Study of the Pyrimidine Nucleobase C5-C6 Bond Reactivity Under Thio-Michael/Aldol Tandem Reaction Conditions

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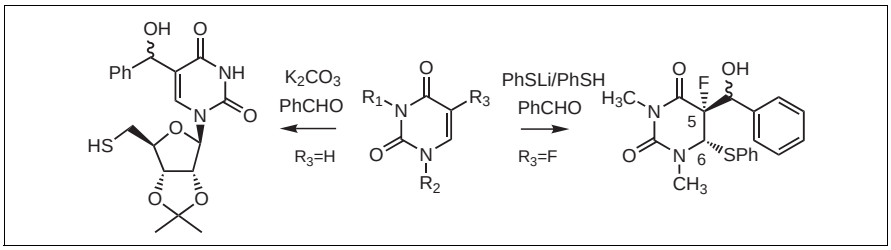
Received October 3, 2005



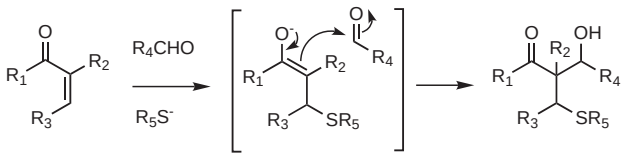
Pyrimidine nucleobases can undergo a Michael/aldol tandem reaction triggered by a thiolate. In an intramolecular context, 5'-deoxy-2',3'-isopropylidene-5'-thiouridine affords the reminiscent Baylis-Hillman adduct after retro-Michael addition whereas its thymine counterpart is unreactive. In an intermolecular context, the conjugate addition-aldol reaction occurs only if the C5-C6 double bond is activated.

J. Heterocyclic Chem., **43**, 1095 (2006).

Introduction.

Nucleophilic addition of thiolates at the β-carbon of α,β-unsaturated esters generates a β-thioenolate intermediate that can subsequently undergo an aldol reaction to give α-alkylthio-alkyl-β-hydroxy esters (Scheme 1, R_1=OR) [1]. The recent extension of this reaction to the less reactive α,β-unsaturated amide series (Scheme 1, R_1=NHR) [2] led us to envisage the preparation of modified dihydropyrimidines of potential biological interest [3] and decided to investigate the possible extension to this tandem reaction in the nucleobase series.

Scheme 1



The possibility of extending the tandem approach for the pyrimidine nucleobase series was supported by the ability, on one hand, of the C5-C6 double bond of uracil or thymine derivatives to undergo a thiol addition at C6 [4], and on the other hand, of the 5,6-dihydrothymine enolate species to undergo an aldol coupling [5]. However, to our knowledge, the thio-Michael/Aldol tandem reaction has not been studied in this series. Only an alkoxide-mediated Michael/Aldol reaction, followed by elimination of the alkoxide (Baylis-Hillman-type reaction) has been reported to provide 5-α-hydroxyalkyl uracil derivatives [6].

We have investigated this one pot tandem reaction in an intermolecular context as well as in a thio-initiated intramolecular Michael addition context using benzaldehyde as a model electrophile.

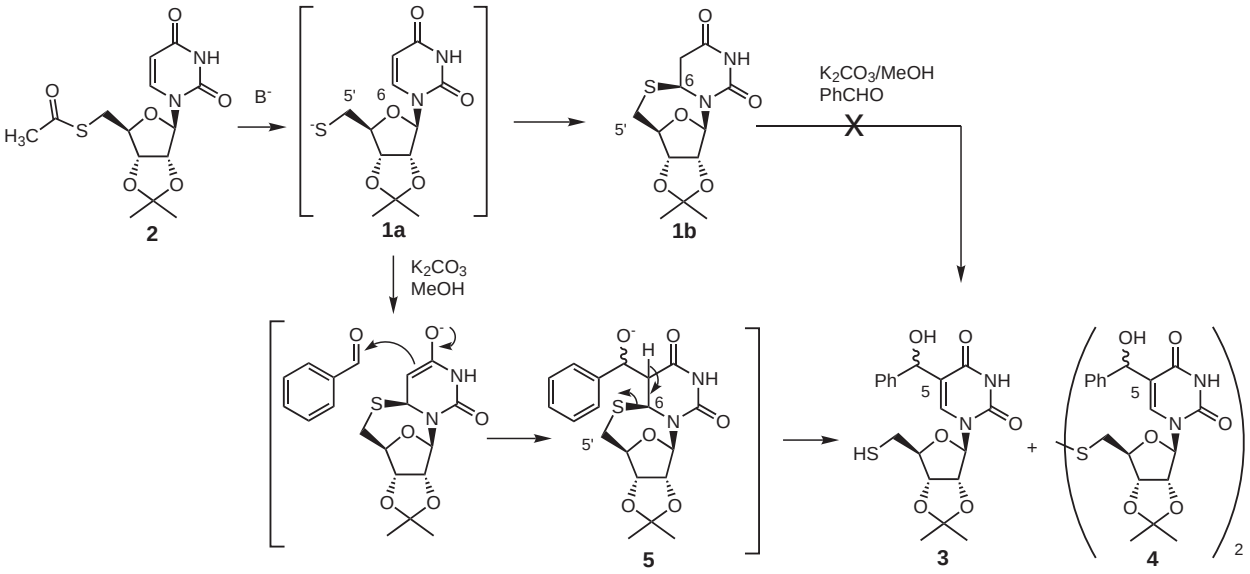
Results and Discussion.

In the intramolecular context, the tandem process was examined on 5'-thio nucleosides containing the 2',3'-*O*-isopropylidene motif known to facilitate the addition of the 5'-thiol group onto the C6 position through conformational assistance [7].

The required thiolate **1a** was generated *in situ* by alkaline hydrolysis of **2** since **1a** spontaneously leads to its isomeric cyclic sulfide form **1b** [8]. Treatment of **2** in methanol and in the presence of K_2CO_3 with benzaldehyde (10 equiv) at room temperature under an argon atmosphere afforded **3** in 79% together with the disulfide derivative **4** in 10%. Upon treatment under the same conditions, **1b** remained unreactive (Scheme 2).

Isolation of **3** clearly indicated that the expected adduct **5** had been formed but that subsequent elimination of the thiol had occurred according to a Baylis-Hillman-type reaction (Scheme 2) as already observed in the case of 2',3'-*O*-isopropylideneuridine [6]. Compared to the thio-Michael/Aldol tandem reactions reported so far, it is likely that in the pyrimidine nucleobase series, at least in an intramolecular context, the tandem adduct undergoes a re-aromatisation reaction.

Scheme 2

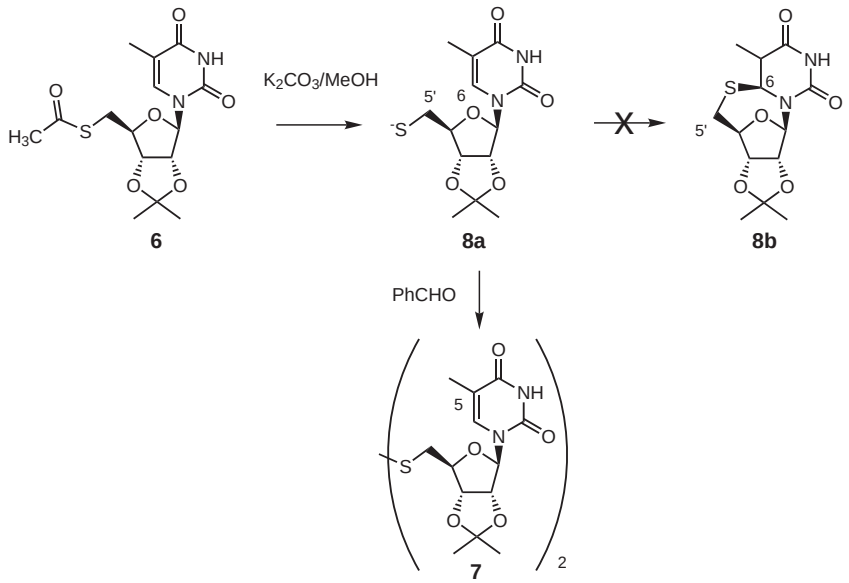


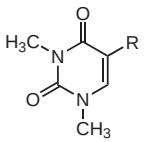
We then evaluated the intramolecular tandem reaction in the thymine nucleobase series. Upon the conditions used for the formation of **3** and **4**, the *S*-acetyl derivative of 5'-deoxy-2',3'-*O*-isopropylidene-5-methyl-5'-thiouridine (**6**) [9] gave rise only to the disulfide derivative **7** (Scheme 3).

Such behavior is likely due to the methyl group at the C5 position that deactivates the C5-C6 double bond Michael acceptor [1b], and is consistent with the occurrence of 5'-deoxy-2',3'-*O*-isopropylidene-5-methyl-5'-thiouridine under its open form (**8a**) [9,10].

We then investigated the intermolecular version of the thio-Michael/Aldol tandem reaction. For the sake of simplicity, we studied it in the *N*1,*N*3-dimethyl pyrimidine base series. As anticipated, treatment of 1,3-dimethylthymine **9** with lithium thiophenolate (1.1 equiv) [1b,2] in CH_2Cl_2 in the presence of benzaldehyde (2 equiv) gave no reaction. More surprisingly, 1,3-dimethyluracil **10** was also found unreactive upon these conditions. To increase the reactivity of the C5-C6 double bond Michael acceptor, we decided to use 1,3-dimethyl-5-fluorouracil **11** as Michael acceptor.

Scheme 3





9 R:CH_3
10 R:H
11 R:F

Treatment of **11** in CH_2Cl_2 with lithium thiophenolate (1.1 eq) in the presence of benzaldehyde (2 equiv) gave no reaction (Table 1, entry 1). In the presence of 10 equiv of PhSH, 1.5 equiv of PhSLi, 5 equiv PhCHO, and in THF [11], the tandem Michael/Aldol adduct **12** was obtained in 11% yield (entry 2) together with 1,3-dimethyl-5-phenylthiouracil **13** and 1,3-dimethyluracil **10** (9% and 7% yield, respectively) (Scheme 4) [12]. The yield of **12** could be increased up to 46% using 10 equiv of PhCHO (entry 4). Toluene was also found a suitable solvent (entry 5) [11a].

Interestingly, for each set of experiments, compound **12** was isolated as a mixture of only two diastereomers. Absence of coupling between H6 and the fluorine atom for both diastereomers demonstrated an eq-eq relationship between these two atoms and consequently a transdiaxial orientation for the two bulky substituants at C5 and C6. Therefore, the stereochemistry depicted in structure **12** was assigned to the tandem adduct.

Attempts to transform **9** and **10** using the optimal conditions determined for the formation of **12** turned out to be unsuccessful confirming the insufficient reactivity of these derivatives.

Conclusion.

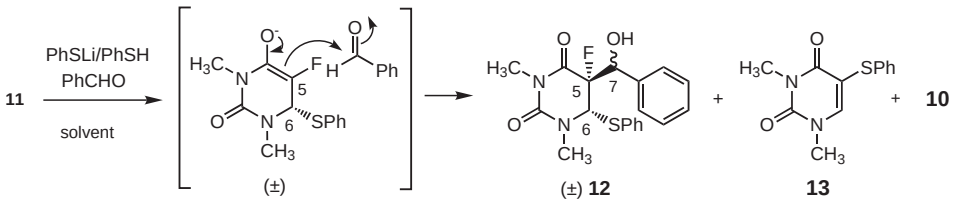
In summary, this work represents the first example of the extension of the Michael-aldol tandem reaction initiated by a thiolate to the pyrimidine nucleobase series. It clearly demonstrates the importance, in this series, of the intra or inter molecular context on the ability of the reaction to proceed and on its issue.

The tandem adduct prepared during this work is of potential biological interest as are derivatives accessible using the sulfur functionality. Biological results will be reported elsewhere.

EXPERIMENTAL

^1H and ^{13}C NMR spectra were measured in $CDCl_3$ and recorded on Bruker Avance 300 or 500. Chemical shifts (δ) are reported in ppm relative to residual solvent peak (δ_H 7.26, δ_C 77.0) or solvent lock reference (δ_F). High resolution mass spectra were recorded on a Micromass LCT (ESI, CH_3OH). Reactions were performed under argon atmosphere. Benzaldehyde was purified as described in the literature [13] and THF was dried over sodium and distilled under argon before use.

Scheme 4



The hypothesis of the capture of the transient thio-Michael adduct enolate with benzaldehyde from the anion species less hindered side (opposite to the bulky SPh group) is consistent with the observed results (Scheme 4).

Table 1

The tandem 1,3-dimethyl-5-fluorouracil-Michael-aldol reaction to benzaldehyde.

Entry	PhSLi	PhSH	PhCHO	Solvent	**12** (%)[a]	**11** recov. (%)[a]
1	1.1	-	2	CH_2Cl_2	-	72
2	1.5	10	5	THF	11	25
3	1.5	10	6	THF	18	44
4	1.5	10	10	THF	46	34
5	1.5	10	10	Toluene	45	40

[a]: Isolated yield.

5-α-Hydroxybenzyl-5'-deoxy-2',3'-*O*-isopropylidene-5'-thiouridine (**3**).

To a solution of **2** [8] (100 mg, 0.3 mmol) in MeOH (1 mL) were added benzaldehyde (0.3 ml, 3 mmol) then a saturated methanolic K_2CO_3 solution (2 mL). The reaction was stirred at room temperature for 3 h then poured dropwise in a saturated aqueous NH_4Cl solution (3 mL). The resulting mixture was extracted with ethylacetate (2x50 mL). The combined organic phases were dried over Na_2SO_4. After filtration and removal of the solvent under reduced pressure, the crude product was purified by silica gel column chromatography under argon atmosphere using a gradient of ethylacetate in CH_2Cl_2 (0 to 60%) to give **3** as an equimolecular mixture of diastereomers [a] and [b] (91 mg, 79% yield). (^1H NMR **(**$CDCl_3$, 300 MHz**)** **δ** 9.66 (sl, 2H, NH), 7.4 -7.3 (m, 10H, Ph), 6.97 (sl, 2H, H6), 5.77 (sl, 2H, H7), 5.62 (d, $J_{1'2'}$ = 2.34 Hz, 1H, H1' [a]), 5.55 (d, $J_{1'2'}$ = 2.16 Hz, 1H, H1' [b]), 4.89 (dd, $J_{2'1'}$ = 2.18 Hz, $J_{2'3'}$ = 6.4 Hz 1H, H2'

[b]), 4.86 (dd, $J_{2'1'}$ = 2.35 Hz, $J_{2'3'}$ = 6.9 Hz, 1H, H2' [a]), 4.71 (dd, $J_{3'4'}$ = 4.11 Hz, $J_{3'2'}$ = 6.59 Hz, 1H, H3' [b]), 4.69 (dd, $J_{3'4'}$ = 4.15 Hz, $J_{3'2'}$ = 6.62 Hz, 1H, H3' [a]), 4.11 (m, 2H, H4'), 3.8 (sl, 2H, OH), 2.7 (m, 4H, H5' + H5''), 1.53 (s, 6H, CH_3 Isop), 1.32 (s, 6H, CH_3 Isop); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 163.5/163.4 (C4), 149.6 (C2), 140.0 (Cq*, Ph), 139.9 (C6* [b]), 139.6 (C6 [a]), 128.7 (CH, Ph), 128.2 (CH, Ph), 126.6 (CH, Ph), 118.0/117.8 (C5), 114.7/114.6 (Cq, Isop), 94.5 (C1' [b]), 93.6 (C1' [a]), 88.0/87.4 (C4'), 84.4/84.3 (C2'), 82.6/82.4 (C3'), 69.1/69.0 (C7), 27.1 (CH_3, Isop), 26.6 (C5'), 25.3 (CH_3, Isop); HRMS (ESI$^+$ mode) *m/z* 429.1078 $(M+Na)^+$ (429.1096 calculated for $C_{19}H_{22}N_2O_6NaS$).

Anal. Calcd. for $C_{19}H_{22}N_2O_6S$: C, 56.14; H, 5.46; N, 6.89. Found: C, 55.77; H, 5.71; N, 6.57.

*: Interchangeable attributions

Compound **12**.

A solution of **11** (100 mg, 0.63 mmol), benzaldehyde (0.63 mL, 0.63 mmol) and PhSH (0.65 mL, 6.3 mmol) was added dropwise over a period of 1 h to an heterogenous mixture of PhS^-Li^+ in THF prepared at 0°C as previously described [1b] from PhSH (0.09 mL, 0.9 mmol), *n*-butyllithium in hexane (1.5 *M*, 0.6 mL, 0.9 mmol) and THF (1.5 mL). The reaction was allowed to warm at room temperature. The reaction mixture was poured to a saturated aqueous NH_4Cl solution (4 mL) and the mixture was extracted with ethyl acetate (2 x 60 ml), the organic phases were combined and dried over Na_2SO_4. After filtration and removal of the solvent under reduced pressure, the residue was purified by silica gel column chromatography using a gradient of ethylacetate (5% to 20%) in heptane to give **12** (equimolecular mixture of diastereomers [a] and [b]) as a white powder (109 mg, 46% yield): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.45-7.20 (m, 20H, Ph), 5.11 (d, J_{H7F5} = 16.2 Hz, 1H, H7 [a]), 5.02 (d, J_{H7F5} = 18.3 Hz, 1H, H7 [b]), 4.77 (s, 1H, H6 [a]), 4.74 (s, 1H, H6 [b]), 3.11 (s, 3H, CH_3, MeN1 [b]), 2.89 (s, 3H, CH_3, MeN1 [a]), 2.71 (s, 3H, CH_3, MeN3 [b]), 2.59 (s, 3H, CH_3, MeN3 [a]); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 165.8/165.5 (C4), 151.9 (C2, [b]), 150.5 (C2, [a]), 136.6/136.4 (CH, Ph), 135.5/135.4 (Cq, Ph), 129.7/129.6/129.2/128.6/128.5/128.3/128.2/126.9/126.0 (CH, Ph), 92.1 (d, $^2J_{C5F}$ = 193 Hz, C5 [b]), 91.1 (d, $^2J_{C5F}$ = 194 Hz, C5 [a]), 75.9 (d, $^3J_{C7F}$ = 26 Hz, C7 [a]), 75.7 (d, $^3J_{C7F}$ = 28 Hz, C7 [b]), 67.1 (d, $^3J_{C6F}$ = 30 Hz, C6 [b]), 66.0 (d, $^3J_{C6F}$ = 29 Hz, C6 [a]), 34.9/34.6 (CH_3, MeN1), 27.6/27.4 (CH_3, MeN3); ^{19}F NMR ($CDCl_3$, 282 MHz) δ -162.1 (J_{5FH7} =16 Hz [a]); -167.6 (J_{5FH7} =18 Hz [b]); HRMS (ESI$^+$ mode) *m/z* 397.1039 $(M+Na)^+$ (397.0998 calculated for $C_{19}H_{19}N_2O_3FNaS$).

Anal. Calcd. for $C_{19}H_{19}N_2O_3FS$: C, 60.95; H, 5.11; N, 7.48. Found: C, 60.89; H, 5.32; N, 7.32.

Acnowlegements

We thank the CNRS for a doctoral fellowship to C. D. and a postdoctoral fellowship to E.V.V.

REFERENCES AND NOTES

[1a] A. Kamimura, H. Mitsudera, S. Asano, A. Kakehi, M. Noguchi, *Chem. Commun*., 1095 (1998); [b] A. Kamimura, H. Mitsudera, S. Asano, S. Kidera, A. Kakehi, *J. Org. Chem*., **64**, 6353 (1999).

[2] A. Kamimura, Y. Omata, H. Mitsudera, A. Kakehi, *J. Chem. Soc. Perkin Trans I*, 4499 (2000).

[3] O. Miyashita, K. Matsumura, T. Kasahara, H. Shimadzu, N. Hashimoto, *Chem. Pharm. Bull*., **30**, 887 (1982).

[4a] P. F. C. Van der Meij, U. K. Pandit, *Tetrahedron Lett*., **29**, 5445 (1988); [b] E. Vega, G. A. Rood, E. R. de Waard, U. K. Pandit, *Tetrahedron*, **47**, 4361 (1991) and references cited.

[5] R. Nicewonger, T. P. Begley, *Tetrahedron Lett*., **38**, 935 (1997).

[6a] H. Sajiki, A. Yamada, K. Yasunaga, T. Tsunoda, M. F. A. Amer, K. Hirota, *Tetrahedron Lett*., **44**, 2179 (2003). For selected examples of C6-addition of other nucleophiles followed by anion trapping with electrophiles see: [b] N. Baret, J.-P. Dulcere, J. Rodriguez, *Nucelosides Nucleotides*, **17**, 1125 (1998); [c] G. Grangier, D. J. Aitken, D. Guillaume, A. Tomas, B. Viossat, H.-P. Husson, *J. Heterocyclic Chem*., **31**, 1707 (1994); [d] K. Hirota, T. Tomishi, M. Sako, Y. Maki, *J. Chem. Soc. Perkin Trans I*, 2227 (1988).

[7] E. J. Reist, A. Benitez, L. Goodman, *J. Org. Chem*., **29**, 554 (1964).

[8] B. Bannister, F. Kagan, *J. Am. Chem. Soc*., **82**, 3363 (1960**)**.

[9] B. Wang, J. R. Kagel, M. P. Mertes, K. Bowman-James, *Biorg. Chem*., **22**, 405 (1994).

[10] V. Skaric, J. Matulic-Adamic, *Helv*. *Chim. Acta*, **63**, 2179 (1980).

[11] For thio-Michael tandem reaction carried out in THF or toluene, see: [a] M. Ono, K. Nishimura, Y. Nagaoka, K. Tomioka, *Tetrahedron Lett*., **40**, 1509 (1999); [b] M. Ono, K. Nishimura, H. Tsubouchi, Y. Nagaoka, K. Tomioka, *J. Org. Chem*., **66**, 8199 (2001).

[12a] T. Chikuma, K. Negishi, H. Hayatsu, *Chem. Pharm. Bull*., **26**, 1746 (1978); [b] B. C. Pal, *J. Am. Chem. Soc*., **100**, 5170 (1978).

[13] D. D. Perrin, W. L. F. Armarego, D. R. Perrin, Purification of Laboratory Chemicals, Pergamon Press, Oxford, 1980, p 117.