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HSAB-driven chemoselective N1-alkylation of pyrimidine bases and their 4-methoxy- or 4-acetylamino-derivatives

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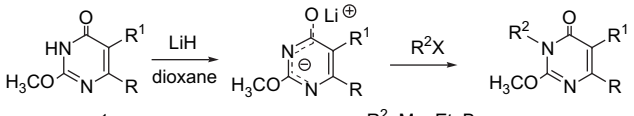
Abstract—The lithium salts of the conjugated bases of 4-methoxy- and 4-acetylamino-2(1*H*)-pyrimidinones **1–3** undergo highly chemoselective N^1-methylation or ethylation when treated with methyl- or ethylsulfate (hard electrophiles) in dry dioxane, while the use of DMF as solvent results in competitive O^2-alkylation. Potassium salts of the same bases in DMF undergo prevalent O^2-attack. Under the same conditions, a similar but less chemoselective behaviour is observed in alkylation of thymine and uracil, where some N^3-attack occurs. This can be rationalised in terms of the HSAB principle.

1. Introduction

N^1-Alkylated nucleic acid bases are widely used as nucleoside analogs to exert anti-viral[1] and anti-inflammatory activities,[2] to inhibit the activity of reverse transcriptase in the HIV virus,[3] as anti-cancer agents,[4] as agonists of AMPA and kainate receptors[5] and as GnRH receptor antagonists.[6] Although N^1-alkylated pyrimidinones can be obtained by intramolecular condensation[6] or by Pd(0)-catalysed reactions,[1a] the most general approach involves an S_N^2 displacement. In this process, the conjugate base of the substrate is usually reacted with electrophiles (alkyl phosphates,[7] halides[3b,5,7b,8] and diazoalkanes[9]) in polar solvents. However, due to the tetradentate nucleophilic nature of the intermediate conjugated base, these reactions suffer from low chemoselectivity and lead to mixtures of N^1-monoalkylated and N^1,N^3-dialkylated products[1a,3b,5,7a,8] along with some products resulting from O^2- and O^4-attack.[7a,9a] In order to avoid these problems, various methodologies using 2,4-dimethoxy-[10] or 2,4-disilyloxy-pyrimidines[11] as starting materials have been developed and are widely applied.[3b,c,e]

Formation of the N^1,N^3-dialkylated products can be easily explained in terms of deprotonation and subsequent realkylation of the N^1-monoalkylated products. However, the lack of N^3-monoalkylation derivatives is difficult to explain in terms of the higher acidity of N^1–H than N^3–H, as stated in the literature.[3e] In fact, Wittenburg[12] and Ganguly and Kundu[13] have shown that first deprotonation of thymine occurs at N^3 with a pK_a value of 9.9, but the N^{3-} species is in equilibrium with the N^{1-} species in a 1:1 ratio. The same behaviour is also operative in uracil, with a pK_a value of 9.5.[13,14] Therefore, the lack of N^3-attack should be related to an intrinsic higher reactivity of N^1.

While investigating the hypothesis that the HSAB principle[15] is responsible for the above regioselectivity, we first studied the chemoselectivity on simplified models: 2-methoxy-4(3*H*)-pyrimidinone derivatives (Scheme 1), where the intermediate tridentate conjugate base can undergo N^1-, N^3- or O^4-attack. We reported[16] that a very high N^3-chemoselectivity is observed when reactions are carried out in a low polarity solvent (dioxane) and both a hard base (LiH) and a hard electrophile (alkylsulfate or tosylate) are used. Under these conditions, tight ionic pairs



Scheme 1.

Keywords: Pyrimidinones; Alkylation; Regioselection; HSAB principle.
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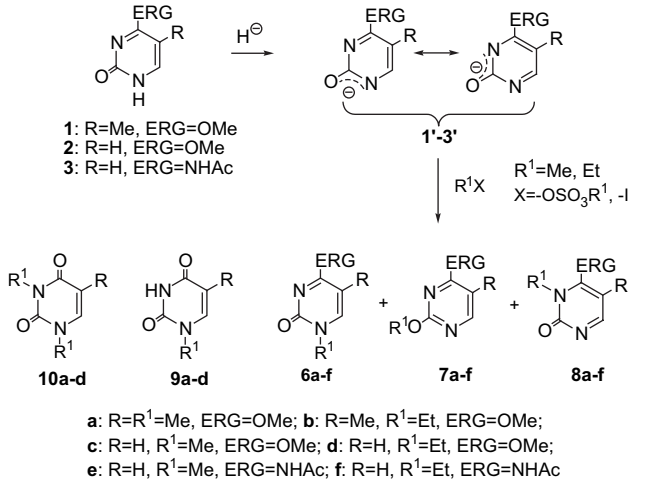
between the O^4-anion and the lithium cation are obtained, so preventing O^4-alkylation, in addition, the electron releasing methoxy group at C_2 enhances the hardness of the N^3-anion with respect to that of N^1, giving rise to a favourable hard–hard match with the hard alkylating agents. Accordingly, the use of either softer counterions (Na^+ or K^+) or a softer alkylating agent (BnBr) results in competitive O^4- or N^1-attack.[16]

The above findings prompted us to further investigate the applicability of the HSAB principle to the selective alkylation of pyrimidinone derivatives. We proposed that, moving the methoxy group from C_2 to C_4 would result in an inversion of the hardness of the two nitrogen in favour of the N^1 position and so give selective N^1-alkylations. Hence, in order to confirm our hypothesis and to take synthetic advantage, we carried out a model study to investigate the effects of different solvents and alkylating agents on the distribution of products arising from S_N^2 alkylation of the 4-methoxy- and the 4-acetylamino-2(1*H*)-pyrimidinones **1**–**3** (Scheme 2). In addition, to obtain information about the real effect of the electron releasing group (ERG) at O^4 (or O^2) on the chemoselectivity, the same studies were extended to the unprotected pyrimidine bases thymine **4** and uracil **5** (Scheme 4).

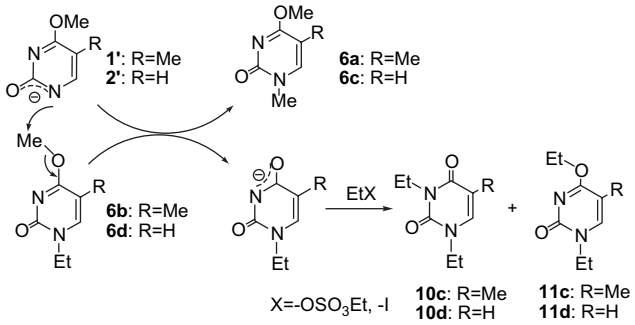


Scheme 2.

2. Results and discussion

Initial experiments were concerned with the chemoselection of the methylation or ethylation of **1**–**3** where, due to the tridentate nucleophilic character of the intermediate metal salts **1′**–**3′**, the monoalkylated compounds **6a–f**, **7a–f** and **8a–f** (Scheme 2) were expected as products, with their relative amounts depending on the metal counterion, the solvent and the alkylating agent. Substrate **3** is commercial, while compounds **1** and **2** were prepared as reported in the literature.[17] Surprisingly, we noticed that substrates **1**–**3** are remarkably less soluble in dioxane than the corresponding, previously studied,[16] 2-methoxy-derivatives (Scheme 1). This feature was found to be in agreement with the calculated logarithms of the partition coefficient for *n*-octanol/water,[18] which are −0.599, −1.098 and −1.680 for compounds **1**, **2** and **3**, respectively, versus 0.133 and −0.385 for the 2-methoxy-derivatives corresponding to **1** and **2**. This lower solubility necessitated the reactions in dioxane to occur in heterogeneous phase, even at 60 °C, and accounted for the low reaction rates observed.

Trial experiments allowed the isolation and characterisation (see Section 4 and Scheme 2) of pure standards of the unprecedented products of N^1- and O^2-alkylation **6b**, **6f**, **7b**, **7d** and **7f**, as well as the known compounds **6a**,**c**,**d**,[9a] **6e**,[19] **7a**,**c**[17] and **7e**,[20] while products **8a–f** were never found. In addition, variable amounts of the commercial compounds **9a**,**c**,**d** and **9b**,[21] resulting from hydrolysis of the methoxy group at C_4 during either workup or chromatographic separation, were isolated from the reaction mixtures of **1** and **2** together with the already known N^1,N^3-double alkylation products **10a–d**.[22–24] Finally, small amounts of the known N^1,O^4-diethyl derivatives **11b**[24] and **11d**[9a] (Scheme 3) were identified in some ethylation experiments.



Scheme 3.

The metal salts **1′**–**3′** were prepared using LiH or KH as hard or soft counterions, respectively, while dry dioxane (ε=2.2) or DMF (ε=38.2) were used as solvents in order to generate either tight or loose ionic couples. Dry dimethylsulfate (DMS) and diethylsulfate (DES) were chosen as hard electrophiles, while the corresponding iodides were used as soft alkylating agents. All reactions were monitored and analysed by HPLC, over 24 h, in the case of substrates **1** and **2**, and over 48 h for substrate **3**. Results are shown in Table 1, where the HPLC percentages of the obtained products **6** (including their hydrolysis products **9**) and **7** are reported in the N^1 and O^2 columns, respectively, together with the corresponding N^1/O^2 ratios. HPLC percentages of the unexpected (vide infra) products **10**, arising from N^1,N^3-double alkylation, are also reported, while minor products (less than 1%) are not shown.

The data in Table 1 support our hypothesis of HSAB-driven chemoselectivity. N^3-Alkylation is always absent so that only N^1 and O^2 compete for the electrophile. This can be explained as a result of the very hard character of N^1 versus N^3. When the LiH/dioxane/alkylsulfate system is used to favour both tight O^-Li^+ pairs and hard–hard nucleophile–electrophile matches, N^1-alkylation is always the main process with chemoselectivities ranging from >200:1 (methylations) or 36:1 (ethylations) for the 4-methoxypyrimidinones **1**–**2**, and up to >30:1 for the acetylcytosine **3**. Accordingly, the use of soft electrophiles, such as iodides, does not affect the chemoselectivity, but results only in a poorer conversion of the substrates.

Alkylation of the N^4-acetylcytosine **3** showed some differences with that of **1** and **2**. The reactivity was always lower

Table 1. HPLC % distribution of products in the S_N^2 alkylation of **1–3** under different conditions

Run	Base	R^1X	Solvent	N^1	O^2	N^1,N^3	Unreacted substrate	N^1/O^2 ratio
4-Methoxy-5-methyl-2(1*H*)-pyrimidinone, **1**[a]								
1	LiH	DMS	Dioxane	93.8	—	—	6.1	>200
2	LiH	MeI	Dioxane	74.0	—	—	26.0	>200
3	LiH	DES	Dioxane	76.2	2.1	—	21.0	36.3
4	LiH	EtI	Dioxane	21.6	Traces	—	75.5	>20
5	KH	DES	Dioxane	51.2	24.5	—	21.2	2.2
6	LiH	DMS	DMF	76.0	8.8	—	14.8	8.6
7	LiH	MeI	DMF	75.2	9.8	0.4	13.6	7.7
8	LiH	DES[b]	DMF	42.1	37.4	3.8	15.5	1.1
9	LiH	EtI[b]	DMF	29.9	24.0	8.0	36.5	1.2
10	KH	DES	DMF	9.8	64.2	—	25.0	0.1
4-Methoxy-2(1*H*)-pyrimidinone, **2**[a]								
11	LiH	DMS	Dioxane	90.0	—	5.0	4.5	>200
12	LiH	MeI	Dioxane	87.6	—	5.1	7.1	>200
13	LiH	DES[c]	Dioxane	89.7	2.5	4.5	3.0	35.9
14	LiH	EtI[c]	Dioxane	29.1	Traces	1.0	69.0	>20
15	KH	DES	Dioxane	46.7	33.7	Traces	18.0	1.4
16	LiH	DMS	DMF	80.1	8.7	4.0	6.0	9.2
17	LiH	MeI	DMF	65.5	8.5	5.0	20.7	7.7
18	LiH	DES[c]	DMF	55.3	37.6	3.3	3.0	1.5
19	LiH	EtI[c]	DMF	43.8	31.2	5.9	18.5	1.4
20	KH	DES	DMF	40.6	54.0	2.1	2.5	0.7
4-Acetylamino-2(1*H*)-pyrimidinone, **3**[d]								
21	LiH	DMS	Dioxane	77.9	2.1	—	17.8	37.1
22	LiH	MeI	Dioxane	17.0	0.5	—	81.9	34.0
23	LiH	DES	Dioxane	54.5	1.8	—	43.5	30.2
24	LiH	EtI	Dioxane	2.6	—	—	97.4	—
25	KH	DES	Dioxane	44.5	30.3	—	24.2	1.5
26	LiH	DMS	DMF	60.0	6.5	—	33.0	9.2
27	LiH	MeI	DMF	56.4	7.0	—	36.2	8.0
28	LiH	DES	DMF	65.8	8.8	—	25.0	7.5
29	LiH	EtI	DMF	55.0	7.9	—	36.8	7.0
30	KH	DES	DMF	37.5	39.6	—	21.0	0.9

[a] Reactions carried out at 60 °C for 24 h.
[b] Small amounts of **6a** and **11b** were detected.
[c] Small amounts of **6c** and **11d** were detected.
[d] Reactions carried out at 60 °C for 48 h.

and small amounts of O^2-alkylation products were always present, even in methylations. This is not unexpected[25] and could be related to the weaker electron releasing effect of the acetylamino group, resulting in a smaller difference in hardness between O^2 and N^1.

In contrast, the use of DMF, where loose O^-Li^+ pairs were expected, gave rise to a considerable amount of O^2-alkylation, which increased with the hardness of the electrophile (compare runs 6, 16 and 26 with runs 8, 18 and 28, respectively). This decrease of the N^1/O^2 chemoselectivity confirms the typical trend of reactions, which obey the HSAB principle. Accordingly, the use of the soft K^+ cation (runs 5, 10, 15, 20, 25 and 30) led to a sharp inversion of chemoselectivity in favour of O^2-alkylation as observed previously.[25]

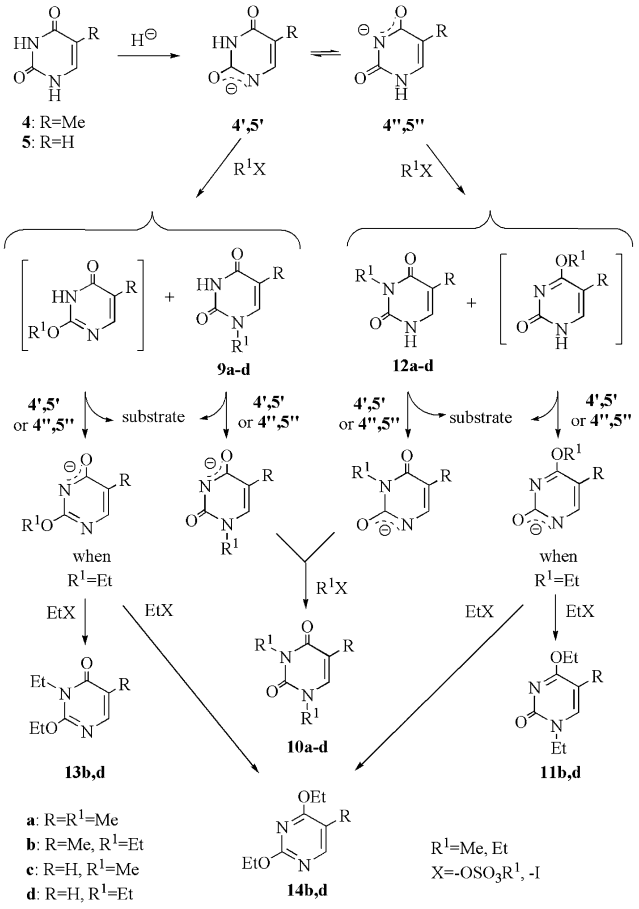
Formation of the unexpected N^1,N^3-double alkylation products **10**, usually reported as important by-products in the literature,[1a,3b,5,7b,8] is the result of a side reaction. N^1,N^3-Dialkylated products occur in very small amounts for the methoxy derivative **1** and increased amounts for substrate **2**, but are absent for the N^4-acetylcytosine **3**. This trend can be rationalised as a result of a competitive nucleophilic displacement of the O^4-methyl group by the suitable conjugated base **1′** (or **2′**), as shown in Scheme 3 for the ethylation reactions.

This side reaction is suppressed by the steric hindrance of the methyl group at C_5 in the thymine derivative **1** and is impossible for the acetylcytosine **3**.

The detection of small amounts of the N^1,O^4-dimethylated products **6a** (or **6c**) and the corresponding diethylated products **11b** (or **11d**), together with the N^1,N^3-derivatives **10b** (or **10d**), in some ethylation reactions (Table 1, runs 8, 9, 13, 14, 18 and 19), supports this hypothesis. In conclusion, N^1-attack was the most prevalent, if not the only, monoalkylation process observed with the LiH/alkylsulfate/dioxane system and this is in favour of a strong activation at N^1 due to a N^1-hardening effect operated by the ERG at C_4.

With the above results in hand, and in order to verify the real effect of the C_4-ERG (or C_2-ERG)[16] in driving chemoselection, we extended the same experiments to the C_4-unprotected pyrimidine bases thymine **4** and uracil **5**. Here, as outlined in Section 1, single proton extraction can give rise to the two different conjugated bases **4′**, **4″** and **5′**, **5″** (Scheme 4) in a ratio of 1:1,[12–14] so that each alkylation could afford, in theory, four monoalkylated compounds. Trial experiments showed that alkylation of **4** and **5** was always very slow giving the monoalkylation products **9a–d** and **12a–d**[16] together with the N^1,N^3-double alkylation derivatives **10a–d**, as major products. O^2- or O^4-Monoalkylation products were never found, but small amounts of the already

mentioned N^1,O^4-diethyl derivatives **11b**,**d** together with the corresponding unprecedented N^3,O^2-isomers **13b**,**d** were identified in some ethylation experiments. The latter compounds were not isolated, but detected by both GC–MS and by their conversion into **12b**,**d**, respectively, after acid hydrolysis of their mixtures.



Scheme 4.

As depicted in Scheme 4, compounds **11** and **13** are most likely derived from an easier re-alkylation of the more reactive and previously formed O^2- or O^4-monoalkylation products, respectively. Therefore, the relative amounts of N,O-bis-alkylation products account for the percentage of O^2- or O^4-attack. The same route is followed for the formation of **10** from **9** or **12**.

Product distributions under different conditions were measured as described above for substrates **1**–**3** and the results are reported in Table 2, where N^1, N^3 and N^1,N^3 columns report the relative amounts of compounds **9**, **12** and **10**, while columns O^2 and O^4 report the amounts of compounds **13** and **11**, respectively. Ratios N^1/N^3, N^1/O^2 and N^1/O^4 are also reported.

The data shown in Table 2 depict a trend similar to that observed for compounds **1**–**3**, but both chemoselection and reactivity are reduced. N^1-Alkylation was certainly the preferred process, but, in contrast to previous reports,[1a,3b,3e,5,7a,8] N^3-attack was always competitive, especially with soft electrophiles and/or in polar solvent. In addition, O^2- and O^4-attack also took place, even in dioxane, when a harder ethyl electrophile was used. Therefore, the N^1 versus N^3-chemoselectivity was still good for alkylations carried out with the LiH/dioxane/sulfate system, but dropped down if softer iodides and/or polar solvents were used.

Irrespective of the hard/soft features of the reagents, N^1,N^3-double alkylation products were always present, their relative amount increasing with polarity. This can be related to an easier proton transfer (Scheme 4) from the monoalkylated products **9** and **12** to the conjugated bases **4′**, **5′** (or **4″**, **5″**). As expected, a sharp increase of both N^3- and O-alkylation products, including the known O^2,O^4-bis-alkylation derivatives **14b**,**c**,[9,24] was observed when K^+ was used as counterion and DMF as solvent (runs 9 and 18 in Table 2). Therefore, the most used reaction conditions for preparative purposes[1a,3b,5,7–9] give rise to the worst chemoselection.

Table 2. HPLC % distribution of products in the S_N^2 alkylation[a] of either lithium or potassium salts of **4** and **5** in polar or apolar solvents

Run	Base	R^1X	Solvent	N^1	N^3	O^2	O^4	N^1,N^3	Unreacted substrate	N^1/N^3 ratio	N^1/O^2 ratio	N^1/O^4 ratio
Thymine, **4**												
1	LiH	DMS	Dioxane	68.7	2.2	—	—	3.6	25.0	31.2	—	—
2	LiH	MeI	Dioxane	47.4	2.2	—	—	1.1	48.5	21.5	—	—
3	LiH	DES	Dioxane	63.2	3.2	1.4	2.8	4.7	23.5	19.7	45.1	22.5
4	LiH	EtI	Dioxane	21.7	2.3	—	—	1.0	68.7	9.4	—	—
5	LiH	DMS	DMF	17.8	4.0	—	—	5.8	71.8	4.5	—	—
6	LiH	MeI	DMF	18.8	5.5	—	—	4.5	69.7	3.4	—	—
7	LiH	DES	DMF	32.1	3.6	1.7	2.0	9.0	49.7	8.9	18.9	16.0
8	LiH	EtI	DMF	30.6	3.9	1.9	2.1	11.5	48.5	7.8	16.1	14.6
9	KH	DES[b]	DMF	34.0	8.7	2.7	6.3	13.8	29.2	3.9	12.6	5.4
Uracil, **5**												
10	LiH	DMS	Dioxane	59.7	2.2	—	—	5.0	32.0	27.1	—	—
11	LiH	MeI	Dioxane	12.2	0.8	—	—	2.4	83.8	15.2	—	—
12	LiH	DES	Dioxane	22.9	1.4	0.6	1.0	2.4	70.8	16.3	37.9	22.9
13	LiH	EtI	Dioxane	16.5	2.3	—	—	7.1	73.2	7.1	—	—
14	LiH	DMS	DMF	29.7	4.7	—	—	10.5	54.6	6.3	—	—
15	LiH	MeI	DMF	41.0	8.2	—	—	2.2	47.5	5.0	—	—
16	LiH	DES	DMF	41.3	5.1	2.0	2.7	12.1	35.9	8.0	20.6	15.3
17	LiH	EtI	DMF	38.8	5.2	2.1	2.8	14.4	36.2	7.5	18.5	13.9
18	KH	DES[c]	DMF	34.4	8.5	2.6	5.8	18.6	25.3	4.0	13.2	5.9

[a] Methylations were carried out at 60 °C for 24 h, ethylations for 72 h.
[b] Compound **14b** (3.2%) was also present.
[c] Compound **14d** (4.0%) was also present.

Despite the already mentioned 1:1 ratio between the two conjugate bases of thymine and uracil,[12–14] the N^1 position was always preferentially alkylated and this behaviour was enhanced when hard–hard matches were set up by the use of the LiH/dioxane/sulfate system. This is true for an HSAB-driven chemoselection, if N^1 is assumed to be harder than N^3, and accounts for the widely reported preferential alkylation at that position.

3. Conclusion

The above results show that chemoselection in S_N^2 alkylation of pyrimidine bases **4**–**5** is driven by the HSAB principle, the N^1 position being intrinsically harder than the N^3. Therefore, the use of both hard alkylating agents, such as sulfates, and apolar solvents results in an increased N^1-attack. Moreover, the intrinsic higher hardness of N^1 is strongly enhanced by the introduction of ERG at C_4, as in compounds **1**–**3**, and this gives rise to both higher reactivity and almost quantitative N^1-chemoselectivity, under the above reaction conditions. In contrast, when the ERG is at C_2, as in the 2-methoxy-pyrimidinones (Scheme 1), the N^3 site becomes harder than that of the N^1 and regioselection is completely reversed to give quantitative N^3-alkylation.[16]

In conclusion, high yielding chemoselective alkylations at either N^1 or N^3 can be obtained if the HSAB principle is applied to pyrimidinone derivatives carrying ERGs at the C_4 or C_2 position, respectively.

4. Experimental

4.1. General

Chromatographic separations were carried out on silica gel (Fluka, 70–230 mesh) washed with 0.1 M HCl and rinsed with hot distilled water, while Fluka silica gel TLC plates (5–17 μm, 0.25 mm) were used for TLC analyses. HPLC analyses were performed on a TSP Spectra Series P200 apparatus equipped with a Thermo Hypersil BDS C18 column (250×4.6 mm, 5 μm) at λ=254 nm.

GC/MS analyses were obtained on a FISON GC 8000 gas-chromatograph equipped with a capillary column (MEGA SE52MS, 30 m-long, ID 0.25 mm, film thickness 0.25 μm) and coupled with a FISON MD800 mass detector. FTIR spectra were recorded in $CHCl_3$ on a Brucker Vector 22 spectrometer. ^{1}H NMR and ^{13}C NMR spectra were performed in CD_3OD on Gemini 200 and VARIAN XL300 spectrometers. HRMS spectra were recorded with Micromass Q-TOF *micro* Mass Spectrometer (Waters).

Substrates **1** and **2** were prepared according to the literature,[17] while substrates **3**–**5** were purchased (Sigma–Aldrich). Alkylsulfates (DMS and DES) were distilled to neutrality over $NaHCO_3$, while alkyliodides (MeI and EtI) were distilled over P_2O_5.

4.2. Products from alkylation of 1–5

LiH (1.2 equiv) was added to a solution of the appropriate substrate (1.0 g) in dry DMF (80 ml) under a dry Ar atmosphere and the mixture was stirred at 60 °C for 0.5 h. Next, 1.2 equiv of the appropriate freshly distilled alkylsulfate (DMS or DES) were added, the solution was stirred at 60 °C and the reaction progress was monitored by TLC. After two days (three days in the case of uracil **5**), the solution was left to cool, neutralised while stirring with 1.2 equiv of solid NH_4Cl and evaporated under reduced pressure at 40 °C. The residue was suspended in *n*-butanol, dried over anhydrous Na_2SO_4, filtered and evaporated to leave a crude mixture, which was separated by chromatography (SiO_2, 1:100; eluent $CHCl_3$/MeOH=99.5:0.5).

All isolated new compounds were determined to be >95% pure by HPLC or GLC and are reported in elution order together with their spectroscopic properties. All isolated known products were identified by comparison with either authentic samples or by their literature described spectroscopic properties and are reported in elution order.

4.2.1. Products from 4-methoxy-5-methyl-2(1*H*)-pyrimidinone 1.

4.2.1.1. Methylation. 5-Methyl-2,4-dimethoxypyrimidine **7a** (110 mg, 10%);[17] 1,3,5-trimethyl-2,4-pyrimidinedione **10a** (12 mg, 1%);[24] 4-methoxy-1,5-dimethyl-2(1*H*)-pyrimidinone **6a** (0.65 g, 59%);[9a] 1,5-dimethyl-2,4(3*H*)-pyrimidindione **9a** (0.15 g, 15%).

4.2.1.2. Ethylation.

*4.2.1.2.1. 2-Ethoxy-4-methoxy-5-methylpyrimidine (**7b**).* Colourless oil (0.42 g, 35%); δ_H 8.05 (1H, q, *J* 1.0 Hz, C_6H), 4.23 (2H, q, *J* 7.0 Hz, O^2-CH_2), 3.95 (3H, s, O^4-CH_3), 2.07 (3H, d, *J* 1.0 Hz, C_5-CH_3), 1.33 (3H, t, *J* 7.0 Hz, CH_3); δ_C 170.8 (C_4), 168.8 (C_2), 161.3 (C_6), 110.2 (C_5), 63.4 (O^2-C), 55.0 (O^4-C), 13.6 (Me), 13.1 (C_5-Me); *m/z* (%) 168 (38), 153 (60), 140 (53), 125 (36), 124 (100), 82 (70), 55 (83); ν_{max} 1570, 1455, 1256, 1065 cm^{-1}; HRMS found, 168.0898. $C_8H_{12}N_2O_2$ requires 168.0899.

*4.2.1.2.2. 1-Ethyl-4-methoxy-5-methyl-2(1H)-pyrimidinone (**6b**).* White solid (0.46 g, 39%); δ_H 7.17 (1H, q, *J* 1.0 Hz, C_6H), 3.96 (3H, s, OCH_3), 3.86 (2H, q, *J* 7.0 Hz, N^1-CH_2), 1.92 (3H, d, *J* 1.0 Hz, C_5-CH_3), 1.31 (3H, t, *J* 7.0 Hz, CH_3); δ_C 170.66 (C_2), 156.60 (C_4), 143.60 (C_6), 104.61 (C_5), 54.53 (O-C), 45.16 (N^1-C), 14.55 (CH_3), 12.10 (C_5-CH_3); *m/z* (%) 168 (100), 167 (20), 140 (60), 125 (22), 110 (78), 82 (20), 55 (19); ν_{max} 1644, 1628, 1545, 1485 cm^{-1}; HRMS found, 168.0896. $C_8H_{12}N_2O_2$ requires 168.0899.

4-Ethoxy-1-ethyl-5-methyl-2(1*H*)-pyrimidinone **11b** (4 mg, 0.3%);[24] 1,3-diethyl-5-methyl-2,4-pyrimidindione **10b** (52 mg, 4%);[22] 1-ethyl-5-methyl-2,4(3*H*)-pyrimidindione **9b** (22 mg, 2%).[21] Traces of **6a** were also found by GC–MS.

4.2.2. Products from 4-methoxy-2(1*H*)-pyrimidinone 2.

4.2.2.1. Methylation. 2,4-Dimethoxypyrimidine **7c** (120 mg, 11%);[17] 1,3-dimethyl-2,4-pyrimidindione **10c** (45 mg, 4%);[24] 4-methoxy-1-methyl-2(1*H*)-pyrimidinone **6c** (0.77 g, 70%);[9a] 1-methyl-2,4(3*H*)-pyrimidindione **9c** (80 mg, 8%).

4.2.2.2. Ethylation.

*4.2.2.2.1. 2-Ethoxy-4-methoxypyrimidine (**7d**).* Colourless oil (0.41 g, 39%); δ_H 8.17 (1H, d, *J* 5.5 Hz, C_6H), 6.42 (1H, d,

J 5.5 Hz, C_5-H), 4.40 (2H, q, *J* 7.0 Hz, O^2-CH_2), 3.97 (3H, s, O^4-CH_3), 1.32 (3H, t, *J* 7.0 Hz, CH_3); δ_C 171.1 (C_4), 169.5 (C_2), 161.1 (C_6), 101.0 (C_5), 62.9 (O^2-C), 54.9 (O^4-C), 14.1 (Me); *m/z* (%) 154 (28), 139 (41), 126 (34), 110 (100), 96 (42), 68 (60); ν_{max} 1580, 1460, 1380, 1268, 1085 cm^{-1}; HRMS found, 154.0744. $C_7H_{10}N_2O_2$ requires 154.0742.

4-Ethoxy-1-ethyl-2(1*H*)-pyrimidinone **11d** (3 mg, 0.2%);[9a] 1,3-diethyl-2,4-pyrimidindione **10d** (46 mg, 3.5%);[23] 1-ethyl-4-methoxy-2(1*H*)-pyrimidinone **6d** (0.63 g, 52%);[9a] 1-ethyl-2,4(3*H*)-pyrimidindione **9d** (33 mg, 3%). Traces of **6c** were also found by GC–MS.

4.2.3. Products from 4-acetylamino-2(1*H*)-pyrimidinone 3.

4.2.3.1. Methylation. 4-Acetylamino-2-methoxypyrimidine **7e** (54 mg, 5%);[20] 4-acetylamino-1-methyl-2(1*H*)-pyrimidinone **6e** (0.52 g, 48%).[19]

4.2.3.2. Ethylation.

*4.2.3.2.1. 4-Acetylamino-2-ethoxypyrimidine (**7f**).* White solid (106 mg, 9%); δ_H 8.22 (1H, d, *J* 5.5 Hz, C_6H), 7.62 (1H, d, *J* 5.5 Hz, C_5H), 4.26 (2H, q, *J* 7.0 Hz, O^2-CH_2), 2.07 (3H, s, N^4-$COCH_3$), 1.27 (3H, t, *J* 7.0 Hz, CH_3); δ_C 172.85 (C_2), 165.99 (C=O), 161.26 (C_4), 161.14 (C_6), 104.63 (C_3), 64.42 (O^2-C), 24.29 (C(O)-CH_3), 14.78 (CH_3); *m/z* (%) 181 (M^+, 20), 166 (47), 137 (47), 111 (47), 95 (100), 68 (28), 43 (83); ν_{max} 3230, 1675, 1390, 1265 cm^{-1}; HRMS found, 181.0849. $C_8H_{11}N_3O_2$ requires 181.0851.

*4.2.3.2.2. 4-Acetylamino-1-ethyl-2(1H)-pyrimidinone (**6f**).* White solid (0.75 g, 63%); δ_H 7.91 (1H, d, *J* 7.0 Hz, C_6H), 7.28 (1H, d, *J* 7.0 Hz, C_5H), 3.84 (2H, q, *J* 7.0 Hz, N^1-CH_2), 2.07 (3H, s, N^4-$COCH_3$), 1.23 (3H, t, *J* 7.0 Hz, CH_3); δ_C 73.04 (C=O), 164.20 (C_4), 158.68 (C=O), 150.70 (C_6), 98.25 (C_5), 41.12 (N^1-C), 24.53 (C(O)-CH_3), 14.50 (CH_3); *m/z* (%) 81 (M^+, 70), 166 (100), 139 (41), 138 (64), 111 (62), 81 (49), 43 (97); ν_{max} 3240, 1700, 1674, 1645, 1495 cm^{-1}; HRMS found, 181.0853. $C_8H_{11}N_3O_2$ requires 181.0851.

4.2.4. Products from thymine 4.

4.2.4.1. Methylation. 1,3,5-Trimethyl-2,4-pyrimidindione **10a** (86 mg, 7%);[24] 3,5-dimethyl-2,4(1*H*)-pyrimidindione **12a** (56 mg, 5%);[16] 1,5-dimethyl-2,4(3*H*)-pyrimidindione **9a** (0.22 g, 20%).

4.2.4.2. Ethylation.

*4.2.4.2.1. 2-Ethoxy-3-ethyl-5-methyl-2(3H)-pyrimidinone (**13b**).* *m/z* (%) 182 (M^+, 40), 154 (55), 126 (100), 110 (83), 83 (55), 82 (58) in 1:5 mixture (0.13 g, 12%) via HPLC with **10b**;[22] the mixture **13b+10b** (50 mg) was dissolved in 2 M HCl (2.5 ml), warmed at 60 °C for 2 h, evaporated, redissolved in MeOH and analysed via HPLC to give a 1:5 mixture of **12b** and **10b**.

4-Ethoxy-1-ethyl-5-methyl-2(1*H*)-pyrimidinone **11b** (43 mg, 3%);[24] 3-ethyl-5-methyl-2,4(1*H*)-pyrimidindione **12b** (50 mg, 4%);[16] 1-ethyl-5-methyl-2,4(3*H*)-pyrimidindione **9b** (0.42 g, 34%).

When KH was used in place of LiH (see run 9 in Table 2) 2,4-diethoxy-5-methylpyrimidine **14b** (3.2%)[24] was detected by HPLC among the other products and recognised by GC–MS coupling; *m/z* (%) 182 (M^+, 27), 167 (18), 154 (100), 138 (20), 126 (64), 110 (54), 96 (50).

4.2.5. Products from uracil 5.

4.2.5.1. Methylation. 1,3-Dimethyl-2,4-pyrimidindione **10c** (0.15 g, 12%); 3-methyl-2,4(1*H*)-pyrimidindione **12c** (56 mg, 5%);[16] 1-methyl-2,4(3*H*)-pyrimidindione **9c** (0.33 g, 30%).[24]

4.2.5.2. Ethylation.

*4.2.5.2.1. 2-Ethoxy-3-ethyl-2(3H)-pyrimidinone (**13d**).* *m/z* (%) 168 (M^+, 57), 140 (56), 112 (53), 96 (40), 82 (100), 69 (30), 68 (31) in 1:6 mixture (0.22 g, 15%) via HPLC with **10d**;[23] the mixture **13d+10d** (50 mg) was dissolved in 2 M HCl (2.5 ml), warmed at 60 °C for 2 h, evaporated, redissolved in MeOH and analysed via HPLC to give a 1:6 mixture of **12d** and **10d**.

4-Ethoxy-1-ethyl-2(1*H*)-pyrimidinone **11d** (45 mg, 3%);[9a] 3-ethyl-2,4(1*H*)-pyrimidindione **12d** (62 mg, 5%);[16] 1-ethyl-2,4(3*H*)-pyrimidindione **9d** (0.52 g, 42%).

When KH was used in place of LiH (see run 18 in Table 2) 2,4-diethoxy-pyrimidine **14d** (4%)[9a] was detected by HPLC among the other products and recognised by GC–MS coupling; *m/z* (%) 168 (M^+, 29), 140 (53), 124 (17), 112 (68), 96 (100), 82 (25), 70 (51).

4.3. HPLC distribution of products

One equivalent of the appropriate metal hydride (LiH or KH) was added under stirring to 5.0 ml of a 0.07 M solution of the substrates **1**–**5** in dioxane or DMF, and the mixture was stirred for 30 min at 60 °C under a dry Ar atmosphere. Subsequently, 1.3 equiv of the appropriate alkylating agent (DMS, DES, MeI or EtI) were added and stirring at 60 °C was continued for the time reported in Tables 1 and 2. After this time, 1 equiv of solid NH_4Cl was added and the resulting suspension was filtered. DMF solutions were analysed directly by HPLC, while reactions in dioxane were evaporated under reduced pressure and redissolved in MeOH. Reaction mixtures from **2** and **5** were analysed through a gradient elution from 100% H_2O (1 min) to 100% MeCN in 15 min (flow=0.7 ml/min), while a gradient elution from H_2O/MeCN=90:10 to 100% MeCN in 20 min (flow=1 ml/min) was used for mixtures from **1**, **3** and **4**. Results are reported in Tables 1 and 2 as average of two independent runs.

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