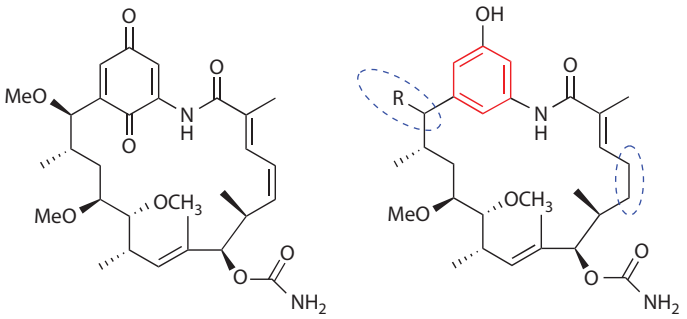

FIGURE 7.10 Macbecin and nonquinone analogs derived from the knock out of a key oxidative function.

altered product. One such example is shown in Figure 7.10 from the work of scientists at Biotica Technologies, Cambridge, UK, where the oxidation state of the aromatic ring in the ansamycin antibiotic macbecin is reduced from quinone to phenol. This was accomplished by inactivation of the gene *macM*, which was found through genetic analysis to code for the specific enzyme responsible for addition of the *para* oxygen to the phenol ring that is further oxidized to the quinone. The macbecins are promising HSP-90 inhibitors with potential in cancer chemotherapy whose off-target effects have been linked to the reactive quinone moiety. The new products lack this reactive unit and are expected to have reduced side effects. This precise alteration in structure was made possible by the identification of the functions associated with the key genes found in the macbecin biosynthetic gene cluster.

7.5.3.1 Mutasynthesis

Another technique that relies upon the knockout of an enzymatic function is known as mutasynthesis. In mutasynthesis, a key step in a biosynthetic sequence is knocked out such that no product is made without the addition of a suitable precursor. In the past, these processes were done by random mutagenesis followed by screening of the resultant mutants for the desired phenotype. Today, it is a straightforward process to obtain the fully annotated genetic map of a biosynthetic pathway and to specifically design experiments to knock out the targeted function. One such example is shown in Figure 7.11 for the microbial product rapamycin. This work was pioneered by Peter Leadlay at the University of Cambridge, England who mapped the biosynthetic gene cluster for rapamycin. As illustrated, knock out of the gene rapL results in the organism's inability to make pipecolic acid which is the usual amino acid incorporated into the rapamycin macrocycle. Supplementing the fermentation medium of the knockout strain with alternative cyclic amino acids, such as substituted proline analogs, results in efficient incorporation of these units yielding selectively modified rapamycin analogs.

7.5.3.2 Polyketide Synthase (PKS) Engineering

In the case of polyketide-derived compounds, the biosynthetic modules that are responsible for the iterative addition of two-carbon units to the nascent chain can be exchanged within sequences to alter both the substitution and oxidation state of the resultant unit. The most studied case of this biosynthetic class is erythromycin. The polyketide assembly of this macrolide antibiotic is illustrated in Figure 7.12, where three multifunctional enzymes DEBS 1, 2 and 3, encoded by *eryAI, II* and *III* genes, assemble a starter unit propionate residue with six propionate extender units to produce the substituted polyketide chain. The chain is indicated in the figure growing as the successive condensations add the propionate units. The resulting keto groups are reduced to alcohols by ketoreductase functions (KR, modules 1, 2, 5, and 6), not reduced at all as in module 3 (note the lack of a reductive loop), or fully reduced to the bare methylene by ketone reduction, enolization, and