET pair employed tetramethylrhodamine (TAMRA) and fluorescein (Fischer, R., et al (2010) *Bioconjug Chem* **21**, 64-73). The maleimide FRET peptide was synthesized by standard Fmoc solid-phase chemistry using a PS3 peptide synthesizer (Protein Technologies, Inc). Briefly, 0.1 mmol of Rink amide resin was used to generate C-terminal carboxamide. Fmoc-Lys(Mtt)-OH at the N- and C-terminal residues was utilized in order to remove the Mtt (monomethoxytrityl) group on the resin and carry out additional side-chain chemistry to attach TAMRA and fluorescein. The CBDK-cit peptidomimetic unit was added between the FRET pair as a cathepsin-cleavable spacer. The crude maleimide FRET peptide or maleimidocaproyl-K(TAMRA)-G- CBDK-cit - K(Fluorescein) cleaved off from the resin was subjected to further purification by reverse-phase HPLC with a Jupiter 5μm C4 column (5 μm, 10 mm x 250 mm, Phenomenex). Our FRET probe allows monitoring not only the intracelluar trafficking of the antibody conjugate, but also the processing of the linker in the phagolysosome. The intact antibody conjugate fluoresces only in red due to the fluorescence resonance energy transfer from the donor. However, upon the substrate cleavage of the FRET peptide in the phagolysosome, the green fluorescence from the donor is expected to appear.

Video Microscopy to detect cleavage of the linker inside macrophages

Murine peritoneal macrophages were plated on chamber slides (Ibidi, Verona, WI. catalog 80826) in complete media as described for the macrphage intracellular killing assay. USA300 was labeled with Cell Tracker Violet (Invitrogen C10094) at 100ug/mL in PBS .1% BSA by incubation for 30 minutes at 37 C. The labeled bacteria were opsonized with the 4497-FRET probe by incubation for 1 hour in HB buffer. Macrophages were washed once immediately prior to addition of the opsonized bacteria, and bacteria were added to cells at $1x10^7$ Bacteria/mL. For no-phagocytosis controls, the macrophages were pre-treated with 60 nM Latrunculin A (Calbiochem) for 30 minutes prior to and during phagocytosis. The slides were placed on the micrscope immediately after addition of bacteria to the cells and movies were acquired with a Leica SP5 confocal microscope equipped with an environmental chamber with CO_2 and Temperature controllers from Ludin. The images were captured every minute for a total time of 30 minutes using a Plan APO CS 40X, N..A: 1.25, oil immersion lens, and the 488nm and 543nm laser lines to excite respectively alexa 488 and TAMRA. Phase images were also recorded using the 543 nm laser line.

Quantification of released antibiotic inside macrophages by mass spectrometry.

Murine peritoneal macrophages were infected in 24 well tissue culture dishes as described below for the intracellular killing assay with MRSA opsonized with AAC at 100 ug/mL in HB. After phagocytosis was complete, the cells were washed and 250uL of complete media + gentamycin was added to wells and the cells were incubated for 1 hour or 3 hours. At each time point the supernatant and cellular fractions were collected and acetonitrile (ACN) was added to 75% final concentration and incubated for 30 minutes. Cell and supernatant extracts were lyophilized by evaporation under N2 (TurboVap) and reconstituted in 100 uL of 50% ACN, filtered and analyzed on Ab Sciex QTRAP 6500 LC/MS/MS system.

In vitro Intracellular Killing Assay.

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). HBMEC cells (Catalog #1000) and ECM media (catalog# 1001) were obtained from SciencCell Research Labs (Carlsbad, CA).

Murine 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 $4x10^5$ cells/well in 24 well culture dishes. Macrophages were cultured over night to permit adherence to the plate.

Human M2 Macrophages: $CD14^+$ Monocytes were purified from normal human blood using a Monocyte Isolation Kit II (Miltenyi, Cat 130-091-153) and plated at $1.5x10^5$ cells/cm^2 on tissue culture dishes pre-coated with Fetal Calf Serum (FCS) and cultured in RPMI 1640 media with 20% FCS + 100 ng/mL rhM-CSF. Media was refreshed on day 1 and on day 7, the media was changed to 5% serum + 20 ng/mL IL-4. Macrophages were used 18 hours later.

Assay protocol: In all experiments bacteria were cultured in Tryptic Soy Broth. To assess intracellular killing with Antibody Antibiotic Conjugates (AACs), USA300 was taken from an exponentially growing culture and washed in HB (Hanks Balanced Salt Solution supplemented with 10 mM HEPES and .1% Bovine Serum Albumin). AACs 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 ($4x10^6$ bacteria in 250 uL 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_2 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 50ug/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 .1% triton-X (lyses the macrophages without damaging the intracellular bacteria). Serial dilutions of the lysate were made in phosphate buffered saline solution supplemented with .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.

EXAMPLES

Example 1 Intracellular MRSA are protected from conventional antibiotics

To confirm the hypothesis that mammalian cells provide a protective niche for *S. aureus* in the presence of antibiotic therapy, the efficacy was compared of three major antibiotics that are currently used as standard of care (SOC) for invasive MRSA infections (vancomycin, daptomycin and linezolid) against extracellular planktonic bacteria versus bacteria sequestered inside murine macrophages (Table 4).

For extracellular bacteria, MRSA was cultured overnight in Tryptic Soy Broth, and the MIC was determined to be the minimum antibiotic dose that prevented growth. For intracellular bacteria, murine peritoneal macrophages were infected with MRSA and cultured in the presence of gentamycin to kill extracellular bacteria. Test antibiotics were added to the culture medium one day post infection, and the total number of surviving intracellular bacteria was determined 24 hours later. The expected serum concentrations for clinically relevant antibiotics was reported in Antimicrobial Agents, Andre Bryskier. ASM Press, Washington DC (2005).

Table 4: Minimum inhibitory concentrations (MIC) for several antibiotics on extracellular bacteria grown in liquid culture *vs.* intracellular bacteria sequestered inside murine macrophages.

Antibiotics (Abx)	Extracellular MRSA MIC (μg/mL)	Intracellular MRSA MIC (μg/mL)	Serum Cmax (μg/mL)
Vancomycin	1	>100	50
Daptomycin	4	>100	60
Linezolid	0.3	>20	20
Rifampicin	0.004	50	20

This analysis with a highly virulent community-acquired MRSA strain USA300 revealed that although extracellular MRSA is highly susceptible to growth inhibition by low concentrations of vancomycin, daptomycin, and linezolid in liquid culture, all three antibiotics failed to kill the same strain of MRSA sequestered inside macrophages exposed to clinically achievable concentrations of the antibiotics. Even rifampicin, thought to be relatively effective at eliminating intracellular pathogens(Vandenbroek, P.V. (1989) Antimicrobial Drugs, Microorganisms, and Phagocytes. *Reviews of Infectious Diseases* **11**, 213-245), required a 6,000-fold higher dose to eliminate intracellular MRSA compared to the dose required to inhibit growth (MIC) of planktonic bacteria (Table 1), consistent with other studies showing that the majority of existing antibiotics are inefficient at killing intracellular *S. aureus* both in vitro and in vivo(Sandberg, A., Hessler, J.H., Skov, R.L., Blom, J. & Frimodt-Moller, N. (2009) "Intracellular activity of antibiotics against Staphylococcus aureus in a mouse peritonitis model" *Antimicrob Agents Chemother* **53**, 1874-1883).

Example 2 Dissemination of infection with intracellular MRSA

These experiments compared the virulence of intracellular bacteria *versus* an equivalent dose of free-living planktonic bacteria, and determined whether the intracellular bacteria are able to establish infection in the presence of vancomycin in vivo. Four cohorts of mice were infected by intravenous injection with roughly equivalent doses of *S. aureus* viable free bacteria (2.9×10^6) taken directly from broth culture or intracellular bacteria (1.8×10^6) sequestered inside host macrophages and neutrophils that were generated by peritoneal infection of donor mice (Fig. 1A) and selected groups were treated with vancomycin immediately after infection and then once per day. Mice were examined 4 days after infection for bacterial colonization in the kidney, an organ that is consistently colonized by *S. aureus* in mice. In three independent experiments, equivalent or higher bacterial burdens in the kidneys of mice infected with intracellular bacteria compared to those infected with an equivalent dose of planktonic bacteria was observed (Fig. 1B). Surprisingly, it was found that infection with intracellular bacteria resulted in more consistent colonization of the brain, an organ that is not efficiently colonized following infection with planktonic bacteria in this model (Fig. 1C). Furthermore, intracellular bacteria, but not planktonic bacteria, were able to establish infection in the face of vancomycin therapy in this model (Fig. 1B, Fig.1C)

Further analyses in vitro addressed more quantitatively the extent to which intracellular survival facilitates antibiotic evasion. To this end, MG63 osteoblasts were infected with either planktonic MRSA or intracellular MRSA, in the presence of vancomycin.

Infection of osteoblasts or HBMEC. MG63 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). HBMEC cells (Catalog #1000) and ECM media (catalog# 1001) were obtained from SciencCell Research Labs (Carlsbad, CA). Cells were plated in 24 well tissue culture plates and cultured to obtain a confluent layer. On the day of the experiment, the cells were washed once in RPMI (without supplements). MRSA or infected peritoneal cells were diluted in complete RPMI-10 and vancomycin was added at 5 ug/mL immediately prior to infection. Peritoneal cells were added to the osteoblasts at $1x10^6$ peritoneal cells/mL. A sample of the cells was lysed with .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.

MRSA (free bacteria) was seeded in media, media + vancomycin, or media + vancomycin and plated on a monolayer of MG63 osteoblasts (Fig.1E) or Human Brain Microvascular Endothelial Cells (HBMEC, Fig.1F). Plates were centrifuged to promote contact of the bacteria with the monolayer. At each time point, the culture supernatant was collected to recover extracellular bacteria or adherent cells were lysed to release intracellular bacteria.

Planktonic bacteria exposed to vancomycin alone were efficiently killed. Surviving bacteria were not recoverd after one day in culture (Fig. 1D). When a similar number of planktonic bacteria were plated on MG63 osteoblasts, a small number of surviving bacteria (approximately 0.06% of input) associated with the MG63 cells one day after infection, which had been protected from vancomycin by invasion of the osteoblasts, was recovered.

MRSA that were sequestered inside peritoneal cells showed a dramatic increase in both survival and efficiency of infection in the presence of vancomycin. About 15% of intracellular MRSA in the leukocytes survived under identical conditions where vancomycin had sterilized the cultures of planktonic bacteria. Intracellular bacteria also were better able to infect the monolayer of MG63 osteoblasts in the presence of vancomycin, resulting in a doubling of the bacteria recovered one day after exposure to vancomycin (Fig. 1D). Moreover, intracellular *S. aureus* were able to increase by almost 10-fold over a 24 hour period in MG63 cells (Fig. 1E), primary human brain endothelial cells (Fig. 1F), and A549 bronchial epithelial cells (not shown) under constant exposure to a concentration of vancomycin that killed free living bacteria. Although protected from antibiotic killing,

bacterial growth did not occur in cultures of infected peritoneal macrophages and neutrophils (not shown). Together these data support that intracellular reservoirs of MRSA in myeloid cells can promote dissemination of infection to new sites, even in the presence of active antibiotic treatment, and intracellular growth can occur in endothelial and epithelial cells, even under conditions of constant antibiotic therapy.

To develop a reagent that specifically kills intracellular *S. aureus*, the antibody and antibiotic components were carefully chosen and optimized for maximal efficacy. The examples below show the experiments and results leading to the choice of anti-wall-teichoic acid beta (anti-WTAβ) antibodies and to certain rifamycin -type antibiotics to be conjugated to form an AAC.

Example 3 Selection of anti-S.aureus monoclonal antibody

The amount of antibiotic delivered by an AAC, and therefore its ultimate efficacy, is limited by the number of antibody binding sites on the surface of the bacterium. Thus, it was essential to select an antibody that binds to a highly abundant antigen that is stably expressed on MRSA during all phases of an in vivo infection. As an initial step, a panel of greater than 40 *anti-S. aureus* antibodies were cloned and purified from B cells derived from peripheral blood of patients recovering from various *S. aureus* infections and screened for binding to MRSA isolated directly from the kidneys of infected mice.

Antibody generation, screening and selection

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).

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 5: 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:

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.

The highest level of antibody binding was found with a human IgG_1 that recognizes β-O-linked GlcNAc sugar modifications on WTA (Table 6). Less binding was achieved with monoclonal antibodies recognizing the α-O-linked GlcNAc; an isotype control antibody

against cytomegalovirus (CMV) gD protein showed some minimum reactivity due to protein A expressed on in-vivo-derived *S. aureus* (Figure 7A). The antigen specificity of the antibodies was determined by genetic means, so that antibodies against α-or β- GlcNAcs sugar modifications on WTA failed to bind to S. aureus strains lacking their respective glycosyltransferases (as exemplified in Figure 7B). Consistent with the extent of antibody binding to in vivo-derived MRSA, AACs made with anti-β-GlcNAc WTA antibodies showed superior efficacy to those made with anti-α-GlcNAc WTA antibodies.

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

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.

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. 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, antibodies were selected 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.

Additionally, the capacity of these mAbs to bind to the following *S. aureus* strains, was assessed and 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.

Example 4 Characterization of antibodies with specificity against wall teichoic acids

Confirming WTA specificity of Abs

Cell wall preparations (CWP) from a *S. aureus* wild-type (WT) strain and a *S. aureus* mutant strain lacking WTA (ΔTagO; 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 μg/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.

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 ΔTagO 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.

i) Flow cytometry to determine extent of mAb binding to MRSA surface

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 C57Bl/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 Tattevin P. et al. Antimicrobial agents and chemotherapy 54: 610-613 (2010). Rabbits were injected intravenously with $5x10^7$ 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 $7x10^7$ CFU stationary-phase

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 μg/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 μg/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 μg/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 μg/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.

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

Table 6 shows equilibrium binding analysis of MRSA antibodies binding to Newman-ΔSPA strain, and the antigen density on the bacterium.

Table 6

MRSA Antibody	Specificity	aveK$_D$,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

The K_D 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.

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 5 Amino acid modifications of anti-WTA antibodies

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. Wild-type sequences were altered at certain positions to improve the antibody stability while maintaining antigen binding as described below. Cysteine engineered Abs (ThioMabs, also referred to as THIOMAB™) were then generated.

i. Linking Variable regions to Constant regions

The VH regions of the WTA beta Abs identified from the human antibody library above were linked to human γ1 constant regions to make full length IgG1 Abs. The L chains were kappa L chains.

ii. Generating stability variants

The WTA Abs in Figure 12, (see in particular, Figures 13A, 13B, 14A, 14B) were engineered to improve certain properties (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'CCAGGGTTCCCTGGCCCCAWTMGTCAAGTCCASCWKCACCTCTTGCACAGTAATAGACAGC- 3' (SEQ ID NO. 154); and

5'- CCTGGCCCCAGTCGTCAAGTCCTCCTTCACCTCTTGCACAGTAATAGACAGC- 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.

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.

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 16) and was altered for stability. CDR H3 is generally critical for antigen binding so several mutants were tested for antigen binding and chemical stability. Mutant D96E (v8) retains binding to antigen, similar to wild-type Ab 4497 (Figure 16), and is stable and does not form iso-aspartic acid.

Staph CWP ELISA

For analysis of 6078 antibody mutants, a lysostaphin-treated USA300 ΔSPA S. aureus cell well preparation (WT) consisting of $1X10^9$ 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')2 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)

Full length ThioMabs were produced by introducing a Cysteine into the H chain (in CH1) or the L chain (Cκ) at a predetermined position as previously taught and described below to allow conjugation of the antibody to a linker-antibiotic intermediate (Cysteine amino acids may be engineered at reactive sites in the heavy chain (HC) or light chain (LC) of 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; WO 2011/156328; WO2009/052249, Shen et al (2012) Nature Biotech., 30(2):184-191; Junutula et al (2008) Jour of Immun. Methods 332:41-52). 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 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 an engineered Cys in each of the H and L chains (HCLCCys) leading to 4 engineered Cys per antibody tetramer, were generated by expressing the desired combination of cys mutant chains and wild type chains.

Figures 13A and 13B 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 of FACS analysis (data not shown), 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 was used as a non-specific negative control antibody.

Example 6: Selection of Antibiotic

Rifamycin-type antibiotics were selected for high potency, unaltered bactericidal activity in low phagolysosomal pH, ability to withstand intracellular insults and the ease with which they can be coupled to a protease-cleavable, non-peptide (PML) linker reagent suitable for conjugation to an anti-WTA antibody. Since the intra-phagocytic bacteria were at most slowly replicating, optimization of the antibiotic also required that it be able to kill non-replicating MRSA when released from the AAC.

MRSA was collected from a stationary phase culture and suspended in phosphate buffered saline containing no antibiotic or $1x10^{-6}$M of rifampin or the rifamycin-derivative (Rifalog) and incubated at 37°C. At the indicated times, a sample of the culture was collected and centrifuged to remove the antibiotic and the total number of surviving bacteria was determined by plating.

Intriguingly, the addition of the rifamycin-type (rifalog) antibiotic but not rifampicin resulted in a more than 1,000-fold decrease in the number of viable, but non-replicating, bacteria after overnight incubation in minimal phosphate-saline buffer (PBS) (see FIG. 8). Similarly, the rifamycin-type antibiotic was for the ability to kill classically defined persister cells, bacteria that presumably enter a dormant state to survive antibiotic treatment (e.g., ciprofloxacin) of growing cultures (data not shown). The addition of rifampicin had no effect on their viability, in agreement with previous observations (Conlon, B.P., *et al.* (2013) *Nature* **503**, 365-370). By contrast, the addition of rifamycin-type antibiotic (rifalog) led to the eradication of persister cells below the limit of detection. These results suggest that the rifamycin-type antibiotics have a remarkable ability to kill dormant, non-dividing cells.

Example 7: Preparation of Anti-WTA Antibody-Antibiotic Conjugates

Anti-wall teichoic acid antibody-antibiotic conjugates (AAC) Table 3 were prepared by conjugating an anti-WTA antibody to a PML 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 teachings of which are incorporated by reference for this purpose. 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.

Reduction/Oxidation of ThioMabs for Conjugation: Full length, cysteine engineered monoclonal antibodies (ThioMabs - Junutula, et al., 2008b Nature Biotech., 26(8):925-932; Doman 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 1/20th 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.

The eluted reduced ThioMab was treated with 15 fold molar excess of DHAA (dehydroascorbic acid) or 200 nM aqueous copper sulfate ($CuSO_4$). 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.

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.

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.

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.

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_2O 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 8: Cleavage and release of the antibiotic

The rifamycin-type antibiotics were tested for the ability to directly kill extracellular bacteria when linked to the anti-β WTA mAb in the AAC format. AAC was incubated in buffer alone or treated with cathepsin B. Serial dilutions of the resulting reaction were added to wells containing MRSA in Tryptic Soy Broth and cultured over night to identify wells containing sufficient active antibiotic to prevent growth.

As predicted, growing, planktonic bacteria were not harmed by overnight incubation with intact anti-MRSA AAC unless the AAC was pre-treated with cathepsin B to release the active antibiotic (Figure 9). Pretreatment of the AAC with cathepsin B released sufficient antibiotic activity to prevent bacterial growth at .6 ug/mL of AAC, which is predicted to contain .006 ug/mL of antibiotic.

To test if antibiotic is released from the AAC only after internalization of AAC-opsonized bacteria into cells, cleavage of the linker can be examined with a Fluorescence Resonance Energy Transfer (FRET)-based probe consisting of the same anti-MRSA antibody conjugated to two dye molecules, using the same linker as in the AAC. MRSA is opsonized with the FRET conjugate and added to macrophage cultures. Uptake of the bacteria and cleavage of the probe is monitored by video microscopy. The linker is cleaved within minutes after uptake of the bacteria by macrophages as visualized by the release of the A488 probe, analogous to the release of rifamycin-type antibiotic on the AACs. On the other hand, the linker remained intact when the bacteria are not internalized due to treatment of the macrophages with latrunculin A, an inhibitor of phagocytosis. Mass spectrometry analysis can also be used to confirm that the free antibiotic is indeed released inside macrophages after uptake of MRSA coated with the actual AACs.

Example 9 Anti-WTAβ PML AAC *in vitro* potency

Anti-WTAβ-CBDK AAC effectively kills *S. aureus* when internalized by primary human and mouse macrophages and several human cell lines *in vitro*.

In vitro Macrophage Assay.

S. aureus (USA300 NRS384 strain) was incubated with various doses (100 u/mL, 10 ug/mL, 1 ug/mL or 0.1 ug/mL) of an anti-WTAβ antibody 4497, Ab4497-CBDK-dimethylpipBOR AAC loaded with 2 antibiotic molecules per antibody (DAR2) or with anti-WTA-CBDK-dimethylpipBOR AAC loaded with 4 antibiotic molecules per antibody (DAR4) 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 ug/mL of gentamycin to kill any remaining extracellular bacteria. The total number of surviving intracellular bacteria were determined 2 days later by plating serial dilutions of the macrophage lysates on Tryptic Soy Agar plates.

Example 10 *In vivo* efficacy of anti-WTAβ-PML AACs

Treatment of *S. aureus* infection in mice reduces the bacterial load in organs by several orders of magnitude.

To determine whether a therapeutic directed specifically to intracellular *S. aureus* would have efficacy during an infection, the WTA-PML AACs were tested in a mouse intravenous infection model. This example demonstrates that the WTA-PML AACs were

effective in greatly reducing or eradicating intracellular S. aureus infections, in the murine intravenous infection model.

Peritonitis Model. 7 week old female A/J mice (Jackson Laboratories) are infected by peritoneal injection with $5x10^7$ CFU of USA300. Mice are sacrificed 2 days post infection and the peritoneum is flushed with 5 mL of cold phosphate buffered saline solution (PBS). Kidneys are homogenized in 5 mL of PBS as described below for the intravenous infection model. Peritoneal washes are centrifuged for 5 minutes at 1,000 rpm at 4°C in a table top centrifuge. The supernatant is collected as the extracellular bacteria and the cell pellet containing peritoneal cells is collected as the intracellular fraction. The cells are treated with 50 μg/mL of lysostaphin for 20 minutes at 37°C to kill contaminating extracellular bacteria. Peritoneal cells are washed 3x in ice cold PBS to remove the lysostaphin prior to analysis. To count the number of intracellular CFUs, peritoneal cells are 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 are made in PBS with 0.05% tween-20.

Murine intravenous infection model

To be clinically relevant, an AAC would need to be able to eliminate already established intracellular infection. To assess this, treatment was delayed until 24h after initiation of bacteremia, a time at which vancomycin treatment is minimally effective. Intracellular infection in neutrophils is established rapidly; in at least one model of bacteremia, 95% of the bacteria in the blood are inside neutrophils within 15 minutes[7], presumably accounting for the decreased efficacy of vancomycin. Under these conditions, treatment with a single dose of AAC was efficacious and proved superior to treatment with an equivalent dose of the free rifamycin-type antibiotic.

S. aureus is a common colonizer of human skin and mucosal surfaces. Preliminary analysis of multiple sources of human serum, including IGIV-GammaGard, a pooled immunoglobulin preparation from ~10,000 humans, demonstrated that human serum contains approximately 300 ug/mL of anti-*S. aureus* antibodies, of which ~70% are directed towards the GlcNAc modifications of WTA. Mouse serum has no appreciable levels of anti-*S aureus* antibody. To determine whether endogenous anti-WTA antibodies found in normal human serum might compete for binding with the AAC, 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 at least 10 mg/mL of human IgG in serum. IGIV was administered with an initial intravenous dose of 30 mg per mouse followed by a second dose of 15 mg/mouse by intraperitoneal (i.p.) injection after 6 hours, and subsequent daily dosings of 15 mg per mouse by intraperitoneal injection for 3 consecutive days. These mice were equally susceptible to infection with MRSA compared to untreated controls.

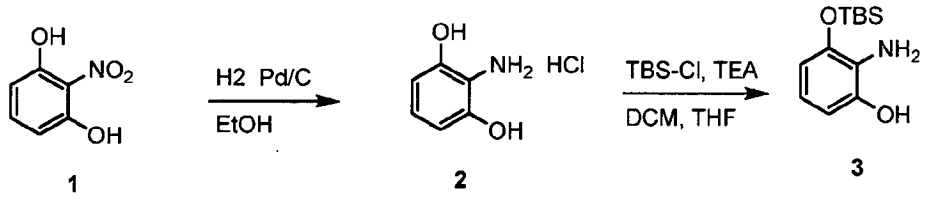
Mice (n=8 for each of antibody or AAC) were infected 4 hours after the first dose of IGIV with $1x10^7$ CFU of MRSA (USA300 NRS384 strain) diluted in phosphate buffered saline by intravenous injection. Infected mice were treated with 50 mg/kg of S4497 naked antibody or S4497 AAC from Table 3. Mice were given a single dose of AAC 24h post infection by intravenous injection, sacrificed on day 4 post infection, and kidneys and hearts 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 organ was determined by plating serial dilutions of the tissue homogenate in PBS .05% Tween on Tryptic Soy Agar with 5% defibrinated sheep blood.

As the results in Figure 10 show, despite the presence of potentially competing antibodies, a single dose of anti-β-GlcNAc WTA AAC (S4497-AAC) greatly reduced or eradicated bacterial counts in infected organs as compared to naked antibody. Figure 10B shows treatment with AAC (DAR2) reduced bacterial load in the kidneys by approximately 7,000-fold. Figure 10C shows that treatment with AAC (DAR2) from Table 3 reduced bacterial burdens in the heart by approximately 500-fold. Treatment with naked antibiotic, dimethylpipBOR alone (at the equal molar concentration to dimethylpipBOR in AAC) in the in vivo infection model was not efficacicou compared to the dimethylpipBOR conjugated to anti-WTA antibody as the AAC.

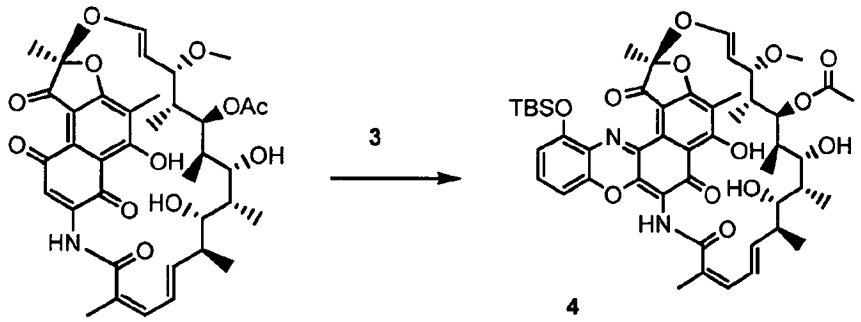
Unconjugated (free) anti-WTA antibodies are not efficacious in vivo

Figure 17 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 $2x10^7$ 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).

Example 11 Piperidyl benzoxazino rifamycin (pipBOR) **5**

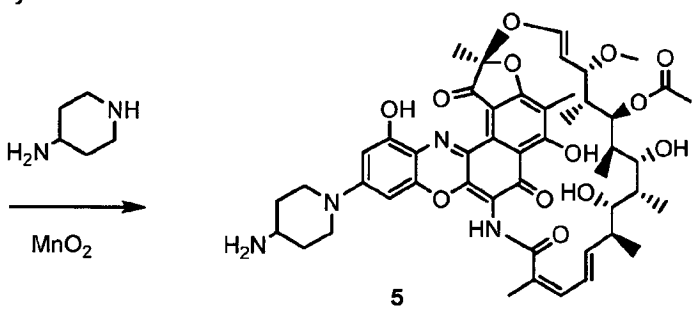


1 2 3



4

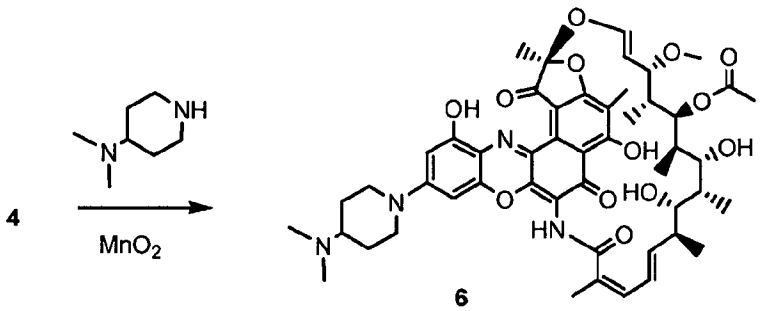
rifamycin S



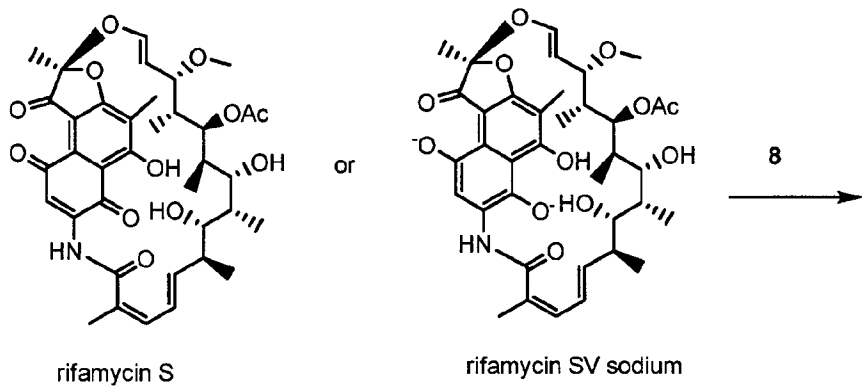
5

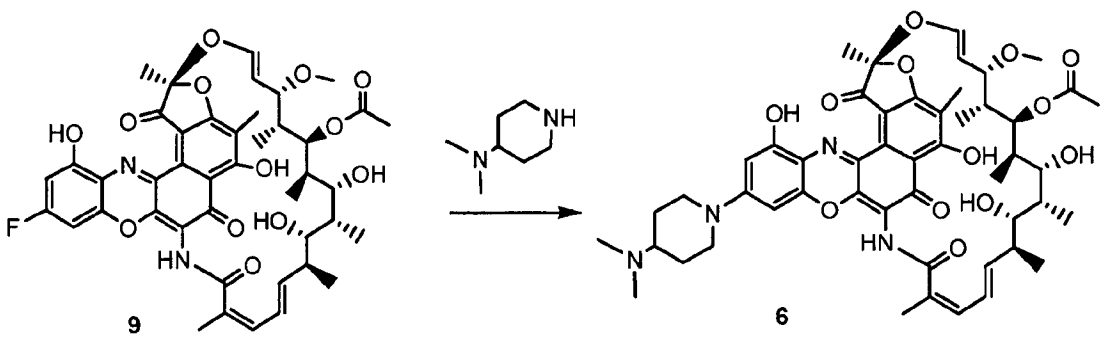
2-Nitrobenzene-1,3-diol **1** was hydrogenated under hydrogen gas with palladium/carbon catalyst in ethanol solvent to give 2-aminobenzene-1,3-diol **2**, isolated as the hydrochloride salt. Mono-protection of **2** with tert-butyldimethylsilyl chloride and triethylamine in dichloromethane/tetrahydrofuran 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**. LCMS (ESI): $M+H^+ = 915.41$. Reaction of **4** with piperidin-4-amine and manganese oxide gave piperidyl benzoxazino rifamycin (pipBOR) **5**. LCMS (ESI): $M+H^+ = 899.40$

Example 12 Dimethyl pipBOR **6**



Reaction of N,N-dimethylpiperidin-4-amine with TBS-protected benzoxazino rifamycin **4** gave dimethylpiperidyl benzoxazino rifamycin (dimethyl pipBOR) **6**



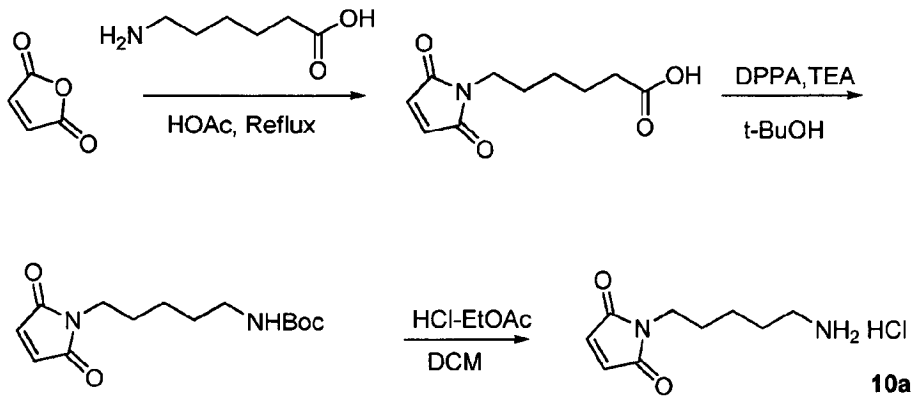


Alternatively, (5-fluoro-2-nitro-1,3-phenylene)bis(oxy)bis(methylene)dibenzene **7** was hydrogenated under hydrogen gas with palladium/carbon catalyst in tetrahydrofuran/methanol solvent to remove the benzyl groups to give 2-amino-5-fluorobenzene-1,3-diol **8**. LCMS (ESI): $M+H^+ = 144.04$. Commercially available Rifamycin

S or Rifamycin SV sodium salt (ChemShuttle Inc., Fremont, CA) was reacted with 2-amino-5-fluorobenzene-1,3-diol **8** by oxidative condensation in air or potassium ferric cyanide in ethylacetate at 60 °C to give fluoro benzoxazino rifamycin **9**. Displacement of fluoro with N,N-dimethylpiperidin-4-amine gave dimethylpipBOR **6**. LCMS (ESI): $M+H^+ = 927.43$

Example 13 (S)-N-(5-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)pentyl)-N-(1-(4-(hydroxymethyl)phenylamino)-1-oxo-5-ureidopentan-2-yl)cyclobutane-1,1-dicarboxamide **10**

Step 1: Preparation of 1-(5-aminopentyl)-1H-pyrrole-2,5-dione hydrochloride **10a**

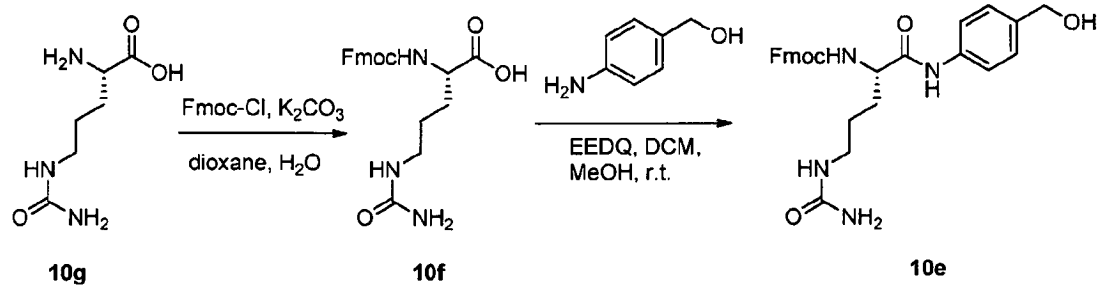


10a

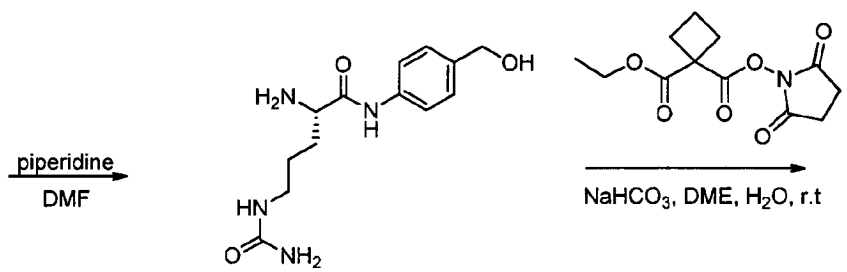
Maleic anhydride, furan-2,5-dione (150 g, 1.53 mol) was added to a stirred solution of 6-aminohexanoic acid (201 g, 1.53 mol) in HOAc (1000 mL). After the mixture was stirred at r.t. for 2 h, it was heated at reflux for 8 h. The organic solvents were removed under reduced pressure and the residue was extracted with EtOAc (500 mL × 3), washed with H_2O. The combined organic layers was dried over Na_2SO_4 and concentrated to give the crude product. It was washed with petroleum ether to give 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoic acid as white solid (250 g, 77.4 %). DPPA (130 g, 473 mmol) and TEA (47.9 g, 473 mmol) was added to a solution of 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoic acid (100 g, 473 mmol) in t-BuOH (200 mL). The mixture was heated at reflux for 8 h under N_2. The mixture was concentrated, and the residue was purified by column chromatography on silica gel (PE:EtOAc= 3:1) to give tert-butyl 5-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)pentylcarbamate (13 g, 10 %). To a solution of tert-butyl 5-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)pentylcarbamate (28 g, 992 mmol) in anhydrous EtOAc (30 mL) was added HCl/EtOAc (50 mL) dropwise. After the mixture was stirred at r.t. for 5 h, it was filtered and the solid was dried to give 1-(5-aminopentyl)-1H-pyrrole-2,5-dione hydrochloride **10a** (16 g,

73.7 %). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.02 (s, 2H), 6.99 (s, 2H), 3.37-3.34 (m, 2H), 2.71-2.64 (m, 2H), 1.56-1.43 (m, 4H), 1.23-1.20 (m, 2H).

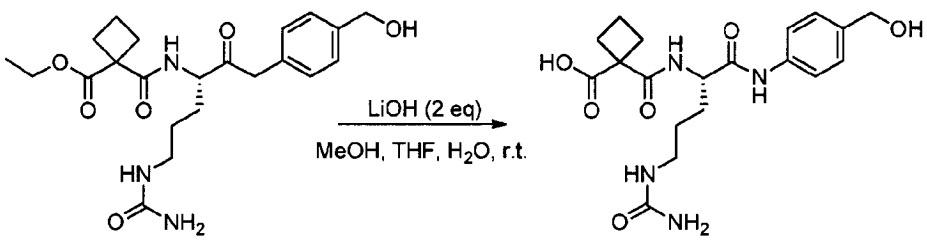
Step 2: Preparation of (S)-1-(1-(4-(hydroxymethyl)phenylamino)-1-oxo-5-ureidopentan-2-ylcarbamoyl)cyclobutanecarboxylic acid **10b**



10g **10f** **10e**



10d



10c **10b**

To a mixture of (S)-2-amino-5-ureidopentanoic acid **10g** (17.50 g, 0.10 mol) in a mixture of dioxane and H_2O (50 mL / 75 mL) was added K_2CO_3 (34.55 g, 0.25 mol). Fmoc-Cl (30.96 g, 0.12 mol) was added slowly at 0 °C. The reaction mixture was warmed to r.t. over 2 h. Organic solvent was removed under reduced pressure, and the water slurry was adjusted to pH = 3 with 6 M HCl solution, and extracted with EtOAc (100 mL × 3). The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-5-ureidopentanoic acid **10f** (38.0 g, 95.6 %). **10f** is commercially available.

To a solution of **10f** (4 g, 10 mmol) in a mixture of DCM and MeOH (100 mL / 50 mL) were added (4-aminophenyl)methanol (1.6 g, 13 mmol, 1.3 eq) and 2-Ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline, EEDQ, Sigma-Aldrich CAS Reg. No. 16357-59-8 (3.2 g, 13 mmol, 1.3 eq). After the mixture was stirred at r.t. for 16 h under N_2, it was concentrated to give a brown solid. MTBE (200 mL) was added and it was stirred at 15°C for 2 h. The solid was collected by filtration, washed with MTBE (50 mL × 2) to give (S)-(9H-fluoren-9-yl)methyl (1-((4-(hydroxymethyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)carbamate **10e** as an orange solid (4.2 g, 84%). LCMS (ESI): m/z 503.0 [M+1].

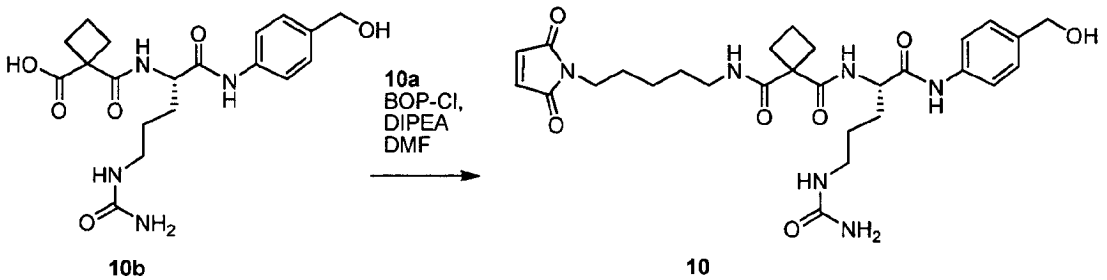
To a stirred solution of **10e** (4.2 g, 8.3 mmol) in dry DMF (20 ml) was added piperidine (1.65 mL, 17 mmol, 2 eq) dropwise at r.t. The mixture was stirred at r.t. for 30 min, and solid precipitate formed. Dry DCM (50 mL) was added, and the mixture became transparent immediately. The mixture was stirred at r.t. for another 30 min, and LCMS showed **10e** was consumed. It was concentrated to dryness under reduced pressure (make sure no piperidine remained), and the residue was partitioned between EtOAc and H_2O (50 mL / 20 mL). Aqueous phase was washed with EtOAc (50 mL × 2) and concentrated to give (S)-2-amino-N-(4-(hydroxymethyl)phenyl)-5-ureidopentanamide **10d** as an oily residual (2.2 g, 94%) (contained small amount of DMF).

Commercially available 1,1-cyclobutanedicarboxylic acid, 1,1-diethyl ester (CAS Reg. No. 3779-29-1) was converted by limited saponification with aqueous base to the half acid/ester 1,1-cyclobutanedicarboxylic acid, 1-ethyl ester (CAS Reg No. 54450-84-9) and activation with a coupling reagent such as TBTU (*O*-(Benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium tetrafluoroborate, also called: *N,N,N',N'*-Tetramethyl-*O*-(benzotriazol-1-yl)uronium tetrafluoroborate, CAS No. 125700-67-6, Sigma-Aldrich B-2903), and N-hydroxysuccinimide to the NHS ester, 1-(2,5-dioxopyrrolidin-1-yl) 1-ethyl cyclobutane-1,1-dicarboxylate.

To a solution of 1-(2,5-dioxopyrrolidin-1-yl) 1-ethyl cyclobutane-1,1-dicarboxylate (8 g, 29.7 mmol) in DME (50 mL) was added a solution of **10d** (6.0 g, 21.4 mmol) and $NaHCO_3$ (7.48 g, 89.0 mmol) in water (30 mL). After the mixture was stirred at r.t. for 16 h, it was concentrated to dryness under reduced pressure and the residue was purified by column chromatography (DCM:MeOH = 10:1) to give (S)-ethyl 1-((1-(4-(hydroxymethyl)phenyl)-2-oxo-6-ureidohexan-3-yl)carbamoyl)cyclobutanecarboxylate **10c** as white solid (6.4 g, 68.7%). LCMS (ESI): m/z 435.0 [M+1]

To a stirred solution of **10c** (6.4 g, 14.7 mmol) in a mixture of THF and MeOH (20 mL / 10 mL) was added a solution of LiOH • H_2O (1.2 g, 28.6 mmol) in H_2O (20 mL) at r.t. After the reaction mixture was stirred at r.t. for 16 h, solvent was removed under reduced pressure, the residue obtained was purified by prep-HPLC to give (S)-1-(1-(4-(hydroxymethyl)phenylamino)-1-oxo-5-ureidopentan-2-ylcarbamoyl)cyclobutanecarboxylic acid **10b** (3.5 g, yield: 58.5%). LCMS (ESI): m/z 406.9 [M+1]. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.86 (d, J = 8.4 Hz, 2 H), 8.51 (d, J = 8.4 Hz, 2 H), 5.88 - 5.85 (m, 1 H), 5.78 (s, 2 H), 4.54 - 4.49 (m, 3 H), 4.38 - 4.32 (m, 1 H), 3.86 - 3.75 (m, 1 H), 3.84 - 3.80 (m, 2 H), 3.28 - 3.21 (m, 1 H), 3.30 - 3.24 (m, 1 H), 3.00 - 2.80 (m, 1 H), 2.37 - 2.28 (m, 2 H).

Step 3: Preparation of S)-N-(5-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)pentyl)-N-(1-(4-(hydroxymethyl)phenylamino)-1-oxo-5-ureidopentan-2-yl)cyclobutane-1,1-dicarboxamide **10**

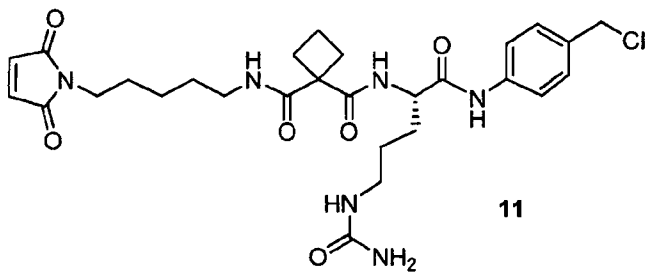


10b **10**

Diisopropylethylamine, DIPEA (1.59 g, 12.3 mmol) and bis(2-oxo-3-oxazolidinyl)phosphinic chloride, BOP-Cl (CAS Reg. No. 68641-49-6, Sigma-Aldrich, 692 mg, 2.71 mmol) was added to a solution of (S)-1-(1-(4-(hydroxymethyl)phenylamino)-1-oxo-5-ureidopentan-2-ylcarbamoyl)cyclobutanecarboxylic acid **10b** (1 g, 2.46 mmol) in DMF (10 mL) at 0 °C, followed by 1-(5-aminopentyl)-1H-pyrrole-2,5-dione hydrochloride 10a (592 mg, 2.71 mmol). The mixture was stirred at 0 °C for 0.5h. The reaction mixture was quenched with citric acid solution (10 mL), extracted with DCM/MeOH (10:1). The organic layer was dried and concentrated, and the residue was purified by column chromatography on silica gel (DCM:MeOH = 10:1) to give to give S)-N-(5-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)pentyl)-N-(1-(4-(hydroxymethyl)phenylamino)-1-oxo-5-ureidopentan-2-yl)cyclobutane-1,1-dicarboxamide 10 (1.0 g, 71 %), also referred to as MC-CBDK-cit-PAB-OH. LCMS (ESI): M+H$^+$ = 571.28. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.00 (s, 1H), 7.82-7.77 (m, 2H), 7.53 (d, J = 8.4 Hz, 2 H), 7.19 (d, J = 8.4 Hz, 2 H), 6.96 (s, 2H), 5.95 (t, J = 6.4 Hz, 1H), 5.39 (s, 2H), 5.08 (t, J = 5.6 Hz, 1H), 4.40-4.35 (m, 3H), 4.09 (d, J = 4.8 Hz, 1 H), 3.01 (d, J = 3.2

Hz, 2 H), 3.05-2.72 (m, 4H), 2.68-2.58 (m, 3H), 2.40-2.36 (m, 4H), 1.72-1.70 (m, 3H), 1.44-1.42 (m, 1H), 1.40-1.23 (m, 6H), 1.21-1.16 (m, 4H).

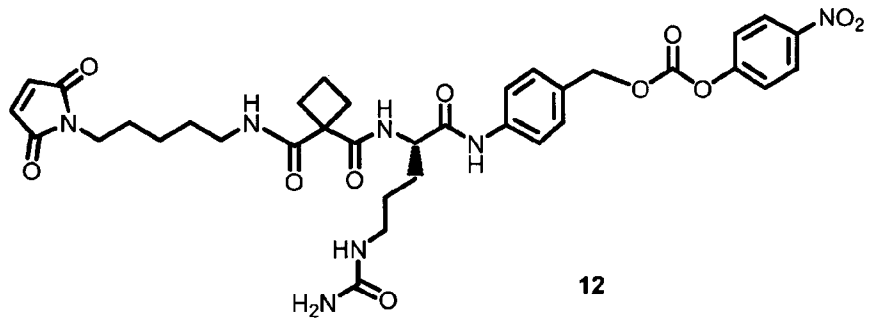
Example 14 (S)-N-(1-(4-(chloromethyl)phenylamino)-1-oxo-5-ureidopentan-2-yl)-N-(5-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)pentyl)cyclobutane-1,1-dicarboxamide **11**



11

A solution of (S)-N-(5-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)pentyl)-N-(1-(4-(hydroxymethyl)phenylamino)-1-oxo-5-ureidopentan-2-yl)cyclobutane-1,1-dicarboxamide **10** (2.0 g, 3.5 mmol) in N,N-dimethylformamide, DMF or N-methylpyrrolidone, NMP (50 mL) was treated with thionyl chloride, $SOCl_2$ (1.25 g, 10.5 mmol) in portions dropwise at 0 °C. The reaction remained yellow. The reaction was monitored by LC/MS indicating >90% conversion. After the reaction mixture was stirred at 20 °C for 30 min or several hours, it was diluted with water (50 mL) and extracted with EtOAc (50 mL x 3). The organic layer was dried, concentrated and purified by flash column (DCM : MeOH = 20 : 1) to to form **11**, also referred to as MC-CBDK-cit-PAB-Cl as a gray solid. LCMS: (5-95, AB, 1.5 min), 0.696 min, m/z = 589.0 $[M+1]^+$.

Example 15 (S)-4-(2-(1-(5-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)pentylcarbamoyl)cyclobutanecarboxamido)-5-ureidopentanamido)benzyl 4-nitrophenyl carbonate **12**

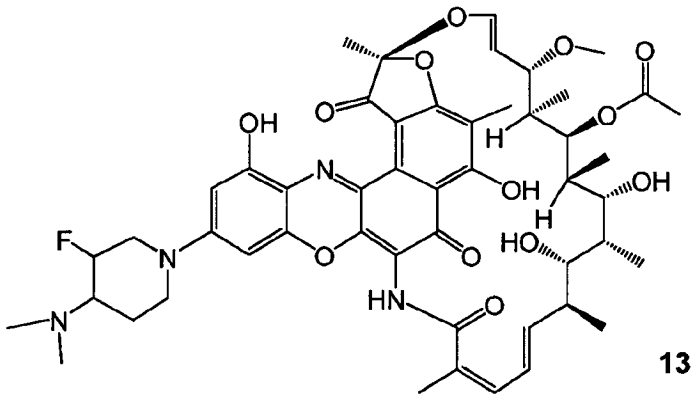


12

To a solution of (S)-N-(5-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)pentyl)-N-(1-(4-(hydroxymethyl)phenylamino)-1-oxo-5-ureidopentan-2-yl)cyclobutane-1,1-dicarboxamide **10**

in anhydrous DMF was added diisopropylethylamine (DIEA), followed by PNP carbonate (bis(4-nitrophenyl) carbonate). The reaction solution was stirred at room temperature (r.t.) for 4 hours and the mixture was purified by prep-HPLC to afford **12**. LCMS (ESI): $M+H^+$ = 736.29.

Example 16 Preparation of MC-(CBDK-cit)-PAB-(dimethyl, fluoropipBOR) - PLA-1



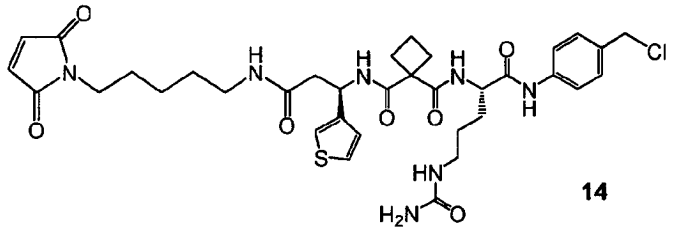
13

Following the procedure for PLA-2, (S)-N-(1-(4-(chloromethyl)phenylamino)-1-oxo-5-ureidopentan-2-yl)-N-(5-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)pentyl)cyclobutane-1,1-dicarboxamide **11** and the fluorinated rifamycin-derivative, dimethylfluoropipBOR **13** (LCMS (ESI): $M+H^+$ = 945.43) were reacted to form MC-(CBDK-cit)-PAB-(dimethyl, fluoropipBOR) - PLA-1, Table 2. LCMS (ESI): $M+H^+$ = 1499.7

Example 17 Preparation of MC-(CBDK-cit)-PAB-(dimethylpipBOR) - PLA-2

(S)-N-(1-(4-(chloromethyl)phenylamino)-1-oxo-5-ureidopentan-2-yl)-N-(5-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)pentyl)cyclobutane-1,1-dicarboxamide **11** (0.035 mmol) in DMF was cooled to 0 °C and dimethylpipBOR **6**, (10 mg, 0.011 mmol) was added. The mixture was diluted with another 0.5 mL of DMF. Stirred open to air for 30 minutes. N,N-diisopropylethylamine (DIEA, 10 µL, 0.05 mmol) was added and the reaction stirred overnight open to air. By LC/MS, 50% of desired product was observed. An additional 0.2 eq N,N-diisopropylethylamine base was added while the reaction stirred open to air for another 6 hours until the reaction appeared to stop progressing. The reaction mixture was diluted with DMF and purified on HPLC (20-60% ACN/HCOOH in H_2O) to give MC-(CBDK-cit)-PAB-(dimethylpipBOR) - PLA-2, Table 2. LCMS (ESI): $M+H^+$ = 1481.8, yield 31%.

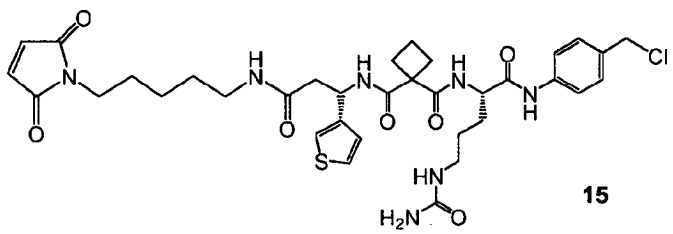
Example 18 Preparation of MC-((*R*)-thiophen-3-yl-CBDK-cit)-PAB-(dimethylpipBOR) (PLA-3)



14

Following the procedure for PLA-2, (N-((S)-1-(4-(chloromethyl)phenylamino)-1-oxo-5-ureidopentan-2-yl)-N-((R)-3-(5-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)pentylamino)-3-oxo-1-(thiophen-3-yl)propyl)cyclobutane-1,1-dicarboxamide **14** (LCMS (ESI): $M+H^+$ = 742.3) and dimethylpipBOR **6** were reacted to give MC-((*R*)-thiophen-3-yl-CBDK-cit)-PAB-(dimethylpipBOR) (PLA-3, Table 2). LCMS (ESI): $M+H^+$ = 1633.9

Example 19 Preparation of MC-((*S*)-thiophen-3-yl-CBDK-cit)-PAB-(dimethylpipBOR) (PLA-4)



15

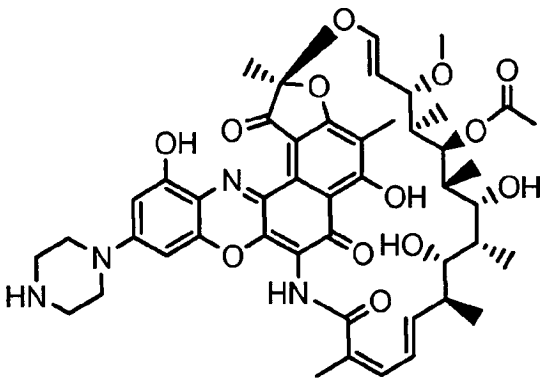
Following the procedure for PLA-2, (N-((R)-1-(4-(chloromethyl)phenylamino)-1-oxo-5-ureidopentan-2-yl)-N-((R)-3-(5-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)pentylamino)-3-oxo-1-(thiophen-3-yl)propyl)cyclobutane-1,1-dicarboxamide **15** (LCMS (ESI): $M+H^+$ = 742.3) and dimethylpipBOR **6** were reacted to give MC-((*R*)-thiophen-3-yl-CBDK-cit)-PAB-(dimethylpipBOR) (PLA-4, Table 2). LCMS (ESI): $M+H^+$ = 1633.9

Example 20 Preparation of MC-(CBDK-cit)-PABC-(pipBOR) (PLA-5)

Piperidyl benzoxazino rifamycin (pipBOR) **5** (15 mg, 0.0167 mmol), and then (S)-4-(2-(1-(5-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)pentylcarbamoyl)cyclobutanecarboxamido)-5-ureidopentanamido)benzyl 4-nitrophenyl carbonate **12** (12 mg, 0.0167 mmol) were weighed into a vial. Dimethylformamide, DMF (0.3 mL) was added, followed by diisopropylethylamine, DIEA (0.006 mL, 0.0334 mmol), and the reaction was allowed to stir at room temperature for 2 h. The reaction solution was directly purified by HPLC (30 to 70%

MeCN/water + 1% formic acid) to give MC-(CBDK-cit)-PABC-(pipBOR) (PLA-5, Table 2). LCMS (ESI): $M+H^+$ = 1496.5

Example 21 Preparation of MC-(CBDK-cit)-PABC-(piperazBTR) (PLA-6)



16

Following the procedures for PLA-5,the piperidine rifamycin derivative, piperazBOR **16** (LCMS (ESI): $M+H^+$ = 885.4) and (S)-4-(2-(1-(5-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)pentylcarbamoyl)cyclobutanecarboxamido)-5-ureidopentanamido)benzyl 4-nitrophenyl carbonate **12** were reacted to give MC-(CBDK-cit)-PABC-(piperazBTR) (PLA-6. Table 2). LCMS (ESI): $M+H^+$ = 1482.5

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. All patents, patent applications, and references cited throughout the specification are expressly incorporated by reference.

CLAIMS

We Claim:

1. An antibody-antibiotic conjugate compound comprising an anti-wall teichoic acid (WTA) antibody, covalently attached by a protease-cleavable, non-peptide linker to a rifamycin-type antibiotic.

2. The antibody-antibiotic conjugate compound of claim 1 having the formula:

$$Ab–(PML–abx)_p$$

wherein:

Ab is the anti-wall teichoic acid antibody;

PML is the protease-cleavable, non-peptide linker having the formula:

$$–Str–PM–Y–$$

where Str is a stretcher unit; PM is a peptidomimetic unit, and Y is a spacer unit;

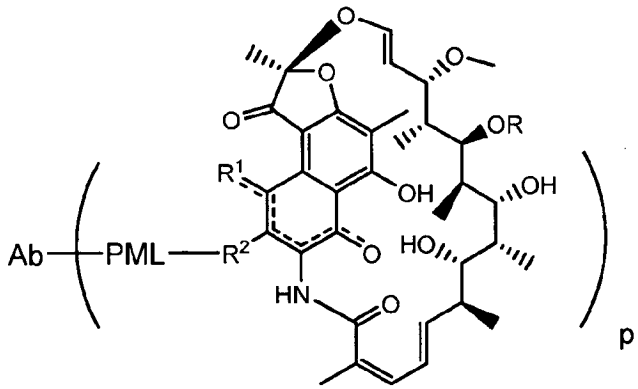
abx is the rifamycin-type antibiotic; and

p is an integer from 1 to 8.

3. The antibody-antibiotic conjugate compound of claim 2 wherein the rifamycin-type antibiotic is a rifalazil-type antibiotic.

4. The antibody-antibiotic conjugate compound of claim 2 wherein the rifamycin-type antibiotic comprises a quaternary amine attached to the protease-cleavable, non-peptide linker.

5. The antibody-antibiotic conjugate compound of claim 2 having Formula I:



I

wherein:

the dashed lines indicate an optional bond;

R is H, C_1–C_{12} alkyl, or $C(O)CH_3$;

R^1 is OH;

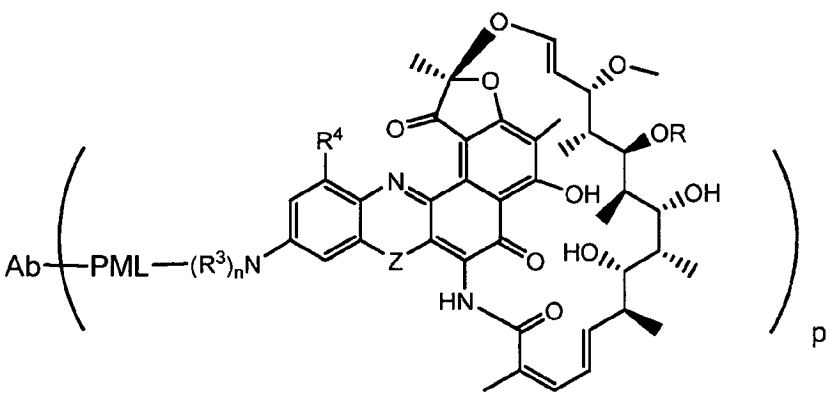
R^2 is CH=N–(heterocyclyl), wherein the heterocyclyl is optionally substituted with one or more groups independently selected from $C(O)CH_3$, C_1–C_{12} alkyl, C_1–C_{12} heteroaryl, C_2–C_{20} heterocyclyl, C_6–C_{20} aryl, and C_3–C_{12} carbocyclyl;

or R^1 and R^2 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_1–C_{12} alkyl, or OH;

PML is the protease-cleavable, non-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.

6. The antibody-antibiotic conjugate compound of claim 5 having the formula:



wherein

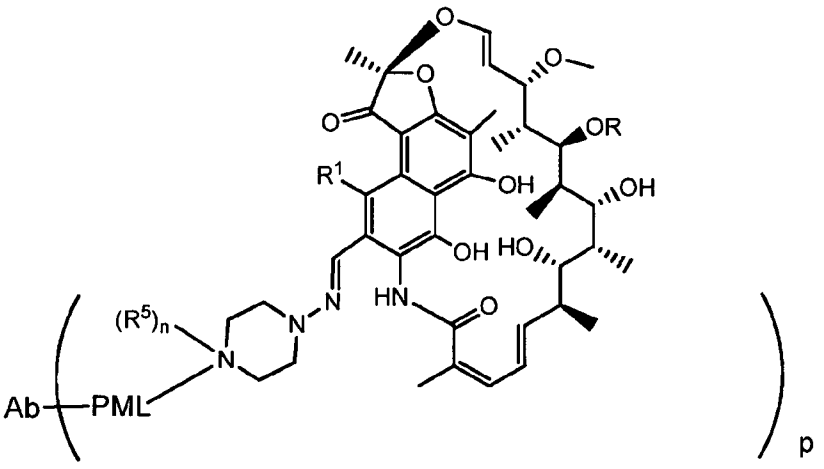
R^3 is independently selected from H and C_1–C_{12} alkyl;

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.

7. The antibody-antibiotic conjugate compound of claim 2 having the formula:

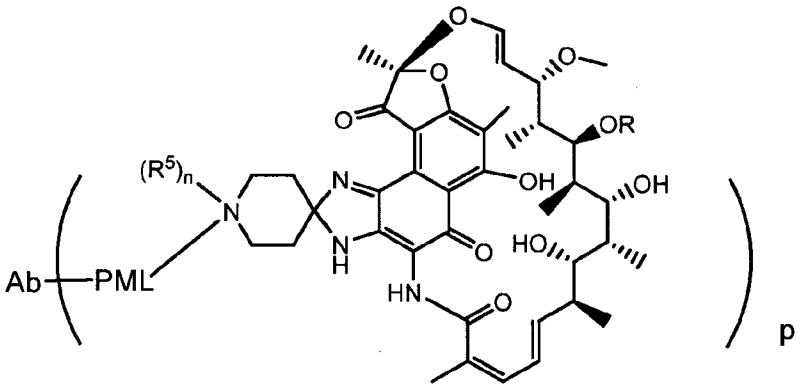


wherein

R^5 is selected from H and C_1–C_{12} alkyl; and

n is 0 or 1.

8. The antibody-antibiotic conjugate compound of claim 2 having the formula:

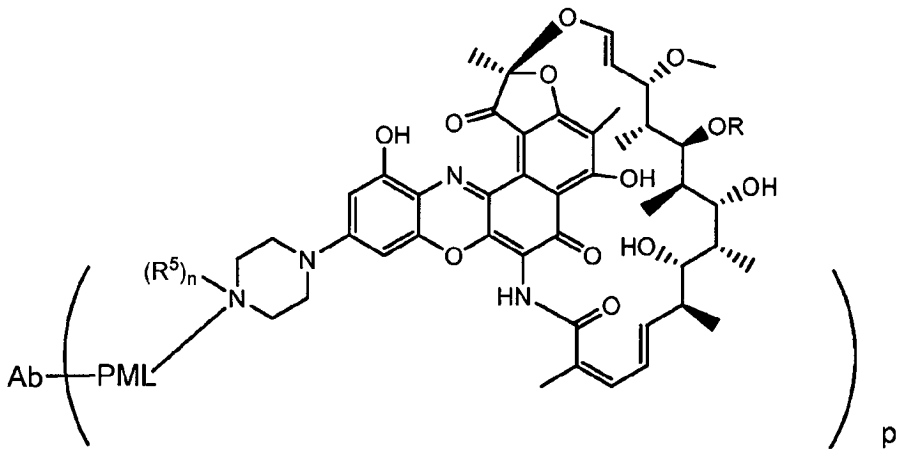


wherein

R^5 is selected from H and C_1–C_{12} alkyl; and

n is 0 or 1.

9. The antibody-antibiotic conjugate compound of claim 2 having the formula:

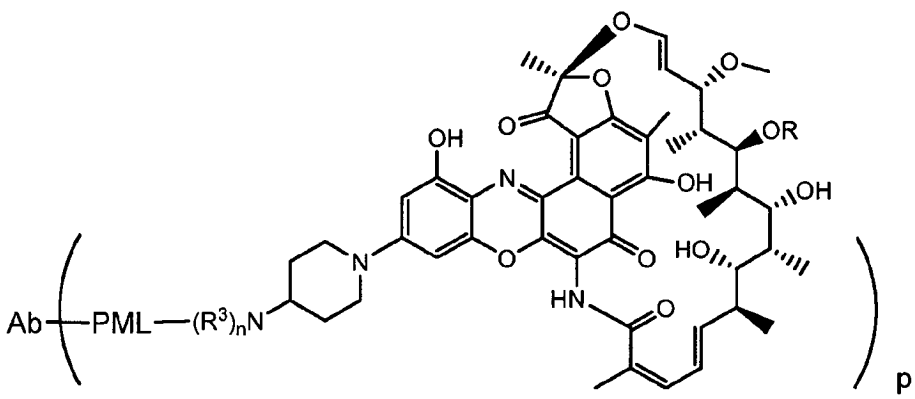


wherein

R^5 is independently selected from H and C_1–C_{12} alkyl; and

n is 0 or 1.

10. The antibody-antibiotic conjugate compound of claim 2 having the formula:

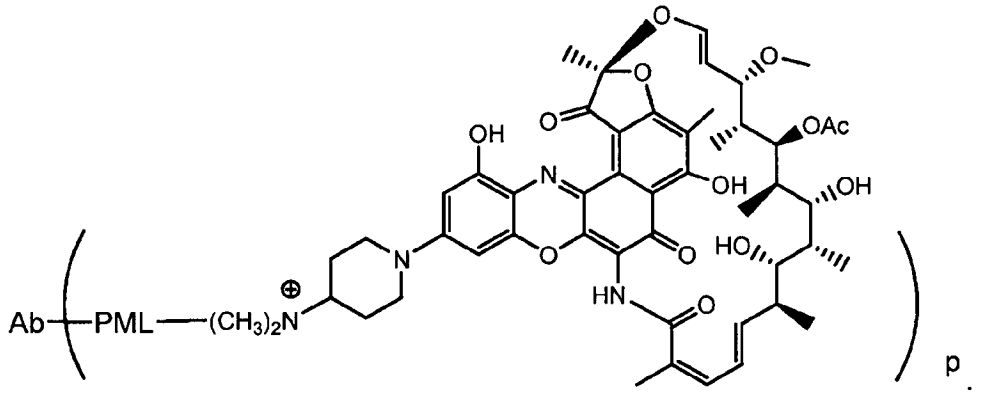


wherein

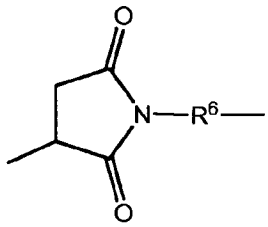
R^3 is independently selected from H and C_1–C_{12} alkyl; and

n is 1 or 2.

11. The antibody-antibiotic conjugate compound of claim 10 having the formula:



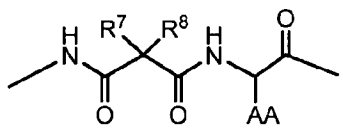
12. The antibody-antibiotic conjugate compound of claim 2 wherein Str has the formula:



wherein R^6 is selected from the group consisting of C_1-C_{12} alkylene, C_1-C_{12} alkylene-C(=O), C_1-C_{12} alkylene-NH, $(CH_2CH_2O)_r$, $(CH_2CH_2O)_r$-C(=O), $(CH_2CH_2O)_r$-CH_2, and C_1-C_{12} alkylene-NHC(=O)CH_2CH(thiophen-3-yl), where r is an integer ranging from 1 to 10.

13. The antibody-antibiotic conjugate compound of claim 12 wherein R^6 is $(CH_2)_5$.

14. The antibody-antibiotic conjugate compound of claim 2 wherein PM has the formula:

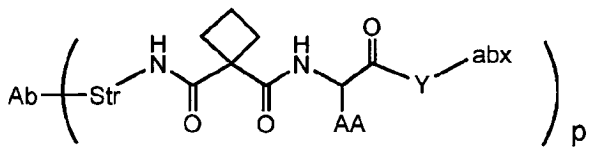


where R^7 and R^8 together form a C_3-C_7 cycloalkyl ring, and

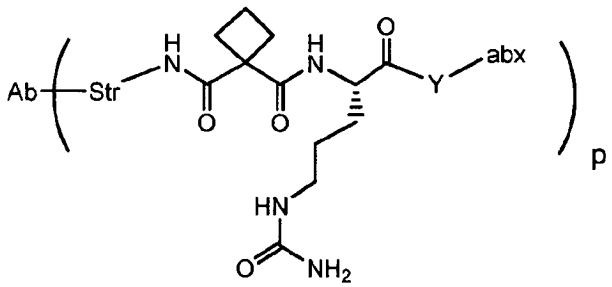
AA is an amino acid side chain selected from H, $-CH_3$, $-CH_2(C_6H_5)$, $-CH_2CH_2CH_2CH_2NH_2$, $-CH_2CH_2CH_2NHC(NH)NH_2$, $-CHCH(CH_3)CH_3$, and $-CH_2CH_2CH_2NHC(O)NH_2$.

15. The antibody-antibiotic conjugate compound of claim 2 wherein Y comprises para-aminobenzyl or para-aminobenzyloxycarbonyl.

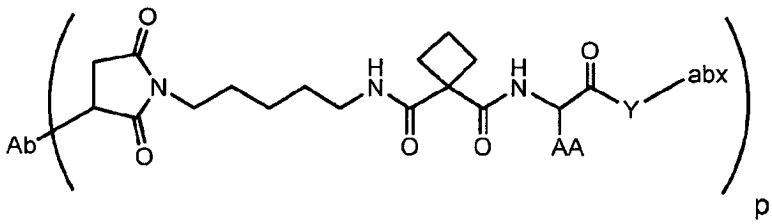
16. The antibody-antibiotic conjugate compound of claim 2 having the formula:



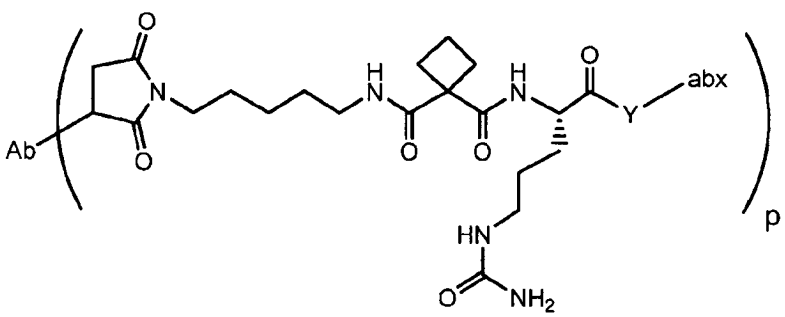
17. The antibody-antibiotic conjugate compound of claim 16 having the formula:



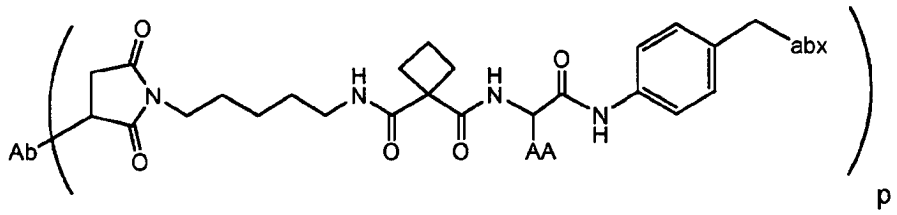
18. The antibody-antibiotic conjugate compound of claim 15 having the formula:



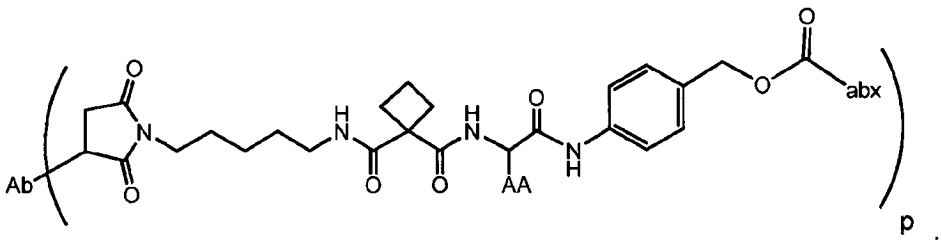
19. The antibody-antibiotic conjugate compound of claim 18 having the formula:



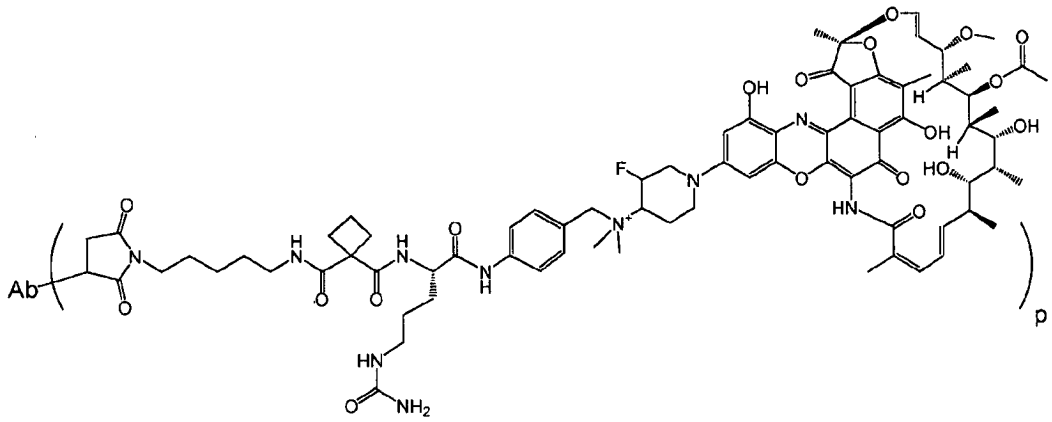
20. The antibody-antibiotic conjugate compound of claim 15 selected from the formulas:



and



21. The antibody-antibiotic conjugate compound of claim 16 selected from the formulas:



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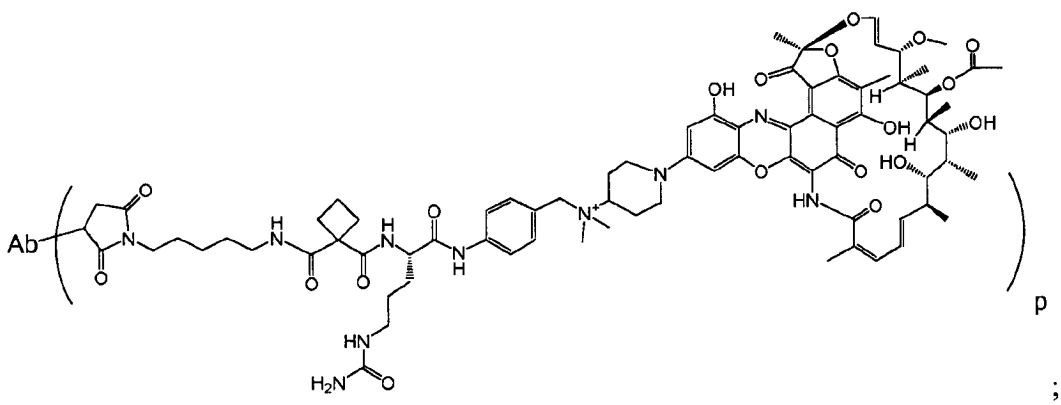

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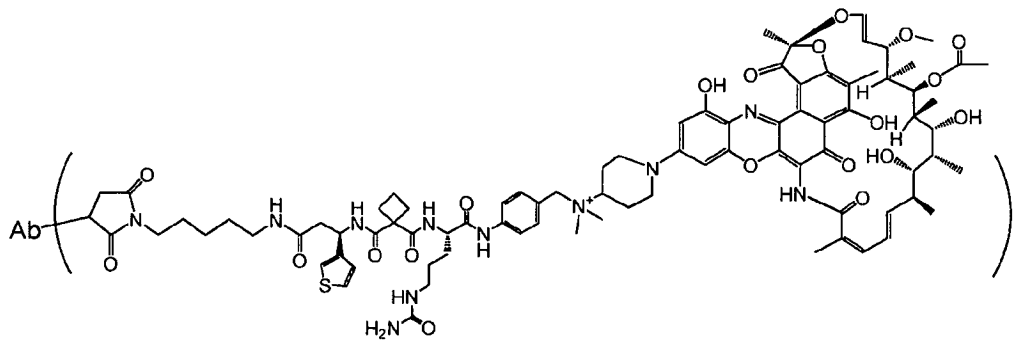

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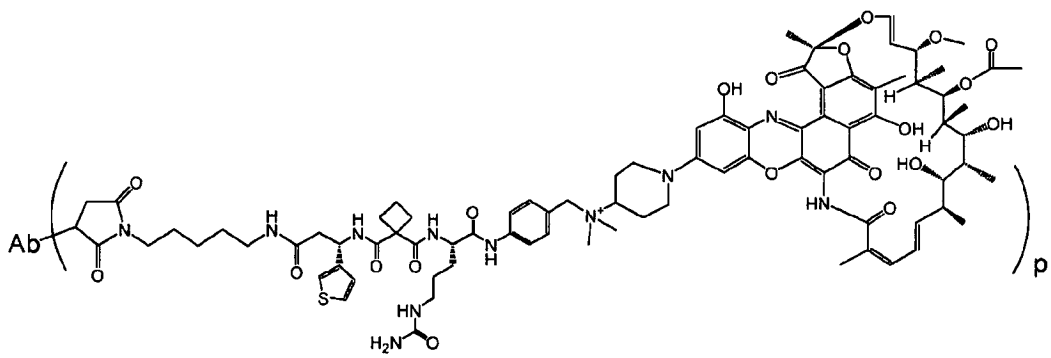

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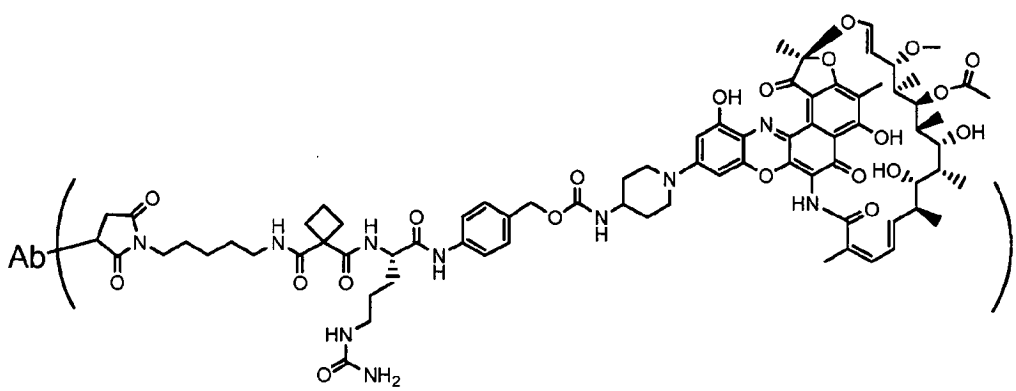
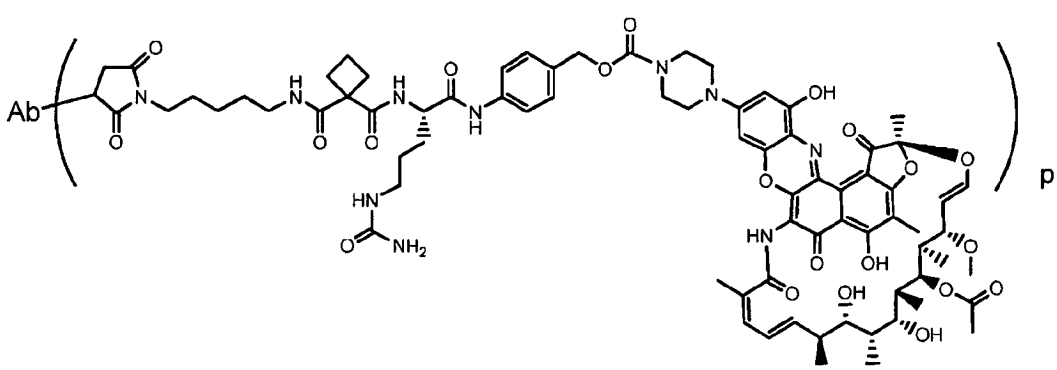


;

and



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22. The antibody-antibiotic conjugate compound of claim 1, wherein the antibody is an anti-WTAα monoclonal antibody.

23. The antibody-antibiotic conjugate of claim 22 wherein the anti-wall teichoic acid (WTA) antibody is an isolated monoclonal antibody comprising a light (L) chain and a heavy (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 1A and 1B.

24. The antibody-antibiotic conjugate of claim 22 wherein the anti-wall teichoic acid (WTA) antibody comprises 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.

25. The antibody-antibiotic conjugate of claim 24, further comprising 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.

26. The antibody-antibiotic conjugate compound of claim 1, wherein the antibody is an anti-WTAβ monoclonal antibody.

27. The antibody-antibiotic conjugate compound of claim 26, wherein the anti-WTAβ antibody comprises 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 corresponding CDRs of each of Abs shown in Figure 12 (SEQ ID NO. 33-110).

28. The antibody-antibiotic conjugate compound of claim 26, wherein the anti-WTAβ 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 corresponding to each of the antibodies 6078, 6263, 4450, 6297, 6239, 6232, 6259, 6292, 4462, 6265, 6253, 4497, and 4487 respectively, as shown in Figure 15A-1, 15A-2, 15A-3 at Kabat positions 1-107.

29. The antibody-antibiotic conjugate compound of claim 28, wherein the anti-WTAβ antibody further comprises 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 Figure 15B-1 to 15B-6 at Kabat positions 1-113.

30. The antibody-antibiotic conjugate compound of claim 29, wherein the VL comprises the sequence of SEQ ID NO. 111 and the VH comprises the sequence of SEQ ID NO. 112 wherein X is Q or E and X_1 is M, I or V.

31. The antibody-antibiotic conjugate compound of claim 26, wherein the antibody light chain contains an engineered cysteine and comprises the sequence of SEQ ID NO. 115 and the H chain comprises the SEQ ID NO. 116 wherein X is M, I or V.

32. The antibody-antibiotic conjugate compound of claim 26, wherein the antibody light chain comprises the sequence of SEQ ID NO. 113 and the H chain contains an engineered cysteine and comprises the SEQ ID NO. 117 wherein X is M, I or V.

33. The antibody-antibiotic conjugate compound of claim 26, wherein the antibody light chain contains an engineered cysteine and comprises the sequence of SEQ ID NO. 115, and the H chain contains an engineered cysteine and comprises the SEQ ID NO. 117 wherein X is M, I or V.

34. The antibody-antibiotic conjugate compound of claim 26, wherein the anti-WTAβ antibody comprises a VH and a VL, wherein the VH comprises at least 95% sequence identity over the length of the VH of SEQ ID NO. 156 and the VL comprises at least 95% sequence identity over the length of the VL of sequence SEQ ID NO. 119.

35. The antibody-antibiotic conjugate compound of claim 26, wherein the anti-WTAβ antibody comprises a VH comprising the sequence of SEQ ID NO. 156 and a VL comprising the sequence of the SEQ ID NO. 119.

36. The antibody-antibiotic conjugate compound of claim 26, wherein the anti-WTAβ antibody comprises a L chain comprising the sequence of SEQ ID NO.121 and a H chain comprising the sequence of SEQ ID NO. 124.

37. The antibody-antibiotic conjugate compound of claim 26, wherein the anti-WTAβ antibody comprises a L chain comprising the sequence of SEQ ID NO. 123 and a H chain comprising the sequence of SEQ ID NO. 157

38. The antibody-antibiotic conjugate compound of claim 26, wherein the anti-WTAβ antibody comprise a L chain comprising the sequence of SEQ ID NO. 123 and a H chain comprising the sequence of SEQ ID NO. 124.

39. The antibody-antibiotic conjugate compound of claim 1 wherein the antibody comprises: i) L chain and H chain CDRs of SEQ ID NOs 99-104 or the L chain and H chain CDRs of SEQ ID NOs. 33-38; 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.

40. The antibody-antibiotic conjugate compound of claim 1 wherein the anti-wall teichoic acid (WTA) antibody binds to *Staphylococcus aureus*.

41. The antibody-antibiotic conjugate compound of any of the preceding claims, wherein the antibody is a F(ab) or a $F(ab')_2$.

42. A pharmaceutical composition comprising the antibody-antibiotic conjugate compound of claim 1, and a pharmaceutically acceptable carrier, glidant, diluent, or excipient.

43. A method of treating a bacterial infection in a patient comprising administering to the patient a therapeutically-effective amount of the antibody-antibiotic conjugate compound of claim 1.

44. A method of killing intracellular Staph aureus in the cells of a staph aureus infected patient without killing the host cells by administering an anti-WTA-antibiotic conjugate of claim 1.

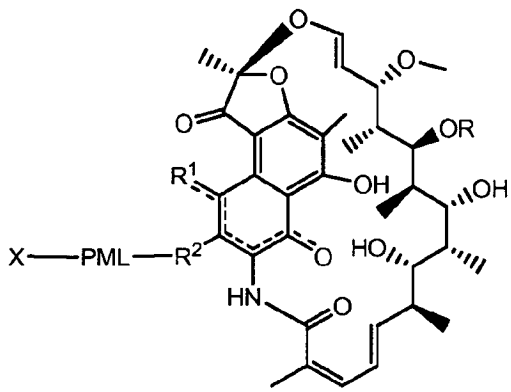
45. A process for making the antibody-antibiotic conjugate compound of claim 1 comprising conjugating a rifamycin-type antibiotic to an anti-wall teichoic acid (WTA) antibody.

46. A kit for treating a bacterial infection, comprising:

a) the pharmaceutical composition of claim 23; and

b) instructions for use.

47. An antibiotic-linker intermediate having Formula II:



II

wherein:

the dashed lines indicate an optional bond;

R is H, C_1–C_{12} alkyl, or $C(O)CH_3$;

R^1 is OH;

R^2 is CH=N–(heterocyclyl), wherein the heterocyclyl is optionally substituted with one or more groups independently selected from $C(O)CH_3$, C_1–C_{12} alkyl, C_1–C_{12} heteroaryl, C_2–C_{20} heterocyclyl, C_6–C_{20} aryl, and C_3–C_{12} carbocyclyl;

or R^1 and R^2 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_1–C_{12} alkyl, or OH;

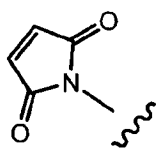
PML is a protease-cleavable, non-peptide linker attached to R^2 or the fused heteroaryl or heterocyclyl formed by R^1 and R^2, and having the formula:

–Str–PM–Y–

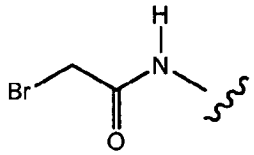
where Str is a stretcher unit; PM is a peptidomimetic unit, and Y is a spacer unit; and

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

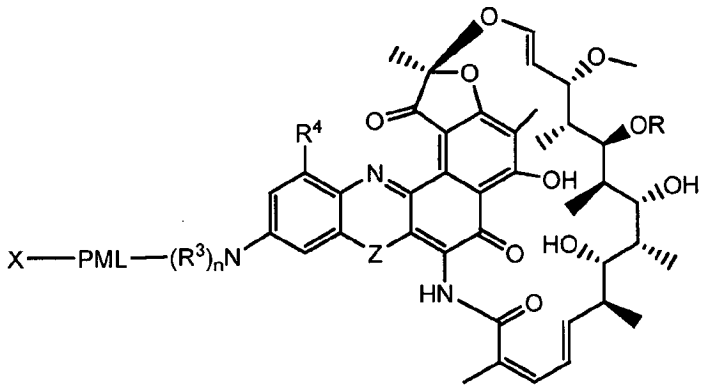
48. The antibiotic-linker intermediate of claim 47 wherein X is



or



49. The antibiotic-linker intermediate of claim 47 having the formula:



wherein

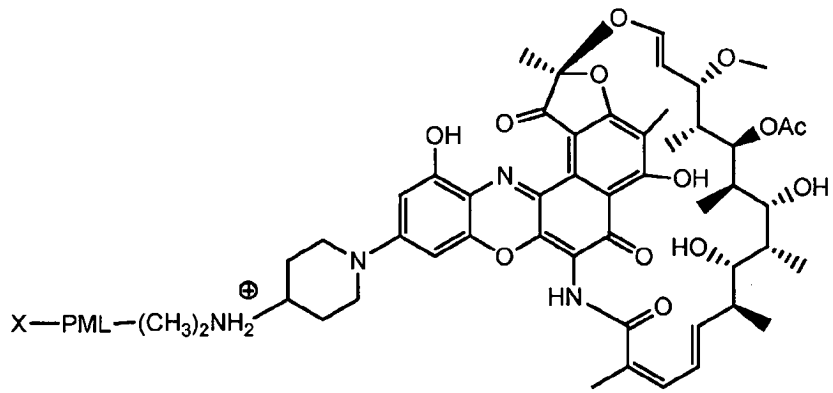
R^3 is independently selected from H and C_1–C_{12} alkyl;

n is 1 or 2;

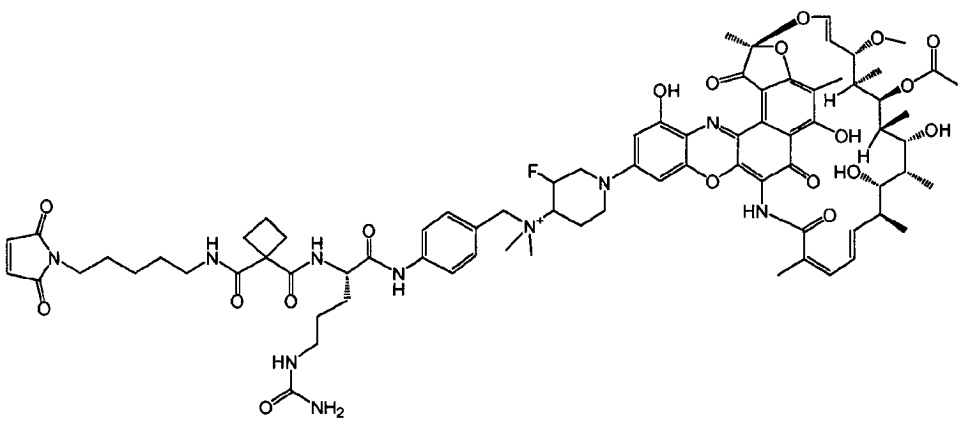
R^4 is selected from H, F, Cl, Br, I, C_1–C_{12} alkyl, and OH; and

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

50. The antibiotic-linker intermediate of claim 47 having the formula:



51. The antibiotic-linker intermediate of claim 47 selected from the formulas:



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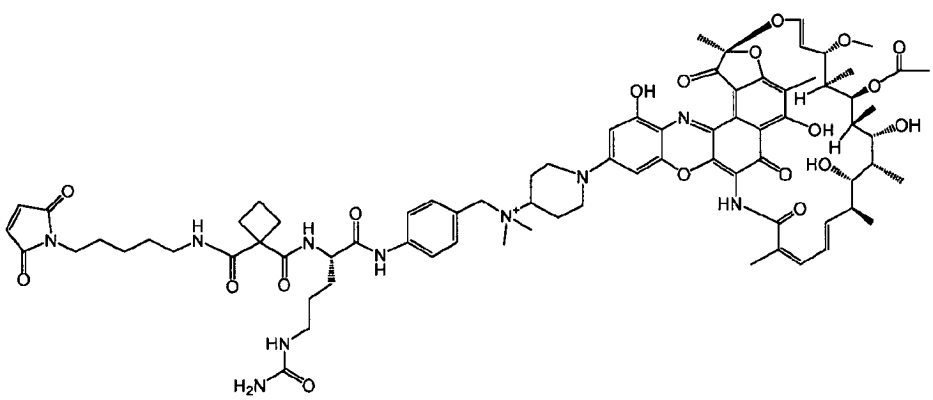

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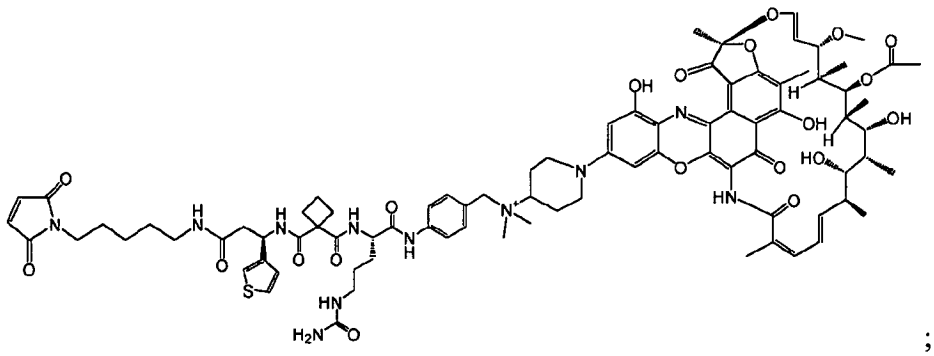

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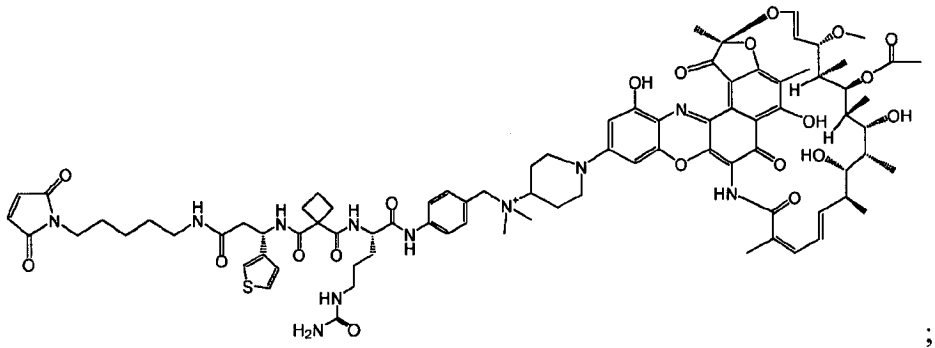

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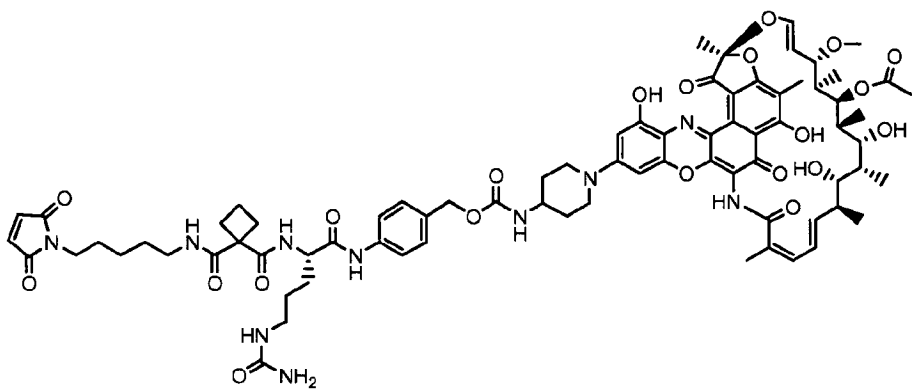
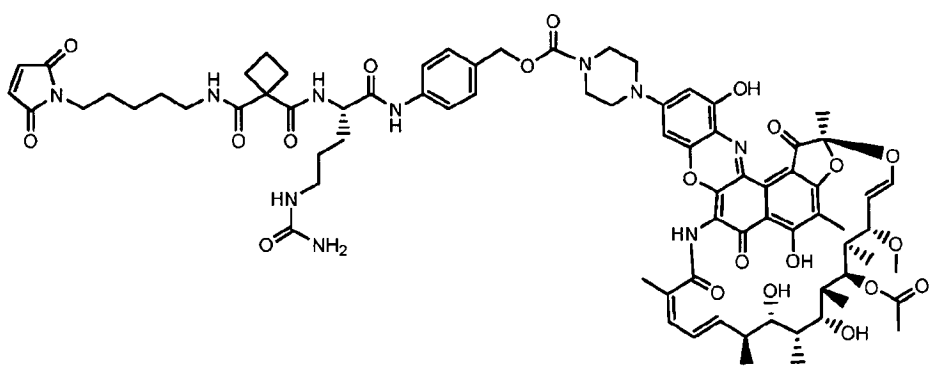


; and



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52. The method of claim 43 wherein the patient is infected with a Staphylococcal type bacteria.

53. The method of claim 52 wherein the patient is infected with Staphylococcus aureus.

54. The method of claim 43 wherein the antibody-antibiotic conjugate compound is administered to the patient at a dose in the range of about 50mg/kg to 100mg/kg.

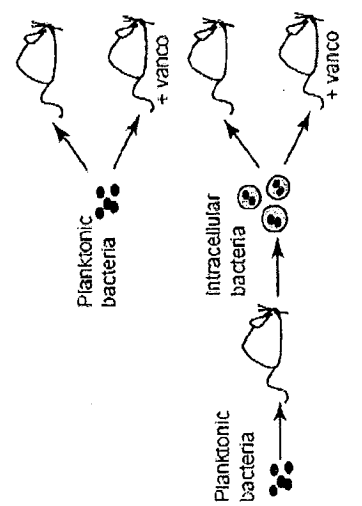


FIG. 1A

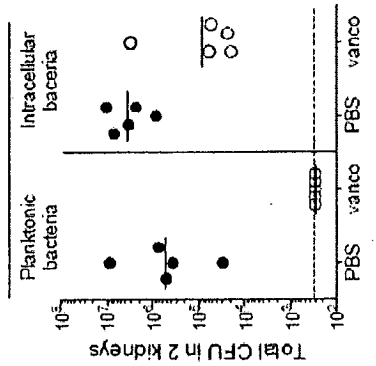


FIG. 1B

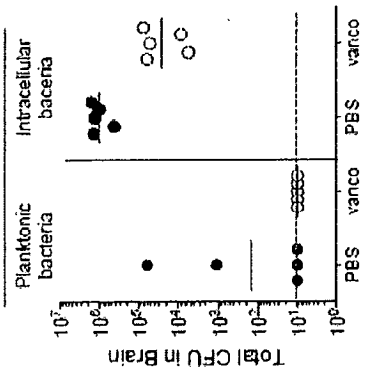


FIG. 1C

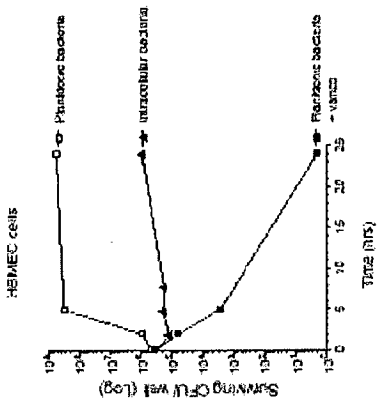


FIG. 1F

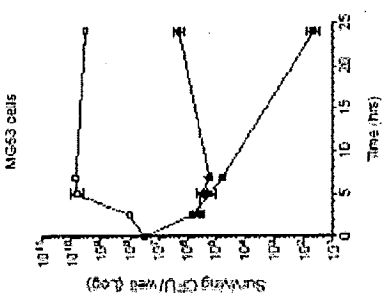


FIG. 1E

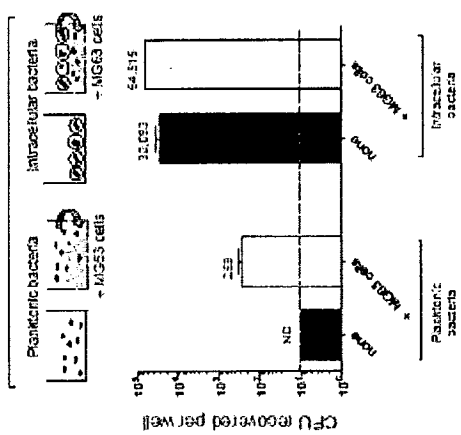


FIG. 1D

- **Concept of TAC:**
  antibiotic is released from TAC by phagolysosomal proteases

FIG. 2

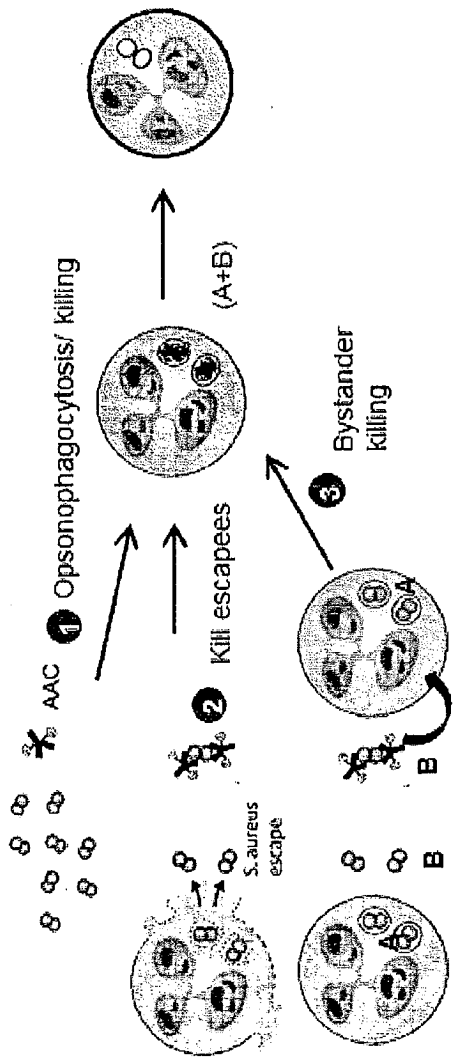

AAC
1 Opsonophagocytosis/ killing
2 Kill escapees
3 Bystander killing
(A+B)
S. aureus escape
A
B

FIG. 3

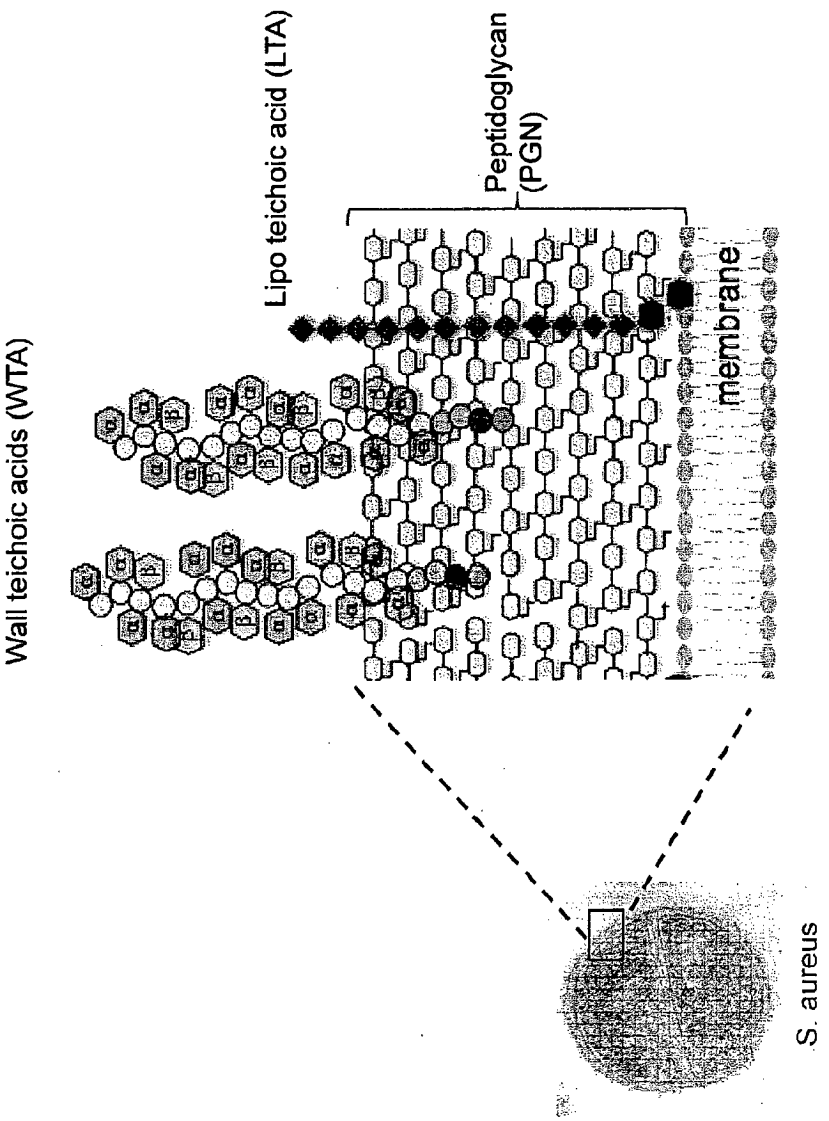

Wall teichoic acids (WTA)
Lipo teichoic acid (LTA)
Peptidoglycan (PGN)
membrane
S. aureus

FIG. 4

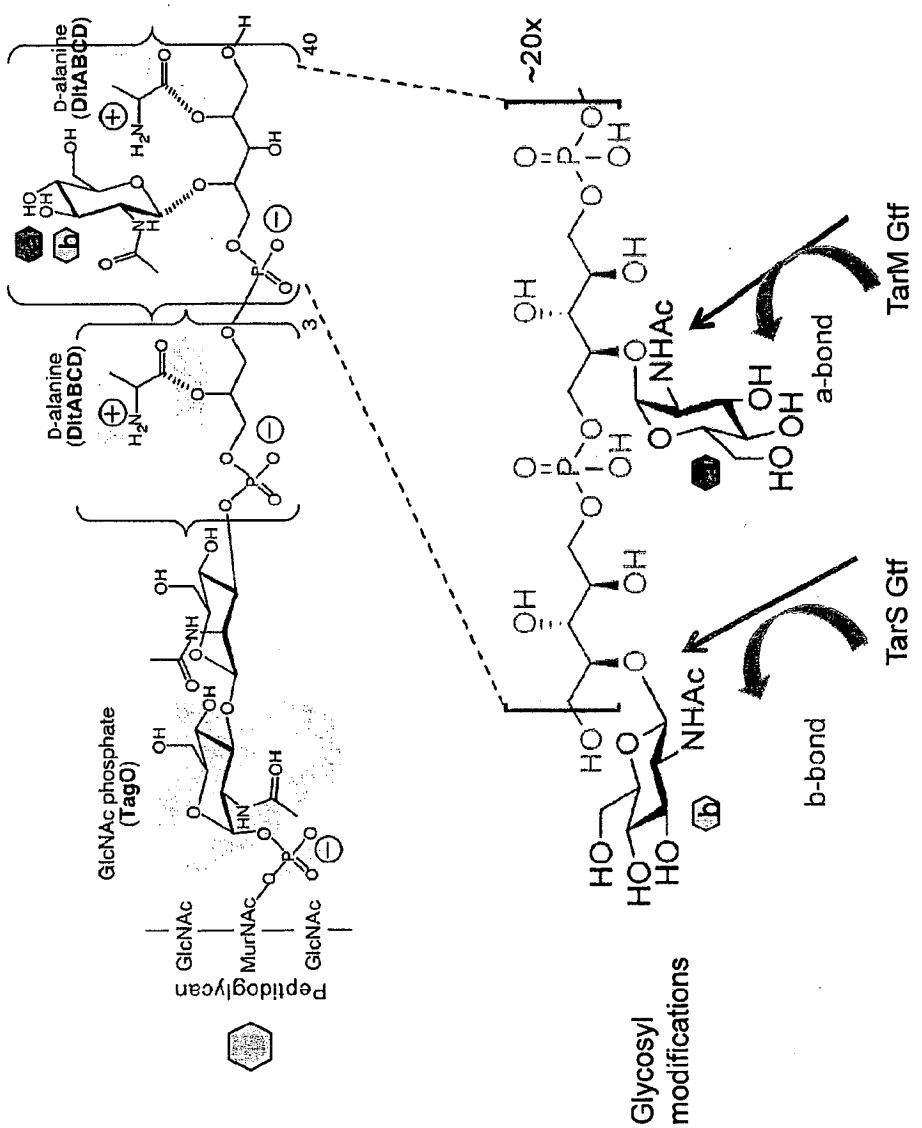

D-alanine (DltABCD)
40
3
~20x
GlcNAc phosphate (TagO)
GlcNAc
MurNAc
GlcNAc
Peptidoglycan
NHAc
a-bond
TarM Gtf
b-bond
TarS Gtf
Glycosyl modifications

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 depi:TSB in 96:4 ratio)
4624(7578)	WTA	alpha	0.4	16	326	sMBC	CW USA300 stat (iron depi:TSB in 96:4 ratio)
4339	WTA	alpha			326	sMBC	CW USA300 stat (iron depi:TSB in 96:4 ratio)
6267	WTA	alpha			350	PB/PC	CW USA300 stat (iron depi:TSB in 96:4 ratio)
rF1	SDR-proteins	?	0.3	1600			
4516(7577)	SDR-proteins	?			327	PB/PC	WTA; PGN; CW USA300 stat (iron depi:TSB in 96:4 ratio)
6234	SDR-proteins	?			350	PB/PC	PGN+WTA (1:1); CW USA300 stat (iron depi:TSB in 96:4 ratio)
6060	SDR-proteins	?			349	sMBC	PGN+WTA (1:1); CW USA300 stat (iron depi: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 depi:TSB in 96:4 ratio)

FIG. 6B

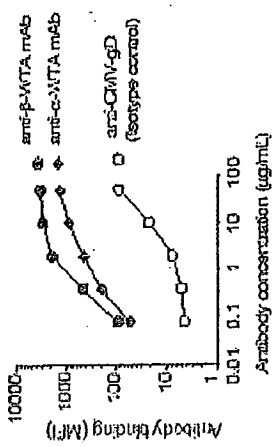

anti-β-NTA mAb
anti-α-NTA mAb
anti-CMV-gD
(isotype control)
Antibody binding (MFI)
10000
1000
100
10
1
0.01
0.1
1
10
100
Antibody concentration (μg/mL)

FIG. 7A

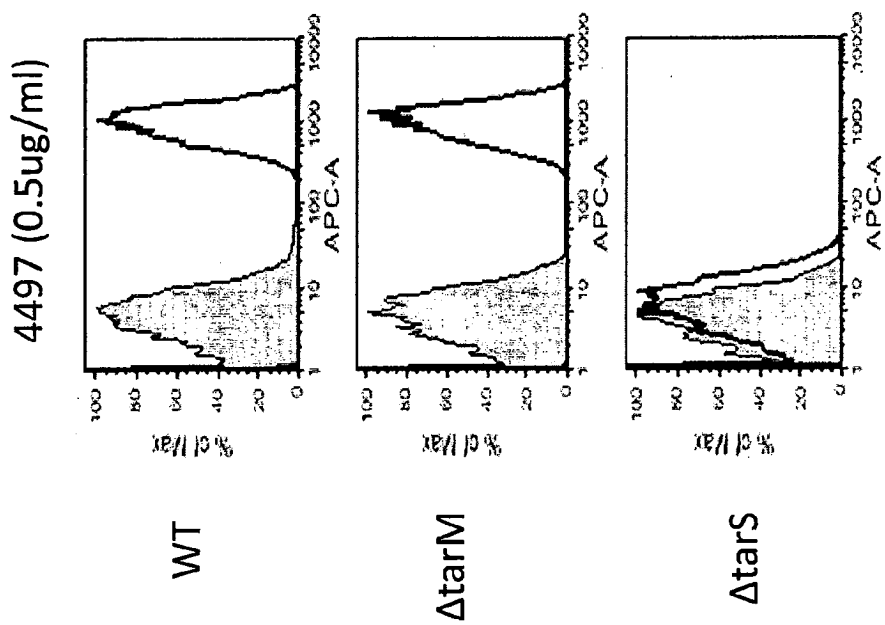

4497 (0.5ug/ml)
WT
ΔtarM
ΔtarS
% of Max
APC-A
0
20
40
60
80
100
1
10
100
1000
10000
100000

FIG. 7B

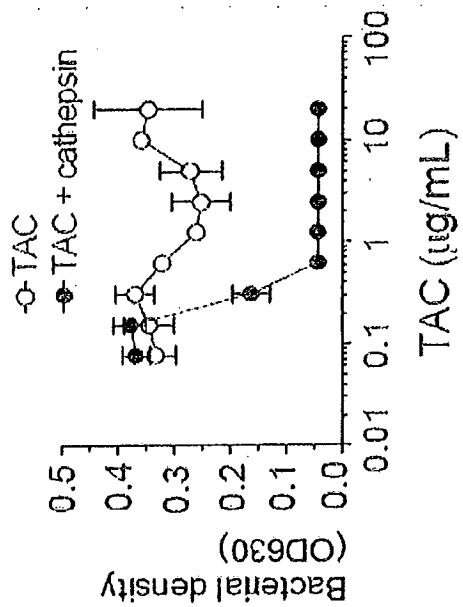

TAC
TAC + cathepsin
Bacterial density
(OD630)
0.5
0.4
0.3
0.2
0.1
0.0
0.01
0.1
1
10
100
TAC (μg/mL)

FIG. 9

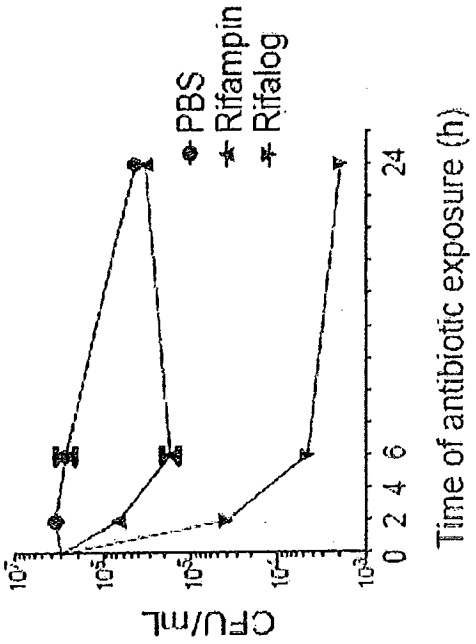

PBS
Rifampin
Rifalog
CFU/mL
0
2
4
6
24
Time of antibiotic exposure (h)

FIG. 8

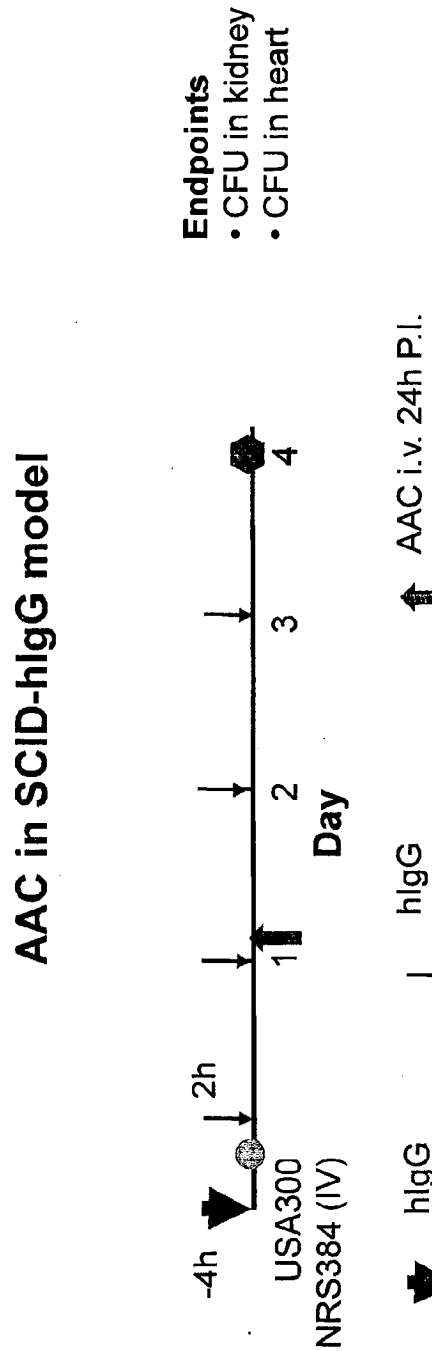

AAC in SCID-hIgG model
Endpoints
• CFU in kidney
• CFU in heart
-4h
2h
USA300
NRS384 (IV)
1
2
3
4
Day
hIgG
30 mg i.v.
hIgG
15 mg i.p.
AAC i.v. 24h P.I.

FIG. 10A

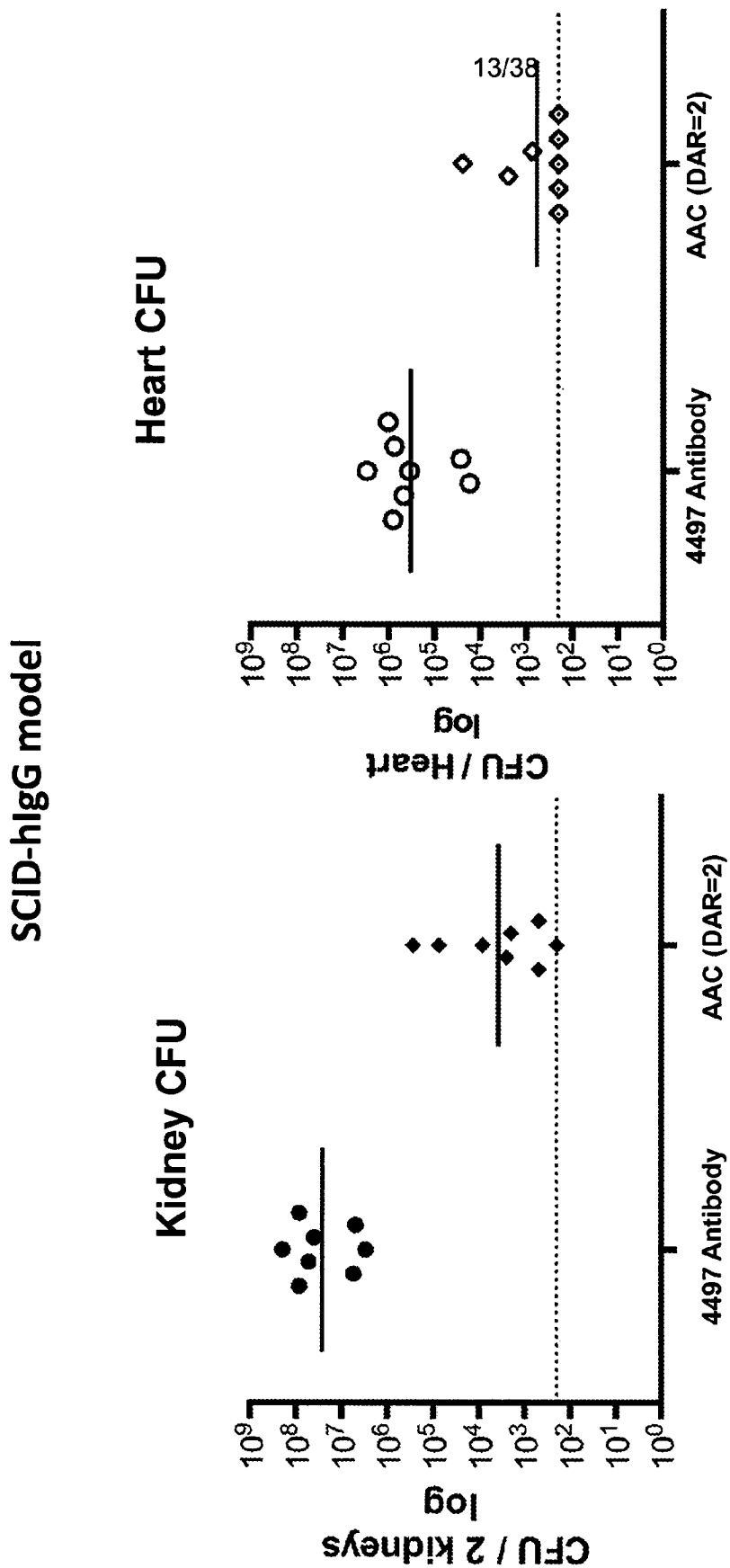


FIG. 10B

FIG. 10C

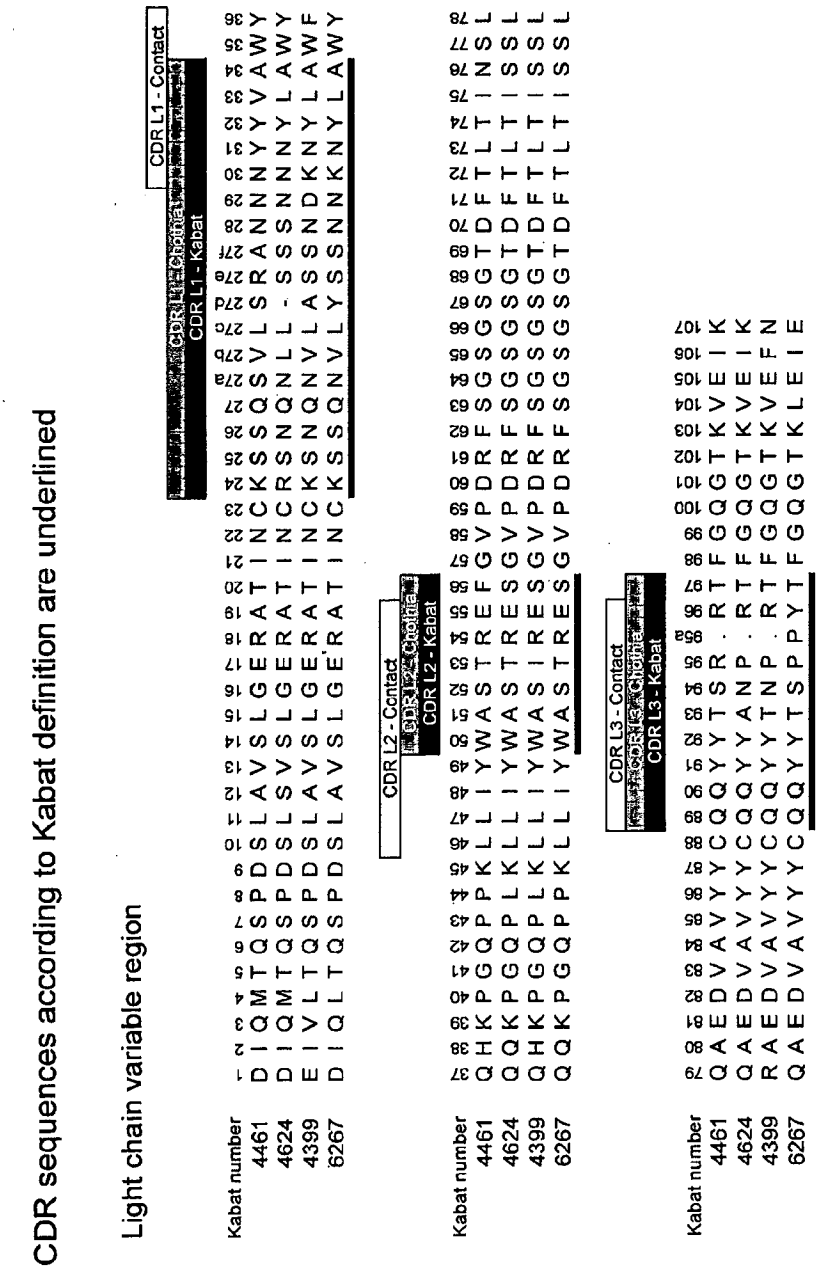

CDR sequences according to Kabat definition are underlined
Light chain variable region
CDR L1 - Contact
CDR L1 - Kabat
CDR L2 - Contact
CDR L2 - Kabat
CDR L3 - Contact
CDR L3 - Kabat
Kabat number
4461
4624
4399
6267

FIG. 11A

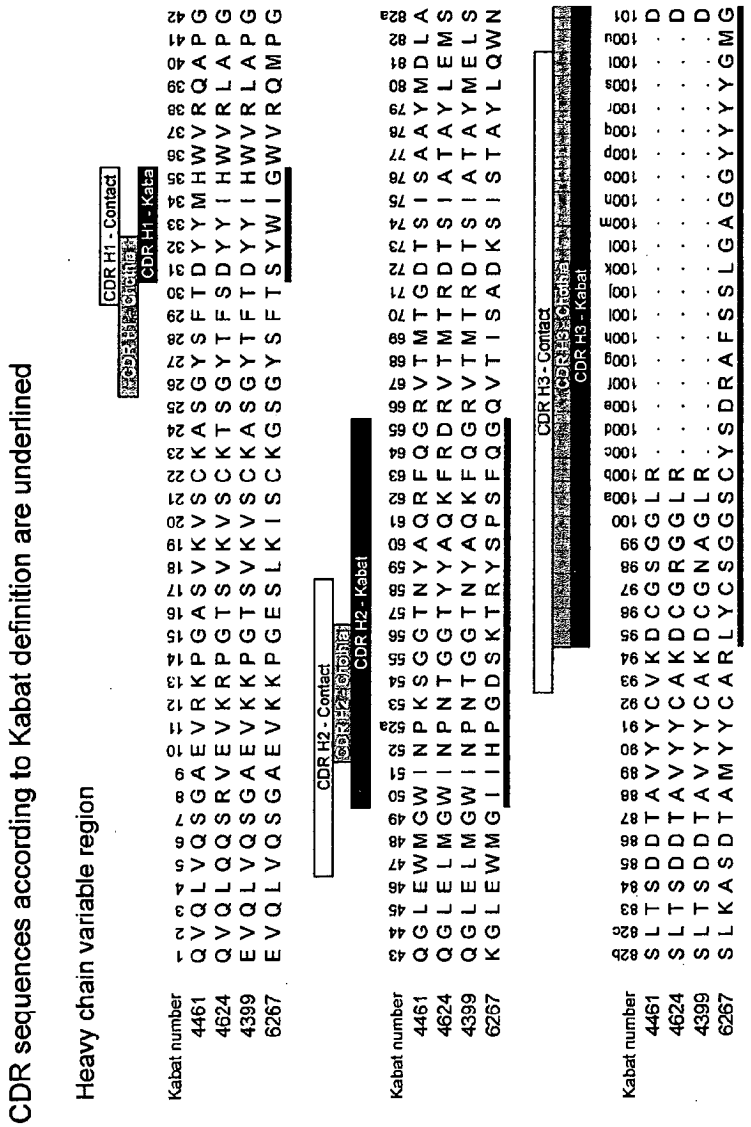

CDR sequences according to Kabat definition are underlined
Heavy chain variable region
CDR H1 - Contact
CDR H1 - Kabat
CDR H2 - Contact
CDR H2 - Kabat
CDR H3 - Contact
CDR H3 - Kabat
Kabat number
4461
4624
4399
6267

FIG. 11B

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)	WMNANSGNTGYAQKFQG (SEQ ID NO:37)	SSILVRGALGRYFDL (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)	WMNANSGNTGYAQKFQG (SEQ ID NO:43)	SSILVRGALGRYFDL (SEQ ID NO:44)
4450	RASQFVSRTSLA (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)	WMNPNSGNTNYAQRFQG (SEQ ID NO:55)	ERWSKDTGHYYYY GMDV (SEQ ID NO:56)
6239	RASLDITNHLA (SEQ ID NO:57)	EASILQS (SEQ ID NO:58)	EKCNSTPRT (SEQ ID NO:59)	NYDIN (SEQ ID NO:60)	WMNPSSGRTGYAPKFRG (SEQ ID NO:61)	GGGYYDSSGNYHIS GLDV (SEQ ID NO:62)
6232	RASQSVGAIYLA (SEQ ID NO:63)	GVSNRAT (SEQ ID NO:64)	QLYTSSRALT (SEQ ID NO:65)	AYAMN (SEQ ID NO:66)	SITKNSDSLYYADSVKG (SEQ ID NO:67)	LAARIMATDY (SEQ ID NO:68)
6259	RASQGIRNGLG (SEQ ID NO:69)	PASTLES (SEQ ID NO:70)	LQDHNYPPT (SEQ ID NO:71)	YYSMI (SEQ ID NO:72)	SIDSSSRYLYYADSVKG (SEQ ID NO:73)	DGDDILSVYRGSGR PFDY (SEQ ID NO:74)
6292	RASQGIRNGLG (SEQ ID NO:75)	PASTLES (SEQ ID NO:76)	LQDHNYPPS (SEQ ID NO:77)	YYSMI (SEQ ID NO:78)	SIDSSSRYRYYTDSVKG (SEQ ID NO:79)	DGDDILSVYQGSGR PFDY (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)	TIIGIDDTTHYADSVRG (SEQ ID NO:85)	NSGIYSF (SEQ ID NO:86)
6265	RASQDIGSSLA (SEQ ID NO:87)	ATSTLQS (SEQ ID NO:88)	QQLNNYVHS (SEQ ID NO:89)	DYAMG (SEQ ID NO:90)	VVTGHSYRTHYADSVKG (SEQ ID NO:91)	RIWSYGDDSFDV (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)	YISSIETIYYADSVKG (SEQ ID NO:97)	DRLVDVPLSSPNS (SEQ ID NO:98)
4497	KSSQSIFRTSRNKN LLN (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	RASQFTNHYLN (SEQ ID NO:105)	VASNLQS (SEQ ID NO:106)	QQSYRTPYT (SEQ ID NO:107)	SGYYN (SEQ ID NO:108)	YILSGAHTDIKASLGS (SEQ ID NO:109)	SGVYSKYSLDV (SEQ ID NO:110)

6263 has same CDR sequences as 6078.

FIG. 12

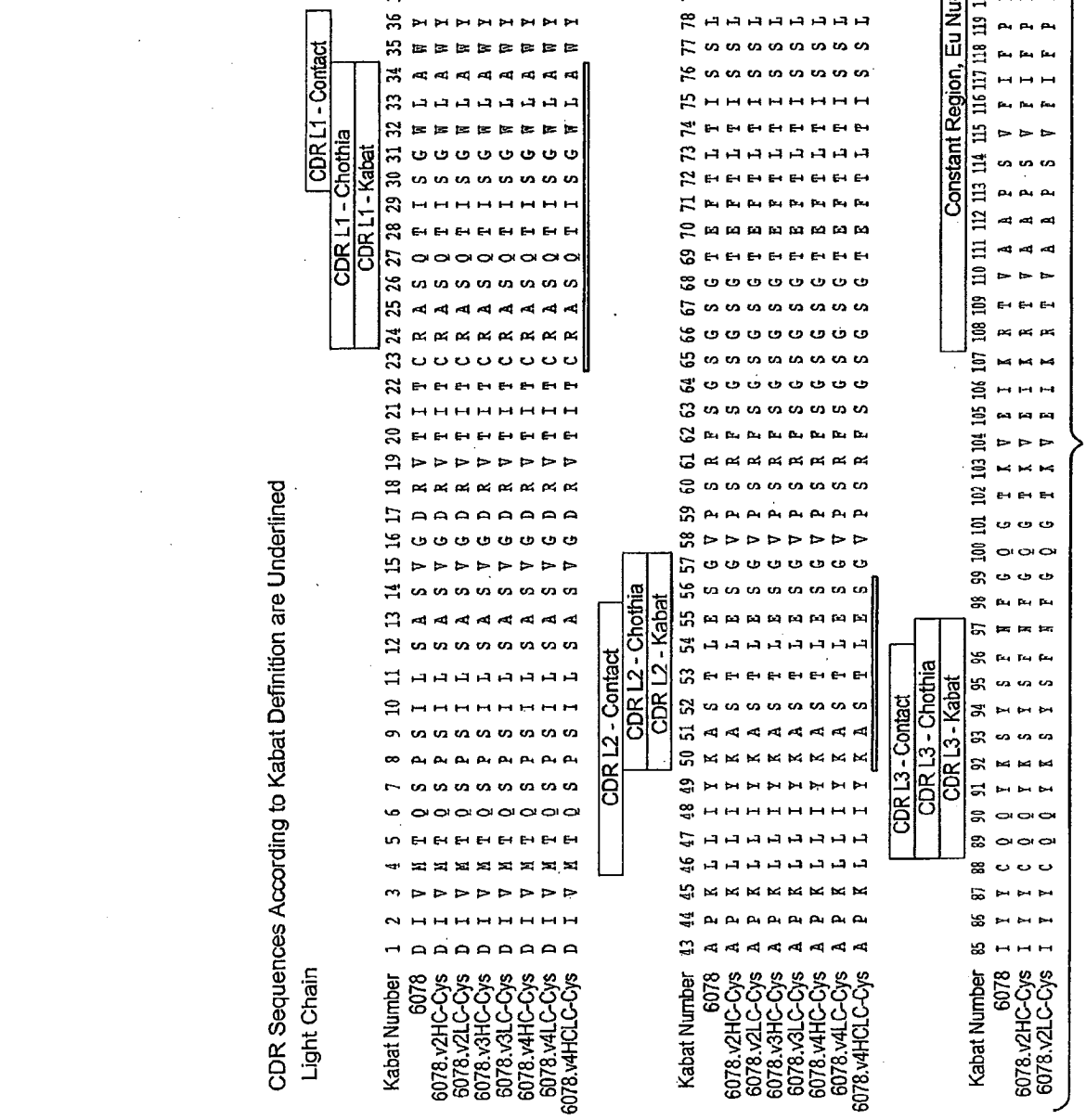

CDR Sequences According to Kabat Definition are Underlined
Light Chain
CDR L1 - Contact
CDR L1 - Chothia
CDR L1 - Kabat
CDR L2 - Contact
CDR L2 - Chothia
CDR L2 - Kabat
CDR L3 - Contact
CDR L3 - Chothia
CDR L3 - Kabat
Constant Region, Eu Number
Kabat Number
6078
6078.v2HC-Cys
6078.v2LC-Cys
6078.v3HC-Cys
6078.v3LC-Cys
6078.v4HC-Cys
6078.v4LC-Cys
6078.v4HCLC-Cys

FIG. 13A-1

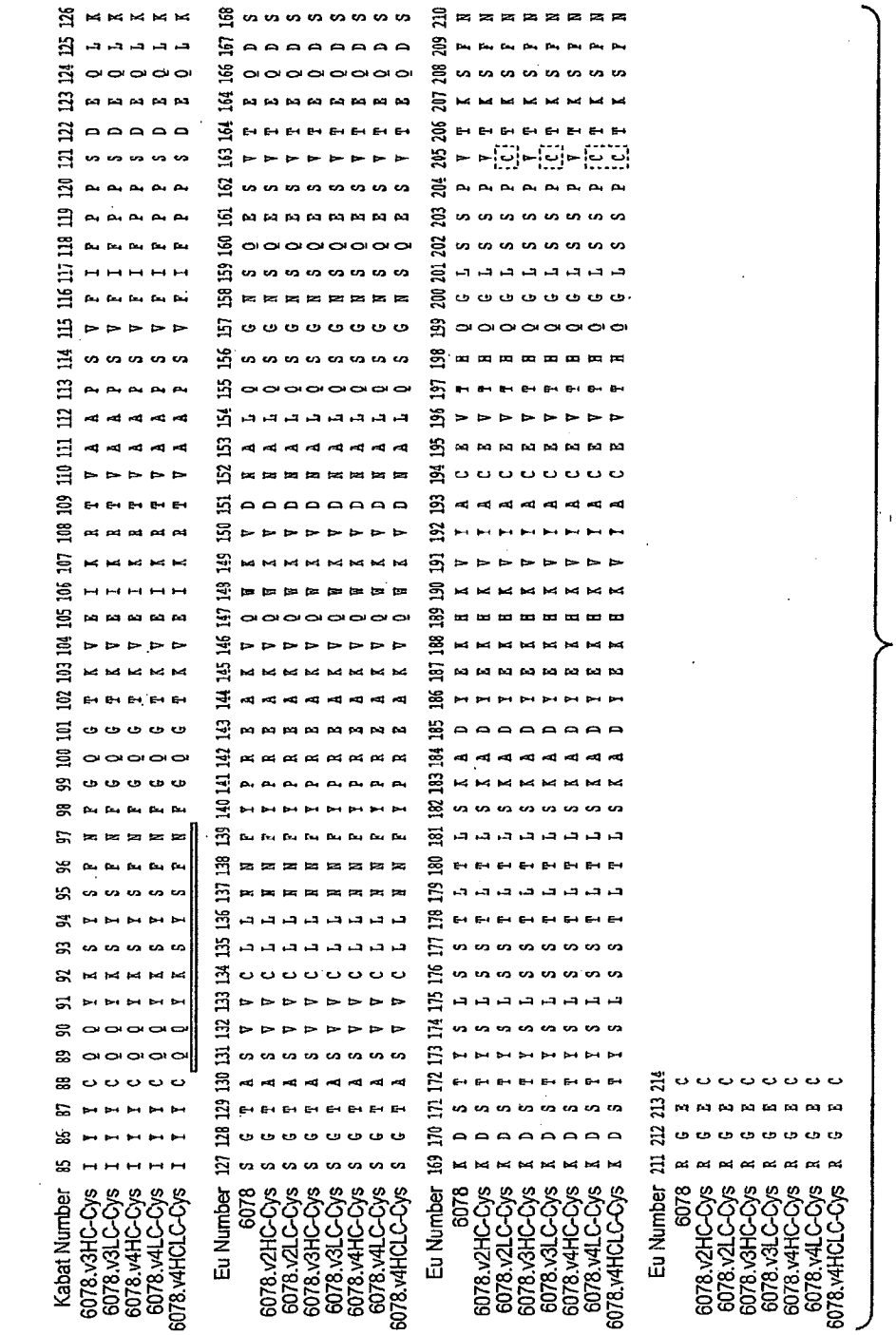


FIG. 13A-2

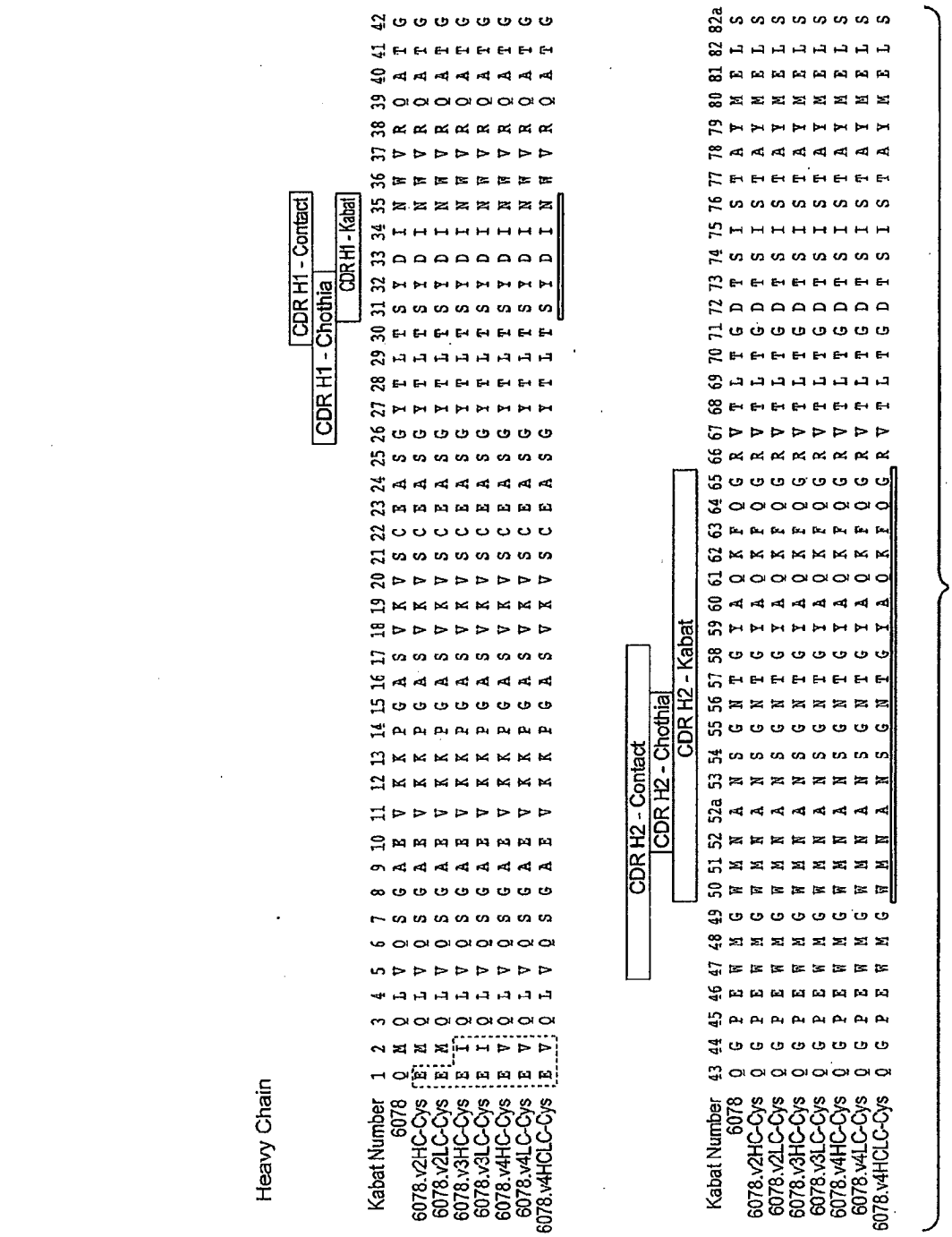

Heavy Chain
CDR H1 - Contact
CDR H1 - Chothia
CDR H1 - Kabat
CDR H2 - Contact
CDR H2 - Chothia
CDR H2 - Kabat
Kabat Number
6078
6078.v2HC-Cys
6078.v2LC-Cys
6078.v3HC-Cys
6078.v3LC-Cys
6078.v4HC-Cys
6078.v4LC-Cys
6078.v4HCLC-Cys

FIG. 13B-1

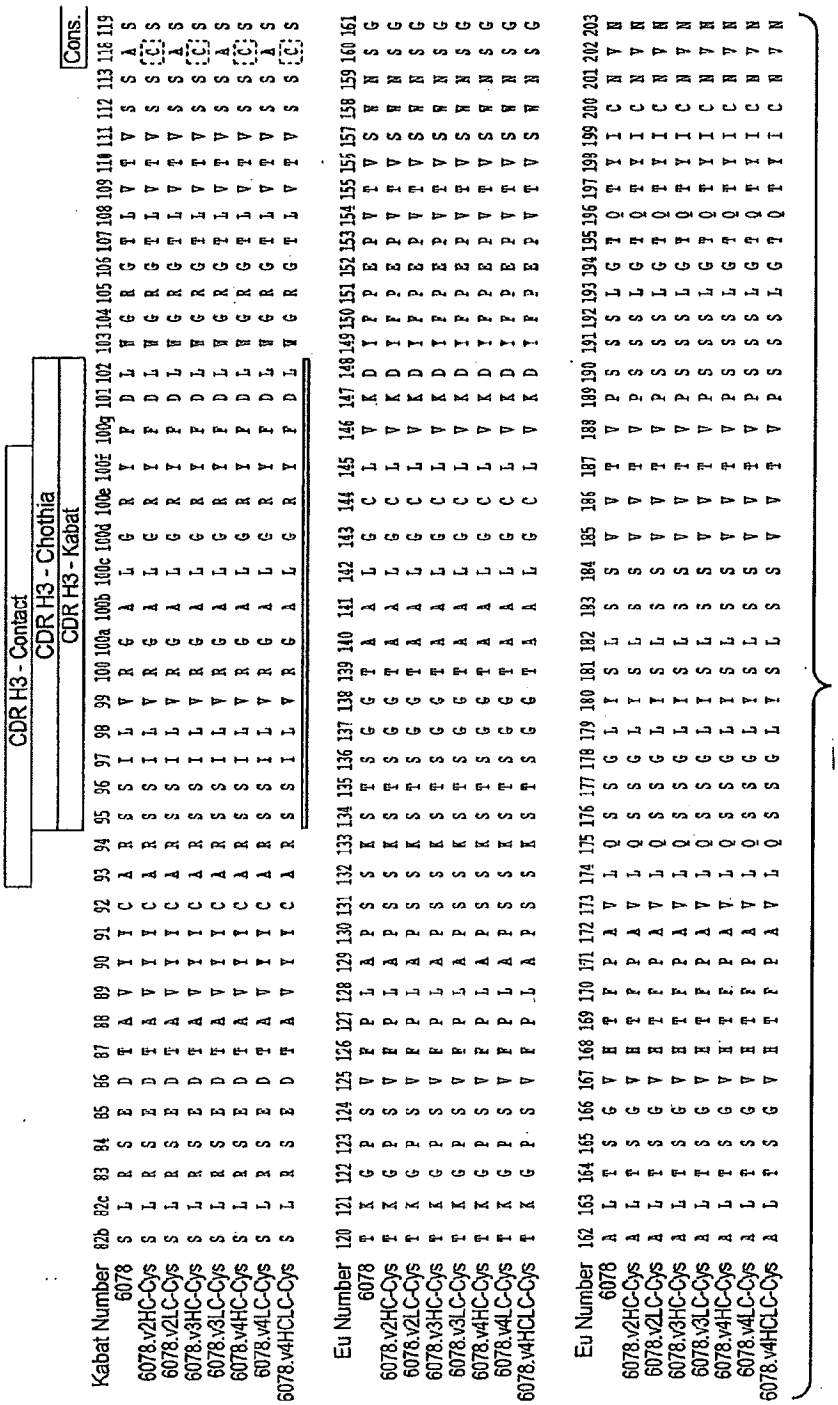

CDR H3 - Contact
CDR H3 - Chothia
CDR H3 - Kabat
Cons.
Kabat Number
Eu Number
6078
6078.v2HC-Cys
6078.v2LC-Cys
6078.v3HC-Cys
6078.v3LC-Cys
6078.v4HC-Cys
6078.v4LC-Cys
6078.v4HCLC-Cys

FIG. 13B-2

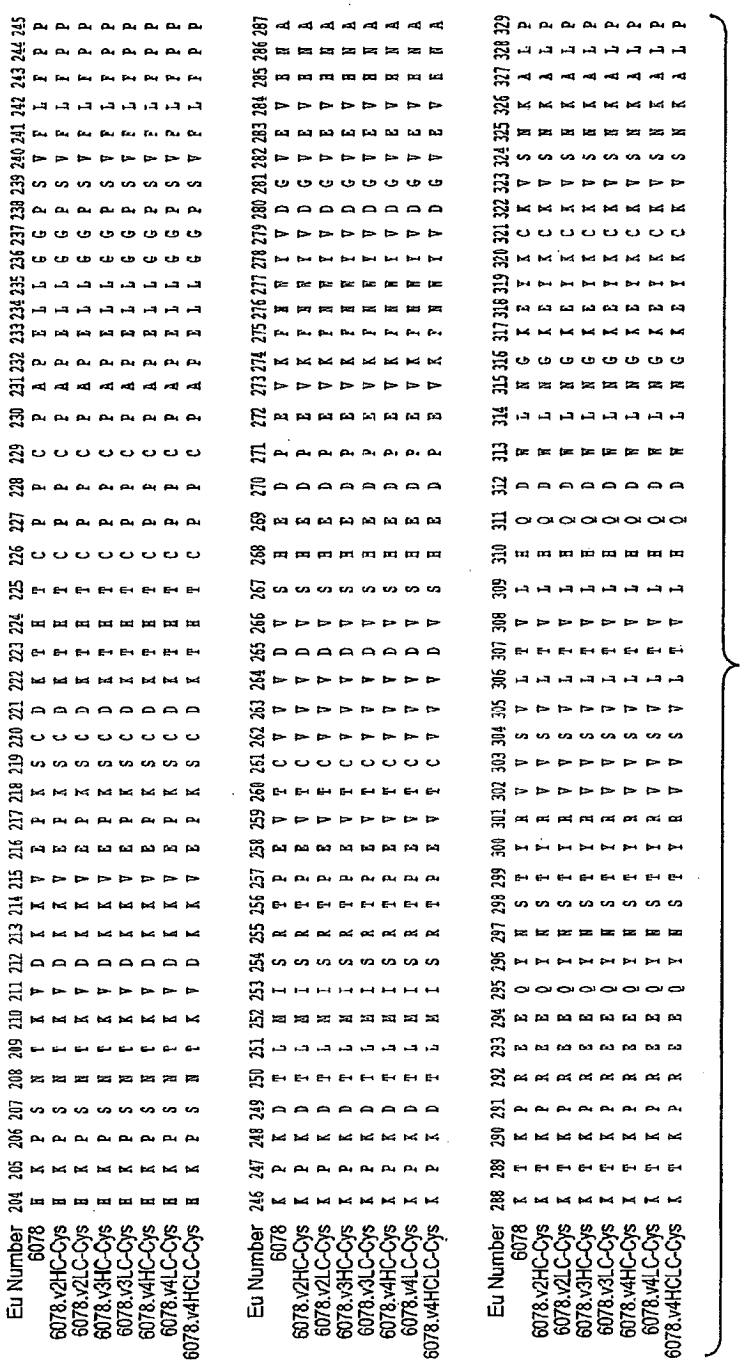


FIG. 13B-3

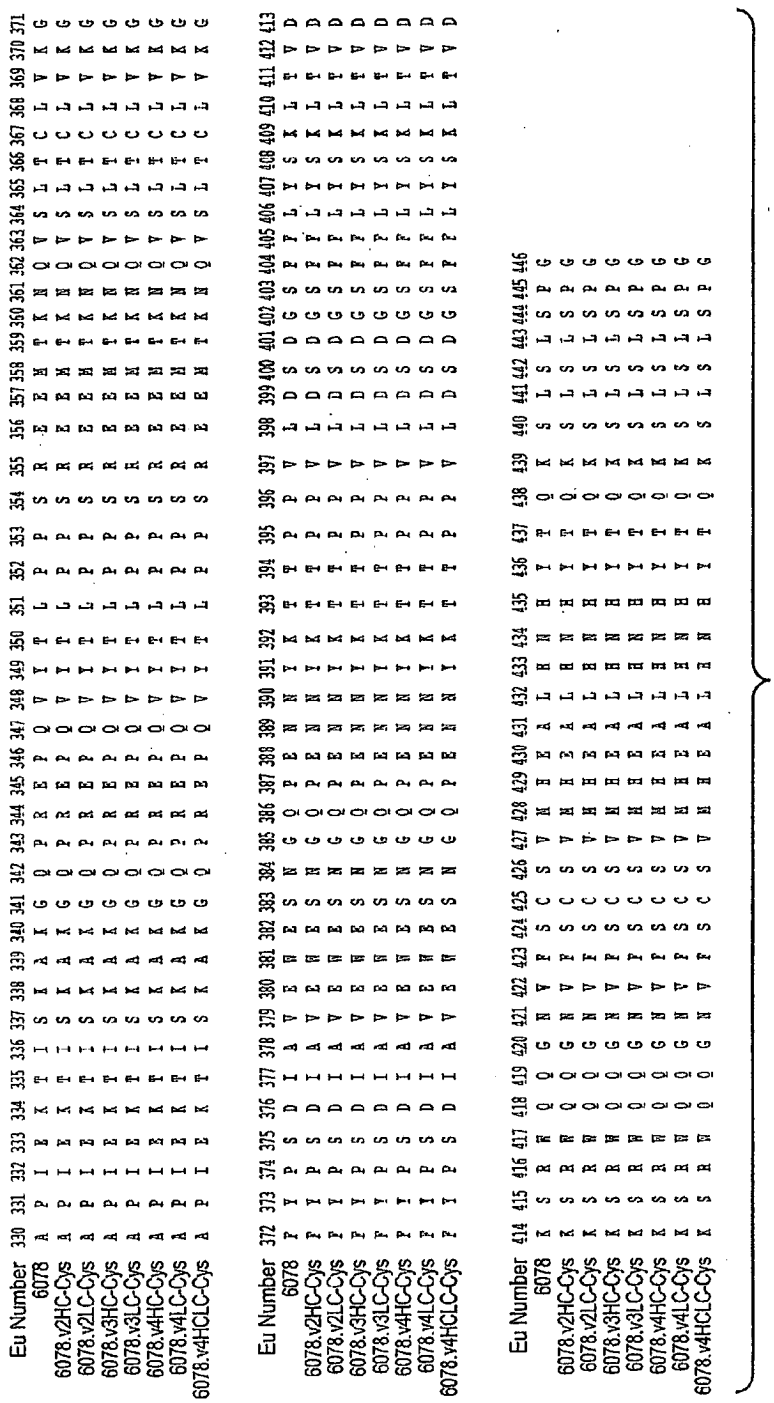

Eu Number 330 331 332 333 334 335 336 337 338 339 340 341 342 343 344 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360 361 362 363 364 365 366 367 368 369 370 371
6078 A P I E K T I S K A K G Q P R E P Q V Y T L P P S R E E M T K N Q V S L T C L V K G
6078.v2HC-Cys A P I E K T I S K A K G Q P R E P Q V Y T L P P S R E E 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 T L P P S R E E 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 T L P P S R E E 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 T L P P S R E E 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 T L P P S R E E 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 T L P P S R E E 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 T L P P S R E E 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 P E N N Y K T T P P V L D S D G S F F L Y S K L T V D
6078.v2HC-Cys F Y P S D I A V 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 K L T V D
6078.v2LC-Cys F Y P S D I A V 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 K L T V D
6078.v3HC-Cys F Y P S D I A V 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 K L T V D
6078.v3LC-Cys F Y P S D I A V 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 K L T V D
6078.v4HC-Cys F Y P S D I A V 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 K L T V D
6078.v4LC-Cys F Y P S D I A V 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 K L T V D
6078.v4HCLC-Cys F Y P S D I A V 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 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 K 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 K 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 K 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 K 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 K 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 K 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 K 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 K S L S L S P G

FIG. 13B-4

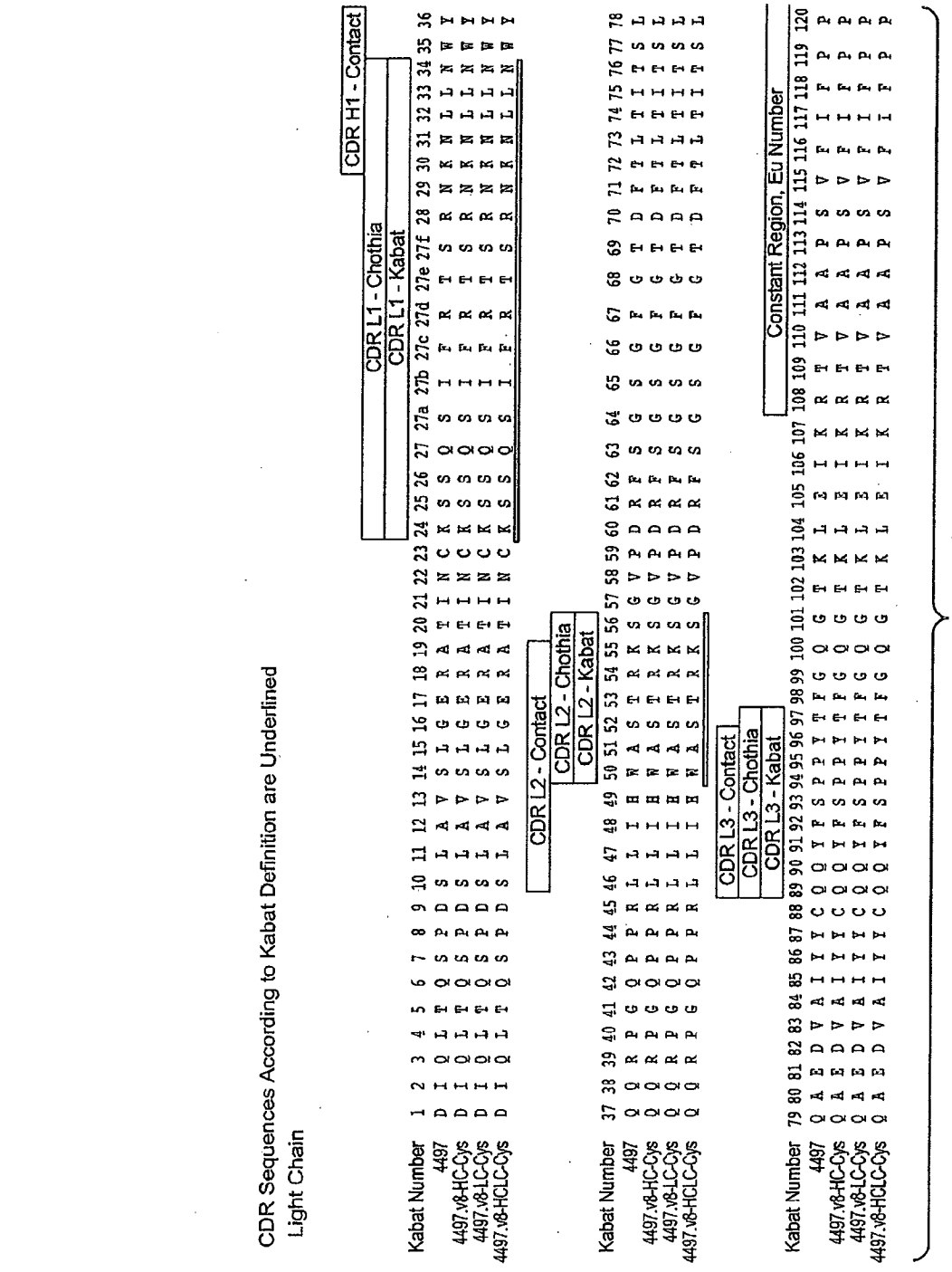

CDR Sequences According to Kabat Definition are Underlined
Light Chain
CDR H1 - Contact
CDR L1 - Chothia
CDR L1 - Kabat
CDR L2 - Contact
CDR L2 - Chothia
CDR L2 - Kabat
CDR L3 - Contact
CDR L3 - Chothia
CDR L3 - Kabat
Constant Region, Eu Number
Kabat Number
4497
4497.v8-HC-Cys
4497.v8-LC-Cys
4497.v8-HCLC-Cys

FIG. 14A-1

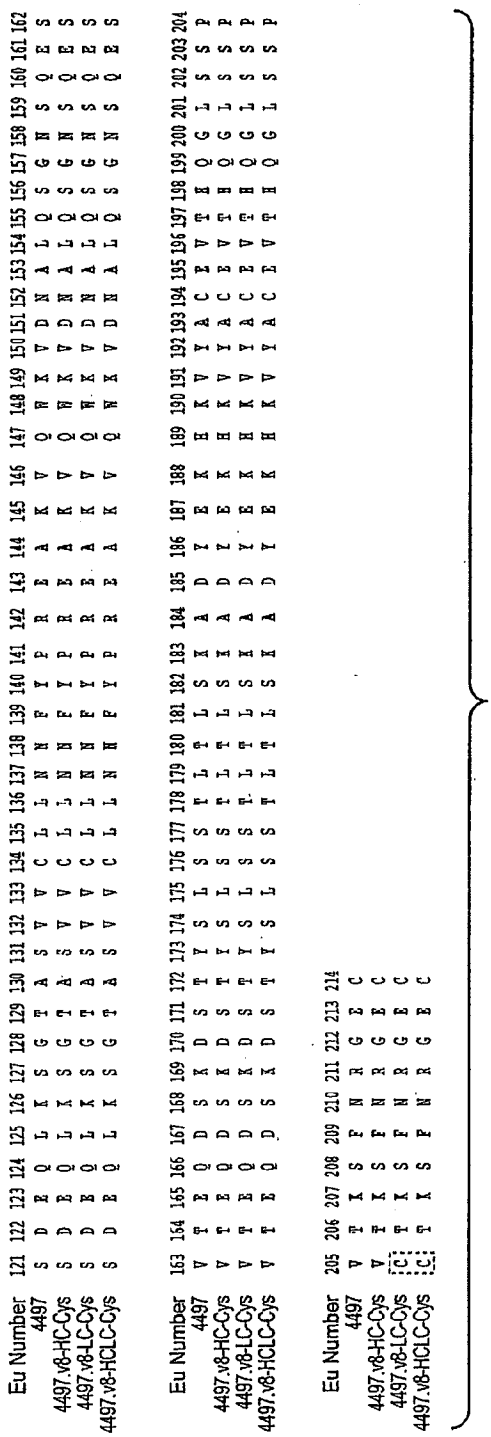

Eu Number 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150 151 152 153 154 155 156 157 158 159 160 161 162
4497 S D E Q L K S G T A S V V C L L N N F Y P R E A K V Q W K V D N A L Q S G N S Q E S
4497.v8-HC-Cys S D E Q L K S G T A S V V C L L N N F Y P R E A K V Q W K V D N A L Q S G N S Q E S
4497.v8-LC-Cys S D E Q L K S G T A S V V C L L N N F Y P R E A K V Q W K V D N A L Q S G N S Q E S
4497.v8-HCLC-Cys S D E Q L K S G T A S V V C L L N N F Y P R E A K V Q W K 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 D S K D S T Y S L S S T 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 P
4497.v8-HC-Cys V T E Q D S K D S T Y S L S S T 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 P
4497.v8-LC-Cys V T E Q D S K D S T Y S L S S T 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 P
4497.v8-HCLC-Cys V T E Q D S K D S T Y S L S S T 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 P
Eu Number 205 206 207 208 209 210 211 212 213 214
4497 V T K S F N R G E C
4497.v8-HC-Cys V T K S F N R G E C
4497.v8-LC-Cys [C:] T K S F N R G E C
4497.v8-HCLC-Cys [C:] T K S P N R G E C

FIG. 14A-2

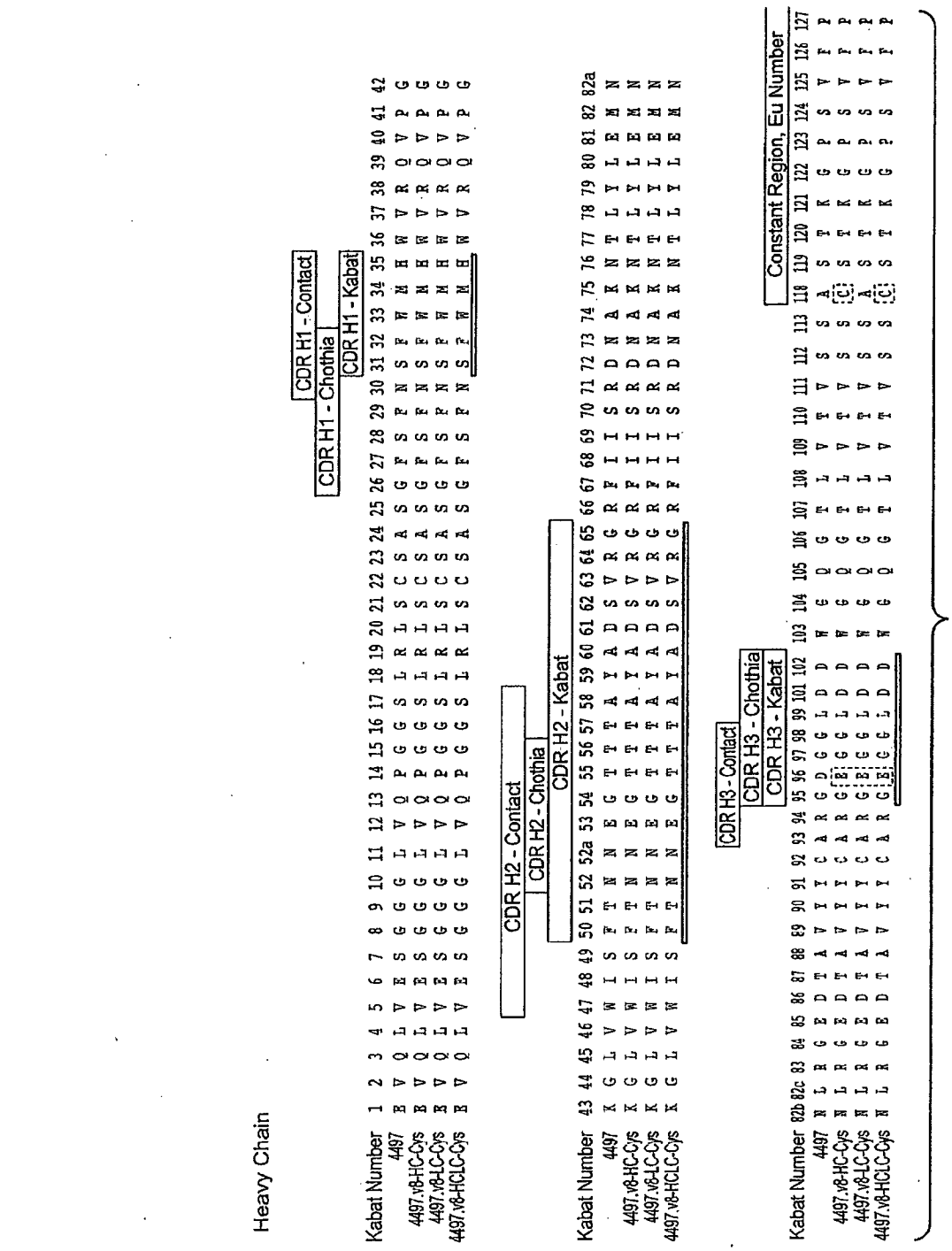

Heavy Chain
CDR H1 - Contact
CDR H1 - Chothia
CDR H1 - Kabat
CDR H2 - Contact
CDR H2 - Chothia
CDR H2 - Kabat
CDR H3 - Contact
CDR H3 - Chothia
CDR H3 - Kabat
Constant Region, Eu Number
Kabat Number
4497
4497.v8-HC-Cys
4497.v8-LC-Cys
4497.v8-HCLC-Cys

FIG. 14B-1

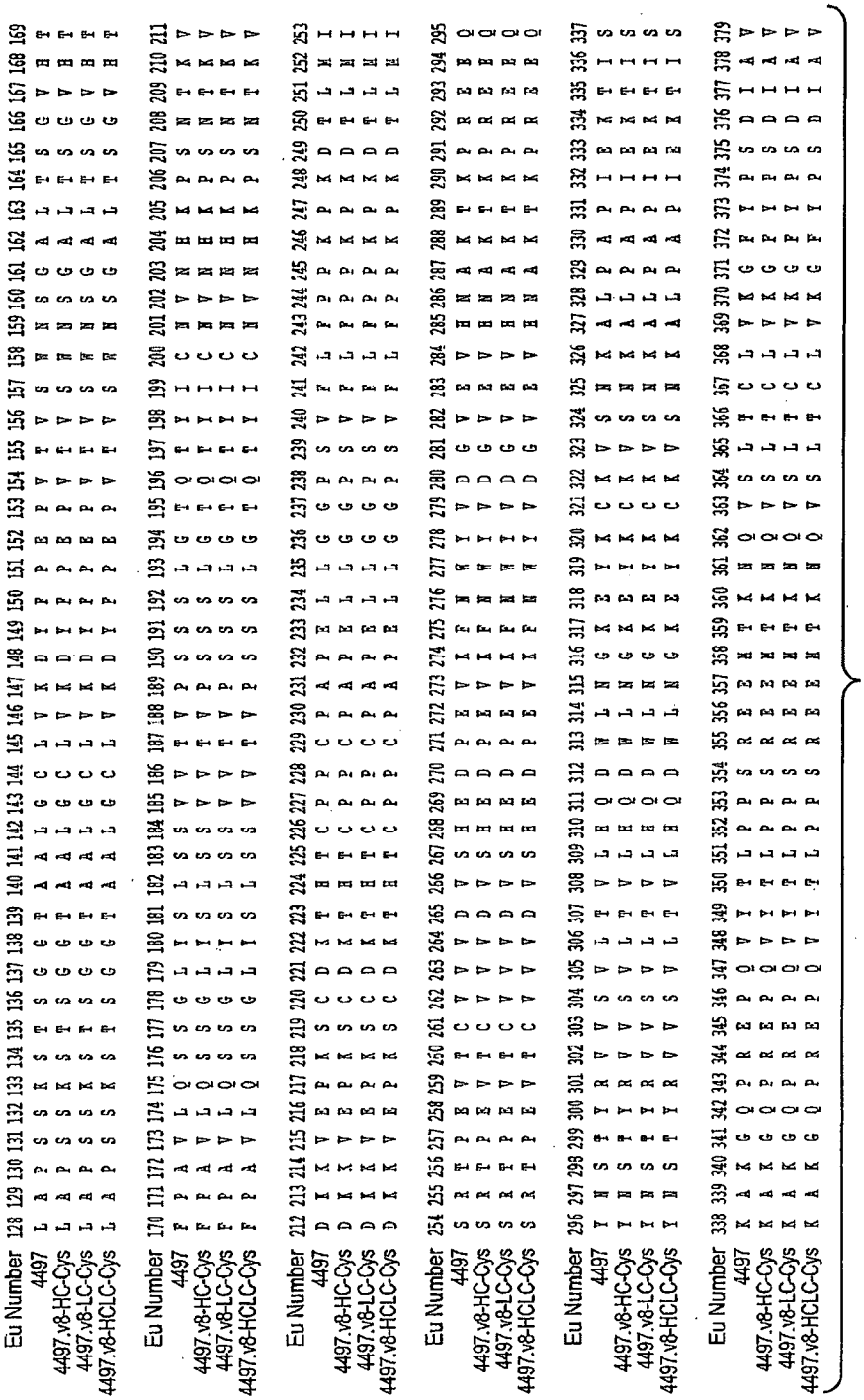


FIG. 14B-2

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	K	L	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	K	L	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	K	L	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	K	L	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	L	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	L	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	L	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	L	S	L	S	P	G

FIG. 14B-3

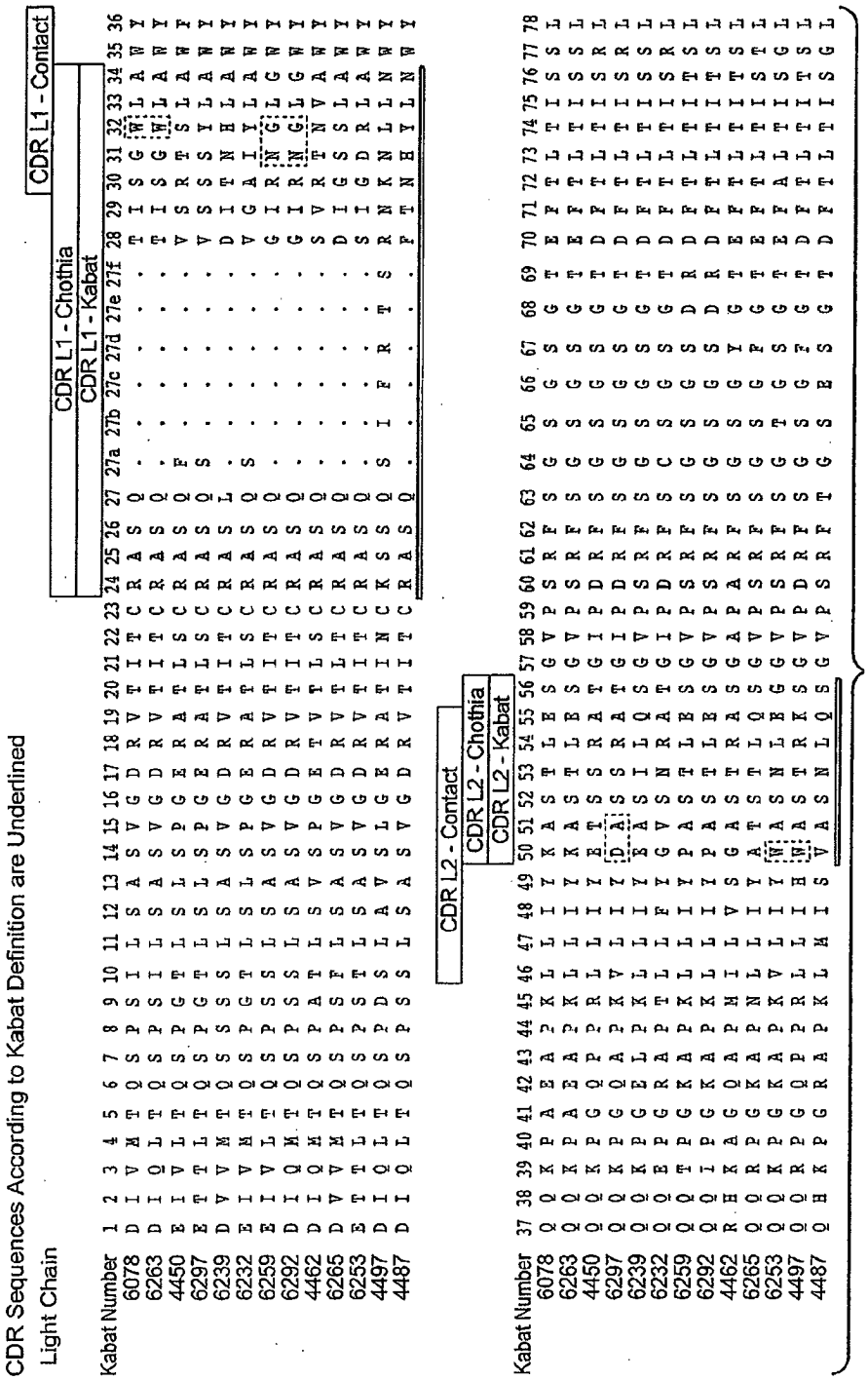

CDR Sequences According to Kabat Definition are Underlined
Light Chain
CDR L1 - Contact
CDR L1 - Chothia
CDR L1 - Kabat
CDR L2 - Contact
CDR L2 - Chothia
CDR L2 - Kabat
Kabat Number

FIG. 15A-1

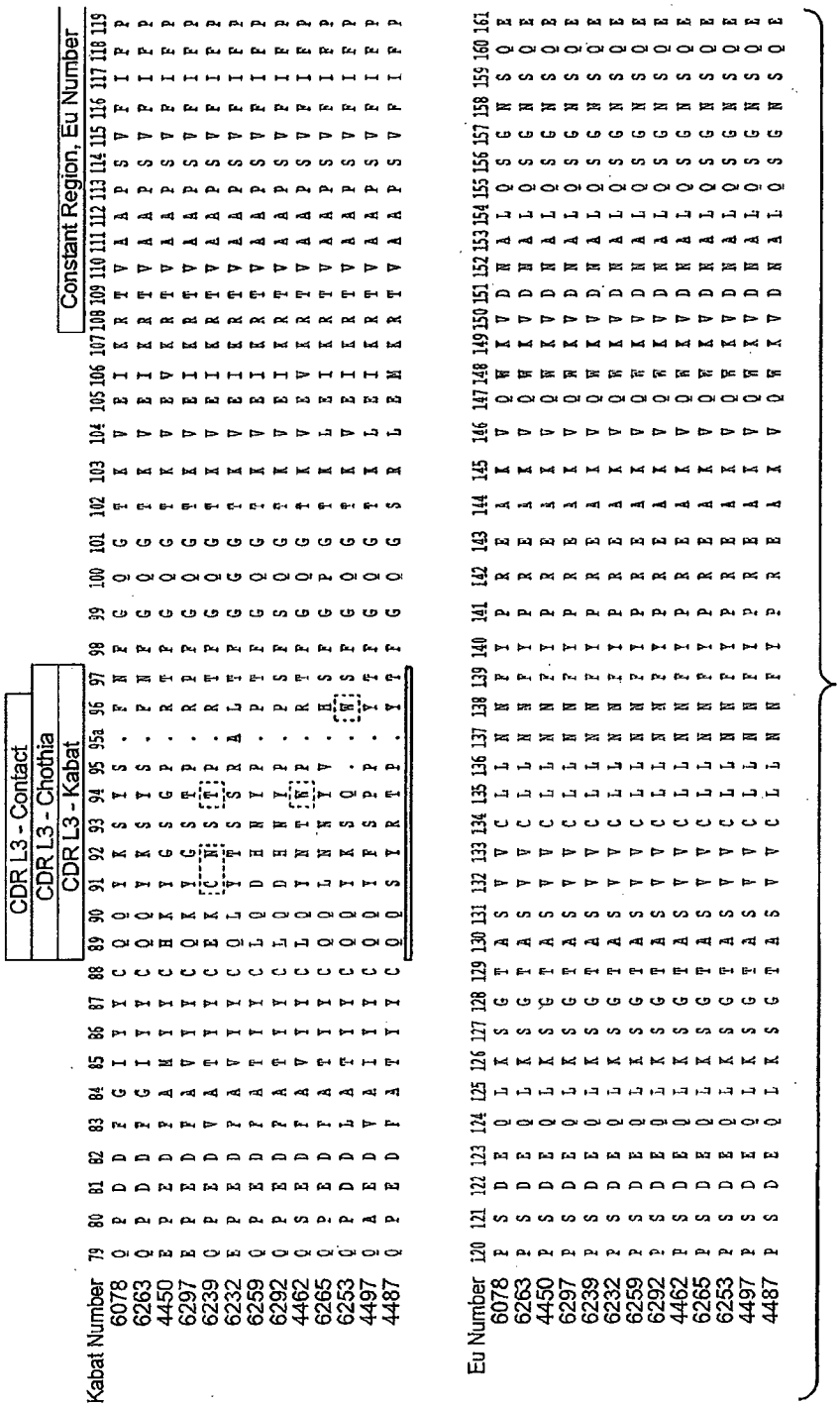
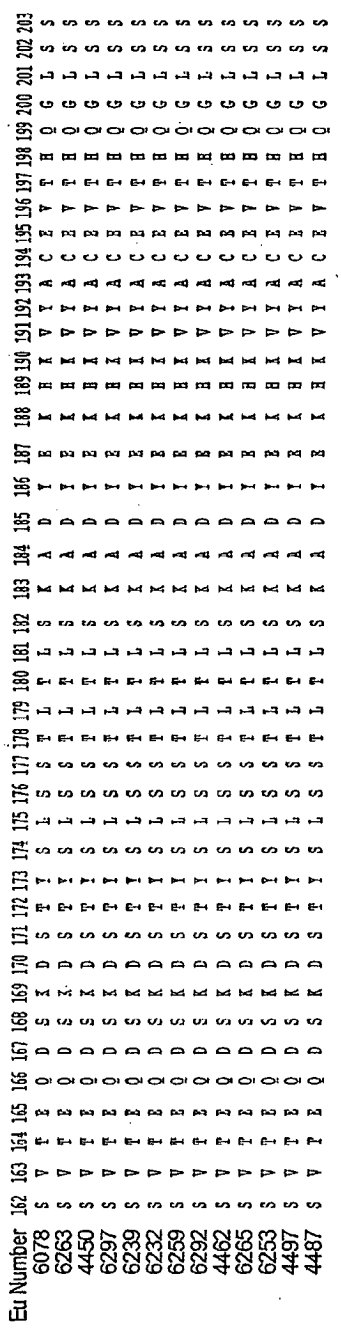

CDR L3 - Contact
CDR L3 - Chothia
CDR L3 - Kabat
Constant Region, Eu Number
Kabat Number
Eu Number

FIG. 15A-2



* Light chain Eu position 205 marked by asterisk can be changed to Cys for drug conjugation.

FIG. 15A-3

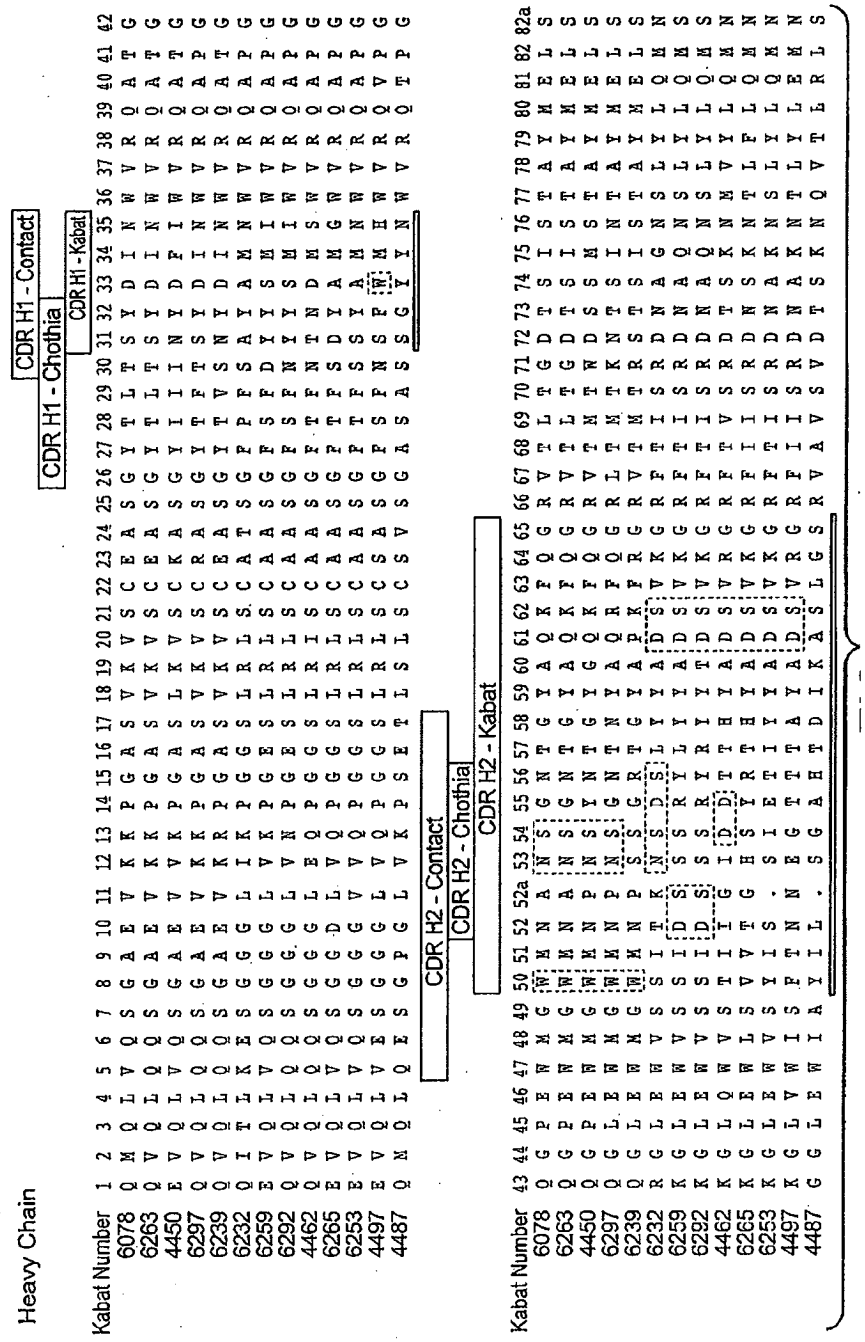

Heavy Chain
CDR H1 - Contact
CDR H1 - Chothia
CDR H1 - Kabat
CDR H2 - Contact
CDR H2 - Chothia
CDR H2 - Kabat
Kabat Number

FIG. 15B-1

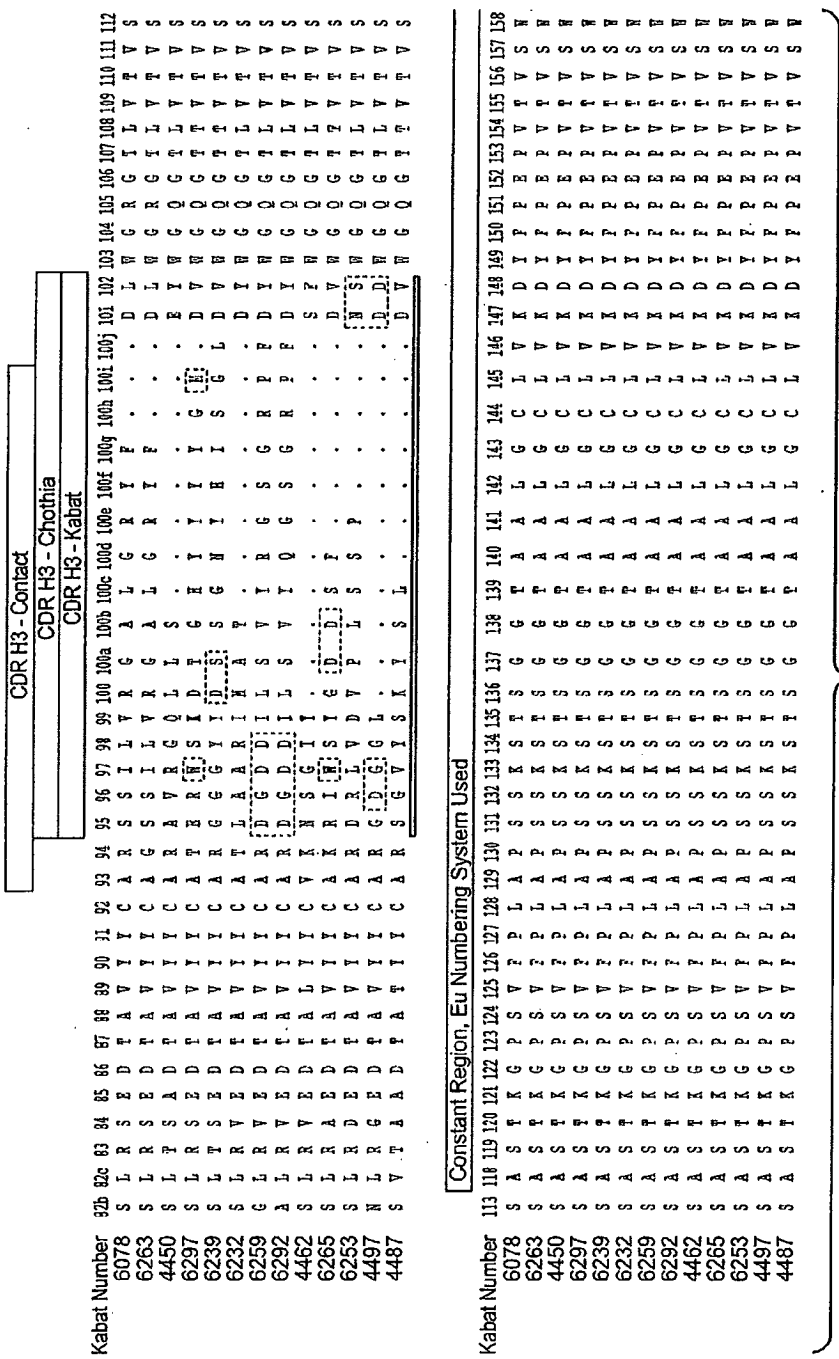

CDR H3 - Contact
CDR H3 - Chothia
CDR H3 - Kabat
Constant Region, Eu Numbering System Used
Kabat Number

FIG. 15B-2

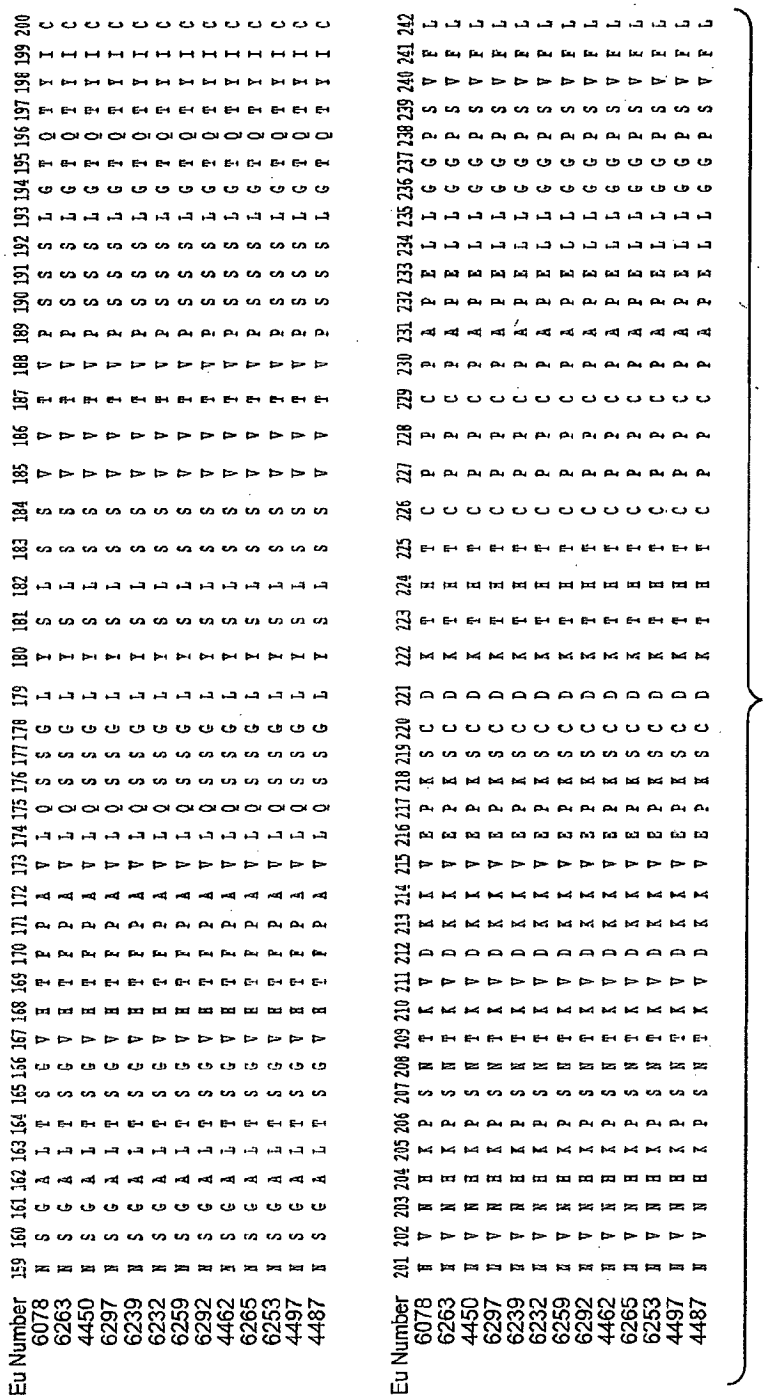

Eu Number
6078
6263
4450
6297
6239
6232
6259
6292
4462
6265
6253
4497
4487

FIG. 15B-3

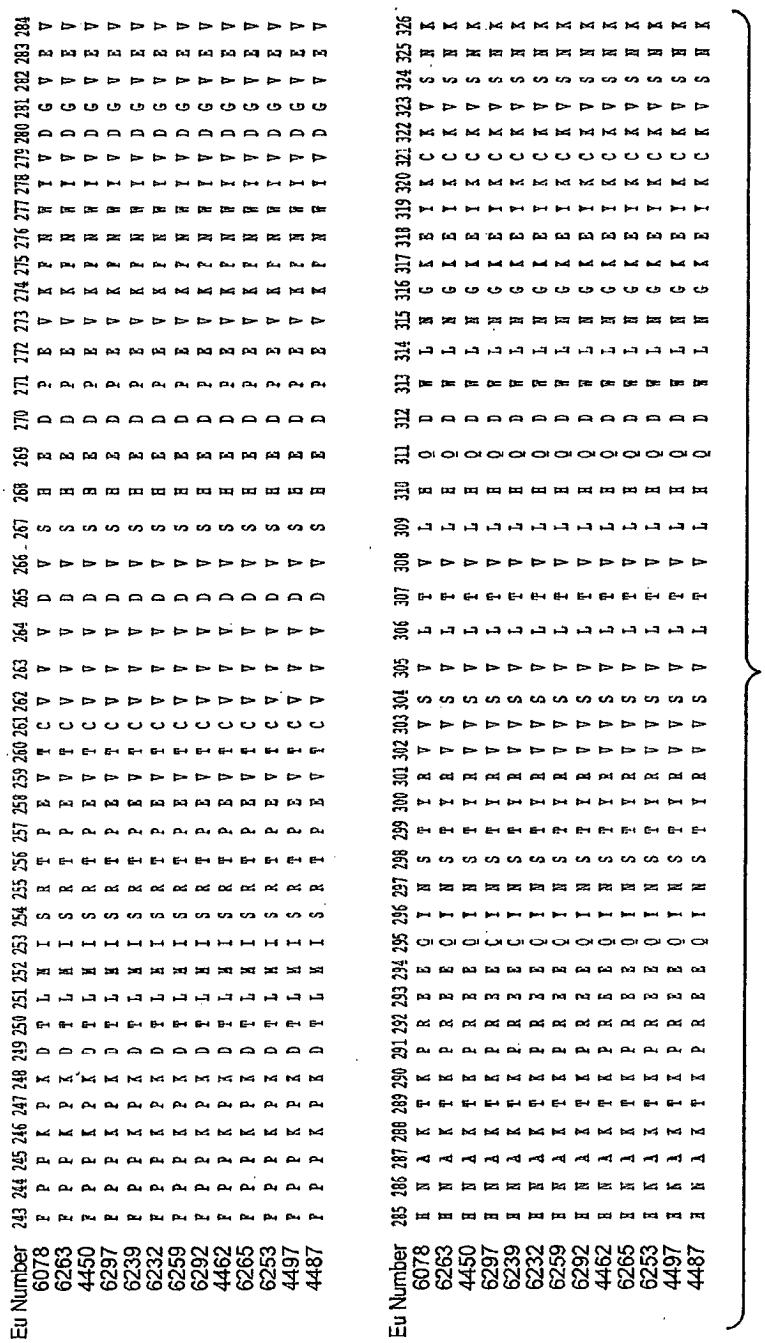


FIG. 15B-4

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	E	K	T	I	S	K	A	K	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	R	E	E	M	T	K	N	Q	V	S	L	T	C	L
6263	A	L	P	A	P	I	E	K	T	I	S	K	A	K	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	R	E	E	M	T	K	N	Q	V	S	L	T	C	L
4450	A	L	P	A	P	I	E	K	T	I	S	K	A	K	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	R	E	E	M	T	K	N	Q	V	S	L	T	C	L
6297	A	L	P	A	P	I	E	K	T	I	S	K	A	K	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	R	E	E	M	T	K	N	Q	V	S	L	T	C	L
6239	A	L	P	A	P	I	E	K	T	I	S	K	A	K	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	R	E	E	M	T	K	N	Q	V	S	L	T	C	L
6232	A	L	P	A	P	I	E	K	T	I	S	K	A	K	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	R	E	E	M	T	K	N	Q	V	S	L	T	C	L
6259	A	L	P	A	P	I	E	K	T	I	S	K	A	K	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	R	E	E	M	T	K	N	Q	V	S	L	T	C	L
6292	A	L	P	A	P	I	E	K	T	I	S	K	A	K	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	R	E	E	M	T	K	N	Q	V	S	L	T	C	L
4462	A	L	P	A	P	I	E	K	T	I	S	K	A	K	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	R	E	E	M	T	K	N	Q	V	S	L	T	C	L
6265	A	L	P	A	P	I	E	K	T	I	S	K	A	K	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	R	E	E	M	T	K	N	Q	V	S	L	T	C	L
6253	A	L	P	A	P	I	E	K	T	I	S	K	A	K	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	R	E	E	M	T	K	N	Q	V	S	L	T	C	L
4497	A	L	P	A	P	I	E	K	T	I	S	K	A	K	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	R	E	E	M	T	K	N	Q	V	S	L	T	C	L
4487	A	L	P	A	P	I	E	K	T	I	S	K	A	K	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	R	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	K	T	T	P	P	V	L	D	S	D	G	S	F	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	K	T	T	P	P	V	L	D	S	D	G	S	F	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	K	T	T	P	P	V	L	D	S	D	G	S	F	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	K	T	T	P	P	V	L	D	S	D	G	S	F	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	K	T	T	P	P	V	L	D	S	D	G	S	F	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	K	T	T	P	P	V	L	D	S	D	G	S	F	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	K	T	T	P	P	V	L	D	S	D	G	S	F	F	L	Y	S	K	L
6292	V	K	G	F	Y	P	S	D	I	A	V	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	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	K	T	T	P	P	V	L	D	S	D	G	S	F	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	K	T	T	P	P	V	L	D	S	D	G	S	F	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	K	T	T	P	P	V	L	D	S	D	G	S	F	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	K	T	T	P	P	V	L	D	S	D	G	S	F	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	K	T	T	P	P	V	L	D	S	D	G	S	F	F	L	Y	S	K	L

FIG. 15B-5

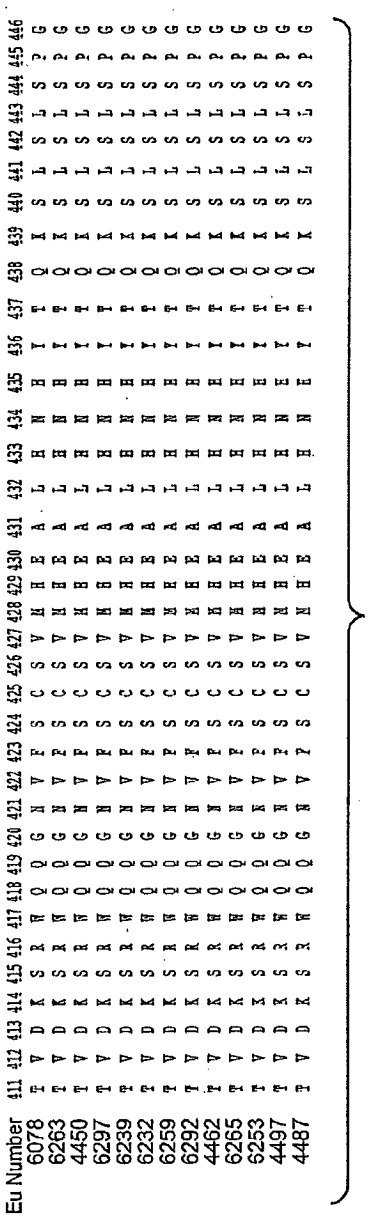

Eu Number 411 412 413 414 415 416 417 418 419 420 421 422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445 446
6078 E V D K S R W Q Q G N V F S C S V W H E A L H N H Y F Q M S L S P G
6263 E V D K S R W Q Q G N V F S C S V W H E A L H N H Y F Q M S L S P G
4450 E V D K S R W Q Q G N V F S C S V W H E A L H N H Y F Q M S L S P G
6297 E V D K S R W Q Q G N V F S C S V W H E A L H N H Y F Q M S L S P G
6239 E V D K S R W Q Q G N V F S C S V W H E A L H N H Y F Q M S L S P G
6232 E V D K S R W Q Q G N V F S C S V W H E A L H N H Y F Q M S L S P G
6259 E V D K S R W Q Q G N V F S C S V W H E A L H N H Y F Q M S L S P G
6292 E V D K S R W Q Q G N V F S C S V W H E A L H N H Y F Q M S L S P G
4462 E V D K S R W Q Q G N V F S C S V W H E A L H N H Y F Q M S L S P G
6265 E V D K S R W Q Q G N V F S C S V W H E A L H N H Y F Q M S L S P G
6253 E V D K S R W Q Q G N V F S C S V W H E A L H N H Y F Q M S L S P G
4497 E V D K S R W Q Q G N V F S C S V W H E A L H N H Y F Q M S L S P G
4487 E V D K S R W Q Q G N V F S C S V W H E A L H N H Y F Q M S L S P G

FIG. 15B-6

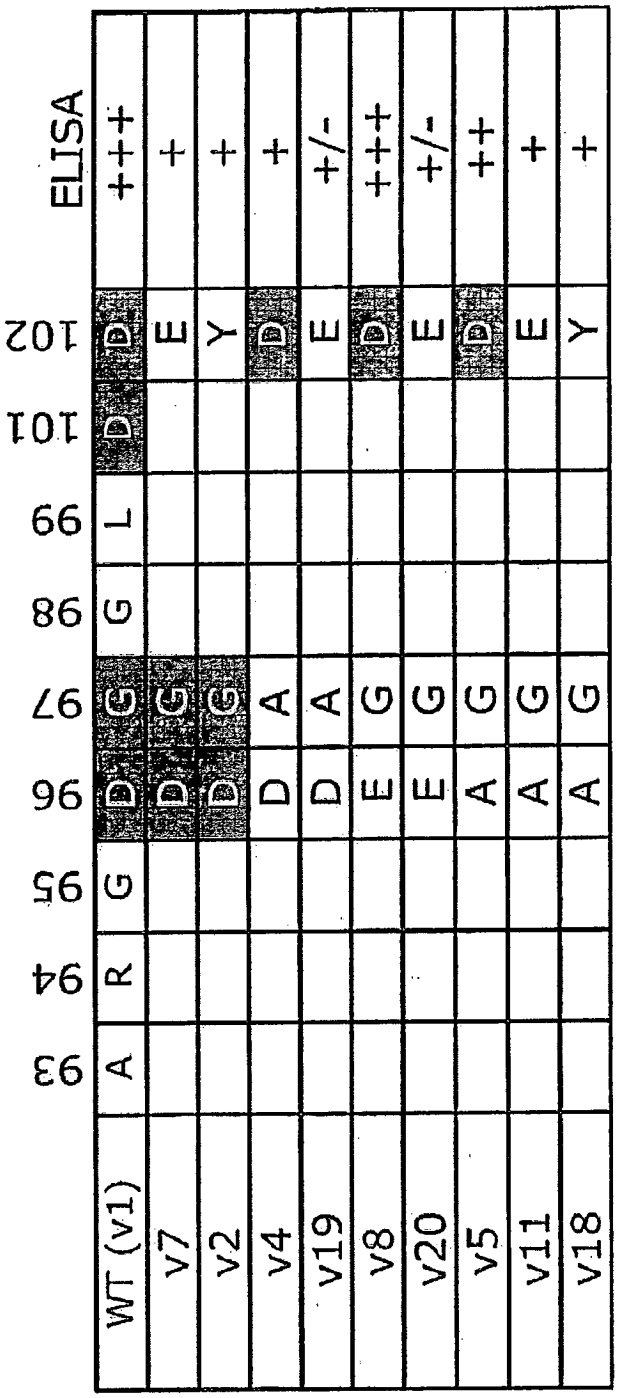

93 94 95 96 97 98 99 101 102 ELISA
A R G D G G L D E +++
A R G D G G L E Y +
A R G D G G L D E +
A R G D A G L D E +
A R G D A G L E E +/-
A R G E G G L D E +++
A R G E G G L E E +/-
A R G A G G L D E ++
A R G A G G L E E +
A R G A G G L E Y +
WT (v1)
v7
v2
v4
v19
v8
v20
v5
v11
v18

FIG. 16

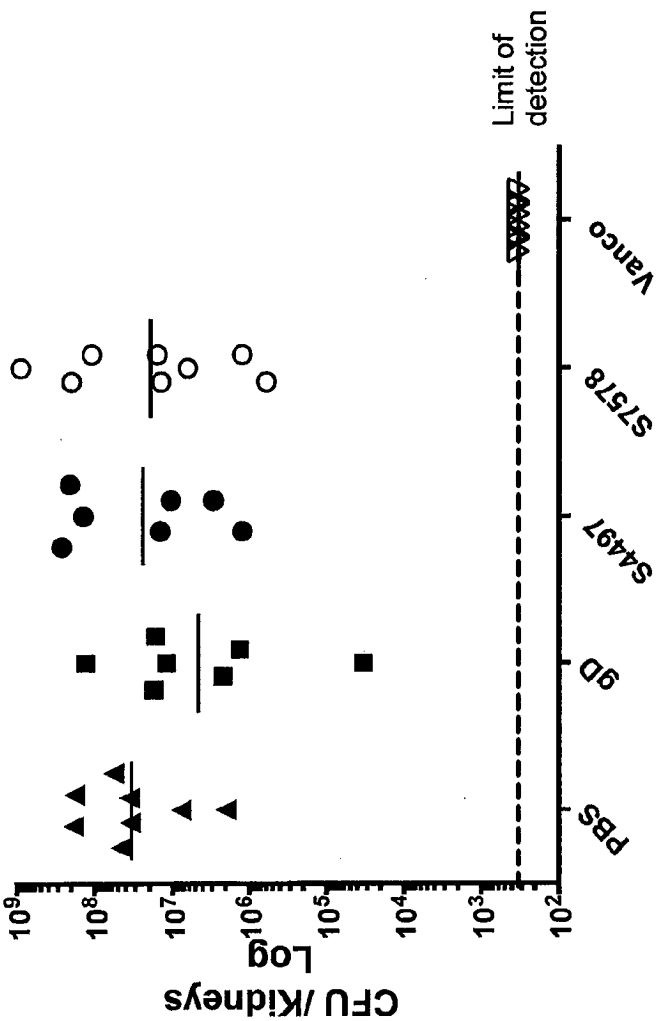

CFU /Kidneys
Log
10^9
10^8
10^7
10^6
10^5
10^4
10^3
10^2
Limit of detection
PBS
9D
SA97
S1578
Vanco

FIG. 17

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/063510

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K47/48
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	WO 2011/008092 A2 (AIMM THERAPEUTICS BV; GENENTECH INC) 20 January 2011 (2011-01-20) pages 19-20; claims 1-7,14-16,25; examples 3,5; sequences 1-10,27-30 pages 2-11	1,22-46, 52-54 1-13, 15-21, 47-51
X A	WO 2014/080251 A1 (HANGZHOU DAC BIOTECH CO LTD) 30 May 2014 (2014-05-30) pages 35-36; claims 14; 11, 12; figures 18,19 pages 43-44	12,13, 15,47,48 1-3,14, 16-20, 22, 40-42, 44,45, 52,54

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 February 2016	03/03/2016

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Kanbier, Titia

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Y	WO 2005/082023 A2 (GENENTECH INC) 9 September 2005 (2005-09-09) pages 14-15; claims 5,6,12,19	1-13, 15-21, 47-51
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A61P 31/04(2006.01)

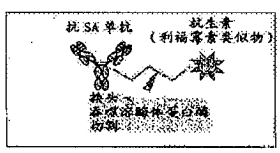
权利要求书13页 说明书83页
序列表76页 附图37页

(54)发明名称

抗金黄色葡萄球菌抗体利福霉素缀合物及其用途

(57)摘要

本发明提供抗金黄色葡萄球菌抗体利福霉素抗生素缀合物以及使用其的方法。



CN 107206102 A

1.一种抗体-抗生素缀合化合物，其包含抗壁磷壁酸(WTA)抗体，所述抗体通过蛋白酶可切割的非肽类接头共价连接利福霉素型抗生素。

2.权利要求1的抗体-抗生素缀合化合物，其具有下式：

Ab-(PML-abx)$_p$

其中：

Ab是抗壁磷壁酸抗体；

PML是具有下式的蛋白酶可切割的非肽类接头：

-Str-PM-Y-

其中Str是延伸单元；PM是拟肽单元，和Y是间隔单元；

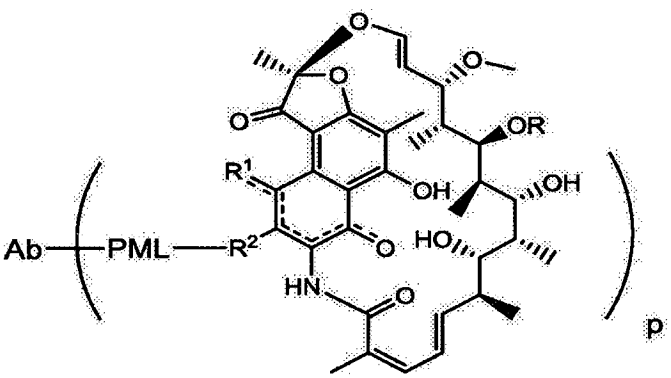
abx是利福霉素型抗生素；并且

p是从1到8的整数。

3.权利要求2的抗体-抗生素缀合化合物，其中利福霉素型抗生素是利福拉齐型抗生素。

4.权利要求2的抗体-抗生素缀合化合物，其中利福霉素型抗生素包含与蛋白酶可切割的非肽类接头连接的季胺。

5.权利要求2的抗体-抗生素缀合化合物，其具有式I：



I

其中：

虚线表示任选的键；

R是H、C_1-C_{12}烷基或C(O)CH_3；

R^1是OH；

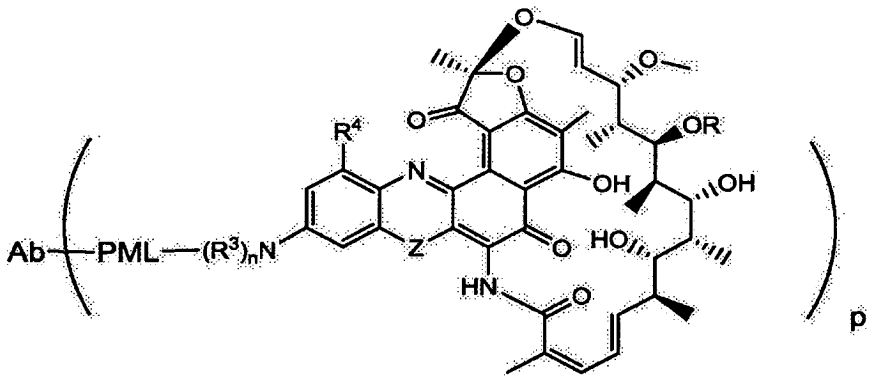
R^2是CH=N-(杂环基)，其中所述杂环基任选地被一个或多个独立地选自C(O)CH_3、C_1-C_{12}烷基、C_1-C_{12}杂芳基、C_2-C_{20}杂环基、C_6-C_{20}芳基和C_3-C_{12}碳环基的基团取代；

或R^1和R^2形成五元或六元稠合杂芳基或杂环基，并且任选地形成螺或稠合的六元杂芳基、杂环基、芳基或碳环基环，其中所述螺或稠合的六元杂芳基、杂环基、芳基或碳环基环任选地被H、F、Cl、Br、I、C_1-C_{12}烷基或OH取代；

PML是连接到R^2或由R^1和R^2形成的稠合杂芳基或杂环基的蛋白酶可切割的非肽类接头；并且

Ab是抗壁磷壁酸(WTA)抗体。

6.权利要求5的抗体-抗生素缀合化合物，其具有下式：



其中，

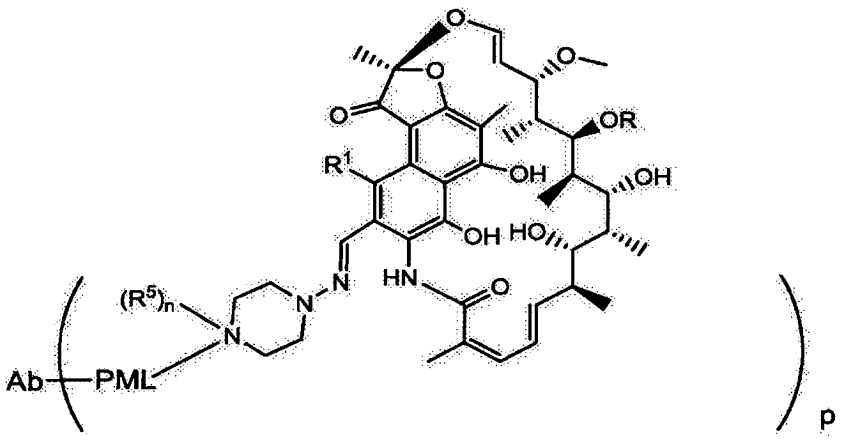
R^3独立地选自H和C_1-C_{12}烷基；

n为1或2；

R^4选自H、F、Cl、Br、I、C_1-C_{12}烷基和OH；并且

Z选自NH、N(C_1-C_{12}烷基)、O和S。

7.权利要求2的抗体-抗生素缀合化合物，其具有下式：

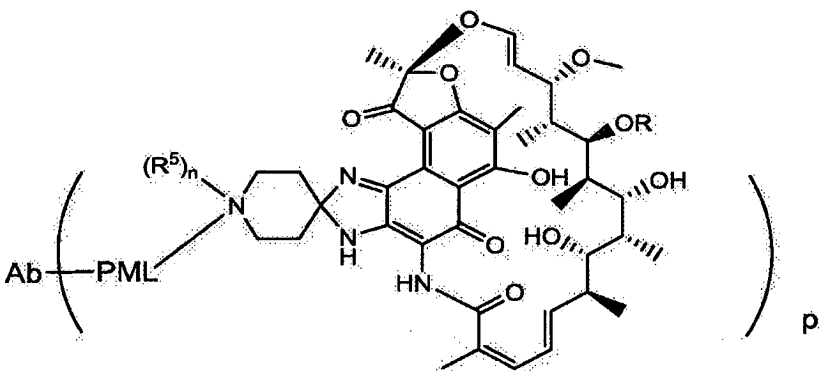


其中，

R^5选自H和C_1-C_{12}烷基；和

n为0或1。

8.权利要求2的抗体-抗生素缀合化合物，其具有下式：

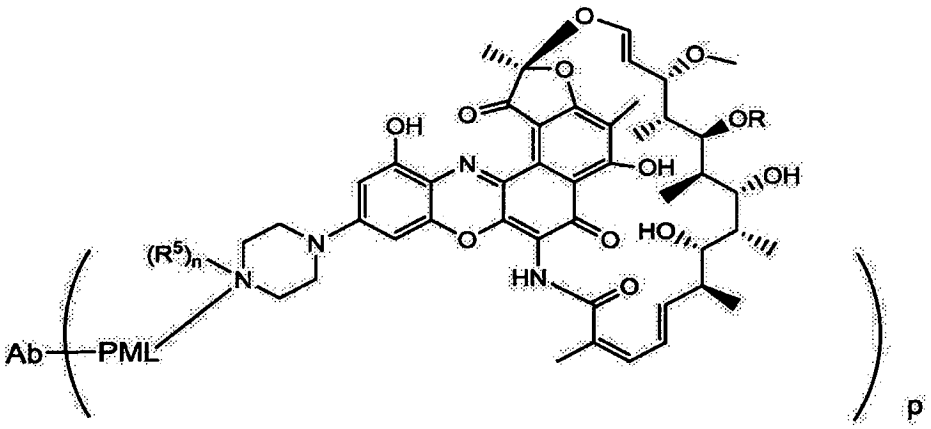


其中，

R^5选自H和C_1-C_{12}烷基；和

n为0或1。

9.权利要求2的抗体-抗生素缀合化合物，其具有下式：

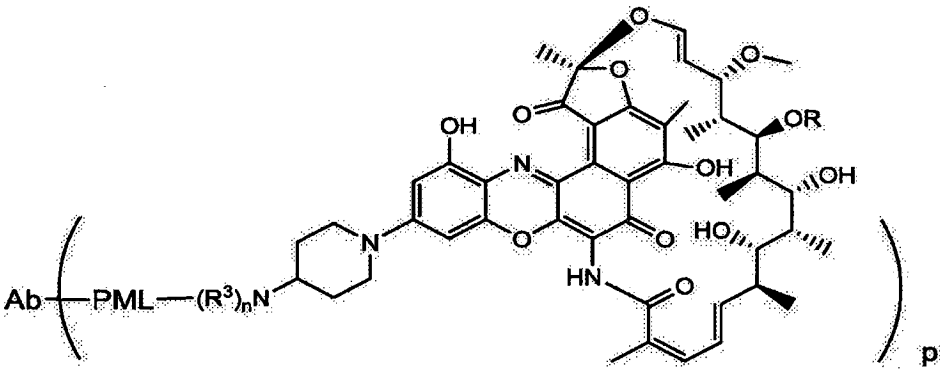


其中，

R^5独立地选自H和C_1-C_{12}烷基；和

n为0或1。

10.权利要求2的抗体-抗生素缀合化合物，其具有下式：

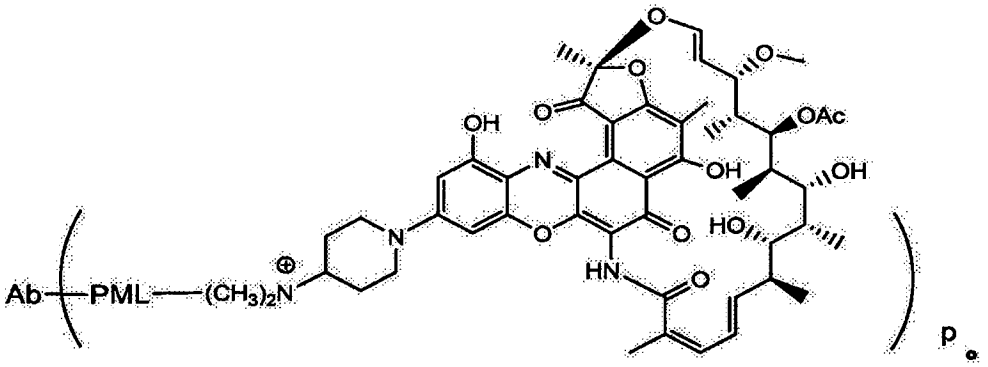


其中，

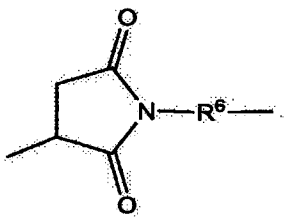
R^3独立地选自H和C_1-C_{12}烷基；和

n为1或2。

11.权利要求10的抗体-抗生素缀合化合物，其具有下式：



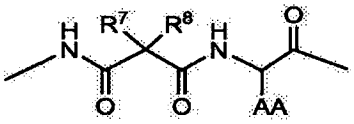
12.权利要求2的抗体-抗生素缀合化合物，其中Str具有下式：



其中R^6选自C_1-C_{12}亚烷基、C_1-C_{12}亚烷基-C(=O)、C_1-C_{12}亚烷基-NH、$(CH_2CH_2O)_r$、$(CH_2CH_2O)_r$-C(=O)、$(CH_2CH_2O)_r$-CH_2、和C_1-C_{12}亚烷基-NHC(=O)CH_2CH(噻吩-3-基)，其中r是1至10的整数。

13.权利要求12的抗体-抗生素缀合化合物，其中R^6是$(CH_2)_5$。

14.权利要求2的抗体-抗生素缀合化合物，其中PM具有下式：

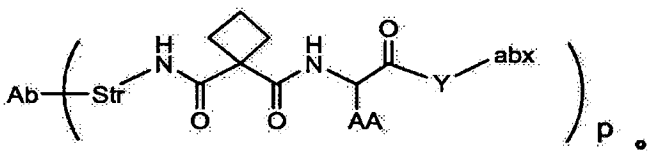


其中R^7和R^8一起形成C_3-C_7环烷基环，和

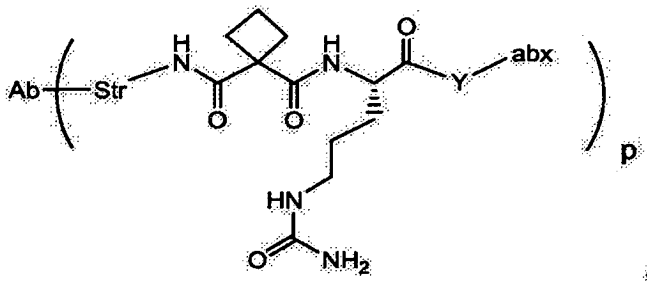
AA是选自H、$-CH_3$、$-CH_2(C_6H_5)$、$-CH_2CH_2CH_2CH_2NH_2$、$-CH_2CH_2CH_2NHC(NH)NH_2$、$-CHCH(CH_3)CH_3$、和$-CH_2CH_2CH_2NHC(O)NH_2$的氨基酸侧链。

15.权利要求2的抗体-抗生素缀合化合物，其中Y包含对氨基苄基或对氨基苄氧基羰基。

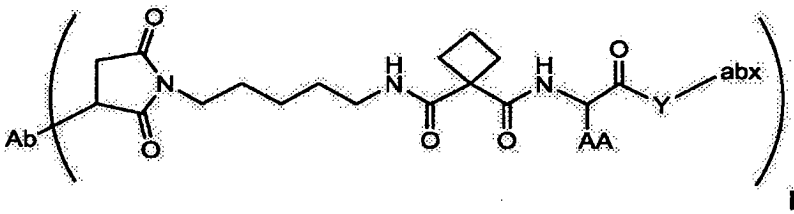
16.权利要求2的抗体-抗生素缀合化合物，其具有下式：



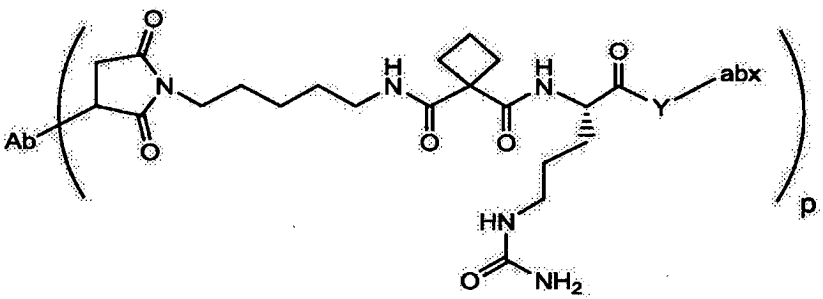
17.权利要求16的抗体-抗生素缀合化合物，其具有下式：



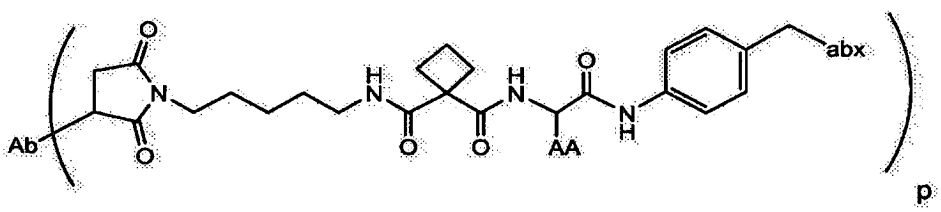
18.权利要求15的抗体-抗生素缀合化合物，其具有下式：



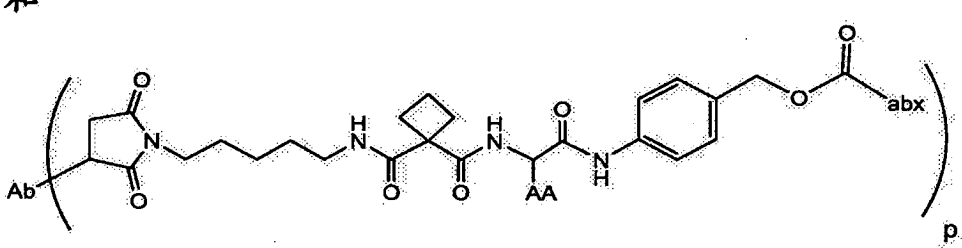
19.权利要求18的抗体-抗生素缀合化合物，其具有下式：



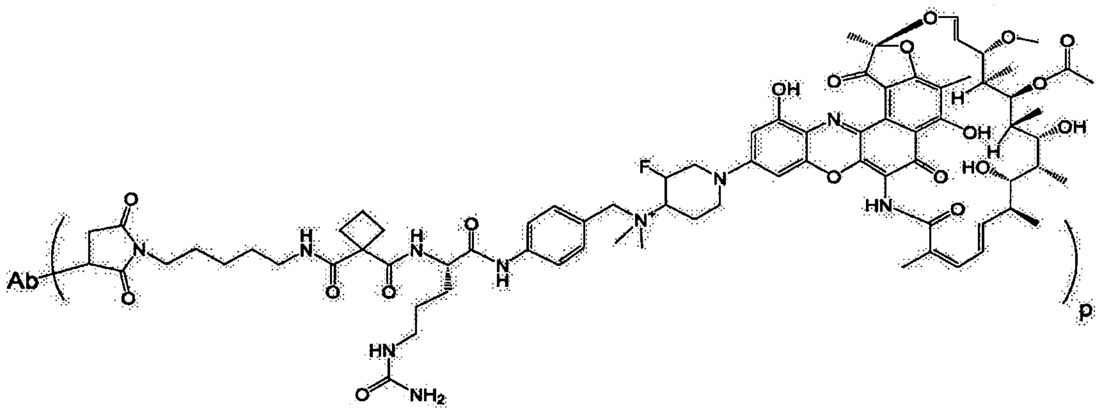
20.权利要求15的抗体-抗生素缀合化合物，其选自下式：



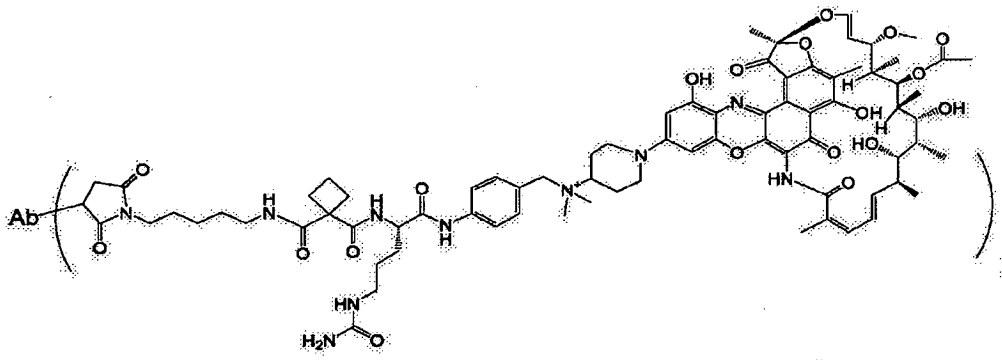
和



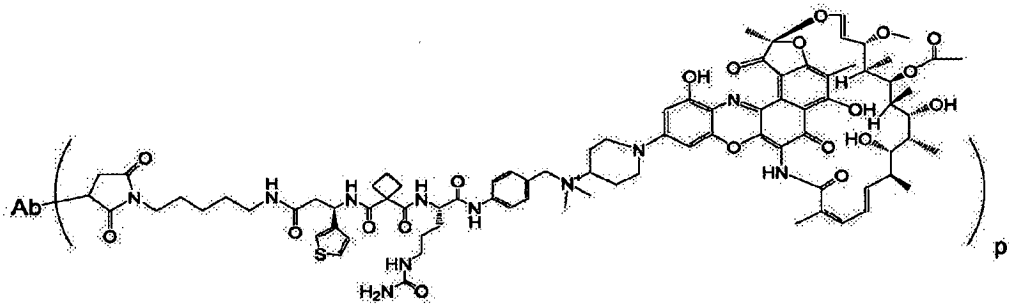
21.权利要求16的抗体-抗生素缀合化合物，其选自下式：


Ab
O
N
H
N
NH2
HN
OH
F
F
N+
O
OH
HO
p

；



;



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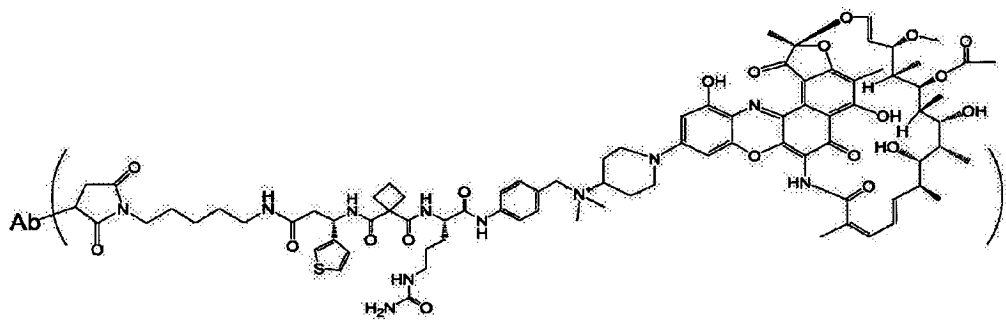
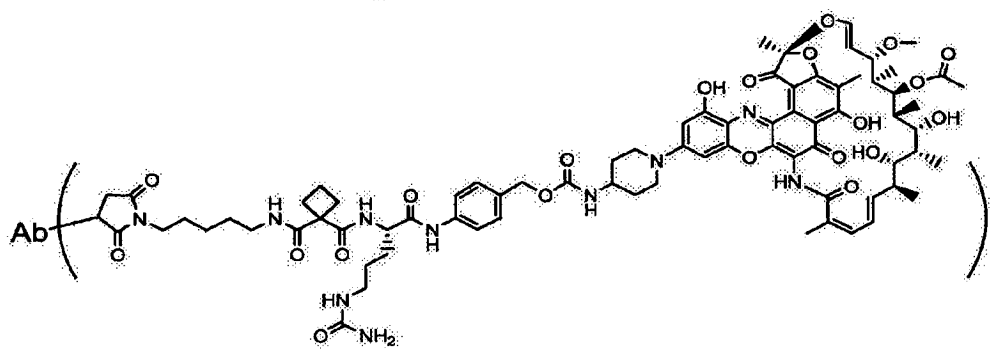
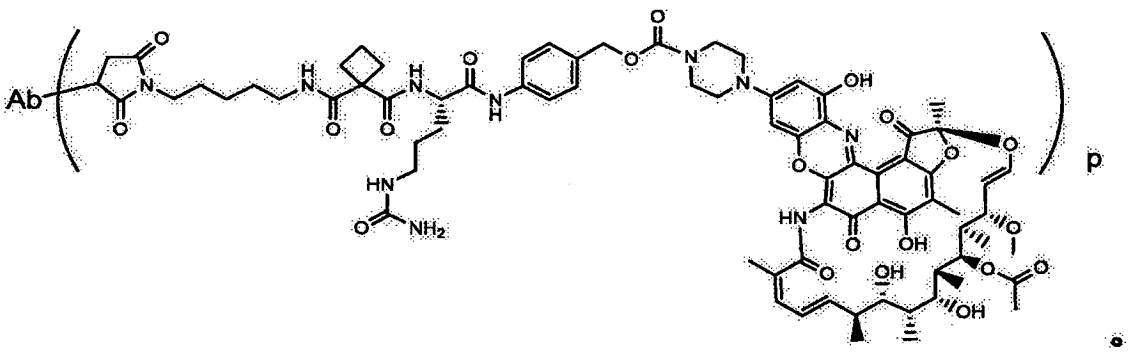


;



;

和



22.权利要求1的抗体-抗生素缀合化合物，其中抗体是抗WTAα单克隆抗体。

23.权利要求22的抗体-抗生素缀合化合物，其中抗壁磷壁酸(WTA)抗体是分离的单克隆抗体，其包含轻(L)链和重(H)链，L链包含CDR L1、CDR L2和CDR L3，且H链包含CDR H1、CDR H2和CDR H3，其中CDR L1、CDR L2和CDR L3与CDR H1、CDR H2和CDR H3，分别包含各个抗体4461(SEQ ID NO.1-6)、4624(SEQ ID NO.7-12)、4399(SEQ ID NO.13-18)和6267(SEQ ID NO.19-24)的CDR的氨基酸序列，如表1A和1B所示。

24.权利要求22的抗体-抗生素缀合物，其中抗壁磷壁酸(WTA)抗体包含重链可变区(VH)，其中VH与分别选自抗体4461、4624、4399和6267的SEQ ID NO.26、SEQ ID NO.28、SEQ ID NO.30、SEQ ID NO.32的VH序列的VH区在长度上包含至少95%的序列同一性。

25.权利要求24的抗体-抗生素缀合物，其进一步包含与分别选自抗体4461、4624、4399和6267的SEQ ID NO.25、SEQ ID NO.27、SEQ ID NO.29、SEQ ID NO.31的VL序列的VL区在长度上包含至少95%的序列同一性。

26.权利要求1的抗体-抗生素缀合化合物，其中该抗体是抗-WTAβ单克隆抗体。

27.权利要求26的抗体-抗生素缀合化合物，其中该抗-WTAβ抗体包含轻链和H链，所述L链包含CDR L1、CDR L2和CDR L3，且所述H链包含CDR H1、CDR H2和CDR H3，其中CDR L1、CDR L2和CDR L3以及CDR H1、CDR H2和CDR H3包含图12所示的每个Ab的相应CDR的氨基酸序列(SEQ ID NO:33-110)。

28.权利要求26的抗体-抗生素缀合化合物，其中该抗-WTAβ抗体包含L链可变区(VL)，其中VL包含相对于选自分别对应于以下每种抗体的VL序列的VL区域长度至少95%的序列同一性：6078、6263、4450、6297、6239、6232、6259、6292、4462、6265、6253、4497和4487，如图15A-1、15A-2、15A-3在Kabat位置1-107处所示。

29.权利要求28的抗体-抗生素缀合化合物，其中该抗-WTAβ抗体还包含重链可变区(VH)，其中VH包含相对于选自分别对应于以下每种抗体的VH序列的VH区域长度至少95%的序列同一性：6078、6263、4450、6297、6239、6232、6259、6292、4462、6265、6253、4497和4487，如图15B-1至15B-6在Kabat位置1-113所示。

30.权利要求29的抗体-抗生素缀合化合物，其中VL包含SEQ ID NO.111的序列，且VH包含SEQ ID NO.112的序列，其中X是Q或E，且X_1是M、I或V。

31.权利要求26的抗体-抗生素缀合化合物，其中抗体轻链含有工程化的半胱氨酸并且包含SEQ ID NO.115序列，且H链包含SEQ ID NO.116，其中X是M、I或V。

32.权利要求26的抗体-抗生素缀合化合物，其中抗体轻链包含SEQ ID NO.113序列，且

H链含有工程化的半胱氨酸，并包含SEQ ID NO.117，其中X是M、I或V。

33.权利要求26的抗体-抗生素缀合化合物，其中抗体轻链含有工程化的半胱氨酸，并且包含SEQ ID NO.115序列，并且H链含有工程化的半胱氨酸，并且包含SEQ ID NO.117，其中X是M、I或V。

34.权利要求26的抗体-抗生素缀合化合物，其中抗WTAβ抗体包含VH和VL，其中VH在SEQ ID NO.156的VH的长度上包含至少95％的序列同一性，并且VL在序列SEQ ID NO.119的VL的长度上包含至少95％的序列同一性。

35.权利要求26的抗体-抗生素缀合化合物，其中抗WTAβ抗体包含VH，其包含SEQ ID NO.156序列，且VL包含SEQ ID NO.119序列。

36.权利要求26的抗体-抗生素缀合化合物，其中抗WTAβ抗体包含含有SEQ ID NO.121序列的L链和含有SEQ ID NO.124序列的H链。

37.权利要求26的抗体-抗生素缀合化合物，其中抗WTAβ抗体包含含有SEQ ID NO.123序列的L链和含有SEQ ID NO.157序列的H链。

38.权利要求26的抗体-抗生素缀合化合物，其中抗WTAβ抗体包含含有SEQ ID NO.123序列的L链和含有SEQ ID NO.124序列的H链。

39.权利要求1的抗体-抗生素缀合化合物，其中该抗体包含：i)SEQ ID NO.99-104的L链和H链CDR、或SEQ ID NO.33-38的L链和H链CDR；或ii)与SEQ ID NO.120或SEQ ID NO.156的VH配对的SEQ ID NO.119或SEQ ID NO.123的VL；或iii)与SEQ ID NO.112的VH配对的SEQ ID NO.111的VL。

40.权利要求1的抗体-抗生素缀合化合物，其中抗壁磷壁酸(WTA)抗体与金黄色葡萄球菌结合。

41.前述权利要求中任一项的抗体-抗生素缀合化合物，其中抗体是F(ab)或$F(ab')_2$。

42.一种药物组合物，其包含权利要求1的抗体-抗生素缀合化合物和药学上可接受的载体、助流剂、稀释剂或赋形剂。

43.一种治疗患者的细菌感染的方法，其包含向患者施用治疗有效量的权利要求1的抗体-抗生素缀合化合物。

44.一种通过施用权利要求1的抗-WTA-抗生素缀合物在金黄色葡萄球菌感染的患者的细胞中杀死细胞内金黄色葡萄球菌、而不杀死宿主细胞的方法。

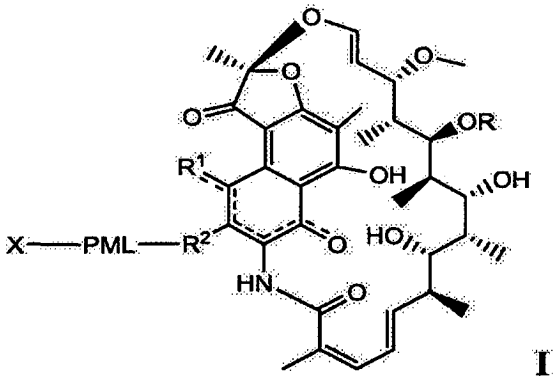
45.一种制备权利要求1的抗体-抗生素缀合化合物的方法，其包括将利福霉素型抗生素与抗壁磷壁酸(WTA)抗体缀合。

46.一种用于治疗细菌感染的药盒，其包含：

a)权利要求23的药物组合物；和

b)使用说明。

47.具有式II的抗生素-接头中间体：



II

其中：

虚线表示任选的键；

R是H、C_1-C_{12}烷基或C(O)CH_3；

R^1是OH；

R^2是CH=N-(杂环基)，其中所述杂环基任选地被一个或多个独立地选自C(O)CH_3、C_1-C_{12}烷基、C_1-C_{12}杂芳基、C_2-C_{20}杂环基、C_6-C_{20}芳基和C_3-C_{12}碳环基的基团取代；

或R^1和R^2形成五元或六元稠合杂芳基或杂环基，并且任选地形成螺或稠合的六元杂芳基、杂环基、芳基或碳环基环，其中所述螺或稠合的六元杂芳基、杂环基、芳基或碳环基环任选地被H、F、Cl、Br、I、C_1-C_{12}烷基或OH取代；

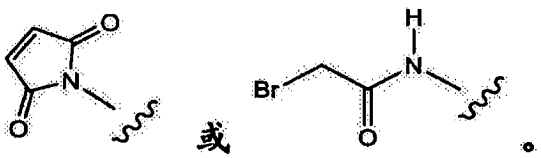
PML是连接到R^2或由R^1和R^2形成的稠合杂芳基或杂环基的蛋白酶可切割的非肽类接头；并具有下式：

-Str-PM-Y-

其中Str是延伸单元；PM是拟肽单元，和Y是间隔单元；并且

X是选自马来酰亚胺、硫醇、氨基、溴化物、溴乙酰氨基、碘乙酰氨基、对甲苯磺酸酯、碘化物、羟基、羧基、吡啶基二硫化物和N-羟基琥珀酰亚胺的反应性官能团。

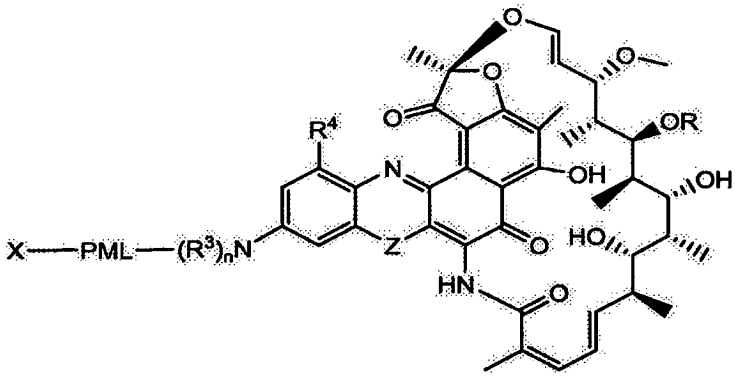
48.权利要求47的抗生素-接头中间体，其中X为



或

。

49.权利要求47的抗生素-接头中间体，其具有下式：



其中，

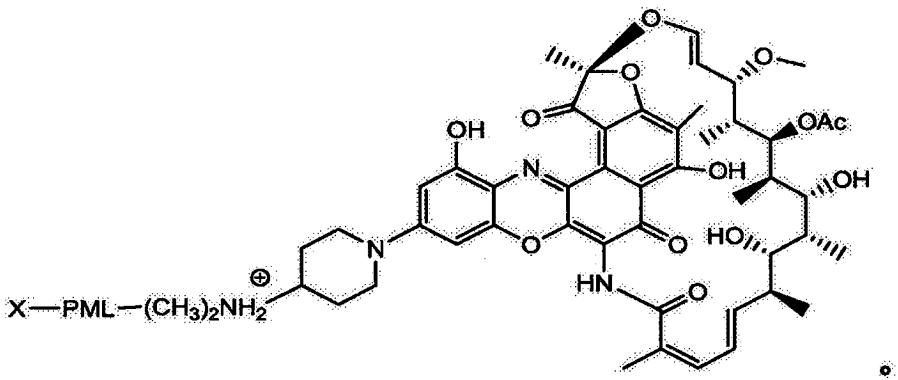
R^3独立地选自H和C_1-C_{12}烷基；

n为1或2；

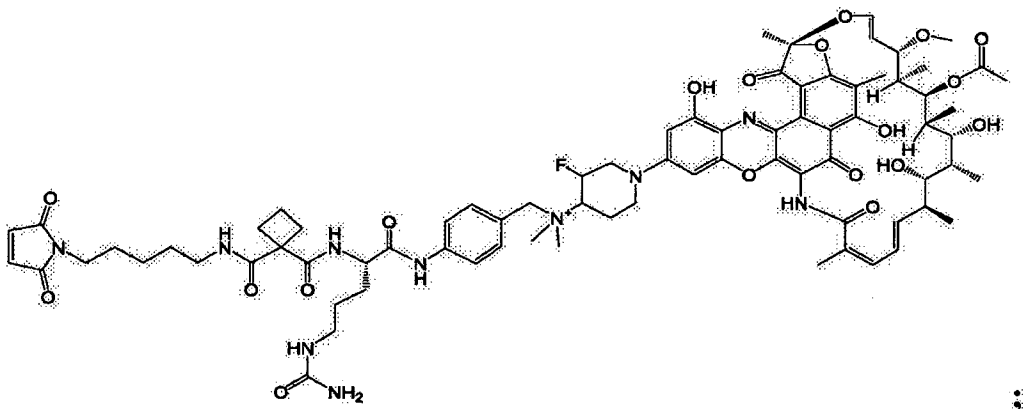
R^4选自H、F、Cl、Br、I、C_1-C_{12}烷基和OH；和

Z选自NH、N(C_1-C_{12}烷基)、O和S。

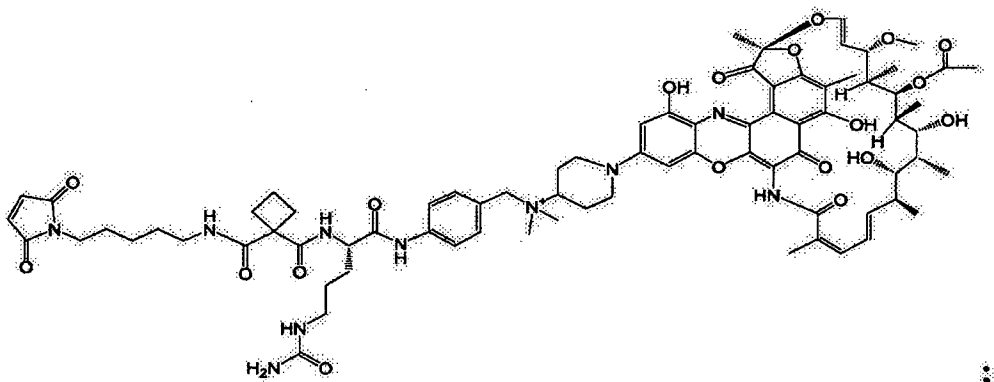
50.权利要求47的抗生素-接头中间体，其具有下式：



51.权利要求47的抗生素-接头中间体，其选自下式：



;



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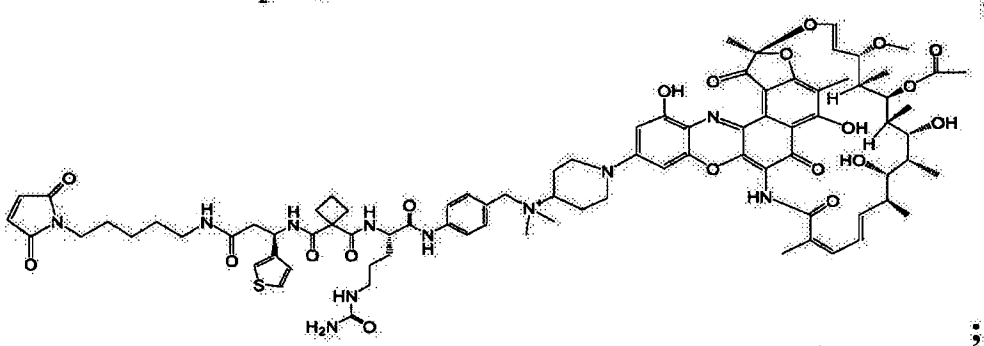

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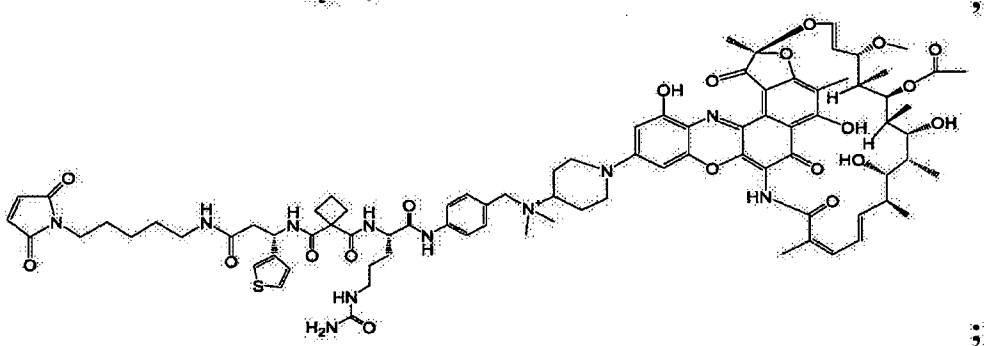

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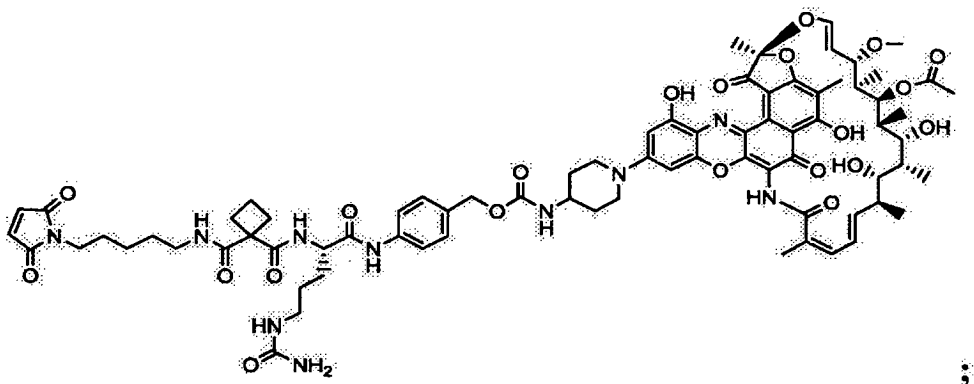
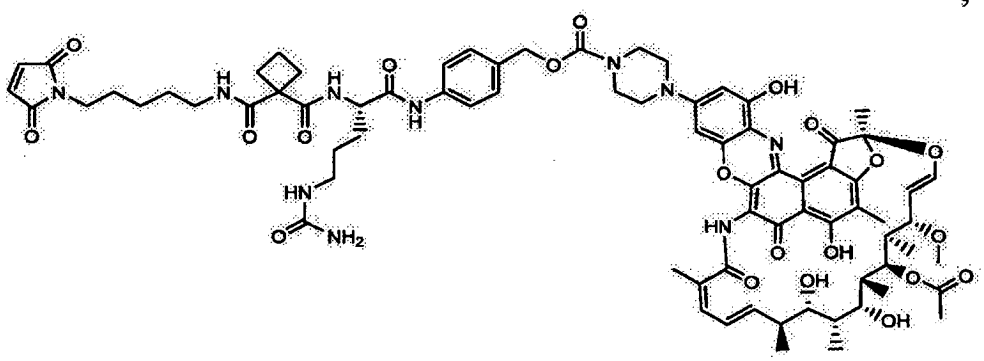


；和



。

52.权利要求43的方法，其中所述患者是被葡萄球菌型细菌感染。

53.权利要求52的方法，其中所述患者是被金黄色葡萄球菌感染。

54.权利要求43的方法，其中所述抗体-抗生素缀合化合物以约50mg/kg至100mg/kg的剂量施用于患者。

抗金黄色葡萄球菌抗体利福霉素缀合物及其用途

[0001] 相关申请的交叉引用

[0002] 本申请要求2014年12月3日提交的美国临时申请号62/087,184的优先权,其全部内容通过引用并入本文,用于所有目的。

[0003] 序列表

[0004] 本申请包含已经以ASCII格式电子提交的序列表,其全部内容通过引用并入本文。所述ASCII副本,于2015年11月20日创建,名称为P32433-WO_SL.txt,且大小为190541字节。

技术领域

[0005] 本发明涉及与利福霉素型抗生素缀合的抗壁磷壁酸("抗WTA")抗体,以及所得抗体-抗生素缀合物在治疗葡萄球菌感染中的用途。

[0006] 发明背景

[0007] 金黄色葡萄球菌(SA)是世界范围内人类细菌感染的重要原因,是医院和社区环境中的主要健康问题。然而,金黄色葡萄球菌不仅仅是一种病原体,并且通常定殖于健康个体的前鼻孔和皮肤。当感染确实发生时,在细菌传播到血液中之后发生最严重的感染例如心内膜炎、骨髓炎、坏死性肺炎和败血症(Lowy,F.D.(1998)"Staphylococcus aureus infections"N Engl J Med 339,520-532)。在过去几十年中,由于耐甲氧西林金黄色葡萄球菌(MRSA)的出现和快速传播,金黄色葡萄球菌的感染变得越来越难以治疗,这种菌对所有已知的β-内酰胺抗生素具有抗性(Boucher,H.W.等人,(2009)"Bad bugs,no drugs:no ESKAPE!An update from the Infectious Diseases Society of America"Clin Infect Dis 48,1-12)。侵入性MRSA感染难以治疗,死亡率约为20%,是美国传染性疾病死亡的主要原因。因此,万古霉素、利奈唑胺和达托霉素成为少数用于治疗侵入性MRSA感染的抗生素(Boucher,H.,Miller,L.G.&Razonable,R.R.(2010)"Serious infections caused by methicillin-resistant Staphylococcus aureus"Clin Infect Dis 51 Suppl 2,S183-197)。然而,在MRSA临床菌株中,已经报道了对万古霉素降低的敏感性和对利奈唑胺和达托霉素的交叉耐药性(Nannini,E.,Murray,B.E.&Arias,C.A.(2010)"Resistance or decreased susceptibility to glycopeptides,daptomycin,and linezolid in methicillin-resistant Staphylococcus aureus"Curr Opin Pharmacol 10,516-521)。随着时间的推移,克服抗性所需的万古霉素剂量已经上升至发生肾毒性的水平。因此,尽管有这些抗生素,侵入性MRSA感染的死亡率和发病率仍然很高。

[0008] 调查显示,金黄色葡萄球菌能够在哺乳动物细胞内侵入和存活,包括负责细菌清除的吞噬细胞(Thwaites,G.E.&Gant,V.(2011)Are bloodstream leukocytes Trojan Horses for the metastasis of Staphylococcus aureus?Nat Rev Microbiol 9,215-222);Rogers.D.E.,Tompsett,R.(1952)"The survival of staphylococci within human leukocytes"J.Exp.Med 95,209-230);Gresham,H.D.,等人(2000)"Survival of Staphylococcus aureus inside neutrophils contributes to infection"J Immunol 164,3713-3722);Kapral,F.A.&Shayegani,M.G.(1959)"Intracellular survival of

staphylococci"J Exp Med 110,123-138;Anwar,S.,等人(2009)"The rise and rise of Staphylococcus aureus:laughing in the face of granulocytes"Clin Exp Immunol 157,216-224);Fraunholz,M.&Sinha,B.(2012)"Intracellular Staphylococcus aureus: live-in and let die"Front Cell Infect Microbiol 2,43);Garzoni,C.&Kelley,W.L.(2011)"Return of the Trojan horse:intracellular phenotype switching and immune evasion by Staphylococcus aureus"EMBO Mol Med 3,115-117)。在静脉感染后几分钟内,金黄色葡萄球菌被宿主吞噬细胞(主要是嗜中性粒细胞和巨噬细胞)摄取(Rogers,D.E.(1956)"Studies on Bacterimia:Mechanisms Relating to the Persistence of Bacteremia in Rabbits Following the Intravanous Injection of Staphylococci"JEM 103,713)。尽管大多数细菌被这些细胞有效地杀死,但血液中吞噬细胞内的金黄色葡萄球菌的不完全清除,可以使这些被感染的细胞作为"特洛伊木马"用于从初始感染部位传播细菌。实际上,正常的嗜中性粒细胞计数患者可能比那些减少的嗜中性粒细胞计数患者更容易传播疾病(Thwaites,G.E.&Gant,V.(2011)见上文)。一旦传递到组织,金黄色葡萄球菌可以侵入各种非吞噬细胞类型,并且组织中的细胞内金黄色葡萄球菌与慢性或复发性感染相关。此外,细胞内细菌暴露于次优的抗生素浓度可能会促进抗生素耐药菌株的出现,从而使临床问题更加突出。与这些观察结果一致,用万古霉素或达托霉素治疗侵入性MRSA感染的患者如菌血症或心内膜炎,与超过50%的失败率相关(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,VG,Jr.等人,Daptomycin versus standard therapy for bacteremia and endocarditis caused by Staphylococcus aureus.The New England journal of medicine 355,653-665(2006);Yoon,YK,Kim,J.Y.,Park,D.W.,Sohn,J.W.和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))。因此,更成功的抗葡萄球菌治疗应包括清除细胞内的细菌。

[0009] 安莎霉素是一类抗生素,包括利福霉素、利福平(rifampin)、利福平(rifampicin)、利福布汀、利福喷汀、利福拉齐、ABI-1657及其类似物,它们抑制细菌RNA聚合酶,并且具有对革兰氏阳性菌和选择性的革兰氏阴性菌的特殊效力(Rothstein,D.M.等人(2003)Expert Opin.Invest.Drugs 12(2):255-271;US 7342011;US 7271165)。

[0010] 已经报道了用于预防和治疗金黄色葡萄球菌(包括MRSA)感染的免疫疗法。US2011/0262477涉及细菌粘附蛋白Eap、Emp和AdsA作为疫苗刺激针对MRSA的免疫应答的应用。WO2000/071585描述了与特异性金黄色葡萄球菌菌株分离物反应的分离的单克隆抗体。US2011/0059085A1提出了利用对一种或多种SA荚膜抗原特异性的IgM抗体的基于抗体的策略,但没有描述实际的抗体。

[0011] 抗体-药物缀合物(ADC)也称为免疫缀合物,是靶向的化学治疗分子,其通过将有效的细胞毒性药物靶向于表达抗原的肿瘤细胞,结合了抗体和细胞毒性药物的理想特性

(Teicher,B.A.(2009)Curr.Cancer Drug Targets 9:982-1004)，从而通过使效力最大化和使靶点外毒性最小化来增强治疗指数(Carter,P.J.和Senter P.D.(2008)The Cancer J.14(3):154-169;Chari,R.V.(2008)Acc.Chem.Res.41:98-107)。ADC包含通过接头单元与细胞毒性药物部分共价连接的靶向抗体。当未缀合的药物的全身给药可能引起对正常细胞和试图清除的肿瘤细胞的不可接受的毒性水平时，免疫缀合物允许将药物部分靶向递送至肿瘤并在其中的细胞内蓄积(Polakis P.(2005)Curr.Opin.Pharmacol.5:382-387)。

[0012] 描述了通过用于治疗败血症的抗生素结合目标细菌表面的非特异性免疫球蛋白-抗生素缀合物(US 5,545,721;US 6,660,267)。描述了抗生素缀合的抗体，其具有对细菌抗原(例如SA荚膜多糖)特异性的抗原结合部分，但缺乏与细菌Fc结合蛋白例如葡萄球菌蛋白A反应的恒定区(US 7569677)。

[0013] 鉴于MRSA对常规抗生素的令人震惊的抗药率，以及侵入性MRSA感染引起的死亡率和发病率，很需要治疗金黄色葡萄球菌感染的新的治疗药物。本发明满足了这一需求，提供了克服当前治疗组合物限制的组合物和方法以及提供从下面详述中显而易见的额外优点。

[0014] 发明概述

[0015] 本发明提供了包括消除细胞内细菌的独特治疗剂。本发明证实，当常规抗生素如万古霉素无效时，这种治疗剂在体内是有效的。

[0016] 本发明提供称为“抗体-抗生素缀合物”或“AAC”的组合物，其包含通过共价连接与一种或多种利福霉素型抗生素部分缀合的抗体。

[0017] 本发明的一个方面是抗体-抗生素缀合物化合物，其包含抗壁磷壁酸(WTA)抗体，其通过蛋白酶可切割的非肽类接头共价连接利福霉素型抗生素。

[0018] 本发明的示例性实施方案是具有下式的权利要求1的抗体-抗生素缀合物：

[0019] $Ab-(PML-abx)_p$

[0020] 其中：

[0021] Ab是抗壁磷壁酸抗体；

[0022] PML是具有下式的蛋白酶可切割的非肽类接头：

[0023] -Str-PM-Y-

[0024] 其中Str是延伸单元；PM是拟肽单元，和Y是间隔单元；

[0025] abx是利福霉素型抗生素；并且

[0026] p是从1到8的整数。

[0027] 任何前述实施方案的抗体-抗生素缀合化合物可以包含本文所述的抗壁磷壁酸(WTA)抗体中的任何一种。这些抗WTA抗体结合金黄色葡萄球菌。在一项实施方案中，所述抗体是抗-WTAα单克隆抗体。在示例性的WTAα抗体中，该Ab是包含轻(L)链和重(H)链的单克隆抗体，L链包含CDR L1、CDR L2和CDR L3，且H链包含CDR H1、CDR H2和CDR H3，其中CDR L1、CDR L2和CDR L3与CDR H1、CDR H2和CDR H3分别包含以下各个抗体4461(SEQ ID NO.1-6)、4624(SEQ ID NO.7-12)、4399(SEQ ID NO.13-18)和6267(SEQ ID NO.19-24)的CDR的氨基酸序列，如表1A和1B所示。

[0028] 在一些实施方案中，抗WTA抗体包含重链可变区(VH)，其中VH分别与选自抗体4461、4624、4399和6267的SEQ ID NO.26、SEQ ID NO.28、SEQ ID NO.30、SEQ ID NO.32的VH序列在VH区长度上包含至少95%的序列同一性。该抗体还可以包含L链可变区(VL)，其中VL

分别与选自抗体4461、4624、4399和6267的SEQ ID NO.25、SEQ ID NO.27、SEQ ID NO.29、SEQ ID NO.31的VL序列在VL区域长度上包含至少95%的序列同一性。

[0029] 在另一个实施方案中，本发明的抗体-抗生素缀合化合物包含抗WTAβ单克隆抗体。示例性的抗WTAβ抗体包含轻链和H链，所述L链包含CDR L1、CDR L2和CDR L3，且所述H链包含CDR H1、CDR H2和CDR H3，其中CDR L1、CDR L2和CDR L3以及CDR H1、CDR H2和CDR H3包含图12(SEQ ID NO:33-110)所示每个抗体的相应CDR的氨基酸序列。

[0030] 可用于产生本发明的AAC的另一个抗WTAβ抗体包含L链可变区(VL)，其中VL在VL区域的长度上包含与选自分别对应于以下每种抗体的VL序列至少95%的序列同一性：6078、6263、4450、6297、6239、6232、6259、6292、4462、6265、6253、4497和4487，如图15A-1、15A-2、15A-3在Kabat位置1-107处所示。该抗体还可以包含重链可变区(VH)，其中VH在VH区域的长度上包含与选自分别对应于以下每个抗体的VH序列至少95%的序列同一性：6078、6263、4450、6297、6239、6232、6259、6292、4462、6265、6253、4497和4487，如图15B-1至15B-6在Kabat位置1-113所示。

[0031] 在另一种抗WTAβ抗体中，VL包含SEQ ID NO.111序列，且VH包含SEQ ID NO.112序列，其中X是Q或E，X_1是M、I或V。

[0032] 本发明提供了可用于产生本发明的AAC的抗WTAβ，其中抗体轻链含有工程化的半胱氨酸并且包含SEQ ID NO.115序列，且H链包含SEQ ID NO.116，其中X是M、I或V。在可选的配对L和H链中，抗体轻链包含SEQ ID NO.113序列，且H链含有工程化的半胱氨酸，并包含SEQ ID NO.117，其中X是M、I或V。Cys可以被设计进入L链和H链中的每一个；在该WTAβ抗体的一个实例中，轻链含有工程化的半胱氨酸，并且包含SEQ ID NO.115序列，并且H链含有工程化的半胱氨酸，并且包含SEQ ID NO.117，其中X是M、I或V。

[0033] 可用于缀合的另一抗WTAβ抗体包含VH和VL，其中VH在SEQ ID NO.156的VH的长度上包含至少95%的序列同一性，并且VL在序列SEQ ID NO.119的VL的长度上包含至少95%的序列同一性。在具体实施方案中，抗WTAβ抗体包含VH和VL，VH包含SEQ ID NO.156的序列，且VL包含SEQ ID NO.119的序列。

[0034] 本发明的抗WTAβ抗体可以包含含有SEQ ID NO.121序列的L链和含有SEQ ID NO.124序列的H链。在另一个实例中，抗WTAβ抗体包含含有SEQ ID NO.123序列的L链和含有SEQ ID NO.157或SEQ ID NO.124序列的H链。

[0035] 在其它实施方案中，该抗体包含：i)SEQ ID NO.99-104的L链和H链CDR、或SEQ ID NO.33-38的L链和H链CDR；或ii)与SEQ ID NO.120或SEQ ID NO.156的VH配对的SEQ ID NO.119或SEQ ID NO.123的VL；或iii)与SEQ ID NO.112的VH配对的SEQ ID NO.111的VL。

[0036] 在本发明的AAC的一些实施方案中，抗体结合与前述任一实施方案的抗体相同的表位。

[0037] 在任何一项前述实施方案中，抗体可以是缺乏Fc区的抗原结合片段。在一些实施方案中，抗体是F(ab)或$F(ab')_2$。在一些实施方案中，抗体还包含重链恒定区和/或轻链恒定区，其中重链恒定区和/或轻链恒定区包含被半胱氨酸残基取代的一个或多个氨基酸。在一些实施方案中，重链恒定区包含氨基酸取代A118C和/或S400C，和/或轻链恒定区包含氨基酸取代V205C，其中所述编号系统根据EU编号。

[0038] 在上述任何抗体的一些实施方案中，抗体不是IgM同种型。在上述任何抗体的一些

实施方案中，抗体是IgG(例如，IgG1、IgG2、IgG3、IgG4)、IgE、IgD或IgA(例如，IgA1或IgA2)同种型。

[0039] 本发明的一个示例性实施方案是包含抗体-抗生素缀合化合物和药学上可接受的载体、助流剂、稀释剂或赋形剂的药物组合物。

[0040] 本发明的抗WTA-AAC可用作有效治疗人和兽医学的葡萄球菌的抗微生物剂，例如金黄色葡萄球菌、腐生性葡萄球菌和溶血性葡萄球菌，以及利斯特菌，例如单核细胞增生利斯特菌。在具体方面，本发明的AAC可用于治疗金黄色葡萄球菌感染。因此，本发明还提供了治疗人或兽医学患者中的葡萄球菌感染的方法，包括向患者施用治疗有效量的前述实施方案中任一项的抗体-抗生素缀合物。在一个实施方案中，所述细菌感染是金黄色葡萄球菌感染。在一些实施方案中，患者被诊断患有金黄色葡萄球菌感染。在一些实施方案中，治疗细菌感染包括减少细菌负荷或计数。在一个实施方案中，所述治疗方法是向包括金黄色葡萄球菌在内的细菌感染导致菌血症的患者施用。在具体实施方案中，该方法用于治疗葡萄球菌性心内膜炎或骨髓炎。在一个实施方案中，抗体-抗生素缀合化合物以约50mg/kg至100mg/kg的剂量施用于感染的患者。

[0041] 还提供了在金黄色葡萄球菌感染的患者细胞中杀死细胞内金黄色葡萄球菌而不杀死宿主细胞的方法，其通过施用任何上述实施方案的抗WTA抗生素缀合化合物。通过使耐药株(persister)细菌与任何前述实施方案的AAC接触，提供了另一种用于在体内杀死耐药株葡萄球菌细菌细胞(例如金黄色葡萄球菌)的方法。

[0042] 在另一个实施方案中，所述治疗方法还包括施用第二治疗剂。在另一个实施方案中，第二治疗剂是抗生素，其包括普遍针对金黄色葡萄球菌或特别是MRSA的抗生素。

[0043] 在一个实施方案中，与本发明的抗体-抗生素缀合化合物组合施用的第二抗生素选自以下结构类别：(i)氨基糖苷类；(ii)β-内酰胺类；(iii)大环内酯/环肽类；(iv)四环素类；(v)氟代喹啉/氟喹诺酮类；(vi)和噁唑烷酮。

[0044] 在一个实施方案中，与本发明的抗体-抗生素缀合化合物组合施用的第二抗生素选自克林霉素、新生霉素、瑞他帕林(retapamulin)、达托霉素、GSK-2140944、CG-400549、西他沙星、替考拉宁、三氯生、萘啶酮(napthyridone)、雷得唑来(radezolid)、多柔比星、氨苄西林、万古霉素、亚胺培南、多利培南、吉西他滨、达巴万星(dalbavancin)和阿奇霉素。

[0045] 在本文的一些实施方案中，感染患者中的细菌负荷在治疗后已经降低到不可检测的水平。在一个实施方案中，与治疗前的阳性血培养物相比，患者的血培养物在治疗后是阴性的。在本文的一些实施方案中，个体中的细菌耐药性是不可检测的或低的。在本文的一些实施方案中，个体对甲氧西林或万古霉素的治疗无反应。

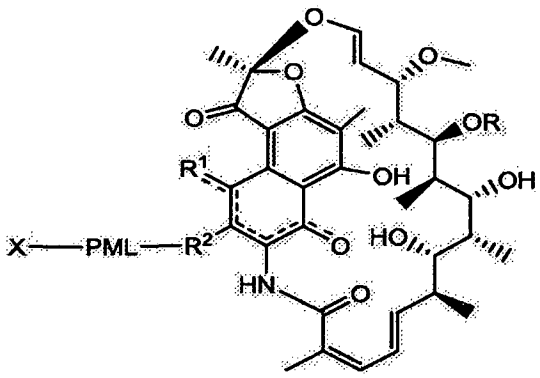
[0046] 本发明的示例性实施方案是制备抗体-抗生素缀合物的方法，包含将利福霉素型抗生素与抗壁磷壁酸(WTA)抗体缀合。

[0047] 本发明的一个示例性实施方案是用于治疗细菌感染的药盒，其包含：

[0048] a)药物组合物，包含抗体-抗生素缀合化合物和药学上可接受的载体、助流剂、稀释剂或赋形剂；和

[0049] b)使用说明。

[0050] 本发明的一个方面是具有式II的抗生素-接头中间体：

[0051] 

II

[0052] 其中：

[0053] 虚线表示任选的键；

[0054] R是H、C_1-C_{12}烷基或C(O)CH_3；

[0055] R^1是OH；

[0056] R^2是CH=N-(杂环基)，其中所述杂环基任选地被一个或多个独立地选自C(O)CH_3、C_1-C_{12}烷基、C_1-C_{12}杂芳基、C_2-C_{20}杂环基、C_6-C_{20}芳基和C_3-C_{12}碳环基的基团取代；

[0057] 或R^1和R^2形成五元或六元稠合杂芳基或杂环基，并且任选地形成螺或稠合的六元杂芳基、杂环基、芳基或碳环基环，其中所述螺或稠合的六元杂芳基、杂环基、芳基或碳环基环任选地被H、F、Cl、Br、I、C_1-C_{12}烷基或OH取代；

[0058] PML是连接到R^2或由R^1和R^2形成的稠合杂芳基或杂环基的蛋白酶可切割的非肽类接头；并具有下式：

[0059] -Str-PM-Y-

[0060] 其中Str是延伸单元；PM是拟肽单元，和Y是间隔单元；并且

[0061] X是选自马来酰亚胺、硫醇、氨基、溴化物、溴乙酰氨基、碘乙酰氨基、对甲苯磺酸酯、碘化物、羟基、羧基、吡啶基二硫化物和N-羟基琥珀酰亚胺的反应性官能团。

[0062] 应当理解，本文描述的各种实施方案的一个、一些或全部特性可以组合以形成本发明的其他实施方案。本发明的这些和其它方面对于本领域技术人员是很明显的。

[0063] 附图简述

[0064] 图1：在体内和体外，MRSA的细胞内储存物被保护免于万古霉素杀死。图1A显示了产生游离细菌(浮游)与细胞内细菌的实验设计的示意图。通过静脉注射大约相同剂量的活的游离细菌或细胞内细菌感染四组小鼠，并且选择的组在感染后立即用万古霉素处理，然后每天一次(参见实施例2)。图1B和图1C分别显示感染后4天的感染小鼠的肾脏和脑部细菌负荷。虚线表示测定的检测限。图1D显示当在单层感染细胞上培养时，MRSA被保护免于万古霉素杀死。(ND=未检测到)。图1E和图1F显示当在单层感染细胞上培养时，MRSA能够在万古霉素存在下生长。将MRSA(游离细菌)接种在培养基、培养基+万古霉素、或培养基+万古霉素并接种在单层MG63成骨细胞(图1E)或人脑微血管内皮细胞(HBMEC，图1F)中。细胞外细菌(游离细菌)在仅有培养基时生长良好，但被万古霉素杀死。在含有单层哺乳动物细胞(细胞内+万古霉素)的孔中，一部分细菌在感染后的前8个小时被保护免于万古霉素杀死，并能在

24小时内在细胞内的区室内扩张。误差线显示一式三份的孔的标准偏差。

[0065] 图2显示了抗体抗生素缀合物(AAC)的概念。在一个实例中，AAC由针对金黄色葡萄球菌表面上的表位的抗体组成，其通过溶酶体蛋白酶切割的接头连接到有效的利福霉素型抗生素(例如Rifalog)上。

[0066] 图3显示抗体-抗生素缀合物(AAC)的药物活化的可能机制。AAC通过抗体的抗原结合结构域(Fab)与细胞外细菌结合，并通过Fc介导的吞噬作用促进调理化细菌的摄取。接头被溶酶体蛋白酶例如组织蛋白酶B切割。在切割接头之后，接头被水解，在吞噬溶酶体内释放游离抗生素。游离的抗生素杀死了调理化细胞和被吞噬的细菌，以及任何以前存在于同一区室中的内化的细菌。

[0067] 图4显示革兰氏阳性细菌例如金黄色葡萄球菌的细胞壁，其具有壁磷壁酸(WTA)、脂磷壁酸(LTA)和肽聚糖(PGN)鞘的漫画显示，它们稳定细胞膜并提供附着位点。

[0068] 图5显示了在定义部分中详细描述的壁磷壁酸(WTA)的化学结构和糖基修饰。

[0069] 图6A和6B总结了来自mAb库的初步筛选的抗体特征，所述mAb显示结合来自USA300或Wood46金黄色葡萄球菌菌株的细胞壁制备物的阳性ELISA，如实施例3所述。在与WTA结合的抗体中，4个特异于WTAα且13个特异性结合WTAβ。

[0070] 图7A显示了在直接从感染的小鼠肾脏分离的MRSA上Alexa-488标记的抗β-GlcNAC WTA或抗α-GlcNAC WTA抗体的滴定。抗CMV-gD抗体作为抗体同种型对照品。图7B显示用于产生AAC的抗体识别了壁磷壁酸上的表位，由糖基转移酶TarS介导。在Wt USA300、USA300-TarM或USA300-TarS上使用抗β-GlcNAC WTA抗体或同种型对照品进行FACS分析。

[0071] 图8显示了选择强效的利福霉素型抗生素(rifalog)二甲基pipBOR，因为其杀死非复制性MRSA的能力。

[0072] 图9：生长抑制测定证明完整的TAC(一种形式的AAC)不会杀死浮游细菌，除非通过用组织蛋白酶B处理而释放抗生素。将TAC在仅有缓冲液(空心圆圈)中孵育或用组织蛋白酶B处理(实心圆圈)。完整的TAC在过夜孵育后不能防止细菌生长。用组织蛋白酶B预处理TAC释放了足够的抗生素活性，以防止细菌在0.6ug/mL TAC中生长，其预计含有0.006μg/mL的抗生素。

[0073] 图10显示了如实施例10所述，与裸抗体相比，用抗-WTA-PML AAC处理金黄色葡萄球菌感染的大鼠大幅减少或根除细菌计数。图10A是显示实施例10中所述的实验和注射时间点的时间线的示意图。图10B显示用表3中的AAC(DAR2)处理，降低肾脏中的细菌负荷约7,000倍。图10C显示用AAC(DAR2)处理，降低心脏中的细菌负荷大约500倍。

[0074] 图11A提供四种人抗WTAα抗体4461、4624、4399、6267的轻链可变区(VL)(分别为SEQ ID NO.25、27、29和31，按出现次序)的氨基酸序列比对。根据Kabat编号的CDR序列CDRL1、L2和L3加下划线。图11B显示图11A的四种人抗WTAα抗体的重链可变区(VH)的氨基酸序列比对。根据Kabat编号的CDR序列CDR H1、H2和H3以下划线表示(分别为SEQ ID NO.26、28、30和32，按出现次序)。

[0075] 图12显示了13种人抗WTAβ抗体的L和H链的CDR序列(SEQ ID NO:33-110)。

[0076] 图13A-1和13A-2显示了抗WTAβAb 6078(未修饰)及其变体v2、v3、v4的全长L链(轻链)的比对(分别为SEQ ID NO.113、113、115、113、115、113、115和115，按出现次序)。根据Kabat编号的CDR序列CDRL1、L2和L3加下划线。框根据Kabat和Chothia显示接触残基和CDR

残基。含有工程化Cys的L链变体由恒定区末端附近的黑框中的C表示(在该情况下为EU残基205)。变体名称,例如v2LC-Cys是指包含引入L链的Cys的变体2。HCLC-Cys是指每个H和L链含有工程化的Cys。变体2、3和4如图13B所示在H链的开始处具有改变。

[0077] 图13B-1、13B-2、13B-3、13B-4显示抗WTAβAb 6078(未修饰)及其变体v2、v3、v4的全长H链(重链)的比对(分别为SEQ ID NO.114、139-144和143,按出现次序),其在H链开始处具有变化。含有工程化Cys的H链变体由恒定区开始端的黑框中的C表示(在该情况下为EU残基118)。

[0078] 图14A-1和14A-2显示抗-WTAβAb 4497(未修饰)和Cys工程化L链(分别为SEQ ID NO.121、123、145和145,按出现次序)的全长L链的比对。根据Kabat编号的CDR序列CDRL1、L2和L3加下划线。框根据Kabat和Chothia显示接触残基和CDR残基。含有工程化Cys的L链变体由恒定区末端附近虚线框中的C表示(在该情况下为EU残基205)。

[0079] 图14B-1、14B-2、14B-3显示抗-WTAβAb 4497(未修饰)及其v8变体(其在CDR H3的96位将D改变为E,含有或不含有工程化的Cys)的全长H链的比对(分别为SEQ ID NO:146-147、157和147,按出现的次序)。含有工程化Cys的H链变体由恒定区开始端的黑框中的C表示(在该情况下为EU残基118)。

[0080] 图15A-1、15A-2、15A-3显示了十三种人抗WTAβ抗体的全长轻链的氨基酸序列比对(分别为SEQ ID NO:113、158-167、121和168,按出现次序)。可变区(VL)跨越Kabat氨基酸位置1-107。根据Kabat编号的CDR序列CDRL1、L2和L3被加下划线。

[0081] 图15B-1至15B-6显示了图15A-1、15A-2、15A-3的十三种人抗WTAβ抗体的全长重链的氨基酸序列比对(分别为SEQ ID NO:114、169-176、133-134、138和127,按出现次序)。可变区(VH)跨越Kabat氨基酸位置1-113。根据Kabat编号的CDR序列CDR H1、H2和H3加下划线。用星号标记的H链Eu位置118可以改为Cys,用于药物缀合。用黑色突出显示的残基可以被不影响抗原结合的其它残基替代,以避免脱酰胺化、天冬氨酸异构化、氧化或N-连接的糖基化。

[0082] 图16显示Ab 4497与突出显示氨基酸位置的其突变体和通过ELISA测试的它们的相对抗原结合强度的比较。图16分别公开SEQ ID NO:177、177、177、178、178、179、179、180、180和180,按出现次序。

[0083] 图17显示在静脉感染模型中用50mg/kg游离抗体的预处理是无效的。在用$2x10^7$CFU的USA300感染之前30分钟通过静脉内注射向Balb/c小鼠施用单剂量的载体对照物(PBS)或50mg/Kg抗体。治疗组包括不与金黄色葡萄球菌结合的同种型对照抗体(gD),其是针对壁磷壁酸(4497)的β修饰的抗体或针对壁磷壁酸(7578)的α修饰的抗体。通过腹膜内注射(万古霉素),用110mg/Kg万古霉素治疗,每天向对照小鼠施用两次。

[0084] 本发明的实施方案详述

[0085] 现在将详细参考本发明的某些实施方案,其实例在所附的结构和分子式中示出。虽然将结合列举的实施方案,包括方法、材料和实例来描述本发明,但是这样的描述是非限制性的,并且本发明旨在覆盖所有替代、修改和等同物,无论它们是一般已知的,还是并入本文。如果一个或多个并入的文献、专利和类似材料与本申请不同或相矛盾,包括但不限于定义的术语、术语使用、所描述的技术等,以本申请为准。除非另有定义,本文使用的所有技术和科学术语具有与本发明所属领域的普通技术人员通常理解的相同含义。本领域技术人

员将认识到可以用于本发明的实践中的与本文所述类似或等同的许多方法和材料。本发明在任何情况下都不限于所述的方法和材料。

[0086] 本文提及的所有出版物、专利申请、专利和其它参考文献通过引用整体并入本文。

[0087] I.一般技术

[0088] 本文描述或参考的技术和方法，通常被本领域技术人员很好的理解和使用常规方法普遍采用，例如，下面描述的广泛使用的方法：Sambrook等人，分子克隆：实验室手册第三版(2001)冷泉港实验室出版社，冷泉港，纽约(Molecular Cloning:A Laboratory Manual 3d edition(2001)Cold Spring Harbor Laboratory Press,Cold Spring Harbor,N.Y.)；分子生物学现代方法(Current Protocols in Molecular Biology)(F.M.Ausubel等人编辑，(2003))；酶学系列方法(the series Methods in Enzymology)(Academic Press,Inc.)；PCR 2:实用方法(PCR 2:A Practical Approach)(M.J.MacPherson,B.D.Hames和G.R.Taylor编辑(1995))，Harlow和Lane编辑(1988)抗体、实验室手册、和动物细胞培养(Antibodies,A Laboratory Manual,and Animal Cell Culture)(R.I.Freshney编辑(1987))；寡核苷酸合成(Oligonucleotide Synthesis)(M.J.Gait编辑，1984)；分子生物学方法(Methods in Molecular Biology)，Humana Press；细胞生物学：实验室手册(Cell Biology:A Laboratory Notebook)(J.E.Cellis编辑，1998)Academic Press；动物细胞培养(Animal Cell Culture)(R.I.Freshney)编辑，1987)；细胞和组织培养介绍(Introduction to Cell and Tissue Culture)(J.P.Mather和P.E.Roberts,1998)Plenum Press；细胞和组织培养：实验室方法(Cell and Tissue Culture:Laboratory Procedures)(A.Doyle,J.B.Griffiths,和D.G.Newell编辑，1993-8)J.Wiley和Sons；实验免疫学手册(Handbook of Experimental Immunology)(D.M.Weir和C.C.Blackwell编辑)；哺乳动物细胞的基因转移载体(Gene Transfer Vectors for Mammalian Cells)(J.M.Miller和M.P.Calos编辑，1987)；PCR：聚合酶链氏反应(PCR:The Polymerase Chain Reaction)，(Mullis等人编辑，1994)；现代免疫学方法(Current Protocols in Immunology)(J.E.Coligan等人编辑，1991)；分子生物学简要方法(Short Protocols in Molecular Biology)(Wiley和Sons,1999)；免疫学(Immunobiology)(C.A.Janeway和P.Travers,1997)；抗体(Antibodies)(P.Finch,1997)；抗生素：实用方法(Antibodies:A Practical Approach)(D.Catty.编辑，IRL Press,1988-1989)；单克隆抗体：实用方法(Monoclonal Antibodies:A Practical Approach)(P.Shepherd和C.Dean编辑，牛津大学出版社(Oxford University Press)，2000)；抗体应用：实验室手册(Using Antibodies:A Laboratory Manual)(E.Harlow和D.Lane，冷泉港实验室出版社(Cold Spring Harbor Laboratory Press)，1999)；抗体(The Antibodies)(M.Zanetti和J.D.Capra编辑，Harwood Academic Publishers,1995)；和癌症：肿瘤学原理与实践(Cancer:Principles and Practice of Oncology)(V.T.DeVita等人编辑，J.B.Lippincott Company,1993)。

[0089] 本申请中使用的术语是基于IUPAC系统命名法，除非另有说明。除非另有定义，本文所用的技术和科学术语具有与本发明所属领域的普通技术人员通常理解的相同含义，并且符合：Singleton等人(1994)微生物和分子生物学词典，第二版(Microbiology and Molecular Biology Dictionary,2nd Ed.)，J.Wiley&Sons,New York,NY；和Janeway,C.,Travers,P.,Walport,M.,Shlomchik(2001)免疫学，第五版(Immunobiology,5th Ed.)，

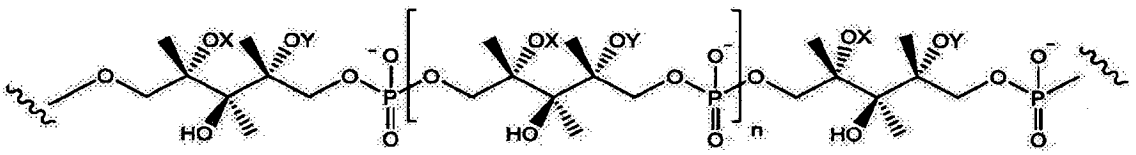
Garland Publishing,New York。

[0090] II.定义

[0091] “抗体抗生素缀合物”或AAC是由通过接头与抗生素化学连接的抗体组成的化合物。抗体结合细菌表面上的抗原或表位，例如细菌细胞壁组分。如本发明所用，接头是蛋白酶可切割的非肽类接头，其被设计为被蛋白酶切割，包括组织蛋白酶B，其是大多数哺乳动物细胞类型中发现的溶酶体蛋白酶(Dubowchik等人(2002)Bioconj.Chem.13:855-869)。具有3个组分的AAC图描述于图2中。“THIOMAB™抗生素缀合物”或“TAC”是AAC的一种形式，其中抗体通过一个或多个半胱氨酸与接头-抗生素单元化学缀合，所述半胱氨酸通常是在抗体的特定位点被重组操作到抗体中的半胱氨酸，以不干扰抗原结合功能。

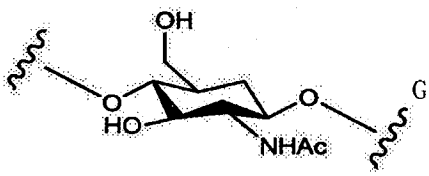
[0092] 术语“壁磷壁酸”(WTA)的含义是指通过磷酸二酯键与N-乙酰胞壁酸糖的C6羟基连接而共价连接到肽聚糖上的阴离子糖聚合物。尽管精细的化学结构可以在生物体之间变化，但在一个实施方案中，WTA是具有位置2上的D-核糖醇和D-丙氨酸基酯的1,5-磷酸二酯键和位置4上的糖基取代基的重复单元的核糖醇磷壁酸。糖基可以是存在于金黄色葡萄球菌中的N-乙酰葡糖胺基α或β。醛醇/糖醇磷酸酯重复序列上的羟基被阳离子D-丙氨酸酯和单糖如N-乙酰葡糖胺取代。一方面，羟基取代基包括D-丙氨酰和α或βGlcNHAc。在一个特定方面，WTA包含下式的化合物：

[0093]



[0094] 其中波浪线表示重复连接单元或聚糖醇-P或肽聚糖的连接位点，其中X是D-丙氨酰基或-H；且Y是α-GlcNHAc或β-GlcNHAc。

[0095]



GlcNHAc

[0096] 在金黄色葡萄球菌中，WTA通过由N-乙酰葡糖胺(GlcNAc)-1-P和N-乙酰甘露糖胺(ManNAc)构成的二糖与N-乙酰胞壁酸(MurNAc)的6-OH共价连接，其随后与两个或三个甘油磷酸酯单位连接。实际的WTA聚合物由11-40个核糖醇-磷酸酯(Rbo-P)重复单元组成。WTA的分步合成首先由称为TagO的酶启动，缺乏TagO基因的金黄色葡萄球菌(通过人工删除该基因)不产生任何WTA。可以通过α-或β-糖苷键用在C2-OH位置的D-丙氨酸(D-Ala)和/或C4-OH位置的N-乙酰葡糖胺(GlcNAc)进一步定制该重复单元。根据金黄色葡萄球菌菌株或细菌的生长期，糖苷键可以是α-、β-或这两个端基异构体的混合物。

[0097] 如本文所用，术语“WTA抗体”是指结合WTA(无论是WTAα或WTAβ)的任何抗体。术语“抗壁磷壁酸α抗体”或“抗-WTAα抗体”或“抗αWTA”或“抗αGlcNacWTA抗体”可互换使用，是指特异性结合壁磷壁酸(WTA)α的抗体。类似地，术语“抗壁磷壁酸β抗体”或“抗WTAβ抗体”或“抗-βWTA”或“抗-βGlcNacWTA抗体”可互换使用，是指特异性结合壁磷壁酸(WTA)β的抗体。

[0098] 术语“抗生素”(abx或Abx)包括在给药浓度和给药间隔下特异性抑制微生物生长

或杀死微生物例如细菌、但是对宿主是非致死性的任何分子。在具体方面，抗生素在给药浓度和给药间隔下对宿主无毒性。对细菌有效的抗生素可以广泛地分类为杀菌(即直接杀死)或抑菌(即防止分裂)。抗细菌抗生素可进一步分类为窄谱或广谱。与窄谱抗生素相反，广谱抗生素对于广泛的细菌是有效的，包括革兰氏阳性和革兰氏阴性细菌，而窄谱抗生素对于较小范围或特定的细菌家族有效。抗生素的例子包括：(i)氨基糖苷类，例如阿米卡星、庆大霉素、卡那霉素、新霉素、奈替米星、链霉素、妥布霉素、巴龙霉素，(ii)安莎霉素类，例如格尔德霉素、除莠霉素，(iii)碳头孢烯类，例如氯碳头孢，(iv)碳青霉烯类，例如厄他培南、多利培南、亚胺培南/西司他丁、美罗培南，(v)头孢菌素类(第一代)，例如头孢羟氨苄、头孢唑啉、头孢噻吩、头孢氨苄，(vi)头孢菌素类(第二代)，例如头孢克洛、头孢羟唑、头孢西丁、头孢丙烯、头孢呋辛，(vi)头孢菌素类(第三代)，例如头孢克肟、头孢地尼、头孢托仑、头孢哌酮、头孢噻肟、头孢泊肟、头孢他啶、头孢布烯、头孢唑肟、头孢曲松，(vii)头孢菌素类(第四代)，例如头孢吡肟，(viii)头孢菌素类(第五代)，例如头孢吡普(ceftobiprole)，(ix)糖肽类，例如替考拉宁、万古霉素，(x)大环内酯类，例如阿奇霉素、克拉霉素、地红霉素、红霉素、罗红霉素、醋竹桃霉素、泰利霉素、壮观霉素，(xi)内酰胺类，例如氨曲南，(xii)青霉素类，例如阿莫西林、氨苄西林、阿洛西林、羧苄西林、氯唑西林、双氯西林、氟氯西林、美洛西林、甲氧西林、萘夫西林、苯唑西林、青霉素、哌拉西林、替卡西林，(xiii)抗生素多肽类，例如杆菌肽、粘菌素、多粘菌素B，(xiv)喹诺酮类，例如环丙沙星、依诺沙星、加替沙星、左氧氟沙星、洛美沙星、莫西沙星、诺氟沙星、氧氟沙星、曲伐沙星，(xv)磺酰胺类，例如磺胺米隆、偶氮磺胺、磺胺乙酰胺、磺胺甲二唑、磺胺、柳氮磺吡啶、磺胺异噁唑、甲氧苄啶、甲氧苄啶-磺胺甲噁唑(TMP-SMX)，(xvi)四环素类，例如地美环素、多西环素、米诺环素、土霉素、四环素，和(xvii)其它如肿凡纳明、氯霉素、克林霉素、林可霉素、乙胺丁醇、磷霉素、夫西地酸、呋喃唑酮、异烟肼、利奈唑胺、甲硝唑、莫匹罗星、呋喃妥因、平板霉素、吡嗪酰胺、奎奴普丁/达福普汀、利福平(rifampin)/利福平(rifampicin)或替硝唑。

[0099] 金黄色葡萄球菌也简称为Staph A或S.aureus。术语“耐甲氧西林金黄色葡萄球菌”(MRSA)也称为多药耐药性金黄色葡萄球菌或耐苯唑西林金黄色葡萄球菌(ORSA)，是指对β-内酰胺抗生素有抗性的金黄色葡萄球菌菌株，其中包括青霉素(如甲氧西林、双氯西林、萘夫西林、苯唑西林等)和头孢菌素类。“甲氧西林敏感型金黄色葡萄球菌”(MSSA)是指对β-内酰胺抗生素敏感的金黄色葡萄球菌的任何菌株。

[0100] 术语“抗金黄色葡萄球菌抗体”和“与金黄色葡萄球菌结合的抗体”是指能够以足够的亲和力结合金黄色葡萄球菌(“S.aureus”)上的抗原的抗体，使得抗体可用作靶向于金黄色葡萄球菌的诊断和/或治疗剂。在一个实施方案中，例如通过放射免疫测定(RIA)测量，抗金黄色葡萄球菌抗体与非相关的非金黄色葡萄球菌蛋白的结合程度不超过抗体与MRSA结合程度的约10％。在某些实施方案中，结合金黄色葡萄球菌的抗体具有≤1μM、≤100nM、≤10nM、≤5Nm、≤4nM、≤3nM、≤2nM、≤1nM、≤0.1nM、≤0.01nM或≤0.001nM(例如10^{-8}M或更小，例如10^{-8}M至10^{-13}M，例如10^{-9}M至10^{-13}M)的解离常数(Kd)。在某些实施方案中，抗金黄色葡萄球菌抗体结合来自不同物种的葡萄球菌中保守的金黄色葡萄球菌的表位。

[0101] 术语“最小抑菌浓度”(MIC)是指在过夜孵育后抑制微生物可见生长的抗微生物剂的最低浓度。测定MIC的试验方法是已知的。一种方法如下面的实施例部分所述。

[0102] 本文中的术语“抗体”以最广泛的含义使用，并且具体涵盖单克隆抗体、多克隆抗

体、二聚体、多聚体、多特异性抗体(例如双特异性抗体)及其抗原结合的抗体片段(Miller等人(2003)J.Immunology 170:4854-4861)。抗体可以是鼠、人、人源化、嵌合或衍生自其他物种。抗体是由能够识别和结合特异性抗原的免疫系统产生的蛋白质(Janeway,C.,Travers,P.,Walport,M.,Shlomchik(2001)Immuno Biology,第五版,Garland Publishing,New York)。靶抗原通常具有多个结合位点,也称为表位,由多个抗体上的CDR识别。特异性结合不同表位的每种抗体具有不同的结构。因此,一种抗原可被多于一种相应的抗体识别和结合。抗体包括全长免疫球蛋白分子或全长免疫球蛋白分子的免疫活性部分,即包含抗原结合位点的分子,其免疫特异性地结合关注的靶点的抗原或其部分,这样的靶点包括但不限于癌细胞或产生与自身免疫疾病相关的自身免疫抗体的细胞、感染的细胞、或微生物如细菌。本文公开的免疫球蛋白(Ig)可以是除IgM以外的任何同种型(例如IgG、IgE、IgD和IgA)和亚类(例如IgG1、IgG2、IgG3、IgG4、IgA1和IgA2)。免疫球蛋白可以来自任何物种。在一方面,Ig是人、鼠或兔来源的。在一个具体实施方案中,Ig是人来源的。

[0103] 抗体的"类"指由其重链拥有的恒定结构域或恒定区的类型。存在五个主要类别的抗体:IgA、IgD、IgE、IgG和IgM,并且这些类别中的几种可以进一步划分成亚类(同种型),例如IgG1、IgG2、IgG3、IgG4、IgA1和IgA2。对应于不同类别免疫球蛋白的重链恒定结构域分别称作α、δ、ε、γ和μ。

[0104] "天然抗体"是指具有不同结构的天然存在的免疫球蛋白分子。举例来说,天然IgG抗体为约150,000道尔顿的异四聚体糖蛋白,由二硫键键合的两个相同轻链和两个相同重链构成。自N末端至C末端,各重链具有可变区(VH),还被称为可变重结构域或重链可变结构域,随后为三个恒定结构域(CH1、CH2及CH3)。类似地,自N末端至C末端,各轻链具有可变区(VL),还被称为可变轻结构域或轻链可变结构域,随后为恒定轻(CL)结构域。抗体的轻链可基于其恒定结构域的氨基酸序列而指定为称为κ(κ)和λ(λ)的两种类型之一。

[0105] 术语"全长抗体"、"完整抗体"和"全抗体"在本文中可互换地用于指具有与天然抗体结构基本相似的抗体,或具有包含本文定义的Fc区的重链的抗体。

[0106] 抗体的"抗原结合片段"是指非完整抗体的分子,其包含完整抗体的一部分,能结合与完整抗体结合的抗原。抗体片段的实例包括但不限于Fv、Fab、Fab'、Fab'-SH、F(ab')2;双体;线性抗体;单链抗体分子(例如scFv);以及由抗体片段形成的多特异性抗体。

[0107] 如本文中所用的术语"单克隆抗体"是指从基本上同质的抗体群体获得的抗体,即,组成群体的单个抗体是相同的和/或结合相同的表位,除了可能的变体抗体之外,例如,含有天然发生的突变或在产生单克隆抗体制备物期间出现(例如,糖基化的天然变异),这类变体通常以微小的量存在。IgG1抗体的一个这样的可能变体是重链恒定区的C末端赖氨酸(K)的切割。与通常包括针对不同决定簇(表位)的不同抗体的多克隆抗体制备物不同,单克隆抗体制备物的每个单克隆抗体针对抗原上的单一决定簇。因此,修饰语"单克隆的"指示该抗体的特征为从基本上同质的抗体群体获得,并且不得解释为要求通过任何特定方法产生该抗体。例如,可以通过多种技术产生根据本发明使用的单克隆抗体,所述技术包括但不限于杂交瘤方法、重组DNA方法、噬菌体展示方法和利用含有全部或部分的人免疫球蛋白基因座的转基因动物的方法,在本文中描述了用于产生单克隆抗体的这类方法和其他示例性方法。除了它们的特异性之外,单克隆抗体是有利的,因为它们可以在不被其它抗体污染的情况下合成。

[0108] 术语“嵌合抗体”是指其中一部分重链和/或轻链衍生自特定来源或物种而重链和/或轻链的其余部分衍生自不同来源或物种的抗体。

[0109] “人抗体”是这样一种抗体，其拥有对应于人或人细胞产生的或衍生自利用人抗体库或其他编码人抗体的序列的非人来源的抗体的氨基酸序列。人抗体的这种定义特别排除包含非人抗原结合残基的人源化抗体。

[0110] “人源化抗体”指包含来自非人HVR的氨基酸残基和来自人FR的氨基酸残基的嵌合抗体。在某些实施方案中，人源化抗体将包含至少1个、并且通常2个可变结构域中的基本上全部，其中HVR(例如，CDR)中的全部或基本上全部与非人抗体中的那些对应，并且FR中的全部或基本上全部与人抗体中的那些对应。人源化抗体任选地可以包含从人抗体衍生的抗体恒定区的至少一部分。抗体(例如，非人抗体)的“人源化形式”指已经进行过人源化的抗体。

[0111] 术语“可变区”或“可变域”是指抗体的重或轻链中牵涉抗体结合抗原的结构域。天然抗体的重链和轻链可变域(分别为VH和VL)一般具有类似的结构，其中每个域包含4个保守的框架区(FR)和3个高变区(HVR)。(参见，例如，Kindt等人Kuby Immunology，第6版，W.H.Freeman和Co.，第91页(2007))。单个VH或VL域可足以赋予抗原结合特异性。此外，可以分别使用来自结合抗原的抗体的VH或VL域以筛选互补VL或VH域的文库，从而分离结合特定抗原的抗体。参见，例如Portolano等人J.Immunol.150:880-887(1993)；Clarkson等人，Nature 352:624-628(1991)。

[0112] 在本文中术语“高变区”，“HVR”或“HV”是指抗体可变区的区域，其在序列上是高变的(“互补决定区”或“CDR”)和/或形成结构上定义的环和/或含有抗原接触残基(“抗原接触”)。通常，抗体包含六个HVR；三个在VH(H1、H2、H3)中，三个在VL(L1、L2、L3)中。在天然抗体中，H3和L3显示了六个HVR中的最大多样性，特别是H3被认为在赋予抗体的特异性方面发挥独特的作用。参见，例如，Xu等人，Immunity 13:37-45(2000)；Johnson和Wu，分子生物学方法(Methods in Molecular Biology)248:1-25(Lo编辑，Human Press，Totowa，NJ，2003)。实际上，仅由重链组成的天然存在的骆驼抗体在轻链不存在的情况下是功能性的和稳定的(Hamers-Casterman等人，(1993)Nature 363:446-448；Sheriff等人，(1996)Nature Struct.Biol.3:733-736)。

[0113] 许多HVR描绘正在使用中并被包括在本文中。Kabat互补决定区(CDRs)基于序列变异性并且是最常用的(Kabat等人，Sequences of Proteins of Immunological Interest，5th Ed.Public Health Service，National Institutes of Health)，Bethesda，MD.(1991))。Chothia改为指结构环的位置(Chothia和Lesk，(1987)J.Mol.Biol.196:901-917)。对于抗原接触，请参阅MacCallum等人J.Mol.Biol.262:732-745(1996)。AbM HVRs代表Kabat HVRs和Chothia结构环之间的折衷，并由Oxford Molecular的AbM抗体建模软件使用。“接触”HVR是基于对可用的复杂晶体结构的分析。这些HVR中每一个的残基如下所述。

[0114]

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

[0115] HVR可以包含以下的“延伸HVR”：在VL中的24-36或24-34 (L1)、46-56或50-56 (L2)和89-97或89-96 (L3)，以及在VH中的26-35 (H1)、50-65或49-65 (H2)和93-102、94-102或95-102 (H3)。除非另有说明，在本文中根据Kabat等人的以上文章来编号HVR残基、CDR残基和可变结构域中的其它残基(例如FR残基)。

[0116] 术语“依照Kabat的可变结构域残基编号”或“依照Kabat的氨基酸位置编号”及其变化形式指Kabat等人的以上文章中的用于抗体编制的重链可变结构域或轻链可变结构域编辑的编号系统。使用此编号系统，实际的线性氨基酸序列可包含较少或另外的氨基酸，对应于可变结构域FR或HVR的缩短或插入。例如，重链可变结构域可包含H2的残基52后的单一氨基酸插入(依照Kabat为残基52a)及重链FR残基82后的插入残基(例如依照Kabat为残基82a、82b和82c等)。对于给定抗体的残基Kabat编号可通过将抗体序列与“标准的”Kabat编号序列对比同源区域来确定。

[0117] “框架”或“FR”是指除高变区 (HVR)残基外的可变结构域残基。一般地，可变结构域的FR由4个FR结构域组成：FR1、FR2、FR3和FR4。因此，HVR和FR序列在VH (或VL) 中一般以如下顺序出现：FR1-H1 (L1) -FR2-H2 (L2) -FR3-H3 (L3) -FR4。

[0118] 为了本文的目的，“受体人框架”为包含由人免疫球蛋白框架或如下文定义的人共有框架衍生的轻链可变域 (VL) 框架或重链可变域 (VH) 框架的氨基酸序列的框架。由人免疫球蛋白框架或人共有框架“衍生”的受体人框架可以包含与其相同的氨基酸序列，或者它可以含有氨基酸序列改变。在一些实施方案中，氨基酸改变的数目是10或更少、9或更少、8或更少、7或更少、6或更少、5或更少、4或更少、3或更少、或2或更少。在一些实施方案中，VL受体人框架与VL人免疫球蛋白框架序列或人共有框架序列在序列上相同。

[0119] “人共有框架”是表示人免疫球蛋白VL或VH框架序列选集中最常存在的氨基酸残基的框架。通常，人免疫球蛋白VL或VH序列选集来自可变域序列亚组。通常，序列的亚组是如Kabat等人，Sequences of Proteins of Immunological Interest,Fifth Edition,NIH Publication 91-3242,Bethesda MD (1991)，第1-3卷中的亚组。在一个实施方案中，对于VL，亚组是如Kabat等人的上述文章中的亚组κI。在一个实施方案中，对于VH，亚组是如Kabat等人的上述文章中的亚组III。

[0120] 本文中的术语“Fc区”用于定义免疫球蛋白重链的C末端区域。该术语包括天然序

列Fc区和变体Fc区。尽管免疫球蛋白重链的Fc区的边界可能变化，但人IgG重链Fc区通常定义为从Cys226或从Pro230位置的氨基酸残基延伸到其羧基末端。Fc区的C末端赖氨酸(根据EU编号系统的残基447-也称为EU指数，如在Kabat等人，Sequences of Proteins of Immunological Interest,5th Ed.Public Health Service,National Institutes of Health,Bethesda,MD,1991中所述)可以除去，例如，在抗体的制备或纯化期间，或通过重组工程化改造编码抗体重链的核酸。因此，完整抗体的组合物可以包含除去所有K447残基的抗体群体、未除去K447残基的抗体群体以及具有和不具有K447残基的抗体混合物的抗体群体。术语"Fc受体"或"FcR"还包括新生儿受体FcRn，其负责将母体IgG转移至胎儿。Guyer等人，J.Immunol.117:587(1976)和Kim等人，J.Immunol.24:249(1994)。测量与FcRn结合的方法是已知的(参见，例如，Ghetie和Ward,Immunol.Today 18:(12):592-8(1997)；Ghetie等人，自然生物技术(Nature Biotechnology)15(7):637-40(1997)；Hinton等人，J.Biol.Chem.279(8):6213-6(2004)；WO 2004/92219Hinton等人)。可以测定FcRn在体内的结合和人FcRn高亲和力结合多肽的血清半衰期，例如，在转基因小鼠或表达人FcRn的转染的人细胞系中，或在其中施用具有变体Fc区的多肽的灵长类动物中。WO 2004/42072(Presta)描述了抗体变体，其改善或减少与FcR的结合。另见，例如Shields等人，J.Biol.Chem.9(2):6591-6604(2001)。

[0121] "亲和力成熟的"抗体是指在一个或多个高变区(HVR)中具有一个或多个改变的抗体，与不具有这种改变的亲本抗体相比，这些改变导致抗体对抗原亲和力的改善。

[0122] 术语"表位"是指抗原分子上与抗体结合的特定位点。

[0123] "结合相同表位的抗体"作为参照抗体，是指在竞争测定中阻断参照抗体与其抗原结合50%以上的抗体，相反地，在竞争测定中参照抗体阻断该抗体与其抗原结合50%以上。本文提供了示例性的竞争测定。

[0124] "裸抗体"是指未与异源部分(例如细胞毒性部分)或放射性标记物缀合的抗体。裸抗体可以存在于药物制剂中。

[0125] "效应子功能"是指归因于抗体Fc区的那些生物活性，其随抗体同种型而变化。抗体效应子功能的实例包括：C1q结合和补体依赖性细胞毒性(CDC)；Fc受体结合；抗体依赖性细胞介导的细胞毒性(ADCC)；吞噬；细胞表面受体(例如B细胞受体)的下调；和B细胞激活。

[0126] "抗体依赖性细胞介导的细胞毒性"或ADCC是指细胞毒性的形式，其中分泌的Ig结合到某些细胞毒性细胞(例如，天然杀伤(NK)细胞、嗜中性粒细胞和巨噬细胞)上存在的Fc受体(FcR)上，使得这些细胞毒性效应细胞特异性结合携带抗原的靶细胞，随后用细胞毒素杀死靶细胞。抗体"武装"细胞毒性细胞，并且是通过这种机制杀死靶细胞所必需的。用于介导ADCC、NK细胞的初级细胞仅表达Fcγ(gamma)RIII，而单核细胞表达Fcγ(gamma)RI、Fcγ(gamma)RII和Fcγ(gamma)RIII。在Ravetch和Kinet,Annu.Rev.Immunol.9:457-92(1991)的第464页表3中总结了造血细胞上的Fc表达。为了评估关注的分子的ADCC活性，可以进行体外ADCC测定，例如可以进行在US 5,500,362或US 5,821,337中描述的那些。用于这种测定的有用的效应细胞包括外周血单核细胞(PBMC)和天然杀伤(NK)细胞。或者或另外，可以在体内评价关注的分子的ADCC活性，例如在Clynes等人，PNAS USA 95:652-656(1998)中公开的动物模型中。

[0127] "吞噬作用"是指病原体被宿主细胞(例如巨噬细胞或嗜中性粒细胞)吞噬或内化

的过程。吞噬细胞通过三种途径介导吞噬作用：(i)直接细胞表面受体(例如，凝集素、整联蛋白和清道夫受体)，(ii)补体增强-使用补体受体(包括CRI、C3b、CR3和CR4的受体)结合和摄取补体调理的病原体，和(iii)抗体增强-使用Fc受体(包括Fc γ gammaRI，Fc γ gammaRIIA和Fc γ gammaRIIIA)结合抗体调理的粒子，然后其被内化并与溶酶体融合成为吞噬溶酶体。在本发明中，认为途径(iii)在将抗MRSA AAC治疗剂递送到感染的白细胞(例如嗜中性粒细胞和巨噬细胞)中起重要作用(微生物的吞噬作用：行动的复杂性(Phagocytosis of Microbes:complexity in Action)D.Underhill和A Ozinsky.(2002)Annual Review of Immunology,Vol 20:825)。

[0128] "补体依赖性细胞毒性"或"CDC"是指在补体存在下裂解靶细胞。经典补体途径的激活由补体系统的第一组分(C1q)与结合于其同源抗原的抗体(适当亚类)结合引发。为了评估补体活化，可以使用CDC测定法，如Gazzano-Santoro等人，J.Immunol.Methods 202:163(1996)中所述。

[0129] 可以改变与Fc区连接的碳水化合物。由哺乳动物细胞产生的天然抗体通常包含支链的双分枝寡糖，其通常通过N-连接与Fc区的CH2结构域的Asn297连接。参见，例如Wright等人(1997)TIBTECH 15:26-32。寡糖可以包括各种碳水化合物，例如甘露糖、N-乙酰氨基葡萄糖(GlcNAc)、半乳糖和唾液酸，以及连接到双分枝寡糖结构的"茎"中的GlcNAc的岩藻糖。在一些实施方案中，可以制备在IgG中的寡糖修饰，以便产生具有某些另外改进特性的IgG。例如，提供具有碳水化合物结构的抗体修饰，其缺乏与Fc区(直接或间接)连接的岩藻糖。这样的修改可能具有改善的ADCC功能。参见，例如US 2003/0157108(Presta,L.)；US 2004/0093621(Kyowa Hakko Kogyo有限公司)。与"去岩藻糖化"或"岩藻糖缺乏"的抗体修饰相关的出版物的实例包括：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等人，J.Mol.Biol.336:1239-1249(2004)；Yamane-Ohnuki等人Biotech.Bioeng.87:614(2004)。能够产生去岩藻糖化抗体的细胞系的实例包括缺乏蛋白质岩藻糖基化的Lee 13CHO细胞(Ripka等人，Arch.Biochem.Biophys.249:533-545(1986)；美国专利申请公开号2003/0157108A1，Presta,L；和WO 2004/056312A1,Adams等人，特别是实施例11)和敲除细胞系，如α-1,6-岩藻糖基转移酶基因、FUT8、敲除的CHO细胞(参见例如Yamane-Ohnuki等人，Biotech.Bioeng.87:614(2004)；Kanda,Y.等人，Biotechnol.Bioeng.,94(4):680-688(2006)；和WO2003/085107)。

[0130] "分离的抗体"是已经与其天然环境的一种成分分开的抗体。在一些实施方案中，将抗体纯化至超过95%或99%纯度，如通过例如电泳(例如，SDS-PAGE、等电聚焦(IEF)、毛细管电泳)或色谱(例如，离子交换或反相HPLC)所测定的。关于评估抗体纯度的方法的综述参见，例如Flatman等人，J.Chromatogr.B 848:79-87(2007)。

[0131] "分离的核酸"是指已经与其天然环境的一种成分分开的核酸分子。分离的核酸包括正常情况下包含该核酸分子的细胞中含有的该核酸分子，但是该核酸分子存在于染色体外或与其天然染色体位置不同的染色体位置。

[0132] "分离的编码抗WTAβ抗体的核酸"是指一种或多种编码抗体重链和轻链的核酸分

子，包括在单一载体或分开的载体中的所述核酸分子，以及存在于宿主细胞中一个或多个位置处的所述核酸分子。

[0133] 如本文所用，术语“特异性结合”或“特异性”是指可测量和可重复的相互作用，例如靶点和抗体之间的结合，其在包括生物分子的异质分子群体存在下确定靶点的存在。例如，特异性结合靶点（其可以是表位）的抗体是以更高的亲和力、亲合力、更容易和/或具有比其它靶点结合更长的持续时间来结合该靶点的抗体。在一个实施方案中，例如通过放射免疫测定法（RIA）测量，抗体与和WTAβ无关的靶点的结合程度低于抗体与靶点结合程度的约10％。在某些实施方案中，特异性结合WTAβ的抗体具有≤1μM、≤100nM、≤10nM、≤1nM或≤0.1nM的解离常数（Kd）。在某些实施方案中，抗体特异性结合来自不同物种的表位。在另一个实施方案中，特异性结合可以包括、但不是必需排他性结合。

[0134] “结合亲和力”通常是指分子（例如，抗体）的单一结合位点与其结合配偶体（例如，抗原）之间的非共价相互作用的总和强度。除非另有说明，如本文所用，“结合亲和力”是指反映结合对成员（例如，抗体和抗原）之间的1:1相互作用的固有结合亲和力。分子X对其配偶体Y的亲和力通常可以由解离常数（Kd）表示。亲和力可以通过本领域已知的常规方法测量，包括本文所述的那些。低亲和力抗体通常缓慢地结合抗原并易于解离，而高亲和力抗体通常更快地结合抗原并且易于保持结合更长时间。测量结合亲和力的各种方法是本领域已知的，其中任何一种可以用于本发明的目的。以下描述用于测量结合亲和力的具体说明性和示例性实施方案。

[0135] 在一个实施方案中，根据本发明的“Kd”或“Kd值”，通过用目标抗体的Fab型及其抗原进行的放射性标记抗原结合测定（RIA）来测量，如以下测定所述。通过在存在未标记抗原的滴定系列的情况中用最小浓度的（^{125}I）-标记抗原而平衡Fab，然后用抗Fab抗体包被的板子捕捉结合的抗原来测量Fab对抗原的溶液结合亲和力（参见例如Chen等人，J.Mol.Biol.293:865-881（1999））。为了建立测定法的条件，微量滴定板（DYNEX Technologies,Inc.）用5μg/ml捕获性抗Fab抗体（Cappel Labs）在50mM碳酸钠（pH9.6）中包被过夜，随后在PBS中用2％（w/v）牛血清白蛋白于室温（约23℃）封闭2-5小时。在非吸附板（Nunc#269620）中，将100pM或26pM[^{125}I]-抗原与连续稀释的目标Fab混合（例如与Presta等人，Cancer Res.57:4593-4599（1997）中抗VEGF抗体Fab-12的评估一致）。然后将目标Fab孵育过夜；然而，孵育可持续更长时间（例如，约65小时）以确保达到平衡。此后，将混合物转移至捕获板，用于室温孵育（例如，1小时）。然后除去溶液，并用PBS中的0.1％吐温-20™表面活性剂洗板8次。平板干燥后，加入150μl/孔的闪烁液（MICROSCINT-20™；Packard），然后在TOPCOUNT™γ计数器（Packard）上对平板计数10分钟。选择给出小于或等于最大结合的20％的各Fab浓度，用于竞争性结合测定法。

[0136] 根据另一个实施方案，采用表面等离子共振测定法，使用**BIACORE®-2000**或**BIACORE®-3000**仪器（BIAcore,Inc.,Piscataway,NJ）于25℃用固定的抗原CM5芯片以约10个响应单位（RU）测量Kd。简言之，根据供应商的说明书，用N-乙基-N'-（3-二甲基氨基丙基）-碳二亚胺盐酸盐（EDC）和N-羟基琥珀酰亚胺（NHS）活化羧甲基化的右旋糖酐生物传感器芯片（CM5,BIAcore公司）。将抗原用10mM乙酸钠pH4.8稀释至5μg/ml（～0.2μM），之后以5μl/min的流速注射以实现大约10个响应单位（RU）的偶联蛋白质。注射抗原之后，注射1M

乙醇胺以封闭未反应基团。对于动力学测量，于25℃以大约25μl/min的流速注射在含0.05％吐温20™表面活性剂的PBS(PBST)中两倍连续稀释的Fab(0.78nM至500nM)。使用简单的一对一Langmuir结合模型(**BIAcore®**Evaluation Software version 3.2)，通过同时拟合结合和解离传感图来计算结合速率(k_{on})和解离速率(k_{off})。以k_{off}/k_{on}比率计算平衡解离常数(Kd)。参见，例如Chen等人，J.Mol.Biol.293:865-881(1999)。如果通过上述表面等离子共振测定法，结合速率超过$10^6M^{-1}s^{-1}$，那么可使用荧光淬灭技术来测定结合速率，所述技术在分光计例如带有断流装置的分光光度计(Aviv Instruments)或8000系列SLM-AMINCO™分光光度计(ThermoSpectronic)中用搅拌比色杯测量的浓度渐增的抗原存在下，测量在PBS pH7.2中20nM抗-抗原的抗体(Fab形式)于25℃的荧光发射强度(激发＝295nm；发射＝340nm，16nm带通)的升高或降低。

[0137] 根据本发明的“结合速率”或“k_{on}”还可以使用**BIACORE®-2000**或**BIACORE®-3000**系统(BIAcore，Inc.，Piscataway，NJ)按如上所述测定。

[0138] 术语“宿主细胞”、“宿主细胞系”和“宿主细胞培养物”可互换使用，并且指已经导入外源核酸的细胞，包括所述细胞的后代。宿主细胞包括“转化体”和“经转化的细胞”，其包括原代的经转化的细胞及由其衍生的后代而不考虑传代的次数。后代在核酸内容物上可以与亲本细胞不完全相同，而是可以含有突变。本文中包括具有与在初始转化细胞中筛选或选择的相同功能或生物学活性的突变体后代。

[0139] 如本文使用的术语“载体”是指能够增殖与其连接的另一种核酸的核酸分子。该术语包括作为自身复制型核酸结构的载体及整合到其已导入的宿主细胞的基因组中的载体。某些载体能够指导与其可操作连接的核酸的表达。所述载体在本文中称为“表达载体”。

[0140] 关于参照多肽序列的“氨基酸序列同一性百分比(％)”定义为候选序列中与参照多肽序列中的氨基酸残基相同的氨基酸残基的百分比，在比对序列并引入间隙之后，如果需要，可以达到最大百分比序列同一性，并且不考虑作为序列同一性的一部分的任何保守取代。用于确定氨基酸序列同一性百分比的比对可以通过本领域技术范围内的各种方式实现，例如，使用诸如BLAST、BLAST-2、ALIGN或Megalign(DNASTAR)软件的公众可用的计算机软件。本领域技术人员可以确定用于比对序列的适当参数，包括在待比较的序列的全长上实现最大比对所需的任何算法。然而，为了本文的目的，使用序列比较计算机程序ALIGN-2产生％氨基酸序列同一性值。Genentech公司编写了ALIGN-2序列比较计算机程序，并且源代码已经在华盛顿特区20559号美国版权局提交了用户文档，以美国版权注册号TXU510087注册。ALIGN-2程序可从加利福尼亚州南旧金山的Genentech公司公开获得，或者可以从源代码编译。ALIGN-2程序应编译为在UNIX操作系统上使用，包括数字UNIX V4.0D。所有序列比较参数由ALIGN-2程序设置，并且不变。

[0141] 在使用ALIGN-2用于氨基酸序列比较的情况下，给定的氨基酸序列A与或相对于给定的氨基酸序列B的氨基酸序列同一性％(其也可被称为具有或包含与或相对于给定氨基酸序列B的某一氨基酸序列同一性％的给定的氨基酸序列A)的计算如下：100乘以分数X/Y，其中X是在该程序的A和B的比对中，通过序列比对程序ALIGN-2评价为相同匹配的氨基酸残基数，并且其中Y是B中氨基酸残基的总数。应当理解，如果氨基酸序列A的长度不等于氨基酸序列B的长度，A与B的氨基酸序列同一性％将不等于B与A的氨基酸序列同一性％。除非另

有特别说明，否则本文使用的所有氨基酸序列同一性%值如上所述获得。

[0142] 术语“利福霉素型抗生素”是指具有利福霉素结构或类似结构的抗生素类或组。

[0143] 术语“利福拉齐型抗生素”是指具有利福拉齐结构或类似结构的抗生素类或组。

[0144] 当指示取代基的数目时，术语“一个或多个”是指从一个取代基到最高可能取代数的范围，即，替换一个氢到通过取代基替换所有氢。术语“取代基”表示替换母体分子上的氢原子的一个原子或一组原子。术语“取代的”表示指定的基团具有一个或多个取代基。当任何基团可以携带多种取代基且提供多种可能的取代基时，独立地选择取代基并且不必相同。术语“未取代的”是指指定的基团不含取代基。术语“任选取代的”是指指定基团是未取代的或被一个或多个取代基取代，其独立地选自可能的取代基的组。当指示取代基的数目时，术语“一个或多个”是指从一个取代基到最高可能的取代数量，即通过取代基替换一个氢直到替换所有氢。

[0145] 本文所用的术语“烷基”是指具有1至12个碳原子(C_1-C_{12})的饱和直链或支链单价烃基，其中烷基可以任选地被一个或多个下述取代基独立地取代。在另一个实施方案中，烷基为1至8个碳原子(C_1-C_8)或1至6个碳原子(C_1-C_6)。烷基的实例包括但不限于甲基(Me，-CH_3)、乙基(Et，-CH_2CH_3)、1-丙基(n-Pr，正丙基，-$CH_2CH_2CH_3$)、2-丙基(i-Pr，异丙基，-$CH(CH_3)_2$)、1-丁基(n-Bu，正丁基，-$CH_2CH_2CH_2CH_3$)、2-甲基-1-丙基(i-Bu，异丁基，-$CH_2CH(CH_3)_2$)、2-丁基(s-Bu，仲丁基，-$CH(CH_3)CH_2CH_3$)、2-甲基-2-丙基(t-Bu，叔丁基，-$C(CH_3)_3$)、1-戊基(正戊基，-$CH_2CH_2CH_2CH_2CH_3$)、2-戊基(-$CH(CH_3)CH_2CH_2CH_3$)、3-戊基(-$CH(CH_2CH_3)_2$)、2-甲基-2-丁基(-$C(CH_3)_2CH_2CH_3$)、3-甲基-2-丁基(-$CH(CH_3)CH(CH_3)_2$)、3-甲基-1-丁基(-$CH_2CH_2CH(CH_3)_2$)、2-甲基-1-丁基(-$CH_2CH(CH_3)CH_2CH_3$)、1-己基(-$CH_2CH_2CH_2CH_2CH_2CH_3$)、2-己基(-$CH(CH_3)CH_2CH_2CH_2CH_3$)、3-己基(-$CH(CH_2CH_3)(CH_2CH_2CH_3)$)、2-甲基-2-戊基(-$C(CH_3)_2CH_2CH_2CH_3$)、3-甲基-2-戊基(-$CH(CH_3)CH(CH_3)CH_2CH_3$)、4-甲基-2-戊基(-$CH(CH_3)CH2CH(CH_3)_2$)、3-甲基-3-戊基(-$C(CH_3)(CH_2CH_3)_2$)、2-甲基-3-戊基(-$CH(CH_2CH_3)CH(CH_3)_2$)、2,3-二甲基-2-丁基(-$C(CH_3)_2CH(CH_3)_2$)、3,3-二甲基-2-丁基(-$CH(CH_3)C(CH_3)_3$)、1-庚基、1-辛基等。

[0146] 本文所用的术语“亚烷基”是指1至12个碳原子(C_1-C_{12})的饱和直链或支链二价烃基，其中亚烷基可以任选地被一个或多个下述取代基独立地取代。在另一个实施方案中，亚烷基是1-8个碳原子(C_1-C_8)或1-6个碳原子(C_1-C_6)。亚烷基的实例包括但不限于亚甲基(-CH_2-)、亚乙基(-CH_2CH_2-)、亚丙基(-$CH_2CH_2CH_2$-)等。

[0147] 术语“烯基”是指具有至少一个不饱和位点即碳-碳sp^2双键的2-8个碳原子(C_2-C_8)的直链或支链一价烃基，其中烯基可以任选地被本文所述的一个或多个取代基独立地取代，并且包括具有“顺式”和“反式”取向，或者“E”和“Z”取向的基团。实例包括但不限于亚乙基或乙烯基(-CH=CH_2)、烯丙基(-CH_2CH=CH_2)等。

[0148] 术语“亚烯基”是指具有至少一个不饱和位点即碳-碳sp^2双键的2-8个碳原子(C_2-C_8)的直链或支链二价烃基，其中亚烯基可以任选地被本文所述的一个或多个取代基独立地取代，并且包括具有“顺式”和“反式”取向，或者“E”和“Z”取向的基团。实例包括但不限于亚乙烯基(-CH=CH-)、烯丙基(-CH_2CH=CH-)等。

[0149] 术语“炔基”是指具有至少一个不饱和位点即碳-碳sp叁键的2-8个碳原子(C_2-C_8)的直链或支链一价烃基，其中炔基可以任选地被本文所述的一个或多个取代基独立地取

代。实例包括但不限于乙炔基(-C≡CH)、丙炔基(炔丙基,-CH_2C≡CH)等。

[0150] 术语"亚炔基"是指具有至少一个不饱和位点即碳-碳sp叁键的2-8个碳原子(C_2-C_8)的直链或支链二价烃基,其中亚炔基可以任选地被本文所述的一个或多个取代基独立地取代。实例包括但不限于亚乙炔基(-C≡C-)、亚丙炔基(亚炔丙基,-CH_2C≡C-)等。

[0151] 术语"碳环"、"碳环基"和"环烷基"是指单价非芳香族饱和或部分不饱和环,具有3-12个碳原子(C_3-C_{12})的作为单环,或具有7至12个碳原子作为双环。具有7至12个原子的双环碳环可以排列成,例如双环[4,5]、[5,5]、[5,6]或[6,6]系统,并且具有9或10个环原子的双环碳环可以排列成双环[5,6]或[6,6]系统,或成桥连系统如双环[2.2.1]庚烷、双环[2.2.2]辛烷和双环[3.2.2]壬烷。螺环部分也包括在本定义的范围内。单环碳环的实例包括但不限于环丙基、环丁基、环戊基、1-环戊-1-烯基、1-环戊-2-烯基、1-环戊-3-烯基、环己基、1-环己-1-烯基、1-环己-2-烯基、1-环己-3-烯基、环己二烯基、环庚基、环辛基、环壬基、环癸基、环十一烷基、环十二烷基等。碳环基基团任选被一个或多个本文所述的取代基独立地取代。

[0152] "芳基"是指通过从母体芳族环系统的单个碳原子除去一个氢原子而衍生的6-20个碳原子(C_6-C_{20})的单价芳族烃基。一些芳基在示例性结构中表示为"Ar"。芳基包括双环基团,其包含与饱和的,部分不饱和的环或芳族碳环稠合的芳香环。典型的芳基包括但不限于衍生自苯(苯基)、取代的苯、萘、蒽、联苯、茚基、茚满基、1,2-二氢萘、1,2,3,4-四氢萘基等的基团。芳基任选地被一个或多个本文所述的取代基独立地取代。

[0153] "亚芳基"是指通过从母体芳族环系统的两个碳原子除去两个氢原子而衍生的6-20个碳原子(C_6-C_{20})的二价芳族烃基。一些亚芳基在示例性结构中表示为"Ar"。亚芳基包括双环基团,其包含与饱和的,部分不饱和的环或芳族碳环稠合的芳香环。典型的亚芳基包括但不限于衍生自苯(亚苯基)、取代苯、萘、蒽、联苯、亚茚基、亚茚满基、1,2-二氢萘、1,2,3,4-四氢萘基等的基团。亚芳基任选地被一个或多个本文所述的取代基取代。

[0154] 术语"杂环"和"杂环基"在本文中可互换使用,是指饱和或部分不饱和(即,在环内具有一个或多个双键和/或叁键)的3至约20个环原子的碳环基,其中至少一个环原子是选自氮、氧、磷和硫的杂原子,剩余的环原子是C,其中一个或多个环原子任选地被一个或多个如下所述的取代基独立地取代。杂环可以是具有3至7个环成员(2至6个碳原子和1至4个选自N,O,P和S的杂原子)的单环或具有7至10个环成员(4至9个碳原子和1至6个选自N,O,P和S的杂原子)的双环,例如:双环[4,5]、[5,5]、[5,6]或[6,6]系统。杂环描述于Paquette,Leo A.,"现代杂环化学原理(Principles of Modern Heterocyclic Chemistry)"(W.A.Benjamin,New York,1968),特别是第1、3、4、6、7和9章;"杂环化合物化学,一系列专著(The Chemistry of Heterocyclic Compounds,A series of Monographs)"(John Wiley&Sons,New York,1950至今),特别是第13、14、16、19和28卷;和J.Am.Chem.Soc.(1960)82:5566。"杂环基"还包括其中杂环基与饱和的,部分不饱和环或芳族碳环或杂环稠合的基团。杂环的实例包括但不限于吗啉-4-基、哌啶-1-基、哌嗪基、哌嗪-4-基-2-酮、哌嗪-4-基-3-酮、吡咯烷-1-基、硫吗啉-4-基、S-二氧代硫吗啉-4-基、氮杂环辛烷-1-基、氮杂环丁烷-1-基、八氢吡啶并[1,2-a]吡嗪-2-基、[1,4]二氮杂环庚-1-基、吡咯烷基、四氢呋喃基、二氢呋喃基、四氢噻吩基、四氢吡喃基、二氢吡喃基、四氢噻喃基、哌啶基、吗啉代、硫代吗啉代、噻咯烷基、哌嗪基、高哌嗪基、氮杂环丁烷基、氧杂环丁烷基、硫杂环丁烷基、高哌啶

基、氧杂环庚烷基、硫杂环庚烷基、氧氮杂䓬基、二氮杂䓬基、硫杂䓬基、2-吡咯啉基、3-吡咯啉基、二氢吲哚基、2H-吡喃基、4H-吡喃基、二噁烷基、1,3-二氧杂环戊烷基、吡唑啉基、二噻烷基、二硫杂环戊烷基、二氢吡喃基、二氢噻吩基、二氢呋喃基、吡唑烷基、咪唑啉基、咪唑烷基、3-氮杂双环[3.1.0]己烷基、3-氮杂二环[4.1.0]庚烷基、氮杂双环[2.2.2]己烷基、3H-吲哚基、喹嗪基和N-吡啶基脲。螺环部分也包括在本定义的范围内。其中2个环原子被氧代(=O)部分取代的杂环基团的实例是嘧啶酮基和1,1-二氧代-硫吗啉基。本文的杂环基团任选地被一个或多个本文所述的取代基独立地取代。

[0155] 术语"杂芳基"是指5-、6-或7-元环的单价芳族基团,并且包括5-20个原子的稠环体系(其中至少一个是芳族的),其独立地含有一个或多个选自氮、氧和硫的杂原子。杂芳基的实例是吡啶基(包括例如,2-羟基吡啶基)、咪唑基、咪唑并吡啶基、嘧啶基(包括例如,4-羟基嘧啶基)、吡唑基、三唑基、吡嗪基、四唑基、呋喃基、噻吩基、异噁唑基、噻唑基、噁二唑基、噁唑基、异噻唑基、吡咯基、喹啉基、异喹啉基、四氢异喹啉基、吲哚基、苯并咪唑基、苯并呋喃基、噌啉基、吲唑基、吲哚基、酞嗪基、哒嗪基、三嗪基、异吲哚基、蝶啶基、嘌呤基、噁二唑基、三唑基、噻二唑基、呋咱基、苯并呋咱基、苯并噻吩基、苯并噻唑基、苯并噁唑基、喹唑啉基、喹喔啉基、萘啶基和呋喃并吡啶基。杂芳基任选被一个或多个本文所述的取代基独立地取代。

[0156] 在可能的情况下,杂环或杂芳基可以是碳(碳连接的)或氮(氮连接的)键合的。作为举例而非限制,碳键合的杂环或杂芳基键合在吡啶的第2、3、4、5或6位,哒嗪的第3、4、5或6位,嘧啶的2、4、5或6位,吡嗪的2、3、5或6位,呋喃、四氢呋喃、噻吩(thiofuran)、噻吩(thiophene)、吡咯或四氢吡咯的2、3、4或5位,噁唑、咪唑或噻唑的2、4或5位,异噁唑、吡唑或异噻唑的3、4或5位,氮丙啶的2或3位,氮杂环丁烷的2、3或4位,喹啉的2、3、4、5、6、7或8位,或异喹啉的1、3、4、5、6、7或8位。

[0157] 举例而言而非限制,氮键合的杂环或杂芳基键合于氮丙啶、氮杂环丁烷、吡咯、吡咯烷、2-吡咯啉、3-吡咯啉、咪唑、咪唑啉、2-咪唑啉、3-咪唑啉、吡唑、吡唑啉、2-吡唑啉、3-吡唑啉、哌啶、哌嗪、吲哚、二氢吲哚、1H-吲唑的1位,异吲哚或异吲哚啉的2位,吗啉的4位,以及咔唑或β-咔啉的9位。

[0158] "代谢物"是特定化合物或其盐通过体内代谢产生的产物。可以使用本领域已知的常规技术来鉴定化合物的代谢物,并且使用本文所述的测试来确定其活性。这样的产物可以例如由所施用的化合物的氧化、还原、水解、酰胺化、脱酰胺化、酯化、脱酯化、酶裂解等产生。因此,本发明包括本发明化合物的代谢物,包括通过包含使本发明的式I化合物与哺乳动物接触足以产生其代谢产物的时间的方法产生的化合物。

[0159] 术语"药物制剂"是指处于如下的形式,使得容许其中含有的活性成分的生物学活性是有效的,且不含对将接受制剂施用的个体具有不可接受的毒性的其他组分的制剂。

[0160] "无菌"制剂是无菌的或不含所有活的微生物及其孢子。

[0161] "稳定"制剂是其中蛋白质在储存时基本上保持其物理和化学稳定性和完整性的制剂。用于测量蛋白质稳定性的各种分析技术在本领域是可获得的,并且在肽和蛋白质药物递送(Peptide and Protein Drug Delivery),247-301,Vincent Lee Ed.,Marcel Dekker,Inc.,New York,New York,Pubs.(1991)和Jones,A.Adv.Drug Delivery Rev.10:29-90(1993)中进行了综述。稳定性可以在所选择的温度下测量所选择的时间段。为了快速

筛选，制剂可以在40℃保持2周至1个月，此时测定稳定性。当制剂在2-8℃下储存时，通常制剂应在30℃或40℃下稳定至少1个月，和/或在2-8℃下稳定至少2年。当制剂在30℃下储存时，通常制剂应在30℃下稳定至少2年，和/或在40℃稳定至少6个月。例如，储存期间的聚集程度可以用作蛋白质稳定性的指标。因此，“稳定”制剂可以是其中小于约10％和优选小于约5％的蛋白质在制剂中以聚集体存在。在其它实施方案中，可以确定在制剂储存过程中聚集体形成的任何增加。

[0162] “等渗”制剂是具有与人血液基本相同的渗透压的制剂。等渗制剂通常具有约250至350mOsm的渗透压。术语“低渗”描述渗透压低于人血液的制剂。相应地，术语“高渗”用于描述渗透压高于人血液的制剂。例如，可以使用蒸气压或冰冻型渗透压计测量等渗性。作为加入盐和/或缓冲液的结果，本发明的制剂是高渗的。

[0163] 本文所用的“载体”包括药学上可接受的载体，赋形剂或稳定剂，其所使用的剂量和浓度对暴露于其中的细胞或哺乳动物是无毒的。生理上可接受的载体通常是含水pH缓冲溶液。生理上可接受的载体的实例包括缓冲液如磷酸盐、柠檬酸盐和其它有机酸；抗氧化剂，包括抗坏血酸；低分子量（少于约10个残基）多肽；蛋白质，如血清白蛋白、明胶或免疫球蛋白；亲水性聚合物如聚乙烯吡咯烷酮；氨基酸如甘氨酸、谷氨酰胺、天冬酰胺、精氨酸或赖氨酸；单糖、二糖和其他碳水化合物，包括葡萄糖、甘露糖或糊精；螯合剂如EDTA；糖醇如甘露醇或山梨糖醇；盐形成抗衡离子如钠；和/或非离子表面活性剂如吐温®，聚乙二醇（PEG）和PLURONICS™。

[0164] “药学上可接受的载体”是指药物制剂中活性成分以外的对个体无毒的成分。药学上可接受的载体包括但不限于缓冲剂、赋形剂、稳定剂或防腐剂。“药学上可接受的酸”包括在配制浓度和方式下无毒的无机酸和有机酸。例如，合适的无机酸包括盐酸、高氯酸、氢溴酸、氢碘酸、硝酸、硫酸、磺酸、亚磺酸、对氨基苯磺酸、磷酸、碳酸等。合适的有机酸包括直链和支链烷基、芳族、环状、脂环族、芳基脂族、杂环、饱和、不饱和、单、二和三羧酸，包括例如甲酸、乙酸、2-羟基乙酸、三氟乙酸、苯乙酸、三甲基乙酸、叔丁基乙酸、邻氨基苯甲酸、丙酸、2-羟基丙酸、2-氧代丙酸、丙二酸、环戊烷丙酸、3-苯基丙酸、丁酸、丁二酸、苯甲酸、3-(4-羟基苯甲酰基)苯甲酸、2-乙酰氧基-苯甲酸、抗坏血酸、肉桂酸、月桂基硫酸、硬脂酸、粘康酸、扁桃酸、琥珀酸、双羟萘酸、富马酸、苹果酸、马来酸、羟基马来酸、丙二酸、乳酸、柠檬酸、酒石酸、乙醇酸、葡萄糖酸、葡糖酸、丙酮酸、乙醛酸、草酸、甲磺酸、琥珀酸、水杨酸、邻苯二甲酸、扑酸、棕榈酸(palmeic acid)、硫氰酸、甲磺酸、乙磺酸、1,2-乙烷二磺酸、2-羟基乙磺酸、苯磺酸、4-氯苯磺酸、萘-2-磺酸、对甲苯磺酸、樟脑磺酸、4-甲基双环[2.2.2]辛-2-烯-1-甲酸、葡庚糖、4,4'-亚甲基双-3-(羟基-2-烯-1-甲酸)、羟基萘甲酸。

[0165] “药学上可接受的碱”包括以配制的浓度和方式无毒的无机和有机碱。例如，合适的碱包括由无机碱形成金属如锂、钠、钾、镁、钙、铵、铁、锌、铜、锰、铝、N-甲基葡糖胺、吗啉、哌啶形成的碱和有机无毒碱包括伯胺、仲胺和叔胺、取代的胺、环胺和碱性离子交换树脂，[例如，$N(R')_4^+$（其中R'是独立地H或C_{1-4}烷基，例如铵、Tris)]，例如异丙胺、三甲胺、二乙胺、三乙胺、三丙胺、乙醇胺、2-二乙氨基乙醇、三甲胺、二环己胺、赖氨酸、精氨酸、组氨酸、咖啡因、普鲁卡因、海巴明、胆碱、甜菜碱、乙二胺、葡糖胺、甲基葡糖胺、可可碱、嘌呤、哌嗪、哌啶、N-乙基哌啶、聚胺树脂等。特别优选的有机无毒碱是异丙胺、二乙胺、乙醇胺、三甲基胺、二环己胺、胆碱和咖啡因。

[0166] 可用于本发明的其它药学上可接受的酸和碱包括衍生自氨基酸的那些，例如组氨酸，甘氨酸，苯丙氨酸，天冬氨酸，谷氨酸，赖氨酸和天冬酰胺。

[0167] “药学上可接受的”缓冲剂和盐包括衍生自上述酸和碱的酸和碱加成盐的那些。特定的缓冲液和/或盐包括组氨酸，琥珀酸盐和乙酸盐。

[0168] “药学上可接受的糖”是当与关注的蛋白质结合时，显著地防止或减少储存时蛋白质的化学和/或物理不稳定性的分子。当制剂被冻干，然后重构时，“药学上可接受的糖”也可以称为“冻干保护剂”。示例性的糖及其相应的糖醇包括：氨基酸，如谷氨酸钠或组氨酸；甲胺如甜菜碱；溶致盐如硫酸镁；多元醇，例如三元或更高分子量的糖醇，例如甘油、葡聚糖、赤藓糖醇、甘油、阿糖醇、木糖醇、山梨醇和甘露醇；丙二醇；聚乙二醇；**PLURONICS®**；及其组合。另外的示例性的冻干保护剂包括甘油和明胶，以及麦芽糖、松三糖、棉子糖、甘露三糖和水苏糖。还原糖的实例包括葡萄糖、麦芽糖、乳糖、麦芽酮糖、异麦芽酮糖和乳果糖。非还原性糖的实例包括选自糖醇和其它直链多元醇的多羟基化合物的非还原性糖苷。优选的糖醇是单糖苷，特别是通过还原二糖，例如乳糖、麦芽糖、乳果糖和麦芽酮糖，获得的那些化合物。糖苷侧基可以是糖苷或半乳糖苷。糖醇的另外的实例是葡萄糖醇、麦芽糖醇、乳糖醇和异麦芽酮糖。优选的药学上可接受的糖是非还原糖海藻糖或蔗糖。将药学上可接受的糖以“保护量”(例如预冻干)加入到制剂中，这意味着蛋白质在储存期间(例如，在重构和储存之后)基本上保持其物理和化学稳定性和完整性。

[0169] 本文所关注的“稀释剂”是药学上可接受的(施用于人类时安全无毒)，并且可用于制备液体制剂，例如冻干后重新配制的制剂。示例性的稀释剂包括无菌水、注射用抑菌水(BWFI)、pH缓冲溶液(例如磷酸盐缓冲盐水)、无菌盐水溶液、林格溶液或葡萄糖溶液。在替代实施方案中，稀释剂可以包括盐和/或缓冲液的水溶液。

[0170] “防腐剂”是可以加入到本文制剂中以减少细菌活性的化合物。例如，添加防腐剂可以促进生产多用途(多剂量)制剂。潜在防腐剂的实例包括十八烷基二甲基苄基氯化铵、氯化六烃季铵、苯扎氯铵(烷基苄基二甲基氯化铵的混合物，其中烷基是长链化合物)和苄索氯铵。其他类型的防腐剂包括芳族醇如苯酚、丁基和苄醇，对羟基苯甲酸烷基酯如对羟基苯甲酸甲酯或对羟基苯甲酸丙酯、邻苯二酚、间苯二酚、环己醇、3-戊醇和间甲酚。本文最优选的防腐剂是苄醇。

[0171] “个体”或“受试者”或“患者”是哺乳动物。哺乳动物包括但不限于驯养的动物(例如，牛、羊、猫、狗和马)，灵长类动物(例如，人类和非人类灵长类动物如猴子)，兔子和啮齿动物(例如，小鼠和大鼠)。在某些实施方案中，个体或受试者是人。

[0172] 如本文所用，“治疗”(及其语法变化例如“治疗”)是指临床干预，旨在临床病理过程中改变待治疗的个体，组织或细胞的自然过程。治疗的期望效果包括但不限于降低疾病进展的速度，改善或缓解疾病状态，以及缓解或改善的预后，所有这些都可由本领域技术人员如医师检测。在一个实施方案中，治疗可以意味着减轻症状，减轻疾病的任何直接或间接的病理后果，降低感染性疾病进展的速度，疾病状态的改善或缓解，以及缓解或改善的预后。在一些实施方案中，本发明的AAC、TAC用于延缓疾病的发展或减缓感染性疾病的进展或减少血流和/或感染的组织和器官中的细菌负荷。

[0173] 如本文所用，“联合”是指除了另一种治疗方式之外施用的一种治疗方式。因此，“联合”是指在向个人施用其他治疗方式之前，期间或之后施用的一种治疗方式。

[0174] 术语“菌血症”是指血液中存在细菌，其是通过血液培养最常检测到的。在手术期间(特别是当涉及粘膜如胃肠道时)，或由于导管和其他异物进入动脉或静脉时，细菌可以进入血液作为感染(如肺炎或脑膜炎)的严重并发症。菌血症有几个后果。对细菌的免疫应答可导致败血症和败血性休克，死亡率相对较高。细菌也可以通过血液传播到身体的其他部位，导致感染远离原始感染部位。实例包括心内膜炎或骨髓炎。

[0175] “治疗有效量”是实现特定病症的可测量改善所需的最小浓度。本文的治疗有效量可以根据诸如患者的疾病状态、年龄、性别和体重等因素，以及抗体在个体中引起所需反应的能力而变化。治疗有效量也是抗体的治疗有益效果超过任何毒性或有害作用的量。在一个实施方案中，治疗有效量是有效降低体内感染中的菌血症的量。在一方面，“治疗有效量”是至少有效地减少从患者样品(例如血液)分离出的相对于给药前至少一个对数的细菌负荷或集落形成单位(CFU)的量。在更具体的方面，减少至少为2个对数。在另一方面，减少量至少为3、4、5个对数。然而在另一方面，使用本领域已知的测定法，包括本文所例示的测定，降低到可检测水平以下。在另一个实施方案中，与感染患者治疗前或治疗开始时的阳性血培养物相比，治疗有效量是在治疗期间给予的一个或多个剂量中的AAC的量，其实现阴性的血培养物(即，不产生作为AAC靶点的细菌)。

[0176] “预防有效量”是指为达到期望的预防结果在所需的剂量和时间段内的有效量。通常但不一定是由于在疾病的早期阶段，甚至在暴露于感染风险升高的条件之前，在个体中使用预防剂量时，预防有效量可以小于治疗有效量。在一个实施方案中，预防有效量是至少有效减少、预防感染从一个细胞到另一个细胞的发生或扩散的量。

[0177] “慢性”给药是指以连续而不是急性模式施用药物，以便在较长时间内维持初始治疗效果(活性)。“间歇性”给药是并非不间断地连续进行的治疗，而是本质上是周期性的。

[0178] 术语“包装插入物”用于指通常包括在治疗产品的商业包装中的说明书，其包含关于使用这种治疗产品的适应症、用途、剂量、给药、组合疗法、禁忌症和/或警告的信息。

[0179] 术语“手性”是指具有镜像配偶体不重叠性质的分子，而术语“非手性”是指在其镜像配偶体上重叠的分子。

[0180] 术语“立体异构体”是指具有相同化学构成但空间中原子或基团的排列不同的化合物。

[0181] “非对映体”是指具有两个或更多个手性中心的立体异构体，其分子不是彼此的镜像。非对映体具有不同的物理性质，例如熔点，沸点，光谱性质和反应性。非对映异构体的混合物可以在高分辨率分析方法如电泳和色谱中分离。

[0182] “对映异构体”是指化合物的两种立体异构体，它们是彼此不重叠的镜像。

[0183] 本文使用的立体化学定义和惯例通常遵循S.P.Parker编辑，McGraw-Hill化学词典(McGraw-Hill Dictionary of Chemical Terms)(1984)McGraw-Hill Book Company，New York；和Eliel，E.和Wilen，S.，有机化合物的立体化学(Stereochemistry of Organic Compounds)(1994)John Wiley&Sons，Inc.，New York。许多有机化合物以光学活性形式存在，即它们具有旋转偏振光平面的能力。在描述光学活性化合物时，前缀D和L，或R和S用于表示分子关于其手性中心的绝对构型。使用前缀d和l或(+)和(-)来表示化合物对偏振光平面的旋转符号，其中(-)或l表示化合物是左旋的。以(+)或d为前缀的化合物是右旋的。对于给定的化学结构，这些立体异构体是相同的，除了它们是彼此的镜像。具体的立体异构体也

可以称为对映异构体，这些异构体的混合物通常称为对映体混合物。将对映异构体的50:50混合物称为外消旋混合物或外消旋物，其可能发生在化学反应或过程中不存在立体选择性或立体定向性的情况。术语“外消旋混合物”和“外消旋物”是指没有光学活性的两种对映体物质的等摩尔混合物。

[0184] 术语“保护基团”是指这样的取代基，其通常用于阻断或保护特定官能团，而其它官能团在化合物上起反应。例如，“氨基保护基”是连接到氨基上的取代基，该氨基阻断或保护化合物中的氨基官能团。合适的氨基保护基包括但不限于乙酰基、三氟乙酰基、叔丁氧羰基(BOC)、苄氧羰基(CBZ)和9-芴基亚甲氧基羰基(Fmoc)。关于保护基及其用途的一般描述，参见T.W.Greene,有机合成中的保护基(Protective Groups in Organic Synthesis)，John Wiley&Sons,New York,1991,或更新版本。

[0185] 本文所用的术语“约”是指本技术领域中的技术人员熟知的相应值的通常误差范围。在此引用“约”值或参数包括(特别描述)针对该值或参数本身的实施方案。

[0186] 如本文和所附权利要求中所使用，单数形式“一种”、“一个”和“该”包括复数参考，除非上下文另有明确指出。例如，提到“抗体”时是指从一个到多个抗体，例如以摩尔量，并且包括本领域技术人员已知的其等价物等。

[0187] III.组成成分和方法

[0188] 抗生素-抗体缀合物(AAC)

[0189] 本文的实验结果强有力地表明，旨在消除细胞内细菌的疗法将提高临床成功率。为了实现这一目的，本发明提供了一种独特的治疗剂，其选择性地杀死已经侵入宿主细胞的细胞内区室的金黄色葡萄球菌。本发明证明这样的治疗剂在常规抗生素如万古霉素失败的体内模型中是有效的。

[0190] 本发明提供了一种抗菌治疗，其旨在通过靶向于逃避常规抗生素治疗的细菌群而防止抗生素逃避。该新型抗菌治疗是通过抗体抗生素缀合物(AAC)实现的，其中对在金黄色葡萄球菌(包括MRSA)上发现的细胞壁组分特异型的抗体与有效的抗生素(利福霉素衍生物)化学连接。该抗生素通过设计为可被蛋白酶切割的蛋白酶可切割的非肽类接头连接到抗体上，所述蛋白酶包括组织蛋白酶B，其是大多数哺乳动物细胞类型中发现的溶酶体蛋白酶(Dubowchik等人(2002)Bioconj.Chem.13:855-869)。图2中显示了具有3个组分的AAC的图。不受任何一种理论的限制，AAC的一种作用机制如图3所示。AAC作为前药，其中抗生素是无活性的(由于抗体的大尺寸)，直到接头被切割为止。由于在天然感染中发现的显著比例的金黄色葡萄球菌在宿主感染过程中的某一时刻被宿主细胞吸收，主要是嗜中性粒细胞和巨噬细胞，在宿主细胞内的时间为细菌提供了逃避抗生素活性的重要机会。本发明的AAC被设计为结合葡萄球菌细菌，并且在细菌被宿主细胞摄取后在吞噬体内部释放抗生素。通过这种机制，AAC能够将活性抗生素专门集中在通过常规抗生素治疗金黄色葡萄球菌不佳的地方。虽然本发明不受特定作用机制的限制或限定，但AAC通过三种潜在的机制改善抗生素活性：(1)AAC在摄取细菌的哺乳动物细胞内递送抗生素，从而增加难以扩散进入细菌隐藏的吞噬溶酶体中的抗生素的效力。(2)AAC调理细菌，从而增加吞噬细胞对游离细菌的吸收，并在局部释放抗生素以便当细菌在吞噬溶酶体中隐藏时杀死细菌。由于成千上万的AAC可以结合单个细菌，因此该平台在这些细胞内的小环境中释放出足够的抗生素，以维持最大的抗菌杀伤力。此外，随着越来越多的细菌从预先存在的细胞内储库中释放出来，这种基于

抗体的治疗方法的快速结合率确保这些细菌立即被“标记”，然后才能逃逸到相邻或远端的细胞，从而减轻感染的进一步扩散。(3)与从血清中快速清除的抗生素相比，AAC通过将抗生素与抗体连接来改善体内抗生素的半衰期(改善的药代动力学)。AAC的改善的药代动力学能够在金黄色葡萄球菌富集的区域中递送足够的抗生素，同时限制需要全身施用的抗生素的总体剂量。该属性将允许用AAC长期治疗以靶向于持续感染，且抗生素副作用最小。

[0191] 抗体-抗生素缀合化合物包含抗壁磷壁酸(WTA)抗体，其通过蛋白酶可切割的非肽类接头共价连接到利福霉素型抗生素。

[0192] 示例性实施方案是具有下式的抗体-抗生素缀合物：

[0193] $Ab-(PML-abx)_p$

[0194] 其中：

[0195] Ab是抗壁磷壁酸抗体；

[0196] PML是具有下式的蛋白酶可切割的非肽类接头：

[0197] -Str-PM-Y-

[0198] 其中Str是延伸单元；PM是拟肽单元，和Y是间隔单元；

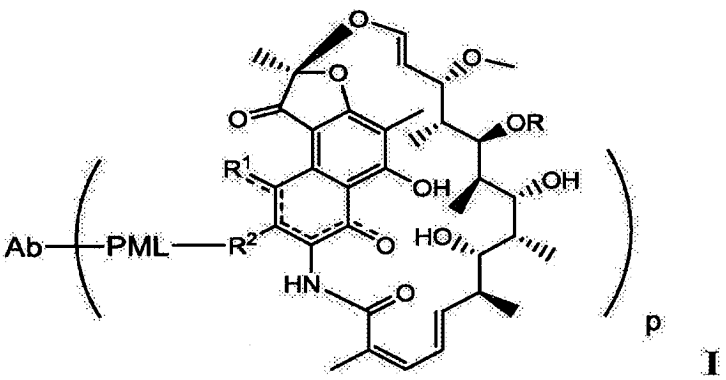
[0199] abx是利福霉素型抗生素；并且

[0200] p是从1到8的整数。

[0201] 利福霉素型抗生素可以是利福拉齐型抗生素。

[0202] 利福霉素型抗生素可以包含与蛋白酶可切割的非肽类接头连接的季胺。

[0203] 抗体-抗生素缀合物的示例性实施方案具有式I：

[0204] 

I

[0205] 其中：

[0206] 虚线表示任选的键；

[0207] R是H、C_1-C_{12}烷基或C(O)CH_3；

[0208] R^1是OH；

[0209] R^2是CH=N-(杂环基)，其中所述杂环基任选地被一个或多个独立地选自C(O)CH_3、C_1-C_{12}烷基、C_1-C_{12}杂芳基、C_2-C_{20}杂环基、C_6-C_{20}芳基和C_3-C_{12}碳环基的基团取代；

[0210] 或R^1和R^2形成五元或六元稠合杂芳基或杂环基，并且任选地形成螺或稠合的六元杂芳基、杂环基、芳基或碳环基环，其中所述螺或稠合的六元杂芳基、杂环基、芳基或碳环基环任选地被H、F、Cl、Br、I、C_1-C_{12}烷基或OH取代；

[0211] PML是连接到R^2或由R^1和R^2形成的稠合杂芳基或杂环基的蛋白酶可切割的非肽类接头；并且

[0212] Ab是抗壁磷壁酸(WTA)抗体。

[0213] 抗生素部分可以通过反应性接头部分与抗体分子缀合，其中抗生素部分的数目可以通过本文所述的方法引入的游离半胱氨酸残基的数量来限制。示例性AAC包含具有1、2、3或4个工程化改造的半胱氨酸氨基酸的抗体(Lyon,R.等人(2012)Methods in Enzym.502:123-138)。

[0214] 抗-壁磷壁酸(WTA)抗体

[0215] 本文公开了与在多种革兰氏阳性细菌(包括金黄色葡萄球菌)上表达的WTA结合的某些抗WTA抗体和缀合的抗WTA抗体。抗WTA抗体可以选择和通过US 8283294;Meijer PJ等人(2006)J Mol Biol.358(3):764-72;Lantto J等人(2011)J Virol.85(4):1820-33和下文实施例3-4中所述方法产生。

[0216] 革兰氏阳性细菌的细胞壁由多个肽聚糖(PGN)鞘的厚层构成，不仅可以稳定细胞膜，而且可以提供许多可以连接其它分子的位点(图4)。这些细胞表面糖蛋白的主要类别是磷壁酸("TA")，其在许多聚糖结合蛋白(GPB)上发现是磷酸酯丰富的分子。TA有两种类型：(1)脂磷壁酸("LTA")，其锚定于质膜并从细胞表面延伸到肽聚糖层中；和(2)壁TA("WTA")，其共价连接到肽聚糖并延伸穿过细胞壁外(图4)。WTA可以占GPB总细胞壁质量的60％。因此，其呈现高度表达的细胞表面抗原。

[0217] WTA的化学结构因生物而异。在金黄色葡萄球菌中，WTA通过由N-乙酰葡萄糖胺(GlcNAc)-1-P和N-乙酰甘露糖胺(ManNAc)构成的二糖与N-乙酰胞壁酸(MurNAc)的6-OH共价连接，其随后与约2或3个甘油磷酸酯(图5)单位连接。实际的WTA聚合物由11-40个核糖醇-磷酸盐(Rbo-P)重复单元组成。WTA的分步合成首先由称为TagO的酶启动，缺乏TagO基因(通过删除该基因)的金黄色葡萄球菌不产生任何WTA。可以通过α-或β-糖苷键用在C2-OH位置的D-丙氨酸(D-Ala)和/或C4-OH位置的N-乙酰葡糖胺(GlcNAc)进一步定制该重复单元。根据金黄色葡萄球菌菌株或细菌的生长期，糖苷键可以是α-、β-或这两个端基异构体的混合物。这些GlcNAc糖修饰由两种特异性金黄色葡萄球菌衍生的糖基转移酶(Gtfs)实现：TarM Gtf介导α-糖苷键，而TarS Gtfs介导β-糖苷键。

[0218] 鉴于MRSA的细胞内储存受到保护以逃避抗生素的显著证据，开发本发明的新型治疗组合物以通过使用金黄色葡萄球菌特异性抗体将抗生素固定在细菌上，使得当细菌在体内被宿主细胞吞噬或以其它方式内化时，其将抗生素带入宿主细胞，从而阻止这种抗生素逃避方法。

[0219] 本发明的AAC的抗WTA抗体可以是抗WTAα或抗WTAβ抗体。从金黄色葡萄球菌感染患者的B细胞克隆了整个说明书中提供的示例性抗WTA抗体(如下文实施例中所述)。在一个实施方案中，抗WTA和抗金黄色葡萄球菌抗体是人单克隆抗体。本发明的AAC或TAC包括包含本发明WTA抗体的CDR的嵌合抗体和人源化抗体。

[0220] 对于本发明的治疗用途，用于缀合抗生素以产生AAC的WTA抗体可以是IgM以外的任何同种型。在一个实施方案中，WTA抗体是人IgG同种型。在更具体的实施方案中，WTA抗体是人IgG1。

[0221] 在整个说明书和附图中，由4位数字(例如4497)指定的Ab也可以用前面的"S"来表示，例如S4497；这两个名称都是指与抗体的野生型(WT)未修饰序列相同的抗体。抗体的变体通过抗体号例如4497v8之后的"v"表示。除非特别指出(例如通过变体号)，所示的氨基酸

序列是原始的、未修饰的/未改变的序列。这些抗体可以在一个或多个残基处改变，例如为了改善pK、稳定性、表达、可制造性(例如，如下文实施例中所述)，同时保持基本上与野生型未经修饰的抗体相比相当或改善的抗原结合亲和力。具有保守氨基酸取代的本发明WTA抗体的变体包括在本发明中。以下除非另有说明，CDR编号是根据Kabat进行，且恒定区编号是根据EU进行编号。

[0222] 为了缀合以产生TAC化合物，本发明的抗WTA抗体可以在L链和H链中的一个中或两个都包含工程化Cys，用于与接头-抗生素中间体缀合，如下所述。

[0223] 图11A和11B分别提供了四种人抗WTAα抗体的轻链可变区(VL)和重链可变区(VH)的氨基酸序列比对。根据Kabat编号，CDR序列CDR L1、L2、L3和CDR H1、H2、H3加有下划线。

[0224] 表1A:抗WTAα的轻链CDR序列。

[0225]

抗体	CDR L1	CDR L2	CDR L3
4461	KSSQSVLSRANNNYYVA (SEQ ID NO.1)	WASTREF (SEQ ID NO.2)	QQYYTSRRT (SEQ ID NO.3)
4624	RSNQNLLSSSNNNYLA (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)

[0226] 表1B:抗WTAα的重链CDR序列。

[0227]

抗体	CDR H1	CDR H2	CDR H3
4461	DYYMH (SEQ ID NO.4)	WINPKSGGTNYAQRFQG (SEQ ID NO.5)	DCGSGGLRDF (SEQ ID NO.6)
4624	DYYIH (SEQ ID NO.10)	WINPNTGGTYYAQKFRD (SEQ ID NO.11)	DCGRGGLRDI (SEQ ID NO.12)
4399	DYYIH (SEQ ID NO.16)	WINPNTGGTNYAQKFQG (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)

[0228] 每对VL和VH的序列如下：

[0229] 4461轻链可变区

[0230]

DIQMTQSPDSLAVSLGERATINCKSSQSVLSRANNNYYVAWYQHKPGQPPKLLIYWASTREFGVPDRFSGSGSGTDF TLTINSLQAEDVAVYYCQQYYTSRRTFGQGTKVEIK(SEQ ID NO.25)

[0231] 4461重链可变区

[0232]

QVQLVQSGAEVRKPGASVKVSCKASGYSFTDYYMHWVRQAPGQGLEWMGWINPKSGGTNYAQRFQGRVTMTGDTSIS AAYMDLASLTSDDTAVYYCVKDCGSGGLRDFWGQGTTVTVSS(SEQ ID NO.26)

[0233] 4624轻链可变区

[0234]

DIQMTQSPDSLSVSLGERATINCRSNQNLLSSSNNNYLAWYQQKPGQPLKLLIYWASTRESGVPDRFSGSGSGTDFT LTISSLQAEDVAVYYCQQYYANPRTFGQGTKVEIK(SEQ ID NO.27)

[0235] 4624重链可变区

[0236]

QVQLQQSRVEVKRPGTSVKVSCKTSGYTFSDYYIHWVRLAPGQGLELMGWINPNTGGTYYAQKFRDRVTMTRDTSIA TAYLEMSSLTSDDTAVYYCAKDCGRGGLRDIWGPGTMVTVSS(SEQ ID NO.28)

[0237] 4399轻链可变区

[0238]

EIVLTQSPDSLAVSLGERATINCKSNQNVLASSNDKNYLAWFQHKPGQPLKLLIYWASIRESGVPDRFSGSGSGTDF TLTISSLRAEDVAVYYCQQYYTNPRTFGQGTKVEFN(SEQ ID NO.29)

[0239] 4399重链可变区

[0240]

EVQLVQSGAEVKKPGTSVKVSCKASGYTFTDYYIHWVRLAPGQGLELMGWINPNTGGTNYAQKFQGRVTMTRDTSIA TAYMELSSLTSDDTAVYYCAKDCGNAGLRDIWGQGTTVTVSS(SEQ ID NO.30)

[0241] 6267轻链可变区

[0242]

DIQLTQSPDSLAVSLGERATINCKSSQNVLYSSNNKNYLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGSGTDF TLTISSLQAEDVAVYYCQQYYTSPPYTFGQGTKLEIE(SEQ ID NO.31)

[0243] 6267重链可变区

[0244]

EVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIGWVRQMPGKGLEWMGIIHPGDSKTRYSPSFQGQVTISADKSIS TAYLQWNSLKASDTAMYYCARLYCSGGSCYSDRAFSSLGAGGYYYYGMGVWGQGTTVTVSS(SEQ ID NO.32).

[0245] 为了生产AAC化合物，本发明提供了一种分离的单克隆抗体，其结合壁磷壁酸α(WTAα)，包含一个轻链和一个H链，该L链包含CDR L1、L2、L3，且该H链包含CDR H1、H2、H3，其中CDR L1、L2、L3和H1、H2、H3分别包含每个抗体4461(SEQ ID NO.1-6)、4624(SEQ ID NO.7-12)、4399(SEQ ID NO.13-18)和6267(SEQ ID NO.19-24)的CDR的氨基酸序列，如上文表1A和表1B中所示。

[0246] 在另一个实施方案中，结合WTAa的分离的单克隆抗体包含H链可变区(VH)和L链可变区(VL)，其中VH分别在抗体4461、4624、4399和6267各自的VH区序列的长度上分别具有至少95％的序列同一性。在另一个特定的方面，该序列同一性是96％、97％、98％、99％或100％。

[0247] 本发明还提供了包含从图12中示例的Ab列表中的抗WTAβ抗体的AAC。在一个实施方案中，该分离的抗WTAβ单克隆抗体包含选自图12中的13个Ab中的每一个的CDR的CDR L1、L2、L3和H1、H2、H3。在另一个实施方案中，本发明提供了在13个抗体中的每一个的V区结构域的长度上包含至少95％序列同一性的分离的抗WTAβ抗体。在另一个具体方面，序列同一性为96％、97％、98％、99％或100％。

[0248] 在13种抗WTAβ抗体中，6078和4497被修饰以产生变体：i)在L链和H链中的一个或两个中具有工程化Cys，用于与接头-抗生素中间体缀合；和ii)其中H链中的第一个残基Q被改变为E(v2)，或者将前两个残基QM改变为EI或EV(v3和v4)。

[0249] 图13A-1和13A-2提供抗WTAβ抗体6078(未修饰)及其变体v2、v3、v4的全长L链的氨基酸序列。含有工程化Cys的L链变体由黑框中的C表示恒定区的末端(在该情况下为EU残基号205)。变体名称，例如v2LC-Cys是指包含引入到L链中的Cys的变体2。HCLC-Cys的含义是抗体的H和L链都含有工程化的Cys。图13B-1至13B-4显示了抗-WTAβ抗体6078(未修饰)及其变体v2、v3、v4的全长H链的比对，其在H链的第一个或第一和二个残基中有变化。包含工程化Cys的H链变体由黑框中的C表示恒定区的末端(在EU残基号118)。

[0250] 6078轻链可变区(VL)

[0251]

DIVMTQSPSILSASVGDRVTITCRASQTISGWLAWYQQKPAEAPKLLIYKASTLESGVPSRFSGSGSGTEFTLTISS LQPDDFGIYYCQQYKSYSFNFGQGTKVEIK(SEQ ID NO.111)

[0252] 6078重链可变区(VH)

[0253]

XX_1QLVQSGAEVKKPGASVKVSCEASGYTLTSYDINWVRQATGQGPEWMGWMNANSGNTGYAQKFQGRVTLTGDTSI STAYMELSSLRSEDTAVYYCARSSILVRGALGRYFDLWGRGTLVTVSS(SEQ ID NO.112)其中X是Q或E;且 X_1是M、I或V。

[0254] 6078轻链

[0255]

DIVMTQSPSILSASVGDRVTITCRASQTISGWLAWYQQKPAEAPKLLIYKASTLESGVPSRFSGSGSGTEFTLTISS LQPDDFGIYYCQQYKSYSFNFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNAL QSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC(SEQ ID NO.113)

[0256] 6078半胱氨酸工程化的轻链

[0257]

DIVMTQSPSILSASVGDRVTITCRASQTISGWLAWYQQKPAEAPKLLIYKASTLESGVPSRFSGSGSGTEFTLTISS LQPDDFG1YYCQQYKSYSFNFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNAL QSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPCTKSFNRGEC(SEQ ID NO.115)

[0258] 6078WT全长重链

[0259]

QMQLVQSGAEVKKPGASVKVSCEASGYTLTSYDINWVRQATGQGPEWMGWMNANSGNTGYAQKFQGRVTLTGDTSIS TAYMELSSLRSEDTAVYYCARSSILVRGALGRYFDLWGRGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTY1CNVNHKPSNTKVDKKVEPKSCDKTH TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG(SEQ ID NO.114)

[0260] 6078变体(v2、v3或v4)全长重链

[0261]

EXQLVQSGAEVKKPGASVKVSCEASGYTLTSYDINWVRQATGQGPEWMGWMNANSGNTGYAQKFQGRVTLTGDTSIS TAYMELSSLRSEDTAVYYCARSSILVRGALGRYFDLWGRGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTH TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG(SEQ ID NO.116)其中X可以是M、I或V。

[0262] 6078变体(v2、v3或v4),Cys-工程化的重链

[0263]

EXQLVQSGAEVKKPGASVKVSCEASGYTLTSYDINWVRQATGQGPEWMGWMNANSGNTGYAQKFQGRVTLTGDTSIS TAYMELSSLRSEDTAVYYCARSSILVRGALGRYFDLWGRGTLVTVSSCSTKGPSVFPLAPSSKSTSGGTAALGCLVK

DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTH TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG(SEQ ID NO.117)其中X可以是M、I或v。

[0264] 在一个实施方案中，本发明提供了分离的抗WTAβ抗体，其包含重链和轻链，其中该重链包含与SEQ ID NO:112至少95％序列同一性的VH。在另外的实施方案中，该抗体还包含与SEQ ID NO:111具有至少95％序列同一性的VL。在一个具体实施方案中，该抗WTAβ抗体包含轻链和重链，其中L链包含SEQ ID NO:111的VL，且H链包含SEQ ID NO:112的VH。在更具体的实施方案中，该分离的抗WTAβ抗体包含SEQ ID NO:113的L链和SEQ ID NO:114的H链。

[0265] 6078Cys工程化的H和L链的变体可以以任何以下组合配对以形成用于与接头-Abx中间体缀合的完整Ab，以产生本发明的抗WTA AAC。未修饰的L链(SEQ ID NO.13)可以与SEQ ID NO:117的Cys工程化的H链变体配对；该变体可以是其中X是M、I或V的化合物。SEQ ID NO.115的Cys工程化的L链可以与以下配对：SEQ ID NO:114的H链；SEQ ID NO.116的H链变体；或SEQ ID NO.117的Cys工程化的H链变体(在该版本中，H和L链均为Cys工程化的)。在一个具体实施方案中，本发明的抗WTAβ抗体和抗WTAβAAC包含SEQ ID NO.115的L链和SEQ ID NO.116的H链。

[0266] 图14A-1和14A-2提供了抗WTAβAb 4497(未修饰)及其v8变体的全长L链。含有工程化Cys的L链变体由恒定区末端附近(在EU残基号205)的黑框中的C表示。图14B-1、14B-2、14B-3显示抗WTAβAb 4497(未修饰)和其v8变体的全长H链比对，v8变体在CDR H3的96位将D改变为E，具有或不具有工程化的Cys。含有工程化Cys的H链变体由恒定区C_H1开始处的黑框中的C表示(在该情况下为EU残基号118)。未修饰的CDR H3是GDGGLDD(SEQ ID NO.104)；4497v8CDR H3是GEGGLDD(SEQ ID NO.118)。

[0267] 4497轻链可变区

[0268]

DIQLTQSPDSLAVSLGERATINCKSSQSIFRTSRNKNLLNWYQQRPGQPPRLLIHWASTRKSGVPDRFSGSGFGTDF TLTITSLQAEDVAIYYCQQYFSPPYTFGQGTKLEIK(SEQ ID NO.119)

[0269] 4497重链可变区

[0270]

EVQLVESGGGLVQPGGSLRLSCSASGFSFNSFWMHWVRQVPGKGLVWISFTNNEGTTTAYADSVRGRFIISRDNAKN TLYLEMNNLRGEDTAVYYCARGDGGLDDWGQGTLVTVSS(SEQ ID NO.120)

[0271] 4497.v8重链可变区

[0272]

EVQLVESGGGLVQPGGSLRLSCSASGFSFNSFWMHWVRQVPGKGLVWISFTNNEGTTTAYADSVRGRFIISRDNAKN TLYLEMNNLRGEDTAVYYCARGEGGLDDWGQGTLVTVSS(SEQ ID NO.156)

[0273] 4497轻链

[0274]

DIQLTQSPDSLAVSLGERATINCKSSQSIFRTSRNKNLLNWYQQRPGQPPRLLIHWASTRKSGVPDRFSGSGFGTDF TLTITSLQAEDVAIYYCQQYFSPPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQW

KVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC(SEQ ID NO.121)

[0275] 4497v.8重链

[0276]

EVQLVESGGGLVQPGGSLRLSCSASGFSFNSFWMHWVRQVPGKGLVWISFTNNEGTTTAYADSVRGRFIISRDNAKN TLYLEMNNLRGEDTAVYYCARGEGGLDDWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG(SEQ ID NO.122)

[0277] 4497-Cys轻链

[0278]

DIQLTQSPDSLAVSLGERATINCKSSQSIFRTSRNKNLLNWYQQRPGQPPRLLIHWASTRKSGVPDRFSGSGFGSDF TLTITSLQAEDVAIYYCQQYFSPPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQW KVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC(SEQ ID NO.123)

[0279] 4497.v8-重链

[0280]

EVQLVESGGGLVQPGGSLRLSCSASGFSFNSFWMHWVRQVPGKGLVWISFTNNEGTTTAYADSVRGRFIISRDNAKN TLYLEMNNLRGEDTAVYYCARGEGGLDDWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG(SEQ ID NO.157;与SEQ ID NO.122相同)。

[0281] 4497.v8-Cys重链

[0282]

EVQLVESGGGLVQPGGSLRLSCSASGFSFNSFWMHWVRQVPGKGLVWISFTNNEGTTTAYADSVRGRFIISRDNAKN TLYLEMNNLRGEDTAVYYCARGEGGLDDWGQGTLVTVSSCSTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG(SEQ ID NO.124)

[0283] 本发明提供的另一种分离的抗WTAβ抗体包含重链和轻链,其中重链包含与SEQ ID NO:120具有至少95%序列同一性的VH。在另外的实施方案中,该抗体还包含与SEQ ID NO:119具有至少95%序列同一性的VL。在一个具体实施方案中,抗WTAβ抗体包含轻链和重链,其中所述L链包含SEQ ID NO:119的VL,且H链包含SEQ ID NO:120的VH。在更具体的实施方案中,该分离的抗WTAβ抗体包含SEQ ID NO:121的L链和SEQ ID NO:122的H链。

[0284] 4497Cys工程化的H和L链变体可以以任何以下组合配对以形成完整的Ab,用于与

接头-Abx中间体缀合，以产生本发明的抗WTA AAC。未修饰的L链(SEQ ID NO.121)可以与SEQ ID NO:124的Cys工程化的H链变体配对。SEQ ID NO:123的Cys工程化的L链可以与以下配对:SEQ ID NO:157的H链变体;或SEQ ID NO.124的Cys工程化H链变体(在该版本中，H和L链都是Cys工程化的)。在一个具体实施方案中，本发明的抗WTAβ抗体和抗WTAβAAC包含SEQ ID NO:123的L链。

[0285] 另一个实施方案是结合与图11A和图11B的抗WTAα抗体中的每一个所结合的相同表位的抗体。还提供了结合与图12、图13A和13B以及图14A和14B的抗WTAβ抗体相同的表位的抗体。

[0286] 抗WTA抗体与WTA的结合受到GlcNAc-糖修饰对WTA的端基异构取向的影响。分别通过TarM糖基转移酶或TarS糖基转移酶，通过α-或β-糖苷键在C4-OH位置通过N-乙酰葡糖胺(GlcNAc)糖修饰来修饰WTA。因此，使用抗WTA的抗体对来自缺乏TarM(ΔTarM)、TarS(ΔTarS)或TarM和TarS(ΔTarM/ΔTarS)的糖基转移酶突变体菌株的细胞壁制备物进行免疫印迹分析。对WTA上的α-GlcNAc修饰特异性的WTA抗体(S7574)不结合来自ΔTarM菌株的细胞壁制备物(Meijer,P.J.等人(2006)"Isolation of human antibody repertoires with preservation of the natural heavy and light chain pairing."Journal of molecular biology 358,764-772)。反之亦然，对WTA上的β-GlcNAc修饰特异性的WTA抗体(S4462)不结合来自ΔTarS菌株的细胞壁制备物。如所预期的那样，这两种抗体都不与缺少糖基转移酶(ΔTarM/ΔTarS)的缺失菌株和缺乏任何WTA(ΔTagO)的菌株的细胞壁制备物结合。根据这样的分析，抗体已经被表征为如图6A和6B中的表中所列的抗α-GlcNAc WTA mAb或者抗β-GlcNAc WTA mAb。

[0287] 半胱氨酸氨基酸可以在抗体中的反应性位点进行工程化，并且不形成链内或分子间二硫键(Junutula等人，2008b Nature Biotech.,26(8):925-932;Dornan等人(2009)Blood 114(13):2721-2729;US 7521541;US 7723485;WO2009/052249,Shen等人(2012)Nature Biotech.,30(2):184-191;Junutula等人(2008)Jour of Immun.Methods 332:41-52)。该工程化的半胱氨酸硫醇可以与具有硫醇反应性的亲电基团如马来酰亚胺或α-卤代酰胺的本发明的接头试剂或接头-抗生素中间体反应，以形成具有半胱氨酸工程化的抗体(THIOMAB™或thioMab)和抗生素(abx)部分的AAC。因此抗生素部分的位置可以被设计、控制并已知。可以控制抗生素负载，因为工程化的半胱氨酸硫醇基团通常以高产率与硫醇反应性接头试剂或接头-抗生素中间体反应。通过在重链或轻链上的单个位点取代引入半胱氨酸氨基酸来制备抗WTA抗体，在对称四聚体抗体上提供两个新的半胱氨酸。可以实现接近2的抗生素负载和缀合产物AAC的近似的均质性。

[0288] 在某些实施方案中，可能需要产生半胱氨酸工程化的抗WTA抗体，例如"thioMAb"，其中抗体的一个或多个残基被半胱氨酸残基取代。在具体实施方案中，该取代的残基出现在抗体的可接近位点处。通过用半胱氨酸取代那些残基，因此反应性硫醇基团定位在抗体的可接近位点，并且可用于将抗体与其它部分(例如抗生素部分或接头-抗生素部分)缀合以产生免疫缀合物，如本文进一步描述。在某些实施方案中，任何一个或多个以下残基可以被半胱氨酸取代，包括轻链的V205(Kabat编号);重链A118(EU编号);和重链Fc区的S400(EU编号)。显示了抗WTA抗体的非限制性的示例性半胱氨酸工程化的重链A118C(SEQ ID NO:149)和轻链V205C(SEQ ID NO:151)突变体。可以如Junutula等人，2008b Nature

Biotech.,26(8):925-932;US 7521541;US-2011/0301334所述产生半胱氨酸工程化的抗WTA抗体。

[0289] 在另一个实施方案中,本发明提供了用于缀合以产生AAC的分离的抗WTA抗体,该抗体包含重链和轻链,其中该重链包含野生型重链恒定区序列或半胱氨酸工程化的突变体(ThioMab),且所述轻链包含野生型轻链恒定区序列或半胱氨酸工程化的突变体(ThioMab)。一方面,该重链具有与以下序列至少95%的序列同一性:

[0290] 重链(IgG1)恒定区,野生型

[0291] ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV TVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[0292] (SEQ ID NO:148)

[0293] 重链(IgG1)恒定区,A118C"ThioMab"

[0294] CSTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV TVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[0295] (SEQ ID NO:149)

[0296] 且轻链具有与以下序列至少95%的序列同一性:

[0297] 轻链(κ)恒定区,野生型

[0298] RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[0299] (SEQ ID NO:150)

[0300] 轻链(κ)恒定区,V205C"ThioMab"

[0301] RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS STLTLSKADYEKHKVYACEVTHQGLSSPCTKSFNRGEC

[0302] (SEQ ID NO:151)

[0303] 本发明的AAC包括半胱氨酸工程化的抗WTA抗体;其中野生型或母体抗WTA抗体的一个或多个氨基酸被半胱氨酸氨基酸替代。任何形式的抗体都可以如此工程化,即突变。例如,可以将母体Fab抗体片段工程化以形成半胱氨酸工程化的Fab,在本文中称为"ThioFab"。类似地,母体单克隆抗体可以被工程化以形成"ThioMab"。应当注意,由于IgG抗体的二聚体性质,单一位点突变在ThioFab中产生单个工程化的半胱氨酸残基,而单一位点突变在ThioMab中产生两个工程化的半胱氨酸残基。对于新引入的工程化半胱氨酸硫醇基团的反应性,评估具有置换的("工程化")半胱氨酸(Cys)残基的突变体。

[0304] 本文所述的抗体可以使用培养基中的宿主细胞产生。可以用包含编码本文所述抗体的一种或多种核酸的载体(表达或克隆载体)转化宿主细胞。所述细胞可以在适于产生抗体的条件下培养,细胞产生的抗体可进一步纯化。用于产生抗体的合适细胞可包括原核、酵

母或更高级的真核(例如哺乳动物)细胞。在一些实施方案中,使用哺乳动物细胞(人或非人哺乳动物细胞)。在一些实施方案中,使用中国仓鼠卵巢(CHO)细胞。

[0305] 可以培养哺乳动物细胞,并且哺乳动物细胞在培养基(组织培养基)中的繁殖已经成为常规方法。哺乳动物宿主细胞系的实例可以包括但不限于由SV40转化的猴肾CV1细胞系(COS-7、ATCC CRL 1651);人胚胎肾细胞系(亚克隆的293或293细胞,用于在悬浮培养基中生长,Graham等人,J.Gen Virol.36:59(1977));小仓鼠肾细胞(BHK,ATCC CCL 10);小鼠塞尔托利细胞(TM4,Mather,Biol.Reprod.23:243-251(1980));猴肾细胞(CV1ATCC CCL 70);非洲绿猴肾细胞(VERO-76,ATCC CRL-1587);人宫颈癌细胞(HELA,ATCC CCL 2);犬肾细胞(MDCK,ATCC CCL 34);水牛鼠肝细胞(BRL 3A,ATCC CRL 1442);人肺细胞(W138,ATCC CCL 75);人肝细胞(Hep G2,HB 8065);小鼠乳腺肿瘤(MMT 060562,ATCC CCL51);TRI细胞(Mather等人,Annals N.Y.Acad.Sci.383:44-68(1982));MRC 5细胞;FS4细胞;和人肝癌细胞系(Hep G2)。其它有用的哺乳动物宿主细胞系包括骨髓瘤细胞系如NS0和Sp2/0。对于适于抗体生成的某些哺乳动物宿主细胞系的综述,参见例如Yazaki和Wu,Methods in Molecular Biology,Vol.248(B.K.C.Lo,ed.,Humana Press,Totowa,N.J.,2003),第255-268页。

[0306] 棉花、玉米、马铃薯、大豆、牵牛花、番茄、浮萍(Leninaceae)、紫花苜蓿(M.truncatula)和烟草的植物细胞培养物也可以用作宿主。

[0307] 用于此目的的合适的原核细胞包括细菌,如革兰氏阴性或革兰氏阳性菌,例如肠杆菌科,如埃希氏菌属,例如大肠杆菌,肠杆菌属、欧文氏菌属、克雷伯菌属、变形杆菌属、沙门菌属,如鼠伤寒沙门菌,沙雷菌属,如粘质沙雷氏菌,和志贺菌属,以及杆菌,例如枯草杆菌和地衣芽孢杆菌(如1989年4月12日出版的DD 266,710中公开的地衣芽孢杆菌41P),假单胞菌属例如铜绿假单胞菌,和链霉菌属。一种优选的大肠杆菌克隆宿主是大肠杆菌294(ATCC 31,446),但其它菌株例如大肠杆菌B、大肠杆菌X1776(ATCC 31,537)和大肠杆菌W3110(ATCC 27,325)也是适合的。这些实例是说明性而非限制性的。

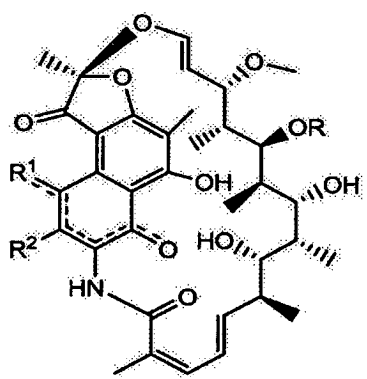
[0308] 除了原核生物之外,真核微生物如丝状真菌或酵母是用于编码抗体的载体的合适的克隆或表达宿主。酿酒酵母或普通面包酵母是较低级真核宿主微生物中最常用的酵母。然而,许多其它属、种和菌株通常是可获得的和在本文中有用的,例如粟酒裂殖酵母菌(schizosaccharomyces prombe);克鲁维酵母属宿主,例如乳酸克鲁维酵母(K.lactis)、脆壁克鲁维酵母(K.fragilis)(ATCC 12,424)、保加利亚克鲁维酵母(K.bulgaricus)(ATCC 16,045)、威克克鲁维酵母(K.wickeramii)(ATCC 24,178)、K.waltii(ATCC 56,500)、果蝇克鲁维酵母(K.drosophilarum)(ATCC 36,906)、耐热克鲁维酵母(K.thermotolerans)和马克思克鲁维酵母(K.marxianus);子囊菌酵母属(EP 402,226);毕赤酵母(EP 183,070);假丝酵母属;瑞氏木霉(EP 244,234);粗糙脉孢菌;许旺酵母属,例如许旺酵母;和丝状真菌,例如链孢霉属、青霉属、弯颈霉属和曲霉菌属的宿主,例如构巢曲菌和黑曲霉。

[0309] 利福霉素型抗生素

[0310] 本发明的抗体-抗生素缀合物(AAC)的抗生素部分(abx)是具有细胞毒性或细胞抑制作用的利福霉素型抗生素或基团。利福霉素是由细菌地中海诺卡氏菌(Nocardia mediterranei)、地中海拟无枝酸菌(Amycolatopsis mediterranei)或人工获得的一组抗生素。它们是抑制细菌RNA聚合酶的较大的安莎霉素家族的亚类(Fujii等人(1995)

Antimicrob.Agents Chemother.39:1489-1492;Feklistov等人(2008)Proc Natl Acad Sci USA,105(39):14820-5),并且对革兰氏阳性菌和选择性革兰氏阴性菌具有效力。利福霉素对分枝杆菌特别有效,且因此用于治疗结核病、麻风病和鸟分枝杆菌复合体(MAC)感染。利福霉素型群组包括"经典的"利福霉素药物以及利福霉素衍生物利福平(rifampicin)(利福平(rifampin),CA登记号13292-46-1)、利福布汀(CA登记号72559-06-9;US 2011/0178001),利福喷汀和利福拉齐(CA登记号129791-92-0,Rothstein等人(2003)Expert Opin.Investig.Drugs 12(2):255-271;Fujii等人(1994)Antimicrob.Agents Chemother 38:1118-1122)。许多利福霉素型抗生素都具有抗发育的有害性质(Wichelhaus等人(2001)J.Antimicrob.Chemother.47:153-156)。在1957年首先从地中海链霉菌(Streptomyces mediterranei)的发酵培养物中分离出利福霉素。发现约七种利福霉素,称为利福霉素A、B、C、D、E、S和SV(US 3150046)。利福霉素B是商业上首次引入的,且在二十世纪六十年代可用于治疗耐药性结核病。利福霉素已被用于治疗许多疾病,最重要的是HIV相关性结核病。由于大量可用的类似物和衍生物,利福霉素已广泛用于消除已经变得对常用抗生素具有抗性的致病菌。例如,利福平以其有效作用和预防耐药性的能力而闻名。它迅速杀死快速分裂的杆菌菌株以及"耐药株"细胞,所述细胞长时间保持生物无活性来使其逃避抗生素活性。此外,利福布汀和利福喷汀均已被用于HIV阳性患者的获得性结核病。

[0311] 式I的抗体-抗生素缀合物的抗生素部分(abx)是具有以下结构的利福霉素型部分:

[0312] 

[0313] 其中:

[0314] 虚线表示任选的键;

[0315] R是H、C_1-C_{12}烷基或C(O)CH_3;

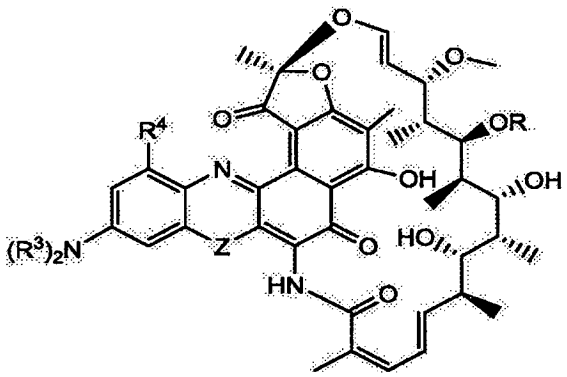
[0316] R^1是OH;

[0317] R^2是CH=N-(杂环基),其中所述杂环基任选地被一个或多个独立地选自C(O)CH_3、C_1-C_{12}烷基、C_1-C_{12}杂芳基、C_2-C_{20}杂环基、C_6-C_{20}芳基和C_3-C_{12}碳环基的基团取代;

[0318] 或R^1和R^2形成五元或六元稠合杂芳基或杂环基,并且任选地形成螺或稠合的六元杂芳基、杂环基、芳基或碳环基环,其中所述螺或稠合的六元杂芳基、杂环基、芳基或碳环基环任选地被H、F、Cl、Br、I、C_1-C_{12}烷基或OH取代;并且

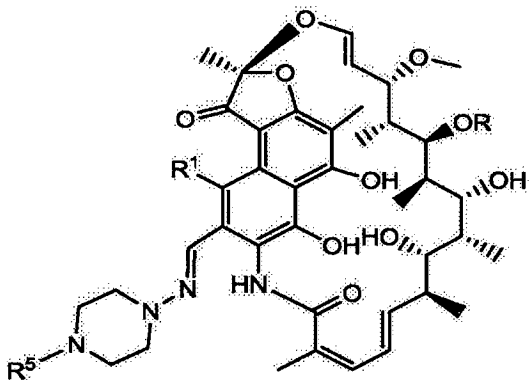
[0319] 其中非肽类接头PML共价连接到R^2上。

[0320] 利福霉素型部分的实施方案是:

[0321] 

[0322] 其中R^3独立地选自H和C_1-C_{12}烷基；R^4选自H、F、Cl、Br、I、C_1-C_{12}烷基和OH；且Z选自NH、N(C_1-C_{12}烷基)、O和S；并且其中非肽类接头PML共价连接到$N(R^3)_2$的氮原子上。

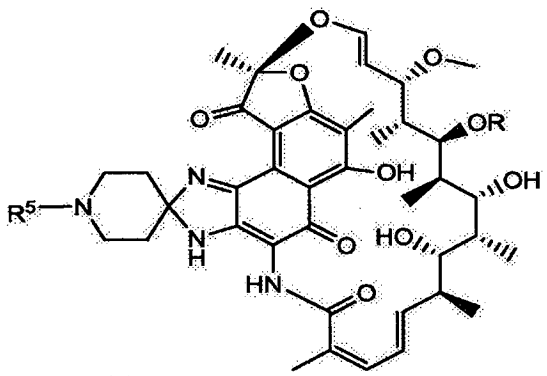
[0323] 利福平型部分的实施方案是：

[0324] 

[0325] 其中，

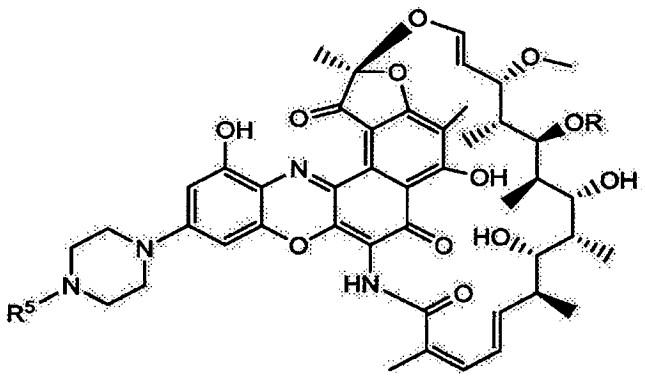
[0326] R^5选自H和C_1-C_{12}烷基；并且其中非肽类接头PML共价连接到NR^5的氮原子上。

[0327] 利福布汀型部分的实施方案是：

[0328] 

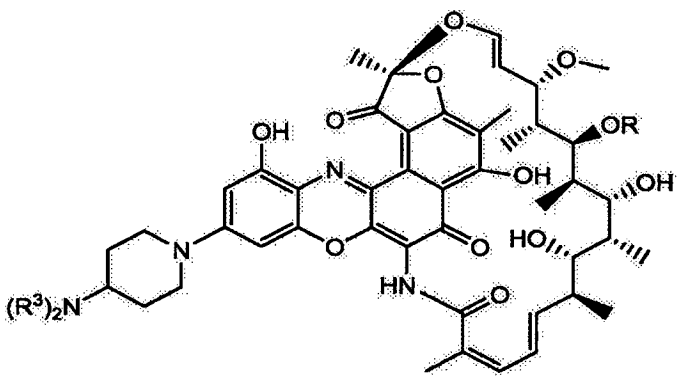
[0329] 其中R^5选自H和C_1-C_{12}烷基；并且其中非肽类接头PML共价连接到NR^5的氮原子上。

[0330] 苯并噁嗪并利福霉素型部分的实施方案是：

[0331] 

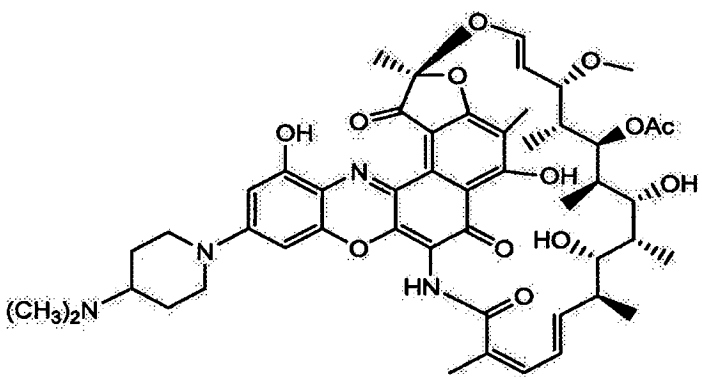
[0332] 其中R^5选自H和C_1-C_{12}烷基；并且其中非肽类接头PML共价连接到NR^5的氮原子上。

[0333] 本文称为pipBOR的苯并噁嗪并利福霉素型部分的实施方案是：

[0334] 

[0335] 其中R^3独立地选自H和C_1-C_{12}烷基；并且其中非肽类接头PML共价连接到$N(R^3)_2$的氮原子上。

[0336] 本文称为二甲基pipBOR的苯并噁嗪并利福霉素型部分的实施方案是：

[0337] 

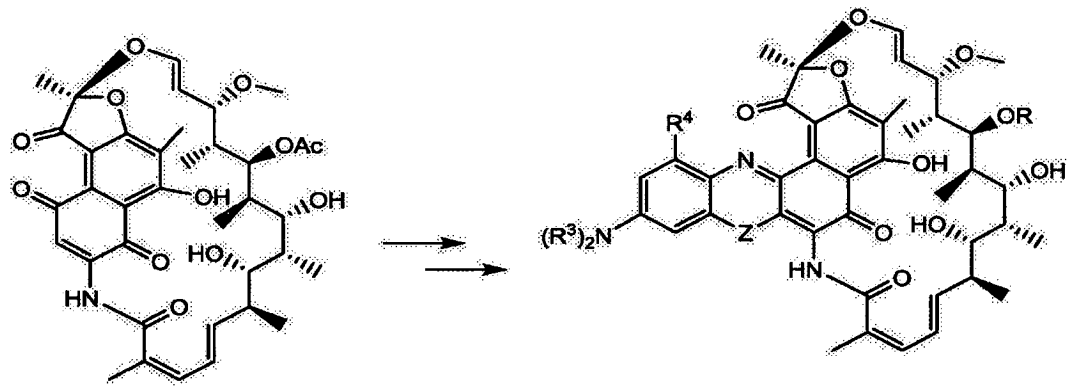
[0338] 其中非肽类接头PML共价连接到二甲基氨基的氮原子上。

[0339] 半合成衍生物利福霉素S，或还原的钠盐型利福霉素SV可以以几个步骤转化为利福拉齐型抗生素，其中R为H或Ac，R^3独立地选自H和C_1-C_{12}烷基；R^4选自H、F、Cl、Br、I、C_1-C_{12}烷基和OH；并且Z选自NH、N(C_1-C_{12}烷基)、O和S(参见，例如WO 2014/194247中的图23A和B，以及图25A和B)。可制备苯并噁嗪并(Z=O)、苯并噻嗪并(Z=S)、苯并二嗪并(Z=NH、N(C_1-C_{12})烷基)利福霉素(US 7271165)。含有取代基的苯并噁嗪并利福霉素(BOR)、苯并噻嗪并利福霉

素(BTR)和苯并二噻并利福霉素(BDR)类似物根据US 7271165第28栏式A中提供的编号方案编号，其通过引用并入本文。“25-O-脱乙酰基”利福霉素是指利福霉素类似物，其中25位的乙酰基已被除去。在该位置进一步衍生化的类似物称为“25-O-脱乙酰基-25-(取代基)利福霉素”，其中在完整化合物名称中用衍生基团的命名替换“取代基”。

[0340] 利福霉素型抗生素部分可以通过在US 4610919;US 4983602;US 5786349;US5981522;US 4859661;US 7271165;US 2011/0178001;Seligson等人,(2001)抗癌药物(Anti-Cancer Drugs)12:305-13;Chem.Pharm.Bull.,(1993)41:148中和在WO 2014/194247中公开的类似方法合成，它们各自通过引用并入本文。通过使用标准的MIC体外测定，通过测量其最小抑菌浓度(MIC)筛选利福霉素型抗生素部分的抗微生物活性(Tomioka等人,(1993)Antimicrob.Agents Chemother.37:67)。

[0341]



利福霉素-S 苯并噁嗪并利福霉素

[0342] 蛋白酶可切割的非肽类接头

[0343] “蛋白酶可切割的非肽类接头”(PML)是双官能或多官能部分，其共价连接到一个或多个抗生素部分(abx)和抗体单元(Ab)以形成式I的抗体-抗生素缀合物(AAC)。AAC中的蛋白酶可切割的非肽类接头是用于细胞内蛋白酶切割的底物，包括在溶酶体条件下。蛋白酶包括各种组织蛋白酶和半胱天冬酶。细胞内AAC的非肽类接头的切割可以释放具有抗菌作用的利福霉素型抗生素。

[0344] 可以使用具有与抗生素(abx)和抗体(Ab)结合的反应官能性的接头试剂或接头-抗生素中间体，方便地制备抗体-抗生素缀合物(AAC)。在一个示例性实施方案中，半胱氨酸工程化改造的抗体(Ab)的半胱氨酸硫醇可以与接头试剂，抗生素部分或抗生素-接头中间体的官能团形成键。

[0345] AAC的PML部分可以包含一个氨基酸残基。

[0346] AAC的PML部分包含拟肽单元。

[0347] 一方面，接头试剂或接头-抗生素中间体具有反应性位点，其具有对存在于抗体上的亲核半胱氨酸具有反应性的亲电子基团。抗体的半胱氨酸硫醇与接头试剂或接头抗生素上的亲电子基团反应，形成共价键。有用的亲电子基团包括但不限于马来酰亚胺和卤代乙酰胺基团。

[0348] 根据Klussman等人(2004)，Bioconjugate Chemistry 15(4):765-773第766页的缀合方法，且根据实施例19的方法，半胱氨酸改造的抗体与接头试剂或接头-抗生素中间

体，与亲电子官能团如马来酰亚胺或α-卤代羰基反应。

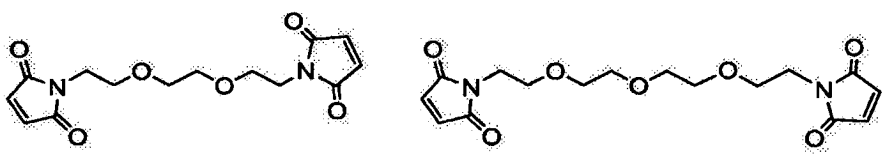
[0349] 在另一个实施方案中，接头试剂或接头-抗生素中间体的反应性基团含有可与抗体的游离半胱氨酸硫醇形成键的硫醇反应性官能团。硫醇反应官能团的实例包括但不限于马来酰亚胺、α-卤代乙酰基，活化酯如琥珀酰亚胺酯、4-硝基苯基酯、五氟苯基酯、四氟苯基酯、酸酐、酰氯、磺酰氯、异氰酸酯和异硫氰酸酯。

[0350] 在另一个实施方案中，接头试剂或抗生素-接头中间体具有反应性官能团，其具有对存在于抗体上的亲电子基团具有反应性的亲核基团。抗体上有用的亲电子基团包括但不限于吡啶基二硫化物、醛和酮羰基。接头试剂或抗生素-接头中间体的亲核基团的杂原子可以与抗体上的亲电子基团反应，并与抗体单元形成共价键。接头试剂或抗生素-接头中间体上有用的亲核基团包括但不限于酰肼、肟、氨基、硫醇、肼、缩氨基硫脲、肼羧酸酯和芳基酰肼。抗体上的亲电子基团提供了用于连接到接头试剂或抗生素-接头中间体的方便位点。

[0351] PML部分可以包含一个或多个接头组分。示例性的接头组分包括单个氨基酸，例如瓜氨酸（"cit"），6-马来酰亚胺基己酰基（"MC"），马来酰亚胺基丙酰基（"MP"）和对氨基苄氧基羰基（"PAB"），N-琥珀酰亚胺基4-(2-吡啶基硫基)戊酸酯（"SPP"）和4-(N-马来酰亚胺基甲基)环己烷-1-羧酸酯（"MCC"）。各种接头组分是本领域已知的，其中一些在下面描述。

[0352] 在另一个实施方案中，接头可以被调节溶解度或反应性的基团取代。例如，带电荷的取代基如磺酸根（$-SO_3^-$）或铵可增加试剂的水溶性，且促进接头试剂与抗体或抗生素部分的偶联反应，或促进Ab-L(抗体-接头中间体)与abx或abx-L(抗生素-接头中间体)与Ab的偶联反应，这取决于用于制备AAC的合成途径。

[0353] 本发明的AAC明确地考虑但不限于用以下接头试剂制备的那些：BMPEO、BMPS、EMCS、GMBS、HBVS、LC-SMCC、MBS、MPBH、SBAP、SIA、SIAB、SMCC、SMPB、SMPH、磺基-EMPS、磺基-GMBS、磺基-KMOS、磺基-MBS、磺基-SIAB、磺基-SMCC、磺基-SMPB、SVSB(琥珀酰亚胺基-(4-乙烯基砜)苯甲酸酯)和双马来酰亚胺试剂如DTME、BMB、BMDB、BMH、BMOE、BM(PEG)$_2$和BM(PEG)$_3$。双马来酰亚胺试剂允许半胱氨酸工程化改造的抗体的硫醇基团以顺序或汇合的方式连接到含硫醇的抗生素部分、标记物或接头中间体。除马来酰亚胺之外，与半胱氨酸工程化改造的抗体、抗生素部分或接头-抗生素中间体的巯基反应的其它官能团包括碘乙酰胺、溴乙酰胺、乙烯基吡啶、二硫化物、吡啶基二硫化物、异氰酸酯和异硫氰酸酯。

[0354] 

BM(PEG)$_2$ BM(PEG)$_3$

[0355] 还可以通过其他商业来源，例如Molecular Biosciences Inc.(Boulder,CO)获得有用的接头试剂，或根据Toki等人(2002)J.Org.Chem.67:1866-1872;Dubowchik等人(1997)Tetrahedron Letters,38:5257-60;Walker,M.A.(1995)J.Org.Chem.60:5352-5355;Frisch等人(1996)Bioconjugate Chem.7:180-186;US 6214345;WO 02/088172;US 2003130189;US2003096743;WO 03/026577;WO 03/043583;和WO 04/032828中描述的方法合成。

[0356] 在另一个实施方案中，AAC的PML部分包含树突状接头，用于将一个以上的抗生素

部分通过支链多官能接头部分共价连接至抗体上(Sun等人(2002)生物有机&药物化学通讯(Bioorganic&Medicinal Chemistry Letters)12:2215;Sun等人(2003)生物有机&药物化学(Bioorganic&Medicinal Chemistry)11:1761-1768)。树突状接头可以增加抗生素与抗体的摩尔比,即负载,其与AAC的效力有关。因此,当半胱氨酸改造的抗体仅具有一个反应性半胱氨酸硫醇基团时,多个抗生素部分可以通过树枝状接头连接。

[0357] 在式I AAC的某些实施方案中,蛋白酶可切割的非肽类接头PML具有下式:

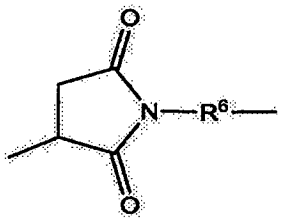
[0358] -Str-PM-Y-

[0359] 其中Str是延伸单元;PM是拟肽单元,和Y是间隔单元;

[0360] abx是利福霉素型抗生素;并且

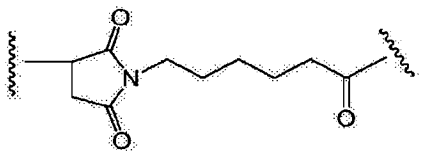
[0361] p是从1到8的整数。

[0362] 在一个实施方案中,延伸单元"Str"具有下式:

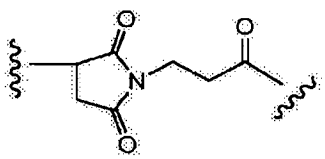
[0363] 

[0364] 其中R^6选自C_1-C_{12}亚烷基、C_1-C_{12}亚烷基-C(=O)、C_1-C_{12}亚烷基-NH、$(CH_2CH_2O)_r$、$(CH_2CH_2O)_r$-C(=O)、$(CH_2CH_2O)_r-CH_2$、和C_1-C_{12}亚烷基-NHC(=O)CH_2CH(噻吩-3-基),其中r是1至10的整数。

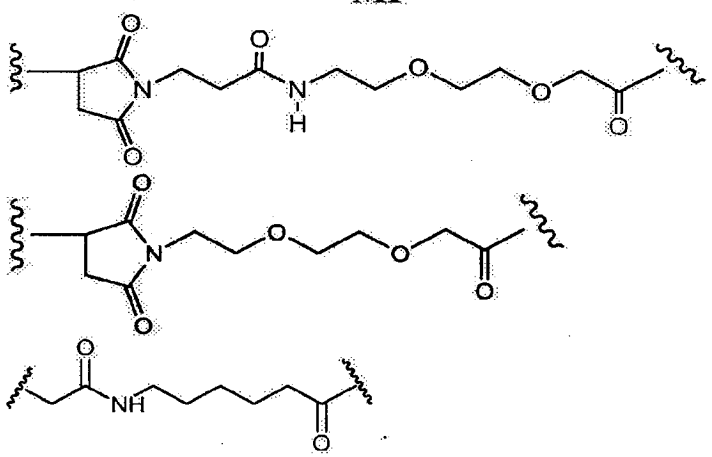
[0365] 示例性的延伸单元如下所示(其中波浪线表示与抗体共价连接的位点):



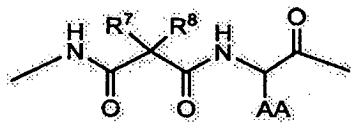
MC



MP

[0366] 

[0367] 在一个实施方案中，PM具有下式：

[0368] 

[0369] 其中R^7和R^8一起形成C_3-C_7环烷基环，和

[0370] AA是选自H、$-CH_3$、$-CH_2(C_6H_5)$、$-CH_2CH_2CH_2CH_2NH_2$、$-CH_2CH_2CH_2NHC(NH)NH_2$、$-CHCH(CH_3)CH_3$、和$-CH_2CH_2CH_2NHC(O)NH_2$的氨基酸侧链。

[0371] 在一个实施方案中，间隔单元Y包含对氨基苄基(PAB)或对氨基苄氧基羰基(PABC)。

[0372] 间隔单元允许释放抗生素部分而没有单独的水解步骤。间隔单元可以是“自发性的(self-immolative)”或“非自发性的”。在某些实施方案中，接头的间隔单元包含对氨基苄基单元(PAB)。在一个这样的实施方案中，对氨基苄醇通过对氨基苄基基团和抗生素部分之间的酰胺键，氨基甲酸酯，甲基氨基甲酸酯或碳酸酯连接到氨基酸单元上(Hamann等人(2005)Expert Opin.Ther.Patents(2005)15:1087-1103)。在一个实施方案中，间隔单元是对氨基苄氧基羰基(PAB)。

[0373] 在一个实施方案中，当与非肽类接头PML的PAB间隔单元连接时，抗生素包含季胺，例如二甲基氨基哌啶基基团。这种季胺接头-抗生素中间体(PLA)的实例是来自表2的PLA-1至4。季胺基团可调节抗生素部分的切割以优化AAC的抗菌作用。在另一个实施方案中，抗生素与非肽类接头PML的PABC间隔单元连接，在AAC中形成氨基甲酸酯官能团。这样的氨基甲酸酯官能团也可以优化AAC的抗菌作用。PABC氨基甲酸酯接头-抗生素中间体(PLA)的实例是来自表2的PLA-5和PLA-6。

[0374] 自发性间隔物的其它实例包括但不限于与PAB基团电子性相似的芳族化合物，例如2-氨基咪唑-5-甲醇衍生物(US7375078;Hay等人(1999)Bioorg.Med.Chem.Lett.9:2237)和邻-或对-氨基苄基缩醛。可以使用在酰胺键水解后进行环化的间隔物，例如取代和未取代的4-氨基丁酸酰胺(Rodrigues等人(1995)Chemistry Biology 2:223)，适当取代的双环[2.2.1]和双环[2.2.2]环系统(Storm等人(1972)J.Amer.Chem.Soc.94:5815)和2-氨基苯基丙酸酰胺(Amsberry等人(1990)J.Org.Chem.55:5867)。在甘氨酸处被取代的含胺药物的消除(Kingsbury等人(1984)J.Med.Chem.27:1447)也是在AAC中有用的自发性间隔物的示例。

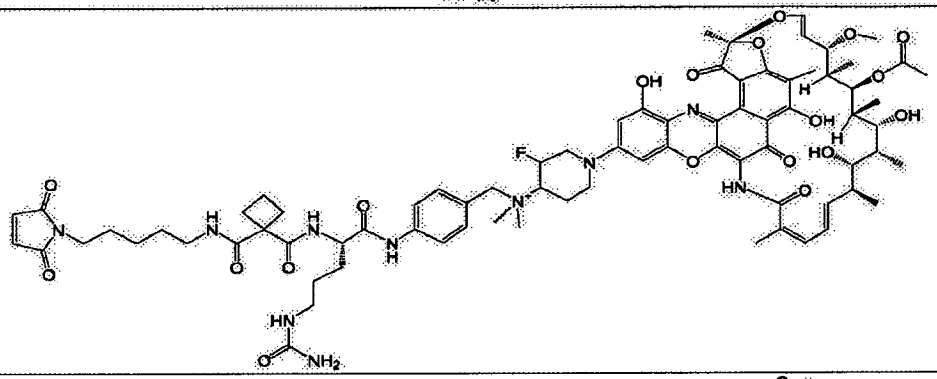
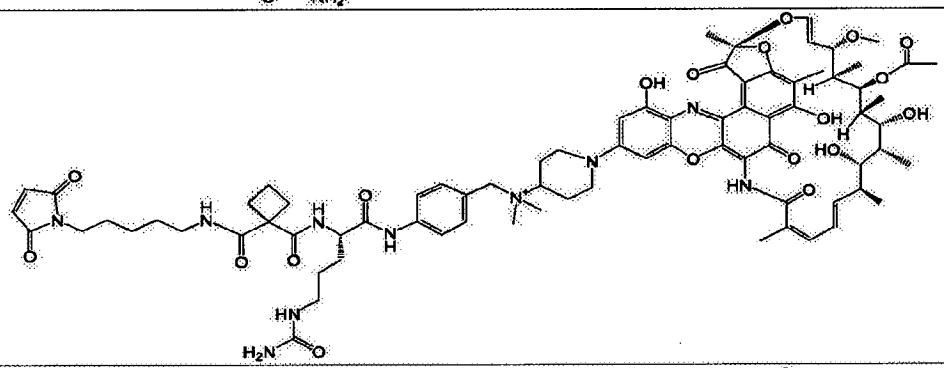
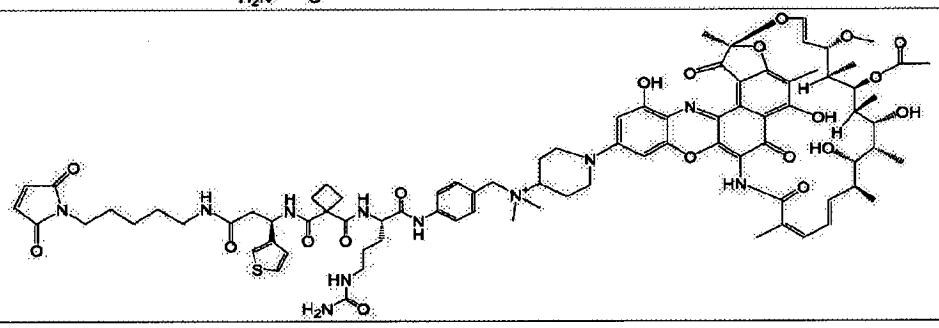
[0375] 从AAC切割释放的活性抗生素的量可以通过实施例8的半胱天冬酶释放试验来测量。

[0376] 对AAC有用的接头-抗生素中间体

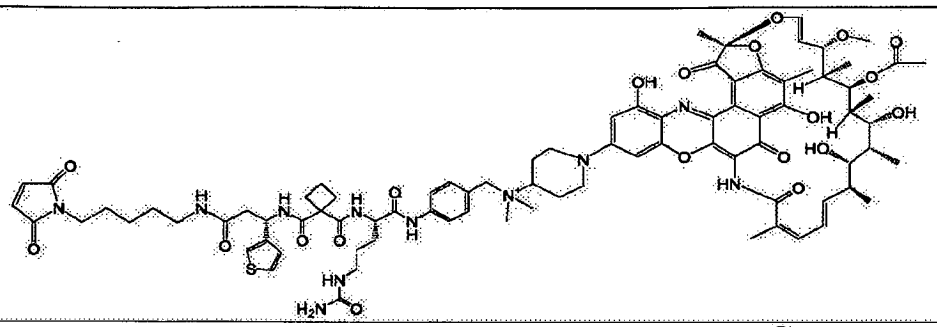
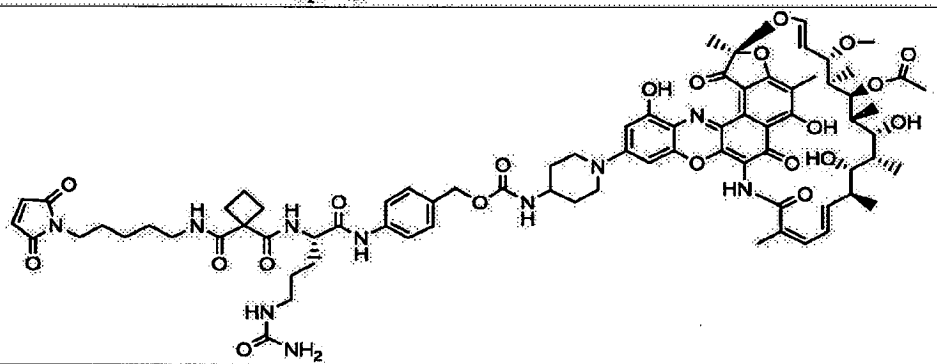
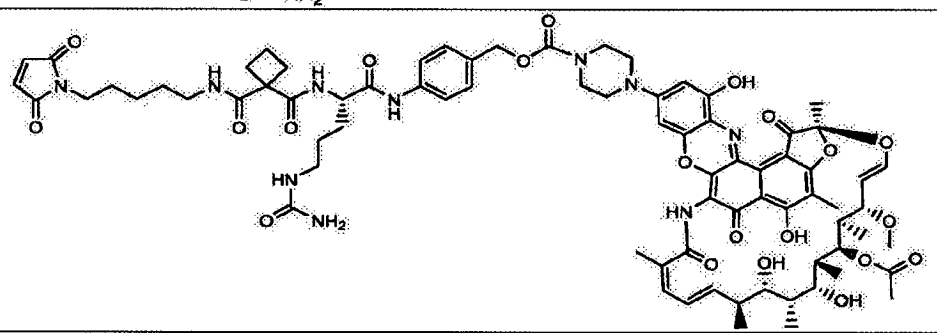
[0377] 实施例11-21通过将利福霉素型抗生素部分与接头试剂偶联制备式II和表2的PML接头-抗生素中间体(PLA)。接头试剂通过WO 2012/113847;US 7659241;US 7498298;US 20090111756;US 2009/0018086;US 6214345;Dubowchik等人(2002)Bioconjugate Chem.13(4):855-869中描述的方法制备。

[0378] 表2 PML接头-抗生素中间体

[0379]

LA 编号	结构
PLA-1	
PLA-2	
PLA-3	

[0380]

PLA-4	
PLA-5	
PLA-6	

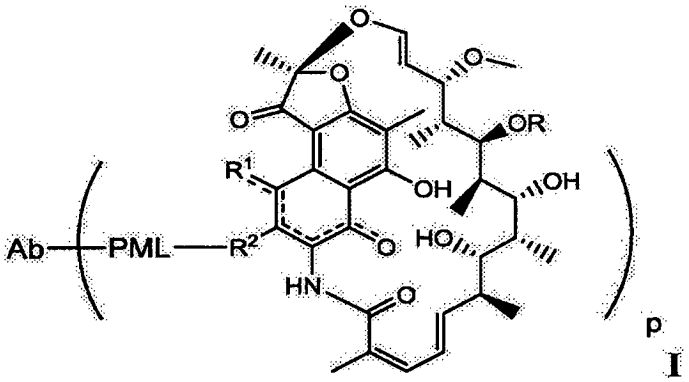
[0381] 抗体-抗生素缀合物的实施方案

[0382] 将半胱氨酸改造的抗WTA抗体通过游离半胱氨酸硫醇基团与利福霉素的衍生物(称为pipBOR等)经蛋白酶可切割的非肽类接头连接,形成表3中的抗体-抗生素缀合物化合物(AAC)。接头设计为被包括组织蛋白酶B、D等的溶酶体蛋白酶切割。实施例11-21详细描述了由抗生素和PML接头等组成的接头-抗生素中间体的产生。设计接头,使得在PAB部分处的酰胺键的切割将抗体与活性状态的抗生素分离。

[0383] 命名为"二甲基pipBOR"的AAC与"pipBOR"AAC相同,除了抗生素上的二甲基化的氨基和接头上的氧羰基。

[0384] 图3显示抗体-抗生素缀合物(AAC)的药物活化的可能机制。活性抗生素(Ab)仅在AAC在哺乳动物细胞内内化后释放。AAC中抗体的Fab部分结合金黄色葡萄球菌,而AAC的Fc部分通过Fc受体介导的结合吞噬细胞(包括嗜中性粒细胞和巨噬细胞)增强细菌摄取。在内化到吞噬溶酶体中后,接头可以通过溶解体蛋白酶裂解,释放吞噬溶酶体内部的活性抗生素。

[0385] 本发明的抗体-抗生素缀合物(AAC)化合物的一个实施方案包括式I:

[0386] 

[0387] 其中：

[0388] 虚线表示任选的键；

[0389] R是H、C_1-C_{12}烷基或C(O)CH_3；

[0390] R^1是OH；

[0391] R^2是CH=N-(杂环基)，其中所述杂环基任选地被一个或多个独立地选自C(O)CH_3、C_1-C_{12}烷基、C_1-C_{12}杂芳基、C_2-C_{20}杂环基、C_6-C_{20}芳基和C_3-C_{12}碳环基的基团取代；

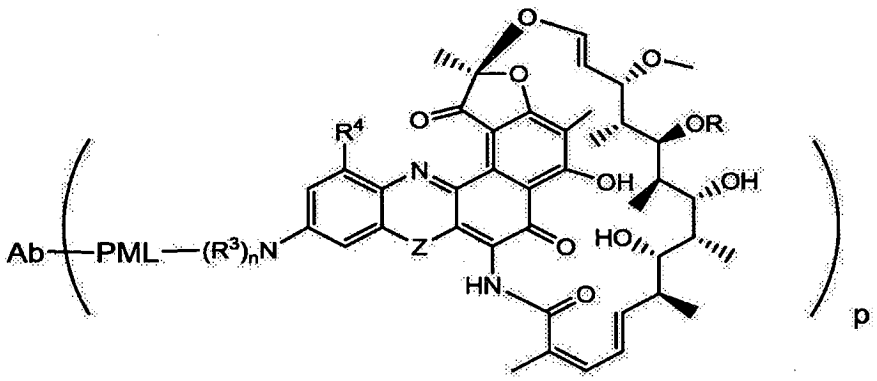
[0392] 或R^1和R^2形成五元或六元稠合杂芳基或杂环基，并且任选地形成螺或稠合的六元杂芳基、杂环基、芳基或碳环基环，其中所述螺或稠合的六元杂芳基、杂环基、芳基或碳环基环任选地被H、F、Cl、Br、I、C_1-C_{12}烷基或OH取代；

[0393] PML是连接到R^2或由R^1和R^2形成的稠合杂芳基或杂环基的蛋白酶可切割的非肽类接头；

[0394] Ab是抗壁磷壁酸(WTA)抗体；并且

[0395] p是从1到8的整数。

[0396] 本发明的抗体-抗生素缀合物(AAC)化合物的另一个实施方案包括下式：

[0397] 

[0398] 其中，

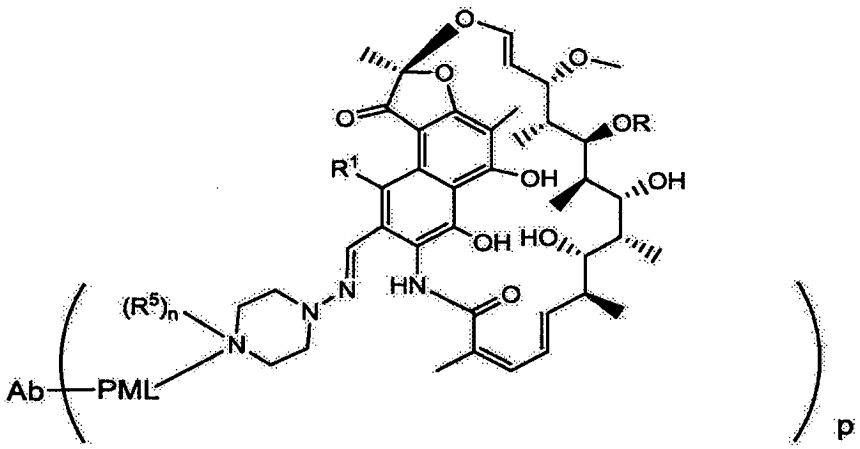
[0399] R^3独立地选自H和C_1-C_{12}烷基；

[0400] n为1或2；

[0401] R^4选自H、F、Cl、Br、I、C_1-C_{12}烷基和OH；和

[0402] Z选自NH、N(C_1-C_{12}烷基)、O和S。

[0403] 本发明的抗体-抗生素缀合物(AAC)化合物的另一个实施方案包括下式：

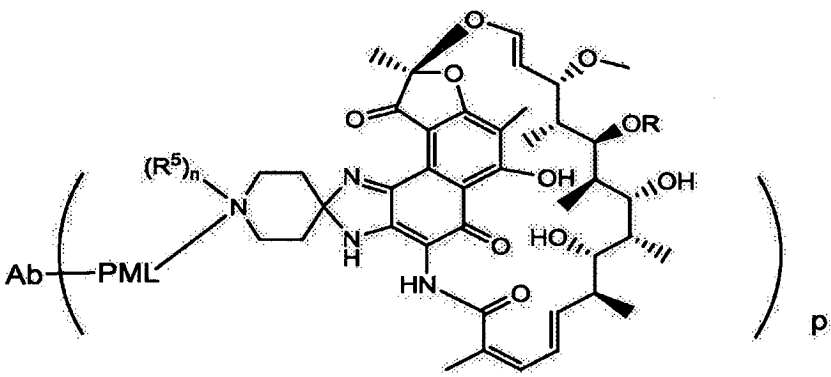
[0404] 

[0405] 其中，

[0406] R^5选自H和C_1-C_{12}烷基；和

[0407] n为0或1。

[0408] 本发明的抗体-抗生素缀合物(AAC)化合物的另一个实施方案包括下式：

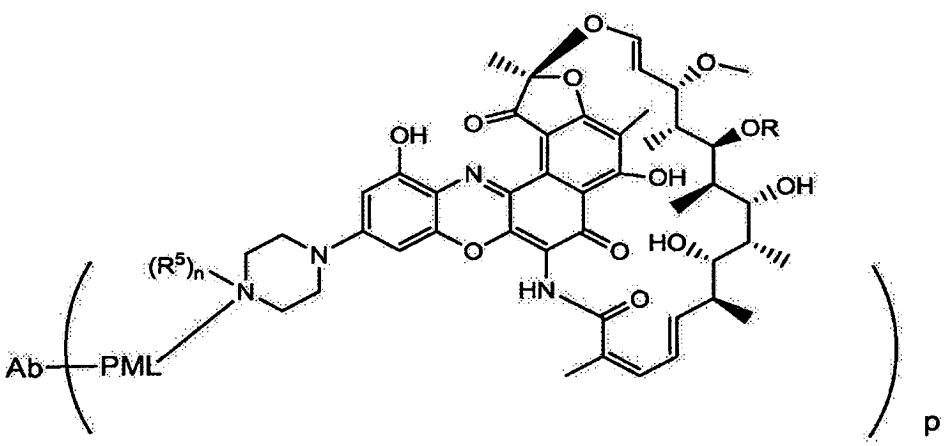
[0409] 

[0410] 其中，

[0411] R^5选自H和C_1-C_{12}烷基；和

[0412] n为0或1。

[0413] 本发明的抗体-抗生素缀合物(AAC)化合物的另一个实施方案包括下式：

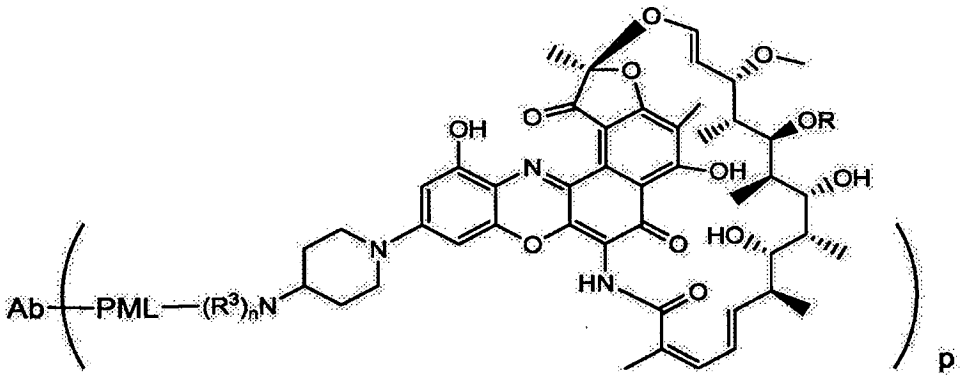
[0414] 

[0415] 其中，

[0416] R^5选自H和C_1-C_{12}烷基；和

[0417] n为0或1。

[0418] 本发明的抗体-抗生素缀合物(AAC)化合物的另一个实施方案包括下式：

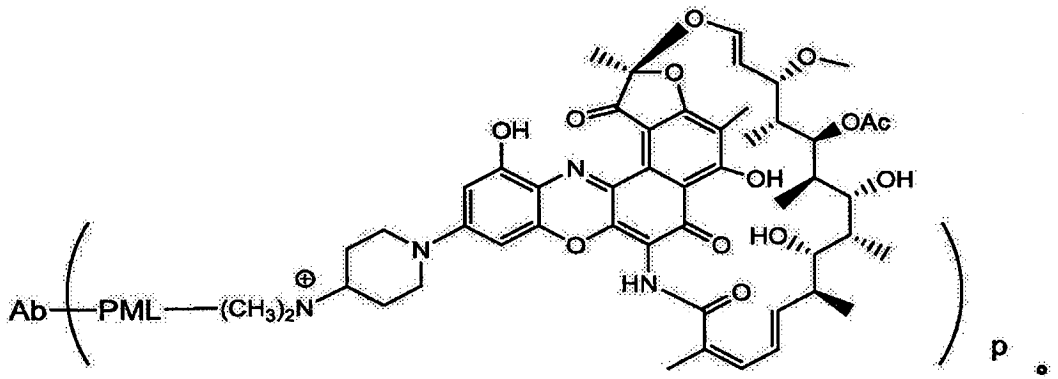
[0419] 

[0420] 其中，

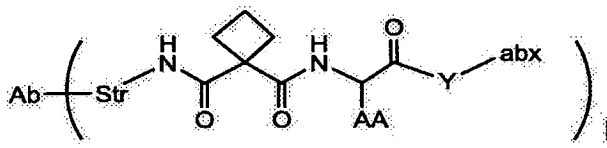
[0421] R^3独立地选自H和C_1-C_{12}烷基；和

[0422] n为1或2。

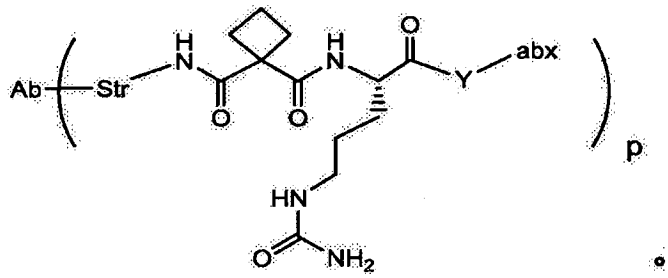
[0423] 本发明的抗体-抗生素缀合物(AAC)化合物的另一个实施方案包括下式：

[0424] 

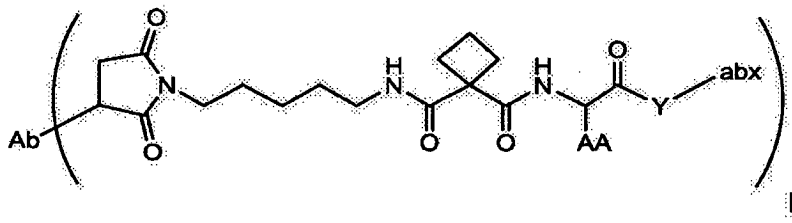
[0425] 本发明的抗体-抗生素缀合物(AAC)化合物的另一个实施方案包括下式：

[0426] 

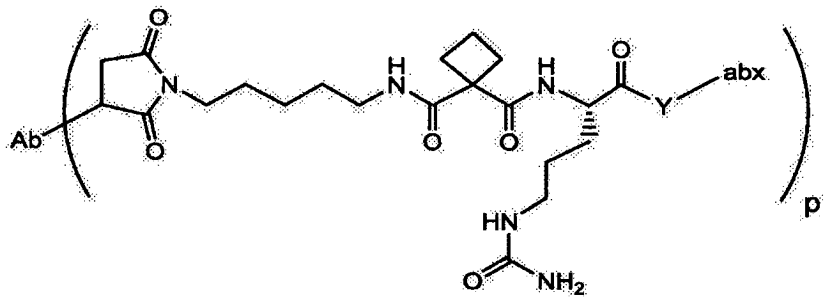
[0427] 本发明的抗体-抗生素缀合物(AAC)化合物的另一个实施方案包括下式：

[0428] 

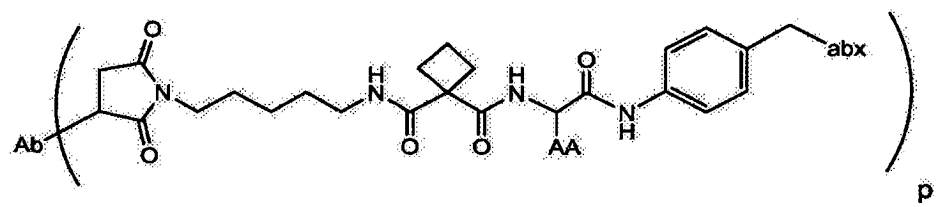
[0429] 本发明的抗体-抗生素缀合物(AAC)化合物的另一个实施方案包括下式：

[0430] 

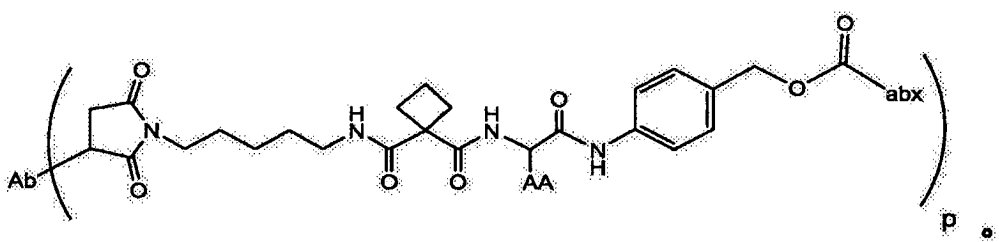
[0431] 本发明的抗体-抗生素缀合物(AAC)化合物的另一个实施方案包括下式：

[0432] 

[0433] 本发明的抗体-抗生素缀合物(AAC)化合物的另一个实施方案包括下式：

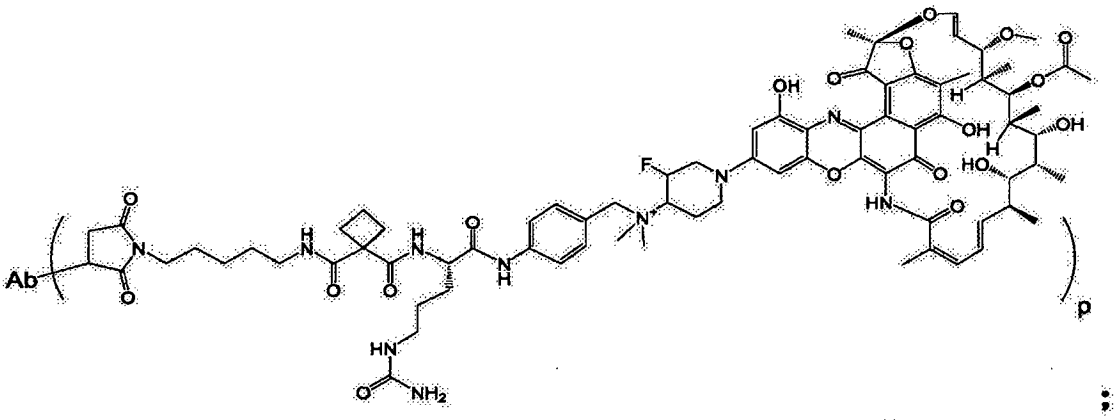
[0434] 

和

[0435] 

[0436] 本发明的抗体-抗生素缀合物(AAC)化合物的另一实施方案包括下式：

[0437]



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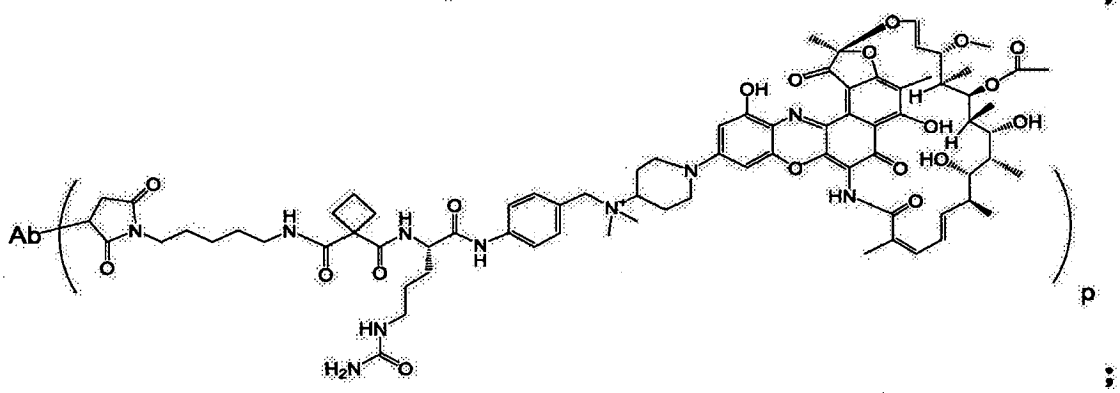
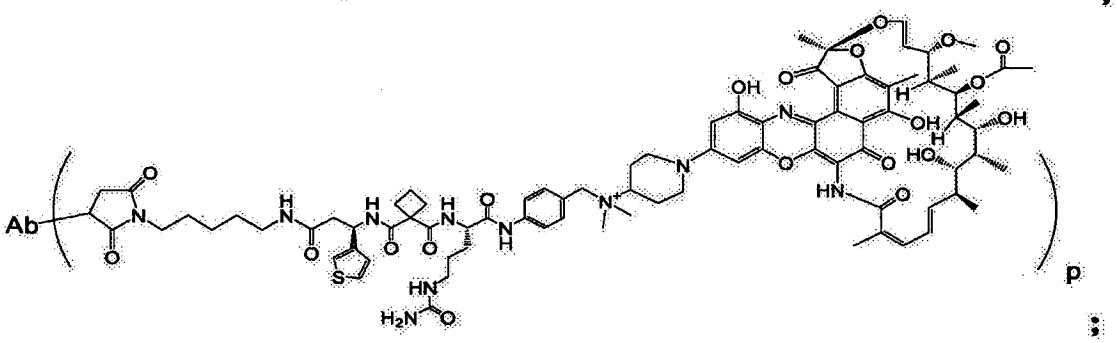
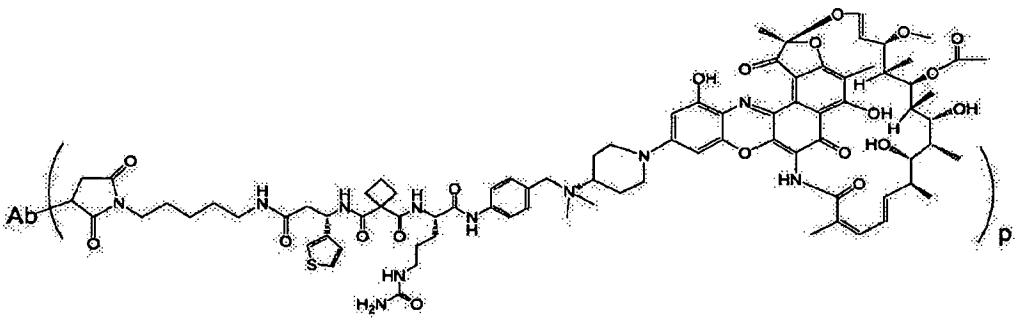


;



;

[0438]



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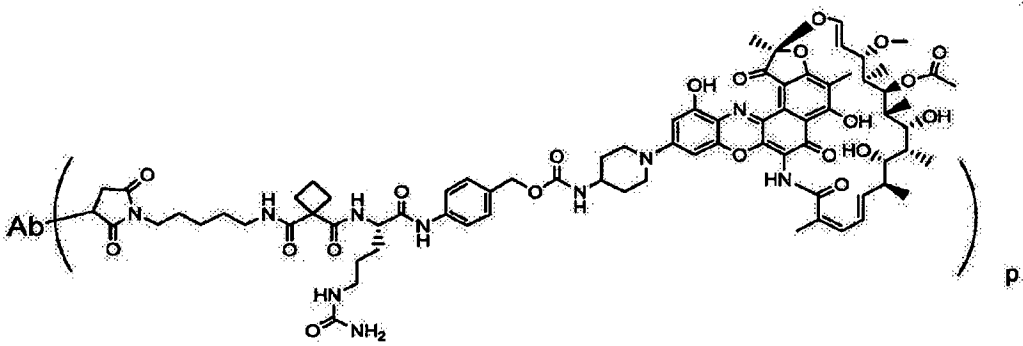
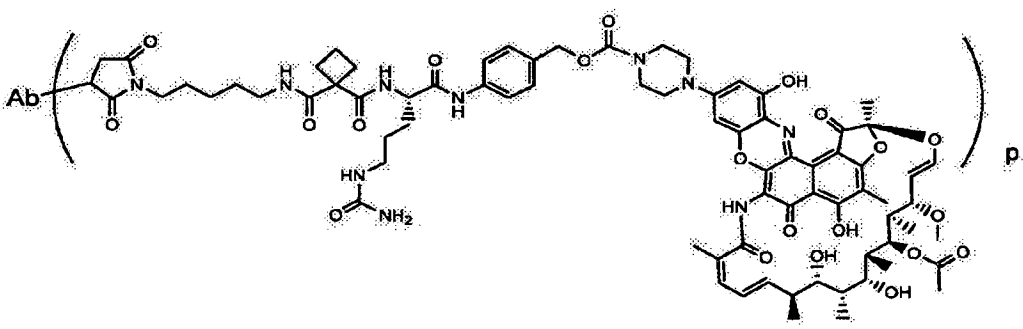


;

和



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[0439] AAC的抗生素负载

[0440] 抗生素负载量由p表示，即，在式I分子中每个抗体的抗生素(abx)部分的数。抗生素负载量可以为每个抗体1至20个抗生素部分(D)。式I的AAC包括与1至20个抗生素部分缀合的抗体集合或一组抗体。来自缀合反应的AAC制剂中每个抗体的抗生素部分的平均数可以通过常规方法表征，例如质谱、ELISA测定和HPLC。也可以确定AAC在p方面的定量分布。在一些情况下，分离、纯化和表征均质的AAC(其中p是来自具有其它抗生素负荷的AAC的某一值)可以通过诸如反相HPLC或电泳的方法来实现。

[0441] 对于一些抗体-抗生素缀合物，p可能受抗体上附着位点数目的限制。例如，如上述示例性实施方案中，当附着点是半胱氨酸硫醇，抗体可以仅具有一个或几个半胱氨酸硫醇基团，或者可以仅具有一个或几个足够反应性的硫醇基团，通过其可连接接头。在某些实施方案中，较高的抗生素负载，例如p>5，可能导致某些抗体-抗生素缀合物的聚集、不溶性、毒性或细胞通透性的丧失。在某些实施方案中，本发明的AAC的抗生素负载量为1至约8；约2至约6；约2至约4；或约3至约5；约4；或约2。

[0442] 在某些实施方案中，在缀合反应期间少于抗生素部分的理论最大值与抗体缀合。抗体可以含有例如不与抗生素-接头中间体或接头试剂反应的赖氨酸残基，如下所述。通常，抗体不含许多可与抗生素部分连接的游离和反应性半胱氨酸硫醇；实际上，抗体中大多数半胱氨酸硫醇残基作为二硫键存在。在某些实施方案中，可以在部分或全部还原条件下，用还原剂例如二硫苏糖醇(DTT)或三羰基乙基膦(TCEP)还原抗体，以产生反应性半胱氨酸硫醇基团。在某些实施方案中，将抗体进行变性条件以显示反应性亲核基团，例如赖氨酸或半胱氨酸。

[0443] 可以以不同的方式控制AAC的负载(抗生素/抗体比例，"AAR")，在本文还称为药物抗体比例(DAR)，例如通过：(i)限制抗生素-接头中间体或接头试剂相对于抗体的摩尔过量，(ii)限制缀合反应时间或温度，和(iii)部分或限制半胱氨酸硫醇修饰的还原条件。

[0444] 应当理解，当多于一个亲核基团与抗生素-接头中间体反应或与接头试剂、随后是抗生素部分试剂反应时，那么所得产物是具有连接到抗体上的一个或多个抗生素部分分布的AAC化合物的混合物。每种抗体的平均抗生素数可以通过双重ELISA抗体测定从混合物中计算出来，该测定对于抗体是特异性的并且对抗生素是特异性的。可以通过质谱在混合物中鉴定单个AAC分子，并通过HPLC分离，例如疏水相互作用层析(参见，例如McDonagh等人(2006)Prot.Engr.Pesign&Selection 19(7):299-307；Hamblett等人(2004)Clin.Cancer Res.10:7063-7070；Hamblett,K.J.等人，"药物负荷对抗CD30抗体-药物缀合物的药理学、药代动力学和毒性的影响(Effect of drug loading on the pharmacology, pharmacokinetics,and toxicity of a anti-CD30antibody-drug conjugate)"，Abstract No.624,美国癌症研究协会,2004年度会议(American Association for Cancer Research,2004Annual Meeting),March 27-31,2004,AACR学报，第45卷，2004年3月(Proceedings of the AACR,Volume 45,March 2004)；Alley,S.C.等人，"控制药物连接在抗体-药物缀合物中的位置(Controlling the location of drug attachment in antibody-drug conjugates)"，Abstract No.627,美国癌症研究协会,2004年度会议(American Association for Cancer Research,2004Annual Meeting),March 27-31,2004,AACR学报，第45卷，2004年3月(Proceedings of the AACR,Volume 45,March 2004))。在某些实施方案中，可以通过电泳或色谱从缀合混合物中分离具有单一负载值的均相AAC。本发明的半胱氨酸改造的抗体能够实现更均质的制备，因为抗体上的反应位点主要限于改造的半胱氨酸硫醇。在一个实施方案中，每个抗体的抗生素部分的平均数目在约1至约20的范围内。在一些实施方案中，选择和控制该范围为约1至4。

[0445] 制备抗体-抗生素缀合物的方法

[0446] 式I的AAC可以通过使用本领域技术人员已知的有机化学反应、条件和试剂的几种途径制备，包括：(1)抗体的亲核基团与二价接头试剂的反应，通过共价键形成Ab-L，随后与抗生素部分(abx)反应；和(2)抗生素部分的亲核基团与二价接头试剂反应，通过共价键形成L-abx，随后与抗体的亲核基团反应。US 7498298描述了通过后一种途径制备式I的AAC的示例性方法，其通过引用明确地并入本文。

[0447] 抗体上的亲核基团包括但不限于：(i)N-末端胺基，(ii)侧链胺基，例如赖氨酸，(iii)侧链硫醇基，例如半胱氨酸，和(iv)抗体被糖基化的糖羟基或氨基。胺、硫醇和羟基是亲核基团，并且能够与接头部分和接头试剂上的亲电子基团反应形成共价键，亲电子基团

包括：(i)活性酯如NHS酯、HOBt酯、卤代甲酸酯和酰基卤；(ii)烷基和苄基卤，如卤代乙酰胺；(iii)醛、酮、羧基和马来酰亚胺基团。某些抗体具有可还原的链间二硫键，即半胱氨酸桥。通过用还原剂如DTT(二硫苏糖醇)或三羰基乙基膦(TCEP)进行处理，使得抗体完全或部分还原，抗体可以与接头试剂缀合反应。因此，每个半胱氨酸桥在理论上将形成两个反应性硫醇亲核试剂。另外的亲核基团可以通过赖氨酸残基的修饰引入抗体，例如通过使赖氨酸残基与2-亚氨基噻吩(Traut's试剂)反应，导致胺转化为硫醇。可以通过引入一个、两个、三个、四个或更多个半胱氨酸残基(例如通过制备包含一个或多个非天然半胱氨酸氨基酸残基的变体抗体)，将反应性硫醇基团引入抗体。

[0448] 本发明的抗体-抗生素缀合物也可以通过抗体上的亲电子基团，例如醛或酮羰基，与接头试剂或抗生素上的亲核基团之间的反应产生。接头试剂上有用的亲核基团包括但不限于酰肼、肟、氨基、肼、缩氨基硫脲、肼羧酸酯和芳基酰肼。在一个实施方案中，修饰抗体以引入能够与接头试剂或抗生素上的亲核取代基反应的亲电子部分。在另一个实施方案中，糖基化抗体的糖可被氧化，例如，与高碘酸氧化试剂形成可与连接试剂或抗生素部分的胺基反应的醛或酮基。所得到的亚胺希夫碱基可以形成稳定的键，或可以被还原。例如由硼氢化物试剂形成稳定的胺键。在一个实施方案中，糖基化抗体的碳水化合物部分与半乳糖氧化酶或偏高碘酸钠反应，可以在抗体中产生可与抗生素上适当基团反应的羰基(醛和酮)基团(Hermanson,Bioconjugate Techniques)。在另一个实施方案中，含有N-末端丝氨酸或苏氨酸残基的抗体可与偏高碘酸钠反应，导致产生醛而代替第一个氨基酸(Geoghegan& Stroh,(1992)Bioconjugate Chem.3:138-146;US 5362852)。这样的醛可以与抗生素部分或接头亲核试剂反应。

[0449] 抗生素部分上的亲核基团包括但不限于：胺、硫醇、羟基、酰肼、肟、肼、缩氨基硫脲、肼羧酸酯和芳基酰肼基团，其能够与接头部分和接头试剂上的亲电子基团形成共价键，包括：(i)活性酯如NHS酯、HOBt酯、卤代甲酸酯和酰基卤；(ii)烷基和苄基卤，如卤代乙酰胺；(iii)醛、酮、羧基和马来酰亚胺基团。

[0450] 根据实施例7中所述的方法，表3中的抗体-抗生素缀合物(AAC)通过所述抗WTA抗体与表2的接头-抗生素中间体缀合制备。通过体外巨噬细胞测定(实施例9)和体内小鼠肾模型(实施例10)测试AAC的功效。

[0451] 表3 WTA抗体-PML-抗生素缀合物(AAC)

[0452]

AAC 编号	AAC 式	接头-抗生素 PLA 编号	AAR *
101	硫代-S6060-HC-WT/LC-cys-MC-(CBDK-cit)-PAB-(二甲基 pipBOR)	PLA-2	1.9
102	硫代-S4497-LC-cys-MC-(CBDK-cit)-PAB-(二甲基 pipBOR)	PLA-2	1.8
103	硫代-S4497-LC-V205C-MC-((*R*)-噻吩-3-基-CBDK-cit)-PAB-(二甲基 pipBOR)	PLA-3	1.8
104	硫代-S4497-LC-V205C-MC-((*S*)-噻吩-3-基-CBDK-cit)-PAB-(二甲基 pipBOR)	PLA-4	1.5
105	硫代-S4497-LC-MC-(CBDK-cit)-PABC-(派噻 BTR)	PLA-6	-
106	硫代-S4497-HC-A118C-MC-(CBDK-cit)-PAB-(二甲基 pipBOR)	PLA-2	1.8
107	硫代-S4497-HC-A118C-MC-(CBDK-cit)-PABC-(pipBOR)	PLA-5	2.0

[0453] *AAR＝抗生素/抗体比例平均值

[0454] 野生型(“WT”)、半胱氨酸改造的突变抗体(“硫代”)、轻链(“LC”)、重链(“HC”)、6-马来酰亚胺基己酰基(“MC”)、马来酰亚胺基丙酰基(“MP”)、环丁基二酮基(“CBDK”)、瓜氨酸(“cit”)、半胱氨酸(“cys”)、对氨基苄基(“PAB”)和对氨基苄氧羰基(“PABC”)

[0455] 用抗体-抗生素缀合物治疗和预防感染的方法

[0456] 本发明的抗-WTA-AAC可用作对人和兽医的葡萄球菌，例如金黄色葡萄球菌、腐生性葡萄球菌(S.saprophyticus)和溶血性葡萄球菌(S.simulans)的有效的抗微生物剂。在具体方面，本发明的AAC可用于治疗金黄色葡萄球菌感染。

[0457] 进入血液后，金黄色葡萄球菌可以在几乎任何器官中引起转移性感染。继发感染发生在治疗开始前约三分之一的病例中(Fowler等人，(2003)Arch.Intern.Med.163:2066-2072)，甚至在治疗开始后的10％的患者中(Khatib等人(2006)Scand.J.Infect.Dis.,38:7-14)。感染的标志是大量的脓液储库、组织破坏和脓疮的形成(所有这些都含有大量的嗜中性粒细胞)。如果菌血症持续超过三天，约40％的患者会发生并发症。

[0458] 上文(在副标题抗体-抗生素缀合物下)已经描述了提出的AAC的作用机制。本发明的抗WTA抗体-抗生素缀合物(AAC)对于治疗细胞内病原体具有显著的治疗优势。AAC接头通过暴露于吞噬溶酶体酶而切割，释放活性抗生素。由于局限的空间和相对较高的局部抗生素浓度(每个细菌约为10^4个)，结果是吞噬溶酶体不再支持细胞内病原体的存活。因为AAC基本上是无活性的前药，抗生素的治疗指数可以相对于游离(非缀合)形式扩展。抗体提供病原体特异性靶向，而可切割接头在病原体的细胞内位置特定的条件下切割。这种作用可以直接关系到调理的病原体以及共定位在吞噬溶酶体中的其他病原体。抗生素耐受性是导

致疾病的病原体抵抗抗生素和其他抗菌药物杀伤的能力，并且在机制上与多药耐药性不同(Lewis K(2007)."耐药株细胞、休眠和感染性疾病"。自然微生物学综述("Persister cells,dormancy and infectious disease".Nature Reviews Microbiology)5(1):48-56.doi:10.1038/nrmicro1557)。相反，这种耐受的形式是由称为耐药株的一小部分微生物细胞引起的(Bigger JW(1944年10月14日))"通过间歇性灭菌用青霉素治疗葡萄球菌感染"，柳叶刀("Treatment of staphylococcal infections with penicillin by intermittent sterilization".Lancet)244(6320):497-500)。这些细胞在经典意义上不是多药耐药性的，而是耐受抗生素治疗的休眠细胞，抗生素可以杀死其遗传上相同的兄弟姐妹。这种抗生素耐受性是由非分裂或非常缓慢分裂的生理状态引起的。当抗菌治疗无法消除这些耐药株细胞时，它们成为复发性慢性感染的储库。本发明的抗体-抗生素缀合物具有杀死这些耐药株细胞的独特性质并且抑制多药耐药细菌群体的出现。

[0459] 在另一个实施方案中，本发明的抗WTA-AAC可用于治疗感染，不管病原体是否存活于细胞内区室中。

[0460] 在另一个实施方案中，本发明的抗WTA-AAC也可用于靶向于以浮游生物或生物膜形式的葡萄球菌。用本发明的抗体-抗生素缀合物(AAC)治疗的细菌感染包括治疗细菌性肺部感染，例如金黄色葡萄球菌肺炎、骨髓炎、复发性鼻窦炎、细菌性心内膜炎、细菌性眼部感染如沙眼和结膜炎、心脏、脑或皮肤感染，胃肠道感染，一般例如旅行者腹泻、溃疡性结肠炎、肠易激综合征(IBS)、克罗恩病和IBD(炎性肠病)，细菌性脑膜炎、以及任何器官如肌肉、肝、脑膜或肺中的脓肿。细菌感染可以在身体其他部位，如尿路、血流、伤口或导管插入部位。本发明的AAC可用于涉及生物膜、植入物或避孕区(例如骨髓炎和假体关节感染)，以及高死亡率感染例如医院获得性肺炎和菌血症的难治疗的感染。可以治疗以预防金黄色葡萄球菌感染的易感患者组包括血液透析患者、免疫受损患者、重症监护病房的患者和某些手术患者。在另一方面，本发明提供了杀死、治疗或预防在动物，优选哺乳动物，最优选人类中的微生物感染的方法，其包括向所述动物施用抗WTA AAC或本发明的AAC药物制剂。本发明进一步特征在于治疗或预防与这种微生物感染相关或由其机会性地引起的疾病。这些治疗或预防的方法可包括本发明组合物的口服、局部、静脉内、肌肉内或皮下给药。例如，在手术或插入IV导管之前、在ICU护理、移植医学、癌症化疗或癌症化疗后，或其它具有高感染风险的活动中，可以施用本发明的AAC以预防感染发作或传播。

[0461] 细菌感染可以由具有活性和非活性形式的细菌引起，并且AAC以治疗细菌感染的活性和非活性潜在形式的量和足够的持续时间施用，后者持续时间长于需要治疗细菌感染的活性形式。

[0462] 多种革兰氏阳性细菌的分析发现WTAβ在全部金黄色葡萄球菌上表达，包括MRSA和MSSA菌株，以及葡萄球菌菌株如腐生性葡萄球菌和溶血性葡萄球菌。WTAα(α-GLcNAc核糖醇WTA)在大多数但不是全部的金黄色葡萄球菌上存在，并且还在单核细胞增生利斯特菌上存在。WTA不存在于革兰氏细菌上。因此，本发明的一个方面是通过施用治疗有效量的本发明的抗WTAβ-AAC来治疗感染了金黄色葡萄球菌、腐生性葡萄球菌或溶血性葡萄球菌的一种或多种的患者的方法。本发明的另一个方面是通过施用治疗有效量的本发明的抗WTAα-AAC，来治疗感染金黄色葡萄球菌和/或单核细胞增多利斯特菌的患者的方法。本发明还考虑了一种通过在医院设置例如外科手术、烧伤患者和器官移植中施用治疗有效量的本发明的抗

WTAβ-AAC，来预防金黄色葡萄球菌或腐生性葡萄球菌或溶血性葡萄球菌感染的方法。

[0463] 由本领域有经验的医师确定的需要治疗细菌感染的患者可能已经被感染、但不需要用他/她感染的细菌类型做诊断。由于患有细菌感染的患者可以在非常快的时间内轮流转，所以在几个小时内，进入医院的患者可以给予本发明的抗WTA-AAC以及一种或多种护理标准抗生素，如万古霉素或环丙沙星。当诊断结果变得可用并且指示在感染中存在例如金黄色葡萄球菌时，患者可以继续用抗WTA AAC进行治疗。因此，在治疗细菌感染或特别是金黄色葡萄球菌感染的方法的一个实施方案中，给予患者治疗有效量的抗WTAβAAC。在本发明的治疗或预防方法中，本发明的AAC可以作为唯一的治疗剂或与其它药剂如下述那些联合给药。在临床前模型中，本发明的AAC显示优于万古霉素对MRSA的治疗。AAC与SOC的比较可以例如通过降低死亡率来测量。被评估的患者将通过各种可测量因素评估对AAC治疗的反应。临床医生可能用来评估患者改善的体征和症状的例子包括：诊断升高时白细胞计数的正常化、诊断升高(发热)时体温的正常化、血液培养物的清除、在伤口中可见的改善包括红斑减少和脓液引流、减少呼吸机要求、如通气的患者需要较少氧气或降低通气率，如果患者在诊断时通气，则完全从呼吸机中脱气，如果在诊断时需要这些药物，则使用较少的药物来支持稳定的血压，如果在诊断时异常，那么提示终末器官功能衰竭例如升高的肌酐或肝功能测试的实验室异常的正常化，和放射学成像(例如以前建议肺炎显示分辨率的胸部X片)的改善。在ICU的患者中，这些因素可能至少每天测量一次。密切监测发烧，包括绝对嗜中性粒细胞计数的白细胞计数以及“左移”(指示响应于主动感染的增加的嗜中性粒细胞产生爆发的出现)的证据已经解决。

[0464] 在本发明的治疗方法的上下文中，如果本领域技术人员的医师评估在至少两个或更多个以前的因素中与治疗前或治疗开始时或诊断时的值、体征或症状相比存在显著的可测量的改善，则认为具有细菌感染的患者被治疗。在一些实施方案中，在3、4、5、6或更多个上述因素中存在可测量的改善。在一些实施方案中，与治疗前的值相比，测量因素的改善为至少50％、60％、70％、80％、90％、95％或100％。通常，如果患者的可测量的改善包括以下内容，则可以将患者视为细菌感染(例如金黄色葡萄球菌感染)被完全治疗：i)重复了不会生长出最初识别的细菌的血液或组织培养物(通常为几种)；ii)发烧正常化；iii)WBC正常化；和iv)对于患者先前存在的并发症，表明终末器官衰竭(心脏、肺、肝、肾、血管塌陷)的证据已经完全或部分地解决。

[0465] 给药。

[0466] 在任何上述方面中，在治疗感染的患者中，AAC的剂量通常为约0.001至1000mg/kg/天。在一个实施方案中，细菌感染的患者以约1mg/kg至约150mg/kg范围内的AAC剂量治疗，通常为约5mg/kg至约150mg/kg，更具体地为约25mg/kg至125mg/kg，50mg/kg至125mg/kg，甚至更具体地为约50mg/kg至100mg/kg。AAC可以每天给予(例如，单剂量为5至50mg/kg/天)或较不频繁(例如，单剂量为5、10、25或50mg/kg/周)。一个剂量可以分开2天，例如一天25mg/kg，第二天25mg/kg。患者可以每3天服用一次剂量(q3D)，每周一次至每隔一周一次(qOW)，持续1-8周。在一个实施方案中，患者通过具有护理标准(SOC)的IV每周一次、持续2-6周给予本发明的AAC，以治疗细菌感染如金黄色葡萄球菌感染。治疗长度将由患者的状况或感染的程度决定，例如，不复杂的菌血症的治疗持续时间为2周，或菌血症伴心内膜炎为6周。

[0467] 在一个实施方案中，AAC以2.5至100mg/kg的初始剂量给药连续1-7天，然后每1-7天一次0.005至10mg/kg的维持剂量，持续一个月。

[0468] 给药途径。

[0469] 为了治疗细菌感染，本发明的AAC可以静脉内(i.v.)或皮下的任何前述剂量给药。在一个实施方案中，静脉内给药WTA-AAC。在一个具体实施方案中，通过静脉内给药WTA-AAC是WTA-βAAC，更加具体地是，其中WTA-β抗体是选自具有如图12、图13A1和A2以及图13B1-B4、图14A1-A2和图14B1-B3和图15A1-A3和图15B1-B6中公开的氨基酸序列的抗体。

[0470] 联合疗法。AAC可以酌情与一种或多种另外的、例如第二治疗或预防剂一起施用，其由治疗患者的医师确定。

[0471] 在一个实施方案中，与本发明的抗体-抗生素缀合化合物组合施用的第二抗生素选自以下结构类别：(i)氨基糖苷类；(ii)β-内酰胺类；(iii)大环内酯/环肽；(iv)四环素；(v)氟喹啉/氟喹诺酮类；(vi)和噁唑烷酮类。参见：Shaw,K.和Barbachyn,M.(2011)Ann.N.Y.Acad.Sci.1241:48-70;Sutcliffe,J.(2011)Ann.N.Y.Acad.Sci.1241:122-152。

[0472] 在一个实施方案中，与本发明的抗体-抗生素缀合化合物组合施用的第二抗生素选自克林霉素、新生霉素、瑞他帕林(retapamulin)、达托霉素、GSK-2140944、CG-400549、西他沙星、替考拉宁、三氯生、萘啶酮(napthyridone)、雷得唑来、多柔比星、氨苄西林、万古霉素、亚胺培南、多利培南、吉西他滨、达巴万星和阿奇霉素。

[0473] 这些另外的治疗或预防剂的其它实例是抗炎剂(例如非甾体抗炎药(NSAID，例如detoprofen、双氯芬酸、二氟尼柳、依托度酸、非诺洛芬、氟比洛芬、布洛芬、吲哚美辛、酮洛芬、甲氯灭酸盐、甲芬那酸、美洛昔康、nabumeone、萘普生钠、奥沙普秦、吡罗昔康、舒林酸、托美汀、塞来昔布、罗非昔布、阿司匹林、水杨酸胆碱、salsalte和水杨酸钠和水杨酸镁)和类固醇(例如，可的松、地塞米松、氢化可的松、甲基强的松龙、泼尼松龙、强的松、去炎松))、抗菌药(例如阿奇霉素、克拉霉素、红霉素、加替沙星、左氧氟沙星、阿莫西林、甲硝唑、青霉素G、青霉素V、甲氧西林、苯唑西林、氯唑西林、双氯西林、萘夫西林、氨苄西林、羧苄西林、替卡西林、美洛西林、哌拉西林、阿洛西林、替莫西林、头孢噻吩、头孢匹林、头孢拉定、头孢噻啶、头孢唑啉、头孢羟唑、头孢呋辛、头孢氨苄、头孢丙烯、头孢克洛、氯碳头孢、头孢西丁、头孢奈唑、头孢噻肟、头孢唑肟、头孢曲松、头孢哌酮、头孢他啶、头孢克肟、头孢泊肟、头孢布烯、头孢地尼、头孢匹罗、头孢吡肟、BAL5788、BAL9141、亚胺培南、厄他培南、美罗培南、氨曲南、克拉维酸、舒巴坦、他唑巴坦、链霉素、新霉素、卡那霉素、巴龙霉素、庆大霉素、妥布霉素、阿米卡星、奈替米星、壮观霉素、西索米星、dibekalin、异帕米星、四环素、金霉素、地美环素、米诺环素、土霉素、甲烯土霉素、多西环素、泰利霉素、ABT-773、林可霉素、克林霉素、万古霉素、奥利万星、达巴万星、替考拉宁、奎奴普丁和达福普汀、磺胺、对氨基苯甲酸、磺胺嘧啶、磺胺异噁唑、磺胺甲噁唑、酞磺胺噻唑、利奈唑胺、萘啶酸、噁喹酸、诺氟沙星、培氟沙星、依诺沙星、氧氟沙星、环丙沙星、替马沙星、洛美沙星、氟罗沙星、格帕沙星、司帕沙星、曲伐沙星、克林沙星、莫西沙星、吉米沙星、西他沙星、达托霉素、加雷沙星、雷莫拉宁、法罗培南、多粘菌素、替加环素、AZD2563、甲氧苄氨嘧啶)、抗细菌抗体，包括来自AAC靶向Ag的相同或不同抗原的抗体、血小板聚集抑制剂(例如阿昔单抗、阿司匹林、西洛他唑、氯吡格雷、双嘧达莫、依替巴肽、噻氯匹定或替罗非班)、抗凝血剂(例如，达肝素、达那肝素、依诺肝素、肝素、亭扎肝素或华法林)、退烧药(例如，对乙酰氨基酚)或降脂药(例如，考来烯胺、考来替

泊、烟酸、吉非贝齐、普罗布考、依泽替米贝或他汀类药物如阿托伐他汀、瑞舒伐他汀、洛伐他汀、辛伐他汀、普伐他汀、西立伐他汀和氟伐他汀)。在一个实施方案中,本发明的AAC与金黄色葡萄球菌(包括耐甲氧西林和甲氧西林敏感菌株)的护理标准(SOC)一起施用。MSSA通常用萘夫西林或苯唑西林治疗,MRSA通常用万古霉素或头孢唑啉治疗。

[0474] 这些另外的试剂可以在给予AAC的14天、7天、1天、12小时或1小时内或与其同时施用。另外的治疗剂可以与AAC相同或不同的药物组合物存在。当存在于不同的药物组合物中时,可以使用不同的给药途径。例如,AAC可以静脉内或皮下给药,而第二药剂可以口服给药。

[0475] 药物制剂

[0476] 本发明还提供含有WTA-AAC的药物组合物,以及使用含有AAC的药物组合物治疗细菌感染的方法。这样的组合物可以进一步包含合适的赋形剂,例如药学上可接受的赋形剂(载体),包括缓冲剂、酸、碱、糖、稀释剂、助流剂、防腐剂等,这在本领域是公知的并且在本文中描述。本发明的方法和组合物可以单独使用或与其它常规方法和/或治疗感染性疾病的试剂组合使用。在一些实施方案中,药物制剂包含:1)本发明的抗WTAβ-AAC,和2)药学上可接受的载体。在一些实施方案中,药物制剂包含:1)本发明的AAC,和任选的2)至少一种另外的治疗剂。

[0477] 通过将具有所需纯度的AAC与任选的生理上可接受的载体、赋形剂或稳定剂(Remington's Pharmaceutical Sciences,16th edition,Osol,A.Ed.(1980))以水溶液或冻干的或其他干燥的制剂形式混合,制备包含本发明的AAC的药物制剂用于储存。可接受的载体、赋形剂或稳定剂在所使用的剂量和浓度上对受者无毒,并且包括缓冲液如磷酸盐、柠檬酸盐、组氨酸和其它有机酸;抗氧化剂,包括抗坏血酸和甲硫氨酸;防腐剂(如十八烷基二甲基苄基氯化铵、氯化六甲双铵、苯扎氯铵、苄索氯铵);苯酚、丁基或苄基醇;对羟基苯甲酸烷基酯如对羟基苯甲酸甲酯或丙酯;邻苯二酚;间苯二酚;环己醇;3-戊醇;和间甲酚);低分子量(小于约10个残基)多肽;蛋白质,如血清白蛋白、明胶或免疫球蛋白;亲水性聚合物如聚乙烯吡咯烷酮;氨基酸如甘氨酸、谷氨酰胺、天冬酰胺、组氨酸、精氨酸或赖氨酸;单糖、二糖和其他碳水化合物,包括葡萄糖、甘露糖或糊精;螯合剂如EDTA;糖类如蔗糖、甘露醇、海藻糖或山梨醇;形成盐的反荷离子如钠;金属络合物(例如Zn-蛋白复合物);和/或非离子表面活性剂如吐温™、PLURONICS™或聚乙二醇(PEG)。用于体内施用的药物制剂通常是无菌的,通过无菌过滤膜过滤容易地完成。

[0478] 活性成分也可以被包埋在例如通过凝聚技术或通过界面聚合制备的微胶囊中,例如分别在胶体药物递送系统(例如,脂质体、白蛋白微球、微乳液、纳米颗粒和纳米胶囊)或在大乳剂中的羟甲基纤维素或明胶微胶囊和聚(甲基丙烯酸甲酯)微胶囊。这种技术在Remington's Pharmaceutical Sciences第16版,Osol,A.Ed.(1980)中公开。

[0479] 可以制备缓释制剂。缓释制剂的合适实例包括含有本发明的抗体或AAC的固体疏水性聚合物的半透性基质,该基质是成形制品例如薄膜或微胶囊形式。缓释基质的实例包括聚酯、水凝胶(例如,聚(2-羟乙基-甲基丙烯酸酯)或聚(乙烯醇))、聚交酯(U.S.专利号3,773,919)、L-谷氨酸和γ乙基-L-谷氨酸盐的共聚物、不可降解的乙烯-乙酸乙烯酯、可降解乳酸-乙醇酸共聚物如LUPRON DEPOT™(由乳酸-乙醇酸共聚物和醋酸亮丙瑞林组成的可注射微球)和聚-D-(-)-3-羟基丁酸。虽然聚合物如乙烯-乙酸乙烯酯和乳酸-乙醇酸能够释放

分子超过100天，但某些水凝胶在较短的时间段释放蛋白质。当封装的抗体或AAC长时间保留在体内时，由于在37℃暴露于水分中，它们可能会变性或聚集，导致生物活性的丧失和免疫原性的可能变化。根据所涉及的机制，可以设计出稳定的合理策略。例如，如果发现聚集机制是通过硫醇-二硫化物交换形成分子间S-S键，则可以通过修饰巯基残基、用酸性溶液冻干、控制含水量、使用合适的添加剂和开发特定的聚合物基质组合物来实现稳定化。

[0480] AAC可以以任何合适的形式配制以递送至靶细胞/组织。例如，AAC可以配制成脂质体，由各种类型的脂质、磷脂和/或表面活性剂组成的小囊泡，其可用于向哺乳动物递送药物。脂质体的组分通常排列成双层形成，类似于生物膜的脂质排列。含有抗体的脂质体通过本领域已知的方法制备，例如在Epstein等人(1985)Proc.Natl.Acad.Sci.USA 82:3688;Hwang等人，(1980)Proc.Natl Acad.Sci.USA 77:4030;US 4485045;US 4544545;WO 97/38731;US 5013556中描述的。

[0481] 特别有用的脂质体可以通过含有磷脂酰胆碱、胆固醇和PEG衍生的磷脂酰乙醇胺(PEG-PE)的脂质组合物的反相蒸发法产生。脂质体通过限定孔径的过滤器挤出以产生具有所需直径的脂质体。

[0482] 材料和方法

[0483] 细菌菌株：

[0484] 所有实验都是使用从NARSA(http://www.narsa.net/control/member/repositories)获得的MRSA-USA300NRS384进行的，除非另有说明。

[0485] 细胞外细菌的MIC测定

[0486] 通过在胰酶解大豆酪蛋白肉汤培养基中连续2倍稀释制备的抗生素来测定细胞外细菌的MIC。抗生素的稀释液在96孔培养皿中一式四份制成。从指数生长培养物取MRSA(USA300的NRS384菌株)并稀释至1×10^4CFU/mL。细菌在抗生素存在下培养18-24小时并在37℃下振荡，通过读取630nM的光密度(OD)测定细菌生长。MIC被确定为将细菌生长抑制>90%的抗生素剂量。

[0487] 细胞内细菌的MIC测定

[0488] 对鼠腹膜巨噬细胞内隐藏的细菌测定细胞内MIC(见下文，用于产生鼠腹膜巨噬细胞)。将巨噬细胞以4×10^5个细胞/mL的密度接种在24孔培养皿中，并以每个巨噬细胞10-20个细菌的比例感染MRSA。将巨噬细胞培养物保持在补充有50μg/mL庆大霉素(仅对细胞外细菌有活性的抗生素)的生长培养基中，以抑制细胞外细菌的生长，并在感染后1天将测试的抗生素加入到生长培养基中。在加入抗生素后24小时评估细胞内细菌的存活。用补充有0.1%牛血清白蛋白和0.1%Triton-X的Hanks缓冲盐水溶液裂解巨噬细胞，并在含有0.05%吐温-20的磷酸缓冲盐水溶液中进行裂解物的连续稀释。通过在含有5%去纤维蛋白的羊血的胰酶解大豆琼脂平板上接种来确定存活的细胞内细菌数。

[0489] 分离腹膜巨噬细胞：

[0490] 从6-8周龄Balb/c小鼠(Charles River Laboratories,Hollister,CA)腹膜中分离腹膜巨噬细胞。为了增加巨噬细胞的产量，通过腹膜内注射1mL巯基乙酸盐培养基(Becton Dickinson)预处理小鼠。巯基乙酸盐培养基以4%的浓度在水中制备，通过高压灭菌消毒，并在使用前老化20天至6个月。通过用冷磷酸盐缓冲盐水洗涤腹腔，用巯基乙酸盐处理4天后收获腹膜巨噬细胞。将巨噬细胞以$4x10^5$细胞/孔的密度在24孔培养盘中接种在

补充有10％胎牛血清和10mM HEPES(不含抗生素)的Dulbecco's Modified Eagle培养基(DMEM)上。将巨噬细胞培养过夜以使其粘附到平板上。该试验也用于测试非吞噬细胞类型中的细胞内杀伤。从ATCC获得MG63(CRL-1427)和A549(CCL185)细胞系，并保持在补充有10mM Hepes和10％胎牛血清(RPMI-10)的RPMI 1640组织培养基中。HUVEC细胞获自Lonza并保持在EGM内皮细胞完全培养基(Lonza,Walkersville,MD)中。

[0491] 用调理的MRSA感染巨噬细胞：

[0492] MRSA的USA300株(NRS384)获自NARSA储存库(Chantilly,Virginia)。一些实验使用了金黄色葡萄球菌的Newman菌株(ATCC25904)。在所有实验中，将细菌在胰酶解大豆酪蛋白肉汤培养基中培养。为了评估AAC的细胞内杀伤，从指数生长的培养物中取出USA300，并在HB(Hanks平衡盐溶液，补充有10mM HEPES和0.1％牛血清白蛋白)中洗涤。将AAC或抗体在HB中稀释，并与细菌一起孵育1小时以允许抗体与细菌结合(调理)，并将该调理的细菌用于以每个巨噬细胞10-20个细菌的比例感染巨噬细胞(每孔250μL的HB中$4x10^6$细菌)。巨噬细胞在感染前立即用无血清DMEM培养基进行预洗，并在37℃下在加湿组织培养培养箱中用5％CO_2孵育进行感染以允许细菌吞噬。2小时后，移除感染混合物并用正常生长培养基(补充有10％胎牛血清、10mM HEPES的DMEM)代替，并加入50μg/ml的庆大霉素以阻止细胞外细菌生长。在孵育期结束时，用无血清培养基洗涤巨噬细胞，并将细胞在补充有0.1％triton-X的HB中裂解(裂解巨噬细胞而不损伤细胞内的细菌)。在一些实验中，通过使用LDH细胞毒性检测试剂盒(产品11644793001,Roche Diagnostics Corp,Indianapolis,IN)检测细胞质乳酸脱氢酶(LDH)在培养基上清液中的释放，从而在培养期结束时评估巨噬细胞的活力。根据制造商的说明书，立即收集和分析上清液。裂解物的系列稀释液在补充有0.05％Tween-20的磷酸盐缓冲盐水溶液(以破坏细菌聚集体)中制备，通过在具有5％去纤维化羊血的胰蛋白酶大豆琼脂上接种以测定存活的细胞内细菌总数。

[0493] MRSA感染腹膜细胞的产生。

[0494] 通过腹膜内注射，用1×10^8CFU的USA300的NRS384菌株感染6-8周龄的雌性A/J小鼠(JAX ™小鼠，Jackson Laboratories)。在感染后1天收获腹膜洗涤液，并在37℃下用补充了0.1％BSA(HB缓冲液)的Hepes缓冲液中稀释的50μg/mL溶葡萄球菌酶处理感染的腹膜细胞30分钟。然后将腹膜细胞在冰冷的HB缓冲液中洗涤2次。在补充有10mM Hepes和10％胎牛血清和5μg/mL万古霉素的RPMI 1640组织培养基中将腹膜细胞稀释至1×10^6细胞/mL。在磷酸盐缓冲盐水溶液中，在4℃下将来自原发感染的游离MRSA保存过夜，作为不经历嗜中性粒细胞杀伤的细胞外细菌的对照。

[0495] 将感染从腹膜细胞转移到成骨细胞：

[0496] MG63成骨细胞系获自ATCC(CRL-1427)，并保持在补充有10mM Hepes和10％胎牛血清(RPMI-10)的RPMI 1640组织培养基中。将成骨细胞接种在24孔组织培养板中并培养以获得汇合层。在实验当天，将成骨细胞在RPMI(无补充物)中洗涤一次。在完全RPMI-10中稀释MRSA或感染的腹膜细胞，并在感染前立即以5μg/mL加入万古霉素。将腹膜细胞以1×10^6个腹膜细胞/mL加入到成骨细胞中。用0.1％triton-x裂解细胞样品以测定感染时活的细胞内细菌的实际浓度。所有感染的实际滴度是通过用5％去纤维蛋白的羊血的胰蛋白酶大豆琼脂上的细菌连续稀释液来测定。

[0497] 将MG63成骨细胞接种在4孔玻璃室载玻片中并在补充有10mM Hepes和10％胎牛血

清(RPMI-10)的RPMI 1640组织培养基中培养,直到形成汇合层。在感染当天,用无血清培养基洗涤各孔,用感染腹膜细胞的悬浮液感染,或用补充有5μg/mL万古霉素的完全RPMI-10中稀释的MRSA的USA300菌株进行感染。感染后一天,用磷酸盐缓冲盐水(PBS)洗涤细胞,并在室温下用含有2%多聚甲醛的PBS固定30分钟。将各孔在PBS中洗涤3次,并在室温下用含有0.1%皂角蛋白的PBS透化30分钟。

[0498] 感染模型的体内转移:

[0499] 制备USA300储备液用于在胰酶解大豆酪蛋白肉汤培养基中积极生长培养物的感染。将细菌在磷酸盐缓冲盐水(PBS)中洗涤3次,并将等分试样在-80℃下在PBS 25%甘油中冷冻。细胞内细菌感染:选择A/J小鼠用于这些实验,因为它们容易被相对低剂量的MRSA($2x10^6$CFU/小鼠)感染。从Jackson Lab获得7周龄的雌性A/J小鼠,并用5×10^7CFU的USA300腹膜内注射感染。在感染后1天处死小鼠,用5mL冷PBS冲洗腹膜。在台式离心机中,将腹膜洗涤液在4℃下以1,000rpm离心5分钟。收集含有腹膜细胞的细胞沉淀,并将细胞用50μg/mL溶葡萄球菌酶(Cell Sciences Inc.MAC MA,CRL 309C)在37℃下处理20分钟以杀死污染的细胞外细菌。将腹膜细胞在冰冷的PBS中洗涤3次以除去溶葡萄球菌酶。汇集来自供体小鼠的腹膜细胞,并通过静脉注射向受体小鼠的尾静脉注射来自每个受体的5个供体的细胞。为了测定活细胞内CFU的数量,将腹膜细胞样品在含有0.1%Triton-X的HB(Hanks平衡盐溶液中补充有10mM HEPES和0.1%牛血清白蛋白)中裂解,并在含有0.05%吐温-20的PBS中制备裂解液的连续稀释液。无细菌感染:使用用于腹膜注射的甘油储备液的新鲜等分试样,将A/J小鼠感染各种剂量的游离细菌。实际感染剂量通过CFU接种确认。对于图1A所示的数据,细胞内细菌的实际感染剂量为1.8×10^6CFU/小鼠,无细菌的实际感染剂量为2.9×10^6CFU/小鼠。选择的小鼠感染后立即静脉注射的单剂量100mg/Kg万古霉素治疗。

[0500] 感染的体外转移到非吞噬细胞。

[0501] MRSA感染腹膜细胞的产生:通过腹腔注射用1×10^8CFU的USA300的NRS384菌株感染6-10周龄的雌性A/J小鼠(Jackson Lab)。在感染后1天收获腹膜洗涤液,在37℃下,用补充了0.1%BSA(HB缓冲液)的Hepes缓冲液中稀释的50μg/mL溶葡萄球菌酶处理感染的腹膜细胞30分钟。然后将腹膜细胞在冰冷的HB缓冲液中洗涤2次。在补充有10mM Hepes和10%胎牛血清和5μg/mL万古霉素的RPMI 1640组织培养基中将腹膜细胞稀释至1×10^6细胞/mL。将原代感染的游离MRSA在4℃下在磷酸缓冲盐水溶液中保存过夜,作为不经历嗜中性粒细胞杀伤的细胞外细菌的对照。

[0502] 成骨细胞或HBMEC感染。MG63细胞系获自ATCC(CRL-1427),并保持在补充有10mM Hepes和10%胎牛血清(RPMI-10)的RPMI 1640组织培养基中。从SciencCell Research Labs(Carlsbad,CA)获得HBMEC细胞(目录号1000)和ECM培养基(目录#1001)。将细胞接种在24孔组织培养板中并培养以获得汇合层。在实验当天,将细胞在RPMI(无补充物)中洗涤一次。将MRSA或感染的腹膜细胞在完全RPMI-10中稀释,并在感染前立即以5ug/mL加入万古霉素。将腹膜细胞以1×10^6个腹膜细胞/mL加入到成骨细胞中。用0.1%triton-x裂解细胞样品以测定感染时活细胞内细菌的实际浓度。所有感染的实际滴度通过在含5%去纤维蛋白的羊血的胰蛋白酶大豆琼脂上的细菌连续稀释液来测定。

[0503] 抗金黄色葡萄球菌抗体的产生。

[0504] 使用单克隆抗体发现技术,从金黄色葡萄球菌感染患者的外周B细胞克隆抗β-

GlcNAc WTA mAb的人IgG抗体，其保护抗体重链和轻链的同源配对[38]。通过转染哺乳动物细胞表达单个抗体克隆(Meijer,P.J.,Nielsen,L.S.,Lantto,J.&Jensen,A.(2009)Human antibody repertoires.Methods Mol Biol 525,261-277,xiv;Meijer,P.J.等人(2006)"Isolation of human antibody repertoires with preservation of the natural heavy and light chain pairing."Journal of molecular biology 358,764-772)。7天后收获含有全长IgG1抗体的上清液，用于筛选ELISA的抗原结合。这些抗体对于结合来自USA300的细胞壁制备物是阳性的。随后在200-ml瞬时转染中产生抗体，并用蛋白A色谱(MabSelect SuRe,GE Life Sciences,Piscataway,NJ)纯化用于进一步测试。这些抗体的分离和使用被区域伦理审查委员会批准。

[0505] 接头药物与抗体的缀合。

[0506] 抗-WTA抗体(Ab)的THIOMAB变体的构建和制备如下进行。在抗-WTA Ab轻链的Val 205位置处将半胱氨酸残基工程化，以产生其THIOMAB™半胱氨酸工程化的抗体变体。该硫代抗WTA与表2中的PML接头-抗生素中间体缀合。在五十倍摩尔过量的DTT存在下将抗体还原过夜。使用HiTrap SP-HP柱(GE Healthcare)将还原剂和半胱氨酸和谷胱甘肽嵌段纯化。在十五倍摩尔过量脱氢抗坏血酸(MP Biomedical)存在下将抗体再氧化2.5小时。通过LC/MS监测链间二硫键的形成。将超过蛋白质的3倍摩尔过量的PML接头抗生素中间体与THIOMAB孵育1小时。通过0.2um SFCA过滤器(Millipore)过滤纯化抗体药物缀合物。通过过滤除去过量的游离接头药物。通过透析将缀合物缓冲液交换成20mM的组氨酸乙酸盐pH5.5/240mM蔗糖。通过LC/MS分析将每mAb的缀合的利福霉素型抗生素的数量定量为抗生素/抗体比(AAR)。还通过尺寸排阻色谱法评估纯度。

[0507] 质谱分析

[0508] 在6530精确质量四极杆飞行时间(Q-TOF)LC/MS(Agilent Technologies)上进行LC/MS分析。将样品在加热至80℃的PRLP-S柱，**1000 Å**，8μm(50mm×2.1mm,Agilent Technologies)上进行色谱分离。使用4.3分钟内30-60%B的线性梯度(溶剂A,水中0.05% TFA;溶剂B,乙腈中的0.04%TFA)，使用电喷雾源直接进行洗脱液离子化。使用Agilent Mass Hunter定性分析软件收集数据并进行解卷积。在LC/MS分析之前，将抗体药物缀合物用赖氨酰内肽酶(Wako)以1:100w/w的酶与抗体比例，在pH8.0和37℃处理30分钟，产生Fab和Fc部分以便于分析。使用MassHunter软件计算的Fab和Fab+1的丰度计算抗生素对抗体比例(AAR)(本文中与药物与抗体比率(DAR)可互换使用)。

[0509] 从感染小鼠分离的细菌分析：通过静脉内注射1×10^7CFU MRSA(USA300)感染Balb/c小鼠，感染后第3天收获肾。使用M-Tubes和程序RNA01.01(Miltenyi Biotec,Auburn,CA)，使用每2个肾5mL体积的GentleMACS解离器将肾均质化。均质化缓冲液是:PBS+0.1%Triton-X100、10ug/mL DNA酶(牛胰腺II级,Roche)和蛋白酶抑制剂(完整的蛋白酶抑制剂鸡尾酒,Roche 11-836-153001)。均质化后，将样品在室温下孵育10分钟，然后用冰冷的PBS稀释，并通过40uM细胞过滤器过滤。将组织匀浆在冰冷的PBS中洗涤2次，然后在HB缓冲液(Hanks平衡盐溶液，补充有10mM HEPES和0.1%牛血清白蛋白)中悬浮于每2个肾0.5mL的体积中。再次过滤细胞悬浮液，每次染色反应取25μL细菌悬浮液。

[0510] 用流式细胞术比较抗MRSA抗体的表达：将流式细胞术的抗体染色细菌($1x10^7$体外生长细菌或25uL上述组织匀浆)悬浮在HB(上述)中，通过用400μg/mL(微克/毫升)小鼠IgG

(SIGMA,I5381)孵育1小时进行阻断。将荧光标记的抗体直接加入到阻断反应中,并在室温下再孵育10-20分钟。将细菌在HB缓冲液中洗涤3次,然后在FACS分析前将其固定在PBS 2%多聚甲醛中。使用胺反应试剂(Invitrogen,Alexa Fluor 488的琥珀酰亚胺酯,NHS-A488)将测试抗体(抗βWTA:4497、抗αWTA:7578或同种型对照:gD)与Alexa-488缀合。将50mM磷酸钠中的抗体与5-10倍摩尔过量的NHS-A488在室温下在黑暗中反应2-3小时。将标记混合物施加到在PBS中平衡的GE Sepharose S200柱上以从缀合抗体中除去过量的反应物。使用制造商所述的UV法测定A488分子/抗体的数量。

[0511] 为了分析组织匀浆中的细菌,使用非竞争性抗金黄色葡萄球菌抗体(rF1-Hazenbos,W.L.等人(2013))新型葡萄球菌糖基转移酶SdgA和SdgB介导了毒力相关细胞壁蛋白的免疫原性和保护作用。PLoS Pathog 9、e1003653与Alexa-647缀合以将金黄色葡萄球菌从相似大小的颗粒中区分。在80ng/mL至50μg/mL的剂量范围内检查测试抗体。使用Beckton Dickson FACS ARIA(BD Biosciences,San Jose CA)进行流式细胞术,并使用FlowJo分析软件(Flow Jo LLC,Ashland OR)进行分析。

[0512] 在非复制细菌上杀死游离抗生素的时间:将金黄色葡萄球菌(USA300)从过夜静止期培养物中取出,在磷酸盐缓冲盐水(PBS)中洗涤一次,并以1×10^{7}CFU/mL悬浮于无抗生素或以1×10^{-6}M抗生素在10mL体积的50mL聚丙烯离心管中的PBS中。将细菌在37℃下振荡过夜。在每个时间点,从每个培养物中取出三个1mL样品,并离心收集细菌。将细菌用PBS洗涤一次以除去抗生素,并通过在琼脂板上接种细菌的连续稀释液来测定存活细菌的总数。

[0513] 通过游离抗生素杀死休眠细胞:将金黄色葡萄球菌(USA300)从过夜静止期培养物中取出,在胰酶解大豆酪蛋白肉汤培养基(TSB)中洗涤一次,然后调节至在10mL总体积的TSB或含有环丙沙星(0.05mM)的TSB中1×10^{7}CFU/ml的终浓度。将培养物在37℃振荡培养6小时,然后加入第二种抗生素,利福平(1μg/ml)或另一种利福霉素型抗生素(1μg/ml)。在指定时间,从每个培养物中取出样品,用PBS洗涤一次以除去抗生素并重新悬浮在PBS中。通过在琼脂板上接种细菌的连续稀释液来测定存活细菌的总数。在最后时间点,收集每个培养物的剩余部分并接种。

[0514] AAC的组织蛋白酶释放试验:为了量化用组织蛋白酶B处理后从AAC释放的活性抗生素的量,将AAC在组织蛋白酶缓冲液(20mM乙酸钠,1mM EDTA,5mM L-半胱氨酸pH 5)中稀释至200ug/mL。以10ug/mL加入组织蛋白酶B(来自牛脾,SIGMA C7800),将样品在37℃下孵育1小时。作为对照,将AAC在缓冲液中单独孵育。通过加入9体积的细菌生长培养基胰酶解大豆酪蛋白肉汤培养基pH 7.4(TSB)来终止反应。为了评估活性抗生素的总释放量,将反应混合物的系列稀释液在TSB中在96孔板中一式四份制成,并以2×10^{3}CFU/mL的最终密度向每个孔中加入MRSA(USA300)。将培养物在37℃下振荡孵育过夜,并使用平板读数器通过读取630nM的吸光度来检测细菌生长。

[0515] 合成S4497抗体FRET缀合物用于吞噬溶酶体处理。

[0516] 合成了马来酰亚胺FRET肽,并与S4497半胱氨酸工程化的THIOMAB™抗体缀合。FRET对使用四甲基罗丹明(TAMRA)和荧光素(Fischer,R.等人(2010)Bioconjug Chem 21,64-73)。通过使用PS3肽合成仪(Protein Technologies,Inc)的标准Fmoc固相化学合成马来酰亚胺FRET肽。简言之,使用0.1mmol Rink酰胺树脂产生C末端甲酰胺。使用N-和C-末端残基处的Fmoc-Lys(Mtt)-OH以除去树脂上的Mtt(单甲氧基三苯甲基)基团,并进行附加的侧链

化学以连接TAMRA和荧光素。作为组织蛋白酶切割间隔物，在FRET对之间添加CBDK-cit肽模拟单元。从树脂上切下的粗的马来酰亚胺FRET肽或马来酰亚氨基己酰基-K(TAMRA)-G-CBDK-cit-K(荧光素)通过反相HPLC进一步纯化，使用Jupiter 5μmC4柱(5μm，10mm x 250mm，Phenomenex)。我们的FRET探针不仅可以监测抗体缀合物的细胞内运输，还可以监测吞噬溶酶体中接头的处理。由于来自供体的荧光共振能量转移，完整抗体缀合物仅发红色荧光。然而，在吞噬溶酶体中的FRET肽的底物切割后，预期出现来自供体的绿色荧光。

[0517] 视频显微镜检测巨噬细胞内接头的裂解

[0518] 将小鼠腹膜巨噬细胞接种在室载玻片(Ibidi，Verona，WI，目录80826)上，在如巨噬细胞细胞内杀伤试验所述的完整培养基中。通过在100μg/mL PBS.1%BSA中在37℃下孵育30分钟，将USA300用Cell Tracker Violet(Invitrogen C10094)标记。通过在HB缓冲液中孵育1小时，用4497-FRET探针调理标记的细菌。立即将巨噬细胞洗涤一次，然后加入调理细菌，且将细菌以1×10^7细菌/mL加入细胞。对于无吞噬作用的对照，巨噬细胞在吞噬前和吞噬期间用60nM Latrunculin A(Calbiochem)预处理30分钟。在将细菌加入细胞后立即将载玻片放置在显微镜上，并用配备有CO_2环境室和来自Ludin的温度控制器的Leica SP5共聚焦显微镜获得视频。使用Plan APO CS 40X，N..A:1.25，油浸透镜和488nm和543nm激光线，分别激发alexa 488和TAMRA，每分钟捕获图像总共30分钟。还使用543nm激光线记录相位图像。

[0519] 通过质谱法测定巨噬细胞内释放的抗生素。

[0520] 将小鼠腹膜巨噬细胞在24孔组织培养皿中感染，如下所述，用在HB中100μg/mL的AAC调理的MRSA进行细胞内杀伤试验。吞噬完成后，洗涤细胞，向各孔中加入250μL完全培养基+庆大霉素，将细胞孵育1小时或3小时。在每个时间点收集上清液和细胞组分，并加入乙腈(ACN)至最终浓度为75%，孵育30分钟。细胞和上清液提取物通过在N2(TurboVap)下蒸发并冷冻干燥，并在100μL的50%ACN中重构，过滤和在Ab Sciex QTRAP 6500LC/MS/MS系统上进行分析。

[0521] 体外的细胞内杀伤试验。

[0522] 非吞噬细胞类型：从ATCC获得MG63(CRL-1427)和A549(CCL185)细胞系，并保持在补充有10mM Hepes和10%胎牛血清(RPMI-10)的RPMI 1640组织培养基中。HUVEC细胞获自Lonza并保持在EGM内皮细胞完全培养基(Lonza，Walkersville，MD)中。从SciencCell Research Labs(Carlsbad，CA)获得HBMEC细胞(目录号1000)和ECM培养基(目录#1001)。

[0523] 鼠巨噬细胞：从6-8周龄Balb/c小鼠(Charles River Laboratories，Hollister，CA)腹膜中分离腹膜巨噬细胞。为了增加巨噬细胞的产量，通过腹膜内注射1mL巯基乙酸盐培养基(Becton Dickinson)预处理小鼠。巯基乙酸盐培养基以4%的浓度在水中制备，通过高压灭菌消毒，并在使用前老化20天至6个月。通过用冷磷酸盐缓冲盐水洗涤腹膜，用巯基乙酸盐处理4天后收获腹膜巨噬细胞。将巨噬细胞接种在补充有10%胎牛血清和10mM HEPES的不含抗生素的Dulbecco改良的Eagle培养基(DMEM)中，在24孔培养盘中以$4x10^5$细胞/孔的密度接种。将巨噬细胞培养过夜使其粘附到平板上。

[0524] 人M2巨噬细胞：使用单核细胞分离试剂盒II(Miltenyi，Cat 130-091-153)从正常人血液中纯化$CD14^+$单核细胞，并以1.5×10^5细胞/cm^2在预先涂覆有胎牛血清(FCS)的组织培养皿上接种，并在含有20%FCS+100ng/mL rhM-CSF的RPMI 1640培养基中培养。在第1天

和第7天更新培养基，将培养基更换为5％血清+20ng/mL IL-4。18小时后使用巨噬细胞。

[0525] 试验方案：在所有实验中，细菌在胰酶解大豆酪蛋白肉汤培养基中培养。为了评估抗体抗生素缀合物(AAC)的细胞内杀伤，将USA300从指数生长培养物中取出，并在HB(Hanks平衡盐溶液，补充有10mM HEPES和0.1％牛血清白蛋白)中洗涤。将AAC或抗体在HB中稀释并与细菌一起孵育1小时以允许抗体与细菌结合(调理)，并且调理细菌用于以每个巨噬细胞10-20个细菌的比例(每孔250uL HB中4×10^6细菌)感染巨噬细胞。巨噬细胞在感染前立即用无血清DMEM培养基进行预清洗，并在37℃下，在含有5％CO_2的加湿组织培养箱中孵育，以允许细菌吞噬。2小时后，除去感染混合物并用正常生长培养基(补充有10％胎牛血清、10mM HEPES)代替，并将庆大霉素以50ug/ml加入以防止细胞外细菌生长。在孵育期结束时，用无血清培养基洗涤巨噬细胞，并将细胞在补充有0.1％triton-X的HB中裂解(裂解巨噬细胞而不损伤细胞内的细菌)。在补充有0.05％Tween-20(破坏细菌聚集体)的磷酸盐缓冲盐水溶液中制备裂解物的系列稀释液，通过在含有5％去纤维蛋白的绵羊血的胰蛋白酶大豆琼脂接种来测定存活的细胞内细菌总数。

实施例

[0526] 实施例1细胞内MRSA受到对抗常规抗生素的保护

[0527] 为了证实哺乳动物细胞在抗生素治疗存在下为金黄色葡萄球菌提供保护性小环境的假说，将目前用作侵入性MRSA感染的护理标准(SOC)的三种主要抗生素(万古霉素、达托霉素和利奈唑胺)对细胞外浮游细菌与小鼠巨噬细胞内隔离细菌的效力进行比较(表4)。

[0528] 对于细胞外细菌，将MRSA在胰酶解大豆酪蛋白肉汤培养基中培养过夜，并确定MIC是防止生长的最小抗生素剂量。对于细胞内细菌，小鼠腹膜巨噬细胞用MRSA感染并在庆大霉素存在下培养，以杀死细胞外细菌。感染后一天将测试抗生素加入到培养基中，24小时后测定存活的细胞内细菌总数。临床相关抗生素的预期血清浓度在抗菌药物(Antimicrobial Agents)，Andre Bryskier.ASM Press，Washington DC(2005)中被报道。

[0529] 表4：几种抗生素对在液体培养物中生长的细胞外细菌与小鼠巨噬细胞内隔离的细胞内细菌的最小抑菌浓度(MIC)。

[0530]

抗生素(Abx)	细胞外 MRSA MIC (μg/mL)	细胞内 MRSA MIC (μg/mL)	血清 Cmax (μg/mL)

[0531]

万古霉素	1	>100	50
达托霉素	4	>100	60
利奈唑胺	0.3	>20	20
利福平	0.004	50	20

[0532] 具有高毒力的社区获得性MRSA菌株USA300的分析显示，虽然细胞外MRSA对液体培养中低浓度的万古霉素、达托霉素和利奈唑胺的生长抑制高度敏感，但所有三种抗生素都不能杀死暴露于临床可达到的抗生素浓度的在巨噬细胞内部隔离的MRSA相同菌株。即使是利福平，被认为相对有效地消除细胞内病原体(Vandenbroek,P.V.(1989)抗菌药物、微生物、吞噬细胞。传染病综述(Antimicrobial Drugs,Microorganisms,Phagocytes.Reviews of Infectious Diseases)11,213-245)，与抑制浮游细菌生长(MIC)所需的剂量比较，仍需要6,000倍更高的剂量来消除细胞内MRSA(表1)，这与其他研究一致，表明大多数现有抗生素在体外和体内杀死细胞内金黄色葡萄球菌都是无效的(Sandberg,A.,Hessler,J.H.,Skov,R.L.,Blom,J.&Frimodt-Moller,N.(2009)“鼠腹膜炎模型中抗生素对金黄色葡萄球菌的细胞内活性(Intracellular activity of antibiotics against Staphylococcus aureus in a mouse peritonitis model)”Antimicrob Agents Chemother 53,1874-1883)。

[0533] 实施例2细胞内MRSA感染的传播

[0534] 这些实验比较了细胞内细菌与等量的游离浮游细菌的毒性，并确定细胞内细菌是否能够在体内在万古霉素存在下建立感染。通过静脉内注射大致相当剂量的金黄色葡萄球菌游离细菌(2.9×10^6)感染四组小鼠，其细菌直接从肉汤培养物，或在由供体小鼠腹膜感染产生的宿主巨噬细胞和嗜中性粒细胞内隔离的细胞内细菌(1.8×10^6)中取出(图1A)，并且选择的组在感染后立即用万古霉素处理，然后每天一次。在感染后4天检查小鼠，细菌定殖在肾脏中，其是在小鼠中一致地被金黄色葡萄球菌定殖的器官[23]。在三次独立实验中，观察到感染细胞内细菌的小鼠肾脏中与那些感染了相同量的浮游细菌相比的相同或更高的细菌负荷(图1B)。令人惊讶的是，发现细胞内细菌的感染导致脑的更一致的定殖，这是在该模型中感染浮游细菌后没有有效定殖的器官(图1C)。此外，在该模型中，细胞内细菌而不是浮游细菌能够在万古霉素治疗的情况下建立感染(图1B、图1C)

[0535] 体外的进一步分析更定量地解决了细胞内存活促进抗生素逃避的程度。为此，在万古霉素存在下，MG63成骨细胞被浮游MRSA或细胞内MRSA感染。

[0536] 成骨细胞或HBMEC感染。从ATCC获得MG63细胞系(CRL-1427)，并保持在补充有10mM Hepes和10％胎牛血清的RPMI 1640组织培养基(RPMI-10)中。从Scienc Cell Research Labs(Carlsbad,CA)获得HBMEC细胞(目录号#1000)和ECM培养基(目录号#1001)。将细胞接种在24孔组织培养板中并培养以获得汇合层。在实验当天，细胞在RPMI(无补充物)中洗涤一次。将MRSA或感染的腹膜细胞在完全RPMI-10中稀释，并在感染前立即加入5ug/mL万古霉

素。将腹膜细胞以1×10^6个腹膜细胞/mL加入到成骨细胞中。用0.1%triton-x裂解细胞样品以确定感染时活的细胞内细菌的实际浓度。所有感染的实际滴度通过在含5%去纤维蛋白的羊血的胰酶解大豆琼脂上连续稀释的细菌接种来测定。

[0537] 将MRSA(游离细菌)接种在培养基、培养基+万古霉素或培养基+万古霉素并接种在单层MG63成骨细胞(图1E)或人脑微血管内皮细胞(HBMEC,图1F)上。将板离心以促进细菌与单层接触。在每个时间点,收集培养物上清液以回收细胞外细菌或裂解粘附细胞以释放细胞内细菌。

[0538] 暴露于仅有的万古霉素的浮游细菌被有效地杀死。在培养一天后,存活细菌未恢复(图1D)。当将类似数量的浮游细菌接种在MG63成骨细胞上时,回收了通过入侵成骨细胞而抵抗万古霉素的感染后一天与MG63细胞相关的少量存活细菌(约0.06%的输入)。

[0539] 隔离在腹膜细胞内的MRSA在万古霉素存在下显示出存活率和感染效率的显著增加。在万古霉素杀死浮游细菌培养物的相同条件下,白细胞中约15%的细胞内MRSA存活。在万古霉素存在下,细胞内的细菌也能更好地感染MG63成骨细胞的单层,导致在暴露于万古霉素后一天回收的细菌增加一倍(图1D)。此外,在MG63细胞(图1E)、原代人脑内皮细胞(图1F)和A549支气管上皮细胞(未显示)中,在持续暴露于杀死游离活细菌的万古霉素浓度下,细胞内金黄色葡萄球菌在24小时内能够增加近10倍。虽然保护免受抗生素杀伤,但在感染的腹膜巨噬细胞和嗜中性粒细胞的培养物中未发生细菌生长(未显示)。这些数据一起支持髓系细胞中MRSA的细胞内储库可以促进感染传播到新的位点,甚至在活性抗生素治疗存在的情况下,并且甚至在持续的抗生素治疗的条件下,细胞内生长可以在内皮和上皮细胞中发生。

[0540] 为了开发特异性杀死细胞内金黄色葡萄球菌的试剂,对抗体和抗生素组分进行谨慎的选择并优化以达到最大效力。以下实施例显示了实验和结果,其导致用于缀合形成AAC的抗壁磷壁酸β(抗-WTAβ)抗体和某些利福霉素型抗生素的选择。

[0541] 实施例3抗-金黄色葡萄球菌单克隆抗体的选择

[0542] 由AAC递送的抗生素的量及其最终功效受到细菌表面上的抗体结合位点数量的限制。因此,在体内感染的所有期间,选择与在MRSA上稳定表达的高度丰富的抗原结合的抗体是重要的。作为初始步骤,从各种金黄色葡萄球菌感染恢复的患者的外周血B细胞中克隆并纯化了一组超过40个抗金黄葡萄球菌抗体,并筛选了与从感染小鼠的肾脏直接分离的与MRSA结合的抗体。

[0543] 抗体产生、筛选和选择

[0544] 缩写:MRSA(甲氧西林抗性金黄色葡萄球菌);MSSA(甲氧西林敏感性金黄色葡萄球菌);VISA(万古霉素中等抗性金黄色葡萄球菌);LTA(脂磷壁酸);TSB(胰酶解大豆酪蛋白肉汤培养基);CWP(细胞壁制备物)。

[0545] 使用Symplex™技术(Symphogen,Lyngby,丹麦)从金黄色葡萄球菌感染患者的外周B细胞克隆人IgG抗体,所述技术保护抗体重链和轻链的同源配对,如US 8,283,294:"Method for cloning cognate antibodies";Meijer PJ等人Journal ofMolecular Biology 358:764-772(2006);和Lantto J等人J Virol.85(4):1820-33(Feb 2011)所述;血浆和记忆细胞被用作重组全长IgG库的基因来源。通过转染哺乳动物细胞表达单个抗体克隆,如Meijer PJ等人Methods in Molecular Biology 525:261-277,xiv.(2009)中所

述。7天后收集含有全长IgG1抗体的上清液，并用于在初筛中筛选通过间接ELISA的抗原结合。产生显示与来自USA300或Wood46金黄色葡萄球菌菌株的细胞壁制备物结合的阳性ELISA的单克隆抗体库。随后在200-ml瞬时转染中产生抗体，并用蛋白A色谱(MabSelect SuRe,GE Life Sciences,Piscataway,NJ)纯化用于进一步试验。对于较大规模的抗体产生，在CHO细胞中产生抗体。将编码VL和VH的载体转染到CHO细胞中，并通过蛋白A亲和色谱从细胞培养基中纯化IgG。

[0546] 表5:用于分离抗体的抗原清单

[0547]

Ag	描述	供应商/来源	包被
WTA	壁磷壁酸(WTA)，来自 Staph A. 目录号 R84500(2mg/瓶)，批号 5E14909.	Meridian Life Sciences	2 μg/ml
PGN	来自金黄色葡萄球菌的肽聚糖；目录号 77140，批号 1396845	Sigma	2 μg/ml
CW #1	CW USA300, RPMI, 铁消耗, 固定相	Genentech, 100x	
CW #3	CW USA300, TSB. 固定相	Genentech, 500X	
CW #4	CW Wood46, TSB. 固定相	Genentech, 500X	

[0548] CW#1和CW#3通常在制备ELISA包被中混合在一起：

[0549] 图6总结了通过ELISA对抗体的初步筛选。当用USA300细胞壁制备物混合物(铁消耗:TSB以96:4比例)进行筛选时，分离所有抗体(除了4569)。所有GlcNAcβ(除了6259)、SDR和PGN(4479)mAb在初次筛选中也对PGN和WTA阳性。通过筛选与USA300 CW混合物的结合，专一地发现所有GlcNAcα。通过在Wood46CWP上筛选发现4569(LTA特异性)。

[0550] 使用识别在WTA上的β-O-连接的GlcNAc糖修饰的人IgG_1发现抗体结合的最高水平(表6)。用识别α-O-连接的GlcNAc的单克隆抗体获得较少的结合；针对巨细胞病毒(CMV)gD蛋白的同种型对照抗体由于在体内衍生的金黄色葡萄球菌中表达的蛋白A而显示出一些最小反应性(图7A)。通过遗传手段确定抗体的抗原特异性，使得针对WTA上的α或β-GlcNAcs糖修饰的抗体不能结合缺少其各自糖基转移酶的金黄色葡萄球菌菌株(如图7B所示)。与抗体结合到体内衍生的MRSA的程度一致，用抗-β-GlcNAc WTA抗体制备的AAC显示出优于用抗α-GlcNAc WTA抗体制备的抗体。

[0551] 使用离体流式细胞术从库中选择抗WTA mAb

[0552] 查询该库中的每个mAb有三个选择标准：(1)与MRSA表面结合的mAb的相对强度，作为有利于高抗生素递送的相应同源抗原的高表达的指示；(2)mAb与从多种感染组织分离的MRSA结合的一致性，作为感染期间体内MRSA表面上同源抗原稳定表达的指示；和(3)mAb与

临床的金黄色葡萄球菌菌株组的结合能力，作为同源表面抗原表达保守性的指示。为此，使用流式细胞术检测库中所有这些预先选择的mAb培养物上清液，检测其与来自不同感染组织和不同金黄色葡萄球菌菌株的金黄色葡萄球菌的反应性。

[0553] 分析了库中所有mAb结合来自感染了MRSA USA300的小鼠感染的肾脏、脾脏、肝脏和肺以及在兔心内膜炎模型中感染了USA300 COL的兔子的心脏或肾脏内的MRSA的能力。抗体从多种感染组织识别金黄色葡萄球菌的能力提高了治疗性抗体在金黄色葡萄球菌的多种不同临床感染中有活性的可能性。在收获器官后立即分析细菌，即不传代培养，以防止由体外培养条件引起的表型变化。几种金黄色葡萄球菌表面抗原在体外培养期间表达，但在感染组织中丧失表达。针对这些抗原的抗体不太可能用于治疗感染。在多种感染组织中对该mAb库进行分析时，证实了大量抗体的观察结果，这些抗体显示出与培养物中金黄色葡萄球菌的显著结合，但不存在与来自任何测试感染组织的细菌结合。一些抗体结合来自部分但不是全部测试感染组织的细菌。因此，选择了能够识别来自测试的所有感染条件下的细菌的抗体。评估的参数为：(1)相对荧光强度，作为抗原丰度的量度；(2)染色阳性的器官数量，作为抗原表达稳定性的量度；和(3)mAb对一组临床的金黄色葡萄球菌菌株的结合能力，作为同源表面抗原表达保守性的指示。测定试验抗体相对于针对非相关抗原的同种型对照抗体例如IgG1 mAb抗疱疹病毒gD:5237(参考下文)的荧光强度。针对WTA-β的mAb不仅显示出最高的抗原丰度，而且还显示出与上述测试和指定的所有感染组织的MRSA非常一致的结合。

[0554] 此外，评估了这些mAb与以下金黄色葡萄球菌菌株结合的能力，并且它们是在体外在TSB中培养：USA300(MRSA)、USA400(MRSA)、COL(MRSA)、MRSA252(MRSA)、Wood46(MSSA)、Rosenbach(MSSA)、Newman(MSSA)和Mu50(VISA)。发现抗WTAβmAb而不是抗WTAαmAb与所有这些菌株都具有反应性。结合不同菌株的分析表明，WTAβ比WTAα更保守，因此更适合于AAC。

[0555] 实施例4具有对壁磷壁酸特异性的抗体的表征

[0556] 确认Ab的WTA特异性

[0557] 通过将40mg沉淀的金黄色葡萄球菌菌株与1mL补充有30％棉子糖、100μg/ml溶葡萄球菌酶(Cell Sciences,Canton,MA)和不含EDTA的蛋白酶抑制剂混合物(Roche,Pleasanton,CA)的10mM Tris-HCl(pH 7.4)在37℃下孵育30分钟，以产生来自金黄色葡萄球菌野生型(WT)菌株和缺乏WTA的金黄色葡萄球菌突变菌株(ΔTagO;无WTA菌株)的细胞壁制备物(CWP)。将裂解物以11,600×g离心5分钟，收集含有细胞壁成分的上清液。对于免疫印迹分析，将蛋白质在4-12％Tris-甘氨酸凝胶上分离，并转移到硝酸纤维素膜(Invitrogen,Carlsbad,CA)上，然后用针对WTA的指定测试抗体或用针对PGN和LTA的对照抗体进行印迹法检测。

[0558] 免疫印迹显示针对WTA的抗体结合来自WT金黄色葡萄球菌的细胞壁制备物，但不与缺乏WTA的ΔTagO菌株的细胞壁制备物结合。抗肽聚糖(抗PGN)和脂磷壁酸(抗LTA)的对照抗体很好地与两种细胞壁制备物结合。这些数据表明测试抗体对WTA的特异性。

[0559] i)流式细胞术确定mAb与MRSA表面的结合程度

[0560] 使用以下方案通过流式细胞术分析来自感染组织的整个细菌的表面抗原表达。对于来自感染的小鼠组织的细菌的抗体染色，将6-8周龄雌性C57Bl/6小鼠(Charles River,Wilmington,MA)静脉内注射在PBS中的10^8CFU的对数相生长的USA300。感染后两天收获小

鼠器官。如先前在Tattevin P.等人,Antimicrobial agents and chemotherapy 54:610-613(2010)中描述的那样建立兔感染性心内膜炎(IE)。将兔子静脉注射5x10^{7}CFU的静止期生长的MRSA菌株COL,并在18小时后收获心脏赘生物。用7x10^{7}CFU固定相感染18小时后每日两次静脉内施用30mg/kg万古霉素治疗。

[0561] 为了裂解小鼠或兔细胞,使用温和的MACS细胞解离器(Miltenyi)在M管(Miltenyi,Auburn,CA)中将组织匀浆,随后在含有0.1%Triton-X100(Thermo)、10μg/mL DNAseI(Roche)和Complete Mini蛋白酶抑制剂混合物(Roche)的PBS中在室温下孵育10分钟。将悬浮液通过40微米过滤器(BD),并用不含酚红的补充有0.1%不含IgG的BSA(Sigma)和10mM Hepes,pH 7.4(HB缓冲液)的HBSS洗涤。然后将细菌悬浮液与300μg/mL的兔IgG(Sigma)在HB缓冲液中室温(RT)下孵育1小时,以阻断非特异性IgG结合。将细菌用2μg/mL的一抗进行染色,包括rF1或同种型对照IgG1 mAb抗疱疹病毒gD:5237(Nakamura GR等人,J Virol 67:6179-6191(1993)),接下来用荧光抗-人IgG二抗(Jackson Immunoresearch,West Grove,PA)染色。为了能够从小鼠或兔器官碎片分辨细菌,使用20μg/mL小鼠mAb 702抗金黄色葡萄球菌肽聚糖(Abcam,Cambridge,MA)和荧光染料标记的抗小鼠IgG二抗(Jackson Immunoresearch)进行双染色。将细菌洗涤并通过FACSCalibur(BD)分析。在流式细胞术分析过程中,从双荧光图中门选mAb 702染色阳性的细菌。

[0562] ii)测量对金黄色葡萄球菌的结合亲和力和MRSA上的抗原密度

[0563] 表6显示与Newman-ΔSPA菌株结合的MRSA抗体的平衡结合分析以及细菌上的抗原密度。

[0564] 表6

[0565]

MRSA抗体	特异性	平均K_D,nM(n=2)	抗原密度,平均位点/细菌
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

[0566] 在以下试验条件下,使用放射性配体细胞结合试验法得到K_D和抗原密度:DMEM+2.5%小鼠血清结合缓冲液;溶液在室温(RT)下结合2小时;并使用400,000个细菌/孔。

[0567] Ab 6263是类似6078的,因为序列非常相似。除了CDR H3中的第二残基(R相对于G)之外,所有其它L和H链CDR序列是相同的。

[0568] 实施例5抗WTA抗体的氨基酸修饰

[0569] 总之,每个抗WTAβAb的VH区被克隆出来并与人H链γ1恒定区连接,VL与κ恒定区连接以表达作为IgG1的Ab。野生型序列在某些位置被改变以提高抗体的稳定性,同时保持如下所述的抗原结合。然后生成半胱氨酸工程化的Ab(ThioMabs,也称为THIOMAB™)。

[0570] i.将可变区连接到恒定区

[0571] 从上述人抗体库鉴定的WTAβAb的VH区与人γ1恒定区连接以制备全长IgG1抗体。L链是κL链。

[0572] ii.产生稳定性变体

[0573] 将图12中的WTA Ab(特别参见图13A、13B、14A、14B)进行工程化以改善某些性质(避免脱酰胺、天冬氨酸异构化、氧化或N-连接的糖基化)并测试抗原结合的保留以及氨基酸置换后的化学稳定性。使用QIAprep Spin M13试剂盒(Qiagen),从生长在大肠杆菌CJ236细胞中的M13KO7噬菌体颗粒纯化编码重链或轻链的克隆的单链DNA。5′磷酸化的合成寡核苷酸具有如下序列:

[0574] 5'-CCCAGACTGCACCAGCTGGATCTCTGAATGTACTCCAGTTGC-3' (SEQ ID NO.152)

[0575] 5'-CCAGACTGCACCAGCTGCACCTCTGAATGTACTCCAGTTGC-3' (SEQ ID NO.153)

[0576] 5' CCAGGGTTCCCTGGCCCCAWTMGTCAAGTCCASCWKCACCTCTTGCACAGTAATAGACAGC-3' (SEQ ID NO.154);和

[0577] 5'-CCTGGCCCCAGTCGTCAAGTCCTCCTTCACCTCTTGCACAGTAATAGACAGC-3' (SEQ ID NO.155) (IUPAC编码)

[0578] 其用于按照如下所述方法通过位点特异性诱变将所述的寡核苷酸定向位点诱变来突变编码抗体的克隆:Kunkel,T.A.(1985),快速有效的位点特异性诱变且无表型选择,Proceedings of the National Academy of Sciences USA 82(2):488-492。诱变的DNA用于转化大肠杆菌XL1-Blue细胞(Agilent Technologies)并接种在含有50μg/ml羧苄青霉素的Luria肉汤平板上。单独挑选菌落并在含有50μg/ml羧苄青霉素的液体Luria肉汤培养基中生长。对Miniprep DNA进行测序以确认是否存在突变。

[0579] 对于Ab 6078,VH中的第二个氨基酸met(met-2)容易氧化。因此met-2突变为Ile或Val,以避免残基的氧化。由于met-2的改变可能影响结合亲和力,因此通过ELISA测试突变体与葡萄球菌CWP的结合。

[0580] 发现CDR H3"DG"或"DD"基序容易转化为异天冬氨酸。Ab 4497在CDR H3位点96和97包含DG(参见图16),并且为了稳定性而改变。CDR H3对于抗原结合通常是关键的,因此测试几种突变体的抗原结合和化学稳定性。突变体D96E(v8)保留与抗原的结合,类似于野生型Ab 4497(图16),并且是稳定的,且不形成异天冬氨酸。

[0581] 葡萄球菌CWP的ELISA

[0582] 对于6078抗体突变体的分析,将溶葡萄球菌酶处理的由$1X10^9$细菌/ml组成的USA300ΔSPA金黄色葡萄球菌细胞壁制备物(WT)在0.05碳酸钠pH 9.6中1/100稀释,并涂覆到384孔ELISA板(Nunc;Neptune,NJ)上,在4℃孵育过夜。用加0.05%吐温20的PBS洗涤平板,并用0.5%牛血清白蛋白(BSA)的PBS孵育2小时来封闭。这次和所有后续孵育在室温下轻轻搅拌进行。将抗体样品在样品/标准稀释缓冲液(PBS,0.5%BSA,0.05%Tween 20,0.25%CHAPS,5mM EDTA,0.35M NaCl,15ppm Proclin,(pH 7.4))中稀释,加入洗涤过的平板中,并孵育1.5-2小时。用在试验缓冲液(PBS,0.5%BSA,15ppm Proclin,0.05%Tween 20)中稀释至40ng/mL的过氧化物酶缀合的山羊抗人IgG(Fc)F(ab')2片段(Jackson ImmunoResearch;West Grove,PA)孵育1小时,期间检测到平板结合的抗金黄色葡萄球菌。最终洗涤后,加入四甲基联苯胺(KPL,Gaithersburg,MD),显色5-10分钟,用1M磷酸终止反应。使用酶标仪在450nm处读取平板,以620nm参考。

[0583] iii.生成Cys工程化突变体(ThioMab)

[0584] 通过如前文教导和下文描述的在预定位置将半胱氨酸引入H链(在CH1)或L链(Cκ)

中，从而生成全长ThioMab，以使抗体与接头-抗生素中间体（半胱氨酸氨基酸可以在抗体的重链（HC）或轻链（LC）中的反应位点处进行工程化，并且不形成链内或分子间二硫键（Junutula等人，2008b Nature Biotech.，26（8）：925-932；Dornan等人（2009）Blood 114（13）：2721-2729；US 7521541；US 7723485；WO 2011/156328；WO2009/052249，Shen等人（2012）Nature Biotech.，30（2）：184-191；Junutula等人（2008）Jour of Immun.Methods 332：41-52）。然后将H和L链克隆到单独的质粒中，并将编码H和L的质粒共转染到293细胞中，在其中它们被表达并组装成完整的Ab。H和L链也可以克隆到相同的表达质粒中。通过表达所需的cys突变体链和野生型链的组合产生IgG1，其具有2个工程化的Cys，每个H链中有一个；或2个工程化的Cys，每个L链中有一个；或每个H链和L链（HCLCCys）中各有一个工程化Cys的组合，导致每个抗体四聚体4个工程化Cys。

[0585] 图13A和13B显示6078WT和具有HC Cys和LC Cys的组合的突变体Ab。还测试了6078突变体结合来自过夜培养物的蛋白A缺陷型USA300金黄色葡萄球菌的能力。从FACS分析结果（数据未显示）可见，与6078WT（未改变）抗体相似，突变体Abs与USA300结合；突变体中的氨基酸改变不影响与金黄色葡萄球菌的结合。使用gD作为非特异性阴性对照抗体。

[0586] 实施例6：抗生素选择

[0587] 选择具有以下特点的利福霉素型抗生素：具有高效能、在低吞噬溶酶体pH中未改变的杀菌活性、能够承受细胞内损伤的能力，以及其可以容易地与适合与抗WTA抗体缀合的蛋白酶可切割的非肽类（PML）接头试剂偶联。由于吞噬细胞内的细菌复制最为缓慢，因此抗生素的优化也要求在从AAC释放时能够杀死非复制性MRSA。

[0588] 从固定相培养物中收集MRSA，并悬浮在不含抗生素或含1×10^{-6}M利福平或利福霉素衍生物（Rifalog）的磷酸盐缓冲盐水中，并在37℃下孵育。在指定时间，收集培养物样品并离心除去抗生素，并通过接种测定存活细菌的总数。

[0589] 有趣地是，在最少量磷酸盐缓冲液（PBS）中过夜孵育后，添加利福霉素型（rifalog）抗生素而不是利福平导致活的但非复制型细菌的数量减少了1,000倍以上（参见图8）。类似地，利福霉素型抗生素有能力杀死常规定义的耐药株细胞，其是可能进入休眠状态以在生长培养物中的抗生素处理（例如环丙沙星）下存活的细菌（数据未显示）。与以前的观察结果一致，利福平的添加对其活力没有影响（Conlon，B.P.等人（2013）Nature 503，365-370）。相比之下，添加利福霉素型抗生素（rifalog）导致根除了低于检测限的耐药株细胞。这些结果表明，利福霉素类抗生素具有显著的杀死休眠、非分裂细胞的能力。

[0590] 实施例7：抗WTA抗体-抗生素缀合物的制备

[0591] 抗壁磷酸抗体-抗生素缀合物（AAC）表3是通过将抗WTA抗体与PML接头-抗生素中间体（包括来自表2的那些）缀合制备的。在缀合之前，根据WO 2004/010957描述的方法的标准方法，使用TCEP部分地还原抗WTA抗体，为此目的，其教导通过引用并入本文作为参考。使用例如在Doronina等人（2003）Nat.Biotechnol.21：778-784和US 2005/0238649 A1中所述的方法的标准方法将部分还原的抗体与接头-抗生素中间体缀合。简言之，将部分还原的抗体与接头-抗生素中间体合并，以使接头-抗生素中间体与抗体的还原半胱氨酸残基缀合。将缀合反应淬灭，并纯化AAC。测定每个AAC的抗生素负荷（每个抗体的抗生素部分的平均数），对于用单个半胱氨酸突变位点工程化的抗壁磷壁酸抗体，其约为1至约2。

[0592] 用于缀合的硫代-单克隆抗体的还原/氧化：将在CHO细胞中表达的全长、半胱氨酸

工程化单克隆抗体(ThioMabs-Junutula等人,2008b Nature Biotech.,26(8):925-932;Dornan等人(2009)Blood 114(13):2721-2729;US 7521541;US 7723485;WO2009/052249,Shen等人(2012)Nature Biotech.,30(2):184-191;Junutula等人(2008)Jour of Immun.Methods 332:41-52)在37℃下用约20-40倍过量的在50mM Tris pH 7.5中的含有2mM EDTA的TCEP(三(2-羧乙基)膦盐酸盐或DTT(二硫苏糖醇))还原3小时,或在室温下过夜(Getz等人(1999)Anal.Biochem.Vol 273:73-80;Soltec Ventures,Beverly,MA)。将还原的ThioMab稀释并加载到10mM醋酸钠pH 5的HiTrap S柱上,并用含有0.3M氯化钠的PBS洗脱。或者,通过加入1/20体积的10%乙酸(用10mM琥珀酸盐pH 5稀释)酸化该抗体,并加载到柱上,然后用10倍柱体积的琥珀酸缓冲液洗涤。将该柱用50mM Tris pH7.5,2mM EDTA洗脱。

[0593] 用15倍摩尔过量的DHAA(脱氢抗坏血酸)或200nM硫酸铜($CuSO_4$)水溶液处理洗脱的还原的ThioMab。链间二硫键的氧化在约3小时或以上完成。环境空气氧化也是有效的。将再氧化的抗体透析至20mM琥珀酸琥珀酸钠pH5、150mM NaCl、2mM EDTA中,并在-20℃冷冻保存。

[0594] 硫代-单克隆抗体与接头-抗生素中间体的缀合:将解封的、再氧化的硫代抗体(ThioMab)与6-8倍摩尔过量的表2的接头-抗生素中间体(来自20mM浓度的DMSO储存液)在50mM Tris,pH8中反应直到反应完成(16-24小时),通过反应混合物的LC-MS分析测定。

[0595] 然后将粗抗体-抗生素缀合物(AAC)在用20mM琥珀酸钠(pH 5)稀释后施加到阳离子交换柱上。用至少10个柱体积的20mM琥珀酸钠(pH 5)洗涤该柱,并用PBS洗脱。使用凝胶过滤柱将AAC配制到含有240mM蔗糖的20mM His/乙酸盐pH 5中。通过UV光谱法对AAC进行表征,以测定蛋白质浓度,在用赖氨酸C内肽酶处理前后进行分析型SEC(尺寸排阻色谱)的聚集分析和LC-MS。

[0596] 使用Shodex KW802.5柱,在0.2M磷酸钾pH6.2中用0.25mM氯化钾和15%IPA以0.75ml/min的流速进行尺寸排阻色谱。AAC的聚集状态通过280nm处洗脱的峰面积吸光度的积分来确定。

[0597] 使用Agilent QTOF 6520ESI仪器进行LC-MS分析。作为一个实例,使用该化学法产生的AAC在37℃在Tris(pH7.5)中用1:500w/w内蛋白酶Lys C(Promega)处理30分钟。将所得切割片段加载到加热至80℃的1000A,8μLPLRP-S柱上,并在5分钟内用30%B至40%B的梯度洗脱。流动相A:含0.05%TFA的H_2O。流动相B:含0.04%TFA的乙腈。流速:0.5ml/min。在电喷雾电离和MS分析之前,通过280nm处的UV吸光度检测来监测蛋白质洗脱。通常实现非缀合Fc片段、残留的非缀合Fab和抗生素-Fab的色谱拆分。使用Mass Hunter™软件(Agilent Technologies)将获得的m/z光谱解卷积,以计算抗体片段的质量。

[0598] 实施例8:切割和抗生素的释放

[0599] 在与抗βWTA mAb以AAC形式连接时,测试利福霉素型抗生素直接杀死细胞外细菌的能力。AAC在单独的缓冲液中孵育或用组织蛋白酶B处理。将所得反应物的系列稀释液加入到在胰酶解大豆酪蛋白肉汤培养基中含有MRSA的孔中,并培养过夜,以鉴定含有足够的活性抗生素以防止生长的孔。

[0600] 如预测的那样,与完整的抗MRSA AAC的过夜孵育不会使生长的浮游细菌受到伤害,除非AAC用组织蛋白酶B预处理以释放活性抗生素(图9)。用组织蛋白酶B预处理AAC释放了足够的抗生素活性,防止细菌在0.6μg/mL的AAC中生长,其预计含有0.006μg/mL的抗生

素。

[0601] 为了测试抗生素是否仅在将AAC调理的细菌内化到细胞后才从AAC中释放出来，可以用荧光共振能量转移(FRET)为基础的探针检测接头的切割，该探针由与两个染料分子缀合的同一个抗MRSA抗体组成，使用与AAC中相同的接头。MRSA用FRET缀合物调理，并加入到巨噬细胞培养物中。通过视频显微镜监测细菌的摄取和探针的切割。接头在巨噬细胞摄取细菌后几分钟内被切割，其通过A488探针的释放而可视化，类似于在AAC上的利福霉素型抗生素的释放。另一方面，当由于巨噬细胞用latrunculin A(一种吞噬作用的抑制剂)处理而使细菌不被内化时，接头保持完整。也可以使用质谱分析来证实，在摄取用实际AAC涂覆的MRSA后在巨噬细胞内确实释放了游离抗生素。

[0602] 实施例9抗WTAβPML AAC体外效力

[0603] 金黄色葡萄球菌在体外被原代人和小鼠巨噬细胞和几种人类细胞系内化时，抗WTAβ-CBDK AAC有效地杀死金黄色葡萄球菌。

[0604] 体外巨噬细胞试验。

[0605] 将金黄色葡萄球菌(USA300NRS384菌株)与各种剂量(100u/mL、10μg/mL、1μg/mL或0.1μg/mL)的抗WTAβ抗体4497，每个抗体载有2个抗生素分子(DAR2)的Ab4497-CBDK-二甲基pipBOR AAC或每个抗体载有4个抗生素分子(DAR4)的抗-WTA-CBDK-二甲基pipBOR AAC持续孵育1小时，以使抗体与细菌结合。将所得的调理细菌饲喂鼠巨噬细胞，并在37℃下孵育以允许吞噬。2小时后，移除感染混合物，并用补充有50μg/mL庆大霉素的正常生长培养基替换，以杀死任何剩余的细胞外细菌。2天后，通过将胰蛋白酶大豆琼脂平板上的巨噬细胞裂解物的连续稀释液接种，测定存活的细胞内细菌总数。

[0606] 实施例10抗WTAβ-PML AAC的体内功效

[0607] 治疗小鼠的金黄色葡萄球菌感染将器官中的细菌负荷降低了几个数量级。

[0608] 为了确定特异性针对细胞内金黄色葡萄球菌的治疗是否会在感染期间具有疗效，在小鼠静脉感染模型中测试WTA-PML AAC。该实施例证明，在鼠静脉感染模型中，WTA-PML AAC有效地大幅减少或消除细胞内金黄色葡萄球菌感染。

[0609] 腹膜炎模型。

[0610] 将7周龄的雌性A/J小鼠(Jackson Laboratories)用5×10^7CFU的USA300腹膜注射感染。在感染后2天处死小鼠，并用5mL冷磷酸盐缓冲盐水溶液(PBS)冲洗腹膜。将肾脏在5mL PBS中匀浆，如下文所述，用于静脉内感染模型。将腹膜洗涤物在4℃下以1,000rpm在台式离心机中离心5分钟。收集上清液作为细胞外细菌，收集含有腹膜细胞的细胞沉淀作为细胞内组分。将细胞在37℃下用50μg/mL溶葡萄球菌酶处理20分钟以杀死污染的细胞外细菌。将腹膜细胞在冰冷的PBS中洗涤3次，以在分析前除去溶葡萄球菌酶。为了计数细胞内CFU的数量，将腹膜细胞在含有0.1%Triton-X的HB(Hanks平衡盐溶液，补充有10mM HEPES和0.1%牛血清白蛋白)中裂解，在含有0.05%tween-20的PBS中制备裂解物的连续稀释液。

[0611] 鼠静脉感染模型

[0612] 为了在临床上相关，AAC需要能够消除已经建立的细胞内感染。为了评估这一点，治疗延迟到菌血症开始24小时后，此时万古霉素治疗的效果最低。嗜中性粒细胞的细胞内感染迅速发展；在至少一种菌血症模型中，血液中的95%的细菌在15分钟内就在嗜中性粒细胞内[7]，这可能是由于万古霉素的功效下降。在这些条件下，单剂量AAC的治疗是有效的，

并证明优于用等量的游离利福霉素型抗生素治疗。

[0613] 金黄色葡萄球菌是人类皮肤和粘膜表面的常见定殖者。人血清多种来源的初步分析，包括IGIV-GammaGard(来自～10,000人的合并免疫球蛋白制剂)，证明人血清含有约300μg/mL的抗金黄色葡萄球菌抗体，其中～70％是针对WTA的GlcNAc修饰。小鼠血清中没有明显的抗金黄色葡萄球菌抗体水平。为了确定在正常人血清中发现的内源抗WTA抗体是否可能与AAC竞争结合，CB17.SCID小鼠(Charles River Laboratories，Hollister，CA)用GammaGard S/D IGIV免疫球蛋白(ASD Healthcare，Brooks KY)重构，其使用优化的剂量方案以在血清中达到至少10mg/mL人IgG的恒定血清水平。施用IGIV的初始静脉注射剂量为30mg/小鼠，然后6小时后通过腹膜内(i.p.)注射15mg/小鼠的第二剂量，随后连续3天通过腹膜内注射向每只小鼠每日给药15mg。与未处理的对照相比，这些小鼠同样容易感染MRSA。

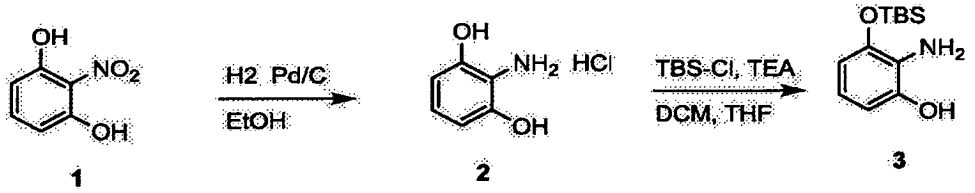
[0614] 在第一次施用IGIV后4小时，通过静脉内注射用磷酸盐缓冲盐水稀释的1×10^7CFU MRSA(USA300NRS384菌株)感染小鼠(n＝8，对于每种抗体或AAC)。感染的小鼠用表3中的50mg/kg S4497裸抗体或S4497AAC处理。感染24小时后通过静脉内注射向小鼠施用单次剂量的AAC，感染后第4天处死小鼠，并在5mL磷酸盐缓冲盐水中收获肾脏和心脏。使用GentleMACS Dissociator™(Miltenyi Biotec，Auburn，CA)将组织样品匀浆。通过将组织匀浆液在PBS 0.05％Tween中的连续稀释液在含有5％去纤维蛋白的羊血的胰蛋白酶大豆琼脂上接种，测定每个器官中回收的细菌总数。

[0615] 如图10的结果所示，尽管存在潜在的竞争性抗体，与裸抗体相比，单剂量的抗-β-GlcNAc WTA AAC(S4497-AAC)大大减少或消除感染器官中的细菌计数。图10B显示用AAC(DAR2)处理，肾脏中的细菌负荷减少约7,000倍。图10C显示用表3中的AAC(DAR2)处理，心脏中的细菌负荷减少大约500倍。与作为AAC的抗WTA抗体缀合的二甲基pipBOR相比，在体内感染模型中单独用裸抗生素，即二甲基pipBOR(与在AAC中的二甲基pipBOR等摩尔浓度)进行处理是无效的。

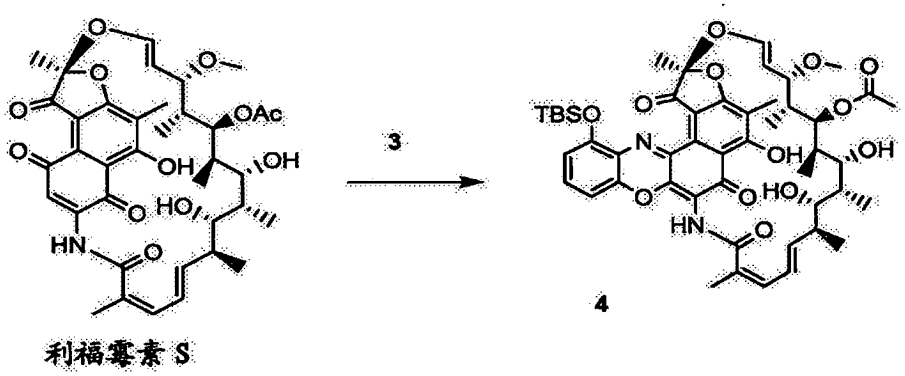
[0616] 未缀合的(游离)抗WTA抗体在体内是无效的

[0617] 图17显示用50mg/kg游离抗体的预处理对静脉内感染模型是无效的。在使用2×10^7CFU的USA300感染30分钟之前，通过静脉注射向Balb/c小鼠施用单剂量的载体对照(PBS)或50mg/Kg抗体。治疗组包括不与金黄色葡萄球菌(gD)结合的同种型对照抗体、针对壁磷壁酸(4497)的β修饰的抗体、或针对壁磷壁酸(7578)的α修饰的抗体。通过腹膜内注射(万古霉素)，用110mg/Kg的万古霉素每天向对照小鼠施用两次。

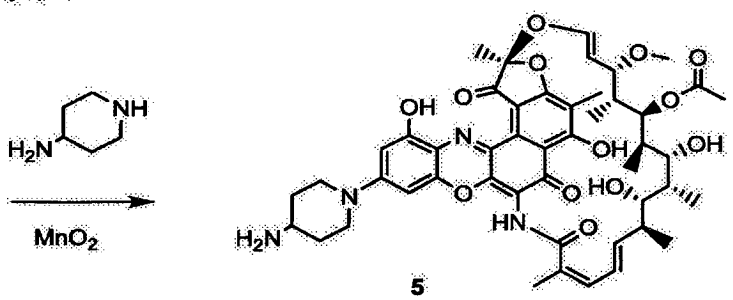
[0618] 实施例11哌啶基苯并噁嗪并利福霉素(pipBOR)5



[0619]



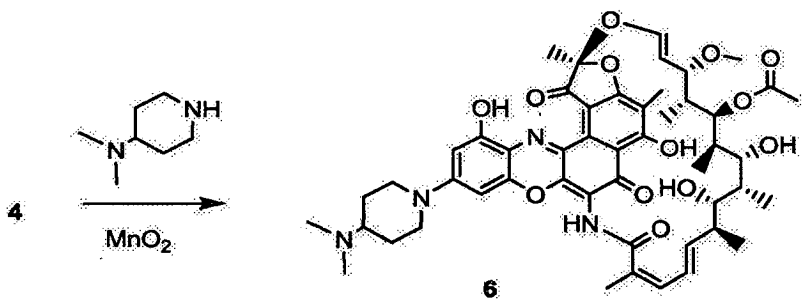
利福霉素 S



[0620] 将2-硝基苯-1,3-二醇1在氢气下用钯/碳催化剂在乙醇溶剂中氢化,得到2-氨基苯-1,3-二醇2,分离为盐酸盐。用叔丁基二甲基硅烷基氯和三乙胺在二氯甲烷/四氢呋喃中单保护2,得到2-氨基-3-(叔丁基二甲基硅烷氧基)苯酚3。利福霉素S(ChemShuttle Inc.,Fremont,CA,US 7342011;US 7271165;US 7547692)与3通过用氧化锰或氧气在甲苯中氧化缩合,在室温下反应得到TBS保护的苯并噁嗪并利福霉素4。LCMS(ESI):M+H$^+$=915.41。4与哌啶-4-胺和氧化锰反应得到哌啶基苯并噁嗪并利福霉素(pipBOR)5。LCMS(ESI):M+H$^+$=899.40

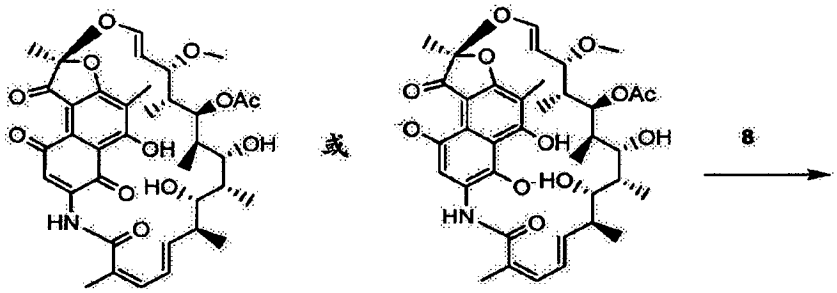
[0621] 实施例12二甲基pipBOR 6

[0622]

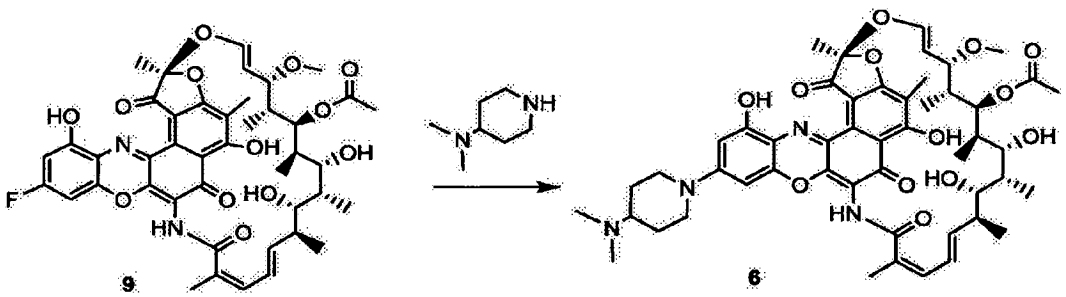


[0623] N,N-二甲基哌啶-4-胺与TBS-保护的苯并噁嗪并利福霉素4反应得到二甲基哌啶基苯并噁嗪并利福霉素(二甲基pipBOR)6

[0624]



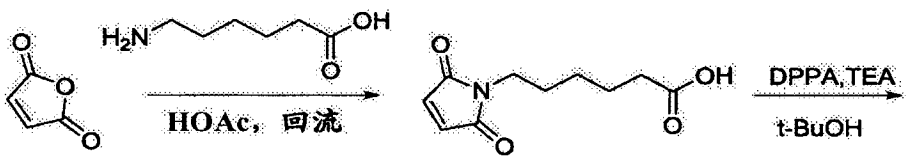
利福霉素S 利福霉素SV钠



[0625] 或者，(5-氟-2-硝基-1,3-亚苯基)双(氧基)双(亚甲基)二苯7在氢气下用钯/碳催化剂在四氢呋喃/甲醇溶剂中氢化以除去苄基，得到2-氨基-5-氟苯-1,3-二醇8。LCMS(ESI)：$M+H^+=144.04$。将市售的利福霉素S或利福霉素SV钠盐(ChemShuttle Inc.,Fremont,CA)与2-氨基-5-氟苯-1,3-二醇8通过在乙酸乙酯中在空气或铁氰化钾中氧化缩合而在60℃下反应，得到氟苯并噁嗪并利福霉素9。用N,N-二甲基哌啶-4-胺置换氟得到二甲基pipBOR6。LCMS(ESI)：$M+H^+=927.43$

[0626] 实施例13(S)-N-(5-(2,5-二氧代-2,5-二氢-1H-吡咯-1-基)戊基)-N-(1-(4-(羟甲基)苯基氨基)-1-氧代-5-脲基戊烷-2-基)环丁烷-1,1-二甲酰胺10

[0627] 步骤1：1-(5-氨基戊基)-1H-吡咯-2,5-二酮盐酸盐10a的制备



[0628]

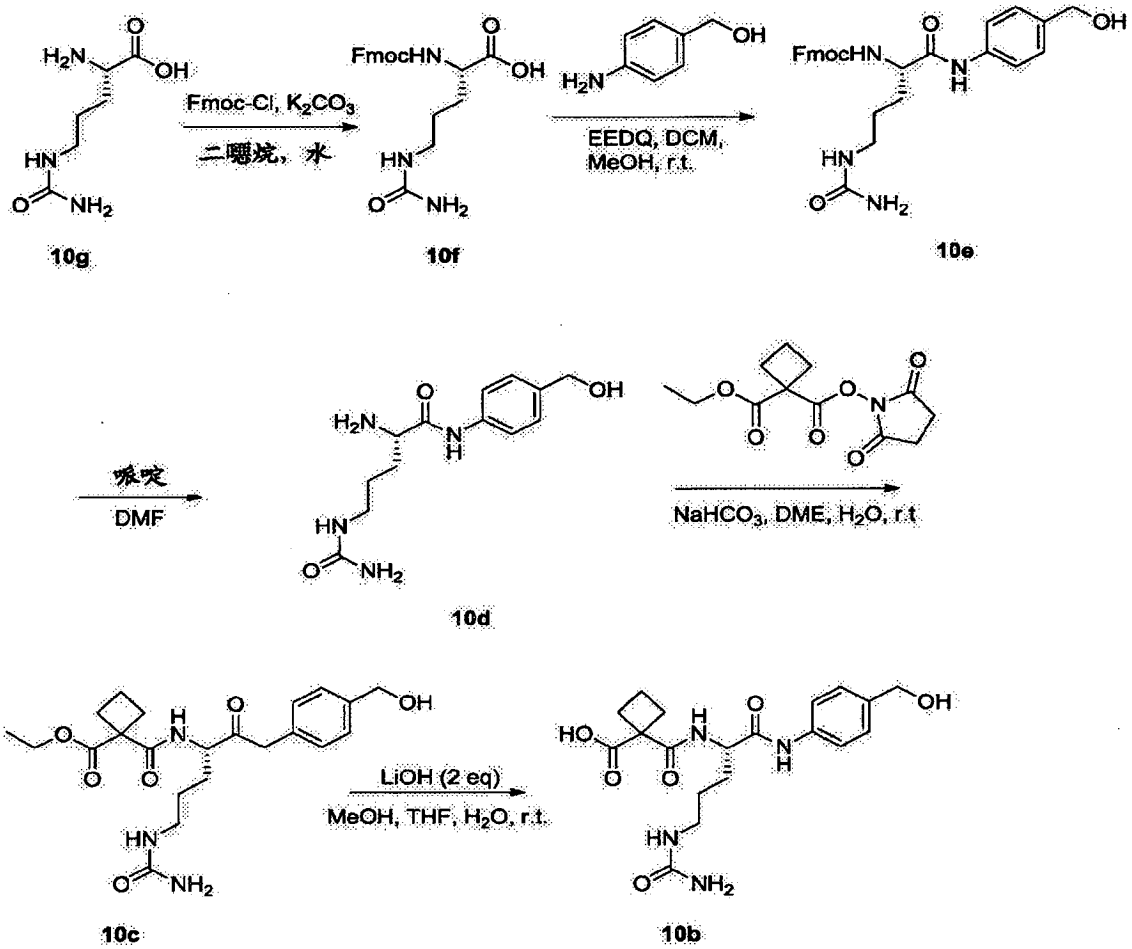
10a

[0629] 将马来酸酐、呋喃-2,5-二酮(150g,1.53mol)加入到在HOAc(1000mL)中搅拌的6-氨基己酸(201g,1.53mol)溶液中。混合物在室温下搅拌2小时后，回流加热8小时。在减压下除去有机溶剂，并且用EtOAc(500mL×3)萃取残余物，用H_2O洗涤。将合并的有机层用Na_2SO_4干燥并浓缩，得到粗产物。用石油醚洗涤，得到白色固体状的6-(2,5-二氧代-2,5-二氢-1H-

吡咯-1-基)己酸(250g,77.4%)。将DPPA(130g,473mmol)和TEA(47.9g,473mmol)加入到在t-BuOH(200mL)中的6-(2,5-二氧代-2,5-二氢-1H-吡咯-1-基)己酸(100g,473mmol)溶液中。将混合物在N_2下加热回流8小时。将混合物浓缩,通过硅胶柱层析(PE:EtOAc=3:1)纯化残余物,得到5-(2,5-二氧代-2,5-二氢-1H-吡咯-1-基)戊基氨基甲酸叔丁酯(13g,10%)。向5-(2,5-二氧代-2,5-二氢-1H-吡咯-1-基)戊基氨基甲酸叔丁酯(28g,992mmol)的无水EtOAc(30mL)溶液中逐滴加入HCl/EtOAc(50mL)。混合物在室温下搅拌5小时后,过滤,将固体干燥,得到1-(5-氨基戊基)-1H-吡咯-2,5-二酮盐酸盐10a(16g,73.7%)。^{1}H NMR(400MHz,DMSO-d_6):δ8.02(s,2H),6.99(s,2H),3.37-3.34(m,2H),2.71-2.64(m,2H),1.56-1.43(m,4H),1.23-1.20(m,2H).

[0630] 步骤2:制备(S)-1-(1-(4-(羟甲基)苯基氨基)-1-氧代-5-脲基戊-2-基氨基甲酰基)环丁烷甲酸10b

[0631]



[0632] 向二噁烷和H_2O(50mL/75mL)混合物中的(S)-2-氨基-5-脲基戊酸10g(17.50g,0.10mol)混合物中加入K_2CO_3(34.55g,0.25mol)。在0℃下缓慢加入Fmoc-Cl(30.96g,0.12mol)。将反应混合物历经2小时温热至室温。减压下除去有机溶剂,用6M HCl溶液将水浆体调至pH=3,用EtOAc(100mL×3)萃取。有机层用Na_2SO_4干燥,过滤并减压浓缩,得到(S)-

2-((((9H-芴-9-基)甲氧基)羰基)氨基)-5-脲基戊酸10f(38.0g,95.6%)。10f可商购。

[0633] 向DCM和MeOH(100mL/50mL)混合物中的10f(4g,10mmol)溶液中加入(4-氨基苯基)甲醇(1.6g,13mmol,1.3当量)和2-乙氧基-1-乙氧基羰基-1,2-二氢喹啉,即EEDQ、Sigma-Aldrich CAS登记号16357-59-8(32g,13mmol,1.3当量)。混合物在N_2下在室温搅拌16小时后,将其浓缩得到棕色固体。加入MTBE(200mL),在15℃下搅拌2小时。通过过滤收集固体,用MTBE(50mL×2)洗涤,得到(1-((4-(羟甲基)苯基)氨基)-1-氧代-5-脲基戊-2-基)氨基甲酸(S)-(9H-芴-9-基)甲基酯10e,为橙色固体(4.2g,84%)。LCMS(ESI):m/z 503.0[M+1]。

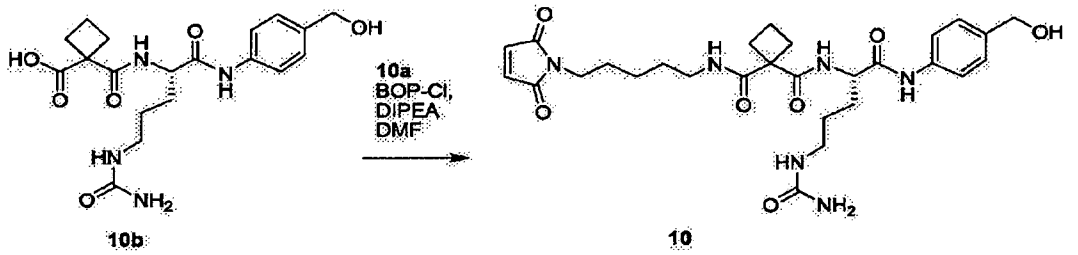
[0634] 在室温下向无水DMF(20ml)中的10e(4.2g,8.3mmol)搅拌溶液中滴加哌啶(1.65mL,17mmol,2当量)。将混合物在室温下搅拌30分钟,形成固体沉淀物。加入无水DCM(50mL),立即使混合物变得透明。将混合物在室温下搅拌另外30分钟,LCMS显示10e被消耗。将其在减压下浓缩至干(确保不存在哌啶),并将残余物在EtOAc和H_2O(50mL/20mL)之间分配。水相用EtOAc(50mL×2)洗涤并浓缩,得到油状残余物(2.2g,94%)(含少量DMF)的(S)-2-氨基-N-(4-(羟甲基)苯基)-5-脲基戊酰胺10d。

[0635] 将市售的1,1-环丁烷二甲酸,1,1-二乙基酯(CAS登记号3779-29-1)通过碱水溶液的限制皂化转化成半酸/酯1,1-环丁烷二甲酸,1-乙酯(CAS登记号5450-84-9),并用偶联试剂如TBTU(O-(苯并三唑-1-基)-N,N,N',N'-四甲基脲四氟硼酸盐,也称为N,N,N',N'-四甲基-O-(苯并三唑-1-基)脲四氟硼酸盐(CAS No.125700-67-6,Sigma-Aldrich B-2903)和N-羟基琥珀酰亚胺活化为NHS酯,即1-(2,5-二氧代吡咯烷-1-基)1-乙基环丁烷-1,1-二甲酸酯。

[0636] 向DME(50mL)中的1-(2,5-二氧代吡咯烷-1-基)1-乙基环丁烷-1,1-二甲酸酯(8g,29.7mmol)的溶液中加入在水(30mL)中的10d(6.0g,21.4mmol)和$NaHCO_3$(7.48g,89.0mmol)溶液。混合物在室温下搅拌16个小时后,减压浓缩至干,残余物通过柱色谱法(DCM:MeOH=10:1)纯化,得到白色固体(6.4g,68.7%)的(S)-乙基1-((1-(4-(羟甲基)苯基)-2-氧代-6-脲基己烷-3-基)氨基甲酰基)环丁烷甲酸酯10c。LCMS(ESI):m/z 435.0[M+1]

[0637] 在室温下向THF和MeOH(20mL/10mL)混合物中的搅拌的10c(6.4g,14.7mmol)溶液中加入在H_2O(20mL)中的$LiOH \cdot H_2O$(1.2g,28.6mmol)溶液。反应混合物在室温下搅拌16小时后,减压除去溶剂,所得残余物通过制备型HPLC纯化,得到(S)-1-(1-(4-(羟甲基)苯基氨基)-1-氧代-5-脲基戊-2-基氨基甲酰基)环丁烷甲酸10b(3.5g,产率:58.5%)。LCMS(ESI):m/z 406.9[M+1].^1H NMR(400MHz,甲醇-d_4)δ8.86(d,J=8.4Hz,2H),8.51(d,J=8.4Hz,2H),5.88-5.85(m,1H),5.78(s,2H),4.54-4.49(m,3H),4.38-4.32(m,1H),3.86-3.75(m,1H),3.84-3.80(m,2H),3.28-3.21(m,1H),3.30-3.24(m,1H),3.00-2.80(m,1H),2.37-2.28(m,2H)。

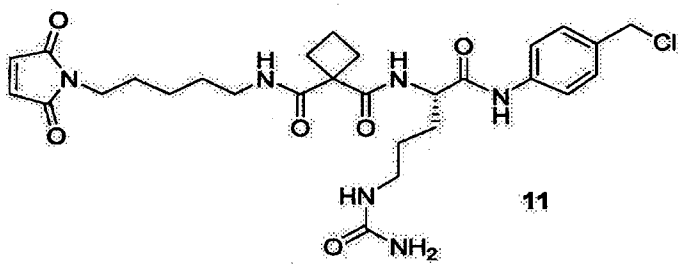
[0638] 步骤3:S)-N-(5-(2,5-二氧代-2,5-二氢-1H-吡咯-1-基)戊基)-N-(1-(4-(羟甲基)苯基氨基)-1-氧代-5-脲基戊-2-基)环丁烷-1,1-二甲酰胺10的制备

[0639] 

10b 10

[0640] 在0℃将二异丙基乙胺、DIPEA(1.59g,12.3mmol)和双(2-氧代-3-噁唑烷基)次膦酰氯、BOP-Cl(CAS登记号68641-49-6,Sigma-Aldrich,692mg,2.71mmol)加入到DMF(10mL)中的(S)-1-(1-(4-(羟甲基)苯基氨基)-1-氧代-5-脲基戊-2-基氨基甲酰基)环丁烷甲酸10b(1g,2.46mmol)溶液中,然后加入1-(5-氨基戊基)-1H-吡咯-2,5-二酮盐酸盐10a(592mg,2.71mmol)。将混合物在0℃下搅拌0.5小时。将反应混合物用柠檬酸溶液(10mL)淬灭,用DCM/MeOH(10:1)萃取。将有机层干燥并浓缩,残余物通过硅胶柱色谱(DCM:MeOH=10:1)纯化,得到S)-N-(5-(2,5-二氧代-2,5-二氢-1H-吡咯-1-基)戊基)-N-(1-(4-(羟甲基)苯基氨基)-1-氧代-5-脲基戊-2-基)环丁烷-1,1-二甲酰胺10(1.0g,71%),也称为MC-CBDK-cit-PAB-OH。LCMS(ESI):M+H+=571.28。^{1}H NMR(400MHz,DMSO-d6):δ10.00(s,1H),7.82-7.77(m,2H),7.53(d,J=8.4Hz,2H),7.19(d,J=8.4Hz,2H),6.96(s,2H),5.95(t,J=6.4Hz,1H),5.39(s,2H),5.08(t,J=5.6Hz,1H),4.40-4.35(m,3H),4.09(d,J=4.8Hz,1H),3.01(d,J=3.2Hz,2H),3.05-2.72(m,4H),2.68-2.58(m,3H),2.40-2.36(m,4H),1.72-1.70(m,3H),1.44-1.42(m,1H),1.40-1.23(m,6H),1.21-1.16(m,4H)。

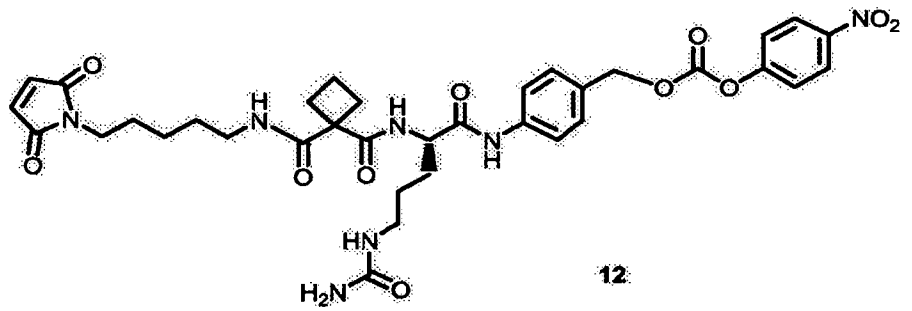
[0641] 实施例14(S)-N-(1-(4-(氯甲基)苯基氨基)-1-氧代-5-脲基戊-2-基)-N-(5-(2,5-二氧代-2,5-二氢-1H-吡咯-1-基)戊基)环丁烷-1,1-二甲酰胺11

[0642] 

11

[0643] 向在N,N-二甲基甲酰胺、DMF或N-甲基吡咯烷酮NMP(50mL)中的(S)-N-(5-(2,5-二氧代-2,5-二氢-1H-吡咯-1-基)戊基)-N-(1-(4-(羟甲基)苯基氨基)-1-氧代-5-脲基戊-2-基)环丁烷-1,1-二甲酰胺10(2.0g,3.5mmol)溶液中,在0℃逐渐滴加亚硫酰氯$SOCl_2$(1.25g,10.5mmol)。反应保持黄色。通过LC/MS监测反应,显示>90%转化。将反应混合物在20℃下搅拌30分钟或数小时后,用水(50mL)稀释,用EtOAc(50mL×3)萃取。将有机层干燥,浓缩并通过快速柱(DCM:MeOH=20:1)纯化,形成11,也称为MC-CBDK-cit-PAB-Cl,为灰色固体。LCMS:(5-95,AB,1.5min),0.696min,m/z=589.0$[M+1]^+$。

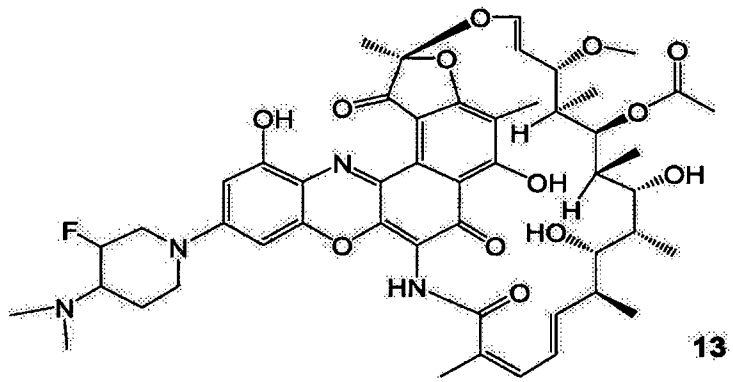
[0644] 实施例15(S)-4-(2-(1-(5-(2,5-二氧代-2,5-二氢-1H-吡咯-1-基)戊基氨基甲酰基)环丁烷甲酰胺基)-5-脲基戊酰氨基)苄基4-硝基苯基碳酸酯12

[0645] 

12

[0646] 向无水DMF中的(S)-N-(5-(2,5-二氧代-2,5-二氢-1H-吡咯-1-基)戊基)-N-(1-(4-(羟基甲基)苯基氨基)-1-氧代-5-脲基戊-2-基)环丁烷-1,1-二甲酰胺10的溶液中加入二异丙基乙胺(DIEA),然后加入PNP碳酸酯(碳酸二(4-硝基苯基)酯)。将反应溶液在室温(r.t.)下搅拌4小时,混合物通过制备型HPLC纯化,得到12。LCMS(ESI):$M+H^+$=736.29。

[0647] 实施例16MC-(CBDK-cit)-PAB-(二甲基,氟代pipBOR)-PLA-1的制备

[0648] 

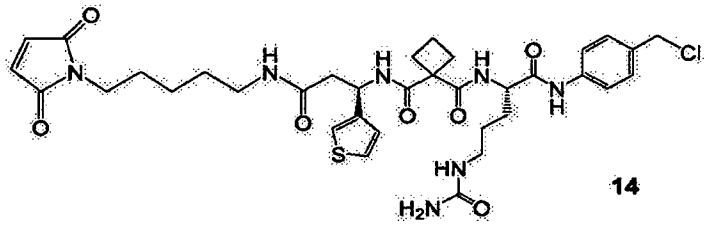
13

[0649] 按照PLA-2的方法,使(S)-N-(1-(4-(氯甲基)苯基氨基)-1-氧代-5-脲基戊-2-基)-N-(5-(2,5-二氧代-2,5-二氢-1H-吡咯-1-基)戊基)环丁烷-1,1-二甲酰胺11和氟代利福霉素衍生物二甲基氟代pipBOR 13(LCMS(ESI):$M+H^+$=945.43)反应形成MC-(CBDK-cit)-PAB-(二甲基,氟代pipBOR)-PLA-1,表2。LCMS(ESI):$M+H^+$=1499.7

[0650] 实施例17MC-(CBDK-cit)-PAB-(二甲基pipBOR)-PLA-2的制备

[0651] 将DMF中的(S)-N-(1-(4-(氯甲基)苯基氨基)-1-氧代-5-脲基戊-2-基)-N-(5-(2,5-二氧代-2,5-二氢-1H-吡咯-1-基)戊基)环丁烷-1,1-二甲酰胺11(0.035mmol)溶液冷却至0℃,加入二甲基pipBOR 6(10mg,0.011mmol)。将混合物用另外0.5mL的DMF稀释。在空气中搅拌30分钟,加入N,N-二异丙基乙胺(DIEA,10μl,0.05mmol),在空气中将反应物搅拌过夜。通过LC/MS观察到50%的所需产物。加入另外的0.2当量N,N-二异丙基乙胺碱,同时在空气中将反应物再搅拌6小时,直到反应停止进行。反应混合物用DMF稀释,并在HPLC(20-60% ACN/HCOOH在H_2O中)中纯化,得到MC-(CBDK-cit)-PAB-(二甲基pipBOR)-PLA-2,表2。LCMS(ESI):$M+H^+$=1481.8,产率31%。

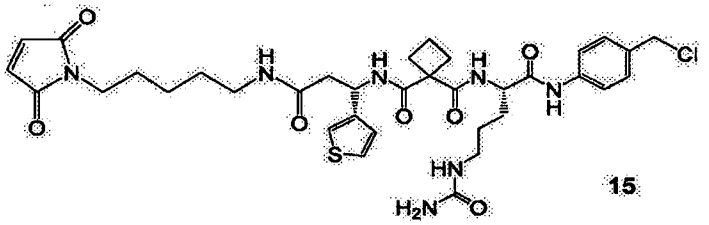
[0652] 实施例18 MC-((R)-噻吩-3-基-CBDK-cit)-PAB-(二甲基pipBOR)(PLA-3)的制备

[0653] 

14

[0654] 按照PLA-2的方法，将(N-((S)-1-(4-(氯甲基)苯基氨基)-1-氧代-5-脲基戊-2-基)-N-((R)-5-(2,5-二氧代-2,5-二氢-1H-吡咯-1-基)戊基氨基)-3-氧代-1-(噻吩-3-基)丙基)环丁烷-1,1-二甲酰胺14(LCMS(ESI):M+H$^+$=742.3)和二甲基pipBOR 6反应得到MC-((R)-噻吩-3-基-CBDK-cit)-PAB-(二甲基pipBOR)(PLA-3,表2)。LCMS(ESI):M+H$^+$=1633.9

[0655] 实施例19 MC-((S)-噻吩-3-基-CBDK-cit)-PAB-(二甲基pipBOR)(PLA-4)

[0656] 

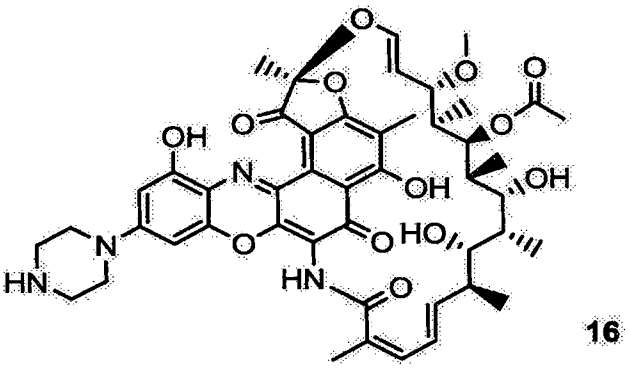
15

[0657] 按照PLA-2的方法，将(N-((R)-1-(4-(氯甲基)苯基氨基)-1-氧代-5-脲基戊-2-基)-N-((R)-5-(2,5-二氧代-2,5-二氢-1H-吡咯-1-基)戊基氨基)-3-氧代-1-(噻吩-3-基)丙基)环丁烷-1,1-二甲酰胺15(LCMS(ESI):M+H$^+$=742.3)和二甲基pipBOR 6反应得到MC-((R)-噻吩-3-基-CBDK-cit)-PAB-(二甲基pipBOR)(PLA-4,表2)。LCMS(ESI):M+H$^+$=1633.9

[0658] 实施例20 MC-(CBDK-cit)-PABC-(pipBOR)(PLA-5)的制备

[0659] 哌啶基苯并噁嗪并利福霉素(pipBOR)5(15mg,0.0167mmol)、然后(S)-4-(2-(1-(5-(2,5-二氧代-2,5-二氢-1H-吡咯-1-基)戊基氨基甲酰基)环丁烷甲酰氨基)-5-脲基戊酰氨基)苄基4-硝基苯基碳酸酯12(12mg,0.0167mmol)称重后加入小瓶中。加入二甲基甲酰胺DMF(0.3mL)，然后加入二异丙基乙胺DIEA(0.006ml,0.0334mmol)，并将反应物在室温下搅拌2小时。反应溶液通过HPLC(30-70%MeCN/水+1%甲酸)直接纯化，得到MC-(CBDK-cit)-PABC-(pipBOR)(PLA-5,表2)。LCMS(ESI):M+H$^+$=1496.5

[0660] 实施例21 MC-(CBDK-cit)-PABC-(哌嗪BTR)(PLA-6)的制备

[0661] 

16

[0662] 按照PLA-5的步骤，哌啶利福霉素衍生物即哌嗪BOR 16(LCMS(ESI):M+H$^+$=885.4)和(S)-4-(2-(1-(5-2,5-二氢-1H-吡咯-1-基)戊基氨基甲酰基)环丁烷甲酰氨基)-5-脲基

戊酰氨基)苄基4-硝基苯基碳酸酯12反应,得到MC-(CBDK-cit)-PABC-(哌嗪BTR)(PLA-6,表2)。LCMS(ESI):$M+H^+$=1482.5

[0663] 虽然为了清楚理解的目的,已经通过说明和示例的方式对前述发明进行了详细描述,但这些描述和实施例不应被解释为限制本发明的范围。在整个说明书中引用的所有专利、专利申请和参考文献通过引用明确地并入本文。

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<110> 基医技术公司等

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[0005]

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Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
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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
115

<210> 29
<211> 113
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<400> 29
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 Glu 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

[0007]

<210> 30
<211> 119
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<400> 30
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Thr
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

<210> 31
<211> 114

<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<400> 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

Tyr Tyr Thr Ser Pro Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu
100 105 110

Ile Glu

<210> 32
<211> 138
<212> PRT
<213> 人工序列

[0008]

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<400> 32
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu
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
115 120 125

Gly Gln Gly Thr Thr Val Thr Val Ser Ser
130 135

<210> 33
<211> 11
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 33
Arg Ala Ser Gln Thr Ile Ser Gly Trp Leu Ala
1 5 10

<210> 34
<211> 7
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

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

<210> 35
<211> 9
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 35
Gln Gln Tyr Lys Ser Tyr Ser Phe Asn
1 5

<210> 36
<211> 5
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

[0009]

<400> 36
Ser Tyr Asp Ile Asn
1 5

<210> 37
<211> 17
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 37
Trp Met Asn Ala Asn Ser Gly Asn Thr Gly Tyr Ala Gln Lys Phe Gln
1 5 10 15

Gly

<210> 38
<211> 15
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 38
Ser Ser Ile Leu Val Arg Gly Ala Leu Gly Arg Tyr Phe Asp Leu
1 5 10 15

<210> 39
<211> 11
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 39
Arg Ala Ser Gln Thr Ile Ser Gly Trp Leu Ala
1 5 10

<210> 40
<211> 7
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 40
Lys Ala Ser Thr Leu Glu Ser
1 5

<210> 41
<211> 9
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 41
Gln Gln Tyr Lys Ser Tyr Ser Phe Asn
1 5

<210> 42
<211> 5
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 42
Ser Tyr Asp Ile Asn
1 5

[0010]

<210> 43
<211> 17
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 43
Trp Met Asn Ala Asn Ser Gly Asn Thr Gly Tyr Ala Gln Lys Phe Gln
1 5 10 15

Gly

<210> 44
<211> 15
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 44
Ser Ser Ile Leu Val Arg Gly Ala Leu Gly Arg Tyr Phe Asp Leu
1 5 10 15

<210> 45
<211> 12
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 45
Arg Ala Ser Gln Phe Val Ser Arg Thr Ser Leu Ala

1 5 10

<210> 46
<211> 7
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 46
Glu Thr Ser Ser Arg Ala Thr
1 5

<210> 47
<211> 9
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 47
His Lys Tyr Gly Ser Gly Pro Arg Thr
1 5

<210> 48
<211> 5
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 48
Asn Tyr Asp Phe Ile
1 5

[0011]

<210> 49
<211> 17
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<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> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 50
Ala Val Arg Gly Gln Leu Leu Ser Glu Tyr
1 5 10

<210> 51
<211> 12
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 51
Arg Ala Ser Gln Ser Val Ser Ser Ser Tyr Leu Ala
1 5 10

<210> 52
<211> 7
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 52
Asp Ala Ser Ser Arg Ala Thr
1 5

<210> 53
<211> 9
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 53
Gln Lys Tyr Gly Ser Thr Pro Arg Pro
1 5

<210> 54
<211> 5
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 54
Ser Tyr Asp Ile Asn
1 5

[0012]

<210> 55
<211> 17
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<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> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<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> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 57
Arg Ala Ser Leu Asp Ile Thr Asn His Leu Ala
1 5 10

<210> 58
<211> 7
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 58
Glu Ala Ser Ile Leu Gln Ser
1 5

<210> 59
<211> 9
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 59
Glu Lys Cys Asn Ser Thr Pro Arg Thr
1 5

<210> 60
<211> 5
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 60
Asn Tyr Asp Ile Asn
1 5

[0013]

<210> 61
<211> 17
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<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> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<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> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 63
Arg Ala Ser Gln Ser Val Gly Ala Ile Tyr Leu Ala
1 5 10

<210> 64
<211> 7
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 64
Gly Val Ser Asn Arg Ala Thr
1 5

<210> 65
<211> 10
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 65
Gln Leu Tyr Thr Ser Ser Arg Ala Leu Thr
1 5 10

<210> 66
<211> 5
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 66
Ala Tyr Ala Met Asn
1 5

[0014]

<210> 67
<211> 17
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<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> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 68
Leu Ala Ala Arg Ile Met Ala Thr Asp Tyr
1 5 10

<210> 69
<211> 11
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 69
Arg Ala Ser Gln Gly Ile Arg Asn Gly Leu Gly
1 5 10

<210> 70

<211> 7
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 70
Pro Ala Ser Thr Leu Glu Ser
1 5

<210> 71
<211> 9
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 71
Leu Gln Asp His Asn Tyr Pro Pro Thr
1 5

<210> 72
<211> 5
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 72
Tyr Tyr Ser Met Ile
1 5

<210> 73
<211> 17
<212> PRT
[0015] <213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<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> 16
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<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> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 75
Arg Ala Ser Gln Gly Ile Arg Asn Gly Leu Gly
1 5 10

<210> 76
<211> 7
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 76
Pro Ala Ser Thr Leu Glu Ser
1 5

<210> 77
<211> 9
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 77
Leu Gln Asp His Asn Tyr Pro Pro Ser
1 5

<210> 78
<211> 5
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 78
Tyr Tyr Ser Met Ile
1 5

<210> 79
<211> 17
<212> PRT
[0016] <213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<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> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<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> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 81
Arg Ala Ser Gln Ser Val Arg Thr Asn Val Ala
1 5 10

<210> 82
<211> 7
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 82
Gly Ala Ser Thr Arg Ala Ser
1 5

<210> 83
<211> 9
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 83
Leu Gln Tyr Asn Thr Trp Pro Arg Thr
1 5

<210> 84
<211> 5
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 84
Thr Asn Asp Met Ser
1 5

[0017]

<210> 85
<211> 17
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<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> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 86
Asn Ser Gly Ile Tyr Ser Phe
1 5

<210> 87
<211> 11
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 87
Arg Ala Ser Gln Asp Ile Gly Ser Ser Leu Ala
1 5 10

<210> 88
<211> 7

<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 88
Ala Thr Ser Thr Leu Gln Ser
1 5

<210> 89
<211> 9
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 89
Gln Gln Leu Asn Asn Tyr Val His Ser
1 5

<210> 90
<211> 5
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 90
Asp Tyr Ala Met Gly
1 5

<210> 91
<211> 17
<212> PRT
<213> 人工序列

[0018]

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<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> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 92
Arg Ile Trp Ser Tyr Gly Asp Asp Ser Phe Asp Val
1 5 10

<210> 93
<211> 11
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 93
Arg Ala Ser Gln Ser Ile Gly Asp Arg Leu Ala
1 5 10

<210> 94
<211> 7
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 94
Trp Ala Ser Asn Leu Glu Gly
1 5

<210> 95
<211> 8
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 95
Gln Gln Tyr Lys Ser Gln Trp Ser
1 5

<210> 96
<211> 5
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 96
Ser Tyr Ala Met Asn
1 5

<210> 97
<211> 16
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

[0019]

<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> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<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> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<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> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 100
Trp Ala Ser Thr Arg Lys Ser
1 5

<210> 101
<211> 9
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 101
Gln Gln Tyr Phe Ser Pro Pro Tyr Thr
1 5

<210> 102
<211> 5
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 102
Ser Phe Trp Met His
1 5

<210> 103
<211> 17
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 103
Phe Thr Asn Asn Glu Gly Thr Thr Thr Ala Tyr Ala Asp Ser Val Arg
1 5 10 15

[0020]

Gly

<210> 104
<211> 7
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 104
Gly Asp Gly Gly Leu Asp Asp
1 5

<210> 105
<211> 11
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 105
Arg Ala Ser Gln Phe Thr Asn His Tyr Leu Asn
1 5 10

<210> 106
<211> 7
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 106
Val Ala Ser Asn Leu Gln Ser

1 5

<210> 107
<211> 9
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 107
Gln Gln Ser Tyr Arg Thr Pro Tyr Thr
1 5

<210> 108
<211> 5
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 108
Ser Gly Tyr Tyr Asn
1 5

<210> 109
<211> 16
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 109
Tyr Ile Leu Ser Gly Ala His Thr Asp Ile Lys Ala Ser Leu Gly Ser
1 5 10 15

[0021]

<210> 110
<211> 11
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 110
Ser Gly Val Tyr Ser Lys Tyr Ser Leu Asp Val
1 5 10

<210> 111
<211> 107
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<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> 134
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<220>
<221> VARIANT
<222> (1)..(1)
<223> /替换="Glu"

<220>
<221> VARIANT
<222> (2)..(2)
<223> /替换="Ile"或"Val"

<220>
<221> MISC_FEATURE
<222> (1)..(124)
<223> /备注="在该序列中给出的变体残基对于在变体位置注解中的那些不具有优选性"

<400> 112
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

[0022]

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> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<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 Asn 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> 114
<211> 453
<212> PRT
<213> 人工序列

[0023]

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<400> 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

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

[0024]

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> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<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

[0025] Phe Asn Arg Gly Glu Cys
210

<210> 116
<211> 453
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<220>
<221> VARIANT
<222> (2)..(2)
<223> /替换="Ile"或"Val"

<220>
<221> MISC_FEATURE
<222> (1)..(453)
<223> /备注="在该序列中给出的变体残基对于在变体位置注解中的那些不具有优选性"

<400> 116
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 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

[0026] 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> 117
<211> 453
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<220>
<221> VARIANT
<222> (2)..(2)
<223> /替换="Ile"或"Val"

<220>
<221> MISC_FEATURE
<222> (1)..(453)
<223> /备注="在该序列中给出的变体残基对于在变体位置注解中的那些不具有优选性"

<400> 117
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

Glu Gly Arg Val Thr Leu Thr Gly Asp Thr Ser Ile Ser Thr Ala Tyr
65 70 75 80

[0027] 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

[0028] Ser Leu Ser Pro Gly
450

<210> 118
<211> 7
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 118
Gly Glu Gly Gly Leu Asp Asp
1 5

<210> 119
<211> 113
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<400> 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 Gln 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> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<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

[0029]

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
115

<210> 121
<211> 220
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<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 Glu Gly Thr Lys Leu Glu Ile
100 105 110

Lys Arg Glu 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> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<400> 122
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Glu Pro Gly Gly
1 5 10 15

[0030]

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

[0031]

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> 123
<211> 220
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<400> 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 Tyr
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 Glu 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> 124
<211> 444
<212> PRT
<213> 人工序列

<220>
<221> 来源
[0032] <223> /备注="人工序列的描述：合成多肽"

<400> 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

[0033] 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

<400> 125
000

<210> 126

<400> 126
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<210> 127
<211> 448
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<220>
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<223> /备注="人工序列的描述：合成多肽"

<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

[0034] 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

<210> 128

<400> 128
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<400> 131
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[0035]

<210> 132

<400> 132
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<210> 133
<211> 450
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<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 Tyr Tyr 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

[0036]

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> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<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

[0037] 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> 135

<400> 135
000

<210> 136

<400> 136
000

<210> 137

<400> 137
000

[0038]

<210> 138
<211> 445
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<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

[0039]

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> 139
<211> 153
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<400> 139
Glu Met Gln Leu Val Glu 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 Gln 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

[0040] 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> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<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

[0041] 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

[0042]

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> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<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

[0043] 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> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<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

[0044]

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> 143
<211> 453
<212> PRT
<213> 人工序列

<220>
<221> 来源
[0045] <223> /备注="人工序列的描述：合成多肽"

<400> 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

[0046] 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> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<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

[0047] 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
105 410 415

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> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<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

[0048]

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> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<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

[0049] 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> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<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
[0050] 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

[0051] 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> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<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

[0052] 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> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<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

[0053]

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> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<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> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<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
[0054] <212> DNA
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成寡核苷酸"

<400> 152
ccagactgc accgctgga tctctgaatg tactccagtt gc 42

<210> 153
<211> 41
<212> DNA
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成寡核苷酸"

<400> 153
ccagactgca ccgctgcac ctctgaatgt actccagttg c 41

<210> 154
<211> 61
<212> DNA
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成寡核苷酸"

<400> 154
ccaggttcc ctggccccaw tmgtccagtc cascwkcacc tcttgcacag taatagacag 60

c 61

<210> 155
<211> 52
<212> DNA
<213> 人工序列

<220>

<221> 来源
<223> /备注="人工序列的描述：合成寡核苷酸"

<400> 155
cctggcccca gtcgtcaagt cctccttcac ctcttgcaca gtaatagaca gc 52

<210> 156
<211> 116
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<400> 156
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

[0055] Thr Val Ser Ser
115

<210> 157
<211> 445
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<400> 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

[0056] 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> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<400> 158
Asp Ile Glu 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

[0057]

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> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<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 Tyr
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> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<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

[0058]

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 Glu 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> 161
<211> 314
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<400> 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

[0059]

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> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<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

[0060]

<210> 163
<211> 214
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<400> 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

Gln 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 Glu 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> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<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

[0061]

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> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<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

[0062]

Glu Ser Val Thr Glu Glu 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> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<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 Glu 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> 人工序列

<220>
<221> 来源
[0063] <223> /备注="人工序列的描述：合成多肽"

<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 Glu Phe Ala Leu Thr Ile Ser Gly Leu Gln Pro
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> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<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

[0064]

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 Glu
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> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<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

[0065] 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 Glu 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> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<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

[0066]

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

[0067]

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

<210> 171
<211> 455
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<400> 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

[0068] 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> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<400> 172
Glu 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

[0069] 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> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<400> 173
Gln Ile Thr Leu Lys Glu Ser Gly Gly Gly Leu Ile Lys Pro Gly Gly
1 5 10 15

[0070]

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 Ala Arg Ile Met Ala Thr Asp 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

[0071]

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

<210> 174
<211> 456
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<400> 174
Glu Val Gln Leu Val Gln Ser Gly Gly Gly Leu Val Lys Pro Gly Glu
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Phe Asp Tyr Tyr
20 25 30

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 Glu 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

[0072] 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> 175
<211> 456
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<400> 175
Glu Val Gln Leu Gln Glu Ser Gly Gly Gly Leu Val Asn Pro Gly Glu
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Phe Asn Tyr Tyr
20 25 30

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 Glu 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

[0073] 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
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 Gln 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> 176
<211> 445
<212> PRT
[0074] <213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成多肽"

<400> 176
Gln Val Gln Leu Gln Gln Ser Gly Gly Gly Leu Glu Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Ile Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Asn
20 25 30

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
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

[0075] 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> 177
<211> 7
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 177
Ala Arg Gly Asp Gly Gly Leu
1 5

<210> 178
<211> 7
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 178
Ala Arg Gly Asp Ala Gly Leu
1 5

<210> 179
<211> 7
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

[0076]

<400> 179
Ala Arg Gly Glu Gly Gly Leu
1 5

<210> 180
<211> 7
<212> PRT
<213> 人工序列

<220>
<221> 来源
<223> /备注="人工序列的描述：合成肽"

<400> 180
Ala Arg Gly Ala Gly Gly Leu
1 5

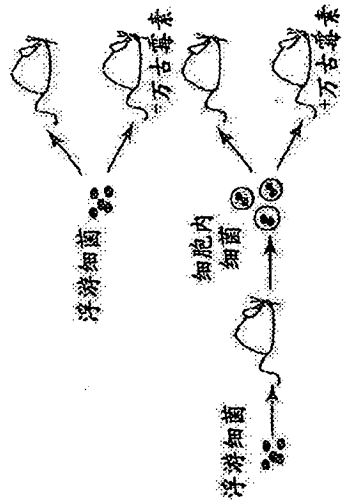

浮游细菌
细胞内细菌
浮游细菌
+万古霉素
+万古霉素

图1A

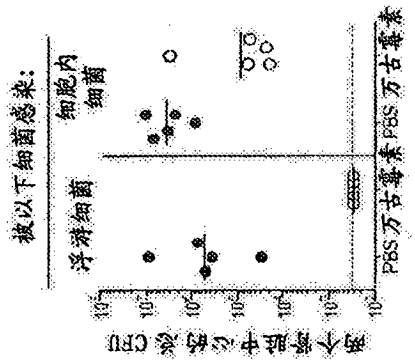

被以下细菌感染:
浮游细菌
细胞内细菌
PBS
万古霉素
PBS
万古霉素
每个肾脏中心的总 CFU

图1B

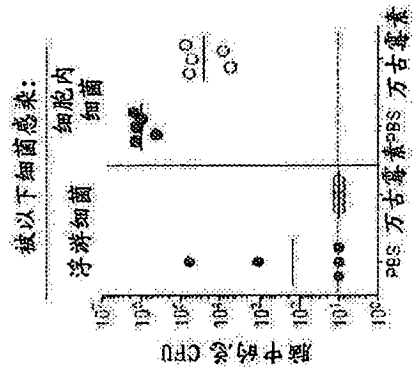

被以下细菌感染:
浮游细菌
细胞内细菌
PBS
万古霉素
PBS
万古霉素
脾中的总 CFU

图1C

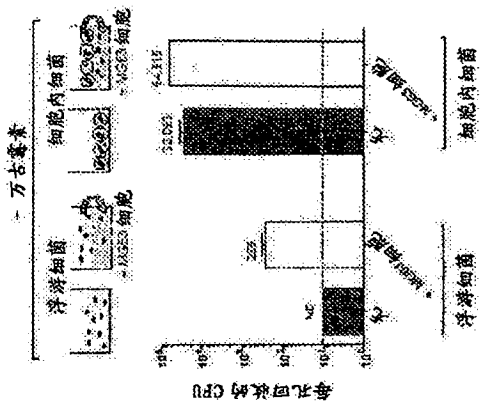


图1D

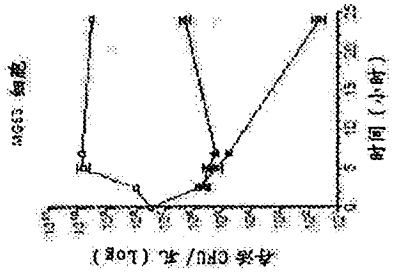


图1E

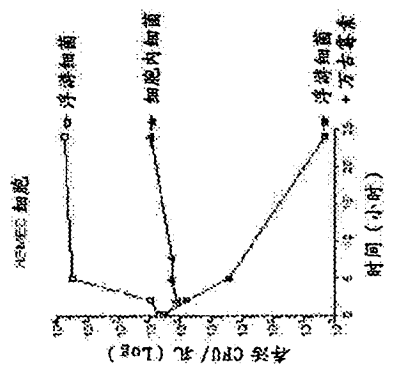


图1F

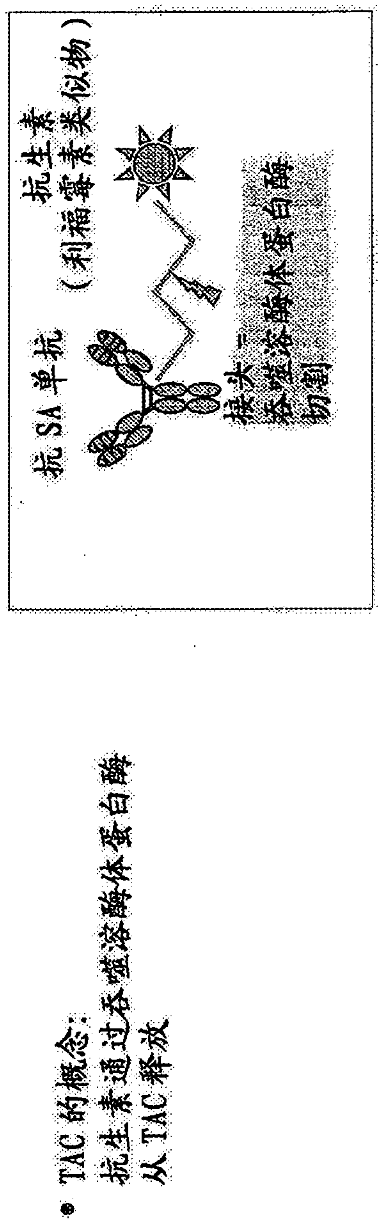

抗SA单抗
抗生素
（利福霉素类似物）
接头=
吞噬溶酶体蛋白酶
切割
• TAC的概念：
抗生素通过吞噬溶酶体蛋白酶
从TAC释放

图2

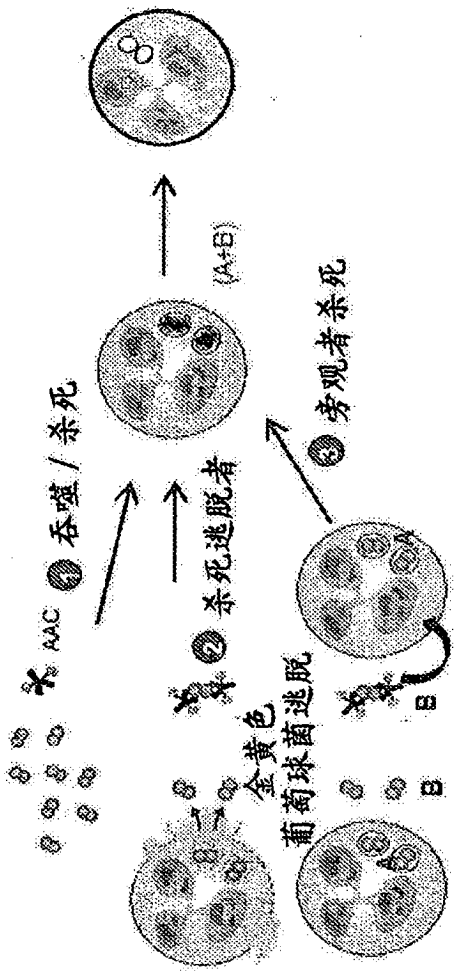


图3

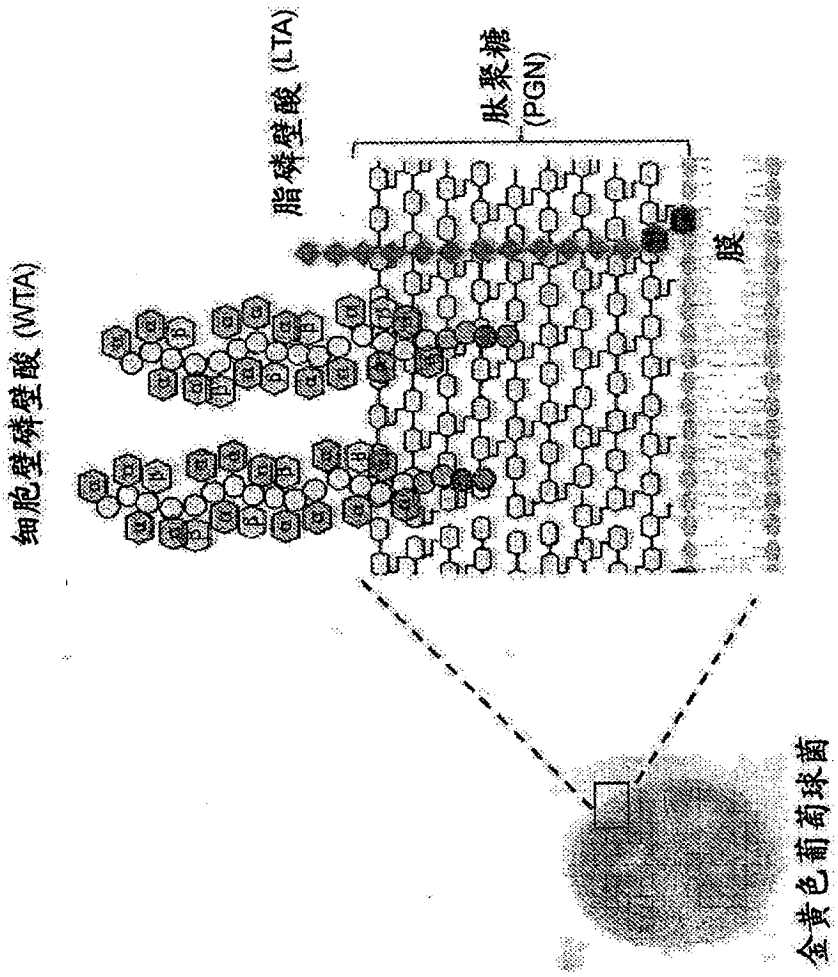


图4

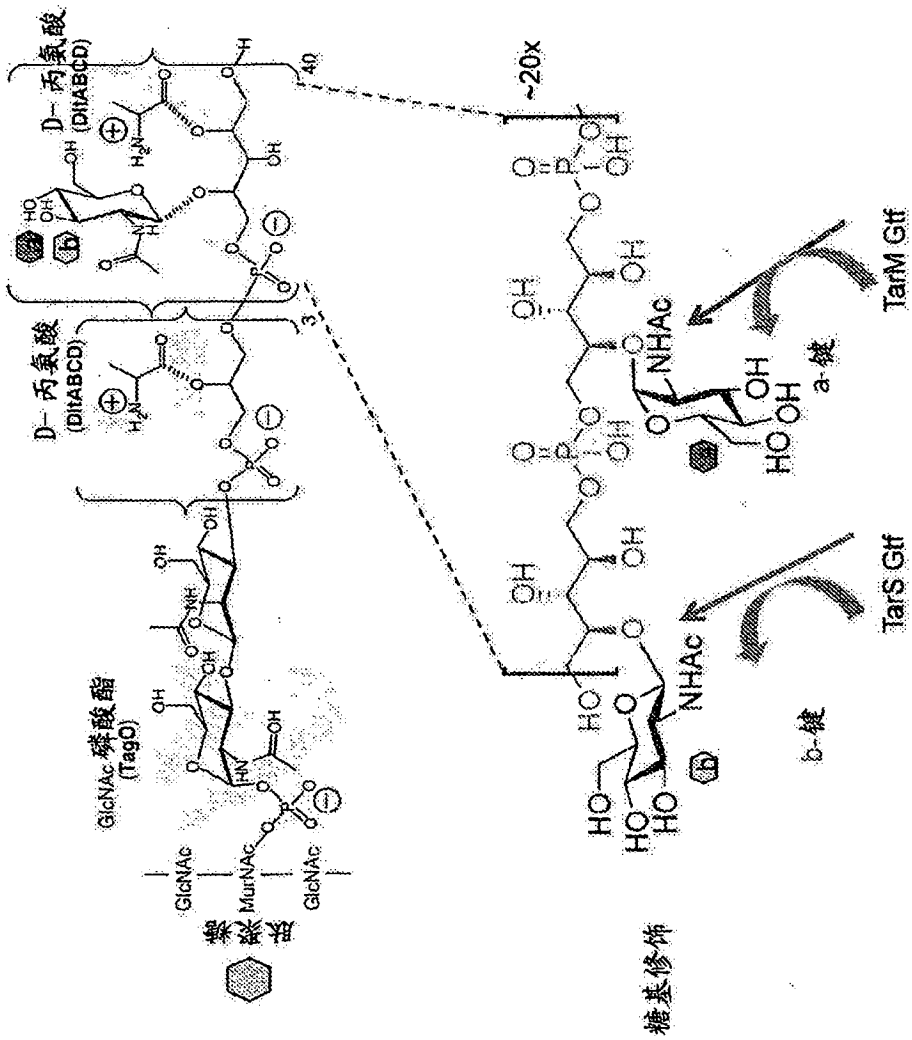

D- 丙氨酸
(DltABCD)
D- 丙氨酸
(DltABCD)
GlcNAc 磷酸酯
(TagO)
GlcNAc
MurNAc
GlcNAc
肽聚糖
~20x
NHAc
NHAc
TarM Gtf
TarS Gtf
a-键
b-键
糖基修饰

图5

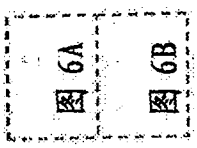
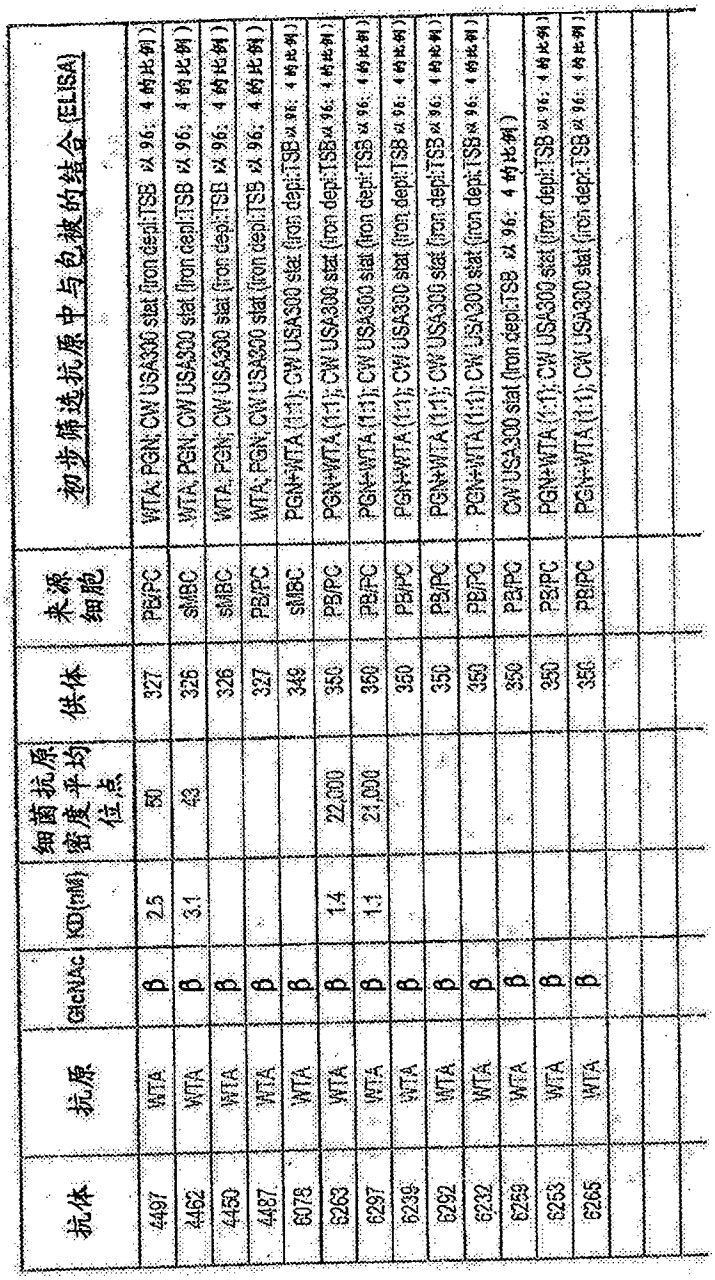


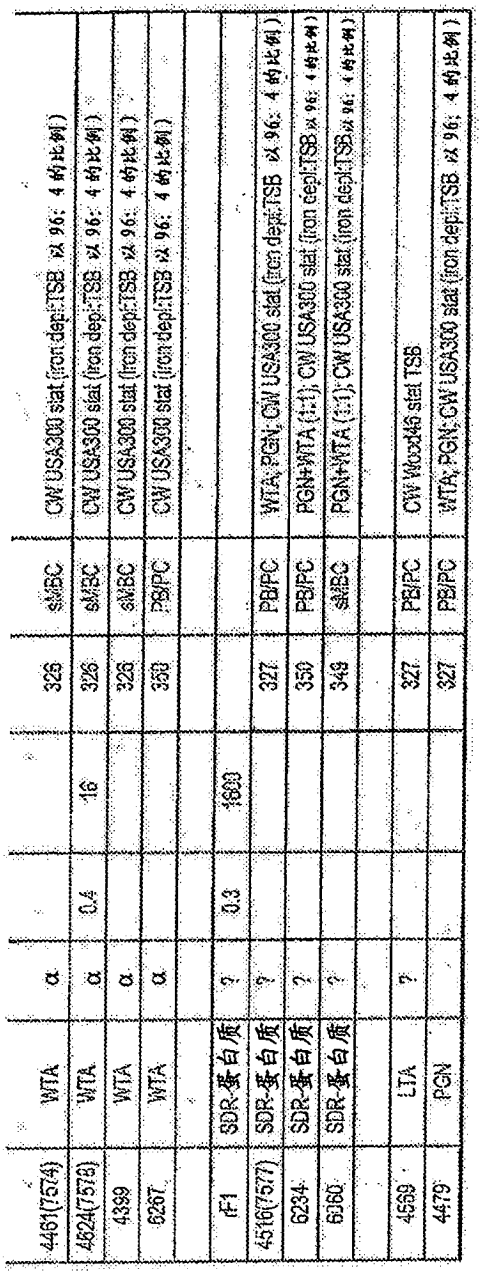
图 6A
图 6B

图6



抗体	抗原	GlcNAc	KD(nM)	细菌抗原密度平均位点	供体	来源细胞	初步筛选抗原中与包被的结合(ELISA)
4497	WTA	β	2.5	50	327	PB/PC	WTA; PGN; CW USA300 stat (iron depl.TSB 以 96: 4 的比例)
4462	WTA	β	3.1	43	326	sMBC	WTA; PGN; CW USA300 stat (iron depl.TSB 以 96: 4 的比例)
4450	WTA	β			326	sMBC	WTA; PGN; CW USA300 stat (iron depl.TSB 以 96: 4 的比例)
4487	WTA	β			327	PB/PC	WTA; PGN; CW USA300 stat (iron depl.TSB 以 96: 4 的比例)
6078	WTA	β			349	sMBC	PGN+WTA (1:1); CW USA300 stat (iron depl.TSB 以 96: 4 的比例)
6263	WTA	β	1.4	22,000	350	PB/PC	PGN+WTA (1:1); CW USA300 stat (iron depl.TSB 以 96: 4 的比例)
6297	WTA	β	1.1	21,000	350	PB/PC	PGN+WTA (1:1); CW USA300 stat (iron depl.TSB 以 96: 4 的比例)
6239	WTA	β			350	PB/PC	PGN+WTA (1:1); CW USA300 stat (iron depl.TSB 以 96: 4 的比例)
6292	WTA	β			350	PB/PC	PGN+WTA (1:1); CW USA300 stat (iron depl.TSB 以 96: 4 的比例)
6232	WTA	β			350	PB/PC	PGN+WTA (1:1); CW USA300 stat (iron depl.TSB 以 96: 4 的比例)
6269	WTA	β			350	PB/PC	CW USA300 stat (iron depl.TSB 以 96: 4 的比例)
6253	WTA	β			350	PB/PC	PGN+WTA (1:1); CW USA300 stat (iron depl.TSB 以 96: 4 的比例)
6265	WTA	β			356	PB/PC	PGN+WTA (1:1); CW USA300 stat (iron depl.TSB 以 96: 4 的比例)

图6A



4461(7574)	WTA	α			326	sMBC	CW USA300 stat (iron dep.:TSB 以 96: 4 的比例)
4824(7578)	WTA	α	0.4	16	326	sMBC	CW USA300 stat (iron dep.:TSB 以 96: 4 的比例)
4389	WTA	α			326	sMBC	CW USA300 stat (iron dep.:TSB 以 96: 4 的比例)
6267	WTA	α			368	PB/PC	CW USA300 stat (iron dep.:TSB 以 96: 4 的比例)
rF1	SDR-蛋白质	?	0.3	1893			
4516(7577)	SDR-蛋白质	?			327	PB/PC	WTA; PGN; CW USA300 stat (iron dep.:TSB 以 96: 4 的比例)
6234	SDR-蛋白质	?			359	PB/PC	PGN+WTA (1:1); CW USA300 stat (iron dep.:TSB 以 96: 4 的比例)
6360	SDR-蛋白质	?			349	sMBC	PGN+WTA (1:1); CW USA300 stat (iron dep.:TSB 以 96: 4 的比例)
4569	LTA	?			327	PB/PC	CW Wood46 stat TSB
4479	PGN				327	PB/PC	WTA; PGN; CW USA300 stat (iron dep.:TSB 以 96: 4 的比例)

图6B

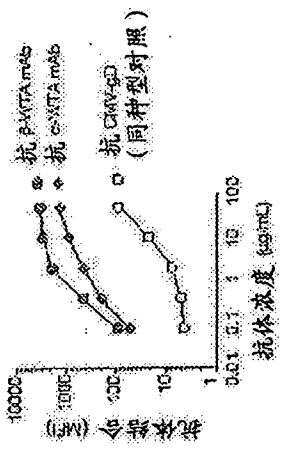

抗 β-VNTA mAb
抗 cVNTA mAb
抗 CMVgD
（同种型对照）
抗体浓度 (μg/mL)

图7A

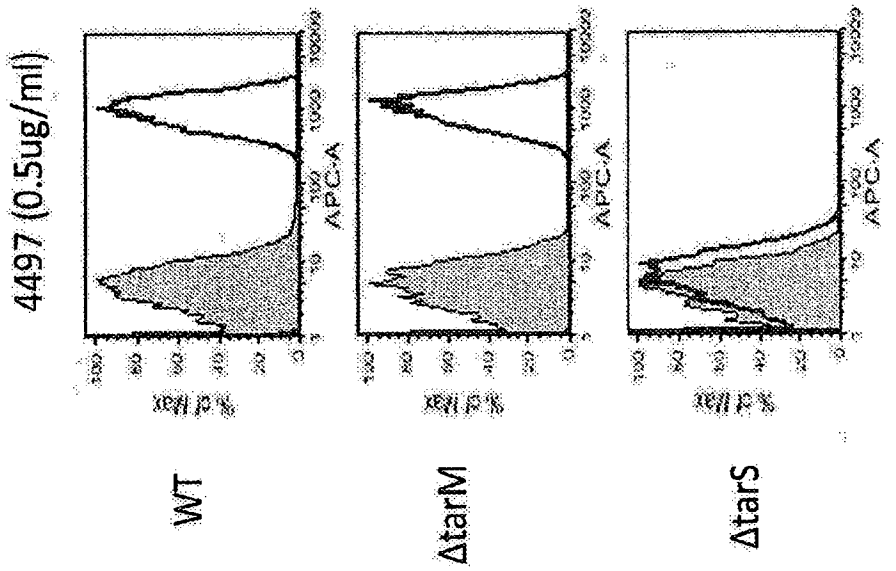

4497 (0.5ug/ml)
APC-A
WT
ΔtarM
ΔtarS

图7B

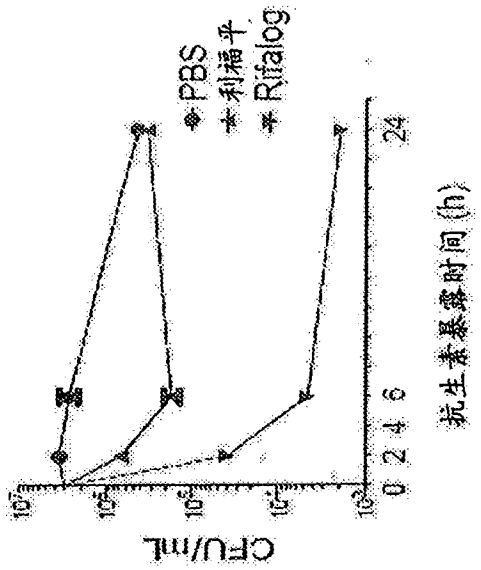

PBS
利福平
Rifalog
CFU/mL
抗生素暴露时间 (h)
0 2 4 6 24

图8

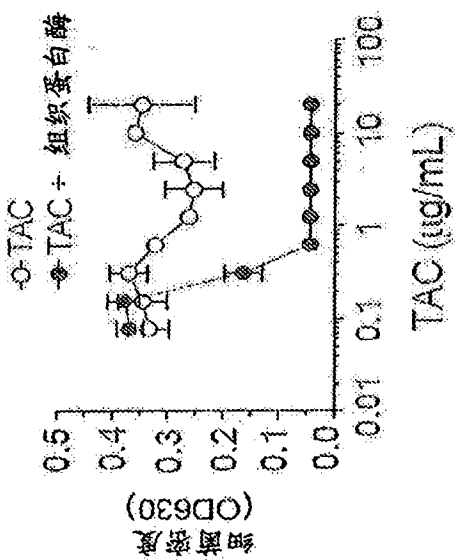

TAC
TAC + 组织蛋白酶
细菌密度 (OD630)
TAC (ug/mL)
0.01 0.1 1 10 100
0.0 0.1 0.2 0.3 0.4 0.5

图9

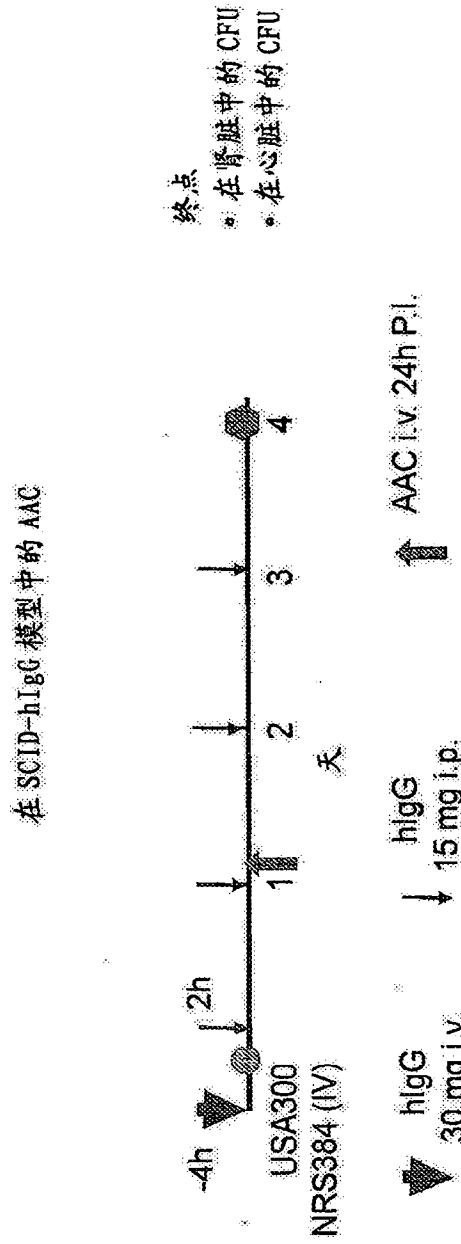

在 SCID-hIgG 模型中的 AAC
终点
• 在肾脏中的 CFU
• 在心脏中的 CFU
-4h
2h
USA300
NRS384 (IV)
1
2
3
4
天
hIgG
30 mg i.v.
hIgG
15 mg i.p.
AAC i.v. 24h P.I.

图10A

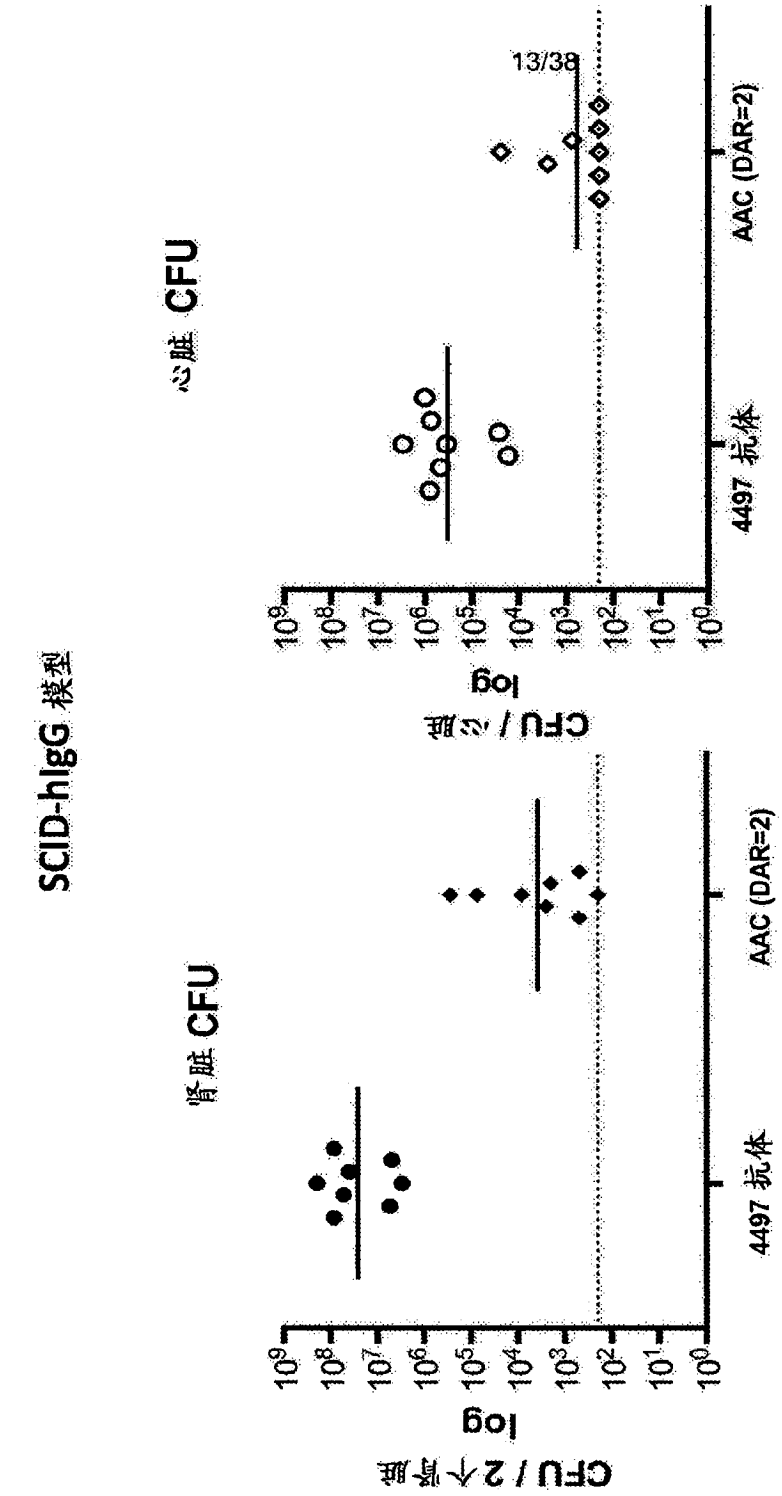

SCID-hIgG 模型
肾脏 CFU
CFU / 2个肾脏
log
4497 抗体
AAC (DAR=2)
心脏 CFU
CFU / 心脏
log
4497 抗体
AAC (DAR=2)
13/38

图 10B

图 10C

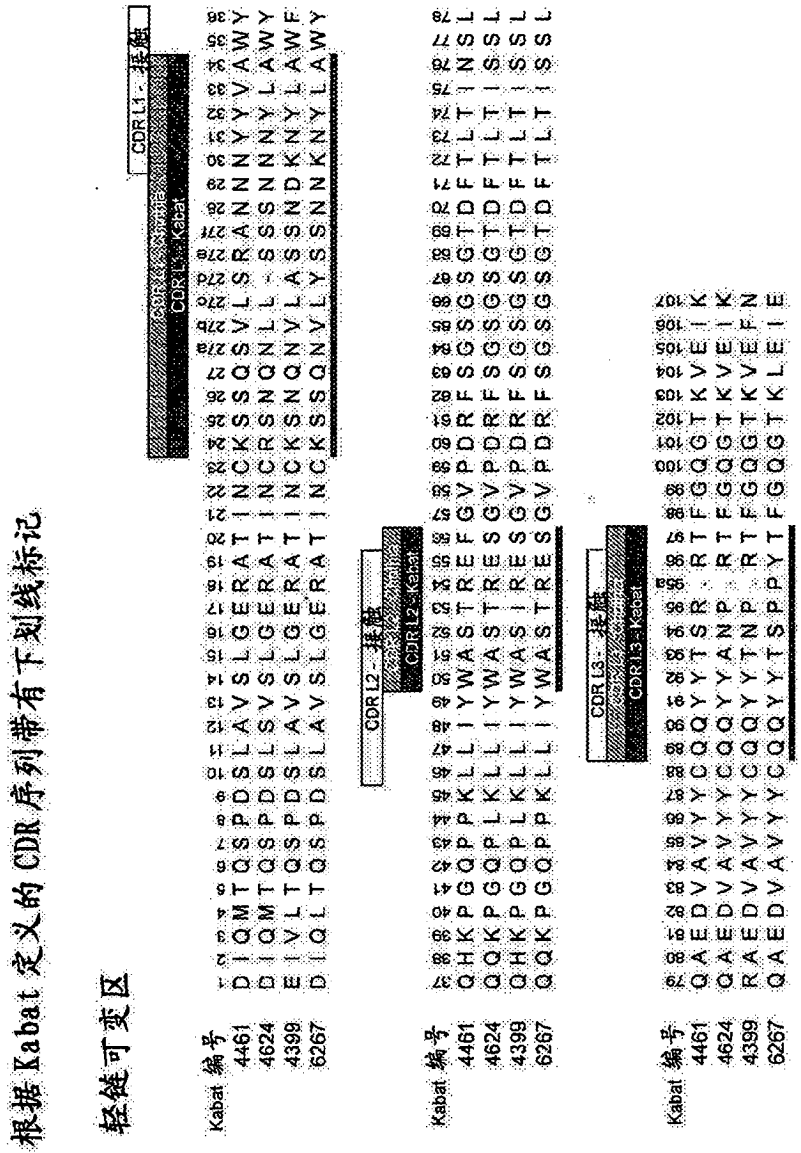

根据 Kabat 定义的 CDR 序列带有下划线标记
轻链可变区
CDR L1 - 接触
CDR L1 - Chothia
CDR L1 - Kabat
CDR L2 - 接触
CDR L2 - Chothia
CDR L2 - Kabat
CDR L3 - 接触
CDR L3 - Chothia
CDR L3 - Kabat
Kabat 编号
4461
4624
4399
6267

图11A

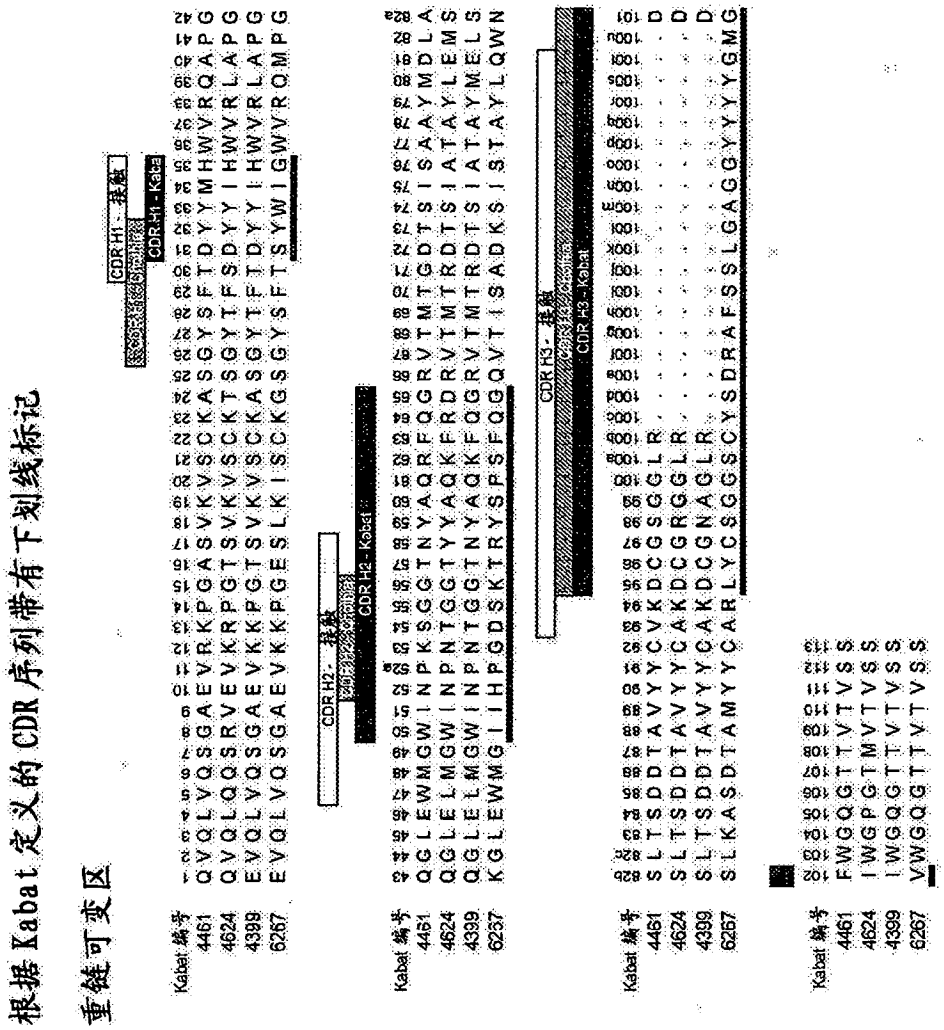

根据 Kabat 定义的 CDR 序列带有下划线标记
重链可变区
CDR H1 - 基板
CDR H1 - Kabat
CDR H2 - 基板
CDR H2 - Kabat
CDR H3 - 基板
CDR H3 - Kabat
Kabat 编号
4461
4624
4399
6267

图11B

抗体	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)	WMNANSGNTGYAQKFQG (SEQ ID NO:37)	SSILVRGALGRYFDL (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)	WMNANSGNTGYAQKFQG (SEQ ID NO:43)	SSILVRGALGRYFDL (SEQ ID NO:44)
4450	RASQFVSRTSLA (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)	WMNPNSGNTNYAQRFQG (SEQ ID NO:55)	ERWSKDTGHYYYY GMDV (SEQ ID NO:56)
6239	RASLDITNHLA (SEQ ID NO:57)	EASILQS (SEQ ID NO:58)	EKCNSTPRT (SEQ ID NO:59)	NYDIN (SEQ ID NO:60)	WMNPSSGRTGYAPKFRG (SEQ ID NO:61)	GGGYYDSSGNYHIS GLDV (SEQ ID NO:62)
6232	RASQSVGAIYLA (SEQ ID NO:63)	GVSNRAT (SEQ ID NO:64)	QLYTSSRALT (SEQ ID NO:65)	AYAMN (SEQ ID NO:66)	SITKNSDSLYYADSVKG (SEQ ID NO:67)	LAARIMATDY (SEQ ID NO:68)
6259	RASQGIRNGLG (SEQ ID NO:69)	PASTLES (SEQ ID NO:70)	LQDHNYPPT (SEQ ID NO:71)	YYSMI (SEQ ID NO:72)	SIDSSSRYLYYADSVKG (SEQ ID NO:73)	DGDDILSVYRGSGR PFDY (SEQ ID NO:74)
6292	RASQGIRNGLG (SEQ ID NO:75)	PASTLES (SEQ ID NO:76)	LQDHNYPPS (SEQ ID NO:77)	YYSMI (SEQ ID NO:78)	SIDSSSRYRYYTDSVKG (SEQ ID NO:79)	DGDDILSVYQGSGR PFDY (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)	TIIGIDDTTHYADSVRG (SEQ ID NO:85)	NSGIYSF (SEQ ID NO:86)
6265	RASQDIGSSLA (SEQ ID NO:87)	ATSTLQS (SEQ ID NO:88)	QQLNNYVHS (SEQ ID NO:89)	DYAMG (SEQ ID NO:90)	VVTGHSYRTHYADSVKG (SEQ ID NO:91)	RIWSYGDDSFDV (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)	YISSIETYYADSVKG (SEQ ID NO:97)	DRLVDVPLSSPNS (SEQ ID NO:98)
4497	KSSQSIFRTSRNKN LLN (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	RASQFTNHYLN (SEQ ID NO:105)	VASNLQS (SEQ ID NO:106)	QQSYRTPYT (SEQ ID NO:107)	SGYYN (SEQ ID NO:108)	YILSGAHTDIKASLKGS (SEQ ID NO:109)	SGVYSKYSLDV (SEQ ID NO:110)

6263 具有与 6078 相同的 CDR 序列

图12

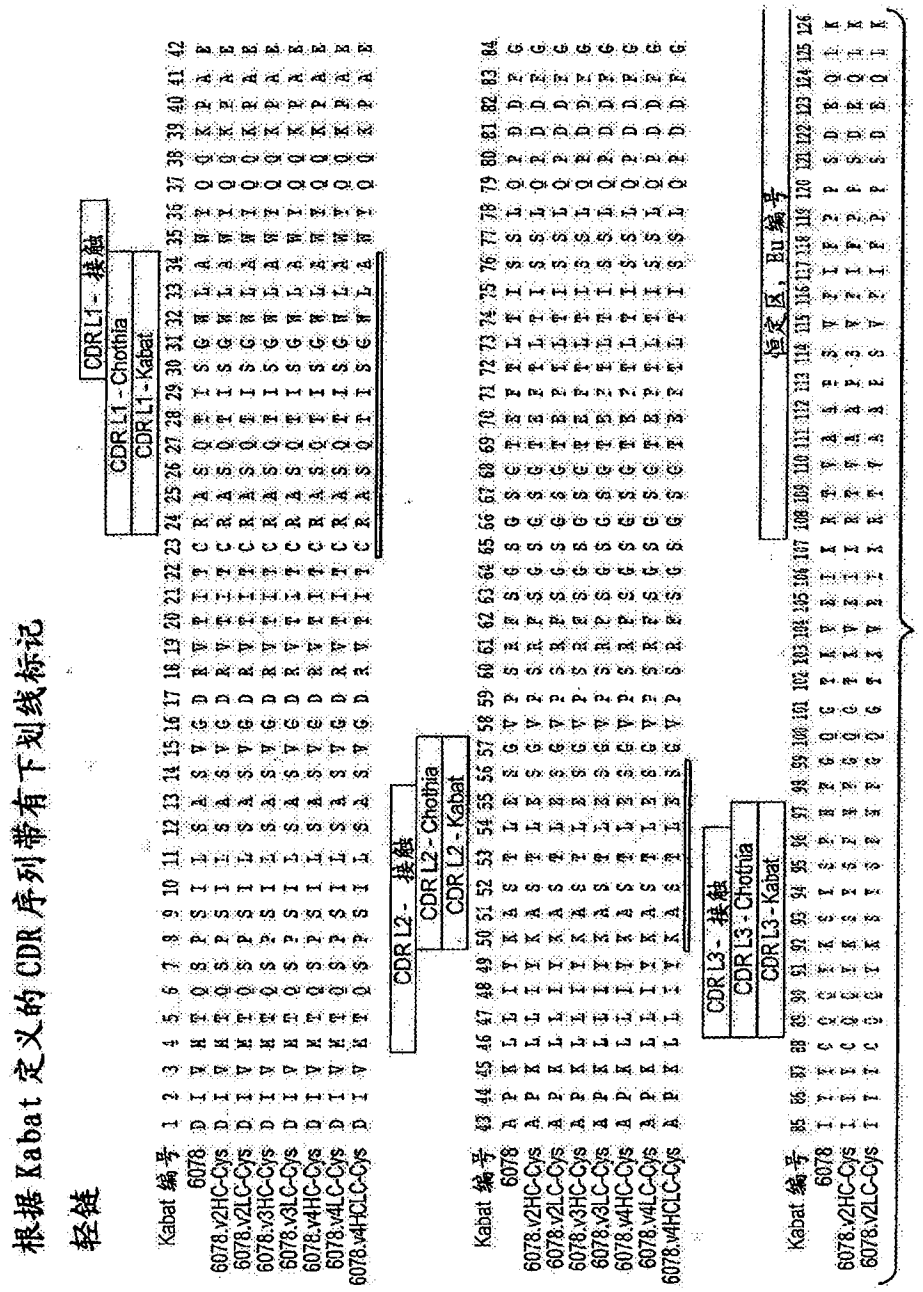

根据 Kabat 定义的 CDR 序列带有下划线标记
轻链
CDR L1 - 接触
CDR L1 - Chothia
CDR L1 - Kabat
CDR L2 - 接触
CDR L2 - Chothia
CDR L2 - Kabat
CDR L3 - 接触
CDR L3 - Chothia
CDR L3 - Kabat
恒定区，Bu 编号
Kabat 编号
6078
6078.v2HC-Cys
6078.v2LC-Cys
6078.v3HC-Cys
6078.v3LC-Cys
6078.v4HC-Cys
6078.v4LC-Cys
6078.v4HCLC-Cys

图13A-1

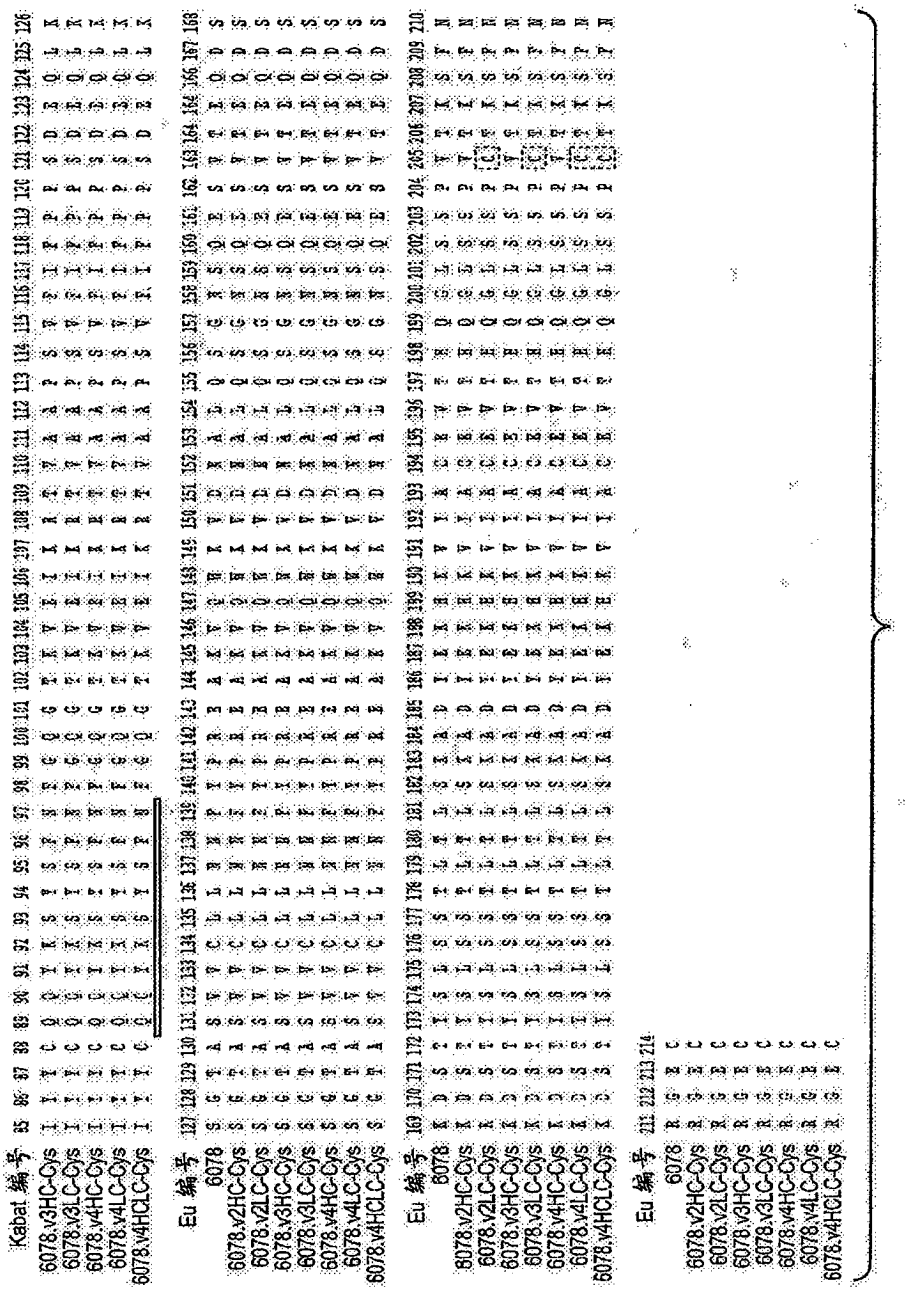


图13A-2

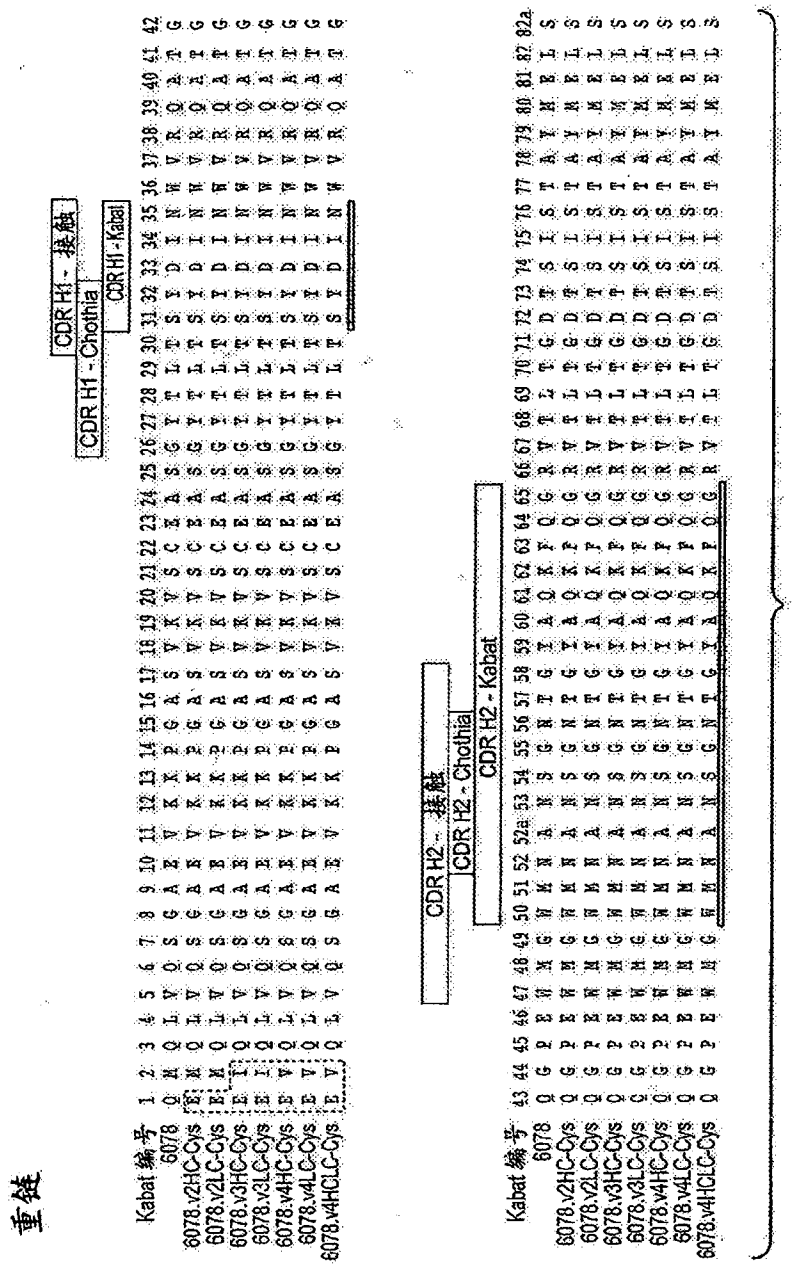

重链
CDR H1 - 接触
CDR H1 - Chothia
CDR H1 - Kabat
CDR H2 - 接触
CDR H2 - Chothia
CDR H2 - Kabat
Kabat 编号
6078
6078.v2HC-Cys
6078.v2LC-Cys
6078.v3HC-Cys
6078.v3LC-Cys
6078.v4HC-Cys
6078.v4LC-Cys
6078.v4HCLC-Cys

图13B-1

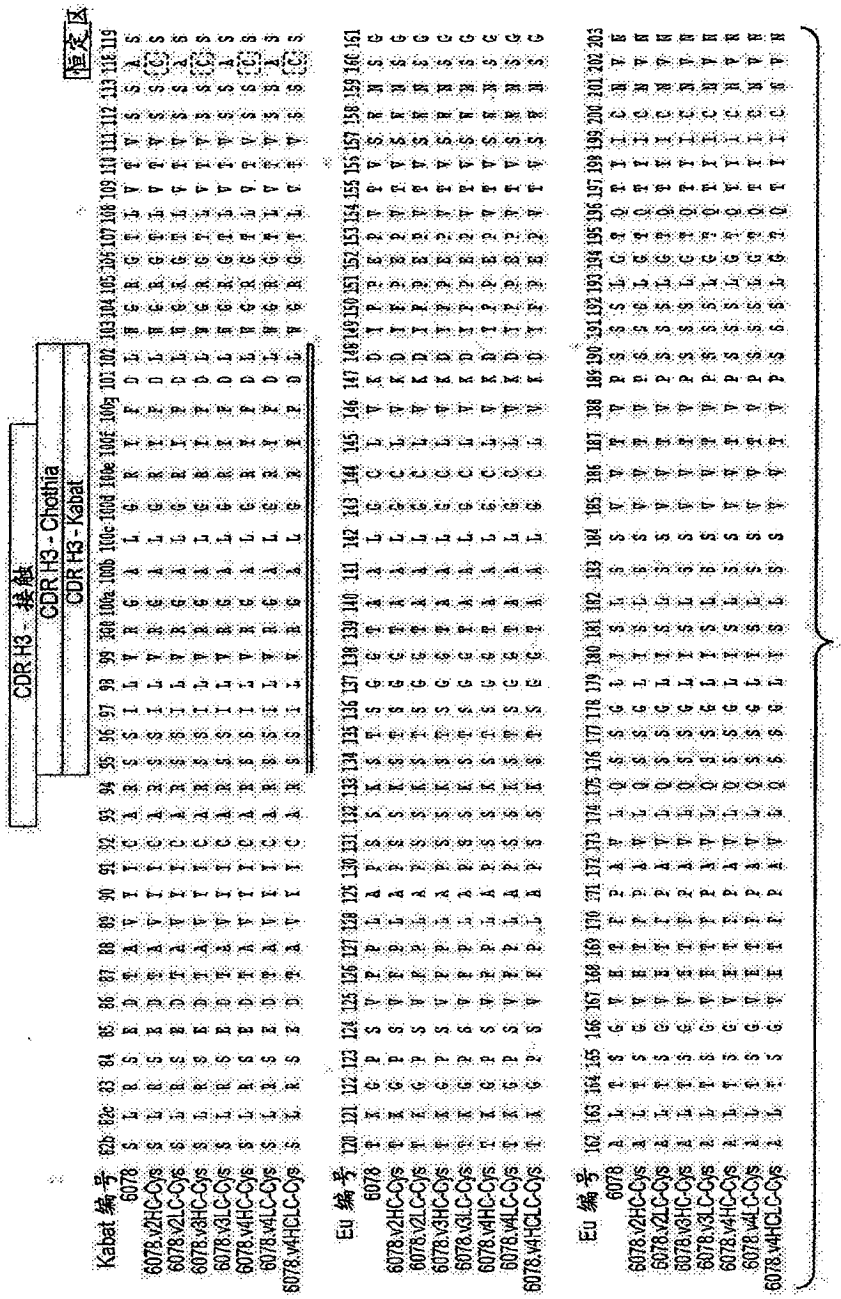

CDR H3 - 接触
CDR H3 - Chothia
CDR H3 - Kabat
恒定区
Kabat 编号
6078
6078.v2HC-Cys
6078.v2LC-Cys
6078.v3HC-Cys
6078.v3LC-Cys
6078.v4HC-Cys
6078.v4LC-Cys
6078.v4HCLC-Cys
Eu 编号
6078
6078.v2HC-Cys
6078.v2LC-Cys
6078.v3HC-Cys
6078.v3LC-Cys
6078.v4HC-Cys
6078.v4LC-Cys
6078.v4HCLC-Cys
Eu 编号
6078
6078.v2HC-Cys
6078.v2LC-Cys
6078.v3HC-Cys
6078.v3LC-Cys
6078.v4HC-Cys
6078.v4LC-Cys
6078.v4HCLC-Cys

图13B-2

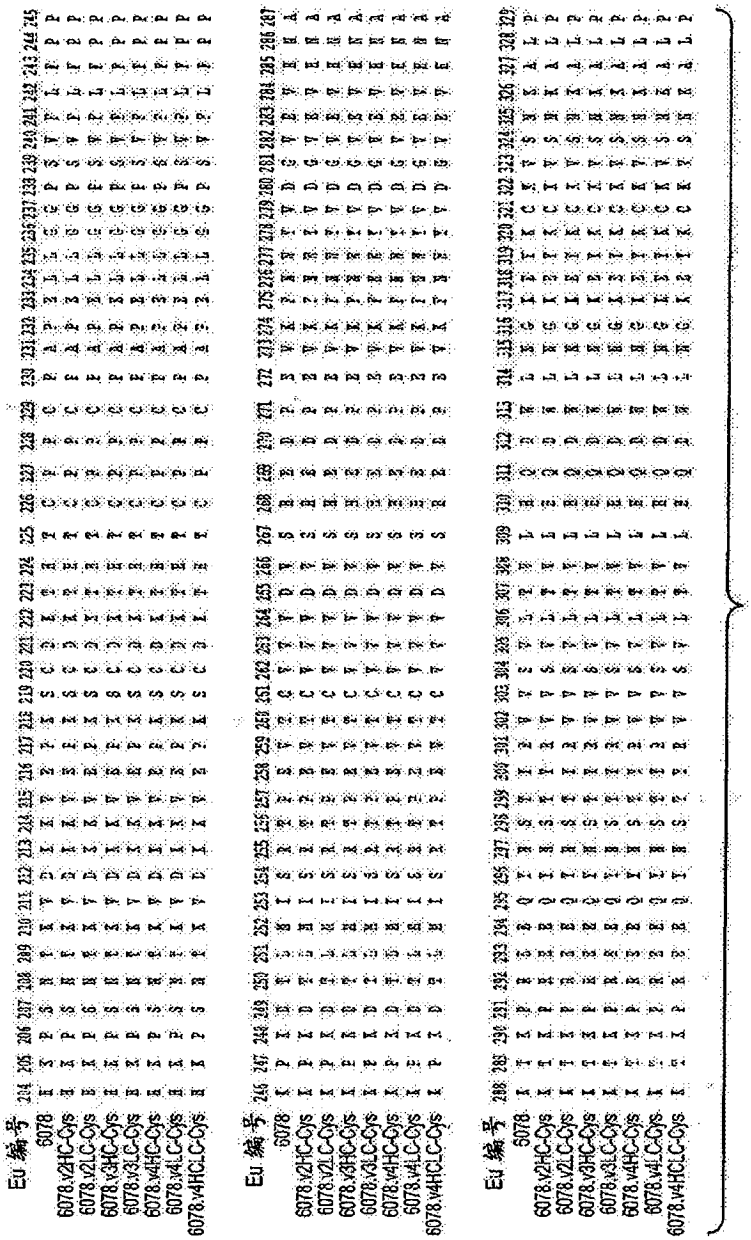


图13B-3

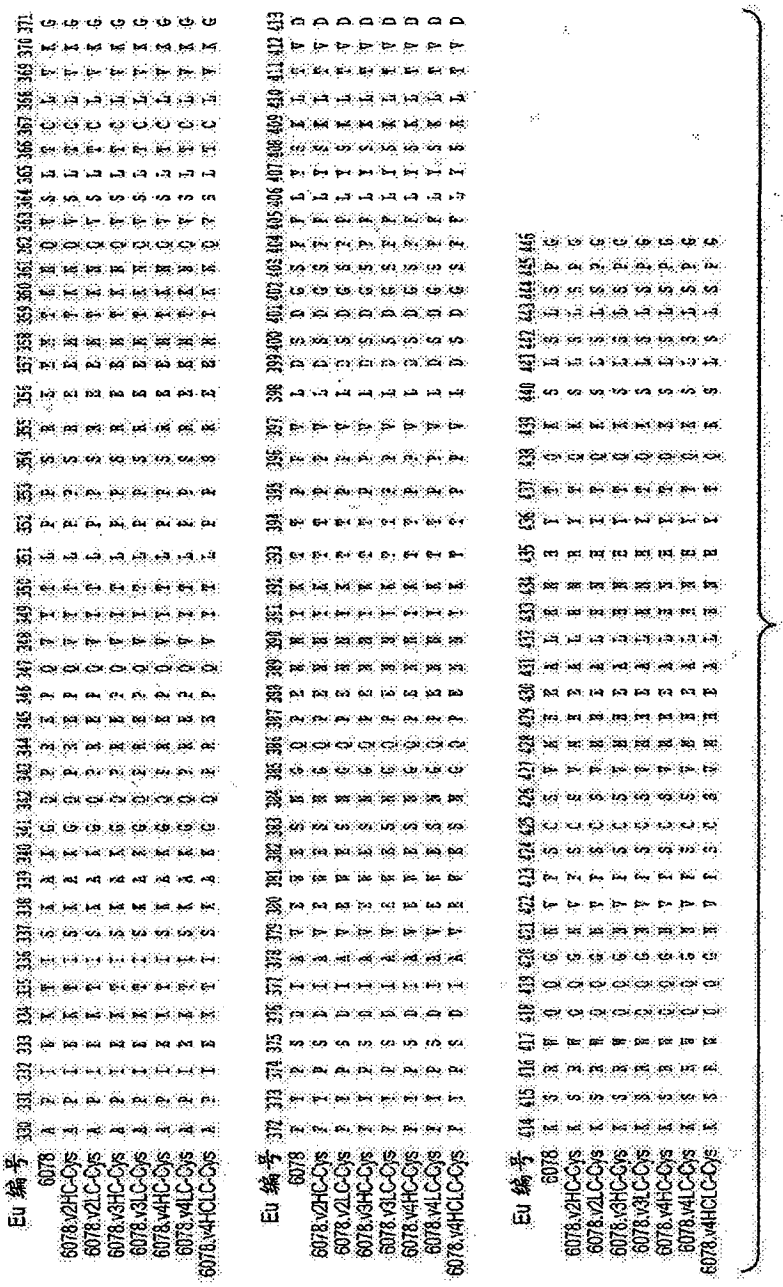

Eu 编号
6078
6078.v2HC-Cys
6078.v2LC-Cys
6078.v3HC-Cys
6078.v3LC-Cys
6078.v4HC-Cys
6078.v4LC-Cys
6078.v4HCLC-Cys

图13B-4

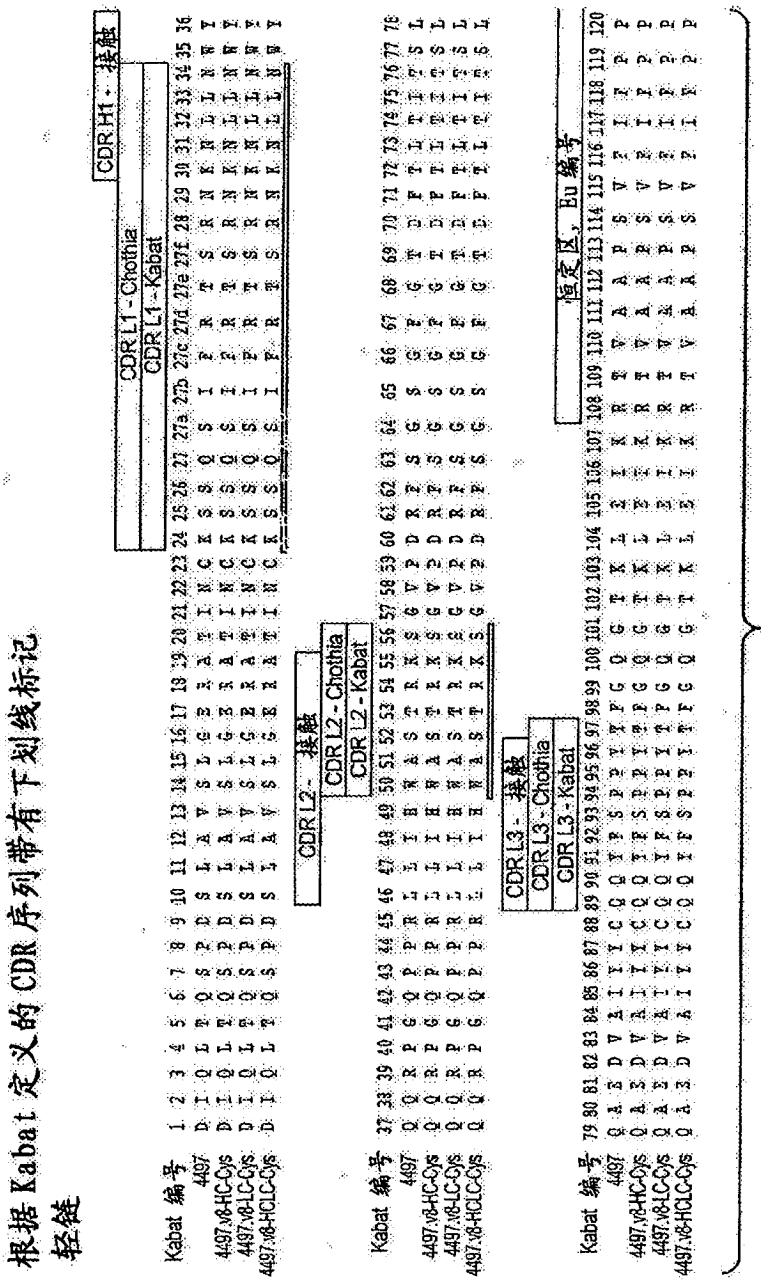

根据 Kabat 定义的 CDR 序列带有下划线标记
轻链
CDR L1 - Chothia
CDR L1 - Kabat
CDR H1 - 接触
CDR L2 - 接触
CDR L2 - Chothia
CDR L2 - Kabat
CDR L3 - 接触
CDR L3 - Chothia
CDR L3 - Kabat
恒定区，Eu 编号
Kabat 编号
4497
4497.v8-HC-Cys
4497.v8-LC-Cys
4497.v8-HCLC-Cys

图14A-1

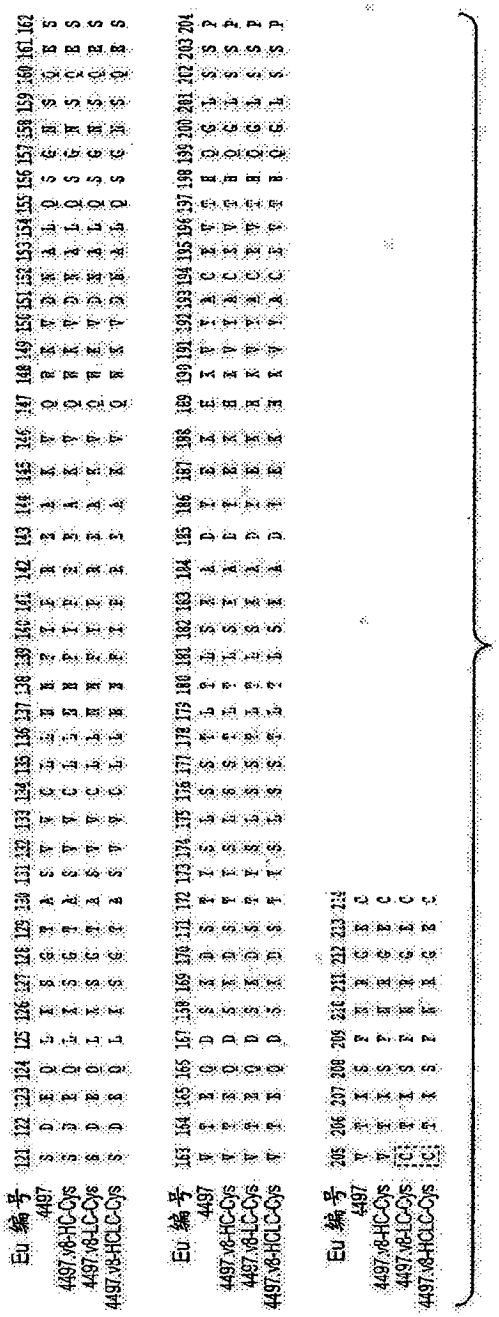


图14A-2

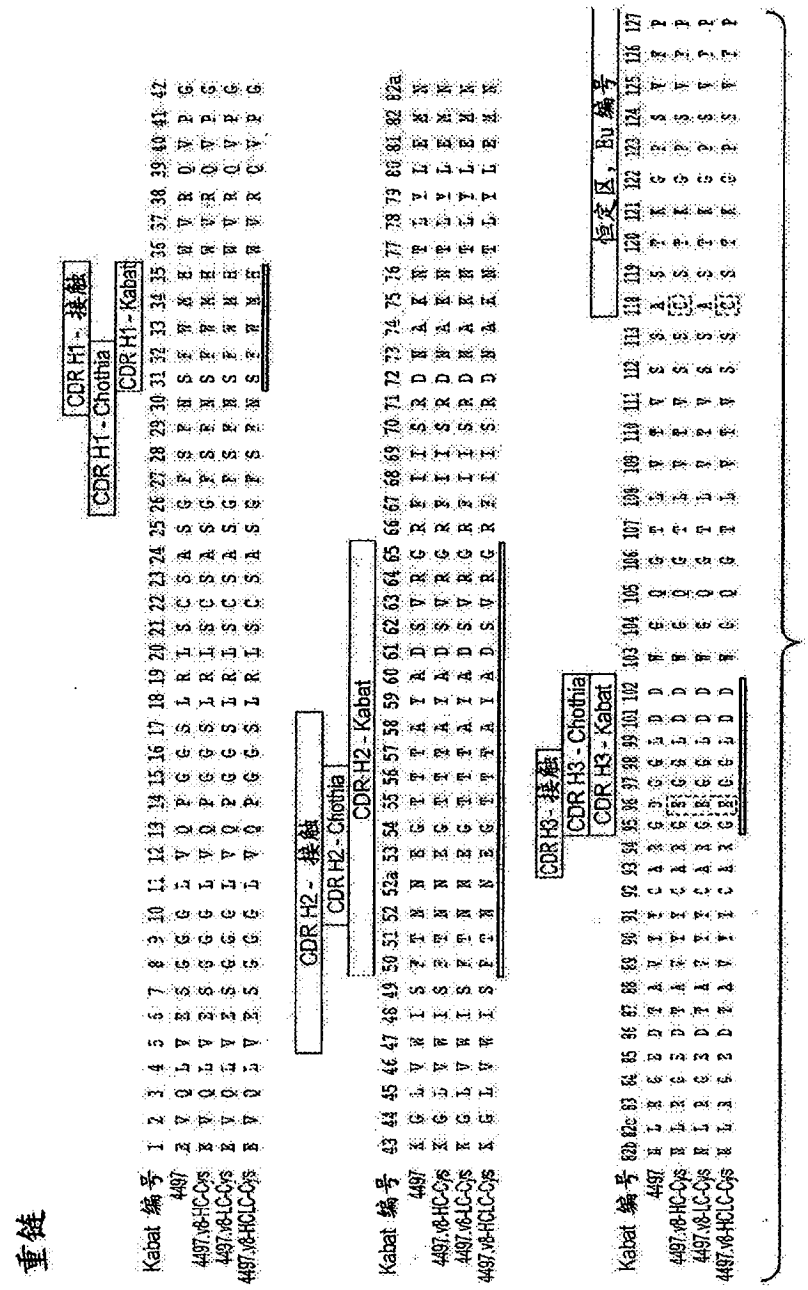

重链
CDR H1 - 接触
CDR H1 - Chothia
CDR H1 - Kabat
CDR H2 - 接触
CDR H2 - Chothia
CDR H2 - Kabat
CDR H3 - 接触
CDR H3 - Chothia
CDR H3 - Kabat
恒定区，Eu 编号
Kabat 编号
4497
4497.γ6-HC-Cys
4497.γ6-LC-Cys
4497.γ6-HCLC-Cys

图14B-1

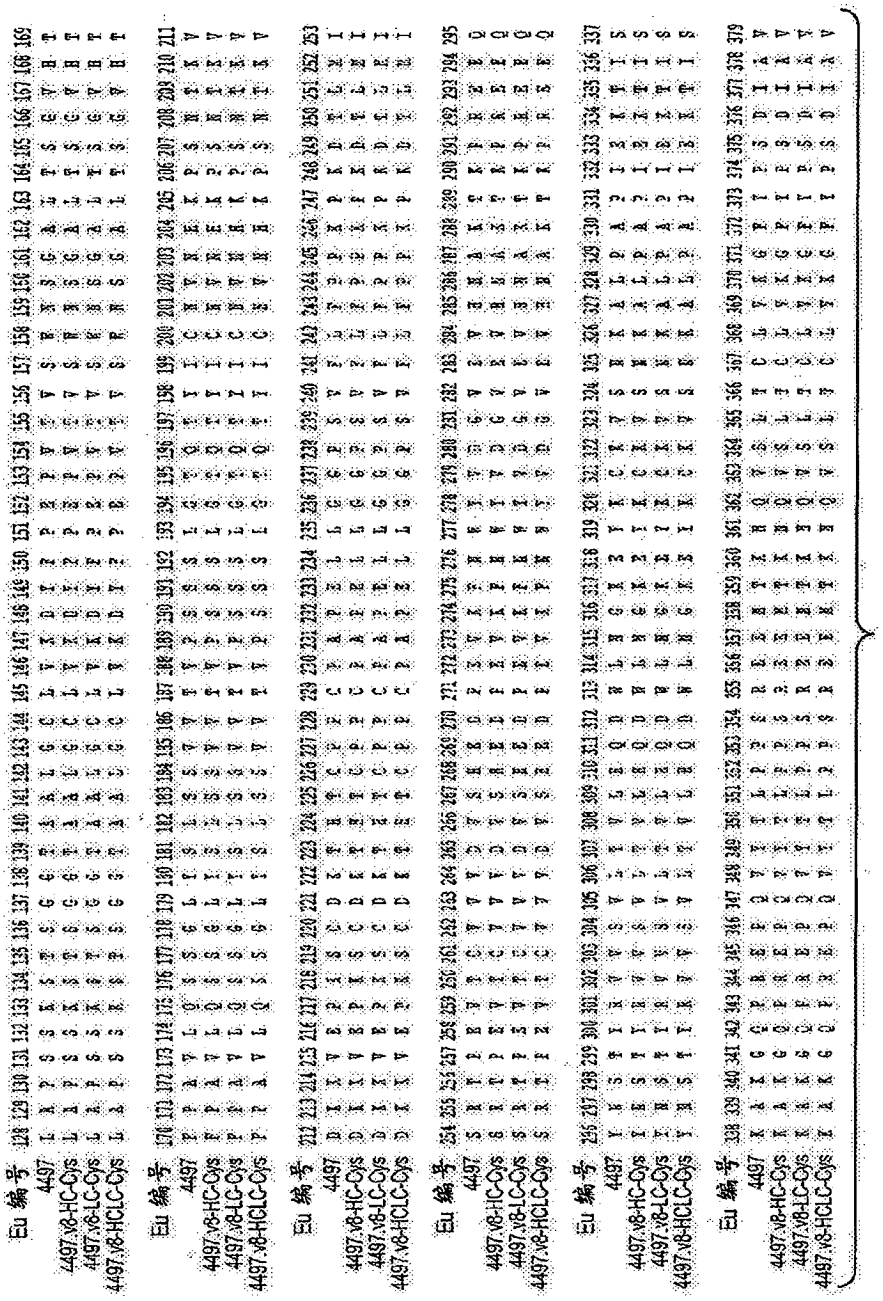


图14B-2

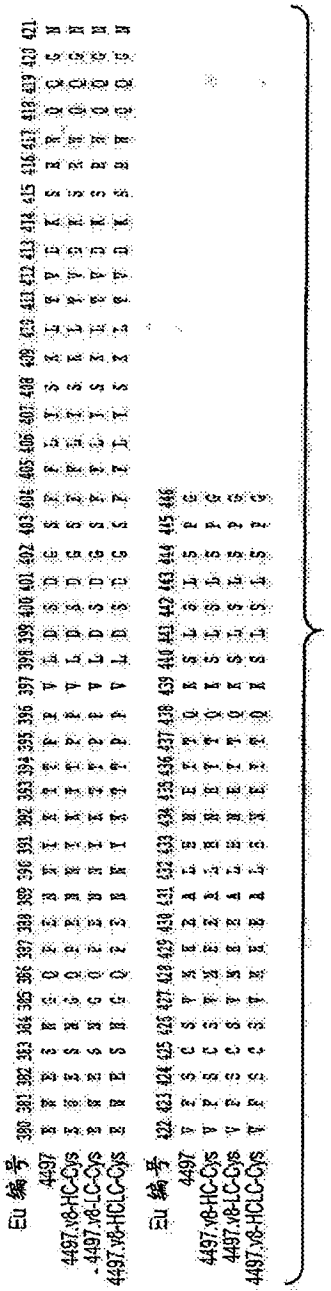


图14B-3

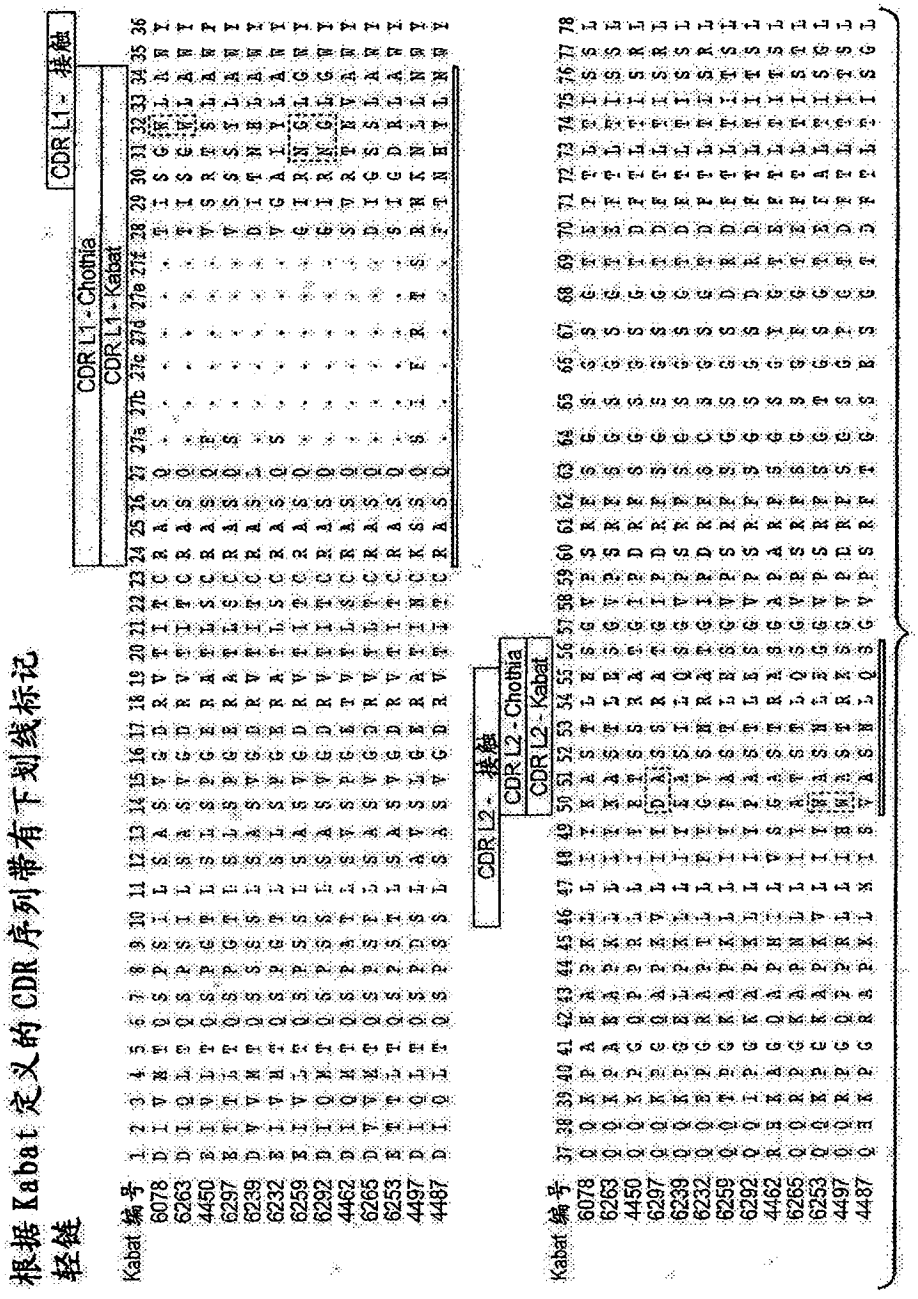

根据 Kabat 定义的 CDR 序列带有下划线标记
轻链
CDR L1 - 接触
CDR L1 - Chothia
CDR L1 - Kabat
CDR L2 - 接触
CDR L2 - Chothia
CDR L2 - Kabat
Kabat 编号
6078
6263
4450
6297
6239
6232
6259
6292
4462
6265
6253
4497
4487

图15A-1

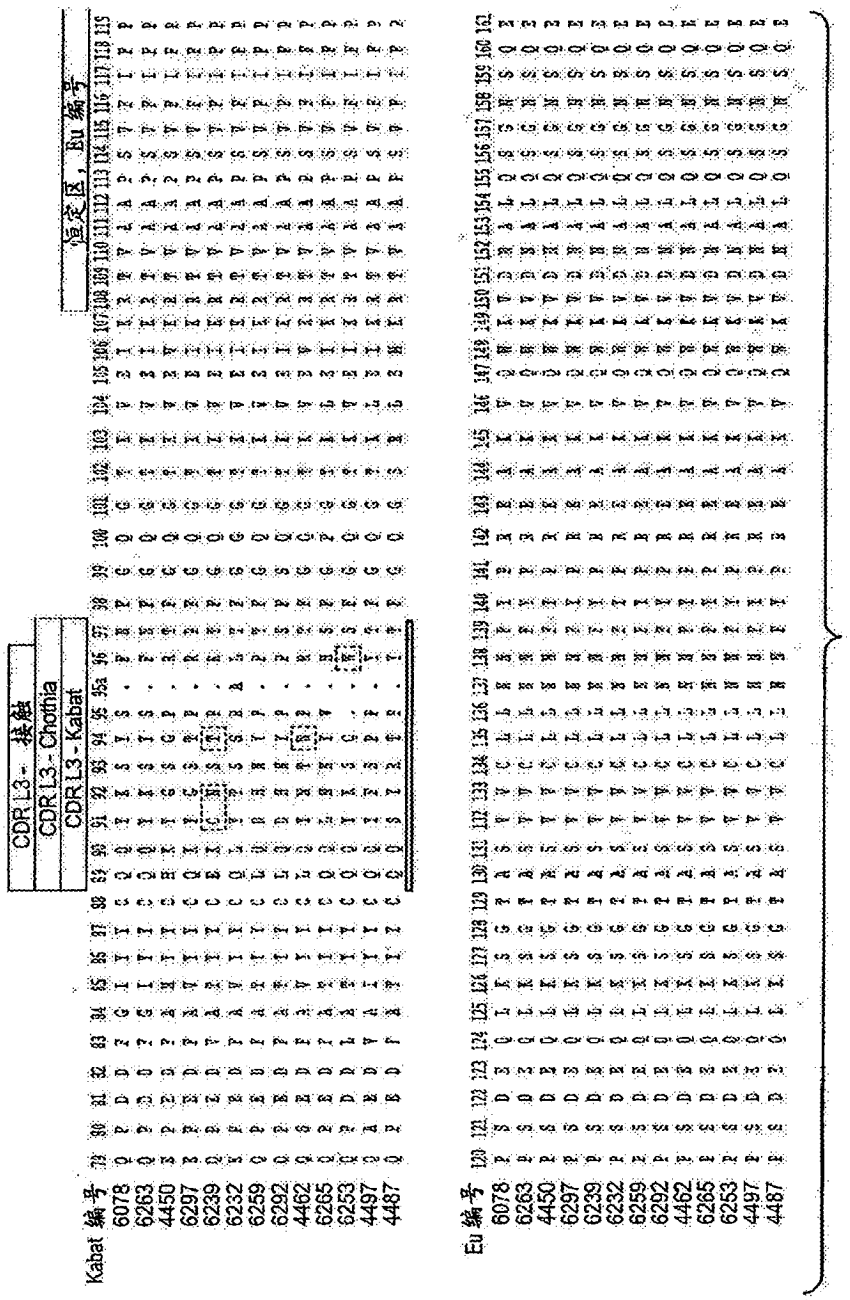

CDR L3 - 接触
CDR L3 - Chothia
CDR L3 - Kabat
恒定区，Eu 编号
Kabat 编号
6078
6263
4450
6297
6239
6232
6259
6292
4462
6265
6253
4497
4487
Eu 编号
6078
6263
4450
6297
6239
6232
6259
6292
4462
6265
6253
4497
4487

图15A-2

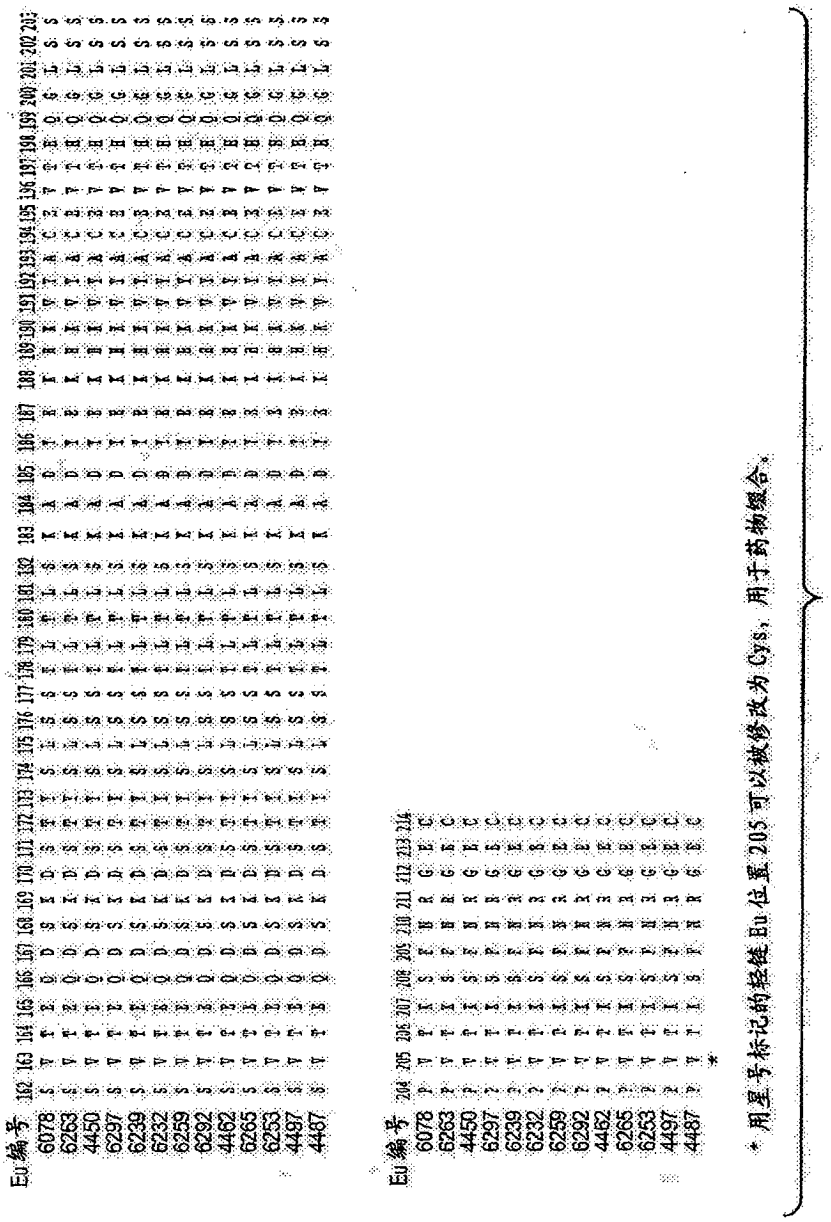

Eu编号
6078
6263
4450
6297
6239
6232
6259
6292
4462
6265
6253
4497
4487
* 用星号标记的经缝 Eu 位置 205 可以被修改为 Cys，用于药物缀合。

图15A-3

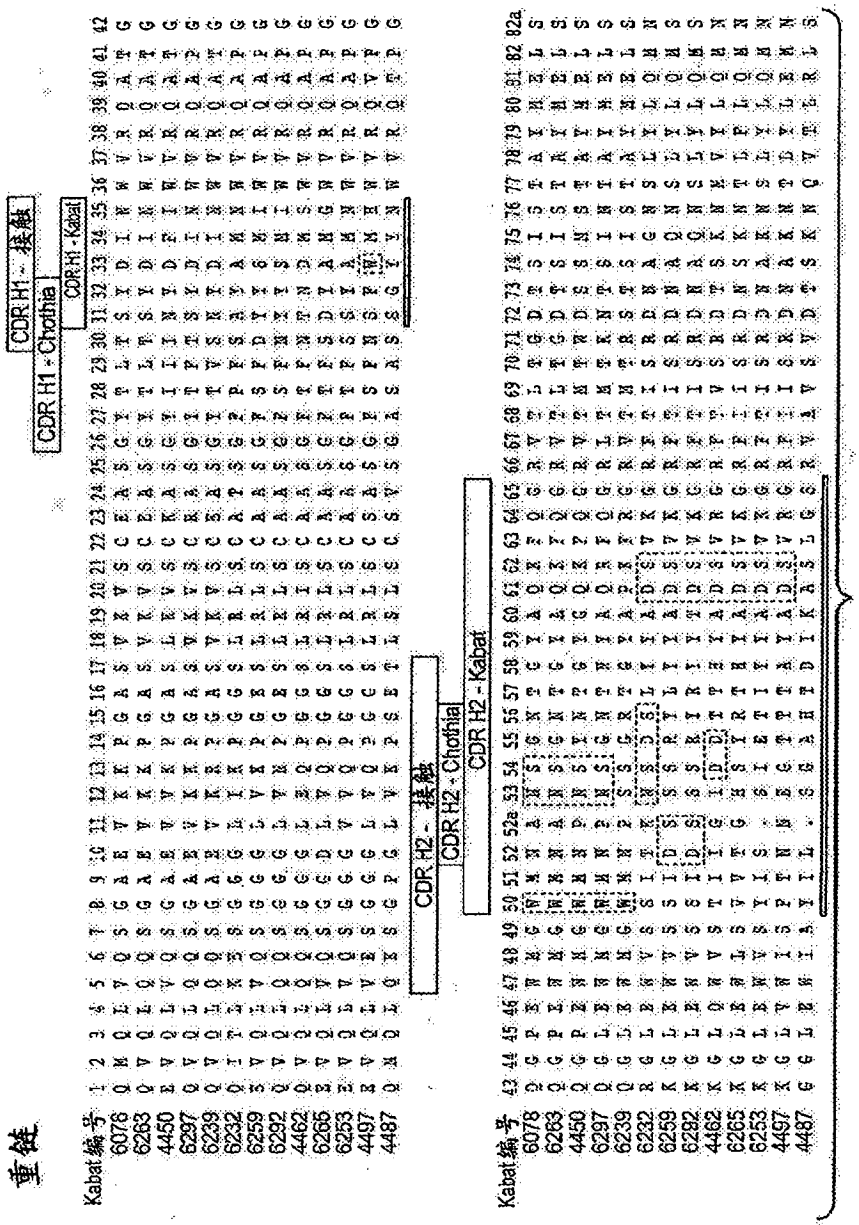

重链
Kabat编号
CDR H1 - 接触
CDR H1 - Chothia
CDR H1 - Kabat
CDR H2 - 接触
CDR H2 - Chothia
CDR H2 - Kabat
6078
6263
4450
6297
6239
6232
6259
6292
4462
6265
6253
4497
4487

图15B-1

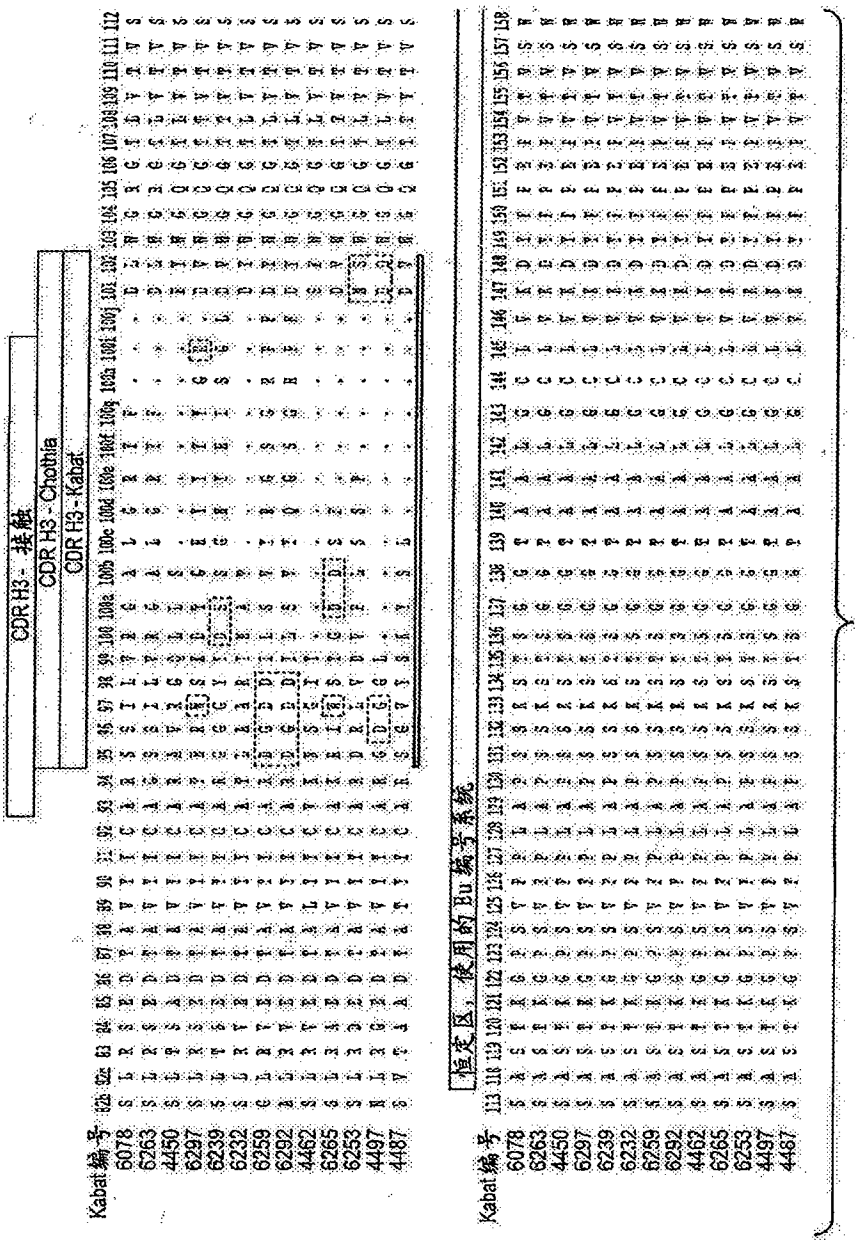

CDR H3 - 接触
CDR H3 - Chothia
CDR H3 - Kabat
恒定区，使用的Eu编号系统
Kabat编号
6078
6263
4450
6297
6239
6232
6259
6292
4462
6265
6253
4497
4487

图15B-2

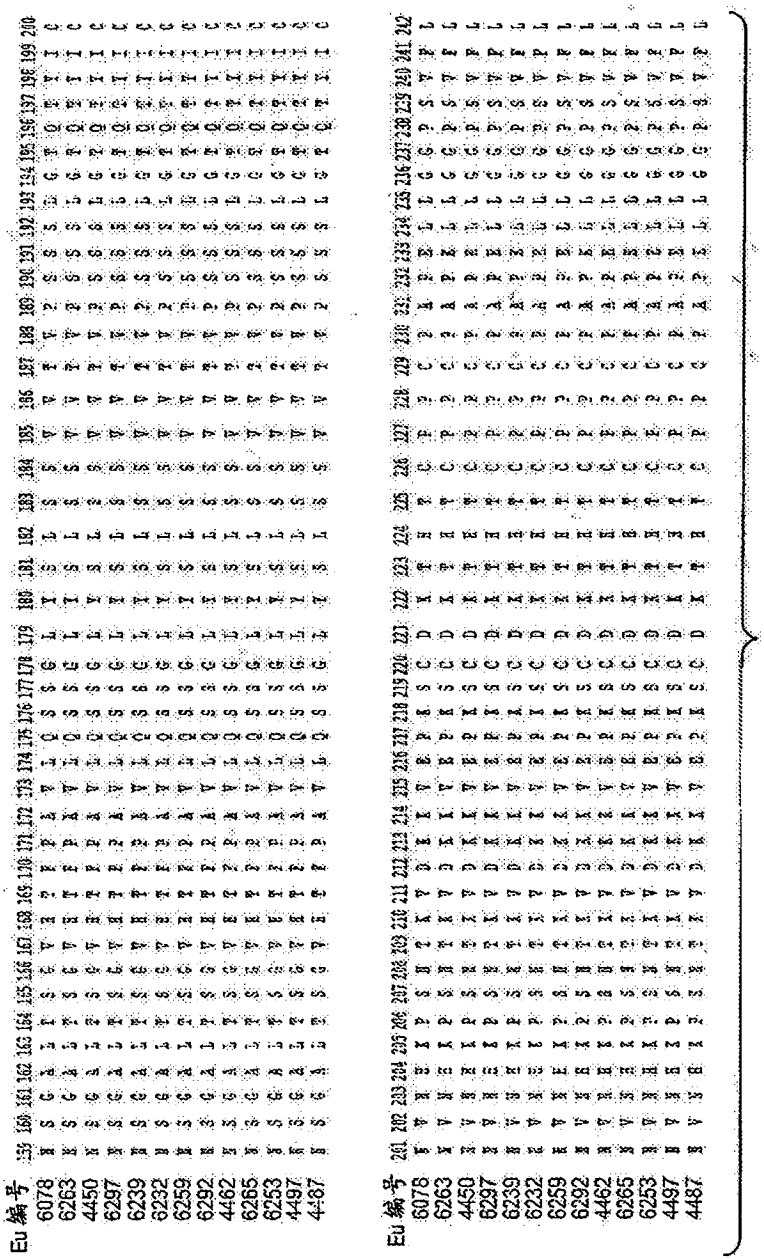

Eu 编号
6078
6263
4450
6297
6239
6232
6259
6292
4462
6265
6253
4497
4487

图15B-3

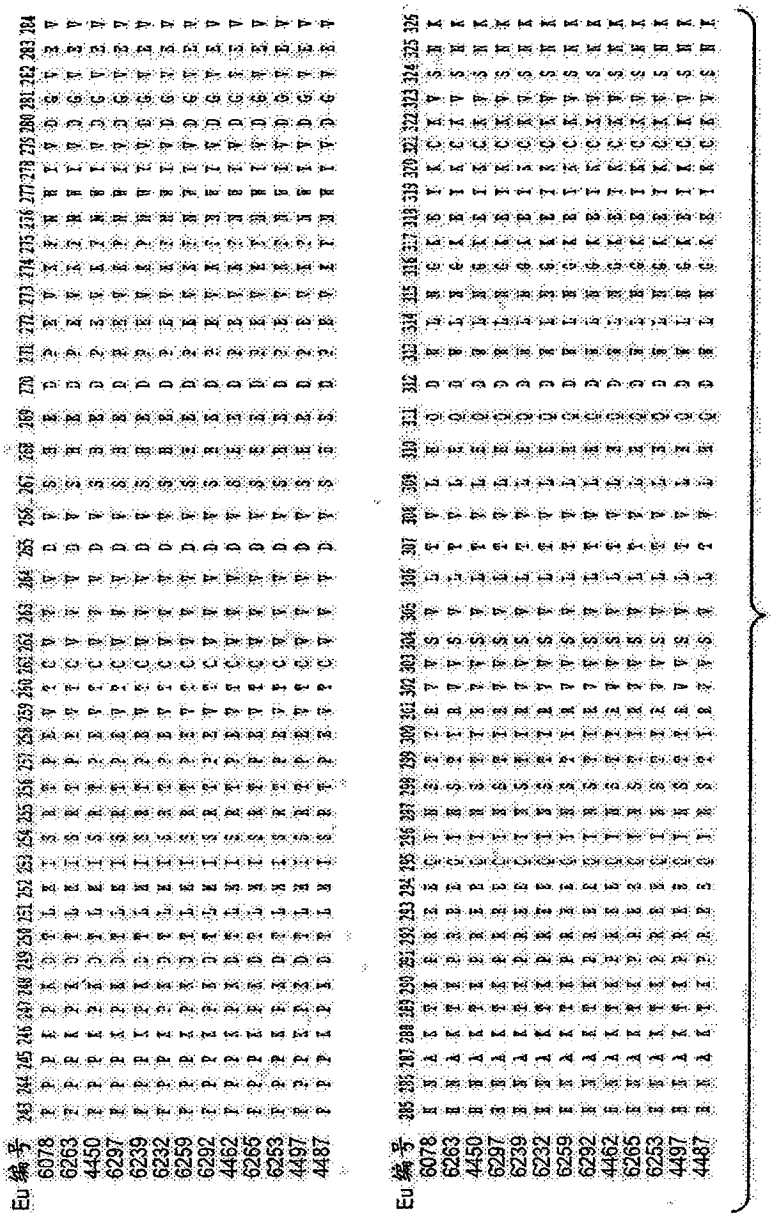

Eu 编号
6078
6263
4450
6297
6239
6232
6259
6292
4462
6265
6253
4497
4487

图15B-4

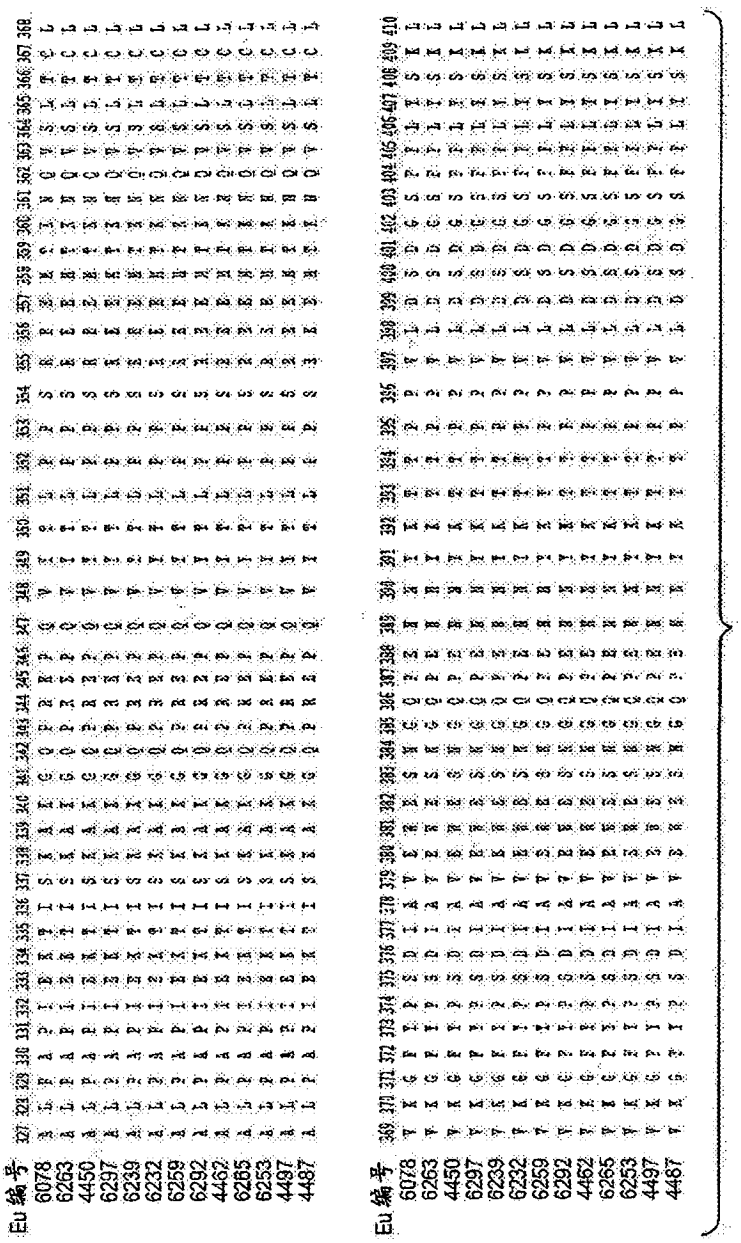


图15B-5

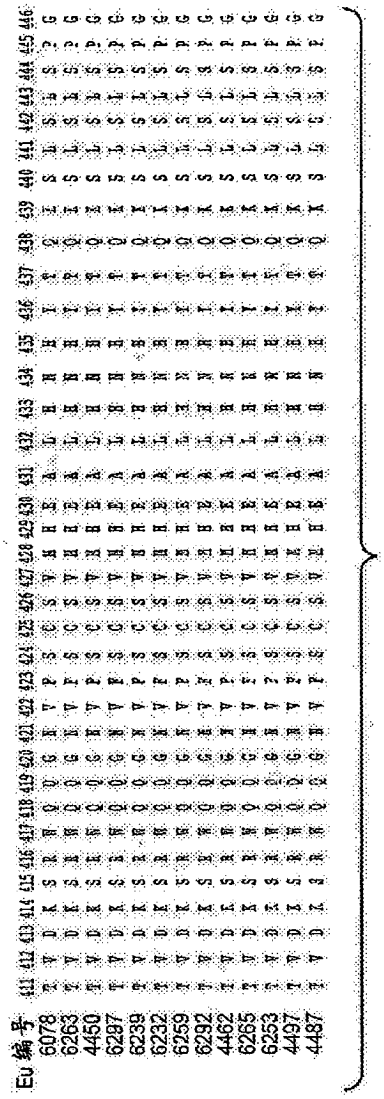


图15B-6

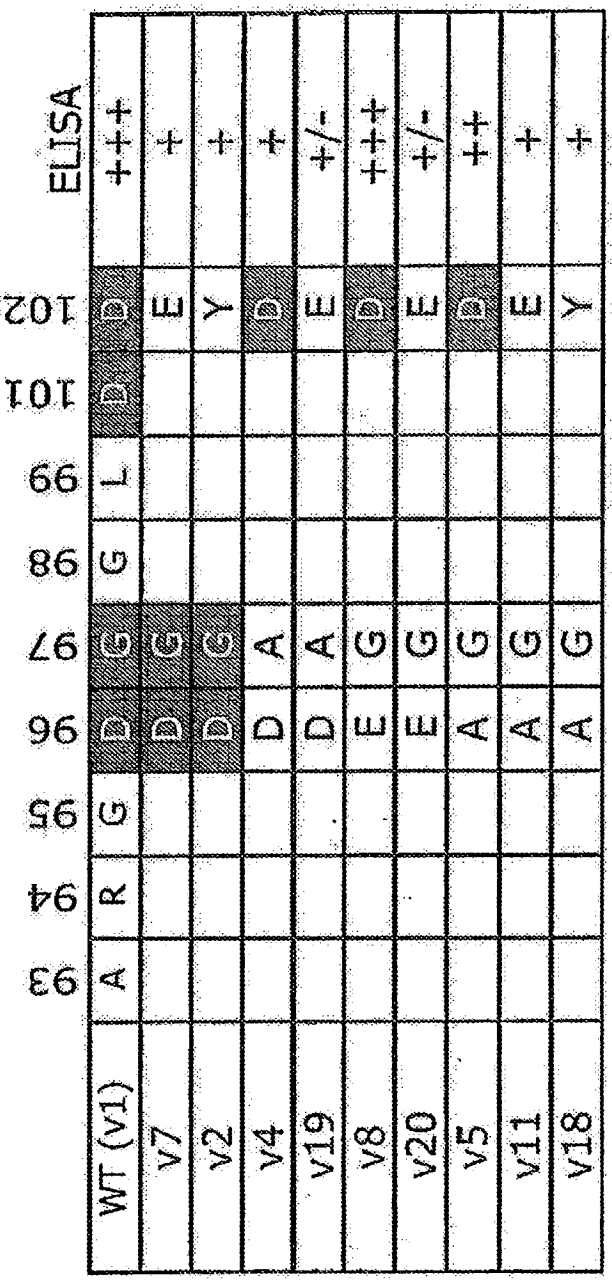

ELISA
93 94 95 96 97 98 99 101 102
WT (v1) A R G D D G L D D
v7 G D E
v2 G D Y
v4 A D D
v19 A D E
v8 G E G
v20 G E E
v5 G A D
v11 G A E
v18 G A Y
+++
+
+
+
+/-
+++
+/-
++
+
+

图16

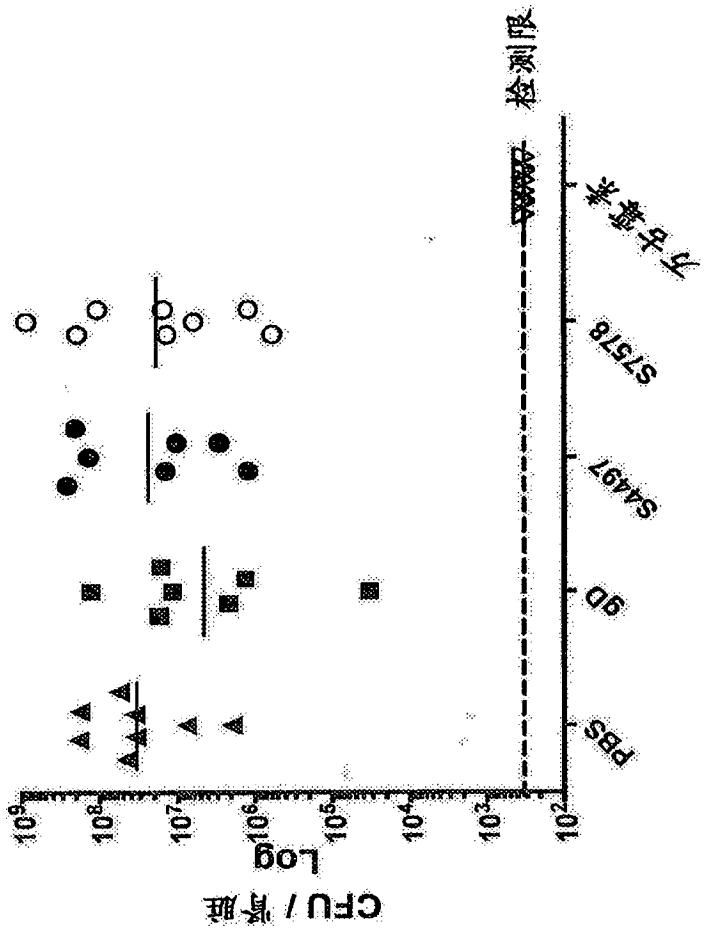

检测限
万古霉素
ST5T8
SA491
QD
PBS
Log
CFU / 肾脏
10^9
10^8
10^7
10^6
10^5
10^4
10^3
10^2

图17

Abstract

The invention provides anti-Staphylococcus aureus antibody rifamycin antibiotic conjugates and methods of using same.