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Apolar carbohydrates as DNA capping agents†‡

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Mono- and disaccharides have been shown to stack on top of DNA duplexes stabilizing sequences with terminal C–G base pairs. Here we present an apolar version of glucose and cellobiose as new capping agents that stack on DNA increasing considerably its stability with respect to their natural polyhydroxylated mono- and disaccharide DNA conjugates.

Non-covalent forces direct molecular interactions between biomolecules and their combination and interplay in biology rules life. DNA being the central molecule of life also gives the chance to study molecular interactions in aqueous media. Aromatic π–π stacking interactions have extensively been studied using DNA as a model. Both natural[1] and non-natural[2–4] aromatic bases attached to the 3′-end or 5′-end of double stranded DNA have shown enhanced stabilization of DNA duplexes, acting as capping agents. These molecular "caps" are usually planar aromatic rings of different size and shape that take advantage of π–π stacking interactions.[5–8] The only non-planar compounds described to stack on DNA are steroids such as cholic acid which showed a high increase in DNA stability *via* CH–π interactions.[9] Recently, binaphthyl and phenylcyclohexyl nucleosides[10,11] with nonplanar aromatic bases have been included inside DNA but no data as capping entities were reported.

Our group has studied carbohydrate–aromatic stacking interactions using carbohydrate oligonucleotide conjugates (COCs) with dangling-ends as a model. First, we evaluated monosaccharide–phenyl interactions as a double dangling motif at the edge of a duplex of DNA.[12] We found that stabilization varies from −0.15 to −0.40 kcal mol^{-1} and depends on the number of hydroxy groups and stereochemistry. Recently, we have shown that highly polar carbohydrates can act as DNA capping molecules. Sugar stacking is observed for mono- and disaccharides on top of C–G or T–A base pairs as the edge of the DNA duplex.[13] Nevertheless, stabilization of the DNA double helix is only observed with C–G or G–C terminal base pairs.

Herein, we report the synthesis of oligonucleotides with permethylated mono- and disaccharides covalently linked to their 5′-end. These apolar carbohydrates act as new capping molecules capable of stacking on double-stranded DNA (Fig. 1). Permethylated glucose and cellobiose were found to stabilize DNA duplexes much more than natural glucose and cellobiose.

Synthesis of the permethylated carbohydrate oligonucleotide conjugates started with the preparation of the corresponding permethylated glucose and cellobiose phosphoramidite derivatives (**5** and **10**, respectively) (Scheme 1). Glycosylation of the *O*-benzyl protected ethylene glycol spacer followed by deprotection of the acetyl groups yielded intermediate **2**. Methylation under standard conditions produced compound **3** in good overall yield (70%, 3 steps). Further hydrogenation and standard phosphoramidite preparation proceeded uneventfully to yield permethylated glucose phosphoramidite **5** (76%, 2 steps). A similar synthetic strategy was followed to prepare permethylated cellobiose phosphoramidite **10** (48% yield, 5 steps).

Preparation of the apolar saccharide oligonucleotide conjugates was carried out by standard solid phase oligonucleotide synthesis using compounds **5** or **10** at the last coupling step. Both apolar carbohydrates were attached to self-complementary sequences CGCGCG, GGCGCC, AGCGCT and TGCGCA. Solutions of the COCs were subjected to UV melting analysis and thermodynamic parameters were calculated (Table 1).

Conjugates containing permethylated glucose and cellobiose on sequences terminated on a C–G base pair (conjugates **15** and **19**) increased considerably their melting points (7.8 °C and 8.3 °C, respectively) over those of the natural control sequence **11**.

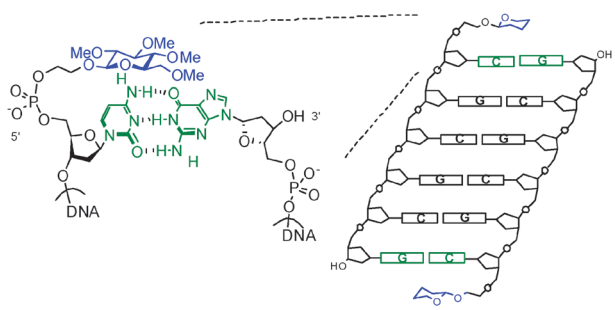

Fig. 1 Schematic drawing of COCs with dangling-ends and details of one of them (permethylated glucose stacking on top of a C–G base pair).

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† Dedicated to Professor Soledad Penadés on her 70th birthday.
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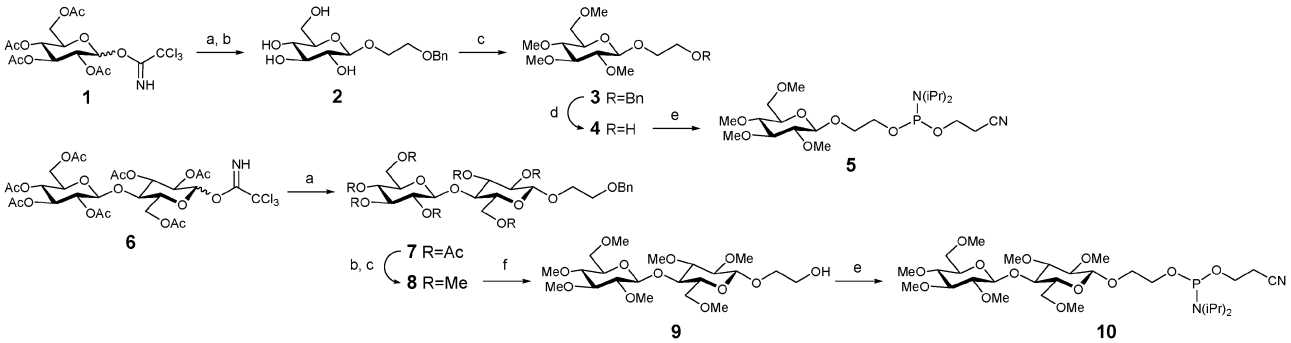

Scheme 1 Synthesis of permethylated glucose and cellobiose phosphoramidites **5** and **10**. Reaction conditions: (a) $BnOCH_2CH_2OH$, $BF_3{\cdot}OEt_2$, CH_2Cl_2; (b) Na_2CO_3, MeOH; (c) MeI, NaH, DMF; (d) H_2, $Pd(OH)_2$, THF–MeOH; (e) 2-cyanoethyl-*N*,*N*′-diisopropylamino-chlorophosphoramidite, DIEA, CH_2Cl_2; (f) H_2, $Pd(OH)_2$, AcOEt–MeOH.

Table 1 Thermodynamic parameters for COCs

X-DNA sequence[a,b,c,d]	T_m[e]/°C	$-\Delta H°$	$-\Delta S°$	$-\Delta G°_{37}$	$\Delta\Delta G°_{37}$
X = none[f]					
CGCGCG **11**	40.9	46.5	123	8.2	—
AGCGCT **12**	33.5	40.3	107	7.1	—
X = glucose–C2[f]					
CGCGCG **13**	44.0	52.1	140	8.7	−0.5
AGCGCT **14**	33.6	37.3	98	7.0	0.1
X = glc(Me)–C2					
CGCGCG **15**	48.7	55.0	147	9.4	−1.2
AGCGCT **16**	34.5	44.8	121	7.2	−0.2
X = cellobiose–C2[f]					
CGCGCG **17**	45.9	49.2	130	8.9	−0.7
AGCGCT **18**	34.4	39.1	103	7.1	0.0
X = cellob(Me)–C2					
CGCGCG **19**	49.2	55.0	146	9.7	−1.5
AGCGCT **20**	37.7	43.9	117	7.7	−0.6

[a] –C2– states for $–CH_2–CH_2–OPO_2^-–$. [b] Buffer: 10 mM Na phosphate, 1 M NaCl, pH 7.0. [c] Estimated errors are: $T_m \pm 0.8$ °C and ±6% in $\Delta G°$. [d] Units for $\Delta H°$ and $\Delta G°$ are kcal mol^{-1} and for $\Delta S°$ are cal K^{-1} mol^{-1}. [e] Average value of three experiments measured at 5 μM conc. [f] From ref. 13.

When conjugates with apolar glucose **15** and apolar cellobiose **19** are compared with their corresponding natural hydroxylated versions glucose–DNA conjugate **13** and cellobiose–DNA conjugate **17**, T_m's are increased by 4.7 °C and 3.3 °C, respectively. A similar trend is observed when ΔG values are compared; conjugates **15** and **19** stabilize CGCGCG duplexes by −1.2 and −1.5 kcal mol^{-1}, respectively, with respect to unmodified CGCGCG. This stabilization is similar to that found for a benzene nucleoside in the same context.[2] As a result, the duplex stabilizations of conjugates with the apolar version of glucose **15** and cellobiose **19** are 2.4 and 2.1 times more stable, respectively, than their corresponding conjugates with natural glucose **13** and cellobiose **17**. The smaller increase in cellobiose may be due to the fact that the increased surface of the apolar version of cellobiose could be too large to fully stack on top of the C–G base pair. Similar results were found when the apolar sugars were attached to the GGCGCC sequence (see ESI‡, Table S3).

In the case of the AGCGCT sequence, both conjugates with permethylated glucose **16** and cellobiose **20** show an increase in T_m (1 °C and 4.2 °C, respectively) and in free energy (−0.1 and −0.6 kcal mol^{-1}, respectively) with respect to the natural sequence **12**. Once again, similar results were found when the apolar carbohydrates were attached to the TGCGCA sequence (see Table S3, ESI‡). This decrease of COC stabilization on sequences with A–T or T–A base pairs at the edge of the duplex with respect to the sequences with C–G or G–C base pairs was also observed for COCs with the natural mono- and disaccharides. This effect may be due to the larger entropy cost of reducing the fraying in the more flexible terminal A–T base pair that counteracts the stabilization obtained with the stacking of the apolar sugar.[13]

The structures of the conjugates containing the permethylated glucose unit **15** and **16** were studied by NMR spectroscopy. Proton assignment was carried out following standard procedures. The DNA duplex structures are barely distorted by the presence of the apolar sugars as can be inferred by comparison of the DNA chemical shifts of the conjugates and the control sequences (see ESI‡, Fig. S2). Chemical shift changes are mostly observed in the neighboring residues of the permethylated glucose (C1 in the CGCGCG sequence and A1 in the AGCGCT sequence), indicating that the carbohydrate is interacting mainly with the terminal residues. This capping interaction is also supported by a significant number of NOEs (see Fig. 2 and Table S2, in ESI‡). The number and intensities of these NOE contacts are comparable with those observed in the disaccharide conjugates studied in our previous work.[13] Strong and medium NOEs are observed between several protons of the terminal base-pairs with H3 and H5 of the apolar glucose unit, suggesting that the permethylated glucose interacts with the terminal base-pair of the duplex predominantly through its α face. In the case of conjugate **16** some low intensity NOEs are also observed with H4 proton. These NOEs may arise from spin-diffusion or from minor species with different carbohydrate conformations, and were not used in the structural calculations. Interestingly, many of the DNA-permethylated glucose NOEs involve exchangeable protons of the terminal base-pair. In both conjugates, these protons exhibit narrow signals, indicating that they are protected from water exchange. As in the case of the natural disaccharide–DNA conjugates studied previously, the capping carbohydrate reduces strongly the internal dynamics of the terminal base-pairs. This effect is especially pronounced in conjugate **16**, where the terminal base-pair is AT.

Restrained molecular dynamics calculations were carried out with the AMBER program. Resulting structures are shown in Fig. 3. In both conjugates **15** and **16**, the carbohydrate and the linker adopt a similar and well-defined structure. Permethylated

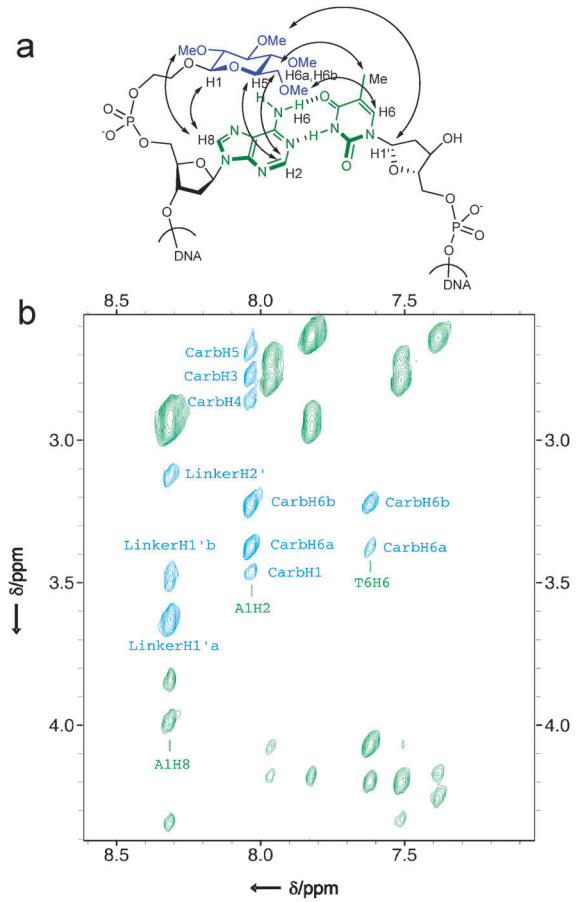

Fig. 2 (a) Schematic drawing of conjugate **16** with arrows indicating important observed NOEs; (b) selected region of NOESY spectra for conjugate **16** (carbohydrate–DNA contacts are shown in cyan).

glucoses stack on top of the terminal base-pair, with their α sides oriented towards the nucleobases. Carbohydrate conformation is the usual $^4C_1$ chair. Permethylation increases the carbohydrate size and allows for an enhanced stacking interaction in which a single monosaccharide covers most of the terminal base-pair surface (Fig. 3). Although the main features of both conjugates are quite similar, minor differences are observed (see Fig. 3, top). These differences are probably due to the different adjacent nucleobase, purine in the case of conjugate **15** and pyrimidine for **16**.

These results are noteworthy since hydrophobic mono- and disaccharides attached to DNA show a relevant increase in stabilization of DNA duplexes especially with terminal C–G or G–C base pairs. In this context, the stability of DNA with apolar sugars 5′-caps is approaching to that found with the traditional aromatic caps. Further improvement may be obtained modulating the hydrophobicity of the carbohydrate. NMR studies confirmed that permethylated sugars stack on top of duplex DNA similarly to other aromatic moieties. Finally, Our results have implications in molecular recognition and may be useful in drug design and in the assembly of supramolecular structures.

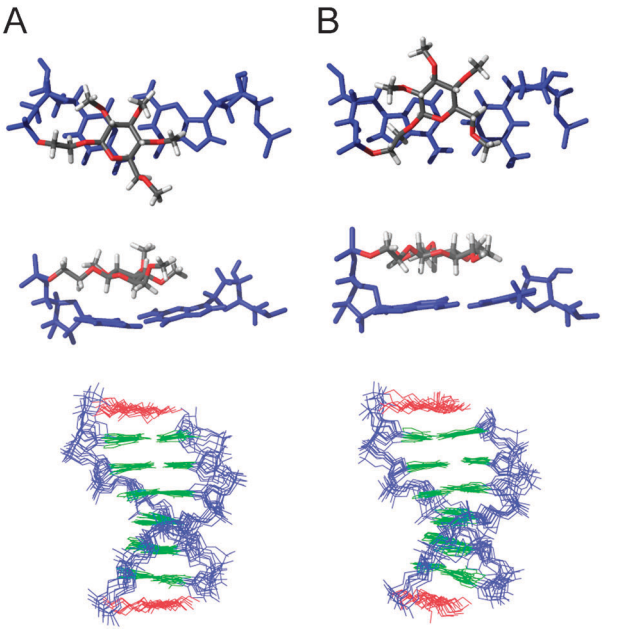

Fig. 3 Structures of conjugate **15** (A), and conjugate **16** (B). Top: details of the stacking. Bottom: superposition of ten calculated structures.

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