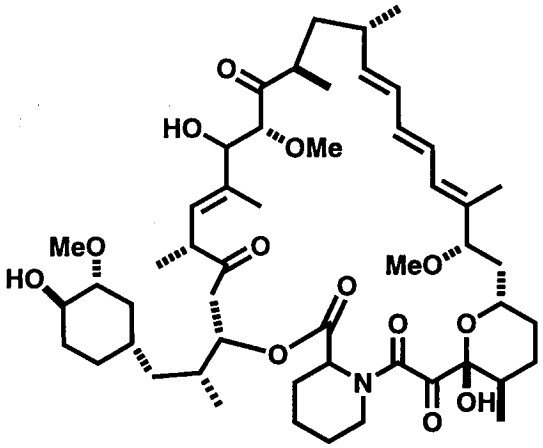
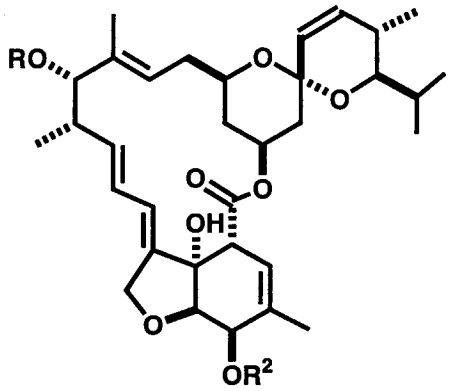
Some Polyketide Natural Products

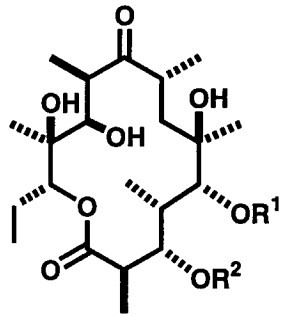
Note diversity of structure and diversity of activity



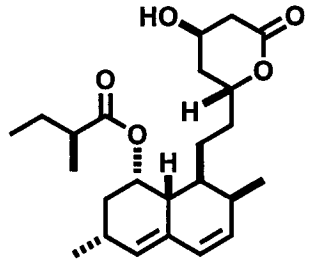
Rapamycin
(Immunosuppressant)



Avermectin
(antiparasitic)

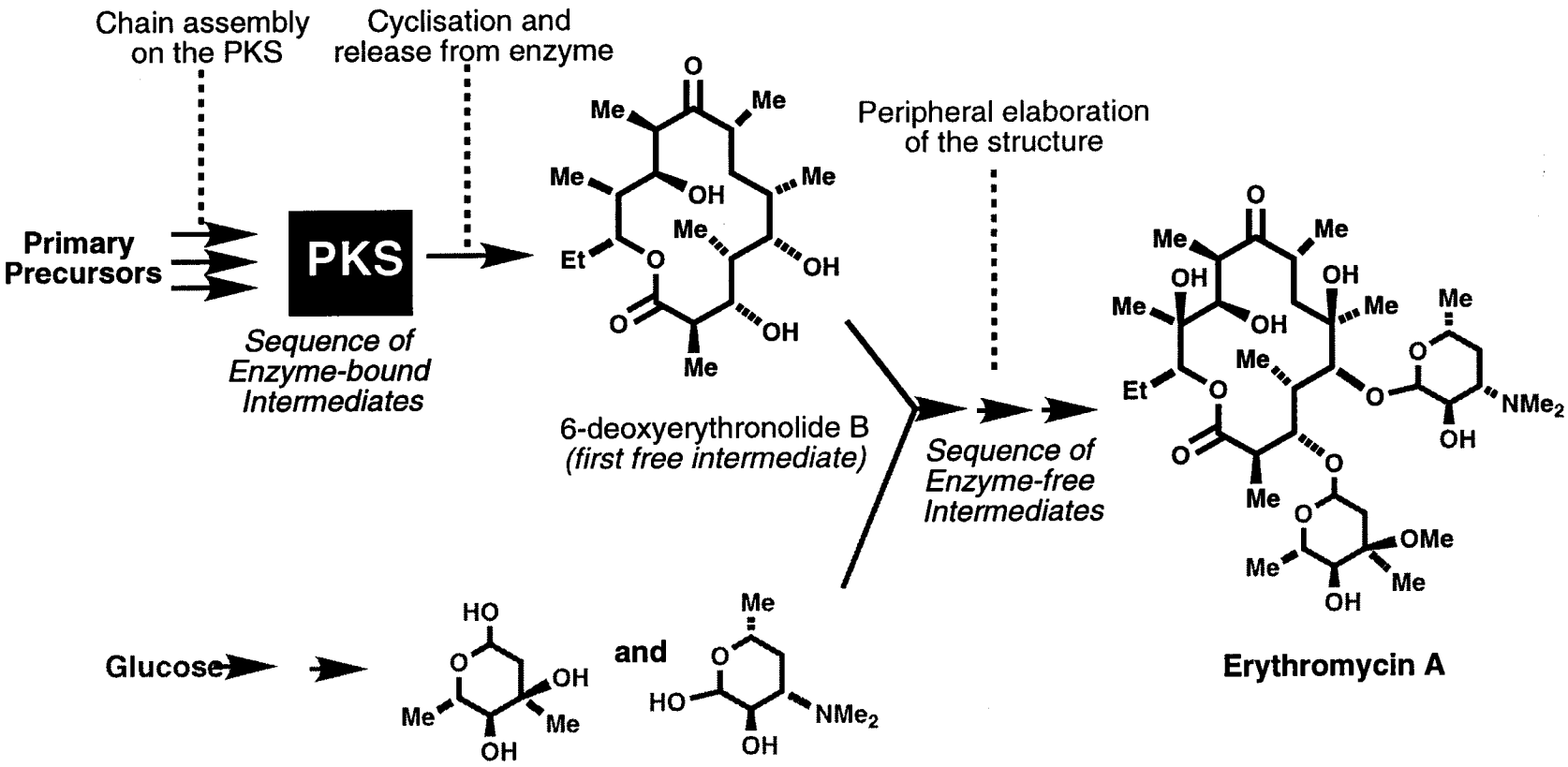


Erythromycin
(antibiotic)

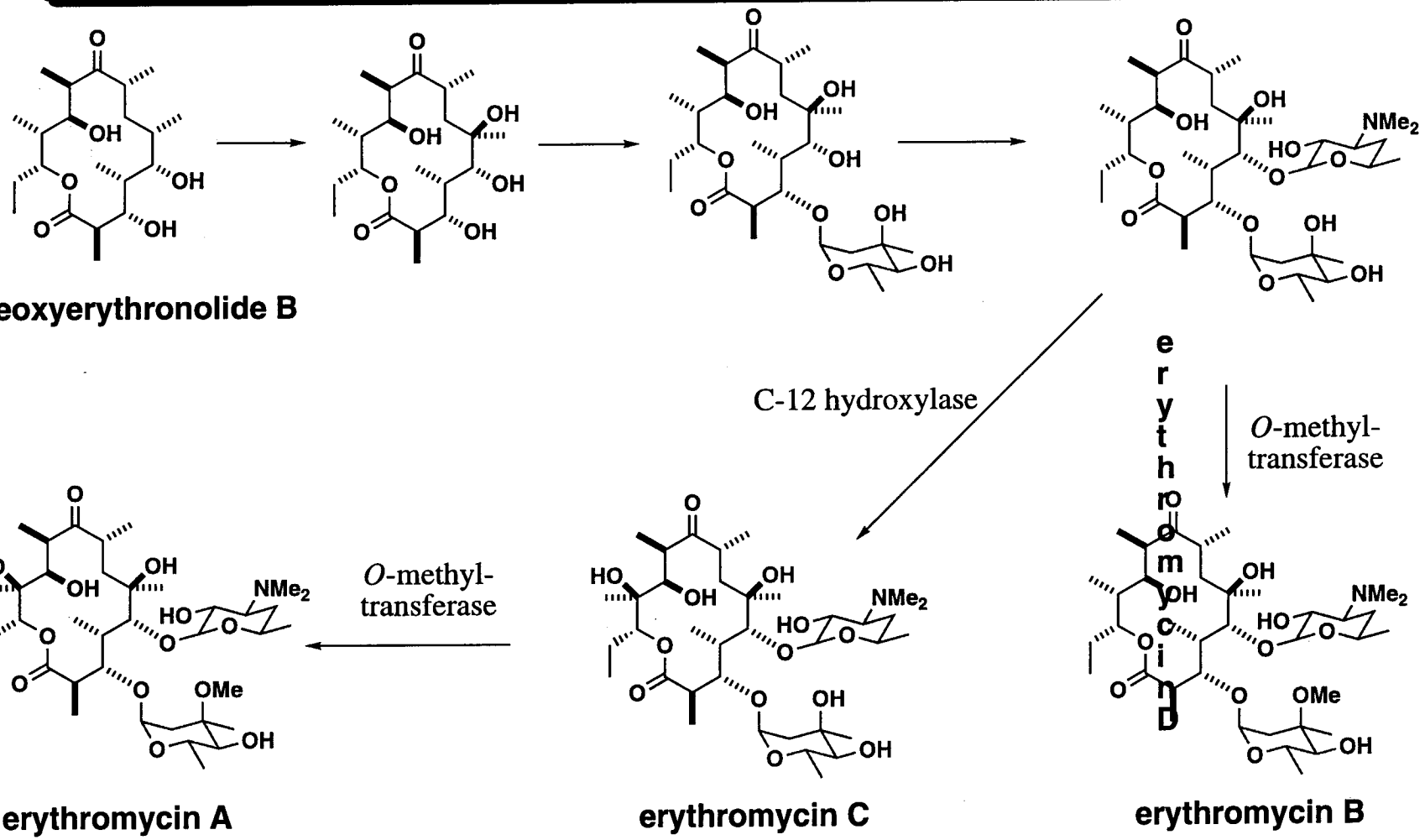


Lovastatin
(cholesterol lowering)

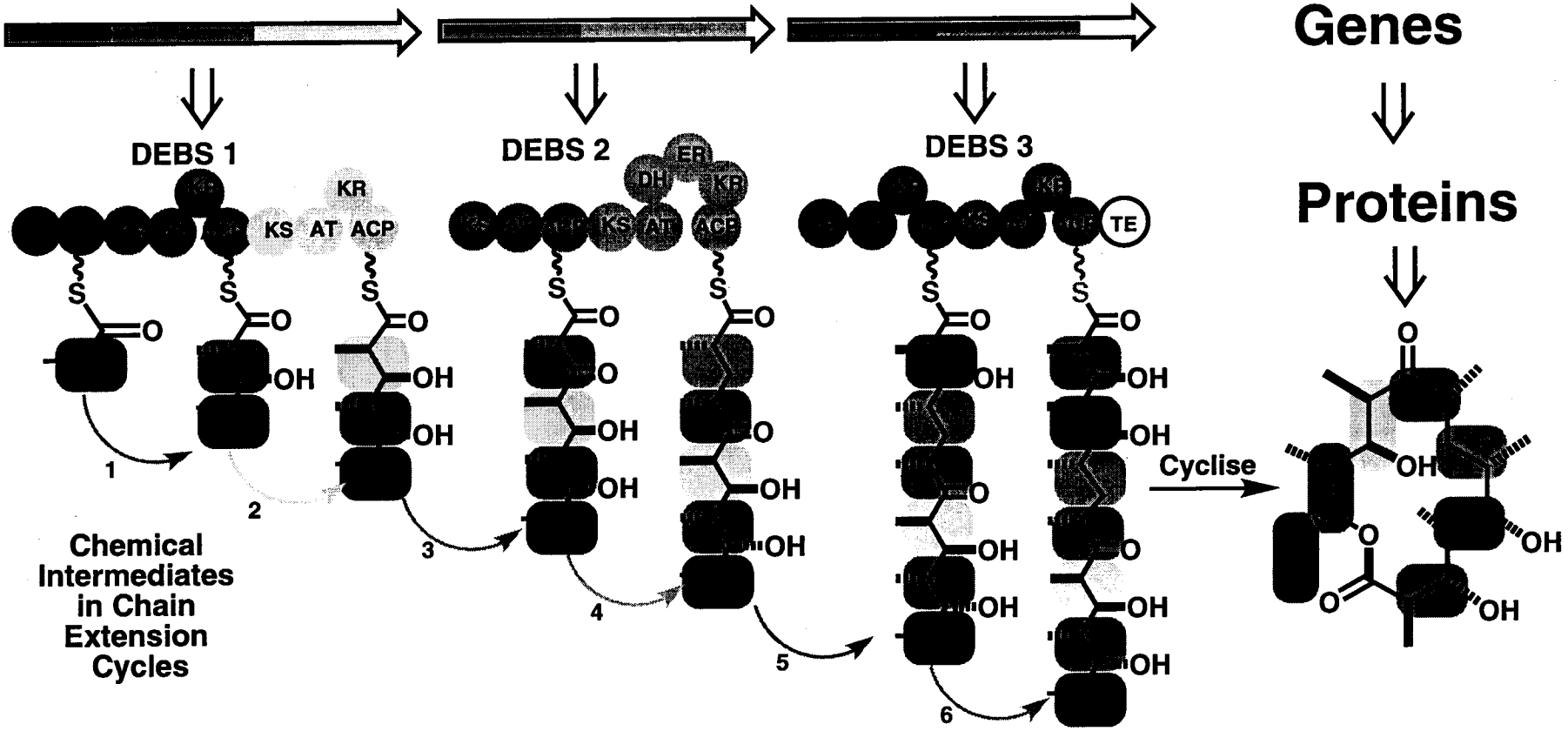
Overview of the Erythromycin Biosynthetic Pathway



Normal Post-PKS Intermediates in Erythromycin Biosynthesis



Organisation of the Erythromycin PKS



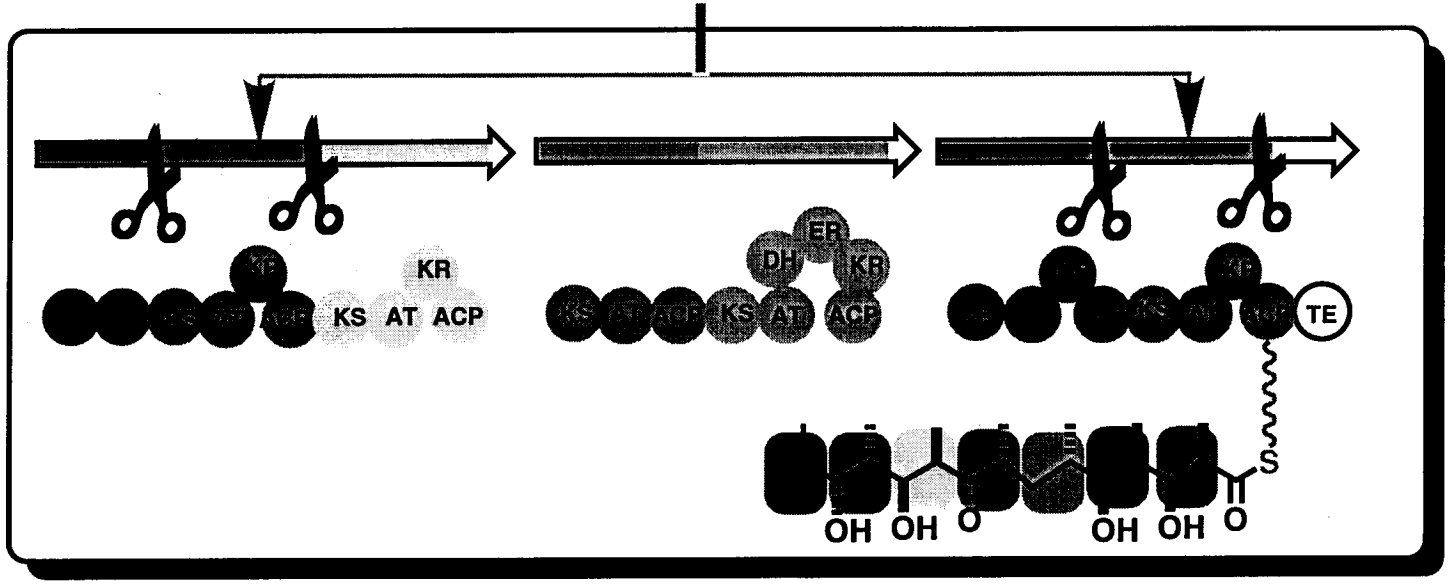
The PKS acts as a highly organised *molecular assembly line*

The various catalytic sites ('domains') are organised into "modules" one for each chain extension cycle

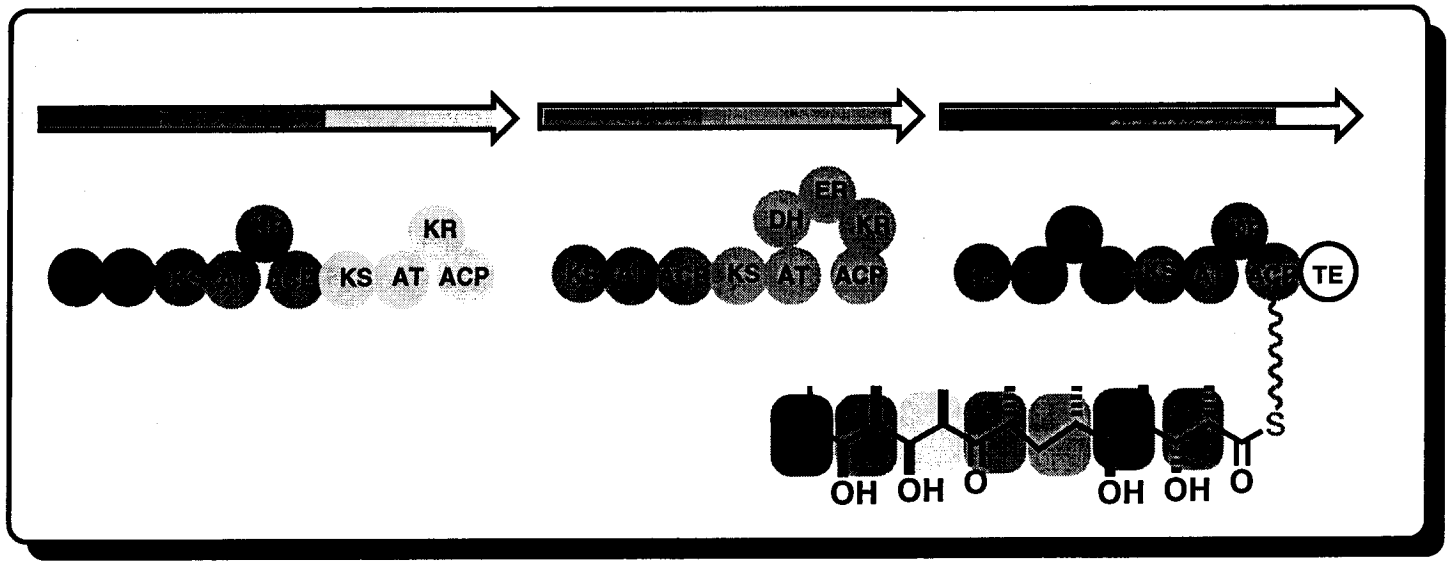
It may be possible to alter the overall synthetic product by altering the composition or order of the modules?

RE-organisation of the Erythromycin PKS!?

Cut and Paste Genes

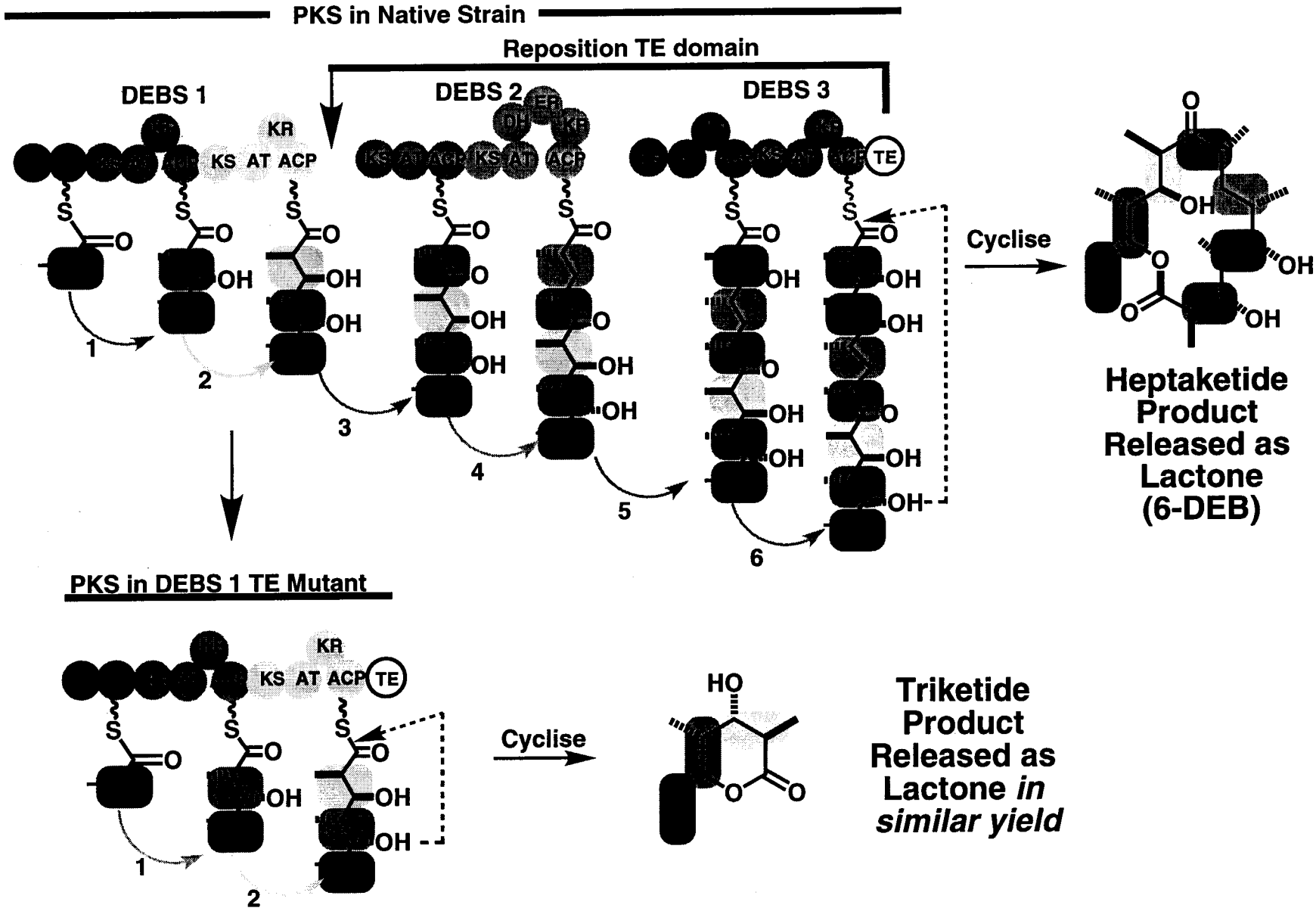


Genes
⇓
Proteins
⇓
Natural Compound

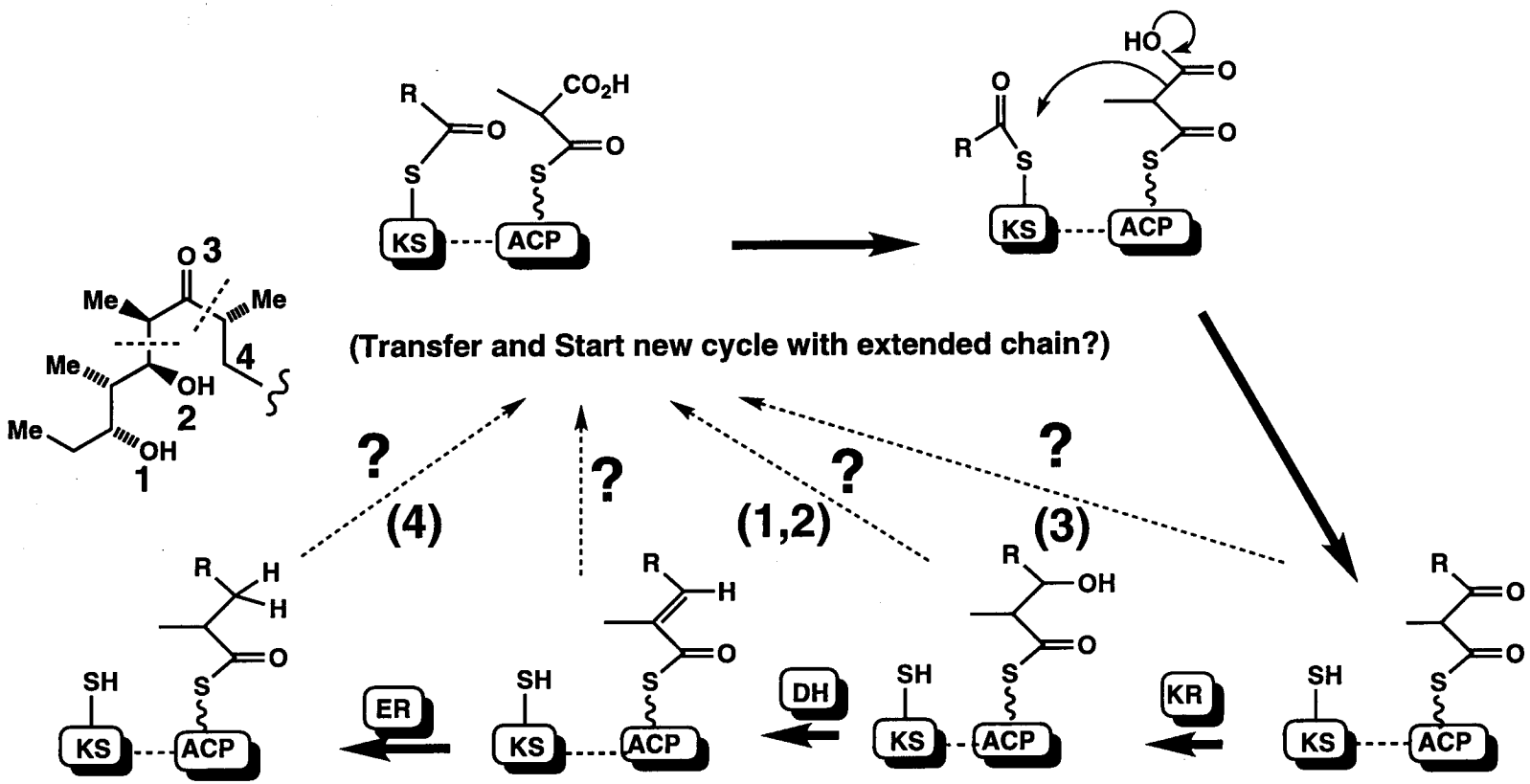


Genes
⇓
Proteins
⇓
Novel Compound!

Genetic Engineering to *Reposition* a Catalytic Domain

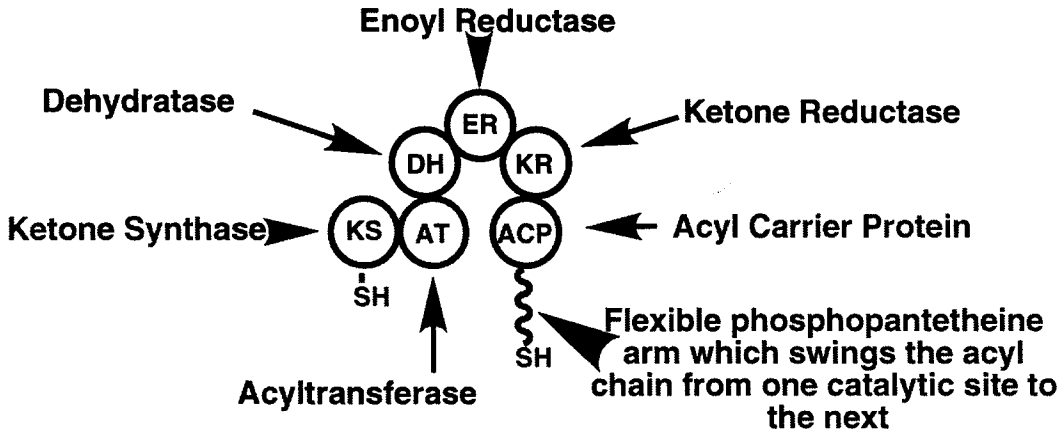


Programming of erythromycin chain extension cycles

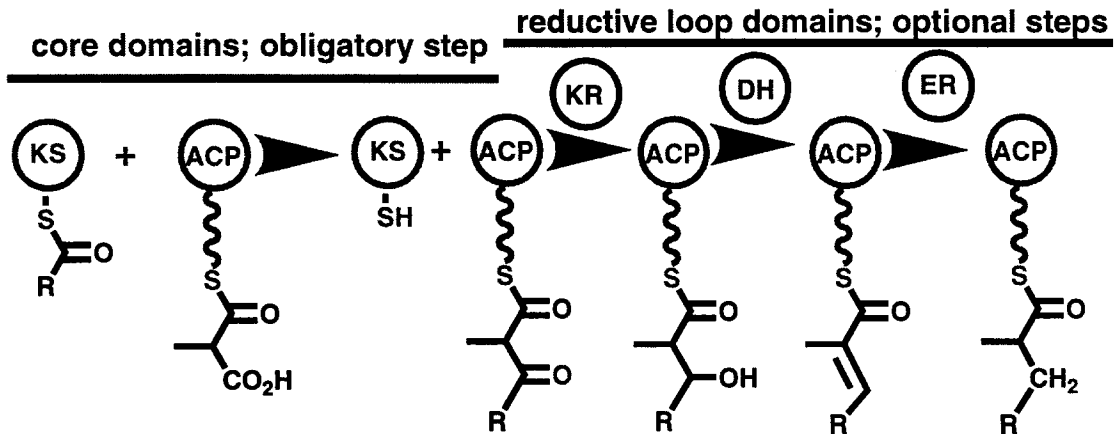


KS = Ketoacyl Synthase; KR = Keto Reductase; DH = Dehydratase; ER = Enoyl Reductase

Role of Domains in a Modular PKS



Sequence of Chain Extension Reactions.



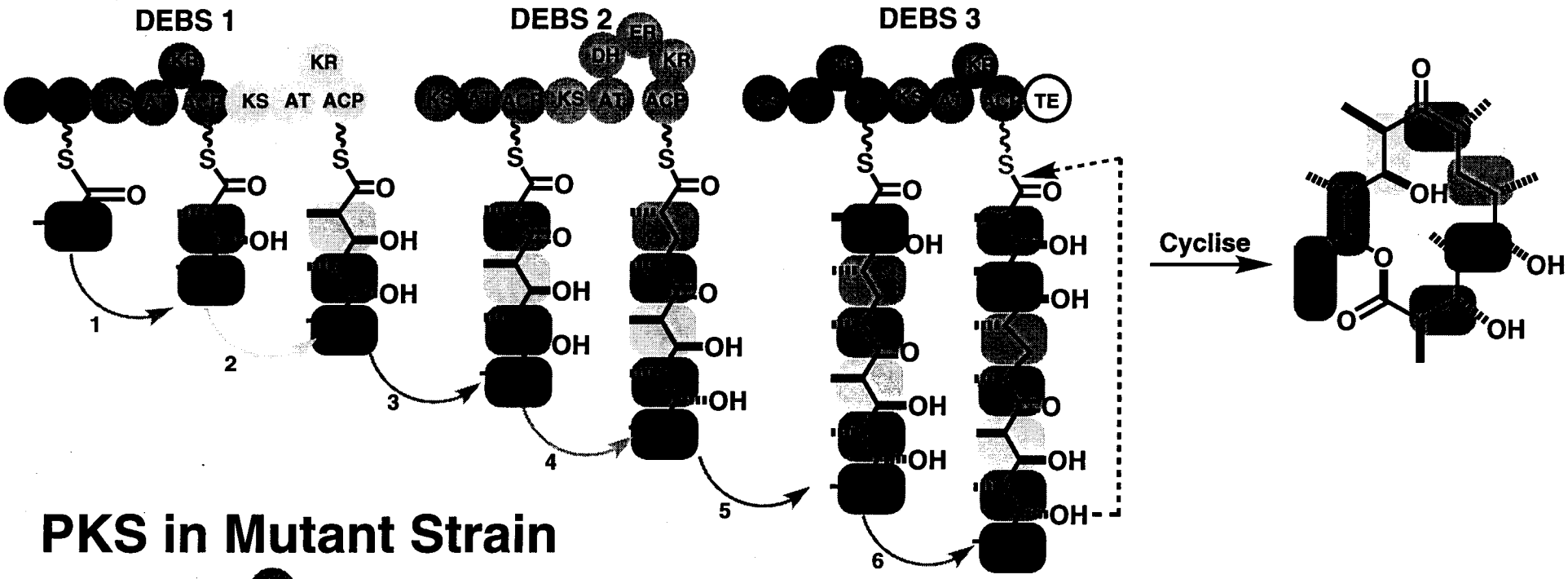
In the first step (not shown) a unit of methyl malonate is loaded onto the active thiol of the ACP.

A condensation reaction then takes place with the acyl group attached to the active thiol of the KS domain to give a ketoester.

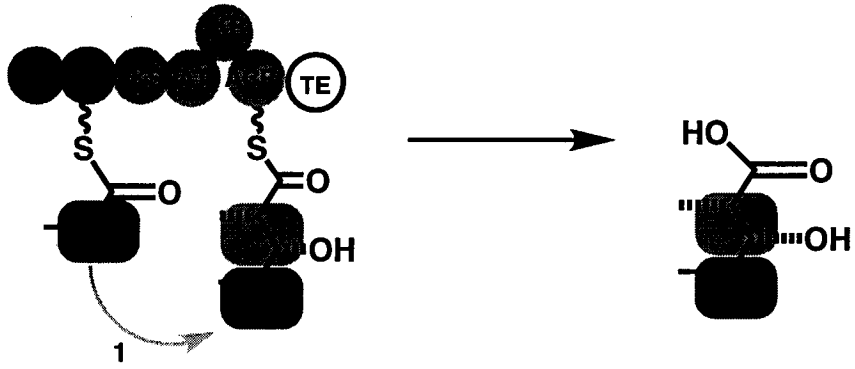
The extended chain remains bound to the thiol of the ACP during the subsequent sequence of reductive operations in which the newly formed keto group is appropriately modified

Diketide Synthase Mutant

PKS in Native Strain

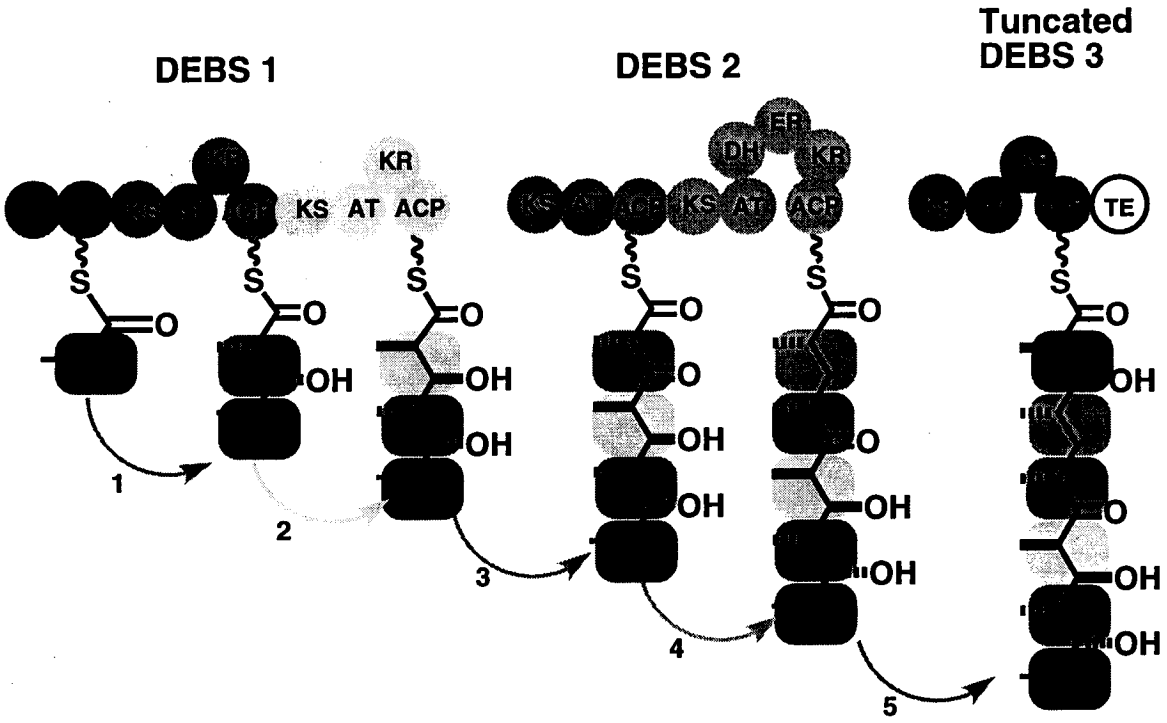


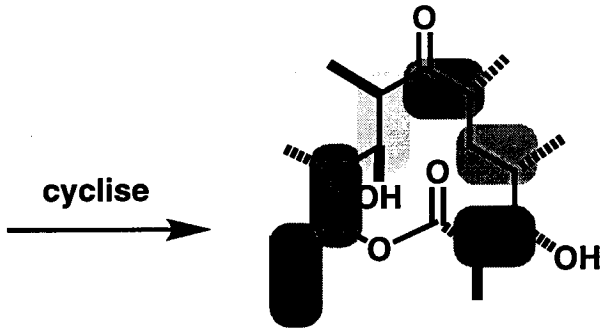
PKS in Mutant Strain



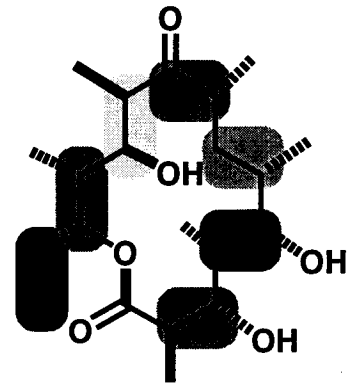
Product identified by GC and accurate mass measurement; Yield to be scaled up

Hexaketide Synthase





Mutant Product



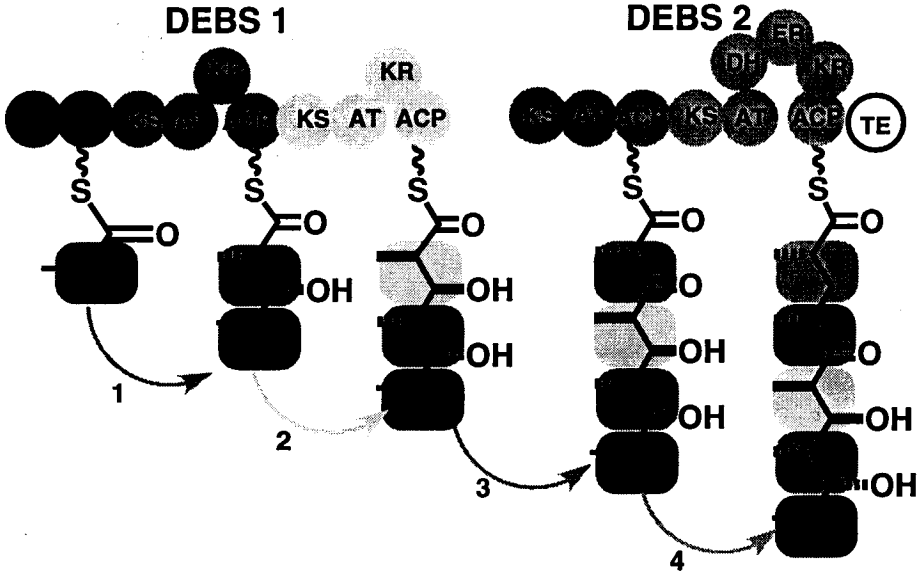
Normal PKS Product

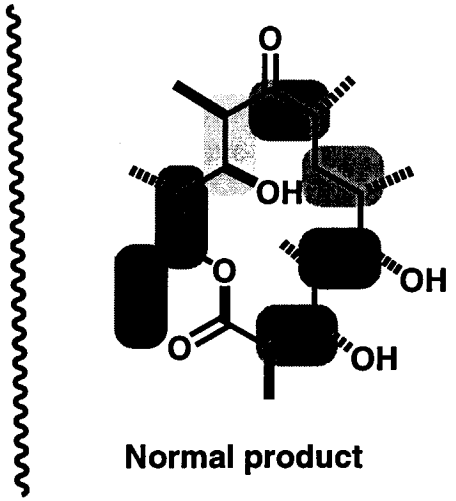
C M Kao, G Luo, L Katz, D E Cane and C Khosla,

J Amer Chem Soc, 1995, 117 9105.

Genetic Engineering to *Reposition* a Catalytic Domain

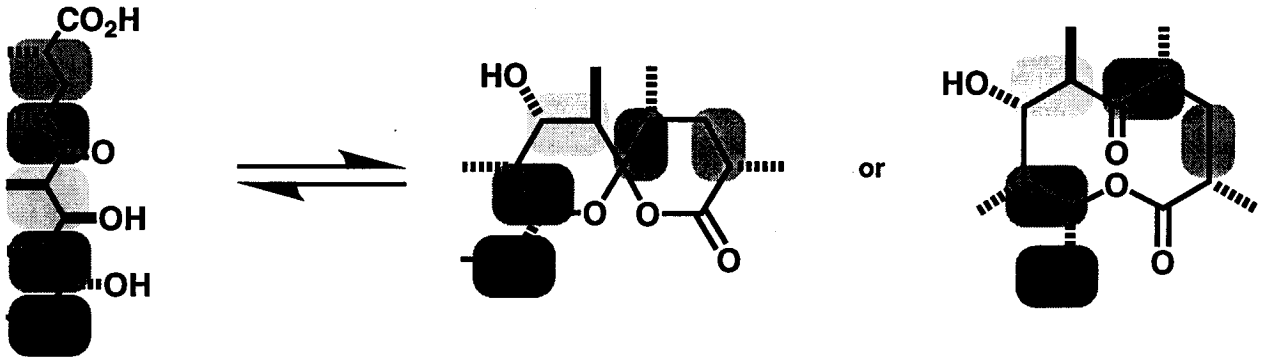
PKS in Pentaketide Synthase Mutant





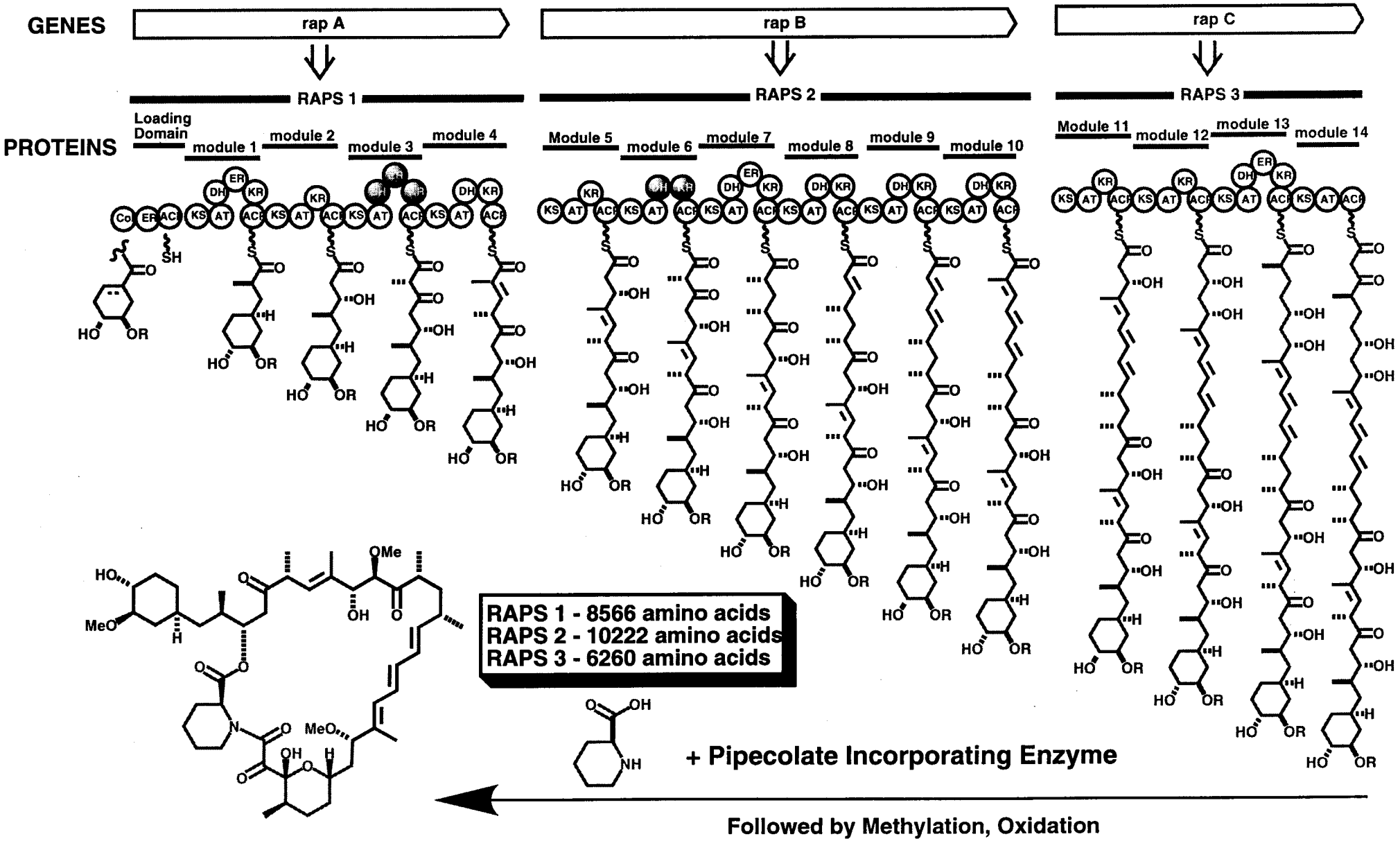
Normal product

Expected mutant products



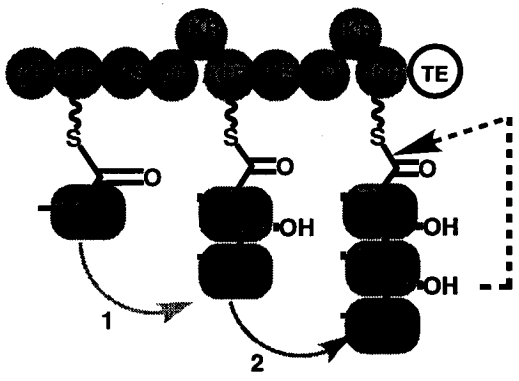
Products identified so far by accurate mass measurement; yields to be scaled up

Proposed Organisation of the Rapamycin Polyketide Synthase

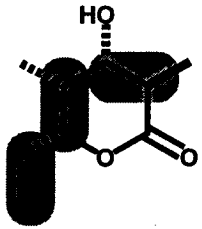


Hybrid PKS Produced by Replacement of a Natural Domain by a Foreign Domain

DEBS1-TE

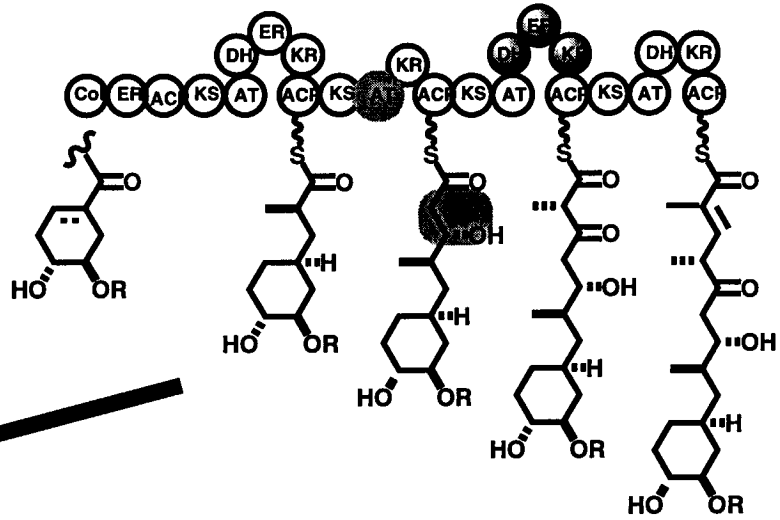


Cyclise

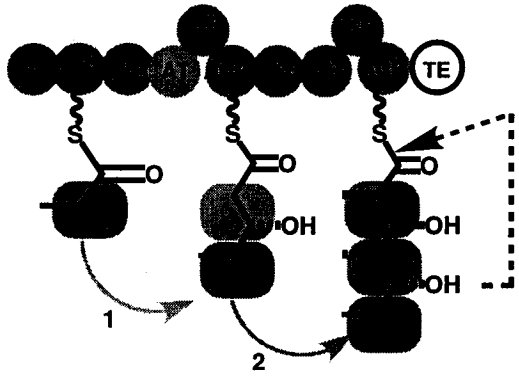


Normal product

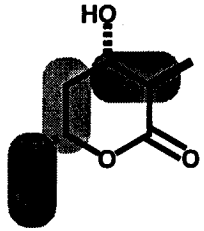
RAPS 1



Hybrid of DEBS1-TE and RAPS1



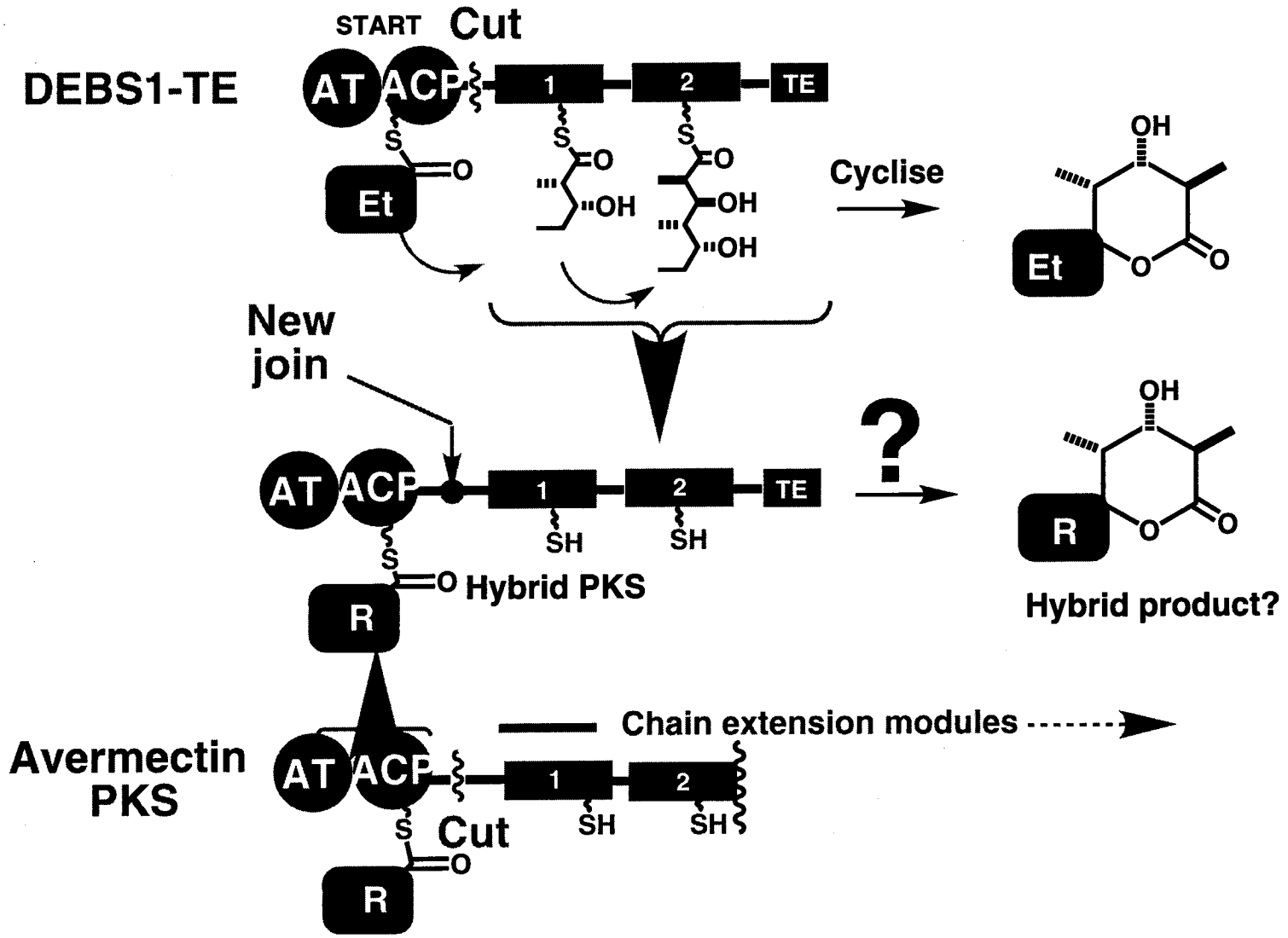
Cyclise



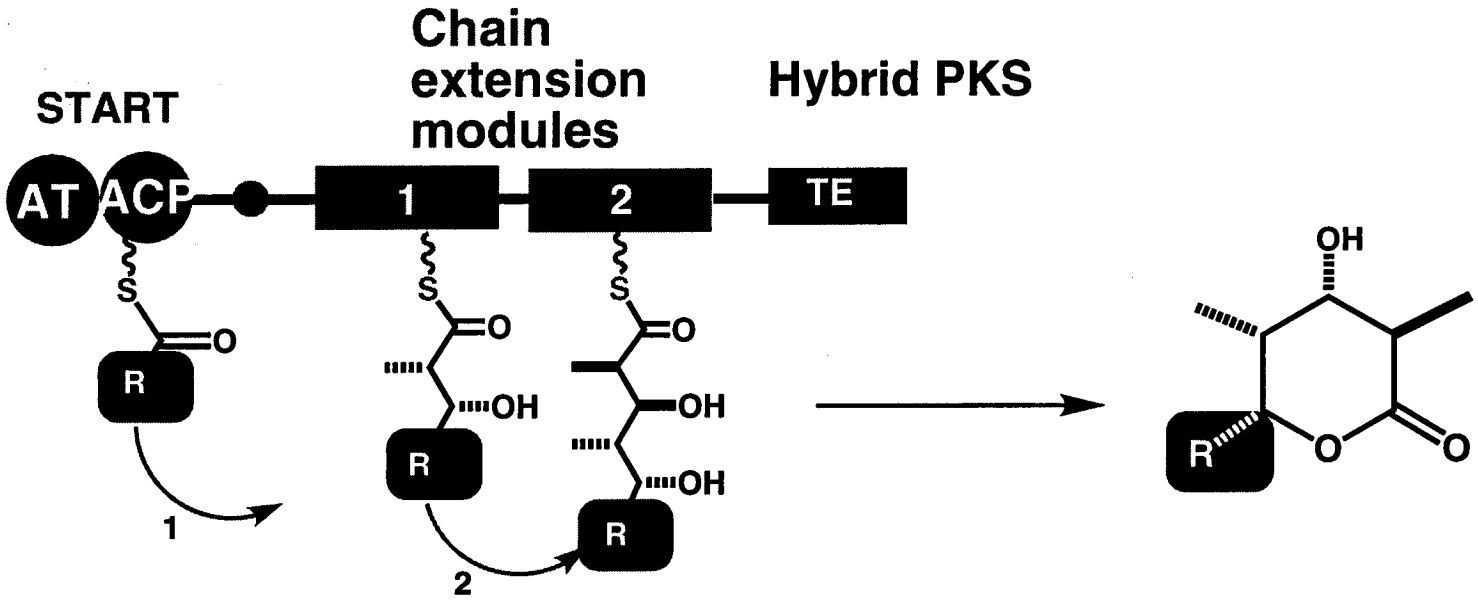
Hybrid product

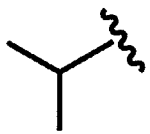
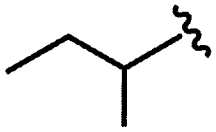
The "predicted" result!
A C_3-unit replaced by a C_2- unit

Construction of *Hybrid* Derivative of the "toy" PKS , DEBS1-TE: erythromycin STARTER domain replaced by avermectin STARTER domains



Productivity of *Hybrid* Derivative of the "toy" PKS , DEBS1-TE: erythromycin STARTER domain replaced by avermectin STARTER domains

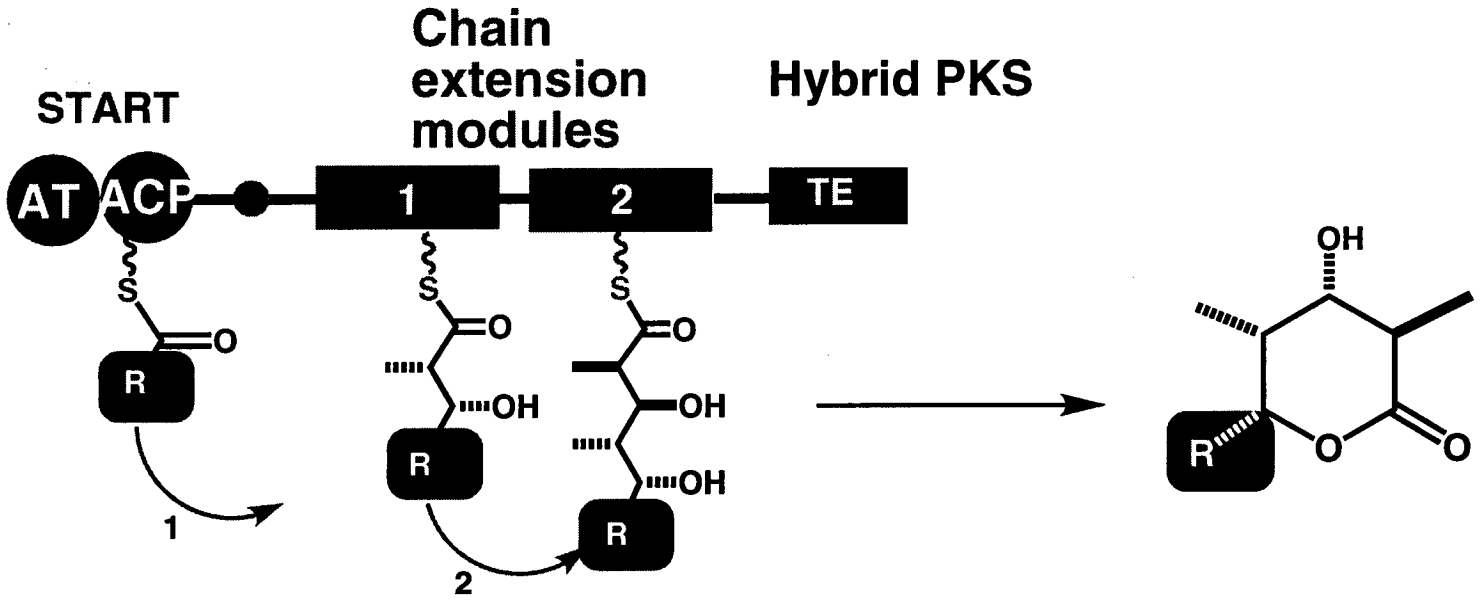


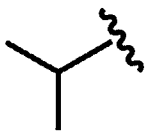
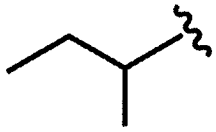
For avermectins R =  and 

erythromycin, R = Et or Me

Results: All four compounds present in similar amounts; total lactone production not significantly depressed; the replacement has worked!

Productivity of *Hybrid* Derivative of the "toy" PKS , DEBS1-TE: erythromycin STARTER domain replaced by avermectin STARTER domains



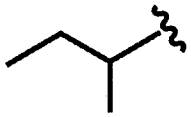
For avermectins R =  and 

erythromycin, R = Et or Me

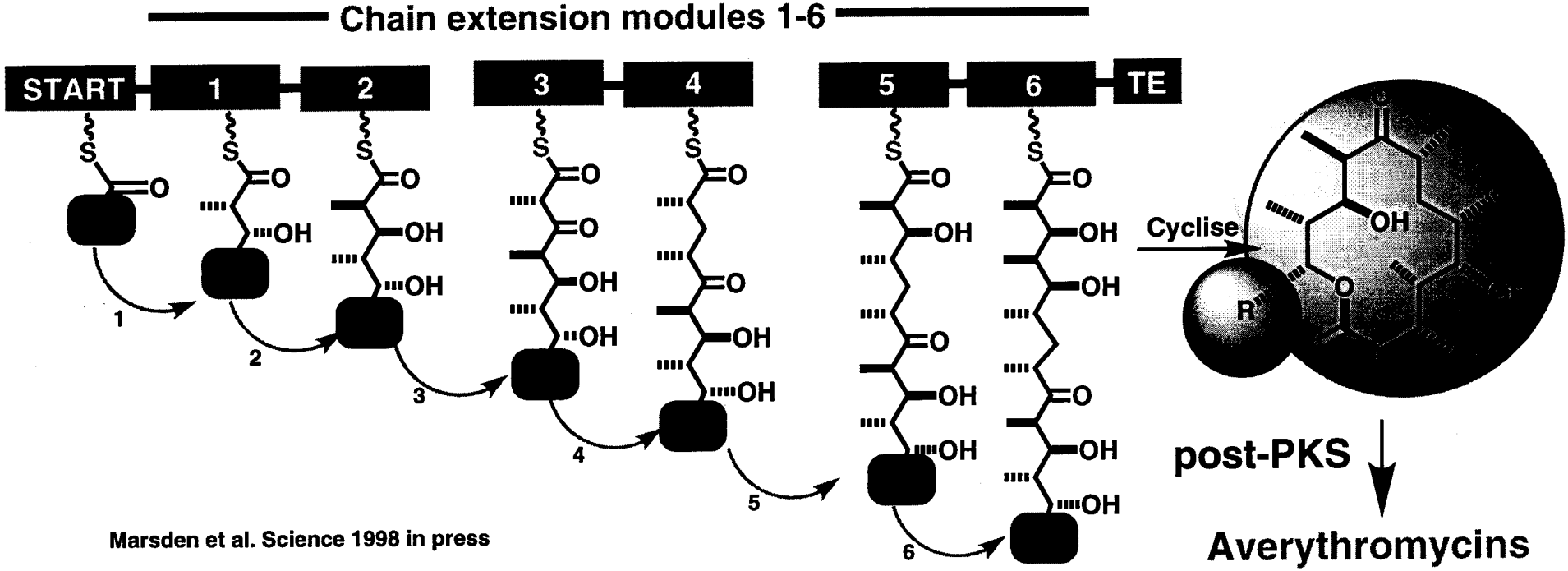
Results: All four compounds present in similar amounts; total lactone production not significantly depressed; the replacement has worked!

Chimeric PKS with erythromycin STARTER domain displaced by avermectin STARTER domain

KS

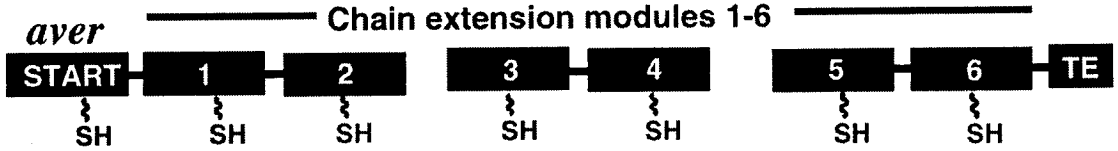
For avermectins R =  and 

erythromycin, R = 

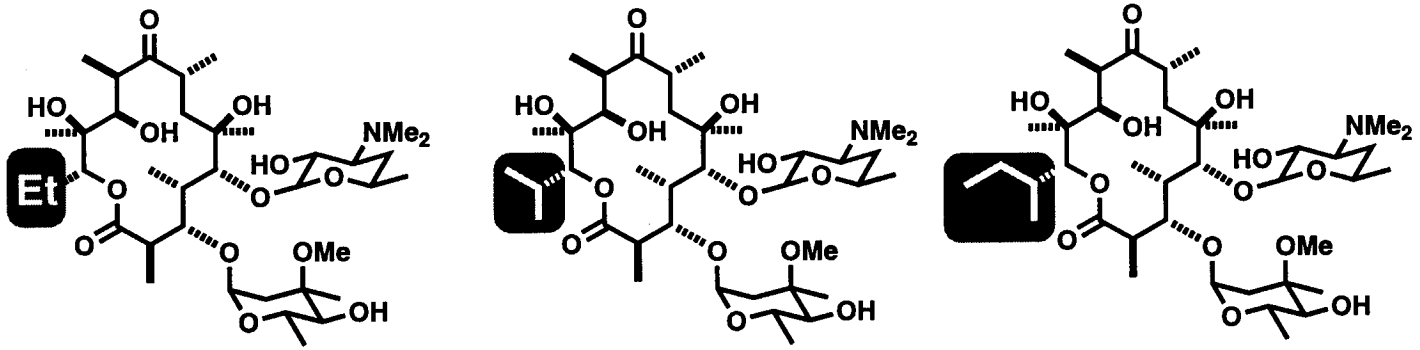


Marsden et al. Science 1998 in press

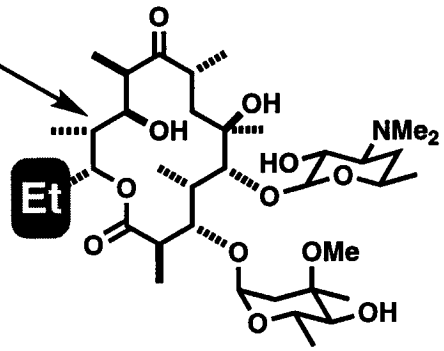
Post-PKS Intermediates from *aver* START mutant



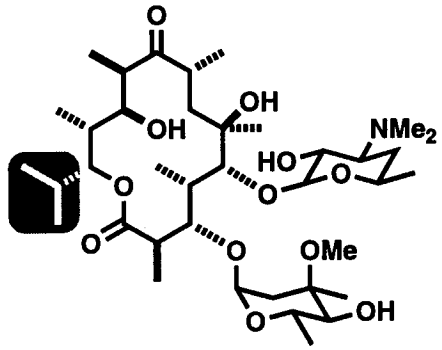
erythromycin A analogues



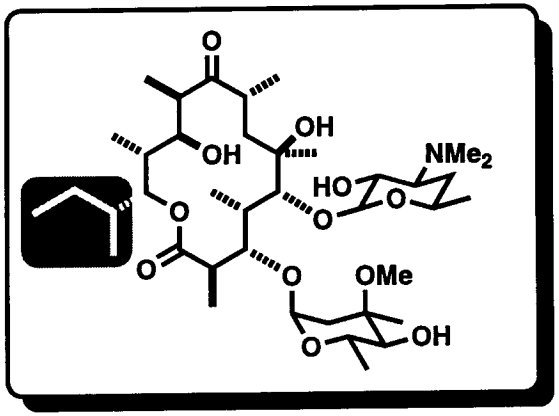
erythromycin B analogues (no hydroxylation)



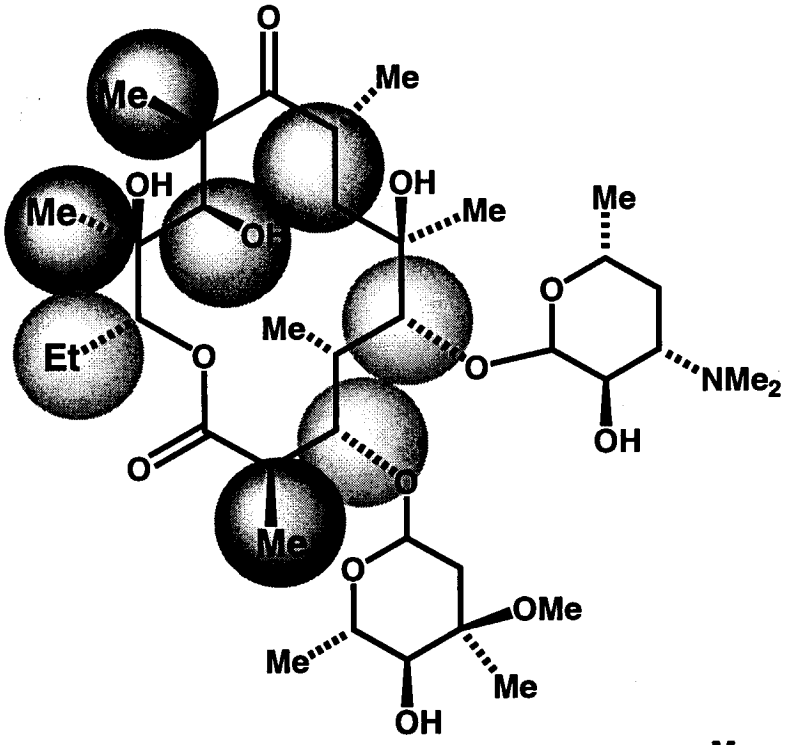
Normal products



Novel products



Novel Erythromycin Templates from Genetic Engineering



Erythromycin A

= altered starter acid

= Me replaced by H

= altered oxidation level

More than 20 altered compounds have been made;

Potentially hundreds more could be made by various combinations of the new technology

Semi-synthetic Derivatives of Erythromycin A with Improved Activity