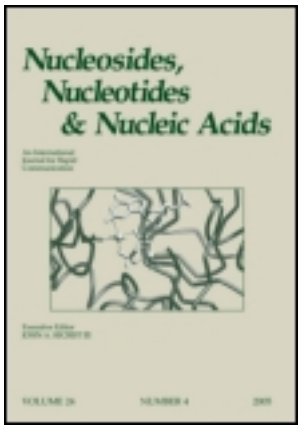

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lncn20

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Published online: 31 Aug 2006.

To cite this article: Stephen C. Holmes & Michael J. Gait (2003) The Synthesis of 2′-O-Methyl G-Clamp Containing Oligonucleotides and Their Inhibition of the HIV-1 Tat-TAR Interaction, Nucleosides, Nucleotides and Nucleic Acids, 22:5-8, 1259-1262, DOI: 10.1081/NCN-120022850

To link to this article: http://dx.doi.org/10.1081/NCN-120022850

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 22, Nos. 5–8, pp. 1259–1262, 2003

The Synthesis of 2′-O-Methyl G-Clamp Containing Oligonucleotides and Their Inhibition of the HIV-1 Tat-TAR Interaction

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ABSTRACT

We have synthesised a 2′-O-methyl riboside phosphoramidite derivative of the cytosine analogue 9-(2-aminoethoxy)-phenoxazine ('G-clamp') and successfully incorporated it into a series of small steric blocking 2′-O-methyl oligonucleotides targeting the stem-loop region of HIV-1 TAR RNA. The 'G-clamp' containing oligonucleotides show significant increases in binding to a model TAR RNA system when the 'G-clamp' is positioned opposite the loop region. The oligonucleotides also display dose-dependent inhibition of Tat-dependent transcription of an HIV DNA template in HeLa cell nuclear cell extract.

Key Words: Steric block; Oligonucleotides; G-clamp; Nucleoside analogue.

HIV-1 transcription is regulated by the trans-activator protein, Tat, binding to its RNA recognition sequence, TAR, a stem loop structure that occurs at the 5′-end of all HIV RNA transcripts.[1] As such, the Tat-TAR interaction has become an important drug target and model system for the development of RNA-protein inhibitors.

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DOI: 10.1081/NCN-120022850
1525-7770 (Print); 1532-2335 (Online)

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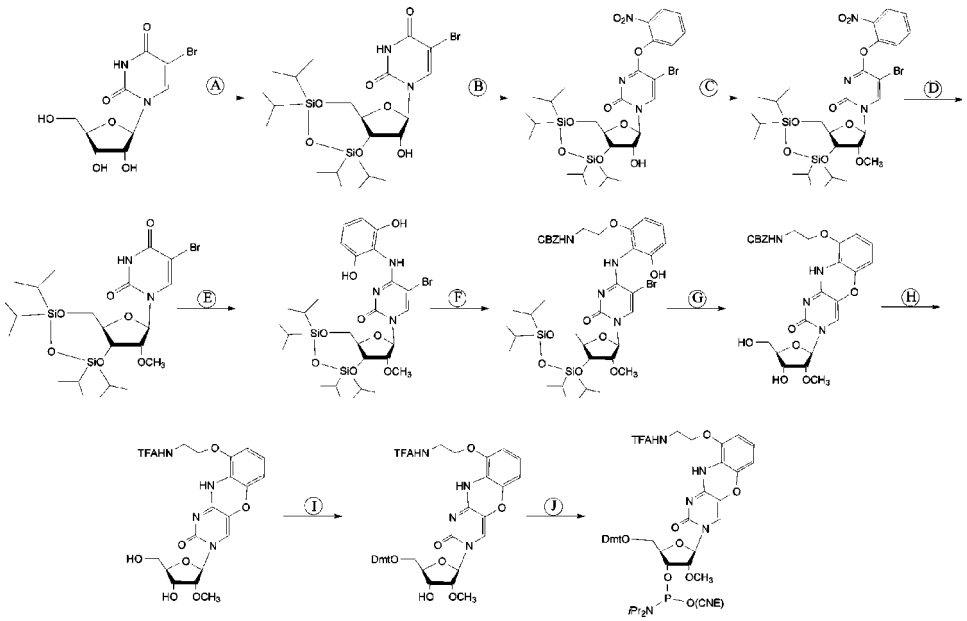

Scheme 1. Synthesis: A: Markiewicz Reagent, Pyridine (95% yield); B: i. TMSCl, Et_3N, DCM. ii. MesCl, Et_3N, DMAP. iii. σ-NO_2-phenol, DBO. iv. 2% Tosic acid, DCM (80%) C: MeI, Ag_2O, Acetone (85%) D: 4-nitrobenzaldoxime, tetramethylguanidine, Dioxane/Water (75%) E: i. CCl_4, PPh_3, DCM, ii. 2-aminoresorcinol (75%) F: Benzyl-N-(2-hydroxyethyl)carbamate, DEAD, PPh_3 (75%) G: KF, EtOH (60%) H: i. H_2, Pd/C, DMF ii. Ethyltrifluoroacetate, DMAP (65%) I. DmtCl, Pyr. (85%) J. Phosphitylating agent, DIPEA, DCM (85%).

Small molecules and peptidomimetics have been previously shown to inhibit the Tat-TAR interaction by binding to TAR-RNA in the region of a U-rich bulge.[2] Furthermore, the system has been used to investigate the ability of short oligonucleotides (and analogues) to strand invade and sterically block TAR RNA function.[3–5]

A recent development within the field of steric blocking and antisense molecules has been the synthesis of a cytosine analogue, 9-(2-aminoethoxy)-phenoxazine ('G-clamp'), capable of enhanced binding and specificity towards its complementary target.[6] A single analogue incorporated into a decamer DNA sequence was found to increase the T_m of its duplex with complementary DNA by 18°C.

We have chemically synthesised a novel 2′-O-methyl riboside phosphoramidite derivative of the 'G-clamp' (Sch. 1) and incorporated it into a series of small steric blocking 2′-O-methyl oligonucleotides targeting the stem-loop region of TAR RNA by solid phase methods previously described.[4,5]

The results from a gel mobility shift assay indicated that the 2′-O-methyl 'G-clamp' containing oligonucleotides showed significant increases in binding to a model TAR RNA system when the base analogue was positioned opposite the TAR RNA loop region. However, positioning the 'G-clamp' analogue to target guanosine residues in the duplex region of TAR RNA resulted in poorer binding than that obtained using an unmodified 2′-O-methyl oligoribonucleotide (Table 1).

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Table 1. 2′-O-methyl oligonucleotide binding to TAR RNA (Kd) and to complementary DNA and RNA (T_m).

Name	Sequence 5′-3′ 'G-clamp' analogue = **C**	Gel mobility shift binding assay		Tm[c]	
		Kd[a]	Kd[b]	DNA (°C ± 1)	RNA (°C ± 1)
12 TAR OMe	5′- CUC CCA GGC UCA	12 ± 1.5	3.2 ± 0.7	58	79
12 TAR P1	5′- **C**UC CCA GGC UCA	20 ± 0.8	3.3 ± 1.6	64	85
12 TAR P3	5′- CU**C** CCA GGC UCA	6.5 ± 0.6	1.5 ± 0.4	71	90
12 TAR P4	5′- CUC **C**CA GGC UCA	1.0 ± 0.1	0.8 ± 0.2	75	94
12 TAR P5	5′- CUC C**C**A GGC UCA	0.9 ± 0.4	0.2 ± 0.1	73	94
12 TAR P9	5′- CUC CCA GG**C** UCA	15 ± 2.0	2.4 ± 1.0	70	87
12 TAR P11	5′- CUC CCA GGC U**C**A	27 ± 1.1	3.1 ± 0.7	67	88

Gel Mobility Shift Assay Buffer Conditions:
[a](TK80 buffer) 50 mM Tris HCl pH 7.4, 80 mM KCl.
[b](Transcription buffer) 20 mM HEPES, 2 mM DTT, 10 μM $ZnSO_4$, 80 mM KCl, 3 mM $MgCl_2$, 10 mM creatine phosphate.
Thermal Melting experiment conditions:
[c]20 mM KCl, 5 mM Na_2HPO_4 pH 7.2, 10 mM $MgCl_2$.

The stabilities of duplexes formed by the modified oligonucleotides with their complementary DNA or RNA targets were also investigated. All duplexes containing a 2′-O-methyl 'G-clamp' analogue showed T_m increases ranging from 4–17 degrees. The magnitude of the increase is dependent on the position of the 'G-clamp' within the duplex and the most stable duplexes are those where the analogue is placed in the interior of the oligonucleotide.

The series of 2′-O-methyl 'G-clamp' containing oligonucleotides all displayed dose-dependent inhibition of Tat promoted transcription of an HIV DNA template in HeLa cell nuclear cell extract.[5] Fifty percent transcription inhibition occurred at between 50-200 nM oligonucleotide concentration with the oligonucleotide 12BRU P5 having the greatest inhibition of in vitro transcription. The investigation revealed a complex relationship between the binding strength of an oligonucleotide and its ability to inhibit in vitro transcription.

We are currently investigating the incorporation of multiple 'G-clamp' substitutions into an oligonucleotide and its effect on binding strength and inhibition of in vitro transcription. The ability of 2′-O-methyl 'G-clamp' modified oligonucleotides to inhibit Tat-dependent transcription in cells is also under investigation.

REFERENCES

1. Karn, J. Tackling Tat. J. Mol. Biol **1999**, *293*, 235–254.
2. Wilson, W.D.; Li, K. Targeting RNA with small molecules. Curr. Med. Chem. **2000**, *7* (1), 73–98.

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3. Ecker, D.J.; Vickers, T.A.; Bruice, T.W.; Freier, S.M.; Jenison, R.D.; Manoharan, M.; Zounes, M. Pseudo half-knot formation with RNA. Science **1992**, *257* (5072), 958–961.
4. Mestre, B.; Arzumanov, A.; Singh, M.; Boulme, F.; Litvak, S.; Gait, M.J. Oligonucleotide inhibition of the interaction of HIV-1 Tat protein with the trans-activation responsive region (TAR) of HIV RNA. BBA-Gene Struct. Expr. **1999**, *1445* (1), 86–98.
5. Arzumanov, A.; Walsh, A.P.; Liu, X.H.; Rajwanshi, V.K.; Wengel, J.; Gait, M.J. Oligonucleotide analogue interference with the HIV-1 Tat protein-TAR RNA interaction. Nucleosides, Nucleotides and Nucl. Acids **2001**, *20* (4–7), 471–480.
6. Lin, K.Y.; Matteucci, M.D. A cytosine analogue capable of clamp-like binding to a guanine in helical nucleic acids. J. Am. Chem. Soc. **1998**, *120* (33), 8351–8352.
7. Arzumanov, A.; Walsh, A.P.; Rajwanshi, V.K; Kumar, R.; Wengel, J.; Gait, M.J. Inhibition of HIV-1 Tat-dependent trans-activation by steric block chimeric 2′-O-methyl/LNA oligoribonucleotides. Biochemistry **2001**, *40* (48), 14,645–14,654.

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