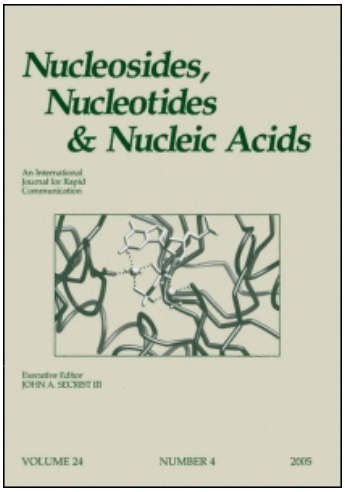

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COMPARISON OF PHYSICOCHEMICAL AND BIOLOGICAL PROPERTIES OF (*o*-CARBORAN-1-YL)METHYLPHOSPHONATE AND METHYLPHOSPHONATE OLIGONUCLEOTIDES.

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ABSTRACT. (*o*-Carboran-1-yl)methylphosphonate (CBMP) oligonucleotides demonstrate advantages in their physicochemical and biological characteristics over the corresponding methylphosphonate oligonucleotides.

INTRODUCTION

The rationale for the synthesis of boron containing oligonucleotides is their potential application as boron rich carriers for boron neutron capture therapy (BNCT) and as antisense oligonucleotides for antisense oligonucleotide technology (AOT).[1,2]

Sood et al.[3] and Shaw et al.[4] described the synthesis of boranophosphate methyl ester and boranophosphate oligonucleotide analogues. Recently we described the synthesis of dinucleotide[5] and oligonucleotides bearing (*o*-carboran-1-yl)methylphosphonate (CBMP) internucleotide group at different locations within the oligonucleotide chain.[6]

In this communication we compare the physical and biological characteristics of CBMP oligonucleotides to the more common methylphosphonate oligomers.

RESULTS

*Lipophilicity of CBMP (**8-10**) and methylphosphonate (**8a-10a**) oligonucleotides.* The dodecathymidylates **8-10** bearing CBMP group at different locations in oligonucleotide chain express substantially increased lipophilicity as measured by affinity to C18 resin (HPLC-R_t) compared to unmodified $d(T)_{12}$ (**7**) and methylphosphonate oligonucleotides **8a-10a**. The lipophilicity increased in the order: $d(T)_{12}$ (**7**) < $dT_{PMe}d(T)_{11}$ (**9a**) ≈ $d(T)_{6PMe}d(T)_6$ (**8a**) = $d(T)_{11PMe}d(T)$ (**10a**) < $d(T)_{6PR}d(T)_6$ (*closo*-**8**) ≈ $dT_{PR}d(T)_{11}$ (*closo*-**9**) < $d(T)_{11PR}d(T)$ (*closo*-**10** Fast) < $d(T)_{11PR}d(T)$ (*closo*-**10** Slow) (PR = CBMP).

T_m measurements. Melting temperature (T_m) measurements of the duplexes between CBMP oligonucleotides **8-10** or methylphosphonate oligonucleotides **8a-10a** and poly r(A) as a complementary sequence, were compared to those formed between unmodified $d(T)_{12}$ (**7**) and template. Significant effects on T_m were noted, depending on the location of the modification within the oligonucleotide chain. The T_m value for all oligonucleotides studied was significantly higher than unmodified $d(T)_{12}$ (**7**). The T_m

increased in the order: $d(T)_{12}$ (**7**) < $d(T)_{6PR}d(T)_6$ (*closo*-**8**) ≈ $dT_{PMe}d(T)_{11}$ (**9a**) < $d(T)_{6PMe}d(T)_6$ (**8a**) < $d(T)_{11PMe}d(T)$ (**10a**) < $d(T)_{PR}d(T)_{11}$ (*closo*-**9**) (PR = CBMP). As anticipated, the bulky CBM group caused more unfavorable steric interactions with duplex components than a methyl group, resulting in generally lower T_m s for **8-10** than for **8a-10a**.

The exception was CBMP oligonucleotide *closo*-**9**, containing the modification at 5'-end which formed a highly stable duplex with a T_m of 38°C. The high stability of the helix observed for *closo*-**9** is probably due to the higher freedom of motion of base pairs at the 5'-end of the helix than in the middle of the duplex or its 3'-end.

*Resistance of CBMP (**8-10**) and methylphosphonate (**8a-10a**) oligonucleotides to 3'-exonuclease activity of SVPDE.* To test resistance of CBMP oligonucleotides toward 3'-exonuclease, phosphodiesterase I from snake venom (SVPDE) was used. A pronounced effect of the carboranyl modification on oligonucleotide resistance toward SVPDE, was observed in the case of 3'-terminal location of CBMP internucleotide linkage. The $t_{1/2}$ for oligonucleotide $d(T)_{11PR}d(T)$ (*closo*-**10** Fast) and (*closo*-**10** Slow) containing a carboranyl modification at 3'-end was about 75% and 100% higher, respectively, than for the corresponding methylphosphonate oligonucleotide. Unmodified oligonucleotide $d(T)_{12}$ (**7**) and oligonucleotides containing methylphosphonate as well as CBMP modification in the middle of the oligonucleotide chain or at the 5'-end were digested rapidly by SVPDE.

As anticipated carboranyl oligonucleotides bearing modification at 5'-end were completely protected from digestion by 5'-exonuclease from bovine spleen (BSPDE).

*Phosphorylation of CBMP oligonucleotides **8-10** and methylphosphonate oligonucleotides **8a-10a** by T4 polynucleotide kinase.* Phosphorylation of CBMP-containing oligonucleotides with T4 polynucleotide kinase was observed for all oligonucleotides **8-10** and **8a-10a** with the exception of oligonucleotide bearing CBMP group at the 5'-end of the oligomer (*closo*-**9**).

The efficacy of phosphorylation was dependent upon the type of modification (*CBMP- vs.* methylphosphonate group) and its location within oligonucleotide chain. The susceptibility to phosphorylation by T4 polynucleotide kinase increased in order: $d(T)_{PR}d(T)_{11}$ (*closo*-**9**) (no phosphorylation), $d(T)_{11PR}d(T)$ (*closo*-**9**) ≈ $d(T)_{6PR}d(T)_6$ (*closo*-**8**) ≈ $d(T)_{PMe}d(T)_{11}$ (**9a**) < $d(T)_{6PMe}d(T)_6$ (**8a**) ≈ $d(T)_{PMe}d(T)_{11}$ (**10a**) ≈ $d(T)_{12}$ (**7**) (PR = CBMP). Interestingly some phosphorylation of **9a** was observed. This is unexpected since it is known that T4 polynucleotide kinase requires a 3'-phosphate group for efficient 5'-end phosphate attachment.

CONCLUSIONS

The advantages of carborane-containing oligonucleotides as compared to methylphosphonate counterparts include increased resistance to enzymatic digestion, increased lipophilicity, and formation of stable duplexes with complementary templates if the location of CBMP modification within the oligonucleotide chain is judiciously chosen.

Due to a prominent effect of carboranyl group on the carrier, the desired modulation of physicochemical and biological characteristics of the oligonucleotide molecule may be achieved with a limited number of modified internucleotide linkages. This, is in contrast to phosphorothioate or methylphosphonate oligonucleotides which usually must contain all internucleotides linkages modified to obtain the more desirable physicochemical properties. This often results in increased toxicity and other adverse effects.

These studies provide evidence of improved physicochemical and biological properties for CBMP oligonucleotides. Studies are undergoing to determine the potential medical utility of these modified oligonucleotides as antisense agents and as boron carriers for BNCT.

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