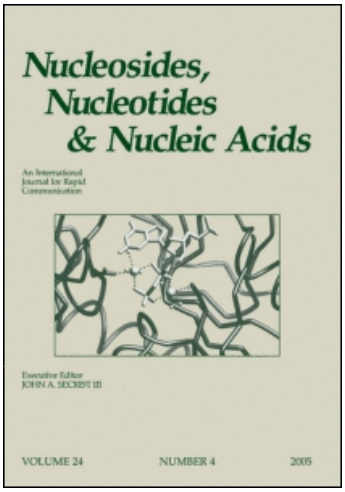

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Aryldiazomethane Derivatives as Reagents for Site Specific Labeling of Nucleic Acids at Phosphate

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Online publication date: 09 August 2003

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 22, Nos. 5–8, pp. 1371–1373, 2003

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ABSTRACT

An efficient and direct labeling method based on direct alkylation of nucleic acids at phosphates by aryldiazomethane derivatives is described.

Key Words: Aryldiazomethane derivatives; Phosphate alkylation; DNA labeling; Synthesis.

Parallel to current progress in DNA chip technology,[1–3] requirements for efficient nucleic acid labeling methods are growing. Most commonly used labeling methods involve enzymatic or chemical incorporation of modified nucleotides bearing a reporter or a reactive group.[4,5] Here we describe an alternative efficient and direct labeling method based on alkylation of nucleic acids at phosphates by aryldiazomethane derivatives.

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DOI: 10.1081/NCN-120022968
1525-7770 (Print); 1532-2335 (Online)

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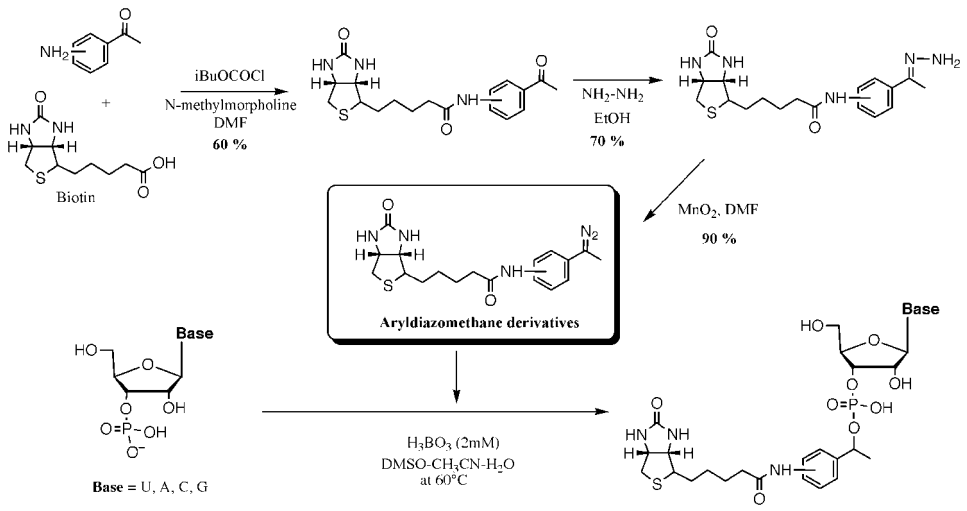

Scheme 1.

Series of aryldiazomethane derivatives tethered to reporter groups (fluorescent dyes or biotin) were synthesized typically as described in Scheme 1. Reaction of 2 mM solutions of the aryldiazomethane reagents with 40 μM nucleotide 3′-UMP in homogenous solution (H_2O-CH_3CN-DMSO: 1/3/1), borate buffer, pH 7.3, 60°C, 2 h yielded quantitatively the 3′-adducts corresponding to selective alkylation of the phosphate groups. The adducts were characterized by ^{1}H and ^{31}P NMR spectroscopy. Selective alkylation at the phosphate was also observed when aryldiazomethane was reacted in the same conditions with the 3′-AMP, 3′-GMP and 3′-CMP nucleotides.

Thus we have demonstrated the high specificity of the alkylation reaction on phosphate by these reagents at the nucleotide level. It is also noteworthy that the chemical properties (stability and solubility) of these reagents can be rationally modulated according to the nature of the substituents of the phenyl ring and/or of linker group.

The validity and efficiency of this new labeling strategy was further demonstrated by detection and identification experiments of PCR amplicons on DNA chips.

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