ORGANIC LETTERS

2000
Vol. 2, No. 12
1693−1696

Synthesis of Aminoglycoside-Modified Oligonucleotides

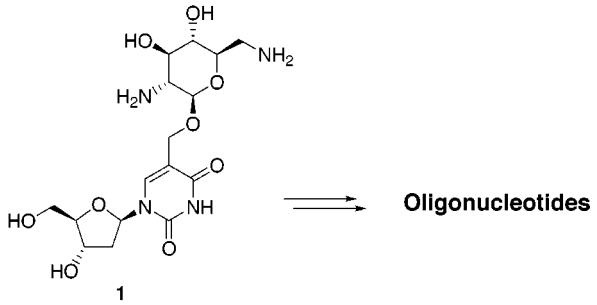
Rolf Tona, Reto Bertolini, and JÜrg Hunziker*

Department of Chemistry and Biochemistry, University of Bern, Freiestrasse 3, 3012 Bern, Switzerland

juerg.hunziker@ioc.unibe.ch

Received March 13, 2000

ABSTRACT

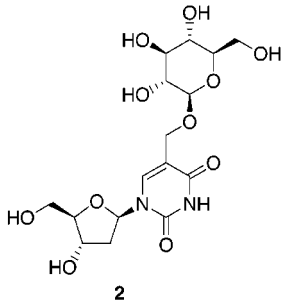


1

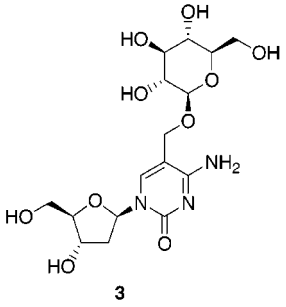
To study the structural requirements of aminoglycoside binding to nucleic acids, compound 1—an analogue of the naturally occurring nucleoside J—was synthesized. When incorporated into oligodeoxynucleotides, 1 leads to thermal stabilization of the resulting duplexes. The increase in pairing affinity is stronger with complementary RNA than with DNA.

Structural modifications to DNA bases, apart from degradative damage, are rarely found in nature.[1] This contrasts the vast abundance of altered nucleobases found in RNA.[2] The most prominent DNA base modification in eukaryotes is cytosine methylation for the silencing of genes on homologous chromosomes.[3] Less conservative changes, such as the glycosylated nucleosides **2** or **3**, are found in the DNA of certain pathogens. *Escherichia coli* bacteriophages of the T-even series exclusively contain 5-hydroxymethyldeoxycytidine instead of deoxycytidine in their DNA.[1] About $^3/_4$ of these 5-hydroxymethylcytosine residues are β-D-glucosylated. A similar modification is found in the genome of *Trypanosoma brucei*—a protozoan causing African sleeping sickness—as well as in phages. Here, the glucose is linked to 5-hydroxymethyldeoxyuridine.[4] This unusual nucleoside replaces thymidines within the long telomeric repeats in *T. brucei*.[4b]

The glucose residues of DNA containing **2** reside within the major groove[5] and render the DNA inaccessible for



2



3

enzymes[6] which helps the pathogen to escape degradation by host restriction enzymes. In the X-ray crystal structure[5] of a short self-complementary duplex, the glucose is arranged such that the primary hydroxy group is pointing toward the sugar−phosphate backbone of the complementary strand.

(1) Kornberg, A.; Baker, T. A. *DNA Replication*; W. H. Freeman Co.: New York, 1992.
(2) http://medlib.med.utah.edu/RNAmods/. Rozenski, J.; Crain, P. F.; McCloskey, J. A. *Nucleic Acids Res.* **1999**, *27*, 196.
(3) Robertson, K. D.; Jones, P. A. *Carcinogenesis* **2000**, *21*, 461.
(4) This modification is also referred to as nucleoside **J**. (a) Gommers-Ampt, J. H.; van Leeuwen, F.; de Beer, A. L. J.; Vliegenthart, J. F. G.; Dizdaroglu, M.; Kowalak, J. A.; Crain, P. F.; Borst, P. *Cell* **1993**, *75*, 1129. (b) van Leeuwen, F.; Wijsman, E. R.; Kuyl-Yeheskiely, E.; van der Marel, G. A.; van Boom, J. H.; Borst, P. *Nucleic Acids Res.* **1996**, *24*, 2476.
(5) Gao, Y.; Robinson, H.; Wijsman, E. R.; van der Marel, G. A.; van Boom, J. H.; Wang, A. H.-J. *J. Am. Chem. Soc.* **1997**, *119*, 1496.
(6) van Leeuwen, F.; de Kort, M.; van der Marel, G. A.; van Boom, J. H.; Borst, P. *Anal. Biochem.* **1998**, *258*, 223.

10.1021/ol005797d CCC: $19.00 © 2000 American Chemical Society
Published on Web 05/16/2000

The secondary hydroxy group in the 2-position is located near a phosphodiester group on the strand containing **2** (Figure 1).

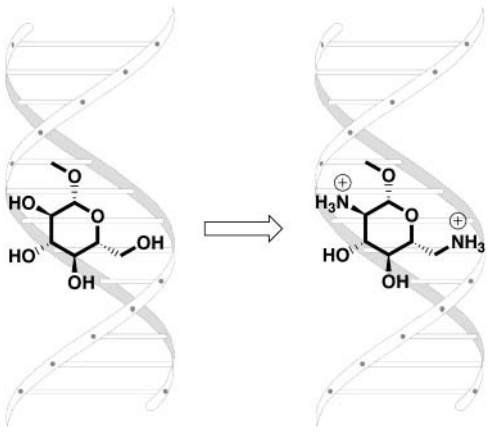
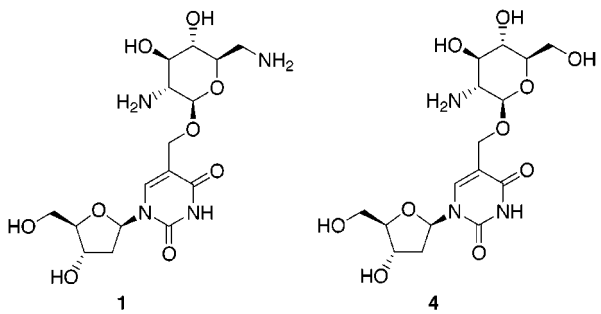


Figure 1. The glucose moiety of nucleoside analogue **2** is located within the major groove of double stranded DNA.[3] By replacing the hydroxy groups at the 2- and 6-position of the glucose by ammonium groups, the two oligonucleotide strands could be clamped together through electrostatic interactions.

Incorporating **2** into oligonucleotide duplexes reduces their thermal stability. The replacement of ordered water in the major groove by hydrogen-bonding contacts between the glucose and nucleobases leads to a favorable entropic contribution which, however, cannot fully compensate for the smaller enthalpy of duplex formation.[7] This seems to be a tolerable tradeoff for the pathogens mentioned, to protect their genome from degradation.

To improve the thermal stability and yet retain the desired protection from nucleases, we sought to synthesize oligodeoxynucleotides (ODNs) containing nucleoside analogues **1** and **4**. The amino groups, positively charged at neutral pH, should improve binding to a complementary strand by electrostatic interactions (Figure 1). However, **4** when built into ODNs behaves in a similar manner to that of **2**: a decrease in melting temperature is observed.[8] Most likely, the 2-ammonium group of **4** is located near a phosphodiester group of the strand it is attached to and thus does not affect duplex formation. Here, we report the synthesis of ODNs containing **1** which show the anticipated behavior.



1 **4**

The synthesis of protected 5-hydroxymethyl-2′-deoxyuridine glycosyl acceptor **12** has already been described.[8,9] However, the rather low yields and high costs for starting materials in these syntheses led us to explore a different route. 5-Hydroxymethyluracil (**6**), obtained in one step from uracil,[10] was coupled with tetraacetylribose **5** to give 5-hydroxymethyluridine (**7**) in 79% yield (Scheme 1).[11] To differentiate the two primary hydroxy groups, **7** was reacted with dimethoxytrityl chloride. After removal of the acetyl groups, the ribose was selectively protected at the 3′- and 5′-hydroxyls with TIPDS to give **10**. Barton−McCombie reduction of the 2′-hydroxy group gave deoxynucleoside **11** in high yield. The DMT protecting group could then easily be removed to give the glycosyl acceptor **12** in 43% overall yield from 5-hydroxymethyluracil (**6**).

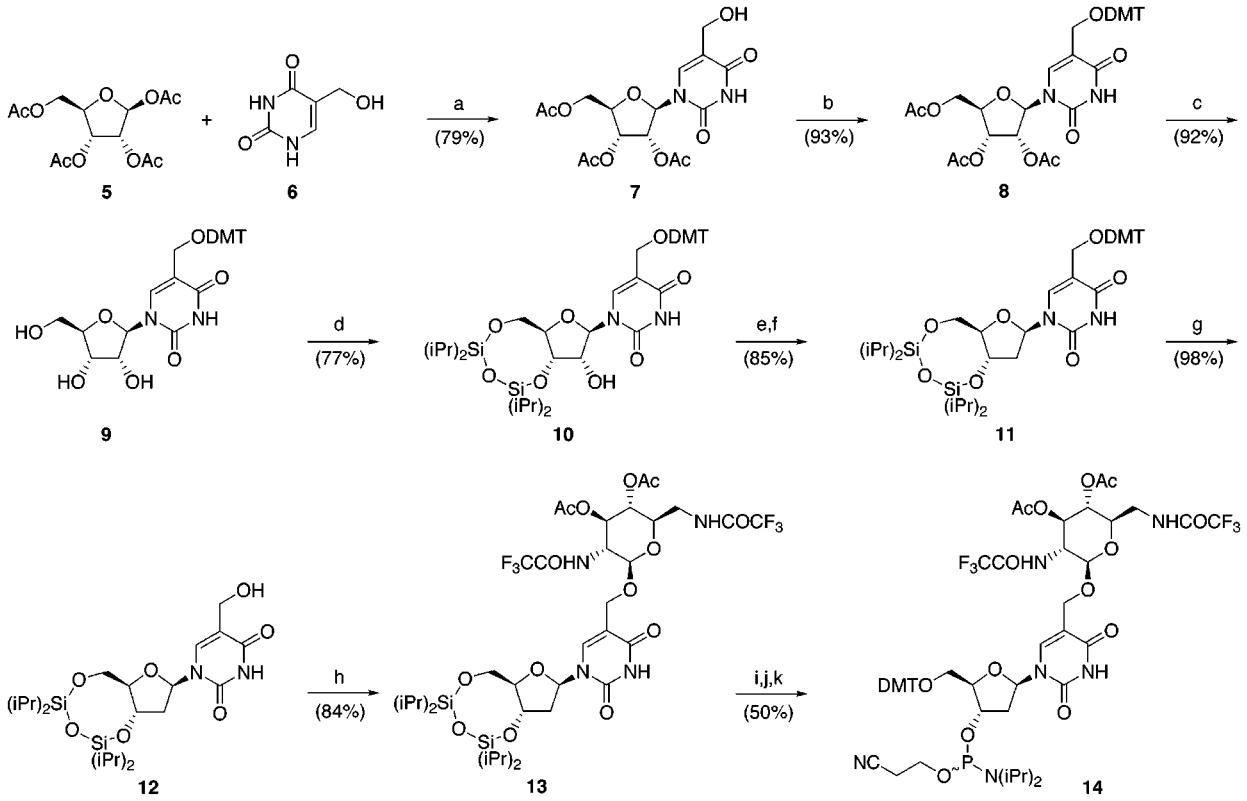
Since we felt that the synthesis of a suitable 2,6-diaminoglucose donor from a commercially available monosaccharide would require eight to ten steps, we chose instead to start from a natural product. The aminoglycoside neomycin B (**15**) contains a 2,6-diamino-2,6-dideoxy-D-glucose subunit and is readily available. The glycosidic linkage between the ribose residue and the aminocyclohexitol 2-deoxystreptamine (2-DOS) can be cleaved selectively under acidic conditions to give neamine hydrochloride **16**.[12] The amino functions in neamine were first protected as trifluoroacetamides and then the hydroxy groups were esterified with acetic anhydride to give **17** in 67% yield (Scheme 2). This neamine derivative could be hydrolyzed with HBr in acetic acid to give a mixture of the desired glycosyl donor **18** and the 2-DOS byproduct **19**.[13]

The coupling of glycosyl bromide **18** and uridine derivative **12** proceeded smoothly under mercury salt activation to give diaminoglucosylated nucleoside **13** in 84% yield. Removal of the silyl protecting groups and standard manipulations[14] then provided phosphoramidite building block **14**. With this, oligonucleotides **22** and **26** (Table 1) were prepared on a 1.3 μmol scale by automated solid-phase synthesis.[15,16]

Replacing one thymidine residue with 2,6-diamino-β-D-glucose-modified **1** at a central position in the reference

(7) Bertolini, R.; Hunziker, J. Unpublished data.
(8) Hunziker, J. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 201.
(9) de Kort, M.; Ebrahimi, E.; Wijsman, E. R.; van der Marel, G. A.; van Boom, J. H. *Eur. J. Org. Chem.* **1999**, 2337.
(10) Cline, R. E.; Fink, R. M.; Fink, K. *J. Am. Chem. Soc.* **1959**, *81*, 2521.
(11) All new compounds were fully characterized by UV, IR, ^{1}H and ^{13}C NMR, and mass spectroscopy.
(12) Park, W. K. C.; Auer, M.; Jaksche, H.; Wong, C.-H. *J. Am. Chem. Soc.* **1996**, *118*, 10150.
(13) The mixture of these two compounds could only be separated by silica gel chromatography with substantial loss of **18**. Nevertheless, the ease of preparation of **18** outweighted the poor yields of this strategy.
(14) Conolly, B. A. In *Oligonucletides and Analogues*; Eckstein, F., Ed.; Oxford University Press: Oxford, 1991; pp 155−183.
(15) Oligonucleotides **20**−**28** were prepared on a Pharmacia Gene Assembler Plus using the standard protocol. The coupling time for **14** was changed from 2 to 6 min. Average coupling yields for aminoglycoside-modified nucleosides (>98%) were in line with unmodified phosphoramidites. The oligonucleotides were purified by reverse-phase HPLC followed by ion-exchange HPLC. The integrity of the isolated oligonucleotides **20**−**28** was subsequently confirmed by MALDI-ToF mass spectrometry: *m*/*z* (monoanion, H^{+}-form) **22**, calcd 3155.0, found 3154.9; **26**, calcd 3363.3, found 3364.4. Matrix conditions as described in the following: Pieles, U.; Zürcher, W.; Schär, M.; Moser, H. E. *Nucleic Acids Res.* **1993**, *21*, 3191.

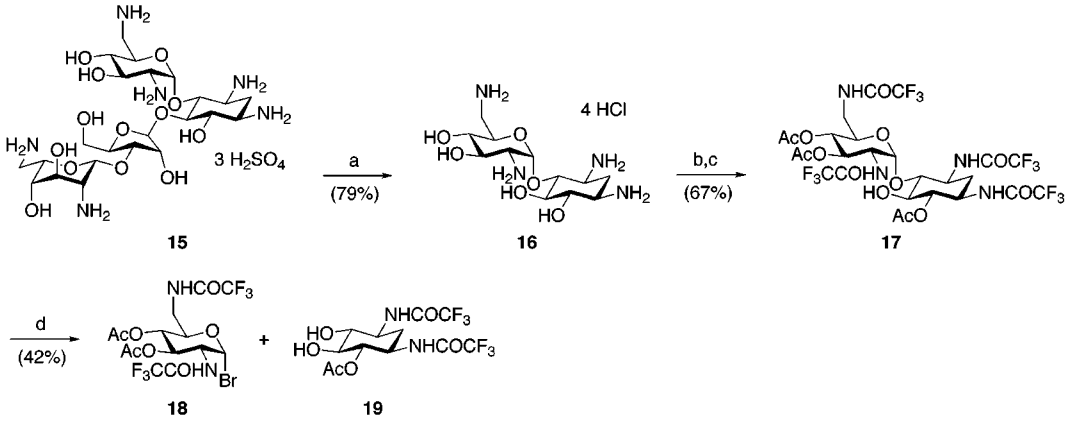
Scheme 1. Synthesis of Diaminoglucose-Modified 5-Hydroxymethyl-2′-deoxyuridine Phosphoramidite **14**[a]



[a] (a) BSA, TMSOTf, CH_3CN, 75 °C, 2 h; (b) DMTCl, DMAP, pyridine, rt, 3 h; (c) Na_2CO_3, MeOH, rt, 17 h; (d) TIPDSCl, pyridine, rt, 22 h; (e) thiocarbonyldiimidazole, CH_3CN, rt, 21 h; (f) Bu_3SnH, AIBN, toluene, 80 °C, 50 min; (g) $Cl_2HCCOOH$, pyrrole, CH_2Cl_2, rt, 15 min; (h) **18**, $Hg(CN)_2$, molecular sieves 3 Å, rt, 16 h; (i) TBAF, AcOH, THF, rt, 30 min; (j) DMTCl, pyridine, rt, 4 h; (k) $(^iPr_2N)(NCCH_2CH_2O)PCl$, $^iPr_2NEt$, THF, rt, 1 h. DMT = 4,4′-dimethoxytrityl.

duplex d(T_{10})•d(A_{10}) leads to a decrease in thermal stability (**22·21** ΔT_m/mod = −5 °C, Table 1). In a mixed sequence context (**26·24**), however, an increase of ΔT_m/mod = +1.5 °C results. The different effects of **1** in these pairing systems might be due to the particular structure of oligo(dA)·oligo(dT) duplexes which have a wide and flat major groove.[17] The same trend is seen for the analogous glucose modification (**23·21** vs **27·25**), although a destabilization is observed in both cases. With a complementary RNA strand, the stabilizing effect of nucleoside **1** is even more pronounced (**26·28** ΔT_m/mod = +3.5 °C). Not surprisingly, the increase in affinity due to the diaminoglucose modification is stronger

Scheme 2. Preparation of 2,6-Diaminoglucose Donor **18**[a]



[a] (a) Aqueous HCl, MeOH, reflux, 18 h; (b) $F_3CCOOEt$, MeOH, Et_3N, rt, 16 h; (c) Ac_2O, pyridine, rt, 2 h; (d) HBr, AcOH, rt, 16 h.

Table 1. Melting Temperatures (T_m) of Oligonucleotides Containing **1**[a]

oligonucleotide duplex	1 M NaCl		0.15 M NaCl	
	T_m [°C][b]	ΔT_m/mod [°C]	T_m [°C][b]	ΔT_m/mod [°C]
d(T_{10})·d(A_{10}) (**20·21**)	33		23	
d(TTTT**1**TTTTT)·d(A_{10}) (**22·21**)	28	−5	20	−3
d(TTTT**2**TTTTT)·d(A_{10}) (**23·21**)	29	−4	nd	nd
d(CTGAATCGAC)·d(GTCGATTCAG) (**24·25**)	51		44	
d(C**1**GAA**1**CGAC)·d(GTCGATTCAG) (**26·25**)	54	+1.5	49	+2.5
d(C**2**GAA**2**CGAC)·d(GTCGATTCAG) (**27·25**)	49	−1	nd	nd
d(CTGAATCGAC)·r(GUCGAUUCAG) (**24·28**)	41		37	
d(C**1**GAA**1**CGAC)·r(GUCGAUUCAG) (**26·28**)	48	+3.5	nd	nd

[a] Oligonucleotide concentration: 4 μM in 10 mM NaH_2PO_4, pH 7.0, and the indicated amount of NaCl. [b] Absorbance detected at 260 nm. Melting temperatures represent the mean value of three melting curves. Heating rate: 0.5 °C/min.

at low ionic strength,[18] which indicates that the amino groups are indeed protonated at pH 7.0.

Aminoglycosides show a marked preference for RNA.[19] The same is seen here for ODNs containing the diaminoglucose-modified nucleoside **1**. In DNA−RNA heteroduplexes and RNA duplexes, the major groove is deep and narrow.[17] The primary ammonium group of **1** might thus be located closer to the phosphodiester groups of the opposing strand if the duplex adopts a more RNA-like conformation. This electrostatic attraction might actually pull the backbones together and alter duplex conformation. In fact, the CD spectrum of the DNA−DNA duplex **26·24** differs more from its unmodified counterpart than the corresponding DNA−RNA duplex **26·28** (data not shown).

In summary, we have successfully synthesized ODNs containing 2,6-amino-β-D-glucose-modified nucleoside **1**. These display increased affinity toward DNA and even more so for RNA complementary strands. More detailed structural and synthetic characterization of this pairing system will be reported in due course.

Acknowledgment. This work was supported by a research grant from the Swiss National Science Foundation (Grant 20-53692.98).

OL005797D

(16) The HPLC trace of crude ODNs **22** and **26** showed that a substantial amount of byproducts was present—presumably not fully deprotected ODNs which arise from the capping step of the oligo synthesis. Acetic anhydride could acylate the trifluoroacetamide functions of the diaminoglucose residues and these acetamides might not be fully deprotected under standard deprotection conditions (concentrated ammonia at 55 °C for 16 h). MALDI-ToF MS analysis of these byproducts was inconclusive.

(17) Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984.

(18) 5-Aminoalkyl- and alkynyl-modified pyrimidine oligonucleotides also bind stronger to complementary DNA under low salt conditions and neutral pH than their unmodified counterparts. (a) Hashimoto, H.; Nelson, M. G.; Switzer, C. *J. Am. Chem. Soc.* **1993**, *115*, 7128. (b) Heystek, L. E.; Zhou, H.; Dande, P.; Gold, B. *J. Am. Chem. Soc.* **1998**, *120*, 12165.

(19) Tor, Y. *Angew. Chem., Intl. Ed.* **1999**, *38*, 1579.