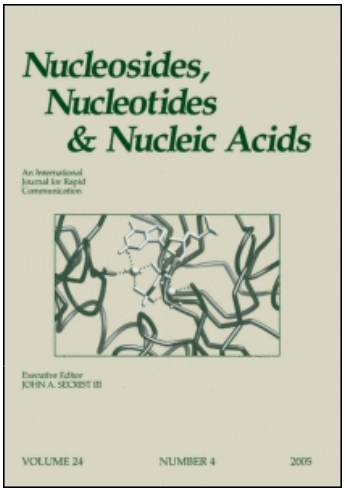


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Branched Polyethylene Glycol (Bpeg) Conjugated Antisense Oligonucleotides

B. Burcovich[a]; F. M. Veronese[a]; V. Zarytova[b]; G. M. Bonora[c]
[a] Dept. of Pharmaceutical Sciences, University of Padova, (Italy) [b] Novosibirsk Institute of Bioorganic Chem., Novosibirsk, (Russia) [c] Pharmaco-Chemico-Technological Dept., University of Cagliari, (Italy)

To cite this Article Burcovich, B. , Veronese, F. M. , Zarytova, V. and Bonora, G. M.(1998) 'Branched Polyethylene Glycol (Bpeg) Conjugated Antisense Oligonucleotides', Nucleosides, Nucleotides and Nucleic Acids, 17: 9, 1567 — 1570
To link to this Article: DOI: 10.1080/07328319808004687
URL: http://dx.doi.org/10.1080/07328319808004687

NUCLEOSIDES & NUCLEOTIDES, 17(9-11), 1567-1570 (1998)

BRANCHED POLYETHYLENE GLYCOL (bPEG) CONJUGATED ANTISENSE OLIGONUCLEOTIDES

B.Burcovich [a], F.M.Veronese [a], V.Zarytova [b] and G.M.Bonora [c *]
a. Dept. of Pharmaceutical Sciences - University of Padova (Italy) b. Novosibirsk Institute of Bioorganic Chem. - Novosibirsk (Russia) c. Pharmaco-Chemico-Technological Dept. - University of Cagliari (Italy)

ABSTRACT : The synthesis of new oligonucleotide conjugates bearing an high molecular weight, branched polyethylene glycol (bPEG) chain is reported.

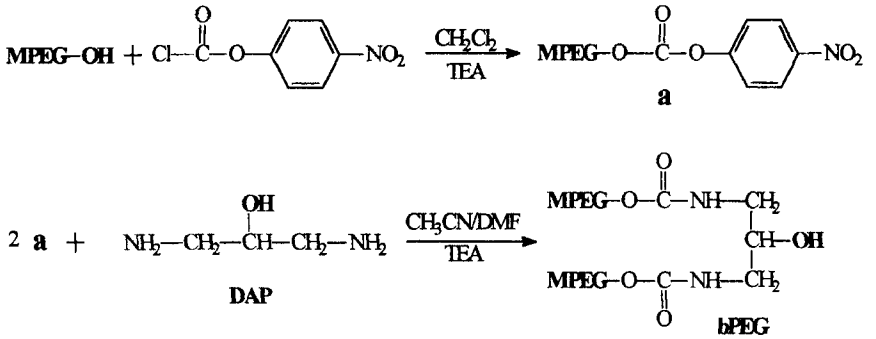
The therapeutic application of oligonucleotides as antisense and antigene drugs calls for their chemical modification to achieve better pharmacological behaviors, as increased *in vivo* stability and cell membrane permeability[1]. Among the different reactions, the conjugation with polymeric molecules, as polyethylene glycols (PEGs) of low-[2] and high-molecular weight[3], has been investigated. PEG is non-toxic, very soluble, and non immunogenic. The PEG-conjugates usually exhibit : i) high protection against the enzymatic degradation, ii) more tendency to penetrate the cell membranes, and iii) a low capability to give aspecific interactions with endogenous proteins[4].

The PEG chains may be introduced before the oligonucleotide synthesis, using a modified solid support[5], or after, with properly activated chains[6]. The positive effects provided by PEG generally increases with its molecular dimensions; however, at the same time, its chemical reactivity decreases, thus making difficult the conjugation synthesis. To overcome this obstacle recently a new synthetic procedure, based on the application of the liquid-phase method HELP has been devised[7]. In this process the original protocol for the oligonucleotide synthesis has been modified by introducing a stable phosphate bond between the growing oligonucleotide and the polymeric chain; thus, the PEG acts both as a polymeric synthetic support and as the final conjugated moiety.

Recently, a new high-molecular weight PEG derivative has been proposed for its use as protein modifier[8]. This polymeric unit has been obtained by reaction of two shorter PEG chain with an appropriate multifunctional linker. This procedure avoids the problems present with high-molecular weight PEGs due to their high polydipersity and low purity level. In this case the reactive moiety is located in the center of the final polymeric chain, instead of at the terminus, if a monomethoxy polyethylenglycol (MPEG) is employed. The final products is therefor a monofunctional, branched derivative (bPEG).

To obtain a new branched PEG with a single OH functionality, to be phosphorylated following the same chemistry previously utilized for the synthesis of linear PEG-conjugated oligonucleotides, we have employed the 1,3-diamino-2-propanol (DAP) as linker. The same approach has been independently proposed, but for different purposes, in a very recent paper[9].

The scheme of the reaction is the following :



The MPEG (M.W. = 5000 Da) activation has been performed by modification of the free OH of the starting molecule with p-nitrophenylchloroformate in basic organic solution. The reaction has been repeated twice to obtain a complete modification of the starting polymer. The reaction of the MPEG-p-nitrophenylcarbonate (**a**) with the DAP unit has then been performed in a CH_3CN/DMF mixture to overcome the low solubility of this molecule. After refluxing for 72 hours, the final product (bPEG) has been collected by precipitation with ether, filtration, and recrystallization from EtOH. The TNBS analysis indicated a 76% modification of the starting NH_2 groups of DAP. A gel-filtration procedure on BioGel P60 has allowed the final purification of product from partially and unreacted MPEG molecules.

The ^1H NMR and GPC analyses have confirmed the identity of the product (FIG. 1).

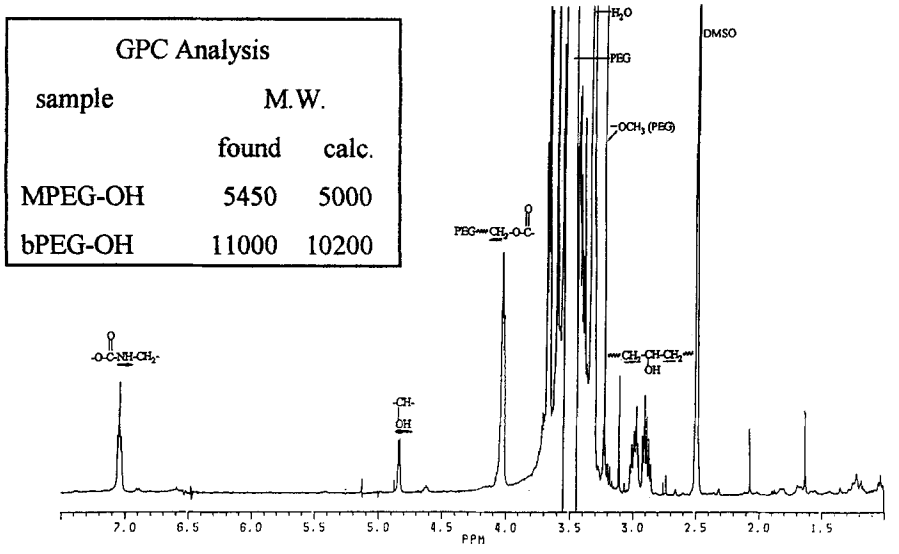


FIG.1 ^{1}H NMR spectrum of bPEG in DMSO-d_6 (GPC data in the inset)

To investigate the effect of a branched PEG chain on the biological properties of its conjugated antisense oligonucleotide, the same 12mer previously obtained on a linear MPEG, of comparable size, has been synthesized. The bPEG moiety has been successfully functionalized by introducing the first nucleotide of the growing chain as its 3'-amidite derivative. A lower reactivity of the free OH group has been observed, when compared to the linear MPEG ; very likely this is due to the steric hindrance of the two MPEG chains surrounding the reactive group. An almost quantitative reaction has been observed only after two repeated condensation reactions, as confirmed by UV and NMR investigations. The 12mer has been synthesized by following the same protocol set up for the linear derivative[7], with the exception of a repeated cycle of the condensation step during the addition of the first three nucleotides, due to the low reactivity of the terminal 5'-OH group of the growing chain at that stage. The final yield was consistent with the same molecule obtained with the linear MPEG ; an average yield of 96.5%, compared to 98%, has been observed. Particular attention has been devoted to the stability of the urethane linkages between the bPEG and the DAP component during the final deblocking procedure of the synthesized oligonucleotides. A partial release of the nucleic

acid component has been observed only when severe condition of time and temperature has been employed, well far from those adopted in the standard deblocking process. The IE HPLC analysis of the final product revealed a less favorable chromatographic pattern than the liner one derivative. The expected product hold for less than 20 % of the entire mixture, 1/3 of the amount previously obtained from the synthesis of the single chain MPEG-conjugated. The identity of the bPEG-12mer has been confirmed by GPC and NMR analyses.

Further investigations are now in progress to verify the effectiveness of this new branched polyethylene glycol in improving the biological properties and the antisense activity of the conjugated oligonucleotide.

REFERENCES

1. De Mesmaeker, A. ; Häver , R.; Martin, P. ; Moser, H.E. *Acc. Chem. Res.*, **1995** ***28***, 366-374.
2. Durand, M. ; Peloille, S. ; Thuong, N.T. ; Maurizot, J.C. *Biochemistry*, **1992** ***31***, 9197-9204.
3. Manoharan, M. ; Tivel, K.L. ; Andrade, L.K. ; Mohan, V. ; Condon, T.P., Frank-Bennet, C. ; Dan Cook, P. *Nucleosides & Nucleotides*, **1995** ***14***, 969-973.
4. Harris, J.M. in *Poly(ethylene glycol) Chemistry : Biotechnical and Biomedical Applications*, Harris, J.M. Ed. ; Plenum Press New York **1997** pp. 1-14.
5. Jäschke, A. ; Fürste, J.P. ; Nordhoff, E. ; Hillenkamp, F. ; Cech, D. ; Erdmann, V.A. *Nucleic Acids Res.*, **1994** ***22***, 4810-4817.
6. Tarasow, T.M. ; Tinnermeier, D. ; Zyzniewski, C. *Bioconjugate Chem.*, **1997** ***8*** 89-93.
7. Bonora, G.M. ; Ivanova, E. ; Zarytova, V. ; Burcovich, B. ; Veronese, F.M. *Bioconjugate Chem.* **1997** in press.
8. Monfaredini, C. ; Schiavon, O. ; Caliceti, P. ; Morpurgo, M. ; Harris, J.M. ; Veronese, F.M. *Bioconjugate Chem.*, **1995** ***6***, 62-69.
9. Martinez, A. ; Pendri, A. ; Xia, J. ; Greenwald, R.B. *Macromol. Chem. Phys.* **1997** ***198***, 2989-2489.