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In this manuscript, we report a detailed and systematic study of the protonation behavior of polycationic poly(propyl ether imine) dendrimers. Both experimental and theoretical studies are utilized so as to derive finer details of this series of dendritic macromolecules, in relevance to their efficient gene transfection vector properties. The study shows that PETIM dendrimers undergo protonation of amine groups, initiating from outer shell primary amine moieties to the inner shell tertiary amine moieties, in a shell-wise sequential pattern. This pattern of protonation is unique to PETIM dendrimers, resulting directly from the molecular feature of flexible ether linkages constituting the dendrimers. The insights accrued from the study aids understanding the ability of PETIM dendrimers to condense nucleic acids efficiently. Further, buffering capacities are derived, so as to relate the endosomal escape properties, essential for an efficient gene delivery.