Tetrahedron Letters,Vol.25,No.36,pp 4003-4006,1984 0040-4039/84 $3.00 + .00
Printed in Great Britain

A CONVENIENT METHOD FOR THE FORMATION OF INTERNUCLEOTIDE LINKAGE

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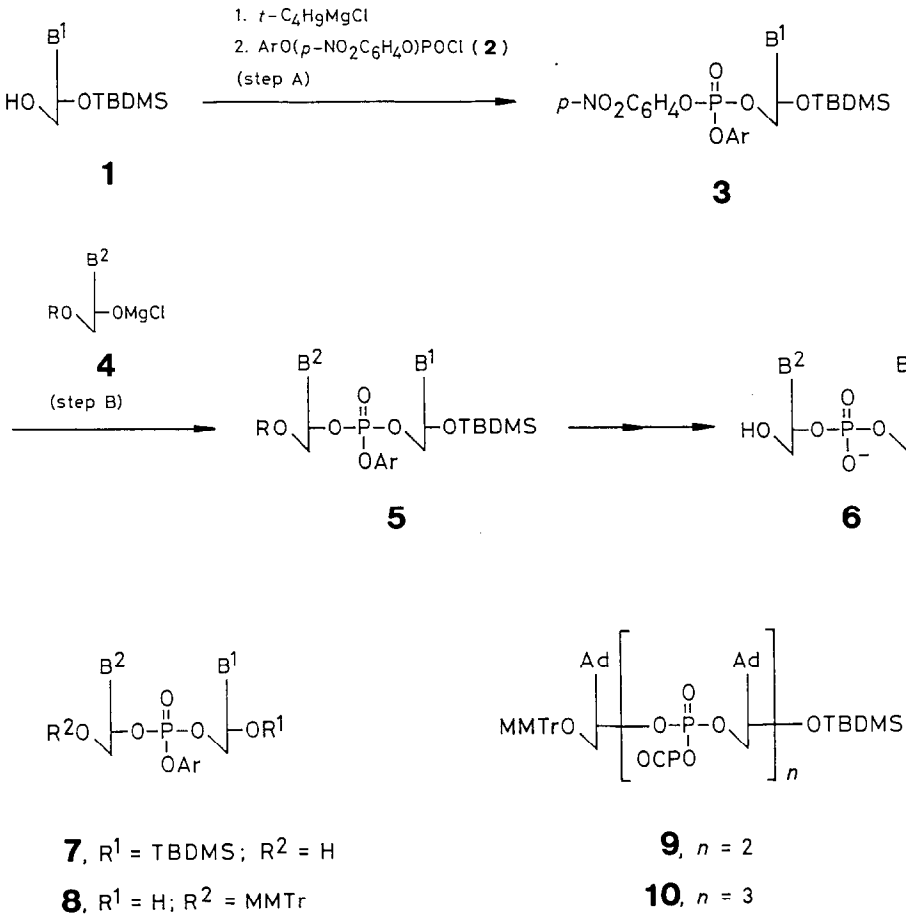
Summary: Internucleotide linkage can be made readily by reaction of N-unprotected nucleosides and phosphorochloridates or p-nitrophenyl phosphates by the assistance of Grignard reagents.

We have demonstrated that exclusive O-phosphorylation of N-unprotected nucleosides is achievable with phosphorochloridates or p-nitrophenyl phosphates by activation of the substrates as oxophilic metal alkoxides.[1] Recently we found that the magnesium-aided procedure[2] was among the most appropriate in view of the wide generality, rapidness, high yield, and operational simplicity. This paper describes the application to formation of internucleotide linkage.[3]

The route to dinucleoside phosphates is illustrated in Scheme I. The first nucleoside 1 was treated with 1 equiv of tert-butylmagnesium chloride and 1—1.1 equiv of o-chlorophenyl p-nitrophenyl phosphorochloridate (2)[4] (step A), and the resulting phosphorotriester intermediate 3, without isolation, was condensed with 0.9—1.1 equiv of the magnesium alkoxide of the second nucleoside, 4, (step B), leading to the phosphate 5 in excellent yield. The nucleotide product could be deprotected to give 6 according to the previously reported operation.[1b] Table I exemplifies the utility. In the preparation of the dinucleoside phosphates using a thymidine or guanosine moiety as the second nucleoside, a mixture of DMF and THF is recommended as the solvent.

Selective deprotection of the nucleotide 5 possessing a 5'-p-methoxytrityl (MMTr) substituent could be done by the known procedures[5] to give 7 and 8, which were usable to preparation of higher nucleotides as the building blocks. Thus, synthesis of trimeric or tetrameric adenyl nucleotides was also accomplished. Treatment of 4 (B^2 = Ade, R = MMTr) with 2 (15 °C, 2 h), followed by the magnesium alkoxide of 7 (B^1 = B^2 = Ade, Ar = o-ClC_6H_4) (15 °C, 12 h, in a 1:5 mixture of DMF and THF) led to the trinucleoside diphosphate 9 in 60% yield. In a similar fashion, condensation of the diadenyl derivative 7 and 2, followed by the second nucleotide 8, afforded the tetramer 10 in 63% yield.

Scheme I



TBDMS = t-$C_4H_9(CH_3)_2Si$; MMTr = p-$CH_3OC_6H_4(C_6H_5)_2C$; OCP = o-ClC_6H_4

Table I. One-Pot Synthesis of the Dinucleoside Phosphate 5

				conditions				yield
				step A	step B			
B^2	B^1	R	Ar	time, h	solvent	temp, °C,	time, h	%[a]
Ade	Ade	TBDMS[b]	C_6H_5	3	THF	15	1.5	85
Ade	Ade	MMTr[c]	o-ClC_6H_4	1	THF	15	2	86
Cyt	Ade	TBDMS	C_6H_5	2.5	THF	60	2	81
Gua[d]	Ade	MMTr	o-ClC_6H_4	2	DMF—THF[e]	15	10	71
Thy[d]	Ade	TBDMS	o-ClC_6H_4	2	DMF—THF[e]	15	12	80

[a] Isolated yield. [b] tert-Butyldimethylsilyl. [c] p-Methoxytrityl. [d] Two equivalents of tert-butylmagnesium chloride were employed. [e] A 1:2 mixture of DMF and THF.

This approach excels the conventionally utilized phosphotriester method[6] in several respects.[7] First of all, no protection of the amino function is necessary. The phosphotriester intermediate of type 3, obtained by the reaction of the bifunctional phosphorylating agent and the first nucleoside (or nucleotide) 1, is reactive enough to undergo the condensation with the second magnesium alkoxide 4. Therefore, the reaction proceeds without activating agents such as arenesulfonyl chlorides or -amides, which are generally expensive and occasionally afford undesired sulfonylated products in the phosphorylation of thymidine[8] or guanosine.[9] Furthermore, the reactive phosphotriester intermediate of type 3 does not require a time-consuming purification and thus all operations for construction of the internucleotide linkage can be carried out simply in one pot.

Typical Procedure for the Preparation of Dinucleotide Phosphates: Synthesis of o-Chlorophenyl 5'-O-p-Methoxytrityl-2'-deoxyadenylyl(3'→5')-3'-O-tert-butyldimethylsilyl-2'-deoxyadenosine (5, $B^1 = B^2$ = Ade, R = MMTr, Ar = o-ClC_6H_4). To a solution of 3'-O-tert-butyldimethylsilyl-2'-deoxyadenosine (1, B^1 = Ade, 1.10 g, 3.00 mmol) in THF (20 mL) was added a 0.58 M solution of tert-butylmagnesium chloride in THF (5.17 mL, 3.00 mmol) at 15 °C. After stirring for 5 min, the reaction mixture was added to a solution of o-chlorophenyl p-nitrophenyl phosphorochloridate (1.04 g, 3.00 mmol) in THF (12 mL) over 45 min under argon. The mixture was stirred at 15 °C for an additional 15 min. In the meanwhile, magnesium alkoxide of 5'-O-p-methoxytrityl-2'-deoxyadenosine (4, B^2 = Ade, R = MMTr) was prepared by the addition of a 0.58 M THF solution of tert-butylmagnesium chloride to a solution of the nucleoside (1.41 g, 2.70 mmol) in THF (12 mL) at 15 °C under argon, and the mixture was stirred at the same temperature for 2 h. The reaction mixture was diluted with dichloromethane (150 mL) and then poured into 10% brine. The resulting emulsion was subjected to cetrifugation (2000 rpm x 5 min) and the separated aqueous layer was extracted with dichloromethane (50 mL, 30 mL x 2). The combined organic layers were dried and concentrated to give a yellow gum (3.9 g). Column chromatography on silica gel (deactivated by addition of 6% of water and 0.5% of triethylamine) using 1:20:7 to 1:10:3 methanol—ethyl acetate—hexane as eluent afforded the title compound[10] (2.46 g, 86% yield, a 1:1 mixture of diastereomeric phosphates) as colorless amorphous solid.

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(Received in Japan 23 May 1984)