Sixteen new asymmetric monomethine cyanine dyes have been synthesized and their spectral characteristics and interaction with double stranded DNA have been investigated. The dyes absorb in the region 453–519 nm and have molar absorptivities in the range 37.900–93.100 lM$^{-1}$ cm$^{-1}$. The dyes do not have intrinsic fluorescence, but in the presence of dsDNA they exhibited a significant enhancement in fluorescence. The most pronounced increase was found for D9, D10, D12 and D16 allowing the recommendation of these dyes as the most sensitive DNA markers. Thermodynamic analysis of cyanine–DNA complexation was carried out using the McGhee & von Hippel non-cooperative excluded site model, and binding parameters have been derived. A hypothesis describing the DNA–dye binding mode has been proposed.