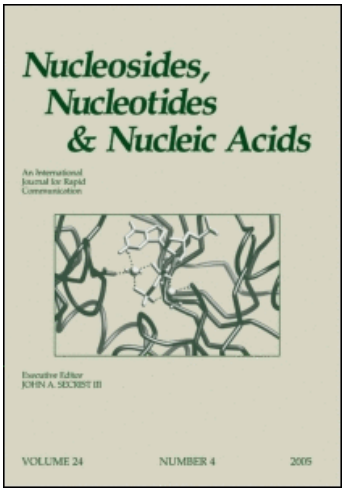

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis and Evaluation Of 2''-Modified MMI Linked Dimers in Antisense Constructs

Didier Peoc'h[a]; Eric E. Swayze[a]; Balkrishen Bhat[a]; Stuart Dimock[a]; Richard Griffey[a]; Yogesh S. Sanghvi[a]
[a] Isis Pharmaceuticals, Carlsbad, CA, USA

To cite this Article Peoc'h, Didier , Swayze, Eric E. , Bhat, Balkrishen , Dimock, Stuart , Griffey, Richard and Sanghvi, Yogesh S.(1997) 'Synthesis and Evaluation Of 2''-Modified MMI Linked Dimers in Antisense Constructs', Nucleosides, Nucleotides and Nucleic Acids, 16: 7, 959 — 962
To link to this Article: DOI: 10.1080/07328319708006115
URL: http://dx.doi.org/10.1080/07328319708006115

NUCLEOSIDES & NUCLEOTIDES, 16(7-9), 959-962 (1997)

SYNTHESIS AND EVALUATION OF 2'-MODIFIED MMI LINKED DIMERS IN ANTISENSE CONSTRUCTS

Didier Peoc'h, Eric E. Swayze, Balkrishen Bhat, Stuart Dimock, Richard Griffey and Yogesh S. Sanghvi*

Isis Pharmaceuticals, 2292 Faraday Avenue, Carlsbad, CA 92008, USA

ABSTRACT: Synthesis of four methylene(methylimino) (MMI) linked dimers modified at the 2'-position with fluoro and/or methoxy groups and their incorporation into different sequences has been accomplished. From these dimers, bis 2'-OMe MMI dimer was selected for further studies based on its synthetic accessibility, conformational study by NMR, and Tm analysis. Several chimeric antisense oligomers containing bis 2'-OMe dimers have been synthesized on a 10 μmol scale for *in vivo* studies.

INTRODUCTION

Replacement of the 3'→5' phosphodiester linkage with a neutral, achiral and non-phosphate linkage has proven to be a powerful strategy for the preparation of antisense oligomers exhibiting a high degree of nuclease stability and enhanced affinity for complement RNA target.[1] Our efforts in this area have lead to the discovery of the MMI linkage as a very promising backbone modification for antisense constructs.[2]

In this study we have extended the SAR of the MMI backbone[3] to include an electronegative substituent at the 2'-position, such as fluoro and/or methoxy with an aim to further enhance the affinity for RNA target. It is well established that the Tms of 2'-OMe or 2'-F modified antisense chimeric oligomers with complement RNA are considerably higher than that of the corresponding DNA·RNA duplexes.[4] In view of this, we have synthesized four MMI dimers **1-4** with a combination of 2'-OMe and 2'-F modifications (Figure 1). The novel MMI-sugar modified dimers **1-4** were subjected to extensive ^{1}H NMR studies which demonstrated their preference for a north-type (C3'-*endo*) puckered conformation. Subsequently, these were incorporated into various oligomeric sequences and their Tms measured with complement RNA.

CHEMISTRY AND DISCUSSION

The dimers **1** and **2** containing a 2'-OMe substituent in the top sugar residue were synthesized in a straightforward manner and in good yields. Dehydrative coupling of a 2'-OMe-3'-C-formyl nucleoside **5** and 2'-Ome (or F) -5'-*O*-amino nucleoside **6** furnished an oxime dimer, which upon *in situ* reduction followed by methylation, provided **1** or **2**. On the other hand, synthesis of dimers containing a 2'-F substituent in the top sugar unit was relatively tricky. The trans positioning of an α-2'-F substituent with an acidic β-3'-H in **5**

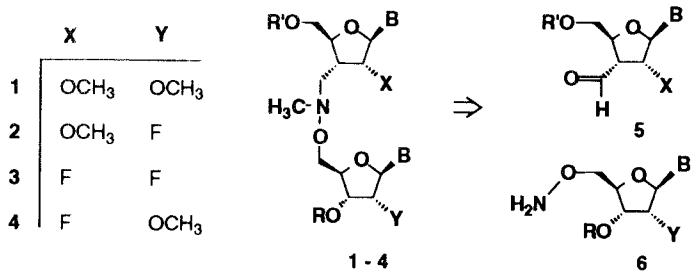

Figure 1: Synthesis of the bis-2′-modifed MMI linked dimeric nucleosides

TABLE 1: Fractional Population (%) of C3′-*endo* pucker in MMI dimers

2′-Sugar Modification	% of *N*-Conformation (2′-substituent)					
	DNA	MMI				
Top Sugar	35 (H)	68 (H)	95 (OMe)	- (OMe)	98 (F)	96 (F)
Bottom Sugar	28 (H)	31 (H)	76 (OMe)	- (F)	71 (OMe)	96 (F)

make it prone to undergo a facile elimination. Therefore, an alternative route for the synthesis of **3** and **4** was developed and will be published elsewhere.

The solution conformation of various dimers (**1-4**) was inferred from the vicinal proton-proton NMR coupling constants and nOe contacts. The results were interpreted in terms of a two state equilibrium between an *N*-type puckered ring and a *S*-type puckered ring using the PSEUROT 6.2 methodology of Altona *et al.*[5] The calculated % of *N*-pucker in each sugar unit of the four dimers **1-4** has been summarized in Table 1. The results indicate that all dimers had a preference for high *N*-type pucker depending upon the nature of the 2′-substituent. A detailed analysis of this study will be the subject matter of another publication. A general conclusion emerging from this study is that an appropriate combination of 2′-sugar and MMI linkage will be very useful in designing high affinity antisense constructs for RNA targets.

The dimers **1-4** were transformed to their corresponding phosphoramidites and incorporated 1-5 times into various oligomers using a standard automated DNA synthesizer. The modified oligos were HPLC purified and fully characterized by CGE and ESMS. The results of the Tm studies with oligomers **A-C** containing dimers **1-4** at various positions are summarized in Table 2. Clearly, all modifications had substantial stabilizing effects in duplex formation compared to the MMI backbone alone or modified DNA. The enhanced stability can be attributed to the hydrophobic interaction between substituents in the minor groove, and particularly to the higher degree of the *N*-puckered sugar conformation. This results in a decreased entropic motion of the sugar while maintaining preorganization favoring an A-type duplex. Based on these results and amenable synthesis[6] of building blocks **5** and **6**, we chose to further explore the utility of oligonucleotides containing bis 2′-OMe MMI linked dimers **1** (B = T, 5MeC, A, G) in *in*

TABLE 2: Hybridization study of 2'-modified MMI dimers

Sequences (5'→3')

A: GCG T*T T*T T*T T*T T*T GCG **B:** CTC GTA CT*T T*TC CGG TCC
C: CTC GTA CC T*T TC CGG TCC

Type of Modification			ΔTm/modification vs. RNA			
Backbone	2'-Top	2'-Bottom	A	B	C	Avg.
DNA	H	H	0.0	0.0	0.0	0.0
MMI	H	H	+0.13	-0.23	+1.51	+0.20
MMI	OMe	OMe	+3.71	+2.78	+1.85	+3.20
MMI	OMe	F	+3.13	+2.50	+1.56	+2.80
MMI	F	OMe	+3.74	+3.01	+1.95	+3.30
MMI	F	F	+3.27	+2.20	+1.47	+2.80

TABLE 3: Analytical and hybridization data of 4189 analogs containing dimer **1**

Sequences (5'→3')

4189 $C_sA_sG_s\ C_sC_sA_s\ T_sG_sG_s\ T_sT_sC_s\ C_sC_sC_s\ C_sC_sA_s\ A_sC$

D. $C^*A_s\ G_sC_sC_s\ A_sT_sG_s\ G_sT_sT_s\ C_sC_sC_s\ C_sC_sC_s\ A_s\ A^*C$

E. $C^*A_o\ G^*C_s\ C_sA_sT_s\ G_sG_sT_s\ T_sC_sC_s\ C_sC_sC_s\ C^*A_o\ A^*C$

F. $C^*A_o\ G^*C_o\ C^*A_s\ T_sG_sG_s\ T_sT_sC_s\ C_sC_sC^*A_o\ C^*A_o\ A^*C$

G. $C^*A_o\ G^*C_o\ C^*A_o\ T^*G_oG^*T_o\ T^*C_o\ C^*C_o\ C^*C_o\ C^*A_o\ A^*C$

s = Phosphorothioate; o = phosphodiester; * = MMI linkage

Oligo	OD units	wt. mg.	CGE % purity	ESMS Ob. (calc'd)	Tm (ΔTm)
D	1076	60	>90	6331.8 (6330.4)	67.4 (+1.0)
E	1241	63	>90	6341.5 (6340.5)	73.7 (+7.3)
F	1163	62	>90	6365.1 (6364.6)	83.3 (+17.1)
G	783	48	>85	6370.4 (6370.8)	>95.0 (+30.0)

vitro and *in vivo* antisense experiments. As a demonstration of the utility of our novel synthetic procedure,[7] we have synthesized all sixteen bis 2'-OMe MMI linked dimers as phosphoramidites and appropriately derivatized solid-supports for automated synthesis.

In order to evaluate the utility of bis 2'-OMe MMI modification *in vivo*, we chose to modify a 20-mer phosphorothioate (Isis 4189) which inhibits the expression of PKC-α in mice.[8] Four oligonucleotide analogs of Isis 4189 containing bis 2'-OMe MMI dimers have been synthesized on a 10 μmol scale to provide 50-60 mg of the final products (Table 3), using an Expedite DNA synthesizer operating under standard phosphoramidite and RNA-cycle protocols. An extended coupling time (900 sec.) and sulfurization (120 sec.) step improved the quality of overall syntheses. The modified oligomers **D-G** have been fully characterized both by CGE and ESMS (Table 3). The Tm data for oligomers **D-G** is summarized in Table 3 and indicates a substantial increase in the affinity for the RNA complement compared to the parent phosphorothioate 4189.

In summary, incorporation of the 2'-OMe substituent on both sugar moieties and MMI linkage has emerged as a very powerful modification to gain affinity towards RNA target. The bis 2'-OMe MMI dimers are now readily accessible and have been successfully incorporated into the biologically relevant sequences to confer additional nuclease stability and affinity. Detailed evaluation of these second generation antisense constructs is in progress.

Acknowledgment: The authors wish to thank Dr. Elena Lesnik for Tm measurements and Dr. P. Dan Cook for encouragement and support.

REFERENCES

1. Sanghvi, Y.S. In "Comprehensive Natural Products Chemistry." Editors-in-Chief, D.H.R. Barton and K. Nakanishi, in Volume 7: *DNA and Aspects of Molecular Biology* Ed. E.T. Kool, Pergamon Press, **1997**, submitted.
2. Sanghvi, Y.S. and Cook, P.D. In *Carbohydrate Modifications in Antisense Research* ACS Symposium Series No. 580, Eds. Y.S. Sanghvi and P.D.Cook, American Chemical Society, Washington, DC, **1994**, 1-22.
3. Sanghvi, Y.S.; Swayze, E.E.; Peoc'h, D.; Bhat, B.; Dimock, S. *Nucleosides Nucleotides* **1997**, *16*, in this issue.
4. Lesnik, E.A.; Guinosso, C.J.; Kawasaki, A. M.; Sasmor, H.; Zounes, M.; Cummins, L.L.; Ecker, D.J.; Cook, P.D.; Freier, S.M. *Biochemistry* **1993**, *32*, 7832-7838.
5. PSEUROT Version 6.2, Van Wijk, J.C.; Altona, C., *et al.* Gorlaeus Laboratories, Leiden, The Netherlands.
6. Dimock, S.; Bhat, B.; Peoc'h, D.; Sanghvi, Y.S.; Swayze, E.E. *Nucleosides Nucleotides* **1997**, *16*, in this issue.
7. Swayze, E.E., Bhat, B.; Peoc'h, D.; Sanghvi, Y.S.; *Nucleosides Nucleotides* **1997**, *16*, in this issue.
8. Dean, N.M.; McKay, R. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 11762-11766.