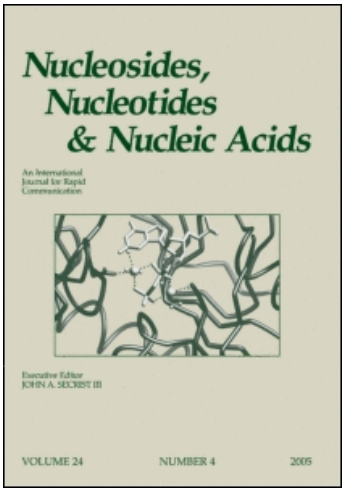

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

^{15}N-Labeled Oligodeoxynucleotides -Useful Probes for ^{1}H-NMR Investigations

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To cite this Article Kieper, Ingo , Schmidt, Thomas , Fera, Brian and Rüterjans, Heinz(1988) '^{15}N-Labeled Oligodeoxynucleotides -Useful Probes for ^{1}H-NMR Investigations', Nucleosides, Nucleotides and Nucleic Acids, 7: 5, 821 — 825
To link to this Article: DOI: 10.1080/07328318808056337
URL: http://dx.doi.org/10.1080/07328318808056337

NUCLEOSIDES & NUCLEOTIDES, 7(5&6), 821-825 (1988)

^{15}N-LABELED OLIGODEOXYNUCLEOTIDES - USEFUL PROBES FOR ^{1}H-NMR INVESTIGATIONS

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Abstract. The amino protons of ^{15}N-labeled deoxyoligonucleotides were studied as possible structural probes for NMR investigations of the interaction between DNA and regulatory proteins. To apply this strategy, 6-$^{15}NH_2$-2'-deoxyadenosine, 4-$^{15}NH_2$-2'-deoxycytidine and 2-$^{15}NH_2$-2'-deoxyguanosine were chemically synthesized. The labeled nucleosides were introduced into distinct positions of oligodeoxy-nucleotides by large-scale DNA synthesis. The behaviour of the ^{15}N-coupled cytidine amino protons in a 18 base pair (bp) *lac* operator sequence were investigated using ^{1}H-^{15}N INDOR spectroscopy .

Introduction. The amino protons of the nucleic acid bases are located in the major or the minor groove of DNA and are possible H-bond donors for the interaction with regulatory proteins and antibiotics. Unfortunately, however, these protons are difficult to detect with NMR methods because of the heavy overlap of resonances originating from both protein and DNA. Even in a NOESY spectrum, separation of these proton signals is complicated. The introduction of an ^{15}N label into the amino group in combination with special NMR pulse techniques allow the selective observation of ^{15}N coupled protons.

Synthesis of ^{15}N-labeled Nucleosides. The introduction of the ^{15}N label into the amino group of 2'-deoxyguanosine was carried out in five steps using 2'-deoxyguanosine as a starting compound (Fig. 1). After blocking the sugar moiety with methoxyacetic anhydride, the *Mitsunobu* reaction (3) using 2-(phenylmercapto)-ethanol led to 2'-deoxy-3',5'-di-O-methoxyacetyl-O^6-[2-(phenylmercapto)-ethyl]-guanosine 3. The protecting groups provide good solubility in organic solvents and better handling in general. Deamination of the 2'-deoxyguanosine derivative 3 was achieved under optimized conditions with sodium nitrite and 48% aqueous fluoroboric acid in acetone solution at -10°C, yielding the

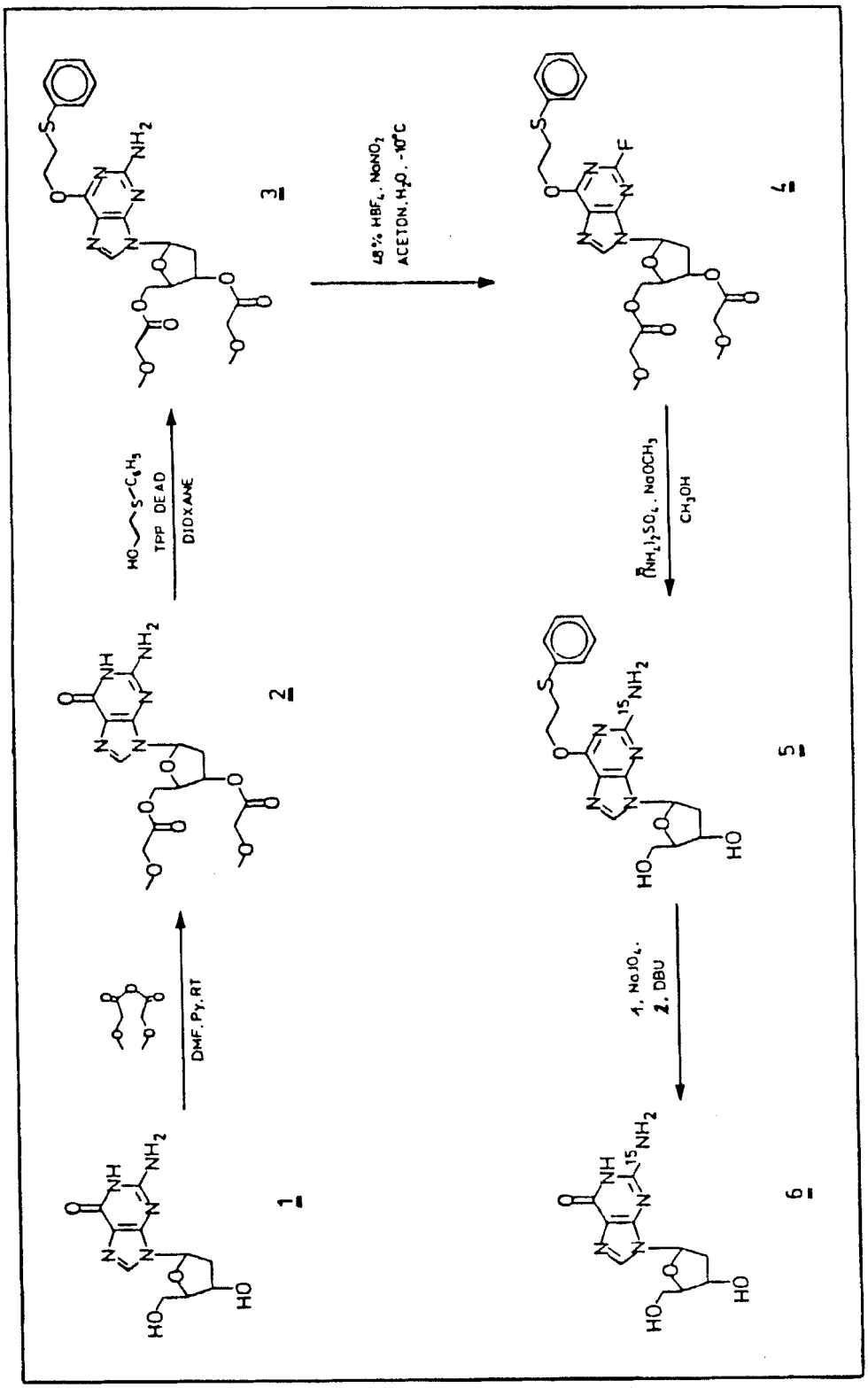

Fig. 1: Synthetic pathway for the preparation of 2-$^{15}NH_2$-2'-deoxyguanosine

2-fluoro-substituted compound. Reaction of 4 with ^{15}N-ammoniumsulfate in methanol resulted in the ^{15}N-labeled derivative 5. Removal of the O^6-protecting group with sodium perjodate and DBU led to 2'-$^{15}NH_2$-2'-deoxyguanosine.

$^{15}NH_2$-2'-deoxyguanosine was converted into the corresponding phosphoramidite (4) and introduced in a distinct position of an 18 bp symmetrical *lac* operator sequence by the *phosphoramidite* method.

NMR Measurements. A wealth of information is available about the DNA imino protons and their exchange behaviour as a structural probe for the helix-coil dynamics of DNA. On the other hand, little is known about the amino protons, which would provide complementary information. In the following, results are presented from the study of an 18 bp symmetric *lac* operator fragment (Fig. 2) labeled in position 14 with 4-$^{15}NH_2$-2'-deoxycytidine. Figure 3a shows the temperature dependence of the ^{1}H chemical shifts of the cytidine-14 Watson-Crick (WC) and non-Watson-Crick (nWC) amino protons and of the guanosine-7 imino proton, which belongs to the same base pair (in 0.01 mM EDTA, 0.02% NaN_3 at pH 7). The ^{1}H shifts of the amino protons were obtained from INDOR spectra (2), which allow selective editing of ^{15}N-bound protons. In Fig. 3b the line widths of the G-7 imino proton and the C-14 WC amino proton derived from ^{15}N-decoupled ^{1}H spectra are shown as a function of temperature.

There is a nearly linear upfield shift of all three resonances over the whole temperature range, reflecting the increasing opening rate of the helix, as well as a simultaneous broadening and disappearance of all three resonances from the spectrum above 320 K. The almost simultaneous disappearance of all three resonances reflects the melting of the double helix, which in contrast to previously investigated tetramers and hexamers (5, 6), occurs in a relatively narrow temperature range owing to the cooperativity of the process for the 18mer. The temperature dependence of the line widths also indicates this. First the line widths decrease up to a temperature of about 300 K, because the decreasing solvent viscosity permits a higher flexibility of the DNA. Upon further increase of the temperature , the line widths increase owing to the exchange of the protons with the solvent. The exchange rates at 325 K are 3 s^{-1} for the imino proton and 11 s^{-1} for the WC amino proton.

The similar behaviour of these inherently very different protons must be caused by a common rate-limiting step of their solvent exchange. A significant contribution to the exchange rate of the nWC amino proton, which is freely accessible to the solvent, from a base-catalyzed exchange mechanism can be excluded from our data, as well as a contribution from rotational exchange of the two amino protons, which has been supposed by McConnell (5). A rotation about the cytidine-14 C4-NH_2 bond would result in a decrease of the distance between the two amino proton resonances in the NMR spectra. Apparently this process is not relevant in longer oligodeoxynucleotides.

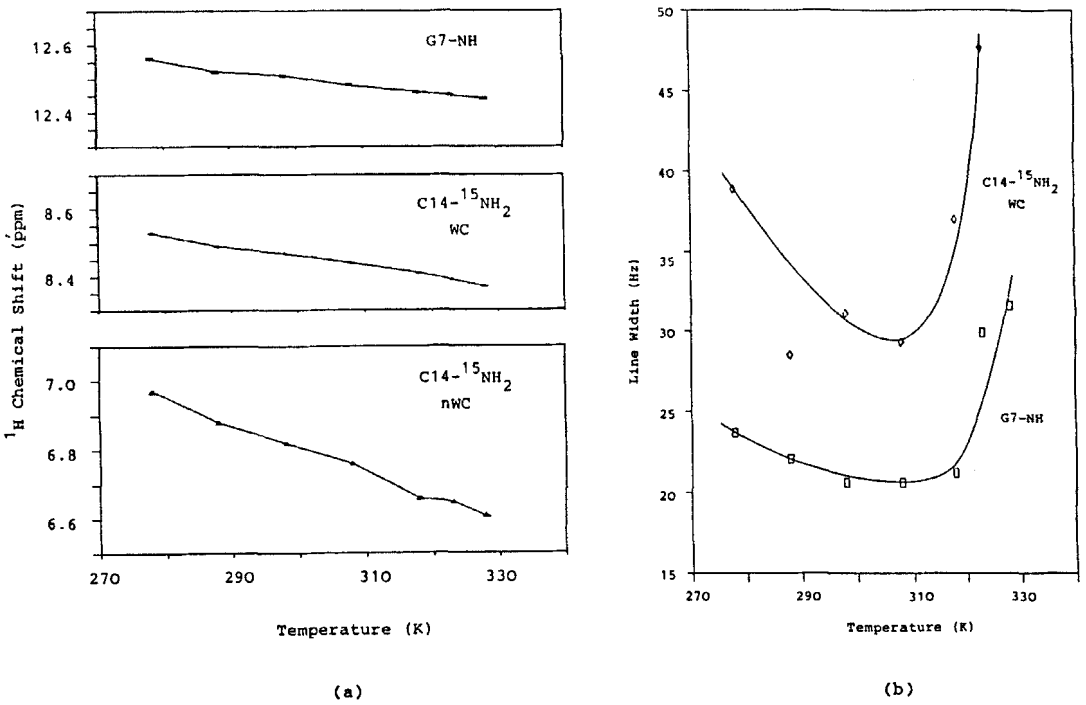

FIG. 3: a) Temperature dependence of the ^{1}H chemical shifts of the ^{15}N-labeled cytidine-14 amino protons and the guanosine-7 imino proton, b) temperature dependence of the line width of the ^{15}N-labeled cytidine-14 WC amino and guanosine-7 imino proton

From studies of the free nucleosides it is known that the cytidine amino protons exchange predominantly through a pathway requiring the protonation of the cytidine ring at N3 (pK 4.3), whereas the guanosine imino proton exchanges directly from the neutral base (7, 8). Thus it is concluded that the opening of the hydrogen bond between the guanosine-N1H and the cytidine-N3, which renders N3 free for protonation, constitutes the rate-limiting step of the exchange of both the guanosine imino and the cytidine amino protons.

Currently we are extending our investigations to the third H-bonded proton in that base pair, the guanosine amino proton, hoping to get a detailed description of the complete network of exchangeable protons of a distinct DNA base pair.

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