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Direct Phosphorylation of Nucleosides by Oxyphosphorane

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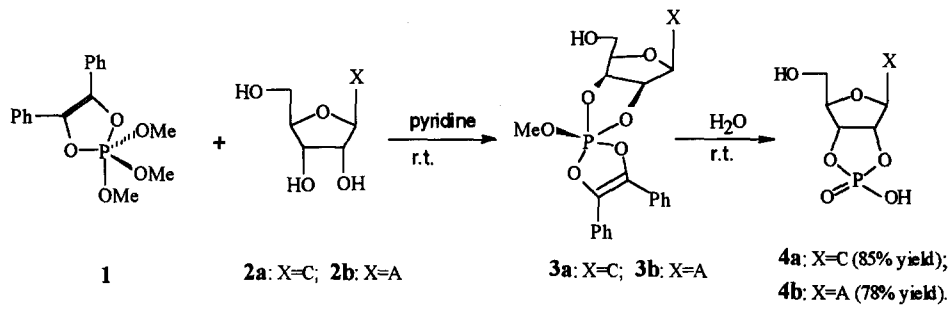
Abstract: Ester exchange reaction of oxyphosphorane **1** with unprotected ribonucleosides cytidine **2a** and adenosine **2b** generated selectively spirooxyphosphoranes **3a** and **3b** respectively. Upon hydrolysis, the labile spirooxyphosphoranes were converted into stable ribonucleoside 2',3'-cyclic monophosphates **4a** and **4b** in good yields. Some polyribonucleotides were also detected from the hydrolytic products. Reaction of **1** with a 2'-deoxyribonucleoside such as thymidine **5** followed by hydrolysis afforded thymidine acyclic monophosphates (**7**, **8**). Hence, the ester exchange reaction of oxyphosphorane might provide an efficient one-pot phosphorylation methodology for ribonucleosides and 2'-deoxyribonucleosides without any protection. © 1997 Elsevier Science Ltd. All rights reserved.

It is well established that most nucleoside analogues become biologically active as a result of cellular conversion to phosphomonoesters. For this reason, there has been increasing interest in the synthesis of nucleoside phosphotriesters, and in some cases, phosphodiesters which might act as membrane soluble prodrugs of the bioactive nucleotides.[1] In the literature, phosphoranes had been proposed as the possible intermediates in the displacement reactions of ATP with nucleophiles,[2,3] as well as in the activation of protein kinases by cAMP for the regulatory subunit.[4-6] Recently, the kinetics and mechanism of the ester exchange reaction of oxyphosphorane (**1**) with nucleosides had been investigated in details in this laboratory.[7] In the present communication, we wish to report an efficient preparation methodology for mononucleotides by the ester exchange reaction of oxyphosphorane.

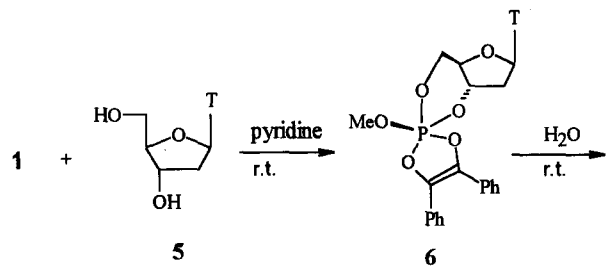
The ester exchange reaction of oxyphosphorane (**1**) with ribonucleosides, such as cytidine (**2a**) and adenosine (**2b**), in pyridine solution at room temperature afforded selectively diastereomeric spirooxyphosphoranes[4,6] composed of two five-membered rings (**3a, 3b**) (**Scheme 1**). The labile compounds **3a** and **3b** could not been isolated, and their structures were determined by their spectral properties in solution.[8] After the

reaction was complete, water was added to the reaction solution to decompose the resulting spiro-oxyphosphoranes (**3a, 3b**). **3a** was converted into cytidine-2',3'-cyclic phosphate **4a** in 85% yield (based on the consumed **2a**), and adenosine-2',3'-cyclic phosphate **4b** resulted from **3b** in 78% yield (based on the consumed **2b**). The ribonucleoside 2',3'-cyclic phosphates were purified by DEAE-cellulose (D-32) column chromatography, eluting with a linear gradient of 0.005-0.05 M NH_4HCO_3 buffer. Their structures were confirmed by their ^{31}P, ^{1}H NMR spectral and MS data, as well as ^{1}H-^{1}H and ^{1}H-^{31}P COSY experiments. The possible mechanism of formation of **4a** and **4b** was proposed in **Scheme 2**. Holy *et al.* had used triethyl phosphite as a phosphorylation agent to synthesize ribonucleoside 2',3'-cyclic phosphates in three steps: by subsequent transesterification of ribonucleosides with triethyl phosphite, the corresponding ribonucleoside phosphites were formed, which were hydrolyzed to a mixture of 2'- and 3'-phosphites, and finally oxidation of the mixture lead to the corresponding 2',3'-cyclic phosphates.[9] The reported yields for the cyclic phosphates were in the range 57-70%, and the preparation procedure involved two purification steps with DEAE cellulose column chromatography.

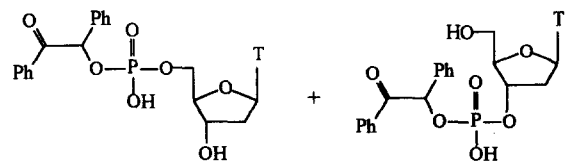
Scheme 1



1 **2a**: X=C; **2b**: X=A **3a**: X=C; **3b**: X=A **4a**: X=C (85% yield); **4b**: X=A (78% yield).



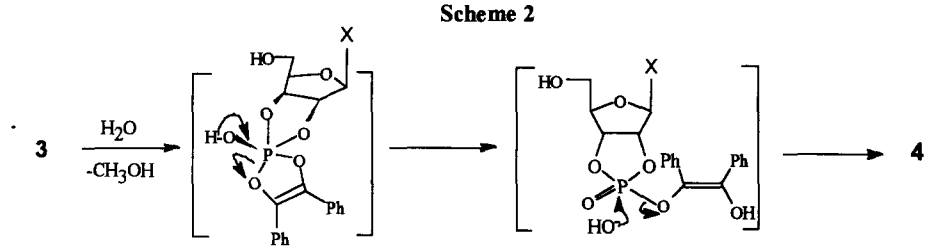
5 **6**



7 (48%) **8** (26%)

It is worthy of noting that besides the 2',3'-cyclic phosphates, considerable amounts of polyribonucleotides were also detected by FAB-MS from the hydrolytic product mixtures. For example, after cytidine-2',3'-cyclic phosphate **4a** has been collected, some higher polar components were obtained by a further elution with a linear gradient of 0.05-0.5 M NH_4HCO_3 buffer from the D-32 column. The negative FAB-MS of the components showed one peak at *m/z* 853, which probably ascribed to cytidine acyclic phosphate trimer CpCpC. Another peak at *m/z* 628 was due to the dimer CpCp. Determination on the exact structures of the polyribonucleotides is in hand.

Scheme 2



2'-Deoxyribonucleosides underwent relatively slower ester exchange reaction toward oxyphosphorane (**1**).[7] When thymidine (**5**) was mixed with **1** in pyridine, a pair of diastereomeric 5-membered--6-membered spirooxyphosphorane **6** resulted. Upon hydrolysis, spirooxyphosphorane **6** was converted into thymidine-5'-yl benzoin phosphate **7** (in 48% yield, based on the consumed **5**) and thymidine-3'-yl benzoin phosphate **8** (in 26% yield) (**Scheme 1**). Compounds **7** and **8** were purified by D-32 column chromatography, and their structures were determined by the ^{31}P, ^{1}H and ^{13}C NMR spectral and HRMS data.[10] The ^{1}H-^{1}H and ^{1}H-^{31}P COSY experiments also supported the structural determinations. But among the hydrolytic products, no cyclic phosphates were detected. This seems to contradict normal behaviour, since it is well known that the hydrolysis of five-membered cyclic esters of phosphoric or phosphonic acids in acid or base proceed millions of times faster than that of their acyclic analogues.[11] But for the hydrolysis of the spirooxyphosphoranes, the saturated five-membered rings in the spirooxyphosphoranes (**3a, 3b**) play a key role during the course of hydrolysis, because a five-membered ring has a significant stabilizing effect on pentacoordinated phosphorus structure,[12] whereas a six-membered ring has no such stabilizing effect.

From the results described above, it could be concluded that the ester exchange reaction of oxyphosphoranes provide an efficient phosphorylation methodology for nucleosides. The advantage of this methodology is that not only this is a one-pot procedure, but also no prior protection for other amino or hydroxyl groups is necessary.

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8. Spectral data for compound **3b** $C_{25}H_{24}N_5O_7P$: δ_P (81 MHz, DMSO-d_6): -23.50 (major), -25.19 (minor) ppm with a ratio of 2:1 (a pair of diastereomeric isomers[4,6]); δ_H (500 MHz, DMSO-d_6) for the major isomer: 3.70 (brs, 1H, 5'-OH), 3.84 (d, 3H, $^3J_{PC}$=13.5 Hz, CH_3O), 3.91 (m, 2H, 5'-H), 4.31 (m, 1H, 4'-H), 4.96 (m, 1H, 3'-H), 5.48 (m, 1H, 2'-H), 6.35 (brs, 1H, 1'-H), 7.45-7.68 (m, 10H, Ar-H), 7.62 (brs, 2H, NH_2), 8.30 (s, 1H, 8-H), 8.55 (s, 1H, 2-H) ppm; δ_C (125 MHz) for the major isomer: 53.82 (d, $^2J_{PC}$=10.8 Hz, CH_3O), 61.31 (5'-C), 73.25 (d, $^2J_{PC}$=4.1 Hz, 3'-C), 75.50 (d, $^2J_{PC}$=4.7 Hz, 2'-C), 86.31 (4'-C), 89.03 (1'-C), 120.64 (5-C), 126.25, 127.78, 128.27, 130.30 (d, $^3J_{PC}$=12.8 Hz) (12C, Ar-C), 133.67 (PhC=CPh), 139.66 (8-C), 149.11 (4-C), 153.33 (2-C), 156.11(6-C) ppm; MS (EI): *m/z* 537 (M^+).
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10. The spectral data for compounds **7** and **8** were obtained from the separated pure components of the crude products of hydrolysis of **6**. **7**: δ_P (D_2O): 1.20 ppm; δ_H (D_2O, OH and NH were exchanged): 1.91 (s, 3H, C_5-CH_3), 2.15-2.30 (m, 2H, 2'-H), 4.12 (m, 2H, 5'-H), 4.21 (m, 1H, 4'-H), 4.90 (m, 1H, 3'-H), 6.18 (dd, 1H, *J*=6.7 Hz, 1'-H), 6.56 (d, 1H, $^3J_{PH}$=9.1 Hz, PhCH), 7.52 (s, 1H, C_6-H), 7.30-7.88 (m, 10H, Ar-H); MS (negative FAB) calcd. for $C_{24}H_{24}N_2O_9P$: *m/z* 515.1219 $(M-1)^-$, found: 515.1200. **8**: δ_P (D_2O): 1.10 (brs); δ_H (D_2O, OH and NH were exchanged) for one isomer: 1.91 (s, 3H, C_5-CH_3), 2.14-2.26 (m, 2H, 2'-H), 3.75 (m, 2H, 5'-H), 4.08 (m, 1H, 4'-H), 4.69 (m, 1H, 3'-H), 6.15 (dd, 1H, *J*=6.7 Hz, 1'-H), 6.71 (d, 1H, $^3J_{PH}$=8.7 Hz, PhCH), 7.56 (s, 1H, C_6-H), 7.33-8.03 (m, 10H, Ar-H); δ_C: 11.00 (C_5-CH_3), 37.28 ($^3J_{PC}$=3.0 Hz, 2'-C), 60.62 (5'-C), 74.54 ($^2J_{PC}$=5.3 Hz, 3'-C), 78.78 ($^2J_{PC}$=4.8 Hz, PhCH), 84.64 (1'-C), 85.23 ($^3J_{PC}$=6.4 Hz, 4'-C), 111.00 (5-C), 127.25, 128.59, 129.03, 133.66, 135.14 (12C, Ar-C), 136.96 (6-C), 151.08 (2-C), 165.93 (4-C), 198.30 ($^3J_{PC}$=1.0 Hz, PhCO); MS (negative FAB) calcd. for $C_{24}H_{24}N_2O_9P$: *m/z* 515.1219 $(M-1)^-$, found: 515.1211.
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