Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No. 2, pp. 237-240, 1994

0960-894X/94 $6.00+0.00

0960-894X(93)E0065-9

SYNTHESIS OF 2'-O-METHYL-6,3'-ETHANOURIDINE AND ITS INTRODUCTION INTO ANTISENSE OLIGONUCLEOTIDES

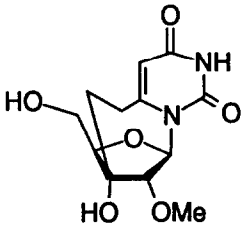
Marc-Olivier Bévierre, Alain De Mesmaeker, Romain M.Wolf and Susan M. Freier#*
Central Research Laboratories, Ciba-Geigy Ltd., CH-4002 Basel, Switzerland
#ISIS pharmaceuticals, 2292 Faraday av., Carlsbad, CA 92008, U.S.A.

Abstract: *A molecular modelling study and a synthesis of title compound **1** are presented. This novel modified nucleoside was introduced into different oligonucleotides and the corresponding melting temperatures of the duplexes formed with the complementary RNA were measured.*

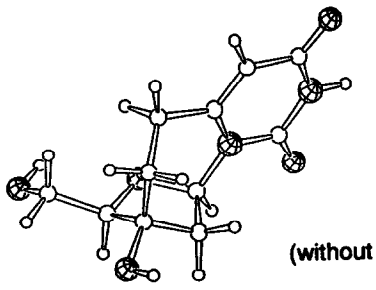
In double stranded nucleic acids, the sugar part of each nucleotide has a defined puckering (measured by the two parameters ν_{max} and P), the base part a defined orientation (measured by the torsion angle χ = [O(4)-C(1')-N(1)-C(2)]) and the backbone part defined torsion angles (α, β, γ,...).[1] By using conformationally restricted nucleoside analogs, certain of these parameters can be fixed at the stage of the monomer. Resulting oligonucleotides preorganised with a 3'-endo type sugar puckering and an appropriate base orientation that is common to the A-family of nucleic acid conformations should therefore, for entropic reasons, possess an enhanced binding affinity against RNA compared with the wild type.[2]

In order to fix as many parameters as possible at the level of the monomer, we decided to concentrate ourselves on modified nucleosides with a covalent carbon bridge between base and sugar. In these compounds, both χ and the sugar puckering are restricted. Nucleosides of this type have been synthesized previously.[3]

Among all the monomers tested by molecular modelling (bridges between C(6) and C(5'), C(6) and C(3'), zero, one, two or three carbon atoms), we have selected the new C(6-3')-ethano-bridged uridine analog **1** (Fig. 1).



1



(without -OMe at C(2'))[13]

Figure 1. Lowest-energy conformer of the bridged nucleoside **1**, obtained from molecular mechanics computations using the AMBER all-atoms force field. The conformational range was scanned, generating fifty structures by high-temperature dynamics, followed by complete minimization ("quenching"). The structure has a base-torsion angle χ = -131°, *i.e.* close to the corresponding torsion angle in standard B-DNA ($\chi \cong$ -140°). The sugar puckering parameters *P* (pseudo rotation) and ν_{max} (maximum degree of pucker) are: *P* = 26.5 (in the C3'-endo range, *i.e.* corresponding to the A-form sugar puckering in nucleic acids) and ν_{max} = 46°. (Two other distinct geometries, higher in energy by 4.5, respectively 6.6 kcal/mol were found during the conformational analysis). No modelling studies were made on RNA/DNA duplexes containing **1**.

Synthesis of 1

The key step consists of a palladium-catalysed cross-coupling between the protected 3'-C-ethinyl sugar **6**[4] and the 2,4-dimethoxy-6-iodopyrimidine **4**[5] (Scheme 1). The choice of the 2,4-dichlorobenzyl group for the protection of the 3'- and 5'-alcohol moieties of **5** was critical:[6,7] it had to stand acidic, basic, as well as the mild hydrogenation conditions required for the selective reduction of the triple bond.[8] After that reduction and changing the 1',2'-O-isopropylidene moiety for two acetate protecting groups, the second ring was formed by means of an intramolecular glycosilation catalysed by $SnCl_4$ giving **10**. As the 4-O-methyl moiety of **10** was sensitive to halide-mediated dealkylations[9], it had to be replaced by a N(3)-benzyloxymethyl protective group.[10] 2'-O-methylation was achieved by the silver oxide method.[11] A stronger base like NaH forms an allylic anion by deprotonation on the bridge at the carbon center which is α to the base; 5-methylated and 5,5-dimethylated side-products were thus isolated from the reaction mixture in that case. Final hydrogenation under more vigourous conditions gave **1** (see step *m* in Scheme 1).

Oligomerisation of 1

The 5'-O-dimethoxytrityl-3'-O-phosphoramidite derivative of **1** was synthesized in good yield and introduced into five DNA sequences; the melting temperatures of the duplexes formed with the complementary RNA strands are summarised in table 1.

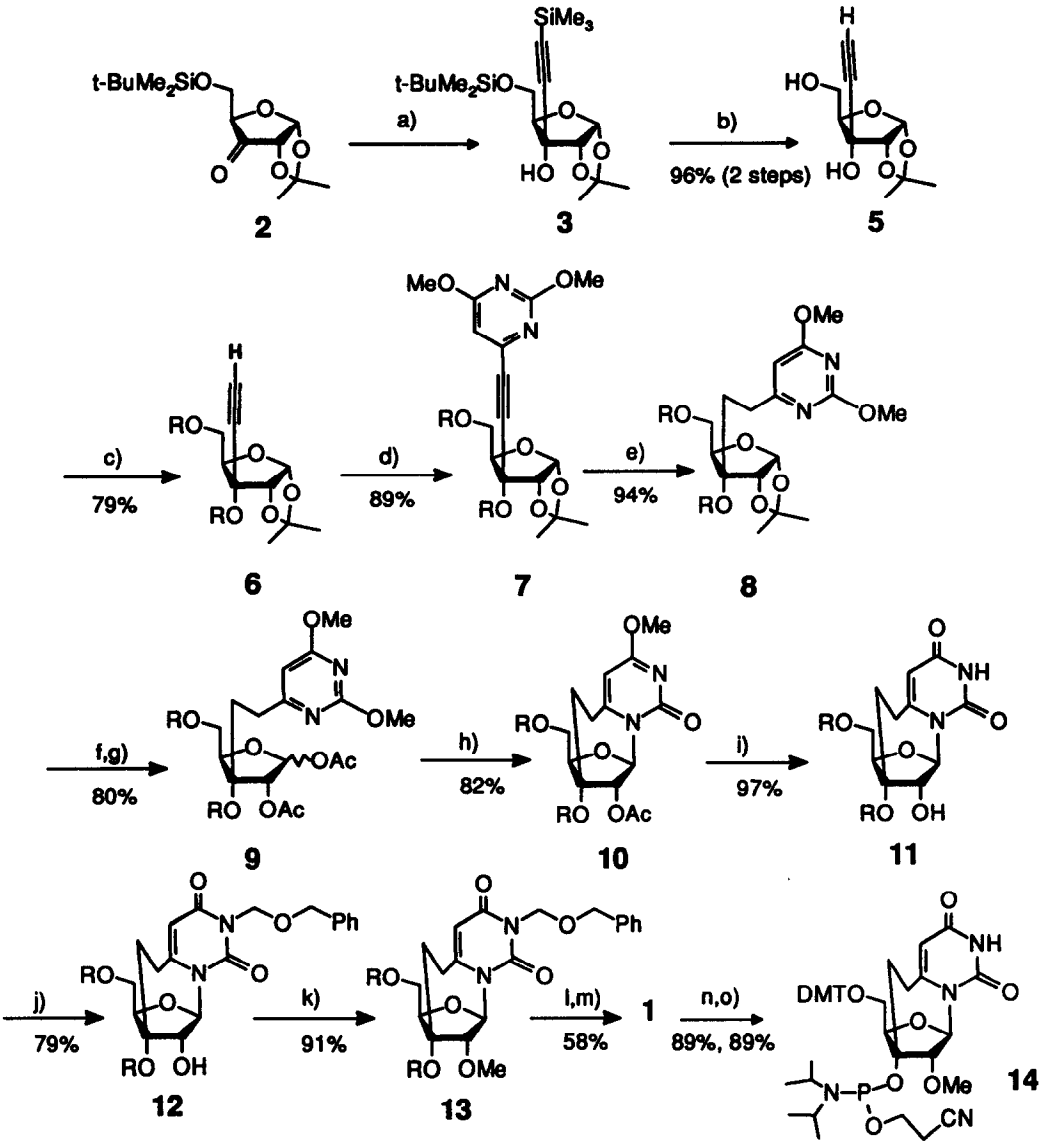
Modified DNA Sequence	T_m[a)]	ΔT_m/mod.[b)]
tCCAGGtGtCCGCAt	43	-4.6
CTCGTACCtTTCCGGTCC	55.2	-8.8
CTCGTACttttCCGGTCC	46.2	-3.9
GCGttttttttttGCG	no duplex formation	
TTTTtCTCTCTCTCT	48.2	-5.1

Table 1. The oligonucleotides were synthesized on an ABI 390 DNA synthesizer using standard phosphoramidite chemistry. Capital letters stand for unmodified natural bases; "t" stands for **1**. a) Melting temperature against RNA in degrees Celsius; the thermal denaturation of DNA/RNA hybrids was performed at 260 nm using a Gilford Response II spectrophotometer (Ciba-Corning Diagnostics Corp., Oberlin, OH); absorbance *vs* temperature profiles were measured at 4 μM of each strand in 10 mM phosphate pH 7.0 (Na salts), 100 mM total [Na^+] (supplemented as NaCl), 0.1 mM EDTA. b) difference of T_m per modification introduced compared with the wild-type DNA-RNA analogous sequence, in degrees Celsius.

Conclusion

The antisense oligonucleotides containing the bridged nucleoside **1** display a low affinity towards the complementary natural RNA strands. This result might be explained by the rigidity of **1** itself. The previous modelling study, which dealt only with the *monomer* nucleoside, suggested that, although very close values were found for the sugar puckering and the base orientation, **1** probably does not have *exactly* the conformation required for the introduction into a DNA-RNA duplex. Because of its rigidity, no room exists in the structure of **1** for conformational adjustment to the local parameters of the duplex; especially since steric interactions between the 5'-O-phosphate and the bridge in **1** would prevent the γ angle from adopting the value of ca 50° required for duplex formation. A destabilization of the double strand is thus observed. A modelling study on the stability of DNA/RNA double strands containing **1** is in progress to confirm this hypothesis.

It has been experimentally evidenced[12] that too much conformational flexibility in a modified DNA strand could induce drastic drops in the binding affinity of this strand towards the complementary RNA sequence. The example reported here indicates that too much rigidity can also be disfavourable.



Scheme 1. (R = 2,4-Dichlorobenzyl); Conditions: a)TMSCCLi (1.5 eq.), THF, -80°C-->RT; b) Bu_4NF/AcOH, THF, RT; c) 2,4-Dichlorobenzyl chloride (3 eq), Bu_4NI (0.4 eq.), NaH (2.2 eq.), DMF, -10°C --> 0°C d) 2,4-Dimethoxy-6-iodopyrimidine (4), $PdCl_2(PPh_3)_2$ (5%), CuI (10%), 2,2,6,6-tetramethylpiperidine; e) H_2/RaNi, MeOH:THF 4:1; f) 80% trifluoroacetic acid, RT; g) Ac_2O, pyridine, RT; h) $SnCl_4$, CH_2Cl_2, 0°C --> RT; i) 2N NaOH:dioxane 1:1, 100°C, 20 min.; j) $PhCH_2OCH_2Cl$, DBU, DMF; k) Ag_2O, MeI, reflux; l) H_2/5% Pd-C, THF; m) 1. H_2/5% Pd-C, AcONa, MeOH; 2. H_2/10%Pd-C, MeOH; n) DMTCl, Pyridine, RT ; o) i-$Pr_2NP(Cl)OCH_2CH_2CN$, i-Pr_2NEt, THF, RT.

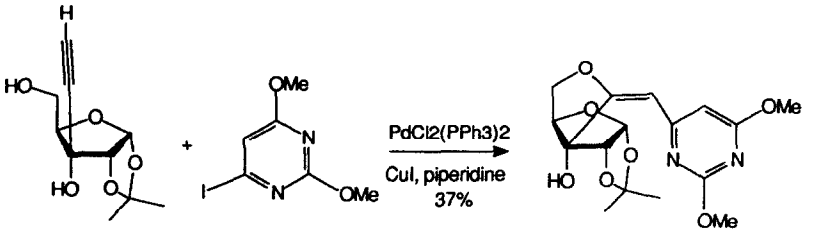
Acknowledgements

We thank Dr H. Moser for helpful discussions and Mr Auer for the synthesis in large amounts of our starting material **2** (for the synthesis of **2**, see ref. 2a). We also thank Drs. U. Pieles and D. Hüsken for the synthesis and the purification of the oligonucleotides, and Dr. T. Winkler for the identification of certain problematic structures.

References and notes

* To whom correspondance should be addressed.

1. Sänger, W., *Principles of Nucleic Acid Structure*, Springer-Verlag New York Inc. (1984).
2. Eschenmoser, A. *Nach. Chem. Tech. Lab.* **39**, 795 (1991).
3. See, for example, a) Yoshimura, Y.; Sano, T.; Matsuda, A.; Ueda, T., *Chem. Pharm. Bull.* **36**, 162 (1988); b) Suzuki, Y.; Matsuda, A.; Ueda, T., *Chem. Pharm. Bull.* **35**, 1808 (1987) and references cited therein; c) M. Tarkoy, M. Bolli, B. Schweizer, C. Leumann, *Helv. Chim. Acta* **76**, 481 (1993).
4. **5** was synthesised according to the procedure of Nakatani, K.; Arai, K., Terashima, S., *J. Chem. Soc. Chem. Comm.* , 289 (1992); the t-butyldimethylsilyl goup was used instead of the pivaloyl group for the protection of the 5'-OH moiety.
5. A synthesis of this compound has been published: Horwitz, J.P.; Tomson, A.J., *J. Org. Chem.* **26**, 3392 (1961); this synthesis gave us only 1,3-dimethyl-6-iodouracil. Another way consists of using the reaction of iodine on 2,4-Dimethoxy-6-lithiopyrimidine, prepared according to Langley's method (Langley, B.W., *J. Am. Chem. Soc.* **78**, 2136 (1956)), which gives 2,4-Dimethoxy-6-iodopyrimidine in high yield (89%).
6. Attempts to perform this coupling reaction without protecting the 3'- and 5'-OH groups gave a bicyclic structure resulting from an addition of the 5'-OH on the triple bond:



5 **4**

This result may be explained by the strong polarisation of the triple bond after its coupling with the electron-withdrawing pyrimidine; the basic reaction medium catalyses the cyclisation of the 5'-OH group. Heating **5** in DMF at 40°C in the presence of the stronger base NaH also leads to such a cyclisation. A similar reaction was published recently on ortho-iodophenol (ref. 7).

7. Kundu, N.G., Pal, M., Mahanty, J.S., Dasgupta, S.K., *J. Chem. Soc. Chem. Comm.* 41 (1992).
8. The paramethoxybenzyl group was initially used, but it did not resist the acidic treatement of step f).
9. Ulbricht, T.L.V., *J. Chem. Soc.* 3345 (1961).
10. Hanessian, S.; Kloss, J.; Sugawara, T., *J. Am. Chem. Soc.* **108**, 2758 (1986).
11. Akiyama, T.; Nishimoto, H.; Ozaki, S., *Bull. Chem. Soc. Jpn.*, 3356 (1990).
12. See for example Schneider, K.C.; Benner, S.A., *J. Am. Chem. Soc.*, **112**, 453 (1990); Augustyns, K.; Van Aerschot, A.; Van Schepdael, A.; Urbanke, C.; Herdewijn, P., *Nucl. Ac. Res.* **19**, 2587 (1991).
13. Due to the rigid bicyclic structure of **1**, the presence of a 2'-OMe would not further influence the puckering of the sugar (found as C(3')-endo). Therefore, the 2'-OMe in 1 was omitted in the calculations.

(*Received in Belgium* 16 *August* 1993; *accepted* 19 *October* 1993)