FINAL PROJECT REPORT

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GRANT TITLE: Preparation of Oligonucleotide Arrays for Hybridization Studies

REPORTING AND AWARD PERIOD: 2/15/92-5/14/96

OBJECTIVE:

This project was intended to develop new methods of DNA synthesis that will permit the production of large oligoarrays. The method used to accomplish this goal is light-directed, spatially-addressable parallel chemical synthesis. Because only the synthesis of peptides had been achieved with this method, the chemistry of photochemical nucleic acid synthesis needed to be developed. The key to modifying a polymer synthesis technique for this (VLSIPS) method is a photochemically-removable protecting group. For both the normal phosphoramidite and phosphotriester methods, this requirement implies a replacement for dimethoxytrityl. Thus, paramount among the needs was a photochemically-removable protecting group for the sugar hydroxyls of nucleosides. However, by modifying the position of the anchor of the oligo to the support, it was also possible to use currently-available photoremovable groups in our newly-developed photochemical inverse phosphotriester method. During the execution of this part of the program, we became involved with the research program of a collaborator, C. Thomas Caskey of the Baylor College of Medicine, who conceived the idea of arrayed-primer extension (APEX) as a method for detecting disease-causing mutations when they are spread throughout a gene. This proposed diagnostic/sequencing method incorporates the strengths and minimizes the weaknesses of SBH technology. The advantage to be offered by our new approach compared to sequencing-by-hybridization is much greater reliability in the analysis of the results; fluorescent termination signals have an additional "fidelity filter" of the DNA polymerase imposed on them and are much less ambiguous than hybridization signals. Since primer extension requires a free 3'-hydroxyl, this necessitated the novel development of inverse photochemical phosphoramidite DNA synthesis.

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ACCOMPLISHMENTS:

We have developed several novel ways to prepare DNA. In each, the deprotection step in each synthesis cycle is accomplished with light. The group that we developed for this purpose is the dimethoxybenzoin (DMB) group which, when attached to acidic functionalities, is readily removed with long wavelength (350 nm) UV irradiation that will not damage the DNA bases. Furthermore, unlike many other photoremovable groups, irradiation of the DMB group gives produces in very high yield a stable dimethoxy-2-phenylbenzofuran. The quantum yield is estimated to be fairly high, ~ 0.6 . The phenylbenzofuran (BF) is a very convenient byproduct in that it is relatively inert chemically. Besides the advantage in avoiding reactive byproducts, DMB groups offer a much more rapid photochemical cleavage (<50 nanoseconds) than nitrobenzyl protecting groups (milliseconds) that can permit photolithographic patterning to be accomplished more quickly by using greater radiance. The BF byproduct has a significant absorption and fluorescence that enables the conversion in a photochemical deprotection to be easily followed. We have demonstrated detection of the BF at concentrations as low as 10^{-6} M with UV (300 nm) and as low as 10^{-9} M with fluorescence (396 nm).

The DMB group was initially used as a phosphate derivative (paper 3), among which were 3'-nucleoside phosphates that could be used for the inverse phosphotriester DNA synthesis method (paper 1). It was concurrently developed for use in normal-direction phosphoramidite DNA synthesis as a 5'-carbonate derivative, and this method was directly compared with conventional acid-based deprotection (paper 2). This was a necessary prerequisite to inversion of the direction of synthesis for the inverse photochemical phosphoramidite-based DNA synthesis, which requires a 3'-carbonate derivative of DMB (unpublished work). These compounds are currently under investigation for the preparation of DNA chains. The preparation of the monomers for each of the foregoing chemistries, with different protecting and coupling groups on the 3' and 5'-hydroxyls of the ribose unit, requires much synthetic manipulation. This has been facilitated by a novel method for the removal of silyl protecting groups with the mild acid reagent, triethylamine trihydrofluoride (paper 4).

SIGNIFICANCE:

This work is significant in that it is the first to directly evaluate the yield and purity of DNA prepared using light-based synthesis. Since these traits affect the ability of the synthesized sequences to serve as either hybridization probes or extension primers, a method for the assessment and increase of purity as provided in our work is important to eventual high-fidelity sequence analysis.

PUBLICATIONS:

1. Michael C. Pirrung, Lara Fallon, Steven W. Shuey, and David C. Lever, "Inverse Phosphotriester DNA Synthesis Using Photochemically-removable Dimethoxybenzoinphosphate Protecting Groups," J. Org. Chem., **61**, 2129 (1996).
2. Michael C. Pirrung and Jean-Claude Bradley, "Comparison of Methods for Photochemical Phosphoramidite-based DNA Synthesis," J. Org. Chem., **60**, 6270 (1995).
3. Michael C. Pirrung and Steve W. Shuey, "Photoremovable Protecting Groups for Phosphorylation of Chiral Alcohols. Asymmetric Synthesis of Phosphotriesters of (-)-3',5'-Dimethoxybenzoin," J. Org. Chem., **59**, 3890 (1994).
4. Michael C. Pirrung, Steven W. Shuey, David C. Lever, and Lara Fallon, "A Convenient Procedure for the Deprotection of Silylated Nucleosides and Nucleotides Using Triethylamine Trihydrofluoride," Bioorg. Med. Chem. Lett., **4**, 1345 (1994).

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