COMMUNICATION

ChemComm
www.rsc.org/chemcomm

Thiation of 2′-deoxy-5,6-dihydropyrimidine nucleosides with Lawesson's reagent: Characterisation of oxathiaphosphepane intermediates†

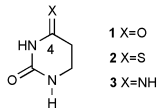
Frédéric Peyrane, Jean-Louis Fourrey and Pascale Clivio*

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

Received (in Cambridge, UK) 19th November 2002, Accepted 4th February 2003
First published as an Advance Article on the web 21st February 2003

Treatment of 2′-deoxy-3′,5′-dithexyldimethylsilyl-5,6-dihydrouridine with Lawesson's reagent led to the expected C4-thiolated derivative together with a number of oxathiaphosphepane isomers which resulted from the heat reversible incorporation of an $AnPS_2$ unit within the 2′-deoxyribose moiety explaining the subsequent anomerisation of the 5,6-dihydropyrimidine nucleosides.

5,6-Dihydrouracil (DHU, **1**) is a modified nucleobase involved in several important biological processes. Whereas in RNA, DHU is believed to play a structural role,[1] it represents in DNA a major and highly mutagenic cytosine-derived oxidative lesion.[2,3] Moreover, as a free base, **1** it is an intermediate of the pyrimidine metabolism.[4] Thus, structural analogues of this



1 X=O
2 X=S
3 X=NH

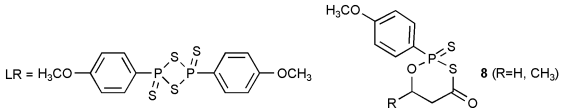
important modified nucleobase are highly desirable molecules for structure/biological function relationship studies. Furthermore, 5,6-dihydro-4-thiouracil (**2**) is a useful synthetic intermediate for the preparation of 5,6-dihydrocytosine products (**3**)[5,6] which are representative of the primary ionising radiation base damage of cytosine.[2] For these reasons, great attention has been paid to the syntheses of 5,6-dihydro-4-thiouracil which so far have been described in the nucleobase[5,7] and ribonucleoside[6,8] series only. Indeed, to the best of our knowledge and despite the potential utility of this class of compounds in the field of DNA damage, thiation of 5,6-dihydrouracil nucleosides has not yet been achieved in the labile deoxyribonucleoside series.

We herein report our results on the synthesis of the 3′,5′-disilyl derivative of 2′-deoxy-5,6-dihydro-4-thiouridine (**4**) together with the formation of products **5** and **6** which highlights the particular reactivity of the 2′-deoxyribose moiety of a 5,6-dihydrouracil nucleoside towards Lawesson's reagent.

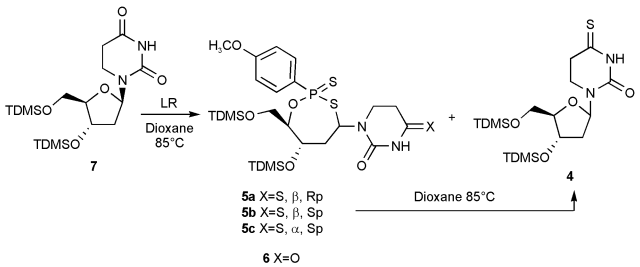
If thiation of the C4 amide function of DHU, in both the base or the ribose series, has been successfully achieved using phosphorus pentasulfide,[5–8] we noticed that complete decomposition of **7**[9] occurred in the presence of this reagent upon heating. This instability towards P_2S_5 is reminiscent of the observations made with the purine nucleosides which, compared to their ribo congeners, showed the corresponding 2′-deoxyribose derivatives to exhibit an increased lability of the glycosidic linkage.[10]

Consequently, we turned our attention to Lawesson's reagent (LR) known to be a smoother thiating agent.[11] It has already been used for thiation of 5,6-dihydrouracils and suggested as a reagent of choice for compounds with labile glycosidic bonds such as 5,6-dihydropyrimidine nucleosides.[12]

When compound **7** was treated with one molar equivalent of LR[13] in dioxane at 85 °C, several compounds were formed after 30 min and found extremely difficult to purify. However, two groups of compounds could be separated. The first group (**4** and **5**), isolated in 40% yield, contained four products thiated at C4 among which three of them (**5a–c**; 36% as estimated by ^{1}H NMR† in 53/42/5 ratio respectively) were isomers. The key structural arguments in favour of structure **5** are the following : a $M+Na^+$ ion at *m*/*z* 755, the presence of a ^{31}P NMR signal around 90 ppm, the observation of the characteristic signals of the LR anisole part on the ^{1}H and ^{13}C NMR spectra, the splitting of the C1′ and C4′ signals attributed to J_{CP} coupling, the shielding of the C1′ signal (*ca*. 26 ppm) compared to that of **7** which we consider to be diagnostic of the oxathiaphosphepane structure and, finally, the presence of both a carbon signal near 202 ppm and of a deshielded singlet proton near 9 ppm which attested to the C4 thioamide structure. Consequently, these isomers were C1′–O4′ thiophosphine ylide insertion products (Scheme 1) and we found that they could be prepared in 93% yield using two molar equiv. of LR (in 60/31/9 ratio respectively).[14] The fourth compound of this group was the target compound **4** (4% as estimated by ^{1}H NMR†).[15] The second group of compounds (**6** and **7**), isolated in 42% yield, consisted of a mixture of three inseparable insertion products which had not undergone thiation at C4 (**6**) (45/45/10 ratio) ($M+Na^+$ at *m*/*z* 739, ^{1}H NMR spectra similar to those of **5** except for the signals due to the H5 and N3H protons) and which was contaminated by the starting material **7** (10% yield as estimated by ^{1}H NMR†). Formation of compounds **4** and **6** clearly indicated a competition between thiation at C4 and insertion of $AnPS_2$ into the sugar ring.

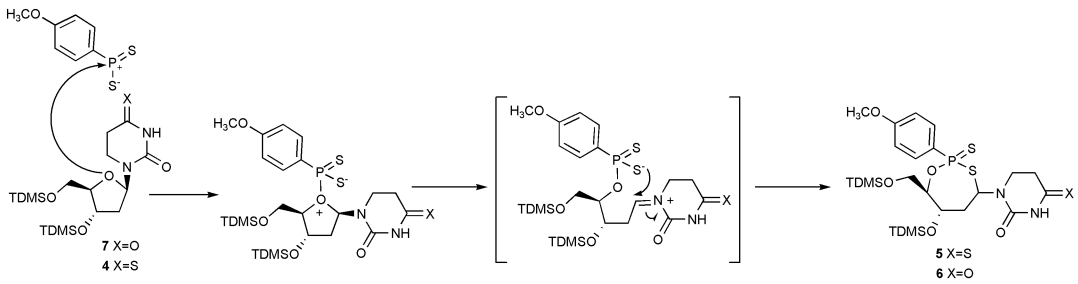


To date there are some precedents reporting the insertion of LR during thionation of some substrates leading to the formation of an O–P–S heterocycle.[11] However, to our knowledge, such an insertion of $AnPS_2$ within an heterocycle is rare and only one example leading to an oxathiaphosphorinane **8** has been reported.[16] Since the LR thiation reaction mechanism involves an oxygen nucleophilic attack on phosphorus,[11,17] it is reasonable to propose that both the O4′ ethereal oxygen and the C4 carbonyl oxygen compete to interact with the electrophilic phosphorus. Thus, the formation of **5** or **6** would occur following an O4′ nucleophilic attack onto the phosphorus ylide which is believed to be the reactive species of Lawesson's reagent.[11,17] This would produce an oxonium intermediate which opens to give an acyclic Schiff base that subsequently cyclises to yield **5** or **6** as a mixture of α and β isomers (Scheme



Scheme 1

DOI: 10.1039/b211405d



Scheme 2

2). The saturation of the base and the deoxyribose nature of the sugar are both crucial to explain the O1′–C4′ ring opening. Indeed, thiation of 2′-deoxyuridine at C4 using LR is nearly quantitative.[18] Hence, compared to the corresponding dihydro series, the difference in reactivity may be due to the π-electron delocalisation at the glycosylated nitrogen atom. This might increase the affinity of the O4 atom for the electrophilic phosphorus species and concomitantly decreases the nucleophilicity of O4′ atom in direction of the phosphorus ylide. Interestingly, thiation of the trisilyl derivative of 5,6-dihydrouridine using one molar equivalent of LR led to 16% of insertion products and 67% of the expected thiated compound (estimated by ^{1}H NMR).[19] Increasing the amount of LR (2 equiv.) led to 65% of insertion products and 9% of the expected thiated derivative. Thus, compared to the deoxy series, the insertion reaction is less favoured in the ribose series. This result can be explained by the electron-withdrawing inductive effect of the 2′-oxygen substituent which reduces the electron density at the anomeric carbon and hence the nucleophilicity of the O4′ atom.

Surprisingly, if the thiation reaction time was extended to 16 h, the insertion products **5** and **6** were not detected. However, under these conditions, the C4 thiated nucleoside **4** (20% yield) was isolated as a mixture of inseparable C1′ anomers whose α/β ratio was estimated by proton NMR to be 1/5. Anticipating that oxathiaphosphepane compounds could eliminate the $AnPS_2$ species, we studied the thermal stability of **5**. When a mixture of insertion products **5a**–**c** was heated to 85 °C for 9 h in neat dioxane, complete conversion to dihydro-4-thionucleosides (**4**) occurred providing a mixture of α/β anomers. In addition, if a pure β insertion product **5** was used as substrate, a mixture of α/β anomers of **4** was recovered, proving the occurrence of epimerisation during the elimination of the $AnPS_2$ moiety. This elimination reaction was also observed in the ribo series, although the rate was slower (53% of conversion after 9 h). Here again, we hypothesised a participation of the glycosylated nitrogen in the process leading to the regeneration of the Schiff base intermediate. Finally, expulsion of the $AnPS_2$ unit followed by recyclisation of the sugar would explain the epimerisation at C1′. Indeed, as for the insertion reaction, the electronegativity of the 2′-substituent in the ribose series may destabilise the Schiff base intermediate explaining the slower reaction rate. The thermal stability of Lawesson's adducts has been addressed in the past but heterocycles similar to **5** were not found reactive[16,20] even to temperatures up to 140 °C. In our case, milder conditions enabled efficient departure of the $AnPS_2$ moiety; this result might be explained by the intrinsic instability of a seven-membered ring.

In conclusion, although thiation of nucleosides and related compounds has been extensively studied in the past, the mild Lawesson's reagent has never been applied to the more labile 2′-deoxy-5,6-dihydrouridines. We found that the thiation at C4 competed with an unexpected insertion of an $AnPS_2$ moiety into the furane ring giving rise to oxathiaphosphepane nucleosides. These compounds could be converted to the target 2′-deoxy-5,6-dihydro-4-thiouridine derivative as a mixture of α and β anomers by thermal treatment. Further work is in progress to control the ring-closure stereoselectivity consecutive to the $AnPS_2$ departure. Considering the sugar insertion reaction, this is the first time that such a reaction is reported in the nucleoside series. In addition, these oxathiaphosphepane nucleosides, which can be obtained in excellent yields, are representatives of a new class of potentially biologically active nucleoside derivatives.

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

Notes and references

1 J. J. Dalluge, T. Hashizume, A. E. Sopchik, J. A. McCloskey and D. R. Davis, *Nucleic Acids Res.*, 1996, **24**, 1073.
2 M. Dizdaroglu, J. Laval and S. Boiteux, *Biochemistry*, 1993, **32**, 12105.
3 J. Liu and P. W. Doetsch, *Nucleic Acids Res.*, 1998, **26**, 1707.
4 D. Dobritzsch, S. Ricagno, G. Schneider, K. D. Schnackerz and Y. Lindqvist, *J. Biol. Chem.*, 2002, **277**, 13155.
5 D. M. Brown and M. J. E. J. Hewlins, *J. Chem. Soc. C*, 1968, 2050.
6 V. Skaric, B. Gaspert, M. Hohnjec and G. Lacan, *J. Chem. Soc., Perkin Trans. 1*, 1974, 267.
7 V. Skaric, B. Gaspert, I. Jerkunica and D. Skaric, *Croat. Chem. Acta*, 1965, **37**, 199.
8 V. Skaric, B. Gaspert, M. Hohnjec and G. Lacan, *J. Chem. Soc., Perkin Trans. 1*, 1970, 2444.
9 Compound **7** was prepared in 94% yield from 2′-deoxy-5,6-dihydrouridine using standard silylation procedures. 2′-deoxy-5,6-dihydrouridine was obtained by catalytic hydrogenation of 2′-deoxyuridine according to the method of Cohn and Doherty: W. E. Cohn and D. G. Doherty, *J. Am. Chem. Soc.*, 1956, **78**, 2863. The experimental details will be described in a full paper.
10 R. H. Iwamoto, E. M. Acton and L. Goodman, *J. Med. Chem.*, 1963, **6**, 684.
11 M. P. Cava and M. I. Levinson, *Tetrahedron*, 1985, **41**, 5061.
12 K. Felczak, M. Bretner, T. Kulikowski and D. Shugar, *Nucleosides Nucleotides*, 1993, **12**, 245.
13 When 0.5 molar equiv. of LR was used, most of the starting material remained. Note that 2 molar equiv. of LR are used for the thiation of 5,6-dihydrouracils (ref. 12).
14 Compounds **5a**–**c** were purified by HPLC to provide pure isomers for characterisation. Configurations at C1′ and at the phosphorus atom were determined by NOESY experiments; the β-configuration of the anomeric center of **5a**–**b** was assigned from a noe correlation between H1′ and H4′ whereas the α-configuration of **5c** was based on a noe correlation between H1′ and H3′. For **5a**, a correlation between H4′ and H-*ortho* of the $AnPS_2$ moiety could be ascertained, leading to a Rp configuration. For **5b**, the correlation between H-*ortho* of $AnPS_2$ and H2′ may be the proof for a Sp configuration. The α compound spectrum showed a correlation between H-*ortho* of the $AnPS_2$ and H1′, which could be the proof for a Sp configuration.
15 Selected data for 2′-deoxy-3′,5′-dithexyldimethylsilyl-5,6-dihydro-4-thiouridine (**4**): ^{1}H ($CDCl_3$) δ_{ppm}: 8.99 (1H, NH); 6.25 (1H, H1′); 4.34 (1H, H3′); 3.79 (1H, H4′); 3.70 (2H, H5′5″); 3.62 (1H, H6a); 3.29 (1H, H6b); 3.02 (2H, H5ab); 1.97 (2H, H2′2″); 1.62 (2H, TDMS); 0.89/0.83 (24H, TDMS); 0.11/0.09 (12H, TDMS). HRMS (MALDI) $(M+Na)^+$ calcd. for $C_{25}H_{50}N_2O_4SSi_2$ 553.29276, found 553.29278.
16 R. Shabana, J. B. Rasmussen and S.-O. Lawesson, *Bull. Soc. Chim. Belg.*, 1981, **90**, 103.
17 T. B. Rauchfuss and G. A. Zank, *Tetrahedron Lett.*, 1986, **27**, 3445.
18 R. S. Coleman and E. A. Kesicki, *J. Am. Chem. Soc.*, 1994, **116**, 11636.
19 Analytical samples were obtained for characterisation.
20 A. A. El-Barbary and S.-O. Lawesson, *Tetrahedron*, 1981, **37**, 2641.