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ON THE STABILITY OF PHOSPHODIESTER-AMIDE INTERNUCLEOTIDE BOND

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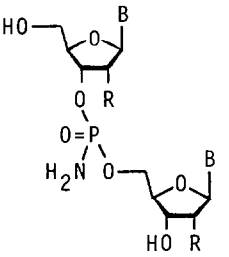
Abstract. The stability of phosphodiester-amide internucleotide bond is compared in the deoxyribo and ribo series. The destabilizing effect of the 2'-OH group in the ribo series is discussed.

Oligodeoxyribonucleotides with phosphodiester-amide internucleotide bonds [e.g. $Tp(NH_2)T$] can be formed when aryl protected internucleotide linkages are unblocked by ammoniacal treatment during the phosphotriester approach of oligo-



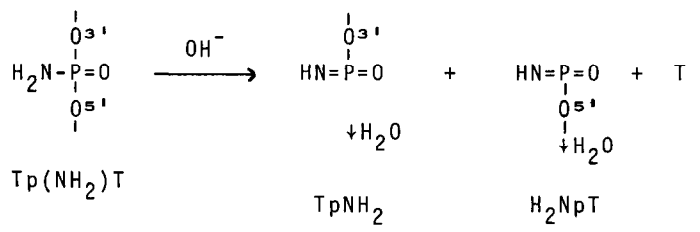
	B	R
$Tp(NH_2)T$	thymin-1-yl	H
$Up(NH_2)U$	uracil-1-yl	OH

nucleotide synthesis[1]. At the same time, the analogous oligoribonucleotide derivatives [e.g. $Up(NH_2)U$] may be expected to be thermodynamically unstable structures as a result of the cis-α 2'-OH groups. For example, in aqueous solution, $Up(NH_2)U$ will probably be decomposed with the loss of NH_3 partly to UpU and partly to a mixture of U > p and U. This assumption is based on the instability of diribonucleoside monophosphates having N(P)-alkylated phosphodiester-amide internucleotide linkages[2] and on our unsuccessful attempts to prepare ribonucleoside 2'(3')-phosphoramidates and phosphorodiamidates.

Instead of these derivatives we could always isolate the respective 2',3'-cyclic phosphates[3,4].

Recently Nemer and Ogilvie reported on the synthesis of $Up(NH_2)U$ by deprotecting the 2'-O-silyl derivative under mild anhydrous conditions[5]. They found the compound to be stable in aqueous solution (during incubation with spleen phosphodiesterase) and hydrolyzable with snake venom phosphodiesterase. The compound had a ^{31}P nmr shift at - 0.50 ppm. This is more characteristic for a phosphodiester however, than for a phosphodiester-amide structure[6]. Since these results are also in marked disagreement with earlier literature data[2-4,6], we tried to prepare $Tp(NH_2)T$ and $Up(NH_2)U$ to make a comparison between their behaviour under different chemical and enzymic hydrolysis conditions.

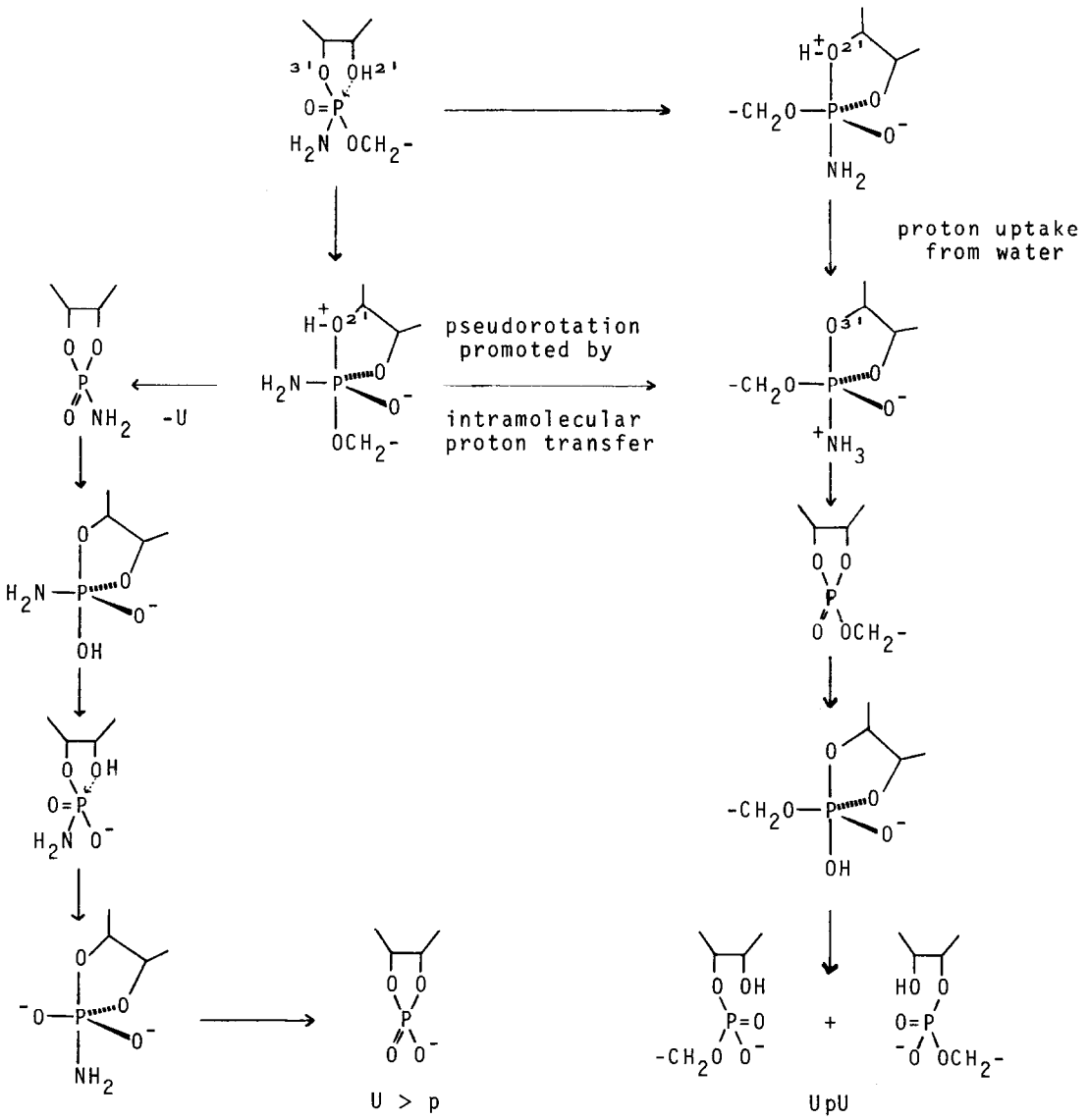
TpT ($Et_3\overset{+}{N}H$ salt, 0.12 mmol) was treated with diphenyl-phosphorochloridate (0.24 mmol) in anhydrous DMF (1.5 ml) in the presence of Bu_3N (0.36 mmol) at room temperature for 2.5 hr. The reaction mixture was poured into 7N NH_4OH (10 ml) under vigorous stirring at 0^o, and the solution was extracted with ether. The aqueous phase, after concentration was passed through a DEAE-cellulose [HCO_3^-] column, and the column was washed with water. Fractions containing UV absorbing material were pooled, evaporated and further purified by partition chromatography on a cellulose column in n-BuOH/EtOH/0.1 M $Et_3N.H_2CO_3$, pH = 7.5 (16:2:5 $^v/_v$) to yield 26 mg (40%)[7] of TLC pure $Tp(NH_2)T$ as a white powder. R_f = 0.25, Silica gel, n-BuOH/H_2O/cc.NH_4OH (86:14:5 $^v/_v$). ^{31}P nmr: δ = +12.08ppm[8] $Tp(NH_2)T$ was readily hydrolyzed both by acid and alkali to the expected products[1,9]. In acid, $Tp(NH_2)T$ is converted to TpT (δ = -1.04 ppm, $t_{1/2} \sim 20$ hr at pH = 1.0 and 25^o). In alkali, $Tp(NH_2)T$ is decomposed to T and an equimolar mixture of $TpNH_2$(δ = +8.85 ppm) and H_2NpT(δ = +9.32 ppm, $t_{1/2} \sim 20$ min at pH = 13.0 and 25^o)[10]. Since $Tp(NMe_2)T$ prepared in similar manner using 7N aqueous $(CH_3)_2NH$ instead of NH_4OH, is stable in alkali, the alkaline hydrolysis of $Tp(NH_2)T$ may be best formulated <u>via</u> metaphosphorimidate intermediates[11]:



Neither spleen phosphodiesterase nor snake venom phosphodiesterase can hydrolyze $Tp(NH_2)T$, (cf. Ref. 1) and pppA - in the presence of T4 polynucleotide kinase does not phosphorylate the 5'-terminus of the molecule.

The synthesis of $Up(NH_2)U$ failed according to this route. Instead of

Up(NH_2)U more than 90% of the starting UpU (δ = -0.64 ppm) could be recovered and about 5-10% of chain cleavage to U > p and U also occured. Since there is no reason to suppose that Up(NH_2)U was not formed, the only explanation is the very fast decomposition of the compound to the thermodynamically more stabl derivatives UpU, U > p and U. A possible mechanism for this decomposition may be given as follows[12]:



On the basis of these results it seems unlikely that the compound described by Nemer and Ogilvie was Up(NH_2)U. Rather, it was probably formed transiently and was transformed into UpU either immediately or after dissolving in water.

Acknowledgements

^{31}P nmr measurements performed by Mr. K. Caster (Gross Chemical Laboratory, Duke University, Durham) and the kind information by Prof. C.B. Reese (University of London King's College) on their unpublished observations are gratefully acknowledged.

REFERENCES AND FOOTNOTES

Abbreviations: Tp(NH_2)T = thymidylyl-(3'-5')-thymidine (P→N) amide, Up(NH_2)U = uridylyl-(3'-5')-uridine (P→N) amide, UpU = uridylyl-[2'(3')-5']-uridine, U > p = uridine 2',3'-cyclic phosphate, U = uridine, TpT = thymidylyl-(3'-5')--thymidine, T= thymidine, TpNH_2 = thymidine 3'-phosphoramidate, H_2NpT = thymidine 5'-phosphoramidate, Tp(NMe_2)T = thymidylyl-(3'-5')-thymidine (P→N) dimethylamide, pppA = adenosine 5'-triphosphate.

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[6] M.L. Nielsen, J.V. Pustinger and J. Strobel, J. Chem. Eng. Data 9, 167 (1964).

[7] The other 60% were recovered from the DEAE-cellulose column as TpT.

[8] ^{31}P nmr measurements were performed on a JEOL FX90Q instrument at 36.2 MHz using 0.02 M solutions of the samples in D_2O. δ values for hydrolysis products were obtained at pD = 1.0 and pD = 13.0, respectively.

[9] A.J. Kirby and S.G. Warren, "The Organic Chemistry of Phosphorus", Elsevier Amsterdam, 1976, p. 294.

[10] For the ^{31}P nmr chemical shifts of isomeric pyrimidine deoxyribonucleoside phosphoramidates see J. Ludwig and J. Tomasz, Synthesis submitted for publication.

[11] Metaphosphorimidate intermediates in alkaline hydrolysis of those phosphoramidates having at least one ionizable hydrogen atom attaching to amide nitrogen atom were first suggested by F.H. Westheimer, Chem. Soc. Spec. Publ., No. 8, 181 (1957).

[12] Metaphosphorimidate intermediates may be also involved.

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