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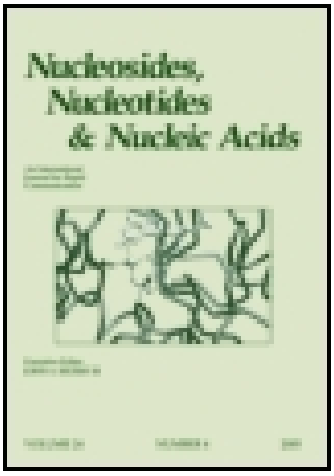

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A Convenient Synthesis of Deoxynebularine Phosphoramidite

Douglas Picken [a] & Vicki Gault [a]
[a] Link Technologies Limited , 2 Napier Court, Cumbernauld, Glasgow, G68 OLG, U.K.
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A CONVENIENT SYNTHESIS OF DEOXYNEBULARINE PHOSPHORAMIDITE

Douglas Picken* and Vicki Gault

Link Technologies Limited, 2 Napier Court, Cumbernauld, Glasgow G68 0LG, U.K.

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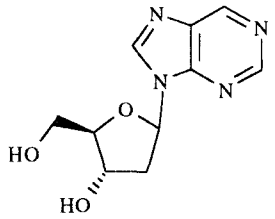
ABSTRACT

Enzyme catalysis by a cell free preparation from *Lactobacillus leichmannii* is used to accomplish the transformation of thymidine to deoxynebularine in the presence of purine. The resulting mixture of nucleosides is then converted to their 5'-dimethoxytrityl derivatives which are easily separated using conventional chromatography. Phosphitylation of the 5'-dimethoxytrityl-deoxynebularine then gives the phosphoramidite derivative suitable for incorporation into oligonucleotides.

INTRODUCTION

Deoxynebularine (9-β-D-2'-deoxyribofuranosylpurine) is the deoxy analogue of the naturally occurring antibiotic nebularine[1]. It is of interest in the systematic study of the structural elements involved in hydrogen bonding[2] and for its ability to form the N..CG motif in triple helical structures[3].



Deoxynebularine

Previous reports of the synthesis of this compound involved either a multi-step 2' deoxygenation sequence[4] starting from very expensive natural nebularine or the deamination of protected 2'-deoxyadenosine[5] in a non-aqueous diazotization reaction involving prolonged treatment at elevated temperatures. This we found to give variable results and led to highly coloured material. Because neither of these alternatives seemed totally satisfactory for the synthesis of multi-gram amounts of

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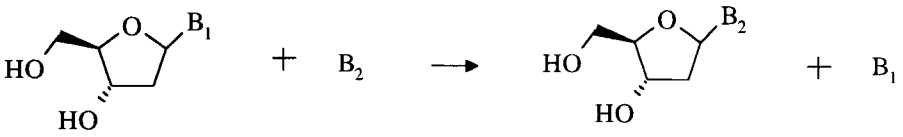
deoxynebularine, we developed a method based on the enzyme system[6] derived from *Lactobacillus leichmannii*. This enzyme system has previously been shown to be of use in the preparation of a variety of nucleoside analogues such as the 2'-deoxy and 2',3'-dideoxynucleosides of 2-aminopurine[7]. A previously reported enzymic preparation of deoxynebularine suffers from the disadvantage of requiring expensive 2-deoxyribose-1-phosphate as substrate[8].

RESULTS AND DISCUSSION

Nucleoside N-deoxyribosyltransferase (E.C. 2.4.2.6) catalyses the transfer between purine and pyrimidine bases and deoxynucleosides[9].



The insensitivity of the enzyme from *Lactobacillus* spp. towards organic solvents allows the use of crude preparations from these sources. The addition of solvents inhibits the competing activities of degradative enzymes while maintaining the activity of interest. We have investigated a variety of water-miscible and immiscible solvents as well as different nucleosides in this reaction (B1=thymidyl, cytidyl, uridyl; B2 = purine). For our preparative purposes, thymidine in the presence of purine dissolved in buffer containing 10% ethanol is transformed into the desired product -deoxynebularine. Using a five-fold excess of nucleoside, the reaction reaches 100% conversion of purine in a period of several days, without other side reactions being detectable by hplc. The resulting crude mixture of nucleosides is then most conveniently converted to their 5'-dimethoxytrityl derivatives by standard methods prior to separation by column chromatography. We have found that this is possible even in the presence of citrate buffer residues. Pure 5'-dimethoxytrityl-deoxynebularine is subsequently phosphitylated to yield the phosphoramidite derivative suitable for incorporation into oligonucleotides by automated synthesisers.

This synthesis has the advantages of being environmentally friendly by minimising the use of organic solvents, using cheap starting material and achieving high conversion of heterocyclic base to product nucleoside.

EXPERIMENTAL

Enzyme preparation. *Lactobacillus leichmannii* (ATCC4797) was grown in 5L MRS broth at 37°C for 24 hours (1% starter inoculum). The cells were harvested by centrifugation at 10000 x g for 15 minutes at 4°C and washed twice by re-suspension and centrifugation in 20mM PIPES buffer (pH 6.5, 0.02% sodium azide). The cell pellet (wet weight approximately 15g) was then frozen and thawed before being passed twice through a French press and made up to a volume of 10ml in the same buffer. After

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centrifugation at 25000 x g for 45 minutes at 4°C the supernatant was dialysed overnight against two changes of 4L PIPES buffer and then stored frozen until required for use.

Enzyme incubation and derivatisation. Thymidine (14.5g, 60mmol) and purine (2.4g, 20mmol) were dissolved in citrate buffer (0.1M, pH 6.0, 0.05% sodium azide) containing 10% ethanol (total volume 495ml) and enzyme extract was added (5ml). After incubation at 40°C for 6 days, the reaction mixture was evaporated to dryness, co-evaporated with pyridine (3 x 100ml) and the resulting solid was suspended in pyridine (100ml). Dimethoxytrityl chloride (20.5g, 60mmol) was added in several portions and the reaction allowed to proceed overnight. Following evaporation and aqueous workup, the mixture of nucleosides was separated by chromatography over silica gel, eluting with ethyl acetate. This yielded pure 5'-dimethoxytrityl-deoxynebularine (3.6g, 7 mmol) whose identity was confirmed by ^{1}H and ^{13}C nmr spectroscopy.

Standard methods were then used to convert this into its cyanoethyl phosphoramidite derivative.

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