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1,1-Dihydroperoxycyclododecane as a new, crystalline non-hygroscopic oxidizer for the chemical synthesis of oligodeoxyribonucleotides

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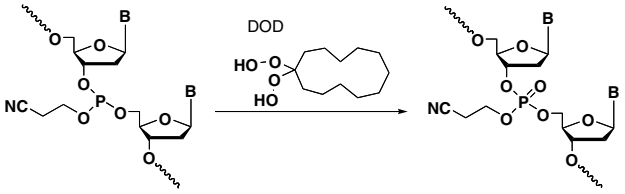
Abstract—A new oxidation method for DNA synthesis was developed by the use of 1,1-dihydroperoxycyclododecane in CH_2Cl_2–EtOAc under anhydrous conditions. This new oxidizer was successfully applied to the synthesis of oligodeoxyribonucleotides that involves an oxidation step for conversion of phosphite intermediates to phosphate derivatives.

A number of methods in molecular biology and DNA-based diagnostics to amplify, detect, analyze, and quantify nucleic acids require chemically synthesized and modified oligonucleotides.[1]

The so-called phosphoramidite method currently used for the synthesis of DNA/RNA has proved to be the most reliable and reproducible.[2] The synthetic cycle of this strategy involves four steps of detritylation: coupling reaction, oxidation, and capping reaction. In this cycle, the oxidation was commonly carried out by the use of iodine in aqueous pyridine as a convenient, economical, and simple reagent. However, this conventional method required an aqueous solution for the complete conversion of phosphite intermediates to phosphate derivatives. Mullah and co-workers.[3] also reported that the dimethylaminomethylene group introduced as the protecting group into the 2-amino group of deoxyguanosine was converted to a nitrile group when a 0.1 M I_2 solution was used for the oxidation. In the DNA/RNA synthesis by use of the phosphoramidite approach, the existence of water in the reaction vessel inhibits the coupling reaction. To overcome this problem, a non-aqueous reaction system for the oxidation should be developed. Actually, a number of methods for the oxidation of phosphite intermediates under anhydrous conditions have been reported by several researchers to date.[4a–g] Most of the non-aqueous oxidizers previously reported were liquid, expensive, and explosive. Particularly, *t*-BuOOH[4a] has been often used in anhydrous solvents for the DNA synthesis. Butanone peroxide was reported to be a mixture of several kinds of peroxide derivatives so that the actual effective components are unclear.[4e]

In this letter, we report 1,1-dihydroperoxycyclododecane as a new, crystalline, and convenient oxidizer and demonstrate the usefulness of this new reagent applying it to DNA synthesis under anhydrous conditions (Scheme 1).

1,1-Dihydroperoxycyclododecane (DOD)[5] has been known as the starting material for the synthesis of an



Scheme 1. New reagent for oxidation of phosphite intermediates in DNA synthesis.

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anti-malaria agent.[6] This compound has two characteristic hydroperoxide functions at the geminal carbon. Although DOD has never been used as an oxidizing agent for certain organic reactions, it is strongly suggested that the *gem*-hydroperoxy structure of DOD has a possibility to act as an oxidant.

DOD can be easily prepared by the reaction of cyclododecanone, a commercially available hydrocarbon-cyclic ketone with a 12-membered ring, with a 30% hydrogen peroxide solution and crystallized as non-hygroscopic white crystals from Et_2O–hexane.[5]

The oxidation ability of DOD was examined by reaction with triphenylphosphine. Consequently, it turned out that this oxidizer could oxidize immediately triphenylphosphine to give triphenylphosphine oxide. This result led us to study further the potential ability of this reagent for the conversion of phosphite intermediates to phosphate derivatives during the automated DNA synthesis. A dodecathymidylate (T_{12}) was synthesized by the use of an ABI 392 DNA/RNA automated synthesizer. A 0.1 M solution of the oxidizer in THF was used. The protected oligothymidylate thus synthesized on CPG via a succinate linker was treated with concd NH_4OH at room temperature for 40 min. The released solution was directly analyzed by reverse-phase HPLC. When the oxidation time was set up to be 15 s, a complex mixture was obtained, as shown in Figure 1A. However, when the oxidation time was prolonged to 90 s, the desired product T_{12} could be obtained as an almost single peak, as shown in Figure 1A.

These results indicated that DOD has an excellent oxidation ability. Therefore, a DNA 12mer, GACTGACTGACT containing all four canonical nucleobases was synthesized under similar conditions. The protected oligodeoxyribonucleotide derivatives synthesized on CPG by the use of the phenoxyacetyl (pac) as the base protecting group was released from the support and deprotected by treatment with concd NH_4OH at room temperature for 8 h. The deprotected crude oligomer was analyzed, as shown in Figure 2A. It was found that the oligomer having the mixed sequence could be obtained as an almost single peak.

Among the solvents tested for dissolving DOD, THF was the best while this reagent was highly soluble in CH_2Cl_2 widely used for the current DNA synthesis. Thereby, the synthesis of the DNA 12mer was carried out by use of THF. However, it was found that DOD becomes freely soluble upon addition of ethyl acetate to CH_2Cl_2. Thus, CH_2Cl_2–EtOAc (9:1) was the choice of solvent. By use of this new oxidation solution, a DNA 30mer, TCTCCATCTGATGAGGCCGAAAGGCCGTAT was similarly synthesized without any difficulty, as shown in Figure 2B. The high purity of this oligomer was confirmed by enzymatic digestion and TOF-mass analysis, as shown in Figures 3 and 4.[7]

In conclusion, we have found 1,1-dihydroperoxycyclododecane (DOD) as a new, safe, and crystalline oxidizer. This new reagent was easily prepared by a simple procedure. The new reagent would be useful for the DNA/RNA synthesis under anhydrous conditions using CH_2Cl_2–EtOAc (9:1) as the solvent.

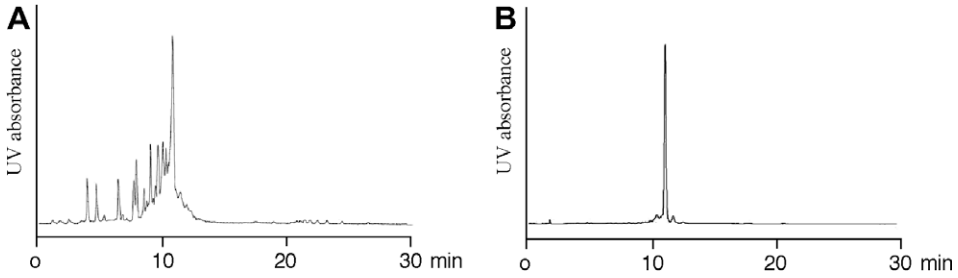

Figure 1. Reverse-phase HPLC profiles of the mixtures containing T_{12} obtained after the DNA synthetic cycle was finished. **A**: The oxidation time, 15 s, **B**: The oxidation time, 90 s.

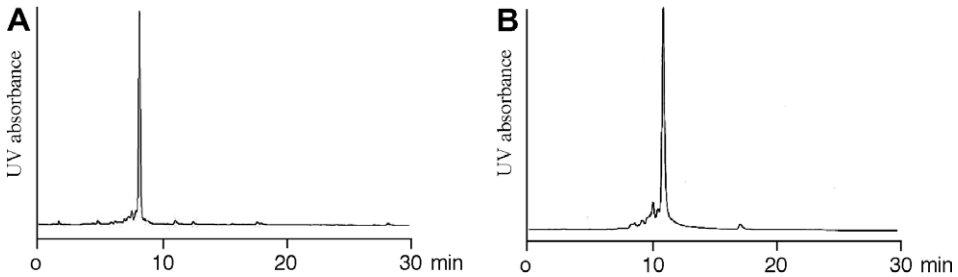

Figure 2. Reverse-phase HPLC profiles of the mixtures containing GACTGACTGACT (panel **A**) and 5′-TCTCCATCTGATGAGGCCGAAAGGCCGTAT (panel **B**) obtained after the synthetic cycle was finished.

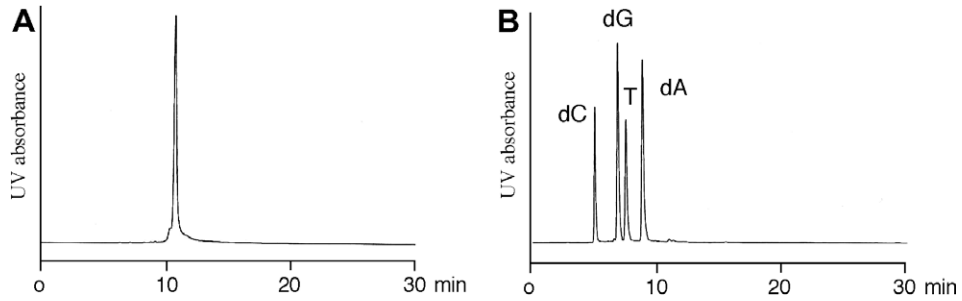

Figure 3. Reverse-phase HPLC profiles of the purified DNA 30mer (A) and the mixture obtained after its enzymatic digestion (B).

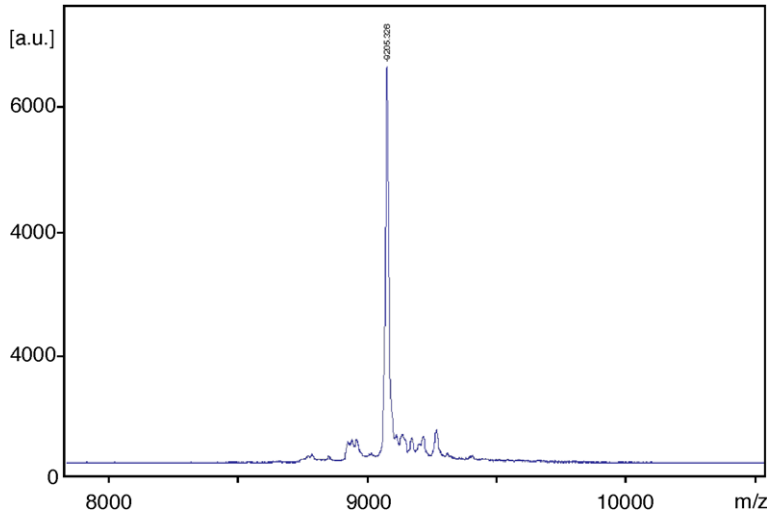

Figure 4. The MALDI-TOF mass analysis of the isolated DNA 30mer.

Acknowledgements

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