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PREPARATION AND PHOSPHORYLATION REACTIVITY OF N-NONACYLATED NUCLEOSIDE PHOSPHORAMIDITES

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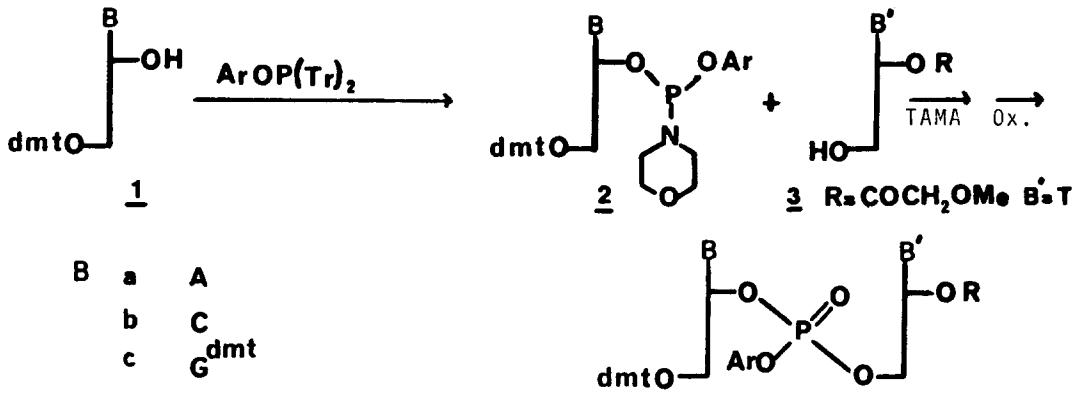
Summmary:
N-nonacylated nucleoside phosphoramidites can be prepared in two steps from 2'-deoxynucleosides. Their use in phosphorylation reactions is exemplified.

During the past several years two different phosphorylation strategies have emerged for the synthesis of oligodeoxynucleotides. Both of these use the same types of protecting groups[1]. Trityl (4,4'-dimethoxy- and 4-methoxy-) and acyl are the most popular groups for O and N-protection, respectively, of deoxynucleosides. Nucleosides protected in this manner exhibit a good solubility in the usual organic solvents and their heterocyclic amino functions are inert towards phosphorylating agents[2]. But in the case of 2'-deoxyadenosine N^6-benzoylation renders the molecule very sensitive to acidic conditions required for the removal of the trityl group[3]. In order to circumvent these problems alternative procedures which involve masking hydroxy and amino functions have been proposed [4]. However, the introduction of different types of suitable protecting groups still remains a tedious task. In a conceptually original approach phosphorylation conditions have been found which are compatible with N-unprotected nucleosides[5].

Letsinger and Ogilvie[6] have previously established that N-acylation is not necessary in the case of the phosphite strategy. Since nucleoside phosphoramidites[7] are now employed as building blocks of choice for this method[8], it became of interest to explore the possibility of obtaining N-nonacylated phosphoramidites.

We now report that such nucleoside arylphosphoramidites 2a-c are readily accessible and that they can be conveniently used in phosphorylation reactions .

To a solution of 2-chlorophenoxydi(triazolyl)phosphine [9](2 mM) in THF (6 ml) maintained at -78°C was slowly added a THF(6 ml) solution of 2'-deoxy-5'-O-dimethoxytrityladenosine 1a[10] (1 mM) followed by an excess of neat morpholine. The cooling bath was removed allowing the reaction mixture to attain room temperature. Working up gave, after column chromatography purification [11], phosphoramidite 2a as a mixture of two isomers. The proposed structure 2a is fully consistent with the spectral data (TABLE). It was also attempted to obtain 2a by running



dmt= 4,4'-dimethoxytrityl

Ar = o-C_6H_4Cl

Tr = Triazolyl

TABLE

^1H NMR Data (δ ppm/TMS ; $CDCl_3$)

	Yields	Heterocyclic base	Deoxyribose			Others		
		H	Me	H-1'	H-3'	H-5'	OCH_2	NCH_2	OCH_3
2a	68%	8.37 8.03	-	6.50	5.07	3.40	3.63	3.23	3.90
2b	68%	8.03 5.43	-	6.40	4.93	3.43	3.60	3.17	3.80
2c	74%	8.27	-	5.93	4.80	3.30	3.60	3.20	3.73
4a	76%	8.37 8.03	1.77	6.43	5.37	4.60	4.10 3.40	-	3.77 3.47
4b	63%	7.67 5.60	1.80	6.33	5.40 5.33	4.47 3.43	4.07	-	3.77 3.43
4c	78%	*	1.74	6.30 5.80	5.27	4.43 3.27	4.06	-	3.70 3.40
5	72%	8.40 8.07	1.85	6.50	5.50 4.93	4.57 3.46	3.73	3.23	3.80

* Not attributed

^{31}P NMR (δ ppm/PO_4H external; $CDCl_3$)

2a :	142.08 140.76	2b :	141.70 141.36	2c :	141.91 140.94	5 :	142.12 141.09	- 7.14 - 7.29

the reaction at 0°C. Then the yield decreased dramatically (40%) although no other side product, in particular N-phosphitylated derivative, could be detected.

In the same manner phosphoramidites 2b and 2c were prepared [11] after treatment of 2'-deoxy-5'-O-dimethoxytritylcytidine 1b [12] and 2'-deoxy-N^2-5'-O-bis(dimethoxytrityl) guanosine 1c [13] under the same conditions (TABLE).

The new phosphoramidites 2a-c which are readily soluble in acetonitrile can serve for expeditious phosphorylation of 5'-OH free nucleoside derivatives. Thus, a mixture of 2a (210 mg, 1.3 eq.) and 3 (64 mg, 1 eq.) which had been coevaporated twice with pyridine was dissolved in acetonitrile (1 ml) and treated with N-methylanilinium trifluoroacetate (TAMA)(106 mg, 2.2 eq.) in the same solvent (0.75 ml)[9]. The resulting phosphite was oxidized in situ (either with iodine or with iodobenzene diacetate [14]) to give the expected dimer 4a (156 mg) (TABLE). The 3'-OH free derivative 4a (R=H) obtained after removal of the methoxyacetyl group (conc. NH_4OH/MeOH; 1/99) was transformed into the phosphoramidite dimer 5 by using the same reaction conditions as for 2a-c. This phosphoramidite 5 could be used satisfactorily to phosphorylate various nucleosidic derivatives. Finally, phosphoramidites 2b and 2c gave the corresponding dimers 4b and 4c as indicated in the TABLE. It is noteworthy that standard conditions (CH_2Cl_2+ 2% trifluoroacetic acid)[15] fully detritylated 4c to give the expected phosphotriester GpT (yield 90%). Its molecular weight was determined by FAB mass spectrometry (MH^+ 754)[16].

In conclusion, we have established a method to prepare stable phosphoramidite derivatives in two steps from 2'-deoxynucleosides (tritylation followed by 3'-O-phosphitylation) which uses simple, easy to handle and commercially available inexpensive chemicals. Such N-nonacylated nucleoside phosphoramidites should be very useful for rapid synthesis of oligodeoxynucleotides at a low cost. From this viewpoint we anticipate that the very versatile cellulose filter disc method [17] would prove to be the most appropriate.

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REFERENCES

1 - For recents reviews see :
S.A. NARANG, Tetrahedron (1983), 39, 3.
K. ITAKURA, J.J. ROSSI and B. WALLACE, Ann. Rev. Biochem.(1984), 53, 323.
2 - R. CHARUBALA and W. PLEIDERER, Heterocycles (1981), 15, 761.

3 - T. TANAKA, R.L. LETSINGER, Nucleic Acids Res. (1982), 10, 3249.
T.P. PATEL, T.A. MILLICAN, C.C. BOSE, R.C. TITMAS, G.A. MOCK, Ibid. (1982), 10, 5605 and references given therein.

4 - S. HONDA, K. TERADA, Y. SATO, M. SEKINE and T. HATA, Chemistry Letters (1982), 15.
A. KUME, M. SEKINE and T. HATA, Tetrahedron Letters (1982), 23, 4365.
L.J. McBRIDE and M.H. CARUTHERS, Ibid. (1983), 24, 2953.

5 - Y. HAYAKAWA, M. UCHIYAMA and R. NOYORI, Ibid. (1984), 25, 4003.
Y. HAYAKAWA, Y. ASO, M. UCHIYAMA and R. NOYORI, Ibid. (1983), 24, 1165 and 5641.

6 - K.K. OGILVIE, A.L. SCHIFMAN and C.L. PENNEY, Can. J. Chem. (1979) , 57, 2230.
K.K. OGILVIE, N. THIERAULT, K.L. SADANA, J. Am. Chem. Soc. (1977) , 99, 7741 and references given therein.

7 - S.L. BEAUCAGE and M.H. CARUTHERS, Tetrahedron Letters (1981), 22, 1859.

8 - For a recent reference see : M.W. SCHWARZ and W. PFLEIDERER, Ibid. (1984), 25, 5513.

9 - J.L. FOURREY and J. VARENNE, Ibid. (1983), 24, 1963 and (1984), 25, 4511.

10- Prepared according to : M. SMITH , D.H. RAMMLER, I.H. GOLDBERG and H.G. KHORANA, J. Am. Chem. Soc. (1963), 84, 3821.

11 - Silica gel: 2a was eluted with ethyl acetate; 2b and 2c were eluted with ethyl acetate : methanol (95:5).

12 - Prepared according to : A.M. MICHELSON and A.R. TODD, J.Chem.Soc.(1954), 34.

13 - H. SCHALLER, G. WEIDMAN, B. LERCH and H.G. KHORANA J. Am. Chem. Soc. (1963), 85, 3821 . We might have used 2'-deoxy-5'-O-dimethoxytritylguanosine. This compound cannot be obtained directly from deoxyguanosine. Moreover 1c shows some advantage due to its high solubility.

14 - J.L. FOURREY and J. VARENNE, Tetrahedron Letters (1985), 26, 1217.

15 - R. CHARUBALA, F. UHLMANN and W. PFLEIDERER Liebigs Ann. Chem. (1981), 2392.

16 - We thank Dr P. VARENNE for the FAB mass spectrometry measurements.

17 - J. OTT and F. ECKSTEIN Nucleic Acids Res. (1984), 12, 9137 .
H.W.D. MATTHES, W.M. ZENKE, T. GRUNDSTROM, A. STAUB, M. WINTZERITH and P. CHAMBON, EMBO J.(1984), 3, 801.
R. FRANK, W. HEIKENS, G. HEISTERBERG-MOUTSIS and H. BLOCKER, Nucleic Acids Res. (1983), 11, 4365.

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