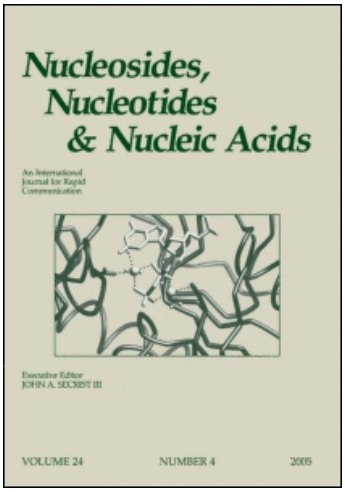

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Acetal Oligonucleotide Conjugates in Antisense Strategy

S. Matysiak[a]; R. Frank[a]; W. Pfleiderer[b]
[a] Department of Molecular Recognition, Mascheroder Weg, National Research Institute for Biotechnologie, Braunschweig [b] University of Konstanz, Faculty of Chemistry, Konstanz

To cite this Article Matysiak, S. , Frank, R. and Pfleiderer, W.(1997) 'Acetal Oligonucleotide Conjugates in Antisense Strategy', Nucleosides, Nucleotides and Nucleic Acids, 16: 5, 855 — 861
To link to this Article: DOI: 10.1080/07328319708002963
URL: http://dx.doi.org/10.1080/07328319708002963

NUCLEOSIDES & NUCLEOTIDES, 16(5&6), 855-861 (1997)

ACETAL OLIGONUCLEOTIDE CONJUGATES IN ANTISENSE STRATEGY

S. Matysiak*/+, R. Frank+, W. Pfleiderer°

Abstract: To enhance the cellular uptake for antisense oligonucleotides or analogues a lipophilic steroid moiety was linked to the 5′-O- or 2′-O-position of appropriate protected thymidine or uridine simply by acid catalysed reaction with cholesterylvinylether. The corresponding cholesteryl-acetals were derivatized to the 3′-O- or 5′-O-phosphoramidites and then introduced as 5′-end terminating agents or incorporated within the sequence of oligodeoxyribonucleotides up to a chain length of 18 bases. 3′-end linkage was achieved by using the corresponding 2′-O-cholesteryluridine building unit, tethered via a 3′-O-/5′-O-succinate-bridge to polystyrene as solid support.

The application of antisense-oligonucleotides (AON′s) as therapeutic agents represents a new paradigm for drug discovery and development [1-4]. Oligonucleotides designed to interact with nucleic acid receptors represent a potentially revolutionary advance in pharmacotherapy . The compositon of the ligand is exactly defined by the complementary nucleic acid sequence of the target, the so called sense strand. The nature of the chemical interactions, Watson-Crick base pairing via hydrogen bonding, results in a ligand-receptor complex with a high selectivity and great affinity. In comparison to classical drugs, small molecules which inhibit certain enzymes with a more or less high potential of side effects, antisense-oligonucleotide-analogues (AONA′s) might show much more efficiency by binding to (sense) pre-RNA via duplex or triplex helices. So the expression of proteins is inhibited at the level of translation. Beside survival in the enzyme-cocktail of the cell the polyanionic antisense-oligonucleotides or analogues first must penetrate the lipophilic cell membrane and then still be able to interact with the target RNA-sequence with the desired specificity. The correlation of chemical or enzymatic stability, cell-uptake and the specificity and affinity of binding is very complex. Therefore it has carefully to be determined, if a manipulation of the structure to improve one parameter (e.g. enhanced enzyme

* to whom correspondence should be adressed; + National Research Institute for Biotechnologie, Department of Molecular Recognition, Mascheroder Weg , D-38124 Braunschweig; ° University of Konstanz, Faculty of Chemistry, D-78434 Konstanz

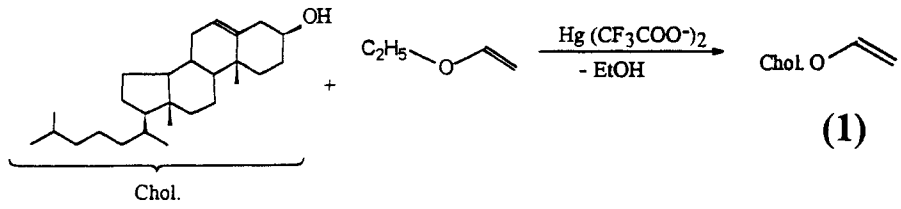

Figure 1: Vinylether interchange reaction

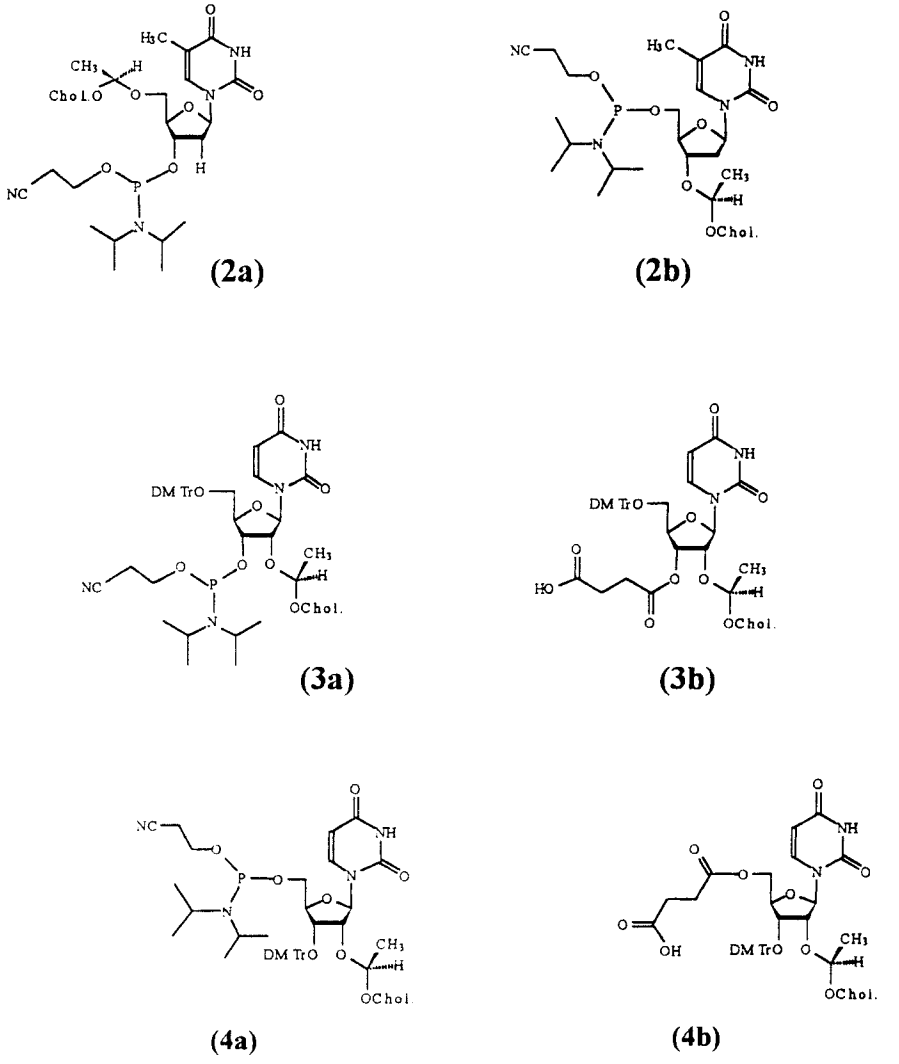

Figure 2: Synthesized monomeric units;

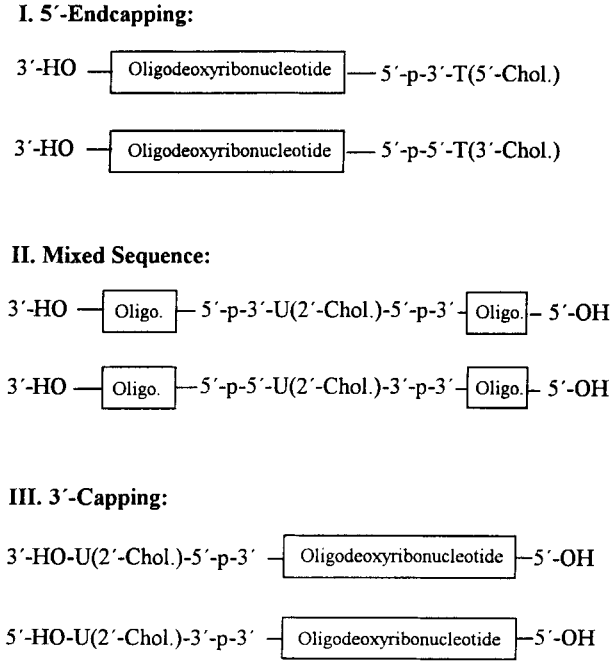

Figure 3: Cholesteryl-conjugates.

stability) is still acceptabel for the desired purpose as a therapeutic agent and does not implicate another parameter in a negative way (e.g. less selectivity and affinity of interaction).

In this communication we describe our attempts to introduce a new handle for cellular uptake of antisense-sequences. Penetration through the membrane in mammalian cells is believed to take place via endocytosis [5] and once in the cell the oligonucleotides display a punctate distribution. To improve the permeability, uptake and bioavailability we have synthesized 5′-O- and 2′-O-acetal linked cholesteryl-conjugates. As e.g. membranes of HIV and HIV infected cells are rich in cholesterol, enhanced uptake is expected. Instead of a phosphate-, ester- or amido-bond [6] we use an acid labile acetal-bridge for tethering the lipohilic steroid moiety. Cholesteryl-vinylether, easily pepared via a modified vinyl-interchange-reaction [7] (figure 1) is quantitatively linked to 3′- or 5′-O-tert.butyldimethylsilyl protected thymidine or 3′,5′-O-(1,1,3,3-tetra-isopropyldisiloxan-1,3-diyl) protected uridine. After deprotection and further routine steps 5′-O-[(1-cholesteryloxy)-ethyl]-thymidine-3′-O-(2-cyanoethyl-N,N-diisopropyl)-phosphoramidite (**2a**) or the regioisomer **2b** and 2′-O-[(1-cholesteryloxy)-ethyl]-5′-O-dimethoxytrityl-uridine-3′-O-(2-

HPLC:

Eluent A: 0,05 N NaOAc/ 20% ACN.
Eluent B: 0,05 N NaOAc/ 1N LiCl/ 20% ACN.
Gradient 1: 0-3′ (100% A), 3-23 (100% B), 23-28 (100% B), 28-32′(100%A), 32-35′(100% A).
Gradient 2: 0-3′ (100% A), 3-23 (50% B), 23-28 (50% B), 28-32′(100%A), 32-35′(100% A).
Column: Nucleogen DEAE 60-7 ET 125/4 (Anion exchange).

Mixed Sequence:

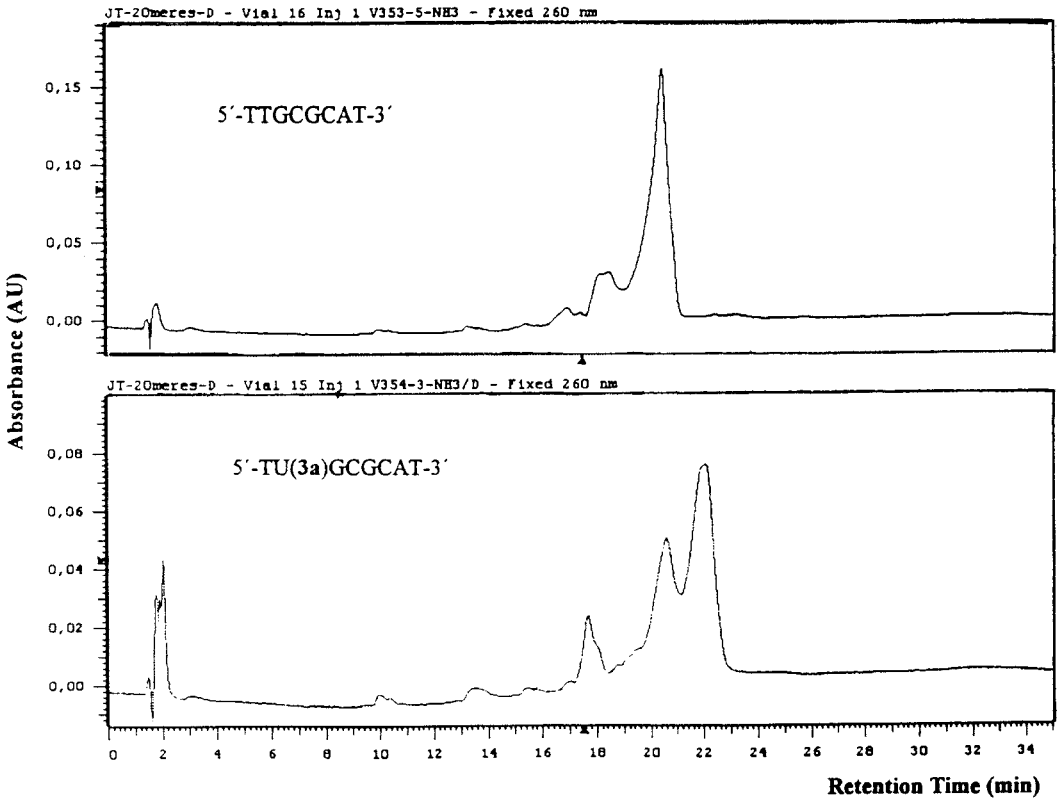

Figure 4: HPLC of the crude products, gradient 2.

cyanoethyl-N,N-diisopropyl)-phosphoramidite (**3a**) or the corresponding isomer (**4a**) could be isolated in good yields (figure 2).

The phosphoramidites **3a** and **4a** then were incorporated to a model sequence either at the end of the oligonucleotide as a 5′-O-terminating unit or within the sequence. Introduction of the steroid moiety to the 3′-O-position was achieved by preparation of the corresponding 3′- or 5′-

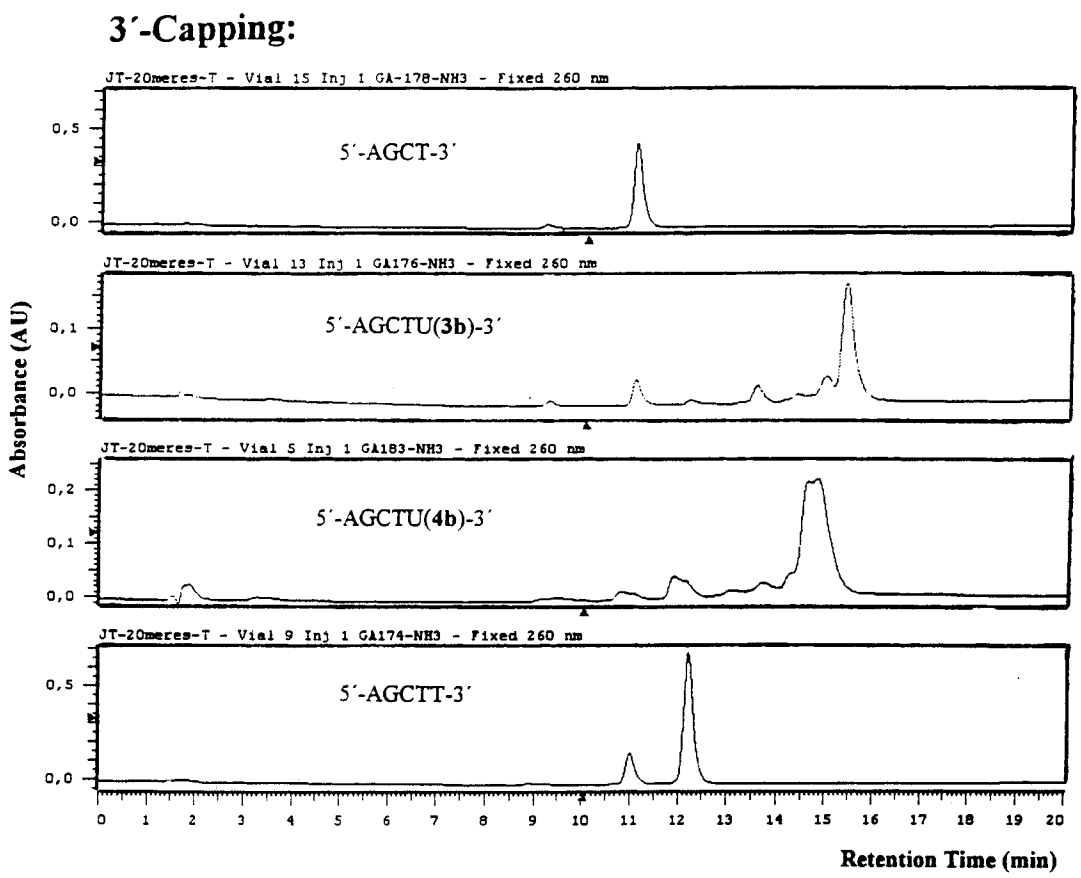

Figure 5: HPLC of the crude products, gradient 1.

O-succinate-derivative (**3b** and **4b**) and coupling with amino-polystyrene-solid support [8]. After capping with Ac_2O/Pyridin/DMAP this support was used to build up the desired sequences using slightly modified phosphoramidite chemistry (2%DCA/DCE for deprotection.of the DMTr-group, extended coupling times). The possible modifications of oligodeoxynucleotides are shown in figure 3 as a diagramm.

Experimental:

Oligonucleotide synthesis was carried out on an Pharmacia Gene Assembler using the standard 0.2 μmol programme . For detritylation 2% DCA in DCE was used instead of 1%TCA/DCE. The 2′-O-cholesteryl-uridine phosphoramidites **3a** and **4a** were reacted for 30 minutes at 25°C with dried polystyrene-tethered oligonucleotides in ACN/DCE (1:2) and tetrazole catalysis. After

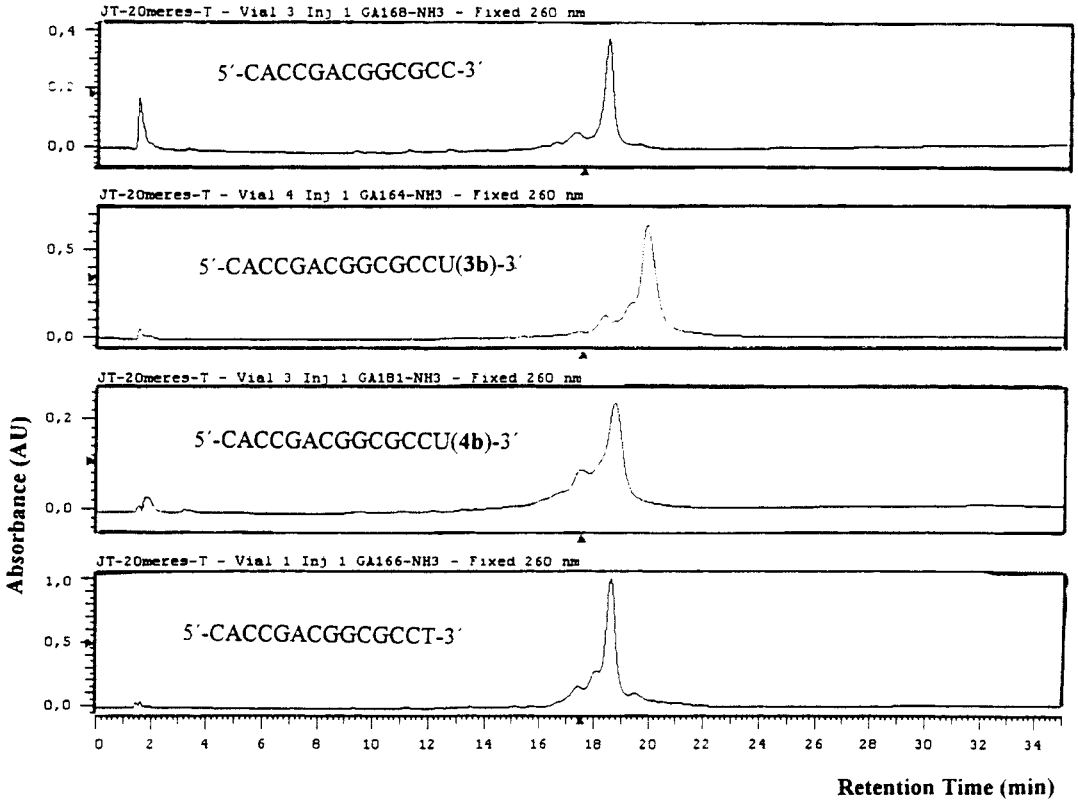

Figure 6: HPLC of the crude products, gradient 2.

oxidation with iodine/THF/H_2O the polystyrene matrix was filtered off via the original reaction vessel, washed with ACN and DCE and the vial sealed with a polypropylene filter. Detritylation and further reactions then took place automatically as mentioned before. The purity of the compounds **1a-4b** was checked by TLC, NMR (300 MHz), HPLC (RP 18) and Mass Spectroscopy (ESI, EI). Oligonucleotides were analysed by HPLC and Agarose Gel.

Summary:

We have successfully synthesized acetal linked building blocks for the synthesis of modified oligodeoxynucleotides. Introduction of a steroid system was possible either at the 5′-end or within the sequence using thymidine or uridine phosphoramidites. 2′-O-tethering was achieved via the corresponding 3′-O-succinate- 5′-O-DMTr-derivatives. Advantages of this method are the easy approach of the monomeric units, the use of standard phosphoramidite chemistry and

the variabel placement of introduction. Due to the mild chemistry this method is suitable for any kind of steroid (e.g testosterone, oestrone, 5a-dihydroteststerone etc.) or similar lipophilic moiety (e.g. vitamine A, B_2, D_2/D_3, E) bearing a primary or secondary hydroxyl function.

By determining the melting temperature of these modified oligonucleotide sequences their binding affinity to complementary DNA or RNA-strands and hence their capability as antisense therapeutics will be elucidated. The next step will involve radioactive labeling of model sequences and investigation of their efficiency to penetrate the membrane and their distribution within the cell. Due to the acidic environment in e.g. lysosomes and endosomes we would expect a rapid cleavage of the acetal bond [9] to give the native oligonucleotide. In a further step the acetal linkage could be exploited to modify enzymatically more stable analogues e.g. phosphorothioates, which then could inhibit protein expression by annealing with the RNA-template [10].

References:

[1] P. C. Zamecnik, M. L. Stephenson, *Proc. Natl. Acad. Sci. U.S.A.*, 75, 280, 1978.

[2] F.Eckstein, Ed., Oligonucleotides and Analogues: A Practical Approach, IRL Press, Oxford University Press, N.Y. 1991.

[3] E. Uhlmann, A. Peymann, *Chem. Rev.* 90, 543, 1990.

[4] J. Goodchild, *Bioconj. Chem.1*, 165, 1990.

[5] J. Darnell, H. Lodish, D. Baltimore in " Molecular Cell Biology", p. 617 Scientific American Books, Inc., 1986.

[6] M. Manohara: "Designer Antisense Oligonucleotides: Conjugation Chemistry and Functionality Placement" in "Antisense Research and Applications", 303-349, S. T. Crooke, B. Lebleu (Ed.), CRC Press 1993.

[7] S.Matysiak, "New Protected 2'-O-Acetal Protecting Groups for the Synthesis of Oligoribonucleotides", Thesis, University of Konstanz, 1995, ISBN 3-931986-28-4.

[8] Pharmacia Polystyrene-Amino-30 HL

[9] P. A. Trail, D. Willner, S. J. Lasch, A. J. Henderson, S. Hofstead, A. M. Casazza, R. A. Firestone, I. Hellström, K. E. Hellström, *Science* 212, 261, 1993.

[10] V. J. Shah, I. D. Kuntz Jr., G. L. Kenyon, *Bioorganic Chemistry*, 24, 194-201, 1996.