

A Chimeric Nucleobase - Phenylazo Derivative as an Intrinsic Nucleobase Quencher

Molecular beacons are important bioanalytical probes which are most often constructed from a single-stranded oligonucleotide which has been labeled at opposite termini with a fluorophore and a quencher. When the fluorophore and quencher are in close proximity, no fluorescence is observed due to FRET (Fluorescence Resonance Energy Transfer). DABCYL (4-dimethylaminoazobenzene-4'-carboxylic acid) has been used as a quencher in the molecular beacon to absorb excitation energy from a fluorophore and to dissipate the energy as heat. However, DABCYL is unable to form a base-pair and is conventionally placed as an overhanging residue. This produces a derivative wherein the chromophore has substantial mobility and limits the types of other conjugates that can be prepared. In order to overcome these limitations, we have embarked on the synthesis of deoxyribonucleoside and peptide nucleic acid (PNA) analogue possessing DMPAU (5-[(4-dimethylaminophenyl) diazenyl]uracil) as the nucleobase. DMPAU has DABCYL-like properties due to the installation of an azo moiety at the 5-position of the uracil base. This base is designed to have the ability to form a complementary base pair with adenosine by canonical hydrogen bonding and also to quench the fluorescence emission in a molecular beacon construct. Both DMPAUridine and DMPAU PNA analogue are determined to have same UV-Vis absorbance ranges as DABCYL and reasonable quenching effect to the fluorophore.

