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A GENERAL APPROACH TO NUCLEOSIDE 3'- AND 5'-MONOPHOSPHATES

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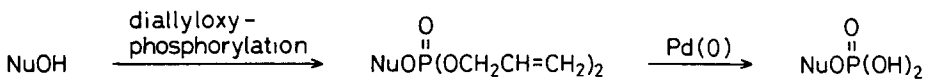
Summary : Diallyloxyphosphorylation of nucleoside hydroxyls followed by palladium(0)-catalyzed deallylation provides a new, general method for the preparation of the 3'- and 5'-monophosphates.

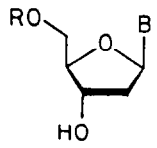
The 3'- and 5'-*O*-monophosphorylation of nucleosides is the significant problem in nucleotide synthesis because the monophosphates serve as the key synthetic intermediates of biologically important nucleoside di- and triphosphates.[1] However, most of the chemical and enzymatic methods developed up to now are applicable only to the 5'-*O*-phosphorylation.[2,3] The appropriate way to phosphorylate the 3'-hydroxyls is still elusive.[4] We disclose herein a general entry to the monophosphates via palladium(0)-mediated deprotection[5] of the diallyl-blocked precursors.



NuOH = nucleoside

The diallyloxyphosphorylation of the hydroxyls, the key step of the present method, is achievable in two different ways. One approach is the Grignard reagent-promoted coupling of nucleosides and diallyl phosphorochloridate[6] (method A).[7] For instance, when the 3'-*O*-free nucleoside **1** [B = Cy ; R = t-$C_4H_9(CH_3)_2Si$ (TBDMS)] was treated with one equiv of t-butylmagnesium chloride and then the diallyloxyphosphorylating agent at room temperature for 0.3 h, the protected nucleoside phosphate **2** (B = Cy ; R = TBDMS) was obtained in 97% isolated yield. The other way to prepare the diallyl phosphates is base-assisted condensation of nucleosides and diallyl phosphorochloridite[8] and oxidation of the resulting phosphite intermediates (method B). Thus, reaction of **1** [B = Th ; R = $C_6H_5(p$-$CH_3OC_6H_4O)_2C$ (DMTr)] and the phosphorochloridite in the presence of 2, 4, 6-collidine (−78 ℃, 0.5 h) followed by oxidation by t-butyl hydroperoxide[9] (0 ℃, 20 min) gave the phosphorotriester **2** (B = Th ; R = DMTr) in 89% yield. Similarly, the 5'-*O*-diallyloxyphosphorylation of **4** was accomplished according to either method. These methods are quite general and applicable to all kinds of nucleosides. Several examples are listed in Table I. Although the reaction using *N*-free cytidine or guanosine derivatives by method B did not give satisfactory yields of the diallyloxyphosphorylated products, the protection of the nucleoside bases improved the efficiency to a considerable extent. We recommend method A for the reaction of simple *N*-unprotected cytidines and guanosines.

Removal of the allyl and other protective groups allows the conversion of the phosphorotriesters, **2** and **5**, to the desired nucleoside 3'-phosphate **3** and 5'-phosphate **6**, respectively. The way of the deblocking was dependent on the



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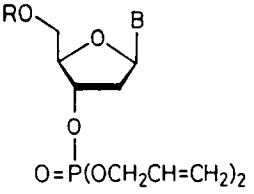

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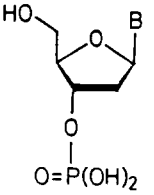

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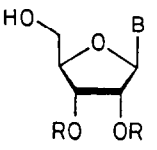

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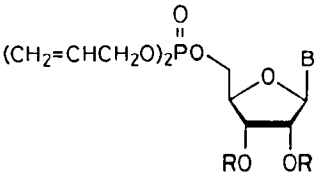

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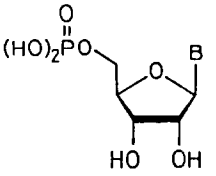

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Table I. Diallyloxyphosphorylation of Nucleoside 3'- and 5'-Hydroxyls

nucleoside		condensation		% yield of 3'-or 5'-phosphate[a]
number (B)	R or R—R	method	time/h	
1 (Ad)	DMTr	A	1	98
1 (Ad)	DMTr	B	4	87
1 (Cy)	TBDMS	A	0.3	97
1 (Cy)	TBDMS	B	2.3	64
1 (AOC-Cy[b])	DMTr	B	0.5	92
1 (Gu)	TBDMS	A[c]	0.7	94
1 (Gu)	TBDMS	B	1.3	37
1 (AOC-Gu[d])	DMTr	B	1	70[e]
1 (Th)	DMTr	A[c]	1	99
1 (Th)	DMTr	B	0.5	89
4 (Ad)	$(CH_3)_2C$	A	2	90
4 (Ad)	$(CH_3)_2C$	B	1	93
4 (Cy)	$(CH_3)_2C$	A	1	91
4 (Gu)	TBDMS	A[c]	1	93
4 (Ur)	$(CH_3)_2C$	A[c]	4.5	86
4 (Ur)	$(CH_3)_2C$	B	0.7	94

[a] Isolated yield. [b] N^4-Allyloxycarbonylcytosinyl [c] Two equivalents of *t*-butylmagnesium chloride was employed. [d] N^2-Allyloxycarbonylguanyl. [e] Yield based on the consumed starting material (ca. 80%).

mode of the protection, and the suitable reaction sequences and conditions were as follows: For the allyl—trityl derivatives, (1) $CHCl_2COOH$ in CH_2Cl_2, 25 ℃, 10 min, (2) 10 mol % $Pd[P(C_6H_5)_3]_4$, 60 mol % $P(C_6H_5)_3$, and n-$C_4H_9NH_2$—HCOOH (1 :4, excess) in THF, 50 ℃ (method C);[10] for the allyl—silyl derivatives, (1) 10 mol % $Pd[P(C_6H_5)_3]_4$, 60 mol % $P(C_6H_5)_3$, and n-$C_4H_9NH_2$—HCOOH (1 :4, excess) in THF, 50 ℃, (2) tetrabutylammonium fluoride (TBAF) in THF, 25 ℃, 1 h (method D); for the allyl—AOC—trityl derivatives, (1) 10 mol % $Pd[P(C_6H_5)_3]_4$, 60 mol % $P(C_6H_5)_3$, and n-$C_4H_9NH_2$—HCOOH (1 :4, excess) in THF, 50 ℃, (2) $CHCl_2COOH$ in CH_2Cl_2, 25 ℃, 2—10 min (method E); for the allyl—isopropylidene derivatives, (1) 10 mol % $Pd[P(C_6H_5)_3]_4$, 60 mol % $P(C_6H_5)_3$, and n-$C_4H_9NH_2$—HCOOH (1 :4, excess) in THF, 50 ℃, (2) 2 N aq HCl, 25 ℃, 12 h (method F). Table II summarizes some examples of the deprotection. The overall process left the whole nucleotide functionalities completely intact.

The present approach provides the first general way capable of preparing all kinds of 2'-deoxyribonucleoside 3'-phosphates, and is advantageous over the existing methods in its applicability to a wide range of nucleosides.

Table II. Deprotection of the Nucleoside Phosphorotriesters, **2** and **5**

nucleotide		deprotection		% yield of **3** or **6**[a]
number (B)	R or R—R	method	Pd treatment/h	
2 (Ad)	DMTr	C	3.7	91
2 (Cy)	TBDMS	D	3	91
2 (AOC-Cy[b])	DMTr	E	4.3	90
2 (Gu)	TBDMS	D	4.3	91
2 (AOC-Gu[c])	DMTr	E	4	83
2 (Th)	DMTr	C	4.3	90
5 (Ad)	$(CH_3)_2C$	F	5	89
5 (Cy)	$(CH_3)_2C$	F	5.7	92
5 (Gu)	TBDMS	D	5.3	100
5 (Ur)	$(CH_3)_2C$	F	5.7	91

[a] HPLC yield of the overall deprotection. [b] N^4-Allyloxycarbonylcytosinyl [c] N^2-Allyloxycarbonylguanyl

Diallyloxyphosphorylation of Nucleosides by Method A. To a solution of **1** or **4** (0.5 mmol) in THF (10 mL) was added a THF solution of t-butylmagnesium chloride (1 equiv for B = Ad, Cy; 2 equiv for B = Gu, Th, Ur) at ambient temperature. After 10 min, diallyl phosphorochloridate (0.5 mmol) was added and the resulting mixture was stirred for the stated period. Usual extractive workup followed by chromatography on silica gel ($CH_3OH/CHCl_3$ = 1/50 to 1/20) gave the phosphorotriester **2** or **5**, respectively.

Diallyloxyphosphorylation of Nucleosides by Method B. A mixture of the nucleoside, **1** or **4** (0.2 mmol), diallyl phosphorochloridite (1.2 mmol), and 2, 4, 6-collidine (1.0 mmol) in THF (1 mL) was stirred at −78 ℃ for the stated period. The mixture was quenched by addition of methanol (0.1 mL) at −78 ℃ and warmed to 0 ℃. To this was added a 1.9 M toluene solution of t-butyl hydroperoxide (2.0 mmol) and stirring was continued for 20 min. Extractive workup and subsequent chromatography as described above afforded **2** or **5**, respectively.

Typical Example of Deprotection. A mixture of **2** (B = Cy, R = TBDMS) (89 mg, 0.18 mmol), $Pd[P(C_6H_5)_3]_4$ (20 mg, 0.018 mmol), $P(C_6H_5)_3$ (27 mg, 0.11 mmol), butylamine (70 μL, 0.71 mmol), and formic acid (106 μL, 2.8 mmol) in THF (2

mL) was kept at 50 ℃ with stirring for 3 h. Concentration gave an oil and this was then treated with a 1 *M* THF solution of TBAF (10 mL, 10 mmol) at ambient temperature for 1 h. Removal of the solvent *in vacuo* gave a viscous residue, which was subjected to HPLC analysis ODS-5 μm, 0.1 *M* KH_2PO_4 + 0.005 *M* $(n\text{-}C_4H_9)_4NBr$ aq buffer/CH_3OH = 95/5, 260 nm, 1 mL/min, 40 ℃, indicating the formation of **3** (B = Cy) in 91% yield. DEAE cellulose (DE52) column chromatography[HCO_3^- form, 3.8 x 12 cm, H_2O to 0.15—1 *M* triethylammonium hydrogencarbonate (pH 7.4)] of the crude material afforded triethylammonium salt of the nucleotide (1436 OD_{270} units, 89% yield) in a pure form.

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8. This phosphorochloridite, bp 78—80 ℃/16 mmHg, was prepared by disproportionation of PCl_3 and $P(OCH_2CH=CH_2)_3$ by heating the 1:2 mixture at 50 ℃ for 2 h.
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10. In the palladium-aided reactions, addition of $P(C_6H_5)_3$ is necessary for obtaining high yields of products. The reaction of **5** [B = Ad; R—R = $(CH_3)_2C$] in the absence of $P(C_6H_5)_3$ gave, after deacetonidation, **6** (B = Ad) in only 66% yield. In place of $Pd[P(C_6H_5)_3]_4$, $Pd_2[(C_6H_5CH=CH)_2CO]_3 \cdot CHCl_3$ and $Pd(OAc)_2$ were also employable, but the yields of products decreased to some extent.

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