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SYNTHESIS OF 5-(2-AMINO-2-DEOXY-β-D-GLUCOPYRANOSYLOXYMETHYL)-2'-DEOXYURIDINE AND ITS INCORPORATION INTO OLIGOTHYMIDYLATES

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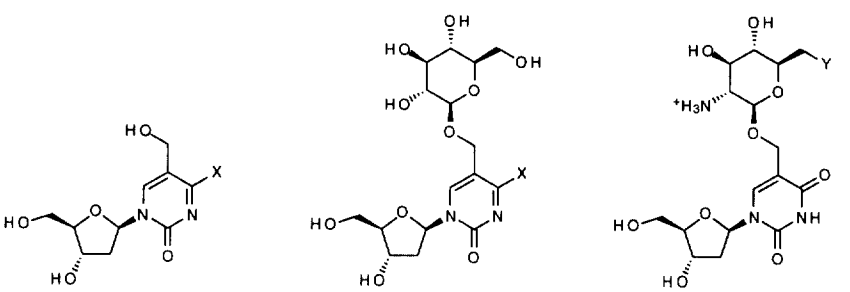
Abstract: The protected 5-(2-amino-2-deoxy-β-D-glucopyranosyloxymethyl)-2'-deoxyuridine phosphoramidite **15** has been prepared from uridine in 12 steps. When incorporated into oligodeoxyribonucleotides the novel nucleoside analog **5** leads to decreased binding affinity. This decrease is larger with a complementary RNA-strand than with a DNA complement.

Among the various non-canonical nucleosides isolated from nucleic acids the majority is found in RNA, only a few unusual components of DNA have been reported.[1] 5-Hydroxymethyl-2'-deoxyuridine (HMdU, **1**), is found in the genomic material of bacteriophages.[1] HMdU has also been identified as degradation product of thymidine when DNA is exposed to hydroxyl radicals generated by chemical agents or ionizing radiation.[2] Likewise, 5-hydroxymethyl-2'-deoxycytidine (HMdC, **2**) is found in the DNA of T-even phages which infect *E. coli*.[1] Here, deoxycytidine is completely replaced by **2**. In addition, both these hydroxymethylated pyrimidine nucleosides are often glycosylated. D-Glucose is linked to the nucleoside via the 5-hydroxymethyl group to form **3** and **4**.[1,3] The glucose moieties cover the major groove of the double helix and thus protect the pathogen's genome from degradation by host endonucleases expressed in response to the infection.[1] These modifications also have a profound effect on DNA structure.[4-6] T2 phage DNA displays a conformation reminiscent of D-DNA with a deep, narrow minor groove and a shallow, wide major groove.

Figure 1



1: X = OH
2: X = NH_2

3: X = OH
4: X = NH_2

5: Y = OH
6: Y = NH_3^+

The synthesis of oligonucleotides containing **1** and **3** have previously been reported.[6-8] However, data describing the effect of these modifications on the pairing properties are not available. This lead us to investigate

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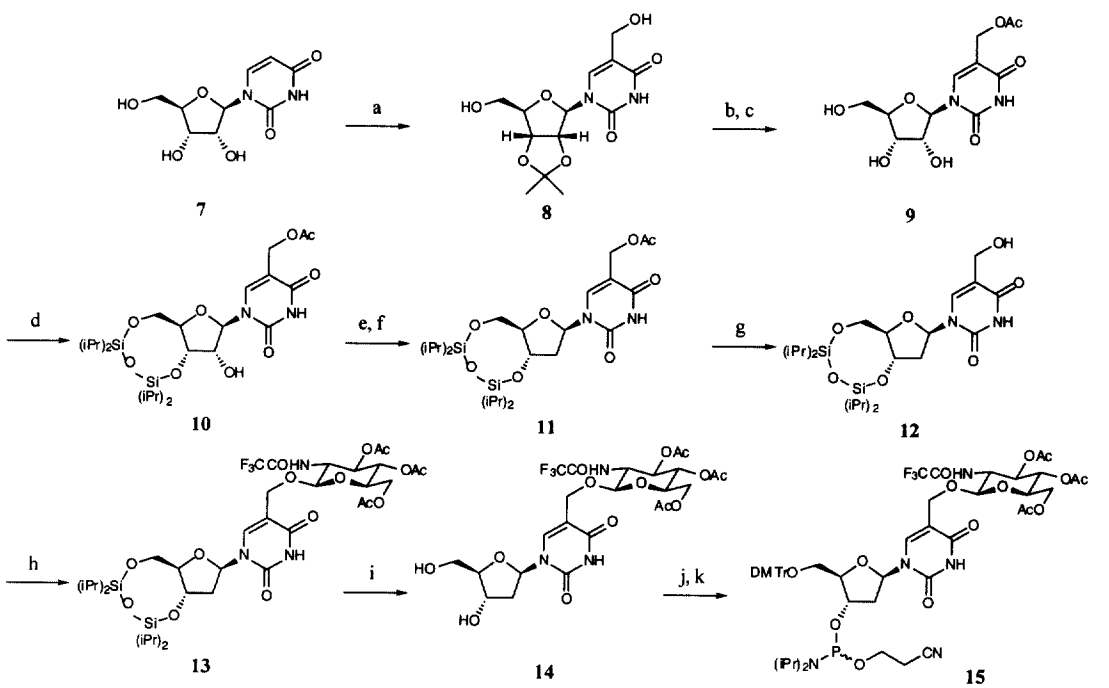
various glycoside modified nucleobases with respect to their properties in antisense oligonucleotides. Glucose moieties which can easily be accomodated within the major groove[6] presumably displace part of the hydration shell and may thus lead to enhanced thermal stability due to positive entropic contributions in the pairing process. Oligonucleotides containing **3** or **4** are also less prone to nuclease degradation.[1] Additionally, the introduction of ammonium groups such as in **5** or **6** may enhance binding through favourable electrostatic interactions. Here, we report on initial results regarding the synthesis and incorporation of 5-(2-amino-2-deoxy-β-D-glucopyranosyloxymethyl)-2'-deoxyuridine (**5**) into oligodeoxyribonucleotides.

Scheme 1



a) Ref. 11, 12; b) CF_3COOH, H_2O, 60°C, 1h, 89%; c) AcOH, cat. CF_3COOH, reflux, 1h, 75%; d) TIPDSCl, imidazole, DMF, r.t., 12h, 66%; e) H_7C_7OCSCl, DMAP, CH_3CN, r.t., 15h; f) Bu_3SnH, AIBN, toluene, 80°C, 3h, 89% (2 steps); g) ^tBuOK, CH_3OH, r.t., 5h, 87%; h) 2-Deoxy-3,4,6-tri-O-acetyl-2-trifluoracetamido-α-D-glucopyranosyl bromide, $HgCN_2$, CH_3CN, r.t., 16h, 85%; i) TBAF, AcOH, THF, r.t., 30min, 86%; j) DMTrCl, pyridine, r.t., 4h, 82%; k) $(iPr_2N)(OCH_2CH_2CN)PCl$, iPr_2NEt, THF, r.t., 1h, 88%

The synthesis of the sugar protected hydroxymethyl-deoxyuridine derivative **12** has been described before.[9] However, the dismal yield[10] in the hydroxymethylation of the rather expensive 2'-deoxyuridine prompted us to explore a route starting from uridine. Our synthesis of a 2-amino-2-deoxy-β-D-glucose modified HMdU derivative suitable for oligonucleotide synthesis is outlined in *scheme 1*. Uridine (**7**) is converted to 5-hydroxymethyl-2',3'-isopropylidene-uridine (**8**) in 89% yield following slightly modified literature procedures.[11,12] The isopropylidene protecting group is removed by mild acid treatment to give **9** in 89% yield. The 5-hydroxymethyl group is then selectively esterified under the conditions described for the synthesis of 5-acetoxymethyl-2'-deoxyuridine.[8] The introduction of the TIPDS protecting was somewhat cumbersome and

resulted in varying yields. The reasons for this problem remained elusive as numerous attempts to improve the reaction conditions did not lead to higher yields. The two step radical reduction of the 2'-hydroxy group in **10** then proceeded smoothly and after deprotection of the 5-hydroxymethyl group **12** was obtained in 30% overall yield from uridine as compared to 9% from 2'-deoxyuridine in the previously reported synthesis.[9] Coupling of **12** with 2-deoxy-3,4,6-tri-O-acetyl-2-trifluoracetamido-α-D-glucopyranosyl bromide[13] under Hg^{2+}-catalysis[14] gave **13** in 85% yield. This result contrasts attempts to couple **12** with tetraacetyl-glucopyranosyl bromide, which mostly lead to epimerization of the nucleosidic bond. After desilylation nucleoside **14** was converted to the phosphoramidite-building block **15** according to standard procedures.[15]

With the suitable building blocks at hand, oligonucleotides **16 - 20** (*table 1*) were prepared on a 1.3 μmol scale by automated solid-phase synthesis.[16] 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 **17 - 20** was subsequently confirmed by MALDI-ToF mass spectrometry.[17]

Replacing one thymidine residue with 2-amino-2-deoxy-β-D-glucose modified HMdU **5** at a central position in the reference duplex $d(T_{10})$•$d(A_{10})$ leads to a decrease in thermal stability (**17**•$d(A_{10})$, ΔTm = -4°C). A similar decrease is observed in the corresponding DNA-RNA heteroduplex **17**•poly(A) (ΔTm = -6°C). This destabilization is even more pronounced with two consecutive modifications (**18**•$d(A_{10})$, ΔTm = -6°C, **18**•poly(A), ΔTm = -6.5°C). As expected substitution of **5** for thymidine towards the end of the duplex, as in sequence **19**, leads to a more moderate decrease in melting temperature as compared to the alternatingly substituted **20**. Changing the pH of the buffer solution to 5.0 does not alter the melting temperatures within ± 1°C (data not shown). If the ionic strength is lowered (150mM NaCl, pH 7.0) melting temperatures decrease by approximately 10°C. However, ΔTm per modification is the same as under high salt conditions (data not shown).

Table1

	oligonucleotide sequence	T_m [°C]		ΔT_m/modification	
		complementary oligonucleotide			
		$d(A_{10})$	poly(A)	$d(A_{10})$	poly(A)
16	$d(T_{10})$	33	37	—	—
17	d(T-T-T-T-**5**-T-T-T-T-T)	29	31	-4	-6
18	d(T-T-T-T-**5**-**5**-T-T-T-T)	21	24	-6	-6.5
19	d(T-**5**-T-T-T-T-T-T-**5**-T)	30	32	-1.5	-2.5
20	d(**5**-T-**5**-T-**5**-T-**5**-T-**5**-T)	15	11	-3.6	-5.2

Concentration of oligonucleotides (duplex) = 4μM in 10mM NaH_2PO_4, 1M NaCl, pH 7.0. Absorbance detected at 260nm. Heating rate: 0.5°C/min (heating-cooling-heating cycle). The accuracy of melting points is estimated to be ± 0.5°C. Melting points represent the mean value of three melting curves.

Previously, it was shown that 5-ω-aminohexyl modified pyrimidine oligonucleotides bind moderately stronger to complementary DNA under low salt conditions and neutral pH than their unmodified counterparts.[18] In contrast to these results, oligonucleotides **17 - 20** uniformly bind weaker to $d(A_{10})$ and poly(A) than $d(T_{10})$. A reason for this behaviour could be that the 2-amino group of the glycoside portion in **5** is not protonated at pH 7.0.[19] However, lowering the pH to 5.0 leads to essentially identical melting

temperatures. An explanation for the decreased thermal stability then might be that the attraction between ammonium and phosphodiester groups induces a conformation which might not be optimal for hydrogen bonding of the corresponding hydroxymethyluracil–adenine base pair. The fact that the destabilization is more pronounced in the case of an RNA complement indicates that the glycoside is still located in the major groove since DNA–DNA duplexes are known to have a wider major groove than DNA–RNA heteroduplexes[5] and should thus better be able to accomodate the glycoside.

In conclusion, we have successfully synthesized oligonucleotides containing 2-amino-2-deoxy-β-D-glucose modified HMdU **5**. These display decreased affinity towards DNA as well as RNA complementary strands. The synthesis of oligonucleotides containing 2,6-diamino-2,6-dideoxy-β-D-glucose modified HMdU **6** is currently under way. In these the 6-amino group might be located near a posphodiester group on the opposite strand, effectively clamping the two strands together.

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16. Oligonucleotides **16 - 20** were synthesized on a Pharmacia Gene Assembler Plus using the standard protocol, only changing the duration of the coupling step with **15** to 6min. Deprotection was carried out in conc. ammonia at 55°C for 5h.
17. m/z (monoanion, H^+-form): **17**: calc. 3156.2, found 3154.7; **18**: calc. 3333.3, found 3333.0; **19**: calc. 3333.3, found 3333.0; **20**: calc. 3864.8, found 3862.7. Matrix conditions as described in: Pieles, U.; Zürcher, W.; Schär, M.; Moser, H. E. *Nucleic Acids Res.* **1993**, *21*, 3191.
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19 The pK_a of 2-amino-2-deoxy-β-D-glucopyranose is 7.3 at 25°C. Neuberger, A.; Fletcher, A. P. *J. Chem. Soc. B* **1969**, 178.