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CHEMOSELECTIVE PHOSPHORYLATION OF N-UNPROTECTED NUCLEOSIDES VIA ALUMINUM ALKOXIDES

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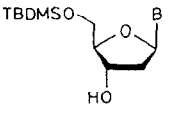
Summary: An O-selective phosphorylation of NH_2-unprotected nucleosides has been achieved via the aluminum alkoxides.

Although a number of phosphorylation methods have been reported for the preparation of nuclectides, most of them have a common problem of chemoselectivity. The reaction of N-unblocked cytidine, adenosine, and guanosine nucleosides forms mixtures of the O- and N-phosphorylated products[1] and therefore the prior N-protection is required to achieve the desired O-phosphorylation. Recently we developed a method via hydroxyl activation by strong bases which provides a partial solution to this perennial problem. This approach, though is particularly useful for the phosphorylation of adenosine,[2] does not yet find general applicability, however. Since the nature of metal cations affects strongly the stability and reactivity of metal alkoxides, we anticipated that metals such as aluminum having greater affinity to oxygen atom than to nitrogen atom[3] would allow selective activation of the hydroxyl function which leads to the desired O-phosphorylation. This expection was indeed realized by employing three types of aluminum alkoxides.

First used was a lithium alkoxytriaryloxyaluminate [$LiAl(OAr)_3OR$, type I alkoxide][4] obtainable by the reaction of $LiAl[N(CH_3)_2]_4$[5] and 1 equiv of a nucleoside followed by 3 equiv of a phenol derivative. The typical example of the 3'-phosphorylation of the deoxyribonucleoside 1 via the aluminate is as follows. To an ice-cooled 0.28 M solution of $LiAl[N(CH_3)_2]_4$ (3.47 mmol) in THF (12.5 mL) was added dropwise a suspension of 5'-O-tert-butyldimethylsilyl-2'-deoxycitidine (1, B = Cyt, 1.18 g, 3.47 mmol) in THF (18 mL) followed by a THF (10 mL) solution of p-nitrophenol (1.45 g, 10.4 mmol). After 60-min stirring, the resulting solution was evaporated to dryness with a vacuum pump[6] to give a foam. This was dissolved in THF (16 mL), and to the resulting homogeneous mixture was added diethyl phosphorochloridate (670 mg, 3.88 mmol) in THF (4 mL) at 0 °C. The mixture was stirred at 0 °C for 6 h and poured into a 0.5 N aqueous NaOH solution (80 mL). The usual extractive workup with dichloro-

methane (80 mL x 2, 40 mL x 2) and chromatographic purification on a silica gel column (1:30 to 1:20 methanol—chloroform mixture) furnished the phosphate 2 (B = Cyt, R = C_2H_5, 1.42 g)[7] in 86% isolated yield (95% by NMR assay). 5'-Phosphates of 3 were prepared as well. An alkoxydiaryloxyaluminum [$Al(OAr)_2OR$, type II alkoxide][4] made from a nucleoside, a phenol, and $Al[N(CH_3)_2]_3$[5] (1:2:1 mol ratio), and $(i\text{-}C_4H_9)_2AlOR$ (type III alkoxide)[4] derived from equimolar amounts of a nucleoside and diisobutylaluminum hydride were other choices of alkoxides. With these reagents, higher reaction temperatures were necessary to obtain reasonable reaction rate.



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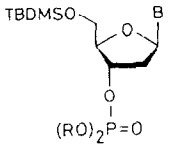

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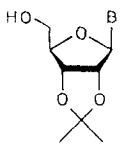


3

TBDMS = $Si(CH_3)_2\text{-}t\text{-}C_4H_9$

This procedure is efficient for not only cytidine nucleosides but also various other nucleosides, as illustrated in Table I. All entries gave no or

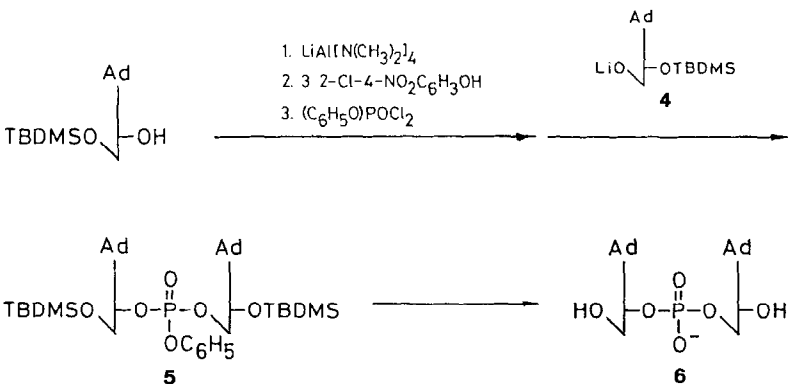
Table I. Phoshorylation of Nucleosides via Aluminum Alkoxides[a]

nucleoside (B)	aluminum alkoxide		R in $(RO)_2POCl$[b]	conditions		% yield of nucleotide[c]
	type	ArO		temp, °C	time, h	
1 (Cyt)	I	p-$NO_2C_6H_4O$	C_2H_5	0	6	95, 86[d]
1 (Cyt)	I	p-$NO_2C_6H_4O$	o-ClC_6H_4	20	6	73, (83)[d,e]
1 (Cyt)	II	o-ClC_6H_4O	o-ClC_6H_4	27	14	62[d]
1 (Thy)	I	p-$NO_2C_6H_4O$	o-ClC_6H_4	0	10	88
1 (Thy)	III	——	C_2H_5	25	3	70
1 (Ade)	I	p-$NO_2C_6H_4O$	o-ClC_6H_4	0	6	82
1 (Ade)	II	o-ClC_6H_4O	o-ClC_6H_4	20	4	67
1 (Ade)	III	——	C_2H_5	25	2	88
1 (Gua)	I	p-$NO_2C_6H_4O$	o-ClC_6H_4	20	11	69
3 (Cyt)	II	o-ClC_6H_4O	C_2H_5	-40 to 0	20	74[d]
3 (Ura)	I	o-ClC_6H_4O	C_2H_5	-20	6	87[d]
3 (Ura^{Me})	I	o-ClC_6H_4O	C_2H_5	-20	3	93[d]
3 (Ura^{Me})	II	o-ClC_6H_4O	C_2H_5	0	4	94, (90)[d,f]
3 (Ade)	I	o-ClC_6H_4O	C_2H_5	-20 to 0	3	97[d]

[a] All reactions were carried out in THF. [b] A slight excess (1.1—1.2 equiv) of the phosphorochloridate was used. [c] Determined by ^1H NMR analysis, unless otherwise stated. [d] Isolated yield. [e] The yield obtained by the reaction using di-o-chlorophenyl-p-nitrophenyl phosphate as the phosphorylating agent (20 °C, 3 h). [f] The yield obtained by the phosphoryaltion with diethyl-2,4-dinitrophenyl phosphate (ArO = p-$NO_2C_6H_4O$, 0 to 20 °C, 24 h).

little (<1%, if formed) N-phosphorylated products. As the phosphorylating agent, not only phosphorochloridates but also p-nitrophenyl or 2,4-dinitrophenyl phosphates are usable. The ArO functions in the aluminum alkoxides affected strongly the product yield and the groups given in Table I showed the satisfactory results.

This chemoselective phosphorylation was successfully used for the formation of internucleotide linkages: Condensation of phenyl phosphorodichloridate with the type-I aluminate, generated from 1 (B = Ade, 1 equiv), $LiAl[N(CH_3)_2]_4$ (1 equiv), and 2-chloro-4-nitrophenol (3 equiv), in THF at -78 to 0 °C for 11 h was followed by reaction with the lithium alkoxide of 3'-O-tert-butyldimethylsilyl-2'-deoxyadenosine (4) (0.7 equiv) in the same pot at -78 to 0 °C for 10 h, to give the phosphorotriester product 5 in 57% yield (based on 4), which could be converted to 6 according to the previously reported procedure.[2]



The present method exhibits two distinctive features. (1) The reaction was accomplished by using an equimolar amount (not excess) of the aluminum reagent to the nucleosides, even in the case of thymidine, uridine, and guanosine derivatives[8] which, in the nucleoside bases, contain a hydrogen(s) more acidic than that of the sugar hydroxyl.[9] (2) Perfectly chemoselective O-phosphorylation was achieved with NH_2-unblocked cytidine, adenosine, and guanosine derivatives. The latter is particularly advantageous, because one can avoid tedious, thriftless blocking-deblocking operations, which have been inevitable in the conventional methods using highly reactive phosphorylating agents such as mixed anhydrides of phosphoric and sulfonic acids. Nucleophilicity of the amino and hydroxyl groups is thus strongly perturbed by the addition of metallic reagents. Evidently, the eminent O vs N selectivity observed in the aluminum alkoxide-promoted procedure is governed primarily by the relative affinities between aluminum and hetero atoms rather than pKa value of the acidic hydrogens.[3,9]

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3. Bond dissociation energy affords a simple guide for affinity of two atoms. The dissociation energies of Al-O and Al-N bonds are 512.1 ± 4.2 and 297 ± 96 KJ/mol, respectively. J. A. Kerr and A. F. Trotman-Dickenson, In "Handbook of Chemistry and Physics", 58th edn, R. C. Weast, Ed., p. F-220, Chemical Rubber Co., Cleaveland (1977), and references cited therein. For chemical evidence of greater affinity of aluminum to oxygen than to nitrogen and its synthetic application, see H. Yamamoto and H. Nozaki, Angew. Chem., Int. Ed. Engl., 17, 169 (1978).
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6. The resulting dimethylamine, a potential nucleophile, was removed from the reaction system by this operation.
7. Foam: IR ($CHCl_3$) 3540 and 3400 (NH_2), 1260 cm^{-1} (P=O); UV λ_{max} (CH_3OH) 272 nm (ε 9900); ^1H NMR ($CDCl_3$) δ 0.10 (s, $Si(CH_3)_2$), 0.90 (s, Si-t-C_4H_9), 1.34 (dt, J = 1.1, 7.1 Hz, 2 $POCH_2CH_3$), 1.9—2.3 (m, H_2,), 2.6—2.8 (m, H_2,), 3.89 (m, 2 H_5,), 4.11 (dq, J = 7.3, 7.1 Hz, 2 $POCH_2CH_3$), 4.27 (m, H_4,), 4.92 (m. H_3,), 5.67 (d, J = 7.4 Hz, H_5), 6.00 (br s, NH_2), 6.38 (dd, J = 1.8, 11.7 Hz, H_1,), 7.90 (d, J = 7.4 Hz, H_6); ^{13}C NMR ($CDCl_3$) δ -6.2, -6.1 ($Si(CH_3)_2$), 15.4 (d, J = 7 Hz, $POCH_2CH_3$), 17.6 ($SiC(CH_3)_3$), 39.4 (d, J = 4 Hz, C_2,), 62.5 (C_5,), 63.4 (d, J = 8 Hz, $POCH_2CH_3$), 77.3 (d, J = 6 Hz, C_4,), 85.0 (d, J = 8 Hz, C_3,), 85.2 (C_1,), 94.7 (C_5), 139.4 (C_6), 155.3 (C_2), 165.5 (C_4).
8. In the phosphorylation promoted by strong bases,[2] 2 equiv of the strong base was needed to achieve the high-yield reaction of N^3-unblocked thymidine or uridine derivatives. Further, guanosine nucleosides failed the phosphorylation reaction.
9. The pKa values of hydroxyl and amino protons of 2'-deoxyribonucleosides reported in literatures or measured in our laboratories are: thymidyl and uracil 3-NH (9.2[10]), guanyl 1-NH (9.2[10]), 5'-OH (ca. 17[11]), 3'-OH (ca. 18[11]), cytocyl 4-NH_2 (ca. 20[12]), and adenyl 6-NH_2 (ca. 20[12]).
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