Pergamon

Tetrahedron Letters, Vol. 37, No. 7, pp. 941-944, 1996

0040-4039/96 $15.00 + 0.00

0040-4039(95)02396-8

Synthesis and Hybridization Properties of an Oligonucleotide Analog Containing a Glucose-derived Conformation-restricted Ribose Moiety and 2', 5' Formacetal Linkages

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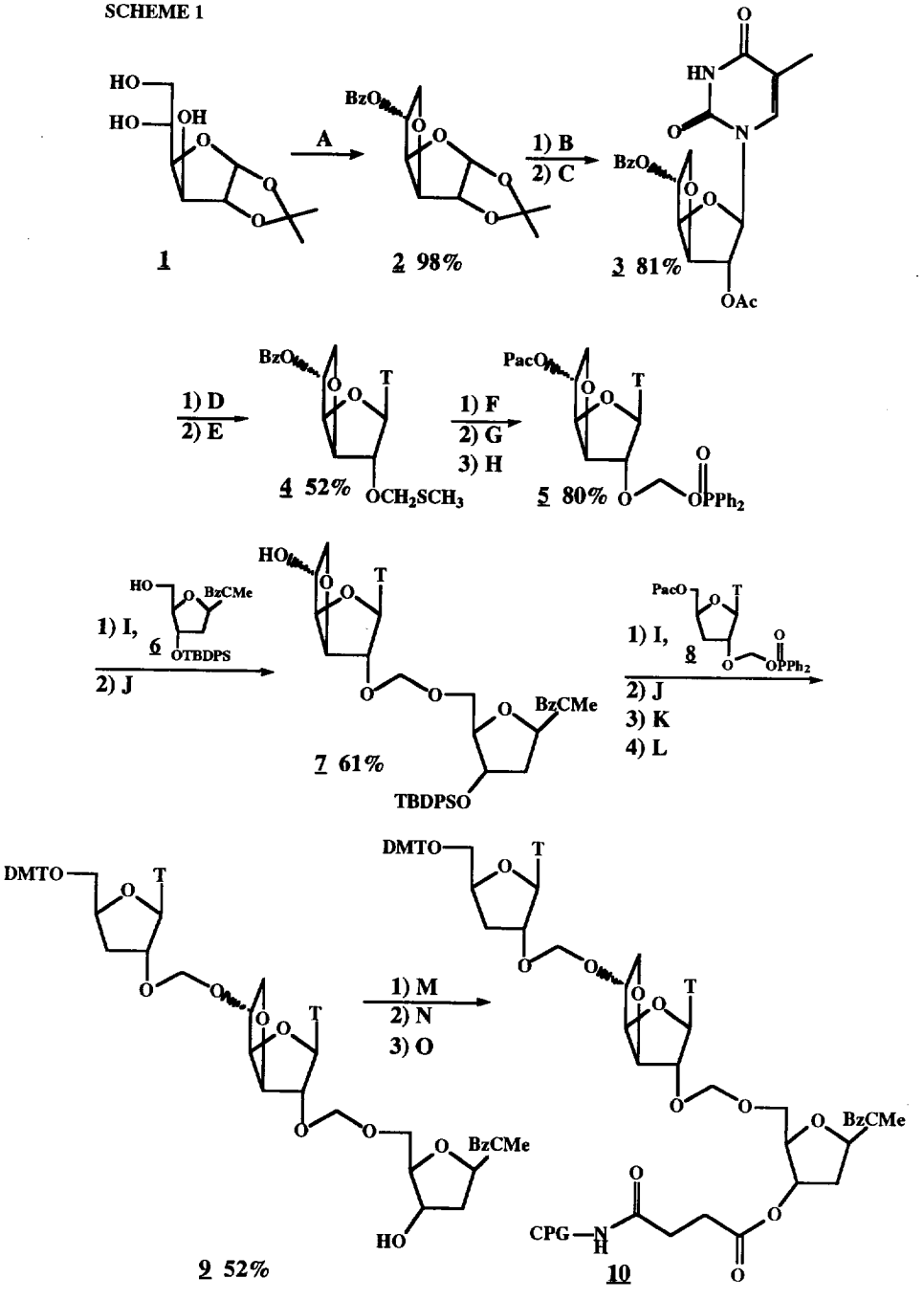
Abstract: A pyrimidine trinucleoside containing a conformationally restricted ribose moiety and two 2', 5' formacetal internucleoside linkages was synthesized. The bicyclic ribose moiety was identified by molecular modeling as a candidate to preorder a nucleoside to resemble the bound duplex conformation. The thymine nucleoside analog was simply synthesized from glucose. The trimer was incorporated at the 3' end of a larger unmodified ON. The hybridization affinity of this chimeric ON analog to a complementary RNA was found to be somewhat inferior relative to a non conformationally restricted control.

Covalent conformational restriction of unbound ligands to resemble their bound state is a proven method for the enhancement of ligand-receptor interactions.[1] This concept is now beginning to be applied toward the enhanced recognition of nucleic acid receptors such as complementary RNA and DNA by oligonucleotides (ONs) ligands.[2] We have previously reported the synthesis and binding properties of ON analogs containing 3', 5' formacetal[3] and 2', 5' formacetal internucleoside[4] linkages. These modifications replace the phosphodiester with a small, non-ionic and non-chiral linkage and confer to the ON analogs comparable binding affinity for ssRNA. In an effort to improve the binding affinity of 2', 5' formacetal ON analogs, a conformation-restricted furanose moiety was simply synthesized in order to pre-ordered the ribose conformation. The common sugar, glucose, was converted into a 5,5 bicyclic ring system which locks the ribose torsion angles of the nucleoside into a conformation resembling the bound form of canonical A form duplex.[5] We report the synthesis of the trinucleoside analog (**9**) (Scheme 1) bearing a 3', 6'-anhydro-glucose derivative, its incorporation into a longer ON, and the hybridization property of the ON analog with a complementary ssRNA sequence.

The synthesis of the trinucleoside **9** is outlined in Scheme 1. Treatment of 1, 2-*O*-isopropylidene-α-D-glucofuranose (**1**) with $PhC(OMe)_3$/TsOH resulted in the formation of a glucose ortho ester intermediate, which underwent concomitant acid-catalyzed rearrangement to give the 3, 6-anhydroglucose derivative (**2**) in 98% yield.[6] Acetolysis of **2** followed by glycosylation with silylated thymine in the presence of trimethylsilyltriflate (TMSOTf) furnished the desired nucleoside **3**. Selective removal of the 2'-*O*-acetyl protecting group of **3** was achieved with NaOMe/THF and subsequent treatment with Me_2S/Bz_2O_2 afforded the methylthiomethyl ether **4**.[7] The 5'-*O*-benzoyl group of **4** was removed and replaced by a phenoxyacetyl (Pac) group to enable its facile, mildly basic, selective removal during the chain elongation process. The 2'-O-methylthiomethyl group **5** was then converted to the 2'-*O*-methyl diphenylphosphinate (**5**).[7,8] Condensation of **5** with 2'-deoxy-N^4-benzoyl-3'-*O*-t-butyldiphenylsilyl-5-methylcytidine (**6**)[10] followed by

SCHEME 1



A: $Ph(COMe)_3$, TsOH, CH_2Cl_2, RT; B: AcOH, Ac_2O, H_2SO_4, RT; C: thymine, BSA, MeCN, TMSOTf, 70°C; D: NaOMe, THF, RT; E: Me_2S, Bz_2O_2, MeCN, 0°C; F: NaOMe, MeOH, RT; G: PacCl, Pyr, CH_2Cl_2, RT; H: NIS, Ph_2PO_2H, DCE, RT; I: TMSOTf, MeCN, DCE, -40°C; J: NH_3/MeOH, 0°C; K: DMTCl, Pyr, CH_2Cl_2, RT: L: TBAF, THF, RT; M: succinic anhydride, Et_3N, DMAP, DCE; N: TEAB; O: Pyr, DMF, diisopropylcarbodiimide

selective deprotection of the 5'-*O*-Pac group with NH_3/MeOH provided the dinucleoside **7**. Further chain elongation with the diphenylphosphinate **8**,[11] replacement of the 5'-*O*-Pac with a dimethoxytrityl (DMT) group, and desilylation at the 3' end gave the target trinucleoside **9**. The trinucleoside **9** was functionalized at the 3' end as a succinate and then coupled onto the controlled pore glass (CPG, amino form). This CPG was used for the solid phase ON synthesis using the H-phosphonate method.[12]

The trinucleoside **9** was incorporated into an ON of the sequence 5'-$C^{M}TTC^{M}ATTTT$***T^gT^C^M***-3', where C^{M} = 5-methyl-2'-deoxycytidine and ***T^gT^C^M*** is **9**, the modified trinucleoside bearing two 2', 5' formacetal linkages and a conformation-restricted glucose monomer. The ONs of the same sequence, 5'-$C^{M}TTC^{M}ATTTTT^T^C^{M}$-3' (where $T^T^C^{M}$ is the non-conformationally restricted 2', 5' formacetal-linked trimer and all other linkages are 3',5' phosphodiester) and 5'-$C^{M}TTC^{M}ATTTTTTC^{M}$-3' (all linkages are 3',5' phosphodiester) were also synthesized as controls in the hybridzation experiments. The formation of the duplex between these ON analogs and the complementary ssRNA target (5'-GAAGAAAAAAUGAAGAAAAU-3') was studied by thermal denaturation experiments using intracellular salt conditions.[13]

The T_m results are listed in Table 1. The T_m of the ON bearing two 2', 5' formacetal linkages was 2.5°C lower (-1.25°C/substitution) than that of the corresponding 3', 5' phosphate. This result was consistent with the previous observation from another 2', 5' formacetal ON (-0.5°C/substitution)[4]. Further modification by restriction of the sugar conformation with a 3', 6'-anhydroglucose moiety resulted in a destabilization (-0.75°C/substitution) of the duplex with the target ssRNA compared to the 2', 5' formacetal control.

ONs have potential application as antisense inhibitors.[14] Formacetal linkages are of interest due to their lack of sensitivity to nucleases and their potential of favorable cellular permeation properties.[3c] This particular attempt at covalent conformational restriction of the ribose moiety in conjunction with a 2',5' formacetal linkage has resulted in somewhat poorer binding affinity. A 5,5 bicyclic ring system is very rigid and causes a clear distortion of the sugar pucker geometries of the parent ribose ring.[2a,2b] The hybridization properties of these ONs may be improved if the extreme rigidity of the 5,5 ring system is relaxed by creating a larger ring fused to the ribose. Work on the synthesis of 2', 5' formacetal ONs bearing such conformation-restricted sugar moieties is currently in progress.

Table 1. T_m Results

ON	Modification	T_m°C
5'-$C^{M}TTC^{M}ATTTTTTC^{M}$-3'	3', 5' Phosphate	41.0
5'-$C^{M}TTC^{M}ATTTTT^T^C^{M}$-3'	3'-Deoxyribo-2', 5' formacetal	38.5
5'-$C^{M}TTC^{M}ATTTTT^gT^C^{M}$-3'	3', 6'-Anhydrogluco-2', 5' formacetal	37.0

Acknowledgments: We thank Terry Terhorst for the solid phase ON synthesis, Theresa Huang for T_m measurements and Mary Hogsett for manuscript preparation.

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5. Modeling studies performed using Biograf software from Molecular Simulations Inc. The principle conformational restriction within this analog is the freezing of rotation about the C5' -O5' bond thereby fixing a gauche relationship between the O4' and O5'. The ribose ring pucker is likely locked in a 3' endo (A helix like) conformation by the 2' oxygen substituent.
6. Compound **2** had been prepared previously from 1, 2, 5, 6-diisopropylidene-D-glucose via a four-step synthesis (Ernie Prisbe, personal communication).
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(*Received in USA* 16 *October* 1995; *accepted* 1 *December* 1995)