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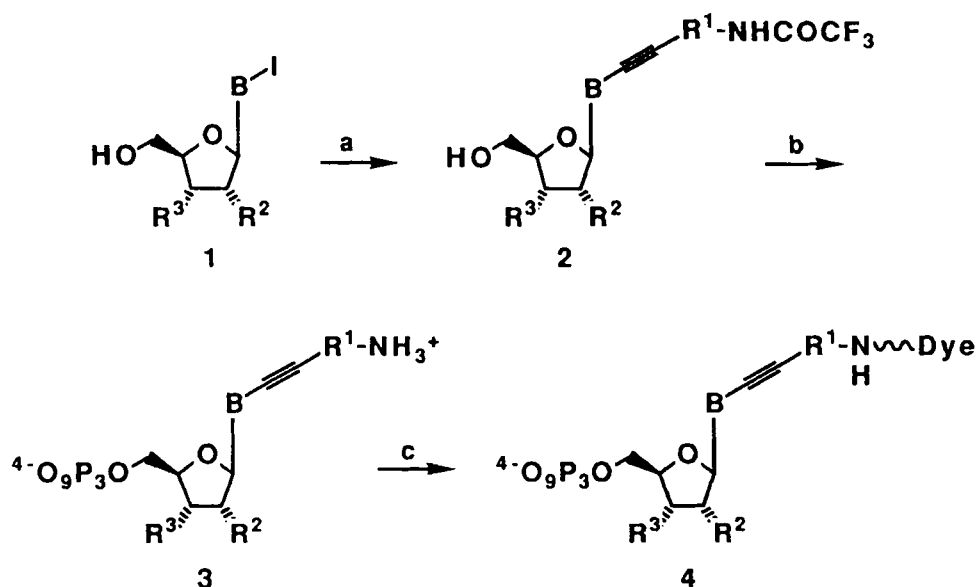
SYNTHESIS OF REAGENTS FOR FLUORESCENCE-TAGGING OF DNA

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Abstract: A general route for the synthesis of alkynylamino nucleoside triphosphates is described. These nucleosides can be selectively coupled through the alkynylamino group to a variety of reporter groups and used to enzymatically label DNA.

The detection and analysis of small quantities of nucleic acids has traditionally been done using radioactive nuclei as reporter groups. In 1981, Ward disclosed a method for enzymatically "biotinylating" hybridization probes¹ and, in 1987, we described a method for automated sequencing of DNA using fluorescence-tagged chain terminators.² These methods take advantage of the ability to detect the presence of as little as 10^{-18} moles of biotinylated or fluorescence-tagged nucleic acids. The key ingredients in both of these methods are nucleoside triphosphates which have been covalently labeled with large reporter groups in such a way that they are still substrates for DNA polymerases. This paper describes a general method for the preparation of "alkynylamino" nucleoside triphosphates **3**, reagents which are useful precursors for the preparation of such labeled polymerase substrates (**4**).

A variety of DNA polymerases tolerate the presence of large substituents located at or near the 5-position of pyrimidine nucleotides and the 7-position of 7-deazapurine nucleotides. Using a reaction first described by Bergstrom³, Ward coupled allylamine to dUTP to introduce an enzymatically acceptable site for biotinylation. In our hands, however, this coupling method worked poorly with cytidine nucleotides and apparently failed with 7-deazapurines. We have therefore developed a method for coupling a variety of alkynylamines to 5-iodopyrimidine nucleosides and 7-iodo-7-deazapurine nucleosides **1**.⁴ The novel 7-iodo-7-deazapurine precursors needed for this method were prepared by total synthesis.⁵ The resulting alkynylamino nucleosides **2** were converted⁶ to the corresponding 5'-triphosphates and deacylated with concentrated ammonium hydroxide. The amino group of the resulting triphosphates (**3**) serves as a site for attachment of a variety of fluorescent dyes and other reporter



a) $\text{HCCCH}_2\text{NHCOCF}_3$, $((\text{C}_6\text{H}_5)_3)_4\text{Pd}$, CuI , Et_3N / DMF, 25° . b) POCl_3 / $\text{PO}(\text{OCH}_3)_3$, then $(n\text{-BuNH}_3)_3\text{HP}_2\text{O}_7$ / DMF, then NH_4OH / H_2O . c) Standard methods.

groups. The resulting labeled nucleoside triphosphates (4) are frequently, but not always, efficiently incorporated into nucleic acids by polymerases. When the dye is a succinylfluorescein derivative, B is one of the four normal bases, and R^2 and R^3 are hydrogen, for example, substrate 4 chain terminates DNA polymerization by AMV reverse transcriptase and T7 DNA polymerase, but not the Klenow fragment of *E. coli* DNA polymerase I, nearly as efficiently as the corresponding unmodified dideoxynucleotide triphosphate. Further work using substrates with structure 4 to enzymatically label nucleic acids is in progress.⁶

REFERENCES

- 1) Langer, P. R.; Waldrop, A. A.; Ward, D. C. Proc. Natl. Acad. Sci. U.S.A. **1981**, 78, 6633.
- 2) Prober, J. M.; Trainor, G. L.; Dam, R. L.; Hobbs, F. W.; Robertson, C. W.; Zagursky, R. J.; Cocuzza, A. J.; Jensen, M. A.; Baumeister, K. Science **1987**, 238, 336.
- 3) Bergstrom, D. E.; Ruth, J. L. J. Am. Chem. Soc. **1976**, 98, 1587.
- 4) Hobbs, F. W. J. Org. Chem. **1988**, 53, 000.
- 5) Cocuzza, A. J. Tetrahedron Lett. **1988**, 29, 4061.
- 6) Trainor, G. L.; Jensen, M. A. submitted to Nucleic Acids Res.