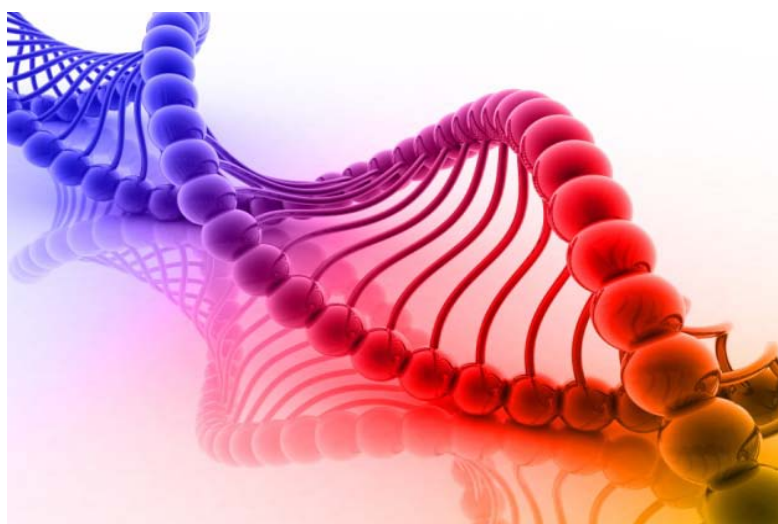


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Reduction of metal ions by boranephosphonate DNA†‡

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Oligodeoxyribonucleotides bearing boranephosphonate linkages (bpDNA) were shown to reduce a number of metal ions and form nanoparticles through a novel reaction pathway that leads to phosphate diesters or phosphate triesters in water or alcohols respectively. The synthetic utility of this reaction was further demonstrated through the synthesis of oligodeoxyribonucleotides containing phosphate triester linkages. This new reactivity also makes bpDNA promising for use in construction of DNA templated metallic nanostructures.

Boranephosphonate DNA (bpDNA) is a DNA derivative where one of the non-bridging oxygens of the phosphate backbone has been replaced by a BH₃ group (Fig. 1a).¹ bpDNA was designed as a hydrolytically stable molecule, capable of mimicking the structural and biochemical properties of DNA. Unlike other DNA/RNA analogs however, the presence of the BH₃ group, which is a well-known reducing agent,² raises interesting possibilities for combining the binding properties expected of a DNA mimic with a chemically functional moiety. Specifically, we wanted to explore the reductive properties of bpDNA towards noble metal ions such as Au(III), Ag(I) and Pt(II). Such reactivity would be of significant interest in the area of DNA templated metal nanostructures where much effort has been expended towards development of methods for site-specific and controlled reduction of conductive metals onto DNA scaffolds.^{3,4} bpDNA, where the reductive moiety is an inherent part of the backbone has several advantages: (1) these linkages can be located at every position on the oligonucleotide or distributed amongst phosphate linkages as required, (2) the boranephosphonate linkage is stable to all conditions used in the preparation of the nanostructures and no *in situ* treatment, as in the case of aldehydes,^{4c} is necessary and (3) these linkages can be introduced using well-established chemical^{5,6} and enzymatic⁷ methods.

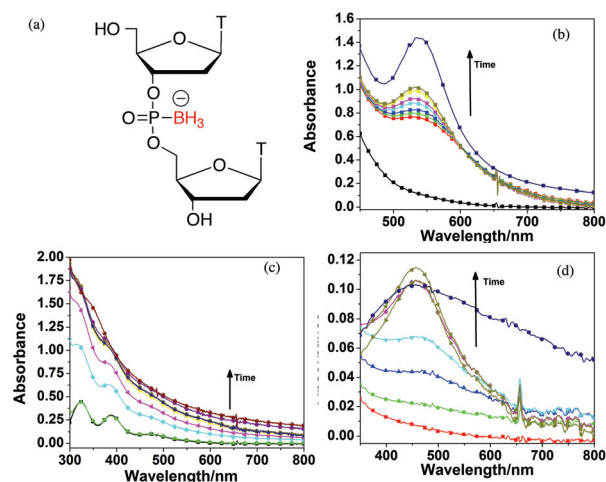


Fig. 1 (a) Structure of dithymidine boranephosphonate (**bpT₂**). Reduction of (b) AuCl₄⁻, (c) PtCl₄²⁻ at room temperature and (d) Ag⁺ at 55 °C by **bpT₂**. **bpT₂** was added at a final concentration of 200 μM to solutions containing the metal ions at a concentration of 5 mM. Absorbance spectra were recorded at 1, 10, 30, 60, 120, 180, 240 min and overnight.

Here we report that bpDNA is able to reduce various metal ions while producing the corresponding nanoparticles. In addition, investigations of the reaction have revealed a heretofore unknown and synthetically useful reaction pathway for boranephosphonates. We have found that during the reduction of metal ions in protic solvents, the B–P bond undergoes solvolysis and generates phosphate diesters (in water) or triesters (in alcohols). As a demonstration of the utility of this reaction we have carried out the synthesis of an oligomer containing an *O*-methyl phosphate triester linkage. Such triesters are base labile⁸ and cannot be prepared *via* usual methods of DNA synthesis. Thus in addition to the potential applications in nanoscience the new reactivity of boranephosphonates described here can be exploited as a general method for the introduction of various functionalities onto the DNA backbone *via* phosphate triesters.

Initial experiments were carried out with a dithymidine boranephosphonate (Fig. 1a, **bpT₂**)^{1b} and a fully boronated 21-mer oligodeoxyribothymidine (**bpT₂₁**). **bpT₂** reduced both AuCl₄⁻ and PtCl₄²⁻ readily to their zero oxidation states. These atoms then aggregate to produce nanoparticles. Formation of Au nanoparticles led to the solution turning pink with characteristic

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† This manuscript is dedicated to H. Gobind Khorana – one of the most influential scientists of the 20th century. Gobind was our lifelong friend and mentor who taught us how to do good science and to enjoy the path it created for each of us.

‡ Electronic supplementary information (ESI) available: Experimental details, additional data. See DOI: 10.1039/c2ob26661j

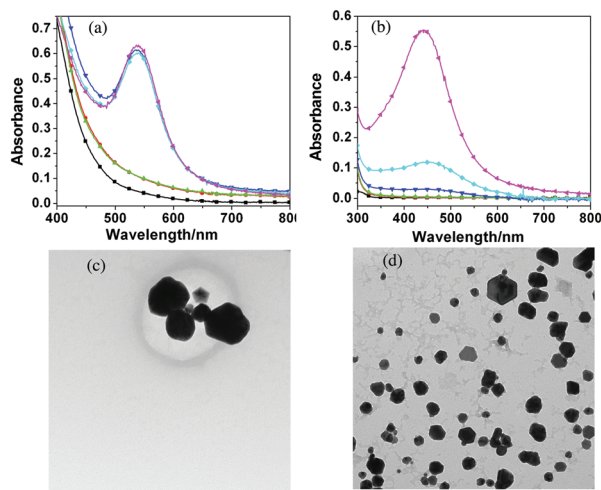


Fig. 2 Reduction of (a) AuCl_4^- and (b) Ag^+ by bpT_{21} . Metal ion concentration was 5 mM whereas the oligomer was added to a final concentration of 2.5 μM . Absorbance spectra were recorded before addition of DNA, then at 30 s, 5 min, 1 h, 4 h and overnight. TEM micrographs of (c) gold and (d) silver nanoparticles produced from reduction by bpT_{21} . The scale bar in both images is 100 nm.

absorbance due to the surface plasmon resonance band at 525 nm (Fig. 1b and S1a†) which increases with time as the reaction progresses. Pt nanoparticles have a broad surface plasmon resonance band located in the ultraviolet region with a tail extending into the visible⁹ (Fig. 1c) that causes a darkening of the solution (Fig. S1b†). In contrast no reaction was observed upon addition of bpT_2 to a solution of AgNO_3 , consistent with the lower reduction potential of Ag^+ . Reduction however did occur slowly, upon warming the mixture to 55 °C (Fig. 1d). The 21-mer (bpT_{21}) on the other hand not only reduced AuCl_4^- readily but the reaction with Ag^+ was also found to proceed at room temperature (Fig. 2a and b respectively). Cu^{2+} which has an even lower reduction potential was not reduced by either bpT_2 or bpT_{21} even upon prolonged heating at 55 °C. The difference between the dimer and the 21-mer with respect to Ag^+ is likely a consequence of additional binding sites for Ag^+ on bpT_{21} , which leads to higher local concentration of the cation, as well as greater stabilization of the growing nanoparticles through coordination of the DNA nucleobases to the metal nanoparticle surface. The gold and silver nanoparticles formed in the above reactions were characterized by transmission electron microscopy (Fig. 2c and 2d). Expectedly, in the absence of any specific templates a broad distribution of shapes and sizes were observed.

Reports from our lab⁵ and others¹⁰ have shown that the BH_3 moiety in boranephosphonates reacts with strong oxidizers such as peroxides and triphenylmethyl cations to form H-phosphonates. Wada and co-workers¹⁰ have utilized this pathway for the synthesis of several phosphate derivatives through further reactions of the resulting H-phosphonate.¹⁰ This led us to investigate whether a similar pathway was valid in the present case as well. We were also concerned about the fate of the internucleotide linkage, as strand cleavage during reduction would be problematic for nanotech applications by compromising the structural integrity of the DNA scaffold. The reaction of bpT_2 with

AuCl_4^- was chosen for further investigations, as it was the strongest oxidant among the metal salts studied here and therefore most likely to cause degradative reactions. A combination of ^{31}P NMR and ESI-MS was used initially to characterize the products of these reactions in water containing an excess of AuCl_4^- . The mixtures were incubated overnight at room temperature to ensure completion of the reaction. Analysis of the ^{31}P NMR spectra showed that upon reacting with AuCl_4^- , the broad resonance of bpT_2 , characteristic of a boron coupled phosphate P atom at 94 ppm (Fig. 3a), disappears completely. In its place, a single new sharp resonance at -1 ppm was observed (Fig. 3b), which indicates cleavage of the P–B bond and formation of a single phosphate species. The identity of this compound was confirmed by comparing the ESI-MS spectrum of the starting material with that of the product mixture (Fig. 3c and 3d). The peak at 543 Da corresponding to the starting material was shifted by 2 units to 545 Da in the product mixture. More importantly the isotope distribution of the two peaks was found to be markedly different. In the starting material the relative isotope distribution of the ^{11}B (80.1%) and ^{10}B (19.9%) gives rise to a spectrum where the $M + 1$ peak is the most intense whereas this effect is absent in the product mixture in accordance with the loss of boron. Comparisons with theoretical isotope distributions confirmed that the resulting compound is a dithymidine having a phosphate internucleotide linkage. The ^{11}B $\{^1\text{H}\}$ NMR (Fig. S2†) further showed that the two diastereomeric peaks of starting material at -40 ppm disappear completely and are replaced by a single peak at 19 ppm which corresponds to $\text{B}(\text{OH})_3$.¹¹

When the same reaction was carried out in other protic solvents such as methanol or *n*-butanol, we found that the product obtained was the corresponding *O*-alkylphosphate triester. This was confirmed by ESI-MS as well as by the appearance of two peaks in the ^{31}P NMR (Fig. S3 and S4†) corresponding to a pair of diastereomers. On the other hand, no reaction occurs in non-protic solvents such as acetonitrile and 1,4-dioxane. We note that the reported reaction of boranephosphonates with trityl cations^{5,10} in contrast, is able to proceed in aprotic solvents (anhydrous dichloromethane) and form H-phosphonates. This implies that the present reaction represents a distinct mode of reactivity that does not involve an H-phosphonate intermediate.

The reported mechanism of reduction by amine–boranes in solvents such as water or alcohols proceeds through solvolysis of the amine–borane complex and formation of the corresponding ammonium salt of boric acid (or ester) and release of dihydrogen (the active reducing agent). In the present case, the requirement of a protic solvent as well as the solvolysis of the P–B bond accompanied with the formation of $\text{B}(\text{OH})_3$ indicates that a similar mechanism may be valid. While further experiments are undoubtedly needed to delineate the exact mechanism, the overall reaction based on the evidence presented here may be depicted as in Scheme 1.

Based on these results we next investigated whether this reaction could be used for the synthesis of oligodeoxynucleotides containing phosphate triester linkages (Scheme 2). Non-ionic phosphate triesters such as the *O*-methyl phosphate triester are believed to have promising biochemical properties. However these phosphate derivatives are unstable to the basic conditions for deprotection and cleavage used routinely following DNA

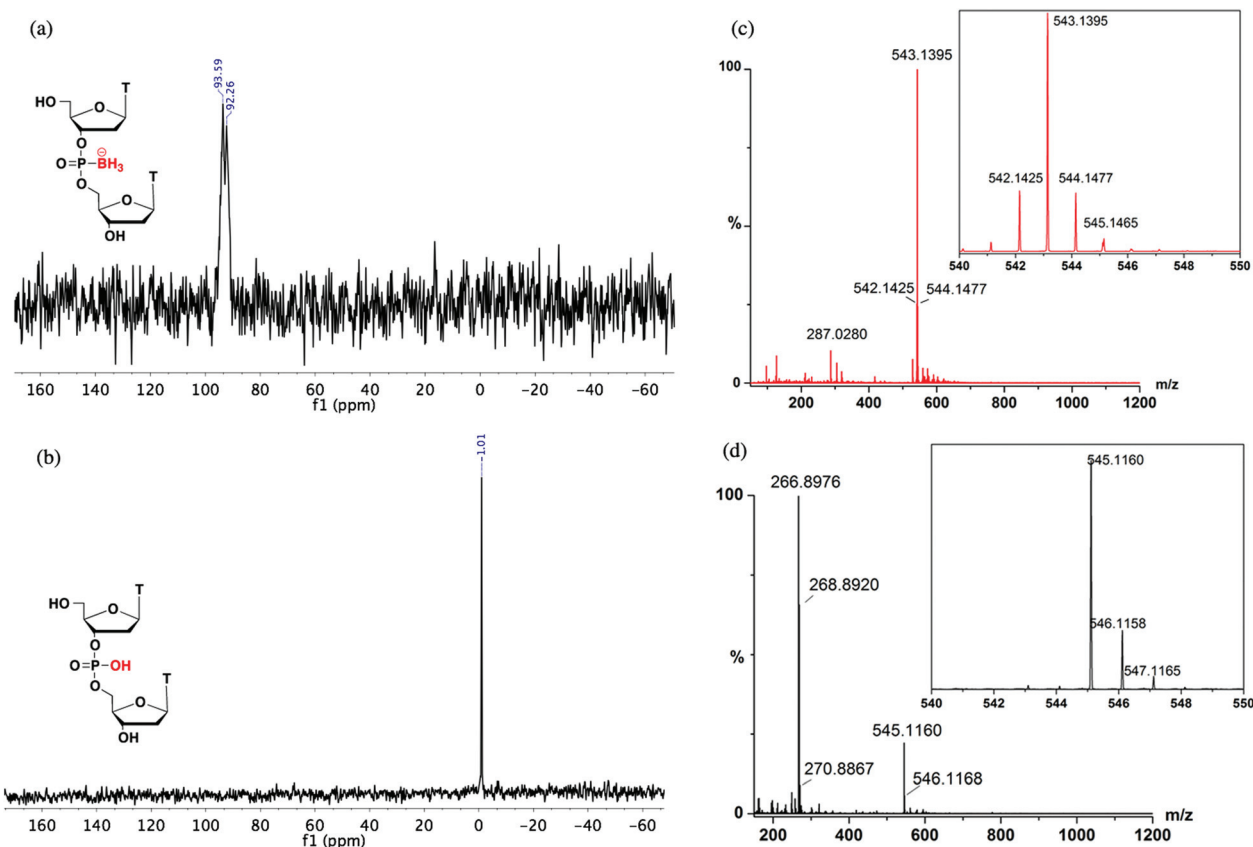


Fig. 3 ^{31}P NMR and ESI-MS analysis of the reaction of bpT_2 with AuCl_4^- in water showing the conversion of the boranephosphonate to phosphate. (a) and (c) correspond to the starting material whereas (b) and (d) were recorded for the product mixture.

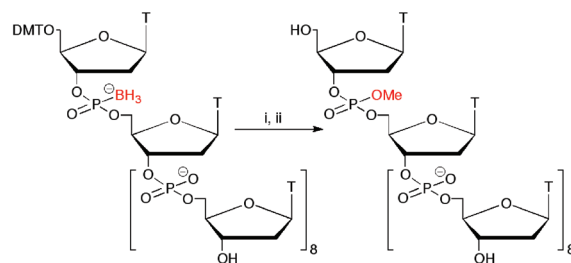
synthesis. Thus to demonstrate as a proof of principle, the usefulness of this reaction in introducing phosphate triester linkages at specific locations in the DNA backbone, we synthesized a decaoxythymidine containing a single boranephosphonate internucleotide linkage (see ESI† for details). The 5' dimethoxytrityl group was left intact to improve solubility in methanol as well as enable DMT on/off purification. This oligodeoxynucleotide was then reacted with AuCl_4^- in methanol. HPLC analysis and MALDI-TOF MS following DMT on/off purification demonstrated exclusive conversion of the boranephosphonate linkage into the *O*-methyl phosphate triester (Fig. S5†).

In conclusion, we have demonstrated that the BH_3 group present in the backbone of bpDNA is able to reduce three noble metal ions. This reactivity represents a novel reaction pathway whereby the boranephosphonate itself is cleanly converted to phosphate without degradation of the internucleotide backbone. This not only makes bpDNA attractive for the construction of DNA templated metal nanostructures, but the reaction is also useful for synthesis of oligomers containing phosphate triesters. As a proof of principle, the synthesis of a DNA oligomer containing a phosphate triester linkage was carried out. Current

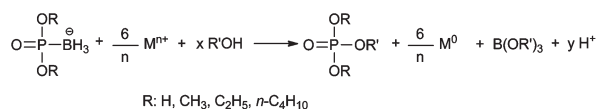
efforts in our laboratory are directed towards construction of metalized nanostructures as well as using other alcohols for introduction of additional functionalities into the DNA backbone as a phosphate triester.

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Scheme 2 Synthesis of oligonucleotide containing an *O*-methyl-phosphate triester linkage. (i) 25 mM HAuCl_4 in anhydrous MeOH. (ii) DMT on/off purification using a Polypak II cartridge.



Scheme 1

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