as the transition state, similar to superacidic solutions.[1b] At the same time, according to literature data both parallel pathways a), d) and b), c) are possible for the formation of pivalic aldehyde (**4**) by the direct formylation of the C–H bond in isobutane with the [HC$^+$O] cation[1b] and by reduction of pivaloyl cation **3** with hydride ion.[1b, 14] Thus, we are not able to demonstrate by which pathway (a) and d) or b) and c)) the intermediate pivalic aldehyde (**4**) preferentially forms.

The observed alkane carbonylation provides new insight into the negative influence of CO on the alkane isomerization over SZ. The data obtained imply that suppression of the alkane isomerization by CO may result not only from the blocking of Lewis acid sites with CO[5] or from the reversible formation of **3** from **2**,[15, 16] but that carbon monoxide can also change the reaction route from isomerization towards carbonylation, thus contributing to the inhibiting effect on the alkane isomerization rate.

In conclusion, the present work represents the first small-alkane carbonylation on a SZ catalyst. The valuable chemical products (carboxylic acids and ketones) have been shown to be selectively produced over SZ at low temperature. This finding opens up a new possibility for the use of SZ-based catalysts for direct carbonylation of alkanes with CO.

Experimental Section

A sample of SZ of the low-temperature tetragonal phase with a surface area of 60 $m^2 g^{-1}$ and 9.9 wt % SO_3 content was prepared by a procedure described earlier.[17] The sample of SZ was calcined at 600 °C in air for 1 h and at 400 °C in vacuum (10^{-5} Torr) for 2 h. Equal amounts of isobutane (ca. 300 μmol g^{-1}) and CO (or isobutane, CO and H_2O) were frozen out on the SZ under vacuum at liquid nitrogen temperature. After sealing the SZ sample inside a glass tube (volume 0.2 cm^3) it was heated at 70 °C for 1 h; the pressure of CO was 8 atm under these conditions. Reaction products were analyzed in situ in the sealed glass tubes by ^{13}C CP/MAS NMR spectroscopy.

The ^{13}C CP/MAS NMR and ^{13}C high-resolution NMR spectra in $CDCl_3$ solution were recorded on a Bruker MSL-400 NMR spectrometer at room temperature (~23 °C). The conditions used for CP experiments are described in refs. [7c, 8], spinning rate was 3–10 kHz. A few thousand scans were collected for each spectrum. Chemical shifts (δ) of the organic compounds adsorbed and in $CDCl_3$ were measured with respect to TMS as external reference.

GC-MS analysis of the reaction products extracted with Et_2O from the SZ sample was made with VG 70–70 mass spectrometer. The fused silica capillary column of 35 m × 0.3 mm internal diameter with SE 30 as the active phase, forming a film of 0.3 μm thickness, was used for the separation of the organic products.

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Nucleoglycoconjugates: Design and Synthesis of a New Class of DNA–Carbohydrate Conjugates**

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Solid-phase DNA synthesis, based upon phosphorus(III) coupling chemistry,[1] has revolutionized the study of nucleic acids. In addition to providing synthetic DNA for molecular biological investigations, these solid-phase strategies have facilitated the synthesis of chemically modified oligonucleotides,[2] which have been used as probes of DNA structure and function[3] and as tools for mechanistic studies in nucleic acid biochemistry.[4] The synthesis of modified DNA also has fueled antisense therapeutics[5] and provided insight into the chemical evolution of nucleic acid structure.[6] The phosphoramidite method offers a general strategy for the conjugation of molecules such as biotin and fluorescein to DNA through

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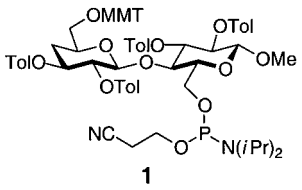
stable terminal or internal phosphodiester linkages.[7] Phosphoramidite chemistry also has been used to prepare phosphorylated biomolecules including sugars,[8] glycopeptides,[9] carbohydrate–nucleoside drug hybrids,[10] and phosphate-linked carbonucleotoid structures.[11]

Herein we describe an efficient phosphoramidite-based method for the synthesis of a new class of DNA–carbohydrate conjugates, which we term nucleoglycoconjugates. Our strategy involves the preparation of mono- and disaccharide phosphoramidite derivatives and conjugation of these derivatives, by solid-phase chemistry, to terminal or internal positions of oligonucleotides. Our approach was motivated by the biological relevance and potential applications of carbohydrate–DNA conjugates. Carbohydrate modifications of DNA nucleobases have been observed in biological systems,[12] and synthetic routes to these modified nucleotides have been demonstrated.[13] Conjugation of carbohydrates to the 5′-end of DNA has been achieved previously by the direct glycosylation of the 5′-hydroxy group of oligonucleotides,[14] and by the use of mannoside mono- and disaccharide phosphoramidite derivatives.[15] However, no general method exists for the linkage of carbohydrates to internal sites of nucleic acids through phosphodiester bonds. Such carbohydrate-modified DNA molecules would be hydrolytically and thermally stable, and would possess the attractive features of both carbohydrates and nucleic acids. As such, nucleoglycoconjugates may have utility in oligonucleotide therapeutic strategies, as substrates for the in vitro evolution of new catalytic RNA and DNA molecules, and in the engineering of DNA-based materials.

We designed and synthesized three carbohydrate phosphoramidite derivatives **1**–**3** as nucleoglycoconjugate building blocks. Phosphoramidite **1**, based upon methyl 4′-deoxylactoside (Me-dLac), was designed to provide access to DNA containing an internal disaccharide linked through its primary hydroxy groups to adjacent DNA sequences. This deoxysugar



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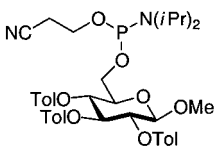

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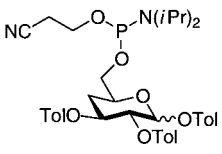

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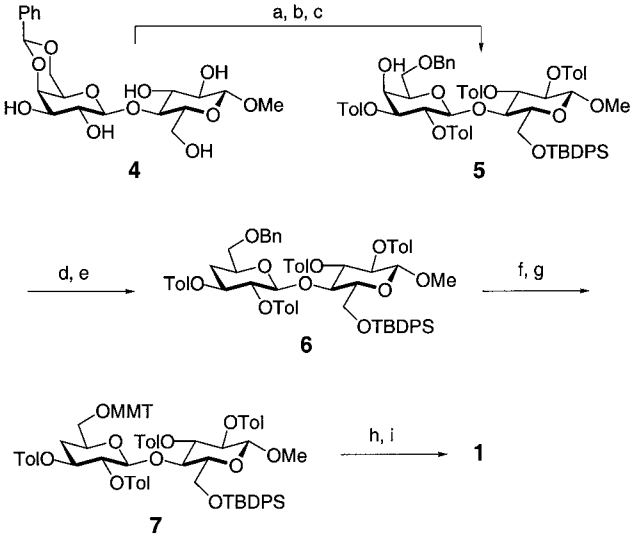
target was chosen over the fully hydroxylated lactose parent structure because, once incorporated into oligonucleotides, the 4′-hydroxy group in lactose could facilitate DNA strand scission at the carbohydrate site; intramolecular attack of the 4′-hydroxy group on the 6′-phosphate group would displace the upstream DNA sequence and form a cyclic phosphate. Thus, despite the increased synthetic challenge, the deoxysugar target **1** was chosen. Compounds **2** and **3** correspond to the reducing and nonreducing monosaccharide fragments, respectively, of the Me-dLac structure, and were designed to permit conjugation of these sugars to either the 5′- or 3′-end of DNA.

Phosphoramidite derivative **1** was prepared starting from the known[16] 4′,6′-benzylidene derivative of methyl-β-D-lactoside (**4**) (Scheme 1). Benzylidene **4** was silylated selectively at



Scheme 1. Synthetic route to **1**. a) TBDPSCl, pyr, DMAP (63%); b) *p*-MePhCOCl, pyr, DMAP (72%); c) $NaCNBH_3$, THF, HCl/Et_2O (94%); d) Im_2CS, CH_3CN (84%); e) nBu_3SnH, AIBN, $PhCH_3$ (80%); f) H_2, $Pd(OH)_2/C$, EtOAc, EtOH (76%); g) MMTCl, pyr, DMAP (86%); h) TBAF, THF, HOAc (73%); i) $[(iPr)_2N]_2POCH_2CH_2CN$, diisopropylammonium tetrazolide, CH_2Cl_2 (82%). AIBN = azobisisobutyronitrile, Im = imidazole, DMAP = 4,4-dimethylaminopyridine, pyr = pyridine.

the 6-position of the glucopyranose ring using *tert*-butyldiphenylsilyl chloride (TBDPSCl). The remaining secondary alcohols were protected as *p*-toluoyl (Tol) esters. The 4′-hydroxy group was revealed by regioselective ring opening of the 4′,6′-benzylidene derivative using sodium cyanoborohydride ($NaCNBH_3$) in HCl/diethyl ether[17] to give the desired 6′-*O*-benzyl derivative **5**. Selective removal of the 4′-hydroxy group of lactose was accomplished by a two-step Barton deoxygenation sequence to produce **6**.

With the Me-dLac skeleton in place, the synthesis of phosphoramidite **1** was completed by manipulation of protecting groups (Scheme 1). The 6′-*O*-benzyl group of **6** was removed by hydrogenolysis with $Pd(OH)_2/C$ (Degussa) catalyst. The monomethoxytrityl (MMT) protecting group was introduced at the 6′-position by reaction with MMTCl in pyridine to afford compound **7**.[18] Desilylation of **7** using tetrabutylammonium fluoride (TBAF) in THF[19] liberated the 6-hydroxy group, which subsequently was converted to the 2-cyanoethyl diisopropylphosphoramidite derivative using the conditions employed by Caruthers and co-workers.[20] Compound **1** was produced as a 1:1 mixture of diastereomers; ^{31}P NMR revealed two signals with chemical shifts of $\delta = 151.2$ and 151.4.

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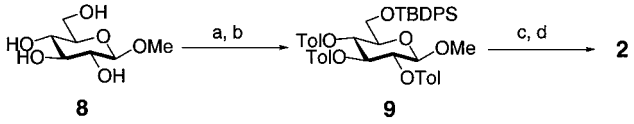
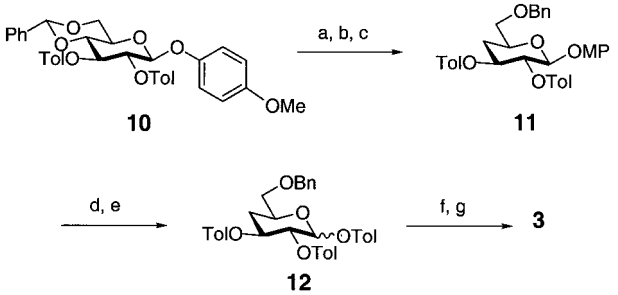
We next prepared phosphoramidite derivatives of the two monosaccharide components of the Me-dLac skeleton: the reducing-end methyl β-D-glucopyranoside (**2**) and the nonreducing-end 4-deoxy-D-glucose (**3**). Phosphoramidite **2** was synthesized in four steps starting from methyl β-D-glucopyranoside (**8**) (Scheme 2). Compound **9** was prepared by



Scheme 2. Synthetic route to **2**. a) TBDPSCl, imidazole, DMF (98%); b) *p*-MePhCOCl, pyr, DMAP (75%); c) CH_3COCl, MeOH, Et_2O (52%); d) $[(i\mathrm{Pr})_2\mathrm{N}]_2\mathrm{POCH_2CH_2CN}$, diisopropylammonium tetrazolide, CH_2Cl_2 (83%).

silylation of **8** at the 6-position with TBDPSCl in DMF[21] and subsequent esterification of the secondary hydroxy groups with *p*-toluoyl chloride. The TBDPS group of **9** was removed under acidic conditions, and the resulting primary hydroxy group was phosphitylated as described for **1** to produce monosaccharide phosphoramidite **2**.

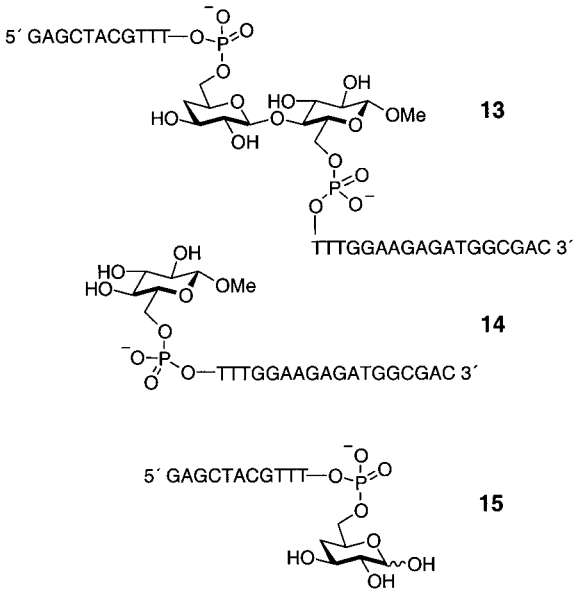
Phosphoramidite **3**, based upon 4-deoxyglucose, was synthesized in a manner similar to **1** (Scheme 3). The starting material, the known[22] *p*-methoxyphenyl glycoside **10**, was



Scheme 3. Synthetic route to **3**. a) $NaCNBH_3$, THF, $HCl\cdot Et_2O$ (69%); b) Im_2CS, CH_3CN, reflux (87%); c) $n\mathrm{Bu_3SnH}$, AIBN, $PhCH_3$ (95%); d) $Ce(NH_4)_2(NO_3)_6$, $PhCH_3$, CH_3CN, H_2O (91%); e) *p*-MePhCOCl, pyr, DMAP (96%); f) H_2, $Pd(OH)_2$/C cat., EtOAc, EtOH (85%); g) $[(i\mathrm{Pr})_2\mathrm{N}]_2\mathrm{POCH_2CH_2CN}$, diisopropylammonium tetrazolide, CH_2Cl_2 (80%).

prepared in five steps from glucose pentaacetate. Regioselective ring opening of the 4,6-benzylidene **10** with $NaCNBH_3$ under acidic conditions afforded the 6-*O*-benzyl sugar. Barton deoxygenation of the revealed 4-hydroxy group yielded the 4-deoxyglucose derivative **11**. The anomeric center of **11** was deprotected by treatment with ceric ammonium nitrate to produce an equimolar mixture of three compounds: the desired α- and β-1-hydroxy anomers, and the α-anomer of the 1-*O*-*p*-toluoyl ester resulting from ester migration from the 2-position. This mixture was converted to the tri-*p*-toluoyl ester **12** by reaction with *p*-toluoyl chloride.[23] The synthesis of amidite **3** was completed by debenzylation of **12** and phosphitylation at the 6-position.

Phosphoramidites **1**–**3** coupled with high yields to DNA oligonucleotides to produce DNA–carbohydrate chimera containing internally embedded (**13**) and terminally conjugated (**14** and **15**) carbohydrates.[24] Oligonucleotides **13** and



14 were prepared by using solid-phase DNA synthesis in the standard 3′-to-5′ direction. Conjugate **15**, which required coupling of phosphoramidite derivative **3** to the 3′-end of the oligonucleotide, was synthesized in the 5′-to-3′ direction using commercially available nucleoside 3′-*O*-DMT-5′-*O*-phosphoramidites.[25] Nucleoglycoconjugates **13**–**15** were cleaved from the solid support and deprotected by treatment with concentrated aqueous ammonia. Subsequently, the modified oligonucleotides were purified by denaturing polyacrylamide gel electrophoresis and their identities were verified by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometric analysis.

These DNA–carbohydrate conjugates behave like standard DNA oligonucleotides and are stable under conditions used in molecular biology experiments. Oligonucleotides **13**–**15** migrate normally in polyacrylamide gels, with each modified sugar-phosphate behaving like a nucleoside phosphate. No cleavage of the β-1,4-linkage of the deoxylactose disaccharide in oligonucleotide **13** was observed after 72 h over the pH range of 5.0–9.0 and the temperature range of 25–50 °C, as expected by recent measurements of glycoside stability.[26] Thus, these carbohydrate-modified DNA molecules should be stable in buffered aqueous solutions and under thermal cycling conditions employed in the polymerase chain reaction (PCR).

The nucleoglycoconjugates form stable duplexes with complementary DNA oligonucleotides as demonstrated by UV-thermal denaturation analysis. Construct **13** was hybridized with a complementary 29mer DNA such that the Me-dLac moiety was unpaired and all of the flanking nucleotides were paired in a Watson–Crick fashion. The thermal stability of this duplex was compared to that of duplexes in which the Me-dLac residue was either replaced by an unpaired thymidylate or deleted to allow perfect complementarity. The fully complementary duplex had a T_m value of 74 °C. It was destabilized slightly by the presence of the unpaired thymidylate ($T_m = 70$ °C), and slightly more by the presence of the Me-dLac residue ($T_m = 66$ °C). A second set of

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melting studies was performed by employing a 20mer rather than 29mer complementary DNA. These gave similar results, but with a T_m value that was 10–15 °C lower in all cases (see Supporting Information). Thus, despite the slight disruption of duplex structure, inclusion of the Me-dLac moiety within a DNA oligonucleotide does not significantly impair its ability to hybridize to a complementary template.

The nucleoglycoconjugates are substrates for common DNA-manipulating enzymes such as kinases and polymerases and can be utilized as primers in template-directed extension reactions. Nucleoglycoconjugates with a free 5′-hydroxy group (**13** and **15**) are efficiently labeled with radioactive [^{32}P]-phosphate by T4 polynucleotide kinase. Sugar-modified oligonucleotides with a free 3′-hydroxy group (**13** and **14**) are effective primers for enzymatic extension reactions,[27] permitting the construction of large DNA molecules with specific internal (with **13**) or terminal (with **14**) carbohydrate modifications. PCR amplification of an 85-nucleotide insert also was possible using either **13** or **14** in concert with a second DNA primer and a thermostable DNA polymerase.

In summary, we have described the design, construction, and preliminary analysis of a new class of carbohydrate–DNA conjugates, which we term nucleoglycoconjugates. These DNA–saccharide hybrids are efficiently synthesized, chemically stable, and serve as substrates for standard DNA-modifying enzymes. We believe that these conjugates will provide a platform for the development of a new form of hybrid biomaterials. These materials could, in principle, be assembled from carbohydrate-modified DNA primers by the enzymes of carbohydrate and nucleic acid metabolism. They would possess the unique coding properties of nucleic acids and the recognition abilities of carbohydrates. In view of the prevalence of cell surface proteins (lectins) that recognize carbohydrate epitopes, these DNA-glycoconjugates may also be useful for increasing the specificity and efficiency of cellular uptake of therapeutic oligonucleotides.

Experimental Section

Oligonucleotides were synthesized using a Pharmacia Gene Assembler Special employing standard methods. Sugar phosphoramidites **1**–**3** were dissolved in absolute CH_3CN to a concentration of 0.1M. Coupling yields for **1**–**3** were calculated by quantitation of DMT and MMT cation effluents. Syntheses employing phosphoramidites **1** and **2** were conducted in the standard 3′-to-5′ direction using 3′-solid-supported nucleosides and nucleoside phosphoramidites containing labile base protecting groups (Glen Research, "Ultramild"). Syntheses employing phosphoramidite **3** were conducted in the reverse 5′-to-3′ direction using 5′-solid-supported nucleosides and "reverse" 3′-*O*-DMT-5′-*O*-*β*-cyanoethyl phosphoramidites (Glen Research). Oligonucleotides were cleaved from the support and deprotected by using concentrated aqueous ammonia (normal synthesis: 8 h, 37 °C; reverse synthesis: 15 h, 55 °C), and were purified by denaturing polyacrylamide gel electrophoresis.

MALDI-TOF mass spectrometric analysis of **13**–**15** was performed on a PerSeptive Biosystems Voyager-STR mass spectrometer. The matrix was 2,4,6-trihydroxyacetophenone for **13** and 3-hydroxypicolinic acid for **14** and **15**. Conjugate **13**: *m*/*z*: calcd 9414.9; found $M_{av} = 9409$; conjugate **14**: *m*/*z*: calcd 5858.6; found $M_{av} = 5859$; conjugate **15**: *m*/*z*: calcd 3572.3; found $M_{av} = 3572$.

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[18] The standard dimethoxytrityl (DMT) protecting group used in DNA synthesis proved too labile at the 6-position of the disaccharide. Thus, the more stable MMT group was employed.

[19] The reaction must be buffered to pH 7 with acetic acid to prevent ester migration to the revealed primary 6-hydroxy group.

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[23] Product **12** was obtained as a 4:1 mixture of α:β-anomers.

[24] Compound **1** coupled to DNA in >95% yield. Extension of DNA after removal of the MMT group proceeded with a coupling yield of >70%. Phosphoramidites **2** and **3** coupled to the termini of DNA in >90% yield to provide **14** and **15**, respectively.

[25] Reverse syntheses using commercially available nucleoside 5′-phosphoramidite derivatives proceeded with stepwise coupling yields of >90%.

[26] R. Wolfenden, X. Lu, G. Young, *J. Am. Chem. Soc.* **1998**, *120*, 6814–6815.

[27] Polymerases that were tested included three reverse transcriptases (AMV, MMLV, and SuperScript II) and three thermostable DNA polymerases (*Taq*, *Pfu*, and *Tth*).