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Synthesis of Oligonucleotides Containing N^2-[2-(Imidazol-4-ylacetamido)ethyl]-2'-deoxyguanosine

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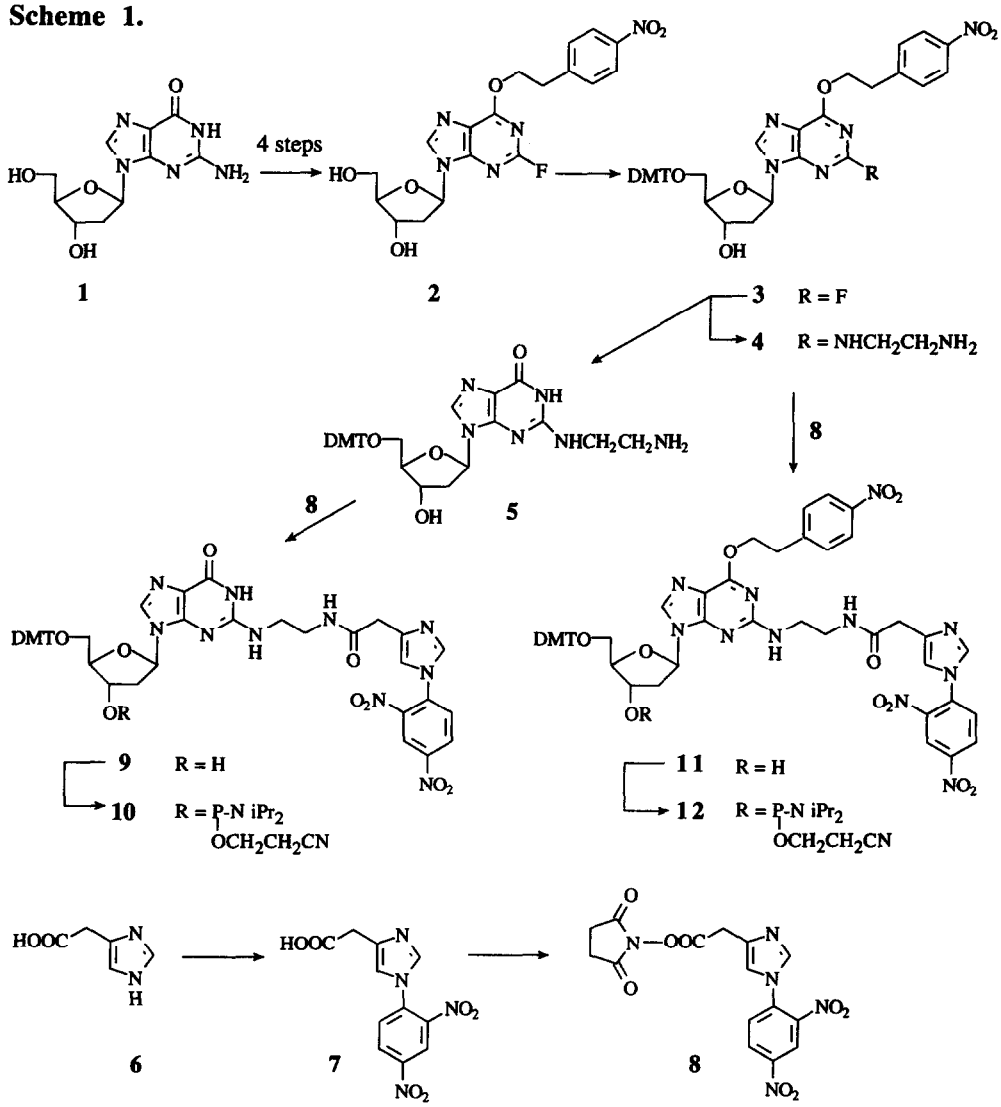
Abstract: Synthesis of 2'-deoxyguanosine tethered through N-2 to an imidazole is described. The modified 2'-deoxyguanosine was converted to two different phosphoramidites, one with and the other without a 6-*O*-protecting group. The phosphoramidites were incorporated into oligonucleotide alone or together with a 2'-deoxyuridine tethered to a bipyridine. Protection and deprotection of the imidazole are also briefly described.

Oligonucleotides tethered to ligands which facilitate nucleic acid cleavage have received considerable attention because of their potential as reagents for the sequence–specific cleavage of nucleic acids.[1-4] Oligonucleotide–imidazole conjugates are attractive candidates because imidazole is known to promote the catalytic hydrolysis of RNA. To accelerate the rate of cleavage to a useful level, it appears necessary to activate the targeted phosphodiester bond.[5] This could conceivably be accomplished by positioning a second ligand, such as bipyridine, to mediate metal complex formation with the phosphodiester anion. As part of our research project directed towards oligonucleotide–based synthetic ribonuclease, we have designed oligonucleotides that contain both of these modifications spaced to interact with the same phosphodiester bond in a target RNA. To test the concept, separate tandem sequences, containing the imidazole conjugate in one segment and the bipyridine conjugate in the other segment, as well as single continuous sequences containing both the imidazole and bipyridine conjugates were synthesized. We report here the synthesis of phosphoramidites of imidazole-conjugated 2'-deoxyguanosine and its incorporation into oligonucleotides alone or together with bipyridine-conjugated 2'-deoxyuridine.

Two different modifications of an imidazole-conjugated 2'-deoxyguanosine were synthesized and transformed into phosphoramidites (Scheme 1). The starting material, **2**, was obtained from 2'-deoxyguanosine by a four-step synthesis according to a known procedure.[6] Nucleoside **2** was treated with 4,4'-dimethoxytrityl chloride to give the 5'-protected derivative **3** in 87% yield. Introduction of a DMT protecting group at an early stage greatly facilitated the subsequent reactions and work up. The coupling reaction of 1,2-diaminoethane with either **2** or with unprotected 2-fluoro-2'-deoxyguanosine gave very polar, insoluble intermediates that were difficult to purify. On the other hand treatment of **3** with 1,2-diaminoethane in ethanol at room temperature for 3 h afforded **4** as the free amine in 91% yield. Compound **4** was further heated with 1,2-diaminoethane at 65 °C for 24 h, and the resulting crude product was purified on silica gel eluting with 10% conc. ammonia in methanol to give **5** in 91% yield. Coupling of **5** with **8** at room temperature gave imidazole–conjugated nucleoside **9** in 69% yield. Compound **8** was prepared in two steps from imidazole-4-acetic acid(**6**). Reaction of **6** with 2,4-dinitrofluorobenzene in the presence of sodium bicarbonate in DMF gave crude **7**, which was purified by chromatography on silica gel eluting with 2% AcOH and 10% MeOH in CH_2H_2 to give a single pure

Scheme 1.

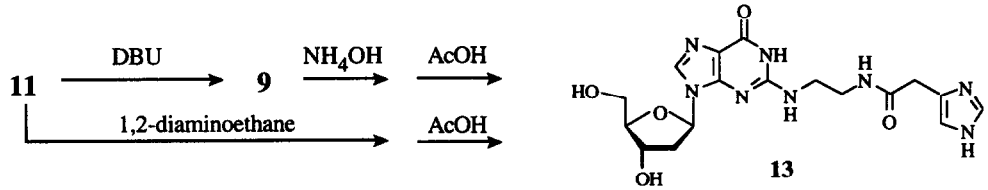


isomer, **7** in 72% yield. Compound **7** was converted to **8** by treatment with *N*-hydroxysuccinimide and DCC. Although **9** could be transformed to phosphoramidite **10** by reaction with 2-cyano-*N*,*N*-diisopropylchlorophosphoramidite, a significant amount of **10** was lost during purification, presumably because of the necessity of using a polar eluent (Et_3N and 5-10% MeOH in CH_2Cl_2) for silica gel chromatography. The isolated yield of purified phosphoramidite **10** was 30–40%. To facilitate purification, a less polar phosphoramidite, **12**, was constructed. Reaction of **4** with **8** gave the less polar deoxyguanosine–imidazole conjugate **11** in 94% yield, which was converted to phosphoramidite **12** in good yield.

The choice of a protecting group for the imidazole–conjugated nucleotide required careful consideration. The 2,4-dinitrophenyl group which has been used as a protecting group for histidine in peptide chemistry can be

deprotected by treatment with mercaptoethanol.[7] We found that this protecting group could be readily removed from imidazole by the standard ammonia treatment used to deprotect synthetic oligonucleotides, and that it was also stable enough to survive the reagents used for DNA synthesis. In a model experiment, treatment of **9** with 30% NH_4OH at room temperature, followed by treatment with acetic acid, gave the desired imidazole-conjugated nucleoside, **13**. Nucleoside **13** was designed to position the imidazole moiety adjacent to a 2'-OH group on a complementary RNA sequence when incorporated into an oligodeoxyribonucleotide. Removal of the *p*-nitrophenylethyl group could be accomplished by treatment with 0.5 M DBU in pyridine at room temperature overnight, or alternatively, by treatment with 1,2-diaminoethane in ethanol at 65 °C for 24 h. Thus, **11** was readily converted to **13** as outlined in Scheme 2.

Scheme 2.



From **10**, **12**, and the phosphoramidite of 5-[2-(4'-methyl-2,-2'-dipyrid-4-ylcarboxamido)ethylthio]-2'-deoxyuridine,[8] eight modified oligonucleotides (Table 1) were synthesized on CPG supports on a MilliGen/Biosearch 8750 Synthesizer by standard phosphoramidite chemistry. The solid supports containing **oligos 1–3** were treated following the normal procedure (conc. NH_4OH, 55 °C), while the solid supports

Table 1. Oligodeoxynucleotides containing imidazole and bipyridine ligands

oligo 1 5'-GT G*GTATTTGTG-3'	**oligo 5** 5'-GTG*GTATTTGTG-3'
oligo 2 5'-AGATCT*CAGT G*GTAT-3'	**oligo 6** 5'-GTGG*TATTTGTG-3'
oligo 3 5'-AGATCT*CAGTGG*TAT-3'	**oligo 7** 5'-AGATCT*CAGTG*GTAT-3'
oligo 4 5'-GTG*GTAT-3'	**oligo 8** 5'-AGATCT*CAGTGG*TAT-3'

G* = **13**; T* = bipyridine modified 2'-deoxyuridine; phosphoramidite **10** was used for the synthesis of **oligos 1–3**; phosphoramidite **12** was used for the synthesis **oligos 4–8**.

bearing **oligos 4–8** were thoroughly dried, treated with 0.5 M DBU in pyridine overnight, washed with acetonitrile, and then treated according to the usual deprotection procedure. The modified oligonucleotides were purified by C_{18} reverse–phase HPLC with 0.1 M triethylammonium acetate buffer (containing 5% acetonitrile, pH 7.0) and acetonitrile as eluents. **Oligos 2–3** and **oligos 7–8** appeared to have secondary structure as implicated by HPLC.[9] Incorporation of the modified nucleosides was confirmed by analysis of the enzyme digestion products of **oligos 1–8**.[10] A mixture of 0.5-0.7 A_{260} units of oligonucleotide, snake venom phosphodiesterase (Boehringer–Mannheim) and bacterial alkaline phosphatase (Pharmacia or Sigma) was incubated at 37 °C for 20 h, lyophilized, and subjected to HPLC analysis on a C_{18} reverse–phase column using the same buffer as described above. Figure 1 shows two representative chromatographs of enzyme digestion products. The peak at 9.5 min. or 10.6 min. (the different retention times can be ascribed to the differences in polarities of the injection volumes due to the different buffer compositions used by the two different brands of alkaline phosphatase) corresponds to that of an authentic sample of **13**. The peak at 17.4 min. corresponds to

that of the bipyridine-conjugated 2'-deoxyuridine, which was severely broadened when it was injected in very small amounts. The peaks at 16.2 and 17.7 min are impurities in the mobile phase. Integration of the peak areas yielded the correct proportion of modified and unmodified nucleosides.

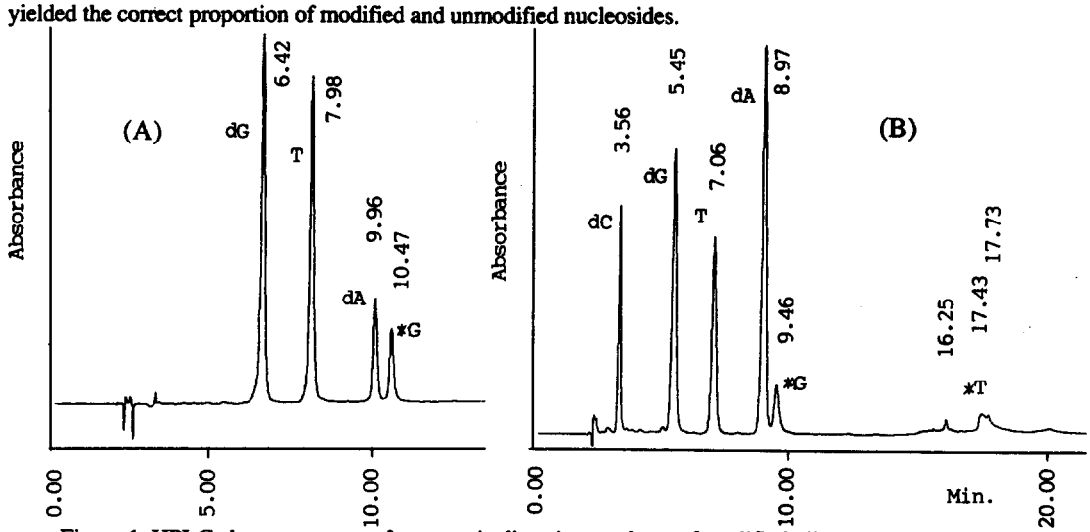


Figure 1. HPLC chromatograms of enzymatic digestion products of modified oligonucleotides (A) **oligo 1**. Gradient: 100-95% 0.1 TEAA buffer and 0-5% acetonitrile from 0-10 min.; and 95-50% 0.1 TEAA buffer and 5-50% acetonitrile from 10-30 min. (B) **oligo 2**. Gradient: 100-95% 0.1 TEAA buffer and 0-5% acetonitrile from 0-10 min.; and 95-70% 0.1 TEAA buffer and 5-30% acetonitrile from 10-12 min., then constant.

In summary, the synthesis of an imidazole–conjugated 2'-deoxyguanosine and its incorporation into oligonucleotides alone, or with bipyridine–conjugated 2'-deoxyuridine are reported. Further characterization of the synthesized oligonucleotides and investigation of their RNA cleaving properties are in progress.

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9. C_{18} reverse–phase HPLC using 0.1M TEAA buffer and acetonitrile as eluents showed different shapes in chromatograms of **oligos 5-6** and **oligos 7-8** before and after their aqueous solutions were frozen overnight. We suspect that this was due to secondary structures of the modified oligonucleotides as described by Gait in *Oligonucleotides and Analogues*; Eckstein, F., ed., IRL Press, **1991**; p. 42.
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