



**FIGURE 7.11** Mutasynthesis of rapamycin analogs. (a) Conversion of lysine to pipecolic acid mediated by rapL. (b) Incorporation of pipecolate in control strain leads to rapamycin. (c) Feeding 4-hydroxyproline to the rapL knockout strain yields a novel analog.

hydrogenation (KR, ER, DH, module 4). The chain is terminated and cyclized through the action of the final active site in DEBS 3, the thioesterase (TE). Additional tailoring enzymes further modify 6-deoxyerythronolide B, by oxidation, glycosylation, and methylation processes to yield the fully functionalized erythromycin A.

In order to modify the alkyl substitution of the macrocycle, one would alter the specificity of the acyltransferase moiety (AT) that is responsible for recruiting these units to join the growing chain. As shown in Figure 7.13, by swapping the genes that encode for the propionyl group to be added in module 4 for another AT domain that is specific for an acetyl group, it is possible to encode for an erythromycin analog that lacks methyl substitution at position 6. In theory, similar modifications in alkyl substitution and oxidation state can be engineered for the other positions on the macrocycle.

#### 7.5.4 STRUCTURE–ACTIVITY RELATIONSHIPS

Such alterations in structure, whether they are done by chemical synthesis or biosynthetically, result in variations in the biological properties of the compound in comparison with the parent. Such structure–activity relationships or SAR reveal the areas of a given structure type that are optimal for driving the potency or selectivity of the series. A very simple example of SAR observed in naturally occurring congeners is illustrated in Figure 7.14 for the antibiotic mannopeptimycin. The mannopeptimycins are ordinarily produced as a mixture of components, some of which contain an isovaleryl ester group on the terminal unit of the di-mannose side chain. The bioassay data in the