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SHAMS — COMBINING CHEMICAL MODIFICATION OF RNA AND MASS SPECTROMETRY TO EXAMINE POLYPURINE TRACT-CONTAINING RNA/DNA HYBRIDS

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Selective 2' hydroxyl acylation analyzed by primer extension (SHAPE) has emerged as a facile method of examining RNA structure both *in vitro* and *in vivo* and exploits accessibility of the ribose 2'-OH to acylation by N-methylisatoic anhydride (NMIA) in a flexible, or unpaired, configuration. Subsequent primer extension terminates at the site of chemical modification, and the products are detected by high resolution gel electrophoresis. When applying SHAPE to investigate structural anomalies associated with the wild type and analog-substituted polypurine tract (PPT)-containing RNA/DNA hybrids, the size of the duplex (20-25 bp) rendered primer extension impractical. We therefore combined NMIA treatment with sequencing by tandem mass spectrometry, relying on the mass increment of covalently-modified RNA fragments (NMIA adduct M_r = 133Da) to identify unambiguously the probed site(s). Using this approach, we demonstrate both specific modification of the HIV-1 PPT RNA primer and variations in this acylation pattern induced by replacing template nucleotides with a non-hydrogen-bonding thymine isostere. The "SHAMS" strategy (selective 2' hydroxyl acylation analyzed by mass spectrometry) should find utility when examining the structure of small RNA fragments or RNA/DNA hybrids where primer extension cannot be performed.