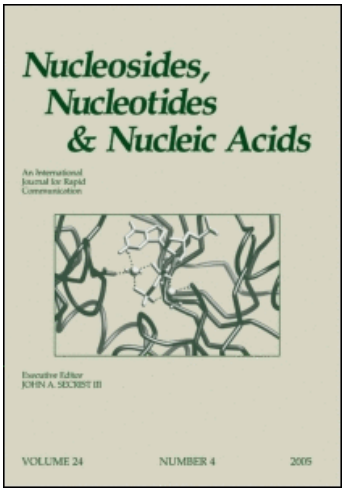

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PHOSPHORAMIDATE ANALOGS OF DINUCLEOTIDES: SYNTHESIS AND ^1H ASSIGNMENT BY TWO DIMENSIONAL NMR SPECTROSCOPY (^1H,^1H-COSY)

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ABSTRACT

The synthesis of several dinucleoside phosphate derivatives which are linked by phosphoramidate bonds 3'-OP(O)NH-5' are described. One of these dimer units can be used in automated solid phase DNA synthesis by the phosphoramidite procedure. In order to study the conformational change which is induced on substituting O-P-O against O-P-N we have also prepared the fully deprotected dimer analog. The constitution of the dimer units were confirmed by means of 2D-300MHz homonuclear chemical shift correlation spectroscopy (^1H,^1H-COSY).

INTRODUCTION

It has previously been shown that oligomers containing nucleoside units linked by 3'-O-P-N-5' or 3'-N-P-O-5' bonds are stable under neutral and alkaline conditions.[1,2] Due to the greater nucleophilicity of aliphatic amino- contrary to hydroxyl-groups, aminonucleosides may be involved in the none-enzymatic synthesis of oligonucleotides in prebiotic time. This fact possibly could be contributed to the origin of live. Our interest in synthesizing dinucleoside phosphate analogs that possess internucleotide phosphoramidate bonds was stimulated by the expectation to yield appropiate building blocks for automated DNA synthesis which we can incorporate into oligodeoxyribonucleotides. In addition we are analysing the conformational change which is induced on substituting O-P-O against O-P-N. Here we describe in part our preliminary results about the synthesis and characterization of several dinucleoside phosphoramidate analogs.

RESULTS

The synthesis of the protected 3'-O-phosphitylated thymidylyl-(3'->5')-5'-amino-2',5'-dideoxyadenosine analog 9 was started from dT and dA as outlined in FIG. 1. The 5'-hydroxyl group from 1 was protected

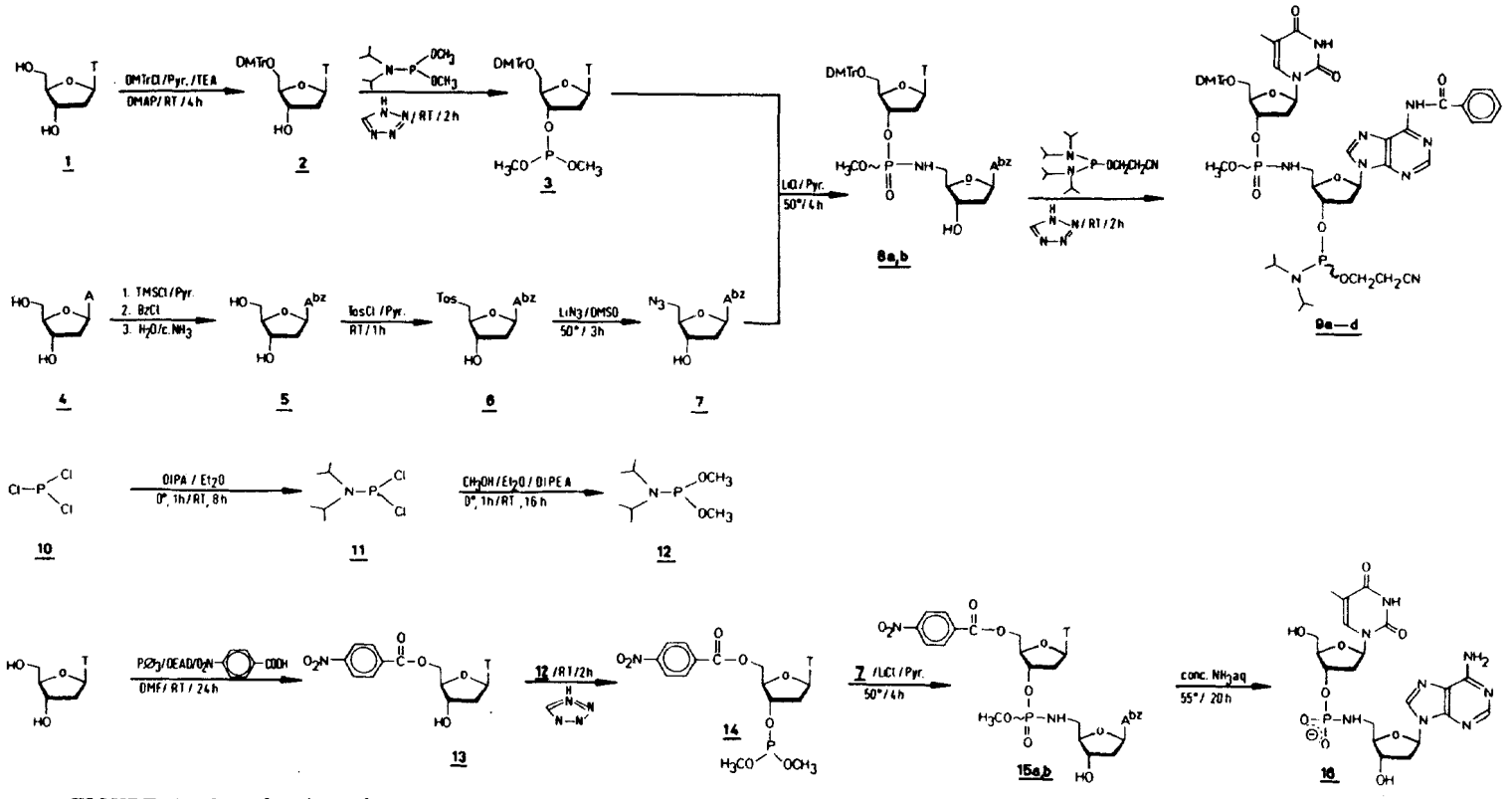

FIGURE 1. Synthesis scheme

EXPERIMENTAL

Spectra were recorded with a Bruker AM 300 WB spectrometer equipped with an Aspect 3000 Computer. Time domain in F2: 1K, in F1: 256 W; Size for calculation in F2: 2K, in F1: 1K; 4 scans/increment; Aquisition time: ca. 0,3s; Relaxation delay:ca. 3,0s; Apodization: unshifted squared sinebell in both directions. Compound number 3 was recorded in $CDCl_3$, compound number 7, 8a,b and 15a,b in DMSO-d_6 and the fully deprotected dimer 16 in D_2O solutions at ambient temperature. Tetramethylsilane was used as internal reference and the cited chemical shifts are given in ppm downfield to this standard. ^{31}P experiments were also performed on the AM 300 WB spectrometer. Spectra were recorded at 121,5MHz using broadband proton noise decoupling. The spectrometer was referenced onto a DMSO sample containing an 85% H_3PO_4 filled glass capillary.

with the acid labile DMT group yielding 2 in 85% yield. A two hour treatment of 2 with the bis-methoxy phosphoramidite 12 (^{31}P-NMR: 151,0ppm) in the presence of tetrazole afforded the phosphite triester 3 (^{31}P-NMR: 141,7ppm) in quantitative yield. For the preparation of 7 dA (4) was converted to 5 using the transient protection method developed by Jones et al.[3] The N^6-protected derivative 5 was reacted with three equivalents of p-toluenesulfonyl chloride in pyridine at r.t. This reaction proceeds in a high regioselectivity (80% 5'-tosylate and 20% 3',5'-ditosylate). The tosylate 6 was readily converted to the azide 7 with a five fold excess lithium azide in DMSO at 50°C. After three hours the nucleoside had reacted completely and no N^3,5'-cyclization product could be observed.[4] The introduction of the azide group could be easily detected by means of IR-spectroscopy, because the N_3 group gives a strong absorption band at 2090 cm^{-1}. The formation of the 3'-O-P-N-5' linkage between unit 3 and 7 to afford the dimer 8a,b is illustrated in FIG. 1. Therefore a solution of 3 in pyridine was treated with 7 in the presence of LiCl at 50°C. The initial phosphite imine is formed by the visible evolution of nitrogen, followed by the conversion to the phosphoramidate by a Michaelis-Arbuzove type reaction which was enhanced by LiCl.[5,6] After flash chromatographic purification and precipitation into cold n-hexane the desired dimer 8a,b (^{31}P-NMR: 11,8 and 11,9 ppm) was obtained as a colorless solid in 60% yield. The reaction requires no coupling or activating reagents and the blocking of the oxygen function at the 3' position of 7 is not necessary. Subsequent phosphitylation[7] afforded the desired building block 9a-d as a mixture of four diastereomers. The reaction mixture was purified by flash chromatography yielding a white powder after precipitation into cold n-hexane. In the case of the thymidylyl-(3'->5')-5'-amino-2',5'-dideoxyadenosine 16, 1 was first protected with a base labile 5'-O protecting group using the Mitsunobu reaction[8] as illustrated in FIG. 1. Successive treatment of thymidine with p-nitrobenzoic acid, triphenylphosphine and diethyl azodicarboxylate afforded 5'-O-p-nitrobenzoylthymidine 13 in 80% yield. Subsequent phosphitylation with 12 which was activated with tetrazole afforded the phosphite triester 14 in quantitative yield. This compound was reacted with 7 as described above for compound 8a,b. After flash chromatographic purification and precipitation into cold n-hexane the desired dimer 15a,b was obtained as a mixture of two diastereomers. The introduction of the base labile p-nitrobenzoyl group is advantageous since it facilitates the deblocking of

the protected dimer 15a,b. This 5'-O protecting group allowed us to deprotect the diastereomeric compounds 15a,b in a single step with conc. ammonia at 55°C during 20 hours. After lyophilisation the residue could be purified either by reversed phase HPLC or ion exchange chromatography yielding 16.

NMR-MEASUREMENTS

To confirm the constitution of the dimer blocks 8a,b, 15a,b and 16 we used 2D-NMR techniques. The complete assignment of the protons in the two sugar- and base-systems in each compound is possible with the $^1H,^1H$-COSY experiment. The chemical shift data of the compounds 3, 7, 8a,b, 15a,b and 16 are given in TAB. 1.

	3	7	8a,b		15a,b		16	
Proton			dTp	p_{NH}dA	dTp	p_{NH}dA	dTp	p_{NH}dA
H-1'	6,40	6,55	6,22	6,45	6,20	6,48	6,03	6,35
H-2'	2,33	3,00	2,45	2,87	2,45	2,88	2,25*	2,61*
H-2''	2,50	2,44	2,45	2,36	2,45	2,36	2,42*	2,87*
H-3'	4,97	4,50	4,94	4,43	5,02	4,47	4,54	4,75
H-4'	4,14	4,05	4,13	3,90	4,35	3,92	4,03	4,13
H-5'	3,35	3,56	3,20	3,00	4,51	3,05	3,73	3,15
H-5''	3,50	3,68	3,20	3,15	4,60	3,20	3,82	3,15
T-CH_3	1,45	-	1,45	-	1,67	-	1,70	-
H-6	7,60	-	7,58	-	7,45	-	7,57	-
OH-3'	-	5,56	-	5,43	-	5,40	-	-
NH-P	-	-	-	5,45	-	5,47	-	-
$POCH_3$	3,48	-	3,53		3,59		-	

TABLE 1. Proton Chemical Shifts (ppm); *: the distinction between the H-2' and H-2'' protons was not possible

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