Low molecular weight thiols safeguard various organisms against toxic oxidants and offer a reducing environment necessary for effective cellular operation. Mycothiol is the principal low molecular weight thiol in actinobacteria, a diverse group of Gram-positive, high-G+C microorganisms that include notable genera as Mycobacterium and Streptomyces. Similar to glutathione, its functional analog in eukaryotes and Gram-negative bacteria, the sulfhydryl group of mycothiol is acquired from cysteine of which the carboxyl and amino groups are blocked to avoid autooxidation. In mycothiol (1), the cysteinyl moiety is N-acetylated and is attached as an amide to D-glucosamine (GlcN), which, in turn, is α1′→1-linked to D-myoinositol (Ins). Mycothiol disulfide (2) is produced upon reaction with oxidants, but is rapidly recycled back by mycothiol disulfide reductase to 1 preserving a high thiol/disulfide ratio within the confines of the cell. The sulfhydryl group could also attack electrophilic xenobiotics leading to an S-conjugated mycothiol (exemplified by the bimane derivative 3), which is further cleaved at the D-glucosaminyl amide bond by mycothiol-S-conjugate amidase to form the cysteine-bound toxin that is excreted out of the cell and the GlcN-Ins pseudodisaccharide that is reused as a substrate for mycothiol biosynthesis. Mycobacterial strains depleted of mycothiol have shown a dramatic increase in susceptibility to oxidative stress and some antitubercular agents. A total synthesis of mycothiol will be presented here.