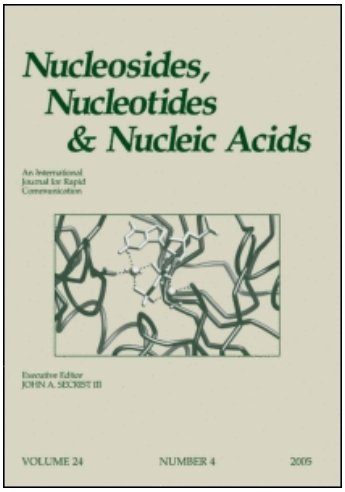

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Synthesis and Biophysical Characterization of G-Rich Oligonucleotides Conjugated with Sugar-Phosphate Tails

J. D'Onofrio[a]; E. Erra[b]; G. Di Fabio[a]; A. Iadonisi[a]; L. Petraccone[b]; L. De Napoli[a]; G. Barone[b]; J. Balzarini[c]; C. Giancola[b]; D. Montesarchio[a]
[a] Dipartimento di Chimica Organica e Biochimica, Università degli Studi di Napoli "Federico II,", Napoli, Italy [b] Dipartimento di Chimica, Universitá degli Studi di Napoli "Federico II,", Napoli, Italy [c] Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium

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SYNTHESIS AND BIOPHYSICAL CHARACTERIZATION OF G-RICH OLIGONUCLEOTIDES CONJUGATED WITH SUGAR-PHOSPHATE TAILS

J. D'Onofrio □ *Dipartimento di Chimica Organica e Biochimica, Università degli Studi di Napoli "Federico II," Napoli, Italy*

E. Erra □ *Dipartimento di Chimica, Università degli Studi di Napoli "Federico II," Napoli, Italy*

G. Di Fabio and A. Iadonisi □ *Dipartimento di Chimica Organica e Biochimica, Università degli Studi di Napoli "Federico II," Napoli, Italy*

L. Petraccone □ *Dipartimento di Chimica, Università degli Studi di Napoli "Federico II," Napoli, Italy*

L. De Napoli □ *Dipartimento di Chimica Organica e Biochimica, Università degli Studi di Napoli "Federico II," Napoli, Italy*

G. Barone □ *Dipartimento di Chimica, Università degli Studi di Napoli "Federico II," Napoli, Italy*

J. Balzarini □ *Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium*

C. Giancola □ *Dipartimento di Chimica, Università degli Studi di Napoli "Federico II," Napoli, Italy*

D. Montesarchio □ *Dipartimento di Chimica Organica e Biochimica, Università degli Studi di Napoli "Federico II," Napoli, Italy*

□ *A number of 5′-and 3′-glycoconjugates of the oligonucleotide $^{5'}$d(TGGGAG)$^{3'}$ have been synthesized, exploiting fully automated, online phosphoramidite-based solid phase strategy, as potential anti-HIV-1 agents. The thermodynamic stability of the resulting quadruplexes has been investigated by thermal denaturation studies, via a detailed CD analysis.*

Keywords Conjugated oligonucleotides; G-quadruplex structures sugar-phosphate tails; anti-HIV-1 agents

Address correspondence to D. Montesarchio, Dipartimento di Chimica Organica e Biochimica, Università degli Studi di Napoli "Federico II," Via Cintia 4, I-80126 Napoli, Italy. E-mail: daniela.montesarchio@unina.it

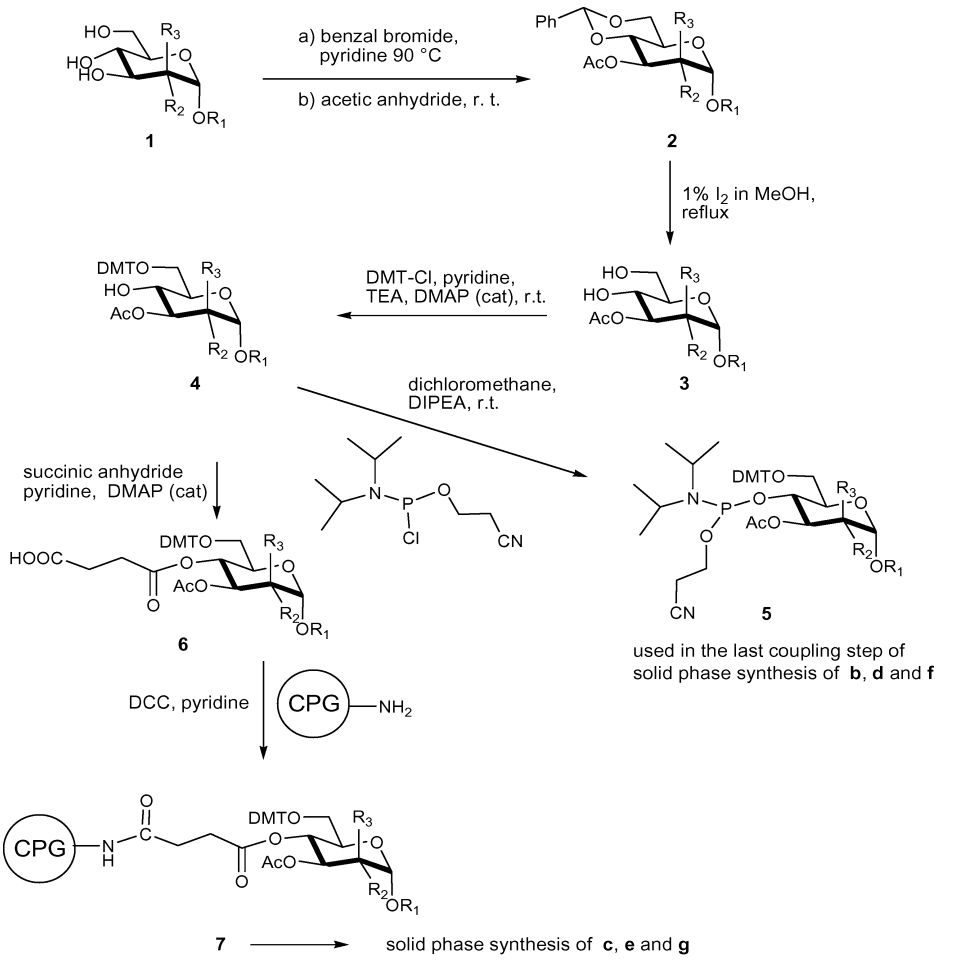
Recently, Hotoda and coworkers demonstrated that a number of G-rich oligonucleotides, decorated with large aromatic groups at their 5′-end, are potent anti-HIV active compounds.[1] Interestingly, the lead sequence $^{5'}$d(TGGGAG)$^{3'}$ was found to be inactive against HIV-1 without end modifications, and active at μM concentrations when 5′-conjugated with aromatic groups.[2,3] This antiviral activity was correlated with the ability of G-rich sequences to form stable quadruplex structures,[4,5] postulated to be the species directly interacting with the in vivo targets.

Among the various modifications proposed to improve the pharmacological profile of oligonucleotides, end-modification with carbohydrates may generate useful hybrids for targeting cells with DNA fragments. Glyco-conjugation increases nucleases resistance and cell uptake and can also reduce unwanted aggregation. Extensive research has been carried out to conjugate peptides to carbohydrate-based ligands; on the contrary, only a few examples of DNA glyco-conjugates are reported in the literature. In this frame, we[6] recently described a straightforward protocol based on the usage of appropriate glucose and sucrose phosphoramidite derivatives, as model compounds for mono- and disaccharide building blocks, which can be easily incorporated in the solid phase in a standard automated oligonucleotide synthesis to generate stable phosphodiester linkages connecting the saccharide residues to one or both OH termini of the oligonucleotide chain.

Here we present the design and synthesis of a set of model glyco-conjugated oligonucleotides carrying Hotoda's sequence d($^{5'}$TGGGAG$^{3'}$), together with preliminary results on their biophysical characterization. We have synthesized 5′- and 3′-conjugated 6-mers **b-g** (Scheme 1 and Table 1) and compared their quadruplex formation abilities with unmodified 6-mer **a**. Glucose and sucrose-containing oligomers **b-e** were prepared essentially following previously reported procedures.[6] This synthetic strategy has been here extended to mannose-linked oligomers **f** and **g**, as examples of glyco-conjugation which can find direct application for

TABLE 1 Synthesized oligonucleotides and Tm values of the resulting quadruplexes, obtained by CD-monitored (at 264 nm) thermal denaturation curves

Sequence (5′-3′)	T_m (°C)
$^{5'}$TGGGAG$^{3'}$ (**a**)	41 ± 1
glucose-$^{5'}$TGGGAG$^{3'}$ (**b**)	26 ± 1
$^{5'}$TGGGAG$^{3'}$-glucose (**c**)	44 ± 1
sucrose-$^{5'}$TGGGAG$^{3'}$ (**d**)	35 ± 1
$^{5'}$TGGGAG$^{3'}$-sucrose (**e**)	36 ± 1
mannose-$^{5'}$TGGGAG$^{3'}$ (**f**)	31 ± 1
$^{5'}$TGGGAG$^{3'}$-mannose (**g**)	45 ± 1



SCHEME 1

lectine recognition. In all cases, starting from 100 mg of commercially available guanosine-functionalized CPG support (0.1 meq/g, used in connection with phosphoramidite **5** for the synthesis of 5′-conjugated oligomers) or with the first sugar residue (**7**, 0.06-0.08 meq/g, used for the 3′-conjugation[7]), on average 80-110 OD units of pure **a-g** could be isolated, after HPLC purification, then characterized by MALDI-TOF MS.

CD studies for all the investigated oligomers showed spectra with a typical negative band at *ca.* 240 nm and a large positive band at 264 nm, diagnostic of tetramolecular, parallel G-quadruplex structures. To determine the thermodynamic stability of the resulting quadruplexes, thermal

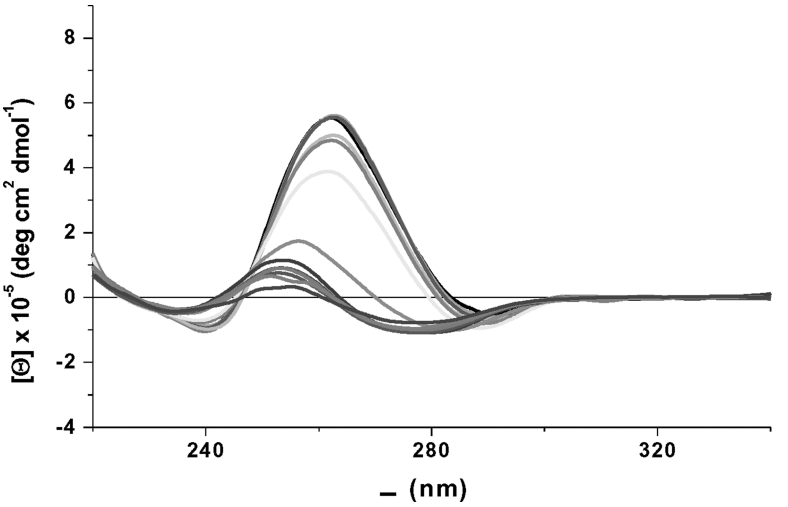


FIGURE 1 Superimposed CD spectra of $^{5'}$TGGGAG$^{3'}$ (**a**) taken in the temperature range 10–75°C.

denaturation studies were carried out *via* detailed CD analyses. Data concerning the unmodified sequence are here reported in Figures 1 and 2. The measured Tm data for all the studied quadruplexes, listed in Table 1, show that the saccharide-phosphate tails in G-rich oligomers do influence the stability of the resulting complexes. In the case of the disaccharide moiety, a destabilization occurred both in the 5′- and 3′-conjugation (**d** and **e**, respectively). The insertion of a monosaccharide-phosphate residue at the 5′ end

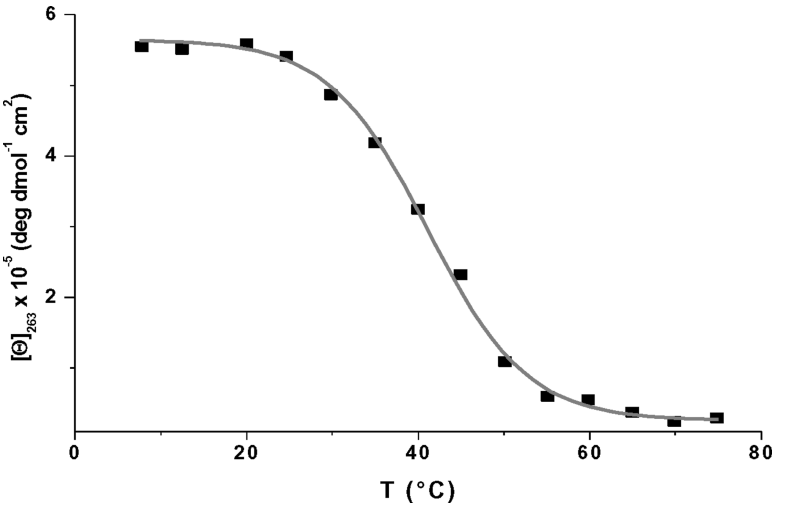


FIGURE 2 Plot of the molar ellipticities of $^{5'}$TGGGAG$^{3'}$ (**a**) at 264 nm as a function of the temperature.

(**b** and **f**) was detrimental to quadruplex formation, while a $\Delta Tm = +3–4°C$ was obtained for the 3′-conjugation (**c** and **g**).

The complete definition of the thermodynamic parameters for the quadruplex complexes, as well as studies on the in vitro antiviral activity of the novel glyco-conjugated oligonucleotides, are currently underway and will be presented in due course. These data can be useful to develop novel non-antisense anti-HIV active oligonucleotide-based lead compounds.

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7. Oligonucleotides **c**, **e**, and **g** could have alternatively synthesized using commercially available universal solid supports and 2′-deoxyribonucleoside 5′-phosphoramidites, in connection with sugar phosphoramidite building block **5**. This strategy was here not adopted on the basis of the overall higher costs and lower expected yields associated with the usage of nucleoside 5′-phosphoramidites.