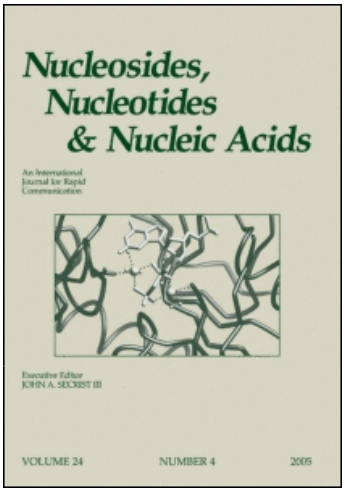

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PREPARATION OF ^{15}N LABELED NUCLEOSIDES AND LARGE SCALE SYNTHESIS OF LABELED OLIGONUCLEOTIDES WITH A NEW TYPE DNA-SYNTHESIZER

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Abstract: Microbiological and chemical methods for the preparation of ^{15}N labeled nucleosides are described. Oligonucleotides are synthesized from the labeled nucleosides on a large scale by the phosphoramidite procedure using a self-developed DNA - Synthesizer. Preliminary ^{15}N-NMR studies are reported.

Preparation of 4-$^{15}NH_2$-2'-Deoxycytidine: The synthesis of 4-$^{15}NH_2$-2'-deoxycytidine is accomplished in five steps using 2'-deoxyuridine as a starting compound (Fig. 1). The reaction of the 3',5'-di-O,O-benzoyl-2'-deoxyuridine with Lawesson Reagent in equimolar quantities yields the 4-thiouracil derivative[1]. The cristalline compound is converted into the ^{15}N-labeled nucleoside in a single reaction sequence without the isolation of intermediates[2]. Hydrolysis of the 2'-deoxyribose protecting groups is followed by permanganate oxydation of the 4-thiouracil moiety[3] in phosphate buffered aqueous solution. The resulting 4-sulfonate provides a good leaving group favouring nucleophilic substitution which

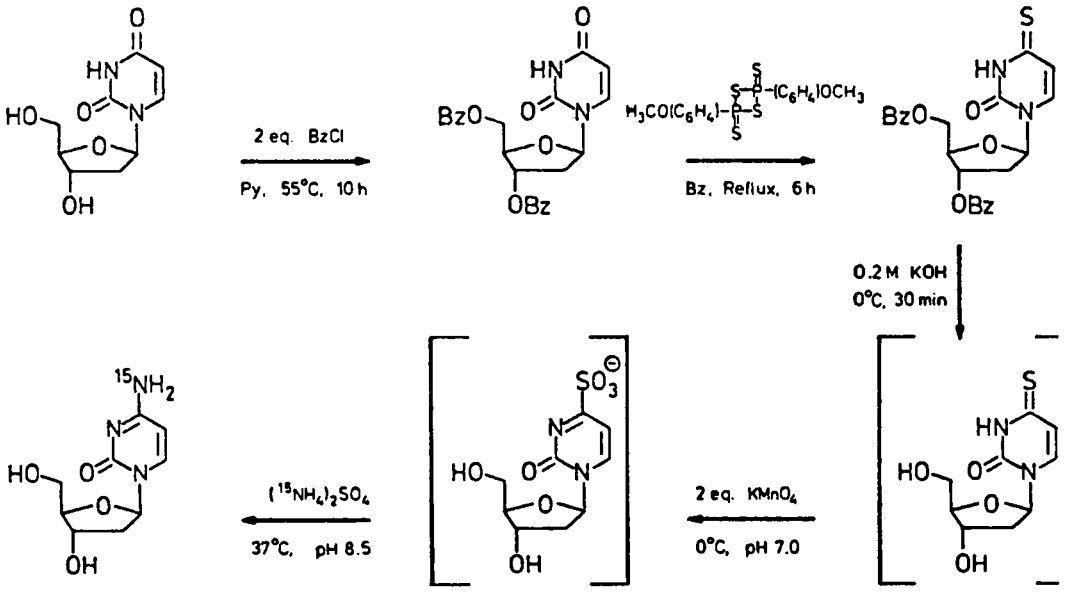

FIG. 1: Synthetic route for the preparation of 4-$^{15}NH_2$-2'-Deoxycytidine

is achieved under mild conditions by a very low (10-fold) excess of in-situ generated ^{15}N-ammonia.

Preparation of 6-$^{15}NH_2$-2'-Deoxyadenosine: 6-Chloropurine reacts with 95% enriched ^{15}N-ammoniumsulfate and sodiummethanolate (n-butanol, 120°C, autoclave, 20 h) leading to 6-$^{15}NH_2$-adenine. The free purine is linked to 2'-deoxyribose by the action of trans-N-deoxyribosylase extracted from Lactobacillus helveticus (Fig. 2). The common nucleobases and many of their derivatives are substrates for the enzyme[4].

Isolation of uniformly labeled ^{15}N-2'-Deoxynucleosides: Hydrolysis of ^{15}N enriched RNA extracted from E.coli grown on ^{15}N-ammoniumsulfate as the only nitrogen source yields uniformly ^{15}N labeled nucleobases. The cleavage of the glycosidic bonds by means of concentrated formic acid is completed within one hour at 175°C in sealed tubes. Purification is carried out by ion exchange chromatography on Sephadex A-25 and Dowex 50 WX-8 resins. Fully ^{15}N labeled 2'-deoxycytidine, 2'-deoxyadenosine, 2'-deoxyguanosine and 2'-deoxyuridine are obtained according to the scheme mentioned above using trans-N-deoxyribosylase (Fig. 2).

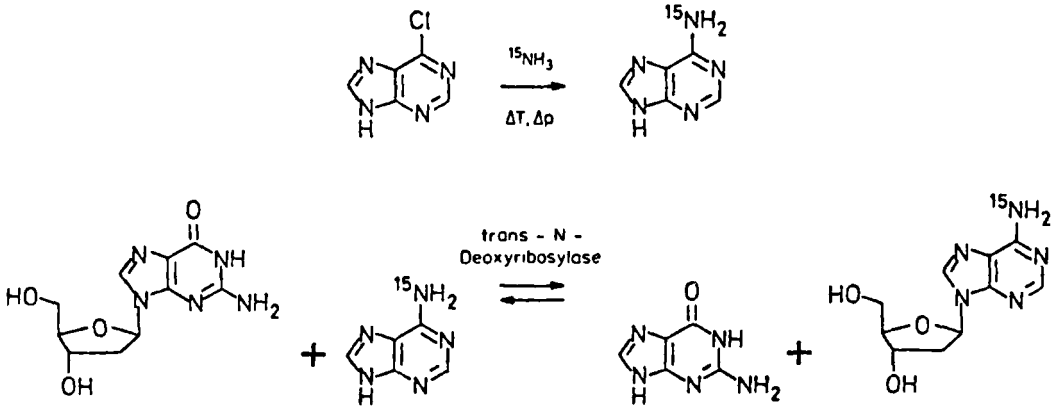

FIG. 2: Combined chemical and microbiological method for the preparation of 6-$^{15}NH_2$-2'-Deoxyadenosine

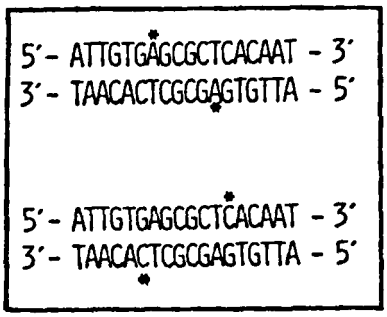

FIG. 3: Two of the synthesized ^{15}N labeled 18-bp-lac-operator oligonucleotides. The positions of the ^{15}N-amino labeled nucleobases are marked by (*).

Synthesis of ^{15}N labeled oligonucletides: All ^{15}N labeled nucleosides are converted into the corresponding phosphoramidites[5,6]. Two 18-mers of the symmetrical lac-operator sequence d(ATTGTGAGCGCTCACAAT)$_2$ amino-labeled in different base positions have been synthesized which are shown in Fig. 3.

The DNA - Synthesizer: A new type DNA - Synthesizer has been developed[7] for the large scale preparation of oligonucleotides with the purpose of homo- and heteronuclear NMR studies. The intention was to offer a system with high flexibility wherein users are assisted by a

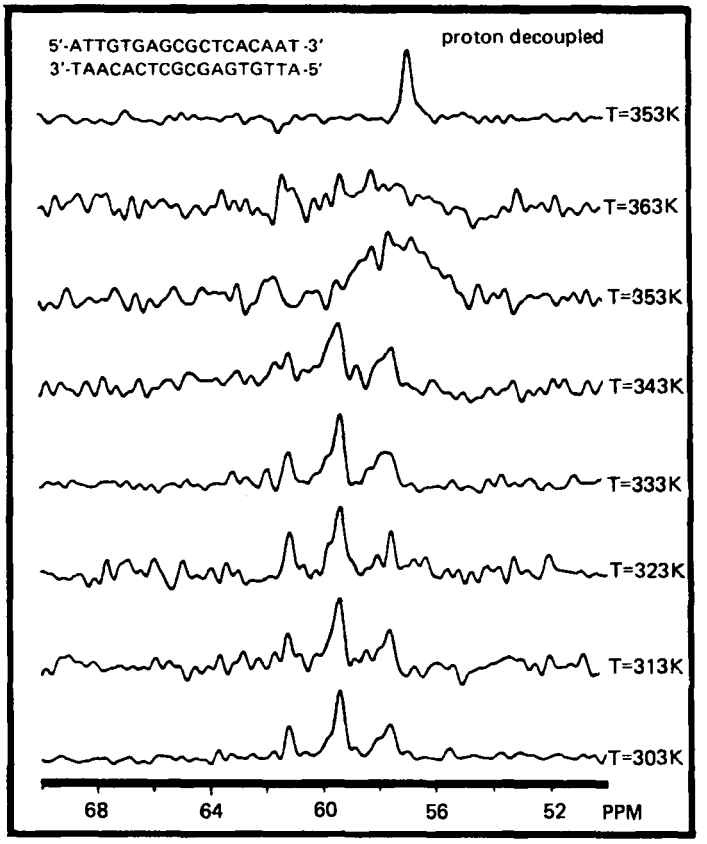

FIG. 4: 50.68 MHz-^{15}N-spectra of the 18-bp-lac-operator sequence at various temperatures (10 mM in 90% H_2O / 10% D_2O, 0.2 M NaCl). Chemical shifts refer to 4N $(^{15}NH_4)_2SO_4$ / 1 N H_2SO_4 (9 : 1). The position of the ^{15}N-amino labeled base is marked by (*).

menutechnique. In contrast to most of the commercially available instruments our system enables the setup of syntheses ranging variably from below 1 umol up to 15 umol. As a striking hardware feature reagents and solvents for washing can be delivered from top or bottom of the reaction column optionally thus increasing the efficiency of washing- and reaction-steps.

NMR measurements: ^{15}N-NMR spectra of one of the 18-bp-oligonucleotides recorded at various temperatures are shown in Fig. 4. Due to the

low gyromagnetic constant of the ^{15}N nucleus a high concentration of the labeled oligonucleotides is required. The melting of the double helix gives rise to an upfield shift of the ^{15}N-resonance. In addition a temperature dependent line broadening of the ^{15}N-signal is observed. This might be interpreted as a temperature dependent exchange of ^{15}N-bound protons with solvent.

Conclusions: ^{15}N labeled lac-operator sequences can be synthesized on a large scale. These oligonucleotides may be subjected to thorough NMR investigations: ^{15}N-NMR spectroscopy is applicable, but also ^{1}H-NMR spectroscopy may be applied for protons bound to ^{15}N-nuclei. These ^{1}H resonances are selectively detectable using ^{15}N-^{1}H-multiple-quantum NMR techniques.

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