Although antiretroviral therapy for HIV has advanced, there remains a critical need for new therapeutics, especially those targeted at resistant viral strains. The Gag polyprotein, and in particular its constituent nucleocapsid protein, NC, represents a prime target for antiretroviral inhibition. We have developed a class of small molecule inhibitors of NC, based upon an S-acyl-2-mercaptobenzamide thioester (SAMT) scaffold, that covalently modify the zinc-binding domains of NCp7 in vitro and in cells. We have used mass spectrometry to investigate the mechanism of viral inactivation of NC and Gag by SAMT, identifying multiple sites of covalent modification that resulted from SAMT reaction. Targeting multiple residues limits the potential for emergence of virus escape mutations, making mercaptobenzamide antiviral compounds a strong starting point for developing a new therapeutic agent against HIV. Indeed, recent results have indicated that combination of SAMT-247 formulated as a microbicide coupled with a vaccine provided a high degree of protection from viral infection in a rhesus macaque model. These studies further suggest the presence of host targets as well, which we have begun to investigate using unbiased mass spectrometry approaches.