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ALLYL PROTECTION OF INTERNUCLEOTIDE LINKAGE

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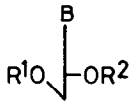
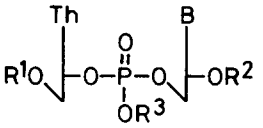
Summary : Allyl group serves as a useful protecting group for an internucleotide bond, which can be removed mildly by brief treatment with a catalytic amount of $Pd[P(C_6H_5)_3]_4$ and various nucleophiles.

A variety of protective groups for internucleotide linkage have been developed.[1] However, removal of the currently used blocking groups such as methyl, cyanoethyl, *o*- or *p*-chlorophenyl, etc., needs strong nucleophiles or bases which sometimes bring about serious side reactions including cleavage of the internucleotide bond. In addition, use of a large excess of the reagents requires tedious operation for obtaining the pure products. We here describe that allyl moiety is easily and cleanly deprotectable by a catalytic amount of a Pd(0) phosphine complex and various nucleophiles. The auxiliary reagents cause no undesired reactions and can be removed by simple workup, thereby avoiding the above annoyances.

First, the phospho(III)triesters could be conveniently made by using a new phosphoramidite coupling agent, $CH_2=CHCH_2OP[N(CH_3)_2]_2$ (**1**).[2] Thus 1*H*-tetrazole-mediated condensation of **1** (1.0 equiv) and the 3′-*O*-free thymidine derivative **2** (1.0 equiv) followed by the 5′-*O*-unprotected thymidine nucleoside **3** (0.95 equiv) in a 1 : 3 THF—acetonitrile mixture[3] gave the corresponding thymidine—thymidine phospho(III)triester intermediate. The product, without isolation, was subsequently oxidized by NO_2[4] in the same solvent at −78 °C to afford the protected dinucleoside phosphate **5**[5] in 86% isolated yield. Removal of the allyl group to the phosphodiester was accomplished almost quantitatively on brief exposure to a mixture of 0.05 equiv of $Pd[P(C_6H_5)_3]_4$, 0.3 equiv of $P(C_6H_5)_3$, and an excess of butylamine in THF. The Pd(0) promoted reaction proceeds via the π-allyl—Pd(II) phosphate intermediate[6] and, as exemplified in Table I, not only butylamine but also various nucleophilic reagents are employable to regenerate the Pd(0) catalyst. Among them use of primary and secondary amines and





2, B = Th; R^1 = MMTr; R^2 = H

3, B = Th; R^1 = H; R^2 = TBDMS

4, B = Ad^{bz}; R^1 = H; R^2 = TBDMS

5, B = Th; R^1 = MMTr; R^2 = TBDMS; R^3 = allyl

6, B = Th; R^1 = R^2 = R^3 = H

7, B = Ad^{bz}; R^1 = MMTr; R^2 = TBDMS; R^3 = allyl

8, B = Ad; R^1 = R^2 = R^3 = H

9, B = Th; R^1 = H; R^2 = TBDMS; R^3 = allyl

10, B = Th; R^1 = MMTr; R^2 = H; R^3 = allyl

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

Table I. Deprotection of the Allyl Dithymidine Phosphate **5**[a]

Pd catalyst	nucleophile (equiv)	time	% yield of deallylated product[b]
$Pd[P(C_6H_5)_3]_4$	n-$C_4H_9NH_2$ (20)	5 min	>95
$Pd[P(C_6H_5)_3]_4$	n-$C_4H_9NH_2$ (2)	75 min	>95
$Pd[P(C_6H_5)_3]_4$	$(C_2H_5)_2NH$ (2)	25 min	>95
$Pd[P(C_6H_5)_3]_4$	$(C_2H_5)_3N$ (2)	12 h	87
$Pd[P(C_6H_5)_3]_4$	C_5H_5N (2)	12 h	>95
$Pd[P(C_6H_5)_3]_4$	CH_3OH (2)	1.5 days	13[c]
$Pd[P(C_6H_5)_3]_4$	HCOOH (2)	2 days	34[d]
$Pd[P(C_6H_5)_3]_4$	CH_3COOH (2)	1.5 days	30[d]
$Pd[P(C_6H_5)_3]_4$	$HCOONH_4$ (2)	12 h	>95
$Pd[P(C_6H_5)_3]_4$	$HCOONH_3$-n-C_4H_9 (2)	50 min	>95
$Pd[P(C_6H_5)_3]_4$	$HCOONH_2(C_2H_5)_2$ (2)	25 min	>95
$Pd[P(C_6H_5)_3]_4$	$HCOONH(C_2H_5)_3$ (2)	12 h	77
$Pd[P(C_6H_5)_3]_4$	$HCOOH-C_5H_5N$ (2)	12 h	>95
$Pd(OCOCH_3)_2$	n-$C_4H_9NH_2$ (20)	1.5 days	52[e]

[a] The reaction was carried out in THF containing **5**, the Pd catalyst (0.05 equiv), $P(C_6H_5)_3$ (0.2−0.3 equiv), and the nucleophile at 20 to 25 °C. [b] The yield was estimated by HPLC (ODS, a 1 : 2 mixture of H_2O and CH_3OH). [c] A *ca.* 70% of the starting material was recovered. [d] A *ca.* 50% of the starting nucleotide was recovered. [e] A *ca.* 40% of the unreacted nucleotide was recovered.

ammonium formates are recommended. $Pd(OCOCH_3)_2$ in place of $Pd[P(C_6H_5)_3]_4$ was less effective for this deblocking reaction. The completely deprotected dinucleoside phosphate **6**[7] was obtained in 84% overall yield through further treatments of **5** with tetrabutylammonium fluoride (TBAF) in THF and dichloroacetic acid in dichloromethane. In a similar manner, the condensation of **1** (2 equiv), the thymidine nucleoside **2** (2 equiv), and the adenosine derivative **4** (1 equiv), followed by NO_2 oxidation, afforded the coupled allyl phosphate **7** in 86% yield. Its deprotection furnished **8**[7] in 80% yield.

The allyl protection is quite stable under conditions to remove ordinary sugar-hydroxyl protecting groups. Thus detritylation of **5** was achieved by dichloroacetic acid in dichloromethane (20 °C, 1 h), giving **9** in 82% isolated yield. While, treatment of **5** with TBAF in THF (20 °C, 1 h) gave 3′-*O*-unprotected dinucleoside phosphate **10** as the single product in 85% yield ; no phosphodiester was obtained. This selective desilylation is in contrast to behavior of the corresponding dinucleoside phosphates having conventional cyanoethyl and *o*- or *p*-chlorophenyl protecting groups which very frequently give the undesired diesters.[8]

Preparation of Thymidylyl(3′→5′)thymidine (6) : A solution of **1** (549 mg, 3.12 mmol), **2** (1.54 g, 3.00 mmol), and 1*H*-tetrazole (252 mg, 3.60 mmol) in a 1 : 3 mixture of THF and acetonitrile (15 mL) was stirred at 20 °C for 80 min. To the mixture were added **3** (1.02 g, 2.86 mmol) and 1*H*-tetrazole (240 mg, 3.42 mmol) and stirring was continued for an additional 2 h. The resulting mixture was treated with 0.43 *N* solution of NO_2[9] in dichloromethane (11 mL, 4.68 mmol) at −78 °C for 30 min and quenched with 0.5 *M* $Na_2S_2O_3$ solution (30 mL). Usual extractive workup followed by silica gel chromatography using a 1 : 30 to 1 : 20 methanol—chloroform mixture as eluent afforded **5** (2.40 g, 86% yield). A mixture of **5** (94 mg, 0.1 mmol), $Pd[P(C_6H_5)_3]_4$ (5 mg, 0.005 mmol), $P(C_6H_5)_3$ (8 mg, 0.03 mmol), and butylamine (146 mg, 2 mmol) in THF (2 mL) was stirred at room temperature for 5 min. After concentration of the mixture, the resulting residue was exposed to 1 *M* TBAF solution in THF (0.15 mL) at room temperature for 17 h followed by dichloroacetic acid (0.3 mL) in dichloromethane (5 mL) at 18 °C for 1.5 h. Quenching with 1 *M* triethylammonium hydrogencarbonate solution (2 mL), extraction with dichloromethane, and evaporation gave gummy material. DEAE-cellulose column chromatography with 0.01 *M* to 0.25 *M* triethylammonium hydrogencarbonate solution (linear gradient) afforded TpT (**6**) (1440 OD, 84% yield).

REFERENCES AND NOTES

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2. Prepared by reaction of $CH_2=CHCH_2OPCl_2$ and an excess of dimethylamine in anhydrous ether at ambient temperature, bp 76—77 °C/12 mmHg. The NMR data of **1** are : ^1H NMR (C_6D_6) δ 2.55 (d, J = 12.8 Hz, 4 NCH_3), 4.15 (ddt, J = 11.0, 4.8, 1.5 Hz, $C=CCH_2$), 5.08 (ddd, J = 10.1, 2.0, 1.5 Hz, cis $CH=CHCH_2$), 5.34 (ddd, J = 15.1, 2.0, 1.5 Hz, trans $CH=CHCH_2$), 5.90 (ddt, J = 15.1, 10.1, 4.8 Hz, $CH_2=CHCH_2$) ; ^{31}P NMR (a 1 : 1 mixture of $CDCl_3$ and C_6H_6) 138.36 ppm downfield from H_3PO_4.
3. H.-J. Lee and S.-H. Moon, *Chem. Lett.*, 1229 (1984).

4. G. S. Bajwa and W. G. Bentrude, *Tetrahedron Lett.*, 421 (1978) ; D. Z. Denney, G. Y. Chen, and D. B. Denney, *J. Am. Chem. Soc.*, **91**, 6838 (1969).
5. A 1 : 1 mixture of the diastereomers : IR ($CHCl_3$) 3350, 2900, 1700, 1680, 1595, 1250 cm^{-1} ; UV (CH_3OH) λ_{max} 266 nm ; ^1H NMR ($CDCl_3$) δ 0.09 (s, $Si(CH_3)_2$), 0.89 (s, Si-*t*-C_4H_9), 1.41, 1.90 (two s's, 2 C(5)CH_3), 2.1—2.8 (m, 4 H_2'), 3.44 (m, 2 H_5' of the 5'-terminal nucleoside), 3.79 (s, OCH_3), 4.0—4.7 (m, 2 H_4', H_3' of the 3'-terminal nucleoside, 2 H_5' of the 3'-terminal nucleoside, $CH_2=CHCH_2$), 5.1—5.5 (m, H_3' of the 5'-terminal nucleoside, $CH_2=$), 5.7—6.1 (m, $CH_2=CH$), 6.1—6.4 (m, 2 H_1'), 6.8 and 7.2—7.5 (m, aromatic protons of MMTr), 7.67 (br s, 2 C(6)H), 9.48 (br s, 2 N(3)H) ; ^{31}P NMR ($CDCl_3$) 9.42 and 10.30 ppm downfield from H_3PO_4.
6. Pd(0)-catalyzed reaction of allyl phosphates was first reported by Murahashi *et al.* See, Y. Tanigawa, K. Nishimura, A. Kawasaki, and S.-I. Murahashi, *Tetrahedron Lett.*, **23**, 5549 (1982). For a comprehensive review of this chemistry, see also, B. M. Trost and T. R. Verhoeven, "Comprehensive Organometallic Chemistry", Vol. 8, G. Wilkinson, Ed., Oxford, pp 799—938 (1982).
7. The product had identical HPLC and electrophoresis characteristics with those of the authentic sample. The structure was also confirmed by enzymatic digestion ; incubation of the product with snake venom phosphodiesterase (pH 7.6, 37 °C, 12 h) gave a 1 : 1 mixture of thymidine and 5'-TMP in **6** or 5'-dAMP in **8**, respectively.
8. For attempts of the selective desilylation reactions : K. K. Ogilvie, S. L. Beaucage, and D. W. Entwistle, *Tetrahedron Lett.*, 1255 (1976).
9. Nitrogen dioxide is *toxic* and should be used in a well-ventilated hood.

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