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SYNTHESIS AND HYBRIDIZATION PROPERTY OF AN OLIGONUCLEOTIDE ANALOG CONTAINING A 1′,3′-DI-*O*-METHYLENE-α-D-FRUCTOSE BACKBONE

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Abstract: Hydrogen phosphonate monomers of T (thymine) and C^m (5-methylcytosine) bearing a 1′,3′-di-*O*-methylene-α-D-fructose sugar moiety were synthesized and incorporated into an oligonucleotide. Hybridization studies by thermal denaturation experiment indicated that this oligonucleotide did not form a duplex with the complementary RNA target. © 1998 Elsevier Science Ltd. All rights reserved.

Antisense oligonucleotides (ONs) are capable of inhibiting gene expression in a sequence specific manner.[1,2] Much effort has focused on the chemical modification of ONs to improve their binding affinity, nuclease stability, and permeability across cell membranes. Modification of ONs by replacing the β-D-deoxyribose moiety with an unnatural α sugar has led to α-ONs, which are more stable to nuclease activity while retaining the ability to bind to the complementary DNA and RNA sequences.[3,4] This α modification has also been introduced into a dodecathymidine phosphoramidate producing a novel ON analog with unexpected high affinity for the DNA and RNA targets.[5]

Preordering unbound ligands by covalent conformation restriction to resemble their bound state is an important method to enhance ligand receptor interaction.[6-8] We have recently applied this method to ON ligand design by preordering the ONs to resemble their bound helical conformations.[9-11] In an attempt to improve the binding affinity of α-ONs, we designed and synthesized an α-ON fully substituted with conformation-restricted α-fructose nucleotide units. The restriction of sugar conformation was achieved by a covalent acetal linkage between the 1′ and 3′ hydroxy groups. We now report the synthesis and hybridization property of a preordered α-ON bearing 1′,3′-di-*O*-methylene-α-D-fructose units.

The syntheses of the T (thymine) and C^m (5-methylcytosine) fructoside monomers are shown in Scheme 1. The peracylated fructose derivative **1** was prepared from D-fructose using a literature method.[12,13] Condensation of **1** with silylated thymine in the presence of $SnCl_4$ gave the α-D-fructose nucleoside **2** in 75% yield. Selective removal of the 1′-*O*- and 3′-*O*-benzoyl protecting groups was effected by portion wise addition of NaOMe in THF and carefully monitoring the reaction progress by TLC. Under these conditions, the 3′-*O*-benzoyl was selectively removed first to generate a 3′-alkoxide intermediate. Subsequent preferential deprotection of the 1′-*O*-benzoyl was probably due to an intramolecular attack of the 3′-*O*-alkoxide on the 1′-*O*-benzoyl resulting in a benzoyl migration followed by the facile debenzoylation of the newly formed 3′ benzoate.

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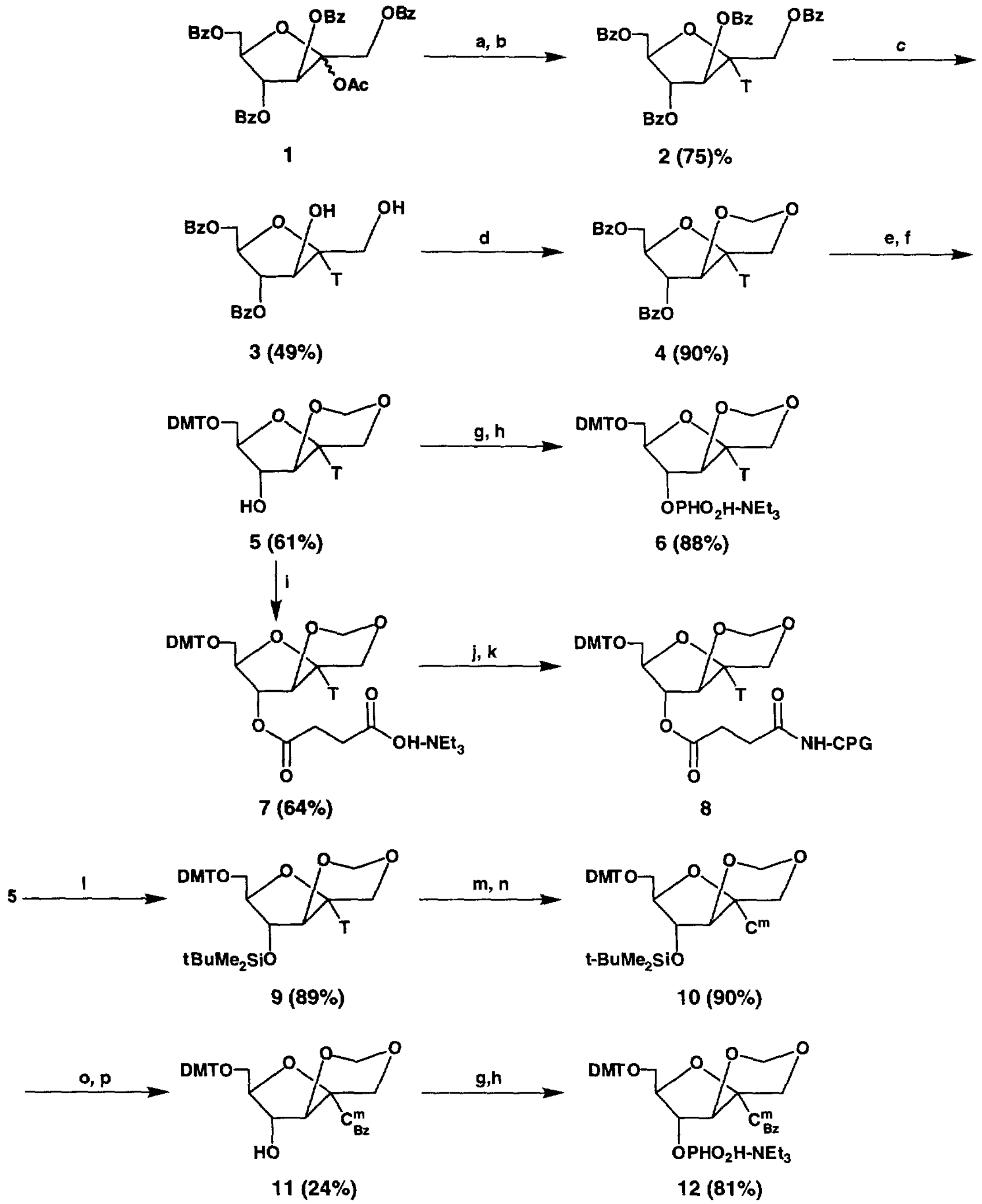

Scheme 1. *Reagents and conditions*: (a) Thymine, BSA, MeCN, 70 °C; (b) **1**, $SnCl_4$, 0 °C to rt; (c) NaOMe, THF; (d) $(CH_2O)n$, $MeSO_3H$, CH_2Cl_2; (e) NaOMe, MeOH; (f) DMTCl, pyridine; (g) PA, pyridine, CH_2Cl_2; (h) TEAB; (i) Succinic anhydride, Et_3N, 50 °C; (j) CPG-NH_2, TBTU; (k) Ac_2O, pyridine; (l) *t*-$BuMe_2SiCl$, imidazole; (m) $POCl_3$, 1,2,4-triazole, Et_3N; (n) Conc. NH_4OH; (o) BzCl, pyridine, CH_2Cl_2; (p) Bu_4NF, AcOH, THF.

This gave the dihydroxy compound **3**, which was treated with paraformaldehyde and $MeSO_3H$ to afford the 1′,3′-di-*O*-methylene derivative **4**. The structure of compound **4** was fully characterized by NMR spectroscopy.[14] Its bicyclic ring system and anomeric configuration were determined by HMBC and ROESY experiments. Deprotection of **4** with NaOMe in MeOH was followed by tritylation with dimethoxytrityl chloride (DMTCl) yielding the 5′-*O*-DMT derivative **5**. Compound **5** was characterized by NMR spectroscopy and FAB MS.[15]

The formacetal ring system has the potential to isomerize under acidic conditions by the attack of the neighboring 5′-OH. This would preclude ON synthesis using standard protocols if this side reaction were to occur under the mild acidic conditions used to remove the DMT group. To test this possibility, compound **5** was treated with 5% dichloroacetic acid in CH_2Cl_2, which is the standard condition used in solid-phase ON synthesis to remove the DMT group. Clean deprotection to the 5′-OH derivative was observed even upon extended treatment (2 h). Compound **5** was converted to its 3′ hydrogen phosphonate monomer **6** by treatment with 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one (PA). Transformation of **5** to the corresponding C^m derivative **11** was achieved via a triazole intermediate. The hydrogen phosphonate monomer of C^m (**12**) was obtained by phosphitylation of **11** under similar conditions.

Compound **5** was coupled onto the controlled pore glass (CPG, amino form) via a succinate linkage. This CPG was employed for the solid-phase synthesis of an ON using the hydrogen phosphonate protocol.[16] The ON synthesized was $5'\text{-}T{\cdot}C^m{\cdot}T{\cdot}C^m{\cdot}T{\cdot}C^m{\cdot}T{\cdot}C^m{\cdot}T$,[17] where T = thymine α-fructoside, C^m = 5-methylcytosine α-fructoside and all linkages were phosphodiester. The hybridization property of this ON with its complementary single stranded RNA target (15-mer, 5′-AAAAAGAGAGAGAGA) was studied by a thermal denaturation experiment (Tm). A control sequence of the corresponding β-9-mer phosphodiester had a Tm of 39.5 °C (buffer conditions: 140 mM KCl, 5 mM Na_2HPO_4, 1 mM $MgCl_2$, pH 7.2). The Tm data indicated that the α-ON did not form a duplex with the ssRNA target under the experimental condition.

This attempt to preorder α-ONs by conformational restriction of the α-ribose moiety with the 1′,3′-di-*O*-methylene fructose linkage resulted in destabilization of the duplex. Model building suggests that the 6 membered formacetal ring locks the ribose ring into a Southern like conformation[18] with the internucleotide linking oxygen at the 4′ position being driven down into an exo or axial conformation. α-Nucleoside analogs that are preordered in the Northern like conformation[18] with the internucleotide oxygen in the endo or equatorial conformer may result in enhanced binding.

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