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β-Selective synthesis of 2′-deoxy-5,6-dihydro-4-thiouridine, a precursor of the unstable nucleoside product of ionising radiation damage 2′-deoxy-5,6-dihydrocytidine

Frédéric Peyrane and Pascale Clivio*

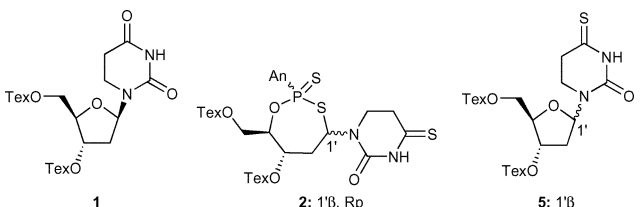
Institut de Chimie des Substances Naturelles, CNRS, 1 Avenue de la Terrasse, 91190, Gif-sur-Yvette, France

Received 15th February 2005, Accepted 10th March 2005
First published as an Advance Article on the web 24th March 2005

4-Thio oxathiaphosphepane nucleosides **2**–**4** undergo a rearrangement in pyridine that leads selectively to the β anomer of the 2′-deoxy-5,6-dihydro-4-thiouridine derivative **5**. This diastereoselective reaction proceeds through a multistep mechanism initiated by the addition of pyridine at the C1′ position of **2**–**4** and concomitant opening of the oxathiaphosphepane. This was confirmed by the trapping of the corresponding intermediate in the closely related DMAP series. In contrast, LR thiation of **1** in pyridine leads to a new class of modified nucleosides **12** containing an oxathiaphospholane moiety. The quantitative conversion of **5** into the corresponding 5,6-dihydrocytosine derivative with NH_3–MeOH is also reported.

Introduction

Exposure of DNA to ionising radiation induces the formation of 5,6-dihydrocytosine (DHC).[1] Under biological conditions, this primary base damage undergoes hydrolytic deamination to give rise to 5,6-dihydrouracil (DHU) which is currently considered to be the species responsible for the mutagenic properties of DHC.[2,3] However, the lifespan of DHC in DNA is unknown, nor are its intrinsic mutagenic properties and its repair. To address these points, DHC-containing oligodeoxynucleotides (ODNs) and, therefore, DHC derivatives are highly desirable. Synthesis of DHC by catalytic hydrogenation of cytosine derivatives is frequently accompanied by concomitant deamination and overhydrogenation.[4] Consequently, alternative strategies have been developed and 5,6-dihydro-4-thiouracil has been proved to be a useful synthetic intermediate in the nucleobase and ribose series.[5] The deoxyribose series is known to be more labile towards thiation reagents than the base or ribonucleoside series.[6] In this series, our first attempts at thiation of the disilyl derivative of 2′-deoxy-5,6-dihydrouridine (**1**) have revealed that the use of P_4S_{10} leads exclusively to degradative material whereas the use of one molar equivalent of Lawesson's reagent (LR) gives rise to the corresponding thiated compound **5**, though in poor yield.[7] However, the use of two molar equivalents of LR leads to oxathiaphosphepanes **2**–**4** (93% yield) whose subsequent thermal rearrangement (dioxane, 85 °C, 2 h) gives rise to **5** and its α anomer **6** (75 : 25 ratio, 58% yield).[7] We now report a modified procedure that allows the diastereoselective synthesis of the 2′-deoxy-5,6-dihydro-4-thiouridine derivative in the β anomeric form (**5**) from oxathiaphosphepanes **2**–**4**. The subsequent quantitative conversion of **5** into the 2′-deoxy-5,6-dihydrocytidine derivative **15** is also reported.



Results and discussion

1 β-Selective synthesis of 5 from oxathiaphosphepanes 2–4

Access to the mixture of oxathiaphosphepanes **2**–**4** in high yield and its possible thermal transformation into 2′-deoxy-5,6-dihydro-4-thiouridine compounds **5** and **6**[7] prompted us to examine this transformation more closely. Though no reaction occurred when the **2**–**4** mixture was allowed to stand at rt for 24 h in dioxane, to our delight we observed that stirring the **2**–**4** mixture in pyridine at rt for 24 h led exclusively to **5** in 57% yield after column chromatography.[8] Interestingly, when **2** (β anomer) was treated in pyridine at rt for 1 h, a mixture composed of **2**–**5** (50, 31, 7 and 12%, respectively) was obtained; after 3 h, the respective yields were 35, 34, 9 and 21%. This clearly indicated that during the process leading to the β-selective formation of **5**, oxathiaphosphepane **2** partially epimerized, leading to its phosphorus diastereomer **3**. Epimerization at the C1′ position led to α anomer **4**, elimination of $AnPS_2$/cyclization led to **5**.

2 Proposed mechanisms

Reactivity in dioxane.

*Formation of oxathiaphosphepanes **2**–**4** in the thiation reaction.* We have previously proposed that reaction between LR and **1** proceeds through a cyclic oxonium intermediate that opens to give Schiff base **7**[7] whose cyclization yields **2** and **3**, or **4** after rotation of the C1′-C2′ bond. However, so far, we have not considered the stereochemical parameters governing the observed yield of each oxathiaphosphepane during the thiation of **1** conducted with two eq. of LR. Since **2** and **3** (both β oxathiaphosphepane anomers) are the major compounds obtained, their favoured formation is likely to be induced by the neighbouring non-participating α-oriented silyl substituant at the C3′ position of **7** and/or consecutively to the stabilisation (by π-stacking and/or ionic bonds) of a conformation in which the base moiety is β-configurated. Additionally, that compound **2** (Rp) is the major isomer formed reflects the preferred *anti* orientation adopted by the An group, with respect to the dihydropyrimidine, in order to avoid steric constraints. Accordingly, in the minor α series, only **4** (dihydropyrimidine and An *anti*) is observed, diastereomer **8** being unfavoured.

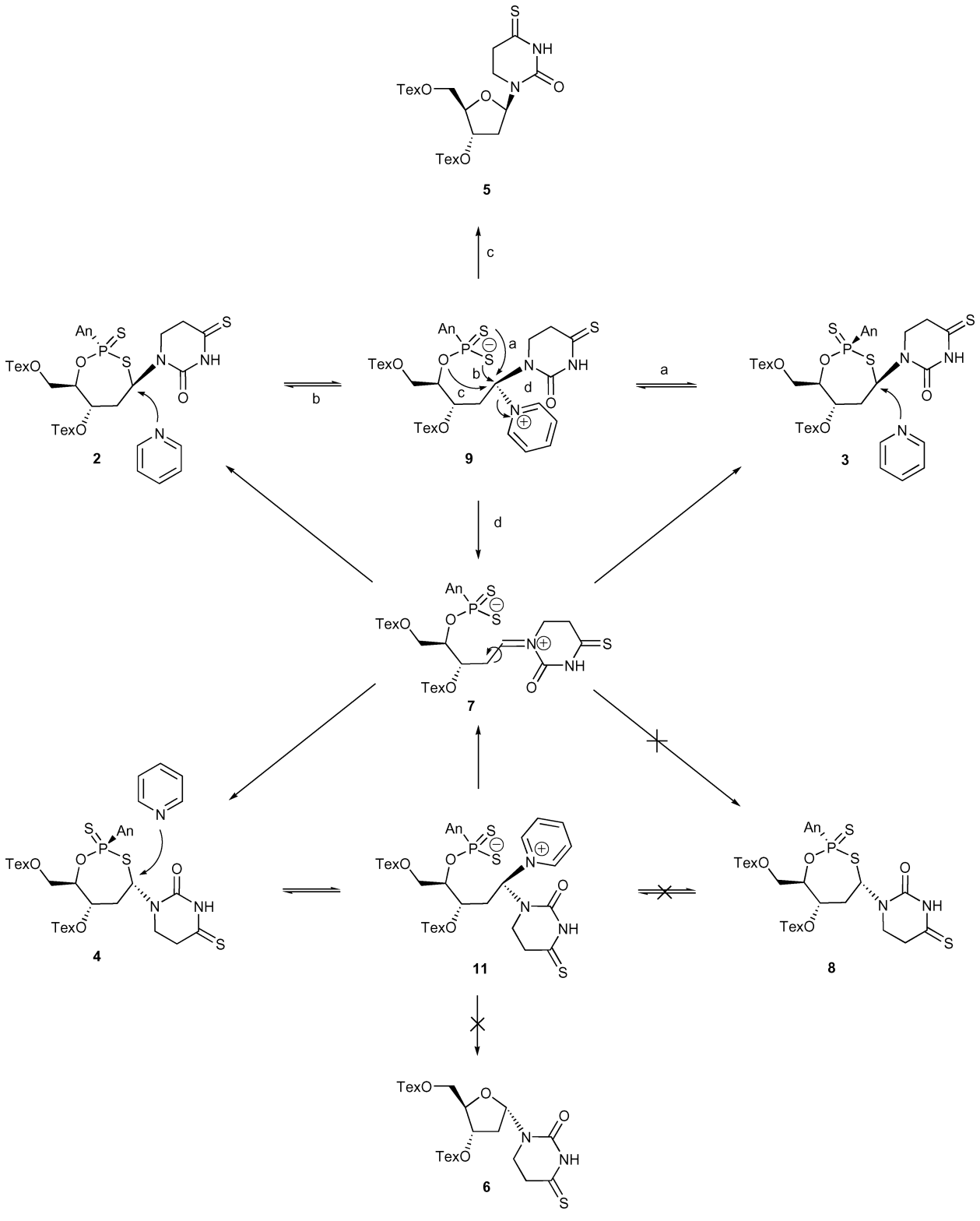
*Thermal transformation of oxathiaphosphepanes **2**–**4**.* The thermal conversion of the **2**–**4** mixture (50 : 35 : 15) into **5** and **6** (58%, 75 : 25, respectively) also involves the participation of the

DOI: 10.1039/b502302e

Schiff base intermediate **7**.[7] It is likely that thermal conditions increase the yield of the less favoured α anomer **6** by allowing or easing the C1′-C2′ bond rotation of **7**.

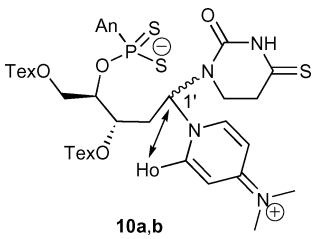
Reactivity in pyridine. Whereas heating (100 °C) of **2**–**4** in dioxane or pyridine led to **5** and **6**, treatment with pyridine for 24 h at rt led exclusively to **5**. It is likely that in this latter case, the precursor of **5** is **9** (Scheme 1) which results from the nucleophilic attack of pyridine on **2** or **3** C1′-position.[9] From **9**, oxathiaphosphepanes **2** and **3** can be obtained consecutively to a *S*-directed cyclization (path a or b, Scheme 1); *O*-directed cyclization and concomitant elimination of $AnPS_2$ leads to **5** (path c, Scheme 1), and pyridine elimination leads to **7** (path d, Scheme 1).

However, **9** could not be isolated. To ascertain the C1′ addition of the pyridine, we investigated the reactivity of the **2**–**4** mixture in the presence of DMAP. Treatment of a dichloromethane solution of the **2**–**4** mixture with DMAP (1 eq.) led to a mixture of two separable isomers (**10a** and **b**) in 6 and 19% yield, respectively, for which dimethylaminopyridinyl insertion was supported by HRMS (**10a**: calcd 877.3448, found 877.3423; **10b**: calcd 877.3448, found 877.3478). Addition of DMAP onto



Scheme 1

the oxathiaphosphepane skeleton C1′ position was evidenced by its downfield ^{13}C chemical shift (**10a** δ 75.5; **10b** δ 74.7 *vs.* δ 57.1–58.6 for **2**–**4**).[10] Observation of a correlation between C1′ and *o*-protons of DMAP on the ^{1}H-^{13}C HMBC spectrum further confirmed the DMAP substitution location. Therefore, compounds **10a** and **10b** are diastereomers at their C1′ position and their isolation fully supports the hypothesis of a C1′ addition of pyridine.

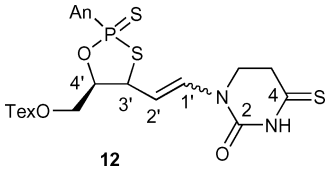


10a,b

Alternatively, if **9** undergoes pyridine elimination, Schiff base **7** is obtained (path d, Scheme 1). Following the mechanism depicted for the reactivity in dioxane, **7** can afford **2**, **3** or **4**. Addition of pyridine on the C1′ position of **4** leads to **11**. The exclusive isolation of **5** in pyridine, whereas **6** is also obtained in dioxane, suggests that the kinetics of the pyridinium elimination or of the *S*-oriented cyclisation are faster than the *O*-oriented cyclisation (the lack of formation of **8** has been previously explained; *vide supra*).

3 Thiation in pyridine

As the treatment of oxathiaphosphepane nucleosides **2**–**4** with pyridine at rt selectively afforded **5**, we investigated the thiation reaction of **1** in this solvent. When treated with 2 eq. of LR at 85 °C in pyridine, **1** remained unaltered. Only the use of 10 eq. of LR at 100 °C allowed the consumption of **1** in 30 min, but instead of **5**, we obtained, as major compounds, a mixture of four isomers **12a**–**d**. The main isomer was isolated in 20% yield. Its NMR data were in good agreement with the presence of a 5,6-dihydro-4-thiouracil moiety (^{13}C: δ 201.6 (C4), 40.2 (C6), 38.4 (C5); ^{1}H: δ 9.10 (NH), 3.18 (H5)), confirming thiation at the C4 position. The HRMS spectrum revealed the occurrence of only one thexyldimethylsilyl group (calcd 595.1320, found 595.1358, $C_{24}H_{37}N_2O_4PS_3Si$) and confirmed the presence of an $AnPS_2$ unit by the signals on the ^{1}H NMR spectrum (δ 7.98, 6.95, 3.88) along with the signal at δ 99.4 on the ^{31}P NMR spectrum. In addition, the presence of an ethylenic moiety in the vicinity of the dihydropyrimidine (^{1}H NMR: δ H1′ 7.36, H2′ 5.04; ^{13}C NMR: δ C1′ 128.3, C2′ 105.0)[10] suggested the oxathiaphospholane nature of **12**.

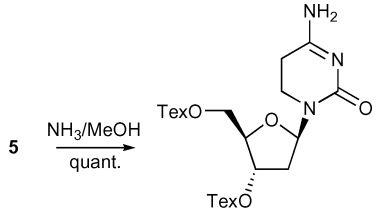


12

Thus, we believe that **12a**–**d** are isomers at their C3′ and P position. A formation mechanism involving an oxonium intermediate at the C3′ position to assist the elimination of the silyloxy group is proposed (Scheme 2). Pyridine-initiated abstraction of an acidic proton at the C2′ position of **13** could lead to olefinic compounds **13a** (path a) or **13b** (path b), both affording **14**. Subsequent cyclisation of **14** would then lead to **12**.

4 Synthesis of the 2′-deoxy-5,6-dihydrocytidine derivative 15 from 5

Displacement of the thiocarbonyl function by NH_3 is known to be very efficient in the 5,6-dihydropyrimidine series.[5] Indeed, when **5** was treated with NH_3–MeOH at rt, its conversion to the 5,6-dihydrocytosine derivative occurred smoothly and **15** [HRMS (calcd 514.3496, found 514.3489); ^{13}C NMR (δ 165.0 (C4), 28.6 (C5))] was obtained in quantitative yield (Scheme 3).



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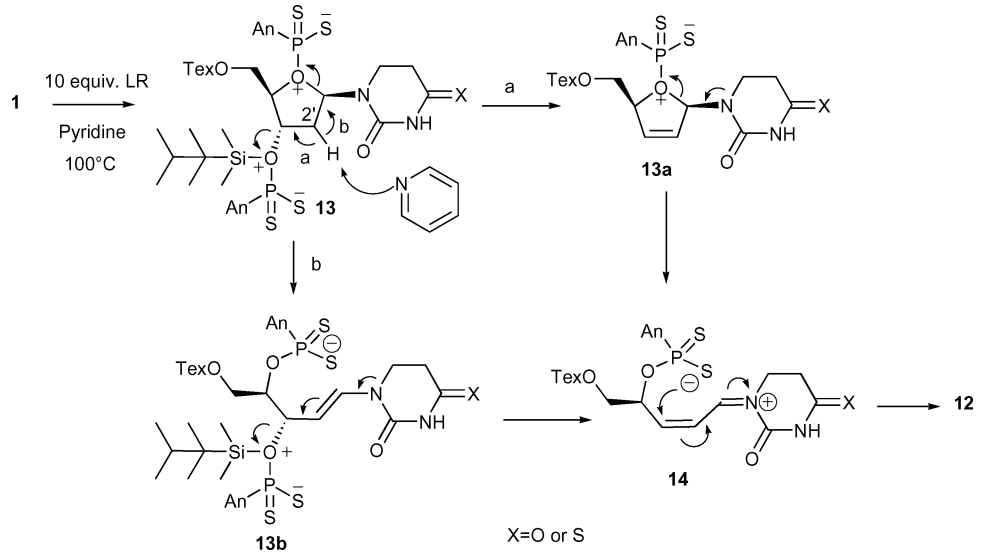
Scheme 3

Conclusion

The β-selective synthesis of the delicate 5,6-dihydrocytosine deoxynucleoside derivative using the thiation/amination pathway can be achieved in three steps and an overall yield of 53%. Further work to prepare ODNs containing site specifically DHC using a suitably protected DHC, or a 4-thio-DHU, derivative is currently in progress in our laboratory.

Experimental

LR was purchased from Acros (Noisy Le Grand, France). Dioxane was freshly distilled under argon over sodium–benzophenone. Pyridine and dichloromethane were dried over



Scheme 2

CaH_2, distilled and stored on 4 Å molecular sieves. Methanol was distilled over magnesium methoxide. Chromatography was performed on Chromagel 60 silica (35–70 μm) from SDS (Peypin, France) and preparative TLC were home-made using Kieselgel 60 PF_{254} silica from Merck. NMR experiments were performed on Bruker AC-250, AM-300, AMX-400 spectrometers. Chemical shifts (δ) are reported in ppm relative to residual solvent peak ($CHCl_3$ δ_H 7.26, δ_C 77.4; CH_3CN δ_H 1.96, δ_C 1.79.) ^{31}P Chemical shifts are reported relative to an external capillary standard of 85% phosphoric acid. High resolution mass spectra were recorded on Micromass LCT (ESI, CH_3OH) and PerSeptive Biosystems Voyager-DE STR (MALDI, 2,5-dihydroxybenzoic acid).

For convenience, the numbering system of the nucleoside series has been used for compounds **2**–**4**, **10**, and **12**. Ho, Hm and Ci, Co, Cm, Cp in **2**–**4**, **10**, and **12** refer to the An group signals, in ortho, meta and ipso, ortho, meta and para, respectively with respect to the phosphorus substitution. Prime index in **10** refers to the DMAP signals with respect to the C1′ substitution. Superscript (a) and (b) labels indicate interchangeable assignments within a compound.

2′-Deoxy-5,6-dihydro-3′,5′-di-*O*,*O*′-thexyldimethylsilyluridine (1)

A suspension of 2′-deoxyuridine (2.0 g, 8.77 mmol) in MeOH (100 mL) containing rhodium on alumina (5%, 500 mg) was hydrogenated at 35 psi for 4 h in a Parr apparatus. Then, the reaction mixture was filtered on celite. The filtrate was concentrated to give a residue, which was repeatedly co-evaporated with diethyl ether to afford 2′-deoxy-5,6-dihydrouridine as a white solid in quantitative yield. δ_H (250 MHz; CD_3OD) 6.26(1H, dd, $J_{1',2'}$ 7.9 Hz, $J_{1',2''}$ 6.4 Hz, H1′), 4.28 (1H, dt, $J_{3',2'}$ 6.5 Hz, $J_{3',2''} = J_{3',4'}$ 3.3 Hz, H3′), 3.76 (1H, m, H4′), 3.65 (2H, m, H5′H5″), 3.57 (1H, m, H6a), 3.42 (1H, m, H6b), 2.62 (2H, m, H5aH5b), 2.20 (1H, ddd, $J_{2',2''}$ 13.5 Hz, $J_{1',2'}$ 7.9 Hz, $J_{3',2'}$ 6.5 Hz, H2″$^{(a)}$), 1.98 (1H, ddd, $J_{1',2''}$ 6.4 Hz, $J_{3',2''}$ 3.3 Hz, $J_{2',2''}$ 13.5 Hz, H2″$^{(a)}$); δ_C (75 MHz; CD_3OD) 173.0 (C4), 154.9 (C2), 87.4 (C4′), 85.3 (C1′), 72.4 (C3′), 63.3 (C5′), 37.5 (C2′), 36.9 (C6), 31.9 (C5); HRMS (ESI) $(M + Na)^+$ Calcd for $C_9H_{14}N_2O_5Na$ 253.0800, found 253.0791.

Dry 2′-deoxy-5,6-dihydrouridine (1.0 g, 4.35 mmol) obtained by repeated coevaporation with anhydrous pyridine was dissolved in anhydrous pyridine (3 mL). To this solution imidazole (1.2 g, 17.4 mmol) and thexyldimethylsilyl chloride (2.52 mL, 13.0 mmol) were added. The mixture was stirred at rt for 16 h. The solvent was then evaporated and the crude material partitioned between dichloromethane and brine. The organic phase was dried with Na_2SO_4 and evaporated to give a residue purified by column chromatography on silica gel using a gradient of EtAc in heptane (10–30%) as the eluent to afford **1** (2.23 g, 99%) as a colourless oil, which solidified after several days at rt. δ_H (250 MHz; $CDCl_3$) 7.96 (1H, s, NH), 6.28 (1H, t, $J_{1',2'} = J_{1',2''}$ 7.0 Hz, H1′), 4.35 (1H, m, H3′), 3.78 (1H, m, H4′), 3.70 (2H, m, H5′H5″), 3.65 (1H, m, H6a), 3.29 (1H, ddd, $J_{6b,6a}$ 12.4 Hz, $J_{6b,5a}$ 9.1 Hz, $J_{6b,5b}$ 5.6 Hz, H6b), 2.59 (2H, m, H5aH5b), 1.97 (2H, m, H2′H2″), 1.62 (2H, m, H-Tex), 0.87 (4 × 3H, d, J = 7.0 Hz, CH_3-Tex), 0.84 (4 × 3H, s, CH_3-Tex); 0.10 (4 × 3H, m, CH_3–Si); δ_C (62.5 MHz; $CDCl_3$)·169.9 (C4), 152.2 (C2), 86.3 (C4′), 83.9 (C1′), 71.8 (C3′), 62.8 (C5′), 37.6 (C2′), 35.4 (C6), 34.2 (CH-Tex), 31.2 (C5), 25.4 and 24.9 (C-Tex), 20.4 and 18.6 (CH_3-Tex), −2.5 and −2.7 (CH_3–Si), −3.4 and −3.5 (CH_3–Si); HRMS (ESI) $(M + Na)^+$ Calcd for $C_{25}H_{50}N_2O_5Na$ 537.3156, found 537.3177.

Preparation of oxathiaphosphepanes 2–4

To a solution of **1** (100 mg, 0.19 mmol) in dioxane (1 mL) was added LR (157 mg, 0.39 mmol). The reaction was stirred at 85 °C for 20 min. After filtration on cotton, the filtrate was concentrated to give a residue, which was briefly chromatographed on silica gel, using EtAc–heptane 10% as the eluent, affording **2**–**4** (133 mg, 93%, 60 : 31 : 9 estimated by ^1H NMR) as a yellow foam. Analytical samples were obtained by HPLC using an Hypersil HS (250 × 10 mm) column and an isocratic eluent consisting of isopropanol–heptane 0.3% at a flow rate of 5 mL min^{-1}. 230 nm detection: **2** rt, 13 min; **3** rt, 29 min; **4** rt, 25 min

Oxathiaphosphepane 2. δ_H (250 MHz; $CDCl_3$) 8.85 (1H, s, NH), 7.93 (2H, dd, $J_{o,m}$ 8.8 Hz, $J_{P,o}$ 14.3 Hz, Ho), 6.93 (2H, dd, $J_{o,m}$ 8.8 Hz, $J_{P,m}$ 3.4 Hz, Hm), 6.43 (1H, m, H1′), 4.90 (1H, m, H4′), 4.18 (1H, m, H6a), 4.13 (1H, m, H3′), 3.95 (2H, m, H5′H5″), 3.85 (3H, s, OCH_3), 3.74 (1H, m, H2′$^{(a)}$), 3.70 (1H, m, H6b), 3.14 (2H, m, H5aH5b), 1.96 (1H, m, H2″$^{(a)}$), 1.65 (2H, m, H-Tex), 0.93–0.83 (8 × 3H, m, CH_3-Tex), 0.14–0.08 (4 × 3H, m, CH_3–Si); δ_C (75 MHz; $CDCl_3$)203.2 (C4), 163.4 ($J_{P,p}$ 3.0 Hz, Cp), 148.5 (C2), 133.3 ($J_{P,o}$ 13.9 Hz, Co), 126.9 ($J_{P,i}$ 121.4 Hz, Ci), 114.0 ($J_{P,m}$ 16.2 Hz, Cm), 85.4 ($J_{P,4'}$ 12.0 Hz, C4′), 67.9 (C3′), 63.7 ($J_{P,5'}$ 10.4 Hz, C5′), 57.1 ($J_{P,1'}$ 3,5 Hz, C1′), 55.6 (OCH_3), 39.7 (C6), 39.5 (C5), 37.2 ($J_{P,2'}$ 5.3 Hz, C2′), 34.1 (CH-Tex), 25.4/24.9 (C-Tex), 20.4/18.6 (CH_3-Tex), −3.0/−3.2 (CH_3–Si); δ_P (121.5 MHz; $CDCl_3$) 89.9; HRMS (MALDI) $(M + Na)^+$ Calcd for $C_{32}H_{57}N_2O_5PS_3Si_2Na$ 755.26035, found 755.26206.

Oxathiaphosphepane 3. δ_H (250 MHz; $CDCl_3$)8.80 (1H, s, NH), 7.91 (2H, dd, $J_{o,m}$ 8.8 Hz, $J_{P,o}$ 13.8 Hz, Ho), 7.00 (2H, dd, $J_{o,m}$ 8.8 Hz, $J_{P,m}$ 3.6 Hz, Hm), 6.16 (1H, td, $J_{4',5'} = J_{P,4'}$ 12.1 Hz, $J_{4',5''}$ 3.0 Hz, H1′), 4.92 (1H, m, H4′), 4.56 (1H, m, H3′), 3.98 (2H, m, H5′H5″), 3.89 (3H, s, OCH_3), 2.80–2.64 (4H, m, H5aH5b, H6aH6b), 2.56 (1H, m, H2′$^{(a)}$), 1.89 (1H, m, H2″$^{(a)}$), 1.67 (2H, m, H-Tex), 0.94–0.89 (24H, m, CH_3-Tex), 0.16–0.10 (12H, m, CH_3–Si); δ_C (75 MHz; $CDCl_3$)202.4 (C4), 162.9 ($J_{P,p}$ 3.3 Hz, Cp), 148.0 (C2), 132.5 ($J_{P,o}$ 13.2 Hz, Co), 128.9 ($J_{P,i}$ 129.5 Hz, Ci), 114.4 ($J_{P,m}$ 16.5 Hz, Cm), 78.5 ($J_{P,4'}$ 7.1 Hz, C4′), 66.3 (C3′), 62.3 ($J_{P,5'}$ 7.7 Hz, C5′), 58.6 ($J_{P,1'}$ 6.6 Hz, C1′), 55.7 (OCH_3), 39.3/38.1 (C6,C5), 37.2 ($J_{P,2'}$ 2.2 Hz, C2′), 34.4/34.2 (CH-Tex), 25.3/25.0 (C-Tex), 20.6/20.4 (CH_3-Tex), 18.8/18.6 (CH_3-Tex), −2.2/−3.3 (CH_3–Si); δ_P (121.5 MHz; $CDCl_3$)89.6; HRMS (MALDI) $(M + Na)^+$ Calcd for $C_{32}H_{57}N_2O_5PS_3Si_2Na$ 755.26035, found 755.26576.

Oxathiaphosphepane 4. δ_H (300 MHz; $CDCl_3$) 8.75 (1H, s, NH), 8.05 (2H, dd, $J_{o,m}$ 8.9 Hz, $J_{P,o}$ 14.5 Hz, Ho), 7.00 (2H, dd, $J_{o,m}$ 8.9 Hz, $J_{P,m}$ 3.7 Hz, Hm), 5.88 (1H, dd, $J_{4',5'}$ 10.3 Hz, $J_{P,4'}$ 7.4 Hz, H4′), 4.77 (1H, m, H1′), 4.19 (1H, m, H4′), 4.04 (1H, m, H5′$^{(a)}$), 3.87 (3H, s, OCH_3), 3.86 (1H, m, H5″$^{(a)}$), 3.46 (2H, m, H6aH6b), 3.06 (2H, m, H5aH5b), 2.69 (1H, m, H2″$^{(b)}$), 2.35 (1H, m, H2′$^{(b)}$), 1.61 (2H, m, H-Tex), 0.90–0.83 (24H, m, CH_3-Tex), 0.18–0.10 (12H, m, CH_3–Si); δ_C (75 MHz; $CDCl_3$)202.1 (C4), 163.3 ($J_{P,p}$ 3.0 Hz, Cp), 148.0 (C2), 133.0 ($J_{P,o}$ 14.2 Hz, Co), 127.0 ($J_{P,i}$ 133.2 Hz, Ci), 114.0 ($J_{P,m}$ 16.9 Hz, Cm), 79.1 ($J_{P,4'}$ 6.8 Hz, C4′), 67.3 ($J_{P,3'}$ 2.2 Hz, C3′), 62.7 (C5′), 57.3 ($J_{P,1'}$ 5.2 Hz, C1′), 55.5 (OCH_3), 46.6 (C2′), 39.3 (C5), 38.8 (C6), 34.1 (CH-Tex), 25.4/25.0 (C-Tex), 20.5/20.3 (CH_3-Tex), 18.7/18.6 (CH_3-Tex), −2.16-(-3.1) (CH_3–Si); δ_P (121.5 MHz; $CDCl_3$) 88.3; HRMS (MALDI) $(M + Na)^+$ Calcd for $C_{32}H_{57}N_2O_5PS_3Si_2Na$ 755.26035, found 755.26357.

Reaction of 2–4 in pyridine: 2′-deoxy-5,6-dihydro-3′,5′-di-*O*,*O*′-thexyldimethylsilyl-4-thiouridine (5)

Compounds **2**–**4** (133 mg, 0.18 mmol) were dissolved in pyridine (10 mL), and the solution was stirred at rt for 24 h. The solvent was evaporated and the residue was purified by column chromatography using a gradient of EtAc in heptane (10–20%) as the eluent yielding **5** (55 mg, 57%) as a yellowish glassy film. δ_H (250 MHz; $CDCl_3$) 8.99 (1H, s, NH), 6.25 (1H, t, $J_{1',2'} = J_{1',2''}$ 7.1 Hz, H1′), 4.34 (1H, m, H3′), 3.79 (1H, m, H4′), 3.70 (2H, m, H5′H5″), 3.62 (1H, m, H6a), 3.29 (1H, ddd, $J_{6b,6a}$ 12.4 Hz, $J_{6b,5a}$ 9.0 Hz, $J_{6b,5b}$ 5.1 Hz, H6b), 3.02 (2H, m, H5aH5b), 1.97 (2H, m, H2′H2″), 1.62 (2H, m, H-Tex), 0.89 and 0.86 (4 × 3H, 2s, CH_3-Tex), 0.83 (4 × 3H, d, J = 7 Hz, CH_3-Tex), 0.11, 0.10, 0.09, 0.08 (4 × 3H, 4s, CH_3–Si); δ_C (75 MHz; $CDCl_3$) 203.6 (C4), 148.8 (C2), 86.6 (C4′), 84.1 (C1′), 71.9 (C3′), 62.8 (C5′), 39.6 (C5), 37.7 (C2′), 36.1 (C6), 34.2/34.1 (CH-Tex), 25.4/24.9 (C-Tex), 20.4

and 20.3 (CH_3-Tex), 18.6 and 18.5 (CH_3-Tex), −2.5 and −2.7 (CH_3–Si), −3.4 and −3.5 (CH_3–Si); HRMS (MALDI) (M + Na)$^+$ Calcd for $C_{25}H_{50}N_2O_4SSi_2Na$ 553.29276, found 553.29278.

Reaction of **2**–**4** with DMAP (**10a** and **b**): To a solution of compounds **2**–**4** (133 mg, 0.19 mmol) in CH_2Cl_2 (3 mL) was added DMAP (23 mg, 0.18 mmol). The mixture was stirred at rt for 40 h, the solvent was evaporated and the crude material was purified on silica gel column chromatography using a gradient of MeOH in CH_2Cl_2 (3–5%) as the eluent. Compounds **10a** and **10b** were separated by preparative TLC (MeOH–CH_2Cl_2 3%, 4 times elution) yielding **10a** (10 mg, 6%) and **10b** (29 mg, 19%) as pale yellow solids.

Compound 10a. δ_H (400 MHz; CD_3CN) 9.81 (1H, s, NH), 8.34 (2H, d, $J_{o',m'}$ 7.9 Hz, Ho′), 8.07 (2H, dd, $J_{P,o}$ 13.2 Hz, $J_{o,m}$ 8.8 Hz, Ho), 6.86 (2H, dd, $J_{o,m}$ 8.8 Hz, $J_{P,m}$ 2.5 Hz, Hm), 6.77 (2H, d, $J_{o',m'}$ 7.9 Hz, Hm′), 6.58 (1H, dd, $J_{1',2''}$ 9.6 Hz, $J_{1',2'}$ 6.2 Hz, H1′), 4.49 (1H, m, H4′), 3.99 (1H, m, H3′), 3.79 (3H, s, OCH_3), 3.75–3.48 (4H, m, H5′H5″, H6aH6b), 3.25–3.17 (7H, m, H2′$^{(a)}$, $N(CH_3)_2$), 3.02 (2H, t, $J_{5,6}$ 6.5 Hz, H5aH5b), 2.53 (1H, m, H2″$^{(a)}$), 1.68–1.49 (2H, m, H-Tex), 0.91–0.75 (24H, m, CH_3-Tex), 0.08-(-0.03) (12H, m, CH_3–Si); δ_C (75 MHz; CD_3CN) 206.1 (C4), 161.6 (Cp), 157.9 (Cp′), 150.9 (C2), 141.9 (Co′), 132.9 ($J_{P,o}$ 13.1 Hz, Co), 113.2 ($J_{P,m}$ 14.2 Hz, Cm), 108.5 (Cm′), 77.6 ($J_{P,4'}$ 7.6 Hz, C4′), 75.5 (C1′), 69.6 ($J_{P,3'}$ 4.3 Hz, C3′), 62.7 (C5′), 56.0 (CH_3O), 41.6 (C6), 40.8 (CH_3N), 40.7 (C5), 37.1 (C2′), 34.9 (CH-Tex), 25.9 (C-Tex), 20.9/20.7 (CH_3-Tex), 18.9/18.8 (CH_3-Tex), −2.9/−3.0 (CH_3–Si); δ_P (121.5 MHz; CD_3CN) 109.5; HRMS (ESI) (M + Na)$^+$ Calcd for $C_{39}H_{67}N_4O_5PS_3Si_2Na$ 877.3448, found 877.3423.

Compound 10b. δ_H (300 MHz; CD_3CN) 9.86 (1H, s, NH), 8.20 (2H, d, $J_{o',m'}$ 7.9 Hz, Ho′), 8.07 (2H, dd, $J_{P,o}$ 13.4 Hz, $J_{o,m}$ 8.8 Hz, Ho), 6.87–6.83 (4H, m, Hm′, Hm), 6.43 (1H, dd, $J_{1',2'}$ 5.3 Hz, $J_{1',2''}$ 10.2 Hz, H1′), 4.58 (1H, ddt, $J_{P,4'}$ 17.3 Hz, $J_{4',3'}$ 7.2 Hz, $J_{4',5'} = J_{4',5''}$ 3.6 Hz, H4′), 4.16 (1H, m, H3′), 3.94–3.79 (5H, m, H6a, H5′$^{(a)}$, OCH_3), 3.66 (1H, dd, $J_{5',4'}$ 3.6 Hz, $J_{5',5''}$ 10.0 Hz, H5″$^{(a)}$), 3.47 (1H, m, H6b), 3.24–3.13 (8H, m, H5a, $N(CH_3)_2$, H2′$^{(b)}$), 3.00 (1H, m, H5b), 2.36 (1H, ddd, $J_{2',2''}$ 14.0 Hz, $J_{1',2''}$ 10.2 Hz, $J_{3',2''}$ 7.8 Hz, H2″$^{(b)}$), 1.68–1.48 (2H, m, H-Tex); 0.91–0.75 (8 × 3H, m, CH_3-Tex); 0.08-(−0.03) (4 × 3H, m, CH_3–Si); δ_C (100 MHz; CD_3CN) 206.2 (C4), 161.5 ($J_{P,p}$ 3.5 Hz, Cp), 157.9 (Cp′), 151.5 (C2), 141.5 (Co′), 138.1 ($J_{P,i}$ 115.0 Hz, Ci), 132.4 ($J_{P,o}$ 13.2 Hz, Co), 113.1 ($J_{P,m}$ 14.6 Hz, Cm), 108.5 (Cm′), 77.0 (C4′), 74.7 (C1′), 70.6 (C3′), 62,6 ($J_{P,5'}$ 3.5 Hz, C5′), 55.9 (OCH_3), 41.1 (C5), 40.8 (CH_3N), 39.8 (C6), 37.2 (C2′), 34.9/34.8 (CH-Tex), 25.9/25.7 (C-Tex), 20.7/20.6 (CH_3-Tex), 18.9/18.7 (CH_3-Tex), −3.0/−3.1 (CH_3–Si); δ_P (121.5 MHz; CD_3CN) 110.8; HRMS (ESI) (M + Na)$^+$ Calcd for $C_{39}H_{67}N_4O_5PS_3Si_2Na$ 877.3448, found 877.3478.

Compound 12. To a solution of **1** (50 mg, 0.01 mmol) in pyridine (0.8 mL) was added LR (393 mg, 0.97 mmol). The mixture was stirred at 100 °C for 30 min. Chromatography on silica gel using CH_2Cl_2 provided a mixture of isomers from which the major one was isolated by preparative TLC (CH_2Cl_2, 3 times elution) yielding **12** (11.5 mg, 20%) as a pale yellow film. δ_H (250 MHz; $CDCl_3$) 9.10 (1H, s, NH), 7.98 (dd, 2H, $J_{P,o}$ 15.0 Hz, $J_{o,m}$ 8.7 Hz, Ho), 7.36 (1H, d, $J_{1',2'}$ 13.8 Hz, H1′), 6.95 (2H, dd, $J_{o,m}$ 8.7 Hz, $J_{P,m}$ 3.4 Hz, Hm), 5.04 (2H, m, H2′,H3′), 4.54 (1H, m, H4′), 4.08 (dd, 1H, $J_{5'',5'}$ 11.7 Hz, $J_{5',4'}$ 2.3 Hz, H5′$^{(a)}$), 3.88 (3H, s, OCH_3), 3.83 (1H, m, H5″$^{(a)}$), 3.62 (2H, t, $J_{6,5}$ 6.5 Hz, H6aH6b), 3.18 (2H, t, $J_{6,5}$ 6.5 Hz, H5aH5b), 1.63 (1H, m, H-Tex), 0.90 (2 × 3H, d, J 7 Hz, CH_3-Tex), 0.89 (2 × 3H, s, CH_3-Tex), 0.11 and 0.09 (2 × 3H, 2s, CH_3–Si); δ_C (75 MHz; $CDCl_3$) 201.6 (C4), 163.3 ($J_{P,p}$ 3.3 Hz, Cp), 146.8 (C2), 133.7 ($J_{P,o}$ 14.8 Hz, Co), 128.3 (C1′), 127.5 ($J_{P,i}$ 122.4 Hz, Ci), 113.9 ($J_{P,m}$ 16.5 Hz, Cm), 105.0 ($J_{P,2'}$ 7.7 Hz, C2′), 85.5 ($J_{P,4'}$ 4.9 Hz, C4′), 60.5 ($J_{P,5'}$ 13.2 Hz, C5′), 56.3 (C3′), 55.6 (OCH_3), 40.2 (C6), 38.4 (C5), 34.2 (CH-Tex), 25.4 (C-Tex), 20.4 and 20.3 (CH_3-Tex), 18.7 and 18.6 (CH_3-Tex), −3.3 and −3.5 (CH_3–Si), δ_P (121.5 MHz; $CDCl_3$) 99.4; HRMS (ESI) (M + Na)$^+$ Calcd for $C_{24}H_{37}N_2O_4PS_3SiNa$ 595.1320, found 595.1358.

2′-Deoxy-5,6-dihydro-3′,5′-di-*O*,*O*′-thexyldimethylsilylcytidine (15)

Compound **5** (35 mg, 0.06 mmol) was treated with a saturated solution of methanolic ammonia (3 mL) for 20 min at rt. The volatile materials were then evaporated to yield **15** (34 mg, quantitative) as a colourless film. δ_H (300 MHz; $CDCl_3$) 6.31 (1H, t, $J_{1',2'} = J_{1',2''}$ 7.1 Hz, H1′), 4.30 (1H, m, H3′), 3.74 (1H, m, H4′), 3.66 (2H, m, H5′H5″), 3.54 (1H, m, H6a), 3.18 (1H, m, H6b), 2.60 (2H, m, H5aH5b), 1.93 (2H, m, H2′H2″), 1.60 (2H, m, H-Tex), 0.86 (4 × 3H, d, J 7 Hz, CH_3-Tex), 0.83 and 0.82 (2 × (2 × 3H), 2s, CH_3-Tex), 0.08 (12H, m, CH_3–Si); δ_C (75 MHz; $CDCl_3$) 165.0 (C4), 156.3 (C2), 86.0 (C4′), 84.0 (C1′), 71.9 (C3′), 62.8 (C5′), 37.3 (C2′), 35.7 (C6), 34.2 (CH-Tex), 28.6 (C5), 25.4/24.9 (C-Tex), 20.3/18.6 (CH_3-Tex), −2.5/−2.7 (CH_3–Si), −3.4/−3.5 (CH_3–Si); HRMS (ESI) (M + H)$^+$ Calcd for $C_{25}H_{52}N_3O_4Si_2$ 514.3496, found 514.3489.

Acknowledgements

We thank the Ligue Nationale Contre le Cancer for a doctoral fellowship to F.P.

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8 The β-configuration of the anomeric center of **5** was deduced from a NOE between H1′ and H4′.
9 Since we did not observed any epimerisation in dioxane at rt, it is unlikely that **9** results from the addition of pyridine to **7**. However, this possibility cannot be totally discarded.
10 Assignments were performed by 2D ^{1}H-^{1}H and ^{1}H-^{13}C NMR experiments.