Coupling of 2′-Deoxyribonucleotides with 2-Pyridinealdehyde Methiodides

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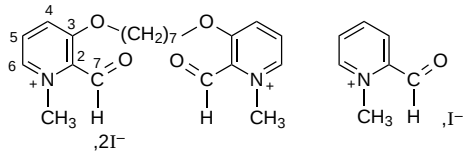
In the course of our search for a simple chemical model that would mimic the reaction of a new class of bridged 2-pyridinealdehyde methiodides (BPA) with DNA,[1] we have synthesized new adducts, tentatively symmetric phosphoesters, whose formation mechanism may be relevant to BPA/DNA interaction and which may have potential synthetic interest in the chemistry of oligonucleotides.

BPAs were shown to bind covalently, though reversibly, to DNA *in vitro* and to cause growth inhibition of cultured human cells.[1] Covalent binding was specific to the aldehyde substituent and was not observed with other functional groups such as oximes, amides, and amines present in the bridged molecule.



1 **2**

We reasoned that information regarding binding of the aldehyde dimers to DNA might be gained from the study of their reaction with single *deoxyribonucleotides*. We have previously shown that, in the interaction of the bridged dimers with DNA, a linking tether of sufficient length was essential to facilitate opening of the double helix and to expose the nucleotides.[1,2] Since *mononucleotides* are readily accessible, they should also react with the monomeric 2-pyridinecarboxaldehyde methiodide (compound **2**), the pyridinium moiety of the bridged compounds. Hence, the reaction of the latter with nucleotides has been also investigated in this study.

Preliminary observations indicate no reaction of the monomeric or dimeric aldehydes with the monodeoxynucleotides *in aqueous solutions in the time and temperature ranges* of this study. This is likely to arise from the fact that reactions involving aldehyde often proceed through water elimination, a reaction not favored in aqueous solution. Experiments were, therefore, preferably conducted in a water-free medium even if the relevance of this approach to the reaction of DNA in water may be questioned.

Results and Discussion

UV absorption spectrum in buffered aqueous solution of the reaction products of compound **1** with d-AMP and d-GMP clearly shows a nucleotide/effector molar ratio of 4 (meaning two nucleotides per pyridinium moiety). No significant shift with respect to the absorbance of authentic **1** and nucleotides is observed. Similarly, the NMR spectrum in buffered D_2O displays the same 2/1 molecular ratio (Figure 1). Only a single set of lines from the nucleotide is observed, thus suggesting either that the species has a 2-fold symmetry or that the overall spectrum simply results from superposition of the spectra of species formed upon hydrolysis. In DMSO, the solvent where synthesis was performed, one also obtains a symmetric NMR spectrum with the 2/1 molar ratio, thus indicating that the spectrum in water is probably that of a single derivative. Spectra in DMSO-d_6 do not show the resonance around 10.3 ppm, seen in pure pyridinium aldehydes and attributed to the aldehydic proton close to the unhydrated carbonyl group. This indicates reaction of the carbonyl group in adduct formation. ^{31}P NMR spectra of adducts in buffered D_2O or DMSO exhibit a single resonance consistant with a 2-fold symmetry. NOESY correlation in D_2O shows numerous intraresidue cross-peaks but no cross-interaction between protons in the nucleotide and pyridinium moieties.

The reaction of the bridged pyridinium aldehydes is strongly reminiscent of the binding of formaldehyde with DNA that leads, in a two-step process, to cross-linking of amino groups in adenine or guanine present in both strands.[3,4] However, and most surprisingly, *adenine* and *2′-deoxyadenosine do not react with the pyridinium aldehydes*. Moreover, the resonance at 7.34 ppm (two protons) in the spectrum of the deoxynucleotide/aldehyde adducts *in DMSO* is also observed in d-AMP, in deoxyadenosine, and in adenine in DMSO and is readily assigned to the NH_2 protons. This unambigously shows that the amino substituent is still present in the adduct. Additional evidence confirms that reaction does not involve the amino group on the base but *rather the 5′-phosphate group*: thymidine 5′-monophosphate (TMP) yields to an adduct with the same stoichiometry adduct as d-AMP and d-GMP.

In the same way as **1**, compound **2** reacts with 2′-deoxyribonucleotides, giving rise to adducts with a nucleotide/effector ratio of 2 (Figure 2).

The adducts are found to be unstable under the conditions of MALDI mass spectrometry. The mass corresponding to the 2/1 adducts is not observed,[5] and the proportions of the various fragments are variable from run to run. However, significant mass peaks are present. The **1**/d-AMP adduct shows three main peaks: mass 331 Da is d-AMP, mass 643 Da corresponds to a d-AMP dimer where a *water molecule has been released*, and mass 684 Da is a 1/1 adduct of d-AMP and **1** with loss of H_2O and two iodide ions. The spectra of adducts of d-AMP and d-GMP with compound **2** display the mass M of the free oligonucleotide and the 2M minus H_2O mass. Electrospray mass spectrometry also fails to show a mass corresponding to the adducts. However, and most interestingly, a mass of 136 Da, that of adenine, is the major fragment, whereas the mass spectrum of d-AMP, run under the same conditions, consists essentially of a

(1) Dodin, G.; Kühnel, J. M.; Demerseman, P.; Kotzyba, J. *Anti-Cancer Drug Des.* **1993**, *8*, 361.

(2) Only the bis-pyridinium aldehyde with a linking tether of seven methylene groups (compound **1**, MW 626), as being representative of the other long chain derivatives, was considered in this study (see ref 1). For the synthesis of BPA, see the reference quoted in ref 1.

(3) Huang, H.; Solomon, M. S.; Hopkins, P. B. *J. Am. Chem. Soc.* **1992**, *114*, 9240.

(4) Huang, H.; Hopkins, P. B. *J. Am. Chem. Soc.* **1993**, *115*, 9402.

(5) The mass spectrum of the species formed from **2**-OH-5′-AMP and **2** at 60 °C (see ref 3) shows high proportions of the 2/1 adduct (797 Da), together with a mass corresponding to diadenosine pyrophosphate.

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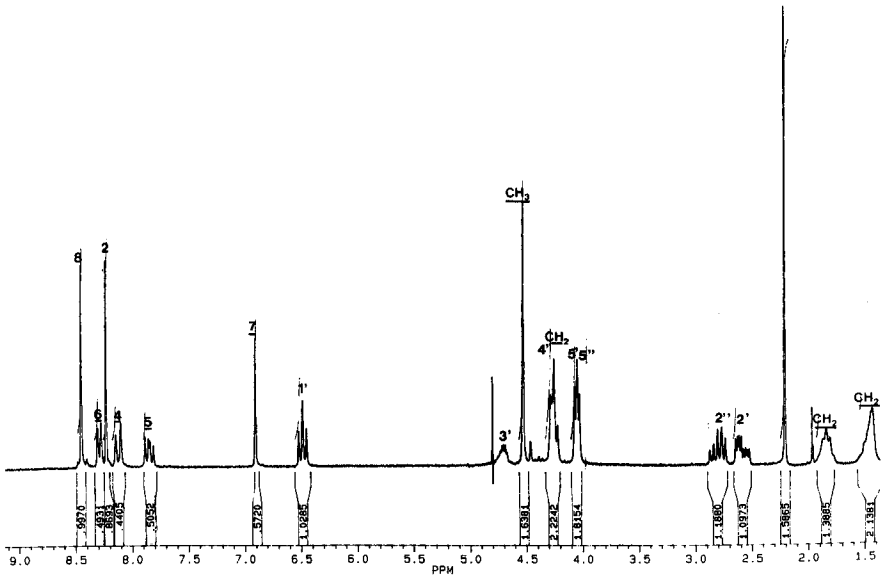


Figure 1. ^{1}H NMR (200 MHz) at room temperature and in buffered D_2O (sodium cacodylate, 50 mM, pH 7.4) of **1**/d-AMP (2.3×10^{-2} M). Numbering of the protons in **1** is underlined.

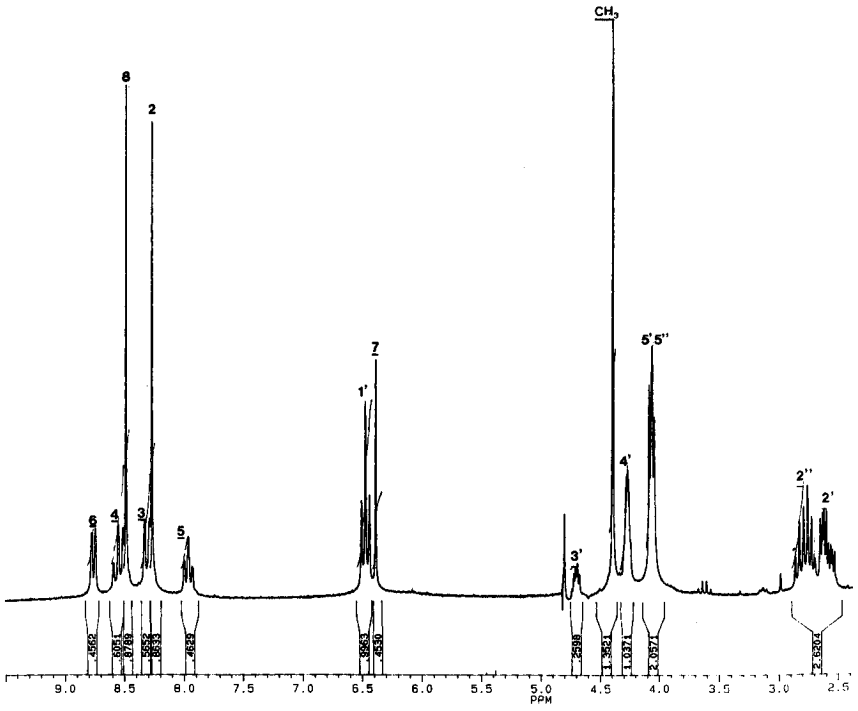


Figure 2. ^{1}H NMR (200 MHz) in buffered D_2O (sodium cacodylate, 50 mM, pH 7.4) of **2**/d-AMP (2.3×10^{-2} M). Numbering of protons in **2** is underlined.

mass of 331 Da, thus indicating easy depurination of the adduct under the conditions of MALDI.

The addition of the pyridinium aldehydes to 2′-deoxyribonucleotides bears a striking resemblance to activation of the phosphate group by various substituents in ribonucleotides.[6−9]

The reaction of pyridinium aldehydes with the D-ribonucleotides leading to a symmetric adduct may proceed as shown in Scheme 1. It is probable that the mass peaks observed in MALDI spectrometry arise from fragmentation and rearrangement of the substituted phosphoester.

Conclusion

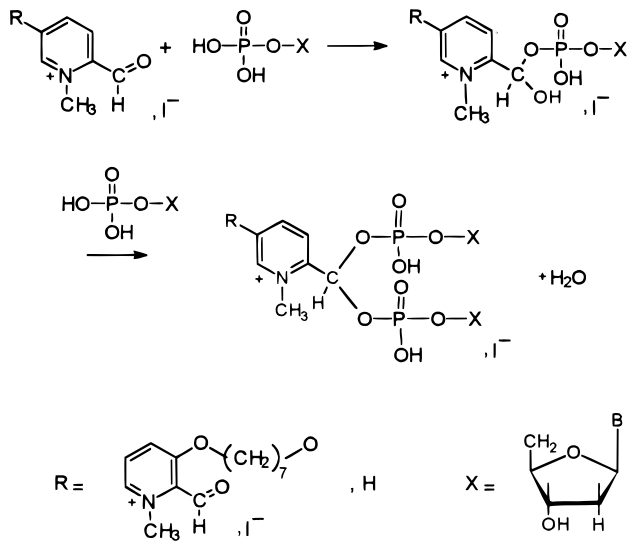
Although the relevance of this mechanism to BPA interaction with DNA in water remains to be established (particularly in regard to the possibility of reaction of the aldehyde derivatives with POH in the 3′−5′ phosphodiester linkage), this study has revealed 2-pyridinecarboxy aldehyde methiodides, both the monomer or the bridged dimers, as new phosphate-activating agents leading to chemical intermediates of potential interest in the chemistry of nucleotides.[6−9]

Experimental Section

Synthesis of 2-Pyridinecarboxaldehyde Methiodide (Compound 2). 2-pyridinecarboxaldehyde (2×10^{-2} mol) was allowed to react with iodomethane (10^{-1} mol) in 30 mL of

(6) Orgel, L. E. *Nature* **1992**, *358*, 2O3.
(7) Wu, T.; Orgel, L. E. *J. Am. Chem. Soc.* **1992**, *114*, 7963.
(8) Ferris, J. P.; Ertem, G. *J. Am. Chem. Soc.* **1993**, *115*, 12270.
(9) Prabahar, K. J.; Cole, T. D. *J. Am. Chem. Soc.* **1994**, *116*, 10914.

Scheme 1



dimethylacetamide for 20 h at 40 °C. The precipitate (yellow needles) was thoroughly washed with cold diethyl ether and dried. ^{1}H NMR 200 MHz, δ (ppm): (i) in DMSO-d_6, 10.27 (s, 1H, aldehyde), 9.12 (d, 1H, Ar), 8.78 (t, 1H, Ar), 8.53 (d, 1H, Ar), 8.33 (d, 1H, Ar), 4.65 (s, 3H, CH_3); (ii) in D_2O, 8.83 (d, 1H, Ar), 8.62 (t, 1H, Ar), 8.37 (d, 1H, Ar), 6.46 (s, 1H, aldehyde hydrate).

Synthesis of 2′-Deoxyribonucleotide Adducts. A 10^{-4} mol of 2′-deoxyadenosine 5′-monophosphate free acid (d-AMP) or 2′-deoxyguanosine 5′-monophosphate free acid (d-GMP) was stirred with 10^{-4} mol of **1** or **2** in 1.5 mL of an argon-flushed DMF/DMSO (2/1) mixture containing 2 μL of piperidine in a capped tube. After 3 h at room temperature the reaction product was precipitated with dry acetone and thoroughly washed with acetone. The absence of unbound aldehyde in the precipitate after washing was assessed from the absence of the aldehyde proton resonance at 10.3 ppm in the NMR spectrum in dry DMSO. The precipitate, dissolved in dry methanol, was found to be homogeneous on TLC (Merck RP18 reversed phase) and was applied to a RP 18 column and eluted with methanol. ^{1}H NMR spectra in D_2O are shown in Figures 1 and 2. Under these mild experimental conditions, 2′-hydroxyadenosine 5′-monophosphate (5′-AMP) failed to react with the aldehydes. However, at 60 °C and after 15 h, a 2/1 stoichiometric adduct was formed with the pyridinium derivatives.

MALDI Mass Spectrometry. Spectra were recorded using a home-built time-of-flight mass spectrometer operated in the negative mode. A mixture of 80% anthranilic acid, 20% nicotinic acid, H_2O, or methanol was used as matrix, and the molar ratio of analyte to matrix was 1/10 000.

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