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(54) **RECOMBINANT NARBONOLIDE POLYKETIDE SYNTHASE**

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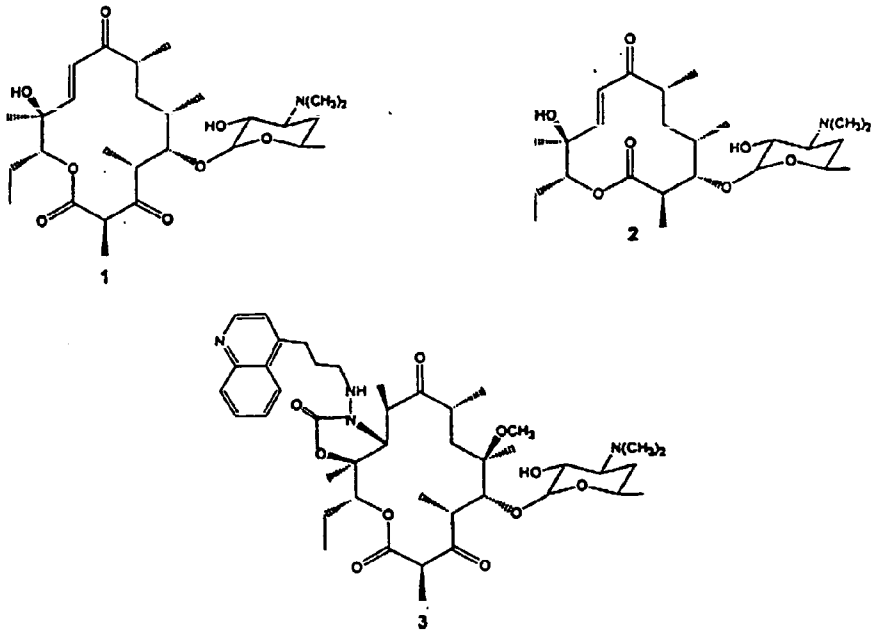
(57) **ABSTRACT**

Recombinant DNA compounds that encode all or a portion of the narbonolide polyketide synthase are used to express recombinant polyketide synthase genes in host cells for the production of narbonolide, narbonolide derivatives, and polyketides that are useful as antibiotics and as intermediates in the synthesis of compounds with pharmaceutical value.


1
2
3

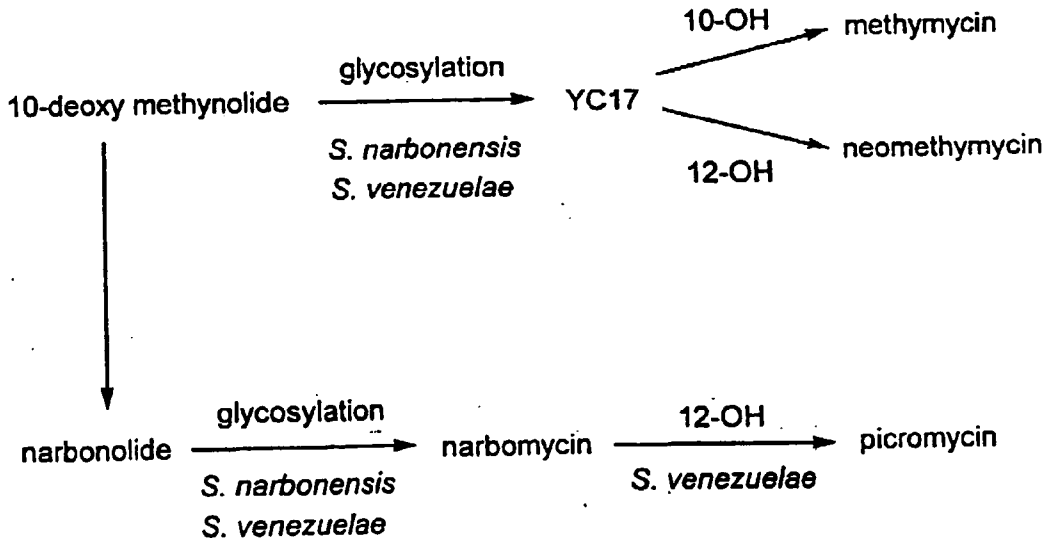

10-deoxy methynolide
glycosylation
S. narbonensis
S. venezuelae
YC17
10-OH
methymycin
12-OH
neomethymycin
narbonolide
glycosylation
S. narbonensis
S. venezuelae
narbomycin
12-OH
S. venezuelae
picromycin

Figure 1

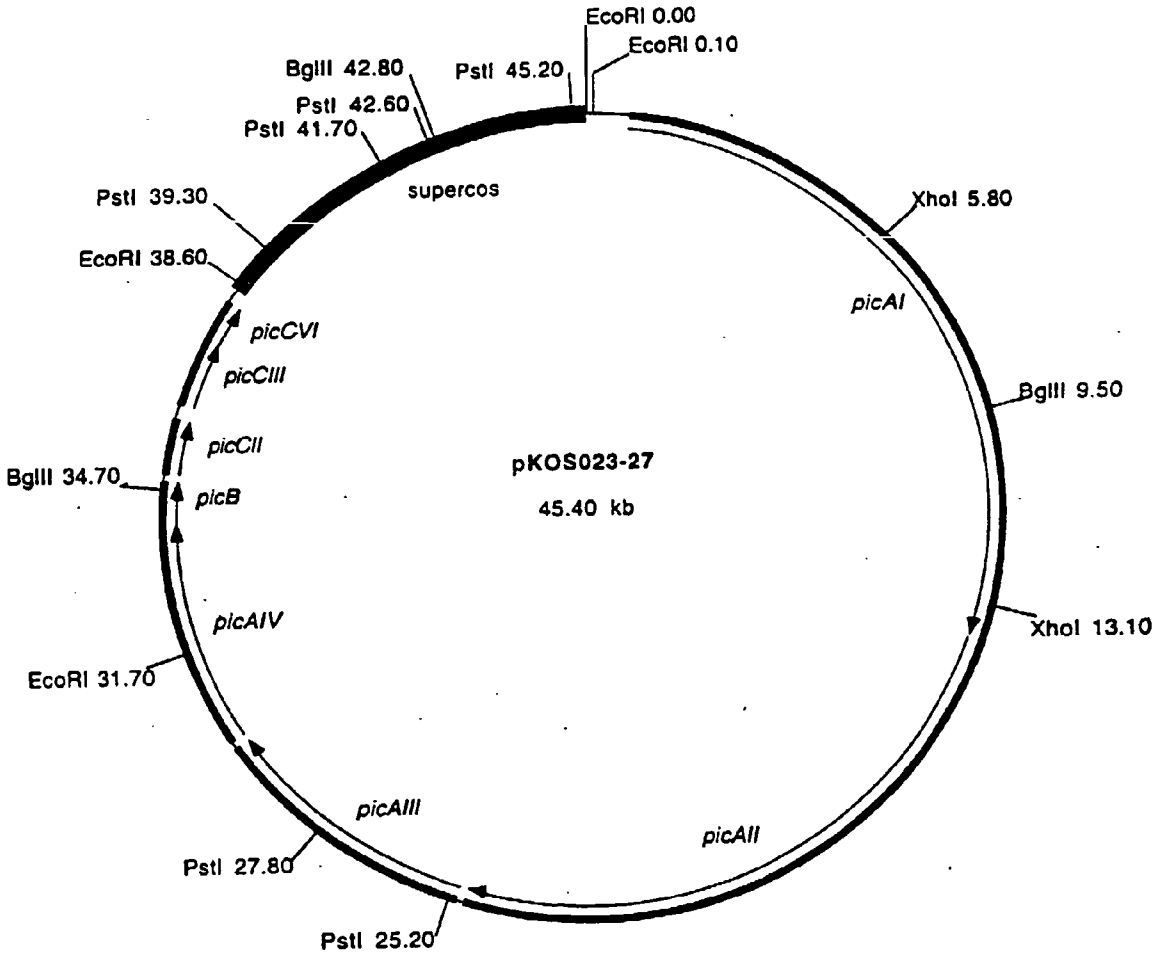

EcoRI 0.00
EcoRI 0.10
PstI 45.20
BglII 42.80
PstI 42.60
PstI 41.70
PstI 39.30
EcoRI 38.60
supercos
XhoI 5.80
picAI
BglII 9.50
picCVI
picCIII
picCII
BglII 34.70
picB
pKOS023-27
45.40 kb
picAIV
XhoI 13.10
EcoRI 31.70
picAIII
picAII
PstI 27.80
PstI 25.20

Figure 2

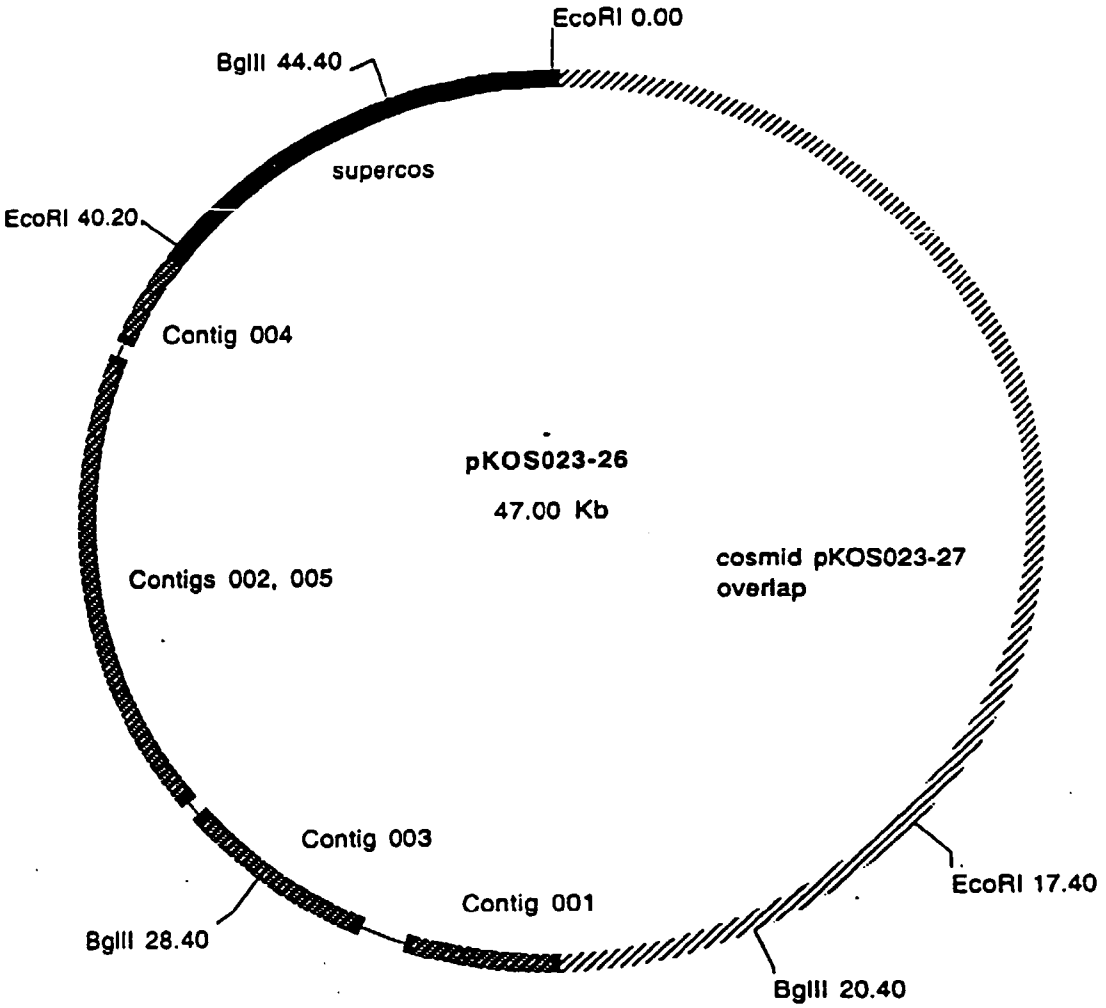

EcoRI 0.00
BglII 44.40
supercos
EcoRI 40.20
Contig 004
pKOS023-26
47.00 Kb
Contigs 002, 005
cosmid pKOS023-27
overlap
Contig 003
Contig 001
EcoRI 17.40
BglII 28.40
BglII 20.40

Figure 3

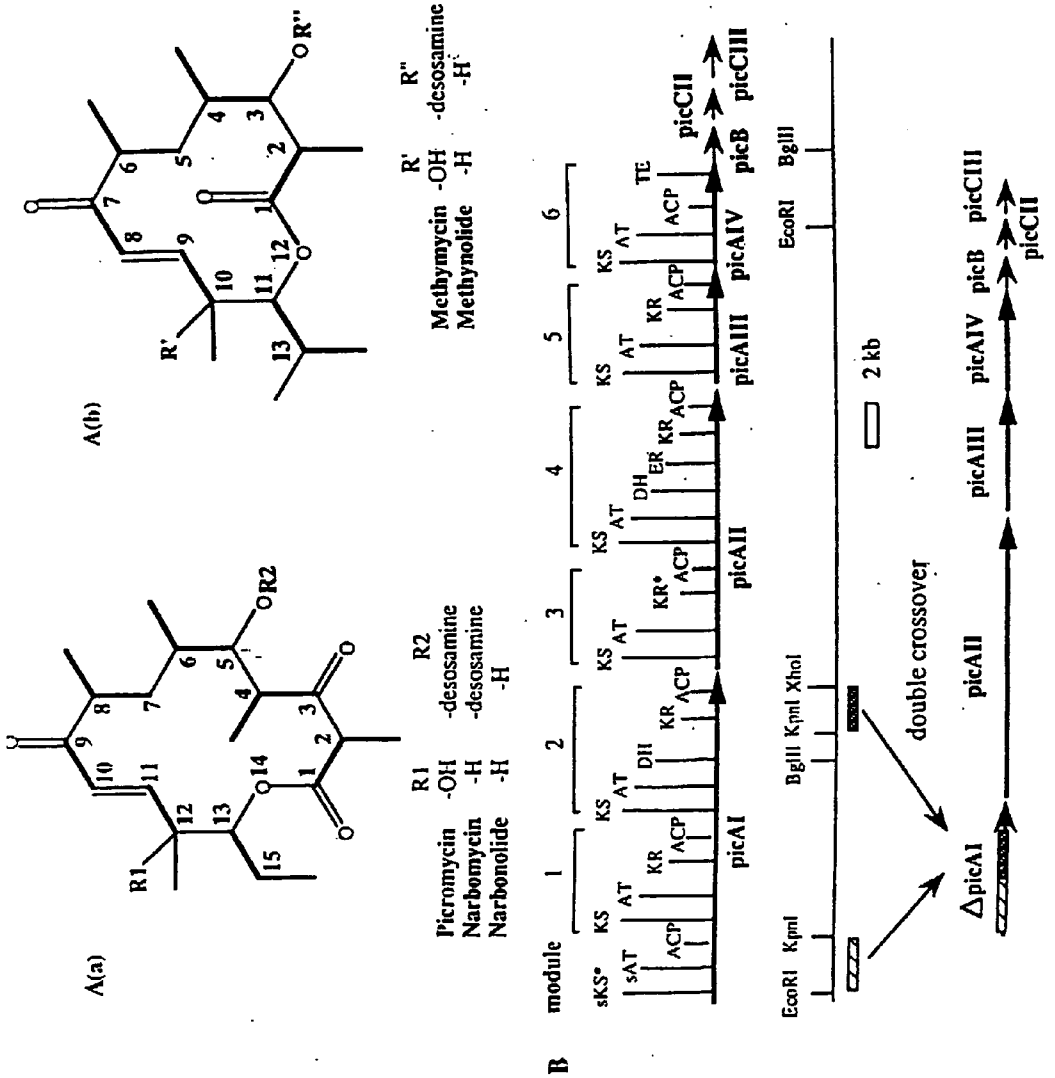

A(a)
A(b)
R1 R2
Picromycin -OH -desosamine
Narbomycin -H -desosamine
Narbonolide -H -H
R' R"
Methymycin -OH -desosamine
Methynolide -H -H
B
module
sKS* sAT ACP
picAI
picAII
picAIII
picAIV
picB
picCII
picCIII
S. venezuelae ATCC15439
pKOS023-27
S. venezuelae K039-03
EcoRI KpnI
BglII KpnI XhoI
EcoRI BglII
2 kb
double crossover
ΔpicAI
picAII
picAIII
picAIV
picB
picCIII
picCII

Figure 4

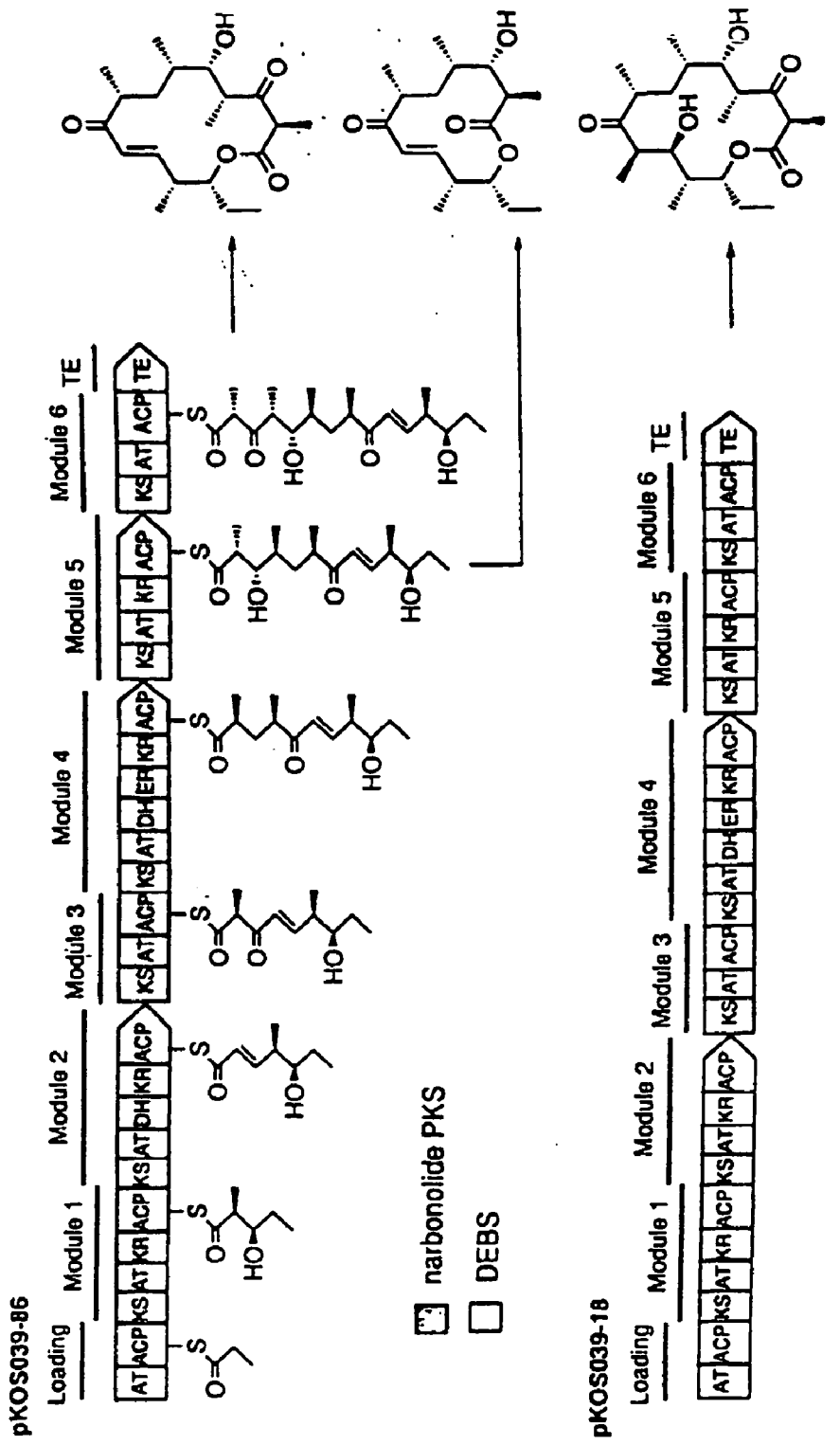
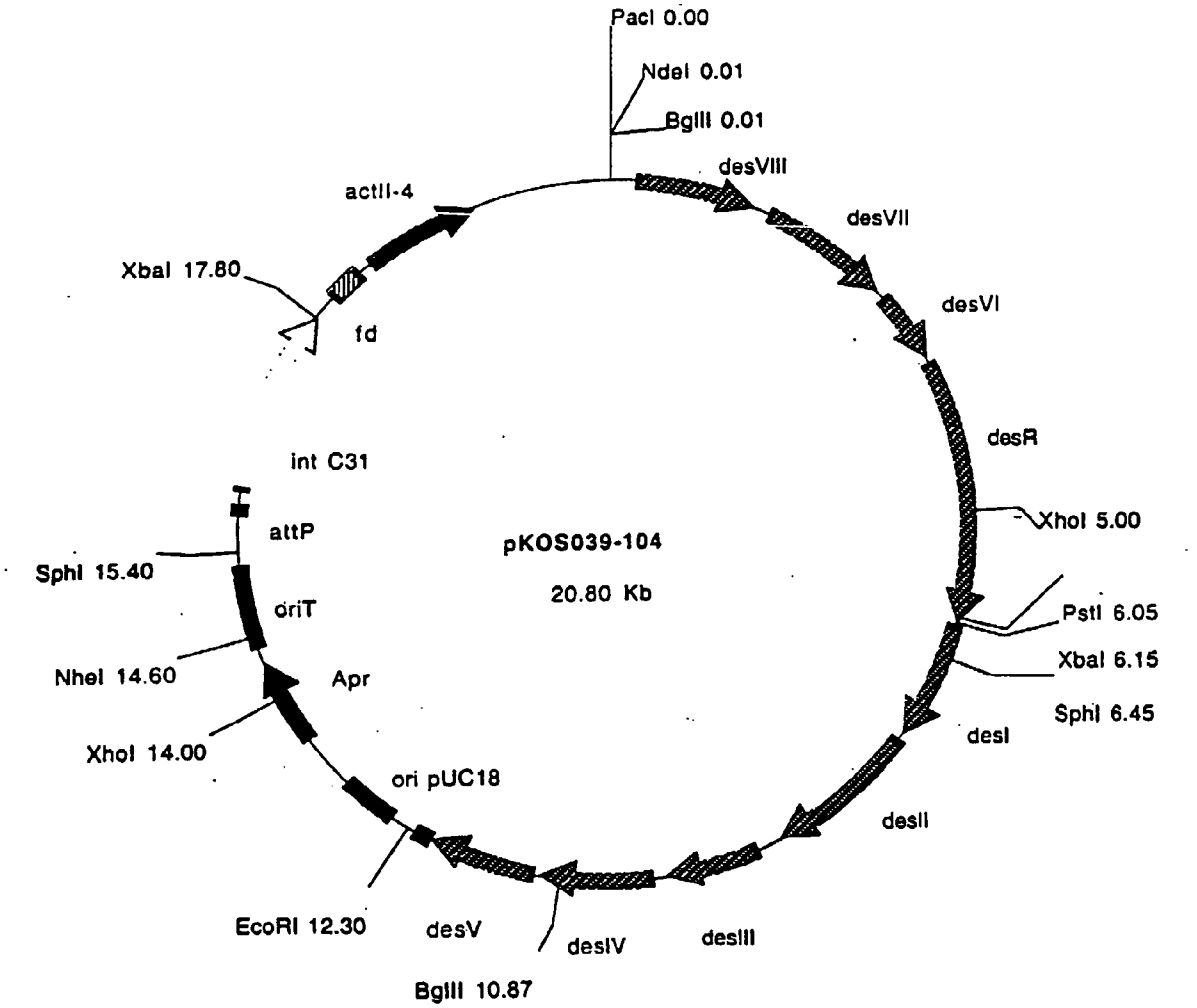

pKOS039-86
Loading
Module 1
Module 2
Module 3
Module 4
Module 5
Module 6
TE
narbonolide PKS
DEBS
pKOS039-18

Figure 5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NdeI 0.01
BglII 0.01
desVIII
desVII
desVI
desR
XhoI 5.00
PstI 6.05
XbaI 6.15
SphI 6.45
desI
desII
desIII
desIV
desV
BglII 10.87
EcoRI 12.30
ori pUC18
XhoI 14.00
NheI 14.60
Apr
oriT
SphI 15.40
attP
int C31
pKOS039-104
20.80 Kb
XbaI 17.80
fd
actII-4

Figure 6

RECOMBINANT NARBONOLIDE POLYKETIDE SYNTHASE

REFERENCE TO GOVERNMENT FUNDING

[0001] This invention was supported in part by SBIR grant 1R43-CA75792-01. The U.S. government has certain rights in this invention.

FIELD OF THE INVENTION

[0002] The present invention provides recombinant methods and materials for producing polyketides by recombinant DNA technology. The invention relates to the fields of agriculture, animal husbandry, chemistry, medicinal chemistry, medicine, molecular biology, pharmacology, and veterinary technology.

BACKGROUND OF THE INVENTION

[0003] Polyketides represent a large family of diverse compounds synthesized from 2-carbon units through a series of condensations and subsequent modifications. Polyketides occur in many types of organisms, including fungi and mycelial bacteria, in particular, the actinomycetes. There are a wide variety of polyketide structures, and the class of polyketides encompasses numerous compounds with diverse activities. Tetracycline, erythromycin, FK506, FK520, narbomycin, picromycin, rapamycin, spinocyn, and tylosin, are examples of such compounds. Given the difficulty in producing polyketide compounds by traditional chemical methodology, and the typically low production of polyketides in wild-type cells, there has been considerable interest in finding improved or alternate means to produce polyketide compounds. See PCT publication Nos. WO 93/13663; WO 95/08548; WO 96/40968; 97/02358; and 98/27203; U.S. Pat. Nos. 4,874,748; 5,063,155; 5,098,837; 5,149,639; 5,672,491; and 5,712,146; Fu et al., 1994, *Biochemistry* 33: 9321-9326; McDaniel et al., 1993, *Science* 262: 1546-1550; and Rohr, 1995, *Angew. Chem. Int. Ed. Engl.* 34(8): 881-888, each of which is incorporated herein by reference.

[0004] Polyketides are synthesized in nature by polyketide synthase (PKS) enzymes. These enzymes, which are complexes of multiple large proteins, are similar to the synthases that catalyze condensation of 2-carbon units in the biosynthesis of fatty acids. PKS enzymes are encoded by PKS genes that usually consist of three or more open reading frames (ORFs). Each ORF typically comprises two or more "modules" of ketosynthase activity, each module of which consists of at least two (if a loading module) and more typically three or more enzymatic activities or "domains." Two major types of PKS enzymes are known; these differ in their composition and mode of synthesis. These two major types of PKS enzymes are commonly referred to as Type I or "modular" and Type II "iterative" PKS enzymes.

[0005] Modular PKSs are responsible for producing a large number of 12, 14, and 16-membered macrolide antibiotics including methymycin, erythromycin, narbomycin, picromycin, and tylosin. These large multifunctional enzymes (>300,000 kDa) catalyze the biosynthesis of polyketide macrolactones through multistep pathways involving decarboxylative condensations between acyl thioesters followed by cycles of varying β-carbon processing activities (see O'Hagan, D. *The polyketide metabolites*; E. Horwood: New York, 1991, incorporated herein by reference).

[0006] During the past half decade, the study of modular PKS function and specificity has been greatly facilitated by the plasmid-based *Streptomyces coelicolor* expression system developed with the 6-deoxyerythronolide B (6-dEB) synthase (DEBS) genes (see Kao et al., 1994, *Science,* 265: 509-512, McDaniel et al., 1993, *Science* 262: 1546-1557, and U.S. Pat. Nos. 5,672,491 and 5,712,146, each of which is incorporated herein by reference). The advantages to this plasmid-based genetic system for DEBS were that it overcame the tedious and limited techniques for manipulating the natural DEBS host organism, *Saccharopolyspora erythaea*, allowed more facile construction of recombinant PKSs, and reduced the complexity of PKS analysis by providing a "clean" host background. This system also expedited construction of the first combinatorial modular polyketide library in *Streptomyces* (see PCT publication No. WO 98/49315, incorporated herein by reference).

[0007] The ability to control aspects of polyketide biosynthesis, such as monomer selection and degree of β-carbon processing, by genetic manipulation of PKSs has stimulated great interest in the combinatorial engineering of novel antibiotics (see Hutchinson, 1998, *Curr. Opin. Microbiol.* 1: 319-329; Carreras and Santi, 1998, *Curr. Opin. Biotech.* 9: 403-411; and U.S. Pat. Nos. 5,712,146 and 5,672,491, each of which is incorporated herein by reference). This interest has resulted in the cloning, analysis, and manipulation by recombinant DNA technology of genes that encode PKS enzymes. The resulting technology allows one to manipulate a known PKS gene cluster either to produce the polyketide synthesized by that PKS at higher levels than occur in nature or in hosts that otherwise do not produce the polyketide. The technology also allows one to produce molecules that are structurally related to, but distinct from, the polyketides produced from known PKS gene clusters.

[0008] The present invention provides methods and reagents relating to the PKS gene cluster for the polyketide antibiotics known as narbomycin and picromycin. Narbomycin is produced in *Streptomyces narbonensis*, and both narbomycin and picromycin are produced in *S. venezuelae*. These species are unique among macrolide producing organisms in that they produce, in addition to the 14-membered macrolides narbomycin and picromycin (picromycin is shown in **FIG. 1**, compound 1), the 12-membered macrolides neomethymycin and methymycin (methymycin is shown in **FIG. 1**, compound 2). Based on the structural similarities between picromycin and methymycin, it was speculated that methymycin would result from premature cyclization of a hexaketide intermediate in the picromycin pathway.

[0009] Glycosylation of the C5 hydroxyl group of the polyketide precursor, narbonolide, is achieved through an endogenous desosaminyl transferase to produce narbomycin. In *Streptomyces venezuelae*, narbomycin is then converted to picromycin by the endogenously produced narbomycin hydroxylase. Thus, as in the case of other macrolide antibiotics, the macrolide product of the narbonolide PKS is further modified by hydroxylation and glycosylation.

[0010] Picromycin (**FIG. 1**, compound 1) is of particular interest because of its close structural relationship to

ketolide compounds (e.g. HMR 3004, **FIG. 1**, compound 3). The ketolides are a new class of semi-synthetic macrolides with activity against pathogens resistant to erythromycin (see Agouridas et al., 1998, *J. Med. Chem.* 41: 4080-4100, incorporated herein by reference). Thus, genetic systems that allow rapid engineering of the narbonolide PKS would be valuable for creating novel ketolide analogs for pharmaceutical applications. Furthermore, the production of picromycin as well as novel compounds with useful activity could be accomplished if the heterologous expression of the narbonolide PKS in *Streptomyces lividans* and other host cells were possible. The present invention meets these and other needs.

SUMMARY OF THE INVENTION

[**0011**] The present invention provides recombinant methods and materials for expressing PKSs derived in whole and in part from the narbonolide PKS and other genes involved in narbomycin and picromycin biosynthesis in recombinant host cells. The invention also provides the polyketides derived from the narbonolide PKS. The invention provides the complete PKS gene cluster that ultimately results, in *Streptomyces venezuelae*, in the production of picromycin. The ketolide product of this PKS is narbonolide. Narbonolide is glycosylated to obtain narbomycin and then hydroxylated at C12 to obtain picromycin. The enzymes responsible for the glycosylation and hydroxylation are also provided in recombinant form by the invention.

[**0012**] Thus, in one embodiment, the invention is directed to recombinant materials that contain nucleotide sequences encoding at least one domain, module, or protein encoded by a narbonolide PKS gene. The invention also provides recombinant materials useful for conversion of ketolides to antibiotics. These materials include recombinant DNA compounds that encode the C12 hydroxylase (the picK gene), the desosamine biosynthesis and desosaminyl transferase enzymes, and the beta-glucosidase enzyme involved in picromycin biosynthesis in *S. venezuelae* and the recombinant proteins that can be produced from these nucleic acids in the recombinant host cells of the invention.

[**0013**] In one embodiment, the invention provides a recombinant expression vector that comprises a heterologous promoter positioned to drive expression of the narbonolide PKS. In a preferred embodiment, the promoter is derived from a PKS gene. In a related embodiment, the invention provides recombinant host cells comprising the vector that produces narbonolide. In a preferred embodiment, the host cell is *Streptomyces lividans* or *S. coelicolor.*

[**0014**] In another embodiment, the invention provides a recombinant expression vector that comprises the desosamine biosynthetic genes as well as the desosaminyl transferase gene. In a related embodiment, the invention provides recombinant host cells comprising the vector that produces the desosamine biosynthetic gene products and desosaminyl transferase gene product. In a preferred embodiment, the host cell is *Streptomyces lividans* or *S. coelicolor.*

[**0015**] In another embodiment, the invention provides a method for desosaminylating polyketide compounds in recombinant host cells, which method comprises expressing the PKS for the polyketide and the desosaminyl transferase and desosamine biosynthetic genes in a host cell. In a preferred embodiment, the host cell expresses a beta-glucosidase gene as well. This preferred method is especially advantageous when producing desosaminylated polyketides in *Streptomyces* host cells, because such host cells typically glucosylate desosamine residues of polyketides, which can decrease desired activity, such as antibiotic activity. By coexpression of beta-glucosidase, the glucose residue is removed from the polyketide.

[**0016**] In another embodiment, the invention provides the picK hydroxylase gene in recombinant form and methods for hydroxylating polyketides with the recombinant gene product. The invention also provides polyketides thus produced and the antibiotics or other useful compounds derived therefrom.

[**0017**] In another embodiment, the invention provides a recombinant expression vector that comprises a promoter positioned to drive expression of a hybrid PKS comprising all or part of the narbonolide PKS and at least a part of a second PKS. In a related embodiment, the invention provides recombinant host cells comprising the vector that produces the hybrid PKS and its corresponding polyketide. In a preferred embodiment, the host cell is *Streptomyces lividans* or *S. coelicolor.*

[**0018**] In a related embodiment, the invention provides recombinant materials for the production of libraries of polyketides wherein the polyketide members of the library are synthesized by hybrid PKS enzymes of the invention. The resulting polyketides can be further modified to convert them to other useful compounds, such as antibiotics, typically through hydroxylation and/or glycosylation. Modified macrolides provided by the invention that are useful intermediates in the preparation of antibiotics are of particular benefit.

[**0019**] In another related embodiment, the invention provides a method to prepare a nucleic acid that encodes a modified PKS, which method comprises using the narbonolide PKS encoding sequence as a scaffold and modifying the portions of the nucleotide sequence that encode enzymatic activities, either by mutagenesis, inactivation, insertion, or replacement. The thus modified narbonolide PKS encoding nucleotide sequence can then be expressed in a suitable host cell and the cell employed to produce a polyketide different from that produced by the narbonolide PKS. In addition, portions of the narbonolide PKS coding sequence can be inserted into other PKS coding sequences to modify the products thereof. The narbonolide PKS can itself be manipulated, for example, by fusing two or more of its open reading frames, particularly those for extender modules 5 and 6, to make more efficient the production of 14-membered as opposed to 12-membered macrolides.

[**0020**] In another related embodiment, the invention is directed to a multiplicity of cell colonies, constituting a library of colonies, wherein each colony of the library contains an expression vector for the production of a modular PKS derived in whole or in part from the narbonolide PKS. Thus, at least a portion of the modular PKS is identical to that found in the PKS that produces narbonolide and is identifiable as such. The derived portion can be prepared synthetically or directly from DNA derived from organisms that produce narbonolide. In addition, the invention provides methods to screen the resulting polyketide and antibiotic libraries.

[**0021**] The invention also provides novel polyketides and antibiotics or other useful compounds derived therefrom. The compounds of the invention can be used in the manufacture of another compound. In a preferred embodiment, the antibiotic compounds of the invention are formulated in a mixture or solution for administration to an animal or human.

[0022] These and other embodiments of the invention are described in more detail in the following description, the examples, and claims set forth below.

BRIEF DESCRIPTION OF THE FIGURES

[0023] **FIG. 1** shows the structures of picromycin (compound 1), methymycin (compound 2), and the ketolide HMR 3004 (compound 3).

[0024] **FIG. 2** shows a restriction site and function map of cosmid pKOS023-27.

[0025] **FIG. 3** shows a restriction site and function map of cosmid pKOS023-26.

[0026] **FIG. 4** has three parts. In Part A, the structures of picromycin (A(a)) and methymycin (A(b)) are shown, as well as the related structures of narbomycin, narbonolide, and methynolide. In the structures, the bolded lines indicate the two or three carbon chains produced by each module (loading and extender) of the narbonolide PKS. Part B shows the organization of the narbonolide PKS genes on the chromosome of *Streptomyces venezuelae*, including the location of the various module encoding sequences (the loading module domains are identified as sKS*, sAT, and sACP), as well as the picB thioesterase gene and two desosamine biosynthesis genes (picCII and picCIII). Part C shows the engineering of the *S. venezuelae* host of the invention in which the picAI gene has been deleted. In the Figure, ACP is acyl carrier protein; AT is acyltransferase; DH is dehydratase; ER is enoylreductase; KR is ketoreductase; KS is ketosynthase; and TE is thioesterase.

[0027] **FIG. 5** shows the narbonolide PKS genes encoded by plasmid pKOS039-86, the compounds synthesized by each module of that PKS and the narbonolide (compound 4) and 10-deoxymethynolide (compound 5) products produced in heterologous host cells transformed with the plasmid. The Figure also shows a hybrid PKS of the invention produced by plasmid pKOS038-18, which encodes a hybrid of DEBS and the narbonolide PKS. The Figure also shows the compound, 3,6-dideoxy-3-oxo-erythronolide B (compound 6), produced in heterologous host cells comprising the plasmid.

[0028] **FIG. 6** shows a restriction site and function map of plasmid pKOS039-104, which contains the desosamine biosynthetic, beta-glucosidase, and desosaminyl transferase genes under transcriptional control of actII-4.

DETAILED DESCRIPTION OF THE INVENTION

[0029] The present invention provides useful compounds and methods for producing polyketides in recombinant host cells. As used herein, the term recombinant refers to a compound or composition produced by human intervention. The invention provides recombinant DNA compounds encoding all or a portion of the narbonolide PKS. The invention also provides recombinant DNA compounds encoding the enzymes that catalyze the further modification of the ketolides produced by the narbonolide PKS. The invention provides recombinant expression vectors useful in producing the narbonolide PKS and hybrid PKSs composed of a portion of the narbonolide PKS in recombinant host cells. Thus, the invention also provides the narbonolide PKS, hybrid PKSs, and polyketide modification enzymes in recombinant form. The invention provides the polyketides produced by the recombinant PKS and polyketide modification enzymes. In particular, the invention provides methods for producing the polyketides 10-deoxymethynolide, narbonolide, YC17, narbomycin, methymycin, neomethymycin, and picromycin in recombinant host cells.

[0030] To appreciate the many and diverse benefits and applications of the invention, the description of the invention below is organized as follows. First, a general description of polyketide biosynthesis and an overview of the synthesis of narbonolide and compounds derived therefrom in *Streptomyces venezuelae* are provided. This general description and overview are followed by a detailed description of the invention in six sections. In Section I, the recombinant narbonolide PKS provided by the invention is described. In Section II, the recombinant desosamine biosynthesis genes, the desosaminyl transferase gene, and the beta-glucosidase gene provided by the invention are described. In Section III, the recombinant picK hydroxylase gene provided by the invention is described. In Section IV, methods for heterologous expression of the narbonolide PKS and narbonolide modification enzymes provided by the invention are described. In Section V, the hybrid PKS genes provided by the invention and the polyketides produced thereby are described. In Section VI, the polyketide compounds provided by use invention and pharmaceutical compositions of those compounds are described. The detailed description is followed by a variety of working examples illustrating the invention.

[0031] The narbonolide synthase gene, like other PKS genes, is composed of coding sequences organized in a loading module, a number of extender modules, and a thioesterase domain. As described more fully below, each of these domains and modules is a polypeptide with one or more specific functions. Generally, the loading module is responsible for binding the first building block used to synthesize the polyketide and transferring it to the first extender module. The building blocks used to form complex polyketides are typically acylthioesters, most commonly acetyl, propionyl, malonyl, methylmalonyl, and ethylmalonyl CoA. Other building blocks include amino acid like acylthioesters. PKSs catalyze the biosynthesis of polyketides through repeated, decarboxylative Claisen condensations between the acylthioester building blocks. Each module is responsible for binding a building block, performing one or more functions on that building block, and transferring the resulting compound to the next module. The next module, in turn, is responsible for attaching the next building block and transferring the growing compound to the next module until synthesis is complete. At that point, an enzymatic thioesterase activity cleaves the polyketide from the PKS.

[0032] Such modular organization is characteristic of the class of PKS enzymes that synthesize complex polyketides and is well known in the art. The polyketide known as 6-deoxyerythronolide B is a classic example of this type of complex polyketide. The genes, known as eryAI, eryAII, and eryAIII (also referred to herein as the DEBS genes, for the proteins, known as DEBS1, DEBS2, and DEBS3, that comprise the 6-dEB synthase), that code for the multisubunit protein known as DEBS that synthesizes 6-dEB are described in U.S. Pat. No. 5,824,513, incorporated herein by reference. Recombinant methods for manipulating modular PKS genes are described in U.S. Pat. Nos. 5,672,491; 5,843,718; 5,830,750; and 5,712,146; and in PCT publication Nos. 98/49315 and 97/02358, each of which is incorporated herein by reference.

[0033] The loading module of DEBS consists of two domains, an acyl-transferase (AT) domain and an acyl

carrier protein (ACP) domain. Each extender module of DEBS, like those of other modular PKS enzymes, contains a ketosynthase (KS), AT, and ACP domains, and zero, one, two, or three domains for enzymatic activities that modify the beta-carbon of the growing polyketide chain. A module can also contain domains for other enzymatic activities, such as, for example, a methyltransferase or dimethyltransferase activity. Finally, the releasing domain contains a thioesterase and, often, a cyclase activity.

[0034] The AT domain of the loading module recognizes a particular acyl-CoA (usually acetyl or propionyl but sometimes butyryl) and transfers it as a thiol ester to the ACP of the loading module. Concurrently, the AT on each of the extender modules recognizes a particular extender-CoA (malonyl or alpha-substituted malonyl, i.e., methylmalonyl, ethylmalonyl, and carboxylglycolyl) and transfers it to the ACP of that module to form a thioester. Once the PKS is primed with acyl- and malonyl-ACPs, the acyl group of the loading module migrates to form a thiol ester (trans-esterification) at the KS of the first extender module; at this stage, extender module 1 possesses an acyl-KS adjacent to a malonyl (or substituted malonyl) ACP. The acyl group derived from the loading module is then covalently attached to the alpha-carbon of the malonyl group to form a carbon-carbon bond, driven by concomitant decarboxylation, and generating a new acyl-ACP that has a backbone two carbons longer than the loading unit (elongation or extension). The growing polyketide chain is transferred from the ACP to the KS of the next module, and the process continues.

[0035] The polyketide chain, growing by two carbons each module, is sequentially passed as covalently bound thiol esters from module to module, in an assembly line-like process. The carbon chain produced by this process alone would possess a ketone at every other carbon atom, producing a polyketone, from which the name polyketide arises. Most commonly, however, additional enzymatic activities modify the beta keto group of each two-carbon unit just after it has been added to the growing polyketide chain but before it is transferred to the next module. Thus, in addition to the minimal module containing KS, AT, and ACP domains necessary to form the carbon-carbon bond, modules may contain a ketoreductase (ICR) that reduces the keto group to an alcohol. Modules may also contain a KR plus a dehydratase (DH) that dehydrates the alcohol to a double bond. Modules may also contain a KR, a DH, and an enoylreductase (ER) that converts the double bond to a saturated single bond using the beta carbon as a methylene function. As noted above, modules may contain additional enzymatic activities as well.

[0036] Once a polyketide chain traverses the final extender module of a PKS, it encounters the releasing domain or thioesterase found at the carboxyl end of most PKSs. Here, the polyketide is cleaved from the enzyme and cyclyzed. The resulting polyketide can be modified further by tailoring enzymes; these enzymes add carbohydrate groups or methyl groups, or make other modifications, i.e., oxidation or reduction, on the polyketide core molecule.

[0037] While the above description applies generally to modular PKS enzymes, there are a number of variations that exist in nature. For example, some polyketides, such as epothilone, incorporate a building block that is derived from an amino acid. PKS enzymes for such polyketides include an activity that functions as an amino acid ligase or as a non-ribosomal peptide synthetase (NRPS). Another example of a variation, which is actually found more often than the two domain loading module construct found in DEBS, occurs when the loading module of the PKS is not composed of an AT and an ACP but instead utilizes an inactivated KS, an AT, and an ACP. This inactivated KS is in most instances called KSQ, where the superscript letter is the abbreviation for the amino acid, glutamine, that is present instead of the active site cysteine required for activity. For example, the narbonolide PKS loading module contains aKS^{Q}. Yet another example of a variation has been mentioned above in the context of modules that include a methyltransferase or dimethyltransferase activity; modules can also include an epimerase activity. These variations will be described further below in specific reference to the narbonolide PKS and the various recombinant and hybrid PKSs provided by the invention.

[0038] With this general description of polyketide biosynthesis, one can better appreciate the biosynthesis of narbonolide related polyketides in *Streptomyces venezuelae* and *S. narbonensis*. The narbonolide PKS produces two polyketide products, narbonolide and 10-deoxymethynolide. Narbonolide is the polyketide product of all six extender modules of the narbonolide PKS. 10-deoxymethynolide is the polyketide product of only the first five extender modules of the narbonolide PKS. These two polyketides are desosaminylated to yield narbomycin and YC17, respectively. These two glycosylated polyketides are the final products produced in *S. narbonensis*. In *S. venezuelae*, these products are hydroxylated by the picK gene product to yield picromycin and either methymycin (hydroxylation at the C10 position of YC7) or neomethymycin (hydroxylation at the C12 position of YC17). The present invention provides the genes required for the biosynthesis of all of these polyketides in recombinant form.

[0039] Section 1: The Narbonolide PKS

[0040] The narbonolide PKS is composed of a loading module, six extender modules, and a thioesterase domain. **FIG. 4**, part B, shows the organization of the narbonolide PKS genes on the *Streptomyces venezuelae* chromosome, as well as the location of the module encoding sequences in those genes, and the various domains within those modules. In the Figure, the loading module is not numbered, and its domains are indicated as sKS*, sAT, and ACP. Also shown in the Figure, part A, are the structures of picromycin and methymycin.

[0041] The loading and six extender modules and the thioesterase domain of the narbonolide PKS reside on four proteins, designated PICAI, PICAII, PICAII, and PICAIV. PICAI includes the loading module and extender modules 1 and 2 of the PKS. PICAII includes extender modules 3 and 4. PICAIII includes extender module 5. PICAIV includes extender module 6 and a thioesterase domain. There is a second thioesterase domain (TEII) on a separate protein, designated PICB. The amino acid sequences of these proteins are shown below.

Amino acid sequence of narbonolide synthase subunit 1, PICAI

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   1 MSTVSKSESE EFVSVSNDAG SAHGTAEPVA VVGISCRVPG ARDPPEFWEL LAAGGQAVTD
  61 VPADRWNAGD FYDPDRSAPG RSNSRWGGFI EDVDRFDAAF FGISPREAAE MDPQQRLALE
 121 LGWEALERAG IDPSSLTGTR TGVFAGAIWD DYATLKHRQG GAAITPHTVT GLHRGIIANR
 181 LSYTLGLRGP SMVVDSGQSS SLVAVHLACE SLRRGESELA LAGGVSLNLV PDSIIGASKF
 241 GGLSPDGRAY TFDARANGYV RGEGGGFVVL KRLSRAVADG DPVLAVIRGS AVNNGGAAQG
 301 MTTPDAQAQE AVLREAHERA GTAPADVRYV ELHGTGTPVG DPIEAAALGA ALGTGRPAGQ
 361 PLLVGSVKTN IGHLEGAAGI AGLIKAVLAV RGRALPASLN YETPNPAIPF EELNLRVNTE
 421 YLPWEPEHDG QRMVVGVSSF GMGGTNAHVV LEEAPGVVEG ASVVESTVGG SAVGGGVVPW
 481 VVSAKSAAAL DAQIERLAAF ASRDRTDGVD AGAVDAGAVD AGAVARVLAG GRAQFEHRAV
 541 VVGSGPDDLA AALAAPEGLV RGVASGVGRV AFVFPGQGTQ WAGMGAELLD SSAVFAAAMA
 601 ECEAALSPYV DWSLEAVVRQ APGAPTLERV DVVQPVTFAV MVSLARVWQH HGVTPQAVVG
 661 HSQGEIAAAY VAGALSLDDA ARVVTLRSKS IAAHLAGKGG MLSLALSEDA VLERLAGFDG
 721 LSVAAVNGPT ATVVSGDPVQ IEELARACEA DGVRARVIPV DYASHSRQVE IIESELAEVL
 781 AGLSPQAPRV PFFSTLEGAW ITEPVLDGGY WYRNLRHRVG FAPAVETLAT DEGFTHFVEV
 841 SAHPVLTMAL PGTVTGLATL RRDNGGQDRL VASLAEAWAN GLAVDWSPLL PSATGHHSDL
 901 PTYAFQTERH WLGEIEALAP AGEPAVQPAV LRTEAAEPAE LDRDEQLRVI LDKVRAQTAQ
 961 VLGYATGGQI EVDRTFREAG CTSLTGVDLR NRINAAFGVR MAPSMIFDFP TPEALAEQLL
1021 LVVHGEAAAN PAGAEPAPVA AAGAVDEPVA IVGMACRLPG GVASPEDLWR LVAGGGDAIS
1081 EFPQDRGWDV EGLYHPDPEH PGTSYVRQGG FIENVAGFDA AFFGISPREA LAMDPQQRLL
1141 LETSWEAVED AGIDPTSLRG RQVGVFTGAM THEYGPSLRD GGEGLDGYLL TGNTASVMSG
1201 RVSYTLGLEG PALTVDTACS SSLVALHLAV QALRKGEVDM ALAGGVAVMP TPGMFVEFSR
1261 QRGLAGDGRS KAFAASADGT SWSEGVGVLL VERLSDARRN GHQVLAVVRG SAVNQDGASN
1321 GLTAPNGPSQ ORVIRRALAD ARLTTSDVDV VEAHGTGTRL GDPIEAQALI ATYGQGRDDE
1381 QPLRLGSLKS NIGHTQAAAG VSGVIKMVQA MRHGLLPKTL HVDEPSDQID WSAGAVELLT
1441 EAVDWPEKQD GGLRRAAVSS FGISGTNAHV VLEEAPVVVE GASVVEPSVG GSAVGGGVTP
1501 WVVSAKSAAA LDAQIERLAA FASRDRTDDA DAGAVDAGAV AHVLADGRAQ FEHRAVALGA
1561 GADDLVQALA DPDGLIRGTA SGVGRVAFVF PGQGTQWAGM GAELLDSSAV FAAAMAECEA
1621 ALSPYVDWSL EAVVRQAPGA PTLERVDVVQ PVTFAVMVSL ARVWQHHGVT PQAVVGHSQG
1681 EIAAAYVAGA LPLDDAARVV TLRSKSIAAH LAGKGGMLSL ALNEDAVLER LSDFDGLSVA
1741 AVNGPTATVV SGDPVQIEEL AQACKADGFR ARIIPVDYAS HSRQVEIIES ELAQVLAGLS
1801 PQAPRVPFFS TLEGTWITEP VLDGTYWYRN LRHRVGFAPA IETLAVDEGF THFVEVSAHP
1861 VLTMTLPETV TGLGTLRREQ GGQERLVTSL AEAWVNGLPV AWTSLLPATA SRPGLPTYAF
1921 QAERYWLENT PAALATGDDW RYRIDWKRLP AAEGSERTGL SGRWLAVTPE DHSAQAAAVL
1981 TALVDAGAKV EVLTAGADDD REALAARLTA LTTGDGFTGV VSLLDGLVPQ VAWVQALGDA
2041 GIKAPLWSVT QGAVSVGRLD TPADPDRAML WGLGRVVALE HPERWAGLVD LPAQPDAAAL
2101 AHLVTALSGA TGEDQIAIRT TGLHARRLAR APLHGRRPTR DWQPHGTVLI TGGTGALGSH
2161 AARWMAHHGA EHLLLVSRSG EQAPGATQLT AELTASGARV TIAACDVADP HAMRTLLDAI
2221 PAETPLTAVV HTAGALDDGI VDTLTAEQVR RAHRAKAVGA SVLDELTRDL DLDAFVLFSS
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2281 VSSTLGIPGQ GNYAPHNAYL DALAARRRAT GRSAVSVAWG PWDGGGMAAG DGVAERLRNH

2341 GVPGMDPELA LAALESALGR DETAITVADI DWDRFYLAYS SGRPQPLVEE LPEVRRIIDA

2401 RDSATSGQGG SSAQGANPLA RRLAAAAPGE RTEILLGLVR AQAAAVLRMR SPEDVAADRA

2461 FKDIGFDSLA GVELRNRLTR ATGLQLPATL VFDHPTPLAL VSLLRSEFLG DEETADARRS

2521 AALPATVGAG AGAGAGTDAD DDPIAIVAMS CRYPGDIRSP EDLWRMLSEG GEGITPFPTD

2581 RGWDLDGLYD ADPDALGRAY VREGGFLHDA AEFDAEFFGV SPREALAMDP QQRMLLTTSW

2641 EAFERAGIEP ASLRGSSTGV FIGLSYQDYA ARVPNAPRGV EGYLLTGSTP SVASGRIAYT

2701 FGLEGPATTV DTACSSSLTA LHLAVRALRS GECTMALAGG VAMMATPHMF VEFSRQRALA

2761 PDGRSKAFSA DADGFGAAEG VGLLLVERLS DARRNGHPVL AVVRGTAVNQ DGASNGLTAP

2821 NGPSQQRVIR QALADARLAP GDIDAVETHG TGTSLGDPIE AQGLQATYGK ERPAERPLAI

2881 GSVKSNIGHT QAAAGAAGII KMVLAMRHGT LPKTLHADEP SPHVDWANSG LALVTEPIDW

2941 PAGTGPRRAA VSSFGISGTN AHVVLEQAPD AAGEVLGADE VPEVSETVAM AGTAGTSEVA

3001 EGSEASEAPA APGSREASLP GHLPWVLSAK DEQSLRGQAA ALHAWLSEPA ADLSDADGPA

3061 RLRDVGYTLA TSRTAFAHRA AVTAADRDGF LDGLATLAQG GTSAHVHLDT ARDGTTAFLF

3121 TGQGSQRPGA GRELYDRHPV FARALDEICA HLDGHLELPL LDVMFAAEGS AEAALLDETR

3181 YTQCALFALE VALFRLVESW GMRPAALLGH SVGEIAAAHV AGVFSLADAA RLVAARGRLM

3241 QELPAGGAML AVQAAEDEIR VWLETEERYA GRLDVAAVNG PEAAVLSGDA DAAREAEAYW

3301 SGLGRRTRAL RVSHAFHSAH MDGMLDGFRA VLETVEFRRP SLTVVSNVTG LAAGPDDLCD

3361 PEYWVRHVRG TVRFLDGVRV LRDLGVRTCL ELGPDGVLTA MAADGLADTP ADSAAGSPVG

3421 SPAGSPADSA AGALRPRPLL VALLRRKRSE TETVADALGR AHAHGTGPDW HAWFAGSGAH

3481 RVDLPTYSFR RDRYWLDAPA ADTAVDTAGL GLGTADHPLL GAVVSLPDRD GLLLTGRLSL

3541 RTHPWLADHA VLGSVLLPGA AMVELAAHAA ESAGLRDVRE LTLLEPLVLP EHGGVELRVT

3601 VGAPAGEPGG ESAGDGARPV SLHSRLADAP AGTAWSCHAT GLLATDRPEL PVAPDRAAMW

3661 PPQGAEEVPL DGLYERLDGN GLAFGPLFQG LNAVWRYEGE VFADIALPAT TNATAPATAN

3721 GGGSAAAAPY GIHPALLDAS LHAIAVGGLV DEPELVRVPF HWSGVTVHAA GAAAARVRLA

3781 SAGTDAVSLS LTDGEGRPLV SVERLTLRPV TADQAAASRV GGLMHRVAWR PYALASSGEQ

3841 DPHATSYGPT AVLGKDELKV AAALESAGVE VGLYPDLAAL SQDVAAGAPA PRTVLAPLPA

3901 GPADGGAEGV RGTVARTLEL LQAWLADEHL AGTRLLLVTR GAVRDPEGSG ADDGGEDLSH

3961 AAAWGLVRTA QTENPGRFGL LDLADDASSY RTLPSVLSDA GLRDEPQLAL HDGTIRLARL

4021 ASVRPETGTA APALAPEGTV LLTGGTGGLG GLVARHVVGE WGVRRLLLVS RRGTDAPGAD

4081 ELVHELEALG ADVSVAACDV ADREALTAVL DAIPAEHPLT AVVHTAGVLS DGTLPSMTTE

4141 DVEHVLRPKV DAAFLLDELT STPAYDLAAF VMFSSAAAVF GGAGQGAYAA ANATLDALAW

4201 RRRAAGLPAL SLGWGLWAET SGMTGELGQA DLRRMSRAGI GGISDAEGIA LLDAALRDDR

4261 HPVLLPLRLD AAGLRDAAGN DPAGIPALFR DVVGARTVRA RPSAASASTT AGTAGTPGTA

4321 DGAAETAAVT LADRAATVDG PARQRLLLEF VVGEVAEVLG HARGHRIDAE RGFLDLGFDS

4381 LTAVELRNRL NSAGGLALPA TLVFDHPSPA ALASHLDAEL PRGASDQDGA GNRNGNENGT

4441 TASRSTAETD ALLAQLTRLE GALVLTGLSD APGSEEVLEH LRSLRSMVTG ETGTGTASGA

4501 PDGAGSGAED RPWAAGDGAG GGSEDGAGVP DFMNASAEEL FGLLDQDPST D

Amino acid sequence of narbonolide synthase subunit 2, PICAII

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   1 VSTVNEEKYL DYLRRATADL HEARGRLREL EAKAGEPVAI VGMACRLPGG VASPEDLWRL
  61 VAGGEDAISE FPQDRGWDVE GLYDPNPEAT GKSYAREAGF LYEAGEFDAD FFGISPREAL
 121 AMDPQQRLLL EASWEAFEHA GIPAATARGT SVGVFTGVMY HDYATRLTDV PEGIEGYLGT
 181 GNSGSVASGR VAYTLGLEGP AVTVDTACSS SLVALHLAVQ ALRKGEVDMA LAGGVTVMST
 241 PSTFVEFSRQ RGLAPDGRSK SFSSTADGTS WSEGVGVLLV ERLSDARRKG HRILAVVRGT
 301 AVNQDGASSG LTAPNGPSQQ RVIRRALADA RLTTSDVDVV EAHGTGTRLG DPIEAQAVIA
 361 TYGQGRDGEQ PLRLGSLKSN IGHTQAAAGV SGVIKMVQAM RHGVLPKTLH VEKPTDQVDW
 421 SAGAVELLTE AMDWPDKGDG GLRRAAVSSF GVSGTNAHVV LEEAPAAEET PASEATPAVE
 481 PSVGAGLVPW LVSAKTPAAL DAQIGRLAAF ASQGRTDAAD PGAVARVLAG GRAEFEHRAV
 541 VLGTGQDDFA QALTAPEGLI RGTPSDVGRV AFVFPGQGTQ WAGMGAELLD VSKEFAAAMA
 601 ECESALSRYV DWSLEAVVRQ APGAPTLERV DVVQPVTFAV MVSLAKVWQH HGVTPQAVVG
 661 HSQGEIAAAY VAGALTLDDA ARVVTLRSKS IAAHLAGKGG MISLALSEEA TRQRIENLHG
 721 LSIAAVNGPT ATVVSGDPTQ IQELAQACEA DGVRARIIPV DYASHSAHVE TIESELAEVL
 781 AGLSPRTPEV PFFSTLEGAW ITEPVLDGTY WYRNLRHRVG FAPAVETLAT DEGFTHFIEV
 841 SAHPVLTMTL PETVTGLGTL RREQGGQERL VTSLAEAWTN GLTIDWAPVL PTATGHHPEL
 901 PTYAFQRRHY WLHDSPAVQG SVQDSWRYRI DWKRLAVADA SERAGLSGRW LVVVPEDRSA
 961 EAAPVLAALS GAGADPVQLD VSPLGDRQRL AATLGEALAA AGGAVDGVLS LLAWDESAHP
1021 GHPAPFTRGT GATLTLVQAL EDAGVAAPLW CVTHGAVSVG RADHVTSPAQ AMVWGMGRVA
1081 ALEHPERWGG LIDLPSDADR AALDRMTTVL AGGTGEDQVA VRASGLLARR LVRASLPAHG
1141 TASPWWQADG TVLVTGAEEP AAAEAARRLA RDGAGHLLLH TTPSGSEGAE GTSGAAEDSG
1201 LAGLVAELAD LGATATVVTC DLTDAEAAAR LLAGVSDAHP LSAVLHLPPT VDSEPLAATD
1261 ADALARVVTA KATAALHLDR LLREAAAAGG RPPVLVLFSS VAAIWGGAGQ GAYAAGTAFL
1321 DALAGQHRAD GPTVTSVAWS PWEGSRVTEG ATGERLRRLG LRPLAPATAL TALDTALGHG
1381 DTAVTIADVD WSSFAPGFTT ARPGTLLADL PEARRALDEQ QSTTAADDTV LSRELGALTG
1441 AEQQRRMQEL VREHLAVVLN HPSPEAVDTG RAFRDLGFDS LTAVELRNRL KNATGLALPA
1501 TLVFDYPTPR TLAEFLLAEI LGEQAGAGEQ LPVDGGVDDE PVAIVGMACR LPGGVASPED
1561 LWRLVAGGED AISGFPQDRG WDVEGLYDPD PDASGRTYCR AGGFLDEAGE FDADFFGISP
1621 REALAMDPQQ RLLLETSWEA VEDAGIDPTS LQGQQVGVFA GTNGPHYEPL LRNTAEDLEG
1681 YVGTGNAASI MSGRVSYTLG LEGPAVTVDT ACSSSLVALH LAVQALRKGE CGLALAGGVT
1741 VMSTPTTFVE FSRQRGLAED GRSKAFAASA DGFGPAEGVG MLLVERLSDA RRNGHRVLAV
1801 VRGSAVNQDG ASNGLTAPNG PSQQRVIRRA LADARLTTAD VDVVEAHGTG TRLGDPIEAQ
1861 ALIATYGQGR DTEQPLRLGS LKSNIGHTQA AAGVSGIIKM VQAMRHGVLP KTLHVDRPSD
1921 QIDWSAGTVE LLTEAMDWPR KQEGGLRRAA VSSFGISGTN AHIVLEEAPV DEDAPADEPS
1981 VGGVVPWLVS AKTPAALDAQ IGRLAAFASQ GRTDAADPGA VARVLAGGRA QFEHRAVALG
2041 TGQDDLAAAL AAPEGLVRGV ASGVGRVAFV FPGQGTQWAG MGAELLDVSK EFAAAMAECE
2101 AALAPYVDWS LEAVVRQAPG APTLERVDVV QPVTFAVMVS LAKVWQHHGV TPQAVVGHSQ
2161 GEIAAAYVAG ALSLDDAARV VTLRSKSIGA HLAGQGGMLS LALSEAAVVE RLAGFDGLSV
2221 AAVNGPTATV VSGDPTQIQE LAQACEADGV RARIIPVDYA SHSAHVETIE SELADVLAGL
2281 SPQTPQVPFF STLEGAWITE PALDGGYWYR NLRHRVGFAP AVETLATDEG FTHFVEVSAH
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2341 PVLTMALPET VTGLGTLRRD NGGQHRLTTS LAEAWANGLT VDWASLLPTT TTHPDLPTYA

2401 FQTERYWPQP DLSAAGDITS AGLGAAEHPL LGAAVALADS DGCLLTGSLS LRTHPWLADH

2461 AVAGTVLLPG TAFVELAFRA GDQVGCDLVE ELTLDAPLVL PRRGAVRVQL SVGASDESGR

2521 RTFGLYAHPE DAPGEAEWTR HATGVLAARA DRTAPVADPE AWPPPGAEPV DVDGLYERFA

2581 ANGYGYGPLF QGVRGVWRRG DEVFADVALP AEVAGAEGAR FGLHPALLDA AVQAAGAGGA

2641 FGAGTRLPFA WSGISLYAVG ATALRVRLAP AGPDTVSVSA ADSSGQPVFA ADSLTVLPVD

2701 PAQLAAFSDP TLDALHLLEW TAWDGAAQAL PGAVVLGGDA DGLAAALRAG GTEVLSFPDL

2761 TDLVEAVDRG ETPAPATVLV ACPAAGPGGP EHVREALHGS LALMQAWLAD ERFTDGRLVL

2821 VTRDAVAARS GDGLRSTGQA AVWGLGRSAQ TESPGRFVLL DLAGEARTAG DATAGDGLTT

2881 GDATVGGTSG DAALGSALAT ALGSGEPQLA LRDGALLVPR LARAAAPAAA DGLAAADGLA

2941 ALPLPAAPAL WRLEPGTDGS LESLTAAPGD AETLAPEPLG PGQVRIAIRA TGLNFRDVLI

3001 ALGMYPDPAL MGTEGAGVVT ATGPGVTHLA PGDRVMGLLS GAYAPVVVAD ARTVARMPEG

3061 WTFAQGASVP VVFLTAVYAL RDLADVKPGE RLLVHSAAGG VGMAAVQLAR HWGVEVHGTA

3121 SHGKWDALRA LGLDDAHIAS SRTLDFESAF RAASGGAGMD VVLNSLAREF VDASLRLLGP

3181 GGRFVEMGKT DVRDAERVAA DHPGVGYRAF DLGEAGPERI GEMLAEVIAL FEDGVLRHLP

3241 VTTWDVRRAR DAFRHVSQAR HTGKVVLTMP SGLDPEGTVL LTGGTGALGG IVARHVVGEW

3301 GVRRLLLVSR RGTDAPGAGE LVHELEALGA DVSVAACDVA DREALTAVLD SIPAEHPLTA

3361 VVHTAGVLSD GTLPSMTAED VEHVLRPKVD AAFLLDELTS TPGYDLAAFV MFSSAAAVFG

3421 GAGQGAYAAA NATLDALAWR RRTAGLPALS LGWGLWAETS GMTGGLSDTD RSRLARSGAT

3481 PMDSELTLSL LDAAMRRDDP ALVPIALDVA ALRAQQRDGM LAPLLSGLTR GSRVGGAPVN

3541 QRRAAAGGAG EADTDLGGRL AAMTPDDRVA HLRDLVRTHV ATVLGHGTPS RVDLERAFRD

3601 TGFDSLTAVE LRNRLNAATG LRLPATLVFD HPTPGELAGH LLDELATAAG GSWAEGTGSG

3661 DTASATDRQT TAALAELDRL EGVLASLAPA AGGRPELAAR LRALAAALGD DGDDATDLDE

3721 ASDDDLFSFI DKELGDSDF

Amino acid sequence of narbonolide synthase subunit 3, PICAIII

1 MANNEDKLRD YLKRVTAELQ QNTRRLREIE GRTHEPVAIV GMACRLPGGV ASPEDLWQLV

61 AGDGDAISEF PQDRGWDVEG LYDPDPDASG RTYCRSGGFL HDAGEFDADF FGISPREALA

121 MDPQQRLSLT TAWEAIESAG IDPTALKGSG LGVFVGGWHT GYTSGQTTAV QSPELEGHLV

181 SGAALGFLSG RIAYVLGTDG PALTVDTACS SSLVALHLAV QALRKGECDM ALAGGVTVMP

241 NADLFVQFSR QRGLAADGRS KAFATSADGF GPAEGAGVLL VERLSDARRN GHRILAVVRG

301 SAVNQDGASN GLTAPHGPSQ QRVIRRALAD ARLAPGDVDV VEAHGTGTRL GDPIEAQALI

361 ATYGQEKSSE QPLRLGALKS NIGHTQAAAG VAGVIKMVQA MRHGLLPKTL HVDEPSDQID

421 WSAGTVELLT EAVDWPEKQD GGLRRAAVSS FGISGTNAHV VLEEAPAVED SPAVEPPAGG

481 GVVPWPVSAK TPAALDAQIG QLAAYADGRT DVDPAVAARA LVDSRTAMEH RAVAVGDSRE

541 ALRDALRMPE GLVRGTSSDV GRVAFVFPGQ GTQWAGMGAE LLDSSPEFAA SMAECETALS

601 RYVDWSLEAV VRQEPGAPTL DRVDVVQPVT FAVMVSLAKV WQHHGITPQA VVGHSQGEIA

661 AAYVAGALTL DDAARVVTLR SKSIAAHLAG KGGMISLALD EAAVLKRLSD FDGLSVAAVN

721 GPTATVVSGD PTQIEELART CEADGVRARI IPVDYASHSR QVEIIEKELA EVLAGLAPQA

781 PHVPFFSTLE GTWITEPVLD GTYWYRNLRH RVGFAPAVET LAVDGFTHFI EVSAHPVLTM

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 841 TLPETVTGLG TLRREQGGQE RLVTSLAEAW ANGLTIDWAP ILPTATGHHP ELPTYAFQTE

 901 RFWLQSSAPT SAADDWRYRV EWKPLTASGQ ADLSGRWIVA VGSEPEAELL GALKAAGAEV

 961 DVLEAGADDD REALAARLTA LTTGDGFTGV VSLLDDLVPQ VAWVQALGDA GIKAPLWSVT

1021 QGAVSVGRLD TPADPDRAML WGLGRVVALE HPERWAGLVD LPAQPDAAAL AHLVTALSGA

1081 TGEDQIAIRT TGLHARRLAR APLHGRRPTR DWQPHGTVLI TGGTGALGSH AARWMAHHGA

1141 EHLLLVSRSG EQAPGATQLT AELTASGARV TIAACDVADP HAMRTLLDAI PAETPLTAVV

1201 HTAGAPGGDP LDVTGPEDIA RILGAKTSGA EVLDDLLRGT PLDAFVLYSS NAGVWGSGSQ

1261 GVYAAANAHL DALAARRRAR GETATSVAWG LWAGDGMGRG ADDAYWQRRG IRPMSPDRAL

1321 DELAKALSHD ETFVAVADVD WERFAPAFTV SRPSLLLDGV PEARQALAAP VGAPAPGDAA

1381 VAPTGQSSAL AAITALPEPE RRPALLTLVR THAAAVLGHS SPDRVAPGRA FTELGFDSLT

1441 AVQLRNQLST VVGNRLPATT VFDHPTPAAL AAHLHEAYLA PAEPAPTDWE GRVRRALAEL

1501 PLDRLRDAGV LDTVLRLTGI EPEPGSGGSD GGAADPGAEP EASIDDLDAE ALIRMALGPR

1561 NT
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Amino acid sequence of narbonolide synthase subunit 4, PICAIV

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   1 MTSSNEQLVD ALRASLKENE ELRKESRRRA DRRQEPMAIV GMSCRFAGGI RSPEDLWDAV

  61 AAGKDLVSEV PEERGWDIDS LYDPVPGRKG TTYVRNAAFL DDAAGFDAAF FGISPREALA

 121 MDPQQRQLLE ASWEVFERAG IDPASVRGTD VGVYVGCGYQ DYAPDIRVAP EGTGGYVVTG

 181 NSSAVASGRI AYSLGLEGPA VTVDTACSSS LVALHLALKG LRNGDCSTAL VGGVAVLATP

 241 GAFIEFSSQQ AMAADGRTKG FASAADGLAW GEGVAVLLLE RLSDARRKGH RVLAVVRGSA

 301 INQDGASNGL TAPHGPSQQR LIRQALADAR LTSSDVDVVE GHGTGTRLGD PIEAQALLAT

 361 YGQGRAPGQP LRLGTLKSNI GHTQAASGVA GVIKMVQALR HGVLPKTLHV DEPTDQVDWS

 421 AGSVELLTEA VDWPERPGRL RRAGVSAFGV GGTNAHVVLE EAPAVEESPA VEPPAGGGVV

 481 PWPVSAKTSA ALDAQIGQLA AYAEDRTDVD PAVAARALVD SRTAMEHRAV AVGDSREALR

 541 DALRMPEGLV RGTVTDPGRV AFVFPGQGTQ WAGMGAELLD SSPEFAAAMA ECETALSPYV

 601 DWSLEAVVRQ APSAPTLDRV DVVQPVTFAV MVSLAKVWQH HGITPEAVIG HSQGEIAAAY

 661 VAGALTLDDA ARVVTLRSKS IAAHLAGKGG MISLALSEEA TRQRIENLHG LSIAAVNGPT

 721 ATVVSGDPTQ IQELAQACEA DGIRARIIPV DYASHSAHVE TIENELADVL AGLSPQTPQV

 781 PFFSTLEGTW ITEPALDGGY WYRNLRHRVG FAPAVETLAT DEGFTHFIEV SAHPVLTMTL

 841 PDKVTGLATL RREDGGQHRL TTSLAEAWAN GLALDWASLL PATGALSPAV PDLPTYAFQH

 901 RSYWISPAGP GEAPAHTASG REAVAETGLA WGPGAEDLDE EGRRSAVLAM VMRQAASVLR

 961 CDSPEEVPVD RPLREIGFDS LTAVDFRNRV NRLTGLQLPP TVVFEHPTPV ALAERISDEL

1021 AERNWAVAEP SDHEQAEEEK AAAPAGARSG ADTGAGAGMF RALFRQAVED DRYGEFLDVL

1081 AEASAFRPQF ASPEACSERL DPVLLAGGPT DRAEGRAVLV GCTGTAANGG PHEFLRLSTS

1141 FQEERDFLAV PLPGYGTGTG TGTALLPADL DTALDAQARA ILRAAGDAPV VLLGHSGGAL

1201 LAHELAFRLE RAHGAPPAGI VLVDPYPPGH QEPIEVWSRQ LGEGLFAGEL EPMSDARLLA

1261 MGRYARFLAG PRPGRSSAPV LLVRASEPLG DWQEERGDWR AHWDLPHTVA DVPGDHFTMM

1321 RDHAPAVAEA VLSWLDAIEG IEGAGK
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Amino acid sequence of typeII thioesterase, PICB

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   1 VTDRPLNVDS GLWIRRFHPA PNSAVRLVCL PHAGGSASYF FRFSEELHPS VEALSVQYPG
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 61 RQDRRAEPCL ESVEELAEHV VAATEPWWQE GRLAFFGHSL GASVAFETAR ILEQRHGVRP

121 EGLYVSGRRA PSLAPDRLVH QLDDRAFLAE IRRLSGTDER FLQDDELLRL VLPALRSDYK

181 AAETYLHRPS AKLTCPVMAL AGDRDPKAPL NEVAEWRRHT SGPFCLRAYS GGHFYLNDQW

241 HEICNDISDH LLVTRGAPDA RVVQPPTSLI EGAAKRWQNP R
```

[0042] The DNA encoding the above proteins can be isolated in recombinant form from the recombinant cosmid pKOS023-27 of the invention, which was deposited with the American Type Culture Collection under the terms of the Budapest Treaty on 20 Aug. 1998 and is available under accession number ATCC 203141. Cosmid pKOS023-27 contains an insert of *Streptomyces venezuelae* DNA of ~38506 nucleotides. The complete sequence of the insert from cosmid pKOS023-27 is shown below. The location of the various ORFs in the insert, as well as the boundaries of the sequences that encode the various domains of the multiple modules of the PKS, are summarized in the Table below. **FIG. 2** shows a restriction site and function map of pKOS023-27, which contains the complete coding sequence for the four proteins that constitute narbonolide PKS and four additional ORFs. One of these additional ORFs encodes the picB gene product, the type II thioesterase mentioned above. PICB shows a high degree of similarity to other type II thioesterases, with an identity of 51%, 49%, 45% and 40% as compared to those of *Amycolatopsis mediterranae, S. griseus, S. fradiae* and *Saccharopolyspora erythraea*, respectively. The three additional ORFs in the cosmid pKOS023-27 insert DNA sequence, from the picCII, picCIII, and picCVI, genes, are involved in desosamine biosynthesis and transfer and described in the following section.

From Nucleotide	To Nucleotide	Description
70	13725	picAI
70	13725	narbonolide synthase 1 (PICAI)
148	3141	loading module
148	1434	KS loading module
1780	2802	AT loading module
2869	3141	ACP loading module
3208	7593	extender module 1
3208	4497	KS1
4828	5847	AT1
6499	7257	KR1
7336	7593	ACP1
7693	13332	extender module 2
7693	8974	KS2

-continued

From Nucleotide	To Nucleotide	Description
9418	10554	AT2
10594	11160	DH2
12175	12960	KR2
13063	13332	ACP2
13830	25049	picAII
13830	25049	narbonolide synthase 2 (PICAII)
13935	18392	extender module 3
13935	15224	KS3
15540	16562	AT3
17271	18071	KR3 (inactive)
18123	18392	ACP3
18447	24767	extender module 4
18447	19736	KS4
20031	21050	AT4
21093	21626	DH4
22620	23588	ER4
23652	24423	KR4
24498	24765	ACP4
25133	29821	picAIII
25133	29821	narbonolide synthase 3 (PICAIII)
25235	29567	extender module 5
25235	26530	KS5
26822	27841	AT5
28474	29227	KR5
29302	29569	ACP5
29924	33964	picAIV
29924	33964	narbonolide synthase 4 (PICAIV)
30026	32986	extender module 6
30026	31312	KS6
31604	32635	AT6
32708	32986	ACP6
33068	33961	PKS thioesterase domain
33961	34806	picB
33961	34806	type II thioesterase homolog
34863	36011	picCII
34863	36011	4-keto-6-deoxyglucose isomerase
36159	37439	picCIII
36159	37439	desosaminyl transferase
37529	38242	picCVI
37529	38242	3-amino dimethyltransferase

[0043]

Sequence of the Insert DNA in Cosmid pKOS023-27

```
  1 GATCATGCGG AGCACTCCTT CTCTCGTGCT CCTACCGGTG ATGTGCGCGC CGAATTGATT

 61 CGTGGAGAGA TGTCGACAGT GTCCAAGAGT GAGTCCGAGG AATTCGTGTC CGTGTCGAAC

121 GACGCCGGTT CCGCGCACGG CACAGCGGAA CCCGTCGCCG TCGTCGGCAT CTCCTGCCGG

181 GTGCCCGGCG CCCGGGACCC GAGAGAGTTC TGGGAACTCC TGGCGGCAGG CGGCCAGGCC

241 GTCACCGACG TCCCCGCGGA CCGCTGGAAC GCCGGCGACT TCTACGACCC GGACCGCTCC

301 GCCCCCGGCC GCTCGAACAG CCGGTGGGGC GGGTTCATCG AGGACGTCGA CCGGTTCGAC
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 361 GCCGCCTTCT TCGGCATCTC GCCCCGCGAG GCCGCGGAGA TGGACCCGCA GCAGCGGCTC
 421 GCCCTGGAGC TGGGCTGGGA GGCCCTGGAG CGCGCCGGGA TCGACCCGTC CTCGCTCACC
 481 GGCACCCGCA CCGGCGTCTT CGCCGGCGCC ATCTGGGACG ACTACGCCAC CCTGAAGCAC
 541 CGCCAGGGCG GCGCCGCGAT CACCCCGCAC ACCGTCACCG GCCTCCACCG CGGCATCATC
 601 GCGAACCGAC TCTCGTACAC GCTCGGGCTC CGCGGCCCCA GCATGGTCGT CGACTCCGGC
 661 CAGTCCTCGT CGCTCGTCGC CGTCCACCTC GCGTGCGAGA GCCTGCGGCG CGGCGAGTCC
 721 GAGCTCGCCC TCGCCGGCGG CGTCTCGCTC AACCTGGTGC CGGACAGCAT CATCGGGGCG
 781 AGCAAGTTCG GCGGCCTCTC CCCCGACGGC CGCGCCTACA CCTTCGACGC GCGCGCCAAC
 841 GGCTACGTAC GCGGCGAGGG CGGCGGTTTC GTCGTCCTGA AGCGCCTCTC CCGGGCCGTC
 901 GCCGACGGCG ACCCGGTGCT CGCCGTGATC CGGGGCAGCG CCGTCAACAA CGGCGGCGCC
 961 GCCCAGGGCA TGACGACCCC CGACGCGCAG GCGCAGGAGG CCGTGCTCCG CGAGGCCCAC
1021 GAGCGGGCCG GGACCGCGCC GGCCGACGTG CGGTACGTCG AGCTGCACGG CACCGGCACC
1081 CCCGTGGGCG ACCCGATCGA GGCCGCTGCG CTCGGCGCCG CCCTCGGCAC CGGCCGCCCG
1141 GCCGGACAGC CGCTCCTGGT CGGCTCGGTC AAGACGAACA TCGGCCACCT GGAGGGCGCG
1201 GCCGGCATCG CCGGCCTCAT CAAGGCCGTC CTGGCGGTCC GCGGTCGCGC GCTGCCCGCC
1261 AGCCTGAACT ACGAGACCCC GAACCCGGCG ATCCCGTTCG AGGAACTGAA CCTCCGGGTG
1321 AACACGGAGT ACCTGCCGTG GGAGCCGGAG CACGACGGGC AGCGGATGGT CGTCGGCGTG
1381 TCCTCGTTCG GCATGGGCGG CACGAACGCG CATGTCGTGC TCGAAGAGGC CCCGGGGGTT
1441 GTCGAGGGTG CTTCGGTCGT GGAGTCGACG GTCGGCGGGT CGGCGGTCGG CGGCGGTGTG
1501 GTGCCGTGGG TGGTGTCGGC GAAGTCCGCT GCCGCGCTGG ACGCGCAGAT CGAGCGGCTT
1561 GCCGCGTTCG CCTCGCGGGA TCGTACGGAT GGTGTCGACG CGGGCGCTGT CGATGCGGGT
1621 GCTGTCGATG CGGGTGCTGT CGCTCGCGTA CTGGCCGGCG GGCGTGCTCA GTTCGAGCAC
1681 CGGGCCGTCG TCGTCGGCAG CGGGCCGGAC GATCTGGCGG CAGCGCTGGC CGCGCCTGAG
1741 GGTCTGGTCC GGGGCGTGGC TTCCGGTGTC GGGCGAGTGG CGTTCGTGTT CCCCGGGCAG
1801 GGCACGCAGT GGGCCGGCAT GGGTGCCGAA CTGCTGGACT CTTCCGCGGT GTTCGCGGCG
1861 GCCATGGCCG AATGCGAGGC CGCACTCTCC CCGTACGTCG ACTGGTCGCT GGAGGCCGTC
1921 GTACGGCAGG CCCCCGGTGC GCCCACGCTG GAGCGGGTCG ATGTCGTGCA GCCTGTGACG
1981 TTCGCCGTCA TGGTCTCGCT GGCTCGCGTG TGGCAGCACC ACGGGGTGAC GCCCCAGGCG
2041 GTCGTCGGCC ACTCGCAGGG CGAGATCGCC GCCGCGTACG TCGCCGGTGC CCTGAGCCTG
2101 GACGACGCCG CTCGTGTCGT GACCCTGCGC AGCAAGTCCA TCGCCGCCCA CCTCGCCGGC
2161 AAGGGCGGCA TGCTGTCCCT CGCGCTGAGC GAGGACGCCG TCCTGGAGCG ACTGGCCGGG
2221 TTCGACGGGC TGTCCGTCGC CGCTGTGAAC GGGCCCACCG CCACCGTGGT CTCCGGTGAC
2281 CCCGTACAGA TCGAAGAGCT TGCTCGGGCG TGTGAGGCCG ATGGGGTCCG TGCGCGGGTC
2341 ATTCCCGTCG ACTACGCGTC CCACAGCCGG CAGGTCGAGA TCATCGAGAG CGAGCTCGCC
2401 GAGGTCCTCG CCGGGCTCAG CCCGCAGGCT CCGCGCGTGC CGTTCTTCTC GACACTCGAA
2461 GGCGCCTGGA TCACCGAGCC CGTGCTCGAC GGCGGCTACT GGTACCGCAA CCTGCGCCAT
2521 CGTGTGGGCT TCGCCCCGGC CGTCGAGACC CTGGCCACCG ACGAGGGCTT CACCCACTTC
2581 GTCGAGGTCA CCGCCCACCC CGTCCTCACC ATGGCCCTCC CCGGGACCGT CACCGGTCTG
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2641 GCGACCCTGC GTCGCGACAA CGGCGGTCAG GACCGCCTCG TCGCCTCCCT CGCCGAAGCA

2701 TGGGCCAACG GACTCGCGGT CGACTGGAGC CCGCTCCTCC CCTCCGCGAC CGGCCACCAC

2761 TCCGACCTCC CCACCTACGC GTTCCAGACC GAGCGCCACT GGCTGGGCGA GATCGAGGCG

2821 CTCGCCCCGG CGGGCGAGCC GGCGGTGCAG CCCGCCGTCC TCCGCACGGA GGCGGCCGAG

2881 CCGGCGGAGC TCGACCGGGA CGAGCAGCTG CGCGTGATCC TGGACAAGGT CCGGGCGCAG

2941 ACGGCCCAGG TGCTGGGGTA CGCGACAGGC GGGCAGATCG AGGTCGACCG GACCTTCCGT

3001 GAGGCCGGTT GCACCTCCCT GACCGGCGTG GACCTGCGCA ACCGGATCAA CGCCGCCTTC

3061 GGCGTACGGA TGGCGCCGTC CATGATCTTC GACTTCCCCA CCCCCGAGGC TCTCGCGGAG

3121 CAGCTGCTCC TCGTCGTGCA CGGGGAGGCG GCGGCGAACC CGGCCGGTGC GGAGCCGGCT

3181 CCGGTGGCGG CGGCCGGTGC CGTCGACGAG CCGGTGGCGA TCGTCGGCAT GGCCTGCCGC

3241 CTGCCCGGTG GGGTCGCCTC GCCGGAGGAC CTGTGGCGGC TGGTGGCCGG CGGCGGGGAC

3301 GCGATCTCGG AGTTCCCGCA GGACCGCGGC TGGGACGTGG AGGGGCTGTA CCACCCGGAT

3361 CCCGAGCACC CCGGCACGTC GTACGTCCGC CAGGGCGGTT TCATCGAGAA CGTCGCCGGC

3421 TTCGACGCGG CCTTCTTCGG GATCTCGCCG CGCGAGGCCC TCGCCATGGA CCCGCAGCAG

3481 CGGCTCCTCC TCGAAACCTC CTGGGAGGCC GTCGAGGACG CCGGGATCGA CCCGACCTCC

3541 CTGCGGGGAC GGCAGGTCGG CGTCTTCACT GGGGCGATGA CCCACGAGTA CGGGCCGAGC

3601 CTGCGGGACG GCGGGGAAGG CCTCGACGGC TACCTGCTGA CCGGCAACAC GGCCAGCGTG

3661 ATGTCGGGCC GCGTCTCGTA CACACTCGGC CTTGAGGGCC CCGCCCTGAC GGTGGACACG

3721 GCCTGCTCGT CGTCGCTGGT CGCCCTGCAC CTCGCCGTGC AGGCCCTGCG CAAGGGCGAG

3781 GTCGACATGG CGCTCGCCGG CGGCGTGGCC GTGATGCCCA CGCCCGGGAT GTTCGTCGAG

3841 TTCAGCCGGC AGCGCGGGCT GGCCGGGGAC GGCCGGTCGA AGGCGTTCGC CGCGTCGGCG

3901 GACCGGACCA GCTGGTCCGA GGGCGTCGGC GTCCTCCTCG TCGAGCGCCT GTCGGACGCC

3961 CGCCGCAACG GACACCAGGT CCTCGCGGTC GTCCGCGGCA GCGCCGTGAA CCAGGACGGC

4021 GCGAGCAACG GCCTCACGGC TCCGAACGGG CCCTCGCAGC AGCGCGTCAT CCGGCGCGCG

4081 CTGGCGGACG CCCGGCTGAC GACCTCCGAC GTGGACGTCG TCGAGGCACA CGGCACGGGC

4141 ACGCGACTCG GCGACCCGAT CGAGGCGCAG GCCCTGATCG CCACCTACGG CCAGGGCCGT

4201 GACGACGAAC AGCCGCTGCG CCTCGGGTCG TTGAAGTCCA ACATCGGGCA CACCCAGGCC

4261 GCGGCCGGCG TCTCCGGTGT CATCAAGATG GTCCAGGCGA TGCGCCACGG ACTGCTGCCG

4321 AAGACGCTGC ACGTCGACGA GCCCTCGGAC CAGATCGACT GGTCGGCTGG CGCCGTGGAA

4381 CTCCTCACCG AGGCCGTCGA CTGGCCGGAG AAGCAGGACG GCGGGCTGCG CCGGGCCGCC

4441 GTCTCCTCCT TCGGGATCAG CGGCACCAAT GCGCATGTGG TGCTCGAAGA GGCCCCGGTG

4501 GTTGTCGAGG GTGCTTCGGT CGTCGAGCCG TCGGTTGGCG GGTCGGCGGT CGGCGGCGGT

4561 GTGACGCCTT GGGTGGTGTC GGCGAAGTCC GCTGCCGCGC TCGACGCGCA GATCGAGCGG

4621 CTTGCCGCAT TCGCCTCGCG GGATCGTACG GATGACGCCG ACGCCGGTGC TGTCGACGCG

4681 GGCGCTGTCG CTCACGTACT GGCTGACGGG CGTGCTCAGT TCGAGCACCG GGCCGTCGCG

4741 CTCGGCGCCG GGGCGGACGA CCTCGTACAG GCGCTGGCCG ATCCGGACGG GCTGATACGC

4801 GGAACGGCTT CCGGTGTCGG GCGAGTGGCG TTCGTGTTCC CCGGTCAGGG CACGCAGTGG

4861 GCTGGCATGG GTGCCGAACT GCTGGACTCT TCCGCGGTGT TCGCGGCGGC CATGGCCGAG

4921 TGTGAGGCCG CGCTGTCCCC GTACGTCGAC TGGTCGCTGG AGGCCGTCGT ACGGCAGGCC
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4981 CCCGGTGCGC CCACGCTGGA GCGGGTCGAT GTCGTGCAGC CTGTGACGTT CGCCGTCATG
5041 GTCTCGCTGG CTCGCGTGTG GCAGCACCAC GGTGTGACGC CCCAGGCGGT CGTCGGCCAC
5101 TCGCAGGGCG AGATCGCCGC CGCGTACGTC GCCGGAGCCC TGCCCCTGGA CGACGCCGCC
5161 CGCGTCGTCA CCCTGCGCAG CAAGTCCATC GCCGCCCACC TCGCCGGCAA GGGCGGCATG
5221 CTGTCCCTCG CGCTGAACGA GGACGCCGTC CTGGAGCGAC TGAGTGACTT CGACGGGCTG
5281 TCCGTCGCCG CCGTCAACGG GCCCACCGCC ACTGTCGTGT CGGGTGACCC CGTACAGATC
5341 GAAGAGCTTG CTCAGGCGTG CAAGGCGGAC GGATTCCGCG CGCGGATCAT TCCCGTCGAC
5401 TACGCGTCCC ACAGCCGGCA GGTCGAGATC ATCGAGAGCG AGCTCGCCCA GGTCCTCGCC
5461 GGTCTCAGCC CGCAGGCCCC GCGCGTGCCG TTCTTCTCGA CGCTCGAAGG CACCTGGATC
5521 ACCGAGCCCG TCCTCGACGG CACCTACTGG TACCGCAACC TCCGTCACCG CGTCGGCTTC
5581 GCCCCCGCCA TCGAGACCCT GGCCGTCGAC GAGGGCTTCA CGCACTTCGT CGAGGTCAGC
5641 GCCCACCCCG TCCTCACCAT GACCCTCCCC GAGACCGTCA CCGGCCTCGG CACCCTCCGT
5701 CGCGAACAGG GAGGCCAAGA GCGTCTGGTC ACCTCGCTCG CCGAGGCGTG GGTCAACGGG
5761 CTTCCCGTGG CATGGACTTC GCTCCTGCCC GCCACGGCCT CCCGCCCCGG TCTGCCCACC
5821 TACGCCTTCC AGGCCGAGCG CTACTGGCTC GAGAACACTC CCGCCGCCCT GGCCACCGGC
5881 GACGACTGGC GCTACCGCAT CGACTGGAAG CGCCTCCCGG CCGCCGAGGG GTCCGAGCGC
5941 ACCGGCCTGT CCGGCCGCTG GCTCGCCGTC ACGCCGGAGG ACCACTCCGC GCAGGCCGCC
6001 GCCGTGCTCA CCGCGCTGGT CGACGCCGGG GCGAAGGTCG AGGTGCTGAC GGCCGGGGCG
6061 GACGACGACC GTGAGGCCCT CGCCGCCCGG CTCACCGCAC TGACGACCGG TGACGGCTTC
6121 ACCGGCGTGG TCTCGCTCCT CGACGGACTC GTACCGCAGG TCGCCTGGGT CCAGGCGCTC
6181 GGCGACGCCG GAATCAAGGC GCCCCTGTGG TCCGTCACCC AGGGCGCGGT CTCCGTCGGA
6241 CGTCTCGACA CCCCCGCCGA CCCCGACCGG GCCATGCTCT GGGGCCTCGG CCGCGTCGTC
6301 GCCCTTGAGC ACCCCGAACG CTGGGCCGGC CTCGTCGACC TCCCCGCCCA GCCCGATGCC
6361 GCCGCCCTCG CCCACCTCGT CACCGCACTC TCCGGCGCCA CCGGCGAGGA CCAGATCGCC
6421 ATCCGCACCA CCGGACTCCA CGCCCGCCGC CTCGCCCGCG CACCCCTCCA CGGACGTCGG
6481 CCCACCCGCG ACTGGCAGCC CCACGGCACC GTCCTCATCA CCGGCGGCAC CGGAGCCCTC
6541 GGCAGCCACG CCGCACGCTG GATGGCCCAC CACGGAGCCG AACACCTCCT CCTCGTCAGC
6601 CGCAGCGGCG AACAAGCCCC CGGAGCCACC CAACTCACCG CCGAACTCAC CGCATCGGGC
6661 GCCCGCGTCA CCATCGCCGC CTGCGACGTC GCCGACCCCC ACGCCATGCG CACCCTCCTC
6721 GACGCCATCC CCGCCGAGAC GCCCGTCACC GCCGTCGTCC ACACCGCCGG CGCGCTCGAC
6781 GACGGCATCG TGGACACGCT GACCGCCGAG CAGGTCCGGC GGGCCCACCG TGCGAAGGCC
6841 GTCGGCGCCT CGGTGCTCGA CGAGCTGACC CGGGACCTCG ACCTCGACGC GTTCGTGCTC
6901 TTCTCGTCCG TGTCGAGCAC TCTGGGCATC CCCGGTCAGG GCAACTACGC CCCGCACAAC
6961 GCCTACCTCG ACGCCCTCGC GGCTCGCCGC CGGGCCACCG GCCGGTCCGC CGTCTCGGTG
7021 GCCTGGGGAC CGTGGGACGG TGGCGGCATG GCCGCCGGTG ACGGCGTGGC CGAGCGGCTG
7081 CGCAACCACG GCGTGCCCGG CATGGACCCG GAACTCGCCC TGGCCGCACT GGAGTCCGCG
7141 CTCGGCCGGG ACGAGACCGC GATCACCGTC GCGGACATCG ACTGGGACCG CTTCTACCTC
7201 GCGTACTCCT CCGGTCGCCC GCAGCCCCTC GTCGAGGAGC TGCCCGAGGT GCGGCGCATC
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7261 ATCGACGCAC GGGACAGCGC CACGTCCGGA CAGGGCGGGA GCTCCGCCCA GGGCGCCAAC

7321 CCCCTGGCCG AGCGGCTGGC CGCCGCGGCT CCCGGCGAGC GTACGGAGAT CCTCCTCGGT

7381 CTCGTACGGG CGCAGGCCGC CGCCGTGCTC CGGATGCGTT CGCCGGAGGA CGTCGCCGCC

7441 GACCGCGCCT TCAAGGACAT CGGCTTCGAC TCGCTCGCCG GTGTCGAGCT GCGCAACAGG

7501 CTGACCCGGG CGACCGGGCT CCAGCTGCCC GCGACGCTCG TCTTCGACCA CCCGACGCCG

7561 CTGGCCCTCG TGTCGCTGCT CCGCAGCGAG TTCCTCGGTG ACGAGGAGAC GGCGGACGCC

7621 CGGCGGTCCG CGGCGCTGCC CGCGACTGTC GGTGCCGGTG CCGGCGCCGG CGCCGGCACC

7681 GATGCCGACG ACGATCCGAT CGCGATCGTC GCGATGAGCT GCCGCTACCC CGGTGACATC

7741 CGCAGCCCGG AGGACCTGTG GCGGATGCTG TCCGAGGGCG GCGAGGGCAT CACGCCGTTC

7801 CCCACCGACC GCGGCTGGGA CCTCGACGGC CTGTACGACG CCGACCCGGA CGCGCTCGGC

7861 AGGGCGTACG TCCGCGAGGG CGGGTTCCTG CACGACGCGG CCGAGTTCGA CGCGGAGTTC

7921 TTCGGCGTCT CGCCGCGCGA GGCGCTGGCC ATGGACCCGC AGCAGCGGAT GCTCCTGACG

7981 ACGTCCTGGG AGGCCTTCGA GCGGGCCGGC ATCGAGCCGG CATCGCTGCG CGGCAGCAGC

8041 ACCGGTGTCT TCATCGGCCT CTCCTACCAG GACTACGCGG CCCGCGTCCC GAACGCCCCG

8101 CGTGGCGTGG AGGGTTACCT GCTGACCGGC AGCACGCCGA GCGTCGCGTC GGGCCGTATC

8161 GCGTACACCT TCGGTCTCGA AGGGCCCGCG ACGACCGTCG ACACCGCCTG CTCGTCGTCG

8221 CTGACCGCCC TGCACCTGGC GGTGCGGGCG CTGCGCAGCG GCGAGTGCAC GATGGCGCTC

8281 GCCGGTGGCG TGGCGATGAT GGCGACCCCG CACATGTTCG TGGAGTTCAG CCGTCAGCGG

8341 GCGCTCGCCC CGGACGGCCG CAGCAAGGCC TTCTCGGCGG ACGCCGACGG GTTCGGCGCC

8401 GCGGAGGGCG TCGGCCTGCT GCTCGTGGAG CGGCTCTCGG ACGCGCGGCG CAACGGTCAC

8461 CCGGTGCTCG CCGTGGTCCG CGGTACCGCC GTCAACCAGG ACGGCGCCAG CAACGGGCTG

8521 ACCGCGCCCA ACGGACCCTC GCAGCAGCGG GTGATCCGGC AGGCGCTCGC CGACGCCCGG

8581 CTGGCACCCG GCGACATCGA CGCCGTCGAG ACGCACGGCA CGGGAACCTC GCTGGGCGAC

8641 CCCATCGAGG CCCAGGGCCT CCAGGCCACG TACGGCAAGG AGCGGCCCGC GGAACGGCCG

8701 CTCGCCATCG GCTCCGTGAA GTCCAACATC GGACACACCC AGGCCGCGGC CGGTGCGGCG

8761 GGCATCATCA AGATGGTCCT CGCGATGCGC CACGGCACCC TGCCGAAGAC CCTCCACGCC

8821 GACGAGCCGA GCCCGCACGT CGACTGGGCG AACAGCGGCC TGGCCCTCGT CACCGAGCCG

8881 ATCGACTGGC CGGCCGGCAC CGGTCCGCGC CGCGCCGCCG TCTCCTCCTT CGGCATCAGC

8941 GGGACGAACG CGCACGTCGT GCTGGAGCAG GCGCCGGATG CTGCTGGTGA GGTGCTTGGG

9001 GCCGATGAGG TGCCTGAGGT GTCTGAGACG GTAGCGATGG CTGGGACGGC TGGGACCTCC

9061 GAGGTCGCTG AGGGCTCTGA GGCCTCCGAG GCCCCCGCGG CCCCCGGCAG CCGTGAGGCG

9121 TCCCTCCCCG GGCACCTGCC CTGGGTGCTG TCCGCCAAGG ACGAGCAGTC GCTGCGCGGC

9181 CAGGCCGCCG CCCTGCACGC GTGGCTGTCC GAGCCCGCCG CCGACCTGTC GGACGCGGAC

9241 GGACCGGCCC GCCTGCGGGA CGTCGGGTAC ACGCTCGCCA CGAGCCGTAC CGCCTTCGCG

9301 CACCGCGCCG CCGTGACCGC CGCCGACCGG GACGGGTTCC TGGACGGGCT GGCCACGCTG

9361 GCCCAGGGCG GCACCTCGGC CCACGTCCAC CTGGACACCG CCCGGGACGG CACCACCGCG

9421 TTCCTCTTCA CCGGCCAGGG CAGTCAGCGC CCCGGCGCCG GCCGTGAGCT GTACGACCGG

9481 CACCCCGTCT TCGCCCGGGC GCTCGACGAG ATCTGCGCCC ACCTCGACGG TCACCTCGAA

9541 CTGCCCCTGC TCGACGTGAT GTTCGCGGCC GAGGGCAGCG CGGAGGCCGC GCTGCTCGAC
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 9601 GAGACGCGGT ACACGCAGTG CGCGCTGTTC GCCCTGGAGG TCGCGCTCTT CCGGCTCGTC
 9661 GAGAGCTGGG GCATGCGGCC GGCCGCACTG CTCGGTCACT CGGTCGGCGA GATCGCCGCC
 9721 GCGCACGTCG CCGGTGTGTT CTCGCTCGCC GACGCCGCCC GCCTGGTCGC CGCGCGCGGC
 9781 CGGCTCATGC AGGAGCTGCC CGCCGGTGGC GCGATGCTCG CCGTCCAGGC CGCGGAGGAC
 9841 GAGATCCGCG TGTGGCTGGA GACGGAGGAG CGGTACGCGG GACGTCTGGA CGTCGCCGCC
 9901 GTCAACGGCC CCGAGGCCGC CGTCCTGTCC GGCGACGCGG ACGCGGCGCG GGAGGCGGAG
 9961 GCGTACTGGT CCGGGCTCGG CCGCAGGACC CGCGCGCTGC GGGTCAGCCA CGCCTTCCAC
10021 TCCGCGCACA TGGACGGCAT GCTCGACGGG TTCCGCGCCG TCCTGGAGAC GGTGGAGTTC
10081 CGGCGCCCCT CCCTGACCGT GGTCTCGAAC GTCACCGGCC TGGCCGCCGG CCCGGACGAC
10141 CTGTGCGACC CCGAGTACTG GGTCCGGCAC GTCCGCGGCA CCGTCCGCTT CCTCGACGGC
10201 GTCCGTGTCC TGCGCGACCT CGGCGTGCGG ACCTGCCTGG AGCTGGGCCC CGACGGGGTC
10261 CTCACCGCCA TGGCGGCCGA CGGCCTCGCG GACACCCCCG CGGATTCCGC TGCCGGCTCC
10321 CCCGTCGGCT CTCCCGCCGG CTCTCCCGCC GACTCCGCCG CCGGCGCGCT CCGGCCCCGG
10381 CCGCTGCTCG TGGCGCTGCT GCGCCGCAAG CGGTCGGAGA CCGAGACCGT CGCGGACGCC
10441 CTCGGCAGGG CGCACGCCCA CGGCACCGGA CCCGACTGGC ACGCCTGGTT CGCCGGCTCC
10501 GGGGCGCACC GCGTGGACCT GCCCACGTAC TCCTTCCGGC GCGACCGCTA CTGGCTGGAC
10561 GCCCCGGCGG CCGACACCGC GGTGGACACC GCCGGCCTCG GTCTCGGCAC CGCCGACCAC
10621 CCGCTGCTCG GCGCCGTGGT CAGCCTTCCG GACCGGGACG GCCTGCTGCT CACCGGCCGC
10681 CTCTCCCTGC GCACCCACCC GTGGCTCGCG GACCACGCCG TCCTGGGGAG CGTCCTGCTC
10741 CCCGGCGCCG CGATGGTCGA ACTCGCCGCG CACGCTGCGG AGTCCGCCGG TCTGCGTGAC
10801 GTGCGGGAGC TGACCCTCCT TGAACCGCTG GTACTGCCCG AGCACGGTGG CGTCGAGCTG
10861 CGCGTGACGG TCGGGGCGCC GGCCGGAGAG CCCGGTGGCG AGTCGGCCGG GGACGGCGCA
10921 CGGCCCGTCT CCCTCCACTC GCGGCTCGCC GACGCGCCCG CCGGTACCGC CTGGTCCTGC
10981 CACGCGACCG GTCTGCTGGC CACCGACCGG CCCGAGCTTC CCGTCGCGCC CGACCGTGCG
11041 GCCATGTGGC CGCCGCAGGG CGCCGAGGAG GTGCCGCTCG ACGGTCTCTA CGAGCGGCTC
11101 GACGGGAACG GCCTCGCCTT CGGTCCGCTG TTCCAGGGGC TGAACGCGGT GTGGCGGTAC
11161 GAGGGTGAGG TCTTCGCCGA CATCGCGCTC CCCGCCACCA CGAATGCGAC CGCGCCCGCG
11221 ACCGCGAACG GCGGCGGGAG TGCGGCGGCG GCCCCCTACG GCATCCACCC CGCCCTGCTC
11281 GACGCTTCGC TGCACGCCAT CGCGGTCGGC GGTCTCGTCG ACGAGCCCGA GCTCGTCCGC
11341 GTCCCCTTCC ACTGGAGCGG TGTCACCGTG CACGCGGCCG GTGCCGCGGC GGCCCGGGTC
11401 CGTCTCGCCT CCGCGGGGAC GGACGCCGTC TCGCTGTCCC TGACGGACGG CGAGGGACGC
11461 CCGCTGGTCT CCGTGGAACG GCTCACGCTG CGCCCGGTCA CCGCCGATCA GGCGGCGGCG
11521 AGCCGCGTCG GCGGGCTGAT GCACCGGGTG GCCTGGCGTC CGTACGCCCT CGCCTCGTCC
11581 GGCGAACAGG ACCCGCACGC CACTTCGTAC GGGCCGACCG CCGTCCTCGG CAAGGACGAG
11641 CTGAAGGTCG CCGCCGCCCT GGAGTCCGCG GGCGTCGAAG TCGGGCTCTA CCCCGACCTG
11701 GCCGCGCTGT CCCAGGACGT GGCGGCCGGC GCCCCGGCGC CCCGTACCGT CCTTGCGCCG
11761 CTGCCCGCGG GTCCCGCCGA CGGCGGCGCG GAGGGTGTAC GGGGCACGGT GGCCCGGACG
11821 CTGGAGCTGC TCCAGGCCTG GCTGGCCGAC GAGCACCTCG CGGGCACCCG CCTGCTCCTG
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11881 GTCACCCGCG GTGCGGTGCG GGACCCCGAG GGGTCCGGCG CCGACGATGG CGGCGAGGAC

11941 CTGTCGCACG CGGCCGCCTG GGGTCTCGTA CGGACCGCGC AGACCGAGAA CCCCGGCCGC

12001 TTCGGCCTTC TCGACCTGGC CGACGACGCC TCGTCGTACC GGACCCTGCC GTCGGTGCTC

12061 TCCGACGCGG GCCTGCGCGA CGAACCGCAG CTCGCCCTGC ACGACGGCAC CATCAGGCTG

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12181 GGCACGGTCC TGCTGACCGG CGGCACCGGC GGCCTGGGCG GACTGGTCGC CCGGCACGTG

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26761 GGACGCCCTG CGGATGCCGG AAGGACTGGT ACGCGGCACG TCCTCGGACG TGGGCCGGGT

26821 GGCGTTCGTC TTCCCCGGCC AGGGCACGCA GTGGGCCGGC ATGGGCGCCG AACTCCTTGA

26881 CAGCTCACCG GAGTTCGCTG CCTCGATGGC CGAATGCGAG ACCGCGCTCT CCCGCTACGT

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38401 CACCACCGTG GCGGCCGCCG CTCCCGGCGC CGCCGACACG GCCAATGTTC AGTACACGAG

38461 CCGGGCGGCG GAGCTCGTCG CCCAGATGAC GCTCGACGAG AAGATC

[0044] Those of skill in the art will recognize that, due to the degenerate nature of the genetic code, a variety of DNA compounds differing in their nucleotide sequences can be used to encode a given amino acid sequence of the invention. The native DNA sequence encoding the narbonolide PKS of *Streptomyces venezuelae* is shown herein merely to illustrate a preferred embodiment of the invention, and the invention includes DNA compounds of any sequence that encode the amino acid sequences of the polypeptides and proteins of the invention. In similar fashion, a polypeptide can typically tolerate one or more amino acid substitutions, deletions, and insertions in its amino acid sequence without loss or significant loss of a desired activity. The present invention includes such polypeptides with alternate amino acid sequences, and the amino acid sequences shown merely illustrate preferred embodiments of the invention.

[0045] The recombinant nucleic acids, proteins, and peptides of the invention are many and diverse. To facilitate an understanding of the invention and the diverse compounds and methods provided thereby, the following description of the various regions of the narbonolide PKS and corresponding coding sequences is provided.

[0046] The loading module of the narbonolide PKS contains an inactivated KS domain, an AT domain, and an ACP domain. The AT domain of the loading module binds propionyl CoA. Sequence analysis of the DNA encoding the KS domain indicates that this domain is enzymatically inactivated, as a critical cysteine residue in the motif TVDACSSSL, which is highly conserved among KS domains, is replaced by a glutamine and so is referred to as a KSQ domain. Such inactivated KS domains are also found in the PKS enzymes that synthesize the 16-membered macrolides carbomycin, spiromycin, tylosin, and niddamycin. While the KS domain is inactive for its usual function in extender modules, it is believed to serve as a decarboxylase in the loading module.

[0047] The present invention provides recombinant DNA compounds that encode the loading module of the narbonolide PKS and useful portions thereof. These recombinant DNA compounds are useful in the construction of PKS coding sequences that encode all or a portion of the narbonolide PKS and in the construction of hybrid PKS encoding DNA compounds of the invention, as described in the section concerning hybrid PKSs below. To facilitate description of the invention, reference to a PKS, protein, module, or domain herein can also refer to DNA compounds comprising coding sequences therefor and vice versa. Also, reference to a heterologous PKS refers to a PKS or DNA compounds comprising coding sequences therefor from an organism other than *Streptomyces venezuelae*. In addition, reference to a PKS or its coding sequence includes reference to any portion thereof.

[0048] The present invention provides recombinant DNA compounds that encode one or more of the domains of each of the six extender modules (modules 1-6, inclusive) of the narbonolide PKS. Modules 1 and 5 of the narbonolide PKS

are functionally similar. Each of these extender modules contains a KS domain, an AT domain specific for methylmalonyl CoA, a KR domain, and an ACP domain. Module 2 of the narbonolide PKS contains a KS domain, an AT domain specific for malonyl CoA, a KR domain, a DH domain, and an ACP domain. Module 3 differs from extender modules f and 5 only in that it contains an inactive ketoreductase domain. Module 4 of the narbonolide PKS contains a KS domain, an AT domain specific for methylmalonyl CoA, a IR domain, a DH domain, an ER domain, and an ACP domain. Module 6 of the narbonolide PKS contains a KS domain, an AT domain specific for methylmalonyl CoA, and an ACP domain.

[0049] In one important embodiment, the invention provides a recombinant narbonolide PKS that can be used to express only narbonolide (as opposed to the mixture of narbonolide and 10-deoxymethynolide that would otherwise be produced) in recombinant host cells. This recombinant narbonolide PKS results from a fusion of the coding sequences of the picAIII and picAIV genes so that extender modules 5 and 6 are present on a single protein. This recombinant PKS can be constructed on the *Streptomyces venezuelae* or *S. narbonensis* chromosome by homologous recombination. Alternatively, the recombinant PKS can be constructed on an expression vector and introduced into a heterologous host cell. This recombinant PKS is preferred for the expression of narbonolide and its glycosylated and/or hydroxylated derivatives, because a lesser amount or no 10-deoxymethynolide is produced from the recombinant PKS as compared to the native PKS. In a related embodiment, the invention provides a recombinant narbonolide PKS in which the picAIV gene has been rendered inactive by an insertion, deletion, or replacement. This recombinant PKS of the invention is useful in the production of 10-deoxymethynolide and its derivatives without production of narbonolide.

[0050] In similar fashion, the invention provides recombinant narbonolide PKS in which any of the domains of the native PKS have been deleted or rendered inactive to make the corresponding narbonolide or 10-deoxymethynolide derivative. Thus, the invention also provides recombinant narbonolide PKS genes that differ from the narbonolide PKS gene by one or more deletions. The deletions can encompass one or more modules and/or can be limited to a partial deletion within one or more modules. When a deletion encompasses an entire module, the resulting narbonolide derivative is at least two carbons shorter than the polyketide produced from the PKS encoded by the gene from which deleted PKS gene and corresponding polyketide were derived. When a deletion is within a module, the deletion typically encompasses a KR, DH, or ER domain, or both DH and ER domains, or both KR and DH domains, or all three KR, DH, and ER domains.

[0051] This aspect of the invention is illustrated in **FIG. 4**, parts B and C, which shows how a vector of the invention, plasmid pKOS039-16 (not shown), was used to delete or "knock out" the picAI gene from the *Streptomyces venezuelae* chromosome. Plasmid pKOS039-16 comprises two segments (shown as cross-hatched boxes in **FIG. 4**, part B) of DNA flanking the picAI gene and isolated from cosmid pKOS023-27 (shown as a linear segment in the Figure) of the invention. When plasmid pKOS039-16 was used to transform *S. venezuelae* and a double crossover homologous recombination event occurred, the picAI gene was deleted. The resulting host cell, designated K039-03 in the Figure, does not produce picromycin unless a functional picAI gene is introduced.

[0052] This *Streptomyces venezuelae* K039-03 host cell and corresponding host cells of the invention are especially useful for the production of polyketides produced from hybrid PKS or narbonolide PKS derivatives. Especially preferred for production in this host cell are narbonolide derivatives produced by PKS enzymes that differ from the narbonolide PKS only in the loading module and/or extender modules 1 and/or 2. These are especially preferred, because one need only introduce into the host cell the modified picAI gene or other corresponding gene to produce the desired PKS and corresponding polyketide. These host cells are also preferred for desosaminylating polyketides in accordance with the method of the invention in which a polyketide is provided to an *S. venezuelae* cell and desosaminylated by the endogenous desosamine biosynthesis and desosaminyl transferase gene products.

[0053] The recombinant DNA compounds of the invention that encode each of the domains of each of the modules of the narbonolide PKS are also useful in the construction of expression vectors for the heterologous expression of the narbonolide PKS and for the construction of hybrid PKS expression vectors, as described further below.

[0054] Section II: The Genes for Desosamine Biosynthesis and Transfer and for Beta-glucosidase

[0055] Narbonolide and 10-deoxymethynolide are desosaminylated in *Streptomyces venezuelae* and *S. narbonensis* to yield narbomycin and YC-17, respectively. This conversion requires the biosynthesis of desosamine and the transfer of the desosamine to the substrate polyketides by the enzyme desosaminyl transferase. Like other *Streptomyces, S. venezuelae* and *S. narbonensis* produce glucose and a glucosyl transferase enzyme that glucosylates desosamine at the 2' position. However, *S. venezuelae* and *S. narbonensis* also produce an enzyme called beta-glucosidase, which removes the glucose residue from the desosamine. The present invention provides recombinant DNA compounds and expression vectors for each of the desosamine biosynthesis enzymes, desosaminyl transferase, and beta-glucosidase.

[0056] As noted above, cosmid pKOS023-27 contains three ORFs that encode proteins involved in desosamine biosynthesis and transfer. The first ORF is from the picCII gene, also known as des VIII, a homologue of enyCII, believed to encode a 4-keto-6-deoxyglucose isomerase. The second ORF is from the picCIII gene, also known as des VII, a homologue of eryCIII, which encodes a desosaminyl transferase. The third ORF is from the picCVI gene, also known as desVI, a homologue of eryCVI, which encodes a 3-amino dimethyltransferase.

[0057] The three genes above and the remaining desosamine biosynthetic genes can be isolated from cosmid pKOS023-26, which was deposited with the American Type Culture Collection on 20 Aug. 1998 under the Budapest Treaty and is available under the accession number ATCC 203141. **FIG. 3** shows a restriction site and function map of cosmid pKOS023-26. This cosmid contains a region of overlap with cosmid pKOS02327 representing nucleotides 14252 to nucleotides 38506 of pKOS023-27.

[0058] The remaining desosamine biosynthesis genes on cosmid pKOS023-26 include the following genes. ORF11, also known as desR, encodes beta-glucosidase and has no ery gene homologue. The picCI gene, also known as desV, is a homologue of eryCI. ORF14, also known as desIV, has no known ery gene homologue and encodes an NDP glucose 4,6-dehydratase. ORF13, also known as desIII, has no known ery gene homologue and encodes an NDP glucose synthase. The picCV gene, also known as desII, a homologue of eryCV is required for desosamine biosynthesis. The picCIV gene also known as desI, is a homologue of eryCIV, and its product is believed to be a 3,4-dehydratase. Other ORFs on cosmid pKOS02326 include ORF12, believed to be a regulatory gene; ORF15, which encodes an S-adenosyl methionine synthase; and ORF16, which is a homolog of the *M. tuberculosis* cbhK gene. Cosmid pKOS023-26 also encodes the picK gene, which encodes the cytochrome P450 hydroxylase that hydroxylates the C12 of narbomycin and the C10 and C12 positions of YC-17. This gene is described in more detail in the following section.

[0059] Below, the amino acid sequences or partial amino acid sequences of the gene products of the desosamine biosynthesis and transfer and beta-glucosidase genes are shown. These amino acid sequences are followed by the DNA sequences that encode them.

Amino acid sequence of PICCI

```
  1VSSRAETPRV PFLDLKAAYE ELRAETDAAI ARVLDSGRYL LGPELEGFEA EFAAYCETDH

 61AVGVNSGMDA LQLALRGLGI GPGDEVIVPS HTYIASWLAV SATGATPVPV EPHEDHPTLD

121PLLVEKAITP RTRALLPVHL YGHPADMDAL RELADRHGLH IVEDAAQAHG ARYRGRRIGA

181GSSVAAFSFY PGKNLGCFGD GGAVVTGDPE LAERLRMLRN YGSRQKYSHE TKGTNSRLDE

241MQAAVLRIRL XHLDSWNGRR SALAAEYLSG LAGLPGIGLP VTAPDTDPVW HLFTVRTERR

301DELRSHLDAR GIDTLTHYPV PVHLSPAYAG EAPPEGSLPR AESFARQVLS LPIGPHLERP

361QALRVIDAVR EWAERVDQA
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Amino acid sequence of 3-keto-6-deoxyglucose isomerase, PICCII

```
  1VADRELGTHL LETRGIHWIH AANGDPYATV LRGQADDPYP AYERVRARGA LSFSPTGSWV

 61TADHALAASI LCSTDFGVSG ADGVPVPQQV LSYGEGCPLE REQVLPAAGD VPEGGQRAVV

121EGIHRETLEG LAPDPSASYA FELLGGFVRP AVTAAAAAVL GVPADRRADF ADLLERLRPL

181SDSLLAPQSL RTVRAADGAL AELTALLADS DDSPGALLSA LGVTAAVQLT GNAVLALLAH

241PEQWRELCDR PGLAAAAVEE TLRYDPPVQL DARVVRGETE LAGRRLPAGA HVVVLTAATG

301RDPEVFTDPE RFDLARPDAA AHLALHPAGP YGPVASLVRL QAEVALRTLA GRFPGLRQAG

361DVLRPRRAPV GRGPLSVPVS SS
```

Amino acid sequence of desosaminyl transferase, PICCIII

```
  1MRVLLTSFAH HTHYYGLVPL AWALLAAGHE VRVASQPALT DTITGSGLAA VPVGTDHLIH

 61EYRVRMAGEP RPNHPAIAFD EARPEPLDWD HALGIEAILA PYFYLLANND SMVDDLVDFA

121RSWQPDLVLW EPITYAGAVA AQVTGAAHAR VLWGPDVMGS ARRKFVALRD RQPPEHREDP

181TAEWLTWTLD RYGASFEEEL LTGQFTIDPT PPSLRLDTGL PTVGMRYVPY NGTSVVPDWL

241SEPPARPRVC LTLGVSAREV LGGDGVSQGD ILEALADLDI ELVATLDASQ RAEIRNYPKH

301TRFTDFVPMH ALLPSCSAII HHGGAGTYAT AVINAVPQVM LAELWDAPVK ARAVAEQGAG

361FFLPPAELTP QAVRDAVVRI LDDPSVATAA HRLREETFGD PTPAGIVPEL ERLAAQHRRP

421PADARH
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Partial amino acid sequence of aminotransferase-dehydrase, PICCIV

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  1VKSALSDLAF FGGPAAFDQP LLVGRPNRID RARLYERLDR ALDSQWLSNG GPLVREFEER

 61VAGLAGVRHA VATCNATAGL QLLAHAAGLT GEVIMPSMTF AATPHALRWI GLTPVFADID

121PDTGNLDPDQ VAAAVTPRTS AVVGVHLWGR PCAADQLRKV ADEHGLRLYF DAAHALGCAV

181DGRPAGSLGD AEVFSFHATK AVNAFEGGAV VTDDADLAAR IRALHNFGFD LPGGSPAGGT

241NAKMSEAAAA MGLTSLDAFP EVIDRNRRNH AXYREHLADL PGVLVADHDR HGLNNHQYVI

301VEIDEATTGI HRDLVMEVLK AEGVHTRAYF S
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Amino acid sequence of PICCV

1 MTAPALSATA PAERCAHPGA DLGAAVHAVG QTLAAGGLVP PDEAGTTARH LVRLAVRYGN

61 SPFTPLEEAR HDLGVDRDAF RRLLALFGQV PELRTAVETG PAGAYWKNTL LPLEQRGVFD

121 AALARKPVFP YSVGLYPGPT CMFRCHFCVR VTGARYDPSA LDAGNAMFRS VIDEIPAGNP

181 SAMYFSGGLE PLTNPGLGSL AAHATDHGLR PTVYTNSFAL TERTLERQPG LWGLHAIRTS

241 LYGLNDEEYE QTTGKKAAFR RVRENLRRFQ QLRAERESPI NLGFAYIVLP GRASRLLDLV

301 DFIADLNDAG QGRTIDFVNI REDYSGRDDG KLPQEERAEL QEALNAFEER VRERTPGLHI

361 DYGYALNSLR TGADAELLRI KPATMRPTAH PQVAVQVDLL GDVYLYREAG FPDLDGATRY

421 IAGRVTPDTS LTEVVRDFVE RGGEVAAVDG DEYFMDGFDQ VVTARLNQLE RDAADGWEEA

481 RGFLR

Amino acid sequence of 3-amino dimethyl transferase, PICCVI

1 VYEVDHADVY DLFYLGRGKD YAAEASDIAD LVRSRTPEAS SLLDVACGTG THLEHFTKEF

61 GDTAGLELSE DMLTHARKRL PDATLHQGDM RDFRLGRKFS AVVSMFSSVG YLKTTEELGA

121 AVASFAEHLE PGGVVVVEPW WFPETFADGW VSADVVRRDG RTVARVSHSV REGNATRMEV

181 HFTVADPGKG VRHFSDVHLI TLFHQAEYEA AFTAAGLRVE YLEGGPSGRG LFVGVPA

Partial amino acid sequence of beta-glucosidase, ORF11

1 MTLDEKISFV HWALDPDRQN VGYLPGVPRL GIPELRAADG PNGIRLVGQT ATALPAPVAL

61 ASTFDDTMAD SYGKVMGRDG RALNQDMVLG PMMNNIRVPH GGRNYETFSE DPLVSSRTAV

121 AQIKGIQGAG LMTTAKHFAA NNQENNRFSV NANVDEQTLR EIEFPAFEAS SKAGAGSFMC

181 AYNGLNGKPS CGNDELLNNV LRTQWGFQGW VMSDWLATPG TDAITKGLDQ EMGVELPGDV

241 PKGEPSPPAK FFGEALKTAV LNGTVPEAAV TRSAERIVGQ MEKFGLLLAT PAPRPERDKA

301 GAQAVSRKVA ENGAVLLRNE GQALPLAGDA GKSIAVIGPT AVDPKVTGLG SAHVVPDSAA

361 APLDTIKARA GAGATVTYET GEETFGTQIP AGNLSPAFNQ GHQLEPGKAG ALYDGTLTVP

421 ADGEYRIAVR ATGGYATVQL GSHTIEAGQV YGKVSSPLLK LTKGTHKLTI SGFAMSATPL

481 SLELGWVTPA AADATIAKAV ESARKARTAV VFAYDDGTEG VDRPNLSLPG TQDKLISAVA

541 DANPNTIVVL NTGSSVLMPW LSKTRAVLDM WYPGQAGAEA TAALLYGDVN PSGKLTQSFP

601 AAENQHAVAG DPTSYPGVDN QQTYREGIHV GYRWFDKENV KPLFPFGHGL SYTSFTQSAP

661 TVVRTSTGGL KVTVTVRNSG KRAGQEVVQA YLGASPNVTA PQAKKKLVGY TKVSLAAGEA

721 KTVTVNVDRR QLQFWDAATD NWKTGTGNRL LQTGSSSADL RGSATVNVW

Amino acid sequence of transcriptional activator, ORF12

1 MNLVERDGEI AHLRAVLDAS AAGDGTLLLV SGPAGSGKTE LLRSLRRLAA ERETPVWSVR

61 ALPGDRDIPL GVLCQLLRSA EQHGADTSAV RDLLDAASRR AGTSPPPPTR RSASTRHTAC

121 TTGCSPSPAG TPFLVAVDDL THADTASLRF LLYCAAHHDQ GGIGFVMTER ASQRAGYRVF

181 RAELLRQPHC RNMWLSGLPP SGVRQLLAHY YGPEAAERRA PAYHATTGGN PLLLRALTQD

241 RQASHTTLGA AGGDEPVHGD AFAQAVLDCL HRSAEGTLET ARWLAVLEQS DPLLVERLTG

301 TTAAAVERHI QELAAIGLLD EDGTLGQPAI REAALQDLPA GERTELHRRA AEQLHRDGAD

361 EDTVARHLLV GGAPDAPWAL PLLERGAQQA LFDDRLDDAF RILEFAVRSS TDNTQLARLA

421 PHLVAASWRM NPHMTTRALA LFDRLLSGEL PPSHPVMALI RCLVWYGRLP EAADALSRLR

481 PSSDNDALEL SLTRMWLAAL CPPLLESLPA TPEPERGPVP VRLAPRTTAL QAQAGVFQRG

541 PDNASVAQAE QILQGCRLSE ETYEALETAL LVLVHADRLD RALFWSDALL AEAVERRSLG

601 WEAVFAATRA MIAIRCGDLP TARERAELAL SHAAPESWGL AVGMPLSALL LACTEAGEYE

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661QAERVLRQPV PDAMFDSRHG MEYMHARGRY WLAXGRLHAA LGEFMLCGEI LGSWNLDQPS

721IVPWRTSAAE VYLRLGNRQK ARALAEAQLA LVRPGRSRTR GLTLRVLAAA VDGQQAERLH

781AEAVDMLHDS GDRLEHARAL AGMSRHQQAQ GDNYRARMTA RLAGDMAWAC GAYPLAEEIV

841PGRGGRRAKA VSTELELPGG PDVGLLSEAE RRVAALAARG LTNRQIARRL CVTASTVEQH

901LTRVYRKLNV TRRADLPISL AQDKSVTA

Amino acid sequence of dNDP-glucose synthase
(glucose-1-phosphate thymidyl transferase), ORF13

1MKGIVLAGGS GTRLHPATSV ISKQILPVYN KPMIYYPLSV LMLGGIREIQ IISTPQHIEL

61FQSLLGNGRH LGIELDYAVQ KEPAGIADAL LVGAEHIGDD TCALILGDNI FHGPGLYTLL

121RDSIARLDGC VLFGYPVKDP ERYGVAEVDA TGRLTDLVEK PVKPRSNLAV TGLYLYDNDV

181VDIAKNIRPS PRGELEITDV NRVYLERGRA ELVNLGRGFA WLDTGTHDSL LRAAQYVQVL

241EERQGVWIAG LEEIAFRMGF IDAEACHGLG EGLSRTEYGS YLMEIAGREG AP

Amino acid sequence of dNDP-glucose 4,6-dehydratase, ORF14

1VRLLVTGGAG FIGSHFVRQL LAGAYPDVPA DEVIVLDSLT YAGNRANLAP VDADPRLRFV

61HGDIRDAGLL ARELRGVDAI VHFAAESHVD RSIAGASVFT ETNVQGTQTL LQCAVDAGVG

121RVVHVSTDEV YGSIDSGSWT ESSPLEPNSP YAASKAGSDL VARAYHRTYG LDVRITRCCN

181NYGPYQHPEK LIPLFVTNLL DGGTLPLYGD GANVREWVHT DDHCRGIALV LAGGRAGEIY

241HIGGGLELTN RELTGILLDS LGADWSSVRK VADRKGHDLR YSLDGGKIER ELGYRPQVSF

301ADGLARTVRW YRENRGWWEP LKATAPQLPA TAVEVSA

Partial amino acid sequence of S-adenosylmethionine
synthase, ORF15

1IGYDSSKKGF DGASCGVSVS IGSQSPDIAQ GVDTAYEKRV EGASQRDEGD ELDKQGAGDQ

61GLMFGYASDE TPELMPLPIH LAHRLSRRLT EVRKNGTIPY LRPDGKTQVT IEYDGDRAVR

121LDTVVVSSQH ASDIDLESLL APDVRKFVVE HVLAQLVEDG IKLDTDGYRL LVNPTGRFEI

181GGPMGDAGLT GRKIIIDTYG GMARHGGGAF SGKDPSKVDR SAAYAMRWVA KNVVAAGLAS

241RCEVQVAYAI GKAEPVGLFV ETFGTHKIET EKIENAIGEV FDLRPAAIIR DLDLLRPIYS

301QTAAYGHFGR ELPDFTWERT DRVDALKKAA GL

Partial amino acid sequence of ORF16
(homologous to *M. tuberculosis cbhK*)

1MRIAVTGSIA TDHLMTFPGR FAEQILPDQL AHVSLSFLVD TLDIRHGGVA ANIAYGLGLL

61GRRPVLVGAV GKDFDGYGQL LRAAGVDTDS VRVSDRQHTA RFMCTTDEDG NQLASFYAGA

121MAEARDIDLG ETAGRPGGID LVLVGADDPE AMVRHTRVCR ELGLRPAADP SQQLARLEGD

181SVRELVDGAE LLFTNAYERA LLLSKTGWTE QEVLARVGTW ITTLGAKGCR

[0060] While not all of the insert DNA of cosmid pKOS02326 has been sequenced, five large contigs shown of **FIG. 3** have been assembled and provide sufficient sequence information to manipulate the genes therein in accordance with the methods of the invention. The sequences of each of these five contigs are shown below.

[0061] Contig 001 from cosmid pKOS023-26 contains 2401 nucleotides, the first 100 bases of which correspond to 100 bases of the insert sequence of cosmid pKOS023-27. Nucleotides 80-2389 constitute ORF11, which encodes 1 beta glucosidase.

1 CGTGGCGGCC GCCGCTCCCG GCGCCGCCGA CACGGCCAAT GTTCAGTACA CGAGCCGGGC

61 GGCGGAGCTC GTCGCCCAGA TGACGCTCGA CGAGAAGATC AGCTTCGTCC ACTGGGCGCT

121 GGACCCCGAC CGGCAGAACG TCGGCTACCT TCCCGGCGTG CCGCGTCTGG GCATCCCGGA

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 181 GCTGCGTGCC GCCGACGGCC CGAACGGCAT CCGCCTGGTG GGGCAGACCG CCACCGCGCT
 241 GCCCGCGCCG GTCGCCCTGG CCAGCACCTT CGACGACACC ATGGCCGACA GCTACGGCAA
 301 GGTCATGGGC CGCGACGGTC GCGCGCTCAA CCAGGACATG GTCCTGGGCC CGATGATGAA
 361 CAACATCCGG GTGCCGCACG GCGGCCGGAA CTACGAGACC TTCAGCGAGG ACCCCCTGGT
 421 CTCCTCGCGC ACCGCGGTCG CCCAGATCAA GGGCATCCAG GGTGCGGGTC TGATGACCAC
 481 GGCCAAGCAC TTCGCGGCCA ACAACCAGGA GAACAACCGC TTCTCCGTGA ACGCCAATGT
 541 CGACGAGCAG ACGCTCCGCG AGATCGAGTT CCCGGCGTTC GAGGCGTCCT CCAAGGCCGG
 601 CGCGGGCTCC TTCATGTGTG CCTACAACGG CCTCAACGGG AAGCCGTCCT GCGGCAACGA
 661 CGAGCTCCTC AACAACGTGC TGCGCACGCA GTGGGGCTTC CAGGGCTGGG TGATGTCCGA
 721 CTGGCTCGCC ACCCCGGGCA CCGACGCCAT CACCAAGGGC CTCGACCAGG AGATGGGCGT
 781 CGAGCTCCCC GGCGACGTCC CGAAGGGCGA GCCCTCGCCG CCGGCCAAGT TCTTCGGCGA
 841 GGCGCTGAAG ACGGCCGTCC TGAACGGCAC GGTCCCCGAG GCGGCCGTGA CGCGGTCGGC
 901 GGAGCGGATC GTCGGCCAGA TGGAGAAGTT CGGTCTGCTC CTCGCCACTC CGGCCCCGCG
 961 GCCCGAGCGC GACAAGGCGG GTGCCCAGGC GGTGTCCCGC AAGGTCGCCG AGAACGGCGC
1021 GGTGCTCCTG CGCAACGAGG GCCAGGCCCT GCCGCTCGCC GGTGACGCCG GCAAGAGCAT
1081 CGCGGTCATC GGCCCGACGG CCGTCGACCC CAAGGTCACC GGCCTGGGCA GCGCCCACGT
1141 CGTCCCGGAC TCGGCGGCGG CGCCACTCGA CACCATCAAG GCCCGCGCGG GTGCGGGTGC
1201 GACGGTGACG TACGAGACGG GTGAGGAGAC CTTCGGGACG CAGATCCCGG CGGGGAACCT
1261 CAGCCCGGCG TTCAACCAGG GCCACCAGCT CGAGCCGGGC AAGGCGGGGG CGCTGTACGA
1321 CGGCACGCTG ACCGTGCCCG CCGACGGCGA GTACCGCATC GCGGTCCGTG CCACCGGTGG
1381 TTACGCCACG GTGCAGCTCG GCAGCCACAC CATCGAGGCC GGTCAGGTCT ACGGCAAGGT
1441 GAGCAGCCCG CTCCTCAAGC TGACCAAGGG CACGCACAAG CTCACGATCT CGGGCTTCGC
1501 GATGAGTGCC ACCCCGCTCT CCCTGGAGCT GGGCTGGGTN ACGCCGGCGG CGGCCGACGC
1561 GACGATCGCG AAGGCCGTGG AGTCGGCGCG GAAGGCCCGT ACGGCGGTCG TCTTCGCCTA
1621 CGACGACGGC ACCGAGGGCG TCGACCGTCC GAACCTGTCG CTGCCGGGTA CGCAGGACAA
1681 GCTGATCTCG GCTGTCGCGG ACGCCAACCC GAACACGATC GTGGTCCTCA ACACCGGTTC
1741 GTCGGTGCTG ATGCCGTGGC TGTCCAAGAC CCGCGCGGTC CTGGACATGT GGTACCCGGG
1801 CCAGGCGGGC GCCGAGGCCA CCGCCGCGCT GCTCTACGGT GACGTCAACC CGAGCGGCAA
1861 GCTCACGCAG AGCTTCCCGG CCGCCGAGAA CCAGCACGCG GTCGCCGGCG ACCCGACCAG
1921 CTACCCGGGC GTCGACAACC AGCAGACGTA CCGCGAGGGC ATCCACGTCG GGTACCGCTG
1981 GTTCGACAAG GAGAACGTCA AGCCGCTGTT CCCGTTCGGG CACGGCCTGT CGTACACCTC
2041 GTTCACGCAG AGCGCCCCGA CCGTCGTGCG TACGTCCACG GGTGGTCTGA AGGTCACGGT
2101 CACGGTCCGC AACAGCGGGA AGCGCGCCGG CCAGGAGGTC GTCCAGGCGT ACCTCGGTGC
2161 CAGCCCGAAC GTGACGGCTC CGCAGGCGAA GAAGAAGCTC GTGGGCTACA CGAAGGTCTC
2221 GCTCGCCGCG GGCGAGGCGA AGACGGTGAC GGTGAACGTC GACCGCCGTC AGCTGCAGTT
2281 CTGGGATGCC GCCACGGACA ACTGGAAGAC GGGAACGGGC AACCGCCTCC TGCAGACCGG
2341 TTCGTCCTCC GCCGACCTGC GGGGCAGCGC CACGGTCAAC GTCTGGTGAC GTGACGCCGT
2401 G
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[**0062**] Contig 002 from cosmid pKOS023-26 contains 5970 nucleotides and the following ORFs: from nucleotide 995 to 1 is an ORF of picCIV that encodes a partial sequence of an amino transferase-dehydrase; from nucleotides 1356 to 2606 is an ORF of picK that encodes a cytochrome P450 hydroxylase; and from nucleotides 2739 to 5525 is ORF12, which encodes a transcriptional activator.

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   1 GGCGAGAAGT AGGCGCGGGT GTGCACGCCT TCGGCCTTCA GGACCTCCAT GACGAGGTCG
  61 CGGTGGATGC CGGTGGTGGC CTCGTCGATC TCGACGATCA CGTACTGGTG GTTGTTGAGG
 121 CCGTGGCGGT CGTGGTCGGC GACGAGGACG CCGGGGAGGT CCGCGAGGTG CTCGCGGTAG
 181 SCGGCGTGGT TGCGCCGGTT CCGGTCGATG ACCTCGGGAA ACGCGTCGAG GGAGGTGAGG
 241 CCCATGGCGG CGGCGGCCTC GCTCATCTTG GCGTTGGTCC CGCCGGCGGG GCTGCCGCCG
 301 GGCAGGTCGA AGCCGAAGTT GTGGAGGGCG CGGATCCGGG CGGCGAGGTC GGCGTCGTCG
 361 GTGACGACGG CGCCGCCCTC GAAGGCGTTG ACGGCCTTGG TGGCGTGGAA GCTGAAGACC
 421 TCGGCGTCGC CGAGGCTGCC GGCGGGCCGG CCGTCGACCG CGCAGCCGAG GGCGTGCGCG
 481 GCGTCGAAGT ACAGCCGCAG GCCGTGCTCG TCGGCGACCT TCCGCAGCTG GTCGGCGGCG
 541 CAGGGGCGGC CCCAGAGGTG GACGCCGACG ACGGCCGAGG TGCGGGGTGT GACCGCGGCG
 601 GCCACCTGGT CCGGGTCGAG GTTGCCGGTG TCCGGGTCGA TGTCGGCGAA GACCGGGGTG
 661 AGGCCGATCC AGCGCAGTGC GTGCGGGGTG GCGGCGAACG TCATCGACGG CATGATCACT
 721 TCGCCGGTGA GGCCGGCGGC GTGCGCGAGG AGCTGGAGCC CGGCCGTGGC GTTGCAGGTG
 781 GCCACGGCAT GCCGGACCCC GGCGAGCCCG GCGACGCGCT CCTCGAACTC GCGGACGAGC
 841 GGGCCGCCGT TGGACAGCCA CTGGCTGTCG AGGGCCCCGT CGAGCCGCTC GTACAGCCTG
 901 GCGCGGTCGA TGCGGTTGGG CCGCCCCACG AGGAGCGGCT GGTCGAAAGC GGCGGGGCCG
 961 CCGAAGAATG CGAGGTCGGA TAAGGCGCTT TTCACGGATG TTCCCTCCGG GCCACCGTCA
1021 CGAAATGATT CGCCGATCCG GGAATCCCGA ACGAGGTCGC CGCGCTCCAC CGTGACGTAC
1081 GACGAGATGG TCGATTGTGG TGGTCGATTT CGGGGGGACT CTAATCCGCG CGGAACGGGA
1141 CCGACAAGAG CACGCTATGC GCTCTCGATG TGCTTCGGAT CACATCCGCC TCCGGGGTAT
1201 TCCATCGGCG GCCCGAATGT GATGATCCTT GACAGGATCC GGGAATCAGC CGAGCCGCCG
1261 GGAGGGCCGG GGCGCGCTCC GCGGAAGAGT ACGTGTGAGA AGTCCCGTTC CTCTTCCCGT
1321 TTCCGTTCCG CTTCCGGCCC GGTCTGGAGT TCTCCGTGCG CCGTACCCAG CAGGGAACGA
1381 CCGCTCCTCC CCCGGTACTC GACCTCGGGG CCCTGGGGCA GGATTTCGCG GCCGATCCGT
1441 ATCCGACGTA CGCGAGACTG CGTGCCGAGG GTCCGGCCCA CCGGGTGCGC ACCCCCGAGG
1501 GGGACGAGGT GTGGCTGGTC GTCGGCTACG ACCGGGCGCG GGCGGTCCTC GCCGATCCCC
1561 GGTTCAGCAA GGACTGGCGC AACTCCACGA CTCCCCTGAC CGAGGCCGAG GCCGCGCTCA
1621 ACCACAACAT GCTGGAGTCC GACCCGCCGC GGCACACCCG GCTGCGCAAG CTGGTGGCCC
1681 GTGAGTTCAC CATGCGCCGG GTCGAGTTGC TGCGGCCCCG GGTCCAGGAG ATCGTCGACG
1741 GGCTCGTGGA CGCCATGCTG GCGGCGCCCG ACGGCCGCGC CGATCTGATG GAGTCCCTGG
1801 CCTGGCCGCT GCCGATCACC GTGATCTCCG AACTCCTCGG CGTGCCCGAG CCGGACCGCG
1861 CCGCCTTCCG CGTCTGGACC GACGCCTTCG TCTTCCCGGA CGATCCCGCC CAGGCCCAGA
1921 CCGCCATGGC CGAGATGAGC GGCTATCTCT CCCGGCTCAT CGACTCCAAG CGCGGGCAGG
1981 ACGGCGAGGA CCTGCTCAGC GCGCTCGTGC GGACCAGCGA CGAGGACGGC TCCCGGCTGA
2041 CCTCCGAGGA GCTGCTCGGT ATGGCCCACA TCCTGCTCGT CGCGGGGCAC GAGACCACGG
2101 TCAATCTGAT CGCCAACGGC ATGTACGCGC TGCTCTCGCA CCCCGACCAG CTGGCCGCCC
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2161 TGCGGGCCGA CATGACGCTC TTGGACGGCG CGGTGGAGGA GATGTTGCGC TACGAGGGCC

2221 CGGTGGAATC CGCGACCTAC CGCTTCCCGG TCGAGCCCGT CGACCTGGAC GGCACGGTCA

2281 TCCCGGCCGG TGACACGGTC CTCGTCGTCC TGGCCGACGC CCACCGCACC CCCGAGCGCT

2341 TCCCGGACCC GCACCGCTTC GACATCCGCC GGGACACCGC CGGCCATCTC GCCTTCGGCC

2401 ACGGCATCCA CTTCTGCATC GGCGCCCCCT TGGCCCGGTT GGAGGCCCGG ATCGCCGTCC

2461 GCGCCCTTCT CGAACGCTGC CCGGACCTCG CCCTGGACGT CTCCCCCGGC GAACTCGTGT

2521 GGTATCCGAA CCCGATGATC CGCGGGCTCA AGGCCCTGCC GATCCGGTGG CGGCGAGGAC

2581 GGGAGGCGGG CCGCCGTACC GGTTGAACCC GCACGTCACC CATTACGACT CCTTGTCACG

2641 GAAGCCCCGG ATCGGTCCCC CCTCGCCGTA ACAAGACCTG GTTAGAGTGA TGGAGGACGA

2701 CGAAGGGTTC GGCGCCCGGA CGAGGGGGGA CTTCCGCGAT GAATCTGGTG GAACGCGACG

2761 GGGAGATAGC CCATCTCAGG GCCGTTCTTG ACGCATCCGC CGCAGGTGAC GGGACGCTCT

2821 TACTCGTCTC CGGACCGGCC GGCAGCGGGA AGACGGAGCT GCTGCGGTCG CTCCGCCGGC

2881 TGGCCGCCGA GCGGGAGACC CCCGTCTGGT CGGTCCGGGC GCTGCCGGGT GACCGCGACA

2941 TCCCCCTGGG CGTCCTCTGC CAGTTACTCC GCAGCGCCGA ACAACACGGT GCCGACACCT

3001 CCGCCGTCCG CGACCTGCTG GACGCCGCCT CGCGGCGGGC CGGAACCTCA CCTCCCCCGC

3061 CGACGCGCCG CTCCGCGTCG ACGAGACACA CCGCCTGCAC GACTGGCTGC TCTCCGTCTC

3121 CCGCCGGCAC CCCGTTCCTC GTCGCCGTCG ACGACCTGAC CCACGCCGAC ACCGCGTCCC

3181 TGAGGTTCCT CCTGTACTGC GCCGCCCACC ACGACCAGGG CGGCATCGGC TTCGTCATGA

3241 CCGAGCGGGC CTCGCAGCGC GCCGGATACC GGGTGTTCCG CGCCGAGCTC CTCCGCCAGC

3301 CGCACTGCCG CAACATGTGG CTCTCCGGGC TTCCCCCCAG CGGGGTACGC CAGTTACTCG

3361 CCCACTACTA CGGCCCCGAG GCCGCCGAGC GGCGGGCCCC CGCGTACCAC GCGACGACCG

3421 GCGGGAACCC GCTGCTCCTG CGGGCGCTGA CCCAGGACCG GCAGGCCTCC CACACCACCC

3481 TCGGCGCGGC CGGCGGCGAC GAGCCCGTCC ACGGCGACGC CTTCGCCCAG GCCGTCCTCG

3541 ACTGCCTGCA CCGCAGCGCC GAGGGCACAC TGGAGACCGC CCGCTGGCTC GCGGTCCTCG

3601 AACAGTCCGA CCCGCTCCTG GTGGAGCGGC TCACGGGAAC GACCGCCGCC GCCGTCGAGC

3661 GCCACATCCA GGAGCTCGCC GCCATCGGCC TCCTGGACGA GGACGGCACC CTGGGACAGC

3721 CCGCGATCCG CGAGGCCGCC CTCCAGGACC TGCCGGCCGG CGAGCGCACC GAACTGCACC

3781 GGCGCGCCGC GGAGCAGCTG CACCGGGACG GCGCCGACGA GGACACCGTG GCCCGCCACC

3841 TGCTGGTCGG CGGCGCCCCC GACGCTCCCT GGGCGCTGCC CCTGCTCGAA CGGGGCGCGC

3901 AGCAGGCCCT GTTCGACGAC CGACTCGACG ACGCCTTCCG GATCCTCGAG TTCGCCGTGC

3961 GGTCGAGCAC CGACAACACC CAGCTGGCCC GCCTCGCCCC ACACCTGGTC GCGGCCTCCT

4021 GGCGGATGAA CCCGCACATG ACGACCCGGG CCCTCGCACT CTTCGACCGG CTCCTGAGCG

4081 GTGAACTGCC GCCCAGCCAC CCGGTCATGG CCCTGATCCG CTGCCTCGTC TGGTACGGNC

4141 GGCTGCCCGA GGCCGCCGAC GCGCTGTCCC GGCTGCGGCC CAGCTCCGAC AACGATGCCT

4201 TGGAGCTGTC GCTCACCCGG ATGTGGCTCG CGGCGCTGTG CCCGCCGCTC CTGGAGTCCC

4261 TGCCGGCCAC GCCGGAGCCG GAGCGGGGTC CCGTCCCCGT ACGGCTCGCG CCGCGGACGA

4321 CCGCGCTCCA GGCCCAGGCC GGCGTCTTCC AGCGGGGCCC GGACAACGCC TCGGTCGCGC

4381 AGGCCGAACA GATCCTGCAG GGCTGCCGGC TGTCGGAGGA GACGTACGAG GCCCTGGAGA

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4441 CGGCCCTCTT GCTCCTCGTC CACGCCGACC GGCTCGACCG GGCGCTGTTC TGGTCGGACG

4501 CCCTGCTCGC CGAGGCCGTG GAGCGGCGGT CGCTCGGCTG GGAGGCGGTC TTCGCCGCGA

4561 CCCGGGCGAT GATCGCGATC CGCTGCGGCG ACCTCCCGAC GGCGCGGGAG CGGGCCGAGC

4621 TGGCGCTCTC CCACGCGGCG CCGGAGAGCT GGGGCCTCGC CGTGGGCATG CCCCTCTCCG

4681 CGCTGCTGCT CGCCTGCACG GAGGCCGGCG AGTACGAACA GGCGGAGCGG GTCCTGCGGC

4741 AGCCGGTGCC GGACGCGATG TTCGACTCGC GGCACGGCAT GGAGTACATG CACGCCCGGG

4801 GCCGCTACTG GCTGGCGANC GGCCGGCTGC ACGCGGCGCT GGGCGAGTTC ATGCTCTGCG

4861 GGGAGATCCT GGGCAGCTGG AACCTCGACC AGCCCTCGAT CGTGCCCTGG CGGACCTCCG

4921 CCGCCGAGGT GTACCTGCGG CTCGGCAACC GCCAGAAGGC CAGGGCGCTG GCCGAGGCCC

4981 AGCTCGCCCT GGTGCGGCCC GGGCGCTCCC GCACCCGGGG TCTCACCCTG CGGGTCCTGG

5041 CGGCGGCGGT GGACGGCCAG CAGGCGGAGC GGCTGCACGC CGAGGCGGTC GACATGCTGC

5101 ACGACAGCGG CGACCGGCTC GAACACGCCC GCGCGCTCGC CGGGATGAGC CGCCACCAGC

5161 AGGCCCAGGG GGACAACTAC CGGGCGAGGA TGACGGCGCG GCTCGCCGGC GACATGGCGT

5221 GGGCCTGCGG CGCGTACCCG CTGGCCGAGG AGATCGTGCC GGGCCGCGGC GGCCGCCGGG

5281 CGAAGGCGGT GAGCACGGAG CTGGAACTGC CGGGCGGCCC GGACGTCGGC CTGCTCTCGG

5341 AGGCCGAACG CCGGGTGGCG GCCCTGGCAG CCCGAGGATT GACGAACCGC CAGATAGCGC

5401 GCCGGCTCTG CGTCACCGCG AGCACGGTCG AACAGCACCT GACGCGCGTC TACCGCAAAC

5461 TGAACGTGAC CCGCCGAGCA GACCTCCCGA TCAGCCTCGC CCAGGACAAG TCCGTCACGG

5521 CCTGAGCCAC CCCCGGTGTC CCCGTGCGAC GACCCGCCGC ACGGGCCACC GGGCCCGCCG

5581 GGACACGCCG GTGCGACACG GGGGCGCGCC AGGTGCCATG GGGACCTCCG TGACCGCCCG

5641 AGGCGCCCGA GGCGCCCGGT GCGGCACCCG GAGACGCCAG GACCGCCGGG ACCACCGGAG

5701 ACGCCAGGGA CCGCTGGGGA CACCGGGACC TCAGGGACCG CCGGGACCGC CCGAGTTGCA

5761 CCCGGTGCGC CCGGGGACAC CAGACCGCCG GGACCACCCG AGGGTGCCCG GTGTGGCCCC

5821 GGCGGCCGGG GTGTCCTTCA TCGGTGGGCC TTCATCGGCA GGAGGAAGCG ACCGTGAGAC

5881 CCGTCGTGCC GTCGGCGATC AGCCGCCTGT ACGGGCGTCG GACTCCCTGG CGGTCCCGGA

5941 CCCGTCGTAC GGGCTCGCGG GACCCGGTGC
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[0063] Contig 003 from cosmid pKOS023-26 contains 3292 nucleotides and the following ORFs: from nucleotide 104 to 982 is ORF13, which encodes dNDP glucose synthase (glucose-1-phosphate thymidyl transferase); from nucleotide 1114 to 2127 is ORF14, which encodes dNDP-glucose 4,6-dehydratase; and from nucleotide 2124 to 3263 is the picCI ORF.

```
   1 ACCCCCCAAA GGGGTGGTGA CACTCCCCCT GCGCAGCCCC TAGCGCCCCC CTAACTCGCC

  61 ACGCCGACCG TTATCACCGG CGCCCTGCTG CTAGTTTCCG ACAATGAAGG GAATAGTCCT

 121 GGCCGGCGGG AGCGGAACTC GGCTGCATCC GGCGACCTCG GTCATTTCGA AGCAGATTCT

 181 TCCGGTCTAC AACAAACCGA TGATCTACTA TCCGCTGTCG GTTCTCATGC TCGGCGGTAT

 241 TCGCGAGATT CAAATCATCT CGACCCCCCA GCACATCGAA CTCTTCCAGT CGCTTCTCGG

 301 AAACGGCAGG CACCTGGGAA TAGAACTCGA CTATGCGGTC CAGAAAGAGC CCGCAGGAAT

 361 CGCGGACGCA CTTCTCGTCG GAGCCGAGCA CATCGGCGAC GACACCTGCG CCCTGATCCT

 421 GGGCGACAAC ATCTTCCACG GGCCCGGCCT CTACACGCTC CTGCGGGACA GCATCGCGCG
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 481 CCTCGACGGC TGCGTGCTCT TCGGCTACCC GGTCAAGGAC CCCGAGCGGT ACGGCGTCGC
 541 CGAGGTGGAC GCGACGGGCC GGCTGACCGA CCTCGTCGAG AAGCCCGTCA AGCCGCGCTC
 601 CAACCTCGCC GTCACCGGCC TCTACCTCTA CGACAACGAC GTCGTCGACA TCGCCAAGAA
 661 CATCCGGCCC TCGCCGCGCG GCGAGCTGGA GATCACCGAC GTCAACCGCG TCTACCTGGA
 721 GCGGGGCCGG GCCGAACTCG TCAACCTGGG CCGCGGCTTC GCCTGGCTGG ACACCGGCAC
 781 CCACGACTCG CTCCTGCGGG CCGCCCAGTA CGTCCAGGTC CTGGAGGAGC GGCAGGGCGT
 841 CTGGATCGCG GGCCTTGAGG AGATCGCCTT CCGCATGGGC TTCATCGACG CCGAGGCCTG
 901 TCACGGCCTG GGAGAAGGCC TCTCCCGCAC CGAGTACGGC AGCTATCTGA TGGAGATCGC
 961 CGGCCGCGAG GGAGCCCCGT GAGGGCACCT CGCGGCCGAC GCGTTCCCAC GACCGACAGC
1021 GCCACCGACA GTGCGACCCA CACCGCGACC CGCACCGCCA CCGACAGTGC GACCCACACC
1081 GCGACCTACA GCGCGACCGA AAGGAAGACG GCAGTGCGGC TTCTGGTGAC CGGAGGTGCG
1141 GGCTTCATCG GCTCGCACTT CGTGCGGCAG CTCCTCGCCG GGGCGTACCC CGACGTGCCC
1201 GCCGATGAGG TGATCGTCCT GGACAGCCTC ACCTACGCGG GCAACCGCGC CAACCTCGCC
1261 CCGGTGGACG CGGACCCGCG ACTGCGCTTC GTCCACGGCG ACATCCGCGA CGCCGGCCTC
1321 CTCGCCCGGG AACTGCGCGG CGTGGACGCC ATCGTCCACT TCGCGGCCGA GAGCCACGTG
1381 GACCGCTCCA TCGCGGGCGC GTCCGTGTTC ACCGAGACCA ACGTGCAGGG CACGCAGACG
1441 CTGCTCCAGT GCGCCGTCGA CGCCGGCGTC GGCCGGGTCG TGCACGTCTC CACCGACGAG
1501 GTGTACGGGT CGATCGACTC CGGCTCCTGG ACCGAGAGCA GCCCGCTGGA GCCCAACTCG
1561 CCCTACGCGG CGTCCAAGGC CGGCTCCGAC CTCGTTGCCC GCGCCTACCA CCGGACGTAC
1621 GGCCTCGACG TACGGATCAC CCGCTGCTGC AACAACTACG GGCCGTACCA GCACCCCGAG
1681 AAGCTCATCC CCCTCTTCGT GACGAACCTC CTCGACGGCG GGACGCTCCC GCTGTACGGC
1741 GACGGCGCGA ACGTCCGCGA GTGGGTGCAC ACCGACGACC ACTGCCGGGG CATCGCGCTC
1801 GTCCTCGCGG GCGGCCGGGC CGGCGAGATC TACCACATCG GCGGCGGCCT GGAGCTGACC
1861 AACCGCGAAC TCACCGGCAT CCTCCTGGAC TCGCTCGGCG CCGACTGGTC CTCGGTCCGG
1921 AAGGTCGCCG ACCGCAAGGG CCACGACCTG CGCTACTCCC TCGACGGCGG CAAGATCGAG
1981 CGCGAGCTCG GCTACCGCCC GCAGGTCTCC TTCGCGGACG GCCTCGCGCG GACCGTCCGC
2041 TGGTACCGGG AGAACCGCGG CTGGTGGGAG CCGCTCAAGG CGACCGCCCC GCAGCTGCCC
2101 GCCACCGCCG TGGAGGTGTC CGCGTGAGCA GCCGCGCCGA GACCCCCCGC GTCCCCTTCC
2161 TCGACCTCAA GGCCGCCTAC GACGAGCTCC GCGCGGAGAC CGACGCCGCG ATCGCCCGCG
2221 TCCTCGACTC GGGGCGCTAC CTCCTCGGAC CCGAACTCGA AGGATTCGAG GCGGAGTTCG
2281 CCGCGTACTG CGAGACGGAC CACGCCGTCG GCGTGAACAG CGGGATGGAC GCCCTCCAGC
2341 TCGCCCTCCG CGGCCTCGGC ATCGGACCCG GGGACGAGGT GATCGTCCCC TCGCACACGT
2401 ACATCGCCAG CTGGCTCGCG GTGTCCGCCA CCGGCGCGAC CCCCGTGCCC GTCGAGCCGC
2461 ACGAGGACCA CCCCACCCTG GACCCGCTGC TCGTCGAGAA GGCGATCACC CCCCGCACCC
2521 GGGCGCTCCT CCCCGTCCAC CTCTACGGGC ACCCCGCCGA CATGGACGCC CTCCGCGAGC
2581 TCGCGGACCG GCACGGCCTG CACATCGTCG AGGACGCCGC GCAGGCCCAC GGCGCCCGCT
2641 ACCGGGGCCG GCGGATCGGC GCCGGGTCGT CGGTGGCCGC GTTCAGCTTC TACCCGGGCA
2701 AGAACCTCGG CTGCTTCGGC GACGGCGGCG CCGTCGTCAC CGGCGACCCC GAGCTCGCCG
2761 AACGGCTCCG GATGCTCCGC AACTACGGCT CGCGGCAGAA GTACAGCCAC GAGACGAAGG
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2821 GCACCAACTC CCGCCTGGAC GAGATGCAGG CCGCCGTGCT GCGGATCCGG CTCGNCCACC
2881 TGGACAGCTG GAACGGCCGC AGGTCGGCGC TGGCCGCGGA GTACCTCTCC GGGCTCGCCG
2941 GACTGCCCGG CATCGGCCTG CCGGTGACCG CGCCCGACAC CGACCCGGTC TGGCACCTCT
3001 TCACCGTGCG CACCGAGCGC CGCGACGAGC TGCGCAGCCA CCTCGACGCC CGCGGCATCG
3061 ACACCCTCAC GCACTACCCG GTACCCGTGC ACCTCTCGCC CGCCTACGCG GGCGAGGCAC
3121 CGCCGGAAGG CTCGCTCCCG CGGGCCGAGA GCTTCGCGCG GCAGGTCCTC AGCCTGCCGA
3181 TCGGCCCGCA CCTGGAGCGC CCGCAGGCGC TGCGGGTGAT CGACGCCGTG CGCGAATGGG
3241 CCGAGCGGGT CGACCAGGCC TAGTCAGGTG GTCCGGTAGA CCCAGCAGGC CG
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[0064] Contig 004 from cosmid pKOS023-26 contains 1693 nucleotides and the following ORFs: from nucleotide 1692 to 694 is ORF15, which encodes a part of S-adenosylmethionine synthetase; and from nucleotide 692 to 1 is ORF16, which encodes a part of a protein homologous to the *M. tuberculosis* cbhK gene.

```
   1 ATGCGGCACC CCTTGGCGCC GAGCGTGGTG ATCCAGGTGC CGACCCGGGC GAGCACCTCC
  61 TGCTCGGTCC AGCCCGTCTT GCTGAGCAGC AGCGCCCGCT CGTAGGCGTT CGTGAACAGC
 121 AGCTCGGCTC CGTCGACGAG CTCCCGGACG CTGTCGCCCT CCAGCCGGGC GAGCTGCTGC
 181 GAGGGGTCCG CGGCCCGGCG GAGGCCCAGC TCGCGGCAGA CCCGCGTGTG CCGCACCATC
 241 GCCTCGGGGT CGTCCGCGCC GACGAGGACG AGGTCGATCC CGCCGGGCCG GCCGGCCGTC
 301 TCGCCCAGGT CGATGTCGCG CGCCTCGGCC ATCGCGCCCG CGTAGAACGA GGCGAGCTGA
 361 TTGCCGTCCT CGTCGGTGGT GCACATGAAG CGGGCGGTGT GCTGACGGTC CGACACCCGC
 421 ACGGAGTCGG TGTCGACGCC CGCGGCGCGG AGCAGCTGCC CGTACCCGTC GAAGTCCTTG
 481 CCGACGGCGC CGACGAGGAC GGGGCGGCGA CCGAGCAGGC CGAGGCCGTA CGCGATGTTG
 541 GCGGCGACGC CGCCGTGCCG GATGTCCAGG GTGTCGACGA GGAACGACAG GGACACGTGG
 601 GCGAGCTGGT CCGGCAGGAT CTGCTCGGCG AAGCGGCCCG GGAAGGTCAT CAGGTGGTCG
 661 GTGGCGATCG ACCCGGTGAC GGCTATACGC ATGTCAGAGC CCCGCGGCCT TCTTCAGGGC
 721 GTCCACGCGG TCGGTGCGCT CCCAGGTGAA GTCCGGCAGC TCGCGGCCGA AGTGGCCGTA
 781 GGCGGCGGTC TGGGAGTAGA TCGGGCGGAG CAGGTCGAGG TCGCGGATGA TCGCGGCCGG
 841 GCGGAGGTCG AAGACCTCGC CGATGGCGTT CTCGATCTTC TCGGTCTCGA TCTTGTGGGT
 901 GCCGAAGGTC TCGACGAAGA GGCCGACGGG CTCGGCCTTG CCGATCGCGT ACGCGACCTG
 961 GACCTCGCAG CGCGAGGCGA GACCGGCGGC GACGACGTTC TTCGCCACCC AGCGCATCGC
1021 GTACGCGGCG GAGCGGTCGA CCTTCGACGG GTCCTTGCCG GAGAAGGCGC CGCCACCGTG
1081 GCGGGCCATG CCGCCGTAGG TGTCGATGAT GATCTTGCGG CCGGTGAGGC CGGCGTCGCC
1141 CATCGGGCCG CCGATCTCGA AGCGACCGGT CGGGTTCACG AGCAGGCGGT AGCCGTCGGT
1201 GTCGAGCTTG ATGCCGTCCT CGACGAGCTG CGCAAGCACG TGCTCGACGA CGAACTTCCG
1261 CACGTCGGGG GCGAGCAGCG ACTCCAGGTC GATGTCCGAG GCGTGCTGCG AGGAGACGAC
1321 GACCGTGTCG AGACGGACCG CCCTGTCGCC GTCGTACTCG ATGGTGACCT GGGTCTTGCC
1381 GTCGGGACGC AGGTACGGGA TGGTCCCGTT CTTGCGGACC TCGGTCAGGC GGCGCGAGAG
1441 ACGGTGCGCG AGGTGGATCG GCAGCGGCAT CAGCTCGGGC GTCTCGTCCG AGGCATAGCC
1501 GAACATCAGG CCCTGGTCAC CGGCGCCCTG CTTGTCGAGC TCGTCCCCCT CGTCCCGCTG
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1561 GGAGGCACCC TCGACCCGCT TCTCGTACGC GGTGTCGACA CCCTGGGCGA TGTCCGGGGA

1621 CTGCGACCCG ATGGACACCG ACACGCCGCA GGAGGCGCCG TCGAAGCCCT TCTTCGAGGA

1681 GTCGTACCCG ATC
```

[0065] Contig 005 from cosmid pKOS023-26 contains 1565 nucleotides and contains the ORF of the picCV gene that encodes PICCV, involved in desosamine biosynthesis.

```
   1 CCCCGCTCGC GGCCCCCCAG ACATCCACGC CCACGATTGG ACGCTCCCGA TGACCGCCCC

  61 CGCCCTCTCC GCCACCGCCC CGGCCGAACG CTGCGCGCAC CCCGGAGCCG ATCTGGGGGC

 121 GGCGGTCCAC GCCGTCGGCC AGACCCTCGC CGCCGGCGGC CTCGTGCCGC CCGACGAGGC

 181 CGGAACGACC GCCCGCCACC TCGTCCGGCT CGCCGTGCGC TACGGCAACA GCCCCTTCAC

 241 CCCGCTGGAG GAGGCCCGCC ACGACCTGGG CGTCGACCGG GACGCCTTCC GGCGCCTCCT

 301 CGCCCTGTTC GGGCAGGTCC CCGAGCTCCG CACCGCGGTC GAGACCGGCC CCGCCGGGGC

 361 GTACTGGAAC AACACCCTGC TCCCGCTCGA ACAGCGCGGC GTCTTCGACG CGGCGCTCGC

 421 CAGGAAGCCC GTCTTCCCGT ACAGCGTCGG CCTCTACCCC GGCCCGACCT GCATGTTCCG

 481 CTGCCACTTC TGCGTCCGTG TGACCGGCGC CCGCTACGAC CCGTCCGCCC TCGACGCCGG

 541 CAACGCCATG TTCCGGTCGG TCATCCACGA GATACCCGCG GGCAACCCCT CGGCGATGTA

 601 CTTCTCCGGC GGCCTGGAGC CGCTCACCAA CCCCGGCCTC GGGAGCCTGG CCGCGCACGC

 661 CACCGACCAC GGCCTGCGGC CCACCGTCTA CACGAACTCC TTCGCGCTCA CCGAGCGCAC

 721 CCTGGAGCGC CAGCCCGGCC TCTGGGGCCT GCACGCCATC CCCACCTCGC TCTACGGCCT

 781 CAACGACGAG GAGTACGAGC AGACCACCGG CAAGAAGGCC GCCTTCCGCC GCGTCCGCGA

 841 GAACCTGCGC CGCTTCCAGC AGCTGCGCGC CGAGCGCGAG TCGCCGATCA ACCTCGGCTT

 901 CGCCTACATC GTGCTCCCGG GCCGTGCCTC CCGCCTGCTC GACCTGGTCG ACTTCATCGC

 961 CGACCTCAAC GACGCCGGGC AGGGCAGGAC GATCGACTTC GTCAACATTC GCGAGGACTA

1021 CAGCGGCCGT GACGACGGCA AGCTGCCGCA GGAGGAGCGG GCCGAGCTCC AGGAGGCCCT

1081 CAACGCCTTC GAGGAGCGGG TCCGCGAGCG CACCCCCGGA CTCCACATCG ACTACGGCTA

1141 CGCCCTGAAC AGCCTGCGCA CCGGGGCCGA CGCCGAACTG CTGCCGATCA AGCCCGCCAC

1201 CATGCGGCCC ACCGCGCACC CGCAGGTCGC GGTGCAGGTC GATCTCCTCG GCGACGTGTA

1261 CCTGTACCGC GAGGCCGGCT TCCCCGACCT GGACGGCGCG ACCCGCTACA TCGCGGGCCG

1321 CGTGACCCCC GACACCTCCC TCACCGAGGT CGTCAGGGAC TTCGTCGAGC GCGGCGGCGA

1381 GGTGGCGGCC GTCGACGGCG ACGAGTACTT CATGGACGGC TTCGATCAGG TCGTCACCGC

1441 CCGCCTGAAC CAGCTGGAGC GCGACGCCGC GGACGGCTGG GAGGAGGCCC GCGGCTTCCT

1501 GCGCTGACCC GCACCCGCCC CGATCCCCC GATCCCCCCC CCACGATCCC CCCACCTGAG

1561 GGCCC
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[0066] The recombinant desosamine biosynthesis and transfer and beta-glucosidase genes and proteins provided by the invention are useful in the production of glycosylated polyketides in a variety of host cells, as described in Section IV below.

[0067] Section III. The picK Hydroxylase Gene

[0068] The present invention provides the picK gene in recombinant form as well as recombinant PicK protein. The availability of the hydroxylase encoded by the picK gene in

recombinant form is of significant benefit in that the enzyme can convert narbomycin into picromycin and accepts in addition a variety of polyketide substrates, particularly those related to narbomycin in structure. The present invention also provides methods of hydroxylating polyketides, which method comprises contacting the polyketide with the recombinant PicK enzyme under conditions such that hydroxylation occurs. This methodology is applicable to large numbers of polyketides.

[0069] DNA encoding the picK gene can be isolated from cosmid pKOS023-26 of the invention. The DNA sequence of the picK gene is shown in the preceding section. This DNA sequence encodes one of the recombinant forms of the enzyme provided by the invention. The amino acid sequence of this form of the picK gene is shown below. The present invention also provides a recombinant picK gene that encodes a picK gene product in which the PicK protein is fused to a number of consecutive histidine residues, which facilitates purification from recombinant host cells.

Amino acid sequence of picromycin/methymycin cytochrome P450 hydroxylase, PicK

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  1VRRTQQGTTA SPPVLDLGAL GQDFAADPYP TYARLRAEGP AHRVRTPEGD EVWLVVGYDR

 61ARAVLADPRF SKDWRNSTTP LTEAEAALNH NMLESDPPRH TRLRKLVARE FTMRRVELLR

121PRVQEIVDGL VDAMLAAPDG RADLMESLAW PLPITVISEL LGVPEPDRAA FRVWTDAFVF

181PDDPAQAQTA MAEMSGYLSR LIDSKRGQDG EDLLSALVRT SDEDGSRLTS EELLGMAHIL

241LVAGHETTVN LIANGMYALL SHPDQLAALR ADMTLLDGAV EEMLRYEGPV ESATYRFPVE

301PVDLDGTVIP AGDTVLVVLA DAHRTPERFP DPHRFDIRRD TAGHLAFGHG IHFCIGAPLA

361RLEARIAVRA LLERCPDLAL DVSPGELVWY PNPMIRGLKA LPIRWRRGRE AGRRTG
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[0070] The recombinant PicK enzyme of the invention hydroxylates narbomycin at the C12 position and YC-17 at either the C10 or C12 position. Hydroxylation of these compounds at the respective positions increases the antibiotic activity of the compound relative to the unhydroxylated compound. Hydroxylation can be achieved by a number of methods. First, the hydroxylation may be performed in vitro using purified hydroxylase, or the relevant hydroxylase can be produced recombinantly and utilized directly in the cell that produces it. Thus, hydroxylation may be effected by supplying the nonhydroxylated precursor to a cell that expresses the hydroxylase. These and other details of this embodiment of the invention are described in additional detail below in Section IV and the examples.

[0071] Section IV: Heterologous Expression of the Narbonolide PKS; the Desosamine Biosynthetic and transferase Genes; the Beta-Glucosidase Gene; and the picK Hydroxylase Gene

[0072] In one important embodiment, the invention provides methods for the heterologous expression of one or more of the genes involved in picromycin biosynthesis and recombinant DNA expression vectors useful in the method. Thus, included within the scope of the invention in addition to isolated nucleic acids encoding domains, modules, or proteins of the narbonolide PKS, glycosylation, and/or hydroxylation enzymes, are recombinant expression systems. These systems contain the coding sequences operably linked to promoters, enhancers, and/or termination sequences that operate to effect expression of the coding sequence in compatible host cells. The host cells are modified by transformation with the recombinant DNA expression vectors of the invention to contain these sequences either as extrachromosomal elements or integrated into the chromosome. The invention also provides methods to produce PKS and post-PKS tailoring enzymes as well as polyketides and antibiotics using these modified host cells.

[0073] As used herein, the term expression vector refers to a nucleic acid that can be introduced into a host cell or cell-free transcription and translation medium. An expression vector can be maintained stably or transiently in a cell, whether as part of the chromosomal or other DNA in the cell or in any cellular compartment, such as a replicating vector in the cytoplasm. An expression vector also comprises a gene that serves to produce RNA, which typically is translated into a polypeptide in the cell or cell extract. To drive production of the RNA, the expression vector typically comprises one or more promoter elements. Furthermore, expression vectors typically contain additional functional elements, such as, for example, a resistance-conferring gene that acts as a selectable marker.

[0074] The various components of an expression vector can vary widely, depending on the intended use of the vector. In particular, the components depend on the host cell(s) in which the vector will be introduced or in which it is intended to function. Components for expression and maintenance of vectors in *E. coli* are widely known and commercially available, as are components for other commonly used organisms, such as yeast cells and *Streptomyces* cells.

[0075] One important component is the promoter, which can be referred to as, or can be included within, a control sequence or control element, which drives expression of the desired gene product in the heterologous host cell. Suitable promoters include those that function in eucaryotic or procaryotic host cells. In addition to a promoter, a control element can include, optionally, operator sequences, and other elements, such as ribosome binding sites, depending on the nature of the host. Regulatory sequences that allow for regulation of expression of the heterologous gene relative to the growth of the host cell may also be included. Examples of such regulatory sequences known to those of skill in the art are those that cause the expression of a gene

to be turned on or off in response to a chemical or physical stimulus. Preferred host cells for purposes of selecting vector components include fungal host cells such as yeast and procaryotic, especially *E. coli* and *Streptomyces*, host cells, but single cell cultures of, for example, mammalian cells can also be used. In hosts such as yeasts, plants, or mammalian cells that ordinarily do not produce polyketides, it may be necessary to provide, also typically by recombinant means, suitable holo-ACP synthases to convert the recombinantly produced PKS to functionality. Provision of such enzymes is described, for example, in PCI publication Nos. WO 97/13845 and 98/27203, each of which is incorporated herein by reference. Control systems for expression in yeast, including controls that effect secretion are widely available and can be routinely used. For *E. coli* or other bacterial host cells, promoters such as those derived from sugar metabolizing enzymes, such as galactose, lactose (lac), and maltose, can be used. Additional examples include promoters derived from genes encoding biosynthetic enzymes, and the tryptophan (trp), the beta-lactamase (bla), bacteriophage lambda PL, and T5 promoters. In addition, synthetic promoters, such as the tac promoter (U.S. Pat. No. 4,551,433), can also be used.

[0076] Particularly preferred are control sequences compatible with *Streptomyces* spp. Particularly useful promoters for *Streptomyces* host cells include those from PKS gene clusters that result in the production of polyketides as secondary metabolites, including promoters from aromatic (Type II) PKS gene clusters. Examples of Type II PKS gene cluster promoters are act gene promoters and tcm gene promoters; an example of a Type I PKS gene cluster promoter is the spiramycin PKS gene promoter.

[0077] If a *Streptomyces* or other host ordinarily produces polyketides, it may be desirable to modify the host so as to prevent the production of endogenous polyketides prior to its use to express a recombinant PKS of the invention. Such hosts have been described, for example, in U.S. Pat. No. 5,672,491, incorporated herein by reference. In such hosts, it may not be necessary to provide enzymatic activities for all of the desired post-translational modifications of the enzymes that make up the recombinantly produced PKS, because the host naturally expresses such enzymes. In particular, these hosts generally contain holo-ACP synthases that provide the pantotheinyl residue needed for functionality of the PKS.

[0078] Thus, in one important embodiment, the vectors of the invention are used to transform *Streptomyces* host cells to provide the recombinant *Streptomyces* host cells of the invention. *Streptomyces* is a convenient host for expressing narbonolide or 10-deoxymethynolide or derivatives of those compounds, because narbonolide and 10-deoxymethynolide are naturally produced in certain *Streptomyces* species, and *Streptomyces* generally produce the precursors needed to form the desired polyketide. The present invention also provides the narbonolide PKS gene promoter in recombinant form, located upstream of the picAI gene on cosmid pKOS023-27. This promoter can be used to drive expression of the narbonolide PKS or any other coding sequence of interest in host cells in which the promoter functions, particularly *S. venezuelae* and generally any *Streptomyces* species. As described below, however, promoters other than the promoter of the narbonolide PKS genes will typically be used for heterologous expression.

[0079] For purposes of the invention, any host cell other than *Streptomyces venezuelae* is a heterologous host cell. Thus, *S. narbonensis*, which produces narbomycin but not picromycin is a heterologous host cell of the invention, although other host cells are generally preferred for purposes of heterologous expression. Those of skill in the art will recognize that, if a *Streptomyces* host that produces a picromycin or methymycin precursor is used as the host cell, the recombinant vector need drive expression of only a portion of the genes constituting the picromycin gene cluster. As used herein, the picromycin gene cluster includes the narbonolide PKS, the desosamine biosynthetic and transferase genes, the beta-glucosidase gene, and the picK hydroxylase gene. Thus, such a vector may comprise only a single ORF, with the desired remainder of the polypeptides encoded by the picromycin gene cluster provided by the genes, on the host cell chromosomal DNA.

[0080] The present invention also provides compounds and recombinant DNA vectors useful for disrupting any gene in the picromycin gene cluster (as described above and illustrated in the examples below). Thus, the invention provides a variety of modified host cells (particularly, *S. narbonensis* and *S. venezuelae*) in which one or more of the genes in the picromycin gene cluster have been disrupted. These cells are especially useful when it is desired to replace the disrupted function with a gene product expressed by a recombinant DNA vector. Thus, the invention provides such *Streptomyces* host cells, which are preferred host cells for expressing narbonolide derivatives of the invention. Particularly preferred host cells of this type include those in which the coding sequence for the loading module has been disrupted, those in which one or more of any of the PKS gene ORFs has been disrupted, and/or those in which the picK gene has been disrupted.

[0081] In a preferred embodiment, the expression vectors of the invention are used to construct a heterologous recombinant *Streptomyces* host cell that expresses a recombinant PKS of the invention. As noted above, a heterologous host cell for purposes of the present invention is any host cell other than *S. venezuelae*, and in most cases other than *S. narbonensis* as well. Particularly preferred heterologous host cells are those which lack endogenous functional PKS genes. Illustrative host cells of this type include the modified *Streptomyces coelicolor* CH999 and similarly modified *S. lividans* described in PCT publication No. WO 96/40968.

[0082] The invention provides a wide variety of expression vectors for use in *Streptomyces*. For replicating vectors, the origin of replication can be, for example and without limitation, a low copy number vector, such as SCP2* (see Hopwood et al., *Genetic Manipulation of Streptomyces: A Laboratory manual* (The John Innes Foundation, Norwich, U.K., 1985); Lydiate et al., 1985, Gene 35: 223-235; and Kieser and Melton, 1988, *Gene* 65: 83-91, each of which is incorporated herein by reference), SLP1.2 (Thompson et al., 1982, *Gene* 20: 51-62, incorporated herein by reference), and pSG5(ts) (Muth et al., 1989, *Mol. Gen. Genet.* 219: 341-348, and Bierman et al., 1992, *Gene* 116: 43-49, each of which is incorporated herein by reference), or a high copy number vector, such as pIJ101 and pJV1 (see Katz et al., 1983, *J. Gen. Microbiol.* 129: 2703-2714; Vara et al., 1989, *J. Bacteriol.* 171: 5782-5781; and Servin-Gonzalez, 1993, *Plasmid* 30: 131-140, each of which is incorporated herein by reference). High copy number vectors are generally, however, not preferred for expression of large genes or multiple genes. For non-replicating and integrating vectors and generally for any vector, it is useful to include at least

an *E. coli* origin of replication, such as from pUC, p1P, p1I, and pBR. For phage based vectors, the phage phiC31 and its derivative KC515 can be employed (see Hopwood et al., supra). Also, plasmid pSET152, plasmid pSAM, plasmids pSE101 and pSE211, all of which integrate site-specifically in the chromosomal DNA of *S. lividans*, can be employed.

[0083] Preferred *Streptomyces* host cell/vector combinations of the invention include *S. coelicolor* CH999 and, *S. lividans* K4-114 host cells, which do not produce actinorhodin, and expression vectors derived from the pRM1 and pRM5 vectors, as described in U.S. Pat. No. 5,830,750 and U.S. patent application Ser. No. 08/828,898, filed 31 Mar. 1997, and Ser. No. 09/181,833, filed 28 Oct. 1998, each of which is incorporated herein by reference.

[0084] As described above, particularly useful control sequences are those that alone or together with suitable regulatory systems activate expression during transition from growth to stationary phase in the vegetative mycelium. The system contained in the illustrative plasmid pRM5, i.e., the actI/actIII promoter pair and the actII-ORF4 activator gene, is particularly preferred. Other useful *Streptomyces* promoters include without limitation those from the ermE gene and the melC1 gene, which act constitutively, and the tipA gene and the merA gene, which can be induced at any growth stage. In addition, the T7 RNA polymerase system has been transferred to *Streptomyces* and can be employed in the vectors and host cells of the invention. In this system, the coding sequence for the T7 RNA polymerase is inserted into a neutral site of the chromosome or in a vector under the control of the inducible merA promoter, and the gene of interest is placed under the control of the T7 promoter. As noted above, one or more activator genes can also be employed to enhance the activity of a promoter. Activator genes in addition to the actII-ORF4 gene described above include dnrI, redD, and ptpA genes (see U.S. patent application Ser. No. 09/181,833, supra).

[0085] Typically, the expression vector will comprise one or more marker genes by which host cells containing the vector can be identified and/or selected. Selectable markers are often preferred for recombinant expression vectors. A variety of markers are known that are useful in selecting for transformed cell lines and generally comprise a gene that confers a selectable phenotype on transformed cells when the cells are grown in an appropriate selective medium. Such markers include, for example, genes that confer antibiotic resistance or sensitivity to the plasmid. Alternatively, several polyketides are naturally colored, and this characteristic can provide a built-in marker for identifying cells. Preferred selectable markers include antibiotic resistance conferring genes. Preferred for use in *Streptomyces* host cells are the ermE (confers resistance to erythromycin and lincomycin), tsr (confers resistance to thiostrepton), aadA (confers resistance to spectinomycin and streptomycin), aacC4 (confers resistance to apramycin, kanamycin, gentamicin, geneticin (G418), and neomycin), hyg (confers resistance to hygromycin), and vph (confers resistance to viomycin) resistance conferring genes.

[0086] To provide a preferred host cell and vector for purposes of the invention, the narbonolide PKS genes were placed on a recombinant expression vector that was transferred to the non-macrolide producing host *Streptomyces lividans* K4-114, as described in Example 3. Transformation of *S. lividans* K4-114 with this expression vector resulted in a strain which produced two compounds in similar yield (5-10 mg/L each). Analysis of extracts by LC/MS followed by ^{1}H-NMR spectroscopy of the purified compounds established their identity as narbonolide (**FIG. 5**, compound 4) and 10-deoxymethynolide (**FIG. 5**, compound 5), the respective 14 and 12-membered polyketide precursors of narbomycin and YC17.

[0087] To provide a host cell of the invention that produces the narbonolide PKS as well as an additional narbonolide biosynthetic gene and to investigate the possible role of the Pik TEII in picromycin biosynthesis, the picB gene was integrated into the chromosome to provide the host cell of the invention *Streptomyces lividans* K39-18. The picB gene was cloned into the *Streptomyces* genome integrating vector pSET152 (see Bierman et al., 1992, Gene 116:43, incorporated herein by reference) under control of the same promoter (PactI) as the PKS on plasmid pKOS039-86.

[0088] A comparison of strains *Streptomyces lividans* K39-18/pKOS039-86 and KS 114/pKOS039-86 grown under identical conditions indicated that the strain containing TEII produced 47 times more total polyketide. This increased production indicates that the enzyme is functional in this strain and is consistent with the observation that yields fall to below 5% for both picromycin and methymycin when picB is disrupted in *S. venezuelae*. Because the production levels of compound 4 and 5 from K39-18/pKOS03986 increased by the same relative amounts, TEII does not appear to influence the ratio of 12 and 14-membered lactone ring formation. Thus, the invention provides methods of coexpressing the picB gene product or any other type II thioesterase with the narbonolide PKS or any other PKS in heterologous host cells to increase polyketide production.

[0089] In accordance with the methods of the invention, picromycin biosynthetic genes in addition to the genes encoding the PKS and Pik TEII can be introduced into heterologous host cells. In particular, the picK gene, desosamine biosynthetic genes, and the desosaminyl transferase gene can be expressed in the recombinant host cells of the invention to produce any and all of the polyketides in the picromycin biosynthetic pathway (or derivatives thereof). Those of skill will recognize that the present invention enables one to select whether only the 12-membered polyketides, or only the 14-membered polyketides, or both 12- and 14-membered polyketides will be produced. To produce only the 12-membered polyketides, the invention provides expression vectors in which the last module is deleted or the KS domain of that module is deleted or rendered inactive. To produce only the 14-membered polyketides, the invention provides expression vectors in which the coding sequences of extender modules 5 and 6 are fused to provide only a single polypeptide.

[0090] In one important embodiment, the invention provides methods for desosaminylating polyketides or other compounds. In this method, a host cell other than *Streptomyces venezuelae* is transformed with one or more recombinant vectors of the invention comprising the desosamine biosynthetic and desosaminyl transferase genes and control sequences positioned to express those genes. The host cells so transformed can either produce the polyketide to be desosaminylated naturally or can be transformed with expression vectors encoding the PKS that produces the desired polyketide. Alternatively, the polyketide can be supplied to the host cell containing those genes. Upon production of the polyketide and expression of the desosamine biosynthetic and desosaminyl transferase genes, the

desired desosaminylated polyketide is produced. This method is especially useful in the production of polyketides to be used as antibiotics, because the presence of the desosamine residue is known to increase, relative to their undesosaminylated counterparts, the antibiotic activity of many polyketides significantly. The present invention also provides a method for desosaminylating a polyketide by transforming an *S. venezuelae* or *S. narbonensis* host cell with a recombinant vector that encodes a PKS that produces the polyketide and culturing the transformed cell under conditions such that said polyketide is produced and desosaminylated. In this method, use of an *S. venezuelae* or *S. narbonensis* host cell of the invention that does not produce a functional endogenous narbonolide PKS is preferred.

[0091] In a related aspect, the invention provides a method for improving the yield of a desired desosaminylated polyketide in a host cell, which method comprises transforming the host cell with a beta-glucosidase gene. This method is not limited to host cells that have been transformed with expression vectors of the invention encoding the desosamine biosynthetic and desosaminyl transferase genes of the invention but instead can be applied to any host cell that desosaminylates polyketides or other compounds. Moreover, while the beta-glucosidase gene from *Streptomyces venezuelae* provided by the invention is preferred for use in the method, any beta-glucosidase gene may be employed. In another embodiment, the beta-glucosidase treatment is conducted in a cell free extract.

[0092] Thus, the invention provides methods not only for producing narbonolide and 10-deoxymethynolide in heterologous host cells but also for producing narbomycin and YC-17 in heterologous host cells. In addition, the invention provides methods for expressing the picK gene product in heterologous host cells, thus providing a means to produce picromycin, methymycin, and neomethymycin in heterologous host cells. Moreover, because the recombinant expression vectors provided by the invention enable the artisan to provide for desosamine biosynthesis and transfer and/or C10 or C12 hydroxylation in any host cell, the invention provides methods and reagents for producing a very wide variety of glycosylated and/or hydroxylated polyketides. This variety of polyketides provided by the invention can be better appreciated upon consideration of the following section relating to the production of polyketides from heterologous or hybrid PKS enzymes provided by the invention.

[0093] Section V: Hybrid PKS Genes

[0094] The present invention provides recombinant DNA compounds encoding each of the domains of each of the modules of the narbonolide PKS, the proteins involved in desosamine biosynthesis and transfer to narbonolide, and the PicK protein. The availability of these compounds permits their use in recombinant procedures for production of desired portions of the narbonolide PKS fused to or expressed in conjunction with all or a portion of a heterologous PKS. The resulting hybrid PKS can then be expressed in a host cell, optionally with the desosamine biosynthesis and transfer genes and/or the picK hydroxylase gene to produce a desired polyketide.

[0095] Thus, in accordance with the methods of the invention, a portion of the narbonolide PKS coding sequence that encodes a particular activity can be isolated and manipulated, for example, to replace the corresponding region in a different modular PKS. In addition, coding sequences for individual modules of the PKS can be ligated into suitable expression systems and used to produce the portion of the protein encoded. The resulting protein can be isolated and purified or can may be employed in situ to effect polyketide synthesis. Depending on the host for the recombinant production of the domain, module, protein, or combination of proteins, suitable control sequences such as promoters, termination sequences, enhancers, and the like are ligated to the nucleotide sequence encoding the desired protein in the construction of the expression vector.

[0096] In one important embodiment, the invention thus provides a hybrid PKS and the corresponding recombinant DNA compounds that encode those hybrid PKS enzymes. For purposes of the invention, a hybrid PKS is a recombinant PKS that comprises all or part of one or more extender modules, loading module, and/or thioesterase/cyclase domain of a first PKS and all or part of one or more extender modules, loading module, and/or thioesterase/cyclase domain of a second PKS. In one preferred embodiment, the first PKS is most but not all of the narbonolide PKS, and the second PKS is only a portion or all of a non-narbonolide PKS. An illustrative example of such a hybrid PKS includes a narbonolide PKS in which the natural loading module has been replaced with a loading module of another PKS. Another example of such a hybrid PKS is a narbonolide PKS in which the AT domain of extender module 3 is replaced with an AT domain that binds only malonyl CoA.

[0097] In another preferred embodiment, the first PKS is most but not all of a non-narbonolide PKS, and the second PKS is only a portion or all of the narbonolide PKS. An illustrative example of such a hybrid PKS includes a DEBS PKS in which an AT specific for methylmalonyl CoA is replaced with the AT from the narbonolide PKS specific for malonyl CoA.

[0098] Those of skill in the art will recognize that all or part of either the first or second PKS in a hybrid PKS of the invention need not be isolated from a naturally occurring source. For example, only a small portion of an AT domain determines its specificity. See U.S. provisional patent application Ser. No. 60/091,526, and Lau et al., infra, incorporated herein by reference. The state of the art in DNA synthesis allows the artisan to construct de novo DNA compounds of size sufficient to construct a useful portion of a PKS module or domain. Thus, the desired derivative coding sequences can be synthesized using standard solid phase synthesis methods such as those described by Jaye et al., 1984, *J. Biol. Chem.* 259: 6331, and instruments for automated synthesis are available commercially from, for example, Applied Biosystems, Inc. For purposes of the invention, such synthetic DNA compounds are deemed to be a portion of a PKS.

[0099] With this general background regarding hybrid PKSs of the invention, one can better appreciate the benefit provided by the DNA compounds of the invention that encode the individual domains, modules, and proteins that comprise the narbonolide PKS. As described above, the narbonolide PKS is comprised of a loading module, six extender modules composed of a KS, AT, ACP, and zero, one, two, or three KR, DH, and ER domains, and a thioesterase domain. The DNA compounds of the invention that encode these domains individually or in combination are useful in the construction of the hybrid PKS encoding DNA compounds of the invention.

[0100] The recombinant DNA compounds of the invention that encode the loading module of the narbonolide PKS and the corresponding polypeptides encoded thereby are useful for a variety of applications. In one embodiment, a DNA compound comprising a sequence that encodes the narbonolide PKS loading module is inserted into a DNA compound that comprises the coding sequence for a heterologous PKS. The resulting construct, in which the coding sequence for the loading module of the heterologous PKS is replaced by that for the coding sequence of the narbonolide PKS loading module provides a novel PKS. Examples include the 6-deoxyerythronolide B, rapamycin, FK506, FK520, rifamycin, and avermectin PKS coding sequences. In another embodiment, a DNA compound comprising a sequence that encodes the narbonolide PKS loading module is inserted into a DNA compound that comprises the coding sequence for the narbonolide PKS or a recombinant narbonolide PKS that produces a narbonolide derivative.

[0101] In another embodiment, a portion of the loading module coding sequence is utilized in conjunction with a heterologous coding sequence. In this embodiment, the invention provides, for example, replacing the propionyl CoA specific AT with an acetyl CoA, butyryl CoA, or other CoA specific AT. In addition, the KS^Q and/or ACP can be replaced by another inactivated KS and/or another ACP. Alternatively, the KS^Q, AT, and ACP of the loading module can be replaced by an AT and ACP of a loading module such as that of DEBS. The resulting heterologous loading module coding sequence can be utilized in conjunction with a coding sequence for a PKS that synthesizes narbonolide, a narbonolide derivative, or another polyketide.

[0102] The recombinant DNA compounds of the invention that encode the first extender module of the narbonolide PKS and the corresponding polypeptides encoded thereby are useful for a variety of applications. In one embodiment, a DNA compound comprising a sequence that encodes the narbonolide PKS first extender module is inserted into a DNA compound that comprises the coding sequence for a heterologous PKS. The resulting construct, in which the coding sequence for a module of the heterologous PKS is either replaced by that for the first extender module of the narbonolide PKS or the latter is merely added to coding sequences for modules of the heterologous PKS, provides a novel PKS coding sequence. In another embodiment, a DNA compound comprising a sequence that encodes the first extender module of the narbonolide PKS is inserted into a DNA compound that comprises coding sequences for the narbonolide PKS or a recombinant narbonolide PKS that produces a narbonolide derivative.

[0103] In another embodiment, a portion or all of the first extender module coding sequence is utilized in conjunction with other PKS coding sequences to create a hybrid module. In this embodiment, the invention provides, for example, replacing the methylmalonyl CoA specific AT with a malonyl CoA, ethylmalonyl CoA, or carboxyglycolyl CoA specific AT; deleting (which includes inactivating) the KR; inserting a DH or a DH and ER; and/or replacing the KR with another KR, a DH and KR, or a DH, KR, and ER. In addition, the KS and/or ACP can be replaced with another KS and/or ACP. In each of these replacements or insertions, the heterologous MS, AT, DH, KR, ER, or ACP coding sequence can originate from a coding sequence for another module of the narbonolide PKS, from a gene for a PKS that produces a polyketide other than narbonolide, or from chemical synthesis. The resulting heterologous first extender module coding sequence can be utilized in conjunction with a coding sequence for a PKS that synthesizes narbonolide, a narbonolide derivative, or another polyketide.

[0104] In an illustrative embodiment of this aspect of the invention, the invention provides recombinant PKSs and recombinant DNA compounds and vectors that encode such PKSs in which the KS domain of the first extender module has been inactivated. Such constructs are especially useful when placed in translational reading frame with the remaining modules and domains of a narbonolide PKS or narbonolide derivative PKS. The utility of these constructs is that host cells expressing, or cell free extracts containing, the PKS encoded thereby can be fed or supplied with N-acetylcysteamine thioesters of novel precursor molecules to prepare narbonolide derivatives. See U.S. patent application Ser. No. 60/117,384, filed 27 Jan. 1999, and PCT publication Nos. WO 99/03986 and 97/02358, each of which is incorporated herein by reference.

[0105] The recombinant DNA compounds of the invention that encode the second extender module of the narbonolide PKS and the corresponding polypeptides encoded thereby are useful for a variety of applications. In one embodiment, a DNA compound comprising a sequence that encodes the narbonolide PKS second extender module is inserted into a DNA compound that comprises the coding sequence for a heterologous PKS. The resulting construct, in which the coding sequence for a module of the heterologous PKS is either replaced by that for the second extender module of the narbonolide PKS or the latter is merely added to coding sequences for the modules of the heterologous PKS, provides a novel PKS. In another embodiment, a DNA compound comprising a sequence that encodes the second extender module of the narbonolide PKS is inserted into a DNA compound that comprises the coding sequences for the narbonolide PKS or a recombinant narbonolide PKS that produces a narbonolide derivative.

[0106] In another embodiment, a portion or all of the second extender module coding sequence is utilized in conjunction with other PKS coding sequences to create a hybrid module. In this embodiment, the invention provides, for example, replacing the malonyl CoA specific AT with a methylmalonyl CoA, ethylmalonyl CoA, or carboxyglycolyl CoA specific AT; deleting (or inactivating) the KR, the DH, or both the DH and KR; replacing the KR or the KR and DH with a KR, a KR and a DH, or a KR, DH, and ER; and/or inserting an ER. In addition, the KS and/or ACP can be replaced with another KS and/or ACP. In each of these replacements or insertions, the heterologous KS, AT, DH, KR, ER, or ACP coding sequence can originate from a coding sequence for another module of the narbonolide PKS, from a coding sequence for a PKS that produces a polyketide other than narbonolide, or from chemical synthesis. The resulting heterologous second extender module coding sequence can be utilized in conjunction with a coding sequence from a PKS that synthesizes narbonolide, a narbonolide derivative, or another polyketide.

[0107] The recombinant DNA compounds of the invention that encode the third extender module of the narbonolide PKS and the corresponding polypeptides encoded thereby are useful for a variety of applications. In one embodiment,

a DNA compound comprising a sequence that encodes the narbonolide PKS third extender module is inserted into a DNA compound that comprises the coding sequence for a heterologous PKS. The resulting construct, in which the coding sequence for a module of the heterologous PKS is either replaced by that for the third extender module of the narbonolide PKS or the latter is merely added to coding sequences for the modules of the heterologous PKS, provides a novel PKS. In another embodiment, a DNA compound comprising a sequence that encodes the third extender module of the narbonolide PKS is inserted into a DNA compound that comprises coding sequences for the narbonolide PKS or a recombinant narbonolide PKS that produces a narbonolide derivative.

[0108] In another embodiment, a portion or all of the third extender module coding sequence is utilized in conjunction with other PKS coding sequences to create a hybrid module. In this embodiment, the invention provides, for example, replacing the methylmalonyl CoA specific AT with a malonyl CoA, ethylmalonyl CoA, or carboxyglycolyl CoA specific AT; deleting the inactive KR; and/or inserting a KR, or a KR and DH, or a KR, DH, and ER. In addition, the KS and/or ACP can be replaced with another KS and/or ACP. In each of these replacements or insertions, the heterologous KS, AT, DH, KR, ER, or ACP coding sequence can originate from a coding sequence for another module of the narbonolide PKS, from a gene for a PKS that produces a polyketide other than narbonolide, or from chemical synthesis. The resulting heterologous third extender module coding sequence can be utilized in conjunction with a coding sequence for a PKS that synthesizes narbonolide, a narbonolide derivative, or another polyketide.

[0109] The recombinant DNA compounds of the invention that encode the fourth extender module of the narbonolide PKS and the corresponding polypeptides encoded thereby are useful for a variety of applications. In one embodiment, a DNA compound comprising a sequence that encodes the narbonolide PKS fourth extender module is inserted into a DNA compound that comprises the coding sequence for a heterologous PKS. The resulting construct, in which the coding sequence for a module of the heterologous PKS is either replaced by that for the fourth extender module of the narbonolide PKS or the latter is merely added to coding sequences for the modules of the heterologous PKS, provides a novel PKS. In another embodiment, a DNA compound comprising a sequence that encodes the fourth extender module of the narbonolide. PKS is inserted into a DNA compound that comprises coding sequences for the narbonolide PKS or a recombinant narbonolide PKS that produces a narbonolide derivative.

[0110] In another embodiment, a portion of the fourth extender module coding sequence is utilized in conjunction with other PKS coding sequences to create a hybrid module. In this embodiment, the invention provides, for example, replacing the methylmalonyl CoA specific AT with a malonyl CoA, ethylmalonyl CoA, or carboxyglycolyl CoA specific AT; deleting any one, two, or all three of the ER, DH, and KR; and/or replacing any one, two, or all three of the ER, DH, and KR with either a KR, a DH and KR, or a KR, DH, and ER. In addition, the KS and/or ACP can be replaced with another KS and/or ACP. In each of these replacements or insertions, the heterologous KS, AT, DH, KR, ER, or ACP coding sequence can originate from a coding sequence for another module of the narbonolide PKS, from a coding sequence for a PKS that produces a polyketide other than narbonolide, or from chemical synthesis. The resulting heterologous fourth extender module coding sequence can be utilized in conjunction with a coding sequence for a PKS that synthesizes narbonolide, a narbonolide derivative, or another polyketide.

[0111] The recombinant DNA compounds of the invention that encode the fifth extender module of the narbonolide PKS and the corresponding polypeptides encoded thereby are useful for a variety of applications. In one embodiment, a DNA compound comprising a sequence that encodes the narbonolide PKS fifth extender module is inserted into a DNA compound that comprises the coding sequence for a heterologous PKS. The resulting construct, in which the coding sequence for a module of the heterologous PKS is either replaced by that for the fifth extender module of the narbonolide PKS or the latter is merely added to coding sequences for the modules of the heterologous PKS, provides a novel PKS. In another embodiment, a DNA compound comprising a sequence that encodes the fifth extender module of the narbonolide PKS is inserted into a DNA compound that comprises the coding sequence for the narbonolide PKS or a recombinant narbonolide PKS that produces a narbonolide derivative.

[0112] In another embodiment, a portion or all of the fifth extender module coding sequence is utilized in conjunction with other PKS coding sequences to create a hybrid module. In this embodiment, the invention provides, for example, replacing the methylmalonyl CoA specific AT with a malonyl CoA, ethylmalonyl CoA, or carboxyglycolyl CoA specific AT; deleting (or inactivating) the KR; inserting a DH or a DH and ER; and/or replacing the KR with another KR, a DH and KR, or a DH, KR, and ER. In addition, the KS and/or ACP can be replaced with another KS and/or ACP. In each of these replacements or insertions, the heterologous KS, AT, DH, KR, ER, or ACP coding sequence can originate from a coding sequence for another module of the narbonolide PKS, from a coding sequence for a PKS that produces a polyketide other than narbonolide, or from chemical synthesis. The restyling heterologous fifth extender module coding sequence can be utilized in conjunction with a coding sequence for a PKS that synthesizes narbonolide, a narbonolide derivative, or another polyketide.

[0113] The recombinant DNA compounds of the invention that encode the sixth extender module of the narbonolide PKS and the corresponding polypeptides encoded thereby are useful for a variety of applications. In one embodiment, a DNA compound comprising a sequence that encodes the narbonolide PKS sixth extender module is inserted into a DNA compound that comprises the coding sequence for a heterologous PKS. The resulting construct, in which the coding sequence for a module of the heterologous PKS is either replaced by that for the sixth extender module of the narbonolide PKS or the latter is merely added to coding sequences for the modules of the heterologous PKS, provides a novel PKS. In another embodiment, a DNA compound comprising a sequence that encodes the sixth extender module of the narbonolide PKS is inserted into a DNA compound that comprises the coding sequences for the narbonolide PKS or a recombinant narbonolide PKS that produces a narbonolide derivative.

[0114] In another embodiment, a portion or all of the sixth extender module coding sequence is utilized in conjunction with other PKS coding sequences to create a hybrid module. In this embodiment, the invention provides, for example, replacing the methylmalonyl CoA specific AT with a malonyl CoA, ethylmalonyl CoA, or carboxyglycolyl CoA specific AT; and/or inserting a KR, a KR and DH, or a KR, DH, and an ER. In addition, the KS and/or ACP can be replaced with another KS and/or ACP. In each of these replacements or insertions, the heterologous KS, AT, DH, KR, ER, or ACP coding sequence can originate from a coding sequence for another module of the narbonolide PKS, from a coding sequence for a PKS that produces a polyketide other than narbonolide, or from chemical synthesis. The resulting heterologous sixth extender module coding sequence can be utilized in conjunction with a coding sequence for a PKS that synthesizes narbonolide, a narbonolide derivative, or another polyketide.

[0115] The sixth extender module of the narbonolide PKS is followed by a thioesterase domain. This domain is important in the cyclization of the polyketide and its cleavage from the PKS. The present invention provides recombinant DNA compounds that encode hybrid PKS enzymes in which the narbonolide PKS is fused to a heterologous thioesterase or a heterologous PKS is fused to the narbonolide synthase thioesterase. Thus, for example, a thioesterase domain coding sequence from another PKS gene can be inserted at the end of the sixth extender module coding sequence in recombinant DNA compounds of the invention. Recombinant DNA compounds encoding this thioesterase domain are therefore useful in constructing DNA compounds that encode the narbonolide PKS, a PKS that produces a narbonolide derivative, and a PKS that produces a polyketide other than narbonolide or a narbonolide derivative. The following Table lists references describing illustrative PKS genes and corresponding enzymes that can be utilized in the construction of the recombinant hybrid PKSs and the corresponding DNA compounds that encode them of the invention. Also presented are various references describing tailoring enzymes and corresponding genes that can be employed in accordance with the methods of the invention.

[0116] Avermectin

[0117] U.S. Pat. No. 5,252,474 to Merck.

[0118] MacNeil et al., 1993, *Industrial Microorganisms: Basic and Applied Molecular Genetics*, Baltz, Hegeman, & Skatrud, eds. (ASM), pp. 245-256, A Comparison of the Genes Encoding the Polyketide Synthases for Avermectin, Erythromycin, and Nemadectin.

[0119] MacNeil et al., 1992, *Gene* 115: 119-125, Complex Organization of the *Streptomyces avermitilis* genes encoding the avermectin polyketide synthase.

[0120] Candicidin (FR008)

[0121] Hu et al., 1994, *Mol. Microbiol.* 14 163-172.

[0122] Epothilone

[0123] U.S. patent application Ser. No. 60/130,560, filed 22 Apr. 1999, and Ser. No. 60/122,620, filed 3 Mar. 1999.

[0124] Erythromycin

[0125] PCT Pub. No. 93/13663 to Abbott.

[0126] U.S. Pat. No. 5,824,513 to Abbott.

[0127] Donadio et al., 1991, *Science* 252:675-9.

[0128] Cortes et al., 8 Nov. 1990, *Nature* 348:176-8, An unusually large multifunctional polypeptide in the erythromycin producing polyketide synthase of *Saccharopolyspora erythraea.*

[0129] Glycosylation Enzymes

[0130] PCT Pat. App. Pub. No. 97/23630 to Abbott.

[0131] FK506

[0132] Motamedi et al., 1998, The biosynthetic gene cluster for the macrolactone ring of the immunosuppressant FK506, *Eur. J. biochem.* 256: 528-534.

[0133] Motamedi et al., 1997, Structural organization of a multifunctional polyketide synthase involved in the biosynthesis of the macrolide immunosuppressant FK506, *Eur. J. Biochem.* 244: 74-80.

[0134] Methyltransferase

[0135] U.S. Pat. No. 5,264,355, issued 23 Nov. 1993, Methylating enzyme from *Streptomyces* MA6858. 31-O-desmethyl-FK506 methyltransferase.

[0136] Motamedi et al., 1996, Characterization of methyltransferase and hydroxylase genes involved in the biosynthesis of the immunosuppressants FK506 and FK520, *J. Bacteriol.* 178: 5243-5248.

[0137] FK520

[0138] U.S. patent application Ser. No. 60/123,810, filed 11 Mar. 1999.

[0139] Nielsen et al., 1991, *Biochem.* 30:5789-96.

[0140] Lovastatin

[0141] U.S. Pat. No. 5,744,350 to Merck.

[0142] Nemadectin

[0143] MacNeil et al., 1993, supra.

[0144] Niddamycin

[0145] Kakavas et al., 1997, Identification and characterization of the niddamycin polyketide synthase genes from *Streptomyces caelestis, J. Bacteriol.* 179: 7515-7522.

[0146] Oleandomycin

[0147] Swan et al., 1994, Characterisation of a *Streptomyces antibioticus* gene encoding a type I polyketide synthase which has an unusual coding sequence, *Mol. Gen. Genet.* 242: 358-362.

[0148] Olano et al., 1998, Analysis of a *Streptomyces antibioticus* chromosomal region involved in oleandomycin biosynthesis, which encodes two glycosyltransferases responsible for glycosylation of the riacrolactone ring, *Mol. Gen. Genet.* 259(3): 299-308.

[0149] U.S. patent application Ser. No. 60/120,254, filed 16 Feb. 1999, and Ser. No. 60/106,000, filed 29 Oct. 1998.

[0150] Platenolide

[0151] EP Pat. App. Pub. No. 791,656 to Lilly.

[0152] Pradimicin

[0153] PCI Pat. Pub. No. WO 98/11230 to Bristol-Myers Squibb.

[0154] Rapamycin

[0155] Schwecke et al., August 1995, The biosynthetic gene cluster for the polyketide rapamycin, *Proc. Natl. Acad. Sci. USA* 92:7839-7843.

[0156] Aparicio et al., 1996, Organization of the biosynthetic gene cluster for rapamycin in *Streptomyces hygroscopicus*: analysis of the enzymatic domains in the modular polyketide synthase, *Gene* 169: 9-16.

[0157] Rifamycin

[0158] August et al., 13 Feb. 1998, Biosynthesis of the ansamycin antibiotic rifamycin: deductions from the molecular analysis of the rif biosynthetic gene cluster of *Amycolatopsis mediterranei* S669, *Chemistry & Biology,* 5(2): 69-79.

[0159] Soraphen

[0160] U.S. Pat. No. 5,716,849 to Novartis.

[0161] Schupp et al., 1995, *J. Bacteriology* 177: 3673-3679. A *Sorangium cellulosum* (Myxobacterium) Gene Cluster for the Biosynthesis of the Macrolide Antibiotic Soraphen A: Cloning, Characterization, and Homology to Polyketide Synthase Genes from Actinomycetes.

[0162] Spiramycin

[0163] U.S. Pat. No. 5,098,837 to Lilly.

[0164] Activator Gene

[0165] U.S. Pat. No. 5,514,544 to Lilly.

[0166] Tylosin

[0167] EP Pub. No. 791,655 to Lilly.

[0168] Kuhstoss et al., 1996, *Gene* 183:231-6., Production of a novel polyketide through the construction of a hybrid polyketide synthase.

[0169] 25. U.S. Pat. No. 5,876,991 to Lilly.

[0170] Tailoring Enzymes

[0171] Merson-Davies and Cundliffe, 1994, *Mol. Microbiol.* 13: 349-355. Analysis of five tylosin biosynthetic genes from the tylBA region of the *Streptomyces fradiae* genome.

[0172] As the above Table illustrates, there are a wide variety of PKS genes that serve as readily available sources of DNA and sequence information for use in constructing the hybrid PKS-encoding DNA compounds of the invention. Methods for constructing hybrid PKS-encoding DNA compounds are described without reference to the narbonolide PKS in U.S. Pat. Nos. 5,672,491 and 5,712,146 and PCT publication No. 98/49315, each of which is incorporated herein by reference.

[0173] In constructing hybrid PKSs of the invention, certain general methods may be helpful. For example, it is often beneficial to retain the framework of the module to be altered to make the hybrid PKS. Thus, if one desires to add DH and ER functionalities to a module, it is often preferred to replace the KR domain of the original module with a KR, DH, and ER domain-containing segment from another module, instead of merely inserting DH and ER domains. One can alter the stereochemical specificity of a module by replacement of the KS domain with a KS domain from a module that specifies a different stereochemistry. See Lau et al., 1999, Dissecting the role of acyltransferase domains of modular polyketide synthases in the choice and stereochemical fate of extender units"*Biochemistry* 38(5):1643-1651, incorporated herein by reference. One can alter the specificity of an AT domain by changing only a small segment of the domain. See Lau et al., supra. One can also take advantage of known linker regions in PKS proteins to link modules from two different PKSs to create a hybrid PKS. See Gokhale et al., 16 Apr. 1999, Dissecting and Exploiting Intermodular Communication in Polyketide Synthases", *Science* 284: 482-485, incorporated herein by reference.

[0174] The hybrid PKS-encoding DNA compounds of the invention can be and often are hybrids of more than two PKS genes. Even where only two genes are used, there are often two or more modules in the hybrid gene in which all or part of the module is derived from a second (or third) PKS gene. Thus, as one illustrative example, the invention provides a hybrid narbonolide PKS that contains the naturally occurring loading module and thioesterase domain as well as extender modules one, two, four, and six of the narbonolide PKS and further contains hybrid or heterologous extender modules three and five. Hybrid or heterologous extender modules three and five contain AT domains specific for malonyl CoA and derived from, for example, the rapamycin PKS genes.

[0175] To construct a hybrid PKS or narbonolide derivative PKS of the invention, one can employ a technique, described in PCT Pub. No. 98/27203, which is incorporated herein by reference, in which the large PKS gene cluster is divided into two or more, typically three, segments, and each segment is placed on a separate expression vector. In this manner, each of the segments of the gene can be altered, and various altered segments can be combined in a single host cell to provide a recombinant PKS gene of the invention. This technique makes more efficient the construction of large libraries of recombinant PKS genes, vectors for expressing those genes, and host cells comprising those vectors.

[0176] The invention also provides libraries of PKS genes, PKS proteins, and ultimately, of polyketides, that are constructed by generating modifications in the narbonolide PKS so that the protein complexes produced have altered activities in one or more respects and thus produce polyketides other than the natural product of the PKS. Novel polyketides may thus be prepared, or polyketides in general prepared more readily, using this method. By providing a large number of different genes or gene clusters derived from a naturally occurring PKS gene cluster, each of which has been modified in a different way from the native cluster, an effectively combinatorial library of polyketides can be produced as a result of the multiple variations in these activities. As will be further described below, the metes and bounds of

this embodiment of the invention can be described on both the protein level and the encoding nucleotide sequence level.

[0177] As described above, a modular PKS "derived from" the narbonolide or other naturally occurring PKS includes a modular PKS (or its corresponding encoding gene(s)) that retains the scaffolding of the utilized portion of the naturally occurring gene. Not all modules need be included in the constructs. On the constant scaffold, at least one enzymatic activity is mutated, deleted, replaced, or inserted so as to alter the activity of the resulting PKS relative to the original PKS. Alteration results when these activities are deleted or are replaced by a different version of the activity, or simply mutated in such a way that a polyketide other then the natural product results from these collective activities. This occurs because there has been a resulting alteration of the starter unit and/or extender unit, and/or stereochemistry, and/or chain length or cyclization, and/or reductive or dehydration cycle outcome at a corresponding position in the product polyketide. Where a deleted activity is replaced, the origin of the replacement activity may come from a corresponding activity in a different naturally occurring PKS or from a different region of the narbonolide PKS. Any or all of the narbonolide PKS genes may be included in the derivative or portions of any of these may be included, but the scaffolding of the PKS protein is retained in whatever derivative is constructed. The derivative preferably contains a thioesterase activity from the narbonolide or another PKS.

[0178] In summary, a PKS derived from the narbonolide PKS includes a PKS that contains the scaffolding of all or a portion of the narbonolide PKS. The derived PKS also contains at least two extender modules that are functional, preferably three extender modules, and more preferably four or more extender modules, and most preferably six extender modules. The derived PKS also contains mutations, deletions, insertions, or replacements of one or more of the activities of the functional modules of the narbonolide PKS so that the nature of the resulting polyketide is altered. This definition applies both at the protein and DNA sequence levels. Particular preferred embodiments include those wherein a KS, AT, KR, DH, or ER has been deleted or replaced by a version of the activity from a different PKS or from another location within the same PKS. Also preferred are derivatives where at least one non-condensation cycle enzymatic activity (KR, DH, or ER) has been deleted or added or wherein any of these activities has been mutated so as to change the structure of the polyketide synthesized by the PKS.

[0179] Conversely, also included within the definition of a PKS derived from the narbonolide PKS are functional PKS modules or their encoding genes wherein at least one portion, preferably two portions, of the narbonolide PKS activities have bean inserted. Exemplary is the use of the narbonolide AT for extender module 2 which accepts a malonyl CoA extender unit rather than methylmalonyl CoA to replace a methylmalonyl specific AT in a PKS. Other examples include insertion of portions of non-condensation cycle enzymatic activities or other regions of narbonolide synthase activity into a heterologous PKS. Again, the derived from definition applies to the PKS at both the genetic and protein levels.

[0180] Thus, there are at least five degrees of freedom for constructing a hybrid PKS in terms of the polyketide that will be produced. First, the polyketide chain length is determined by the number of modules in the PKS. Second, the nature of the carbon skeleton of the PKS is determined by the specificities of the acyl transferases that determine the nature of the extender units at each position, e.g., malonyl, methylmalonyl, ethylmalonyl, or other substituted malonyl. Third, the loading module specificity also has an effect on the resulting carbon skeleton of the polyketide. The loading module may use a different starter unit, such as acetyl, butyryl, and the like. As noted above and in the examples below, another method for varying loading module specificity involves inactivating the KS activity in extender module 1 (KS1) and providing alternative substrates, called diketides that are chemically synthesized analogs of extender module 1 diketide products, for extender module 2. This approach was illustrated in PCT publication Nos. 97/02358 and 99/03986, incorporated herein by reference, wherein the KS1 activity was inactivated through mutation. Fourth, the oxidation state at various positions of the polyketide will be determined by the dehydratase and reductase portions of the modules. This will determine the presence and location of ketone and alcohol moieties and C—C double bonds or C—C single bonds in the polyketide. Finally, the stereochemistry of the resulting polyketide is a function of three aspects of the synthase. The first aspect is related to the AT/KS specificity associated with substituted malonyls as extender units, which affects stereochemistry only when the reductive cycle is missing or when it contains only a ketoreductase, as the dehydratase would abolish chirality. Second, the specificity of the ketoreductace may determine the chirality of any beta-OH. Finally, the enoylreductase specificity for substituted malonyls as extender units may influence the result when there is a complete KR/DH/ER available.

[0181] Thus, the modular PKS systems, and in particular, the narbonolide PKS system, permit a wide range of polyketides to be synthesized. As compared to the aromatic PKS systems, a wider range of starter units including aliphatic monomers (acetyl, propionyl, butyryl, isovaleryl, etc.), aromatics (aminohydroxybenzoyl), alicyclics (cyclohexanoyl), and heterocyclics (thiazolyl) are found in various macrocyclic polyketides. Recent studies have shown that modular PKSs have relaxed specificity for their starter units (Kao et al., 1994, *Science*, supra). Modular PKSs also exhibit considerable variety with regard to the choice of extender units in each condensation cycle. The degree of beta-ketoreduction following a condensation reaction has also been shown to be altered by genetic manipulation (Donadio et al., 1991, *Science*, supra; Donadio et al., 1993, *Proc. Natl. Acad. Sci. USA* 90: 7119-7123). Likewise, the size of the polyketide product can be varied by designing mutants with the appropriate number of modules (Kao et al., 1994, *J. Am. Chem. Soc.* 116:1612-11613). Lastly, these enzymes are particularly well known for generating an impressive range of asymmetric centers in their products in a highly controlled manner. The polyketides and antibiotics produced by the methods of the invention are typically single stereoisomeric forms. Although the compounds of the invention can occur as mixtures of stereoisomers, it may be beneficial in some instances to generate individual stereoisomers. Thus, the combinatorial potential within modular PKS pathways based on any naturally occurring modular, such as the narbonolide, PKS scaffold is virtually unlimited.

[0182] The combinatorial potential is increased even further when one considers that mutations in DNA encoding a polypeptide can be used to introduce, alter, or delete an activity in the encoded polypeptide. Mutations can be made to the native sequences using conventional techniques. The substrates for mutation can be an entire cluster of genes or only one or two of them; the substrate for mutation may also be portions of one or more of these genes. Techniques for mutation include preparing synthetic oligonucleotides including the mutations and inserting the mutated sequence into the gene encoding a PKS subunit using restriction endonuclease digestion. See, e.g., Kunkel, 1985, *Proc. Natl. Acad. Sci. USA* 82: 448; Geisselsoder et al., 1987, *BioTechniques* 5:786. Alternatively, the mutations can be effected using a mismatched primer (generally 10-20 nucleotides in length) that hybridizes to the native nucleotide sequence, at a temperature below the melting temperature of the mismatched duplex. The primer can be made specific by keeping primer length and base composition within relatively narrow limits and by keeping the mutant base centrally located, See Zoller and Smith, 1983, *Methods Enzymol.* 100:468. Primer extension is effected using DNA polymerase, the product cloned, and clones containing the mutated DNA, derived by segregation of the primer extended strand, selected. Identification can be accomplished using the mutant primer as a hybridization probe. The technique is also applicable for generating multiple point mutations. See, e.g., Dalbie-McFarland et al., 1982, *Proc. Natl. Acad. Sci. USA* 79: 6409. PCR mutagenesis can also be used to effect the desired mutations. Random mutagenesis of selected portions of the nucleotide sequences encoding enzymatic activities can also be accomplished by several different techniques known in the art, e.g., by inserting an oligonucleotide linker randomly into a plasmid, by irradiation with X-rays or ultraviolet light, by incorporating incorrect nucleotides during in vitro DNA synthesis, by error-prone PCR mutagenesis, by preparing synthetic mutants, or by damaging plasmid DNA in vitro with chemicals. Chemical mutagens include, for example, sodium bisulfite, nitrous acid, nitrosoguanidine, hydroxylamine, agents which damage or remove bases thereby preventing normal base-pairing such as hydrazine or formic acid, analogues of nucleotide precursors such as 5-bromouracil, 2-aminopurine, or acrdine intercaculating agents such as proflavine, acriflavine, quinacrine, and the like. Generally, plasmid DNA or DNA fragments are treated with chemicals, transformed into *E. coli* and propagated as a pool or library of mutant plasmids.

[0183] In constructing a hybrid PKS of the invention, regions encoding enzymatic activity, i.e., regions encoding corresponding activities from different PKS synthases or from different locations in the same PKS, can be recovered, for example, using PCR techniques with appropriate primers. By "corresponding" activity encoding regions is meant those regions encoding the same general type of activity. For example, a KR activity encoded at one location of a gene cluster "corresponds" to a KR encoding activity in another location in the gene cluster or in a different gene cluster. Similarly, a complete reductase cycle could be considered corresponding. For example, KR/DH/ER corresponds to KR alone.

[0184] If replacement of a particular target region in a host PKS is to be made, this replacement can be conducted in vitro using suitable restriction enzymes. The replacement can also be effected in vivo using recombinant techniques involving homologous sequences framing the replacement gene in a donor plasmid and a receptor region in a recipient plasmid. Such systems, advantageously involving plasmids of differing temperature sensitivities are described, for example, in PCT publication No. WO 96/40968, incorporated herein by reference. The vectors used to perform the various operations to replace the enzymatic activity in the host PKS genes or to support mutations in these regions of the host PKS genes can be chosen to contain control sequences operably linked to the resulting coding sequences in a manner such that expression of the coding sequences can be effected in an appropriate host.

[0185] However, simple cloning vectors may be used as well. If the cloning vectors employed to obtain PKS genes encoding derived PKS lack control sequences for expression operably linked to the encoding nucleotide sequences, the nucleotide sequences are inserted into appropriate expression vectors. This need not be done individually, but a pool of isolated encoding nucleotide sequences can be inserted into expression vectors, the resulting vectors transformed or transfected into host cells, and the resulting cells plated out into individual colonies.

[0186] The various PKS nucleotide sequences can be cloned into one or more recombinant vectors as individual cassettes, with separate control elements, or under the control of, e.g., a single promoter. The PKS subunit encoding regions can include flanking restriction sites to allow for the easy deletion and insertion of other PKS subunit encoding sequences so that hybrid PKSs can be generated. The design of such unique restriction sites is known to those of skill in the art and can be accomplished using the techniques described above, such as site-directed mutagenesis and PCR.

[0187] The expression vectors containing nucleotide sequences encoding a variety of PKS enzymes for the production of different polyketides are then transformed into the appropriate host cells to construct the library. In one straightforward approach, a mixture of such vectors is transformed into the selected host cells and the resulting cells plated into individual colonies and selected to identify successful transformants. Each individual colony has the ability to produce a particular PKS synthase and ultimately a particular polyketide. Typically, there will be duplications in some, most, or all of the colonies; the subset of the transformed colonies that contains a different PKS in each member colony can be considered the library. Alternatively, the expression vectors can be used individually to transform hosts, which transformed hosts are then assembled into a library. A variety of strategies are available to obtain a multiplicity of colonies each containing a PKS gene cluster derived from the naturally occurring host gene cluster so that each colony in the library produces a different PKS and ultimately a different polyketide. The number of different polyketides that are produced by the library is typically at least four, more typically at least ten, and preferably at least 20, and more preferably at least 50, reflecting similar numbers of different altered PKS gene clusters and PKS gene products. The number of members in the library is arbitrarily chosen; however, the degrees of freedom outlined above with respect to the variation of starter, extender units, stereochemistry, oxidation state, and chain length is quite large.

[0188] Methods for introducing the recombinant vectors of the invention into suitable hosts are known to those of skill in the art and typically include the use of $CaCl_2$ or agents such as other divalent cations, lipofection, DMSO, protoplast transformation, infection, transfection, and electroporation. The polyketide producing colonies can be identified and isolated using known techniques and the produced polyketides further characterized. The polyketides produced by these colonies can be used collectively in a panel to represent a library or may be assessed individually for activity.

[0189] The libraries of the invention can thus be considered at four levels: (1) a multiplicity of colonies each with a different PKS encoding sequence; (2) colonies that contain the proteins that are members of the PKS library produced by the coding sequences; (3) the polyketides produced; and (4) antibiotics or compounds with other desired activities derived from the polyketides. Of course, combination libraries can also be constructed wherein-members of a library derived, for example, from the narbonolide PKS can be considered as a part of the same library as those derived from, for example, the rapamycin PKS or DEBS.

[0190] Colonies in the library are induced to produce the relevant synthases and thus to produce the relevant polyketides to obtain a library of polyketides. The polyketides secreted into the media can be screened for binding to desired targets, such as receptors, signaling proteins, and the like. The supernatants per se can be used for screening, or partial or complete purification of the polyketides can first be effected. Typically, such screening methods involve detecting the binding of each member of the library to receptor or other target ligand. Binding can be detected either directly or through a competiton assay. Means to screen such libraries for binding are well known in the art. Alternatively, individual polyketide members of the library can be tested against a desired target. In this event, screens wherein the biological response of the target is measured can more readily be included. Antibiotic activity can be verified using typical screening assays such as those set forth in Lehrer et al., 1991, *J. Immunol. Meth.* 137:167-173, incorporated herein by reference, and in the examples below.

[0191] The invention provides methods for the preparation of a large number of polyketides. These polyketides are useful intermediates in formation of compounds with antibiotic or other activity through hydroxylation and glycosylation reactions as described above. In general, the polyketide products of the PKS must be further modified, typically by hydroxylation and glycosylation, to exhibit antibiotic activity. Hydroxylation results in the novel polyketides of the invention that contain hydroxyl groups at C6, which can be accomplished using the hydroxylase encoded by the erF gene, and/or C12, which can be accomplished using the hydroxylase encoded by the picK or eryK gene. The presence of hydroxyl groups at these positions can enhance the antibiotic activity of the resulting compound relative to its unhydroxylated counterpart.

[0192] Gycosylation is important in conferring antibiotic activity to a polyketide as well. Methods for glycosylating the polyketides are generally known in the art; the glycosylation may be effected intracellularly by providing the appropriate glycosylation enzymes or may be effected in vitro using chemical synthetic means as described herein and in PCT publication No. WO 98/49315, incorporated herein by reference. Preferably, glycosylation with desosamine is effected in accordance with the methods of the invention in recombinant host cells provided by the invention. In general, the approaches to effecting glycosylation mirror those described above with respect to hydroxylation. The purified enzymes, isolated from native sources or recombinantly produced may be used in vitro. Alternatively and as noted, glycosylation may be effected intracellularly using endogenous or recombinantly produced intracellular glycosylases. In addition, synthetic chemical methods may be employed.

[0193] The antibiotic modular polyketides may contain any of a number of different sugars, although D-desosamine, or a close analog thereof, is most common. Erythromycin, picromycin, narbomycin and methymycin contain desosamine. Erythromycin also contains L-cladinose (34-methyl mycarose). Tylosin contains mycaminose (4-hydroxy desosamine), mycarose and 6-deoxy-D-allose. 2-acetyl-1-bromodesosamine has been used as a donor to glycosylate polyketides by Masamune et al., 1975, *J. Am. Chem. Soc.* 97: 3512-3513. Other, apparently more stable donors include glycosyl fluorides, thioglycosides, and trichloroacetimidates; see Woodward et al., 1981, *J. Am. Chem. Soc.* 103: 3215; Martinet al., 1997, *J. Am. Chem. Soc.* 119: 3193; Toshima et al., 1995, *J. Am. Chem. Soc.* 117: 3717; Matsumoto et al., 1988, *Tetrahedron Lett.* 29: 3575. Glycosylation can also be effected using the polyketide aglycones as starting materials and using *Saccharopolyspora erythraea* or *Streptomyces venezuelae* to make the conversion, preferably using mutants unable to synthesize macrolides.

[0194] To provide an illustrative hybrid PKS of the invention as well as an expression vector for that hybrid PKS and host cells comprising the vector and producing the hybrid polyketide, a portion of the narbonolide PKS gene was fused to the DEBS genes. This construct also allowed the examination of whether the TE domain of the narbonolide PKS (pikTE) could promote formation of 12-membered lactones in the context of a different PKS. A construct was generated, plasmid pKOS039-18, in which the pikTE ORF was fused with the DEBS genes in place of the DEBS TE ORF (see **FIG. 5**). To allow the TE to distinguish between substrates most closely resembling those generated by the narbonolide PKS, the fusion junction was chosen between the AT and ACP to eliminate ketoreductase activity in DEBS extender module 6 (KR6). This results in a hybrid PKS that presents the TE with a β-ketone heptaketide intermediate and a β-(S)-hydroxy hexaketide intermediate to cyclize, as in narbonolide and 10-deoxymethynolide biosynthesis.

[0195] Analysis of this construct indicated the production of the 14-membered ketolide 3,6-dideoxy-3-oxo-erythronolide B (**FIG. 5**, compound 6). Extracts were analyzed by LC/MS. The identity of compound 6 was verified by comparison to a previously authenticated sample (see PCT publication No. 98/49315, incorporated herein by reference). The predicted 12-membered macrolactone, (8R,9S)-8,9-dihydromethyl-9-hydroxy-10-deoxymethynolide (see Kao et al, 1995, *J. Am. Chem. Soc.* 127, incorporated herein by reference) was not detected. This result, along with others reported herein, suggests that protein interactions between the narbonolide PKS modules play a role in formation of the 12 and 14-membered macrolides.

[0196] The above example illustrates also how engineered PKSs can be improved for production of novel compounds. Compound 6 was originally produced by deletion of the KR6 domain in DEBS to create a 3-ketolide producing PKS (see U.S. patent application Ser. No. 09/073,538, filed 6 May

1998, and PCT publication No. WO 98/49315, each of which is incorporated herein by reference). Although the desired molecule was made, purification of compound 6 from this strain was hampered by the presence of 2-desmethyl ketolides that could not be easily separated. Extracts from *Streptomyces lividans* KS 114/pKOS039-18, however, do not contain the 2-desmethyl compounds, greatly simplifying purification. Thus, the invention provides a useful method of producing such compounds. The ability to combine the narbonolide PKS with DEBS and other modular PKSs provides a significant advantage in the production of macrolide antibiotics.

[0197] Two other hybrid PKSs of the invention were constructed that yield this same compound. These constructs also illustrate the method of the invention in which hybrid PKSs are constructed at the protein, as opposed to the module, level. Thus, the invention provides a method for constructing a hybrid PKS which comprises the coexpression of at least one gene from a first modular PKS gene cluster in a host cell that also expresses at least one gene from a second PKS gene cluster. The invention also provides novel hybrid PKS enzymes prepared in accordance with the method. This method is not limited to hybrid PKS enzymes composed of at least one narbonolide PKS gene, although such constructs are illustrative and preferred. Moreover, the hybrid PKS enzymes are not limited to hybrids composed of unmodified proteins; as illustrated below, at least one of the genes can optionally be a hybrid PKS gene.

[0198] In the first construct, the eryAI and eryAI genes were coexpressed with picAIV and a gene encoding a hybrid extender module 5 composed of the KS and AT domains of extender module 5 of DEBS3 and the KR and ACP domains of extender module 5 of the narbonolide PKS. In the second construct, the picAIV coding sequence was fused to the hybrid extender module 5 coding sequence used in the first construct to yield a single protein. Each of these constructs produced 3-deoxy-3-oxo-6-deoxyerythronolide B. In a third construct, the coding sequence for extender module 5 of DEBS3 was fused to the picAIV coding sequence, but the levels of product produced were below the detection limits of the assay.

[0199] A variant of the first construct hybrid PKS was constructed that contained an inactivated DEBS1 extender module 1 KS domain. When host cells containing the resultant hybrid PKS were supplied the appropriate diketide precursor, the desired 13-desethyl-13-propyl compounds were obtained, as described in the examples below.

[0200] Other illustrative hybrid PKSs of the invention were made by coexpressing the picAI and picAII genes with genes encoding DEBS3 or DEBS3 variants. These constructs illustrate the method of the invention in which a hybrid PKS is produced from coexpression of PKS genes unmodified at the modular or domain level. In the first construct, the enjAIII gene was coexpressed with the picAI and picAII genes, and the hybrid PKS produced 10-desmethyl-10,11-anhydro-6-deoxyerythronolide B in *Streptomyces lividans*. Such a hybrid PKS could also be constructed in accordance with the method of the invention by transformation of *S. venzuelae* with an expression vector that produces the enyAIII gene product, DEBS3. In a preferred embodiment, the *S. venezuelae* host cell has been modified to inactivate the picAIII gene.

[0201] In the second construct, the DEBS3 gene was a variant that had an inactive KR in extender module 5. The hybrid PKS produced 5,6-dideoxy-5-oxo-10-desmethyl-10, 11-anhydroerythronolide B in *Streptomyces lividans.*

[0202] In the third construct, the DEBS3 gene was a variant in which the KR domain of extender module 5 was replaced by the DH and KR domains of extender module 4 of the rapamycin PKS. This construct produced 5,6-dideoxy-5-oxo-10-desmethyl-10,11-anhydroerythronolide B and 5,6-dideoxy-4,5-anhydro-10-desmethyl-10,11-anhydroerythronolide B in *Streptomyces lividans*, indicating that the rapamycin DH and KR domains functioned only inefficiently in this construct.

[0203] In the fourth construct, the DEBS3 gene was a variant in which the KR domain of extender module 5 was replaced by the DH, KR, and ER domains of extender module 1 of the rapamycin PKS. This construct produced 5,6-dideoxy-5-oxo-10-desmethyl-10,11-anhydroerythronolide B as well as 5,6-dideoxy-10-desmethyl-10,11-anhydroerythronolide B in *Streptomyces lividans*, indicating that the rapamycin DH, KR, and ER domains functioned only inefficiently in this construct.

[0204] In the fifth construct, the DEBS3 gene was a variant in which the KR domain of extender module 6 was replaced by the DH and KR domains of extender module 4 of the rapamycin PKS. This construct produced 3,6-dideoxy-2,3-anhydro-10-desmethyl-10,11-anhydroerythronolide B in *Streptomyces lividans.*

[0205] In the sixth construct, the DEBS3 gene was a variant in which the AT domain of extender module 6 was replaced by the AT domain of extender module 2 of the rapamycin PKS. This construct produced 2,10-didesmethyl-10,11-anhydro-6-deoxyerythronolide B in *Streptomyces lividans.*

[0206] These hybrid PKSs illustrate the wide variety of polyketides that can be produced by the methods and compounds of the invention. These polyketides are useful as antibiotics and as intermediates in the synthesis of other useful compounds, as described in the following section.

[0207] Section VI: Compounds

[0208] The methods and recombinant DNA compounds of the invention are useful in the production of polyketides. In one important aspect, the invention provides methods for making ketolides, polyketide compounds with significant antibiotic activity. See Griesgraber et al., 1996, *J. Antibiot.* 49: 465-477, incorporated herein by reference. Most if not all of the ketolides prepared to date are synthesized using erythromycin A, a derivative of 6-dEB, as an intermediate. While the invention provides hybrid PKSs that produce a polyketide different in structure from 6-dEB, the invention also provides methods for making intermediates useful in preparing traditional, 6-dEB-derived ketolide compounds.

[0209] Because 6-dEB in part differs from narbonolide in that it comprises a 10-methyl group, the novel hybrid PKS genes of the invention based on the narbonolide PKS provide many novel ketolides that differ from the known ketolides only in that they lack a 10-methyl group. Thus, the invention provides the 10-desmethyl analogues of the ketolides and intermediates and precursor compounds described in, for example, Griesgraber et al., supra; Agouridas et al., 1998, *J. Med. Chem.* 41: 4080-4100, U.S. Pat. Nos. 5,770,579; 5,760,233; 5,750,510; 5,747,467; 5,747,466; 5,656,607; 5,635,485; 5,614,614; 5,556,118; 5,543,

400; 5,527,780; 5,444,051; 5,439,890; 5,439,889; and PCT publication Nos. WO 98/09978 and 98/28316, each of which is incorporated herein by reference. Because the invention also provides hybrid PKS genes that include a methylmalonyl-specific AT domain in extender module 2 of the narbonolide PKS, the invention also provides hybrid PKS that can be used to produce the 10-methyl-containing ketolides known in the art.

[0210] Thus, a hybrid PKS of the invention that produces 10-methyl narbonolide is constructed by substituting the malonyl-specific AT domain of the narbonolide PKS extender module 2 with a methylmalonyl specific AT domain from a heterologous PKS. A hybrid narbonolide PKS in which the AT of extender module 2 was replaced with the AT from DEBS extender module 2 was constructed using boundaries described in PCT publication No. 98/49315, incorporated herein by reference. However, when the hybrid PKS expression vector was introduced into *Streptomyces venezuelae*, detectable quantities of 10-methyl picromycin were not produced. Thus, to construct such a hybrid PKS of the invention, an AT domain from a module other than DEBS extender module 2 is preferred. One could also employ DEBS extender module 2 or another methylmalonyl specific AT but utilize instead different boundaries than those used for the substitution described above. In addition, one can construct such a hybrid PKS by substituting, in addition to the AT domain, additional extender module 2 domains, including the KS, the KR, and the DH, and/or additional extender module 3 domains.

[0211] Although modification of extender module 2 of the narbonolide PKS is required, the extent of hybrid modules engineered need not be limited to module 2 to make 10-methyl narbonolide. For example, substitution of the KS domain of extender module 3 of the narbonolide PKS with a heterologous domain or module can result in more efficient processing of the intermediate generated by the hybrid extender module 2. Likewise, a heterologous TE domain may be more efficient in cyclizing 10-methyl narbonolide.

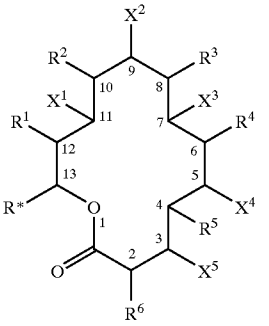
[0212] Substitution of the entire extender module 2 of the narbonolide PKS with a module encoding the correct enzymatic activities, i.e., a KS, a methylmalonyl specific AT, a KR, a DH, and an ACP, can also be used to create a hybrid PKS of the invention that produces a 10-methyl ketolide. Modules useful for such whole module replacements include extender modules 4 and 10 from the rapamycin PKS, extender modules 1 and 5 from the FK506 PKS, extender module 2 of the tylosin PKS, and extender module 4 of the rifamycin PKS. Thus, the invention provides many different hybrid PKSs that can be constructed starting from the narbonolide PKS that can be used to produce 10-methyl narbonolide. While 10-methyl narbonolide is referred to in describing these hybrid PKSs, those of skill recognize that the invention also therefore provides the corresponding derivatives produces by glycosylation and hydroxylation. For example, if the hybrid PKS is expressed in *Streptomyces narbonensis* or *S. venezuelae*, the compounds produced are 10-methyl narbomycin and picromycin, respectively. Alternatively, the PKS can be expressed in a host cell transformed with the vectors of the invention that encode the desosamine biosynthesis and desosaminyl transferase and picK hydroxylase genes.

[0213] Other important compounds provided by the invention are the 6-hydroxy ketolides. These compounds include 3-deoxy-3-oxo erythronolide B, 6-hydroxy narbonolide, and 6-hydroxy-10-methyl narbonolide. In the examples below, the invention provides a method for utilizing EryF to hydroxylate 3-ketolides that is applicable for the production of any 6-hydroxy-3-ketolide.

[0214] Thus, the hybrid PKS genes of the invention can be expressed in a host cell that contains the desosamine biosynthetic genes and desosaminyl transferase gene as well as the required hydroxylase gene(s), which may be either picK (for the C12 position) or eryK (for the C12 position) and/or eryF (for the C6 position). The resulting compounds have antibiotic activity but can be further modified, as described in the patent publications referenced above, to yield a desired compound with improved or otherwise desired properties. Alternatively, the aglycone compounds can be produced in the recombinant host cell, and the desired glycosylation and hydroxylation steps carried out in vitro or in vivo, in the latter case by supplying the converting cell with the aglycone.

[0215] The compounds of the invention are thus optionally glycosylated forms of the polyketide set forth in formula (2) below which are hydroxylated at either the C6 or the C12 or both. The compounds of formula (2) can be prepared using the loading and the six extender modules of a modular PKS, modified or prepared in hybrid form as herein described. These polyketides have the formula:

(2)



[0216] including the glycosylated and isolated stereoisomeric forms thereof;

[0217] wherein R* is a straight chain, branched or cyclic, saturated or unsaturated substituted or unsubstituted hydrocarbyl of 1-4C;

[0218] each of R^1-R^6 is independently H or alkyl (1-4C) wherein any alkyl at R^1 may optionally be substituted;

[0219] each of X^1-X^5 is independently two H, H and OH, or =O; or

[0220] each of X^1-X^5 is independently H and the compound of formula (2) contains a double-bond in the ring adjacent to the position of said X at 2-3, 4-5, 6-7,8-9 and/or 10-11;

[0221] with the proviso that:

[0222] at least two of R^1-R^6 are alkyl (1-4C).

[0223] Preferred compounds comprising formula 2 are those wherein at least three of R^1-R^5 are alkyl (1-4C),

preferably methyl or ethyl; more preferably wherein at least four of R^1-R^5 are alkyl (1-4C), preferably methyl or ethyl. Also preferred are those wherein X^2 is two H, =O, or H and OH, and/or X^3 is H, and/or X^1 is OH and/or X^5 is OH and/or X^5 is OH. Also preferred are compounds with variable R* when R^1-R^5 is methyl, X^2 is =O, and X^1, X^4 and X^5 are OH. The glycosylated forms of the foregoing are also preferred.

[0224] The invention also provides the 12-membered macrolides corresponding to the compounds above but produced from a narbonolide-derived PKS lacking extender modules 5 and 6 of the narbonolide PKS.

[0225] The compounds of the invention can be produced by growing and fermenting the host cells of the invention under conditions known in the art for the production of other polyketides. The compounds of the invention can be isolated from the fermentation broths of these cultured cells and purified by standard procedures. The compounds can be readily formulated to provide the pharmaceutical compositions of the invention. The pharmaceutical compositions of the invention can be used in the form of a pharmaceutical preparation, for example, in solid, semisolid, or liquid form. This preparation will contain one or more of the compounds of the invention as an active ingredient in admixture with an organic or inorganic carrier or excipient suitable for external, enteral, or parenteral application. The active ingredient may be compounded, for example, with the usual non-toxic, pharmaceutically acceptable carriers for tablets, pellets, capsules, suppositories, solutions, emulsions, suspensions, and any other form suitable for use.

[0226] The carriers which can be used include water, glucose, lactose, gum acacia, gelatin, mannitol, starch paste, magnesium trisilicate, talc, corn starch, keratin, colloidal silica, potato starch, urea, and other carriers suitable for use in manufacturing preparations, in solid, semi-solid, or liquefied form. In addition, auxiliary stabilizing, thickening, and coloring agents and perfumes may be used. For example, the compounds of the invention may be utilized with hydroxypropyl methylcellulose essentially as described in U.S. Pat. No. 4,916,138, incorporated herein by reference, or with a surfactant essentially as described in EPO patent publication No. 428,169, incorporated herein by reference.

[0227] Oral dosage forms may be prepared essentially as described by Hondo et al., 1987, *Transplantation Proceedings XIX*, Supp. 6: 17-22, incorporated herein by reference. Dosage forms for external application may be prepared essentially as described in EPO patent publication No. 423,714, incorporated herein by reference. The active compound is included in the pharmaceutical composition in an amount sufficient to produce the desired effect upon the disease process or condition.

[0228] For the treatment of conditions and diseases caused by infection, a compound of the invention may be administered orally, topically, parenterally, by inhalation spray, or rectally in dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvant, and vehicles. The term parenteral, as used herein, includes subcutaneous injections, and intravenous, intramuscular, and intrasternal injection or infusion techniques.

[0229] Dosage levels of the compounds of the invention are of the order from about 0.01 mg to about 50 mg per kilogram of body weight per day, preferably from about 0.1 mg to about 10 mg per kilogram of body weight per day. The dosage levels are useful in the treatment of the above-indicated conditions (from about 0.7 mg to about 3.5 mg per patient per day, assuming a 70 kg patient). In addition, the compounds of the invention may be administered on an intermittent basis, i.e., at semi-weekly, weekly, semi-monthly, or monthly intervals.

[0230] The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. For example, a formulation intended for oral administration to humans may contain from 0.5 mg to 5 gm of active agent compounded with an appropriate and convenient amount of carrier material, which may vary from about 5 percent to about 95 percent of the total composition. Dosage unit forms will generally contain from about 0.5 mg to about 500 mg of active ingredient. For external administration, the compounds of the invention may be formulated within the range of, for example, 0.00001% to 60% by weight, preferably from 0.001% to 10% by weight, and most preferably from about 0.005% to 0.8% by weight.

[0231] It will be understood, however, that the specific dose level for any particular patient will depend on a variety of factors. These factors include the activity of the specific compound employed; the age, body weight, general health, sex, and diet of the subject; the time and route of administration and the rate of excretion of the drug; whether a drug combination is employed in the treatment; and the severity of the particular disease or condition for which therapy is sought.

[0232] A detailed description of the invention having been provided above, the following examples are given for the purpose of illustrating the invention and shall not be construed as being a limitation on the scope of the invention or claims.

EXAMPLE 1

General Methodology

[0233] Bacterial strains, plasmids, and culture conditions. *Streptomyces coelicolor* CH999 described in WO 95/08548, published 30 Mar. 1995, or *S. lividans* K4-114, described in Ziermann and Betlach, Jan. 99, Recombinant Polyketide Synthesis in *Streptomyces*: Engineering of Improved Host Strains, BioTechniques 26:106-110, incorporated herein by reference, was used as an expression host. DNA manipulations were performed in *Escherichia coli* XL1-Blue, available from Stratagene. *E. coli* MC1061 is also suitable for use as a host for plasmid manipulation. Plasmids were passaged through *E. coli* ET12567 (dam dcm hsdS Cm^r) (MacNeil, 1988, *J. Bacteriol.* 170: 5607, incorporated herein by reference) to generate unmethylated DNA prior to transformation of *S. coelicolor*. *E. coli* strains were grown under standard conditions. *S. coelicolor* strains were grown on R2YE agar plates (Hopwood et al., *Genetic manipulation of Streptomyces. A laboratory manual.* The John Innes Foundation: Norwich, 1985, incorporated herein by reference).

[0234] Many of the expression vectors of the invention illustrated in the examples are derived from plasmid pRM5, described in WO 95/08548, incorporated herein by reference. This plasmid includes a colEI replicon, an appropri-

ately truncated SCP2* *Streptomyces* replicon, two act-promoters to allow for bidirectional cloning, the gene encoding the actII-ORF4 activator which induces transcription from act promoters during the transition from growth phase to stationary phase, and appropriate marker genes. Engineered restriction sites in the plasmid facilitate the combinatorial construction of PKS gene clusters starting from cassettes encoding individual domains of naturally occurring PKSs. When plasmid pRM5 is used for expression of a PKS, all relevant biosynthetic genes can be plasmid-borne and therefore amenable to facile manipulation and mutagenesis in *E. coli*. This plasmid is also suitable for use in *Streptomyces* host cells. *Streptomyces* is genetically and physiologically well-characterized and expresses the ancillary activities required for in vivo production of most polyketides. Plasmid pRM5 utilizes the act promoter for PKS gene expression, so polyketides are produced in a secondary metabolite-like manner, thereby alleviating the toxic effects of synthesizing potentially bioactive compounds in vivo.

[**0235**] Manipulation of DNA and organisms. Polymerase chain reaction (PCR) was performed using Pfu polymerase (Stratagene; Taq polymerase from Perkin Elmer Cetus can also be used) under conditions recommended by the enzyme manufacturer. Standard in vitro techniques were used for DNA manipulations (Sambrook et al. *Molecular Cloning: A Laboratory Manual* (Current Edition)). *E. coli* was transformed using standard calcium chloride-based methods; a Bio-Rad *E. coli* pulsing apparatus and protocols provided by Bio-Rad could also be used. *S. coelicolor* was transformed by standard procedures (Hopwood et al. *Genetic manipulation of Streptomyces. A laboratory manual*. The John Innes Foundation: Norwich, 1985), and depending on what selectable marker was employed, transformants were selected using 1 mL of a 1.5 mg/mL thiostrepton overlay, 1 mL of a 2 mg/mL apramycin overlay, or both.

EXAMPLE 2

Cloning of the Picromycin Biosynthetic Gene Cluster from *Streptomyices venezuelae*

[**0236**] Genomic DNA (100 μg) isolated from *Streptomyces venezuelae* ATCC15439 using standard procedures was partially digested with Sau3AI endonuclease to generate fragments ~40 kbp in length. SuperCosI (Stratagene) DNA cosmid arms were prepared as directed by the manufacturer. A cosmid library was prepared by ligating 2.5 μg of the digested genomic DNA with 1.5 μg of cosmid arms in a 20 μL reaction. One microliter of the ligation mixture was propagated in *E. coli* XL1-Blue MR (Stratagene) using a GigapackIII XL packaging extract kit (Stratagene). The resulting library of ~3000 colonies was plated on a 10×150 mm agar plate and replicated to a nylon membrane.

[**0237**] The library was initially screened by direct colony hybridization with a DNA probe specific for ketosynthase domain coding sequences of PKS genes. Colonies were alkaline lysed, and the DNA was crosslinked to the membrane using UV irradiation. After overnight incubation with the probe at 42° C., the membrane was washed twice at 25° C. in 2×SSC buffer+0.1% SDS for 15 minutes, followed by two 15 minute washes with 2×SSC buffer at 55° C. Approximately 30 colonies gave positive hybridization signals with the degenerate probe. Several cosmids were selected and divided into two classes based on restriction digestion patterns. A representative cosmid was selected from each class for further analysis. The representative cosmids were designated pKOS023-26 and pKOS023-27. These cosmids were determined by DNA sequencing to comprise the narbonolide PKS genes, the desosamine biosynthesis and transferase genes, the beta-glucosidase gene and the picK hydroxylase gene.

[**0238**] These cosmids were deposited with the American Type Culture Collection in accordance with the terms of the Budapest Treaty. Cosmid pKOS023-26 was assigned accession number ATCC 203141, and cosmid pKOS023-27 was assigned accession number ATCC 203142.

[**0239**] To demonstrate that the narbonolide PKS genes had been cloned and to illustrate how the invention provides methods and reagents for constructing deletion variants of narbonolide PKS genes, a narbonolide PKS gene was deleted from the chromosome of *Streptomyces venezuelae*. This deletion is shown schematically in **FIG. 4**, parts B and C. A ~2.4 kb EcoRI-KpnI fragment and a ~2.1 kb KpnI-XhoI fragment, which together comprise both ends of the picAI gene (but lack a large portion of the coding sequence), were isolated from cosmid pKOS023-27 and ligated together into the commercially available vector pLitmus 28 (digested with restriction enzymes EcoRI and XhoI) to give plasmid pKOS039-07. The ~4.5 kb HindIII-SpeI fragment from plasmid pKOS039-07 was ligated with the 2.5 kb HindIII-NheI fragment of integrating vector pSET152, available from the NRRL, which contains an *E. coli* origin of replication and an apramycin resistance-conferring gene to create plasmid pKOS039-16. This vector was used to transform *S. venezuelae*, and apramycin-resistant transformants were selected.

[**0240**] Then, to select for double-crossover mutants, the selected transformants were grown in TSB liquid medium without antibiotics for three transfers and then plated onto non-selective media to provide single colony isolates. The isolated colonies were tested for sensitivity to apramycin, and the apramycin-sensitive colonies were then tested to determine if they produced picromycin. The tests performed included a bioassay and LC/MS analysis of the fermentation media. Colonies determined not to produce picromycin (or methymycin or neomethymycin) were then analyzed using PCR to detect an amplification product diagnostic of the deletion. A colony designated K3903 was identified, providing confirmation that the narbonolide PKS genes had been cloned. Transformation of strain K39-03 with plasmid pKOS039-27 comprising an intact picA gene under the control of the ermE* promoter from plasmid pWHM3 (see Vara et al., 1989, *J. Bact.* 171: 5872-5881, incorporated herein by reference) was able to restore picromycin production.

[**0241**] To determine that the cosmids also contained the picK hydroxylase gene, each cosmid was probed by Southern hybridization using a labeled DNA fragment amplified by PCR from the *Saccharopolyspora erythraea* C12-hydroxylase gene, eryK. The cosmids were digested with BamHI endonuclease and electrophoresed on a 1% agarose gel, and the resulting fragments were transferred to a nylon membrane. The membrane was incubated with the enyK probe overnight at 42° C., washed twice at 25° C. in 2×SSC buffer with 0.1% SDS for 15 minutes, followed by two 15 minute washes with 2×SSC buffer at 50° C. Cosmid

pKOS023-26 produced an ~3 kb fragment that hybridized with the probe under these conditions. This fragment was subcloned into the PCRscript™ (Stratagene) cloning vector to yield plasmid pKOS023-28 and sequenced. The ~1.2 kb gene designated picK above was thus identified. The picK gene product is homologous to eryK and other known macrolide cytochrome P450 hydroxylases.

[0242] By such methodology, the complete set of picromycin biosynthetic genes were isolated and identified. DNA sequencing of the cloned DNA provided further confirmation that the correct genes had been cloned. In addition, and as described in the following example, the identity of the genes was confirmed by expression of narbomycin in heterologous host cells.

EXAMPLE 3

Heterologous Expression of the Narbonolide PKS and the Picromycin Biosynthetic Gene Cluster

[0243] To provide a preferred host cell and vector for purposes of the invention, the narbonolide PKS was transferred to the non-macrolide producing host *Streptomyces lividans* K4-114 (see Ziermann and Betlach, 1999, *Biotechniques* 26, 106-110, and U.S. patent application Ser. No. 09/181,833, filed 28 Oct. 1998, each of which is incorporated herein by reference). This was accomplished by replacing the three DEBS ORFs on a modified version of pCK7 (see Kao et al., 1994, *Science* 265, 509-512, and U.S. Pat. No. 5,672,491, each of which is incorporated herein by reference) with all four narbonolide PKS ORFs to generate plasmid pKOS039-86 (see **FIG. 5**). The pCK7 derivative employed, designated pCK7'Kan', differs from pCK7 only in that it contains a kanamycin resistance conferring gene inserted at its HindIII restriction enzyme recognition site. Because the plasmid contains two selectable markers, one can select for both markers and so minimize contamination with cells containing rearranged, undesired vectors.

[0244] Protoplasts were transformed using standard procedures and transformants selected using overlays containing antibiotics. The strains were grown in liquid R5 medium for growth/seed and production cultures at 30° C. Transformed strains produced two compounds in similar yield (~5-10 mg/L each). Polyketides produced in the host cells were analyzed by bioassay against *Bacillus subtilis* and by LC/MS analysis. Analysis of extracts by LC/MS followed by ^{1}H-NMR spectroscopy of the purified compounds established their identity as narbonolide (**FIG. 5**, compound 4; see Kaiho et al., 1982, *J. Org. Chem.* 47: 1612-1614, incorporated herein by reference) and 10-deoxymethynolide (**FIG. 5**, compound 5; see Lambalot et al., 1992, *J. Antibiotics* 45, 1981-1982, incorporated herein by reference), the respective 14 and 12-membered polyketide aglycones of YC17, narbomycin, picromycin, and methymycin.

[0245] The production of narbonolide in *Streptomyces lividans* represents the expression of an entire modular polyketide pathway in a heterologous host. The combined yields of compounds 4 and 5 are similar to those obtained with expression of DEBS from pCK7 (see Kao et al., 1994, *Science* 265: 509-512, incorporated herein by reference). Furthermore, based on the relative ratios (~1:1) of compounds 4 And 5 produced, it is apparent that the narbonolide PKS itself possesses an inherent ability to produce both 12 and 14-membered macrolactones without the requirement of additional activities unique to *S. venezuelae*. Although the existence of a complementary enzyme present in *S. lividans* that provides this function is possible, it would be unusual to find such a specific enzyme in an organism that does not produce any known macrolide.

[0246] To provide a heterologous host cell of the invention that produces the narbonolide PKS and the picB gene, the picB gene was integrated into the chromosome of *Streptomyces lividans* harboring plasmid pKOS039-86 to yield *S. lividans* K39-18/pKOS039-86. To provide the integrating vector utilized, the picB gene was cloned into the *Streptomyces* genome integrating vector pSET152 (see Bierman et al., 1992, *Gene* 116, 43, incorporated herein by reference) under control of the same promoter (Pact1) as the PKS on plasmid pKOS039-86.

[0247] A comparison of strains K39-18/pKOS39-86 and K4-114/pKOS039-86 grown under identical conditions indicated that the strain containing TEII produced 47 times more total polyketide. Each strain was grown in 30 mL of R5 (see Hopwood et al., *Genetic Manipulation of Streptomyces: A Laboratory Manual*; John Innes Foundation: Norwich, UK, 1985, incorporated herein by reference) liquid (with 20 μg/mL thiostrepton) at 30° C. for 9 days. The fermentation broth was analyzed directly by reverse phase HPLC. Absorbance at 235 nm was used to monitor compounds and measure relative abundance. This increased production indicates that the enzyme is functional in this strain. As noted above, because the production levels of compound 4 and 5 from K39-18/pKOS03986 increased by the same relative amounts, TEII does not appear to influence the ratio of 12 and 14-membered lactone ring formation.

[0248] To express the glycosylated counterparts of narbonolide (narbomycin) and 10-deoxymethynolide (YC17) in heterologous host cells, the desosamine biosynthetic genes and desosaminyl transferase gene were transformed into the host cells harboring plasmid pKOS039-86 (and, optionally, the picB gene, which can be integrated into the chromosome as described above).

[0249] Plasmid pKOS039-104, see **FIG. 6**, comprises the desosamine biosynthetic genes, the beta-glucosidase gene, and the desosaminyl transferase gene. This plasmid was constructed by first inserting a polylinker oligonucleotide, containing a restriction enzyme recognition site for PacI, a Shine-Dalgarno sequence, and restriction enzyme recognition sites for NdeI, BglII, and HindIII, into a pUC19 derivative, called pKOS2447, to yield plasmid pKOS039-98.

[0250] An ~0.3 kb PCR fragment comprising the coding sequence for the N-terminus of the desI gene product and an ~0.12 kb PCR fragment comprising the coding sequence for the C-terminus of the desR gene product were amplified from cosmid pKOS23-26 (ATCC 203141) and inserted together into pLitmus28 treated with restriction enzymes NsiI and EcoRI to produce plasmid pKOS039-101. The ~6 kb SphI-PstI restriction fragment of pKOS23-26 containing the desI, desII, desIII, desIV, and desV genes was inserted into plasmid pUC19 (Stratagene) to yield plasmid pKOS039-102. The ~6 kb SphI-EcoRI restriction fragment from plasmid pKOS039-102 was inserted into pKOS039-101 to produce plasmid pKOS039-103. The ~6 kb BglII-PstI fragment from pKOS23-26 that contains the desR, desVI,

desVII, and desVIII genes was inserted into pKOS39-98 to yield pKOS39-100. The ~6 kb PacI-PstI restriction fragment of pKOS39-100 and the 6.4 kb NsiI-EcoRI fragment of pKOS39-103 were cloned into pKOS39-44 to yield pKOS39-104.

[0251] When introduced into *Streptomyces lividans* host cells comprising the recombinant narbonolide PKS of the invention, plasmid pKOS39-104 drives expression of the desosamine biosynthetic genes, the beta-glucosidase gene, and the desosaminyl transferase gene. The glycosylated antibiotic narbomycin was produced in these host cells, and it is believed that YC17 was produced as well. When these host cells are transformed with vectors that drive expression of the picK gene, the antibiotics methymycin, neomethymycin, and picromycin are produced.

[0252] In similar fashion, when plasmid pKOS039-18, which encodes a hybrid PKS of the invention that produces 3-deoxy-3-oxo-6-deoxyerythronolide B was expressed in *Streptomyces lividans* host cells transformed with plasmid pKOS39-104, the 5-desosaminylated analog was produced. Likewise, when plasmid pCK7, which encodes DEBS, which produces 6-deoxyerythronolide B, was expressed in *Streptomyces lividans* host cells transformed with plasmid pKC639-104, the 5-desosaminylated analog was produced. These compounds have antibiotic activity and are useful as intermediates in the synthesis of other antibiotics.

EXAMPLE 4

Expression Vector for Desosaminyl Transferase

[0253] While the invention provides expression vectors comprising all of the genes required for desosamine biosynthesis and transfer to a polyketide, the invention also provides expression vectors that encode any subset of those genes or any single gene. As one illustrative example, the invention provides an expression vector for desosaminyl transferase. This vector is useful to desosaminylate polyketides in host cells that produce NDP-desosamine but lack a desosaminyl transferase gene or express a desosaminyl transferase that does not function as efficiently on the polyketide of interest as does the desosaminyl transferase of *Streptomyces venezuelae*. This expression vector was constructed by first amplifying the desosaminyl transferase coding sequence from pKOS023-27 using the primers:

```
N3917:  5'-CCCTGCAGCGGCAAGGAAGGACACGACGCCA-3';
and

N3918:  5'-AGGTCTAGAGCTCAGTGCCGGGCGTCGGCCGG-3',
```

[0254] to give a 1.5 kb product. This product was then treated with restriction enzymes PstI and XbaI and ligated with HindIII and XbaI digested plasmid pKOS039-06 together with the 7.6 kb PstI-HindIII restriction fragment of plasmid pWHM1104 to provide plasmid pKOS039-14. Plasmid pWHM1104, described in Tang et al., 1996, *Molec. Microbiol.* 22(5): 801-813, incorporated herein by reference, encodes the ermE* promoter. Plasmid pKOS039-14 is constructed so that the desosaminyl transferase gene is placed under the control of the ermE* promoter and is suitable for expression of the desosaminyl transferase in *Streptomyces, Saccharopolyspora erythraea*, and other host cells in which the ermE* promoter functions.

EXAMPLE 5

Heterologous Expression of the picK Gene Product in *E. coli*

[0255] The picK gene was PCR amplified from plasmid pKOS023-28 using the oligonucleotide primers:

```
N024-36B (forward):
5'-TTGCATGCATATGCGCCGTACCCAGCAGGGAACGACC;
and

N024-37B (reverse):
5'-TTGAATTCTCAACTAGTACGGCGGCCCGCCTCCCGTCC.
```

[0256] These primers alter the *Streptomyces* GTG start codon to ATG and introduce a SpeI site at the C-terminal end of the gene, resulting in the substitution of a serine for the terminal glycine amino acid residue. The blunt-ended PCR product was subcloned into the commercially available vector pCRscript at the SrfI site to yield plasmid pKOS023-60. An ~1.3 kb NdeI-XhoI fragment was then inserted into the NdeI/XhoI sites of the T7 expression vector pET22b (Novagen, Madison, Wis.) to generate pKOS023-61. Plasmid pKOS023-61 was digested with restriction enzymes SpeI and EcoRI, and a short linker fragment encoding 6 histidine residues and a stop codon (composed of oligonucleotides 30-85a: 5'-CTAGTATGCATCATCATCATCATCATTAA-3'; and 30-85b: 5'-AATTTTAATGATGATGATGATGATGCATA-3') was inserted to obtain plasmid pKOS023-68. Both plasmid pKOS023-61 and pKOS023-68 produced active PicK enzyme in recombinant *E. coli* host cells.

[0257] Plasmid pKOS023-61 was transformed into *E. coli* BL21-DE3. Successful transformants were grown in LB-containing carbenicillin (100 μg/ml) at 37° C. to an OD_{600} of 0.6. Isopropyl-beta-D-thiogalactopyranoside (G) was added to a final concentration of 1 mM, and the cells were grown for an additional 3 hours before harvesting. The cells were collected by centrifugation and frozen at −80° C. A control culture of BL21-DE3 containing the vector plasmid pET21c (Invitrogen) was prepared in parallel.

[0258] The frozen BL21-DE3/pKOS023-61 cells were thawed, suspended in 2 μL of cold cell disruption buffer (5 mM imidazole, 500 mM NaCl, 20 mM Tris/HCl, pH 8.0) and sonicated to facilitate lysis. Cellular debris and supernatant were separated by centrifugation and subjected to SDSPAGE on 10-15% gradient gels, with Coomassie Blue staining, using a Pharmacia Phast Gel Electrophoresis system. The soluble crude extract from BL21-DE3/pKOS023-61 contained a Coomassie stained band of M_r~46 kDa, which was absent in the control strain BL21-DE3/pET21c.

[0259] The hydroxylase activity of the picK protein was assayed as follows. The crude supernatant (20 μL) was added to a reaction mixture (100 μL total volume) containing 50 mM Tris/HCl (pH 7.5), 20 μM spinach ferredoxin, 0.025 Unit of spinach ferredoxin:$NADP^+$oxidoreductase, 0.8 Unit of glucose-6-phosphate dehydrogenase, 1.4 mM $NADP^+$, 7.6 mM glucose-6 phosphate, and 20 mmol of narbomycin. The narbomycin was purified from a culture of *Streptomyces narbonensis*, and upon LC/MS analysis gave a single peak of $[M+H]^+$=510. The reaction was allowed to proceed for 105 minutes at 30° C. Half of the reaction mixture was

loaded onto an HPLC, and the effluent was analyzed by evaporative light scattering (ELSD) and mass spectrometry. The control extract (BL21-DE3/pET21c) was processed identically. The BL21-DE3/pKOS023-61 reaction contained a compound not present in the control having the same retention time, molecular weight and mass fragmentation pattern as picromycin ($[M+H]^+$=526). The conversion of narbomycin to picromycin under these conditions was estimated to be greater than 90% by ELSD peak area.

[0260] The poly-histidine-linked PicK hydroxylase was prepared from pKOS023-68 transformed into *E. coli* BL21 (DE3) and cultured as described above. The cells were harvested and the PicK protein purified as follows. All purification steps were performed at 4° C. *E. coli* cell pellets were suspended in 32 μL of cold binding buffer (20 mM Tris/HCl, pH 8.0, 5 mM imidazole, 500 mM NaCl) per mL of culture and lysed by sonication. For analysis of *E. coli* cell-free extracts, the cellular debris was removed by low-speed centrifugation, and the supernatant was used directly in assays. For purification of PicK/6-His, the supernatant was loaded (0.5 mL/min.) onto a 5 mL HiTrap Chelating column (Pharmacia, Piscataway, N.J.), equilibrated with binding buffer. The column was washed with 25 μL of binding buffer and the protein was eluted with a 35 μL linear gradient (5-500 mM imidazole in binding buffer). Column effluent was monitored at 280 nm and 416 nm. Fractions corresponding to the 416 nm absorbance peak were pooled and dialyzed against storage buffer (45 mM Tris/HCl, pH 7.5, 0.1 mM EDTA, 0.2 mM DTT, 10% glycerol). The purified 46 kDa protein was analyzed by SDSPAGE using Coomassie blue staining, and enzyme concentration and yield were determined.

[0261] Narbomycin was purified as described above from a culture of *Streptomyces narbonensis* ATCC19790. Reactions for kinetic assays (100 μL) consisted of 50 mM Tris/HCl (pH 7.5), 100 μM spinach ferredoxin, 0.025 Unit of spinach ferredoxin:$NADP^+$ oxidoreductase, 0.8 U glucose-6-phosphate dehydrogenase, 1.4 mM NADP+, 7.6 mM glucose-6-phosphate, 20-500 μM narbomycin substrate, and 50-500 nM of PicK enzyme. The reaction proceeded at 30° C., and samples were withdrawn for analysis at 5, 10, 15, and 90 minutes.

[0262] Reactions were stopped by heating to 100° C., for 1 minute, and denatured protein was removed by centrifugation. Depletion of narbomycin and formation of picromycin were determined by high performance liquid chromatography (HPLC, Beckman C-18 0.46×15 cm column) coupled to atmospheric pressure chemical ionization (APCI) mass spectroscopic detection (Perkin Elmer/Sciex API 100) and evaporative light scattering detection (Alltech 500 ELSD).

EXAMPLE 6

Expression of the picK Gene Encoding the Hydroxalase in *Streptomyces narbonensis*

[0263] To produce picromycin in *Streptomyces narbonensis*, a host that produces narbomycin but not picromycin, the methods and vectors of the invention were used to express the picK gene in this host.

[0264] The picK gene was amplified from cosmid pKOS023-26 using the primers:

```
N3903:      5'-TCCTCTAGACGTTTCCGT-3';
and

N3904:      5'-TGAAGCTTGAATTCAACCGGT-3'
```

[0265] to obtain an ~1.3 kb product. The product was treated with restriction enzymes XbaI and HindIII and ligated with the 7.6 kb XbaI-HindIII restriction fragment of plasmid pWHM1104 to provide plasmid pKOS039-01, placing the picK gene under the control of the ermE* promoter The resulting plasmid was transformed into purified stocks of *S. narbonensis* by protoplast fusion and electroporation. The transformants were grown in suitable media and shown to convert narbomycin to picromycin at a yield of over 95%.

EXAMPLE 7

Construction of a Hybrid DEBS/Narbonolide PKS

[0266] This example describes the construction of illustrative hybrid PKS expression vectors of the invention. The hybrid PKS contains portions of the narbonolide PKS and portions of rapamycin and/or DEBS PKS. In the first constructs, pKOS039-18 and pKOS039-19 the hybrid PKS comprises the narbonolide PKS extender module 6 ACP and thioesterase domains and the DEBS loading module and extender modules 1-5 as well as the KS and AT domains of DEBS extender module 6 (but not the KR domain of extender module 6). In pKOS039-19, the hybrid PKS is identical except that the KS1 domain is inactivated, i.e., the ketosynthase in extender module 1 is disabled. The inactive DEBS KS1 domain and its construction are described in detail in PCT publication Nos. WO 97/02358 and 99/03986, each of which is incorporated herein by reference. To construct pKOS039-18, the 2.33 kb BamHI-EcoRI fragment of pKOS023-27, which contains the desired sequence, was amplified by PCR and subcloned into plasmid pUC9. The primers used in the PCR were:

```
N3905:    5'-TTTATGCATCCCGCGGGTCCCGGCGAG-3';
and

N3906:    5'-TCAGAATTCTGTCGGTCACTTGCCCGC-3'.
```

[0267] The 1.6 kb PCR product was digested with PstI and EcoRI and cloned into the corresponding sites of plasmid pKOS015-52 (this plasmid contains the relevant portions of the coding sequence for the DEBS extender module 6) and commercially available plasmid pLitmus 28 to provide plasmids pKOS039-12 and pKOS039-13, respectively. The BglII-EcoRI fragment of plasmid pKOS039-12 was cloned into plasmid pKOS011-77, which contains the functional DEBS gene cluster and into plasmid pJRJ2, which contains the mutated DEBS gene that produces a DEBS PKS in which the KS domain of extender module I has been rendered inactive. Plasmid pJRJ2 is described in PCI publication Nos. 99/03986 and 97/02358, incorporated herein by reference.

[0268] Plasmids pKOS039-18 and pKOS039-19, respectively, were obtained. These two plasmids were transformed into *Streptomyces coelicolor* CH999 by protoplast fusion.

The resulting cells were cultured under conditions such that expression of the PKS occurred. Cells transformed with plasmid pKOS039-18 produced the expected product 3-deoxy-3-oxo-6-deoxyerythronolide B. When cells transformed with plasmid pKOS039-19 were provided (2S,3R)-2-methyl-3-hydroxyhexanoate NACS, 13-desethyl-13-propyl-3-deoxy-3-oxo-6-deoxyerythronolide B was produced.

EXAMPLE 8

6-Hydroxylation of 3,6-dideoxy-3-oxoerythronolide B Using the eryF Hydroxylase

[0269] Certain compounds of the invention can be hydroxylated at the C6 position in a host cell that expresses the eryF gene. These compounds can also be hydroxylated in vitro, as illustrated by this example.

[0270] The 6-hydroxylase encoded by eryF was expressed in *E. coli*, and partially purified. The hydroxylase (100 pmol in 10 μL) was added to a reaction mixture (100 μl total volume) containing 50 mM Tris/HCl (pH 7.5), 20 μM spinach ferredoxin, 0.025 Unit of spinach ferredoxin-:$NADP^+$ oxidoreductase, 0.8 Unit of glucose-6-phosphate dehydrogenase, 1.4 mM $NADP^+$, 7.6 mM glucose-6-phosphate, and 10 nmol 6-deoxyerythronolide B. The reaction was allowed to proceed for 90 minutes at 30° C. Half of the reaction mixture was loaded onto an HPLC, and the effluent was analyzed by mass spectrometry. The production of erythronolide B as evidenced by a new peak eluting earlier in the gradient and showing $[M+H]^+$=401. Conversion was estimated at 50% based on relative total ion counts.

[0271] Those of skill in the art will recognize the potential for hemiketal formation in the above compound and compounds of similar structure. To reduce the amount of hemiketal formed, one can use more basic (as opposed to acidic) conditions or employ sterically hindered derivative compounds, such as 5-desosaminylated compounds.

EXAMPLE 9

Measurement of Antibacterial Activity

[0272] Antibacterial activity was determined using either disk diffusion assays with *Bacillus cereus* as the test organism or by measurement of minimum inhibitory concentrations (MIC) in liquid culture against sensitive and resistant strains of *Staphylococcus pneumoniae*.

Example 10

Construction of Desosamine Containing Polyketide Libraries Using a Glycosyltransferase with Broad Substrate Specificity

[0273] Desosamine is an important deoxyaminosugar present on a number of structurally related macrolide antibiotics such as erythromycin and is the only glycoside present on picromycin, methymycin, and the highly potent semisynthetic ketolides. In this example, a set of nine deoxysugar biosynthetic and auxiliary genes from the picromycin/methymycin (pik) cluster was integrated in the chromosome of *Streptomyces lividans* to create a host that synthesizes TDP-D-desosamine and can be used in combination with PKS expression plasmids to generate libraries of desosaminylated polyketides. The versatility of the DesVII desosaminyltransferase is demonstrated by formation of desosaminylated macrolides from more than twenty different 14-membered lactones. The attachment of desosamine is sufficient to confer antibiotic activity to each of the otherwise inactive aglycones, reinforcing the belief that this sugar plays a critical role in the molecular binding properties of erythromycin and related macrolides. This host and others that can be engineered to produce deoxysugar and polyketide tailoring pathways in accordance with the methods of the invention are valuable tools for expanding the size and diversity of polyketides that can be generated by combinatorial biosynthesis. References cited in this example are indicated by a reference number; the numbered list of references is located at the end of this example. All references cited are incorporated herein by reference.

[0274] Much of the structural diversity and complexity among polyketides can be attributed to the chemistry performed by PKSs (1), and the modular architecture of catalytic domains within PKSs has been exploited by different rational and combinatorial engineering approaches to create polyketide diversity (24). However, structural variability among polyketides can also result from post-PKS biosynthetic steps, including oxidation and/or glycosylation with unique deoxy and amino sugars. Such modifications are often necessary to impart or enhance the specific biological activity of the molecule. For example, erythromycin A contains two deoxysugar moieties, L-cladinose and D-desosamine, that are required for antibacterial activity and the absence of either carbohydrate results in loss of potency. Although some chemical modifications to erythromycin have been discovered that can ameliorate the loss of the cladinose residue (5-7), there has been no substitution found for desosamine. This important deoxyaminosugar is also present in other macrolide antibiotics, such as oleandomycin and megalomicin, and is the only glycoside necessary to confer antibacterial activity to picromycin, methymycin, and the semisynthetic ketolide pharmacophores.

[0275] Polyketide libraries generated by genetic modification of macrolide PKSs in which enzymatic domains and entire protein subunits were removed, added, or exchanged in various combinations have been produced (3, 4, 8). Because these libraries were constructed in heterologous hosts lacking glycosylation pathways, only the corresponding aglycones were produced. The methods and reagents of the present invention can be used to expand the capabilities of the combinatorial biosynthesis strategies described to incorporate post-PKS tailoring steps, in particular the addition of deoxysugar components.

[0276] Some experiments have been performed in which structurally modified macrolactones are subsequently glycosylated in their native hosts (9-13), and also in bioconversion experiments in which a modified aglycone is fed to a PKS blocked mutant strain (14). These experiments indicate that glycosyltransferases are able to accept polyketide substrates with some amount of structural alteration. However, neither of these approaches is well-suited for the production and biological screening of large numbers of compounds, because most polyketide host organisms are difficult to manipulate genetically and the bioconversion of aglycones requires a tedious initial purification step.

[0277] A more practical approach is the heterologous expression of deoxysugar biosynthetic pathways in hosts

that have been developed for library expression. Although the effort to clone entire deoxysugar biosynthetic pathways in a heterologous organism can be a significant initial investment (most deoxysugars require six or more enzymatic steps whose genes are typically scattered within a polyketide gene cluster), these expression vectors, once made, can be easily combined with those containing PKSs to engineer glycosylated libraries rapidly. Olano et al. recently utilized a two-plasmid system to produce L-daunosamine, the deoxyaminosugar of daunorubicin and doxorubicin, in *Streptomyces lividans* (15).

[0278] Here we report the development of a single expression vector for the production of desosaminylated macrolides in *Streptomyces*. Desosamine was selected as the sugar constituent, because it was believed that addition of this single deoxysugar would be sufficient to confer antibacterial activity upon macrolactones to which it was attached. The expression vector was combined with a library of existing PMS expression plasmids to produce several novel glycosylated macrolide compounds in *S. lividans*, providing the first examples in which both polyketide and deoxysugar pathways have been placed in a single heterologous host.

[0279] A. Material and Methods

[0280] (i) Strains, Culture Conditions, and DNA Manipulation

[0281] DNA manipulation was performed in *Escherichia coli* XL1-Blue (Stratagene) using standard protocols (16). *Bacillis subtilis* was grown in LB at 37° C. PCR was performed with Pfu polymerase (Stratagene) under conditions recommended by the manufacturer. *S. lividans* K4-114 (17) was used as the host for expression of engineered PKS and desosamine genes. *S. lividans* strains were maintained on R2YE agar plates (18) with appropriate antibiotic selection. *S. lividans* protoplasts were transformed by the standard procedure (18) and transformants were selected using 1 ml of a 1 mg/ml thiostrepton and/or 1 ml of a 2 mg/ml apramycin overlay on R2YE regeneration plates.

[0282] (ii) Construction of Expression Plasmids

[0283] Expression plasmid pKOS39-104 was constructed as follows. The 6.0 kb Bgl II-Pst I fragment containing the picromycin des VIII, des VII, desVI and desR (partial) genes from cosmid pKOS23-26 (19) was subcloned into the Bgl II-Pst I sites of pKOS39-98, a pUC19 derivative with a redesigned multiple cloning site. The resulting plasmid, pKOS39-100, contains a Pac I site upstream of the Bgl II site which is used in a later cloning step. The 6 kb Sph I-Pst I fragment containing the desI (partial), desII, desIII, desIV and des V genes from pKOS23-26 was subcloned into the Sph I-Pst I of pUC19 to make pKOS39-102. The remaining 3'-end of the desR gene and 5'-end of the desI gene were PCR amplified from cosmid pKOS23-26 with the following oligonucleotides (restriction sites shown in italics):

desR gene:
forward 5'-*AGATGCAT*TTCTGGGATGCCGCCACGGA;
and

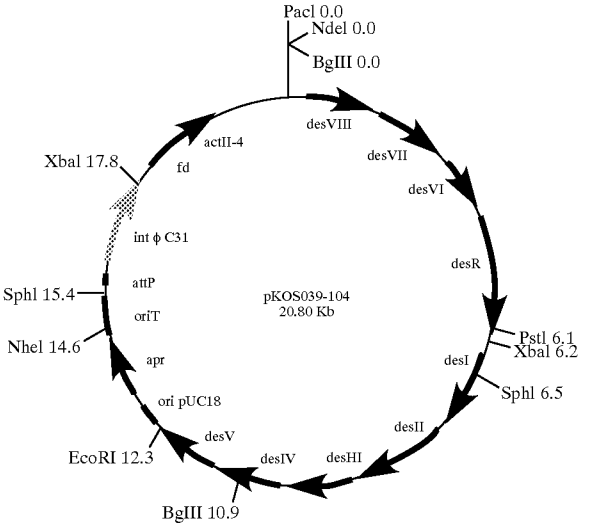
reverse 5'-*CGTCTAGA*CGTCACCAGACGTTGACCGTG;

desI gene:

-continued
forward 5'-TTT*CTAGA*CGGTGGCCCGGAGGGAACATC;
and

reverse 5'-CGG*AATTC*CGCAGCTGGTCGGCGGCGCA.

[0284] The two PCR fragments were digested with Nsi I-Xba I and Xba I-EcoR I, respectively, and ligated with Nsi I-EcoR I digested Litmus 28 (New England Biolabs) to obtain pKOS39-101B. The 6 kb Sph I-EcoR I fragment of pKOS39-102 was inserted into pKOS39-101B to make pKOS39-103. The 6.4 kb Nsi I-EcoR I fragment of pKOS39-103 and the 6 kb Pac I-Pst I fragment of pKOS39-100 were then ligated together with the 8.5 kb Pac I-EcoR I fragment of pKOS39-44 (20), yielding the final expression plasmid pKOS39-104. A restriction site and function map of this plasmid is shown below.



[0285] (iii) Production and Analysis of Compounds

[0286] All stains were grown in 5 ml liquid R2YE medium at 30° C. and analyzed following 5 days growth. For bioconversion experiments, aglycones (~10 mg/liter) were fed at the start of fermentation. Fermentation broth was analyzed directly by liquid chromatography/mass spectrometry (LC/MS) and evaporative light scattering detection (ELSD) as previously described (20). An authentic sample of narbomycin prepared from *Streptomyces narbonensis* (19) was used to validate production of this compound. For LC/MS analysis of strains containing PKS expression plasmids the cultures were extracted twice with 5 ml of ethyl acetate/triethylamine (99:1), concentrated to dryness and resuspended in 0.5 ml of acetonitrile.

[0287] (iv) Antibacterial Assays

[0288] Extracts prepared from the culture broths as above were assayed for biological activity against. *B. subtilis* using an agar plate diffusion method (see Example 9). Samples (5 μl) from each of the extracts were pipetted to sterile filter disks, dried, and placed on an LB plate spread with 20 μl of an overnight culture of *B. subtilis*. The plates were incubated overnight at 37° C. to visualize zones of growth inhibition.

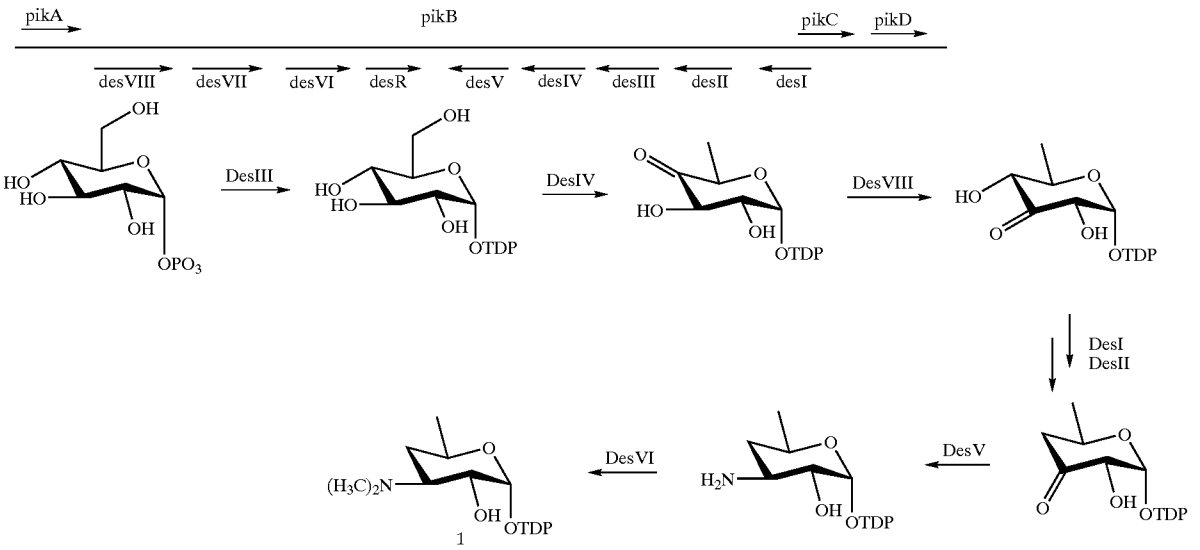
[0289] B. Results

[0290] (i) Construction and Validation of a Desosamine Expression System

[0291] The picromycin/methymycin (pik) gene cluster from *Streptomyces venezuelae* (21) was chosen as the source of desosamine biosynthetic genes rather than other available clusters (i.e. erythromycin, oleandomycin, or megalomicin) for several reasons. First, all of the genes required for biosynthesis of TDP desosamine from glucose-1-phosphate, a primary metabolite, as well as the desosaminyl transferase are present in the pik cluster whereas one or more of the genes are missing or not yet identified in each of the other clusters. Second, the genes from the pik cluster are comprised in a single contiguous segment of DNA (the des cluster), compared to those in other clusters which are dispersed among other genes, facilitating cloning and plasmid construction. The organization of these genes in the picromycin biosynthetic gene cluster is shown below, followed by the depiction of the biosynthetic pathway.

mycin, picromycin, and oleandomycin, causes inactivation and provides self-resistance to these compounds which are reactivated by a 13-glucosidase upon export (24, 25). *S. lividans* is known to possess at least two such glucosyltransferases which inactivate erythromycin and picromycin by the same mechanism (26). Therefore, it was important to include this gene for expression in *S. lividans* to produce desosaminylated compounds without the glucose modification.

[0294] The expression system used here was adopted from the multi-vector system developed for separate expression of erythromycin PKS, or 6-deoxyerythronolide B synthase (DEBS), subunits in *Streptomyces* (4, 27; see also U.S. Pat. No. 6,033,883). Plasmid pKOS39-104 contains the des genes cloned in a single orientation under control of the actI promoter and actII-44 activator. Since pKOS39-104 is a derivative of pSET152 (28), it contains the phiC31-int-attP loci for chromosomal integration in *Streptomyces* and can be used in conjunction with the pRM5-based PKS expression



[0292] Third, the natural substrates for the desosaminyl transferase from the pik gene cluster, narbonolide and 10-deoxymethynolide, are themselves aglycones; in each of the other cases, desosamine is attached subsequent to addition of at least one other sugar. Furthermore, the difference in macrolactone ring sizes between narbonolide and 10-deoxymethynolide (14 and 12 atoms, respectively) suggests that the desosaminyl transferase from this cluster is somewhat forgiving towards its polyketide substrate.

[0293] Seven genes in the des cluster, desI, desII, desIII, desIV, desV, desVI, and des VIII, are presumed to be responsible for the biosynthesis of TDP-D-desosamine (22). Also present is the des VII gene encoding the glycosyltransferase. In addition to catalyzing the transfer of desosamine to both 12- and 14-membered macrolactones, it has been shown that DesVII is able to incorporate non-natural deoxysugar substrates (22, 23). The desR gene encodes a β-glucosidase that removes a glucose residue attached to the C-2' hydroxyl of desosamine (24). It is believed that the glucosylation of desosamine containing macrolides like methy-

plasmid library (3; see also U.S. Pat. No. 5,672,491). *S. lividans* K4114 was transformed with pKOS39-104 and designated K39-22. Confirmation that this strain produced TDP-D-desosamine was performed by feeding aglycones to the strain and looking for the presence of desosaminylated compounds by LC/MS analysis.

[0295] Four aglycones (10 mg/liter each) were fed to liquid fermentations of *S. lividans* K39-22: narbonolide and 10-deoxymethynolide, the natural substrates for DesVII, 3-keto-6-deoxyerythronolide B (dEB), and 6-dEB. Fermentation broth from all four aglycone fed strains displayed antibacterial activity against *B. subtilis* whereas *S. lividans* K39-22 alone produced no detectable activity. LC/MS analysis demonstrated that each of the corresponding desosaminylated compounds narbomycin, 10-deoxymethymycin (YC17), 3-keto-5-O-desosaminyl-6-dEB, and 5-O-desosaminyl-6-dEB were produced. In each case, the parent ion ($M+H^+$) of the expected compound was detected in addition to a characteristic ion at 158 amu produced by the desosamine fragment. Production of narbomycin in the narbono-

lide fed strain was further confirmed by comparison to authentic narbomycin obtained from *S. narbonensis*. LC/MS also revealed that a significant amount (50-90%) of the aglycone remained unconverted in each of the samples.

[**0296**] These results established that the des expression vector was functional and that the DesVII glycosyltransferase was able to glycosylate non-natural macrolactone substrates. The bioassay results also confirmed that desosamine is sufficient to confer antibacterial activity to these macrolactones. There were no 2'-O-glucosyl derivatives detected, which indicates that the DesR glucosidase included in pKOS39-104 was also operational, although minor glucosylated products were putatively found in subsequent experiments with the strain (see below).

[**0297**] (ii) Co-Expression of Desosamine and Aglycone Pathways in *S. lividans*.

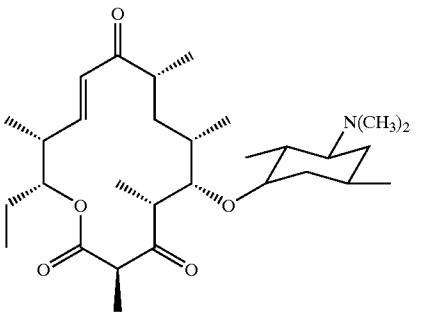
[**0298**] Although expression of both a modular polyketide pathway and a deoxysugar pathway together in a heterologous host has not been reported, the bioconversion results suggested that transformation of *S. lividans* K39-22 with plasmids encoding macrolide PKSs would lead to production of desosaminylated compounds. Plasmids encoding the PKSs that, in *S. lividans*, produce the same four aglycones used in the bioconversion studies were therefore transformed into *S. lividans* K39-22. Plasmid pKOS39-86 contains the picromycin/methymycin PKS and produces both narbonolide and 10-deoxymethynolide (20). Plasmid pKAO127 contains DEBS and produces 6-dEB (17). Plasmid pKOS39-18 contains DEBS with a modified terminal module that produces 3-keto dB (20).

[**0299**] Culture broth from each of the transformed strains displayed activity against *B. subtilis*. LC/MS analysis as above confirmed the presence of each of the expected desosaminylated compounds as well as their aglycone precursors and minor amounts of the corresponding 2'-O-glucosyl derivatives. The total yield of narbomycin and 10-deoxymethymycin in *S. lividans* K39-22/pKOS3986 was approximately 1 mg/liter each and represents about a 20% conversion of the total aglycone produced. Thus, although both PKS and deoxysugar pathways function as expected, complete glycosylation of even the natural substrates for DesVII did not occur under these conditions. *S. lividans* K39-22 contains a copy of the ermE macrolide resistance gene, and no obvious growth defects were observed with production of the biologically active compounds. These results suggest that a limiting amount of TDP-desosamine is being produced by the strain under these conditions.

[**0300**] (iii) Production and Biological Screening of a Glycosylated Macrolide Library

[**0301**] Over 50 PKS expression plasmids have been constructed and tested in using DEBS and other macrolide PKS genes (3, 8, 20). These PKSs produce a variety of 14-membered macrolactones in which single or multiple carbon centers have been altered. Each plasmid contains the same pRM5-based vector as above, providing a convenient opportunity to expand and diversify any existing aglycone library by routine transformation of *S. lividans* K39-22. Because a C-5 hydroxyl would be necessary for glycosylation, a subset of 19 additional plasmids encoding PKSs that produce compounds containing this functional group was selected and tested. The desired desosaminylated polyketides would theoretically possess antibiotic activity, and the transformed strains can therefore be readily analyzed in a simple bioassay for production of glycosylated macrolides.

[**0302**] All of the strains transformed and tested displayed antimicrobial activity against *B. subtilis*. The presumed structures of the desosamine containing compounds, based on the structures of the aglycones produced by the PKS on each plasmid, are shown below.



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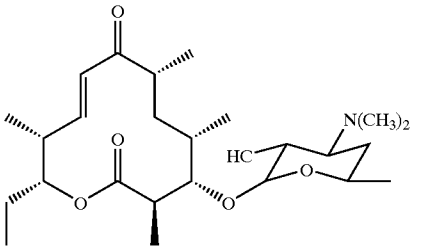

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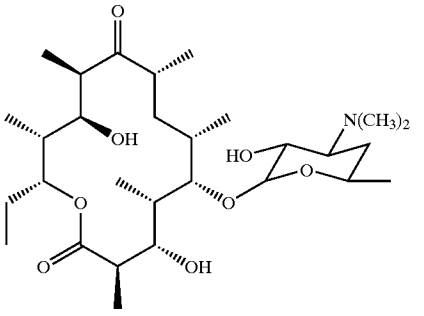

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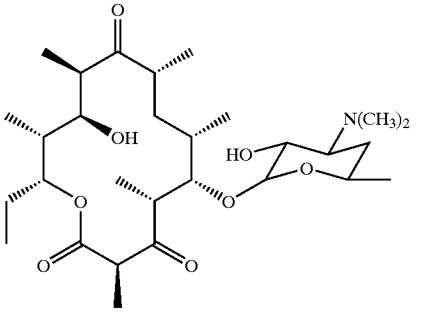

KOS39-18

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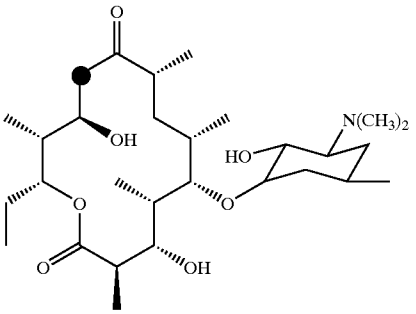

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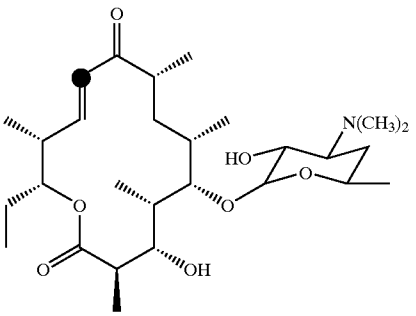

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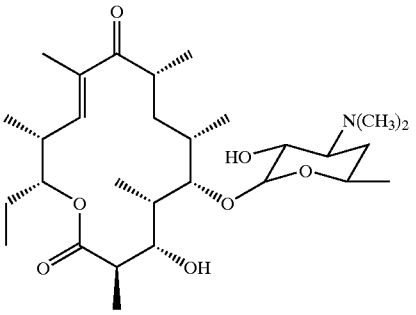

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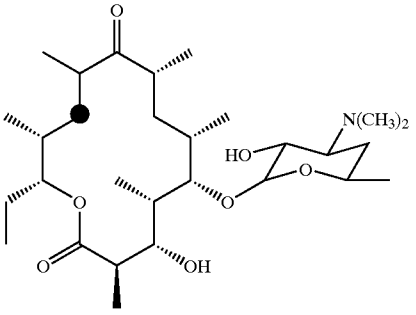

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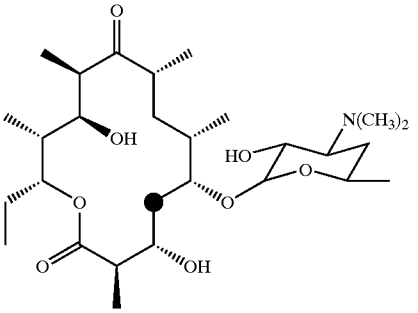

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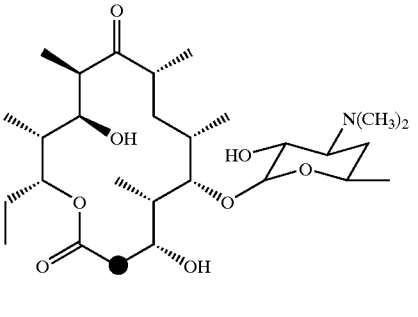

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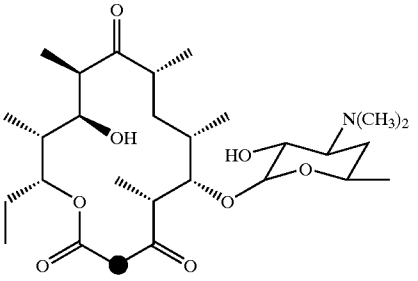
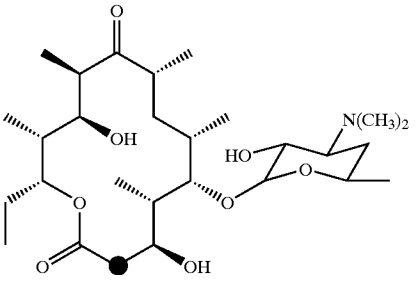


KOS15-106
KOS15-109



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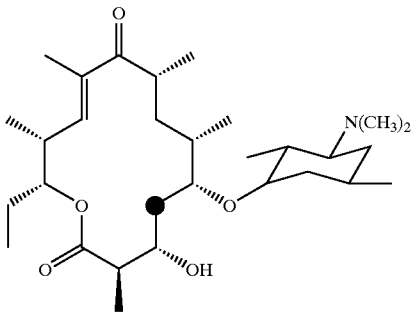

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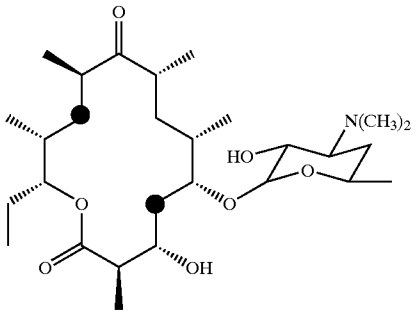

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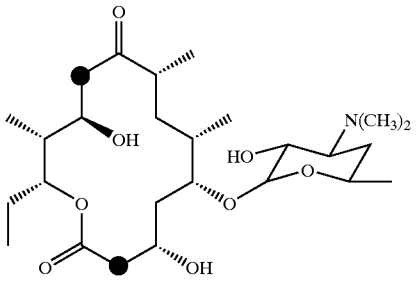

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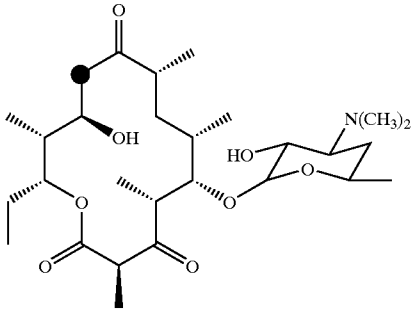

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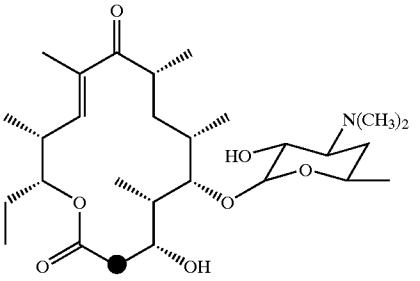

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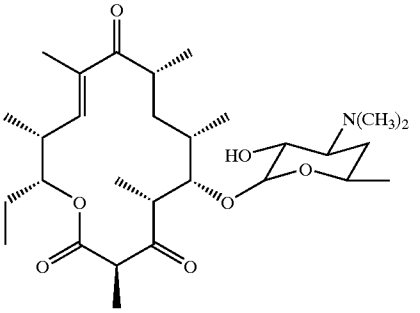

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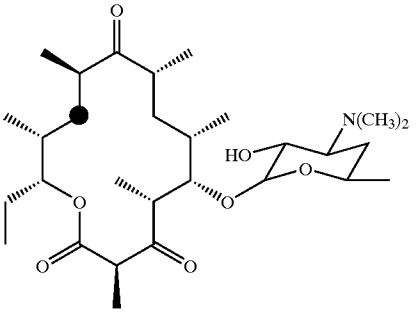

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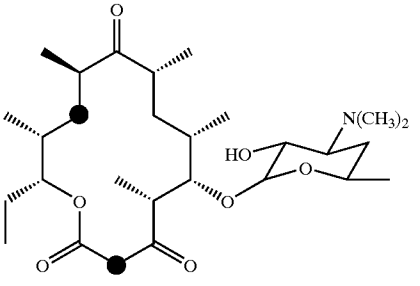

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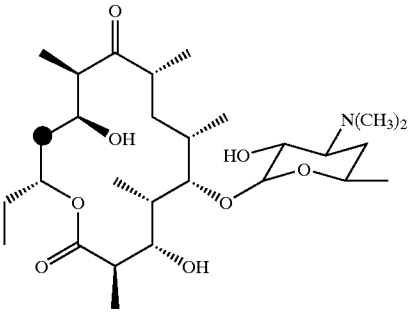

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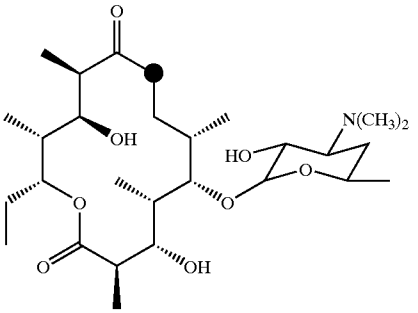

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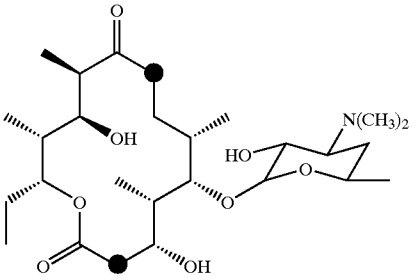

KOS15-34

[0303] Culture extracts from six of these stains (those containing plasmids pKOS15-22, pKOS15-106, pKOS39-20, pKOS1142, pKOS15-30, and pKOS2415) were examined by LC/MS and, in each case, the expected parent ion was found along with the 158 amu desosamine fragment. Two compounds were detected in the strain containing pKOS15-106 with molecular weights corresponding to 3-hydroxy and 3-keto derivatives. This is consistent with both aglycones being produced by plasmid pKOS15-109 in *S. lividans*. Two compounds were also detected in the strain with pKOS112, the predicted molecule, 5-O-desosaminyl-10-desmethyl-6-dEB, and a putative dehydrated derivative at carbons C-10 and C-11. Both aglycones were also produced when the plasmid was originally analyzed in *S. lividans* K4-114 (3), although only the former was reported at that time. As with the first set of plasmids tested, small amounts of 2'-O-glucosylated derivatives could also be detected in some of the culture extracts. The yields of the desosamine containing compounds were too low to determine absolute titers (<1 mg/L) and, therefore, the relative antibacterial activity of the compounds could not be determined from these assays.

[0304] C. Discussion

[0305] This example demonstrates that a minimal set of seven genes (desI, II, III, IV, V, VI, VIII) is sufficient for biosynthesis of TDP-desosamine from glucose-1-phosphate in *S. lividans*. The apparent low abundance of TDP-desosamine in the engineered host could be due either to the availability of glucose-1-phosphate in this host or to poor expression of the sugar biosynthesis and/or transferase genes. Alternatively, it is interesting to note that narbonolide and 10-deoxymethynolide are present in the natural picromycin/methymycin producing organism, *S. venezuelae*, and could therefore reflect that one or more of the enzymes from the des cluster is relatively inefficient. One can increase the amount of TDP-desosamine either by increasing expression levels of these genes and/or by complementing one or more of the enzymes in the pathway with homologs from other clusters such as erythromycin or oleandomycin.

[0306] Expression of the minimal desosamine biosynthesis genes together with the DesVII desosaminyltransferase in *S. lividans* has enabled the production of more than 20 glycosylated macrolides with detectable antibacterial activity. The structures of the macrolides that were glycosylated highlight both the remarkable substrate tolerance of the DesVII glycosyltransferase as well as the ability of desosamine to impart biological activity to structurally diverse macrolactones. In addition to their antibacterial properties the desosamine containing compounds presented here may possess additional biological properties that are associated with erythromycin and other macrolides, including motilin antagonism and anti-inflammatory activities. Furthermore, the demonstration by others that DesVII and other glycosyltransferases can also tolerate modifications of the sugar substituent (22, 23, 29) opens the door to manipulation of both polyketide and deoxysugar pathways for the production of 'unnatural' natural product libraries.

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[0336] The invention having now been described by way of written description and example, those of skill in the art will recognize that the invention can be practiced in a variety of embodiments and that the foregoing description and examples are for purposes of illustration and not limitation of the following claims.

SEQUENCE LISTING

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<212> TYPE: PRT
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Gly Ile Ser Cys Arg Val Pro Gly Ala Arg Asp Pro Arg Glu Phe Trp
         35                  40                  45

Glu Leu Leu Ala Ala Gly Gly Gln Ala Val Thr Asp Val Pro Ala Asp
     50                  55                  60

Arg Trp Asn Ala Gly Asp Phe Tyr Asp Pro Asp Arg Ser Ala Pro Gly
 65                  70                  75                  80

Arg Ser Asn Ser Arg Trp Gly Gly Phe Ile Glu Asp Val Asp Arg Phe
                 85                  90                  95
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Ala Gly Ile Asp Pro Ser Ser Leu Thr Gly Thr Arg Thr Gly Val Phe
    130                 135                 140

Ala Gly Ala Ile Trp Asp Asp Tyr Ala Thr Leu Lys His Arg Gln Gly
145                 150                 155                 160

Gly Ala Ala Ile Thr Pro His Thr Val Thr Gly Leu His Arg Gly Ile
                165                 170                 175

Ile Ala Asn Arg Leu Ser Tyr Thr Leu Gly Leu Arg Gly Pro Ser Met
            180                 185                 190

Val Val Asp Ser Gly Gln Ser Ser Ser Leu Val Ala Val His Leu Ala
        195                 200                 205

Cys Glu Ser Leu Arg Arg Gly Glu Ser Glu Leu Ala Leu Ala Gly Gly
    210                 215                 220

Val Ser Leu Asn Leu Val Pro Asp Ser Ile Ile Gly Ala Ser Lys Phe
225                 230                 235                 240

Gly Gly Leu Ser Pro Asp Gly Arg Ala Tyr Thr Phe Asp Ala Arg Ala
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Asn Gly Tyr Val Arg Gly Glu Gly Gly Gly Phe Val Val Leu Lys Arg
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Thr Pro Val Gly Asp Pro Ile Glu Ala Ala Ala Leu Gly Ala Ala Leu
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Thr Asn Ile Gly His Leu Glu Gly Ala Ala Gly Ile Ala Gly Leu Ile
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385                 390                 395                 400

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785                 790                 795                 800

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865                 870                 875                 880

Gly Leu Ala Val Asp Trp Ser Pro Leu Leu Pro Ser Ala Thr Gly His
                885                 890                 895

His Ser Asp Leu Pro Thr Tyr Ala Phe Gln Thr Glu Arg His Trp Leu
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            900                 905                 910

Gly Glu Ile Glu Ala Leu Ala Pro Ala Gly Glu Pro Ala Val Gln Pro
        915                 920                 925

Ala Val Leu Arg Thr Glu Ala Ala Glu Pro Ala Glu Leu Asp Arg Asp
    930                 935                 940

Glu Gln Leu Arg Val Ile Leu Asp Lys Val Arg Ala Gln Thr Ala Gln
945                 950                 955                 960

Val Leu Gly Tyr Ala Thr Gly Gly Gln Ile Glu Val Asp Arg Thr Phe
                965                 970                 975

Arg Glu Ala Gly Cys Thr Ser Leu Thr Gly Val Asp Leu Arg Asn Arg
            980                 985                 990

Ile Asn Ala Ala Phe Gly Val Arg Met Ala Pro Ser Met Ile Phe Asp
        995                 1000                1005

Phe Pro Thr Pro Glu Ala Leu Ala Glu Gln Leu Leu Leu Val Val His
    1010                1015                1020

Gly Glu Ala Ala Ala Asn Pro Ala Gly Ala Glu Pro Ala Pro Val Ala
1025                1030                1035                1040

Ala Ala Gly Ala Val Asp Glu Pro Val Ala Ile Val Gly Met Ala Cys
                1045                1050                1055

Arg Leu Pro Gly Gly Val Ala Ser Pro Glu Asp Leu Trp Arg Leu Val
            1060                1065                1070

Ala Gly Gly Gly Asp Ala Ile Ser Glu Phe Pro Gln Asp Arg Gly Trp
        1075                1080                1085

Asp Val Glu Gly Leu Tyr His Pro Asp Pro Glu His Pro Gly Thr Ser
    1090                1095                1100

Tyr Val Arg Gln Gly Gly Phe Ile Glu Asn Val Ala Gly Phe Asp Ala
1105                1110                1115                1120

Ala Phe Phe Gly Ile Ser Pro Arg Glu Ala Leu Ala Met Asp Pro Gln
                1125                1130                1135

Gln Arg Leu Leu Leu Glu Thr Ser Trp Glu Ala Val Glu Asp Ala Gly
            1140                1145                1150

Ile Asp Pro Thr Ser Leu Arg Gly Arg Gln Val Gly Val Phe Thr Gly
        1155                1160                1165

Ala Met Thr His Glu Tyr Gly Pro Ser Leu Arg Asp Gly Gly Glu Gly
    1170                1175                1180

Leu Asp Gly Tyr Leu Leu Thr Gly Asn Thr Ala Ser Val Met Ser Gly
1185                1190                1195                1200

Arg Val Ser Tyr Thr Leu Gly Leu Glu Gly Pro Ala Leu Thr Val Asp
                1205                1210                1215

Thr Ala Cys Ser Ser Ser Leu Val Ala Leu His Leu Ala Val Gln Ala
            1220                1225                1230

Leu Arg Lys Gly Glu Val Asp Met Ala Leu Ala Gly Gly Val Ala Val
        1235                1240                1245

Met Pro Thr Pro Gly Met Phe Val Glu Phe Ser Arg Gln Arg Gly Leu
    1250                1255                1260

Ala Gly Asp Gly Arg Ser Lys Ala Phe Ala Ala Ser Ala Asp Gly Thr
1265                1270                1275                1280

Ser Trp Ser Glu Gly Val Gly Val Leu Leu Val Glu Arg Leu Ser Asp
                1285                1290                1295

Ala Arg Arg Asn Gly His Gln Val Leu Ala Val Val Arg Gly Ser Ala
            1300                1305                1310
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Val Asn Gln Asp Gly Ala Ser Asn Gly Leu Thr Ala Pro Asn Gly Pro
        1315                1320                1325

Ser Gln Gln Arg Val Ile Arg Arg Ala Leu Ala Asp Ala Arg Leu Thr
    1330                1335                1340

Thr Ser Asp Val Asp Val Val Glu Ala His Gly Thr Gly Thr Arg Leu
1345                1350                1355                1360

Gly Asp Pro Ile Glu Ala Gln Ala Leu Ile Ala Thr Tyr Gly Gln Gly
                1365                1370                1375

Arg Asp Asp Glu Gln Pro Leu Arg Leu Gly Ser Leu Lys Ser Asn Ile
            1380                1385                1390

Gly His Thr Gln Ala Ala Ala Gly Val Ser Gly Val Ile Lys Met Val
        1395                1400                1405

Gln Ala Met Arg His Gly Leu Leu Pro Lys Thr Leu His Val Asp Glu
    1410                1415                1420

Pro Ser Asp Gln Ile Asp Trp Ser Ala Gly Ala Val Glu Leu Leu Thr
1425                1430                1435                1440

Glu Ala Val Asp Trp Pro Glu Lys Gln Asp Gly Gly Leu Arg Arg Ala
                1445                1450                1455

Ala Val Ser Ser Phe Gly Ile Ser Gly Thr Asn Ala His Val Val Leu
            1460                1465                1470

Glu Glu Ala Pro Val Val Val Glu Gly Ala Ser Val Val Glu Pro Ser
        1475                1480                1485

Val Gly Gly Ser Ala Val Gly Gly Gly Val Thr Pro Trp Val Val Ser
    1490                1495                1500

Ala Lys Ser Ala Ala Ala Leu Asp Ala Gln Ile Glu Arg Leu Ala Ala
1505                1510                1515                1520

Phe Ala Ser Arg Asp Arg Thr Asp Asp Ala Asp Ala Gly Ala Val Asp
                1525                1530                1535

Ala Gly Ala Val Ala His Val Leu Ala Asp Gly Arg Ala Gln Phe Glu
            1540                1545                1550

His Arg Ala Val Ala Leu Gly Ala Gly Ala Asp Asp Leu Val Gln Ala
        1555                1560                1565

Leu Ala Asp Pro Asp Gly Leu Ile Arg Gly Thr Ala Ser Gly Val Gly
    1570                1575                1580

Arg Val Ala Phe Val Phe Pro Gly Gln Gly Thr Gln Trp Ala Gly Met
1585                1590                1595                1600

Gly Ala Glu Leu Leu Asp Ser Ser Ala Val Phe Ala Ala Ala Met Ala
                1605                1610                1615

Glu Cys Glu Ala Ala Leu Ser Pro Tyr Val Asp Trp Ser Leu Glu Ala
            1620                1625                1630

Val Val Arg Gln Ala Pro Gly Ala Pro Thr Leu Glu Arg Val Asp Val
        1635                1640                1645

Val Gln Pro Val Thr Phe Ala Val Met Val Ser Leu Ala Arg Val Trp
    1650                1655                1660

Gln His His Gly Val Thr Pro Gln Ala Val Val Gly His Ser Gln Gly
1665                1670                1675                1680

Glu Ile Ala Ala Ala Tyr Val Ala Gly Ala Leu Pro Leu Asp Asp Ala
                1685                1690                1695

Ala Arg Val Val Thr Leu Arg Ser Lys Ser Ile Ala Ala His Leu Ala
            1700                1705                1710
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Gly Lys Gly Gly Met Leu Ser Leu Ala Leu Asn Glu Asp Ala Val Leu
        1715                1720                1725

Glu Arg Leu Ser Asp Phe Asp Gly Leu Ser Val Ala Ala Val Asn Gly
    1730                1735                1740

Pro Thr Ala Thr Val Val Ser Gly Asp Pro Val Gln Ile Glu Glu Leu
1745                1750                1755                1760

Ala Gln Ala Cys Lys Ala Asp Gly Phe Arg Ala Arg Ile Ile Pro Val
                1765                1770                1775

Asp Tyr Ala Ser His Ser Arg Gln Val Glu Ile Ile Glu Ser Glu Leu
            1780                1785                1790

Ala Gln Val Leu Ala Gly Leu Ser Pro Gln Ala Pro Arg Val Pro Phe
        1795                1800                1805

Phe Ser Thr Leu Glu Gly Thr Trp Ile Thr Glu Pro Val Leu Asp Gly
    1810                1815                1820

Thr Tyr Trp Tyr Arg Asn Leu Arg His Arg Val Gly Phe Ala Pro Ala
1825                1830                1835                1840

Ile Glu Thr Leu Ala Val Asp Glu Gly Phe Thr His Phe Val Glu Val
                1845                1850                1855

Ser Ala His Pro Val Leu Thr Met Thr Leu Pro Glu Thr Val Thr Gly
            1860                1865                1870

Leu Gly Thr Leu Arg Arg Glu Gln Gly Gly Gln Glu Arg Leu Val Thr
        1875                1880                1885

Ser Leu Ala Glu Ala Trp Val Asn Gly Leu Pro Val Ala Trp Thr Ser
    1890                1895                1900

Leu Leu Pro Ala Thr Ala Ser Arg Pro Gly Leu Pro Thr Tyr Ala Phe
1905                1910                1915                1920

Gln Ala Glu Arg Tyr Trp Leu Glu Asn Thr Pro Ala Ala Leu Ala Thr
                1925                1930                1935

Gly Asp Asp Trp Arg Tyr Arg Ile Asp Trp Lys Arg Leu Pro Ala Ala
            1940                1945                1950

Glu Gly Ser Glu Arg Thr Gly Leu Ser Gly Arg Trp Leu Ala Val Thr
        1955                1960                1965

Pro Glu Asp His Ser Ala Gln Ala Ala Ala Val Leu Thr Ala Leu Val
    1970                1975                1980

Asp Ala Gly Ala Lys Val Glu Val Leu Thr Ala Gly Ala Asp Asp Asp
1985                1990                1995                2000

Arg Glu Ala Leu Ala Ala Arg Leu Thr Ala Leu Thr Thr Gly Asp Gly
                2005                2010                2015

Phe Thr Gly Val Val Ser Leu Leu Asp Gly Leu Val Pro Gln Val Ala
            2020                2025                2030

Trp Val Gln Ala Leu Gly Asp Ala Gly Ile Lys Ala Pro Leu Trp Ser
        2035                2040                2045

Val Thr Gln Gly Ala Val Ser Val Gly Arg Leu Asp Thr Pro Ala Asp
    2050                2055                2060

Pro Asp Arg Ala Met Leu Trp Gly Leu Gly Arg Val Val Ala Leu Glu
2065                2070                2075                2080

His Pro Glu Arg Trp Ala Gly Leu Val Asp Leu Pro Ala Gln Pro Asp
                2085                2090                2095

Ala Ala Ala Leu Ala His Leu Val Thr Ala Leu Ser Gly Ala Thr Gly
            2100                2105                2110

Glu Asp Gln Ile Ala Ile Arg Thr Thr Gly Leu His Ala Arg Arg Leu
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        2115                2120                2125

Ala Arg Ala Pro Leu His Gly Arg Arg Pro Thr Arg Asp Trp Gln Pro
    2130                2135                2140

His Gly Thr Val Leu Ile Thr Gly Gly Thr Gly Ala Leu Gly Ser His
2145                2150                2155                2160

Ala Ala Arg Trp Met Ala His His Gly Ala Glu His Leu Leu Leu Val
                2165                2170                2175

Ser Arg Ser Gly Glu Gln Ala Pro Gly Ala Thr Gln Leu Thr Ala Glu
            2180                2185                2190

Leu Thr Ala Ser Gly Ala Arg Val Thr Ile Ala Ala Cys Asp Val Ala
        2195                2200                2205

Asp Pro His Ala Met Arg Thr Leu Leu Asp Ala Ile Pro Ala Glu Thr
    2210                2215                2220

Pro Leu Thr Ala Val Val His Thr Ala Gly Ala Leu Asp Asp Gly Ile
2225                2230                2235                2240

Val Asp Thr Leu Thr Ala Glu Gln Val Arg Arg Ala His Arg Ala Lys
                2245                2250                2255

Ala Val Gly Ala Ser Val Leu Asp Glu Leu Thr Arg Asp Leu Asp Leu
            2260                2265                2270

Asp Ala Phe Val Leu Phe Ser Ser Val Ser Ser Thr Leu Gly Ile Pro
        2275                2280                2285

Gly Gln Gly Asn Tyr Ala Pro His Asn Ala Tyr Leu Asp Ala Leu Ala
    2290                2295                2300

Ala Arg Arg Arg Ala Thr Gly Arg Ser Ala Val Ser Val Ala Trp Gly
2305                2310                2315                2320

Pro Trp Asp Gly Gly Gly Met Ala Ala Gly Asp Gly Val Ala Glu Arg
                2325                2330                2335

Leu Arg Asn His Gly Val Pro Gly Met Asp Pro Glu Leu Ala Leu Ala
            2340                2345                2350

Ala Leu Glu Ser Ala Leu Gly Arg Asp Glu Thr Ala Ile Thr Val Ala
        2355                2360                2365

Asp Ile Asp Trp Asp Arg Phe Tyr Leu Ala Tyr Ser Ser Gly Arg Pro
    2370                2375                2380

Gln Pro Leu Val Glu Glu Leu Pro Glu Val Arg Arg Ile Ile Asp Ala
2385                2390                2395                2400

Arg Asp Ser Ala Thr Ser Gly Gln Gly Gly Ser Ser Ala Gln Gly Ala
                2405                2410                2415

Asn Pro Leu Ala Glu Arg Leu Ala Ala Ala Ala Pro Gly Glu Arg Thr
            2420                2425                2430

Glu Ile Leu Leu Gly Leu Val Arg Ala Gln Ala Ala Ala Val Leu Arg
        2435                2440                2445

Met Arg Ser Pro Glu Asp Val Ala Ala Asp Arg Ala Phe Lys Asp Ile
    2450                2455                2460

Gly Phe Asp Ser Leu Ala Gly Val Glu Leu Arg Asn Arg Leu Thr Arg
2465                2470                2475                2480

Ala Thr Gly Leu Gln Leu Pro Ala Thr Leu Val Phe Asp His Pro Thr
                2485                2490                2495

Pro Leu Ala Leu Val Ser Leu Leu Arg Ser Glu Phe Leu Gly Asp Glu
            2500                2505                2510

Glu Thr Ala Asp Ala Arg Arg Ser Ala Ala Leu Pro Ala Thr Val Gly
        2515                2520                2525
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Ala Gly Ala Gly Ala Gly Ala Gly Thr Asp Ala Asp Asp Asp Pro Ile
    2530                2535                2540

Ala Ile Val Ala Met Ser Cys Arg Tyr Pro Gly Asp Ile Arg Ser Pro
2545                2550                2555                2560

Glu Asp Leu Trp Arg Met Leu Ser Glu Gly Gly Glu Gly Ile Thr Pro
                2565                2570                2575

Phe Pro Thr Asp Arg Gly Trp Asp Leu Asp Gly Leu Tyr Asp Ala Asp
            2580                2585                2590

Pro Asp Ala Leu Gly Arg Ala Tyr Val Arg Glu Gly Gly Phe Leu His
        2595                2600                2605

Asp Ala Ala Glu Phe Asp Ala Glu Phe Phe Gly Val Ser Pro Arg Glu
    2610                2615                2620

Ala Leu Ala Met Asp Pro Gln Gln Arg Met Leu Leu Thr Thr Ser Trp
2625                2630                2635                2640

Glu Ala Phe Glu Arg Ala Gly Ile Glu Pro Ala Ser Leu Arg Gly Ser
                2645                2650                2655

Ser Thr Gly Val Phe Ile Gly Leu Ser Tyr Gln Asp Tyr Ala Ala Arg
            2660                2665                2670

Val Pro Asn Ala Pro Arg Gly Val Glu Gly Tyr Leu Leu Thr Gly Ser
        2675                2680                2685

Thr Pro Ser Val Ala Ser Gly Arg Ile Ala Tyr Thr Phe Gly Leu Glu
    2690                2695                2700

Gly Pro Ala Thr Thr Val Asp Thr Ala Cys Ser Ser Ser Leu Thr Ala
2705                2710                2715                2720

Leu His Leu Ala Val Arg Ala Leu Arg Ser Gly Glu Cys Thr Met Ala
                2725                2730                2735

Leu Ala Gly Gly Val Ala Met Met Ala Thr Pro His Met Phe Val Glu
            2740                2745                2750

Phe Ser Arg Gln Arg Ala Leu Ala Pro Asp Gly Arg Ser Lys Ala Phe
        2755                2760                2765

Ser Ala Asp Ala Asp Gly Phe Gly Ala Ala Glu Gly Val Gly Leu Leu
    2770                2775                2780

Leu Val Glu Arg Leu Ser Asp Ala Arg Arg Asn Gly His Pro Val Leu
2785                2790                2795                2800

Ala Val Val Arg Gly Thr Ala Val Asn Gln Asp Gly Ala Ser Asn Gly
                2805                2810                2815

Leu Thr Ala Pro Asn Gly Pro Ser Gln Gln Arg Val Ile Arg Gln Ala
            2820                2825                2830

Leu Ala Asp Ala Arg Leu Ala Pro Gly Asp Ile Asp Ala Val Glu Thr
        2835                2840                2845

His Gly Thr Gly Thr Ser Leu Gly Asp Pro Ile Glu Ala Gln Gly Leu
    2850                2855                2860

Gln Ala Thr Tyr Gly Lys Glu Arg Pro Ala Glu Arg Pro Leu Ala Ile
2865                2870                2875                2880

Gly Ser Val Lys Ser Asn Ile Gly His Thr Gln Ala Ala Ala Gly Ala
                2885                2890                2895

Ala Gly Ile Ile Lys Met Val Leu Ala Met Arg His Gly Thr Leu Pro
            2900                2905                2910

Lys Thr Leu His Ala Asp Glu Pro Ser Pro His Val Asp Trp Ala Asn
        2915                2920                2925
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Ser Gly Leu Ala Leu Val Thr Glu Pro Ile Asp Trp Pro Ala Gly Thr
    2930                2935                2940

Gly Pro Arg Arg Ala Ala Val Ser Ser Phe Gly Ile Ser Gly Thr Asn
2945                2950                2955                2960

Ala His Val Val Leu Glu Gln Ala Pro Asp Ala Ala Gly Glu Val Leu
                2965                2970                2975

Gly Ala Asp Glu Val Pro Glu Val Ser Glu Thr Val Ala Met Ala Gly
            2980                2985                2990

Thr Ala Gly Thr Ser Glu Val Ala Glu Gly Ser Glu Ala Ser Glu Ala
        2995                3000                3005

Pro Ala Ala Pro Gly Ser Arg Glu Ala Ser Leu Pro Gly His Leu Pro
    3010                3015                3020

Trp Val Leu Ser Ala Lys Asp Glu Gln Ser Leu Arg Gly Gln Ala Ala
3025                3030                3035                3040

Ala Leu His Ala Trp Leu Ser Glu Pro Ala Ala Asp Leu Ser Asp Ala
                3045                3050                3055

Asp Gly Pro Ala Arg Leu Arg Asp Val Gly Tyr Thr Leu Ala Thr Ser
            3060                3065                3070

Arg Thr Ala Phe Ala His Arg Ala Ala Val Thr Ala Ala Asp Arg Asp
        3075                3080                3085

Gly Phe Leu Asp Gly Leu Ala Thr Leu Ala Gln Gly Gly Thr Ser Ala
    3090                3095                3100

His Val His Leu Asp Thr Ala Arg Asp Gly Thr Thr Ala Phe Leu Phe
3105                3110                3115                3120

Thr Gly Gln Gly Ser Gln Arg Pro Gly Ala Gly Arg Glu Leu Tyr Asp
                3125                3130                3135

Arg His Pro Val Phe Ala Arg Ala Leu Asp Glu Ile Cys Ala His Leu
            3140                3145                3150

Asp Gly His Leu Glu Leu Pro Leu Leu Asp Val Met Phe Ala Ala Glu
        3155                3160                3165

Gly Ser Ala Glu Ala Ala Leu Leu Asp Glu Thr Arg Tyr Thr Gln Cys
    3170                3175                3180

Ala Leu Phe Ala Leu Glu Val Ala Leu Phe Arg Leu Val Glu Ser Trp
3185                3190                3195                3200

Gly Met Arg Pro Ala Ala Leu Leu Gly His Ser Val Gly Glu Ile Ala
                3205                3210                3215

Ala Ala His Val Ala Gly Val Phe Ser Leu Ala Asp Ala Ala Arg Leu
            3220                3225                3230

Val Ala Ala Arg Gly Arg Leu Met Gln Glu Leu Pro Ala Gly Gly Ala
        3235                3240                3245

Met Leu Ala Val Gln Ala Ala Glu Asp Glu Ile Arg Val Trp Leu Glu
    3250                3255                3260

Thr Glu Glu Arg Tyr Ala Gly Arg Leu Asp Val Ala Ala Val Asn Gly
3265                3270                3275                3280

Pro Glu Ala Ala Val Leu Ser Gly Asp Ala Asp Ala Ala Arg Glu Ala
                3285                3290                3295

Glu Ala Tyr Trp Ser Gly Leu Gly Arg Arg Thr Arg Ala Leu Arg Val
            3300                3305                3310

Ser His Ala Phe His Ser Ala His Met Asp Gly Met Leu Asp Gly Phe
        3315                3320                3325

Arg Ala Val Leu Glu Thr Val Glu Phe Arg Arg Pro Ser Leu Thr Val
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    3330                3335                3340

Val Ser Asn Val Thr Gly Leu Ala Ala Gly Pro Asp Asp Leu Cys Asp
3345                3350                3355                3360

Pro Glu Tyr Trp Val Arg His Val Arg Gly Thr Val Arg Phe Leu Asp
                3365                3370                3375

Gly Val Arg Val Leu Arg Asp Leu Gly Val Arg Thr Cys Leu Glu Leu
            3380                3385                3390

Gly Pro Asp Gly Val Leu Thr Ala Met Ala Ala Asp Gly Leu Ala Asp
        3395                3400                3405

Thr Pro Ala Asp Ser Ala Ala Gly Ser Pro Val Gly Ser Pro Ala Gly
    3410                3415                3420

Ser Pro Ala Asp Ser Ala Ala Gly Ala Leu Arg Pro Arg Pro Leu Leu
3425                3430                3435                3440

Val Ala Leu Leu Arg Arg Lys Arg Ser Glu Thr Glu Thr Val Ala Asp
                3445                3450                3455

Ala Leu Gly Arg Ala His Ala His Gly Thr Gly Pro Asp Trp His Ala
            3460                3465                3470

Trp Phe Ala Gly Ser Gly Ala His Arg Val Asp Leu Pro Thr Tyr Ser
        3475                3480                3485

Phe Arg Arg Asp Arg Tyr Trp Leu Asp Ala Pro Ala Ala Asp Thr Ala
    3490                3495                3500

Val Asp Thr Ala Gly Leu Gly Leu Gly Thr Ala Asp His Pro Leu Leu
3505                3510                3515                3520

Gly Ala Val Val Ser Leu Pro Asp Arg Asp Gly Leu Leu Leu Thr Gly
                3525                3530                3535

Arg Leu Ser Leu Arg Thr His Pro Trp Leu Ala Asp His Ala Val Leu
            3540                3545                3550

Gly Ser Val Leu Leu Pro Gly Ala Ala Met Val Glu Leu Ala Ala His
        3555                3560                3565

Ala Ala Glu Ser Ala Gly Leu Arg Asp Val Arg Glu Leu Thr Leu Leu
    3570                3575                3580

Glu Pro Leu Val Leu Pro Glu His Gly Gly Val Glu Leu Arg Val Thr
3585                3590                3595                3600

Val Gly Ala Pro Ala Gly Glu Pro Gly Gly Glu Ser Ala Gly Asp Gly
                3605                3610                3615

Ala Arg Pro Val Ser Leu His Ser Arg Leu Ala Asp Ala Pro Ala Gly
            3620                3625                3630

Thr Ala Trp Ser Cys His Ala Thr Gly Leu Leu Ala Thr Asp Arg Pro
        3635                3640                3645

Glu Leu Pro Val Ala Pro Asp Arg Ala Ala Met Trp Pro Pro Gln Gly
    3650                3655                3660

Ala Glu Glu Val Pro Leu Asp Gly Leu Tyr Glu Arg Leu Asp Gly Asn
3665                3670                3675                3680

Gly Leu Ala Phe Gly Pro Leu Phe Gln Gly Leu Asn Ala Val Trp Arg
                3685                3690                3695

Tyr Glu Gly Glu Val Phe Ala Asp Ile Ala Leu Pro Ala Thr Thr Asn
            3700                3705                3710

Ala Thr Ala Pro Ala Thr Ala Asn Gly Gly Gly Ser Ala Ala Ala Ala
        3715                3720                3725

Pro Tyr Gly Ile His Pro Ala Leu Leu Asp Ala Ser Leu His Ala Ile
    3730                3735                3740
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Ala Val Gly Gly Leu Val Asp Glu Pro Glu Leu Val Arg Val Pro Phe
3745                3750                3755                3760

His Trp Ser Gly Val Thr Val His Ala Ala Gly Ala Ala Ala Ala Arg
                3765                3770                3775

Val Arg Leu Ala Ser Ala Gly Thr Asp Ala Val Ser Leu Ser Leu Thr
            3780                3785                3790

Asp Gly Glu Gly Arg Pro Leu Val Ser Val Glu Arg Leu Thr Leu Arg
        3795                3800                3805

Pro Val Thr Ala Asp Gln Ala Ala Ala Ser Arg Val Gly Gly Leu Met
    3810                3815                3820

His Arg Val Ala Trp Arg Pro Tyr Ala Leu Ala Ser Ser Gly Glu Gln
3825                3830                3835                3840

Asp Pro His Ala Thr Ser Tyr Gly Pro Thr Ala Val Leu Gly Lys Asp
                3845                3850                3855

Glu Leu Lys Val Ala Ala Ala Leu Glu Ser Ala Gly Val Glu Val Gly
            3860                3865                3870

Leu Tyr Pro Asp Leu Ala Ala Leu Ser Gln Asp Val Ala Ala Gly Ala
        3875                3880                3885

Pro Ala Pro Arg Thr Val Leu Ala Pro Leu Pro Ala Gly Pro Ala Asp
    3890                3895                3900

Gly Gly Ala Glu Gly Val Arg Gly Thr Val Ala Arg Thr Leu Glu Leu
3905                3910                3915                3920

Leu Gln Ala Trp Leu Ala Asp Glu His Leu Ala Gly Thr Arg Leu Leu
                3925                3930                3935

Leu Val Thr Arg Gly Ala Val Arg Asp Pro Glu Gly Ser Gly Ala Asp
            3940                3945                3950

Asp Gly Gly Glu Asp Leu Ser His Ala Ala Ala Trp Gly Leu Val Arg
        3955                3960                3965

Thr Ala Gln Thr Glu Asn Pro Gly Arg Phe Gly Leu Leu Asp Leu Ala
    3970                3975                3980

Asp Asp Ala Ser Ser Tyr Arg Thr Leu Pro Ser Val Leu Ser Asp Ala
3985                3990                3995                4000

Gly Leu Arg Asp Glu Pro Gln Leu Ala Leu His Asp Gly Thr Ile Arg
                4005                4010                4015

Leu Ala Arg Leu Ala Ser Val Arg Pro Glu Thr Gly Thr Ala Ala Pro
            4020                4025                4030

Ala Leu Ala Pro Glu Gly Thr Val Leu Leu Thr Gly Gly Thr Gly Gly
        4035                4040                4045

Leu Gly Gly Leu Val Ala Arg His Val Val Gly Glu Trp Gly Val Arg
    4050                4055                4060

Arg Leu Leu Leu Val Ser Arg Arg Gly Thr Asp Ala Pro Gly Ala Asp
4065                4070                4075                4080

Glu Leu Val His Glu Leu Glu Ala Leu Gly Ala Asp Val Ser Val Ala
                4085                4090                4095

Ala Cys Asp Val Ala Asp Arg Glu Ala Leu Thr Ala Val Leu Asp Ala
            4100                4105                4110

Ile Pro Ala Glu His Pro Leu Thr Ala Val Val His Thr Ala Gly Val
        4115                4120                4125

Leu Ser Asp Gly Thr Leu Pro Ser Met Thr Thr Glu Asp Val Glu His
    4130                4135                4140
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Val Leu Arg Pro Lys Val Asp Ala Ala Phe Leu Leu Asp Glu Leu Thr
4145                4150                4155                4160

Ser Thr Pro Ala Tyr Asp Leu Ala Ala Phe Val Met Phe Ser Ser Ala
                4165                4170                4175

Ala Ala Val Phe Gly Gly Ala Gly Gln Gly Ala Tyr Ala Ala Ala Asn
            4180                4185                4190

Ala Thr Leu Asp Ala Leu Ala Trp Arg Arg Arg Ala Ala Gly Leu Pro
        4195                4200                4205

Ala Leu Ser Leu Gly Trp Gly Leu Trp Ala Glu Thr Ser Gly Met Thr
    4210                4215                4220

Gly Glu Leu Gly Gln Ala Asp Leu Arg Arg Met Ser Arg Ala Gly Ile
4225                4230                4235                4240

Gly Gly Ile Ser Asp Ala Glu Gly Ile Ala Leu Leu Asp Ala Ala Leu
                4245                4250                4255

Arg Asp Asp Arg His Pro Val Leu Leu Pro Leu Arg Leu Asp Ala Ala
            4260                4265                4270

Gly Leu Arg Asp Ala Ala Gly Asn Asp Pro Ala Gly Ile Pro Ala Leu
        4275                4280                4285

Phe Arg Asp Val Val Gly Ala Arg Thr Val Arg Ala Arg Pro Ser Ala
    4290                4295                4300

Ala Ser Ala Ser Thr Thr Ala Gly Thr Ala Gly Thr Pro Gly Thr Ala
4305                4310                4315                4320

Asp Gly Ala Ala Glu Thr Ala Ala Val Thr Leu Ala Asp Arg Ala Ala
                4325                4330                4335

Thr Val Asp Gly Pro Ala Arg Gln Arg Leu Leu Leu Glu Phe Val Val
            4340                4345                4350

Gly Glu Val Ala Glu Val Leu Gly His Ala Arg Gly His Arg Ile Asp
        4355                4360                4365

Ala Glu Arg Gly Phe Leu Asp Leu Gly Phe Asp Ser Leu Thr Ala Val
    4370                4375                4380

Glu Leu Arg Asn Arg Leu Asn Ser Ala Gly Gly Leu Ala Leu Pro Ala
4385                4390                4395                4400

Thr Leu Val Phe Asp His Pro Ser Pro Ala Ala Leu Ala Ser His Leu
                4405                4410                4415

Asp Ala Glu Leu Pro Arg Gly Ala Ser Asp Gln Asp Gly Ala Gly Asn
            4420                4425                4430

Arg Asn Gly Asn Glu Asn Gly Thr Thr Ala Ser Arg Ser Thr Ala Glu
        4435                4440                4445

Thr Asp Ala Leu Leu Ala Gln Leu Thr Arg Leu Glu Gly Ala Leu Val
    4450                4455                4460

Leu Thr Gly Leu Ser Asp Ala Pro Gly Ser Glu Glu Val Leu Glu His
4465                4470                4475                4480

Leu Arg Ser Leu Arg Ser Met Val Thr Gly Glu Thr Gly Thr Gly Thr
                4485                4490                4495

Ala Ser Gly Ala Pro Asp Gly Ala Gly Ser Gly Ala Glu Asp Arg Pro
            4500                4505                4510

Trp Ala Ala Gly Asp Gly Ala Gly Gly Gly Ser Glu Asp Gly Ala Gly
        4515                4520                4525

Val Pro Asp Phe Met Asn Ala Ser Ala Glu Glu Leu Phe Gly Leu Leu
    4530                4535                4540

Asp Gln Asp Pro Ser Thr Asp
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4545 4550

<210> SEQ ID NO 2
<211> LENGTH: 3739
<212> TYPE: PRT
<213> ORGANISM: Streptomyces venezuelae

<400> SEQUENCE: 2

Val Ser Thr Val Asn Glu Glu Lys Tyr Leu Asp Tyr Leu Arg Arg Ala
1 5 10 15

Thr Ala Asp Leu His Glu Ala Arg Gly Arg Leu Arg Glu Leu Glu Ala
20 25 30

Lys Ala Gly Glu Pro Val Ala Ile Val Gly Met Ala Cys Arg Leu Pro
35 40 45

Gly Gly Val Ala Ser Pro Glu Asp Leu Trp Arg Leu Val Ala Gly Gly
50 55 60

Glu Asp Ala Ile Ser Glu Phe Pro Gln Asp Arg Gly Trp Asp Val Glu
65 70 75 80

Gly Leu Tyr Asp Pro Asn Pro Glu Ala Thr Gly Lys Ser Tyr Ala Arg
85 90 95

Glu Ala Gly Phe Leu Tyr Glu Ala Gly Glu Phe Asp Ala Asp Phe Phe
100 105 110

Gly Ile Ser Pro Arg Glu Ala Leu Ala Met Asp Pro Gln Gln Arg Leu
115 120 125

Leu Leu Glu Ala Ser Trp Glu Ala Phe Glu His Ala Gly Ile Pro Ala
130 135 140

Ala Thr Ala Arg Gly Thr Ser Val Gly Val Phe Thr Gly Val Met Tyr
145 150 155 160

His Asp Tyr Ala Thr Arg Leu Thr Asp Val Pro Glu Gly Ile Glu Gly
165 170 175

Tyr Leu Gly Thr Gly Asn Ser Gly Ser Val Ala Ser Gly Arg Val Ala
180 185 190

Tyr Thr Leu Gly Leu Glu Gly Pro Ala Val Thr Val Asp Thr Ala Cys
195 200 205

Ser Ser Ser Leu Val Ala Leu His Leu Ala Val Gln Ala Leu Arg Lys
210 215 220

Gly Glu Val Asp Met Ala Leu Ala Gly Gly Val Thr Val Met Ser Thr
225 230 235 240

Pro Ser Thr Phe Val Glu Phe Ser Arg Gln Arg Gly Leu Ala Pro Asp
245 250 255

Gly Arg Ser Lys Ser Phe Ser Ser Thr Ala Asp Gly Thr Ser Trp Ser
260 265 270

Glu Gly Val Gly Val Leu Leu Val Glu Arg Leu Ser Asp Ala Arg Arg
275 280 285

Lys Gly His Arg Ile Leu Ala Val Val Arg Gly Thr Ala Val Asn Gln
290 295 300

Asp Gly Ala Ser Ser Gly Leu Thr Ala Pro Asn Gly Pro Ser Gln Gln
305 310 315 320

Arg Val Ile Arg Arg Ala Leu Ala Asp Ala Arg Leu Thr Thr Ser Asp
325 330 335

Val Asp Val Val Glu Ala His Gly Thr Gly Thr Arg Leu Gly Asp Pro
340 345 350

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Ile Glu Ala Gln Ala Val Ile Ala Thr Tyr Gly Gln Gly Arg Asp Gly
        355                 360                 365

Glu Gln Pro Leu Arg Leu Gly Ser Leu Lys Ser Asn Ile Gly His Thr
    370                 375                 380

Gln Ala Ala Ala Gly Val Ser Gly Val Ile Lys Met Val Gln Ala Met
385                 390                 395                 400

Arg His Gly Val Leu Pro Lys Thr Leu His Val Glu Lys Pro Thr Asp
                405                 410                 415

Gln Val Asp Trp Ser Ala Gly Ala Val Glu Leu Leu Thr Glu Ala Met
            420                 425                 430

Asp Trp Pro Asp Lys Gly Asp Gly Gly Leu Arg Arg Ala Ala Val Ser
        435                 440                 445

Ser Phe Gly Val Ser Gly Thr Asn Ala His Val Val Leu Glu Glu Ala
    450                 455                 460

Pro Ala Ala Glu Glu Thr Pro Ala Ser Glu Ala Thr Pro Ala Val Glu
465                 470                 475                 480

Pro Ser Val Gly Ala Gly Leu Val Pro Trp Leu Val Ser Ala Lys Thr
                485                 490                 495

Pro Ala Ala Leu Asp Ala Gln Ile Gly Arg Leu Ala Ala Phe Ala Ser
            500                 505                 510

Gln Gly Arg Thr Asp Ala Ala Asp Pro Gly Ala Val Ala Arg Val Leu
        515                 520                 525

Ala Gly Gly Arg Ala Glu Phe Glu His Arg Ala Val Val Leu Gly Thr
    530                 535                 540

Gly Gln Asp Asp Phe Ala Gln Ala Leu Thr Ala Pro Glu Gly Leu Ile
545                 550                 555                 560

Arg Gly Thr Pro Ser Asp Val Gly Arg Val Ala Phe Val Phe Pro Gly
                565                 570                 575

Gln Gly Thr Gln Trp Ala Gly Met Gly Ala Glu Leu Leu Asp Val Ser
            580                 585                 590

Lys Glu Phe Ala Ala Ala Met Ala Glu Cys Glu Ser Ala Leu Ser Arg
        595                 600                 605

Tyr Val Asp Trp Ser Leu Glu Ala Val Val Arg Gln Ala Pro Gly Ala
    610                 615                 620

Pro Thr Leu Glu Arg Val Asp Val Val Gln Pro Val Thr Phe Ala Val
625                 630                 635                 640

Met Val Ser Leu Ala Lys Val Trp Gln His His Gly Val Thr Pro Gln
                645                 650                 655

Ala Val Val Gly His Ser Gln Gly Glu Ile Ala Ala Ala Tyr Val Ala
            660                 665                 670

Gly Ala Leu Thr Leu Asp Asp Ala Ala Arg Val Val Thr Leu Arg Ser
        675                 680                 685

Lys Ser Ile Ala Ala His Leu Ala Gly Lys Gly Gly Met Ile Ser Leu
    690                 695                 700

Ala Leu Ser Glu Glu Ala Thr Arg Gln Arg Ile Glu Asn Leu His Gly
705                 710                 715                 720

Leu Ser Ile Ala Ala Val Asn Gly Pro Thr Ala Thr Val Val Ser Gly
                725                 730                 735

Asp Pro Thr Gln Ile Gln Glu Leu Ala Gln Ala Cys Glu Ala Asp Gly
            740                 745                 750

Val Arg Ala Arg Ile Ile Pro Val Asp Tyr Ala Ser His Ser Ala His
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        755                 760                 765

Val Glu Thr Ile Glu Ser Glu Leu Ala Glu Val Leu Ala Gly Leu Ser
    770                 775                 780

Pro Arg Thr Pro Glu Val Pro Phe Phe Ser Thr Leu Glu Gly Ala Trp
785                 790                 795                 800

Ile Thr Glu Pro Val Leu Asp Gly Thr Tyr Trp Tyr Arg Asn Leu Arg
                805                 810                 815

His Arg Val Gly Phe Ala Pro Ala Val Glu Thr Leu Ala Thr Asp Glu
            820                 825                 830

Gly Phe Thr His Phe Ile Glu Val Ser Ala His Pro Val Leu Thr Met
        835                 840                 845

Thr Leu Pro Glu Thr Val Thr Gly Leu Gly Thr Leu Arg Arg Glu Gln
    850                 855                 860

Gly Gly Gln Glu Arg Leu Val Thr Ser Leu Ala Glu Ala Trp Thr Asn
865                 870                 875                 880

Gly Leu Thr Ile Asp Trp Ala Pro Val Leu Pro Thr Ala Thr Gly His
                885                 890                 895

His Pro Glu Leu Pro Thr Tyr Ala Phe Gln Arg Arg His Tyr Trp Leu
            900                 905                 910

His Asp Ser Pro Ala Val Gln Gly Ser Val Gln Asp Ser Trp Arg Tyr
        915                 920                 925

Arg Ile Asp Trp Lys Arg Leu Ala Val Ala Asp Ala Ser Glu Arg Ala
    930                 935                 940

Gly Leu Ser Gly Arg Trp Leu Val Val Val Pro Glu Asp Arg Ser Ala
945                 950                 955                 960

Glu Ala Ala Pro Val Leu Ala Ala Leu Ser Gly Ala Gly Ala Asp Pro
                965                 970                 975

Val Gln Leu Asp Val Ser Pro Leu Gly Asp Arg Gln Arg Leu Ala Ala
            980                 985                 990

Thr Leu Gly Glu Ala Leu Ala Ala Ala Gly Gly Ala Val Asp Gly Val
        995                 1000                1005

Leu Ser Leu Leu Ala Trp Asp Glu Ser Ala His Pro Gly His Pro Ala
    1010                1015                1020

Pro Phe Thr Arg Gly Thr Gly Ala Thr Leu Thr Leu Val Gln Ala Leu
1025                1030                1035                1040

Glu Asp Ala Gly Val Ala Ala Pro Leu Trp Cys Val Thr His Gly Ala
                1045                1050                1055

Val Ser Val Gly Arg Ala Asp His Val Thr Ser Pro Ala Gln Ala Met
            1060                1065                1070

Val Trp Gly Met Gly Arg Val Ala Ala Leu Glu His Pro Glu Arg Trp
        1075                1080                1085

Gly Gly Leu Ile Asp Leu Pro Ser Asp Ala Asp Arg Ala Ala Leu Asp
    1090                1095                1100

Arg Met Thr Thr Val Leu Ala Gly Gly Thr Gly Glu Asp Gln Val Ala
1105                1110                1115                1120

Val Arg Ala Ser Gly Leu Leu Ala Arg Arg Leu Val Arg Ala Ser Leu
                1125                1130                1135

Pro Ala His Gly Thr Ala Ser Pro Trp Trp Gln Ala Asp Gly Thr Val
            1140                1145                1150

Leu Val Thr Gly Ala Glu Glu Pro Ala Ala Ala Glu Ala Ala Arg Arg
        1155                1160                1165
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Leu Ala Arg Asp Gly Ala Gly His Leu Leu Leu His Thr Thr Pro Ser
    1170                1175                1180

Gly Ser Glu Gly Ala Glu Gly Thr Ser Gly Ala Ala Glu Asp Ser Gly
1185                1190                1195                1200

Leu Ala Gly Leu Val Ala Glu Leu Ala Asp Leu Gly Ala Thr Ala Thr
                1205                1210                1215

Val Val Thr Cys Asp Leu Thr Asp Ala Glu Ala Ala Ala Arg Leu Leu
            1220                1225                1230

Ala Gly Val Ser Asp Ala His Pro Leu Ser Ala Val Leu His Leu Pro
        1235                1240                1245

Pro Thr Val Asp Ser Glu Pro Leu Ala Ala Thr Asp Ala Asp Ala Leu
    1250                1255                1260

Ala Arg Val Val Thr Ala Lys Ala Thr Ala Ala Leu His Leu Asp Arg
1265                1270                1275                1280

Leu Leu Arg Glu Ala Ala Ala Ala Gly Gly Arg Pro Pro Val Leu Val
                1285                1290                1295

Leu Phe Ser Ser Val Ala Ala Ile Trp Gly Gly Ala Gly Gln Gly Ala
            1300                1305                1310

Tyr Ala Ala Gly Thr Ala Phe Leu Asp Ala Leu Ala Gly Gln His Arg
        1315                1320                1325

Ala Asp Gly Pro Thr Val Thr Ser Val Ala Trp Ser Pro Trp Glu Gly
    1330                1335                1340

Ser Arg Val Thr Glu Gly Ala Thr Gly Glu Arg Leu Arg Arg Leu Gly
1345                1350                1355                1360

Leu Arg Pro Leu Ala Pro Ala Thr Ala Leu Thr Ala Leu Asp Thr Ala
                1365                1370                1375

Leu Gly His Gly Asp Thr Ala Val Thr Ile Ala Asp Val Asp Trp Ser
            1380                1385                1390

Ser Phe Ala Pro Gly Phe Thr Thr Ala Arg Pro Gly Thr Leu Leu Ala
        1395                1400                1405

Asp Leu Pro Glu Ala Arg Arg Ala Leu Asp Glu Gln Gln Ser Thr Thr
    1410                1415                1420

Ala Ala Asp Asp Thr Val Leu Ser Arg Glu Leu Gly Ala Leu Thr Gly
1425                1430                1435                1440

Ala Glu Gln Gln Arg Arg Met Gln Glu Leu Val Arg Glu His Leu Ala
                1445                1450                1455

Val Val Leu Asn His Pro Ser Pro Glu Ala Val Asp Thr Gly Arg Ala
            1460                1465                1470

Phe Arg Asp Leu Gly Phe Asp Ser Leu Thr Ala Val Glu Leu Arg Asn
        1475                1480                1485

Arg Leu Lys Asn Ala Thr Gly Leu Ala Leu Pro Ala Thr Leu Val Phe
    1490                1495                1500

Asp Tyr Pro Thr Pro Arg Thr Leu Ala Glu Phe Leu Leu Ala Glu Ile
1505                1510                1515                1520

Leu Gly Glu Gln Ala Gly Ala Gly Glu Gln Leu Pro Val Asp Gly Gly
                1525                1530                1535

Val Asp Asp Glu Pro Val Ala Ile Val Gly Met Ala Cys Arg Leu Pro
            1540                1545                1550

Gly Gly Val Ala Ser Pro Glu Asp Leu Trp Arg Leu Val Ala Gly Gly
        1555                1560                1565
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Glu Asp Ala Ile Ser Gly Phe Pro Gln Asp Arg Gly Trp Asp Val Glu
    1570                1575                1580

Gly Leu Tyr Asp Pro Asp Pro Asp Ala Ser Gly Arg Thr Tyr Cys Arg
1585                1590                1595                1600

Ala Gly Gly Phe Leu Asp Glu Ala Gly Glu Phe Asp Ala Asp Phe Phe
                1605                1610                1615

Gly Ile Ser Pro Arg Glu Ala Leu Ala Met Asp Pro Gln Gln Arg Leu
            1620                1625                1630

Leu Leu Glu Thr Ser Trp Glu Ala Val Glu Asp Ala Gly Ile Asp Pro
        1635                1640                1645

Thr Ser Leu Gln Gly Gln Gln Val Gly Val Phe Ala Gly Thr Asn Gly
    1650                1655                1660

Pro His Tyr Glu Pro Leu Leu Arg Asn Thr Ala Glu Asp Leu Glu Gly
1665                1670                1675                1680

Tyr Val Gly Thr Gly Asn Ala Ala Ser Ile Met Ser Gly Arg Val Ser
                1685                1690                1695

Tyr Thr Leu Gly Leu Glu Gly Pro Ala Val Thr Val Asp Thr Ala Cys
            1700                1705                1710

Ser Ser Ser Leu Val Ala Leu His Leu Ala Val Gln Ala Leu Arg Lys
        1715                1720                1725

Gly Glu Cys Gly Leu Ala Leu Ala Gly Gly Val Thr Val Met Ser Thr
    1730                1735                1740

Pro Thr Thr Phe Val Glu Phe Ser Arg Gln Arg Gly Leu Ala Glu Asp
1745                1750                1755                1760

Gly Arg Ser Lys Ala Phe Ala Ala Ser Ala Asp Gly Phe Gly Pro Ala
                1765                1770                1775

Glu Gly Val Gly Met Leu Leu Val Glu Arg Leu Ser Asp Ala Arg Arg
            1780                1785                1790

Asn Gly His Arg Val Leu Ala Val Val Arg Gly Ser Ala Val Asn Gln
        1795                1800                1805

Asp Gly Ala Ser Asn Gly Leu Thr Ala Pro Asn Gly Pro Ser Gln Gln
    1810                1815                1820

Arg Val Ile Arg Arg Ala Leu Ala Asp Ala Arg Leu Thr Thr Ala Asp
1825                1830                1835                1840

Val Asp Val Val Glu Ala His Gly Thr Gly Thr Arg Leu Gly Asp Pro
                1845                1850                1855

Ile Glu Ala Gln Ala Leu Ile Ala Thr Tyr Gly Gln Gly Arg Asp Thr
            1860                1865                1870

Glu Gln Pro Leu Arg Leu Gly Ser Leu Lys Ser Asn Ile Gly His Thr
        1875                1880                1885

Gln Ala Ala Ala Gly Val Ser Gly Ile Ile Lys Met Val Gln Ala Met
    1890                1895                1900

Arg His Gly Val Leu Pro Lys Thr Leu His Val Asp Arg Pro Ser Asp
1905                1910                1915                1920

Gln Ile Asp Trp Ser Ala Gly Thr Val Glu Leu Leu Thr Glu Ala Met
                1925                1930                1935

Asp Trp Pro Arg Lys Gln Glu Gly Gly Leu Arg Arg Ala Ala Val Ser
            1940                1945                1950

Ser Phe Gly Ile Ser Gly Thr Asn Ala His Ile Val Leu Glu Glu Ala
        1955                1960                1965

Pro Val Asp Glu Asp Ala Pro Ala Asp Glu Pro Ser Val Gly Gly Val
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    1970                1975                1980

Val Pro Trp Leu Val Ser Ala Lys Thr Pro Ala Ala Leu Asp Ala Gln
1985                1990                1995                2000

Ile Gly Arg Leu Ala Ala Phe Ala Ser Gln Gly Arg Thr Asp Ala Ala
                2005                2010                2015

Asp Pro Gly Ala Val Ala Arg Val Leu Ala Gly Gly Arg Ala Gln Phe
            2020                2025                2030

Glu His Arg Ala Val Ala Leu Gly Thr Gly Gln Asp Asp Leu Ala Ala
        2035                2040                2045

Ala Leu Ala Ala Pro Glu Gly Leu Val Arg Gly Val Ala Ser Gly Val
    2050                2055                2060

Gly Arg Val Ala Phe Val Phe Pro Gly Gln Gly Thr Gln Trp Ala Gly
2065                2070                2075                2080

Met Gly Ala Glu Leu Leu Asp Val Ser Lys Glu Phe Ala Ala Ala Met
                2085                2090                2095

Ala Glu Cys Glu Ala Ala Leu Ala Pro Tyr Val Asp Trp Ser Leu Glu
            2100                2105                2110

Ala Val Val Arg Gln Ala Pro Gly Ala Pro Thr Leu Glu Arg Val Asp
        2115                2120                2125

Val Val Gln Pro Val Thr Phe Ala Val Met Val Ser Leu Ala Lys Val
    2130                2135                2140

Trp Gln His His Gly Val Thr Pro Gln Ala Val Val Gly His Ser Gln
2145                2150                2155                2160

Gly Glu Ile Ala Ala Ala Tyr Val Ala Gly Ala Leu Ser Leu Asp Asp
                2165                2170                2175

Ala Ala Arg Val Val Thr Leu Arg Ser Lys Ser Ile Gly Ala His Leu
            2180                2185                2190

Ala Gly Gln Gly Gly Met Leu Ser Leu Ala Leu Ser Glu Ala Ala Val
        2195                2200                2205

Val Glu Arg Leu Ala Gly Phe Asp Gly Leu Ser Val Ala Ala Val Asn
    2210                2215                2220

Gly Pro Thr Ala Thr Val Val Ser Gly Asp Pro Thr Gln Ile Gln Glu
2225                2230                2235                2240

Leu Ala Gln Ala Cys Glu Ala Asp Gly Val Arg Ala Arg Ile Ile Pro
                2245                2250                2255

Val Asp Tyr Ala Ser His Ser Ala His Val Glu Thr Ile Glu Ser Glu
            2260                2265                2270

Leu Ala Asp Val Leu Ala Gly Leu Ser Pro Gln Thr Pro Gln Val Pro
        2275                2280                2285

Phe Phe Ser Thr Leu Glu Gly Ala Trp Ile Thr Glu Pro Ala Leu Asp
    2290                2295                2300

Gly Gly Tyr Trp Tyr Arg Asn Leu Arg His Arg Val Gly Phe Ala Pro
2305                2310                2315                2320

Ala Val Glu Thr Leu Ala Thr Asp Glu Gly Phe Thr His Phe Val Glu
                2325                2330                2335

Val Ser Ala His Pro Val Leu Thr Met Ala Leu Pro Glu Thr Val Thr
            2340                2345                2350

Gly Leu Gly Thr Leu Arg Arg Asp Asn Gly Gly Gln His Arg Leu Thr
        2355                2360                2365

Thr Ser Leu Ala Glu Ala Trp Ala Asn Gly Leu Thr Val Asp Trp Ala
    2370                2375                2380
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Ser Leu Leu Pro Thr Thr Thr Thr His Pro Asp Leu Pro Thr Tyr Ala
2385                2390                2395                2400

Phe Gln Thr Glu Arg Tyr Trp Pro Gln Pro Asp Leu Ser Ala Ala Gly
                2405                2410                2415

Asp Ile Thr Ser Ala Gly Leu Gly Ala Ala Glu His Pro Leu Leu Gly
            2420                2425                2430

Ala Ala Val Ala Leu Ala Asp Ser Asp Gly Cys Leu Leu Thr Gly Ser
        2435                2440                2445

Leu Ser Leu Arg Thr His Pro Trp Leu Ala Asp His Ala Val Ala Gly
    2450                2455                2460

Thr Val Leu Leu Pro Gly Thr Ala Phe Val Glu Leu Ala Phe Arg Ala
2465                2470                2475                2480

Gly Asp Gln Val Gly Cys Asp Leu Val Glu Glu Leu Thr Leu Asp Ala
                2485                2490                2495

Pro Leu Val Leu Pro Arg Arg Gly Ala Val Arg Val Gln Leu Ser Val
            2500                2505                2510

Gly Ala Ser Asp Glu Ser Gly Arg Arg Thr Phe Gly Leu Tyr Ala His
        2515                2520                2525

Pro Glu Asp Ala Pro Gly Glu Ala Glu Trp Thr Arg His Ala Thr Gly
    2530                2535                2540

Val Leu Ala Ala Arg Ala Asp Arg Thr Ala Pro Val Ala Asp Pro Glu
2545                2550                2555                2560

Ala Trp Pro Pro Pro Gly Ala Glu Pro Val Asp Val Asp Gly Leu Tyr
                2565                2570                2575

Glu Arg Phe Ala Ala Asn Gly Tyr Gly Tyr Gly Pro Leu Phe Gln Gly
            2580                2585                2590

Val Arg Gly Val Trp Arg Arg Gly Asp Glu Val Phe Ala Asp Val Ala
        2595                2600                2605

Leu Pro Ala Glu Val Ala Gly Ala Glu Gly Ala Arg Phe Gly Leu His
    2610                2615                2620

Pro Ala Leu Leu Asp Ala Ala Val Gln Ala Ala Gly Ala Gly Gly Ala
2625                2630                2635                2640

Phe Gly Ala Gly Thr Arg Leu Pro Phe Ala Trp Ser Gly Ile Ser Leu
                2645                2650                2655

Tyr Ala Val Gly Ala Thr Ala Leu Arg Val Arg Leu Ala Pro Ala Gly
            2660                2665                2670

Pro Asp Thr Val Ser Val Ser Ala Ala Asp Ser Ser Gly Gln Pro Val
        2675                2680                2685

Phe Ala Ala Asp Ser Leu Thr Val Leu Pro Val Asp Pro Ala Gln Leu
    2690                2695                2700

Ala Ala Phe Ser Asp Pro Thr Leu Asp Ala Leu His Leu Leu Glu Trp
2705                2710                2715                2720

Thr Ala Trp Asp Gly Ala Ala Gln Ala Leu Pro Gly Ala Val Val Leu
                2725                2730                2735

Gly Gly Asp Ala Asp Gly Leu Ala Ala Ala Leu Arg Ala Gly Gly Thr
            2740                2745                2750

Glu Val Leu Ser Phe Pro Asp Leu Thr Asp Leu Val Glu Ala Val Asp
        2755                2760                2765

Arg Gly Glu Thr Pro Ala Pro Ala Thr Val Leu Val Ala Cys Pro Ala
    2770                2775                2780
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Ala Gly Pro Gly Gly Pro Glu His Val Arg Glu Ala Leu His Gly Ser
2785                2790                2795                2800

Leu Ala Leu Met Gln Ala Trp Leu Ala Asp Glu Arg Phe Thr Asp Gly
                2805                2810                2815

Arg Leu Val Leu Val Thr Arg Asp Ala Val Ala Ala Arg Ser Gly Asp
            2820                2825                2830

Gly Leu Arg Ser Thr Gly Gln Ala Ala Val Trp Gly Leu Gly Arg Ser
        2835                2840                2845

Ala Gln Thr Glu Ser Pro Gly Arg Phe Val Leu Leu Asp Leu Ala Gly
    2850                2855                2860

Glu Ala Arg Thr Ala Gly Asp Ala Thr Ala Gly Asp Gly Leu Thr Thr
2865                2870                2875                2880

Gly Asp Ala Thr Val Gly Gly Thr Ser Gly Asp Ala Ala Leu Gly Ser
                2885                2890                2895

Ala Leu Ala Thr Ala Leu Gly Ser Gly Glu Pro Gln Leu Ala Leu Arg
            2900                2905                2910

Asp Gly Ala Leu Leu Val Pro Arg Leu Ala Arg Ala Ala Ala Pro Ala
        2915                2920                2925

Ala Ala Asp Gly Leu Ala Ala Ala Asp Gly Leu Ala Ala Leu Pro Leu
    2930                2935                2940

Pro Ala Ala Pro Ala Leu Trp Arg Leu Glu Pro Gly Thr Asp Gly Ser
2945                2950                2955                2960

Leu Glu Ser Leu Thr Ala Ala Pro Gly Asp Ala Glu Thr Leu Ala Pro
                2965                2970                2975

Glu Pro Leu Gly Pro Gly Gln Val Arg Ile Ala Ile Arg Ala Thr Gly
            2980                2985                2990

Leu Asn Phe Arg Asp Val Leu Ile Ala Leu Gly Met Tyr Pro Asp Pro
        2995                3000                3005

Ala Leu Met Gly Thr Glu Gly Ala Gly Val Val Thr Ala Thr Gly Pro
    3010                3015                3020

Gly Val Thr His Leu Ala Pro Gly Asp Arg Val Met Gly Leu Leu Ser
3025                3030                3035                3040

Gly Ala Tyr Ala Pro Val Val Val Ala Asp Ala Arg Thr Val Ala Arg
                3045                3050                3055

Met Pro Glu Gly Trp Thr Phe Ala Gln Gly Ala Ser Val Pro Val Val
            3060                3065                3070

Phe Leu Thr Ala Val Tyr Ala Leu Arg Asp Leu Ala Asp Val Lys Pro
        3075                3080                3085

Gly Glu Arg Leu Leu Val His Ser Ala Ala Gly Gly Val Gly Met Ala
    3090                3095                3100

Ala Val Gln Leu Ala Arg His Trp Gly Val Glu Val His Gly Thr Ala
3105                3110                3115                3120

Ser His Gly Lys Trp Asp Ala Leu Arg Ala Leu Gly Leu Asp Asp Ala
                3125                3130                3135

His Ile Ala Ser Ser Arg Thr Leu Asp Phe Glu Ser Ala Phe Arg Ala
            3140                3145                3150

Ala Ser Gly Gly Ala Gly Met Asp Val Val Leu Asn Ser Leu Ala Arg
        3155                3160                3165

Glu Phe Val Asp Ala Ser Leu Arg Leu Leu Gly Pro Gly Gly Arg Phe
    3170                3175                3180

Val Glu Met Gly Lys Thr Asp Val Arg Asp Ala Glu Arg Val Ala Ala
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3185                3190                3195                3200

Asp His Pro Gly Val Gly Tyr Arg Ala Phe Asp Leu Gly Glu Ala Gly
                3205                3210                3215

Pro Glu Arg Ile Gly Glu Met Leu Ala Glu Val Ile Ala Leu Phe Glu
            3220                3225                3230

Asp Gly Val Leu Arg His Leu Pro Val Thr Thr Trp Asp Val Arg Arg
        3235                3240                3245

Ala Arg Asp Ala Phe Arg His Val Ser Gln Ala Arg His Thr Gly Lys
    3250                3255                3260

Val Val Leu Thr Met Pro Ser Gly Leu Asp Pro Glu Gly Thr Val Leu
3265                3270                3275                3280

Leu Thr Gly Gly Thr Gly Ala Leu Gly Gly Ile Val Ala Arg His Val
                3285                3290                3295

Val Gly Glu Trp Gly Val Arg Arg Leu Leu Leu Val Ser Arg Arg Gly
            3300                3305                3310

Thr Asp Ala Pro Gly Ala Gly Glu Leu Val His Glu Leu Glu Ala Leu
        3315                3320                3325

Gly Ala Asp Val Ser Val Ala Ala Cys Asp Val Ala Asp Arg Glu Ala
    3330                3335                3340

Leu Thr Ala Val Leu Asp Ser Ile Pro Ala Glu His Pro Leu Thr Ala
3345                3350                3355                3360

Val Val His Thr Ala Gly Val Leu Ser Asp Gly Thr Leu Pro Ser Met
                3365                3370                3375

Thr Ala Glu Asp Val Glu His Val Leu Arg Pro Lys Val Asp Ala Ala
            3380                3385                3390

Phe Leu Leu Asp Glu Leu Thr Ser Thr Pro Gly Tyr Asp Leu Ala Ala
        3395                3400                3405

Phe Val Met Phe Ser Ser Ala Ala Ala Val Phe Gly Gly Ala Gly Gln
    3410                3415                3420

Gly Ala Tyr Ala Ala Ala Asn Ala Thr Leu Asp Ala Leu Ala Trp Arg
3425                3430                3435                3440

Arg Arg Thr Ala Gly Leu Pro Ala Leu Ser Leu Gly Trp Gly Leu Trp
                3445                3450                3455

Ala Glu Thr Ser Gly Met Thr Gly Gly Leu Ser Asp Thr Asp Arg Ser
            3460                3465                3470

Arg Leu Ala Arg Ser Gly Ala Thr Pro Met Asp Ser Glu Leu Thr Leu
        3475                3480                3485

Ser Leu Leu Asp Ala Ala Met Arg Arg Asp Asp Pro Ala Leu Val Pro
    3490                3495                3500

Ile Ala Leu Asp Val Ala Ala Leu Arg Ala Gln Gln Arg Asp Gly Met
3505                3510                3515                3520

Leu Ala Pro Leu Leu Ser Gly Leu Thr Arg Gly Ser Arg Val Gly Gly
                3525                3530                3535

Ala Pro Val Asn Gln Arg Arg Ala Ala Ala Gly Gly Ala Gly Glu Ala
            3540                3545                3550

Asp Thr Asp Leu Gly Gly Arg Leu Ala Ala Met Thr Pro Asp Asp Arg
        3555                3560                3565

Val Ala His Leu Arg Asp Leu Val Arg Thr His Val Ala Thr Val Leu
    3570                3575                3580

Gly His Gly Thr Pro Ser Arg Val Asp Leu Glu Arg Ala Phe Arg Asp
3585                3590                3595                3600
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Thr Gly Phe Asp Ser Leu Thr Ala Val Glu Leu Arg Asn Arg Leu Asn
                3605                3610                3615

Ala Ala Thr Gly Leu Arg Leu Pro Ala Thr Leu Val Phe Asp His Pro
            3620                3625                3630

Thr Pro Gly Glu Leu Ala Gly His Leu Leu Asp Glu Leu Ala Thr Ala
        3635                3640                3645

Ala Gly Gly Ser Trp Ala Glu Gly Thr Gly Ser Gly Asp Thr Ala Ser
    3650                3655                3660

Ala Thr Asp Arg Gln Thr Thr Ala Ala Leu Ala Glu Leu Asp Arg Leu
3665                3670                3675                3680

Glu Gly Val Leu Ala Ser Leu Ala Pro Ala Ala Gly Gly Arg Pro Glu
                3685                3690                3695

Leu Ala Ala Arg Leu Arg Ala Leu Ala Ala Ala Leu Gly Asp Asp Gly
            3700                3705                3710

Asp Asp Ala Thr Asp Leu Asp Glu Ala Ser Asp Asp Asp Leu Phe Ser
        3715                3720                3725

Phe Ile Asp Lys Glu Leu Gly Asp Ser Asp Phe
    3730                3735


<210> SEQ ID NO 3
<211> LENGTH: 1562
<212> TYPE: PRT
<213> ORGANISM: Streptomyces venezuelae

<400> SEQUENCE: 3

Met Ala Asn Asn Glu Asp Lys Leu Arg Asp Tyr Leu Lys Arg Val Thr
  1               5                  10                  15

Ala Glu Leu Gln Gln Asn Thr Arg Arg Leu Arg Glu Ile Glu Gly Arg
             20                  25                  30

Thr His Glu Pro Val Ala Ile Val Gly Met Ala Cys Arg Leu Pro Gly
         35                  40                  45

Gly Val Ala Ser Pro Glu Asp Leu Trp Gln Leu Val Ala Gly Asp Gly
     50                  55                  60

Asp Ala Ile Ser Glu Phe Pro Gln Asp Arg Gly Trp Asp Val Glu Gly
 65                  70                  75                  80

Leu Tyr Asp Pro Asp Pro Asp Ala Ser Gly Arg Thr Tyr Cys Arg Ser
                 85                  90                  95

Gly Gly Phe Leu His Asp Ala Gly Glu Phe Asp Ala Asp Phe Phe Gly
            100                 105                 110

Ile Ser Pro Arg Glu Ala Leu Ala Met Asp Pro Gln Gln Arg Leu Ser
        115                 120                 125

Leu Thr Thr Ala Trp Glu Ala Ile Glu Ser Ala Gly Ile Asp Pro Thr
    130                 135                 140

Ala Leu Lys Gly Ser Gly Leu Gly Val Phe Val Gly Gly Trp His Thr
145                 150                 155                 160

Gly Tyr Thr Ser Gly Gln Thr Thr Ala Val Gln Ser Pro Glu Leu Glu
                165                 170                 175

Gly His Leu Val Ser Gly Ala Ala Leu Gly Phe Leu Ser Gly Arg Ile
            180                 185                 190

Ala Tyr Val Leu Gly Thr Asp Gly Pro Ala Leu Thr Val Asp Thr Ala
        195                 200                 205

Cys Ser Ser Ser Leu Val Ala Leu His Leu Ala Val Gln Ala Leu Arg
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    210                 215                 220

Lys Gly Glu Cys Asp Met Ala Leu Ala Gly Gly Val Thr Val Met Pro
225                 230                 235                 240

Asn Ala Asp Leu Phe Val Gln Phe Ser Arg Gln Arg Gly Leu Ala Ala
                245                 250                 255

Asp Gly Arg Ser Lys Ala Phe Ala Thr Ser Ala Asp Gly Phe Gly Pro
            260                 265                 270

Ala Glu Gly Ala Gly Val Leu Leu Val Glu Arg Leu Ser Asp Ala Arg
        275                 280                 285

Arg Asn Gly His Arg Ile Leu Ala Val Val Arg Gly Ser Ala Val Asn
    290                 295                 300

Gln Asp Gly Ala Ser Asn Gly Leu Thr Ala Pro His Gly Pro Ser Gln
305                 310                 315                 320

Gln Arg Val Ile Arg Arg Ala Leu Ala Asp Ala Arg Leu Ala Pro Gly
                325                 330                 335

Asp Val Asp Val Val Glu Ala His Gly Thr Gly Thr Arg Leu Gly Asp
            340                 345                 350

Pro Ile Glu Ala Gln Ala Leu Ile Ala Thr Tyr Gly Gln Glu Lys Ser
        355                 360                 365

Ser Glu Gln Pro Leu Arg Leu Gly Ala Leu Lys Ser Asn Ile Gly His
    370                 375                 380

Thr Gln Ala Ala Ala Gly Val Ala Gly Val Ile Lys Met Val Gln Ala
385                 390                 395                 400

Met Arg His Gly Leu Leu Pro Lys Thr Leu His Val Asp Glu Pro Ser
                405                 410                 415

Asp Gln Ile Asp Trp Ser Ala Gly Thr Val Glu Leu Leu Thr Glu Ala
            420                 425                 430

Val Asp Trp Pro Glu Lys Gln Asp Gly Gly Leu Arg Arg Ala Ala Val
        435                 440                 445

Ser Ser Phe Gly Ile Ser Gly Thr Asn Ala His Val Val Leu Glu Glu
    450                 455                 460

Ala Pro Ala Val Glu Asp Ser Pro Ala Val Glu Pro Pro Ala Gly Gly
465                 470                 475                 480

Gly Val Val Pro Trp Pro Val Ser Ala Lys Thr Pro Ala Ala Leu Asp
                485                 490                 495

Ala Gln Ile Gly Gln Leu Ala Ala Tyr Ala Asp Gly Arg Thr Asp Val
            500                 505                 510

Asp Pro Ala Val Ala Ala Arg Ala Leu Val Asp Ser Arg Thr Ala Met
        515                 520                 525

Glu His Arg Ala Val Ala Val Gly Asp Ser Arg Glu Ala Leu Arg Asp
    530                 535                 540

Ala Leu Arg Met Pro Glu Gly Leu Val Arg Gly Thr Ser Ser Asp Val
545                 550                 555                 560

Gly Arg Val Ala Phe Val Phe Pro Gly Gln Gly Thr Gln Trp Ala Gly
                565                 570                 575

Met Gly Ala Glu Leu Leu Asp Ser Ser Pro Glu Phe Ala Ala Ser Met
            580                 585                 590

Ala Glu Cys Glu Thr Ala Leu Ser Arg Tyr Val Asp Trp Ser Leu Glu
        595                 600                 605

Ala Val Val Arg Gln Glu Pro Gly Ala Pro Thr Leu Asp Arg Val Asp
    610                 615                 620
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Val Val Gln Pro Val Thr Phe Ala Val Met Val Ser Leu Ala Lys Val
625                 630                 635                 640

Trp Gln His His Gly Ile Thr Pro Gln Ala Val Val Gly His Ser Gln
                645                 650                 655

Gly Glu Ile Ala Ala Ala Tyr Val Ala Gly Ala Leu Thr Leu Asp Asp
            660                 665                 670

Ala Ala Arg Val Val Thr Leu Arg Ser Lys Ser Ile Ala Ala His Leu
        675                 680                 685

Ala Gly Lys Gly Gly Met Ile Ser Leu Ala Leu Asp Glu Ala Ala Val
    690                 695                 700

Leu Lys Arg Leu Ser Asp Phe Asp Gly Leu Ser Val Ala Ala Val Asn
705                 710                 715                 720

Gly Pro Thr Ala Thr Val Val Ser Gly Asp Pro Thr Gln Ile Glu Glu
                725                 730                 735

Leu Ala Arg Thr Cys Glu Ala Asp Gly Val Arg Ala Arg Ile Ile Pro
            740                 745                 750

Val Asp Tyr Ala Ser His Ser Arg Gln Val Glu Ile Ile Glu Lys Glu
        755                 760                 765

Leu Ala Glu Val Leu Ala Gly Leu Ala Pro Gln Ala Pro His Val Pro
    770                 775                 780

Phe Phe Ser Thr Leu Glu Gly Thr Trp Ile Thr Glu Pro Val Leu Asp
785                 790                 795                 800

Gly Thr Tyr Trp Tyr Arg Asn Leu Arg His Arg Val Gly Phe Ala Pro
                805                 810                 815

Ala Val Glu Thr Leu Ala Val Asp Gly Phe Thr His Phe Ile Glu Val
            820                 825                 830

Ser Ala His Pro Val Leu Thr Met Thr Leu Pro Glu Thr Val Thr Gly
        835                 840                 845

Leu Gly Thr Leu Arg Arg Glu Gln Gly Gly Gln Glu Arg Leu Val Thr
    850                 855                 860

Ser Leu Ala Glu Ala Trp Ala Asn Gly Leu Thr Ile Asp Trp Ala Pro
865                 870                 875                 880

Ile Leu Pro Thr Ala Thr Gly His His Pro Glu Leu Pro Thr Tyr Ala
                885                 890                 895

Phe Gln Thr Glu Arg Phe Trp Leu Gln Ser Ser Ala Pro Thr Ser Ala
            900                 905                 910

Ala Asp Asp Trp Arg Tyr Arg Val Glu Trp Lys Pro Leu Thr Ala Ser
        915                 920                 925

Gly Gln Ala Asp Leu Ser Gly Arg Trp Ile Val Ala Val Gly Ser Glu
    930                 935                 940

Pro Glu Ala Glu Leu Leu Gly Ala Leu Lys Ala Ala Gly Ala Glu Val
945                 950                 955                 960

Asp Val Leu Glu Ala Gly Ala Asp Asp Asp Arg Glu Ala Leu Ala Ala
                965                 970                 975

Arg Leu Thr Ala Leu Thr Thr Gly Asp Gly Phe Thr Gly Val Val Ser
            980                 985                 990

Leu Leu Asp Asp Leu Val Pro Gln Val Ala Trp Val Gln Ala Leu Gly
        995                 1000                1005

Asp Ala Gly Ile Lys Ala Pro Leu Trp Ser Val Thr Gln Gly Ala Val
    1010                1015                1020
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Ser Val Gly Arg Leu Asp Thr Pro Ala Asp Pro Asp Arg Ala Met Leu
1025                1030                1035                1040

Trp Gly Leu Gly Arg Val Val Ala Leu Glu His Pro Glu Arg Trp Ala
                1045                1050                1055

Gly Leu Val Asp Leu Pro Ala Gln Pro Asp Ala Ala Ala Leu Ala His
            1060                1065                1070

Leu Val Thr Ala Leu Ser Gly Ala Thr Gly Glu Asp Gln Ile Ala Ile
        1075                1080                1085

Arg Thr Thr Gly Leu His Ala Arg Arg Leu Ala Arg Ala Pro Leu His
    1090                1095                1100

Gly Arg Arg Pro Thr Arg Asp Trp Gln Pro His Gly Thr Val Leu Ile
1105                1110                1115                1120

Thr Gly Gly Thr Gly Ala Leu Gly Ser His Ala Ala Arg Trp Met Ala
                1125                1130                1135

His His Gly Ala Glu His Leu Leu Leu Val Ser Arg Ser Gly Glu Gln
            1140                1145                1150

Ala Pro Gly Ala Thr Gln Leu Thr Ala Glu Leu Thr Ala Ser Gly Ala
        1155                1160                1165

Arg Val Thr Ile Ala Ala Cys Asp Val Ala Asp Pro His Ala Met Arg
    1170                1175                1180

Thr Leu Leu Asp Ala Ile Pro Ala Glu Thr Pro Leu Thr Ala Val Val
1185                1190                1195                1200

His Thr Ala Gly Ala Pro Gly Gly Asp Pro Leu Asp Val Thr Gly Pro
                1205                1210                1215

Glu Asp Ile Ala Arg Ile Leu Gly Ala Lys Thr Ser Gly Ala Glu Val
            1220                1225                1230

Leu Asp Asp Leu Leu Arg Gly Thr Pro Leu Asp Ala Phe Val Leu Tyr
        1235                1240                1245

Ser Ser Asn Ala Gly Val Trp Gly Ser Gly Ser Gln Gly Val Tyr Ala
    1250                1255                1260

Ala Ala Asn Ala His Leu Asp Ala Leu Ala Ala Arg Arg Arg Ala Arg
1265                1270                1275                1280

Gly Glu Thr Ala Thr Ser Val Ala Trp Gly Leu Trp Ala Gly Asp Gly
                1285                1290                1295

Met Gly Arg Gly Ala Asp Asp Ala Tyr Trp Gln Arg Arg Gly Ile Arg
            1300                1305                1310

Pro Met Ser Pro Asp Arg Ala Leu Asp Glu Leu Ala Lys Ala Leu Ser
        1315                1320                1325

His Asp Glu Thr Phe Val Ala Val Ala Asp Val Asp Trp Glu Arg Phe
    1330                1335                1340

Ala Pro Ala Phe Thr Val Ser Arg Pro Ser Leu Leu Leu Asp Gly Val
1345                1350                1355                1360

Pro Glu Ala Arg Gln Ala Leu Ala Ala Pro Val Gly Ala Pro Ala Pro
                1365                1370                1375

Gly Asp Ala Ala Val Ala Pro Thr Gly Gln Ser Ser Ala Leu Ala Ala
            1380                1385                1390

Ile Thr Ala Leu Pro Glu Pro Glu Arg Arg Pro Ala Leu Leu Thr Leu
        1395                1400                1405

Val Arg Thr His Ala Ala Ala Val Leu Gly His Ser Ser Pro Asp Arg
    1410                1415                1420

Val Ala Pro Gly Arg Ala Phe Thr Glu Leu Gly Phe Asp Ser Leu Thr
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1425                1430                1435                1440

Ala Val Gln Leu Arg Asn Gln Leu Ser Thr Val Val Gly Asn Arg Leu
                1445                1450                1455

Pro Ala Thr Thr Val Phe Asp His Pro Thr Pro Ala Ala Leu Ala Ala
            1460                1465                1470

His Leu His Glu Ala Tyr Leu Ala Pro Ala Glu Pro Ala Pro Thr Asp
        1475                1480                1485

Trp Glu Gly Arg Val Arg Arg Ala Leu Ala Glu Leu Pro Leu Asp Arg
    1490                1495                1500

Leu Arg Asp Ala Gly Val Leu Asp Thr Val Leu Arg Leu Thr Gly Ile
1505                1510                1515                1520

Glu Pro Glu Pro Gly Ser Gly Gly Ser Asp Gly Gly Ala Ala Asp Pro
                1525                1530                1535

Gly Ala Glu Pro Glu Ala Ser Ile Asp Asp Leu Asp Ala Glu Ala Leu
            1540                1545                1550

Ile Arg Met Ala Leu Gly Pro Arg Asn Thr
        1555                1560


<210> SEQ ID NO 4
<211> LENGTH: 1346
<212> TYPE: PRT
<213> ORGANISM: Streptomyces venezuelae

<400> SEQUENCE: 4

Met Thr Ser Ser Asn Glu Gln Leu Val Asp Ala Leu Arg Ala Ser Leu
  1               5                  10                  15

Lys Glu Asn Glu Glu Leu Arg Lys Glu Ser Arg Arg Arg Ala Asp Arg
             20                  25                  30

Arg Gln Glu Pro Met Ala Ile Val Gly Met Ser Cys Arg Phe Ala Gly
         35                  40                  45

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

Asp Leu Val Ser Glu Val Pro Glu Glu Arg Gly Trp Asp Ile Asp Ser
 65                  70                  75                  80

Leu Tyr Asp Pro Val Pro Gly Arg Lys Gly Thr Thr Tyr Val Arg Asn
                 85                  90                  95

Ala Ala Phe Leu Asp Asp Ala Ala Gly Phe Asp Ala Ala Phe Phe Gly
            100                 105                 110

Ile Ser Pro Arg Glu Ala Leu Ala Met Asp Pro Gln Gln Arg Gln Leu
        115                 120                 125

Leu Glu Ala Ser Trp Glu Val Phe Glu Arg Ala Gly Ile Asp Pro Ala
    130                 135                 140

Ser Val Arg Gly Thr Asp Val Gly Val Tyr Val Gly Cys Gly Tyr Gln
145                 150                 155                 160

Asp Tyr Ala Pro Asp Ile Arg Val Ala Pro Glu Gly Thr Gly Gly Tyr
                165                 170                 175

Val Val Thr Gly Asn Ser Ser Ala Val Ala Ser Gly Arg Ile Ala Tyr
            180                 185                 190

Ser Leu Gly Leu Glu Gly Pro Ala Val Thr Val Asp Thr Ala Cys Ser
        195                 200                 205

Ser Ser Leu Val Ala Leu His Leu Ala Leu Lys Gly Leu Arg Asn Gly
    210                 215                 220
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Asp Cys Ser Thr Ala Leu Val Gly Gly Val Ala Val Leu Ala Thr Pro
225                 230                 235                 240

Gly Ala Phe Ile Glu Phe Ser Ser Gln Gln Ala Met Ala Ala Asp Gly
                245                 250                 255

Arg Thr Lys Gly Phe Ala Ser Ala Ala Asp Gly Leu Ala Trp Gly Glu
            260                 265                 270

Gly Val Ala Val Leu Leu Leu Glu Arg Leu Ser Asp Ala Arg Arg Lys
        275                 280                 285

Gly His Arg Val Leu Ala Val Val Arg Gly Ser Ala Ile Asn Gln Asp
    290                 295                 300

Gly Ala Ser Asn Gly Leu Thr Ala Pro His Gly Pro Ser Gln Gln Arg
305                 310                 315                 320

Leu Ile Arg Gln Ala Leu Ala Asp Ala Arg Leu Thr Ser Ser Asp Val
                325                 330                 335

Asp Val Val Glu Gly His Gly Thr Gly Thr Arg Leu Gly Asp Pro Ile
            340                 345                 350

Glu Ala Gln Ala Leu Leu Ala Thr Tyr Gly Gln Gly Arg Ala Pro Gly
        355                 360                 365

Gln Pro Leu Arg Leu Gly Thr Leu Lys Ser Asn Ile Gly His Thr Gln
    370                 375                 380

Ala Ala Ser Gly Val Ala Gly Val Ile Lys Met Val Gln Ala Leu Arg
385                 390                 395                 400

His Gly Val Leu Pro Lys Thr Leu His Val Asp Glu Pro Thr Asp Gln
                405                 410                 415

Val Asp Trp Ser Ala Gly Ser Val Glu Leu Leu Thr Glu Ala Val Asp
            420                 425                 430

Trp Pro Glu Arg Pro Gly Arg Leu Arg Arg Ala Gly Val Ser Ala Phe
        435                 440                 445

Gly Val Gly Gly Thr Asn Ala His Val Val Leu Glu Glu Ala Pro Ala
    450                 455                 460

Val Glu Glu Ser Pro Ala Val Glu Pro Pro Ala Gly Gly Gly Val Val
465                 470                 475                 480

Pro Trp Pro Val Ser Ala Lys Thr Ser Ala Ala Leu Asp Ala Gln Ile
                485                 490                 495

Gly Gln Leu Ala Ala Tyr Ala Glu Asp Arg Thr Asp Val Asp Pro Ala
            500                 505                 510

Val Ala Ala Arg Ala Leu Val Asp Ser Arg Thr Ala Met Glu His Arg
        515                 520                 525

Ala Val Ala Val Gly Asp Ser Arg Glu Ala Leu Arg Asp Ala Leu Arg
    530                 535                 540

Met Pro Glu Gly Leu Val Arg Gly Thr Val Thr Asp Pro Gly Arg Val
545                 550                 555                 560

Ala Phe Val Phe Pro Gly Gln Gly Thr Gln Trp Ala Gly Met Gly Ala
                565                 570                 575

Glu Leu Leu Asp Ser Ser Pro Glu Phe Ala Ala Ala Met Ala Glu Cys
            580                 585                 590

Glu Thr Ala Leu Ser Pro Tyr Val Asp Trp Ser Leu Glu Ala Val Val
        595                 600                 605

Arg Gln Ala Pro Ser Ala Pro Thr Leu Asp Arg Val Asp Val Val Gln
    610                 615                 620

Pro Val Thr Phe Ala Val Met Val Ser Leu Ala Lys Val Trp Gln His
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625                 630                 635                 640

His Gly Ile Thr Pro Glu Ala Val Ile Gly His Ser Gln Gly Glu Ile
                645                 650                 655

Ala Ala Ala Tyr Val Ala Gly Ala Leu Thr Leu Asp Asp Ala Ala Arg
            660                 665                 670

Val Val Thr Leu Arg Ser Lys Ser Ile Ala Ala His Leu Ala Gly Lys
        675                 680                 685

Gly Gly Met Ile Ser Leu Ala Leu Ser Glu Glu Ala Thr Arg Gln Arg
    690                 695                 700

Ile Glu Asn Leu His Gly Leu Ser Ile Ala Ala Val Asn Gly Pro Thr
705                 710                 715                 720

Ala Thr Val Val Ser Gly Asp Pro Thr Gln Ile Gln Glu Leu Ala Gln
                725                 730                 735

Ala Cys Glu Ala Asp Gly Ile Arg Ala Arg Ile Ile Pro Val Asp Tyr
            740                 745                 750

Ala Ser His Ser Ala His Val Glu Thr Ile Glu Asn Glu Leu Ala Asp
        755                 760                 765

Val Leu Ala Gly Leu Ser Pro Gln Thr Pro Gln Val Pro Phe Phe Ser
    770                 775                 780

Thr Leu Glu Gly Thr Trp Ile Thr Glu Pro Ala Leu Asp Gly Gly Tyr
785                 790                 795                 800

Trp Tyr Arg Asn Leu Arg His Arg Val Gly Phe Ala Pro Ala Val Glu
                805                 810                 815

Thr Leu Ala Thr Asp Glu Gly Phe Thr His Phe Ile Glu Val Ser Ala
            820                 825                 830

His Pro Val Leu Thr Met Thr Leu Pro Asp Lys Val Thr Gly Leu Ala
        835                 840                 845

Thr Leu Arg Arg Glu Asp Gly Gly Gln His Arg Leu Thr Thr Ser Leu
    850                 855                 860

Ala Glu Ala Trp Ala Asn Gly Leu Ala Leu Asp Trp Ala Ser Leu Leu
865                 870                 875                 880

Pro Ala Thr Gly Ala Leu Ser Pro Ala Val Pro Asp Leu Pro Thr Tyr
                885                 890                 895

Ala Phe Gln His Arg Ser Tyr Trp Ile Ser Pro Ala Gly Pro Gly Glu
            900                 905                 910

Ala Pro Ala His Thr Ala Ser Gly Arg Glu Ala Val Ala Glu Thr Gly
        915                 920                 925

Leu Ala Trp Gly Pro Gly Ala Glu Asp Leu Asp Glu Glu Gly Arg Arg
    930                 935                 940

Ser Ala Val Leu Ala Met Val Met Arg Gln Ala Ala Ser Val Leu Arg
945                 950                 955                 960

Cys Asp Ser Pro Glu Glu Val Pro Val Asp Arg Pro Leu Arg Glu Ile
                965                 970                 975

Gly Phe Asp Ser Leu Thr Ala Val Asp Phe Arg Asn Arg Val Asn Arg
            980                 985                 990

Leu Thr Gly Leu Gln Leu Pro Pro Thr Val Val Phe Glu His Pro Thr
        995                 1000                1005

Pro Val Ala Leu Ala Glu Arg Ile Ser Asp Glu Leu Ala Glu Arg Asn
    1010                1015                1020

Trp Ala Val Ala Glu Pro Ser Asp His Glu Gln Ala Glu Glu Glu Lys
1025                1030                1035                1040
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Ala Ala Ala Pro Ala Gly Ala Arg Ser Gly Ala Asp Thr Gly Ala Gly
                1045                1050                1055

Ala Gly Met Phe Arg Ala Leu Phe Arg Gln Ala Val Glu Asp Asp Arg
            1060                1065                1070

Tyr Gly Glu Phe Leu Asp Val Leu Ala Glu Ala Ser Ala Phe Arg Pro
        1075                1080                1085

Gln Phe Ala Ser Pro Glu Ala Cys Ser Glu Arg Leu Asp Pro Val Leu
    1090                1095                1100

Leu Ala Gly Gly Pro Thr Asp Arg Ala Glu Gly Arg Ala Val Leu Val
1105                1110                1115                1120

Gly Cys Thr Gly Thr Ala Ala Asn Gly Gly Pro His Glu Phe Leu Arg
                1125                1130                1135

Leu Ser Thr Ser Phe Gln Glu Glu Arg Asp Phe Leu Ala Val Pro Leu
            1140                1145                1150

Pro Gly Tyr Gly Thr Gly Thr Gly Thr Gly Thr Ala Leu Leu Pro Ala
        1155                1160                1165

Asp Leu Asp Thr Ala Leu Asp Ala Gln Ala Arg Ala Ile Leu Arg Ala
    1170                1175                1180

Ala Gly Asp Ala Pro Val Val Leu Leu Gly His Ser Gly Gly Ala Leu
1185                1190                1195                1200

Leu Ala His Glu Leu Ala Phe Arg Leu Glu Arg Ala His Gly Ala Pro
                1205                1210                1215

Pro Ala Gly Ile Val Leu Val Asp Pro Tyr Pro Pro Gly His Gln Glu
            1220                1225                1230

Pro Ile Glu Val Trp Ser Arg Gln Leu Gly Glu Gly Leu Phe Ala Gly
        1235                1240                1245

Glu Leu Glu Pro Met Ser Asp Ala Arg Leu Leu Ala Met Gly Arg Tyr
    1250                1255                1260

Ala Arg Phe Leu Ala Gly Pro Arg Pro Gly Arg Ser Ser Ala Pro Val
1265                1270                1275                1280

Leu Leu Val Arg Ala Ser Glu Pro Leu Gly Asp Trp Gln Glu Glu Arg
                1285                1290                1295

Gly Asp Trp Arg Ala His Trp Asp Leu Pro His Thr Val Ala Asp Val
            1300                1305                1310

Pro Gly Asp His Phe Thr Met Met Arg Asp His Ala Pro Ala Val Ala
        1315                1320                1325

Glu Ala Val Leu Ser Trp Leu Asp Ala Ile Glu Gly Ile Glu Gly Ala
    1330                1335                1340

Gly Lys
1345


<210> SEQ ID NO 5
<211> LENGTH: 281
<212> TYPE: PRT
<213> ORGANISM: Streptomyces venezuelae

<400> SEQUENCE: 5

Val Thr Asp Arg Pro Leu Asn Val Asp Ser Gly Leu Trp Ile Arg Arg
  1               5                  10                  15

Phe His Pro Ala Pro Asn Ser Ala Val Arg Leu Val Cys Leu Pro His
             20                  25                  30

Ala Gly Gly Ser Ala Ser Tyr Phe Phe Arg Phe Ser Glu Glu Leu His
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         35                  40                  45

Pro Ser Val Glu Ala Leu Ser Val Gln Tyr Pro Gly Arg Gln Asp Arg
     50                  55                  60

Arg Ala Glu Pro Cys Leu Glu Ser Val Glu Glu Leu Ala Glu His Val
 65                  70                  75                  80

Val Ala Ala Thr Glu Pro Trp Trp Gln Glu Gly Arg Leu Ala Phe Phe
                 85                  90                  95

Gly His Ser Leu Gly Ala Ser Val Ala Phe Glu Thr Ala Arg Ile Leu
            100                 105                 110

Glu Gln Arg His Gly Val Arg Pro Glu Gly Leu Tyr Val Ser Gly Arg
        115                 120                 125

Arg Ala Pro Ser Leu Ala Pro Asp Arg Leu Val His Gln Leu Asp Asp
    130                 135                 140

Arg Ala Phe Leu Ala Glu Ile Arg Arg Leu Ser Gly Thr Asp Glu Arg
145                 150                 155                 160

Phe Leu Gln Asp Asp Glu Leu Leu Arg Leu Val Leu Pro Ala Leu Arg
                165                 170                 175

Ser Asp Tyr Lys Ala Ala Glu Thr Tyr Leu His Arg Pro Ser Ala Lys
            180                 185                 190

Leu Thr Cys Pro Val Met Ala Leu Ala Gly Asp Arg Asp Pro Lys Ala
        195                 200                 205

Pro Leu Asn Glu Val Ala Glu Trp Arg Arg His Thr Ser Gly Pro Phe
    210                 215                 220

Cys Leu Arg Ala Tyr Ser Gly Gly His Phe Tyr Leu Asn Asp Gln Trp
225                 230                 235                 240

His Glu Ile Cys Asn Asp Ile Ser Asp His Leu Leu Val Thr Arg Gly
                245                 250                 255

Ala Pro Asp Ala Arg Val Val Gln Pro Pro Thr Ser Leu Ile Glu Gly
            260                 265                 270

Ala Ala Lys Arg Trp Gln Asn Pro Arg
        275                 280


<210> SEQ ID NO 6
<211> LENGTH: 379
<212> TYPE: PRT
<213> ORGANISM: Streptomyces venezuelae
<220> FEATURE:
<221> NAME/KEY: 251
<222> LOCATION: unsure
<223> OTHER INFORMATION: unsure of amino acid at this position

<400> SEQUENCE: 6

Val Ser Ser Arg Ala Glu Thr Pro Arg Val Pro Phe Leu Asp Leu Lys
  1               5                  10                  15

Ala Ala Tyr Glu Glu Leu Arg Ala Glu Thr Asp Ala Ala Ile Ala Arg
             20                  25                  30

Val Leu Asp Ser Gly Arg Tyr Leu Leu Gly Pro Glu Leu Glu Gly Phe
         35                  40                  45

Glu Ala Glu Phe Ala Ala Tyr Cys Glu Thr Asp His Ala Val Gly Val
     50                  55                  60

Asn Ser Gly Met Asp Ala Leu Gln Leu Ala Leu Arg Gly Leu Gly Ile
 65                  70                  75                  80

Gly Pro Gly Asp Glu Val Ile Val Pro Ser His Thr Tyr Ile Ala Ser
                 85                  90                  95
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Trp Leu Ala Val Ser Ala Thr Gly Ala Thr Pro Val Pro Val Glu Pro
            100                 105                 110

His Glu Asp His Pro Thr Leu Asp Pro Leu Leu Val Glu Lys Ala Ile
        115                 120                 125

Thr Pro Arg Thr Arg Ala Leu Leu Pro Val His Leu Tyr Gly His Pro
    130                 135                 140

Ala Asp Met Asp Ala Leu Arg Glu Leu Ala Asp Arg His Gly Leu His
145                 150                 155                 160

Ile Val Glu Asp Ala Ala Gln Ala His Gly Ala Arg Tyr Arg Gly Arg
                165                 170                 175

Arg Ile Gly Ala Gly Ser Ser Val Ala Ala Phe Ser Phe Tyr Pro Gly
            180                 185                 190

Lys Asn Leu Gly Cys Phe Gly Asp Gly Gly Ala Val Val Thr Gly Asp
        195                 200                 205

Pro Glu Leu Ala Glu Arg Leu Arg Met Leu Arg Asn Tyr Gly Ser Arg
    210                 215                 220

Gln Lys Tyr Ser His Glu Thr Lys Gly Thr Asn Ser Arg Leu Asp Glu
225                 230                 235                 240

Met Gln Ala Ala Val Leu Arg Ile Arg Leu Xaa His Leu Asp Ser Trp
                245                 250                 255

Asn Gly Arg Arg Ser Ala Leu Ala Ala Glu Tyr Leu Ser Gly Leu Ala
            260                 265                 270

Gly Leu Pro Gly Ile Gly Leu Pro Val Thr Ala Pro Asp Thr Asp Pro
        275                 280                 285

Val Trp His Leu Phe Thr Val Arg Thr Glu Arg Arg Asp Glu Leu Arg
    290                 295                 300

Ser His Leu Asp Ala Arg Gly Ile Asp Thr Leu Thr His Tyr Pro Val
305                 310                 315                 320

Pro Val His Leu Ser Pro Ala Tyr Ala Gly Glu Ala Pro Pro Glu Gly
                325                 330                 335

Ser Leu Pro Arg Ala Glu Ser Phe Ala Arg Gln Val Leu Ser Leu Pro
            340                 345                 350

Ile Gly Pro His Leu Glu Arg Pro Gln Ala Leu Arg Val Ile Asp Ala
        355                 360                 365

Val Arg Glu Trp Ala Glu Arg Val Asp Gln Ala
    370                 375


<210> SEQ ID NO 7
<211> LENGTH: 382
<212> TYPE: PRT
<213> ORGANISM: Streptomyces venezuelae

<400> SEQUENCE: 7

Val Ala Asp Arg Glu Leu Gly Thr His Leu Leu Glu Thr Arg Gly Ile
  1               5                  10                  15

His Trp Ile His Ala Ala Asn Gly Asp Pro Tyr Ala Thr Val Leu Arg
             20                  25                  30

Gly Gln Ala Asp Asp Pro Tyr Pro Ala Tyr Glu Arg Val Arg Ala Arg
         35                  40                  45

Gly Ala Leu Ser Phe Ser Pro Thr Gly Ser Trp Val Thr Ala Asp His
     50                  55                  60

Ala Leu Ala Ala Ser Ile Leu Cys Ser Thr Asp Phe Gly Val Ser Gly
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 65                  70                  75                  80

Ala Asp Gly Val Pro Val Pro Gln Gln Val Leu Ser Tyr Gly Glu Gly
                 85                  90                  95

Cys Pro Leu Glu Arg Glu Gln Val Leu Pro Ala Ala Gly Asp Val Pro
            100                 105                 110

Glu Gly Gly Gln Arg Ala Val Val Glu Gly Ile His Arg Glu Thr Leu
        115                 120                 125

Glu Gly Leu Ala Pro Asp Pro Ser Ala Ser Tyr Ala Phe Glu Leu Leu
    130                 135                 140

Gly Gly Phe Val Arg Pro Ala Val Thr Ala Ala Ala Ala Ala Val Leu
145                 150                 155                 160

Gly Val Pro Ala Asp Arg Arg Ala Asp Phe Ala Asp Leu Leu Glu Arg
                165                 170                 175

Leu Arg Pro Leu Ser Asp Ser Leu Leu Ala Pro Gln Ser Leu Arg Thr
            180                 185                 190

Val Arg Ala Ala Asp Gly Ala Leu Ala Glu Leu Thr Ala Leu Leu Ala
        195                 200                 205

Asp Ser Asp Asp Ser Pro Gly Ala Leu Leu Ser Ala Leu Gly Val Thr
    210                 215                 220

Ala Ala Val Gln Leu Thr Gly Asn Ala Val Leu Ala Leu Leu Ala His
225                 230                 235                 240

Pro Glu Gln Trp Arg Glu Leu Cys Asp Arg Pro Gly Leu Ala Ala Ala
                245                 250                 255

Ala Val Glu Glu Thr Leu Arg Tyr Asp Pro Pro Val Gln Leu Asp Ala
            260                 265                 270

Arg Val Val Arg Gly Glu Thr Glu Leu Ala Gly Arg Arg Leu Pro Ala
        275                 280                 285

Gly Ala His Val Val Val Leu Thr Ala Ala Thr Gly Arg Asp Pro Glu
    290                 295                 300

Val Phe Thr Asp Pro Glu Arg Phe Asp Leu Ala Arg Pro Asp Ala Ala
305                 310                 315                 320

Ala His Leu Ala Leu His Pro Ala Gly Pro Tyr Gly Pro Val Ala Ser
                325                 330                 335

Leu Val Arg Leu Gln Ala Glu Val Ala Leu Arg Thr Leu Ala Gly Arg
            340                 345                 350

Phe Pro Gly Leu Arg Gln Ala Gly Asp Val Leu Arg Pro Arg Arg Ala
        355                 360                 365

Pro Val Gly Arg Gly Pro Leu Ser Val Pro Val Ser Ser Ser
    370                 375                 380


<210> SEQ ID NO 8
<211> LENGTH: 426
<212> TYPE: PRT
<213> ORGANISM: Streptomyces venezuelae

<400> SEQUENCE: 8

Met Arg Val Leu Leu Thr Ser Phe Ala His His Thr His Tyr Tyr Gly
  1               5                  10                  15

Leu Val Pro Leu Ala Trp Ala Leu Leu Ala Ala Gly His Glu Val Arg
             20                  25                  30

Val Ala Ser Gln Pro Ala Leu Thr Asp Thr Ile Thr Gly Ser Gly Leu
         35                  40                  45
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```
Ala Ala Val Pro Val Gly Thr Asp His Leu Ile His Glu Tyr Arg Val
     50                  55                  60

Arg Met Ala Gly Glu Pro Arg Pro Asn His Pro Ala Ile Ala Phe Asp
 65                  70                  75                  80

Glu Ala Arg Pro Glu Pro Leu Asp Trp Asp His Ala Leu Gly Ile Glu
                 85                  90                  95

Ala Ile Leu Ala Pro Tyr Phe Tyr Leu Leu Ala Asn Asn Asp Ser Met
            100                 105                 110

Val Asp Asp Leu Val Asp Phe Ala Arg Ser Trp Gln Pro Asp Leu Val
        115                 120                 125

Leu Trp Glu Pro Thr Thr Tyr Ala Gly Ala Val Ala Ala Gln Val Thr
    130                 135                 140

Gly Ala Ala His Ala Arg Val Leu Trp Gly Pro Asp Val Met Gly Ser
145                 150                 155                 160

Ala Arg Arg Lys Phe Val Ala Leu Arg Asp Arg Gln Pro Pro Glu His
                165                 170                 175

Arg Glu Asp Pro Thr Ala Glu Trp Leu Thr Trp Thr Leu Asp Arg Tyr
            180                 185                 190

Gly Ala Ser Phe Glu Glu Glu Leu Leu Thr Gly Gln Phe Thr Ile Asp
        195                 200                 205

Pro Thr Pro Pro Ser Leu Arg Leu Asp Thr Gly Leu Pro Thr Val Gly
    210                 215                 220

Met Arg Tyr Val Pro Tyr Asn Gly Thr Ser Val Val Pro Asp Trp Leu
225                 230                 235                 240

Ser Glu Pro Pro Ala Arg Pro Arg Val Cys Leu Thr Leu Gly Val Ser
                245                 250                 255

Ala Arg Glu Val Leu Gly Gly Asp Gly Val Ser Gln Gly Asp Ile Leu
            260                 265                 270

Glu Ala Leu Ala Asp Leu Asp Ile Glu Leu Val Ala Thr Leu Asp Ala
        275                 280                 285

Ser Gln Arg Ala Glu Ile Arg Asn Tyr Pro Lys His Thr Arg Phe Thr
    290                 295                 300

Asp Phe Val Pro Met His Ala Leu Leu Pro Ser Cys Ser Ala Ile Ile
305                 310                 315                 320

His His Gly Gly Ala Gly Thr Tyr Ala Thr Ala Val Ile Asn Ala Val
                325                 330                 335

Pro Gln Val Met Leu Ala Glu Leu Trp Asp Ala Pro Val Lys Ala Arg
            340                 345                 350

Ala Val Ala Glu Gln Gly Ala Gly Phe Phe Leu Pro Pro Ala Glu Leu
        355                 360                 365

Thr Pro Gln Ala Val Arg Asp Ala Val Val Arg Ile Leu Asp Asp Pro
    370                 375                 380

Ser Val Ala Thr Ala Ala His Arg Leu Arg Glu Glu Thr Phe Gly Asp
385                 390                 395                 400

Pro Thr Pro Ala Gly Ile Val Pro Glu Leu Glu Arg Leu Ala Ala Gln
                405                 410                 415

His Arg Arg Pro Pro Ala Asp Ala Arg His
            420                 425
```

<210> SEQ ID NO 9
<211> LENGTH: 331
<212> TYPE: PRT

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<213> ORGANISM: Streptomyces venezuelae
<220> FEATURE:
<221> NAME/KEY: 272
<222> LOCATION: unsure
<223> OTHER INFORMATION: unsure of amino acid at this position

<400> SEQUENCE: 9

```
Val Lys Ser Ala Leu Ser Asp Leu Ala Phe Phe Gly Gly Pro Ala Ala
  1               5                  10                  15

Phe Asp Gln Pro Leu Leu Val Gly Arg Pro Asn Arg Ile Asp Arg Ala
             20                  25                  30

Arg Leu Tyr Glu Arg Leu Asp Arg Ala Leu Asp Ser Gln Trp Leu Ser
         35                  40                  45

Asn Gly Gly Pro Leu Val Arg Glu Phe Glu Glu Arg Val Ala Gly Leu
     50                  55                  60

Ala Gly Val Arg His Ala Val Ala Thr Cys Asn Ala Thr Ala Gly Leu
 65                  70                  75                  80

Gln Leu Leu Ala His Ala Ala Gly Leu Thr Gly Glu Val Ile Met Pro
                 85                  90                  95

Ser Met Thr Phe Ala Ala Thr Pro His Ala Leu Arg Trp Ile Gly Leu
            100                 105                 110

Thr Pro Val Phe Ala Asp Ile Asp Pro Asp Thr Gly Asn Leu Asp Pro
        115                 120                 125

Asp Gln Val Ala Ala Ala Val Thr Pro Arg Thr Ser Ala Val Val Gly
    130                 135                 140

Val His Leu Trp Gly Arg Pro Cys Ala Ala Asp Gln Leu Arg Lys Val
145                 150                 155                 160

Ala Asp Glu His Gly Leu Arg Leu Tyr Phe Asp Ala Ala His Ala Leu
                165                 170                 175

Gly Cys Ala Val Asp Gly Arg Pro Ala Gly Ser Leu Gly Asp Ala Glu
            180                 185                 190

Val Phe Ser Phe His Ala Thr Lys Ala Val Asn Ala Phe Glu Gly Gly
        195                 200                 205

Ala Val Val Thr Asp Asp Ala Asp Leu Ala Ala Arg Ile Arg Ala Leu
    210                 215                 220

His Asn Phe Gly Phe Asp Leu Pro Gly Gly Ser Pro Ala Gly Gly Thr
225                 230                 235                 240

Asn Ala Lys Met Ser Glu Ala Ala Ala Ala Met Gly Leu Thr Ser Leu
                245                 250                 255

Asp Ala Phe Pro Glu Val Ile Asp Arg Asn Arg Arg Asn His Ala Xaa
            260                 265                 270

Tyr Arg Glu His Leu Ala Asp Leu Pro Gly Val Leu Val Ala Asp His
        275                 280                 285

Asp Arg His Gly Leu Asn Asn His Gln Tyr Val Ile Val Glu Ile Asp
    290                 295                 300

Glu Ala Thr Thr Gly Ile His Arg Asp Leu Val Met Glu Val Leu Lys
305                 310                 315                 320

Ala Glu Gly Val His Thr Arg Ala Tyr Phe Ser
                325                 330
```

<210> SEQ ID NO 10
<211> LENGTH: 485
<212> TYPE: PRT
<213> ORGANISM: Streptomyces venezuelae

-continued

<400> SEQUENCE: 10

```
Met Thr Ala Pro Ala Leu Ser Ala Thr Ala Pro Ala Glu Arg Cys Ala
  1               5                  10                  15

His Pro Gly Ala Asp Leu Gly Ala Ala Val His Ala Val Gly Gln Thr
             20                  25                  30

Leu Ala Ala Gly Gly Leu Val Pro Pro Asp Glu Ala Gly Thr Thr Ala
         35                  40                  45

Arg His Leu Val Arg Leu Ala Val Arg Tyr Gly Asn Ser Pro Phe Thr
     50                  55                  60

Pro Leu Glu Glu Ala Arg His Asp Leu Gly Val Asp Arg Asp Ala Phe
 65                  70                  75                  80

Arg Arg Leu Leu Ala Leu Phe Gly Gln Val Pro Glu Leu Arg Thr Ala
                 85                  90                  95

Val Glu Thr Gly Pro Ala Gly Ala Tyr Trp Lys Asn Thr Leu Leu Pro
            100                 105                 110

Leu Glu Gln Arg Gly Val Phe Asp Ala Ala Leu Ala Arg Lys Pro Val
        115                 120                 125

Phe Pro Tyr Ser Val Gly Leu Tyr Pro Gly Pro Thr Cys Met Phe Arg
    130                 135                 140

Cys His Phe Cys Val Arg Val Thr Gly Ala Arg Tyr Asp Pro Ser Ala
145                 150                 155                 160

Leu Asp Ala Gly Asn Ala Met Phe Arg Ser Val Ile Asp Glu Ile Pro
                165                 170                 175

Ala Gly Asn Pro Ser Ala Met Tyr Phe Ser Gly Gly Leu Glu Pro Leu
            180                 185                 190

Thr Asn Pro Gly Leu Gly Ser Leu Ala Ala His Ala Thr Asp His Gly
        195                 200                 205

Leu Arg Pro Thr Val Tyr Thr Asn Ser Phe Ala Leu Thr Glu Arg Thr
    210                 215                 220

Leu Glu Arg Gln Pro Gly Leu Trp Gly Leu His Ala Ile Arg Thr Ser
225                 230                 235                 240

Leu Tyr Gly Leu Asn Asp Glu Glu Tyr Glu Gln Thr Thr Gly Lys Lys
                245                 250                 255

Ala Ala Phe Arg Arg Val Arg Glu Asn Leu Arg Arg Phe Gln Gln Leu
            260                 265                 270

Arg Ala Glu Arg Glu Ser Pro Ile Asn Leu Gly Phe Ala Tyr Ile Val
        275                 280                 285

Leu Pro Gly Arg Ala Ser Arg Leu Leu Asp Leu Val Asp Phe Ile Ala
    290                 295                 300

Asp Leu Asn Asp Ala Gly Gln Gly Arg Thr Ile Asp Phe Val Asn Ile
305                 310                 315                 320

Arg Glu Asp Tyr Ser Gly Arg Asp Asp Gly Lys Leu Pro Gln Glu Glu
                325                 330                 335

Arg Ala Glu Leu Gln Glu Ala Leu Asn Ala Phe Glu Glu Arg Val Arg
            340                 345                 350

Glu Arg Thr Pro Gly Leu His Ile Asp Tyr Gly Tyr Ala Leu Asn Ser
        355                 360                 365

Leu Arg Thr Gly Ala Asp Ala Glu Leu Leu Arg Ile Lys Pro Ala Thr
    370                 375                 380

Met Arg Pro Thr Ala His Pro Gln Val Ala Val Gln Val Asp Leu Leu
```

-continued

```
385                 390                 395                 400

Gly Asp Val Tyr Leu Tyr Arg Glu Ala Gly Phe Pro Asp Leu Asp Gly
                405                 410                 415

Ala Thr Arg Tyr Ile Ala Gly Arg Val Thr Pro Asp Thr Ser Leu Thr
            420                 425                 430

Glu Val Val Arg Asp Phe Val Glu Arg Gly Gly Glu Val Ala Ala Val
        435                 440                 445

Asp Gly Asp Glu Tyr Phe Met Asp Gly Phe Asp Gln Val Val Thr Ala
    450                 455                 460

Arg Leu Asn Gln Leu Glu Arg Asp Ala Ala Asp Gly Trp Glu Glu Ala
465                 470                 475                 480

Arg Gly Phe Leu Arg
                485


<210> SEQ ID NO 11
<211> LENGTH: 237
<212> TYPE: PRT
<213> ORGANISM: Streptomyces venezuelae

<400> SEQUENCE: 11

Val Tyr Glu Val Asp His Ala Asp Val Tyr Asp Leu Phe Tyr Leu Gly
  1               5                  10                  15

Arg Gly Lys Asp Tyr Ala Ala Glu Ala Ser Asp Ile Ala Asp Leu Val
             20                  25                  30

Arg Ser Arg Thr Pro Glu Ala Ser Ser Leu Leu Asp Val Ala Cys Gly
         35                  40                  45

Thr Gly Thr His Leu Glu His Phe Thr Lys Glu Phe Gly Asp Thr Ala
     50                  55                  60

Gly Leu Glu Leu Ser Glu Asp Met Leu Thr His Ala Arg Lys Arg Leu
 65                  70                  75                  80

Pro Asp Ala Thr Leu His Gln Gly Asp Met Arg Asp Phe Arg Leu Gly
                 85                  90                  95

Arg Lys Phe Ser Ala Val Val Ser Met Phe Ser Ser Val Gly Tyr Leu
            100                 105                 110

Lys Thr Thr Glu Glu Leu Gly Ala Ala Val Ala Ser Phe Ala Glu His
        115                 120                 125

Leu Glu Pro Gly Gly Val Val Val Val Glu Pro Trp Trp Phe Pro Glu
    130                 135                 140

Thr Phe Ala Asp Gly Trp Val Ser Ala Asp Val Val Arg Arg Asp Gly
145                 150                 155                 160

Arg Thr Val Ala Arg Val Ser His Ser Val Arg Glu Gly Asn Ala Thr
                165                 170                 175

Arg Met Glu Val His Phe Thr Val Ala Asp Pro Gly Lys Gly Val Arg
            180                 185                 190

His Phe Ser Asp Val His Leu Ile Thr Leu Phe His Gln Ala Glu Tyr
        195                 200                 205

Glu Ala Ala Phe Thr Ala Ala Gly Leu Arg Val Glu Tyr Leu Glu Gly
    210                 215                 220

Gly Pro Ser Gly Arg Gly Leu Phe Val Gly Val Pro Ala
225                 230                 235


<210> SEQ ID NO 12
<211> LENGTH: 769
```

-continued

<212> TYPE: PRT
<213> ORGANISM: Streptomyces venezuelae

<400> SEQUENCE: 12

```
Met Thr Leu Asp Glu Lys Ile Ser Phe Val His Trp Ala Leu Asp Pro
  1               5                  10                  15

Asp Arg Gln Asn Val Gly Tyr Leu Pro Gly Val Pro Arg Leu Gly Ile
             20                  25                  30

Pro Glu Leu Arg Ala Ala Asp Gly Pro Asn Gly Ile Arg Leu Val Gly
         35                  40                  45

Gln Thr Ala Thr Ala Leu Pro Ala Pro Val Ala Leu Ala Ser Thr Phe
     50                  55                  60

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

Arg Ala Leu Asn Gln Asp Met Val Leu Gly Pro Met Met Asn Asn Ile
                 85                  90                  95

Arg Val Pro His Gly Gly Arg Asn Tyr Glu Thr Phe Ser Glu Asp Pro
            100                 105                 110

Leu Val Ser Ser Arg Thr Ala Val Ala Gln Ile Lys Gly Ile Gln Gly
        115                 120                 125

Ala Gly Leu Met Thr Thr Ala Lys His Phe Ala Ala Asn Asn Gln Glu
    130                 135                 140

Asn Asn Arg Phe Ser Val Asn Ala Asn Val Asp Glu Gln Thr Leu Arg
145                 150                 155                 160

Glu Ile Glu Phe Pro Ala Phe Glu Ala Ser Ser Lys Ala Gly Ala Gly
                165                 170                 175

Ser Phe Met Cys Ala Tyr Asn Gly Leu Asn Gly Lys Pro Ser Cys Gly
            180                 185                 190

Asn Asp Glu Leu Leu Asn Asn Val Leu Arg Thr Gln Trp Gly Phe Gln
        195                 200                 205

Gly Trp Val Met Ser Asp Trp Leu Ala Thr Pro Gly Thr Asp Ala Ile
    210                 215                 220

Thr Lys Gly Leu Asp Gln Glu Met Gly Val Glu Leu Pro Gly Asp Val
225                 230                 235                 240

Pro Lys Gly Glu Pro Ser Pro Pro Ala Lys Phe Phe Gly Glu Ala Leu
                245                 250                 255

Lys Thr Ala Val Leu Asn Gly Thr Val Pro Glu Ala Ala Val Thr Arg
            260                 265                 270

Ser Ala Glu Arg Ile Val Gly Gln Met Glu Lys Phe Gly Leu Leu Leu
        275                 280                 285

Ala Thr Pro Ala Pro Arg Pro Glu Arg Asp Lys Ala Gly Ala Gln Ala
    290                 295                 300

Val Ser Arg Lys Val Ala Glu Asn Gly Ala Val Leu Leu Arg Asn Glu
305                 310                 315                 320

Gly Gln Ala Leu Pro Leu Ala Gly Asp Ala Gly Lys Ser Ile Ala Val
                325                 330                 335

Ile Gly Pro Thr Ala Val Asp Pro Lys Val Thr Gly Leu Gly Ser Ala
            340                 345                 350

His Val Val Pro Asp Ser Ala Ala Ala Pro Leu Asp Thr Ile Lys Ala
        355                 360                 365

Arg Ala Gly Ala Gly Ala Thr Val Thr Tyr Glu Thr Gly Glu Glu Thr
    370                 375                 380
```

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```
Phe Gly Thr Gln Ile Pro Ala Gly Asn Leu Ser Pro Ala Phe Asn Gln
385                 390                 395                 400

Gly His Gln Leu Glu Pro Gly Lys Ala Gly Ala Leu Tyr Asp Gly Thr
                405                 410                 415

Leu Thr Val Pro Ala Asp Gly Glu Tyr Arg Ile Ala Val Arg Ala Thr
            420                 425                 430

Gly Gly Tyr Ala Thr Val Gln Leu Gly Ser His Thr Ile Glu Ala Gly
        435                 440                 445

Gln Val Tyr Gly Lys Val Ser Ser Pro Leu Leu Lys Leu Thr Lys Gly
    450                 455                 460

Thr His Lys Leu Thr Ile Ser Gly Phe Ala Met Ser Ala Thr Pro Leu
465                 470                 475                 480

Ser Leu Glu Leu Gly Trp Val Thr Pro Ala Ala Ala Asp Ala Thr Ile
                485                 490                 495

Ala Lys Ala Val Glu Ser Ala Arg Lys Ala Arg Thr Ala Val Val Phe
            500                 505                 510

Ala Tyr Asp Asp Gly Thr Glu Gly Val Asp Arg Pro Asn Leu Ser Leu
        515                 520                 525

Pro Gly Thr Gln Asp Lys Leu Ile Ser Ala Val Ala Asp Ala Asn Pro
    530                 535                 540

Asn Thr Ile Val Val Leu Asn Thr Gly Ser Ser Val Leu Met Pro Trp
545                 550                 555                 560

Leu Ser Lys Thr Arg Ala Val Leu Asp Met Trp Tyr Pro Gly Gln Ala
                565                 570                 575

Gly Ala Glu Ala Thr Ala Ala Leu Leu Tyr Gly Asp Val Asn Pro Ser
            580                 585                 590

Gly Lys Leu Thr Gln Ser Phe Pro Ala Ala Glu Asn Gln His Ala Val
        595                 600                 605

Ala Gly Asp Pro Thr Ser Tyr Pro Gly Val Asp Asn Gln Gln Thr Tyr
    610                 615                 620

Arg Glu Gly Ile His Val Gly Tyr Arg Trp Phe Asp Lys Glu Asn Val
625                 630                 635                 640

Lys Pro Leu Phe Pro Phe Gly His Gly Leu Ser Tyr Thr Ser Phe Thr
                645                 650                 655

Gln Ser Ala Pro Thr Val Val Arg Thr Ser Thr Gly Gly Leu Lys Val
            660                 665                 670

Thr Val Thr Val Arg Asn Ser Gly Lys Arg Ala Gly Gln Glu Val Val
        675                 680                 685

Gln Ala Tyr Leu Gly Ala Ser Pro Asn Val Thr Ala Pro Gln Ala Lys
    690                 695                 700

Lys Lys Leu Val Gly Tyr Thr Lys Val Ser Leu Ala Ala Gly Glu Ala
705                 710                 715                 720

Lys Thr Val Thr Val Asn Val Asp Arg Arg Gln Leu Gln Phe Trp Asp
                725                 730                 735

Ala Ala Thr Asp Asn Trp Lys Thr Gly Thr Gly Asn Arg Leu Leu Gln
            740                 745                 750

Thr Gly Ser Ser Ser Ala Asp Leu Arg Gly Ser Ala Thr Val Asn Val
        755                 760                 765

Trp
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<210> SEQ ID NO 13
<211> LENGTH: 928
<212> TYPE: PRT
<213> ORGANISM: Streptomyces venezuelae
<220> FEATURE:
<221> NAME/KEY: 694
<222> LOCATION: unsure
<223> OTHER INFORMATION: unsure of amino acid at this position

<400> SEQUENCE: 13

```
Met Asn Leu Val Glu Arg Asp Gly Glu Ile Ala His Leu Arg Ala Val
  1               5                  10                  15

Leu Asp Ala Ser Ala Ala Gly Asp Gly Thr Leu Leu Leu Val Ser Gly
             20                  25                  30

Pro Ala Gly Ser Gly Lys Thr Glu Leu Leu Arg Ser Leu Arg Arg Leu
         35                  40                  45

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

Asp Arg Asp Ile Pro Leu Gly Val Leu Cys Gln Leu Leu Arg Ser Ala
 65                  70                  75                  80

Glu Gln His Gly Ala Asp Thr Ser Ala Val Arg Asp Leu Leu Asp Ala
                 85                  90                  95

Ala Ser Arg Arg Ala Gly Thr Ser Pro Pro Pro Pro Thr Arg Arg Ser
            100                 105                 110

Ala Ser Thr Arg His Thr Ala Cys Thr Thr Gly Cys Ser Pro Ser Pro
        115                 120                 125

Ala Gly Thr Pro Phe Leu Val Ala Val Asp Asp Leu Thr His Ala Asp
    130                 135                 140

Thr Ala Ser Leu Arg Phe Leu Leu Tyr Cys Ala Ala His His Asp Gln
145                 150                 155                 160

Gly Gly Ile Gly Phe Val Met Thr Glu Arg Ala Ser Gln Arg Ala Gly
                165                 170                 175

Tyr Arg Val Phe Arg Ala Glu Leu Leu Arg Gln Pro His Cys Arg Asn
            180                 185                 190

Met Trp Leu Ser Gly Leu Pro Pro Ser Gly Val Arg Gln Leu Leu Ala
        195                 200                 205

His Tyr Tyr Gly Pro Glu Ala Ala Glu Arg Arg Ala Pro Ala Tyr His
    210                 215                 220

Ala Thr Thr Gly Gly Asn Pro Leu Leu Leu Arg Ala Leu Thr Gln Asp
225                 230                 235                 240

Arg Gln Ala Ser His Thr Thr Leu Gly Ala Ala Gly Gly Asp Glu Pro
                245                 250                 255

Val His Gly Asp Ala Phe Ala Gln Ala Val Leu Asp Cys Leu His Arg
            260                 265                 270

Ser Ala Glu Gly Thr Leu Glu Thr Ala Arg Trp Leu Ala Val Leu Glu
        275                 280                 285

Gln Ser Asp Pro Leu Leu Val Glu Arg Leu Thr Gly Thr Thr Ala Ala
    290                 295                 300

Ala Val Glu Arg His Ile Gln Glu Leu Ala Ala Ile Gly Leu Leu Asp
305                 310                 315                 320

Glu Asp Gly Thr Leu Gly Gln Pro Ala Ile Arg Glu Ala Ala Leu Gln
                325                 330                 335

Asp Leu Pro Ala Gly Glu Arg Thr Glu Leu His Arg Arg Ala Ala Glu
            340                 345                 350
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```
Gln Leu His Arg Asp Gly Ala Asp Glu Asp Thr Val Ala Arg His Leu
        355                 360                 365

Leu Val Gly Gly Ala Pro Asp Ala Pro Trp Ala Leu Pro Leu Leu Glu
    370                 375                 380

Arg Gly Ala Gln Gln Ala Leu Phe Asp Asp Arg Leu Asp Asp Ala Phe
385                 390                 395                 400

Arg Ile Leu Glu Phe Ala Val Arg Ser Ser Thr Asp Asn Thr Gln Leu
                405                 410                 415

Ala Arg Leu Ala Pro His Leu Val Ala Ala Ser Trp Arg Met Asn Pro
            420                 425                 430

His Met Thr Thr Arg Ala Leu Ala Leu Phe Asp Arg Leu Leu Ser Gly
        435                 440                 445

Glu Leu Pro Pro Ser His Pro Val Met Ala Leu Ile Arg Cys Leu Val
    450                 455                 460

Trp Tyr Gly Arg Leu Pro Glu Ala Ala Asp Ala Leu Ser Arg Leu Arg
465                 470                 475                 480

Pro Ser Ser Asp Asn Asp Ala Leu Glu Leu Ser Leu Thr Arg Met Trp
                485                 490                 495

Leu Ala Ala Leu Cys Pro Pro Leu Leu Glu Ser Leu Pro Ala Thr Pro
            500                 505                 510

Glu Pro Glu Arg Gly Pro Val Pro Val Arg Leu Ala Pro Arg Thr Thr
        515                 520                 525

Ala Leu Gln Ala Gln Ala Gly Val Phe Gln Arg Gly Pro Asp Asn Ala
    530                 535                 540

Ser Val Ala Gln Ala Glu Gln Ile Leu Gln Gly Cys Arg Leu Ser Glu
545                 550                 555                 560

Glu Thr Tyr Glu Ala Leu Glu Thr Ala Leu Leu Val Leu Val His Ala
                565                 570                 575

Asp Arg Leu Asp Arg Ala Leu Phe Trp Ser Asp Ala Leu Leu Ala Glu
            580                 585                 590

Ala Val Glu Arg Arg Ser Leu Gly Trp Glu Ala Val Phe Ala Ala Thr
        595                 600                 605

Arg Ala Met Ile Ala Ile Arg Cys Gly Asp Leu Pro Thr Ala Arg Glu
    610                 615                 620

Arg Ala Glu Leu Ala Leu Ser His Ala Ala Pro Glu Ser Trp Gly Leu
625                 630                 635                 640

Ala Val Gly Met Pro Leu Ser Ala Leu Leu Leu Ala Cys Thr Glu Ala
                645                 650                 655

Gly Glu Tyr Glu Gln Ala Glu Arg Val Leu Arg Gln Pro Val Pro Asp
            660                 665                 670

Ala Met Phe Asp Ser Arg His Gly Met Glu Tyr Met His Ala Arg Gly
        675                 680                 685

Arg Tyr Trp Leu Ala Xaa Gly Arg Leu His Ala Ala Leu Gly Glu Phe
    690                 695                 700

Met Leu Cys Gly Glu Ile Leu Gly Ser Trp Asn Leu Asp Gln Pro Ser
705                 710                 715                 720

Ile Val Pro Trp Arg Thr Ser Ala Ala Glu Val Tyr Leu Arg Leu Gly
                725                 730                 735

Asn Arg Gln Lys Ala Arg Ala Leu Ala Glu Ala Gln Leu Ala Leu Val
            740                 745                 750
```

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```
Arg Pro Gly Arg Ser Arg Thr Arg Gly Leu Thr Leu Arg Val Leu Ala
        755                 760                 765

Ala Ala Val Asp Gly Gln Gln Ala Glu Arg Leu His Ala Glu Ala Val
    770                 775                 780

Asp Met Leu His Asp Ser Gly Asp Arg Leu Glu His Ala Arg Ala Leu
785                 790                 795                 800

Ala Gly Met Ser Arg His Gln Gln Ala Gln Gly Asp Asn Tyr Arg Ala
                805                 810                 815

Arg Met Thr Ala Arg Leu Ala Gly Asp Met Ala Trp Ala Cys Gly Ala
            820                 825                 830

Tyr Pro Leu Ala Glu Glu Ile Val Pro Gly Arg Gly Gly Arg Arg Ala
        835                 840                 845

Lys Ala Val Ser Thr Glu Leu Glu Leu Pro Gly Gly Pro Asp Val Gly
    850                 855                 860

Leu Leu Ser Glu Ala Glu Arg Arg Val Ala Ala Leu Ala Ala Arg Gly
865                 870                 875                 880

Leu Thr Asn Arg Gln Ile Ala Arg Arg Leu Cys Val Thr Ala Ser Thr
                885                 890                 895

Val Glu Gln His Leu Thr Arg Val Tyr Arg Lys Leu Asn Val Thr Arg
            900                 905                 910

Arg Ala Asp Leu Pro Ile Ser Leu Ala Gln Asp Lys Ser Val Thr Ala
        915                 920                 925


<210> SEQ ID NO 14
<211> LENGTH: 292
<212> TYPE: PRT
<213> ORGANISM: Streptomyces venezuelae

<400> SEQUENCE: 14

Met Lys Gly Ile Val Leu Ala Gly Gly Ser Gly Thr Arg Leu His Pro
  1               5                  10                  15

Ala Thr Ser Val Ile Ser Lys Gln Ile Leu Pro Val Tyr Asn Lys Pro
             20                  25                  30

Met Ile Tyr Tyr Pro Leu Ser Val Leu Met Leu Gly Gly Ile Arg Glu
         35                  40                  45

Ile Gln Ile Ile Ser Thr Pro Gln His Ile Glu Leu Phe Gln Ser Leu
     50                  55                  60

Leu Gly Asn Gly Arg His Leu Gly Ile Glu Leu Asp Tyr Ala Val Gln
 65                  70                  75                  80

Lys Glu Pro Ala Gly Ile Ala Asp Ala Leu Leu Val Gly Ala Glu His
                 85                  90                  95

Ile Gly Asp Asp Thr Cys Ala Leu Ile Leu Gly Asp Asn Ile Phe His
            100                 105                 110

Gly Pro Gly Leu Tyr Thr Leu Leu Arg Asp Ser Ile Ala Arg Leu Asp
        115                 120                 125

Gly Cys Val Leu Phe Gly Tyr Pro Val Lys Asp Pro Glu Arg Tyr Gly
    130                 135                 140

Val Ala Glu Val Asp Ala Thr Gly Arg Leu Thr Asp Leu Val Glu Lys
145                 150                 155                 160

Pro Val Lys Pro Arg Ser Asn Leu Ala Val Thr Gly Leu Tyr Leu Tyr
                165                 170                 175

Asp Asn Asp Val Val Asp Ile Ala Lys Asn Ile Arg Pro Ser Pro Arg
            180                 185                 190
```

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```
Gly Glu Leu Glu Ile Thr Asp Val Asn Arg Val Tyr Leu Glu Arg Gly
        195                 200                 205

Arg Ala Glu Leu Val Asn Leu Gly Arg Gly Phe Ala Trp Leu Asp Thr
    210                 215                 220

Gly Thr His Asp Ser Leu Leu Arg Ala Ala Gln Tyr Val Gln Val Leu
225                 230                 235                 240

Glu Glu Arg Gln Gly Val Trp Ile Ala Gly Leu Glu Glu Ile Ala Phe
                245                 250                 255

Arg Met Gly Phe Ile Asp Ala Glu Ala Cys His Gly Leu Gly Glu Gly
            260                 265                 270

Leu Ser Arg Thr Glu Tyr Gly Ser Tyr Leu Met Glu Ile Ala Gly Arg
        275                 280                 285

Glu Gly Ala Pro
    290


<210> SEQ ID NO 15
<211> LENGTH: 337
<212> TYPE: PRT
<213> ORGANISM: Streptomyces venezuelae

<400> SEQUENCE: 15

Val Arg Leu Leu Val Thr Gly Gly Ala Gly Phe Ile Gly Ser His Phe
  1               5                  10                  15

Val Arg Gln Leu Leu Ala Gly Ala Tyr Pro Asp Val Pro Ala Asp Glu
             20                  25                  30

Val Ile Val Leu Asp Ser Leu Thr Tyr Ala Gly Asn Arg Ala Asn Leu
         35                  40                  45

Ala Pro Val Asp Ala Asp Pro Arg Leu Arg Phe Val His Gly Asp Ile
     50                  55                  60

Arg Asp Ala Gly Leu Leu Ala Arg Glu Leu Arg Gly Val Asp Ala Ile
 65                  70                  75                  80

Val His Phe Ala Ala Glu Ser His Val Asp Arg Ser Ile Ala Gly Ala
                 85                  90                  95

Ser Val Phe Thr Glu Thr Asn Val Gln Gly Thr Gln Thr Leu Leu Gln
            100                 105                 110

Cys Ala Val Asp Ala Gly Val Gly Arg Val Val His Val Ser Thr Asp
        115                 120                 125

Glu Val Tyr Gly Ser Ile Asp Ser Gly Ser Trp Thr Glu Ser Ser Pro
    130                 135                 140

Leu Glu Pro Asn Ser Pro Tyr Ala Ala Ser Lys Ala Gly Ser Asp Leu
145                 150                 155                 160

Val Ala Arg Ala Tyr His Arg Thr Tyr Gly Leu Asp Val Arg Ile Thr
                165                 170                 175

Arg Cys Cys Asn Asn Tyr Gly Pro Tyr Gln His Pro Glu Lys Leu Ile
            180                 185                 190

Pro Leu Phe Val Thr Asn Leu Leu Asp Gly Gly Thr Leu Pro Leu Tyr
        195                 200                 205

Gly Asp Gly Ala Asn Val Arg Glu Trp Val His Thr Asp Asp His Cys
    210                 215                 220

Arg Gly Ile Ala Leu Val Leu Ala Gly Gly Arg Ala Gly Glu Ile Tyr
225                 230                 235                 240

His Ile Gly Gly Gly Leu Glu Leu Thr Asn Arg Glu Leu Thr Gly Ile
```

-continued

```
               245                 250                 255

Leu Leu Asp Ser Leu Gly Ala Asp Trp Ser Ser Val Arg Lys Val Ala
            260                 265                 270

Asp Arg Lys Gly His Asp Leu Arg Tyr Ser Leu Asp Gly Gly Lys Ile
        275                 280                 285

Glu Arg Glu Leu Gly Tyr Arg Pro Gln Val Ser Phe Ala Asp Gly Leu
    290                 295                 300

Ala Arg Thr Val Arg Trp Tyr Arg Glu Asn Arg Gly Trp Trp Glu Pro
305                 310                 315                 320

Leu Lys Ala Thr Ala Pro Gln Leu Pro Ala Thr Ala Val Glu Val Ser
                325                 330                 335

Ala


<210> SEQ ID NO 16
<211> LENGTH: 332
<212> TYPE: PRT
<213> ORGANISM: Streptomyces venezuelae

<400> SEQUENCE: 16

Ile Gly Tyr Asp Ser Ser Lys Lys Gly Phe Asp Gly Ala Ser Cys Gly
  1               5                  10                  15

Val Ser Val Ser Ile Gly Ser Gln Ser Pro Asp Ile Ala Gln Gly Val
             20                  25                  30

Asp Thr Ala Tyr Glu Lys Arg Val Glu Gly Ala Ser Gln Arg Asp Glu
         35                  40                  45

Gly Asp Glu Leu Asp Lys Gln Gly Ala Gly Asp Gln Gly Leu Met Phe
     50                  55                  60

Gly Tyr Ala Ser Asp Glu Thr Pro Glu Leu Met Pro Leu Pro Ile His
 65                  70                  75                  80

Leu Ala His Arg Leu Ser Arg Arg Leu Thr Glu Val Arg Lys Asn Gly
                 85                  90                  95

Thr Ile Pro Tyr Leu Arg Pro Asp Gly Lys Thr Gln Val Thr Ile Glu
            100                 105                 110

Tyr Asp Gly Asp Arg Ala Val Arg Leu Asp Thr Val Val Val Ser Ser
        115                 120                 125

Gln His Ala Ser Asp Ile Asp Leu Glu Ser Leu Leu Ala Pro Asp Val
    130                 135                 140

Arg Lys Phe Val Val Glu His Val Leu Ala Gln Leu Val Glu Asp Gly
145                 150                 155                 160

Ile Lys Leu Asp Thr Asp Gly Tyr Arg Leu Leu Val Asn Pro Thr Gly
                165                 170                 175

Arg Phe Glu Ile Gly Gly Pro Met Gly Asp Ala Gly Leu Thr Gly Arg
            180                 185                 190

Lys Ile Ile Ile Asp Thr Tyr Gly Gly Met Ala Arg His Gly Gly Gly
        195                 200                 205

Ala Phe Ser Gly Lys Asp Pro Ser Lys Val Asp Arg Ser Ala Ala Tyr
    210                 215                 220

Ala Met Arg Trp Val Ala Lys Asn Val Val Ala Ala Gly Leu Ala Ser
225                 230                 235                 240

Arg Cys Glu Val Gln Val Ala Tyr Ala Ile Gly Lys Ala Glu Pro Val
                245                 250                 255

Gly Leu Phe Val Glu Thr Phe Gly Thr His Lys Ile Glu Thr Glu Lys
```

-continued

```
            260                 265                 270

Ile Glu Asn Ala Ile Gly Glu Val Phe Asp Leu Arg Pro Ala Ala Ile
        275                 280                 285

Ile Arg Asp Leu Asp Leu Leu Arg Pro Ile Tyr Ser Gln Thr Ala Ala
    290                 295                 300

Tyr Gly His Phe Gly Arg Glu Leu Pro Asp Phe Thr Trp Glu Arg Thr
305                 310                 315                 320

Asp Arg Val Asp Ala Leu Lys Lys Ala Ala Gly Leu
                325                 330


<210> SEQ ID NO 17
<211> LENGTH: 230
<212> TYPE: PRT
<213> ORGANISM: Streptomyces venezuelae

<400> SEQUENCE: 17

Met Arg Ile Ala Val Thr Gly Ser Ile Ala Thr Asp His Leu Met Thr
  1               5                  10                  15

Phe Pro Gly Arg Phe Ala Glu Gln Ile Leu Pro Asp Gln Leu Ala His
             20                  25                  30

Val Ser Leu Ser Phe Leu Val Asp Thr Leu Asp Ile Arg His Gly Gly
         35                  40                  45

Val Ala Ala Asn Ile Ala Tyr Gly Leu Gly Leu Leu Gly Arg Arg Pro
     50                  55                  60

Val Leu Val Gly Ala Val Gly Lys Asp Phe Asp Gly Tyr Gly Gln Leu
 65                  70                  75                  80

Leu Arg Ala Ala Gly Val Asp Thr Asp Ser Val Arg Val Ser Asp Arg
                 85                  90                  95

Gln His Thr Ala Arg Phe Met Cys Thr Thr Asp Glu Asp Gly Asn Gln
            100                 105                 110

Leu Ala Ser Phe Tyr Ala Gly Ala Met Ala Glu Ala Arg Asp Ile Asp
        115                 120                 125

Leu Gly Glu Thr Ala Gly Arg Pro Gly Gly Ile Asp Leu Val Leu Val
    130                 135                 140

Gly Ala Asp Asp Pro Glu Ala Met Val Arg His Thr Arg Val Cys Arg
145                 150                 155                 160

Glu Leu Gly Leu Arg Arg Ala Ala Asp Pro Ser Gln Gln Leu Ala Arg
                165                 170                 175

Leu Glu Gly Asp Ser Val Arg Glu Leu Val Asp Gly Ala Glu Leu Leu
            180                 185                 190

Phe Thr Asn Ala Tyr Glu Arg Ala Leu Leu Leu Ser Lys Thr Gly Trp
        195                 200                 205

Thr Glu Gln Glu Val Leu Ala Arg Val Gly Thr Trp Ile Thr Thr Leu
    210                 215                 220

Gly Ala Lys Gly Cys Arg
225                 230


<210> SEQ ID NO 18
<211> LENGTH: 416
<212> TYPE: PRT
<213> ORGANISM: Streptomyces venezuelae

<400> SEQUENCE: 18

Val Arg Arg Thr Gln Gln Gly Thr Thr Ala Ser Pro Pro Val Leu Asp
```

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```
  1               5                  10                  15

Leu Gly Ala Leu Gly Gln Asp Phe Ala Ala Asp Pro Tyr Pro Thr Tyr
             20                  25                  30

Ala Arg Leu Arg Ala Glu Gly Pro Ala His Arg Val Arg Thr Pro Glu
         35                  40                  45

Gly Asp Glu Val Trp Leu Val Val Gly Tyr Asp Arg Ala Arg Ala Val
     50                  55                  60

Leu Ala Asp Pro Arg Phe Ser Lys Asp Trp Arg Asn Ser Thr Thr Pro
 65                  70                  75                  80

Leu Thr Glu Ala Glu Ala Ala Leu Asn His Asn Met Leu Glu Ser Asp
                 85                  90                  95

Pro Pro Arg His Thr Arg Leu Arg Lys Leu Val Ala Arg Glu Phe Thr
            100                 105                 110

Met Arg Arg Val Glu Leu Leu Arg Pro Arg Val Gln Glu Ile Val Asp
        115                 120                 125

Gly Leu Val Asp Ala Met Leu Ala Ala Pro Asp Gly Arg Ala Asp Leu
    130                 135                 140

Met Glu Ser Leu Ala Trp Pro Leu Pro Ile Thr Val Ile Ser Glu Leu
145                 150                 155                 160

Leu Gly Val Pro Glu Pro Asp Arg Ala Ala Phe Arg Val Trp Thr Asp
                165                 170                 175

Ala Phe Val Phe Pro Asp Asp Pro Ala Gln Ala Gln Thr Ala Met Ala
            180                 185                 190

Glu Met Ser Gly Tyr Leu Ser Arg Leu Ile Asp Ser Lys Arg Gly Gln
        195                 200                 205

Asp Gly Glu Asp Leu Leu Ser Ala Leu Val Arg Thr Ser Asp Glu Asp
    210                 215                 220

Gly Ser Arg Leu Thr Ser Glu Glu Leu Leu Gly Met Ala His Ile Leu
225                 230                 235                 240

Leu Val Ala Gly His Glu Thr Thr Val Asn Leu Ile Ala Asn Gly Met
                245                 250                 255

Tyr Ala Leu Leu Ser His Pro Asp Gln Leu Ala Ala Leu Arg Ala Asp
            260                 265                 270

Met Thr Leu Leu Asp Gly Ala Val Glu Glu Met Leu Arg Tyr Glu Gly
        275                 280                 285

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Phe Cys Ile Gly Ala Pro Leu Ala Arg Leu Glu Ala Arg Ile Ala Val
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ccgcggccgc accacgccca gggccttcac cctggccgtc gtcggcaccc tgctggcggg 38400

caccaccgtg gcggccgccg ctcccggcgc cgccgacacg gccaatgttc agtacacgag 38460

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<210> SEQ ID NO 20
<211> LENGTH: 2401
<212> TYPE: DNA
<213> ORGANISM: Streptomyces venezuelae
<220> FEATURE:
<221> NAME/KEY: 1540
<222> LOCATION: unsure
<223> OTHER INFORMATION: unsure of nucleotide at this position

<400> SEQUENCE: 20

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cagcccgaac gtgacggctc cgcaggcgaa gaagaagctc gtgggctaca cgaaggtctc 2220

gctcgccgcg ggcgaggcga agacggtgac ggtgaacgtc gaccgccgtc agctgcagtt 2280

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g 2401

<210> SEQ ID NO 21
<211> LENGTH: 5970
<212> TYPE: DNA
<213> ORGANISM: Streptomyces venezuelae
<220> FEATURE:
<221> NAME/KEY: 4139 and 4819
<222> LOCATION: unsure
<223> OTHER INFORMATION: unsure of nucleotides at these positions

<400> SEQUENCE: 21

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<210> SEQ ID NO 22
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<212> TYPE: DNA
<213> ORGANISM: Streptomyces venezuelae
<220> FEATURE:
<221> NAME/KEY: 2875
<222> LOCATION: unsure
<223> OTHER INFORMATION: unsure of nucleotide at this position

<400> SEQUENCE: 22

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<212> TYPE: DNA
<213> ORGANISM: Streptomyces venezuelae

<400> SEQUENCE: 23

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<213> ORGANISM: Artificial sequence
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<223> OTHER INFORMATION: Primer

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<210> SEQ ID NO 26
<211> LENGTH: 32
<212> TYPE: DNA
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aggtctagag ctcagtgccg ggcgtcggcc gg 32

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<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
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<400> SEQUENCE: 27

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<213> ORGANISM: Artificial sequence
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<223> OTHER INFORMATION: Primer (reverse)

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<212> TYPE: DNA
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<213> ORGANISM: Artificial sequence
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<223> OTHER INFORMATION: Oligonucleotides 30-85b

<400> SEQUENCE: 30

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<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 31

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<210> SEQ ID NO 32
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<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 32

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<210> SEQ ID NO 33
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
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<400> SEQUENCE: 33

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<210> SEQ ID NO 34
<211> LENGTH: 27
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<400> SEQUENCE: 34

tcagaattct gtcggtcact tgcccgc 27

<210> SEQ ID NO 35
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<210> SEQ ID NO 36
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<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer (reverse)

<400> SEQUENCE: 36

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<210> SEQ ID NO 37
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Primer (forward)

<400> SEQUENCE: 37

tttctagacg gtggcccgga gggaacatc 29

<210> SEQ ID NO 38
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<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer (reverse)

<400> SEQUENCE: 38

cggaattccg cagctggtcg gcggcgca 28

<210> SEQ ID NO 39
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Streptomyces venezuelae

<400> SEQUENCE: 39

Thr Val Asp Ala Cys Ser Ser Ser Leu
1 5

1. An isolated recombinant DNA compound that comprises a coding sequence for a domain of a narbonolide PKS.

2. The isolated recombinant DNA compound of claim 1, wherein said domain is selected from the group consisting of a thioesterase domain, a KSQ domain, an AT domain, a KS domain, an ACP domain, a KR domain, a DH domain, and an ER domain.

3. The isolated recombinant DNA compound of claim 2 that comprises the coding sequence for a loading module, thioesterase domain, and all six extender modules of the narbonolide PKS.

4. An isolated recombinant DNA compound that comprises a coding sequence for a desosamine biosynthetic gene or a desosaminyl transferase gene or a beta-glucosidase gene of *Streptomyces venezuelae*.

5. An isolated recombinant DNA compound that comprises a coding sequence for a picK hydroxylase gene of *Streptomyces venezuelae*.

6. An isolated DNA compound of any of claim 1 that further comprises a promoter operably linked to said coding sequence.

7. The isolated recombinant DNA compound of claim 6, wherein said promoter is a promoter derived from a cell other than a *Streptomyces venezuelae* cell.

8. The isolated recombinant DNA compound of claim 7 that is a recombinant DNA expression vector.

9. The recombinant DNA expression vector of claim 8 that expresses a PKS in *Streptomyces* host cells.

10. The recombinant DNA expression vector of claim 9 that encodes a hybrid PKS comprising at least a portion of a narbonolide PKS gene and at least a portion of a second PKS gene for a macrolide aglycone other than narbonolide.

11. The recombinant DNA compound of claim 10, wherein said second PKS gene is a DEBS gene.

12. The recombinant DNA compound of claim 11, wherein said hybrid PKS is composed of a loading module

and extender modules 1 through 6 of DEBS excluding a KR domain of extender module 6 of DEBS and an ACP of extender module 6 and a thioesterase domain of the narbonolide PKS.

13. A recombinant host cell, which in its untransformed state does not produce 10-deoxymethynolide or narbonolide, that comprises a recombinant DNA expression vector of claim 9 that encodes a narbonolide PKS and said cell produces 10-deoxymethynolide or narbonolide.

14. The recombinant host cell of claim 13 that further comprises a picB gene.

15. The recombinant host cell of claim 13 that further comprises desosamine biosynthetic genes and a gene for desosaminyl transferase and produces YC17 or narbomycin.

16. The recombinant host cell of claim 15 that further comprises a picK gene and produces methymycin, neomethymycin, or picromycin.

17. The recombinant host cell of any of claim 16 that is *Streptomyces coelicolor* or *Streptomyces* lividans.

18. A recombinant host cell other than a *Streptomyces venezuelae* cell that expresses a picK hydroxylase gene of *S. venezuelae* encoded by the DNA compound of claim 5.

19. A recombinant host cell other than a *Streptomyces venezuelae* host cell that expresses a desosamine biosynthetic gene or desosaminyl transferase gene of *S. venezuelae* encoded by the DNA compound of claim 4.

20. A method for increasing the yield of a desosaminylated polyketide in a cell, which method comprises transforming the cell with a recombinant expression vector that encodes a functional beta-glucosidase gene.

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