



**Figure 5.** Catalytic mechanism of sirtuins (Class III). Proposed mechanism of the NAD<sup>+</sup>-dependent deacetylase reaction. The first step of the reaction involves nucleophilic addition of the acetamide oxygen to the C1' position of the nicotinamide ribose to form a C1'-O-alkylamidate intermediate and free nicotinamide. Next, the 2'-hydroxy group of the NAD<sup>+</sup> ribose is activated by an active site histidine residue that, in turn, attacks the C1'-O-alkylamidate to form the 1',2'-cyclic intermediate. The 1',2'-cyclic intermediate is then attacked by an activated water molecule resulting in the formation of deacetylated lysine and 2'-O-acetyl-ADP ribose. 2'-O-acetyl-ADP ribose can be readily converted to 3'-O-acetyl-ADP ribose in aqueous solution by nonenzymatic intramolecular transesterification. Thus, nicotinamide, the deacetylated peptide, and a mixture of 2'- and 3'-O-acetyl-ADP ribose are the final reaction products.