Pergamon

Tetrahedron Letters, Vol. 35, No. 28, pp. 5085-5088, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $7.00+0.00

0040-4039(94)00976-7

Acid-catalysed Hydrolysis of Adenosine 5'-phosphorodithiomorpholidate

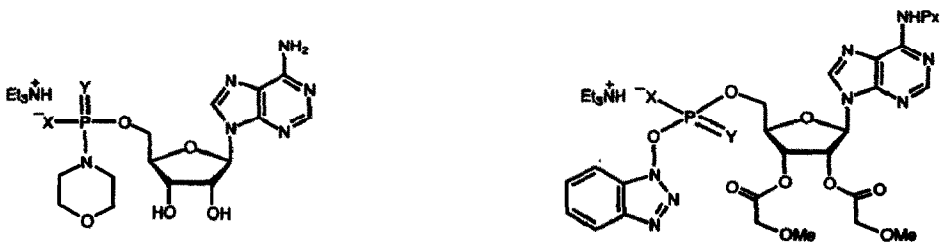
Colin B. Reese*, Louise H. K. Shek and Zhengyun Zhao

Department of Chemistry, King's College London, Strand, London WC2R 2LS, England

Abstract: Adenosine 5'-phosphorodithiomorpholidate **1c** is quantitatively converted into adenosine **5** within 1 hr in acetic acid - water (95:5 v/v) solution at room temperature.

Esters of phosphoromorpholidic acid, such as adenosine 5'-phosphoromorpholidate[1] **1a** have proved to be valuable phosphorylating agents in the synthesis[2] of biologically-important esters of di- and tri-phosphoric acids such as the nucleotide coenzymes and nucleoside 5'- di- and tri-phosphates. A characteristic reaction of nucleoside 5'-phosphoramidates (e.g. **1a**) is that they undergo acid-catalysed hydrolysis with resulting cleavage of the P-N bond to give the corresponding 5'-phosphates (e.g. **4a**). Thus the half-time of hydrolysis of compound **1a** in 0.05 mol dm^{-3} sulfuric acid was reported[1] to be *ca.* 10 min at room temperature and we later found[3] the half-time for this reaction to be *ca.* 20 min in 0.01 mol dm^{-3} hydrochloric acid (pH 2.0) at room temperature. Much interest has been shown in phosphorothioate analogues of nucleotides especially in studies directed towards the elucidation of the mechanisms of phosphoryl-transfer and phosphorylytic enzymes[4], and also in phosphorothioate analogues of oligonucleotides[5] as potential inhibitors of gene expression. More recently achiral phosphorodithioate analogues[6] of oligonucleotides have been synthesized and examined in the latter context. Our recent interest[7] in activated nucleoside phosphorothioates and phosphorodithioates has led us to investigate the chemistry of adenosine 5'-phosphorothio- and phosphorodithio-morpholidates (**1b** and **1c**, respectively).

6-*N*-(9-Phenylxanthen-9-yl)adenosine 5'-phosphorothiomorpholidate **3b** [δ_P[$(CD_3)_2SO$] 60.8, 61.0] was obtained in quantitative yield (HPLC) by allowing the corresponding 1-hydroxybenzotriazole derivative[7] **2b** to react overnight with a large (*ca.* 40-fold) excess of morpholine in dry tetrahydrofuran (THF); it was isolated as a colourless precipitated solid following work-up and short column chromatography of the products on silica gel. Treatment of the latter intermediate **3b** with acetic acid - water (95:5 v/v) at room temperature for 2 min gave adenosine 5'-phosphorothiomorpholidate **1b** [$\delta_P(D_2O)$ 60.8, 61.0] in high (>85% by HPLC) yield; when intermediate **3b** was allowed to stand first in acetic acid - water (95:5 v/v) solution at room temperature for 1 hr and then in acetic acid - water (20:80 v/v) solution for 4 days, adenosine 5'-phosphorothioate **4b** [δ_P (D_2O) 44.0] was obtained as expected[3] and in very high (> 93% by HPLC) yield; it was isolated as its pure triethylammonium salt following chromatography on DEAE Sephadex A25. The crude products were contaminated with a small quantity (< 2%) of a component with the same R_t (Jones APEX ODS 5μ column) as adenosine. In our earlier studies[3], we had found that the half-time of hydrolysis of adenosine 5'-phosphorothiomorpholidate **1b** in 0.01 mol dm^{-3} hydrochloric acid (pH 2.0) at room temperature was *ca.* 6 h, that is 17 times greater than that for adenosine 5'-phosphoromorpholidate **1a**.



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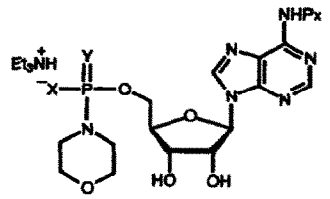

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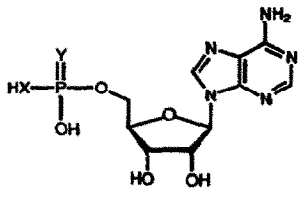
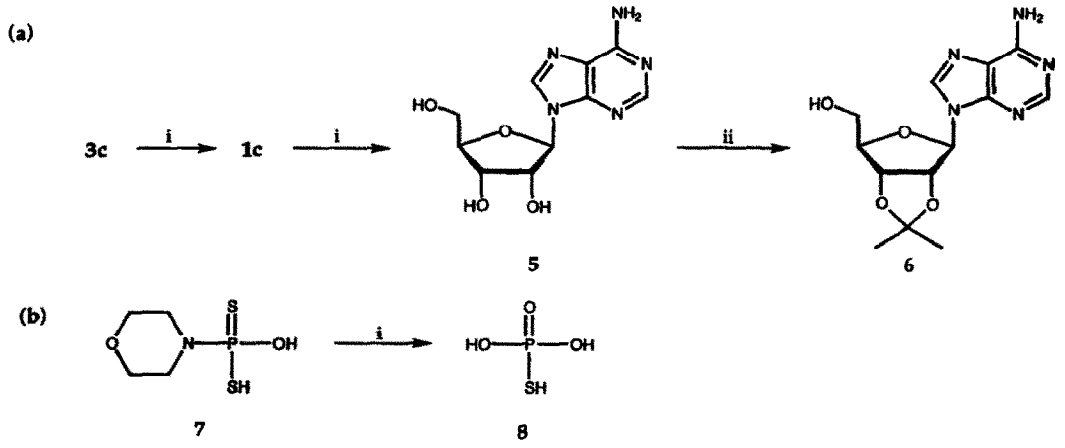


4

a; X = Y = O; b; X = S, Y = O; c; X = Y = S; Px = 9-phenylxanthen-9-yl

6-*N*-(9-Phenylxanthen-9-yl)adenosine 5'-phosphorodithiomorpholidate **3c** [$\delta_P[(CD_3)_2SO]$ 114.0] was similarly obtained in quantitative yield (HPLC) by allowing compound **2c**[7] to react overnight at room temperature with a large excess of morpholine in THF, and was also isolated as a colourless precipitated solid. Very surprisingly, when a *ca.* 0.025 mol dm^{-3} solution of compound **3c** in acetic acid - water (95:5 v/v) was allowed to stand at room temperature (Scheme 1), it was quantitatively converted within 1 h into adenosine **5** as the sole nucleoside or nucleotide product. After reaction times of 3 min and 30 min under the same conditions, the products contained (HPLC) adenosine 5'-phosphorodithiomorpholidate **1c** [$\delta_P(D_2O)$ 111.5] and adenosine **5** in the approximate molar proportions of 91 : 9 and 3 : 97, respectively. In another experiment



Scheme 1 *Reagents and conditions*: i, AcOH - H_2O (95 : 5 v/v), RT; ii, $Me_2C(OMe)_2$, TsOH, MeCN, RT

Table 1. Decomposition of **3c** in CD_3CO_2D - D_2O (95:5 v/v)[a] at 22°C

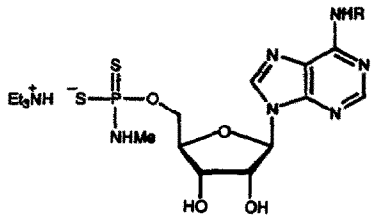
Time (min)	Chemical shifts of ^{31}P resonance signals of decomposition products[b]			
	δ 119.2	δ 46.1	δ 39.7[c]	δ 17.8
5	89	7	-	4
10	65	10	12	13
20	52	6	33	9
30	35	3	53	9
60	11	-	85	4
80	4	-	96	-[d]

[a]The initial concentration of substrate was *ca* 0.03 mol dm^{-3}. [b]NMR Spectra were measured at 145.8 MHz. The numbers represent the relative percentages indicated by signal integrals. [c]The chemical shift of triethylammonium phosphorothioate in CD_3CO_2D - D_2O (95:5 v/v) is 39.6 ppm. [d]After 80 min, the signal at δ 17.8 was too small to integrate.

(Scheme 1a), a *ca.* 0.025 mol dm^{-3} solution of compound **3c** in acetic acid - water (95:5 v/v) was allowed to stand at room temperature for 17.4 h and the products were then concentrated to dryness under reduced pressure. The residue was extracted with hot methanol and the evaporated extracts were allowed to react with 2,2-dimethoxypropane in acetonitrile solution to give 2',3'-*O*-isopropylideneadenosine **6**. The latter compound, which was identical to authentic material[8], was isolated as a crystalline solid in 88% overall yield based on compound **3c**.

The acid-catalysed decomposition [in CD_3CO_2D - D_2O (95:5 v/v) at 22°C] of compound **3c** was also monitored by ^{31}P NMR spectroscopy (Table 1). It is clear from HPLC studies (see above) that **3c** is very rapidly converted into adenosine 5'-phosphorodithiomorpholidate **1c** in acetic acid - water (95:5 v/v) at room temperature. It may therefore be assumed that the ^{31}P resonance signal at δ 119.2 (Table 1) relates to compound **1c**. It is also clear from HPLC studies that no nucleoside or nucleotide intermediate can be detected in the conversion of **1c** into adenosine **5**. It is therefore tempting to suggest that, in acetic acid - water (95:5 v/v) solution, **1c** undergoes P-O bond cleavage[9] to give adenosine **5** and what must be assumed to be phosphorodithiomorpholidic acid **7** directly. Furthermore, unless the phosphorus resonance signals of **1c** and **7** have identical chemical shifts, it would appear that the putative phosphorodithiomorpholidic acid **7** (Scheme 1b) is too unstable to be detected in CD_3CO_2D - D_2O (95:5 v/v) solution at 22°C. On the basis of the chemical shift (δ 39.7) of its resonance signal, it seems probable that the main phosphorus containing decomposition product (96% after 80 min; Table 1) of adenosine 5'-phosphorodithiomorpholidate **1c** in acetic acid - water (95:5 v/v) is thiophosphoric acid **8**. The minor intermediate products (revealed by the resonance signals at δ 46.1 and δ 17.8) have not been identified and the mechanisms of the reactions involved have not so far been elucidated.

The hydrolysis of adenosine 5'-phosphorodithiomorpholidate **1c** proceeded much more slowly in acetic acid - water (20:80 v/v) but again no adenosine 5'-phosphorodithioate was detected in the products. When compound **3c** was allowed to stand in acetic acid - water (20:80 v/v) at room temperature for 30 min, it was converted into **1c** and adenosine **5** in the approximate molar proportions of 89:10 (HPLC); after 15.6 h, the approximate molar ratio of **1c** to **5** was found to be 15:83. The acid-catalysed conversion of nucleoside



9 a; R = Px
b; R = H

phosphorodithioamidates into the corresponding nucleosides appears to be a general reaction[10]. Thus when 6-*N*-(9-phenylxanthen-9-yl)adenosine 5'-(*N*-methylphosphorodithioamidate)[7] **9a** was allowed to stand in acetic acid - water (95:5 v/v) at room temperature for 1 h, it was converted into **9b** [$\delta_P(D_2O)$ 107.1, 2.5%] and adenosine **5** (97.5%). This reaction was also monitored by ^{31}P NMR spectroscopy [CD_3CO_2D - D_2O (95:5 v/v)] and the principal phosphorus-containing decomposition product was again believed to be thiophosphoric acid **8**. When compound **9a** was allowed to stand in acetic acid - water (20:80 v/v) at room temperature for 30 min, it was quantitatively converted into **9b**. However, after 21 h, a *ca.* 3 : 2 mixture of **9b** and adenosine **5** was obtained.

It seems clear from the present studies that nucleoside phosphorodithioamidates (such as **1c**) are most unlikely to react in the same way as the corresponding phosphoramidates (such as **1a**) with ortho- and diphosphoric acids and their esters, and thereby prove to be valuable intermediates in the synthesis of dithio-analogues of condensed phosphates.

Acknowledgements: We thank the K. C. Wong Foundation and the University of London for the award of research scholarships (to Z.Z. and L.H.K.S., respectively).

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9. Cleavage of the P-N bond would lead to adenosine 5'-phosphorodithioate **4c** which was not detected either by HPLC or by ^{31}P NMR spectroscopy; cleavage of a P-S bond would lead to adenosine 5'-phosphorothiomorpholidate **1b** (see above) which was also not detected in the products.
10. Similarly, 5'-*O*-(9-phenylxanthen-9-yl)thymidine 3'-phosphorodithiomorpholidate was found[11] to decompose in aqueous acetic acid solution at room temperature to give thymidine as the sole nucleoside or nucleotide product.
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(*Received in UK* 6 *April* 1994; *accepted* 20 *May* 1994)