Chemical modifications to histones play a pivotal role in the epigenome and the regulation of gene expression, and their abnormality has been tightly linked to numerous disorders in humans. Therefore, chemical tools to manipulate histone modifications hold promise for both therapy and the elucidation of epigenetic mechanisms. We previously developed the chemical catalyst LANA-DSH, which binds to nucleosomes via a LANA peptide ligand and selectively acylates lysine 120 (K120) of histone H2B, a lysine residue proximal to the catalyst moiety, by activating acyl-CoAs (Amamoto Y et al. J. Am. Chem. Soc. 2017). Thus far, however, in-cell histone acylation by a catalyst system has not yet been demonstrated. Here, we report a chemical catalyst system, composed of a nucleosome-binding catalyst (PEG-LANA-DSH) and a cell-permeable thioester acyl donor (NAC-acyl), that can promote regioselective lysine acylation of histones in living cells. Our data suggest that while LANA-DSH is immediately decomposed in cells, the addition of polyethylene glycol (PEG) to the catalyst can prevent this. We found that increasing the size of PEG conferred LANA with greater in-cell stability, but reduced its activity as a histone acylation catalyst, indicating that there is an optimum PEG length balancing its stability and catalyst activity. The optimized PEG-LANA-DSH catalyst efficiently promoted H2BK120 acetylation in living cells, which subsequently suppressed ubiquitination of H2B and gene transcription. Thus, our chemical catalyst system can be used as a unique tool to manipulate the epigenome for therapeutic purposes or further understanding epigenetic mechanisms.