Aminoethyl-Phenoxazine-deoxycytosine (AP-dC), sometimes referred to as “G-clamp”, pairs with dG, and when substituted for dC in an oligonucleotide, is able to form both Watson-Crick and Hoogsteen hydrogen bonds with the guanine base. A total of four hydrogen bonds form between AP-dC and dG: the usual three Watson-Crick hydrogen bonds and a Hoogsteen hydrogen bond between the protonated amine of AP-dC’s aminoethyl side-chain and the O6 position of the dG. As a result, an AP-dC:dG base pair significantly increases the stability of the resulting duplex relative to the comparable unmodified form. The increase in stability can be quite dramatic; in one study, a single incorporation of AP-dC in a 10-mer polypyrimidine oligonucleotide raised the Tm of the corresponding duplex by 18 degC over a control duplex containing 5-Me-dC at the same position (1). Moreover, the additional, specific presence of the Hoogsteen hydrogen bond leads to high specificity of AP-dC for dG over the other three bases (1). Thus, AP-dC may be useful in any application in which the ability to discriminate dG in a target is necessary.

Flanagan and co-workers tested AP-dC for its utility in anti-sense oligos. Based on studies of AP-dC-modified anti-sense oligos for sequence-context dependence, activity mismatch, sensitivity, RNAse-H cleavage, and hybridization kinetics, they concluded that AP-dC is a very potent, mis-match sensitive analog for dC, with high potential for improving the potency of anti-sense oligonucleotides (2). In another study, oligos containing one AP-dC at the 3’-end confer resistance to 3’-exonuclease digestion (3).

AP-dC is an excellent choice of modification whenever a large increase in duplex stability and/or specificity for dG in a target is required. References