Pseudomonas syringae pv. *syringae* (*Pss.*) strains infect many food crops worldwide. The extracellular signaling molecule leudiazen, isolated from *Pss.* UMAF0158, regulates the virulence of this strain by controlling production of mangotoxin, a toxin causing necrosis of mango leaves. The biosynthesis of leudiazen is coordinated by *Pseudomonas virulence factor* (*pvf*), composed of the genes *pvfABCD*. While leudiazen has been identified as a chemical signal, the relationship between its stereochemistry and bioactivity was unknown. Furthermore, the biosynthetic route to leudiazen remains to be elucidated, specifically regarding the functions of the enzymes PvfA and PvfD. Here, we synthesized and tested (*R*)- and (*S*)-leudiazen enantiomers in a β-galactosidase reporter assay, comparing the relative abilities of these enantiomers to induce the expression of mangotoxin biosynthesis. We determined that (*S*)-leudiazen is 100-fold more potent than (*R*)-leudiazen in activating mangotoxin expression. We also purified the uncharacterized enzyme PvfA for biochemical study. We found that PvfA performs a six-electron oxidation of *p*-aminobenzoic acid to *p*-nitrobenzoic acid in an iron(II)-dependent manner. While the role of PvfA in leudiazen biosynthesis remains to be determined, these results demonstrate PvfA is an iron-dependent oxidase, which may be used to produce similar diazeniumdiolate molecules to leudiazen for industrial or medical purposes. Additionally, understanding the regulation of *Pss.* UMAF0158 virulence by leudiazen could guide strategies to mitigate the effects of *Pss.* infection on crops.