

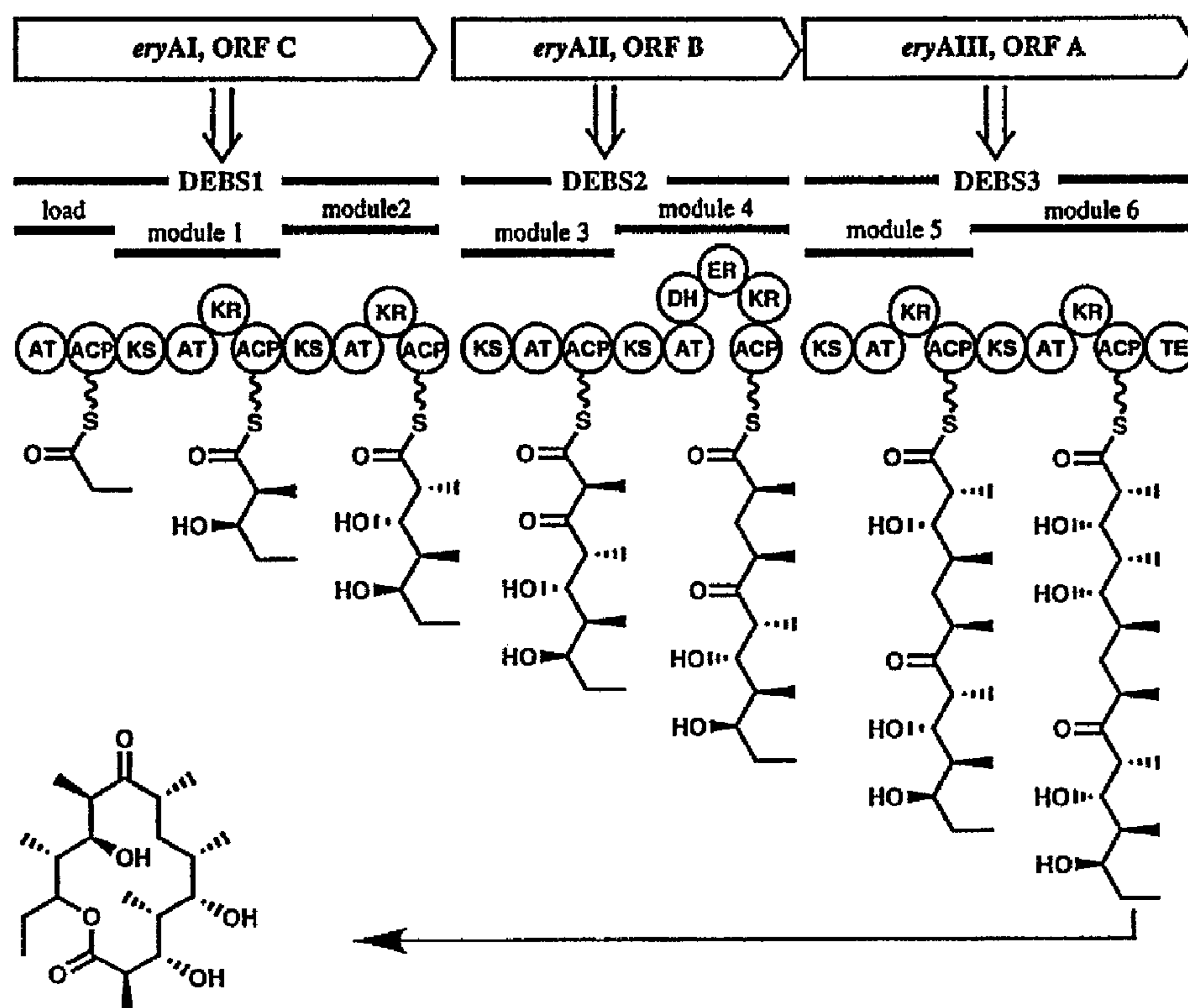


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The erythromycin PKS



(57) Abrégé/Abstract:

A polyketide synthase ("PKS") of Type I is a complex multienzyme including a loading domain linked to a multiplicity of extension domains. The first extension module receives an acyl starter unit from the loading domain and each extension module adds a



(57) **Abrégé(suite)/Abstract(continued):**

further ketide unit which may undergo processing (e.g. reduction). We have found that the Ksq domain possessed by some PKS's has decarboxylating activity, e.g. generating (substituted) acyl from (substituted) malonyl. The CLF domain of type II PKS's has similar activity. By inserting loading modules including such domains into PKS's not normally possessing them it is possible to control the starter units used.

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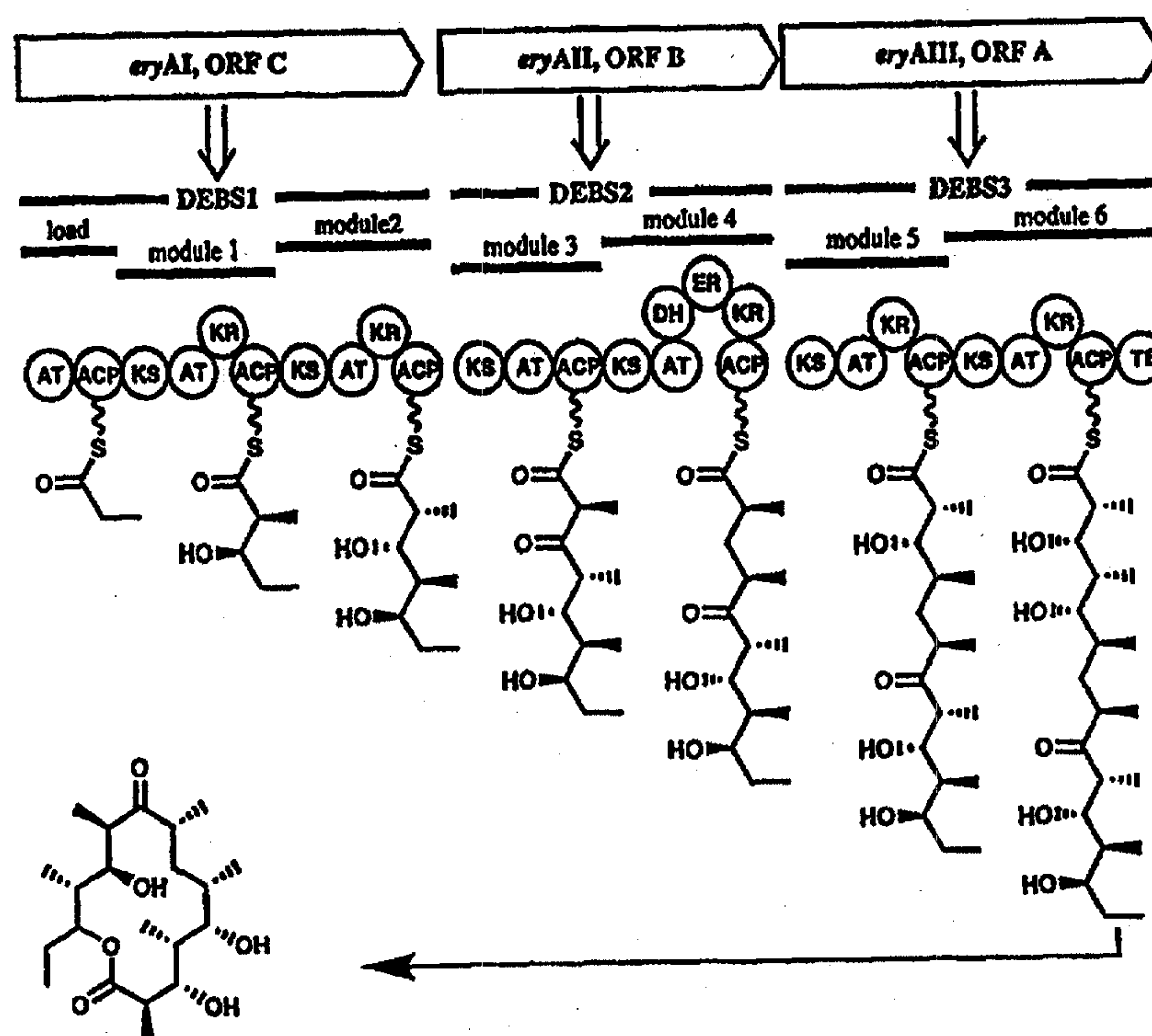
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(54) Title: POLYKETIDES AND THEIR SYNTHESIS

(57) Abstract

A polyketide synthase ("PKS") of Type I is a complex multienzyme including a loading domain linked to a multiplicity of extension domains. The first extension module receives an acyl starter unit from the loading domain and each extension module adds a further ketide unit which may undergo processing (e.g. reduction). We have found that the Ksq domain possessed by some PKS's has decarboxylating activity, e.g. generating (substituted) acyl from (substituted) malonyl. The CLF domain of type II PKS's has similar activity. By inserting loading modules including such domains into PKS's not normally possessing them it is possible to control the starter units used.

The erythromycin PKS

Polyketides and their Synthesis

The present invention relates to processes and materials (including enzyme systems, nucleic acids, vectors and cultures) for preparing novel polyketides, particularly 12-, 14- and 16-membered ring macrolides, by recombinant synthesis and to the novel polyketides so produced. Polyketide biosynthetic genes or portions of them, which may be derived from different polyketide biosynthetic gene clusters are manipulated to allow the production of specific novel polyketides, such as 12-, 14- and 16-membered macrolides, of predicted structure. The invention is particularly concerned with the replacement of genetic material encoding the natural starter unit with other genes in order to prepare macrolides with preferentially an acetate starter unit; or preferentially a propionate unit; or preferentially with an unusual starter unit, in each case minimising the formation of by-products containing a different starter unit.

Polyketides are a large and structurally diverse class of natural products that includes many compounds possessing antibiotic or other pharmacological properties, such as erythromycin, tetracyclines, rapamycin, avermectin, monensin, epothilones and FK506.

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In particular, polyketides are abundantly produced by *Streptomyces* and related actinomycete bacteria. They are synthesised by the repeated stepwise condensation of acylthioesters in a manner analogous to that of fatty acid biosynthesis. The greater structural diversity found among natural polyketides arises from the selection of (usually) acetate or propionate as "starter" or "extender" units; and from the differing degree of processing of the β -keto group observed after each condensation. Examples of processing steps include reduction to β -hydroxyacyl-, reduction followed by dehydration to 2-enoyl-, and complete reduction to the saturated acylthioester. The stereochemical outcome of these processing steps is also specified for each cycle of chain extension.

The biosynthesis of polyketides is initiated by a group of chain-forming enzymes known as polyketide synthases. Two classes of polyketide synthase (PKS) have been described in actinomycetes. One class, named Type I PKSs, represented by the PKSs for the macrolides erythromycin, oleandomycin, avermectin and rapamycin, consists of a different set or "module" of enzymes for each cycle of polyketide chain extension. For an example see Figure 1 (Cortés, J. et al. Nature (1990) 348:176-178; Donadio, S. et al. Science (1991) 2523:675-679;

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Swan, D.G. et al. Mol. Gen. Genet. (1994) 242:358-362;
MacNeil, D. J. et al. Gene (1992) 115:119-125; Schwecke,
T. et al. Proc. Natl. Acad. Sci. USA (1995) 92:7839-
7843).

5 The term "extension module" as used herein refers to
the set of contiguous domains, from a β -ketoacyl-ACP
synthase ("KS") domain to the next acyl carrier protein
("ACP") domain, which accomplishes one cycle of
polyketide chain extension. The term "loading module" is
used to refer to any group of contiguous domains which
10 accomplishes the loading of the starter unit onto the PKS
and thus renders it available to the KS domain of the
first extension module. The length of polyketide formed
has been altered, in the case of erythromycin
biosynthesis, by specific relocation using genetic
15 engineering of the enzymatic domain of the erythromycin-
producing PKS that contains the chain releasing
thioesterase/cyclase activity (Cortés et al. Science
(1995) 268:1487-1489; Kao, C.M. et al. J. Am. Chem. Soc.
(1995) 117:9105-9106).

20 In-frame deletion of the DNA encoding part of the
ketoreductase domain in module 5 of the erythromycin-
producing PKS (also known as 6-deoxyerythronolide B
synthase, DEBS) has been shown to lead to the formation
of erythromycin analogues 5,6-dideoxy-3- α -mycarosyl-5-

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oxoerythronolide B, 5,6-dideoxy-5-oxoerythronolide B and 5,6-dideoxy, 6 β -epoxy-5-oxoerythronolide B (Donadio, S. et al. Science (1991) 252:675-679). Likewise, alteration of active site residues in the enoylreductase domain of module 4 in DEBS, by genetic engineering of the
5 corresponding PKS-encoding DNA and its introduction into *Saccharopolyspora erythraea*, led to the production of 6,7-anhydroerythromycin C (Donadio, S. et al. Proc Natl. Acad. Sci. USA (1993) 90:7119-7123).

International Patent Application number WO 93/13663
10 describes additional types of genetic manipulation of the DEBS genes that are capable of producing altered polyketides. However many such attempts are reported to have been unproductive (Hutchinson, C. R. and Fujii, I. Annu. Rev. Microbiol. (1995) 49:201-238, at p. 231). The
15 complete DNA sequence of the genes from *Streptomyces hygroscopicus* that encode the modular Type I PKS governing the biosynthesis of the macrocyclic immunosuppressant polyketide rapamycin has been disclosed (Schwecke, T. et al. (1995) Proc. Natl. Acad. Sci. USA
20 92:7839-7843). The DNA sequence is deposited in the EMBL/Genbank Database under the accession number X86780.

The second class of PKS, named Type II PKSs, is represented by the synthases for aromatic compounds. Type II PKSs contain only a single set of enzymatic

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activities for chain extension and these are re-used as appropriate in successive cycles (Bibb, M. J. et al. EMBO J. (1989) 8:2727-2736; Sherman, D. H. et al. EMBO J. (1989) 8:2717-2725; Fernandez-Moreno, M.A. et al. J. Biol. Chem. (1992) 267:19278-19290). The "extender" units for the Type II pKSs are usually acetate units, and the presence of specific cyclases dictates the preferred pathway for cyclisation of the completed chain into an aromatic product (Hutchinson, C. R. and Fujii, I. Annu. Rev. Microbiol. (1995) 49:201-238). Hybrid polyketides have been obtained by the introduction of clones Type II PKS gene-containing DNA into another strain containing a different Type II PKS gene cluster, for example by introduction of DNA derived from the gene cluster for actinorhodin, a blue-pigmented polyketide from *Streptomyces coelicolor*, into an anthraquinone polyketide-producing strain of *Streptomyces galileus* (Bartel, P. L. et al. J. Bacteriol. (1990) 172:4816-4826).

The minimal number of domains required for polyketide chain extension on a Type II PKS when expressed in a *Streptomyces coelicolor* host cell (the "minimal PKS") has been defined for example in International Patent Application Number WO 95/08548 as containing the following three polypeptides which are

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products of the *act I* genes: first KS; secondly a polypeptide termed the CLF with end-to-end amino acid sequence similarity to the KS but in which the essential active site residue of the KS, namely a cysteine residue, is substituted either by a glutamine residue, or in the case of the PKS for a spore pigment such as the *whiE* gene product (Chater, K. F. and Davis, N. K. Mol. Microbiol. (1990) 4:1679-1691) by a glutamic acid residue (Figure 2); and finally an ACP. The CLF has been stated for example in International Patent Application Number WO 95/08548 to be a factor that determines the chain length of the polyketide chain that is produced by the minimal PKS. However it has been found (Shen, B. et al. J. Am. Chem. Soc. (1995) 117:6811-6821) that when the CLF for the octaketide actinorhodin is used to replace the CLF for the decaketide tetracenomycin in host cells of *Streptomyces glaucescens*, the polyketide product is not found to be altered from a decaketide to an octaketide, so the exact role of the CLF remains unclear. An alternative nomenclature has been proposed in which KS is designated $KS\alpha$ and CLF is designated $KS\beta$, to reflect this lack of knowledge (Meurer, G. et al. Chemistry and Biology (1997) 4:433-443). The mechanism by which acetate starter units and acetate extender units are loaded onto the Type II PKS is not known, but it is

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speculated that the malonyl-CoA: ACP acyltransferase of the fatty acid synthase of the host cell can fulfil the same function for the Type II PKS (Revill, W. P. et al. J. Bacteriol. (1995) 177:3946-3952).

International Patent Application Number WO 95/08548 describes the replacement of actinorhodin PKS genes by heterologous DNA from other Type II PKS gene clusters, to obtain hybrid polyketides. The same International Patent Application WO 95/08548 describes the construction of a strain of *Streptomyces coelicolor* which substantially lacks the native gene cluster for actinorhodin, and the use in that strain of a plasmid vector pRM5 derived from the low-copy number vector SCP2* isolated from *Streptomyces coelicolor* (Bibb, M. J. and Hopwood, D. A. J. Gen. Microbiol. (1981) 126:427) and in which heterologous PKS-encoding DNA may be expressed under the control of the divergent act I/ act III promoter region of the actinorhodin gene cluster (Fernandez-Moreno, M.A. et al. J. Biol. Chem. (1992) 267:19278-19290). The plasmid pRM5 also contains DNA from the actinorhodin biosynthetic gene cluster encoding the gene for a specific activator protein, ActII-orf4. The Act II-orf4 protein is required for transcription of the genes placed under the control of the actI/ act II bidirectional promoter and activates gene expression during the

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transition from growth to stationary phase in the vegetative mycelium (Hallam, S. E. et al. Gene (1988) 74:305-320).

Type II clusters in *Streptomyces* are known to be activated by pathway-specific activator genes (Narva, K. E. and Feitelson, J. S. J. Bacteriol. (1990) 172:326-333; Stutzman-Engwall, K. J. et al. J. Bacteriol. (1992) 174:144-154; Fernandez-Moreno, M.A. et al. Cell (1991) 66:769-780; Takano, E. et al. Mol. Microbiol. (1992) 6:2797-2804; Takano, E. et al. Mol. Microbiol. (1992) 7:837-845), The DnrI gene product complements a mutation in the *actII-orf4* gene of *S. coelicolor*, implying that DnrI and ActII-orf4 proteins act on similar targets. A gene (*srmR*) has been described (EP 0 524 832 A2) that is located near the Type I PKS gene cluster for the macrolide polyketide spiramycin. This gene specifically activates the production of the macrolide antibiotic spiramycin, but no other examples have been found of such a gene. Also, no homologues of the ActII-orf4/DnrI/RedD family of activators have been described that act on Type I PKS genes.

Although large numbers of therapeutically important polyketides have been identified, there remains a need to obtain novel polyketides that have enhanced properties or possess completely novel bioactivity. The complex

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polyketides produced by Type I PKSs are particularly valuable, in that they include compounds with known utility as anthelmintics, insecticides, immunosuppressants, antifungal or antibacterial agents.

Because of their structural complexity, such novel polyketides are not readily obtainable by total chemical synthesis, or by chemical modifications of known polyketides.

There is also a need to develop reliable and specific ways of deploying individual modules in practice so that all, or a large fraction, of hybrid PKS genes that are constructed, are viable and produce the desired polyketide product.

Pending International Patent Application number

WO 98/01546 discloses that a PKS gene assembly (particularly of Type I) encodes a loading module which is followed by at least one extension module. Thus Figure 1 shows the organisation of the DEBS genes. The first open reading frame encodes the first multi-enzyme or cassette (DEBS 1) which consists of three modules: the loading module (ery-load) and two extension modules (modules 1 and 2). The loading module comprises an acyltransferase and an acyl carrier protein. This may be contrasted with Fig. 1 of WO 93/13663 (referred to above). This shows ORF1 to consist of only two modules,

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the first of which is in fact both the loading module and the first extension module.

WO 98/01546 describes in general terms the production of a hybrid PKS gene assembly comprising a loading module and at least one extension module.

5 WO 98/01546 also describes (see also Marsden, A. F. A. et al. Science (1998) 279:199-202) construction of a hybrid PKS gene assembly by grafting the wide-specificity loading module for the avermectin-producing polyketide synthase onto the first multienzyme component (DEBS 1) for the erythromycin PKS in place of the normal loading
10 module. Certain novel polyketides can be prepared using the hybrid PKS gene assembly, as described for example in pending International Patent Application number WO 98/01571. Patent Application WO 98/01546
15 further describes the construction of a hybrid PKS gene assembly by grafting the loading module for the rapamycin-producing polyketide synthase onto the first multienzyme component (DEBS 1) for the erythromycin PKS in place of the normal loading module. The loading
20 module of the rapamycin PKS differs from the loading modules of DEBS and the avermectin PKS in that it comprises a CoA ligase domain, an enoylreductase ("ER") domain and an ACP, so that suitable organic acids including the natural starter unit 3,4-

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dihydroxycyclohexane carboxylic acid may be activated in situ on the PKS loading domain, and with or without reduction by the ER domain transferred to the ACP for intramolecular loading of the KS of extension module 1 (Schwecke, T. et al. Proc. Natl. Acad. Sci. USA (1995) 92:7839-7843).

The DNA sequences have been disclosed for several Type I PKS gene clusters that govern the production of 16-membered macrolide polyketides, including the tylosin PKS from *Streptomyces fradiae* (EP 0 791 655 A2), the niddamycin PKS from *Streptomyces caelestis* (Kavakas, S. J. et al. J. Bacteriol. (1998) 179:7515-7522) and the spiramycin PKS from *Streptomyces ambofaciens* (EP 0791 655 A2). All of these gene sequences have in common that they show the loading module of the PKS to differ from the loading module of DEBS and of the avermectin PKS in that they consist of a domain resembling the KS domains of the extension modules, an AT domain and an ACP (Figure 3). The additional N-terminal KS-like domain has been named KSq because it differs in each case from an extension KS by the specific replacement of the active site cysteine residue essential for β -ketoacyl-ACP synthase activity by a glutamine (Q in single letter notation) residue. The function of the KSq domain is unknown (Kavakas, S. J. et al. J. Bacteriol. (1998)

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179:7515-7522), but its presence in these PKSs for 16-membered macrolides is surprising because the starter units of tylosin, niddamycin and spiramycin appear to be propionate, acetate and acetate respectively, that is, the same type of starter unit as in DEBS. The AT
5 adjacent to the KSq domain is named here the ATq domain.

When the entire loading module of the tylosin PKS was used to replace the analogous loading module in the spiramycin PKS in *S. ambofaciens* (Kuhstoss et al. Gene (1996) 183:231-236), the nature of the starting unit was
10 stated to be altered from acetate to propionate. Since the role of the KSq domain was not understood, no specific disclosure was made that revealed either the importance of the KSq domain, or the possible utility of these KSq-containing loading modules in ensuring the
15 purity of the polyketide product in respect of the starter unit, even at high levels of macrolide production. The interpretation for their results was stated as: "Therefore we believe that the experiments described here provide strong experimental support for
20 the hypothesis that the AT domains in Type I PKS systems select the appropriate substrate at each step in synthesis" (Kuhstoss et al. Gene (1996) 183:231-236, at p. 235). These authors noted the analogy with the CLF protein in Type II PKS systems and that the latter

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protein is thought to be involved in determining the chain length. They state: "KSq may serve a similar function, although it is unclear why such a function would be necessary in the synthesis of these 16-membered polyketides when it is not needed for the synthesis of other complex polyketides such as 6-DEB or rapamycin. In any case the KSq is unlikely to be involved in substrate choice at each step of synthesis." (Kuhstoss et al. Gene (1996) 183:231-236).

It has been shown that when genetic engineering is used to remove the loading module of DEBS, the resulting truncated DEBS in *S. erythraea* continues to produce low levels of erythromycins containing a propionate starter unit (Pereda, A. et al. Microbiology (1995) 144:543-553). The same publication shows that when in this truncated DEBS the methylmalonyl-CoA -specific AT of extension module 1 was replaced by a malonyl-CoA-specific AT from an extension module of the rapamycin PKS, the products were also low levels of erythromycins containing a propionate starter unit, demonstrating that the origin of the starter units is not decarboxylation of the (methyl)malonyl groups loaded onto the enzyme by the AT of module 1, but from direct acylation of the KS of extension module 1 by propionyl-CoA. This is in contrast to a previous report, using partially purified DEBS1+TE,

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a truncated bimodular PKS derived from DEBS (Kao, C. M. et al. J. Am. Chem. Soc. (1995) 117:9105-9106) and functionally equivalent to DEBS1-TE (Brown, M. J. B. et al., J. Chem. Soc. Chem. Commun. (1995) 1517-1518; Cortés, J. et al. Science (1991) 2523:675-679), which
5 stated that the origins of the starter units for DEBS can include methylmalonate units which are loaded onto module 1 and are decarboxylated by the KS of module 1 (Pieper, R. et al. Biochemistry (1997) 36:1846-1851). It has now been found that when the DEBS1-TE protein is fully
10 purified from extracts of recombinant *S. erythraea* it contains no such specific decarboxylase activity (Weissmann, K. et al. Biochemistry, (1998) 37, 11012-11017), further confirming that starter units do not in fact arise from decarboxylation of extension units
15 mediated by the KS of extension module 1 .

It is known that the DEBS loading module has a slightly broader specificity than propionate only, and in particular acetate starter units are used both in vitro and in vivo, when the PKS containing this loading module
20 is part of a PKS that is expressed either in *S. erythraea* the natural host for erythromycin production (see for example Cortés, J. et al. Science (1995) 268:1487-1489), or in a heterologous host such as *S. coelicolor* (Kao, C. M. et al. J. Am. Chem. Soc. (1994) 116:11612-11613;

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Brown, M. J. B. et al. J. Chem. Soc. Chem. Commun. (1995) 1517-1519). In vitro experiments using purified DEBS1-TE have demonstrated that propionyl-CoA and acetyl-CoA are alternative substrates that efficiently supply propionate and acetate units respectively to the loading module

5 (Wiessmann, K. E. H. et al. Chemistry and Biology (1995) 2:583-589; Pieper, R. et al. J. Am. Chem. Soc. (1995)

117:11373-11374). The outcome of the competition between acetate and propionate starter units is influenced by the respective intracellular concentrations of propionyl-CoA

10 and acetyl-CoA prevailing in the host cell used (see for example Kao, C. M. et al. Science (1994) 265:509-512;

Pereda, A. et al. Microbiology (1995) 144:543-553). It is also determined by the level of expression of the host PKS, so that as disclosed for example in Pending

15 International Patent Application number PCT/GB97/01819, when recombinant DEBS or another hybrid PKS containing the DEBS loading module is over-expressed in *S.*

erythraea, the products are generally mixtures whose components differ only in the presence of either an

20 acetate or a propionate starter unit.

There is a need to develop reliable methods for avoiding the formation of mixtures of polyketides with both acetate and propionate starter units, and to allow the specific incorporation of unusual starter units. It

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has now been found, surprisingly, that the role of the loading domains in the PKSs for the 16-membered macrolides tylosin, niddamycin and spiramycin is different from that of the loading domains of the avermectin PKS and of DEBS. It has been realised that the KSq domain of the tylosin PKS and the associated AT domain, which is named here ATq, together are responsible for the highly specific production of propionate starter units because the ATq is specific for the loading of methylmalonyl-CoA and not propionyl-CoA as previously thought; and the KSq is responsible for the highly specific decarboxylation of the enzyme-bound methylmalonate unit to form propionate unit attached to the ACP domain of the loading module and appropriately placed to be transferred to the KS of extension module 1 for the initiation of chain extension. In a like manner the ATq of the spiramycin and niddamycin PKSs, and the adjacent KSq, are responsible for the specific loading of malonate units rather than acetate units as previously believed, and for their subsequent specific decarboxylation to provide acetate starter units for polyketide chain extension.

It has also now been found here that not only the PKSs for the above-mentioned 16-membered macrolides, but also the PKSs for certain 14-membered macrolides

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particularly the oleandomycin PKS from *Streptomyces*
antibioticus (Figure 4) and also the PKSs for certain
polyether ionophore polyketides particularly the putative
monensin PKS from *Streptomyces cinnamonensis* (Figure 4),
possess a loading domain comprising a KSq domain, an ATq
5 domain, and an ACP. In Figure 4 is shown a sequence
alignment of the KSq domains and of the adjacent linked
ATq domains that have been identified, showing the
conserved active site glutamine (Q) residue in the KSq
domains, and an arginine residue which is conserved in
10 all extension AT domains and is also completely conserved
in ATq domains. This residue is characteristically not
arginine in the AT domains of either DEBS or of the
avermectin PKS loading modules, where the substrate for
the AT is a non-carboxylated acyl-CoA ester (Haydock, S.
15 F. et al. FEBS Letters (1995) 374:246-248) . The
abbreviation ATq is used here to simply to distinguish
the AT domains found immediately C-terminal of Ksq from
extension ATs, and the label has no other significance.

In one aspect the invention provides a PKS
20 multienzyme or part thereof, or nucleic acid (generally
DNA) encoding it, said multienzyme or part comprising a
loading module and a plurality of extension modules,
wherein

(a) the loading module is adapted to load a malonyl

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or substituted malonyl residue and then to effect decarboxylation of the loaded residue to provide an acetyl or substituted acetyl (which term encompasses propionyl) residue for transfer to an extension module; and

5 (b) the extension modules, or at least one thereof (preferably at least the one adjacent the loading module), are not naturally associated with a loading module that effects decarboxylation of an optionally substituted malonyl residue.

10 Generally the loading module will also include an ACP (acyl carrier protein) domain.

Preferably the decarboxylating functionality of the loading module is provided by a KS (ketosynthase)-type domain. Suitably this differs from a KS of a
15 conventional extension module by possessing a glutamine residue in place of the essential cysteine residue in the active site. It is termed Ksq. It may be "natural" or genetically engineered, e.g. resulting from site-directed mutagenesis of nucleic acid encoding a different KS such
20 as a KS of an extension module.

Alternatively the decarboxylating functionality can be provided by a CLF-type domain of the general type occurring in Type II PKS systems.

Preferably the loading functionality is provided by

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an AT (acyltransferase)-type domain which resembles an AT domain of a conventional extension module in having an arginine residue in the active site, which is not the case with the AT domains of loader modules which load acetate or propionate, e.g. in DEBS or avermectin PKS systems. It may be termed Atq. Once again, it may be "natural" or genetically engineered, e.g. by mutagenesis of an AT of an extension module.

Usually the loading module will be of the form:

10 Ksq-ATq-ACP

where ACP is acyl carrier protein.

In one aspect the invention provides a polyketide synthase which comprises a loading module and a plurality of extension modules wherein: said loading module loads an optionally substituted malonyl and then effects decarboxylation of the loaded moiety to provide a corresponding optionally substituted acetyl moiety for transfer to the first of the extension modules and wherein the loading module is of the form:

(decarbox) - (AT) - (ACP) wherein: (ACP) is an acyl carrier protein domain, (AT) is an acyltransferase domain which has an arginine residue in the active site and loads an optionally substituted malonyl; and (decarbox) is a domain which effects decarboxylation of a loaded optionally substituted malonyl and

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is selected from (i) a ketosynthase-type domain herein designated KSq which differs from a KS domain of an extension module by having a glutamine residue in place of a cysteine in the active site: and ii) chain length factor (CLF), and b) at least the first of said extension modules is not naturally associated with a loading module that effects decarboxylation of an optionally substituted malonyl, with the proviso that 1) the synthase is not composed of the loading module of the tylosin polyketide synthase coupled to the spiramycin polyketide synthase minus its natural loading module, and 2) the polyketide produced by the polyketide synthase is not a 14-membered macrolide having a 13-methyl group due to incorporation of an unsubstituted acetate starter.

15 In another aspect the invention provides a method of synthesising a polyketide having substantially exclusively a desired starter unit by providing a PKS multienzyme incorporating a loading module as defined above which specifically provides the desired starter unit. This may 20 comprise providing nucleic acid encoding the multienzyme and introducing it into an organism where it can be expressed.

 In another aspect, the invention provides a process for producing a polyketide which comprises culturing a transformed

- 19(b) -

cell described herein and recovering the polyketide.

In another aspect, the invention provides a process for producing a type II polyketide comprising culturing an
5 organism containing a type II polyketide synthase ("PKS") wherein a) the wild type PKS comprises a CLF domain which effects decarboxylation to produce an undesired starter; and
(b) said wild type PKS has been genetically engineered to suppress the decarboxylating activity of the CLF by mutating
10 the active site glutamine residue to an alanine residue.

In further aspects the invention provides vectors and transformant organisms and cultures containing nucleic acid encoding the multienzyme. A preferred embodiment is a culture which produces a polyketide having a desired starter unit
15 characterised by the substantial absence of polyketides with different starter

-20-

units. Thus, for example, erythromycin can be produced substantially free from analogues resulting from the incorporation of acetate starter units in place of propionate.

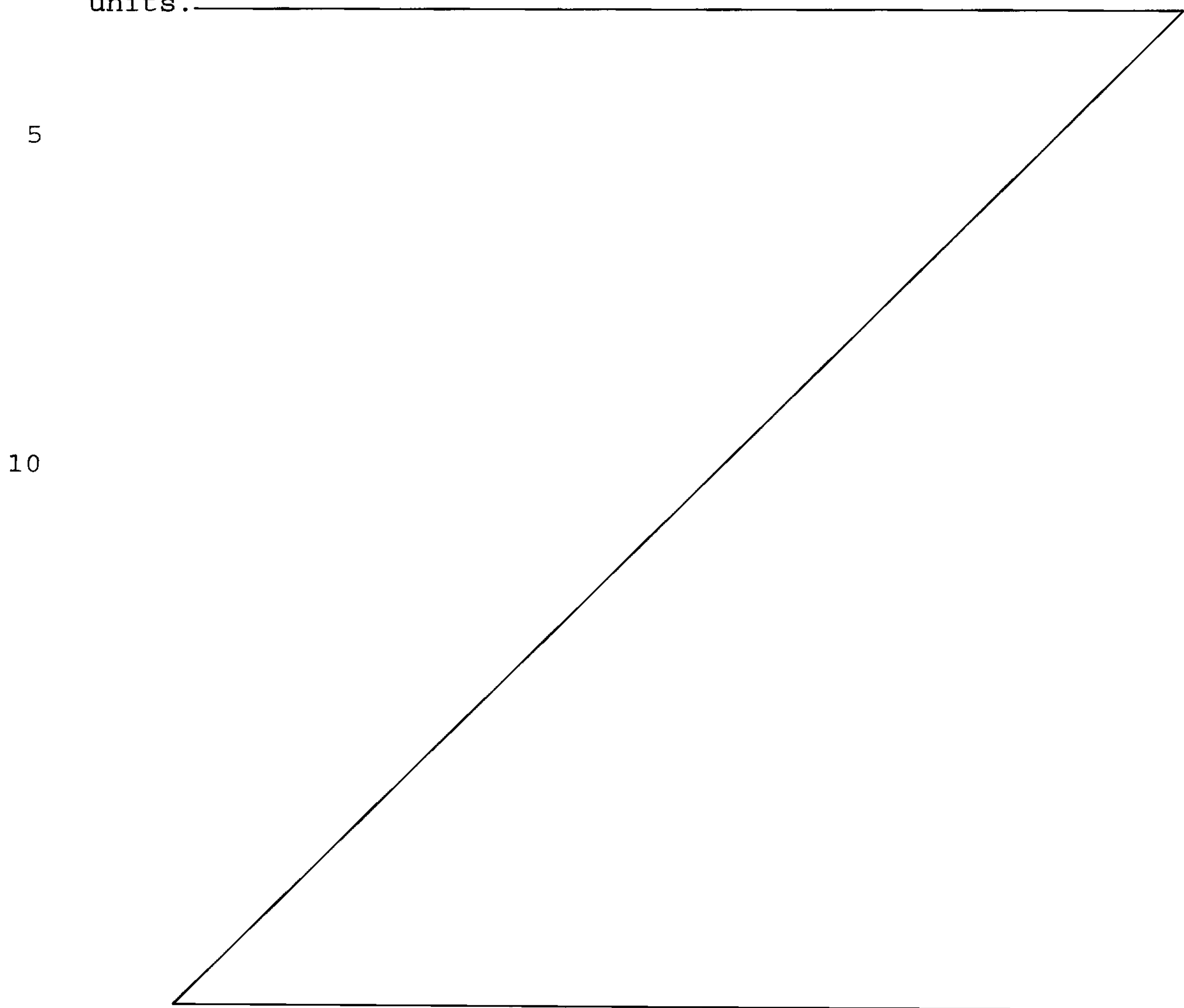
5 In yet another aspect, the invention provides nucleic acid encoding the polyketide synthase, a vector containing the nucleic acid or a transformed cell comprising the nucleic acid, as described herein.

10 Preferably the hybrid PKS encodes a loading module and from 2 to 7 extension modules and a chain terminating enzyme (generally a thioesterase).

It is particularly useful to provide a loading module of the type KSq - ATq-ACP for a PKS gene assembly which produces a 12-, 14- or 16-membered macrolide in order to prepare a 12-,
15 14- or 16-membered macrolide which contains exclusively or almost exclusively an acetate starter unit, even when such PKS gene assembly is expressed at high levels in an actinomycete host cell. Particularly suitable PKSs for this purpose are the components of PKSs for the biosynthesis of erythromycin,
20 methymycin, oleandomycin, tylosin, spiramycin, midecamycin, and niddamycin for all of which the gene and modular organisation is known at least in part. Particularly suitable sources of the genes encoding a loading module of the type KSq - ATq-ACP are the loading modules of oleandomycin, spiramycin, niddamycin,
25 methymycin and monensin which are specific for the loading of

- 20(a) -

malonate units which are then decarboxylated to acetate starter
units.



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Similarly it is particularly useful to provide a loading module of the type KSq - ATq-ACP for a PKS gene assembly which produces the macrolides rifamycin, avermectin, rapamycin, immunomycin and FK506 whose loading modules possess an unusual specificity (and for all of which the gene and modular organisation is known at least in part) in order to prepare such macrolides containing exclusively or almost exclusively an acetate starter unit, even when such PKS gene assembly is expressed at high levels in an actinomycete host cell. Particularly suitable sources of the genes encoding a loading module of the type KSq - ATq-ACP are the loading modules of oleandomycin, spiramycin, niddamycin, methymycin and monensin which are specific for the loading of malonate units which are decarboxylated to acetate starter units.

It is similarly useful to provide a loading module of the type KSq - ATq-ACP for a PKS gene assembly which produces a 12-, 14- or 16-membered macrolide in order to prepare a 12-, 14- or 16-membered macrolide which contains exclusively or almost exclusively a propionate starter unit, even when such PKS gene assembly is expressed at high levels in an actinomycete host cell. Particularly suitable PKSs for this purpose are the components of PKSs for the biosynthesis of erythromycin,

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methymycin, oleandomycin, tylosin, spiramycin,
midecamycin, and niddamycin for all of which the gene and
modular organisation is known at least in part. A
particularly suitable source of the genes encoding a
loading module of the type KSq-ATq-ACP is the loading
5 module of tylosin which is specific for the loading of
methylmalonate units which are decarboxylated to
propionate starter units.

Similarly it is particularly useful to provide a
loading module of the type KSq - ATq-ACP for a PKS gene
10 assembly which produces the macrolides rifamycin,
avermectin, rapamycin, immunomycin and FK506 whose
loading modules possess an unusual specificity (and for
all of which the gene and modular organisation is known
at least in part) in order to prepare such macrolides
15 containing exclusively or almost exclusively a propionate
starter unit, even when such PKS gene assembly is
expressed at high levels in an actinomycete host cell. A
particularly suitable source of the genes encoding a
loading module of the type KSq - ATq-ACP is the loading
20 module of tylosin which is specific for the loading of
methylmalonate units which are decarboxylated to
propionate starter units.

In the loading module of the type KSq - ATq-ACP the
domains or portions of them may be derived from the same

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or from different sources, and comprise either natural or engineered domains. For example the ATq domain can be replaced by an AT domain derived from any extension module of a Type I PKS, having specificity either for loading of malonate units or for loading of methylmalonate units respectively, so long as the KSq domain is chosen to have a matching specificity towards either methylmalonate or malonate units respectively.

Alternatively, the KSq domain in the loading module provided of the type KSq - ATq-ACP may be substituted by the CLF polypeptide of a Type II PKS. It is now apparent that in contrast to its previous identification as a factor uniquely determining chain length, the CLF, in addition to any other activities that it may possess, is the analogue of the KSq domain and can act as a decarboxylase towards bound malonate units.

The appreciation that the CLF domain of Type II PKS's has decarboxylating activity has led us to devise useful interventions in Type II systems, e.g. to enhance the yields obtainable in some fermentations. Many high-yielding industrial fermentations tend to give mixtures, owing to the incorporation of undesired starters. This is particularly the case in systems which have auxiliary genes for generating unusual starters. CLF genes may act to produce undesired acyl species, leading to products

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incorporating the undesired acyl units.

For example the production of oxytetracycline involves an unusual malonamido starter. However the undesired activity of a CLF domain causes some decarboxylation, leading to the incorporation of acetyl instead. Daunomycin synthesis likewise involves an unusual starter which is liable to the "parasitic" activity of a CLF domain.

The active site (for decarboxylation) of a CLF domain generally includes a glutamine residue. We find that the decarboxylating activity of the domain can be removed by a mutation by which the Gln residue is converted into (for example) Ala.

Thus in a further aspect the invention provides a system and process for synthesis of a type II (aromatic) polyketide, in which a gln residue of a CLF domain of the type II PKS is mutated to suppress decarboxylation activity. Techniques of site-specific mutagenesis by which this can be achieved are by now well known to those skilled in the art.

The loading module of the type KSq - ATq-ACP may be linked to a hybrid PKS produced for example as in WO 98/01546 and WO 98/01571. It is particularly useful to link such a loading module to gene assemblies that encode hybrid PKSs that produce novel derivatives of

- 25 -

14-membered macrolides as described for example in
WO 98/01546 and WO 98/01571:

The invention further provides such PKS assemblies
furnished with a loading module of the type KSq - ATq-
ACP, vectors containing such assemblies, and transformant
5 organisms that can express them. Transformant organisms
may harbour recombinant plasmids, or the plasmids may
integrate. A plasmid with an *int* sequence will integrate
into a specific attachment site (*att*) of the host's
chromosome. Transformant organisms may be capable of
10 modifying the initial products, eg by carrying out all or
some of the biosynthetic modifications normal in the
production of erythromycins (as shown in Figure 5) and
for other polyketides. Use may be made of mutant
organisms such that some of the normal pathways are
15 blocked, e.g. to produce products without one or more
"natural" hydroxy-groups or sugar groups. The invention
further provides novel polyketides as producible,
directly or indirectly, by transformant organisms. This
includes polyketides which have undergone enzymatic
20 modification.

In a further aspect the invention provides both
previously-obtained polyketides and novel polyketides in
a purer form with respect to the nature of the starter
unit, than was hitherto possible. These include 12- ,

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14- and 16-membered ring macrolides which are either "natural" or may differ from the corresponding "natural" compound:

5 a) in the oxidation state of one or more of the ketide units (ie selection of alternatives from the group: -CO-, -CH(OH)-, alkene -CH-, and -CH₂-) where the stereochemistry of any -CH(OH)- is also independently selectable;

10 b) in the absence of a "natural" methyl side-chain; or

c) in the stereochemistry of "natural" methyl; and/or ring substituents other than methyl.

15 It is also possible to prepare derivatives of 12-, 14- and 16-membered ring macrolides having the differences from the natural product identified in two or more of items a) to c) above.

20 Derivatives of any of the afore-mentioned polyketides which have undergone further processing by non-PKS enzymes, eg one or more of hydroxylation, epoxidation, glycosylation and methylation may also be prepared.

The present invention provides a novel method of obtaining both known and novel complex polyketides

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without the formation of mixtures of products differing only in having either an acetate or a propionate starter unit. In addition the present invention provides a method to obtain novel polyketides in which the starter unit is an unusual starter unit which is derived by the action of a KSq domain on the enzyme-bound product of an AT of unusual specificity derived from an extension module of a natural Type I PKS. In particular the AT of extension module 4 of the FK506 PKS gene cluster preferentially incorporates an allyl side-chain; the AT of extension module 6 of the niddamycin PKS gene cluster preferentially incorporates a sidechain of structure HOCH₂-; and the ATs of extension module 5 of spiramycin and of extension module 5 of monensin incorporate an ethyl side chain. In each case the KSq domain is preferentially one that is naturally propionate-specific. Alternatively, any KS from an extension module of a Type I PKS may be converted into a KSq domain capable of decarboxylating a bound carboxylated acyl thioester, by site-directed mutagenesis of the active site cysteine residue to replace it by another residue, preferably glutamine. It is known that the animal fatty acid synthase, which shares many mechanistic features with Type I PKS, in the absence of acetyl-CoA, has a demonstrable malonyl-CoA decarboxylase activity (Kresze,

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G. B. et al. Eur. J. Biochem. (1977) 79:191-199). When treated with an alkylating agent such as iodoacetamide the fatty acid synthase is inactivated by specific modification of the active site cysteine of the KS, and the resulting protein has an enhanced malonyl-CoA decarboxylase activity. The conversion of a fatty acid KS domain into a decarboxylase mirrors the genetically-determined change between the KS domains and the KSq domain in Type I PKSs. Indeed, the size and polarity characteristics of a glutamine side chain very closely approximate those of carboxamido-cysteine. The KSq to be used for decarboxylation of an unusual alkylmalonate unit is preferably selected from the same extension module of the same Type I PKS that provides the unusual AT, in order to optimise the decarboxylation of the unusual alkylmalonate, and the ACP to be used is preferably also the ACP of the same extension module.

Suitable plasmid vectors and genetically engineered cells suitable for expression of PKS genes incorporating an altered loading module are those described in PCT/GB97/01819 as being suitable for expression of hybrid PKS genes of Type I. Examples of effective hosts are *Saccharopolyspora erythraea*, *Streptomyces coelicolor*, *Streptomyces avermitilis*, *Streptomyces griseofuscus*, *Streptomyces cinnamonensis*, *Streptomyces fradiae*,

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Streptomyces longisporoflavus, *Streptomyces*
hygroscopicus, *Micromonospora griseorubida*, *Streptomyces*
lasaliensis, *Streptomyces venezuelae*, *Streptomyces*
antibioticus, *Streptomyces lividans*, *Streptomyces*
rimosus, *Streptomyces albus*, *Amycolatopsis mediterranei*,
5 and *Streptomyces tsukubaensis*. These include hosts in
which SCP2*-derived plasmids are known to replicate
autonomously, such as for example *S. coelicolor*, *S.*
avermitilis and *S. griseofuscus*; and other hosts such as
Saccharopolyspora erythraea in which SCP2*-derived
10 plasmids become integrated into the chromosome through
homologous recombination between sequences on the plasmid
insert and on the chromosome; and all such vectors which
are integratively transformed by suicide plasmid vectors.

Some embodiments of the invention will now be
15 described with reference to the accompanying drawings in
which:

Fig 1 is a diagram showing the functioning of 6-
deoxyerythronolide B synthase (DEBS), a modular PKS
producing 6-deoxyerythronolide B (6-DEB) a precursor of
20 erythromycin A.

Fig 2 gives the amino acid sequence comparison of
the KS domains and the CLF domains of representative Type
II PKS gene clusters. The active site Cysteine (C) of
the KS domains is arrowed in the Figure and aligns with

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the Glutamine (Q) or glutamic acid (E) of the CLF domains. The abbreviations used, and the relevant Genbank/EMBL accession numbers are: GRA: granaticin from *Streptomyces violaceoruber* (X63449); HIR: unknown polyketide from *Saccharopolyspora hirsuta* (M98258); ACT, 5 actinorhodin from *Streptomyces coelicolor* (X63449); CIN: unknown polyketide from *Streptomyces cinnamonensis* (Z11511); VNZ: jadomycin from *Streptomyces venezuelae* (L33245); NOG: anthracyclines from *Streptomyces nogalater* (Z48262); TCM: tetracenomycin from *S. glaucescens* 10 (M80674); DAU: daunomycin from *Streptomyces* sp. C5 (L34880); PEU, doxorubicin from *Streptomyces peucetius* (L35560); WHI: WhiE spore pigment from *Streptomyces coelicolor* (X55942).

Fig 3 shows the gene organisation of the PKSs for 15 three 16-membered ring macrolides, tylosin, spiramycin and niddamycin.

Fig 4 shows the amino acid sequence alignment of KSq-ATq loading didomains of the PKSs for niddamycin, platenolide(spiramycin), monensin, oleandomycin and 20 tylosin. The sequences for the monensin and oleandomycin loading didomains have not been previously disclosed.

Fig. 5 The enzymatic steps that convert 6-deoxyerythronolide B into erythromycin A in *Saccharopolyspora erythraea*

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Fig. 6 is a diagram showing the construction of plasmid pJLK117.

Fig. 7 shows the structures of two oligonucleotides.

The present invention will now be illustrated, but is not intended to be limited, by means of some examples.

5 All NMR spectra were measured in CDCl₃ using a Bruker 500MHz DMX spectrometer unless otherwise indicated and peak positions are expressed in parts per million (ppm) downfield from tetramethysilane. The atom number shown in the NMR structure is not representative of standard
10 nomenclature, but correlates NMR data to that particular example.

HPLC methods

Method 1

Column	Waters Symmetry 5_C18 2.1mm x 150mm
Flow	0.29 ml/min
Mobile phase	Gradient: A:B (22.78) to A:B (38:62) over 12 minutes, then to A:B (80:20) by minute 15. Maintain for 1 minute. Re-equilibrate before next sample. Where A = acetonitrile and B = 0.01M ammonium acetate in 10% acetonitrile and 0.02% TFA

Method B

Column	Waters Symmetry 5_ C18 2.1mm X 150mm
Flow	0.29 ml/min
Mobile phase	Gradient: 28:72 acetonitrile: 10mM NH ₄ OAc to 50:50 in 18 minutes. 50:50 until 25 minutes. back to 28:72, re-equilibrate for 7 minutes
Instrument	Acquired with Hewlett Packard 1100 LC/MS with APCI source

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Tap Water medium

glucose	5g/litre
tryptone	5g/litre
yeast extract	2.5g/litre
EDTA	36mg/litre
Tap water to 1L total volume	

ERY - P medium

dextrose	50g/litre
Nutrisoy TM flour	30g/litre
(NH ₄) ₂ SO ₄	3g/litre
NaCl	5g/litre
CaCO ₃	6g/litre
Tap water to 1L total volume	
pH adjusted to 7.0	

Example 1**Construction of the Recombinant Vector pPFL43**

5 Plasmid pCJR24 was prepared as described in
PCT/GB97/01819. pPFL43 is a pCJR24-based plasmid
containing the gene encoding a hybrid polyketide synthase
that contains the putative monensin PKS loading module
(isolated from *S. cinnamomensis*) the DEBS extension
10 modules 1 and 2 and the chain-terminating thioesterase.
Plasmid pPFL43 was constructed as follows:

The following synthetic oligonucleotides: 5'-

CCATATGGCCGCATCCGCGTCAGCGT-3' and 5'-

15 GGCTAGCGGGTCCTCGTCCGTGCCGAGGTCA-3'

are used to amplify the DNA encoding the putative
monensin-producing loading module using a cosmid that

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contains the 5' end of the putative monensin-producing
PKS genes from *S. cinnamomensis* or chromosomal DNA of *S.*
cinnamomensis as template. The PCR product of 3.3 kbp is
purified by gel electrophoresis, treated with T4
polynucleotide kinase and ligated to plasmid pUC18, which
5 has been linearised by digestion with *Sma* I and then
treated with alkaline phosphatase. The ligation mixture
was used to transform electrocompetent *E.coli* DH10B cells
and individual clones were checked for the desired
plasmid pPFL40. Plasmid pPFL40 was identified by
10 restriction pattern and sequence analysis.

Plasmid pHD30His is a derivative of pNEWAVETE
(PCT/GB97/01810) which contains the avermectin loading
module, erythromycin extension modules 1 and 2 and the
ery thioesterase domain. Plasmid pNEWAVETE was cut with
15 *Eco*RI and *Hin*DIII and a synthetic oligonucleotide linker
was inserted that encodes the addition of a C-terminal
polyhistidine tail to the polypeptide. The following
oligonucleotides:

5'-AATTCACATCACCATCACCATCACTAGTAGGAGGTCTGGCCATCTAGA-3'

20 and

5'-AGCTTCTAGATGGCCAGACCTCCTACTAGTGATGGTGATGGTGATGTG-3'

were annealed together and the duplex was ligated to
*Eco*RI-and *Hin*DIII-cut pNEWAVETE. The resulting plasmid
was cut with *Nde*I and *Xba*I and ligated into plasmid

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pCJR24 that had been previously cut with same two enzymes, to produce plasmid pND30His.

Plasmid pPFL40 was digested with Nde I and Nhe I and the 3.3 kbp fragment was purified by gel electrophoresis and ligated to pND30-His previously digested with Nde I and Nhe I and treated with alkaline phosphatase. The ligation mixture was used to transform electrocompetent *E.coli* DH10B cells and individual clones were checked for the desired plasmid pPFL43. Plasmid pPFL43 was identified by restriction analysis.

10 Example 2

Construction of *S. erythraea* JC2/ pPFL43

Plasmid pPFL43 was used to transform *S.erythraea* JC2 protoplasts. The construction of strain JC2 from which the resident DEBS genes are substantially deleted is given in Pending Patent Application PCT/GB97/01819. Thiostrepton resistant colonies were selected in R2T20 medium containing 10 µg/ml of thiostrepton. Several clones were tested for the presence of pPFL43 integrated into the chromosome by Southern blot hybridisation of their genomic DNA with DIG-labelled DNA containing the mon PKS fragment encoding for the loading module.

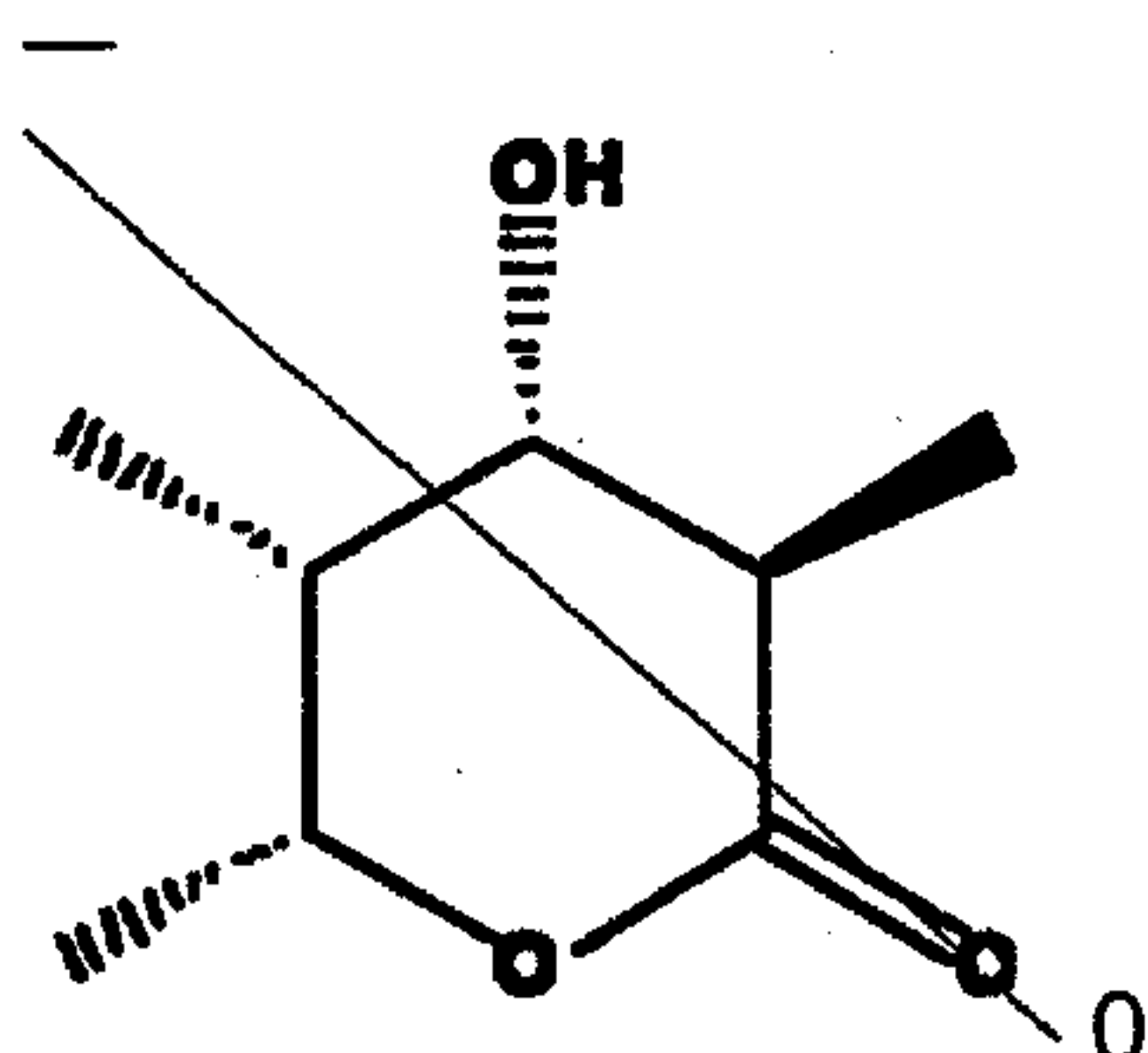
20 Example 3

Production of polyketides using *S. erythraea* JC2/pPFL43

A frozen suspension of strain *S. erythraea* JC2/

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pPFL43 was inoculated in eryP medium, containing 5 μ g/ml of thiostrepton. The inoculated culture was allowed to grow for seven days at 28-30°C. After this time the broth was filtered to remove mycelia and the pH adjusted to pH=3.0. The broth was extracted twice with two
5 volumes of ethyl acetate and the combined extracts were washed with an equal volume of saturated sodium chloride, dried over anhydrous sodium sulphate, and the ethyl acetate was removed under reduced pressure, to give crude product. The product was shown to have the structure
10 shown below, and by MS, GC-MS and ^1H NMR was found to be identical to an authentic sample.



Example 4

15 Construction of *S. erythraea* NRRL2338/pPFL43

Plasmid pPFL43 was used to transform *S. erythraea* NRRL2338 protoplasts. Thiostrepton resistant colonies

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were selected in R2T20 medium containing 10 µg/ml of thiostrepton. Several clones were tested for the presence of pPFL43 integrated into the chromosome by Southern blot hybridisation of their genomic DNA with DIG-labelled DNA containing the *mon* PKS fragment encoding for the loading module. A clone with an integrated copy of pPFL43 was selected in this way.

Example 5a

Production of 13-methyl-erythromycin A and B using *Sacch. erythraea* NRRL 2338/pPFL43

10 The culture *Saccharopolyspora erythraea* NRRL2338(pPFL43), constructed with the wild-type loading domain displaced by a monensin loader-D1TE DNA insert, produced as described in Example 2, was inoculated into 30ml Tap Water medium with 50 ug/ml thiostrepton in a 15 300ml Erlenmeyer flask. After three days incubation at 29°C, this flask was used to inoculate 300 ml of ERY-P medium in a 300 ml flask. The broth was incubated at 29°C at 200 rpm for 6 days. After this time, the whole broth was adjusted to pH 8.5 with NaOH, then extracted 20 with equal volume of ethyl acetate. The ethyl acetate extract was evaporated to dryness at 45°C under a nitrogen stream using a Zymark TurboVap LV Evaporator, then reconstituted in 0.0625 volumes methanol to concentrate the extract 16-fold. The structures of the 25 products were confirmed by LC/MS, Method A. A 4.0 min

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retention time peak was observed as the major component, with m/z value of 720 $(M+H)^+$, required for 13-methyl-erythromycin A. A second peak was observed with a retention time of 6.4 min and with m/z value of 704 $(M+H)^+$, required for 13-methyl-erythromycin B.

5

Example 5bProduction and Recovery of 13-methyl-erythromycin A and B using *Sacch. erythraea* NRRL-2338 (pPFL43) at 8L scale

Saccharopolyspora erythraea NRRL2338 (pPFL43) was inoculated into 1000mls Tap Water medium with 50 ug/ml thiostrepton in a 2.8l Fernbach flask. After three days incubation at 29°C, this flask was used to inoculate 8l of ERY-P medium in a 14l Microferm fermentor jar (New Brunswick Scientific Co., Inc., Edison, NJ). The broth was incubated at 28°C with an aeration rate of 8l/min, stirring at 800 rpm and with pH maintained between 6.9 and 7.3 with NaOH or H₂SO₄ (15%). Water was added to maintain volume at the 24 hour volume level. The fermentation was continued for 167 hours. After this time, presence of 13-methyl- erythromycin A and B were confirmed by adjusting a broth sample from the fermentor to pH 8.5 with NaOH, then extracting with equal volume of ethyl acetate. The ethyl acetate extract was evaporated to dryness at 45°C under a nitrogen stream using a Zymark TurboVap LV Evaporator, then reconstituted in 0.25

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volumes methanol to concentrate the extract 4-fold. The structures of the products were confirmed by LC/MS, Method A. A 4.1 min retention time peak was observed as the major component, with m/z value of 720 $(M+H)^+$, required for 13-methyl-erythromycin A. A second peak was
5 observed with a retention time of 6.6 min and with m/z value of 704 $(M+H)^+$, required for 13-methyl-erythromycin B.

About 35 liters of broth containing approximately 2.8 grams of 13-methyl- erythromycin A were processed for
10 recovery of product. Broth was filtered through a pilot sized Ceraflo ceramic unit and loaded onto a 500ml XAD-16 resin column. The product was eluted using 100% methanol. A 175ml CG-161 adsorption column was prepared and equilibrated with 20% methanol/water. A portion of the
15 product solution was adjusted to 20% methanol and loaded onto the column, no breakthrough of product was observed. Washing of the column with up to 40% methanol/water failed at removing any significant level of impurities. Elution
20 with 50% methanol/water achieved chromatographic separation of the product from the two major impurities, 13-methyl-erythromycin B and a degradation product, 13-methyl-dehydroerythromycin A. The purest cuts were combined and reduced in volume by approximately 75% using evaporation to achieve <10% methanol concentration. To enhance 13-methyl-
25 erythromycin A extraction, solid sodium bicarbonate was added until a total concentration of 250mM was obtained.

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The aqueous product layer was extracted 2x with methylene chloride, using one-half the total volume each time. The volume was reduced to light yellow solids by evaporation. The 13-methyl-erythromycin A was purified by dissolving the crude crystals into methylene chloride at ambient temperature and diluting to 15% methylene chloride with hexane. The cloudy solution is placed at -10°C for ~30 minutes when the liquid is decanted to a 2nd flask, leaving the majority of impurities behind as an oil. The flask is left overnight at -10°C, followed by filtration of off-white 13-methyl-erythromycin A crystals the next day. Approximately 300 milligrams of 13-methyl-erythromycin A were isolated from the partial work-up of the 35l broth volume.

Approximately 100 grams of evaporated mother liquor were utilized further to isolate 13-methyl-erythromycin B. Residual 13-methyl-erythromycin A was removed with repetitive extraction of the initial sample with aqueous acetic acid (pH 5). The subsequent methylene chloride layer was chromatographed on 700 g of silica gel using 20% methanol in methylene chloride. The 13-methyl-erythromycin B enriched fractions, as determined by LC/MS, were combined and evaporated to yield ~11.0 grams of dark oil. The oil was dissolved in a minimal amount of methanol and loaded onto 500 ml of Amberchrom CG-161 resin. The 13-methyl-erythromycin B was eluted at 2 bed volumes per hour with 40% methanol in deionized water. One bed volume fractions

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were collected and assayed by LC/MS. Fractions 42 through 62 were combined, diluted to ~20% methanol with deionized water, and neutralized to pH 7.5 with sodium bicarbonate. The resulting solution was extracted once with 4l of methylene chloride, concentrated to ~500 ml, and dried over anhydrous magnesium sulfate. After removal of the MgSO₄ by filtration the filtrate was evaporated to give ~110 mg of light brown solids. The 110 mg of crude 13-methyl-erythromycin B was dissolved in ~ 3.0 milliliters of HPLC grade acetonitrile and loaded onto a 20cm x 20cm, 2mm thick, silica gel preparative thin layer chromatography (PTLC) plate. The plate was developed with 60:40 methanol:acetonitrile. The desired portion of silica from the PTLC plate (iodine visualisation) was removed and extracted with HPLC grade acetone. The acetone extract was evaporated to give 12.1 mg of clear solid.

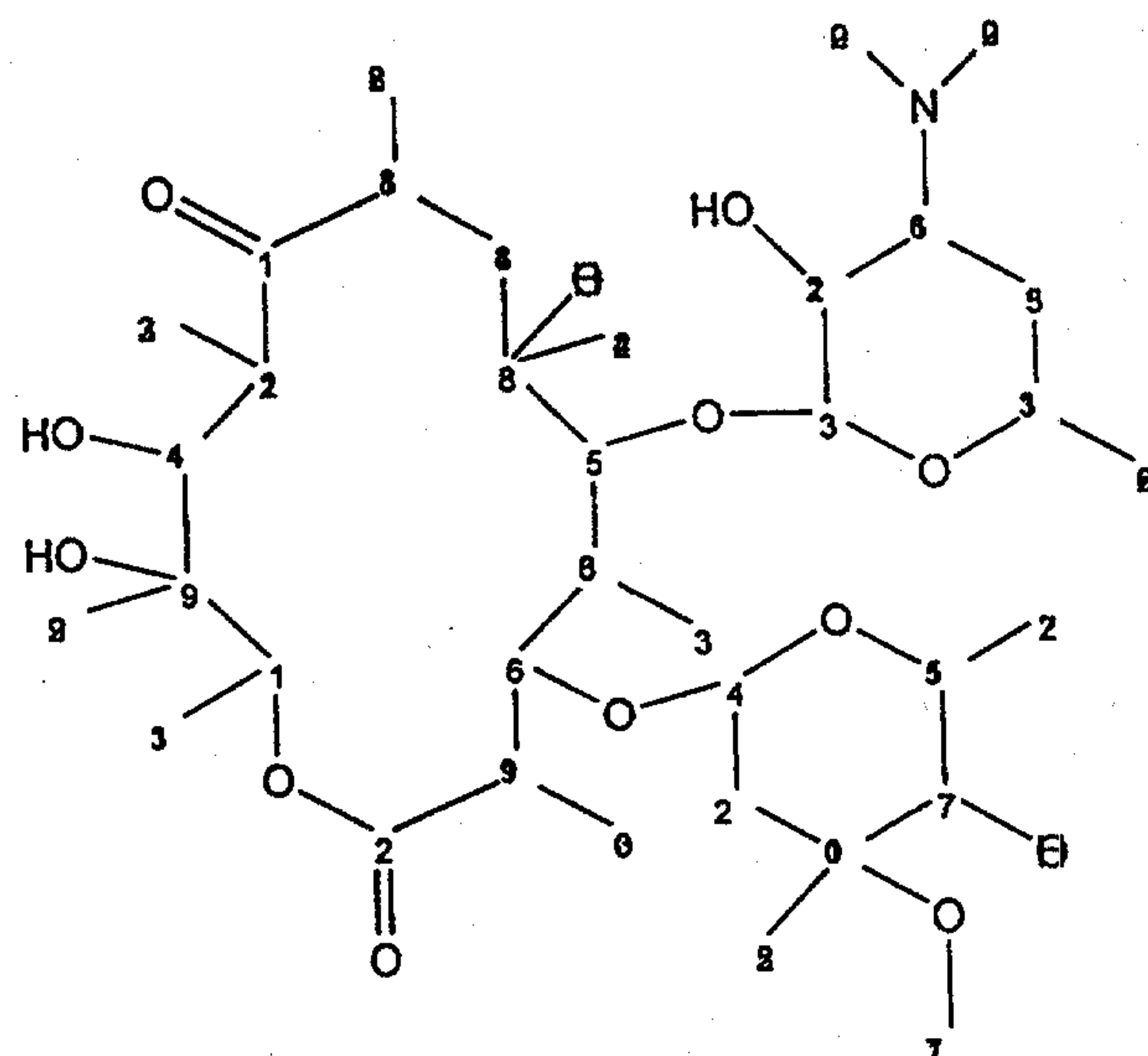
Identification of the 13-methyl-erythromycin A and 13-methyl-erythromycin B samples were confirmed by mass spectroscopy (LC/MS Method B) and NMR spectroscopy. The 13-methyl-erythromycin A sample peak had a 4.7 min retention time, with m/z value of 720 (M+H)⁺, required for 13-methyl-erythromycin A. The 13-methyl-erythromycin B sample peak had a 7.6 min retention time, with m/z value of 704 (M+H)⁺, required for 13-methyl-erythromycin B.

NMR, 13-methyl-erythromycin A:

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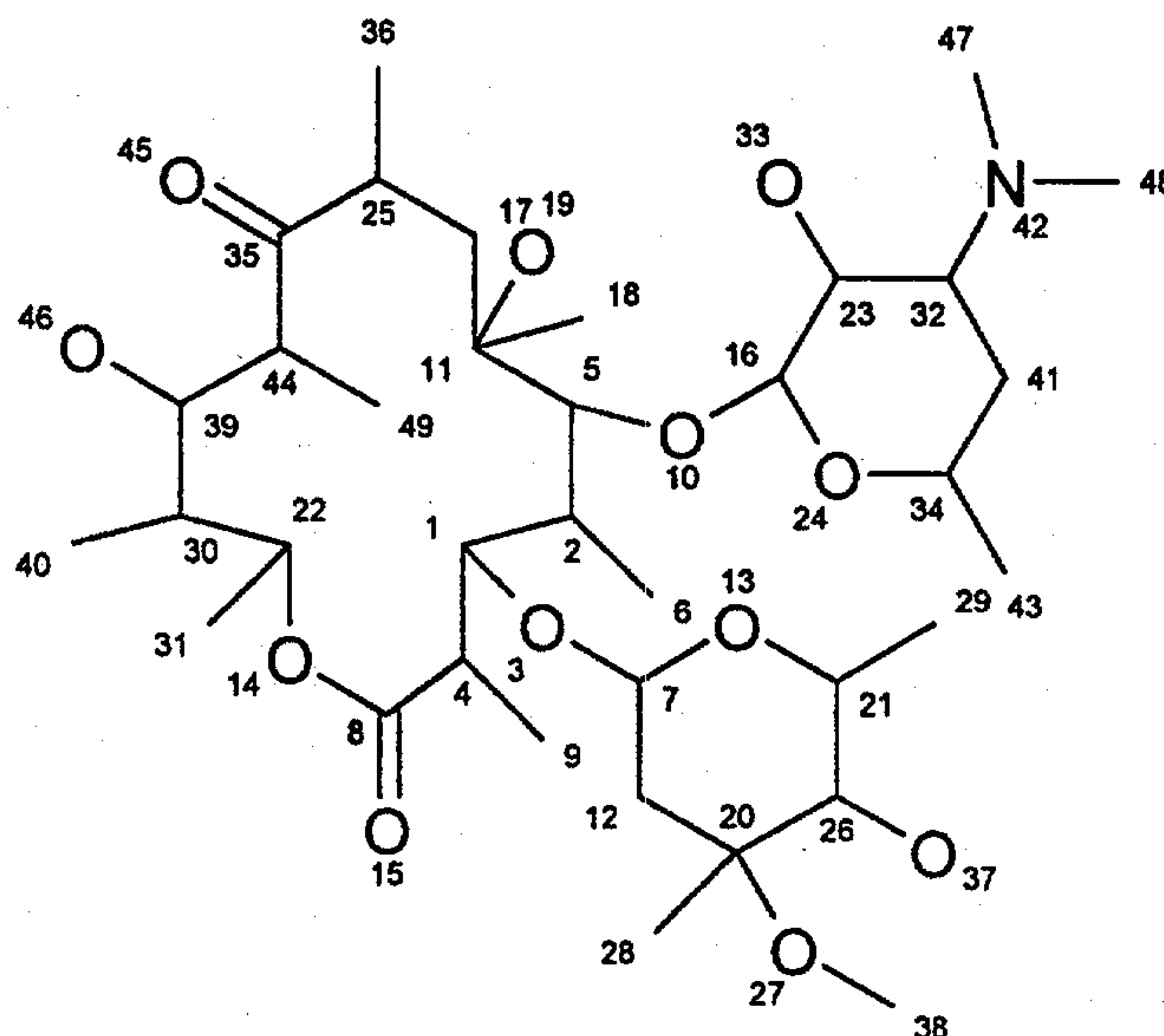
- 41 -



	#	¹³ C - ppm	#H	¹ H - ppm
10	1	221.91	0	
	2	175.99	0	
	3	103.63	1	4.45
	4	96.81	1	4.88
15	5	83.76	1	3.60
	6	79.86	1	4.10
	7	78.36	1	3.05
	8	75.50	0	
	9	74.87	0	
20	10	73.07	0	
	11	72.25	1	5.19
	12	71.25	1	3.26
	13	69.53	1	3.53
	14	69.24	1	3.97
25	15	66.16	1	4.06
	16	65.96	1	2.48
	17	49.96	3	3.36
	18	45.36	1	2.79
	19	45.07	1	2.81
30	20	40.73	3	2.32
	21	39.00	1	3.15
	22	35.30	2	2.42/1.61
	24	27.20	3	1.50
	25	21.92	3	1.28
35	26	21.82	3	1.27
	27	18.99	3	1.32
	28	18.60	3	1.22
	29	16.07	3	1.19
	30	15.08	3	1.19
40	31	14.23	3	1.26
	32	12.12	3	1.19
	33	9.60	3	1.15
	34	39.00	2	1.98/1.75
	35	28.90	2	1.72/1.27
45	36	40.94	1	2.05

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NMR, 13-methyl-erythromycin B:



#	13C - PPM	#H attached	1H - PPM
1	80.50	1	4.15
2	40.62	1	2.15
4	45.17	1	2.84
5	84.08	1	3.62
6	9.86	3	1.18
7	97.26	1	4.88
8	176.48	0	
9	15.25	3	1.22
11	75.98	0	
12	35.43	2	2.42/1.61
16	103.75	1	4.46
17	38.77	2	2.09/1.72
18	27.67	3	1.51
20	73.09	0	
21	66.20	1	4.06
22	70.27	1	5.58
23	71.24	1	3.28
25	45.49	1	2.81
26	78.29	1	3.06
28	21.91	3	1.28
29	19.03	3	1.33
30	41.61	1	1.65

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31	18.73	3	1.29
32	65.94	1	2.53
34	69.52	1	3.55
35	219.92	0	
36	19.03	3	1.21
38	49.97	3	3.36
39	70.17	1	3.88
40	9.27	3	0.95
41	29.12	2	1.73/1.28
43	21.80	3	1.27
44	39.87	1	3.07
47	40.74	3	2.35
48	40.74	3	2.35
49	9.62	3	1.04

Example 6

Construction of the Recombinant Vector pPFL42

Plasmid pPFL42 is a pCJR24-based plasmid containing the gene encoding a hybrid polyketide synthase that contains the tylosin-producing PKS loading module, the erythromycin extension modules 1 and 2 and the chain-terminating thioesterase. Plasmid pPFL42 was constructed as follows:

The following synthetic oligonucleotides:

5'-CCATATGACCTCGAACACCGCTGCACAGAA-3' and

5'-GGCTAGCGGCTCCTGGGCTTCGAAGCTCTTCT-3'

were used to amplify the DNA encoding the tylosin-producing loading module using either cos6T (a cosmid that contains the tylosin-producing PKS genes from *S. fradiae*) or chromosomal DNA from *S. fradiae* as template. The PCR

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product of 3.3 kbp was purified by gel electrophoresis, treated with T4 polynucleotide kinase and ligated to plasmid pUC18, which had been linearised by digestion with *Sma* I and then treated with alkaline phosphatase. The ligation mixture was used to transform electrocompetent *E.coli* DH10B cells and individual clones were checked for the desired plasmid pPFL39. Plasmid pPFL39 was identified by restriction and sequence analysis.

Plasmid pPFL39 was digested with *Nde* I and *Nhe* I and the 3.3 kbp fragment was purified by gel electrophoresis and ligated to pND30 previously digested with *Nde* I and *Nhe* I and treated with alkaline phosphatase. The ligation mixture was used to transform electrocompetent *E.coli* DH10B cells and individual clones were checked for the desired plasmid pPFL42. Plasmid pPFL42 was identified by restriction analysis.

Example 7

Construction of *S. erythraea* JC2/pPFL42

Plasmid pPFL42 was used to transform *S. erythraea* JC2 protoplasts. Thiostrepton resistant colonies were selected in R2T20 medium containing 10 µg/ml of thiostrepton. Several clones were tested for the presence of pPFL42 integrated into the chromosome by Southern blot hybridisation of their genomic DNA with DIG-labelled DNA containing the *tyl* PKS fragment encoding for the loading

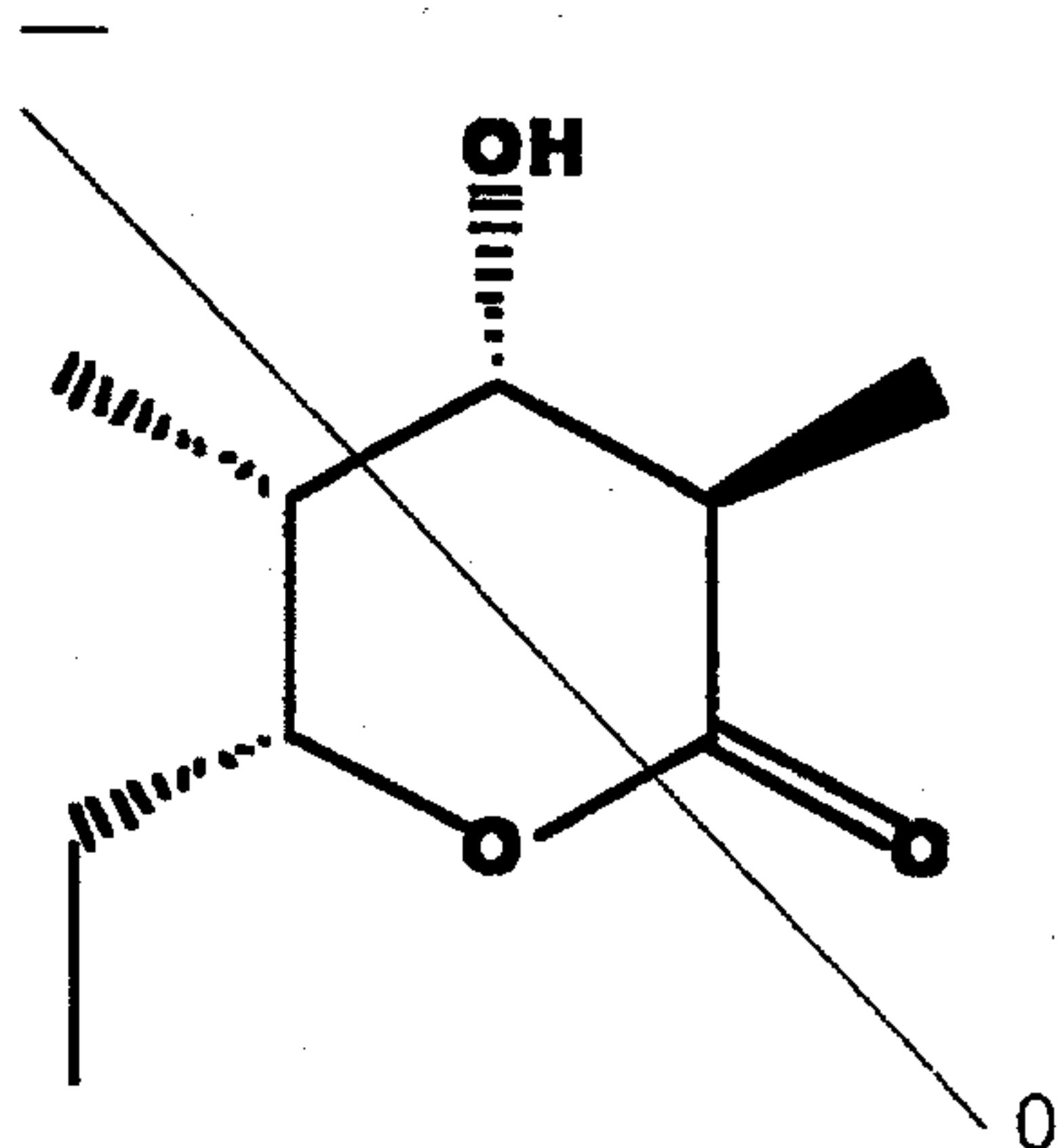
- 45 -

module. A clone with an integrated copy of pPFL42 was identified in this way,

Example 8

Production of polyketides using *S. erythraea* JC2/pPFL42

A frozen suspension of strain *S. erythraea* JC2/pPFL42 was used to inoculate eryP medium containing 5 $\mu\text{g/ml}$ of thiostrepton and allowed to grow for seven days at 28-30°C. After this time the broth was filtered to remove mycelia and the pH adjusted to pH=3. The broth was extracted twice with two volumes of ethyl acetate and the combined extracts were washed with an equal volume of saturated sodium chloride, dried over anhydrous sodium sulphate, and the ethyl acetate was removed under reduced pressure, to give crude product. The product was shown to have the structure shown below, and was identical, as judged by MS, GC-MS, and ^1H NMR with an authentic sample:.



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Example 9Construction of *S. erythraea* NRRL2338/pPFL42

Plasmid pPFL42 was used to transform *S. erythraea* NRRL2338 protoplasts. Thiostrepton resistant colonies were selected in R2T20 medium containing 10 µg/ml of
5 thiostrepton. Several clones were tested for the presence of pPFL42 integrated into the chromosome by Southern blot hybridisation of their genomic DNA with DIG-labelled DNA containing the *tyl* PKS fragment encoding for the loading module. A clone with an integrated copy of pPFL42 was
10 identified in this way.

Example 10Production of polyketides using *S. erythraea*NRRL2338/pPFL42

A frozen suspension of strain *S. erythraea*
15 NRRL2338/pPFL42 was used to inoculate eryP medium containing 5 µg/ml of thiostrepton and allowed to grow for seven days at 28-30°C. After this time the broth was filtered to remove mycelia and the pH adjusted to pH=9. The supernatant was then extracted three times with an
20 equal volume of ethyl acetate and the solvent was removed by evaporation. Products were analysed by HPLC/MS and a macrolide was identified with the following structure, identical with that of authentic erythromycin A (together with other products, which were identified as the

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corresponding erythromycins B and D, the result of incomplete post-PKS processing):

Example 11

Construction of plasmid pPFL35

Plasmid pPFL35 is a pCJR24-based plasmid containing a
5 PKS gene comprising a loading module, the first and second
extension modules of DEBS and the chain terminating
thioesterase. The loading module comprises the KSq domain
DNA from the loading module of the oleandomycin PKS fused
to the malonyl-CoA-specific AT of module 2 of the rapamycin
10 PKS, in turn linked to the DEBS loading domain ACP. Plasmid
pPFL35 was constructed via several intermediate plasmids as
follows:

A 411 bp DNA segment of the *eryAI* gene from
S. erythraea extending from nucleotide 1279 to nucleotide
15 1690 (Donadio, S. et al., Science (1991) 2523:675-679) was
amplified by PCR using the following synthetic
oligonucleotide primers:-

5'-TGGACCGCCGCCAATTGCCTAGGCGGGCCGAACCCGGCT-3' and

5'-CCTGCAGGCCATCGCGACGACCGCGACCGGTTCGCC-3'

20 The DNA from a plasmid designated pKSW, derived from
pT7-7 and DEBS1-TE in which new *Pst* I and *Hind*III sites had
been introduced to flank the KS1 of the first extension
module, was used as a template. The 441 bp PCR product was
treated with T4 polynucleotide kinase and ligated to

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plasmid pUC18, which had been linearised by digestion with *Sma* I and then treated with alkaline phosphatase. The ligation mixture was used to transform electrocompetent *E.coli* DH10B cells and individual clones were checked for the desired plasmid, pPFL26. The new *Mfe* I/*Avr* II sites bordering the insert are adjacent to the *Eco* RI site in the polylinker of pUC18. Plasmid pPFL26 was identified by restriction pattern and sequence analysis.

An *Mfe* I restriction site is located 112 bp from the 5' end of the DNA encoding the propionyl-CoA:ACP transferase of the loading module of DEBS. Plasmid pKSW was digested with *Mfe* I and *Pst* I and ligated with the 411 bp insert obtained by digesting plasmid pPFL26 with *Mfe* I and *Pst* I. The ligation mixture was used to transform electrocompetent *E.coli* DH10B cells and individual clones were checked for the desired plasmid, pPFL27. Plasmid pPFL27 contains a PKS gene comprising the DEBS loading module, the first and second extension modules of DEBS and the DEBS chain terminating thioesterase. Plasmid pPFL27 was identified by its restriction pattern.

Plasmid pPFL27 was digested with *Nde* I and *Avr* II and ligated to a 4.6kbp insert derived from digesting plasmid pMO6 (PCT/GB97/01819) with *Nde* I and *Avr* II. Plasmid pMO6 contains a PKS gene comprising the DEBS loading module, the first and second extension modules of DEBS and the DEBS

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chain terminating thioesterase, except that the DNA segment encoding the methylmalonate-specific AT within the first extension module has been specifically substituted by the DNA encoding the malonate-specific AT of module 2 of the rap PKS. The ligation mixture was used to transform electrocompetent *E. coli* DH10B cells and individual clones were checked for the desired plasmid, pPFL28. Plasmid pPFL28 contains a hybrid PKS gene comprising the DEBS loading module, the malonate-specific AT of module 2 of the rap PKS, the ACP of the DEBS loading module, followed by the first and second extension modules of DEBS and the DEBS chain terminating thioesterase. Plasmid pPFL28 was identified by restriction analysis.

A DNA segment encoding the KSq domain from the *oleA* gene of *S. antibioticus* extending from nucleotide 1671 to nucleotide 3385 was amplified by PCR using the following synthetic oligonucleotide primers:-

5'-CCACATATGCATGTCCCCGGCGAGGAA-3' and

5'-CCCTGTCCGGAGAAGAGGAAGGCGAGGCCG-3'

and chromosomal DNA from *Streptomyces antibioticus* as a

template. The PCR product was treated with T4 polynucleotide kinase and ligated to plasmid pUC18, which had been linearised by digestion with *Sma* I and then treated with alkaline phosphatase. The ligation mixture was used to transform electrocompetent *E. coli* DH10B cells and

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individual clones were checked for the desired plasmid, pPFL31. The new *Nde* I site bordering the insert is adjacent to the *Eco* RI site of the pUC18 polylinker while the new *Bsp* EI site borders the *Hin* dIII site of the linker region. Plasmid pPFL31 was identified by restriction and sequence analysis.

Plasmid pPFL31 was digested with *Nde* I and *Avr* II and the insert was ligated with plasmid pPFL28 that had been digested with *Nde* I and *Avr* II. The ligation mixture was used to transform electrocompetent *E.coli* DH10B cells and individual clones were checked for the desired plasmid, pPFL32. Plasmid pPFL32 was identified by restriction analysis.

Plasmid pPFL32 was digested with *Nde* I and *Xba* I and the insert was ligated to plasmid pCJR24, which had been digested with *Nde* I and *Xba* I and purified by gel electrophoresis. The ligation mixture was used to transform electrocompetent *E.coli* DH10B cells and individual clones were checked for the desired plasmid, pPFL35. Plasmid pPFL35 was identified by restriction analysis.

Example 12

Construction of *S. erythraea* JC2 / pPFL35

Plasmid pPFL35 was used to transform *S. erythraea* JC2 protoplasts. Thiostrepton resistant colonies were selected in R2T20 medium containing 10 µg/ml of thiostrepton.

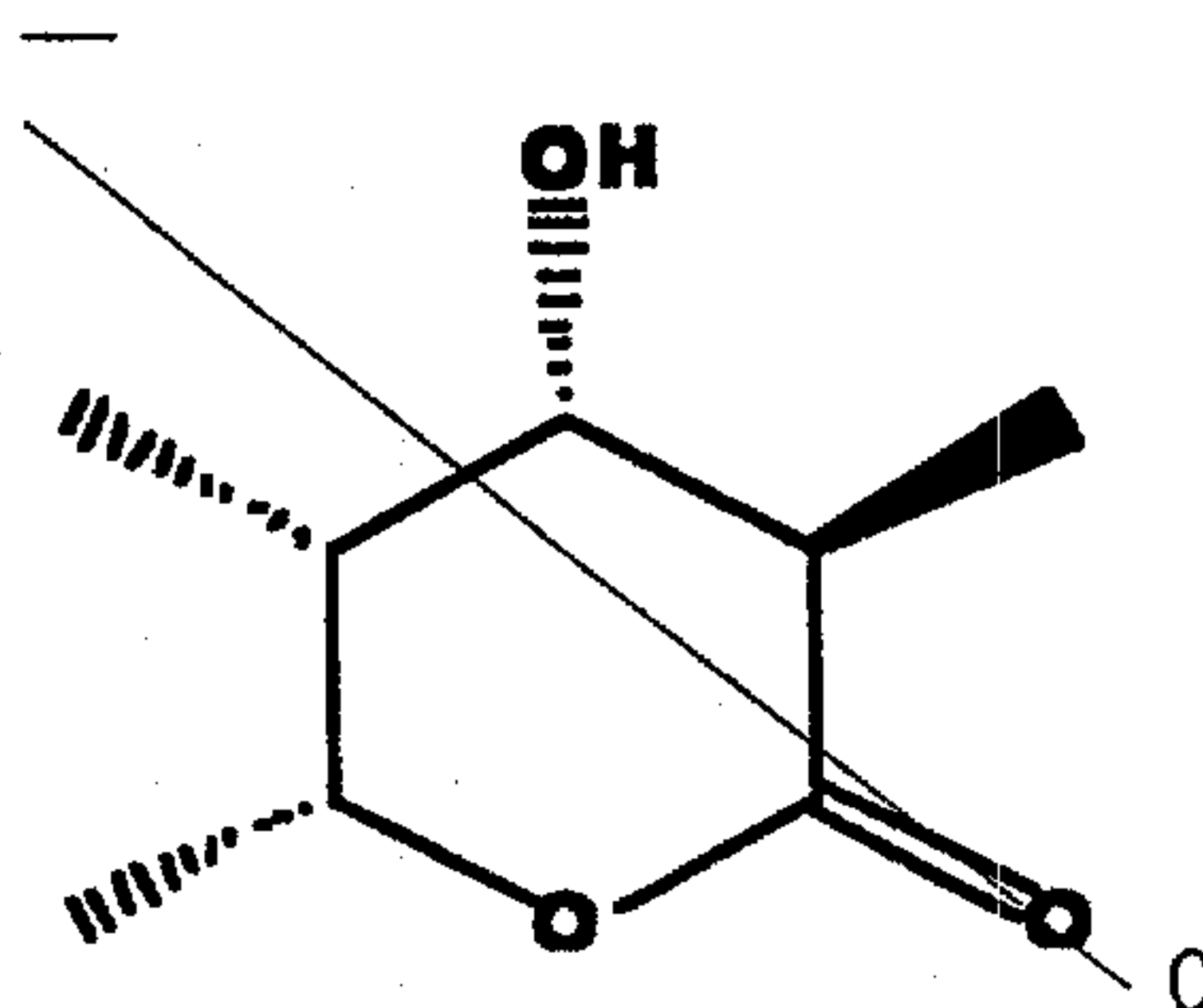
- 51 -

Several clones were tested for the presence of pPFL35 integrated into the chromosome by Southern blot hybridisation of their genomic DNA with DIG-labelled DNA containing the *rap* PKS fragment encoding for module 2 acyltransferase. A clone with an integrated copy of pPFL35 was identified in this way.

Example 13

Production of polyketides using *S. erythraea* JC2 / pPFL35

A frozen suspension of strain *S. erythraea* JC2 / pPFL35 was used to inoculate eryP medium containing 5 µg/ml of thiostrepton and allowed to grow for seven days at 28-30°C. After this time the broth was filtered to remove mycelia and the pH adjusted to pH=3. The broth was extracted twice with two volumes of ethyl acetate and the combined extracts were washed with an equal volume of saturated sodium chloride, dried over anhydrous sodium sulphate, and the ethyl acetate was removed under reduced pressure, to give crude product. The product was shown to have the following structure, and was found by MS, GC-MS and ¹H NMR to be identical to authentic material:



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Example 14Construction of *S. erythraea* NRRL2338/pPFL35

Plasmid pPFL35 was used to transform *S.erythraea* NRRL2338 protoplasts. Thiostrepton resistant colonies were selected in R2T20 medium (Yamamoto et al.) containing 10 μ g/ml of thiostrepton. Several clones were tested for the presence of pPFL35 integrated into the chromosome by Southern blot hybridisation of their genomic DNA with DIG-labelled DNA containing the rap PKS fragment encoding for module 2 AT. A clone with an integrated copy of pPFL35 was identified in this way.

Example 15Production of 13-methyl-erythromycin A and B using*Sacch.erythraea* NRRL-2338 (pPFL35)

The culture *Saccharopolyspora erythraea* NRRL2338 (pPFL35), constructed with the wild-type loading domain displaced by an oleandomycin KSQ-rapamycin AT2- D1TE DNA insert, prepared as described in Example 14, was inoculated into 30ml Tap Water medium with 50 μ g/ml thiostrepton in a 300ml Erlenmeyer flask. After two days incubation at 29°C, this flask was used to inoculate 300 ml of ERY-P medium in a 300ml flask. The broth was incubated at 29°C at 200 rpm for 6 days. After this time, the whole broth was adjusted to pH 8.5 with NaOH, then extracted with an equal volume of ethyl acetate. The ethyl acetate extract was evaporated to

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dryness at 45°C under a nitrogen stream using a Zymark TurboVap LV Evaporator, then reconstituted in 0.25 volumes methanol to concentrate the extract 4-fold. The structure of the products were confirmed by LC/MS, Method A. A peak was observed with a retention time of 4.0 min and with an m/z value of 720 (M+H)⁺, required for 13-methyl-erythromycin A (C₃₆H₆₅NO₁₃). A second peak was observed with a retention time of 6.4 min and with m/z value of 704 (M+H)⁺, required for 13-methyl-erythromycin B (C₃₆H₆₅NO₁₂).

Example 16

10 Construction of Recombinant Vector pPFL44

Plasmid pPFL44 is a pCJR24- based plasmid containing the gene encoding a hybrid polyketide synthase that contains the spiramycin PKS loading module, the erythromycin extension modules 1 and 2 and the chain-terminating thioesterase. Plasmid pPFL44 was constructed as follows:

The following synthetic oligonucleotides:

5'-CCATATGTCTGGAGAACTCGCGATTTCGCCAGT-3' and

20 5'-GGCTAGCGGGTCGTCGTCGTCGCCGGCTG-3'

were used to amplify the DNA encoding the spiramycin-producing loading module using chromosomal DNA from the spiramycin producer *S. ambofaciens* prepared according to the method described by Hopwood et al. (1985). The PCR

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product of 3.3 kbp was purified by gel electrophoresis, treated with T4 polynucleotide kinase and ligated to plasmid pUC18, which had been linearised by digestion with Sma I and then treated with alkaline phosphatase. The ligation mixture was used to transform electrocompetent *E.coli* DH10B cells and individual clones were checked for the desired plasmid pPFL41. Plasmid pPFL41 was identified by restriction pattern and sequence analysis.

Plasmid pPFL41 was digested with Nde I and Nhe I and the 3.3 kbp fragment was purified by gel electrophoresis and ligated to pND30 (a plasmid derived from plasmid pCJR24 having as insert the ave PKS loading module and extension modules 1 and 2 or DEBS and the DEBS thioesterase) (PCTGB97/01810) previously digested with Nde I and Nhe I and treated with alkaline phosphatase. The ligation mixture was used to transform electrocompetent *E.coli* DH10B cells and individual clones checked for the desired plasmid pPFL44. Plasmid pPFL44 was identified by restriction analysis.

20 Example 17

Construction of *S. erythraea* JC2/pPFL44

Plasmid pPFL44 was used to transform *S.erythraea* JC2 protoplasts. Thiostrepton resistant colonies were selected in R2T20 medium containing 10 µg/ml of thiostrepton.

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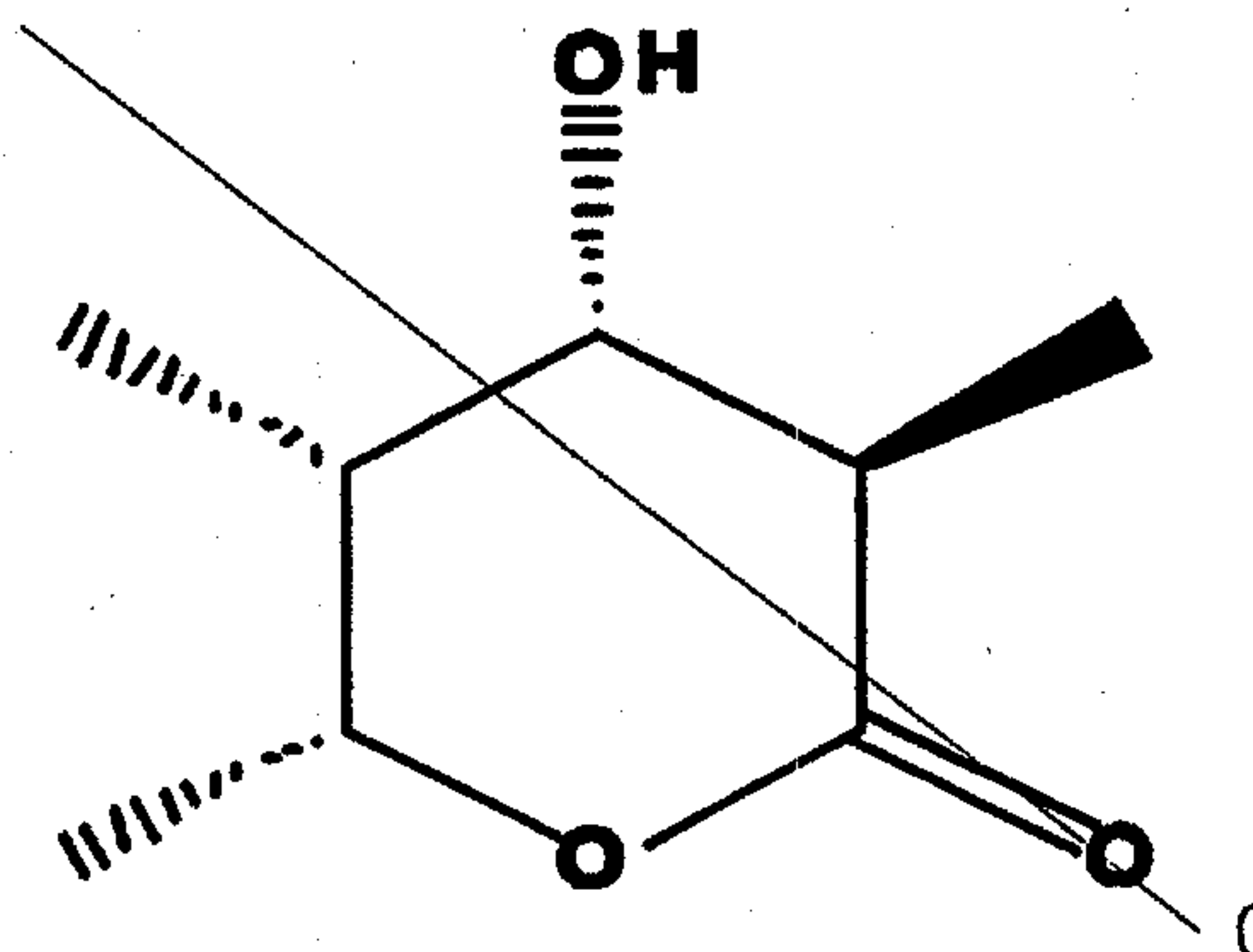
Several clones were tested for the presence of pPFL44 integrated into the chromosome by Southern blot hybridisation of their genomic DNA with DIG-labelled DNA containing the srm PKS fragment encoding for the loading module. A clone with an integrated copy of pPFL44 was identified in this way.

Example 18

Production of polyketides using *S. erythraea* JC2/pPFL44

A frozen suspension of strain *S. erythraea* JC2/pPFL44 was used to inoculate eryP medium containing 5 µg/ml of thiostrepton and allowed to grow for seven days at 28-30°C. After this time the broth was filtered to remove mycelia and the pH adjusted to pH=3. The broth was extracted twice with two volumes of ethyl acetate and the combined extracts were washed with an equal volume of saturated sodium chloride, dried over anhydrous sodium sulphate, and the ethyl acetate was removed under reduced pressure, to give crude product. The product was shown to have the structure shown below and by GC-MS and ¹H NMR analysis was identical to authentic material:

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Example 19

Construction of *S. erythraea* NRRL2338/pPFL44

Plasmid pPFL44 was used to transform *S.erythraea*

5 NRRL2338 protoplasts. Thiostrepton resistant colonies were selected in R2T20 medium containing 10 μ g/ml of thiostrepton. Several clones were tested for the presence of pPFL44 integrated into the chromosome by Southern blot hybridisation of their genomic DNA with DIG-labelled DNA

10 containing the spiramycin PKS fragment encoding for the loading module. A clone with an integrated copy of pPFL44 was identified in this way.

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Example 20Production of 13 methyl-erythromycin A and B using *Sacch. erythraea* NRRL-2338 (pPFL44)

The culture *Saccharopolyspora erythraea* NRRL2338 (pPFL44), constructed with the wild-type loading domain displaced by spiramycin loader-DlTE DNA insert, was inoculated into 30ml Tap Water medium with 50 μ g/ml thiostrepton in a 300ml Erlenmeyer flask. After three days incubation at 29°C, this flask was used to inoculate 300 ml of ERY-P medium in a 300 ml flask. The broth was incubated at 29°C at 200 rpm for 6 days. After this time, the whole broth was adjusted to pH 8.5 with NaOH, then extracted with equal volume of ethyl acetate. The ethyl acetate extract was evaporated to dryness at 45°C under a nitrogen stream using Zymark TurboVap LV Evaporator, then reconstituted in 0.0625 volumes methanol to concentrate the extract 16-fold. The structures of the products were confirmed by LC/MS, Method A. A 4.0 min retention time peak was observed as the major component, with m/z value of 720 (M+H)⁺, required for 13-methyl-erythromycin A (C₃₆H₆₅NO₁₃). A second peak was observed with a retention time of 6.4 min and with m/z value of 704 (M+H)⁺, required for 13-methyl-erythromycin B (C₃₆H₆₅NO₁₂).

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Example 21**Construction of plasmid pJLK114**

Plasmid pJLK114 is a pCJR24 based plasmid containing a PKS
5 gene comprising the ery loading module, the first and the
second extension modules of the ery PKS and the ery chain-
terminating thioesterase except that the DNA segment
between the end of the acyltransferase and the beginning of
the ACP of the second ery extension module has been
10 substituted by a synthetic oligonucleotide linker
containing the recognition sites of the following
restriction enzymes: AvrII, BglII, SnaBI, PstI, SpeI, NsiI,
Bsu36I and HpaI. It was constructed via several
intermediate plasmids as follows (Figure 6).

15

Construction of plasmid pJLK02

The approximately 1.47 kbp DNA fragment of the eryAI gene
of *S. erythraea* was amplified by PCR using as primers the
20 synthetic oligonucleotides:

5'-TACCTAGGCCGGGCCGGACTGGTCGACCTGCCGGGTT-3' and

5'-ATGTTAACCGGTCGCGCAGGCTCTCCGTCT-3' and plasmid pNTEP2

(Oliynyk, M. et al., Chemistry and Biology (1996) 3:833-

839; WO98/01546) as template. The PCR product was treated

25

with T4 polynucleotide kinase and then ligated with plasmid
pUC18, which had been linearised by digestion with SmaI and

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then treated with alkaline phosphatase. The ligation mixture was used to transform electrocompetent *E. coli* DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pJLK02 was identified by its restriction pattern and DNA sequencing.

5

Construction of plasmid pJLK03

The approximately 1.12 kbp DNA fragment of the *eryAI* gene of *S. erythraea* was amplified by PCR using as primers the synthetic oligonucleotides:

10

5'-ATGTTAACGGGTCTGCCGCGTGCCGAGCGGAC-3' and

5'-CTTCTAGACTATGAATTCCCTCCGCCCAGC-3' and plasmid pNTEPH as template. The PCR product was treated with T4

15

polynucleotide kinase and then ligated with plasmid pUC18,

which had been linearised by digestion with *Sma*I and then treated with alkaline phosphatase. The ligation mixture was used to transform electrocompetent *E. coli* DH10B cells and individual colonies were checked for their plasmid content.

20

The desired plasmid pJLK03 was identified by its restriction pattern and DNA sequencing.

Construction of plasmid pJLK04

Plasmid pJLK02 was digested with *Pst*I and *Hpa*I and the 1.47 kbp insert was ligated with plasmid pJLK03 which had been digested with *Pst*I and *Hpa*I. The ligation mixture was used

25

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to transform electrocompetent E. coli DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pJLK04 was identified by its restriction pattern.

5 Construction of plasmid pJLK05

Plasmid pJLK01 (PCT/GB97/01819) was digested with PstI and AvrII and the 460 bp insert was ligated with plasmid pJLK04 which had been digested with PstI and AvrII. The ligation mixture was used to transform electrocompetent E. coli DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pJLK05 was identified by its restriction pattern.

15 Construction of plasmid pJLK07

Plasmid pJLK05 was digested with ScaI and XbaI and plasmid pNTEPH was digested with NdeI and ScaI and these two fragments were ligated with plasmid pCJR24 which had been digested with NdeI and XbaI. The ligation mixture was used to transform electrocompetent E. coli DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pJLK07 was identified by its restriction pattern.

25

Construction of plasmid pJLK114

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The two synthetic oligonucleotides Plf and Plb (Figure 7) were each dissolved in TE-buffer. 10 μ l of each solution (0.5nmol/ μ l) were mixed and heated for 2 minutes to 65C and then slowly cooled down to room temperature. Plasmid pJLK07 was digested with AvrII and HpaI and ligated with the annealed oligonucleotides. The ligation mixture was used to transform electrocompetent E. coli DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pJLK114 was identified by its restriction pattern.

10

Plasmid pJLK117 is a pCJR24 based plasmid containing a PKS gene comprising the ery loading module, the first and the second extension modules of the ery PKS and the ery chain-terminating thioesterase except that the DNA segment between the end of the acyltransferase and the beginning of the ACP of the second ery extension module has been substituted by a synthetic oligonucleotide linker containing the recognition sites of the following restriction enzymes. AvrII, BglII, SnaBI, PstI, SpeI, NsiI, Bsu36I and NheI.

20

It was constructed via several intermediate plasmids as follows (Figure 6).

25

Construction of plasmid pJLK115

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Plasmid pJLK114 was digested with NdeI and XbaI and the approximately 9.9 kbp insert was ligated with plasmid pUC18 which had been digested with NdeI and XbaI. The ligation mixture was used to transform electrocompetent E. coli DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pJLK115 was identified by its restriction pattern.

Construction of plasmid pJLK116

Plasmid pJLK13 (PCT/GB97/01819) was digested with Bsu36I and XbaI and the 1.1 kbp fragment was ligated with plasmid pJLK115 which had been digested with Bsu36I and XbaI. The ligation mixture was used to transform electrocompetent E. coli DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pJLK116 was identified by its restriction pattern.

Construction of plasmid pJLK117

Plasmid pJLK116 was digested with NdeI and XbaI and the 9.9 kbp fragment was ligated with plasmid pCJR24 which had been digested with NdeI and XbaI. The ligation mixture was used to transform electrocompetent E. coli DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pJLK117 was identified by its restriction pattern.

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Example 11**Construction of plasmid pJLK29**

5 Plasmid pJLK29 is a pJLK117 based plasmid except that the DNA fragment encoding the reductive loop of module 10 of the rap PKS has been inserted into the mcs. It was constructed via several intermediate plasmids as follows. (Figure 5)

10 Construction of plasmid pJLK121.1

The approximately 2.2 kbp DNA segment of the rapB gene of *S. hygroscopicus* encoding the reductive loop of module 10 was amplified by PCR using as primers the synthetic
15 oligonucleotides:

5'-TAAGATCTTCCGACGTACGCGTTCCAGC-3' and

5'-ATGCTAGCCACTGCGCCGACGAATCACCGGTGG-3' and as template an approximately 7 kbp fragment, which has been obtained by digestion of cosmid cos 26 (Schwecke, T. et al. (1995)

20 Proc. Natl. Acad. Sci. USA 92:7839-7843) with ScaI and SphI. The PCR product was treated with T4 polynucleotide kinase and then ligated with plasmid pUC18, which had been linearised by digestion with SmaI and then treated with alkaline phosphatase. The ligation mixture was used to
25 transform electrocompetent *E. coli* DH10B cells and individual colonies were checked for their plasmid content.

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The desired plasmid pJLK121.1 was identified by its restriction pattern and DNA sequencing.

Construction of plasmid pJLK29

- 5 Plasmid pJLK121.1 was digested with BglII and NheI and the 2.2 kbp fragment was ligated with plasmid pJLK117 which had been digested with BglII and NheI. The ligation mixture was used to transform electrocompetent *E. coli* DH10B cells and individual colonies were checked for their plasmid content.
- 10 The desired plasmid pJLK29 was identified by its restriction pattern.

Example 24

- 15 Construction of Plasmid pJLK50

The approximately 6.1 kbp DNA segment of the erythromycin PKS gene cluster of *S. erythraea* encoding the DNA fragment from the beginning of the ACP of module 2 to the beginning of the ACP of module 3 was amplified by PCR using as

20 primers the synthetic oligonucleotides:

5'-TACCTGAGGGACCGGCTAGCGGGTCTGCCGCGTG-3' and

5'-ATGCTAGCCGTTGTGCCGGCTCGCCGGTCCGTCC-3' and plasmid pBAM25 (published pBK25 by Best, D J et al. *Eur J Biochem* (1992)

- 25 204: 39-49) as template. The PCR product was treated with T4 polynucleotide kinase and then ligated with plasmid

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pUC18, which had been linearised by digestion with SmaI and then treated with alkaline phosphatase. The ligation mixture was used to transform electrocompetent *E. coli* DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pJLK50 was identified by its restriction pattern and DNA sequencing.

Example 25

Construction of *S.erythraea* strain JLK10

Strain JLK10 is a variant of strain NRRL2338 in which the reductive loop of ery module 2 (i.e. the KR domain) is replaced by the reductive loop of the rapamycin module 10. It was constructed using plasmid pJLK54 which was constructed as follows.

Construction of plasmid pJLK54

Plasmid pJLK54 is a pJLK29 based plasmid containing a PKS gene comprising the ery loading module, the first, the second and the third extension modules of the ery cluster and the ery chain-terminating thioesterase except that the DNA segment between the end of the acyltransferase and the beginning of the ACP of the second ery extension module has been substituted by the equivalent segment of module 10 of the rapamycin PKS.

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It was constructed as follows.

Plasmid pJLK50 was digested with NheI and the 6.1 kbp insert was ligated with plasmid pJLK29 which had been digested with NheI. The ligation mixture was used to transform electrocompetent *E. coli* DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pJLK54 was identified by its restriction pattern.

10 Use of plasmid pJLK54 for construction of *S. erythraea* NRRL2338/pJLK54 and the production of TKL derivatives

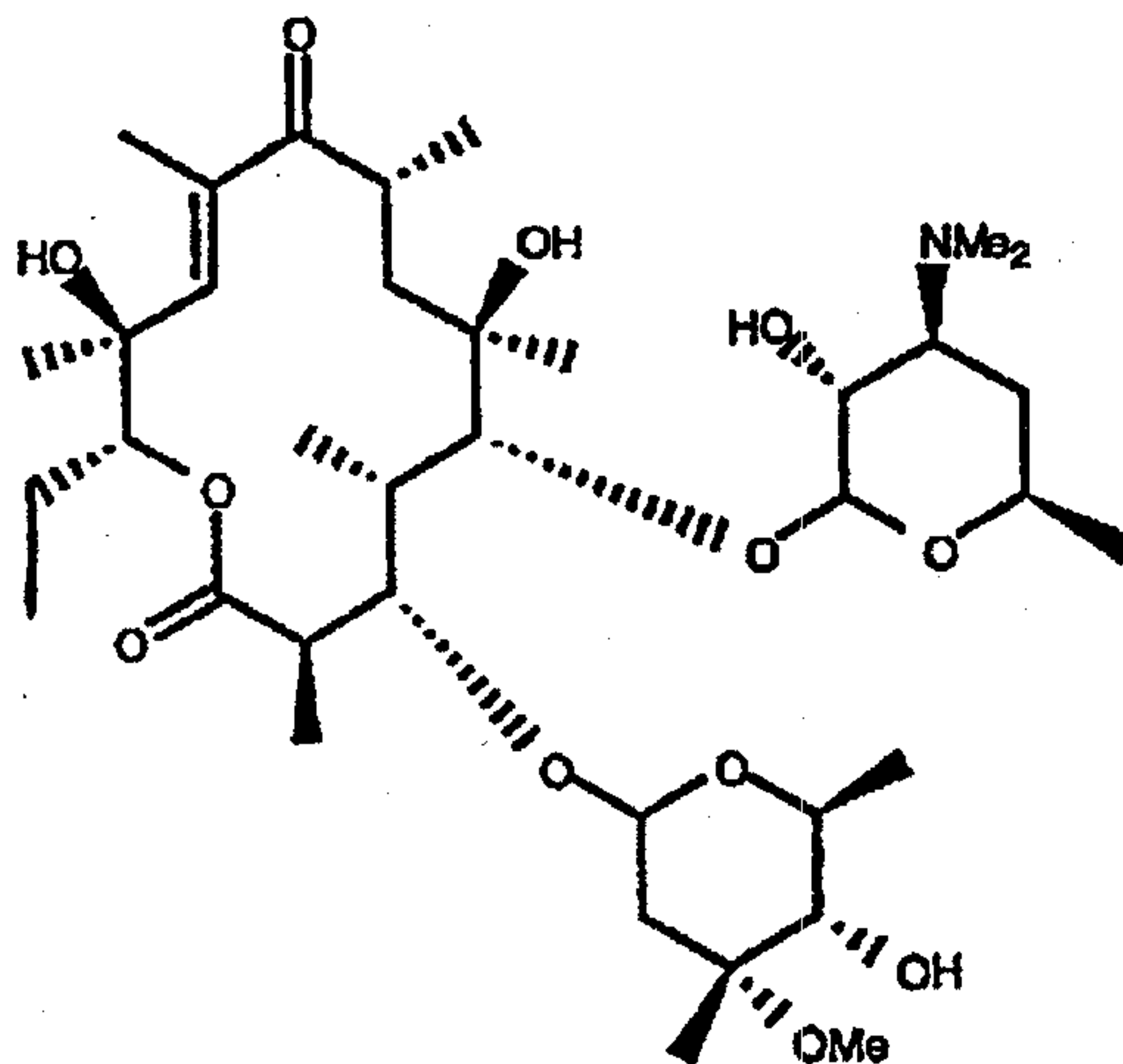
Approximately 5 µg plasmid pJLK54 were used to transform protoplasts of *S. erythraea* NRRL2338 and stable thioestrepton resistant colonies were isolated. From several colonies total DNA is obtained and analysed by Southern blot hybridisation, to confirm that the plasmid has integrated into the TE.

20 Construction of *S.erythraea* strain JLK10 and its use in production of 13-methyl-10,11-dehydro-erythromycin A

S. erythraea strain JLK10 is a mutant of *S. erythraea* NRRL2338 in which the 'reductive loop' of ery module 2 i.e. the ketoreductase domain is substituted by the 'reductive

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loop' of rapamycin module 10. It was constructed starting from *S. erythraea* NRRL2338 into which plasmid pJLK54 had been integrated. *S. erythraea* NRRL2338/pJLK54 was subjected to several rounds of non-selective growth which resulted in second crossover concomitant with the loss of the integrated plasmid. Clones in which replacement of the erythromycin gene coding for DEBS1 with the mutant version had occurred, were identified by Southern blot hybridisation. One of these was named *S. erythraea* strain JLK10 and was used to inoculate SM3 medium (eryP medium gave similar results), and allowed to grow for seven to ten days at 28-30°C. After this time the broth was centrifuged and the pH of the supernatant adjusted to pH 9. The supernatant was then extracted three times with an equal volume of ethyl acetate and the solvent was removed by evaporation. Products were analysed by HPLC/MS, MS/MS and ¹H-NMR. The following macrolide C-13 methyl erythromycin A was identified (accompanied by products of incomplete processing by post-PKS enzymes).



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Example 26**Construction of plasmid pPFL50**

Plasmid pPFL50 is a pPFL43-based plasmid from which a DNA fragment encoding KR1 (in part), ACP1 and module 2 of the erythromycin PKS and the erythromycin TE, has been removed.

5 It was constructed as follows. Plasmid pPFL43 was digested with SfuI and XbaI to remove a 6.5 kb fragment. The 5' overhangs were filled in with Klenow fragment DNA Polymerase I and the plasmid was recircularised. The ligation mixture was used to transform electrocompetent E. coli DH10B cells and individual colonies were checked for
10 their plasmid content. The desired plasmid pPFL50 was identified by its restriction pattern.

Construction of *S. erythraea* JLK10/pPFL50

15 Approximately 5 µg plasmid pPFL50 were used to transform protoplasts of *S. erythraea* strain JLK10 and stable thiostrepton resistant colonies were isolated. From several colonies total DNA was obtained and analysed by Southern blot hybridisation, to confirm that the plasmid had
20 integrated into the homologous chromosomal DNA region. *S. erythraea* strain JLK10/pPFL50 was used to inoculate SM3 medium containing 5 µg/ml thiostrepton (eryP medium containing 5 µg/ml thiostrepton gave similar results) and allowed to grow for seven to ten days at 28-30°C. After

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this time the broth was centrifuged and the pH of the supernatant adjusted to pH 9. The supernatant was then extracted three times with an equal volume of ethyl acetate and the solvent was removed by evaporation. Products were analysed by HPLC/MS, MS/MS and ¹H-NMR. The macrolide C-13 methyl 10,11-dehydro-erythromycin A was identified (accompanied by products of incomplete processing by post-PKS enzymes)

Construction of *S. erythraea* NRRL2338/pPFL50

Approximately 5 µg plasmid pPFL50 were used to transform protoplasts of *S. erythraea* NRRL2338 and stable thiostrepton resistant colonies were isolated. From several colonies total DNA was obtained and analysed by Southern blot hybridisation, to confirm that the plasmid had integrated into the homologous region of the chromosomal DNA. *S. erythraea* NRRL2338/pPFL50 was used to inoculate SM3 medium containing 5 µg/ml thiostrepton (eryP medium containing 5 µg/ml thiostrepton gives similar results) and allowed to grow for seven to ten days at 28-30°C. After this time the broth was centrifuged and the pH of the supernatant adjusted to pH 9.5. The supernatant was then extracted three times with an equal volume of ethyl acetate and the solvent was removed by evaporation. Products were analysed by HPLC/MS, MS/MS and ¹H-NMR. The macrolide C-13

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methyl erythromycin A was identified (accompanied by products of incomplete processing by post-PKS enzymes).

Construction of plasmid pCB121

5 Plasmid pCB121 is a plasmid containing the monensin loading module and KS of monensin module 1 followed by the erythromycin module 1 AT and part of the erythromycin module 1 KR. It was constructed via several intermediate plasmids as follows.

10 Construction of plasmid pPFL45

The approximately 1.8 kbp DNA segment of the monensin PKS gene cluster of *Streptomyces cinnamonensis* encoding part of the ACP of the loading module and KS of module 1 was amplified by PCR using as primers the synthetic
15 oligonucleotides:

5'-CGTTCCTGAGGTCGCTGGCCCAGGCGTA-3'

5'-CGAAGCTTGACACCGCGGCGGCGCGG-5'

and a cosmid containing the 5' end of the monensin PKS genes from *S. cinnamonensis* or alternatively chromosomal
20 DNA of *S. cinnamonensis* as template. The PCR product was treated with T4 polynucleotide kinase and then ligated with plasmid pUC18, which had been linearised by digestion with *Sma*I and then treated with alkaline phosphatase. The ligation mixture was used to transform electrocompetent *E.*

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coli DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pPFL45 was identified by its restriction pattern.

Construction of plasmid pPFL47

Plasmid pPFL45 was digested with NdeI and Bsu36I and the approximately 2.6 kbp fragment was ligated into plasmid pPFL43 which had been digested with NdeI and Bsu36I. The ligation mixture was used to transform electrocompetent E. coli DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pPFL47 was identified by its restriction pattern.

5 **Construction of plasmid pCB135**

Plasmid pCJR24 was digested with HindIII, the 5' overhang was filled in with Klenow fragment DNA Polymerase I and religated. The ligation mixture was used to transform electrocompetent E. coli DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pCB135 was identified by its restriction pattern, lacking the recognition site for HindIII.

10 **Construction of plasmid pKSW1**

15 Plasmid pKS1W is a pNTEP2 (GB97/01810)-derived vector containing a DEBS1TE-derived triketide synthase with the unique restriction sites introduced at the limits of KS1. Plasmid pKS1W is obtained via several intermediate plasmids as follows.

Construction of plasmids pMO09, pMO10 and pMO13

For the PCR amplification for plasmid pMO09, the following synthetic oligonucleotides were used as mutagenic primers, one containing a MunI site and the other a PstI site:

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5' -GCGCGCCAATTGCGTGCACATCTCGAT- 3'

and 5' -CCTGCAGGCCATCGCGACGACCGCGACCGGTTCGCCG- 3'

For the PCR amplification for plasmid pMO10, the following
synthetic oligonucleotides were used as mutagenic primers,
one containing a HindIII site and the other an EcoRV site:

5' -GTCTCAAGCTTCGGCATCAGCGGCACCAA- 3'

and 5' -CGTGCGATATCCCTGCTCGGCGAGCGCA-3'

For the PCR amplification for plasmid pMO13, the following
synthetic oligonucleotides were used as mutagenic primers,
one containing a PstI site and the other a HindIII site:

5' -GATGGCCTGCAGGCTGCCCCGGCGGTGTGAGCA- 3'

and 5' -GCCGAAGCTTGAGACCCCCGCCCCGGCGCGGTTCGC- 3'

15

PCR was carried out on pNTEP2 (GB97/01810) as template
using Pwo DNA polymerase and one cycle of: 96°C (1min);
annealing at 50°C (3min); and extension at 72°C (1min), and
25 cycles of: 96°C (1min); annealing at 50°C (1min); and
extension at 72°C (1min) in the presence of 10% (vol/vol)
dimethylsulphoxide. The products were end-repaired and
cloned into pUC18 digested with SmaI and the ligation
mixture was transformed into E. coli DH 10B. Plasmid DNA
was prepared from individual colonies. The desired plasmids

20

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for pMO09 (3.8kbp), pMO10 (3.9 kbp) and pMO13 (4.3 kbp) were identified by their restriction pattern and DNA sequencing.

Construction of plasmid pMO11

5 Plasmid pMO13 was digested with HindIII, and the 1.2 kbp insert was cloned into pMO10 which had been digested with HindIII. The ligation mixture was transformed into E. coli DH 10B. The desired plasmid (5.0 kbp) was identified by its restriction pattern and designated pMO11.

10

Construction of plasmid pMO12

Plasmid pMO09 was digested with PstI, and the 1.6 kbp insert was cloned into pMO11 which had been digested with PstI. The ligation mixture was transformed into E. coli DH 15 10B. The desired plasmid (6.6 kbp) was identified by its restriction pattern and designated pMO12.

Construction of pKS1W

20 Plasmid pMO12 was digested with MunI and EcoRV, and the 3.9 kbp fragment was cloned into pNTEPH (see below) which had been digested with MunI and EcoRV. The ligation mixture was transformed into E. coli DH 10B. The desired plasmid (13. kbp) was identified by its restriction pattern and designated pKS1W.

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Construction of pNTEPH

Plasmid pNTEPH was obtained from pNTEP2 by removing the HindIII site. pNTEP2 was digested with HindIII, the 5' overhang was filled in with Klenow Fragment DNA Polymerase I and religated. The desired plasmid (13.6 kbp) was identified by its restriction pattern.

Construction of plasmid pCB136

Plasmid pKSW1 was digested with NdeI and XbaI and the approximately 11.2 kbp fragment was ligated with plasmid pCB135 which had been digested with NdeI and XbaI. The ligation mixture was used to transform electrocompetent E. coli DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pCB136 was identified by its restriction pattern.

Construction of plasmid pCB137

Plasmid pCB136 was digested with SfuI and XbaI to remove a 6.5 kb fragment, the 5' overhangs were filled in with Klenow Fragment DNA Polymerase I and religated. The ligation mixture was used to transform electrocompetent E. coli DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pCB137 was identified by its restriction pattern.

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Construction of plasmid pCB121

Plasmid pPFL47 was digested with NdeI and HindIII and the approximately 4.4 kbp insert was ligated with plasmid pCB137 which had been digested with NdeI and HindIII. The ligation mixture was used to transform electrocompetent E. coli DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pCB121 was identified by its restriction pattern.

Example**10 Construction of *S.erythraea* JLK10/pCB121**

Approximately 5 µg plasmid pCB121 were used to transform protoplasts of *S. erythraea* JLK10 and stable thiostrepton resistant colonies were isolated. From several colonies total DNA was obtained and analysed by Southern blot hybridisation, to confirm that the plasmid had integrated into the homologous chromosomal DNA region. *S. erythraea* strain JLK10/pCB121 was used to inoculate SM3 medium containing 5 µg/ml thiostrepton (eryP medium containing 5 µg/ml thiostrepton gave similar results) and allowed to grow for seven to ten days at 28-30°C. After this time the broth was centrifuged and the pH of the supernatant adjusted to pH 9. The supernatant was then extracted three times with an equal volume of ethyl acetate and the solvent was removed by evaporation. Products were analysed by

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HPLC/MS, MS/MS and ¹H-NMR. The macrolide C13-methyl-10,11-dehydro-erythromycin A was identified (accompanied by products of incomplete processing by post-PKS enzymes):

Example

5 Construction of *S. erythraea* NRRL2338/pCB121

Approximately 5 µg plasmid pCB121 were used to transform protoplasts of *S. erythraea* NRRL2338 and stable thiostrepton resistant colonies were isolated. From several colonies total DNA was obtained and analysed by Southern blot hybridisation, to confirm that the plasmid had integrated into the homologous chromosomal DNA region. *S. erythraea* NRRL2338/pPFL50 was used to inoculate SM3 medium containing 5 µg/ml thiostrepton (eryP medium containing 5 µg/ml thiostrepton gave similar results) and allowed to grow for seven to ten days at 28-30°C. After this time the broth was centrifuged and the pH of the supernatant adjusted to pH=9.. The supernatant was then extracted three times with an equal volume of ethyl acetate and the solvent was removed by evaporation. Products were analysed by HPLC/MS, MS/MS and ¹H-NMR. The macrolide C13-erythromycin A was identified (accompanied by products of incomplete processing by post-PKS enzymes):

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Although the present invention is illustrated by the examples listed above, they should not be regarded as limiting the scope of the invention. The above descriptions illustrate for the first time the construction of a Type I PKS gene assembly containing a wholly or partly heterologous Ksq-containing loading module and its use to obtain polyketide products of utility as synthetic intermediates or as bioactive materials such as antibiotics. It will readily occur to the person skilled in the art that a wholly or partly heterologous Ksq-containing loading module from other PKS gene sets could be used to replace the loading module of DEBS, or indeed into a quite different PKS gene assembly. It will also readily occur to the person skilled in the art that the additional specificity provided by the more efficient discrimination made between methylmalonyl-CoA and malonyl-CoA by an Atq, followed by specific decarboxylation by a Ksq, is preferable to the imperfect discrimination between propionyl-CoA and acetyl-CoA that is a feature of the DEBS loading module and of many other PKS loading modules, in that it maximises the production of a single product rather than a mixture differing from each other in the nature of the starter unit. The avoidance of such mixtures increases yields and avoids the need for tedious and difficult separation procedures.

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Although the present invention is illustrated by the examples listed above, they should not be regarded as limiting the scope of the invention. The above descriptions illustrate for the first time the construction of a Type I PKS gene assembly containing a wholly or partly

5 heterologous KSq-containing loading module and its use to obtain polyketide products of utility as synthetic intermediates or as bioactive materials such as antibiotics. It will readily occur to the person skilled in the art that a wholly or partly heterologous KSq-

10 containing loading module from other PKS gene sets could be used to replace the loading module of DEBS, or indeed into a quite different PKS gene assembly. It will also readily occur to the person skilled in the art that that the additional specificity provided by the more efficient

15 discrimination made between methylmalonyl-CoA and malonyl-CoA by an ATq, followed by specific decarboxylation by a KSq, is preferable to the imperfect discrimination between propionyl-CoA and acetyl-CoA that is a feature of the DEBS loading module and of many other PKS loading modules, in

20 that it maximises the production of a single product rather than a mixture differing from each other in the nature of the starter unit. The avoidance of such mixtures increases yields and avoids the need for tedious and difficult separation procedures.

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Ile	Glu	Ala	Ala	Ala	Ile	Arg	Glu	Leu	Phe	Gly	Pro	Ser	Gly	Val	Pro			
305					310					315					320			
Val	Thr	Ala	Pro	Lys	Thr	Met	Thr	Gly	Arg	Leu	Tyr	Ser	Gly	Gly	Gly			
				325					330					335				
Pro	Leu	Asp	Leu	Val	Ala	Ala	Leu	Leu	Ala	Ile	Arg	Asp	Gly	Val	Ile			
			340					345					350					
Pro	Pro	Thr	Val	His	Thr	Ala	Glu	Pro	Val	Pro	Glu	His	Gln	Leu	Asp			
		355					360					365						
Leu	Val	Thr	Gly	Asp	Pro	Arg	His	Gln	Gln	Leu	Gly	Thr	Ala	Leu	Val			
	370					375					380							
Leu	Ala	Arg	Gly	Lys	Trp	Gly	Phe	Asn	Ser	Ala	Val	Val	Val	Arg	Gly			
385					390					395					400			
Val	Thr	Gly																

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<210> 5

<211> 415

<212> PRT

<213> Streptomyces violaceoruber

<400> 5

Met	Ser	Thr	Pro	Asp	Arg	Arg	Arg	Ala	Val	Val	Thr	Gly	Leu	Ser	Val
1				5					10					15	

Ala	Ala	Pro	Gly	Gly	Leu	Gly	Thr	Glu	Arg	Tyr	Trp	Lys	Ser	Leu	Leu
			20					25					30		

Thr	Gly	Glu	Asn	Gly	Ile	Ala	Glu	Leu	Ser	Arg	Phe	Asp	Ala	Ser	Arg
		35					40					45			

Tyr	Pro	Ser	Arg	Leu	Ala	Gly	Gln	Ile	Asp	Asp	Phe	Glu	Ala	Ser	Glu
	50					55					60				

His	Leu	Pro	Ser	Arg	Leu	Leu	Pro	Gln	Thr	Asp	Val	Ser	Thr	Arg	Tyr
65					70					75					80

Ala	Leu	Ala	Ala	Ala	Asp	Trp	Ala	Leu	Ala	Asp	Ala	Gly	Val	Gly	Pro
				85					90					95	

Glu	Ser	Gly	Leu	Asp	Asp	Tyr	Asp	Leu	Gly	Val	Val	Thr	Ser	Thr	Ala
			100					105					110		

Gln	Gly	Gly	Phe	Asp	Phe	Thr	His	Arg	Glu	Phe	His	Lys	Leu	Trp	Ser
		115					120					125			

Gln	Gly	Pro	Ala	Tyr	Val	Ser	Val	Tyr	Glu	Ser	Phe	Ala	Trp	Phe	Tyr
	130					135					140				

Ala	Val	Asn	Thr	Gly	Gln	Ile	Ser	Ile	Arg	Asn	Thr	Met	Arg	Gly	Pro
145					150					155					160

Ser	Ala	Ala	Leu	Val	Gly	Glu	Gln	Ala	Gly	Gly	Leu	Asp	Ala	Ile	Gly
			165						170					175	

His	Ala	Arg	Arg	Thr	Val	Arg	Arg	Gly	Pro	Gly	Trp	Cys	Ser	Ala	Val
			180					185					190		

Ala	Ser	Thr	Arg	Arg	Ser	Thr	Arg	Gly	Ala	Ser	Ser	Ser	Gln	Leu	Ser
		195					200					205			

Gly	Gly	Leu	Val	Ser	Thr	Val	Ala	Asp	Pro	Glu	Arg	Ala	Tyr	Leu	Pro
	210					215					220				

Phe	Asp	Val	Asp	Ala	Ser	Gly	Tyr	Val	Pro	Gly	Glu	Gly	Gly	Ala	Val
225					230					235					240

Leu	Ile	Val	Glu	Asp	Ala	Asp	Ser	Ala	Arg	Ala	Arg	Gly	Ala	Glu	Arg
				245					250					255	

Ile	Tyr	Val	Arg	Ser	Pro	Leu	Arg	Arg	Asp	Pro	Ala	Pro	Gly	Ser	Gly
		260						265					270		

Arg	Pro	Pro	Ala	Leu	Gly	Arg	Ala	Ala	Glu	Leu	Ala	Leu	Ala	Glu	Ala
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

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275		280		285
Gly Leu Thr Pro Ala Asp Ile Ser Val Val Phe Ala Asp Gly Ala Gly				
290		295		300
Val Pro Glu Leu Asp Arg Ala Glu Ala Asp Thr Leu Ala Arg Leu Phe				
305		310		315
Gly Pro Arg Gly Val Pro Val Thr Ala Pro Lys Ala Leu Thr Gly Arg				
	325		330	335
Leu Cys Ala Gly Gly Gly Pro Ala Asp Leu Ala Ala Ala Leu Leu Ala				
	340		345	350
Leu Arg Asp Gln Val Ile Pro Ala Thr Gly Arg His Arg Ala Val Pro				
	355		360	365
Asp Ala Tyr Ala Leu Asp Leu Val Thr Gly Arg Pro Arg Glu Ala Ala				
	370		375	380
Leu Ser Ala Ala Leu Val Leu Ala Arg Gly Arg His Gly Phe Asn Ser				
385		390		395
Ala Val Val Val Thr Leu Arg Gly Ser Asp His Arg Arg Pro Thr				
	405		410	415

<210> 6

<211> 409

<212> PRT

<213> Streptomyces nogalater

<400> 6

Met Thr Ala Ala Val Val Val Thr Gly Leu Gly Val Val Ala Pro Thr				
1		5		10
				15
Gly Leu Gly Val Arg Glu His Trp Ser Ser Thr Val Arg Gly Ala Ser				
	20		25	30
Ala Ile Gly Pro Val Thr Arg Phe Asp Ala Gly Arg Tyr Pro Ser Lys				
	35		40	45
Leu Ala Gly Glu Val Pro Gly Phe Val Pro Glu Asp His Leu Pro Ser				
	50		55	60
Arg Leu Met Pro Gln Thr Asp His Met Thr Arg Leu Ala Leu Val Ala				
	65		70	75
				80
Ala Asp Trp Ala Phe Gln Asp Ala Ala Val Asp Pro Ser Lys Leu Pro				
	85		90	95
Glu Tyr Gly Val Gly Val Val Thr Ala Ser Ser Ala Gly Gly Phe Glu				
	100		105	110
Phe Gly His Arg Glu Leu Gln Asn Leu Trp Ser Leu Gly Pro Gln Tyr				
	115		120	125
Val Ser Ala Tyr Gln Ser Phe Ala Trp Phe Tyr Ala Val Asn Thr Gly				
	130		135	140

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Gln Val Ser Ile Arg His Gly Leu Arg Gly Pro Gly Gly Val Leu Val
 145 150 155 160
 Thr Glu Gln Ala Gly Gly Leu Asp Ala Leu Gly Gln Ala Arg Arg Gln
 165 170 175
 Leu Arg Arg Gly Leu Pro Met Val Val Ala Gly Ala Val Asp Gly Ser
 180 185 190
 Pro Cys Pro Trp Gly Trp Val Ala Gln Leu Ser Ser Gly Gly Leu Ser
 195 200 205
 Thr Ser Asp Asp Pro Arg Arg Ala Tyr Leu Pro Phe Asp Ala Ala Ala
 210 215 220
 Gly Gly His Val Pro Gly Glu Gly Gly Ala Leu Leu Val Leu Glu Ser
 225 230 235 240
 Asp Glu Ser Ala Arg Ala Arg Gly Val Thr Arg Trp Tyr Gly Arg Ile
 245 250 255
 Asp Gly Tyr Ala Ala Thr Phe Asp Pro Pro Pro Gly Ser Gly Arg Pro
 260 265 270
 Pro Asn Leu Leu Arg Ala Ala Gln Ala Ala Leu Asp Asp Ala Glu Val
 275 280 285
 Gly Pro Glu Ala Val Asp Val Val Phe Ala Asp Ala Ser Gly Thr Pro
 290 295 300
 Asp Glu Asp Ala Ala Glu Ala Asp Ala Val Arg Arg Leu Phe Gly Pro
 305 310 315 320
 Tyr Gly Val Pro Val Thr Ala Pro Lys Thr Met Thr Gly Arg Leu Ser
 325 330 335
 Ala Gly Gly Ala Ala Leu Asp Val Ala Thr Ala Leu Leu Ala Leu Arg
 340 345 350
 Glu Gly Val Val Pro Pro Thr Val Asn Val Ser Arg Pro Arg Pro Glu
 355 360 365
 Tyr Glu Leu Asp Leu Val Leu Ala Pro Arg Arg Thr Pro Leu Ala Arg
 370 375 380
 Ala Leu Val Leu Ala Arg Gly Arg Gly Gly Phe Asn Ala Ala Met Val
 385 390 395 400
 Val Ala Gly Pro Arg Ala Glu Thr Arg
 405

<210> 7

<211> 409

<212> PRT

<213> Streptomyces glaucescens

<400> 7

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Met	Ser	Ala	Pro	Ala	Pro	Val	Val	Val	Thr	Gly	Leu	Gly	Ile	Val	Ala	
1				5					10					15		
Pro	Asn	Gly	Thr	Gly	Thr	Glu	Glu	Tyr	Trp	Ala	Ala	Thr	Leu	Ala	Gly	
			20					25					30			
Lys	Ser	Gly	Ile	Asp	Val	Ile	Gln	Arg	Phe	Asp	Pro	His	Gly	Tyr	Pro	
		35					40					45				
Val	Arg	Val	Gly	Gly	Glu	Val	Leu	Ala	Phe	Asp	Ala	Ala	Ala	His	Leu	
	50					55					60					
Pro	Gly	Arg	Leu	Leu	Pro	Gln	Thr	Asp	Arg	Met	Thr	Gln	His	Ala	Leu	
65					70					75					80	
Val	Ala	Ala	Glu	Trp	Ala	Leu	Ala	Asp	Ala	Gly	Leu	Glu	Pro	Glu	Lys	
				85					90					95		
Gln	Asp	Glu	Tyr	Gly	Leu	Gly	Val	Leu	Thr	Ala	Ala	Gly	Ala	Gly	Gly	
			100					105					110			
Phe	Glu	Phe	Gly	Gln	Arg	Glu	Met	Gln	Lys	Leu	Trp	Gly	Thr	Gly	Pro	
		115					120					125				
Glu	Arg	Val	Ser	Ala	Tyr	Gln	Ser	Phe	Ala	Trp	Phe	Tyr	Ala	Val	Asn	
	130					135					140					
Thr	Gly	Gln	Ile	Ser	Ile	Arg	His	Gly	Met	Arg	Gly	His	Ser	Ser	Val	
145					150					155					160	
Phe	Val	Thr	Glu	Gln	Ala	Gly	Gly	Leu	Asp	Ala	Ala	Ala	His	Ala	Ala	
				165					170					175		
Arg	Leu	Leu	Arg	Lys	Gly	Thr	Leu	Asn	Thr	Ala	Leu	Thr	Gly	Gly	Cys	
			180					185					190			
Glu	Ala	Ser	Leu	Cys	Pro	Trp	Gly	Leu	Val	Ala	Gln	Ile	Pro	Ser	Gly	
		195					200					205				
Phe	Leu	Ser	Glu	Ala	Thr	Asp	Pro	His	Asp	Ala	Tyr	Leu	Pro	Phe	Asp	
	210					215					220					
Ala	Arg	Ala	Ala	Gly	Tyr	Val	Pro	Gly	Glu	Gly	Gly	Ala	Met	Leu	Val	
225					230					235				240		
Ala	Glu	Arg	Ala	Asp	Ser	Ala	Arg	Glu	Arg	Asp	Ala	Ala	Thr	Val	Tyr	
				245					250					255		
Gly	Arg	Ile	Ala	Gly	His	Ala	Ser	Thr	Phe	Asp	Ala	Arg	Pro	Gly	Thr	
			260					265					270			
Gly	Arg	Pro	Thr	Gly	Pro	Ala	Arg	Ala	Ile	Arg	Leu	Ala	Leu	Glu	Glu	
		275					280					285				
Ala	Arg	Val	Ala	Pro	Glu	Asp	Val	Asp	Val	Val	Tyr	Ala	Asp	Ala	Ala	
	290					295					300					
Gly	Val	Pro	Ala	Leu	Asp	Arg	Ala	Glu	Ala	Glu	Ala	Leu	Ala	Glu	Val	
305					310					315					320	

<400> 8															
Met	Thr	Pro	Val	Ala	Val	Thr	Gly	Met	Gly	Ile	Ala	Ala	Pro	Asn	Gly
1				5					10					15	
Leu	Gly	Arg	Pro	Thr	Thr	Gly	Arg	Pro	Pro	Trp	Ala	Pro	Arg	Ala	Ala
			20					25					30		
Ser	Ala	Ala	Ser	Thr	Arg	Phe	Asp	Pro	Ser	Gly	Tyr	Pro	Ala	Gln	Leu
		35					40					45			
Ala	Gly	Glu	Ile	Pro	Gly	Phe	Arg	Ala	Ala	Glu	His	Leu	Pro	Gly	Arg
	50					55					60				
Leu	Val	Pro	Gln	Thr	Asp	Arg	Val	Thr	Arg	Leu	Ser	Leu	Ala	Ala	Ala
65					70					75					80
Asp	Trp	Ala	Leu	Ala	Asp	Ala	Gly	Val	Glu	Val	Ala	Ala	Phe	Asp	Pro
				85					90					95	
Leu	Asp	Met	Gly	Val	Val	Thr	Ala	Ser	His	Ala	Gly	Gly	Phe	Glu	Phe
			100					105					110		
Gly	Gln	Asp	Glu	Leu	Gln	Lys	Leu	Leu	Gly	Gln	Gly	Gln	Pro	Val	Leu
		115					120					125			
Ser	Ala	Tyr	Gln	Ser	Phe	Ala	Trp	Phe	Tyr	Ala	Val	Asn	Ser	Gly	Gln
	130					135					140				
Ile	Ser	Ile	Arg	His	Gly	Met	Lys	Gly	Pro	Ser	Gly	Val	Val	Val	Ser
145					150					155					160
Asp	Gln	Ala	Gly	Gly	Leu	Asp	Ala	Leu	Ala	Gln	Ala	Arg	Arg	Leu	Val
				165					170					175	
Arg	Lys	Gly	Thr	Pro	Leu	Ile	Val	Cys	Gly	Ala	Val	Glu	Pro	Arg	Ser

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180					185					190					
Ala	Pro	Gly	Ala	Gly	Ser	Pro	Ser	Ser	Pro	Ala	Gly	Gly	Met	Ser	Asp
		195					200					205			
Ser	Asp	Glu	Pro	Asn	Arg	Ala	Tyr	Leu	Pro	Phe	Asp	Arg	Asp	Gly	Arg
	210					215					220				
Gly	Tyr	Val	Pro	Gly	Gly	Gly	Arg	Gly	Val	Val	Pro	Pro	Leu	Glu	Arg
225					230					235					240
Ala	Glu	Ala	Ala	Pro	Ala	Arg	Gly	Ala	Glu	Val	Tyr	Gly	Glu	Ala	Gly
				245					250					255	
Pro	Leu	Ala	Arg	Leu	Pro	Ala	Pro	His	Ser	Gly	Arg	Gly	Ser	Thr	Arg
			260					265					270		
Ala	His	Ala	Ile	Arg	Thr	Ala	Leu	Asp	Asp	Ala	Gly	Thr	Ala	Pro	Gly
		275					280					285			
Asp	Ile	Arg	Arg	Val	Phe	Ala	Asp	Gly	Gly	Gly	Arg	Tyr	Pro	Asn	Asp
	290					295					300				
Arg	Ala	Glu	Ala	Glu	Ala	Ile	Ser	Glu	Val	Phe	Gly	Pro	Gly	Arg	Val
305					310					315					320
Pro	Val	Thr	Cys	Pro	Arg	Thr	Met	Thr	Gly	Arg	Leu	His	Ser	Gly	Ala
				325					330					335	
Ala	Pro	Leu	Asp	Val	Ala	Cys	Ala	Leu	Leu	Ala	Met	Arg	Ala	Gly	Val
			340					345					350		
Ile	Pro	Pro	Thr	Val	His	Ile	Asp	Pro	Cys	Pro	Glu	Tyr	Asp	Leu	Asp
		355					360					365			
Leu	Val	Leu	Tyr	Gln	Val	Arg	Pro	Ala	Ala	Leu	Arg	Thr	Ala	Leu	Gly
	370					375					380				
Gly	Ala	Arg	Gly	His	Gly	Gly	Phe	Asn	Ser	Ala	Leu	Val	Val	Arg	Ala
385					390					395					400
Gly Gln															

<210> 9

<211> 404

<212> PRT

<213> Streptomyces venezuelae

<400> 9

Met	Ser	Ala	Ser	Val	Val	Val	Thr	Gly	Leu	Gly	Val	Ala	Ala	Pro	Asn
1				5					10					15	

Gly	Leu	Gly	Arg	Glu	Asp	Phe	Trp	Ala	Ser	Thr	Leu	Gly	Gly	Lys	Ser
			20					25					30		

Gly	Ile	Gly	Pro	Leu	Thr	Arg	Phe	Asp	Pro	Thr	Gly	Tyr	Pro	Ala	Arg
		35					40					45			

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Leu Ala Gly Glu Val Pro Gly Phe Ala Ala Glu Glu His Leu Pro Ser
 50 55 60

Arg Leu Leu Pro Gln Thr Asp Arg Met Thr Arg Leu Ala Leu Val Ala
 65 70 75 80

Ala Asp Trp Ala Leu Ala Asp Ala Gly Val Arg Pro Glu Glu Gln Asp
 85 90 95

Asp Phe Asp Met Gly Val Val Thr Ala Ser Ala Ser Gly Gly Phe Glu
 100 105 110

Phe Gly Gln Gly Glu Leu Gln Lys Leu Trp Ser Gln Gly Ser Gln Tyr
 115 120 125

Val Ser Ala Tyr Gln Ser Phe Ala Trp Phe Tyr Ala Val Asn Ser Gly
 130 135 140

Gln Ile Ser Ile Arg Asn Gly Met Lys Gly Pro Ser Gly Val Val Val
 145 150 155 160

Ser Asp Gln Ala Gly Gly Leu Asp Ala Val Ala Gln Ala Arg Arg Gln
 165 170 175

Ile Arg Lys Gly Thr Arg Leu Ile Val Ser Gly Gly Val Asp Ala Ser
 180 185 190

Leu Cys Pro Trp Gly Trp Val Ala His Val Ala Ser Asp Arg Leu Ser
 195 200 205

Thr Ser Glu Glu Pro Ala Arg Gly Tyr Leu Pro Phe Asp Arg Glu Ala
 210 215 220

Gln Gly His Val Pro Gly Glu Gly Gly Ala Ile Leu Val Met Glu Ala
 225 230 235 240

Ala Glu Ala Ala Arg Glu Arg Gly Ala Arg Ile Tyr Gly Glu Ile Ala
 245 250 255

Gly Tyr Gly Ser Thr Phe Asp Pro Arg Pro Gly Ser Gly Arg Glu Pro
 260 265 270

Gly Leu Arg Lys Ala Ile Glu Leu Ala Leu Ala Asp Ala Gly Ala Ala
 275 280 285

Pro Gly Asp Ile Asp Val Val Phe Ala Asp Ala Ala Ala Val Pro Glu
 290 295 300

Leu Asp Arg Val Glu Ala Glu Ala Leu Asn Ala Val Phe Gly Thr Gly
 305 310 315 320

Ala Val Pro Val Thr Ala Pro Lys Thr Met Thr Gly Arg Leu Tyr Ser
 325 330 335

Gly Ala Ala Pro Leu Asp Leu Ala Ala Ala Phe Leu Ala Met Asp Glu
 340 345 350

Gly Val Ile Pro Pro Thr Val Asn Val Glu Pro Asp Ala Ala Tyr Gly
 355 360 365

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Leu Asp Leu Val Val Gly Gly Pro Arg Thr Ala Glu Val Asn Thr Ala
 370 375 380

Leu Val Ile Ala Arg Gly His Gly Gly Phe Asn Ser Ala Met Val Val
 385 390 395 400

Arg Ser Ala Asn

<210> 10

<211> 424

<212> PRT

<213> Streptomyces coelicolor

<400> 10

Met Ser Gly Pro Gln Arg Thr Gly Thr Gly Gly Gly Ser Arg Arg Ala
 1 5 10 15

Val Val Thr Gly Leu Gly Val Leu Ser Pro His Gly Thr Gly Val Glu
 20 25 30

Ala His Trp Lys Ala Val Ala Asp Gly Thr Ser Ser Leu Gly Pro Val
 35 40 45

Thr Arg Glu Gly Cys Ala His Leu Pro Leu Arg Val Ala Gly Glu Val
 50 55 60

His Gly Phe Asp Ala Ala Glu Thr Val Glu Asp Arg Phe Leu Val Gln
 65 70 75 80

Thr Asp Arg Phe Thr His Phe Ala Leu Ser Ala Thr Gln His Ala Leu
 85 90 95

Ala Asp Ala Arg Phe Gly Arg Ala Asp Val Asp Ser Pro Tyr Ser Val
 100 105 110

Gly Val Val Thr Ala Ala Gly Ser Gly Gly Gly Glu Phe Gly Gln Arg
 115 120 125

Glu Leu Gln Asn Leu Trp Gly His Gly Ser Arg His Val Gly Pro Tyr
 130 135 140

Gln Ser Ile Ala Trp Phe Tyr Ala Ala Ser Thr Gly Gln Val Ser Ile
 145 150 155 160

Arg Asn Asp Phe Lys Gly Pro Cys Gly Val Val Ala Ala Asp Glu Ala
 165 170 175

Gly Gly Leu Asp Ala Leu Ala His Ala Ala Leu Ala Val Arg Asn Gly
 180 185 190

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

Ser Ile Val Cys Gln Leu Gly Tyr Pro Glu Leu Ser Arg Ala Thr Glu
 210 215 220

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Pro Asp Arg Ala Tyr Arg Pro Phe Thr Glu Ala Ala Cys Gly Phe Ala
 225 230 235 240

Pro Ala Glu Gly Gly Ala Val Leu Val Val Glu Glu Glu Ala Ala Ala
 245 250 255

Arg Glu Arg Gly Ala Asp Val Arg Ala Thr Val Ala Gly His Ala Ala
 260 265 270

Thr Phe Thr Gly Ala Gly Arg Trp Ala Glu Ser Arg Glu Gly Leu Ala
 275 280 285

Arg Ala Ile Gln Gly Ala Leu Ala Glu Ala Gly Cys Arg Pro Glu Glu
 290 295 300

Val Asp Val Val Phe Ala Asp Ala Leu Gly Val Pro Glu Ala Asp Arg
 305 310 315 320

Ala Glu Ala Leu Ala Leu Ala Asp Ala Leu Gly Pro His Ala Ala Arg
 325 330 335

Val Pro Val Thr Ala Pro Lys Thr Gly Thr Gly Arg Ala Tyr Cys Ala
 340 345 350

Ala Pro Val Leu Asp Val Ala Thr Ala Val Leu Ala Met Glu His Gly
 355 360 365

Leu Ile Pro Pro Thr Pro His Val Leu Asp Val Cys His Asp Leu Asp
 370 375 380

Leu Val Thr Gly Arg Ala Arg Pro Ala Glu Pro Arg Thr Ala Leu Val
 385 390 395 400

Leu Ala Arg Gly Leu Met Gly Ser Asn Ser Ala Leu Val Leu Arg Arg
 405 410 415

Gly Ala Val Pro Pro Glu Gly Arg
 420

<210> 11

<211> 421

<212> PRT

<213> Streptomyces violaceoruber

<400> 11

Met Thr Arg Arg Val Val Ile Thr Gly Val Gly Val Arg Ala Pro Gly
 1 5 10 15

Gly Ser Gly Thr Lys Glu Phe Trp Asp Leu Leu Thr Ala Gly Arg Thr
 20 25 30

Ala Thr Arg Pro Ile Ser Phe Phe Asp Ala Ser Pro Phe Arg Ser Arg
 35 40 45

Ile Ala Gly Glu Ile Asp Phe Asp Ala Val Ala Glu Gly Phe Ser Pro
 50 55 60

Arg Glu Val Arg Arg Met Asp Arg Ala Thr Gln Phe Ala Val Ala Cys

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65					70					75					80
Thr	Arg	Asp	Ala	Leu	Ala	Asp	Ser	Gly	Leu	Asp	Thr	Gly	Ala	Leu	Asp
				85					90					95	
Pro	Ser	Arg	Ile	Gly	Val	Ala	Leu	Gly	Ser	Ala	Val	Ala	Ser	Ala	Thr
			100					105					110		
Ser	Leu	Glu	Asn	Glu	Tyr	Leu	Val	Met	Ser	Asp	Ser	Gly	Arg	Glu	Trp
		115					120					125			
Leu	Val	Asp	Pro	Ala	His	Leu	Ser	Pro	Met	Met	Phe	Asp	Tyr	Leu	Ser
	130					135					140				
Pro	Gly	Val	Met	Pro	Ala	Glu	Val	Ala	Trp	Ala	Ala	Gly	Ala	Glu	Gly
145					150					155				160	
Pro	Val	Thr	Met	Val	Ser	Asp	Gly	Cys	Thr	Ser	Gly	Leu	Asp	Ser	Val
				165					170					175	
Gly	Tyr	Ala	Val	Gln	Gly	Thr	Arg	Glu	Gly	Ser	Ala	Asp	Val	Val	Val
			180					185					190		
Ala	Gly	Ala	Ala	Asp	Thr	Pro	Val	Ser	Pro	Ile	Val	Val	Ala	Cys	Phe
		195					200					205			
Asp	Ala	Ile	Lys	Ala	Thr	Thr	Pro	Arg	Asn	Asp	Asp	Pro	Ala	His	Ala
	210					215					220				
Ser	Arg	Pro	Phe	Asp	Gly	Thr	Arg	Asn	Gly	Phe	Val	Leu	Ala	Glu	Gly
225					230					235				240	
Ala	Ala	Met	Phe	Val	Leu	Glu	Glu	Tyr	Glu	Ala	Ala	Gln	Arg	Arg	Gly
				245					250					255	
Ala	His	Ile	Tyr	Ala	Glu	Val	Gly	Gly	Tyr	Ala	Thr	Arg	Ser	Gln	Ala
			260					265					270		
Tyr	His	Met	Thr	Gly	Leu	Lys	Lys	Asp	Gly	Arg	Glu	Met	Ala	Glu	Ser
		275					280					285			
Ile	Arg	Ala	Ala	Leu	Asp	Glu	Ala	Arg	Leu	Asp	Arg	Thr	Ala	Val	Asp
	290					295					300				
Tyr	Val	Asn	Ala	His	Gly	Ser	Gly	Thr	Lys	Gln	Asn	Asp	Arg	His	Glu
305					310					315				320	
Thr	Ala	Ala	Phe	Lys	Arg	Ser	Leu	Gly	Glu	His	Ala	Tyr	Ala	Val	Pro
				325					330					335	
Val	Ser	Ser	Ile	Lys	Ser	Met	Gly	Gly	His	Ser	Leu	Gly	Ala	Ile	Gly
			340					345					350		
Ser	Ile	Glu	Ile	Ala	Ala	Ser	Val	Leu	Ala	Ile	Glu	His	Asn	Val	Val
		355					360					365			
Pro	Pro	Thr	Ala	Asn	Leu	His	Thr	Pro	Asp	Pro	Glu	Cys	Asp	Leu	Asp
	370					375					380				
Tyr	Val	Pro	Leu	Thr	Ala	Arg	Glu	Gln	Arg	Val	Asp	Thr	Val	Leu	Thr

<400> 12															
Met	Thr	Arg	Arg	Val	Val	Ile	Thr	Gly	Val	Gly	Val	Arg	Ala	Pro	Gly
1				5					10					15	
Gly	Leu	Gly	Ala	Lys	Asn	Phe	Trp	Glu	Leu	Leu	Thr	Ser	Gly	Arg	Thr
			20					25					30		
Ala	Thr	Arg	Arg	Ile	Ser	Phe	Phe	Asp	Pro	Thr	Pro	Asn	Arg	Ser	Gln
		35					40					45			
Ile	Ala	Ala	Glu	Cys	Asp	Phe	Asp	Pro	Glu	His	Glu	Gly	Leu	Ser	Pro
	50					55					60				
Arg	Glu	Ile	Arg	Arg	Met	Asp	Arg	Ala	Ala	Gln	Phe	Ala	Val	Val	Cys
65					70					75					80
Thr	Arg	Asp	Ala	Val	Ala	Asp	Ser	Gly	Leu	Glu	Phe	Glu	Gln	Val	Pro
				85					90					95	
Pro	Glu	Arg	Ile	Gly	Val	Ser	Leu	Gly	Ser	Ala	Val	Ala	Ala	Ala	Thr
			100					105					110		
Ser	Leu	Glu	Gln	Glu	Tyr	Leu	Val	Leu	Ser	Asp	Gly	Gly	Arg	Glu	Trp
		115					120					125			
Gln	Val	Asp	Pro	Ala	Tyr	Leu	Ser	Ala	His	Met	Phe	Asp	Tyr	Leu	Ser
	130					135					140				
Pro	Gly	Val	Met	Pro	Ala	Glu	Val	Ala	Trp	Thr	Val	Gly	Ala	Glu	Gly
145					150					155					160
Pro	Val	Ala	Met	Val	Ser	Asp	Gly	Cys	Thr	Ser	Gly	Leu	Asp	Ser	Leu
				165				170						175	
Ser	His	Ala	Cys	Ser	Leu	Ile	Ala	Glu	Gly	Thr	Thr	Asp	Val	Met	Val
			180					185					190		
Ala	Gly	Ala	Ala	Asp	Thr	Pro	Ile	Thr	Pro	Ile	Val	Val	Ser	Cys	Phe
		195					200					205			
Asp	Ala	Ile	Lys	Ala	Thr	Thr	Pro	Arg	Asn	Asp	Asp	Pro	Glu	His	Ala
	210					215					220				
Ser	Arg	Pro	Phe	Asp	Asn	Ser	Arg	Asn	Gly	Phe	Val	Leu	Ala	Glu	Gly
225					230					235					240

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Ala Ala Leu Phe Val Leu Glu Glu Leu Glu His Ala Arg Ala Arg Gly
 245 250 255
 Ala His Val Tyr Ala Glu Ile Ser Gly Cys Ala Thr Arg Leu Asn Ala
 260 265 270
 Tyr His Met Thr Gly Leu Lys Thr Asp Gly Arg Glu Met Ala Glu Ala
 275 280 285
 Ile Arg Val Ala Leu Asp Leu Ala Arg Ile Asp Pro Thr Asp Ile Asp
 290 295 300
 Tyr Ile Asn Ala His Gly Ser Gly Thr Lys Gln Asn Asp Arg His Glu
 305 310 315 320
 Thr Ala Ala Phe Lys Arg Ser Leu Gly Glu His Ala Tyr Arg Thr Pro
 325 330 335
 Val Ser Ser Ile Lys Ser Met Val Gly His Ser Leu Gly Ala Ile Gly
 340 345 350
 Ser Ile Glu Val Ala Ala Cys Ala Leu Ala Ile Glu His Gly Val Val
 355 360 365
 Pro Pro Thr Ala Asn Leu His Glu Pro Asp Pro Glu Cys Asp Leu Asp
 370 375 380
 Tyr Val Pro Leu Thr Ala Arg Glu Gln Arg Val Asp Thr Val Leu Ser
 385 390 395 400
 Val Gly Ser Gly Phe Gly Gly Phe Gln Ser Ala Met Val Leu Arg Arg
 405 410 415
 Leu Gly Gly Ala Asn Ser
 420

<210> 13
 <211> 424
 <212> PRT
 <213> Streptomyces coelicolor

<400> 13
 Met Lys Arg Arg Val Val Ile Thr Gly Val Gly Val Arg Ala Pro Gly
 1 5 10 15
 Gly Asn Gly Thr Arg Gln Phe Trp Glu Leu Leu Thr Ser Gly Arg Thr
 20 25 30
 Ala Thr Arg Arg Ile Ser Phe Phe Asp Pro Ser Pro Tyr Arg Ser Gln
 35 40 45
 Val Ala Ala Glu Ala Asp Phe Asp Pro Val Ala Glu Gly Phe Gly Pro
 50 55 60
 Arg Glu Leu Asp Arg Met Asp Arg Ala Ser Gln Phe Ala Val Ala Cys
 65 70 75 80

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Ala	Arg	Glu	Ala	Phe	Ala	Ala	Ser	Gly	Leu	Asp	Pro	Asp	Thr	Leu	Asp	85	90	95	
Pro	Ala	Arg	Val	Gly	Val	Ser	Leu	Gly	Ser	Ala	Val	Ala	Ala	Ala	Thr	100	105	110	
Ser	Leu	Glu	Arg	Glu	Tyr	Leu	Leu	Leu	Ser	Asp	Ser	Gly	Arg	Asp	Trp	115	120	125	
Glu	Val	Asp	Ala	Ala	Trp	Leu	Ser	Arg	His	Met	Phe	Asp	Tyr	Leu	Val	130	135	140	
Pro	Ser	Val	Met	Pro	Ala	Glu	Val	Ala	Trp	Ala	Val	Gly	Ala	Glu	Gly	145	150	155	160
Pro	Val	Thr	Met	Val	Ser	Thr	Gly	Cys	Thr	Ser	Gly	Leu	Asp	Ser	Val	165	170	175	
Gly	Asn	Ala	Val	Arg	Ala	Ile	Glu	Glu	Gly	Ser	Ala	Asp	Val	Met	Phe	180	185	190	
Ala	Gly	Ala	Ala	Asp	Thr	Pro	Ile	Thr	Pro	Ile	Val	Val	Ala	Cys	Phe	195	200	205	
Asp	Ala	Ile	Arg	Ala	Thr	Thr	Ala	Arg	Asn	Asp	Asp	Pro	Glu	His	Ala	210	215	220	
Ser	Arg	Pro	Phe	Asp	Gly	Thr	Arg	Asp	Gly	Phe	Val	Leu	Ala	Glu	Gly	225	230	235	240
Ala	Ala	Met	Phe	Val	Leu	Glu	Asp	Tyr	Asp	Ser	Ala	Leu	Ala	Arg	Gly	245	250	255	
Ala	Arg	Ile	His	Ala	Glu	Ile	Ser	Gly	Tyr	Ala	Thr	Arg	Cys	Asn	Ala	260	265	270	
Tyr	His	Met	Thr	Gly	Leu	Lys	Ala	Asp	Gly	Arg	Glu	Met	Ala	Glu	Thr	275	280	285	
Ile	Arg	Val	Ala	Leu	Asp	Glu	Ser	Arg	Thr	Asp	Ala	Thr	Asp	Ile	Asp	290	295	300	
Tyr	Ile	Asn	Ala	His	Gly	Ser	Gly	Thr	Arg	Gln	Asn	Asp	Arg	His	Glu	305	310	315	320
Thr	Ala	Ala	Tyr	Lys	Arg	Ala	Leu	Gly	Glu	His	Ala	Arg	Arg	Thr	Pro	325	330	335	
Val	Ser	Ser	Ile	Lys	Ser	Met	Val	Gly	His	Ser	Leu	Gly	Ala	Ile	Gly	340	345	350	
Ser	Leu	Glu	Ile	Ala	Ala	Cys	Val	Leu	Ala	Leu	Glu	His	Gly	Val	Val	355	360	365	
Pro	Pro	Thr	Ala	Asn	Leu	Arg	Thr	Ser	Asp	Pro	Glu	Cys	Asp	Leu	Asp	370	375	380	
Tyr	Val	Pro	Leu	Glu	Ala	Arg	Glu	Arg	Lys	Leu	Arg	Ser	Val	Leu	Thr	385	390	395	400

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Val Gly Ser Gly Phe Gly Gly Phe Gln Ser Ala Met Val Leu Arg Asp
 405 410 415

Ala Glu Thr Ala Gly Ala Ala Ala
 420

<210> 14

<211> 420

<212> PRT

<213> Streptomyces cinnamonensis

<400> 14

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

Gly Gly Leu Gly Arg Lys Glu Phe Trp Gln Leu Leu Ser Glu Gly Arg
 20 25 30

Thr Ala Thr Arg Gly Ile Thr Phe Phe Asp Pro Ala Pro Phe Arg Ser
 35 40 45

Lys Val Ala Ala Glu Ala Asp Phe Cys Gly Leu Glu Asn Gly Leu Ser
 50 55 60

Pro Gln Glu Val Arg Arg Met Asp Arg Ala Ala Gln Phe Ala Val Val
 65 70 75 80

Thr Ala Arg Ala Val Glu Asp Ser Gly Ala Glu Leu Ala Ala His Pro
 85 90 95

Pro His Arg Ile Gly Val Val Val Gly Ser Ala Val Gly Ala Thr Met
 100 105 110

Gly Leu Asp Asn Glu Tyr Arg Val Val Ser Asp Gly Gly Arg Leu Asp
 115 120 125

Leu Val Asp His Arg Tyr Ala Val Pro His Leu Tyr Asn Tyr Leu Val
 130 135 140

Pro Ser Ser Phe Ala Ala Glu Val Ala Trp Ala Val Gly Ala Glu Gly
 145 150 155 160

Pro Ser Thr Val Val Ser Thr Gly Cys Thr Ser Gly Ile Asp Ala Val
 165 170 175

Gly Ile Ala Val Glu Leu Val Arg Glu Gly Ser Val Asp Val Met Val
 180 185 190

Ala Gly Ala Val Asp Ala Pro Ile Ser Pro Ile Pro Cys Val Leu Asp
 195 200 205

Ala Ile Lys Ala Thr Thr Pro Arg His Asp Ala Pro Ala Thr Ala Ser
 210 215 220

Arg Pro Phe Asp Ser Thr Arg Asn Gly Phe Val Leu Gly Glu Gly Ala
 225 230 235 240

Ala Phe Phe Val Leu Glu Glu Leu His Ser Ala Arg Arg Arg Gly Ala

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	245		250		255
His Ile Tyr	Ala Glu Ile Ala Gly Tyr	Ala Thr Arg Ser Asn Ala Tyr			
	260		265		270
His Met Thr Gly Leu Arg Asp Gly Ala Glu Met Ala Glu Ala Ile Arg					
	275		280		285
Leu Ala Leu Asp Glu Ala Arg Leu Asn Pro Glu Gln Val Asp Tyr Ile					
	290		295		300
Asn Ala His Gly Ser Gly Thr Lys Gln Asn Asp Arg His Glu Thr Ala					
305		310		315	320
Ala Phe Lys Lys Ala Leu Gly Glu His Ala Tyr Arg Thr Pro Val Ser					
	325		330		335
Ser Ile Lys Ser Met Val Gly His Ser Leu Gly Ala Ile Gly Ser Ile					
	340		345		350
Glu Ile Ala Ala Ser Ala Leu Ala Met Glu Tyr Asp Val Val Pro Pro					
	355		360		365
Thr Ala Asn Leu His Thr Pro Asp Pro Glu Cys Asp Leu Asp Tyr Val					
	370		375		380
Pro Leu Thr Ala Arg Asp Gln Arg Val Asp Ser Val Leu Thr Val Gly					
385		390		395	400
Ser Gly Phe Gly Gly Phe Gln Ser Ala Met Val Leu Thr Ser Ala Gln					
	405		410		415
Arg Ser Thr Val					
	420				

<210> 15

<211> 422

<212> PRT

<213> Streptomyces venezuelae

<400> 15

Met Thr Ala Arg Arg Val Val Ile Thr Gly Ile Glu Val Leu Ala Pro														
1				5				10					15	
Gly Gly Thr Gly Ser Lys Ala Phe Trp Asn Leu Leu Ser Glu Gly Arg														
			20					25					30	
Thr Ala Thr Arg Gly Ile Thr Phe Phe Asp Pro Thr Pro Phe Arg Ser														
			35				40					45		
Arg Val Ala Ala Glu Ile Asp Phe Asp Pro Glu Ala His Gly Leu Ser														
	50				55				60					
Pro Gln Glu Ile Arg Arg Met Asp Arg Ala Ala Gln Phe Ala Val Val														
65				70				75					80	
Ala Ala Arg Ala Val Ala Asp Ser Gly Ile Asp Leu Ala Ala His Asp														
			85				90						95	

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Pro	Tyr	Arg	Val	Gly	Val	Thr	Val	Gly	Ser	Ala	Val	Gly	Ala	Thr	Met	100	105	110	
Gly	Leu	Asp	Glu	Glu	Tyr	Arg	Val	Val	Ser	Asp	Gly	Gly	Arg	Leu	Asp	115	120	125	
Leu	Val	Asp	His	Ala	Tyr	Ala	Val	Pro	His	Leu	Tyr	Asp	Tyr	Met	Val	130	135	140	
Pro	Ser	Ser	Phe	Ser	Ala	Glu	Val	Ala	Trp	Ala	Val	Gly	Ala	Glu	Gly	145	150	155	160
Pro	Asn	Thr	Val	Val	Ser	Thr	Gly	Cys	Thr	Ser	Gly	Leu	Asp	Ser	Val	165	170	175	
Gly	Tyr	Ala	Arg	Gly	Glu	Leu	Ile	Arg	Glu	Gly	Ser	Ala	Asp	Val	Met	180	185	190	
Ile	Ala	Gly	Ser	Ser	Asp	Ala	Pro	Ile	Ser	Pro	Ile	Thr	Met	Ala	Cys	195	200	205	
Phe	Asp	Ala	Ile	Lys	Ala	Thr	Thr	Asn	Arg	Tyr	Asp	Asp	Pro	Ala	His	210	215	220	
Ala	Ser	Arg	Pro	Phe	Asp	Gly	Thr	Arg	Asn	Gly	Phe	Val	Leu	Gly	Glu	225	230	235	240
Gly	Ala	Ala	Val	Phe	Val	Leu	Glu	Glu	Leu	Glu	Ser	Ala	Arg	Ala	Arg	245	250	255	
Gly	Ala	His	Ile	Tyr	Ala	Glu	Ile	Ala	Gly	Tyr	Ala	Thr	Arg	Ser	Asn	260	265	270	
Ala	Tyr	His	Met	Thr	Gly	Leu	Arg	Pro	Asp	Gly	Ala	Glu	Met	Ala	Glu	275	280	285	
Ala	Ile	Arg	Val	Ala	Leu	Asp	Glu	Ala	Arg	Met	Asn	Pro	Thr	Glu	Ile	290	295	300	
Asp	Tyr	Ile	Asn	Ala	His	Gly	Ser	Gly	Thr	Lys	Gln	Asn	Asp	Arg	His	305	310	315	320
Glu	Thr	Ala	Ala	Phe	Lys	Lys	Ser	Leu	Gly	Asp	His	Ala	Tyr	Arg	Thr	325	330	335	
Pro	Val	Ser	Ser	Ile	Lys	Ser	Met	Val	Gly	His	Ser	Leu	Gly	Ala	Ile	340	345	350	
Gly	Ser	Ile	Glu	Ile	Ala	Ala	Ser	Ala	Leu	Ala	Met	Glu	His	Asn	Val	355	360	365	
Val	Pro	Pro	Thr	Gly	Asn	Leu	His	Thr	Pro	Asp	Pro	Glu	Cys	Asp	Leu	370	375	380	
Asp	Tyr	Val	Arg	Ser	Cys	Arg	Glu	Gln	Leu	Thr	Asp	Ser	Val	Leu	Thr	385	390	395	400
Val	Gly	Ser	Gly	Phe	Gly	Gly	Phe	Gln	Ser	Ala	Met	Val	Leu	Ala	Arg	405	410	415	

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Pro Glu Arg Lys Ile Ala
420

<210> 16
<211> 430
<212> PRT
<213> Streptomyces nogalater

<400> 16
Met Lys Glu Ser Ile Asn Arg Arg Val Val Ile Thr Gly Ile Gly Ile
1 5 10 15
Val Ala Pro Asp Ala Thr Gly Val Lys Pro Phe Trp Asp Leu Leu Thr
20 25 30
Ala Gly Arg Thr Ala Thr Arg Thr Ile Thr Ala Phe Asp Pro Ser Pro
35 40 45
Phe Arg Ser Arg Ile Ala Ala Glu Cys Asp Phe Asp Pro Leu Ala Glu
50 55 60
Gly Leu Thr Pro Gln Gln Ile Arg Arg Met Asp Arg Ala Thr Gln Phe
65 70 75 80
Ala Val Val Ser Ala Arg Glu Ser Leu Glu Asp Ser Gly Leu Asp Leu
85 90 95
Gly Ala Leu Asp Ala Ser Arg Thr Gly Val Val Val Gly Ser Ala Val
100 105 110
Gly Cys Thr Thr Ser Leu Glu Glu Glu Tyr Ala Val Val Ser Asp Ser
115 120 125
Gly Arg Asn Trp Leu Val Asp Asp Gly Tyr Ala Val Pro His Leu Phe
130 135 140
Asp Tyr Phe Val Pro Ser Ser Ile Ala Ala Glu Val Ala His Asp Arg
145 150 155 160
Ile Gly Ala Glu Gly Pro Val Ser Leu Val Ser Thr Gly Cys Thr Ser
165 170 175
Gly Leu Asp Ala Val Gly Arg Ala Ala Asp Leu Ile Ala Glu Gly Ala
180 185 190
Ala Asp Val Met Leu Ala Gly Ala Thr Glu Ala Pro Ile Ser Pro Ile
195 200 205
Thr Val Ala Cys Phe Asp Ala Ile Lys Ala Thr Thr Pro Arg Asn Asp
210 215 220
Thr Pro Ala Glu Ala Ser Arg Pro Phe Asp Arg Thr Arg Asn Gly Phe
225 230 235 240
Val Leu Gly Glu Gly Ala Ala Val Phe Val Leu Glu Glu Phe Glu His
245 250 255

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Ala Arg Arg Arg Gly Ala Leu Val Tyr Ala Glu Ile Ala Gly Phe Ala
 260 265 270
 Thr Arg Cys Asn Ala Phe His Met Thr Gly Leu Arg Pro Asp Gly Arg
 275 280 285
 Glu Met Ala Glu Ala Ile Gly Val Ala Leu Ala Gln Ala Gly Lys Ala
 290 295 300
 Pro Ala Asp Val Asp Tyr Val Asn Ala His Gly Ser Gly Thr Arg Gln
 305 310 315 320
 Asn Asp Arg His Glu Thr Ala Ala Phe Lys Arg Ser Leu Gly Asp His
 325 330 335
 Ala Tyr Arg Val Pro Val Ser Ser Ile Lys Ser Met Ile Gly His Ser
 340 345 350
 Leu Gly Ala Ile Gly Ser Leu Glu Ile Ala Ala Ser Val Leu Ala Ile
 355 360 365
 Thr His Asp Val Val Pro Pro Thr Ala Asn Leu His Glu Pro Asp Pro
 370 375 380
 Glu Cys Asp Leu Asp Tyr Val Pro Leu Arg Ala Arg Ala Cys Pro Val
 385 390 395 400
 Asp Thr Val Leu Thr Val Gly Ser Gly Phe Gly Gly Phe Gln Ser Ala
 405 410 415
 Met Val Leu Cys Gly Pro Gly Ser Arg Gly Arg Ser Ala Ala
 420 425 430

<210> 17

<211> 426

<212> PRT

<213> Streptomyces glaucescens

<400> 17

Met Thr Arg His Ala Glu Lys Arg Val Val Ile Thr Gly Ile Gly Val
 1 5 10 15
 Arg Ala Pro Gly Gly Ala Gly Thr Ala Ala Phe Trp Asp Leu Leu Thr
 20 25 30
 Ala Gly Arg Thr Ala Thr Arg Thr Ile Ser Leu Phe Asp Ala Ala Pro
 35 40 45
 Tyr Arg Ser Arg Ile Ala Gly Glu Ile Asp Phe Asp Pro Ile Gly Glu
 50 55 60
 Gly Leu Ser Pro Arg Gln Ala Ser Thr Tyr Asp Arg Ala Thr Gln Leu
 65 70 75 80
 Ala Val Val Cys Ala Arg Glu Ala Leu Lys Asp Ser Gly Leu Asp Pro
 85 90 95
 Ala Ala Val Asn Pro Glu Arg Ile Gly Val Ser Ile Gly Thr Ala Val

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100					105					110					
Gly	Cys	Thr	Thr	Gly	Leu	Asp	Arg	Glu	Tyr	Ala	Arg	Val	Ser	Glu	Gly
		115					120					125			
Gly	Ser	Arg	Trp	Leu	Val	Asp	His	Thr	Leu	Ala	Val	Glu	Gln	Leu	Phe
	130					135					140				
Asp	Tyr	Phe	Val	Pro	Thr	Ser	Ile	Cys	Arg	Glu	Val	Ala	Trp	Glu	Ala
145						150					155				160
Gly	Ala	Glu	Gly	Pro	Val	Thr	Val	Val	Ser	Thr	Gly	Cys	Thr	Ser	Gly
				165					170					175	
Leu	Asp	Ala	Val	Gly	Tyr	Gly	Thr	Glu	Leu	Ile	Arg	Asp	Gly	Arg	Ala
			180					185					190		
Asp	Val	Val	Val	Cys	Gly	Ala	Thr	Asp	Ala	Pro	Ile	Ser	Pro	Ile	Thr
		195					200					205			
Val	Ala	Cys	Phe	Asp	Ala	Ile	Lys	Ala	Thr	Ser	Ala	Asn	Asn	Asp	Asp
	210					215					220				
Pro	Ala	His	Ala	Ser	Arg	Pro	Phe	Asp	Arg	Asn	Arg	Asp	Gly	Phe	Val
225						230					235				240
Leu	Gly	Glu	Gly	Ser	Ala	Val	Phe	Val	Leu	Glu	Glu	Leu	Ser	Ala	Ala
				245					250					255	
Arg	Arg	Arg	Gly	Ala	His	Ala	Tyr	Ala	Glu	Val	Arg	Gly	Phe	Ala	Thr
			260					265					270		
Arg	Ser	Asn	Ala	Phe	His	Met	Thr	Gly	Leu	Lys	Pro	Asp	Gly	Arg	Glu
		275					280					285			
Met	Ala	Glu	Ala	Ile	Thr	Ala	Ala	Leu	Asp	Gln	Ala	Arg	Arg	Thr	Gly
	290					295					300				
Asp	Asp	Leu	His	Tyr	Ile	Asn	Ala	His	Gly	Ser	Gly	Thr	Arg	Gln	Asn
305						310					315				320
Asp	Arg	His	Glu	Thr	Ala	Ala	Phe	Lys	Arg	Ser	Leu	Gly	Gln	Arg	Ala
				325					330					335	
Tyr	Asp	Val	Pro	Val	Ser	Ser	Ile	Lys	Ser	Met	Ile	Gly	His	Ser	Leu
			340					345					350		
Gly	Ala	Ile	Gly	Ser	Leu	Glu	Leu	Ala	Ala	Cys	Ala	Leu	Ala	Ile	Glu
		355					360					365			
His	Gly	Val	Ile	Pro	Pro	Thr	Ala	Asn	Tyr	Glu	Glu	Pro	Asp	Pro	Glu
	370					375					380				
Cys	Asp	Leu	Asp	Tyr	Val	Pro	Asn	Val	Ala	Arg	Glu	Gln	Arg	Val	Asp
385						390					395				400
Thr	Val	Leu	Ser	Val	Gly	Ser	Gly	Phe	Gly	Gly	Phe	Gln	Ser	Ala	Ala
				405					410					415	
Val	Leu	Ala	Arg	Pro	Lys	Glu	Thr	Arg	Ser						

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420

425

<210> 18

<211> 418

<212> PRT

<213> Streptomyces sp. C5

<400> 18

Met	Asn	Arg	Arg	Val	Val	Ile	Thr	Gly	Met	Gly	Val	Val	Ala	Pro	Gly
1				5					10					15	

Ala	Ile	Gly	Ile	Lys	Ser	Phe	Trp	Glu	Leu	Leu	Leu	Ser	Gly	Thr	Thr
			20					25					30		

Ala	Thr	Arg	Ala	Ile	Thr	Thr	Phe	Asp	Ala	Thr	Pro	Phe	Arg	Ser	Arg
		35					40					45			

Ile	Ala	Ala	Glu	Cys	Asp	Phe	Asp	Pro	Val	Ala	Ala	Gly	Leu	Ser	Ala
50						55					60				

Glu	Gln	Ala	Arg	Arg	Leu	Asp	Arg	Ala	Gly	Gln	Phe	Ala	Leu	Val	Ala
65					70					75					80

Gly	Gln	Glu	Ala	Leu	Thr	Asp	Ser	Gly	Leu	Arg	Ile	Gly	Glu	Asp	Ser
				85					90					95	

Ala	His	Arg	Val	Gly	Val	Cys	Val	Gly	Thr	Ala	Val	Gly	Cys	Thr	Gln
			100					105					110		

Lys	Leu	Glu	Ser	Glu	Tyr	Val	Ala	Leu	Ser	Ala	Gly	Gly	Ala	Asn	Trp
		115					120					125			

Val	Val	Asp	Pro	His	Arg	Gly	Ala	Pro	Glu	Leu	Tyr	Asp	Tyr	Phe	Val
	130					135					140				

Pro	Ser	Ser	Leu	Ala	Ala	Glu	Val	Ala	Trp	Leu	Ala	Gly	Ala	Glu	Gly
145					150					155					160

Pro	Val	Asn	Ile	Val	Ser	Ala	Gly	Cys	Thr	Ser	Gly	Ile	Asp	Ser	Ile
			165						170					175	

Gly	Tyr	Ala	Cys	Glu	Leu	Ile	Arg	Glu	Gly	Thr	Val	Asp	Val	Met	Leu
			180					185					190		

Ala	Gly	Gly	Val	Asp	Ala	Pro	Ile	Ala	Pro	Ile	Thr	Val	Ala	Cys	Phe
		195					200					205			

Asp	Ala	Ile	Arg	Val	Thr	Ser	Asp	His	Asn	Asp	Thr	Pro	Glu	Thr	Leu
	210					215					220				

Ala	Pro	Phe	Ser	Arg	Ser	Arg	Asn	Gly	Phe	Val	Leu	Gly	Glu	Gly	Gly
225					230					235					240

Ala	Ile	Val	Val	Leu	Glu	Glu	Ala	Glu	Ala	Ala	Val	Arg	Arg	Gly	Ala
				245				250						255	

Arg	Ile	Tyr	Ala	Glu	Ile	Gly	Gly	Tyr	Ala	Ser	Arg	Gly	Asn	Ala	Tyr
			260					265					270		

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His Met Thr Gly Leu Arg Ala Asp Gly Ala Glu Met Ala Ala Ala Ile
 275 280 285
 Thr Ala Ala Leu Asp Glu Ala Arg Arg Asp Pro Ser Asp Val Asp Tyr
 290 295 300
 Val Asn Ala His Gly Thr Ala Thr Arg Gln Asn Asp Arg His Glu Thr
 305 310 315 320
 Ser Ala Phe Lys Arg Ser Leu Gly Asp His Ala Tyr Arg Val Pro Ile
 325 330 335
 Ser Ser Val Lys Ser Met Ile Gly His Ser Leu Gly Ala Ala Gly Ser
 340 345 350
 Leu Glu Val Ala Ala Thr Ala Leu Ala Val Glu Tyr Gly Ala Ile Pro
 355 360 365
 Pro Thr Ala Asn Leu His Asp Pro Asp Pro Glu Leu Asp Leu Asp Tyr
 370 375 380
 Val Pro Leu Thr Ala Arg Glu Lys Arg Val Arg His Ala Leu Thr Val
 385 390 395 400
 Gly Ser Gly Phe Gly Gly Phe Gln Ser Ala Met Leu Leu Ser Arg Pro
 405 410 415

Glu Arg

<210> 19
 <211> 419
 <212> PRT
 <213> Streptomyces peucetius

<400> 19
 Met Asn Arg Arg Ile Val Ile Thr Gly Ile Gly Val Val Ala Pro Gly
 1 5 10 15
 Ala Val Gly Thr Lys Pro Phe Trp Glu Leu Leu Leu Ser Gly Thr Thr
 20 25 30
 Ala Thr Arg Ala Ile Ser Thr Phe Asp Ala Thr Pro Phe Arg Ser Arg
 35 40 45
 Ile Ala Ala Glu Cys Asp Phe Asp Pro Val Ala Ala Gly Leu Ser Ala
 50 55 60
 Glu Gln Ala Arg Arg Leu Asp Arg Ala Gly Gln Phe Ala Leu Val Ala
 65 70 75 80
 Gly Gln Glu Ala Leu Ala Asp Ser Gly Leu Arg Ile Asp Glu Asp Ser
 85 90 95
 Ala His Arg Val Gly Val Cys Val Gly Thr Ala Val Gly Cys Thr Gln
 100 105 110

[illegible]

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<210> 20
 <211> 423
 <212> PRT
 <213> Streptomyces coelicolor

<400> 20

Met	Thr	Arg	Arg	Arg	Val	Ala	Val	Thr	Gly	Ile	Gly	Val	Val	Ala	Pro
1				5					10					15	
Gly	Gly	Ile	Gly	Thr	Pro	Gln	Phe	Trp	Arg	Leu	Leu	Ser	Glu	Gly	Arg
		20						25					30		
Thr	Ala	Thr	Arg	Arg	Ile	Ser	Leu	Phe	Asp	Pro	Ser	Gly	Leu	Arg	Ser
		35					40					45			
Gln	Ile	Ala	Ala	Glu	Cys	Asp	Phe	Glu	Pro	Ser	Asp	His	Gly	Leu	Gly
	50					55					60				
Leu	Ala	Thr	Ala	Gln	Arg	Cys	Asp	Arg	Tyr	Val	Gln	Phe	Ala	Leu	Val
65					70					75					80
Ala	Ala	Ser	Glu	Ala	Val	Arg	Asp	Ala	Asn	Leu	Asp	Met	Asn	Arg	Glu
				85					90					95	
Asp	Pro	Trp	Arg	Ala	Gly	Ala	Thr	Leu	Gly	Thr	Ala	Val	Gly	Gly	Thr
			100					105					110		
Thr	Arg	Leu	Glu	His	Asp	Tyr	Val	Leu	Val	Ser	Glu	Arg	Gly	Ser	Arg
		115					120					125			
Trp	Asp	Val	Asp	Asp	Arg	Arg	Ser	Glu	Pro	His	Leu	Glu	Arg	Ala	Phe
	130					135					140				
Thr	Pro	Ala	Thr	Leu	Ser	Ser	Ala	Val	Ala	Glu	Glu	Phe	Gly	Val	Arg
145					150					155					160
Gly	Pro	Val	Gln	Thr	Val	Ser	Thr	Gly	Cys	Thr	Ser	Gly	Leu	Asp	Ala
				165					170					175	
Val	Gly	Tyr	Ala	Tyr	His	Ala	Val	Ala	Glu	Gly	Arg	Val	Asp	Val	Cys
			180					185					190		
Leu	Ala	Gly	Ala	Ala	Asp	Ser	Pro	Ile	Ser	Pro	Ile	Thr	Met	Ala	Cys
		195					200					205			
Phe	Asp	Ala	Ile	Lys	Ala	Thr	Ser	Pro	Asn	Asn	Asp	Asp	Pro	Ala	His
	210					215					220				
Ala	Ser	Arg	Pro	Phe	Asp	Ala	Asp	Arg	Asn	Gly	Phe	Val	Met	Gly	Glu
225					230					235					240
Gly	Ala	Ala	Val	Leu	Val	Leu	Glu	Asp	Leu	Glu	His	Ala	Arg	Ala	Arg
				245					250					255	
Gly	Ala	Asp	Val	Tyr	Cys	Glu	Val	Ser	Gly	Tyr	Ala	Thr	Phe	Gly	Asn
			260					265					270		
Ala	Tyr	His	Met	Thr	Gly	Leu	Thr	Lys	Glu	Gly	Leu	Glu	Met	Ala	Arg

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275	280	285
Ala Ile Asp Thr Ala Leu Asp Met Ala Glu Leu Asp Gly Ser Ala Ile		
290	295	300
Asp Tyr Val Asn Ala His Gly Ser Gly Thr Gln Gln Asn Asp Arg His		
305	310	315
Glu Thr Ala Ala Val Lys Arg Ser Leu Gly Glu His Ala Tyr Ala Thr		
	325	330
Pro Met Ser Ser Ile Lys Ser Met Val Gly His Ser Leu Gly Ala Ile		
	340	345
Gly Ser Ile Glu Leu Ala Ala Cys Val Leu Ala Met Ala His Gln Val		
	355	360
Val Pro Pro Thr Ala Asn Tyr Thr Thr Pro Asp Pro Glu Cys Asp Leu		
	370	375
Asp Tyr Val Pro Arg Glu Ala Arg Glu Arg Thr Leu Arg His Val Leu		
385	390	395
Ser Val Gly Ser Gly Phe Gly Gly Phe Gln Ser Ala Val Val Leu Ser		
	405	410
Gly Ser Glu Gly Gly Leu Arg		
	420	

<210> 21
 <211> 871
 <212> PRT
 <213> Streptomyces caelestis

<400> 21
 Met Ala Gly His Gly Asp Ala Thr Ala Gln Lys Ala Gln Asp Ala Glu
 1 5 10 15
 Lys Ser Glu Asp Gly Ser Asp Ala Ile Ala Val Ile Gly Met Ser Cys
 20 25 30
 Arg Phe Pro Gly Ala Pro Gly Thr Ala Glu Phe Trp Gln Leu Leu Ser
 35 40 45
 Ser Gly Ala Asp Ala Val Val Thr Ala Ala Asp Gly Arg Arg Arg Gly
 50 55 60
 Thr Ile Asp Ala Pro Ala Asp Phe Asp Ala Ala Phe Phe Gly Met Ser
 65 70 75 80
 Pro Arg Glu Ala Ala Ala Thr Asp Pro Gln Gln Arg Leu Val Leu Glu
 85 90 95
 Leu Gly Trp Glu Ala Leu Glu Asp Ala Gly Ile Val Pro Glu Ser Leu
 100 105 110
 Arg Gly Glu Ala Ala Ser Val Phe Val Gly Ala Met Asn Asp Asp Tyr
 115 120 125

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Ala	Thr	Leu	Leu	His	Arg	Ala	Gly	Ala	Pro	Thr	Asp	Thr	Tyr	Thr	Ala
130						135					140				
Thr	Gly	Leu	Gln	His	Ser	Met	Ile	Ala	Asn	Arg	Leu	Ser	Tyr	Phe	Leu
145					150					155					160
Gly	Leu	Arg	Gly	Pro	Ser	Leu	Val	Val	Asp	Thr	Gly	Gln	Ser	Ser	Ser
				165					170					175	
Leu	Val	Ala	Val	Ala	Leu	Ala	Val	Glu	Ser	Leu	Arg	Gly	Gly	Thr	Ser
			180					185					190		
Gly	Ile	Ala	Leu	Ala	Gly	Gly	Val	Asn	Leu	Val	Leu	Ala	Glu	Glu	Gly
		195					200					205			
Ser	Ala	Ala	Met	Glu	Arg	Val	Gly	Ala	Leu	Ser	Pro	Asp	Gly	Arg	Cys
	210					215					220				
His	Thr	Phe	Asp	Ala	Arg	Ala	Asn	Gly	Tyr	Val	Arg	Gly	Glu	Gly	Gly
225					230					235					240
Ala	Ile	Val	Val	Leu	Lys	Pro	Leu	Ala	Asp	Ala	Leu	Ala	Asp	Gly	Asp
				245					250					255	
Arg	Val	Tyr	Cys	Val	Val	Arg	Gly	Val	Ala	Thr	Gly	Asn	Asp	Gly	Gly
			260					265					270		
Gly	Pro	Gly	Leu	Thr	Val	Pro	Asp	Arg	Ala	Gly	Gln	Glu	Ala	Val	Leu
		275					280					285			
Arg	Ala	Ala	Cys	Asp	Gln	Ala	Gly	Val	Arg	Pro	Ala	Asp	Val	Arg	Phe
	290					295					300				
Val	Glu	Leu	His	Gly	Thr	Gly	Thr	Pro	Ala	Gly	Asp	Pro	Val	Glu	Ala
305					310					315					320
Glu	Ala	Leu	Gly	Ala	Val	Tyr	Gly	Thr	Gly	Arg	Pro	Ala	Asn	Glu	Pro
				325					330					335	
Leu	Leu	Val	Gly	Ser	Val	Lys	Thr	Asn	Ile	Gly	His	Leu	Glu	Gly	Ala
			340					345					350		
Ala	Gly	Ile	Ala	Gly	Phe	Val	Lys	Ala	Ala	Leu	Cys	Leu	His	Glu	Arg
		355					360					365			
Ala	Leu	Pro	Ala	Ser	Leu	Asn	Phe	Glu	Thr	Pro	Asn	Pro	Ala	Ile	Pro
	370					375					380				
Leu	Glu	Arg	Leu	Arg	Leu	Lys	Val	Gln	Thr	Ala	His	Ala	Ala	Leu	Gln
385					390					395					400
Pro	Gly	Thr	Gly	Gly	Gly	Pro	Leu	Leu	Ala	Gly	Val	Ser	Ala	Phe	Gly
				405					410					415	
Met	Gly	Gly	Thr	Asn	Cys	His	Val	Val	Leu	Glu	Glu	Thr	Pro	Gly	Gly
			420					425					430		
Arg	Gln	Pro	Ala	Glu	Thr	Gly	Gln	Ala	Asp	Ala	Cys	Leu	Phe	Ser	Ala
		435					440					445			

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Ser	Pro	Met	Leu	Leu	Leu	Ser	Ala	Arg	Ser	Glu	Gln	Ala	Leu	Arg	Ala			
	450					455					460							
Gln	Ala	Ala	Arg	Leu	Arg	Glu	His	Leu	Glu	Asp	Ser	Gly	Ala	Asp	Pro			
465					470					475					480			
Leu	Asp	Ile	Ala	Tyr	Ser	Leu	Ala	Thr	Thr	Arg	Thr	Arg	Phe	Glu	His			
				485					490					495				
Arg	Ala	Ala	Val	Pro	Cys	Gly	Asp	Pro	Asp	Arg	Leu	Ser	Ser	Ala	Leu			
			500					505					510					
Ala	Ala	Leu	Ala	Ala	Gly	Gln	Thr	Pro	Arg	Gly	Val	Arg	Ile	Gly	Ser			
		515					520					525						
Thr	Asp	Ala	Asp	Gly	Arg	Leu	Ala	Leu	Leu	Phe	Thr	Gly	Gln	Gly	Ala			
	530					535					540							
Gln	His	Pro	Gly	Met	Gly	Gln	Glu	Leu	Tyr	Thr	Thr	Asp	Pro	His	Phe			
545					550					555					560			
Ala	Ala	Ala	Leu	Asp	Glu	Val	Cys	Glu	Glu	Leu	Gln	Arg	Cys	Gly	Thr			
				565				570						575				
Gln	Asn	Leu	Arg	Glu	Val	Met	Phe	Thr	Pro	Asp	Gln	Pro	Asp	Leu	Leu			
			580					585					590					
Asp	Arg	Thr	Glu	Tyr	Thr	Gln	Pro	Ala	Leu	Phe	Ala	Leu	Gln	Thr	Ala			
		595					600					605						
Leu	Tyr	Arg	Thr	Leu	Thr	Ala	Arg	Gly	Thr	Gln	Ala	His	Leu	Val	Leu			
	610					615					620							
Gly	His	Ser	Val	Gly	Glu	Ile	Thr	Ala	Ala	His	Ile	Ala	Gly	Val	Leu			
625					630					635					640			
Asp	Leu	Pro	Asp	Ala	Ala	Arg	Leu	Ile	Thr	Ala	Arg	Ala	His	Val	Met			
				645					650					655				
Gly	Gln	Leu	Pro	His	Gly	Gly	Ala	Met	Leu	Ser	Val	Gln	Ala	Ala	Glu			
			660					665					670					
His	Asp	Leu	Asp	Gln	Leu	Ala	His	Thr	His	Gly	Val	Glu	Ile	Ala	Ala			
		675					680					685						
Val	Asn	Gly	Pro	Thr	His	Cys	Val	Leu	Ser	Gly	Pro	Arg	Thr	Ala	Leu			
	690					695					700							
Glu	Glu	Thr	Ala	Gln	His	Leu	Arg	Glu	Gln	Asn	Val	Arg	His	Thr	Trp			
705					710					715					720			
Leu	Lys	Val	Ser	His	Ala	Phe	His	Ser	Ala	Leu	Met	Asp	Pro	Met	Leu			
				725					730					735				
Gly	Ala	Phe	Arg	Asp	Thr	Leu	Asn	Thr	Leu	Asn	Tyr	Gln	Pro	Pro	Thr			
			740					745					750					
Ile	Pro	Leu	Ile	Ser	Asn	Leu	Thr	Gly	Gln	Ile	Ala	Asp	Pro	Asn	His			
		755					760					765						

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Leu Cys Thr Pro Asp Tyr Trp Ile Asp His Ala Arg His Thr Val Arg
 770 775 780
 Phe Ala Asp Ala Val Gln Thr Ala His His Gln Gly Thr Thr Thr Tyr
 785 790 795 800
 Leu Glu Ile Gly Pro His Pro Thr Leu Thr Thr Leu Leu His His Thr
 805 810 815
 Leu Asp Asn Pro Thr Thr Ile Pro Thr Leu His Arg Glu Arg Pro Glu
 820 825 830
 Pro Glu Thr Leu Thr Gln Ala Ile Ala Ala Val Gly Val Arg Thr Asp
 835 840 845
 Gly Ile Asp Trp Ala Val Leu Cys Gly Ala Ser Arg Pro Arg Arg Val
 850 855 860
 Glu Leu Pro Thr Tyr Ala Phe
 865 870

<210> 22

<211> 890

<212> PRT

<213> Streptomyces ambofaciens

<400> 22

Met Ser Gly Glu Leu Ala Ile Ser Arg Ser Asp Asp Arg Ser Asp Ala
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 Ala Glu Phe Trp Lys Leu Leu Thr Asp Gly Arg Asp Ala Ile Gly Arg
 35 40 45
 Asp Ala Asp Gly Arg Arg Arg Gly Met Ile Glu Ala Pro Gly Asp Phe
 50 55 60
 Asp Ala Ala Phe Phe Gly Met Ser Pro Arg Glu Ala Ala Glu Thr Asp
 65 70 75 80
 Pro Gln Gln Arg Leu Met Leu Glu Leu Gly Trp Glu Ala Leu Glu Asp
 85 90 95
 Ala Gly Ile Val Pro Gly Ser Leu Arg Gly Glu Ala Val Gly Val Phe
 100 105 110
 Val Gly Ala Met His Asp Asp Tyr Ala Thr Leu Leu His Arg Ala Gly
 115 120 125
 Ala Pro Val Gly Pro His Thr Ala Thr Gly Leu Gln Arg Ala Met Leu
 130 135 140
 Ala Asn Arg Leu Ser Tyr Val Leu Gly Thr Arg Gly Pro Ser Leu Ala
 145 150 155 160

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Val	Asp	Thr	Ala	Gln	Ser	Ser	Ser	Leu	Val	Ala	Val	Ala	Leu	Ala	Val	
				165					170					175		
Glu	Ser	Leu	Arg	Ala	Gly	Thr	Ser	Arg	Val	Ala	Val	Ala	Gly	Gly	Val	
			180					185					190			
Asn	Leu	Val	Leu	Ala	Asp	Glu	Gly	Thr	Ala	Ala	Met	Glu	Arg	Leu	Gly	
		195					200					205				
Ala	Leu	Ser	Pro	Asp	Gly	Arg	Cys	His	Thr	Phe	Asp	Ala	Arg	Ala	Asn	
	210					215					220					
Gly	Tyr	Val	Arg	Gly	Glu	Gly	Gly	Ala	Ala	Val	Val	Leu	Lys	Pro	Leu	
225					230					235					240	
Ala	Asp	Ala	Leu	Ala	Asp	Gly	Asp	Pro	Val	Tyr	Cys	Val	Val	Arg	Gly	
				245					250					255		
Val	Ala	Val	Gly	Asn	Asp	Gly	Gly	Gly	Pro	Gly	Leu	Thr	Ala	Pro	Asp	
			260					265					270			
Arg	Glu	Gly	Gln	Glu	Ala	Val	Leu	Arg	Ala	Ala	Cys	Ala	Gln	Ala	Arg	
		275					280					285				
Val	Asp	Pro	Ala	Glu	Val	Arg	Phe	Val	Glu	Leu	His	Gly	Thr	Gly	Thr	
	290					295					300					
Pro	Val	Gly	Asp	Pro	Val	Glu	Ala	His	Ala	Leu	Gly	Ala	Val	His	Gly	
305					310					315					320	
Ser	Gly	Arg	Pro	Ala	Asp	Asp	Pro	Leu	Leu	Val	Gly	Ser	Val	Lys	Thr	
				325					330					335		
Asn	Ile	Gly	His	Leu	Glu	Gly	Ala	Ala	Gly	Ile	Ala	Gly	Leu	Val	Lys	
			340					345					350			
Ala	Ala	Leu	Cys	Leu	Arg	Glu	Arg	Thr	Leu	Pro	Gly	Ser	Leu	Asn	Phe	
		355					360					365				
Ala	Thr	Pro	Ser	Pro	Ala	Ile	Pro	Leu	Asp	Gln	Leu	Arg	Leu	Lys	Val	
	370					375					380					
Gln	Thr	Ala	Ala	Ala	Glu	Leu	Pro	Leu	Ala	Pro	Gly	Gly	Ala	Pro	Leu	
385					390					395					400	
Leu	Ala	Gly	Val	Ser	Ser	Phe	Gly	Ile	Gly	Gly	Thr	Asn	Cys	His	Val	
				405					410					415		
Val	Leu	Glu	His	Leu	Pro	Ser	Arg	Pro	Thr	Pro	Ala	Val	Ser	Val	Ala	
			420					425					430			
Ala	Ser	Leu	Pro	Asp	Val	Pro	Pro	Leu	Leu	Leu	Ser	Ala	Arg	Ser	Glu	
		435					440					445				
Gly	Ala	Leu	Arg	Ala	Gln	Ala	Val	Arg	Leu	Gly	Glu	Thr	Val	Glu	Arg	
	450					455					460					
Val	Gly	Ala	Asp	Pro	Arg	Asp	Val	Ala	Tyr	Ser	Leu	Ala	Ser	Thr	Arg	
465					470					475					480	

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Thr	Leu	Phe	Glu	His	Arg	Ala	Val	Val	Pro	Cys	Gly	Gly	Arg	Gly	Glu			
				485					490						495			
Leu	Val	Ala	Ala	Leu	Gly	Gly	Phe	Ala	Ala	Gly	Arg	Val	Ser	Gly	Gly			
			500					505					510					
Val	Arg	Ser	Gly	Arg	Ala	Val	Pro	Gly	Gly	Val	Gly	Val	Leu	Phe	Thr			
		515					520					525						
Gly	Gln	Gly	Ala	Gln	Trp	Val	Gly	Met	Gly	Arg	Gly	Leu	Tyr	Ala	Gly			
	530					535					540							
Gly	Gly	Val	Phe	Ala	Glu	Val	Leu	Asp	Glu	Val	Leu	Ser	Met	Val	Gly			
545					550					555					560			
Glu	Val	Asp	Gly	Arg	Ser	Leu	Arg	Asp	Val	Met	Phe	Gly	Asp	Val	Asp			
				565					570					575				
Val	Asp	Ala	Gly	Ala	Gly	Ala	Asp	Ala	Gly	Ala	Gly	Ala	Gly	Ala	Gly			
			580					585					590					
Val	Gly	Ser	Gly	Ser	Gly	Ser	Val	Gly	Gly	Leu	Leu	Gly	Arg	Thr	Glu			
		595					600					605						
Phe	Ala	Gln	Pro	Ala	Leu	Phe	Ala	Leu	Glu	Val	Ala	Leu	Phe	Arg	Ala			
	610					615					620							
Leu	Glu	Ala	Arg	Gly	Val	Glu	Val	Ser	Val	Val	Leu	Gly	His	Ser	Val			
625					630					635					640			
Gly	Glu	Val	Ala	Ala	Ala	Thr	Val	Ala	Gly	Val	Leu	Ser	Leu	Gly	Asp			
				645					650					655				
Ala	Val	Arg	Leu	Val	Val	Ala	Arg	Gly	Gly	Leu	Met	Gly	Gly	Leu	Pro			
			660					665					670					
Val	Gly	Gly	Gly	Met	Trp	Ser	Val	Gly	Ala	Ser	Glu	Ser	Val	Val	Arg			
		675					680					685						
Gly	Val	Val	Glu	Gly	Leu	Gly	Glu	Trp	Val	Ser	Val	Ala	Ala	Val	Asn			
	690					695					700							
Gly	Pro	Arg	Ser	Val	Val	Leu	Ser	Gly	Asp	Val	Gly	Val	Leu	Glu	Ser			
705					710					715					720			
Val	Val	Ala	Ser	Leu	Met	Gly	Asp	Gly	Val	Glu	Tyr	Arg	Arg	Leu	Asp			
				725					730					735				
Val	Ser	His	Gly	Phe	His	Ser	Val	Leu	Met	Glu	Pro	Val	Leu	Gly	Glu			
			740					745					750					
Phe	Arg	Gly	Val	Val	Glu	Ser	Leu	Glu	Phe	Gly	Arg	Val	Arg	Pro	Gly			
		755					760					765						
Val	Val	Val	Val	Ser	Gly	Val	Ser	Gly	Gly	Val	Val	Gly	Ser	Gly	Glu			
						775					780							
Leu	Gly	Asp	Pro	Gly	Tyr	Trp	Val	Arg	His	Ala	Arg	Glu	Ala	Val	Arg			
785					790					795					800			

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Phe Ala Asp Gly Val Gly Val Val Arg Gly Leu Gly Val Gly Thr Leu
 805 810 815
 Val Glu Val Gly Pro His Gly Val Leu Thr Gly Met Ala Gly Glu Cys
 820 825 830
 Leu Gly Ala Gly Asp Asp Val Val Val Val Pro Ala Met Arg Arg Gly
 835 840 845
 Arg Ala Glu Arg Glu Val Phe Glu Ala Ala Leu Ala Thr Val Phe Thr
 850 855 860
 Arg Asp Ala Gly Leu Asp Ala Thr Ala Leu His Thr Gly Ser Thr Gly
 865 870 875 880
 Arg Arg Ile Asp Leu Pro Thr Thr Pro Phe
 885 890

<210> 23
 <211> 920
 <212> PRT
 <213> Streptomyces cinnamonensis

<400> 23
 Met Ala Ala Ser Ala Ser Ala Ser Pro Ser Gly Pro Ser Ala Gly Pro
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 Asp Pro Ile Ala Val Val Gly Met Ala Cys Arg Leu Pro Gly Ala Pro
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 Asp Pro Asp Ala Phe Trp Arg Leu Leu Ser Glu Gly Arg Ser Ala Val
 35 40 45
 Ser Thr Ala Pro Pro Glu Arg Arg Arg Ala Asp Ser Gly Leu His Gly
 50 55 60
 Pro Gly Gly Tyr Leu Asp Arg Ile Asp Gly Phe Asp Ala Asp Phe Phe
 65 70 75 80
 His Ile Ser Pro Arg Glu Ala Val Ala Met Asp Pro Gln Gln Arg Leu
 85 90 95
 Leu Leu Glu Leu Ser Trp Glu Ala Leu Glu Asp Ala Gly Ile Arg Pro
 100 105 110
 Pro Thr Leu Ala Arg Ser Arg Thr Gly Val Phe Val Gly Ala Phe Trp
 115 120 125
 Asp Asp Tyr Thr Asp Val Leu Asn Leu Arg Ala Pro Gly Ala Val Thr
 130 135 140
 Arg His Thr Met Thr Gly Val His Arg Ser Ile Leu Ala Asn Arg Ile
 145 150 155 160
 Ser Tyr Ala Tyr His Leu Ala Gly Pro Ser Leu Thr Val Asp Thr Ala
 165 170 175
 Gln Ser Ser Ser Leu Val Ala Val His Leu Ala Cys Glu Ser Ile Arg

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180					185					190					
Ser	Gly	Asp	Ser	Asp	Ile	Ala	Phe	Ala	Gly	Gly	Val	Asn	Leu	Ile	Cys
		195					200					205			
Ser	Pro	Arg	Thr	Thr	Glu	Leu	Ala	Ala	Ala	Arg	Phe	Gly	Gly	Leu	Ser
	210					215					220				
Ala	Ala	Gly	Arg	Cys	His	Thr	Phe	Asp	Ala	Arg	Ala	Asp	Gly	Phe	Val
225					230					235					240
Arg	Gly	Glu	Gly	Gly	Gly	Leu	Val	Val	Leu	Lys	Pro	Leu	Ala	Ala	Ala
				245					250					255	
Arg	Arg	Asp	Gly	Asp	Thr	Val	Tyr	Cys	Val	Ile	Arg	Gly	Ser	Ala	Val
			260					265					270		
Asn	Ser	Asp	Gly	Thr	Thr	Asp	Gly	Ile	Thr	Leu	Pro	Ser	Gly	Gln	Ala
		275					280					285			
Gln	Gln	Asp	Val	Val	Arg	Leu	Ala	Cys	Arg	Arg	Ala	Arg	Ile	Thr	Pro
	290					295					300				
Asp	Gln	Val	Gln	Tyr	Val	Glu	Leu	His	Gly	Thr	Gly	Thr	Pro	Val	Gly
305					310					315					320
Asp	Pro	Ile	Glu	Ala	Ala	Ala	Leu	Gly	Ala	Ala	Leu	Gly	Gln	Asp	Ala
				325					330					335	
Ala	Arg	Ala	Val	Pro	Leu	Ala	Val	Gly	Ser	Ala	Lys	Thr	Asn	Val	Gly
			340					345					350		
His	Leu	Glu	Ala	Ala	Ala	Gly	Ile	Val	Gly	Leu	Leu	Lys	Thr	Ala	Leu
		355					360					365			
Ser	Ile	His	His	Arg	Arg	Leu	Ala	Pro	Ser	Leu	Asn	Phe	Thr	Thr	Pro
	370					375					380				
Asn	Pro	Ala	Ile	Pro	Leu	Ala	Asp	Leu	Gly	Leu	Thr	Val	Gln	Gln	Asp
385					390					395					400
Leu	Ala	Asp	Trp	Pro	Arg	Pro	Glu	Gln	Pro	Leu	Ile	Ala	Gly	Val	Ser
				405					410					415	
Ser	Phe	Gly	Met	Gly	Gly	Thr	Asn	Gly	His	Val	Val	Val	Ala	Ala	Ala
			420					425					430		
Pro	Asp	Ser	Val	Ala	Val	Pro	Glu	Pro	Val	Gly	Val	Pro	Glu	Arg	Val
		435					440					445			
Glu	Val	Pro	Glu	Pro	Val	Val	Val	Ser	Glu	Pro	Val	Val	Val	Pro	Thr
	450					455					460				
Pro	Trp	Pro	Val	Ser	Ala	His	Ser	Ala	Ser	Ala	Leu	Arg	Ala	Gln	Ala
465					470					475					480
Gly	Arg	Leu	Arg	Thr	His	Leu	Ala	Ala	His	Arg	Pro	Thr	Pro	Asp	Ala
				485					490					495	
Ala	Arg	Val	Gly	His	Ala	Leu	Ala	Thr	Thr	Arg	Ala	Pro	Leu	Ala	His

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500					505					510					
Arg	Ala	Val	Leu	Leu	Gly	Gly	Asp	Thr	Ala	Glu	Leu	Leu	Gly	Ser	Leu
		515					520					525			
Asp	Ala	Leu	Ala	Glu	Gly	Ala	Glu	Thr	Ala	Ser	Ile	Val	Arg	Gly	Glu
		530				535					540				
Ala	Tyr	Thr	Glu	Gly	Arg	Thr	Ala	Phe	Leu	Phe	Ser	Gly	Gln	Gly	Ala
545					550					555					560
Gln	Arg	Leu	Gly	Met	Gly	Arg	Glu	Leu	Tyr	Ala	Val	Phe	Pro	Val	Phe
				565					570					575	
Ala	Asp	Ala	Leu	Asp	Glu	Ala	Phe	Ala	Ala	Leu	Asp	Val	His	Leu	Asp
			580					585					590		
Arg	Pro	Leu	Arg	Glu	Ile	Val	Leu	Gly	Glu	Thr	Asp	Ser	Gly	Gly	Asn
		595					600					605			
Val	Ser	Gly	Glu	Asn	Val	Ile	Gly	Glu	Gly	Ala	Asp	His	Gln	Ala	Leu
		610				615					620				
Leu	Asp	Gln	Thr	Ala	Tyr	Thr	Gln	Pro	Ala	Leu	Phe	Ala	Ile	Glu	Thr
625					630					635					640
Ser	Leu	Tyr	Arg	Leu	Ala	Ala	Ser	Phe	Gly	Leu	Lys	Pro	Asp	Tyr	Val
				645					650					655	
Leu	Gly	His	Ser	Val	Gly	Glu	Ile	Ala	Ala	Ala	His	Val	Ala	Gly	Val
			660					665					670		
Leu	Ser	Leu	Pro	Asp	Ala	Ser	Ala	Leu	Val	Ala	Thr	Arg	Gly	Arg	Leu
		675					680					685			
Met	Gln	Ala	Val	Arg	Ala	Pro	Gly	Ala	Met	Ala	Ala	Trp	Gln	Ala	Thr
		690				695					700				
Ala	Asp	Glu	Ala	Ala	Glu	Gln	Leu	Ala	Gly	His	Glu	Arg	His	Val	Thr
705					710					715					720
Val	Ala	Ala	Val	Asn	Gly	Pro	Asp	Ser	Val	Val	Val	Ser	Gly	Asp	Arg
				725					730					735	
Ala	Thr	Val	Asp	Glu	Leu	Thr	Ala	Ala	Trp	Arg	Gly	Arg	Gly	Arg	Lys
			740					745					750		
Ala	His	His	Leu	Lys	Val	Ser	His	Ala	Phe	His	Ser	Pro	His	Met	Asp
			755				760					765			
Pro	Ile	Leu	Asp	Glu	Leu	Arg	Ala	Val	Ala	Ala	Gly	Leu	Thr	Phe	His
			770			775					780				
Glu	Pro	Val	Ile	Pro	Val	Val	Ser	Asn	Val	Thr	Gly	Glu	Leu	Val	Thr
785					790					795					800
Ala	Thr	Ala	Thr	Gly	Ser	Gly	Ala	Gly	Gln	Ala	Asp	Pro	Glu	Tyr	Trp
				805					810					815	
Ala	Arg	His	Ala	Arg	Glu	Pro	Val	Arg	Phe	Leu	Ser	Gly	Val	Arg	Gly

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820	825	830
Leu Cys Glu Arg Gly Val Thr Thr Phe Val Glu Leu Gly Pro Asp Ala		
835	840	845
Pro Leu Ser Ala Met Ala Arg Asp Cys Phe Pro Ala Pro Ala Asp Arg		
850	855	860
Ser Arg Pro Arg Pro Ala Ala Ile Ala Thr Cys Arg Arg Gly Arg Asp		
865	870	875
Glu Val Ala Thr Phe Leu Arg Ser Leu Ala Gln Ala Tyr Val Arg Gly		
	885	890
Ala Asp Val Asp Phe Thr Arg Ala Tyr Gly Ala Thr Ala Thr Arg Arg		
	900	905
Phe Pro Leu Pro Thr Tyr Pro Phe		
915	920	

<210> 24
 <211> 928
 <212> PRT
 <213> Streptomyces antibioticus

<400> 24
 Met His Val Pro Gly Glu Glu Asn Gly His Ser Ile Ala Ile Val Gly
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 20 25 30
 Leu Leu Ala Asp Ser Ala Asp Ala Leu Asp Glu Pro Pro Ala Gly Arg
 35 40 45
 Phe Pro Thr Gly Ser Leu Ser Ser Pro Pro Ala Pro Arg Gly Gly Phe
 50 55 60
 Leu Asp Ser Ile Asp Thr Phe Asp Ala Asp Phe Phe Asn Ile Ser Pro
 65 70 75 80
 Arg Glu Ala Gly Val Leu Asp Pro Gln Gln Arg Leu Ala Leu Glu Leu
 85 90 95
 Gly Trp Glu Ala Leu Glu Asp Ala Gly Ile Val Pro Arg His Leu Arg
 100 105 110
 Gly Thr Arg Thr Ser Val Phe Met Gly Ala Met Trp Asp Asp Tyr Ala
 115 120 125
 His Leu Ala His Ala Arg Gly Glu Ala Ala Leu Thr Arg His Ser Leu
 130 135 140
 Thr Gly Thr His Arg Gly Met Ile Ala Asn Arg Leu Ser Tyr Ala Leu
 145 150 155 160
 Gly Leu Gln Gly Pro Ser Leu Thr Val Asp Thr Gly Gln Ser Ser Ser
 165 170 175

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Leu	Ala	Ala	Val	His	Met	Ala	Cys	Glu	Ser	Leu	Ala	Arg	Gly	Glu	Ser	180	185	190	
Asp	Leu	Ala	Leu	Val	Gly	Gly	Val	Asn	Leu	Val	Leu	Asp	Pro	Ala	Gly	195	200	205	
Thr	Thr	Gly	Val	Glu	Arg	Phe	Gly	Ala	Leu	Ser	Pro	Asp	Gly	Arg	Cys	210	215	220	
Tyr	Thr	Phe	Asp	Ser	Arg	Ala	Asn	Gly	Tyr	Ala	Arg	Gly	Glu	Gly	Gly	225	230	235	240
Val	Val	Val	Val	Leu	Lys	Pro	Thr	His	Arg	Ala	Leu	Ala	Asp	Gly	Asp	245	250	255	
Thr	Val	Tyr	Cys	Glu	Ile	Leu	Gly	Ser	Ala	Leu	Asn	Asn	Asp	Gly	Ala	260	265	270	
Thr	Glu	Gly	Leu	Thr	Val	Pro	Ser	Ala	Arg	Ala	Gln	Ala	Asp	Val	Leu	275	280	285	
Arg	Gln	Ala	Trp	Glu	Arg	Ala	Arg	Val	Ala	Pro	Thr	Asp	Val	Gln	Tyr	290	295	300	
Val	Glu	Leu	His	Gly	Thr	Gly	Thr	Pro	Ala	Gly	Asp	Pro	Val	Glu	Ala	305	310	315	320
Glu	Gly	Leu	Gly	Thr	Ala	Leu	Gly	Thr	Ala	Arg	Pro	Ala	Glu	Ala	Pro	325	330	335	
Leu	Leu	Val	Gly	Ser	Val	Lys	Thr	Asn	Ile	Gly	His	Leu	Glu	Gly	Ala	340	345	350	
Ala	Gly	Ile	Ala	Gly	Leu	Leu	Lys	Thr	Val	Leu	Ser	Ile	Lys	Asn	Arg	355	360	365	
His	Leu	Pro	Ala	Ser	Leu	Asn	Phe	Thr	Ser	Pro	Asn	Pro	Arg	Ile	Asp	370	375	380	
Leu	Asp	Ala	Leu	Arg	Leu	Arg	Val	His	Thr	Ala	Tyr	Gly	Pro	Trp	Pro	385	390	395	400
Ser	Pro	Asp	Arg	Pro	Leu	Val	Ala	Gly	Val	Ser	Ser	Phe	Gly	Met	Gly	405	410	415	
Gly	Thr	Asn	Cys	His	Val	Val	Leu	Ser	Glu	Leu	Arg	Asn	Ala	Gly	Gly	420	425	430	
Asp	Gly	Ala	Gly	Lys	Gly	Pro	Tyr	Thr	Gly	Thr	Glu	Asp	Arg	Leu	Gly	435	440	445	
Ala	Thr	Glu	Ala	Glu	Lys	Arg	Pro	Asp	Pro	Ala	Thr	Gly	Asn	Gly	Pro	450	455	460	
Asp	Pro	Ala	Gln	Asp	Thr	His	Arg	Tyr	Pro	Ala	Leu	Ile	Leu	Ser	Ala	465	470	475	480
Arg	Ser	Asp	Ala	Ala	Leu	Arg	Ala	Gln	Ala	Glu	Arg	Leu	Arg	His	His	485	490	495	

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Leu	Glu	His	Ser	Pro	Gly	Gln	Arg	Leu	Arg	Asp	Thr	Ala	Tyr	Ser	Leu
			500					505					510		
Ala	Thr	Arg	Arg	Gln	Val	Phe	Glu	Arg	His	Ala	Val	Val	Thr	Gly	His
		515					520					525			
Asp	Arg	Glu	Asp	Leu	Leu	Asn	Gly	Leu	Arg	Asp	Leu	Glu	Asn	Gly	Leu
	530					535					540				
Pro	Ala	Pro	Gln	Val	Leu	Leu	Gly	Arg	Thr	Pro	Thr	Pro	Glu	Pro	Gly
545					550					555					560
Gly	Leu	Ala	Phe	Leu	Phe	Ser	Gly	Gln	Gly	Ser	Gln	Gln	Pro	Gly	Met
				565					570					575	
Gly	Lys	Arg	Leu	His	Gln	Val	Phe	Pro	Gly	Phe	Arg	Asp	Ala	Leu	Asp
			580					585					590		
Glu	Val	Cys	Ala	Glu	Leu	Asp	Thr	His	Leu	Gly	Arg	Leu	Leu	Gly	Pro
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Ala	His	Ser	Ala	Leu	Leu	Ser	Glu	Thr	His	Tyr	Thr	Gln	Ala	Ala	Leu
625					630					635					640
Phe	Ala	Leu	Glu	Thr	Ala	Leu	Phe	Arg	Leu	Leu	Val	Gln	Trp	Gly	Leu
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Lys	Pro	Asp	His	Leu	Ala	Gly	His	Ser	Val	Gly	Glu	Ile	Ala	Ala	Ala
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His	Ala	Ala	Gly	Ile	Leu	Asp	Leu	Ser	Asp	Ala	Ala	Glu	Leu	Val	Ala
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Thr	Arg	Gly	Ala	Leu	Met	Arg	Ser	Leu	Pro	Gly	Gly	Gly	Val	Met	Leu
	690					695					700				
Ser	Val	Gln	Ala	Pro	Glu	Ser	Glu	Val	Ala	Pro	Leu	Leu	Leu	Gly	Arg
705					710					715					720
Glu	Ala	His	Val	Gly	Leu	Ala	Ala	Val	Asn	Gly	Pro	Asp	Ala	Val	Val
				725					730					735	
Val	Ser	Gly	Glu	Arg	Gly	His	Val	Ala	Ala	Ile	Glu	Gln	Ile	Leu	Arg
			740					745					750		
Asp	Arg	Gly	Arg	Lys	Ser	Arg	Tyr	Leu	Arg	Val	Ser	His	Ala	Phe	His
		755					760					765			
Ser	Pro	Leu	Met	Glu	Pro	Val	Leu	Glu	Glu	Phe	Ala	Glu	Ala	Val	Ala
	770					775					780				
Gly	Leu	Thr	Phe	Arg	Ala	Pro	Thr	Thr	Pro	Leu	Val	Ser	Asn	Leu	Thr
785					790					795					800
Gly	Ala	Pro	Val	Asp	Asp	Arg	Thr	Met	Ala	Thr	Pro	Ala	Tyr	Trp	Val
				805					810					815	

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Arg His Val Arg Glu Ala Val Arg Phe Gly Asp Gly Ile Arg Ala Leu
 820 825 830
 Gly Lys Leu Gly Thr Gly Ser Phe Leu Glu Val Gly Pro Asp Gly Val
 835 840 845
 Leu Thr Ala Met Ala Arg Ala Cys Val Thr Ala Ala Pro Glu Pro Gly
 850 855 860
 His Arg Gly Glu Gln Gly Ala Asp Ala Asp Ala His Thr Ala Leu Leu
 865 870 875 880
 Leu Pro Ala Leu Arg Arg Gly Arg Asp Glu Ala Arg Ser Leu Thr Glu
 885 890 895
 Ala Val Ala Arg Leu His Leu His Gly Val Pro Met Asp Trp Thr Ser
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 Val Leu Gly Gly Asp Val Ser Arg Val Pro Leu Pro Thr Tyr Ala Phe
 915 920 925

<210> 25
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 <212> PRT
 <213> Streptomyces fradiae

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 20 25 30
 Asp Val Asp Gly Pro Asp Ser Thr His Gly Gly Glu Ile Ala Val Val
 35 40 45
 Gly Met Ser Cys Arg Leu Pro Gly Ala Ala Gly Val Glu Glu Phe Trp
 50 55 60
 Glu Leu Leu Arg Ser Gly Arg Gly Met Pro Thr Arg Gln Asp Asp Gly
 65 70 75 80
 Thr Trp Arg Ala Ala Leu Glu Asp His Ala Gly Phe Asp Ala Gly Phe
 85 90 95
 Phe Gly Met Asn Ala Arg Gln Ala Ala Ala Thr Asp Pro Gln His Arg
 100 105 110
 Leu Met Leu Glu Leu Gly Trp Glu Ala Leu Glu Asp Ala Gly Ile Val
 115 120 125
 Pro Gly Asp Leu Thr Gly Thr Asp Thr Gly Val Phe Ala Gly Val Ala
 130 135 140

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Ser	Asp	Asp	Tyr	Ala	Val	Leu	Thr	Arg	Arg	Ser	Ala	Val	Ser	Ala	Gly
145					150					155					160
Gly	Tyr	Thr	Ala	Thr	Gly	Leu	His	Arg	Ala	Leu	Ala	Ala	Asn	Arg	Leu
				165					170					175	
Ser	His	Phe	Leu	Gly	Leu	Arg	Gly	Pro	Ser	Leu	Val	Val	Asp	Ser	Ala
			180					185					190		
Gln	Ser	Ala	Ser	Leu	Val	Ala	Val	Gln	Leu	Ala	Cys	Glu	Ser	Leu	Arg
		195					200					205			
Arg	Gly	Glu	Thr	Ser	Leu	Ala	Val	Ala	Gly	Gly	Val	Asn	Leu	Ile	Leu
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Thr	Glu	Glu	Ser	Thr	Thr	Val	Met	Glu	Arg	Met	Gly	Ala	Leu	Ser	Pro
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Asp	Gly	Arg	Cys	His	Thr	Phe	Asp	Ala	Arg	Ala	Asn	Gly	Tyr	Val	Arg
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Gly	Glu	Gly	Gly	Gly	Ala	Val	Val	Leu	Lys	Pro	Leu	Asp	Ala	Ala	Leu
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Ala	Asp	Gly	Asp	Arg	Val	Tyr	Cys	Val	Ile	Lys	Gly	Gly	Ala	Val	Asn
		275					280					285			
Asn	Asp	Gly	Gly	Gly	Ala	Ser	Leu	Thr	Thr	Pro	Asp	Arg	Glu	Ala	Gln
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Glu	Ala	Val	Leu	Arg	Gln	Ala	Tyr	Arg	Arg	Ala	Gly	Val	Ser	Thr	Gly
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Ala	Val	Arg	Tyr	Val	Glu	Leu	His	Gly	Thr	Gly	Thr	Arg	Ala	Gly	Asp
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Pro	Val	Glu	Ala	Ala	Ala	Leu	Gly	Ala	Val	Leu	Gly	Ala	Gly	Ala	Asp
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Ser	Gly	Arg	Ser	Thr	Pro	Leu	Ala	Val	Gly	Ser	Val	Lys	Thr	Asn	Val
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Gly	His	Leu	Glu	Gly	Ala	Ala	Gly	Ile	Val	Gly	Leu	Ile	Lys	Ala	Thr
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Leu	Cys	Val	Arg	Lys	Gly	Glu	Leu	Val	Pro	Ser	Leu	Asn	Phe	Ser	Thr
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Glu	Arg	Gln	Glu	Trp	Asn	Glu	Glu	Asp	Asp	Arg	Pro	Arg	Val	Ala	Gly
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Val	Ser	Ser	Phe	Gly	Met	Gly	Gly	Thr	Asn	Val	His	Leu	Val	Ile	Ala
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Glu	Ala	Pro	Ala	Ala	Ala	Gly	Ser	Ser	Gly	Ala	Gly	Gly	Ser	Gly	Ala
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Gly	Ser	Gly	Ala	Gly	Ile	Ser	Ala	Val	Ser	Gly	Val	Val	Pro	Val	Val	
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Val	Ser	Gly	Arg	Ser	Arg	Val	Val	Val	Arg	Glu	Ala	Ala	Gly	Arg	Leu	
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Ala	Glu	Val	Val	Glu	Ala	Gly	Gly	Val	Gly	Leu	Ala	Asp	Val	Ala	Val	
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Thr	Met	Ala	Asp	Arg	Ser	Arg	Phe	Gly	Tyr	Arg	Ala	Val	Val	Leu	Ala	
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Arg	Gly	Glu	Ala	Glu	Leu	Ala	Gly	Arg	Leu	Arg	Ala	Leu	Ala	Gly	Gly	
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Asp	Pro	Asp	Ala	Gly	Val	Val	Thr	Gly	Ala	Val	Leu	Asp	Gly	Gly	Val	
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Val	Val	Gly	Ala	Ala	Pro	Gly	Gly	Ala	Gly	Ala	Ala	Gly	Gly	Ala	Gly	
				565				570						575		
Ala	Ala	Gly	Gly	Ala	Gly	Gly	Gly	Gly	Val	Val	Leu	Val	Phe	Pro	Gly	
			580					585					590			
Gln	Gly	Thr	Gln	Trp	Val	Gly	Met	Gly	Ala	Gly	Leu	Leu	Gly	Ser	Ser	
		595					600					605				
Glu	Val	Phe	Ala	Ala	Ser	Met	Arg	Glu	Cys	Ala	Arg	Ala	Leu	Ser	Val	
	610					615					620					
His	Val	Gly	Trp	Asp	Leu	Leu	Glu	Val	Val	Ser	Gly	Gly	Ala	Gly	Leu	
625					630					635					640	
Glu	Arg	Val	Asp	Val	Val	Gln	Pro	Val	Thr	Trp	Ala	Val	Met	Val	Ser	
				645					650					655		
Leu	Ala	Arg	Tyr	Trp	Gln	Ala	Met	Gly	Val	Asp	Val	Ala	Ala	Val	Val	
			660					665					670			
Gly	His	Ser	Gln	Gly	Glu	Ile	Ala	Ala	Ala	Thr	Val	Ala	Gly	Ala	Leu	
		675					680					685				
Ser	Leu	Glu	Asp	Ala	Ala	Ala	Val	Val	Ala	Leu	Arg	Ala	Gly	Leu	Ile	
	690					695					700					
Gly	Arg	Tyr	Leu	Ala	Gly	Arg	Gly	Ala	Met	Ala	Ala	Val	Pro	Leu	Pro	
705					710					715					720	
Ala	Gly	Glu	Val	Glu	Ala	Gly	Leu	Ala	Lys	Trp	Pro	Gly	Val	Glu	Val	
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Ala	Ala	Val	Asn	Gly	Pro	Ala	Ser	Thr	Val	Val	Ser	Gly	Asp	Arg	Arg	
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Ala	Val	Ala	Gly	Tyr	Val	Ala	Val	Cys	Gln	Ala	Glu	Gly	Val	Gln	Ala	
		755					760					765				
Arg	Leu	Ile	Pro	Val	Asp	Tyr	Ala	Ser	His	Ser	Arg	His	Val	Glu	Asp	
	770					775					780					

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Leu Lys Gly Glu Leu Glu Arg Val Leu Ser Gly Ile Arg Pro Arg Ser
785 790 795 800

Pro Arg Val Pro Val Cys Ser Thr Val Ala Gly Glu Gln Pro Gly Glu
805 810 815

Pro Val Phe Asp Ala Gly Tyr Trp Phe Arg Asn Leu Arg Asn Arg Val
820 825 830

Glu Phe Ser Ala Val Val Gly Gly Leu Leu Glu Glu Gly His Arg Arg
835 840 845

Phe Ile Glu Val Ser Ala His Pro Val Leu Val His Ala Ile Glu Gln
850 855 860

Thr Ala Glu Ala Ala Asp Arg Ser Val His Ala Thr Gly Thr Leu Arg
865 870 875 880

Arg Gln Asp Asp Ser Pro His Arg Leu Leu Thr Ser Thr Ala Glu Ala
885 890 895

Trp Ala His Gly Ala Thr Leu Thr Trp Asp Pro Ala Leu Pro Pro Gly
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His Leu Thr Thr Leu Pro Thr Tyr Pro Phe
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<211> 122

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tt 122

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<211> 118

<212> DNA

<213> Artificial Sequence

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agccagaacc gcttgccctg gaaaggatac gtaggcagat ctaccagtcc ggcccggc 118

<210> 28

<211> 26

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<212> DNA
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39

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36

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- 126 -

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<210> 39

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<210> 40

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32

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<212> DNA

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30

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33

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34

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- 128 -

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28

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32

<210> 53

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<210> 54

<211> 30

<212> DNA

<213> Artificial Sequence

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30

<210> 55

<211> 32

<212> DNA

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32

CLAIMS

1. A polyketide synthase which comprises a loading module
5 and a plurality of extension modules wherein:

a) said loading module loads an optionally substituted
malonyl and then effects decarboxylation of the loaded
moiety to provide a corresponding optionally substituted
10 acetyl moiety for transfer to the first of said
extension modules and wherein said loading module is of
the form: (decarbox) - (AT) - (ACP) wherein:

(ACP) is an acyl carrier protein domain,
15 (AT) is an acyltransferase domain which has an
arginine residue in the active site and loads an
optionally substituted malonyl; and
(decarbox) is a domain which effects
decarboxylation of a loaded optionally substituted
20 malonyl and is selected from
(i) a ketosynthase-type domain herein
designated KSq which differs from a KS domain
of an extension module by having a glutamine
residue in place of a cysteine in the active
25 site; and
(ii) chain length factor (CLF), and

b) at least the first of said extension modules is not
naturally associated with a loading module that effects
30 decarboxylation of an optionally substituted malonyl,

with the proviso that

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1) the synthase is not composed of the loading module of the tylosin polyketide synthase coupled to the spiramycin polyketide synthase minus its natural loading module, and
5 (2) the polyketide produced by the polyketide synthase is not a 14-membered macrolide having a 13-methyl group due to incorporation of an unsubstituted acetate starter.

2. A polyketide synthase as defined in claim 1, wherein said
10 (decarbox) domain is a KSq domain.

3. A polyketide synthase as defined in claim 2, wherein said
15 KSq domain is derived from a KS domain from an extension module wherein the active site cysteine residue has been mutated to a glutamine residue.

4. A polyketide synthase as defined in claim 1, wherein said (decarbox) domain is a CLF domain.

20 5. A polyketide synthase according to any one of claims 1 to 4, wherein the AT domain is a natural extension module AT domain.

25 6. A polyketide synthase according to claim 5, wherein the KSq domain is derived from a KS domain from an extension module by mutation of the active site cysteine residue of the KS domain to a glutamine residue; and wherein the KS and AT domains naturally occur together in said extension module.

30 7. A polyketide synthase according to any one of claims 1 to 6, wherein said AT domain is specific for loading with malonyl.

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8. A polyketide synthase according to any one of claims 1 to 6, wherein said AT domain is specific for loading with methylmalonyl.

5

9. A polyketide synthase according to any one of claims 1 to 6, wherein said AT domain is selected from the AT domain of module 5 of the monensin polyketide synthase and the AT domain of module 5 of the spiramycin polyketide synthase.

10

10. A polyketide synthase according to any one of claims 1 to 3 or 5 to 8, wherein at least the KSq domain is derived from the loading module of a polyketide synthase multienzyme selected from: oleandomycin, spiramycin, niddamycin, methymycin and monensin.

15

11. Nucleic acid encoding the polyketide synthase of any one of claims 1 to 10.

20

12. A vector containing the nucleic acid as defined in claim 11.

13. A transformed cell comprising the nucleic acid of claim 12.

25

14. A process for producing a polyketide which comprises culturing a transformed cell according to claim 13 and recovering the polyketide.

30

15. A polyketide synthase according to any of claims 1 to 10, wherein said polyketide synthase is able to synthesize polyketide selected from:

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a) 12- and 16- membered macrolides with acetate starter units;

(b) 12- and 14- and 16- membered macrolides with propionate starter units;

(c) variants of rifamycin, avermectin, rapamycin, immunomycin and FK506 which differ from the natural compound in the incorporation of acetate starter units or propionate starter units; and

(d) a polyketide wherein the starter unit gives rise to a sidechain selected from allyl and hydroxymethyl.

16. A polyketide synthase according to claim 15 (d) wherein the AT domain of the loading module is selected from:

a) the AT of extension module 6 of the niddamycin polyketide synthase

(b) the AT of extension module 4 of the FK506 polyketide synthase.

17. A process for producing a type II polyketide comprising culturing an organism containing a type II polyketide synthase ("PKS") wherein

(a) the wild type PKS comprises a CLF domain which effects decarboxylation to produce an undesired starter; and

(b) said wild type PKS has been genetically engineered to suppress the decarboxylating activity of said CLF by mutating the active site glutamine residue to an alanine residue.

The erythromycin PKS

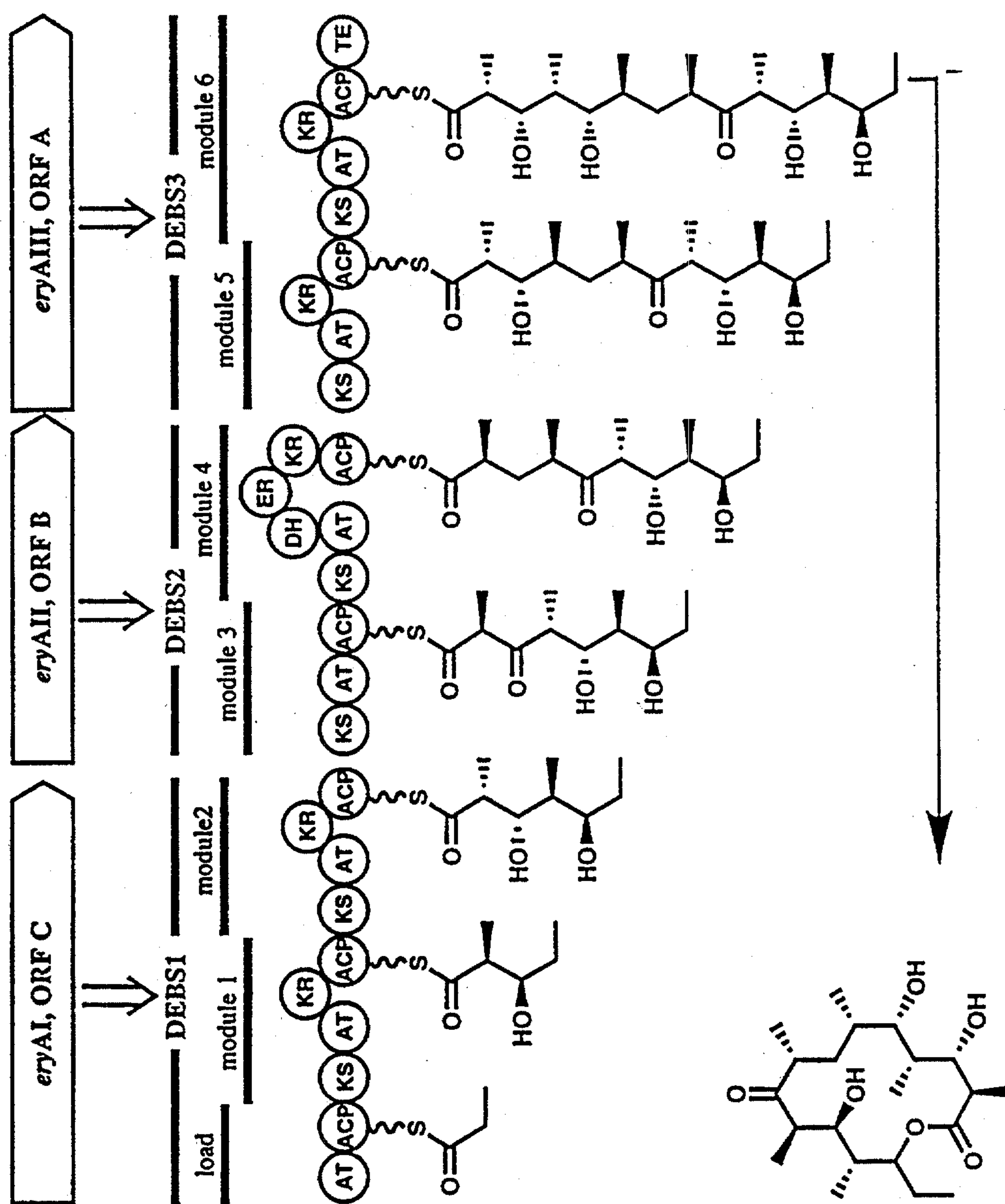


Fig. 1

KCLFDAU
KCLFPEU
KCLFACT
KCLFHIR
KCLFGRA
KCLFNOG
KCLFTCM
KCLFCIN
KCLFVNZ
KCLFWHIE
KSGRA
KSHIR
KSACT
KSCIN
KSVNZ
KSNOG
KSTCM
KSDAU
KSPEU
KSWHI

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MTGTAARTASSQLHASPAGRRGLRGRAVVTGLGIVAPNGLGVGAYWDAVLNGRNGIGPLR
-----MSVLITGVGVVAPNGLGLAPYWSAVLDGRHGLGPVT
-----MSTWVTGMGVVAPNGLGADDHWAATLKGRHGISRLS
-----MSTPDRRRRAVVTGLSVAAPGGLGTERYWKSLLTGENGIAELS
-----MTAAVVVTGLGVVAPTGLGVREHWSSTVRGASAI GPVT
-----MSAPAPVVVTGLGIVAPNGTGT EEWAAATLAGKSGIDVTIQ
-----MTP-VAVTGMGIAAPNGLGRPTTGRPPWAPRAASAAS
-----MSASVVVTGLGVAAPNGLGREDFWASTLGGKSGIGPLT
-----MSGPQRTGTGGGSRRAVVTGLGVLSPHGTGVEAHWKAVADGTSSLGPVT
-----MTRRVVITGVGVRAPGGSGTKEFWDLLTAGRTATRPIS
-----MTRRVVITGVGVRAPGGGLGAKNFWELLTSGRTATRRIS
-----MKRRVITGVGVRAPGGNGTRQFWELLTSGRTATRRIS
-----MTQRRVAITGIEVLAPGGLGRKEFWOLLSEGRTATRGIT
-----MTARRVITGIEVLAPGGTGSKAFWNLLSEGRTATRGIT
-----MKESINRRVITGIGIVAPDATGVKPFWDLLTAGRTATRTIT
-----MTRHAEKRVTITGIGVRAPGGAGTAAFWDLLTAGRTATRTIS
-----MNRRVITGMGVVAPGAIGIKSFWELLSGTTATRAIT
-----MNRRVITGIGVVAPGAVGTPKFWE LLSGTTATRAIS
-----MTRRRVAVTIGIVVAPGGIGTPQFWRL LSEGRTATRRIS
: ** : : * . *

KCLFDAU
KCLFPEU
KCLFACT
KCLFHIR
KCLFGRA
KCLFNOG
KCLFTCM
KCLFCIN
KCLFVNZ
KCLFWHIE
KSGRA
KSHIR
KSACT
KSCIN
KSVNZ
KSNOG
KSTCM
KSDAU
KSPEU
KSWHI

RFADDGRLGRLAGEVSDFVP-EDHLPKRLLVQTDPM TQMTALAAAEWALREAGCAPSS--
RFTGDGRLGRLAGEVSDFVP-EDHLPKRLLAQTDPM TQY-ALAAAEWALRESGCSPSS--
RFDVSRYPATLAGQIDDFHA-PDHI PGRLLPQTD PSTRL-ALTAADWALQDAKADPES-L
RFDPTGYPAELAGQVLD FDA-TEHLPKRLLPQTDV STRF-ALAAA WALADA EVDPAE-L
RFDASRYPSRLAGQIDDFEA-SEHLP SRLLPQTDV STRY-ALAAADWALADAGVGPESGL
RFDAGRYPSKLAGVPGFVP-EDHLP SRLMPQTDH MTRL-ALVAADWAFQDAAVDPSK-L
RFDPHGYPV RVGGEVLAFDA-AAHLPGRLLPQTD RMTQH-ALVAAEWALADAGLEPEK-Q
RFDPSGYPAQLAGEI PGFRA-AEHLPGRLVPQTD RVTRL-SLAAADWALADAGVEVAA-F
RFDPTGYPARLAGEVPGFAA-EEHLP SRLLPQTD RMTRL-ALVAADWALADAGVRPEE-Q
REGCAHLPLRVAGEVHG FDA-AETVEDRFLVQTD RFTHF-ALSATQH ALADARFGRADVD
FFDASPFERSRIAGEI-DFDAVAEGFSPREVRMDRATQF-AVACTRDALADSGLD TGA-L
FFDPTPNRSQIAAEC-DFDPEHEGLSPREIRMDR AAQF-AVVCTRDAVADSGLEFEQ-V
FFDPSPYRSQVAAEA-DFDPVAEGFGPRELDRMDRASQF-AVACAREAF AASGLDPDT-L
FFDPAPFRSKVAAEA-DFCGLENGLSPOEVRRMDR AAQF-AVVTAR-AVEDSGAELAA-H
FFDPTPFRSRVAAEI-DFDPEAHGLSPQEI RMDR AAQF-AVVAAR-AVADSGIDLAA-H
AFDPSPFERSRI AEC-DFDPLAEG LTPQQI RMDRATQF-AVVSARESLED SG L DLGA-L
LFDAAPYRSRIAGEI-DFDPIGEGLSPRQASTYDRATQL-AVVCAREALKDSGLDPAA-V
TFDATPFRSRI AEC-DFDPVAAGLSAEQARRLD RAGQF-ALVAGQEAL TDSGLRIGE-D
TFDATPFRSRI AEC-DFDPVAAGLSAEQARRLD RAGQF-ALVAGQEALADSGLRIDE-D
LFDPSGLRSQIAAEC-DFEPSDHGLGLATAQRCDRYVQF-ALVAASEAVRDANLDMNR-E
: . . : * . * : : : . . :

Fig 2A

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KCLFDAU
KCLFPEU
KCLFACT
KCLFHIR
KCLFGRA
KCLFNOG
KCLFTCM
KCLFCIN
KCLFVNZ
KCLFWHIE
KSGRA
KSHIR
KSACT
KSCIN
KSVNZ
KSNOG
KSTCM
KSDAU
KSPEU
KSWHI

-PLEAGVITASASGGFASGQRELQNLWSKG-----PAHVSAYMSFAWFY-AVNTGQIAIR
-PLEAGVITASASGGFAFGQRELQNLWSKG-----PAHVSAYMSFAWFY-AVNTGQIAIR
TDYDMGVVTANACGGFDFTHREFRKLWSEG-----PKSVSVYESFAWFY-AVNTGQISIR
PEYGTGVITSNATGGFEFTHREFRKLWAG-----PEFVSVYESFAWFY-AVNTGQISIR
DDYDLGVVTSTAQGGFDFTHREFHKLWSQG-----PAYVSVYESFAWFY-AVNTGQISIR
PEYGVGVVTASSAGGFEFGHRELQNLWSLG-----PQYVSAYQSFAWFY-AVNTGQVSIR
DEYGLGVLTAAGAGGFEFGQREMOKLWGTG-----PERVSAYQSFAWFY-AVNTGQISIR
DPLDMGVVTASHAGGFEFGQDELQKLLGQG-----QPVLSAYQSFAWFY-AVNSGQISIR
DDFDMGVVTASASGGFEFGQDELQKLLWSQG-----SQYVSAYQSFAWFY-AVNSGQISIR
SPYSVGVTAAAGSGGFEFGQRELQNLWGHG-----SRHVGPYQSIWFY-AASTGQVSIR
DPSRIGVALGSAVASATSLENEYLVMSDSGREWLVDPAHLSPMMFDYLSPGVMPAEVAWA
PPERIGVSLGSAVAAATSLEQEYLVLSDGGREWQVDPAYLSAHMFDYLSPGVMPAEVAWT
DPAVGVSLGSAVAAATSLEREYLLLSDSGRDWEVDAAWLSRHMFYDYLPSVMPAEVAWA
PPHRIGVVGSAGVATMGLDNEYRVVSDGGRLDLVDHRYAVPHLYNYLVPSSFAAEVAWA
DPYRVGVTVGSAGVATMGLDEEYRVVSDGGRLDLVDHAYAVPHLYDYMPSSFAAEVAWA
DASRTGVVGSAGVCTTSLEEEYAVVSDSGRNWLVDGAYVPHLYDYFVPSSIAAEVAHD
NPERIGVSI GTAVGCTTGLDREYARVSEGGSRWLVDHTLAVEQLFDYFVPSSICREVAWE
SAHRVGVCVGTAVGCTQKLESEYVALSAGGANWVDPHARGAPELYDYFVPSSLAAEVAWL
SAHRVGVCVGTAVGCTQKLESEYVALSAGGAHWVDPGRGSELYDYFVPSSLAAEVAWL
DPWRAGATLGTAVGGTTRLEHDYVLVSEGRSWDVRSEPHLERAFPTATLSSAAVAEE

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KCLFDAU
KCLFPEU
KCLFACT
KCLFHIR
KCLFGRA
KCLFNOG
KCLFTCM
KCLFCIN
KCLFVNZ
KCLFWHIE
KSGRA
KSHIR
KSACT
KSCIN
KSVNZ
KSNOG
KSTCM
KSDAU
KSPEU
KSWHI

-HDLRGPVGVVVAEQAGGLDALAHAR-RKVRGGAE-LIVSGAMDSSLCP-YGMAAQVRSG
-HDLRGPVGVVVAEQAGGLDALAHAR-RKVRGGAE-LIVSGAVDSSLCP-YGMAAQVKSG
-HGMRGPSALVAEQAGGLDALGHAR-RTIRRGTP-LVVSGGVDSALDP-WGWVSQIASG
-HGLRGPVSVLVAEQAGGLDAVGHGG--AVRNGTP-MVVTGGVDSSFDLP-WGWVSHVSSG
-NIMRGPSAALVGEQAGGLDAIGHAR-RTVRRGPG-WCSAVASTRRSTR-GASSSQLSGG
-HGLRGPVGVVLTVEQAGGLDALGOAR-RQLRRGLP-MVVGAVDGSPPCP-WGWVAQLSSG
-HGMRGHSSVFVTEQAGGLDAAAHAA-RLLRKGTNTALTGGCEASLCP-WGLVAQIPSG
-HGMKGPSGVVVSQAGGLDALAQAR-RLVRKGTP-LIVCGAVEPRAPGAGSPSSPAGG
-NGMKGPSGVVVSQAGGLDAVAQAR-RQIRKGTR-LIVSGGVDSALCP-WGWVAHVASD
-NDFKGPCGVVADEAGGLDALAHAA-LAVRNGTD-TVVCGATEAPLAP-YSIVCQLGYP
-AGAEGPVTMVSDGCTSGLDVSGYAV-QGTREGSADVVAAGAADTPVSPIVVACFDAIKA
-VGAEGPVAMVSDGCTSGLDVSLSHAC-SLIAEGTTDVMVAGAADTPITPIVVSCFDAIKA
-VGAEGPVTMVSTGCTSGLDVSGNAV-RAIEEGSADVMFAGAADTPITPIVVACFDAIRA
-VGAEGPSTVSTGCTSGIDAVGLAV-ELVREGSVDVMVAGAVDAPISPIPI-CVLDAIKA
-VGAEGPNTVSTGCTSGLDVSGYARGELIREGSADVMVLAGSSDAPISPIITMACFDAIKA
RIGAEGPVSLVSTGCTSGLDVAVGRAA-DLIAEGAADVMLAGATEAPISPIITVACFDAIKA
-AGAEGPVTVSTGCTSGLDVAVGYGT-ELIRDGRADVVCATDAPISPIITVACFDAIKA
-AGAEGPVNIVSAGCTSGIDSIGYAC-ELIREGTVDVMLAGGVDAPIAPITVACFDAIRV
-AGAEGPVNIVSAGCTSGIDSIGYAC-ELIREGTVDAMVAGGVDAPIAPITVACFDAIRA
-FGVRGPVQTVSTGCTSGLDVAVGYAY-HAVAEGRVDVCLAGAADSPISPIITMACFDAIKA

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KCLFDAU
KCLFPEU
KCLFACT
KCLFHIR
KCLFGRA
KCLFNOG
KCLFTCM
KCLFCIN
KCLFVNZ
KCLFWHIE

RLSGSDDPTAGYLPFDRRAAGHVPGE-GAILAVEDAERVAERG-GKVYGSIAGT-ASFD
RLSGSDNPTAGYLPFDRRAAGHVPGE-GAILTVEDAERAAERG-AKVYGSIAGYGASFD
RISTATDPDRAYLPFDERAAGYVPGE-GAILVLEDSAAAEARGRHDAYGELAGCASTFD
RVSRATDPGRAYLPFDVAANGYVPGE-GAILLLEDAESAKARG-ATGYGEIAGYAATFD
LVSTVADPERAYLPFDVDASGYVPGE-GAVLIVEDADSARARG---AERIYVRSPLRRD
GLSTSDDPRRAYLPFDAAAGHVPGE-GALLVLESDSARARGVTRWYGRIDGYAATFD
FLSEATDPHDAYLPFDARAAGYVPGE-GAMLVAERADSARERDAATVYGRIGHASTFD
-MSDSDEPNRAYLPFDRDGRGYVPGGGRGVVPLERAEAPARG-AEYGE-AGPLARL-
RLSTSEEPARGYLPFDREAQGHVPGE-GAILVMEAAEAARERG-ARIYGEIAGYGSTFD
ELSRATEPDRAYRPFTEAACGFAPAEG-GAVLVVEEEAAARERG-ADVRA TVAGHAATFT

Fig 2b

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KSGRA
KSHIR
KSACT
KSCIN
KSVNZ
KSNOG
KSTCM
KSDAU
KSPEU
KSWHI

TTPRNDDPAHASRPFDRNGFVLAEG-AAMFVLEEEYEAQRRG-AHIYAEVGGYATRSQ
TTPRNDDPEHASRPFDRNSRNGFVLAEG-AALFVLEEEHARARG-AHVYAEISGCATRLN
TTARNDDPEHASRPFDRNGFVLAEG-AAMFVLEEDYDSALARG-ARIHAEISGYATRCN
TTPRHDA PATASRPFDRNGFVLGEG-AAFFVLEELHSARRRG-AHIYAEIAGYATRSN
TINRYDDPAHASRPFDRNGFVLGEG-AAVFLVEELESARARG-AHIYAEIAGYATRSN
TTPRNDTPAEASRPFDRTRNGFVLGEG-AAVFLVEEFHARRRG-ALVYAEIAGFATRCN
TSANNDPAHASRPFDRNRDGFVLGEG-SAVFVLEELSAARRRG-AHAYAEVRGFATRSN
TSDHNDTPETLA-PFSRSRNGFVLGEG-GAIVVLEEEAAVRRG-ARIYAEIGGYASRGN
TSDHNDTPETASRPFDRSRNGFVLGEG-GAIVVLEEEAAVRRG-ARIYAEIGGYASRGN
TSPNNDPAHASRPFDRNGFVMGEG-AAVLVLEEDLEHARARG-ADVCEVSGYATFGN
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KCLFDAU
KCLFPEU
KCLFACT
KCLFHIR
KCLFGRA
KCLFNOG
KCLFTCM
KCLFCIN
KCLFVNZ
KCLFWHIE
KSGRA
KSHIR
KSACT
KSCIN
KSVNZ
KSNOG
KSTCM
KSDAU
KSPEU
KSWHI

-PPPGSGRP---SALARAVETALADAGLDRSDIAVVFADGAA-VGELDVAEAEALASVFG
-PPPGSGRP---SALARAVETALADAGLDGSDIAVVFADGAA-VPELDAAEAEALASVFG
-PAPGSGRP---AGLERAIRLALNDAGTGPEDVDVVFADGAG-VPELDAAEARAIGRVFG
-PAPGSRP---PALRRAIELALADAELRPEQVDVVFADAAAG-VAELDAIEAAAIREFLG
-PAPGSGRP---PALGRAELALAEAGLTPADISVVFADGAG-VPELDRAEADTLARLFG
-PPPGSGRP---PNLLRAAQALDDAEVGPEDVDVVFADAGS-TPDEDAEADAVRRLFG
-ARPGTGRP---TGPARAIRLALAEARVAPEDVDVVFADAAAG-VPALDRAEAEALAEVFG
-PAPHSGRG---STRAHAIRLALDDAGTAPGDIRRVFADGGGRYPN-DRAEAEAISEVFG
-PRPGSGRE---PGLRKAIELALADAGAAPGDIDVVFADAAA-VPELDRVEAEALNAVFG
GAGRWAESR---EGLARAIQALAEAGCRPEEVDVVFADALG-VPEADRAEALALADALG
-AYHMTGLKKGREMAESIRALDEARLDRTAVDYVNAHGSG-TKQNDRHETAFAFKRSLG
-AYHMTGLKTDGREMAEIRVALDLARIDPTDIDYINAHGSG-TKQNDRHETAFAFKRSLG
-AYHMTGLKADGREMAETIRVALDESRTDATDIDYINAHGSG-TRQNDRHETAAYKRALG
-AYHMTGLR-DGAEMAEAIRLALDEARLNPEQVDYINAHGSG-TKQNDRHETAFAFKKALG
-AYHMTGLRPDGAEMAEIRVALDEARMNPTEIDYINAHGSG-TKQNDRHETAFAFKKSLG
-AFHMTGLRPDGREMAEAI GVALAQAGKAPADVVDYVNAHGSG-TRQNDRHETAFAFKRSLG
-AFHMTGLKPDGREMAEAI TAALDQARRTGDDLHYINAHGSG-TRQNDRHETAFAFKRSLG
-AYHMTGLRADGAEMAAAI TAALDEARRDP SDVDYVNAHGTA-TRQNDRHETSAFKRSLG
-AYHMTGLRADGAEMAAAI TAALDEARRDP SDVDYVNAHGTA-TKQNDRHETSAFKRSLG
-AYHMTGLTKEGLEMARAI DTALDMAELDGSADYVNAHGSG-TQQNDRHETAFAVKRSLG
. : : ** : : * . . * * : : *

Fig 2c

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KCLFDAU
KCLFPEU
KCLFACT
KCLFHIR
KCLFGRA
KCLFNOG
KCLFTCM
KCLFCIN
KCLFVNZ
KCLFWHIE
KSGRA
KSHIR
KSACT
KSCIN
KSVNZ
KSNOG
KSTCM
KSDAU
KSPEU
KSWHI

P--HRVPVTVPKTLTGRLYSGAGPLDVATGLLALRDEVVPATGHVH-PDPDLPLDVVTGR
P--RRVPVTVPKTLTGRLYSGAGPLDVATALLALRDEVVPATAHVD-PDPDLPLDVVTGR
R--EGVPVTVPKTTTGRLYSGGGPLDVVTALMSLREGVIAPTAGVTSVPREYGIDLVLGE
P--SGVPVTAPKMTGTGRLYSGGGPLDLVAALLAIRDGVIPPTVHTAEPVPEHQDLVLTGD
P--RGVPVTAPKALTGRLCAGGGPADLAAALLALRDQVIPATGRHRAVPDAYALDLVTGR
P--YGVVTVAPKMTGTGRLSAGGAALDVATALLALREGVVPPTVNVSRPRPEYELDLVLA-
P--GAVPVTAPKMTGTGRLYAGGAALDVATALLSIRDCVVPPTVGTGAPAPGLGIDLVLHQ
P--GRVPVTCPRMTGTGRLHSGAAPLDVACALLAMRAGVIPPTVHID-PCPEYDLDLVLYQ
T--GAVPVTAPKMTGTGRLYSGAAPLDLAAFLAMDEGVIPPTVNVE-PDAAYGLDLVVGG
PHAARVPVTAPKGTGTGRAYCAAPVLDVATAVLAMEHGLIPPTPHVL--DVCHDLDLVTGR
EHAYAVPVSSIKSMGGHSLGAIGSIEIAASVLAIEHNVVPPTANLHTPDPECDLDYVPLT
EHAYRTPVSSIKSMVGHSLGAIGSIEVAACALAIHGVVPPTANLHEPDPECDLDYVPLT
EHARRTPVSSIKSMVGHSLGAIGSLEIAACVLALEHGVVPPTANLRTSDPECDLDYVPLE
EHAYRTPVSSIKSMVGHSLGAIGSIEIAASALAMEYDVVPPTANLHTPDPECDLDYVPLT
DHAYRTPVSSIKSMVGHSLGAIGSIEIAASALAMEHNVVPPTGNLHTPDPECDLDYVR-S
DHAYRVFVSSIKSMIGHSLGAIGSLEIAASVLAITHDVVPPTANLHEPDPECDLDYVPLR
QRAYDVPVSSIKSMIGHSLGAIGSLELAACALAIHGVIPPTANYEEDPECDLDYVNV
DHAYRVPISSVKSMMIGHSLGAAGSLEVAATALAVEYGAIPPTANLHDPDPDELDDYVPLT
EHAYRVPISSIKSMIGHSLGAVGSLEVAATALAVEYGVIPPTANLHDPDPDELDDYVPLT
EHAYATPMSSIKSMVGHSLGAIGSIEIAACVLAHAHQVVPPTANYTTTPDPECDLDYVPRE

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KCLFDAU
KCLFPEU
KCLFACT
KCLFHIR
KCLFGRA
KCLFNOG
KCLFTCM
KCLFCIN
KCLFVNZ
KCLFWHIE
KSGRA
KSHIR
KSACT
KSCIN
KSVNZ
KSNOG
KSTCM
KSDAU
KSPEU
KSWHI

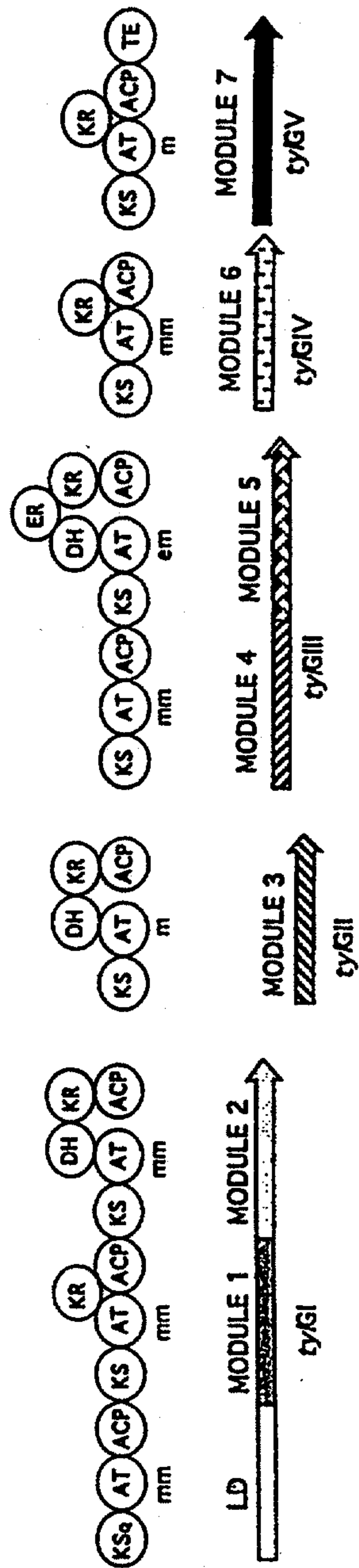
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PRSLADARAALLVARGYGGFNSALVVRGAA-----
PRSTAPRTA-LVLARGRWGFNSAAVLRRAFPTP----
PRHQQLGTA-LVLARGKWGFNSAVVVRGVGTG-----
PREAALSAA-LVLARGRHGFNSAVVVTLRGSDHRRPT
PRRTPLARA-LVLARGRGGFNAAMVVAGPRAETR---
PRELRVDTA-LVVARGMGGFNSALVVRRHG-----
VRPAALRTA-LGGARGHGGFNSALVVRAGQ-----
PRTAEVNTA-LVIARGHGGFNSAMVVRSAN-----
ARPAEPRTA-LVLARGLMGSNSALVLRRGAVPPEGR-
AREQRVDTV-LTVGSGFGGFQSAMVLRPEEAA-----
AREQRVDTV-LSVGSGFGGFQSAMVLRRLGGANS---
ARERKLRVS-LTVGSGFGGFQSAMVLRDAETAGAAA-
ARDQRVDSV-LTVGSGFGGFQSAMVLTSAQ---RSTV
CREQLTDSV-LTVGSGFGGFQSAMVLRARPE---RKIA
ARACPVDTV-LTVGSGFGGFQSAMVLCGPGSRGRSAA
AREQRVDTV-LSVGSGFGGFQSAVLRARPKETRS---
AREKRVRA-LTVGSGFGGFQSAMLLSRPER-----
AREKRVRA-LTVGSGFGGFQSAMLLSRLER-----
ARERTLRHV-LSVGSGFGGFQSAVVLSGSEGGLR---

* . * . * * : * : *

mole:~/ks2%

Fig 2D

ORGANISATION OF THE TYLOSIN-PRODUCING POLYKETIDE SYNTHASE



ORGANISATION OF THE SPIRAMYCIN-PRODUCING POLYKETIDE SYNTHASE

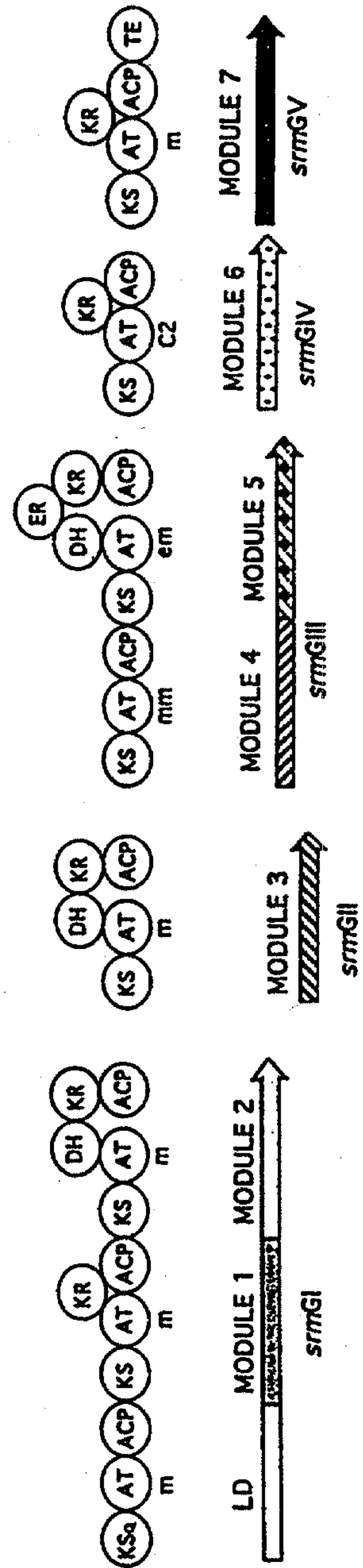


Fig 3A

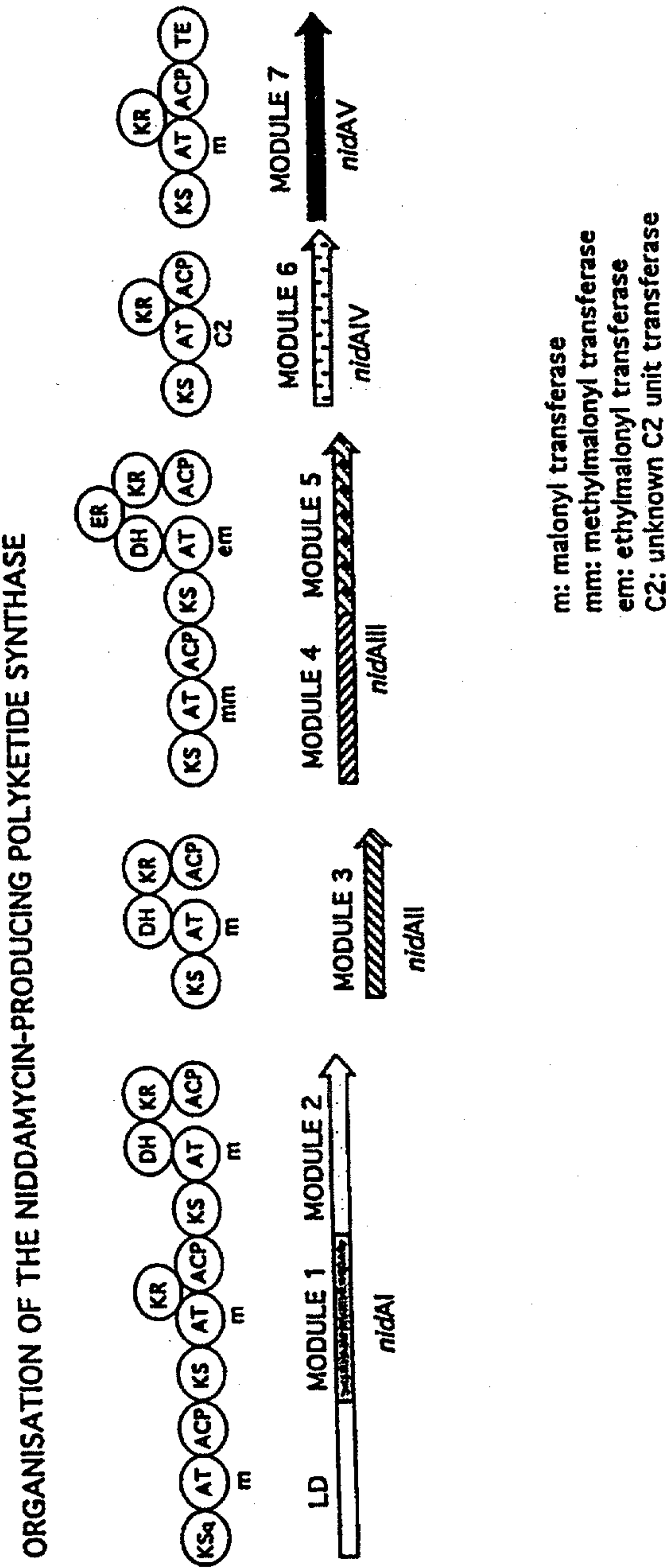


Fig 3B

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	1		50
niddamycin	-----	MAGHGDATAQ KAQDAEKSED GSDAIAVIGM	
platenolide	-----	-----MS GELAISRSD RSDAVAVVGM	
monensin	-----	-----MAAS ASASPSGPSA GPDPIAVVGM	
oleandomycin	-----	-----MHVPGEE NGHSIAIVGI	
tylosin	MSSALRRVQ SNCGYGDLMT SNTAAQNTGD QEDVDGPDST HGGEIAVVGM		
	51		100
niddam...	SCRFPGAPGT AEFWQLLSSG ADAVVTAADG RRR.....	GTIDA	
platenol.	ACRFPGAPGI AEFWKLLTDG RDAIGRDADG RRR.....	GMIEA	
monensin	ACRLPGAPDP DAFWRLLSEG RSAVSTAPPE RRRADSG LHG P...	GGYLDR	
oleandom	ACRLPGSATP QEFWRLLADS ADALDEPPAG RFFTGSLSSP	PAPRGGFLDS	
tylosin	SCRLPGAAGV EEFWELLRSG RGMPTRODDG TWRAA.....	LED	
	101		150
niddam...	PADFDAFFG MSPREAAATD PQORLVLELG WEALEDAGIV	PESLRGEAAS	
platenol.	PGDFDAFFG MSPREAAETD PQORLMLELG WEALEDAGIV	PGSLRGEAVG	
monensin	IDGFDADFFH ISPREAVAMD PQORLLELS WEALEDAGIR	PPTLARSRTG	
oleandom	IDTFDADFFN ISPREAGVLD PQORLLELG WEALEDAGIV	PRHLRGTRTS	
tylosin	HAGFDAGFFG MNARQAAATD PQHRLMLELG WEALEDAGIV	PGDLTGTDG	
	151		200
niddam...	VFVGAMNDY ATLLH.RAGA PTDTYTATGL QHSMIANRLS	YFLGLRGPSL	
platenol.	VFVGAMHDDY ATLLH.RAGA PVGPHTATGL QRAMLANRLS	YVLGTRGPSL	
monensin	VFVGAFWDDY TDVLNLRAPG AVTRHTMTGV HRSILANRIS	YAYHLAGPSL	
oleandom	VFMGAMWDDY AHLAHARGEAL ALTRHSLTGT HRGMIANRLS	YALGLQGPSL	
tylosin	VFAGVASDDY A.VLTRRSV SAGGYTATGL HRALAANRLS	HFLGLRGPSL	
	201		250
niddam...	VVDTGQSSSL VAVALAVESL RGGTSGIALA GGVNLVLAEE	GS.AAMERVG	
platenol.	AVDTAQSSSL VAVALAVESL RAGTSRVAVA GGVNLVLADE	GT.AAMERLG	
monensin	TVDTAQSSSL VAVHLACESI RSGDSIAFA GGVNLICSPR	TTELAARFG	
oleandom	TVDTGQSSSL AAVHMACESE ARGESDLALV GGVNLVLDPA	GT.TGVERFG	
tylosin	VVDSAQSASL VAVQLACESL RRGESLAVA GGVNLILTEE	ST.TVMERMG	
	251		300
niddam...	ALSPDGRCHT FDARANGYVR GEGGAIVVLK PLADALADGD	RVYCVVRGVA	
platenol.	ALSPDGRCHT FDARANGYVR GEGGAIVVLK PLADALADGD	PVYCVVRGVA	
monensin	GLSAAGRCHT FDARADGFVR GEGGGLVVLK PLAAARRDGD	TVYCVIRGSA	
oleandom	ALSPDGRCHT FDSRANGYAR GEGGVVVVLK PTHRALADGD	TVYCEILGSA	
tylosin	ALSPDGRCHT FDARANGYVR GEGGGAIVVLK PLDAALADGD	RVYCVIKGGA	
	301		350
niddam...	TGNDGGGPGL TVPDRAGQEA VLRAACDQAG VRPADVRFVE	LHGTGTPAGD	
platenol.	VGNDGGGPGL TAPDREGQEA VLRAACAQAR VDPAEVRFVE	LHGTGTPVGD	
monensin	VNSDGTDDGI TLPSGQAQD VVRLACRRAR ITPDQVQYVE	LHGTGTPVGD	
oleandom	LNNDGATEGL TVPSARAQAD VLRQAWERAR VAPTDVQYVE	LHGTGTPAGD	
tylosin	VNNDGGGASL TTPDREAQEA VLRQAYRRAG VSTGAVRYVE	LHGTGTRAGD	

Fig 4A

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	351		400
niddam...	PVEAEALGAV YGTGRP..AN	EPLLVGSVKT NIGHLEGAAG	IAGFVKAALC
platenol.	PVEAHALGAV HGSGRP..AD	DPLLVGSVKT NIGHLEGAAG	IAGLVKAALC
monensin	PIEAAALGAA LGQDAA..RA	VPLAVGSAKT NVGHLEAAAG	IVGLLKTALS
oleandom	PVEAEGLGTA LGTARP..AE	APLLVGSVKT NIGHLEGAAG	IAGLLKTVLS
tylosin	PVEAAALGAV LGAGADSGRS	TPLAVGSVKT NVGHLEGAAG	IVGLIKATLC
	401		450
niddam...	LHERALPASL NFETPNPAIP	LERLRLKVQT AHAALQPGTG	GGPLLAGVSA
platenol.	LRERTLPDSL NFATPSPAIP	LDQLRLKVQT AAAELPLAPG	GAPLLAGVSS
monensin	IHHRRLAPSL NFFTTPNPAIP	LADLGLTVQO DLADWP..RP	EQPLIAGVSS
oleandom	IKNRHLPASL NFTSPNPRID	LDALRLRVHT AYGPPW..SP	DRPLVAGVSS
tylosin	VRKGELVPSL NFSTPNPDIP	LDDLRLRVQT ERQEW.NEED	DRPRVAGVSS
	451		500
niddam...	FGMGGTNCHV VLEETPGG..		...RQPAE.T
platenol.	FGIGGTNCHV VLEHLPSR..		...PTPAV.S
monensin	FGMGGTNCHV VVA....AAP	DSVAVPEPVG VPERVEVPEP	VVSEPVVVP
oleandom	FGMGGTNCHV VLSELNAGG	DGAGKGPYTG TEDRLGATEA	EKRDPDPATGN
tylosin	FGMGGTNVHL VIAEAPAAAG	SSGAGGSGAG SGAGISAVSG	VV.....
	501		550
niddam...	GQADACLFSA SPMLLLSARS	EQALRAQAAR LREHL..EDS	GADPLDIAYS
platenol.	VAAS...LPD VPPLLLSARS	EGALRAQAVR LGETV..ERV	GADPRDVAYS
monensin	TPWP.....	VSAHS ASALRAQAGR	LRTHLAAHRP TPDAARVGH
oleandom	GPDPAQDTHR YPALILSARS	DAALRAQAER LRHHL.EHSP	GQRLRDTAYS
tylosin		..PVVVSGRS RVVVREAAGR	LAE..VVEAG GVGLADVAVT
	551		600
niddam...	LATTRTRFEH RAAVPCGDPD	RLSSALAALA AGQTPRGVRI	GS..TDADGR
platenol.	LASTRTLFEH RAVVPCGGRG	ELVAALGGFA AGRVSGGVRS	GR..A.VPGG
monensin	LATTRAPLAH RAVLLGGDTA	ELLGSLDALA EGAETASIVR	GEAYT..EGR
oleandom	LATTRQVFER HAVVTGHDRE	DLNGLRDLE NGLPAPQVLL	GRTPTPEPGG
tylosin	MAD.RSRFGY RAVVLARGEA	ELAGRLRALA GGDPDAGVVT	G...AVLDGG
	601		650
niddam...	LALLFTGQGA QHPGMGQELY	TTDPHFAAAL DEVCEELQRC	GTQNLREVMF
platenol.	VGVLFSGQGA QWVGMGRGLY	AGGGVFAEVL DEVLSMVGEV	DGRSLRDVMF
monensin	TAFLESGQGA QRLGMGRELY	AVFPVFADAL DEAFALDVH	LDRPLREIVL
oleandom	LAFLESGQGS QQPGMGKRLH	QVFPGFRDAL DEVCAELDTH	LGRLL.....
tylosin	VVGAAPGGA GAAGGAGAAG	GAGGGGVVLV FPGQGTQWVG	MGAGLLGSSE
	651		700
niddam...	TPDQPD.....		LLDRTEYTQP ALFALQTALY
platenol.	GDVDVDAGAG ADAGAGAGAG	VSGSGSVGG LLGRTEFAQP	ALFALEVALF
monensin	GETDSGGNVS GENVIGEGA.	DHQA LLDQTAYTQP	ALFAIETSLY
oleandom	.GPEAGPPLR DVMFAERGT.	AHSA LLSETHYTQA	ALFALETALF
tylosin	VFAASMRECA RALSVHVGWD	LLEVVSAGGAG .LERVDVVQP	VTWAVMVSIA
	701		750
niddam...	RTLTARGETQA HLVLGHSVGE	ITAAHIAGVL DLPDAARLIT	ARAHVMGQLP
platenol.	RALEARGVEV SVVLGHSVGE	VAAATVAGVL SLGDAVRLVV	ARGGLMGGLP
monensin	RLAASFGLKP DYVLGHSVGE	IAAAHVAGVL SLPDASALVA	TRGRLMQAVR
oleandom	RLLVQWGLKP DHLGHSVGE	IAAAHAAGIL DLSDAAELVA	TRGALMRSIP
tylosin	RYWQAMGVDV AAVVGHSQGE	IAAATVAGAL SLEDAAVVA	LRAGLIGRYL

↑ Fig 4B

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	751		800
niddam...	HG.GAMLSVQ	AAEHDLDQLA	HTHG..VEIA AVNGPTHCVL SGPRTALEET
platenol.	VG.GGMWSVG	ASESVVRGVV	EGLGEWVSV AVNGPRSVVL SGDVGVLSEV
monensin	AP.GAMAAWQ	ATADEAAEQ	AGHERHVTVA AVNGPDSVVV SGDRATVDEL
oleandom	GG.GVMLSVQ	APESVAPLL	LGREAHVGLA AVNGPDAVVV SGERGHVAAI
tylosin	AGRGAMAAVP	LPAGEVEAGL	.AKWPGVEVA AVNGPASTVV SGDRRAVAGY
	801		850
niddam...	AQHLREQNVR	HTWLKVSHAF	HSALMDPMLG AFRDTLNTLN Y..QPPTIPL
platenol.	VASLMGDGVE	YRRLDVSHGF	HSVLMEPVLG EFRGVVESLE FGRVRPGVVV
monensin	TAAWRGRGRK	AHHLKVSHAF	HSPHMDPILD ELRAVAAGLT FHE..PVIPV
oleandom	EQILRDRGRK	SRYLRVSHAF	HSPLMEPVLE EFAEAVAGLT FRA..PTTPL
tylosin	VAVCQAEQVQ	ARLIPVDYAS	HSRHVEDLKG ELERVLSGI. .RPRSPRPV
	851		900
niddam...	ISNLTGQIA.	DPNHL	CTPDYWIDHA RHTVRFADAV QTAHHQGTIT
platenol.	VSGVSGGVV.	GSGEL	GDPGYWVRHA REAVRFADGV GVVRGLGVGT
monensin	VSNVTGELVT	ATATGSGAGQ	ADPEYWARHA REPVRFLSGV RGLCERGVTT
oleandom	VSNLTG....	..APVDDRTM	ATPAYWVRHV REAVRFGDGI RALGKLGTGS
tylosin	CSTVAGEQPG	EPVF.....	.DAGYWFRNL RNRVEFSAVV GGLLEEGRH
	901		950
niddam...	YLEIGPHPTL	TTLHHTL..	.DNP..... T TIPTLHRERP
platenol.	LVEVGPHGVL	TGMAGECLGA	GDDV..... V VVPAMRRGRA
monensin	FVELGPDAPL	SAMARDCFPA	P..... .ADRSRPRPA AIATCRRGRD
oleandom	FLEVGPDGVL	TAMARACVTA	APEPGHRGEQ GADADAHTAL LLPALRRGRD
tylosin	FIEVSAHPVL	V.....	HAIEQ TAEAADRSVH ATGTLRRQDD
	951		
niddam...	EPETLTQAIA	AVGVRTDGID	WAVLCGASRP RRVELPTYAF
platenol.	EREVFEEALA	TVFTRDAGLD	ATALHTGSTG RRIDLPTTPF
monensin	EVATFLRSLA	QAYVRGADV	FTRAYGATAT RRFPLPTYPF
oleandom	EARSLTEAVA	RLHLHGVPMD	WTSVLGGDVS .RVPLPTYAF
tylosin	SPHRLTSTA	EAWAHGATLT	WDPAL..PPG HLTTLPPTYPF

niddam: niddamycin; platenol: platenolide I (spiramycin); oleandom: oleandomycin.

Fig 4c

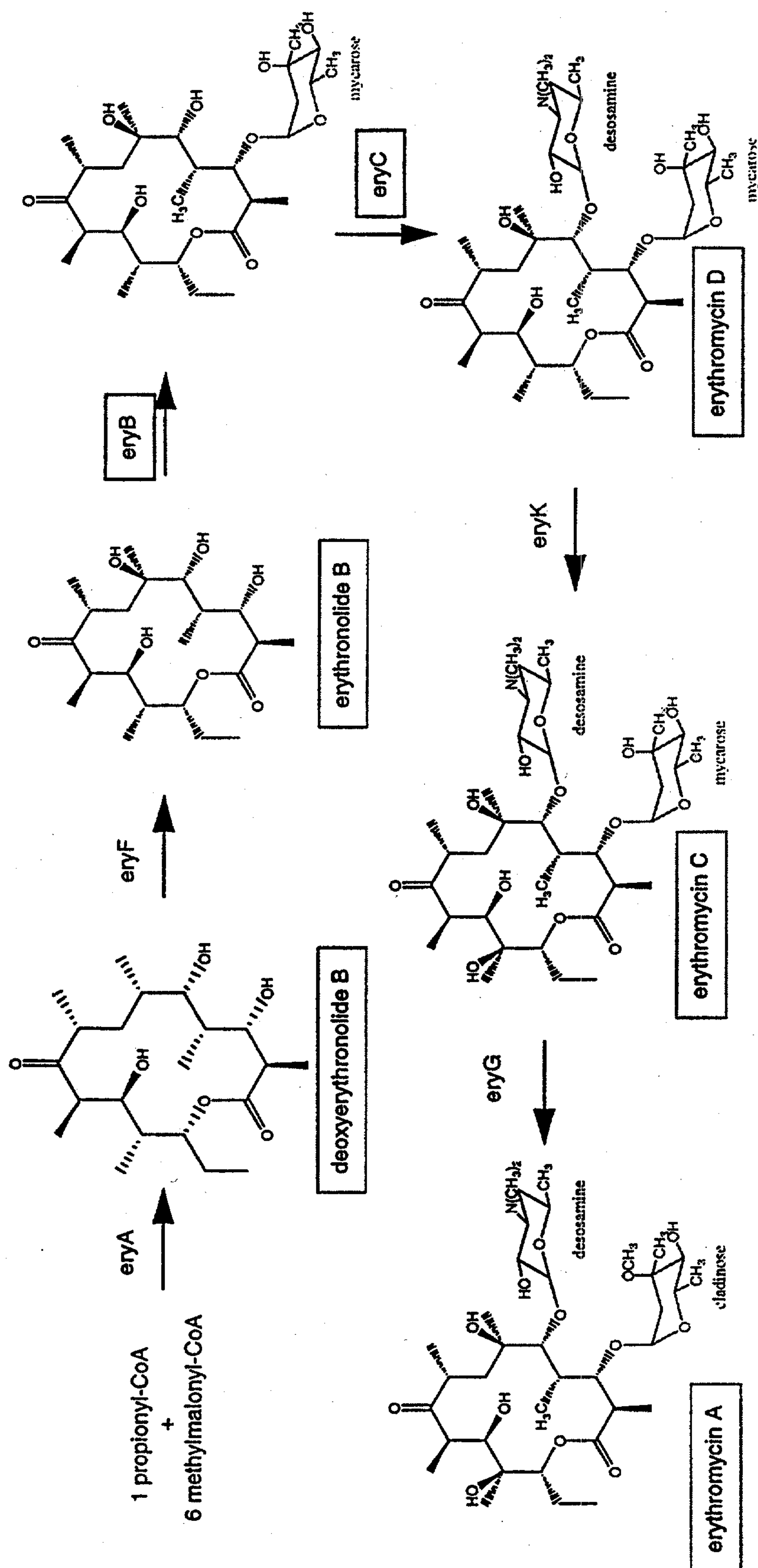


Fig. 5

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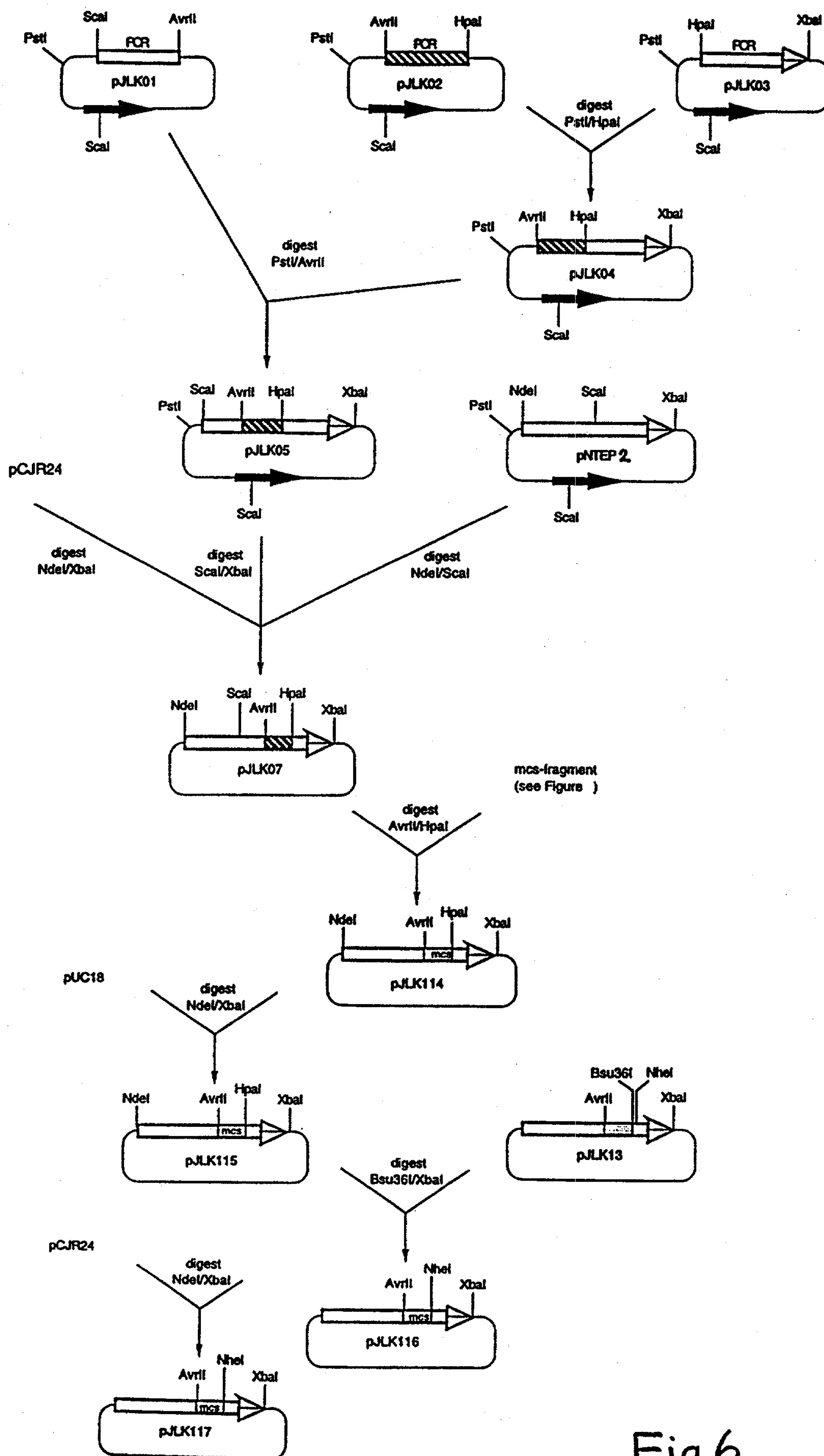


Fig 6

Figure 7

forward (Plf):

5'--CTA GCC CGG GCC GGA CTG GTA GAT CTG CCT ACC TAT CCT TTC CAG GCG AAG CGG TTC TGG CTG CAG CCG GAC CGC ACT AGT CCT CGT GAC GAG

GGG GAT GCA TCG AGC CTG AGG GAC CGG TT-3'

backward (Plb):

... CCG TCC CAC CTC ACC CAC TCC CCG TCC CCG CTA GTG CGG TCC GGC TGC AGC CAG AAC CGC TTG CCC TGG AAA GGA TAC GTA

GGC AGA TCT ACC AGT CCG GCC CGG C-3'

oligos annealed:

----- BglII SnaBI PstI SpeI NsiI Bsu36I HpaI
 CTAGGCCGGCCGGACTGGTAGATCTGCCCTACGTATCCTTTCCAGGGCAAGCGGTTCTGGCTGCAGCCGGACCCGCACTAGTCCCTCGTGACGAGGAGATGATCGAGCCCTGAGGGACCGGTT
 CGGCCCGGCCCTGACCATCTAGACGGATGATAGGAAGAGTCCGGTTCGCCAAGACCGACGTGGCCCTGGCGGTGATCAGGAGCACTGCTCCCTCTACGTAGCTCGGACTCCCTGGCCAA

The erythromycin PKS

