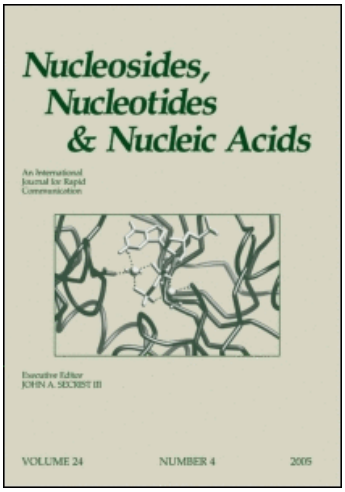

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Use of [4,6-Di-^{14}C]-5'-DMT-thymidine Phosphoramidite Reagent for the Radiolabeling of Synthetic Oligonucleotides

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To cite this Article Guzaev, Andrei , Boyode, Brunel , Balow, Guity and Manoharan, Muthiah(1999) 'Use of [4,6-Di-^{14}C]-5'-DMT-thymidine Phosphoramidite Reagent for the Radiolabeling of Synthetic Oligonucleotides', Nucleosides, Nucleotides and Nucleic Acids, 18: 6, 1389 — 1390
To link to this Article: DOI: 10.1080/07328319908044726
URL: http://dx.doi.org/10.1080/07328319908044726

NUCLEOSIDES & NUCLEOTIDES, 18(6&7), 1389–1390 (1999)

USE OF [4,6-DI-^{14}C]-5'-DMT-THYMIDINE PHOSPHORAMIDITE REAGENT FOR THE RADIOLABELING OF SYNTHETIC OLIGONUCLEOTIDES

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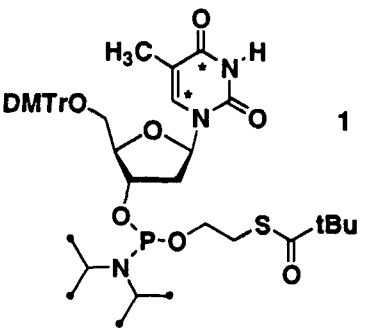
ABSTRACT: A novel synthesis of 5'-radiolabeled oligonucleotides is described. The labeling is carried out by the phosphoramidite method with the aid of building block **1**. The feasibility of the method is demonstrated by preparation of 5'-radiolabeled 3'-phosphorylated dodecathymidylate phosphorothioate.

Labeling with radioactive isotopes provides an efficient tool for studying pharmacological properties of antisense oligonucleotides. As with other classes of drug compounds, this novel class of therapeutics requires high sensitivity radiodetection for evaluation of distribution of antisense agents in tissues and assessment of their metabolic fate. Several methods to introduce ^{35}S, ^{3}H, or ^{14}C into synthetic oligonucleotides have been reported. Among these labels, ^{14}C offers the highest specific activity and the longest half-life. Considering catabolism of nucleic acids, labeling with ^{14}C at the C-2 position of thymidine results in formation of $^{14}CO_2$ which is cumbersome to trap and analyze. On the other hand, labeling at either the C-4 or C-6 position leads to β-aminoisobutyric acid as the metabolite which is much more convenient to analyze.



1

In this communication, the incorporation of radioisotope is achieved with the aid of phosphoramidite reagent **1** that is derived from [4,6-di-^{14}C]thymidine and thus possesses high specific activity.[2] Phosphoramidite **1** features the *S*-pivaloyl 2-mercaptoethyl

(SPME) group which has recently been reported as an enzyme-labile phosphate protecting group.[1] As the first step in our study of bioreversible protection of the internucleosidic (thio)phosphate moiety, we applied **1** to the preparation of phosphorothioate oligonucleotides.

To synthesize phosphoramidite reagent **1**, *bis*(diisopropylamino)chlorophosphine, was reacted with *S*-pivaloyl-2-mercaptoethanol to give corresponding bisamidite. (125.3 ppm; 70-75% yield). In the next step, [4,6-di-^{14}C]-DMT-T was treated with crude bisamidite in the presence of 1*H*-tetrazole followed by standard work-up.

Half-life of non-radioactive **1** (**1a**) in 80% aq MeCN was found to be 58 h, which permits safe isolation of **1** in aqueous media. Accordingly, crude product was purified by RP HPLC to give **1** in 78.8% yield.

In optimal condition, coupling procedure with **1** consisted of 3 main steps. First, a solid support bound oligonucleotide (10 μmol) was dried by treatment with a mixture of **1a** (10 μmol) and 1*H*-tetrazole for 1 min. This step was designed to result in a very low coupling yield (5 to 10%) due to the low concentration of the phosphoramidite. However, it efficiently consumed any traces of water that were not removed by washing with acetonitrile and extended drying. Next, solution of **1** (20 μmol) was delivered to the solid support followed by 1*H*-tetrazole, and the reaction mixture was kept for 20 min. Washing the support and performing the sulfurization reaction completed the step. As determined by DMT assay, coupling efficiency of **1** was 83% (41% yield with respect to **1**). Finally, the solid support was coupled with **1a** (50 μmol) and excess 1*H*-tetrazole in order to obtain uniform full-length support-bound oligonucleotide. After sulfurization, the product was treated with concentrated ammonia. Crude radiolabeled T*p$(Tp)_{11}$ was isolated by RP HPLC in 60% yield. The labeled product was analyzed by MALDI-TOF mass spectrometry and anion exchange HPLC to confirm the presence of homogeneous oligonucleotide free from any radioactive impurities.

REFERENCES

1. Tosquellas, G.; Alvarez, K.; Dell'Aquila, K.; Morvan, F.; Vasseur, J.-J.; Imbach, J.-L.; Rayner, B. (1998) *Nucleic Acids Res.*, **26**, 2069-2074, and references cited therein.
2. Guzaev, A.; Boyode, B.; Balow, G.; Tivel, K.L.; and Manoharan, M. (1998) *Bioorg. Med. Chem. Lett.*, **8**, 1123-1126.