Automated Solid-Phase Synthesis of Site-Specifically Labeled Ruthenium−Oligonucleotides

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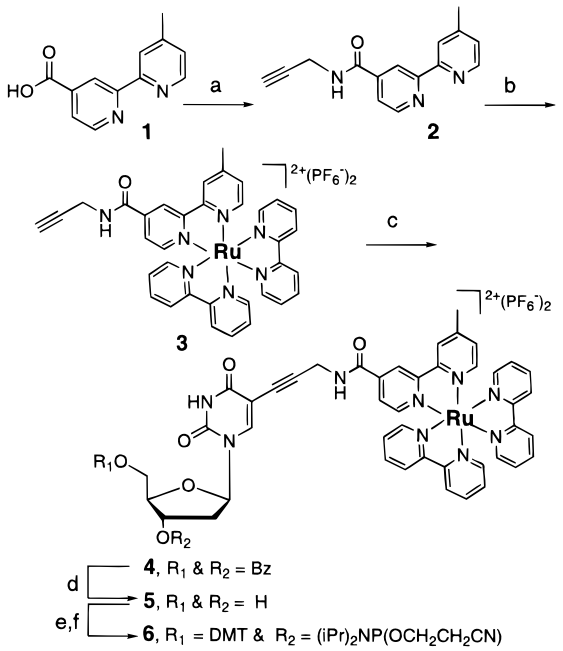
Modifying a nucleoside, nucleotide, or oligonucleotide with a specific chemical functionality, such as a transition or lanthanide metal complex, is of widespread interest for analytical applications (sequencing, hybridization assays), therapeutic uses (anticancer, antiviral pharmaceuticals), and mechanistic studies (electron transfer, structure−function).[1−5] Synthetic strategies toward these supramolecular bioassemblies focus primarily on postmodification of the synthesized nucleic acid single strand or complementary duplex. A number of researchers[6−10] use this strategy to link a metal complex, usually as the activated succinimidyl ester, to the terminus of the nucleic acid single strand containing an alkylamine. Alternatively, an amino- or diimine-modified (e.g., phenanthroline) oligonucleotide is reacted with a metal to form the desired complex.[11−15] A strategy that offers clear synthetic advantages over post-modification is to use DNA solid-phase synthetic methodologies for the site-specific labeling of an oligonucleotide with a transition metal complex. A number of groups, including ours, are currently exploring this approach.[16−22] Herein, we report the first solid-phase synthesis of a ruthenium-labeled oligonucleotide using a novel ruthenium−nucleoside phosphoramidite and a fully automated protocol.

A number of requirements must be met to ensure successful incorporation of a functional photoactive transition metal complex (e.g., $Ru(bpy)_3^{2+}$) in an oligonucleotide. The metallonucleoside phosphoramidite approach to the synthesis of labeled oligonucleotides requires (1) straightforward reactions and purification steps to the precursor metal complex and metallonucleoside phosphoramidite, (2) sufficient solubility of the metallonucleoside phosphoramidite in acetonitrile for solid-phase reactions, (3) high stability of the metal complex during oligonucleotide synthesis and deprotection reactions to prevent undesired side reactions and decomposition, and, finally, (4) efficient coupling of the metallonucleoside phosphoramidite using standard automated DNA synthesis.[23,24] Tris-diimine metal complexes are of interest since they possess a number of favorable photochemical properties for study including high stability, inertness to ligand exchange reactions, tunable electronic structures, long lifetimes in fluid solution ($\tau \approx 1\ \mu s$), and high quantum yields.[25,26]

With these issues in mind, we synthesized the ruthenium-nucleoside phosphoramidite, **6**, as shown in Scheme 1. Specifically, $Ru(bpy)_3^{2+}$ was selected as the target molecule rather than a phenanthroline analogue since bipyridine complexes, in general,

Scheme 1



Reagents: (a) propargyl amine·HCl, DCC, HOBt, DIPEA, DMF, 76% yield; (b) $Ru(bpy)_2Cl_2$, 70% aq. C_2H_5OH, 82% yield; (c) $Pd(PPh_3)_4$, CuI, TEA, 2'-deoxy-3',5'-dibenzoyloxy-5-iodouridine, DMF, 79% yield; (d) NH_3/CH_3OH, 90% yield; (e) DMT-Cl, C_5H_5N, 81%; and (f) $(iPr)_2NP(Cl)OCH_2CH_2CN$, DIPEA, CH_3CN, 90% yield.

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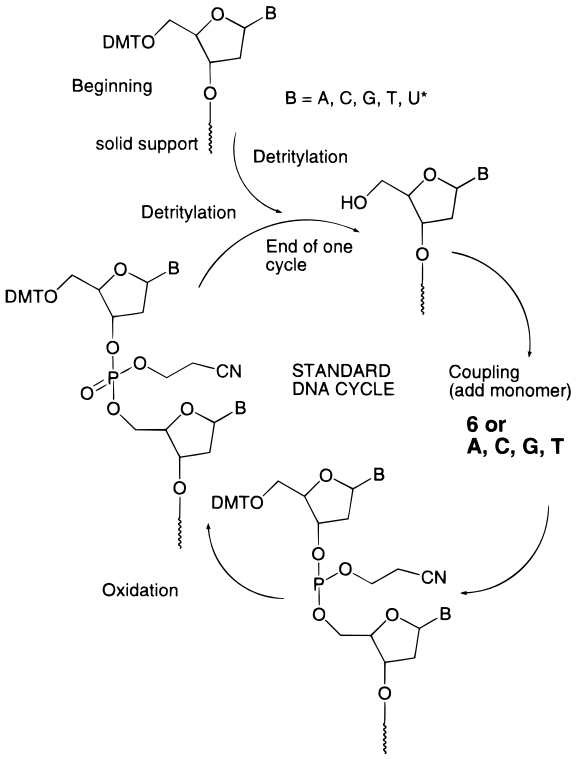
Scheme 2



7 5'-TCA ACA G**U***T TGT AGC A-3'
8 5'-**U***CA ACA GTT TGT AGC A-3'
9 5'-TCA ACA GTT TGU* AGC A-3'
10 5'-TCA ACA GTT TGT AGC A-3'
11 5'-TGC TAC AAA CTG TTG A-3'
12 5-TGC TAC AAA CTG TTG A-NH_2-3'
U* = ruthenium-modified uridine

are stronger oxidants in their excited state. The presence of an amide bond formed between the 4′-carboxylic acid of bipyridine and propargylamine should favor localization of the excited-state electron on that particular bipyridine. This functionality has been previously shown to enhance the electronic coupling between adjacent $M(bpy)_3^{2+}$ (where M = Ru or Os) in a covalently cross-linked metallobipyridine polymeric system.[27] An alkynyl group was introduced on the bipyridine to efficiently cross-couple the metal complex to a halonucleoside using well-precedented Pd(0) chemistry.[28,29] Finally, to ensure sufficient solubility in organic solvents and to aid in purification and recrystallization steps, a large counterion such as PF_6^- was selected.[30]

The site-specific incorporation of the ruthenium-nucleoside in a 16-mer oligonucleotide was accomplished using an automated ABI 392 DNA/RNA synthesizer (Scheme 2).[23,24] Using the standard coupling protocol, the metallonucleoside phosphoramidite was incorporated at different positions within an oligonucleotide.[31] After synthesis, the ruthenium-modified oligonucleotide was cleaved from the column without detritylation of the 5′-terminus. The nitrogenous bases and phosphate groups were subsequently deprotected in 30% ammonium hydroxide at 55 °C for 12 h. Finally, the ruthenium-labeled oligonucleotide was purified and the 5′-DMT grouped removed using a standard Poly-Pak cartridge followed by reverse-phase HPLC (C18 column; 0.1 M TEAA/CH_3CN; 10−50% gradient over 50 min; monitoring at 254 and/or 450 nm).[32]

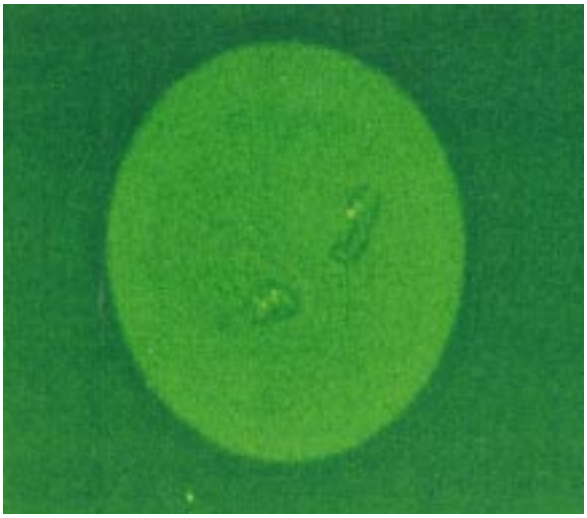


Figure 1. Confocal micrograph of a ruthenium modified duplex (**7·12**) anchored to a glass slide.

Thermal denaturation experiments on the unmodified and modified oligonucleotide duplexes show a moderate decrease in thermal stability when the ruthenium is introduced into the middle of the oligonucleotide sequence compared to an unmodified duplex (150 mM sodium phosphate; pH = 7; monitoring at 260 nm). The melting temperature (T_m) of the unmodified duplex, **10·11**, is 60 °C compared to 51 °C for the ruthenium-labeled duplex, **7·11**. The T_m for **7·11** is slightly greater than observed for a duplex with a single mismatch. When the metal complex is attached to the terminal nucleotide of the oligonucleotide sequence the Tm is unchanged (**8·11**, T_m = 60 °C).

The absorption spectrum of the ruthenium-labeled oligonucleotide single strand, **7**, exhibits the characteristic metal-to-ligand charge-transfer band (^{1}MLCT-$^1A_1$), centered at 450 nm, analogous to $Ru(bpy)_3^{2+}$. Excitation of the MLCT band of **7** produces an emission centered at 660 nm corresponding to the ^{3}MLCT excited state. This emission is essentially unchanged with the duplex, **7·11**. As shown in Figure 1, this emissive metallo-oligonucleotide can be attached to a surface and visualized with a confocal microscope (excitation at 488 nm, emission monitored at 600 nm; see Supporting Information for experimental details).

In summary, a facile, solid-phase, and fully automated method for the construction of site-specifically metallolabeled oligonucleotides is reported using a novel metallonucleoside phosphoramidite. Importantly, these results demonstrate that ruthenium-modified oligonucleotides form stable luminescent duplexes at room temperature which are amenable to further photophysical studies.

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Supporting Information Available: Detailed synthesis and characterization data for the ruthenium modified oligonucleotides are available (5 pages). Ordering information is given on any current masthead page.

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(30) An HPLC trace of the ruthenium-modified 2′-deoxyuridine, **5**, shows one peak, and its elution time is greater compared to 2′-deoxyuridine. We observe the $MH^+ - PF_6^-$ (1036) and $-2PF_6^-$ (891) peaks in the FAB-MS, confirming formation of the product.
(31) Please see the Supporting Information for details about the DNA synthesis.
(32) The ruthenium-modified oligonucleotides exhibit one peak in an HPLC trace, with retention times greater than the corresponding unmodified oligonucleotide. MALDI mass spectrometry of the metallo-oligonucleotide also confirms formation [e.g., ruthenium-modified oligonucleotide **8**, MALDI (5058.25; 5055.6; calculated: found ±3)].