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Further studies with adenosine 5′-dithiophosphoromorpholidate. A convenient preparation of nucleoside phosphorodithioates by a 'triester' approach

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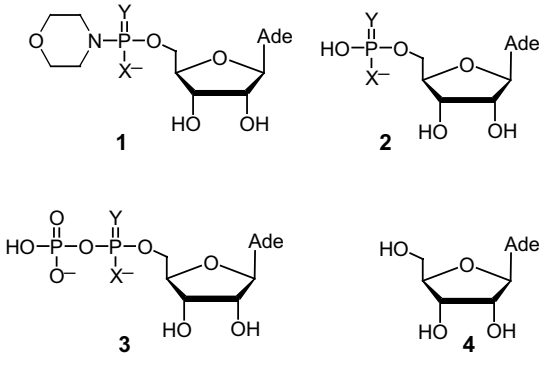
Abstract—Adenosine 5′-dithiophosphoromorpholidate **1c** reacts with orthophosphate to give adenosine 5′-(α,α-dithio)diphosphate **3c** but is not converted into adenosine 5′-phosphorodithioate **2c** by acidic hydrolysis. A new approach to the synthesis of nucleoside phosphorodithioates is described.

Nucleoside 5′-phosphoromorpholidates,[1] such as the adenosine derivative **1a**, are important intermediates in the synthesis of condensed phosphates (e.g., adenosine 5′-di- and tri-phosphates[1]) and nucleotide coenzymes (e.g., coenzyme A[2]); they also readily undergo acid-catalysed hydrolysis[1,3] to give the corresponding nucleoside 5′-phosphates (e.g., adenosine 5′-phosphate **2a**). In the same way, adenosine 5′-thiophosphoromorpholidate[3,4] **1b** reacts with orthophosphate to give adenosine 5′-(α-thio)-diphosphate[3] **3b** and undergoes acid-catalysed hydrolysis to give adenosine 5′-phosphorothioate[3,4] **2b**. Several years ago, we were surprised to find that adenosine 5′-dithiophosphoromorpholidate[4,5] **1c** underwent hydrolysis in 95% acetic acid at room temperature to give adenosine **4** as the sole nucleoside or nucleotide product. Shortly after the publication of our study,[4] Caruthers and his co-workers reported[6] the first synthesis of adenosine 5′-phosphorodithioate **2c**, and found that it was readily converted into adenosine **4** under mild conditions of acidic hydrolysis. We had originally concluded[4] that adenosine 5′-dithiophosphoromorpholidate **1c** was converted *directly* into adenosine **4** by acidic hydrolysis. However, it was clearly possible that acid-labile adenosine 5′-phosphorodithioate **2c** was an intermediate, which, under the prevailing acidic conditions, was rapidly converted into adenosine **4**. This possibility has prompted us to reconsider whether adenosine 5′-dithiophosphoromorpholidate **1c** is a suitable starting material for the synthesis of condensed α,α-dithiophosphates, such as adenosine 5′-(α,α-dithio)-diphosphate **3c**.



We now report that adenosine 5′-(α,α-dithio)diphosphate **3c** was indeed obtained in ca. 25% yield when adenosine 5′-dithiophosphoromorpholidate **1c** was heated with an excess of mono-(tri-*n*-propylammonium) phosphate in anhydrous pyridine solution.[7] The modest yield obtained may possibly have been due to the difficulty encountered in excluding moisture in a relatively small scale reaction. Although it would be premature to conclude that this is a particularly satisfactory approach

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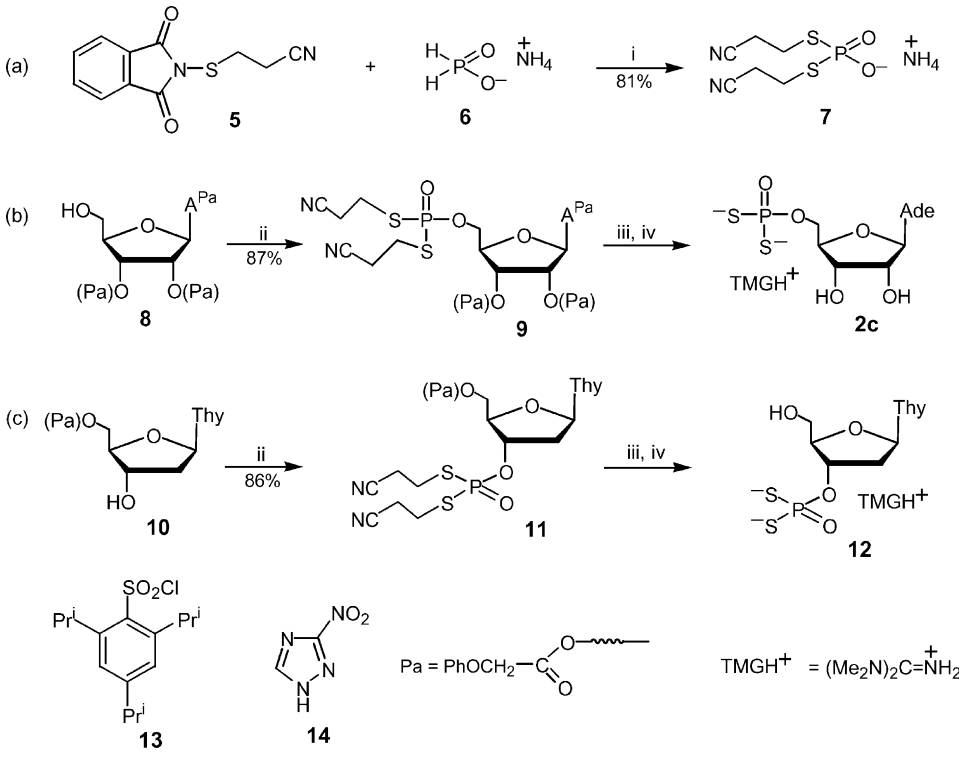


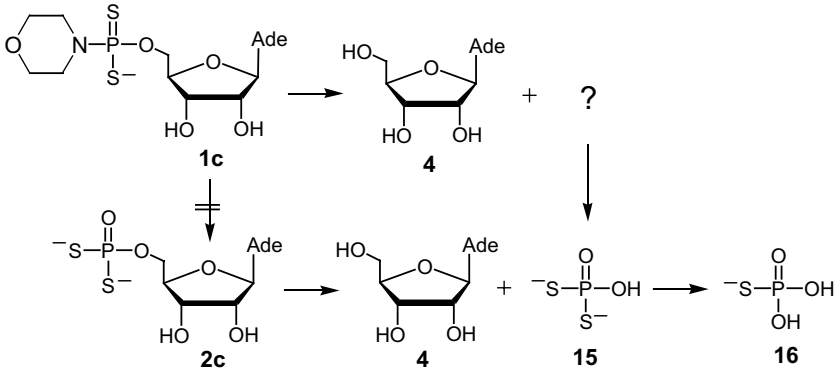
Ade = adenin-9-yl; Thy = thymin-1-yl; A^{Pa} = 6-*N*-(phenoxyacetyl)adenin-9-yl

Scheme 1. Reagents and conditions: (i) (a) $(Me_3Si)_2NH$, CH_2Cl_2, rt, 24 h, (b) H_2O, C_5H_5N, rt, 1 h, (c) $(NH_4)_2CO_3$, MeOH, rt, 10 min; (ii) **7**, **13**, **14**, C_5H_5N, rt, 1 h; (iii) Me_3SiCl, $(MeN)_2C=NH$ (TMG), MeCN, 60 °C, 6 h; (iv) NH_3, H_2O, MeCN, rt, 5 min.

to the synthesis of the dithio-diphosphate **3c** and related α,α-dithio condensed phosphates, this result led us to re-examine whether adenosine 5′-phosphorodithioate **2c** was indeed an intermediate in the acid-catalysed conversion of adenosine 5′-dithiophosphoromorpholidate **1c** into adenosine **4**. In order to further investigate this matter, we required a sample of adenosine 5′-phosphorodithioate **2c**. Although several procedures have been described in the literature for the preparation of nucleoside phosphorodithioates,[6,8–10] none of them appeared to us to be particularly convenient experimentally. For this reason, we set out to develop an alternative approach involving triester intermediates.

Ammonium bis-*S*-(2-cyanoethyl) phosphorodithioate **7** was obtained[11] (Scheme 1a) as a pure crystalline solid in 81% isolated yield by allowing ammonium phosphinate **6** to react with *N*-[(2-cyanoethyl)sulfanyl]phthalimide[12] **5** in the presence of hexamethyldisilazane. Treatment of 6-*N*,2′-*O*,3′-*O*-tri(phenoxyacetyl)adenosine[13] **8** with the phosphorodithioate salt **7**, 2,4,6-triisopropylbenzenesulfonyl chloride **13** and 3-nitro-1,2,4-1*H*-triazole **14** in pyridine solution[16] (Scheme 1b) gave the intermediate triester **9** in high yield.[17] When this product **9** was treated first with an excess each of chlorotrimethylsilane and 1,1,3,3-tetramethylguanidine (TMG)[18,19] in acetonitrile solution at 60 °C to remove both 2-cyanoethyl protecting groups and then with aqueous ammonia to remove the phenoxyacetyl protecting groups,[20] adenosine 5′-phosphorodithioate **2c** was obtained[21] as its 1,1,3,3-tetramethylguanidinium ($TMGH^+$) salt in virtually quantitative yield. In the same way, 5′-*O*-(phenoxyacetyl)thymidine[22] **10** was converted (Scheme 1c) into the intermediate triester **11** in almost quantitative yield. Following the same deblocking procedure, thymidine 3′-phosphorodithioate **12** was obtained[25] as its $TMGH^+$ salt, also in high yield.

With adenosine 5′-phosphorodithioate **2c** now in hand, a study of its decomposition both in acetic acid–water (95:5 v/v) (the medium in which the acid-catalysed hydrolysis of adenosine 5′-dithiophosphoromorpholidate **1c** was originally examined[4]) and acetic acid–water (5:95 v/v) (pH 2.3) was undertaken. The decomposition reactions were monitored first by HPLC at 25 °C, and adenosine **4** was found to be the sole nucleoside or nucleotide product. The half-times for the conversion of the phosphorodithioate **2c** into adenosine **4** were found to be ca. 24 and 40 min, respectively, under these conditions. The decomposition reactions of adenosine 5′-phosphorodithioate **2c** were then monitored by ^{31}P NMR spectroscopy both in CD_3CO_2D–D_2O (95:5 v/v) and CD_3CO_2D–D_2O (5:95 v/v) solution at 23 °C. In 95% CD_3CO_2D, the substrate **2c** (δ_P 86.6) was converted first into dithiophosphate **15** (δ_P 68.1),[26] which was itself then converted into thiophosphate **16** (δ_P 38.1).[26] Integration of the resonance signals suggested that $t_{1/2}$ of the substrate **2c** under these conditions was ca. 9 min. After 50 min, thiophosphate **16** was virtually the sole phosphorus-containing species present in the products. Decomposition in 5% CD_3CO_2D occurred more slowly: $t_{1/2}$ of the substrate **2c** (δ_P 102.4) appeared to be more than 30 min, and the ^{31}P chemical shifts of dithio- and thio-phosphate (**15** and **16**, respectively), were found to be 92.9 and 47.3 ppm.



Scheme 2. Decomposition of **1c** and **2c** in CD_3CO_2D–D_2O (95:5 v/v) at 23 °C.

The decomposition of adenosine 5′-dithiophosphoromorpholidate **1c** in 95% acetic acid was then re-examined. Our previous conclusion[4] that adenosine **4** was the sole nucleoside or nucleotide product and that adenosine 5′-phosphorodithioate **2c** was not an intermediate was confirmed first by reversed phase HPLC: $t_{1/2}$ of the substrate **1c** was found to be between 10 and 15 min at 25 °C. ^{31}P NMR spectroscopic studies in CD_3CO_2D–D_2O (95:5 v/v) at 23 °C were then repeated: $t_{1/2}$ of substrate (δ_P 119.9) appeared to be between 15 and 20 min and it was clear that adenosine 5′-phosphorodithioate **2c** (δ_P 86.6) was not an intermediate in its decomposition. What are believed to be dithio- and thio-phosphate (**15** and **16**, respectively; δ_P 68.1 and 38.1 ppm) appeared to be the main phosphorus-containing products[28] and, after 80 min, thiophosphate **16** was confirmed[4] to be virtually the sole phosphorus-containing product. Adenosine 5′-dithiophosphoromorpholidate **1c** was found to decompose very slowly indeed in CD_3CO_2D–D_2O (5:95 v/v) at 23 °C. The decomposition reactions of adenosine 5′-dithiophosphoromorpholidate **1c** and adenosine 5′-phosphorodithioate **2c** in 95% CD_3CO_2D are summarised in Scheme 2. The nature of the initial phosphorus-containing decomposition product (or products) of the dithiophosphoromorpholidate **1c** in 95% CD_3CO_2D has not yet been elucidated. The most surprising and as yet unexplained conclusion of this study is that although the substrate **1c** reacts with orthophosphate to give adenosine 5′-(α,α-dithio)diphosphate **3c**, albeit in modest yield, it does not undergo hydrolysis in 95% acetic acid to give adenosine 5′-phosphorodithioate **2c**.

Acknowledgements

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mp 133–134 °C; δ_C [$(CD_3)_2SO$] 18.97, 27.80, 120.21; δ_P [$(CD_3)_2SO$] 28.77.
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