Nucleosides play essential roles in human biology by serving as precursors for DNA biosynthesis, metabolic signaling molecules, and modulators of membrane receptor activity. Equilibrative nucleoside transporters (ENTs) transport nucleosides across cell membranes and regulate the efficacy of a broad range of human therapeutics for a range of disease states. The underlying ENT transport mechanism is unknown and no structures have been determined for this family of membrane transporter from any eukaryotic organism. This work has been hampered by the inability to obtain purified, and functional, ENT protein. Work outlined in this thesis provides the first example of obtaining purified, and functional, ENT protein followed by detailed functional analysis and optimization. A structural model was developed and used to guide mutational studies to propose a transport mechanism. In addition, human therapeutic and cellular substrates were studied to identify functional elements involved in substrate recognition and transport. This work amounts to a major advancement for the ENT biology field.