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One-Pot Synthesis of Deoxyadenosine 3'-Thiophosphates

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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 was 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.

Keywords: enzyme inhibitors; thiophosphates; nucleosides; nucleotides.

Continuing our studies on structure activity relationship of inhibitors of adenylyl cyclase, we were interested in synthesizing 3'-thiophosphate analogs of 2'-d-3'AMP and 2',5'-dd-3'AMP, two inhibitors of this enzyme.[1,2] Thiophosphate derivatives are powerful tools for biochemical and pharmacological studies since they are more stable than are the respective phosphates, present a chirality useful in studies of enzyme mechanisms, and can be easily conjugated to reactive functionalities to create probes for affinity or fluorescence labeling.[3-6] The synthesis of polynucleotides that contain thiophosphate linkages is well described, however the synthesis of the unsubstituted 3'-nucleotide thiophosphates has received considerably less attention.[7,8]

The first preparation of 2'-d-3'AMPS was achieved in 3 steps from di-protected 2'-deoxyadenosine **1** by modification of known procedures.[9-11] In the first step, **1** was converted to phosphite via phosphoramidate and then oxidized with elemental sulfur to yield triester **2**. The complete deprotection of **2** provided thiophosphate **3** in an overall yield of 71 % (Scheme 1).

This approach, however, could not be applied to the synthesis of 2',5'-dd-3'AMPS. The protection of the exocyclic amino group of 2',5'-dideoxyadenosine (2',5'-dd-Ado) would be required and classical methods of protecting this functionality were unsuccessful due to extensive depurination. Since this nucleoside is significantly less stable than 2'-deoxyadenosine (2'-d-Ado), we sought a milder method for 3'-thiophosphorylation. This report describes an efficient method of introducing thiophosphate group in one step without protection of the exocyclic amino group of adenine. The method can be applied to the synthesis of nucleotides sensitive to depurination.

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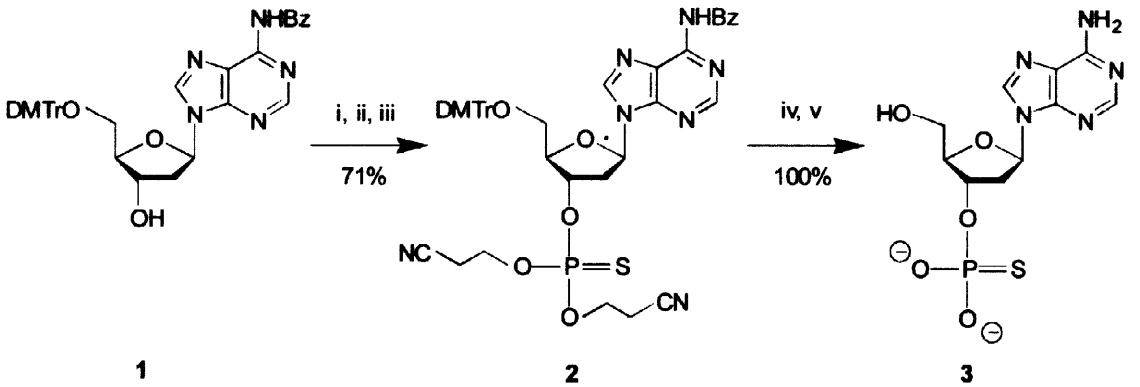
Scheme 1



1 2 3

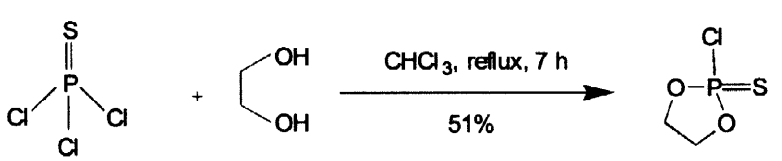
Reagents: i, $(i\text{-}Pr)_2NPCl(OCH_2CH_2CN)$, $i\text{-}Pr_2NEt$, THF, rt, 35 min; ii, 1-H-tetrazole, $HOCH_2CH_2CN$, CH_3CN,rt,16 h; iii, S_8, pyr,rt , 45 min; iv, conc.aq NH_3; v, 80% AcOH, rt, 30 min.

The synthesis is based on thiophosphorylation of nucleoside magnesium alkoxide by 2-chloro-2-thio-1,3,2-dioxaphospholane **5**. 2-Chloro-2-thio-1,3,2-dioxaphospholane **5** was prepared in 51% yield by refluxing a mixture of ethylene glycol and thiophosphoryl chloride in chloroform (Scheme 2).[12] Sequential treatment of *N*-unprotected 2',5'-dd-Ado[13] **4a** with *tert*-butylmagnesium chloride and 2-chloro-2-thio-1,3,2-dioxaphospholane **5** yielded the thiophosphate **6a** (Scheme 3). The cyclic triester **6a** could be easily deprotected by treatment with NaCN in DMSO under vacuum followed by ethanolic NaOH solution.[14] By application of this strategy, **6a** furnished 2',5'-dd-3'AMPS **7** in 61% overall yield.[15]

The general applicability of the above thiophosphorylation procedure was verified by the syntheses of 2'-d-3'AMPS **3** and 5'AMPS **10** from 5'-O-TBDMS-2'-deoxyadenosine[16] **4b** and 2',3'-O-isopropylideneadenosine **8**, respectively (Schemes 3 and 4). Final deprotection of **6b** was carried out with 1 M TBAF in THF and of **9** with 60 % HCOOH, to give 2'-d-3'AMPS **3** (31% overall yield) and 5'AMPS **10** (47% overall yield). The structures of compounds **3**, **7**, **9**, and **10** were confirmed by ^{1}H-NMR, ^{31}P-NMR, MS and UV spectroscopy analyses.[17]

In conclusion, the utility and efficiency of the thiophosphorylation method by use of **5** has been demonstrated by its application to the synthesis of 2',5'-dd-3'AMPS. This one-pot method requires only 2 steps and avoids protection and deprotection of the exocyclic amino group, generally required for such syntheses. Although the method can be used for the synthesis of naturally occurring nucleotides, its main value resides in the potential for the synthesis of labile nucleotides unavailable by the phosphoramidite approach. This procedure, due to its simplicity, should find general application in the synthesis of nucleotide analogs prone to depurination.

Scheme 2



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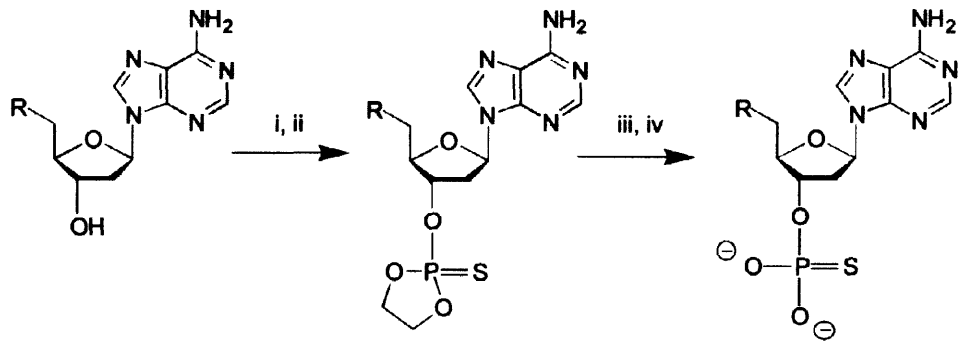
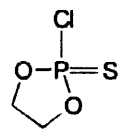
Scheme 3



4a, R=H
b, R=TBDMSO

6a, R=H
b, R=TBDMSO

7, R=H
3, R=OH



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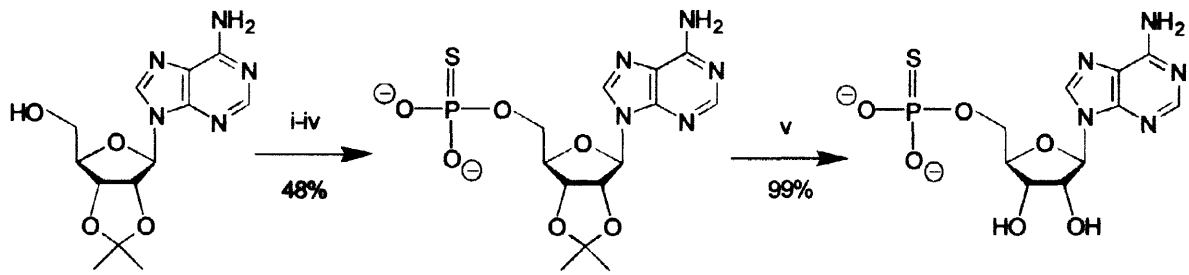
Reagents: i, *tert*-BuMgCl, THF, rt, 5 min; ii, **5**, 1.5 h; iii, NaCN, DMSO, rt, 18h; iv, NaOH, EtOH, rt, 2h and TBAF in THF, rt, 6h in case of **6b**. Overall yields: 61% for **7** and 31% for **3**.

Scheme 4



8 **9** **10**

Reagents: i, *tert*-BuMgCl, THF, rt; ii, **5**, 1.5h; iii, NaCN, DMSO, rt, 21h; iv, NaOH, EtOH, rt, 2h; v, 60% HCOOH, rt, 24h.

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REFERENCES AND NOTES

Abbreviations used: 2'-d-3'AMP, 2'-deoxyadenosine-3'-monophosphate; 2',5'-dd-3'AMP, 2',5'-dideoxyadenosine-3'-monophosphate; 2'-d-3'AMPS, 2'-deoxyadenosine-3'-thiophosphate; 2',5'-dd-3'AMPS, 2',5'-dideoxyadenosine-3'-thiophosphate; DMSO, dimethylsulfoxide; Bz, benzoyl-; THF, tetrahydrofuran; HPA, 4-hydroxypicolinic acid; DMF, dimethylformamide; TEAB, triethylammonium bicarbonate.

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15. *tert*-Butylmagnesium chloride (3 mmol) was added dropwise to a solution of 2',5'-dd-Ado **4a** (3 mmol) in the mixture of THF (40 ml) and DMF (3 ml). After 5 min, a solution of **5** (3.75 mmol) in benzene was added and the mixture was stirred at room temperature for an additional 1.5 h. The reaction mixture was concentrated, diluted with 50 ml water and extracted with ethyl acetate. After evaporation of solvent, **6a** was dissolved in 30 ml DMSO containing 4 equivalents of NaCN. The solution was stirred overnight under vacuum. Then the mixture was treated for 2 h with 4.5 mmoles of NaOH in 20 ml of EtOH. Final compound **7** was purified on DEAE-Sephadex ion-exchange column using TEAB as an eluent. Isolated triethylammonium salt of **7** was converted to its sodium salt by treatment with 1 M NaI in acetone.
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17. Selected spectra:
    Compound **3**: ^1H NMR (250 MHz, D_2O) δ 2.67-2.79 (m, 2H, H-2'), 3.77 (m, 2H, H-5'), 4.27 (m, 1H, H-4'), 4.92 (m, 1H, H-3'), 6.38 (t, 1H, J=6.8 Hz, H-1'), 8.10 (s, 1H, H-2), 8.24 (s, 1H, H-8). ^{31}P NMR (101 MHz, D_2O) δ 31.78. MALDI-TOF (HPA, negative ionization) m/z 346.3 (M+1 calcd for $C_{10}H_{12}N_5O_5PS$: 346.3).
    Compound **7**: ^1H NMR (250 MHz, D_2O) δ 1.37 (d, 3H, J=6.6 Hz, H-5'), 2.79 (m, 1H, H-2'), 2.89 (m, 1H, H-2''), 4.38 (m, 1H, H-4'), 4.40 (m, 1H, H-3'), 6.40 (t, 1H, J=6.9 Hz, H-1'), 8.18 (s, 1H, H-2), 8.36 (s, 1H, H-8). ^{31}P NMR (101 MHz, D_2O) δ 47.26. MALDI-TOF (HPA, negative ionization) m/z 330.5 (M+1 calcd for $C_{10}H_{12}N_5O_4PS$: 330.3).
    Compound **9**: ^1H NMR (250 MHz, MeOH-d_4) δ 1.38 and 1.61 (2s, 6H, $C(CH_3)_2$), 3.98-4.17 (m, 2H, H-5'), 4.53 (m, 1H, H-4'), 5.23-5.33 (m, 2H, H-3' and H-2'), 6.23 (d, 1H, J=3.5 Hz, H-1'), 8.16 (s, 1H, H-8), 8.83 (s, 1H, H-2). ^{31}P NMR (101 MHz, D_2O) δ 52.02. MALDI-TOF (HPA, negative ionization) m/z 402.0 (M+1 calcd for $C_{13}H_{16}N_5O_6PS$: 402.3).
    Compound **10**: ^1H NMR (250 MHz, D_2O) δ 4.18 (m, 2H, H-5'), 4.42 (m, 1H, H-4'), 4.55-4.77 (m, 2H, H-3' and H-2'), 6.12 (d, 1H, J=5.5 Hz, H-1'), 8.22 (s, 1H, H-8), 8.62 (s, 1H, H-2). ^{31}P NMR (101 MHz, D_2O) δ 51.90. MALDI-TOF (HPA, negative ionization) m/z 362.1 (M+1 calcd for $C_{10}H_{12}N_5O_6PS$: 362.3).