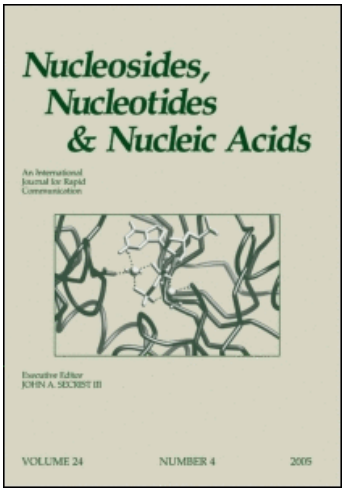

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Synthesis of Nucleoside 3'-Thiophosphates in One Step Procedure

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SYNTHESIS OF NUCLEOSIDE 3'-THIOPHOSPHATES IN ONE STEP PROCEDURE

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ABSTRACT:

A mild and efficient one-step method of thiophosphorylation was devised for acid-sensitive nucleosides. The procedure is based on thiophosphorylation of nucleoside magnesium alkoxide by 2-chloro-2-thio-1,3,2-dioxaphospholane. The utility and efficiency of this method combined with deprotection of the resulting cyclic triester were demonstrated by its application to the synthesis of both adenosine 3'- and 5'-thiophosphates. The procedure does not require protection of the exocyclic amino group and can be successfully used for the thiophosphorylation of nucleosides that are unusually sensitive to depurination.

Continuing our studies on the structure activity relationship of inhibitors of adenylyl cyclase, we synthesized 3'-thiophosphate analogs of 2'-d-3'AMP and 2',5'-dd-3'AMP, two modestly potent inhibitors of this enzyme.[1-2]

A new, efficient method for introduction of a thiophosphate group in one step was devised. The procedure is based on thiophosphorylation of nucleoside magnesium alkoxide by 2-chloro-2-thio-1,3,2-dioxaphospholane. The utility and efficiency of this method combined with deprotection of the resulting cyclic triester has been demonstrated by its application to the synthesis of both adenosine 3'- and 5'-thiophosphates. The procedure does not require protection of the exocyclic amino group and can be successfully used for the preparation of labile nucleotides such as 2',5'-dd-3'AMPS, unavailable via the phosphoramidite approach.

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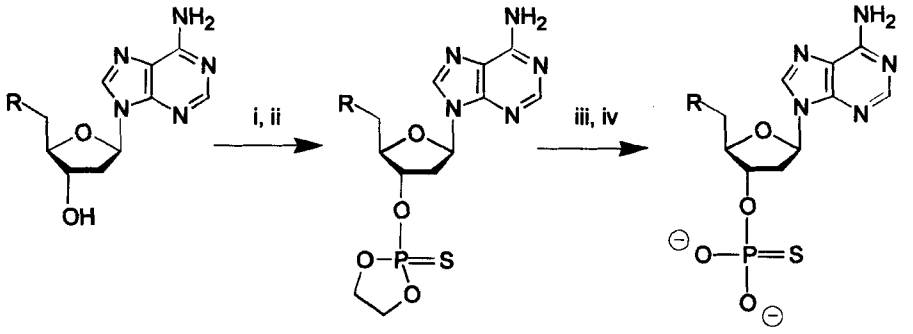
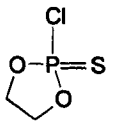
Scheme



1a , R=H
b , R=TBDMSO

2a , R=H
b , R=TBDMSO

3a , R=H
b , R=OH



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Reagents : i, *tert* -BuMgCl, THF, rt, 5 min; ii, **4** , 1.5 h; iii, NaC DMSO, rt, 18h; iv, NaOH, EtOH, rt, 2h and TBAF in THF, rt, 6h in case of **2b** . Overall yields: 61% for **3a** and 31% for **3b** .

Sequential treatment of *N*-unprotected 2',5'-dd-Ado[3] **1a** with *tert*-butylmagnesium chloride and 2-chloro-2-thio-1,3,2-dioxaphospholane[4] **4** yielded the thiophosphate **2a** (Scheme). The cyclic triester **2a** could be easily deprotected by treatment with NaCN in DMSO under vacuum followed by ethanolic NaOH solution to give 2',5'-dd-3'AMPS **3a** in 61% overall yield.[5] Analogous treatment of 5'-TBDMS-2'-deoxyadenosine **1b** and 2',3'-O-isopropylideneadenosine gave 2'-d-3'AMPS and 5'AMPS in overall yields 31% and 47%, respectively. The structures of all products were confirmed by ^{1}H-NMR, ^{31}P-NMR, MS, and UV spectroscopic analyses.[6]

As expected, 2'-d-3'AMPS and 2',5'-dd-3'AMPS were found to inhibit adenylyl cyclase, with potencies comparable to those of the respective 3'-phosphates.[7] Both 2'-d-3'AMPS and 2',5'-dd-3'AMPS were stable to rat liver hydrolytic enzymes, which agrees with the generally higher stability of thiophosphates as opposed to phosphates. But, somewhat surprisingly, the 3'-thiophosphates were not effective competitive inhibitors of the hydrolysis of 2'-d-3'AMP.

The availability of these thiophosphates can lead to the preparation of tools for biochemical and pharmacological studies. This includes the synthesis of stable, chiral

derivatives useful in studies of enzyme mechanism or conjugation to reactive functionalities to create unique probes such as affinity labels.

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7. IC_{50} values are as follows: 2'-d-3'AMP: 1.2 μM, 2'-d-3'AMPS: 3.1 μM, 2',5'-dd-3'AMP: 0.46 μM, 2',5'-dd-3'AMPS: 0.60 μM.