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Effects of Sugar Functional Groups, Hydrophobicity, and Fluorination on Carbohydrate–DNA Stacking Interactions in Water

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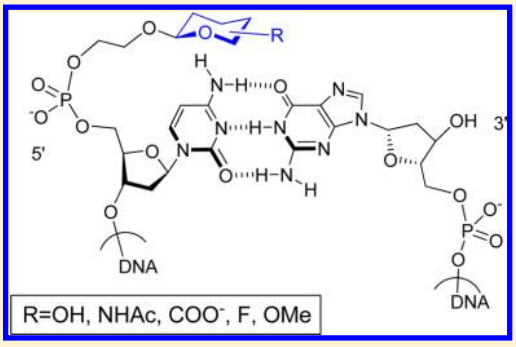
Supporting Information

ABSTRACT: Carbohydrate–aromatic interactions are highly relevant for many biological processes. Nevertheless, experimental data in aqueous solution relating structure and energetics for sugar–arene stacking interactions are very scarce. Here, we evaluate how structural variations in a monosaccharide including carboxyl, *N*-acetyl, fluorine, and methyl groups affect stacking interactions with aromatic DNA bases. We find small differences on stacking interaction among the natural carbohydrates examined. The presence of fluorine atoms within the pyranose ring slightly increases the interaction with the C–G DNA base pair. Carbohydrate hydrophobicity is the most determinant factor. However, gradual increase in hydrophobicity of the carbohydrate does not translate directly into a steady growth in stacking interaction. The energetics correlates better with the amount of apolar surface buried upon sugar stacking on top of the aromatic DNA base pair.



INTRODUCTION

Molecular interactions between biomolecules rule most of the biological processes inside the cell. A detailed understanding of these interactions provides information on these processes, and also allows their exploitation in a variety of applications such as in protein engineering or drug design. Stacking between aromatics is among the most studied interactions, whereas full comprehension of carbohydrate–aromatic stacking interactions is still to be reached.[1] This binding motif is commonly found in carbohydrate–protein interactions such as in lectins[2] and is also found in aminoglycoside-RNA recognition.[3]

Approaches to study carbohydrate–aromatic stacking interactions include molecular biology tools,[4] NMR spectroscopy,[5] IR spectroscopy,[6] computational methods,[7] and model systems based in supramolecular architectures[8] or in biomolecules.[9] These studies have revealed that the hydrophobic effect and CH–π interactions comprising dispersion and electrostatic forces play a critical role. A recent work by Chen et al.[10] has used an enhanced aromatic sequon (sequence of amino acids with an attached oligosaccharide) to study the relative contributions of each of these forces. The authors have found that the hydrophobic effect contributes significantly to carbohydrate–aromatic stacking and is supplemented by CH–π interactions with a dominating dispersive component.

A model based on carbohydrate–oligonucleotide conjugates (COCs) has been employed by our group to study carbohydrate–aromatic stacking interactions. We calculated monosaccharide–phenyl interactions using a double dangling motif at the 5′-end of a self-complementary CGCGCG sequence.[11] Energies of −0.15 to −0.4 kcal mol^{-1} were found depending on the stereochemistry and number of hydroxyl groups of the sugar. We have also observed mono- and disaccharide stacking on top of DNA base pairs in COCs where the sugar is directly linked to the 5′-end to DNA via an ethylene glycol spacer.[12] Extra stabilization of the DNA duplex due to sugar stacking was observed on sequences with terminal C–G and G–C base pairs (e.g., up to −0.25 kcal mol^{-1} for glucose/DNA unit and −0.45 kcal mol^{-1} for cellobiose/DNA unit). However, no stabilization was found on top of terminal A–T or T–A base pairs most probably due to the enhanced entropic cost of reducing the breathing of these more flexible terminal pairs.

In an effort to increase the energetics of carbohydrate–DNA stacking, we synthesized COCs containing hydrophobic versions of natural sugars (permethylated glucose or cellobiose)

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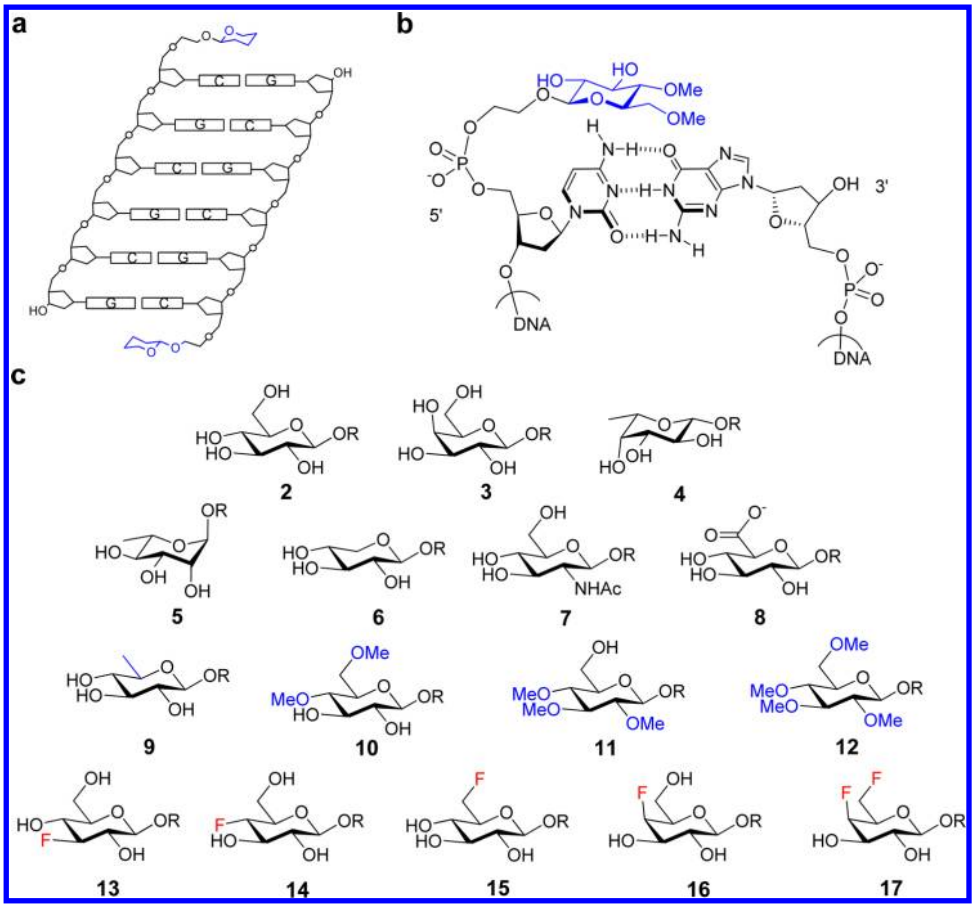

Figure 1. Description of the oligonucleotide conjugates under study. (a) Schematic drawing of the dangling-ended DNA designed to study carbohydrate−DNA interactions. (b) Enlarged view of the dangling-end area of a monosaccharide−oligonucleotide conjugate. (c) Carbohydrate−oligonucleotide conjugates included in the study. R = $-OCH_2CH_2-OPO_2^-$−CGCGCG.

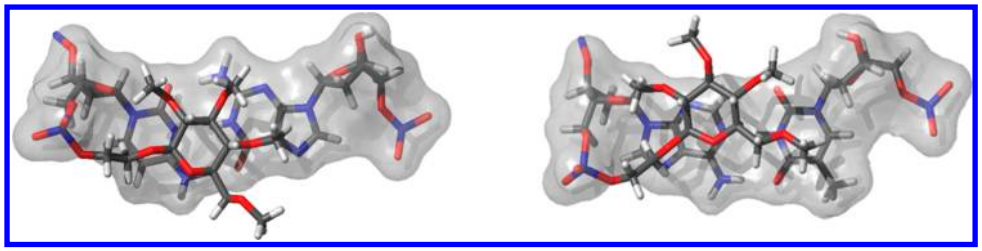

Figure 2. Top view of self-complementary DNA conjugates forming a double helix, permethylated glucose−$CH_2CH_2-OPO_2^-$−CGCGCG (left) and permethylated glucose−$CH_2CH_2-OPO_2^-$−AGCGCT (right). The apolar carbohydrate stacks on top of a C−G base pair (left) and the apolar carbohydrate stacks on top of an A−T base pair (right).

at the edge.[13] In this case, the stability of the DNA double helix increased significantly (e.g., up to −0.8 kcal mol^{-1} for glc(Me)/DNA unit and −0.9 kcal mol^{-1} for cellob(Me)/DNA unit). Additionally, these apolar carbohydrates were able also to stabilize dsDNA with A−T or T−A terminal base-pairs. We postulate that CH−π interactions together with the higher hydrophobicity imparted by the methyl groups are responsible for duplex stabilization.

In this work we decided to deepen our understanding carbohydrate−DNA stacking interactions by studying three different groups of carbohydrates in our dangling end COC model (Figure 1). First, we studied a group of COCs containing natural monosaccharides **5**−**8** (rhamnose, xylose, *N*-acetylglucosamine, and glucuronic acid) to explore the relevance of other functionalities on the pyranose. Second, the influence on carbohydrate−DNA stacking of increasing hydrophobicity in the pyranose unit was studied with a series of COCs with partially hydrophobic glucose units **9**−**12**. We have observed in the NMR structures of permethylated glucose−DNA conjugates (Figure 2) that one or two methyl groups apparently do not participate contacting the DNA base pair during stacking. The question arising was whether a gradual increase in hydrophobicity of the sugar will gradually increase stacking or whether the hydrophobic area contacting the aromatic DNA bases will be more determinant. Finally, we selected a group of COCs with fluorinated sugars **13**−**17** in order to study the influence of this electron-withdrawing group in the stacking interaction. At the same time, the reduced hydrophilicity upon OH → F change could also have a role in the carbohydrate−DNA interactions.

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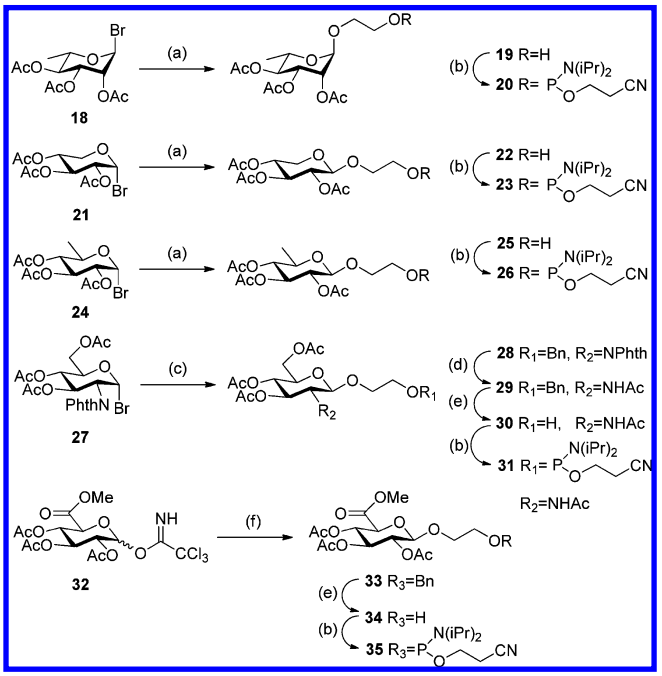
RESULTS AND DISCUSSION

Synthesis of Carbohydrate−Oligonucleotide Conjugates. The preparation of carbohydrate−oligonucleotide conjugates was carried out using standard solid phase automated oligonucleotide synthesis as reported previously.[11,12] Different monosaccharides were attached via an ethylene glycol spacer to the 5′-end of the self-complementary CGCGCG sequence using the corresponding protected carbohydrate phosphoramidite derivatives. Their synthesis was carried out by glycosylation of the spacer followed by standard phosphoramidite preparation (Scheme 1).

Scheme 1. Synthesis of Rhamnose, Xylose, 6-Deoxyglucose, *N*-Acetylglucosamine and Glucuronate Methyl Ester Phophoramidites 20, 23, 26, 31, and 35[a]



[a]Reagents and conditions: (a) ethylene glycol, Ag_2CO_3, CH_2Cl_2; (b) 2-cyanoethyl-*N*,*N*′-diisopropyamino-chlorophosphoramidite, DIPEA, CH_2Cl_2; (c) 2-benzyloxyethanol, Ag_2CO_3, CH_2Cl_2; (d) $MeNH_2$, THF then pyridine/acetic anhydride, DMAP; (e) H_2, $Pd(OH)_2$, EtOAc/MeOH (1:1); (f) ethylene glycol, $BF_3{\cdot}OEt_2$, CH_2Cl_2.

In the case of the rhamnose, xylose, and 6-deoxyglucose, their sugar phosphoramidites **20**, **23**, and **26** were synthesized by attachment of ethylene glycol by Koenigs−Knorr glycosylation with the corresponding acetobromosugars.[11,14] The promoter used was silver carbonate, and the isolated yields were between 44 to 83% (Scheme 1). Reaction of the obtained alcohols with 2-cyanoethyl-*N*,*N*′-diisopropylamino-chlorophosphoramidite in the presence of base resulted in the carbohydrate phosphoramidite derivatives **20**, **23**, and **26** in high yields (75−90%).

The *N*-acetylglucosamine phosphoramidite **31** was synthesized by glycosylation of 2-benzyloxyethanol with acetobromo *N*-phthalimide glucose **27**.[15] Next, phthalimide deprotection with methylamine in THF and acetylation yielded the corresponding *N*-acetamido derivative **29**. Finally, hydrogenation of the benzyl protecting group and phosphoramidite incorporation produced GlcNAc derivative **31**. Glucuronate methyl ester phosphoramidite **35** was synthesized from the corresponding trichloroacetimidate glycosyl donor **32**.[16] When ethylene glycol was used as the glycosyl acceptor, very low yields of glycosylated product were obtained. Then, 2-benzyloxyethanol was reacted with **32** to give **33** (30% yield) and subsequent hydrogenation resulted in alcohol **33** (46% yield). Synthesis of the corresponding phosphoramidite compound **35** was carried out as described above.

The synthesis of 6-*O*-acetyl-2,3,4-*O*-trimethyl glucose phosphoramidite **41** started by differentiating the primary hydroxyl group of 2-benzyloxyethyl β-D-glucopyranoside **36**[13] with a TBDPS group (Scheme 2). Further methylation of the remaining OH groups, hydrogenation and phosphoramidite synthesis yielded derivative **41**. In the case of 2,3-*O*-diacetyl-4,6-*O*-dimethyl glucose phosphoramidite **44** its preparation was carried out from the reported 1,2,3-*O*-triacetyl-4,6-*O*-dimethyl α,β-glucopyranoside **42**[17] by reaction of its corresponding α-bromo derivative with ethylene glycol. Then, hydrogenation and phosphoramidite preparation in similar conditions as described above resulted in compound **44**.

Several mono- and difluorinated carbohydrate phosphoramidite derivatives were synthesized from the corresponding acetylated α-bromide compounds (Scheme 3).[18] Again classical Koenigs−Knorr glycosylation was used and the alcohol derivatives were obtained in moderate to good yields (44−78%). The phosphoramidite derivatives of 3-fluoro-3-deoxy-glucose **47**, 4-fluoro-4-deoxy-glucose **50**, 6-fluoro-6-deoxy-glucose **53**, 4-fluoro-4-deoxy-galactose **56**, and 4,6-difluoro-4,6-dideoxy-galactose **59** were synthesized using the same reaction conditions used previously.

After standard solid phase automated oligonucleotide synthesis, the resulting carbohydrate oligonucleotide conjugates were cleaved from the resin, deprotected with ammonia, HPLC purified, and characterized by MALDI-TOF mass spectrometry (Supporting Information). In the case of glucuronic acid DNA conjugate **8**, preliminary treatment with Na_2CO_3 in MeOH/H_2O (2:1) for 24 h was carried out followed by normal treatment with ammonia and HPLC purification.

Energetics of Carbohydrate−DNA Interactions. DNA duplex stability was measured by UV-monitored thermal denaturation experiments in a pH 7.0 phosphate buffer containing 1 M NaCl. Thermodynamic parameters were calculated from melting curve fitting and from van't Hoff plots ($1/T_m$ versus ln[conjugate]).[19] Thermodynamic parameters for the carbohydrate−oligonucleotide conjugates containing natural sugars at the 5′-end **1**−**8** are shown in Table 1. It is important to clarify that the thermodynamic results obtained are for each COC double helix that incorporates two carbohydrate−DNA binding motifs within its structure. We observed previously that the presence of glucose, galactose, and fucose at the edge stabilized the DNA duplex by 3.1−3.5 °C and by 0.3−0.6 kcal·mol^{-1}.[12] DNA stabilization by xylose and *N*-acetylglucosamine is in the same range as for the previous monosaccharides, whereas rhamnose and glucuronic acid are less stabilizing, possibly due to the presence of an axial OH and a negative charge in the pyranose, respectively.

In the series of hydrophobic glucose DNA conjugates **9**−**12**, all carbohydrates increased DNA stability with respect to the natural glucose DNA conjugate **2** (Table 2). Whereas COC double helix containing 6-deoxyglucose units showed a small increase in T_m of 0.7 °C and 0.2 kcal·mol^{-1} with respect to glucose, the other apolar sugar DNA conjugates containing 4,6-dimethylated glucose, 2,3,4-trimethylated glucose, and 2,3,4,6-

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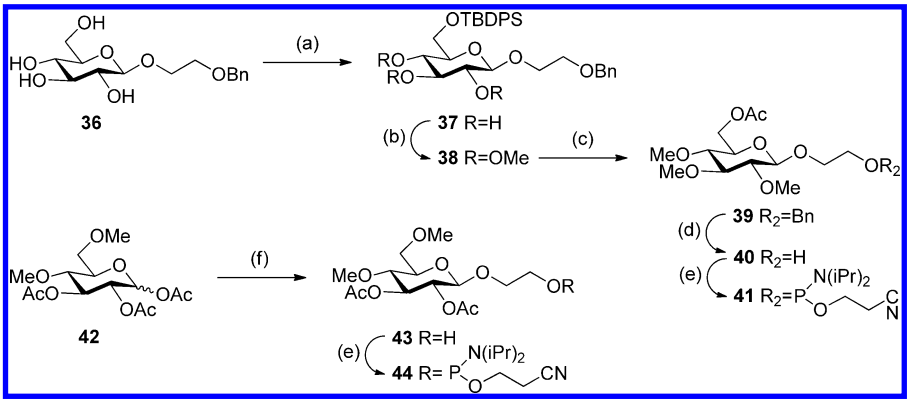
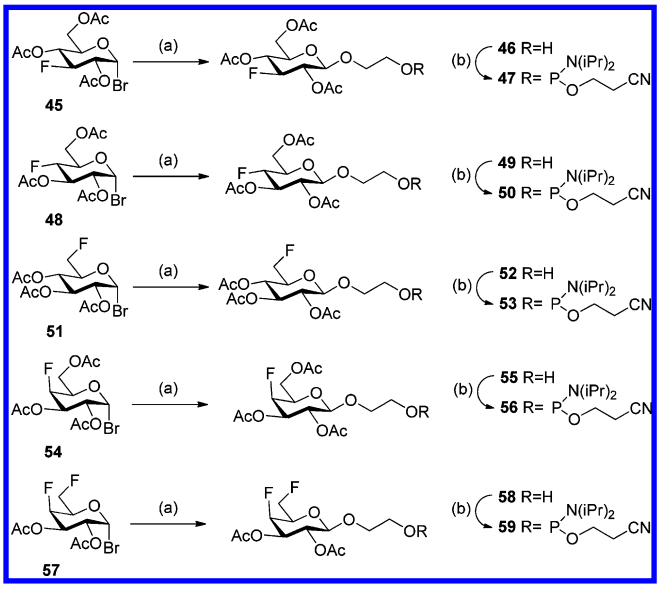
Scheme 2. Synthetic Route for the Preparation of Partially Methylated Glucose Phophoramidites 41 and 44[a]



[a]Reagents and conditions: (a) TBDPSCl, DIPEA, NEt_3, DMF; (b) NaH, MeI, DMF; (c) TBAF 1.0 M, THF then pyridine/acetic anhydride, DMAP; (d) H_2, $Pd(OH)_2$, EtOAc/MeOH (8:1); (e) 2-cyanoethyl-*N*,*N*′-diisopropyamino-chlorophosphoramidite, DIPEA, CH_2Cl_2; (f) HBr, AcOH, CH_2Cl_2 then ethylene glycol, Ag_2CO_3, CH_2Cl_2.

Scheme 3. Synthetic Route for the Preparation of Fluoro Monosaccharide Phophoramidites 47, 50, 53, 56, and 59[a]



[a]Reagents and conditions: (a) ethylene glycol, Ag_2CO_3, CH_2Cl_2; (b) 2-cyanoethyl-*N*,*N*′-diisopropyamino-chlorophosphoramidite, DIPEA, CH_2Cl_2.

tetramethylated glucose derivatives were more stable than **2** by 3.7−4.7 °C and by 0.7−0.9 kcal·mol^{-1}.

It is interesting to observe that the large increase in DNA stability from the dimethylated glucose does not correlate with the gradual increase in hydrophobicity in this series as can be deduced from the log *P* values of the glucose derivatives (Table 3). In fact, it does not correlate either with the increase in surface area of the apolar sugars. We estimated the actual surface area buried as the result of the sugar stacking on top of the C−G final DNA base pair for each of the glucose derivatives. A good correlation between the buried surface and the DNA stability is found since adding third and fourth methyl groups to the glucose unit does not increase the stacking area in the conjugate but slightly reduces it. In fact, a graph plotting free energy of COCs (containing glucose, **2**, and hydrophobic sugars, **9**−**12**) versus stacking area showed a good linear fit (the coefficient of determination is R^2 = 0.9193; see Figure S4, Supporting Information). This seems to indicate that the free energy of the conjugate is also directly related with the number of water molecules displaced by the hydrophobic carbohydrates from the area of contact with the DNA base pair and not with the hydrophobicity of the carbohydrates. A similar result was found by Kool et al. when studying $\pi-\pi$ aromatic interactions with nonpolar DNA bases (e.g., benzene, naphthalene, pyrene, etc.) at the edge of dsDNA.[20] Log *P* values were a poor predictor of stacking interaction, whereas surface area of

Table 1. Melting Temperatures and Thermodynamic Parameters for Carbohydrate Oligonucleotide Conjugate Duplexes Containing Natural Carbohydrates and the CGCGCG Sequence

dangling moiety in 5′-CGCGCG[a,b,c]	T_m (°C)	$-\Delta S°$ (van't Hoff) (cal/k mol)	$-\Delta H°$ (van't Hoff) (kcal/mol)	$-\Delta G°_{37}$ (van't Hoff) (kcal/mol)	$\Delta G°_{37}$ (fits) (kcal/mol)	$\Delta\Delta G°_{37}$[d] (kcal/mol)
(none) **1**	40.9 ± 0.4	114 ± 5	43.5 ± 2	8.2 ± 0.3	8.2 ± 0.1	–
β-D-glucose-C2[e] **2**	44.0 ± 0.7	140 ± 4	52.1 ± 2	8.7 ± 0.3	8.7 ± 0.0	−0.5
β-D-galactose-C2[e] **3**	44.4 ± 0.4	113 ± 8	43.5 ± 3	8.5 ± 0.5	8.7 ± 0.1	−0.3
β-L-fucose-C2[e] **4**	44.4 ± 0.4	136 ± 3	51.1 ± 1	8.8 ± 0.2	8.8 ± 0.0	−0.6
α-L-rhamnose-C2 **5**	42.7 ± 0.2	137 ± 7	50.9 ± 2	8.6 ± 0.4	8.5 ± 0.0	−0.4
β-D-xylose-C2 **6**	43.6 ± 0.4	156 ± 9	57.2 ± 3	8.8 ± 0.6	8.6 ± 0.1	−0.6
N-acetyl-*β*-D-glucosamine-C2 **7**	44.6 ± 0.3	136 ± 7	50.8 ± 3	8.7 ± 0.5	8.7 ± 0.0	−0.5
β-D-glucuronic acid-C2 **8**	42.4 ± 0.7	141 ± 2	52.2 ± 1	8.5 ± 0.1	8.5 ± 0.0	−0.3

[a]C2 stands for $-CH_2-CH_2-$. [b]Buffer: 10 mM Na phosphate, 1 M NaCl, pH 7.0. [c]Average value of three experiments measured at 5 μM conc. [d]$\Delta\Delta G°_{37} = \Delta G_{vh} - \Delta G$ (CGCGCG control). [e]Data from Lucas et al.[12]

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Table 2. Melting Temperatures and Thermodynamic Parameters for Carbohydrate Oligonucleotide Conjugate Duplexes Containing Hydrophobic Sugars and the CGCGCG Sequence

dangling moiety in 5′-CGCGCG[a,b,c]	T_m (°C)	$-\Delta S°$ (van't Hoff) (cal/k mol)	$-\Delta H°$ (van't Hoff) (kcal/mol)	$-\Delta G°_{37}$ (van't Hoff) (kcal/mol)	$\Delta G°_{37}$ (fits) (kcal/mol)	$\Delta\Delta G°_{37}$[d] (kcal/mol)
(none) **1**	40.9 ± 0.4	114 ± 5	43.5 ± 2	8.2 ± 0.3	8.2 ± 0.1	–
β-D-glucose-C2[e] **2**	44.0 ± 0.7	140 ± 4	52.1 ± 2	8.7 ± 0.3	8.7 ± 0.0	−0.5
β-D-6-deoxyglucose-C2 **9**	44.7 ± 0.2	145 ± 5	53.8 ± 2	8.9 ± 0.3	8.8 ± 0.0	−0.7
β-D-4,6-dimethyl-glc-C2 **10**	47.7 ± 0.2	157 ± 11	58.3 ± 4	9.6 ± 0.8	9.4 ± 0.1	−1.4
β-D-2,3,4-trimethyl-glc-C2 **11**	48.2 ± 0.1	146 ± 11	54.7 ± 4	9.4 ± 0.8	9.4 ± 0.1	−1.2
β-D-2,3,4,6-tetramethyl-glc-C2[f] **12**	48.7 ± 0.2	147 ± 10	55.1 ± 4	9.4 ± 0.6	9.4 ± 0.1	−1.2

[a]C2 stands for $-CH_2-CH_2-$. [b]Buffer: 10 mM Na phosphate, 1 M NaCl, pH 7.0. [c]Average value of three experiments measured at 5 μM conc. [d]$\Delta\Delta G°_{37} = \Delta G_{vh} - \Delta G$ (CGCGCG control). [e]Data from Lucas et al.[12] [f]Data from Lucas et al.[13]

Table 3. Molecular Weight and Partition Coefficient Data for Dangling Moieties Studied

dangling moiety in 5′-CGCGCG		MW	calc log P[a]	surface area (Å^2)[b]	S-area folded	S-area unfolded	stacking area (Å^2)[c]
glucose	**2**	194.2	−2.01	186	2918	3093	175
6-deoxyglucose	**9**	178.2	−1.16	179	2905	3082	177
4,6-dimethyl-glucose	**10**	222.2	−1.29	207	2946	3147	201
2,3,4-trimethyl-glucose	**11**	236.3	−0.93	227	2954	3143	189
2,3,4,6-tetramethyl-glucose	**12**	250.3	−0.57	243	2981	3170	189

[a]Log P values were calculated using the Crippen's fragmentation[21] in the ChemBioDraw Ultra 11.0 software. [b]Half of the calculated surface area of base as Connolly accessible area using Chem 3D Pro software. [c]Estimated as buried surface by subtracting the unfolded from the folded carbohydrate−DNA base pair.

Table 4. Melting Temperatures and Thermodynamic Parameters for Carbohydrate Oligonucleotide Conjugate Duplexes Containing Fluorosugars and the CGCGCG Sequence

dangling moiety in 5′-CGCGCG[a,b,c]	T_m (°C)	$-\Delta S°$ (van't Hoff) (cal/k mol)	$-\Delta H°$ (van't Hoff) (kcal/mol)	$-\Delta G°_{37}$ (van't Hoff) (kcal/mol)	$\Delta G°_{37}$ (fits) (kcal/mol)	$\Delta\Delta G°_{37}$[d] (kcal/mol)
(none) **1**	40.9 ± 0.4	114 ± 5	43.5 ± 2	8.2 ± 0.3	8.2 ± 0.1	–
β-D-glc-C2 **2**	44.0 ± 0.7	140 ± 4	52.1 ± 2	8.7 ± 0.3	8.7 ± 0.0	−0.5
3-F-β-D-glc-C2 **13**	44.5 ± 0.1	141 ± 2	52.4 ± 1	8.8 ± 0.1	8.8 ± 0.0	−0.6
4-F-β-D-glc-C2 **14**	44.7 ± 1.3	141 ± 6	52.5 ± 2	8.9 ± 0.4	8.9 ± 0.1	−0.7
6-F-β-D-glc-C2 **15**	44.3 ± 0.0	150 ± 5	55.3 ± 2	8.9 ± 0.3	8.8 ± 0.0	−0.7
β-D-gal-C2 **3**	44.4 ± 0.4	113 ± 8	43.6 ± 3	8.5 ± 0.5	8.7 ± 0.1	−0.3
4-F-β-D-gal-C2 **16**	44.6 ± 0.9	138 ± 14	51.2 ± 5	8.6 ± 1.0	8.6 ± 0.0	−0.4
4,6-diF-β-D-gal-C2 **17**	45.0 ± 0.5	135 ± 9	50.4 ± 3	8.6 ± 0.6	8.6 ± 0.1	−0.4

[a]C2 stands for $-CH_2-CH_2-$. [b]Buffer: 10 mM Na phosphate, 1 M NaCl, pH 7.0. [c]Average value of three experiments measured at 5 μM conc. [d]$\Delta\Delta G°_{37} = \Delta G_{vh} - \Delta G$ (CGCGCG control).

overlap (buried surface by aromatic stacking) was a much better predictor.

In the series of fluorinated sugars **13**−**17**, DNA stability of the carbohydrate oligonucleotide conjugates showed a minor increase with respect to glucose and galactose derivatives **2** and **3** (0.3−0.7 °C in T_m and 0.1−0.2 kcal·mol^{-1} in ΔG, see Table 4). The incorporation of one or two electron-withdrawing agents such as fluorine atoms in the pyranose could modify CH−π interactions between the pyranose ring and the aromatic DNA bases, but this effect seems to have little influence in the carbohydrate−aromatic stacking interaction observed. These results fit well with those obtained recently by Asensio et al.[22] in a dynamic combinatorial approach using a disaccharide model system. The authors observe a minor strengthening of the carbohydrate/aromatic forces by incorporating electron-withdrawing substituents at the anomeric position (−F or $-CH_2CF_3$).

If we consider the relevance of stereochemistry of fluorosugars in the interaction with the DNA base pair, for example by comparison of 4-fluoroglucose (4FGlc) and 4-fluorogalactose (4FGal), we observe no difference in energetics since the values are within the experimental error. This is a similar situation to that found when comparing glucose and galactose.

Finally, a couple of explicit comparisons between the three groups of monosaccharides may yield some information on the relevance of different modifications in the carbohydrate when stacking with DNA bases. If we compare glucose, 6-deoxyglucose and 6-fluoroglucose, we observe that replacement of the 6-OH with a proton or a fluorine atom yields only a small increase in stability, probably due to the small decrease in hydrophilicity with this modification. In contrast, a large difference in energetics is observed between 4,6-difluorogalactose and 4,6-dimethyl-glucose (the latest 1.0 kcal·mol^{-1} in ΔG more stable). Bearing in mind the different stereochemistry of the carbohydrate, the increased hydrophobic surface in the apolar carbohydrate has much more influence in the stacking interaction than increasing the polarity of the −CH− protons of the pyranose by the presence of the fluorine atoms.

Structural Features of Carbohydrate Oligonucleotide Conjugates. As in previous NMR studies in related systems,[12,13] no distortion of the DNA double helix due to

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the presence of the fluorinated carbohydrates is observed. Two dimensional NMR spectra were recorded for conjugates **13** to **16**. Complete resonance assignment was performed, and we found significant chemical shift differences with the control duplex only for some protons of the terminal base pair. Observed NOEs between the sugar units and the terminal C−G base pair were very similar to those observed for the corresponding nonfluorinated natural sugars (see Table S2, Supporting Information). A structural model of conjugate **13** was built on the basis of the observed experimental NOEs (see Figure S5, Supporting Information). The carbohydrate stacks on top of the terminal C−G base pairs mainly through its alpha face. The fluorine atom is oriented toward the solvent and does not participate in the interaction with the DNA, explaining the little effect of the fluorine substitutions in the thermal stability of these conjugates. Consequently, we conclude that the structures of the fluorinated conjugates are very similar to those reported previously, and no further modeling was carried out.

CONCLUSION

Evaluation of stacking of natural monosaccharides on top of a DNA base pair in water using our COC model system have shown small differences due to changes in sugar stereochemistry and to the presence of typical functional groups such as *N*-acetyl or carboxylic acid in the pyranose ring. At the same time, replacement of a hydroxyl group with a fluorine atom should increase −CH− polarization and local hydrophobicity, but none of these aspects showed large differences on stacking of the carbohydrate derivatives. Finally, increasing the hydrophobicity of the sugars through methylation exhibited a considerable enhancement in carbohydrate aromatic stacking. Nevertheless, it is important to emphasize that energetics seem to correlate better with the amount of surface contact area between the carbohydrate and the aromatic DNA base pair than with a net increase in sugar hydrophobicity. These results appear to point out the relevance of the number of water molecules displaced from the surface of contact between the carbohydrate and the aromatics.

EXPERIMENTAL SECTION

General Methods. All chemicals were obtained and used without further purification, unless otherwise noted. All reactions were monitored by TLC on precoated silica gel 60 plates F254 and detected by heating with 5% sulfuric acid in ethanol or Mostain (500 mL of 10% H_2SO_4, 25 g of $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 1 g of $Ce(SO_4)_2{\cdot}4H_2O$). Products were purified by flash chromatography with silica gel 60 (200−400 mesh). Low resolution mass spectra were obtained on an ESI/ion trap mass spectrometer. High resolution mass spectra were obtained on an ESI/quadrupole mass spectrometer. NMR spectra were recorded on a 300, 400, or 500 MHz [300 or 400 MHz (1H), 75 or 100 (^{13}C)] NMR spectrometers, at room temperature for solutions in $CDCl_3$, D_2O, or CD_3OD. Chemical shifts are referred to the solvent signal. 2D experiments (COSY, TOCSY, ROESY, and HMQC) were done when necessary to assign the carbohydrate derivatives and the conjugates. Chemical shifts are in ppm. In all experiments the ^{1}H carrier frequency was kept at the water resonance. Data were processed using manufacturer software, raw data were multiplied by shifted exponential window function prior to Fourier transform, and the baseline was corrected using polynomial fitting.

Synthetic Procedures. *General Procedure for Glycosylation of Ethylene Glycol.* Ag_2CO_3 (1.7 g, 6.25 mmol) was added to a solution of the acetyl protected glycosylbromide (2.5 mmol) and ethylenglycol (1.3 mL, 25 mmoL) previously dried over molecular sieves, in anhydrous CH_2Cl_2 (10 mL). The reaction was stirred for 24 h; the mixture was then filtered over Celite and washed with CH_2Cl_2. The solvent was removed, and the crude product was purified by flash column chromatography (hexane:ethyl acetate mixtures) to afford the corresponding glycosylated ethylene glycol.

*2-Hydroxyethyl 2,3,4-tri-O-acetyl-β-L-rhamnopyranose (**19**).* 2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl bromide **18** (972 mg, 2.76 mmol) was reacted following the general glycosylation procedure. The crude product was purified by flash column chromatography (hexane:ethyl acetate from 2:1 to 1:2) to afford **19** (592 mg, 64%) as glassy solid: $[\alpha]_D^{22}$ −3.3 (*c* 1 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 5.26−5.19 (m, 1H, H_2), 5.02−4.96 (t, J = 9.8 Hz, 1H, H_3), 4.72 (s, 1H, H_1), 3.90−3.83 (m, 1H, H_4), 3.74−3.69 (m, 3H, H_5, CH_2), 3.51−3.55 (m, 2H, CH_2), 2.08, 1.99, 1.92 (3s, 9H, 3 × $OCOCH_3$), 1.17−1.15 (d, J = 8 Hz, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 170.1, 170.0, 169.9 (3 × CO), 97.6 (C_1), 70.9 (C_3), 69.7 (C_2), 69.6 (CH_2), 69.10 (C_5), 66.4 (C_4), 61.3 (CH_2), 20.8, 20.7, 20.6 (3 × OCH_3), 17.3 (CH_3); HRMS (FAB$^+$) Calcd. for $C_{14}H_{22}O_9Na$ (M + Na) 357.1162, found 357.1152.

*2-Hydroxyethyl 2,3,4-tri-O-acetyl-β-D-xylopyranose (**22**).* 2,3,4-Tri-*O*-acetyl-α-D-xylopyranosyl bromide **21** (850 mg, 2.50 mmol) was reacted following the general glycosylation procedure. The crude product was purified by flash column chromatography (hexane:ethyl acetate from 2:1 to 1:2) to afford **22** (540 mg, 66%) as glassy solid: $[\alpha]_D^{22}$ −48.5 (*c* 1 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 5.18−5.12 (t, J = 9.0 Hz, 1H, H_3), 4.97−4.88 (m, 2H, H_2, H_4), 4.50−4.48 (d, J = 7.2 Hz, 1H, H_1), 4.14−4.08 (dd, J = 5.4/5.1 Hz, 1H, H_5), 3.84−3.79 (m, 4H, 2 × CH_2), 3.39−3.32 (dd, J = 5.3 Hz, 1H, $H_{5'}$), 2.03−2.00 (3s, 9H, 3 × $OCOCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 170.4, 170.3, 169.9 (3 × CO), 100.5 (C_1), 72.0 (C_3), 71.8 (C_2), 71.3 (CH_2), 69.1 (C_4), 62.5 (CH_2), 61.9 (C_5), 21.0. 20.9 ($OCCH_3$); HRMS (FAB$^+$) Calcd. for $C_{13}H_{20}O_9Na$ (M + Na) 343.0999, found 343.1005.

*2-Hydroxyethyl 2,3,4-tri-O-acetyl-β-D-6-deoxyglucopyranose (**25**).* 2,3,4-Tri-*O*-acetyl-6-deoxy-α-D-glucosylbromide **24** (0.5 g, 1.50 mmol) was reacted following the general glycosylation procedure. The crude product was purified by flash column chromatography (hexane:ethyl acetate from 1:1 to 1:2) to afford **25** (196 mg, 44%) as glassy solid: $[\alpha]_D^{22}$ −23.8 (*c* 1 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 5.11 (t, J = 9.0 Hz, 1H, H_3), 4.92 (dd, J = 9.6 Hz, 1H, H_2), 4.77 (t, J = 9.5 Hz, 1H, H_4), 4.46 (d, J = 8.1 Hz, 1H, H_1), 3.72−3.69 (m, 2H, CH_2), 3.57−3.52 (m, 1H, H_5), 2.40 (s, 1H, OH), 1.98, 1.97, 1.93 (3 × $OCOCH_3$), 1.18 (d, 3H, J = 6.3 Hz, CH_3) ; ^{13}C NMR (75 MHz, $CDCl_3$) δ = 170.2, 169.6, 169.5 (CO), 100.9 (C_1), 73.2 (C_4), 72.7 (C_3), 72.1 (CH_2), 71.7 (C_2), 70.0 (C_5), 61.7 (CH_2), 20.7, 20.6, 20.5 ($OCCH_3$), 17.3 (CH_3); HRMS (FAB$^+$) Calcd. for $C_{14}H_{22}O_9Na$ (M + Na) 357.1162, found 357.1168.

*2-Benzyloxyethyl 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose (**28**).* Ag_2CO_3 (1.38 g, 5.01 mmol) was added to a solution of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide **27**[15] (1 g, 2.0 mmol) and 2-benzyloxyethanol (2.85 mL, 20.0 mmoL) in anhydrous CH_2Cl_2 (12 mL). The reaction was stirred for 16 h and the mixture was then filtered over Celite and washed with CH_2Cl_2. The solvent were removed and the crude purified by flash column chromatography (hexane:ethyl acetate from 4:1 to 1:1) to afford **28** (800 mg, 70%) as glassy solid: $[\alpha]_D^{22}$ +19.7 (*c* 1 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.74−7.71 (m, 2H, Harom, Phth), 7.63−7.59 (m, 2H, Harom, Phth), 7.17−7.00 (m, 5H, Ph), 5.74 (dd, J = 9.0/10.8 Hz, 1H, H_3), 5.39 (d, J = 8.4 Hz, 1H, H_1), 5.11 (dd, J = 9.0/10.0 Hz, 1H, H_4), 4.32−4.22 (m, 2H, H_2, H_6), 4.17 (s, 2H, CH_2Ph), 4.09 (dd, J = 2.4/12.3 Hz, 1H, $H_{6'}$), 3.92−3.77 (m, 2H, CH_2, H_5), 3.69−3.60 (m, 1H, CH_2), 3.42−3.39 (m, 2H, CH_2), 2.03, 1.95, 1.80 (3s, 9H, 3 × $OCOCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 170.7, 170.2, 169.5 (3 × CO), 138.0, 134.2, 131.4, 128.4, 128.3, 127.8, 127.4, 127.3, 123.5, 98.3 (C_1), 72.9 (CH_2), 71.8 (C_5), 70.7 (C_3), 69.3 (C_4), 69.0, 68.7 (2 × CH_2), 62.0 (C_6), 54.6 (C_2), 20.8. 20.6, 20.5 ($OCCH_3$); HRMS (FAB$^+$) Calcd. for $C_{29}H_{31}NO_{11}Na$ (M + Na) 592.1795, found 592.1786.

*2-Benzyloxyethyl 3,4,6-tri-O-acetyl-2-acetylamino-2-deoxy-β-D-glucopyranose (**29**).* $MeNH_2$ (40% aq, 3 mL) was added to a solution of **28** (390 mg, 0.68 mmol) in THF (6 mL). The reaction was stirred for 16 h. The solvent was then removed and the reaction

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mixture was coevaporated with dry toluene. The crude was dissolved in dry pyridine (10 mL) and acetic anhydride (3 mL) and a catalytic amount of DMAP was added. The reaction mixture was stirred at room temperature for 24 h. The solvent was removed in vacuo and the crude product was purified by flash column chromatography (hexane:ethyl acetate from 1:4 to 0:1) to afford **29** (200 mg, 62% two steps) as a brown solid: $[\alpha]_D^{22}$ −24.5 (*c* 1 in MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.30−7.19 (m, 5H, Ph), 5.76 (d, *J* = 8.7 Hz, 1H, NH), 5.16 (t, *J* = 9.3 Hz, 1H, H_3), 4.99 (t, *J* = 9.3 Hz, 1H, H_4), 4.69 (d, *J* = 7.2 Hz, 1H, H_1), 4.46 (s, 2H, CH_2Ph), 4.21−4.18 (m, 1H, H_6), 4.03−3.92 (m, 1H, $H_{6'}$), 3.95−3.50 (m, 6H, 2 × CH_2, H_2, H_5), 1.99, 1.95, 1.94, 1.78 (3s, 12H, 3 × $OCOCH_3$, N-$COCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 170.8, 170.7, 170.3, 169.4 (4 × CO), 138.0, 128.5, 127.8, 127.6 (C_{arom}), 100.9 (C_1), 73.1 (CH_2), 72.6 (C_3), 71.8 (C_5), 69.3 (CH_2), 68.8 (C_4), 68.6 (CH_2), 62.1 (C_6), 54.5 (C_2), 23.2 ($NCOCH_3$), 20.8. 20.7, 20.6 ($OCCH_3$); HRMS (FAB$^+$) Calcd. for $C_{23}H_{31}NO_{10}Na$ (M + Na) 504.1846, found 504.1852.

*2-Hydroxyethyl 3,4,6-tri-O-acetyl-2-acetylamino-2-deoxy-β-D-glucopyranose (**30**).* A solution of compound **29** (180 mg, 0.37 mmol) in ethyl acetate-MeOH (1:1, 6 mL) and $Pd(OH)_2$ in catalytic amount was stirred under an atmosphere of hydrogen for 48 h. The mixture was filtered off over Celite and solvents were removed. The crude product was purified by silica gel column chromathography using as eluent (hexane:ethyl acetate, from 1:4 to 0:1) to give **30** (120 mg, 83%): $[\alpha]_D^{22}$ −2.4 (*c* 1 in $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD) δ ppm 5.22 (t, *J* = 9.6 Hz, 1H, H_3), 5.00 (t, *J* = 9.6 Hz, 1H, H_4), 4.70 (d, *J* = 8.4 Hz, 1H, H_1), 4.29 (d, *J* = 4.8/12.4 Hz, 1H, H_6), 4.15 (d, *J* = 2.0/12.4 Hz, 1H, $H_{6'}$), 3.91−3.80 (m, 3H, H_2, H_5, OCH_2), 3.70−3.64 (m, 3H, OCH_2, CH_2OH), 2.08, 2.02, 1.99, 1.93 (4s, 12H, 3 × $OCOCH_3$, N-$COCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 172.2, 170.9, 170.4, 169.9 (4 × CO), 100.8 (C_1), 72.8 (C_3), 71.5 (C_5), 70.9 (CH_2O), 68.8 (C_4), 61.9 (C_6), 60.8 (CH_2OH), 54.1 (C_2), 21.4, 19.3, 19.2, 19.1; HRMS (FAB$^+$) Calcd. for $C_{16}H_{25}NO_{10}Na$ (M + Na) 414.1376, found 414.1375.

*2-Benzyloxyethyl-(methyl 2,3,4-tri-O-acetyl-β-D-glucopyranosyluronate) (**33**).* $BF_3{\cdot}OEt_2$ (50 mL, 0.67 mmol) was added to a solution of trichloroacetimidate **32**[16] (800 mg, 1.67 mmol) and 2-benzyloxyethanol (356 mL, 2.50 mmol) in anhydrous CH_2Cl_2 (8 mL). After stirring at room temperature for 1 h under argon atmosphere, triethylamine (0.3 mL) was added. Solvents were removed and the crude product was purified by silica gel column chromatography (toluene:acetone, from 10:1 to 6:1) to give the **33** (240 mg, 30%) as glassy solid: $[\alpha]_D^{22}$ −20.1 (*c* 1 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.35−7.28 (m, 5H, Ph), 5.27−5.23 (m, 2H, H_4, H_3), 5.05 (t, *J* = 8.4 Hz, 1H, H_2), 4.67(d, *J* = 7.8 Hz, 1H, H_1), 4.58−4.50 (m, 2H, CH_2Ph), 4.06−3.96 (m, 2H, CH_2, H_5), 3.81−3.75 (m, 4H, CH_3O, CH_2), 3.66−3.63 (m, 2H, CH_2), 2.06, 2.03, 199 (3s, 9H, 3 × $OCOCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 170.0, 169.3, 169.2, 167.1 (4 × CO), 137.9 (Cq-$_{arom}$), 128.3, 127.5, 127.4 (C_{arom}), 101.9 (C_1), 73.1 (CH_2), 72.4 (C_5), 71.9 (C_3), 71.0 (C_2), 70.3 (C_4), 69.9, 69.3 (2 × CH_2), 52.7 (CH_3O), 20.5. 20.4 ($OCCH_3$); HRMS (FAB$^+$) Calcd. for $C_{13}H_{20}O_9Na$ (M + Na) 343.0999, found 343.1005.

*2-Hydroxyethyl-(methyl 2,3,4-tri-O-acetyl-β-D-glucopyranosyluronate) (**34**).* A solution of compound **33** (480 mg, 1.024 mmol) in ethyl acetate-MeOH (1:1, 10 mL) and $Pd(OH)_2$ in catalytic amount was stirred under an atmosphere of hydrogen for 18 h. The mixture was filtered off over Celite and solvents were removed. The crude product was purified by silica gel column chromathography using as eluent (hexane:ethyl acetate, 1:2) to give **34** (180 mg, 46%) as a syrup: $[\alpha]_D^{22}$ −19.0 (*c* 1 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 5.31−5.23 (m, 2H, H_4, H_3), 5.04 (t, *J* = 8.0 Hz, 1H, H_2), 4.63 (d, *J* = 7.5 Hz, 1H, H_1), 4.10 (d, *J* = 9.0 Hz, 1H, H_5), 3.90−92 (m, 2H, CH_2), 3.77 (s, 3H, CH_3O) 3.76−3.73 (m, 2H, CH_2), 2.07, 2.05, 2.04 (3s, 9H, 3 × $OCOCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 170.1, 169.5, 169.4, 167.1 (4 × CO), 101.2 (C_1), 72.9 (C_5), 72.3 (C_3), 71.9 (C_2), 71.3 (C_4), 69.1, 61.9 (2 × CH_2), 53.0 (CH_3O), 20.7, 20.6, 20.5 ($OCCH_3$); HRMS (FAB$^+$) Calcd. for $C_{15}H_{22}O_{11}Na$ (M + Na) 401.1060, found 401.1060.

*2-Benzyloxyethyl 6-tert-butyldiphenylsilyl-β-D-glucopyranose (**37**).* Dimethylaminopyridine (catalytic amount) and triethylamine (0. 476 mL, 4.64 mmoL) were added to a cooled solution of 2-benzyloxyethyl β-D-glucopyranoside **36**[13] (1.325 g, 4.22 mmol) in anhydrous DMF under argon atmosphere. Then *tert*-butyldiphenylsilyl chloride (1.21 mL, 4.64 mmoL) was slowly added to the mixture and the reaction was left to at room temperature until starting material had disappeared in 1h. Ethyl acetate (300 mL) was then added to the mixture and the organic layer was washed with brine (2 × 100 mL), dried with anhydrous Na_2SO_4 and concentrated to dryness. The crude product was purified by silica gel column chromatography using ethyl acetate 100% as eluent to give **37** (1.3 g, 57%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.63−7.57 (m, 5H, Ph), 7.32−7.25 (m, 5H, Ph), 7.23−7.14 (m, 5H, Ph), 4.52−4.35 (m, 2H, CH_2), 4.20 (d, *J* = 7.6 Hz, 1H, H_1) 3.84 (m, 2H, CH_2), 3.76 (dd, *J* = 10.9, 5.3 Hz, 1H), 3.64−3.36 (m, 5H, CH_2, H_5, H_6, $H_{6'}$), 3.35−3.25 (m, 2H, H2, H), 0.95 (s, 9H, 3 × $CH_{3isopropyl}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 137.7 (Cq-$_{arom}$), 135.7 (2 × C_{arom}), 135.6 (2 × C_{arom}),133.3(C_{arom}), 129.8(2 × C_{arom}), 128.5 (2 × C_{arom}), 127.9 (2 × C_{arom}), 127.8 (2 × C_{arom}), 127.7 (4 × C_{arom}), 102.6 (C_1), 76.4(CH_2), 75.4, 73.4, 73.1, 71.4, 69.0, 68.3, 64.5 (C_2−C_5, 2 × CH_2), 26.8 (3 × CH_3 iPr), 19.3(CCH_3 iPr); HRMS (FAB$^+$) Calcd. for $C_{31}H_{40}O_7NaSi$ (M + Na) 575.2441, found 575.2451.

*2-Benzyloxyethyl 2,3,4-tri-O-methyl-6-tert-butyldiphenylsilyl-β-D-glucopyranose (**38**).* Sodium hydride (1.25g, 60% conc., 30.8 mmol) was slowly added to a cooled (0 °C) solution of 2-benzyloxyethyl 6-*O*-*tert*-butyldiphenylsilyl-β-D-glucopyranose (**37**) (2.13 g, 3.86 mmol) in anhydrous DMF (20 mL). After stirring at 0 °C for 10 min under argon atmosphere, methyl iodide (3.6 mL, 58 mmol) was added and the reaction was left to process overnight at room temperature. The reaction was quenched with 2-propanol and treated with 50 mL sat. NH_4Cl. The aqueous layer was extracted with ethyl acetate (2 × 50 mL). The organic layer was then washed with brine and $Na_2S_2O_3$, dried with anhydrous Na_2SO_4 and concentrated to dryness. The crude product was purified by silica gel column chromatography (hexane:ethyl acetate, from 4:1 to 2:1) to give **38** (1.5 g, 67%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.68−7.59 (m, 5H, Ph), 7.33−7.14 (m, 10H, Ph), 4.49 (s, 2H, CH_2), 4.22 (d, *J* = 7.6 Hz, 1H, H_1) 3.95 (m, 1H), 3.79 (d, *J* = 2.8 Hz, 2H, CH_2), 3.68−3.59 (m, 3H), 3.55 (s, 3H, OCH_3), 3.52 (s, 3H, OCH_3), 3.47 (s, 3H, OCH_3), 3.27 (t, 1H,), 3.09 (ddd, *J* = 8.9, 5.7, 2.8 Hz, 2H), 2.97 (t, 1H, H2), 0.96 (s, 9H, 3 × CH_3 iPr); ^{13}C NMR (101 MHz, $CDCl_3$) δ =, 138.4 (Cq-$_{arom}$), 135.9 (2 × C_{arom}), 135.6 (2 × C_{arom}),133.8(C_{arom}), 133.3(C_{arom}), 129.6(2 × C_{arom}), 128.4 (2 × C_{arom}), 127.8 (2 × C_{arom}), 127.72 (2 × C_{arom}), 127.69 (2 × C_{arom}), 127.6 (3 × C_{arom}), 103.4 (C_1), 86.6, 83.8 (C_2), 79.1, 75.6, 73.2, 69.4, 68.7, 62.8, 60.9, 60.5(2 × C), 26.8 (3 × CH_3 iPr), 19.4(CCH_3 iPr); HRMS (FAB$^+$) Calcd. for $C_{34}H_{46}O_7NaSi$ (M + Na) 617.2911, found 617.2899.

*2-Benzyloxyethyl 2,3,4-tri-O-methyl-6-O-acetyl-β-D-glucopyranose (**39**).* TBAF (1 M in THF, 4.38 mL, 4.38 mmol) was slowly added to a solution of 2-benzyloxyethyl 2,3,4-tri-*O*-methyl-6-*O*-*tert*-butyldiphenylsilyl-β-D-glucopyranose (**38**) (1.3 g, 2.19 mmol) in anhydrous THF (10 mL). The reaction was stirred for 90 min under argon atmosphere at room temperature. 50 mL of CH_2Cl_2 were added to the reaction mixture and the organic layer was then washed with brine and NH_4Cl, dried with anhydrous $MgSO_4$ and concentrated to dryness. The crude product was purified by silica gel column chromatography using as eluent (hexane:ethyl acetate, from 1:2 to 1:4) to give 2-benzyloxyethyl 2,3,4-tri-*O*-methyl-β-D-glucopyranose (650 mg, 1.83 mmol, 83%) as a white solid. The product was then solved in anhydrous pyridine (10 mL) and acetic anhydride (1.38 mL, 14.6 mmol) and dimethylaminopyridine (catalytic amount) was added to the reaction mixture. The reaction was left to process overnight at room temperature under argon atmosphere. CH_2Cl_2 was then added to the reaction mixture (50 mL) and the organic layer was washed with brine and HCl (5% v/v), dried with anhydrous Na_2SO_4 and concentrated to dryness. The crude product was purified by silica gel column chromatography (hexane:ethyl acetate, from 1:1 to 1:3) to give **39** (705 mg, 97%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.35−7.13 (m, 5H, Ph), 4.48 (s, 2H, CH_2), 4.26 (d, *J* = 4.8 Hz, 1H, H_1), 4.22 (d, 1H), 4.11 (dd, 1H), 3.99−3.86 (m, 1H), 3.66 (dd, 1H), 3.62−3.56 (m, 2H), 3.54 (s, 3H, OCH_3), 3.50 (s, 3H, OCH_3),

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3.48 (s, 3H, OCH_3), 3.28 (ddd, 1H, H_5), 3.14−2.90 (m, 3H), 1.98 (s, 3H, $OCOCH_3$); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 170.7 (CO), 138.2 (Cq_{arom}), 128.3 (2 × C_{arom}), 127.6 (2 × C_{arom}), 127.55 (C_{arom}), 103.5 (C_1), 86.3(CH_2), 83.6, 79.4, 73.1, 72.7, 69.25, 69.05, 63.3 (C_2), 60. 8, 60.44, 60.37, 24.7, 20.8($OCOCH_3$); HRMS (FAB^+) Calcd. for $C_{20}H_{30}O_8Na$ (M + Na) 421.1838, found 421.1850.

2-Hydroxyethyl 2,3,4-tri-O-methyl-6-O-acetyl-β-D-glucopyranose (40). Palladium hydroxyde (catalytic amount) was added to a solution of 2-benzyloxyethyl 2,3,4-tri-*O*-methyl-6-*O*-acetyl-β-D-glucopyranose (**39**) (705 mg, 1.77 mmol) in ethyl acetate (4 mL) and methanol (0.5 mL). The reaction was stirred at room temperature under H_2 (g) atmosphere (4 psi) for 3 h. The mixture was then filtered over Celite and washed with CH_2Cl_2 and methanol. The solvents were then removed and the crude product was purified by flash column chromatography (hexane:ethyl acetate, from 2:1 to 1:20) to afford **40** (518 mg, 95%) as a white solid: ^1H NMR (400 MHz, $CDCl_3$) δ ppm 4.32 (dd, 1H, $H_{6'}$), 4.22 (d, J = 7.8 Hz, 1H, H_1), 4.07 (dd, 1H, H_6), 3.87 (ddd, 1H), 3.76 (ddd, 1H), 3.70−3.61 (m, 2H), 3.56 (s, 3H, OCH_3), 3.53 (s, 3H, OCH_3), 3.45 (s, 3H, OCH_3), 3.37 (ddd, 1H, H_5), 3.12 (t, 1H, H_3), 3.04−2.91 (m, 2H, H_2, H_4), 2.03 (s, 3H, $OCOCH_3$); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 170.8 (CO), 104.0 (C_1), 86.4(C_3), 83.6 (C_2), 79.7 (C_4), 73.7 (CH_2), 72.8 (C_5), 63.3 (C_6), 62.4 (CH_2), 60.9, 60.7, 60.6 (3 × OCH_3), 20.7 ($OCOCH_3$); HRMS (FAB^+) Calcd. for $C_{13}H_{24}O_8Na$ (M + Na) 331.1369, found 331.1367.

2-Hydroxyethyl 2,3-di-O-acetyl-4,6-di-O-methyl-β-D-glucopyranose (43). HBr (2 mL, 33% in AcOH) were added dropwise to a solution of 1,2,3-tri-*O*-acetyl-4,6-di-*O*-methyl-α/β-D-glucopyranose **42**[17] (1.0 g, 2.99 mmol) in anhydrous CH_2Cl_2 (5 mL). The reaction was stirred at room temperature for 2 h. The mixture was then washed with ice−water and $NaHCO_3$, dried over Na_2SO_4 and concentrated. 2,3-Di-*O*-acetyl-4,6-di-*O*-methyl-β-D-glucopyranose bromide **42b** (1.06 g) was obtained as a white solid and used without further purification for next step. Ag_2CO_3 (2.06 g, 7.48 mmol) was added to a solution of 2,3-di-*O*-acetyl-4,6-di-*O*-methyl-β-D-glucopyranose bromide **42b** (1.06 g, 2.99 mmol) and ethylene glycol (167 μL, 2.99 mmol), previously dried over molecular sieves, in anhydrous CH_2Cl_2 (10 mL). The reaction was then stirred at room temperature for 16 h under argon atmosphere. The mixture was then filtered over Celite and washed with CH_2Cl_2. The solvent was then removed and the crude purified by flash column chromatography (hexane:ethyl acetate, from 1:2 to 1:20) to afford **43** (520 mg, 52%) as white solid: ^1H NMR (400 MHz, $CDCl_3$) δ ppm 5.15 (t, 1H, H_3), 4.90 (t, 1H, H_2), 4.48 (d, J= 7.96 Hz, 1H, H_1), 3.89−3.76 (m, 2H, CH_2), 3.76−3.55 (m, 4H, CH_2, H_6, $H_{6'}$), 3.49 (m, 1H, H_5), 3.43−3.41 (m, 6H, 2 × OCH_3), 3.40−3.35 (m, 1H, H_4), 2.89 (t, 1H, OH), 2.07−2.04 (m, 6H, 2 × $OCOCH_3$); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 170.1, 169.8, (CO), 101.2 (s, C_1), 77.6 (s, C_4), 74.9 (s, C_3), 74.4 (s, C_5), 73.3 (s, CH_2), 72.0 (s, C_2), 71.0 (s, C_6), 62.2 (CH_2), 60.3, 59.3 (OCH_3), 20.8, 20.7 ($OCOCH_3$); HRMS (FAB^+) Calcd. for $C_{14}H_{24}O_9$ (M + Na) 359.1318, found 359.1313.

2-Hydroxyethyl 2,4,6-tri-O-acetyl-3-deoxy-3-fluoro-β-D-glucopyranose (46). 2,4,6-Tri-*O*-acetyl-3-deoxy-3-fluoro-α-D-glucopyranosyl bromide **45**[18d] (1.05 g, 2.85 mmol) was reacted following the general glycosylation procedure. The crude product was purified by flash column chromatography (hexane:ethyl acetate, from 1:2 to 1:3) to afford **46** (688 mg, 68%) as a glassy solid: $[\alpha]_D^{22}$ −14.6 (*c* 1 in $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ ppm 5.06−4.93 (m, 2H, H_4, H_2), 4.37−4.31 (m, 2H, H_1, H_3), 4.06−4.04 (m, 2H, H_6, $H_{6'}$), 3.70−3.52 (m, 5H, 2 × CH_2, H_5), 2.53 (s, 1H, OH), 1.98, 1.97, 1.95 (3 × $OCOCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 170.9, 169.6, 169.5 (CO), 101.0 (d, $J_{C,F}$ = 11.1 Hz, C_1), 91.8 (d, $J_{C,F}$ = 189.9 Hz, C_3), 73.1 (CH_2), 71.5 (d, $J_{C,F}$ = 18.7 Hz, C_4), 71.2 (d, $J_{C,F}$ = 7.65 Hz, C_5), 68.6 (d, $J_{C,F}$ = 18.6 Hz, C_2), 62.1 (C_6), 62.0 (CH_2), 20.9, 20.8 ($OCCH_3$); ^{19}F NMR (CDCl3, 323.6 MHz) −119.6 ppm; HRMS (FAB^+) Calcd. for $C_{14}H_{21}O_9FNa$ (M + Na) 375.1067, found 375.1075.

2-Hydroxyethyl 2,3,6-tri-O-acetyl-4-deoxy-4-fluoro-β-D-glucopyranose (49). 2,3,6-Tri-*O*-acetyl-4-deoxy-4-fluoro-α-D-glucopyranosyl bromide **48**[18f] (2.0 g, 5.00 mmol) was reacted following the general glycosylation procedure. The crude product was purified by flash column chromatography (hexane:ethyl acetate, from 1:2 to 1:4) to afford **49** (1.06 g, 60%) as glassy solid: $[\alpha]_D^{22}$ −12.5 (*c* 1 in $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ ppm 5.03 (dt, $J_{H,F}$= 15.0, J = 9.0 Hz, 1H, H_3), 4.63 (dd, J= 7.8/9.6 Hz, 1H, H_2), 4.30 (d, J= 7.8 Hz, 1H, H_1), 4.26−4.07 (m, 2H, H_6, H_4), 3.94−3.88 (ddd, J= 1.1/5.5/12.2 Hz, 1H, $H_{6'}$), 3.58−3.47 (m, 4H, 2 × CH_2), 3.41−3.38 (m, 1H, H_5), 2.43 (br.s, 1H, OH), 1.80, 1.75, 1.73 (3 × $OCOCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 170.7, 170.1, 169.8 (CO), 101.3 (C_1), 87.0 (d, $J_{C,F}$ = 184.0 Hz, C_4), 72.9 (OCH_2), 72.6 (d, $J_{C,F}$ = 19.6 Hz, C_3), 71.5 (d, $J_{C,F}$ = 10.05 Hz, C_5), 71.3 (d, $J_{C,F}$ = 5.47 Hz, C_2), 62.2 (C_6), 61.9 (CH_2OH), 20.8 ($OCCH_3$); ^{19}F NMR (CDCl3, 323.6 MHz) −123.75 ppm; HRMS (FAB^+) Calcd. for $C_{14}H_{21}O_9FNa$ (M + Na) 375.1067, found 375.1073.

2-Hydroxyethyl 2,3,4-tri-O-acetyl-6-deoxy-6-fluoro-β-D-glucopyranose (52). 2,3,4-Tri-*O*-acetyl-6-deoxy-6-fluoro-α-D-glucopyranosyl bromide **51**[18e] (742 mg, 2.00 mmol) was reacted following the general glycosylation procedure. The crude product was purified by flash column chromatography (hexane:ethyl acetate, from 1:2 to 1:3) to afford **52** (310 mg, 44%) as glassy solid: $[\alpha]_D^{22}$ −2.2 (*c* 1 in $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ ppm 5.08 (t, J = 9.5 Hz, 1H, H_3), 4.90−4.79 (m, 2H, H_4, H_2), 4.44 (d, J= 8.0 Hz, 1H, H_1), 4.40−4.22 (dm, $J_{H,F}$= 47.0 Hz, 2H, H_6, $H_{6'}$), 3.75−3.53 (m, 5H, 2 × CH_2, H_5), 2.46 (br.s, 1H, OH), 1.90, 1.88, 1.84 (3 × $OCOCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 170.0, 169.4, 169.3 (CO), 100.9 (C_1), 81.1 (d, $J_{C,F}$ = 174.0 Hz, C_6), 72.5 (d, $J_{C,F}$ = 19.3 Hz, C_5), 72.4 (C_3), 72.1 (OCH_2), 71.1 (C_2), 67.8 (d, $J_{C,F}$ = 6.82 Hz, C_4), 61.5 (CH_2OH), 20.5, 20.4 ($OCCH_3$); ^{19}F NMR (CDCl3, 323.6 MHz) −155.60 ppm; HRMS (FAB^+) Calcd. for $C_{14}H_{21}O_9FNa$ (M + Na) 375.1067, found 375.1071.

2-Hydroxyethyl 2,3,6-tri-O-acetyl-4-deoxy-4-fluoro-β-D-galactopyranose (55). 2,3,6-Tri-*O*-acetyl-4-deoxy-4-fluoro-α-D-galactopyranosyl bromide **54**[18a,c] (600 mg, 1.62 mmol) was reacted following the general glycosylation procedure. The crude product was purified by flash column chromatography (hexane:ethyl acetate, from 2:3 to 1:3) to afford **55** (447 mg, 78%) as an oil: ^1H NMR (400 MHz, $CDCl_3$) δ ppm 5.22 (dd, J = 8.0, 10.4 Hz,1H, H_2), 4.94 (ddd, J = 2.4, 10.4, 27.6 Hz, 1H, H_3), 4.81 (dd, J = 2.8, 50 Hz, 1H, H_4), 4.51 (d, J = 7.9 Hz, H_1), 4.32−4.20 (m, 2H, H_6, $H_{6'}$), 3.89−3.75 (m, 5H, 2 × CH_2, H_5), 2.74 (s, 1H, OH), 2.04, 2.02, 2.00 (3s, 9H, 3 × $OCOCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 170.5, 170.3, 169.5 (CO), 101.4 (C_1), 85.8 (d, $J_{C,F}$ = 185.1 Hz, C_4), 72.6 (CH_2), 71.2 (d, $J_{C,F}$ = 17.6 Hz, C_5), 70.9 (d, $J_{C,F}$ = 18.1 Hz, C_3), 68.7 (C_2), 61.8 (CH_2), 61.5 (d, $J_{C,F}$ = 5.6 Hz, C_6), 20.7, 20.6 ($OCCH_3$); ^{19}F NMR (CDCl3, 323.6 MHz, internal reference TFT) −216.1 ppm; HRMS (FAB^+) Calcd. for $C_{14}H_{21}O_9FNa$ (M + Na) 375.1067, found 375.1075.

2-Hydroxyethyl 2,3-di-O-acetyl-4,6-dideoxy-4,6-difluoro-β-D-galactopyranose (58). 2,3-Di-*O*-acetyl-4,6-dideoxy-4,6-difluoro-α-D-galactopyranosyl bromide **57**[18b] (253 mg, 0.77 mmol) was reacted following the general glycosylation procedure. The crude product was purified by flash column chromatography (hexane:ethyl acetate, from 1:1 to 1:2) to afford **58** (158 mg, 66%): $[\alpha]_D^{22}$ +5.8 (*c* 1 in $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ ppm 5.20 (t, J = 9.6 Hz, 1H, H_2), 5.011−4.79 (m, 2H, H_3, H_4), 4.56 (dd, $J_{H,F}$= 46.4, J= 6.4 Hz, 2H, H_6, $H_{6'}$), 4.55 (d, J= 8.0 Hz, 1H, H_1), 3.96−3.83 (m, 2H, H_5, CH), 3.74−3.66 (m, 3H, CH_2), 2.72 (br.s, 1H, OH), 2.07, 2.03 (2 × $OCOCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 170.3, 169.6 (CO), 101.2 (C_1), 85.5 (dd, J = 186.0, 5.0 Hz, C_4), 80.4 (dd, J = 170.0, 6.0 Hz, C_6), 72.9 (OCH_2), 72.1 (CH_2), 71.6 (dd, J = 24.0, 18.0 Hz, C_5), 71.0 (d, J = 17.0 Hz, C_3), 68.7 (C_2), 61.6 (CH_2OH), 20.7, 20.6 (OCH_3); ^{19}F NMR (CDCl3, 323.6 MHz) −140.7, −155.1 ppm; HRMS (FAB^+) Calcd. for $C_{12}H_{18}O_7F_2Na$ (M + Na) 335.0918, found 335.0917.

General Procedure for Synthesis of Carbohydrate Phosphoramidites. To a solution of 2-hydroxyethyl acetyl protected carbohydrate (0.5 mmol) in dry CH_2Cl_2 (5 mL), DIEA (0.35 mL, 2.0 mmol) and 2-cyanoethyl-*N*,*N*′-diisopropylamino-chlorophosphoramidite (0.174 mL, 0.76 mmoL) were added at room temperature under argon atmosphere. After 1 h the solvent was evaporated to dryness. The product was purified by silica gel column chromathography using a mixture of hexane, ethyl acetate and triethylamine.

2-(2,3,4-Tri-O-acetyl-β-L-rhamnopyranosyloxy)ethyl(2-cyanoethyl)(N,N-diisopropyl)phosphoramidite (20). 2-Hydroxyethyl 2,3,4-tri-*O*-acetyl-β-L-rhamnopyranose **19** (0.12 g, 0.358 mmol) was reacted

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following the general procedure. The crude product was purified by flash column chromatography (hexane:ethyl acetate, 1:1 with 1% NEt_3) to obtain compound **20** as a syrup (165 mg, 86%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.25−5.16 (m, 2H, H_2, H_3), 4.99 (t, J = 9.9 Hz, 1H, H_4), 4.71 (dd, J = 3.7/1.6 Hz, 1H, H_1), 3.94−3.48 (m, 9H, 2 × OCH_2, OCH_2CH_2CN, 2 × $CH_{isopropyl}$, H_5), 2.63−2.57 (m, 2H, CH_2CN), 2.07, 1.98, 1.91 (3s, 3H, 3 × $OCOCH_3$), 1.19−1.11 (m, 15H, CH_3, 4 × $CH_{3isopropyl}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 170.1, 170.0, 169.9 (CO), 117.8 (CN), 97.7 (C_1), 71.1, 69.7, 69.0, 67.8, 67.5, 63.3, 62.4, 62.2, 58.5, 43.2 ($NCH_{isopropyl}$), 24.6, 24.5 ($CH_{3isopropyl}$), 20.9, 20.8 ($OCCH_3$), 20.6 (CH_2CN), 17.4 (CH_3); HRMS (FAB^+) Calcd. for $C_{23}H_{39}N_2O_{10}PNa$ (M + Na) 557.2240, found 557.2222.

*2-(2,3,4-Tri-O-acetyl-β-D-xylopyranosyloxy)ethyl(2-cyanoethyl)-(N,N-diisopropyl)phosphoramidite (**23**).* 2-Hydroxyethyl 2,3,4-tri-*O*-acetyl-β-D-xylopyranose **22** (0.06 g, 0.197 mmol) was reacted following the general procedure. The crude product was purified by silica gel column chromathography (hexane:ethyl acetate, 1:1 with 1% NEt_3) to give compound **23** as a syrup (80 mg, 83%): ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 5.08 (t, J = 8.5 Hz, 1H, H_3), 4.88−4.82 (m, 2H, H_2, H_4), 4.51 (m, 1H, H_1), 4.06 (dd, J = 5.3/11.8 Hz, 1H, H_5), 3.89−3.46 (m, 8H, 2 × OCH_2, OCH_2CH_2CN, 2 × $CH_{isopropyl}$), 3.34−3.26 (m, 1H, $H_{5'}$), 2.61−2.54 (m, 2H, CH_2CN), 1.99, 1.98, 1.97 (3s, 9H, 3 × $OCOCH_3$), 1.11, 1.10 (2d, 12H, 4 × $CH_{3isopropyl}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 170.1, 169.9, 169.4 (3 × CO), 117.7 (CN), 100.7 (C_1), 71.4, 70.6, 69.1, 68.8, 62.5, 62.3, 61.9, 58.5, 58.3, 43.1 ($NCH_{isopropyl}$), 24.6, 24.5 ($CH_{3isopropyl}$), 20.8, 20.6 ($OCCH_3$), 20.5 (CH_2CN); HRMS (FAB^+) Calcd. for $C_{22}H_{37}KN_2O_{10}P$ (M + K) 559.1823, found 559.1827.

*2-(2,3,4-Tri-O-acetyl-6-deoxy-β-D-glucopyranosyloxy)ethyl(2-cyanoethyl)(N,N-diisopropyl)phosphoramidite (**26**).* 2-Hydroxyethyl 2,3,4-tri-*O*-acetyl-6-deoxy-β-D-glucopyranoside **25** (0.140 g, 0.418 mmol) was reacted following the general procedure. The crude product was purified by silica gel column chromathography using as eluent (hexane:ethyl acetate, 1:1 with 1% NEt_3) to give compound **26** as a syrup (200 mg, 90%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.90 (t, J = 9.6 Hz, 1H, H_3), 4.74−4.680 (m, 1H, H_2), 4.57 (t, J = 9.6 Hz, 1H, H_4), 4.36 (dd, $J_{H,P}$= 14.5, J = 8.1 Hz, 1H, H_1), 3.70−3.40 (m, 6H, 2 × OCH_2, OCH_2CH_2CN), 3.39−3.29 (m, 3H, 2 × $CH_{isopropyl}$, H_5), 2.67−2.62 (m, 2H, CH_2CN), 2.05, 2.03, 1.99 (3s, 3H, 3 × $OCOCH_3$), 1.23 (d, 3H, J = 6.0 Hz, CH_3), 1.17 (t, 12H, $4CH_{3isopropyl}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 170.2, 169.9, 169.6 (CO), 118.0 (CN), 100.8 (C_1), 73.6 (C_4), 73.1 (C_3), 71.9 (C_2), 70.2 (C_5), 69.5, 62.8, 58.7 (3 × CH_2), 43.3 ($NCH_{isopropyl}$), 25.0, 24.9, 24.8, 24.7 ($CH_{3isopropyl}$), 21.0, 20.9, 20.8 ($OCCH_3$), 20.6 (CH_2CN), 17.6 (CH_3); HRMS (FAB^+) Calcd. for $C_{23}H_{39}N_2O_{10}PNa$ (M + Na) 557.2240, found 557.2243.

*2-(3,4,6-Tri-O-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosyloxy)ethyl(2-cyanoethyl)(N,N-diisopropyl)phosphoramidite (**31**).* 2-Hydroxyethyl 3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranoside **30** (0.20 g, 0.50 mmol) was reacted following the general procedure. The crude product was purified by silica gel column chromathography (hexane:ethyl acetate, 1:1 with 1% NEt_3) to give compound **31** as a syrup (265 mg, 90%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.92 (dd, J = 3.0/8.7 Hz, 1H, NH), 4.96 (t, J = 9.6 Hz, 1H, H_3), (td, J = 2.7/9.6 Hz, 1H, H_4), 4.61 (t, J = 8.4 Hz, 1H, H_1), 3.98 (dd, J = 4.8/12.3 Hz, 1H, H_6), 3.84 (dd, J = 2.7/12.3 Hz, 1H, $H_{6'}$), 3.72−3.40 (m, 9H, 2 × OCH_2, OCH_2CH_2CN, H_2, H_5), 3.37−3.26 (m, 2H, 2 × $CH_{isopropyl}$), 2.43−2.39 (m, 2H, CH_2CN), 1.81, 1.73, 1.67 (3s, 12H, 3 × $OCOCH_3$), 0.92−0.89 (m, 12H, 4 × $CH_{3isopropyl}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 170.9, 170.6, 169.6 (CO), 118.4 (CN), 101.3 (C_1), 73.0 (C_3), 72.0 (C_4), 69.6, 68.9 (CH_2), 62.9 (CH_2), 62.4 (C_6), 58.5 (CH_2), 54.6 (C_2), 43.3 ($NCH_{isopropyl}$), 24.9 ($CH_{3isopropyl}$), 21.0, 20.9, 20.8 ($OCCH_3$), 20.7 (CH_2CN); HRMS (FAB^+) Calcd. for $C_{25}H_{42}N_3NaO_{11}P$ (M + Na) 614.2455, found 614.2440.

*2-(Methyl 2,3,4-tri-O-acetyl-β-D-glucopyranosyluronate-oxy)-ethyl(2-cyanoethyl)(N,N-diisopropyl)phosphoramidite (**35**).* 2-Hydroxyethyl methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate **34** (0.14 g, 0.37 mmol) was reacted following the general procedure. The crude product was purified by silica gel column chromathography (hexane:ethyl acetate, 1:1 with 1% NEt_3) to give compound **35** as a syrup (160 mg, 75%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.21−5.11 (m, 2H, H_3, H_4), 4.97−4.90 (m, 2H, H_2), 4.61 (dd, J = 7.8/9.6 Hz, 1H, H_1), 4.00−3.65 (m, 10H, 2 × OCH_2, OCH_2CH_2CN, CH_3O, H_5), 3.60−3.48 (m, 2H, 2 × $CH_{isopropyl}$), 2.59−2.55 (m, 2H, CH_2CN), 2.04, 1.99 (2s, 9H, 3 xOCOCH$_3$), 1.15−1.09 (m, 12H, 4 × $CH_{3isopropyl}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 170.1, 169.4, 169.3, 167.2 (4 × CO), 117.8 (CN), 100.8 (C_1), 72.5 (C_5), 72.0 (C_3), 71.1 (C_2), 69.6 (CH_2), 69.4 (C_4), 62.4, 58.5, 52.8 (3 × CH_2), 43.0 ($NCH_{isopropyl}$), 24.6, 24.5 ($CH_{3isopropyl}$), 20.7, 20.5, 20.4 ($OCCH_3$), 20.3 (CH_2CN); HRMS (FAB^+) Calcd. for $C_{24}H_{39}N_2O_{12}PNa$ (M + Na) 601.2134, found 601.2138.

*2-(2,3,4-Tri-O-methyl-6-O-acetyl-β-D-glucopyranosyloxy)ethyl(2-cyanoethyl)(N,N-diisopropyl)phosphoramidite (**41**).* 2-Hydroxyethyl 2,3,4-tri-*O*-methyl-6-*O*-acetyl-β-D-glucopyranose **40** (165 mg, 0.535 mmol) was reacted following the general procedure. The crude product was purified by silica gel column chromatography (hexane:ethyl acetate, from 3:2 to 1:2 with 1% NEt_3) to give compound **41** as a white foam (155 mg, 57%): ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 4.30−4.20 (m, 2H, H6′, H1), 4.13 (dd, 1H, H6), 3.90 (m, 1H, CH_2), 3.85−3.73 (m, 3H, CH iPr, CH_2), 3.67 (dtt, 2H, CH_2), 3.55 (s, 5H, OCH_3+2H), 3.51 (s, 3H, OCH_3), 3.44 (s, 3H, OCH_3), 3.30 (ddd, 1H, H5), 3.10 (t, 1H, H3), 3.02 (t, 1H, H4), 2.92 (ddd, 1H, H2), 2.58 (t, 2H, CH_2), 2.02 (s, 3H, $OCOCH_3$), 1.14−1.08 (m, 12H, 4 × $CH_{3\ isopropyl}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 170.8 (CO), 117.7 (CN), 103.5 (C1), 86.3, 83.6, 79.4, 72.7, 69.6 (dd), 63.3, 62.4 (dd), 60.8, 60.6−60.2 (m, 2C), 58.4 (dd), 43.0 (dd, 2C), 24.7−24.4 (m, 4C), 20.8, 20.3; HRMS (FAB^+) Calcd. for $C_{22}H_{42}N_2O_9P$ (M + H) 509.2628, found 509.2653.

*2-(2,3-Di-O-acetyl-4,6-di-O-methyl-β-D-glucopyranosyloxy)ethyl-(2-cyanoethyl)(N,N-diisopropyl)phosphoramidite (**44**).* 2-Hydroxyethyl 2,3-di-*O*-acetyl-4,6-di-*O*-methyl-β-D-glucopyranose **43** (0.150 g, 0.45 mmol) was reacted following the general procedure. The crude product was purified by silica gel column chromathography (hexane:ethyl acetate, 1:1 with 1% NEt_3) to give compound **44** as a white foam (111 mg, 53%): ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 5.14 (m, 1H, H_3), 4.89 (ddd, 1H, H_2), 4.55 (dd, 1H, H_1), 3.98 (m, 2H, CH_2), 3.83 (m, 2H, CH_2), 3.73 (m, 2H, CH_2), 3.68−3.56 (m, 5H, H5, H6/$H_{6'}$, 2 × $CH_{isopropyl}$), 3.48−3.40 (m, 7H, H_4, 2 × OCH_3), 2.66 (m, 2H, CH_2), 2.06 (m, 6H, 2 × $OCOCH_3$), 1.19 (m, 12H, 4 × $CH_{3isopropyl}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 170.2, 169.7 (CO), 117.8 (CN), 100.7 (C_1), 75.1, 74.7, 71.9, 70.8, 69.3, 69.1, 62.5, 62.3, 60.3, 59.4, 58.6, 58.4, 43.1, 43.0, 24.7, 24.6, 24.5, 20.8, 20.4; HRMS (FAB^+) Calcd. for $C_{23}H_{41}N_2O_{10}PNa$ (M + Na) 559.2392, found 559.2397.

*2-(2,4,6-Tri-O-acetyl-3-deoxy-3-fluoro-β-D-glucopyranosyloxy)-ethyl(2-cyanoethyl)(N,N-diisopropyl)phosphoramidite (**47**).* 2-Hydroxyethyl 2,4,6-tri-*O*-acetyl-3-deoxy-3-fluoro-β-D-glucopyranose **46** (0.220 g, 0.62 mmol) was reacted following the general procedure. The crude product was purified by silica gel column chromathography (hexane:ethyl acetate, from 3:2 to 1:1 with 1% NEt_3) to give compound **47** as a syrup (260 mg, 76%): ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 5.11−4.91 (m, 2H, H_4, H_2), 4.53−4.28 (m, 2H, H_1, H_3), 4.12 (ddd, J= 2.0/4.7/12.4 Hz, 1H, H_6), 3.99 (br. d, J= 12.3 Hz, 1H, $H_{6'}$), 3.86−3.53 (m, 6H, 2 × OCH_2, OCH_2CH_2CN) 3.52−3.40 (m, 3H, 2 × $CH_{isopropyl}$, H_5), 2.53−2.49 (m, 2H, CH_2CN), 1.99, 1.97, 1.96 (s, 9H, 3 × $OCOCH_3$), 1.05, 1.03 (2d, 12H, $4CH_{3isopropyl}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 170.9, 169.5, 169.4 (CO), 118.0 (CN), 101.0 (d, $J_{C,F}$ = 10.65 Hz, C_1), 91.9 (d, $J_{C,F}$ = 189.1 Hz, C_3), 71.4 (d, $J_{C,F}$ = 18.5 Hz, C_2), 71.1 (d, $J_{C,F}$ = 7.65 Hz, C_5), 69.8 (CH_2), 68.5 (d, $J_{C,F}$ = 18.3 Hz, C_4), 62.6 (CH_2), 62.0 (C_6), 58.6 (CH_2), 43.3 ($NCH_{isopropyl}$), 24.9, 24.8 ($CH_{3\ isopropyl}$), 21.1, 21.0, 20.9 ($OCCH_3$), 20.6 (CH_2CN). ^{19}F NMR ($CDCl_3$, 323.6 MHz, internal reference TFT) −195.3 ppm; HRMS (FAB^+) Calcd. for $C_{23}H_{38}N_2O_{10}FNaP$ (M + Na) 575.2146, found 575.2158.

*2-(2,3,6-Tri-O-acetyl-4-deoxy-4-fluoro-β-D-glucopyranosyloxy)-ethyl(2-cyanoethyl)(N,N-diisopropyl)phosphoramidite (**50**).* 2-Hydroxyethyl 2,3,6-tri-*O*-acetyl-4-deoxy-4-fluoro-β-D-glucopyranose **49** (0.365 g, 1.036 mmol) was reacted following the general procedure. The crude product was purified by silica gel column chromathography (hexane:ethyl acetate, from 3:2 to 1:1 with 1% NEt_3) to give compound **50** as a syrup (434 mg, 76%): ^{1}H NMR (300 MHz,

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$CDCl_3$) δ ppm 5.03 (dt, $J_{H,F}$= 15.0, J = 9.0 Hz, 1H, H_3), 4.68 (dd, J= 7.8/9.6 Hz, 1H, H_2), 4.40 (dd, J= 7.8/10.5 Hz, 1H, H_1), 4.37−4.13 (m, 2H, H_6, H_4), 4.02−3.97 (dd, J= 5.5/12.2 Hz, 1H, $H_{6'}$), 3.74−3.43 (m, 7H, 2 × OCH_2, OCH_2CH_2CN, H_5), 3.40−3.28 (m, 2H, 2 × $CH_{isopropyl}$), 2.43−2.37 (m, 2H, CH_2CN), 1.87, 1.83, 1.80 (3s, 9H, 3 × $OCOCH_3$), 0.95, 0.93 (m, 12H, $4CH_{3isopropyl}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 170.4, 169.8, 169.3 (CO), 117.7 (*C*N), 101.6 (C_1), 86.6 (d, $J_{C,F}$ = 186.7 Hz, C_4), 72.4 (d, $J_{C,F}$ = 19.5 Hz, C_3), 71.2 (d, $J_{C,F}$ = 11.1 Hz, C_5), 70.9 (d, $J_{C,F}$ = 4.35 Hz, C_2), 69.5, 62.3 (CH_2), 61.9 (C_6), 58.3 (CH_2), 42.9 ($NCH_{isopropyl}$), 24.5, 24.4 ($CH_{3isopropyl}$), 20.6, 20.3 ($OCCH_3$), 20.2 (CH_2CN). ^{19}F NMR ($CDCl_3$, 323.6 MHz, internal reference TFT) −199.9 ppm; HRMS (FAB^+) Calcd. for $C_{23}H_{38}N_2O_{10}FNaP$ (M + Na) 575.2146, found 575.2164.

*2-(2,3,4-Tri-O-acetyl-6-deoxy-6-fluoro-β-D-glucopyranosyloxy)-ethyl(2-cyanoethyl)(N,N-diisopropyl)phosphoramidite (**53**).* 2-Hydroxyethyl 2,3,4-tri-*O*-acetyl-6-deoxy-6-fluoro-β-D-glucopyranose **52** (0.150 g, 0.42 mmol) was reacted following the general procedure. The crude product was purified by silica gel column chromathography (hexane:ethyl acetate, from 3:2 to 1:1 with 1% NEt_3) to give compound **53** as a syrup (150 mg, 65%): ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 5.08 (td, J = 3.0/9.5 Hz, 1H, H_3), 5.05 (t, J = 9.5 Hz, 1H, H_4), 5.00−4.97 (m, 1H, H_2), 4.66 (dd, J= 8.0/12.0 Hz, 1H, H_1), 4.40−4.22 (dm, $J_{H,F}$= 47.0 Hz, 2H, H_6, $H_{6'}$), 4.00−3.69 (m, 7H, 2 × OCH_2, OCH_2CH_2CN, H_5), 3.64−3.57 (m, 2H, 2 × $CH_{isopropyl}$), 2.67−2.63 (m, 2H, CH_2CN), 2.08, 2.06, 2.02 (3 × $OCOCH_3$), 1.19 (t, 12H, $4CH_{3isopropyl}$); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 170.3, 169.5, 169.3 (CO), 118.0 (*C*N), 100.9 (C_1), 81.1 (d, $J_{C,F}$ = 174.0 Hz, C_6), 72.7 (d, $J_{C,F}$ = 4.3 Hz, C_3), 72.5 (d, $J_{C,F}$ = 19.6 Hz, C_5), 71.2 (C_2), 69.4 (CH_2), 68.1 (d, $J_{C,F}$ = 6.75 Hz, C_4), 62.4, 58.5 (CH_2), 43.3 ($NCH_{isopropyl}$), 24.6, 24.5 ($CH_{3isopropyl}$), 20.7, 20.6 ($OCCH_3$), 20.4 (CH_2CN). ^{19}F NMR ($CDCl_3$, 323.6 MHz, internal reference TFT) −231.4 ppm; HRMS (FAB^+) Calcd. for $C_{23}H_{38}N_2O_{10}FNaP$ (M + Na) 575.2146, found 575.2117.

*2-(2,3,6-Tri-O-acetyl-4-deoxy-4-fluoro-β-D-galactopyranosyloxy)-ethyl(2-cyanoethyl)(N,N-diisopropyl)phosphoramidite (**56**).* 2-Hydroxyethyl 2,3,6-tri-*O*-acetyl-4-deoxy-4-fluoro-β-D-galactopyranose **55** (0.120 g, 0.34 mmol) was reacted following the general procedure. The crude product was purified by silica gel column chromathography (hexane:ethyl acetate, 1:1 with 1% NEt_3) to give compound **56** as a syrup (121 mg, 64%): ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 5.25−5.22 (m, 1H, H_2), 5.01−4.90 (m, 1H, H_3), 4.83 (d, J= 50.4 Hz, 1H, H_4), 4.60 (dd, J= 8.0/11.8 Hz, 1H, H_1), 4.37−4.32 (m, 1H, H_5), 4.23−4.18 (dd, J= 6.8/11.3 Hz, 1H, H_6), 3.96−3.70 (m, 7H, 2 × OCH_2, OCH_2CH_2CN, $H_{6'}$), 3.62−3.54 (m, 2H, 2 × $CH_{isopropyl}$), 2.66−2.62 (m, 2H, CH_2CN), 2.1, 2.08, 2.06 (3s, 9H, 3 × $OCOCH_3$), 1.17 (t, J = 6.6 Hz, 12H, 4 × $CH_{3isopropyl}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 170.4, 170.3, 169.3 (CO), 117.7 (*C*N), 100.9 (C_1), 85.8 (dd, $J_{C,P}$ = 6.8 Hz, $J_{C,F}$ = 185.1 Hz, C_4), 71.3 (dd, J = 4.7 Hz, J = 19.5 Hz), 70.8 (d, J = 18.0 Hz), 69.1 (dd, J = 25.2, 7.37 Hz), 68.6 (d, J = 4.8 Hz), 62.5 (dd, J = 7.7, 16.6 Hz), 61.3, 58.4 (d, J = 19.0 Hz, CH_2), 42.9 (dd, J = 4.2, 12.39 Hz, $NCH_{isopropyl}$), 24.5, 24.4 ($CH_{3isopropyl}$), 20.6, 20.3 ($OCCH_3$), 20.2 (CH_2CN). ^{19}F NMR ($CDCl_3$, 323.6 MHz, internal reference TFT) −216.1 ppm; HRMS (FAB^+) Calcd. for $C_{23}H_{38}N_2FO_{10}NaP$ (M + Na) 575.2161, found 575.2146.

*2-(2,3-Di-O-acetyl-4,6-dideoxy-4,6-difluoro-β-D-galacto-pyranosyloxy)ethyl(2-cyanoethyl)(N,N-diisopropyl)phos-phoramidite (**59**).* 2-Hydroxyethyl 2,3-di-*O*-acetyl-4,6-dideoxy-4,6-difluoro-β-D-galactopyranose **58** (0.120 g, 0.38 mmol) was reacted following the general procedure. The crude product was purified by silica gel column chromathography (hexane:ethyl acetate, 1:1 with 1% NEt_3) to give compound **59** as a syrup (125 mg, 62%): ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 5.23−5.21 (m, 1H, H_2), 5.00−4.93 (m, 1H, H_3), 4.86 (d, J= 50.4 Hz, 1H, H_4), 4.62−4.52 (m, 3H, H_6, H_1, $H_{6'}$), 3.97−3.65 (m, 7H, H_5, 2 × OCH_2, OCH_2CH_2CN), 3.63−3.53 (m, 2H, 2 × $CH_{isopropyl}$), 2.66−2.61 (m, 2H, CH_2CN), 2.09−2.07 (m, 6H, 2 × $OCOCH_3$), 1.20 (t, J = 6.6 Hz, 12H, $4CH_{3isopropyl}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 170.3, 169.3 (CO), 117.9 (*C*N), 100.9 (C_1), 85.7 (dd, J = 5.0 Hz, 186 Hz, C_4), 71.3 (dd, J = 4.7, 19.5 Hz, C_6), 71.6, 71.4, 71.3, 71.2, 69.3, 68.2, 62.5, 58.4 (d, J = 19.6 Hz, CH_2), 42.9 ($NCH_{isopropyl}$), 24.6, 24.5 ($CH_{3isopropyl}$), 20.8, 20.7 ($OCCH_3$), 20.3 (CH_2CN). ^{19}F NMR ($CDCl_3$, 323.6 MHz, internal reference TFT) −217.1, −231.0 ppm; HRMS (FAB^+) Calcd. for $C_{21}H_{35}N_2O_8F_2NaP$ (M + Na) 535.1997, found 535.2005.

Synthesis of Carbohydrate−Oligonucleotide Conjugates. Carbohydrate−oligonucleotide conjugates **6**, **8**, and **14** were synthesized on a DNA automatic synthesizer by using standard β-cyanoethylphosphoramidite chemistry. Conjugate **5**, **7**, **9**, **10**, **11**, and **13**−**17** were prepared by *Biomers* following the same methodology. Oligonucleotide conjugates were synthesized either on low-volume 200 nmols (LV200) or 1.0 μmol scale and using the DMT-off procedure. Oligonucleotide supports were treated with 33% aqueous ammonia for 16 h at 55 °C, and then the ammonia solutions were evaporated to dryness and the conjugates were purified by reversed-phase HPLC in a Waters Alliance separation module with a PDA detector. HPLC conditions were as follows: Nucleosil 120 C18, 250 × 8 mm, 10 μm column; flow rate: 3 mL/min. A 27 min linear gradient 0−30% B (solvent A: 5% CH3CN/95% 100 mM triethylammonium acetate (TEAA; pH 6.5); solvent B: 70% CH3CN/30% 100 mM TEAA (pH 6.5)).

Thermodynamic Measurements. Melting curves for the DNA conjugates were measured in an UV/vis spectrophotometer at 280 nm while the temperature was raised from 10 to 80 °C at a rate of 1.0 °C min^{-1}. Curve fits were excellent, with c^2 values of 10^6 or better, and the van't Hoff linear fits were quite good (r^2 = 0.98) for all oligonucleotides. ΔH, ΔS, and ΔG errors were calculated as described previously.[19]

NMR Spectroscopy. Samples of all the conjugates were purified by HPLC, ion-exchanged with Dowex 50W resin and then suspended in 500 μL of either D_2O or H_2O/D_2O 9:1 in phosphate buffer, 100 mM NaCl, pH 7. NMR spectra were acquired in 600 or 800 MHz NMR spectrometers. DQF-COSY, TOCSY and NOESY experiments were recorded in D_2O. The NOESY spectra were acquired with mixing times of 150 and 300 ms, and the TOCSY spectra were recorded with standard MLEV-17 spin-lock sequence, and 80-ms mixing time. NOESY spectra in H_2O were acquired with 100 ms mixing time. In 2D experiments in H_2O, water suppression was achieved by including a WATERGATE[23] module in the pulse sequence prior to acquisition. Two-dimensional experiments in D_2O were carried out at temperatures ranging from 5 to 25 °C, whereas spectra in H_2O were recorded at 5 °C to reduce the exchange with water. The spectral analysis program Sparky[24] was used for semiautomatic assignment of the NOESY cross-peaks and quantitative evaluation of the NOE intensities.

Conjugate Modeling and Buried Surface Area Calculations. Computer models of conjugates **9**−**12** were built from the solution structure of the permethylated conjugate **12** determined in a previous study.[13] The computer package Sybyl was used to perform the adequate $-CH_3$ to $-OH$ substitutions. The coordinates were energy minimized before surface area calculations. Models of the "open" conjugates were built by manually changing the torsion angles of the linking phosphate to locate the carbohydrate in a position where it is completely exposed to the solvent.

The buried surface area is obtained as the difference between de surface area of the "open" and the "folded" model of the corresponding conjugate. Surface areas were calculated with the program MOLMOL.[25]

■ ASSOCIATED CONTENT

Supporting Information

Copies of ^{1}H NMR and ^{13}C NMR spectra for compounds **19**−**59**. HPLC traces of carbohydrate oligonucleotide conjugates (COCs) **5**−**17**. Melting and van't Hoff curves of the COCs. NMR proton assignments, relevant NOEs, and chemical shift changes for COCs **13**−**17**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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