Abstract

An efficient methodology of construction of base-modified nucleosides bearing oligopyridine ligands, based on the Sonogashira or Suzuki cross-coupling reaction of halogenated nucleosides, was developed. This methodology was then successfully employed in construction of base-modified DNA bearing oligopyridine ligands which were studied in post-synthetic complexation with labile transition metals. The first step in construction of modified DNA is the synthesis of deoxynucleoside triphosphate (dNTPs) bearing various metal chelating groups, which are in second step enzymatically incorporated into DNA by primer extension experiment. The first task was the synthesis of dNTPs bearing different oligopyridine ligands, which could be done by aqueous phase cross-coupling reaction with suitable building blocks or by triphosphorylation of oligopyridine-modified deoxynucleosides. Both ways were successfully used. Aqueous phase Sonogashira cross-coupling was used for synthesis of dNTPs bearing oligopyridine ligands attached via short and rigid acetylene tether, while classical triphosphorylation of modified nucleosides was used for construction of dNTPs bearing oligopyridine ligands attached via long and flexible octadiyne linker. Sonogashira cross-coupling reaction was also used for preparation of both types of oligopyridine-modified nucleosides (with acetylene or octadiyne linker), used as model compounds. Oligopyridine-modified dNTPs were tested as substrates for several thermostable DNA polymerases and were successfully incorporated into DNA by primer extension experiments and then tested for post-synthetic complexation. DNA bearing 2,2':6',2"-terpyridine forms stable complexes with Fe^{2+} ions which were detected by polyacrylamide gel electrophoresis and by UV/Vis spectroscopy. While DNA bearing 2,2':6',2"-terpyridine attached via rigid acetylene tether can form only the inter-strand complexes, DNA bearing 2,2':6',2"-terpyridine attached via flexible octadiyne linker can form inter-strand as well intra-strand complexes. Formation of intra-strand complex was clearly showed by faster mobility on gel in comparison to twice as large inter-strand complex formed from DNA bearing 2,2':6',2"-terpyridine attached via acetylene tether. Formation of intra-strand DNA complex was also confirmed by MALDI, CD spectroscopy and modeling.